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The Oxidation of Glycosides

VIII.* The Degradation of Methyl α-D-3-oxo-glucopyranoside, Methyl β-D-3-oxo-glucopyranoside and Methyl β-D-2-oxo-glucopyranoside by Lime-water **

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On treatment with lime-water methyl α-D-3-oxo-glucopyranoside, methyl β-D-3-oxo-glucopyranoside and methyl β-D-2-oxo-glucopyranoside were very rapidly degraded. The principal route involved the elimination of the methoxyl group and, in all cases, led initially to the formation of a dicarbonyl intermediate which then gave acids. The dicarbonyl intermediate was proved to be 1,5-anhydro-2,3-dioxo-D-glucitol. On reduction it gave all four expected 1,5-anhydro-D-glycitols including the hitherto unknown 1,5-anhydro-D-allitol.

It is commonly believed that the pronounced sensitivity of oxidised cellulose to alkali depends upon the presence of oxo-glucose residues. The alkaline degradation of carbohydrates to saccharinic acids has recently been reviewed by Sowden [1]. A probable ionic mechanism for those transformations, which has been advanced by Isbell [2], involves the following successive steps: (i) the formation and ionisation of an enediol; (ii) the β-elimination of a hydroxyl, alkoxyl or glycosyl group; (iii) a rearrangement to an α-dicarbonyl intermediate; and (iv) a benzilic acid type of rearrangement to give a saccharinic acid. In Fig. 1 this mechanism is shown applied to the alkaline degradation of those oxo-glucosides studied in the present investigation. Haskins and Hogsed [3] were the first to suggest that it was probable that the alkaline degradation of oxidised cellulose involved the scission of β-glucosylcarbonylresidues.

Oxo-glucosides are now available [4,5] and these were used as models simulating oxo-glucose residues in oxidised cellulose or in oxidised starch. Alkaline treatment with lime-water was used to enable a comparison to be made with the results obtained by Kenner *et al.*[1], who used oxygen-free, saturated lime-

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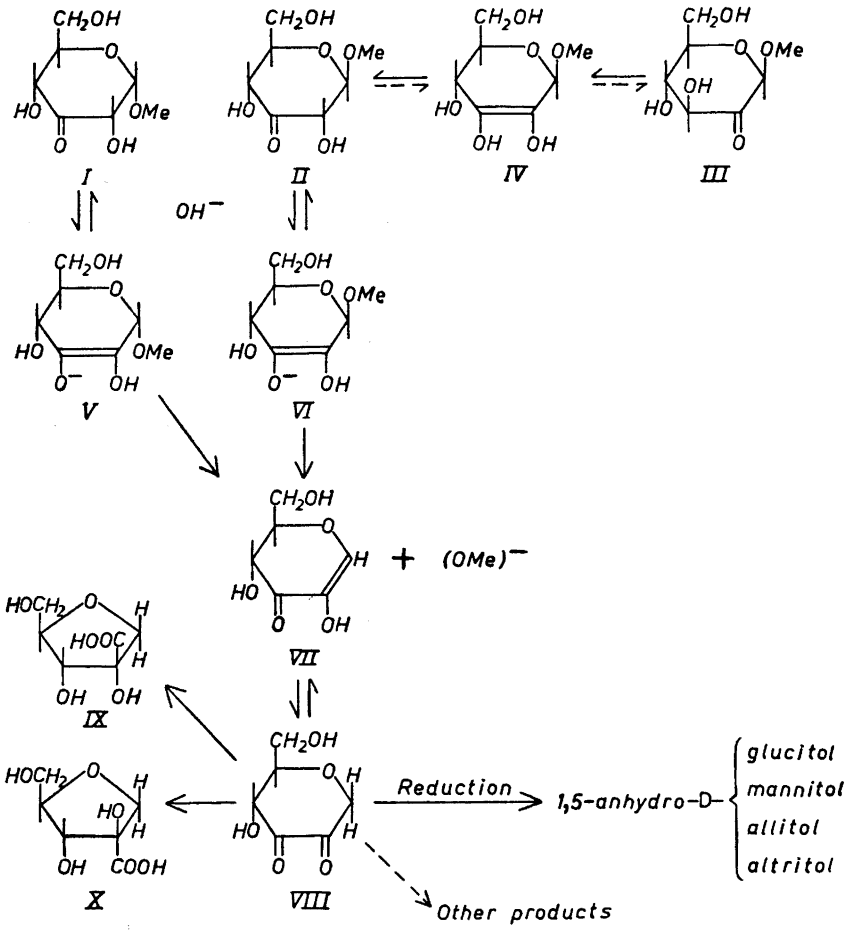

Fig. 1. Treatment of methyl α-3-oxo-glucoside (I), methyl β-3-oxo-glucoside (II) and methyl β-2-oxo-glucoside (III) with lime-water and reduction of 1,5-anhydro-2,3-dioxo-glucitol(VIII).

water (at 25°) in their extensive studies of the role played by substituents during the alkaline degradation of monosaccharides, oligosaccharides or polysaccharides.

When methyl β-D-3-oxo-glucopyranoside (II) was treated with saturated lime-water (at 25°) the optical rotation altered very rapidly from a negative to a high positive value. Fractions were withdrawn after short periods of treatment and immediately acidified and then examined on paper chromatograms. It was found that, associated with a rapid decrease of II, there was an increase in the amounts of the acids. A considerable quantity of a compound, giving distinctive colorations with the various spray reagents, was

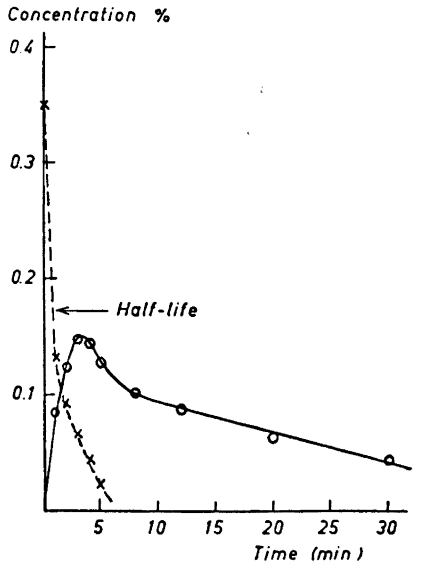

Fig. 2. Formation of 1,5-anhydro-2,3-dioxo-glucitol (—O—O—) on treatment of methyl β-3-oxo-glucoside (– – × – – × – –) with lime-water (0.042 N) at 25°.

observed. It was shown to be the α-dicarbonyl intermediate (VIII) that would be expected to be formed if the degradation involved scission of the glycosidic ether linkage. Both II and VIII were semi-quantitatively determined throughout the reaction period (Fig. 2). The extremely short half-life of II (about one minute) is roughly one hundreth of those of the most alkali-labile sugars previously studied (laminaribiose and turanose [6]) and is about 2 000 times less than that of cellobiose [7].

Dilute lime-water (0.02 N) was used to enable the optical rotation to be followed during the degradation of methyl α-D-3-oxo-glucopyranoside (I), methyl β-D-3-oxo-glucopyranoside (II) and methyl β-D-2-oxo-glucopyranoside (III). All three oxo-glucosides gave a maximum positive rotation which was correlated, by paper chromatographic examination, with the presence of the principal component (compound VIII). The 2-oxo-glucoside (III) was degraded more slowly than were the two anomers of 3-oxo-glucoside, and by the paper chromatographic examination, it was shown that it was first transformed into a compound having the same R_F-values as II. There was also a component present which gave a pink spot with resorcinol, and which had an R_F-value between those of the two oxo-glucosides. This spot may have been due to the enediol IV. But no epimerisation of II to III was detected when the former was treated with alkali (Fig. 3).

In several experiments compound VIII (prepared from II) was isolated. The amorphous product, in aqueous solution at pH 5, had $[\alpha]_D^{22}$ + 155° and ε_{max} 3 100 at 293 mμ (methyl β-3-oxo-glucoside: $\varepsilon_{max.}$ 90 at 285 mμ). Small amounts were obtained crystalline (m.p. 53—55°). The amorphous and crystalline products gave single clearly defined spots which were both chromatographically and electrophoretically indistinguishable from one another.

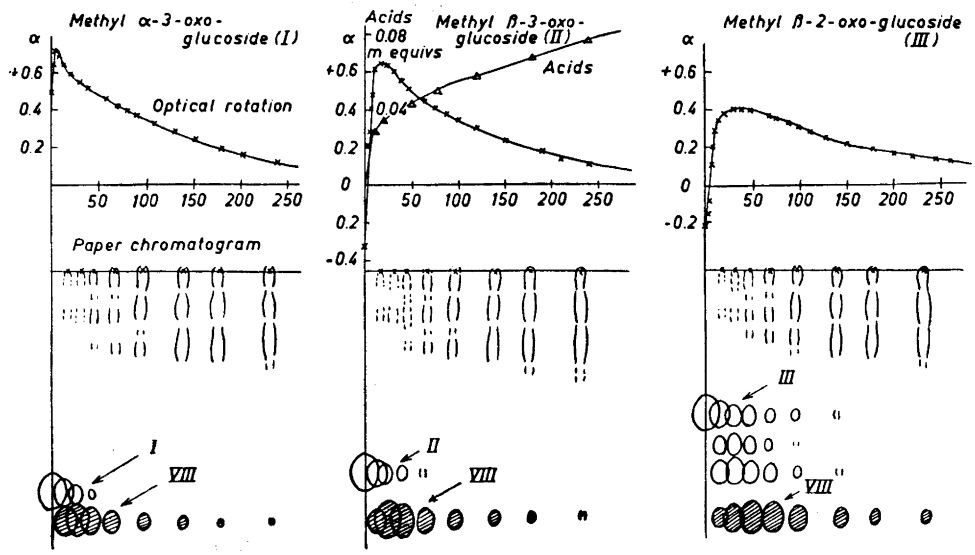

Fig. 3. Diagram of paper chromatograms (irrigant B) showing spots observed and relating them to the fall in optical rotation when methyl α-3-oxo-glucoside, methyl β-3-oxo-glucoside and methyl β-2-oxo-glucoside were separately treated with lime-water (0.02 N) at 22°. Abscissa = time in minutes.

The dicarbonyl compound rapidly formed a precipitate with 2,4-dinitrophenylhydrazine, but did not give a satisfactory crystalline derivative with this reagent or with *o*-phenylenediamine. It carried no methoxyl group, but carbonyl determination, by the borohydride method, indicated the presence of two carbonyl groups (assuming M.W. 160).

The mixture of products obtained on the reduction of the dicarbonyl intermediate with Raney nickel or with borohydride was separated. The four products obtained were: amorphous 1,5-anhydro-D-altritol (characterised as its crystalline tetra-acetate) and crystalline 1,5-anhydro-D-glucitol, 1,5-anhydro-D-mannitol and a substance having m.p. 147—148°, $[\alpha]_D^{22}$ + 26° (in water). This last substance gave an analysis consistent with the formula $C_6H_{12}O_5$ and, from the evidence presented below, it was concluded to be 1,5-anhydro-D-allitol. Its specific rotation, +26°, accords reasonably well with those calculated from the specific rotations of methyl β-D-alloside and the methyl β-D-glycosides and 1,5-anhydro-D-glycitol derivatives of glucose, altrose and gulose ($[\alpha]_D$ = +20°, 22° and 28°). The values calculated from the rotations of the corresponding derivatives of mannose and galactose however were markedly different. Foster [8] has shown that 1,5-anhydro-glycitols and the corresponding methyl-glycosides behave similarly on electrophoresis. The M_G value of the above unknown compound was similar to that of methyl β-alloside (Table 1); a strong indication of the former being 1,5-anhydro-D-allitol. No 1,5-anhydro-galactitol could be detected among the reduction products.

Table 1. Paper electrophoretic mobilities in borate (M_G-values) of methyl D-glycopyranosides and 1,5-anhydro-D-glycitols.

	M_G
Methyl β-alloside	0.49
1,5-Anhydro-allitol	0.48
1,5-Anhydro-altritol	0.63
Methyl α-glucoside	0.10
Methyl β-glucoside	0.16
1,5-Anhydro-glucitol	0.18
Methyl α-mannoside	0.39
Methyl β-mannoside	0.30
1,5-Anhydro-mannitol	0.38
Methyl α-galactoside	0.35
Methyl β-galactoside	0.37
1,5-Anhydro-galactitol	0.36

It is of interest to note that the proportions of the four reduction products varied with the choice of reducing conditions. When Raney nickel was used (at pH 5) there was a much higher yield of anhydro-mannitol than of anhydro-glucitol but this order was reversed when borohydride was used (at pH 9.5). There was no marked difference in the relative yields of anhydro-allitol, but that of anhydro-altritol decreased to traces when borohydride was used. The difference might be due both to the nature of the reductants as such, and to a preferential formation of the dioxo form (VIII) at pH 5 and of the enolic form (VII) at pH 9.5. At pH 5, the dicarbonyl intermediate gave no coloration with ferric chloride. UV spectroscopic studies at different pH values strongly indicate that at pH 9.5 a keto-enol equilibrium exists (Fig. 4). At that pH the UV absorption is compounded of a contribution given by the absorption peak noted at 290 mμ at pH 5 and of the peak noted at *ca.* 340 mμ at pH 12 (a ketone having an αβ-ethylenic bond exhibits an absorption peak at *ca.* 330 mμ [9]). A compatible observation was made when II was treated with lime-water and the reaction stopped, by acidification of the mixture, at that time at which VIII was present in maximum amount. The peak initially observed in the UV spectra at 320 mμ then vanished and a new peak appeared at 280 mμ; simultaneously the optical rotation fell by about 50 %. The UV absorption reverted to the former type on making the solution alkaline.

The isolation of the four 1,5-anhydro-glycitols (in the relatively high yield of 60 %) proves that the structure of the intermediate is 1,5-anhydro-2,3-dioxo-D-glucitol. This is the first case reported of isolation and identification of a dicarbonyl intermediate in the alkaline degradation of carbohydrates.

Recently Blears, Machell and Richards [10] reported that when 4-*O*-substituted glucose derivatives were degraded by dilute sodium hydroxide solution, an intermediate was formed which was believed to be a dicarbonyl derivative. It was not pure, although it gave a crystalline osazone. Evidence was also obtained of the presence of fragmentation products, possibly formed from dicarbonyl intermediates.

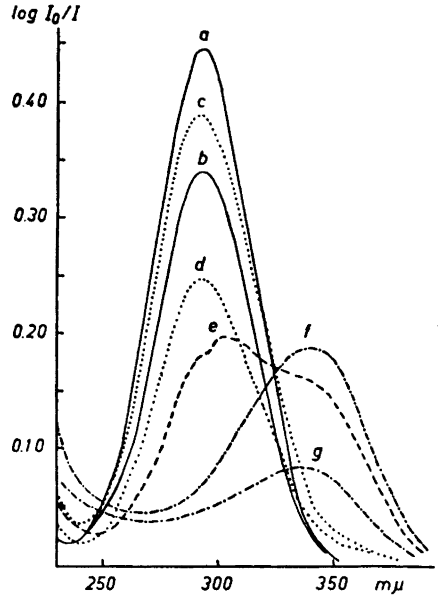

Fig. 4. Ultraviolet absorption spectra of 1,5-anhydro-2,3-dioxo-glucitol in aqueous solutions at different pH-values at 22°. (All four solutions were made from the same stock solution by diluting it to $1.5 \cdot 10^{-4}$ M).

pH 5.0 (0.05 M acetate buffer):	*a*, directly		after	pH	adjustment
	b, 1 day		»	»	»
pH 7.5 (0.05 M phosphate buffer):	*c*, 10—30	min.	»	»	»
	d, 130—150	»	»	»	»
pH 9.5 (0.05 M borate buffer):	*e*, 10—30	»	»	»	»
pH 12.1 (0.02 N lime-water):	*f*, 35—50	»	»	»	»
	g, 110—125	»	»	»	»

From the reaction-mixture produced when methyl 3-oxo-glucoside was treated with lime-water the various neutral products were isolated at that time at which the product VIII was present in maximum amount. The overall yield of neutral products was 65 % and of this three fifths were VIII, one fifth was unreacted methyl 3-oxo-glucoside and one fifth a complex mixture of components. Scission of a carbon-oxygen bond could take place at three sites in β-position to the oxo-group, namely at the two ring-oxygen linkages or at the glycosidic link (see Fig. 1). It is apparent that the reaction proceeded, to a very large extent by elimination of the glycosidic methoxyl to give the intermediate VIII. This view is supported by the fact that the acids were present in roughly the same proportions as were those obtained when a sample of VIII was treated with lime-water; in both cases the mixtures of acids were electrophoretically similar. They have not yet been studied in detail. On treatment of VIII with lime-water also some neutral products were formed. The dicarbonyl compound VIII was unstable over the entire pH range investi-

gated (1.5—12) but most labile on the alkaline side. The reaction-mixtures were studied by paper chromatography and by UV spectroscopy (Fig. 4). These studies indicated that different types of reactions take place under acidic and under basic conditions.

The mixture of neutral products obtained on treating II with lime-water would be expected to be highly complex as numerous reactions will have taken place. In addition to the principal reaction discussed, there may be, for instance, reactions involving epimerisation, molecular fragmentation or the scission of ring-oxygen linkage. From the mixture small amounts of a compound were isolated, which were chromatographically and electrophoretically indistinguishable from methyl β-D-4-oxo-glucopyranoside. On reduction and subsequent hydrolysis it gave glucose and galactose, indicating that epimerisation had taken place at C_4. Two other compounds were isolated having higher R_F-values than VIII. One had a methoxyl group, while the other did not. The latter compound crystallised (m.p. 157—159°) and gave a violet coloration with ferric chloride.

Investigations are continuing on the alkaline degradation of oxo-glucosides. Experiments now in progress show that the degradation of II and III via VIII is of importance even under slightly acidic conditions.

EXPERIMENTAL

Melting points are corrected. The ultraviolet spectra were determined in aqueous solution with a Beckman DU instrument. All solvents were removed by distillation under reduced pressure. Whatman No. 1 paper was normally used for paper chromatography and electrophoresis. Preparative separations were carried out on Whatman No. 3 MM or 31 paper. The following reagents were used in sprays: (a) silver nitrate-sodium hydroxide, (b) *p*-anisidine hydrogen chloride, (c) resorcinol-hydrochloric acid, (d) bromocresol green and (e) ferric chloride.

The irrigants and buffers used were:
A. Ethyl acetate-acetic acid-water, 3:1:3 (upper phase).
B. Butanol-ethanol-water, 10:3:5.
C. Hydrogen sulphite buffer pH 4.7, 0.1 M. (used at 50°) [11].
D. Borate buffer pH 10.0, 0.1 M.
E. Acetate buffer pH 4.0, 0.1 M.

Kinetic studies of the action of lime-water on methyl α-D-3-oxo-glucopyranoside (I), methyl β-D-3-oxo-glucopyranoside (II) and methyl β-D-2-oxo-glucopyranoside (III)

The oxo-glucosides were prepared as previously [4,5] described. The lime-water used was freed from oxygen by bubbling a stream of nitrogen through it.

A sample (35 mg) of II was treated with saturated lime-water (50 ml; 0.042 N; pH 12.3) at 25°. The pH remained practically constant throughout the treatment and fractions were withdrawn at short intervals and immediately shaken with an excess of Amberlite resin IR-120 (H^+) to form the free acids from the calcium salts of the reaction products. Samples of the fractions were placed, with cold air drying of the solutions, on paper chromatograms, some of which were then run in irrigant A and some in irrigant B. One paper of each was treated with spray *a* and one with spray *c* and quantitative estimations were made by visual comparison of the area of the spots produced with those, produced by a series of reference spots corresponding to known amounts of II and of VIII. The

average values obtained are given in Fig. 2. The sample of VIII used for the preparation of the reference solution was freshly isolated from a pure fraction on thick paper chromatograms. The concentration in an aliquot of the stock solution was determined gravimetrically following evaporation of the solvent. The amount of contaminating decomposition products formed between the time of elution of VIII and that of its application to the chromatograms was probably < 5 %.

In another series of experiments samples (63 mg of each) of I, II and III were treated with dilute lime-water (25 ml; 0.019 N, pH 12.1) at 22°. The change in optical rotation was noted (2 dm tube) and samples were withdrawn from time to time and, after acidification (by treatment with Amberlite IR-120, see above), were examined on paper chromatograms. The number of equivalents of acids formed during the alkaline treatment of II was estimated in a separate experiment by back-titration after the addition of an excess of sulphuric acid. The results are given in Fig. 3.

In all three cases the solution became pale yellow and turned cloudy shortly after the maximum optical rotations were observed. This cloudiness may have been due to sparingly soluble calcium salts. The precipitated material was filtered off and nitrogen again passed through the solution. The optical rotations taken before and after the development of the cloudiness all lay on a smooth continuous curve.

Action of lime-water on methyl β-D-3-oxo-glucopyranoside (II) and fractionation of the products

Methyl β-3-oxo-glucoside was treated batchwise (0.2—1.0 g) with oxygen-free lime-water (40 ml 0.02 N/0.1 g of II) at 25° and the products were then fractionated. The course of the reaction was followed polarimetrically and when the maximum rotation was observed the degradation was normally stopped by acidification as previously, giving a pH of *ca.* 4. In a few cases before paper chromatographic separation the solution was rapidly passed through a column containing an excess of Amberlite IR-4 B (free base) from which the acids could then be recovered as ammonium salts. It was found that a better yield of VIII could be obtained if the last ion exchange step was omitted. The acidic products accounted for 25 % and the neutral products for 65 % of the starting material. The solution was concentrated to a volume small enough to be suitable for application to chromatographic papers (the last step of this concentration was by lyophilisation). The separation was carried out on Whatman No. 31 papers by irrigating them with A for 1 3/4 h. After drying the sheets, the positions of the various components were determined in the usual way and they were then eluted. The total time for those procedures was *ca.* 1 h. Four fractions were obtained:

A. A complex mixture of compounds, principally acids, having R_F-values less than that of II.
B. Unreacted II (*ca.* 20 % of the neutral products).
C. Chromatographically pure VIII (*ca.* 60 % of the neutral products).
D. A mixture of compounds having R_F-values higher than that of VIII and containing two major components. (Two minor components giving brown spots were noted on unsprayed chromatograms on which this fraction was examined.)

From fraction A a small amount of a component, indistinguishable from methyl β-D-4-oxo-glucopyranoside, was separated by paper chromatography (irrigant A). On reduction with borohydride and subsequent hydrolysis it gave compounds chromatographically indistinguishable from glucose and galactose.

From fraction D the two principal neutral products were chromatographically separated (each representing about 5 % of the starting II) on Whatman No. 3 MM paper (irrigant A). One of them, which contained no methoxyl gave a strong violet coloration with spray *e*, crystallised (m.p. 157—159°) from aqueous ethanol and the other contained *ca.* 15 % OCH_3.

Characterisation and properties of 1,5-anhydro-2,3-dioxo-D-glucitol (VIII)

Fraction C was slightly contaminated by traces of acetic acid (pH of solution 3—4). Generally the solution was used directly in order to minimize the danger of decomposition. The concentration was determined gravimetrically after evaporating the solvent from an aliquot and drying the amorphous material over P_2O_5. The optical rotations and UV-absorptions of the fresh eluates were determined in several cases (the pH was adjusted to 5). The average values obtained were $[\alpha]_D^{22}$ +155° and $\varepsilon_{max.}$ 3 100 at 293 mμ. In three experiments the solutions were lyophilised in a flask externally cooled to −10°. The syrup obtained, partly crystallised. By treatment of aqueous slurries of this material with either acetone, *n*-propanol or methyl ethyl ketone and acetic acid, small amounts of chromatographically and electrophoretically pure crystalline material (m.p. 53—55°) were obtained. The compound decomposed so readily that it was not possible to crystallise it in the usual way from warmed solvents (even at 0° the crystals decomposed).

Assuming the molecular weight of VIII to be 160, the compound was estimated, by borohydride reduction [12], to contain 1.84 carbonyl groups. It had no methoxyl groups. On paper chromatographic examination it was found to react with silver nitrate, even in the absence of sodium hydroxide, and to give distinctive yellow spots with sprays *b* or *c* but no coloration with sprays *d* or *e*. The compound has $R_{Glucose}$ 4.42 and 2.31 (irrigants A and B, resp.); M_V, 1.62 (buffer C); M_G, 0.63 and 0 (buffers D and E, resp.).

The results of UV-studies on VIII at different pH-values are given in Fig. 4. Products formed from VIII on treating it for various times and at different pH-values (1.5—12) were examined paper chromatographically. A sample of VIII was treated for 30 min with lime-water (0.02 N) and the ammonium salts of the acids formed were compared on electrophoretograms (buffer E) with the ammonium salts mentioned earlier (those obtained on the alkaline treatment of II). The two mixtures were electrophoretically similar, and contained one component which predominated in each case and which had $M_{Gluconic\ acid}$, 1.30.

Reduction of 1,5-anhydro-2,3-dioxo-D-glucitol and fractionation of the products

Samples of VIII (present in freshly isolated eluates) were reduced either by boiling them under reflux with Raney nickel in 70 % ethanol (pH 5) for 3 h, or by treating them with borohydride (pH 9.5) at room temperature for 6 h. After deionisation and evaporation of the solvents the products were chromatographically fractionated (irrigant A) on Whatman No. 3 MM paper (previously washed with water).

Two fractions (1 and 2) were obtained (60 % yield from VIII). The first one was subfractionated by chromatography (irrigant A) and the second one by electrophoresis in borate buffer to give four fractions (1a, 1b, 2a and 2b).

Fraction 1a was treated with charcoal and then crystallised from aqueous ethanol. It was chromatographically and electrophoretically indistinguishable from 1,5-anhydro-D-glucitol and had m.p. and mixed m.p. 141—142° and $[\alpha]_D^{22}$ +43° (*c*, 0.5 in water).

Fraction 1b was crystallised in the same way as above. The product was chromatographically and electrophoretically indistinguishable from 1,5-anhydro-D-mannitol and had m.p. and mixed m.p. 156—157° and $[\alpha]_D^{22}$ −54° (*c*, 0.5 in water).

Fraction 2a was amorphous. The product was chromatographically and electrophoretically indistinguishable from 1,5-anhydro-D-altritol. It had $[\alpha]_D^{22}$ +25 (*c*, 0.5 in water) (Ref.[13] +28.4). The product was acetylated and then gave 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-altritol which had m.p. and mixed m.p. 102—103°.

Fraction 2b was crystallised from aqueous ethanol. The product had m.p. 147—148° $[\alpha]_D^{22}$ +26 (*c*, 0.5 in water) and C 43.8; H 7.28. Calc. for $C_6H_{12}O_5$: C 43.9; H 7.37.

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