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EXAMINATION OF THE DIPHENYLPROPANOIDS OF NUTMEG AS THEIR TRIMETHYLSILYL, TRIETHYLSILYL AND TRI-*n*-PROPYLSILYL DERIVATIVES USING COMBINED GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

Ethyl acetate extracts of thirteen samples of nutmeg and one sample of mace were examined as trimethylsilyl, triethylsilyl and tri-*n*-propylsilyl derivatives by gas chromatography and mass spectrometry. Eleven compounds derived from two types of diphenylpropanoid were identified; the relative proportions and quantities of these compounds varied considerably in the different samples. Single ion chromatograms were used to obtain the relative concentration of one series of compounds. Preparation of the triethylsilyl and tri-*n*-propylsilyl derivatives resulted in greatly increased separation of the diphenylpropanoids over trimethylsilyl derivatives, which tended to produce a group of compounds with many unresolved peaks.

INTRODUCTION

Nutmeg and mace, both obtained from the plant *Myristica fragrans* Houtt. have long been known to contain a hallucinogenic principle, once thought to be myristicin[1]. Recent work by Forrest and Heacock[2] has shown a similarity in the thin-layer chromatographic properties of both nutmeg extracts and organic extracts of *Cannabis sativa* L. Both plants give positive results with Fast Blue B dye, although few plants are known which do this. Previous investigations of organic extracts of nutmeg[3-9] and mace[10] have led to the identification of many components, including terpenes, phenyl propanoids and, recently, two sets of compounds derived from di-(phenylpropanoids)[11,12]. We have recently examined the constituents of a number of samples of *Cannabis sativa* L.[13] and, because of the apparent similarities in the chromatographic properties between extracts of this plant and those of nutmeg, have now extended this work to include *Myristica fragrans* H.

EXPERIMENTAL

Whole nutmeg and ground nutmeg and mace samples were obtained from local supermarkets.

Extraction and preparation of derivatives

100-mg samples of nutmeg were ground as finely as possible and allowed to stand in ethyl acetate for 1 h with occasional shaking. The solid material was filtered off and washed well with ethyl acetate. The combined extracts were then concentrated to about 1 ml and kept at 0° to complete precipitation of triglycerides (chromatograms of the complete extracts indicated that the diphenylpropanoids remained in solution). The ethyl acetate was removed from the filtered solution with a stream of nitrogen and derivatives were prepared by the addition of 200 μl of the appropriate reagents (described below) and allowing the mixture to stand overnight at room temperature.

Trimethylsilyl derivatives. Trimethylsilyl (TMS) derivatives were prepared by the addition of a pre-prepared mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (80 μl), acetonitrile (80 μl) and trimethylchlorosilane (40 μl).

d_9-*TMS derivatives*[14]. d_9-TMS derivatives were prepared from 0.01 ml of solution with d_9-N,O-bis-trimethylsilylacetamide (10 μl), acetonitrile (10 μl) and a trace of trimethylchlorosilane as a catalyst.

Triethylsilyl derivatives[15]. The reagent was prepared by mixing triethylchlorosilane (1 ml), pyridine (2 ml) and diethylamine (0.5 ml) in a centrifuge tube, cooling the resulting mixture in ice water and centrifuging to remove the white precipitate. The clear supernatant was removed with a pipette and used for derivative preparation.

Tri-n-propylsilyl derivatives. The reagent was prepared as above with tri-n-propylchlorosilane.

For the estimation of the quantity of the diphenylpropanoids in each sample, 0.5 ml of a solution of cholesterol (1 mg/ml) was added to each sample following the extraction and the compounds were converted into TMS derivatives as described above. Response factors were not determined as the individual diphenylpropanoids were not isolated. However, because of the similarity in the composition of the cannabinoids and diphenylpropanoids, these compounds were assumed to have similar response factors. Cannabinol response factors were determined previously[13]. Gas chromatographic peak areas were obtained by the weighing technique. Removal of the triglycerides by prior crystallization was not carried out with these samples.

Gas chromatography

Gas chromatography was carried out with a Varian 2400 gas chromatograph fitted with flame ionization detectors and two 6 ft. $\times$ 2 mm glass columns packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.). Nitrogen at 30 ml/min was used as the carrier gas and the column oven was programmed from 150–330° at 4°/min. The temperature was then kept at 330° for about 30 min to complete the elution of the triglycerides. Injector and detector temperatures were maintained at 270°.

Mass spectrometry

Mass spectra were recorded at 22.5 eV with a VG-Micromass 12B mass spectrometer, interfaced via a glass jet separator to a single-column gas chromatographic system similar to that described above. The ion source was maintained at 220° and the spectra were obtained for each chromatographic peak using a 3-sec scan and an accelerating voltage of 2.5 kV. For the single-ion chromatograms, the acce-
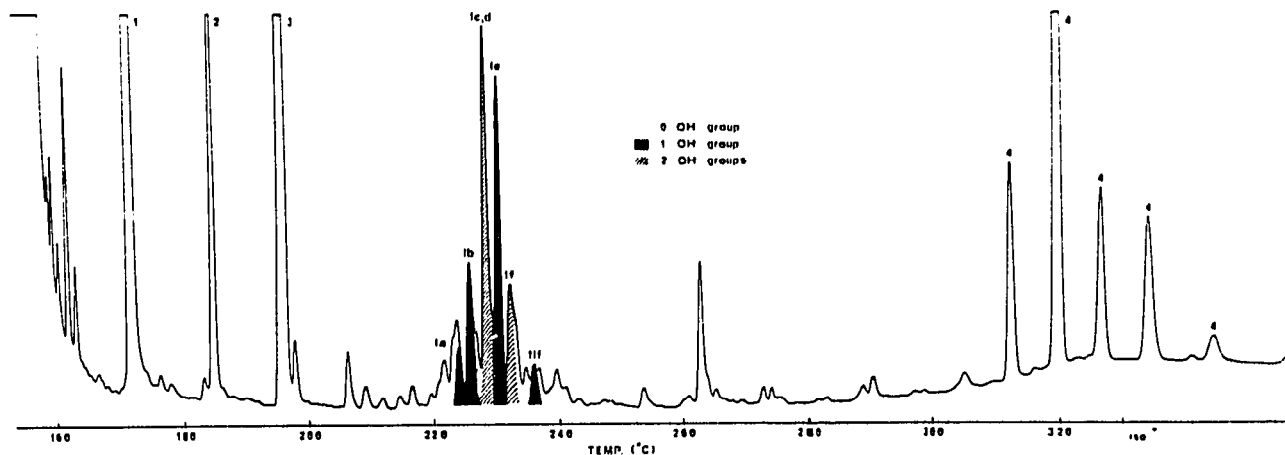
Fig. 1. Separation of the ethyl acetate extract of nutmeg (sample 2) as TMS derivatives on a 6 ft. × 2 mm glass column packed with 3 % SE-30 on Gas-Chrom Q and temperature programmed at 4°/min from 150°. Compounds identified include myristic acid (1), palmitic acid (2), oleic and linoleic acids (3), several triglycerides (4) and the diphenylpropanoids (compounds I and II).

lerating voltage was held at 2.5 kV and the magnetic field adjusted to focus the ion in question. The resolving slit was opened to give flat-top peaks and the output was recorded on a Servoscribe 1S single-channel flat-bed recorder.

RESULTS AND DISCUSSION

The lower-boiling constituents of the nutmeg extracts, consisting mainly of monoterpenes and phenylpropanoids, were not examined as the gas chromatograms of this fraction have already been reported[4,5,8,9]. Fig. 1 shows the gas chromatogram on 3 % SE-30 of an ethyl acetate extract of a typical sample of nutmeg over the range
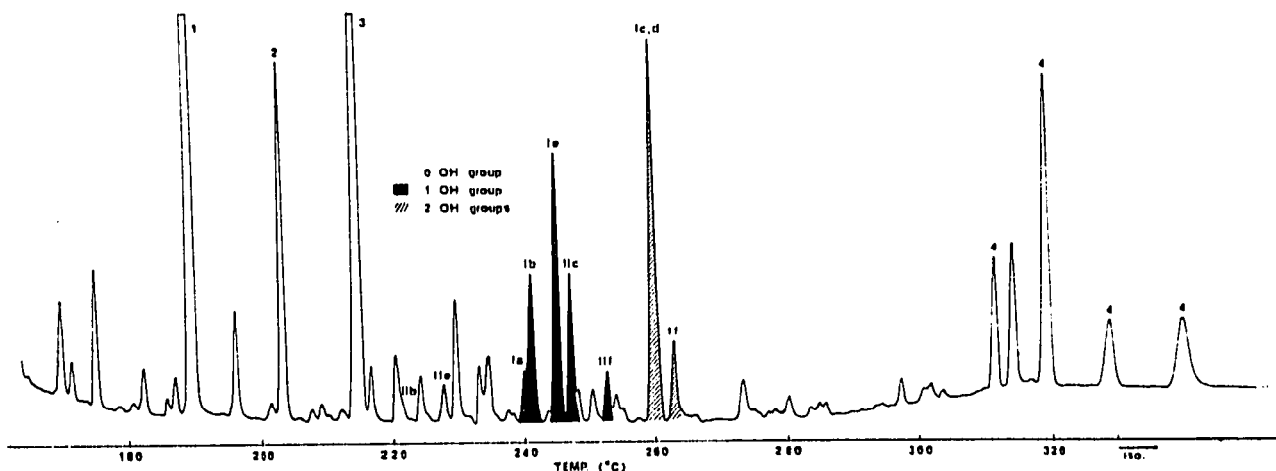

Fig. 2. Separation of the ethyl acetate extract of nutmeg (sample 2) as triethylsilyl derivatives. Conditions, as for Fig. 1.
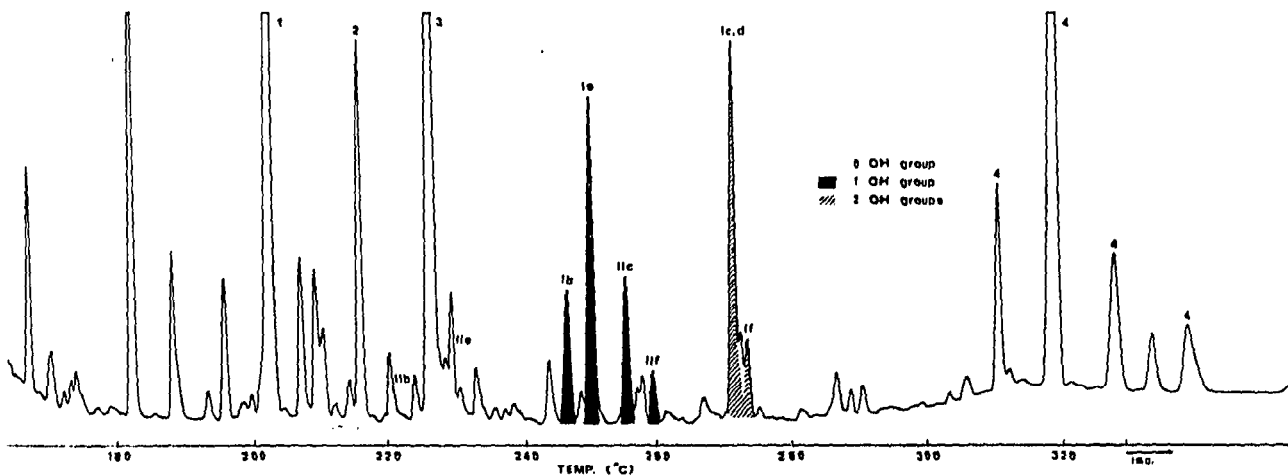
Fig. 3. Separation of the ethyl acetate extract of nutmeg (sample 2) as tripropylsilyl derivatives. Conditions as for Fig. 1.

150–330°. Three groups of compounds were identified: fatty acids, mainly myristic, palmitic and oleic acids, all known constituents of nutmeg[3,8,9]; triglycerides, also known constituents; and a group of compounds derived from diphenylpropanoids. Different samples of nutmeg and mace (fourteen samples were examined) showed the same general pattern, but the proportions and quantities of the various diphenyl-propanoids were observed to vary considerably (Figs. 4 and 5). These compounds were present to the extent of 0.4–2 % of the total weight of the nutmeg depending on the sample. Table I lists the samples examined, the amount of each sample left after extraction with ethyl acetate, and the per cent of diphenylpropanoid found. The major components of the organic extracts were triglycerides.

Mass spectrometry showed that most of the gas chromatographic peaks in the diphenylpropanoid region were produced by several compounds. Two sets of com-
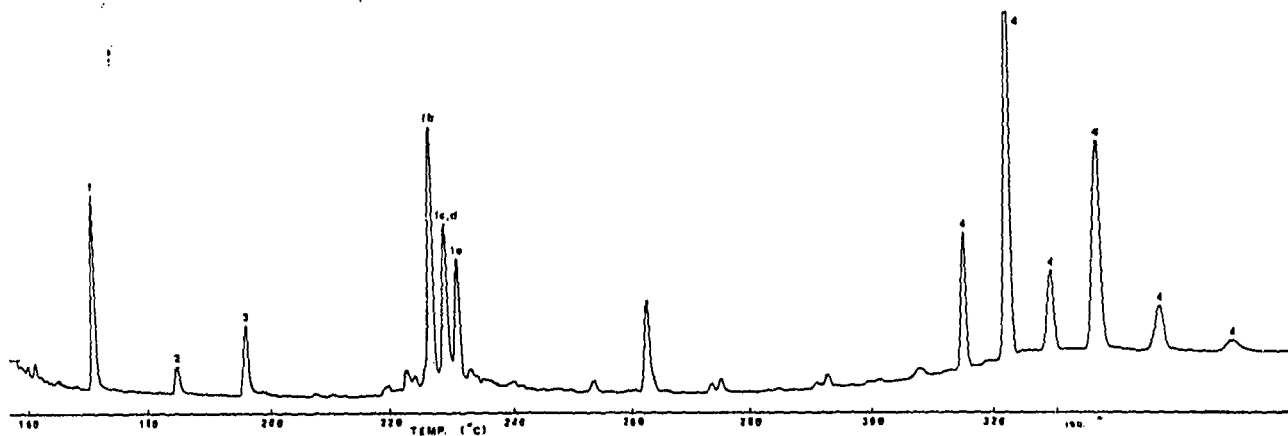

Fig. 4. Separation of the TMS derivatives of the diphenylpropanoids of nutmeg sample 1. Conditions, as for Fig. 1.
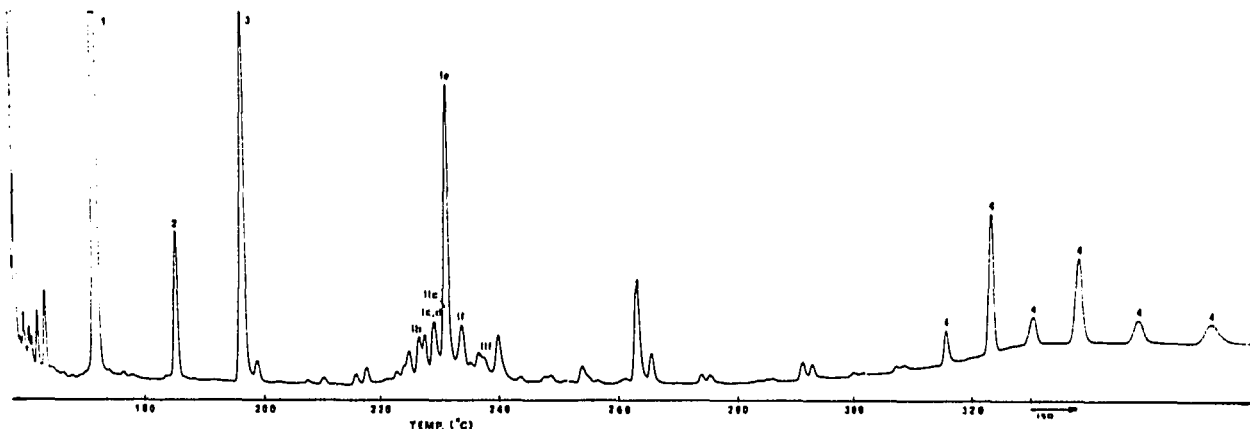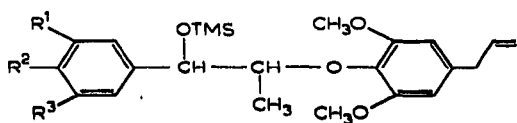
Fig. 5. Separation of the TMS derivatives of the diphenylpropanoids of nutmeg sample 7. Conditions, as for Fig. 1.

pounds were distinguished, and identified by their mass spectral properties as belonging to the two groups of diphenylpropanoids I and II isolated by Forrest et al.[12].

Compound no	R^1	R^2	R^3
Ia	—O—CH_2—O—		H
Ib	OCH_3	OCH_3	H
Ic	OCH_3	OH	H
Id	OH	OCH_3	H
Ie	OCH_3	OCH_3	OCH_3
If	OCH_3	OH	OCH_3
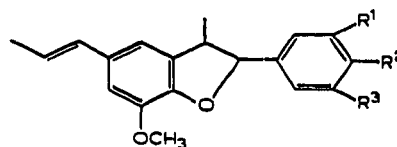
Compound no	R^1	R^2	R^3
IIa	—O—CH_2—O—		H
IIb	OCH_3	OCH_3	H
IIc	OCH_3	OH	H
IIe	OCH_3	OCH_3	OCH_3
IIf	OCH_3	OH	OCH_3

The mass spectra of the compounds of type I (Table II) were dominated by four fragment ions, a–d. Molecular and, in most cases, $[M-15]^+$ ions were also present, but in low abundance. Ions a, b and c contained only one of the aromatic rings (termed ring A). The position of these ions in the spectra of the various compounds therefore indicated the change of substitution in this ring. Also the observed shift in the position of these ions in the spectra of the d_9-derivatives was indicative of the number of hydroxyl groups present.

Ions a and b were the result of simple cleavage of the molecule, whereas ion c was produced by a hydrogen migration to the neutral fragment.

Ion d was a rearrangement ion produced by migration of the aliphatic TMS group and expulsion of ring A, presumably as a substituted styrene oxide type of
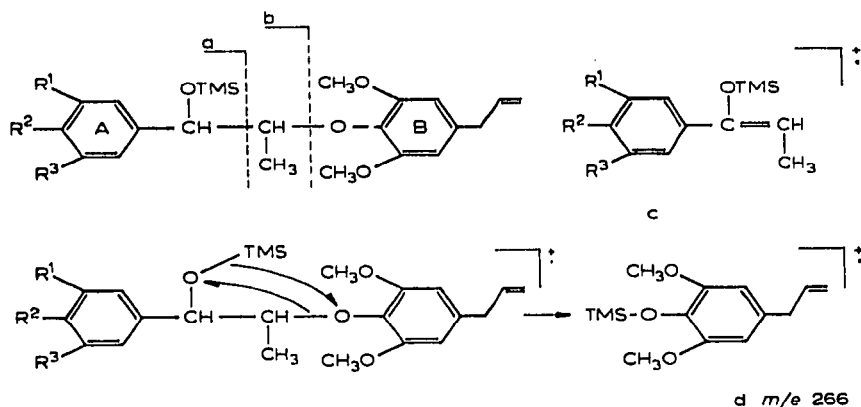
d m/e 266

neutral fragment. All of the compounds of type I found in the nutmeg sample gave ion d at m/e 266, thus indicating no change in the substitution pattern of its aromatic ring (termed ring B). All of these compounds therefore had the same basic structure and differed only in the substitution on ring A.

The compounds of type I found in the various samples are given below the scheme of the type I diphenylpropanoids. The positions of the substituents around the aromatic ring were based on reported syntheses[12] and on biochemical considerations; the corresponding phenylpropanoids, for example eugenol (III, R^1 = OMe, R^2 = OH, R^3 = H), have previously been reported as constituents of the essential oil of nutmeg[10]. Compounds of type I may be regarded as derivatives of the iso-phenylpropanoids, e.g. isoeugenol (IV, R^1 = OMe, R^2 = OH, R^3 = H), and iso-phenylpropanoids corresponding to the substitution present in Ib, Ic and Id have also been reported as constituents of nutmeg oil[10]. Isomyristicin (IV, R^1, R^2 = $-O-CH_2-O-$, R^3 = OMe) has not been reported although it had been looked for[6], and this may be related to the fact that although myristicin (III, R^1, R^2 = $-O-CH_2-O-$, R^3 = OMe) is the major phenylpropanoid in most nutmeg samples examined[9], its corresponding diphenylpropanoid (type I) was not found. Again, compound Ia, derived from isosafrole (IV, R^1, R^2 = $-O-CH_2-O-$, R^3 = H), was usually present in only small amounts, possibly reflecting the apparent absence of a report of the detection of isosafrole in nutmeg.

Compounds Ic and Id had very similar retention indices and mass spectra and were thus probably isomeric around ring A. Compound Ic has been reported previously[12]. No phenylpropanoid containing the substitution pattern of Id has been reported from nutmeg although it (chavibetol) is known in other species. Another possible structure for Id could be the diastereoisomer of the erythro[12] compound Ic, but this is unlikely in view of the apparent absence of corresponding isomers of the other compounds.

TABLE I

CHARACTERISTICS OF SAMPLES

Sample No.	Type	State	Dry weight* (%)	Diphenylpropanoids** (%)
1	Nutmeg	Whole	44.0	2.1
2	Nutmeg	Ground	48.8	2.2
3	Mace	Ground	59.5	1.7
4	Nutmeg	Ground	49.0	0.9
5	Nutmeg	Ground	50.0	2.1
6	Nutmeg	Ground	53.2	1.9
7	Nutmeg	Ground	51.6	1.1
8	Nutmeg	Whole	42.0	0.8
9	Nutmeg	Whole	45.0	0.4
10	Nutmeg	Whole	44.5	0.9
11	Nutmeg	Whole	41.1	0.6
12	Nutmeg	Whole	39.65	0.8
13	Nutmeg	Whole	44.5	1.8
14	Nutmeg	Whole	45.3	1.4

   * After separation by ethyl acetate.
 ** % of total sample.

No isomer of If was observed; this compound also has mixed substituents in ring A. Several of these compounds, in particular Ib ($R^1 = R^2 = OMe$, $R^3 = H$) have not been reported previously. It was interesting that compound Ib was the major constituent of sample 1.

Because ion d (m/e 266) was produced by all of the compounds of this type and not by the other components, single ion chromatograms of m/e 266 were used to examine the proportion of compounds of type I in each sample. Very clear chromatograms consisting essentially of only compounds of type I were produced. The con-

TABLE II

PARTIAL MASS SPECTRA OF COMPOUNDS OF TYPE I (TMS DERIVATIVES)

Ion	Ia	Ib	Ic	Id	Ie	If
M+	m/e 444 (1.4)*	m/e 460 (2.1)	m/e 518 (1.3)	m/e 518 (0.7)	m/e 490 (5)	m/e 548 (2.1)
[M − 15]+	m/e 429 (0.25)	m/e 445 —	m/e 503 (0.9)	m/e 503 (0.7)	m/e 475 (0.15)	m/e 533 (1.25)
a	m/e 223 (100)	m/e 239 (100)	m/e 297 (100)	m/e 297 (100)	m/e 269 (100)	m/e 327 (100)
b	m/e 251 (80.5)	m/e 267 (65)**	m/e 325 (44)	m/e 325 (26)	m/e 297 (71)	m/e 355 (47)
c	m/e 250 (38)	m/e 266 ***	m/e 324 (19.5)	m/e 324 (11)	m/e 296 (30)	m/e 354 (21)
d	m/e 266 (75)	m/e 266 (86)§	m/e 266 (73)	m/e 266 (41.5)	m/e 266 (88)	m/e 266 (85)

   * Relative abundances are given in parentheses.
  ** Isotope contribution from ion d.
 *** Ions c and d.
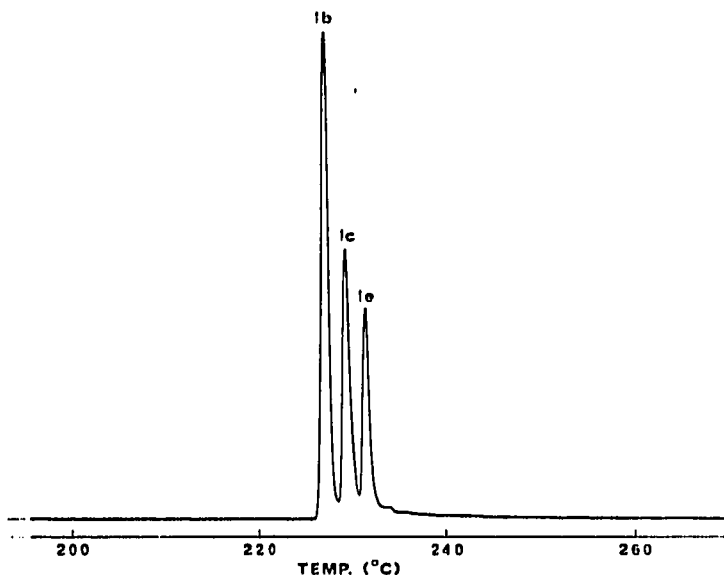   § Contains contribution from ion c.

Fig. 6. Single-ion chromatogram of *m/e* 266 (ion d) for nutmeg sample 1. Conditions, as for Fig. 1.

siderable variation in the relative concentration of each component is apparent by comparing Figs. 6–8 and the relative proportions given in Table III. As the percentage of the total ion current carried by ion d was similar for each compound, comparison of concentration could be made directly.
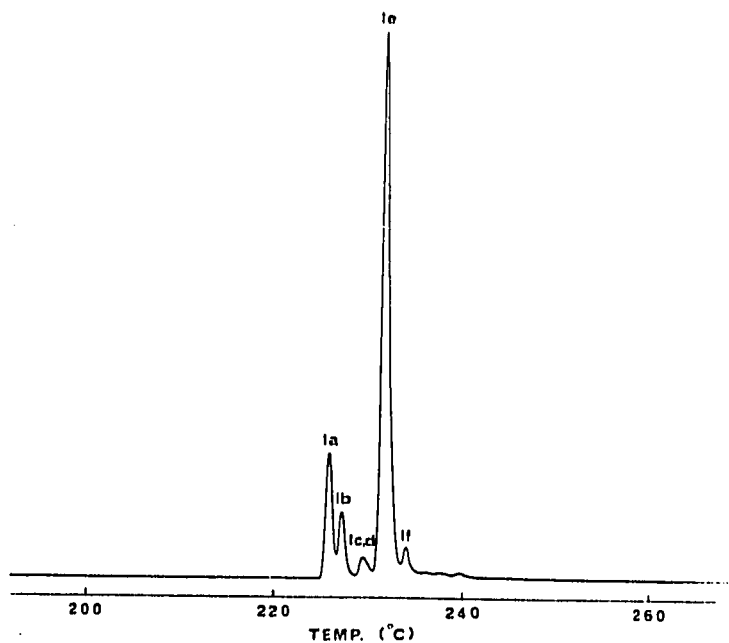

Fig. 7. Single-ion chromatogram of *m/e* 266 (ion d) for nutmeg sample 12. Conditions, as for Fig. 1.
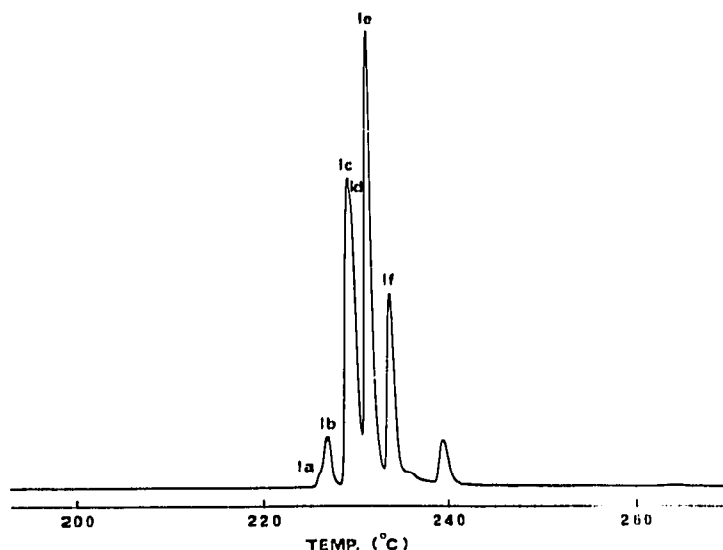
Fig. 8. Single-ion chromatogram of m/e 266 (ion d) for nutmeg sample 14. Conditions, as for Fig. 1.

Compounds of type II, related to diisophenylpropanoids, were present in smaller quantities than those of type I, but no abundant characteristic ion was found which was suitable for single-ion monitoring of the group as a whole. Five compounds, three of which (IIa, IIb and IIe) have not been reported previously, were identified by mass spectrometry. These contained the same aromatic substituents as the type I compounds with the exception of Id. Single-ion chromatograms of the molecular ion

TABLE III

PROPORTIONS* OF THE COMPOUNDS OF TYPE I FOUND IN EACH SAMPLE

Sample	Ia	Ib	Ic, Id**	Ie	If
1	—	100	55	46	—
2	7.5	40	100	77	19
3	11	26	19	100	15
4	11	21	18	100	20
5	2	30	100	37	10.5
6	3	35	100	56	14.5
7	9.5	22	22.5	100	21
8	6.5	18.5	7.5	100	13.5
9	—	48	25	100	4.5
10	27	5	36	100	63.5
11	4	12	3	100	5
12	25	14	5	100	6.5
13	3.5	10	0.5	100	—
14	0.05	11	77	100	45

* Expressed as a percentage of the major component. Quantitation is based on peak area measurement.
** The gas–liquid chromatographic peak contained both components. Compound Id was much less abundant in all cases.

(the base peak in all cases) in addition to complete mass spectral scans were used to confirm the presence of these compounds. Again, considerable variation in the relative abundances of these compounds was noted for the different samples, and again the derivative of myristicin was absent.

The presence, in these compounds, of various degrees of hydroxylation (zero, one or two hydroxyl groups) offered a second method of deconvoluting the group of poorly resolved gas chromatographic peaks produced by the TMS derivatives. Preparation of the triethylsilyl or tri-*n*-propylsilyl derivatives[15] introduced a larger retention increment than the TMS derivatives and this was multiplied where several hydroxyl groups were present. Separation of these compounds into three groups according to their degree of hydroxylation could thus be made. This is shown in Figs. 2 and 3, which show the gas chromatograms of the triethylsilyl and tri-*n*-propylsilyl derivatives, respectively, of nutmeg samples whose TMS derivatives are shown in Fig. 1. The underivatized compounds IIb and IIe did not shift their position but the mono- and dihydroxy derivatives separated into two distinct groups at higher retention index values. Retention indices are listed in Table IV. The largest change in retention increment was observed between the TMS and triethylsilyl derivatives, a feature shared with the same derivatives of other compounds such as the cannabinoids.[15] In addition, some improvement in the separation between compounds of type I and II was obtained, for example between Ie and IIc (Figs. 1 and 2). A slightly larger increase in relative retention was produced with compounds of type II. This is shown graphically in Fig. 9.

TABLE IV

RETENTION INDICES OF THE DIPHENYLPROPANOIDS

Compound	Free	TMS	Et₃Si	Pr₃Si
Ia	--	2630	2940	--*
Ib	--	2655	2955	3065
Ic	--	2700	3300	3540
Id	--	2710	3310	*(Ic)
Ie	--	2740	3020	3125
If	--	2760	3360	3595
IIa	2623	--	--	--
IIb	*(Ib)	--	--	--
IIc	--	2725	3060	3215
IIe	2770	--	--	--
IIf	--	2825	3160	3310

* Peak not resolved: interfering compound in parentheses.

As the geographical origin and the age of the sample were unknown, it was not possible to determine the reason for the variation in the abundance of the diphenylpropanoids. Geographical dependence has previously been demonstrated for nutmeg constituents[5,9]. Similar changes are found in samples of cannabis depending to a large extent on geographical origin. Aging of cannabis samples produces an increase in the proportion of fully aromatic compounds such as cannabinol[16]. It is possible that a similar situation occurs in aged nutmeg samples, but although aromatic compounds, particularly those derived from the type II diphenylpropanoids, were
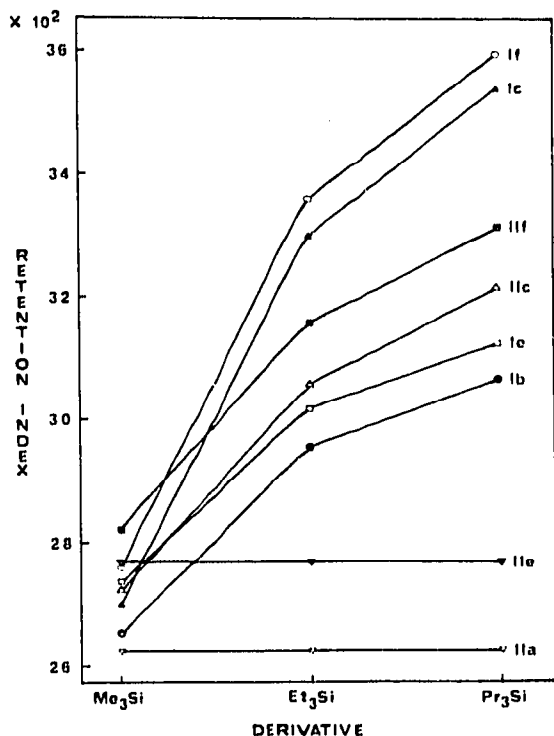
Fig. 9. Retention index plotted as a function of the derivative for the diphenylpropanoids. Three populations are present (ignoring compounds IIa and IIe which are not derivatized), the monohydroxy compounds of type I, those of type II which have a slightly greater change in retention index, and the dihydroxy compounds Ic, Id and If.

looked for, none were found. Aromatic compounds of this type do, however, occur in other species[17].

One sample of mace (sample 3) was examined, but its chromatogram showed no significant differences in diphenylpropanoid content from a number of the nutmeg samples (Table III). Greater differences were found between the individual nutmeg samples themselves.

ACKNOWLEDGEMENTS

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