476. *The Reaction of 2-Amino-2-deoxy-D-glucose Hydrochloride with Aqueous Ammonia.*

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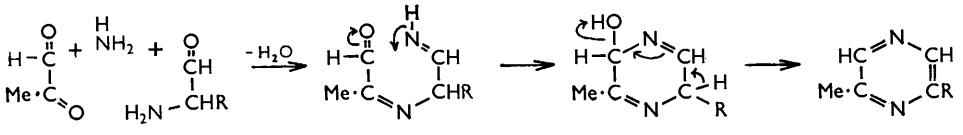
The products of the action of aqueous ammonia on 2-amino-2-deoxy-D-glucose hydrochloride at room temperature have been separated by chromatography on cellulose. They include 2-methyl-6-D-*arabo*-tetrahydroxybutyl-, 2-methyl-5-D-*arabo*-tetrahydroxybutyl-3-D-*erythro*-trihydroxypropyl-, and 2,5-bis-(D-*arabo*-tetrahydroxybutyl)-pyrazine.

SUGAR solutions containing ammonia develop a colour which changes from pale cream to dark brown,[1] a reaction attributed both to the alkaline reaction [2] of ammonia and to its effect as an amino-compound.[1,3] The alkaline action leads to rearrangement [4] and fragmentation,[5] while condensation of ammonia with the products yields nitrogen-containing heterocyclic compounds.[1] With non-amino-bases amino-sugars, *e.g.*, 2-amino-2-deoxy-D-glucose at room temperature, undergo deamination,[6] accompanied by epimerisation of the deaminated sugar, yielding D-glucose, D-fructose, and saccharinic acids. I

[1] Hough, Jones, and Richards, *J.*, 1952, 3854; 1953, 2005; 1954, 295; Ling and Nanji, *J. Soc. Chem. Ind.*, 1922, **41**, 151T.
[2] Whistler and Bemiller, *Adv. Carbohydrate Chem.*, 1958, **13**, 289; Wolfrom, Cavalieri, and Cavalieri, *J. Amer. Chem. Soc.*, 1947, **69**, 241.
[3] Ellis, *Adv. Carbohydrate Chem.*, 1959, **14**, 63.
[4] Lobry De Bruyn and Alberda Van Ekerstein, *Rec. Trav. chim.*, 1895, **14**, 150, 195; 1899, **18**, 147; and other papers.
[5] Nef, *Annalen*, 1907, **357**, 294; 1910, **376**, 1; 1913, **403**, 204; Schmidt, *Chem. Rev.*, 1935, **17**, 137.
[6] Tracey, *Biochem. J.*, 1952, **52**, 265.

have therefore, investigated the action of aqueous ammonia on 2-amino-2-deoxy-D-glucose.

2-Amino-2-deoxy-D-glucose hydrochloride and aqueous ammonia were allowed to react for six months at room temperature. The optical rotation of the mixture decreased to zero in seven days, after which the intensity of the brown colour prevented estimation of the rotation. Meanwhile the absorption at 2750 Å increased continuously. Paper chromatography revealed at least five components. Chromatography on a column of cellulose afforded five materials, one of them being unchanged 2-amino-2-deoxy-D-glucose. The second component was crystalline 2-methyl-6-D-*arabo*-tetrahydroxybutylpyrazine (IV) which had been obtained by Hough, Jones, and Richards [1] by a similar reaction of D-glucose with aqueous ammonia. It is probable that the pyrazine derivative (IV) is formed by condensation (I—IV) of *aldehydo*-2-amino-2-deoxy-D-glucose with ammonia and methylglyoxal. The glyoxal could arise from 2-amino-2-deoxy-D-glucose by fragmentation and transformation.

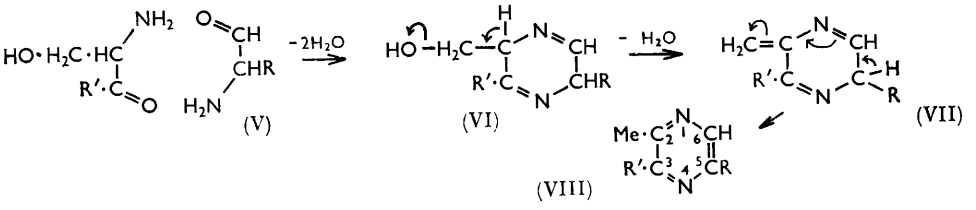


(I) (II) (III) (IV)

R = D-*arabo*-Tetrahydroxybutyl.

The alternative condensation product, 2-methyl-5-D-*arabo*-tetrahydroxybutylpyrazine was not isolated.

The third component was a crystalline non-reducing compound $C_{12}H_{20}N_2O_7$ (Y) containing one *C*-methyl group, giving a crystalline heptabenzoate, and containing a pyrazine nucleus [$\lambda_{max.}$ 2800 Å (ε 9420) in water]. Oxidation by periodate afforded two mol. of formaldehyde, indicating the presence of two carbon chains each ending with at least two hydroxyl groups on adjacent carbon atoms. One of the periodate products was isolated (in 9·7% yield) as the crystalline 2,4-dinitrophenylhydrazone $C_{10}H_{15}IN_6O_4$ (Z) containing no pyrazine nucleus (as indicated by the ultraviolet absorption) but one *C*-methyl group; the high melting point (192°) suggested that it was a hydriodide and indeed with aqueous ammonia it afforded a base $C_{10}H_{14}N_6O_4$. Since compound (Z) is derived from a pyrazine derivative, the two nitrogen atoms must be linked to adjacent carbon atoms and it is evident from the empirical formula that they can only be present as amino-groups. This gains some support from the fact that primary amino-groups were detected during the oxidation of compound (Y) by periodate. Compound (Z) is, therefore, probably 2,3-diaminobutanal 2,4-dinitrophenylhydrazone hydriodide. Its isolation shows that the pyrazine nucleus of its parent (Y) is destroyed by periodate, in conformity with the high uptake (8 mols.). It also shows that the methyl group of the parent compound (Y) is attached directly to the pyrazine ring, and that the carbon atom carrying the methyl group



(V) (VI) (VII) (VIII)

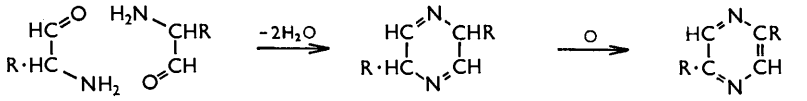
R = D-*arabo*-Tetrahydroxybutyl. R′ = D-*erythro*-Trihydroxypropyl.

is adjacent to one carrying one of the carbon chains (R in VIII). The second carbon chain, therefore, must be attached to the other side of the pyrazine ring.

It is evident that compound (Y) ($C_{12}H_{20}N_2O_7$) could be formed from two molecules

of 2-amino-2-deoxy-D-glucose ($C_6H_{13}NO_5$) by the loss of three molecules of water, *e.g.*, (V—VIII). This condensation would result in a molecule with six carbon atoms on either side of the plane of the nitrogen atoms which would allow a three-carbon chain on the side of the pyrazine nucleus carrying the methyl group, and a four-carbon chain on the other side of the nucleus. The four-carbon chain will necessarily be located at position 5. From all these considerations it is suggested that compound (Y) is 2-methyl-5-D-*arabo*-tetrahydroxybutyl-3-D-*erythro*trihydroxypropylpyrazine (VIII).

The fourth component was crystalline 2,5-bis-(D-*arabo*-tetrahydroxybutyl)pyrazine (XI) which was reported [7] to be formed in aqueous solutions of 2-amino-2-deoxy-D-glucose. It yielded a crystalline octa-acetate and octabenzoate and its ultraviolet absorption curve was similar to those of compounds (IV) and (VIII) [$\lambda_{max.}$ 2750 Å (ε 9410) in water]. On oxidation by potassium permanganate, compound (XI) yielded pyrazine-2,5-dicarboxylic acid; it consumed six mols. of periodate with the simultaneous liberation of four mols. of formic acid and two of formaldehyde. It is probably formed by condensation of two molecules of *aldehydo*-2-amino-2-deoxy-D-glucose, yielding the dihydro-compound (X) which is oxidised by air.



(IX) (X) (XI)

R = D-*arabo*-Tetrahydroxybutyl.

The fifth component was a dark brown amorphous substance which is under investigation.

Experimental

Paper chromatography was made on Whatman No. 1 filter paper, by the descending method with ethyl acetate–acetic acid–water (9 : 2 : 2 v/v) as mobile phase. The separated substances were detected with (*a*) a 4% solution of silver nitrate containing an excess of ammonia,[8] or (*b*) 0·1% ninhydrin in butan-1-ol. Solutions were evaporated under reduced pressure. Optical rotations refer to room temperature.

Reaction of 2-Amino-2-deoxy-D-glucose Hydrochloride with Aqueous Ammonia.—A mixture of 2-amino-2-deoxy-D-glucose hydrochloride (50 g.) and aqueous ammonia (500 ml.; *d* 0·88) was kept at room temperature. At intervals samples (1 ml.) were diluted ten-fold with distilled water, and their optical rotations estimated. Of the diluted solution 1 ml. was further diluted forty-fold before its absorption at 2750 Å was determined (Unicam S.P. 500). After 6 months, the remainder (*ca.* 98%) of the reaction mixture was concentrated to dryness to remove the excess of ammonia. The product was dissolved in water (200 ml.), deionised by Permutit " Deacidite H " (100 g.) and then Permutit ZeoKarb 225 (100 g.), and concentrated to a brown syrup (*ca.* 36 g.) which, when examined on paper chromatograms with spray (*a*), was observed to contain at least four products (R_F 0·705, 0·673, 0·46, 0·0). The syrup was fractionated on a cellulose column with butan-1-ol–water (10 : 1 v/v) as mobile phase. Three fractions were obtained.

Fraction 1 gave crystals of 2-methyl-6-D-*arabo*-tetrahydroxybutylpyrazine (*ca.* 0·63 g.) which after recrystallisation from ethanol had m. p. 170°, $[\alpha]_D$ −70° (*c* 1·0 in H_2O), R_F 0·705 (Found: C, 50·3; H, 6·65; N, 13·1; *C*-Me, 6·3. Calc. for $C_9H_{14}N_2O_4$: C, 50·5; H, 6·5; N, 13·1; *C*-Me, 7·0%). Hough, Jones, and Richards [1] record m. p. 170°, $[\alpha]_D$ −74° (in H_2O).

Fraction 2 yielded crystalline 2-*methyl*-5-D-arabo-*tetrahydroxybutyl*-3-D-erythro-*trihydroxypropylpyrazine* (*ca.* 2·2 g.) which on recrystallisation from ethanol had m. p. 152°, $[\alpha]_D$ −100° (*c* 1·32 in H_2O), R_F 0·673 (Found: C, 47·3; H, 6·7; N, 9·3; *C*-Me, 4·2. $C_{12}H_{20}N_2O_7$ requires C, 47·3; H, 6·6; N, 9·2; *C*-Me, 4·9%).

Fraction 3 afforded 2,5-bis-(D-*arabo*-tetrahydroxybutyl)pyrazine as crystals (*ca.* 1·8 g.), m. p. 235° (from ethanol), $[\alpha]_D$ −76° (*c* 1·4 in H_2O), R_F 0·46 (Found: C, 44·85; H, 6·35; N, 9·0.

[7] Stolte, *Beitr. Chem. Physiol*, 1908, **11**, 19.
[8] Partridge, *Biochem. J.*, 1948, **42**, 238.

Calc. for $C_{12}H_{20}N_2O_8$: C, 45·0; H, 6·25; N, 8·75%). Stolte [7] recorded m. p. 232·5°, $[\alpha]_D$ −71° (in H_2O).

A fourth fraction was obtained by washing off the brown material, which had been stationary on top of the cellulose column, with water. On concentration the washings yielded a dark brown amorphous solid (*ca.* 18·6 g.). It is under investigation.

2-*Methyl*-6-D-arabo-*tetra-acetoxybutylpyrazine*.—A mixture of 2-methyl-6-D-*arabo*-tetrahydroxybutylpyrazine (fraction 1) (22 mg.), dry pyridine (0·5 ml.), and acetic anhydride (0·5 ml.) was set aside at room temperature for 24 hr. and then poured into ice water (*ca.* 10 ml.). The *acetate* was extracted with chloroform (3 × 2 ml.), and the combined extracts were washed successively with 2N-hydrochloric acid (2 × 2 ml.), 2N-sodium hydrogen carbonate (2 × 2 ml.), and water, and dried (Na_2SO_4). Subsequent concentration gave a colourless syrup (44 mg.) (Found: C, 53·5; H, 5·9; N, 7·2; Ac, 41·8. $C_{17}H_{22}N_2O_8$ requires C, 53·4; H, 5·8; N, 7·3; Ac, 44·7%).

2-*Methyl*-5-D-arabo-*tetrabenzoyloxybutyl*-3-D-erythro-*tribenzoyloxypropylpyrazine*.—2-Methyl-5-D-*arabo*-tetrahydroxybutyl-3-D-*erythro*-trihydroxypropylpyrazine (fraction 2) (0·1 g.), dry pyridine (2 ml.), and benzoyl chloride (2 ml.) was left at room temperature overnight and poured into ice-cold saturated sodium hydrogen carbonate solution (*ca.* 20 ml.). The oily product which separated was dissolved in chloroform (10 ml.), washed successively with 2N-hydrochloric acid (2 × 10 ml.), sodium hydrogen carbonate (2 × 10 ml.), and water, and dried (Na_2SO_4). On concentration the chloroform solution afforded the *heptabenzoate* as needles (0·214 g.) which after recrystallisation from acetone–light petroleum (b. p. 40—60°) had m. p. 163° (Found: C, 71·0; H, 4·9; N, 2·8. $C_{61}H_{48}N_2O_{14}$ requires C, 71·0; H, 4·65; N, 2·8%).

2,3-*Diaminobutanal 2,4-Dinitrophenylhydrazone Hydriodide*.—Sodium metaperiodate (3 g.) in water (50 ml.) was added with stirring to 2-methyl-5-D-*arabo*-tetrahydroxybutyl-3-D-*erythro*-trihydroxypropylpyrazine (fraction 2) (0·5 g.). After 3 hr. the product was extracted with chloroform (10 ml.), washed with 1% sodium thiosulphate solution (2 × 10 ml.) and then with water, dried, and concentrated. The syrupy product was heated with 2,4-dinitrophenylhydrazine (0·1 g.) in ethanol (10 ml.) under reflux for 5 min., after which the mixture was cooled and concentrated hydrochloric acid (0·5 ml.) was added. The *dinitrophenylhydrazone* (65 mg.) formed needles (from ethanol), m. p. 192° (Found: C, 29·1; H, 3·8; I, 29·6; N, 20·4; *C*-Me, 2·5. $C_{10}H_{15}IN_6O_4$ requires C, 29·3; H, 3·7; I, 31·0; N, 20·5; *C*-Me, 3·7%).

2,3-*Diaminobutanal 2,4-dinitrophenylhydrazone* (*ca.* 5 mg.) was obtained from the hydriodide (23 mg.) by recrystallisation from aqueous ethanol (5 ml.) containing ammonia solution (1 drop; *d* 0·88). It had m. p. 135° (Found: C, 42·9; H, 4·9. $C_{10}H_{14}N_6O_4$ requires C, 42·55; H, 5·0%).

2,5-*Bis*-(D-arabo-*tetra-acetoxybutyl*)*pyrazine*.—The crystalline *octa-acetate* (44 mg.) was obtained from 2,5-bis-(D-*arabo*-tetrahydroxybutyl)pyrazine (fraction 3) (40 mg.) as above. On recrystallisation from acetone–light petroleum (b. p. 40—60°) it had m. p. 175° (Found: C, 51·2; H, 6·3; N, 4·5; Ac, 54·9. $C_{28}H_{36}N_2O_{16}$ requires C, 51·2; H, 6·2; N, 4·3; Ac, 52·5%).

The *octabenzoate* (24 mg.) was also obtained from the pyrazine derivative (fraction 3) (15 mg.) as above. It afforded needles (from ethanol), m. p. 120° (Found: C, 72·6; H, 4·7; N, 2·3. $C_{68}H_{52}N_2O_{16}$ requires C, 72·5; H, 4·5; N, 2·4%).

Pyrazine-2,5-dicarboxylic Acid.—2,5-Bis-(D-*arabo*-tetrahydroxybutyl)pyrazine (*ca.* 0·5 g.) in 5N-sulphuric acid (10 ml.) was titrated dropwise with saturated potassium permanganate solution until the pink colour persisted. The product was extracted with chloroform (3 × 5 ml.), washed with water, and dried (Na_2SO_4). Concentration of the chloroform extract afforded crystalline pyrazine-2,5-dicarboxylic acid (85 mg.). On purification by sublimation [9] this had m. p. 253° (sealed tube). It gave a bluish-violet colour with ferrous sulphate solution (Found: C, 42·8; H, 2·4; N, 17·0. Calc. for $C_6H_4N_2O_4$: C, 42·9; H, 2·4; N, 16·7%). Stoehr [9] records m. p. 255—256°.

Periodate Oxidations.—In each case, a mixture of *ca.* 0·3N-sodium metaperiodate (10 ml.) and the compound (*ca.* 100 mg., accurately weighed) was made up to 100 ml. with distilled water and stored in the dark. A control containing none of the compound was handled concurrently. At intervals, the periodate uptake was estimated by transferring samples (5 ml.) from the oxidation mixture and from the control into mixtures of phosphate buffer (pH 6·98; 25 ml.) and 20% potassium iodide solution (2 ml.), and the liberated iodine was

[9] Stoehr, *J. prakt. Chem.*, 1893, **47**, 487; 1895, **51**, 464.

titrated with 0·01N-sodium thiosulphate with starch as indicator.[10] Acid liberated during the oxidation was determined [11] by taking samples from the oxidation mixture and from the blank, adding ethylene glycol (2 ml.), and after 10 min. titrating the whole with 0·01N-sodium hydroxide (Methyl Red, screened with Methylene Blue). Formaldehyde liberated was determined colorimetrically with chromotropic acid,[12] D-glucose being used as standard. Primary amines formed during the oxidation of 2-methyl-5-D-*arabo*-tetrahydroxybutyl-3-D-*erythro*-trihydroxypropylpyrazine was estimated [13] by transferring samples (1 ml. portions) from the oxidation mixture and from the control into centrifuge tubes containing 10% aqueous lead dithionate solution (1 ml.). After mixing and centrifugation, 1 ml. portions of the supernatant liquor were placed in boiling tubes containing distilled water (5 ml.). 0·1% Ethanolic ninhydrin solution (1 ml. portions) was added and the tubes were left in boiling water for 20 min. in the dark. The tubes were cooled to room temperature and their contents were transferred into graduated flasks (10 ml.) which were then filled to the mark with distilled water. The absorption at 570 mμ was read on a colorimeter (Ilford, filter 626). Ethylenediamine was used for the preparation of the standard curve. The results are given in the Table.

Results of periodate oxidation.

	Time (hr.):	1/2	1	2	4	6	9	CH_2O *
(IV)	Uptake *	2·74	2·77	2·80	2·93	2·97	3·17	0·985
	Acid *	1·59	1·79	1·79	1·79	1·99	2·10	
(VIII)	Uptake	6·83	7·17	7·60	7·90	8·00	8·15	1·98
	Acid	3·49	3·91	4·04	4·18	4·67	4·80	
	NH_2 †	—	0·0	0·18	0·35	0·24	0·05	
(XI)	Uptake	5·10	5·35	5·37	5·42	5·55	5·86	1·67
	Acid	3·10	3·22	3·22	3·37	3·70	3·85	

* Moles/mole of the compound oxidised. † Equiv./mole of the compound oxidised.

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[10] Neumüller and Vasseur, *Arkiv Kemi*, 1953, **5**, 235.
[11] Halsall, Hirst, and Jones, *J.*, 1947, 1427.
[12] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.
[13] Moore and Stein, *J. Biol. Chem.*, 1948, **176**, 367.