Synthesis of Unsaturated Fatty Acids. Positional Isomers of Linoleic Acid[1]

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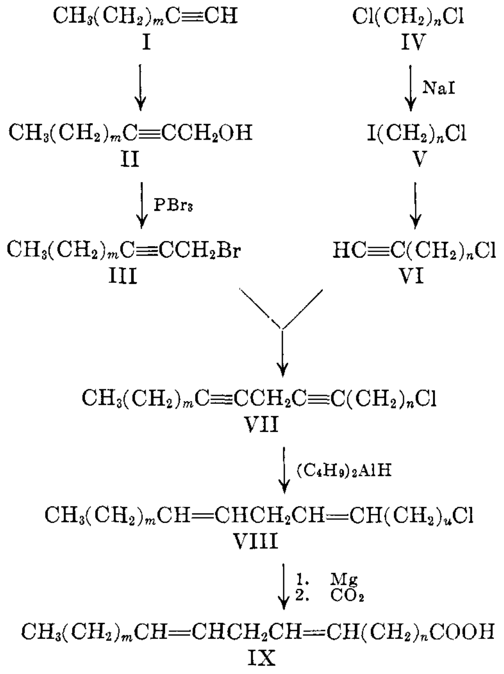
Analogs of linoleic acid, an "essential fatty acid," have been synthesized. Coupling 1-bromo-2-alkynes with the Grignard derivative of appropriate chloroalkylacetylenes in the presence of cuprous chloride formed three 17-carbon compounds, 1-chloro-7,10-, 1-chloro-9,12-, and 1-chloro-10,13-heptadecadiyne. Diisobutylaluminum hydride reacted with these diynes to give adducts, which on protonation yielded 1-chloro-*cis,cis*-heptadecadienes. Adding another carbon atom by carbonating the Grignard reagents from the chloroheptadecadienes furnished the desired 18-carbon acids. In this way the following skipped (methylene-interrupted) unsaturated isomers of linoleic acid were obtained: *cis*-8,*cis*-11-octadecadienoic, *cis*-10,*cis*-13-octadecadienoic, and *cis*-11,*cis*-14-octadecadienoic acids. The acids were characterized by their melting points, indices of refraction, and the melting points of their tetrabromides. The 18-carbon compounds and their ozonolysis products were examined with the help of gas–liquid chromatography.

Only three of the approximately twenty octadecadienoic acids in the literature incorporate the methylene-interrupted or skipped unsaturated system. These acids, all biologically active,[3] are *cis*-9,*cis*-12-octadecadienoic, *cis*-8,*cis*-11-octadecadienoic,[4] and *cis*-6,*cis*-9-octadecadienoic acid.[5] Only the familiar *cis*-9,*cis*-12 isomer (linoleic acid) has been synthesized.[6] Since more information in this area is highly desirable, we undertook to synthesize and characterize three closely related isomers, the *cis*-8,*cis*-11-octadecadienoic, *cis*-10,*cis*-13-octadecadienoic, and *cis*-11,*cis*-14-octadecadienoic acids (IX). The present paper reports our results.

Syntheses.—The same general approach served for all the syntheses. Each proceeded through a 17-carbon diacetylenic intermediate VII, which was half-reduced to the diethylenic analog VIII and then extended to the final 18-carbon acid IX. The 17-carbon intermediates VII were prepared by combining 1-bromo-2-alkynes (III) with chloroalkynes (VI). These starting materials were reached by brominating 1-hydroxy-2-alkynes (II) with phosphorus tribromide and by alkylating sodium acetylide with chloralkyl iodides (V). Combination of the fragments III and VI was effected by adding cuprous chloride as catalyst[7,8] to a mixture of the bromide III and the Grignard derivative of chloroalkyne VI. With ether as solvent, the yield of coupling product VII was as high as or even higher than with tetrahydrofuran. However, tetrahydrofuran was preferred, since this solvent shortened the reaction time fifteenfold.[9]



We planned to arrive at the final dienoic acids by way of the corresponding 18-carbon diynoic acids, which should be available by carbonating the Grignard reagents derived from the 17-carbon diacetylenic chlorides VII. Actually, in one experiment, carbonation of the Grignard reagent from 1-chloro-9,12-heptadecadiyne (VII, $m = 4$, $n = 8$) gave the desired 10,13-octadecadiynoic acid (40–50%). But this experiment could not be repeated; thereafter all attempts to prepare the Grignard derivative failed. Although this remarkable inertness to magnesium[10] was not observed with 1-iodo-9,12-heptadecadiyne, the main process with the iodo compound was Wurtz coupling. Since replacing the halogen in chloro compounds VII or in 1-iodo-9,12-heptadecadiyne with a nitrile group was also unsatisfactory,[11] this approach was abandoned.

We then turned to the VII–VIII–IX sequence and so to the half-reduction of the triple bonds of chlorides

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[3] *Cf.* H. J. Deuel, Jr., "The Lipids," Vol. III, Interscience Publishers, Inc., New York, N. Y., 1957.

[4] A. J. Fulco and J. F. Mead, *J. Biol. Chem.*, **235**, 3379 (1960).

[5] A. J. Fulco and J. F. Mead, *ibid.*, **234**, 1411 (1959); W. Stoffel and E. H. Ahrens, Jr., *J. Lipid Res.*, **1**, 139 (1960); J. F. Mead in K. Bloch's "Lipide Metabolism," John Wiley and Sons, Inc., New York, N. Y., 1960, p. 41.

[6] The most recent synthesis is by M. de Gaudemaris and P. Arnaud, *Bull. soc. chim. France*, 315 (1962).

[7] *Cf.* W. J. Gensler and G. R. Thomas, *J. Am. Chem. Soc.*, **73**, 4601 (1951).

[8] *Cf.* W. J. Gensler and A. P. Mahadevan, *ibid.*, **77**, 3076 (1955).

[9] See among others S. N. Eğe, R. Wolovsky, and W. J. Gensler, *ibid.*, **83**, 3080 (1961).

[10] Search of the literature revealed no examples of Grignard reagents prepared from chlorides containing acetylenic bonds. The unsuccessful attempts include trials with 1-chloro-6,9-pentadecadiyne [W. R. Taylor and F. M. Strong, *ibid.*, **72**, 4263 (1950)], 1-chloro-5-hexadecen-8-yne [L. Crombie and A. G. Jacklin, *J. Chem. Soc.*, 1632 (1957)], and 1-chloro-4,7,10,13-nonadecatetrayne [A. I. Rachlin, N. Wasyliw, and M. W. Goldberg, *J. Org. Chem.*, **26**, 2688 (1961)]. Propargyl chlorides such as 1,4-dichloro-2-butyne [A. W. Johnson, *J. Chem. Soc.*, 1009 (1946)], 1-chloro-1,1-dialkyl-2-alkyne [K. N. Campbell and L. T. Eby, *J. Am. Chem. Soc.*, **62**, 1798 (1940)], and 1-chloro-2-heptyne (even with the entrainment method) [M. S. Newman and J. H. Wotiz, *ibid.*, **71**, 1292 (1949)] also fail to react.

[11] Sodium cyanide in alcohol gave tars, while cuprous cyanide in xylene [W. D. Celmer and I. A. Solomons, *ibid.*, **75**, 3430 (1953)] or in acetonitrile [B. C. L. Weedon, *J. Chem. Soc.*, 4168 (1954)] gave only starting material.

VII. Neither hydrogenation over a palladium-on-calcium carbonate catalyst[12,13] nor reduction with a zinc–copper alloy in alcohol[14] proved suitable. Diisobutylaluminum hydride,[15] on the other hand, reacted smoothly with the diynes to give the desired chlorodienes VIII. Whether necessary or not, we followed the practice of routinely reducing each diacetylene twice so as to eliminate the possibility of finding unchanged triple bonds in the dienes VIII. This method was used satisfactorily for all the VII to VIII conversions.

The last step, conversion of the 17-carbon chlorodienes VIII to the octadecadienoic acids IX, proceeded without difficulty. In sharp contrast to the reaction with chlorodiyne VII, a mixture of ethyl bromide[16] and chlorodiene VIII reacted smoothly with magnesium. Carbonation of the Grignard reagent with gaseous carbon dioxide gave analytically pure acids IX in yields of 50–60% or better.

The acids IX were converted to stearic acid by catalytic hydrogenation so that any question of the presence of a branched chain could be dismissed. Each unsaturated acid had the double bonds in the assigned positions, since ozonolysis of the methyl esters led to the expected mono- and dicarboxylic acid cleavage fragments.[17] The infrared absorptions curves of acids IX and their methyl esters gave no indication of *trans*-ethylenic materials; estimates based on the absorption at 10.3 μ limited the *trans* content to considerably less than 1%. The ultraviolet absorption at 233 mμ showed that conjugated unsaturation was present at a concentration lower than 1%.[18] Gas–liquid chromatographic analyses of the methyl esters, by indicating homogeneities of 98–99%,[19,20] were consistent with the results of the ultraviolet and infrared analyses.

Discussion

With the exception of the 7,10 isomer, a series of skipped diunsaturated fatty acids from *cis*-6,*cis*-9- through *cis*-11,*cis*-14-octadecadienoic acid has now been reported. *In vivo* fatty acid transformations can occur by dehydrogenating unsaturated fatty acids between the point of unsaturation and the carboxyl group and by extending the chain at the carboxyl end.[4,5,21] Test of the generality of these processes by finding whether the new acids generate new families of skipped polyunsaturated acids is of considerable interest. The parent acids and their biosynthesized derivatives are also of importance in connection with essential fatty acid activity,[3,22] and even more so in the controversial area of the relation between polyunsaturated acids, blood cholesterol, and atherosclerosis.[3,23]

With several of the diunsaturated acids IX at hand, comparison of some of their properties became possible. Table VII shows that the boiling points are closely bunched and that the indices of refraction are very similar. A clean separation of the acids by fractional distillation or a clear-cut distinction based on index of refraction appears unlikely. The melting points fall lower than those of the monounsaturated *cis*-octadecenoic acids.[24] The 8,11 acid has the lowest melting point, the 11,14 acid the highest. Our data are not precise enough to prove or disprove a generalization that the melting points go up as the unsaturation moves away from the carboxyl group or to show whether the zig-zag pattern observed for the melting points of the monounsaturated acids[24] has its counterpart in the diunsaturated acids IX.

Bromine added to acids IX to give crystalline tetrabromides, whose melting points differ by no more than 7° (Table VIII). The tetrabromo derivatives from adjacent members of the series show little depression in their mixture melting points. A tentative generalization may be drawn: only non-adjacent members of the series of tetrabromides will give appreciable depressions and ranges in their mixture melting points. Accordingly, caution is indicated before relying on the sharpness of melting point of a derived tetrabromo derivative as an indication of the absence of adjacent isomers.

None of the methyl esters of compounds IX showed more than one peak on gas–liquid chromatographic analysis. Table IX shows that the retention times on a 5-ft. silicone column all lie between 28.2 and 29.4 min. (230°). A 5-ft. polyester packing permitted the esters to pass through more rapidly, retention times of 15.5–19 min. (200°) being observed. Differences of 3–4 min. should permit at least partial separation. Unexpectedly, the order of retention time on the polyester column was not regular, although the shortest time was observed for the 8,11 isomer and the longest time for the 11,14 isomer. Better separation was obtained with longer packed columns and especially with long capillary columns.[20]

Homogeneity measures based on gas–liquid chromatography of the ozonolysis products indicated a 3–5.7% content of positional isomers in the methyl esters of acids IX. These measures accordingly were not in line with the abovementioned gas–liquid chromatography results nor with estimates based on ultraviolet and infrared absorption. Actually, by the same kind of cleavage analysis, the methyl ester of Hormel "high purity" linoleic acid furnished dimethyl azeleate with no less than 9.7% in homogeneity. We feel that assays based on ozonolysis products are better regarded as minimum, instead of true, measures of homogeneity.[25]

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(12) H. Lindlar, *Helv. Chim. Acta*, **35**, 446 (1952).

(13) The degree of selectivity and stereospecificity in catalytic half-hydrogenation of triple to double bonds can vary. [*Cf.* N. A. Dobson, G. Elinton, M. Krishnamurti, R. A. Raphael, and R. G. Willis, *Tetrahedron*, **16**, 16 (1961).]

(14) B. S. Rabinowitz and F. S. Looney, *J. Am. Chem. Soc.*, **75**, 2652 (1953); A. J. Clarke and L. Crombie, *Chem. Ind.* (London), 143 (1957).

(15) G. Wilke and H. Müller, *Chem. Ber.*, **89**, 444 (1956); also *Ann.*, **629**, 222 (1960). Note that F. Bohlmann, E. Inhoffen, and J. Politt, *ibid.*, **604**, 207 (1957), using diethylaluminum hydride, failed to generate an ethylenic bond from an acetylenic bond, possibly because ether was used as solvent.

(16) M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Nonmetallic Substances," Prentice-Hall, Inc., New York, N. Y., 1954, pp. 38–45.

(17) J. G. Keppler, *Rec. trav. chim.*, **76**, 49 (1957).

(18) See R. T. Holman in "Methods of Biochemical Analysis," Vol. IV, D. Glick, ed., Interscience Publishers, Inc., New York, N. Y., 1957, p. 99.

(19) Personal communications from R. A. Landowne.

(20) R. A. Landowne and S. R. Lipsky, *Biochim. Biophys. Acta*, **46**, 1 (1961).

(21) *Cf.* E. Klenk and H. Debuch, *Ann. Rev. Biochem.*, **28**, 39 (1939).

(22) *Cf.* H. J. Thomasson, *Intern. Rev. Vitamin Research*, **25**, 62 (1953). Also note "Essential Fatty Acids," Proc. Intern. Conf. Biochem. Problems of Lipids, Oxford, 1957 [*Chem. Abstr.*, **53**, 17277 (1959)].

(23) Council Report, *J. Am. Med. Assoc.*, **181**, 411 (1962); J. Enselme, "Unsaturated Fatty Acids in Atherosclerosis," Pergamon Press, New York, N. Y., 1962.

(24) W. F. Huber, *J. Am. Chem. Soc.*, **73**, 2730 (1951).

(25) Other indications that the method of ozonolysis and analysis of the products is not free of complications have been found by Keppler,[17] R. R. Allen [*J. Org. Chem.*, **21**, 143 (1956)], F. L. Benton, A. A. Kiess, and H. J. Harwood [*J. Am. Oil Chemists' Soc.*, **36**, 457 (1959)], and E. Ucciani, J. Pasero, and M. Naudet [*Bull. soc. chim. France*, 1209 (1962)].

TABLE I

1-HYDROXY-2-ALKYNES (II)

$CHO_3(CH_2)_mC{\equiv}CCH_2OH$

m	Yield, %	B.p., °C.	$n^{25}D$		Calcd. C	Calcd. H	Found C	Found H
5^a	72	62–63 (2 mm.)	1.4541	$C_9H_{16}O$	77.10	11.50	77.1	11.5
$3^{b,c}$	82	80–81 (9 mm.)	1.4520					
2^c	77	73–74 (15–16 mm.)	1.4507^d	$C_6H_{10}O$	73.40	10.27	73.2	10.3

[a] Ch. Moureu and H. Desmots, *Bull. soc. chim.*, [3] **27,** 360 (1902). [b] W. J. Gensler and G. R. Thomas, *J. Am. Chem. Soc.*, **73,** 4601 (1951). [c] M. S. Newman and J. H. Wotiz, *ibid.*, **71,** 1292 (1949). [d] At 26°.

TABLE II

1-BROMO-2-ALKYNES (III)

$CH_3(CH_2)_mC{\equiv}CCH_2Br$

m	B.p., °C.	$n^{25}D$	Yield, %
5^a	67–68 (2.5 mm.)	1.4831	88
3^b	43–44 (4 mm.)	1.4878	82
2^c	76–78 (35 mm.)	1.4903	98

[a] *Anal.* Calcd. for $C_9H_{15}Br$: C, 53.20; H, 7.45; Br, 39.38. Found: C, 52.9; H, 7.3; Br, 39.7. [b] M. S. Newman and J. H. Wotiz, *J. Am. Chem. Soc.*, **71,** 1292 (1949), report b.p. 104–105° (56 mm.); W. J. Gensler and G. R. Thomas, *ibid.*, **73,** 4601 (1951), report b.p. 76–80° (15 mm.) and nD 1.491; J. H. Wotiz, *ibid.*, **72,** 1639 (1950), reports b.p. 54° (4 mm.) and n^{25}D 1.4844. [c] M. S. Newman and J. H. Wotiz, *ibid.*, **71,** 1292 (1949), report b.p. 97–98° (80 mm.) and n^{25}D 1.4884; J. H. Wotiz, *ibid.*, **72,** 1639 (1950), reports b.p. 38° (4 mm.) and n^{25}D 1.4886.

Experimental[26]

1-Bromo-2-alkynes (III).—Table I gives the results of combining appropriate acetylenic Grignard reagents with formaldehyde to form the intermediate propargyl alcohols (II).[27] Phosphorus tribromide with pyridine in catalytic quantities converted the propargyl alcohols (II) to 1-bromo-2-alkynes[28] (See Table II). One-mole quantities could be handled without difficulty.

Chloroalkynes (VI).—The chloroiodoalkanes (V) were prepared from the corresponding dichloroalkanes.[7,29] Table III summarizes the data. The directions used for alkylating sodium acetylide with the chloroiodoalkanes to give chloroalkynes VI are an adaption and modification of a procedure reported before for 9-chloro-1-nonyne.[30] The results in Table IV were obtained when sodium acetylide from 0.66 mole of sodamide was allowed to react with 0.26 mole of chloroiodoalkane in 100 ml. of ether plus 1.2 l. of liquid ammonia.

1-Chloroheptadecadiynes (VII).—The three diynes (VII) were obtained from the appropriate 1-bromo-2-alkyne (III) and chloroalkyne (VI) by following essentially the same procedure, which is given below for 1-chloro-7,10-heptadecadiyne (VII, $m = 5$, $n = 6$).

The reaction was carried out under dry nitrogen in a carefully dried three-necked flask equipped with dropping funnel and magnetic stirring bar. The tetrahydrofuran solvent was purified by treatment with potassium hydroxide and then distillation from lithium aluminum hydride. A solution of ethyl Grignard reagent was formed by dropping 20 ml. (23.0 g.; 0.211 mole) of dry ethyl bromide in 50 ml. of tetrahydrofuran into a stirred mixture of dry magnesium shavings (5.07 g.; 0.211 g.-atom; Dow sublimed metal) and 150 ml. of tetrahydrofuran. Bringing the mixture initially to a boil started the reaction, which then proceeded without external heating during the remainder of the addition. After another hour of boiling, the Grignard solution was treated with a solution of 29.25 g. (0.201 mole) of 8-chloro-1-octyne (VI, $n = 6$) in 50 ml. of tetrahydrofuran. The dropwise addition required 0.5 hr. The spontaneously boiling mixture evolved a gas. The reaction mixture was boiled for an additional hour and then allowed to stand overnight under nitrogen.

Dry cuprous chloride (0.7 g.) was added, and the mixture was boiled for 1 hr. 1-Bromo-2-nonyne (III, $m = 5$; 40.8 g.; 0.201 mole) in 50 ml. of tetrahydrofuran was added to the stirred solution, which was then boiled for 2–4 hr. Approximately 88% of the initial Grignard content was consumed in the first hour, 94% after 2 hr.

The reaction mixture, containing a heavy, bright green precipitate, was poured over 500 g. of crushed ice plus 50 ml. of concentrated sulfuric acid. The aqueous layer was extracted with several 100-ml. portions of ether. The combined organic phases were washed with water until the washings were neutral to litmus and then dried with magnesium sulfate. Removal of solvent left the oily product, which was fractionated through a 5-cm. Vigreux column. The water-white 1-chloro-7,10-heptadecadiyne (VII) was distributed in ampoules, which were sealed without releasing the vacuum. In some preparations the distilled material contained small amounts of terminal acetylenic impurities as shown by a small absorption peak at 3.04 μ. Such impurities were readily precipitated by shaking the diyne (10 g.) with a saturated methanolic solution (50 ml.) of silver nitrate. Table V presents the data for the 1-chloroheptadecadiynes.

The infrared absorption spectra of the three compounds, taken as neat layers, were practically identical. The major absorption peaks occur at 4.35 (vw), 4.42 (vw), 4.48 (vw), 6.8 (s), 7.61 (s), 13.8, and 15.35 μ (s). No peaks were noted at 3 μ (acetylenic hydrogen) or at 5.1 μ (allenic unsaturation).

1-Chloroheptadecadienes (VIII) by Diisobutylaluminum Hydride Reduction of 1-Chloroheptadecadiynes (VII).—The reagent was prepared[31] by boiling 160 ml. of a 25% solution of triisobutylaluminum in heptane for 2–3 hr. Distillation afforded 27 ml. of colorless diisobutylaluminum hydride, b.p. 80–90° (0.05 mm.) The recommended precautions for handling organoaluminum compounds were observed.[15,32]

The reduction of 1-chloro-7,10-heptadecadiyne is given here as illustrative. The diyne (13.76 g.; 0.052 mole) was placed in a 300-ml. three-necked flask fitted with a mercury-sealed stirrer, a vertical condenser, and a dropping funnel. Dry, oxygen-free nitrogen, introduced through the top of the condenser under a slight positive pressure, blanketted the reagent and the reaction mixture at all times. Approximately 21 g. (0.15 mole) of diisobutylaluminum hydride was added over a period of 1 hr. to the stirred mixture at 0°. After residual reagent was rinsed into the flask with a small volume of dry heptane, stirring was continued at room temperature for 12–15 hr.

The condenser was replaced with a tube leading to a trap held at Dry Ice–acetone temperature. With the stirred reaction mixture in a bath at −5°, 60 ml. of a methanol–petroleum ether (b.p. 30–60°) solution (2:3) was carefully added in 1 hr. Then just enough ice-cold 20% sulfuric acid was slowly added to dissolve the precipitated aluminum methoxide. After the condensate in the trap was transferred to the acidified mixture with 50 ml. of ether, the aqueous layer was separated and extracted twice with ether. The combined organic layers were washed in succession with water, saturated sodium bicarbonate solution, and again with water until the washings were neutral to litmus. The solution was dried with magnesium sulfate and then warmed on the steam bath under water-pump vacuum to remove all solvent.

To eliminate all possibility of acetylenic impurities, the oil was treated routinely with a second portion of reagent in a repetition of the above procedure.

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[26] Analyses were performed by Carol K. Fitz, Needham Heights, Mass.

[27] *Cf.* Taylor and Strong, *J. Am. Chem. Soc.*, **72,** 4263 (1950); Tchao Yin Lai, *Bull. soc. chim.*, [4] **53,** 682 (1933); R. A. Raphael and F. Sondheimer, *J. Chem. Soc.*, 2100 (1950).

[28] M. S. Newman and J. H. Wotiz, *J. Am. Chem. Soc.*, **71,** 1292 (1949). *Cf.* Taylor and Strong[27] as well as Tchao Yin Lai, ref. 27 and *Bull. soc. chim.*, [4] **53,** 1533 (1933).

[29] *Cf.* R. A. Raphael and F. Sondheimer.[27]

[30] W. J. Gensler and C. B. Abrahams, *J. Am. Chem. Soc.*, **80,** 4593 (1958).

[31] K. Ziegler, *Chem. Abstr.*, **51,** 15081 (1957) [British Patent 778,098 (1957)]; K. Ziegler, H. G. Geller, H. Lehmkuhl, W. Pfohl, and K. Zosel, *Ann.*, **629,** 1 (1960).

[32] *Cf.* "Handling and Properties of Triisobutylaluminum," Hercules Powder Co., Wilmington, Del.

TABLE III

CHLOROIODOALKANES (V)

$I(CH_2)_nCl$

n	B.p., °C.	$n^{25}D$		Calcd. C	Calcd. H	Calcd. I	Found C	Found H	Found I
6[a]	55.5–57 (0.25 mm.)	1.5176							
8[b]	85–89 (0.25 mm.)	1.5089	$C_8H_{16}ClI$	35.00	5.88	46.20	35.0	5.8	46.4
9[c]	96–98 (0.25 mm.)	1.5066	$C_9H_{18}ClI$	37.50	6.28	43.95	37.7	6.3	43.8

[a] W. J. Gensler and G. R. Thomas, *J. Am. Chem. Soc.*, **73**, 4601 (1951), report b.p. 112–116° (12 mm.) and $n^{25}D$ 1.5220; R. A. Raphael and F. Sondheimer, *J. Chem. Soc.*, 2100 (1950), report b.p. 73–74° (0.7 mm.) and $n^{24}D$ 1.5248; W. F. Huber, *J. Am. Chem. Soc.*, **73**, 2730 (1951), reports b.p. 96–98° (6 mm.) and $n^{25}D$ 1.5214. [b] W. F. Huber, *ibid.*, **73**, 2730 (1951), b.p. 101–105° (2.5 mm.), and $n^{25}D$ 1.5113. [c] K. Ahmad, F. M. Bumpus, and F. M. Strong, *ibid.*, **70**, 3391 (1948), report b.p. 123–124° (2.8–2.9 mm.) and $n^{25}D$ 1.5060; W. F. Huber, *ibid.*, **73**, 2730 (1951), reports b.p. 123–126° (4 mm.) and $n^{25}D$ 1.5074.

TABLE IV

CHLOROALKYNES (VI)

$HC{\equiv}C(CH_2)_nCl$

n	Yield, %	B.p., °C.	$n^{25}D$		Calcd. C	Calcd. H	Calcd. Cl	Found C	Found H	Found Cl
6[a]	77	40–42 (2.5 mm.)	1.4507	$C_8H_{13}Cl$	66.40	9.06	24.50	66.3	9.1	24.3
8	88	56–59 (2.5 mm.)	1.4528	$C_{10}H_{17}Cl$	69.60	9.93	20.55	69.6	9.8	21.4
9	80	57–58 (0.15–0.2 mm.)	1.4538	$C_{11}H_{19}Cl$	70.80	10.25		70.9	10.5	

[a] R. A. Raphael and F. Sondheimer, *J. Chem. Soc.*, 2100 (1950), report b.p. 73–76° (10 mm.) and $n^{12}D$ 1.4590; W. J. Gensler and G. R. Thomas, *J. Am. Chem. Soc.*, **73**, 4601 (1951), report b.p. 73–76° (11 mm.) and $n^{25}D$ 1.4548.

TABLE V

1-CHLOROHEPTADECADIYNES (VII)

$CH_3(CH_2)_mC{\equiv}CCH_2C{\equiv}C(CH_2)_nCl$

m	n	B.p., °C.	$n^{25}D$	Yield, %	Found[a] C	Found[a] H	Found[a] Cl
5	6	103–107 (0.5 × 10^{-3} mm.)	1.4784	70	76.6	10.2	13.5
3	8	106–109 (1 × 10^{-3} mm.)	1.4779	68[b]	76.6	10.3	13.1
2	9	101–111 (0.5 × 10^{-3} mm.)	1.4780	62	76.7	10.3	13.2

[a] Calcd. for $C_{17}H_{27}Cl$: C, 76.51; H, 10.20; Cl, 13.29. [b] With a 96-hr. reaction period in ether solvent, the yield was 81%.

The light yellow product (13.0 g.) dissolved in petroleum ether (b.p. 30–60°) was placed on a column of 130 g. of 28–200-mesh silica gel (Davison Chemical Co.). The first 250 ml. of eluate (petroleum ether solvent) contained 0.4 g. of a fatty solid which was discarded. The next 500 ml. of eluate (petroleum ether–ether solvent, 4:1) furnished 11.4 g. (82%) of pale yellow, solvent-free product. Distillation afforded colorless to faintly yellow 1-chloro-7,10-heptadecadiene (VIII), which was collected and stored *in vacuo* in sealed vials.

The 9,12 and 10,13 isomers were prepared in a similar way as colorless oils. The three isomers all showed very similar infrared absorption curves, with peaks evident at 3.32 (m), 6.05 (w), 7.15 (m), 7.26 (m), 7.64 (m), 7.8 (m), 10.3 (vw), 11.0 (m), 13.8 (s), and 15.3 (s) μ. The absorption curve of the 7,10 isomer was taken before as well as after distillation. The close similarity in the two curves suggested that the chromatographed material was practically pure, and that distillation (presumably with the other isomers as well), with its accompanying losses, may have been unnecessary.

Table VI presents the data.

TABLE VI

cis,cis-CHLOROHEPTADECADIENES (VIII)

$CH_3(CH_2)_mCH{=}CHCH_2CH{=}CH(CH_2)_nCl$

m	n	B.p., °C.	$n^{25}D$	Yield, % a	Yield, % b	Found[c] C	Found[c] H	Found[c] Cl
5	6	87–91 (1 × 10^{-4} mm.)	1.4683	65	82			
3	8	87–89 (1 × 10^{-4} mm.)	1.4678	83	94	75.8	11.6	12.7
2	9	75–77 (0.5 × 10^{-4} mm.)	1.4678	70	87	75.3	11.5	

[a] After distillation. [b] Before distillation. [c] Calcd. for $C_{17}H_{31}Cl$: C, 75.37; H, 11.54; Cl, 13.09.

***cis,cis*-Octadecadienoic Acids (IX).**—The appropriate 1-chloroheptadecadiene was converted to its Grignard reagent and then carbonated. The procedure, the same for the three acids, is given below for 8,11-octadecadienoic acid (IX, $m = 5, n = 6$).

A 250-ml. three-necked flask was fitted with a vertical condenser. Dry, oxygen-free nitrogen, which was supplied under a slight positive pressure through the top of the condenser, blanketted the reaction during the entire experiment. All glassware was carefully dried. Turnings of Dow sublimed magnesium (2.28 g. or 0.094 g.-atom) were dried briefly in the flask by brushing the flask with a soft flame. A mixture of 1-chloro-7,10-heptadecadiene (VIII, $m = 5$, $n = 6$) (5.00 g.; 0.0184 mole) and carefully dried ethyl bromide (6.05 g.; 0.0555 mole) was placed in a dropping funnel and was diluted with 45 ml. of ether distilled directly into the dropping funnel from lithium aluminum hydride. Approximately 80 ml. of ether, distilled directly into the flask, covered the magnesium. The ethereal halide solution was added to the boiling, magnetically stirred mixture over a period of 2 hr. After the addition, the mixture was boiled for 18 hr.

Gaseous carbon dioxide, after passing through a tower of concentrated sulfuric acid and then through an empty flask, was introduced into the cooled mixture through a tube reaching almost to the bottom of the flask. The inside temperature, initially −60°, climbed to −5° and then rapidly dropped to −60°. Solidification was noted.

Enough cold 10% sulfuric acid was added to dissolve the excess magnesium. The aqueous layer was separated and extracted with several portions of ether. The combined ether layers were shaken with several portions of water and then dried with magnesium sulfate. All material volatile below 50–60° (*ca.* 0.5 mm.) was removed, and the yellow residual oil (5.07 g.), diluted with an equal volume of light petroleum ether, was placed on a 2.5 × 33 cm. column of 28–200-mesh silica gel (100 g.). Passing 300 ml. of petroleum ether through the column removed 0.3 g. of material, which was discarded. The solvent was changed to petroleum ether–ether (4:1, v./v.), and 500 ml. was collected. Removal of solvent left 4.23 g. of 8,11-octadecadienoic acid, which was distilled through a 5-cm. vacuum-jacketed Vigreux column. The practically colorless main fraction was distributed in several small ampoules, which were sealed without breaking the vacuum.

Melting points were determined by observing 0.1–0.2 g. of sealed solidified material as it was allowed to warm slowly in a bath. The infrared absorption curves of the acids IX, taken with neat samples, were all very similar and corresponded closely to the curve for "high purity" linoleic acid (Hormel). All showed absorption peaks at 3.76 (m), 5.85 (s), 6.04 (w, sh), 7.17 (m), 7.26 (m), 7.8 (s), 8.1 (m), 10.7 (s), and 13.85 (s) μ. Ultraviolet absorption curves were determined with *ca.* 1.8 × 10^{-3} M methanolic solutions of the acids. The extinction at the 233 mμ maximum (log ϵ 2.2–2.5) served as a measure of the amount of conjugated impurities.[18] Neutralization equivalents were determined by titration of the acids in 95% alcohol under nitrogen with 0.05 N aqueous sodium hydroxide to a phenolphthalein end point. The values were slightly high (0.43–0.90%); but pure stearic acid by the same procedure also gave high results (0.18–

TABLE VII

cis,cis-OCTADECADIENOIC ACID (IX)

$CH_3(CH_2)_mCH{=}CHCH_2CH{=}CH(CH_2)_nCOOH$

Isomer	m	n	B.p., °C. (10^{-4} mm.)	M.p., °C. (approx.)	$n^{25}D$	Yield,[a] %	Conjugation, %	Found[b] C	Found[b] H	Neut.[c] equiv.	Iodine[d] no.
$\Delta^{8,11}$	5	6	118–120	−12.5 to −9.5	1.4663	58	0.62	77.1	11.5	283	185, 186
$\Delta^{9,12}$	4	7		−5.1 to −5.4[e]	1.4690[e]		0.45				185, 185 184[f]
$\Delta^{10,13}$	3	8	121–128	−9 to −6.5	1.4670	51	0.87	77.2	11.5	282	184
$\Delta^{11,14}$	2	9	112–115	6.0 to 8.0	1.4664	60	0.73	77.3	11.5	282	185

[a] The figures refer to distilled material. The chromatographed acids were obtained in 20–30% higher yield. Much of the decreased yield of distilled material is due to mechanical losses. [b] Calcd. for $C_{13}H_{32}O_2$: C, 77.09; H, 11.50. [c] Molecular weight: 280.44. [d] Theoretical iodine number: 181. [e] Reported by B. Sreenivasan, J. B. Brown, E. P. Jones, V. L. Davidson, and J. Nowakowska, *J. Am. Oil Chemists' Soc.*, **39**, 255 (1962). The index of refraction refers to 20°. Synthetic linoleic acid was reported recently with b.p. 115–120° (0.001 mm.), m.p. −5°, and $n^{22}D$ 1.4670 [M. de Gaudemaris and P. Arnaud, *Bull. soc. chim. France*, 315 (1962)]. [f] The Wijs iodine number of this Hormel "high purity" linoleic acid was found by the supplier to be 181, the theoretical value.

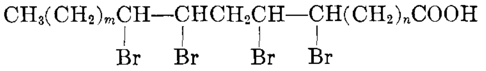
0.7%). Iodine numbers were obtained by the Kaufmann method.[33] Table VII summarizes the results.

Hydrogenation of *cis,cis*-Octadecadienoic Acids (IX) to Stearic Acid.—A mixture of the unsaturated acid (*ca.* 0.1 g.), platinum oxide (*ca.* 0.06 g.), and 95% alcohol (20 ml.) was magnetically stirred at room temperature in an atmosphere of hydrogen until absorption of hydrogen ceased. After filtration, the solution was concentrated under reduced pressures to a volume of 2–3 ml. Cooling the concentrated solution gave white crystals, which were collected and dried *in vacuo*. Stearic acid, melting within the range 68–70°, was obtained in better than 97% yield from each of the three acids IX. Mixtures of the hydrogenation stearic acids with authentic stearic acid (m.p. 69.5–70°) melted at 68–70°.

Tetrabromostearic Acids from Skipped Octadecadienoic Acids (IX).—Bromine was added dropwise to a stirred solution of 0.1–0.5 g. of unsaturated acid in 6–7 ml. of low boiling petroleum ether at temperatures held below 0°. When the yellow color persisted, the precipitated solids were collected and washed on the funnel with cold petroleum ether. The compounds were recrystallized two or three times from methylene chloride–pentane or methylene chloride–cyclohexane and then dried *in vacuo* at 56°. Table VIII summarizes the results. From the reported melting point of linoleic acid tetrabromide (m.p. 113.2–113.8°[34]; 115.4–115.5°[5]), we judge that the melting points in Table VIII might be slightly low.

TABLE VIII

TETRABROMO DERIVATIVES OF OCTADECADIENOIC ACIDS (IX)



$$CH_3(CH_2)_m\underset{Br}{\underset{|}{C}}H{-}\underset{Br}{\underset{|}{C}}HCH_2\underset{Br}{\underset{|}{C}}H{-}\underset{Br}{\underset{|}{C}}H(CH_2)_nCOOH$$

Starting isomer	m	n	Yield, %	M.p.,[a] °C.	Found[b] C	Found[b] H	Found[b] Br
$\Delta^{8,11}$	5	6	20	106–107	35.9	5.2	
$\Delta^{9,12c}$	4	7	35	111.5–112.5	36.3	5.1	53.3
$\Delta^{10,13}$	3	8	22	111–111.5	36.1	5.1	53.4
$\Delta^{11,14}$	2	9	26	112–112.5	36.2	5.2	53.5

[a] The mixture melting points are as follows: [8,11 + 9,12] 106–107°; [9,12 + 10,13] 107.5–112.5°; [9,12 + 11,14] 94–98.5°; [10,13 + 11,14] 108–111°. [b] Calcd. for $C_{18}H_{32}Br_4O_2$: C, 36.02; H, 5.38; Br, 53.26. [c] Hormel "high purity" linoleic acid.

Methyl *cis,cis*-Octadecadienoates.—The acids IX were esterified by treating 0.1–0.2 g. samples in 10–15 ml. of dry ether with distilled ethereal diazomethane until a yellow color persisted. An atmosphere of pure nitrogen was maintained in the flask. After filtration through thin layers of a filter aid (Celite), anhydrous magnesium sulfate, and activated charcoal, the solution was warmed in a stream of nitrogen to remove solvent. The residual colorless esters in several vials were exposed without delay to a 0.01-mm. vacuum. The vials, sealed without breaking the vacuum, were stored in the cold away from light.

Infrared absorption curves of the esters were taken with 0.04-mm. thicknesses of the neat oils. All showed maxima at 3.33 (m), 3.49 (m), 6.05 (w), 7.15 (m), 7.26 (m), 7.34 (m), 7.58 (w), 8.05 (m), 8.36 (s), 8.55 (s), 9.85 (m), 10.1 (w), 10.98 (w), and 13.85 (m) μ. The minor differences in the 8–9- and 11–12.5-μ region offer little encouragement in the use of infrared to distinguish the several esters. Except for these differences, all the esters including methyl linoleate showed practically the same curves. The curves showed no characteristic *trans* double bond absorption at 10.3 μ. Since a distinct shoulder was observed here when a test mixture of methyl linoleate containing 1% of methyl *trans*-11-octadecenoate was scanned under the same conditions, the content of *trans* double bonds in the synthetic compounds was substantially less than 1%.

Gas–Liquid Chromatographic Analysis of the Octadecadienoic Acids IX and Esters.—Table IX gives the results of gas–liquid chromatography with the methyl octadecadienoates. Only one peak appeared for each ester. The retention times for the esters on a column with a polyester stationary phase show significant differences, but the trend of retention time with the position of the skipped unsaturation proved not to be regular.

TABLE IX

GAS–LIQUID CHROMATOGRAPHIC ANALYSIS[a] OF THE METHYL ESTERS OF OCTADECADIENOIC ACIDS (IX)

$CH_3(CH_2)_mCH{=}CHCH_2CH{=}CH(CH_2)_nCOOCH_3$

Isomer	m	n	Silicone column Temp., °C.	Silicone column Retention time, min.	Polyester column Temp., °C.	Polyester column Retention time, min.
$\Delta^{8,11}$	5	6	231	29.4	201	15.5
$\Delta^{9,12b}$	4	7	230	29.0	201	18.3
$\Delta^{10,13}$	3	8	231	28.2	201	15.6
$\Delta^{11,14}$	2	9	235	28.6	201	19.1

[a] An "Aerograph" unit with catherometer detector was used with two 5-ft. columns, one packed with silicone (sample size, 10 μl.) and the second with a polyester ("LAC-446"; sample size, 1–3 μl.). The carrier gas was helium at a constant flow rate (rotameter reading, 85.0 mm.). [b] Methyl ester of Hormel "high purity" linoleic acid.

Longer packed columns and, even better, capillary columns gave more effective separation. These results are fully documented elsewhere.[20] With the more efficient columns, the four methyl esters showed 98–99% homogeneity. The maximum single impurity was observed in the methyl linoleate.[19,20]

After approximately 6 months of storage at −5° *in vacuo* away from light, the acids themselves were examined. The analyses were performed at the Unilever Research Laboratory (Vlaardingen) through the courtesy of Dr. R. K. Beerthuis. A column (120 cm. long; 0.4-cm. diameter) packed with 10% Apiezon L on diatomaceous earth (Celite) was used. At a flow rate of 20 ml. per min. of argon and at a column temperature of 182° the retention times of the 18-carbon acids were just over 2 hr. The column gave only partial separation of the 8,11 isomer (with the shortest retention time) from the other acids. Homogeneity estimates were made as follows: for the 8,11-acid, *ca.* 94%; for the 10,13 acid, *ca.* 100%; for the 11,14 acid, *ca.* 96%. Whether the appearance of inhomogeneity in the 8,11 and 11,14 isomer is

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[33] N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," 2nd ed., Interscience Publishers, Inc., New York, N. Y., 1957, p. 736.

[34] H. M. Walborsky, R. H. Davis, and D. R. Howton, *J. Am. Chem. Soc.*, **73**, 2590 (1951).

TABLE X

GAS-LIQUID CHROMATOGRAPHIC ANALYSIS[a] OF THE OZONOLYSIS METHYL ESTERS DERIVED FROM OCTADECADIENOIC ACIDS

Octadecadienoic acid IX			Retention time, min.						
			Column temperature, 100°			Column temperature, 190°			
Isomer	*m*	*n*	Reference methyl ester		Cleavage ester	Reference methyl ester		Cleavage ester	Impurity, %
$\Delta^{8,11}$	5	6	Heptanoate	4.28	4.32	Octanedioate	7.50	7.25	2.9
$\Delta^{9,12}$[b]	4	7	Hexanoate	2.60	2.55	Nonanedioate	9.4	8.5[c]	9.7
$\Delta^{10,13}$	3	8	Pentanoate	1.6	1.6	Decanedioate	13.2	11.8[c]	5.7
$\Delta^{11,14}$	2	9	Butanoate	0.91	0.92	Undecanedioate	17.7	17.4	3.8
						Malonate	1.4		

[a] An "Aerograph" unit with a catherometer detector was used. Samples (2–3 μl.) were injected into a 5-ft. packed helical column containing a polyester stationary phase ("LAC-446"). Helium was used at a constant flow rate (rotameter reading, 85.0 mm.). [b] Methyl ester of Hormel "high purity" linoleic acid. [c] Column temperature: 195°.

due to deterioration on storage, or to partial isomerization of the acids[35] during the 2-hr. period on the column is not known.

Cleavage Analysis of Skipped Unsaturated Acids IX.—The procedure given below for the ozonolysis and analysis of methyl *cis*-10,*cis*-13-octadecadienoate was followed for all the methyl esters.

Ozonized oxygen was passed through a solution of 0.2 g. of the methyl ester of 10,13-octadecadienoic acid (IX, $m = 3$, $n = 8$) in 10 ml. of pure chloroform in a bath at $-18°$. The gas flow was interrupted when the emergent gases developed a brown color in an aqueous potassium iodide solution, and the reaction mixture was then allowed to stand at room temperature for 0.5 hr.

Solvent was removed at 30° by distillation *in vacuo*, and the residual oil was treated with a slurry of freshly prepared silver oxide (0.9 g.), 10 ml. of water, and 1.6 ml. of 10% aqueous sodium hydroxide in several portions. The mixture was stirred vigorously and heated at 90–95° during and for 1 hr. after the addition.[17] Hydrochloric acid (20%) was added to pH 2. The mixture was extracted several times with ether, and the combined extracts, after one rinsing with water, were dried with magnesium sulfate. Treatment of the solution with diazomethane, as described above for the preparation of the methyl octadecadienoates, esterified all free carboxylic acid groups. When volatile material was removed at steam temperatures under a moderately reduced pressure, 0.2 g. of residue consisting largely of methyl valerate and dimethyl sebacate remained. Yields ranging from 80–95% were obtained at this point. No dimethyl malonate was recovered.

Table X summarizes the results of gas-liquid chromatographic analysis of the mixture. One or more minor peaks revealed the presence of lower, homologous diesters. The diester tracings were used to obtain the percentage impurities listed in Table X. Whether the values obtained in this way in fact reflect the degree of inhomogeneity in the 18-carbon acids, or whether the values are artifacts or the consequence of the degradation of initially homogeneous cleavage products[25] was not determined.

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[35] The methyl esters of polyunsaturated acids suffer no significant change during gas-liquid chromatography at 197° with Apiezon M as the stationary phase [W. Stoffel, W. Insul, Jr., and E. H. Ahrens, Jr., *Chem. Abstr.*, **53**, 3973 (1959); *Proc. Soc. Exptl. Biol. Med.*, **99**, 238 (1958)].

Yerba Buena. II. The Identification of Micromerol as Ursolic Acid[1]

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It has been shown that micromerol is mainly ursolic acid, mixed, in some cases, with smaller amounts of oleanolic acid. The three-dimensional crystal structure of methylmicromerol bromoacetate (methyl ursolate bromoacetate) has been determined in the course of this study, providing an independent confirmation of the stereochemistry of the α-amyrin system.

Among the numerous products isolated by Power and Salway[2] from *yerba buena* (*Satureia douglassii*) were two colorless alcohols to which were assigned the formulas $C_{33}H_{52}O_3 \cdot 2H_2O$ and $C_{30}H_{46}O_4 \cdot 2H_2O$ and the names micromerol and micromeritol. Of these, micromerol was present in significantly greater quantities. Although the proposed formulas were not in agreement, the properties described for these compounds suggested strongly that they were triterpene carboxylic acids.

In the course of our isolation of xanthomicrol[1] we obtained by cooling the crude, concentrated ethereal extract of *yerba buena* a copious greenish white precipitate. This showed on thin-layer chromatography (t.l.c.)[3] two major components having the color reactions of triterpenes.[4] The less polar material was present in considerably larger amounts and proved to be a colorless hydroxy acid, whose properties were in agreement with those reported for micromerol. Our sample and its derivatives, however, gave analyses compatible with the formula $C_{30}H_{48}O_3$, *i.e.*, a mono hydroxylated triterpene acid.

Methylation of micromerol with diazomethane gave a methyl ester which showed the same melting points for the hydrated and dried forms as reported by Powers. Since the ester appeared to be rather more easily crystallized and purified than micromerol itself, most of our material was isolated in this form by chromatography

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[1] Previous paper, G. H. Stout and V. F. Stout, *Tetrahedron*, **14**, 296 (1961).

[2] F. B. Power and A. H. Salway, *J. Am. Chem. Soc.*, **30**, 251 (1908).

[3] We wish to thank Mr. Erich Gauglitz, U. S. Bureau of Commercial Fisheries, Seattle, for first pointing out to us the striking advantages of this technique.

[4] C. R. Noller, R. A. Smith, G. H. Harris, and J. W. Walker, *J. Am. Chem. Soc.*, **64**, 3047 (1942).