408. *The Reaction of Amino-compounds with Sugars. Part II.**
The Action of Ammonia on Glucose, Maltose, and Lactose.

By L. HOUGH, J. K. N. JONES, and E. L. RICHARDS.

D-Psicose has been isolated from the products formed by reaction of D-glucose with aqueous ammonia at 37°. Lactose and maltose in aqueous ammonia are isomerised to lactulose and maltulose respectively. Alkaline degradation of lactose takes place with the production of D-galactose and its isomerisation products, while maltose produces D-glucose and its isomerisation products.

LOBRY DE BRUYN and VAN EKENSTEIN (*Rec. Trav. chim.*, 1895, **14**, 195) reported that weak alkali causes isomerisation of reducing sugars at room temperature, glucose, for example, being transformed *via* the 1 : 2-enediol to a mixture of glucose, mannose, and fructose. In addition, a second ketose, *pseudo*fructose, which is probably formed *via* the 2 : 3-enediol, was obtained in an impure state (*idem, ibid.*, 1897, **16**, 257, 274); subsequent authors have named this sugar D-psicose and D-allulose. The identity of Lobry de Bruyn's product with D-psicose has been in doubt because of their observation that *pseudo*fructose is fermentable by yeast, whereas synthetic D-psicose is not (Steiger and Reichstein, *Helv. Chim. Acta*, 1936, **19**, 184). Other workers (de Whalley, Albon, and Gross, *Analyst*, 1951, **76**, 287; Schneider and Erlemann, *Zucker-Beihefte*, 1951, **3**, 41) have confirmed this formation of a second ketohexose, which Zerban, Sattler, Rosenthal, and Glaubach (*Sugar*, 1952, **47**, [2], 33) isolated and characterised as D-psicosazone. D-Psicose has been found in very small amounts in distillery slop (Zerban and Sattler, *Ind. Eng. Chem.*, 1942, **34**, 1180) where it may be an artefact formed by the lime used in sugar manufacture. Zerban, Sattler, Rosenthal, and Glaubach (*loc. cit.*) recently showed that D-psicose is formed even when a solution of D-fructose is heated. In Part I, the complex mixture resulting from the reaction of D-glucose with aqueous ammonia at 37° was described as containing a ketohexose which was indistinguishable from psicose on paper chromatograms. This identification was confirmed by the preparation of the crystalline phenylosazone which was identical with D-psicosazone, and of crystalline 1 : 2-3 : 4-di*iso*propylidene D-psicofuranose, which had properties identical with those described by Steiger and Reichstein (*loc. cit.*). It was observed that D-psicose was not fermented by yeast.

Either lactose or maltose with aqueous ammonia develops a reddish-brown colour.

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* Part I, *J.*, 1952, 3854.

By following the course of the reactions on paper chromatograms it was observed that the disaccharides (aldobioses) were rapidly isomerised to glycosylketoses and that simultaneous alkaline fission took place, galactose and glucose (and their epimerisation products) being formed in good yield from lactose and maltose respectively.

The glycosylketose formed from lactose was isolated as a syrup and converted into the crystalline osazone which was identified as lactosazone. On hydrolysis the glycosylketose yielded D-galactose, characterised as the methylphenylhydrazone, and D-fructose characterised as the 2 : 3-4 : 5-diisopropylidene derivative. It is thus lactulose [4-(β-D-galactosyl)-D-fructose] which was prepared previously by the action of lime water on lactose (Montgomery and Hudson, *J. Amer. Chem. Soc.*, 1930, **52**, 2101).

Crystalline D-galactose was isolated from the lactose ammonia mixture and further characterised as the methylphenylhydrazone (cf. Lobry de Bruyn and van Ekenstein, *Rec. Trav. chim.*, 1899, **18**, 147; Corbett, Kenner, and Richards, *Chem. and Ind.*, 1953, 154); glucose was not detected. This mixture also contained a sugar which is tentatively identified as lyxose by paper chromatography; it has the same optical rotation as D-lyxose. However, as the sugar could be neither crystallised nor converted into a crystalline derivative, this identification as D-lyxose requires confirmation. The glycosylketose formed by isomerisation of maltose was isolated as the crystalline monohydrate. It gave maltosazone and, on hydrolysis, gave D-glucose, characterised as its osazone, and D-fructose characterised as described above. It is thus maltulose [4-(α-D-glucopyranosyl)-D-fructose]. Maltulose has been obtained as a syrup by the action of salivary α-amylase on the glycogen from livers of pregnant does and by the action of lime water on maltose (Peat, Roberts, and Whelan, *Biochem. J.*, 1952, **51**, xvii). Our product was indistinguishable on paper chromatograms from a specimen kindly supplied by Professor Peat, and has the same optical rotation. In addition to maltulose, the reaction of ammonia with maltose led to the formation of another glycosylketose which after hydrolysis gave spots on paper chromatograms corresponding to D-glucose, and to a ketohexose which was indistinguishable from psicose, but distinct from fructose, sorbose, and tagatose. This glycosylketose is thus very probably 4-(α-D-glucopyranosyl)-D-psicose, formed from maltose as psicose is formed from glucose.

D-Glucose from the alkaline fission of maltose was isolated and characterised as were mannose and fructose, and from paper chromatograms it appears that arabinose also is present. Lobry de Bruyn and van Ekenstein (*loc. cit.*, 1899), Kolb (*Biochem. Z.*, 1914, **63**, 1), and Evans and Benoy (*J. Amer. Chem. Soc.*, 1930, **52**, 294) have previously described the isolation of D-mannose phenylhydrazone from the products produced by the alkaline degradation of a solution of maltose.

These observations suggest that in the presence of ammonia an aldobiose is first isomerised to a glycosylketose and that the disaccharide is then degraded, perhaps to a compound of the type (I), which on hydrolysis would afford a reducing hexose, namely, glucose from maltose and galactose from lactose (cf. Isbell, *J. Res. Nat. Bur. Stand.*, 1941,

(I)
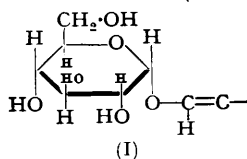
26, 35). As well as epimerisation, isomerisation, and fission products, strongly fluorescent materials were present in the products of the reaction of ammonia with lactose or maltose. These, in some cases, gave bright red colours when held over ammonia after being sprayed with diazotised sulphanilic acid, suggesting the presence of glyoxaline derivatives (see Part I).

EXPERIMENTAL

Analyses are by Mr. B. S. Noyes of Bristol. Evaporations were carried out under reduced pressure. The following solvent mixtures were used to separate the sugars by chromatography on Whatman No. 1 paper : (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), (*b*) butanol–pyridine–water (10 : 3 : 3), and (*c*) butanol–ethanol–water (40 : 11 : 19).

Isolation of D-*Psicose* (D-*Allulose*).—Glucose (100 g.) was dissolved in water (1·6 l.) and ammonia (400 ml.; d 0·88). The solution was kept in a stoppered vessel (2 l.) at 37° for 48 hr. It was then concentrated to a syrup which was shaken with bromine water until a sample showed the absence of aldoses. Bromine was then removed from the solution by aeration, and lead acetate solution added dropwise until there was no further precipitation. The solution was then filtered, and dilute sulphuric acid added to the filtrate until no further precipitate was produced. The solution was again filtered and the filtrate freed from ions by treatment with Amberlite resins IR-120 and IR-4B. The neutral solution was concentrated to a syrup (*ca.* 25 g.), and a portion (7 g.) was transferred to a cellulose column and fractionated (cf. Hough, Jones, and Richards, *J.*, 1952, 3854). Concentration of the fraction of eluate which contained sugar with the characteristics of psicose gave a syrup A (0·2 g.), $[\alpha]_D^{20} +2°\pm1°$ (*c*, 1·9 in H_2O). On paper chromatograms, it showed the same colour reactions as a ketohexose and moved at the same rate as psicose, its position being charateristic since the other three keto-hexoses moved more slowly (solvent *c*; see Part I). A solution of the sugar (0·17 g.) in water (4 ml.), phenylhydrazine (0·5 ml.), glacial acetic acid (0·6 ml.), and saturated sodium hydrogen sulphite solution (0·5 ml.) was heated on the boiling-water bath for 30 min. On cooling, yellow crystals (0·14 g.) separated. After two recrystallisations from aqueous ethanol, and a third from benzene they had m. p. 166°, undepressed on admixture with an authentic specimen of D-psicosazone (m. p. 166°) (Found : C, 60·2; H, 6·25; N, 15·2. Calc. for $C_{18}H_{22}O_4N_4$: C, 60·4; H, 6·15; N, 15·6%). When recrystallised from aqueous ethanol, they showed $[\alpha]_D^{19} -30°$ [*c*, 0·52 in EtOH (2·5 ml.) and pyridine (0·1 ml.)] $\longrightarrow$ $-20°\pm3°$ (equilm., 24 hr.). Steiger and Reichstein (*Helv. Chim. Acta*, 1936, **19**, 184) record $[\alpha]_D^{20} -19·2°\pm4°$ (*c*, 1·0 in absolute EtOH), and Zerban and Sattler (*Ind. Eng. Chem.*, 1942, **34**, 1184) give $[\alpha]_D^{25} -41·8°$ [in EtOH (1·5 pts.) and pyridine (1 pt.)] $\longrightarrow$ $-22·1°$ (equilm., 24 hr.). On recrystallisation from benzene, different values were obtained, *viz.*, $[\alpha]_{17}^D -19°$ [*c*, 0·96 in EtOH (1·5 ml.) and pyridine (1 ml.)] $\longrightarrow$ $-45°$ (equilm., 24 hr.). D-Psicose was obtained in lower yield when the bromine oxidation was omitted.

1 : 2-3 : 4-*Diiso*propylidene D-*Psicofuranose*.—A second sample of the syrup A (0·3 g.) was dissolved in methanol containing glass balls, then evaporated, and the residue was warmed under reduced pressure for 3 hr., to give a dry syrup. Dry acetone (10 ml.), anhydrous copper sulphate (0·1 g.), and concentrated sulphuric acid (0·03 g.) were then added. The mixture was shaken for 4 days and set aside for a further 3 days. The 1 : 2-3 : 4-di*iso*propylidene compound was then isolated as described by Bell (*J.*, 1947, 1461) for 2 : 3-4 : 5-di*iso*propylidene fructose. The syrup obtained crystallised at *ca.* −40°, and recrystallised from light petroleum (b. p. 40—60°) as plates, m. p. 57—58°, $[\alpha]_D^{18} -93°$ (*c*, 0·23 in $COMe_2$) (Found : C, 55·4; H, 7·7. Calc. for $C_{12}H_{20}O_6$: C, 55·3; H, 7·7%). Steiger and Reichstein (*Helv. Chim. Acta*, 1936, **19**, 184) record m. p. 57—58·5° and $[\alpha]_D^{20} -98·2°$ (*c*, 2·124 in $COMe_2$).

Fermentation of Reaction Mixture.—Glucose, fructose, and mannose were removed from a portion of the brown reaction mixture, from which ammonia had been removed by evaporation under reduced pressure, by fermentation with baker's yeast. The residual unfermentable ketohexose was isolated by separation on sheets of filter paper (solvent *c*) as described earlier (Hough, Jones, and Richards, *loc. cit.*). The syrup, $[\alpha]_D^{22} 0°\pm2°$ (*c*, 1·0 in H_2O), moved at the same rate and gave the same colour reactions as psicose on paper chromatograms and yielded a phenylosazone, m. p. 163°, with a crystalline form indistinguishable from that of psicosazone.

Reaction of Lactose with Ammonia.—Lactose (10 g.) was dissolved in water (200 ml.) and ammonia (50 ml.; d 0·88). The solution was kept in a stoppered vessel (250 ml.) at 37° for 2 days in the absence of air (rigorous exclusion of air was however not attempted), gradually becoming brown. Paper chromatographic examination showed it to be an exceedingly complex mixture. Evaporation of the reaction mixture left a brown syrup (9·2 g.) which was transferred to a column of cellulose and fractionated by partition chromatography. The effluent was collected portionwise and after examination on paper chromatograms, grouped into six fractions, which were concentrated.

Fraction 1. This (0·32 g.) consisted of materials which trailed on paper chromatograms, were strongly fluorescent, and gave red colours with ammonia after being sprayed with diazotised sulphanilic acid, suggesting the presence of glyoxalines.

Fraction 2. This (0·2 g.), a light brown syrup, gave two spots on a paper chromatogram (solvent *c*). It was separated on sheets of filter paper (solvent *c*) as described by Hough, Jones, and Richards (*loc. cit.*), into two fractions. Fraction A (0·12 g.) consisted of materials giving a pink colour with *p*-anisidine hydrochloride spray, and also after being sprayed with diazotised sulphanilic acid a red colour with ammonia. It was dissolved in water (5 ml.) and shaken

with Amberlite resins IR-120 and IR-4B until the solution no longer gave a reaction with the diazotised sulphanilic acid spray, indicating the absence of glyoxalines. It was concentrated to a syrup (0·075 g.) which gave the colour reactions characteristic of a pentose and moved to the same position as xylose and/or lyxose (solvent *c*). In phenol–water, it behaved as lyxose, but differently from xylose (cf. Hough and Jones, *J.*, 1951, 1122). The syrup $\{[\alpha]_D^{15} -13°$ (*c*, 1·3 in H$_2$O)} did not crystallise. Fraction B (0·045 g.) was a syrup which on paper chromatograms, moved to the same position as, and gave colour reactions characteristic of, tagatose.

Fraction 3. This material (1·5 g.) crystallised as the solvent was removed. On paper chromatograms (solvents *a*, *b*, and *c*) it was indistinguishable from D-galactose; it had m. p. 163°, $[\alpha]_D^{20} +105°$ (*c*, 1·0 in H$_2$O) $\longrightarrow +80°$ (equilm., 24 hr.); the methylphenylhydrazone had m. p. and mixed m. p. 189° (Found : N, 10·2. Calc. for C$_{13}$H$_{26}$O$_5$N$_2$: N, 9·9%).

Fraction 4. This, a syrup (1·2 g.), $[\alpha]_D^{19} -12°$ (*c*, 0·69 in H$_2$O), gave the red colour with the resorcinol–hydrochloric acid spray characteristic of a ketohexose. A solution of the sugar (0·063 g.) in methanol (10·5 ml.) containing hydrogen chloride (0·03 g.) showed $[\alpha]_D^{19} +15°$ ($\frac{3}{4}$ hr.) $\longrightarrow +18°$ (2 hr.) $\longrightarrow +9°$ (4 hr.) $\longrightarrow +2·5°$ (2 days). The sugar, when heated with aqueous phenylhydrazine acetate, gave lactosazone, m. p. and mixed m. p. 204°, the sugar being thus identified as lactulose.

Hydrolysis of lactulose : The sugar (0·14 g.) was hydrolysed with 2% oxalic acid at 100° overnight. The acid was removed by shaking the solution with Amberlite resin IR-4B, and the solution filtered and concentrated. Paper chromatographic analyses of the concentrate indicated the presence of galactose and fructose, which were separated on sheets of paper (cf. Hough, Jones, and Richards, *loc. cit.*). The ketose fraction (0·04 g.) was converted into 2 : 3-4 : 5-di*iso*propylidene fructose (by Bell's method, *J.*, 1947, 1461), m. p. and mixed m. p. 93°, $[\alpha]_D^{20} -22° \pm 6°$ [*c*, 0·94 in H$_2$O (0·5 ml.)]. The aldose fraction gave D-galactose methylphenyl-hydrazone, m. p. 185°, mixed m. p. 187°.

Fraction 5. This (2·3 g.) was largely unchanged lactose which gave a phenylosazone, m. p. and mixed m. p. 204°.

Fraction 6. After the lactose had been removed completely, the column was eluted with ethanol and then with water, and the combined eluates were evaporated to a brittle brown glass (1·1 g.). This did not move on paper chromatograms (solvent *c*) and in solution gave a bluish green fluorescence.

Reaction of Maltose with Ammonia.—Maltose (10 g.), dissolved in water (200 ml.) and ammonia (50 ml. ; *d*, 0·88), was kept in a stoppered vessel (250 ml.) at 37° for 3 days during which it gradually developed a reddish-brown colour. Paper chromatographic examination showed it to be an exceedingly complex mixture. The reaction mixture was concentrated to a syrup which was fractionated as described above.

Fraction 1. This (0·82 g.) was found on paper chromatograms to consist of at least three components of R_G 0·68, 0·57, and 0·47—0·27 (solvent *c*), which moved more slowly than tetra-methyl glucose (R_G 1). The compound with R_G 0·68, after being sprayed with diazotised sulphanilic acid, gave a bright red colour with ammonia, and a brown spot with the ammoniacal silver nitrate spray, and was weakly fluorescent. The material of R_G 0·57 behaved similarly except that it was strongly fluorescent. The materials which moved at rates between R_G 0·47 and 0·27 gave a bright red band with a yellow centre at R_G 0·40 when held over ammonia after being sprayed with diazotised sulphanilic acid and with ammoniacal silver nitrate spray gave a brown band with a white centre at R_G 0·40. The band was also weakly fluorescent.

Fraction 2. This (0·9 g.) showed on paper chromatograms a trace of a material giving the colour reactions of a pentose and moving to the same position as arabinose. The fraction consisted mainly of D-fructose and D-mannose. D-Fructose was characterised as its 2 : 3-4 : 5-di*iso*propylidene derivative, m. p. and mixed m. p. 92°, $[\alpha]_D^{20} -32°$ (*c*, 0·74 in H$_2$O). Mannose was characterised as the insoluble mannose phenylhydrazone, m. p. 193°.

Fraction 3. This (1·1 g.) showed the presence of glucose only, on paper chromatograms. The syrup crystallised from methanol–butanol, and then had m. p. 146—147°, $[\alpha]_D^{18} +92°$ (*c*, 1·66 in H$_2$O) $\longrightarrow +50°$ (equilm., 24 hr.). It gave glucosazone, m. p. 201°; and a glucoso-triazole, m. p. 193—194°, $[\alpha]_D^{18} -81°$ [*c*, 0·67 in pyridine (cf. Hann and Hudson, *J. Amer. Chem. Soc.*, 1944, 66, 735)].

Fraction 4. This (0·5 g.) contained maltose, and a glycosylketose moving at a rate similar to that of maltose on paper chromatograms; this was identified as maltulose. In order to isolate a quantity of the sugar and identify it, the conditions of preparation were slightly modified. The mixture described above was kept for only 24 hr. and then concentrated. The solution was shaken with bromine in the presence of an excess of barium benzoate for 3 days.

The ketoses were then isolated as described for psicose. *Maltulose monohydrate* (R_G 0·074) (3·17 g.) crystallised from dioxan–methanol when the solvent was allowed to evaporate slowly at room temperature (Found : C, 40·1; H. 6·7. $C_{12}H_{22}O_{11},H_2O$ requires C, 40·0; H, 6·7%), and had m. p. 113—115° (decomp.), $[\alpha]_D^{20} +58°$ (*c*, 1·58 in H_2O) $\longrightarrow +64°$ (equilm.). When it was heated with phenylhydrazine acetate solution, maltosazone (m. p. and mixed m. p. 202°) was produced in characteristic crystalline form.

Hydrolysis of Maltulose.—The sugar was hydrolysed with 0·1N-sulphuric acid at 100° overnight. The solution was neutralised by shaking it with Amberlite resin IR-4B, filtered, and concentrated to a syrup, chromatographic analysis of which (solvents *a*, *b*, and *c*) indicated the presence of glucose and fructose. These were separated on sheets of filter paper (solvent *c*). The glucose was characterised as the phenylosazone, m. p. and mixed m. p. 205°, identical with D-glucosazone, and fructose as its 2 : 3-4 : 5-di*iso*propylidene derivative, m. p. and mixed m. p. 93°, $[\alpha]_D^{19} -32°$ (*c*, 0·62 in H_2O).