

**QUANTITATIVE STRUCTURE-GAS CHROMATOGRAPHIC
RETENTION INDEX RELATIONSHIP OF LONG-CHAIN
ESTERS: THE CASE OF *SCANDIX PECTEN-VENERIS*
L. ESSENTIAL-OIL CONSTITUENTS[†]**

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Abstract. *Motivated by a recent identification of two homologous series of branched butanoates and pentanoates in *S. pecten-veneris* essential oil, with an apparently regular change of their retention index (RI) values, we decided to examine the generality of such structure-chromatographic property relationship. Based on the experimentally obtained retention data (RI values of in total 20 compounds) of hexyl, decyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, heneicosyl and tricosyl isobutanoates and 3-methylbutanoates and selected 2-methylbutanoates, a model was built up that correlates the total number of carbon atoms, Wiener (WI), Balaban (BI) and molecular topological (MTI) indices of the mentioned esters and their RI data ($RI = 240.5 + 91.2 \times C + 2.94 \times WI + 4.6 \times 10^{-5} \times BI - 0.381 \times MTI$). The obtained equation represents a new and simple tool for the prediction of gas chromatographic (retention indices) data for esters of straight long-chain fatty alcohols and branched short aliphatic acids.*

Key words: *Scandix pecten-veneris L., essential oil, structure-retention index relationship, esters, fatty alcohols*

1. INTRODUCTION

Recent GC and GC/MS analyses of seven *Scandix pecten-veneris* L. essential-oil samples revealed a series of minor constituents that were tentatively identified as long-chain esters of butanoic and pentanoic acids (Radulović et al., 2014). Some of these esters were also detected in the essential oil of *S. balansae* Reut. ex Boiss (Radulović et

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Dedicated to Professor Radosav Palić on the occasion of his 70th birthday.

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al., 2013). Since the isolation of single compounds from the available essential-oil samples was impossible (these compounds were present in plant tissues in amounts lower than 0.001%), a successful identification was achieved through the application of a synthetic approach that also enabled the determination of their potential biological role (Radulović et al., 2013, 2014). The identified esters of straight long-chain alcohols with 6 to 23 carbon atoms were either new or represented very rare plant secondary metabolites (Radulović et al., 2014). We believe that one of the reasons for their infrequent identification as plant/animal metabolites is the result of the absence of the corresponding chromatographic (RI) data in the literature.

Usually, in the course of a GC/MS analysis of an essential oil, in the absence of a positive match in a mass spectral library or when there is a lack of literature RI data, phytochemists tend to disregard the detected constituent completely or, in the best case scenario provide a mass spectrum and state that such a compound remained unidentified. Thus, the accumulation of experimental and/or theoretical calculation of gas chromatographic retention index values represents an important task since it will enable a great number of researchers to complete their analyses with ease.

The interaction between the stationary phase in the gas chromatography capillary column and the gas-phase molecules reflects the electronic and geometrical properties of the molecule in question. Mathematical modeling of these interactions enables chemists to predict the retention index (RI) of even new compounds. Building retention prediction models may be initiated by a correlation analysis of known structural descriptors, which can be a simple number of present atoms or a more elaborate topological index, with the experimentally obtained values of RI. Quantitative structure-retention relationships (QSRR) are most popular. A number of reports that deal with QSRR retention index calculation of different compounds have appeared in the literature (Goryński et al. 2013; Kalisz 1992; Radulović et al. 2015).

Thus, in the continuation of our previous studies of GC and GC/MS identification of the volatile plant constituents, in this work we investigated a structure-chromatographic behavior relationship with the aim of finding a general mathematical expression connecting structure descriptors of long-chain esters and their chromatographic retention data (retention indices). We hope that the data given in this work will make future identification of related natural compounds a straightforward task.

2. MATERIALS AND METHODS

2.1. Chemicals

For the determination of retention indices, two hydrocarbon mixtures (Sigma-Aldrich, USA) ranging from heptane to eicosane and from heneicosane to tetracontane were used. All solvents (HPLC grade), were purchased from Sigma-Aldrich. The following esters of eight straight long-chain alcohols and three aliphatic acids: hexyl 2-methylpropanoate, decyl 2-methylpropanoate, decyl 3-methylbutanoate, tridecyl 2-methylpropanoate, tridecyl 2-methylbutanoate, tridecyl 3-methylbutanoate, tetradecyl 2-methylpropanoate, tetradecyl 3-methylbutanoate, pentadecyl 2-methylpropanoate, pentadecyl 2-methylbutanoate, pentadecyl 3-methylbutanoate, hexadecyl 2-methylpropanoate, hexadecyl 3-methylbutanoate, heptadecyl 2-methylpropanoate, heptadecyl 2-methyl-butanoate, heptadecyl 3-methylbutanoate,

octadecyl 2-methylpropanoate, octadecyl 3-methylbutanoate, heneicosyl 2-methylpropanoate, tricosyl 2-methylpropanoate, were prepared according to the following general Steglich procedure (*N,N'*-dicyclohexylcarbodiimide (DCC)/4-(dimethylamino)pyridine (DMAP)) as described previously (Radulović et al., 2014). The resulting residue was purified by MPLC chromatography using silica gel and mixtures of increasing polarity of hexane and diethyl ether as the eluent and the purity of the ester fractions was checked using TLC (diethyl ether-hexane, 1:20, v/v).

2.2. Gas chromatography/Mass spectrometry (GC/MS) analyses

The GC/MS analyses (three repetitions) were carried out using a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 °C and 300 °C, respectively. Oven temperature was raised from 70 °C to 290 °C at a heating rate of 5 °Cmin⁻¹ and then the heating program ended with an isothermal period of 10 min. As a carrier, gas helium at 1.0 mLmin⁻¹ was used. The samples, 1 μL of the solutions of compounds in diethyl ether (1 mg per 1 mL), were injected in a pulsed split mode (the flow was 1.5 mLmin⁻¹ for the first 0.5 min and then set to 1.0 mLmin⁻¹ throughout the remainder of the analysis; split ratio 40:1). MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35-650, scan time 0.32 s.

2.3. Calculations

Geometry optimizations of esters and calculation of their Wiener (WI), Balaban (BI) and molecular topological (MTI) indices were performed using the MM2 molecular mechanics force field method incorporated in ChemBio 3D Ultra 12.0 software package. Linear regression analysis was done using Microsoft® Office Excel 2003 worksheet functions.

3. RESULTS AND DISCUSSION

Previously, we encountered two homologous series of isobutanoates and isopentanoates in *S. pecten-veneris* essential oil that displayed an apparently regular change of their retention index values. This motivated us to examine the generality of such structure-retention property relationship. First, the dependence of RI values (DB-5MS column) on the total number of carbon atoms of the esters within a homologous series was plotted (Fig. 1A-C). A linear dependence of RI values was observed for the esters of acid and different n-alcohols. The derived equations ($RI = 41.8 + 106.14 \times C$ for isobutanoates, $RI = 50.3 + 103.75 \times C$ for 2-methylbutanoates and $RI = 102.8 + 101.39 \times C$ for 3-methylbutanoates, where C is the total number of carbon atoms in the molecules) had the correlation coefficients (R^2) higher than 0.99.

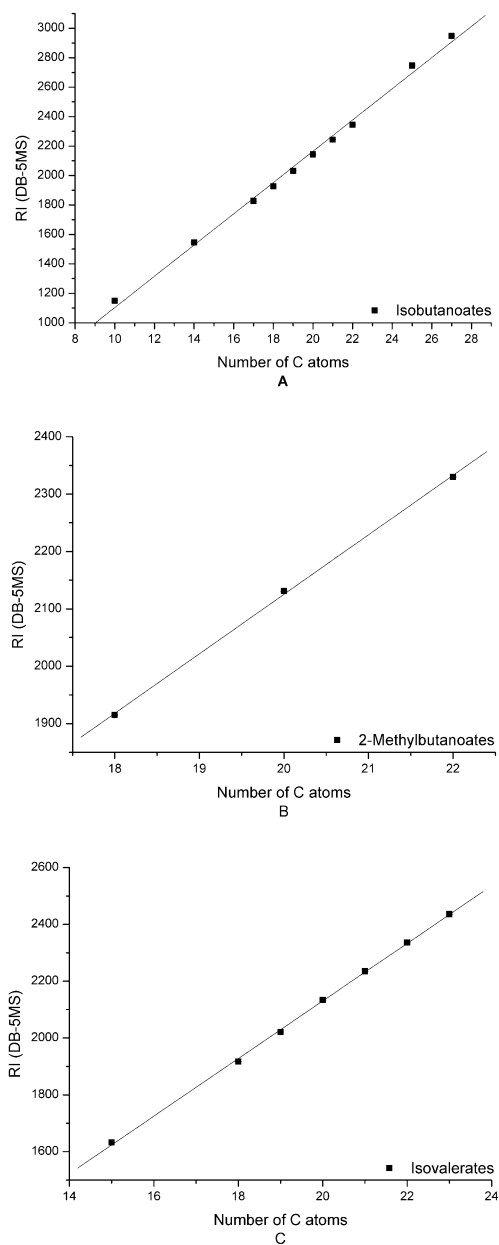


Fig. 1 The dependence of RI values (DB-5MS column) on the number of carbon atoms of the esters of the same acid (**A** – isobutanoic ($RI = 41.8 + 106.14 \times C$ ($R^2 = 0.9997$)), **B** - 2-methylbutanoic ($RI = 50.3 + 103.75 \times C$ ($R^2 = 0.9996$)), **C** - 3-methylbutanoic (isovaleric; $RI = 102.8 + 101.39 \times C$ ($R^2 = 0.9997$)) acid) and different *n*-alcohols

A closer inspection of the RI increments within a series revealed that lower molecular weight members of the series had a much less regular change in Δ RI between neighboring members (being in the range 95-112) when compared with those higher than pentadecyl esters (Δ RI = 100-101). This appears to hold for all series as can be seen from Table 1. The slope of the three linear dependences should correspond to the average increment of RI per CH_2 in the series, and the values (*ca.* 106, 104 and 101, for isobutanoates, 2-methylbutanoates and 3-methylbutanoates, respectively) are in a general agreement with those assigned to an n-alkane series (RI increment of 100). This might be explained by the differing extent of molecular change introduced by an extra methylene group in the lower and higher members of the series. One might speculate that the chain of more than fifteen carbon atoms is sufficient to make the RI increment of isomeric butanoates and pentanoates essentially the same as for the n-alkane series.

The values of y-intercepts, however, are significantly different for the isobutanoates and 2-methylbutanoates (41.8 and 50.3, respectively) on one side and 3-methylbutanoates (102.8) on the other. This means that the α -methyl branch present in isobutanoates and 2-methylbutanoates determines the base value of the RI and that the formal introduction of a methylene to the molecule of the two ester series is not strongly dependent on the elongation site (alcohol or acid moieties). Changing the position of the branch to β drastically alters the y-intercept (by a factor of *ca.* 2). Thus, a direct regioisomeric relationship between the branch and the ester carbonyl group is an important structural feature that determines the value of RI.

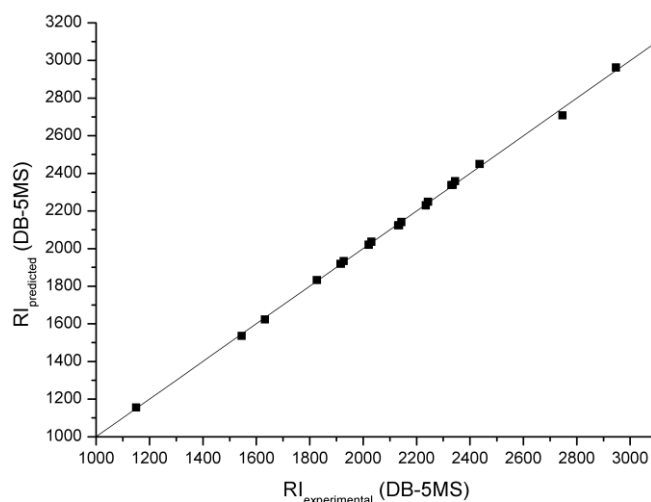


Fig. 2 The correlation between the experimental and calculated RI values (according to the equation $\text{RI} = 240.5 + 91.2 \times \text{C} + 2.94 \times \text{WI} + 4.6 \times 10^{-5} \times \text{BI} - 0.381 \times \text{MTI}$, $R^2 = 0.9997$)

After these considerations, a number of topological indices (Wiener (Wiener, 1947), Balaban (Balaban, 1983) and the molecular topological index (Schultz, 1989)), which would take into account the existence of methyl branches and the branch place in the acid part as the obvious structure differences among the esters, were included in the models in the hope of deriving a single equation that could explain the retention behavior of all compounds at hand.

The correlation of a single topological index with the RI values gave unsatisfactory predicted RIs, but the combination of these and the inclusion of the total number of carbon atoms resulted in an equation ($RI = 240.5 + 91.2 \times C + 2.94 \times WI + 4.6 \times 10^{-5} \times BI - 0.381 \times MTI$, where C is the total number of carbon atoms in the molecules, WI is Wiener index, BI is Balaban index and MTI is the molecular topological index) that predicts RI within an error of ± 18 units ($R^2 = 0.9997$) except for heneicosyl isobutanoate (difference between the experimental and calculated values was 38.5, plot given in Fig. 2).

Table 1 Esters of long-chain *n*-alkanols and isobutanoic, 2-methylbutanoic and isovaleric acids: their (experimental and predicted) retention indices (RI)

Compounds	RI ^{a)}	RI ^{b)}
Hexyl 2-methylpropanoate	1150	1155
Decyl 2-methylpropanoate	1545	1536
Decyl 3-methylbutanoate	1632	1623
Tridecyl 2-methylpropanoate	1827	1832
Tridecyl 2-methylbutanoate	1915	1918
Tridecyl 3-methylbutanoate	1917	1919
Tetradecyl 2-methylpropanoate	1927	1933
Tetradecyl 3-methylbutanoate	2021	2020
Pentadecyl 2-methylpropanoate	2031	2036
Pentadecyl 2-methylbutanoate	2131	2123
Pentadecyl 3-methylbutanoate	2134	2124
Hexadecyl 2-methylpropanoate	2143	2141
Hexadecyl 3-methylbutanoate	2235	2229
Heptadecyl 2-methylpropanoate	2243	2248
Heptadecyl 2-methylbutanoate	2330	2337
Heptadecyl 3-methylbutanoate	2336	2338
Octadecyl 2-methylpropanoate	2340	2358
Octadecyl 3-methylbutanoate	2436	2449
Heneicosyl 2-methylpropanoate	2746	2708
Tricosyl 2-methylpropanoate	2947	2962

^{a)}Retention indices on the apolar DB-5MS column.

^{b)}Predicted RI values according to the equation

$$RI = 240.5 + 91.2 \times C + 2.94 \times WI + 4.6 \times 10^{-5} \times BI - 0.381 \times MTI (R^2 = 0.9997).$$

The equation, unfortunately, is not applicable for the differentiation between 2-methylbutanoates and 3-methylbutanoates since these isomeric compounds elute one close to the other (ΔRI ca. 5 units). However, if one encounters both isomers in the same chromatogram, the faster eluting one is always 2-methylbutanoate. However, this equation still successfully discriminates the retention indices of the isomeric butanoates and pentanoates, even the isomeric isobutanoates and 3-methylbutanoates (average ΔRI predicted

ca. 10 units), i.e. those esters having the same molecular formula but differing in the length of both the acid and alcohol moieties. Thus, if a mass spectral fragmentation indicates an ester of butanoic or pentanoic acids and straight chain n-alcohols, one could, through the use of this equation, even if the molecular ion is absent from the MS in question, deduce the total number of C atoms of the ester and, at least, the number of C atoms of the acidic moiety if not guess the full identity of the ester.

4. CONCLUSIONS

The present study describes a new, fast and simple way (using the derived equations) for the theoretical prediction of gas chromatographic (retention indices) data for straight long-chain esters. The experimentally obtained retention data gave us the opportunity for the construction of equations, with the coefficients of correlation higher than 0.99, for the calculation of retention indices of esters for the branched short aliphatic acids and different straight long-chain 1-alkanols. We hope that the resulting equations $RI = 41.8 + 106.14 \times C$ for isobutanoates, $RI = 50.3 + 103.75 \times C$ for 2-methylbutanoates and $RI = 102.8 + 101.39 \times C$ for isovalerates, as well as a single equation $RI = 240.5 + 91.2 \times C + 2.94 \times WI + 4.6 \times 10^{-5} \times BI - 0.381 \times MTI$ would make the identification of these series of biologically and pharmacologically significant esters much easier.

REFERENCES

- Balaban, A.T., 1983. Pure Appl. Chem. 55, 199–206. DOI: 10.1351/pac198855020199
Goryński, K., Bojko, B., Nowaczyk, A., Buciński, A., Pawliszyn, J., Kaliszczan, R., 2013. Anal. Chim. Acta. 797, 13–19. DOI: 10.1016/j.aca.2013.08.025
Kaliszczan R., 1992. Anal. Chem. 64, 619–631. DOI: 10.1021/ac00035a001
Radulović, N.S., Milojević A.B., Stojković M.B., Blagojević, P.D., 2015. Food Res. Int. 78, 1–10. DOI: 10.1016/j.foodres.2015.11.019
Radulović, N.S., Mladenović M.Z., Blagojević, P.D., 2013. Chem. Biodivers. 10, 1202–1219. DOI: 10.1002/cbdv.201300106
Radulović, N.S., Mladenović M.Z., Stojanović-Radić, Z.Z., 2014. Flavour Frag. J. 29, 255–266. DOI 10.1002/ffj.3205
Schultz, H.P., 1989. J. Chem. Inf. Comp. Sci. 29, 227–228. DOI: 10.1021/ci00063a012
Wiener, H., 1947. J. Am. Chem. Soc. 69, 17–20. DOI: 10.1021/ja01193a700

KVANTITATIVNA VEZA STRUKTURE I RETENCIONI INDEKSA U GASNOJ HROMATOGRAFIJI DUGOLANČANIH ESTARA: SLUČAJ SASTOJAKA ETARSKOG ULJA BILJNE VRSTE *SCANDIX PECTEN-VENERIS* L.

Motivisani nedavnom identifikacijom dve homologe serije račvastih butanoata i pentanoata u etarskom ulju biljne vrste Scandix pecten-veneris L., sa pravilnom promenom vrednosti retencionih indeksa (RI), odlučili smo da detaljnije ispitamo vezu između njihove strukture i hromatografskih karakteristika. Na osnovu eksperimentalno dobivenih retencionih podataka (RI vrednostima ukupno 20 jedinjenja) heksil, decil, tridecil, tetradecil, pentadecil, heksadecil, heptadecil, oktadecil, heneikozil i trikozil izobutanoata i 3-metilbutanoata, i odabranih 2-metilbutanoata, kreiran je matematički model na osnovu koga korelišu ukupan broj ugljenikovih atoma, Vinerov (Wiener (WI)), Balabanov (Balaban

(BI) i molekularni topološki indeks (MTI) pomenutih estara i njihovi RI podaci ($RI = 240.5 + 91.2 \times C + 2.94 \times WI + 4.6 \times 10^{-5} \times BI - 0.381 \times MTI$). Dobivena jednačina predstavlja novo i jednostavno oruđe za predviđanje gasno-hromatografskih (retencionih indeksa) podataka estara linearnih, dugolančanih alkohola i kratkolančanih, račvastih, alifatičnih kiselina.

Ključne reči: *Scandix pecten-veneris L.*, etarsko ulje, odnos strukture jedinjenja i njihovih retencionih indeksa, estri, dugolančani alkoholi