Phosphorylated Sugars. Part XVIII.[1] Synthesis of D-*glycero*-D-*gulo*-Heptose 4-(Dihydrogen Phosphate)

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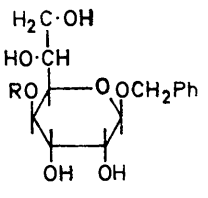
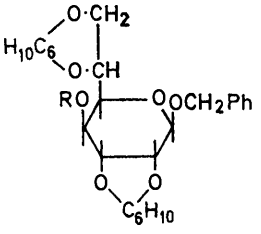
Condensation of benzyl β-D-*glycero*-D-*gulo*-heptopyranoside with cyclohexanone gave the 2,3:6,7-di-*O*-cyclohexylidene derivative, the structure of which was established by mass spectrometry of its benzoate. Phosphorylation of the dicyclohexylideneheptoside, followed by acidic hydrolysis to remove the cyclohexylidene groups, yielded the benzyl heptoside 4-phosphate, which was de-*O*-benzylated by hydrogenolysis.

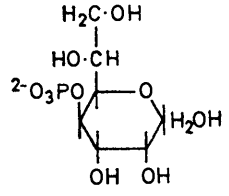
In continuation of our work [2] on the synthesis of phosphorylated heptoses for the purposes of studying their behaviour in the various reactions used to determine the



(I) R = H
(V) R = PO_3^{2-}



(II) R = H
(III) R = Bz
(IV) R = PO_3^{2-}



(VI)

structures of bacterial lipopolysaccharides containing such esters, we now report the synthesis of D-*glycero*-D-*gulo*-heptose 4-(dihydrogen phosphate).

Condensation of benzyl β-D-*glycero*-D-*gulo*-heptopyranoside [3] (I) with cyclohexanone gave a single dicyclohexylidene derivative (II). Mass spectrometry of its benzoate (III) showed one of the cyclohexylidene groups to be at the 6,7-position (cleavage between C-5 and -6 to give two major peaks, *m/e* 423 and 141). As the 3- and 4-hydroxy-groups are *trans*, it was concluded that the second cyclohexylidene group was attached to the *cis* hydroxy-groups on C-2 and -3. The negative Cotton effect observed in o.r.d.[4] was in accord with the presence of a 4-*O*-benzoyl group.

The dicyclohexylideneheptoside was phosphorylated with phosphoric trichloride to yield the 4-phosphate (IV), from which the cyclohexylidene groups were removed by acidic hydrolysis. Since under acidic conditions the possibility of phosphate migration exists, the structure of the phosphorylated benzyl heptoside was proved by periodate oxidation. The ester reduced exactly 2 mol. equiv. of periodate rapidly, no more periodate being reduced during the following 48 h; 1 mol. equiv. of formaldehyde was formed simultaneously. Of all the possible esters of the benzyl heptoside, only the 2- and the 4-phosphates would behave in this way. The former, however, would then suffer over-oxidation,[5] which was not observed in the present case. Hydrogenolysis of the phosphorylated benzyl heptoside (V) yielded D-*glycero*-D-*gulo*-heptose 4-(dihydrogen phosphate) (VI), isolated as its barium salt.

The amounts of inorganic phosphate liberated by this and by other phosphorylated sugars under three sets of conditions of acidic hydrolysis (*cf.* ref. 2) are shown in the Table.

EXPERIMENTAL

All evaporations were carried out under reduced pressure below 40°. M.p.s were taken on a Kofler hot-stage apparatus. Optical rotations were obtained with a Perkin-Elmer 141 polarimeter, o.r.d. spectra with a Fica Spectropol 1 spectropolarimeter, and mass spectra with an Atlas CH4

Acidic hydrolyses of sugar phosphates; yields (%) of inorganic phosphate

Phosphorylated sugar	N-H_2SO_4 (3 h; 100°)	0·1N-H_2SO_4 (3 h; 100°)	$CF_3{\cdot}CO_2H$, pH 2·43 (50 h; 50°)
D-*glycero*-D-*gulo*-Heptose 4-phosphate	21·6	9·7	2·5
Methyl α-D-glucoside 4-phosphate [a]	15	7·7	2·1
Methyl β-D-galactoside 4-phosphate [a]	15·2	9·1	2·3
D-*glycero*-D-*galacto*-Heptose 6-phosphate [b]	9·3	4·2	2·4
D-Glucose 6-phosphate	5·5	3·8	1·6
Methyl α-D-glucoside 6-phosphate [a]	4·2	2·5	1·1
Methyl β-D-galactoside 6-phosphate [a]	5	4·2	1·5
D-*glycero*-L-*manno*-Heptose 7-phosphate [c]	4·5	3·4	1·5
Methyl β-D-*glycero*-L-*manno*-heptoside 7-phosphate [c]	2·6	1·7	1

[a] P. Szabó and L. Szabó, *J. Chem. Soc.*, 1960, 3762. [b] D. R. Strobach and L. Szabó, *J. Chem. Soc.*, 1963, 3970. [c] Ref. 2.

[1] Part XVII, F. Trigalo, M. Level, and L. Szabó, preceding paper.
[2] P. Szabó, *J.C.S. Perkin I*, 1974, 920.
[3] E. Glaser and N. Zuckermann, *Z. physiol. Chem.*, 1927, **166**, 103.
[4] J. H. Brewster, *Tetrahedron*, 1961, **13**, 106; N. Harada, M. Ohashi, and K. Nakanishi, *J. Amer. Chem. Soc.*, 1968, **90**, 7349.
[5] J. E. Barnett, R. E. Brice, and D. L. Corina, *Biochem. J.*, 1970, **119**, 183.

spectrometer. Preparative t.l.c. was performed on glass plates coated with Merck silica gel 60 PF_{254} (1·5 mm) with ethyl acetate–hexane (1 : 3 v/v) as developing solvent. Benzoylation was carried out as previously described.[2] All compounds were dried *in vacuo* over phosphorus pentoxide. Periodate was estimated by the method of Avigad [6] and formaldehyde by the method of MacFadyen.[7]

Benzyl β-D-glycero-D-gulo-*Heptopyranoside* (I).—This compound, prepared from benzyl 2,3,4,6,7-penta-*O*-acetyl-β-D-*glycero*-D-*gulo*-heptopyranoside as previously described,[3] had m.p. 139—141° (lit.,[3] 147—148°), $[\alpha]_D^{26}$ −55° (*c* 1·19 in H_2O) {lit.,[3] $[\alpha]_D^{18}$ −43° (*c* 1·19 in H_2O)} (Found: C, 55·9; H, 7·0. Calc. for $C_{14}H_{20}O_7$: C, 56·0; H, 6·65%).

Benzyl 2,3:6,7-*Di*-O-*cyclohexylidene*-β-D-glycero-D-gulo-*heptopyranoside* (II).—A suspension of compound (I) (2 g) in cyclohexanone (3 ml) containing concentrated sulphuric acid (0·13 ml) was vigorously shaken overnight. Heptane (10 ml) was added and the solution was decanted from a small amount of oil, which was washed with heptane. The heptane solution was washed with aqueous sodium hydrogen carbonate, then with water, dried (Na_2SO_4), and evaporated. The syrupy residue (3 g) was dissolved in ethyl acetate–hexane (1 : 3 v/v; 4 ml) and chromatographed on a column (3·5 cm diam.) of silicic acid (Mallinkrodt 100 mesh) (120 g) which was eluted with the same solvent mixture at 40 ml h^{-1}. The fractions (4 ml) containing the dicyclohexylideneheptoside were pooled and concentrated to dryness. The *heptoside* (2·4 g) had $[\alpha]_D^{22}$ −85° (*c* 1·26 in EtOH) (Found: C, 67·85; H, 8·25. $C_{26}H_{36}O_7$ requires C, 67·85, H, 7·85%).

Benzyl 4-O-*Benzoyl*-2,3:6,7-*di*-O-*cyclohexylidene*-D-glycero-D-gulo-*heptopyranoside* (III).—Compound (II) (0·1 g) in pyridine (4 ml) was treated with benzoyl chloride (0·1 ml). The precipitate was purified by preparative t.l.c. The *benzoate* had $[\alpha]_D^{22}$ −100° (*c* 1 in $CHCl_3$) (Found: C, 70·15; H, 7·1. $C_{33}H_{40}O_8$ requires C, 70·2; H, 7·1%).

Benzyl β-D-glycero-D-gulo-*Heptopyranoside* 4-(*Dihydrogen Phosphate*) (V).—Phosphoric trichloride (0·8 g) in pyridine (15 ml) cooled to −30° was added dropwise to a stirred solution of the dicyclohexylideneheptoside (II) (2 g) in pyridine (16 ml) also at −30°. The mixture was stirred for 12 h at room temperature and a few drops of water were added. The pyridine was removed and the syrupy residue was triturated several times with cold water until the washings remained neutral. The residue was then triturated with water to which 0·5N-barium hydroxide solution was added until the pH remained stable at 9. Trituration was continued until all the syrup had been transformed into a white precipitate (2·4 g), which was then centrifuged off, washed with water, and dried. This salt (0·5 g) was suspended in water (17 ml) and stirred at 100° for 1 h 40 min with Amberlite IR120 (H^+) resin. The resin was filtered from the cooled solution and the pH of the filtrate was adjusted to 6·9 with N-lithium hydroxide. The solution was concentrated to dryness and the residue (0·3 g) was dissolved in water. The solution was treated with IR120 (H^+) resin, neutralised with pyridine, and chromatographed on a column (3·5 × 30 cm) of Whatman CC 31 microcrystalline cellulose powder, which was eluted with propan-1-ol–pyridine–water (12 : 1 : 7) at 6 ml h^{-1}. The fractions (3 ml) containing the benzyl heptoside 4-phosphate were pooled and concentrated to dryness. The residue was dissolved in water and 0·5N-barium hydroxide solution was added (to pH 10). The solution was evaporated to dryness and the residue was triturated with ethanol. The *precipitate* (0·1 g) was centrifuged off, washed with ethanol, dried, and equilibrated in air. It had $[\alpha]_D^{22}$ +5° (*c* 0·5 in 0·5N-HCl) (Found: C, 30·0; H, 3·9; P, 5·55. $C_{14}H_{19}BaO_{10}P,2H_2O$ requires C, 30·5; H, 4·15; P, 5·6%).

D-glycero-D-gulo-*Heptose* 4-(*Dihydrogen Phosphate*) (VI).—The heptoside phosphate (V) (70 mg) in water (5 ml) was treated with IR120 (H^+) resin and hydrogenated over palladium. The pH of the solution was adjusted to 6·9 with 0·4N-barium hydroxide and the solution was concentrated to dryness. The residue was triturated with ethanol, centrifuged off, dried, and equilibrated in air. The 4-*phosphate* had $[\alpha]_D^{22}$ +2·8° at equilibrium (*c* 0·5 in 0·1N-HCl) (Found: C, 17·9; H, 3·9; P, 6·6. $C_7H_{13}BaO_{10}P,2·5H_2O$ requires C, 17·9; H, 3·8; P, 6·6%).

The acidic hydrolyses of the sugar phosphates were carried out as previously described.[2]

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[6] G. Avigad, *Carbohydrate Res.*, 1969, **11**, 119.
[7] D. A. MacFadyen, *J. Biol. Chem.*, 1945, **158**, 107.