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Note

Gas chromatographic analysis of total fatty acids extracted from *Schinus terebenthifolius* berries

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Schinus terebenthifolius is a member of the Anacardiaceae family. It is cultivated as an ornamental garden tree in many countries with warm climates. The berries are rich in essential oil which has been used in the perfume industry.

Most published work on the determination of fatty acids has employed their methyl esters for separation by gas chromatography (GC). Various investigators have studied the quantitative aspects of this conversion using, for example, methanol–sulphuric acid as described by Forman[1] or the methoxy esters resulting from the reduction of methoxy mercuration products as introduced by Abely *et al.*[2]. In 1979, Eyers[3] described a method in which the methyl esters of the acids were prepared by transesterification using sodium methoxide or by refluxing the saponified oil or fat with methanol–sulphuric acid–ammonium chloride. Another modified esterification procedure was developed by Chapman[4] who determined free fatty acids in small vegetable oil samples by GC after a modified boron trichloride or trifluoride and methanol esterification procedure using methylurea.

No previous work has been carried out on *Schinus terebenthifolius* berries as a possible source of fatty acids.

EXPERIMENTAL

The berries of *Schinus terebenthifolius* ornamental plant were collected from the garden of the Faculty of Science. The trees of this plant are of medium height (7–15 ft.), and have green leaves of all times of the year. The leaf is a compound one, comprised of seven leaflets. While the flower is white and appears only in the summer, the berries are scarlet.

All organic solvents used were of analytical grade. The standard fatty acids were obtained from Sigma Chemical Company. Three separate 50-g portions of clean crushed *Schinus terebenthifolius* berries were soaked in light petroleum, diethyl ether or chloroform–methanol (2:1, v/v) for 1 week with intermittent shaking. Each mixture was then filtered through a büchner funnel. The meals (extracted or defated berries) were washed with several portions of the solvent used, and evaporated under reduced pressure. Methanolysis was performed according to the method of Chalvardjian[5] on these residues as well as on a standard fatty acids mixture. Thus a 2-mg amount of each sample was heated 80–90°C with 10 ml of freshly prepared dry

TABLE I

CONDITIONS FOR GC ANALYSIS

Glass column	DEGS (15%, w/w) on Chromosorb W HP (100–120 mesh), 8 ft. × ¼ in. I.D.
Detector	Flame ionization
Temperature (°C)	110–190
Programme rate (°C/min)	6
Air pressure (kg/cm²)	1
Hydrogen pressure (p.s.i.)	30
Nitrogen flow-rate (ml/min)	35
Chart speed (cm/min)	1
Attenuation	32/100

methanol–benzene–concentrated sulphuric acid (8.6:1:0.4, v/v/v) for 2 h in a stream of nitrogen. After cooling, 15 ml distilled water were added and the methyl esters were extracted five times with 3-ml portions of *n*-hexane. The combined extracts were dried over anhydrous sodium sulphate and then subjected to GC analysis. A 5-μl portion of each methylated sample (2 mg/ml *n*-hexane) was injected into the gas chromatograph (Perkin-Elmer, Sigma 3B) using the conditions shown in Table I.

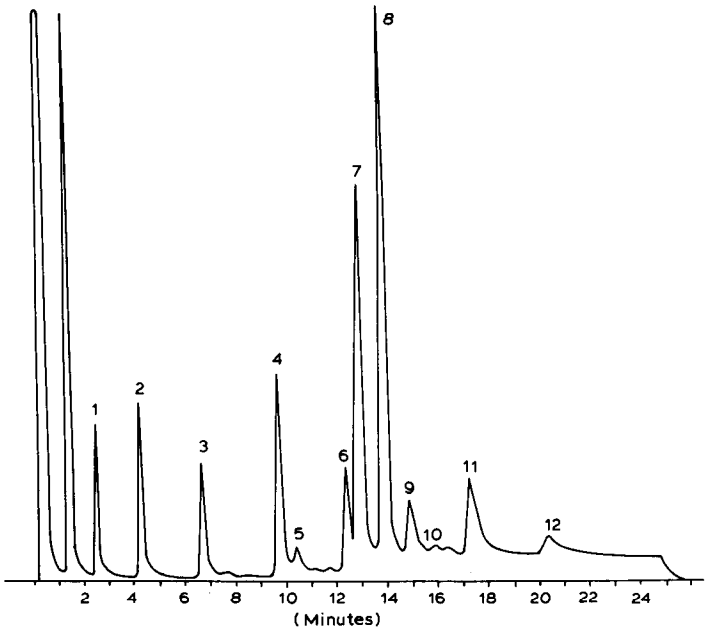

Fig. 1. Gas chromatogram of standard fatty acid methyl esters on a 8 ft. × ¼ in. I.D. glass column loaded with 15% DEGS on Chromosorb W HP (100–120 mesh). Column temperature: 110–190°C. Flow-rate: 35 ml/min. Peaks: 1 = $C_{10:0}$; 2 = $C_{12:0}$; 3 = $C_{14:0}$; 4 = $C_{16:0}$; 5 = $C_{16:1}$; 6 = $C_{18:0}$; 7 = $C_{18:2}$; 8 = $C_{18:2}$; 9 = $C_{20:0}$; 10 = $C_{18:3}$; 11 = $C_{22:0}$; 12 = $C_{24:0}$.

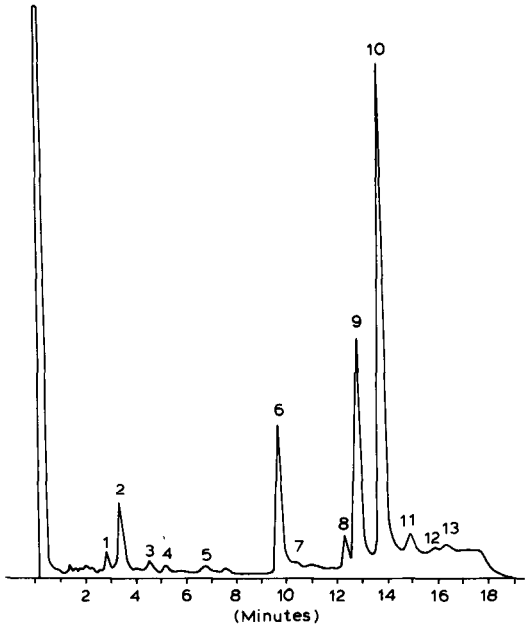

Fig. 2. Gas chromatogram of total fatty acid methyl esters from the light petroleum extract. Conditions as in Fig. 1. Peaks: 1 = $C_{10:0}$; 2, 4, 13 = unknowns; 3 = $C_{12:0}$; 5 = $C_{14:0}$; 6 = $C_{16:0}$; 7 = $C_{16:1}$; 8 = $C_{18:0}$; 9 = $C_{18:1}$; 10 = $C_{18:2}$; 11 = $C_{20:0}$; 12 = $C_{18:3}$.

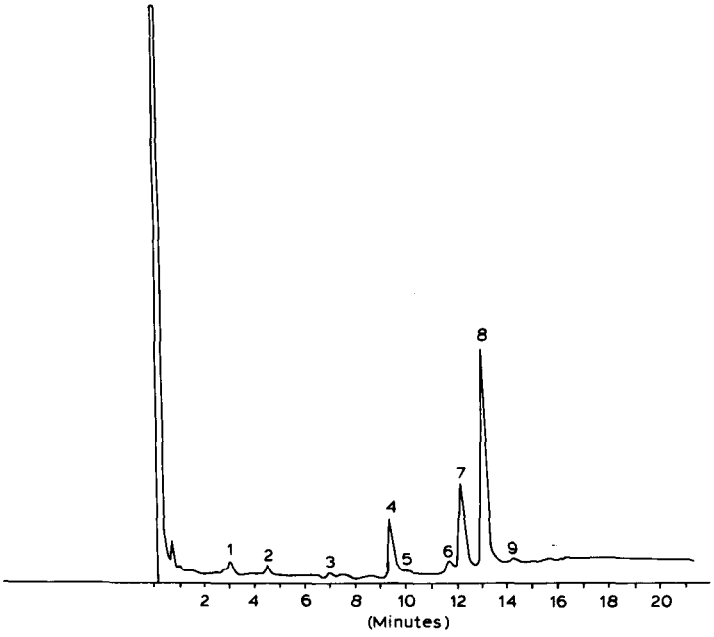

Fig. 3. Gas chromatogram of total fatty acid methyl esters from the diethyl ether extract. Conditions as in Fig. 1. Peaks: 1 = $C_{10:0}$; 2 = $C_{12:0}$; 3 = $C_{14:0}$; 4 = $C_{16:0}$; 5 = $C_{16:1}$; 6 = $C_{18:0}$; 7 = $C_{18:1}$; 8 = $C_{18:2}$; 9 = $C_{20:0}$.

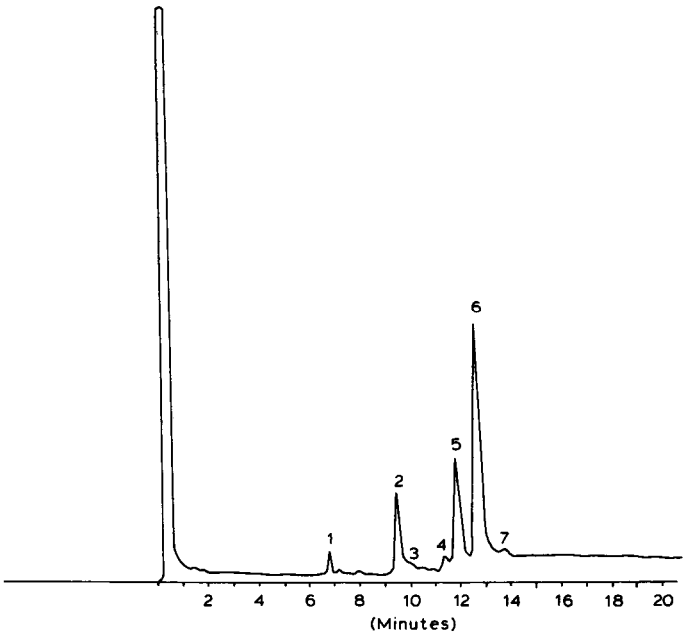

Fig. 4. Gas chromatogram of total fatty acid methyl esters from the chloroform–methanol (2:1, v/v) extract. Conditions as in Fig. 1. Peaks: 1 = $C_{14:0}$; 2 = $C_{16:0}$; 3 = $C_{16:1}$; 4 = $C_{18:0}$; 5 = $C_{18:1}$; 6 = $C_{18:2}$; 7 = $C_{20:2}$.

RESULTS AND DISCUSSION

The fatty acids were converted into their methyl esters to eliminate tailing[6]. The procedures recommended by Chalvardjian[5] were followed throughout this study.

In the present work, the total fatty acid methyl esters (FAME) of light petroleum, diethyl ether and chloroform–methanol (2:1, v/v) extracts as well as of a standard mixture of fatty acids were separated on the polar diethylene glycol succinate (DEGS) column with temperature programming. Chromosorb W was used as a support as according to Krupcik *et al.*[7] it does not adsorb fatty acid methyl esters as do Chromosorb P and other supports used earlier[8–12].

The behaviour of the methyl carbon atoms in methyl esters in the flame ionization detector has not been definitely established, but it appears that primary scission in the flame splits the carbon–oxygen bond, permitting this carbon atom to give the full response of an active carbon. It has also been reported that the carbonyl carbon atom does not give any response with such detectors[13,14].

Figs, 1, 2, 3 and 4 shows gas chromatograms of the methyl esters from the standard fatty acids mixture, and the light petroleum, diethyl ether and chloroform–methanol (2:1, v/v) extracts.

From Table II, it is seen that the percentage of total saturated fatty acids separated from the light petroleum extract (30%) is higher than those from diethyl ether (13.86%) and chloroform–methanol (13.62%). Also, the percentage of the total unsaturated fatty acids separated from the light petroleum extract (70%) is higher

TABLE II

PERCENTAGES OF TOTAL PEAK AREA FOR FATTY ACID METHYL ESTERS EXTRACTED FROM *SCHINUS TEREBENTHIFOLIUS* BERRIES WITH LIGHT PETROLEUM, DIETHYL ETHER AND CHLOROFORM–METHANOL (2:1, v/v)

Fatty acid	*Light petroleum*		*Diethyl ether*		*Chloroform–methanol*	
	Area (mm^2)*	*%*	*Area (mm^2)*	*%*	*Area (mm^2)*	*%*
Capric $C_{10:0}$	0.17	2.06	0.12	1.45	–	–
Lauric $C_{12:0}$	0.075	0.91	0.04	0.48	–	–
Myristic $C_{14:0}$	0.06	0.72	0.04	0.48	0.09	1.09
Palmitic $C_{16:0}$	1.55	18.85	0.75	9.12	0.68	8.26
Palmitoleic $C_{16:1}$	0.063	0.76	0.10	1.21	0.105	1.28
Stearic $C_{18:0}$	0.42	5.1	0.15	1.82	0.21	2.55
Oleic $C_{18:1}$	1.74	21.16	0.875	10.64	1.05	12.77
Linoleic $C_{18:2}$	3.9	47.43	2.175	26.45	2.3	27.97
Linolenic $C_{18:3}$	0.07	0.85	–	–	–	–
Arachidic $C_{20:0}$	0.175	2.12	0.04	0.48	0.14	1.7

* The peak area was calculated by multiplying the height by the width at half height.

than that from chloroform–methanol (42.02%) and diethyl ether (38.31%). The percentage of linoleic acid separated from the light petroleum extract (47.43%) is also higher than from the other two extracts.

The higher percentage of total fatty acids from the chloroform–methanol (2:1, v/v) extract than from diethyl ether may be due to the presence of greater amounts of polar lipids in the former which were hydrolysed by the methanolysis procedure.

REFERENCES

1 L. Forman, *Prum. Potravin*, 21 (1970) 121.
2 P. Abley, F. J. Mequillin, D. E. Mrnikin, K. Moskens and N. Polgar, *Chem. Commun.*, (1970) 348.
3 L. Eyers, *Chem. N. Z.*, 43 (1979) 237.
4 G. W. Chapman, *J. Am. Oil Chem. Soc.*, 56 (1979) 77.
5 A. M. Chalvardjian, *Biochem. J.*, 90 (1964) 518.
6 D. M. Ottenstein and W. R. Supina, *J. Chromatogr.*, 91 (1974) 119.
7 K. Krupcik, K. Tesarik, D. Liska, J. Nemec and I. P. Duchesne, *Chromatographia*, 5 (1972) 252.
8 H. J. Dutten and T. L. Mounts, *J. Catal.*, 3 (1964) 363.
9 T. L. Mounts and H. J. Dutton, *Anal. Chem.*, 37 (1965) 641.
10 M. Beroza and R. Sarmiento, *Anal. Chem.*, 37 (1965) 1040.
11 M. Beroza and R. Sarmiento, *Anal. Chem.*, 38 (1966) 1042.
12 V. Koman, *J. Chromatogr.*, 45 (1969) 311.
13 G. Jr. Perkins, G. M. Rouahayeb, L. D. Lvely and W. C. Hamilton, in N. Brenner, J. E. Callen and M. D. Weriss (Editors), *Gas Chromatography*,. Academic Press, New York, 1962, pp. 269–285.
14 L. S. Ettre, in N. Brenner, J. E. Callen and M. D. Weriss (Editors), *Gas Chromatography*, Academic Press, New York., 1964, p. 307.