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ESSENTIAL OIL ANALYSIS OF THE MYRICACEAE OF THE EASTERN UNITED STATES*

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Abstract—The essential oil content of several members of the Myricaceae were examined for chemotaxonomic purposes. The analysis of the essential oils corroborates the suggestion that the Myricaceae should be divided into three genera. The study also suggests that *Myrica pusilla* and *M. macfarlanei* are not valid species. The analyses were carried out on the oil obtained from steam distillation of the foliage. Specific oil constituents were identified by GLC and IR.

INTRODUCTION

THE FAMILY Myricaceae consists of about 50 species of trees and shrubs growing mainly in subtropical to mild-temperate regions of the world. It is represented in North America by eight or nine species, seven or eight varieties, one natural hybrid and one artificially produced hybrid. More than 45 names have been applied to these plants which indicates the variable nature of the members of this family. Moreover, there is a general lack of agreement concerning the generic delimitations within the family. The number of genera recognized by various authorities ranges from one to four including the genus *Canacomyrica* which is of rather doubtful affinity.[1-3]

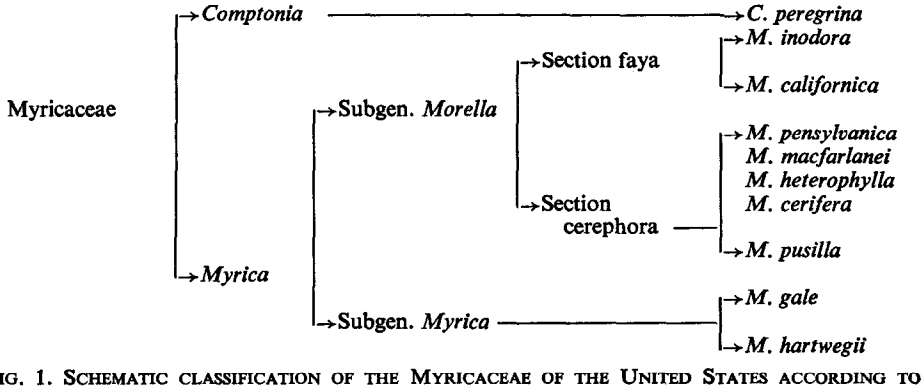

FIG. 1. SCHEMATIC CLASSIFICATION OF THE MYRICACEAE OF THE UNITED STATES ACCORDING TO ELIAS.[1]

Recently the genera of the Myricaceae of the eastern United States were reviewed by Elias[1] and he presented the taxonomic scheme shown in Fig. 1. Also, in a recently completed

* Part I in the projected series "Chemotaxonomy of the Myricaceae".
[1] ELIAS, T. S. (1971) *Arnold Arb.* **52**, 305.
[2] BAIRD, J. R. (1969) Ph.D. Thesis. University of North Carolina, 164 pp.
[3] RANDFORD, A. E., AHLES, H. E. and BELL, C. R. (1968) *Manual of the Vascular Flora of the Carolinas*, p. 360, University of North Carolina Press, Chapel Hill.

study in which the nomenclatural history of the Myricaceae was carefully reviewed, Baird[2] on the basis of anatomical, morphological and cytological evidence clearly defined the genera and species of North American members of the Myricaceae. Baird[2] raised the two subgenera, *Morella* and *Myrica* (Fig. 1), to generic ranks. Thus, he recognizes three genera within the family. The genus *Comptonia*, which was established by L'Heritier when he provided a description for Aiton's *Hortus Kewensis* in 1789 and which is regarded as monotypic by Baird.[2] The genus *Myrica*, viz. Linnaeus, *Genera Plantarum* (1753) with two clearly defined species in North America. One of the species *M. hartwegii* is quite stable in most of its characteristics throughout its range whereas *M. gale* is quite variable throughout its circumboreal range. The genus *Morella* was first described by Loureiro in 1790 in his *Flora Cochinchinensis* and according to Baird[2] there are five species represented in North America north of Mexico. These species are *M. heterophylla*, *M. pensylvanica*, *M. cerifera*, *M. inodora* and *M. californica*. Baird has reduced *M. pusilla* and *M. macfarlanei* to synonomy with *M. cerifera* and *M. pensylvanica* respectively.

One of the characteristic features of most members of the Myricaceae is the presence of aromatic foliage and it was thought that an investigation of the essential oils produced by selected species of the Myricaceae might have some chemotaxonomic value. Essential oils have been used in a number of taxonomic studies in recent years.[4–10]

In spite of the world-wide distribution of the Myricaceae, little attention has been given to their essential oil content. The essential oil of *M. gale* was investigated in a preliminary fashion by several authors.[11–13] The possible occurrence of α-pinene, α-phellandrene, cineole and caryophyllene was claimed by Enklar.[14] A preliminary analysis of the oil of *M. cerifera* was made by Rabak.[15]

RESULTS AND DISCUSSION

In order to avoid confusion the taxonomic scheme proposed by Baird[2] will be used in the following discussion.

The composition of the essential oils found in the various species investigated in this study is presented in Table 1. While a comparison of the total oil content in each species is valuable, a closer examination shows that certain compounds are of particular importance for comparative purposes. These compounds are shown in the bar histogram (Fig. 2).

An examination of the total oil content (Table 1) shows some characteristic features for all of the species examined. For example, in the sesquiterpene fraction caryophyllene and α-humulene are predominent; both these compounds are presumably synthesized through the 1/11 cyclization of *trans–trans*-farnesyl pyrophosphate.[16] Table 1 also shows that most

[4] MIROV, N. T. (1961) in *Recent Advances in Botany*, p. 72, Vol. 1, University of Toronto Press, Toronto.
[5] VON RUDLOFF, E. (1969) in *Recent Advances in Phytochemistry* (SEIKEL, M. K. and RUNECKLES, V. C., eds.), p. 127, Appleton–Century–Crofts, New York.
[6] HEFENDEHL, F. (1962) *Planta Med.* **10,** 240.
[7] DE WET, J. M. and SCOTT, B. D. (1965) *Botn. Gaz.* **126,** 209.
[8] SCORA, R. W. and MALIK, M. N. (1970) *Taxon* **19,** 215.
[9] ZAVARIN, E. and SNAJBERK, K. (1965) *Phytochemistry* **4,** 141.
[10] SMEDMAN, L. A., SNAJBERK, K., ZAVARIN, E. and MON, T. R. (1969) *Phytochemistry* **8,** 1471.
[11] CHEVALIER, J. (1909) *Compt. Rend. Soc. Biol.* **68,** 738.
[12] PERROT, L. E. (1911) *Bull. Sci. Pharmacol.* 253; *idem.* (1910) *Chem. Zentr.* **11,** 324.
[13] SCHOOFS, F. (1921) *Bull. Acad. Roy. Med. Belg.* **5,** 1, 367.
[14] ENKLAR, C. J. (1911) *Chem. Weekblad.* **9,** 218.
[15] RABAK, F. (1911) *Midland Drugg. Pharm. Rev.* **45,** 484.
[16] PARKERS, W., ROBERTS, J. S. and RAMAGE, R. (1967) *Quart. Rev. (London)* **21,** 331.

of the identified compounds were found in all of the species examined and that the hydrocarbon fraction constituted more than two-thirds of the oil in each species. Several of the compounds were present in approximately the same amounts as minor components in all of the species, e.g. camphene, β-pinene, terpinolene, terpinen-4-ol, α-terpineol, citronellol, the cadinenes, calamenene and farnesol.

TABLE 1. PERCENTAGES* OF ESSENTIAL LEAF OIL COMPOUNDS IN SELECTED MYRICACEOUS SPECIES

Compound		*M. pensylvanica*	*M. macfarlanei*	*M. heterophylla*	*M. cerifera*	*M. pusilla*	*M. gale*	*Comptonia peregrina*
α-Pinene	1†	4·44	4·99	19·36	32·18	44·43	4·70	7·00
Camphene		0·13	0·19	0·41	0·31	1·17	0·11	0·11
β-Pinene		1·35	1·00	0·67	1·04	0·95	0·30	1·16
Myrcene	2†	16·02	18·80	1·10	0·61	0·27	29·12	9·90
α-Terpinene		0·74	0·77	0·27	0·61	0·42	5·58	0·90
Limonene	3†	3·10	3·51	1·19	1·23	2·01	14·57	2·25
Cineole	4†	1·38	2·18	8·16	11·28	9·34	0·05	17·80
cis-Ocimene	5†	8·24	7·37	0·27	0·08	—	3·62	3·50
trans-Ocimene	6†	5·89	4·62	0·31	0·12	—	4·42	3·90
γ-Terpinene	7†	7·84	6.84	1·80	2·09	1·96	—	14·70
p-Cymene		2·27	3·60	0·41	0·61	1·47	5·52	3·35
Terpinolene		1·34	1·02	0·27	0·50	0·59	0·15	1·00
cis-3-Hexenol		0·11	0·05	0·15	0·04	—	0·21	—
Linalool	8†	2·80	2·07	7·56	5·01	0·96	1·33	7·13
Terpinen-4-ol		0·25	0·49	1·10	1·23	1·18	0·29	1·30
Caryophyllene	9†	19·31	15·86	14·52	10·00	8·33	5·50	8·91
α-Terpineol		1·35	1·87	2·77	2·25	2·48	0·38	1·70
α-Humulene	10†	6·98	5·95	14·04	9·48	4·11	3·44	4·17
Eremophillene		—	—	—	—	—	0·88	—
β-Selinene / α-Selinene	11†	1·25	1·53	6·54	8·70	9·23	0·87	0·75
Citronellol		0·04	0·30	1·19	0·13	0·12	0·49	Trace
α-Citronellol		—	—	—	—	—	0·49	—
Geranyl acetate		0·44	—	—	—	—	—	—
δ-Cadinene / γ-Cadinene		0·68	0·81	3·06	1·64	1·61	0·29	2·75
Nerol		0·28	0·30	0·28	0·14	0·12	0·07	—
Selina-4(14),7(11) diene		0·53	2·54	—	—	—	—	—
Geraniol		0·22	0·08	0·51	0·05	0·12	0·05	—
Calamenene		0·09	Trace	0·27	Trace	0·12	0·14	0·16
Nerolidol	12†	7·13	8·27	6·55	5·31	2·43	0·84	1·50
γ-Eudesmol	13†	Trace	0·29	0·33	—	0·10	3·53	Trace
α-Eudesmol / β-Eudesmol	14†	0·89	0·46	0·50	0·65	0·54	3·63	0·39
Farnesol		0·19	Trace	0·24	0·20	0·81	0·10	0·18
Total C-10 hydrocarbons		52·6	52·8	26·1	39·7	53·8	71·1	49·82
Total C-15 hydrocarbons		30·1	28·3	40·1	30·8	25·7	15·8	17·72
Total oxygenated		17·3	18·9	33·7	29·5	20·5	13·1	32·46
Unidentified C-10 hydrocarbons		<3	<1	<1	<1	<1	≈4	<1
Unidentified C-15 hydrocarbons		<4	<6	<5	<4	<9	<30	<3
Unidentified oxygenated		<12	<14	<13	<13	<12	<13	<5

* Peak areas were determined by the height × half-width method.
† These numbers refer to the bars in Fig. 2.

There is some confusion in the literature concerning the status of *M. pusilla*. Some authors regarded it as a distinct species, while others[3] have preferred to classify it as a variety of *M. cerifera* (*M. cerifera* var. *pumila* Michx.). The character most commonly ascribed to *M. pusilla* is the colonial habit, i.e. spreading by rhizomes, while other species of the subgenus Morella are said to be non-colonial and therefore lack rhizomes. Thieret[17] however, reported an example of a clone comprised of both 'typical' *M. cerifera* and *M. pusilla* all connected with numerous rhizomes. Consequently he regarded *M. pusilla* to be merely a small xeric form of *M. cerifera*. Baird[2] reported that the presence of rhizomes and the resulting colonial habit is widespread among all the Morella species and is of no taxonomic value. On the basis of the rhizomatous and morphological characters examined, Baird agreed with Thieret that *M. pusilla* represents only extreme expressions of several characters of the highly variable *M. cerifera* and should not be given taxonomic recognition.

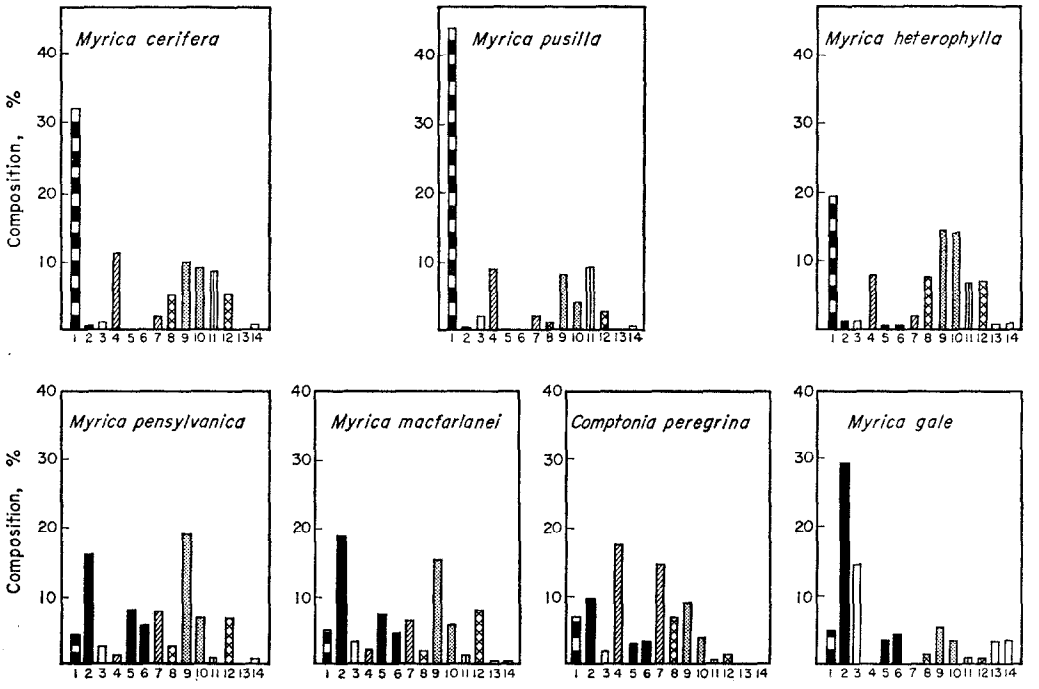

FIG. 2. BAR HISTOGRAMS OF TAXONOMICALLY SIGNIFICANT ESSENTIAL OIL COMPONENTS OF SEVEN MYRICACEAE SPECIES.

In sequence α-pinene (1), myrcene (2), limonene (3), cineole (4), *cis*-ocimene (5), *trans*-ocimene (6), γ-terpinene (7), linalool (8), caryophyllene (9), α-humulene (10), α- and β-selinene (11), nerolidol (12), γ-eudesmol (13) and α- and β-eudesmol (14).

An examination of Table 1 and Fig. 2 shows that the essential oil content of *M. pusilla* and *M. cerifera* is very similar. Acyclic terpenes represent a minor fraction of the whole oil while the cyclic monoterpenes constitute more than 50% of the oil with α-pinene and cineole as the predominant compounds. In the sesquiterpene hydrocarbon fraction of both

[17] THIERET, J. W. (1966) *Castanea* **31**, 183.

species the major constituents are caryophyllene and α-humulene (>50%). Other compounds contributing significantly to the sesquiterpene fraction are α- and β-selinenes (>30%). Nerolidol is the major sesquiterpene alcohol. The presence of much lesser amounts of linalool in *M. pusilla* is so far the only chemical characteristic distinguishing the two species. These findings would tend to substantiate Thieret's[17] and Baird's[2] contention that *M. pusilla* is synonymous with *M. cerifera.*

Although *M. macfarlanei* is reported to be a hybrid between *M. pensylvanica* and *M. cerifera,*[18] a comparison of the essential oil content of this species (Table 1 and Fig. 2) does not support this view; instead, *M. macfarlanei* bears a remarkable similarity to *M. pensylvanica.* In both *M. macfarlanei* and *M. pensylvanica* the acylic terpenes comprise about 34% of the total oil, with myrcene, *cis*-ocimene, *trans*-ocimene and linalool representing the major constituents in this fraction. There are several cyclic monoterpenoids present in both species but no one compound predominates in the mixture, although this fraction does represent about 24% of the total oil. In the sesquiterpene hydrocarbon fraction caryophyllene and α-humulene are the major constituents and nerolidol is by far the major sesquiterpene alcohol. The only clear differences between these two species is the presence of a larger amount of selin-4(14),7(11)-diene in *M. macfarlanei* as compared to *M. pensylvanica* and also the absence of geranyl acetate in *M. macfarlanei.* Morphologically *M. macfarlanei* is difficult to separate from *M. pensylvanica* and the authors are in agreement with Baird[2] who regards this species as synonymous with *M. pensylvanica.*

Myrica heterophylla is generally treated as a distinct species by most authors but Gleason and Cronquist[19] have suggested that it may be a hybrid of *M. pensylvanica* and *M. cerifera.* While the authors are not prepared to draw any conclusions at this time, the examination of the essential oil composition of *M. heterophylla* does show that chemically this species appears to be related to *M. cerifera.* As in *M. cerifera* the acyclic monoterpenes comprise about 11% of the oil with relatively large amounts of linalool present. The cyclic monoterpenes comprise about 36% of the oil and α-pinene and cineole represent the major constituents.

Myrica gale has been classified by some workers within the subgenus Myrica; however, other workers have preferred to regard it as a distinct genus *Gale palustria* (Fam) Chev.[3] The essential oil content of *M. gale* differs significantly from that of other species of *Myrica* and the authors feel that the morphological and chemical evidence when considered together justify the view that *M. gale* should be separated at the generic level. In contrast to all the other species of *Myrica* examined cineole and γ-terpinene were barely detectable in *M. gale* (see Table 1 and Fig. 2). Conversely α-terpinene and limonene were present in large amounts and α-citronellol was found in only this species. Myrcene was the predominant compound comprising about 29% of the total oil. All the sesquiterpenes found in the other species examined were found in *M. gale*; however, eremophillene was detected only in *M. gale.* In addition, four other unidentified sesquiterpenes found only in *M. gale* were detected. Finally, unique to this species is the presence of large amounts of α-, β- and γ-eudesmols in the sesquiterpene oxygenated fraction, replacing the large amount of nerolidol found in the other species.

The composition of the oil of *Comptonia peregrina* has been reported in a previous

[18] YOUNGKEN, H. W. (1923) *J. Am. Pharm. Assoc.* **12,** 484.
[19] GLEASON, H. A. and CRONQUIST, A. (1963) *Manual of the Vascular Plants of Northeastern United States and Adjacent Canada,* 310 pp., Van Nostrand, Princeton, New Jersey.

publication[20] but for comparative purposes the data are included in this study. *Comptonia* is generally regarded as a distinct genus within the Myricaceae and the chemical evidence supports this view. The most characteristic feature of the oil of *C. peregrina* is the presence of large amounts of cineole and of γ-terpinene rather than α-pinene or myrcene in the monoterpene fraction (see Table 1 and Fig. 2).

When essential oils are chosen for chemotaxonomic study several factors which might influence the qualitative and/or quantitative composition of the oil must be taken into consideration. These include the natural variability within the taxon, seasonal variation and geographical location. These factors were tested in the case of *M. pensylvanica*, *M. gale* and *C. peregrina* where different foliage samples collected from the same or different sites in Connecticut on the same or different weeks were always found to produce a consistent essential oil pattern. Similarly, the essential oil pattern from a sample of *M. cerifera* collected from Virginia in late July revealed an almost identical composition with that of a sample of *M. cerifera* collected from New Jersey in late June. Moreover, since most of the species in the Myricaceae are dioecious, separate analyses of the oils of both male and female plants were made of *M. gale*, *M. pensylvanica*, *M. cerifera* and *M. macfarlanei* and no qualitative or significant quantitative differences were found. To eliminate the variation, if any, due to the seasonal factor, all the examined species were harvested only during the fruiting season, i.e. from mid-June to mid-August. *Myrica heterophylla* and *M. pusilla* were collected from Virginia and North Carolina respectively. In each case the analyses were conducted on a single sample and therefore any variations due to seasonal or population effects were not determined.

EXPERIMENTAL

Plant material. Myrica pensylvanica (Lam.), and *M. gale* (L.) were collected near Storrs, Connecticut. *M. heterophylla* (Raf.) was collected near Littleton, Virginia and *M. cerifera* (L.) was collected near Cape May, New Jersey. *M. macfarlanei* Youngken (*M. pensylvanica*) was collected near Dewey Beach in Delaware while *M. pusilla* Raf. (*M. cerifera*) was collected by Mr. Harry Ahles in North Carolina. All identifications were confirmed by Mr. H. Ahles of the University of Massachusetts and Dr. Thomas Elias, of Harvard University. Voucher specimens are on deposit in the Herbarium at the University of Connecticut.

Isolation and separation procedures. The volatile leaf oil was recovered by steam distillation at atmospheric pressure using a cohobation still. The resulting oil was separated into a hydrocarbon and an oxygenated fraction by column chromatography on deactivated silicic acid.

Separation of the terpene from the sesquiterpene hydrocarbons was accomplished by GLC. The gas chromatograph used (Aerograph Autoprep Model 700) was equipped with a thermal conductivity detector. Separation was achieved using a 240 × 0·6 cm o.d. stainless steel column packed with 15% Carbowax 20 M on acid washed, DMCS treated Chromosorb W (80–100 mesh). Operating conditions were: injection port temp. 200°; detector temp. 175°; column temp. 60° for the monoterpenes and 130° for the sesquiterpenes; helium flow rate 40 ml/min; injection vol. was 50 μl. The various fractions were collected as they emerged from the gas chromatograph in glass tubes immersed in a bath of dry ice and acetone. The sesquiterpenes were further fractionated by TLC using plates coated with silica gel G and impregnated with $AgNO_3$. The solvent was C_6H_6–Me_2CO (39:1) or C_6H_6–hexane (3:7). After developments, the plates were sprayed with $SbCl_3$ in $CHCl_3$.

The compounds present in the oxygenated hydrocarbon fractions were separated by GLC. The analyses were made in an F & M Model 5750B gas chromatograph using a FID and an effluent splitter with a 1/25 split ratio. In all cases the columns used were stainless steel and the solid support was 80–100 mesh, acid washed silanized chromosorb W. Operating conditions were: temp., variable; helium flow rate, 30 ml/min; hydrogen flow rate, 40 ml/min; air flow rate, 400 ml/min; sample size, variable. Other column parameters are summarized in Table 2. Identification was made by the comparison of the retention time data and IR spectra with those of known terpenes. The instrumentation and operating procedures were essentially the same as those that have been previously described.[20]

[20] HALIM, A. F. and COLLINS, R. P. (1970) *Lloydia* **33**, 7.

TABLE 2. COLUMNS USED IN THE ANALYSIS OF THE ESSENTIAL OILS OF THE DIFFERENT SPECIES OF MYRICACEAE

Liquid phase	Column dimensions, (cm × 0·3 o.d.)	Column used for Analytical	Preparative
Carbowax 20 M (10%) + PDEAS (1%)*	240	†, ‡, §	†, ‡, §
Apiezon L (10%)	180	†, ‡, §	‡, §
Didecyl phthalate (10%)	180	‡	
DEGS (10%)	180	†	†

* Phenyldiethanol amine succinate.
† Oxygenated fraction.
‡ Terpene hydrocarbon fraction.
§ Sesquiterpene hydrocarbon fraction.

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