13. P. K. Sarkar, B. Wells, and J. T. Jang, J. Mol. Biol., 25, 563 (1967).
14. P. K. Sarkar and J. T. Jang, in: Conformation of Biopolymers, G. N. Ramachandran, ed., Vol. 1 (1967), p. 197.
15. V. C. Bode and L. A. Machattie, J. Mol. Biol., 32, 673 (1968).

AN INVESTIGATION OF THE COMPOSITION OF AN ETHEREAL EXTRACT OF THE FRUIT STONES OF *Anisophyllea laurina*

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It has been shown by the methods of IR and ^1H and ^{13}C NMR spectroscopy and also by spectrometry that ether extracts a fat from the kernels of the fruit stones of *Anisophyllea laurina* (family *Rhizophoraceae*, Guinea). The amount of fat is about 16% of the weight of the kernels. The acid composition of the fat has been studied by gas chromatography and mass spectrometry. It contains the following acids (%): myristic (traces), palmitoleic (26.1), palmitic (54.4), linoleic (5.5), oleic (10.1), vaccenic (3.1), and stearic (0.8).

The drupaceous tree *Anisophyllea laurina* (family *Rhizophoraceae*) the fruit of which is used as food, grows in west Africa, including Guinea. The stones of the fruit have never yet been used. In order to determine the possibility of their utilization, we have investigated the composition of an ethereal extract of the kernels of the stones. After evaporation of the ether, a white solid was obtained (about 16% of the weight of the kernels) which after some days changed into a brownish pasty mass. Recrystallization of this mass from a mixture of methanol and acetone gave a white substance which did not change on prolonged storage.

It was shown by the methods of elementary analysis, IR spectroscopy, and ^1H and ^{13}C NMR spectroscopy that the purified substance consisted of tripalmitin while the initial unpurified product contained free acids, in addition to glycerides of carboxylic acids. From the ratio of the intensities in the IR spectrum of the absorption bands of the carbonyl groups of the glycerides ($\nu_{C=O}$ = 1740 cm^{-1}) and of carboxylic acids ($\nu_{C=O}$ = 1720 cm^{-1}) it followed that the amount of the latter in the initial material was 25-30%. The presence of free acids in the unpurified material is most probably due to the splitting of triglycerides taking place during storage.

A mass-spectrometric analysis of the initial material showed that it contained glycerides including residues not only of palmitic acid but also of hexadecenoic, octadecadienoic, octadecenoic, and octadecanoic acids. They were identified by the ions of the corresponding acyloxy residues (m/z = 253, 255, 279, 281, and 283).

The acid composition of the glycerides of the stones of *Anisophyllea laurina* was studied in more detail by converting the unpurified material into methyl esters and analyzing the latter with the aid of chromato-mass spectrometry and gas chromatography. The chromato-mass spectrometric analysis of the methyl esters confirmed the acid composition of the initial material given above. With the aid of this method it was also shown that the material contained trace amounts of tetradecanoic acid.

The mass-spectrometric analysis did not give unambiguous information on the structure of the skeletons of the acids or on the positions of the multiple bonds in the unsaturated acids. The acids present in the initial material were therefore identified in the form of their methyl esters by comparing their Kovats indices (Table 1) with the corresponding indices of standard esters and those given in the literature [1]. The close values of the indices

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TABLE 1. Kovats Indices for the Methyl Esters of the Acids from the Glycerides of the Stones of *Anisophyllea laurina*

Methyl ester	I_{OV-101}^{200} for the corresponding ester	I_{OV-101}^{200*}	$I_{SE-30}^{200\dagger}$
Methyl myristate	1712	1712	1713
Methyl palmitoleate	1888	—	1888
Methyl palmitate	1911	1910	1913
Methyl linoleate	2073	2074	2074
Methyl oleate	2082	2083	2081
Methyl vaccenate	2088	—	2089
Methyl stearate	2111	2114	2113

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*Determined in the present investigation.

†[1].

permitted us to deduce the presence of myristic acid (14:0), palmitoleic acid (cis-16:1ω7), palmitic (16:0), linoleic acid (cis,cis-18:2ω9,12), oleic acid (cis-18:1ω9), vaccenic acid (trans-18:1ω7), and stearic acid (18:0) in the glycerides of the kernels. A methyl ester in the mixture analyzed having a Kovats index of 2088, which was closer to the index of methyl vaccenate (2089) than to the index of the methyl elaidate (2086 [1]) was assigned to methyl vaccenate also because natural glycerides contain residues of elaidic acid (trans-18:1ω9) extremely rarely [2-4].

The quantitative acid composition of the stone fat was also determined by the chromatographic analysis of the methyl esters using the method of internal standardization:

Acid	Amount, %
Myristic	traces
Palmitoleic	26.1
Palmitic	54.4
Linoleic	5.5
Oleic	10.1
Vaccenic	3.1
Stearic	0.8

As can be seen from the figures given, the main components of the fat were palmitic and palmitoleic acids. The other acids were present in the fat in far smaller amounts.

EXPERIMENTAL

IR spectra were recorded on a UR-20 spectrometer in hexachlorobutadiene.

NMR spectra ($CDCl_3$, internal standard tetramethylsilane) were taken on Varian spectrometers: ^1H — XL-100; ^{13}C — CFT-20; ^{31}P — FT-80A.

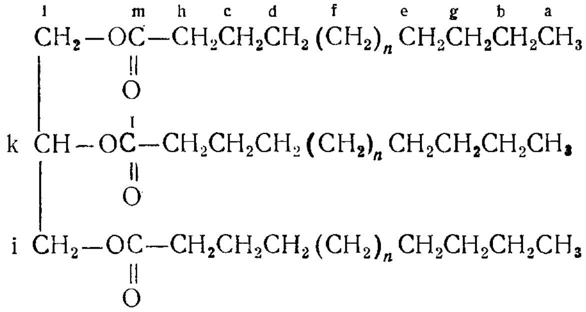
The mass spectra of the initial material and of the purified preparation were taken on a MAT-44S chromato-mass spectrometer with direct introduction of the sample into the ionization source (70 eV). The fatty acid methyl esters were analyzed on a M-80A chromato-mass spectrometer (glass column 2 m × 1 mm, 5% OV-101 on Chromosorb W-HP). Since in this column the methyl esters of the 18:1 and the 18:2 acids have close retention times and are therefore eluted as a single broad peak, the chromatographic peaks of these esters were separated mass-fragmentographically on the basis of the molecular ions with m/z 296 and 294, and their mass spectra were recorded in the non-overlapping sections of the mass-fragmentographic peaks.

The analysis of the fatty acid methyl esters was performed on a Chrom-5 gas chromatograph using a fused silica capillary column (50 m × 0.2 mm, OV-101). The esters were identified by means of their Kovats indices.

Preparation of an Ethereal Extract of the Fruit Stones of *Anisophyllea laurina*. The kernels of the stones (25 g) were ground in a mortar and extracted with 300 ml of ether in a Soxhlet apparatus for 3 h. The white solid product obtained after the evaporation of the ether changed after storage for some days into a brownish pasty mass. IR spectrum (cm^{-1}):

727 (C–H), 1185 (C–O), 1477 (C–H), 1720 (C=O), 1740 (C=O), 2860 (C–H), 2930 (C–H), 2500-3600 (O–H). There were no signals in the ^{31}P NMR spectrum. Found, %: C 76.06; H 12.18; N 0.00.

Recrystallization of this material from methanol–acetone (1:1) gave a white solid with mp 62.5°C. The melting point of tripalmitin is 65.8°C [5]. Found, %: C 75.68; H 12.18. Calculated for tripalmitin, %: C 75.50; H 12.42. IR spectrum (cm^{-1}): 727 (C–H), 1185 (C–O), 1477 (C–H), 1740 (C=O), 2860 (C–H), 2930 (C–H). PMR spectrum (δ, ppm): 1.00 (CH_3), 1.48 (CH_2); 2.56 (–CH_2COO); 5.1 (CH of the glycerol skeleton). ^{13}CNMR spectrum (δ, ppm): 14.0(a); 22.6(b);



22.7(f); 24.9(c); 29.1 and 29.3(d and e); 31.9(g); 34.1(h); 62.1(i), 68.8(k); 172.8(l); 173.2(m). The assignement of the signals in the PMR and ^{13}C NMR spectra was made on the basis of the spectra of tristearin [6] and of methyl myristate [7], respectively.

The methyl esters both of the initial material and of the purified preparation were obtained by hydrolysis with a 0.5 M solution of caustic soda in methanol followed by treatment with a solution of boron trifluoride in methanol [8]. The standard methyl esters were obtained by esterifying saturated acids in methanol in the presence of sulfuric acid and unsaturated acids with a solution of boron trifluoride in methanol.

CONCLUSIONS

1. An ethereal extract of the kernels of the fruit stones of *Anisophyllea laurina* has been analyzed. It has been shown that it includes glycerides of saturated and unsaturated acids.

2. The qualitative and quantitative acid compositions of the fat from the kernels of the stones have been determined.

LITERATURE CITED

1. R. V. Golovnya, V. P. Uralets, and T. E. Kuz'menko, Zh. Anal. Khim., 32, 340 (1977).
2. Handbook on Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry. General Methods of Investigating Fats and Fat-Containing Products (Chemistry and Analysis) [in Russian], Leningrad, Vol. 1, 1st edn. (1967), p. 23.
3. M. Kates, Techniques of Lipidology, North-Holland, Amsterdam (1972).
4. M. I. Goryaev and N. A. Evdakova, Handbook on the Gas–Liquid Chromatography of Organic Acids [in Russian], Alma-Ata (1977).
5. R. W. Growe and C. P. Smyth, J. Am. Chem. Soc., 72, 5281 (1950).
6. High Resolution NMR Spectra Catalog, Varian, Palo Alto, Calif., (1962).
7. L. R. F. Johnson and W. C. Jankowski, Carbon-13 NMR Spectra, Wiley-Interscience, New York (1972).
8. L. D. Metcalfe, A. A. Schmitz, and J. R. Pelka, Anal. Chem., 38, 154 (1966).