Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 80 (6) 749–754 (2015)
JSCS–4754
UDC 547.657:576.314:541.132.3
Short communication

SHORT COMMUNICATION

The influence of naphthenic acids and their fractions on cell membrane permeability

KSENIJA PAVLOVIĆ[1*#], LJUBICA GRBOVIĆ[1#], BOJANA VASILJEVIĆ[1#], ANDREA ŽUPUNSKI[2], MARINA PUTNIK-DELIĆ[2], IVANA MAKSIMOVIĆ[2] and SLAVKO KEVREŠAN[2**#]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, Serbia and* [2]*Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, Serbia*

(Received 1 December 2014, revised 29 January, accepted 3 February 2015)

Abstract: The influence of naphthenic acids (NAs) mixture and their narrow fractions (called NA pH 4, pH 8 and pH 10) on the permeability of beetroot cell membrane was examined. The results showed that the effect depends on the treatment duration, concentration and the structures of the NAs. Longer treatment of plant cell membranes with sodium naphthenate (Na-naph) resulted in an increase in the membrane permeability (*e.g.*, a 4-hour treatment with Na-naph (c = 100 μmol L^{-1}) increased the membrane permeability by about 3 times, while prolongation of treatment to 24 h resulted in an 18-fold increase in the effect). NAs in the concentration range from 0.1 to 10 μmol L^{-1} did not change membrane permeability, while the membrane permeability increased linearly with increasing concentration from 10–100 μmol L^{-1}. The fraction pH 8, where bi- and tricyclic carboxylic acids are the most abundant, expressed the strongest effect. These structures were predominant in the total NAs mixture as well. This could explain the similar, but slightly weaker effect of the mixture, compared to the effect of the NAs present in fraction pH 8. The effect of NAs on beetroot cell membrane was between the effects of the tested anionic (SDS and LS) and non-ionic surfactants (Triton X-100).

Keywords: naphthenic acids (NAs); naphthenic acid fractions; physiological activity; cell membranes permeability.

INTRODUCTION

Crude oil contains 95–98 % of structurally similar hydrocarbon molecules, while the rest consists of oxygen, sulfur and nitrogen compounds. Carboxylic

*,** Corresponding authors. E-mail: (*)ksenija.pavlovic@dh.uns.ac.rs, (**)kevresan@polj.uns.ac.rs
Serbian Chemical Society active member.
doi: 10.2298/JSC141201012P

acids present in the oil are mostly alkane and cycloalkane compounds, called naphthenic acids (NAs). NAs present chemically stabile compounds, while, depending of the oil source, their relative molecular mass could be in a wide range and the structures quite different. Carboxylic acids are present in all types of oil, occurring in concentrations of 2.5–0.5 % or even less, on average. NAs represent a "finger print" for each type of oil, giving very important data about the genesis and nature of a particular oil.

The NAs isolated from Vojvodina crude oil Velebit[1,2] represent mixtures of alkyl-substituted cycloaliphatic and acyclic monocarboxylic acids, with bi- and tricyclic carboxylic acids being predominant.[3]

Fractionation of the total NAs, based on their different solubilities in water and modification of the solution pH afforded narrow fractions of the acids. The concentrating of the active structures into narrow fractions leads to differences in physiological activity of the fractions, mimicking the activity of certain plant hormones of the auxin and gibberellin type, which could be attributed to the structural differences of the examined NAs, determined based on high-resolution mass spectroscopy.[3]

There are very few data about the influence of NAs on cation uptake.[4,5] There are only data about their influence on the uptake of glucose[6] and phosphate ions[7] of bean plants. Previous results suggested that sodium naphthenate (Na-naph) in low concentrations acted at the level of the root cell membrane, causing subtle modifications of the membrane and changes in its permeability for cadmium ions.[8] Furthermore, low-concentration treatment of young soybean plants with Na-naph (10^{-7} mol L^{-1}) affected the accumulation of some essential elements (Mn, Fe, Zn, Ni, K and Na).[5]

Furthermore, being amphiphilic molecules, molecules that have both hydrophilic (carboxylic group) and hydrophobic (hydrocarbon) parts, similarly to detergents, NAs could act as surfactants. It is well known that detergents could cause changes in cell function and transport through the membrane by changing the membrane structures and membrane exchange processes.[9] Thanks to their physicochemical similarities with detergents, it could be assumed that NAs could act as surfactants as well, by specific modification and thus influence the permeability of cell membranes.

The aim of this research was to study the influence of total NAs and their fractions on cell membrane permeability, using beetroot (*Beta vulgaris*) as a model-system,[10,11] and to compare the effects of NAs with the effects of amphiphilic ionic (*N*-lauroylsarcosine, sodium salt, LS and sodium dodecyl sulfate, SDS) and non-ionic (polyethylene glycol, 4-(1,1,3,3-tetramethylbutyl)phenyl-ether, Triton X-100) surfactants.

The study of changes in the permeability of plant cell membrane was performed by measuring the absorbance of samples containing betanin, originating from treated segments of beetroot.[11]

EXPERIMENTAL

Isolation, fractionalization and characterization of naphthenic acids from Vojvodina crude oil Velebit was described previously.[1-3]

Determination of the effects of NAs on the beetroot cell membrane permeability

Comparative tests of the effects of Na-naph, LS, SDS and Triton X-100 were performed with concentration of the tested compounds corresponding to one *CMC* (critical micellar concentration; 1 $CMC_{\text{Na-naph}}$ = 2.4 mmol L^{-1}, 1 CMC_{LS} = 14.6 mmol L^{-1}, 1 CMC_{SDS} = 8.2 mmol L^{-1} and 1 $CMC_{\text{Triton X-100}}$ = 0.23 mmol L^{-1}).

For all calculations and solution preparation, the average molar masses of total NAs (M_{NAs} = 280.8 g mol^{-1}) and the corresponding fractions ($M_{\text{NAs pH 4}}$ = 328 g mol^{-1}, $M_{\text{NAs pH 8}}$ = = 425 g mol^{-1} and $M_{\text{NAs pH 10}}$ = 285 g mol^{-1}) were used.

Stock (1 mmol L^{-1}) of solution Na-naph was prepared by dissolving the necessary amount of total NAs in an equimolar solution of sodium hydroxide. The solutions of the Na-naph for test were prepared by dilution with water, as well as the Na salts of the NAs fractions obtained at pH 4, 8 or 10.

Cylinders (diameter of 6 mm) removed from beetroot were cut into discs of 2 mm thickness. The obtained uniform segments were washed with water for 6 h, and then left overnight in water at 4 °C. Twenty beetroot segments were then treated with the required test solution (20 mL; experiments were repeated three times. Standard deviations of the mean results were within ±5%), and the betanin concentrations were estimated by measuring absorbance at betanin-characteristic maximum (538 nm,[11] UV/Vis 6105 Jenway, UK, spectrophotometer) immediately after adding the test solution (zero time) and after 2, 4, 6, 8, 10, 12 and 24 h.

RESULTS AND DISCUSSION

The results of this study showed that NAs changed permeability of the cell membrane of beetroot. Namely, as can be seen from Fig. 1, the effect of Na-naph on beetroot cell membrane permeability practically laid between the effects of the tested non-ionic (Triton X-100) and ionic (LS and SDS) surfactants.

These comparative studies indicated that NAs at a 1 *CMC* concentration influenced significantly the cell membrane permeability, which prompted a study of the effect of lower concentrations of NAs on the membrane permeability. It was found that there was practically no change in the membrane permeability with increasing concentration from 0.1 to 10 μmol L^{-1}, while thereafter, linear increases in permeability were evidenced with further concentration increases. Based on these facts, further tests were performed with Na-naph in the concentration range from 10 to 100 μmol L^{-1}. The results presented on Fig. 2, show that the absorbance of the solutions at all tested concentrations increased with prolongation of the treatment. Thus, the membrane permeability had increased by about 3 times after 4 h of treatment with NAs (c = 100 μmol L^{-1}) and nearly 18 times after 24-h treatment.

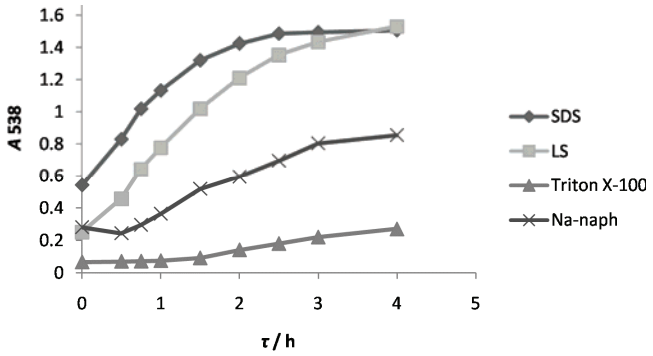

Fig. 1. Time dependencies of the cell permeabilities, estimated by the betaine-characteristic absorbance maximum (538 nm) of 1 *CMC* solutions of non-ionic (Triton X-100) and ionic (LS and SDS) surfactants and Na-naph.

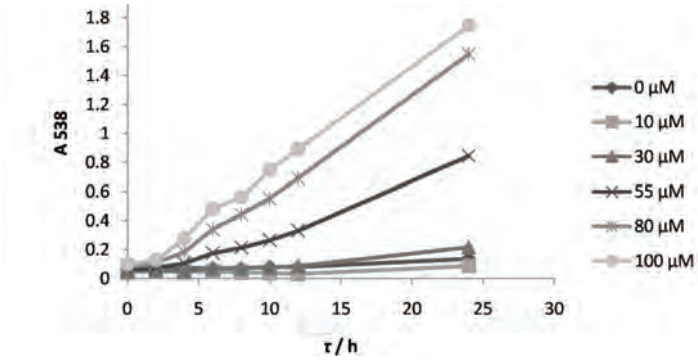

Fig. 2. Time dependency of the cell permeabilities, estimated at the betaine-characteristic absorbance maximum (538 nm) of different concentrations of total NAs salts (Na-naph).

It is evident that the strongest effect on cell membrane permeability showed the most concentrated test solution (c = 100 μmol L^{-1}). Comparing to the solution c = 10 μmol L^{-1} during 24 h, this was an about 20 times stronger effect on the membranes.

Similar tendencies of increasing membrane permeability as a function of time and concentration were exhibited by aqueous solutions of Na–naph fractions. By comparing the data obtained after 24-h treatment (Fig. 3), it could be seen that the strongest effect on membrane permeability was expressed by the fraction isolated from total NAs at pH 8, and that this effect was even stronger than the effect of the total NAs. The effects on membrane permeability, based on these results could be correlated to the structures of the tested NAs.[3] Namely, in the fraction of NAs labeled as pH 8, the most common NAs were bi- and tricyclic carboxylic acids, which were also the most common in the mixture of the total NAs. The fraction isolated at pH 4, consisting predominantly of tricyclic carboxylic acids, showed a somewhat lower effect on membrane permeability. Finally,

NAs fraction isolated at pH 10, with acyclic carboxylic acids being predominant, exhibited the lowest effect.

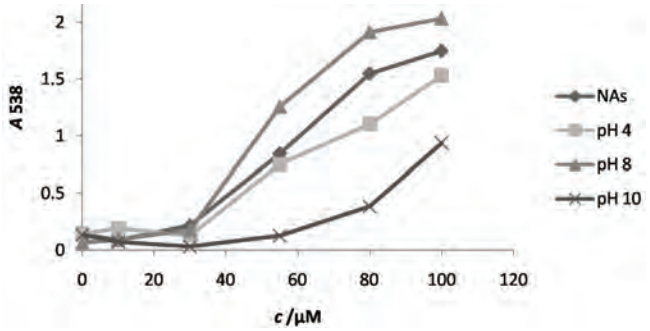

Fig. 3. Estimation of cell permeabilities 24 h after treatment with different concentrations of Na-NAs solutions of total NAs and NAs isolated at pH 4, pH 8 or pH 10, using the betaine-characteristic absorbance maximum (538 nm) as the probe.

Connected with these results, it was observed that the biggest differences in membrane permeability are expressed at c = 80 μmol L^{-1} (Fig. 3), whereby fraction pH 8 showed (by about 24 %) stronger and the fraction pH 4 (by about 29 %) and pH 10 (by about 75 %) lower effects compared to the mixture of total NAs.

Similar effects on membrane permeability were observed in the experiments with Na-naph solutions at c = 55 and 100 μmol L^{-1} (Fig. 3).

CONCLUSION

Generally, it could be concluded that the effect of total NAs and its fractions on cell membrane permeability increased with increasing concentration and prolongation of treatment. The results showed that some differences in membrane permeability could be correlated with the structures of carboxylic acids present predominantly in the total NAs and its fractions. Namely, the fraction of NAs isolated at pH 8 had the most similar chemical composition to that of the total NAs; hence, it could be concluded that bi- and tricyclic carboxylic acids from this fraction are mostly responsible for increasing cell membrane permeability. However, the effect of the fraction isolated at pH 8 was slightly more expressed compared to the effect of the total NAs. Considering this fact and the fact that fraction pH 4, with tricyclic carboxylic acids as most common, showed something lower effect, comparing to total NAs, the conclusion is that bicyclic carboxylic acids are the mostly responsible for changes in cell membranes. Finally, fraction pH 10, with the lowest influence on cell membrane, consisted predominantly of acyclic carboxylic acids. The obtained results indicated that a possible cause of phytotoxicity of NAs could be their influence on cell membrane permeability.

Acknowledgements. This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No. 172006 and Project No. TR31036.

И З В О Д

УТИЦАЈ НАФТНИХ КИСЕЛИНА И ЊИХОВИХ ФРАКЦИЈА НА ПРОПУСТЉИВОСТ ЋЕЛИЈСКИХ МЕМБРАНА

КСЕНИЈА ПАВЛОВИЋ[1], ЉУБИЦА ГРБОВИЋ[1], БОЈАНА ВАСИЉЕВИЋ[1], АНДРЕА ЖУПУНСКИ[2], МАРИНА ПУТНИК-ДЕЛИЋ[2], ИВАНА МАКСИМОВИЋ[2] и СЛАВКО КЕВРЕШАН[2]

[1]Департман за хемију, биохемију и заштиту животне средине, Природно–математички факултет, Универзитет у Новом Саду, Трг Доситеја Обрадовића 3, Нови Сад и [2]Пољопривредни факултет, Универзитет у Новом Саду, Трг Доситеја Обрадовића 8, Нови Сад

Испитиван је утицај смеше нафтних киселина (naphthenic acids; NAs) и њихових ужих фракција (означених као pH 4, pH 8 и pH 10) на пропустљивост ћелијских мембрана цвекле. Резултати су показали да ефекат зависи од времена деловања, концентрације и структуре NAs. Дужи контакт натријум-нафтената (Na-naph) са биљним мембранама доводи до пораста мембранске пропустљивости (нпр. третман Na-naph (c = 100 μmol L^{-1}) за време од 4 сата повећава пропустљивост мембрана око три пута док продужавањем третмана на 24 сата поменути ефекат порасте око осамнаест пута). NAs у опсегу концентрација од 0,1 до 10 μmol L^{-1} не изазивају промене у пропустљивости мембрана а порастом концентрације од 10–100 μmol L^{-1} пропустљивост линеарно расте. Најснажнији ефекат испољила је фракција pH 8 у којој су најзаступљеније би- и трицикличне структуре карбоксилних киселина. Ове структуре су истовремено доминантне и у укупној смеши NAs чиме би се могао објаснити њихов најближи али нешто слабији ефекат у односу на карбоксилне киселине концентрисане у фракцији pH 8. Ефекат NAs на пропустљивост ћелијских мембрана цвекле је између ефекта који имају анјонски сурфактанти и нејонски сурфактант.

(Примљено 1. децембра 2014, ревидирано 29. јануара, прихваћено 3. фебруара 2015)

REFERENCES

1. V. Ćirin-Novta, K. Kuhajda, S. Kevrešan, J. Kandrač, Lj. Radić, *Acta Period. Technol.* **33** (2002) 135
2. V. S. Ćirin-Novta, S. E. Kevrešan, K. N. Kuhajda, J. E. Kandrač, Lj. M. Radić, P. A. Rodić, *Acta Period. Technol.* **34** (2003) 49
3. Lj. Grbović, K. Pavlović, B. Prekodravac, K. Kuhajda, S. Kevrešan, M. Popsavin, J. Milić, V. Ćirin-Novta, *J. Serb. Chem. Soc.* **77** (2012) 147
4. G. G. Slivinskii, A. F. Artamonov, M. I. Goryaev, *Tsitologiya* **17** (1975) 1283
5. S. Kevrešan, V. Ćirin-Novta, K. Kuhajda, J. Kandrač, N. Petrović, Lj. Grbović, Ž. Kevrešan, *Belg. J. Bot.* **138** (2005) 11
6. J. G. Severson, *Phytochemistry* **11** (1972) 71
7. D. J. Wort, J. G. Severson, D. R. Peirson, *Plant Physiol.* **52** (1973) 162
8. S. Kevrešan, V. Ćirin-Novta, K. Kuhajda, J. Kandrač, N. Petrović, Lj. Grbović, Ž. Kevrešan, *Biol. Plant.* **48** (2004) 453
9. M. H. Mohamed, L. D. Wilson, K. M. Peru, J. V. Headley, *J. Colloid Interface Sci*. **395** (2013) 104
10. C. Grunwald, *Plant Physiol.* **43** (1968) 484
11. D. L. Sutton, C. L. Foy, *Bot. Gaz. (Chicago)* **132** (1971) 299.