CROATICA CHEMICA ACTA CCACAA **74** (2) 431–439 (2001)

ISSN-0011-1643
CCA-2742 *Original Scientific Paper*

Composition of Free and Glycosidically Bound Volatiles of *Mentha aquatica* L.

Igor Jerković and Josip Mastelić*

Department of Organic Chemistry, Faculty of Chemical Technology, University of Split, N. Tesle 10/V, 21 000 Split, Croatia

Received May 2, 2000; revised January 17, 2001; accepted January 31, 2001

Free and glycosidically bound volatiles of *Mentha aquatica* L. were investigated. Free volatile compounds were isolated from the dried plant material by hydrodistillation and glycosides by extraction with water during hydrodistillation. Free volatile compounds (essential oil) as well as the aglycones obtained after the hydrolysis of glycosides were analyzed by gas chromatography-mass spectrometry (GC-MS). The essential oil was composed of monoterpene hydrocarbons (10.47%), sesquiterpene hydrocarbons (20.09%) and oxygen containing compounds (68.42%). The major components of the essential oil were: menthofuran (3978.08 mg kg^{-1}), 1,8-cineole (1716.44 mg kg^{-1}) and *trans*-caryophyllene (1109.20 mg kg^{-1}). After enzymatic hydrolysis, the major volatile aglycones were: 1-octen-3-ol (30.22 mg kg^{-1}), eugenol (10.21 mg kg^{-1}), 2-phenylethanol (6.81 mg kg^{-1}), 3-hexen-1-ol (5.35 mg kg^{-1}), perilla alcohol (4.13 mg kg^{-1}), 1-hexanol (3.58 mg kg^{-1}), and 3-octanol (2.44 mg kg^{-1}). Moderate similarity was found between volatile aglycones and the corresponding free volatiles in the essential oil. Acidic hydrolysis, after enzymatic hydrolysis, liberated additional amounts of aglycones. Approx. 38% of the glycosides were not hydrolyzed by the used enzyme.

Key words: Mentha aquatica L., *Lamiaceae*, essential oil, volatile aglycones, menthofuran, 1,8-cineole, 1-octen-3-ol, eugenol.

INTRODUCTION

Mentha aquatica L., (watermint) grows wild on wet ground, near rivers, in many parts of Europe. The essential oil of this plant is characterized by

* Author to whom correspondence should be addressed. (E-mail: igor@ktf-split.hr)

domination of menthofuran.[1,2] Terpinen-4-ol and 1,8-cineole were also identified among the main constituents of watermint volatiles as well as among the volatiles of *Mentha denticulata* from the same systematic section of Capitatae.[3] In his paper, Maffei[3] investigated the qualitative and quantitative variations of the essential oil composition of several *Mentha* species in relation to enviromental factors (temperature and rainfall). The morphology of watermint as well as the chemical composition of its essential oil have been found to be very stable. The published results[4–6] indicate that the essential oils of *Mentha aquatica* hybrids often contain menthofuran as their major component. Menthofuran, which has sweet menthol-like flavour, is used for flavouring non-alcoholic beverages, ice creams and candies. A recent study of Khojastehbakht *et al.*[7] revealed that this furan is a potent inactivator of human liver cytochrome P450 2A6.

Glycosidically bound volatile compounds were detected in many aromatic plants of the *Lamiaceae* family.[8–10] However, these compounds have not been explored in watermint so far. The aim of this study was to determine the chemical composition of the essential oil as well as to identify the volatile aglycones from *Mentha aquatica* L. of Croatian origin. The qualitative and quantitative composition of the obtained free and glycosidically bound volatiles was compared.

EXPERIMENTAL

Plant Material

Mentha aquatica was collected in the submediterranean region of south Croatia (near the river Cetina) in August 1999. The plant material was air-dried at room temperature in a shaded place for 10 days. Two replicates were used. The voucher specimen is deposited in our laboratory.

Isolation of the Essential Oil

The plant material (100 g) was submitted to hydrodistillation in a Clevenger type apparatus for two hours. $CaCO_3$ (3 g) was added into water intended for hydrodistillation. The obtained essential oil was separated and stored at –18 °C.

Isolation and Hydrolysis of the Glycosides

The glycosides of volatile compounds were extracted with water during hydrodistillation. After the hydrodistillation, the aqueous extract was separated and the residual plant material was extracted with boiling water twice more. The pooled extracts were concentrated to 50 mL in a rotating evaporator under reduced pressure. Precipitation of ballast compounds was carried out by addition of 50 mL ethanol. Further purification of the glycosidic fraction was performed with ammonia-ethanol[9] and by flash chromatography on a silica gel column.[10] The obtained glycosidic frac-

tion was concentrated to dryness and dissolved in a citrate buffer (pH 5.5; 5 mL). The aqueous solution was washed with 5 × 5 mL of pentane-dichloromethane 2:1 V/V and with 10 × 5 mL of pure pentane to remove the possibly existing hydrophobic compounds.

Enzyme β-glucosidase from almonds (»Fluka«, 20 mg) was added to the glycosidic solution. The hydrolysis was carried out at 37 °C for 48 hours. Duration of the enzymatic hydrolysis was determined according to the studies of a number of authors[8,11] as well as our previous papers. [12,13] After the hydrolysis, the liberated aglycones were extracted with pentane (10 × 2 mL). Combined pentane extracts were concentrated to a 0.5 mL volume, and 1μL was used for GC-MS analysis. After the enzymatic hydrolysis and extraction of the aglycones, the residue was submitted to acidic hydrolysis (HCl, pH 1) at 65 °C for one hour under a hexane layer. After the acidic hydrolysis, the hexane layer was separated and the aqueous layer was extracted with 10 × 5 mL of hexane. Combined hexane extracts were concentrated to a 0.5 mL volume and analyzed by GC-MS.

Gas Chromatography-Mass Spectrometry Analysis

The samples were analyzed by gas chromatography-mass spectrometry (Hewlett-Packard, model 5890, with a mass selective detector, model 5971A) on two columns. GC operating conditions: column HP-20M (Carbowax 20M), 50 m × 0.2 mm i.d., film thickness 0.2 μm; column temperature programmed from 70 °C isothermal for 4 minutes, then increased to 180 °C at a rate of 4 °C min^{-1}; column HP-101 (Dimethylpolysiloxane fluid), 25 m × 0.2 mm i.d., film thickness 0.2 μm; column temperature programmed from 70 °C isothermal for 2 minutes, then increased 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 μL; split ratio: 1:50. MS conditions: ionization voltage: 70 eV; ion source temperature: 280 °C; mass range: 30–300 mass units.

Quantization and Identification

The individual peaks were identified by comparison of their retention indices to those of authentic samples, as well as by comparing their mass spectra with the Wiley library mass spectral database. The percentage composition of the samples was computed from the GC peak areas. For quantization, the internal standard (octyl-β-glucoside) was added before the enzymatic hydrolysis. Quantization of the aglycones liberated by the acidic hydrolysis was done by addition of thymol. Preliminary GC-MS analysis revealed the absence of 1-octanol and thymol among the watermint volatile aglycones.

RESULTS AND DISCUSSION

Essential Oil

The yield of the essential oil obtained by hydrodistillation of the plant material was $w = 0.94\%$. Twenty-nine components were identified, corresponding to 98.98% of the overall oil. The identified compounds with their

TABLE I

Components and their concentrations in the essential oil of *Mentha aquatica*

No.	Identified compound	Content[a] / mg kg^{-1}	SD	Mode of identification
1.	α-pinene	105.28	15.98	I_1, I_2, MS
2.	sabinene + β-pinene	236.88	27.26	I_1, I_2, MS
3.	β-myrcene	168.26	0.94	I_1, I_2, MS
4.	1,8-cineole	1716.44	75.2	I_1, I_2, MS
5.	*cis*-β-ocimene	191.76	28.2	I_1, I_2, MS
6.	δ-3-carene	141.00	1.88	I_1, I_2, MS
7.	alloocimene	141.00	2.82	I_1, I_2, MS
8.	1-octen-3-ol	37.60	0.94	I_1, I_2, MS
9.	menthofuran	3978.08	34.78	I_1, I_2, MS
10.	β-bourbonene	18.80	0.94	I_1, I_2, MS
11.	linalol	111.86	5.64	I_1, I_2, MS
12.	menthyl acetate	41.36	2.82	I_1, –, MS
13.	calarene	37.60	0.94	–, I_2, MS
14.	β-elemene	38.54	1.88	I_1, I_2, MS
15.	α-gurjunene	47.00	0.94	–, I_2, MS
16.	*trans*-caryophyllene	1109.20	30.08	I_1, I_2, MS
17.	menthol	47.94	2.82	I_1, I_2, MS
18.	aromadendrene	47.00	0.94	I_1, I_2, MS
19.	α-terpineol	71.44	1.88	I_1, I_2, MS
20.	α-humulene	59.22	5.64	I_1, I_2, MS
21.	ledene	38.54	2.82	I_1, I_2, MS
22.	γ-cadinene	338.40	23.50	I_1, I_2, MS
23.	δ-cadinene	76.14	4.70	I_1, I_2, MS
24.	α-muurolene	10.34	0.94	I_1, I_2, MS
25.	β-farnesene	67.68	0.94	I_1, I_2, MS
26.	veridiflorol	320.54	7.52	–, I_2, MS
27.	nerolidol	47.94	0.94	I_1, I_2, MS
28.	spathulenol	11.28	1.88	I_1, –, MS
29.	cadinol	47.00	0.94	I_1, I_2, MS
	Total identified	9304.12 mg kg^{-1}		

[a]Average values of the percentages obtained by two columns for two replicates of two sampled individuals.

I_1, retention indicies on HP-20M; I_2, retention indicies on HP-101; MS, mass spectra; SD, standard deviation.

percentages are ordered in Table I according to the retention indices on a polar HP-20M column. The essential oil was composed of 30.56% of hydrocarbons (monoterpenes 10.47% and sesquiterpenes 20.09%) and 68.42% of oxygen containing compounds. The main oxygenated monoterpenes of this essential oil were menthofuran (3978.08 mg kg^{-1}) and 1,8-cineole (1716.44 mg kg^{-1}). *trans*-Caryophyllene (1109.20 mg kg^{-1}) and γ-cadinene (338.40 mg kg^{-1}) were identified as the major sesquiterpene hydrocarbons of the isolated oil. Veridiflorol (320.54 mg kg^{-1}) was the main sesquiterpene alcohol. This essential oil also contained smaller amounts of *cis*-β-ocimene, α-pinene, β-pinene, sabinene, β-myrcene as well as other compounds, Table I. Menthol and menthyl acetate were identified in very small amounts. The obtained results are in good agreement with those reported by other authors.[2,3] Since the essential oil of *Mentha aquatica* contains menthofuran as the major component, it has been suggested that high menthofuran production can often be used to detect the hybridization of *Mentha aquatica* with other *Mentha* species.[14]

Glycosidically Bound Volatiles

This is the first report on the glycosidically bound volatiles in *Mentha aquatica*. For this purpose, two methods of glycoside hydrolysis were applied. β-Glucosidase was chosen for its selectivity toward β-glucosides, which are more common in plants than those with α-glycosidic linkage. Plant β-glucosidases are generally specific to natural glycosides and β-glucosidase from bitter almonds is commonly used for hydrolysis of most β-glycosides.[8,15]

After the enzymatic hydrolysis, the obtained aglycones were extracted with pentane and analyzed by GC-MS. The content of glycosidically bound volatile compounds in the dried plant material was 81.0 mg kg^{-1}, representing only about one hundredth of the essential oil content. Most of these compounds (89.18%) were identified. The mixture of liberated aglycones is composed of aliphatic alcohols, derivatives of phenylpropanes and terpene compounds. Seventeen aglycones were identified. The results are shown in Table II, column A. The compounds are ordered with respect to the elution time on HP-20M column. The main aglycones were: 1-octen-3-ol (30.22 mg kg^{-1}), eugenol (10.21 mg kg^{-1}), 2-phenylethanol (6.81 mg kg^{-1}), 3-hexen-1-ol (5.35 mg kg^{-1}), perilla alcohol (4.13 mg kg^{-1}) and 1-hexanol (3.58 mg kg^{-1}). Benzyl alcohol, thymoquinone, α-terpineol, limonyl alcohol and benzaldehyde were identified in small quantities. The presence of aldehydes and ketones among the aglycones of plant glycosides was already reported by Stahl-Biskup *et al*.[8] Thymoquinone is probably the product of thymoquinol oxidation during the enzymatic hydrolysis.[16] Some volatiles identified among

TABLE II

Percentage composition of the volatile aglycones of *Mentha aquatica* liberated by the enzymatic and acidic hydrolyses

No.	Identified compound	A		B		Mode of idetification
		Content[a] / mg kg^{-1}	SD	Content[a] / mg kg^{-1}	SD	
1.	1-heptanol	1.30	0.01	–	/	I_1, –, MS
2.	1-hexanol	3.58	0.02	–	/	I_1, I_2, MS
3.	3-hexen-1-ol	5.35	0.08	t	/	I_1, I_2, MS
4.	3-octanol	2.44	0.02	1.24	0.01	I_1, I_2, MS
5.	2-hexen-1-ol	1.47	0.01	–	/	I_1, I_2, MS
6.	1-octen-3-ol	30.22	0.23	3.81	0.12	I_1, I_2, MS
7.	linalool oxide	–	/	0.25	0.02	I_1, I_2, MS
8.	benzaldehyde	0.83	0.01	–	/	I_1, I_2, MS
9.	cyclopentanol	0.34	0.01	–	/	I_1, –, MS
10.	*p*-menth-3-en-1-ol	–	/	1.25	0.02	I_1, I_2, MS
11.	cyclooctanol	–	/	3.09	0.15	I_1, –, MS
12.	terpineol	1.05	0.02	2.55	0.24	I_1, I_2, MS
13.	thymoquinone	1.14	0.01	–	/	I_1, I_2, MS
14.	myrtenol	0.34	0.02	–	/	I_1, I_2, MS
15.	nerol	0.51	0.02	–	/	I_1, I_2, MS
16.	benzyl alcohol	1.46	0.02	1.88	0.05	I_1, I_2, MS
17.	2-phenylethanol	6.81	0.09	3.61	0.34	I_1, I_2, MS
18.	limonyl alcohol	1.06	0.02	0.73	0.01	I_1, I_2, MS
19.	2-hexenoic acid	–	/	2.01	0.05	I_1, I_2, MS
20.	perilla alcohol	4.13	0.17	–	/	I_1, –, MS
21.	bisabolol oxide	–	/	1.71	0.10	I_1, I_2, MS
22.	eugenol	10.21	0.20	3.49	0.40	I_1, I_2, MS
23.	1-(2,3,6-trimethyl-phenyl)-2-butanone	–	/	1.78	0.20	I_1, –, MS
24.	1-(2,3,6-trimethyl-phenyl)-3-buten-2-one	–	/	1.15	0.02	I_1, –, MS

TABLE II (cont.)

No.	Identified compound	A		B		Mode of idetification
		Content[a] / mg kg^{-1}	SD	Content[a] / mg kg^{-1}	SD	
25.	α-(2,3,6-trimethyl phenyl)propanol	–	/	0.66	0.01	I_1, I_2, MS
26.	benzoic acid	–	/	1.75	0.04	I_1, –, MS
27.	hexadecanoic acid	–	/	1.43	0.06	I_1, –, MS
28.	4-hydroxy-3-methyl-2-cyclopenten-1-one	–	/	0.78	0.07	I_1, –, MS
	Total identified	72.24 mg kg^{-1}		33.17 mg kg^{-1}		

[a]Average values of the percentages obtained by two columns for two replicates of two sampled individuals.
A, enzymatic hydrolisis; B, acidic hydrolysis after enzymatic hydrolysis; I_1, retention indicies on HP-20M; I_2, retention indicies on HP-101; MS, mass spectra; SD, standard deviation; –, compound not detected; /, not calculated.

the liberated aglycones are present in the essential oil of *Mentha aquatica*, such as 1-octen-3-ol and α-terpineol. Other detected aglycones were not present or structurally similar to the components of the watermint essential oil. Aliphatic alcohols such as 2-phenylethanol, benzyl alcohol, eugenol, nerol, α-terpineol and terpinen-4-ol are common compounds among the volatile aglycones.[3] In previous studies[9,10] we identified eugenol as the main aglycon in eight plants of the family *Lamiaceae*. 1-Hexanol as a volatile aglycone was identified in *Lavandula hybrida*.[17]

After the enzymatic hydrolysis and extraction of the liberated aglycones, the aqueous residue was submitted to acidic hydrolysis with hydrocloric acid (pH 1). The acidic hydrolysis was performed under hexane to reduce the formation of artifacts. The liberated volatiles were GC-MS analyzed. The results are shown in Table II, column B. After the acidic hydrolysis, the yield of the obtained aglycones was 50.0 mg kg^{-1}, of which 66.32% were identified. A mixture of oxygenated sesquiterpenes mainly constituted the unidentified parts of the chromatogram. The obtained aglycones had only partial similarity with those liberated by the enzymatic hydrolysis. Most of them were aliphatic alcohols, monoterpenes and phenylpropane derivatives. Some of the liberated aglycones are probably artifacts due to the reactions of elimination and/or rearrangement in acidic media. The main components were: 1-octen-3-ol (3.81 mg kg^{-1}), terpineol (2.55 mg kg^{-1}), 2-phe-

nylethanol (3.61 mg kg^{-1}) and 2-hexenoic acid (2.01 mg kg^{-1}). Other oxygenated compounds in smaller percentages were also identified, Table II, column B. It is interesting that, after the acidic hydrolysis, one third of identified compounds were ketones and acids, while only one aldehyde (benzaldehyde) was detected after the enzymatic hydrolysis.

The total amount of liberated aglycones showed that approx. 38% of the glycosides were not hydrolyzed by the used enzyme, suggesting that those aglycones are probably not bound to a glucose unit. This hypothesis is made on the assumption that the enzymatic hydrolysis was complete.

Acknowledgements. – The authors are grateful to the Ministry of Technology and Science of the Republic of Croatia for financial support. We also thank Mr. Paladin for technical assistance.

REFERENCES

1. E. Guenther, *The Essential Oils*, Vol. III, D. van Nostrand Co., Princeton, 1964.
2. T. Sacco and M. Maffei, *Development in Food Science*, (*Flavors and Fragrances),* 18, Elsevier, Amsterdam, 1988, pp. 141–145.
3. M. Maffei, *Flavour Fragr. J.* **3** (1988) 79–84.
4. T. Sacco, M. Maffei, and F. Chialva, *Proceedings of 11th Int. Congresess of Essential Oils, Fragrances and Flavours*, Oxord and IBTT Publ. Co., New Delhi, 1989, pp. 27–34.
5. N. Mimica-Dukić, O. Gasić, R. Jančić, and G. Kite, *J. Essent. Oil Res.* **10** (1998) 502–506.
6. A. Pinarosa, S. Giusy, and C. Giorgio, *Sci. Pharm.* **63** (1995) 223–230.
7. S. C. Khojastehbakht, L. L. Koenings, R. M. Peter, W. F. Trager, and S. D. Nelson, *Drug Metabolism & Disposition* **26** (1998) 701–704.
8. E. Stahl-Biskup, F. Intert, J. Holthuijzen, M. Stengele, and G. Schulz, *Flavour Fragr. J.* **8** (1993) 61–80.
9. J. Mastelić, *The Study of the Relations of Terpenes and Terpene Glycosides of the Aromatic Plants Belonging to the Family* Lamiaceae (Labiatae), Ph. D. Thesis, University of Zagreb, 1995.
10. J. Mastelić, M. Miloš, D. Kuštrak, and A. Radonić, *Croat. Chem. Acta* **71** (1998) 147–154.
11. A. G. Pérez, A. Cert, J. J. Ríos, and J. M. Olías, *J. Agric. Food Chem*. **45** (1998) 4393–4397.
12. M. Miloš and J. Mastelić, *Flavour Fragr. J*. **13** (1998) 248–250.
13. M. Miloš and A. Radonić, *Food Chem*. **68** (2000) 333–338.
14. S. Kokkini, *Advances in Labiatae Science*, Royal Botanic Gardens, Kew, 1992, pp. 325–334.
15. E. Stahl-Biskup, *Flavour Fragr. J*. **2** (1987) 75–82.
16. M. Miloš and A. Radonić, *Acta Pharm*. **46** (1996) 309–314.
17. J. Mastelić and D. Kuštrak, *Acta Pharm.* **47** (1997) 133–138.

SAŽETAK

Sastav slobodnih i glikozidno vezanih hlapljivih spojeva *Mentha aquatica* L.

Igor Jerković i Josip Mastelić

Istraženi su slobodni i glikozidno vezani hlapljivi spojevi biljke *Mentha aquatica* L. Slobodni hlapljivi spojevi izolirani su iz sušenog biljnog materijala hidrodestilacijom, a glikozidi ekstrakcijom vodom za vrijeme hidrodestilacije. Slobodni hlapljivi spojevi (eterično ulje) kao i aglikoni, dobiveni nakon hidrolize glikozida, analizirani su vezanim sustavom plinska kromatografija – masena spektrometrija (GC-MS). Eterično ulje sadržavalo je monoterpenske ugljikovodike (10,47%), seskviterpenske ugljikovodike (20,09%), te spojeve s kisikom (68,42%). Glavne komponente eteričnog ulja bile su: mentofuran (3978,08 mg kg^{-1}), 1,8-cineol (1716,44 mg kg^{-1}) i *trans*-kariofilen (1109,20 mg kg^{-1}). Nakon enzimske hidrolize, glavni hlapljivi aglikoni bili su: 1-okten-3-ol (30,22 mg kg^{-1}), eugenol (10,21 mg kg^{-1}), 2-feniletanol (6,81 mg kg^{-1}), 3-heksen-1-ol (5,35 mg kg^{-1}), perila alkohol (4,13 mg kg^{-1}), 1-heksanol (3,58 mg kg^{-1}) i 3-oktanol (2,44 mg kg^{-1}). Uočena je umjerena sličnost između hlapljivih aglikona i odgovarajućih slobodnih hlapljivih spojeva u eteričnom ulju. Kiselinskom hidrolizom, provedenom nakon enzimske, oslobodile su se dodatne količine aglikona. Približno 38% glikozida nije bilo hidrolizirano upotrijebljenim enzimom.