192. *The Action of Bromine–Sulphuric Acid Mixtures on Methyl α-D-Glucopyranoside.*

By A. B. Foster and S. V. Vardheim.

Methyl α-D-glucopyranoside is hydrolysed on dissolution in bromine–sulphuric acid. Some of the liberated D-glucose is oxidised to D-gluconic acid but the major part undergoes reversion (polymerisation) and sulphation. Omission of the bromine leads to extensive decomposition of the carbohydrate.

Use of bromine–sulphuric acid for oxidative hydrolysis of barium hydrogen heparinate, barium chondroitin sulphate, and sodium mucoitin sulphate has been described by Wolfrom *et al.*[1] From barium hydrogen heparinate, monopotassium D-glucarate (saccharate), derived from the uronic acid moiety, was isolated whereas normal acidic hydrolysis led to total destruction of the released D-glucuronic acid.[2] Wolfrom and Rice [1] reasoned that, in the presence of bromine, the released D-glucuronic acid would be oxidised and hence stabilised. As first step in a study of the general applicability of the bromine–sulphuric acid reagent we have examined its action on methyl α-D-glucopyranoside.

Methyl α-D-glucopyranoside dissolved in 27N-sulphuric acid saturated with bromine did not char during several days at room temperature; omission of the bromine resulted in charring after *ca.* 5 hr. It is not known why charring should be retarded or inhibited by the presence of bromine. Increase in the sulphuric acid concentration above 27N caused rapid charring and decrease below 27N retarded the reactions to be described. The changes in optical rotation attending the dissolution of D-glucose, methyl α-D-glucopyranoside, and D-gluconic acid are shown in Fig. 1. Other information was obtained by

[1] Wolfrom and Rice, *J. Amer. Chem. Soc.*, 1946, **68**, 532; 1947, **69**, 1833.; Wolfrom and Brock Neely, *ibid.*, 1953, **75**, 2778.

[2] Foster and Huggard, *Adv. Carbohydrate Chem.*, 1955, **10**, 335.

determination of reducing power of the mixture as a function of time. The appearance and disappearance, respectively, of reducing power when methyl α-D-glucopyranoside and D-glucose were dissolved in bromine–sulphuric acid are represented in Fig. 2. (curves *A* and *B*). If the reducing material was hydrolysed by acid then an increase in reducing power occurred (Fig. 2; curves *C* and *D* which are related to curves *A* and *B*, respectively).

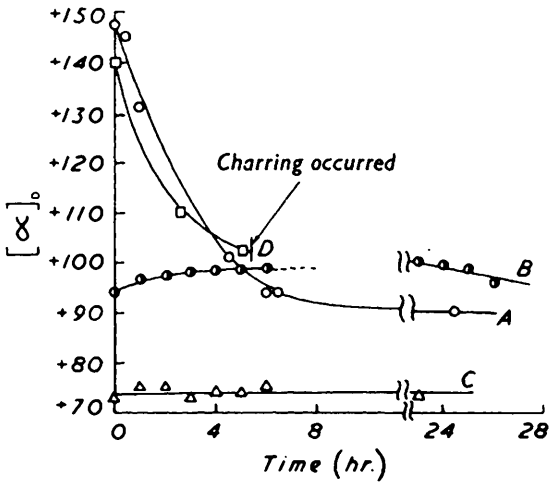

FIG. 1. *Changes in optical rotation (degrees) on the dissolution of derivatives of* D-*glucose in bromine–sulphuric acid.*

A, Methyl α-D-*glucopyranoside.*
B, D-*Glucose.*
C, D-*Gluconic acid.*
D, Methyl α-D-*glucopyranoside in the bromine-free reagent.*

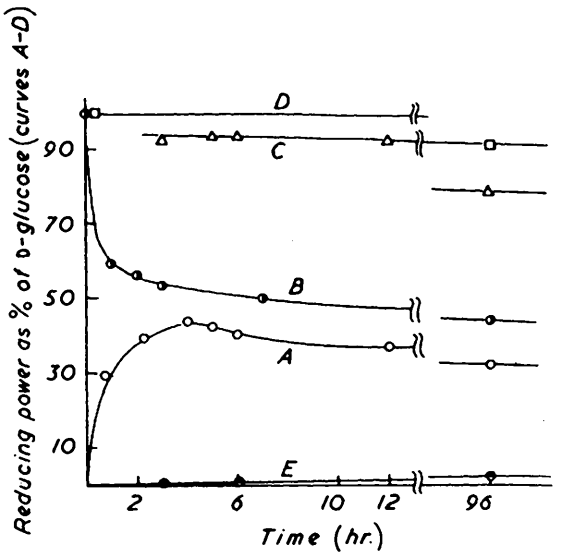

FIG. 2. *Reducing power of solutions of* D-*glucose and methyl* α-D-*glucopyranoside in bromine–sulphuric acid.*

Methyl α-D-*glucopyranoside*:
A, Reducing power of the diluted reaction mixture.
C, Reducing power after hydrolysis of the reducing substances in the diluted reaction solution.
E, Formation of D-*gluconic acid in the mixture.*

D-*Glucose*:
B as A.
D as C.

Paper ionophoretic examination [3] of the mixture after 24 hr. and after hydrolysis revealed a single reducing component with an M_G value [3] identical to that of D-glucose, and 1 : 2 : 3 : 4 : 6-penta-*O*-acetyl-β-D-glucose was subsequently isolated in 69% yield. This result indicates that little D-gluconic acid was produced and this was confirmed by ionophoretic separation of the D-gluconic acid and its determination by reaction with periodate. After 96 hr. only 6% of the methyl α-D-glucopyranoside had been converted into D-gluconic acid (Fig. 2; curve *E*). It is of interest that, after the reaction of barium hydrogen heparinate with bromine–27N-sulphuric acid [1] at 0° for 7 days, 64% of monopotassium

[3] Foster, *Chem. and Ind.*, 1952, 1050; Foster, Newton-Hearn, and Stacey, *J.*, 1956, 30, and references cited therein.

D-glucarate and 59% of 2-amino-2-deoxy-D-gluconic acid (D-glucosaminic acid) could be isolated. The extent of oxidation here is clearly much greater than in the methyl α-D-glucopyranoside reaction.

Other experiments gave some indication of the fate of the D-glucose following its hydrolytic release from methyl α-D-glucopyranoside in bromine–sulphuric acid. A small amount of non-dialysable, and presumably polymeric, material could be isolated which gave D-glucose on total acidic hydrolysis, and traces of a series of oligosaccharides on graded acidic hydrolysis similar to that obtainable from dextran. Infrared analysis indicated that α-1 : 6-links might be present but other properties of the non-dialysable material suggested that it was not a simple poly-D-glucose. It is of interest that acid reversion of D-glucose yields a number of disaccharides in which *iso*maltose (α-1 : 6-link) and gentiobiose (β-1 : 6-link) predominate.[4]

Paper ionophoresis[3] of the mixture after 24 hr. revealed neutral substances and sulphates. The presence of ester sulphate corresponding to 85% of D-glucose monosulphate was determined by hydrolysis of the diluted mixture. Attempts to fractionate the products on charcoal–Celite[5] were only partially successful. A mixture of D-glucose and D-glucose monosulphate (as revealed by paper ionophoresis) was readily eluted from the column with water and had reducing power equivalent to 25% of the theoretical maximum D-glucose content of the mixture and contained ester sulphate corresponding to 18% of D-glucose monosulphate calculated on the same basis. Much material clearly had been retained by the column but application of an alcohol–water gradient elution[6] displaced a negligible amount of material. This result is surprising since it is known that anionic substances are released more readily from charcoal–Celite than are neutral substances[7] and large amounts of the material absorbed by the column must be sulphated.

After 24 hr. the mixture contained a little ether-soluble material with maximal light absorption (in the mixture) at 245 mμ. It is well known[8] that carbohydrates are dehydrated in strong solutions of sulphuric acid to yield ultraviolet-absorbing substances. The formation of this material together with the production of D-gluconic acid appears to account for the slow disappearance of D-glucose from the mixture (Curve *C*, Fig. 2).

Unlike its action on barium hydrogen heparinate, bromine–sulphuric acid therefore does not cause extensive oxidation of the reducing sugar which had been hydrolytically released from methyl α-D-glucopyranoside but brings about polymerisation (reversion) and/or sulphation.

Solutions of methyl α-D-glucopyranoside in bromine–sulphuric acid did not char during 5 hr. at 64° but an apparently different reaction pattern appeared to be operating (see Experimental).

Experimental

All operations were carried out at room temperature unless otherwise stated.

Action of Bromine–27N-Sulphuric Acid on Methyl α-D-Glucopyranoside.—The following mixtures were used: *A*, methyl α-D-glucopyranoside (1·0 g.) plus bromine-saturated 27N-sulphuric acid (25 ml.); *B*, the glycoside (3·0 g.) plus the acid reagent (25 ml.); *C*, the glycoside (2·5 g.) plus the acid reagent (75 ml.). In each case the glycoside was added to the acid reagent; it dissolved within a few minutes.

(a) *Optical activity of the solution.* The change in the optical activity of mixture *A* is shown in curve *A* of Fig. 1 ($[\alpha]_D$ calc. as methyl α-D-glucopyranoside). D-Glucose and D-gluconic acid similarly gave curves *B* and *C* (Fig. 1). Dissolution of methyl α-D-glucopyranoside in 27N-sulphuric acid gave curve *D* (Fig. 1); charring occurred after *ca.* 5 hr.

(b) *Determination of the reducing power.* Aliquot portions were withdrawn from mixture *A* and freed from bromine by aeration. A portion (3 ml.) was diluted with water (45 ml.),

[4] Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309.
[5] Whistler and Durso, *ibid.*, 1950, **72**, 677.
[6] Lindberg and Wickberg, *Acta Chem. Scand.*, 1954, **8**, 569.
[7] Barker, Bourne, and Theander, *J.*, 1955, 4276.
[8] Rice and Fishbein, *J. Amer. Chem. Soc.*, 1956, **78**, 1005.

neutralised (phenolphthalein) by titration with 2N-sodium hydroxide, and made up to 100 ml. The reducing power of this solution was determined by Shaffer and Hartmann's method;[9] curve *A* of Fig. 2 was obtained in this way. Determination of the reducing power of D-glucose was not affected by the presence of excess of sodium sulphate or sodium bromide. Curve *B* (Fig. 2) was similarly obtained for D-glucose.

In separate experiments aliquot portions were withdrawn from mixture *A* and aerated, and a portion (3 ml.) diluted with water (45 ml.). The solution was heated on a boiling-water bath for 3 hr., and then neutralised, and the reducing power determined as described above. Curve *C* (Fig. 2) was thus obtained and similarly curve *D* for D-glucose.

Paper ionophoresis of the diluted and neutralised solutions [the apparatus and technique previously described being used [3] with borate (pH 10) and glycine (pH 11) buffers and development with aniline hydrogen phthalate [10]] indicated the presence of neutral carbohydrates (including D-glucose) and anionic substances (sulphates) before hydrolysis and D-glucose after hydrolysis. Similar results were obtained with mixture *A* after it had been stored at 0°.

Mixture *B* was put aside for 24 hr., then diluted, aerated, and neutralised. The solution was concentrated, freed from precipitated sodium sulphate, concentrated (to *ca.* 10 ml.), made 2N with respect to sulphuric acid, and heated on a boiling-water bath for 3 hr. The residue obtained after neutralisation and concentration of the solution was acetylated (acetic anhydride and sodium acetate) and gave 1 : 2 : 3 : 4 : 6-penta-*O*-acetyl-β-D-glucose (4 g., 69%), m. p. 129—130°.

(c) *Determination of ester sulphate.* After 24 hr. a mixture *B* was diluted, aerated, and neutralised in the usual way. Free sulphate ion was removed by the addition of an excess of barium chloride, and the solution remaining was evaporated to dryness. The residue was boiled with nitric acid (3 : 2-concentrated nitric acid–water; 25 ml.) for 1 hr. and the barium sulphate (3·08 g., corresponding to 85% of D-glucose monosulphate) isolated in the usual way. Hydrolysis with 2N-hydrochloric acid at 95—100° for 3 hr. gave a similar yield (3·15 g.) of the barium sulphate confirming that curve *C* (Fig. 2) represents total release of reducing power.

(d) *Attempted fractionation of the mixture.* A mixture *B* after 24 hr. was diluted, aerated, neutralised, and concentrated (to *ca.* 25 ml.) at 25—30°(bath)/12—15 mm. Precipitated sodium sulphate was removed and the remaining solution, which contained only a small amount of free sulphate ions, was introduced on a charcoal–Celite column (5 × 50 cm.), and eluted with water. Unidentified material (maximal absorption at 257 mμ and 245 mμ) and sodium sulphate were eluted in the first 225 ml. The next 875 ml. was optically active and contained reducing power equivalent to 25% of the D-glucose theoretically derivable from the initial methyl α-D-glucopyranoside and ester sulphate corresponding to 18% of D-glucose monosulphate calculated on the same basis. Paper-ionophoretic examination in borate buffer (pH 10) and development with aniline hydrogen phthalate [10] revealed two reducing components with M_G values [3] identical with those of D-glucose and D-glucose 3-sulphate.

Further elution of the column with an ethanol–water gradient [6] up to 30% alcohol caused the release of negligible amounts of material.

(e) *Determination of the D-gluconic acid produced.* D-Gluconic acid is readily determined in 0·1—1·0 mg. amounts by reaction with periodate,[11] and a good recovery from Whatman No. 3 paper after ionophoresis in glycine buffer (pH 11) was obtained.

Mixture *A* (3 ml.) was diluted, aerated, neutralised, and made up to 25 ml. A portion (2 ml.) was introduced on to Whatman No. 3 paper (*ca.* 57 × 13 cm.) in a suitably located zone (*ca.* 8 × 1 cm.) (cf. Foster [3]) flanked by reference spots of D-gluconic acid. After ionophoresis in glycine buffer (pH 11) for 1·5 hr. under an applied potential of 1000 v the reference spots were located with the silver nitrate–sodium hydroxide reagents,[12] the D-gluconic acid zone from the reaction mixture eluted from the paper, and its content determined by titration with periodate. D-Gluconic acid formation is shown by curve *E* (Fig. 2).

(f) *Isolation of non-dialysable material.* After 24 hr. a mixture *B* was diluted, neutralised, and dialysed against running tap water for 5 days. When the solution was freeze-dried the non-dialysable material (0·115 g.; equivalent to *ca.* 4% of the potential D-glucose content of the mixture) was obtained as a hygroscopic powder, incompletely soluble in water. Hydrolysis

[9] Shaffer and Hartmann, *J. Biol. Chem.*, 1920, **45**, 365.
[10] Partridge, *Nature*, 1949, **164**, 443.
[11] Jackson, *Org. Reactions*, 1944, **2**, 341.
[12] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.

with 2N-sulphuric acid for 3 hr. at 95—100° gave mainly D-glucose (detected chromatographically). Partial acidic hydrolysis (N-sulphuric acid; 1·75 hr. at 95—100°) followed by chromatography of the *N*-benzylglycosylamine derivatives [13] indicated traces of an oligosaccharide series similar to that obtained by the partial acidic hydrolysis of dextran. Infrared analysis (Nujol mull) of the non-dialysable material revealed peak absorptions at 1645(w), 971(s), 938(m), 920(m), 896(m), 872(s), 843(w), 800(w), and 767 cm.$^{-1}$ (m). The absorptions at 920, 843, and 767 cm.$^{-1}$ would be expected for a polyglucosan containing 1 : 6α-links.[14]

(g) *Development of ultraviolet absorption properties.* A mixture *C* after 24 hr. was aerated and a portion (3 ml.) withdrawn and diluted with water (97 ml.). This solution had maximal absorption at 245 mμ; exhaustive extraction with ether removed material (maximal absorption at 226 mμ) and left a weak residual absorption at 257 mμ.

In a separate experiment a mixture *A* was set aside at room temperature for 24 hr. and then heated at 65° for 5 hr. There was no charring but a tremendous increase occurred in the optical density at 245 mμ. The reducing power determined as in (b) was unchanged (20%) after hydrolysis.

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CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM, 15. [*Received, July 20th,* 1956.]

[13] Bayly and Bourne, *ibid.*, 1953, **171**, 385.
[14] Barker, Bourne, Stephens, and Whiffen, *J.*, 1954, 3468; Barker, Bourne, Stacey, and Whiffen, *J.*, 1954, 171.