JSCSEN 75(12)1617-1762(2010)

Journal of the Serbian Chemical Society

VOLUME 75

No 12

BELGRADE 2010

Available on line at



www.shd.org.rs/JSCS/







 $JSCS\text{-}info@shd.org.rs \bullet www.shd.org.rs/JSCS$

J. Serb. Chem. Soc. Vol. 75, No. 12 (2010)

CONTENTS

Organic Chemistry
Y. Xia, Y. Guo and Y. Wen: The total synthesis of cannabisin G 1617
R. Kasimogullari, B. Zengin, M. Maden, S. Mert and C. Kazaz: Synthesis of new deri-
vatives of 1-(3-aminophenyl)-4-benzoyl-5-phenyl-1 <i>H</i> -pyrazole-3-carboxylic acid 1625
<i>B. Fejin, Lj. vujisic, M. Sabovijevic, A. Subovijevic, V. Tesevic</i> and <i>V. vujis.</i> Ferininiary analysis of fatty acid chemistry of <i>Kindhergia praelonga</i> and <i>Kindhergia stokesii</i>
(Brachytheciaceae) (Short communication)
Biochemistry and Biotechnology
D. Gođevac, V. Tešević, M. Veličković, Lj. Vujisić, V. Vajs and S. Milosavljević: Poly-
phenolic compounds in seeds from some grape cultivars grown in Serbia 1641
N. S. Radulović, N. D. Dorđević and R. M. Palić: Volatiles of Pleurospermum austri-
acum (L.) HOIIM. (Apiaceae)
composition and antibacterial activity of the essential oil of <i>Levisticum officinale</i>
Koch flowers and fruits at different developmental stages
Theoretical Chemistry
Lj. Andjelković, S. Grubišić, I. Djordjević, M. Zlatar, S. Niketić and M. Gruden-Pavlović:
Consistent force field for metalloporphyrins
Electrochemistry
A. A. Ensafi, S. Dadkhah-Tehrani and B. Rezaei: Voltammetric determination of dopa-
mine in the presence of uric acid using a 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)- -naphthalin-4-sulfonic acid modified glassy carbon electrode
Analytical Chemistry
D. Kostić, S. Mitić, G. Miletić, S. Despotović and A. Zarubica: The concentrations of Fe.
Cu and Zn in selected wines from South-East Serbia 1701
Polymers
M. B. Milovanović, M. Avramović, L. Katsikas and I. G. Popović: Simplification of the
synthesis of the reversible addition-fragmentation chain transfer agent 2-(2-cya-
nopropyl)-ditniobenzoate
Thermodynamics
R. Chanda, A. Banerjee and M. N. Roy: Studies of viscous antagonism, excess molar volumes viscosity deviation and isentropic compressibility of ternary mixtures
containing <i>N</i> , <i>N</i> -dimethylformamide, benzene and some ethers at 298.15 K
Environmental
L. D. Gomidželović, E. D. Požega and V. K. Trujić: The possibilities of the utilization of
the polymetallic concentrate Čoka Marin
Contents of Volume 75 1743
Author index 1755

Published by the Serbian Chemical Society Karnegijeva 4/III, 11000 Belgrade, Serbia Printed by the Faculty of Technology and Metallurgy Karnegijeva 4, P.O. Box 35-03, 11120 Belgrade, Serbia

Available online at www.shd.org.rs/JSCS/







J. Serb. Chem. Soc. 75 (12) 1617–1623 (2010) JSCS–4081 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 633.888:542.913 Original scientific paper

The total synthesis of cannabisin G

YAMU XIA*, YINGLAN GUO and YANLING WEN

College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China

(Received 26 October 2009, revised 29 June 2010)

Abstract: A convenient method for the synthesis of lignanamide cannabisin G, starting from vanillin, was developed. The convergent synthesis was based on the Stobbe reaction as C–C bond-forming steps to give the skeleton of lignan, which was condensed with a derivative of tyramine to obtain synthetic cannabisin G for the first time.

Keywords: synthesis; lignanamide; stobbe reaction; cannabisin G.

INTRODUCTION

Cannabisin G (1) was first isolated from the fruits of *Cannabis sativa* in 1995.¹ *C. sativa* is an annual plant which belongs to the family Cannabaceae from Central Asia.² *C. sativa* has been utilized as an anti-asthma, anticonstipation, anthelminthic drug in traditional Chinese medicine, and these uses are still well-rooted in folk medicine today.^{3,4} Cannabinoids, flavonoids, stilbenoids, terpenoids, alkaloids and lignanamides are some of the secondary metabolites present in *C. sativa*.⁵ Cannabisin G belongs to the lignanamide group and is classified as lignans of the arylnaphthalene derivative type. Natural products of the lignanamide family displayed interesting and diverse biological activities, including feeding deterrent activity and insecticidal effects.^{6,7} In 2002, it was first reported that cannabisin G showed cytotoxic activity against human prostate cancer LNCaP cells.⁸

A synthetic approach to the lignanamide family has not hitherto been reported. Herein, full details of the total synthesis of the lignanamide cannabisin G(1) are provided.

In the retrosynthetic analysis (Scheme 1), cannabisin G must be developed for the coupling of (E,E)-2 with tyramine. The key intermediate (E,E)-2 is obtained by the condensation of vanillin with diethyl succinate.

1617

Available online at www.shd.org.rs/JSCS/



^{*}Corresponding author. E-mail: xiaym@qust.edu.cn doi: 10.2298/JSC091016128X

XIA, GUO and WEN



Scheme 1. In the retrosynthetic analysis, cannabisin G must be developed by the coupling of (E,E)-2 with tyramine. The key intermediate (E,E)-2 was obtained by the condensation of vanillin and diethyl succinate, involving double Stobbe reactions.

As shown in Scheme 2, the synthesis involved the Stobbe reaction to construct the skeleton of lignan (C_6 - C_4 - C_6), followed by condensation with tyramine to obtain cannabisin G (1).



Scheme 2. Reaction scheme for the preparation of cannabisin G starting from vanillin.

Available online at www.shd.org.rs/JSCS/



EXPERIMENTAL

General

Melting points were taken on a Gallenkamp melting point apparatus and were uncorrected. The infrared spectra were recorded on a Nicolet Nexus 670 FTIR spectrometer. The ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker AM–500 MHz spectrometer. The mass spectra were recorded on a ZAB–HS spectrometer. HRMS were obtained on a Bruker Daltonics APEXII47e spectrometer. Flash column chromatography was performed on silica gel (200–300 mesh) and TLC inspections on silica gel GF254 plates.

4-Benzyloxy-3-methoxybenzaldehyde (3)

A mixture of vanillin (60.8 g, 400 mmol), benzyl bromide (68.4 g, 400 mmol) and anhydrous potassium carbonate (55.2 g, 400 mmol) in acetone (200 ml) was stirred overnight at room temperature. The reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was crystallized from EtOH to give the compound **3** as yellow crystals (92.0 g).

(E)-2-(4-benzyloxy-3-methoxybenzylidene)succinic acid (4)

Compound **3** (72.6 g, 300 mmol) and diethyl succinate (52.2 g, 300 mmol) were added to a solution of NaOEt (40.8 g, 600 mmol) in EtOH (500 mL). The mixture was heated under N₂ and was refluxed for 4 h, and then the ethanol was removed. The residue was cooled and acidified with HCl (5 mol L⁻¹, 60 ml). This was then extracted with EtOAc (3×70 mL). The EtOAc layer was then re-extracted with saturated NaHCO₃ solution (300 mL). Acidification of the aq. NaHCO₃ extract with HCl (5 mol L⁻¹, 60 ml) provided an oily layer, which was again extracted with EtOAc (3×70 mL). The combined organic layer was dried over MgSO₄ and concentrated *in vacuo*. This residue was added to a solution of 20 % aqueous NaOH (500 mL) and refluxed for 3 h. After cooling to room temperature, the mixture was washed with EtOAc (3×70 mL). After decolorizing with active carbon, the mixture was acidified with HCl (5 mol L⁻¹, 60 ml) whereby a white solid was obtained. The crude product was crystallized from EtOH to give the diacid **4** as a yellow crystal (120.0 g).

(E)-Dimethyl 2-(4-benzyloxy-3-methoxybenzylidene)succinate (5)

The diacid 4 (68.4 g, 200 mmol) was added to an ice-cold solution containing an excess of CH_2N_2 in Et_2O . The mixture was stirred for 12 h, and concentrated *in vacuo*. Flash column chromatography of the residue gave diester **5** as a yellow oil (71.8 g).

(E,E)-2,3-bis(4-benzyloxy-3-methoxybenzylidene)succinic acid (6)

Diester 5 (37.0 g, 100 mmol) on Stobbe condensation (following the above-mentioned procedure) with compound 3 (24.2 g, 100 mmol) provided a light-yellow solid which was purified by recrystallization from MeOH to yield product 6 (42.5 g).

4-Benzyloxybenzaldehyde (7)

A mixture of 4-hydroxybenzaldehyde (24.4 g, 200 mmol), benzyl bromide (34.0 g, 200 mmol) and anhydrous potassium carbonate (27.6 g, 200 mmol) in acetone(100 ml) were stirred overnight at room temperature. The reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was crystallized from EtOH to give the compound **7** as yellow crystals (39.4 g).

1-Benzyloxy-4-(2-nitroethenyl)benzene (8)

To a mixture of nitromethane (9.2 g, 150 mmol) and compound **7** (31.8 g, 150 mmol) in MeOH (200 mL) was added dropwise sodium hydroxide (8.0 g, 200 mmol) in water (200 mL) under an ice bath. The reaction mixture was stirred for 5 h and then poured into 500 mL of



XIA, GUO and WEN

HCl (2 M) in water. The mixture was filtered and the yellow crystalline mass was washed with water and crystallized from EtOH to give product **8** as yellow crystals (32.1 g).

o-Benzyltyramine (9)

Compound **8** (12.8 g, 50 mmol) in dry Et₂O was added to the solution of LiAlH₄ (5.0 g, 132 mmol) in Et₂O. The mixture was heated at reflux for 4 h under nitrogen. Then the reaction was quenched with ice water and the resulting mixture filtered. The filtrate was dried over anhydrous MgSO₄ and concentrated *in vacuo*. Flash column chromatography of the residue gave product **9** (8.8 g).

Cannabisin G(1)

A solution of compound **6** (2.8 g, 5 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to a solution of compound **9** (2.3 g, 10 mmol), *N*,*N*⁻dicyclohexylcarbodiimideline, DCC (2.1 g, 10 mmol), and 4-dimethylaminopyridineline, DMAP (1.3 g, 10 mmol), in dry CH_2Cl_2 (100 mL) at 0 °C for 2 h under nitrogen. After stirring the mixture overnight at room temperature, the reaction mixture was filtrated and the solvent was distilled off. Flash column chromatography (petroleumether: ethyl acetate = 6:1) of the residue gave product **10** (4.2 g).

Product **10** (3.0 g, 3.0 mmol) was dissolved in 50 mL MeOH and stirred under a hydrogen atmosphere (1 atm) for 7 h in the presence of 5 % Pd/C (1.5 g). The reaction mixture was filtered through a pad of celite and then the solvent was removed *in vacuo*. Flash column chromatography (petroleumether: ethyl acetate = 5:1) of the residue gave an amorphous powder cannabisin G (**1**) (1.4 g).

RESULTS AND DISCUSSION

The analytic and spectroscopic data of cannabisin G and the intermediate products are given below.

4-Benzyloxy-3-methoxybenzaldehyde (3). Yield: 95 %; m.p. 65–67 °C. IR (KBr, cm⁻¹): 3014, 2845, 1679, 1671, 1587, 1505, 1466, 1385, 1276, 1134, 1032. ¹H-NMR (500 MHz, DMSO– d_6 , δ / ppm): 3.84 (3H, *s*, OCH₃), 5.16 (2H, *s*, ArCH₂O), 6.87–7.54 (8H, *m*, ArH), 9.85 (1H, *s*, ArCHO). EI–MS (*m*/*z*, (%)): 242 (M⁺) (12), 214 (7), 151 (2), 91 (100), 67 (13).

(E)-2-(4-Benzyloxy-3-methoxybenzylidene)succinic acid (4). Yield: 83 %; m.p. 131–133 °C. IR (KBr, cm⁻¹): 3250, 3060, 2912, 1709, 1615, 1497, 1484. ¹H-NMR (500 MHz, CDCl₃, δ / ppm): 3.57 (2H, *s*, CH₂), 3.86 (3H, *s*, OCH₃), 5.15 (2H, *s*, ArCH₂O), 6.68–7.43 (8H, *m*, ArH), 7.87 (1H, *s*, ArCH=C). EI–MS (*m*/*z*, (%)): 342 (M⁺) (26), 324 (12), 297 (27), 175 (16), 91 (100).

(E)-Dimethyl 2-(4-benzyloxy-3-methoxybenzylidene)succinate (5). Yield: 97 %; IR (KBr, cm⁻¹): 3080, 2908, 1712 (CH₂COOCH₃), 1641 (COOCH₃), 1503, 1465. ¹H-NMR (500 MHz, CDCl₃, δ / ppm): 3.69 (3H, *s*, COOCH₃), 3.78 (3H, *s*, COOCH₃), 3.83 (3H, *s*, OCH₃), 3.56 (2H, *s*, CH₂COOCH₃), 5.15 (2H, *s*, ArCH₂O), 6.68–7.45 (8H, *m*, ArH), 7.88 (1H, *s*, ArCH=C). EI–MS (*m*/*z*, (%)): 370 (M⁺) (36), 338 (18), 307 (14), 175 (23), 91 (100).

(E,E)-2,3-Bis(4-benzyloxy-3-methoxybenzylidene)succinic acid (**6**). Yield: 75 %; m.p. 151–153 °C. IR (KBr, cm⁻¹): 3350, 2900, 1740 (2×COOCH₃), 1496, 1241, 1042. ¹H-NMR (500 MHz, CDCl₃, δ / ppm): 3.78 (6H, *s*, 2×OCH₃), 5.16 (4H, *s*,

Available online at www.shd.org.rs/JSCS/



2×ArCH₂O), 6.79 (2H, *d*, ArH, J = 8.5 Hz), 7.07 (2H, *dd*, ArH, J = 2.0 and 8.5 Hz), 7.19 (2H, *d*, ArH, J = 2.0 Hz), 7.28–7.40 (10H, *m*, ArH), 7.96 (2H, *s*, 2×ArCH=C). ¹³C-NMR (CDCl₃, 125 MHz, δ / ppm): 55.7 (2×OCH₃), 70.7 (2×ArCH₂), 112.7, 113.1, 123.3, 124.9, 127.2, 127.3, 128.0, 128.6 (2×ArCH=C), 136.4 (2×ArCH=C), 144.2, 149.2, 150.1, 172.7 (2×C=O). EI–MS (*m*/*z*, (%)): 566 (M⁺) (2.1), 549 (4.3), 325 (11), 175 (35), 151 (5.2), 91 (100). HRMS Calcd. for C₃₄H₃₁O₈ (M+H⁺): 567.2014, found: 567.2012.

4-Benzyloxybenzaldehyde (7). Yield: 93 %; m.p. 72–74 °C. IR (KBr, cm⁻¹): 3034, 2840, 2739, 1690, 1601, 1570, 1510, 1453, 1321, 1262, 1166, 1021. ¹H--NMR (500 MHz, CDCl₃, δ / ppm): 5.16 (2H, *s*, ArCH₂O), 7.05–7.84 (9H, *m*, ArH), 9.88 (1H, *s*, ArCHO). EI–MS (*m*/*z*, (%)): 212 (M⁺) (17), 182 (0.8), 151 (1.8), 121 (1.5), 91 (100), 65 (12).

1-Benzyloxy-4-(2-nitroethenyl)benzene (8). Yield: 84 %; m.p. 113–115 °C. IR (KBr, cm⁻¹): 3110, 3042, 2960, 1635, 1607, 1550, 1510, 1346, 1265, 1164. ¹H-NMR (500 MHz, CDCl₃, δ / ppm): 5.15 (2H, *s*, ArCH₂O), 7.04 (2H, *d*, ArH, *J* = 8.5 Hz), 7.35–7.50 (7H, *m*, ArH), 7.52 (1H, *d*, ArCH=CH, *J* = 13.5 Hz), 7.98 (1H, *d*, ArCH=CH, *J* = 13.5 Hz). EI–MS (*m*/*z*, (%)): 255 (M⁺) 1.4), 238 (2.5), 226 (3.2), 151 (2.8), 121 (5.3), 91 (100), 65 (7.1).

o-*Benzyltyramine* (9). Yield: 78 %; m.p. 203–206 °C. IR (KBr, cm⁻¹): 3285, 3050, 3025, 2932, 2867, 2586, 1615, 1597, 1518, 1460, 1259, 1030. ¹H--NMR (500 MHz, CDCl₃, δ / ppm): 2.82–2.85 (2H, *m*, ArCH₂CH₂), 2.94–2.98 (2H, *m*, ArCH₂CH₂), 5.08 (2H, *s*, ArCH₂O), 6.95 (2H, *d*, ArH, *J* = 8.5 Hz), 7.18 (2H, *d*, ArH, *J* = 8.5 Hz), 7.31–7.45 (5H, *m*, ArH). ¹³C-NMR (125 MHz, CDCl₃, δ / ppm): 32.5 (ArCH₂CH₂), 39.8 (ArCH₂CH₂), 69.6 (ArCH₂O), 115.4, 128.1, 128.3, 128.9, 129.9, 130.2, 137.6, 157.6. EI–MS (*m*/*z*, (%)): 227 (M⁺) (8.7), 198 (1.2), 151 (4.9), 121 (23.6), 91 (100); HRMS Calcd. for C₁₅H₁₈NO (M+H⁺): 228.1383, found: 228.1389.

4,4',4",4"'-tetrabenzyloxy cannabisin G (10). Yield: 85 %. ¹H-NMR (500 MHz, CDCl₃, δ / ppm): 2.48 (2H, dt, H-7" α , H-7"" α , J = 13.5, 7.0 Hz), 2.55 (2H, dt, H-7" β , H-7"" β , J = 13.5 and 7.0 Hz), 3.28 (2H, dt, H-8" α , H-8"" α , J = 13.5, 7.0 Hz), 3.52 (2H, dt, H-8" β , H-8"" β , J = 13.5, 7.0 Hz), 3.78 (6H, s, 2×OCH₃), 5.15 (4H, s, 2×ArCH₂), 5.21 (4H, s, 2×ArCH₂), 6.51–7.48 (34H, m, ArH), 7.96 (2H, s, 2×ArCH=C). HRMS Calcd. for C₆₄H₆₄N₃O₈ (M+NH₄⁺): 1002.4688, found: 1002.4683.

Cannabisin G (1). Yield: 75 %. IR (KBr, cm⁻¹): 3356, 2910, 1659, 1615, 1517, 1194. ¹H-NMR (500 MHz, CDCl₃, δ / ppm): 2.43 (2H, *dt*, H-7" α , H-7" α , *J* = 13.6, 6.5 Hz), 2.51 (2H, *dt*, H-7" β , H-7" β , *J* = 13.6, 6.5 Hz), 3.25 (2H, *dt*, H-8" α , H-8" α , *J* = 13.6, 6.5 Hz), 3.50 (2H, *dt*, H-8" β , H-8" β , *J* = 13.6, 6.5 Hz), 3.75 (6H, *s*, 2×OCH₃), 6.82–7.31 (14H, *m*, ArH), 7.89 (2H, *s*, 2×ArCH=C). ¹³C-NMR (125 MHz, CDCl₃, δ / ppm): 35.6 (C-7", C-7""), 42.7 (C-8"", C-8""), 56.8 (2×OCH₃), 113.1, 116.2, 125.4, 127.6, 127.8 (C-8, C-8"), 130.2, 130.7, 140.8 (C-7, 7.2)

Available online at www.shd.org.rs/JSCS/



XIA, GUO and WEN

C-7'), 148.3, 149.2, 156.5, 166.8 (C-9, C-9'). EI–MS (m/z, (%)): 624 (M⁺) (0.2), 339 (18), 337 (9), 151 (23), 91 (100); HRMS Calcd. for C₃₆H₄₀N₃O₈ (M+NH₄⁺): 642.2810, found: 642.2814. The data are consistent with the literature.¹

As is shown in Scheme 2, vanillin was used as the raw material and the 4-hydroxyl group of vanillin was protected with benzyl chloride to afford product **3**. Compound **3** underwent Stobbe condensation with diethyl succinate in the presence of sodium ethoxide in ethanol to produce compound **4**. The (*E*)-configuration of the olefinic double bond was evident from the appearance of the deshielded vinylic proton at δ 7.87 in its ¹H-NMR spectrum.⁹ Compound **4** was methylated with diazomethane in diethyl ether to yield the diester **5**. The second Stobbe condensation of **5** with **3** in methanol in the presence of sodium methoxide yielded the key intermediate **6**. The deshielded vinylic proton at δ 7.96 in the ¹H-NMR spectrum of **6** indicated the (*E*)-configuration for both olefinic double bonds.^{10,11}

4-Hydroxybenzaldehyde was protected with benzyl chloride to give product 7. Condensation of 7 with nitromethane in the presence of sodium hydroxide gave compound 8, which was followed by reduction with $LiAlH_4$ to afford intermediate 9.

The intermediate **6** was condensed with compound **9** in CH_2Cl_2 in the presence of DCC and DMAP, followed by hydrogenolysis with 5 % palladium on charcoal catalyst at room temperature to remove the benzyl group and obtain the target product cannabisin G (1). Although it is possible to affect cleavage of the benzyl group in the presence of an olefin, in general, the degree of selectivity is dependent upon the substitution pattern and the level of steric hindrance. Good selectivity was achieved for hydrogenolysis of a benzyl group in the presence of a trisubstituted conjugated olefin.¹²

CONCLUSIONS

In summary, an efficient, high-yielding and convergent synthesis of a lignanamide cannabisin G with an overall yield of 22.3 % was developed. The synthesis was based on the Stobbe reaction for the C–C bond-formation steps to give the skeleton of lignan, which afforded the key intermediate diacid, which was condensed with a derivative of tyramine to obtain the natural product cannabisin G for the first time. The present method is a new avenue for the synthesis of a variety of useful and biologically active lignanamides.

Acknowledgements. This work was supported by Shandong Provincial Natural Science Foundation, China (No. ZR2010HM023) and Specialized Research Foundation for the Doctoral Program of Higher Education, China (No. 20093719120004).





TOTAL SYNTHESIS OF CANNABISIN G

ИЗВОД

ТОТАЛНА СИНТЕЗА КАНАБИСИНА G

YAMU XIA, YINGLAN GUO и YANLING WEN

College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China

Развијена је погодна синтеза лигнанамида канабисина G, полазећи од ванилина. Конвергентна синтеза заснива се на Стобеовој реакцији, у којој се формира угљеник–угљеник веза скелета лигнана, који је повезан са дериватом тирамина. Овим поступком први пут је добијен синтетички канабисин G.

(Примљено 26. октобра 2009, ревидирано 29. јуна 2010)

REFERENCES

- 1. I. Sakakibara, Y. Ikeya, K. Hayashi, M. Okada, M. Maruno, *Phytochemistry* 38 (1995) 1003
- H. Jiang, X. Li, Y. X. Zhao, D. K. Ferguson, F. Hueber, S. Bera, Y. F. Wang, L. C. Zhao, C. J. Liu, C. S. Li, *J. Ethnopharmacol.* 108 (2006) 414
- 3. R. Mechoulam, N. K. McCallum, S. Burstein, Chem. Rev. 76 (1976) 75
- 4. G. Appendino, S. Gibbons, A. Giana, A. Pagani, G. Grassi, M. Stavri, E. Smith, M. M. Rahman, *J. Nat. Prod.* **71** (2008) 1427
- 5. I. J. Flores-Sanchez, R. Verpoorte, Phytochem. Rev. 7 (2008) 615
- 6. L. Lajide, P. Escoubas, J. Mizutani, Phytochemistry 40 (1995) 1105
- 7. E. S. Garcia, P. Azambuja, Toxicon 44 (2004) 431
- 8. C. Y. Ma, W. K. Liu, C. T. Che, J. Nat. Prod. 65 (2002) 206
- 9. J. Liu, N. R. Brooks, Org. Lett. 4 (2002) 3521
- 10. P. K. Datta, C. Yau, T. S. Hooper, B. L. Yvon, J. L. Charlton, J. Org. Chem. 66 (2001) 8606
- 11. H. Miyazaki, H. Ohmizu, T. Ogiku, Org. Process Res. Dev. 13 (2009) 760
- 12. D. Caine, T. L. Smith Jr., J. Am. Chem. Soc. 102 (1980) 7568.







J. Serb. Chem. Soc. 75 (12) 1625–1635 (2010) JSCS–4082 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 547.77+547.857.8+547.852+ 547-304.4:66.095.3.095.252 Original scientific paper

Synthesis of new derivatives of 1-(3-aminophenyl)-4-benzoyl--5-phenyl-1*H*-pyrazole-3-carboxylic acid

¹Department of Chemistry, Art and Science Faculty, Dumlupinar University, Kutahya and ²Department of Chemistry, Science Faculty, Atatürk University, Erzurum, Turkey

(Received 18 October, revised 6 December 2010)

Abstract: 1-(3-Aminophenyl)-4-benzoyl-5-phenyl-1*H*-pyrazole-3-carboxylic acid (1) was synthesized according to the literature.¹ 2-(3-Aminophenyl)-2,6-dihyd-ro-3,4-diphenyl-7*H*-pyrazolo[3,4-*d*]pyridazin-7-one (5) was obtained by the cyclocondensation reaction of 1 with hydrazine hydrate. New pyrazole derivatives of compounds 1 and 5 were synthesized by their reaction with β -diketones, β -ketoesters, β -naphthol, phenol and various other reagents. The structures of the synthesized compounds were characterized by ¹H-NMR, ¹³C-NMR, IR and mass spectroscopy, as well as elemental analysis.

Keywords: pyrazole-3-carboxylic acid; pyridazin; diazonium salts; cyclocon-densation.

INTRODUCTION

It is known that pyrazole derivatives having heteroaryl groups attached as substituents exhibit significant biological activity and that some pyrazolo-pyridazine compounds containing heteroaryl groups are used to treat many diseases.^{2–4} On the other hand, diazonium salts have been the focus of great interest for a long time since they play a crucial role in organic syntheses and are commercially important coloring agents.^{5,6}

Arene diazonium groups not only couple to activated aromatic carbon atoms, but may also undergo coupling reactions with aliphatic compounds containing active methylene groups. The facilitated abstraction of the acidic proton in β -diketones and β -ketoesters leads to the formation of a resonance stable anion, which can, therefore, behave as a good nucleophile. Thus, the coupling of this anion with aryl diazonium chlorides gives 2-arylazo-hydrazo derivatives, from which azo-hydrazo substituted heterocyclic compounds can be obtained. These



^{*}Corresponding author. E-mail: rahmikasimoglu@hotmail.com doi: 10.2298/JSC101018135K

KASIMOGULLARI et al

compounds play a significant role in the dye industry and, in addition, enable the synthesis of heterocyclic compounds with different biological activities. Hence, these coupling products are among the most investigated groups of compounds.^{2,7–9}

Commencing from these facts, an attempt was made to expand the research on the preparation of different derivatives of pyrazole carboxylic acid compounds, which are biologically very important and exhibit pharmaceutical activities.^{2,5,7}

RESULTS AND DISCUSSION

In this study, first the diazonium salts from compounds 1 and 5, which contain aromatic primary amine groups, were prepared *in situ* (Schemes 1 and 2).^{2,5,6} For this purpose, compounds 1 and 5 were dissolved in an ethanol–water mixture (50 %) containing sodium acetate and the temperature was kept constant (0–5 °C). Three moles of acid were used per mole of the amine compound in the diazotization reaction.^{10,11} In the experiments, different pH ranges were tested and the best yield was observed in the pH range 3.5-4.0.



Scheme 1. The synthesis of compounds 2a-f, 3a-b and 4a-d.

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS



PYRAZOLE CARBOXYLIC ACIDS

In this study, as a result of the coupling reactions of **1** with various β -dicarbonyl compounds that contained an active aliphatic C–H group, derivatives **2a–f** were synthesized in 42–86 % yield. In all these compounds, due to the unpaired electron pairs on the nitrogen atoms, the proton that transferred to the base was subject to resonance. Thus, the products formed may be in the form of azo (–N=N–) or hydrazo (–NH–N=C) tautomeric structures.^{12–17} However, in the present study, an examination of the ¹H-NMR and IR spectra clearly revealed that the signals belonging to compounds **2a–d** at δ 11.90–11.20 ppm and in the range of 3475–3414 cm⁻¹ stem from the hydrogen on the nitrogen in the corresponding hydrazo forms (–NH–N=C) (Fig. 1).



Fig. 1. Possible tautomeric structures for compounds 2a-f.

On reaction of compound 1 with phenol and β -naphthol, derivatives 3a (74 % yield) and 3b (45% yield) were obtained, respectively. The structures of the compounds were verified by their spectral data (see EXPERIMENTAL). An examination of their resonance structures revealed that although the aryl diazonium ion, bearing partial positive charges on both nitrogen atoms, exhibits weak electrophilic character, it normally formed azo compounds in the diazo coupling reaction with the quite active aromatic compounds phenol and β -naphthol, giving the corresponding diazo compounds 3a and 3b. The mechanism of coupling reactions is the same as those of electrophilic aromatic replacement reactions. In the first step, the electrophile binds to the carbon of the nucleophilic substrate through a covalent bond and an intermediate product is formed. Subsequently, a proton transfer to the base occurs. In the phenol and β -naphthol derivatives, coupling occurs almost exclusively in the para position if the para position is free. If the para position is occupied, then coupling occurs in the ortho position.^{18,19}

In the syntheses, the Sandmeyer reaction²⁰ was employed, in which Cu(I) salts as catalysts together with the potassium salts of Br^- and CN^- were used to obtain derivatives **4a** and **b** in 46 and 88 % yield, respectively.²⁰ For replacement



KASIMOGULLARI et al

by I⁻, having a strong nucleophilic character, the KI alone was sufficient without any necessity for a catalyst and thus, compound **4c** was obtained. On heating the diazo compound with H₂O to 100 °C, derivative **4d** was obtained in high yield (85 %). In addition, the cyclocondensation of compound **1** with anhydrous hydrazine hydrate yielded a pyrazolo[3,4-*d*]pyridazin-7-one (**5**).^{1,21} During the reaction of intermediary **5** with different β -diketones, compounds **6a**–**c** were obtained. Derivatives **7a** and **7b** were synthesized by the coupling reactions of **5** with phenol and β -naphthol, respectively (Scheme 2).



Scheme 2. The synthesis of compounds 6a-c and 7a-b.

The yields, melting points, analytic data and spectral data of the prepared compounds are given below.

4-Benzoyl-1-(3-(2-(1-benzoyl-2-ethoxy-2-oxoethylidene)hydrazinyl)phenyl)--5-phenyl-1H-pyrazole-3-carboxylic acid (**2a**). Yield: 42 %; m.p. 147–148 °C. Anal. Calcd. for C₃₄H₂₆N₄O₆: C, 69.62; H, 4.47; N, 9.55 %. Found: C, 69.45; H, 4.58; N, 9.50 %. IR (KBr, cm⁻¹): 2500–3500 (COOH), 3060 (Ar CH), 2901 and 2835 (aliphatic CH), 1724 and 1666 (C=O), 1607–1463 (Ar C=C and C=N). ¹H--NMR (400 MHz, DMSO– d_6 , δ / ppm): 12.70 (1H, br s, COOH), 11.90 (1H, br s, NH–N=C), 7.20–7.90 (19H, m, ArH), 4.30 (2H, q, J = 7.1 Hz, OCH₂), 1.30

Available online at www.shd.org.rs/JSCS/



(3H, *t*, *J* = 7.0 Hz, CH₃). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 191.65 and 189.27 (benzoyl C=O), 163.46 (ester C=O), 162.75 (acid C=O), 145.60 (NH–N=C), 142.85 (pyrazole C–3), 61.79 (OCH₂), 14.33 (CH₃), 140.13, 138.34, 137.20, 133.67, 133.08, 130.38, 130.04, 129.83, 129.70, 129.65, 129.57, 129.53, 129.01, 128.96, 128.73, 128.61, 128.36, 128.25, 123.31, 120.35.

4-Benzoyl-1-(3-(2-(1-benzoyl-2-oxopropylidene)hydrazinyl)phenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (**2b**). Yield: 86 %; m.p. 126–128 °C. Anal. Calcd. for C₃₃H₂₄N₄O₅: C, 71.21; H, 4.35; N, 10.07 %. Found: C, 69.32; H, 4.65; N, 9.57 %. IR (KBr, cm⁻¹): 2600–3500 (COOH), 3061 (Ar CH), 2950 (aliphatic CH), 1680 and 1665 (C=O), 1601–1461 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 13.09 (1H, br s, COOH), 11.23 (1H, br s, NH–N=C), 6.90–7.90 (19H, m, ArH), 2.50 (3H, s, CH₃). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 196.49 (acetyl C=O), 195.43 and 191.30 (benzoyl C=O), 162.78 (acid C=O), 144.32 (NH–N=C), 143.09 (pyrazole C–3), 25.40 (CH₃), 140.13, 139.97, 138.09, 135.93, 133.93, 130.63, 130.02, 129.81, 129.53, 129.33, 129.21, 129.14, 129.04, 128.98, 128.91, 128.40, 128.28, 127.41, 123.50, 112.19; MS (CI) (m/z): 557.0 (M+1).

4-Benzoyl-1-(3-(2-(1-(tert-butoxycaronyl)-2-oxopropylidene)hydrazinyl)phenyl)-5-phenyl-IH-pyrazole-3-carboxylic acid (**2c**). Yield: 49 %; m.p. 230–231 °C. Anal. Calcd. for $C_{31}H_{28}N_4O_6$: C, 67.38; H, 5.11; N, 10.14 5. Found: C, 67.25; H, 5.15; N, 10.17 %. IR (KBr, cm⁻¹): 2500–3500 (COOH), 3415 (NH), 3059 (Ar CH), 2900 and 2835 (aliphatic CH), 1718 and 1666 (C=O), 1605–1461 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 12.70 (1H, br s, COOH), 11.20 (1H, br s, NH–N=C), 6.90–7.90 (14H, m, ArH), 2.60 (9H, s, OC(CH₃)₃), 1.50 (3H, s, CH₃). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 195.47 (acetyl C=O), 191.86 (benzoyl C=O), 185.38 (acid C=O), 163.86 (ester C=O), 146.96 (NH–N=C), 145.31 (pyrazole C–3), 82.63 (OC(CH₃)₃), 28.39 (C(CH₃)₃), 21.58 (CH₃), 142.54, 140.18, 138.49, 135.70, 133.52, 130.60, 130.32, 129.97, 129.48, 129.20, 128.95, 128.77, 127.50, 123.19, 121.95, 115.12.

4-Benzoyl-1-(3-(2-(1-(ethoxycaronyl)-2-oxopropylidene)hydrazinyl)phenyl)--5-phenyl-IH-pyrazole-3-carboxylic acid (2d). Yield: 47 %; m.p. 240–242 °C. Anal. Calcd. for C₂₉H₂₄N₄O₆: C, 66.41; H, 4.61; N, 10.68 %. Found: C, 66.29; H, 4.65; N, 10.65 %. IR (KBr, cm⁻¹): 2600–3500 (COOH), 3415 (NH), 3060 (Ar CH), 2883 (aliphatic CH), 1665 (C=O), 1609–1460 (Ar C=C and C=N). ¹H--NMR (400 MHz, DMSO–d₆, δ / ppm): 12.80 (1H, br s, COOH), 11.60 (1H, br s, NH–N=C), 7.80–7.10 (14H, m, ArH), 4.30 (2H, q, J = 7.1 Hz, OCH₂), 2.26 (3H, s, CH₃), 1.26 (3H, t, J = 7.1 Hz, OCH₂CH₃). ¹³C-NMR (100 MHz, DMSO-d₆, δ / ppm): 195.47 (acetyl C=O), 191.86 (benzoyl C=O), 185.38 (acid C=O), 163.86 (ester C=O), 145.90 (NH–N=C), 144.21 (pyrazole C–3), 60.93 (OCH₂), 24.40 (O=CCH₃), 14.20 (CH₂CH₃), 142.35, 139.15, 138.45, 135.50, 134.01, 130.65, 130.40, 130.12, 129.45, 129.32, 128.85, 128.53, 127.60, 123.29, 112.10.

KASIMOGULLARI et al

4-Benzoyl-1-(3-(2-(1-benzoyl-2-oxo-2-phenylethylidene)hydrazinyl)phenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (2e). Yield: 55 %; m.p. 205–206 °C. Anal. Calcd. for $C_{38}H_{26}N_4O_5$: C, 73.78; H, 4.24; N, 9.06 %. Found: C, 73.67; H, 4.30; N, 9.11 %. IR (KBr, cm⁻¹): 2500–3500 (COOH), 3422 (NH), 3059 (Ar CH), 1725 and 1664 (C=O), 1599–1460 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 13.70 (1H, br s, COOH), 13.20 (1H, br s, NH–N=C), 7.75–7.15 (24H, m, ArH). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 197.46, 196.79 and 191.28 (benzoyl C=O), 162.78 (acid C=O), 143.43 (NH–N=C), 143.21 (pyrazole C–3), 113.60 (pyrazole C–4), 143.12, 142.99, 140.13, 139.96, 138.09, 134.53, 133.94, 130.71, 130.49, 130.11, 130.04, 129.82, 129.57, 129.13, 129.03, 128.97, 128.45, 128.27, 128.18, 123.48, 122.38, 118.30, 116.96.

1-(3-(2-(1-Acetyl-2-oxo-propylidene)hydrazinyl)phenyl)-4-benzoyl-5-phenyl--IH-pyrazole-3-carboxylic acid (**2***f*). Yield: 55 %; m.p. 222–224 °C. Anal. Calcd. for C₂₈H₂₂N₄O₅: C, 68.01; H, 4.48; N, 11.33 %. Found: C, 67.89; H, 4.53; N, 11.35 %. IR (KBr, cm⁻¹): 2500–3500 (COOH), 3422 (NH), 3059 (Ar CH), 1725 and 1664 (C=O), 1599–1460 (Ar C=C and C=N); ¹H-NMR (400 MHz, DMSO–d₆, δ / ppm): 13.75 (1H, br s, COOH), 13.15 (1H, br s, NH–N=C), 7.80–7.20 (14H, m, ArH), 2.45 and 2.29 (6H, s, 2CH₃). ¹³C-NMR (100 MHz, DMSO–d₆, δ / ppm): 197.49 and 196.78 (acetyl C=O), 191.25 (benzoyl C=O), 162.70 (acid C=O), 143.43 (NH–N=C), 143.19 (pyrazole C–3), 113.57 (pyrazole C-4), 31.67 and 26.88 (CH₃), 142.99, 140.11, 138.06, 134.59, 133.94, 130.72, 130.02, 129.81, 129.54, 129.13, 129.02, 128.16, 123.48, 122.36, 116.96. MS (CI) (*m*/*z*): 495.0 (M+1).

4-Benzoyl-1-(3-((4-hydroxyphenyl)diazenyl)phenyl)-5-phenyl-1H-pyrazole--3-carboxylic acid (**3a**). Yield: 74 %; m.p. 286–288 °C. Anal. Calcd. for C₂₉H₂₀N₄O₄: C, 71.30; H, 4.13; N, 11.47 %. Found: C, 71.17; H, 4.18; N, 11.45 %. IR (KBr, cm⁻¹): 2700–3600 (COOH), 3416 (NH), 3060 (Ar CH), 1663 (C=O), 1607–1494 (Ar C=C and C=N), 1354 (ArOH); ¹H-NMR (400 MHz, DMSO–d₆, δ / ppm): 12.95 (1H, *br s*, COOH), 7.85–7.15 (18H, *m*, ArH), 6.95 (1H, *d*, *J* = 8.7 Hz, ArOH). ¹³C-NMR (100 MHz, DMSO–d₆, δ / ppm): 192.38 (benzoyl C=O), 164.92 (acid C=O), 150.13 (C=C–OH), 149.50 (ArC–N=N), 145.15 (pyrazole C–3), 96.37 (pyrazole C–4), 143.25, 141.87, 140.34, 138.82, 135.40, 133.24, 130.75, 130.41, 130.15, 129.86, 129.44, 129.18, 128.97, 128.82, 128.40, 122.99, 116.74.

4-Benzoyl-1-(3-((2-hydroxynaphthalen-1-yl)diazenyl)phenyl)-5-phenyl-1H--pyrazole-3-carboxylic acid (**3b**). Yield: 45 %; m.p. 261–263 °C. Anal. Calcd. for C₃₃H₂₂N₄O₄: C, 73.60; H, 4.12; N, 10.40 %. Found: C, 73.48; H, 4.19; N, 10.47 %. IR (KBr, cm⁻¹): 2500–3600 (COOH), 3415 (NH), 3064 (Ar CH), 1665 (C=O), 1609–1493 (Ar C=C and C=N), 1354 (ArOH). ¹H-NMR (400 MHz, DMSO–*d*₆, δ / ppm): 15.54 (1H, *s*, ArOH), 13.30 (1H, *br s*, COOH), 8.26–6.86 (20H, *m*, ArH). ¹³C-NMR (100 MHz, DMSO–*d*₆, δ / ppm): 191.27 (benzoyl C=O), 171.37

Available online at www.shd.org.rs/JSCS/

(acid C=O), 162.77 (ArC–OH), 145.47 (pyrazole C–3), 143.47, 143.37, 141.33, 140.28, 138.12, 133.96, 132.93, 130.87, 130.15, 129.92, 129.90, 129.62, 129.35, 129.33, 129.14, 129.05, 128.31, 128.29, 126.67, 124.63, 124.50, 123.64, 121.99, 119.83, 114.90. MS (CI) (*m*/*z*): 539.0 (M+1).

4-Benzoyl-1-(3-bromophenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (4a). Yield: 46 %; m.p. 273–274 °C. Anal. Calcd. for C₂₃H₁₅BrN₂O₃: C, 61.76; H, 3.38; N, 6.26 %. Found: C, 61.55; H, 3.45; N, 6.22 %. IR (KBr, cm⁻¹): 2500–3500 (COOH), 3062 (Ar CH), 1666 (C=O), 1611–1448 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 13.20 (1H, br s, COOH), 7.82–7.21 (14H, m, ArH). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 190.52 (benzoyl C=O), 165.28 (acid C=O), 144.47 (pyrazole C–3), 142.55, 141.30, 139.67, 138.25, 137.64, 134.06, 130.85, 130.26, 129.60, 129.30, 129.25, 129.08, 126.27, 123.75, 123.40, 122.50.

4-Benzoyl-1-(3-cyanophenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (**4b**). Yield: 88 %; m.p. 255–256 °C. Anal. Calcd. for C₂₄H₁₅N₃O₃: C, 73.27; H, 3.84; N, 10.68 %. Found: C, 73.15; H, 3.87; N, 10.74 %. IR (KBr, cm⁻¹): 2800–3600 (COOH), 3061 (Ar CH), 2130 (CN), 1661 (C=O), 1601–1426 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 13.80 (1H, br s, COOH), 7.95–6.70 (14H, m, ArH). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 190.52, (benzoyl C=O), 165.35, (acid C=O), 144.48 (pyrazole C–3), 116.13 (CN), 143.25, 141.12, 139.68, 138.82, 137.63, 134.06, 130.53, 130.27, 129.62, 129.30, 129.08, 128.97, 127.88, 126.26, 123.07, 122.92.

4-Benzoyl-1-(3-iodophenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (4c). Yield: 70 %; m.p. 265–266 °C. Anal. Calcd. for C₂₃H₁₅IN₂O₃: C, 55.89; H, 3.06; N, 5.67 %. Found: C, 55.78; H, 3.11; N, 5.64 %. IR (KBr, cm⁻¹): 2500–3500 (COOH), 3059 (Ar CH), 1664 (C=O), 1608–1427 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 13.10 (1H, br s, COOH), 7.80–7.17 (14H, m, ArH). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 191.28 (benzoyl C=O), 162.79 (acid C=O), 143.38 (pyrazole C–3), 95.20 (ArC–I), 139.96, 138.09, 133.92, 130.49, 130.10, 129.80, 129.56, 129.12, 128.97, 128.28, 127.10, 124.95, 123.44, 118.66, 107.62. MS (CI) (m/z): 495.0 (M+1).

4-Benzoyl-1-(3-hydroxyphenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (4d). Yield: 85 %; m.p. 235–237 °C. Anal. Calcd. for $C_{23}H_{16}N_2O_4$: C, 71.87; H, 4.20; N, 7.29 %. Found: C, 71.79; H, 4.26; N, 7.32 %. IR (KBr, cm⁻¹): 2700–3600 (COOH), 3059 (Ar CH), 1665 (C=O), 1607–1428 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 12.70 (1H, br s, COOH), 10.15 (1H, br s, Ar–OH) 7.74–7.15 (14H, m, ArH). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 190.52 (benzoyl C=O), 165.30 (acid C=O), 152.13 (ArC–OH), 144.48 (pyrazole C–3), 143.22, 139.70, 137.61, 134.07, 130.55, 130.26, 129.89, 129.77, 129.62, 129.08, 128.91, 127.89, 127.41, 123.08, 114.96.

KASIMOGULLARI et al

2-(3-(2-(1-Acetyl-2-oxopropylidene)hydrazinyl)phenyl)-2,6-dihydro-3,4-diphenyl-7H-pyrazolo[3,4-d]pyridayin-7-one (**6a**). Yield: 79 %; m.p. 214–216 °C. Anal. Calcd. for $C_{28}H_{22}N_6O_3$: C, 68.56; H, 4.52; N, 17.13 %. Found: C, 68.39; H, 4.54; N, 17.13 %. IR (KBr, cm⁻¹): 3456 and 3155 (NH), 3021 (Ar–CH), 2969 (aliphatic CH), 1740 (acetyl C=O), 1663 (amide C=O), 1608–1476 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 7.31 (1H, *br s*, NH), 7.56–6.92 (14H, *m*, ArH), 2.41 and 2.26 (6H, *s*, 2CH₃). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 197.67 and 196.95 (tautomeric acetyl C=O), 156.79 (amide C=O), 144.28 (ArC–NH–N), 143.16 (pyrazole C–3), 140.58 (pyrazole C–5), 31.82 and 27.07 (tautomeric CH₃), 134.92, 131.20, 131.17, 129.66, 129.61, 128.96, 128.81, 128.77, 128.52, 128.47, 128.41, 128.38, 128.01, 123.17, 117.37, 117.35, 114.30.

2-(3-(2-(1-Benzoyl-2-oxo-2-phenylethylidene)hydrazinyl)phenyl)-2,6-dihydro-3,4-diphenyl-7H-pyrazolo[3,4-d]pyridayin-7-one (**6b**). Yield: 88 %; m.p. 234– -236 °C. Anal. Calcd. for $C_{38}H_{26}N_6O_3$: C, 74.25; H, 4.26; N, 13.67 %. Found: C, 74.12; H, 4.28; N, 13.65 %. IR (KBr, cm⁻¹): 3461 and 3156 (NH), 3023 (Ar CH), 1740 (benzoyl C=O), 1670 (amide C=O), 1601–1475 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO–d₆, δ / ppm): 11.60 (1H, br s, NH), 8.15–6.82 (24H, m, ArH). ¹³C-NMR (100 MHz, DMSO–d₆, δ / ppm): 186.02 (benzoyl C=O), 156.81 (amide C=O), 144.47 (ArC–NH–N), 144.27 (NH–N=C), 142.78 (pyrazole C–3), 140.88 (pyrazole C–5), 140.32, 135.29, 134.92, 133.69, 133.15, 131.15, 130.76, 130.61, 129.73, 129.61, 129.52, 129.29, 129.17, 128.94, 128.89, 128.79, 128.47, 128.40, 128.31, 128.09, 128.01, 117.37, 93.97.

2-(3-(2-(1-Benzoyl-2-oxopropylidene)hydrazinyl)phenyl)-2,6-dihydro-3,4-diphenyl-7H-pyrazolo[3,4-d]pyridayin-7-one (**6c**). Yield: 50 %; m.p. 256–257 °C. Anal. Calcd. for $C_{33}H_{24}N_6O_3$: C, 71.73; H, 4.38; N, 15.21 %. Found: C, 71.58; H, 4.41; N, 15.22 %; IR (KBr, cm⁻¹): 3393–3241 (NH), 3067 and 3028 (Ar CH), 1666 (C=O), 1608–1477 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– $d_6, \delta /$ / ppm): 12.61 (1H, br s, NH), 7.45–6.92 (19H, m, ArH), 2.47 (3H, s, CH₃). ¹³C--NMR (100 MHz, DMSO– $d_6, \delta /$ ppm): 199.50 (acetyl C=O), 188.25 (benzoyl C=O), 156.81 (amide C=O), 144.28 (ArC–NH–N), 142.78 (pyrazole C–3), 140.89 (pyrazole C–5), 20.24 (CH₃), 140.33, 134.92, 131.33, 131.17, 130.77, 130.61, 129.61, 129.43, 129.09, 128.94, 128.78, 128.54, 128.47, 128.41, 128.01, 127.60, 123.86, 122.57, 117.37, 95.27.

2,6-Dihydro-2-(3-((4-hydroxyphenyl)diazenyl)phenyl)-3,4-diphenyl-7H-pyrazolo[3,4-d]pyridazin-7-one (7a). Yield: 68 %; m.p. 278–279 °C. Anal. Calcd. for C₂₉H₂₀N₆O₂: C, 71.89; H, 4.16; N, 17.35 %. Found: C, 71.78; H, 4.16; N, 17.37 %. IR (KBr, cm⁻¹): 3622 (OH), 3446–3160 (NH), 3062 and 3024 (Ar CH), 1662 (amide C=O), 1608–1476 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO– d_6 , δ / ppm): 12.54 (1H, br s, NH), 10.31 (1H, s, OH), 7.84–6.91 (18H, m, ArH). ¹³C-NMR (100 MHz, DMSO– d_6 , δ / ppm): 162.28 (ArC–OH), 156.82 (amide C=O), 153.00 and 145.70, (ArC–N=N), 144.29, 142.90, 141.07, 140.39,

134.91, 131.29, 130.55, 129.72, 128.98, 128.82, 128.56, 128.38, 128.18, 128.01, 125.86, 124.35, 119.31, 117.41, 116.74.

2,6-Dihydro-2-(3-((2-hydroxynaphthalen-1-yl)diazenyl)phenyl)-3,4-diphenyl-7H-pyrazolo[3,4-d]pyridazin-7-one (**7b**). Yield: 70 %; m.p. 234–235 °C. Anal. Calcd. for C₃₃H₂₂N₆O₂: C, 74.14; H, 4.15; N, 15.72 %. Found: C, 73.98; H, 4.18; N, 15.73 %. IR (KBr, cm⁻¹): 3362 and 3170 (NH and OH), 3061 and 3027 (Ar CH), 1665 (amide C=O), 1606–1476 (Ar C=C and C=N). ¹H-NMR (400 MHz, DMSO–d₆, δ / ppm): 8.25 (1H, br s, NH), 5.56–5.23 (1H, br s, OH), 7.31–6.90 (20H, m, ArH). ¹³C-NMR (100 MHz, DMSO–d₆, δ / ppm): 156.80 (amide C=O), 156.68 (ArC–OH), 156.59 and 144.09 (ArC–N=N), 142.54 (pyrazole C–3), 140.66 (pyrazole C–5), 140.29, 140.08, 134.80, 134.67, 131.08, 130.94, 130.87, 130.41, 130.15, 129.75, 129.49, 129.41, 129.23, 128.73, 128.58, 128.26, 128.18, 127.81, 121.95, 122.30, 117.15, 116.84, 113.80.

EXPERIMENTAL

The chemical compounds used in this research were of analytical grade purity and the solvents were purified using appropriate purifying agents and distillation. All melting points were measured using a Barnstead Electrothermal 9200 apparatus, and are reported uncorrected. The IR spectra of the compounds in KBr pellets were recorded on a Mattson 1000 FT–IR spectrometer. The ¹H-NMR and ¹³C-NMR spectra were recorded on Bruker DPX–400, (400 MHz), and high performance digital FT–NMR (100 MHz) spectrometers. The mass spectra were obtained using Varian Mat III 80 eV spectrometer. At the end of the each experiment, TLC was performed using DC Alufolien Kieselgel 60F/254 Merck and a Camag TLC device. The elemental analyses were performed on a Leco CHNS–932 instrument.

General procedure for the syntheses of compounds 2a-f, 3a,b, 6a-c and 7a,b

To an aqueous solution of sodium acetate (3.0 g, 37 mmol) was added 2 ml HCl and then 1 mmol the required amine compound. Subsequently, ethanol was added until complete dissolution. The prepared solution was cooled to 0 °C on an ice bath. To this solution, a solution of 1.2 mmol NaNO₂ in 2 ml water was slowly added taking care that the temperature did not exceed 5 °C. Thus, the diazonium salt solution was prepared.

An aromatic or β -dicarbonyl compound (1 mmol) was dissolved in a sufficient amount of ethanol, then cooled and added dropwise into the prepared diazonium salt solution. The resulting colored precipitate was filtered under vacuum and the crude product purified by crystallization from an ethanol–water mixture.

Synthesis of 4-benzoyl-1-(3-bromophenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (4a)

CuBr solution was prepared according to the procedure given in literature²² and was slowly added by stirring continuously into the diazonium salt solution of compound **1** (prepared as described in the general procedure). The resulting colored precipitate was filtered and purified by crystallization from ethanol–water mixture (9:1).

Synthesis of 4-benzoyl-1-(3-cyanophenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (4b)

A CuCN + KCN solution, which had been prepared in accordance with a procedure given in the literature,²² was cooled to 0 °C and added dropwise under continuous stirring into a diazonium salt solution of compound **1** (prepared as described in the general procedure). Following the addition, the cold mixture is allowed to warm up to room temperature. When



KASIMOGULLARI et al

the temperature reached about 15 °C, the formation of nitrogen gas began. Then the solution was placed on a steam bath and heated at 50 °C for 15 min to complete the decomposition. The pH was adjusted to 3-4 and left for 12 h at room temperature; the resulting precipitate was filtered under vacuum and dried. The residue was purified by crystallization from an ethanol–water mixture (8:2).

Synthesis of 4-benzoyl-1-(3-iodophenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid (4c)

KI (0.166 g, 1 mmol) was dissolved in 10 ml water and the solution was cooled to 0 $^{\circ}$ C. Then, it was added dropwise under continuous stirring into a diazonium salt solution of compound **1**, which had been prepared in accordance with the general procedure. The pH was adjusted to 3–4 and after standing for 12 h at room temperature, the resulting colored precipitate was filtered under vacuum and purified by crystallization from an ethanol–water mixture (8:2).

Synthesis of 4-benzoyl-1-(3-hydroxyphenyl)-5-phenyl-IH-pyrazole-3-carboxylic acid (4d)

A diazonium salt solution of **1** was prepared according to the general procedure. This solution was brought to room temperature and then heated in a steam bath at 100 °C to allow for the release of nitrogen gas (approximately 15 min). Then some more water was added to the mixture and the pH adjusted to 4. The solution was kept for about 24 h. The formed yellow-colored precipitate was collected by filtration and purified by crystallization from an ethanol–water mixture (9:1).

Acknowledgements. This study was funded by The Scientific and Research Council of Turkey (TUBITAK) and Dumlupinar University, Scientific Research and Project Department. In addition, the authors would like to thank the Faculty of Science of Atatürk University, Turkey, for spectral analysis.

ИЗВОД

СИНТЕЗА НОВИХ ДЕРИВАТА 1-(3-АМИНО-ФЕНИЛ)-4-БЕНЗОИЛ-5-ФЕНИЛ-1*Н*--ПИРАЗОЛ-3-КАРБОКСИЛНЕ КИСЕЛНЕ

RAHMI KASIMOGULLARI¹, BELMA ZENGIN¹, MAKBULE MADEN¹, SAMET MERT¹ ${\tt m}$ Cavit Kazaz²

¹Department of Chemistry, Art and Science Faculty, Dumlupinar University, Kutahya u²Department of Chemistry, Science Faculty, Atatürk University, Erzurum, Turkey

Синтеза 4-бензоил-1-(3-аминофенил)-5-фенил-1*H*-пиразол-3-карбоксилне киселине (1) извршена је према поступку описаном у литератури.¹ Производ 2-(3-аминофенил)-3,4-дифенил-2*H*-пиразол[3,4-*d*]пиридазин-7(6*H*)-он (5) добијен је циклокондензационом реакцијом киселине 1 и хидразин-хидрата. Нови пиразолски деривати добијени су реакцијом 1 и 5 са β -дикетонима, β -кетоестрима, β -нафтолом, фенолом и другим реагенсима. Добијена једињења окарактерисана су ¹H-NMR, ¹³C-NMR, ICи MS спектрима и микроанализом.

(Примљено 18. октобра, ревидирано 6. децембра 2010)

REFERENCES

- A. Şener, R. Kasımoğulları, M. K. Şener, I. Bildirici, Y. Akçamur, J. Heterocycl. Chem. 39 (2002) 869
- 2. R. Kasımoğulları, M. Bülbül, H. Günhan, H. Güleryüz, Bioorg. Med. Chem. 17 (2009) 3295
- D. K. Dodiya, A. R. Trivedi, S. H. Jarsania, S. J. Vaghasia, V. H. Shah, J. Serb. Chem. Soc. 73 (2008) 683

Available online at www.shd.org.rs/JSCS/



PYRAZOLE CARBOXYLIC ACIDS

- P. T. Chovatia, J. D. Akabari, P. K. Kachhadia, P. D. Zalavadia, H. S. Joshi, J. Serb. Chem. Soc. 71 (2007) 713
- 5. R. Kasımoğulları, M. Bülbül, B. S. Arslan, B. Gökçe, Eur. J. Med. Chem. 45 (2010) 4769
- 6. R. Kumar, Y. C. Joshi, J. Serb. Chem. Soc. 73 (2008) 937
- 7. M. Bülbül, R. Kasımoğulları, Ö. İ. Küfrevioğlu, J. Enzyme Inhib. Med. Chem. 23 (2008) 895
- 8. B. K. Kaymakçıoğlu, S. Rollas, Farmaco 57 (2002) 595
- 9. N. Ergenç, B. Durgun, G. Otuk, *Pharmazie* 47 (1992) 495
- 10. H. A. J. Schoutissen, Rec. Trav. Chim. Pays-Bas 40 (1921) 763
- 11. G. Çapan, PhD. Thesis, İ.Ü. Sağlık Bil. Enst, İstanbul, Turkey, 1990, p. 7 (in Turkish)
- 12. C. J. Lestina, T. H. Regan, J. Org. Chem. 34 (1969) 1685
- 13. H. Yashuda, H. Midorikawa, J. Org. Chem. 31 (1966) 1722
- 14. M. F. Abdel Megeed, Spectrosc. Lett. 20 (1987) 291
- 15. L. Antonov, S. Stoyanov, Dyes Pigm. 5 (1995) 83
- 16. H. Zollinger, Color Chemistry, Wiley-VCH, New York, 1991, p. 135
- 17. J. Kelemen, S. Moss, S. Glitsch, Dyes Pigm. 5 (1984) 83
- 18. I. Wawer, V. Koleva, Magn. Reson. Chem. 31 (1993) 375
- 19. A. Lycka, H. Murstroph, J. Prakt. Chem./Chem-Ztg. 331 (1989) 11
- 20. A. R. Katritzky, P. Barczynski, D. L. Ostercamp, J. Chem. Soc. Perk. Trans. 2 (1987) 1214
- 21. Y. Akçamur, A. Şener, A. M. İpekoğlu, G. Kollenz, J. Heterocycl. Chem. 34 (1997) 221
- 22. A. I. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, Longman, London, 1989, p. 428.







J. Serb. Chem. Soc. 75 (12) 1637–1640 (2010) JSCS–4083 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC *Kindbergia+631.445.1:582.32: 581.184+543 Short communication

SHORT COMMUNICATION **Preliminary analysis of fatty acid chemistry of** *Kindbergia praelonga* and *Kindbergia stokesii* (Brachytheciaceae)

BORIS PEJIN¹*[#], LJUBODRAG VUJISIĆ^{2#}, MARKO SABOVLJEVIĆ³, ANETA SABOVLJEVIĆ³, VELE TEŠEVIĆ^{1#} and VLATKA VAJS^{2#}

¹Department of Organic Chemistry, Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade ²Center of Chemistry, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade and ³Institute of Botany and Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

(Received 9 February, revised 5 September 2010)

Abstract: Moss species of the family Brachytheciaceae, *Kindbergia praelonga* (Hedw.) Ochyra and *Kindbergia stokesii* (Turn.) Ochyra, were preliminarily analysed for their fatty acid composition with the aim of studying the chemical relationship of these two entities. Fatty acid methyl esters were examined by GC and GC–MS in their methanol extracts. Thirteen fatty acids were identified. It is likely that the mosses are chemically distinguishable and should be treated as separate entities. However, additional chemical constituents of various moss samples, such as phenolic acids, their derivatives and flavonoids, must be also analyzed in order to support the re-examination of the relationship between these two species.

Keywords: bryophytes; mosses; *Kindbergia*; fatty acids; chemotaxonomy.

INTRODUCTION

Kindbergia praelonga (Hedw.) Ochyra (typified by *Hypnum praelongum* Hedw.) and *Kindbergia stokesii* (Turn.) Ochyra (typified by *Hypnum stokesii* Turn.) belong to the subgenus *Oxyrrhynchium*. The latter species is now usually considered as a synonym for or variety of *K. praelonga*. The genus *Kindbergia* Ochyra was recently treated as a separate genus from the *Eurhynchium* Schimp.^{1,2} According to Hill *et al.*,¹ in Europe only one species is present within the genus: *K. praelonga* (Hedw.) Ochyra (syn. *Eurhynchium praelongum* var. *stokesii* (Turner) Dixon and *Eurhynchium stokesii* (Turner) Schimp. as a syno-

1637



^{*} Corresponding author. E-mail: borispejin@yahoo.com

[#] Serbian Chemical Society member.

doi: 10.2298/JSC100209129P

PEJIN et al.

nym for *K. praelonga*. These two entities were separated into the genus *Stokesiella* (Kindb.) H. Rob., which was later considered as an incorrect homonym of the algal generic name *Stokesiella* Lemmerm. Ochyra renamed the genus *Kindbergia* Ochyra. Now molecular results support this generic independence.³

Worldwide, eleven taxa found at various locations are known, namely *K. africana* (Herz.) Ochyra, *K. altaica* Ignatov, *K. arbuscula* (Broth.) Ochyra, *K. brittoniae* (Grout) Ochyra, *K. dumosa* Mitt., *K. kenyae* (Dix. Ex Tosco & Piovano) O'Schea *et* Ochyra, *K. oedogonium* (C. Müll.) Ochyra, *K. oregana* (Sull.) Ochyra, *K. praelonga*, *K. stokesii* and *K. squarrifolia* Broth. *ex* Iishiba.

The main reason for the repeated nomenclatural confusion and the resulting complications surrounding *Oxyrrhynchium* is that the name *E. praelongum* was widely used with two different meanings. Schimper employed it for *E. hians* (syn. *Hypnum hians, Oxyrrhynchium hians* (Hedw.) Loeske) including the conspecific *E. swartzii* Turn. (syn. *Hypnum swartzii* Turn.) and several closely related elements) for varietal synonymy, whereas his *Eurhynchium stokesii* corresponded to *E. praelongum*, or following the recent nomenclature *K. praelonga*. Although many bryologists knew of the problem, frequently the use of the name *E. praelongum*, instead of *E. hians*, continued.⁴ Thus, *K. stokesii* was often easily overlooked.

Analyses were performed to test if chemotaxonomy can be helpful in the assignment of the two entity species, *K. praelonga* and *K. stokesii*.

EXPERIMENTAL

Both moss species, which were available as fresh material, were collected in Germany in December 2007: *Kindbergia praelonga* (Hedw.) Ochyra (BEOU4701) in Kologne and *Kindbergia stokesii* (Turn.) Ochyra (BEOU4703) in the surroundings of Bonn. Voucher specimens were deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia (bryophyte collection – BEOU).

The moss samples were carefully selected and cleaned from soil and other contaminants. The gametophyte tips were used for the extraction. Air-dried parts of both mosses were ground (1 g) and extracted 3 times with 90 % MeOH for 1 h at room temperature. The extracts were evaporated to dryness and were further transesterified with 5 % H_2SO_4 in MeOH (v/v) for 4 h at 80 °C. The resulting methyl esters of the fatty acids were analysed by comparing their GC-FID chromatograms with the chromatogram of a standard mixture (Supelco 37) obtained under the same conditions, and/or by analysis of GC–MS data using NIST 5 and Wiley 7 libraries.

The GC analyses were performed on an Agilent 7890A GC system equipped with a 5975C MSD and an FID, using a DB-23 column (30 m×0.25 mm×0.25 μ m). The injection volume was 1 μ L and injector temperature was 220 °C with 10:1 split ratio. The carrier gas was He at a flow rate 0.9 ml min⁻¹, while the column temperature was linearly programmed in the range of 150–240 °C at a rate of 4 °C min⁻¹ and held at 240 °C for 10 min. The transfer line was maintained at 240 °C. The FID detector temperature was 300 °C. The EI mass spectra (70 eV) were acquired in the *m/z* range 40–500.



RESULTS

For *K. stokesii*, 13 fatty acids were identified (Fig. 1): palmitic acid (C16:0, 25.04 %), arachidonic acid (C20:4,*n*-6, 18.29 %), linolelaidic acid (C18:2,*n*-6*t*, 14.57 %), α -linolenic acid (C18:3,*n*-3, 11.13 %), *cis*-5,8,11,14,17-eicosapentaenoic acid (C20:3,*n*-6, 8.94 %), elaidic acid (C18:1,*n*-9*t*, 6.32 %), behenic acid (C22:0, 3.04 %), lignoceric acid (C24:0, 2.90 %), palmitoleic acid (C16:1, 2.76 %), stearic acid (C18:0, 2.31 %), myristic acid (C14:0, 1.31 %), oleic acid (C18:1,*n*-9*c*, 1.30 %) and arachidic acid (C20:0, 1.28 %). *K. praelongum* showed less variety in these constituents: only two fatty acid constituents were found (Fig. 2), palmitic acid (C16:0, 88.58 %) and stearic acid (C18:0, 11.42 %).







(*RT* 13.167 min) and stearic acid (*RT* 16.212 min).

Available online at www.shd.org.rs/JSCS/



PEJIN et al.

CONCLUSIONS

The fatty acid composition of the two related and often synonymized *Kindbergia* species strongly suggested that they are chemically distinguishable and, thus, could be treated as separate entities. This, however, has still to be confirmed by the analyses of additional chemical constituents, such as phenolic acids and their derivatives as well as flavonoids,⁵ of various moss samples in order to support a re-examination of the relationship between them. For a general consideration of the quality of fatty acid profiling for chemotaxonomy, more replicates and additional species would have to be included in a follow-up study.

Acknowledgment. This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia (Research grants No. 142053 and 143015).

ИЗВОД

ПРЕЛИМИНАРНА АНАЛИЗА ХЕМИЈЕ МАСНИХ КИСЕЛИНА ВРСТА Kindbergia praelonga И Kindbergia stokesii (BRACHYTHECIACEAE)

БОРИС ПЕЈИН¹, ЉУБОДРАГ ВУЈИСИЋ², МАРКО САБОВЉЕВИЋ³, АНЕТА САБОВЉЕВИЋ³, ВЕЛЕ ТЕШЕВИЋ¹ и ВЛАТКА ВАЈС²

¹Кайиедра за органску хемију, Хемијски факулшеш, Универзишеш у Београду, Сшуденшски шрг 16, 11000 Београд, ²Ценшар за хемију, Инсшишуш за хемију, шехнологију и мешалургију, Његошева 12, 11000 Београд и ³Инсшишуш за бошанику и бошаничка башша, Биолошки факулшеш, Универзишеш у Београду, Таковска 43, 11000 Београд

Прелиминарно је испитиван састав виших масних киселина две маховине из фамилије Brachytheciaceae, *Kindbergia praelonga* (Hedw.) Ochyra и *Kindbergia stokesii* (Turn.) Ochyra, са хемотаксономским циљем. Укупно је идентификовано 13 виших масних киселина GC и GC–-MS анализом. На основу добијених експерименталних резултата се може закључити да се наведене биљне врсте значајно хемијски разликују и да би се могле сматрати засебним ентитетима уколико се то потврди и додатним анализама.

(Примљено 9. фебруара, ревидирано 5. септембра 2010)

REFERENCES

- M. O. Hill, N. Bell, M. A. Bruggemann-Nannenga, M. Brugues, M. J. Cano, J. Enroth, K. I. Flatberg, J.-P. Frahm, M. T. Gallego, R. Garilleti, J. Guerra, L. Hedenäs, D. T. Holyoak, J. Hyvönen, M. S. Ignatov, F. Lara, V. Mazimpaka, J. Munoz, L. Söderström, J. Bryol. 28 (2006) 198
- M. Sabovljević, R. Natcheva, G. Dihoru, E. Tsakiri, S. Dragićević, A. Erdag, B. Papp, *Phytol. Balcan.* 14 (2008) 159
- 3. M. S. Ignatov, S. Huttunen, Arctoa 11 (2002) 245
- 4. M. Ignatov, P. Isoviita, *Taxon* **52** (2003) 352
- N. Jocković, P. B. Andrade, P. Valentao, M. Sabovljević, J. Serb. Chem. Soc. 73 (2008) 1161.

Available online at www.shd.org.rs/JSCS/







J. Serb. Chem. Soc. 75 (12) 1641–1652 (2010) JSCS–4084 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 547.56:634.84:581.48: 547.972.3(497.11) Original scientific paper

Polyphenolic compounds in seeds from some grape cultivars grown in Serbia

DEJAN GOĐEVAC^{1*#}, VELE TEŠEVIĆ^{2#}, MILOVAN VELIČKOVIĆ³, LJUBODRAG VUJISIĆ², VLATKA VAJS^{1#} and SLOBODAN MILOSAVLJEVIĆ^{2#}

¹Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade ²Faculty of Chemistry, Studentski trg 16, 11000 Belgrade and ³Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia

(Received 19 May, revised 26 July 2010)

Abstract: Seed extracts from eight grape cultivars (*Vitis vinifera*) growing in Serbia were screened for their polyphenolic composition by means of HPLC//PDA/ESI/MS analysis. The study revealed 34 phenolic compounds belonging to the following groups: flavan-3-ol monomers, proanthocyanidins, flavonols, hydroxycinnamic acid and hydroxybenzoic acid derivatives. The quantities of the main constituents were determined using PDA/HPLC. Qualitative and quantitative differences among the cultivars were observed.

Keywords: *Vitis vinifera*; grape seeds; HPLC/PDA/ESI/MS; flavanol monomers; proanthocyanidins; flavonols; hydroxycinnamic acid; hydroxybenzoic acid derivatives.

INTRODUCTION

Many agricultural by-products are composed of plant tissues rich in phytochemicals, with valuable chemical and biological properties. Examples are by--products from wine processing,^{1–3} such as marcs, stems, dregs (a sludgy residue deposited on the bottom of fermentation vats) and grape-seeds, which represent rich sources of polyphenolics.

Phenols represent the third most abundant constituent in grapes after carbohydrates and fruit acids.⁴ The composition of phenolics depends on whether the extraction is performed on whole grape, pulp, skin or seeds. The total extractable phenolics in grapes are present at only about 10 % or less in pulp, 60–70 % in the seeds and 28–35 % in the skin. The phenol content of seeds may range from 5 to 8 % by weight.⁵

doi: 10.2298/JSC100519131G

1641



^{*} Corresponding author. E-mail: dgodjev@chem.bg.ac.rs

[#] Serbian Chemical Society member.

GOĐEVAC et al

The phenolic compounds in grapes can be divided into two main groups: phenolic acids (localized mainly in the skin and pulp) and flavonoids. The most common phenolic acids in grape include cinnamic and benzoic acid derivatives. Flavonoids include colorless flavan-3-ols, flavonols and red and blue anthocyanins.⁵ The most abundant phenolics isolated from grape seeds and skins are flavan-3-ols (catechin and epicatechin) and their oligomers and polymers (proanthocyanidins). The outer seed coat contains the majority of both the monomeric and polymeric flavan-3-ols (2 to 5 times more than the endosperm).⁶ Grape skins also contain anthocyanins which contribute to their red or blue color.^{7,8}

Various conditions (time, solvent, and the manner) for the extraction of polyphenols from grape seeds are described in the literature. Due to the acidic lability of interflavan linkages within proanthocyanidins and the susceptibility of polyphenols to oxidation, a valid extraction method should provide for the complete as possible extraction of the polyphenolics while limiting their degradation.⁹ Methanol/water^{10,11} or acetone/water systems¹² are the common solvents used for extracting polyphenols from grape seeds. In particular, lower molecular weight polyphenols, such as phenolic acids, anthocyanins, and flavanol monomers and oligomers, are well extracted with methanol, while the higher molecular weight flavanols are better extracted with aqueous acetone than with methanol.^{13–16}

Several methods for the analysis of polyphenols have been proposed in the literature. Most of them are based on high performance liquid chromatography (HPLC) coupled with either a photodiode array (PDA) detector or a mass spectrometer (MS). Reverse phase columns are favorable, using acetonitrile and acidic water solutions as eluents.¹⁷ Since UV detection depends upon the chemical structure of a molecule, several wavelengths could be selected for monitoring. Red-colored anthocyanins show an absorbance maximum at around 520 nm; yellow-colored flavonols display an absorbance maximum at around 360 nm; hydroxycinnamic acids can be specifically detected by their high absorbance around 320 nm. Flavan-3-ols show no specific absorbance and have a maximum around 280 nm, as do all the above-mentioned phenolics.¹⁸

Many studies proved that procyanidins and other polyphenolics from grape seed could be the key compounds responsible for various beneficial effects for human health.^{19,20} These effects are mainly associated with the antioxidant activity of the phenolic compounds, which act as reducing agents by trapping free radicals, by acting as chelators, by donating hydrogen, and by quenching singlet oxygen. These highly reactive species are present in biological systems and may oxidize lipids, proteins, nucleic acids, which may initiate degenerative heart disease. In addition, grape seed polyphenolics possess various potent biological effects, such as antitumor, antibacterial, antiviral, anti-inflammatory, enzyme-inhibiting effects.^{21–24} Waste products of the winery and grape juice industry derived from grape seeds represent a rich source of polyphenols.^{5,25} It is well

Available online at www.shd.org.rs/JSCS/



1643

known that the concentration of polyphenolic compounds in grapes depends on the grape cultivar,^{26,27} and other factors, such as ripening time, climate, soil and location of growth.²⁸

The aim of this study was to determine the polyphenolic composition of grape seed extracts from Vitis vinifera L. cv., Smederevka, Prokupac, Serbian original varieties, and Italian Riesling, Traminer, Black Burgundy, Gamay Noir, Muscat Hamburg and Gamay Bojadiser, all grown in the same geographical area and vintage. Two grape cultivars, Italian Riesling and Traminer, have a yellow--green colored grape berry used for production of high-quality white wines. The cultivar Smederevka is an autochthonous grape cultivar of Serbia, with lightly vellow and green colored berries. This cultivar is used for production of quality white wines as well as for all kinds of blending, because the grapes accumulate a high level of acids. Since the grape is well transportable and the pulp is crispy, it is also used fresh. Black Burgundy and Gamay Noir are purple-colored grape cultivars, used in production of high-quality red wines. Muscat Hamburg is the most widespread table grape in Serbia. This grape can be used fresh as well as for wine and grape brandy production. Prokupac is also an autochthonous grape cultivar of Serbia. Its berries are navy-blue colored with plenty of dots, and it is used for the production of quality rosé wines. Gamay Bojadiser grapes are full of colored materials, and it is mostly used for blending. An HPLC/PDA/MS method was used for the polyphenols analysis. The similarities and differences between the polyphenolic compositions of grape seed extracts from different cultivars are discussed.

EXPERIMENTAL

Plant material

Seeds from eight grape cultivars, including Italian Riesling, Traminer, Smederevka, Black Burgundy, Gamay Noir, Muscat Hamburg, Prokupac and Gamay Bojadiser were examined. All studied cultivars were grown in the vicinity of Belgrade (experimental orchard of Radmilovac, property of Faculty of Agriculture, University of Belgrade). The experimental vineyard was raised in 1995 (cultivars Smederevka and Gamay Noir), and 1996 (cultivars Italian Riesling, Traminer, Black Burgundy, Muscat Hamburg, Prokupac and Gamay Bojadiser). The distance of sowing was 3×1 m, with two rows support, and the training system was a "double-branched asymmetrical cordone",²⁹ the tree being 90 cm high. Approximately 20 clusters (about 5 kg of grape) were collected in late summer 2008, from 10 different plants. All the samples were collected when the Brix values were in range 22.5–24.5°.

Chemicals

Gallic acid, catechin, epicatechin, caffeic acid, ellagic acid, and rutin were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals and solvents were of analytical grade. The HPLC water was purified by a Milli-Q System.

Sample preparation

The seeds from the berries were manually separated from pulp and dried on filter paper. The samples of whole, dried seeds (20 g) were macerated in 120 mL of 50 % MeOH, and 1

GOĐEVAC et al.

mL of rutin solution (2.78 mg mL⁻¹ in MeOH, internal standard) was added. The mixtures were sonicated in an ultrasonic bath for 8 h. The extracts were filtered through filter paper, evaporated (to 1 mL) at 45 °C under reduced pressure and filtered through a 0.45 mm cellulose filter (Millipore). The filtrate was then transferred into a vial and filled up with 50 % MeOH to a volume of 1.5 mL.

HPLC/PDA Analysis

HPLC analysis of extracts was performed using an Agilent 1200 chromatograph equipped with a PDA model G1315B, a Bin pump model G1312A, an autosampler model G1313A and a RR Zorbax Eclipse Plus C18 column (1.8 μ m, 150 mm×4.6 mm). The mobile phase A was 0.2 % formic acid in water and the mobile phase B was acetonitrile. Elution was performed at 0.95 mL min⁻¹ with the following gradient program of solvent B: 0–20 min, 5–16 %; 20–28 min, 16–40 %; 28–32 min, 40–70 %; 32–36 min, 70–99 %; 36–45 min, 99 % and 45–46, min. 99–5 %.³⁰ The injection volume was 10 μ L. Wavelengths of 280 nm (for flavan-3-ols and benzoic acid derivatives) and 360 nm (for flavonols and cinnamic acid derivatives) were selected for detection.

Quantification of the compounds was realized using calibration curves obtained by HPLC of pure standards: gallic acid, caffeic acid, (+)-catechin, (-)-epicatechin, and ellagic acid. Rutin was used as an internal standard. Some compounds were quantified as equivalents of the most similar chemical structures: gallic acid for gallic acid glucoside, gentisic acid glucoside, proto-catechuic acid, *p*-hydroxybenzoic acid and methyl gallate; caftaric acid as caffeic acid; (+)-catechin for proanthocyanidin dimers and trimers and their monogallates; (–)-epicatechin for epicatechin gallate; ellagic acid for ellagic acid pentoside.

LC/MS analysis

LC/MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC, using the same column and gradient program as were employed for the HPLC/ /PDA analysis. Mass spectra were acquired using an Agilent ESI-MSD TOF. The drying gas (N2) flow was 12 L min⁻¹; the nebulizer pressure was 310.264 kPa and the drying gas temperature was 350 °C. For ESI analysis, the parameters were: capillary voltage, 4000 V; fragmentor, 140 V; skimmer, 60 V; Oct RF V 250 V, for negative modes. The mass range was from 100 to 2000 *m*/*z*. Data processing was realized with the software Molecular Feature Extractor and Mass Profiler.

Statistical analysis

All the experiments were performed in triplicate. Significant differences between the means were separated by analysis of variance (ANOVA) followed by Tukey's test. Computations were realized using Origin software package version 7.0.

RESULTS AND DISCUSSION

The rapid resolution HPLC column and the appropriate gradient program afforded the separation of some 34 phenolic compounds in less than 30 min. Identification of the compounds was based on the UV spectra and molecular formula obtained from accurate mass measurements, both measured on the HPLC//PDA/ESI/MS equipment, which also involved comparison of these data with those of the metabolites previously reported for grape seed extracts.^{23,31} The identified phenolic compounds could be classified into the following groups: flavanol monomers (catechin and epicatechin), proanthocyanidins, flavonols,

Available online at www.shd.org.rs/JSCS/



hydroxycinnamic acids, and hydroxybenzoic acid derivatives (Table I, Fig. 1.). However, owing to the unavailability of authentic compounds, with exception of gallic acid, ellagic acid, catechin and epicatechin, the peaks could be tentatively assigned but without determination of stereochemistry.

Peak	t _R	Compound	Class of	λ_{\max}	Species	Mass	Molecular
	min		compound"	nm	1	150 0015	formula
I	3.2	Gallic acid	HB	220, 272	M–H,	170.0215	$C_7H_6O_5$
•		D 1	D.C.	2 00 2 10 2 20 1	2M-H	0.66.00.00	a 11 o
2	4.0	Proanthocyanidin	PC	200, 218, 228 <i>sh</i> ,	M-2H,	866.2058	$C_{45}H_{38}O_{18}$
•		trimer		236sh, 280	M–H		a 11 o
3	4.3	Gallic acid glucoside	HB	218, 256	M–H,	332.0744	$C_{13}H_{16}O_{10}$
	50		UD	216 252	2M-H	016 0704	
4	5.0	Gentisic acid	HB	216, 252	M–H,	316.0794	$C_{13}H_{16}O_9$
-	- 0	glucoside		210 2 0 2 0 1	2M-H	1	<i>a</i> 11 o
5	5.8	Protocatechuic acid	HB	218, 260, 292 <i>sh</i>	M–H,	154.0266	$C_7H_6O_4$
				200 1 224	2M-H	010 0404	a 11 o
6	7.3	Caftaric acid	HC	300 <i>sh</i> , 324	M–H,	312.0481	$C_{13}H_{12}O_9$
_	~ •				2M-H		~ ~ ~ ~
7	8.2	<i>p</i> -Hydroxybenzoic	HB	278, 312	M–H,	138.0317	$C_7H_6O_3$
0	~ ~	acid	D.C.	200 214	2M-H		a 11 o
8	8.3	Proanthocyanidin	PC	200, 216,	M–H,	578.1424	$C_{30}H_{26}O_{12}$
	~ -	dimer		228 <i>sh</i> , 280	2M-H		a
9	9.5	Proanthocyanidin	PC	200, 216,	M–H,	578.1424	$C_{30}H_{26}O_{12}$
		dimer		228 <i>sh</i> , 280	2M-H		a a
10	9.8	Methyl gallate	HB	220, 272	M–H,	184.0372	$C_8H_8O_5$
				••••	2M-H		a a
11	10.1	(+)-Catechin	FM	200, 218,	М–Н,	290.0790	$C_{15}H_{14}O_6$
				226sh, 278	2M-H		
12	10.9	Proanthocyanidin	PC	200, 218, 228 <i>sh</i> ,	M–2H,	866.2058	$C_{45}H_{38}O_{18}$
		trimer		236 <i>sh</i> , 280	M–H		
13	11.3	Proanthocyanidin	PC	200, 218, 228 <i>sh</i> ,	M–2H,	866.2058	$C_{45}H_{38}O_{18}$
		trimer		236 <i>sh</i> , 280	M–H		
14	11.7	Proanthocyanidin	PC	200, 216,	М–Н,	578.1424	$C_{30}H_{26}O_{12}$
		dimer		228 <i>sh</i> , 280	2M-H		
15	11.8	Caffeic acid	HC	246, 298 <i>sh</i> , 326	M–H	180.0423	$C_9H_8O_4$
16	12.7	Proanthocyanidin	PC	200, 216,	М–Н,	578.1424	$C_{30}H_{26}O_{12}$
		dimer		228sh, 280	2M-H		
17	13.9	Proanthocyanidin	PC/HB	200, 218, 278	M–2H,	1018.2168	$C_{52}H_{42}O_{22}$
		trimer monogallate			M–H		
18	14.3	(–)-Epicatechin	FM	200, 218,	М–Н,	290.0790	$C_{15}H_{14}O_6$
				226sh, 278	2M-H		
19	15.4	Proanthocyanidin	PC/HB	200, 218,	М–Н,	730.1534	$C_{37}H_{30}O_{16}$
		dimer monogallate		278	2M-H		
20	15.8	Proanthocyanidin	PC	200, 218, 228sh,	M–2H,	866.2058	$C_{45}H_{38}O_{18}$
		trimer		236sh, 280	M–H		

TABLE I. LC/MS Data of grape seed extracts (GSEs)

Available online at www.shd.org.rs/JSCS/



GOĐEVAC et al.

Peak	t _R min	Compound	Class of compound ^a	λ _{max} nm	Species	Mass	Molecular formula
21	16.5	Proanthocyanidin	PC	200, 218, 228sh,	M–2H,	866.2058	C ₄₅ H ₃₈ O ₁₈
		trimer		236sh, 280	M–H		
22	17.4	Proanthocyanidin	PC/HB	200, 218,	M–H,	730.1534	$C_{37}H_{30}O_{16}$
		dimer monogallate		278	2М-Н		
23	17.5	Syringic acid	HB	276	M–H,	198.0528	$C_9H_{10}O_5$
					2М-Н		
24	20.6	Ellagic acid	HB	254, 300sh,	M–H,	434.0485	$C_{19}H_{14}O_{12}$
		pentoside		360	2М-Н		
25	21.9	Ellagic acid	HB	254, 298sh, 368	M–H	302.0063	$C_{14}H6O_8$
26	21.9	(-)-Epicatechin	FM/HB	200, 218,	M–H,	442.0900	$C_{22}H_{18}O_{10}$
		gallate		278	2М-Н		
27	22.0	Taxifolin	FL	232, 254,	М–Н,	304.0583	$C_{15}H_{12}O_7$
				290, 330sh	2М-Н		
28	22.7	Quercetin-3-O-	FL	256, 264sh,	М–Н,	478.0747	$C_{21}H_{18}O_{13}$
		glucuronide		356	2М-Н		
29	23.1	Astilbin	FL	292, 326sh	M–H,	450.1162	$C_{21}H_{22}O_{11}$
					2М-Н		
30	22.9	Quercetin-3-O-	FL	256, 268sh,	M–H,	464.0955	$C_{21}H_{20}O_{12}$
		glucoside		300sh, 360	2М-Н		
31	23.8	Kaempferol	FL	266, 320sh,	M–H	594.1585	$C_{27}H_{30}O_{15}$
		rutinoside		350			
32	24.7	Isorhamnetin-3-O-	FL	256, 266sh,	М–Н,	478.1111	$C_{22}H_{22}O_{12}$
		glucoside		302sh, 350	2М-Н		
33	24.3	Quercetin 3-O-	FL	256, 266sh,	M–H,	448.1006	$C_{21}H_{20}O_{11}$
		rhamnoside		302sh, 350	2М-Н		
34	27.4	Quercetin	FL	256, 268sh,	М–Н,	302.0427	$C_{15}H_{10}O_7$
				300sh. 370	2M-H		

TABLE I. Continued

1646

^aHB – hydroxybenzoic acid derivative, FM – flavanol monomers, PC – proanthocyanidins, FL – flavonols, HC – hydroxycinnamic acids

All the identified compounds exhibited quasi-molecular ion $[M-H]^-$, as the dominant ion species in the mass spectrum. The exception were procyanidin trimers, where doubly charged $[M-2H]^{2-}$ species were dominant. Cluster ions, such as $[2M-H]^-$, were also observed for most of the compounds.

The amounts of phenolic compounds are presented in Tables II and III. The range of free gallic acid varied from 4 to 23 mg per 100 g of grape seeds. While white grape cultivars (Italian Riesling, Traminer and Smederevka) showed high gallic acid contents (over 17 mg per 100 g), the colored cultivars possessed significantly lower contents (below 10 mg per 100 g). This is in accordance with data published for some white and red grape varieties from Spain.³² Except in Italian Riesling, glucosides of gallic acid were found in all the studied cultivars. In addition, protocatechuic acid was detected in the white grape cultivars and Gamay Noir. The presence of ellagic acid or ellagic acid glycoside was con-



firmed in Muscat Hamburg and Prokupac seeds. This finding is surprising because it was hitherto believed that the presence of ellagic acid is unique for muscadine grapes (*Vitis rotundifolia*) among the *Vitis* varieties.^{33,34}



Fig. 1. LC/UV Chromatograms of grape seed extracts ($\lambda = 280$ nm).

In comparison with other classes of polyphenolic compounds, hydroxycinnamic acid derivatives were present in lower amounts in the seeds. This is in accordance with data from the literature claiming that hydroxycinnamic acids are localized mainly in the skin and pulp.⁵ No hydroxycinnamic acids derivatives were detected in the cultivar Smederevka, while caftaric acid (ester of caffeic acid with tartaric acid) was found in the remaining cultivars. Underivatized caffeic acid was only found in the seeds of Traminer.

The presence of taxifolin (dihydroquercetin) and its glycoside astilbin was confirmed in Italian Riesling and Traminer cultivars, while in Black Burgundy only astilbin was detected. It should be noted that the presence of such types of flavonols is very rare in grape extracts.³⁵ Kaempferol rutinoside, quercetin or their glycosides were found in all the studied cultivars. Isorhamnetin-3-*O*-gluco-

Sme- er)		SD	0.05	0.10	0.05		I	0.11	Ι		0.05	Ι	I	I	T	Ι	Ι		I	I		I		I				1	, with
SM – S 30jadis	GE	Value	9.45	5.10	1.25		pu	6.62	pu		1.35	pu	pu	pu	pu	pu	pu		pu	Traces		Traces		Traces		pu		pu	<i>b</i> < 0.01
sling, may I		SD	0.10	0.10	0.05		I	0.08	I		0.10	I	I	0.05	0.05	I	Ι		I	I		1				I		1	isk or
lian Rie GB - Ga	PR	Value	8.12	1.29AB*	0.54B		pu	1.87	pu		2.10	pu	pu	1.24	1.26	pu	Traces		pu	Traces		Traces		pu		pu		Traces	hout aster
R - Ita upac, (-	SD	0.05	0.10	I		I	0.05	Ι		0.06	I	I	1	0.05	I	Ι		I	I		I		Ι		I		1	05, wit
iSEs (II – Proku	IM	Value	5.84	4.50	pu		pu	7.04	pu		3.06	pu	pu	pu	2.36	pu	pu		pu	Traces		Traces		pu		pu		Traces	11 (<i>p</i> < 0.
t in G g, PR		SD	0.05	0.05	0.06		0.05	0.10	1		0.08	ł	I	I	I	ł	I		I	I		I		Ι		ł		1	lifferer
s conten Iamburg	G	Value	5.15	$1.54B^{*}$	0.64AB		0.84A	2.90B	pu		1.42	pu	pu	pu	pu	pu	Traces		pu	pu		Traces		pu		pu		Traces	ificantly o
/onols scat E	~	SD	0.10	0.06	I		I	0.15	0.05		0.05	Ι	I	I	T	I	Ι		I	I		I		I		I		I	ot sign
and flav H - Mu	BE	Value	4.30	$1.04A^{*}$	pu		pu	2.67B	0.24		1.44	pu	pu	pu	pu	pu	Traces		Traces	pu		pu		pu		pu		pu	ow are ne
tives ; ir, M		SD	0.41	0.04	Ι		0.03	0.01	I		I	0.03	I	I	I	I	I		I	I		I		I				1	the ro
derivat nay No	T	Value	22.48	0.59	pu		2.44	0.81A	pu		Traces	2.04	Traces	pu	pu	Traces	Traces		Traces	pu		Traces		pu		pu		pu	ers withir
acids - Gar	1	SD	0.35	0.10	0.03		0.10	I	0.15		0.10	I	I	I	T	I	Ι		I	I		I		I		I		I	ne lette
namic dy, GN	SN	Value	18.62	3.51	0.76A		0.78	pu	1.43		1.49	pu	pu	pu	pu	pu	pu		pu	pu		Traces		pu		pu		pu	h the san
xycir Irgun(SD	0.23	I	I		0.05	0.04	Ι		I	I	I	I	I	I	I		I	I		I		I				Ι	les wit
l, hydrc lack Bu	B	Value ^a	17.67	pu	pu		1.96A	1.04A	pu		pu	pu	Traces	pu	pu	Traces	Traces		Traces	Traces		Traces		pu		Traces		pu	eds. Valı
Hydroxybenzoic acic – Traminer, BB - Bl	Commond	Compound	Gallic acid	Gallic acid glucoside	Gentisic acid	glucoside	Protocatechuic acid	Caftaric acid	p-Hydroxybenzoic	acid	Methyl gallate	Caffeic acid	Syringic acid	Ellagic acid pentoside	Ellagic acid	Taxifolin	Quercetin-3-0-glu-	curonide	Astilbin	Quercetin-3-0-glu-	coside	Kaempferol	rutinoside	Isorhamnetin-3-0-	-glucoside	Quercetin 3-0-	-rhamnoside	Quercetin	mg/100g of dry grape set
LE II. I ka, TR	t _R	min	3.2	4.3	5.0		5.8	7.3	8.2		9.8	11.8	17.5	20.6	21.9	22.0	22.7		23.1	22.9		23.8		24.7		24.3		27.4	ssed in 1 ()
TABI derev]	Dool:	reak	-	e.	4		5	9	2		10	15	23	24	25	27	28		29	30		31		32		33		34	^a Expre: asterisk

GOĐEVAC et al.

÷ : a Ë Ť E, **CCF**e _ Ψ 4 7

1648

Available online at www.shd.org.rs/JSCS/



, MH – Muscat Hamburg, PR – Prokupac, GB – Gamay Bojadiser)	IR SM TR BB GN MH PR GB	Value ^a SD Value SD Value SD Value SD Value SD Value SD Value SD Value SD Value SD	trimer 4.48 0.08 Traces - Traces - 2.45 0.05 3.34 0.05 nd - 1.40 0.10 nd -	dimer 29.50 0.59 0.75 0.05 6.85 0.09 11.27A 0.25 10.42A 0.33 17.38 0.13 5.25 0.05 15.29 0.10	dimer 5.22 0.06 Traces - 2.18 0.07 7.76 0.08 17.34 0.15 1.56 0.06 4.24 0.05 8.65 0.05	n 42.41 0.52 6.85 0.10 17.21 0.01 134.82 1.11 145.04 1.00 107.81 1.41 26.14 0.15 37.26 0.64	trimer nd - nd - 5.14 0.12 nd - nd - nd - 4.35 0.05 17.28 0.20	trimer 14.08 0.37 0.96 0.05 Traces - 6.37 0.08 13.06 0.08 nd - nd - nd -	dimer 10.37 0.55 1.52 0.08 Traces - 8.82 0.08 22.04 0.05 23.25 0.15 5.45 0.05 15.46 0.15	dimer 21.35 0.56 2.90 0.10 8.36 0.05 10.40 0.20 16.69 0.10 29.30 0.19 6.11 0.10 32.74 0.25	trimer 14.38 0.54 0.78 0.11 nd - nd - 8.00 0.11 nd - Traces - 25.98 0.43	1	iii 29.47 0.50 39.18 0.76 9.83 0.06 60.39 0.54 91.67 0.59 86.31 0.16 23.49 0.50 57.65 0.48	dimer 14.49 0.50 0.88 0.08 nd - 4.36 0.05 5.75 0.05 16.25 0.13 traces - nd -	0	trimer nd - nd - nd - 4.05 0.05 5.65 0.05 nd - traces - nd -	trimer 50.59 1.28 Traces - Traces - 8.27 0.11 13.24 0.25 Traces - 4.27 0.08 27.68 0.16	dimer 50.77 1.15 1.35 0.05 20.24 0.20 16.70 0.10 40.62 0.78 nd - 12.15 0.15 32.08 0.11		adlate 21.56 0.51 1.07 0.08 Traces – 15.54 0.25 9.80 0.19 7.90 0.10 12.85 0.31 nd –	71.88 1.02 46.03 0.86 27.04 0.06 195.21 1.63 236.72 1.58 194.13 1.56 49.63 0.65 94.91 1.05	1.44 0.01 0.17 0.00 1.75 0.01 2.23 0.00 1.58 0.00 1.25 0.01 1.11 0.02 0.65 0.01	dimer 66.45 1.73 5.17 0.23 17.39 0.21 38.25 0.60 66.49 0.63 71.49 0.52 21.05 0.25 72.13 0.55	I 101.20 2.71 4.07 0.31 20.24 0.20 36.59 0.40 64.18 1.13 24.15 0.23 24.99 0.46 58.06 0.54	lins	and and Mahaa mide the same state of the second stratic field and the second second second second second second
-Muscat Haml	IR	Value ^a SD	4.48 0.08	29.50 0.59	5.22 0.06	42.41 0.52	- pu	14.08 0.37	10.37 0.55	21.35 0.56	14.38 0.54		29.47 0.50	14.49 0.50		- pu	50.59 1.28	50.77 1.15		21.56 0.51	71.88 1.02	1.44 0.01	66.45 1.73	101.20 2.71		de Walnes mith
undy, GN – Gamay Noir, MH –	fR Commenter	min Compound	4 Proanthocyanidin trimer	8.3 Proanthocyanidin dimer	9.5 Proanthocyanidin dimer	10.1 (+)-Catechin	10.9 Proanthocyanidin trimer	11.3 Proanthocyanidin trimer	11.7 Proanthocyanidin dimer	12.7 Proanthocyanidin dimer	13.9 Proanthocyanidin trimer	monogallate	14.3 (–)-Epicatechin	15.4 Proanthocyanidin dimer	monogallate	15.8 Proanthocyanidin trimer	16.5 Proanthocyanidin trimer	17.4 Proanthocyanidin dimer	monogallate	21.9 (–)-Epicatechin gallate	C+E	C/E	Proanthocyanidin dimer	Galloylated	proantocyanidins	and in ma/100c of Ami more and
Burg	1.10	eak		~	~	[]	12	3	4	(e	17		8	6		0	1	22		26						

POLYPHENOLICS IN SERBIAN GRAPE SEEDS

TABLE III. Flavanol monomers and Proanthocyanidins content in GSEs (IR - Italian Riesling, SM - Smederevka, TR - Traminer, BB - Black

Available online at www.shd.org.rs/JSCS/

GOĐEVAC et al.

side was detected only in Gamay Bojadiser (Table II). Such flavonols have been already reported in grapes extracts.^{32,36}

The most abundant phenolic compounds in the grape seed extracts were monomeric flavan-3-ols and proanthocyanidins, as found by other authors.^{26,32,37} Generally, the content of flavan-3-ol monomers (catechin and epicatechin) was higher in the colored than in the white grape cultivars (Table III). Only Smederevka and Gamay Bojadiser possessed greater amounts of epicatechin than catechin, while the catechin/epicatechin ratio for most cultivars was between 1 and 2. The exception was Black Burgundy which contained more than two times more catechin than epicatechin. Muscat Hamburg, Gamay Bojadiser, Italian Riesling and Gamay Noir were the richest in proanthocyanidin dimers, while Italian Riesling possessed a high amount of galloylated proantocyanidins. On the other hand, Smederevka possessed a very low amount of proanthocyanidin dimers and galloylated proanthocyanidins.

CONCLUSIONS

Statistically significant difference in the contents of some polyphenolic compounds between the studied cultivars was noticed. From these findings, it may be concluded that the amounts and distribution of various phenolic compounds in grape seeds depend directly on the cultivar, as the other factors, such as ripening time, climate, soil and location of growth, were the same for all the studied cultivars. This is the first time the presence of ellagic acid or ellagic acid glycoside in some *Vitis vinifera* cultivars was evidenced. The variation of the composition of the phenolic compounds from certain cultivar could be used in industry to make specific food additives or dietary supplements.

ИЗВОД

ПОЛИФЕНОЛНА ЈЕДИЊЕЊА ИЗ СЕМЕНКИ ОСАМ СОРТИ ГРОЖЂА ГАЈЕНИХ У СРБИЈИ

ДЕЈАН ГОЂЕВАЦ¹, ВЕЛЕ ТЕШЕВИЋ², МИЛОВАН ВЕЛИЧКОВИЋ³, ЉУБОДРАГ ВУЈИСИЋ², ВЛАТКА ВАЈС¹ и СЛОБОДАН МИЛОСАВЉЕВИЋ²

¹Инс*йийуй за хемију, йехнологију и мейалургију,* Нјегошева 12, 11000, Београд, ²Хемијски факулйей, Сйуденйски йрг 16, 11000, Београд и ³Пољойривредни факулией, Немањина 6, 11080, Земун

Помоћу HPLC/PDA/ESI/MS анализе је испитан полифенолни састав екстраката семенки осам сорти грожђа (*Vitis vinifera*) гајених у Србији. Утврђено је присуство 34 фенолна једињења која припадају следећим групама: флаванолски мономери, проантоцијанидини, флавоноли, деривати хидроксициметне и деривати хидроксибензоеве киселине. Квантитативни садржај главних састојака је одређен уз помоћ PDA/HPLC. Примећене су квалитативне и квантитативне разлике између појединих сорти.

(Примљено 19. маја, ревидирано 26. јула 2010)

Available online at www.shd.org.rs/JSCS/


REFERENCES

- 1. F. Bonilla, M. Mayen, J. Merida, M. Medina, Food Chem. 66 (1999) 209
- 2. J. L. Torres, R. Bobet, J. Agric. Food Chem. 49 (2001) 4627
- J. L. Torres, B. Varela, M. T. García, J. Carilla, C. Matito, J. J. Centelles, M. Cascante, X. Sort, R. Bobet, J. Agric. Food Chem. 50 (2002) 7548
- 4. V. L. Singleton, in *Proceedings of the University of California, Davis, Grape and Wine Centenary Symposium*, University of California, Davis, CA, 1980, p. 215
- 5. J. Shi, J. Yu, J. E. Pohorly, Y. Kakuda, J. Med. Food 6 (2003) 291
- 6. J. H. Thorngate, V. L. Singleton, Am. J. Enol. Viticult. 45 (1994) 259
- 7. R. C. Silva, J. Rigaud, V. Cheynier, A. Chemina, Phytochemistry 30 (1991) 1259
- 8. C. Prieur, J. Rigaud, V. Cheynier, M. Moutounet, Phytochemistry 36 (1994) 781
- 9. H. Zou, P. Kilmartin, M. Inglis, A. Frost, Aust. J. Grape Wine Res. 8 (2002) 163
- 10. C. F.-A. E. M. Santos-Buelga, M. T. Escribano-Bailon, Food Chem. 53 (1995) 197
- 11. D. Rusjan, Z. Korosec-Korusa, Acta Chim. Slov. 54 (2007) 114
- 12. G. Spagna, R. N. Barbagallo, P. G. Pifferi, J. Agric. Food Chem. 48 (2000) 4619
- 13. L. Foo, L. Porter, J. Sci. Food Agric. 32 (1981) 711
- 14. R. Hemingway, G. McGraw, J. Wood Chem. Technol. 3 (1983) 421
- 15. I. McMurrough, D. Madigan, M. R. Smyth, J. Agric. Food Chem. 44 (1996) 1731
- S. Guyot, N. Marnet, D. Laraba, P. Sanoner, J. F. Drilleau, J. Agric. Food Chem. 46 (1998) 1698
- 17. S. Muñoz, M. Mestres, O. Busto, J. Guasch, Anal. Chim. Acta 628 (2008) 104
- S. Gómez-Alonsoa, E. García-Romeroa, I. Hermosín-Gutiérrez, J. Food Comp. Anal. 20 (2007) 618
- 19. P. M. Aron, J. A. Kennedy, Mol. Nutr. Food Res. 52 (2008) 79
- S. E. Rasmussen, H. Frederiksen, K. S. Krogholm, L. Poulsen, *Mol. Nutr. Food Res.* 49 (2005) 159
- X. Terra, J. Valls, X. Vitrac, J. M. Merrillon, L. Arola, A. Ardevol, C. Blade, J. Fernandez-Larrea, G. Pujadas, J. Salvado, M. Blay, *J. Agric. Food Chem.* 55 (2007) 4357
- C. Agarwal, R. Veluri, M. Kaur, S. C. Chou, J. A. Thompson, R. Agarwal, *Carcinogenesis* 28 (2007) 1478
- M. Stanković, V. Tešević, V. Vajs, N. Todorović, S. Milosavljević, D. Gođevac, *Planta Med.* 74 (2008) 730
- 24. N. Nair, S. Mahajan, R. Chawda, C. Kandaswami, T. C. Shanahan, S. A. Schwartz, *Clin. Diagn. Lab. Immunol.* 9 (2002) 470
- 25. I. S. Arvanitoyannis, D. Ladas, A. Mavromatis, Int. J. Food Sci. Techol. 41 (2006) 475
- 26. E. Bakkalbaşı, O. Yemiş, D. Aslanova, N. Artık, Eur. Food Res. Technol. 221 (2005) 792
- 27. T. Fuleki, J. M. R. da Silva, J. Agric. Food Chem. 45 (1997) 1156
- 28. J. A. Kennedy, M. A. Matthews, A. L. Waterhouse, Phytochemistry 55 (2000) 77
- 29. A. Nakalamić, Jugoslovensko vinogradarstvo i vinarstvo 4 (1991) 7 (in Serbian)
- D. Gođevac, V. Tešević, V. Vajs, S. Milosavljević, M. Stanković, Food Chem. Toxicol. 47 (2009) 2853
- 31. E. Cantos, J. C. Espin, F. A. Tomas-Barberan, J. Agric. Food Chem. 50 (2002) 5691
- 32. R. Rodriguez Montealegre, R. Romero Peces, J. L. Chacon Vozmediano, J. Martinez Gascueña, E. Garcia Romero, *J. Food Compos. Anal.* **19** (2006) 687
- S. U. Mertens-Talcott, J. H. Lee, S. S. Percival, S. T. Talcott, J. Agric. Food Chem. 54 (2006) 5336



GOĐEVAC et al.

- 34. J. H. Lee, J. V. Johnson, S. T. Talcott, J. Agric. Food Chem. 53 (2005) 6003
- 35. C. Cavaliere, P. Foglia, R. Gubbiotti, P. Sacchetti, R. Samperi A. Lagana, *Rapid Com*mun. Mass Spectrom. 22 (2008) 3089
- E. Obreque-Slier, A. Pena-Neira, R. Lopez-Solis, F. Zamora-Marin, J. M. Ricardo-da Silva, O. Laureano, J. Agric. Food Chem. 58 (2010) 3591
- 37. A. I. Mandić, S. M. Đilas, G. S. Ćetković, J. M. Čanadanović-Brunet, V. T. Tumbas, *Int. J. Food Prop.* **11** (2008) 713.

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS







J. Serb. Chem. Soc. 75 (12) 1653–1660 (2010) JSCS–4085 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC *Pleurospermum austriacum: 541.18.045:665.52 Original scientific paper

Volatiles of Pleurospermum austriacum (L.) Hoffm. (Apiaceae)

NIKO S. RADULOVIĆ*, NEVENKA D. ĐORĐEVIĆ and RADOSAV M. PALIĆ#

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

(Received 23 March, revised 12 May 2010)

Abstract: This work presents the first results of detailed GC and GC–MS analyses of the volatile constituents from the fresh leaves, fruits and stalks of an endangered plant species *Pleurospermum austriacum* (L.) Hoffm. (Apiaceae). Sesquiterpene hydrocarbons made up *ca*. 4/5 of the oils obtained in low yield (0.003–0.086 %, w/w). The major volatiles detected were germacrene D (66.5, 62.4 and 49.0 % in fruits, leaves and stalks, respectively), β -caryophyllene (3.1–5.7 %), δ -cadinene (3.6–5.0 %) and (*E*)- β -farnesene (1.0–1.5 %).

Keywords: Pleurospermum austriacum; Apiaceae; essential oil; germacrene D.

INTRODUCTION

Pleurospermum Hoffm. (Apiaceae) is a widespread, heterogeneous genus of complex and controversial taxonomy.^{1–3} Russian authors³ delimit *Pleurospermum sensu stricto* by only two species, *P. austriacum* (that is according to the *Flora Europaea* a sub-boreal Euroasian floral element^{1,2}), and *P. uralense*, referring the other species to *Aulacospermum*, *Hymenidium*, *Hymenolaena*, *Physospermopsis* and *Pterocyclus*. Other morphologically similar genera, where generic boundaries become indistinct, include *Trachydium* and *Pseudotrachydium* Pimenov and Kljuykov.³ As yet, this rather radical classification has not gained widespread acceptance, and the proponents admit that this is a taxonomic hypothesis, and (particularly for some groups) a more natural classification will only be possible following critical revision in the field, herbarium³ and possibly a more general approach, including the use of chemical markers as *principium divisionis*.

Phytochemically speaking, *Pleurospermum* taxa were investigated on a few occasions. The chemistry regarding the volatile compounds of these taxa has been neglected (essential oil investigations), and only *P. lindleyanum* was studied

doi: 10.2298/JSC100323127R

1653



^{*} Corresponding author. E-mail: vangelis0703@yahoo.com

[#] Serbian Chemical Society member.

in this sense.⁴ Most of the phytochemical work realized in this field resulted in the isolation of a number of coumarins (mono-, di-, and trimers) and related phenylpropanoids and concerned only the non-volatile compounds. Some of these molecules were found for the first time as naturally occurring metabolites.^{5–14} Surprisingly, the previously mentioned analyses⁴ of the oil of *P. lindleyanum* resulted in the identification of certain anthropogenic compounds as oil constituents.

The aim of this work was to provide the very first and detailed GC and GC– –MS analyses of the volatile constituents from the leaves, fruits and stalks of the endangered plant species *Pleurospermum austriacum* (L.) Hoffm.¹⁵

EXPERIMENTAL

Plant material

Fresh plants (fruits, leaves and stalks) were collected in July, 2007 at the mountain Stara planina (Babin zub), at an altitude of 1707 m above sea level. The plant was identified by Niko Radulović. A voucher specimen (number 200705) is deposited at the Herbarium of the Faculty of Science and Mathematics, Niš.

Extraction of essential oils

Fresh plant material (three batches of about 250 g of each sample) was subjected to hydrodistillation with *ca*. 2 L of distilled water for 2.5 h using an original Clevenger-type apparatus. Due to the small sample size of the isolated essential oils, which were not completely liquid, the volume of the oils was not measured, and hence the yields are expressed as weight of essential oils per weight of plant material. The obtained oils were separated by extraction with freshly distilled diethyl ether (Merck, Germany) and dried over anhydrous magnesium sulfate (Aldrich, USA). The solvent was evaporated under a gentle stream of nitrogen at room temperature, in order to exclude any loss of the essential oils, and stored at 4 $^{\circ}$ C until analyzed. When the oil yields were determined, after the bulk of the ether had been removed under a stream of N₂, the residue was exposed to vacuum at room temperature for a short period to eliminate the solvent completely. The pure oil was then measured on an analytical balance and multiple gravimetric measurements were taken during 24 h to ensure that all of the solvent had evaporated.

GC-MS

The chemical composition of the oils was investigated by GC and GC–MS. The GC–MS analyses (three repetitions) were realized using a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column HP-5MS (5 % phenylmethylsiloxane, 30 m×0.25 mm, film thickness 0.25 μ m, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface operated at 250 °C and 300 °C, respectively. The oven temperature was increased from 70–290 °C at a heating rate of 5.0 °C min⁻¹ and then isothermally held for 10 min. Helium at a flow rate of 1.0 ml min⁻¹ was used as the carrier gas. The sample, 1 μ l of the oil solution in diethyl ether (1:100), was injected in a pulsed split mode (the flow was 1.5 ml min⁻¹ for the first 0.5 min and then set to 1.0 ml min⁻¹ throughout the remainder of the analysis; split ratio 40:1). The MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35–500, scan time 0.32 s.

Available online at www.shd.org.rs/JSCS/



Gas chromatographic analyses were performed using an HP 5890 gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector (Agilent Technologies, USA). The separation was achieved using a HP-5 (5 % diphenyl- and 95 % dimethylpolysiloxane) fused silica capillary column, 30 m×0.25 mm i.d., 0.25 µm film thickness. The GC oven temperature was programmed from 70 to 290 °C at a rate of 5.0 °C/min. Hydrogen was used as the carrier gas at a flow rate of 1.6 mL min⁻¹ at 45 °C. The injector temperature was 250 °C and the detector temperature: 280 °C; the injection mode was splitless. The percentage composition of the oils was computed from the GC (FID) peak areas without any corrections. The relative standard deviation (*RSD*) of repeated measurements (independent sample preparation and GC–FID) was for all substances below 1 %. The only exceptions which had higher *RSD* were minor components such as methyl salicylate, α -cubebene, 1-hexyl isovale-rate, bicycloelemene and germacrene A, for the *RSD* was 2, 3, 5, 9 and 10 %, respectively.

Oil constituents were identified by comparison of their linear retention indices (relative to C_7 – C_{29} alkanes¹⁶ on the HP-5MS column) with literature values¹⁷ and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known oils, and wherever possible, by co-injection with an authentic sample.

RESULTS AND DISCUSSION

Hydrodistillation of fresh plant material yielded 0.003 % (w/w, based on weight of fresh plant material) of essential oil in the stalks, 0.012 % (w/w) in the leaves and 0.086 % (w/w) in the fruits. The chemical compositions of the essential oils from different parts of P. austriacum are reported in Table I, together with a summation of the compounds according to their respective compound class (percentage and number of detected constituents). Two hundred and five different constituents were identified in the volatile fraction of P. austriacum (156 in fruits, 116 in leaves, 117 in stalks) amounting to 94.5–98.6 % of the total oils. In all samples, the sesquiterpenoid fractions were the most abundant (85.7--90.5 %). About one half of all the identified components belonged to this class, with hydrocarbons in a greater number compared to the oxygenated derivatives. Within this class, the germacranes and related sesquiterpenoids (SGE) were the major ones (71.9 % in the fruits, 63.2 % in the leaves and 52.2 % in the stalks). The major components detected were germacrene D (66.5 % in the fruits, 62.4 % in the leaves and 49.0 % in the stalks), β -caryophyllene (3.1–5.7 %), δ -cadinene (3.6-5.0%) and (E)- β -farnesene (1.0-1.5%). Additional worth mentioning constituents were: (Z)-3-hexen-1-ol (8.8 %), 1-hexanol (1.3 %) and epi-cubebol (2.4 %) in the leaf oil, β -phellandrene (2.3 %), bicyclogermacrene (3.3 %) in the fruit oil and humulene (1.4 %), α -cadinol (3.0 %) and hexadecanoic acid (5.2 %) in the stalk oil. Shyobunol (0.3 %), α -phellandrene (0.3 %), *cis*-muurola-3,5-diene (0.1 %), aristolone (0.1 %) and cinnamyl valerate (0.1 %) were found only in the fruit oil, while 1-phenylethyl 3-methylbutanoate (0.2 %) was only detected in the leaf oil. y-Muurolene (0.8 %), gossonorol (0.3 %), cis-calamenen-10-ol (0.5 %), α -bisabolol (0.9 %), tetradecanoic acid (0.5 %) and 14-hydroxy- δ -cadinene (0.4 %) were present only in the stalk oil and were completely absent from the other



RADULOVIĆ, ĐORĐEVIĆ and PALIĆ

two oils. An additional dissimilarity between these oils was the content of "green leaf" 18 (G) volatiles (volatile aliphatic aldehydes, alcohols, and their esters formed through the hydroperoxide lyase pathway of oxylipin metabolism, plants start to form Gs after disruption of their tissues and after suffering biotic or abio-tic stresses), being predominant in the leaf oil (10.9%), as expected.

		-				
RI^{b}	Component	Fruit oil	Leaf oil	Stalk oil	Class	Method ^c
861	(Z)-3-Hexen-1-ol	0.2	8.8	1.4	G	a, b
863	1-Hexanol	tr^{d}	1.3	0.4	G	a, b, c
1034	β -Phellandrene	2.3	tr	-	Μ	a, b
1383	α-Copaene	1.0	1.0	0.7	SCO	a, b
1428	β -Caryophyllene	3.1	5.7	4.9	SC	a, b, c
1460	(E) - β -Farnesene	1.0	1.2	1.5	SA	a, b
1462	Humulene	0.4	0.6	1.4	SC	a, b, c
1498	Germacrene D	66.5	62.4	49.0	SGE	a, b, c
1504	epi-Cubebol	tr	2.4	tr	SCA	a, b
1507	Bicyclogermacrene	3.3	tr	2.3	SGE	a, b
1511	(E,E) - α -Farnesene	0.6	1.3	1.7	SA	a, b
1514	β -Bisabolene	_	0.5	1.0	SBI	a, b, c
1532	δ -Cadinene	3.9	3.6	5.0	SCA	a, b
1650	τ -Muurolol (syn. ^e <i>epi-a</i> -muurolol)	0.5	0.3	1.0	SCA	a, b
1664	α -Cadinol	1.4	0.9	3.0	SCA	a, b
1962	Hexadecanoic acid	tr	_	5.2	Ο	a, b, c
2027	Isopropyl hexadecanoate	0.3	-	1.0	0	a, b, c
Total i	dentified	95.1	98.6	94.5		
Numb	er of identified comounds	156	116	117		
Monot	terpenoids	$2.8(27)^{\rm f}$	tr (8)	tr (5)		
Hydro	carbons	2.8 (15)	tr (6)	tr (3)		
Oxyge	enated derivatives	tr (12)	tr (2)	tr (2)		
Sesqui	terpenoids	90.5 (66)	85.7 (58)	85.7 (67)		
Hydro	carbons	85.8 (40)	79.5 (37)	72.6 (36)		
Oxyge	enates derivatives	4.7 (26)	6.2 (21)	13.1 (31)		
Acycli	c sesquiterpene (SA)	1.8 (7)	2.6 (7)	4.1 (8)		
Bisabo	planes (SBI)	0.5 (5)	2.0(7)	4.3 (10)		
Caryo	phyllanes and related (SC)	3.8 (3)	6.8 (3)	7.2 (3)		
Cadina	anes and related (SCA)	8.1 (25)	8.6 (19)	14.6 (24)		
Copaa	nes and related (SCO)	1.3 (3)	1.3 (3)	1.1 (3)		
Germa	cranes and related (SGE)	71.9 (5)	63.2 (5)	52.2 (3)		
Other	unclassified sesquiterpenes (S)	3.1 (18)	1.2 (14)	2.2 (16)		
Diterp	enes (DT)	0.1 (2)	0.9 (5)	tr (2)		
"Green	n leaf" volatiles (G)	0.7 (27)	10.9 (24)	1.8 (16)		
Others	s (O)	1.0 (34)	1.1 (21)	7.0 (27)		

TABLE I. Percentage composition of Pleurospermum austriacum oils^a

1656

^aThe remainder of the identified constituents (minor, that have not reached 1 % in any of the samples) of the investigated essential oils are summarized in the following format bellow: *RI*, Component name, Relative percentage of the component in the fruit oil, Leaf oil and stalk oil, Class of the constituent, Method of identification; -743, pyridine, -, tr^d, -, O, a, b, c; 744, (*E*)-2-pentenal, -, tr, -, G, a, b; 762, 1-pentanol, -, -, tr, G, a, b, c; 765, (*Z*)-



2-penten-1-ol, tr, 0.2, tr, G, a, b; 780, (±)-2,3-butandiol, -, -, tr, O, a, b; 785, meso-2,3-butandiol, -, -, tr, O, a, b; 801, hexanal, tr, tr, tr, G, a, b; 834, furfural, tr, tr, tr, O, a, b, c; 852, (E)-3-hexen-1-ol, -, 0.1, tr, G, a, b; 885, 2--butylfuran, tr, tr, tr, G, a, b; 895, (2E,4Z)-2,4-hexadienal, tr, -, -, G, a; 900, nonane, tr, -, tr, O, a, b, c; 903, cyclohexanone, -, tr, -, O, a, b, c; 913, (2E,4E)-2,4-hexadienal, -, tr, -, G, a, b; 930, α-thujene, tr, -, -, M, a, b; 939, α-pinene, 0.1, -, tr, M, a, b, c; 955, camphene, tr, -, -, M, a, b, c; 957, (E)-2-heptenal, tr, tr, -, G, a, b; 967, benzaldehyde, tr, tr, tr, O, a, b, c; 978, sabinene, 0.1, tr, tr, M, a, b; 984, β-pinene, tr, -, -, M, a, b, c; 993, myrcene, tr, -, -, M, a, b, c; 995, 2-pentylfuran, tr, tr, G, a, b; 999, 2,4,6-trimethylpyridine, -, tr, -, O, a, b, c; 1001, mesitylene, tr, -, -, O, a, b, c; 1005, octanal, tr, tr, tr, G, a, b; 1008, (E)-3-hexenyl acetate, 0.1, 0.2, tr, G, a, b; 1010, α-phellandrene, 0.3, -, -, M, a, b; 1013, 1-hexyl acetate, -, tr, -, G, a, b, c; 1014, (2E,4E)-2,4-heptadienal, -, tr, tr, G, a, b; 1021, α-terpinene, tr, -, -, M, a, b, c; 1029, p-cymene, tr, tr, M, a, b, c; 1033, limonene, tr, tr, -, M, a, b, c; 1044, β-isophorone, tr, -, -, O, a, b, c; 1048, phenylacetaldehyde, tr, 0.2, tr, O, a, b, c; 1048, (E)-β-ocymene, tr, -, -, M, a, b; 1059, (Z)-2-octenal, tr, -, -, G, a, b; 1062, γ-terpinene, tr, tr, -, M, a, b; 1062, artemisia ketone, tr, -, -, M, a, b, c; 1070, 1-octanol, -, tr, -, G, a, b, c; 1072, acetophenone, -, -, tr, O, a, b, c; 1077, cis-linalool oxide (furanoid), tr, -, -, M, a, b, c; 1094, terpinolene, tr, -, -, M, a, b; 1094, trans-linalool oxide (furanoid), tr, -, -, M, a, b, c; 1098, 3-isopropyl-2-methoxypyrazine, tr, -, -, O, a, b; 1100, undecane, tr, tr, tr, O, a, b, c; 1103, linalool, tr, tr, tr, M, a, b, c; 1107, nonanal, 0.1, 0.1, -, G, a, b; 1107, hotrienol, tr, -, -, M, a, b; 1109, 2-methylbutyl isovalerate, tr, -, -, O, a, b; 1118, 3-methyl-3-butenyl 3-methylbutanoate, tr, -, -, O, a, b; 1120, endo-fenchol, tr, -, -, M, a, b; 1127, dehydrosabina ketone, tr, -, -, M, a, b; 1127, α-isophorone, tr, -, -, O, a, b; 1162, (E)-2-nonenal, tr, -, -, G, a, b; 1184, terpinen-4-ol, tr, -, -, M, a, b, c; 1191, naphthalene, tr, -, -, O, a, b, c; 1194, cryptone, tr, -, -, M, a, b; 1199, (Z)-4-decenal, tr, -, -, G, a, b; 1201, methyl salicylate, tr, 0.2, tr, O, a, b, c; 1208, decanal, tr, tr, -, G, a, b, c; 1225, *endo*-fenchyl acetate, tr, tr, -, M, a, b; 1227, β-cyclocitral, -, tr, -, O, a, b, c; 1227, (2E,4E)-2,4-nonadien-1-ol, tr, -, -, G, a, b; 1234, (Z)-3-hexenyl 2-methylbutanoate, tr, tr, -, G, a, b; 1238, (Z)-3-hexenyl 3-methylbutanoate, 0.1, 0.2, tr, G, a, b; 1238, 1-hexyl 2-methylbutanoate, tr, -, -, G, a, b; 1238, methyl thymol, tr, -, -, M, a, b, c; 1243, 1-hexyl isovalerate, 0.2, tr, tr, G, a, b; 1248, methyl carvacrol, tr, -, -, M, a, b, c; 1265, (E)-2-decenal, tr, -, -, G, a, b; 1294, 1-tridecene, tr, -, -, O, a, b; 1297, (2E,4Z)-2,4decadienal, tr, -, -, G, a, b; 1300, tridecane, tr, tr, -, O, a, b, c; 1300, 2-methylnaphtalene, tr, -, -, O, a, b; 1309, undecanal, tr, -, -, G, a, b; 1317, (E)-3-hexenyl tiglate, tr, tr, -, G, a, b; 1319, p-vinylguaiacol, -, tr, -, O, a, b; 1321, (2E,4E)-2,4-decadienal, tr, tr, G, a, b; 1323, 1-hexyl senecioate (hexyl 2-methyl-2-butenoate), tr, tr, -, G, a, b; 1325, (E)-9-undecenal, -, -, tr, G, a, b; 1343, bicycloelemene, 0.1, tr, -, S, a, b; 1355, α-cubebene, 0.1, tr, tr, S, a, b; 1374, cyclosativene, tr, -, -, S, a, b; 1378, α-ylangene, tr, tr, tr, S, a, b; 1390, geranyl acetate, tr, tr, tr, M, a, b, c; 1392, β-bourbonene, tr, tr, tr, S, a, b; 1397, β-cubebene, 0.6, 0.8, 0.6, S, a, b; 1398, β-elemene, 0.5, tr, tr, S, a, b; 1411, dodecanal, tr, tr, tr, G, a, b; 1419, a-gurjunene, 0.2, tr, -, S, a, b; 1421, cis-a-bergamotene, -, -, tr, SBI, a, b; 1437, β -copaene, 0.3, 0.3, 0.4, SCO, a, b; 1441, trans- α -bergamotene, -, tr, tr, SBI, a, b; 1443, β --gurjunene, 0.8, tr, tr, S, a, b; 1448, aromadendrene, tr, tr, -, S, a, b; 1454, cis-muurola-3,5-diene, 0.1, -, -, SCA, a, b; 1472, cis-muurola-4(14),5-diene, 0.3, 0.2, 0.3, SCA, a, b; 1484, y-muurolene, -, -, 0.8, SCA, a, b; 1485, y--curcumene, -, -, tr, SBI, a, b; 1488, herbertene, -, -, tr, SBI, a, b; 1496, 1-phenylethyl 3-methylbutanoate, -, 0.2, -, O, a, b; 1498, 10,11-epoxy-calamenene, -, -, tr, SCA, a, b; 1500, β-selinene, tr, -, 0.2, S, a, b; 1502, α-zingiberene, tr, 0.4, -, SBI, a, b; 1503, *trans*-muurola-4(14),5-diene, 0.4, tr, 0.6, SCA, a, b; 1507, α-muurolene, tr, tr, tr, SCA, a, b; 1511, α -cuprenene, tr, tr, tr, SBI, a, b; 1515, germacrene A, 0.6, tr, -, SGE, a, b; 1515, δ -amorphene, tr, -, -, SCA, a, b; 1517, β-curcumene, 0.2, 0.4, 0.8, SBI, a, b; 1523, γ-cadinene, 0.5, 0.3, 0.5, SCA, a, b; 1524, cubebol, tr, tr, -, SCA, a, b; 1538, (E)-y-bisabolene, 0.2, 0.4, 0.7, SBI, a, b; 1540, trans-cadina-1,4-diene, 0.1, tr, tr, SCA, a, b; 1546, α-cadinene, 0.1, tr, tr, SCA, a, b; 1552, α-calacorene, tr, tr, 0.2, SCA, a, b; 1557, cis--muurola-5-en-4 β -ol, tr, -, -, SCA, a, b; 1557, dodecanoic acid, -, -, tr, O, a, b, c; 1564, *cis*-cadinene ether, tr, -, -, SCA, a, b; 1565, *cis*-muurola-5-en-4α-ol, tr, -, -, SCA, a, b; 1568, (*E*)-nerolidol, 0.1, 0.1, 0.5, SA, a, b; 1571, β--calacorene, tr, tr, tr, SCA, a, b; 1574, γ-undecalactone, tr, -, -, O, a, b; 1576, mint oxide, 0.1, 0.1, 0.3, S, a, b; 1582, (E)-dendrolasin, tr, tr, ts, SA, a, b; 1584, germacrene D-4-ol, 0.9, 0.3, -, SGE, a, b; 1587, spathulenol, tr, 0.1, 0.2, S, a, b, c; 1590, 10-epi-junenol, -, -, tr, S, a, b; 1593, caryophyllene oxide, 0.3, 0.5, 0.9, SC, a, b, c; 1595, β-copaen-4α-ol, tr, -, -, SCO, a, b; 1597, cis-β-elemenone, -, -, tr, S, a, b; 1603, salvial-4(14)-en-1-one, 0.1, tr, tr, S, a, b; 1610, 3-phenylpropyl 2-methylbutanoate, tr, -, -, O, a, b; 1611, rosifoliol, -, -, tr, S, a, b; 1613, 3-phenylpropyl 3-methylbutanoate, tr, -, -, O, a, b; 1615, tetradecanal, 0.1, 0.3, O.3, O, a, b, c; 1623, 1,10-di-epi--cubenol, tr, tr, 0.5, SCA, a, b; 1629, junenol, 0.2, 0.1, 0.6, S, a, b; 1630, α -corocalene, -, -, tr, SCA, a, b; 1632, nor-copaanone, -, tr, tr, SCO, a, b; 1637, 1-epi-cubenol, 0.1, 0.2, 0.2, SCA, a, b; 1645, gossonorol, -, -, 0.3, S, a, b; 1650, τ-cadinol (syn.^e epi-α-cadinol), 0.5, 0.3, 0.9, SCA, a, b; 1650, τ-muurolol (syn. epi-α-muurolol), 0.5, 0.3, 1.0, SCA, a, b; 1655, a-muurolol, 0.2, 0.2, 0.7, SCA, a, b; 1667, cis-calamenen-10-ol, -, -, 0.5, SCA, a, b;

cc) 🛈 🕲 🗉

RADULOVIĆ, ĐORĐEVIĆ and PALIĆ

1678, trans-calamenen-10-ol, tr, 0.2, tr, SCA, a, b; 1688, cinnamyl valerate (correct isomer not determined), 0.1, -, -, O, a; 1690, epi-α-bisabolol, 0.1, 0.3, 0.9, SBI, a, b; 1692, α-bisabolol, -, -, 0.9, SBI, a, b, c; 1696, germacra-4(15),5,10(14)-trien-1a-ol, 0.6, 0.5, 0.9, SGE, a, b; 1701, shyobunol, 0.3, -, -, S, a, b; 1701, 3phenylpropyl hexanoate (correct isomer not determined), tr, -, -, O, a; 1705, (2Z,6Z)-farnesol, -, -, tr, SA, a, b; 1713, amorpha-4,9-dien-2α-ol, tr, -, tr, SCA, a, b; 1717, pentadecanal, tr, -, tr, O, a, b; 1720, (2E,6Z)-farnesal, tr, tr, tr, SA, a, b; 1727, (2Z,6E)-farnesol, 0.1, tr, 0.4, SA, a, b; 1747, (2E,6E)-farnesal, tr, tr, tr, SA, a, b; 1749, mint sulfide, tr, 0.1, tr, S, a, b; 1763, tetradecanoic acid, -, -, 0.5, O, a, b, c; 1772, aristolone, 0.1, -, -, S, a, b; 1782, 14-hydroxy-α-muurolene, tr, tr, tr, SCA, a, b; 1811, 14-hydroxy-δ-cadinene, -, -, 0.4, SCA, a, b; 1818, hexadecanal, tr, tr, tr, O, a, b, c; 1841, neophytadiene, isomer II, 0.1, 0.8, tr, DT, a, b; 1862, pentadecanoic acid, -, -, tr, O, a, b; 1900, nonadecane, tr, -, -, O, a, b, c; 1928, methyl hexadecanoate, -, -, tr, O, a, b, c; 1944, (Z)-9--hexedecenoic acid (palmitoleic acid), -, -, tr, O, a, b, c; 1950, isophytol, -, tr, -, DT, a, b; 1971, (Z,Z)-geranyl linalool, -, tr, -, DT, a, b; 1996, ethyl hexadecanoate, -, -, tr, O, a, b, c; 2000, eicosane, tr, -, -, O, a, b, c; 2034, (*E,E*)-geranyl linalool, tr, 0.1, tr, DT, a, b; 2083, 1-octadecanol, -, -, tr, O, a, b, c; 2100, heneicosane, 0.1, tr, -, O, a, b, c; 2117, (E)-phytol, -, tr, -, DT, a, b; 2200, docosane, tr, tr, -, O, a, b, c; 2281, 1-eicosanol, tr, -, -, O, a, b, c; 2300, tricosane, 0.2, 0.1, tr, O, a, b, c; 2396, 1-tetracosene, -, tr, tr, O, a, b; 2400, tetracosane, tr, tr, tr, O, a, b, c; 2500, pentacosane, 0.2, 0.1, tr, O, a, b, c; 2600, hexacosane, -, -, tr, O, a, b, c; 2700, heptacosane, -, tr, -, O, a, b, c; 2833, (all *E*)-squalene, -, -, tr, O, a, b.; ^bexperimentally determined retention indices by co-injection of a homologous series of *n*-alkanes (C_7-C_{29}) ; ^ca – constituent identified by mass spectra comparison, b – constituent identified by retention index matching, c – constituent identity confirmed by co-injection of an authentic sample; d trace (<0.05 %); e synonym; f the value in brackets represents the number of identified compounds belonging to this specific class; M - monoterpenoids; SA - acyclic sesquiterpenes; SBI bisabolanes; SC - caryophyllanes and related sesquiterpenoids; SCA - cadinanes and related sesquiterpenoids; SCO - copaanes and related sesquiterpenoids; SGE - germacranes and related sesquiterpenoids; S - other unclassified sesquiterpenes; DT - diterpenes; G - "green leaf" volatiles (aliphatic aldehydes, alcohols, and their esters formed as the plants response after suffering biotic or abiotic stresses); O - other unclassified constituents

The only previously published investigated essential oil from a *Pleuro-spermum* species was the one from *P. lindleyanum* (according to Pimenov and Kljuykov,³ this taxon is transferred to the genus *Hymenidium* (*H. stellatum*)).⁴ The authors state that 73 oil constituents were identified, and among them were: 1-propoxy-2-propanol, myristicin, 1,2,3-trimethoxy-5-(2-propenyl)-benzene, *cis*-asarone, *n*-hexane, apiol, dimethyl ether, 1,2-dimethoxy-4-(2-propenyl)benzene, ethyl acetate, spathulenol, α, α' ,4-trimethyl-benzenemethanol, *trans*-methyl *iso*-eugenol, and β -phellandrene. Apparently a number of the listed compounds represent either misidentifications or identification of a solvent contaminant introduced during the work-up of the essential oil sample (1-propoxy-2-propanol, dimethyl ether, *n*-hexane, ethyl acetate). Apart from this, the authors refer to the content (20.77 %) of cycloserine "as the highest" in conjunction with the essential oil.⁴ The compounds in common with the present *P. austriacum* oil were spathulenol and β -phellandrene.

The sesquiterpenoid fraction of the oils of *P. austriacum* was mostly comprised of germacranes and highly related types of compounds (Table I) that are the first steps in the biosynthesis pathway of sesquiterpenes from farnesyl diphosphate. Sesquiterpene biosynthesis seems to be complex since the formation *via* either pathway (mevalonic or methylerythrytol) or a combination of both has been reported.¹⁹ However, these appear to be omnipresent in plant taxa and some insects, and is related to the cytosol–mitochondria. A previous investigation showed

1659

a direct correlation between the amount and chemical composition of the essential oils.¹⁸ The results of these statistical analyses strongly suggest that the main volatiles of essential oil-poor species (yields less than 0.1 %) are fatty acid- and carotenoid-derived compounds, while the essential oil rich taxa (essential oil yields much higher than 0.1 %) are generally characterized by the specific production of monotepenoids and/or phenylpropanoids.¹⁸ Two facts, the monoterpenes that accounted only for 0.0–2.8 % and that the yield of each oil was quite low (0.003-0.086 %, w/w), corroborate the previously introduced hypothesis concerning a possible link between the oil yield and the corresponding oil composition. It is, then, directly possible to conclude that if some plant is rich in oil, it will probably have a big portion of monoterpenes, or the phenylpropanoids are predominant. A related hypothesis concerning a possible link between oil yield and composition was already proposed.²⁰ Lawrence suggested that the oil-poor species of the family Lamiaceae produce essential oils rich in hydrocarbons, with germacrene D often being one of the predominant components.²⁰ This does not seem to stand for the Lamiaceae alone. For example, in the oil-poor (0.18-0.57 %) aerial parts of Tamarix boveana (Tamaricaceae), germacrene D (7.69-31.43 %) with its congeners represented the predominant class of volatile compounds.²¹ In some previous investigations of *Lippia alba* (Verbenaceae), possible connections between the morphological characteristics and chemotypes were discussed, and a large content of germacrene D was a feature of one of the chemotypes.²² More strikingly, a study on Artemisia annua showed that the glandless leaves were estimated to contain more than twice as much total sesquiterpenes per unit of fresh weight as the glanded leaves. In this way, the absence of monoterpenes in the steam-distilled oil of the glandless biotype is a good indication that the monoterpenes accumulate exclusively in specialized tissue structures, the glandular trichomes.²³ Thus, one may expect to find germacrene D and/or its hydrocarbon congeners as major constituents of essential oil in plant species not possessing an elaborate biosynthetic apparatus for the production of volatile monoterpenes or phenylpropanoids, as *P. austriacum*.

Acknowledgements. The financial support of this work by the Ministry of Science and Technological Development of the Republic of Serbia is gratefully acknowledged (Project No. 172061).

ИЗВОД

ИСПАРЉИВИ САСТОЈЦИ БИЉНЕ ВРСТЕ *Pleurospermum austriacum* (L.) Hoffm. (APIACEAE)

НИКО С. РАДУЛОВИЋ, НЕВЕНКА Д. ЂОРЂЕВИЋ и РАДОСАВ М. ПАЛИЋ

Одсек за хемију, Природно-машемашички факулшеш, Универзишеш у Нишу, Вишеградска 33, 18000 Ниш

Резултати детаљних GC и GC–MS анализа испарљивих састојака свежих листова, стабљика и плодова угрожене биљне врсте *Pleurospermum austriacum* (L.) Hoffm. (Apiaceae) приказани су у овом раду по први пут. Око 4/5 уља, добијених у ниском приносу (0,003–0,086

RADULOVIĆ, ĐORĐEVIĆ and PALIĆ

mas. %), је било сачињено од сесквитерпенских угљоводоника. Главне компоненте, идентификоване у уљима, су гермакрен Д (66,5 % у плоду, 62,4 % у лишћу и 49,0 % у стабљици), β -кариофилен (3,1–5,7 %), δ -кадинен (3,6–5,0 %) и (*E*)- β -фарнезен (1,0–1,5 %).

(Примљено 23. марта, ревидирано 12. маја 2010)

REFERENCES

- T. G. Tutin, in *Flora Europaea* 2, T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, D. A. Webb, Eds., Cambridge University Press, London, 1968, p. 343.
- 2. P. Zehui, F. M. Watson, Flora Chn. 14 (2005) 40

1660

- 3. M. G. Pimenov, E. V. Kljuykov, Feddes Rep. 111 (2000) 517
- 4. H. A. Aisa, Q. Y. Lu, Tianran Chanwu Yanjiu Yu Kaifa (Nat. Prod. Res. Dev.) 14 (2002) 46
- 5. J. J. Tan, C. H. Tan, Y. Q. Wang, S. H. Jiang, D. Y. Zhu, Helv. Chim. Acta 89 (2006) 117
- J. J. Tan, S. H. Jiang, D. Y. Zhu, *Tianran Chanwu Yanjiu Yu Kaifa (Nat. Prod. Res. Dev.)* 17 (2005) 267
- 7. Y. G. Luo, B. G. Li, G. L. Zhang, J. Asian Nat. Prod. Res. 4 (2002) 155
- 8. M. Taniguchi, Y. Hada, K. Baba, Y. Q. Xiao, in *Proceeding of 42nd Symposium on the Chemistry of Natural Products*, (2000), Okinawa, Japan, 2000, p. 529.
- M. Taniguchi, Y. Q. Xiao, X. H. Liu, A. Yabu, Y. Hada, K. Baba, *Chem. Pharm. Bull.* 46 (1998) 1946
- 10. M. Taniguchi, Y. Q. Xiao, K. Baba, Chem. Pharm. Bull. 48 (2000) 1246
- M. Taniguchi, Y. Q. Xiao, X. H. Liu, A. Yabu, Y. Hada, L. Q. Guo, Y. Yamazoe, K. Baba, *Chem. Pharm. Bull.* 47 (1999) 713
- M. Shibano, H. Naito, M. Taniguchi, N. H. Wang, K. Baba, Chem. Pharm. Bull. 54 (2006) 717
- 13. U. Mahmood, S. B. Singh, R. S. Thakur, Phytochemistry 22 (1983) 774
- 14. U. Mahmood, R. S. Thakur, Indian J. Pharm. Sci. 43 (1981) 157
- 15. O. Gerdjikov, *Biological diversity act*, National Assembly, Republic of Bulgaria, Promulgated, *State gazette* no. 77/9.08.2002
- 16. H. Van Den Dool, P. D. Kratz, J. Chromatogr. 11 (1963) 463
- 17. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography and Mass Spectrometry*, 4th ed., Allured Publishing Corporation, Carol Stream, IL, 2007
- 18. N. S. Radulović, P. D. Blagojević, R. M. Palić, Nat. Prod. Commun. 4 (2009) 405
- 19. D. Umlauf, J. Zapp, H. Becker, K. P. Adam, Phytochemistry 65 (2004) 2463
- B. M. Lawrence, *Essential Oil 1988-1991*, Allured Publishing Corporation, Carol Stream, IL, 1992, p. 194
- D. Saïdana, M. A. Mahjoub, O. Boussaada, J. Chriaa, I. Chéraif, M. Daami, Z. Mighri, A. N. Helal, *Microbiol. Res.* 163 (2008) 445
- 22. T. Hennebelle, S. Sahpaz, C. Dermont, H. Joseph, F. Bailleul, *Chem. Biodivers.* **3** (2006) 1116
- 23. R. M. Tellez, C. Canel, M. A. Rimando, O. S. Duke, *Phytochemistry* 52 (1999) 1035.







J. Serb. Chem. Soc. 75 (12) 1661–1669 (2010) JSCS–4086 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC **Levisticum officinale:665.52:615.281–188 Original scientific paper

The composition and antibacterial activity of the essential oil of *Levisticum officinale* Koch flowers and fruits at different developmental stages

MOHAMMAD HOSSEIN MIRJALILI^{1*}, PEYMAN SALEHI¹, ALI SONBOLI¹, JAVAD HADIAN¹, SAMAD NEJAD EBRAHIMI¹ and MORTEZA YOUSEFZADI²

¹Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C., Evin, Tehran and ²Department of Marine Biology, Faculty of Science, Hormozgan University, Bandar Abbas, Iran

(Received 24 May, revised 9 July 2010)

Abstract: The composition and antibacterial activity of the essential oil of Levisticum officinale Koch at different developmental stages (flower, immature fruit, green mature fruit and ripened fruit) is reported. The essential oils were obtained by hydrodistillation of air-dried samples and their antibacterial activities were tested against seven bacteria. The yield of oil (w/w %) in different stages was in the order: immature fruit (1.5 %) > green mature fruit (1.0 %) > > ripened fruit (0.6 %) > flower (0.1 %). The essential oils were analyzed by GC and GC-MS. In total, 27, 31, 28 and 26 constituents were identified and quantified in the mentioned samples, respectively. Monoterpene hydrocarbons were the main group of compounds in the green mature fruit (79.2 %), immature fruit (78.4 %), ripened fruit (75.2 %) and flower (44.0 %). The antibacterial activity of the oils was evaluated by the disk diffusion method using Müller-Hinton agar and determination of inhibition zones. The results of the bioassays showed some variations between the three tested oils in their inhibitory activity against the tested bacteria at a 10 µl disc⁻¹ concentration. The oils from mature and ripened fruit exhibited potent antibacterial activity against Bacillus subtilis, with minimum inhibitory concentration (MIC) values of 0.90 mg ml⁻¹ in mature and ripened fruits.

Keywords: Levisticum officinale Koch; Apiaceae; essential oil; antibacterial activity; reproductive stage.

INTRODUCTION

Lovage (*Levisticum officinale* Koch) is a perennial herbaceous plant from the Apiaceae family with origins in Iran and Afghanistan; it can now be found throughout the world.^{1–4} The plant has been alternatively classified as *Ligus*-

1661

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS



^{*}Corresponding author. E-mail: m-mirjalili@sbu.ac.ir doi: 10.2298/JSC100524126M

MIRJALILI et al

ticum levisticum L., Hipposelinum levisticum Britt. and Angelica levisticum Baillon.⁵ The essential oil of roots, seeds and leaves of lovage are used in a wide variety of applications including food flavoring, medicinal preparations, aromatherapy, perfumery and industrial fragrances.^{2,6,7} Moreover, the plant is used in Iranian folk medicine for the treatment of several gastrointestinal, nervous and rheumatic disorders.^{2,8} The essential oil composition of the plant was previously studied in different countries and more than 190 compounds were reported in its root, seed or leaf oil.⁹ It was found that the chemical compositions of the essential oils distilled from separate botanical parts of this plant are rather different.^{10–12} The chemical constituents of lovage root oil are mainly phthalides including *n*-butylidene phthalide and *n*-butyl-phthalide, sedanonic anhydride, terpenoids such as α -terpineol, carvacrol, phenylpropanoids such as eugenol and volatile acids.^{5,13,14} Polyacetylenes as antimycobacterial compounds have also been reported from the plant.¹⁵ The effect of harvesting time, plant age, cutting frequency and the method of plantation establishment on the essential oil yield and components in different parts of L. officinale was investigated previously.^{7,11,16,17} It was found that the flowers and seeds produced the highest yields of the oil with β -phellandrene (40.8 and 61.5 %, respectively) as the main constituent, while α -terpinyl acetate (≈ 70.0 %) was reported as the principal constituent of the leaves and stems oils.⁷ In another study, the oil of lovage fruits contained β -phellandrene (69.3 %), α -terpinenyl acetate (4.2 %) and α -terpineol (2.1 %) as the major components.¹² It was reported that the essential oil content was similar in roots, stems, petioles, leaves and inflorescence, while the highest content was found in seeds (1.9 %).18 Seasonal variations in the composition of headspace volatiles were also determined,¹⁰ of which, β -phellandrene was the most abundant component in all plant parts except for root. Samiee et al. reported terpinyl acetate (40.5 %) and β -phellandrene (16.7 %) as the main constituents in the essential oil and β -phellandrene (23.0 %), naphthalene (20.6 %) and γ -terpinene (12.1 %) as the major components in the methanol extract of the plant from Iran.¹⁹ Recently, (Z)-falcarinol, *n*-octanal, palmitic acid, (Z)-ligustilide, (Z)--3-butylidenephthalide, *trans-\beta*-farnesene have been reported as the main compounds of the essential oil of hairy root cultures of L. officinale.²⁰⁻²² Variations in the essential oil composition of roots and leaves of L. officinale from different European countries have also been studied. Ten compounds, including trans-p--mentha-2,8-dien-1-ol, iso-thujyl alcohol, p-mentha-1,5-dien-8-ol, bicyclo[3.2.0]heptan-3-ol, 2-methylene-6,6-dimethyl, trans-carveol, perillaldehyde, sabinyl acetate, perillyl alcohol, the methyl ester of methylpentadecanoic acid and methylhexadecadienoic acid, were introduced for the first time.²³ To the best of our knowledge, there is no previous report on the essential oil analysis and antibacterial activity of L. officinale at different developmental stages. Thus, in this pa-

Available online at www.shd.org.rs/JSCS/

per, the composition and antibacterial activity of the essential oils of this plant at different stages of its development are reported.

EXPERIMENTAL

Plant material

These experiments were conducted during 2007–2009 at the field of the Medicinal Plants and Drugs Research Institute of Shahid Beheshti University, located in Evin (35°48' N, 51°23' E at an altitude of 1785 m) in the north of Tehran, Iran. The plant seeds were obtained from the seed bank of the Medicinal Plants and Natural Products Research Institute, Iranian Academic Center for Education, Culture and Research (ACECR) and were sown in a greenhouse in the last week of February, 2007. Nine-week-old seedlings were transplanted at 50 cm row-to-row and 30 cm plant-to-plant spacing in the experimental field in May, 2007. The sampling was realized from a 2-year-old cultivated population by the random collection of 10 individuals for each developmental stage. For the collection of the flowers, all of them on the inflorescence were opened. The samples at the fruiting stage were collected at three different times of fruit maturation, *i.e.* immature (infructescence with young fruits 15 days after flowering), mature (infructescence with solid and dark green colored fruit) and ripened (infructescence with yellowish fruits just in the deciduous time). Voucher specimens (No. 200364-7) representative of each sample were deposited at the Medicinal Plants and Drugs Research Institute Herbarium (MPH), Shahid Beheshti University of Tehran.

Essential oil isolation procedure

The essential oil of air-dried samples (100 g) of each stage was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (1993).²⁴ The isolated oils were dried over anhydrous sodium sulfate and stored in dark tightly closed vials at 4 °C until analysis.

Essential oil analysis procedure

GC analysis was conducted using a Varian CP-3800 instrument equipped with a DB-1 fused silica capillary column (25 m×0.25 mm i.d., film thickness 0.25 μ m). Nitrogen was used as the carrier gas at a constant flow rate of 1.1 ml min⁻¹. The oven temperature was held at 60 °C for 1 min, then programmed to 250 °C at a rate of 4 °C min⁻¹, and then held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively. GC//MS analysis was realized on a Thermoquest-Finnigan Trace GC/MS instrument equipped with a DB-1 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 μ m). The oven temperature was raised from 60 to 250 °C at a rate of 5 °C min⁻¹ and then held at 250 °C for 10 min; the transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml min⁻¹; the split ratio was 1/50. The quadrupole mass spectrometer was scanned over the 45–465 amu range with an ionizing voltage of 70 eV and an ionization current of 150 μ A.

Identification and quantification of the oil components

The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C6–C24) and the oil on a DB-1 column under the same chromatographic condition. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds (purchased from Sigma-Aldrich and Merck) or with those reported in the literature.²⁵ For quantification purpose, the relative area percentages obtained by FID were used without the use of correction factors.

MIRJALILI et al

Antibacterial activity

The antibacterial activity of the oils was evaluated by the disk diffusion method using Müller-Hinton agar²⁶ and determination of the inhibition zones. The essential oils were tested at a concentration of 10 µl per disk. The microorganisms used were as follows: *Bacillus sub-tilis* ATCC 9372, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas ae-ruginosa* ATCC 27852, and *Klebsiella pneumoniae* ATCC 3583. For the determination of the minimum inhibitory concentration (*MIC*), a microdilution broth susceptibility assay was used, as recommended by NCCLS.²⁷ The technical data were described previously.²⁸ Ampicillin was used as the standard reference for the positive control.

RESULTS AND DISCUSSION

Essential oil analysis

The essential oils had a light yellow color with distinct sharp odor. The yield of the essential oils (w/w %) of the plant at different developmental stages were in the order: immature fruit (1.5 %) > green mature fruit (1.0 %) > ripened fruit (0.6 %) > flower (0.1 %). The qualitative and quantitative analytical results are listed in Table I together with the retention indices of the identified compounds, where all the constituents are arranged in order of their elution on the DB-1 column. In total 27, 31, 28 and 26 constituents, respectively, were identified and quantified in the studied samples representing 95.9, 99.9, 98.7 and 92.7 % of the total oil, respectively. A comparison among the composition of the essential oils revealed both quantitative and qualitative differences. The GC and GC-MS analyses showed that the distribution of saturated hydrocarbons of the oil from flower was remarkably different from that of the oils at the fruiting stage. The results revealed that the saturated hydrocarbons from the flower (12.3 %) were present in higher amount than in the other samples. Heneicosane (6.0 %) and tricosane (3.0 %) were found only in the oil of the flower. The major constituent of the oil of the flower was β -pinene (17.7 %), but it was found that this compound decreased gradually in subsequent developmental stages. The β -pinene and α -pinene contents were highest in the essential oil of the first harvest and decreased with progressive maturation of the fruit. On the contrary, β -phellandrene was found as the principal component of the oil after fruit initiation, *i.e.*, it constituted 11.7 % of the oil of the flower but increased remarkably in the fruit oils, constituting 62.4, 60.5 and 56.4 % of the green mature, immature and ripened fruit oils, respectively. β -Phellandrene has already been reported as the main constituent in the essential oil from the flowers and fruits in previous reports.^{7,10,12} β -Gurjunene (2.8 %), globulol (0.7 %) and geranyl acetate (3.3 %) were found only in the oil of the flower. α -Phellandrene, δ -elemene and germacrene-D were absent completely in the oil of flower but were present in trace or low amounts in the other samples. The essential oil obtained from immature fruit contained the highest contents of sabinene (2.3 %), isomenthol (5.6 %), cis-dihydrocarvon (0.6



%), germacrene-D (0.6 %), elemol (0.5 %) and *trans*-nerolidol (1.6 %) compared with the other samples.

TABLE I. Composition of the essential oil of *Levisticum officinale* at different developmental stages

D r ^a	Commoned	From flowers	In frui	iting stag	es, %	Identification
KI	Compound	%	Immature	Mature	Ripened	methods
0935	α -Pinene	5.3	4.6	4.3	2.9	RI, MS ^b , CoI ^c
0969	Sabinene	1.7	2.3	1.5	1.1	RI, MS
0976	β -Pinene	17.7	11.5	4.1	2.9	RI, MS, CoI
0982	Myrcene	1.3	t ^d	t	t	RI, MS
1002	α -Phellandrene	_	t	t	t	RI, MS
1010	δ -3-Carene	3.0	2.8	5.7	6.8	RI, MS
1017	ortho-Cymene	1.4	t	t	t	RI, MS
1026	β -Phellandrene	11.7	56.4	62.4	60.5	RI, MS
1038	cis-Ocimene	1.9	0.8	0.8	0.8	RI, MS
1083	Terpinolene	_	_	0.4	0.2	RI, MS, CoI
1111	cis-p-Menth-2-en-1-ol	_	0.3	_	0.2	RI, MS
1162	Isomenthol	1.8	5.6	3.9	2.1	RI, MS
1166	4-Terpineol	0.5	0.6	_	_	RI, MS
1168	cis-Dihydrocarvone	_	0.6	_	_	RI, MS
1217	Cuminyl aldehyde	_	0.5	t	_	RI, MS
1265	p-Cymene-7-ol	_	0.4	t	_	RI, MS
1339	δ -Elemene	_	0.4	0.3	0.3	RI, MS
1358	Geranyl acetate	3.3	_	_	_	RI, MS, CoI
1385	α-Copaene	1.3	0.3	0.3	0.2	RI, MS
1392	β -Elemene	1.0	1.2	1.3	1.3	RI, MS
1433	α -Humulene	0.9	0.4	0.3	0.2	RI, MS
1436	β -Gurjunene	2.8	_	_	_	RI, MS
1446	(Z) - β -Farnesene	4.3	0.2	0.2	0.2	RI, MS
1473	γ-Curcumene	8.0	0.2	0.2	0.3	RI, MS
1484	Germacrene-D	_	0.6	0.3	t	RI, MS
1489	Zingiberene	0.8	0.5	0.8	t	RI, MS
1500	Germacrene-B	0.8	1.6	t	1.2	RI, MS
1503	β -Bisabolene	1.6	0.8	1.8	-	RI, MS
1518	β -Sesquiphellandrene	2.1	1.2	2.4	5.1	RI, MS
1541	Elemol	_	0.5	_	_	RI, MS
1548	trans-Nerolidol	0.8	1.6	_	_	RI, MS
1562	γ-Elemene	_	0.8	0.8	1.2	RI, MS
1574	Spathulenol	8.9	1.1	1.9	2.7	RI, MS
1593	Globulol	0.7	_	_	_	RI, MS
1601	Hexadecane	3.3	2.1	2.5	2.2	RI, MS
1691	3-iso-Thujopsanone	_	_	2.5	0.3	RI, MS
2097	Heneicosane	6.0	-	_	-	RI, MS
2301	Tricosane	3.0	-	-	-	RI, MS
-	Monoterpene hydrocarbons	44.0	78.4	79.2	75.3	_

Available online at www.shd.org.rs/JSCS/

6



1665

2010 Copyright (CC) SCS

MIRJALILI et al.

DIa	Compound	From flowers	In frui	iting stag	es, %	Identification
Π	Compound	%	Immature	Mature	Ripened	methods
	Oxygenated	8.9	8.0	3.5	2.3	
_	monoterpenes					
	Sesquiterpene	20.3	9.0	9.1	10.7	
_	hydrocarbons					
	Oxygenated	10.4	2.4	4.4	2.2	
_	sesquiterpenes					
	Other	12.3	2.1	2.5	2.2	
	Total identified	95.9	99.9	98.7	92.7	

TABLE I. Continued

1666

^aRetention indices relative to C_{6} - C_{24} *n*-alkanes on a DB-1 column; ^bmass spectroscopy; ^cco-injection with authentic compounds; ^dtrace, less than 0.1 %

The classification of the identified compounds based on functional groups is summarized at the end of Table I and shows that monoterpene hydrocarbons were the main group of compounds in all samples. The monoterpene hydrocarbons content was the lowest in the flower and increased with subsequent harvesting times to reach maximum in the mature fruit and then decreased in the ripened fruit. In this study, the oil from green mature fruit contained β -phellandrene in higher amount (62.4 %) than the oils from ripened fruit (60.5 %), immature fruit (56.4 %) and flower (11.7 %). The other major monoterpene hydrocarbons which were found in all samples were β -pinene, α -pinene and δ -3-carene, while in another study, α -terpinenyl acetate, α -terpineol, limonene and myrcene were reported as the major monoterpenes.¹² Monoterpene hydrocarbons identified in the oil of flower were present in lower amount than in the oil of other stages. On the other hand, in the essential oil of flower, sesquiterpenes were one of the dominant fraction with spathulenol (8.9 %) as the major compound and their percentage decreased with progressive fruit maturation.

Antibacterial activity

The disk diffusion method, used in preliminary screening of the antibacterial activity, showed that the oils from the three different fruiting stages of *L. officinale* were active against all the tested bacteria. Moreover, the oils proved to be highly active against the tested Gram-positive bacteria, especially *B. subtilis* that was more sensitive than others, and the Gram-negative bacterium, *E. coli* (Table II). The oils were moderately active against *K. pneumoniae* and *P. aeruginosa*. The antibacterial activities of the oils were also determined using the microtiter 96-well dilution method, by measuring the minimal inhibitory concentration (*MIC*) against the tested bacteria (Table II). The essential oils of mature and ripened fruits exhibited the highest activity against *B. subtilis* with an *MIC* value of 0.90 mg ml⁻¹. In addition, the highest activity of the oil of mature fruit was observed against *S. epidermidis*, with an *MIC* value of 0.90 mg ml⁻¹. The oils showed the



lowest activity against *K. pneumoniae* and *P. aeruginosa*, with the *MIC* values ranging from 14.4 to 15.2 mg ml⁻¹.

TA	BLI	ΞII	. An	tibacterial	l activity	of L.	officinal	e essential	oil	at dif	ferent	fruiting sta	ages
----	-----	-----	------	-------------	------------	-------	-----------	-------------	-----	--------	--------	--------------	------

			- Amniaillin ^b					
Microorganism	Immat	ure	Mat	ure	Ripe	Ripened		
	DD^{c}	MIC^{d}	DD	MIC	DD	MIC	DD	
B. subtilis	25.0±0.9	3.8	36.0±0.5	0.9	35.0±0.4	0.9	14.0±0.7	
E. faecalis	19.0 ± 0.7	15.2	17.0 ± 0.4	7.5	13.0±0.4	7.2	11.0 ± 0.4	
S. aureus	17.0 ± 0.5	3.8	21.0 ± 0.5	3.7	16.0 ± 0.5	3.6	13.0±0.6	
S. epidermidis	23.0±0.7	1.9	26.0 ± 0.5	0.9	25.0±0.6	1.8	19.0±0.5	
E. coli	19.0 ± 0.6	15.2	18.0 ± 0.4	7.2	15.0 ± 0.7	7.2	12.0±0.5	
K. pneumoniae	10.0 ± 0.8	>15.2	10.0 ± 0.7	>14.4	9.0 ± 0.4	>14.4	_	
P. aeruginosa	11.0 ± 0.7	>15.2	8.0 ± 0.6	>14.4	9.0±0.5	>14.4	9.7±0.7	
-		1 1-			1 -			

^aTested at a concentration of 10 μ l disc⁻¹; ^btested at a concentration of 10 μ g disc⁻¹; ^cdiameter of inhibition zone (mm) including disk diameter of 6 mm: inactive (–), moderately active (7–14) and highly active (>14); ^dminimum inhibitory concentration, values as mg ml oil⁻¹

CONCLUSIONS

The study of a plant as a source of aromatic and flavoring compounds requires the analysis of not only the whole plant but also its individual parts at their different developmental stages. While the best harvesting time of lovage to obtain sharp odorant compounds such as α - and β -pinene is the flowering stage, β -phellandrene as the main compound of the plant with a peppery-minty and slightly citrusy odor was achieved at the fruiting stage. Chemical characterization and antibacterial screening studies on the plant-based essential oils could also lead to the discovery of new natural antibacterial agents. In addition to perfume and tobacco products, the essential oil of lovage is used as a flavor agent in major food products, such as beverages, frozen dairy desserts, candy, gelatins and pudding, and meat and its products. Although the antimycobacterial activity of polyacetylenes, such as falcarinol and falcarindiol, from the plant has recently been studied,¹⁵ the present study is the first report on the antibacterial activity of the essential oil from fruits of L. officinale at different developmental stages. The oils showed promising antibacterial activity against, B. subtilis and S. epidermidis. The present results revealed that the essential oils tested represent an inexpensive source of natural antibacterial substances for use in pathogenic systems to prevent the growth of bacteria and extend the shelf life of processed food.

Acknowledgement. We are grateful to Shahid Beheshti University Research Council for financial support of this work.



MIRJALILI et al.

ИЗВОД

САСТАВ И АНТИБАКТЕРИЈСКА АКТИВНОСТ ЕТАРСКОГ УЈЪА ЦВЕТА И ПЛОДА БИЈЪКЕ *Levisticum officinale* Koch У РАЗЛИЧИТИМ РАЗВОЈНИМ ФАЗАМА

MOHAMMAD HOSSEIN MIRJALILI¹, PEYMAN SALEHI¹, ALI SONBOLI¹, JAVAD HADIAN¹, SAMAD NEJAD EBRAHIMI¹ 14 MORTEZA YOUSEFZADI²

¹Medicinal Plants and Drugs Research Institute, Shahid Beheshti University,G. C., Evin, Tehran u²Department of Marine Biology, Faculty of Science, Hormozgan University, Bandar Abbas, Iran

Одређиван је састав и антибактеријска активност етарског уља биљке *Levisticum officinale* Косh у различитим развојним фазама: цвет, незрео плод, зелени зрео плод и потпуно зрео плод. Етарско уље је добијено из сувих узорака дестилацијом воденом паром, а антибактеријска активност је одређивана спрам седам врста бактерија. Принос уља (масени %) у различитим фазама је био следећи: незрео плод (1,5 %) > зелени зрео плод (1,0 %) > потпуно зрео плод (0,6 %) > цвет (0,1 %). Састав етарског уља је одређиван методама GC и GC–MS. У ова четири узорка је идентификовано и квантификовано редом 27, 31, 28 и 26 састојака. Монотерпенски угљоводоници су чинили главну групу једињења: 79,2 % у зеленом зрелом плоду, 78,4 % у незрелом плоду, 75,2 % у потпуно зрелом плоду и 44,0 % у цвету. Антибактеријска активност уља је одређивана методом прстенасте дифузије у Милер-Хинтоновом агару мерећи зону инфибиције. Коришћено је $10\times10 \ \mu$ l уља за инхибицију и резултати су били различити за уља добијена из биљке у различитим развојним фазама. Најјача антибактеријска активност је испољена спрам *Bacillus subtilis. MIC* вредност је била 0,90 mg ml⁻¹ са уљем потпуно зрелог плода.

(Примљено 24. маја, ревидирано 9. јула 2010)

REFERENCES

- 1. K. H. Rechinger, in *Flora Iranica*, K. H. Rechinger, Ed., Akademische Drucku Verlagsanstalt, Graz, 1987
- 2. M. H. Mirjalili, J. Javanmardi, in *Handbook of Herbs and Spices*, K. V. Peter, Ed., Woodhead Publishing Ltd., Abington, 2006
- 3. V. Mozaffarian, A Dictionary of Iranian plant names, Farhang Moaser, Tehran, 1996
- 4. E. Daukšas, R. P. Venskutonis, B. Sivik, J. Supercrit. Fluids 22 (2002) 201
- 5. J. E. Simon, A. F. Chadwick, L. E. Craker, *The Scientific Literature on Selected Herbs, Aromatic and Medicinal Plants of the Temperate Zone*, Archon Books, Hamden, CT, 1984
- 6. B. Toulemonde, I. Noleau, Dev. Food Sci. 18 (1988) 641
- 7. E. Bylaite, R. P. Venskutonis, J. P. Roozen, J. Agric. Food Chem. 46 (1998) 3735
- 8. A. Zargari, *Medicinal plants*, Tehran University Publications, Tehran, 1990
- 9. E. Daukšas, R. P. Venskutonis, B. Sivik, T. Nilson, J. Supercrit. Fluids 15 (1999) 51
- E. Bylaite, J. P. Roozen, A. Legger, R. P. Venskutonis, M. A. Posthumus, J. Agric. Food Chem. 48 (2000) 6183
- 11. I. Novak, E. Nemeth, Acta Hort. 576 (2002) 311
- 12. J. Dyduch, A. Najda, T. Wolski, S. Kwiatkowski, Folia Hort. 15 (2003) 141
- 13. M. J. M. Gijbels, J. J. C. Scheffer, A. B. Svendsen, Planta Med. 44 (1982) 207
- 14. M. E. Ibrahim, Egypt. J. Hort. 26 (1999) 177
- 15. A. Schinkovitz, M. Stavri, S. Gibson, F. Bucar, Phytother. Res. 22 (2008) 681
- 16. C. Rohricht, Z. Arznei-Gewurzpflanzen 14 (2009) 105
- 17. S. Andruszczak, Acta Sci. Pol.-Hortoru. 6 (2007) 21



ESSENTIAL OIL OF Levisticum officinale Koch

- 18. K. Seidler-Lozykowska, K. Kazmierczak, Herba Polonica 44 (1998) 11
- 19. K. Samiee, M. R. Akhgar, A. Rustaiyan, S. Masoudi J. Essent. Oil Res. 18 (2006) 19
- S. Nunes, J. M. S. Faria, A. C. Figueiredo, L. G. Pedro, H. Trindade, J. G. Barroso *Planta* Med. 75 (2009) 387
- M. Costa, A. C. Figueiredo, J. G. Barroso, L. G. Pedro, S. G. Deans, J. J. C. Scheffer Biotechol. Lett. 30 (2008) 1265
- P. A. G. Santos, A. C. Figueiredo, M. M. Oliviera, J. G. Baroso, L. G. Pedro, S. G. Deans, J. J. C. Scheffer, *Plant Sci.* 168 (2005) 1089
- 23. A. Raal, E. Arak, A. Orav, T. Kailas, M. Müürisepp, J. Essent. Oil Res. 20 (2008) 318
- 24. British pharmacopoeia, HMSO, London, 1993
- 25. T. Shibamoto, in *Capillary Gas Chromatography in Essential Oil Analysis*, P. Sandra, C. Bicchi. Eds., Hüthig Verlag, New York, 1987, p. 259
- E. J. Baron, S. M. Finegold, *Bailey and Scott's Diagnostic Microbiology*, 8th ed., C.V. Mosby Co., St. Louis, MO, 1990
- National Committee for Clinical Laboratory Standards, *Performance standards for antimicrobial susceptibility testing*, 9th international supplement, M100-S9, National Committee for Clinical Laboratory Standards, Wayne, PA, 1999
- 28. D. Azaz, F. Demirci, F. Satil, M. Kurkcuoglu, K. H. C. Baser, Z. Naturforsch. 57c (2002) 817.







J. Serb. Chem. Soc. 75 (12) 1671–1683 (2010) JSCS–4087 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 547.979.733:539.194:532.14 Original scientific paper

Consistent force field for metalloporphyrins

LJUBICA ANDJELKOVIĆ¹, SONJA GRUBIŠIĆ^{1#}, IVANA DJORDJEVIĆ¹, MATIJA ZLATAR¹, SVETOZAR NIKETIĆ^{1#} and MAJA GRUDEN-PAVLOVIĆ^{2*#}

¹Center for Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11001 Belgrade and ²Faculty of Chemistry, University of Belgrade, Studentski Trg 12–16, 11001 Belgrade, Serbia

(Received 1 July 2010)

Abstract: Molecular mechanics (MM) calculations to analyze the puckering of metalloporphyrins isolated and adsorbed on a graphite layer (0001) as a function of metal ion size and the peripheral substitution are presented. The Consistent Force Field (CFF) program was used with new parameters for metalloporphyrins, which included an out-of-plane bending function. Normal-coordinate structural decomposition (NSD) analysis was performed on the equilibrium structures obtained by the MM calculations. The conformers were also stereo-chemically characterized and compared with available experimental data and with conformers obtained in a previous MM study.

Keywords: porphyrins; graphite (0001) surface; molecular mechanics; non-planar conformations.

INTRODUCTION

Metalloporphyrins not only have important biological functions, but they can also be applied in many different industries. Among other applications, they were successfully employed for olefin epoxidation and alkane hydroxylation reactions as remarkably effective catalysts.¹

It is well known that the porphyrins macrocycle displays a range of distorted non-planar shapes.^{2–5} The non-planarity of the porphyrin core has profound consequences on the spectral, electrochemical and other properties of porphyrins, including functionality.⁶ It was revealed that factors which induce non-planarity can be divided into at least four categories: peripheral substitution, the central metal, axial ligation and the environment of the porphyrin.⁷ Previous studies showed that the type and degree of non-planar deformation can be controlled by the peripheral substitution pattern, the steric bulkiness of substituents and the size

1671



^{*} Corresponding author. E-mail: gmaja@chem.bg.ac.rs

[#] Serbian Chemical Society member.

doi: 10.2298/JSC100701095A

ANDJELKOVIĆ et al

of central metal ion of the macrocycle.^{8,9} Furthermore, it was also shown that adsorption of metalloporphyrins on a graphite layer influences conformational changes of the porphyrin core.¹⁰

Theoretically, the complete set of normal coordinates for a macrocycle forms a basis for describing any distortion of a porphyrin core. For a porphyrin of D_{4h} symmetry, the distortion can be divided into in-plane and out-of-plane deformations. Only a few of the lowest frequency modes are required to adequately describe the out-of-plane distortions.¹¹ These are: ruffing (ruf, B_{1u}), saddling (sad, B_{2u}), doming (dom, A_{2u}), two wavings (wav_x, wav_y, E_g) and propellering (pro, A_{1u}), which are shown in Fig. 1. Many experimental techniques have been used for distinguishing the magnitude of non-planar distortion, although distinguishing the different types of distortion (ruf, sad, dom, wav and pro) has proved to be more challenging.¹² Molecular modeling, developed for the investigation of non-planar porphyrins, provides information about the energetics of non-planar distortions, as well as, additional structural information.¹³ In this work, molecular mechanics (MM) calculations were used to study the effects of peripheral substitution, the nature of the central metal atom and the influence of the graphite (0001) surface on the non-planar distortions of metalloporphyrins. Although, some of the porphyrin molecules mentioned in this paper have already been analyzed, recently force field was supplemented with a new function and hence, all parameters were reoptimized in order to improve their reliability, in other words, to improve their accuracy in producing a physically meaningful potential energy surface.



Fig. 1. Non-planar porphyrin-core conformations. Bold and dashed lines represent core fragments lying below and above the mean plane, respectively.

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS



COMPUTATIONAL DETAILS

Molecular mechanics calculations were performed with the 2007/PC version of the Consistent force field (CFF) conformational program.¹⁴ Conformational energy was defined in the usual way as given in Eq. (1):

$$E_{\text{tot}} = \sum E_{\text{b}} + \sum E_{\theta} + \sum E_{\varphi} + \sum E_{\text{nb}}^{(\text{intramol.+intermol.})} + \sum E_{\text{e}}^{(\text{intramol.+intermol.})} + \sum E_{\text{oop}}$$
(1)

where the terms on the right-hand side represent bond-stretching, angle-bending, torsional, non-bonded, Coulomb, and out-of-plane bending contributions, respectively. Bond-stretching and angle-bending contributions were treated with simple harmonic functions (Eq. (2)). Torsional contributions were represented as Fourier series that accounts for all four torsions involving a double bond, or nine torsions involving a single bond.

Non-bonded intramolecular van der Waals interactions were modeled using the Lennard-Jones "12-6" potential function with parameters which were chosen by extrapolation based on the available parameter sets, imposing their consistency with the original force field.^{15,16}

Non-bonded intramolecular electrostatic contributions were modeled with the Coulomb function.

Point charges were obtained from *ab-initio* calculations (Gaussian 98).¹⁷

Out-of-plane bending was defined by using the height of the pyramid formed by the four atoms (Fig. 2, Eq. (2)). This function was added to the CFF conformational program where hoop is the perpendicular distance of atom i from the plane defined by atoms jkl, and koop is the force constant.¹⁸

$$E_{\text{tot}} = \sum_{r} \frac{1}{2} k_{r} (r - r^{0})^{2} + \sum_{\theta} \frac{1}{2} k_{\theta} (\theta - \theta^{0})^{2} + \sum_{\varphi} \frac{1}{2} (1 + \cos n\varphi) + \sum_{ij} \left[\frac{2}{3} \varepsilon \left(\frac{r^{*}}{r} \right)^{12} - \varepsilon \left(\frac{r^{*}}{r} \right)^{6} \right] + \sum_{ij} \frac{q_{i}q_{j}}{\varepsilon_{ii}r_{ii}} + \sum_{k \text{ oop}} h_{\text{oop}}^{2} + \sum_{ij} \sum_{r} \frac{1}{3} q_{i} (e^{T} \theta_{j} e)$$

$$(2)$$

Intermolecular van der Waals interactions between the porphyrin macrocycle and the graphite C atoms were treated with the Lennard-Jones "12-6" type function, with the same parameters as for intramolecular potential.

Modeling of the graphite layer was described in a previous paper.¹⁰



Fig. 2. Out-of-plane bending functions: $NC_mC_\beta C_\alpha$, $C_\alpha HC_\beta C_\beta$ and $C_\alpha HC_\alpha C_m$. Atom types used in defining the force field parameters for the molecular mechanics calculation.

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS



ANDJELKOVIĆ et al.

Intermolecular electrostatic interactions were treated as monopole–quadrupole interactions between point charges located on the atomic positions of all metalloporphyrin atoms and uniaxial quadrupoles defined on each C atom of the graphite layer:

$$E^{(e,Q)} = \sum_{i} \sum_{j} \frac{1}{r_{ij}^3} q_i \left(e^T \theta_j e \right)$$
(3)

where q_i is the point charge on the *i*-th porphyrin atom; r_{ij} is the interatomic distance; *e* is the unit vector along r_{ij} ; θ_j is the quadrupole moment tensor of the j-th C atom on the graphite layer (Eq. (3)).¹⁰

Non-bonded cut-off was treated with a cubic spline switching function with the spline-on distance of 7 Å and the spline width of 2 Å.

Geometry optimizations were realized using combinations of the steepest-descent, Davidon–Fletcher–Powell and Newton–Raphson methods.¹⁴ Geometry optimizations were performed to the energy rms gradient of $< 10^{-6}$ kJ mol⁻¹ Å⁻¹.

For each of the equilibrium structures obtained by the energy minimization procedure, normal-coordinate structural decomposition (NSD) analysis was performed using the software available at http://jasheln.unm.edu/. The NSD method provides a unique way for characterizing the distortions of the macrocycle and gives detailed analysis of the type of deformations present in the calculated porphyrin structures.

Force field parametrization

In this work, new function which describes out-of-plane bending interaction was added in the CFF conformational program for the first time and hence all the force field parameters on the available X-ray structures of Ni(II)porphyrin, Ni(II)mono-*tert*-butylporphyrin, Ni(II)di-*tert*-butylporphyrin, Ni(II)tetraphenylporphyrin and Ni(II)octaethyl-tetraphenylporphyrin were reoptimized by adjusting the appropriate parameters on a trial-and-error basis until the RMSD values between the calculated and crystallographically observed bond lengths and valence angels were ≤ 0.03 Å and $\leq 3^{\circ}$, respectively.

The force field was parametrized based the four different types of carbon atoms (α and β pyrrole carbons, *meso* carbon of the porphyrin ring, aromatic carbon of the phenyl substituents and sp³-hybridized C atom of the alkyl substituents), one type of hydrogen and of nitrogen, central metal atoms and the halogen substituents (chlorine and bromine).

The aim of involving the out-of-plane bending function was to assure persistency of planarity of selected fragments of metalloporphyrins in the optimized structures, as is indicated in Fig. 2. In earlier works, higher values of the torsional parameters were used in order to keep planarity of selected fragments in the porphyrin molecules, giving better agreement with the X-ray data. Hence, there was a need to introduce a new function in the CFF conformational program so that the torsional parameters could approach more realistic lower values.

The conformers obtained by MM calculations were stereochemically characterized, compared with available X-ray crystal structures, analyzed by the NSD method and also compared to the results obtained in a previous MM study without the out-of-plane bending function. Comparison of selected structural data for the molecules used in parametrization are given in Table I. These results indicate better agreement between calculated and X-ray structures, in comparison with previous results, establishing the reliability of the present force field, but did not affect any of previous conclusions.

A list of all force field parameters is given in Table S-I in the Supplementary material.

Available online at www.shd.org.rs/JSCS/



1675

TABLE I. Selected average bond lengths (Å), valence and torsion angles (°) for the equilibrium metalloporphyrins structures and for available crystal structures. Missing values are not reported (–) in the cited sources

Method	Ni–N	$C_{\beta} - C_{\beta}$	$C_{\alpha} - C_m$	$N-C_{\alpha}$	N–Ni–N	C_{α} -N- C_{α}	$N-C_{\alpha}-C_{\beta}-C_{\beta}N$	$V - C_{\alpha} - C_m - C_{\alpha}$
					NiP			
X-Ray ^a	1.951	1.347	1.371	1.379	179.3	104.3	0.3	0.8
MM ^b	1.912	1.336	1.366	1.377	179.9	103.8	0.02	0.0
MM	1.945	1.340	1.375	1.375	180.0	105.5	0.9	1.2
				Nil	MtBuP			
X-Ray ^c	1.901	1.342	1.380	1.380	178.9	106.3	3.0	11.1
$\mathbf{M}\mathbf{M}^{d}$	1.917	1.338	1.372	1.377	177.3	105.2	4.5	14.3
MM	1.944	1.338	1.377	1.376	177.3	103.5	0.86	1.19
				Ni	DtBuP			
\overline{X} -Ray ^e ($\alpha\alpha$)	1.900	1.353	1.394	1.383	177.0	106.2	2.9	14.8
$MM^{d}\alpha\alpha$	1.900	1.341	1.379	1.373	177.6	106.0	5.4	25.1
ΜΜαα	1.934	1.338	1.380	1.376	176.8	106.3	8.8	23.6
$MM^{d}\alpha\beta$	1.910	1.337	1.378	1.377	179.9	103.9	6.1	19.1
ΜΜαβ	1.934	1.340	1.379	1.375	177.2	103.3	18.26	21.02
				N	iTPP			
X-Ray ^f	1.931	1.340	1.383	1.377	180.0	104.9	2.6	9.6
MM ^b	1.930	1.337	1.370	1.377	180.0	104.9	1.4	11.8
MM	1.964	1.334	1.376	1.378	176.2	106.3	4.2	8.7
				NiC)EtTPP			
X-ray ^g	1.906	1.365	1.395	1.381	90.6	105.9	—	_
MM ^ĥ	1.875	1.341	1.375	1.375	90.4	106.3	_	_
MM	1.924	1.347	1.348	1.376	90.6	108.2	_	_

^aW. Jentzen, I. Turowska-Tyrk, W. R. Scheidt, J. A. Schelnutt, *Inorg. Chem.* **35** (1996) 3559; ^bM. Gruden-Pavlović, S. Grubišić, S. R. Niketić, *J. Inorg. Biochem.* **98** (2004) 1293; ^cX.-Z. Song, W. Jentzen, L. Jaquinod, R. G. Khoury, C. J. Medforth, S.-L. Jia, J.-G. Ma, K. M. Smith, J. A. Shelnutt, *Inorg. Chem.* **37** (1998) 2117; ^dM. Gruden, S. Grubišić, A. G. Coutsolelos, S. R. Niketić, *J. Mol. Struct.* **595** (2001) 209; ^eX. Z. Song, W. Jentzen, S. L. Jia, L. Jaquinod, D. J. Nurco, C. J. Medforth, K. M. Smith, J. A. Shelnutt, *J. Am. Chem. Soc.* **118** (1996) 12975; ^fA. L. MacLean, G. J. Foran, B. J. Kennedy, P. Turner, T. W. Hambley *Austral. J. Chem.* **49** (1996) 1273; ^gK. M. Barkigia, M. W. Renner, L. R. Furenlid, C. J. Medforth, K. M. Smith, J. Fajer, J. *Am. Chem. Soc.* **115** (1993) 3627; ^hUnpublished work

RESULTS AND DISCUSSION

The effects of peripheral substitution of various substituents, coordination of central metal ions of different radii and adsorption of molecules on the graphite layer (0001) on the conformations of porphyrin macrocycle of octa- and tetrahalogenated (chloro and bromo) tetraphenylporphyrin (TPP) derivates with Ni(II) an Tb(III) metal ions were studied. Ni(II) metal ion and Tb(III) metal ion were chosen to represent central metal atoms with a relatively small and large radius, respectively. As in a previous study, the initial structures for tetrahalogeno TPP were chosen based on the most symmetrical substitution pattern of the halogens and comprised: 2,8,12,18-tetrahalogeno-TPP (trans-trans, or tt)

isomer; 2,3,12,13-tetrahalogeno-TPP (cis-trans, or ct); 2,7,12,17-tetrahalogeno-TPP (windmill, or wm) isomer.

All the stable conformers for the series of porphyrins were obtained by energy minimization starting from five (plan, sad, ruf, dom, wav) initial structures. Furthermore, for the porphyrin macrocycles adsorbed on the graphite surface, two different positions of the metal (one with the metal atom directly above a given graphite carbon atom and the other with the metal located above the hole of the graphite hexagon) and various intermolecular distances (3–8 Å, in steps of 0.5 Å) were considered.

It is noteworthy that the geometrical optimization resulted in one unique equilibrium conformation for all the investigated porphyrin structures, regardless of the initial structures that were started with.

Isolated structures

The structural parameters for the energy-minimized conformers, as well as a comparison with the available crystal structures, are given in Table II; the

Method	M–N	N–C _a	$C_{\beta}-C_{\beta}$	C _a -C _m	$X^{a}-C_{\beta}$	N-M-N	C_{α} –N– C_{α}
			Ni(II)I	Br _x TPP			
X-Ray ^b	1.900	1.380	1.340	1.380	1.870	168.5	106.6
Br ₈	1.924	1.374	1.349	1.384	1.911	166.5	108.7
ct-Br ₄	1.951	1.337	1.334	1.379	1.903	90.8	105.3
tt-Br ₄	1.944	1.376	1.340	1.380	1.898	90.4	106.7
wm-Br ₄	1.948	1.376	1.339	1.379	1.896	90.5	107.6
			Ni(II)	Cl _x TPP			
X-Ray ^c	1.904	1.384	1.348	1.394	1.705	90.3	107.3
Cl ₈	1.932	1.376	1.345	1.383	1.737	90.7	108.3
ct-Cl ₄	1.953	1.378	1.338	1.378	1.733	90.6	105.8
tt-Cl ₄	1.947	1.376	1.338	1.379	1.730	90.4	107.3
wm-Cl ₄	1.950	1.377	1.338	1.379	1.730	90.4	107.5
			Tb(III)	Br _x TPP			
Br ₈	2.311	1.377	1.342	1.386	1.899	86.1	105.2
ct-Br ₄	2.309	1.378	1.340	1.386	1.902	86.7	105.9
tt-Br ₄	2.307	1.376	1.341	1.387	1.901	85.6	106.6
wm-Br ₄	2.308	1.377	1.340	1.386	1.896	86.1	107.3
			Tb(III)	Cl _x TPP			
Cl ₈	2.304	1.376	1.346	1.388	1.738	85.1	107.1
ct-Cl ₄	2.308	1.376	1.342	1.386	1.733	85.3	108.4
tt-Cl ₄	2.308	1.377	1.340	1.387	1.732	85.7	106.8
wm-Cl ₄	2.308	1.377	1.340	1.386	1.729	86.1	107.3

TABLE II. Selected average bond lengths (Å), valence and torsion angles (°) for the equilibrium, isolated metalloporphyrins structures and for crystal structures

^aBr,Cl; ^bX-Ray data of Ni(II)Br₈TPP, L. M. Henling, W. P. Schaefer, J. A. Hodge, M. E. Hughes, H. B. Gray, *Acta Cryst. C* **49** (1993) 1743; ^cX-Ray data of Ni(II)Cl₈TPP Ni, G. A. Spyroulias, A. P. Despotopoulos, C. P. Raptopoulou, A. Terzis, D. de Montauzon, R. Poilblance, A. G. Coutsolelos, *Org. Chem.* **41** (2002) 2648

Available online at www.shd.org.rs/JSCS/



calculated energy distributions are listed in Table III and the results of the NSD analysis for all the optimized molecules are presented in Table IV.

TABLE III. Energy contributions (in kcal mol⁻¹) for the isolated, equilibrium TPP conformationsSpecies E_{tot} E_{bond} E_{cop} E_{vdw} E_c E_{nb}

Species	$E_{ m tot}$	E_{bond}	E_{angle}	$E_{ m torsion}$	$E_{ m oop}$	$E_{ m vdw}$	$E_{ m c}$	$E_{\rm nb}$
			N	i(II)Br _x TP	Р			
Br ₈	55.28	4.72	15.55	18.83	1.39	26.53	-11.74	14.79
ct-Br ₄	35.83	1.97	9.99	16.14	1.32	17.95	-11.55	6.40
tt-Br ₄	35.51	1.90	8.56	16.71	1.42	18.68	-11.76	6.93
wm-Br ₄	33.81	1.80	8.90	14.83	1.51	18.48	-11.72	6.76
			N	li(II)Cl _x TP	Р			
Cl ₈	54.09	3.05	12.36	17.83	1.61	23.53	-4.29	19.24
ct-Cl ₄	36.32	1.68	8.64	14.90	1.25	18.59	-8.73	9.86
tt-Cl ₄	34.64	1.70	8.11	15.98	1.37	19.19	-11.70	7.48
wm-Cl ₄	32.91	1.61	8.36	14.51	1.49	18.84	-11.90	6.95
			T	$b(III)Br_xTF$	P			
Br ₈	75.17	5.20	22.30	38.21	4.55	13.83	-8.92	4.91
ct-Br ₄	56.07	4.97	20.1	21.97	2.06	15.73	-8.75	6.98
tt-Br ₄	61.21	5.39	24.18	18.59	2.61	19.25	-8.79	10.45
wm-Br ₄	57.48	4.92	23.01	19.08	2.52	16.74	-8.78	7.96
			Т	b(III)Cl _x TP	P			
Cl ₈	84.93	6.83	31.06	19.59	2.94	25.31	-0.81	24.50
ct-Cl ₄	64.69	5.30	25.7	16.10	2.51	20.25	-5.16	15.09
tt-Cl ₄	60.08	5.11	23.56	17.46	2.49	19.7	-8.24	11.46
wm-Cl ₄	56.7	4.79	22.59	17.98	2.38	17.33	-8.36	8.97

The NSD analysis revealed that the structure of Ni(II)Br8TPP is pure saddle and it shows the largest deviation from planarity (Table IV). The ct--isomer of Ni(II)Br4TPP is a mixture of sad and dom non-planar deformations, the tt-isomer is almost pure saddle with a minor contribution of the pro mode, while the optimized structure of the wm-isomer indicated that it consisted of sad and a small amount of ruf deformations. For the tetrabromo-TPP–Ni(II) complexes, the wm-isomer is the most stable one (Table III).

The Ni(II)Cl8TPP complex is also highly puckered, and the NSD analysis indicated an almost pure saddled macrocycle. All three isomers of Ni(II)Cl4TPP possess sad as the dominant deformation, with contributions of dom, pro and ruf distortions for ct-, tt- and wm-isomer, respectively.

For the octa- and tetrabromo substituted structures with the very large Tb(III) ion, the dom deformation was present in all structures as was expected (Table IV). The NSD analysis revealed that the structure of the Tb(III)Br8TPP complex is a mixture of dom, sad, ruf and pro deformations. In the case of the ct-isomer of Tb(III)Br4TPP, the metal ion favors dom distortion, and saddling is favored by the substituents. An almost equal ratio of sad and dom deformations

ANDJELKOVIĆ et al.

was found in the tt-isomer and in the case of wm-isomer, the sad distortion dominates. The ct-isomer is the most stable one, but the difference between total energy of the ct- and wm-isomer is not so significant (Table III).

TABLE IV. Results of the normal-coordinate structural decomposition (NSD) analysis for the equilibrium, isolated TPP conformations. D_{oop} is the total out-of-plane distortion (in Å)

Species	D_{oop}	sad	ruf	dom	wav(x)	wav(y)	pro				
			Ni(II)	Br _x TPP							
Br ₈	3.7365	3.7365	0.0083	0.0057	0.0057	0.0084	0.0030				
ct-Br ₄	2.5544	2.3495	0.0171	1.0021	0.0153	0.0144	1.0100				
tt-Br ₄	2.4576	2.4426	0.0058	0.0105	0.0091	0.0143	0.2704				
wm-Br ₄	2.3550	2.3236	0.3825	0.0164	0.0080	0.0162	0.0028				
	Ni(II)Cl _x TPP										
Cl ₈	3.3397	3.3397	0.0065	0.0057	0.0059	0.0080	0.0037				
ct-Cl ₄	2.2829	2.1277	0.0141	0.8270	0.0115	0.0121	0.0079				
tt-Cl ₄	2.3360	2.3253	0.0053	0.0102	0.0084	0.0138	0.2219				
wm-Cl ₄	2.1913	2.1475	0.4354	0.0147	0.0074	0.0151	0.0022				
			Tb(III)	Br _x TPP							
Br ₈	2.2838	1.4672	0.5068	1.6167	0.0446	0.0322	0.4353				
ct-Br ₄	1.8914	0.7306	0.0156	1.7440	0.0311	0.0225	0.0206				
tt-Br ₄	1.9571	1.4579	0.0341	1.2637	0.0121	0.0027	0.3263				
wm-Br ₄	3.0589	2.7552	0.0416	1.3281	0.0139	0.0033	0.0088				
			Tb(III))Cl _x TPP							
Cl ₈	3.0589	2.7552	0.0416	1.3281	0.0139	0.0033	0.0088				
ct-Cl ₄	1.5314	0.9842	0.0115	1.1732	0.0087	0.0066	0.0053				
tt-Cl ₄	2.9150	2.1099	0.5525	1.1933	0.5995	1.3628	0.3152				
wm-Cl ₄	3.0589	2.7552	0.0416	1.3281	0.0139	0.0033	0.0088				

In optimized structure of the Tb(III)Cl8TPP complex, sad and dom normal modes were observed (Table IV). The NSD analysis of the ct-isomer of Tb(III)Cl4TPP shows an almost equal ratio of dom and sad distortions. The shape of optimized conformation of the tt-isomer is a combination of the five most commonly observed distortions, with domination of the sad deformation mode. In the case of the wm-isomer, the ratio of the sad and dom deformations is almost the same as for the wm-isomer of Tb(III)Br4TPP. As in the case of the tetrachloro substituted TPP isomers, the wm isomer is the most stable (Table III).

Adsorbed structures

1678

The structural parameters for the energy-minimized conformers are presented in Table V, the calculated energy distributions are listed in Table VI and the results of NSD analysis for all the investigated porphyrins adsorbed on a graphite layer are listed in Table VII.



1679

M-N $X^a - C_\beta$ Species $N-C_{\alpha}$ $C_{\beta}-C_{\beta}$ $C_{\alpha}-C_{m}$ N-M-N $C_{\alpha} - N - C_{\alpha}$ Ni(II)Br_xTPP Br_8 1.925 1.375 1.348 1.385 1.911 166.7 108.80 1.962 1.379 1.378 1.902 90.02 107.54 ct-Br₄ 1.338 tt-Br₄ 1.968 1.376 1.335 1.376 1.901 90.42 106.80 wm-Br₄ 1.971 1.376 1.334 1.374 1.899 89.98 107.54 Ni(II)Cl_rTPP Cl_8 1.945 1.378 1.341 1.384 1.739 90.6 108.60 ct-Cl₄ 1.972 1.379 1.334 1.376 1.733 89.98 106.89 tt-Cl₄ 1.965 1.376 1.334 1.374 1.735 90.51 106.82 wm-Cl 1.971 1.380 1.334 1.372 1.738 90.23 106.72 Tb(III)Br_xTPP Br_8 2.300 1.377 1.344 1.392 1.899 86.5 108.9 ct-Br₄ 2.303 1.376 1.340 1.392 1.901 86.8 109.7 tt-Br₄ 2.303 1.375 1.339 1.388 1.902 86.2 109.8 109.7 wm-Br₄ 2.302 1.378 1.340 1.388 1.899 86.3 Tb(III)Cl_xTPP Cl_8 2.300 1.382 1.343 1.396 1.740 86.6 112.3 ct-Cl₄ 2.303 1.379 1.343 1.389 1.737 85.9 109.6 tt-Cl₄ 2.300 1.378 1.339 1.390 1.731 85.5 108.5 109.2 wm-Cl₄ 2.302 1.379 1.342 1.388 1.740 85.6

TABLE V. Selected average bond lengths (Å), valence and torsion angles (°) for the equilibrium, adsorbed metalloporphyrins structures

^aBr,Cl

NSD Pattern for structure of Ni(II)Br8TPP adsorbed on graphite surface is almost the same as for isolated molecule, because there is no significant intermolecular interaction (Table VI).

Distance of Ni(II) ion from graphite is approximately 6 Å. Large distance is explained by the presence of bulky groups and repulsive interaction between negative charges, hence the porphyrin macrocycle can not approach more closely to the graphite layer. Geometry optimization for three isomers of Ni(II)Br4TPP leads to parallel orientation of porphyrin macrocycle on graphite, where in all cases the metal ion is above the center of C–C bond. In comparison to the isolated structures, all three isomers adsorbed on a graphite layer are less puckered (Tables IV and VII). In the ct-isomer, an almost equal ratio of dom and sad distortions is observed (Table VII). The ruf and dom deformations dominate in the tt-isomer, with presence of the pro deformation mode, while the wm-isomer can be described with dom deformation and a small contribution of pro distortion.

After geometry optimization of the various initial conformations of the adsorbed molecule Ni(II)Cl8TPP, starting from the various distances from the graphite layer and different initial positions of metal ion in relation to graphite, the end result was a unique conformation, very similar to the isolated structure, with the sad mode as dominant, and with a small contribution of the dom dis-

ANDJELKOVIĆ et al.

tortion. Final position of the metal ion is exactly above center of the C–C bond of the graphite layer. The position of the metal ion in relation to the graphite layer of the three isomers of Ni(II)Cl4TPP is the same as in the case of the tetrabromo derivatives. NSD analysis revealed that all three isomers are more planar in comparison to the isolated structures (Tables IV and VII), which enhance the flattening π -stacking interactions. NSD analysis shows that in the ct-isomer, dom and sad deformations dominate. Almost all of the five most commonly observed distortions occur in the equilibrium conformation of the tt-isomer with domination of the ruf and dom modes. In the case of the wm-isomer, dom deformation is the dominant one, with a small contribution of pro distortion (Table VII).

TABLE VI. Energy contributions (in kcal mol⁻¹) for the equilibrium, adsorbed metallopor-phyrins conformations

Species	$E_{\rm tot}$	$E_{\rm bond}$	E_{angle}	E_{torsion}	E_{oop}	$E_{\rm vdw}$	$E_{ m c}$	$E_{\rm nb}$	$E_{\rm intervdw}$	E_{interc}	Einternb
					Ni(II	$Br_{x}TP$	Р				
Br ₈	46.33	4.61	16.15	18.36	1.26	27.11	-11.74	15.37	-5.83	-3.59	-9.42
ct-Br ₄	-3.80	2.94	9.94	20.01	2.20	24.29	-11.11	13.17	-19.72	-32.33	-52.06
tt-Br ₄	2.32	2.50	12.36	24.62	2.31	21.58	-11.28	10.29	-18.64	-31.12	-49.77
wm-Br ₄	-5.40	2.22	11.83	19.67	2.09	20.92	-11.17	9.76	-20.41	-32.71	-53.11
	Ni(II)Cl _x TPP										
Cl ₈	23.48	3.65	16.54	17.44	1.40	32.53	-4.00	28.52	-29.81	-14.28	-44.09
ct-Cl ₄	-16.41	2.58	10.35	19.16	2.12	23.16	-7.58	15.58	-20.07	-46.13	-66.21
tt-Cl ₄	-15.74	2.26	12.08	25.1	2.13	27.76	-10.54	10.22	-18.25	-49.29	-67.55
wm-Cl ₄	-17.69	2.09	13.24	22.42	1.88	20.81	-10.79	10.01	-19.7	-47.65	-67.35
					Tb(II	I)Br _x TF	PP				
Br ₈	12.66	6.2	24.28	35.49	3.03	13.81	-8.66	5.15	-29.81	-31.67	-61.48
ct-Br ₄	16.84	10.35	29.84	19.09	1.48	20.51	-8.87	11.65	-19.55	-36.02	-55.57
tt-Br ₄	16.04	7.59	25.64	21.26	2.02	14.68	-8.84	5.84	-17.68	-28.64	-46.32
wm-Br ₄	12.28	9.23	30.76	17.01	1.28	17.5	-8.94	8.56	-19.88	-34.68	-54.56
					Tb(II	I)Cl _x TF	PP				
Cl ₈	36.32	8.81	36.07	23.57	2.89	35.05	-0.08	35.07	-19.71	-50.39	-70.09
ct-Cl ₄	-4.48	5.32	30.33	22.05	3.15	19.01	-3.81	15.19	-17.88	-62.65	-80.54
tt-Cl ₄	-4.84	5.00	32.45	25.5	2.94	17.29	-6.89	10.39	-17.24	-63.89	-81.14
wm-Cl ₄	-7.54	4.82	31.75	24.44	2.77	16.17	-7.11	9.06	-18.37	-62.02	-80.39

NSD Analysis of the adsorbed octa- and tetra-halogeno TPP derivates with the Tb(III) ion indicates that all the optimized porphyrin macrocycle conformations are less puckered in comparison to the isolated structures, with domination of the dom distortion mode, as is expected from the metal ion size (Tables IV and VII). In all cases of tetrahalogeno substituted Tb(III) complexes, the metal is located exactly above the hole of the graphite hexagon, at a very small distance, which is explained by the strong attractive $M-\pi$ interactions.

Available online at www.shd.org.rs/JSCS/



1681

TABLE VII. Results of the normal-coordinate structural decomposition (NSD) analysis for the equilibrium, adsorbed metalloporphyrins conformations. D_{oop} is the total out-of-plane distortion (in Å)

Species	D _{oop}	sad	ruf	dom	wav(x)	wav(y)	pro				
			Ni(II)B	r _x TPP							
Br ₈	3.7438	3.7419	0.0109	0.1210	0.0046	0.0070	0.0037				
ct-Br ₄	0.9487	0.6100	0.0244	0.7253	0.0212	0.0258	0.0120				
tt-Br ₄	1.0535	0.0218	0.8121	0.5954	0.0085	0.0072	0.3083				
wm-Br ₄	0.7426	0.0140	0.0250	0.6912	0.0251	0.0144	0.2683				
Ni(II)Cl _x TPP											
Cl ₈	3.2494	3.2282	0.0127	0.3700	0.0077	0.0145	0.0035				
ct-Cl ₄	0.8474	0.5568	0.0264	0.6379	0.0138	0.0122	0.0152				
tt-Cl ₄	1.0333	0.0812	0.7823	0.6282	0.0145	0.0109	0.2326				
wm-Cl ₄	0.7060	0.0142	0.0331	0.6283	0.0279	0.0129	0.3186				
			Tb(III)E	Br _x TPP							
Br ₈	1.7730	0.4255	0.2486	1.6281	0.3444	0.2889	0.2185				
ct-Br ₄	1.0064	0.3849	0.0166	0.9108	0.0140	0.1859	0.0043				
tt-Br ₄	1.1030	0.0035	0.3914	1.0310	0.0146	0.0138	0.0067				
wm-Br ₄	1.0332	0.0081	0.0173	1.0159	0.0161	0.0086	0.1865				
			Tb(III)C	Cl _x TPP							
Cl ₈	0.9799	0.3491	0.0067	0.8116	0.4237	0.0078	0.0112				
ct-Cl ₄	1.1660	0.3334	0.0179	1.1171	0.0101	0.0057	0.0127				
tt-Cl ₄	1.2808	0.0147	0.4852	1.1894	0.0092	0.0077	0.0223				
wm-Cl ₄	0.7344	0.0382	0.0075	0.7097	0.0147	0.0074	0.1841				

Again, the octahalogeno derivates of Tb(III) cannot approach close to the graphite surface, resulting in a similar conformation of the porphyrin core in comparison to the isolated structures.

CONCLUSIONS

The main goal of this study was the development of a new force field for metalloporphyrins using the CFF conformational program. This means that the previous force field was supplemented with a new function which describes the deviation of the atoms from planarity, i.e., an out-of-plane bending function. This function was parameterized and all other parameters were reoptimized in order to improve the consistency of the entire force field. The force field was parameterized on planar and highly puckered complexes, whereby the obtained structural results are in good agreement with the published MM results and with the available crystal structures.8–10 The very good agreement between the calculated and experimental structures is the best indicator of the successful augmentation of the new function and the valid reoptimization of the force field. With essentially the same force field, the effects of the peripheral substitution and the nature of the central metal atom as well as the influence of a presence of graphite (0001) surface on the non-planar distortions of octa- and tetrahalogeno

ANDJELKOVIĆ et al.

TPP derivates with Ni(II) and Tb(III) ions were analyzed. It was shown that both metallation and peripheral substitution determine the type and degree of nonplanar deformations of porphyrin macrocycle, as a result of various strainrelieving mechanisms. An increase of the radius of the central metal atom enhances the doming of the porphyrin macrocycle, thus dom deformation is present in all of the isomers of the Tb(III) ion. Dodeca-substituted porphyrins showed a large deviation from planarity and relieved their strain predominantly by saddling of the porphyrin core, irrespective of the size of the central metal atom and the nature of the halogeno substituents. The position of the four halogeno atoms in the tetrahalogeno TPP structures has profound consequences on the mode and amount of normal distortions. In almost all cases, the wm isomer is the most stable one, except for the tetrabromo TPP Tb(III) species, when the ct isomer was found to be the most stable, but the energy difference between this and the wm isomer is small. Octahalogeno TPP derivates of Ni(II) and Tb(III) adsorbed on a graphite layer have similar conformations of porphyrin core in comparison to the isolated structures, because they cannot approach close to the graphite surface. All tetrahalogeno TPP structures are more planar in comparison to the isolated structures, as the result of π -stacking interactions. NSD analysis revealed that dom distortion is present in almost all of the structures adsorbed on a graphite layer and the origin of this distorsion can be explained by M- π interactions. If an analyzed molecule is closer to the substrate, doming is more significant. These results indicate that changes of physical and chemical properties of porphyrin adsorbed on a surface (in the present case a graphite layer) can be explained not only by the adsorption process, but also by specific conformational changes.

SUPPLEMENTARY MATERIAL

CFF parameters for metalloporphyrins are available electronically from http:////www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. This work was financially supported by the Ministry of Science and Technological Development of the Republic of Serbia.

извод

КОНЗИСТЕНТНО ПОЉЕ СИЛА ЗА МЕТАЛОПОРФИРИНЕ

ЉУБИЦА АНЂЕЛКОВИЋ¹, СОЊА ГРУБИШИЋ¹, ИВАНА ЂОРЂЕВИЋ¹, МАТИЈА ЗЛАТАР¹, СВЕТОЗАР НИКЕТИЋ¹ и МАЈА ГРУДЕН-ПАВЛОВИЋ²

¹Ценійар за хемију, Инсійшійуій за хемију, ійехнологију и мешалургију, Универзийией у Београду, Нјегошева 12, 11001 Београд и ²Хемијски факулійей, Универзийией у Београду, 11001 Београд

Конзистентно поље сила (CFF) за молекулско моделирање металопорфирина, које укључује новоуведену *out-of-plane* функцију, параметризовано је на основу кристалних структура Ni(II)-порфирина, никал(II)-моно-терцбутилпорфирина, никал(II)-ди-терцбутилпорфирина, никал(II)-тетрафенилпорфирина и никал(II)-октаетил-тетрафенилпорфирина. Оно је употребљено за проучавање утицаја величине централног металног јона, периферне супсти-

Available online at www.shd.org.rs/JSCS/

туције као и утицаја присутног супстрата на конформације порфиринског језгра код окта- и тетрахалогено-тетрафенилпорфирина са Ni(II) и Tb(III). Јединствене равнотежне структуре, добијене на основу молекулско-механичких прорачуна великог броја почетних структура, показују добро слагање са кристалним структурама, као и са претходно публикованим резултатима, не само у метричким подацима, већ и у начину набирања порфиринског језгра. Метод нормалне-координатне структурне декомпозиције (NSD) омогућава да се на једнозначан начин опише свака оптимизована конформација добијена молекулско-механичким прорачунима.

(Примљено 1. јула 2010)

REFERENCES

- I. D. Kostas, A. G. Coutsolelos, G. Charalambidis, A. Skondra, *Tetrahedron Lett.* 48 (2007) 6688
- 2. Y. Higuchi, M. Kusunoki, N. Yasuoka, M. Kakudo, J. Mol. Biol. 172 (1984) 109
- 3. G. Fermi, M. F. Perutz, B. Shaanan, R. Fourme, J. Mol. Biol. 175 (1984) 159
- 4. B. C. Finzel, T. L. Poulos, J. Kraut, J. Biol. Chem. 259 (1984) 13027
- 5. G. V. Louie, G. D. Brayer, J. Mol. Biol. 214 (1990) 527
- 6. M. O. Senge, in *The Porphyrin Handbook*, Vol. 1, K. M. Kadish, K. M. Smith, R. Guilard, Eds., Academic Press, New York, 2000, p. 239
- X. Song, W. Jentzen, L. Jaquinod, R. G. Khoury, C. J. Medforth, S. L. Jia, J. G. Ma, K. M. Smith, J. A. Shelnutt, *Inorg. Chem.* 37 (1998) 2117
- 8. M. Gruden, S. Grubišić, A. G. Coutsolelos, S. R. Niketić, J. Mol. Struct. 595 (2001) 209
- 9. M. Gruden-Pavlović, S. Grubišić, S. R. Niketić, J. Inorg. Biochem. 98 (2004) 1293
- 10. M. Gruden-Pavlović, S. Grubišić, M. Zlatar, S. R. Niketić, Int. J. Mol. Sci. 8 (2007) 810
- 11. W. Jentzen, X. Z. Song, J. A. Shelnutt, J. Phys. Chem. B 101 (1997) 1684
- J. A. Shelnutt, X. Z. Song, J. G. Ma, S. L. Jia, W. Jentzen, C. J. Medforth, *Chem. Soc. Rev.* 27 (1998) 31
- 13. M. Zimmer, Coord. Chem. Rev. 253 (2009) 817
- 14. S. R. Niketić, K. Rasmussen, *The Consistent Force Field: A Documentation, Lecture Notes in Chemistry*, Springer, Berlin, 1977
- 15. K. Rasmussen, Potential Energy Functions in Conformational Analysis, Lecture Notes in Chemistry, Springer, Berlin, 1985
- 16. N. Raos, S. R. Niketić, V. Simeon, J. Inorg. Biochem. 16 (1982) 1
- 17. Gaussian 98 Pople, Revision A.6, Gaussian Inc., Pittsburgh, PA, 1998
- A. K. Rappé, C. J. Casewit, *Molecular Mechanics across Chemistry*, University Science Books, South Orange, NJ, 1997, p. 37.







J. Serb. Chem. Soc. 75 (12) S1-S3 (2010)

JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS Supplementary material

SUPPLEMENTARY MATERIAL TO Consistent force field for metalloporphyrins

LJUBICA ANDJELKOVIĆ¹, SONJA GRUBIŠIĆ^{1#}, IVANA DJORDJEVIĆ¹, MATIJA ZLATAR¹, SVETOZAR NIKETIĆ^{1#} and MAJA GRUDEN-PAVLOVIĆ^{2*#}

¹Center for Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11001 Belgrade and ²Faculty of Chemistry, University of Belgrade, Studentski Trg 12–16, 11001 Belgrade, Serbia

J. Serb. Chem. Soc. 75 (12) (2010) 1671-1683

CFF PARAMETERS FOR METALLOPORPHYRINS

T.	A	BL	Æ	S-	I.	Bond	stretching	parameters
----	---	----	---	----	----	------	------------	------------

Bond	$k_{\rm r}$ / kcal mol ⁻¹ Å ⁻²	r_0 / Å	Bond	$k_{\rm r}$ / kcal mol ⁻¹ Å ⁻²	<i>r</i> ₀ / Å
Ni–N	359.50	1.940	C _β –Cl	487.48	1.720
Tb–N	359.50	2.360	C_{β} –Br	355.86	1.881
$N-C_{\alpha}$	1594.74	1.375	$C_p - C_p$	1380.48	1.394
$C_{\alpha} - C_{\beta}$	1380.48	1.337	C _p -H	661.80	1.084
C _β –H	661.00	1.101	$C_p - C_m$	632.72	1.497
$C_{sp3} - C_{sp3}$	633.72	1.523	$C_m - C_\alpha$	1409.24	1.370
C _{sp3} –H	661.48	1.113	$C_{\beta}-C_{sp3}$	1409.29	1.470

TABLE S-II. Angle bending parameters

Angle	k_{θ} / kcal mol ⁻¹ rad ⁻²	$ heta_0$ / rad	Angle	k_{θ} / kcal mol ⁻¹ rad ⁻²	$ heta_0$ / rad
N–M–N(cis)	5.00	1.571	C _{sp3} –C _{sp3} –H	20.84	1.902
N-M-N(trans)	30.32	3.146	H-C _{sp3} -H	46.08	1.902
$M-N-C_{\alpha}$	129.60	2.182	$C_{\alpha} - N - C_{\alpha}$	61.92	1.832
$C_{\alpha}-C_{\beta}-H$	129.60	2.182	C _p -C _p -H	51.84	2.094
$C_{\beta}-C_{\beta}-H$	129.60	2.182	$C_p - C_p - C_m$	61.92	2.094
$N-C_{\alpha}-C_{\beta}$	129.60	2.182	C_{α} – C_m – C_{α}	61.92	2.120
$C_{\alpha} - C_{\beta} - C_{\beta}$	129.60	2.182	$N-C_{\alpha}-C_{m}$	61.92	2.175
$C_m - C_\alpha - C_\beta$	129.60	2.182	C_{α} – C_{β} – Cl	134.00	2.094
$C_{\beta}-C_{\beta}-Cl$	129.60	2.182	$C_p - C_p - C_p$	61.92	2.094
$C_{\alpha} - C_m - C_p$	129.60	2.182	$C_{\beta}-C_{\beta}-Br$	109.90	2.094
C_{α} – C_{β} – Br	109.90	2.094	$C_{\beta}-C_{\beta}-C_{sp3}$	134.00	2.094
C_{sp3} – C_{sp3} – C_{sp3}	61.92	2.094	$C_{\beta}-C_{sp3}-C_{sp3}$	80.80	1.902

*Corresponding author. E-mail: gmaja@chem.bg.ac.rs

[#] Serbian Chemical Society member.

S1



ANDJELKOVIĆ et al.

TABLE S-III. Torsion angle parameters

S2

Angle	$k_{\Phi}/\text{kcal mol}^{-1}$	п	Angle	$k_{\Phi}/\text{kcal mol}^{-1}$	n
$C_m - C_\alpha - N - M$	1.57	-2.0	$C_{\beta}-C_{\beta}-C_{\alpha}-C_{m}$	10.00	-2.0
$C_{\alpha} - C_{\beta} - C_{\beta} - C_{\alpha}$	3.00	-2.0	$H - C_{\beta} - C_{\beta} - H$	3.00	-2.0
$H - C_{\beta} - C_{\beta} - C_{\alpha}$	3.00	-2.0	$C_p - C_m - C_\alpha - C_\beta$	10.00	-2.0
$C_p - C_p - C_m - C_\alpha$	0.50	-2.0	$C_p - C_p - C_p - C_p$	7.50	-2.0
$C_m - C_p - C_p - H$	7.50	-2.0	H-Cp-Cp-H	7.50	-2.0
$Cl-C_{\beta}-C_{\beta}-Cl$	3.00	-2.0	$Cl-C_{\beta}-C_{\beta}-C_{\alpha}$	3.00	-2.0
$Cl-C_{\beta}-C_{\beta}-H^{a}$	3.00	-2.0	$H-C_{\beta}-C_{\beta}-C_{\alpha}$	3.00	-2.0
C_{α} -N- C_{α} - C_{m}	3.00	-2.0	$H-C_{sp3}-C_{sp3}-C_m$	0.50	3.0
$H-C_{sp3}-C_{sp3}-C_{sp3}$	0.00	0.0	$Cl-C_{\beta}-C_{\beta}-Cl^{a}$	3.00	-2.0
$H-C_{sp3}-C_{sp3}-C_{\beta}$	0.50	3.0	$H-C_{sp3}-C_{\beta}-C_{\beta}$	0.24	6.0

^aIf the substituent is Br, the torsion angle parameters are the same

TABLE S-IV. Out-of-plane bending parameters

Conformation	$k_{\omega}/\text{kcal mol}^{-1}$		
$N-C_{\beta}-C_{m}.C_{\alpha}$	10		
C_{α} -H- C_{α} . C_{m}	90		
C_{β} -H- C_{α} · C_{β}	10		
$C_{\alpha} - C_{p} - C_{\alpha} \cdot C_{m}$	90		
C_{α} - C_{sp3} - C_{α} . C_{m}	90		
$C_{\beta}-X-C_{\alpha}.C_{\beta}$	10		

Bond	$\mathcal{E}/\text{kcal mol}^{-1}$	<i>r</i> * / Å	Bond	$\mathcal{E}/\text{kcal mol}^{-1}$	<i>r</i> * / Å
M–C _β	0.130	3.24	M–H	0.134	2.70
M-C _m	0.130	3.24	M–Cl	0.115	3.28
M-C _p	0.130	3.24	N–H	0.051	3.32
$N-C_{\beta}$	0.049	3.76	N–C _a	0.049	3.76
M–C _m	0.049	3.76	H–H	0.047	3.00
$C_{\alpha} - C_{\beta}$	0.044	3.88	$C_{\alpha}-C_{\alpha}$	0.044	3.88
$C_{\beta}-C_{\beta}$	0.044	3.88	C _m –H	0.046	3.34
$C_m - C_\alpha$	0.046	3.88	$C_m - C_\beta$	0.046	3.88
$C_p - C_p$	0.044	3.88	N–Cl	0.115	3.85
$H-C_{\beta}$	0.046	3.34	H–C _a	0.046	3.34
Cl–C _β	0.103	3.97	Cl–C _a	0.103	3.97
Cl-Cl	0.240	4.00	H–Cl	0.106	3.53
Cl–C _m	0.103	3.97	N–N	0.055	3.64
H-C _p	0.046	3.34	$C_p - C_p$	0.044	3.88
$C_p - C_m$	0.044	3.88	$C_p - C_\alpha$	0.044	3.88
$C_p - C_\beta$	0.044	3.88	C _p -N	0.049	3.74
C _p -Cl	0.103	3.97	C ^a –Br	0.119	4.12
Br–Br	0.320	4.36	M–Br	0.349	3.38
N–Br	0.133	4.00	H–Br	0.122	3.68
C _{sp3} –H	0.046	3.34	$C_{sp3}-C_{sp3}$	0.044	3.88
$C_{sp3}-C_m$	0.044	3.88	$C_{sp3}-C_{\beta}$	0.044	3.88
C _{sp3} –M	0.131	3.10	C _{sp3} –N	0.049	3.72


Species	Esu
M	0.543
Cl	-0.315
C_{α}	0.285
C _p	-0.080
Br	0.032
Ν	-0.665
C _m	0.334
C_{β}	0.285
H	0.285
H ^a	0.070

TABLE S-VI. Electrostatic parameters

^aThis value was used for CFF calculation of Br-substituted TPP molecules

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS







J. Serb. Chem. Soc. 75 (12) 1685–1699 (2010) JSCS–4088 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 547.857.5+547.269.3:543.552+ 541.135.5–033.5 Original scientific paper

Voltammetric determination of dopamine in the presence of uric acid using a 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)--naphthalin-4-sulfonic acid modified glassy carbon electrode

ALI ASGHAR ENSAFI*, SAMIRA DADKHAH-TEHRANI and BEHZAD REZAEI

Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran

(Received 11 March, revised 30 July 2010)

Abstract: A polymerized film of 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid (HHNANSA) was prepared at the surface of a glassy carbon electrode by electropolymerization. The modified electrode was used for the simultaneous determination of dopamine (DA) and uric acid (UA). The electrochemical behaviors of the compounds at the surface of the modified electrode were studied using cyclic voltammetry, chronoamperometry, and square wave voltammetry (SWV). The experimental results indicated that the modified electrode exhibited an efficient electrocatalytic activity towards the oxidation of DA and UA, with a peak separation of about 140 mV at pH 5.0. Using chronoamperometry, the catalytic reaction rate constant was measured and found to equal to 1.23×10⁴ mol⁻¹ L s⁻¹. At pH 5.0, the catalytic peak currents linearly depended on the DA and/or UA concentrations in the range of 1.0-300 µmol L⁻¹ DA (two linear segments with different slopes) and 6.7-20 µmol L⁻¹ UA, using SWV. The detection limits for DA and UA were 0.25 and 1.17 μ mol L⁻¹, respectively. The RSD % for 40.0 and 140.0 μ mol L⁻¹ DA were 1.9 and 2.2 %, respectively, whereas for 10.0 and 20.0 µmol L⁻¹ UA, they were 1.8 and 1.2 %, respectively. The modified electrode showed good sensitivity, selectivity, and stability. It was successfully applied for the determination of DA and UA in real samples, such as drugs and urine.

Keywords: 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid; simultaneous determination; dopamine and uric acid; voltammetry.

INTRODUCTION

Dihydroxyphenylethylamine, also commonly known as dopamine (DA), is one of the most significant catecholamines and is an important neurotransmitter in the mammalian central nervous system. It is currently the subject of intensive research by neuroscientists and chemists and rapid and simple methods for the

1685



^{*}Corresponding author. E-mail: ensafi@cc.iut.ac.ir doi: 10.2298/JSC100311134E

determination of its concentration are sought.^{1–3} It has also been suggested that DA plays a role in drug addiction and some manifestations of HIV.^{4–6} Dopamine is not just a precursor of norepinephrine (noradrenaline) and epinephrine (adrenaline) but a neurotransmitter as well. Therefore, the determination of this compound is very necessary.

Uric acid (2,6,8-trihydroxypurine, UA) is a compound of great biomedical interests and has important roles in human metabolism. UA, the end metabolic product of purine⁷ via the liver, is present in blood and urine. Monitoring UA in blood or urine can be used as an indicator for an early warning sign of kidney diseases. Abnormal UA level in a human body could be caused by several diseases, such as hyperuricemia, gout, cardiovascular disorder, Lesch–Nyan syndrome, and chronic renal disease. DA and UA are co-present in biological fluids, such as urine and blood. Therefore, it is important to develop new techniques for the selective detection of DA in the presence of UA in a routine assay. The direct electro-oxidation of DA and UA at bare electrodes requires high overpotentials. In addition, they have overlapped signals because the oxidation peaks of DA and UA at a bare electrode are at nearly the same potential, making their discrimination very difficult.^{8,9}

Several papers have reported using modified electrodes for the simultaneous determination of DA and UA.^{10–17,21} Recently, polymer film modified electrodes have been used as electrochemical sensors in the wide application fields of chemical sensors and biosensors.^{18–21} Comparisons of the proposed method with the similar electrochemical methods are presented in Table I. Although, previous studied based on poly(3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid¹⁷ and sulfonazo III film²¹ modified electrodes have better limits of detection, the proposed modified electrode based on 2-hydroxy-1-(1-

Sensitivity		Limit of detection		Linear dynamic range		
μΑ μπ	nol⁻¹ L	μmo	1 L-1	μmo	ol L⁻¹	Ref.
DA	UA	DA	UA	DA	UA	
0.3316	0.0929	0.075	0.021	0.2-45.8	0.06–166.0	10
1.0728	0.0910	0.02	1.0	0.1 - 200	10.0-130.0	11
Not reported	17.00	0.0027	0.2	0.0-6.0	0.5 - 100.0	12
Not reported	Not reported	1.0	1.0	2.0 - 1500	2.0-220	13
1.0	Not reported	0.5	Not reported	2.0-10.0	Not reported	14
1.741	0.7360	0.12	0.6	0.2 - 80.8	1.2 - 100.0	15
Not reported	Not reported	Not reported	5.0	Not reported	5.0-53.0	16
0.0808	0.1013	0.03	0.11	0.05-470	0.2 - 100	17
0.0572	0.3533	0.29	0.016	5.0-280	0.1 - 18.0	21
1.5659	1.1707	0.25	1.17	1.0-20	6.7–20	This work
0.0856	_	_	_	20-300	—	This work

TABLE I. A comparison of the efficiency of some modified electrodes in the determination of DA and UA

Available online at www.shd.org.rs/JSCS/



-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid has a longer linear dynamic range and is free from interference by aspirin.

To the best of our knowledge, no work has been reported on the use of 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid (HHNANSA, Scheme 1) modified glassy carbon electrode (HHNANSA–GCE) for the electrocatalytic determination of DA and UA. Consequently, in this study, a GCE was modified with a HHNANSA polymer film and then the electrochemical behavior of DA and UA at the surface of the modified electrode was studied. Using the modified electrode, a sensitive and selective method was established for the simultaneous determination of DA and UA.



Scheme 1. Structure of 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid.

EXPERIMENTAL

Chemicals

All chemicals were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments. Uric acid was purchased from Sigma-Aldrich. Dopamine was obtained from Merck.

Phosphate buffer solutions (PBS) with different pH values were prepared by mixing 0.10 mol L^{-1} Na₂HPO₄ and 0.10 mol L^{-1} NaH₂PO₄. The pH values were adjusted by addition of 1.0 mol L^{-1} H₃PO₄ and/or NaOH solution.

A stock solution of dopamine (0.010 mol L^{-1}) was prepared daily by dissolving dopamine in water. Uric acid solution (0.010 mol L^{-1}) was prepared by dissolving the solid in a small volume of 0.1 mol L^{-1} NaOH solution and diluted with water to obtain the desired concentration. Other dilute standard solutions were prepared by appropriate dilution of the stock solutions in PBS, pH 5.0.

Apparatus

A Corning pH-meter, Model 140, with a glass electrode (conjugated with an Ag/AgCl reference electrode, Model 6.0232.100) was used to determine the pH of the solutions.

Voltammograms were obtained using an EG & G instrument, Model 384B processor, with three electrodes consisting of a platinum wire as the auxiliary electrode, a HHNANSA–GCE as the working electrode and Ag/AgCl (3 mol L^{-1} KCl) as the reference electrode.

Preparation of the poly-2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid film-modified GC electrode

Prior to each experiment, the glassy carbon electrode (GCE) was polished with 0.05 μ m alumina in a water surrey using a polishing cloth. The GCE was subsequently sonicated in a mixture of water/ethanol (90/10 v/v) after each polishing step to be electrochemically pre-treated later by cycling at a scan rate of 100 mV s⁻¹ 10 times in 0.1 mol L⁻¹ H₂SO₄ solution in the potential range of -0.40 to 1.50 V, to obtain a stable background current. Subsequently,

the electrode was placed in a solution containing 0.2 mol L⁻¹ NaOH and 10.0 mmol L⁻¹ 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid (HHNANSA) and a cyclic potential sweep was applied in the range of -0.10 to 1.00 at a scan rate of 33 mV s⁻¹ for 30 times (the anodic peak potential and current tended to be stable after 25 scans). After the polymerization, the modified electrode was washed with water and scan cycled 5 times at pH 5.0 (PBS) between 0.00 and 1.00 V to eliminate untreated HHNANSA monomer, and thus increase its reproducibility and stability.

Procedure

Five milliliters of the buffer solution (pH 5.0) were transferred into the electrochemical cell using the three-electrode system. Then, the SW voltammogram was recorded from 0.00 to 1.00 V at a frequency of 100 Hz and a pulse height of 20 mV with a potential scan rate of 33 mV s⁻¹. The peak current was measured and recorded as the blank signal (I_{pb}). After the background voltammogram had been obtained, aliquots of the sample solution containing DA, and/or UA were introduced into the cell. Then, the SW voltammogram was recorded as described above to give the sample peak current. The peak current was measured and recorded as the sample signal (I_{ps}). All the data were obtained at room temperature. The difference in the currents ($I_{ps} - I_{pb}$) was considered as a net signal (ΔI_p) for each of the experiments. Calibration graphs were prepared by plotting the net peak currents *vs*. the DA and/or UA concentrations in the solution.

Real sample preparation

Dopamine hydrochloride injection solution (40 mg mL⁻¹) was analyzed directly after diluting 100 times with water. 0.10 mL of the diluted solution was injected into a 10-mL volumetric flask and made up to volume with buffer solution (pH 5.0). The test solution was then transferred into the electrochemical cell and the DA content was measured according to the recommended procedure.

Urine samples were analyzed directly after diluting 25 times with buffer solution (pH 5.0) without any further pretreatment. Then, 5 mL of this test solution was transferred into the electrochemical cell and the DA and UA contents were determined according to the recommended procedure.

RESULTS AND DISCUSSION

Electrochemical properties of the poly (2-hydroxy-1-(1-hydroxynaphthyl-2-azo)--naphthalin-4-sulfonic acid) film-modified GCE

The behavior of poly(2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4--sulfonic acid) (PHHNANSA) at the surface of a GCE is shown in Fig. 1. As can be seen in the cyclic voltammograms, an anodic peak appeared at about 0.10 V due to the oxidation of HHNANSA monomer with cycles, whereas on the reversed scan, a cathodic wave formed at a potential of -0.10 V. These anodic and cathodic peak potentials tended to be stable after 25 scans. This suggest that the initially-formed PHHNANSA film underwent a leaching process during the scan cycles up to 25 cycles, which may imply a self-adjustment of the polymer film thickness at the GCE.

The electrochemical properties of the modified electrode were studied by cyclic voltammetry (CV) in the buffer solution (pH 5.0). The CVs of the mo-

Available online at www.shd.org.rs/JSCS/





Fig. 1. Electropolymerization graph of HHNANSA in 0.2 mol L^{-1} NaOH and 10 mmol L^{-1} HHNANSA at a scan rate of 100 mV s⁻¹.

dified electrode at various scan rates (10–100 mV s⁻¹) are shown in the inset to Fig. 2. The experimental results show well-defined and reproducible anodic and cathodic peaks, with a peak separation potential of about $\Delta E_p (E_{pa} - E_{pc}) = 200$ mV. These CVs were used to examine the variation of the peak currents *vs.* potential scan rates. The plot of the anodic peak currents was linearly dependent on *v* with a correlation coefficient of 0.9966 at all scan rates (Fig. 2A). Therefore, the peak current must be related to the surface concentration of electroactive species, Γ , by:

$$I_{\rm P} = n^2 F^2 A \Gamma v / 4RT \tag{1}$$

where *n* represents the number of electrons involved in the reaction (n = 1), *A* is the surface area of the electrode (0.031 cm²), *I*_P is the peak current, Γ represents the surface coverage concentration (mol cm⁻²), and *v* is the scan rate. From the slope of the anodic peak currents *vs*. the scan rate, the calculated surface concentration of 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid was 4.46×10^{-8} mol cm⁻². The cyclic voltammograms of the bare GCE in PBS (pH 5.0) at various scan rates (between 10 to 110 mV s⁻¹) are shown in Fig. 2B.

The electrochemical response of the HHNANSA–GCE depended on the pH value of the supporting electrolyte solution. By increasing the pH of the supporting electrolyte (from 2.0 to 7.0), the redox and oxidation peak potentials shifted negatively and the anodic peak potential (E_{pa}) depended linearly on the pH value.

Electro-oxidation of DA and UA at the surface of the HHNANSA-GCE

The oxidations of DA and UA at the surface of a bare GCE and HHNANSA– -GCE in two different concentrations are shown in Fig. 3. The results showed that both compounds were oxidized with well-defined and distinguishable sharp peaks potential at the HHNANSA–GCE. On the other hand, the indistinguishable

1690



Fig. 2. A) Dependence of peak current on the scan rate for the poly(HHNANSA)-modified GCE in PBS (pH 5.0). Inset: cyclic voltammograms of the poly(HHNANSA)-modified GCE in PBS (pH 5.0) at various scan rates: a) 10; b) 30; c) 50; d) 70; e) 90; f) 100 mV s⁻¹;
B) Cyclic voltammograms of the bare GCE in PBS (pH 5.0) at various scan rates: a) 10; b) 30; c) 50; d) 70; e) 80; f) 100; g) 110 mV s⁻¹.

and broad peak potentials at the bare GCE indicate slow kinetics of electron transfer. The oxidation peak potentials of DA and UA at the modified electrode separated completely into two well-defined peaks at 0.32 and 0.46 V *vs*. Ag/AgCl, respectively, whereas at the bare GCE, the oxidation peak potentials were at about 0.40 and 0.50 V for DA and UA, respectively at pH 5.0. In addition, both the two peaks potential at the HHNANSA–GCE exhibited negative potential shifts. These shifts in the oxidation peaks potential and enhanced currents of the





Fig. 3. Cyclic voltammograms of: A) 20.0 and 80.0 μ mol L⁻¹ DA; B) 7.0 and 9.0 μ mol L⁻¹ UA at the surface of a) the modified and b) a bare glassy carbon electrode.

oxidation peaks potential with the HHNANSA–GCE indicate that the modified electrode had a catalytic effect on the oxidation of DA and UA. Moreover, the oxidation current of DA increased linearly with the square root of the scan rate, which demonstrates a diffusion controlled electrochemical process (Fig. 4).

The influence of solution pH on the DA and UA peaks current were studied with 50.0 μ mol L⁻¹ DA and 50.0 μ mol L⁻¹ UA. The results showed that the UA peak current increased when the solution pH increased from 2.0 to 5.0, whereas at higher pH values, the peak current decreased. For DA, the peak current increased sharply on increasing the pH to 5.0, then leveled off up to pH 7.0 and then decreased at higher pH values. As is known, the pK_a values of R–SO₃H (R = aryl group) are usually about 4; therefore, the –SO₃Na of the poly(2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid) film could dissociate favorably into the negatively charged –SO₃⁻ group under alkaline conditions. The alkaline –NH₂ group of DA (pK_a 8.9) could obtain a proton and form positive DA ions. These have a great affinity toward the poly(2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid) film.



ENSAFI, DADKHAH-TEHRANI and REZAEI



Fig. 4. Variation of electrocatalytic current (*I_p*) with square scan rate. Inset: cyclic voltammograms of poly(HHNANSA) modified GCE in PBS (pH 5.0) containing 100.0 μmol L⁻¹ DA at different scan rates. The number of 1 to 7 corresponds to 10, 30, 70, 90, 100, 120 and 150 mV s⁻¹, respectively.

Chornoamperometric studies

For the determination of the diffusion coefficient of DA, single potential step chronoamperometry was used with the HHNANSA–GCE. The current–time curves of the HHNANSA–GCE obtained by setting the electrode potential at 270 mV (*vs.* Ag|AgCl|KCl_{sat}) for different concentrations of DA are shown in Fig. 5. The linearity of the electrocatalytic current *vs.* $v^{1/2}$ showed that the current is controlled by diffusion of DA from the bulk solution toward the surface of the



Fig. 5. Chronoamperograms of the poly(HHNANSA) modified GCE in PBS (pH 5.0) in the presence of (in direction of *I*-axis): 0.0; 60.0; 100.0; 160.0; 260.0 and 300.0 μmol L⁻¹ of DA. Inset shows Cottrell plots derived from the chronoamperometric data.

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS



electrode, which caused near Cottrellian behavior. The slope of the linear region of a Cottrell plot can be used to estimate the diffusion coefficients. A plot of *I vs.* $t^{-1/2}$ at the HHNANSA–GCE in the presence of DA gave a straight line, the slope of which can be used to estimate the diffusion coefficient (*D*) of DA in the range of 60 to 300 µmol L⁻¹. The mean value of the *D* for DA was found to be 1.30×10^{-5} cm² s⁻¹ (Fig. 5, inset A). This value is different to the previously obtained value of 6.40×10^{-6} cm² s⁻¹).¹⁷ This is due to difference in the solution pH, which affects the net charge of DA in the solutions and hence affects the diffusion coefficient.

In addition, chronoamperometry can be performed to evaluate the rate of an electrocatalyzed oxidation. The rate constant for the chemical reaction between DA and redox cites of 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid can be evaluated according to the method of Galus:²²

$$I_{\rm C}/I_{\rm L} = \gamma^{1/2} (\pi^{1/2} \operatorname{erf} (\gamma^{1/2}) + \exp (-\gamma)/\gamma^{1/2})$$
(2)

where $I_{\rm C}$ is the catalytic current of the HHNANSA–GCE in the presence of DA, $I_{\rm L}$ is the limited current in the absence of DA and $\gamma = k_{\rm h}C_{\rm b}t$ ($C_{\rm b}$ is the bulk concentration of DA, mol L⁻¹) is the argument of the error function. In cases where γ exceeds 2, the error function is almost equal to 1 and Eq. (2) can be reduced to:

$$I_{\rm C}/I_{\rm L} = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} \left(k_{\rm h} C_{\rm h} t \right)^{1/2} \tag{3}$$

where k_h and t are the catalytic rate constant (mol⁻¹ L s⁻¹) and time elapsed, respectively. Equation (3) can be used to calculate the rate constant of the catalytic process, k_h . From the slope of $I_C/I_L vs. t^{1/2}$, the value of k_h can be simply calculated for a given concentration of the substrate. The calculated value of k_h was equal to 1.23×10^4 mol⁻¹ L s⁻¹. This value of k_h explains the sharp feature of the catalytic peak observed for the catalytic oxidation of DA at HHNANSA– –GCE. These methods have already been used for an estimation of D and k_h for some compounds.^{23–26}

Simultaneous determination of DA and UA

As the obtained results showed, the HHNANSA–GCE possessed excellent electrocatalytic activity for the oxidation of DA and UA. The difference in the oxidation peak potentials for DA–UA was 140 mV, which is large enough to allow for the simultaneous determination of DA and UA in a mixture. The electro-oxidation processes of DA and UA in the mixture were evaluated by varying the concentration of the individual analytic species. The result showed two linear segments with different slopes for the DA concentration; namely, for 1.0–20.0 µmol L⁻¹ DA with a regression equation of I_p (µA) = (1.566±0.050) c_{DA} – (0.4211±0.0080) (r^2 = 0.9944, n = 8) (Fig. 6A), while for 20.0–300.0 µmol L⁻¹ DA, the regression equation was I_p (µA) = (0.0856±0.0050) c_{DA} + (31.019±



 ± 0.090) ($r^2 = 0.9897$, n = 7) (Fig. 6B). In addition, for 6.7–20.0 µmol L⁻¹ UA, the regression equation was I_p (µA) = $(1.1707\pm0.05021)c_{\text{UA}} - (3.3652\pm0.07010)$ ($r^2 = 0.9920$, n = 5) (Fig. 6C), where *c* is the concentration of the substance (µmol L⁻¹) and I_p is the net peak current (the sample peak current minus the blank peak current).



Fig. 6. A) Plot of the oxidation current vs. the concentration of DA in the range of 1.0–20.0 μmol L⁻¹; B) as a (A), in the range of 20–300 μmol L⁻¹ using SWV; C) calibration curve for UA in the range of 6.7–20.0 μmol L⁻¹ using SWV.

The detection limits were determined at 0.25 and 1.17 μ mol L⁻¹ for DA and UA, respectively, according to the definition of $Y_{\text{LOD}} = Y_{\text{B}} + 3\sigma^{27}$.

In order to check for the presence of any intermolecular effects between DA and UA, two different experiments were performed under the optimum conditions at pH 5.0. In each experiment, the concentration of one of the two compounds was changed while the concentration of the other compound was kept constant. The results are shown in Figs. 7A and 7B. The results showed no any intermolecular interactions during the oxidation of the compounds at the surface of the HHNANSA–GCE.

The sensitivities towards DA in the absence and presence of UA were found to be $0.0856\pm0.005 \ \mu\text{A} \ \mu\text{mol} \ \text{L}^{-1}$ (in the absence of UA) and $0.0960\pm0.006 \ \mu\text{A} \ \mu\text{mol} \ \text{L}^{-1}$ (in the presence of UA) (Fig. 8A). However, for UA, the obtained sensitivities were 1.1707 ± 0.0901 (in the absence of DA) and $1.1691\pm0.0240 \ \mu\text{A} \ \mu\text{mol} \ \text{L}^{-1}$ (in the presence of DA) (Fig. 8B). It is interesting to note that the

Available online at www.shd.org.rs/JSCS/



sensitivities of the HHNANSA–GCE to DA and UA in the absence and presence of the other compound were very similar, which indicates that the oxidation processes of DA and UA at the HHNANSA-GCE were independent. Therefore, the simultaneous or independent measurements of the two analytes are possible without any interference.



Fig. 7. A) SWV of: 1) 20.0; 2) 50.0; 3) 110.0; 4) 160.0; 5) 220.0 μ mol L⁻¹ DA in the presence of 60.0 μ mol L⁻¹ UA; B) SWV of: 1) 8.0; 2) 10.0; 3) 12.0; 4) 15.0; 5) 17.0; 6) 19.0 μ mol L⁻¹ UA in the presence of 100.0 μ mol L⁻¹ DA.

Interference study

The influence of various substances as compounds that could potentially interfere with the determination of DA and UA were studied under the optimum conditions with 100.0 μ mol L⁻¹ DA and 14.0 μ mol L⁻¹ UA at pH 5.0. The potentially interfering substances were chosen from substances commonly found with DA and UA in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5 % for the determination of DA and UA. The results are presented in Table II. These results showed the high selectivity of the HHNANSA–GCE for the determination of DA and UA.







Fig. 8. A) Plot of different concentrations of DA in the presence of a fixed amount of UA; B) Plot of different concentrations of UA in the presence of a fixed amount of DA.

Table II. Interference of some foreign substances on the determination of 100.0 μ mol L⁻¹ DA and 14.0 μ mol L⁻¹ UA under the optimized conditions

Species	w(substance)/w(DA)	w(substance)/w(UA)
Glucose, sucrose, urea, fructose	400	1000
Citric acid	100	1000
Ca ²⁺ , Mg ²⁺ , F ⁻ , Cl ⁻ , Na ⁺ , K ⁺ , SO ₄ ²⁻	100	800
Carbonate	200	600
Tryosin, aspirin	20	100
Histidine, cysteine, ascorbic acid	2	100

Real sample analysis

1696

In order to evaluate the applicability of the proposed method for the determination of DA and UA in real samples, the utility of the developed method was tested by analysis of these compounds in mixed synthetic and in real samples using standard addition methods. The results are summarized in Table III. The good recoveries of the mixture samples indicate the successful application of the proposed method for the simultaneous determination of DA and UA. For further



investigation, the recovery of DA was determined for dopamine injection. The dopamine injection solution (specified content of DA was 40.0 mg mL⁻¹) was diluted to 100 mL with water, then a different amount of the diluted solution was transferred into each of a series of 10-mL volumetric flasks and diluted to the mark with phosphate buffer. Then, 10 mL aliquot of this test solution was placed in the electrochemical cell and the DA content was measured by the proposed method. This procedure was repeated five times and the relative standard deviation was found as 1.6 %. Different standard concentrations of DA were added to the diluted DA injection solution, with recoveries between 96.5 and 103.2 % for five measurements (Table III).

Sampla	Added,	Added, µmol L ⁻¹		umol L ⁻¹	Recovery, %	
Sample	DA	UA	DA	UA	DA	UA
1	20.0	10.0	20.9±0.8	9.9±0.2	104.6	99.0
2	50.0	14.0	51.6±0.6	13.4±0.5	103.1	96.1
3	70.0	20.0	67.9±0.7	19.4±0.4	97.1	97.1
			DA injection			
4	10.0	-	9.6±0.5	_	96.5	_
5	15.0	-	15.1±0.7	_	100.8	_
6	50.0	-	51.6±0.6	_	103.2	_

Table III. Determination of DA and UA in human urine and DA injection samples

CONCLUSIONS

In this study, the electrochemical behavior of DA and UA at a glassy carbon electrode modified with a polymerized film of 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-naphthalin-4-sulfonic acid was investigated using cyclic voltammetry and square wave voltammetry. The modified electrode separated the anodic oxidation peak potential of DA and UA with a well-defined peak separation in the presence of each other to measure DA and/or UA separately or simultaneously without any intermolecular effects. The catalytic reaction rate constant, k_h was calculated $(1.23 \times 10^4 \text{ mol}^{-1} \text{ L s}^{-1})$ using chronoamperometry. The sensitivity of the proposed material was higher than those of reported results.^{10,11,14,17,21} Moreover, the proposed method was successfully applied for the determination of these compounds in real samples.

Acknowledgements. The authors gratefully acknowledge support of this work by the Research Council of the Isfahan University of Technology (IUT) and the Center of Excellence in Sensor and Green Chemistry.

ENSAFI, DADKHAH-TEHRANI and REZAEI

ИЗВОД

ВОЛТАМЕТРИЈСКО ОДРЕЂИВАЊЕ ДОПАМИНА У ПРИСУСТВУ МОКРАЋНЕ КИСЕЛИНЕ НА ЕЛЕКТРОДИ ОД СТАКЛАСТОГ УГЉЕНИКА МОДИФИКОВАНОГ 2-ХИДРОКСИ-1(1-ХИДРОКСИ НАФТИЛ-2-АЗО)-НАФТАЛИН-4-СУЛФОНСКОМ КИСЕЛИНОМ

ALI ASGHAR ENSAFI, SAMIRA DADKHAH-TEHRANI и В. REZAEI

Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran

Полимерни филм 2-хидрокси-(1-хидроксинафтил-2-азо)-нафталин-4-сулфонске киселине је формиран на електроди од стакластог угљеника поступком електрохемијске полимеризације. Тако модификована електрода је коришћена за истовремено одређивање допамина (ДА) и мокраћне киселине (МК). Електрохемијско понашање ових једињења на модификованој електроди је испитивано цикличном волтаметријом, хроноамперометријом и волтаметријом са правоугаоним сигналом. Експериментални резултати показују да модификована електрода представља ефикасан катализатор за оксидацију ДА и МК уз сепарацију пикова од око 140 mV при pH 5,0. Константа брзине катализоване реакције одређена је методом хроноамперометрије и износи 1,23×104 mol-1 L s-1. У мерењима волтаметријом са правоугаоним сигналом у раствору pH 5,0 струјни пикови су показали линеарну зависност од концентрације ДА и/или МК у опсегу 1,0-300 µmol L⁻¹ ДА (две линеарне области са различитим нагибима) и 6,7–20 μ mol L⁻¹ МК. Границе детекције за ДА и МК су биле 0,25 и 1,17 μ mol L⁻¹, редом. Стандардна девијација за 140,0 µmol L⁻¹ ДА је износила 1,9 и 2,2%, а за 10,0 и 20,0 µmol L⁻¹ МК 1,8 и 1,2%, редом. Модификована електрода је показала високу осетљивост, селективност и стабилност. Такође је успешно примењена и за одређивање ДА и МК у реалним узорцима као што су лекови и урин.

(Примљено 11. марта, ревидирано 30. јула 2010)

REFERENCES

- N. Q. Jia, Z. Y. Wang, G. F. Yang, H. B. Shen, L. Z. Zhu, *Electrochem Commun.* 9 (2007) 233
- 2. J. W. Mo, B. Ogorevc, Anal. Chem. 73 (2001) 1196
- R. M. Wightman, C. Amatorh, R. C. Engstrom, P. D. Hale, E. W. Kristensen, W. G. Kuhr, L. J. May, *Neuroscience* 25 (1998) 513
- 4. R. M. Wightman, L. J. May, A. C. Michael, Anal. Chem. 60 (1988) 769
- 5. A. Heinz, H. Przuntek, G. Winterer, A. Pietzcker, Nervenarzt 66 (1995) 662
- 6. F. M. Benes, Trends Pharmacol. Sci. 22 (2001) 46.
- J. K. Baillie, M. G. Bates, A. A. Thompson, W. S. Waring, R. W. Partridge, M. F. Schnopp, A. Simpson, F. Gulliver-Sloan, S. R. Maxwell, D. J. Webb, *Chest* 131 (2007) 1473
- 8. Y. Y. Sun, K. B. Wu, S. S. Hu, Chem. J. Chin. Universities 23 (2002) 2067
- M. Mazloum-Ardakani, H. Beitollahi, B. Ganjipour, H. Naeimi, M. Nejati, *Bioelectro-chem.* 75 (2009) 1
- 10. A. A. Ensafi, M. Taei, T. Khayamian, Int. J. Electrochem. Sci. 5 (2010) 116
- 11. H. Yao, Y. Sun, X. Lin, Y. Tang, L. Huang, Electrochim. Acta 52 (2007) 6165
- 12. J. M. Zen, P. J. Chen, Anal. Chem. 69 (1997) 5087
- 13. A. Safavi, N. Maleki, O. Moradlou, F. Tajabadi, Anal. Biochem. 359 (2006) 224
- 14. A. Balamurugan, S. M. Chen, Anal. Chim. Acta 596 (2007) 92
- 15. P. Wang, Y. Li, X. Huang, L. Wang, Talanta 73 (2007) 431

Available online at www.shd.org.rs/JSCS/



VOLTAMMETRIC DETERMINATION OF DOPAMINE

- 16. P. Ramesh, S. Sampath, *Electroanalysis* 16 (2004) 866
- 17. A. A. Ensafi, M. Taei, T. Khayamian, A. Arabzadeh, Sens. Actuators B 147 (2010) 213
- 18. J. B. Raoof, R. Ojani, S. Rashid-Nadimi, Electrochim. Acta 50 (2005) 4694
- 19. H. Zhao, Y. Zhang, Y. Yuan, Analyst 126 (2001) 358
- 20. T. Selvaraju, R. Ramaraj, Electrochem. Comm. 5 (2003) 667
- 21. A. A. Ensafi, M. Taei, T. Khayamian, J. Electroanal. Chem. 633 (2009) 212
- 22. Z. Galus, Fundumentals of Electrochemical Analysis, Ellis Horwood, New York, 1999
- 23. E. Mirmomtaz, A. A. Ensafi, H. Karimi-Maleh, *Electroanalysis* 20 (2008) 1973
- 24. H. Karimi-Maleh, A. A. Ensafi, H. R. Ensafi, J. Braz. Chem. Soc. 20 (2009) 880
- 25. H. Karimi-Maleh, A. A. Ensafi. A. R Allafchian, J. Solid State Electrochem. 14 (2010) 9
- H. Yghoubian, H. Karimi-Maleh, M. A. Khalilzadeh, F. Karimi, J. Serb. Chem. Soc. 74 (2009) 1443
- 27. J. N. Miller, J. C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, 4th ed., Pearson Education Ltd., Harlow, Essex, 2000.





J. Serb. Chem. Soc. 75 (12) 1701–1709 (2010) JSCS–4089 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 663.2+543.421:546.72+ 546.56+546.47(497.11) Original scientific paper

The concentrations of Fe, Cu and Zn in selected wines from South-East Serbia

DANIJELA KOSTIĆ[#], SNEŽANA MITIĆ[#], GORDANA MILETIĆ, SAŠA DESPOTOVIĆ and ALEKSANDRA ZARUBICA^{*#}

Department of Chemistry, Faculty of Science, University of Niš, 18000 Niš, Serbia

(Received 4 January, revised 30 July 2010)

Abstract: Fruits and vegetables constitute the cheapest source of essential trace elements for the majority of people living in developing countries. The Cu, Fe and Zn contents in twenty selected wine samples produced in the South-East region of Serbia were determined by flame atomic absorption spectrometry. The Cu concentrations varied from 0.07 to 0.57 ppm in wines, and the Fe concentrations fluctuated from 2.93 to 36.2 ppm, while the Zn levels were in the range from 0.21 to 0.67 ppm. The established contents of Cu and Zn showed that wines from this part of the world could serve as good dietary sources of the essential trace metals, and the determined values were within the allowed metals levels in wines for human consumption.

Keywords: AAS; Fe, Cu and Zn contents; South-East Serbian wines; wine analysis.

INTRODUCTION

Several spectroscopy techniques can be commonly used for the evaluation of food and/or drink quality¹ as well as of pharmaceutical samples.² Wine is a popular and worldwide consumed alcoholic beverage, which has been well-known since the early periods of civilization. The moderate consumption of wine, especially red wines, was shown to improve health and longevity.^{3,4} From an analytical chemical point of view, wine is referred to as a complex matrix with a varying content of inorganic compounds (*e.g.*, traces of dissolved alkaline and alkaline earth elements, and transition metals), as well as organic substances (*e.g.*, polyphenols, polyhydroxy alcohols, proteins, amino acids, and polysaccharides) dissolved and/or dispersed in an aqueous solution of ethanol.⁵

doi: 10.2298/JSC100104133K

1701



^{*} Corresponding author. E-mail: zarubica2000@yahoo.com

[#] Serbian Chemical Society member.

KOSTIĆ et al

Scientific discussions concerning human exposure to the trace metals contents of various beverages and dietary products, including wines, have received raising attention, since the consumption of wines, especially reasonably large volumes, may significantly contribute to the daily dietary intake of trace elements by humans.⁵ Moreover, some of these trace elements (*e.g.*, Cu, Fe, and Mn) have an organoleptic effect, and also contribute to the haze and taste of wines.⁶ The regional variations of the content trace metal in wines can also be used for identification purposes, *i.e.* to verify authenticity.⁷ Considering all the above points, the determination of toxic (*e.g.*, As, Cd and Pb) and essential trace elements (*e.g.*, Cu) in wines appears to be an important and challenging analytical task, which requires multi-element methods of good selectivity, sensitivity, and robustness. The origin of Cu in wines is associated with copper-based vineyard sprays, whereas the As, Cd and Pb contents reflect differences in grape variety, environmental factors (*e.g.*, soil and climate), and the wine-processing method (an anthropogenic impact).⁵

However, nutritional metals such as Cu and Zn occur naturally in fruits and vegetables, as essential trace elements necessary for good health, but they could be toxic when their concentrations exceed limits of safe exposure.^{3,4,8} In addition to toxicity problems, Cu and Zn deficiency may also be experienced; hence, as stated earlier,³ knowledge of heavy metal contents in crops is important for the identification of adequate, sub-adequate and marginal intake levels for humans and animals, so that diseases related to trace element deficiency can be overcome. A large number of symptoms/ailments, comprising anemia, depressed growth, dermatitis, dwarfism, electrolyte-imbalance, gastro-intestinal and neurological disorders, lethargy and nausea, have been associated with Cu and Zn deficiency in humans, as well as with toxicity due to excessive intake.^{6,8–11} Furthermore, the presence of trace elements in fruits and vegetables has been ascribed to their absorption from the soil and sources such as fertilizer, agricultural chemicals and contaminants.^{8,12} Other sources of heavy metals contamination of most foodstuffs may also include agricultural mechanization procedures, sprays, seed preservatives,¹³ and components from the global pollution process. Hence, the need to determine and/or monitor the Cu and Zn contents of fruits and vegetables have become imperative due to their principal role as essential or detrimental trace elements. A survey of the literature indicates that such a study is scarce, particularly in the southeast region of Serbia.

Trace elements in wine samples can be measured by different techniques, such as stripping voltammetry,^{4–6,8–13} instrumental neutron activation analysis,¹⁴ ICP–AES, ICP–MS^{15–17} and/or UV–Vis spectrophotometry.^{18–21}

However, the official methods for the determination of heavy metals in wine established by the Office International de la Vigne et du Vin and the American Society of Enologists are essentially based on atomic absorption spectrometry

Available online at www.shd.org.rs/JSCS/



(AAS).²² Similarly, Flame-AAS is the official method of analysis for the determination of Na, K, Mg, Ca, Fe, Ag and Zn in wine according to EU regulations.¹ Some elements with relatively high concentrations in wine could be analyzed by flame atomic absorption spectrometry (FAAS).^{23–25} However, for some elements present in wine in low concentrations, hydride generation techniques using FAAS (HG–AAS)^{26–28} or mostly electrothermal atomic absorption spectrometry (ETAAS).^{29–31} are used. The ETAAS method was applied in order to directly determine some trace elements (or using simple dilution) in wine.^{32,33}

Flame-AAS is largely employed in wine analysis mainly due to the low cost of instrumentation, which makes the technique easily accessible to most oenological laboratories. Considering the compromise between the cost and sensitivity required, flame-AAS can be considered as the technique of choice for determination for alkaline and alkali-earth metal in wine. It is also well suited for Cu, Fe, Mn and Zn determinations, with respect to the concentration ranges of these metals in wines. On the other hand, it is not suitable for the determination of toxic or undesirable elements, such as As, Cd, Cr, and Hg and Pb, with the exception of highly contaminated samples and/or application of preconcentration procedures.

The analysis precision was usually very good, being on average above 1 % for all the elements considered at the mg L^{-1} concentration level.^{14,15}

In the present study, a scheme was developed for the determination of Fe, Cu and Zn in different wine fractions. Flame and electrothermal (ET) atomic absorption spectrometry were used for the quantitative determination of the metals, depending on their levels.^{4–6,8–13,34}

EXPERIMENTAL

Reagents and materials

All reagents used were of analytical grade (Merck, Germany). Stock standard solutions were prepared daily by the appropriate dilution of Titrisol standards (Merck) containing 1000 mg L^{-1} Fe, Cu or Zn. High purity water from a Milli-Q apparatus was used to prepare the standard solutions.

Sample preparation

Several types of wine samples were investigated: Serbian wine samples were given from vineries and only filtered in the further procedure. Several other commercially bottled wines (Serbian wines) were purchased from the market. Labels descriptions of the analyzed wine samples are presented in Table I. In each case, aliquots of samples were withdrawn with a glass pipette 10 cm below surface level of the liquid.

An aliquot of 5 ml of wine sample was mixed with 1.0 mL of 2 M HCl solution and further diluted to 10 mL with distilled water, and then directly nebulized in an air-acetylene flame under the optimal instrumental parameters (background correction for zinc was required). Fe, Cu and Zn were determined by AAS in the air-acetylene flame using standard calibration curves. All determinations were performed on untreated wine samples; only nitric

KOSTIĆ et al.

acid was added to lower the pH (1.0 mL of concentrated acid to 100 mL sample, the resulting pH being 1.5).

TABLE I. Label description of the wine samples

Sample No.	Sample label	Year	Vinery
1	Vranac	2001	Župa
2	Rubinova ružica	2002	Rubin
3	Cabernet Sauvignon	2001	Rubin
4	As	2003	Župa
5	Merlot	2005	Navip
6	Car Lazar	2001	Rubin
7	Medveđa krv	2006	Rubin
8	Pinot Noir	2006	Rubin
9	Kratošija	2003	Župa
10	Međaš crni	2004	Župa
11	Župski rizling	2006	Župa
12	Međaš beli	2001	Župa
13	Vranac	2004	Rubin
14	Navipovo crno Rojal	2001	Navip
15	Rubinovo crno	2003	Rubin
16	Graševina	2005	Rubin
17	Pinot Noir	2001	Navip
18	Terra Lazarica	2006	Navip
19	Jagodinska Ružica	2004	Navip
20	Rose	2002	Župa

Apparatus/analysis

The atomic absorption measurements were realized with a Varian Spectra A 10 atomic absorption spectrometer equipped with a deuterium background corrector and single element hollow cathode lamp of Cu, Fe and Zn. An air–acetylene flame was utilized for all the elements. The calibration range, wavelengths and slit values are reported in Table II.

TABLE II. Calibration range, wavelength and slit value

Characteristics of analysis	Fe	Zn	Cu
Wavelength, nm	248.3	213.9	324.8
Slit, nm	0.2	1.0	0.5
Calibration range, mg L ⁻¹	0.06-15.0	0.01 - 2.0	0.03-10.0

RESULTS AND DISCUSSION

The determined concentrations of heavy metals in the examined wine samples originating from the southeast region of Serbia are reported in Table III.

The measured concentrations of iron (Table III) show that these varied in range from 2.93 ("Cabernet Sauvignon") to 11.21 mg L⁻¹ ("Rose") with the exception being the wine sample named as "As". This wine sample had an iron content of 36.2 mg L⁻¹. The concentrations of copper in the different wines also differed a lot, *i.e.*, from 0.07 mg L⁻¹ (Graševina and Župski rizling) to 0.57 mg

Available online at www.shd.org.rs/JSCS/



 L^{-1} ("Merlot"), while the zinc concentrations covered a somewhat narrower range, from 0.21 ("Navipovo crno Royal") to 0.67 mg L^{-1} ("Cabernet Sauvignon").

TABLE III. Average content of Fe, Cu and Zn in the examined wine samples (*ia* – inaccuracy of measurement (standard deviation at the 95 % confidence level))

Sample No	Sampla labal		$c\pm ia / \text{mg L}^{-1}$	
Sample No.	Sample laber	Fe(III)	Cu(II)	Zn(II)
1	Vranac	6.40±0.19	0.40 ± 0.008	0.48±0.03
2	Rubinova ružica	5.84 ± 0.17	0.39 ± 0.007	0.65 ± 0.04
3	Cabernet Sauvignon	2.93 ± 0.09	0.11 ± 0.002	0.67 ± 0.01
4	As	36.2 ± 1.08	0.14 ± 0.003	0.57±0.03
5	Merlot	6.62±0.19	0.57 ± 0.011	0.62 ± 0.04
6	Car Lazar	4.69 ± 0.14	0.12 ± 0.002	0.61 ± 0.04
7	Medveđa krv	3.17±0.09	0.10 ± 0.001	0.54 ± 0.03
8	Pinot Noir	4.62 ± 0.14	0.17 ± 0.003	0.55 ± 0.03
9	Kratošija	9.78±0.29	0.12 ± 0.002	0.55 ± 0.03
10	Međaš crni	5.03 ± 0.15	0.10 ± 0.001	0.57 ± 0.03
11	Župski rizling	5.37±0.16	0.07 ± 0.001	0.38 ± 0.02
12	Međaš beli	6.82 ± 0.20	0.08 ± 0.001	0.52 ± 0.03
13	Vranac	3.69 ± 0.11	0.16 ± 0.003	0.49 ± 0.03
14	Navipovo crno Royal	4.17 ± 0.12	0.19 ± 0.004	0.21 ± 0.01
15	Rubinovo crno	3.53±0.10	0.22 ± 0.004	0.57±0.03
16	Graševina	4.75 ± 0.14	0.07 ± 0.001	0.47 ± 0.03
17	Pinot Noir	4.22±0.13	0.44 ± 0.009	0.35 ± 0.02
18	Terra Lazarica	6.60±0.19	0.32 ± 0.006	0.64 ± 0.04
19	Jagodinska Ružica	5.51±0.16	0.26 ± 0.005	0.31 ± 0.02
20	Rose	11.21±0.34	0.26 ± 0.005	0.49±0.03

The allowed levels of metal in wines are defined by standards. The established allowed values of the standards differ from country to country, even though there are common standards prescribed by the International Office for Grapes and Wines.³ An insight into the accepted limits of the content of metals in wines in different countries and also those given by the Office International de la Vigne et du Vin (OIV) are listed in Table IV.

TABLE IV. The accepted limits of the metals content, mg L^{-1} , in wines in different countries

Country	Al	As	Cd	Cu	Na	Pb	Ti	Zn
Australia	-	0.10	0.05	5.00	-	0.20	-	5.00
Germany	8.00	0.10	0.01	5.00	-	0.30	1.00	5.00
Italy	_	-	-	10.00	_	0.30	-	5.00
OIV	_	0.20	0.01	1.00	60.00	0.20	_	5.00

The results obtained in the current study are comparable with previously reported values showing high degree of agreement.⁸ Here, the estimated data (Table III) demonstrate that the contents of the major metal, *i.e.*, Fe, and the

KOSTIĆ et al

1706

selected trace elements, *i.e.*, Cu and Zn, in wine samples from different parts of Serbia are considerably smaller than the maximum concentrations allowed according to the OIV, Tables III and IV. In addition, the contents of these trace metals in the studied Serbian wines were significantly lower than in some European wines, but similar to those found in some Slovenian and Hungarian wines.^{34,35} Namely, it was reported earlier that the contents of Cu and Zn in some selected Slovenian red wine labels are up to 1.0 and 0.6 mg L⁻¹, respectively.³⁵ It is obvious that determined metal levels in the present study are completely comparable to the ones in the Slovenian wine samples, whereby the contents of Zn are especially similar, Table III. The estimated concentrations of Zn in Serbian wines are also analogous to those discussed formerly in literature data.³⁶

The Hungarian national legislation according to OIV recommendations allows a maximum level of 1 mg L^{-1} Cu in wines and other food products.³⁴ The corresponding Cu contents varied in the range from 0.02 to 0.64 mg L^{-1} being greatly below the cited limit in all the studied Hungarian wine samples.³⁴ The results of the present study evidenced fluctuation of Cu contents over a similar range as in Hungarian wines, whereas these contents reported to be a bit higher in the case of later wines. On the contrary, in German red wines,³⁷ the Cu content was found to be even five times higher than the highest concentration of this element in Hungarian wines and almost the same as in Serbian wines. Similarly, for Australian wines,³⁸ but white ones, the Cu content ranged within limits almost four times higher than in the selected Serbian wines. Unexpectedly, the Cu level ranged from 0.03 to 0.17 mg L⁻¹ in Russian and Italian red and white wines, as well,³⁹ being noticeably lower than the Cu content in wines originating from other European countries, including Serbian wines. On the other hand, in Russian and Italian wines,³⁹ the Zn content was reported to be between 0.14 and 0.76 mg L^{-1} , which is completely in accordance to values found in wines produced from other European regions. Opposed to other papers, one review article¹ reported very wide ranges of the Fe, Cu and Zn contents in Italian wines. Somewhat curious is/are the highest/higher value/values of the Fe content/s in selected Serbian wines (Table III), being even higher than all reported values in the review article concerning Canadian wines.

Wine samples from Jordan show relatively high values of metal contents,⁴⁰ especially the maximal determined levels, but most of the estimated values were below the toxic limit in food (Swiss Standard, 1993). This may be explained by the homemade production procedure applied.

The presence of some other trace metals, such as: lead, manganese, cadmium and nickel, was not detected in the examined wine samples.

The concentration of metals in alcoholic beverages may vary widely depending on the plant origin and the technology used (home-made or by an official producer). Moreover, it was recently reported that some home-produced alcoholic



beverages and spirits contain rather high concentrations of metals.⁸ In addition; it was observed that alcoholic beverages from Africa, India and Canada possess higher metal concentrations, *i.e.*, 58, 68 and 245 mg L^{-1} of Cu, Zn and Fe, respectively.⁴¹

The concentrations of metals and trace metals in wines depend on the metal content in the vineyard soil, which primarily determines the degree of metal uptake by the grape plant. In addition, anthropogenic impact plays a vital role in determining the metal content in wines. Namely, as was stated above before, some home-made wines showed a higher trace element content, probably due to contamination during the wine-making process and/or the mixing/storage procedure, and also additives used.

It is worth emphasizing that the allowed limits for metal contents in alcoholic beverages are higher than those imposed for water envisaged for human consumption.⁸ This may be related to the lower predicted intake of alcoholic beverages.

The variation of the discussed levels of metals concentrations in the present study as well as those reported earlier by other authors suggest a necessity for the establishment of common limits.

CONCLUSIONS

Different metals occur in wines at the mg L^{-1} and/or µg L^{-1} level not directly influencing the taste of the end product. Nevertheless, their content should be determined because excess is undesirable due to potential toxicity and risks to human health, consequently imposing the maximal allowed values and/or prohibited limits. The contents of the investigated metals (Fe, Cu and Zn) in wine samples from different areas of Serbia are considerably lower than the maximum concentrations allowed according to the OIV. The contents of selected trace metals were significantly lower than those of some European wines, but similar to the values found in some Slovenian and Hungarian wines. Additionally, these contents were compared with known literature values. Somewhat curious is the relatively high Fe content in the studied Serbian wines ("As" and "Rose"). The variation in the metal content in the studied Serbian wines may be related to the metal content in the vineyard soil, *i.e.*, soil type, and/or an anthropogenic impact arising from the wine-making process and/or storage procedure.

Acknowledgement. The financial support of the Ministry of Science and Technological Development of the Republic of Serbia (Project No. ON 142051) is highly appreciated.



KOSTIĆ et al.

ИЗВОД

КОНЦЕНТРАЦИЈА Fe, Cu И Zn У ОДАБРАНИМ ВИНИМА ЈУГОИСТОЧНЕ СРБИЈЕ

ДАНИЈЕЛА КОСТИЋ, СНЕЖАНА МИТИЋ, ГОРДАНА МИЛЕТИЋ, САША ДЕСПОТОВИЋ и АЛЕКСАНДРА ЗАРУБИЦА

Природно-машемашички факулшеш, Универзишеш у Нишу, 18000 Ниш

Воће и поврће представљају најјефтинији извор есенцијалних метала, који се јављају у траговима, за највећи део становништва развијеног света. Садржај Сu, Fe и Zn је одређен пламеном атомско апсорпционом спектрометријом у двадесет узорака одабраних вина, пореклом из региона Југоисточне Србије. Концентрација Cu варира од 0,07 до 0,57 ppm у испитиваним винима, гвожђа од 2,93 до 36,2 ppm, док је ниво Zn у интервалу од 0,21 до 0,67 ppm. Установљен садржај Cu и Zn показује да вина из овог дела света могу бити добар дијететски извор есенцијалних метала, заступљених у траговима; одређени садржаји метала су у границама дозвоњених вредности у људској исхрани.

(Примљено 4. јануара, ревидирано 30. јула 2010)

REFERENCES

- 1. M. Aceto, O. Abollino, M. C. Bruzzoniti, E. Mentasti, C. Sarzanini, M. Malandrino, *Food Addit. Contam.* **19** (2002) 126
- 2. S. Mitić, J. Vučetić, S. Miletić, D. Kostić, J. Serb. Chem. Soc. 65 (2000) 595
- 3. B. Zoecklein, K. Fugelsang, B. Gump, F. Nury, *Wine Analysis and Production*, Chapman & Hall, New York, 1994
- 4. J. Wang, S. Mannino, Analyst 114 (1989) 643
- 5. C. Marin, P. Ostapczuk, Fresenius J. Anal. Chem. 343 (1992) 881
- 6. H. Eschnauer, P. Ostapczuk, Wein-Wiss. 47 (1992) 206
- 7. S. Ražić, Dj. Čokeša, S. Sremac, J. Serb. Chem. Soc. 72 (2007) 1487
- J. Ibanez, A. Carreon-Alvarez, M. Barcena-Soto, N. Casillas, J. Food Composition Anal. 21 (2008) 672
- 9. M. Arcos, M. Ancin, J. Echeverria, A. Gonzales, J. Garrido, J. Agric. Food Chem. 41 (1993) 2333
- 10. P. Ostapczuk, Anal. Chim. Acta 273 (1993) 35
- 11. E. Zakharova, V. Pichugina, T. Tolmacheva, Zh. Anal. Khim. 51 (1996) 918
- 12. S. Sanllorente, O. Cruz, M. J. Arcos, Analyst 123 (1998) 513
- 13. O. Domingez, S. Sanllorente, M. Arcos, *Electroanalysis* 1 (1999) 1273
- 14. S. May, J. Leroy, D. Piccot, G. Pinte, J. Radioanal. Chem. 72 (1982) 305
- 15. H. Eschnauer, L. Jakob, H. Meierer, R. Neeb, Microchim. Acta 3 (1989) 291
- 16. J. Goossens, T. De Smaele, L. Moens, R. Dams, Fresenius J. Anal. Chem. 347 (1993) 119
- S. Augagneur, B. Medina, J. Bernard, Szpunar, R.Lobinski, J. Anal. At. Spectrom. 11 (1996) 713
- 18. G. Weber, G. Schwedt, Anal. Chim. Acta 134 (1982) 81
- 19. C. Garcia-Jahres, M. Lage-Yusty, J. Simal-Lozano, Fresenius J. Anal. Chem. 338 (1990) 703
- 20. K. Ohzeki, I. Nukatsuka, K. Ichimura, F. Kumagai, M. Kogawa, Microchem. J. 49 (1994) 256
- 21. S. Cherubin, S. Buiatti, F. Battistutta, R. Zironi, Wein-Wiss. 49 (1994) 78
- 22. T. Stafilov, J. Cvetkovic, S. Arpadjan, I. Karadjova, BAÜ Fen Bil. Enst. Dergisi 4.2 (2002) 90
- 23. R. Farre, M. Lagarda, J. Micronutr. Anal. 2 (1986) 201



- 24. J. Korkisch, A. Sorio, I. Steffan, *Talanta* 23 (1976) 289
- 25. U. Anders, G. Hailer, Fresenius J. Anal. Chem. 278 (1976) 203
- 26. D. Siemer, R. Vitek, P. Koteel, H. Prabhakaran, W. Houser, Anal. Lett. 10 (1977) 357
- 27. J. Sanz, P. Basterra, J. Galban, J. R. Castillo, Mikrochim. Acta 1 (1989) 271
- 28. M. Segura, Y. Madrid, C. Camara, J. Anal. At. Spectrom. 14 (1999) 131
- 29. M. Segura, J. Grgic, Z. Grgic, B. Segura, Dtsch. Lebensm.-Rundsch. 94 (1998) 336
- 30. E. Lendinez, M. Lopez, C. Cabrera, M. Lorenzo, J. AOAC Int. 81 (1998) 1043
- 31. C. Cabrera-Vigne, P. Teissedre, M. Cabanis, J. Cabanis, Am. J. Enol. Vitic. 51 (2000) 103
- 32. B. Kildahl, W. Lund, Fresenius J. Anal. Chem. 354 (1996) 93
- C. Cabrera-Vique, P.-L. Teissedre, M.-T. Cabanis, J. Cabanis, J. Agric. Food Chem. 45 (1997) 1808
- Z. Ajtony, S. Zoboszlai, E. Klaudia Susko, P. Mezei, K. Gyorgy, L. Bencs, *Talanta* 76 (2008) 627
- 35. J. Kristl, M. Veber, M. Slekovec, Acta Chim. Slov. 50 (2003) 123
- 36. K. Bauer, S. Hinkel, R. Neeb, R. Eicher, H. Eschnauer, Vitic. Enol. Sci. 49 (1994) 209
- 37. C. Wiese, G. Schwedt, Fresenius J. Anal. Chem. 358 (1997) 718
- 38. L. Sauvage, D. Frank, J. Stearne, M. B. Millikan, Anal. Chim. Acta 458 (2002) 223
- Kh. Z. Brainina, N. Yu. Stozhko, G. Belysheva, O. Inzhevatova, L. Kolyadina, C. Cremisini, M. Galletti, Anal. Chim. Acta 514 (2004) 227
- 40. F. Al Nasir, A. Jiries, M. Batarseh, F. Beese, Environ. Monit. Assess. 66 (2001) 253
- 41. C. Reilly, J. Sci. Food Agric. 23 (1972) 1143.







J. Serb. Chem. Soc. 75 (12) 1711–1719 (2010) JSCS-4090 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 678.744+661.8'078.2:542.913 Original scientific paper

Simplification of the synthesis of the reversible addition–fragmentation chain transfer agent 2-(2-cyanopropyl)-dithiobenzoate

MILOŠ B. MILOVANOVIĆ^{1#}, MILENA AVRAMOVIĆ², LYNNE KATSIKAS²* and IVANKA G. POPOVIĆ^{2#}

¹Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade and ²Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia

(Received 30 June 2010)

Abstract: The general literature procedure for the preparation of the reversible addition–fragmentation chain transfer (RAFT) agent 2-(2-cyanopropyl)-dithiobenzoate (CPDB) was modified by omitting the recrystallisation of the intermediate di(thiobenzoyl)disulphide. The yield of the CPDB in the simplified synthesis was increased four times compared to the standard one. The behaviour of the CPDB obtained by the modified procedure and by the standard one in the polymerisation of methyl methacrylate was investigated. The CPDB synthesized by the simplified procedure showed itself to be a good RAFT agent, giving excellent control over the polymerisation of methyl methacrylate and it behaved in the same manner as the CPDB prepared by the literature method. The obtained poly(methyl methacrylate) had a narrow molecular weight distibution (PD = 1.1).

Keywords: 2-(2-cyanopropyl)-dithiobenzoate; preparation; reversible addition– –fragmentation chain transfer; poly(methyl methacrylate).

INTRODUCTION

In recent years, much effort has been focused on the synthesis of polymers with controlled molar masses and very narrow molar mass distributions. With the development of several methods of controlled radical polymerisation, well-defined polymers with complex architectures, including block,^{1,2} graft³ and star^{4,5} structures, could be prepared. The development of these methods was promoted by the growing need for truly living radical polymerisation systems that would offer all the benefits of ionic polymerisations without the serious disadvantages inherent to such systems. Among them, RAFT (reversible addition–fragmen-

doi: 10.2298/JSC100628082M

1711



^{*} Corresponding author. E-mail: Lynne@tmf.bg.ac.rs

[#] Serbian Chemical Society member.

MILOVANOVIĆ et al

tation chain transfer) polymerisation has proven itself to be the most versatile one, since it is applicable to a wide range of monomers and can be performed in a wide variety of solvents over a broad range of experimental conditions. RAFT polymerisations are now being used successfully by an ever-growing number of research groups around the world. The key to successful RAFT polymerisations is the presence of a highly efficient dithioester chain transfer agent. Among numerous RAFT agents, only a few are commercially available (carboxymethyl dithiobenzoate, for example). Regarding the importance of the synthesis of RAFT agents of different structures, a large number of procedures for the synthesis of dithioester compounds have been developed. As the syntheses of these RAFT agents are usually costly and require multi-step reactions, the loss of polymerisation mediator throughout the RAFT polymerisation process may be an issue when scaling-up the process. A simple reaction that leads to the full removal of the thiocarbonyl-thio end group from the polymeric chains and recovery of the chain transfer agent has already been reported.⁶ In addition, simplifications of the syntheses of RAFT agents would promote scaling-up the RAFT polymerisation processes.

This study focused on the synthesis of 2-(2-cyanopropyl)-dithiobenzoate (CPDB) which, together with cumyl dithiobenzoate, is one of the most frequently employed RAFT agents. CPDB was reported to be an efficient RAFT agent in the polymerisation of a number of monomers.^{7–10} 2-(2-Cyanopropyl)-dithiobenzoate can be synthesized in two ways. One method is a single-step procedure, by reaction of Davy reagent or P_4S_{10} with benzoic acid. The obtained CPDB was used to control *in situ* the free-radical polymerisation of styrene and alkyl (meth)acrylates.¹¹ The isolation of pure CPDB demands a multi-step procedure.¹² One of the steps in this preparation is the synthesis of dithiobenzoic acid. Dithiobenzoic acid is unstable and should be stored at low temperatures (< -20 °C)¹³ or used immediately. For this reason, it is usually transferred into di(thiobenzoyl)disulphide. Di(thiobenzoyl)disulphide is used not only for the synthesis of CPDB, but also for the syntheses of many different RAFT agents.¹⁴

In the present study, the standard procedure for the preparation of CPDB was modified and simplified by omitting the intermediate step of the recrystallisation of di(thiobenzoyl)disulphide in order to avoid great loss of material in this step. The behaviour of the CPDB synthesized by the simplified procedure in the polymerisation of methyl methacrylate was investigated and compared with that of the CPDB synthesized by the standard method.

EXPERIMENTAL

Methyl methacrylate, MMA, (Fluka) was distilled under reduced pressure after removal of the inhibitor with a 10 % aqueous NaOH solution. Azobis(isobutyronitrile), AIBN, (Aldrich), was purified by recrystallisation from methanol. Benzene, thiophene free, (Fluka) was distilled before use.



The RAFT agent, 2-(2-cyanopropyl)-dithiobenzoate (CPDB), was prepared by a standard method described in the literature.¹⁵ To a thoroughly dried, three-necked round-bottomed flask equipped with a magnetic bar, addition funnel, thermometer and condenser was added elemental sulphur (6.4 g, 0.20 mol), 25 % sodium methoxide solution in methanol (40 g) and anhydrous methanol (40 g). Benzyl chloride (12.6 g, 0.10 mol) was the added dropwise via an addition funnel over a period of 90 min at room temperature. The resulting violet-brown solution was then heated and allowed to reflux overnight. After cooling to room temperature, the mixture was filtered to remove the white solid (sodium chloride) which was formed as a by--product during the reaction. The methanol was removed by rotary evaporation at 40 °C. The resulting violet-brown solid was then re-dissolved in distilled water (100 ml) and transferred to a separation funnel. The crude sodium dithiobenzoate solution was washed with diethyl ether (3×50 ml). A final layer of ether (50 ml) was added to the solution and the two-phase mixture was then acidified with 32 % aqueous HCl until the aqueous layer lost its characteristic violet-brown colour and the top, ether, layer was deep purple. The ether layer containing dithiobenzoic acid was extracted. Deionized water (120 ml) and 1.0 M NaOH (240 ml) were added, and sodium dithiobenzoate was extracted into the aqueous layer. This washing process was repeated two times more to yield a final solution containing sodium dithiobenzoate (360 ml).

The next step was the synthesis of di(thiobenzoyl)disulphide. Potassium ferricyanide (13.17 g, 0.040 mol) was dissolved in deionized water (200 ml). Potassium ferricyanide solution (140 ml) was transferred to a conical flask equipped with a magnetic bar. Potassium ferricyanide solution was added dropwise to the sodium dithiobenzoate *via* an addition funnel over a period of 1 h under vigorous stirring. The red precipitate was filtered and washed with deionized water until the washings become colourless. The solid was dried under vacuum at room temperature. The product was recrystallized from anhydrous ethanol.

The target compound was prepared in the reaction of di(thiobenzoyl)disulphide with azobisisobutyronitrile (AIBN). A solution of AIBN (2.90 g, 0.018 mol) and di(thiobenzoyl)disulphide (3.60 g, 0.012 mol) in ethyl acetate (70 ml) was heated at reflux for 18 h. The ethyl acetate was removed under vacuum. The crude product, CPDB-1, was subjected to column chromatography on a 25×2.6 cm column filled with silica gel (Woelm mesh size 0.063–0.2 mm, ICN Pharmaceuticals, Germany) as the stationary phase and ethyl acetate: *n*-hexane (0.2:0.98) as the eluent, at a flow rate of 1.0 mL min⁻¹.

Due to the large loss of di(thiobenzoyl)disulphide in the recrystallisation step, the synthesis of di(thiobenzoyl)disulphide was repeated but this time it was used in the subsequent reaction without recrystallisation to yield CPDB-2.

The structure of the CPDB from the both synthesis was confirmed by ¹³C-NMR spectroscopy using a Varian-Gemini-200 (200 MHz) instrument.

In order to determine whether CPDB-2 was equally effective as a RAFT agent as CPDB-1, methyl methacrylate was polymerised using both RAFT agents.

The polymerisations of methyl methacrylate, MMA, were performed in a three-necked round-bottomed flask equipped with a magnetic stirring bar, a condenser, a thermometer, an inlet for nitrogen and a rubber septum for removing samples. The flask was charged with MMA (30 ml, 0.28 mol), benzene (10 ml, 0.11 mol), AIBN (40 mg, 0.24 mmol) and CPDB (104 mg, 0.470 mmol). Nitrogen was bubbled through the reaction mixture for 15 min at room temperature before starting the polymerisation, while during the polymerisation the nitrogen stream was directed over the top of the condenser, thus keeping the reaction mixture under a nitrogen atmosphere. A preheated oil bath was employed to commence the polymerisations. The polymerisations were performed at 60 °C. Samples were removed from the flask every 2 h



MILOVANOVIĆ et al.

via a needle and syringe and precipitated into methanol. The polymer samples were reprecipitated from chloroform solution into methanol and dried to constant mass at room temperature under vacuum.

The number and weight average molar masses, M_n and M_w , respectively, and the polydispersity index, *PD*, of the obtained polymers were determined at 30 °C by gel permeation chromatography, SEC, using a Waters instrument fitted with four analytical columns (Waters HR 2, HR 3, HR 4 and HR 5E) and a refractive index detector. THF was used as the solvent at a flow rate of 1.0 ml min⁻¹. The obtained chromatograms were analyzed with Waters Breeze software using a calibration curve of narrow molar mass distribution PMMA standards (PSS Polymer Standards Service GmbH, Mainz, Germany).

RESULTS AND DISCUSSION

The RAFT agent, CPDB, was prepared in two syntheses which differed in the recrystallisation of the intermediate di(thiobenzoyl)disulphide. The final products (CPDB1 and CPDB2, with and without recrystallisation, respectively) from both syntheses were subjected to column chromatography with silica gel as the stationary phase and ethyl acetate:*n*-hexane (0.20:0.98) as eluent. The crude product made from the di(thiobenzoyl)disulphide recrystallized in ethanol gave seven fractions: green (which did not enter the column), yellow and pink which stayed on the column, and yellow (eluted first), pink, red and purple which eluted last. The main purple fraction gave 2-(2-cyanopropyl)-dithiobenzoate as a red--purple liquid after evaporation of the eluent. The yield of the CPDB-1 was extremely low when the pure substance was obtained (5 % of the theoretical yield).

The crude product from the second synthesis made from the di(thiobenzoyl)disulphide without recrystallisation was reddish coloured, but after the column chromatography it gave the characteristic purple coloured fraction as the main product. The crude product from this synthesis gave four fractions: yellow and orange, which stayed on the column, and yellow and the main purple one, which eluted last. After evaporation of the eluent, the main purple fraction from the second synthesis gave CPDB-2 as a red-purple liquid.

The fact that more fractions were obtained during the column chromatography of CPDB-1 than during the chromatography of CPDB-2 indicates that during recrystallisation not only was di(thiobenzoyl)disulphide inherently lost, but also that it decomposed. It was observed that a resinous material was formed during the recrystallisation, which when removed was insoluble in ethanol. This could be the explanation of the green fraction which did not enter the column.

The ¹³C-NMR spectrum and the numbering of the C atoms of the prepared CPDB-1 and CPDB-2 are shown in Figs. 1 and 2, respectively, from which it can be seen that all the expected peaks were present. One additional peak was found in the spectrum of the CPDB2 (at 23 ppm). This could arise by the recombination of primary radicals from the decomposition of AIBN, used in the last step of the synthesis. It was reported¹² that the highest level of impurity found in CPDB could be attributed to recombined radicals arising from the decomposition of

Available online at www.shd.org.rs/JSCS/

AIBN. This recombination compound was found to be difficult to remove from the RAFT agent, even after column chromatography, but it is inert to any radical reaction. The peak of the carbon atoms from the four equivalent CH₃ groups of such a compound is expected at 23 ppm in a ¹³C-NMR spectrum. It is important to emphasise that the yield of the CPDB in the second synthesis was increased to 20 % of theoretical yield, which is four times more compared to the first one.



Fig. 1. ¹³C-NMR Spectrum of CPDB-1 (inset: structure of CPDB with C atoms numbered).



Fig. 2. ¹³C-NMR Spectrum of CPDB-2 (inset structure of CPDB with C atoms numbered).

A change in the standard method of preparation of CPDB was previously reported in the literature.¹⁶ The alteration concerned of the same step in the

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS



MILOVANOVIĆ et al.

procedure of CPDB synthesis to which attention was paid in this study. The purification di(thiobenzoyl)disulphide by the recrystallisation in ethanol was substituted by column chromatography using ethyl acetate/petroleum ether (1:1) as the eluent. The yield of CPDB was 50 % of the theoretical one, which is 2.5 times larger than one obtained in the present study. However, it must be emphasised that the purification di(thiobenzoyl)disulphide was completely avoided in synthesis procedure employed in this study, which is extremely important for laboratory or industrial scale-ups.

Two RAFT polymerisation of methyl methacrylate were performed using CPDB from the two different syntheses. Samples were taken from the reaction flask every 2 h. The polymer obtained after the RAFT polymerisation was pink due to the attachment of CPDB to the chain ends. Two series of PMMA samples were obtained: PMMA-1 from the polymerisation using the CPDB-1 and PMMA-2 from the polymerisation using the CPDB-2. The number and weight average molar masses, M_n and M_w , and the polydispersity index, PD, of the obtained polymers are given in Table I. The polymerisations mediated by CPDB-1 and CPDB-2 exhibited very similar kinetics, *i.e.*, the polymerisation rates were very similar (Fig. 3a).

PMMA-1 and PMMA-2 samples							
T . 1		PMMA-1			PMMA-2		
Time, h	$M_{\rm n}$ / g mol ⁻¹	$M_{\rm W}$ / g mol ⁻¹	PD	$M_{\rm n}$ / g mol ⁻¹	$M_{\rm w}$ / g mol ⁻¹	PD	
-							

TABLE I. Number and weight average molar mass and polydispersidity index, PD, of the

TT' 1		PMMA-1			PMMA-2		
Time, h	$M_{\rm n}$ / g mol ⁻¹	$M_{\rm w}$ / g mol ⁻¹	PD	$M_{\rm n}$ / g mol ⁻¹	$M_{\rm w}$ / g mol ⁻¹	PD	
2	16625	18415	1.108	15161	16674	1.100	
4	19481	21676	1.112	20872	23001	1.102	
6	25126	27972	1.113	26071	28901	1.109	

1.119

1.122

31958

39412

35648

44310

1.115

1.124

34062

41800

30436

37271

8

10

A comparison of M_{W} of PMMA-1 and PMMA-2 samples as a function of polymerisation time is shown in Fig. 3b, from which it can be seen that the molar mass increased with polymerisation time, as is to be expected for RAFT-controlled polymerisations, and that the rate of increases were very similar. The SEC chromatograms revealed good control of the CPDB-mediated polymerisations with narrow molar mass distributions. The comparison of An SEC chromatogram of a PMMA sample obtained using CPDB-2, is compared with that of the corresponding PMMA sample prepared using CPDB-1, in Fig. 4. As can be seen, the chromatograms are very similar.

The polydispersities of the PMMA samples from both series (Table I) were also very similar for the same polymerisation time, indicating that CPDB-1 and CPDB-2 affected the same polymerisations.

Available online at www.shd.org.rs/JSCS/





Fig. 3. a) MMA Conversion vs. polymerisation time in the presence of CPDB-1 and CPDB-2.b) Weight average molar mass of the PMMA-1 and PMMA-2 samples vs. polymerisation time.



Fig. 4. SEC Chromatogram of PMMA samples obtained by polymerisation of MMA in the presence of CPDB-1 and in the presence of CPDB-2 for 4 h.

As can be seen, the CPDB obtained by the simplified procedure exerted the same effects in the polymerisation of PMMA as that obtained by the literature procedure.

CONCLUSIONS

The procedure of CPDB preparation was modified and simplified by omitting the recrystallisation of the intermediate di(thiobenzoyl)disulphide. The yield of the CPDB in the simplified synthesis was increased by a factor of four comparing to the literature method. The CPDB synthesized by the simplified procedure provided very good control of the polymerisation of methyl methacrylate, yielding PMMA samples with narrow molar mass distibutions, and behaved in the same manner as the CPDB prepared by the standard literature procedure. The simplification of the synthesis of CPDB introduced in this study is very important from the points of view of time, scale-up and amounts of solvents necessary.



MILOVANOVIĆ et al.

Acknowledgement. This investigation was financed by the Ministry of Science and Technological Development of the Republic of Serbia, through Project No.142023.

ИЗВОД

ПОЈЕДНОСТАВЉЕЊЕ ПОСТУПКА СИНТЕЗЕ RAFT АГЕНСА 2-(2-ЦИЈАНОПРОПИЛ)-ДИТИОБЕНЗОАТА

МИЛОШ Б. МИЛОВАНОВИЋ¹, МИЛЕНА АВРАМОВИЋ², LYNNE KATSIKAS² и ИВАНКА Г. ПОПОВИЋ²

¹Инсійшійуій за хемију, ійехнологију и мешалургију, Универзийней у Београду, Београд ²Технолошко-мешалуршки факулиней, Универзийней у Београду, Београд

Стандардни поступак синтезе 2-(2-цијанопропил)-дитиобензоата (СРDВ) агенса из литературе за RAFT полимеризацију, односно за реверзибилну адиционо-фрагментациону трансфер полимеризацију, је модификован изостављањем прекристализације међупроизвода бис-(тиобензоил)дисулфида. Принос СРDВ-а синтетисаног поједностављеним поступком је четири пута већи у односу на принос СРDВ-а синтетисаног стандардним поступком. Испитано је понашање СРDВ-а добијеног модификованим и оног добијеног стандардним поступком у полимеризацији метил метакрилата. СРDВ добијен поједностављеним поступком показао се као добар RAFT агенс који успоставља одличну контролу полимеризације метил метакрилата и који се понаша на исти начин као СРDВ синтетисан стандардним поступком. Добијени поли(метил метакрилат) има уску ширину расподеле молских маса (PD = 1.1).

(Примљено 30. јуна 2010)

REFERENCES

- S. Kulkarni, C. Schilli, B. Grin, A. H. E. Muller, A. S. Hoffman, P. S. Stayton, *Biomacro-molecules* 7 (2006) 2736
- G. Moad, R. T. A. Mayadunne, E. Rizzardo, M. Skidmore, S. H. Thang, *Macromol. Symp.* 192 (2003) 1
- 3. H. Bottcher, M. L. Hallensleben, S. Nub, H. Wurm, Polym. Bull. 44 (2000) 223
- 4. A. Blencowe, J. F. Tan, T. K. Goh, G. G. Qiao, Polymer 50 (2009) 5
- J. Bernard, X. Hao, T. P. Davis, C. Barner-Kowollik, M. H. Stenzel, *Biomacromolecules* 7 (2006) 232
- 6. S. Perrier, P. Takolpuckdee, C. A. Mars, Macromolecules 38 (2005) 2033
- J. B. McLeary, F. M. Calitz, J. M. McKenzie, M. P. Tonge, R. D. Sanderson, B Klumperman, *Macromolecules* 37 (2004) 2383
- 8. M. Benaglia, E. Rizzardo, A. Alberti, M. Guerra, Macromolecules 38 (2005) 3129
- L. Katsikas, M. Avramović, R. D. B. Cortes, M. B. Milovanović, M. T. Kalagasidis Krusić, I. G. Popović, J. Serb. Chem. Soc. 73 (2008) 915
- J. B. McLeary, J. M. McKenzie, M. P. Tonge, R. D. Sandersona, B. Klumperman, *Chem. Commun.*(2004) 1950
- 11. A. Dureault, Y. Gnanou, D. Taton, M. Destarac, F. Leising, Angew. Chem. Int. Ed. 42 (2003) 2869
- S. Perrier, C. Barner-Kowollik, J. F. Quinn, P. Vana, T. P. Davis, *Macromolecules* 35S (2002) 30
- Y. K. Chong, J. Krstina, Tam P. T. Le, G. Moad, Almar Postma, E. Rizzardo, S. H. Thang, *Macromolecules* 36 (2003) 2256

Available online at www.shd.org.rs/JSCS/



- Y. Mitsukami, M. S. Donovan, A. B. Lowe, C. L. McCormick, *Macromolecules* 34 (2001) 2248
- S. Perrier, C. Barner-Kowollik, J. F. Quinn, P. Vana, T. P. Davis, *Macromolecules* 35 (2002) 8300
- 16. Uma Adash, *PhD Thesis*, University of Canterbury, New Zealand, 2005, http:////ir.canterbury.ac.nz/bitstream/10092/1282/1/thesis_fulltext.pdf (last accessed on 18.06.2010).






J. Serb. Chem. Soc. 75 (12) 1721–1732 (2010) JSCS–4091 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 532.13:541.25:539.196 Original scientific paper

Studies of viscous antagonism, excess molar volumes, viscosity deviation and isentropic compressibility of ternary mixtures containing N,N-dimethylformamide, benzene and some ethers at 298.15 K

RIJU CHANDA, ASHIS BANERJEE and MAHENDRA NATH ROY*

Department of Chemistry, North Bengal University, Darjeeling-734013, India

(Received 6 August 2009, revised 27 August 2010)

Abstract: The densities (ρ) and viscosities (η) for ternary liquid mixtures of N,N-dimethylformamide + benzene + an ether were measured as a function of composition at 298.15 K. From experimental measurements, the excess molar volumes $(V^{\rm E})$, viscosity deviation $(\Delta \eta)$, antagonic interaction index $(I_{\rm A})$ and Gibbs free energy of activation for viscous flow $(\Delta G^{*{\rm E}})$ were evaluated. The speeds of sound were also measured and excess isentropic compressibilities $(K_{\rm s}^{\rm E})$ were calculated at the experimental temperature. The results are discussed and interpreted in terms of molecular package and specific interaction predominated by hydrogen bonding.

Keywords: viscous antagonism; viscosity deviations; excess molar volumes; isentropic; compressibility; molecular interactions.

INTRODUCTION

The grouping of solvents into classes is often based on the nature of intermolecular forces because the manner in which solvent molecules are associated with each other has a marked effect on the resulting properties. Rheology is the branch of science¹ that studies material deformation and flow, and is increasingly applied to analyze the viscous behaviors of many pharmaceutical products,² and to establish their stability and even bio-availability, since it has been firmly established that viscosity influences the absorption rate of a drug in the body. The increasing use of the solvents: *N*,*N*-dimethylformamide (DMF), benzene, 1,3-dioxolane, 1,4-dioxane, tetrahydrofuran, 1,2-dimethoxyethane, di-isopropyl ether, diethyl ether, 2-methoxyethanol and 2-ethoxyethanol and their mixtures in many industrial processes, such as battery, pharmaceutical and cosmetics, has greatly stimulated the need for extensive information on their various properties. Hence,



^{*}Corresponding author. E-mail: mahendraroy2002@yahoo.co.in doi: 10.2298/JSC090806132C

the present study gives extensive information on the various properties when these ethers are taken with DMF and benzene as mixed solvent systems.^{1–7} The determination of density, viscosity and speed of sound are valuable tools to learn about the liquid state^{8,9} because of the close connection between liquid structure and macroscopic properties.

The viscosity and density of these ternary liquid mixtures were used to understand the molecular interactions between the components of the mixture to develop new theoretical models and also for engineering applications.^{10,11} In systematic investigations, the viscosities, densities and speeds of sound of different solvents and their mixtures were reported in previous papers.^{12–18}

The present work contributes and extends the study of density (ρ) , viscosity deviations $(\Delta \eta)$, viscous antagonism, excess molar volumes $(V^{\rm E})$ and isentropic compressibility $(K_{\rm s}^{\rm E})$ to mixtures of DMF and benzene with some ethers, where DMF is represented as A, benzene as B and the ethers as C.

EXPERIMENTAL

DMF (C₃H₇NO) was obtained from Merck and LR, and further purified by standard methods.¹⁹ Benzene (S. D. Fine Chemicals, purity > 99 %) was further purified by means of a simple distillation technique with the first and last 20 % of the distillate being discarded^{20–22} and finally the density and viscosity value were compared with the literature.²³ The final purities of the obtained solvents were >99.5 %.

The experimental ethers, namely 1,3-dioxolane (1,3-DO), 1,4-dioxane (1,4-DO), tetrahydrofuran (THF), 1,2-dimethoxyethane(1,2-DME), di-isopropyl ether (DIE), diethyl ether (DEE), 2-methoxy ethanol (2-ME) and 2-ethoxyethanol (2-EE) were obtained from Merck and LR. These were further purified by standard methods.^{24–27} The purities of the liquids was ascertained by GLC and also by comparing the experimental values of densities, viscosities and sound velocities with those reported in the literature,^{24,27–35} as listed in Table I. The purities of the finally obtained solvents were >99 %.

Colvert	ho / g c	m ⁻³	η / ml	Pa s	$u / m s^{-1}$		
Solvent	Experimental	Literature	Experimental	Literature	Experimental	Literature	
DMF	0.94609	0.9447^{27}	0.8586	0.802^{27}	1465.2	1465.0^{27}	
Benzene	0.8735	0.8735^{23}	0.5920	0.5920^{23}	1252.7		
1,3-Dioxolane	1.0577	1.0586^{33}	0.5878	0.5873^{21}	1338.2	1338.8 ³³	
1,4-Dioxane	1.0287	1.0279^{33}	1.1779	1.196^{25}	1344.4	1345.5 ³³	
Tetrahydrofuran	0.8807	0.8811^{25}	0.463	0.460^{25}	1292.2	1294.0 ²⁹	
1,2-Dimethoxy ethane	0.8615	0.8611 ³⁵	0.4089	0.4089 ³⁵	1146.2	_	
Di-isopropyl ether	0.7250	0.7250 ²¹	0.379	0.3791 ²¹	1050.1	_	
Diethyl ether	0.7134	0.7134^{25}	0.224	0.22404^{25}	1080.8	_	
2-Methoxy ethanol	0.95979	0.9600 ³⁴	1.543	1.5414 ³⁴	1339.4	-	
2-Ethoxy ethanol	0.92497	0.9256^{27}	1.8277	1.851 27	1308.0	1308.0 ²⁷	

TABLE I. Physical properties of the pure solvents at 298.15 K



1723

Densities (ρ) were measured with an Ostwald–Sprengel type pycnometer having a bulb volume of 25 cm³ and an internal diameter of the capillary of about 0.1 cm. The pycnometer was calibrated at the experimental temperature with triply distilled water and DMF. The measurements were realized in a thermostated water bath controlled to ± 0.01 K.² The weighings were performed on a Mettler electronic balance (AG-285) with a precision of ± 0.01 mg. The viscosities (η) were measured by means of a suspended Ubbelohde type viscometer⁷, which was calibrated at the desired temperature with triply distilled water and purified methanol using density and viscosity values from the literature. The ultrasonic speeds (u) were determined using a single-crystal variable path ultrasonic interferometer (Mittal Enterprises, New Delhi) working at 5 MHz,^{32,36} which was calibrated with water, methanol and benzene. The temperature stability was maintained within ±0.01 K by circulating thermostatic water around the cell with a circulating pump. The solutions were prepared by mixing known volumes of pure liquids in air-tight stoppered bottles at 298.15 K. The precisions of the speed of sound, density and viscosity measurements were $\pm 0.2 \text{ m s}^{-1}$, $\pm 3 \times 10^{-4} \text{ g cm}^{-3}$ and $\pm 2 \times 10^{-4} \text{ mPa s}$, respectively. The estimated uncertainty for the excess molar volume (V^{E}), viscosity deviation $(\Delta \eta)$, antagonic interaction index (I_A) and excess isentropic compressibilities $(K_s^{\rm E})$ were $\pm 0.5 \times 10^{\text{-4}} \text{ m}^3 \text{ mol}^{\text{-1}}, \pm 0.0004 \text{ mPa s}, \pm 0.002 \text{ and } \pm 0.2 \text{ Pa}^{\text{-1}},$ respectively.

RESULTS AND DISCUSSION

The measured density (ρ), viscosity (η) and the speeds of sound (u) data for mixtures of (DMF) (A), benzene (B), and the ethers (C), *i.e.*, 1,3-dioxolane, 1,4-dioxane, tetrahydrofuran, 1,2-dimethoxyethane, di-isopropyl ether, diethyl ether, 2-methoxy ethanol and 2-ethoxyethanol were used to calculate the excess molar volume (V^{E}), viscosity deviation ($\Delta \eta$) and excess isentropic compressibility (K_{s}^{E}).

Viscous antagonism is the term used in respect to the interaction between the components of a system that cause the total viscosity of the latter to be less than the sum of the viscosities of the individual components in the system. The method compares the viscosity of the system determined experimentally, η_{exp} , with that expected in the absence of interaction, η_{calcd} . Viscous antagonism is exists when, $\eta_{exp} < \eta_{calcd}$. This procedure is used when Newtonian fluids are involved.³⁷

Quantitatively, as per the absolute reaction rates theory,³⁸ the deviations of viscosities from the ideal mixture values for a three-component system can be calculated as:

$$\Delta \eta = \eta - \sum_{i=1}^{3} x_i \eta_i \tag{1}$$

where η is the viscosity of the mixture, x_i and η_i are the mole fraction and viscosity of the pure components, respectively. The $\Delta \eta$ values for all the studied ternaries were found to be negative over the whole composition range at 298.15 K, as depicted by a representative plot in Fig. 1 as a function of the mole fraction of both DMF (x_A) and benzene (x_B). The $\Delta \eta$ values for the ternary mixtures (A) + (B) + 1,3-DO, 1,4-DO, THF, 1,2-DME, DIE, DEE, 2-ME and 2-EE (C) are plotted against the mole fraction of DMF (x_A) in Fig. 2, from which it can be



1724

observed that $\Delta \eta$ increases as the mole fraction of DMF increases. The mixtures have a tendency of maximization, indicating a strong specific interaction between the unlike molecules. From the values of $\Delta \eta$, given in Table II, it can be concluded that the affinity of the molecules of the ethers towards benzene molecules in the presence of DMF is enhanced in the following order:

A + B + 2-EE > 2-ME > DEE > DIE > 1,2-DME > THF > 1,4-DO > 1,3-DO

Here, dispersion and dipolar interactions between the DMF, benzene and 1,3-DO, 1,4-DO, THF, 1,2-DME, DIE, DEE, 2-ME and 2-EE molecules are operative, resulting in negative $\Delta \eta$ but with the increasing x_A , the dipolar interactions dominate giving rise to less negative values of $\Delta \eta$.³¹



Fig. 2. Viscosity deviations $(\Delta \eta)$ of: **•**) DMF (A) + benzene (B) + 1,3-DO (C); **•**) DMF (A) + benzene (B) + 1,4-DO (C); **•**) DMF (A) + benzene (B) + THF (C); **•**) DMF (A) + benzene (B) + 1,2-DME (C); **□**) DMF (A) + benzene (B) + DIE (C); **○**) DMF (A) + benzene (B) + DEE (C); +) DMF (A) + benzene (B) + 2-ME (C); Δ) DMF (A) + benzene (B) + 2-EE (C) mixtures with mole fraction of DMF (x_A) at 298.15 K.

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS



1725

TABLE II. Experimental density (ρ), viscosity (η), viscosity deviations ($\Delta \eta$), antagonic interaction index (I_A), excess molar volumes (V^E), Gibbs energy of activation (ΔG^{*E}) of viscous flow, experimental sound velocities (u), isentropic compressibility (K_s) and excess isentropic compressibility (K_s^E) of N,N-dimethylformamide (A) + benzene (B) + 1,3-DO, 1,4-DO, THF, 1,2-DME, DIE, DEE, 2-ME and 2-EE (C) at 298.15 K

		ρ	η	$\Delta \eta$		$V^{\rm E}$	$\varDelta G^{*E}$	и	Ks	$K_{\rm s}^{\rm E}$
$x_{\rm A}$	$x_{\rm B}$	g cm ⁻³	mPa s	mPa s	$I_{\rm A}$	cm ³ mol ⁻¹	J mol ⁻¹	m s ⁻¹	10 ⁻¹² Pa ⁻¹	10 ⁻¹² Pa ⁻¹
		N	N-Dime	ethylform	amide (A) + benzene	e(B) + 1,3	8-DO (C)	
0.0000	0.4868	0.9584	0.3650	-0.2248	0.38118	-0.1310	-1174.47	1302.1	615.4	340.2
0.1036	0.4363	0.9583	0.4296	-0.1881	0.30349	-0.2120	-871.83	1326.2	593.3	301.0
0.2064	0.3863	0.9578	0.4859	-0.1594	0.24506	-0.2630	-665.66	1336.7	584.3	275.0
0.3083	0.3367	0.9568	0.5393	-0.1334	0.19566	-0.2690	-504.20	1339.8	582.2	256.0
0.4095	0.2874	0.9557	0.5862	-0.1137	0.15942	-0.2600	-393.34	1339.8	583.0	240.0
0.5099	0.2386	0.9542	0.6320	-0.0949	0.12741	-0.2290	-301.30	1338.9	584.6	225.0
0.6094	0.1901	0.9527	0.6775	-0.0761	0.09797	-0.1890	-222.05	1344.1	581.0	205.0
0.7082	0.1420	0.9509	0.7235	-0.0567	0.07006	-0.1310	-151.39	1359.0	569.4	177.0
0.8062	0.0943	0.9492	0.7682	-0.0383	0.04552	-0.0840	-94.27	1387.0	547.6	139.0
0.9035	0.0470	0.9476	0.8148	-0.0179	0.02040	-0.0360	-39.35	1444.6	505.7	81.0
1.0000	0.0000	0.9461	0.8586	0.0000	0.00000	0.0000	0.00	1465.2	440.7	0.0
		N	N-Dime	ethylform	amide (A) + benzene	e(B) + 1,4	I-DO (C)	
0.0000	0.5301	0.9472	0.6569	-0.2104	0.25767	-0.2210	-548.86	1097.5	876.5	314.0
0.1118	0.4708	0.9479	0.6990) -0.1674	0.20782	-0.2770	-408.52	1130.9	824.9	276.0
0.2207	0.4131	0.9486	6 0.7290) -0.1364	0.17129	-0.3170	-317.23	1157.7	786.6	251.0
0.3268	0.3568	0.9487	0.7539	-0.1106	5 0.14044	-0.3130	-245.96	1181.8	754.7	232.0
0.4303	0.3020	0.9486	0.7750) -0.0886	5 0.11372	-0.2940	-189.09	1204.9	726.1	216.0
0.5311	0.2485	0.9484	0.7900) -0.0727	0.09381	-0.2540	-152.49	1226.6	700.8	203.0
0.6295	0.1964	0.9479	0.8027	/ -0.0591	0.07641	-0.2000	-123.44	1263.5	660.8	175.0
0.7255	0.1455	0.9474	0.8180) -0.0430	0.05598	-0.1430	-87.32	1301.5	623.1	149.0
0.8192	0.0958	0.9469	0.8329	-0.0273	0.03588	-0.0860	-53.23	1350.9	578.7	116.0
0.9107	0.0473	0.9464	0.8483	3 -0.0111	0.01500	-0.0380	-18.61	1435.8	512.6	61.0
1.0000	0.0000	0.9461	0.8586	5 0.0000	0.00000	0.0000	0.00	1465.2	440.7	0.0
		1	V, <i>N</i> -Din	nethylfori	mamide (A	A) + benzei	ne(B) + T	HF (C)		
0.0000	0.4800	0.8797	0.4022	2 -0.1227	0.23749	-0.2530	-645.90	1165.1	837.5	296
0.1023	0.4309	0.8868	0.4507	/ -0.1084	0.19612	-0.3150	-491.81	1210.0	770.1	268
0.2041	0.3820	0.8939	0.4957	/ -0.0973	0.16504	-0.3570	-382.37	1238.0	729.9	244
0.3054	0.3334	0.9009	0.5413	3 -0.0855	5 0.13641	-0.3840	-290.26	1263.2	695.7	226
0.4061	0.2851	0.9078	0.5843	3 -0.0761	0.11455	-0.3900	-225.66	1290.5	661.5	210
0.5064	0.2370	0.9145	0.6285	5 -0.0654	0.09315	-0.3790	-169.18	1322.2	625.4	190
0.6061	0.1891	0.9211	0.6744	-0.0528	3 0.07132	-0.3360	-117.21	1357.2	589.4	168
0.7053	0.1414	0.9276	6 0.7191	-0.0412	0.05294	-0.2860	-80.15	1392.0	556.4	140
0.8041	0.0941	0.9338	0.7681	-0.0251	0.03062	-0.2000	-37.14	1430.3	523.4	104
0.9023	0.0469	0.9400	0.8153	3 -0.0107	0.01235	-0.1090	-9.35	1484.8	482.5	58
1.0000	0.0000	0.9461	0.8586	5 0.0000	0.00000	0.0000	0.00	1465.2	440.7	0
		N,1	V-Dimet	thylforma	mide (A)	+ benzene	(B) + 1,2-	DME (C)	
0.0000	0.5357	0.8702	0.3970) -0.1100	0.20674	-0.3050	-564.64	1137.8	887.7	285.0
0.1128	0.4752	0.8782	0.4473	3 -0.0994	0.16596	-0.3780	-418.36	1176.0	823.4	239.0
0.2225	0.4165	0.8862	0.4939	-0.0913	0.13661	-0.4290	-318.28	1203.8	778.6	212.0

Available online at www.shd.org.rs/JSCS/



2010 Copyright (CC) SCS

CHANDA, BANERJEE and NATH ROY

TABL	E II. Co	ntinued								
		ρ	η	$\Delta \eta$	I	$V^{\rm E}$	$\varDelta G^{*E}$	и	Ks	$K_{\rm s}^{\rm E}$
$\lambda_{\rm A}$	$\lambda_{\rm B}$	g cm ⁻³	mPa s	mPa s	$I_{\rm A}$	$cm^3 mol^{-1}$	J mol ⁻¹	m s ⁻¹	10 ⁻¹² Pa ⁻¹	10 ⁻¹² Pa ⁻¹
		N,N	-Dimeth	nylformai	nide (A) -	+ benzene ((B) + 1,2-	DME (C	C)	
0.3291	0.3594	0.8943	0.5376	-0.0851	0.11561	-0.4650	-250.96	1228.2	741.3	192.0
0.4329	0.3038	0.9022	0.5813	-0.0779	0.09698	-0.4760	-196.86	1243.8	716.5	184.0
0.5338	0.2498	0.9100	0.6257	-0.0690	0.07926	-0.4690	-150.92	1264.5	687.2	171.0
0.6320	0.1971	0.9177	0.6735	-0.0557	0.05849	-0.4320	-101.41	1282.7	662.3	162.0
0.7276	0.1459	0.9251	0.7155	-0.0473	0.04743	-0.3600	-81.04	1319.6	620.8	136.0
0.8208	0.0960	0.9324	0.7593	-0.0363	0.03519	-0.2690	-60.68	1367.3	573.7	104.0
0.9115	0.0474	0.9395	0.8061	-0.0214	0.02029	-0.1600	-36.31	1437.6	515.0	60.0
1.0000	0.0000	0.9461	0.8586	0.0000	0.00000	0.0000	0.00	1465.2	440.7	0.0
		Ν	√, <i>N</i> -Dim	ethylforn	namide (A	A) + benzer	ne(B) + E	DIE (C)		
0.0000	0.5668	0.7958	0.3997	-0.1000	0.17669	-0.4880	-441.50	1219.2	845.3	261.0
0.1186	0.4995	0.8103	0.4506	-0.0917	0.13814	-0.6450	-306.30	1261.7	775.3	208.0
0.2324	0.4350	0.8248	0.4975	-0.0856	0.11174	-0.7330	-217.97	1287.0	732.0	181.0
0.3417	0.3731	0.8394	0.5424	-0.0800	0.09219	-0.7680	-157.51	1301.5	703.3	168.0
0.4467	0.3136	0.8541	0.5858	-0.0742	0.07703	-0.7550	-115.90	1305.3	687.2	167.0
0.5478	0.2563	0.8690	0.6274	-0.0689	0.06644	-0.7070	-92.30	1312.8	667.7	162.0
0.6450	0.2012	0.8840	0.6700	-0.0612	0.05550	-0.6140	-72.20	1326.7	642.7	151.0
0.7386	0.1481	0.8993	0.7161	-0.0487	0.04100	-0.5050	-47.43	1351.0	609.2	131.0
0.8289	0.0970	0.9145	0.7608	-0.0364	0.02957	-0.3430	-33.58	1394.5	562.3	97.0
0.9160	0.0476	0.9302	0.8069	-0.0215	0.01747	-0.1810	-21.97	1450.8	510.8	58.0
1.0000	0.0000	0.9461	0.8586	0.0000	0.00000	0.0000	0.00	1465.2	440.7	0.0
		Ν	V,N-Dim	ethylforn	namide (A	A) + benzer	ne(B) + D	EE (C)		
0.0000	0.4869	0.7902	0.3396	-0.0636	0.16770	-0.5930	-149.83	1231.1	835.0	232.0
0.1036	0.4364	0.8051	0.3936	-0.0568	0.13130	-0.7240	-6.68	1273.2	766.2	180.0
0.2064	0.3864	0.8201	0.4449	-0.0523	0.10686	-0.8030	76.39	1292.9	729.5	160.0
0.3084	0.3367	0.8353	0.4944	-0.0492	0.08974	-0.8420	118.97	1308.8	698.9	146.0
0.4096	0.2875	0.8508	0.5420	-0.0477	0.07860	-0.8510	128.90	1321.0	673.5	137.0
0.5099	0.2386	0.8662	0.5884	-0.0470	0.07088	-0.7940	117.13	1321.3	661.2	141.0
0.6095	0.1901	0.8819	0.6353	-0.0455	0.06354	-0.7110	92.89	1332.0	639.1	135.0
0.7083	0.1420	0.8978	0.6863	-0.0394	0.05125	-0.5970	71.94	1347.9	613.0	125.0
0.8063	0.0943	0.9137	0.7364	-0.0340	0.04178	-0.4260	35.94	1377.1	577.1	105.0
0.9035	0.0470	0.9298	0.7947	-0.0200	0.02321	-0.2290	15.69	1433.5	523.4	67.0
1.0000	0.0000	0.9461	0.8586	0.0000	0.00000	0.0000	0.00	1465.2	440.7	0.0
		N	,N-Dime	ethylform	amide (A) + benzene	e(B) + 2-	ME (C)		
0.0000	0.4932	0.9229	1.0157	-0.0583	0.04851	-0.7550	116.78	1228.2	718.3	172.6
0.1048	0.4415	0.9269	1.0006	-0.0508	0.04393	-0.8290	107.22	1267.4	671.7	137.0
0.2085	0.3903	0.9306	0.9806	-0.0485	0.04400	-0.8770	84.83	1305.2	630.8	107.0
0.3111	0.3397	0.9343	0.9593	-0.0477	0.04532	-0.9170	57.87	1325.7	609.0	96.0
0.4127	0.2896	0.9375	0.9336	-0.0515	0.05114	-0.9120	18.99	1340.8	593.4	91.0
0.5131	0.2401	0.9402	0.9105	-0.0530	0.05459	-0.8690	-14.16	1349.7	583.8	92.0
0.6125	0.1911	0.9427	0.8891	-0.0530	0.05636	-0.8030	-43.84	1360.2	573.4	92.0
0.7109	0.1426	0.9444	0.8743	-0.0466	0.05101	-0.6720	-54.50	1376.2	559.0	88.0
0.8083	0.0946	0.9457	0.8623	-0.0376	0.04229	-0.5090	-57.14	1403.5	536.8	76.0
0.9046	0.0470	0.9462	0.8597	-0.0194	0.02245	-0.2770	-31.15	1460.1	495.7	45.0

Available online at www.shd.org.rs/JSCS/



2010 Copyright (CC) SCS

TABLI	E II. Co	ntinued								
XΔ	XB	ρ_{r^3}	η	$\Delta \eta$	I_{Δ}	$V^{\rm E}$	ΔG^{*E}	<i>u</i>	$K_{\rm s}$	$K_{\rm s}^{\rm E}$
11	Б	g cm ³	mPa s	mPa s		cm ³ mol ⁴	J mol '	m s '	10 ¹² Pa ¹	10 ¹² Pa ¹
		N	,N-Dime	ethylform	amide (A) + benzene	e(B) + 2-	ME(C)		
1.0000	0.0000	0.9461	0.8586	0.0000	0.00000	0.0000	0.00	1465.2	440.7	0.0
		N	,N-Dim	ethylform	namide (A) + benzene	e(B) + 2	EE (C)		
0.0000	0.5354	0.9066	1.1640	-0.0022	0.03794	-0.8320	357.25	1280.9	672.3	123.1
0.1128	0.4750	0.9139	1.1302	-0.0013	0.03793	-1.0870	323.16	1315.6	632.2	95.2
0.2224	0.4163	0.9202	1.0944	-0.0033	0.03962	-1.2200	283.41	1342.6	602.9	77.8
0.3290	0.3592	0.9252	1.0574	-0.0076	0.04265	-1.2330	238.98	1362.6	582.1	68.6
0.4327	0.3037	0.9297	1.0205	-0.0126	0.04571	-1.1800	191.86	1371.6	571.8	69.5
0.5336	0.2497	0.9331	0.9855	-0.0165	0.04707	-1.0320	147.35	1383.5	559.9	68.6
0.6318	0.1971	0.9361	0.9526	-0.0192	0.04651	-0.8560	104.14	1394.6	549.2	68.6
0.7275	0.1459	0.9390	0.9222	-0.0202	0.04333	-0.6680	63.44	1415.4	531.6	61.3
0.8207	0.0960	0.9414	0.8968	-0.0170	0.03456	-0.4490	33.23	1441.3	511.4	51.2
0.9115	0.0474	0.9437	0.8744	-0.0114	0.02160	-0.2200	8.82	1483.6	481.4	31.1
1.0000	0.0000	0.9461	0.8586	0.0000	0.00000	0.0000	0.00	1465.2	440.7	0.0

In order to secure more comparable viscous antagonic results, the so called antagonic interaction index (I_A) , introduced by Howell,³⁷ was taken into account:

$$I_{\rm A} = (\eta_{\rm cal} - \eta_{\rm exp}) / \eta_{\rm cal} \tag{2}$$

1727

The antagonic interaction index (I_A) values at 298.15 K for the ternary mixtures (A) + (B)+ 1,3-DO, 1,4-DO, THF, 1,2-DME, DIE, DEE, 2-ME and 2-EE (C) are plotted against x_A in Fig. 3. It was found that each mixture had a maxi-



Fig. 3. Antagonic Index (I_A) of: **•**) DMF (A) + benzene (B) + 1,3-DO (C); **•**) DMF (A) + benzene (B) + 1,4-DO (C); **•**) DMF (A) + benzene (B) + THF (C); **•**) DMF (A) + benzene (B) + 1,2-DME (C); **•**) DMF (A) + benzene (B) + DIE (C); **•**) DMF (A) + benzene (B) + DEE (C); +) DMF (A) + benzene (B) + 2-ME (C); Δ) DMF (A) + benzene (B) + 2-EE (C) mixtures with mole fraction of DMF (x_A) at 298.15 K.

2010 Copyright (CC) SCS



CHANDA, BANERJEE and NATH ROY

1728

mum at $x_A = 0.0$, which then decreased with increasing x_A . A perusal of Table II shows that the experimentally determined viscosities, η_{exp} for all mixtures for various mole fractions at the experimental temperature are lower than those of their calculated values, η_{calcd} , which demonstrates viscous antagonism in the eight mixtures studied herein. The explanation of this behavior is based on the known phenomenon of molecular dissociation, as a consequence of the weak-ening of the non-covalent bonding formed between the molecules, causing a decrease in the size of the molecular package, which logically implies an increase in I_A .^{1,12} The maxima observed indicate strong specific interaction between the unlike molecules, which is predominated by non-covalent interaction. Thus, the molecular package increases gradually with the addition of DMF to the mixtures, which implies a decrease in I_A .

The excess molar volumes, V^{E} were calculated from the density data according to the following equation:³⁹

$$V^{\rm E} = \sum_{i=1}^{3} x_i M_i \left(\frac{1}{\rho - 1} \rho_i \right)$$
(3)

where M_i , and ρ_i are the molar mass and density of the pure components, respectively, and ρ is the density of the mixture. For the ternary systems, in general, the V^E values were found to be negative over the whole composition range under study at 298.15 K, which is depicted as a representative plot in Fig. 4 as a function of the mole fraction of both DMF (x_A) and benzene (x_B). The V^E values for the eight ternary mixtures under examination are presented in Fig. 5. The values at first decreases to minima and then increase with increasing x_A . The eight ternary mixtures show minima at the same point, *i.e.*, at $x_A = 0.3$. The trend is: (A)+(B)+1,3-DO > 1,4-DO > THF >1,2-DME > DIE > DEE > 2-ME > 2-EE



Fig. 4. 3D mesh plots of excess molar volumes (V^{E}) of: DMF (A) + benzene (B) + 1,3-DO (C) mixtures with mole fraction of DMF (x_{A}) and benzene (x_{B}) at 298.15 K.

The negative values of V^{E} indicate the presence of strong molecular interaction between the components of the mixture. Volume changes for a mixed system result from changes in the free volume of the liquids, since the bond



lengths and bond distances in the molecules themselves do not change. The optimum packing condition is directly related to differences in molecular sizes and intermolecular attractions, in particular when hydrogen bonding occurs between unlike molecules creating association complexes, as well as being effected by the breaking of interactions between like molecules.³² Several effects contribute to the value of V^{E} , such as: dipolar interaction, interstitial accommodation and possible hydrogen bonded interactions between unlike molecules.⁴⁰ The actual volume change would, therefore, depend on the relative strength of these three effects. Similar results have been reported earlier.^{41,42}



Fig. 5. Excess molar volumes (V^E) of: •) DMF (A) + benzene (B) + 1,3-DO (C); •) DMF (A) + benzene (B) + 1,4-DO (C); •) DMF (A) + benzene (B) + THF (C); •) DMF (A) + benzene (B) + 1,2-DME (C); □) DMF (A) + benzene (B) + DIE (C); ○) DMF (A) + benzene (B) + DEE (C); (+), DMF (A) + benzene (B) + 2-ME (C); △) DMF (A) + benzene (B) + 2-EE (C) mixtures with mole fraction of DMF (x_A) at 298.15 K.

Based on the theory of absolute reaction rates,³⁸ the excess Gibbs energy, ΔG^{*E} , of viscous flow for a ternary system was calculated from:

$$\Delta G^{*E} = RT \ln(\eta M / \rho) - RT \sum_{i=1}^{3} x_i \ln(\eta_i M_i / \rho_i)$$
(4)

where *M* and *M_i* are the molar mass of the mixture and of the pure components i. According to the literature, positive ΔG^{*E} values indicate specific interactions, while negative values indicate the dominance of dispersion forces.^{43,44} From the ΔG^{*E} values recorded in Table II, it can be seen that for all the ternary mixtures, these values are negative or positive keeping similarity with the $\Delta \eta$ values and thereby supports the conclusion drawn from the *V*^E and $\Delta \eta$ considerations.



CHANDA, BANERJEE and NATH ROY

Table II contains the sound velocity (*u*), the isentropic compressibility (K_s) and excess isentropic compressibility (K_s^E) data for the observed mixtures, which were calculated using the following equations: ⁴⁵

$$K_{\rm s} = \left(u^2 \rho_{\rm exp}\right)^{-1} \tag{5}$$

$$K_{s}^{E} = K_{s} - \sum_{i=1}^{3} x_{i} K_{s,i}$$
(6)

where, $K_{s,i}$ gives the isentropic compressibility for the pure components of the mixture.

Figure 6 predicts the curves for the ternary mixtures of K_s^E . The values are positive in all the cases and decreases as the mole fraction of DMF increases. There is a parallel in the qualitative behavior of the K_s^E and V^E curves. The K_s^E values follow the sequence:

(A) + (B) + 1, 3 - DO > 1, 4 - DO > THF > 1, 2 - DME > DIE > DEE > 2 - ME > 2 - EE



Fig. 6. Excess isentropic compressibility (Ks^E) of: **•**) DMF (A) + benzene (B) + 1,3-DO (C); **•**) DMF (A) + benzene (B) + 1,4-DO (C); •) DMF (A) + benzene (B) + THF (C); •) DMF (A) + benzene (B) + 1,2-DME (C); \Box) DMF (A) + benzene (B) + DIE (C); \circ) DMF (A) + benzene (B) + DEE (C); (+), DMF (A) + benzene (B) + 2-ME (C); Δ) DMF (A) + benzene (B) + 2-EE (C) mixtures with mole fraction of DMF (X_A) at 298.15 K.

The positive K_s^E values are due to the breaking of interactions and the corresponding disruption of molecular order in the pure components.²⁴ The donor–acceptor interaction between the component molecule plays an important part for the mixtures containing open chain ethers, especially those having a hydroxyl group, where there is strong specific interaction between the component molecules leading to lower value of K_s^E . Interactions between the molecules of DMF,

Available online at www.shd.org.rs/JSCS/



benzene or ethers are broken in the mixing process; the breaking leads to positive K_s^E values for the mixture containing cyclic ethers as compared to the open chain ethers. Similar results were reported earlier by some authors.^{24,32}

CONCLUSIONS

After a thorough study of the behavior of ethers in *N*,*N*-dimethylformamide + benzene mixtures, a clear idea about the type and amount of molecular interactions between them was obtained and an idea about antagonism was given. The similarity in the working formula of the antagonic interaction index and viscosity deviation would probably indicate that the two properties are similar but a close comparison between these two parameters gave a clear distinction.

Acknowledgments. The authors are thankful to the Departmental Special Assistance Scheme under the University Grants Commission, New Delhi (No. 540/6/DRS/2007, SAP-1) and Department of Chemistry, North Bengal University for the instrumental and financial assistance.

ИЗВОД

ИСПИТИВАЊЕ ВИСКОЗНОСТИ, ДОПУНСКЕ МОЛАРНЕ ЗАПРЕМИНЕ, ПРОМЕНЕ ВИСКОЗНОСТИ И ИЗЕНТРОПСКЕ КОМПРЕСИБИЛНОСТИ ТЕРНЕРНИХ СИСТЕМА *N,N*-ДИМЕТИЛФОРМАМИДА, БЕНЗЕНА И ЕТАРА НА 298,15 К

RIJU CHANDA, ASHIS BANERJEE и MAHENDRA NATH ROY

Department of Chemistry, North Bengal University, Darjeeling-734013, India

Извршена су мерења густина (ρ) и вискозности (η) тернерних смеша *N*,*N*-диметилформамид + бензен + етар, у функцији састава и на температури од 298,15 К. Из експерименталних података су израчунате вредности допунске моларне запремине (V^E), промене вискозности ($\Delta \eta$), интеракциони индекс (I_A) и Гибсову слободну енергију активације (ΔG^{*E}). Такође, на температури на којој су извршени експерименти, мерене су и брзине звука и израчунате допунске изентропске компресибилности (K_s^E). Добијени резултати су анализирани у функцији молекулског паковања и специфичних интеракција проузрокаваних присуством водоничних веза.

(Примљено 6. августа 2009, ревидирано 27. августа 2010)

REFERENCES

- 1. J. V. Herraez, R. Belda, J. Solution Chem. 33 (2004) 117
- 2. R. Belda, J. V. Herraez, O. Diez, Phys. Chem. Liquids. 42 (2004) 467
- 3. S. L. Oswal, H. S. Desai, Fluid Phase Equilib. 161 (1999) 191
- 4. S. L. Oswal, H. S. Desai, Fluid Phase Equilib. 186 (2001) 81
- 5. P. Dimitrios, P. Constantinos, J. Chem. Eng. Data 40 (1995) 202
- 6. G. Czechowski, B. Zywucki, J. Jadzyn, J. Chem. Eng. Data 33 (1998) 55
- 7. R. Belda, J. V. Herraez, O. Diez, Phys. Chem. Liquids 43 (2005) 91
- 8. M. N. Roy, D. K. Hazra. North Bengal Univ. Rev. 8 (1997) 54
- C. R. Reid, B. E. Poling, *The Properties of Gases and Liquids.*, Ch. 1, McGraw-Hill, New York, 1998



CHANDA, BANERJEE and NATH ROY

- 10. C. Lafuente, B. Ginar, A. Villares, I. Gascon, P. Cea, Int. J. Thermophys. 25 (2004) 1735
- 11. P. S. Nikam, L. N. Shirsat, M. Hasan, J. Ind. Chem. Soc. 77 (2000) 244
- 12. M. N. Roy, A. Choudhury, A. Sinha, J. Teach. Res. Chem. 11 (2004) 12
- 13. D. K. Hazra, M. N. Roy, B. Das, Ind. J. Chem. Technol. 1 (1994) 93
- 14. M. N. Roy, A. Jha, R. Dey, J. Chem. Eng. Data 46 (2001)1327
- 15. M. N. Roy, A. Sinha, B. Sinha, J. Sol. Chem. 34 (2005) 1319

1732

- 16. M. N. Roy, S. R. Choudhury, A. Jha, J. Ind. Chem. Soc. 79 (2002) 623
- 17. M. N. Roy, A. Jha, A. Choudhury, J. Chem. Eng. Data 49 (2004) 291
- 18. M. N. Roy, B. B. Gurung, A. Choudhury, J. Ind. Chem. Soc. 81 (2004)1
- 19. D. D. Perrin, W. L. F. Armarego. *Purification of Laboratory Chemicals*, 3rd ed., Pergamon, Oxford, 1988
- 20. A. Vogel, Practical Organic Chemistry, 4th ed., Longman, London, 1978
- J. A. Riddick, W. B. Bunger, T. K. Sokano, Organic Solvents: Physical Properties and Methods of Purifications, Vol. 2, 4th ed., Wiley-Interscience, New York, 1986
- 22. S. J. Kharat, P. S. Nikam, J. Chem. Eng. Data 48 (2003) 1202
- 23. H. Casas, L. Segade, C. Franjo, E. Jiménez, J. Chem. Eng. Data 43 (1998) 756
- 24. T. M. Aminabhavi, B. Gopalkrishna, J. Chem. Eng. Data 40 (1995) 856
- A. K. Covington, T. Dickinson, *Physical Chemistry of Organic Solvent Systems*, Plenum, New York, 1973
- 26. M. N. Roy, A. Sinha, B. Sinha, J. Solution Chem. 34 (2005) 1319
- 27. I. Johnson, M. Kalidoss, R. Srinivasamoorthy, J. Chem. Eng. Data 47 (2002) 1388
- 28. TRC Tables, Selected Values of Properties of Chemical Compounds, Thermodynamic Research Center, A & M University, College Station, TX, 1974
- 29. T. M. Aminabhavi, V. B. Patil, J. Chem. Eng. Data 43 (1998) 497
- 30. D. R. Lide, CSIR Handbook of Chemistry and Physics, 7th ed., 1990-1991
- 31. M. N. Roy, B. Sinha, J. Mol. Liq. 133 (2007) 89
- 32. M. N. Roy, M. Das, Phys. Chem. Liq. 44 (2006) 663
- 33. I. Gascón, S. Martín, P. Cea, M. C. López, F. M. Royo, J. Sol. Chem. 31 (2002) 905
- 34. D. K. Hazra, B. Das, J. Phys. Chem. 99 (1995) 269
- 35. J. Barthel, R. Neueder, H. Roch, J. Chem. Eng. Data 45 (2000) 1007
- 36. M. N. Roy, A. Jha, A. Choudhury, J. Chem. Eng. Data 49 (2004) 291
- 37. N. K. Howell, in Proceedings of the 7th International Conference, Wales, 1993
- 38. S. Glasstone, K. J. Laidler, H. Eyring, *The Theory of Rate Process*, McGraw-Hill, New York, 1941, p. 514
- B. Djordjević, I. Radović, M. Kijevčanin, A. Tasić, S. Šerbanović, J. Serb. Chem. Soc. 74 (2009) 477
- 40. P. S. Nikam, S. J. Kharat, J. Chem. Eng. Data 50 (2005) 455
- 41. P. Brocos, E. Calvo, A. Pineiro, R. Bravo, A. Amigo, J. Chem. Eng. Data 44 (1999) 1341
- 42. A. Amigo, R. Bravo, M. Pintos, J. Chem. Eng. Data 38 (1993) 141
- 43. T. M. Reed, T. E. Taylor, J. Phys. Chem. 63 (1959) 58
- 44. R. Meyer, M. Meyer, J. Metzer, A. Peneloux, Chem. Phys. 62 (1971) 406
- 45. C. Lafuente, B. Giner, A. Villares, I. Gascon, P. Cea, Int. J. Thermophys. 25 (2004) 1735.







J. Serb. Chem. Soc. 75 (12) 1733–1741 (2010) JSCS-4092 JSCS-info@shd.org.rs • www.shd.org.rs/JSCS UDC 669.3+669.187.25:504.75:615.9 Original scientific paper

The possibilities of the utilization of the polymetallic concentrate Čoka Marin

LIDIJA D. GOMIDŽELOVIĆ*#, EMINA D. POŽEGA# and VLASTIMIR K. TRUJIĆ

Mining and Metallurgy Institute, Zeleni bulevar 33, 19210 Bor, Serbia

(Received 14 July 2009, revised 30 June 2010)

Abstract: This paper presents the results of calculations of the composition of composite concentrates used as the charge in the Copper Smelter in Bor, from the aspect of the behavior of zinc, lead, arsenic, cadmium and mercury. These elements have extremely harmful effects on the environment and human health; hence it is crucial to comply with legal values of their emission into the environment.

Keywords: copper smelting; impurities; concentrate; ecology; distribution.

INTRODUCTION

Zinc, lead, arsenic, cadmium and mercury are toxic for most living organisms on the Earth. Even very small concentrations of these elements (expressed in ppm) have serious toxic effects.¹ The behavior and distribution of harmful elements in the production process in the Copper Smelter in Bor, have been observed for many years by the experts of Copper Institute (now Mining and Metallurgy Institute Bor).¹ These elements are major pollutants in the environment and have negative effect on the quality of the produced copper and sulfuric acid, which necessitates the continuous monitoring of their content in the starting raw materials. Based on previous studies in the Copper Smelter from the reverberatory furnace, with the gas phase, more than 50 % arsenic, 40–50 % lead and 41 % zinc present in starting charge are emitted into the atmosphere, while the rest is deposited in the slag.² During smelter treatment of concentrate, 48.7 % of the mercury is emitted with gases during roasting, and 47.3 % into the gases during smelting.²

Marin with a lot of impurities presents a demanding charge that could be treated using the existing technology in the reverberatory furnace only if the allowed limits of any harmful elements in starting mixture are not exceed. In this way, the risk of environmental accidents would be reduced to a minimum.

1733



^{*} Corresponding author. E-mail: lgomidzelovic@yahoo.com

[#] Serbian Chemical Society member.

doi: 10.2298/JSC090714085G

GOMIDŽELOVIĆ, POŽEGA and TRUJIĆ

The increased industrialization has been followed all over the world by the extraction and redistribution of mineral substances from their natural deposits. Passing through the processes of treatment and use, the mineral raw materials arrive *via* waste water, gases, dust and waste dumps into the air, water and land, and, thus, indirectly into the food chain.³

Lead is a typical accumulative poison. It causes the prevention of hemoglobin synthesis, neurological problems (aggressive and destructive behavior), kidney damage and even permanent brain damage.⁴

Zinc is one of the micro-elements necessary for the proper functioning of the body functioning, but in excessive doses can lead to problems in growth and reproduction.¹

Arsenic, a metalloid that can enter into the body through the lungs and gastrointestinal tract, has negative influence on the process of protein coagulation, and could form complex compounds with co-enzymes.⁵

Cadmium is also an accumulative toxin that adversely affects important enzymes; causes bone disease and kidney damage. Inhalation of dust and gases containing cadmium leads to lung failure due to the accumulation of water in them. $^{6-8}$

Mercury enters into the body by ingestion and inhalation and transfers *via* the blood to the brain where could pass through the blood–brain barrier and causes insomnia, depression and irritability. It also leads to kidney damage.⁹ It is only temporarily deposited in the body and a large part is eliminated through the digestive system.

The sampling method for copper concentrates is defined by the SRPS Standard BG-3:451.

The technological analysis of a concentrate includes the determination of content the required elements for process optimization and technological process management, while the environmental analysis involves the determination of the contents of Pb, Zn, As, Sb, Cd, Se, Hg and Cl, which are used to perform an environmental assessment of the concentrate.

Lead and zinc are usually present in the copper ore in the form of PbS and ZnS, arsenic is present in the form of Cu_3AsS_4 , FeAsS and Cu_3AsS_4 minerals,¹⁰ while mercury is present in the form of HgS and cadmium in the form of CdS.

The associated elements in a copper concentrate are distributed during smelting according to their physico-chemical properties and are concentrated in the intermediates and products of the pyrometallurgical treatment. The behavior of each element depends on several factors, the most important of which are: the form in which they are present in the raw materials, the technological parameters of the applied process, their inter-relationship with other elements, the concentration of certain elements, *etc.* The parameters of the distribution each element can only be reliably determined by raw material treatment using the specific technology.¹¹

Available online at www.shd.org.rs/JSCS/

A block diagram of copper concentrate treatment, including treatment of the off-gases and sulfuric acid production, is given in Fig. 1.



Fig. 1. Block diagram of copper concentrate treatment with pollution sources (1 - roasting; 2 - smelting; 3 - converting; 4 - flame refinement).

The distributions of zinc, lead and arsenic in the output products (anode copper, slag, sludge and water, gas) were calculated based on their emission in the period from 1991 to 1997.¹²

For the cadmium and mercury distribution, there are no available data about their emission, hence a critical analysis of the available data on their distribution through the output products of the copper concentrate treatment^{2,3,13} was performed. The obtained distributions of Zn, Pb, As, Cd and Hg, determined as given above, are presented in Table I.

Phase	Pb	Zn	Hg	Cd	As
Batch	100	100	100	100	100
Anode copper	1.95	0.05	0	1.37	2.44
Slag	63.76	54.39	5	8.45	12.06
Sludge and water	4.73	4.49	31.6	5.18	34.31
Gas	29.63	41.08	63.4	85	51.19

TABLE I. Distribution of Pb, Zn, Hg, Cd and As in the reverberatory furnace in %

RESULTS AND DISCUSSION

Monitoring of the contents of impurities in the raw mixture

Using the polymetallic ore from the deposit "Čoka Marin", the collective concentrate (7.92 % Cu, 26 g t⁻¹ Au, 127.2 g t⁻¹ Ag, 3.92 % Zn, 1.1 % Pb, 0.98 % As, 0.0011 % Cd, 12.1 g t⁻¹ Hg) is produced. This concentrate should be



GOMIDŽELOVIĆ, POŽEGA and TRUJIĆ

1736

combined with the already used concentrates for the preparation the mixture in reverberatory furnace (the composition in relation to the contents of the impurities is given in Table II), in such a way that the composition of obtained concentrate mixture meets the allowed limits for the content of zinc, lead, arsenic, cadmium and mercury. The compositions of used imported concentrates (1-ASSAREL and 2-ELATZITE) are given in Table III. The allowed limits for the contents of zinc, lead, arsenic, cadmium and mercury, estimated based on the prescribed contents of these metals in the output gases of Copper Smelter, are listed in Table IV, from which it is possible to conclude that these impurities in the composite concentrate have to be significantly lower than in the concentrates delivered in the reverberatory furnace and used for the preparation of the mixture.

TABLE II. Input contents of impurities in the copper concentrate formed from the mixture (the amount of concentrate Čoka Marin was varied within the limits of 0-10 wt. %)

Concentrate	Weight (dmt)	Content, %						
Concentrate	weight (unit) –	Zn	Pb	As	Cd	Hg		
Bor	36050	0.68	0.15	0.19	0.0025	0.000104		
Krivelj	86265	0.06	0.009	0.012	0.0027	0.000018		
Majdanpek	47510	0.52	0.16	0.01	0.0026	0.000023		
Import	30000	0.03	0.2	0.02	0.0025	0.00008		
Čoka Marin	_	3.92	1.1	0.98	0.011	0.00121		

Concentrate -			Content, %		
	Zn	Pb	As	Cd	Hg
1	0.04	0.4	0.009	0.0025	0.00006
2	0.02	0.03	0.03	0.0025	0.0001

TABLE III Composition of the used import concentrates (ratio 1:1)

TABLE IV Allowed limits of the contents of heavy metals (Zn, Pb, As, Cd and Hg; internal limits applied in the Copper Smelter in Bor, based on the allowed values of these elements in the output gases)

Element	Individual concentrate, %	Composite concentrate, %
Zn+Pb	3	1.5
As	0.2	0.1
Cd	0.01	0.0025
Hg	0.0005	0.0002

Using the percentage amounts and the weight of the concentrates from Table II, the calculation was realized based on the obtained data for the contents of Zn, As, Pb, Cd and Hg in the domestic and imported concentrates. A detailed analysis of the results is shown in Figs. 2a–2d.

The changes in the contents of zinc and lead in the concentrate mixture containing varying amounts of Čoka Marin concentrate, within the limits 0-10 %, are presented in Fig. 2a. Analyzing this figure, it is possible to conclude that the

combined contents of these two metals did not exceed the allowed limit of 1.5 % (Zn+Pb), even when the maximum 10 % of Čoka Marin concentrate was present in the mixture.



Fig. 2. Change of impurities content in the concentrate mixture depending on the varying contents of Čoka Marin concentrate (a – Zn+Pb; b – As; c – Cd and d – Hg); *Ccmb* – percentage of concentrate Čoka Marin in batch; C_{Zn+Pb} – percentage of zinc and lead in concentrate mixture; C_{As} – percentage of arsenic in concentrate mixture; C_{Cd} – percentage of cadmium in concentrate mixture; C_{Hg} – percentage of mercury in concentrate mixture.

The change in the content of arsenic in the concentrate mixture containing varying amounts of Čoka Marin concentrate, within the limits 0-10 % is shown in Fig. 2b. Analyzing this figure, it is possible to conclude that the content of this element exceeds the prescribed limit of 0.1 % when the amount of Čoka Marin concentrate in the mixture exceeded about 6 %.

The change in the content of cadmium in the concentrate mixture containing varying amounts of Čoka Marin concentrate, within the limits 0-10 %, is presented in Fig. 2c. Analyzing this figure, it is possible to conclude that content of this metal is above the allowed limit of 0.0025 % Cd when more than 2 % of



Čoka Marin concentrate was added to the mixture. Concentrate mixtures containing less than 2.5 % Čoka Marin concentrate met the given ecological conditions for their use.

The change of the content mercury in the concentrate mixture containing varying amounts of Čoka Marin concentrate, within the limits 0-10 %, is shown in Fig. 2d. Analyzing this figure, it is possible to conclude that content of this metal in the mixture did not exceed the allowed limit of 0.0002 % Hg, even when the amount of Čoka Marin concentrate was the maximum of 10 %.

Monitoring the content of impurities through the anode copper and pollution sources

Taking into account the data listed in Table I, which considers only the content of impurities in the mixture, the original calculations are extended. Additionally, the obtained results included a distribution of impurities between the anode copper and the by-products (slag, sludge and water and gas) from the smelting of the copper concentrate, which cause different types of pollution. These are shown in Fig. 3.

The distribution of zinc between the anode copper and the by-products (slag, sludge and water, gas) obtained during the processing of mixtures composed of the copper concentrates listed in Table II is presented in Fig. 3a. Analyzing this Figure, it is possible to conclude that the largest amount of zinc from the process is contained in the slag and, in second place, in the gas phase. The anode copper contains a negligible quantity of zinc.

The distribution of arsenic between the anode copper and the by-products (slag, sludge and water, gas) obtained during the processing of mixtures composed of the copper concentrates listed in Table II is presented in Fig. 3b. Maximum amount of arsenic from process exits in the gas phase and the rest mostly in waste water, while the slag bonds a small amount of this element. The anode copper contains 2.44 % As (Table I).

The distribution of cadmium between the anode copper and the by-products (slag, sludge and water, gas) obtained during the processing of mixtures composed of the copper concentrates listed in Table II is shown in Fig. 3c. The largest part of cadmium from the process exits in the gas phase, while the other by products contain smaller amounts of this element; about 1 %. remains in the anode copper.

The distribution of mercury between the anode copper and the by-products (slag, sludge and water, gas) obtained during the processing of mixtures composed of the copper concentrates listed in Table II is shown in Fig. 3d. Mercury is completely removed from the anode copper and the largest part of this element exits with the gases and waste water.

Available online at www.shd.org.rs/JSCS/



POLYMETALLIC CONCENTRATE ČOKA MARIN

1739



The distribution of lead between the anode copper and the by-products (slag, sludge and water, gas) obtained during the processing of mixtures composed of the copper concentrates listed in Table II is presented in Fig. 3e. For the most

2010 Copyright (CC) SCS



GOMIDŽELOVIĆ, POŽEGA and TRUJIĆ

part, lead remains in the slag, but a significant portion exits in the gas phase, while about 2 % is contained in the anode copper.

Based on the calculation results in the first part of this study, it can be seen that the content of cadmium is the main problem, if the set limits for the contents of impurities are to be met. The use of 0 to 10 % Čoka Marin concentrate in the mixture is not limited by the emission of zinc, lead or mercury into the atmosphere.

Arsenic allows the use of Čoka Marin concentrate up to about 6 % in the mixture.

The cadmium in the Čoka Marin concentrate allows the participation of this concentrate in the mixture only up to about 2.5 %, which limits its practical use and does not allow the high profitability its gold content to be expressed.

The use of Čoka Marin concentrate in the existing process is also limited by the cumulative increase of the content of impurities in the atmosphere during the exploitation. This indicates that the use of this concentrate in other modern processes may be possible under strict control.

Based on the results obtained in this part of the work, the distribution of the impurities (As, Pb, Zn, Cd and Hg) can be monitored through the anode copper and the by-products (slag, sludge and water, gas).

Based on the obtained results, it could be concluded that significant amounts of Zn, As, Cd, Hg and Pb exits with gases and for this reason, special attention should be to paid to the process of capture and purification of the gases and also to the provision of a hermetic gas line.

The waste water carries significant amounts of As and Hg, which necessitates the employment of suitable procedures for its purification.

With the slag, larger quantities of Zn and Pb are removed that necessitates the determination of the form in which these elements exist and that regulations are adhered to for the postponement of such material, to reduce soil degradation.

CONCLUSIONS

Taking into account all the mentioned influences of zinc, lead, arsenic, cadmium and mercury and their content in possible mixtures for smelting in the reverberatory furnace, such a solution cannot be recommended. For the health reasons, it would be better to export this type of concentrate to allow it to be smelted now using appropriate contemporary technology, or to wait for the installation of new technology in the RTB Bor Smelter Plant.



POLYMETALLIC CONCENTRATE ČOKA MARIN

ИЗВОД

МОГУЋНОСТ КОРИШЋЕЊА ПОЛИМЕТАЛИЧНОГ КОНЦЕНТРАТА ЧОКА МАРИН СА АСПЕКТА УТИЦАЈА САДРЖАЈА ШТЕТНИХ ЕЛЕМЕНАТА НА ОКОЛИНУ

ЛИДИЈА Д. ГОМИЏЕЛОВИЋ, ЕМИНА Д. ПОЖЕГА и ВЛАСТИМИР К. ТРУЈИЋ

Инсшишуш за рударсшво и мешалургију, Зелени булевар 33, 19210 Бор

У раду су изложени резултати прорачуна састава композитних концентрата који се користе као шаржа у Топионици бакра у Бору са аспекта понашања штетних елемената: цинка, олова, арсена, кадмијума и живе. Ови елементи показују веома штетно дејство на околину и здравље људи, тако да је од изузетне важности да се испоштују законом порписане вредности њихове емисије у околину.

(Примљено 14. јула 2009, ревидирано 30. јуна 2010)

REFERENCES

- M. D. Dimitrijević, A. I. Kostov, V. M. Tasić, N. M. Milošević, J. Hazard. Mater. 164 (2009) 892
- N. T. Mitevska, Ž. D. Živković, in *Proceedings of Sulfide Smelting*, Seattle, NY, 2002, p. 547
- D. M. Vučurović, Č. N. Knežević, in *Copper Metallurgy*, Copper Institute, Bor, 2000, p. 250 (in Serbian)
- 4. R. R. Jones, Lancet 16 (1989) 669
- 5. J. Maslowska, M. Ahmadi, Przemysl Spozywczy 45 (1991) 201 (in Polish)
- 6. F. Gazza, Ann. della Facolta di Med. Vet. 10 (1990) 171
- J. P. Groten, E. J. Sinkeldam, J. B. Luten, P. J. Van Bladeren, Food Chem. Toxicol. 28 (1990) 435
- 8. A. O. Igwegbe, H. M. Belhaj, T. M. Hassan, A. S. Gibali, J. Food Safety 13 (1992) 7
- 9. W. L. Chang, Biomed. Environ. Sci. Res. 3 (1990) 125
- 10. N. T. Mitevska, Ž. D. Živković, J. Min. Metall. B 38 (2002) 93
- 11. C. R. Fountain, M. D. Coulter, J. S. Edwards, *Minor Element Distribution in the Copper Isasmelt Process, Copper, 91*, Volume IV, Pegamon Press, New York, 1991, p. 359
- V. M. Tasić, D. R. Milivojević, N. M. Milošević, D. V. Karabašević, in *Proceedings of 36th IOC on Mining and Metallurgy*, (2004), Bor Lake, Serbia, 2004, p. 371 (in Serbian)
- I. N. Mihajlović, N. D. Štrbac, Ž. D. Živkovic, R. M. Kovačević, M. M. Šteharnik, *Miner*. Eng. 20 (2007) 26
- 14. E. D. Požega, L. D. Gomidželović, V. K. Trujić, M. M. Ćirković, in *Proceedings of Ecological Truth*, (2009), Kladovo, Serbia, (2009), p. 108 (in Serbian).







JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. 75 (12) 1743–1754 (2010)

Contents of Volume 75

NUMBER 1

Organic Chemistry				
<i>R. Grigg, S. Husinec</i> and <i>V. Savić</i> : Stereoselective cyclo-addition reactions of azomethine ylides catalysed by <i>in situ</i> generated Ag(I)/bisphosphine complexes	1			
<i>M. Abass, M. M. Ismail, W. R. Abdel-Monem</i> and <i>A. S. Mayas</i> : Substituted pyridopyrimidi- nones. Part IV. 2-Chloro-4 <i>H</i> -pyrido[1,2- <i>a</i>]pyrimidin-4-one as a synthone of some new heterotricycles	11			
Biochemistry and Biotechnology				
<i>L. Burazer, K. Milovanović, T. Ćirković-Veličković</i> and <i>M. Gavrović-Jankulović</i> : Stability evaluation of house dust mite vaccines for sublingual immunotherapy	19			
<i>M. Pavel, M. Ristić</i> and <i>T. Stević</i> : Essential oils of <i>Thymus pulegioides</i> and <i>Thymus glabrescens</i> from Romania: chemical composition and antimicrobial activity	27			
T. Stević, K. Šavikin, M. Ristić, G. Zdunić, T. Janković, D. Krivokuća-Đokić and T. Vulić: Composition and antimicrobial activity of the essential oil of the leaves of black currant (<i>Ribes nigrum</i> L.) cultivar Čačanska crna	35			
<i>V. Ivanova, M. Stefova</i> and <i>F. Chinnici</i> : Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods	45			
Inorganic Chemistry				
M. S. S. Babu, P. G. Krishna, K. H. Reddy and G. H. Philip: Synthesis, characterization and DNA cleavage activity of nickel(II) adducts with aromatic heterocyclic bases	61			
<i>S. Sharma</i> , <i>D. Dalwadi</i> and <i>M. Neog</i> : A study of the formation constants of ternary and quaternary complexes of some bivalent transition metals				
Theoretical Chemistry				
<i>B. Furtula, I. Gutman, S. Jeremić</i> and <i>S. Radenković</i> : Effect of a ring on the cyclic conjugation in another ring: applications to acenaphthylene-type polycyclic conjugated molecules.	83			
A. Moghani, S. N. Sedeh and M. R. Sorouhesh: The Fujita combinatorial enumeration for the non-rigid group of 2,4-dimethylbenzene	91			
Chemical Engineering				
X. Ling, D. Lu, J. Wang, M. Liang, S. Zhang, W. Ren, J. Chen and P. Ouyang: Investi- gation of the kinetics and mechanism of the glycerol chlorination reaction using gas chromatography-mass spectrometry	101			
Environmental				
<i>S. Murko, R. Milačič, M. Veber</i> and <i>J. Ščančar</i> : Determination of Cd, Pb and As in sediments of the Sava River by electrothermal atomic absorption spectrometry	113			

1743



JOURNAL OF THE SERBIAN CHEMICAL SOCIETY

M. Karajić, A. Lapanje, J. Razinger, A. Zrimec and D. Vrhovšek: The effect of the appli-	
cation of halotolerant microorganisms on the efficiency of a pilot-scale constructed	
wetland for saline wastewater treatment	129
2009 List of referees	143

NUMBER 2

Organic Chemistry	
<i>B. Jović</i> , <i>A. Nikolić</i> , <i>E. Davidović</i> and <i>S. Petrović</i> : N–H···O hydrogen bonding. An FT-IR, NIR study of <i>N</i> -methylformamide–ether systems	157
M. A. Hussain, D. Shahwar, M. N. Tahir, M. Sher, M. N. Hassan and Z. Afzal: An efficient acetylation of dextran using <i>in situ</i> activated acetic anhydride with iodine	165
Biochemistry and Biotechnology	
K. Pithawala, N. Mishra and A. Bahadur: Immobilization of urease in alginate, paraffin and lac	175
V. Leskovac, S. Trivić, D. Peričin, M. Popović and J. Kandrač: Binding of coenzymes to yeast alcohol dehydrogenase	185
J. Filipović, N. Filipović and V. Filipović: The effects of commercial fibres on frozen bread dough	195
D. N. Olennikov, L. V. Dudareva, S. N. Osipenko and T. A. Penzina: Chemical compo- sition of <i>Rhododendron aureum</i> (gold rosebay) essential oil from Pribaikal'e (Rus- sian Federation)	209
Inorganic Chemistry	
D. Singh, K. Kumar, R. Kumar and J. Singh: Template synthesis and characterization of biologically active transition metal complexes comprising 14-membered tetraaza-macrocyclic ligand	217
A. Kriza, L. V. Ababei, N. Cioatera, I. Rău and N. Stănică: Synthesis and structural stu- dies of complexes of Cu, Co, Ni and Zn with isonicotinic acid hydrazide and isoni- cotinic acid (1-naphthylmethylene)hydrazide	229
Theoretical Chemistry	
S. Špirtović-Halilović and D. Završnik: Computer programs for calculating pK_a : a comparative study for 3-(3-(2-nitrophenyl)prop-2-enoyl)-2H-1-benzopyran-2-one	243
Physical Chemistry	
<i>K. Wang</i> and <i>P. Zhong</i> : A kinetic study of CO oxidation over the perovskite-like oxide LaSrNiO ₄	249
J. Xu, G. Liang, L. Wang, W. Xu, W. Cui, H. Zhang and Z. Li: DFT Studies on the elec- tronic structures of indoline dyes for dye-sensitized solar cells	259
Electrochemistry	
Z. J. Lin, X. B. Hu, Y. J. Huai and Z. H. Deng: Preparation and characterization of a new carbonaceous material for electrochemical systems	271
Thermodynamics	
I. R. Radović, M. Lj. Kijevčanin, A. Ž. Tasić, B. D. Djordjević and S. P. Šerbanović: De- rived thermodynamic properties of alcohol + cyclohexylamine mixtures	283
Errata	295



VOLUME 74: CONTENTS

1745

NUMBER 3

Editor's Note	297
Organic Chemistry	
H. Ghasemnejad-Bosra, M. Faraje, S. Habibzadeh and F. Ramzanian-Lehmali: An efficient one-pot synthesis of highly substituted furans catalyzed by N-bromosuccinimide	299
A. Hasaninejad, M. A. Zolfigol, G. Chehardoli and M. Mokhlesi: Molybdatophosphoric acid as an efficient catalyst for the catalytic and chemoselective oxidation of sulfides to sulfoxides using urea hydrogen peroxide as a commercially available oxidant	307
Biochemistry and Biotechnology	
<i>A. Divac, B. Tomić</i> and <i>J. Kušić</i> : The role of adenosine triphosphate in the function of human origin recognition complex 4 protein	317
V. D. Dragičević, S. D. Sredojević and M. B. Spasić: Introduction of the interdependence between the glutathione half-cell reduction potential and thermodynamic parame- ters during accelerated aging of maize seeds	323
<i>M. Voicescu, R. Ion</i> and <i>A. Meghea</i> : Evaluation of the oxidative activity of some free base porphyrins by a chemiluminescence method	333
<i>R. S. Verma, L. U. Rahman, C. S. Chanotiya, R. K. Verma, A. Chauhan, A. Yadav, A. Singh</i> and <i>A. K. Yadav</i> : Essential oil composition of <i>Lavandula angustifolia</i> Mill. cultivated in the mid hills of Uttarakhand, India (Short communication)	343
Inorganic Chemistry	
A. S. Munde, A. N. Jagdale, S. M. Jadhav and T. K. Chondhekar: Synthesis, characteri- zation and thermal study of some transition metal complexes of an asymmetrical tetradentate Schiff base ligand	349
Theoretical Chemistry	
A. R. Ashrafi and M. Ghorbani: Enumeration of a class of IPR hetero-fullerenes	361
Polymers	
<i>Y. Liu, L. Wang, X. Tuo</i> and <i>S. Li</i> : An SEM and EDS study of the microstructure of nitrate ester plasticized polyether propellants	369
J. Gao, Z. Ma, J. Guo, Y. Huai, Z. Deng and J. Suo: Surface-charged polyacrylonit- rile/poly(vinyl alcohol) (PAN/PVA) colloids used to prepare proton conducting ma- terials (Short communication)	377
Materials	
<i>Z. Li, T. Shi</i> and <i>L. Guo</i> : Preparation and morphology of porous SiO ₂ ceramics derived from fir flour templates	385
Chemical Engineering	
J. Ivanović, D. Mišić, M. Ristić, O. Pešić and I. Žižović: Supercritical CO ₂ extract and essential oil of bay (<i>Laurus nobilis</i> L.) – chemical composition and antibacterial activity	395
Environmental	
F. J. Rojas Moreno, J. M. Cardenete López, R. Marín Galvín, M. J. Martínez Cordón and J. M. Rodríguez Mellado: On the removal of s-triazine herbicides from waters using commercial low-cost granular carbons	405



JOURNAL OF THE SERBIAN CHEMICAL SOCIETY

1746

S. P. Agarwal, M. D. Khalid Anwer, R. Khanna, A. Ali and Y. Sultana: Humic acid from	
Shilajit – a physico-chemical and spectroscopic characterization	413

NUMBER 4

Organic Chemistry	
B. Tamami, A. N. Shirazi and K. Parvanak Borujeni: Polystyrene-supported aluminum chloride as an efficient and reusable catalyst for condensation of indole with various carbonyl compounds	423
Biochemistry and Biotechnology	
M M Poša and K N Kuhaida: Influence of hile acids on the adsorption of lidocaine and	
veranamil in an <i>in vitro</i> experiment	433
R. S. Verma, R. K. Verma, A. Chauhan and A. K. Yadav: Changes in the essential oil composition of <i>Majorana hortensis</i> Moench. cultivated in India during plant ontogeny.	441
<i>M. S. Milašinović</i> , <i>M. M. Radosavljević</i> and <i>Lj. P. Dokić</i> : Effects of autoclaving and pullulanase debranching on the resistant starch yield of normal maize starch	449
Inorganic Chemistry	
P. S. Zhao, X. Wang, F. F. Jian, J. L. Zhang and H. L. Xiao: Crystal engineered acid-base complexes with 2D and 3D hydrogen bonding systems using <i>p</i> -hydroxybenzoic acid as the building block	459
D. Singh and K. Kumar: Macrocyclic complexes: synthesis and characterization	475
Theoretical Chemistry	
S. Erić, M. Kalinić, A. Popović, H. Makić, E. Civić and M. Bektašević: The importance of the accuracy of the experimental data for the prediction of solubility	483
Physical Chemistry	
<i>R. F. de Farias</i> and <i>C. Airoldi</i> : Hexamethylenetetramine reaction with graphite oxide (GO) as a strategy to increase the thermal stability of GO: synthesis and characterization of a compound	497
Electrochemistry	
L. Wang, S. Chen, B. Yuan, F. Meng, J. Wang, C. Wang and L. Li: Digital holographic reconstruction detection of localized corrosion arising from scratches	505
Analytical Chemistry	
<i>R. M. Baošić</i> , <i>A. D. Radojević</i> and <i>Ž. Lj. Tešić</i> : Prediction of the retention of β -diketo- nato complexes in TLC systems on silica gel by quantitative structure–retention re- lationships	513
Chemical Engineering	
Z. Arsenijević, Ž. Grbavčić, B. Grbić, N. Radić, R. Garić-Grulović, S. Miletić, G. Savčić and B. Đorđević: Fluidized bed combustion of pesticide-manufacture liquid wastes	523
Materials	
<i>R. E. Sabzi, K. Rezapour</i> and <i>N. Samadi</i> : Polyaniline–multi-wall-carbon nanotube nano- composites as a dopamine sensor	537
Environmental	
V. K. Gochev, Z. I. Velkova and M. S. Stoytcheva: Hexavalent chromium removal by waste mycelium of Aspergillus awamori	551

@0\$∋

VOLUME 74: CONTENTS

 M. B. Ninković, R. D. Petrović and M. D. Laušević: Removal of organochlorine pesticides from water using virgin and regenerated granular activated carbon D. Marinović, M. Stojanović and D. Popović: Purification of waters and elimination of organochloric insecticides by means of active coal 	
EuCheMS News	
B. Karlberg, P. Worsfold and J. E. T. Andersen: European Analytical Column no. 38 from the Division of Analytical Chemistry (DAC) of the European Association for Che- mical and Molecular Sciences (EuCheMS), January 2010	587

NUMBER 5

Organic Chemistry	
O. Farsa, P. Doležal and A. Hrabálek: Esters and amides of hexanoic acid substituted with tertiary amino group in terminal position and their activity as transdermal	
 B. C. Dixit, H. M. Patel, R. B. Dixit and D. J. Desai: Synthesis, characterization and dyeing assessment of novel acid azo dyes and mordent acid azo dyes based on 2-hydroxy-4-methoxybenzophenone on wool and silk fabrics 	595 605
Biochemistry and Biotechnology	
V. J. Sovilj, J. L. Milanović, J. M. Katona and L. B. Petrović: Preparation of micro- capsules containing different contents of different kinds of oils by a segregative coacervation method and their characterization	615
Inorganic Chemistry	
 G. Kumar, D. Kumar, C. P. Singh, A. Kumar and V. B. Rana: Synthesis, physical characterization and antimicrobial activity of trivalent metal Schiff base complexes K. Krishnankuttu, M. P. Unwerthur and D. K. Bahu, Bangothianakulaga derivatives of 	629
K. Krishnankuity, M. B. Chimainur and D. K. Babu. Benzonnazorgiazo derivatives of some β -dicarbonyl compounds and their Cu(II), Ni(II) and Zn(II) complexes	639
Theoretical Chemistry	
X. Tan, P. Li, W. Wang, G. Zheng and Q. Wang: A theoretical study of the mechanism of the addition reaction between carbene and azacyclopropane	649
Physical Chemistry	
A. Antić-Jovanović, M. Momčilović, V. Bojović, M. A. Khakoo and R. R. Laher: Franck– –Condon factors and observed band strength distribution in the vibrational structure of the Ag ₂ D-X band system	659
Analytical Chemistry	
<i>T. Madrakian</i> , <i>M. A. Zolfigol</i> and <i>F. Aboulghazi</i> : Preconcentration of Co, Ni, Cd and Zn on naphthalene–2,4,6-trimorpholino-1,3,5-triazin adsorbent and flame atomic absorption determination	669
Z. J. Papp, V. J. Guzsvány, S. Kubiak, A. Bobrowski and L. J. Bjelica: Voltammetric de- termination of the neonicotinoid insecticide thiamethoxam using a tricresyl phos- phate-based carbon paste electrode (Short communication)	681
Polymers	
I. Gavrilović-Grmuša, O. Nešković, M. Điporović-Momčilović and M. Popović: Molar- mass distribution of urea-formaldehyde resins of different degrees of polymeri- sation by MALDI-TOF mass spectrometry	689

යා මාමා

JOURNAL OF THE SERBIAN CHEMICAL SOCIETY

Environmental

1748

S. S. Nenadović, Lj. Lj. Matović, M. M. Milanović, S. V. Janićević, J. D. Grbović Nova- ković and M. A. Lješević: Impacts of some meteorological parameters on the SO ₂ concentrations in the City of Obrenovac, Serbia	703
I. Gržetić and N. Čamprag: The evolution of the trophic state of the Palić Lake (Serbia)	717
NUMBER 6	
Organic Chemistry Riochemistry and Riotechnology	
M Kaltenhauser E P Ellmerer and C Zidorn: Rhamnonyranosylvitexin derivatives	
from Celtis australis	733
D. S. Bisht, R. C. Padalia, L. Singh, V. Pande, P. Lal and C. S. Mathela: Constituents and antimicrobial activity of the essential oils of six Himalayan Nepeta species	739
Inorganic Chemistry	
G. Rajendran, C. S. Amritha, R. J. Anto and V. T. Cheriyan: Synthesis, thermal and antitumour studies of Th(IV) complexes with furan-2-carboxaldehyde4-phenyl-3-thiosemicarbazone	749
D. P. Singh, V. Malik, R. Kumar and K. Kumar: Template synthesis of macrocyclic complexes of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II): spectroscopic, antibacterial	
and antifungal studies	763
<i>N. Raman</i> and <i>S. Sobha</i> : Synthesis, characterization, DNA interaction and antimicrobial screening of isatin-based polypyridyl mixed-ligand Cu(II) and Zn(II) complexes	773
Physical Chemistry	
I. Lukić, J. Krstić, S. Glišić, D. Jovanović and D. Skala: Biodiesel synthesis using K ₂ CO ₃ /Al–O–Si aerogel catalysts	789
Analytical Chemistry	
S. Kravić, Z. Suturović, J. Švarc-Gajić, Z. Stojanović and M. Pucarević: Determination of trans fatty acids in foodstuffs by gas chromatography-mass spectrometry after simultaneous microwave-assisted extraction-esterification	803
for the determination of hydrogen cyanide in air by a modified König method	813
Polymers	
J. M. Katona, V. J. Sovilj, L. B. Petrović and N. Z. Mucić: Tensiometric investigation of the interaction and phase separation in a polymer mixture–ionic surfactant ternary system	823
Materials	
<i>T. B. Novaković</i> , <i>Lj. S. Rožić</i> , <i>S. P. Petrović</i> , <i>Z. M. Vuković</i> and <i>V. T. Dondur</i> : Pore surface fractal analysis of PEG and La(III)-doped mesoporous alumina obtained by	
the sol-gel method	833
Environmental	
H. Z. Mousavi, A. Hosseinifar and V. Jahed: Removal of Cu(II) from wastewater by waste tire rubber ash	845
M. Smelcerović, D. Đorđević, M. Novaković and M. Mizdraković: Decolorization of a	055
textile vat dye by adsorption on waste ash	855

Available online at www.shd.org.rs/JSCS/

cc) (€)(©)(=)

VOLUME 74: CONTENTS

1749

NUMBER 7

Organic Chemistry, Biochemistry and Biotechnology	
J. Wang, D. Lu, H. Zhao, B. Jiang, J. Wang, X. Ling, H. Chai and P. Ouyang: Dis- crimination and classification of tobacco wastes by identification and quantification of polyphenols with LC–MS/MS	875
<i>Djurendić-Brenesel</i> , <i>N. Ajduković</i> , <i>K. Štajnic-Ristić</i> , <i>V. Pilija</i> and <i>I. Veselinović</i> : Δ^9 - -Tetrahydrocannabinol content in cannabis samples seized in Novi Sad during 2008	893
<i>R. Gevrenova</i> : Determination of natural colorants in plant extracts by high-performance liquid chromatography	903
Inorganic Chemistry	
<i>K. Singh</i> and <i>D. Pal</i> : Synthetic, structural and biological studies of organosilicon(IV) complexes of Schiff bases derived from pyrrole-2-carboxaldehyde	917
I. Khosravi and M. Yazdanbakhsh: Preparation and characterization of novel oxo-cen- tered basic <i>p</i> -chlorobenzoic bridging trinuclear complexes	929
S. Chandra, M. Tyagi and S. Agarwal: Synthesis and characterization of a tetraaza macrocyclic ligand and its cobalt(II), nickel(II) and copper(II) complexes	935
Theoretical Chemistry	
S. Jeremić, S. Radenković and I. Gutman: Cyclic conjugation in benzo-annelated tri- phenylenes	943
Physical Chemistry	
A. Magda, R. Pode, C. Muntean, M. Medeleanu and A. Popa: Synthesis and characte- rization of ammonium phosphate fertilizers with boron	951
S. P. Jovanović, Z. M. Marković, D. N. Kleut, V. D. Trajković, B. S. Babić-Stojić, M. D. Dramićanin and B. M. Todorović Marković: Singlet oxygen generation by higher fullerene-based colloids	965
Analytical Chemistry	
D. M. Milenović and Z. B.Todorović: Development and application of a validated HPLC method for the analysis of dissolution samples of mexiletine hydrochloride capsules	975
Polymers	
<i>Y. Liu, L. Wang, X. Tuo, S. Li</i> and <i>W. Yang</i> : A study on the microstructure of a nitrate ester plasticized polyether propellant dissolved in HCl and KOH solutions	987
Thermodynamics	
J. D. Jovanović and D. K. Grozdanić: Reliable prediction of heat of vaporization of <i>n</i> -alkanes at 298.15 K (Note)	997
Environmental	
Dj. Petrović, M. Todorović, D. Manojlović and V. D. Krsmanović: A simulation experi- ment as a method for the investigation of the mobility of heavy metals from inundated land	1005

NUMBER 8

Organic Chemistry

A. S.	. Alimmari, A. D. Marinković, D. Ž. Mijin, N. V. Valentić, N. Todorović and G.S. Uš-	
	ćumlić: Synthesis, structure and solvatochromic properties of 3-cyano-4,6-diphenyl-	
	-5-(3- and 4-substituted phenylazo)-2-pyridones	1019

@0©≡

JOURNAL OF THE SERBIAN CHEMICAL SOCIETY

V. V. Dabholkar and T. D. Ravi: Synthesis of Biginelli products of thiobarbituric acids and their antimicrobial activity (Short communication)
Biochemistry and Biotechnology
 L. Izrael-Živković, G. Gojgić-Cvijović and I. Karadžić: Isolation and partial characterization of protease from <i>Pseudomonas aeruginosa</i> ATCC 27853
Inorganic Chemistry
 V. M. Leovac, V. Divjaković, M. D. Joksović, Lj. S. Jovanović, Lj. S. Vojinović-Ješić, V. I. Češljević and M. Mlinar: Transition metal complexes with thiosemicarbazide-based ligands. Part 57. Synthesis, spectral and structural characterization of dioxovana- dium(V) and dioxomolybdenum(VI) complexes with pyridoxal S-methylisothio- semicarbazone
M. Imran, L. Mitu, S. Latif, Z. Mahmood, I. Naimat, S. S. Zaman and S. Fatima: Antibac-
terial Co(II), Ni(II), Cu(II) and Zn(II) complexes with biacetyl-derived Schiff bases 1075 A. Manohar, K. Ramalingam, G. Bocelli and A. Cantoni: Synthesis, spectral and single crystal X-ray structural studies on bis(2,2'-bipyridine)sulphidoM(II) (M = Cu or
Zn) and diaqua 2,2'-bipyridine zinc(II)sulphate dihydrate
Theoretical Chemistry
D. Vukičević, J. Đurđević and I. Gutman: On the number of Kekulé structures of fluo- ranthene congeners
Physical Chemistry
 S. V. Mahamuni, P. P. Wadgaonkar and M. A. Anuse: Liquid–liquid extraction and recovery of gallium(III) from acid media with 2-octylaminopyridine in chloroform: analysis of bauxite ore
Environmental
 I. Živadinović, K. Ilijević, I. Gržetić and A. Popović: Long-term changes in the eco-chemical status of the Danube River in the region of Serbia
Z. P. Višak: Liquid–liquid equilibria in solutions with potential ecological importance (Extended abstract)

NUMBER 9

Organic Chemistry

1750

J. B.	. Popović-Djordjević, Lj. I. Došen-Mićović, I. O. Juranić and B. J. Drakulić: Anti-	
	proliferative activity of NCI-DTP glutarimide derivatives. An alignment inde-	
	pendent 3D QSAR study	1167

VOLUME 74: CONTENTS

D. Azarifar, M. Pirhayati, B. Maleki, M. Sanginabadi and R. N. Yami: Acetic acid-pro- moted condensation of o-phenylenediamine with aldehydes into 2-aryl-1-(aryl- methyl)-1H-benzimidazoles under microwave irradiation
Biochemistry and Biotechnology
 Y. Zhang, X. Wang and L. Ding: Interaction between tryptophan-vanillin Schiff base and herring sperm DNA
Inorganic Chemistry
<i>Dj. U. Miodragović, D. Jovanović, G. A. Bogdanović, D. Mitić</i> and <i>K. Andjelković</i> : Synthesis and crystal structure of 1,2,3,4-tetrahydro-9-aminoacridine tetrachlorozincate(II) monohydrate
 P. S. Zhao, J.Song, R. C. Shangguan and F. F. Jian: Synthesis, crystal structure of and DFT calculations on bisglycinato-bis[p-(hydroxymethyl)pyridine]nickel(II)
Theoretical Chemistry
S. Marković, J. Đurđević, S. Jeremić and I. Gutman: Diradical character of some fluo- ranthenes
Physical Chemistry
<i>M. S. Hadnađev-Kostić</i> , <i>T. J. Vulić</i> and <i>R. P. Marinković-Nedučin</i> : A study of thermally activated Mg–Fe layered double hydroxides as potential environmental catalysts 1251
Electrochemistry
<i>X. B. Hu, Z. J. Lin, L. Liu, Y. J. Huai</i> and <i>Z. H. Deng</i> : Effects of the LiFePO ₄ content and the preparation method on the properties of (LiFePO ₄ +AC)/Li ₄ Ti ₅ O ₁₂ hybrid battery–capacitors
Materials
T. Žák, N. M. Talijan, V. R. Ćosović, J. T. Stajić-Trošić and A. S. Grujić: An overstoi- chiometric Nd–Fe–B hard magnetic material
Environmental
 T. M. Zeremski-Škorić, P. D. Sekulić, I. V. Maksimović, S. I. Šeremešić, J. M. Ninkov, S. B. Milić and J. R. Vasin: Chelate-assisted phytoextraction: effect of EDTA and EDDS on copper uptake by Brassica napus L
and spatial variability of cyanobacterial toxins microcystins in three interconnected
freshwater reservoirs
Errata

NUMBER 10

Organic Chemistry

A. Zare, A. Hasaninejad, A. Parhami, A. R. Moosavi-Zare, F. Khedri, Z. Parsaee, M. Abdolalipoor-Saretoli, M. Khedri, M. Roshankar and H. Deisi: Ionic liquid 1-butyl-

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS

JOURNAL OF THE SERBIAN CHEMICAL SOCIETY

1752

-3-methylimidazolium bromide ([bmim]Br): a green and neutral reaction media for the efficient, catalyst-free synthesis of quinoxaline derivatives
Y. Xia, Y. Zhang, W. Wang, Y. Ding and R. He: Synthesis and bioactivity of erythro- -nordihydroguaiaretic acid, threo-(-)-saururenin and their analogues
Biochemistry and Biotechnology
 B. Nikolić, V. Tešević, I. Đorđević, M. Jadranin, M. Todosijević, S. Bojović and P. D. Marin: n-Alkanes in the needle waxes of Pinus heldreichii var. pančići
of <i>Stachys</i> species
M. B. Hassanpouraghdam, G. R. Gohari, S. J. Tabatabaei and M. R. Dadpour: Inflores- cence and leaves essential oil composition of hydroponically grown Ocimum ba- silicum L
Inorganic Chemistry
 D. P. Singh, V. Malik, R. Kumar, K. Kumar and S. S. Dhiman: Synthesis, characterization and antibacterial and antifungal studies of some tetraazamacrocyclic complexes 1369 H. de Santana, L. Pelisson, D. R. Janiaski, C. T. B. V. Zaia and D. A. M. Zaia: UV Ra-
diation and the reaction between ammonium and thiocyanate under prebiotic chemistry conditions
Theoretical Chemistry
 M. Haghdadi and M. H. Fatemi: Artificial neural network prediction of the psychometric activities of phenylalkylamines using DFT-calculated molecular descriptors
Electrochemistry
 V. V. Panić, A. B. Dekanski, V. B. Mišković-Stanković, S. K. Milonjić and B. Ž. Nikolić: Differences in the electrochemical behavior of ruthenium and iridium oxide in electrocatalytic coatings of activated titanium anodes prepared by the sol-gel pro- cedure.
H. R. Zare, R. Samimi, N. Nasirizadeh and M. Mazloum-Ardakani: Preparation and elec- trochemical application of rutin biosensor for differential pulse voltammetric de- termination of NADH in the presence of acetaminophen
M. D. Obradović: The electrochemical properties of carbon nanotubes and carbon XC-72R and their application as Pt supports (Extended abstract) 1435
Analytical Chemistry
Lj. Solomun, S. Ibrić, V. Vajs, I. Vučković and Z. Vujić: Methylprednisolone and its related substances in freeze-dried powders for injections
Environmental
 K. M. Šućur, M. P. Aničić, M. N. Tomašević, D. Z. Antanasijević, A. A. Perić-Grujić and M. Dj. Ristić: Urban deciduous tree leaves as biomonitors of trace element (As, V and Cd) atmospheric pollution in Belgrade, Serbia

@0€

VOLUME 74: CONTENTS

NUMBER 11

Organic	Chemistry
0	•

<i>S. F. Barbuceanu, O. D. Cretu, G. Saramet</i> and <i>C. Draghici</i> : Synthesis and characteri- zation of some 1,2,4-triazole-3-thiones obtained from intramolecular cyclization of new 1-(4-(4-X-nhenvlsulfonvl)henzovl)-4-(4-iodophenvl)-3-thiosemicarbazides 1463
 H. M. Dalloul and A. S. Abu Samaha: Synthesis of nitrogen-containing dispirohete- rocycles using nitrilimines (II).
Biochemistry and Biotechnology
D. Lagundžin, R. Masnikosa, G. Miljuš, D. Robajac and O. Nedić: An investigation of the different molecular forms of IGFBP-1 using immobilised metal-, immuno- and lectin-affinity chromatography
 I. Erdogan-Orhan, E. Baki, S. Şenol and G. Yilmaz: Sage-called plant species sold in Turkey and their antioxidant activities
composition and antioxidant activities of basil from Thailand using retention indices and comprehensive two-dimensional gas chromatography
Inorganic Chemistry
<i>M. L. Dianu, A. Kriza, N. Stanica</i> and <i>A. M. Musuc</i> : Transition metal M(II) complexes with isonicotinic acid 2-(9-anthrylmethylene)-hydrazide
Theoretical Chemistry
J. E. V. Ferreira, A. F. Figueiredo, J. P. Barbosa, M. G. G. Cristino, W. J. C. Macedo, O. P. P. Silva, B. V. Malheiros, R. T. A. Serra and J. Ciriaco-Pinheiro: A study of new antimalarial artemisinins through molecular modeling and multivariate analysis 1533
Physical Chemistry
S. M. Ghag and S. D. Pawar: Extraction and separation of U(VI) and Th(IV) from hydrobromic acid media using Cyanex-923 extractant
Electrochemistry
 A. V. Tripković, J. D. Lović and K. Dj. Popović: Comparative study of ethanol oxidation at Pt-based nanoalloys and UPD-modified Pt nanoparticles
Analytical Chamistry
 M. Ž. Milenković, V. D. Marinković, P. S. Sibinović, R. M. Palić and D. M. Milenović: An HPLC method for the determination of digoxin in dissolution samples
Materials
S. R. Grujić, N. S. Blagojević, M. B. Tošić, V. D. Živanović and Z. S. Aćimović-Pavlović: Crystal growth of K_2 TiGe ₃ O ₉ in the glass
Environmental
V. P. Beškoski, M. Takić, J. Milić, M. Ilić, G. Gojgić-Cvijović, B. Jovančićević and M. M. Vrvić: Change of isoprenoids, steranes and terpanes during <i>ex situ</i> bioremediation of mazut on the industrial scale

Available online at www.shd.org.rs/JSCS/

JOURNAL OF THE SERBIAN CHEMICAL SOCIETY

1754

NUMBER 12

Organic Chemistry
Y. Xia, Y. Guo and Y. Wen: The total synthesis of cannabisin G 1617
R. Kasimogullari, B. Zengin, M. Maden, S. Mert and C. Kazaz: Synthesis of new deri-
R Peiin Li Vuiisić M Saboyliević A Saboyliević V Tešević and V Vais: Preliminary
analysis of fatty acid chemistry of <i>Kindbergia praelonga</i> and <i>Kindbergia stokesii</i>
(Brachytheciaceae) (Short communication)
Biochemistry and Biotechnology
D. Gođevac, V. Tešević, M. Veličković, Lj. Vujisić, V. Vajs and S. Milosavljević: Poly-
phenolic compounds in seeds from some grape cultivars grown in Serbia
<i>acum</i> (L.) Hoffm. (Apiaceae)
M. H. Mirjalili, P. Salehi, A. Sonboli, J. Hadian, S. N. Ebrahimi and M. Yousefzadi: The
composition and antibacterial activity of the essential oil of Levisticum officinale
Koch flowers and fruits at different developmental stages
Theoretical Chemistry
<i>Lj. Andjelković, S. Grubišić, I. Djordjević, M. Zlatar, S. Niketić</i> and <i>M. Gruden-Pavlović</i> : Consistent force field for metalloporphyrins
Electrochemistry
 A. A. Ensafi, S. Dadkhah-Tehrani and B. Rezaei: Voltammetric determination of dopa- mine in the presence of uric acid using a 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)- -naphthalin-4-sulfonic acid modified glassy carbon electrode
Analytical Chemistry
D. Kostić, S. Mitić, G. Miletić, S. Despotović and A. Zarubica: The concentrations of Fe, Cu and Zn in selected wines from South-East Serbia
Polymers
M. B. Milovanović, M. Avramović, L. Katsikas and I. G. Popović: Simplification of the synthesis of the reversible addition-fragmentation chain transfer agent 2-(2-cya- nopropyl)-dithiobenzoate
Thermodynamics
R. Chanda, A. Banerjee and M. N. Roy: Studies of viscous antagonism, excess molar
volumes, viscosity deviation and isentropic compressibility of ternary mixtures containing <i>N</i> , <i>N</i> -dimethylformamide, benzene and some ethers at 298.15 K 1721
Environmental
<i>L. D. Gomidželović, E. D. Požega</i> and <i>V. K. Trujić</i> : The possibilities of the utilization of the polymetallic concentrate Čoka Marin
Contents of Volume 75 1743
Author index 1755

@ (9)⊜

Author index

Ababei, L. V., 229 Abass, M., 11 Abdel-Monem, W. R., 11 Abdolalipoor-Saretoli, M., 1315 Aboulghazi, F., 669 Abu Samaha, A. S., 1473 Aćimović-Pavlović, Z. S., 1595 Adamovský, O., 1303 Afzal, Z., 165 Agarwal, S. P., 413 Agrawal, S., 935 Airoldi, C., 497 Ajduković, N., 893 Akgul, C., 1203 Ali, A., 413 Alimmari, A. S., 1019 Amritha, C. S., 749 Andjelković, K., 1209 Andjelković, Lj., 1671 Aničić, M. P., 1453 Antanasijević, D. Z., 1453 Antić-Jovanović, A., 659 Anto, R. J., 749 Anuse, M. A., 1099 Anwer Khalid, M. D., 413 Arambašić, J., 1149 Arsenijević, Z., 523 Ashrafi, A. R., 361 Avramović, M., 1711 Azarifar, D., 1181 Babica, P., 1303

Babića, P., 1505 Babić-Stojić, B. S., 965 Babu, D. K., 639 Bahadur, A., 175 Baki, E., 1491 Baošić, R. M., 513

Banerjee, A., 1721 Barbosa, J. P., 1533 Barbuceanu, S. F., 1463 Bektašević, M., 483 Beškoski, V. P., 1605 Bisht, D. S., 739 Bjelica, L. J., 681 Blagojević, N. S., 1595 Bláha, l., 1303 Bláhová, l., 1303 Blinova, i., 1291 Bobrowski, A., 681 Bocelli, G., 1085 Bogdanović, G. A., 1209 Bogojević, D., 1149 Bojović, S., 1337 Bojović, V., 659 Borujeni, K. P., 423 Burazer, L., 19 Cantoni, A., 1085 Chai, H., 875 Chanda, R., 1721 Chandra, S., 935 Chanotiya, C. S., 343 Chauhan, A., 343, 441 Chehardoli, G., 307 Chen, J., 101 Chen, S., 505 Cheriyan, V. T., 749 Chinnici, F., 45 Chondhekar, T. K., 349 Chumpolsri, W., 503 Cioatera, N., 229 Ciriaco-Pinheiro, J., 1533 Civić, E., 483 Cordón Martínez, M. J., 405

1755


Cretu, O. D., 1463 Cristino, M. G. G., 1533 Cui, W., 259 Čamprag, N., 717 Češljević, V. I., 1063 Ćirković-Veličković, T., 19 Ćosović, V. R., 1271 Dabholkar, V. V., 1033 Dadkhah-Tehrani, S., 1685 Dadpour, M. R., 1361 Dalloul, H. M., 1473 Dalwadi, D., 75 Davidović, E., 157 de Farias, R. F., 497 de Santana, H., 1381 Deisi, H., 1315 Dekanski, A. B., 1413 Deng, H., 1405 Deng, Z. H., 271, 377, 1259 Desai, D. J., 605 Despotović, S., 1701 Dhiman, S. S., 1369 Dianu, M. L., 1515 Ding, L., 1191 Ding, Y., 1325 Dinić, S., 1149 Divac, A., 317 Divjaković, V., 1063 Dixit, B. C., 605 Dixit, R. B., 605 Dobričić, J. D., 1053 Dokić, Lj. P., 449 Doležal, P., 595 Dondur, V. T., 833 Došen-Mićović, Lj, I., 1167 Draghici, C., 1463 Dragičević, V. D., 323 Drakulić, B. J., 1167 Dramićanin, M. D., 965 Dudareva, L. V., 209

1756

Djordjević, B. D., 283 Djordjević, I., 1671

Djurendić-Brenesel, M., 893 Điporović-Momčilović M., 689 Đorđević, B., 523 Đorđević, D., 855 Đorđević, I., 1337 Đorđević, N. D., 1653 Đurđević, J., 1093, 1241 Džudović, R. M., 1575 Ebrahimi, S. N., 1661 Ellmerer, E. P., 733 Ensafi, A. A., 1685 Erdogan-Orhan, I., 1491 Erić, S., 483 Faraje, M., 299 Farsa, O., 595 Fatemi, M. H., 1391 Fatima, S., 1075 Ferreira, J. E. V., 1533 Figueiredo, A. F., 1533 Filipović, J., 195 Filipović, N., 195 Filipović, V., 195 Furtula, B., 83 Galvín Marín, R., 405 Gao, J., 377 Garić-Grulović, R., 523 Gavrilović-Grmuša, I., 689 Gavrović-Jankulović, M., 19 Gevrenova, R., 903 Ghag, S. M., 1549 Ghasemnejad-Bosra, H., 299 Ghorbani, M., 361 Glišić, S., 789 Gochev, V. K., 551 Gođevac, D., 1641 Gohari, G. G., 1361 Gojgić-Cvijović, G., 1041, 1605 Gomidželović, L. D., 1733 Grbavčić, Ž., 523 Grbić, B., 523 Grbović Novaković, J. D., 703



Grdović, N., 1149 Grigg, R., 1 Grigorov, I., 1149 Grozdanić, D. K., 997 Grubišić, S., 1671 Gruden-Pavlović, M., 1671 Grujić, A. S., 1271 Grujić, S. R., 1595 Gržetić, I., 717, 1125 Guo, J., 377 Guo, L., 385 Guo, Y., 1617 Gutman, I., 83, 943, 1093, 1241 Guzsvány, V. J., 681 Habibzadeh, S., 299 Hadian, J., 1661 Hadnađev-Kostić, M. S., 1251 Haghdadi, M., 1391 Halámek, E., 813 Hasaninejad, A., 307, 1315 Hassan, M. N., 165 Hassanpouraghdam, M. B., 1361 He, R., 1325 Hosseinifar, A., 845 Hrabálek, A., 595 Hu, X. B., 271, 1259 Huai, Y. J., 271, 377, 1259 Husinec, S., 1 Hussain Reddy, K., 61 Hussain, M. A., 165 Ibrić, S., 1441

llić, M., 1605 Ilijević, K., 1125 Imran, A., 1075 Ion, R., 333 Ismail, M. M., 11 Ivanova, V., 45 Ivanović, J., 395 Ivanović-Matić, S., 1149 Izrael-Živković, L., 1041

Jadhav, S. M., 349 Jadranin, M., 1337 Jagdale, A. N., 349 Jahed, V., 845 Jakovljević, K. V., 1053 Jakšić, Lj. N., 1583 Janiaski, D. R., 1381 Janićević, S. V., 703 Janković, R. N., 1053 Janković, T., 35 Jeremić, S., 83, 943, 1241 Jian, F. F., 459, 1219 Jiang, B., 875 Joksimović, D., 1149 Joksović, M. D., 1063 Jovančićević, B., 1605 Jovanović, Dr., 1209 Jovanović, Du, 789 Jovanović, J. D., 997 Jovanović, Lj. S., 1063 Jovanović, S. P., 965 Jović, B., 157 Juranić, I. O., 1167 Kahru, a., 1291 Kalinić, M., 483 Kaltenhauser, M., 733 Kandrač, J., 185 Karadžić, I., 1041 Karajić, M., 129 Katona, J. M., 615, 823 Katsikas, L., 1711 Kasimogullari, R., 1625 Kazaz, C., 1625 Khakoo, M. A., 659 Khanna, R., 413 Khedri, F., 1315 Khedri, M., 1315 Khosravi, I., 929 Kijevčanin, M. Lj., 283 Kleut, D. N., 965 Kobliha, Z., 813 Kohoutek, J., 1303 Kostić, D., 1701 Kravić, S., 803 Krishna, P. G., 61 Krishnankutty, K., 639 Krivokuća, A. M., 1053 Krivokuća-Đokić, D., 35

Available online at www.shd.org.rs/JSCS/



1757

Kriza, A., 229, 1515 Krsmanović, V. D., 1005 Krstić, J., 789 Kubiak, S., 681 Kuhajda, K. N., 433 Kumar, A., 629 Kumar, D., 629 Kumar, G., 629 Kumar, K., 217, 475, 763, 1369 Kumar, R., 217, 763, 1369 Kurtoglu, M., 1231 Kušić, J., 317 Labus-Blagojević, S., 1149 Lagundžin, D., 1481 Laher, R. R., 659 Lal, P., 739 Lapanje, A., 129 Latif, S., 1075 Laušević, M. D., 565 Lazarević, J. S., 1347 Leovac, V. M., 1063 Leskovac, V., 185 Li, L., 505 Li, P., 649 Li, S., 369, 987 Li, Z., 259, 385 Liang, G., 259 Liang, M., 101 Lin, Z. J., 271, 1259 Ling, X., 101, 875 Liu, L., 1259 Liu, Y., 369, 987 López Cardenete, J. M., 405 Lović, J. D., 1559 Lu, D., 101, 875 Lukić, I., 789 Lješević, M. A., 703 Ma. Z., 377

1758

Ma, Z., 377 Macedo, W. J. C., 1533 Maden, M., 1625 Madrakian, T., 669 Magda, A., 951 Mahamuni, S. V., 1099

Mahmood, Z., 1075 Makić, H., 483 Maksimović, I. V., 1279 Maleki, B., 1181 Malheiros, B. V., 1533 Malik, V., 763, 1369 Mališić, E. J., 1053 Manohar, A., 1085 Manojlović, D., 1005 Marcu, I-. C., 1115 Marin, P. D., 1337 Marinković, A. D., 1019 Marinković, V. D., 1583 Marinković-Nedučin, R. P., 1251 Marinović, D., 575 Marković, S., 1241 Marković, Z. M., 965 Maršálek, B., 1303 Martinović, V., 1149 Masnikosa, R., 1481 Mathela, C. S., 739 Matović, Lj. Lj., 703 Mayas, A. S., 11 Mazloum-Ardakani, M., 1421 Medeleanu, M., 951 Meghea, A., 333 Mellado Rodríguez, J. M., 405 Meng, F., 505 Mert, S., 1625 Mihailović, M., 1149 Mijin, D. Ž., 1019 Milačič, R., 113 Milanović, J. L., 615 Milanović, M. M., 703 Milašinović, M. S., 449 Milenović, D. M., 1583 Milenović, D. M., 975 Miletić, G., 1701 Miletić, S., 523 Milić, J., 1605 Milić, S. B., 1279 Miljuš, G., 1481 Milonjić, S. K., 1413 Milosavljević, S., 1641 Milovanović, K., 19 Milovanović, M. B., 1711



Miodragović, Dj. U., 1209 Mirjalili, M. H., 1661 Mishra, N., 175 Mišić, D., 395 Mišković-Stanković, V. B., 1413 Mitić, D., 1209 Mitić, S., 1701 Mitran, G., 1115 Mitu, L., 1075 Mizdraković, M., 855 Mlinar, M., 1063 Moghani, A., 91 Mokhlesi, M., 307 Momčilović, M., 659 Moosavi-Zare, A. R., 1315 Moreno Rojas, F. J., 405 Mortimer, M., 1291 Mousavi, H. Z., 845 Mucić, N. Z., 823 Munde, A. S., 349 Muntean, C., 951 Murko, S., 113 Musuc, A. M., 1515 Naimat, I., 1075 Nasirizadeh, N., 1421 Nedić, O., 1481 Nenadović, S. S., 703 Neog, M., 75 Nešković, O., 689 Niketić, S., 1671 Nikolić, A., 157 Nikolić, B. Ž., 1413 Nikolić, B., 1337 Ninkov, J. M., 1279 Ninković, M. B., 565 Novaković, M., 855 Novaković, T. B., 833 Obradović, M. D., 1435 Olennikov, D. N., 209

Osipenko, S. N., 209 Ouyang, P., 101, 875 Padalia R. C. 739

Padalia, R. C., 739 Pal, D., 917 Palić, R. M., 1347, 1583, 1653 Pande, V., 739 Panić, V. V., 1413 Papp, Z. J., 681 Parhami, A., 1315 Parsaee, Z., 1315 Patel, H. M., 605 Pavel, M., 27 Pawar, S. D., 1549 Pejin, B., 1637 Pelisson, L., 1381 Penzina, T. A., 209 Perić-Grujić, A. A., 1453 Peričin, D., 185 Pešić, O., 395 Petrović, DJ., 1005 Petrović, L. B., 615, 823 Petrović, M., 1149 Petrović, R. D., 565 Petrović, S. P., 833 Petrović, S., 157 Philip, G. H., 61 Pilija, V., 893 Pirhayati, M., 1181 Pithawala, K., 175 Pitschmann, V., 813 Pode, R., 951 Popa, A., 951 Popović, A., 483, 1125 Popović, D., 575 Popović, I. G., 1711 Popović, K. Dj., 1559 Popović, M., 185, 689 Popović-Djordjević, J. B., 1167 Poša M. M., 433 Poznanović, G., 1149 Požega, E. D., 1733 Pripdeevech, P., 1503 Pucarević, M., 803

Radenković, S., 83, 943 Radić, N., 523 Radojević, A. D., 513 Radosavljević, M. M., 449 Radović, I. R., 283 Radulović, N. R., 1347



Radulović, N. S., 1653 Rahman, L. U., 343 Rajendran, G., 749 Ramalingam, K., 1085 Raman, N., 773 Ramzanian-Lehmali, F., 299 Rana, V. B., 629 Rău, I., 229 Ravi, T. D., 1033 Razinger, J., 129 Ren, W., 101 Rezaei, B., 1685 Rezapour, K., 537 Ristić, M. Dj., 1453 Ristić, M., 27, 35, 395 Ristić, N. R., 1347 Robajac, D., 1481 Roshankar, M., 1315 Roy, M. N., 1721 Rožić, Lj. S., 833 Sabovljević, A., 1637 Sabovljević, M., 1637 Sabzi, R. E., 537 Salehi, P., 1661 Samadi, N., 537 Samimi, R., 1421 Săndulescu, I., 1115 Sanginabadi, M., 1181 Saramet, G., 1463 Savčić, G., 523 Savić, V., 1 Sedeh, S. N., 91 Sekulić, P. Đ., 1279 Şenol, S., 1491 Serra, R. T. A., 1533 Shahwar, D., 165 Shangguan, R. C., 1219 Sharma, S., 75 Sher, M., 165 Shi. T., 385 Shirazi, A. N., 423 Sibinović, P. S., 1583 Sihtmäe, M., 1291 Silva, O. P. P., 1533 Singh, A., 343

1760

Singh, C. P., 629 Singh, D. P., 217, 475, 763, 1369 Singh, J., 217 Singh, K., 917 Singh, L., 739 Skala, D., 789 Sobha, S., 773 Solomun, Lj., 1441 Sonboli, A., 1661 Song, J., 1219 Sorouhesh, M. R., 91 Sovilj, V. J., 615, 823 Spasić, M. B., 323 Spasić, M. R., 1053 Sredojević, S. D., 323 Stajić-Trošić, J. T., 1271 Stănică, N., 229, 1515 Stefova, M., 45 Stević, T., 27, 35 Stojanović, G. S., 1347 Stojanović, M., 575 Stojanović, Z., 803 Stoytcheva, M. S., 551 Sultana, Y., 413 Suo, J., 377 Surendra Babu, M. S., 61 Suttiarporn, P., 1503 Suturović, Z., 803 Šavikin, K., 35 Ščančar, J., 113 Šerbanović, S. P., 283 Šeremešić, S. I., 1279 Šmelcerović, M., 855 Špirtović-Halilović, S., 243

Štajnic-Ristić, K., 893 Šućur, K. M., 1453 Švarc-Gajić, J., 803 Tabatabaei, S. J., 1361 Tahir, M. N., 165

Takić, M., 1605 Talijan, N. M., 1271 Tamami, B., 423 Tan, X., 649 Tasić, A. Ž., 283



Tešević, V., 1337, 1637, 1641 Tešić, Ž. Lj., 513 Todorović Marković, B. M., 965 Todorović, M., 1005 Todorović, N., 1019 Todorović, Z. B., 975 Todosijević, M., 1337 Tomašević, M. N., 1453 Tomić, B., 317 Tošić, M. B., 1595 Trajković, V. D., 965 Tripković, A. V., 1559 Trivić, S., 185 Trujić, V. K., 1733 Tuo, X., 369, 987 Tušarová, I., 813 Tyagi, M., 935 Ummathur, M. B., 639 Urdă. A., 1115 Ušćumlić, G. S., 1019 Uskoković, A., 1149 Vajs, V., 1441, 1637, 1641 Valentić, N. V., 1019 Vasin, J. R., 1279 Veber, M., 113 Veličković, M., 1641 Velkova, Z. I., 551 Verma, R. K., 343, 441 Verma, R. S., 343, 441 Veselinović, I., 893 Vidaković, M., 1149 Višak, Z. P., 1161 Voicescu, M., 333 Vojinović-Ješić, Lj. S., 1063 Vrhovšek, D., 129 Vrvić, M. M., 1605 Vučković, I., 1441 Vujić, Z., 1441 Vujisić, Lj., 1637, 1641 Vukičević, D., 1093 Vuković, Z. M., 833 Vulić, T. J., 1251 Vulić, T., 35

Wadgaonkar, P.P., 1099 Wang, C., 505 Wang, J., 101, 505, 875 Wang, K., 249 Wang, Liang, 505 Wang, Luoxin (Beijing), 369 Wang, Luoxin (Wuhan), 259, 987 Wang, Q., 649 Wang, Wei, 1325 Wang, Weihua, 649 Wang, Xian, 459 Wang, Xingming, 1191 Wen, Y., 1617 Wongpornchai, S., 1503 Xia, Y., 1325, 1617 Xiao, H. L., 459 Xiao, H., 1405 Xu, J., 259 Xu. W., 259 Yadav, A. K., 343, 441 Yadav, A., 343 Yami, R. N., 1181 Yang, W., 987 Yazdanbakhsh, M., 929 Yildirim, M., 1203 Yilmaz, G., 1491 Yousefzadi, M., 1661 Yuan, B., 505 Zaia, C. T. B. V., 1381 Zaia, D. A. M., 1381 Zaman, S. S., 1075 Zare, A., 1315 Zare, H. R., 1421 Zarubica, A., 1701 Završnik, D., 243 Zdunić, G., 35 Zengin, B., 1625 Zeremski-Škorić, T. M., 1279 Zhang, H., 259 Zhang, J. L., 459 Zhang, S., 101 Zhang, Yan., 1191 Zhang, Yuanyuan, 1325



Zhao, H., 875 Zhao, P. S., 459, 1219 Zheng, G., 649 Zhong, P., 249 Zidorn, C., 733 Zlatar, M., 1671 Zolfigol, M. A., 307, 669

1762

Zrimec, A., 129

Žák, T., 1271 Živadinović, I., 1125 Živanović, V. D., 1595 Žižović, I., 395

Subject Index of Vol. **75** and *List of Referees* in the year 2010 are given at the Internet address of the Journal of the Serbian Chemical Society (http:////www.shd.org.rs/jscs/).

End of Volume 75.

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS





Volume 75 (2010)



JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

Subject index

Absorption spectra, of solvatochromic compounds, 1019 Accumulation lake, pollutants in, 1005 Acenaphthylene-type hydrocarbons, 83 Acetaminophen, 1421 Acetic anhydride, in acetylation of dextran, 165 Acetylcholinesterase, 1491 Acetoacetate esters, 299 Acetylation, of dextran, 165 Acid azo dye, on wool and silk fabrics, 605 Activated carbon, granular, in removal of organochlorine pesticides, 565 Activated titanium anodes, 1413 Active coal, in elimination of organochloric insecticides, 575 1-Acylthiosemicarbazide, in intramolecular cyclization, 1463 Addition reaction, 649 Aerogel, in biodiesel synthesis, 789 Affinity chromatography, 1481 Ag₂ D-X band system, 659 Ag(I) complexes, 1 Alkane with k methyl groups, 1405 *n*-Alkanes, heat of vaporization of, 997 in the needle waxes of pines, 1337 Allergen extract, 19 Alignment-independent 3D QSAR, 1167 Alumina/silica, as catalyst support, 789 Aluminum chloride, as catalyst, 423 ω -Amino acid derivatives, as transdermal permeation enhancers, 595 in Ni complexes, 1219 2-Amino-4-ethyl-5-hydroxybenzaldehyde, in synthesis of ligands, 629

3-Aminopyridine, in synthesis of oligomers/polymers, 1203 Ammonium dihydrogen phosphate, as fertilizer, 951 Ammonium thiocyanate, synthesis of by UV, 1381 Anionic clays, 1251 Antibacterial activity, of Co(II), Ni(II), Cu(II) and Zn(II) complexes, 1075 of essential oil of bay, 395 of essential oil of Levisticum officinale Koch, 1661 of metal complexes with oxime, 1231 of oligomers/polymers, 1203 of organosilicon(IV) complexes, 917 Antifungal activity, of macrocyclic complexes, 475, 763, 935, 1369 of metal complexes with oxime, 1231 of organosilicon(IV) complexes, 917 Antimicrobial activity, of Biginelli products of thiobarbituric acids, 1033 of Cu(II) and Zn(II) complexes, 773 of complexes with 14-membered tetraazamacrocyclic ligand, 217, 1369 of essential oils from six Himalayan Nepeta species, 739 of extracts of Stachys species, 1347 of macrocyclic complexes, 763 of Ribes nigrum L., 35 of Thymus glabrescens and Thymus pulegioides, 27 of transition metal complexes, 349 of trivalent metal Schiff base complexes, 629 Antioxidant capacity,

1



of extracts of Stachys species, 1347 of sage, 1491 Antiproliferative agents, 1167 Antitumour activity, of thorium(IV) complexes, 749 Apigenin, in plant extracts, 903 Aqueous solubility prediction, 483 Aromatic aldehydes, 1033 Artemisinin, 1533 Artificial neural network, prediction by, 1391 2-Aryl-1-(arylmethyl)-1H-benzimidazoles, synthesis of, 1181 Arylazo pyridone dyes, 1019 Asymmetrical tetradentate Schiff base, as ligand, 349 Aspergillus awamori, waste mycelium of, 551 Atmosphere analysis, 813 Atomic absorption spectrometry, 1701 Atomic force microscopy, 965 Autoprotolysis constant, determination of, 1575 Azacyclopropane, 649 Azo-enol form, 639 Azomethine ylides, cyclo-addition reaction of, 1

Band strength distribution, 659 Bauxite ore, analysis of, 1099 Bay, essential oil of, 395 Benzo-annelated triphenylene, 943 Benzothiazolylazo- β -dicarbonyls, as ligands, 639 Berry extraction, 45 Biacetyl-derived complexes, 1075 Bile acids, in the in vitro adsorption, 433 Binary mixtures, thermodynamic properties of, 283 Bioactivity, against the HIV gene, 1325 Biodiesel synthesis, 789 Biomonitoring, 1453 Bioremediation, 1605 Biosensor, of rutin, 1421 2,2'-Bipyridine, 1085 Bis-indolylmethanes, 423

Black currant leaves of cultivar Čačanska crna, essential oil of, 35 Brassica napus L., in phytoextraction of heavy metals, 1279 Bread quality, 195 N-Bromosuccinimide, as catalyst, 299 2,3-Butanedione, in synthesis of the ligands of macrocyclic complexes, 475 Calarene, 209 Calcinations, of porous SiO₂ ceramics, 385 Calcium alginate gel spheres, for urease ntrapment, 175 Cannabinoids, 893 Cannabisin G, synthesis of, 1617 Carbene, 649 Carbon nanotubes, electrochemical properties of, 1435 Carbonaceous materials, 271 Carbon paste electrode, 681 Carboxylic ligand, 929 Celtis australis, methanolic extract from the leaves of, 733 Chemiluminescence, as a method for evaluation of the oxidative activity, 333 Chemoselective oxidation, of sulfides, 307 Chemotaxonomy, 1637 Chlorogenic acids, 875 Cleavage activity, of DNA, 61 CO oxidation, kinetics of, 249 Coacervation, 615, 823 Coenzyme binding, 185 Complete active space calculation, on fluoranthenes, 1241 Complexes of factor-binding protein 1, 1481 Composite solid propellants, 369 Compressibility, of ternary mixtures, 1721 Computer programs for calculating pK_a , 243 Constructed wetlands, for saline wastewater treatment, 129 Container closure system, 1441 Copper smelting, 1733 Coulometry, 1575

Available online at www.shd.org.rs/JSCS/



2

VOLUME 75: SUBJECT INDEX

Coumarin-based compounds, 243 Cr(VI) Removal, 551 Crop age, in variation of major essential oil constituents, 441 Crystal growth of K₂TiGe₃O₉, 1595 Crystal structure, of acid-base complexes, 459 of Ni complexes, 1219 of Zn complexes, 1209 CSpKaPredictor and ADME/ToxWEB programs, 243 Cyanex-923 extractant, 1549 Cyanobacterial toxins, in freshwater reservoirs, 1303 Cyanogen chloride, 813 2-(2-Cyanopropyl)-dithiobenzoate, synthesis of, 1711 Cyclic conjugation, in benzo-annelated triphenylenes, 943 ring effects on. 83 energy effect of, 83, 943 PCP-rule for, 83 Cycle index, of IPR hetero-fullerenes, 361 Cycle life, of batteries and capacotirs, 1259 Cyclic voltammograms test, 271 Cyclo-addition reaction, of azomethine ylides, 1 1,4-Cyclohexanedione oxime, in dispiroheterocycles synthesis, 1473 Cyclohexylamine, 283 CYP1A enzyme, 1149 Danube River, eco-chemical status of, 1125 Decolorization, of textile, 855 Degree of polymerisation, 689 Density functional theory, 259, 1391 Dermatophagoides pteronyssinus, in

sublingual immunotherapy, 19 Determination of Cd, Pb and As, in river sediments, 113 Dextran, acetylation of, 165 DFT Calculation, on Ni complexes, 1219 1,2-Diamine, in synthesis of ionic liquids, 1315

Diaminonaphthalene, 217 Dichloropropanol, as product of glycerol chlorination, 101 Differential pulse voltammetry, 681 Digital holography, in detection of localized corrosion, 505 Digoxin, determination of, 1583 Dihydropyrimidine, synthesis of, 1033 β -Diketonato complexes, 513 1,2-Diketone, condensation with 1,2-diamines, 1315 Dioxomolybdenum(VI) complexes, 1063 Dioxovanadium(V) complexes, 1063 Dipyridopyrimidine, as synthone for heterotricycles, 11 Diradical, singlet and triplet, 1241 Dispiroheterocycles, synthesis of, 1473 Dithiocarbamates, synthesis of, 1085 DNA Binding and cleavage, 773 Dopamine. determination of, 1685 sensor for, 537 Double hydroxides of Mg-Fe, layered, 1251 DPPH Radical scavenging activity, 1503 Dye-sensitized solar cells, 259 Dynamic light scattering, 965 Eco-chemical status, of shallow lake, 717 Ecotoxicity, of anilines, 1291 Electrocatalyst for oxygen reduction, 1435 Electron paramagnetic resonance spectroscopy, 965 Electrochemical impedance spectroscopy, 1413 Electrothermal atomic absorption spectro-

metry, for determination of heavy metals, 113 ELISA Inhibition, 19 Emulsions, 615 Ethanol oxidation, 1559 Eutrophication, 717 Excess molar volumes, in ternary mixtures, 1721

Fertilizer, synthesis of, 951



Fibrex, 195 Flavonoids, 733 Flavanol monomers, 1641 Fluidized bed incinerator, 523 Fluoranthenes. Kekulé structures of, 1093 diradical character of, 1241 Foodstuffs, 803 Forensic samples, of cannabis, 893 Formation constants, of complexes, 75 Franck-Condon factors, 659 Free base porphyrins, oxidative activity of, 333 Free thiols, during maize seeds aging, 323 Freeze-dried powder, for injections, 1441 Frozen dough, effect of commercial fibres on, 195 Fuel cell, with polymer electrolyte, 377 Full non-rigid group, of 2,4-dimethylbenzene. 91 Fullerene, higher, 965 Fullerene-based colloids, 965 Furan-2-carboxaldehyde4-phenyl-3-thiosemicarbazone, as ligand, 749

Gallium(III), extraction and recovery of, 1099 Gas chromatography-mass spectrometry, 101, 343, 441, 565, 575, 803, 1337, 1347, 1361, 1503 GCD Analysis, 893 Gene expression patterns, in blood, 1053 Germacrene D, 1653 Gibbs free energy, of coenzyme binding, 185 Glass, 1595 Glassy carbon electrode, modified, 1685 Glutarimides, activity of, 1167 Glutathione, half-cell reduction potential of. 323 Glycerol, chlorination of, 101 C-Glycosides, 733 Grape seeds, polyphenolic compounds in, 1641 Graphite oxide, 497

Graphite (0001) surface, 1671 Granular carbon, in removal of s-triazine herbicides from waters, 405 GRIND descriptors, 1167 Halotolerant microorganisms, for saline wastewater treatment, 129 Heat of vaporization, prediction of, 997 Heavy metals, as pollutants, 1005 Hetero-fullerene, enumeration of a class of, 361 Heuristic method, in prediction of solubility, 483 Hexamethylenetetramine, in reaction with graphite oxide, 497 Hexanoic acid, esters and amides of, 595 Highly substituted furans, synthesis of, 299 HPLC, 875, 903, 975, 1303, 1583, 1641 Humic acid, characteritzation of, 413 Hybrid battery-capacitor, 1259 Hydrogen bonding, in N-methylformamide-ether systems, 157 in acid-base complexes, 459 Hydrogen cyanide, in air analysis of, 813 Hydroponical growth, 1361 Hydrotalcites, 1251 *p*-Hydroxybenzoic acid, as the building block in acid-base complexes, 459 2-Hydroxy-4-methoxybenzophenone, in dyes, 605 Hydroxycinnamic acid, 1641 ICP-MS, 1453 Ion exchange capacity, of polymers, 377 Indigotin, in plant extracts, 903 Indole, condensation of, 423 Indoline dyes, 259 Inflorescence, of lavender, 343 Insulin-like growth factor-binding protein 1.1481 Intramolecular cyclization, 1463 Inulin, 195

Inundated soil, 1005 Iodine, for activation of acetic anhydride, 165

Available online at www.shd.org.rs/JSCS/



4

VOLUME 75: SUBJECT INDEX

Ionic liquids, as solvents, 1161 netral, synthesis of, 1315 Iridoids, 739 Isobutene, oxidative dehydrogenation of, 1115 Isoiridomyrmecin, 739 Isoniazid, 229 Isonicotinic acid (1-naphthylmethylene)hydrazide, 229 Isonicotinoylhydrazone, as ligand, 1515 Isoprenoids, 1605 Isotherm, in removal of Cu(II) from wastewater, 845 Isotopic effect, 659

Juglone, in plant extracts, 903

K₂CO₃, as catalyst, 789 K₂TiGe₃O₉ in the glass, 1595 Kekulé structure, of fluoranthene congeners, 1093 *Kindbergia* species, fatty acid chemistry of, 1637 König method, 813

Lake trophicity, 717 Langmuir model, for dye adsorption on waste ash, 855 Laurus nobilis, essential oil of, 395 Lavandula angustifolia, essential oil composition of, 343 Lawsone, in plant extracts, 903 Leukocytes, gene expression patterns in, 1053 Levisticum officinale Koch, essential oil of, 1661 Lidocaine, adsorption of, 433 LiFePO₄ content, in hybrid batterycapacitor, 1259 Light fastness, 605 Lignanamide, 1617 Liquid-liquid extraction, of gallium(III), 1099 Liquid waste, of pesticide manufacture, 523

Lithium insertion capacity, 271 Long-term spatial and temporal trends, 1125 Luminol, 333

Macrocyclic complexes, 217, 475, 763 Maize seeds, aging of, 323 Maize starch, resistant starch yield of, 449 Majorana hortensis, essential oil composition of, 441 Malaria, 1533 Markaracter Table, 91 Matrix-assisted laser desorption/ionisation time-of-flight, in mass spectromentry, 689 Mazut, as pollutant, 1605 Mesoporous alumina, pore surface fractal analysis of, 833 Metal complexes, with oxime, 1231 Metallothionein, 1149 Meteorological data, 703 *N*-Methylformamide, FT-IR and NIR study, 157 Method validation, 975 Methylprednisolone sodium succinate, 1441 Metalloporphyrins, consistent force field for, 1671 Mexiletine hydrochloride, dissolution of the capsules of, 975 Mg-Fe mixed oxides, as environmental catalysts, 1251 Microencapsulation, 615 Microcystin, as pollutant, 1303 Micronutrient, 951 Microwave-assisted extractionesterification, 803 Microwave-assisted digestion, 113 Microwave irradiation, in synthesis of organic compounds, 1181 Mixed-valence complexes, 929 Model training, 483 Molecular complex of ethers, as O-electron donors, 157 Molecular descriptors, in multiple linear regression analyses, 513 Molecular docking, 1533



Molecular electrostatic potential maps, 1533 Molecular mechanics, 1671 Molybdatophosphoric acid, as catalyst, 307 Monochloropropanediol, as product of glycerol chlorination, 101 Mössbauer phase analysis, of magnetic materials, 1271 Multitrophic test battery, 1291 Multivariate statistical analysis, 875 Multi-wall-carbon nanotubes, 537 Muslin cloth, impregnated, for urease immobilization, 175 Naphthalene, as adsorbent, 669 NEPE Propellants, 987 Nepeta, essential oils of, 739 Nepetalactone, 739 Nd-Fe-B-based hard magnetic material, 1271 Ni(II) complexes, with amino acids, 1219 with benzothiazolylazo derivatives, 639 with biacetyl-derived Schiff bases, 1075 with heterocyclics, 61 with isonicotinic acid hydrazide and isonicotinic acid (1naphthylmethylene)hydrazide, 229 with oxime, 1231 with tetraaza macrocyclic ligand, 935, 1369 Nicotiana tabacum L., waste of, 875 Nicotinamide adenine dinucleotide, determination of, 1421 Nitrate ester plasticized polyether propellant, 987 Nitrilimines, 1473 Non-planar conformations, 1671 Nordihydroguaiaretic acid, synthesis of, 1325 Numerical reconstruction, in localized corrosion, 505

Ocimum basilicum L., essential oil composition of, 1361, 1503 Octogen, element analysis of, 369 2-Octylaminopyridine, as extractant, 1099 Oligomer, antistaphylococcal activities of, 1203 One-pot reactions, 299 Organochloric insecticides, 575 Organochlorine pesticides, 565 Origin of life, 1381 Overstoichiometry, 1271 Oxidative dehydrogenation, 1115 Oximes, as ligands in Ni(II) complexes, 61 Oxo-centered complexes, 929 Perovskite-like oxides, as catalysts, 249 Pesticide production plant, 523 1,10-Phenanthroline/2,2'-bipyridine, as ligand, 773 Phase separation, in polymer mixtureionic surfactant ternary system, 823 Phenylalkylamines, psychometric activities of, 1391 o-Phenylenediamine, condensation of, 1181 Phosphine, chiral, 1 Phytoextraction, of copper, 1279 Pinus heldreichii, needle waxes of, 1337 Platinum-ruthenium nanocatalyst, 1559 Platinum-tin nanocatalyst, 1559 Pleurospermum austriacum, volatiles of, 1653 Pólya theorem, 361 Polyacrylonitrile, in proton conducting materials, 377 Polyaniline, in nanocomposites, 537 Poly(ethylene glycol), as solvent, 1161 Polychlorinated biphenyls, in fish hepatopancreas, 1149 Polycyclic aromatic hydrocarbons, 1093 Polymer-surfactant interaction, 823 Polymetallic concentrate, 1733 Poly(methyl methacrylate), 1711 Polyphenol contents in wines and grapes, 45

Available online at www.shd.org.rs/JSCS/



6

VOLUME 75: SUBJECT INDEX

Polysaccharide, 165 Polystyrene, as catalyst support, 423 Poly(vinyl alcohol), in proton conducting materials, 377 Porous silicon ceramics, 385 Porous structure, of mesoporous alumina, 833 Potable (drinking) water, 575 Potentiometry, 1575 Prebiotic chemistry, 1381 Prometon, as pollutant, 405 Prometryn, as pollutant, 405 Propanediols, as solvents, 1161 Propazine, as pollutant, 405 Protease, from Pseudomonas aeruginosa, 1041 Protease, purification of, 1041 Proton conductivity, of polymers, 377 Pseudocapacitance, 1413 Pseudomonas aeruginosa, protease from, 1041 Pt nanoparticles, 1435, 1559 Pullulanase, debranching of, 449 Pyrazole-3-carboxylic acid, synthesis of, 1625 Pyridopyrimidine, as synthone for heterotricycles, 11 Pyridazin, 1625 Pyridopyrimidopyrimidine, 11 pyridoxal S-methylisothiosemicarbazone, as ligand, 1063 Pyrolytic carbon, 271

Quantitative structure–retention relationship, 513 Quantitative structure–property relationship, 1391 Quaternary complexes, of bivalent transition metals, 75 Quinoxaline derivatives, synthesis of, 1315

Rapid quenched magnetic material, 1271 Relative acidity scale, 1575 Resistant starch, yield of, 449 Reversible addition–fragmentation chain transfer, 1711 River water, pollution of, 1291 *Rhododendron aureum*, essential oil of, 209 RNA Isolation, 1053 Rutin, 875, 1421

Salicylaldoxime ligand, 1231 Salicylic acid, as ligand, 75 Saline wastewater, removal of pollutants from. 129 Saururenin, 1325 Scanning electron microscopy, of plasticized polyether propellants, 369, 987 Scratch corrosion, 505 Scrap tire rubber, as carbon source, 271 Seed germination ability, 323 Self-purification, of rivers, 1125 Sensor, 537 Sequential extraction, of heavy metals, 1005 Serbian wines, from South-East, 1701 Shallow lake, 717 Shilajit, humic acid from, 413 Sideritis species, as antioxidants, 1491 Silicon complexes, 917 Simultaneous distillation and extraction (SDE), 1503 Single or double point mutations, of enzymes, 185 Sodium borate pentahydrate, in synthesis of phosphate fertilizers with boron, 951 Sol-gel process, in preparation of anodes, 1413 in SiO₂ preparation, 385 in PEG and La(III)-doped mesoporous alumina preparation, 833 Solid-liquid extraction, 669 Solvatochromism, solvent effect on, 1019 Solvent extraction, of uranium(VI) and thorium(IV), 1549 Spectral studies, 639 Spectrophotometry, for polyphenols determination, 45 Stachys species, extracts of, 1347



Steranes and terpanes, 1605 Stereoselectivity, of reactants, 1 Stobbe reaction, 1617 Storage stability, of enzyme, 175 Sulfoxides, preparation of, from sulfides, 307 Sulfur dioxide, as pollutant, 703 Supercritical extraction, of essential oil, 395 Supramolecular buildings, 459 Surface fractal dimension, 833 Surfactant properties, 413 Sustainable solvents, 1161 Swelling, 987 Symmetry-broken method, unrestricted, 1241

Tacrine hydrochloride, in synthesis of Zn complexes, 1209 Tautomeric equilibration, 1019 Tbp geometry, 1085 Tensiometry, 823 Template synthesis, of macrocyclic complexes, 475 Tetraaza macrocycle, 935, 1369 Textile vat dye, 855 Thermal analysis, of thorium(IV) complexes, 749 of transition metal complexes, 349 Thermal power plant, 703 Thermal stability, of graphite oxide, 497 of Ni complexes with amino acids, 1219 of transition metal complexes, 1515 Thiamethoxam, determination of, 681 Thin layer chromatography, 513 Thiobarbituric acid, Biginelli products of, 1033 Thiocarbohydrazide, in synthesis of ligands, 629 Thiosemicarbazide-based ligands, 1063 Thorium(IV) complexes, 749 Thymus glabrescens, essential oil of, 27 Thymus pulegioides, essential oil of, 27

Topological index, of molecular graphs of alkanes, 1405 Trace elements, as atmospheric pollution, 1453 Trans fatty acids, in foodstuffs determination of, 803 Transdermal permeation enhancers, 595 Transition metal complexes, 349, 1515 2,4,6-Trimorpholino-1,3,5-triazi, as adsorbent, 669 Tree leaves, pollution of, 1453 Triazine herbicides, removal of, from waters, 405 1,2,4-Triazole-3-thione, synthesis of, 1463 Tricresyl phosphate, 681 Trinuclear complexes, 929 Triphenylene, 943 Tristimulus colorimetry, 813 Trivalent metal Schiff base complexes, 629 TRI Reagent[®], 1053 Trophic state index, 717 L-Tryptophan, in Schiff base, 1191

Underpotential deposition, 1559 Unit subduced cycle index, 91 Urea-formaldehyde resins, 689 Urea hydrogen peroxide, as oxidant, 307 Urease, immobilization of, 175

Vaccines, for house dust mite, 19 Vanadia–molybdena catalysts, in oxidative dehydrogenation of isobutene, 1115 Vanillin, in Schiff base, 1191 Verapamil, adsorption of, 433 Viscosity deviations, in ternary mixtures, 1721 Viscous antagonism, in ternary mixtures, 1721 *Vitis vinifera*, Polyphenolic compounds in, 1641 Voltammetry, 1685 Volumetric properties, 283

Waller index, 893 Washing fastness, 605



VOLUME 75: SUBJECT INDEX

Waste ash, 855 Waste fungal mycelium, 551 Waste tire rubber ash, 845 Waste water, 575, 845 Weight hourly space velocity of the reaction, 249 Wiener polarity index, 1405 Wine analysis, 1701

Yeast alcohol dehydrogenase, 185

Zymogenous microbial consortia, 1605

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS





Volume 75 (2010)



JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

2010 List of referees

Editorial Board of the Journal is grateful to the following referees for reviewing the manuscripts during 2010:

Bakr F. Abdel-Wahab, Applied Organic Chemistry Department, National Research Center, Dokki, Giza, Egypt Biljana Abramović, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia Hazem M. Abu Shawish, Faculty of Science, Research Center, Al-Aqsa University, Gaza, Palestine M. Adharvana Chari, Department of Applied Chemistry, Kyung Hee University, Suwon, Yongin-si, South Korea Mara Aleksić, Faculty of Pharmacy, University of Belgrade, Serbia Ahmet Alim, Public Health Laboratory, Sivas Directorate of Health, Sivas, Turkey Ivana Aljančić, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Mohammad Amir, Faculty of Pharmacy, Hamdard University, New Delhi , India Aldo Andreani, Dipartimento di Scienze Farmaceutiche, Università di Bologna, Bologna Italy Deana Andrić, Faculty of Chemistry, University of Belgrade, Serbia Katarina Anđelkovič, Faculty of Chemistry, University of Belgrade, Serbia Goran Angelovski, Max Planck Institute for Biological Cybernetics, Tübingen, Germany Mališa Antić, Faculty of Agriculture, University of Belgrade, Serbia Vesna Antić, Faculty of Agriculture, University of Belgrade, Serbia Dušan Antonović, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Farooq Anwar, University of Agriculture, Faisalabad, Pakistan Item J. Atangwho, Depatment of Biochemistry, University of Calabar, Nigeria Amir Azam, Department of Chemistry, Jamia Millia Islamia, New Delhi, India Milka Avramov Ivić, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Nada Babovič, Faculty of Applied Ecology, Belgrade, Serbia Petr Babula, ÚPL - University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic Alessia Bacchi, Dipartimento di Chimica Generale ed Inorganica, University of Parma, Parma, Italy Goran Bačić, Faculty of Physical Chemistry, University of Belgrade, Serbia

1



2

Wojciech Bal, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw. Poland Etienne Balan, Institut de Minéralogie et Physique des Milieux Condensés (IMPMC), Paris, France Wojciech Baran, Department of General and Inorganic Chemistry, Medical University of Silesia, Sosnowiec, Poland Bamdad Barari, Shiraz University.Shiraz, Iran Véronique J. Barthet, Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Canada Nikola Basarić, Ruđer Bošković Institute, Zagreb, Croatia Rufina Bastida, Departamento de Química Inorgánica, Universidad de Santiago de Compostela, Santiago de Compostela, Spain Ivana Beara, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia Kiss Bela, Faculty of Pharmacy, University of Medicine and Pharmacy, Cluj, Romania Mohandas Bhat S, M S Ramaiah Institute of Technology, MSR Nagar, Bangalore, Karnataka, India. Vjera Bilušić Vundac, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia Srđan Blagojević, Faculty of Agriculture, University of Belgrade, Serbia Biljana Blažeković, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia Gao Bo, School of Stomatology, The Fourth Military Medical University, Xi'an, China Samir Bondock, Faculty of Science, Mansoura University, Mansoura, Egypt Marie-Lise Bourguet-Kondracki, Molécules de Communication et Adaptation des Microorganismes, Museum National d'Histoire Naturelle, Paris, France Aurelien Blanc, Université de Strasbourg, Laboratoire des Matériaux, Strasbourg Cedex 2, France Biljana Blažeković, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia Ilija Brčeski, Faculty of Chemistry, University of Belgrade, Serbia Tomasz Breczewski, Departamento de Fisica Aplicada II, Facultad de Ciencias y Tecnologia, UPV/EHU, Spain George N Breit, Denver Federal Center Mail Stop 964, Denver, USA André Brodkorb, Teagasc Food Research Centre, Moorepark, Fermoy Co. Cork, Ireland Gianluigi Broggini, Dipartimento di Scienze Chimiche e Ambientali, Università dell'Insubria, Como, Italy Wolfgang Buchberger, Department of Anlytical Chemistry, Universitat Linz, Linz, Austria Ana Bucić-Kojić, Faculty of Food Technology, Osijek, Croatia Zorica Bugarčić, Department of Chemistry, Faculty of Science, University of Kraguievac, Serbia Živojin Bugarčić, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Ishfaq Bukhari, Section Pharmacology, Shifa College of Medicine, Islamabad, Pakistan Klaudija Carović-Stanko, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia

Eduardo Castro, Universidad Nacional de La Plata, La Plata, Argentina

Veronica S. Chedea, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Božidar Cekić, Vinča Institute of Nuclear Sciences, Belgrade, Serbia

Veronica S. Chedea, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Yuru Chen, College of Life Science, Nanjing Normal University, Nanjing, China Chinpiao Chen, Department of Chemistry, National Dong Hwa University, Shoufeng, Taiwan, Republic of China

Fanica Cimpoesu, Institute of Physical-Chemistry, Bucharest, Romania Gianni Ciofani, Italian Institute of Technology, Smart Materials Lab, Center for Micro-BioRobotic, Pontedera (Pisa), Italy

Francesco Crea, Dipartimento di Chimica Inorganica, Università di Messina, Messina, Italy

Janos Csanadi, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia Janoš Čanadi, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia Valerija I. Češljević, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia Željko Čupić, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia

Gordana Ćirić Marjanović, Faculty of Physical Chemistry, University of Belgrade, Serbia Tanja Ćirković Veličković, Faculty of Chemistry, University of Belgrade, Serbia Aleksandra Daković, Institute for Technology of Nuclear and other Raw Materials, Belgrade, Serbia

Ljiljana Damjanović, Faculty of Physical Chemistry, University of Belgrade, Serbia José S. Dambolena, Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), Córdoba, Argentina

Inês da Silva, *Technical University of Denmark, Lyngby, Denmark* Andrés de Blas, *Facultad de Ciencias, Universidade da Coruna, Coruna, Spain* Henrique Guilhon de Castro, *Universidade Federal do Tocantins, Gurupi-TO, Brasil* Silvia Dei, *Dipartimento di Scienze Farmaceutiche, Università di Firenze, Sesto Fiorentino (FI), Italy*

Aleksandar Dekanski, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia

Dragana Dekanski, Biomedical Research, R&D Institute, Galenika a.d., Belgrade, Serbia Thereza Christina Monteiro De Lima, Department of Pharmacology, Center of Biological Science (CCB), Universidade Federal de Santa Catarina, Brazil

Ahmet Demirbas, Department of Chemistry, Karadeniz Technical University, Trabzon, Turkey

Brás Heleno de Oliveira, *Universidade Federal do Paraná, Parana, Brazil* Sergio Paulo Severo de Souza Diniz, *Department of Biochemistry, University of Maringa, Parana, Brazil*

Concetta De Stefano, Dipartimento di Chimica Inorganica, Università di Messina, Messina (Vill. S. Agata), Italy

Radovan Dimitrijević, Faculty of Mining and Geology, University of Belgrade, Serbia Benoit Divol, Institute for Wine Biotechnology, Stellenbosch University, Matieland, South Africa

4

Leila Dhouibi, Unité de Recherche Corrosion et Protection des Métalliques, Ecole Nationale d'Ingénieurs de Tunis, Tunis, Tunisia Hans Dolhaine, Henkel, Dusseldorf, Germany Ljiljana I. Došen-Mićović, Faculty of Chemistry, University of Belgrade, Serbia Snežana Dragović, Institute for the Application of Nuclear Energy - INEP, Beograd-Zemun, Serbia Branko Drakulič, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Deepak Kumar Dubey, Process Technology Development Division, Defence R&D Establishment, Gwalior, MP, India Svetlana Đogo, Institute of Analytical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia Jasna Đonlagić, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Dragana Đorđević, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Emila Đorđević, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Bojan Đorđević, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Miloš I. Đuran, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Predrag Đurđević, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Ahmed Fawzy Abdel-Hamid El-Asmy, Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt Fereshteh Eftekhar, Shahid Beheshti University, Faculty of Biological Sciences, Microbiology Department, Teheran, Iran Wael A. El-Sayed, Department of Photohemistry, National Research Center, El-Dokki, Cairo, Egypt Ilkay Erdogan-Orhan, Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey Istvan Fabian, Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary Mohsen Fathi Najafi, Razi Vaccine and Serum Research Institute, Ahmadabad Ave-Mashhad. Iran Juliana Feijó de Souza Daniel, Universidade Tecnológica Federal do Paraná, Campus Cornélio Procópio, Brazil Zorana Ferjančić, Faculty of Chemistry, University of Belgrade, Serbia Ana Cristina Figueiredo, Faculdade de Ciências da Universidade de Lisboa, Departamento de Biologia Vegetal, Lisboa, Portugal Ana Clara Fortio Mourato Teixeira Grosso, Faculty of Farmacy, University of O'Porto, Portugal Octávio Luiz Franco, Centro de Análises Proteômicas e Bioquímicas, Universidade Católica de Brasília, Brasilia - DF, Brazil Boris Furtula, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Mario Gabričević, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia



Deepak Ganjewala, Amity Institute of Biotechnology, Noida, India Ali Gargouri, Laboratoire de Génétique Moléculaire des Eucaryotes, Centre de Biotechnologie de Sfax, Sfax, Tunisia Renata Gašparová, University of Ss. Cyril and Methodius in Trnava, Trnava, Slovakia Katya Georgieva, M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria Athina Geronikaki, School of Pharmacy, University of Thessaloniki, Thessaloniki, Greece Charansingh Gill, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India Shanmugam Girija, Department Of Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu, India Dejan Gođevac, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Gordana Gojgić-Cvijović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Sandra Cristina Gonçalves Gouveia, Centro de Química da Madeira, Universidade da Madeira, Portugal Malgorzata Grabarczyk, Faculty of Chemistry, Maria Curie-Sklodowska University, Lublin. Poland Branimir Grgur, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Sanja Grgurić-Šipka, Faculty of Chemistry, University of Belgrade, Serbia Laurence Grimaud, Laboratoire Chimie et Procédés, Ecole Nationale Supérieure de Techniques Avancées, Paris, France Dragoljub Grubišić, Institute for Biological Research Siniša Stanković, University of Belgrade, Beograd žAlisa Gruden-Movsesijan, Institute for the Application of Nuclear Energy - INEP, Beograd-Zemun, Serbia Ivan Gržetić, Faculty of Chemistry, University of Belgrade, Serbia Ivan Gutman, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Valeria Guzsvany, Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia Béla Gyurcsik, Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary Milica Gvozdenović, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Abraha Habtemariam, Department of Chemistry, University of Warwick, Coventry, U.K. Sajjad Haider, Department of Chemical Engineering, College of Engineering, King Saud University, Rivadh, Saudi Arabia Reza Hajian, College of Science, Islamic Azad University Branch of Gachsaran, Gachsaran, Iran Hans-Jürgen Hamann, Institut für Chemie, Humboldt-Universität zu Berlin, Berlin, Germany Bingfang He, College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing, China



Alžbeta Hegedűsová, Constantine the Philosopher University in Nitra, Department of Chemistry, Nitra, Slovakia

Stéphanie Hesse, Institut Jean Barriol, Université Paul Verlaine—Metz, Metz Technopôle, France

Ivanka Holclajtner-Antunović, Faculty of Physical Chemistry, University of Belgrade, Serbia

Jongki Hong, College of Pharmacy, Kyung Hee University, Hoegi-Dong, Seoul, South Korea

Marijana Hranjec, Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Croatia

Roger Hunter, Department of Chemistry, University of Cape Town, Rondebosch, South Africa

Nenad Ignjatović, Institute of Technical Sciences of the Serbian Academy of Sciences and Arts, Belgrade, Serbia

Violeta Ivanova, Department for Enology, Institute of Agriculture, Skopje, Macedonia Milovan Ivanović, Faculty of Chemistry, University of Belgrade, Serbia

Ivana Ivanović-Burmazović, University of Erlangen-Nürnberg, Germany

Jernej Iskra, Department of Physical and Organic Chemistry, Jožef Stefan Institute, Ljubljana, Slovenia

Raquel G. Jacob, Instituto de Química e Geociências, Universidade Federal de Pelotas, Pelotas, Brazil

Xiaobin Jia, Key Laboratory of New Drug Delivery System of Chinese Meteria Medica, Nanjing, P.R. China

Andreja Jakas, Ruđer Bošković Institute, Zagreb, Croatia

Đorđe Janaćković, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Branimir Jelenković, Institute of Physics, Belgrade, Serbia

Stanka Jerosimić, Faculty of Physical Chemistry, University of Belgrade, Serbia

Xiaobin Jia, Key Laboratory of New Drug Delivery System of Chinese Meteria Medica,

Jiangsu Provincial Academy of Chinese Medicine, Nanjing, China

Milan Joksović, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia

Sian B. Jones, Division of Chemistry and Materials, Manchester Metropolitan University, Manchester, United Kingdom

Daniel Joulain, Robertet S.A., Grasse Cedex, France

Jovan Jovanovič, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Ljiljana Jovanović, Department of Chemistry, Faculty of Science, University of Novi Sad, Serbia

Vladislava M. Jovanović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia

Vesna Jović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia

Ivan Juranić, Faculty of Chemistry, University of Belgrade, Serbia

Zorica D. Juranić, Institute of Oncology and Radiology of Serbia, Belgrade, Serbia

Emmanuel Kalogeris, National Technical University of Athens, Greece

Željko Kamberović, Faculty of Technology and Metallurgy, University of Belgrade, Serbia



Emiko Kaneko, Tohoku University, Japan

Ivanka Karadžić, Department of Chemistry, School of Medicine, University of Belgrade, Serbia Ioannis Karapanagiotis, Ormylia Art Diagnosis Center, Sacred Convent of the Annunciation, Ormylia, Greece Fikret Karcı, Pamukkale University, Faculty of Science and Arts, Denizli, Turkey Fatma Karipcin, Science and Arts Faculty, Süleyman Demirel University, Isparta-Turkey Petr Kaspar, CTU FEE, Technical University, Praha, Czech Republic Arvind M. Kayastha, School of Biotechnology, Banaras Hindu University, Varanasi, India Alan Kelly, Graduate Studies and School of Food and Nutritional Sciences, University College Cork, Cork, Ireland Shane Kendell, School of Science and Technology, University of New England, Armidale, Australia Hanan Mohammed Khairy Moustafa, National Institute of Oceanography and Fisheries, Alexandria, Egypt Nadeem Khan, Department of Diagnostic Radiology, Dartmouth Medical School, Hanover, USA Mirjana Kijevčanin, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Edward Cameron Kirby, Resource Use Institute, Kinglassie, Fife, Scotland Goran Klobučar, Department of Zoology, Faculty of Science, University of Zagreb, Croatia Koffi Koba, Ecole Supérieure d'Agronomie, Université de Lomé, Lomé, Togo Ljljana Kolar Anić, Faculty of Physical Chemistry, University of Belgrade, Serbia Ulrich Kortz, School of Engineering and Science, International UniVersity Bremen, Bremen, Germany Velizar Kostadinov Gochev, Biological Faculty, "Paisii Hilendarski" University of Plovdiv, Plovdiv, Bulgaria Nenad M. Kostić, Chemistry Department, Texas A & M University-Commerce, Texas, USA Aleksandar Kremenović, Faculty of Mining and Geology, University of Belgrade, Serbia Antoniana U. Krettli, Centro de Pesquisas René Rachou, Fiocruz & Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil Svjetlana Krištafor, Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Croatia Nedeljko Krstajić, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Turibio Kuiate Tabopda, Faculté de Pharmacie, Université de Bourgogne, Dijon Cedex, France Zevnel Kılıc, Department of Chemistry, Ankara University, Ankara, Turkey Vesna Kuntić, Faculty of Pharmacy, University of Belgrade, Serbia Marc Lamshöft, Institute of Environmental Research (INFU) of the Faculty of Chemistry, TU Dortmund, Germany Eduardo A. Castro, Facultad de Ciencias Exactas de la Universidad Nacional de La

Peter Langer, Institut für Chemie, Universitat Rostock, Rostock, Germany

Plata, La Plata, Argentina

8

Eun Yeol Lee, College of Engineering, Kyung Hee University, Yongin-si, Gyeonggi-do, South Korea Ivan Lefkovits, Department of Biomedicine, University Hospital Basel, Basel, Switzerland Ari Lehtonen, Department of Chemistry, University of Turku, Turku, Finland Ji-Tai Li, College of Chemistry and Environmental Science, Hebei University, China Zhiping Li, Department of Chemistry, Renmin University of China, Beijing, China Donald R. Love, Diagnostic Genetics, LabPLUS, Auckland City Hospital, Auckland, New Zealand Žika Lepojević, Faculty of Technology, Novi Sad, Serbia Milivoj Lovrić, Ruđer Bošković Institute, Zagreb, Croatia Branislava Lakušić, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia Barbara Machura, Institute of Chemistry, University of Silesia, Katowice, Poland Ramón Macías Maza, Instituto Universitario de Catálisis Homogénea, University of Zaragoza, Spain J. Madhusudana Rao, Natural Products Laboratory, Indian Institute of Chemical Technology, Hyderabad, India Filippo Maggi, University of Camerino, School of Pharmacy, Pharmaceutical Botany Unit, Camerino, Italy Kata Majerski, Ruđer Bošković Institute, Zagreb, Croatia Milka Maksimović, Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina Xavier Malcata, Instituto Superior da Maia, Maia - Avioso (São Pedro) Portugal Jorge Mancini Filho, Pharmaceutical Science Faculty, Sao Paulo University, Sao Paulo, Brazil Emilia Mancini, Department of Pharmaceutical Science, University of Salerno, Fisciano, Italy Dragan Manojlović, Faculty of Chemistry, University of Belgrade, Serbia Petar Marin, Faculty of Biology, University of Belgrade, Serbia Dr Davor Margetić, Ruđer Bošković Institute, Zagreb, Croatia Dragan A. Marković, Faculty of Applied Ecology, University Singidunum Belgrade, Serbia Svetlana Marković, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Veselin Maslak, Faculty of Chemistry, University of Belgrade, Serbia Romana Masnikosa, Institute for the Application of Nuclear Energy - INEP, Beograd-Zemun, Serbia Milan Matavulj, Department of Biology and Ecology, Faculty of Natural Sciences, Novi Sad Radomir Matović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Zoran Matović, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Anderson Maxwell, Department of Chemistry, University of the West Indies, St. Augustine, Trinidad and Tobago Mauro Mazzei, Department of Pharmaceutical Sciences, University of Genoa, Italy



Liping Meng, Department of Chemistry, University of California, Davis, California, USA Slavko Mentus, Faculty of Physical Chemistry, University of Belgrade, Serbia Katalin Mészáros Szécsényi, Department of Chemistry, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia Anne S Meyer, Technical University of Denmark, Lyngby, Denmark Slawomir Mielcarek, Faculty of Physics, Adam Mickiewicz University, Poznan, Poland Nevena Mihailović, Institute for the Application of Nuclear Energy - INEP, Beograd-Zemun, Serbia Spiro Mihaylov Konstantinov, Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria Dušan Mijin, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Miloš Milčić, Faculty of Chemistry, University of Belgrade, Serbia Šćepan Miljanić, Faculty of Physical Chemistry, University of Belgrade, Serbia Nenad Milosavić, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Slobodan Milosavljevicć, Faculty of Chemistry, University of Belgrade, Serbia Neda Mimica-Dukić, Faculty of Sciences, University of Novi Sad, Serbia Ubavka Mioč, Faculty of Physical Chemistry, University of Belgrade, Serbia Valentin Mirčeski, Institute of Chemistry, UKIM, Skopje, R. Macedonia Tsutomu Mivasaka, Toin University of Yokohama, Yokohama, Japan Kristina Mladenovska, Institute of Pharmaceutical Technology, Faculty of Pharmacy, Skopje, Macedonia Mosaad S. Mohamed, Faculty of Pharmacy, Helwan University, Helwan, Egypt Vesna Mrvić, Institute of Soil Science, Belgrade, Serbia Mahmoud Nasrollahzadeh, Laboratory of Organic Chemistry, Moshashimi Company, Babol, Iran Olgica Nedić, Institute for the Application of Nuclear Energy - INEP, Beograd-Zemun, Serbia Éva Németh-Zámbori, Corvinus University of Budapest, Department of Medicinal and Aromatic Plants, Budapest, Hungary Emily D. Niemeyer, Department of Chemistry and Biochemistry, Southwestern University, Georgetown, USA Svetozar Niketić, Faculty of Chemistry, University of Belgrade, Serbia Aleksandra Nikolić, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Branislav Nikolić, Serbian Chemical Society, Belgrade, Serbia Li Niu, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China Jasmina Novaković, Apotex Inc, Toronto, Canada Maciej J. Nowak, Institute of Physics, Polish Academy of Sciences, Warsaw, Poland Maja Obradović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Isiaka A. Ogunwande, Natural Product Research Unit, Lagos State University, Ojo, Lagos, Nigeria

Krishan G. Ojha, Department of Chemistry, M.D.S. University, Ajmer (Raj.), India

10

Anthony I Okoh, Department of Biochemistry & Microbiology, University of Fort Hare, Alice. South Africa Antonije Onjia, Vinča Institute of Nuclear Sciences, Belgrade, Serbia Feyyaz Onur, Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Ancara, Turkey Dejan Opsenica, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Anne Orav, Institute of Chemistry, Tallinn University of Technology, Estonia Oscar Osorno, Departamento de Química, Universidad Nacional de Colombia, Bogotá, Colombia Hakan Ozer, Faculty of Agriculture, Ataturk University, Erzurum, Turkey Berrin Özçelik, Faculty of Pharmacy, Gazi University, Ankara, Turkey Banu Özden Tuncer, Faculty of Science, Department of Biology, Ankara University, Ankara, Turkey Gavin F. Painter, Carbohydrate Chemistry, Industrial Research Limited, New Zealand Jitendra Pandey, Department of Botany, Banaras Hindu University, Varanasi, India Milica Pavlović, Department of Pharmacognosy, Faculty of Pharmacy, University of Belgrade, Serbia Mirjana Pavlović, Vinča Institute of Nuclear Sciences, Belgrade, Serbia Vladimir Pavlović, Faculty of Chemistry, University of Belgrade, Serbia Keshav C. Patel, Department of Chemistry, Veer Narmad South Gujarat University, Gujarat. India Julia Pérez-Prieto, Instituto de Ciencia Molecular (ICMOL), Paterna, Valencia, Spain Miljenko Perić, Faculty of Physical Chemistry, University of Belgrade, Serbia Guido Persoone, Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium Biljana Petrović, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Silvana Petrović, Faculty of Pharmacy, University of Belgrade, Serbia Slobodan Petrović, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Andrej Pevec, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia Gianluca Picariello, Istituto di Scienze dell'Alimentazione - CNR, Avellino, Italy Anna Picinelli Lobo, SERIDA, Asturias, Spain Jorge A. Pino, Instituto de Investigaciones para la Industria Alimentaria, La Habana, Cuba Franco Piozzi, Department of Organic Chemistry, University of Palermo, Palermo, Italy Bruno Pitteri, Dipartimento di Chimica, Universita, Ca Foscari" di Venezia, Venezia, Italv Janez Plavec, Slovenian NMR centre, National Institute of Chemistry, Ljubljana, Slovenia Viktor Pocajt, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Georg Pohnert, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Germany Vesna Poleksić, Faculty of Agriculture, University of Belgrade Serbia Dejan Poleti, Faculty of Technology and Metallurgy, University of Belgrade, Serbia

Natalija Polović, Faculty of Chemistry, University of Belgrade, Serbia

Angelina Popova, Department of Physical Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

Mirjana Popsavin, Department of Chemistry, Faculty of Sciences, University of Novi Sad, Serbia

Velimir Popsavin, Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia

Lenuta Profire, Faculty of Pharmacy, "Gr. T. Popa" Medicine and Pharmacy University, Romania

Maria Prokopova Geneva, Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Bulgaria

Susana Puntarulo, Physical Chemistry-PRALIB, University of Buenos Aires-CONICET, Argentina

Ain Raal, Department of Pharmacy, University on Tartu, Tartu, Estonia Dušanka Radanović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia

Bojan Radak, Vinča Institute of Nuclear Sciences, Belgrade, Serbia

Ivona Radović, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Niko Radulović, Department of Chemistry, Faculty of Science, University of Niš, Serbia Siniša Radulović, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia Velimir Radmilović, Lawrence Berkeley National Laboratory, University of California, Berkeley, USA

Raghavachary Raghunathan, Department of Organic Chemistry, University of Madras, Madras, India

Karlo Raić, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Pravin Ramchandra Puranik School of Life Sciences, North Maharashtra University, Jalgaon, MS, India

Natarajan Raman, Department of Chemistry, VHNSN College, Virudhunagar, India Nevenka Rajić, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Vesna Rakić, Faculty of Agriculture, University of Belgrade Serbia Slavica Ražić, Faculty of Pharmacy, University of Belgrade, Serbia

C. Devendranath Reddy, Department of Chemistry, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

Daniela Rigano, Department of Chemistry of Natural Compounds, University Federico II, Napoli, Italy

Mihailo Ristić, Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade, Serbia

Goran Roglić, Faculty of Pharmacy, University of Belgrade, Serbia

Marin Roje, Ruđer Bošković Institute, Zagreb, Croatia

Romeo Romagnoli, Dipartimento di Scienze Farmaceutiche, Université di Ferrara, Ferrara, Italy

Josep Ros, Departament de Química, Universitat Autònoma de Barcelona, Barcelona, Spain

Tudor Rosu, University of Bucharest, Chemistry Faculty, Bucharest, Romania Sergio Rosselli, Department of Organic Chemistry, University of Palermo, Palermo, Italy

Zofia. Rzaczynska, Faculty of Chemistry, Maria Curie-Sklodowska University, Lublin, Poland Radomir N. Saičić, Faculty of Chemistry, University of Belgrade, Serbia Victoria Samanidou, Department of Chemistry, Aristotle University of Thessaloniki, Greece Yaghoub Sarrafi, Department of Chemistry, University of Mazandaran, Babolsar, Iran Vladimir Savić, Faculty of Pharmacy, University of Belgrade, Serbia Hamid Reza Shaterian, Department of Chemistry, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran Xueguang Shao, College of Chemistry, Nankai University, China Hamid Reza Shaterian, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan. Iran Mohamed M. Shoukry, Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt Claudio C. Silveira, Departamento de Química, Universidade Federal de Santa Maria, Brazil Arun K. Sinha, Natural Plant Products Division, Institute of Himalayan Bioresource Technology, Palampur, India Slavica Solujić, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Imre Sóvágó, Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary Sofija Sovilj, Faculty of Chemistry, University of Belgrade, Serbia Vladimir Srdić, Faculty of Technology, University of Novi Sad, Serbia Rohit Srivastava, Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai, India Dragomir Stanisavljev, Faculty of Physical Chemistry, University of Belgrade, Serbia Milena Jelikić-Stankov, Faculty of Pharmacy, University of Belgrade, Serbia Slaviša Stanković, Faculty of Biology, University of Belgrade, Serbia Radosław Starosta, Faculty of Chemistry, University of Wroclaw, Wroclaw, Poland Vladimir Stilinović, Chemistry Department, Faculty of Science, University of Zagreb, Croatia Ksenija Stojanović, Faculty of Chemistry, University of Belgrade, Serbia Danuta Czakis-Sulikowska, Institute of General and Ecological Chemistry, Technical University of Łódź, Łódź, Poland Slavica Sunarić, School of Medicine, University of Niš, Serbia Zorica Svirčev, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia Zoran Šaponjić, Vinča Institute of Nuclear Sciences, Belgrade, Serbia Slobodan Šerbanović, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Bogdan Šolaja, Faculty of Chemistry, University of Belgrade, Serbia Vukić Šoškić, ProteoSys AG, Mainz, Germany Selma Špirtović, Faculty of Pharmacy, University of Sarajevo, Sarajevo Bosnia and

Herzegovina

12



Vladimir Šukalović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Manish Tadhani, Sardar Patel University, Gujarat, India Hassan Tajik, Department of Chemistry, Guilan University, Rasht, Iran Hamdi Temel, Department of Chemistry,, Faculty of Education, Dicle University, Divarbakir, Turkey Vesna Tepavčević, Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia Vele Tešević, Faculty of Chemistry, University of Belgrade, Serbia Živoslav Tešić, Faculty of Chemistry, University of Belgrade, Serbia Demetrius G. Themelis, Department of Chemistry, Aristotle University of Thessaloniki, Greece Nina Todorović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Vera Todorović, School of Dentistry, Pančevo, University Business Academy, Novi Sad, Serbia Xinli Tong, Department of Chemistry, Tianjin University, Tianjin, PR China Snežana Trifunović, Faculty of Chemistry, University of Belgrade, Serbia Vessela Tsakova, Institute of Physical Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria Sachiko Tsukamoto, Kumamoto University, Kumamoto, Japan Srećko Trifunović, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Nenad Trinajstić, Ruđer Bošković Institute, Zagreb, Croatia Lemi Turker, Middle East Technical University, Ankara, Turkey Ljerka Tušek-Božić, Division of Physical Chemistry, Ruđer Bošković Institute, Zagreb, Croatia Mustafa Tuzen, Gaziosmanpasa University, Tokat, Turkey Paraskevas Tzanavaras, Department of Chemistry, Aristotle University of Thessaloniki, Greece Aysel Ugur, Department of Biology, Faculty of Arts and Sciences, Mugla University, Mugla, Turkey Gordana Ušćimlić, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Suresh B. Waghmode, Department of Chemistry, Ganeshkhind, University of Pune, India Karon Waldron, Department of Chemistry, University of Montréal, Montréal, Canada Dun-Jia Wang, Department of Chemistry and Environmental Engineering, Hubei Normal University, PR China Bradley G. Williams, Department of Chemistry, University of Johannesburg, South Africa Jill K. Winkler-Moser, USDA/ARS Research Cemist, Functional Foods Research, Peoria, IL. USA Dorota Woźniak, University of Medicine, Department of Biology and Botany, Wrocław, Poland Longhou Wu, Institute of Molecular Medicine, Huaqiao University, China

Longhou Wu, Institute of Molecular Medicine, Huaqiao University, China Wu Xu, Pacific Northwest National Laboratory, USA Shi Ping Yan, Nankai University, Nankai , China

14

Yumin Yang, Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Jiangsu, China Min Ye, School of Pharmaceutical Sciences, Peking University, Beijing, China Hu Yan Yun, University of Science and Technology of China, Hefei, Anhui, China Ozlem Yesil Celiktas, Faculty of Engineering, Ege University, Bornova-Izmir, Turkey Xiao-Qi Yu, College of Chemistry, Sichuan University, Chengdu, China Lilyana Yurukova, Institute of Botany, Bulgarian Academy of Science, Sofia, Bulgaria Yufang Xu, School of Pharmacy, East China University of Science and Technology, Shangha, China Vlatka Vajs, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia Adelina Vallribera, Department of Chemistry, Universitat Autònoma de Barcelona, Cerdanyola, Barcelona, Spain Vesna Vasić, Vinča Institute of Nuclear Sciences, Belgrade, Serbia V. Vijayakumar, Organic Chemistry Division, School of Science and Humanities, VIT University, India Angela Vogts, Institute of Chemistry and Biology of the Marin Environment (ICBM), Carl von Ossietzky University of Oldenburg, Oldenburg, Germany Tatjana Volkov Husović, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Ljiljana Vračar, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Višnja Vrdoljak, Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia žMiroslav M. Vrvić, Faculty of Chemistry, University of Belgrade, Serbia Gordana Vučković, Faculty of Chemistry, University of Belgrade, Serbia Zoran Vujčić, Faculty of Chemistry, University of Belgrade, Serbia Marija Vukčević, Faculty of Technology and Metallurgy, University of Belgrade, Serbia Rastko Vukićević, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia Nenad Vuković, Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia George Zachariadis, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece Ennio Zangrando, Department of Chemistry, University of Trieste, Trieste, Italy Snežana Zarić, Faculty of Chemistry, University of Belgrade, Serbia Davorka Završnik, Faculty of Pharmacy, University of Sarajevo, Sarajevo Bosnia and Herzegovina Gordana Zdunić, Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade, Serbia Mira Zečević, Faculty of Pharmacy, University of Belgrade, Serbia Jian-Jun Zhang, Experimental Center, Hebei Normal University, Shijiazhuang, China Linxiang Zhao, Shenvang Pharmaceutical University, Shenvang, China Si-yuan Zhou, Department of Pharmaceutics, Fourth Military Medical University, Xi'an, Shaanxi, China

Guonian Zhu, Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou, China

Muhammad Zia-ur-Rehman, Pakistan Council of Scientific & Industrial Research Laboratories Complex, Lahore, Pakistan.
Jiri Zima, Department of Analytical Chemistry, UNESCO Laboratory of Enviromental Electrochemistry, Charles University in Prague, Czech Republic
Mario Zlatović, Faculty of Chemistry, University of Belgrade, Serbia
Liu Zuliang, School of Chemical Engineering, Nanjing University of Science & Technology, Nanjing, China
Branka Žarković, Faculty of Agriculture, University of Belgrade, Serbia
Emila Živković, Faculty of Technology and Metallurgy, University of Belgrade, Serbia
Jelena Živković, Medical Faculty of Niš, Department of Pharmacy, Niš, Serbia

Available online at www.shd.org.rs/JSCS/

2010 Copyright (CC) SCS

