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New Acetylenic Fatty Acids from *Acanthosyris spinescens* Seed Oil*

R. G. Powell and C. R. Smith, Jr.

ABSTRACT: The seed oil of *Acanthosyris spinescens* contains a number of previously unknown acetylenic fatty acids. These include 17-octadecen-9-ynoic acid, 18%; *trans*-10,16-heptadecadien-8-ynoic acid, 10%; and *trans*-11,17-octadecadien-9-ynoic acid, 4%. One other nonoxygenated C_{17} acid (9%) is also present but was not fully characterized.

A. spinescens is the first of the higher plants found to contain straight-chain C_{17} acids in more than very small amounts.

Investigations of the seed oil of *A. spinescens* (Mart. et Eich.) Griseb., family *Santalaceae*, by procedures conventionally applied to seed oils revealed that a number of unusual fatty acids were present. Infrared and ultraviolet spectra of the oil indicated the presence of hydroxyl, acetylene, terminal methylene, and conjugated enyne groupings. Gas–liquid partition chromatographic (glpc)[1] analyses of the fatty acid methyl esters revealed the presence of a number of unfamiliar components.

Previously, fatty acids containing terminal double bonds had been found only in the seed oil of *Onguekoa gore* (family *Olacaceae*), commonly referred to as isano oil, and in *Santalum acuminatum* (Bu'Lock and Smith, 1963). Conjugated enynoid fatty acids had been found in oils of a few genera in the *Santalaceae* and *Olacaceae* (Sørenson, 1963; Gunstone and Sealy, 1963) and in one member of the *Compositae*, *Helichrysum bracteatum*. 9-Hydroxy-*trans*-10-octadecen-12-ynoic acid has been characterized as a constituent of the seed oil of the latter (Powell *et al.*, 1965).

It appeared to us that investigation of *Acanthosyris* oil would yield results that might be significant biogenetically. Isolation and characterization of the nonhydroxylated acetylenic fatty acids are discussed in this paper. The structure of one of the hydroxyacetylenic acids has been indicated in a preliminary communication (Powell and Smith, 1965). The entire group of hydroxyacetylenic acids from *A. spinescens* oil will be the subject of a forthcoming paper from this laboratory.

Results

Separation of Nonoxygenated and Hydroxy Acids. Separation of *Acanthosyris* free fatty acids into hydroxy and nonhydroxy fractions was accomplished by using modifications of a procedure described by Frankel

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[1] Abbreviations used in this work: glpc, gas–liquid partition chromatographic; tlc, thin layer chromatography; nmr, nuclear magnetic resonance.

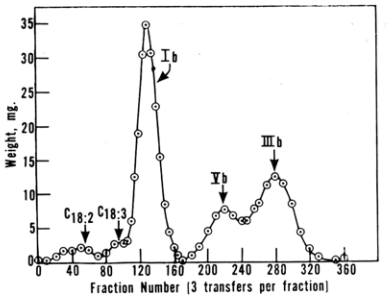


FIGURE 1: Countercurrent distribution of *Acanthosyris* nonhydroxy terminal methylene methyl esters. Fractions 0–360 were derived from transfers 200–1280 using the single withdrawal procedure.

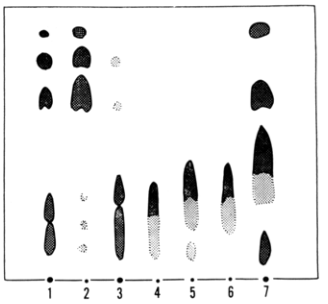


FIGURE 2: Thin layer chromatography of *Acanthosyris* methyl ester fractions (25% $AgNO_3$–silica gel G, benzene). 1, Complete nonhydroxy fraction; 2, nonterminal methylene fraction; 3, terminal methylene fraction; 4, analytical sample of Ib; 5, analytical sample of Vb; 6, analytical sample of IIIb; 7, soybean methyl ester standard containing, in order of increasing R_F: linolenate, linoleate, oleate, and saturates (palmitate and stearate). The plate was sprayed with 2′,7′-dichlorofluorescein, and the spots were outlined while being viewed under ultraviolet light.

et al. (1962). Adsorbosil[2] treated with 20% methanol in benzene served as the stationary phase, and 2% methanol in benzene was the eluting solvent. Nonhydroxy acids made up 80% of the total acids and hydroxy acids made up 20%.

Glpc analyses of the nonhydroxy fraction, as methyl esters, indicated the composition shown in Table I. Significant amounts of five unusual acids are readily apparent. When a portion of this fraction was hydrogenated (see Table I), methyl heptadecanoate was a major product. From these data it was clear that at least two of the unusual acids possessed normal C_{17} skeletons.

Isolation of a Terminal Methylene Fraction. Methyl esters of the nonhydroxy acid fraction were subjected to chromatography on a silica gel column impregnated with 25% silver nitrate using benzene as the eluting solvent (deVries, 1962). This procedure gave a fraction consisting primarily of esters containing terminal double bonds as indicated by maxima at 990 and 913 cm^{-1} in the infrared.

Separation of Terminal Methylenes Ib, IIIb, and Vb. The mixture of terminal methylene containing esters obtained by chromatography on silver nitrate impregnated silica was subjected to a 1400-transfer countercurrent distribution in an acetonitrile–hexane system. Ib was obtained as a single peak (Figure 1). IIIb and Vb were only partially resolved; however, samples sufficiently pure for characterization purposes were obtained from selected fractions. The nonhydroxy, terminal methylene, and nonterminal methylene containing fractions (as well as analytical samples of Ib, IIIb, and Vb) were examined by thin layer chromatography (tlc) on silver nitrate impregnated silica gel (deVries and Jurriens, 1963) (Figure 2).

Characterization of Ib. Peaks at 913 and 990 cm^{-1} in the infrared spectrum of Ib showed it to have a $CH_2{=}$ CH– terminal double bond (Bellamy, 1962). Characterization of Ib by chemical means is outlined in Scheme I. Permanganate–periodate cleavage (von

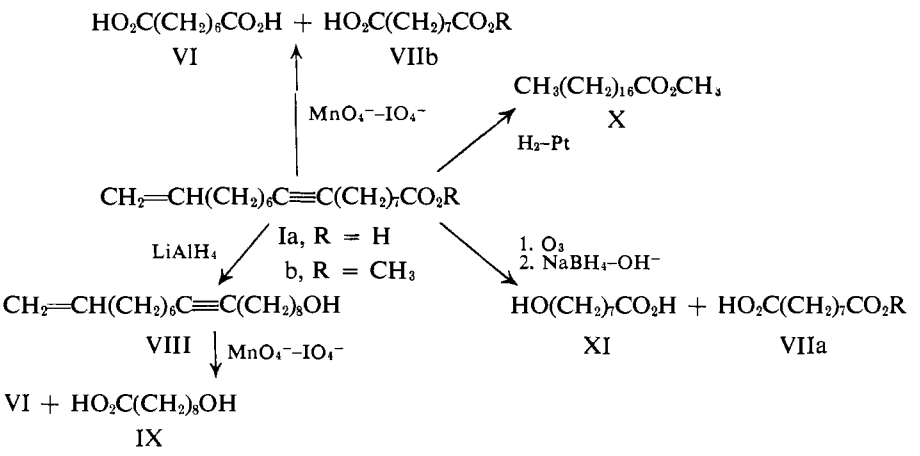
[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

TABLE I: Gas–Liquid Partition Chromatographic Analyses of Methyl Esters of Fractions Derived from *Acanthosyris* Oil.

Type of Acid[a]	Equivalent Chain Lengths (Miwa, 1960)		Acid (Area %)		
	Apiezon-L	LAC-2-R-446	Nonhydroxy Fraction	Hydrogenated Non-OH Fraction[b]	Total in Oil (Calcd)[c]
$C_{16:0}$	16.0	16.0	2.6	3.4	2
$C_{16:1}$	15.7	16.4	0.8	...	1
$C_{17:0}$	17.0	17.0	...	26.0	0
$C_{18:0}$	18.0	18.0	1.7	64.0	1
$C_{18:1}$	17.7	18.4	33.4	2.0	27
$C_{18:2}$	17.6	18.9	1.5	0.8	1
$C_{18:3}$[d]	17.6	19.8	5.9	...	5
Ia	17.9	20.4	22.9	0.3	18
IIa	17.9	20.9	11.3	...	9
IIIa	17.9	21.6	12.8	...	10
IVa	18.9	21.9	1.2	...	1
Va	18.9	22.6	5.4	...	4

[a] Subscripts indicate the number of carbon atoms and the number of double bonds in the fatty acids. [b] $C_{17:1}$ (0.8%) was among other products not listed. This result indicated that hydrogenation was not quite complete. [c] As the nonhydroxy acid fraction accounts for only 80% of the total acids in *Acanthosyris* oil, the figures in the last column were calculated as follows: column four (area %, nonhydroxy fraction) × 0.8 = total in oil (to the nearest whole percentage). [d] This is possibly a mixture of linolenate and stearolate.

SCHEME I



Rudloff, 1956) gave VI and VIIb which, after preparation of methyl esters, were identified as octanedioate and nonanedioate by glpc analyses. Lithium aluminum hydride reduction of Ib gave an alcohol (VIII). When VIII was cleaved with permanganate–periodate, the products formed were octanedioic (VI) and 9-hydroxynonanoic (IX) acids. Ib took up 2.97 moles of hydrogen to give methyl stearate (X).

A portion of Ib was also ozonized and subsequently cleaved reductively with alkaline sodium borohydride (Diaper and Mitchell, 1960). This procedure is known to produce alcohols from cleavage of double bonds. Glpc analyses of the cleavage products, as methyl esters, showed that nonanedioic (VII) and 8-hydroxyoctanoic (XI) acids were the primary products. Thus, we demonstrated that carboxylic acids are produced when triple bonds are cleaved by this procedure.

The nuclear magnetic resonance (nmr) spectrum of Ib is summarized in Table II. It is particularly significant that there are no protons due to a terminal methyl group (τ 9.08), that only three vinyl protons are present, and that eight protons appear at τ 7.83 (protons α to unsaturated centers). The chemical shifts and splittings of the vinyl protons were compared to those observed in the spectra of 1-hexene and 10-undecenoic acid (Hopkins, 1961) and found to be identical with these. Thus, Ia can only be 17-octadecen-9-ynoic acid.

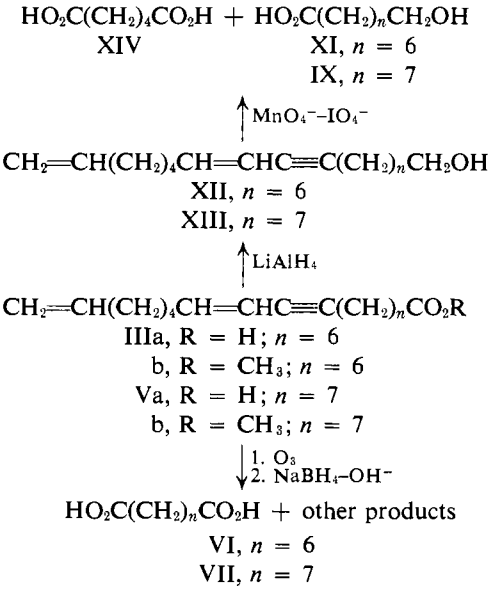
Characterization of IIIb. Peaks at 913 and 990 cm^{-1}, due to a terminal double bond, are also present in the infrared spectrum of IIIb. In addition there is a peak at 955 cm^{-1} characteristic of *trans*-conjugated enynes (Ahlers and Ligthelm, 1952). A maximum at 228 mμ (ϵ 16,400) in the ultraviolet spectrum of IIIb also shows the presence of a conjugated enyne chromophore (Ligthelm *et al.*, 1952). Reduction with lithium aluminum hydride gave XII (see Scheme II). When XII was cleaved with permanganate–periodate reagent the products were hexanedioic (XIV) and 8-hydroxyoctanoic (XI) acids. When IIIb was ozonized and the ozonides were cleaved reductively with borohydride, the major product identified was octanedioic acid (VI). This was accompanied by a product tentatively identified as 10-hydroxy-8-decynoic acid (XV). No 8-hydroxy-

TABLE II: Nmr Data Observed for Ib, IIIb, and Vb.

Assignment	τ Value	Number of Protons Ib	IIIb	Vb
CH_2 in chain	8.60	18	12	14
CH_2 α to unsaturation	7.83	8	8	8
OCH_3	6.33	3	3	3
CH_2=CH—R[a]	5.17–3.67	3	3	3
Olefinic H, α to acetylene	4.70–4.35	0	1	1
Olefinic H, β to acetylene	4.20–3.65	0	1	1

[a] Chemical shifts and splitting identical with that observed in 1-hexene.

SCHEME II



octanoic acid was found and the other major product expected, 1,6-hexanediol (XVII), was probably lost during work-up due to its high solubility in water. We therefore conclude that IIIa has a double bond at the 10,11 position and a triple bond at the 8,9 position, *i.e.*, that it is *trans*-10,16-heptadecadien-8-ynoic acid.

The nmr spectrum of IIIb is in agreement with the assigned structure and is summarized in Table II. The protons of C-10 and C-11 show coupling expected for a *trans* configuration (J = 15.8 cps, δ = 35.4 cps). The signal due to the C-11 proton is further split into a pair of triplets by coupling with the two methylene protons on C-12 (J = 6.7 cps) and the doublet due to the C-10 proton consists of two rather ill-defined peaks apparently because of long-range couplings to protons at C-12 and C-7. Except for the number of methylene protons (τ 7.83) the remainder of the spectrum of IIIb is much the same as that of Ib.

Characterization of Vb. Infrared and ultraviolet spectra of Vb were almost identical with those of IIIb. The nmr spectrum of Vb differed from that of IIIb only in that the former had two more methylene protons (Table II). Vb was also characterized according to Scheme II. Lithium aluminum hydride reduction produced an alcohol (XIII). When XIII was cleaved with permanganate–periodate, the products were hexanedioic and 9-hydroxynonanoic acids. Ozonolysis followed by reductive cleavage with borohydride gave nonanedioic acid as the major product along with what was apparently 11-hydroxy-9-decynoic acid (XVI). No 9-hydroxynonanoic acid was found and 1,6-hexanediol, the other expected product, was again lost during work-up. From these observations, we conclude that Va has a double bond at the 11,12 position and a triple bond at the 9,10 position, and that it is *trans*-11,17-octadecadien-9-ynoic acid.

The Nature of Acids IIa and IVa. An examination of glpc data in Table I shows that IVb has equivalent chain lengths very close to those reported for ximenynate (Mikolajczak *et al.*, 1953), 18.9 (Apiezon-L), and 22.1 (LAC-2-Resoflex-446). Thus, it may be surmised that IVa is ximenynic acid. Equivalent chain lengths for

$$CH_3(CH_2)_5CH{=}CHC{\equiv}C(CH_2)_nCO_2R$$

IIa, n = 6; R = H IVa, n = 7; R = H
b, n = 6; R = CH_3 b, n = 7; R = CH_3

IIb are exactly one unit less on both columns, showing it to be a C_{17} homolog of IVb. Considering the biogenetic patterns observed thus far in *Acanthosyris*, we suggest that IIa probably is *trans*-10-heptadecen-8-ynoic acid.

Discussion

A. spinescens seed oil contains a unique array of fatty acids. Four of the nonhydroxylated acetylenic acids present are previously unknown. The fifth, tentatively identified as ximenynic acid (IVa), was first reported by Ligthelm *et al.* (1952).

The nonconjugated acetylenic acid (Ia) is the third fatty acid with an isolated triple bond to be found in nature, and is the first with this feature in combination with a terminal double bond. 6-Octadecynoic acid has long been known as a component of the seed fat of *Picramnia tariri* (*Simarubaceae*) (Arnaud, 1892). 9-Octadecynoic (stearolic) acid, the acetylenic analog of oleic, has been reported quite recently as a component of *Pyrularia pubera* (*Santalaceae*) seed oil (Hopkins and Chisholm, 1964). An acetylenic analog of linoleic acid has also come to light since the summaries of Sørensen and Gunstone were compiled. *cis*-9-Octadecen-12-ynoic acid is a constituent of *Crepis foetida* (*Compositae*) seed oil (Mikolajczak *et al.*, 1964).

To our knowledge, *A. spinescens* seed oil has a much larger concentration of fatty acids with normal C_{17} chains than that of any higher plant source reported previously. Probably it is biogenetically significant that the homologous pair of enynes IIIa and Va are identical, except for the number of methylene groups between the unsaturated center and the carboxyl end of the chain. A strikingly similar situation exists in the case of sterculic (9,10-methyleneoctadec-9-enoic) and malvalic (8,9-methyleneheptadec-8-enoic) acids which occur together (Wilson *et al.*, 1961); malvalic acid has one less CH_2 group between the cyclopropene ring and the carboxyl group than sterculic acid. Smith and Bu'Lock (1964) have presented evidence that malvalic and sterculic acids have the same biosynthetic precursors, but that a one-carbon chain shortening occurs at some stage during the formation of malvalic acid. One-carbon degradations of fatty acids in plants were observed previously by Martin and Stumpf (1959) and by Hitchcock and James (1964). A similar one-carbon degradation mechanism may well be operative in the biogenesis of the C_{17} acids of *Acanthosyris*.

Experimental Section

General Methods. Gas–liquid partition chromatographic analyses were carried out with a Burrell Kromotog K-5, and retention values were treated as described by Miwa *et al.* (1960). Infrared spectra were determined with an Infracord Model 137 spectrophotometer on 1% carbon tetrachloride solutions unless otherwise specified. Ultraviolet spectra were determined with a Beckman DK-2A far ultraviolet spectrophotometer. The nmr spectra were determined with a Varian A-60 spectrometer on deuteriochloroform solutions containing 1% tetramethylsilane. Melting points were determined with a Fisher-Johns block and are uncorrected.

Isolation and Saponification of Acanthosyris Oil. Seed kernels of *A. spinescens* were crushed in a mortar and extracted overnight with petroleum ether (bp 30–60°) in a Soxhlet apparatus. Almost all the solvent was removed on a steam bath under a stream of nitrogen, and the remainder was removed *in vacuo* with a rotary evaporator. The yield of oil was 74.5%. The oil (neat) had infrared maxima at 3490 (OH), 2200 (C≡C), 1635 (C=C), 953 (*trans*-enyne), and 909 cm^{-1} (CH_2=

CH–); its ultraviolet spectrum showed λ_{max}^{EtOH} 228 mμ ($E_{1cm}^{1\%}$ 304).

Free acids were prepared by refluxing 17.9 g of oil with 500 ml of 1 N potassium hydroxide in ethanol under nitrogen for 1.5 hr. The mixture was concentrated with a rotary evaporator, diluted with an equal volume of water, and extracted with ether to remove unsaponifiables. The aqueous layer was then acidified with 6 N hydrochloric acid and extracted repeatedly with ethyl ether. The extracts were dried over anhydrous sodium sulfate. Ether was removed under a stream of nitrogen and 12.9 g of free acids, λ_{max}^{EtOH} 228 mμ ($E_{1cm}^{1\%}$ 320), was obtained.

Separation of Nonoxygenated and Hydroxy Acids. Preparation of Methyl Esters. A chromatographic column (34-mm i.d.) was prepared with a slurry containing 200 ml of 20% methanol in benzene and 100 g of Adsorbosil, 100–140 mesh (Applied Science Laboratories, State College, Pa.). Before use, 200 ml of 2% methanol in benzene was passed through the column (Frankel *et al.*, 1962). *Acanthosyris* free acids, 10.5 g, were then chromatographed in 2.6-g batches by using 2% methanol in benzene as the eluting solvent. Essentially quantitative separation was achieved. The nonoxygenated portion amounted to 8.4 g (80%). Characterization of the hydroxy acids (2.1 g) will be discussed in a future paper.

A portion of the nonhydroxy acid fraction, 6.0 g, was esterified by refluxing 3 hr with 70 ml of 4% HCl in methanol (under nitrogen). The reaction mixture was then diluted with 250 ml of water and extracted repeatedly with ethyl ether. Combined ether extracts were dried over sodium sulfate and afforded 5.9 g of methyl esters. Glpc analyses of this ester mixture yielded the data given in Table I. A portion of the ester mixture was hydrogenated (10% palladium on charcoal in acetic acid) and glpc analyses of the hydrogenation product are also given in Table I.

Isolation of Terminal Methylene Containing Esters from the Nonhydroxy Fraction. A chromatographic column (34-mm i.d.) was prepared from 100 g of Adsorbosil–CABN (25% silver nitrate on silica gel, 100–140 mesh). The nonhydroxy esters (5.8 g) were then chromatographed in four batches of approximately 1.4 g each. Mixtures of benzene and ethyl ether were used as eluting solvents. Three peaks were obtained in each case. The first peak, combined material from four runs amounting to 3.0 g, was shown by glpc analyses to be a mixture of $C_{16:0}$ (5%), $C_{16:1}$ (2%), $C_{18:0}$ (2%), $C_{18:1}$ (54%), $C_{18:2}$ (2%), $C_{18:3}$ (6%), IIb (21%), and IVb (6%). Linolenate would complex strongly with silver ion and consequently would not be expected in this fraction. Stearolate has glpc retention values very similar to those of linolenate, and may well be present instead of the apparent $C_{18:3}$. The combined second peak (2.4 g) consisted of terminal methylenes Ib, IIIb, and Vb (greater than 90%) along with small amounts of $C_{18:2}$ and $C_{18:3}$. The remaining material, 0.3 g, was primarily $C_{18:3}$. Over-all recovery was 96%. The terminal methylene fraction had $\lambda_{max}^{cyclohexane}$ 228 mμ ($E_{1cm}^{1\%}$ 257).

Countercurrent Distribution of Terminal Methylene Esters. Terminal methylene containing esters (2.4 g) were dissolved in mutually saturated hexane (10 ml) and acetonitrile (40 ml) and placed in the first tube of a 200-tube automatic Craig-Post apparatus. A 1400-transfer distribution was then carried out with 10 ml of upper phase and 40 ml of lower phase used throughout the operation. As upper phase progressed past tube 200, it was decanted into an automatic fraction collector; three transfers per tube were combined and successively collected until 400 fractions had been obtained. Solvent was evaporated under reduced pressure from the contents of selected tubes. The weight distribution obtained is shown in Figure 1. Fractions 110–160 were combined giving 0.82 g of a colorless liquid shown by glpc analyses to be 98% Ib. Fractions 180–220 were combined giving 0.16 g of liquid shown to be 98% Vb. Fractions 280–330 were combined giving 0.32 g shown to be 98% IIIb. All subsequent characterization work on esters Ib, IIIb, and Vb was performed on these materials.

Characterization of Ib. PRELIMINARY ANALYSES. The infrared spectrum of Ib had peaks at 1635 (C=C) and at 913 and 990 cm^{-1} (CH_2=CH–). It was essentially transparent in the ultraviolet from 200 to 360 mμ. Data obtained from the nmr spectrum are given in Table II. Equivalent chain lengths (Miwa *et al.*, 1960) on both Apiezon-L and LAC-2-R-446 columns are given in Table I. Ib absorbed 2.97 moles of hydrogen (T. K. Miwa *et al.*, submitted for publication) to give methyl stearate, mp 37.5–38.0°. Tlc (Figure 2) indicates that an isolated acetylene (Ib) is more strongly bound to silver nitrate than is a *trans*-enyne (IIIb or Vb).

Anal. Calcd for $C_{19}H_{32}O_2$ (292): C, 78.03; H, 11.03. Found: C, 77.75; H, 11.01.

PERMANGANATE–PERIODATE OXIDATION OF IB. A 57-mg sample of Ib was stirred for 17 hr with 0.4 g of potassium carbonate, 15 ml of *t*-butyl alcohol, and 25 ml of stock oxidant solution prepared according to von Rudloff (1956). The reaction was terminated by the addition of excess sodium metabisulfite and made strongly alkaline with potassium hydroxide. Solvent was removed on a rotary evaporator and the residue acidified with concentrated hydrochloric acid. The free acids obtained by ether extraction (49 mg) were esterified with an ether solution of diazomethane and subjected to glpc analysis. The products were found to be octanedioate (48%) and nonanedioate (47%).

LITHIUM ALUMINUM HYDRIDE REDUCTION OF IB. A 65-mg portion of Ib in 3 ml of dry ethyl ether was added dropwise to a suspension of 400 mg of lithium aluminum hydride in 8 ml of ether and the mixture refluxed for 3 hr. Excess reagent was destroyed with moist ether followed by dilute sulfuric acid. The mixture was then extracted repeatedly with ethyl ether and the combined extracts were dried over sodium sulfate. A yield of 49 mg of the alcohol VIII was obtained. Crude VIII had a new band in the infrared at 3620 cm^{-1} (OH) and showed only a trace of remaining ester carbonyl.

PERMANGANATE–PERIODATE OXIDATION OF VIII. Crude

VIII, 49 mg, was oxidized as described for Ib and free acids were recovered in the same manner. The oxidation products (50 mg), after having been esterified with diazomethane, were subjected to glpc analysis and found to be octanedioate (62%) and 9-hydroxynonanoate (33%).

OZONOLYSIS OF IB. An excess of ozonized oxygen was bubbled through a solution of 95 mg of Ib in absolute methanol at 0°. The ozonide solution was then added dropwise to an ice-cold solution of sodium hydroxide (0.3 g) and sodium borohydride (0.3 g) in 5 ml of 50% ethanol. A gentle evolution of hydrogen resulted and the mixture was stirred at room temperature for 17 hr (Diaper and Mitchell, 1960). Twenty milliliters of water was then added, and the alcohols were removed with a rotary evaporator. The aqueous residue was added dropwise to an excess of dilute hydrochloric acid, and the resulting mixture was extracted repeatedly with ethyl ether. The combined ether extracts were dried over sodium sulfate. A white solid (80 mg) was obtained upon removal of solvent. This solid material was esterified by refluxing it for 1 hr with 10 ml of 1 N sulfuric acid in methanol. The methyl esters were recovered by diluting the reaction mixture with 20 ml of water and extracting repeatedly with ether. Ether extracts were combined and dried over sodium sulfate, and the remaining material was subjected to glpc analysis. The following methyl esters were identified: nonanedioate (52%), 8-hydroxyoctanoate (30%), 9-hydroxynonanoate (13%), and octanedioate (3%).

Characterization of IIIb. PRELIMINARY ANALYSES. The infrared spectrum of IIIb had peaks at 2210 (C≡C), 1635 (C=C), 990 and 913 (CH_2=CH–), and 955 cm^{-1} (*trans* C=C, conjugated enyne). The ultraviolet spectrum gave $\lambda_{max}^{cyclohexane}$ 228 mμ (ε 16,400). Data obtained from the nmr spectrum are given in Table II. Equivalent chain lengths on both Apiezon-L and LAC-2-R-446 columns are given in Table I.

Anal. Calcd for $C_{18}H_{28}O_2$ (276): C, 78.24; H, 10.20. Found: C, 78.01; H, 10.22.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF IIIB. A 48-mg portion of IIIb in 3 ml of dry ethyl ether was added dropwise to a suspension of 400 mg of lithium aluminum hydride in 8 ml of ether and the resulting mixture refluxed for 3 hr. Excess hydride was destroyed with moist ether followed by 10 ml of 5% sulfuric acid. The mixture was then extracted repeatedly with ethyl ether, and the combined extracts were dried over sodium sulfate. A yield of 37 mg of the alcohol XII was obtained. Crude XII had a new band in the infrared at 3620 cm^{-1} (OH) and showed only a trace of remaining ester carbonyl.

PERMANGANATE–PERIODATE OXIDATION OF XII. Crude XII, 37 mg, was oxidized as described for Ib, and free acids were recovered and esterified similarly. Methyl esters of the oxidation products, 22 mg, were subjected to glpc analysis and found to be hexanedioate (42%) and 8-hydroxyoctanoate (21%) along with some degradation products of each.

OZONOLYSIS OF IIIB. An excess of ozonized oxygen was bubbled through a solution of 47 mg of IIIb in absolute methanol at 0°. The resulting ozonide solution was added dropwise to an ice-cold solution of sodium hydroxide (0.2 g) and sodium borohydride (0.2 g) in 5 ml of 50% ethanol. A gentle evolution of hydrogen resulted, and the mixture was stirred at room temperature for 17 hr. Ten milliliters of water was added, and the alcohols were removed by a rotary evaporator. The aqueous residue was added dropwise to an excess of dilute hydrochloric acid and the resulting mixture extracted repeatedly with ethyl ether. The combined ether extracts were dried over sodium sulfate and yielded 42 mg of a white solid after removal of solvent. The solid material was esterified by refluxing for 1 hr with 10 ml of 1 N sulfuric acid in methanol. The methyl esters were recovered by diluting the reaction mixture with 20 ml of water and extracting repeatedly with ether. Ether extracts were combined and dried over sodium sulfate; the residue was subjected to glpc analysis. The following products were identified: octanedioate (57%), 10-hydroxy-8-decynoate (26%), and 6-hydroxyhexanoate (4%). The identification of 10-hydroxy-8-decynoate was tentative, based on equivalent chain lengths of 13.0 (Apiezon-L) and 20.5 (LAC-2-Resoflex-446). The other expected major cleavage product, hexanediol, was presumed to have been lost during work-up because it is very soluble in water. 8-Hydroxyoctanoate was not present among the cleavage products.

Characterization of Vb. A. PRELIMINARY ANALYSES. The infrared spectrum of Vb was very similar to that of IIIb in having peaks at 2210 (C≡C), 1635 (C=C), 990 and 913 (CH_2=CH–), and 955 cm^{-1} (*trans*-enyne). The ultraviolet spectrum showed $\lambda_{max}^{cyclohexane}$ 228 mμ (ε 15,200). In Figure 2 tlc indicated a minor impurity which might account for the somewhat low ε value; however, the sample was 98% Vb by glpc analysis. The nmr spectrum of Vb (summarized in Table II) was identical with that of IIIb except for two more methylene protons at τ 8.60.

B. LITHIUM ALUMINUM HYDRIDE REDUCTION OF VB. A 48-mg portion of Vb was reduced as described earlier for IIIb, and the product (XIII) was recovered in the same manner. Crude XIII has a new band in the infrared at 3620 cm^{-1} (OH) and showed only a trace of remaining ester carbonyl.

C. PERMANGANATE–PERIODATE OXIDATION OF XIII. Crude XIII, 42 mg, was oxidized, and the oxidation products (21 mg) were recovered and esterified as described earlier for XII. The methyl esters were analyzed by glpc and found to be hexanedioate (42%) and 9-hydroxynonanoate (35%) along with some degradation products of both.

D. OZONOLYSIS OF VB. An ozonolysis of Vb was carried out as described for IIIb. The following products were identified: octanedioate (68%), 11-hydroxy-9-undecynoate (15%), and 6-hydroxyhexanoate (5%). The identification of 11-hydroxy-9-undecynoate was tentative, based on equivalent chain lengths of 14.0 (Apiezon-L) and 21.7 (LAC-2-Resoflex-446). Hexanediol was apparently lost in the work-up as before and

9-hydroxynonanoate was not present among the cleavage products.

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