*Carbohydrate Research*, 49 (1976) 489–493


## Note

# Formation of 1,6-anhydro-L-*glycero*-D-*manno*-heptopyranose during acidic hydrolysis and methanolysis of a polysaccharide from *Bordetella pertussis**

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(Received December 4th, 1975; accepted for publication, January 13th, 1976)

It has been observed[1,2] that during acidic hydrolyses of L-*glycero*-D-*manno*-heptose-containing bacterial lipopolysaccharides, the heptose gives rise to two peaks when the resulting alditol acetates are analysed by g.l.c. The same authors noted that when the enantiomeric (and more readily accessible) D-*glycero*-L-*manno*-heptose was subjected to the same hydrolytic conditions, two peaks were also observed. The peak having the higher retention time was assigned to the heptitol peracetate; the other was not identified. Angyal and Dawes[3] have reported that acidic treatment of D-*glycero*-L-*manno*-heptose followed by acetylation gave, in addition to two peaks for the sugar, a peak having a lower retention time. It was assumed that this peak could be assigned to an anhydroheptose to which, from theoretical stereochemical considerations, they ascribed the 1,7-anhydro structure.

By using g.l.c.–m.s. of the alditol acetates, we have now investigated the products of acidic hydrolysis of an L-*glycero*-D-*manno*-heptose-containing polysaccharide[4] from *Bordetella pertussis* endotoxin and have also found that the heptose gave rise to two peaks. The same two peaks were obtained by similar acidic treatment of D-*glycero*-L-*manno*-heptose. Moreover, methanolysis of the polysaccharide gave, after acetylation of the reaction mixture, in addition to peaks for the acetylated methyl heptosides, the same unknown peak although in lesser amounts than for acidic hydrolysis. The same results were obtained upon methanolysis of D-*glycero*-L-*manno*-heptose and of its methyl glycoside.

The mass spectrum of the unknown peak suggested it to be a 1,6-anhydroheptose, but because Angyal and Dawes[3] had postulated the formation of a 1,7-anhydride for this sugar, definite proof of structure was sought. This was obtained by comparison of the mass spectrum with those of authentic peracetylated 1,6- and 1,7-anhydrides of D-*glycero*-D-*gulo*-heptose. The latter sugar is known[5] to give a mixture of the two anhydrides on acidic treatment. The mixture was prepared as described by Angyal and Dawes[3], and the acetates were subjected to g.l.c.–m.s. The

*Dedicated to the memory of Professor Edward J. Bourne.

peak corresponding to the 1,7-anhydride was identified by cochromatography with an authentic sample[5] which had been acetylated. Angyal and Dawes[3] reported yields of 8.9 and 66% for the 1,6- and 1,7-anhydro-D-*glycero*-D-*gulo*-heptopyranoses. Using their conditions, we found that the ratio of 1,6- to 1,7-anhydride was ~2:1 and that the combined yield of anhydroheptoses did not exceed 20% of the residual heptose.

The main differences between the spectra of the 1,6- and the 1,7-anhydro-D-*glycero*-D-*gulo*-heptopyranoses reside in the different, relative intensities of certain

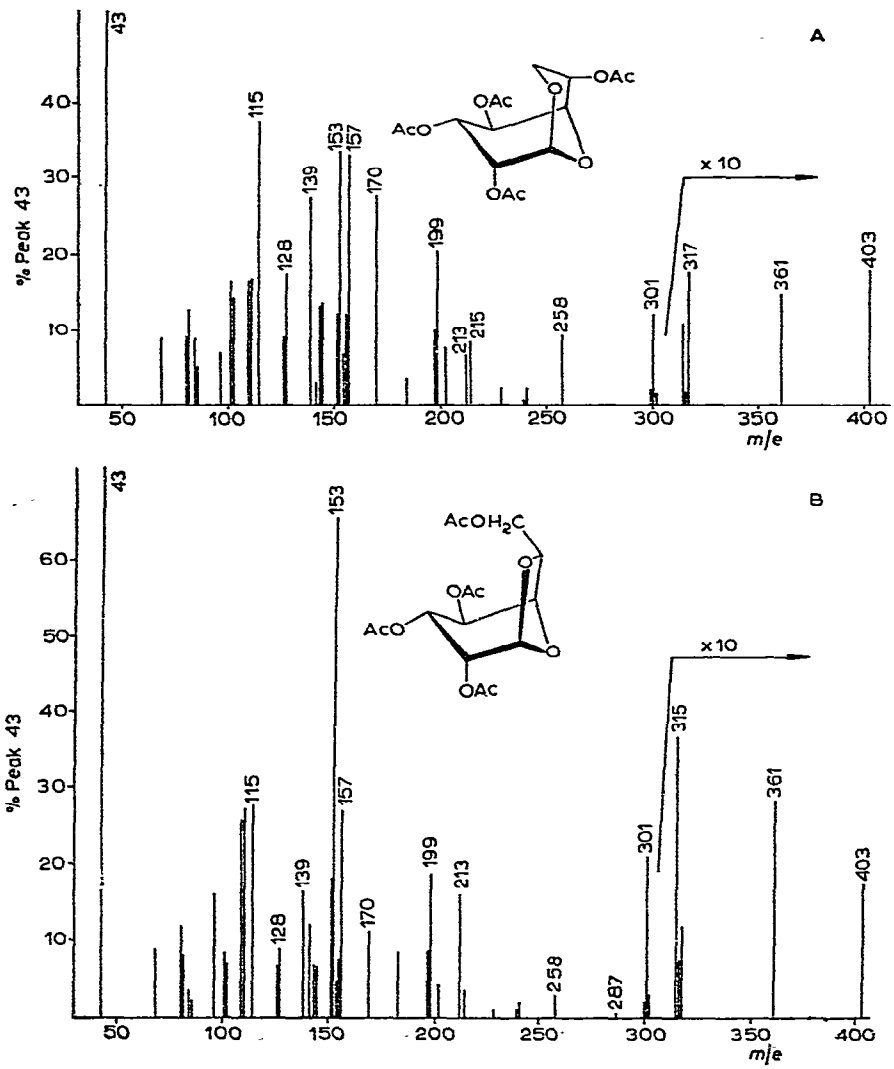
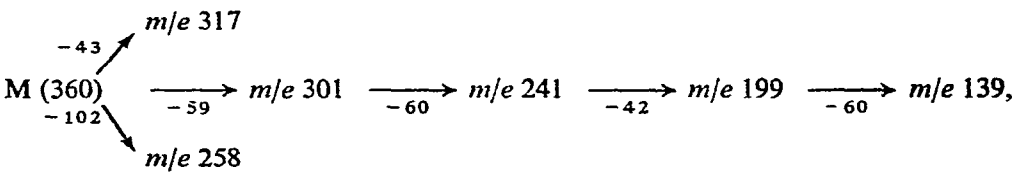
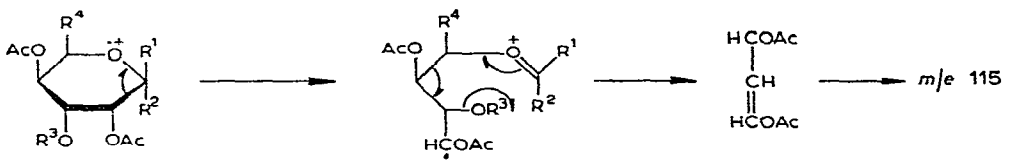



Fig. 1. Mass spectra of the tetra-acetates of 1,7-anhydro (A) and 1,6-anhydro (B) derivatives of D-*glycero*-D-*gulo*-heptose.

series of peaks (Fig. 1). Some peaks, arising from the following fragmentations, are common to both spectra:

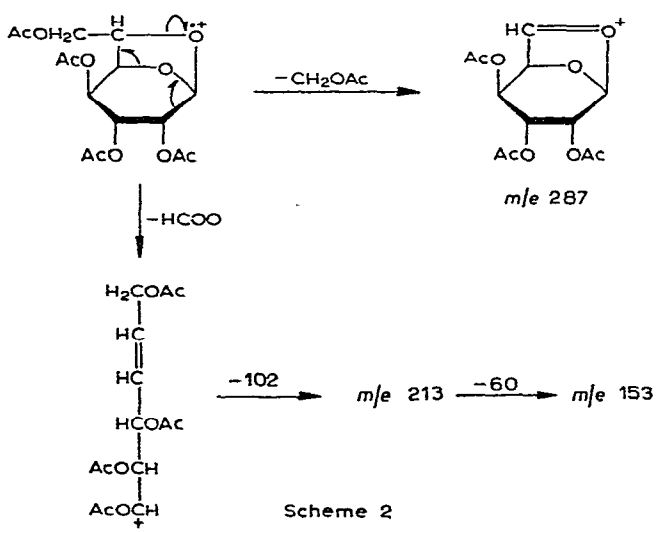



as are the peaks *m/e* 157 and 115 which are found in the spectra of all aldopyranoses acetylated in positions 3 and 4 and which are formed as shown in Scheme 1.

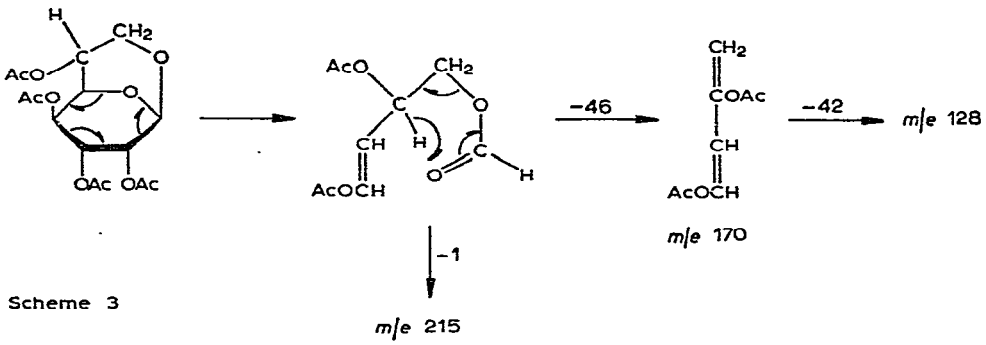



Scheme 1

Certain peaks that are more intense in the spectrum of the 1,6- than in that o the 1,7-anhydroheptose may be explained by the fragmentations shown in Scheme 2, whereas those that are more intense in the spectrum of the 1,7-anhydride may arise as shown in Scheme 3. When large quantities of substance are present in the source of the mass spectrometer, peaks at M+1 and M+43 are observed. These disappear as the quantities of substance in the source diminish. This phenomenon has already been noted for other acetylated derivatives[6].




Scheme 2




Scheme 3

EXPERIMENTAL

*General.* — Mass spectra were recorded with a DuPont 21-492 B spectrometer coupled to a Varian Aerograph gas–liquid chromatograph. The spectra were obtained by electron impact at 70 eV at a source temperature of 250°. G.l.c. was performed on (*a*) a column (1/8 in. × 5 ft) packed with 3% of methyl silicone SE 30 on Varaport 30 and (*b*) a column (1/8 in. × 10 ft) packed with 5% of XE 60 on Varaport 30. Acetylations were performed with acetic anhydride–sodium acetate at 110° for 1 h, acetic anhydride was removed by co-distillation with toluene, and solutions of the residues in ethyl acetate were used for g.l.c.–m.s.

*1,6- and 1,7-Anhydro*-D-glycero-D-gulo-*heptopyranoses.* — D-*glycero*-D-*gulo*-Heptose[7] (10 mg) in 0.25M sulphuric acid (4 ml) was heated in an oil bath at 100° for 28 h. After working-up, the mixture of acetates was chromatographed on column (*a*) using a programme of 2°/min from 170° to 250°. The retention times of the 1,7- and 1,6-anhydroheptoses (present in a ratio of ~1:2) with respect to the major peak of the peracetylated heptose were 0.60 and 0.65, respectively.

*Acid treatment of* D-glycero-L-manno-*heptose.* — The heptose[8] (10 mg) was heated at 100° for 1 h with 4M hydrochloric acid (5 ml). The solution was evaporated to dryness, water was removed by co-distillation with toluene, and the residue was acetylated. By g.l.c., 36% of the residual sugar was shown to be present as the 1,6-anhydroheptose, identified by its mass spectrum. On column (*a*), with a programme of 8°/min from 100° to 250°, it has a retention time (*T*) of 0.91; on column (*b*) heated isothermally at 240°, $T = 0.59$ with respect to the major peak of the corresponding peracetylated heptose, and 1.0 with respect to peracetylated D-glucitol.

*Methanolysis.* — (*a*) After incubation of dry polysaccharide (2 mg) with 2M anhydrous, methanolic hydrogen chloride (1 ml) at 90° for 24 h, concentration of the solution, and acetylation of the residue, 7% of residual heptose was present as the 1,6-anhydride.

(*b*) Under the same conditions, D-*glycero*-L-*manno*-heptose (1.75 mg) and methyl β-D-*glycero*-L-*manno*-heptopyranoside[8] (1.56 mg), each in 5 ml of methanolic hydrogen chloride, gave, respectively, 6 and 8% of 1,6-anhydroheptose.

ACKNOWLEDGMENTS

We thank Dr. N. K. Richtmyer for a gift of D-*glycero*-L-*manno*-heptose, and Mr. E. Zissis for a sample of 1,7-anhydro-D-*glycero*-D-*gulo*-heptose.

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