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IDENTIFICATION OF THE MAJOR COMPONENTS OF THE ESSENTIAL OIL OF MACE*

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SUMMARY

Gas chromatographic and combined gas chromatographic–mass spectrometric analysis of the essential oil of mace has shown that it consists of a mixture of approximately 87.5 % monoterpenes, 5.5 % monoterpene alcohols, 6.5 % aromatic ethers, together with 0.5 % other components. Nine monoterpene hydrocarbons, six monoterpene alcohols, two aromatic hydrocarbons, one sesquiterpene and six aromatic ethers have been identified.

INTRODUCTION

The common household spice mace is the dried aril which enwraps the seed (known as nutmeg) of the tree *Myristica fragrans* Houtt.

One of the earliest reports of the chemical analysis of the essential oil of mace was that of SEMMLER in 1890, who showed it was composed of a terpene fraction, an unidentified fraction which he termed "myristicol" and a higher boiling fraction containing myristicin[1]. Some of the earliest studies on the chemical composition of the essential oil of the closely related spice nutmeg were those of POWER AND SALWAY[2,3], which were carried out in the early 1900's. These investigations, together with those of other workers, carried out during this era are summarised in the treatise on essential oils by GUENTHER[4].

The essential oil of nutmeg, has lately been studied using gas–liquid chromatography (GLC), by a number of workers including: LEE *et al.*[5]; IKEDA *et al.*[6]; JAURÉGUIBERRY AND WOLFF[7]; BEJNAROWICZ AND KIRCH[8]; SHULGIN *et al.*[9–11]; ITTY AND NIGAM[12]; SAMMY AND NAWAR[13]; and SANFORD AND HEINZ[14], who confirmed and extended the earlier findings of POWER AND SALWAY[2]. Basically the oil was shown to consist of a monoterpene hydrocarbon fraction (~80 %), a monoterpene alcohol fraction (~4 %) and an aromatic ether fraction (~11 %), together with small quantities of miscellaneous other compounds. α-Terpinene[6,13,14], γ-terpinene[6,11,14], α-pinene[5,6,8,11–14], β-pinene[5,6,8,11–14], myrcene[6,13,14], terpinolene[6,8,11,13,14], camphene[4–6,8,11,13,14], limonene[4–6,8,12–14], sabinene[6,7,11,14], α-phellandrene[6,13,14],

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β-phellandrene[6,14], Δ3-carene[6,14] and α-thujene[6,14], have been definitely identified as members of the first group. The following monoterpene alcohols have been positively identified: borneol[2,14], geraniol[4,8,13,14], 4-terpineol [8,11,13,14], α-terpineol[8,11,13,14], β-terpineol[5,13,14], citronellol[12,14] and linalool[4,5,8,11–14]. *Cis*- and *trans*-sabinene hydrates have recently been reported to be present in the volatile constituents of nutmeg[14]. The sesquiterpene β-caryophyllene[5,13,14], the aromatic hydrocarbons *p*-cymene[6,8,11,13,14], and toluene[11] and the terpinic esters geranyl acetate[11,14], linalyl acetate[12,14], and bornyl acetate[12,14] have been identified with a fair degree of certainty whilst cumene[5], cyclamen aldehyde[5], camphor[8], menthone[5] and menthyl isovalerate[5] have been provisionally identified on the basis of retention times only. The following aromatic ethers have also been positively identified in the essential oil of nutmeg: methyl eugenol[11,13], myristicin[4,9–14], elemicin[9–11,14], methyl isoeugenol[9,10,14], methoxyeugenol[10,11,14], safrole[4,5,8,11–14], eugenol[4,5,8,11,14], isoeugenol[4,5,10,11,14] and isoelemicin[10,11,13,14]. SHULGIN *et al.* and SANFORD AND HEINZ have recently shown that the volatile oil of nutmeg also contains small quantities of fatty acids, *e.g.* myristic acid[11,14].

The only studies reported recently on the composition of the essential oil of mace are those of SHULGIN *et al.*, who reported the results of a comparative study of the aromatic fractions of several samples of the volatile oils of nutmeg and mace obtained from different geographical locations[11]. One sample of the mace oil contained as much as 18.2% aromatic ethers whilst in other cases the aromatic content was in the order of 7–8% (ref. 11). In all cases myristicin was the major component with smaller quantities of safrole and elemicin also being present[11].

The present communication reports the results of the GLC analysis of a commercial sample of East Indian oil of mace. The majority of the major components have been identified by a combination of chromatographic and spectroscopic procedures.

EXPERIMENTAL

Materials

Essential oil of mace (East Indian Select) was obtained from Fritzche, Dodge and Olcott Inc., New York, U.S.A. Reference compounds were obtained as gifts or purchased from commercial sources and where necessary were purified by either preparative GLC or preparative thin-layer chromatography (TLC) immediately prior to use.

Gas chromatography

A Hewlett-Packard Model 5750 dual column gas chromatograph was operated as a single column instrument fitted with a flame ionization detector and a Hewlett-Packard 3370A digital integrator. The operating conditions which gave the best separation of components are shown in Table I.

Combined gas chromatography–mass spectrometry

The gas chromatograph used in these experiments was a Varian Aerograph "HY-FI" 600-D equipped with a Varian Aerograph linear temperature programmer. The mass spectrometer was a Dupont Model 21-491 fitted with a Bell and Howell

5-124A recording oscillograph. The ion beam current was recorded and used as the gas chromatograph trace. Operating parameters are recorded in Table II.

The carrier gas was separated from the components of the analysed mixture by passing the effluent from the column through a heated (270°) hypodermic needle into a heated (270°) stainless-steel capillary tube linked to the mass spectrometer through a single stage jet separator. The enriched effluent from the gas chromatograph was fed continuously to the ion source of the mass spectrometer. Mass spectra (at ~70 eV) were then recorded to correspond to the peak maxima as detected by the ion beam monitor. In some instances spectra were also recorded at the leading and tailing edges of the peaks.

TABLE I

OPERATING CONDITIONS FOR HEWLETT-PACKARD MODEL 5750 DUAL COLUMN GAS CHROMATOGRAPH

Stationary liquid phase	XE-60[a] (4 %)
Solid support	Chromosorb W, a.w. DMCS-treated 80–100 mesh
Column length	9 ft.
Column diameter	1/8 in. (O.D.)
Column material	stainless steel
Column temp.	
initial	50°
final	220°
Programme rate	2°/min
Detector	flame ionization
Injection port temp.	260–270°
Detector oven temp.	260–270°
Carrier gas (He) flow rate	66 ml/min
Hydrogen flow rate	28 ml/min
Air flow rate	375 ml/min
Sample size	1.0 μl

[a] Nitrile gum.

TABLE II

OPERATING CONDITIONS FOR VARIAN-AEROGRAPH "HY-FI" 600-D-INSTRUMENT

Stationary liquid phase	XE-60 (4 %)
Solid support	Chromosorb W. a.w. DMCS-treated 80–100 mesh
Column length	9 ft.
Column diameter	1/8 in. (O.D.)
Column material	stainless steel
Column temp.	
initial	50°
final	200°
Programme rate	2°/min
Detector	[a]
Injection port temp.	255°
Carrier gas (He) flow rate	*ca.* 40 ml/min
Sample size	10.0 μl

[a] In this case the mass spectrometer ion beam monitor was effectively used as a detector for the gas chromatographic system.

Compound identification

The various peaks were identified wherever possible by the peak enrichment technique, in which standard compounds were added individually to the sample being chromatographed and compared in each instance to the chromatogram obtained using the oil alone.

The combined gas chromatographic–mass spectrometric procedure enabled the mass spectra of the components present in the majority of the peaks to be recorded and compared with those of authentic samples.

In several instances samples of the individual components were collected by preparative GLC and the identity of these products verified by comparison of their infrared (IR) and nuclear magnetic resonance (NMR) spectra with those of authentic samples. With some compounds, notably some of the aromatic ethers, comparisons of their TLC properties with those of authentic samples provided further evidence for the structures of the compounds in question.

In cases where collection of the components of individual peaks were carried out a larger diameter (3/8 in. O.D.) and longer (15 ft.) aluminium column was employed. The same support and stationary phase was used.

RESULTS AND DISCUSSION

Typical gas chromatograms of the essential oil of mace are shown in Figs. 1 and 2. The constituents, as determined by peak enrichment with authentic samples and combined gas chromatography–mass spectrometry, are listed in Table III. The percentage composition of the constituents as determined with the aid of a digital integrator and comparison of peak areas is shown in Table III.

The current investigation has shown that the sample of the essential oil of mace used in this case consists of approximately 87.5 % monoterpenes, 5.5 % monoterpene alcohols, 6.5 % aromatic ethers and approximately 0.5 % miscellaneous and unidentified products, including small quantities of some sesquiterpenes.

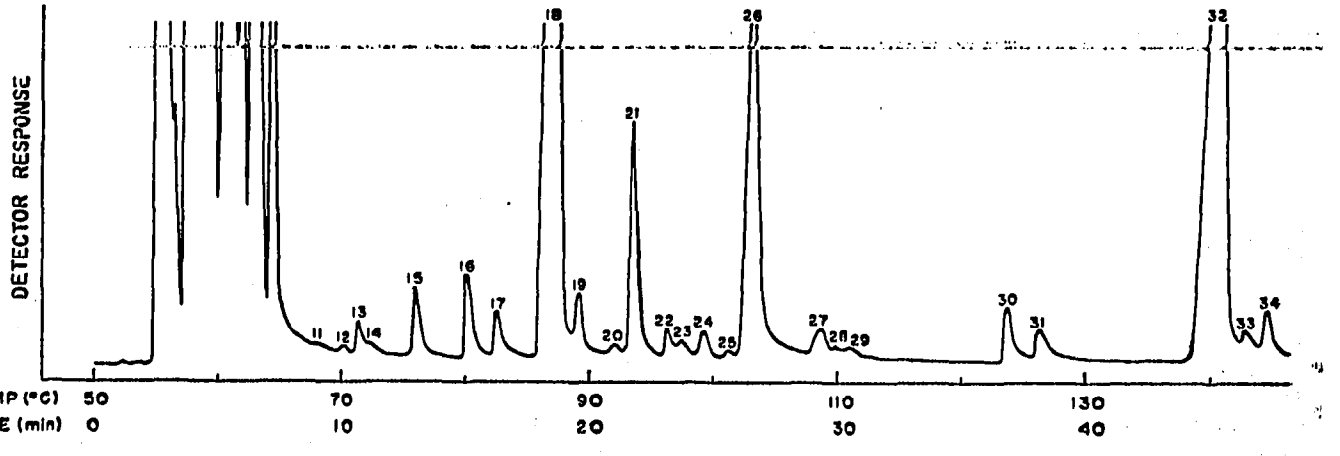

Fig. 1. A typical chromatogram of the essential oil of mace (1.0 μl). The chromatogram was obtained using a 9 ft. × 1/8 in. stainless-steel column containing 4 % XE-60 on a.w. DMCS-treated Chromosorb W. Initial temperature, 50°; programme rate, 2°/min. The peak numbers correspond to those given in Table III.

Gas chromatographic analysis of the mace oil using an 1/8 in. (O.D.) 4% XE-60 (on an a.w. DMCS-treated Chromosorb W support) column showed thirty-four separate peaks, as shown in Figs. 1 and 2. Of these peaks nine were shown to be due to monoterpenes, seven to monoterpene alcohols, two to aromatic hydrocarbons, two to sesquiterpenes and six to aromatic ethers. The remaining eight peaks

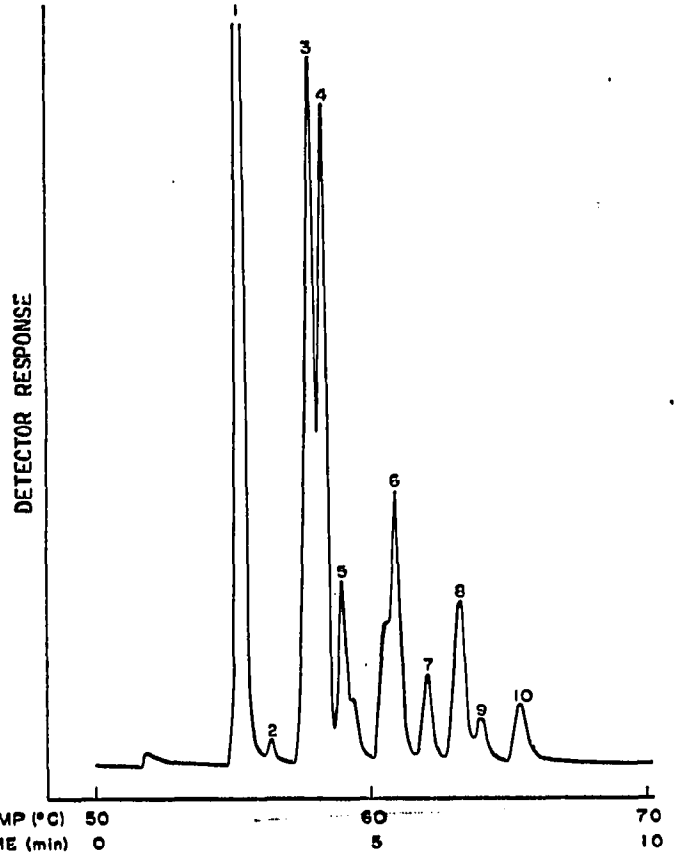

Fig. 2. Chromatogram of the monoterpene hydrocarbons of the essential oil of mace (0.2 μl). The chromatogram was obtained using a 9 ft. × 1/8 in stainless-steel column containing 4% XE-60 on a.w. DMCS-treated Chromosorb W. Initial temperature 50°; programme rate, 2°/min. The peak numbers correspond to those given in Table III.

were not identified. The shoulders on peaks 5 and 6 which can be seen in the chromatogram of the terpene fraction (Fig. 2) are due to other unidentified monoterpenes. Peaks 5 and 8 were classified as monoterpenes on the basis of their retention times and the appearance of strong peaks at *m/e* 136 in their mass spectra. These were the highest mass peaks observed in these spectra. The monoterpenes so far identified in the essential oil of mace have previously been shown to be present in the essential oil of nutmeg, although the percentages of the various components present vary somewhat.

The major components of the aromatic ether fraction of the essential oil of mace (peak numbers 26, 30–34) were present in the same general concentration order as that previously reported (*cf.* ref. 11).

A mass spectrum recorded of the tailing edge of the peak due to safrole ex-

TABLE III

COMPOSITION OF THE ESSENTIAL OIL OF MACE

Abbreviations: PE = peak enrichment; MS = mass spectrum; NMR = nuclear magnetic resonance spectrum; IR = infrared spectrum; TLC = thin layer chromatography.

Peak No.	*Compound*	*Percentage*	*Evidence for identity*
1	α-Pinene	26.7	PE, MS
2	Camphene	0.5	PE, MS
3	β-Pinene (and myrcene)	20.7	PE, MS
4	Sabinene	14.5	PE, MS
5	Unidentified monoterpene	4.8	MS, (M^+ 136)
6	Limonene	9.4	PE, MS
7	β-Phellandrene	2.3	PE, MS
8	Unidentified monoterpene	4.9	MS, (M^+ 136)
9	*p*-Cymene	0.9	PE, MS, NMR
10	Terpinolene	2.1	PE, MS
11	Unidentified	<0.01	—
12	Unidentified	0.01	—
13	*p*-Methylisopropenylbenzene (tentative)	0.02	MS, (M^+ 132)
14	Unidentified	0.02	—
15	Unidentified monoterpinol	0.2	MS
16	Linalool	0.2	PE, MS
17	Unidentified monoterpinol	0.1	MS
18	Terpinen-4-ol	4.4	PE, MS, NMR
19	Unidentified sesquiterpene	0.2	MS, (M^+ 204)
20	Unidentified	0.03	—
21	α-Terpineol	0.7	PE, MS, NMR
22	Unidentified monoterpinol	0.08	MS
23	Unidentified	0.05	—
24	β-Caryophyllene	0.08	PE, MS
25	Unidentified	0.02	—
26	Safrole	1.9	PE, MS, NMR, IR
27	Geraniol	0.1	PE
28	Unidentified	0.02	—
29	Unidentified	<0.01	—
30	Eugenol methyl ether	0.2	PE, MS
31	Eugenol	0.1	PE, MS
32	Myristicin	3.8	PE, MS, NMR, TLC, IR
33	Isoeugenol	0.1	PE
34	Elemicin	0.2	PE, MS

hibited significant ion peaks at *m/e* 204 and 161, indicating the presence of a third sesquiterpene as a minor constituent of that particular fraction. The total percentage of sesquiterpenes present is low, but it is in the same order as that reported for the β-caryophyllene content of oil of nutmeg.

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REFERENCES

1 F. W. SEMMLER, *Ber.*, 23 (1890) 1803.
2 F. B. POWER AND A. H. SALWAY, *J. Chem. Soc.*, 91 (1907) 2037.
3 F. B. POWER AND A. H. SALWAY, *Amer. J. Pharm.*, 80 (1908) 563.
4 E. GUENTHER, *The Essential Oils*, Vol. 5, Van Nostrand, Princeton, N. J., 1952, p. 59.
5 G. D. LEE, F. L. KAUFFMAN, J. W. HARLAN AND W. NIEZABITOWSKI, *Int. Gas Chromatogr. Symp., 3rd, 1961* (pub. 1962) 475; *C.A.*, 58 (1963) 4974b.
6 R. M. IKEDA, W. L. STANLEY, S. H. VANNIER AND E. M. SPITLER, *J. Food Sci.*, 37 (1962) 455.
7 G. JAURÉGUIBERRY AND R. WOLFF, *Bull. Soc. Chim. Fr.*, (1962) 1985.
8 E. A. BEJNAROWICZ AND E. R. KIRCH, *J. Pharm Sci.*, 52 (1963) 988.
9 A. T. SHULGIN, *Nature*, 197 (1963) 397.
10 A. T. SHULGIN AND H. O. KERLINGER, *Naturwissenschaften*, 51 (1964) 360.
11 A. T. SHULGIN, T. SARGENT AND C. NARANJO, in D. H. EFRON, B. HOLMSTEDT AND N. S. KLINE (Editors), *Ethnopharmacologic Search for Psychoactive Drugs*, U. S. Public Health Service Publ. No. 1645, 1967, p. 202.
12 M. I. ITTY AND S. S. NIGAM, *Riechst. Aromen. Koerperpflegem.*, 16 (1966) 399; *C. A.*, 66 (1967) 58802s.
13 G. M. SAMMY AND W. H. NAWAR, *Chem. Ind. (London)*, (1968) 1279.
14 K. J. SANDFORD AND D. E. HEINZ, *Phytochemistry*, 10 (1971) 1245.
15 A. T. SHULGIN, *J. Chromatogr.*, 30 (1967) 54.