

1101. *Fatty Acids. Part XII.* The Acetylenic Acids of Isano (Boleko) Oil.*

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Onguekoa gore seed oil contains nine acetylenic C₁₈ acids, comprising almost three-quarters of the total triglyceride acids. Four of these acids contain a hydroxyl group attached at position 8.

THE seed oil of the tree *Onguekoa gore* (syn. *O. klaineana* Pierre), known as isano (or boleko) oil, though the subject of many investigations, is still not fully characterised in respect of its component acids. It is reported to contain substantial proportions of polyacetylenic acids, including isanic (erythrogonic) acid, bolekiic acid, and one or more hydroxy-acids. Isanic acid is known, from degradative and synthetic studies, to have the structure (I).¹ Bolekiic acid and the hydroxy-acids are of uncertain structure though the former is believed to be a *cis*-octadecenediynoic acid,² whilst structures (II) and (III) have been proposed for the hydroxy-acids.³⁻⁵



The species *O. gore* belongs to the family Olacaceae and it is of interest, in view of the results to be reported here, that a number of other acetylenic acids⁶⁻⁹ have recently been discovered in other species of this family and in species of the closely related Santalaceae family. These findings will be discussed below.

The present study has shown that *O. gore* seed oil contains in its triglycerides [in addition to the usual range of saturated (C₁₄, C₁₆, and C₁₈) and unsaturated acids (oleic and linoleic)] five acetylenic acids, four hydroxy-derivatives of acetylenic acids, and a dihydroxy-stearic acid.

Isolation Procedures.—The mixed acids were divided into three fractions by partition between light petroleum and 80% aqueous methanol.¹⁰ One fraction (P; 50%) contained saturated and olefinic acids along with some acetylenic acids; a second fraction (M₂; 12%) contained acetylenic and monohydroxy-acids; a third fraction (M₁; 38%) was almost entirely mono- and di-hydroxy acids.

The M₁ esters were separated on a column of neutral alumina¹¹ into non-hydroxy-esters (M₁A₁; 12%, eluted with benzene), monohydroxy-esters (M₁A₂; 80%, eluted with ether), and dihydroxy-esters (M₁A₃; 8%, eluted with ether containing 5% of methanol). The non-hydroxy-esters, submitted to repeated crystallisation with urea, gave a non-adduct-forming fraction (10%) rich in enediynoic esters, and an adduct-forming fraction (90%) containing practically none of these esters. Each of these was separated on a

* Part XI, *J.*, 1963, 759.

¹ Black and Weedon, *J.*, 1953, 1785 and earlier references cited there.

² Meade, unpublished observation reported by Meade in "Progress in the Chemistry of Fats and Other Lipids," eds. Holman, Lundberg, and Malkin, London, 1957, Vol. IV, p. 50.

³ Riley, *J.*, 1951, 1346.

⁴ Kaufmann, Baltes, and Herminghaus, *Fette u. Seifen*, 1951, 53, 537.

⁵ Seher, *Annalen*, 1954, 589, 222.

⁶ (a) Lighthelm, Schwartz, and von Holdt, *J.*, 1952, 1088; Lighthelm, Horn, Schwartz, and von Holdt, *J. Sci. Food Agric.*, 1954, 5, 281; (b) Gunstone and Russell, *J.*, 1955, 3782; (c) Hatt and Schoenfeld, *J. Sci. Food Agric.*, 1956, 7, 130.

⁷ Hatt, Triffett, and Wailes, *Austral. J. Chem.*, 1959, 12, 190; 1960, 13, 488.

⁸ Lighthelm, *Chem. and Ind.*, 1954, 249.

⁹ Bu'Lock and Smith, *Biochem. J.*, 1962, 85, 35P.

¹⁰ Gunstone, *J.*, 1954, 1611.

¹¹ Meakins and Swindells, *J.*, 1959, 1044.

column of silica impregnated with silver nitrate¹² into esters containing, and esters not containing, a vinyl group. The monohydroxy-esters were similarly divided into vinyl and non-vinyl compounds.

These separations were monitored by ultraviolet spectroscopy by means of which the strongly absorbing enediyne chromophore was easily recognised, the measured absorption being compared with values quoted by Meade^{2,13} and by Crombie and Williams.¹⁴ Infrared spectroscopy was used to detect the vinyl group: in some fractions absorption at 1645 cm.⁻¹ was practically nil, in others it was such that the ratio of intensity of absorption at the vinyl peak to that at the ester peak (1739 cm.⁻¹) was close to that measured with methyl undec-10-enoate. Gas-liquid chromatography was used only in a limited way because these closely related acids were not always adequately separated. Thin-layer chromatography with silica gel, especially after treatment of the gel with silver nitrate, gave useful information and was extensively used.

The Dihydroxy-acid.—The fraction (M₁A₃) rich in dihydroxy-esters was hydrolysed to give *threo*-9,10-dihydroxystearic acid. This did not depress the melting point of an authentic sample and was oxidised by potassium periodate and potassium permanganate¹⁵ to nonanoic and azelaic acid. This dihydroxy-acid, comprising 2% of the mixed acids, has not previously been reported in this seed oil though it may be related to the epoxy-acid discovered by Morris.¹⁶

TABLE I.

Acid no., non-hydroxy- acids	Vinyl group (%) *	Ultraviolet absorption ($\lambda_{\max.}$ and $E_{1\text{cm.}}^{1\%}$)						Degradation products (acids)			Structure †
		214.5	227	239	252.5	267	282.5	monobasic	dibasic		
1	Nil	1180	112	175	317	478	378	C ₅	C ₉	9a, 11a, 13c	
2	100	1250	118	184	334	504	398	—	C ₄ , C ₉	9a, 11a, 13c, 17e	
3	Nil	—	13	12	4	—	—	C ₇	C ₉	9a, 11a	
4	100	—	13	12	4	—	—	—	C ₈ , C ₉	9a, 11a, 17e	
5	—	$\lambda_{\max.}$ 229 m μ ($E_{1\text{cm.}}^{1\%}$ 290)						—	—		
Hydroxy-acids											
6 (& 7)	Nil	212	37	45	52	67	52	C ₇	C ₈	9a, 11a, and (?) 9a, 11a, 13c	
8 (& 9)	100	197	35	46	57	70	57	—	C ₈ , C ₈	9a, 11a, 17e and (?) 9a, 11a, 13c, 17e	

* The infrared spectrum showed the absence of *trans*-olefinic groups in all the esters examined.

† These are all C₁₈ acids. The symbols indicate acetylenic bond (a), *cis*-ethylenic bond (c), ethylenic bond of unspecified configuration (e), *trans*-ethylenic bond (t).

The Non-hydroxy-acids.—The separation procedures described above yielded four non-hydroxy acids, information about which is summarised in Table I (acids 1—4). The ultraviolet and infrared spectra, the products of oxidative degradation,¹⁵ and the fact that they are hydrogenated to stearic acid lead to the structures indicated.

The fifth acid was recognised in a fraction obtained from the P acids which were loaded on to a reversed-phase chromatographic column of paraffin and kieselguhr.¹⁷ After other acetylenic acids had been eluted with 48% aqueous acetone a fraction rich in a C₁₈-enynoic acid was obtained with this solvent. This may be the 9-yn-11-enoic acid but we have not been able to prove this.

The Monohydroxy acids.—Hydrogenation of the monohydroxy-ester fraction (M₁A₂) gave a mixture of methyl stearate, 8-hydroxystearate, and 8-oxostearate. The position of the oxygen functions was determined by converting the esters through ketones, oximes,

¹² de Vries, *Chem. and Ind.*, 1962, 1049; paper communicated to Internat. Society for Fat Research, London, April 1962.

¹³ Meade, unpublished observation reported by Pitt and Morton (ref. 4, p. 242).

¹⁴ Crombie and Williams, *J.*, 1962, 2449.

¹⁵ von Rudloff, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 126.

¹⁶ Morris, personal communication.

¹⁷ Gunstone and Sykes, *J.*, 1960, 5050.

and amides into a mixture of degradation fragments.^{3,18} It is not considered, however, that the unhydrogenated esters contain oxo-esters. It has been reported¹⁹ that reduction of unsaturated hydroxy-acids gives some keto-acid, presumably through double-bond migration, and we found that some methyl oxostearate resulted during catalytic hydrogenation of methyl ricinoleate, methyl ricinostearolate, methyl 8-hydroxyximenynate (isolated for the first time from *Santalum album* seed oil), and from isano hydroxy-esters even after reduction with sodium borohydride. In addition, attempts to separate keto-compounds from unhydrogenated esters by Girard's reagent⁵ were unsuccessful.

Fractions containing monohydroxy-esters always showed the characteristic ultraviolet absorption of the enediyne chromophore to the extent of 9% and we were unable markedly to increase or decrease this value. The silica-silver nitrate column separated the hydroxy-esters into vinyl and non-vinyl compounds, but each fraction still contained some hydroxy-enediynoic ester. The main hydroxy acids (6 and 8) must have the structures shown in Table 1 on the basis of the spectroscopic and degradative evidence. The shorter-chain degradation fragments from the small amount of enediynoic acids were not adequately recognised. The hydroxy-acid (9) can only be the 9a,11a,13c,17e or the 10a,12a,14c,17e C₁₈ acid and we prefer the former structure.* By analogy, hydroxy-acid (7) is probably the 9a,11a,13c C₁₈ acid, and the four hydroxy-acids are thus the 8-hydroxy-derivatives of the acetylenic acids (1-4).

This conclusion differs from that of Seher⁵ who considers isano oil to contain a hydroxy-acid with the 10a,12a,14c structure shown in (III). After Oppenauer oxidation of the mixed esters, he isolated the unsaturated keto-ester, purified it on an alumina column, ozonised it, and recognised butyraldehyde, glyoxal, and 8-oxononanedioic acid as degradation products. The material submitted to ozonolysis represented 14% of the total esters; we found no evidence for an acid of this structure in such large amount. The two hydroxy-enediynoic acids of uncertain structure in the oil that we examined did not exceed 3% of the total.

TABLE 2.

Natural C₁₈ acetylenic acids in Olacaceae and Santalaceae species.

Triple bonds	Double bonds	Structure †	Sources	Triple bonds	Double bonds	Structure g	Sources
1	1	*9a,11t	a, b, c, d, g	2	2	9a,11a,13e,15e	e, g
1	1	9a,11e	e, f, g	2	2	9a,11a,13c,17e	f, g
2	0	*9a,11a	f	3	1	*9a,11a,13a,15e	e
1	2	9a,11e,13c	e	4	1	9a,11a,13a,15a,17e	e
1	2	9a,11t,13t	d, g				
2	1	9a,11a,13t	c, d, g				
2	1	*9a,11a,13c	f, g				
2	1	9a,11a,13e	e				
2	1	*9a,11a,17e	f, g				

* These acids also occur as hydroxy-derivatives and all have the OH group attached to C-8 except one, the position of which is not reported.

† For significance of a, c, e, t see footnote to Table 1.

Sources: (a) *Ximenia* spp. (ref. 6a & 8), (b) *Santalum* spp. (ref. 6b & c), (c) *Exocarpos cupressiformis* & *E. stricta* (ref. 7a), (d) *Ximenia americana*, *Santalum acuminatum*, & *Leptomeria aphylla* (ref. 7b), (e) Sweet Quandong (ref. 9), (f) *Onguekoa gore* (present work), and (g) synthetic (refs. 1, 14; Grigor, MacInnes, McLean, and Hogg, *J.*, 1955, 1069; Crombie and Jacklin, *J.*, 1957, 1622, 1632; Crombie and Griffin, *J.*, 1958, 4435).

Other reactions carried out to settle the structure of this acid gave inconclusive results, mainly because of the difficulty of recovering and recognising small amounts of reaction products from reactants which contained always more than 90% of diyne acids and less than 10% of enediynoic acid.

* For significance of a, c, e, t see footnote to Table 1.

¹⁸ Gunstone, *J.*, 1952, 1274.

¹⁹ Smith, jun., Wilson, Melvin, and Wolff, *J. Amer. Chem. Soc.*, 1960, **82**, 1417; Applewhite, Diamond, and Goldblatt, *J. Amer. Oil Chemists' Soc.*, 1961, **38**, 609.

Since long-chain acids containing an $\alpha\beta$ -unsaturated hydroxyl group are readily dehydrated by hot acetic acid,¹⁹ this reaction was carried out on the mixed hydroxy-esters (M_1A_2) as a possible means of distinguishing between $\alpha\beta$ - and $\beta\gamma$ -unsaturated alcohols. After reaction, no change in the ultraviolet spectrum could be observed, even though most of the fraction is known to consist of the $\alpha\beta$ -acetylenic hydroxy-compounds. It appears that acetylenic hydroxy-compounds differ from their ethylenic analogues in this respect, for after semi-hydrogenation in the presence of Lindlar's catalyst, the ester was dehydrated and the product showed strong triene absorption but no tetraene absorption. It is uncertain whether this indicates that the enediynoic acids have their hydroxyl group β to the unsaturation or whether, in the partial hydrogenation, the small amounts of hydroxy-enediynoic esters have been reduced beyond the hydroxy-triene stage.

Lithium aluminium hydride reduces α -acetylenic alcohols to *trans*-olefinic alcohols⁶⁰ but does not react with β -acetylenic alcohols. When the mixed hydroxy-esters (M_1A_2) were reduced with lithium aluminium hydride there was spectroscopic evidence of a new enyne chromophore resulting from the diynoic hydroxy-esters but it was impossible to decide whether there had been any change from the enediyne chromophore to a dienyne chromophore.

Oxidation of the hydroxy-esters with chromium trioxide-pyridine¹⁹ and with manganese dioxide²⁰ has also been examined. Though the hydroxy-group was oxidised to a keto-group the change in ultraviolet absorption was not indicative of the chromophore $-\text{CH}=\text{CH}\cdot\text{C}\equiv\text{C}\cdot\text{C}\equiv\text{C}\cdot\text{C}=\text{O}$. The product was examined by gas-liquid chromatography after hydrogenation, and four peaks were observed, due to stearate, oxostearate, hydroxystearate, and an unknown compound of "carbon number"²¹ 18-6 (Apiezon L) and 19-1 (QF-1). This ester was unaffected by sodium borohydride and was isolated by adsorption chromatography as a liquid of probable molecular formula $\text{C}_{19}\text{H}_{36}\text{O}_3$. It was oxidised by chromium trioxide mainly to C_8 and C_7 dibasic acids and probably contains a 5- or 6-membered oxygen heterocycle.

TABLE 3.
Component acids of isano oil.

	Wt.-%
Saturated acids (C_{14} 1%, C_{16} 4%, C_{18} 1%)	6
Olefinic acids (oleic 14%, linoleic 5%)	19
Acetylenic acids (9a,11e 1%; 9a,11a 10%; 9a,11a,17e 32%, 9a,11a,13c 2%; 9a,11a,13c,17e 6%)	51
8-Hydroxyacetylenic acids (9a,11a 4%; 9a,11a,13c 1%; 9a,11a,17e 15%; 9a,11a,13c,17e 2%)	22
9,10-Dihydroxystearic acid	2

Discussion.—The occurrence of acetylenic C_{18} acids in the seed oils of various species of Olacaceae and Santalaceae is summarised in Table 2. These clearly form a series of acids containing one or more acetylenic groups; they show conjugated unsaturation; and olefinic groups, when present, seem to occur in *cis*- or *trans*-configuration. It is of interest that many of these also occur as their 8-hydroxy-derivatives. An 8a,10a,12e,14e C_{18} acid has also been reported.¹⁴

Details of a quantitative study of this oil will be reported elsewhere²² but the results are summarised in Table 3.

EXPERIMENTAL

Light petroleum is the fraction of boiling range 40–60°. Ultraviolet absorption was measured on a Unicam S.P. 700 spectrophotometer for methanolic solutions, and infrared absorption for thin films with a Grubb-Parsons GS2A double-beam grating instrument. Gas-liquid chromatography was carried out with a Pye Argon chromatograph incorporating 4 ft. columns with Apiezon L (10%) or QF-1 (10%) as stationary phase on Celite; these were used at

²⁰ Evans, *Quart. Rev.*, 1959, **13**, 61.

²¹ Woodford and van Gent, *J. Lipid Res.*, 1960, **1**, 188.

²² Badami and Gunstone, *J. Sci. Food Agric.*, 1963, **14**, 479, 481.

temperatures between 100° and 200°. Relative retention times are reported as "carbon numbers."²¹ In thin-layer chromatography, the plates were coated with Merck's silica gel G, developed with 3 : 2 light petroleum-ether, and exposed to iodine vapour. Other plates, also sprayed with saturated methanolic silver nitrate and developed with the same solvent mixture, were sprayed with a 0.2% ethanolic 2',7'-dichlorofluorescein and observed under ultraviolet light.

Partition of Isano Acids.—Isano oil (37.5 g.) was refluxed with 0.5N-alcoholic potassium hydroxide (500 ml.) for 1 hr. in an atmosphere of nitrogen. After addition of water (1 l.) unsaponifiable material (2.25 g.) was removed by extraction with ether and, after acidification by 5N-hydrochloric acid) the mixed fatty acids were recovered.

Light petroleum was equilibrated with water and methanol (1 : 4) in a separatory funnel.¹⁸ Isano acids (33.2 g.) were dissolved in light petroleum (500 ml.) and shaken with aqueous methanol (200 ml.). The extract was then passed through three further separatory funnels each containing light petroleum (200 ml.), and the whole operation was repeated with three more portions of aqueous methanol. The acids were recovered from the first aqueous methanol extract (M_1 ; 12.53 g., 38%), from the second, third, and fourth methanol extracts (M_2 ; 4.15 g., 12%), and from the four light petroleum solutions (P; 16.52 g., 50%). The ultraviolet absorptions of these fractions are summarised in Table 4.

Further Separation by Adsorption Chromatography and Urea Fractionation.—A column of alumina (15 g.), neutralised by treatment with ethyl acetate,¹¹ was used to separate the M_1 esters (200 mg.). Fractions were eluted with benzene (M_1A_1 ; 22 mg., 12%), with ether (M_1A_2 ; 151 mg., 80%), and with ether containing 5% of methanol (M_1A_3 ; 15 mg., 8%).

The esters (fraction M_1A_1 ; 2.6 g.), dissolved in hot methanol (78 ml.) along with urea (13 g.), gave a urea adduct when cooled to 0°. After treatment of the esters which did not form an adduct four more times with urea, the non-adduct-forming esters (M_1A_1Z ; 250 mg.) and the adduct-forming esters (M_1A_1Y ; 2.24 g.) were recovered. The ultraviolet absorption of these fractions is summarised in Table 4.

Silver nitrate was deposited on silicic acid (Mallinckrodt; 100 mesh) by suspending the latter (100 g.) in water (1 l.) containing silver nitrate (500 g.).¹² The mixture was heated at 100° for 30 min., cooled to room temperature, filtered, and dried at 120° for 16 hr. The adsorbent (containing 27% of silver nitrate) was ready for use after grinding. To make a column it (10 g.) was mixed with Hyflo Supercel (5 g.), suspended in light petroleum (50 ml.), boiled and stirred for 5 min., then cooled to room temperature, and the resulting slurry was brought into a chromatograph tube, maintained at constant temperature by circulating tap water and protected from light with black paper.

The non-adduct-forming esters (M_1A_1Z ; 250 mg.) were loaded on such a column with light petroleum-ether (4 : 1) and eluted with this solvent mixture. One fraction (acid 1, Table 1; 60 mg.) was eluted with the first 40 ml. of solvent, and the second fraction (acid 2; 181 mg.) with the next 60 ml. of solvent. The adduct-forming esters (M_1A_1Y ; 250 mg.) were similarly divided into non-vinyl esters (acid 3; 57 mg.) and vinyl esters (acid 4; 176 mg.). Spectroscopic details are given in Table 1.

TABLE 4.
Ultraviolet absorption (λ_{\max} and $E_{1\text{cm.}}^{1\%}$) of various fractions and products.

λ_{\max} (m μ)	214.5	227	239	252.5	267	282.5
<i>cis</i> -Enediyonic acid:						
Meade ²	—	—	—	—	850	679
Meade ¹³	—	112	230	427	635	496
Crombie & Williams ¹⁴	—	142	254	526	796	642
Fraction M_1	260	67	72	78	89	80
M_2	295	63	69	87	109	89
P	152	32	35	37	50	41
M_1A_1	290	60	64	82	100	75
M_1A_2	201	55	56	61	68	56
M_1A_3	98	20	23	29	35	30
M_1A_1Z	1250	118	184	334	504	398
M_1A_1Y	—	13	12	4	—	—
M_1A_2 after dehydration (AcOH)	210	37	45	52	66	51
M_1A_2 after oxidation (CrO ₃)	363	136	157	186	228	193
M_1A_2 after oxidation (MnO ₂)	300	134	155	184	223	190

The monohydroxy-esters (M_1A_2 ; 250 mg.) were similarly divided into non-vinyl esters (acids 6 and 7; 48 mg.) and vinyl esters (acids 8 and 9; 194 mg.) by using 3 : 2 light petroleum-ether as eluting solvent.

A C₁₈-enynoic Acid.—The P acids (100 mg.) were loaded on a column of kieselguhr and paraffin (75 g.) which had been made with 70% aqueous acetone and then washed with 35% acetone.¹⁷ Acetylenic acids were eluted with 48% aqueous acetone, and the second 250 ml. of eluate was enriched in an acid of "carbon number" 18.5 (Apiezon L) with an absorption maximum at 229 μ ($E_{1\text{cm}}^{1\%}$ 290).

threo-9,10-Dihydroxystearic Acid.—The esters (M_1A_3) were hydrolysed with an excess of 0.5N-methanolic sodium hydroxide, and the recovered acid [29, 14 mg. after crystallisation from ethyl acetate (1.5 ml.)] melted at 94—94.5° alone or when mixed with authentic *threo-9,10*-dihydroxystearic acid. Oxidation with periodate and permanganate (details given below) gave nonanoic and nonanedioic acid which were recognised, after esterification, by gas-liquid chromatography.

*von Rudloff Oxidation of Unsaturated Acids.*¹⁵—Ester fractions, obtained as already described, were converted into acids (60 mg.), which were dissolved in a solution of potassium carbonate (380 mg.) in water (32 ml.), to which a solution of potassium permanganate (15 mg.) and potassium periodate (1.3 g.) in water (64 ml.) was then added. This mixture was shaken at room temperature for 24 hr., whereafter the excess of oxidant was reduced with sulphur dioxide. (When products of low molecular weight were expected, this solution was neutralised with potassium carbonate and reduced in volume to ~10 ml. on a rotary-film evaporator.) The acidified solution was saturated with sodium chloride and thoroughly extracted with ether. The extracted acids, esterified with methanolic hydrogen chloride, were examined by gas-liquid chromatography on both Apiezon L and QF-1 columns at appropriate temperatures. This oxidation procedure was applied to acids 1—4, 6 and 7, and 8 and 9 with the results shown in Table I.

Hydrogenation of Isano Hydroxy-esters and of Other Unsaturated Hydroxy-esters. When isano hydroxy-esters (M_1A_2 ; 250 mg.) were hydrogenated in methanol in the presence of 10% palladium-charcoal (50 mg.) the product was a mixture of three compounds with "carbon numbers" (Apiezon L) of 18.0 (stearate), 19.3 (oxostearate), and 19.6 (hydroxystearate). These designations were based on the following evidence: (i) after acetylation (acetic anhydride, pyridine) the product showed three peaks with "carbon numbers" 18.0, 19.3, and 19.75 (acetoxystearate), (ii) after oxidation (chromium trioxide, acetic acid) the product contained only two compounds of "carbon numbers" 18.0 and 19.3, and (iii) after reduction (sodium borohydride) the product showed only "carbon numbers" of 18.0 and 19.6.

The hydrogenated esters (250 mg.) were separated into their three components on a column of alumina (15 g.) with the annexed results. All these fractions showed an ester peak (1727 cm^{-1}) in their infrared spectra but only the second fraction showed a carbonyl peak (at 1698 cm^{-1}).

Eluant	Wt. (mg.)	M. p. of ester (lit.)		M. p. of acid (lit.)		Ester
Light petroleum	25	38.5—39°	(37.8°)	71.2—71.7°	(70.1°)	Stearate
Benzene	38	45.5—46	(46.5—46.9) ²³	83—83.5	(83.6—83.8) ²³	8-Oxostearate
Benzene-ether (1 : 1)	158	55—55.5	(55.3—55.6) ²³	80.5—81	(81.5—81.7) ²³	8-Hydroxystearate

The catalytic hydrogenation of (i) isano hydroxy-esters after reduction with sodium borohydride, (ii) methyl ricinoleate prepared by partition of castor mixed acids, (iii) methyl ricinostearolate,²⁴ and (iv) methyl 8-hydroxyximenynate isolated from *Santalum album* seed oil, was studied. In all cases the major product (hydroxystearate) was accompanied by small amounts of stearate and of oxostearate. The content of oxostearate was greater from the α -acetylenic hydroxy-compounds than from the β -olefinic or acetylenic hydroxy-compounds.

Degradation of Hydroxystearate and Oxostearate.—The hydroxystearate (60 mg.) in acetic acid (0.6 ml.) was oxidised by a 10% solution of chromium trioxide in acetic acid (0.24 ml.) at 15—20°. After 30 min. the mixture was diluted with water (30 ml.), the excess of oxidant

²³ Bergström, Aulin-Erdtman, Rolander, Stenhagen, and Östling, *Acta Chem. Scand.*, 1952, **6**, 1157.

²⁴ Grigor, MacInnes, McLean, and Hogg, *J.*, 1955, 1069.

destroyed with sulphur dioxide, and the oxostearate (57 mg.) extracted with ether. This keto-ester (95 mg.) in ethanol (2 ml.) was converted into its oximes by refluxing it for 2 hr. with hydroxylamine hydrochloride (60 mg.), sodium acetate (90 mg.), and water (0.2 ml.). The oximes were rearranged by heating them at 100° with concentrated sulphuric acid (1 ml.) for 1 hr. and the amides were hydrolysed by refluxing them for a further 4 hr. after addition of water (1 ml.). After further dilution (20 ml.), the solution was extracted with ether (5 × 20 ml.) and the resulting acids (79 mg.) were shown to contain undecanoic and octanedioic acid by gas-liquid chromatography of their esters.

The oxostearate formed during hydrogenation gave the same degradation products.

Direct oxidative fission of the hydroxystearate with potassium permanganate²⁵ led to the same conclusions, but less definitely.

*Dehydration of the Unsaturated Hydroxy-esters.*¹⁹—The hydroxy-esters (M_1A_2 ; 100 mg.) were refluxed with glacial acetic acid (12 ml.) for 4 hr. but the product, after recovery, showed an almost unchanged ultraviolet absorption spectrum.

Semi-hydrogenation with Lindlar's catalyst²⁶ (300 mg.) in ethyl acetate (10 ml.) containing quinoline (50 mg.), furnished olefinic hydroxy-esters (289 mg.) which showed a single absorption peak at 235 $m\mu$ ($E_1^{1\%}$, 725). These esters were successfully dehydrated when heated with acetic acid and the product showed peaks at 265, 275 ($E_1^{1\%}$, 1245), and 287 $m\mu$ in its ultraviolet spectrum.

Reduction of the Acetylenic Hydroxy-esters with Lithium Aluminium Hydride.—A solution of the hydroxy-esters (M_1A_2 ; 300 mg.) in ether was added dropwise to a suspension of lithium aluminium hydride in ether, and the mixture was boiled for 2 hr. Next day the excess of hydride was decomposed with wet ether, and the ethereal solution, after being washed with sodium hydroxide solution, gave a mixture of alcohols (286 mg.), the ultraviolet absorption of which showed a maximum at 229 $m\mu$ ($E_1^{1\%}$, 637) and an inflexion at 240 $m\mu$.

*Oxidation of Acetylenic Hydroxy-esters with Chromium Trioxide-Pyridine*¹⁹ and with Manganese Dioxide.²⁰—The hydroxy-esters (M_1A_2 ; 600 mg.) in dry pyridine (3.5 ml.) were shaken with a solution of chromium trioxide (700 mg.) in the same solvent (7 ml.) for 24 hr. at room temperature. The product (558 mg.) was isolated by ether-extraction and purified by passage through a column of neutral alumina. It had the ultraviolet absorption reported in Table 4 and infrared peaks at 1727 (ester group) and 1667 cm^{-1} ($\alpha\beta$ -unsaturated carbonyl).

A similar product (Table 4) was formed when the hydroxy-esters (M_1A_2 ; 620 mg.) were oxidised with manganese dioxide (6.2 g.) in benzene (100 ml.) for 3 hr.

The oxidation products, after hydrogenation, contained four products of "carbon numbers" 18.0 and 18.0 (stearate), 18.6 and 19.1 (unidentified compound), 19.3 and 23.2 (oxostearate); and 19.6 and 22.0 (hydroxystearate) on Apiezon L and QF-1, respectively. The unidentified compound was unaffected by reduction with sodium borohydride and could then be separated from the other products on a column of neutral alumina. Light petroleum eluted methyl stearate (10 mg.), benzene eluted the unknown compound (52 mg.), and benzene-ether (1:1) eluted methyl hydroxystearate (179 mg.). The infrared spectrum of the unknown ester showed no unusual features (Found: C, 72.4; H, 11.2. Calc. for $C_{19}H_{36}O_3$: C, 73.0; H, 11.5%); when treated with chromium trioxide-acetic acid it was degraded, giving mainly C_8 and C_7 dibasic acids.

Isolation and Identification of 8-Hydroxyximenynic Acid from Santalum album Seed Oil.—By the method described in this paper *Santalum* mixed acids were isolated and partitioned between light petroleum and 80% aqueous methanol. The methanolic extract (9%), after esterification, was absorbed on a column of neutral alumina (15 g.); methyl ximenynate (196 mg.) was eluted with benzene, and a hydroxy-ester (35 mg.) with ether. The hydroxy-acid, λ_{max} , 229 $m\mu$ ($E_1^{1\%}$, 448), ν_{max} , 960 (*trans*-CH=CH), 1725 (CO_2R), and 3620 cm^{-1} (OH), "carbon number" 19.9 (Apiezon L), was hydrogenated to a hydroxystearate (mainly). Degradation of this gave undecanoic and octanedioic acid. Ozonolysis of the unsaturated hydroxy-acid furnished heptanal which was recognised as its dimethyl acetal by gas-liquid chromatography.

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