Phytochemistry, 1974, Vol 13, pp 1531 to 1535 Pergamon Press Printed in England

COMPOSITION OF THE ESSENTIAL OIL OF *MENTHA AQUATICA*

THEO M. MALINGRÉ
Laboratory for Pharmacognosy and Galenical Pharmacy, University of Groningen, The Netherlands

and

HENK MAARSE
Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands

(*Received* 4 *November* 1973 *Accepted* 18 *January* 1974)

Key Word Index—*Mentha aquatica*, Labiatae, essential oils, germacrene-D, bicyclogermacrene, viridiflorol

Abstract—The overall composition of the essential oil of a botanically described *Mentha aquatica* ($2n = 96$) has been determined Limonene, caryophyllene and germacrene-D are the major hydrocarbons and bicyclogermacrene and viridiflorol, an oxygenated compound, are present in substantial amounts The presence of small amounts of menthones, mentholes and menthyl acetates is indicated.

INTRODUCTION

In THE course of a study of the biogenesis of the volatile oils of the parents of *Mentha piperita* L, the composition of the volatile oil of *M. aquatica* L was investigated. This oil has also been investigated in relation to terpene biogenesis by Hefendehl,[1] who identified a number of mostly monoterpene components. Moreover, Hefendehl and Murray[2,3] published the results of a study of the changes in monoterpene composition of *M. aquatica* produced by gene substitution from other mint species.

The object of the present work was to obtain information particularly on the sesquiterpene composition of the volatile oil of *M. aquatica.* On account of the polymorphy of this species, special attention has been paid to the description of the botanical identity of the plant material.

RESULTS AND DISCUSSION

Identification of the strain of Mentha aquatica *L used*

M. aquatica L should be regarded as one form only of a very polymorphic group with numerous combinations of characters and very wide and gradual variations. In accordance with Graham[4] and also on cytological grounds it does not seem reasonable to divide the species into grades of "varieties" and "forma". One strain (No. 23), obtained from seeds of the botanical garden of Pisa (Italy), turned out to be in good agreement with the description given for *M. aquatica* L, and this strain was used for the investigation of the volatile

---

[1] HEFENDEHL, F W (1967) *Arch Pharmaz* **300,** 438

[2] HEFENDEHL, F W and MURRAY, M J (1972) *Phytochemistry* **11,** 189

[3] MURRAY, M J. and HEFENDEHL, F W (1972) *Phytochemistry* **11,** 2469

[4] GRAHAM, R A. (1954) *Watsonia* **3,** 109

oil. Recent publications of chromosome counts for *M aquatica* L by different authors[5-8] give a number of $2n = 96$. This same number was found for our plant material

Description of Mentha aquatica *"Strain* No. 23"

Stem green, branched, hairy at the nodes, otherwise almost glabrous below but becoming more hirsute upwards with retrorse white hairs, internodes up to 6 cm *Leaves* ovate, subacute at the apex, abruptly rounded at the base with a small unequal wedge at the junction with the petiole, up to 5 5 × 3 5 cm, generally subglabrous but with white hairs on the nerves of both surfaces, serratures shallow, more or less blunt, forward directed, up to 10 in number *Petioles* up to 16 mm with short white hairs *Influorescence* consisting of a terminal, ± rounded capitulum with a medium dia of 18 mm, with two pairs of underlying, non-contiguous, axillary, shortly-pedunculate verticils *Calyx* ribbed and tubular, 10 nerves, shortly ciliate, the teeth lanceolate 1/4 the length of the tube *Pedicels* with white hairs especially at the start of the calyx *Corolla* lilac with a few hairs externally and a ring of hairs in the throat *Stamens* exserted The plants are fertile, reach a height up to 70 cm, have a strong odour and develop runners.

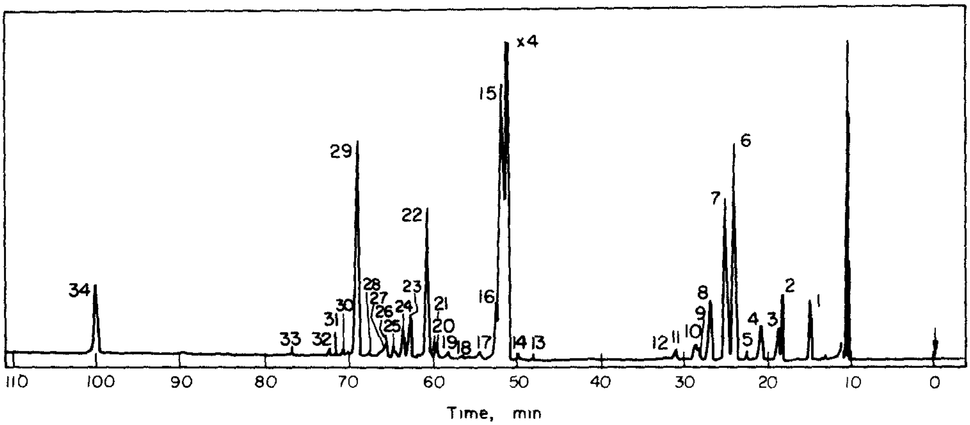

FIG 1 GAS CHROMATOGRAM OF *Mentha aquatica* OIL

Conditions 150 m × 0·75 mm (i d) Carbowax 20 M column, temperature isothermal at 80° for 20 min, then programmed at 2°/min to 170° and run isothermal thereafter, chart speed 15 in/hr range 100 attenuation 1 or as indicated

Composition of the oil

The results of the qualitative analysis of the oil are presented in Table 1. The peak numbers correspond to those in the chromatogram (Figure 1), this chromatogram was obtained by injecting 0·2 μl of a 5% solution of the oil in n-heptane on the Carbowax capillary column.

Limonene, caryophyllene and germacrene-D are the major hydrocarbons in the oil. A number of the other sesquiterpene hydrocarbons identified are chemically and possibly biogenetically related to germacrene-D (α-copaene, β-bourbonene, γ-muurolene and δ-cadinene[9])

[5] MORTON, J K (1956) *Watsonia* **3,** 244

[6] MURRAY M J (1958) *Proc X Int Congr Genet* **2,** 201

[7] BAQUAR, S R and REESE, G (1965) *Pharmazie* **20,** 159

[8] OUWENEEL W J (1968) *Proc R Natl Acad Sci Ser C* **71,** 184

[9] YOSHIHARA, K , OHTA Y , SAKAI, T and HIROSE Y (1969) *Tetrahedron Letters* 2263

TABLE 1 COMPONENTS IDENTIFIED IN *Mentha aquatica* OIL (STRAIN NO 23)

Peak no	Component	Identification		
			Kovats Index‡	
		Spectral	Carb 20M	SF96 (50)
1*	α-thujene*		1038	931
1†	α-pinene	MS	1038	939
2	β-pinene	MS	1120	978
3	sabinene	MS	1130	972
4	myrcene	MS	1168	984
5	α-terpinene	MS	1189	1016
6	limonene	MS	1210	1024
7	1,8-cineol	MS	1223	1025
8	cis-β-ocimene	MS	1238	1027
9	γ-terpinene	MS,IR	1253	1053
10	trans-β-ocimene	MS	1257	1042
11	p-cymene	MS	1275	1018
12	terpinolene	MS	1289	1081
13	α-cubenene	MS	1481	1362
14	menthon	MS	1518	1158
15*	menthofurane	MS	1503	1147
15†	α-copaene	MS	1520	1392
16	isomenthon	MS,IR	1528	1156
17	β-bourbonene	MS	1544	1407
18	β-cubebene	MS	1560	1400
19	isomenthyl acetate	MS	1579	1283
20	neoisomenthyl acetate	MS	1602	1297
21	β-elemene	MS	1608	1400
22	caryophyllene	MS	1618	1436
23	neoisomenthol + unknown	MS,IR	1634	1180
24	menthol	MS,IR	1640	1168
25*	alloaromadendrene	MS	1660	1475
25†	isomenthol	MS	1667	1182
26	trans-β-farnesene	MS	1671	1426
27	α-humulene	MS	1680	1466
28	γ-muurolene + unknown	MS	1695	1486
29	germacrene-D	MS	1718	1488
30	bicyclogermacrene†	MS	1738	§
31	trans-trans-α-farnesene	MS	1756	1501
32	δ-cadinene	MS	1760	1520
33	cuparene	MS	1811	1506
34	viridiflorol‡	MS,IR	2103	1588

* Identification only by comparison of Kovats Indices

† Identification only by comparison of data other than Kovats Indices, no KI have been published and reference compounds were not available

‡ Conditions for KI determinations.

Carbowax 20 M column:	
peak 1–12	column temp 70°
peak 13–33	column temp 130°
peak 34	column temp 180°
SF96 (50) column	
peak 1–12	column temp. 100°
oxygenated monoterpenes	column temp 130°
sesquiterpene hydrocarbons and peak 34	column temp 150°

§ Bicyclogermacrene decomposes under the conditions of the analysis on the SF96 (50) column

A very labile sesquiterpene hydrocarbon in the oil is component 30 (Table 1). It isomerizes during GC at high injection port and detector temperatures (> 160°); resulting in the formation of aromadendrene. The MS of component 30 is identical with that of bicyclogermacrene.[10] The identity was ascertained by Nishimura[11] who isolated this same compound from the cold pressed oil of *Citrus junos*, it was also found to be present in the oil of *Origanum vulgare* L ssp *vulgare*.[12]

Bicycloelemene, an isomerization product of bicyclogermacrene was found by Ognyanov and Vlahov[13] and by Lawrence *et al*[14] in *M. piperita*.

Menthofuran, viridiflorol and 1,8-cineol are the major oxygenated compounds in the oil. Viridiflorol is also a labile compound, which decomposes to give a sesquiterpene hydrocarbon at high injection port temperatures (190°).

Microhydrogenolysis[15] of viridiflorol resulted in a number of saturated hydrocarbons, the main product was identified by its IR spectrum[16] as aromadendrane. This result supports the evidence obtained by comparing the IR spectrum with a published spectrum of viridiflorol.[17] Accurate mass measurement gave the molecular formula $C_{15}H_{26}O$.

Ledol, a stereoisomer of viridiflorol was reported recently by Lawrence *et al.*[14] to be a minor constituent of the oil of *M. piperita* L. From the CGLC–MS analysis of the oxygenated fractions it became evident that two menthones, and a number of menthols and menthyl acetates were present in the oil as well. The mass spectra of the menthols and menthyl acetates show only minor differences so that more evidence was needed for further identification. Therefore, the Kovats Indices were compared with those of reference compounds and IR spectra of a number of the components were recorded (Table 1).

EXPERIMENTAL

Plant material. *Mentha aquatica* from seeds of different botanical gardens or gathered from growing wild plants, was propagated for 5 yr at the experimental garden at Buitenpost (The Netherlands). The identification of the plant material took place by means of the determination key of Ouweneel[18] and of the description as given by Graham.[4]

Counting of chromosomes. Chromosome counts were made from preparations of root tips of germinating seeds, using the squash technique. The preparations were prefixed in a mixture of 1% chromic acid–HOAc–formalin (10:1:4) for a period of 2 or 3 hr and subsequently coloured for 15 mins in a lacto-orcine soln (1 g orcine, 9.4 ml lactic acid, 24.4 ml HOAc, 16.2 ml H_2O, reflux for 1 hr and filter) and finally squashed in 45% HOAc.

Isolation of the volatile oil. The oil was isolated by steam distillation on semi-technical scale (16.7 kg of the fresh herb yielded 7.36 g of the oil).

Column chromatography. The oil was separated by column chromatography over silica gel (Merck 7733, 0.2–0.5 mm) into hydrocarbons and oxygenated components, hexane and EtOH were used as the eluents.

GC. The hydrocarbon fraction was separated into 1 monoterpene and 4 sesquiterpene fractions on a 5 m × 4 mm (i.d.) aluminium column packed with 20% Lac-1-R-296 on Chromosorb W-AW, 60/80 mesh, at a column temp of 110°. These subfractions were further analysed on a CGLC–MS combination. The fraction of oxygenated components was separated on the same Lac column into six subfractions. From these subfractions, which were collected in melting point tubes [30 cm × 0.1 cm (i.d.)], IR and MS were recorded.

Kovats indices of all identified components, measured on two 150 m × 0.75 mm (i.d.) stainless steel capillary columns coated with Carbowax 20 m and SF 96 (50) respectively were compared with Kovats indices of reference compounds or with Kovats Indices taken from the literature.

GC–MS. A 150 m × 0.75 mm (i.d.) stainless steel capillary column coated with Carbowax 20 M was connected with a mass spectrometer (CH4, Varian Mat, Bremen) by means of a membrane separator.

---

[10] NISHIMURA, K. personal communication.

[11] NISHIMURA, K., SHINODA, N. and HIROSE, Y. (1969) *Tetrahedron Letters* 3097.

[12] MAARSE, H. and VAN OS, F. H. L., *Flavour Ind.*, in press.

[13] OGNYANOV, I. and VLAHOV, R. (1967) *Riechstoffen Aromen Korperpflegemittel* **17**, 315.

[14] LAWRENCE, B. M., HOGG, J. W. and TERHUNE, S. J. (1972) *Flavour Ind.* **3**, 467.

[15] KEPNER, R. E. and MAARSE, H. (1972) *J. Chromatog.* **66**, 229.

[16] PLIVA, J., HORAK, M., HEROUT, V. and SORM, F. (1963) *Terpenspektren. Sammlung der Spektren und Physikalischen Konstanten. Band II.* Akademie Verlag.

[17] ibid, Band I.

[18] OUWENEEL, W. J. (1967) *Gorteria* **3**, 213.

Micro-hydrogenolysis One component (peak 34) was hydrogenolysed using the technique described by Kepner and Maarse[15] for the hydrogenolysis of monoterpenes

Spectrometry IR spectra were measured on a Perkin–Elmer 257 and the Varian Mat type 731 MS was used for accurate mass measurement