738. *The Reaction of Amino-compounds with Sugars. Part I. The Action of Ammonia on* D-*Glucose.*

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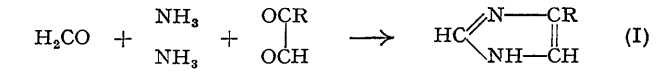
From the complex mixture resulting from the reaction of D-glucose with aqueous ammonia, 4(5)-methylglyoxaline and 2-methyl-5- and 2-methyl-6(?)-(D-*arabo*tetrahydroxybutyl)pyrazine have been isolated and identified.

A MIXTURE of glucose or fructose with aqueous ammonia develops a brown colour (Ling and Nanji, *J. Soc. Chem. Ind.*, 1922, **41**, 151T) and, because of the similarity between this reaction and the " browning " reaction with amino-acids, we have investigated the products. Epimerisation to fructose, mannose, and a ketose (allulose?) occurred rapidly, and then gradually, as the brown colour developed, a very complex mixture resulted. Two strongly coloured zones were observed on the paper chromatogram, one travelling with the solvent front and the other remaining close to the starting line. These brown products have not yet been investigated, attention being confined to the numerous remaining components of the mixture in the hope that they might afford some clue as to the mechanism of formation of the coloured materials.

Fractionation was effected by continuous extraction with chloroform; partition chromatography then gave three fractions, the investigation of two of them being described here.

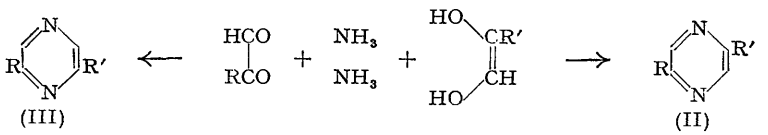
Examination of the first fraction on filter-paper chromatograms revealed the presence of a material that gave a bright red colour when held in ammonia vapour after having been sprayed with diazotised sulphanilic acid (Ames and Mitchell, *J. Amer. Chem. Soc.*, 1952, **74**, 252). This reaction appears to be a characteristic of glyoxalines. On paper chromatograms, the unknown was indistinguishable from 4(5)-methylglyoxaline. Proof of its identity was obtained by the isolation of a crystalline picrate which was identical with authentic 4(5)-methylglyoxaline picrate. Several glyoxalines have been isolated from the reaction between glucose, ammonia, and inorganic bases such as zinc hydroxide, with which glyoxalines form insoluble complexes, as in the preparation of 4(5)-methylglyoxaline (I; R = Me) (Windaus and Knoop, *Ber.*, 1905, **38**, 1166; Windaus, *Ber.*, 1907, **40**, 799). When air is bubbled through the reaction mixture, the main product is 4(5)-hydroxymethylglyoxaline (I; R = CH_2·OH) (Parrod, *Ann. Chim.*, 1933, **19**, 205), and 4(5)-(D-*arabo*-tetrahydroxybutyl)glyoxaline {I; R = $[CH(OH)]_3$·CH_2·OH} is also formed. In order

to account for the formation of 4(5)-methylglyoxaline, Windaus and Knoop postulated fission of the hexose into two molecules of glyceraldehyde, and subsequent conversion of this into methylglyoxal. The latter condenses with ammonia and formaldehyde, which is also formed from fragmentation of hexoses, to form 4(5)-methylglyoxaline. Similarly, hydroxymethylglyoxal and glucose would yield 4(5)-hydroxymethylglyoxaline (I; R = CH_2·OH) and 4(5)-(D-*arabo*tetrahydroxybutyl)glyoxaline respectively.



(I)

From the third fraction of the chloroform extract, fractional crystallisation afforded two isomeric crystalline compounds, $C_9H_{14}O_4N_2$ (*A*, m. p. 201°; *B*, m. p. 170°). Evidence for the presence of a pyrazine nucleus in each was obtained from the ultra-violet absorption curve [principal max. at 2750 A (ε 9540) in water]. Further evidence was obtained by permanganate oxidation of *A* which yields pyrazine-2 : 5-dicarboxylic acid. Both *A* and *B* consumed three mols. of sodium metaperiodate with the simultaneous formation of two mols. of formic acid and one of formaldehyde, thus proving the presence of a tetrahydroxybutyl side chain. Analysis showed the presence of one *C*-methyl group in each. The substance *A* is thus 2-methyl-5-(tetrahydroxybutyl)pyrazine {II; R = Me; R′ = [CH(OH)]$_3$·CH_2·OH}, and *B* is the corresponding 2 : 3- or 2 : 6-compound. The evidence available favours the 2 : 6-configuration {III; R = Me; R′ = [CH(OH)]$_3$·CH_2·OH}. On permanganate oxidation, material was isolated in low yield which sublimed at the temperature and gave the colour with ferrous sulphate expected of pyrazine-2 : 6-dicarboxylic acid and different from those for pyrazine-2 : 3-dicarboxylic acid. As the



(III) (II)

compounds are derived from glucose, the tetrahydroxybutyl side chain would be expected to be D-*arabo* in configuration. This was confirmed by evaluation of the molecular rotations (*A*, $[M]_D^{13}$ −13,360; *B*, $[M]_D^{13}$ −15,820) (cf. Richtmyer and Hudson, *J. Amer. Chem. Soc.*, 1942, **64**, 1612). Reaction of fructose or glucose with alcoholic ammonia affords fructosazine (2 : 5-bistetrahydroxybutyl)pyrazine {II; R = R′ = [CH(OH)]$_3$·CH_2·OH} (Lobry du Bruyn, *Rec. Trav. chim.*, 1899, **18**, 72; Stolte, *Chem. Zentr.*, 1908, I, 224). It is probable that the pyrazine derivatives we have isolated are formed by the condensation of *aldehydo*-glucose with ammonia and methylglyoxal. This mechanism would give rise to two isomers, the 2 : 6- and the 2 : 5-substituted pyrazine.

Experimental

M. p.s are uncorrected. Analyses for carbon and hydrogen are by Drs. Weiler and Strauss of Oxford, and for nitrogen by W. M. Eno of Bristol.

In the following experiments, sheet-paper partition chromatography was carried out on Whatman No. 1 filter paper by the descending method (Partridge, *Biochem. J.*, 1948, **42**, 238), *n*-butanol–ethanol–water (40 : 11 : 19 v/v) being used, unless otherwise stated, as the mobile phase. After separation, the paper chromatograms were dried, sprayed with one of the following reagents, and, with sprays (*a*)—(*d*), the paper was heated: (*a*) Partridge's ammoniacal silver nitrate reagent (*loc. cit.*); (*b*) *p*-anisidine hydrochloride in butanol–water (Hough, Jones, and Wadman, *J.*, 1950, 1702); (*c*) resorcinol–hydrochloric acid in butanol (Forsyth, *Nature*, 1948, **161**, 239); (*d*) ninhydrin in butanol; (*e*) diazotised sulphanilic acid solution (Ames and Mitchell, *J. Amer. Chem. Soc.*, 1952, **74**, 252). Papers sprayed with (*e*) (not heated) were held over ammonia solution. Solutions were evaporated under reduced pressure unless otherwise stated.

Reaction of D-*Glucose with Ammonia.*—D-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 2 weeks; the solution gradually became dark brown, but no insoluble material was produced. It was concentrated to 300 ml. and examined qualitatively on paper chromatograms. Spray (*a*) showed

the concentrate to contain at least fifteen components with the following approximate R_G values: * 0·99, 0·85, 0·68, 0·51, 0·49, 0·43, 0·37, 0·33, 0·28, 0·23, 0·17, 0·14, 0·096, 0·078, 0·053, 0·031. The component with R_G 0·99 gave a white spot with reagent (*a*), in contrast to the usual brown spots given by all the other substances, indicating the presence of anionic material. This component with spray (*e*) gave a white spot at the head of an intense red trail. An intense red colour with (*e*) was found to be a characteristic of glyoxalines. When butanol–acetic acid–water (2 : 1 : 1 v/v) was used as the mobile phase, this component moved as a discrete round spot which gave with (*e*) a white centre surrounded by red. The spot of R_G 0·28 gave a yellow colour with (*b*) and a pink colour with (*c*) and showed the same colour reactions and moved to the same position on the chromatogram as allulose. The component of R_G 0·23 gave a brown colour with (*b*) and a pink with (*c*), and moved on the chromatogram to the same position as fructose and mannose. The component of R_G 0·17 gave a brown colour with (*b*) and moved to the same position as glucose. With spray (*d*), a blue streak formed from R_G 0·022 to 0·132 with more intensely blue zones at R_G's 0·033, 0·066, and 0·129. Spray (*e*), as well as showing the component of R_G 0·99, indicated the presence of small quantities of glyoxalines, which appeared as ill-defined elongated spots ranging from the component of R_G 0·99 to the starting line. The course of the reaction was followed by taking samples from the reaction mixture at intervals and examining them on paper chromatograms. Epimerisation took place rapidly, as was indicated by the formation of fructose, mannose, and allulose (?) when the first sample was taken after 16 hours. Other spots had become detectable after 40 hours, and by 100 hours the full pattern had developed.

Fractionation of the Chloroform Extract.—The solution was extracted continuously with chloroform for a week, then evaporated, leaving a thick dark brown syrup (5·9 g.), which was thinned with *n*-butanol, transferred to a column of cellulose, and fractionated by partition chromatography with *n*-butanol, half saturated with water, as the mobile phase (Hough, Jones, and Wadman, *J.*, 1949, 2511). The effluent was collected portionwise and, after examination on chromatograms, grouped into three fractions which were concentrated.

Fraction 1. This (2·5 g.) yielded a brown syrup which showed only one component (R_G 0·99) on the paper chromatogram, but the presence of dark materials, not detectable by the sprays used, was unmistakable. The syrup was dissolved in a mixture of water (20 ml.) and saturated picric acid (30 ml.), boiled, and kept at 0°. Small dark brown needles (0·33 g.) separated and were filtered off (m. p. 153°). Recrystallisation from water gave orange needles (0·2 g.), m. p. 158°, undepressed on admixture with an authentic specimen of 4(5)-methylglyoxaline picrate prepared according to Windaus and Knoop (*Ber.*, 1905, **38**, 1166) (Found: C, 38·6; H, 3·1; N, 22·3. Calc. for $C_{10}H_9O_7N_5$: C, 38·5; H, 2·9; N, 22·5%). Authentic 4(5)-methylglyoxaline behaved in the same way as the component of R_G 0·99 on a paper chromatogram and gave a bright red colour with spray (*e*).

Fraction 2. This (1·4 g.) was a syrup in which the components of R_G 0·85 and 0·68 were detected on a paper chromatogram, along with small amounts of materials giving red colours with spray (*e*). This fraction was not further investigated.

Fraction 3. This (0·4 g.), on evaporation, gave crystals and eventually there remained a mixture of crystals in a light brown syrup. The crystalline material (0·2 g.) was filtered off and identified as 2-*methyl*-5-(D-arabo*tetrahydroxybutyl*)*pyrazine* (II), m. p. 196°, raised to 201° on recrystallising twice from absolute ethanol, $[\alpha]_D^{13}$ −62·4° (*c*, 0·42 in water) (Found: C, 50·4; H, 6·4; N, 13·1; *C*-Me, 6·8. $C_9H_{14}O_4N_2$ requires C, 50·5; H, 6·54; N, 13·1; *C*-Me, 7·1%), The optical rotation varies appreciably with the temperature. On the paper chromatogram, this material reduced spray (*a*) to give a brown spot (R_G 0·49), but no colour was produced with spray (*b*) or (*e*).

Periodate Oxidation of 2-*Methyl*-5-(D-arabo*tetrahydroxybutyl*)*pyrazine* (II).—The pyrazine (5 mg.) in water (5 ml.) was treated with sodium metaperiodate (0·2M; 1 ml.) for 3 hours at room temperature. Addition of neutral sodium arsenite (5 c.c., 0·1N), followed by back-titration with 0·01N-iodine (26 c.c.), showed that 2·8 moles of periodate were consumed per mole of (II). A control determination was carried out on 1 ml. of periodate (0·2M) and 5 c.c. of arsenite solution (0·1N), and required 13 ml. of 0·01N-iodine. Similar oxidation of another portion (5·9 mg.) with sodium metaperiodate (0·2M; 1 ml.) gave formic acid equivalent to 2·45 c.c. of 0·02N-sodium hydroxide, corresponding to 1·9 moles of formic acid per mole of (II). Similar oxidation of another portion (9·8 mg.) gave formaldehyde, identified as the dimedone derivative (12·7 mg.), m. p. 186° (Bell, *J.*, 1948, 992), equivalent to 0·78 mole of formaldehyde per mole of (II).

* 2 : 3 : 4 : 6-Tetramethyl glucose (R_G 1·0) is used as the reference substance (Hirst, Hough, and Jones, *J.*, 1949, 928).

Pyrazine-2 : 5-dicarboxylic Acid.—2-Methyl-5-(D-*arabo*tetrahydroxybutyl)pyrazine (II) (0·2 g.) was heated in 2% potassium permanganate solution (75 ml.) under reflux for 18 hours. The solution was filtered, concentrated to 15 c.c., and acidified with dilute nitric acid. The white precipitate (19 mg.) was dissolved in dilute aqueous ammonia, filtered, and reprecipitated (14 mg.) by dilute nitric acid. The dicarboxylic acid sublimed at 265—270° and gave a violet-blue colour with ferrous sulphate solution (Found: C, 43·1; H, 2·54; N, 16·6. Calc. for $C_6H_4O_4N_2$: C, 42·9; H, 2·38; N, 16·7%). An *X*-ray powder photograph of the crystals was identical with that of an authentic specimen of pyrazine-2 : 5-dicarboxylic acid obtained by the oxidation of 2 : 5-dimethylpyrazine (Gabriel and Pinkus, *Ber.*, 1893, **26**, 2197), the best results being obtained by dissolving 2 : 5-dimethylpyrazine (0·41 g.) in 2% potassium permanganate solution (150 ml.) and treating it as above (yield, 0·165 g.).

2-Methyl-6-(D-arabo*tetrahydroxybutyl)pyrazine* (III).—The mother-liquor, after the removal of (II), was evaporated further to about a quarter of its volume. Needles gradually separated (0·1 g.; m. p. 166°) which were recrystallised from absolute ethanol, then having m. p. 170°, $[\alpha]_D^{13}$ −74° (*c*, 0·425 in water) (Found: C, 50·5; H, 6·22; N, 13·2; *C*-Me, 7·7. $C_9H_{14}O_4N_2$ requires C, 50·5; H, 6·54; N, 13·1; *C*-Me, 7·1%). The optical rotation of this compound also exhibits considerable variance with temperature. On a paper chromatogram, this material gave a discrete brown spot (R_G 0·51) with spray (*a*).

Periodate oxidation. By the methods described above, it was found that 3·08 moles of periodate were consumed per mole of (III), with the formation of 2 moles of formic acid and 0·85 mole of formaldehyde.

Permanganate oxidation. The pyrazine (III) (0·2 g.) was heated with 2% potassium permanganate solution (75 ml.) as described above for (II). On concentration and acidification a small amount (4 mg.) of a pale yellow material separated. This was isolated on the filter; it sublimed at 220° and gave a reddish-blue colour with ferrous sulphate solution, different from the violet-blue colour given by pyrazine-2 : 3-dicarboxylic acid [prepared by the oxidation of quinoxaline by Gabriel and Sonn's method (*Ber.*, 1907, **40**, 4850)]. The m. p.s of the pyrazine-dicarboxylic acids in sealed tubes were unsatisfactory and the temperature of sublimation was found to be more characteristic.

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