tion and the yellow product recovered by extraction with ether. It afforded 0.082 g. (24%) of Ia, m.p. 145–147°, still slightly yellow after three recrystallizations from ethyl acetate.

In the same manner, 0.721 g. (2 mmoles) of IIIb yielded 0.316 g. (46%) of the fairly pure but yellow IIa, m.p. $147-150^{\circ}$.

 20α -Dimethylamino- 5α -pregnan- 3β -ol Tosylate (Id) and 3α -Azido-20 α -dimethylamino-5 α -pregnane (IVb).--A solution of 3.5 g. of recrystallized p-toluenesulfonyl chloride in 10 ml. of anhydrous pyridine was added in portions with cooling to 1.74 g. (5 mmoles) of Ib in 30 ml. of the same solvent. The solution immediately turned yellow, then light red. Some solid also formed. After having been kept at 0° for 13 days, the reaction mixture was poured into cold dilute sodium bicarbonate solution containing crushed ice and was then extracted with a mixture of benzene and petroleum ether (b.p. 35-60°). The combined extracts, after having been dried over anhydrous sodium sulfate, were evaporated under reduced pressure at room temperature, giving 2.10 g. of slightly yellow residue. The pure tosylate was isolated and characterized in another run. It crystallized from acetone in needles, m.p. $151-151.5^{\circ}$, $[\alpha]D$ $+11^{\circ} (c \ 1.00).$

Anal. Caled. for $C_{30}H_{47}NO_3S$ (501.76): C, 71.81; H, 9.44; N, 2.79; S, 6.39. Found: C, 72.04; H, 9.57; N, 2.67; S, 6.17.

A mixture of the crude tosylate (2.10 g.) and 4.45 g. of sodium azide in 70 ml. of dimethyl sulfoxide was heated in an oil bath at 95–100° with stirring for 6 hr. The mixture was then poured into ice water and extracted with petroleum ether. The extracts were evaporated *in vacuo* and the residue crystallized from acetone, giving 1.00 g. of the azide IVb as needles, m.p. 153–155°. An additional 0.15 g. was obtained from the mother liquor making the total yield 62%. The purified product melted at 156–158°, $[\alpha]D + 17° (c 0.83)$.

Anal. Calcd. for $C_{23}H_{40}N_4$ (372.58): C, 74.14; H, 10.82; N, 15.04. Found: C, 73.99; H, 11.04; N, 14.78.

20 β -Dimethylamino-5 α -pregnan-3 β -ol Tosylate (IId) and 3α -Azido-20 β -dimethylamino-5 α -pregnane (IVd).—To a solution of 4.40 g. of recrystallized *p*-toluenesulfonyl chloride in 30 ml. of anhydrous pyridine was added 1.95 g. (5.6 mmoles) of IIb with cooling. The resulting solution was allowed to stand at 0° for

5 days. No solid was formed during this period, but the solution became red. The reaction product was isolated as described for Id to yield 2.50 g. (89%) of slightly yellow tosylate, m.p. 166°. The pure tosylate formed needles (from acetone), m.p. 166-167°, $[\alpha] p \ 0^{\circ} (c \ 1.01)$.

Anal. Calcd. for $C_{30}H_{47}NO_3S$ (501.76): C, 71.81; H, 9.44; N, 2.79; S, 6.39. Found: C, 71.78; H, 9.65; N, 2.78; S, 6.45.

The azide IVd was prepared in the same manner as IVd; yield, 77%. After recrystallization it formed plates (from acetone), m.p. 139-141°, $[\alpha]D$ +8° (c 1.02).

Anal. Calcd. for $C_{23}H_{40}N_4$ (372.58): C, 74.14; H, 10.82; N, 15.04. Found: C, 74.35; H, 10.98; N, 15.24.

 3α -Amino-20 α -dimethylamino- 5α -pregnane (IVa) and 3α -Amino-20 β -dimethylamino- 5α -pregnane (IVc).—A solution of 0.65 g. of the azide IVb in 150 ml. of anhydrous ether was reduced with 0.9 g. of lithium aluminum hydride. Nitrogen gas evolved immediately on addition of the hydride. The reaction mixture was stirred at room temperature overnight. After the excess of hydride had been consumed by slow addition of water, the mixture was treated with concentrated sodium hydroxide solution and extracted with ether. The combined extracts were washed with water and dried over potassium carbonate and evaporated. The residue crystallized from ethyl acetate to yield 0.52 g. (86%) of the desired amine IVa, m.p. 148–150°. After further recrystallization from the same solvent it melted at 153–153.5°, [α]D +29° (c 1.00). When mixed with the 3 β epimer (Ia) this melted at 120–125°.

The azide IVd (1.12 g. 3 mmoles), on reduction with an excess of lithium aluminum hydride in a similar manner, afforded 0.719 g. (69%) of IVc, m.p. 161-163°. The analytical sample melted at 163-164°, $[\alpha]_D$ +17° (c 1.00). The mixture melting point with IIa was depressed to below 130°.

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A New Type of Naturally Occurring Polyunsaturated Fatty Acid

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The seed oil of Crepis foetida L., a member of the plant family Compositae, contains 60% of a fatty acid that has been shown to be cis-9-octadecen-12-ynoic acid. For convenience it is called crepenynic acid. This nonconjugated polyunsaturated acid is the first known member of a new class of naturally occurring acetylenic fatty acids, analogous to linoleic acid in containing methylene-interrupted unsaturation. The new compound may find considerable importance in mechanistic studies of fatty acid biosynthesis and of fatty acid metabolism. Crepenynic acid readily autoxidizes on standing. Two derivatives have been synthesized, cis-9,10-epoxyoctadec-12-ynoic and three-9,10-dihydroxyoctadec-12-ynoic acids.

During analytical investigation of the seed oil of *Crepis foetida* L., family Compositae, by procedures conventionally applied to seed oils,² it became apparent that the oil must contain compound(s) of novel structure. The presence of 88% of linolenic acid in the oil was indicated by an analysis based on conjugation developed after isomerization in alkali and measured by ultraviolet absorption.³ However, gas-liquid chromatographic (g.l.c.) analyses of fatty acid methyl esters derived from the oil revealed no linolenic acid, but showed 60% of a component that had retention

characteristics unlike those of any of the common naturally occurring fatty acids. Many of the observed properties could be rationalized by assuming a nonconjugated enynic structure for this new acid component, but precedents for this type of compound are lacking.

In this paper we report the isolation, purification, and proof of structure of this new fatty acid from *Crepis* oil, for which we suggest the name crepenynic acid, since the postulated presence of both olefinic and acetylenic unsaturation has been confirmed.

Results

Neither infrared nor ultraviolet spectral analyses provided evidence relevant to the structure of crepenynic acid since the parent oil gave spectra much like

A laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.
 R. W. Miller and F. R. Earle, presentation before the American Oil Chemists' Society, Atlanta, Ga., April 22-24, 1963.

⁽³⁾ American Oil Chemists' Society, "Official and Tentative Methods." Ed. 2. Rev., 1959, cd 7-58.

those of soybean oil which contains predominantly linoleic and oleic acids, together with smaller quantities of linolenic, palmitic, and other saturated acids. Therefore, countercurrent distribution of the fatty acid methyl esters derived from *Crepis* oil was undertaken to obtain a purified sample of the new acid for structural studies.

A 730-transfer countercurrent distribution of the mixed methyl esters, using a hexane-acetonitrile system in a Craig-Post⁴ apparatus, yielded nearly pure methyl crepenynate (Fig. 1, peak C). Figure 1 is the weight distribution plot obtained after removing solvent from selected fractions. G.l.c. analyses of the material in peak A showed that it contained methyl palmitate, stearate, oleate, and a trace of linoleate. Peak B contained predominantly methyl linoleate.

During the course of this work pure methyl crepenynate (I) was stored under nitrogen at -18° . In spite of these precautions the later experiments showed that autoxidation had taken place. The infrared spectrum of the autoxidized ester indicated hydroxyl absorption, 3420 cm.⁻¹, and conjugated trans-envnic absorption, 95 cm. The ultraviolet spectrum showed conjugated unsaturation, $\lambda_{max} 234 \text{ m}\mu$. G.l.c. analyses indicated no unusual long-chain contaminants, but a number of short-chain materials were present. These probably resulted from cleavage of hydroperoxides formed by autoxidation. Little is known about the autoxidation of acetylenic fatty acids. However, Khan, et al.,⁵ have shown that stearolic acid (9-octadecynoic acid) has no induction period and that it autoxidizes more rapidly than oleic acid. The rapid autoxidation of this envnic acid is, therefore, not surprising.

Methyl crepenynate took up 1.08 moles of hydrogen using Lindlar catalyst.^{6,7} The partially reduced free acid (III) on the basis of treatment with lipoxidase as described by MacGee⁸ assayed 60.1% cis,cis methylene interrupted unsaturation. Using identical assay conditions, crepenynic acid (IV) was not a substrate for lipoxidase.

Methyl crepenynate was optically inactive and took up 3.06 moles of hydrogen using platinum oxide catalyst. G.l.c. analyses of the recovered product (II) showed only methyl stearate. The recovered material was further identified as methyl stearate by melting point and mixture melting point. These data establish that crepenynic acid has a straight-chain C_{18} skeleton.

Methyl crepenynate was converted to the corresponding acid (IV) by cold saponification. The infrared and ultraviolet spectra⁷ of the free acid were similar to the spectra of the usual *cis* C₁₈-unsaturated acids. The neutralization equivalent of IV was 279.1 and the iodine value (Wijs, 1 hr.) was 175.6. This iodine value is consistent with formulation IV since under the conditions used acetylenic bonds are known to absorb only 1 mole of iodine.⁹ The calculated iodine value on the basis of this assumption is 182.3. The acid IV

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(8) J. MacGee, Anal. Chem., 31, 298 (1959).



Fig. 1.—Countercurrent distribution of *Crepis foetida* L. methyl esters in acetonitrile-hexane.



Fig. 2.—Infrared spectrum of steam-volatile aldehydes (2.5%) in carbon tetrachloride) obtained by acid periodate oxidation of *threo*-9,10-dihydroxyoctadec-12-ynoic acid (IX).

was oxidized with periodate-permanganate,¹⁰ and g.l.c. analyses of methyl esters prepared from the crude oxidation mixture showed predominantly nonanedioic acid (VIII, 52.5%) and *n*-hexanoic acid (VI, 30.6%). The monobasic acid (VI) obtained by steam distillation, was identified as *n*-hexanoic acid by melting point and the mixture melting point of its anilide. These data place the unsaturation at C-9 and C-12.

The double bond in IV was epoxidized with peracetic acid.¹¹ The purity of the epoxide was determined by titration with hydrobromic acid in glacial acetic acid according to Durbetaki.¹² This acid (V) has not been reported in the literature.

The epoxidized acetylenic acid (V) was converted to the *threo*-acetylenic diol¹³ (IX) by acetylation and saponification. After purification, this acid absorbed 2.04 moles of hydrogen. The product was identified as *threo*-9,10-dihydroxystearic acid by mixture melting point and by oxidative cleavage.

The dihydroxy acetylenic acid (IX) also was cleaved by acid periodate as described by King.¹⁴ The steam volatile aldehydes (X) were not identified; however, the infrared spectrum (Fig. 2) shows a band at 2205 cm.⁻¹ (A) and a weaker band at 2275 cm.⁻¹. One band is probably due to an α,β -acetylenic aldehyde and the other to a β,γ -acetylenic aldehyde.¹⁵ The band at 1940 cm.⁻¹ (B) is probably due to an allene.¹⁶ Precedents for bond migration during acid periodate

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⁽⁴⁾ Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

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 ⁽⁷⁾ R. A. Raphael, "Acetylenic Compounds in Organic Synthesis,"
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 ⁽¹⁰⁾ R. U. Lemieux and E. von Rudloff, Can. J. Chem., 33, 1701 (1955);
 E. von Rudloff, J. Am. Oil Chemists' Soc., 33, 126 (1956).

⁽¹¹⁾ J. G. Sharefkin and E. M. Boghosian, Anal. Chem., 33, 640 (1961).





Fig. 3.—Nuclear magnetic resonance spectrum of methyl crepenynate.

oxidation of β, γ olefins are known^{17.18}; however, 'the only acid periodate oxidation of an acetylenic diol involved α, β unsaturation.¹⁹ Thin layer chromatography of the 2,4-dinitrophenylhydrazones of the steam volatile aldehydes also demonstrated that a mixture was obtained.

The aldehyde acid (XI) was obtained by extraction of the steam nonvolatile residue and identified as azelaaldehydic acid by melting point and mixture melting point of its 2,4-dinitrophenylhydrazone. These results establish that the *cis* double bond is in the 9,10-position, and that the triple bond is in the 12,13-position. Therefore, the structure of crepenynic acid is the previously unknown *cis*-9-octadecen-12-ynoic acid.

The n.m.r. spectrum of methyl crepenynate (see Fig. 3) is in accord with the structure assigned on chemical grounds. The observed peaks for methyl crepenynate were assigned based on structure I, and the number of protons was determined by the relative areas. Table I summarizes the data.

		TABLE 1			
Shifts,	Assignments	, and Number	OF PROTONS	Observed in	
The Nuclear Magnetic Resonance Spectrum of I^a					

Assignment	r-values	Number of protons
CH ₃ terminal	9.08	3
CH_2 in chain	8.64	16
$CH_2 \alpha$ to unsaturated carbons	7.9	4
$CH_2 \alpha$ to carboxyl	7.77	2
CH_2 of 1,4-unsaturation	7.16	2
OCH ₃	6.37	3
Olefinic H	4.58	2

^a The chemical shifts are given in terms of τ -values as defined by G. V. D. Tiers, J. Phys. Chem., 62, 1151 (1958).

The appearance of a poorly defined doublet for the methylene of the 1,4-enyne system (τ 7.16) contrasts to the usual triplet obtained from the methylene of the 1,4-diene of methyl linoleate.²⁰

Discussion

Crepenynic acid has not been reported in the literature, although in recent years numerous syntheses of long-chain acids containing triple bonds have been accomplished using acetylenic intermediates. Osbond²¹ and de Gaudemaris and Arnaud²² synthesized *cis*-12octadecen-9-ynoic acid, and the latter authors also reported preparation of the *trans* isomer of crepenynic acid.

Meade²³ has published a comprehensive review of the known naturally occurring acetylenic fatty acids. Since appearance of that review, ximenynic (*trans*-11octadecen-9-ynoic) acid has been found in numerous seed oils of the Santalaceae.²⁴⁻²⁷ In general, previously known naturally occurring acetylenic fatty acids fall in one of two classes: The acetylenic group either comprises the only center of unsaturation in the

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⁽¹⁷⁾ F. D. Gunstone, J. Chem. Soc., 1611 (1954).

⁽¹⁸⁾ C. R. Smith, Jr., M. O. Bagby, R. L. Lohmar, C. A. Glass, and I. A. Wolff, J. Org. Chem., 25, 218 (1960).

⁽¹⁹⁾⁽a) F. D. Gunstone and W. C. Russell, J. Chem. Soc., 3782 (1955); (b) NOTE ADDED IN PROOF.—Since this paper was submitted. Winter has reported a periodate oxidation of a β_{γ} -acetylenic diol that resulted in formation of a β_{γ} -acetylenic aldehyde, a conjugated allenic aldehyde, and the enol form of the acetylenic aldehyde [M. Winter, Helv. Chim. Acta, 46, 1749 (1963)].

molecule or is conjugated with another double or triple bond. The discovery of crepenynic acid provides a new class of natural acetylenic acids having close relationship to the nutritionally important and widely occurring' polyolefinic fatty acids, such as linoleic and linolenic acid, which contain methylene interrupted unsaturation. The positions of unsaturation in crepenvnic acid follow the biogenetic patterns proposed by Lovern²⁸ and by Klenk and Debuch,²⁹ whereas the previously known naturally occurring polyunsaturated³⁰ acetylenic fatty acids do not. Crepenvnic acid may be biosynthetically related to such other acids as linoleic or vernolic (cis-12,13-epoxyoleic) acids, since both of these also occur frequently in substantial quantities in seed oils of Compositae. It is conceivable that the dihydroxyoleic acid speculated to be an intermediate in vernolic acid biosynthesis³¹ may in this plant be dehydrated enzymatically to crepenynic acid. However, no experimental evidence has yet been obtained on its mode of biosynthesis.

Limited screening for antimicrobial activity showed that crepenvnic acid methyl ester has good inhibition toward Trichophyton rubrum and Microsporum nanum.³²

Further research is in progress in the authors' laboratory on the mechanism of the rearrangement of crepenynic acid which occurs under the influence of alkali. The existence of this acid in substantial quantities in Crepis seed oil suggests that cultivation of the plant be tried to provide a convenient source of the acid for nutritional experiments, synthesis of labeled olefinic acids, studies of biosynthesis of unsaturated fatty acids, and for study of its possible utility as an industrial raw material.

Experimental³³

Preparation and Isomerization of Crepis foetida L. Seed Oil.-Ground seeds of Crepis foetida were extracted overnight with petroleum ether (b.p. 30-60°) in a Soxhlet apparatus. Most of the solvent was removed on a steam bath in a stream of nitrogen. and the remainder was removed in vacuo with a rotary evapora-The ground seeds yielded 22.0% oil. tor.

Alkali isomerization³ of Crepis oil gave ultraviolet absorption equivalent to 88% of conjugated linolenic acid after the 25-min. reaction time. An aliquot removed after only 3 min. gave absorption equivalent to 142% of conjugated linolenic acid, λ_{max}^{EtOH} 268 $m\mu (E_{1 cm}^{1\%} 721).$

Isolation of Methyl cis-9-Octadecen-12-ynoate (I) from Crepis foetida L. Seed Oil.--Methyl esters were prepared by refluxing 14.1 g. of oil with 110 ml. of ca. 4% hydrochloric acid in methanol, and 80 ml. of benzene under nitrogen for 3 hr. The reaction mixture was concentrated, diluted with four volumes of water and extracted with ethyl ether. The extract was dried over

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(29) E. Klenk and H. Debuch, "Annual Reviews of Biochemistry," Vol. 28, J. M. Luck, et al., Ed., Annual Reviews, Inc., Palo Alto, Calif., p. 39. (30) For convenience, polyunsaturated acids refer to all acids with two or more unsaturated centers.

(32) Private communication, A. K. Novak, Department of Food, Science, and Technology, Louisiana State University, Baton Rouge, La

anhydrous sodium sulfate. G.l.c. analyses of the methyl esters (13.7 g.) gave the following composition³⁴: C_{14:0}, 0.1%, $C_{16:0}$, 4.8%; $C_{16:1}$, trace; $C_{18:0}$, 2.9%; $C_{18:1}$, 4.3%; $C_{18:2}$, 27.8%; $C_{20:1}$, 0.3%; and methyl crepenynate, 59.8%. The equivalent chain lengths³⁵ of methyl crepenynate are 18.10 from an Apiezon L column and 20.65 from an LAC-2-R446 column (both 20% liquid phase on 60-80-mesh Celite). Infrared examination of the esters as a film on sodium chloride plates showed no unusual bands, and ultraviolet analysis of an ethanolic solution of the esters showed 1.6% conjugated diene and 0.7% conjugated triene.

The mixed methyl esters (13.5 g.) were divided evenly among the first six tubes of a 200-tube countercurrent distribution apparatus with 40 ml. of lower phase (acetonitrile) and 12.5 ml. of upper phase (n-hexane). The remaining tubes each contained 40 ml. of acetonitrile. The instrument was set to deliver 12.5 ml. of upper phase into the first tube after each transfer. Fraction collection began with transfer 201, allowing two transfers (25 ml.) to collect in each tube. A total of 530 transfers was collected, and the solvent was removed from selected fractions to obtain the weight distribution plot (Fig. 1). Based on g.l.c. analyses of significant fractions, transfers 481-500 were combined to give 0.82 g. of material containing 99.1% of methyl crepenynate and 0.9% of apparent methyl linoleate. Likewise, transfers 501-582 were combined to give 5.20 g. of methyl esters containing 99.8% of the acetylenic material. Ultraviolet analysis indicated only a trace of absorption in the diene region. Total recovery of the acetylenic ester from countercurrent distribution was 6.75 g. of essentially pure material. Recovery of all material was 12.2 g. or 90.5%

Hydrogenation of Methyl cis-9-Octadecen-12-ynoate (I). A. With Platinum Oxide Catalyst .-- A 0.024-g. portion of I. dissolved in ethanol, absorbed 3.06 moles of hydrogen using platinum oxide catalyst. G.l.c. analyses of the reduced material (II, 0.018 g.) showed only methyl stearate. This was confirmed by melting point (35.5-36.0°) and mixture melting point (35.0- 35.5°) with an authentic specimen melting at 34-35

B. With Lindlar Catalyst.-A 0.049-g. portion of I in ethanol was hydrogenated at room temperature and atmospheric pressure using 20 mg. of freshly prepared Lindiar catalyst and 0.1 mg. of quinoline. The reaction ceased after 1.08 moles of hydrogen per mole of I had been taken up. G.l.c. analyses of the reduced product indicated 71.4% of apparent linoleate, 11.1% of I, 3.1% of conjugated triene, and 14.4% of short-chain material. Crude III, obtained by saponifying the reduction mixture with ethanolic potassium hydroxide, had 60.1% of cis, cis methylene interrupted unsaturation as determined by lipoxidase isomerization.⁸ Crepenynic acid (IV) showed no reaction with lipoxidase. The differences in apparent per cent linoleate can probably be accounted for by the formation of trans unsaturation during the reduction³⁶ and by autoxidation of methyl crepenynate (I).

Saponification of I.-A 1.0-g. sample of I was saponified by stirring with 25 ml. of 0.5 N ethanolic potassium hydroxide for 2.5 hr. under nitrogen at room temperature. The saponification mixture was acidified, extracted with ethyl ether, and dried over sodium sulfate. The recovered acid (IV) had a neutralization equivalent of 279.1, (theoretical, 278.4) and an iodine value (Wijs, 1 hr.) of 175.6 (theoretical, 182.3). Infrared and ultraviolet spectra showed no unusual absorption.

Periodate-Permanganate Oxidation of cis-9-Octadecen-12ynoic Acid (IV).--A 0.204-g. portion of IV was stirred at room temperature for 19 hr. with 300 ml. of an aqueous solution containing 0.023 g. of potassium permanganate, 2.570 g. of sodium periodate, and 0.404 g. of potassium carbonate. The resulting solution was treated with sodium metabisulfite and acidified. The acidified solution was saturated with sodium chloride and extracted exhaustively with ethyl ether; a yield of 0.190 g. of products was obtained. Methyl esters were prepared from a portion of this mixture by refluxing with ca. 4% hydrochloric acid in methanol, recovered, and subjected to g.l.c. analysis. The following composition was obtained: methyl hexanoate, 30.6%; methyl nonanedioate, 52.5%; other dibasic acid esters, 12.6%; and unidentified components, 4.2%.

⁽³¹⁾ T. K. Miwa, F. R. Earle, G. C. Miwa, and I. A. Wolff, J. Am. Oil. Chemists' Soc., 40, 225 (1963).

⁽³³⁾ Infrared spectra were obtained with a Perkin-Elmer Model 137-0001 spectrophotometer. Ultraviolet spectra were obtained with either a Beckman DU or a Cary recording spectrophotometer. The n.m.r. spectrum was determined with a Varian A-60 spectrometer on a carbon tetrachloride solution containing 1.2% of tetramethylsilane. G.l.c. analyses were obtained using a Burrell Kromo-Tog K-5 equipped with both Apiezon L and LAC-2-R 446 columns. All g.l.c. identifications are based on retention characteristics of the materials in both polar and nonpolar columns as compared to the retention characteristics of similar known compounds (see ref. 35). Per cents of components obtained by g.l.c. analyses are area per cents. Melting points were determined on a Fisher-Johns block and are uncorrected.

⁽³⁴⁾ Subscripts indicate the number of carbon atoms and the number of double bonds in the fatty acids

⁽³⁵⁾ T. K. Miwa, K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, Anal. Chem., 32, 1739 (1960).

⁽³⁶⁾ R. R. Allen, J. Am. Oil Chemists' Soc., 33, 301 (1956).

The remaining oxidation mixture was steam distilled. The distillate was saturated with sodium chloride and extracted with ethyl ether to yield 0.025 g. of VI, which was reacted with 0.050 g. of aniline according to McElvain.³⁷ The anilide that separated as an oil was extracted from ethanol with 1:1 benzene-petroleum ether solution. After recrystallization from this solvent system, the anilide of VI melted at $92-94^{\circ}$. After two additional recrystallizations, 0.008 g. of product was obtained, m.p. $94.0-94.5^{\circ}$. The melting point of this material mixed with authentic caproanilide (m.p. 95.5°) was $94.5-95.0^{\circ}$.

The steam nonvolatile residue was extracted with ethyl ether to yield 0.065 g. of crude VIII, m.p. $80-86^{\circ}$. No further work was done with this mixture of dibasic acids. The malonic acid fragment was not recovered.

Epoxidation of IV.—A sample of IV (0.900 g.) was stirred at 23–25° for 4 hr. with 7.0 ml. of a 9.0% solution of peracetic acid in glacial acetic acid. The mixture was diluted with an equal volume of ice water, and the oil that separated was extracted with ethyl ether. The extract was concentrated to 15 ml., washed thoroughly with water, and dried over sodium sulfate. A light yellow oil (0.917 g.) remained after removal of solvent. The Durbetaki titration with hydrobromic acid¹² showed 4.6% of oxirane oxygen (equivalent to 85.5% epoxyacetylenic acid) in the crude product (V). Recrystallization from petroleum ether (b.p. 30–60°) at 0° yielded 0.632 g. of a white solid, m.p. 35–37°. A second recrystallization from petroleum ether at 8° gave nearly pure V, m.p. 37.5–38°, hydrobromic acid titration equivalent to 99.8% of epoxyacetylenic acid.

Anal. Calcd. for C18H30O3: C, 73.42; H, 10.27. Found: C, 73.5; H, 10.4.

Preparation of *threo-9*,10-Dihydroxyoctadec-12-ynoic Acid (IX). —A sample of V (0.346 g.) was refluxed with 10 ml. of glacial acetic acid; the product was recovered and saponified essentially as described by Smith, *et al.*¹⁸ The crude IX obtained (0.296 g.) melted at 69–71.5°. The crude dihydroxyacetylenic acid was recrystallized first from ethyl acetate-petroleum ether at -18° and then from ethyl acetate at -18° to yield 0.217 g. of product, m.p. 72–72.5°.

Anal. Calcd. for $C_{18}H_{32}O_4$: C, 69.19; H, 10.32. Found: C, 69.5; H, 10.3.

Preparation of threo-9,10-Dihydroxystearic Acid (XII).—A 0.049-g. portion of IX was hydrogenated at room temperature and atmospheric pressure using platinum oxide catalyst. Compound IX took up 2.04 moles of hydrogen per mole of acid. The catalyst was removed by filtration and the solvent was evaporated *in vacuo* to give 0.044 g. of crude XII, m.p. 93°. Compound XII mixed with *threo*-9,10-dihydroxystearic acid (m.p. 92-93°) melted at 92-93°. An admixture of XII with *threo*-12,13-dihydroxystearic acid (m.p. 94-96°) melted at 82-90°.

Periodate-Permanganate Oxidation of XII.—The remaining portion (0.041 g.) of XII was oxidized and recovered as described for IV, except that the reaction time was 16 hr. Methyl esters of the crude oxidation mixture (0.012 g.) were prepared by refluxing with hydrochloric acid-methanol. G.l.c. analyses of the methyl esters showed 36.8% of methyl nonanoate (XIII, pelargonate), 59.2% of methyl nonanedioate (XIV, azelate), and 4.0% of minor components. Compound XIII was identified only by its g.l.c. retention characteristics.³⁶ The remaining oxidation mixture was triturated with petroleum ether (b.p. $30-60^{\circ}$) to give crude XIV, m.p. $92-99^{\circ}$. After recrystallization from a large volume of ethyl acetate-petroleum ether at 5°, compound XIV melted at $106.5-107^{\circ}$. An admixture with authentic nonanedioic acid (m.p. $104.5-106^{\circ}$) melted at $105-106^{\circ}$.

Periodate Oxidation of IX.—A portion (0.026 g.) of IX was dissolved in 3 ml. of ethanol, 0.022 g. of sodium metaperiodate in 2.5 ml. of 1 N sulfuric acid was added and the solution stirred at $40 \pm 2^{\circ}$ for 15 min. The mixture was diluted with ice water and extracted with ethyl ether. Solvent was removed *in vacuo* at room temperature, and the product was steam distilled. The steam nonvolatile residue was extracted with ethyl ether and 0.013 g. of crude XI was obtained. The steam volatile portion (X) was shown by infrared spectroscopy to be a complex mixture (Fig. 2). Attempts to prepare the 2,4-dinitrophenylhydrazone resulted in an oil which was shown to be a mixture by thin layer chromatography.

The entire 0.013 g. of crude XI was redissolved in ethyl ether (5 ml.), and 2.5 ml. of this solution was placed in a test tube. The ether was removed with a stream of nitrogen and the 2,4-dinitrophenylhydrazone was prepared.³⁸ The mixture was allowed to stand at room temperature, and a yellow precipitate formed in 3 min. The crystals were filtered off by suction, washed well with water, and dried to yield 0.010 g. of a yellow crystalline product, m.p. 106-112°. Two recrystallizations from benzene-petroleum ether (b.p. 30-60°) yielded 0.006 g. of product, m.p. 120-121°, lit.³⁹ m.p. 122-122.5°. The mixture melting point of this product with authentic azelaaldehyde 2,4-dinitrophenylhydrazone (m.p. 120-121°) was also 120-121°.

Purification of Autoxidized I.—Carbon and hydrogen analyses of I were not obtained until after autoxidation had begun; hence a portion was purified for these analyses. Pure methyl crepenynate was separated from the oxidation products by countercurrent distribution between acetonitrile and *n*-hexane in a 30-tube apparatus. The course of the separation was followed by thin layer chromatography on silica gel G plates developed in 3% ethyl ether-petroleum ether. The chromatograms were charred with 50% sulfuric acid. Pure methyl crepenynate was obtained in transfers 40-65, and the oxidized material remained in the first 12 tubes. The combined fractions, 40-65, were placed in a nitrogen atmosphere, and the solvent was removed *in vacuo* under subdued light. Carbon and hydrogen analyses as well as the n.m.r. spectrum (Fig. 3) were obtained on this purified material.

Anal. Caled. for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03. Found: C, 77.8; H, 11.0.

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