754. *Phosphorylated Sugars. Part VII.*[1] *Acid-catalysed Acetyl Group Migration in* 1,2,3,4,6-*Penta*-O-*acetyl*-β-D-glycero-D-galacto-*heptose and the Synthesis of* D-glycero-D-galacto-*Heptose*-6-*phosphate.*[2]

By Donald R. Strobach and L. Szabó.

1,2,3,4,6-Hexa-*O*-acetyl-7-*O*-trityl-β-D-*glycero*-D-*galacto*-heptose, when detritylated with hydrobromic acid in acetic acid, yields the 1,2,3,4,7-penta-acetate by an acid-catalysed acetyl migration. By phosphorylation of this penta-acetate and removal of the protecting groups, D-*glycero*-D-*galacto*-heptose 6-phosphate is obtained.

Aldoheptoses have recently been detected as components of specific polysaccharides and lipo-polysaccharides in Gram-negative bacteria. A detailed discussion of their occurrence in different micro-organisms is given by Davies.[3] Although paper-chromatographic techniques have shown several aldoheptoses to be present in the different micro-organisms, only L-*glycero*-D-*manno*-heptose [4] and D-*glycero*-D-*galacto*-heptose [5] have been unequivocally identified. To our knowledge, neither the biosynthesis nor the metabolic fate of any aldoheptose is known. That phosphate esters are likely to be intermediates of their metabolism is, however, suggested by the general biochemistry of sugars and, in particular, by the work of Slein and Schnell, who demonstrated that L-*glycero*-D-*manno*-heptose, isolated from the polysaccharide of *Shigella flexneri*, was esterified with phosphoric acid.[4] The chemical synthesis of phosphate esters of naturally occurring aldoheptoses is, therefore, clearly of interest and, accordingly, the synthesis of D-*glycero*-D-*galacto*-heptose 7-phosphate was attempted.

[1] Part VI, Rivaille and Szabó, *Bull. Soc. chim. France*, 1963, 716.

[2] Presented in part at the Vth International Congress of Biochemistry, Moscow, 1961; in the Abstracts of Communications the title appears incorrectly as D-glycero-D-galacto-heptose 7-phosphate (Abstract No. 1.66); the corrected paper was presented at this Congress.

[3] Davies, *Adv. Carbohydrate Chem.*, 1960, **15**, 271.

[4] Slein and Schnell, *Proc. Soc. Exp. Biol. N.Y.*, 1953, **82**, 734; Weidel, Koch, and Bobosch, *Z. Naturforsch.*, 1954, **9**b, 303.

[5] Maclennan and Davies, *Biochem. J.*, 1957, **66**, 562.

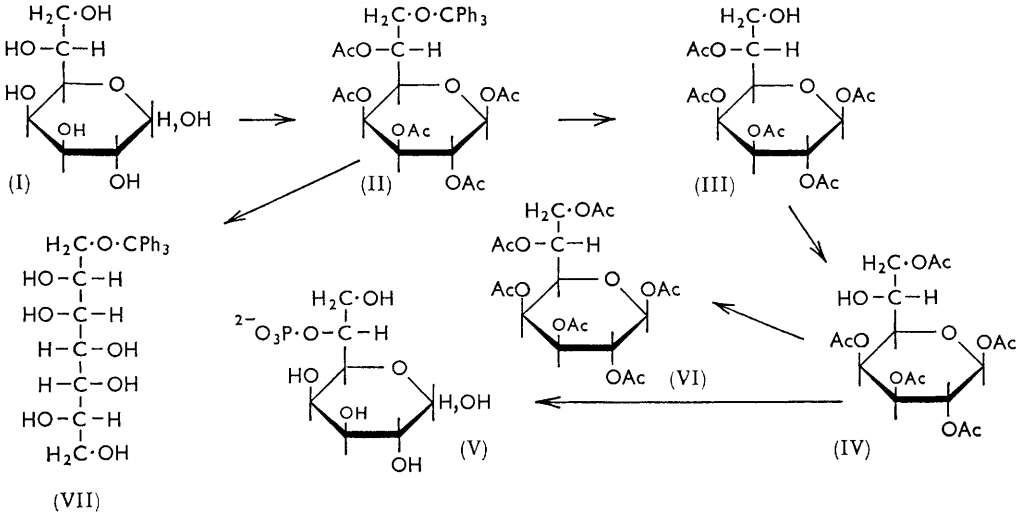
D-*glycero*-D-*galacto*-Heptose [6] (I) was converted, by successive treatments with triphenylmethyl chloride and acetic anhydride, into a mixture of the anomeric penta-*O*-acetyl-7-*O*-tritylheptoses, which were separated by crystallisation. Their molecular rotations ($[M]_\alpha = 56{,}990°$; $[M]_\beta = 12{,}175°$) are in reasonable agreement with those of the penta-*O*-acetyl-D-galactopyranoses ($[M]_\alpha = 41{,}610°$; $[M]_\beta = 9750°$) [7] and quite different from those of the corresponding galactofuranoses ($[M]_\alpha = 23{,}870°$; $[M]_\beta = -16{,}225°$) [7] or the aldehydo-sugar ($[M]_{ald.} = -9750°$) [7] and, therefore, the penta-*O*-acetyl-7-*O*-trityl-α- and -β-D-*glycero*-D-*galacto*-heptopyranose structures are assigned to these compounds. The infrared spectrum (KBr pellet) of the β-anomer (II) supports this assignment, showing a fairly strong band at 1126 cm.$^{-1}$, which, as Isball and his co-workers state,[8] may be indicative of an equatorial acetoxy-group of sugar acetates having a D-galactose configuration. A weak band at 852 cm.$^{-1}$ is ascribed to the α-anomeric C–H deformational vibration, while bands appearing at 676 and 905 cm.$^{-1}$ are considered to arise, respectively, from the symmetrical and the antisymmetrical vibration of the pyranose ring.[9] Further, detritylation, followed by acetylation of the crystalline penta-acetate thus obtained, yielded a hexa-acetate (see below), having physical constants very similar to those reported for " β-d-[α-mannoheptose] hexa-acetate " (β-D-*glycero*-D-*galacto*-heptose hexa-acetate),[10] thus confirming the postulated structure. The detailed characterisation of the penta-*O*-acetyl-7-*O*-tritylheptoses was considered necessary, as D-*glycero*-D-*galacto*-heptose, when acetylated with sodium acetate and acetic anhydride, may yield substantial amounts of the hexa-acetyl-*aldehydo*-sugar.[10]

Attempts to remove the trityl group of the β-anomer (II) by hydrogenation with palladium,[11] 5% palladium–carbon,[12] and palladium black [13] failed, unchanged starting material being recovered in each case. Detritylation with hydrobromic acid in acetic acid yielded a crystalline penta-acetylheptose, which was phosphorylated with diphenyl phosphorochloridate; the resulting oily, neutral ester was then submitted to hydrogenolysis to cleave the phenyl groups. After removal of the acetyl groups by treatment with sodium methoxide, a heptose monophosphate was obtained and isolated as the barium salt, which gave a correct elemental analysis and travelled as a single spot on paper chromatograms, showing the expected mobility when compared with ribose 5-phosphate and glucose 6-phosphate. From ion-exchange columns (Dowex 2 × 10, monochloroacetate form; elution with a solution 0·01M in acid and 0·01M in sodium monochloroacetate) the compound was eluted as a single, symmetrical peak, and was, therefore, considered to be pure. However, when treated with sodium periodate, the compound did not reduce the expected five mol. of this reagent, but only slightly less than four mol., an equal number of mol. of formic acid and no formaldehyde being formed. Of the heptose monophosphates only one, the 6-phosphate, is expected to behave in the above manner and, consequently, the heptose monophosphate obtained from the reaction sequence outlined above must be D-*glycero*-D-*galacto*-heptose 6-phosphate (V).

As the deacetylated sugar phosphate was exposed to acid only for a very short time, the 6-phosphate was not considered to have been formed by acid-catalysed phosphate migration. However, the 6-phosphate could have been formed if acetyl migration had occurred during the detritylation, yielding 1,2,3,4,7-penta-*O*-acetyl-β-D-*glycero*-D-*galacto*-heptose (IV), in which the 6-hydroxyl group is free. That this is probably the case is strongly indicated by the following experiments. The penta-*O*-acetyl-*O*-tritylheptose (II) was detritylated and the penta-*O*-acetylheptose isolated, all operations being carried out

[6] Sowden and Schaffer, *J. Amer. Chem. Soc.*, 1951, **73**, 4662.
[7] Pigman and Goepp, " Chemistry of the Carbohydrates," Academic Press Inc., New York, 1948, p. 152.
[8] Isbell, Smith, Creitz, Frush, Moyer, and Steward, *J. Res. Nat. Bur. Stand.*, 1957, **59**, 41.
[9] Barker, Bourne, Stacey, and Whiffen, *J.*, 1954, 171.
[10] Montgomery and Hudson, *J. Amer. Chem. Soc.*, 1934, **56**, 2463.
[11] *Org. Synth.*, Coll. Vol. I, 1941, p. 463.
[12] *Org. Synth.*, Coll. Vol. III, 1955, p. 685.
[13] Tausz and von Putnoky, *Ber.*, 1919, **52**, 1573.

in the presence of acetic acid so as to avoid base-catalysed acetyl migration. The product was identical with that obtained in previous experiments. When dissolved in pyridine and left at room temperature for a few hours, no change in optical rotation was observed and the penta-acetate was recovered in good yield, indicating that the acetyl migration, considered to be responsible for formation of the 6-phosphate, did not occur during the



phosphorylation which has been carried out in pyridine solution. The penta-acetate was next treated with triphenylmethyl chloride in pyridine and, after 70 hours, acetic anhydride was added to acetylate any remaining free hydroxyl groups. From the resulting mixture, over 90% of the original penta-acetate was isolated as the pure 1,2,3,4,6,7-hexa-*O*-acetyl-β-D-*glycero*-D-*galacto*-heptose.* Hence, it is highly unlikely that the 7-hydroxyl group is free in the penta-acetylheptose. Finally, in order to verify that in the penta-acetyltrityl-heptose (II) the trityl group does, in fact, block the primary hydroxyl group, this compound was deacetylated and then reduced with potassium borohydride. The crystalline *O*-tritylheptitol obtained (VII) reduced slightly less than five mol. of sodium periodate, which is the amount expected to react with a 1-tritylheptitol.

In view of these results, we consider that the 1,2,3,4,6-penta-*O*-acetyl-β-D-*glycero*-D-*galacto*-heptose (III), formed by detritylation of the penta-*O*-acetyl-7-*O*-tritylheptose (II), undergoes an acid-catalysed acetyl group migration to yield the stable 1,2,3,4,7-penta-*O*-acetylheptose (IV).

Although acid-catalysed *N*→*O*-acyl migrations are known to occur in amino-sugars [14] and amino-cyclitols,[15] examples of the corresponding *O*→*O*-acyl migration in the carbohydrate field are rare. The authors are aware of only two such cases. The first is the conversion of 1,6-di-*O*-benzoyl-D-mannitol into a mixture consisting of 1,4:3,6-dianhydro-D-mannitol 2,5-dibenzoate, 1,4-anhydro-2,6(or 3,6)-di-*O*-benzoyl-D-mannitol, and 2,5-anhydro-1,6-di-*O*-benzoyl-D-glucitol [16] in boiling tetrachloroethane in the presence of toluene-*p*-sulphonic acid; the second † is the rearrangement of 2,3,4,3′,4′-penta-*O*-acetylsucrose in

* In one experiment, which however could not be repeated, besides this amount of hexa-acetate, 9% of the penta-acetate was recovered as the original penta-acetyl-7-tritylheptose.

† The authors thank the Referees for drawing their attention to the acid-catalysed acetyl migration occurring in sucrose penta-acetate.

[14] Fodor and Ötvös, *Chem. Ber.*, 1956, **89**, 701.

[15] McCasland, *J. Amer. Chem. Soc.*, 1951, **73**, 2295; Quadbeck and Röhm, *Chem. Ber.*, 1956, **89**, 1645.

[16] Brigl and Grüner, *Ber.*, 1933, **66**, 1945; 1934, **67**, 1582; Hockett, Fletcher, jun., Sheffield, and Goepp, jun., *J. Amer. Chem. Soc.*, 1946, **68**, 927; Hockett, Fletcher, jun., Sheffield, Goepp, jun., and Soltzberg, *ibid.*, p. 930; Hockett, Zief, and Goepp, jun., *ibid.*, p. 935.

98% acetic acid [17] or glacial acetic acid at 40° [18] into the 2,3,6,3′,4′-penta-acetate. The conditions for these reactions are quite different from those prevailing in the present work and it is, indeed, surprising that reaction conditions which effect detritylation in the hexoses, in the hexitols, and in the case of sucrose, should bring about a smooth and rapid acetyl migration, in good yield, in this case. It remains to be seen whether it is an isolated example or a general reaction of aldoheptoses.

Experimental

Chloroform solutions were dried over sodium sulphate before removal of the solvent. Unless otherwise stated, evaporations were conducted under reduced pressure.

Periodate Titrations.—The compound (0·1 mmole) to be titrated was dissolved in a little water. If it was a barium salt, an equivalent amount of sodium sulphate in aqueous solution was added. Then acetate buffer (2·5 ml.; pH 3·2; acetic acid–sodium acetate, 1M) and twice the quantity of periodate expected to be reduced were added and the volume was made up to 25 ml. with water. A blank, in which the substrate only was omitted, was similarly prepared. At regular intervals an aliquot part of the solution (2 ml.) was added to a mixture of 0·01N-sodium arsenite (5 ml.) and saturated sodium hydrogen carbonate solution (5 ml.). A fresh, 20% solution of potassium iodide (0·5 ml.) was added, the mixture was set aside for 20 min. and the excess of arsenite was then titrated with 0·01N-iodine in the presence of a starch indicator. A similar quantity of the blank was also titrated to compensate for the spontaneous decomposition of periodate. The titration values were plotted as a function of time and the reaction was considered to be complete when two or more successive titrations give the same value. When this was not possible (*e.g.*, tritylheptitol), the titration values at given time intervals are indicated.

For the determination of the formic acid formed during treatment with periodate, the oxidation was carried out as above, but without addition of buffer and with an amount of periodate slightly in excess of that required for the oxidation of the compound in buffered medium. When two successive titrations showed that the periodate uptake was complete, the excess of periodate was destroyed with ethylene glycol and the acid formed was titrated, in an aliquot part, with standard base.

1,2,3,4,6-*Penta*-O-*acetyl*-7-O-*trityl*-D-glycero-D-galacto-*heptose*.—D-*glycero*-D-*galacto*-Heptose (32·1 g.) was added in one portion to dry pyridine (650 ml.) containing triphenylmethyl chloride (57·8 g.). The mixture was warmed to about 60° and shaken until all the heptose had dissolved. The solution was left overnight at room temperature. Acetic anhydride (170 ml.) was then added in small portions and the mixture again left overnight. It was then poured into vigorously stirred cold water (5 l.). The solid formed was filtered off, washed with water, dried, and dissolved in the minimum quantity of boiling ethanol. Slow cooling of this solution gave crystals (33·4 g.), having m. p. 181—184°. Recrystallisation of a small sample from ethanol yielded pure 1,2,3,4,6-*penta*-O-*acetyl*-7-O-*trityl*-β-D-glycero-D-galacto-*heptopyranose* (II), m. p. 182·5—184·5°, $[\alpha]_D^{20}$ +18·4° (*c* 2 in $CHCl_3$) (Found: C, 64·9; H, 6·0. $C_{36}H_{38}O_{12}$ requires C, 65·25; H, 5·8%).

The filtrates from the above were concentrated to an oil which was taken up in chloroform, washed, first, with dilute hydrochloric acid, then with water, and dried. The chloroform was removed and the residue dissolved in warm ethanol (300 ml.). Slow concentration of this solution produced crystals which recrystallised from ethanol to give pure 1,2,3,4,6-*penta*-O-*acetyl*-7-O-*trityl*-α-D-glycero-D-galacto-*heptopyranose* (4·0 g.), m. p. 105—107°, $[\alpha]_D^{20}$ +86° (*c* 2·5 in $CHCl_3$) (Found: C, 65·1; H, 5·6%).

1,2,3,4,7-*Penta*-O-*acetyl*-β-D-glycero-D-galacto-*heptopyranose* (IV).—The above β-isomer (5 g.) was dissolved in hot acetic acid (20 ml.), and the solution was cooled to 10°. To the stirred solution was added dry hydrogen bromide (0·61 g.) in acetic acid (4 ml.). After 45 sec., the mixture was filtered into cold water (250 ml.), and the precipitated triphenylmethyl bromide washed with cold acetic acid (5 ml.). The aqueous solution containing the detritylated compound was extracted with chloroform (3 × 50 ml.), and the chloroform layer was then washed with water (4 × 100 ml.) and dried. Concentration of this solution followed by addition of pentane to turbidity produced crystals. Further portions of pentane were then added until

[17] McKeown, Serenius, and Hayward, *Canad. J. Chem.*, 1957, **35**, 32; Lemieux and Barrette, *J. Amer. Chem. Soc.*, 1958, **80**, 2243.
[18] Bredereck, Zinner, Wagner, Faber, Greiner, and Huber, *Chem. Ber.*, 1958, **91**, 2824.

the last addition did not produce turbidity. The resulting crystals (2·72 g.) when filtered off and washed with pentane had m. p. 142—145°. The compound was recrystallised by dissolving it in a small amount of chloroform and slowly adding pentane. The *penta-acetate* had m. p. 145—146°, $[\alpha]_D^{20}$ +26·8° (*c* 2 in EtOH) (Found: C, 48·4; H, 5·9. $C_{17}H_{24}O_{12}$ requires C, 48·6; H, 5·8%).

*Attempted Tritylation of the Penta-*O*-acetylheptose* (IV).—A mixture of the above penta-acetate (153 mg.), triphenylmethyl chloride (130 mg.), and pyridine (1·5 ml.) was left at room temperature for 70 hr. Acetic anhydride (0·2 ml.) was then added followed, an hour later, by a few drops of water. The mixture was then taken up in chloroform, the solution washed with dilute hydrochloric acid and then with water. After removal of the solvent, hot di-isopropyl ether (5 ml.) was added to the residue. Crude hexa-*O*-acetyl heptose (VI) (see below) (159 mg., 94%) crystallised, having m. p. 106—109° and $[\alpha]_D^{20}$ +26° (*c* 2 in $CHCl_3$).

D-glycero-D-galacto-*Heptose* 6-(*Dihydrogen Phosphate*) (V).—To the above penta-*O*-acetylheptose (1 g.), dissolved in dry pyridine (7 ml.), was added, with stirring, diphenyl phosphorochloridate (0·7 g.) in dry pyridine (4 ml.). The mixture was left overnight at room temperature, then cooled in an ice-bath, and several drops of water were added to destroy the excess of phosphorylating agent. After an hour, the pyridine was removed. Water was added to the residue and removed, and this operation was repeated. The residue was then taken up in chloroform, washed with dilute hydrochloric acid and with water, and dried. After removal of the chloroform, methanol was added to the residue, and the methanol was removed. This operation was repeated several times. The resulting oil was then dissolved in methanol (20 ml.) and hydrogenated in the presence of platinum oxide (100 mg.). Hydrogen uptake (420 ml.) ceased after 4—5 hr.; the catalyst was then filtered off. To the filtrate, which was dried over calcium sulphate for 2 hr., a solution from sodium (0·3 g.) in methanol (7 ml.) was added; a white precipitate was formed immediately. The mixture was kept at room temperature for 36 hr. and poured on to a slight excess of Amberlite IR-120 (H^+) resin. Water was added to bring any undissolved product into solution, the resin was filtered off and washed with water, and the combined filtrates were immediately brought to pH 6·9 with aqueous barium hydroxide. Concentration of this solution to a small volume and addition of ethanol (*ca.* 10 vol.) gave a white precipitate which crystallised when heated and scratched. The *barium salt* (0·93 g.) was filtered off from the cold solution and washed with ethanol. It had $[\alpha]_D^{20}$ +26·8° (equil.; *c* 1 in H_2O) (Found: C, 19·9; H, 3·4; Ba, 32·0. $C_7H_{13}BaO_{10}P$ requires C, 19·8; H, 3·1; Ba, 32·3%). The heptose phosphate had the following R_F values: propan-1-ol–water–aqueous ammonia (*d* 0·88) (6 : 3 : 1), 0·24; ethanol–water–N-acetic acid buffer (pH 3·5) (80 : 9·2 : 0·8), 0·21; propan-1-ol–pyridine–water (1 : 1 : 1), 0·41; t-butyl alcohol–water–picric acid (80 ml. : 40 ml. : 4 g.), 0·16. The barium salt of the heptose monophosphate consumed 3·85 mol. of sodium periodate and liberated 3·82 mol. of formic acid; no formaldehyde was produced.

1,2,3,4,6,7-*Hexa*-O-*acetyl*-β-D-glycero-D-galacto-*heptopyranose* (VI).—The β-penta-*O*-acetyl-*O*-tritylheptose (8·34 g.) was detritylated as above. The penta-acetate was not isolated, but, after removal of the chloroform, was directly acetylated with acetic anhydride–pyridine. The mixture was worked up in the usual way and the product crystallised from isopropyl ether to give the hexa-acetate (5·0 g.), m. p. 108·5—110°. The filtrates gave additional material (0·5 g.), having m. p. 107—109°. The compound was recrystallised twice from isopropyl ether to give the pure hexa-acetate, m. p. 109—110°, $[\alpha]_D^{20}$ +30·4° (*c* 1 in $CHCl_3$) {lit.,[16] m. p. 107°, $[\alpha]_D^{20}$ +34·1° (*c* 1 in $CHCl_3$)} (Found: C, 49·7; H, 5·7. Calc. for $C_{19}H_{26}O_{13}$: C, 49·35; H, 5·7%). When this hexa-acetate was de-acetylated with sodium in methanol, the resulting heptose showed the same chromatographic behaviour on paper as authentic D-*glycero*-D-*galacto*-heptose.[19]

1-O-*Trityl*-L-glycero-D-manno-*heptitol* (7-O-*trityl*-D-glycero-D-galacto-*heptitol*) (VII).—The β-penta-*O*-acetyl-*O*-tritylheptose (0·50 g.) was suspended in methanol (3 ml.), and a trace of sodium in methanol was added. The mixture was shaken until dissolution was complete (about 90 min.) and then placed in the cold room overnight. This solution was then added, dropwise, to a stirred solution of potassium borohydride (35 mg.) in methanol (2 ml.) containing enough water to dissolve the borohydride. After complete addition, the mixture was left for 6 hr. during which an oil separated (this crystallised when triturated with methanol; it had m. p. >230° and left an ash on ignition; it was not further examined). The supernatant liquid was poured off and diluted with water. The oil which separated, crystallised after two

[19] Davies, *Biochem. J.*, 1957, **67**, 253.

days and recrystallised from water giving 1-O-*trityl*-L-glycero-D-manno-*heptitol* (75 mg.), m. p. 136—137°, $[\alpha]_D^{20}$ −1·6° (*c* 1 in pyridine) (Found: C, 67·9; H, 6·8. $C_{26}H_{30}O_7,\frac{1}{3}H_2O$ requires C, 67·8; H, 6·7%). The compound consumed the following amounts of sodium periodate:

Time (hr.)	1·5	8·25	22·5
$NaIO_4$ consumed (mol.)	4·72	4·87	5·37

The authors thank the U.S. Department of Health, Education, and Welfare for a Postdoctoral Fellowship award to D. R. S.

CENTRE DE RECHERCES SUR LA CELLULE NORMALE ET CANCÉREUSE,
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE, VILLEJUIF,
SEINE, FRANCE. [*Received, December 12th*, 1962.]