HYDROXY FATTY ACID GLYCOSIDES OF SOPHOROSE FROM TORULOPSIS MAGNOLIAE[1]

P. A. J. Gorin, J. F. T. Spencer, and A. P. Tulloch

ABSTRACT

The oil formed during fermentation by a strain of *Torulopsis magnoliae* consisted mainly of partly acetylated 2-*O*-β-D-glucopyranosyl-D-glucopyranose units attached β-glycosidically to 17-L-hydroxyoctadecanoic and 17-L-hydroxy-9-octadecenoic acids.

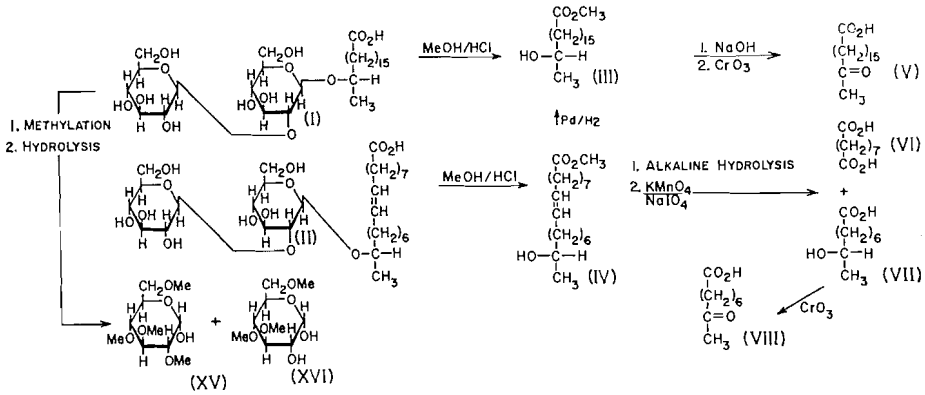
A strain of osmophilic yeast, *Torulopsis magnoliae*, isolated from sow thistle petals, was found to produce (1) an extracellular, heavier than water oil. The main components of the oil are now shown to be glycolipids (I and II) consisting of 2-*O*-β-D-glucopyranosyl-D-glucopyranose units linked β-glycosidically to 17-L-hydroxyoctadecanoic and 17-L-hydroxy-9-octadecenoic acids, the sugar moieties being partly acetylated.

The oil was labile both to acid and alkali. Hot methanolic sodium methoxide removed the acetyl groups, which constituted about 1.5 substituent groups per mole (the positions of these substituents were not determined). The deacetylated product, after fractionation, yielded a solid (I and II),which contained almost equal quantities of lactonic and acidic glycolipids, and it was on this mixture that the characterization studies of the sugar portion were carried out.

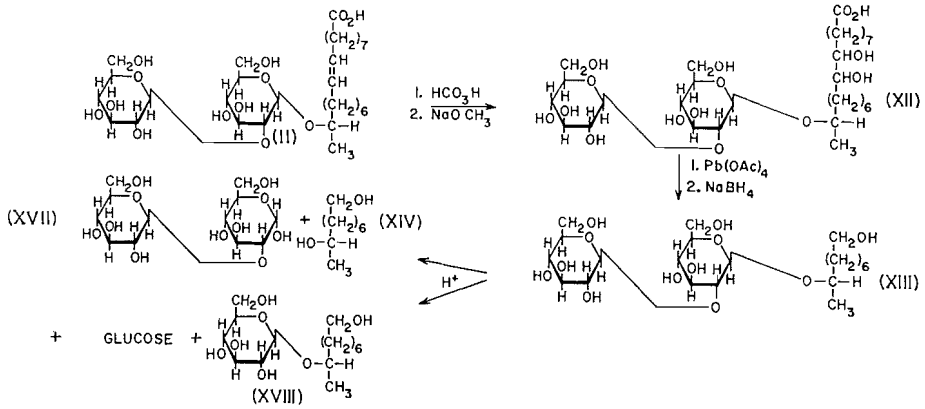
Acid methanolysis of the deacetylated oil yielded 2 moles of methyl-α-D-glucopyranoside and 1 mole of a mixture of fatty acid methyl esters. Gas – liquid phase chromatography of the esters on a silicone column showed one principal peak at the C_{20} saturated position with a shoulder suggesting partial resolution of saturated and unsaturated esters (2). However, on a butanediol succinate column (3) no peaks appeared after 2 hours (normal emergence time for a C_{20} ester was 30 minutes). The properties suggested that the major compounds present were C_{18} esters containing a polar substituent, such as a hydroxyl group (4) and the presence of the latter group was confirmed by infrared spectroscopy. From the quantity of permanganate–periodate reagent (5) consumed, the mixture was shown to contain 65% of monoolefinic ester. Fractional crystallization of the methyl esters also yielded 65% unsaturated and 35% saturated ester. However, later fermentations contained only about 10% of the saturated material. Catalytic hydrogenation showed that the unsaturated C_{18} ester (IV) contained one double bond and the product obtained was identical with the saturated C_{18} component (III). The position of the hydroxyl group in these compounds was shown to be at C_{17}, by the formation of 17-oxostearic acid (V) when the saturated acid was oxidized with chromium trioxide. An authentic specimen of V was synthesized by the method of Bergström *et al.* (6) from 15-bromopentadecanoic acid. The latter was prepared from 15-hydroxypentadecanoic acid which was in turn obtained from the 15,16-dihydroxypalmitic acid described by Lemieux (7).

The unsaturated component (IV) was shown to be 17-hydroxy-9-octadecenoic acid methyl ester since the acid on oxidation with permanganate–periodate (5) yielded azelaic

[1]*Manuscript received December 30, 1960.*
Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan. Presented at the 43rd Canadian Chemical Conference of The Chemical Institute of Canada, Ottawa, June 1960.
Issued as N.R.C. No. 6199.



1*a*



1*b*

FIGS. 1*a* and 1*b*. Degradation of sophorosides of 17-hydroxyoctadecanoic and 17-hydroxy-9-octadecenoic acids.

(VI) and 8-hydroxy-pelargonic acids (VII). These oxidation products could not be separated by crystallization and an attempt to separate the mixture of monomethyl azelate and hydroxy acid obtained by oxidation of the methyl ester in aqueous tertiary butanol (8) was also unsuccessful. Dimethyl azelate and methyl hydroxypelargonate were not well resolved by gas chromatography on a silicone column but conversion to the corresponding *n*-butyl esters gave a mixture which was easily separated preparatively. Oxidation of sirupy 8-hydroxypelargonic acid gave crystalline 8-oxopelargonic acid (VIII) with the same melting point as that reported by Barger *et al.* (9) for the synthetic acid. The double bond (IV) was considered to have the cis configuration as the infrared spectrum had no band in the 10.36 μ region (10).

The asymmetric center at carbon-17 has been provisionally assigned the L-configuration since the specific rotation of 17-hydroxyoctadecanoic acid was +4.4°. Assuming that the carboxyl group at the end of a long methylene chain has no more effect on the specific rotation than a methyl group the saturated C_{18} hydroxy acid can be regarded as a D-carbinol (IX) of which all known members of the type depicted are dextrorotatory (XI) (11). When the structure is written as (X) it has the L-configuration according to the usual rules of nomenclature (12). Further the acid might be expected to have the

same configuration as synthetic 12-L-hydroxyoctadecanoic acid ($[\alpha]_D$ +0.8°) (13) as the sign of rotation is the same.

15-D-16-Dihydroxy- and 2-D-15-D-16-trihydroxyhexadecanoic acids isolated from ustilagic acid also possess a hydroxyl group on the penultimate carbon atom and all

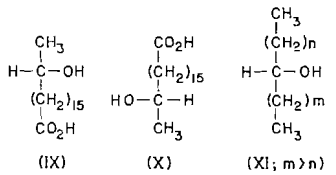


FIG. 2. Hydroxyl configuration in 17-L-hydroxydecanoic acid when regarded as an alcohol.

the optical centers were assigned the D-configuration (7), otherwise acids hydroxylated in this position do not seem to have been found in nature. For saturated acids the common positions for hydroxyl substitution are C_2, C_9, C_{10}, and the terminal carbon atom (14). Only two hydroxy monoenoic C_{18} acids have been reported, 12-hydroxy-9-octadecenoic acid, the major component of castor oil (14), and also occurring in ergot oil (15), which has been assigned the D-configuration (13), and 9-hydroxy-12-octadecenoic acid which is found in the seed fat of *Strophanthus* species (16).

To characterize the linkage types and configurations of the glycosidic linkages in the two disaccharide-C_{18} hydroxy acid glycolipids (I and II) it was necessary to separate them. Since fractionation was difficult the unsaturated portion was modified by oxidation. Treatment of the mixture with performic acid, followed by alkaline hydrolysis, gave *threo*-9,10-dihydroxy derivatives (XII) which were then oxidized by lead tetraacetate in acetic acid. The uptake of oxidant was almost immediate and indicated that 30% of the mixture was olefinic.* The resulting aldehydes were reduced with sodium borohydride, mainly to ω-hydroxypelargonic acid and a crystalline nonanediol sophoroside (XIII). These components were readily separable from the water-insoluble solid containing the 17-hydroxyoctadecanoic acid sophoroside (I). Complete acid hydrolysis of the nonanediol sophoroside yielded glucose and syrupy 1,8-nonanediol (XIV) from which a crystalline bis-*p*-nitrobenzoate was derived. The specific rotation of the diol was positive (+6.5°) indicating an L-hydroxy configuration (11) thus agreeing with the L-configuration already assigned to 17-hydroxy-9-octadecenoic acid. Complete methylation of the nonanediol sophoroside followed by acid hydrolysis gave equimolar amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose (XV) and 3,4,6-tri-*O*-methyl-D-glucose (XVI), which would be expected from two glucose units linked 1,2-glycosidically. Partial acid hydrolysis of nonanediol sophoroside furnished a mixture, from which sophorose (XVII; 2-*O*-β-D-glucopyranosyl-D-glucose) and 1,8-L-nonanediol-β-D-glucopyranoside (XVIII) were isolated by cellulose chromatography (17). The negative rotation of the glycoside indicated a β-configuration for the glucose – fatty acid linkage, as well as that of the glucose-to-glucose linkage. This agrees with the observation that the nonanediol sophoroside was totally hydrolyzed by emulsin, a β-glucosidase.

The amorphous material containing mainly 17-hydroxyoctadecanoic acid sophoroside (I) was similarly methylated and hydrolyzed to yield equimolar amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose (XV), 3,4,6-tri-*O*-methyl-D-glucose (XVI), and 17-L-hydroxyoctadecanoic acid. Although this evidence showed an analogous structure to the 17-L-hydroxy-9-octadecenoic acid sophoroside, the sodium salt was not hydrolyzed by emulsin.

**Since the proportion of unsaturated acid in the solid glycolipid is much less than in the parent oil (65%) it was evident that fractionation had taken place during isolation.*

However, Pigman and Richtmyer (18) note that the effect of emulsin on *n*-alkyl glucosides begins to decrease when the alcoholic aglycones have more than seven carbon atoms. The molecular rotations of the 17-hydroxyoctadecanoic acid (−14,200°, sodium salt) and 1,8-nonanediol sophorosides (−14,500°) agree closely and point to the likelihood that all the glucosidic linkages present are of the β-type.

The 2-*O*-β-D-glucopyranosyl-D-glucopyranose disaccharide unit is a unique structure in microbial metabolites. However, polysaccharides consisting of 1,2-β-D-glucopyranose units are known to be formed by various species of agrobacteria (19, 20, 21). The glycolipid molecule is similar in over-all structure to the ustilagic acids of Haskins (22) characterized by Lemieux *et al.* (7, and references cited therein) as partly acylated β-cellobiosides of 15-D-16-dihydroxyhexadecanoic and 2-D-15-D-16-trihydroxyhexadecanoic acids. Also similar is the acidic glycolipid formed by *Pseudomonas aeruginosa* which was isolated and identified by Jarvis and Johnson (23). This compound consists of a 3-*O*-L-rhamnopyranosyl-L-rhamnopyranose moiety attached glycosidically to a dimeric form of 3-D-hydroxydecanoic acid.

EXPERIMENTAL

Evaporations were carried out under reduced pressure using a bath temperature of 50° C. Optical rotations were measured at 26° C.

Method of Oil Production

The osmophilic yeast, obtained from the petal portion of a sow thistle, was shown to be a strain of *Torulopsis magnoliae* by carbon assimilation tests (1), and was designated strain N_1c.

The medium used consisted of glucose (20%), yeast extract (1.25%), and urea (0.2%) and was agitated at 385 r.p.m. for 8–10 days with an air flow rate of 500 ml/min, and incubated at 30° C in 5-l. New Brunswick fermentors (3-l. working volume). The maximum yield of the oil was about 5% of the volume of the medium and was recovered by allowing it to stand in a separatory funnel until it all settled out.

The aqueous portion of the solution was shown to contain D-mannitol, which is a common product of *Torulopsis magnoliae*, and this was obtained by crystallization of the evaporated residue from methanol. It had m.p. and mixed m.p. 166–167° C.

Deacetylation of the Oil

The oil (240 g), which contained a considerable proportion of water, was dissolved in 10 volumes of ethyl acetate. The solution was dried with $MgSO_4$, filtered, and evaporated to a yellow-brown viscous acidic syrup (148 g); C, 57.2%; H, 8.2%. It was shown to contain C—H, O—H, and carbonyl groups by infrared analysis. When saponified with alkali in aqueous ethanol it had a sap. equiv. of 226. The hydrolyzate was acidified and distilled to give acetic acid (10.2% of oil by weight).

The purified oil (115 g) was dissolved in refluxing methanol (2 l.) and sodium metal was added until the solution became alkaline. Refluxing was continued for 30 minutes, then the solution was evaporated to a syrup and the methanol distillate, which contained the methyl ester of the acetic acid, was collected in a dry-ice trap. Potassium hydroxide (1 g) dissolved in water (5 ml) was added to part of the methanol distillate and refluxed for 2 hours; after the methanol was taken off the residue was diluted with water and made just acid to phenol red by addition of hydrochloric acid. This solution was allowed to react with S-benzyl thiouronium chloride and gave S-benzyl thiouronium acetate, m.p. 131–134° C, which was not depressed by an authentic sample.

The deacetylated syrup was dissolved in water and acidified with formic acid. A gelatinous precipitate formed and after storage of the liquid at 3° C for a week it was filtered off, washed with cold water, and dried *in vacuo*. The light brown powder (44 g) had m.p. 137–139° C and analyzed as a hexose disaccharide attached glycosidically to a C_{18} hydroxy fatty acid. Roughly half of the material appeared to be in a lactonic form. Calculated for the saturated acid glycoside $C_{30}H_{56}O_{13}$: C, 57.6%; H, 9.0%; sap. equiv., 624. Found: C, 58.2%; H, 8.9%; sap. equiv., 652; neutral equiv., 1213.

Acid Hydrolysis of the Deacetylated Oil

The deacetylated syrup was refluxed with a large excess of 4% methanolic hydrogen chloride for 18 hours, then diluted with water and the fatty acid esters extracted with chloroform. The aqueous layer was neutralized (silver carbonate), filtered, and concentrated to a syrup which on crystallization from ethanol gave methyl-α-D-glucopyranoside, m.p. and mixed m.p. 166–167° C and $[\alpha]_D$ +160° (*c*, 1.0, H_2O).

Gas–liquid chromatography of the crude methyl ester on 1:6 silicone on Celite in a 6 ft × $\frac{1}{4}$ in. copper column at 235° C suggested the presence of 75% saturated and unsaturated hydroxy C_{18} esters. On oxidation with permanganate–periodate the crude acid, obtained by saponification, consumed 2.6 moles of oxidant corresponding to 65% of unsaturated esters.

17-Hydroxystearic Acid

A solution of the crude fatty acid methyl esters (15.62 g) in petroleum (b.p. 60–80° C) (225 ml) was stored at +2° C for 2 days and a crystalline product (3.97 g) was obtained. This solid was distilled, b.p./0.08 mm 180° C (bath), and repeatedly crystallized from petroleum (b.p. 60–80° C) but did not yield a completely pure ester. Saponification, isolation of the acid, and crystallization from acetone gave 17-hydroxystearic acid, m.p. 78–80° C. Calculated for $C_{18}H_{36}O_3$: C, 71.9%; H, 12.1%; neutral equiv., 300.5. Found: C, 71.8%; H, 11.95%; neutral equiv., 298; $[\alpha]_D$ +4.4° (*c*, 7.9, CH_3COOH). Methyl-17-hydroxystearate was prepared from the acid with diazomethane and crystallized from petroleum (b.p. 60–80° C) as platelets, m.p. 51–53° C. Calculated for $C_{19}H_{38}O_3$: C, 72.6%; H, 12.2%. Found: C, 72.2%; H, 12.1%; $[\alpha]_D$ +4.6° (*c*, 7.3, MeOH).

17-Hydroxy-cis-9-octadecenoic Acid

The solvent was removed from part of the mother liquors of the first crystallization of the crude saturated ester and the residue distilled, b.p./0.1 mm 150° C. After saponification a further small quantity of saturated acid was removed by crystallization from petroleum (b.p. 60–80° C) containing 8% acetone at 0° C and crystallization at −20° C gave almost pure unsaturated acid, m.p. 5° C. Distillation gave the pure acid as a colorless viscous oil, b.p./0.04 mm 160° C (bath). Calculated for $C_{18}H_{34}O_3$: C, 72.4%; H, 11.5%. Found: C, 72.35%; H, 11.3%; $[\alpha]_D$ +4.3° (*c*, 6.1, MeOH). There was no absorption band in the 10.36 μ region of the infrared spectrum. The methyl ester was prepared and had b.p./0.04 mm 145° C (bath). Calculated for $C_{19}H_{36}O_3$: C, 73.0%; H, 11.6%. Found: C, 72.8%; H, 11.5%; $[\alpha]_D$ +3.5° (*c*, 6.5, MeOH).

Hydrogenation of Methyl-17-hydroxy cis-9-Octadecenoate

The crude unsaturated ester (4.95 g) was recovered from the solvent remaining after crystallization of the saturated ester and was hydrogenated in ethanol (60 ml) in the presence of 5% palladium on charcoal (0.5 g). The volume of hydrogen absorbed at N.T.P. was 293 ml; calculated for the reduction of one double bond 353 ml. 17-Hydroxystearic acid was obtained on saponification and crystallized from acetone, m.p. 78.5–80.5° C,

mixed m.p. with natural saturated acid was 78–80° C. Calculated for $C_{18}H_{36}O_3$: C, 71.9%; H, 12.1%; neutral equiv., 300.5. Found: C, 71.8%; H, 11.95%; neutral equiv., 300; $[\alpha]_D$ +4.1° (*c*, 10.0, CH_3COOH). The derived methyl ester had a melting point of 50.5–51.5° C and the mixed melting point with natural saturated ester was 50.5–52.5° C. Calculated for $C_{19}H_{38}O_3$: C, 72.6%; H, 12.2%. Found: C, 72.4%; H, 12.0%; $[\alpha]_D$ +4.2° (*c*, 9.5, MeOH).

17-Oxostearic Acid

A solution of chromic oxide (2.4 g) in water (5 ml) was diluted with glacial acetic acid (25 ml) and added portionwise to a solution of 17-hydroxystearic acid (8.0 g), prepared by hydrogenation of the unsaturated ester, in acetic acid (80 ml) maintained at room temperature. After 30 minutes the mixture was poured into water (500 ml), the excess oxidant was reduced with sulphur dioxide and the product (6.8 g) was collected, washed, and dried. Crystallization from ethanol gave the pure acid, m.p. 86–87.5° C. Calculated for $C_{18}H_{34}O_3$: C, 72.4%; H, 11.5%. Found: C, 72.2%; H, 11.35%. The acid gave a positive iodoform test. A mixed melting point with 15-oxostearic acid (24) (m.p. 83° C) was depressed to 76–85° C. The melting point was not depressed by synthetic 17-oxostearic acid (m.p. 86.5–87.5° C) and the X-ray powder photographs were indistinguishable. The methyl ester was prepared and crystallized from petroleum as leaflets, m.p. 55–57.3° C, undepressed by admixture with the synthetic ester of m.p. 54.5–56.5° C. Calculated for $C_{19}H_{36}O_3$: C, 73.0%; H, 11.6%. Found: C, 72.9%; H, 11.5%. The infrared spectra and the X-ray powder photographs of the synthetic and natural esters were indistinguishable. 17-Oxostearic acid was similarly prepared from the naturally saturated acid and did not depress the melting point of the above oxo acid.

Oxidation of 17-Hydroxy-cis-9-octadecenoic Acid

The acid (1.73 g) was dissolved in 2% potassium carbonate solution (200 ml) and added all at once to the stock oxidant solution (400 ml, containing 20.86 g sodium metaperiodate and 0.4 g potassium permanganate per liter (5)) diluted with water (1400 ml) and shaken for 4 hours. The excess oxidant was destroyed with sodium metabisulphite, the mixture acidified with sulphuric acid, and the products extracted with ether and the ether removed without drying leaving the mixture of acids (1.88 g). The mixture was taken up in ethyl acetate (10 ml) when part of the azelaic acid (0.25 g) crystallized, recrystallization from the same solvent gave the pure dicarboxylic acid, m.p. 104–107° C undepressed when mixed with authentic azelaic acid. After removing the ethyl acetate the remaining acids were converted to the *n*-butyl esters by 4 hours' reflux with excess *n*-butanol containing 4% HCl. The butyl 8-hydroxypelargonate was separated from the dibutyl azelate by gas chromatography of 100-mg batches using 1:6 silicone on Celite (60–80 mesh) in a 6 ft$\times\frac{1}{4}$ in. copper column. The apparatus was operated at 200° C with injector at 225° C, and a flow rate of 75 ml of helium per minute. The *n*-butyl 8-hydroxypelargonate was distilled, b.p./0.05 mm 115° C (bath). Calculated for $C_{13}H_{26}O_3$: C, 67.8%; H, 11.4%. Found: C, 67.2%; H, 11.3%; $[\alpha]_D$ +4.8° (*c*, 6.8, MeOH). Alkaline hydrolysis of this ester yielded 8-hydroxypelargonic acid as a colorless syrup, b.p./0.04 mm 110° C (bath); $[\alpha]_D$ +6.7° (*c*, 10.1, MeOH). Calculated for $C_9H_{18}O_3$: C, 62.0%; H, 10.4%. Found: C, 62.1%; H, 10.3%. Oxidation of the acid with chromic oxide in the same way as for 17-hydroxystearic acid gave 8-oxopelargonic acid, m.p. 41–42° C (lit. (9) gives m.p. 40–41° C). Calculated for $C_9H_{16}O_3$: C, 62.8%; H, 9.4%. Found: C, 62.9%; H, 9.4%. The semicarbazone crystallized from aqueous methanol and had a melting point of 136.5–138.5° C (lit. (10) gives m.p. 136° C).

Hydroxylation of the Solid Glycolipid Mixture (I and II)

The mixed glycosides (44 g) were dissolved in formic acid (120 cc) and then 30% hydrogen peroxide (10 cc) added. After storage at 0° C for 18 hours the solution was evaporated to a dry crust which was then dissolved in refluxing methanol (1 l.). Sodium metal was added until the solution became alkaline and after 30 minutes the solvent was removed by evaporation. The solid was then dissolved in water and cations removed by shaking with Amberlite IR-120. Precipitation of glycosides on the resin took place, but redissolution was effected by adding an excess of methanol. Filtration and evaporation afforded a crusty light-yellow solid (39.5 g) containing unaffected glycoside and its *threo*-dihydroxy counterparts.

The amount of olefin in the original glycoside mixture was then estimated by lead tetraacetate oxidation of the hydroxylated product. The solid (100 mg) was dissolved in acetic acid (5 cc) and to this solution 1% lead tetraacetate (10 cc) was added. The uptake of oxidant after 3, 10, and 25 minutes corresponded to oxidation equivalents, based on 1 molar uptake of lead tetraacetate, of 2260, 2140, and 1940 respectively indicating 30% of olefinic glycoside.

Isolation of 17-Hydroxyoctadecanoic Acid β-Sophoroside, 1,8-Nonanediol β-Sophoroside, and ω-Hydroxypelargonic Acid

The mixed dihydroxy- and unhydroxylated 17-hydroxy-octadecanoic acid sophorosides (23 g) containing 30% of the former was dissolved in acetic acid (250 cc). Lead tetraacetate (13 mM.) in acetic acid (500 cc) was added and after 30 minutes oxalic acid dihydrate (13 mM.) in acetic acid (50 cc) was added and the precipitate filtered off. Evaporation to a residue and addition of water gave a solution from which 17-hydroxyoctadecanoic acid β-sophoroside precipitated. This material was isolated, dried, and boiled in ether to remove fatty acid fragments. It (11.1 g) had a melting point of 139–140° C and $[\alpha]_D$ −22° (*c*, 1.3, aqueous $NaHCO_3$). Calculated for $C_{30}H_{56}O_{13}$: C, 57.6%; H, 9.0%; sap. equiv., 624. Found: C, 57.7%; H, 9.0%; sap. equiv., 566; neutral equiv., 1050. The product was methanolyzed with 4% methanolic hydrogen chloride overnight under reflux. The methyl ester, obtained by neutralization (Ag_2CO_3), filtration, and evaporation, consisted mainly of the C_{18} ester with traces of other hydroxy methyl esters as shown by gas – liquid phase chromatography.

The mother liquor of the above glycoside precipitation was evaporated to a syrup and reduced with sodium borohydride (1.0 g) in water (100 cc). After 1 hour excess reductant was destroyed with acetic acid, cations removed with Amberlite IR-120, and the resulting filtrate evaporated to a crust. Boric acid was removed by repeated evaporation of the solid with methanol to give nonanediol β-sophoroside, which was recrystallized twice from ethanol to give material (3.2 g) with m.p. 188–190° C and $[\alpha]_D$ −30° (*c*, 1.0, H_2O). Calculated for $C_{21}H_{40}O_{12}$: C, 52.1%; H, 8.3%. Found: C, 51.9%; H, 8.3%.

In order to characterize the other fission fragment of the olefin 858 mg of the mixed hydroxylated glycosides was oxidized with lead tetraacetate in a similar manner to that described above, except that after filtration of the lead oxalate the filtrate was evaporated to a syrup to which sodium borohydride (250 mg) in water (25 cc) was added. After 1 hour the mixture was worked up and the product extracted with boiling ether to give a syrup (0.13 g) which crystallized. Two recrystallizations from ether afforded ω-hydroxypelargonic acid, m.p. and mixed m.p. 48–50° C. Calculated for $C_9H_{18}O_3$: C, 62.0%; H, 10.4%. Found: C, 62.1%; H, 10.1%. The material not extractable by ether was dissolved in methanol and precipitated by addition of excess water. Filtration

afforded a powder which gave on methanolysis (4% MeOH–HCl under reflux overnight) mainly two ether-soluble compounds corresponding to a C_{18} hydroxy methyl ester and an octadecanediol (by G.L.P.C.) thus indicating that reduction of a lactone had taken place.

Hydrolysis of 1,8-Nonanediol Sophoroside

The sophoroside (296 mg) was hydrolyzed by heating on a steam bath for 18 hours in *N* sulphuric acid (5 cc). The solution was neutralized ($BaCO_3$), filtered, and evaporated to a syrup which was extracted with boiling ether. Evaporation of the ether afforded the nonanediol (85 mg) with $[\alpha]_D$ +6.5° (*c*, 2.3, H_2O) and 40 mg of this was dissolved in pyridine (2 cc) containing *p*-nitrobenzoyl chloride (300 mg), and kept at 80° C for 30 minutes. To this an excess of aqueous sodium bicarbonate was added and after shaking for 2 hours the precipitate was collected, washed, and dried, and twice recrystallized from ethyl acetate – hexane. The 1,8-nonanediol bis-*p*-nitrobenzoate (93 mg) had a melting point of 94–96° C and $[\alpha]_D$ +21° (*c*, 2.1, $CHCl_3$). Calculated for $C_{23}H_{26}O_8N_2$: C, 60.3%; H, 5.7%; N, 6.1%. Found: C, 60.4%; H, 5.7%; N, 6.1%.

The residual glucose (233 mg) was crystallized from methanol to give crystals (168 mg) with m.p. and mixed m.p. 146–149° C.

Partial Hydrolysis of 1,8-Nonanediol Sophoroside

Nonanediol sophoroside (2.0 g) was partially hydrolyzed by keeping a solution in *N* sulphuric acid (10 cc) for 4 hours at 75° C. After neutralization ($BaCO_3$), filtration, and evaporation the residual syrup was chromatographed on a cellulose column using *n*-butanol one-half saturated with water as the mobile phase. The solvent eluted firstly 1,8-nonanediol β-D-glucopyranoside (396 mg), which was recrystallized twice from ethyl acetate to give material with m.p. 102–104° C and $[\alpha]_D$ −27° (*c*, 1.8, H_2O). Calculated for $C_{15}H_{30}O_7$: C, 55.9%; H, 9.4%. Found: C, 55.7%; H, 9.4%. This was followed by unhydrolyzed glycoside (670 mg) and glucose (396 mg), which were not positively identified. *n*-Butanol – ethanol – water (4:1:1 v/v) was then passed through the column to elute sophorose (163 mg), which after two crystallizations from methanol–ethanol, gave 80 mg of the α-anomer with m.p. 198–202° C (25) and $[\alpha]_D$ +27° → 19° (*c*, 0.9, H_2O; constant, 42 hours). Calculated for $C_{12}H_{22}O_{11}$: C, 42.1%; H, 6.5%. Found: C, 41.9%; H, 6.5%. Acetylation of the disaccharide gave the β-octaacetate with m.p. 192–195° C (25).

Methylation of 1,8-Nonanediol β-Sophoroside

Nonanediol sophoroside (647 mg) was methylated six times by the dimethyl sulphate – aqueous sodium hydroxide method (26). At the end of the methylation excess reagent was destroyed by heating the solution on a steam bath for 3 hours. It was then cooled and neutralized with aqueous sulphuric acid and then shaken three times with chloroform. The extract was dried ($MgSO_4$), filtered, and evaporated to a syrup (754 mg) which was methylated further with silver oxide – methyl iodide under reflux (27). The methylated product (623 mg) had —OCH_3: 39.1% (calculated for $C_{21}H_{32}O_4(OCH_3)_8$: OCH_3, 41.6%), n_D^{27} 1.4583 and $[\alpha]_D$ −4° (*c*, 2.6, hexane).

The fully methylated glycoside was refluxed overnight in 4% methanolic hydrogen chloride (20 cc) and then *N* hydrochloric acid (10 cc) was added and the methanolic component boiled off. After 20 hours heating on a steam bath the solution was shaken with hexane to remove 1-methoxy-8-nonanol (57 mg), which had n_D^{24} 1.4410 and $[\alpha]_D$ +8° (*c*, 1.9, hexane) after vacuum distillation. The aqueous portion was neutralized (Ag_2CO_3), filtered, and taken down to a syrup (443 mg) which was chromatographed

on cellulose using benzene–ethanol–water (500:50:1 v/v) as solvent. 2,3,4,6-Tetra-*O*-methyl-D-glucose (164 mg) was eluted and recrystallized twice from hexane to give crystals, m.p. and mixed m.p. 80–81° C. Calculated for $C_6H_8O_2(OCH_3)_4$: OCH_3, 51.6%. Found: OCH_3, 51.4%. 3,4,6-Tri-*O*-methyl-D-glucose (144 mg) (28) came off later and two recrystallizations from ether–hexane gave 37 mg of product with m.p. 77–79° C and $[\alpha]_D$ +107° (3 minutes) → 75° (*c*, 0.9, H_2O; 16 hours, constant value). Calculated for $C_6H_9O_3(OCH_3)_3$: OCH_3, 41.9%. Found: OCH_3, 41.4%. On treatment with 1% lead tetraacetate in acetic acid (0.9 molar equivalents) the sugar consumed 0.24, 0.47, and 0.68 molar equivalents of oxidant after 3, 10, and 25 minutes respectively, thus indicating that the 1 and 2 positions of the sugar are not substituted with methoxyl groups (29).

Methylation of 17-Hydroxyoctadecanoic Acid β-Sophoroside

The glycoside (2 g) was methylated with dimethyl sulphate – sodium hydroxide and Purdie's reagent as described for the nonanediol sophoroside. The fully methylated product (1.94 g) n_D^{25} 1.4620 had OCH_3, 32.9%. Calculated for $C_{30}H_{28}O_5(OCH_3)_8$: OCH_3, 33.7%. The fully methylated glycoside was also hydrolyzed under similar conditions and the insoluble residue which accumulated was washed with water and hydrolyzed for 2 hours in 50% aqueous ethanol (20 cc) containing sodium hydroxide (0.5 g). The solution was added to excess water which was acidified (dil. H_2SO_4) and extracted with ether which was dried ($MgSO_4$), filtered, and evaporated to a solid (767 mg). Two recrystallizations from acetone gave 310 mg of 17-hydroxyoctadecanoic acid with m.p. 77–79° C and $[\alpha]_D$ +4.3° (*c*, 10.4, acetic acid). Calculated for $C_{18}H_{36}O_3$: C, 71.9%; H, 12.1%. Found: C, 71.6%; H, 12.0%. Two further recrystallizations from acetone elevated the melting point to 80.5–82° C (constant m.p.).

The aqueous portion of the hydrolyzate was neutralized and chromatographed on a cellulose column as described for the previous methylation. The products consisted of 2,3,4,6-tetra-*O*-methyl-D-glucose (0.43 g), which was crystallized twice from hexane to give a product, m.p. and mixed m.p. 79–80° C, and 3,4,6-tri-*O*-methyl-D-glucose (0.30 g), m.p. and mixed m.p. 77–80° C (two recrystallizations from ether – *n*-hexane).

ACKNOWLEDGMENTS

The authors wish to thank Mr. N. R. Gardner, Mr. L. L. Hoffman, and Miss S. F. Lubin for technical assistance, Miss I. M. Gaffney for infrared measurements and X-ray diffraction diagrams, and Mr. M. Mazurek for microanalytical determinations.

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