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3,7,11,15-Tetramethylhexadecanoic acid, a constituent of butterfat*

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SUMMARY

By subjecting the fatty acid methyl esters from butterfat to fractional distillation, urea fractionation, and preparative gas-liquid chromatography, a fatty acid was isolated that was characterized and identified as 3,7,11,15-tetramethylhexadecanoic acid.

During the past two decades, many new fatty acids have been isolated from animal sources. A number of these fatty acids do not belong to the well-known, even-numbered, straight-chain class. Some have an odd number of carbon atoms; in others, chain branching occurs. In this field, excellent work has been done in New Zealand by Shorland and Hansen (1), who were able to prove the presence of particular acids in the fats of ruminants and in butterfat. In 1952, Hansen and Shorland reported the occurrence of a C_{20} acid in butter (2). In subsequent experiments, they showed that this acid was saturated and contained more than two branchings (3). During an investigation of butter fatty acids in our laboratory, we have probably come across the C_{20} acid reported by Hansen and Shorland. In this paper, we describe the elucidation of its structure.

EXPERIMENTAL METHODS AND RESULTS

Methods of Concentration. The starting material was obtained by melting 400 g of butter. The fat phase was decanted and filtered through cloth in order to remove small amounts of suspended proteinaceous matter. The clear fat was saponified with 130 g KOH dissolved in 3 liters of 70% methanol by refluxing for 1 hr. After acidification, the fatty acids were recovered and esterified with methanol containing 5% H_2SO_4 by refluxing for 2 hr. The methyl esters obtained were distilled at a pressure of 0.05 mm, using a 2.5 × 100-cm Vigreux column. A fraction of 105 g C_{18} methyl esters, containing small amounts of higher homologues, was obtained. Subsequently, this fraction was dissolved in methanol and treated with urea as described by Allen (4). Table 1 shows the results of the urea fractionation. The adducts, after removing the urea, were subjected to gas-liquid chromatographic (GLC) analysis. As was to be expected, adducts 1 to 4 consisted mainly of methyl stearate and octadecenoate. In adduct 5, small amounts of di- and triunsaturated C_{18} methyl esters could be detected. After removing urea from the final nonadduct, the resulting mixture (4.9 g) of branched-chain and polyunsaturated esters was saponified with 3 g KOH in 10 ml of 70% methanol.

The recovered free fatty acids were subjected to counter-current distribution. We used a fully automatic 200-tube apparatus, each tube containing 25 ml of each phase. The solvent system was heptane–methanol–formamide–acetic acid 3:1:1:1 as described by Ahrens and Craig (5). After a total of 520 transfers, a distribution was obtained as shown in Figure 1. The upper phases of tubes 495–499 were separated, and the lower phases were diluted with an equal volume of water and extracted with heptane. The combined heptane solutions (fraction E) yielded 75 mg of acids on evaporation.

A portion of the acids was esterified with diazomethane. By using GLC, with polyethyleneglycol adipate as immobile phase, it was found that the main peak represented a carbon number of 17.1 (6) and thus practically coincided with the peak of *n*-heptadecanoic acid (Fig. 2). After hydrogenation of the esters, no shift in the retention time of the main peak was observed. Since the straight-chain, saturated compounds

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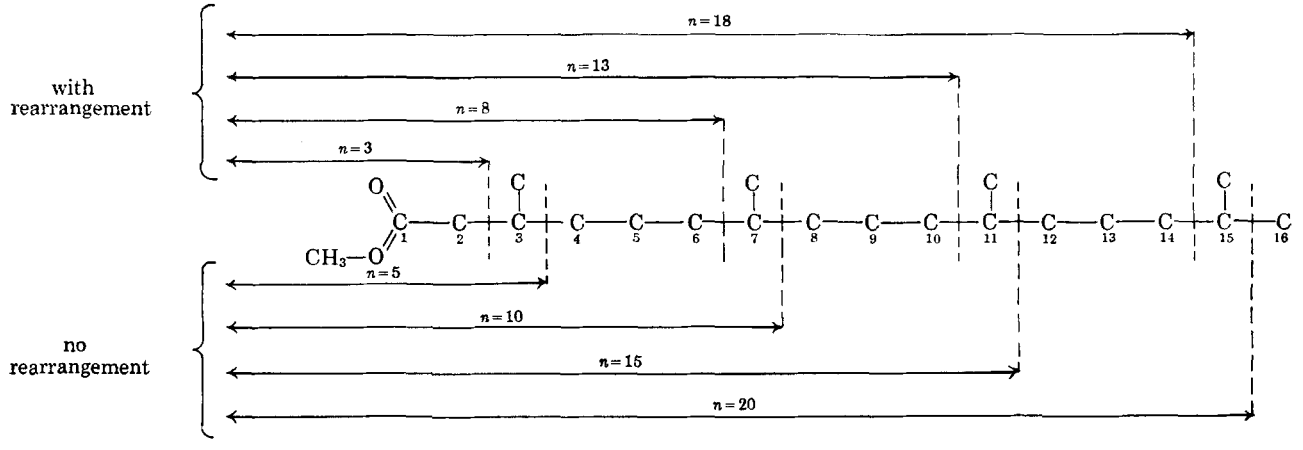
had been largely removed by the urea treatment, we assumed that the unknown main peak was due to a saturated branched-chain ester.

Structure Determination. In order to obtain reliable data for the structure determination of the unknown acid, GLC was used for further purification. Since it was already known that the acid was saturated, fraction E from the counter-current procedure was hydrogenated with Adams' catalyst in methanol. This facilitates purification because unsaturated impurities would tend to spoil the GLC separations. Several aliquots of the hydrogenated preparation (total of about 70 mg) were fractionated on a 1 × 130-cm column (immobile phase: 20% Apiezon on Celite 120/150, temperature 215°, pressure 53 cm). On this nonpolar phase, the methyl ester has a carbon number (6) of 17.5. In all, 41 mg of the purified ester was collected.[1]

Mass spectrometry of the ester showed a parent peak at a mass number of 326. This points to the methyl ester of a saturated C_{20} fatty acid ($C_{21}H_{42}O_2$). To get more information on the structure, we considered the m/e ratio of the ions possessing two oxygen atoms, giving rise to the peaks (a), $m/e = 45 + (n - 1)\ 14$; and to the corresponding rearrangement and isotope peaks (b), which are $m/e = 46 + (n - 1)\ 14$, where n is the number of carbon atoms. Degradation on both sides of a chain branching leads to peaks of high intensity, while rupture on the carboxyl side of the branching gives rearrangement (7). Table 2 shows the observed values for $a + b$, for $n = 1$ up to and including 21, and b/a and the calculated isotope ratios of the maxima of $(a + b)$. It appears that for $n = 3$, 8, 13, and 18, the value found for b/a is much higher than the calculated normal isotope ratio. This is caused by bond rupture with rearrangement. For $n = 5$, 10, 15, and 20, the values found agree with the calculations. The evidence is clearly in favor of methyl branchings on the carbon atoms 3, 7, and 11 of the straight-chain part of the molecule. The ion with $n = 20$ can also arise from the molecule ion, in which one of the methyl groups is split off, but the high value of b/a for $n = 18$ indicates a methyl branching at carbon atom 15. The infrared (IR) spectrum of the free acid on a Unicam SP 100 (equipped with gratings) confirmed the presence of branchings and revealed the presence of the isopropyl group by a doublet at 1370–1380 cm^{-1} (8).

Chemical evidence for the latter grouping was obtained by the CrO_3-oxidation, according to Archer and Hickinbottom (9), whereby acetone was formed. To 1.9 mg of the acid dissolved in a mixture of 0.2 ml acetic acid and 0.1 ml acetic anhydride, 50 mg CrO_3 was added and the mixture stirred for 16 hr at 0°. After stirring for another 4 hr at room temperature and then diluting with 12 ml water, the excess of chromic acid was removed by the careful addition of sodium bisulphite. Of this mixture, 12 ml was distilled into a receiver containing a solution of 60 mg 2,4-dinitrophenyl-hydrazine in 9 ml 2 N HCl. The hydrazones were extracted with light petroleum and, after removal of the solvent, subjected to partition chromatography over a silica gel/nitromethane column (10), the yield being 40 mg of acetone.[2] As a control, similar treatment of 2 mg of myristic acid yielded only 5 mg of acetone.



A nuclear magnetic resonance (NMR) spectrum of the methyl ester also showed four branchings. The ester-methyl and the chain-methyl absorptions fall wide apart and do not interfere. The chain absorption comprises 2 strong bands: one is due to protons in

[1] The acid obtained by saponification of this ester is liquid at −10°.

[2] Checked by comparing the R_f of the dinitrophenylhydrazone on paper with that of the known compound. The maximum of the UV spectrum in chloroform was 362 mμ.

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TABLE 1. UREA FRACTIONATION

105 g C_{18} methyl esters from butterfat
↓ 100 g Urea in 1 liter methanol
→ Adduct 1: 23.4 g methyl ester
Filtrate 1
↓ 85 g Urea in 850 ml methanol
→ Adduct 2: 26.4 g methyl ester
Filtrate 2
↓ 68 g Urea in 680 ml methanol
→ Adduct 3: 25.3 g methyl ester
Filtrate 3
↓ 53 g Urea in 530 ml methanol
→ Adduct 4: 15.0 g methyl ester
Filtrate 4
↓ 42.6 g Urea in 426 ml methanol
→ Adduct 5: 7.0 g methyl ester
Residue 4.9 g

TABLE 2. RELATIVE INTENSITIES OF ION PEAKS

Number of C-Atoms	$a + b$	b/a	Isotope Ratio Calculated for Nonbranched Chain
1	0.81		
2	1.29		
3	69.44	16.32 R*	0.034
4	7.17		
5	106.95	0.0695	0.056
6	2.82		
7	3.00		
8	8.21	0.170 R	0.090
9	0.77		
10	12.57	0.117	0.112
11	0.79		
12	0.89		
13	2.61	0.204 R	0.145
14	0.33		
15	1.85	0.178	0.168
16	0.60		
17	0.77		
18	1.66	0.537 R	0.201
19	0.62		
20	2.26	0.242	0.233
21	3.96		

* R denotes rearrangement, following from the high value of b/a.

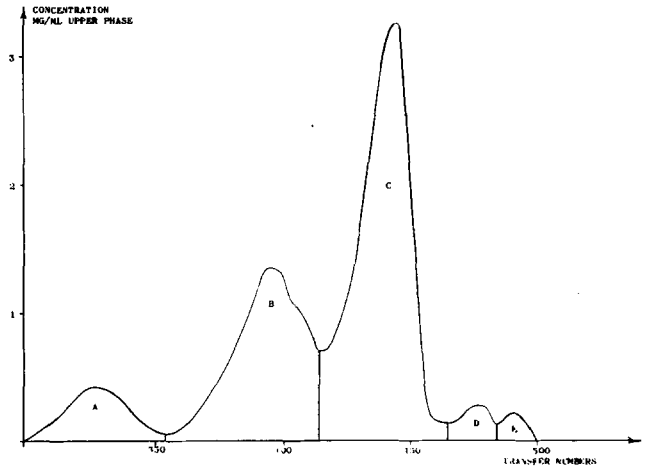

FIG. 1. Countercurrent distribution of C_{18} fatty acids of butterfat after urea fractionation. A = trienoic acids, B = dienoic acids, C = monoenoic acids, D = saturated acids, E = the acid under investigation.

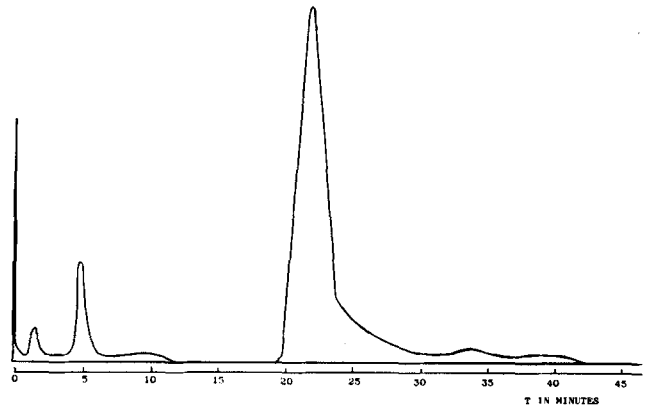

FIG. 2. Gas-liquid chromatography of methyl esters from fraction E countercurrent distribution. Pressure, 52 cm Hg; nitrogen flow rate, 42 ml/min; temperature, 197°; column, 115 × 0.4 cm; support, Celite (150–178 μ); stationary phase, polyethylene glycol adipate 30%.

CH_3 groups, the other to protons in CH_2 and CH groups. In a compound with n carbon atoms containing m branchings, the ratio between the number of protons in CH_3 groups and the number of protons in CH_2 and CH equals

$$\frac{3(m+1)}{2n-3m-4}.$$

Four curves were made, one of which is shown in Figure 3.

From the peak areas, the abovementioned ratios were calculated to the 101/163, 105/172, 98/171, 102/170. If it is assumed on the evidence of the mass spectrum that the total number of carbon atoms in the chain is $n = 20$, it then follows that $m = 4.0$, 3.9, 3.7, and 3.9, respectively. The average value of 3.9

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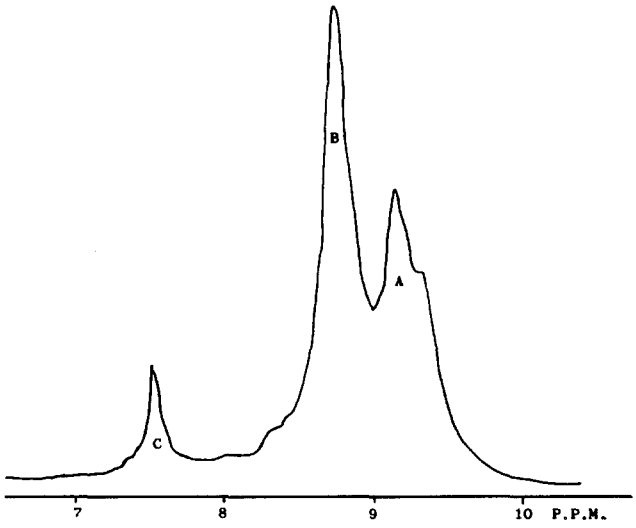

FIG. 3. NMR spectrum of methyl ester of fraction E of countercurrent distribution. A = chain CH_3, B = bulk of CH_2 and CH, C = CH_2 in α-position to the carboxyl group. The scale in ppm has been measured against tetramethylsilane = 10.

supports the evidence of 4 branchings (excluding the end-methyl groups).

Optical Rotation. A solution of 40 mg of the methyl ester in 0.5 ml methanol gave α_D + 0.2°, from which an $[\alpha]_D^{20}$ of + 1.2° was calculated. For the free acid, a value for $[\alpha]_D^{18.5}$ of +1.1° is given by Hansen and Shorland (3).

Synthesis of 3,7,11,15-Tetramethylhexadecanoic Acid. The starting material was commercially available phytol (3,7,11,15-tetramethylhexadecen-2-ol-1), which was found by GLC to have a purity of about 50%. A solution of 3.6 g phytol in 200 ml light petroleum was oxidized[3] with 7.2 g MnO_2 according to Attenburrow et al. (11) The crude α,β-unsaturated aldehyde obtained was dissolved in 50 ml ethanol and hydrogenated at 18° by using 3.6 g palladium catalyst (4% Pd on $BaSO_4$), 61% of the calculated volume of H_2 being used. An aliquot of the hydrogenated product was converted to its 2,4-dinitrophenylhydrazone, the yield being 61%, calculated as 3,7,11,15-tetramethylhexadecanal. The reaction product, dissolved in a mixture of 36 ml dioxan and 8 ml water, was oxidized with 3.55 g freshly precipitated Ag_2O at 60° according to Mitchell and Smith (12). From the reaction products, 300 mg acid was isolated. The unsaponifiable part was 1.3 g. As the conversion was considered to have been unsatisfactory, this unsaponifiable material was again treated with 3.5 g Ag_2O, and an additional 500 mg acid was collected. The total amount of acid, 860 mg, was converted with diazomethane to the methyl ester. The latter was found by GLC to have a purity of 73%, the major component having the same retention time as the compound isolated from butter. The IR, mass, and NMR-spectra of the synthetic compound, purified by means of GLC, were identical with those of the natural compound. The acid was liquid at −10°. The elementary analysis was in good agreement with calculated values: $C_{21}H_{42}O_2$ (326.55).

Calculated	C 77.23%	H 12.96%
Found	C 77.07%	H 12.95%

[3] By this method, "activated" manganese dioxide converts allylic alcohols into the corresponding aldehydes.

DISCUSSION

The presence of 3,7,11,15-tetramethylhexadecanoic acid in butterfat in an estimated amount of 0.05%, calculated on the total fatty acids, has been demonstrated. It has not been established whether this acid occurs as free fatty acid, as a constituent of glycerides or phospholipids, or whether it is bound in other esters. From the biological point of view, the origin of the branched fatty acid might be interesting. In this respect, reference is made to a publication by Christophe and Popják (14), stating that liver enzyme preparations are able to convert mevalonic acid into "prenoic acids" such as, for example, farnesoic acid (3,7,11-trimethyldodecatrienoic acid).

The results of the present investigation once more illustrate the danger of drawing conclusions about the number of carbon atoms solely from the GLC retention volume of an ester (even when saturated). Without the aid of mass spectroscopy, it would not have become evident that the unknown acid had twenty carbon atoms.[4]

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[4] Shortly before this manuscript was submitted for publication, we read in an article by Stenhagen (13) that Biurstam et al. have also isolated the acid described above from butter in the course of investigations that have not yet been published.

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