230. *Fatty Acids. Part I. 9-Hydroxyoctadec-12-enoic Acid,* a New Hydroxy-acid occurring in* Strophanthus sarmentosus *Seed Oil.*

By F. D. Gunstone

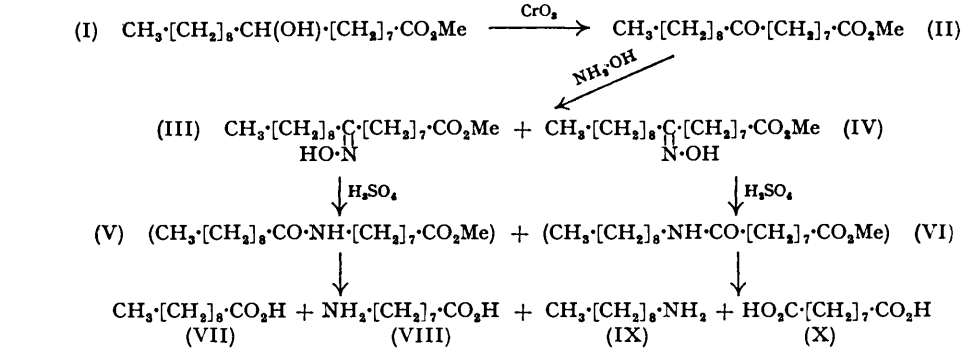
The unsaturated hydroxy-acid which forms 6·6% of the component acids of *Strophanthus sarmentosus* seed oil has been shown to be 9-hydroxyoctadec-12-enoic acid.

The steroid heart-poison aglycone, sarmentogenin, has been the subject of considerable study. It contains an oxygen atom at the 11-position and is therefore a potential starting point for the partial synthesis of cortisone (see Callow, Meikle, and Taylor, *Chem. and Ind.*, 1951, 336, who give earlier references). The oil present in the seeds, however, does not appear to have been examined although details for seed oils from other species of this genus have been reported (Matthes and Rath, *Arch. Pharm.*, 1914, **252**, 683; Tocco and Sanna, *Boll. Soc. ital. Biol. sper.*, 1935, **9**, 350; van Itallie, *Pharm. Weekbl.*, 1929, **66**, 677; Kühn, *Arch. Pharm.*, 1932, **270**, 395). Through the kindness of Dr. R. K. Callow of the Medical Research Council some of this seed oil has been made available. The results of its analysis by modern techniques are reported elsewhere (*J. Sci. Food Agric.*, in the press). Preliminary analysis disclosed an unusual acid. This remained with the more unsaturated acids which were soluble in acetone at −50°; its methyl ester distilled at a higher temperature than the C_{18} unsaturated esters; it behaved as though it were highly unsaturated (but see below), and was solid at room temperature and insoluble in light petroleum (b. p. 40—60°). These properties are those to be expected of an unsaturated hydroxy-acid and catalytic hydrogenation of a concentrate gave a saturated C_{18} hydroxy-acid. Subsequent oxidation

* Geneva numbering (CO_2H = 1).

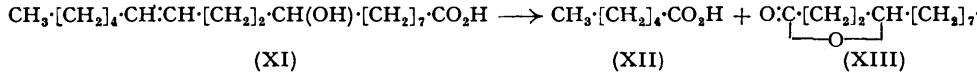
gave the corresponding keto-acid. The best known acid of this type is ricinoleic acid (12-hydroxyoctadec-9-enoic acid) but the saturated hydroxy-acid obtained from *S. sarmentosus* seed oil was different from 12-hydroxystearic acid obtained from castor oil.

Concentrates of the unsaturated hydroxy-acid were obtained by a combination of low-temperature crystallisation and distillation. The acid has been shown to be 9-hydroxyoctadec-12-enoic acid by investigations which have revealed (*a*) the position of the hydroxyl group and (*b*) the position of the double bond.



By the method first described by Baruch (*Ber.*, 1894, **27**, 172) and subsequently used by Goldsobel (*Ber.*, 1894, **27**, 3121), Ross, Gebhart, and Gerecht (*J. Amer. Chem. Soc.*, 1949, **71**, 283), and Riley (*J.*, 1951, 1346) a concentrate of the methyl ester of this new acid was reduced catalytically to methyl hydroxystearate (I), then oxidised to the corresponding keto-ester (II), and the mixed oximes (III and IV) from this were subjected to a Beckmann rearrangement and subsequent hydrolysis. Previous workers have hydrolysed the mixed amides with strong alkali under pressure but in this work it was conveniently effected by boiling 50% sulphuric acid. Of the four hydrolysis products three (VII, IX, and X) were isolated and two (VII and X) identified. The amine, unfortunately, decomposed before it could be identified. The monobasic and the dibasic acid were shown to be decanoic and azelaic acid respectively, indicating that the hydroxyl group was originally attached to the ninth carbon atom.

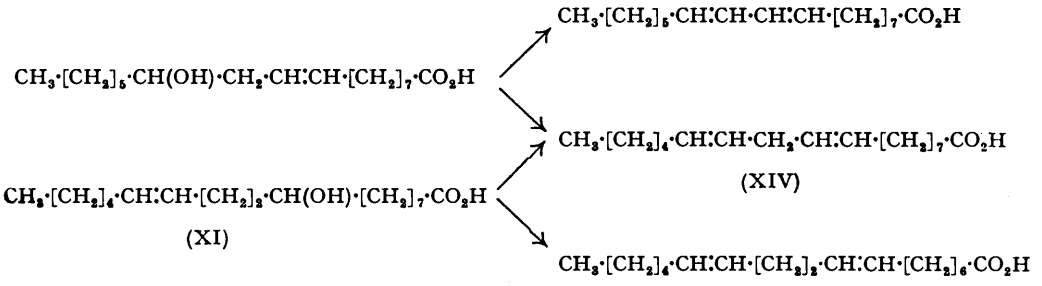
Despite the high iodine values obtained in the preliminary analysis the hydrogenation indicated that the acid contained only one ethylenic linkage. This was shown to be between the $C_{(12)}$ and $C_{(13)}$ by oxidation with potassium permanganate in acetone (Armstrong and Hilditch, *J. Soc. Chem. Ind.*, 1925, **44**, 43T). The monobasic acid (XII) was



identified as hexanoic acid. The dibasic acid fraction, after repeated crystallisation, gave a solid of empirical formula C_3H_5O. The equivalent showed that this solid was both a lactone and an acid and agreed well with the value $C_{12}H_{20}O_4$. The hydroxyl group has not been located but it should be in the γ-position and would consequently be expected to form a lactone very readily.

Long-chain hydroxy-acids are not common amongst the component acids of fats though they occur more frequently in waxes and are also known to be present in brain lipids. Ricinoleic acid is the best known acid of this type and occurs in *Ricinus* seed fats (*e.g.*, castor oil) and has also been reported in the seed fats of *Cephalocroton cordolfanus* (Henry and Grindley, *J. Soc. Chem. Ind.*, 1943, **62**, 60), *Agonandra brasiliensis* (Gurgel and de Amorim, *Mem. Inst. Chim. Rio de Janeiro*, 1929, **2**, 31), and from other unconfirmed sources. A hydroxy-acid present in *Vernonia anthelmintica* may be 11-hydroxyoctadec-9-enoic acid (Vidyarthi and Mallya, *J. Indian Chem. Soc.*, 1939, **16**, 479; Vidyarthi, *Patna Univ. J.*, 1945, **1**, 51; *Chem. Abstr.*, 1946, **40**, 4899), and Riley (*loc. cit.*) has recently shown that the

hydroxy-acid(s) present in *Onguekea Gore* (Engler) is a derivative of 8-hydroxystearic acid. Hydroxy-acids have also been claimed in other vegetable oils but these claims have been disproved. Both ricinoleic acid and our new hydroxy-acid could be dehydrated to give two diethenoid acids. It is perhaps significant that in both cases one product would be the widely distributed linoleic acid (XIV) and it may be that both hydroxy-acids represent precursors in the biogenesis of linoleic acid.



Oxidation with dilute alkaline potassium permanganate converts the acid into 9 : 12 : 13-trihydroxystearic acid. By following Kass and Radlove's procedure (*J. Amer. Chem. Soc.*, 1942, **64**, 2253) two forms of this acid have been isolated melting at 112—119° and 147·7—148·8°. The former, isolated in the smaller quantity, was not pure; a solid dibromide could not be isolated. The acid contains an asymmetric centre but attempts to determine whether it is optically active were inconclusive, the actual rotations measured being too small to be definite.

Throughout this work difficulty was encountered in determining the iodine value of fractions containing this hydroxy-acid. The values obtained were higher than expected and less concordant than usual and it appears that the hydroxyl group reacts with Wijs reagent. This does not occur with ricinoleic acid to any marked extent but Vidyarthi and Mallya (*loc. cit.*) reported the same difficulty with (?)11-hydroxyoctadec-9-enoic acid in which the hydroxyl group is adjacent to the double bond. They also claim that this difficulty does not occur when the hydroxyl group is acetylated and the author has confirmed that more satisfactory results are obtained with methyl 9-acetoxyoctadec-12-enoate. The effect with the hydroxy-acid is quite marked (see Experimental) but no explanation is offered for the different behaviour of 9-hydroxyoctadec-12-enoic and 12-hydroxyoctadec-9-enoic acid.

Experimental

Preparation of a Concentrate of the Unsaturated Hydroxy-acid.—The seed oil (600 g.) from *S. sarmentosus* was hydrolysed, the unsaponifiable portion (3·72 g.) was removed, and the resulting mixed acids (541·5 g.) were crystallised from acetone (5·4 l.) at −50°. The unsaturated hydroxy-acid remained in the soluble portion (209·3 g.). One concentrate was obtained by crystallising a portion (81 g.) of these acids from light petroleum (b. p. 40—60°; 10 ml. per g.) at −50° and by recrystallising the precipitated solids from the same solvent at −20°. The concentrate (*A*) (5·85 g.) so obtained was a viscous liquid slowly solidifying at room temperature. The remainder of the soluble acids, along with the material remaining in the mother-liquor from *A* (total, 198 g.), was methylated and distilled under reduced pressure, the fraction (*B*) (27·7 g.) boiling higher than the main fraction (unsaturated C_{18} esters) being retained. A portion of this material was acetylated (21 g.) and redistilled, the following fractions (*C*) being obtained [I.V. (Wijs) of methyl acetoxyoctadecenoate = 71·6]:

No.	C_1	C_2	C_3	C_4	C_5
Wt. (g.)	3·36	3·29	4·59	4·55	3·13
I.V.	132·6	96·2	81·9	76·2	73·3

A fourth concentrate (*D*) (1·89 g.)—possibly the purest—was obtained by hydrolysis of fraction C_5 and subsequent crystallisation of the acids from light petroleum (b. p. 40—60°; 10 ml. per g.) at −20°. This fraction was solid at room temperature and when subjected to alkali isomerisation (Hilditch, Morton, and Riley, *Analyst*, 1945, **70**, 67) gave no absorption band. A solution

in alcohol gave a value for $[\alpha]_D^{15}$ of $-0.3°$ but the observed rotation (0·02°) was no greater than the variation in repeated determinations.

Determination of the Position of the Hydroxyl Group.—(a) *Hydrogenation of the unsaturated hydroxy-ester.* The concentrate *B* (7·4 g.) in ethanol (50 ml.) was hydrogenated in the presence of palladium–charcoal (uptake, 592 ml. at N.T.P. Calc. 530 ml.). After removal of the solvent, 7·5 g. of saturated esters were recovered.

(b) *Preparation of the mixed oximes* (III *and* IV). The hydroxystearate (I) in acetic acid (75 ml.) was oxidised by a 10% solution of chromium trioxide in acetic acid (32 ml.) at room temperature. After 30 minutes the solution was poured into water, excess of chromic acid reduced with sulphur dioxide, and the ketostearate (II) (7·2 g.) extracted with ether. Oximation was effected in boiling aqueous ethanol (130 ml.; 80%) with hydroxylamine hydrochloride (6 g.) and sodium acetate (9 g.) for 2 hours. After removal of some of the alcohol the mixed oximes (7·4 g.) were extracted with ether. They solidified at room temperature.

(c) *Beckmann rearrangement and hydrolysis of the mixed amides* (V *and* VI). The mixed oximes were heated in concentrated sulphuric acid (45 ml.) at 100° for 1 hour. The mixture was then diluted with water (80 ml.) to about 50% sulphuric acid and refluxed for 3 hours (slightly stronger acid or a longer reflux period would probably have given more satisfactory results). After further dilution the mixture was steam-distilled and the distillate (3 l.), extracted with ether, gave the monobasic acid fraction (VII) (0·70 g.). The distillation residue on extraction with ether yielded ether-soluble material separated into acidic (4·94 g.) and non-acidic (0·05 g.) portions. The aqueous residue was neutralised by potassium hydroxide and steam-distilled; the distillate (1 l.) gave the amine fraction (IX) (0·43 g.) on extraction; the distillation residue containing the amino-acid (VIII) was not further examined. The amine fraction decomposed (probably to the alkyl carbamate) before it could be examined.

(d) *Monobasic acid fraction* (VII). This fraction solidified at 0° and remained solid at room temperature. Distillation under reduced pressure gave fractions (*a*) 0·309 g., m. p. 26—29°, and (*b*) 0·096 g., m. p. 28—32° (lit., 31·3°), both of which gave rather high equivalent weights (203·1 and 185·1 respectively). The combined acids recovered from the equivalent-weight determination readily gave *p*-bromophenacyl decanoate, m. p. 64—65·5° (softens at 62°) (lit., 67·0°), undepressed when mixed with an authentic specimen (Found: C, 58·4; H, 6·6; Br, 21·8. Calc. for $C_{18}H_{25}O_3Br$: C, 58·5; H, 6·8; Br, 21·6%).

(e) *Dibasic acid fraction* (X). The non-volatile acidic component (4·94 g.) was extracted with boiling water (4 × 100 ml.). The insoluble portion (4·3 g.) was probably a mixture of unchanged amide and saturated acids. The combined aqueous extracts, when concentrated to *ca.* 20 ml. and cooled to 0°, yielded fairly pure azelaic acid (X) (0·50 g.), m. p. 103—106°, raised only to 104—106° (lit., 106°) when crystallised from water and from ethyl acetate and undepressed when mixed with an authentic specimen [Found: equiv., 94·8. Calc. for $C_7H_{14}(CO_2H)_2$: equiv., 94·1].

Determination of the Position of the Double Bond.—(a) *Oxidation of the unsaturated hydroxy-acid.* To the concentrate C_4 (4·4 g.) in boiling acetone (44 ml.) powdered potassium permanganate (17·6 g.) was added portionwise at such a rate as to maintain boiling. The solution was then refluxed for 4 hours, the acetone was removed, water and sulphuric acid (25%) were added, and the suspension was decolourised with sulphur dioxide and extracted with ether. The ethereal extract, after being washed with 10% aqueous potassium hydroxide and water, contained only unoxidised material (0·95 g.). The alkaline residue was heated, acidified, and steam-distilled. The distillate (2 l.) yielded the monobasic acid fraction (XII) (0·94 g.), and the residue gave the dibasic acid fraction (XIII) (1·93 g.) when extracted with ether.

(b) *Monobasic acid fraction* (XII). A portion (0·2 g.) of this fraction was converted very readily into its *p*-bromophenacyl ester, m. p. 69—70·5° (lit., 72·0°) undepressed when mixed with a sample prepared from authentic hexanoic acid (Found: C, 53·4; H, 5·4; Br, 25·4. Calc. for $C_{14}H_{17}O_3Br$: C, 53·7; H, 5·5; Br, 25·5%). Distillation under reduced pressure gave two fractions the equivalents of which (109·5 and 112·5) were slightly low.

(c) *Dibasic acid fraction* (XIII). The dibasic acid fraction was completely soluble in boiling water (200 ml.), and the solution, when concentrated to *ca.* 40 ml. and cooled to 0°, deposited a low-melting solid (0·90 g.). The m. p. after several crystallisations from aqueous methanol was raised to 66—67·5° (softens at 47°) (Found: C, 63·1; H, 8·9. $C_{12}H_{20}O_4$ requires C, 63·1; H, 8·8%). The compound behaves as a lactone and an acid giving two equivalents (228·8 and 114·1) by direct titration and by the procedure used for the equivalents of esters ($C_{12}H_{20}O_4$ requires 228·3 and 114·1): it is probably the lactone of 3-hydroxydecane-1 : 10-dicarboxylic acid.

Other Reactions of 9-Hydroxyoctadec-12-enoic acid.—(a) *9-Hydroxy- and 9-keto-stearic acid.* Catalytic reduction of a concentrate of the unsaturated hydroxy-acid gave a product which after crystallisation from benzene melted at 81—82·5° (softened at 75°) (Tomecko and Adams, *J. Amer. Chem. Soc.*, 1927, **49**, 522, give m. p. 74—75° for the synthetic and therefore inactive 9-hydroxystearic acid) (Found: C, 71·8; H, 11·9. Calc. for $C_{18}H_{36}O_3$: C, 72·0; H, 12·1%). Reaction with methyl alcohol and anhydrous hydrogen chloride affords methyl 9-hydroxystearate, m. p. 50—53° (Tomecko and Adams, *loc. cit.*, give m. p. 45—46°) depressed to 45—51° when mixed with a sample of methyl 12-hydroxystearate (m. p. 57—59°) obtained from castor oil (Found: C, 72·6; H, 12·3. Calc. for $C_{19}H_{38}O_3$: C, 72·6; H, 12·2%). Oxidation (chromic oxide–acetic acid) of the hydroxy-acid gives 9-ketostearic acid, m. p. 79·5—81·0° (lit., 83°; Robinson and Robinson, *J.*, 1926, 2204; Behrend, *Ber.*, 1896, **29**, 807). An attempt to prepare methyl 9-acetoxystearate by reduction of the unsaturated ester (C_3) gave a compound crystallising from solution at 0° but melting below room temperature. Hydrolysis gave the same 9-hydroxystearic acid as that obtained above.

(b) *9 : 12 : 13-Trihydroxystearic acid.* A concentrate (*A*) of the unsaturated hydroxy-acid (1·23 g.) was oxidised with alkaline potassium permanganate by Kass and Radlove's method (*loc. cit.*). The dried oxidation product was extracted with boiling light petroleum (b. p. 40—60°) (yielding 0·04 g.) and then with boiling chloroform (yielding 0·19 g.). After crystallisation from aqueous ethanol, aqueous acetic acid, and aqueous ethanol it melted at 112—119° (softened at 105°) and was probably an impure isomer of 9 : 12 : 13-trihydroxystearic acid (Found: C, 65·9; H, 10·6%). The chloroform-insoluble portion (0·61 g.) crystallised from ethanol, aqueous acetic acid, and finally ethanol and gave *9 : 12 : 13-trihydroxystearic acid*, m. p. 147·7—148·8° (Found: C, 65·1; H, 11·0. $C_{18}H_{36}O_5$ requires C, 65·0; H, 10·9%).

(c) *Iodine value of 9-hydroxyoctadec-12-enoic acid.* The iodine value of a concentrate (*A*) of 9-hydroxyoctadec-12-enoic acid, which had not been distilled, was determined after 15, 30, 60, and 120 minutes, the liberated iodine being titrated *immediately* after addition of potassium iodide. Similar determinations were made on the mixed acids of castor oil, the results being:

Minutes	15	30	60	120
" Ricinoleic acid "	89·2	89·5	90·6	91·7
" 9-Hydroxyoctadec-12-enoic acid "	99·3	111·5	125·4	134·5

Methyl 9-acetoxyoctadec-12-enoate does not show this property. Of two similar (but not identical) concentrates of methyl 9-hydroxyoctadec-12-enoate one was acetylated. The iodine values (30 minutes) were 117·8 ± 0·6 and 72·9 ± 0·1 for the hydroxy- and acetoxy-esters respectively (Calc.: 81·2 and 71·6).

The author thanks Dr. R. K. Callow for supplying *S. sarmentosus* seed oil, Dr. J. P. Riley for the sample of methyl 12-hydroxystearate, and Mr. J. M. L. Cameron and Miss R. H. Kennaway for the microanalyses.

THE UNIVERSITY OF GLASGOW. [*Received, November 30th*, 1951.]