MONOSACCHARIDES

COMMUNICATION 30. SYNTHESIS OF D-GLYCERO-L-MANNO-HEPTOSO 4-PHOSPHATE

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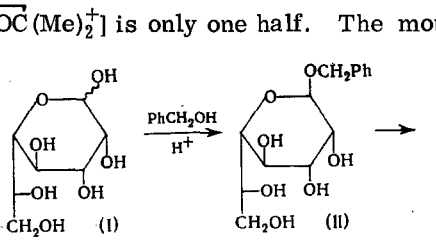
The polysaccharide chain of the O-antigens of gram-negative bacteria contains L-glycero-D-manno-heptoso 4-phosphate, the structure of which was recently proved by a series of chemical transformations directly on the polysaccharide chain [1]. The heptoso phosphate was isolated in the free form only in chromatographic amounts and its properties were not described [2]. As a continuation of studying the synthetic use of the isopropylidene derivatives of heptose [3], in the present paper we studied the acetonation of β-benzyl-D-glycero-L-manno-heptopyranoside (II) and, on the basis of the obtained data, synthesized D-glycero-L-manno-heptoso 4-phosphate (X), an enantiomer of the natural heptoso phosphate.

The starting D-glycero-L-manno-heptose (I) was obtained by the nitromethane method for D-galactose [4]. The crystalline heptoside (II) was obtained from heptose (I) and benzyl alcohol by the Fischer method in 42% yield, the presence of a benzyl group in which was confirmed by the characteristic absorption maximum at 258.5 nm [5], while the β-configuration of the glycoside linkage was proved by the shape of the ORD (optical rotation dispersion) curve [6].

The acetonation of benzylheptoside (II) in the presence of anhydrous $CuSO_4$ leads mainly to 2,3:6,7-di-O-isopropylidene-β-benzyl-D-glycero-L-manno-heptopyranoside (IIIa), along with the formation of a small amount of the 2,3:4,7-di-O-isopropylidene derivative (IVa). Information on the structure of both acetonides was obtained from the mass spectra and analysis of the methylation products. The fragmentation of (IIIa) and (IVa) is typical for the isopropylidene derivatives of sugars [7]: together with the M^+ ion with m/e 380 are present ions with m/e 365 ($M-CH_3$), 289 ($M-PhCH_2$), 273 ($M-PhCH_2O$), and a series of ions that are formed by the cleavage of $(CH_3)_2CO$, CH_3COOH, $CH_2=C=O$, and H_2O molecules from the primary fragments.

The methylation of (IIIa) gave methyl ether (IIIb), the removal of the benzyl group in which by refluxing with Raney Ni in alcohol leads to the di-O-isopropylidene-4-O-methyl-heptopyranose (IIIc) in high yield. The acid hydrolysis of the (IIIc) derivative, and subsequent reduction with KBH_4 and trifluoroacetylation, gives the hexa-O-trifluoroacetyl(TFA)-4-O-methyl-heptitol (VI), the structure of which unequivocally follows from its mass spectrum. Only the structure of (IVa) corresponds to the structure of hexa-O-TFA-6-O-methyl-heptitol (VII), which was obtained by a similar scheme from the minor acetonide of (IVa), and also using $NaBD_4$, and which was identified by the mass spectrum. Additional data also speak in support of this structure. The mass spectrum of the diacetonemethylheptopyranose (IVc), which is formed by the debenzylation of (IVb), differs from the mass spectrum of (IIIc) and, in particular, the relative intensity of the ion with m/e 101 $[\overline{OCH_2CHOC}(Me)_2^+]$ is only one half. The monomethyl-heptose obtained by the



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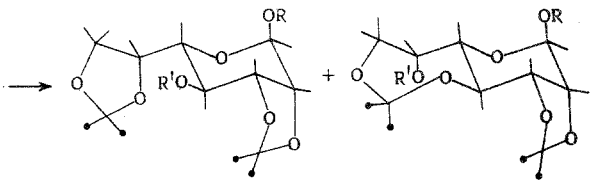
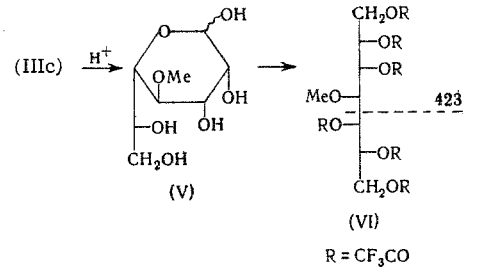


(III) R = $PhCH_2$; R' = H (a)
R = $PhCH_2$; R' = Me (b)
R = H; R' = Me (c)

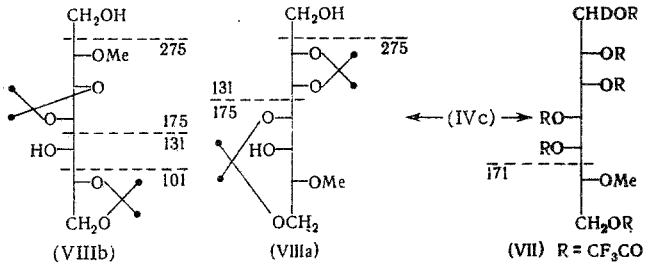
(IV) R = $PhCH_2$; R' = H (a)
R = $PhCH_2$; R' = Me (b)
R = H; R' = Me (c)



(V)

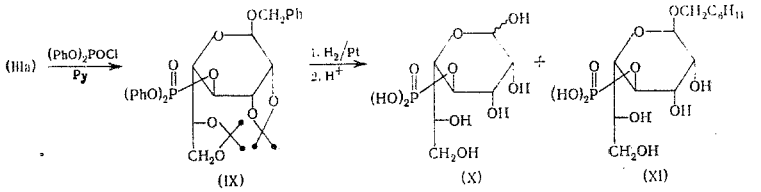
(VI)

R = CF_3CO



(VIIIb) (VIIIa) (VII) R = CF_3CO

acid hydrolysis of (VIc) gives a color with triphenyltetrazole, which is not characteristic for 2-O-substituted aldoses. The peaks of the ions with m/e 101 and 75, which are characteristic for the structure of di-O-isopropylidene-2-O-methyl-heptitol (VIIIb), are absent in the mass spectrum of the borohydride reduction product (VIc), while the ions with m/e 275, 175, and 131, which are characteristic for both heptitols (VIIIa) and (VIIIb), are present in the spectrum



(IX) (X) (XI)

Acetonide (IIIa) was phosphorylated using diphenyl chlorophosphate,* as a result of which the diphenyl 4-O-phosphate (IX) was obtained in high yield, the structure of which was proved by the NMR and mass spectral data. The NMR spectrum of diphenyl phosphate (IX) has the signals of the protons of four CH_3 groups, which correspond to two isopropylidene moieties, and of three phenyl rings, which correspond to the benzyl and two phenyl groups. The mass spectrum of (IX) has characteristic ions with m/e 612 (M^+), 597 ($M-CH_3$), 539 (597 $-(CH_3)_2CO$), 521 ($M-C_6H_5CH_2$), 505 ($M-C_6H_5CH_2OH$), 463 (521 $-(CH_3)_2CO$), 251 $[(C_6H_5O)_2P(OH)_2^+]$, 233 $[(C_6H_5O)_2P{=}O^+]$, and also a number of rearranged fragments that contain phosphorus.

Next we attempted to successively remove the protective groups from diphenyl phosphate (IX). Unexpectedly, it proved that the benzyl group in (IX) is stable under hydrogenolysis conditions on Pd/C, while the compound is irreversibly adsorbed on the catalyst when it is refluxed in alcohol with Raney Ni. Consequently, we ran the hydrogenolysis of (IX) on Adams catalyst, although a priori it could be expected that the removal of the benzyl group will be incomplete due to hydrogenation of the aromatic ring [9]. Actually, based on the paper chromatography data, the hydrogenation of diphenyl phosphate (IX) over Pt and subsequent removal of the isopropylidene groups gives two compounds in the hydrogenolysis products, which are

* Initially acetonide (IIIa) was phosphorylated using o-phenylene chlorophosphate, but the subsequent removal of the o-phenylene protection as described in [8] gave a complex mixture of phosphorus-containing products in the given case.

easily separated by fractionation on a cellulose column and were isolated in respective yields of 39 and 59%. The less mobile substance is heptoso phosphate (X), since during detection on paper it reacts both as a reducing sugar and as a phosphate, while during electrophoresis it behaves like a monosubstituted sugar phosphate. The more mobile compound (XI) proved to be a nonreducing phosphate-containing sugar, which on acid hydrolysis under the conditions for the cleavage of glycoside linkages [10] was converted to heptoso phosphate (X), and failed to display the maximum characteristic for benzyl groups in the UV spectrum. From the presented data it follows that (XI) is the cyclohexylmethylheptoside, which is formed by the hydrogenation of the aromatic ring of the benzyl group in derivative (IX). When the cyclohexylammonium (CHA) salts of both phosphates were prepared we isolated the monosubstituted CHA salts as amorphous powders, although usually sugar phosphates form the disubstituted CHA salts. To prove the position of the phosphate group in heptoside (XI) we oxidized its CHA salt with $Pb(OCOCH_3)_4$ [11]. Two equivalents of oxidizing agent are absorbed during oxidation and 0.9 mole of CH_2O is liberated, which was identified as the dimedon derivative. These results indicate that the phosphate is attached at C_4 in (XI). But since heptoside (XI) on hydrolysis is converted to heptoso phosphate (X), then the position of the phosphate group in the latter may be considered proved.

EXPERIMENTAL METHOD

The chromatography on FN 11 paper (PC) was run by the ascending method in the system: butanol–alcohol–water, 3:2:2 (A). To detect the reducing sugars on the paper we used aniline acid phthalate, for the nonspecific detection we used $AgNO_3$ and alkali, while the sugar phosphates were detected using the molybdenum reagent for phosphorus [12]. The $R_{heptose}$ values are given relative to D-glycero-L-manno-heptose. The TLC was run on plates covered with a loose layer of SiO_2 in the following system: aqueous butanol–ethyl acetate, 6:4 (B), and chloroform–methanol, 97:3 (C), 99:1 (D), and 95:5 (E). The compounds were detected on the plates using conc. H_2SO_4. The chromatographic separation of the compounds on SiO_2 columns was run in $CHCl_3$. The electrophoresis was run on FN 11 paper in 0.25 M triethylammonium carbonate buffer solution at a potential gradient of 15 V/cm. The GLC analysis was run on a Pye Argon chromatograph instrument, equipped with a β-ionization detector, on 120 × 4 mm glass columns that were packed with 5 and 10% SE-30 deposited on silanized Chromosorb W (60-80 mesh) (respectively columns A and B), at an argon flow rate of 60 ml/min. The NMR spectrum was taken on a Varian DA-60-IL instrument in CCl_4 relative to HMDS (δ scale), while the mass spectra were taken on a Varian CH-6 instrument at an ionizing voltage of 70 eV. The specific rotations were determined on a model 141 Perkin–Elmer polarimeter; the melting points were taken on a Kofler microblock. The solvents were evaporated in vacuo at 40-50°.

β-Benzyl-D-glycero-L-manno-heptopyranoside (II). A mixture of 5 g of D-glycero-L-manno-heptose (I) (mp 78-80°, $[\alpha]_D^{20}$ −14.5° (C 2, water)) and 90 ml of $C_6H_5CH_2OH$, which contained 3% of HCl, was stirred at 60° until solution was complete. After 24 h at 20° the mixture was neutralized with Dowex anionite (CO_3^{2-}) and evaporated in vacuo. We obtained ~7 g of a syrup that, based on the TLC data, contained mainly a substance with R_f 0.57 (B) and a number of secondary products. To remove any possible furanosides the mixture was hydrolyzed with 75 ml of 0.2 N H_2SO_4 solution in 75 ml of MeOH (100°, 1 h). Heptose (I) with R_f 0.27 (B) was detected in the hydrolyzate. The syrup obtained after neutralization was chromatographed on a column containing 250 g of SiO_2, with elution using a gradient mixture of ethyl acetate–aqueous butanol, and here syrupy (II) was isolated, which was recrystallized from a 5:3 absolute alcohol–ethyl acetate mixture. We obtained 2.98 g (41.7%) of (II), mp 129-131°; $[\alpha]_D^{20}$ −93°; $[\alpha]_{578}$ −97.3°; $[\alpha]_{546}$ −110°; $[\alpha]_{436}$ −185.5°; $[\alpha]_{365}$ −288° (C, 1.93, CH_3OH). Based on the PC (R_f 0.76, $R_{heptose}$ 2, A) and GLC (as the trimethylsilyl derivative, column A, 210°, retention time 5.9 min) data the substance is homogeneous; UV spectrum of (II) (in 95% alcohol): λ_{max} 258.5 nm. Found: C 55.98; H 6.69%. $C_{14}H_{20}O_7$. Calculated: C 56.01; H 6.71%.

Acetonation of β-Benzyl-D-glycero-L-manno-heptopyranoside (II). To a suspension of 1.88 g of (II) in 75 ml of absolute acetone were added 10 g of anhydrous $CuSO_4$ and 2-3 drops of CH_3CHO. The mixture was stirred for 60 h, the precipitate was filtered and washed with acetone, and the combined filtrate was passed through a bed of filter cel and evaporated. We obtained 2.4 g of a syrup, which contained a main component with R_f 0.6 and a minor component with R_f 0.7 (TLC, two passes in system C). The mixture was separated by preparative TLC on 20 plates (18 × 24 cm) (two passes in the system: $CHCl_3$–MeOH, 98:2). We isolated 1.71 g (72%) of (IIIa) and 220 mg (9.2%) of (IVa). 2,3:6.7-Di-O-isopropylidene-β-benzyl-D-glycero-L-manno-heptopyranoside (IIIa) has mp 86-88° (heptane), $[\alpha]_D^{20}$ −31° (C 2, $CHCl_3$).

Found: C 63.21; H 7.50%. $C_{20}H_{28}O_7$. Calculated: C 63.14; H 7.42%. 2,3:4,7-Di-O-isopropylidene-β-benzyl-D-glycero-L-manno-heptopyranoside (IVa) has mp 97-98° (hexane–CCl_4), $[\alpha]_D^{20}$ −35.5° (C 2.1, $CHCl_3$). Found: C 63.08; H 7.38%. $C_{20}H_{28}O_7$. Calculated: C 63.14; H 7.42%.

Establishing the Structure of Acetonides (IIIa) and (IVa). A stirred mixture of 40 mg of (IIIa), 4 ml of CH_3I, and 200 mg of Ag_2O was heated at 40-50° for 4 h, the Ag salt was filtered and washed with $CHCl_3$, and the filtrate was evaporated. The residue was chromatographed on an SiO_2 column. We isolated 23 mg of (IIIb), $[\alpha]_D^{20}$ −38.2° (C 1.53, $CHCl_3$); based on the TLC (R_f 0.5, D) and GLC (column B, 209°, retention time 6.7 min) data the substance is homogeneous. Mass spectrum (m/e): 394 (M^+), 379 ($M-CH_3$), 303 ($M-C_6H_5CH_2$), 287 ($M-C_6H_5CH_2O$), and a series of derivative ions. A stirred mixture of 21 mg of (IIIb) and 7 ml of alcohol was refluxed with Raney Ni; based on the TLC data, the starting substance disappeared after 3 h and a substance with R_f 0.15 (D) appeared. The mixture was filtered through a bed of filter cel and evaporated. The residue was chromatographed on an SiO_2 column. We isolated 16.5 mg of a syrup (IIIc), $[\alpha]_D^{20}$ −8.5° (C 1.07, $CHCl_3$). Mass spectrum (m/e): 304 (M^+), 289 ($M-CH_3$), 231 [289 $-(CH_3)_2CO$], 171 (231 $-(CH_3COOH)$. To a solution of 14 mg of (IIIc) in 1 ml of glacial CH_3COOH was added 0.5 ml of water. The mixture was heated at 100° for 1 h, cooled, and lyophilized. We obtained 9 mg of a chromatographically homogeneous substance (V) (Pc, $R_{heptose}$ 1.46, A), $[\alpha]_D^{20}$ −20.3° (C 0.69, MeOH). The reduction of 9 mg of (V) with KBH_4 was run in methanol (20°, 24 h). After the usual workup we obtained 9 mg of the 4-O-methyl-heptitol, $[\alpha]_D^{20}$ −7.2° (C 0.69, MeOH), $R_{heptose}$ 1.3 (PC, A), which was acylated with 0.3 ml of $(CF_3CO)_2O$ in the presence of 1-2 mg of CF_3COONa (60-65°, 1 h). The mixture was evaporated and extracted twice with absolute benzene. Evaporation gave the trifluoroacetate (VI). Mass spectrum (m/e): 783 (M–F), 733 ($M-CF_3$), 689 ($M-CF_3COO$), 423 (C_1-C_4 and C_4-C_7 fragments), and secondary fragments that are formed from the primary fragments by the cleavage of CF_3COOH.

The methylation of 75 mg of (IVa) with 5 ml of CH_3I was run in the presence of 400 mg of Ag_2O for 5 h, after which an additional 5 ml of CH_3I and 400 mg of Ag_2O were added, and after 4 h the Ag salt was filtered and washed with $CHCl_3$, while the combined filtrate was evaporated. We obtained 77.5 mg of (IVb), mp 83-85° (petroleum ether), $[\alpha]_D^{20}$ −35.3° (C 4, $CHCl_3$). Based on the TLC (R_f 0.8, C) and GLC (column B, 209°, retention time 8.1 min) data the substance is homogeneous. Mass spectrum (m/e): 394 (M^+), 379 ($M-CH_3$), 303 ($M-C_6H_5CH_2$), 287 ($M-C_6H_5CH_2O$). A stirred mixture of 71 mg of (IVb) and Raney Ni in 20 ml of alcohol was refluxed for 3-4 h, after which the mixture was filtered through a bed of filter cel and evaporated. The residue was chromatographed on an SiO_2 column. We isolated 54.8 mg of (IVc) as a syrup, $[\alpha]_D^{27}$ +10.6° (C 2.74, $CHCl_3$), R_f 0.4 (TLC, C). The reduction of 20 mg of (IVc) with KBH_4 was run in alcohol (27°, 15 h). The mixture was evaporated, the residue was extracted with $CHCl_3$, and the extract was evaporated. The residue was chromatographed on an SiO_2 column. We isolated 18.9 mg of 2,3:4,7-di-O-isopropylidene-6-O-methyl-heptitol (VIIIa) as a syrup, $[\alpha]_D^{27}$ −55.5° (C 1.45, $CHCl_3$), R_f 0.38 (TLC, D). Mass spectrum (m/e): 291 ($M-CH_3$), 275, 175, 131, and a series of derivative ions. To a solution of 18 mg of the obtained heptitol in 3 ml of MeOH was added 3 ml of 2 N HCl solution and the mixture was refluxed for 2 h. After cooling, the mixture was neutralized with Dowex anionite (CO_3^{2-}) and evaporated. We obtained 13.4 mg of the 6-O-methyl-heptitol ($[\alpha]_D^{26}$ −1.5° (C 1, MeOH), $R_{heptose}$ 1.25, PC, A) as a syrup, the trifluoroacetylation of which gave hexatrifluoroacetate (VII), whose mass spectrum has the peaks of the ions with (m/e): 783 (M–F), 689 ($M-CF_3COO$), 675 (C_2–C_7 fragment), 171 (C_1–C_2 fragment), and secondary fragments that are formed from the primary fragments by the cleavage of CF_3COOH and $(CF_3CO)_2O$.

Diphenyl 2,3:6,7-Di-O-isopropylidene-β-benzyl-D-glycero-L-manno-heptopyranoside 4-O-Phosphate (IX). To a solution of 1.28 g of (IIIa) in 30 ml of pyridine, distilled over CaH_2, was added 3.5 ml of $(C_6H_5O)_2POCl$ and the mixture was kept at 20° for 48 h. Then the mixture was cooled to 0° and 4 ml of water was added, the pyridine was removed after 1 h with toluene in vacuo below 30°, and the residue was dissolved in 300 ml of benzene and washed in succession with cold water, 1% HCl solution (3 × 70 ml), 2% $NaHCO_3$ solution (3 × 70 ml), and cold water (2 × 50 ml), and dried over $MgSO_4$. After evaporation we obtained 1.55 g (75%) of (IX) as a syrup, $[\alpha]_D^{21}$ −20.3° (C 1.85, $CHCl_3$), R_f 0.4 (TLC, D). NMR spectrum: three singlets in the 1.18-1.4 ppm region (12 H, 2 $C(CH_3)_2$), singlet of H_1 (4.97 ppm), and 15 aromatic protons in the 7.1-7.2 ppm region. Found: C 62.69; H 5.91; P 4.91%. $C_{32}H_{37}PO_{10}$. Calculated: C 62.73; H 6.00; P 5.06%.

Hydrogenolysis of Diphenyl Phosphate (IX). A solution of 1.6 g of (IX) in 40 ml of alcohol was hydrogenated over 600 mg of Adams catalyst for 2 h, after which an additional 400 mg of catalyst was added, and the hydrogenation was continued for another 12 h (based on the TLC data, the starting substance disappeared completely). After separating the catalyst and evaporation we obtained 1.1 g of a syrupy product that did not show an absorption maximum in the 250-270 nm region.

Hydrolysis of Hydrogenolysis Product of Diphenyl Phosphate (IX). To 500 ml of a 1% aqueous CF_3COOH solution was added a solution of 1.1 g of the hydrogenolysis product of (IX) in 40 ml of MeOH and the mixture was refluxed for 45 min. The hydrolyzate was evaporated in vacuo and the residue was evaporated several times with toluene to remove traces of CF_3COOH, after which the residue was dissolved in a little water and neutralized with NH_4OH. Two phosphorus-containing substances were detected when the material was subjected to paper chromatography, the ammonium salt of (X) with R_f 0.25 (detected with aniline acid phthalate) and the ammonium salt of (XI) with R_f 0.52 (A). The mixture was fractionated on a cellulose column (40 × 4 cm) in the system: butanol–alcohol–water, 7:4:4. We isolated 345 mg (38.5%) of the ammonium salt of (X), $[\alpha]_D^{20}$ −22° (C 3.51, water), and 650 mg (59%) of the ammonium salt of (XI).

Preparation of Cyclohexylammonium Salts of Phosphates (X) and (XI). An aqueous solution of the ammonium salt of (X) was passed through a column that contained 25 ml of KU-2 (H^+). The eluate and wash waters were neutralized with a chilled emulsion of excess $C_6H_{11}NH_2$ in 7 ml of water, and the excess $C_6H_{11}NH_2$ was extracted with ether (3 × 50 ml). The aqueous layer was evaporated, the residue was dissolved in a little aqueous MeOH, and precipitation with acetone gave 400 mg of the CHA salt of (X) as an amorphous powder, $[\alpha]_D^{20}$ −3.5° (C 2, MeOH), $R_{heptose}$ 1.25 (PC, A). When subjected to electrophoresis the substance has the mobility of a glucoso 6-phosphate. Found: C 40.15; H 7.31; N 3.69; P 8.12%. $C_{13}H_{28}NPO_{10}$. Calculated: C 40.10; H 7.25; N 3.60; P 7.96%.

To obtain the CHA salt of (XI) we passed a solution of the ammonium salt of (XI) in a 10:5:2 alcohol $-CHCl_3$–water mixture through a column that contained KU-2 (H^+), and the eluate was treated with $C_6H_{11}NH_2$. Precipitation with acetone from $CHCl_3$ solution gave the CHA salt of (XI) as an amorphous powder, $[\alpha]_D^{20}$ −52° (C 2.3, MeOH), $R_{heptose}$ 1.75 (PC, A). The substance when subjected to electrophoresis has the mobility of a glucoso 6-phosphate. Found: C 49.58; H 8.36; N 3.18; P 6.50%. $C_{20}H_{40}NPO_{10}$. Calculated: C 49.49; H 8.31; N 2.89; P 6.38%.

Acid Hydrolysis of Phosphate (XI). The CHA salt of (XI) (50 mg) was passed through a column that contained 10 ml of KU-2 (H^+). The eluate was evaporated, and the residue was dissolved in 2.5 ml of 2 N HCl solution, followed by the addition of 2.5 ml of water. The mixture was then heated in a sealed ampule at 100° for 3 h. After cooling, the mixture was extracted with ether, evaporated in vacuo, and the residue was evaporated several times with water and then treated with $C_6H_{11}NH_2$. Precipitation with acetone from aqueous MeOH solution gave 38 mg of the CHA salt of (X), which in its mobility on paper and during electrophoresis, and also in its optical rotation, $[\alpha]_D^{20}$ −3.3° (C 2, MeOH), was identical with the above-described specimen of the CHA salt of (X).

Oxidation of Heptoside (XI) with Lead Tetraacetate. The CHA salt of (XI) was oxidized with $Pb(OCOCH_3)_4$ in glacial CH_3COOH as described in [11]. After 40 h the $Pb(OCOCH_3)_4$ consumption was 1.95 moles per mole of heptoside. The excess $Pb(OCOCH_3)_4$ was reduced with anhydrous $(HCOOH)_2$ and separated as lead oxalate. The amount of CH_2O in the filtrate was determined as the dimedon derivative (mp 190-192°) as described in [13]; here 1 mole of the CHA salt of (XI) gave 0.89 mole of CH_2O.

CONCLUSIONS

1. The acetonation of β-benzyl-D-glycero-L-manno-heptopyranoside gives mainly the 2,3:6,7-di-O-isopropylidene derivative.

2. The phosphorylation of the indicated acetonide and subsequent removal of the protective groups gave D-glycero-L-manno-heptoso 4-phosphate, which is an enantiomer of the natural heptose phosphate.

LITERATURE CITED

1. V. Lehmann, O. Lüdertiz, and O. Westphal, Europ. J. Biochem., 21, 339 (1971).
2. P. Müllradt, ibid., 11, 241 (1969).
3. B. A. Dmitriev, A. Ya. Chernyak, O. S. Chizhov, and N. K. Kochetkov, Izv. Akad. Nauk SSSR, Ser. Khim., 2298 (1972).
4. J. C. Sowden and H. O. L. Fischer, J. Am. Chem. Soc., 82, 954 (1960).
5. M. E. Tate and C. T. Bishop, Canad. J. Chem., 41, 1801 (1963).
6. J. Listowsky, G. Avigad, and S. Englard, J. Am. Chem. Soc., 87, 1765 (1965).
7. D. C. DeJongh and K. Biemann, ibid., 86, 67 (1964).
8. T. A. Khawaja and C. B. Reese, ibid., 88, 3446 (1966).
9. K. R. Farrar, J. Chem. Soc., 3131 (1949).

10. V. Lehmann, G. Hämmerling, M. Nurminen, J. Minner, E. Ruschmann, O. Lüderitz, T. T. Kuo, and A. D. Stocker, Europ. J. Biochem., 32, 268 (1973).
11. R. C. Hockett, M. T. Dienes, and H. E. Ramsden, J. Am. Chem. Soc., 65, 1474 (1943).
12. C. S. Hanes and F. A. Ischerwood, Nature, 164, 1107 (1949).
13. C. Brice and A. S. Perlin, Canad. J. Biochem. Physiol., 35, 7 (1957).