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Free and Glycosidically Bound Volatiles of *Mentha citrata* Ehrh.

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Free and glycosidically bound volatiles of surficial parts of *Mentha citrata* were investigated. Free volatile compounds were isolated from dried plant material by two methods: hydrodistillation and extraction with pentane used as control. Free volatile compounds as well as the aglycones obtained after enzymatic hydrolysis of glycosides were analyzed by gas chromatography-mass spectrometry (GC-MS). A significant difference in qualitative and quantitative composition of volatile compounds of the essential oil and pentane extract was found. The major components of the essential oil and pentane extract were linalyl acetate (21.46%; 42.02%), linalool (13.68%; 22.66%), 1,8-cineole (12.51%; 6.40%), β-myrcene (8.10%; 2.87%), α-terpineol (7.38%; 0.73%) and geranyl acetate (8.66%; 1.76%). The major components of the volatile aglycones were 1-octen-3-ol (40.28%), eugenol (12.29%), 3-octanol (7.09%), linalool (4.59%), benzyl alcohol (3.71%), 2-phenylethanol (3.67%), 3-hexene-1-ol (2.87%) and 1-heptene-3-ol (2.88%). Compared with free volatile compounds (essentia1 oil: 1.56%; pentane extract: 1.64%), the glycosidically bound volatiles were present in a lower concentration, about 0.0068%.

Key words: Mentha citrata, volatile compounds, changes during hydrodistillation, glycosidically bound volatiles, GC-MS.

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INTRODUCTION

Mentha citrata Ehrh. (bergamot mint) is a hybrid between *Mentha viridis* L. and *Mentha aquatica* L. *Mentha* species often contain menthol, menthylacetate, menthone, pulegone, menthofurane and related *p*-menthane compounds as main components. On the other hand, *Mentha citrata* contains linalyl acetate and linalool as main components.[1–3] These compounds are very unstable. Namely, they can transform to some artefacts during the isolation of the essential oil by hydrodistillation.[4] In this paper, the isolation of free volatile compounds was performed by two methods: hydrodistillation (essential oil) and extraction by pentane.[5,6] Pentane extract was used as control. Composition of the essential oil was compared with the composition of pentane extract, and finally with the composition of glycosidically bound volatiles.

The essential oil or volatile compounds of aromatic plants were extensively investigated. On the other hand, glycosidically bound volatiles of these plants have been unsufficiently studied.[7,8]

EXPERIMENTAL

Plant Material

Mentha citrata was cultivated in southern Croatia, Split. Surficial parts of the flowering plant were harvested in August 1998 and dried at room temperature in shaded place. A voucher specimen is deposited at the Department of Organic Chemistry, Faculty of Chemical Technology, University of Split.

Isolation of Volatile Compounds

Volatile compounds of *Mentha citrata* were isolated from plant material by two methods: hydrodistillation and extraction with pentane.

Essential Oil

Essential oil was isolated from 100 g of plant material by hydrodistillation in a modified Clevenger-type apparatus during three hours. The obtained essential oil was dried by anhydrous sodium sulphate and stored under argon in a sealed vial at –20 °C, as in the previous paper.[9]

Pentane Extract

Free volatile componds were isolated from 1 g of ground plant material by exhaustive percolation with pentane at room temperature. After percolation, pooled extracts were concentrated to 0.5 mL using a rotating evaporator under reduced pressure.

Isolation of Glycosides

Glycosides were isolated by percolation with ethyl acetate from 100 g of ground plant material. 500 mg of octyl-β-glucoside was added to ethyl acetate as the inter-

nal standard. After exhaustive percolation and evaporation of the solvent, the residue was dissolved in 50 mL of ethanol. Ballast components were removed from ethanol solution by addition of 50 mL water in the form of precipitate. The obtained ethanol-water extract was then concentrated to dryness, dissolved in 30 mL of absolute ethanol and finally the acid ballast components were precipitated with a few drops of concentrated ammonia. The final purification of glycosides was performed by »flash« column chromatography, as in the previous paper.[9]

Enzymatic Hydrolysis

The pooled glycosidic fractions were further concentrated to dryness and the residue was dissolved in 5 mL of 0.1 mol dm^{-3} citrate buffer, pH = 5.5. The aqueous solution was washed with 5 x 5 mL of pentane-dichloromethane 2 : 1 v/v and subsequently with 5 x 5 mL of pentane to remove free terpenes and other hydrophobic compounds, if any. After being concentrated to a few drops, the last pentane extract (5 mL) had to be without traces of free terpene and other hydrophobic compounds (tested by TLC, GC). Then, 20 mg β-glucosidase from almonds (»Fluka«) was added to the glycosidic solution and 3 mL of pentane was added to trap the aglycones. Hydrolysis was carried out at 30 °C for 72 hours, with the mixture being shaken occasionally. After hydrolysis, the pentane layer was separated. The remaining aglycones from aqueous layer were extracted with 4 x 5 mL of pentane. The combined pentane extracts were dried over anhydrous sodium sulphate and concentrated to a final volume of 0.5 mL.

Gas Chromatography-Mass Spectrometry

Analysis was performed with a GC-MS Hewlett-Packard model 5890 with a mass selective detector model 5971A, using two columns with different polarity of stationary phases. GC operating conditions:

– column HP-20M (Carbowax 20M), 50 m x 0.2 mm i.d., film thickness 0.2 mm; column temperature programmed from 70 °C isothermal for 4 minutes to 180 °C at a rate of 4 °C min^{-1};

– column HP–101 (dimethylpolysiloxane), 25 m x 0.2 mm i.d., film thickness 0.2 mm; column temperature programmed from 70 °C isothermal for 2 minutes to 200 °C at a rate of 3 °C min^{-1};

Carrier gas: helium; flow rate: 1 mL min^{-1}; injector temperature: 250 °C ; volume injected: 1 mL; split ratio: 1 : 50.

MS conditions: ionization voltage: 70 eV; ion source temperature: 280 °C; mass range: 30–300 mass units.

Individual peaks were identified by comparing their retention indices to those of authenthic samples, as well as by comparing their mass spectra with those stored in the data-base (Wiley library). Determination of the percentage composition was based on peak area normalization without using correction factors. The content of volatile compounds in pentane extract were calculated from GC peak areas related to the GC peak area of internal standard. An exact quantity of menthol was added into pentane extract as internal standard. The content of aglycones was calculated from GC peak areas related to the GC-peak area of 1-octanol (liberated from octyl-β-glucoside).

RESULTS AND DISCUSSION

Free Volatile Compounds

The essential oil yield obtained by hydrodistillation of dry plant material was 1.56% (mass fraction) and the essential oil yield obtained by percolation with pentane was 1.64% (mass fraction). In order to ensure that none of the components was transformed during hydrodistillation, pentane ex-

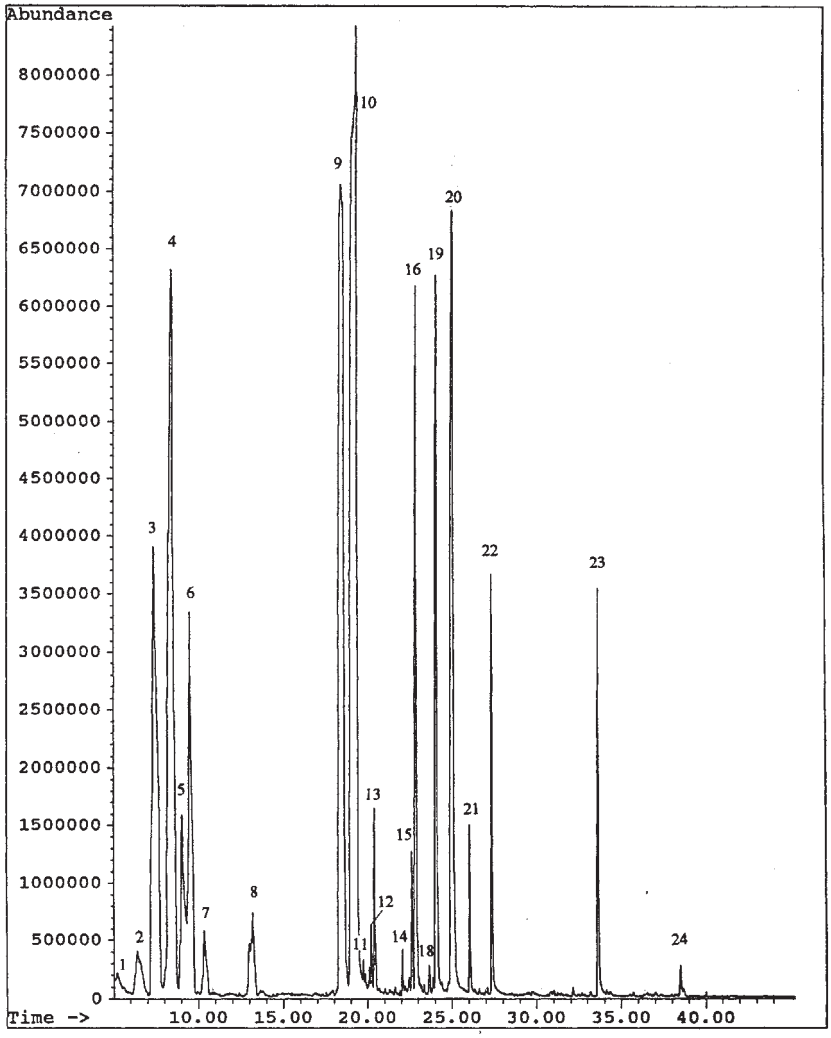


Figure 1. Chromatogram of the essential oil of *Mentha citrata* (on HP-20M). Identification and content of numbered peaks are given in Table I, column A.

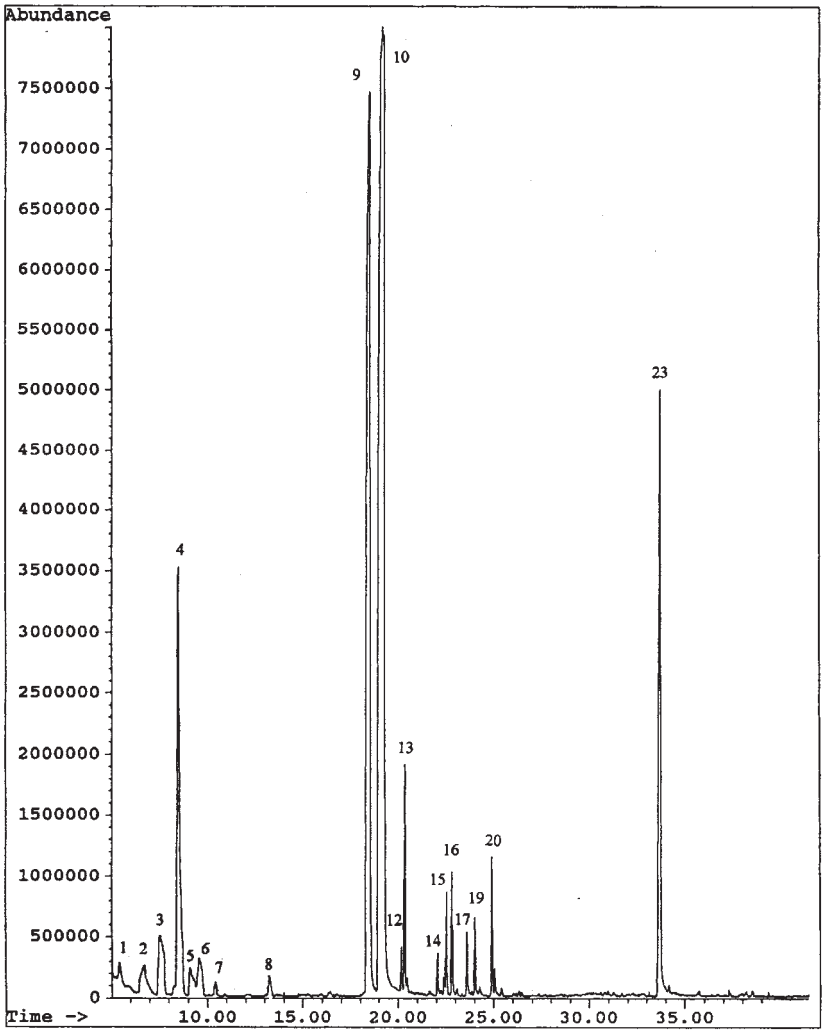


Figure 2. Chromatogram of the pentane extract of *Mentha citrata* (on HP-20M). Identification and content of numbered peaks are given in Table I, column B.

tract was used as control. The chromatograms of essential oil and pentane extract are shown in Figures 1 and 2. The chemical composition and content are given in Table I. The structure of identified volatile compounds is shown in Figure 3.

Twenty-three compounds were identified in essential oil and twenty compounds in pentane extract. A significant qualitative and quantitative difference was found. The major components of the essential oil and pentane extract were linalyl acetate (**10**) (21.46%; 42.02%), linalool (**9**) (13.68%; 22.66%),

TABLE I

Identified constituents and percentage composition of volatile compounds of *Mentha citrata* isolated by two different methods[a]

Peak no.	Compound	A		B		Methods of identification
		$\overline{X}$	σ	$\overline{X}$	σ	
		%		%		
1	α-pinene	0.31	0.02	0.32	0.01	I_1, I_2, MS
2	β-pinene	1.69	0.04	1.01	0.02	I_1, I_2, MS
3	β-myrcene	8.10	0.07	2.87	0.02	I_1, I_2, MS
4	1,8-cineole	12.51	0.29	6.40	0.26	I_1, I_2, MS
5	*cis*-β-ocimene	2.02	0.04	1.08	0.03	I_1, I_2, MS
6	*trans*-β-ocimene	5.27	0.06	1.32	0.02	I_1, I_2, MS
7	α-terpinolene	1.03	0.02	t	/	I_1, –, MS
8	alloocimene	1.18	0.03	t	/	I_1, –, MS
9	linalool	13.68	0.28	22.66	0.23	I_1, I_2, MS
10	linalyl acetate	21.46	0.33	42.02	0.33	I_1, I_2, MS
11	terpinen-4-ol	0.13	0.03	–	/	I_1, I_2, MS
12	β-elemene	0.31	0.03	0.32	0.02	I_1, I_2, MS
13	caryophyllene	1.62	0.06	2.16	0.03	I_1, I_2, MS
14	α-humulene	0.21	0.03	0.22	0.02	I_1, I_2, MS
15	β-farnesene	0.50	0.02	0.87	0.01	–, I_2, MS
16	α-terpineol	7.38	0.05	0.73	0.02	I_1, I_2, MS
17	β-cubebene	–	/	0.58	0.03	I_1, I_2, MS
18	geranial	0.11	0.01	t	/	I_1, –, MS
19	neryl acetate	3.56	0.03	1.03	0.02	I_1, I_2, MS
20	geranyl acetate	8.66	0.02	1.76	0.03	I_1, I_2, MS
21	nerol	0.98	0.02	–	/	I_1, I_2, MS
22	geraniol	3.18	0.02	–	/	I_1, I_2, MS
23	hedycaryol[b]	4.20	0.03	9.88	0.06	–, –, MS
24	eudesmol	0.47	0.02	–	/	I_1, –, MS
	total	98.25		95.23		

[a] Methods used: A, hydrodistillation; B, percolation with pentane.
$\overline{X}$, peak area (mean value, average values of percentages obtained by two columns);
σ, standard deviation.
I_1, retention indices on HP-20M; I_2, retention indices on HP-101; MS, mass spectra.
–, not detected; /, not calculated; t, traces < 0.1 %;

[b] tentative identification based on MS only. The compounds are ordered according to retention indices on HP-20M.

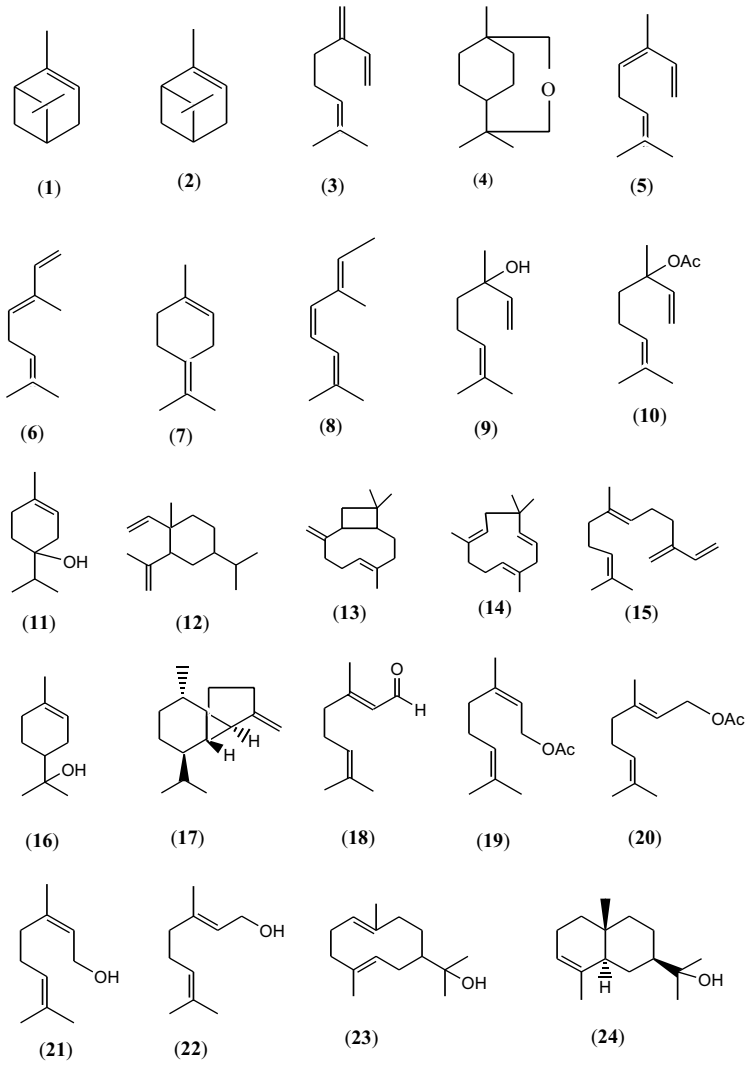
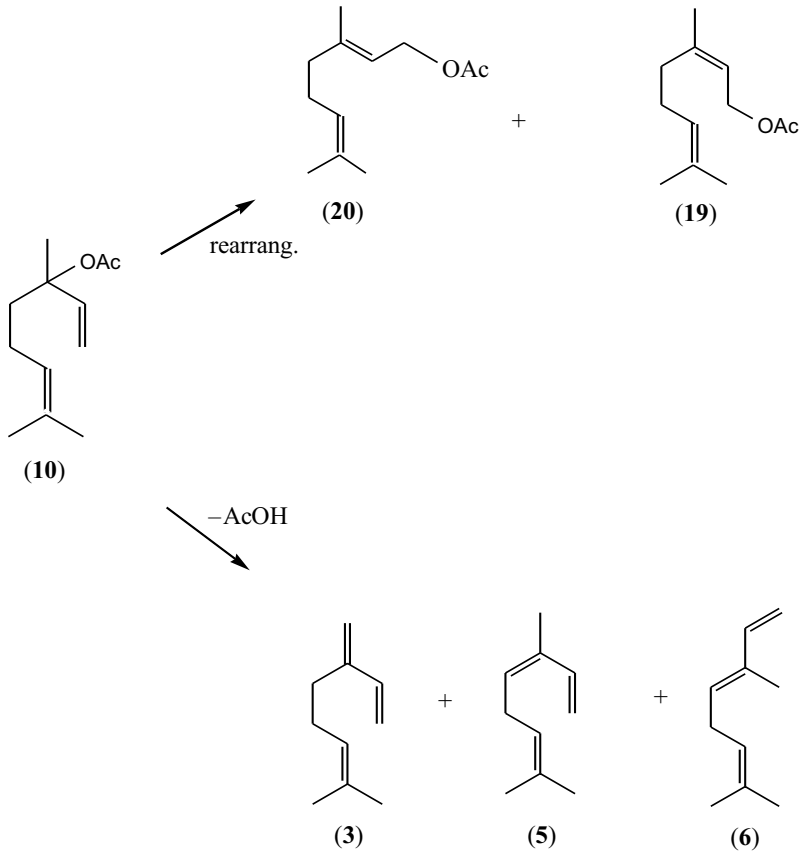


Figure 3. The structure of identified compounds in the essential oil and pentane extract. (Numbers of compounds are the same as in Table I.)

1,8-cineole (**4**) (12.51%; 6.40%), β-myrcene (**3)** (8.10%; 2.87%), α-terpineol (**16**) (7.38%; 0.73%) and geranyl acetate (**20**) (8.66%; 1.76%), trans-β-ocimene (**6**) (5.27%; 1.32%), neryl acetate (**19**) (3.56%; 1.03%), geraniol (**22**) (3.18%; 0.0%), nerol (**21**) (0.98%; 0.0%). The essential oil contained hedycaryol (**23**) in a smaller amount (4.20%) than the pentane extract (9.88%). This is probably due to the low volatility of hedycaryol (high boiling sesquiterpene alcohol) and into good extraction in pentane. Hedycaryol was identi-

fied only with mass spectra. It gave the the following ion fragments, m/z: 204(8), 189(13), 163(10), 161(37), 149(9), 135(20), 119(14), 107(32), 95(22), 93(59), 80(14), 79(22), 67(23), 59(100)%. Furthermore, hedycaryol and elemol have very similar mass spectra, because hedycaryol gives elemol by Cope rearrangement.[10,11] This reaction is known to occur during the isolation of essential oil, but in the ion source, as well. The essential oil and pentane extract have a high concentration of linalyl acetate and linalool, but in very different proportions. During hydrodistillation, the plant material and water are acidic (pH = 5.5–6.5), so the decomposition of linalyl acetate and linalool is promoted.[12] For example, linalyl acetate (**10**) can be converted by allylic rearrangement into geranyl acetate (**20**) and neryl acetate (**19**), and by elimination into acyclic monoterpene hydrocarbons β-myrcene (**3**), *cis*-β-ocimene (**5**) and *trans*-β-ocimene (**6**), Scheme 1.



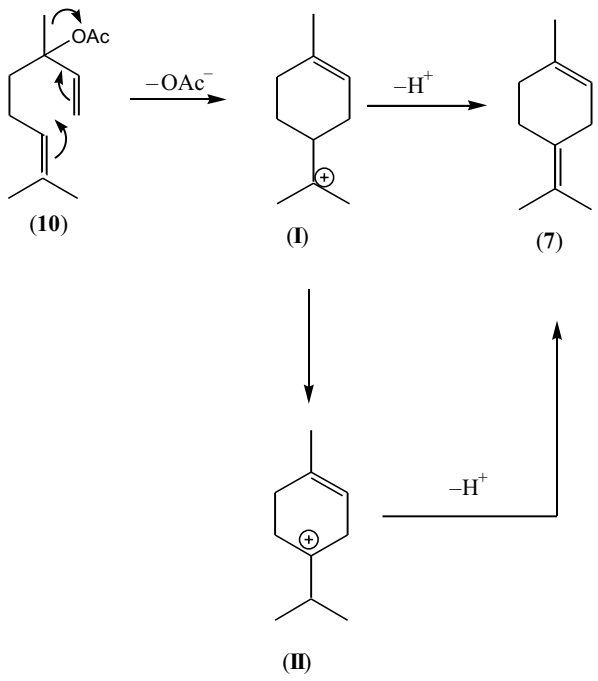
Scheme 1.

Under the same conditions, the formation of monocyclic monoterpene hydrocarbon terpinolene (**7**), as the main product from linalyl acetate (**10**) *via* the α-terpinyl cation (**I**) and terpinene-4-yl cation (**II**), is described in Scheme 2.

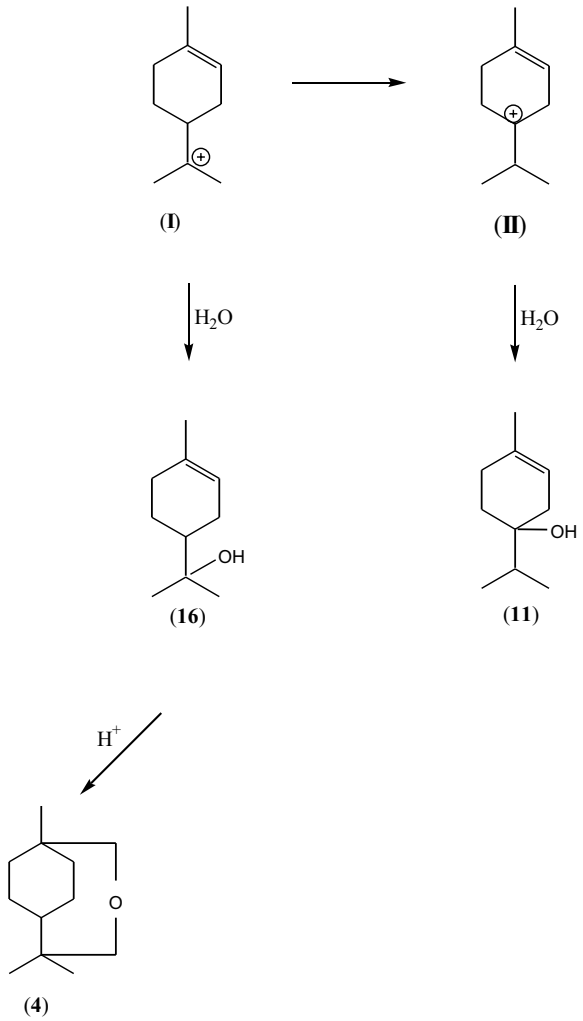
α-Terpineol (**16**), terpinene-4-ol (**11**) and 1,8-cineol (**4**) can be formed by hydration of the α-terpinyl cation (**I**) and terpinene-4-yl cation (**II**), Scheme 3.

Linalool (**9**) shows similar reactions of rearrangement and elimination under the same conditions as well.

Nerol, geraniol and terpinen-4-ol were not identified in the pentane extract. They were originated during the hydrodistillation. On the other hand, the contents of β-myrcene, *trans*-β-ocymene, neryl acetate were increased by three times, geranyl acetate four times and the contents of 1,8-cineol, *cis*-β-ocymene twice in the essential oil compared to the pentane extract. The content of α-terpineol was as much as ten times increased. At the same time, the contents of linalyl acetate and linalool in the essential oil decreased by half of their contents in pentane extract. All aromatic plants with a high content of linalyl acetate can give a significant amount of artifacts dur-



Scheme 2.



Scheme 3.

ing hydrodistillation of the essential oil. In this case, it is recommendable to isolate volatile compounds by pentane extraction for control.

The essential oil of *Mentha citrata* might be a source of natural linalyl acetate and linalool. No menthol, menthon, menthyl acetate, menthofurane, or *p*-menthane compounds were detected in this essential oil. They often exist in oils of many *Mentha* species as their main components.

Glycosidically Bound Volatiles of Mentha citrata

The content of glycosidically bound volatiles was 68 mg kg^{-1} (0.0068%) of dry plant material. The GC-MS analysis of the aglycone fraction after enzymatic hydrolysis revealed 17 compounds. The chromatogram of aglycones is shown in Figure 4. Their chemical composition and content are given in Table II and the structure is shown in Figure 5.

The major components of aglycones were 1-octen-3-ol (**5**) (40.28%), eugenol (**17**) (12.29%), 3-octanol (**4**) (7.09%), linalool (**7**) (4.59%), benzyl alcohol (**14**) (3.71%) and 2-phenylethanol (**15**) (3.67%).

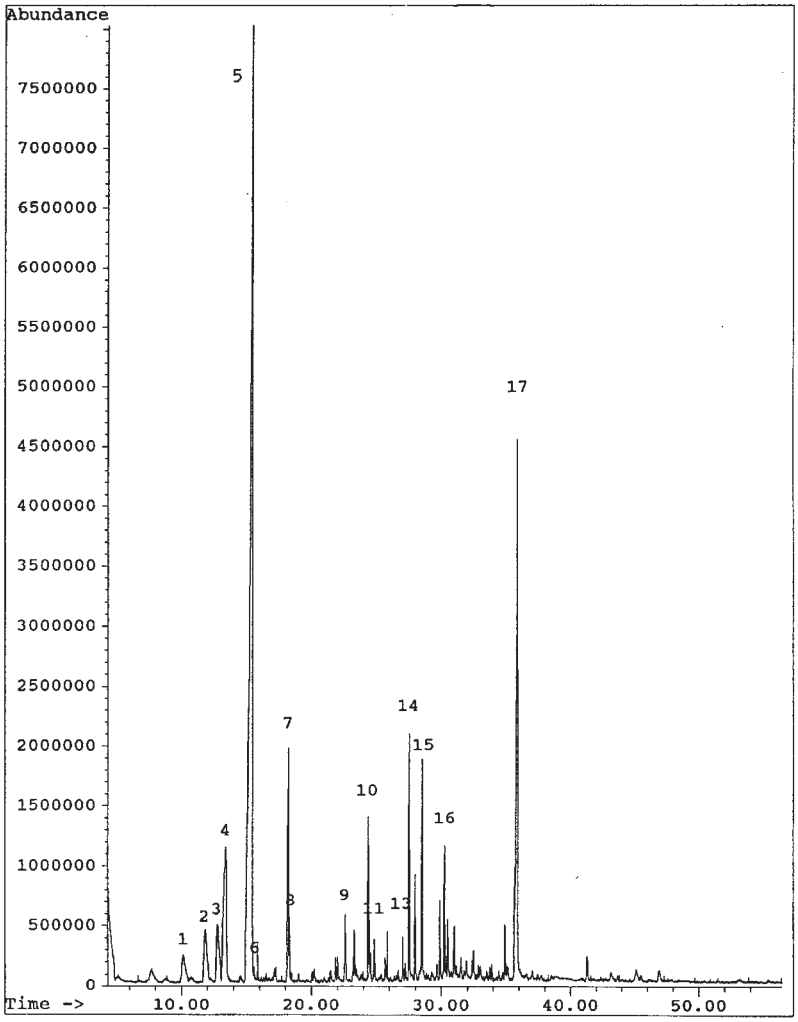


Figure 4. Chromatogram of the volatile aglycones of *Mentha citrata* (on HP-20M). Identification and content of numbered peaks are given in Table II.

TABLE II

Identified constituents and percentage composition of volatile aglycones of *Mentha citrata*

Peak no.	Compound	$\overline{X}$	σ	Methods of identification
		%		
1	3-heptanol	1.60	0.02	I_1, I_2, MS
2	1-hepten-3-ol	2.88	0.03	I_1, I_2, MS
3	3-heksen-1-ol	2.87	0.02	I_1, I_2, MS
4	3-octanol	7.09	0.06	I_1, I_2, MS
5	1-octen-3-ol	40.28	0.28	I_1, I_2, MS
6	linalool oxide	0.29	0.02	I_1, I_2, MS
7	linalool	4.59	0.08	I_1, I_2, MS
8	1-nonen-3-ol	0.60	0.02	–, –, MS
9	α-terpineol	1.21	0.03	I_1, I_2, MS
10	2-butenoic acid	2.62	0.02	I_1, I_2, MS
11	methyl salycilate	0.71	0.03	I_1, I_2, MS
12	nerol	0.22	0.01	I_1, I_2, MS
13	geraniol	0.52	0.01	I_1, I_2, MS
14	benzyl alcohol	3.71	0.02	I_1, I_2, MS
15	2-phenylethanol	3.67	0.07	I_1, I_2, MS
16	2-hexenoic acid	1.12	0.03	–, –, MS
17	eugenol	12.29	0.28	I_1, I_2, MS
	total	86.27		

[a] $\overline{X}$, peak area (mean value, average values of percentages obtained by two columns); σ, standard deviation.
I_1, retention indices on HP-20M; I_2, retention indices on HP-101; MS, mass spectra.
The compounds are ordered according to retention indices on HP–20M.

J. M. van den Dries and A. Baerheim Svendsen[13] did not detect linalool, linalool oxide, α-terpineol, 2-butenoic acid, methyl salycilate, nerol, geraniol and 2-hexenoic acid as aglycones of *Mentha citrata*. On the other hand, we did not detect thymol and carvacrol in aglycones, which they did. Some of the glycosidically bound volatile compounds do not appear in essential oil of the investigated plant material.

Linalool, α-terpineol, geraniol and nerol were identified in both the essential oil and the volatile aglycones of *Mentha citrata.* This supports once more the assumption that, if alcohols or phenols are the main components of essential oil, the corresponding glycosides can also be detected.[14] Our re-

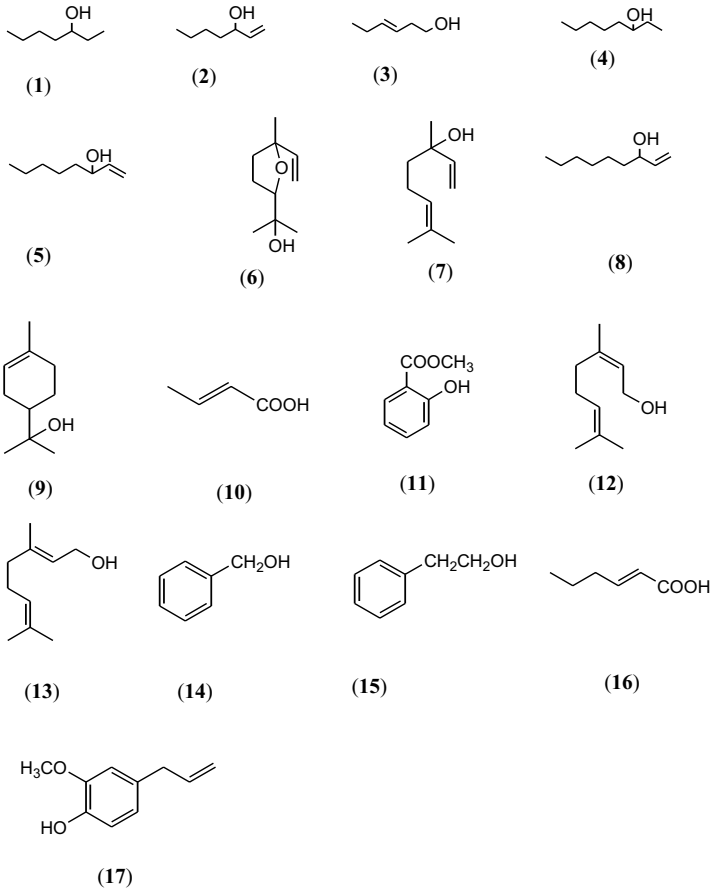


Figure 5. The structure of identified volatile aglycones. (Numbers of compounds are the same as in Table II.)

sults show a moderate correlation between the structures of free and glycosidically bound volatiles.

Aglycones, such as aliphatic alcohols, 2-phenylethanol, eugenol and terpenes, linalool and α-terpineol, can, more or less, be considered to be ubiquitous in the aglycone fraction.[15]

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SAŽETAK

Slobodni i glikozidno vezani hlapljivi spojevi biljke *Mentha citrata* Ehrh.

Josip Mastelić, Mladen Miloš i Danica Kuštrak

Istraživani su slobodni i glikozidno vezani hlapljivi spojevi iz nadzemnih dijelova biljke *Mentha citrata.* Slobodni hlapljivi spojevi izolirani su iz suhog biljnog materijala na dva načina: hidrodestilacijom i ekstrakcijom pentanom. Slobodni hlapljivi spojevi kao i aglikoni dobiveni poslije enzimske hidrolize glikozida analizirani su vezanim sustavom plinska kromatografija-masena spektrometrija (GC-MS). Nađena je znatna razlika u kvalitativnom i kvantitativnom sastavu hlapljivih spojeva eteričnog ulja i pentanskog ekstrakta. Glavne komponente eteričnog ulja i pentanskog ekstrakta bile su linalil-acetat (21,46%; 42,02%), linalol (13,68%; 22,66%), 1,8-cineol (12,51%; 6,40%), β-mircen (8,10%; 2,87%), α-terpineol (7,38%; 0,73%) i geranil-acetat (8,66%; 1,76%). Glavne komponente hlapljivih aglikona bili su 1-okten-3-ol (40,28%), eugenol (12,29%), 3-oktanol (7,09%), linalol (4,59%), benzil-alkohol (3,71%), 2-feniletanol (3,67%), 3-heksen-1-ol (2,87%) i 1-hepten-3-ol (2,88%). U usporedbi sa slobodnim hlapljivim spojevima (1,56%; 1,64%), glikozidno vezani hlapljivi spojevi prisutni su u nižoj koncentraciji (oko 0,0068%).