J. Serb. Chem. Soc. 72 (10) 953–959 (2007)
JSCS–3627

UDC 634.46:665.666.5:577.1
Original scientific paper

Biochemical changes in cuttings of *Robinia pseudoacacia* after treatment with naphthenate

SLAVKO KEVREŠAN[1]*#, BRANISLAV KOVAČEVIĆ[2], VERA ĆIRIN–NOVTA[3#], KSENIJA KUHAJDA[3#], JULIJAN KANDRAČ[1#], KSENIJA PAVLOVIĆ[3#] and LJUBICA GRBOVIĆ[3#]

[1]*Faculty of Agriculture, University of Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad,* [2]*Institute of Lowland Forestry and Environment, Antona Čehova 1, 21000 Novi Sad and* [3]*Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, 21000 Novi Sad, Serbia*

(Received 18 December 2006, revised 27 February 2007)

Abstract: Naphthenic acids were isolated from gas oil fractions (distillation interval 168–290 °C) of Vojvodina crude oil "Velebit", characterized and their biological activity evaluated by the biochemical changes in cuttings of *Robinia pseudoacacia* after treatment with naphthenate. The activities of IAA peroxidase, total peroxidases and amylase, as well as the contents of reducing sugars and total proteins, were determined in the basal parts of soft wood cuttings of black locust after treatment with sodium naphthenate or the sodium salt of 1-naphthaleneacetic acid (NAA), concentration 10^{-7} mol dm^{-3} for 3 or 6 h. High activities of IAA oxidase and amylase, together with a low activity of peroxidase (which is known as being stimulatory for the initiation and activation of primordia) were obtained after the three-hour treatment with sodium naphthenate. Six-hour treatment had an inhibitory effect on the examined biochemical markers. The effects of three- and six-hour treatments with NAA were between those of the corresponding treatment with naphthenic acids.

Keywords: naphthenate, rooting, biochemical markers, black locust.

INTRODUCTION

Naphthenic acids represent a complex mixture of cycloalkyl and alkyl carboxylic acids which are found in raw oil and fractions obtained by its distillation. In previous studies, it was shown that naphthenic acids from the fraction boiling in the temperature range 168–290 °C during the atmospheric distillation temperatures of Vojvodina crude oil "Velebit" exhibited certain biological activity in respect of the uptake of various ions,[1,2] as well as an activity similar to that of the plant hormones auxin and gibberelline.[3] Naphthenic acids from this oil fraction stimulate the rooting of cuttings and the lateral branches of sunflower,[4] as well as of poplar hardwood cuttings.[5]

* Corresponding author. E-mail: kevresan@polj.ns.ac.yu
# Serbian Chemical Society member.
doi: 10.2298/JSC0710953K

On the other hand, in higher concentrations, naphthenic acids and their salts show harmful effects on plants, for example, they inhibit leaf growth, stomatal conductance and net photosynthesis in aspen (*Populus tremuloides*) seedlings.[6]

The effect on rooting could be also examined by the activity of some enzymes, such as IAA oxidase, amylase and peroxidase,[7–10] as well as by the dynamics of glucose and myoinositol.[11]

The aim of this study was to investigate the biochemical changes occurring in softwood cuttings of black locust in the presence of sodium naphthenate.

Naphthenic acids from Vojvodina crude oil "Velebit" have the general chemical formula $C_nH_{2n+z}O_2$, where n represents the carbon number and z is the homologue series number related to the number of five- or six-carbon atom rings within the structure (Fig. 1).[12]

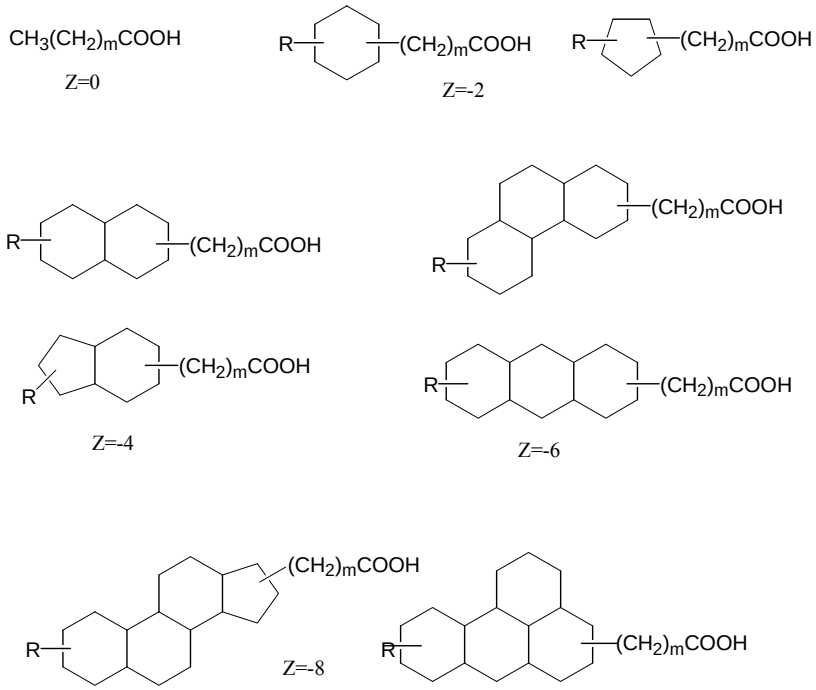

Fig. 1. Typical structures of naphthenic acids in the z homologue series. R represents an alkyl group and *m* represents the length of the alkyl chain.

EXPERIMENTAL

Isolation and structural analysis of naphthenic acids

The naphthenic acids were isolated from the atmospheric gas oil fraction distilling in the temperature interval 168−290 °C of Vojvodina crude oil "Velebit" by an optimized alkaline extraction procedure.[13]

Structural analyses of the isolated naphthenic acids were carried out using naphthenic acids or methyl esters. The esterification method is described in published works.[3,12] The structure and pu-

rity of the investigated acids and esters were determined using ASTM-standardized methods,[14,15] elemental microanalysis performed according to Densted, structural analysis by the standard n-d-M-analysis.[14,15] The density was measured using a DE 40 Density meter, Mettler, at 25 °C and the refraction index on an Abe refractometer, Oficine Galileo.

The FTIR spectra were obtained using a Nicolet termo IR 670 Spectrometer and the band positions (λ_{max}) are given in cm^{-1}. Low resolution mass spectra were obtained on a Varian MAT-311A mass spectrometer, using chemical ionization (CI) as the ion source. The spectra recorded employing a CI source gave almost exclusively signals of the $[M+1]^+$ ions. In this case, the sample introduced into the chamber was diluted with a large quantity of gas carrier (isobutane). The resulting sample concentration was approximately 1 % and the pressure was about 0.07–0.12 kPa.

Plant material

Softwood cuttings were taken from adult trees of black locust, genotype Rozaszin-AC. The softwood cuttings were soaked in a solution of sodium naphthenate, concentration of 10^{-7} mol dm^{-3}, for 3 h for the first group of cuttings and 6 h for the second group. Subsequently, the cuttings were transferred into distilled water, whereas control cuttings were kept all the time in distilled water. For comparison, the cuttings were also treated in the same manner with 1-naphthaleneacetic acid (NAA) of the same concentration as sodium naphthenate. The cuttings were kept in a greenhouse at 25 °C and relative humidity of 80 %. After 1, 3 and 6 days, the lower 2 cm of the cuttings were taken for biochemical analyses.

Biochemical parameters and statistics

Samples were subjected to extraction,[16] to determine the peroxidase activity,[16] amylase activity[17] and activity of IAA oxidase[18] in the extract. One unit of peroxidase activity (U) was defined as the increase of one unit of absorbance per minute under the assay conditions, the enzymatic activity being referred to fresh mass. The content of soluble proteins was determined,[19] as was the content of reducing sugars after Miller.[20] The experiments were carried out in five replicates and the data were treated using the analysis of the variance and LSD test.

RESULTS AND DISCUSSION

The physicochemical characteristics and structural n-d-M analysis of the raw material and isolated naphthenic acids are shown in Table I. The raw material used for the isolation of crude naphthenic acids is the fraction with characteristics very close to those of diesel fuel (Table I). The content of naphthenic acids in the raw oil distillate, calculated on the basis of the acid number, average molecular mass and density of the raw material, was 0.25 % (2.12 g dm^{-3}). The average molecular mass of the naphthenic acids was determined to be 266 and this value was used to prepare solutions and for the biological experiments. The composition of the mixture of naphthenic acids was determined on the basis of low resolution mass spectra, which showed that the largest portion of the naphthenic acids belonged to the bicyclic class of carboxylic acids (Table II).

In all cases, the activities of IAA oxidase and amylase increased until the third day and thereafter decreased. The effect was more pronounced after the three-hour treatment with sodium naphthenate, compared to the six-hour treatment and control (Fig. 2A and 2B). The total peroxidase activity was increased on the first day after the treatment, but exhibited a decrease three days thereafter. The effect was the most pronounced after the three-hour treatment with sodium

naphthenate (Fig. 2C). For all treatments, including control as well, the content of soluble proteins increased one day after the treatment, decreased to the third and again increased to the sixth day, except for the six-hour treatment with sodium naphthenate, when the effect was completely the opposite (Fig. 3A). The changes in the content of reducing sugars were not so clear (Fig. 3B). The effects of three- and six-hour treatments with NAA were between those of the corresponding treatments with sodium naphthenate.

TABLE I. Physicochemical characteristics and structural n-d-M analysis of the raw material and isolated naphthenic acids

	Oil fraction	Naphthenic acids
Distillation interval, °C	168–290	190–390
Density, kg m^{-3}	848.2	947.1
Refraction index	1.4639	1.4903
Acid number, mg KOH g^{-1}	0.51	201.15
Structural n-d-M analysis (Content, mass %)		
C_A	–	1.1
C_N	–	59.7
C_P	–	39.2
Average molecular C-atoms	16	16–17
Average relative molecular mass	248	266
Elemental microanalysis (Content, mass %)		
C		76.69
H		11.28
O		12.00
Average molecular formula		$C_{17}H_{30}O_2$

TABLE II. Low resolution mass spectra of naphthenic acids using the CI method

Class of carboxylic acids	*Z* series $C_nH_{2n+z}O_2$	Series of molecular peaks $(M+1)^+$	No. of C-atoms in molecule *Z*-series	*Z*-series content in total acid mixture mass %
Aliphatic	$Z = 0$ $C_nH_{2n}O_2$	257(0.3); 271(0.5); 285(1.0); 299(0.2)	15–18	2.0
Monocyclic	$Z = -2$ $C_nH_{2n-2}O_2$	241(1.0); 255(2.6); 269(2.8); 283(3.2); 297(3.6); 311(3.0); 325(2.5); 339(1.4); 353(0.7)	14–22	20.8
Bicyclic	$Z = -4$ $C_nH_{2n-4}O_2$	225(1.2); 239(3.0); 253(5.5); 267(7.3); 281(8.0); 295(6.2); 309(5.0); 323(3.2); 337(1.7); 351(0.8)	13–22	41.9
Tricyclic	$Z = -6$ $C_nH_{2n-6}O_2$	237(1.2); 251(2.6); 265(4.4); 279(6.4); 293(7.1); 307(4.6); 321(2.1); 335(0.8); 349(0.5)	14–22	29.7
Tetracyclic	$Z = -8$ $C_nH_{2n-8}O_2$	263(1.4); 277 (1.6); 291(1.9);305(1.2)	16–19	6.1

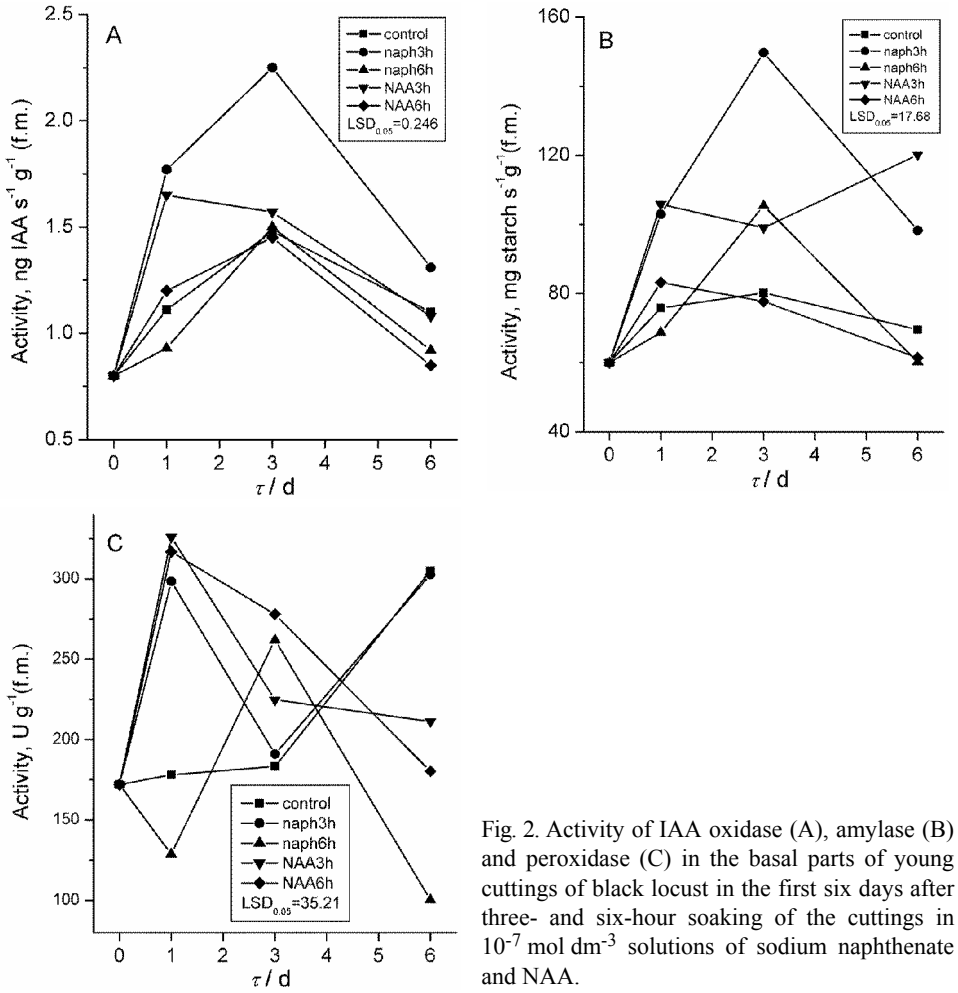

Fig. 2. Activity of IAA oxidase (A), amylase (B) and peroxidase (C) in the basal parts of young cuttings of black locust in the first six days after three- and six-hour soaking of the cuttings in 10^{-7} mol dm^{-3} solutions of sodium naphthenate and NAA.

The results obtained three days after the treatment show that the highest activities of IAA oxidase and amylase and the lowest activity of peroxidase were obtained after the three-hour treatment with 10^{-7} mol dm^{-3} solution of sodium naphthenate. Such an effect is well known as a stimulatory for the initiation and activation of primordial.[7,8,21] The effect was more pronounced compared to that of the treatment with NAA. These results are also in concordance with the results of Loh and Severson,[22] who observed a stimulatory effect of a one-day treatment with potassium naphthenate on IAA oxidase. There were no clear changes in the content of reducing sugars, which is probably caused by the nature of the softwood cuttings (constant income of assimilates from the leaves). It is opposite to the observations of Tschaplinski and Blake,[11] who worked with poplar hardwood cuttings which had no leaves at the beginning of rooting and the content of

reducing sugars depended on the intensity of activation of the starch reserves. However, Severson[23] found that potassium naphthenates stimulated glucose uptake in beans roots.

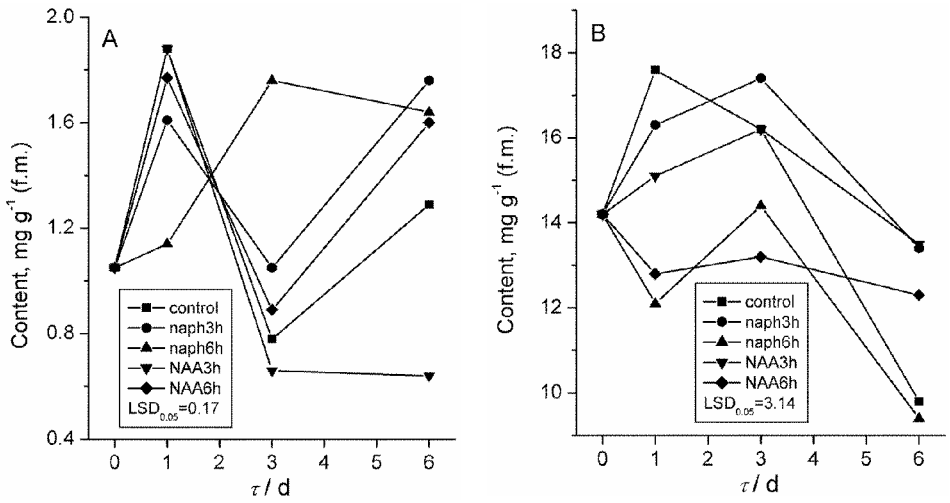

Fig. 3. Contents of soluble proteins (A) and reducing sugars (B) in the basal parts of young cuttings of black locust in the first six days after three- and six-hour soaking of the cuttings in 10^{-7} mol dm^{-3} solutions of sodium naphthenate and NAA.

In contrast to the stimulatory effect of a three-hour treatment, a six-hour treatment had an inhibitory effect on the examined biochemical markers (*e.g.*, the lowest level of soluble proteins one day after the treatment), which is in agreement with previous results.[5,24] Thus, despite of its low concentration, sodium naphthenate during prolonged treatment showed some harmful effects.

The presented results suggest that sodium naphthenate can exhibit a stimulatory effect on several biochemical markers of rooting. Complete information could be obtained with experiments in a greenhouse and with tissue cultures.

Acknowledgement: This study was supported by the Ministry of Science of the Republic of Serbia, project No. 142005B.

ИЗВОД

БИОХЕМИЈСКЕ ПРОМЕНЕ У РЕЗНИЦАМА *Robinia pseudoacacia* НАКОН ТРЕТМАНА СА НАФТЕНАТОМ

СЛАВКО КЕВРЕШАН[1], БРАНИСЛАВ КОВАЧЕВИЋ[2], ВЕРА ЋИРИН–НОВТА[3], КСЕНИЈА КУХАЈДА[3], ЈУЛИЈАН КАНДРАЧ[1], КСЕНИЈА ПАВЛОВИЋ[3] И ЉУБИЦА ГРБОВИЋ[3]

[1]*Poqoprivredni fakul te t, Univerzi te t u Novom Sadu, Trg D. Obradovi}a 8, Novi Sad,* [2] *Ins t i tut za nizijsko {umars tvo i za{ t i tu ` ivo t ne sredine, An t ona ^ehova 1, Novi Sad i* [3]*Depar t man za hemiju, Pri rodno–ma t ema t i~ki fakul te t, Univerzi te t u Novom Sadu, Trg D. Obradovi}a 8, Novi Sad*

Нафтенске киселине су изоловане из гасне фракције (интервал дестилације 168–290 °C) војвођанске нафте “Велебит”, окарактерисане и њихова биолошка активност испитана путем

биохемијских промена у резницама *Robinia pseudoacacia* након третмана са натријум-нафтенатом. Активности IAA пероксидазе, укупна пероксидазна активност и активност амилазе заједно са садржајем редукујућих шећера и протеина одређене су у базалним деловима резница багрема након третмана са натријум-нафтенатом и натријумовом соли (1-нафтил)сирћетне киселине у концентрацији од 10^{-7} mol dm^{-3} у току 3 или 6 сати. Високе активности IAA оксидазе и амилазе праћене ниском активиношћу пероксидазе (што је познато као стимулативно за иницијацију и активацију коренских примордија) добијене су након трочасовног третмана натијум-нафтенатом. Шесточасовни третман је испољио инхибиторни ефекат на испитане биохемијске маркере. Ефекти трочасовног и шесточасовног третмана са NAA су били између одговарајућих третмана са нафтенским киселинама.

(Примљено 18. децембра 2006, ревидирано 27. фебруара 2007)

REFERENCES

1. S. Kevrešan, V. Ćirin–Novta, K. Kuhajda, J. Kandrač, N. Petrović, Lj. Grbović, Ž. Kevrešan, *Biol. Plant*. **48** (2004) 453
2. S. Kevrešan, V. Ćirin–Novta, K. Kuhajda, J. Kandrač, N. Petrović, Lj. Grbović, Ž. Kevrešan, *Belgian J. Bot.* **138** (2005) 11
3. V. Ćirin–Novta, K. Kuhajda, S. Kevrešan, J. Kandrač, Lj. Radić, *Acta Period. Technol.* **33** (2002) 135
4. S. Kevrešan, V. Ćirin–Novta, D. Vasić, K. Kuhajda, J. Kandrač, N. Petrović, Lj. Radić, *Helia* **26** (2003) 18
5. S. Kevrešan, V. Ćirin–Novta, B. Kovačević, D. Vasić, K. Kuhajda, J. Kandrač, N. Petrović, Lj. Radić, *Topola* **171/172** (2003) 63
6. M. Kamaluddin, J. J. Zwiazek, *Tree Physiol*. **22** (2002) 1265
7. N. C. Bhattacharya, in *Adventitious root formation in cuttings,* T. D. Davis, B. E. Haissig, N. Sanklila, Eds., Dioscorides Press, Portland, 1989, p. 88
8. T. Güneş, *Turk. J. Bot*. **24** (2000) 97
9. A. N. Molassiotis, K. Dimassi, G. Diamantidis, I. Therios, *Biol. Plant*. **48** (2004) 1
10. C. Saxena, S. Samantaray, G. R. Rout, P. Das, *Biol. Plant.* **43** (2000) 121
11. T. J. Tschaplinski, T. J. Blake, *Can. J. Bot*. **67** (1989) 2168
12. V. Ćirin–Novta, K. Kuhajda, S. Kevrešan, J. Kandrač, Lj. Grbović, P. Rodić, *Acta Period. Technol.* **35** (2004) 87
13. V. Ćirin–Novta, S. Kevrešan, K. Kuhajda, J. Kandrač, Lj. Radić, P. Rodić, *Acta Period. Technol.* **34** (2003) 49
14. R. V. Anbroh, *Petrol. Chem.* **12** (1972) 230
15. D. M. Shopov, K. I. Kovaceva, N. P. Dimov, *Petrol. Chem.* **24** (1984)169
16. U. C. Basak, A. B. Das, P. Das, *Marine Biol.* **136** (2000) 185
17. B. Panjabi, R. N. Basu, *Indian Biologist* **10** (1978) 65
18. M. A. Quesada, C. Sanchez–Roldan, A. Heredia, V. Valpuesta, M. Bukovac, *J. Plant Growth Regul.* **11** (1992) 1
19. M. M. Bradford, *Anal. Biochem*. **72** (1976) 248
20. G. L. Miller, *Anal. Chem*. **31** (1959) 426
21. T. Gaspar, M. Hofinger, in *Adventitious root formation in cuttings*, T. D. Davis, B. E. Haissig, N. Sanklila, Eds., Dioscorides Press, Portland, 1989, p. 117
22. J. W. C. Loh, J. G. Severson, *Phytochemistry* **14** (1975) 1265
23. J. G. Severson, *Phytochemistry* **11** (1972) 71
24. S. Kevrešan, B. Kovačević, D. Vasić, V. Ćirin–Novta, K. Kuhajda, *Cercetari stiintifice – Horticultura*, Seria A IX-A, Partea a II-a (2005) 1.