Gas Chromatographic and Mass Spectrometric Characterization of Trimethylsilyl Derivatives of Some Terpene Alcohol Phenylpropenoids

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This paper reports mass spectra and linear temperature programmed retention indices (I^T) for 31 esters of mono- and sesquiterpene alcohols with hydroxycinnamic acids. In this study, 14 phenylpropenoids were synthesized by esterification of terpenols with *p*-coumaric, ferulic, caffeic and sinapinic acids. Other phenylpropenoids were semi-quantitatively isolated by column chromatography from exudates covering the buds of two birch species (*Betula pubescens* and *B. litwinowii*). Main diagnostic ions in phenylpropenoids mass spectra were determined by GC/MS analysis. The possibility of predicting I^T values was demonstrated with standard error of prediction between 3 and 11 of retention index units.

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Introduction

Esters of cinnamic acids with terpene alcohols belonging to phenolic substances were discovered rather late in the plant kingdom and, owing to this, are not sufficiently characterized both chromatographically as well as mass spectrometrically. The first discovered compound from this class of substances, geranyl (*E*)-*p*-coumarate, was identified in the buds of North American balsam poplar (*Populus balsamifera*).¹ Later, the same authors found the second terpene phenylpropenoid, geranyl caffeate, in the exudate from Himalayan poplar (*P. ciliate*) buds,² as well as in the poplar type propolis collected in Oxfordshire, UK.³ On the basis of the detailed study of propolis composition, the authors drew a conclusion that its plant precursor is an exudate of an American and European poplar species hybrid, widespread in England.

In the following years, evidence has been collected that phenylpropenoid esters are more widespread in plant exudates than it was assumed. In particular, (*Z*)- and (*E*)-docosyl *p*-coumarate isomers were isolated from aerial parts of tubbak (*Psiadia punctulata*), a plant used in traditional medicine in tropical regions of Africa⁴ and ferulates of aliphatic alcohols with C numbers from 16 to 30 were also found in potato pulp.⁵ Recently, Russian authors⁶ isolated *p*-coumarates of 14-hydroxy- β -caryophyllene, 6-hydroxy- β -caryophyllene, 14-hydroxy- α -humulene and 9,9-dimethyl-2,5-dimethylenebicyclo[6.2.0]decane-4-methanol from silver birch (*Betula pendula*) buds, typical for the Eurasian continent. In the presented GC/MS studies of white birch (*B. pubescens*) and Litvinov birch (*B. litwinowii*) bud exudates, not only *p*-coumarates, but also a series of ferulates and caffeates of sesquiterpene alcohols of caryophyllane and humulane types were found.⁷

Cinnamic acids and their esters show different biological

activities.^{8–10} It was demonstrated that caffeates of two terpene alcohols (geraniol and farnesol) show an inhibitory effect on nitric oxide (which induces inflammation) production. These phenylpropenoids are also potential cytotoxic agents. Thus, they may exhibit antitumor activity.¹¹ Indeed, *in vitro* experiments showed that synthetic esters of ferulic and caffeic acids have an ability to inhibit the development of colon, gastric and breast cancer cells. Apart from that, phenylpropenoids are active in lipid peroxidation inhibition and prevent DNA damage due to oxidation.¹² Because of the discovery of these properties, interest in natural phenylpropenoids and their synthetic analogues has grown in recent years.^{13–15}

Therefore, the current aim is to characterize terpene alcohol phenylpropenoids by gas chromatography and mass spectrometry, and to predict their chromatographic retention index¹⁶ values when standards are not available. The material for this work are phenylpropenoids found in plant material, as well as their analogues synthesized especially for this purpose.

Experimental

Chemicals

Commercial *p*-coumaric, ferulic, caffeic and sinapinic acids, and terpene alcohols: nerol, geraniol and (*E,E*)-farnesol, as well as *n*-hexane, pyridine, oxalyl chloride, 1,1'-carbonyldiimidazole (CDI), 4-*N,N*-dimethylaminopyridine (DMAP) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane were purchased from Sigma-Aldrich (Poznań, Poland). Ethyl acetate, tetrahydrofuran (THF) and diethyl ether were purchased from POCH SA (Gliwice, Poland). Dry THF was obtained by distillation over Na/benzophenone.

Synthesis of phenylpropenoid esters and their TMS derivatives

The esterification reactions (Fig. 1) were performed under argon at room temperature using the Steglich procedure.¹⁷ The appropriate acid (0.25 mmol), CDI (0.5 mmol) and catalytic

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amount of DMAP were stirred in freshly distilled THF (4 mL) for 10 min at room temperature. Then, the solution of corresponding terpene alcohol (0.25 mmol) in dry THF (2 mL) was added. The resulting mixture was stirred overnight. The

Fig. 1 Synthesis scheme and structures of *p*-coumarates, ferulates, caffeates and sinapinates of terpene alcohols.

Fig. 2 EIMS spectra and main fragmentation patterns of phenylpropanoid terpenols: (a) TMS derivative of 14-hydroxy- β -caryophyllene *p*-coumarate, (b) TMS derivative of 14-hydroxy-4,5-dihydro- β -caryophyllene ferulate, (c) TMS derivative of geranyl caffeate, (d) TMS derivative of neryl sinapinate.

Table 1 Retention indices of the TMS derivatives of terpene alcohols and their phenylpropenoid esters

Alcohol	Formula of ester	MW _{ester}	<i>I</i> _{T alcohol}	<i>I</i> _{T ester}
Esters of <i>p</i>-coumaric acid				
Myrtenol	C ₂₂ H ₃₀ O ₃ Si	370	1194	2634
Nerol	C ₂₂ H ₃₂ O ₃ Si	372	1229	2673
Geraniol	C ₂₂ H ₃₂ O ₃ Si	372	1255	2699
Caryophylladiene-β-ol ^a	C ₂₇ H ₃₈ O ₃ Si	438	1635	3085
Caryophylladiene-α-ol ^a	C ₂₇ H ₃₈ O ₃ Si	438	1640	3091
6-Hydroxy-β-caryophyllene ^a	C ₂₇ H ₃₈ O ₃ Si	438	1682	3137
14-Hydroxy-β-caryophyllene ^{a,b}	C ₂₇ H ₃₈ O ₃ Si	438	1707	3165
14-Hydroxy-α-humulene ^{a,b}	C ₂₇ H ₃₈ O ₃ Si	438	1759	3210
(<i>E,E</i>)-Farnesol	C ₂₇ H ₄₀ O ₃ Si	440	1722	3172
14-Hydroxy-4,5-dihydro-β-isocaryophyllene ^b	C ₂₇ H ₄₀ O ₃ Si	440	1734	3190
14-Hydroxy-4,5-dihydro-β-caryophyllene ^b	C ₂₇ H ₄₀ O ₃ Si	440	1762	3220
<i>I</i> _{T ester} = 1414(±6) + 1.023(±0.004)· <i>I</i> _{T alcohol} ; <i>n</i> = 11; <i>R</i> ² = 0.9999; SE = 3; SEP _{CV-LOO} = 3				
Esters of ferulic acid				
Nerol	C ₂₃ H ₃₄ O ₄ Si	402	1229	2806
Geraniol	C ₂₃ H ₃₄ O ₄ Si	402	1255	2831
Caryophylladiene-β-ol ^a	C ₂₈ H ₄₀ O ₄ Si	468	1635	3230
Caryophylladiene-α-ol ^a	C ₂₈ H ₄₀ O ₄ Si	468	1640	3234
6-Hydroxy-β-caryophyllene ^a	C ₂₈ H ₄₀ O ₄ Si	468	1682	3279
14-Hydroxy-β-caryophyllene ^a	C ₂₈ H ₄₀ O ₄ Si	468	1707	3305
14-Hydroxy-α-humulene ^{a,b}	C ₂₈ H ₄₀ O ₄ Si	468	1759	3342
(<i>E,E</i>)-Farnesol	C ₂₈ H ₄₂ O ₄ Si	470	1722	3293
14-Hydroxy-4,5-dihydro-β-caryophyllene ^b	C ₂₈ H ₄₂ O ₄ Si	470	1762	3346
<i>I</i> _{T ester} = 1554(±27) + 1.020(±0.017)· <i>I</i> _{T alcohol} ; <i>n</i> = 9; <i>R</i> ² = 0.9981; SE = 10; SEP _{CV-LOO} = 11				
Esters of caffeic acid				
Nerol	C ₂₅ H ₄₀ O ₄ Si ₂	460	1229	2871
Geraniol	C ₂₅ H ₄₀ O ₄ Si ₂	460	1255	2896
14-Hydroxy-β-caryophyllene ^a	C ₃₀ H ₄₆ O ₄ Si ₂	526	1707	3362
(<i>E,E</i>)-Farnesol	C ₃₀ H ₄₈ O ₄ Si ₂	528	1722	3364
14-Hydroxy-4,5-dihydro-β-caryophyllene ^b	C ₃₀ H ₄₈ O ₄ Si ₂	528	1762	3412
<i>I</i> _{T ester} = 1622(±16) + 1.015(±0.010)· <i>I</i> _{T alcohol} ; <i>n</i> = 5; <i>R</i> ² = 0.9997; SE = 5; SEP _{CV-LOO} = 7				
Esters of sinapinic acid				
Nerol	C ₂₄ H ₃₆ O ₅ Si	432	1229	2986
Geraniol	C ₂₄ H ₃₆ O ₅ Si	432	1255	3012
(<i>E,E</i>)-Farnesol	C ₂₉ H ₄₄ O ₅ Si	500	1722	3465
<i>I</i> _{T ester} = 1793(±2) + 0.971(±0.001)· <i>I</i> _{T alcohol} ; <i>n</i> = 3; <i>R</i> ² = 1.0000; SE = 1; SEP _{CV-LOO} = 8				

SE, standard error; SEP_{CV-LOO}, standard error of prediction (CV-LOO, cross validation method with leave-one-out).

a. Identified in the exudate from white birch (*B. pubescens*) buds. b. Identified in the exudate from Litvinov birch (*B. litwinowii*) buds.

solvent was evaporated to dryness and the residue was dissolved in ethyl acetate, washed with NaHCO₃ and with water, dried over MgSO₄, filtered with a Celite pad and concentrated in a stream of nitrogen. The crude product was dissolved in pyridine (220 μL) in a vial (2 mL) and BSTFA (80 μL) was added. This mixture was heated at 60°C for 0.5 h. The obtained TMS derivatives were subjected to GC/MS analysis.

Isolation of sesquiterpene phenylpropenoids from birch buds

The buds of two birch species (*Betula pubescens* and *B. litwinowii*) were collected from an arboretum, owned by Institute of Dendrology, Polish Academy of Sciences. The 5 g of buds was dried at 40°C, crushed and extracted by three portions (25 mL each) of diethyl ether while stirring constantly. The joint extracts were dried over anhydrous Na₂SO₄, filtered through a paper filter and the solvent was removed to a volume of ca. 1 mL on a rotor evaporator. These extracts were subjected to GC/MS analysis. Then, they were fractionated by column chromatography on a silica column. Next, *n*-hexane was used to elute mixtures of non-polar and slightly polar compounds. Then, gradient elution (*n*-hexane/ethyl acetate) was used to isolate fractions containing phenylpropenoid esters of terpene alcohols. In this study, we did not try to isolate a pure compound and the only task of using column chromatography was to

obtain the fractions that contained compounds of interest. All the fractions were analyzed by GC/MS and, after solvent evaporation, those that contained phenylpropenoids were subjected to derivatization (as above).

GC/MS analysis

TMS derivatives isolated from birch buds and synthesized phenylpropenoids were analyzed on an Agilent 6890 gas chromatograph with a mass selective detector MSD 5973, which was fitted with an autosampler HP-7683, electronic pressure control and split/splitless injector. Separation was performed on an HP-5ms (30 m × 0.25 mm; 0.25 μm film thickness) fused silica column with helium flow rate of 1 mL/min. The injector worked in a split (1:50) mode; injector temperature was 250°C. EIMS spectra were obtained at 70 eV ionization energy, at the source temperature 230°C and that of quadrupole 150°C. The MSD was set to scan *m/z* 40–600. Chromatograms were acquired using a linear temperature program from 50 to 320°C at 3°C per minute.

The hexane solution of C₁₀–C₄₀ *n*-alkanes was separated under the above conditions. Linear temperature programmed retention indices (*I*^T) were calculated from the results for this mixture and for solutions of TMS derivatives of terpene alcohols and TMS derivatives of phenylpropenoids.

Table 2 Results of the retention index values estimation for 14-hydroxy- β -isocaryophyllene phenylpropenoid ($I^T_{\text{alcohol}} = 1723$)

Ester	Formula	MW _{ester}	I^T_{ester} calc.	I^T_{ester} exp.	ΔI^T
<i>p</i> -Coumarate	C ₂₇ H ₃₈ O ₃ Si	438	3178	3177	-1
Ferulate	C ₂₈ H ₄₀ O ₄ Si	468	3312	3321	9
Caffeate	C ₃₀ H ₄₆ O ₄ Si ₂	526	3372	3373	1

Model: $I^T_{\text{ester}} = b_0 + b_1 \cdot I^T_{\text{alcohol}}$

Results and Discussion

Mass spectrometric characterization of phenylpropenoid esters

Figure 2 presents EIMS spectra and fragmentation patterns of two phenylpropenoid esters (their TMS derivatives) found in the buds: 14-hydroxy- β -caryophyllene *p*-coumarate and 14-hydroxy-4,5-dihydro- β -caryophyllene ferulate, as well as synthesized geranyl caffeate and neryl sinapinate. The molecular ion was found in the spectra of all phenylpropenoids. The low-mass region of the spectra shows the ion at m/z 73 (TMS group) and, with relatively low intensity, ions derived from fragmentation of terpene parts. Mass spectra of phenylpropenoid esters formed by acyclic terpene alcohols: nerol, geraniol and farnesol contain peaks at m/z 69 (C₅H₉⁺), typical for EIMS fragmentation of these alcohols. Main diagnostic ions at m/z 219, 249, 279 and 307 are derived from the phenylpropenoic moieties (as the result of ester bond cleavage) in the spectra of *p*-coumaroyl, feruloyl, sinapinyl and caffeoyl esters, respectively. The loss of acyloxy parts from M⁺ with synchronous H-rearrangement gives rise to additional characteristic ions at m/z 236, 266, 296 and 324, with the intensity increasing in this order. Mass spectra of TMS derivatives of phenylpropenoid esters of terpene alcohols are presented in the supplementary information in the edition of this Journal.

Gas chromatographic characterization of phenylpropenoid esters

Table 1 presents experimental values of retention indices for TMS derivatives of 11 terpenols (3 mono-, 8 sesquiterpenols) and 28 phenylpropenoid esters (11 *p*-coumarates, 9 ferulates, 5 caffeates and 3 sinapinates). Despite the fact that the use of fused silica capillary columns with immobilized low- and medium-polar stationary phases allows for separation of high-boiling polar compounds without preliminary derivatization, we chose to present the retention indices of silanized phenylpropenoids of terpenols. One of the reasons for this is the fact that chromatographic retention times (t_R) of high-boiling polar substances not subjected to derivatization are quite sensitive to fluctuations of separation conditions (temperature and carrier gas flow rate). Besides that, when low polarity stationary phases are used, polar substances are eluted from chromatographic columns in the form of quite wide peaks, which makes the accurate determination of t_R values difficult. Therefore, the values of chromatographic retention indices of such substances, even if determined in one laboratory, are characterized by a higher deviation than the analogous values of their derivatives. For example, analysis of birch bud extracts and fractions collected during their separation on a silica column results with I^T values of 1667 ± 8 ($n = 8$) and 3152 ± 18 i.u. ($n = 6$) for non-silanized 14-hydroxy- β -caryophyllene and its *p*-coumarate ester, respectively, whereas for TMS derivatives of these compounds the I^T values are 1707 ± 1 ($n = 11$) and 3165 ± 3 i.u. ($n = 8$), respectively.

The use of derivatization is convenient also because phenylpropenoids in natural objects such as plant tissue and propolis are parts of complex mixtures that contain a large number of polar substances of different classes. Successful analysis of such mixtures is possible only after derivatization of these substances.^{7,18}

It was interesting to examine the possibility of I^T value prediction for terpene phenylpropenoids (their TMS derivatives) that were not subject to the experiment. Table 1 contains the calculated coefficients of linear equation $I^T_{\text{ester}} = b_0 + b_1 \cdot I^T_{\text{alcohol}}$ for all the groups of phenylpropenoids: *p*-coumarates, ferulates, caffeates and sinapinates. It is visible that equations with calculated values of coefficients b_0 and b_1 are characterized by low standard errors (SE) and standard errors of prediction SEP_{CV-LOO} is in the range 3 – 11 i.u. Table 2 contains the results of I^T value calculations for three phenylpropenoids of 14-hydroxy- β -isocaryophyllene for which the experimental values of I^T were determined but they were not used to calculate the coefficients in the above equations. It can be seen that the greatest deviation from experimental values for *p*-coumarates, ferulates and caffeates does not exceed 10 i.u. This accuracy is on the level of inter-laboratory deviation of experimental I^T values. Therefore, it is possible to predict retention indices of other numerous mono- and sesquiterpene alcohols phenylpropenoids with acceptable accuracy.

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Supporting Information

The mass spectra of TMS derivatives of terpene alcohol phenylpropenoids. This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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