Isolation and Structure of the C₁₆ Unsaturated Fatty Acids in Menhaden Body Oil[1]

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A mixture of C₁₆ unsaturated fatty acids was isolated from menhaden body oil by fractional crystallization, followed by high vacuum fractional distillation. Countercurrent distribution yielded pure tetraene, triene, diene and monoene fractions. The structures of these acids were determined by oxidative and reductive ozonolysis with identification of the fragments by reversed phase, paper and gas–liquid chromatographic analysis. These several acids were identified: *n*-hexadeca-6,9,12,15-tetraenoic acid, *n*-hexadeca-4,7,10,13-tetraenoic acid, *n*-hexadeca-6,9,12-trienoic acid, *n*-hexadeca-7,10,13-trienoic acid, *n*-hexadeca-6,9-dienoic acid, *n*-hexadeca-9,12-dienoic acid, *n*-hexadeca-9-monoenoic acid and *n*-hexadeca-8-monoenoic acid. Gas–liquid chromatographic characteristics of these acids are described.

In recent years a number of polyenoic fatty acids with chain lengths C₁₈, C₂₀ and C₂₂ have been isolated from natural sources in the laboratories of Klenk,[2-8] Riemenschneider[9] and Lundberg.[10] Structural studies of these acids suggest certain common pathways in their biosynthesis, for in widely diverse systems (triglycerides and phosphatides of liver and brain in marine and mammalian

species) the polyenoic acids have two characteristics in common: (1) the double bonds are arranged in the divinyl-methane rhythm, often called methylene-interrupted or "skipped" double bonds, and (2) each of these acids belongs either to the linoleic or to the linolenic acid family, *i.e.*, with the double bond farthest from the carboxyl group six or three carbons from the terminal methyl group, respectively.

Studies of the C₁₆ unsaturated acids have been much less comprehensive. Smith and Brown[11] in 1945 were the first to note the presence of a C₁₆ tetraene as a component in menhaden oil and in 1958 Mangold and Schlenk[12] confirmed the presence in menhaden oil of C₁₆ mono-, di-, tri- and tetraenes. A C₁₆ tetraene also was noted by Paschke

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(2) E. Klenk and W. Bongard, *Z. physiol. Chem.*, **291**, 104 (1952).

(3) E. Klenk and W. Bongard, *ibid.*, **290**, 181 (1952).

(4) E. Klenk and W. Montag, *Ann.*, **604**, 4 (1957).

(5) E. Klenk and D. Eberhagen, *Z. physiol. Chem.*, **307**, 42 (1957).

(6) E. Klenk and F. Lindlar, *ibid.*, **299**, 74 (1955).

(7) E. Klenk and H. J. Tomuschat, *ibid.*, **308**, 165 (1957).

(8) E. Klenk and H. Brockerhoff, *ibid.*, **307**, 272 (1957).

(9) S. F. Herb, R. W. Riemenschneider and G. Donaldsen, *J. Am. Oil Chem. Soc.*, **28**, 55 (1957).

(10) E. G. Hammond and W. O. Lundberg, *ibid.*, **30**, 438 (1953).

(11) F. A. Smith and J. B. Brown, *Oil and Soap*, **22**, 280 (1945).

(12) H. K. Mangold and H. Schlenk, *J. Biol. Chem.*, **229**, 731 (1957.)

and Wheeler[13] in *Chlorella* lipides in 1954. However, the double bond structure of the C$_{16}$ polyenes remained undefined until Shorland[14] characterized all-*cis-n*-hexadeca-7,10,13-trienoic acid in rapeseed oil, and Silk and Hahn[15] positively identified *n*-hexadeca-6,9,12,15-tetraenoic acid in South African pilchard oil.

The present report on C$_{16}$ unsaturated acids is part of a larger study defining the total fatty acid composition of menhaden body oil, a program made desirable by nutritional studies recently published.[16] There were also other considerations which demanded the isolation of pure polyenoic acids, particularly the need for extinction coefficients in the ultraviolet range after isomerization with alkali and other physical constants useful in the interpretation of gas–liquid chromatography patterns of highly unsaturated fatty acid esters (retention volumes on various stationary phases, stability, etc.).

The scheme for the isolation and identification of polyenoic fatty acids, applied here to menhaden body oil, is shown in Fig. 1. The source of the oil and its preparation is considered in detail elsewhere.[16] The most highly unsaturated acids were concentrated by low temperature crystallization. Then this mixture was resolved into homogeneous chain length groups by fractional distillation at high vacuum, and each group was further subdivided by countercurrent distribution (CCD) into monoene, diene, etc., subgroups. The precise double bond pattern of the individual acids in each of these subgroups finally was determined by degradative studies. All steps were carried out under nitrogen whenever possible. Successful avoidance of artifactual changes was verified at every stage: ultraviolet and infrared spectrography demonstrated the absence of conjugation or *cis–trans* isomerization of double bonds.

Degradations were carried out by oxidative and reductive ozonolysis. Lengths of the fragments from the carboxyl group to the nearest double bond (the "carboxyl fragment") and of those portions between double bonds (the "intermediate fragments") were identified after converting these portions of the molecule to dicarboxylic acids by oxidative ozonolysis. In this process the fragment from the terminal methyl group to the double bond nearest to it (the "terminal fragment") is converted to the monocarboxylic acid. After separation of mono- from dicarboxylic acids, the individual dicarboxylic acids were isolated, identified and quantitated. The terminal fragments also were converted to aldehydes by reductive ozonolysis and these aldehydes identified as 2,4-dinitrophenylhydrazones. Numerous chromatographic procedures were used in separating and measuring the fragments produced, of which gas–liquid chromatography (GLC) proved easily the most valuable.

Table I lists the degradation products of the C$_{16}$ unsaturated fatty acids in menhaden body oil, as well as the structures of the acids proven to be present in the C$_{16}$ mixture by means of these degradation

(13) R. F. Paschke and D. H. Wheeler, *J. Am. Oil Chem. Soc.*, **31**, 81 (1954).

(14) F. B. Shorland, *Nature* (London), **156**, 269 (1945).

(15) M. H. Silk and H. H. Hahn, *Biochem. J.*, **57**, 582 (1954).

(16) E. H. Ahrens, Jr., *et al.*, *Lancet*, in press.

Triglycerides
↓ Saponification
　Extraction
Sodium salts ──→ Unsaponifiable materials
↓ (petroleum ether)
　Acidification
Fatty acids
↓ Low temp. crystallization

−20° 　−40° 　−70° 　　Polyunsatd. fatty acids
　　　　　　　　　　　　　Esterification
Least unsatd. fatty acids
　　　　　　　　Fatty acid methyl esters
　　　　　　　　↓ Fractional high
　　　　　　　　　vacuum distillation

C$_{14}$　　C$_{16}$　　C$_{18}$　　C$_{20}$　　C$_{22}$

Countercurrent distribution (CCD) of each chain length group

Sat.　Mono-　Di-　Tri-　Tetra　etc.-enoic acid fractions

Degradative studies
↓
Oxidative ozonolysis　　　　Reductive ozonolysis

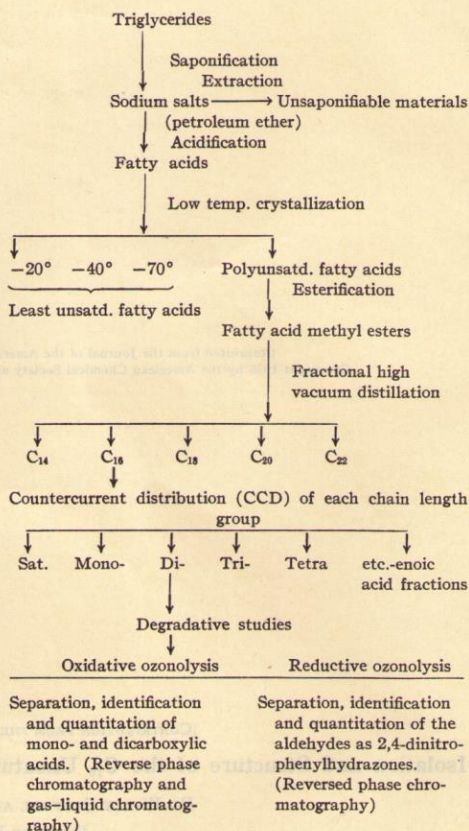| Separation, identification and quantitation of mono- and dicarboxylic acids. (Reverse phase chromatography and gas–liquid chromatography) | Separation, identification and quantitation of the aldehydes as 2,4-dinitrophenylhydrazones. (Reversed phase chromatography) |

Fig. 1.—Scheme for the isolation and identification of the most unsaturated fatty acids in a complex natural mixture.

data. Although Japanese investigators[17,18] reported the presence of ethylene-interrupted double bond systems in the polyenoic acids of sardine oil, Hilditch[19] concluded on the basis of Farmer and Van den Heuvel's observations[20] that the Japanese workers had been led astray by the structural alterations caused by their isolation procedures. In the present study all evidence pointed to the occurrence only of methylene-interrupted double bonds in all the polyenoic acids isolated. An estimate of the quantitative relationships of these acids in the total mixture and to each other was obtained by a combination of preparative and analytical gas–liquid chromatography and has been presented elsewhere.[16]

(17) T. Tutiya, *J. Chem. Soc. Japan*, **60**, 717, 867, 1188 (1940); **62**, 10, 552 (1941).

(18) Y. Toyama and T. Tschudiya, *Bull. Chem. Soc. Japan*, **3**, 299 (1928).

(19) T. P. Hilditch, "The Chemical Constitution of Natural Fats," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, pp. 513, 539.

(20) E. H. Farmer and F. A. Van den Heuvel, *J. Chem. Soc.*, 427 (1938).

<div align="center">TABLE I</div>

SUMMARY OF RESULTS OF DEGRADATIVE STUDIES ON C_{16} UNSATURATED FATTY ACIDS OF MENHADEN BODY OIL

Fraction	Oxidative ozonolysis Mono- and dicarboxylic acids	Moles	Reductive ozonolysis 2,4-Dinitrophenyl-hydrazones of	Moles	Structure indicative by degradative data
C_{16} tetraenoic acids	Adipic	0.90	Acetaldehyde[a]	2.43	n-Hexadeca-6,9,12,15- and (trace) -4,7,10,13-
	Malonic	2.04			tetraenoic acids
	Succinic	Trace			
C_{16} trienoic acids	Adipic	0.58	Butyraldehyde	0.42	n-Hexadeca-6,9,12- and -7,10,13-trienoic
	Pimelic	0.12	Propionaldehyde	0.11	acids
	Malonic	1.15	Acetaldehyde[a]	1.04	
C_{16} dienoic acids	Adipic	0.16	Butyraldehyde	0.57	n-Hexadeca-6,9- and 9,12-dienoic acids
	Azelaic	.59			
	Malonic	.74	Acetaldehyde[a]	0.55	
	Heptylic	..			
C_{16} monoenoic acids	Suberic	.15			n-Hexadeca-8- and -9-monoenoic acids
	Capyrlic	.15			
	Azelaic	.85			
	Heptylic	.85			

[a] Results as a secondary reaction product by decomposition of malonic dialdehyde.

The biosynthetic origins of the various acids in the C_{16} unsaturated group are not readily apparent. However hazardous it may be to reason from structure alone, it is tempting to relate the acids to the well-known linoleic and linolenic types. Such a postulate presupposes prior removal of two carbons at the terminal methyl or alternatively at the carboxyl end of the chain. In a recent study of herring oil not yet reported, Klenk[21] found the same two C_{16} tetraenes as we have shown in menhaden oil. However, in herring oil the 4,7,10,13-isomer constituted about 25% of the total C_{16} tetraene, whereas in menhaden oil this acid was present only in traces (less than 5%). The C_{16} monoenes also deserve comment. The hexadeca-9-enoic acid is undoubtedly related biosynthetically to the long list of 9-monoenes of varying chain lengths isolated from various sources,[19] but the 8-enoic acid fails to fit into any obvious scheme.

After isomerization with alkali our C_{16} acids showed unusually high extinctions in the ultraviolet at the tetraene (314 and 300 mμ) region, but almost no absorption in the diene (232 mμ) region. These findings, noted also by Silk and Hahn,[15] suggest that the 6,9,12,15-C_{16}-tetraene is very readily and completely conjugated. The presence of a terminal vinyl group in the acids isolated by the two laboratories was proven by strong absorption at 910 and 990 cm.$^{-1}$; this absorption was seen distinctly after isolation of the C_{16}-tetraene fraction.

Gas–liquid chromatography of the fatty acid methyl esters[22] has proven a most valuable adjunct to these studies. It provided rapid proof of purity of fractions at various stages in the procedure, and in addition afforded critical identifications of mono- and dicarboxylic acid fragments formed by ozonolysis. Relative retention volumes (V_R^0) on two stationary phases are reported herein; the stability of these and other highly unsaturated acids during chromatography at high temperatures (197°) is a matter of separate report.[33]

(21) E. Klenk, private communication.
(22) A. T. James and A. J. P. Martin, *Biochem. J.*, **63**, 114 (1956).

Experimental

1. Isolation of C_{16} Unsaturated Fatty Acids.—Five hundred g. of menhaden body oil was saponified by refluxing for 1 hour with 15 parts (w.v.) of a 0.5 N NaOH–methanol solution. The reaction mixture was allowed to stand overnight at 4°. The saturated soaps which crystallized were sucked off, the filtrate diluted with 2 volumes of water and the unsaponifiable material extracted with petroleum ether (30–60°). The aqueous fraction containing the soaps of the more unsaturated acids was acidified while cooling in ice-water, and the fatty acids extracted with ether. After evaporation of solvent the mixed acids were separated by low temperature crystallization into fractions of varying degrees of saturation. A 10–15% acetone solution of the mixed acids was chilled successively to −20, −40 and −70° with removal of crystals at each stage. The most highly unsaturated fatty acids remained soluble in acetone at −70° in a yield of 137 g. of a light yellow oil with iodine value[23] (I.V.) of 300. These acids were esterified by refluxing with 15 volumes of a 5% dry HCl in dry MeOH solution for 2 hours. The esters were recovered in 95–97% yield as a slightly yellow oil.

Fractional high vacuum distillation[24] (column length 750 mm., 10^{-4} mm. pressure at the distillation head) was carried out on 100 g. of the mixed esters in order to separate the mixture of homologs into chain length groups (C_{14}, C_{16}, C_{18}, C_{20}). The fractions rich in C_{16} acids were combined and redistilled in 9 fractions. Small samples of each were hydrogenated with Adams catalyst and the purity checked by GLC. Fractions 4–7 were thus shown to be exclusively C_{16} and were colorless oils, I.V. 180–185. Their ultraviolet spectra showed no conjugated acids; after isomerization the presence of tetraenoic, trienoic and dienoic acids (Fig. 3a) was shown.

II. Isolation of Isomer Groups by Countercurrent Distribution (CCD).—Six g. of fraction 5 (C_{16} unsaturated esters free of higher and lower homologs) were saponified, then separated by CCD into groups of acids differing in number of double bonds. A 200-tube all-glass fully automatic machine with 10 ml. lower phase volume[25] was used, with solvent systems previously described.[26] Figure 2 shows a distribution in the formamide system with complete separation of four bands after only 375 transfers. Analysis of aliquots of each peak by isomerization with

(23) K. W. Rosenmund and W. Z. Kuhnhenn, *Unters. Nahrungs. u. Genussmittel*, **46**, 154 (1923).
(24) E. Klenk in Hoppe–Seyler–Thierfelder "Handbuch der physiologisch- und pathologischchemischen Analyse," 10th Ed., Springer Verlag, Berlin, 1955, p. 447.
(25) L. C. Craig, W. Hausmann, E. H. Ahrens, Jr., and E. J. Harfenist, *Anal. Chem.*, **23**, 1236 (1951).
(26) E. H. Ahrens, Jr., and L. C. Craig, *J. Biol. Chem.*, **195**, 299 (1952).
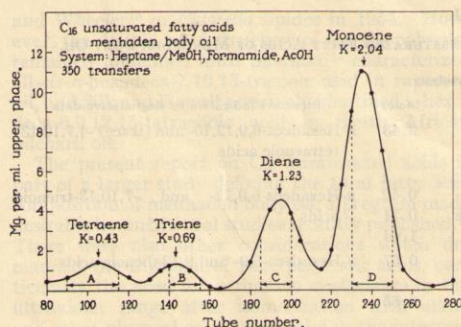
Fig. 2.—Countercurrent distribution (CCD) pattern of 6 g. of C$_{16}$ unsaturated fatty acids from menhaden body oil. Theoretical and experimental curves were superimposable. Fractions A–D were harvested as shown. Separation factors between adjacent fractions varied from 1.60 to 1.79.

alkali[27] showed that A was the tetraene group, B triene, C diene and D monoene (Fig. 3). These spectra showed the absence of contamination of each band by its more highly unsaturated neighbor; additional proof of purity of each band was given by the superimposition of theoretical and experimental CCD curves. Homogeneous isomer groups were collected from the CCD run as shown in Fig. 2; each was a colorless oil.

III. Definition of Double-bond Patterns by Degradative Studies. 1. Methods. (a) Oxidative Ozonolysis.^{2,3} —Eighty to one hundred mg. of acid dissolved in 3 ml. of methyl acetate and 2 ml. of glacial acetic acid was ozonized at −10°. Then 3 ml. of glacial acetic acid and 3 ml. of hydrogen peroxide (33%) were added and the mixture incubated for 3 days at 37°. Finally, the reaction mixture was brought to its boiling point briefly, and the solvent evaporated at 25° in vacuo through a small Widmer column. (Despite careful evaporation of solvent there are losses of the short chain monocarboxylic acids, formic to butyric.) The remaining monocarboxylic acids were separated out of the mixture by solution in petroleum ether (30–60°) at −70°, at which temperature the dicarboxylic acids crystallize on standing. These crystals were washed twice with petrol ether to remove the last traces of monocarboxylic acids. The petrol ether washes then were extracted with water to recover dicarboxylic acids (1 ml. water per 5 ml. petrol ether). The crystals and water washes were combined and the dicarboxylic acids concentrated by freeze-drying. The residue was dissolved in 1% butanol in chloroform and an aliquot taken for column chromatography.^{2} The various dicarboxylic acids were quantitated by titration of the effluent and were further identified by paper chromatography[28] of the individual acids.

In the course of these studies it became apparent that the mono- and dicarboxylic acids could be identified and quantitated more reliably and rapidly by GLC, with a smaller requirement of starting material. Samples of 10–25 mg. were oxidized as described above. The acid residues were esterified with diazomethane and then subjected to GLC with Apiezon-M as stationary phase. Columns of 120 cm. were used with nitrogen or argon as moving phase at 68 cm. inlet pressure. The methyl esters of C$_1$–C$_8$ monocarboxylic acids were most advantageously chromatographed at 25°; the C$_5$–C$_9$ monocarboxylic methyl esters and C$_2$–C$_7$ dicarboxylic methyl esters at 78°; and the C$_7$ and longer dicarboxylic methyl esters at 197°. In all cases corrected retention volumes (V_R^0) were compared with those of known standards. It was recognized that analyses of the short-chain monocarboxylic acids were not quantitative due to losses during evaporation of solvent after ozonolysis; however, the results here were qualitatively useful.

(27) R. T. Holman in D. Glick, "Methods of Biochemical Analysis," Vol. IV, Interscience Publishers, Inc., New York, N. Y., 1957, p. 126.
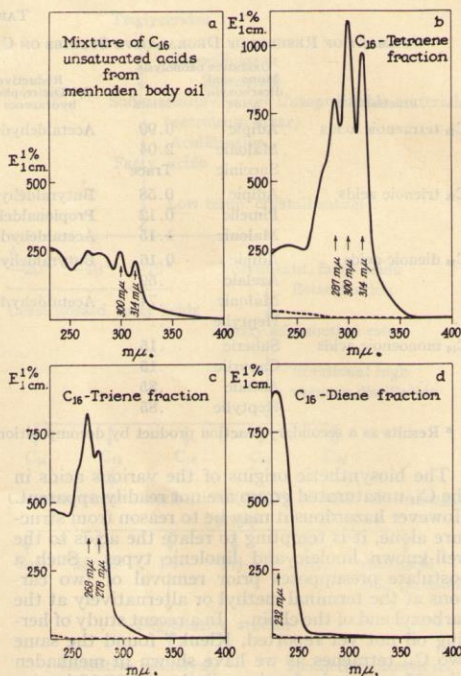(28) J. W. H. Lugg and B. T. Overell, Nature (London), 160, 87 (1947).

Fig. 3.—Ultraviolet spectra before (dotted line) and after (solid line) isomerization with alkali[27] of: (a) the C$_{16}$ acid mixture; (b) the C$_{16}$ tetraenes (CCD peak A); (c) the C$_{16}$ trienes (CCD peak B) and (d) the C$_{16}$ dienes (CCD peak C). The positions of the peaks are designated in italics.

(b) Reductive Ozonolysis.[29]—Samples of about 25 mg. were ozonized in 2 ml. of methyl acetate and 1 ml. of acetic acid at −10° and then hydrogenated with Adams catalyst (about 5 mg.) until the calculated amount of hydrogen was taken up. The solvents and aldehydes were distilled directly into 20 ml. of 0.025% 2,4-dinitrophenyl-hydrazine (0.25 g. of 2,4-dinitrophenylhydrazine in 50 ml. of concd. H$_2$SO$_4$ made up to 1 l. with water). The precipitated hydrazones were extracted with benzene. After drying the residue over sodium sulfate, it was transferred quantitatively into a 25-ml. vol. flask and made to volume with 2 parts benzene:1 part ligroin (60–90°).

One ml. of this solution was chromatographed on a silica gel–Hyflo Super-Cel column according to Roberts and Green.[30] The fractions were concentrated and the residue dissolved in 100 ml. of benzene. The extinction (E) was measured at λ 366 mμ in a 1 cm. cuvette. The following calculations allowed the determination of the number of moles of aldehyde per mole polyenoic acid

$$\text{moles aldehyde} = \frac{\text{mg. hydrazone} \times \text{mol. wt. polyenoic acid}}{\text{mg. sample} \times \text{mole wt. hydrazone}}$$

where mg. hydrazone = $E \times 25 \times f$, in which f equals an empirically determined factor (acetaldehyde 1.27, propionaldehyde 1.31, capronaldehyde 1.48).

The aldehydes were identified and the purity of the fractions controlled by paper chromatography according to Tunmann[31] with acetic acid:H$_2$O:MeOH:petroleum ether (60–80°) (2:1:8:6).

(29) E. Klenk and H. Brockerhoff, Z. physiol. Chem., in press.
(30) J. D. Roberts and C. Green, Ind. Eng. Chem., Anal. Ed., 18, 335 (1946).
(31) P. Tunmann, Arch. Pharm., 289, 329 (1956).

2. Results of Degradative Studies (Table I).—(a) C_{16}-tetraenoic acid fraction (fraction A in Fig. 2): colorless oil, $n^{25}D$ 1.4886, I.V. = 405 (calcd. for $C_{16}H_{24}O_2$, 421); $E_{1cm}^{1\%}$ 1090 at λ_{max} 300 mμ (Fig. 2b), ϵ_{max} 27000. Oxidative ozonolysis yielded mainly adipic and malonic acids, and thus the major tetraene contained double bonds in 6, 9, 12 and 15-positions. This structure was confirmed by the infrared spectrum (Fig. 4) which showed a terminal vinyl group (strong absorptions at 910 and 990 cm.$^{-1}$). In addition, a small amount of succinic acid was demonstrated by GLC, and thus about 5% of the tetraene fraction was the 4,7,10,13-acid.
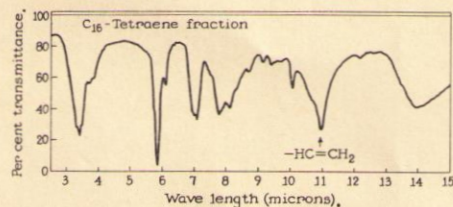

Fig. 4.—Infrared spectrum of C_{16} tetraenoic acid fraction, prepared as an oil film. The terminal vinyl group absorption is shown.

(b) C_{16}-trienoic acid fraction (fraction B in Fig. 2): colorless oil, $n^{25}D$ 1.4731, I.V. = 302 (calcd. for $C_{16}H_{26}O_2$, 313), $E_{1cm}^{1\%}$ 825 at λ_{max} 264 mμ (Fig. 3c), ϵ_{max} 21200. Oxidative ozonolysis yielded pimelic, adipic and malonic acids. On reductive ozonolysis butyraldehyde, propionaldehyde and acetaldehyde were released. These results indicated the presence of 6,9,12- and 7,10,13-trienes in proportions of 5:1.

(c) C_{16}-dienoic acid fraction (fraction C in Fig. 2): colorless oil, $n^{25}D$ 1.4611, I.V. = 202 (calcd. for $C_{16}H_{28}O_2$, 207); $E_{1cm}^{1\%}$ 920 at λ_{max} (Fig. 3d), ϵ_{max} 23200. On oxidative ozonolysis azelaic, adipic and malonic acids were produced. GLC showed these acids and, in addition, butyric and heptylic acids. Reductive ozonolysis released butyraldehyde and heptylic aldehyde. The long chain aldehyde could not be identified by paper chromatography, but it was clearly distinguished by GLC as heptylic acid after oxidative ozonolysis. These results indicated the presence in this fraction of the 6,9- and 9,12-dienes in proportions of 1:4.

(d) C_{16} = monoenoic acid fraction (fraction D in Fig. 2): colorless oil, $n^{25}D$ 1.4563, I.V. = 103 (calcd. for $C_{16}H_{30}O_2$, 104). The analysis of oxidation products was most effectively accomplished by GLC. Azelaic, heptylic, suberic and caprylic acids were identified in amounts which indicated a mixture of 8- and 9-monoenes in proportions of 1:6.

IV. Gas–Liquid Chromatographic Characteristics of the C_{16} Unsaturated Fatty Acids.—Small samples (5–15 mg.) of pure tetraenoic, trienoic, dienoic and monoenoic acids were esterified with HCl–methanol, as described above. The esters were purified by micro-sublimation with an over-all yield of 95% of the theoretical. These esters were chromatographed as single compounds and also admixed in methyl palmitate as reference standard. Apiezon M (high boiling hydrocarbon)[22] and Reoplex 400 (polyglycol adipic acid ester)[32] were used as stationary phases (column temperature 197°, N_2 pressure 68 mm., gas density

(32) C. H. Orr and J. E. Callen, THIS JOURNAL, **80**, 249 (1958).

TABLE II

RELATIVE RETENTION VOLUMES (V_R^0) OF C_{16} UNSATURATED FATTY ACID METHYL ESTERS DURING GAS–LIQUID CHROMATOGRAPHY ON A NON-POLAR (APIEZON-M) AND A POLAR (REOPLEX 400) STATIONARY PHASE

Acids	Retention vol. relative to methyl palmitate ($V_R^0 = 1$)	
	Apiezon M	Reoplex 400
n-Hexadecatetraenoic	0.75	1.95
n-Hexadecatrienoic	.78	1.60
n-Hexadeca-9,12-dienoic	.80	1.46
n-Hexadeca-6,9-dienoic	.86	1.30
n-Hexadecamonoenoic	.88	1.14

balance detector). Table II summarizes the relative retention volumes compared to methyl palmitate ($V_R^0 = 1$). No separations of isomers were seen except in the case of the C_{16} dienes, where the 6,9- and 9,12-dienes clearly separated on both stationary phases (separation factor of 1.04 in each case). Figure 5 shows the relation between
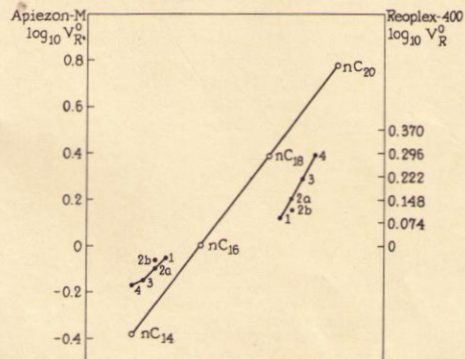

Fig. 5.—Log retention volumes of the C_{16} unsaturated fatty acid methyl esters on two stationary phases, Apiezon M (non-polar) and Reoplex 400 (polar). The logarithms of data in Table II are plotted relative to methyl palmitate, for which log $V_R^0 = 0$ on both vertical scales. The diagonal line shows the linear relationship of log V_R^0 with number of carbon atoms. The unsaturated acids are plotted according to number of double bonds on an arbitrary horizontal scale, the data to the left of the diagonal line relating to Apiezon M, to the right Reoplex 400. 1,2,3,4 = number of double bonds; 2a = n-hexadeca-9,12-dienoic, 2b = n-hexadeca-6,9-dienoic acid.

numbers of double bonds and log retention volumes of the C_{16} unsaturated acids. The aberrant behavior of the C_{16} tetraene and of the 6,9-diene is apparent.

Studies on the remarkable stability of various polyenoic acids from C_{16} to C_{22} during GLC at 197° will be presented elsewhere.[33]

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(33) W. Stoffel, to be published.