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A Synthesis of Phytosphingosines from D-Galactose

By Jill Gigg and Roy Gigg

D-*ribo*-2-Amino-1,3,4-trihydroxyoctadecane (C18-phytosphingosine) and D-*ribo*-2-amino-1,3,4-trihydroxyeicosane (C20-phytosphingosine) have been synthesised by a Wittig reaction between 2,3,5-tri-*O*-benzyl-4-deoxy-4-phthalimidoaldehydo-L-ribose and the appropriate long-chain phosphorane.

WE have described [1] a synthesis of phytosphingosines from 2-amino-2-deoxy-D-glucose. Before the oxazoline derivative used in that route was available we investigated other stereospecific syntheses of phytosphingosines using carbohydrate intermediates, and we describe elsewhere [2] a synthesis of the enantiomer of C20-phytosphingosine *via* 1,2,3,4-tetrahydroxyeicosane.

During the course of these syntheses we introduced the allyl ether as a protecting group for carbohydrates and this made possible the preparation of 1,3,4-tri-*O*-benzyl-L-galactitol (3,4,6-tri-*O*-benzyl-D-galactitol) [3] from D-galactose in good yield. This intermediate has been used to prepare 1,3,4-tri-*O*-benzyl-2-deoxy-2-phthalimido-L-talitol (VII) which has the D-*ribo* configuration required for phytosphingosine at carbon atoms 3, 4, and 5, and which could be converted into a derivative of phytosphingosine after oxidation with sodium metaperiodate and application of the Wittig reaction [4] to the aldehyde produced.

1,3,4-Tri-*O*-benzyl-L-galactitol was converted into the isopropylidene derivative (I) which gave the crystalline methanesulphonate (II). Treatment of the latter compound with sodium azide in dimethylformamide caused slow replacement of the methanesulphonyloxy-group by the azido-group with inversion of configuration.[5] The reaction proceeded more rapidly when dimethyl sulphoxide was used as solvent but unsaturated by-products were also formed, and therefore dimethylformamide was preferred. Reduction of the azide (III) with lithium aluminium hydride gave the amine (IV) which was characterised by conversion into the crystalline 2-acetamido-1,3,4-tri-*O*-benzyl-2-deoxy-L-talitol (VI). Treatment of this compound with sodium metaperiodate gave a syrup whose infrared spectrum showed an amide I peak at 1640 cm.$^{-1}$ but no peaks corresponding to amide II or aldehyde. The aldehyde formed therefore exists in the furanose form (VIII) with nitrogen in the ring.[6] Treatment of the syrup with toluene-*p*-sulphonic acid in methanol gave the methyl glycosides (IX) and subsequent hydrogenolysis of the benzyl groups gave methyl 4-acetamido-4-deoxy-L-ribofuranoside (X), which was characterised as the tri-*p*-nitrobenzoate (XI). Compound (XI) had the same melting point as that recorded for the D-isomer.[7]

To protect the amino-group, 2-amino-1,3,4-tri-*O*-benzyl-2-deoxy-5,6-*O*-isopropylidene-L-talitol (IV) was converted into the phthalimide (V). Nefkens and coworkers [8] have described the use of *N*-ethoxycarbonylphthalimide for the preparation of phthaloyl derivatives of amino-acids in aqueous solution, but as the amine (IV) was insoluble in water we used this reagent successfully with triethylamine as solvent.

Mild acid hydrolysis of the phthalimide (V) gave the glycol (VII), which was cleaved by treatment with sodium metaperiodate. The aldehyde formed was used in a Wittig reaction with the phosphorane derived from triphenyl-n-tridecyl- or n-pentdecyltriphenyl-phosphonium bromide to synthesise the D-ribo-1,3,4-tribenzyloxy-2-phthalimidoalk-5-enes (XII) and (XIII). Hydrogenation of these compounds gave the D-ribo-1,3,4-trihydroxy-2-phthalimido-alkanes (XIV and XV) which were

[1] J. Gigg, R. Gigg, and C. D. Warren, preceding Paper.
[2] R. Gigg and C. D. Warren, following Paper.
[3] J. Gigg and R. Gigg, *J. Chem. Soc.* (*C*), 1966, 82.
[4] A. Maercker, *Org. Reactions*, 1965, **14**, 270.
[5] B. R. Baker and A. H. Haines, *J. Org. Chem.*, 1963, **28**, 442.
[6] W. A. Szarek and J. K. N. Jones, *Canad. J. Chem.*, 1964, **42**, 20.
[7] E. J. Reist, D. E. Gueffroy, and L. Goodman, *J. Amer. Chem. Soc.*, 1965, **87**, 677.
[8] G. H. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. Tran. chim.*, 1960, **79**, 688.

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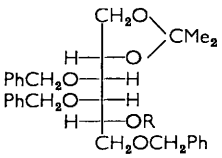
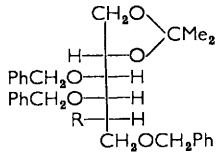
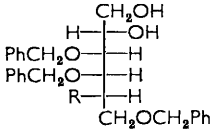
treated with hydrazine hydrate in ethanol to remove the phthaloyl group.[9] The D-ribo-2-amino-1,3,4-trihydroxyalkanes (XVI and XVII) formed were characterised as the " acetone compounds " and *N*-benzoyl derivatives. The melting points and infraredspectra of the derivatives of the synthetic C18-compound (XVI) were identical



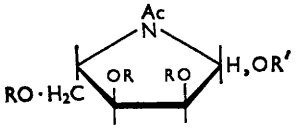
(I) R = H
(II) R = SO_2Me



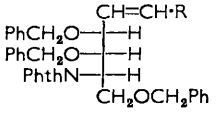
(III) R = N_3
(IV) R = NH_2
(V) R = NPhth



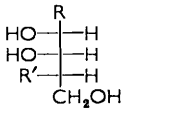
(VI) R = NHAc
(VII) R = NPhth



(VIII) R′ = H; R = CH_2Ph
(IX) R′ = Me; R = CH_2Ph
(X) R′ = Me; R = H
(XI) R′ = Me; R = *p*-NO_2·C_6H_4·CO_2

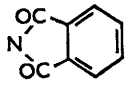


(XII) R = $[CH_2]_{11}$·CH_3
(XIII) R = $[CH_2]_{13}$·CH_3



(XIV) R = $[CH_2]_{13}$·CH_3; R′ = NPhth
(XV) R = $[CH_2]_{15}$·CH_3; R′ = NPhth
(XVI) R = $[CH_2]_{13}$·CH_3; R′ = NH_2
(XVII) R = $[CH_2]_{15}$·CH_3; R′ = NH_2
(XVIII) R = $[CH_2]_{13}$·CH_3; R′ = NH·COPh
(XIX) R = $[CH_2]_{15}$·CH_3; R′ = NH·COPh

NPhth =



with those of the corresponding derivatives of natural C18-phytosphingosine kindly donated by Dr. H. E. Carter and Dr. P. W. O'Connell.

The " acetone compound " formed when natural phytosphingosine is recrystallised from acetone has been described previously [10,11] but no structure has been proposed. The ready formation of these compounds under non-acidic conditions and the lability to aqueous sodium hydroxide excludes a 1,3-dioxolan structure and the compound is assumed to be an oxazolidine.[12]

This route to phytosphingosine is not as convenient as that described in the previous Paper because several intermediates are syrups requiring chromatographic purification. However, as other 1,3,4-tri-*O*-benzyl-2-deoxy-2-phthalimidohexitols can be prepared by similar methods this route affords a general stereospecific preparation of 2-amino-1,3,4-trihydroxyalkanes, and as such it is superior to that described in the following Paper since the number of stages after the Wittig reaction is considerably reduced.

EXPERIMENTAL

For general details see preceding Paper.[1]

1,3,4-*Tri*-O-*benzyl*-5,6-O-*isopropylidene*-L-*galactitol* (I).—A solution of 1,3,4-tri-*O*-benzyl-L-galactitol [3] (20 g.) and toluene *p*-sulphonic acid (250 mg.) in acetone (500 ml.) was kept at room temperature for 3 hr. The solution was neutralised with ammonium hydroxide and the acetone evaporated in the presence of potassium carbonate. The residue was extracted with ether (2 × 250 ml.) and the ether solution was washed with water, dried (K_2CO_3), and evaporated to give the *isopropylidene derivative* (I) as an oil (21·5 g.). For analysis a sample was chromatographed on silica gel using ether–light petroleum (1 : 1) for elution, $[\alpha]_D^{21}$ −20·5° (*c* 2 in chloroform) (Found: C, 73·0; H, 7·1. $C_{30}H_{36}O_6$ requires C, 73·1; H, 7·4%).

1,3,4-*Tri*-O-*benzyl*-5,6-O-*isopropylidene*-2-O-*methanesulphonyl*-L-*galactitol* (II).— Methanesulphonylchloride (6 ml.) was added dropwise to a solution of 1,3,4-tri-*O*-benzyl-5,6-*O*-isopropylidene-L-galactitol (20·5 g.) in dry pyridine (100 ml.) at 0° and the mixture was set aside at room temperature for 3 hr. Water and ice were added and the mixture was extracted with ether (3 × 150 ml.). The ether was washed with saturated aqueous potassium chloride, sufficient ice cold N-hydrochloric acid to remove the pyridine, and saturated aqueous sodium hydrogen carbonate. After drying ($MgSO_4$), evaporation of the ether gave an oil which crystallised on standing. Recrystallisation from methanol gave the *methanesulphonate* (20·5 g.) as prisms, m. p. 80—81°, $[\alpha]_D^{23}$ −5° (*c* 2 in chloroform) (Found: C, 65·25; H, 6·45; S, 5·4. $C_{31}H_{38}O_8S$ requires C, 65·2; H, 6·7; S, 5·6%).

2-*Azido*-1,3,4-*tri*-O-*benzyl*-2-*deoxy*-5,6-O-*isopropylidene*-L-*talitol* (III).—A mixture of 1,3,4-tri-*O*-benzyl-5,6-*O*-isopropylidene-2-*O*-methanesulphonyl-L-galactitol (20 g.) and sodium azide (8 g.) in dry *NN*-dimethylformamide (100 ml.) was stirred at 100° for 10 hr. when thin-layer chromatography (t.l.c.) [ether–light petroleum (1 : 1) as mobile phase] showed complete conversion of the methanesulphonate (R_f 0·5) to the azide (R_f 0·8). The mixture was diluted with ice–water and extracted with light petroleum (3 × 100 ml.). The extract was washed with saturated aqueous sodium hydrogen carbonate, dried (K_2CO_3), and evaporated. Chromatography of the crude product on alumina using ether–light petroleum mixtures for elution gave the *azide* as an oil (11·5 g.), $[\alpha]_D^{24}$ −3·3° (*c* 0·7 in chloroform) (Found: C, 69·4; H, 7·0; N, 8·2. $C_{30}H_{35}N_3O_5$ requires C, 69·6; H, 6·8; N, 8·1%).

2-*Amino*-1,3,4-*tri*-O-*benzyl*-2-*deoxy*-5,6-O-*isopropylidene*-L-*talitol* (IV).—The azide (III) (18 g.) in dry ether (100 ml.) was added slowly to a stirred suspension of lithium aluminium hydride (1·3 g.) in ether (100 ml.) at 0°. After the addition, the mixture was heated under reflux for 2 hr. Ethyl acetate was added to decompose the excess of lithium aluminium hydride followed by sufficient water to decompose the complex. After filtration, the ether solution was dried and evaporated to give the amine (IV) as an oil (16·5 g.) which was used without further purification.

[9] H. R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 1926, 2348; H. J. Barber and W. R. Wragg, *ibid.*, 1947, 1331.
[10] F. Reindel, A. Weickmann, S. Picard, K. Luber, and P. Turula, *Annalen*, 1940, **544**, 116.
[11] H. E. Carter, W. D. Celmer, W. E. M. Lands, K. L. Mueller, and H. H. Tomizawa, *J. Biol. Chem.*, 1954, **206**, 613.
[12] E. D. Bergman, *Chem. Rev.*, 1953, **53**, 309.

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2-*Acetamido*-1,3,4-*tri*-O-*benzyl*-2-*deoxy*-L-*talitol* (VI).—The amine (IV) (2 g.) was acetylated using acetic anhydride in pyridine, and, after hydrolysis with 0·1N-hydrochloric acid in aqueous methanol to remove the isopropylidene group, the *acetate* (VI) was obtained as needles (1·2 g.) from aqueous methanol, m. p. 119—121°, $[\alpha]_D^{23}$ −6° (*c* 2·5 in chloroform) (Found: C, 70·6; H, 7·05; N, 2·9. $C_{29}H_{35}NO_6$ requires C, 70·6; H, 7·15; N, 2·8%).

Methyl 4-*Acetamido*-4-*deoxy*-L-*ribofuranoside* (X).—The diol (VI) (2 g.) was treated with sodium metaperiodate (1 g.) in 25% aqueous methanol (100 ml.) for 2 hr. The methanol was evaporated and the residue extracted with ether. The extract was washed with water, dried ($MgSO_4$), and evaporated yielding the furanose (VIII) as a syrup (R_F 0·3, in ether), $\nu_{max.}$ 1640 cm.$^{-1}$. This product was heated under reflux in methanol (50 ml.) with toluene-*p*-sulphonic acid (0·2 g.). After neutralisation with sodium hydrogen carbonate the methanol was evaporated and the methyl glycosides (IX) (R_f 0·65 in ether) were isolated with ether and chromatographed on silica gel using ether as eluent. The product (1·8 g.) in glacial acetic acid (100 ml.) was hydrogenated at 22°/1 atm. with 10% palladium on charcoal catalyst until uptake was complete. Removal of the catalyst and evaporation of the solvent gave methyl 4-acetamido-4-deoxy-L-ribofuranoside (X) as a syrup. This was converted into the tri-*p*-nitrobenzoate, which was recrystallised from ethyl acetate–light petroleum, m. p. 175°, $[\alpha]_D^{24}$ −114·7° (*c* 0·4 in chloroform) (Found: C, 53·2; H, 3·7; N, 8·5. $C_{29}H_{24}N_4O_{14}$ requires C, 53·4; H, 3·7; N, 8·6%) (lit.,[7] m. p. 175·5—177° for the D-isomer).

1,3,4-*Tri*-O-*benzyl*-2-*deoxy*-5,6-O-*isopropylidene*-2-*phthalimido*-L-*talitol* (V).—A solution of the amine (IV) (16·5 g.) and *N*-ethoxycarbonylphthalimide (10 g.) in dry triethylamine (50 ml.) was kept at 50° for 2 hr. The triethylamine and some urethane were removed by distillation under reduced pressure and the residue was dissolved in ether, washed with water, ice-cold *N*-hydrochloric acid, and saturated aqueous sodium hydrogen carbonate, and then dried and evaporated. Chromatography of the residue on alumina, eluting with ether–light petroleum (2 : 1) gave the *product* (16 g.) as an oil, $[\alpha]_D^{25}$ −29·5° (*c* 0·9 in chloroform) (Found: C, 73·1; H, 6·6; N, 2·4. $C_{38}H_{39}NO_7$ requires C, 73·4; H, 6·3; N, 2·25%).

D-ribo-1,3,4-*Tribenzyloxy*-2-*phthalimido*-*octadec*-5-*ene* (XII).—A solution of the phthalimido compound (V) (16 g.) in methanol (250 ml.) and *N*-hydrochloric acid (25 ml.) was heated under reflux for 30 min., cooled, and neutralised with sodium hydrogen carbonate solution. After evaporation of the acetone and methanol the residue was extracted with ether (3 × 100 ml.) and the extract was washed with saturated aqueous potassium chloride, dried ($MgSO_4$), and evaporated to yield 1,3,4-tri-*O*-benzyl-2-deoxy-2-phthalimido-L-talitol (VII) (14·7 g.) as an oil. Thin-layer chromatography (ether as mobile phase) indicated that the product (R_f 0·65) did not require purification. The diol (VII) (11·5 g.) was oxidised with sodium metaperiodate (4·5 g.) in 25% aqueous methanol (400 ml.) at room temperature for 1 hr. Water (50 ml.) was added and the methanol evaporated. Extraction of the residue with chloroform gave the aldehyde (R_f 0·9, ether as mobile phase) which was dried under high vacuum for 2 hr. and then used immediately. The Wittig reaction using the aldehyde in dry tetrahydrofuran (50 ml.) and the phosphorane, prepared in dry tetrahydrofuran (200 ml.) from triphenyl-n-tridecylphosphonium bromide (13 g.) and phenyl-lithium (25 ml.; N-solution in ether), was carried out, as described previously.[1] The crude product was chromatographed on alumina, eluting with light petroleum–ether mixtures. The fractions were examined by t.l.c. [ether–light petroleum (1 : 3) as mobile phase] and the *product* (XII) (R_f 0·5) was obtained as an oil (7 g.) $[\alpha]_D^{21}$ −59·3° (*c* 0·6 in chloroform) (Found: C, 78·7; H, 8·1; N, 2·0. $C_{47}H_{57}NO_5$ requires C, 78·8; H, 8·0; N, 2·0%).

D-ribo-1,3,4-*Trihydroxy*-2-*phthalimido*-*octadecane* (XIV).—The tribenzyl ether (XII) (7 g.) in glacial acetic acid (50 ml.) was hydrogenated at 22°/1 atm. in the presence of 10% palladium–charcoal until the calculated quantity of hydrogen had been consumed. After removal of the catalyst and solvent the residue was dissolved in ether and washed with saturated aqueous sodium hydrogen carbonate. The ether was evaporated, and crystallisation of the residue from n-hexane followed by aqueous methanol gave the product (3·1 g.), m. p. 87—89°, $[\alpha]_D^{25}$ −38·9° (*c* 1 in pyridine) (Found: C, 69·4; H, 8·9; N, 3·2. $C_{26}H_{41}NO_5$ requires C, 69·75; H, 9·2; N, 3·1%).

D-ribo-2-*Amino*-1,3,4-*trihydroxyoctadecane* (XVI).—The phthaloyl derivative (XIV) (0·57 g.) was heated under reflux in ethanol (100 ml.) containing hydrazine hydrate (100%; 1 ml.) for 2 hr. The solution was poured into water and the amine (XVI) separated as a fine precipitate, which was washed with water and dried. Recrystallisation from acetone gave the *acetone compound* (0·37 g.) as needles, m. p. 108—109°, undepressed on admixture with natural C18 phytosphingosine "acetone compound," $[\alpha]_D^{23}$ +21° (*c* 1 in pyridine), $[\alpha]_D^{24}$ +15·4° (*c* 1 in chloroform) (Found: C, 70·3; H, 11·8; N, 3·9. $C_{21}H_{43}NO_3$ requires C, 70·5; H, 12·1; N, 3·9%) [lit.,[10] m. p. 108—109°; $[\alpha]_D$ +15·5° (*c* 1 in chloroform); lit.,[11] m. p. 105—107°, $[\alpha]_D$ +18°]. The infrared spectra of the "acetone compounds" of the base (XVI) and natural C18-phytosphingosine were identical.

D-ribo-2-*Benzamido*-1,3,4-*trihydroxyoctadecane* (XVIII).—Treatment of the base (XVI) with benzoyl chloride in pyridine and subsequent hydrolysis of the *O*-benzoyl groups with saturated aqueous potassium hydroxide in methanol gave D-*ribo*-2-benzamido-1,3,4-trihydroxyoctadecane, which crystallised as needles from ethyl acetate, m. p. 132—133°, $[\alpha]_D^{28}$ +4° (*c* 3 in pyridine) (Found: C, 70·85; H, 10·1; N, 3·3. Calc. for $C_{25}H_{43}NO_4$: C, 71·2; H, 10·3; N, 3·3%) (see preceding Paper for lit. values).

D-ribo-2-*Amino*-1,3,4-*trihydroxyeicosane* (XVII).—This compound was prepared by the above method using the phosphorane derived from n-pentadecyltriphenylphosphonium bromide [13] in the Wittig reaction. The following compounds were characterised:

D-ribo-1,3,4-*Tribenzyloxy*-2-*phthalimidoeicos*-5-*ene* (XIII); $[\alpha]_D^{24}$ −57° (*c* 3·5 in chloroform) (Found: C, 78·7; H, 8·2; N, 2·0. $C_{49}H_{61}NO_5$ requires C, 79·1; H, 8·3; N, 1·9%).

D-ribo-1,3,4-*Trihydroxy*-2-*phthalimidoeicosane* (XV): m. p 88—90°, $[\alpha]_D^{24}$ −0·7° (*c* 0·35 in chloroform), $[\alpha]_D^{23}$ −35·8 (*c* 1 in pyridine) (Found: C, 70·6; H, 9·6; N, 3·0. $C_{28}H_{45}NO_5$ requires C, 70·7; H, 9·5; N, 2·9%).

Acetone Compound of Base (XVII): m. p. 108—110°, $[\alpha]_D^{25}$ +21·7 (*c* 0·5 in pyridine), $[\alpha]_D^{24}$ +18·4° (*c* 0·6 in chloroform) (Found: C, 71·35; H, 12·4; N, 3·6. $C_{23}H_{47}NO_3$ requires C, 71·6; H, 12·3; N, 3·6%).

D-ribo-2-*Benzamido*-1,3,4-*trihydroxyeicosane* (XIX): m. p.

[13] J. Cunningham and R. Gigg, *J. Chem. Soc.*, 1965, 2968.

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134—136°, $[\alpha]_D^{25}$ +3·7° (*c* 0·4 in pyridine) (Found: C, 72·4; H, 10·5; N, 3·1. $C_{27}H_{47}NO_4$ requires C, 72·1; H, 10·5; N, 3·1%) [lit.,[1] m. p. 136—137°, $[\alpha]_D^{24}$ +3·6° (*c* 1 in pyridine)]. The infrared spectra of this compound and the C18-compound (XVIII) were identical with the spectra of the corresponding compounds prepared as described in the preceding Paper.

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