The Reaction of Amino-compounds with Sugars. Part III. The Action of Ammonia on Melibiose.*

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Melibiose in aqueous ammonia is isomerised to melibiulose and 6-*O*-α-D-galactopyranosyl-β-D-mannose. Alkaline degradation of melibiose then takes place with the production of D-tagatose and D-galactose.

In Part II,* ammonia was shown to isomerise maltose and lactose to maltulose and lactulose and then to cause fission of these 1 : 4-linked disaccharides to D-glucose and D-galactose respectively. It was of interest therefore to investigate the action of ammonia on melibiose, which contains a 1 : 6-linkage, since it may afford some clue as to the mechanism of this degradation. Corbett and Kenner (*J.*, 1953, 2245) find that, with lime-water, lactose is split to give D-galactose and α- and β-*iso*saccharinic acid, and they suggest that lactose is converted into lactulose, a β-hydroxy-carbonyl compound, followed by degradation through the αβ-unsaturated ketone.

As with other reducing sugars, a solution of melibiose in aqueous ammonia developed a reddish-brown colour. By following the course of the reaction on paper chromatograms, it was observed that melibiose was isomerised to other disaccharides and that alkaline fission took place, galactose and its isomer tagatose being formed. The mixture was separated by partition chromatography on a cellulose column.

Many heterocyclic compounds were produced during the reaction of ammonia with melibiose, but 4(5)-methylglyoxaline was the only one identified conclusively. A glycosyl-ketose and a second aldobiose were obtained in a mixed fraction from which the aldobiose crystallised. The latter was characterised as 6-*O*-α-D-galactopyranosyl-β-D-mannose, which had been isolated previously by Whistler and Durso (*J. Amer. Chem. Soc.*, 1951, **73**, 4189) on the partial acid hydrolysis of the mannogalactan of the endosperm mucilage guaran. When the aldobioses were removed from the glycosyl-ketose, by bromine oxidation followed by treatment with ion-exchange resins, the glycosyl-ketose was left as a syrup. It was characterised by conversion into melibiosazone. On acid hydrolysis it gave galactose and fructose. The glycosyl-ketose is thus melibiulose (6-*O*-α-D-galactopyranosyl-D-fructose).

D-Galactose was obtained from the melibiose–ammonia mixture as a syrup, which, when vigorously dried, crystallised in the β-form. From damp methanol it crystallised in the better known α-form. The sugar was further characterised as the methylphenylhydrazone. D-Tagatose was also isolated and crystallised; its identification was confirmed by the preparation from it of D-galactosazone.

D-Galactose probably arises by fragmentation of the reducing portion of the disaccharide, as is known to occur with the hexoses (cf. *Annalen*, 1907, **357**, 294). One might then expect to obtain 1-, 2-, 3-, 4-, and 5-carbon fragments, depending on the structure of the disaccharide. Thus the disaccharide could be degraded to an active form of the type postulated by Isbell (*J. Res. Nat. Bur. Stand.*, 1941, **26**, 35) and undergo hydrolysis. Thus a nonose containing a galactose residue could be further split by the β-hydroxy-carbonyl mechanism to give galactose and a triose unit.

In the light of Corbett and Kenner's results (*loc. cit.*), the melibiose–ammonia mixture was examined for the presence of saccharic acids, but only small amounts of acids were detected.

Experimental

Analyses are by Mr. B. S. Noyes of Bristol. Evaporations were carried out under reduced pressure. Details of chromatographic separations on filter paper and columns of cellulose are given in Part I. R_G values were determined with *n*-butanol–ethanol–water (40 : 11 : 19), unless otherwise stated.

* Part II, *J.*, 1953, 2005.

Reaction of Melibiose with Ammonia.—Melibiose (7 g.), prepared from raffinose by a modification of Hudson and Harding's method (*J. Amer. Chem. Soc.*, 1915, **37**, 2734), was dissolved in water (200 ml.) and ammonia (50 ml.; *d* 0·88), and kept in a stoppered vessel (250 ml.) at 37° for 3 days in the absence of air. Rigorous exclusion of air was not attempted. The course of the reaction was followed by examining samples on paper chromatograms. After 3 hr., there were, in addition to melibiose (R_G 0·038), spots at R_G 0·064 and 0·155 with the characteristics of melibiulose and galactose. After 18 hr., the spots at R_G 0·064 and 0·155 were much stronger, and a spot had appeared at R_G 0·22, corresponding to tagatose. This pattern was very little changed after 44 hr. The solution was concentrated to a thin syrup which was transferred to a column of cellulose and fractionated, with butanol half-saturated with water as the mobile phase.

Fraction 1. A syrup (0·46 g.), which on paper chromatograms sprayed with diazotised sulphanilic acid spray gave a bright red spot at R_G 1·1 at the head of a red trail and at R_G 1·0, a red spot with a yellow centre and red trail, suggesting the presence of glyoxalines. The latter spot also gave a brown colour and trail with the ammoniacal silver nitrate spray. This spray also gave a strong spot at R_G 0·53 and weak spots at R_G 0·89, 0·67, and 0·61.

The basic glyoxalines were isolated by passing the fraction dissolved in water down a column of Amberlite resin IR-120. The column was washed with distilled water, and the effluent and washings were concentrated to a syrup (0·05 g.) which consisted mainly of the compound R_G 0·53, but which also contained compounds with R_G 0·89, 0·67, and 0·61. The column was then washed with 2N-ammonia until the washings no longer gave a colour with the diazotised sulphanilic acid. The ammoniacal washings were then concentrated to a syrup (0·3 g.), which was a mixture of glyoxalines. This was separated by chromatography on sheets of Whatman No. 1 filter paper, to give a syrup (0·1 g.) from which a picrate was prepared, m. p. 161°, which on admixture did not depress the m. p. of 4(5)-methylglyoxaline picrate (m. p. 158°).

Fraction 2 (0·1 g.) contained mainly a compound of R_G 0·38 which gave a brown spot with the ammoniacal silver nitrate and a very pale yellow spot with the *p*-anisidine spray, but no colour with diazotised sulphanilic acid. The fraction was not further investigated.

Fraction 3 (0·19 g.) was indistinguishable from tagatose (R_G 0·22) on paper chromatograms. The syrup was dissolved in water and passed down a small column of charcoal–Celite (1 : 1 parts w/w; 1·5 g.). The eluate was concentrated to a colourless syrup (0·08 g.), which was dried, thinned with methanol, and seeded. At 0° it crystallised to give crystals of m. p. 147° (138° when admixed with genuine D-tagatose, m. p. 135°). With aqueous phenylhydrazine it yielded an osazone, m. p. 185°, not depressed on admixture with genuine D-galactosazone (m. p. 185°) and with a crystalline form identical with that of D-galactosazone.

Fraction 4 (0·62 g.) behaved chromatographically as galactose (R_G 0·16). The syrup was dried by repeated evaporation from methanol; it then crystallised from absolute methanol at 0°, to give β-D-galactose, m. p. 149°, $[\alpha]_D$ +63° ⟶ +78° ± 4° (equil.) (*c*, 0·945 in H_2O). When the sugar was crystallised from damp methanol, it yielded α-D-galactose, m. p. and mixed m. p. 162°, $[\alpha]_D$ +97° ⟶ +78° (equil.) (*c*, 1·28 in H_2O). The sugar gave D-galactose methylphenylhydrazone, m. p. and mixed m. p. 185°.

Fraction 5 (0·06 g.) on chromatographic analysis was found to contain galactose (R_{Gal} 1·0), a compound R_{Gal} 0·5, and a compound R_{Gal} 0·62 which gave a pinkish-brown spot with the *p*-anisidine spray, suggestive of a dissacharide containing a pentose unit. The fraction was further separated on sheets of filter paper with butanol–ethanol–water as the mobile phase, and the strips containing the sugar R_{Gal} 0·62 extracted with methanol to give a syrup (0·015 g.), $[\alpha]_D$ +91° ± 2° (*c*, 0·77 in H_2O). The sugar was hydrolysed with N-sulphuric acid at 100° for 4 hr. The solution was neutralised by shaking it with Amberlite resin IR-4B. Chromatographic analysis of the concentrated eluate revealed the presence of galactose and a pentose.

Fraction 6 (0·48 g.) contained a glycosyl-ketose (R_{Gal} 0·5) and a compound R_{Gal} 0·44, which gave a brown colour with the ammoniacal silver nitrate and *p*-anisidine sprays. The syrup was dissolved in methanol, filtered, and gradually gave crystals (0·19 g.) which, on recrystallisation from methanol, gave 6-*O*-α-D-galactopyranosyl-β-D-mannopyranose, m. p. 197°, $[\alpha]_D$ +123° ⟶ 127·5° (equil.) (*c*, 0·76 in H_2O) (Found: C, 42·2; H, 6·6. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·4%). Whistler and Durso (*J. Amer. Chem. Soc.*, 1951, **73**, 4189) report m. p. 201—201·5°, $[\alpha]_D$ +121° ⟶ 125° (*c*, 2·15 in H_2O). The disaccharide (0·03 g.) was hydrolysed with N-sulphuric acid at 100° for 4 hr., neutralised by shaking it with Amberlite resin IR-4B, and concentrated. Paper chromatography of this concentrate showed equal parts of galactose and mannose.

Fraction 7 (2·9 g.) on chromatographic analysis was observed to be a mixture of the glycosyl-ketose (R_{Gal} 0·5), 6-*O*-α-D-galactopyranosyl-D-mannose (R_{Gal} 0·44), and melibiose (R_{Gal} 0·34). The syrup was dissolved in bromine-water and kept at room temperature, with shaking in the presence of excess of barium benzoate, for 3 days. Aldonic acids were removed and the ketose was isolated as described in the preparation of maltulose (Part II). The product (0·38 g.) was purified by chromatography on sheets of Whatman No. 1 filter paper; melibiulose (0·19 g.), $[\alpha]_D$ +125° (*c*, 1·8 in H_2O), was separated from a small amount of a compound moving just ahead of the glycosyl-ketose and giving a pinkish-brown spot with the *p*-anisidine spray. With aqueous phenylhydrazine acetate the glycosyl-ketose gave an osazone, m. p. 175° not depressed on admixture with genuine melibiosazone, m. p. 176—177°, and with an identical crystalline form. The disaccharide was hydrolysed by heating it in a sealed tube with 2% oxalic acid at 100° overnight. The hydrolysis mixture was neutralised with Amberlite resin IR-4B and concentrated. Examination on paper chromatograms showed the presence of only galactose and fructose when run in butanol–ethanol–water and ethyl acetate–acetic acid–formic acid–water, and sprayed with the *p*-anisidine or resorcinol sprays.

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