

# Essential Oils and their Constituents XXIII.

## Chemotaxonomy of the Genus *Mentha*

By K. L. HANDA\*, D. M. SMITH, I. C. NIGAM, and LEO LEVI

Essential oils derived from *Mentha aquatica* L.; *M. citrata* Ehrh. (bergamot mint); *M. pulegium* L. (European and North African pennyroyal); *Hedeoma pulegioides* (L.) Pers. (American pennyroyal); *M. rotundifolia* and *M. sylvestris* L. were examined by gas-liquid partition chromatography using a polar (SAIB) and nonpolar (Ucon) substrate, respectively. Quantitative data illustrating the extent of compositional differences between these products are presented and compared with similar criteria of authenticity for *M. arvensis* (mint); *M. piperita* (peppermint); *M. cardiaca*, *M. spicata*, and *M. viridis* (spearmints). The occurrence of isopiperitenone as a major constituent of *M. rotundifolia* derived from a French mutant strain and the detection of several terpenoids occurring as trace constituents in these species are reported for the first time. Correlation of the chemical data illustrates their value in plant classification.

THE CLASSIFICATION of aromatic plants considered to belong to the genus *Mentha*—an important source of basic raw materials for the food, drug, and cosmetic industries—has always proved a difficult task to the botanist, geneticist, and physiologist engaged in natural products research. As yet, the many species of this genus cannot be unambiguously identified by a single technique. It is the purpose of this paper to demonstrate the value of using chemical analysis of the essential oils they yield for the recognition of botanical origins.

### EXPERIMENTAL

Apparatus and analytical methods were described in previous publications (1-4).

**Essential Oils.**—The *Mentha aquatica*, *M. citrata*, *M. sylvestris*, and *Hedeoma pulegioides* used were obtained through the courtesy of Dr. F. J. Cramer, A. M. Todd Co., Kalamazoo, Mich. Plants used as the source of these oils were grown under the direction of Dr. M. J. Murray—herbarium specimens available at the Cornell University Herbarium and the Missouri Botanical Gardens, St. Louis, Mo.; *M. rotundifolia* was obtained through the courtesy of Dr. Sumio Shimizu, Laboratory of Agricultural Chemistry, Shinshu University, Ina-Nagano Ken, Japan. Plants used as the source of these oils were grown at the Okayama Agricultural Experiment Station and Laboratory of Plant Breeding under the direction of Drs. Sumio Shimizu and Nagamori Ikeda. *Mentha pulegium*—authenticated commercial products were obtained through the courtesy of Dr. E. Guenther, Fritzsche Brothers, Inc., New York, N. Y., Lautier Fils, New York, N. Y., and Destilaciones Bordas Chinchurreta, S. A., Seville, Spain.

### RESULTS AND DISCUSSION

Essential oils are typical end-products of plant metabolism and as such their composition reflects all biogenetic and biochemical processes associated with and characteristic of plant life. Thus, essential oils analysis can assume a key role in plant authentication.

Table I lists the various constituents occurring in the products examined. Relative retention times

and quantitative data for the components are also given. They show that distinct qualitative and quantitative differences as well as marked compositional similarities exist between essential oils of different botanical provenance. The value of specific ratios of biochemically related constituents as characteristic criteria of authenticity for several essential oils of the genus *Mentha* has already been demonstrated. Limonene-cineole ratios, for example, were shown to distinguish readily *M. arvensis* from *M. piperita* (major constituent menthol) and provide reliable parameters of geographical provenance as well. They permitted, likewise, unambiguous distinctions between *M. cardiaca*, *M. spicata*, and *M. viridis* (major constituent carvone) of different geographical origins (1, 2). The present study shows the specificity of this ratio for *M. aquatica* (0.29); *M. sylvestris* (0.48); *M. citrata* (5.5); and *M. rotundifolia*, French mutant strain (4.8), Italian strain (9.7). These oils can, moreover, be readily distinguished from one another by analysis for their major constituents, e.g., *M. aquatica*, menthofuran (51.3%); *M. citrata*, linalyl acetate (45.6%); *M. pulegium*, pulegone (77.6 ± 6.3%); *M. rotundifolia*, French strain, neo-iso-isopulegol (52.3%), Italian strain, piperitenone oxide (87.3%); *M. sylvestris*, piperitenone oxide (66.3%).

The occurrence of isopiperitenone in *M. rotundifolia* (French strain) was established following isolation and purification of the compound by preparative gas chromatography. Hydrogenation of the isolate with Adams catalyst in methanol showed the presence of two double bonds in the molecule and yielded a product consisting of isomenthone (71%), menthone (17%), and isomeric menthols (12%). Ultraviolet examination ( $\lambda_{\text{max}}^{\text{EtOH}}$  233.5  $\mu\mu$ ) suggested the presence of an  $\alpha,\beta$ -unsaturated keto group. Infrared analysis of the compound (see Fig. 1) confirmed this deduction (1670  $\text{cm}^{-1}$  absorption) and showed, moreover, the presence of a terminal methylene group in a side chain (3080, 1787, 1640, and 888  $\text{cm}^{-1}$ ), as well as a trisubstituted double bond conjugated with a keto group (3035, 1617, and 787  $\text{cm}^{-1}$ ).

One of the oils examined, *Hedeoma pulegioides* (L.) Pers. (American pennyroyal), generally considered belonging to a different genus of the Labiatae family, contained all constituents present in oil of *M. pulegium* L. (European pennyroyal). Yet characteristic quantitative differences were detected, e.g., limonene-cineole ratio for *H. pulegioides* 6.1;

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TABLE I.—ESSENTIAL OILS OF THE GENUS *Mentha*

No.	Constituent	Retention time (relative to menthol) UCON SAIB	Essential Oil Composition %											
			<i>M. aquatica</i> (5, 6, 11)	<i>M. citrata</i> <sup>b</sup> (5, 6, 11)	<i>H. pulegi-</i> <i>oides</i> (6, 11)	<i>M. pulegium</i> (5, 6, 11)	French Strain (7, 8, 11)	Italian Strain (7, 8, 11)	<i>M. syl-</i> <i>vestris</i> (6, 6, 11)	<i>M. arvensis</i> (1, 3, 11)	<i>M. piperita</i> (1, 3, 9, 11)	<i>M. cardiaca</i> (2, 9, 11)	<i>M. spicata</i> (2, 9, 11)	<i>M. viridis</i> (2, 11)
1	$\alpha$ -Pine	0.18	0.9 <sup>a</sup>	0.6 <sup>a</sup>	0.4	0.7 to 1.2	0.9 <sup>a</sup>	0.7 <sup>a</sup>	1.3 <sup>a</sup>	0.2 to 0.5	0.2 to 1.0	0.5 to 1.0	0.5 to 0.9	0.7 to 0.8
2	$\beta$ -Pine	0.23	0.9 <sup>a</sup>	0.9 <sup>a</sup>	0.2	0.7 to 0.8	2.1 <sup>a</sup>	1.7 <sup>a</sup>	4.5 <sup>a</sup>	0.9 to 1.3	0.7 to 1.9	1.3 to 2.6	1.8 to 4.4	4.4 to 4.5
3	Limonene	0.27	6.4 <sup>a</sup>	1.1 <sup>a</sup>	0.6	0.1 to 0.6	2.9 <sup>a</sup>	2.9 <sup>a</sup>	4.3 to 6.0	4.3 to 6.0	2.7 to 6.8	17.7 to 29.7	13.3 to 2.5	1.9 to 16.2
4	Cineole	0.30	22.4	0.2 <sup>a</sup>	0.1 <sup>a</sup>	0.2 to 0.8 <sup>a</sup>	0.6 <sup>a</sup>	0.3 <sup>a</sup>	10.2	0.4 to 0.8	5.2 to 13.5	1.8 to 2.5	2.9 to 4.4	6.0 to 6.8
5	3-Octanol	0.36	1.9 <sup>a</sup>	...	{0.6 <sup>a</sup> }	{1.0 to 1.3 <sup>a</sup> }	{0.9 <sup>a</sup> }	0.7 <sup>a</sup>	0.2 <sup>a</sup>	0.2 to 0.8	...	1.6 to 2.6	0.7 to 1.4	0.6 to 0.7
6	3-Octyl acetate	0.36	0.46	0.3 <sup>a</sup>	...	...	...	0.5 <sup>a</sup>	...	...	0.3	0.2 to 0.5	0.2 to 0.5	0.3
7	Sabinene hydrate	0.49	0.94	...	...	...	...	...	...	0.3 to 0.9	{0.1 to 0.4}	{0.8 to 1.5}	...	...
8	Sabinene acetate	0.49	0.94	...	...	...	...	...	...	...	0.0 to 0.4	0.0 to 0.4	0.1 to 0.2	...
9	Linalool	0.62	0.67	...	32.4	...	...	...	0.2 <sup>a</sup>	0.0 to 0.8	0.0 to 0.4	0.0 to 0.4	<0.1	<0.1
10	Menthofuran	0.65	0.68	51.3	0.1	0.2 to 0.3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
11	Menthone	0.67	0.75	...	...	...	...	...	...	9.9 to 17.1	0.9 to 2.2	0.1 to 0.5	0.1 to 0.5	0.2
12	Isomenthone	0.70	0.78	...	...	...	...	0.2	...	2.7 to 4.2	0. to 0.3	0.0 to 0.1	0.0 to 0.1	0.0
13	Linalyl acetate	0.81	0.94	...	45.0	0.6	1.7 to 4.1	...	0.1 <sup>a</sup>	1.6 to 2.2	2.8 to 5.3	...	...	...
14	Neomenthol	0.86	0.94	...	...	...	...	...	...	4.7 to 6.9	3.8 to 11.6	...	...	...
15	Carvomenthone	0.88	1.03	...	...	...	...	...	...	...	0.1 to 0.6	0.7 to 1.3	0.2	...
16	Menthyl acetate	0.94	1.06	6.2 <sup>a</sup>	...	...	...	...	...	...	...	...	...	...
17	Neo-iso-pulegol	0.98	1.00	...	...	...	...	52.3	0.2 <sup>a</sup>	...	...	...	...	...
18	Dihydrocarvone	1.00	1.11	...	...	...	...	...	...	...	2.5 to 6.9	3.4 to 5.5	3.5 to 4.6	...
19	Menthol	1.00	1.00	2.6	...	0.1	0.2 to 0.7	...	...	61.5 to 68.6	33.2 to 48.7	...	...	...
20	Pulegone	1.16	1.24	1.8	8.1	82.3	71.3 to 83.9	...	...	0.2 to 1.1	0.5 to 4.6	0.0 to 0.9	0.1 to 0.9	1.7 to 1.9
21	Neo-iso-pulegyl acetate	1.30	1.38	...	...	...	...	7.2	0.1	...	...	0.4 to 1.3	0.5 to 4.1	4.4 to 7.7
22	Dihydrocarveyl acetate	1.32	1.59	...	...	...	...	13.4 <sup>a</sup>	...	...	...	...	...	...
23	Isopiperitenone	1.44	1.39	...	...	...	...	...	...	...	...	0.0 to 0.1	0.5 to 0.8	4.8
24	Dihydrocarveol	1.45	1.38	...	...	...	...	...	...	...	...	53.9 to 68.2	59.1 to 68.1	58.2 to 62.1
25	Carvone	1.45	1.59	0.3	...	...	...	...	...	...	...	0.7 to 0.9	0.8 to 1.0	0.9
26	<i>trans</i> -Carveyl acetate	1.45	1.68	...	...	...	...	...	...	1.8 to 2.7	0.5 to 4.1	...	...	...
27	Piperitone	1.48	1.67	0.8	4.2	0.9	0.3 to 1.7	7.3	0.1 <sup>a</sup>	0.3	<0.1	...	...	...
28	Piperitone oxide	1.63	1.70	...	3.0	...	...	...	66.3	...	...	0.0 to 1.6	0.5 to 2.6	0.9 to 1.7
29	<i>cis</i> -Carveyl acetate	1.66	1.92	...	...	...	...	...	...	...	...	0.2	0.2	0.5
30	<i>trans</i> -Carveol	1.79	1.59	...	...	...	...	...	...	...	...	0.0 to 0.9	0.0 to 1.0	0.2
31	<i>cis</i> -Carveol	1.84	1.77	...	...	...	...	...	...	...	...	...	...	...
32	Piperitenone	2.33	2.65	...	...	4.7	1.1 to 12.6	8.6	2.4	...	...	...	...	...
33	Piperitenone oxide	3.30	3.42	...	...	...	...	87.3	2.7	...	...	...	...	...
34	Unaccounted	...	...	2.6	3.8	7.2	5.6	3.7	2.8	2.5	0.0 to 0.9	0.6 to 1.8	0.0 to 0.8	0.0 to 0.7

<sup>a</sup> Presence of this constituent reported for first time. <sup>b</sup> Presence of 0.6% citral in this oil established by the BAC-method of analysis (10).

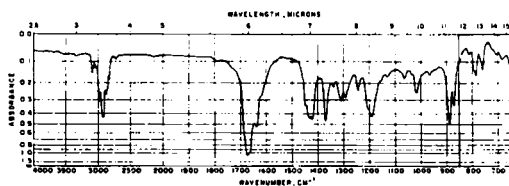


Fig. 1.—Infrared spectrum of isopiperitenone.  
4000–850  $\text{cm}^{-1}$   $\text{CCl}_4$  solution;  
850–650  $\text{cm}^{-1}$  contact film.

for *M. pulegium* 0.12 to 0.30. The somewhat higher linalyl acetate content of the European oil may likewise be a valuable indicator of botanical identity.

None of the essential oils was fractionated or pretreated prior to analysis. All samples were injected directly as the crude natural products obtained by conventional steam distillation. Coupled gas liquid–thin layer chromatography was used successfully for the detection of trace constituents. Similar analyses of fractions isolated by rectification, column chromatography, and/or other separation techniques would undoubtedly establish the presence

in these oils of many more terpene compounds still unaccounted for as shown in Table I.

The experimental data presented summarize results of a series of compositional studies on essential oils of the genus *Mentha*. They serve to illustrate further that distinct biochemical relationships exist between constituents synthesized by its different species and that such data, obtained exclusively by physiochemical analysis, provide important criteria for species characterization and classification via qualitative and quantitative chemotaxonomy.

#### REFERENCES

- (1) Smith, D. M., and Levi, L., *J. Agr. Food Chem.*, **9**, 230(1961).
- (2) Smith, D. M., Skakum, W., and Levi, L., *ibid.*, **11**, 268(1963).
- (3) Nigam, I. C., Sahasrabudhe, M., and Levi, L., *Can. J. Chem.*, **41**, 1535(1963).
- (4) Nigam, I. C., et al., *Perfumery Essent. Oil Record*, **53**, 614(1962).
- (5) Reitsema, H., *THIS JOURNAL*, **43**, 414(1954); *ibid.*, **47**, 265, 267(1958).
- (6) Guenther, E., "Essential Oils," Vol. III, D. Van Nostrand Co., Inc., New York, N. Y., 1949, pp. 575–687.
- (7) Shimizu, S., *Bull. Agr. Chem. Soc. Japan*, **20**, 2(1956).
- (8) Shimizu, S., and Ikeda, N., *ibid.*, **22**, 3(1958).
- (9) Nigam, I. C., and Levi, L., *J. Agr. Food Chem.*, **11**, 276(1963).
- (10) Levi, L., and Laughton, P. M., *ibid.*, **7**, 850(1959).
- (11) Nigam, I. C., and Levi, L., *THIS JOURNAL*, **53**, 1008(1964).

## Quantitative Evaluation of Infiltration Anesthetics in Albino Mice

By W. R. JONES, T. L. KERLEY, and L. C. WEAVER

**Infiltration anesthetics were quantitatively evaluated using a test procedure based on the vocalization of mice in response to electrical stimulation.**

**M**OST LABORATORIES today prefer to use the mouse as a laboratory animal for preliminary toxicity work and primary pharmacodynamic evaluation of chemical compounds. The use of this species for the evaluation of local anesthetic activity is advantageous because of economy and the opportunity to compare more tests in the same species. Weidmann and Petersen (1) first used the mouse to study surface anesthesia, and a slight modification of this method has been used in our laboratories (2). In addition, mice have been used for the evaluation of anesthetics injected directly into the tissues (3, 4). A method using mice for the quantitative assessment of infiltration anesthetics is described in this communication.

#### EXPERIMENTAL

Male Swiss-Webster albino mice were used in the experiments. A constant volume (0.03 ml.) of drug solution was injected beneath the skin on the plantar surface of one hind foot, and an equal volume of 0.9% sodium chloride solution was injected similarly into the opposite foot. Ten minutes after injection, the control foot of each animal was stimulated rapidly and repeatedly until the animal

vocalized, then continued to vocalize in response to 10 successive stimulations. Immediately afterward, the treated foot was stimulated five times, and any animal that failed to vocalize one or more times was classified as anesthetic. An electrical current (100 v. d.c.), delivered by a Grass model S-4 stimulator, was used as the stimulus. Because of tissue damage resulting from the intense stimuli, each animal was used only once. To facilitate conduction, each foot was moistened with 10% sodium chloride solution just prior to contact with the stimulating electrodes. Cocaine hydrochloride, procaine hydrochloride, dibucaine hydrochloride, and dyclonine hydrochloride were administered to groups of 10 animals at a minimum of three different concentrations to establish dose response curves. The anesthetic dose for 50% of mice ( $AD_{50}$ ) was calculated for each drug and relative potency determined with respect to cocaine hydrochloride (5, 6).

#### RESULTS

The  $AD_{50}$  for each of the drugs was determined on four separate occasions, and the results obtained are presented in Table I. Experiments A, B, and C were each completed during a single day for all of the local anesthetics. For the preliminary experiment, the data (except for cocaine) were obtained over a period of 2 days. The results obtained show that the  $AD_{50}$  values were consistent for procaine and dyclonine. There was one  $AD_{50}$  value that was slightly low for dibucaine and one high for cocaine.

The potency of these agents relative to cocaine

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