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ANALYSIS OF NUTMEG OIL USING CHROMATOGRAPHIC METHODS

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SUMMARY

Various chromatographic methods were used for the analysis of nutmeg oil. In addition to the fourteen known monoterpene hydrocarbons, α,*p*-dimethylstyrene has been identified together with sixteen monoterpene alcohols, seven esters and eight sesquiterpene hydrocarbons. The occurrence of the missing *trans*-isomer of *p*-menth-2-en-1-ol and a bifunctional derivative of the same type (*trans*-*p*-menth-2-ene-1,4-diol) are of special interest. Besides the terpenoid constituents, two unsaturated aliphatic compounds, namely, (*E*)-3-methyl-4-decen-1-ol and its acetate, were detected for the first time in a natural substrate.

INTRODUCTION

The characteristic odours of natural substrates (spices, essential oils, foods in general) are mainly caused by volatile components. Gas–liquid chromatography (GLC) is the most important tool for the analysis of these compounds. Older analytical methods worked well only for the main constituents, but were unsuitable for the trace components which so often are responsible for the overall flavour or odour. The new methods developed for all kinds of chromatographic techniques allow much easier qualitative and quantitative monitoring of compositions. The rapid development of capillary columns in gas chromatography has facilitated the investigation of complex mixtures such as essential oils.

In the field of spicy essential oils, nutmeg oil and mace oil play an important rôle in the flavour and fragrance industry. Owing to their known content of noxious substances such as safrol and myristicin[1], it was desirable to obtain more information about the qualitative and quantitative compositions in relation to the odour and flavour characteristics of these oils. Based on the compilation of Van Straaten[2], we have now confirmed all of the components already known and added to the list of nutmeg constituents the following compounds: one monoterpene hydrocarbon; six sesquiterpene hydrocarbons; six monoterpene alcohols; three esters; vanillin and two aliphatic compounds detected for the first time in nature, (*E*)-3-methyl-4-decen-1-ol and its acetate (see Table I).

EXPERIMENTAL

Nutmeg oil of commercial grade (Roure-Bertrand, Grasse, France) was first distilled *in vacuo*. Certain fractions were combined and then chromatographed on silica gel 60 (E. Merck, Darmstadt, G.F.R.) using hexane–diethyl ether in various ratios (100:0 to 0:100) in order to isolate specific components of the oil. Thus, 30 g of a distillation fraction (b.p. 34–39°C/0.04 mmHg; 1.8% of the total oil) were first chromatographed on 150 g of silica gel 60. After elimination of the hydrocarbons, hexane–diethyl ether (10:1) was used to elute 4.3 g of a complex mixture which was rechromatographed on 130 g of silica gel 60. Elution with hexane–diethyl ether (10:1) then afforded a mixture (0.6 g) consisting mainly of the known *trans*-sabinene hydrate, *cis*- and *trans-p*-menth-2-en-1-ol. The previously unknown *trans*-isomer was isolated in pure form (64 mg) by means of preparative GLC.

Another distillation fraction (b.p. 49–65°C/0.03 mmHg; 34 g, 1.9% of the total oil) was treated in the same way to yield 200 mg of (*E*)-3-methyl-4-decen-1-ol (purity 80%) using hexane–diethyl ether (5:1) as eluent. Final elution with diethyl ether afforded *trans-p*-menth-2-ene-1,4-diol in 70% purity. Both constituents were further purified by preparative GLC.

All GLC measurements were conducted on a Carlo Erba GI apparatus. A glass column (3.8 m × 11 mm I.D.) packed with 10–5% Carbowax 20M (loading decreasing stepwise with increasing distance from column entrance) on Supelcoport, treated with terephthalic acid, was used for preparative work. Temperature: isothermal, 180 or 200°C. Carrier gas: nitrogen, 200 ml/min. A glass column (3 m × 3 mm I.D.) packed with 2% Carbowax 20M on Chromosorb G HP (80–100 mesh), treated with terephthalic acid, was used for the quantitative determination of myristicin. Temperature: programmed from 60 to 230°C at 6°C/min. Carrier gas: helium, 55 ml/min. Capillary columns (50 m × 0.31 mm I.D.) were coated with UCON HB 5100 or Carbowax 1000, respectively. Temperature: programmed from 50 to 185°C at 3°C/min. Carrier gas: helium, 2.5 ml/min.

Spectroscopic data of isolated compounds were recorded on Perkin-Elmer Model 157 (infrared), Bruker WH-360 (^{1}H nuclear magnetic resonance, NMR) and Varian XL-100A (^{13}C NMR) spectrophotometers. A Varian-MAT CH-5 or 212 in combination with Varian SS 100 or Finnigan Incos 2400 computer systems was used for mass spectrometry (MS) and GLC–MS coupling experiments.

RESULTS AND DISCUSSION

Monoterpene hydrocarbons represent about 70% of nutmeg oil. In GLC experiments, only Carbowax 1000 resolved all fifteen monoterpene hydrocarbons. With this stationary phase even 1,8-cineol is well separated from the adjacent hydrocarbons (limonene and β-phellandrene). α,*p*-Dimethylstyrene, already known to occur in other natural sources, was newly detected as a trace component in nutmeg oil by GLC–MS coupling experiments.

The capillary columns used showed efficient separation of sesquiterpene hydrocarbons from monoterpene alcohols, *i.e.*, α-copaene from *trans*-sabinene hydrate or caryophyllene from *trans-p*-menth-2-en-1-ol. They also enabled the detection of a sesquiterpene hydrocarbon which seems to decompose during the pretreatment via silica gel column chromatography. We assume that this is a germacrene-type sesquiterpene hydrocarbon in view of the other identified compounds which are related to a common intermediate (Fig. 1). Germacrenes are known to be very sensitive and prone to skeletal rearrangements under acidic conditions[3–5].

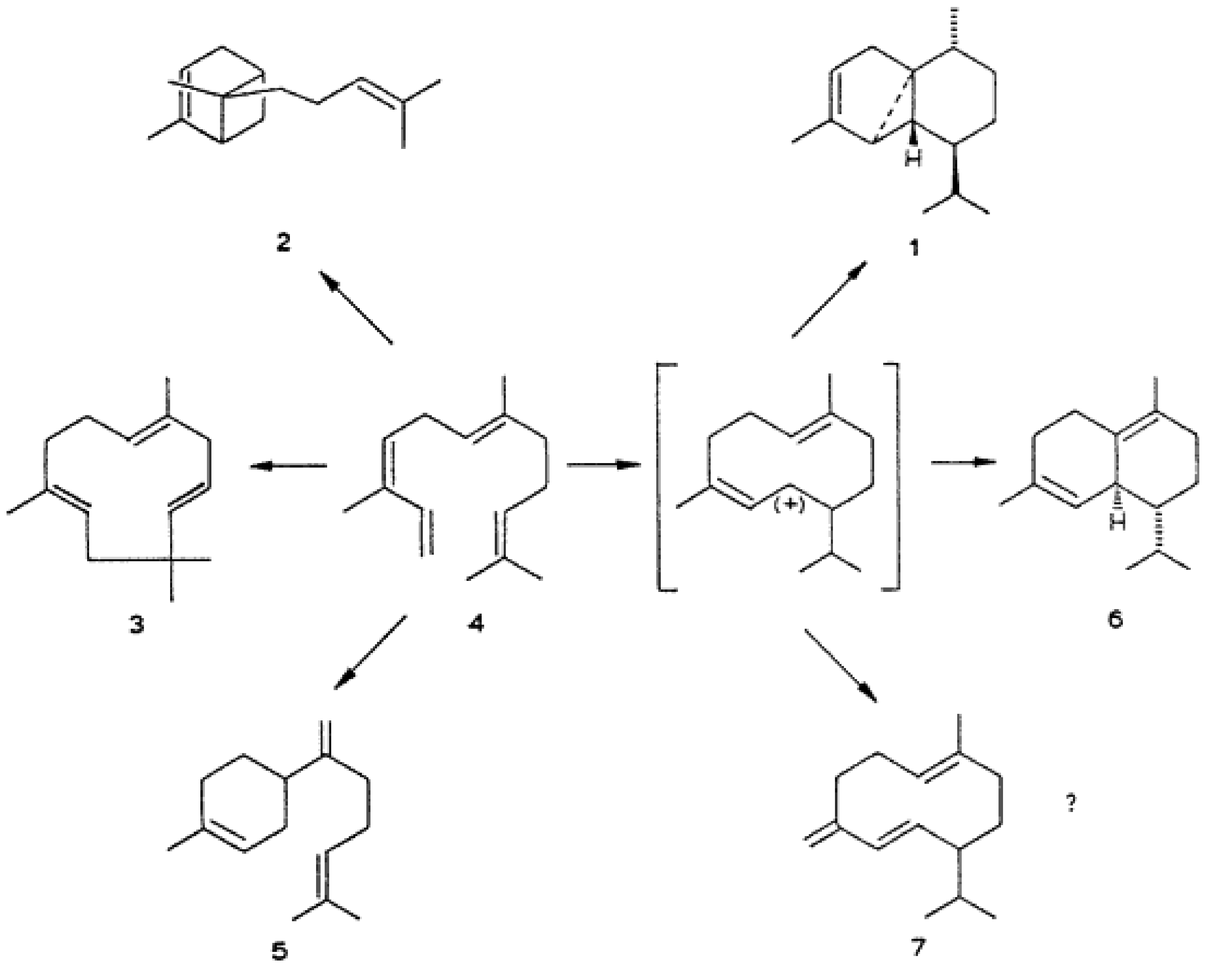

Fig. 1. New sesquiterpene hydrocarbons in nutmeg.

Two pairs of *cis*/*trans*-isomeric monoterpene alcohols usually occur together in essential oils of several spices (especially in Laminaceae-derived oils, for instance rosemary oil[6]), namely, *cis*/*trans*-sabinene hydrate and *cis*/*trans-p*-menth-2-en-1-ol. Three of the compounds are known to occur in nutmeg[7]. The missing fourth compound (*trans-p*-menth-2-en-1-ol) has now also been identified via its isolation and comparison of spectra with an authentic specimen[8].

Another interesting finding was the identification of *trans-p*-menth-2-ene-1,4-diol in nutmeg. This diol, already known as a constituent of Mitcham peppermint oil[9], is the first bifunctional monoterpene derivative detected in nutmeg oil. Its structure was assigned by comparison with synthetic specimens of known stereochemistry: ascaridol was reduced with triphenylphosphine[10] and gave after hydrolysis the *trans*-isomer identical in all respects with the isolated material. In contrast, reduction of ascaridol with lithium aluminium hydride[11] leads to the *cis*-isomer, as yet undetected in our natural substrate.

The occurrence of (*E*)-3-methyl-4-decen-1-ol ($[\alpha]^{22} - 32.05°$, $c = 1.045$, CHCl$_3$) in a natural material is here reported for the first time. The structure of the isolated alcohol follows from spectroscopic data and comparison with an authentic, synthetic sample[12]. This represents a good example of the successful isolation and structure elucidation by combination of a tedious chromatographic step with modern spectroscopic means. The natural alcohol seems to be optically pure, because no additional lines were observed when an optically active Eu(III)-shift reagent was added. In contrast, the synthetic specimen shows doubling of the characteristic signals due to the presence of both possible isomers.

Additionally, 3-methyl-4-decen-1-yl acetate was detected as a trace component by GLC–MS where the mass spectrometer was operated in the chemical ionization (CI) mode: the CI spectrum using isobutane as reaction gas exhibits intense signals at $m/e = 153$ and 213, whereas the normally recorded electron impact spectrum completely lacks the molecular ion at $m/e = 212$.

TABLE I

COMPOSITION OF NUTMEG OIL

The newly identified compounds are marked with an asterisk (*).

Name	*Content (%)*	*Name*	*Content (%)*
Monoterpene hydrocarbons		*Aromatics*	
α-Pinene	17.2	*trans*-Methylisoeugenol	
α-Thujene	0.9	5-Methoxyeugenol	
Camphene	0.4	Myristicin	14.0
β-Pinene	14.8	Elemicin	
Sabinene	21.0	*cis*-Isoelemicin	
Δ^3-Carene	1.4	*trans*-Isoelemicin	
Myrcene	1.6		
α-Phellandrene	1.6	*Monoterpene alcohols*	
α-Terpinene	1.4	Linalool	1.0
Limonene	4.1	Fenchyl alcohol* (α-fenchol)	
β-Phellandrene	2.0	Borneol	
γ-Terpinene	2.1	Terpinen-4-ol	6.3
p-Cymene	0.5	α-Terpineol	1.4
Terpinolene	1.0	Citronellol	
α,*p*-Dimethylstyrene*		Nerol*	
		Geraniol	
Oxides		*cis*-Piperitol	
1,8-Cineol		*trans*-Piperitol*	
		cis-Sabinene hydrate	
Sesquiterpene hydrocarbons		*trans*-Sabinene hydrate	
Total	1.0	*cis-p*-Menth-2-en-1-ol	
α-Copaene (0.3)		*trans-p*-Menth-2-en-1-ol*	
α-Cubebene* 1		*trans-p*-Menth-2-ene-1,4-diol*	
α-Bergamotene* 2		*p*-Cymen-8-ol*	
Carophyllene			
α-Humulene* 3		*Esters*	
α-Farnesene* 4		Bornyl acetate	
β-Bisabolene* 5		Linalyl acetate	
δ-Cadinene* 6		Citronellyl acetate*	
Germacrene D* 7		Neryl acetate*	
		Geranyl acetate	
Aromatics		Terpinen-4-yl acetate	
Safrol	3.3	α-Terpenyl acetate*	
Eugenol	0.3		
cis-Isoeugenol		*Aliphatics*	
trans-Isoeugenol		3-Methyl-4-decen-1-ol*	
Methyleugenol	0.3	3-Methyl-4-decenyl acetate*	
Vanillin*			

The analysis of the so-called mace oil, the oil of the dried arillode of nutmeg reveals only quantitative but no significant qualitative differences when compared with the real nutmeg oil. The higher percentage of caryophyllene and the slightly lower amounts of safrol and myristicin are to be noted. For the latter, a noxious constituent with psycgoactive, toxic properties, a quantitative GLC method was developed using diethyl phthalate as internal standard. Our interest in developing such a method was aroused by the fact that the literature[7] values obtained by GLC measurements show some discrepancy. The results from our method correspond well to the values obtained by conventional work-up (13–14%): nutmeg 13.9 ± 0.5%; mace 10.1 ± 0.5%.

In Table I the approximate composition of nutmeg oil is given for the main components, thus underlining the predominance (70%) of monoterpene hydrocarbons.

REFERENCES

1 D. A. Kalbhen, *Angew. Chem.*, 83 (1971) 392.
2 S. van Straaten (Editor), *Volatile Compounds in Food*, Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands, 1979.
3 K. Yoshihara, Y. Ohta, T. Sakai and Y. Hirose, *Tetrahedron Lett.*, (1969) 2263.
4 K. Morikawa and Y. Hirose, *Tetrahedron Lett.*, (1969) 1799.
5 A. J. Weinheimer, W. W. Youngblood, P. H. Washecheck, T. K. B. Karns and L. S. Ciereszko, *Tetrahedron Lett.*, (1970) 497.
6 D. Lamparsky and H. P. Schenk, *11. Arbeitstagung über Vorkommen und Analytik ätherischer Öle, Groningen, 1980.*
7 J. Baldry, J. Dougan, W. S. Matthews, J. Nabney, G. R. Pickering and F. V. Robinson, *Int. Flavours Food Addit.*, 7 (1976) 28.
8 G. O. Schenck, K. Gollnick, G. Buchwald, S. Schroeter and G. Ohloff, *Justus Liebigs Ann. Chem.*, 674 (1964) 93.
9 J. Katsuhara, K. Ishibashi, K. Hashimoto and M. Kobayashi, *Kogyo Kagaku Zasshi*, 69 (1966) 1170; *C.A.*, 65 (1966) 17006 h.
10 G. O. Pierson and O. A. Runquist, *J. Org. Chem.*, 34 (1969) 3654.
11 J. A. Chan, M. Nagasawa, C. Takeguchi and Ch. J. Sih, *Biochemistry*, 14 (1978) 2987.
12 E. Demole and P. Enggist, *Helv. Chim. Acta*, 52 (1969) 933.