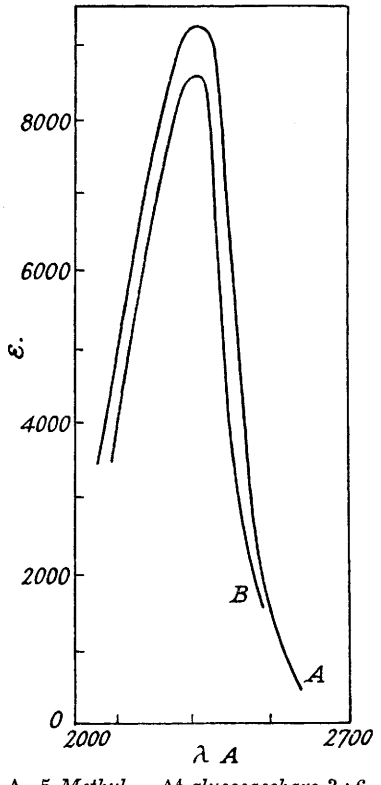
174. *Lactones of Glucosaccharic Acid. Part IV. The Enol of 5-Keto-4-deoxyglucosaccharo-3 : 6-lactone, an Analogue of Ascorbic Acid.*

By (Miss) D. Heslop and F. Smith.

Glucosaccharo-1 : 5-3 : 6-dilactone (V), glucosaccharo-1 : 4-3 : 6-dilactone (IV), glucosaccharo-1 : 4-lactone 6-methyl ester (II; R = Me), and glucosaccharo-3 : 6-lactone 1-methyl ester (III; R = Me) all reduce Fehling's solution. The peculiar reducing activity of these four substances is shown to be due to isomerisation brought about by alkaline reagents whereby there is produced from each of them the highly reducing enol of 5-keto-4-deoxyglucosaccharo-3 : 6-lactone (VIII; R = R′ = H), the structure of which is established. Ozonisation of this lactone gave oxalic acid and *l*-threouronic acid (IX), the latter being identified by its oxidation to dihydroxy-*l*-threosuccinic acid (*d*-tartaric acid) (IX; but CO_2H for CHO). Methylation of (VIII; R = R′ = H) with diazomethane yielded the corresponding 5-methyl ether of the ester lactone (VIII; R = Me, R′ = H), and this was converted by the agency of silver oxide and methyl iodide into the characteristic crystalline 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VIII; R = R′ = Me).

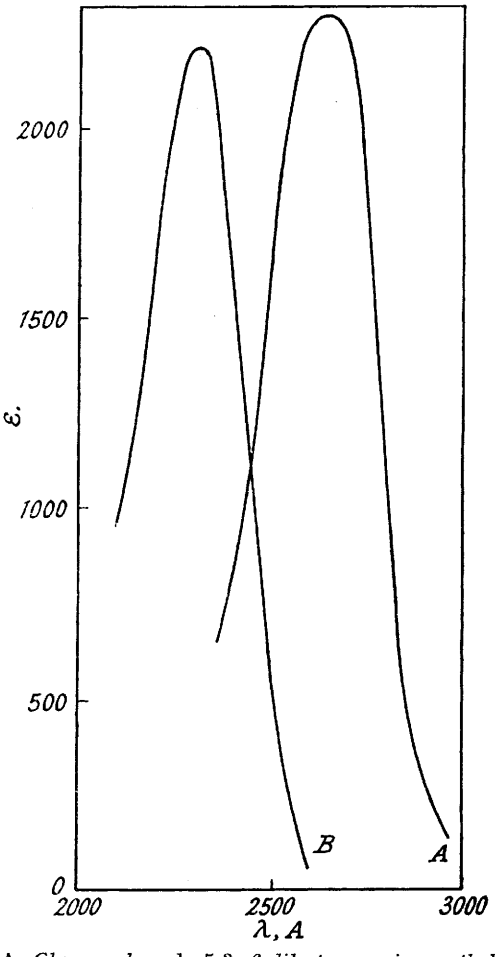
Lactonisation of saccharic acid by concentration of an aqueous solution yielded two monolactones, glucosaccharo-1 : 4- (II; R = H) and -3 : 6-lactone (III; R = H), the latter being obtained in much the greater

Fig. 1.



A. 5-*Methyl* Δ^4-*glucosaccharo*-3 : 6-*lactone* 1-*methyl ester in water* (4 *mg.* %).
B. 2 : 5-*Dimethyl* Δ^4-*glucosaccharo*-3 : 6-*lactone* 1-*methyl ester in water* (4 *mg.* %).

Fig. 2.

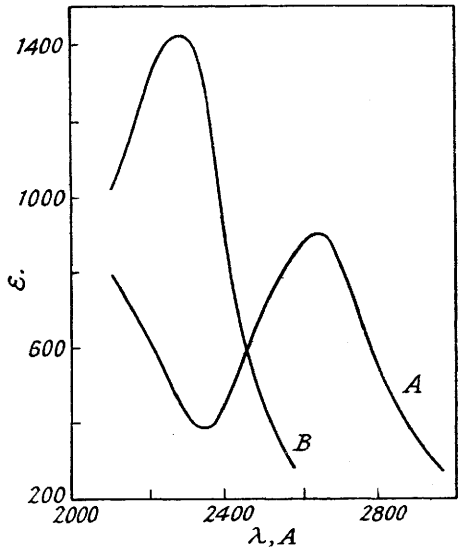


A. *Glucosaccharo*-1 : 5-3 : 6-*dilactone in ethyl alcohol, to which was added aqueous sodium hydroxide* (10 *mg.* %).
B. *Solution used for* A *acidified* (10 *mg.* %).

proportion. By means of diazomethane these two lactones were converted into the corresponding ester lactones (II; R = Me) and (III; R = Me) and by heating in a vacuum at 100° the two monolactones (II; R = H) and (III; R = H) afforded glucosaccharo-1 : 5-3 : 6-dilactone (V) and glucosaccharo-1 : 4-3 : 6-dilactone (IV) respectively (Smith, this vol., p. 510). It was pointed out that the four substances (II; R = Me), (III; R = Me), (IV), and (V) had the peculiar property of reducing Fehling's solution, a phenomenon also displayed

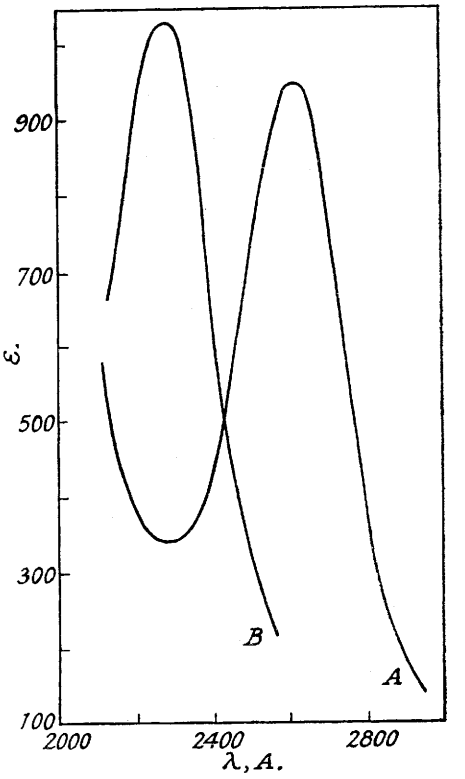
by mannosaccharo-1 : 4-3 : 6-dilactone (VI) (Kiliani, *Ber.*, 1887, **20**, 2710; Fischer, *Ber.*, 1891, **24**, 539). It was demonstrated by the present authors that the reducing activity of the latter was due to the production by alkaline reagents of an unsaturated isomer of the dilactone which proved to be the enol of 5-keto-4-deoxy-mannosaccharo-3 : 6-lactone (VII) (this vol., p. 224). The work herein described proves that a compound analogous to (VII) is responsible for the reducing activity shown by the two glucosaccharodilactones (IV) and (V), and the two ester lactones (II; R = Me) and (III; R = Me). The evidence which led to this conclusion is as follows : Methylation of all these four substances gave rise to **2 : 5**-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VIII; R = R′ = Me), an analogue of ascorbic acid displaying a selective absorption band at λ 2290 A. (Fig. 1). When these four lactones were separately treated with alkaline reagents, isomerisation took

FIG. 3.



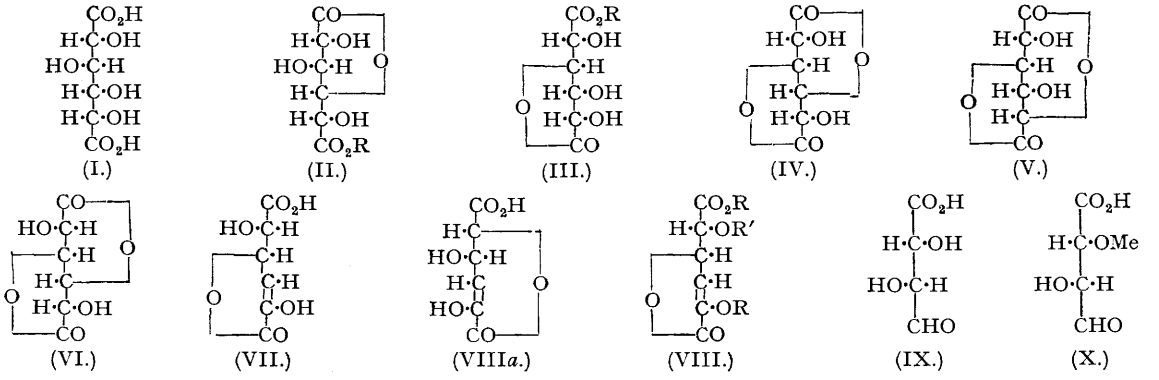
A. *Glucosaccharo*-1 : 4-3 : 6-*dilactone in aqueous alkali* (19 *mg.* %).
B. *Solution used for* A *acidified* (19 *mg.* %).

FIG. 4.



A. *Glucosaccharo*-3 : 6-*lactone* 1-*methyl ester in aqueous alkali* (40 *mg.* %).
B. *Solution used for* A *acidified* (26 *mg.* %).

place with the formation of a substance which exhibits in alkaline solution a band at λ 2630 A. Acidification of each of these solutions caused the band to move to λ 2290 A. (Figs. 2, 3, 4, 5), the position of the band

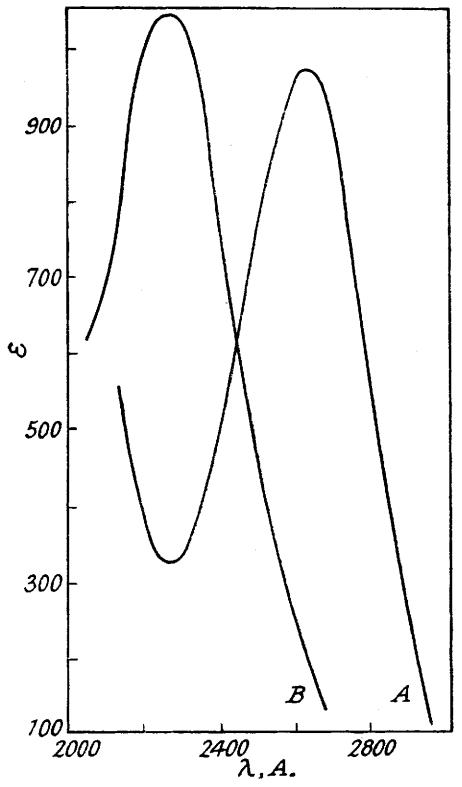


shown by (VIII; R = R′ = Me). Such acidified solutions showed reducing activity to a marked degree; *e.g.*, they reacted immediately with chlorine or bromine, rapidly decolorised potassium permanganate, and

reduced Fehling's solution actively. The substance formed from these four compounds was thought to be the same in each case and closely related to 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VIII; R = R′ = Me), and indeed it was suggested that the reactive reducing substance was the unmethylated form of this (Smith, this vol., p. 510).

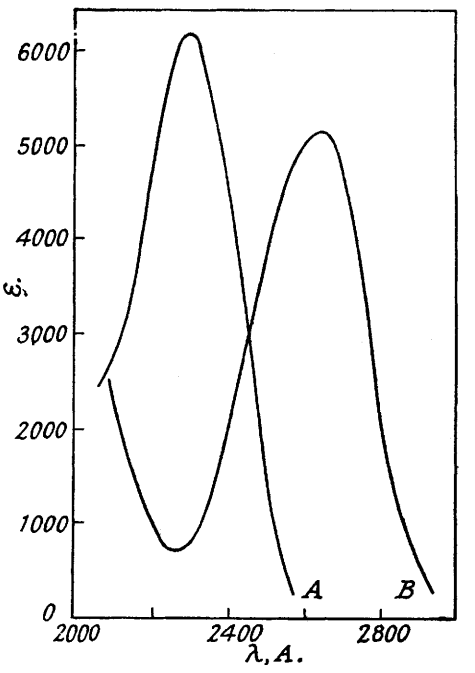
When glucosaccharo-1 : 5-3 : 6-dilactone (V) was treated with methyl-alcoholic sodium methoxide, followed by methyl-alcoholic hydrogen chloride, there was formed a syrup which displayed all the reducing properties referred to above and had an absorption band at λ 2290 A. moving to λ 2630 A. on addition of sodium hydroxide. Methylation of this syrup with silver oxide and methyl iodide gave in good yield 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VIII; R = R′ = Me); these facts suggested, but did not prove, that the unmethylated form of (VIII; R = R′ = Me) was present in the syrup before methylation. Ozonisation of this syrup in glacial acetic acid gave oxalic acid and an aldehydic acid (IX), which upon oxidation with bromine gave rise to dihydroxy-*l*-threosuccinic acid (IX; but CO_2H for CHO). There was thus good evidence for the existence of a compound, in the syrup examined, possessing the reducing system ·C(OH):CH·

FIG. 5.



A. *Glucosaccharo*-1 : 4-*lactone* 6-*methyl ester in aqueous alkali* (41 *mg.* %).
B. *Solution used for* A *acidified* (27 *mg.* %).

FIG. 6.



A. *Enol of* 5-*keto*-4-*deoxyglucosaccharo*-3 : 6-*lactone in water* (5 *mg.* %).
B. *Enol of* 5-*keto*-4-*deoxyglucosaccharo*-3 : 6-*lactone in aqueous alkali* (5 *mg.* %).

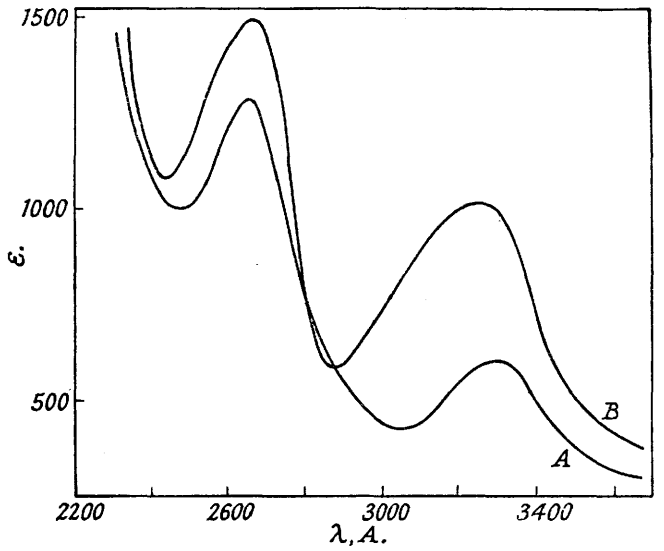
and one, probably the same substance, which affords 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VIII; R = R′ = Me) upon methylation.

Careful control of the conditions for the isomerisation of the dilactone (V) by the agency of sodium methoxide resulted in the isolation of the reactive substance itself in the crystalline form. By a similar procedure the same crystalline substance was obtained from the second dilactone (IV) and the two ester lactones (II; R = Me) and (III; R = Me) (see Figs. 2, 3, 4, 5, 6). The crystalline, reactive substance to which the structure (VIII; R = R′ = H) has been given was acid, and displayed mutarotation in aqueous solution; it showed a strong selective absorption band at λ 2290 A., moving to λ 2630 A. upon addition of sodium hydroxide (Fig. 6), and it reacted in the cold with 2 equivs. of sodium hydroxide, thus indicating the presence of two acid groups. In aqueous solution (VIII; R = R′ = H), like the enol of 5-keto-4-deoxymannosaccharolactone (VII) (Heslop and Smith, *loc. cit.*), reacted with four atomic proportions of chlorine, and in alkaline solution with six atomic proportions of iodine. When subjected to ozonolysis in glacial acetic acid (VIII; R = R′ = H) gave oxalic acid and the highly reducing, aldehydic acid, threuronic acid (IX), characterised by its oxidation with bromine to dihydroxy-*l*-threosuccinic acid (dextro-tartaric acid) (IX; but CO_2H for CHO). No glyoxylic acid was formed. These facts located the position of the double bond between C_4 and C_5 and proved that the system

·CH:C(OH)·CO was present in the crystalline reducing enolic compound (VIII; R = R′ = H). The isolation of the tartaric acid also proved that the stereochemical arrangement of H and OH groups at C_2 and C_3 was the same as that obtaining in this acid. Although these observations lent strong support to the view that the reactive crystalline substance had structure (VIII; R = R′ = H), they did not enable a choice to be made between this and the other possible formula (VIII*a*), for both these structures explained the results of ozonolysis. The following series of transformations, however, proved conclusively that the furone structure (VIII; R = R′ = H) was correct.

Treatment of the reactive compound with ethereal diazomethane effected the smooth introduction of two methyl groups, one at C_1 and the other at C_5, giving crystalline *5-methyl* Δ^4*-glucosaccharo-3 : 6-lactone 1-methyl ester* (VIII; R = Me, R′ = H). By means of silver oxide and methyl iodide the latter was converted into the known crystalline 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VIII; R = R′ = Me). In view of the fact that the original substance and these two derivatives all show in aqueous solution the same selective absorption band at λ 2290 A. (see Figs. 6 and 1), it was evident that the transformation of (VIII; R = R′ = H) into (VIII; R = R′ = Me) *via* (VIII; R = Me, R′ = H) had been performed without any change in structure. It follows, therefore, that the isolation of the known 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VIII; R = R′ = Me) proved that a 3 : 6- and not a 2 : 6-lactone ring was present in all three substances. Hence, the structure (VIII; R = R′ = H) was confirmed for the reducing compound derived as above, and this *enol* of *5-keto-4-deoxyglucosaccharo-3 : 6-lactone* is the substance responsible for the curious reducing activity displayed by the substances from which it is derived.

FIG. 7.



A. *Ethyl glucosaccharate in dilute sodium hydroxide* (370 *mg.*/100 *c.c.*).
B. *Solution used for* B *acidified with dilute sulphuric acid.*

It is noteworthy that saccharic acid (I) and glucosaccharo-1 : 4- (II; R = H) and -3 : 6-lactones (III; R = H) do not reduce Fehling's solution. This curious property of reducing Fehling's solution which is due to the isomerisation to the enol (VIII; R = R′ = H) brought about by the agency of alkaline reagents is shown by those four derivatives referred to above, in each of which both carboxyl groups are esterified either as internal esters, *i.e.*, (IV) and (V), or esterified partly in this manner and partly with methyl alcohol, *i.e.*, (II; R = Me) and (III; R = Me). The isomerisation of these substances under the influence of alkaline reagents is not therefore dependent upon the presence of two interlocking rings (Rehorst, *Ber.*, 1938, **71**, 923). In addition, although it is not surprising that saccharo-1 : 4-3 : 6- and -1 : 5-3 : 6-dilactones, (IV) and (V), and the 3 : 6-lactone 1-methyl ester (III; R = Me), all containing the 3 : 6-lactone ring, should give rise to the reducing enol of 5-keto-4-deoxyglucosaccharo-3 : 6-lactone, yet it is remarkable that this enol is obtained also from the 1 : 4-lactone ester (II; R = Me), for this transformation must involve the scission of the 1 : 4-ring and the introduction of the 3 : 6-lactone ring.

In this connection it should be noted that ethyl glucosaccharate, which has both carboxyl groups esterified, also shows reducing activity towards Fehling's solution, though to a less extent than the four substances in question, but whether the same enol (VIII; R = R′ = H) is produced from this ester with alkaline reagents is not yet known. The reaction is clearly not so simple in this case because the absorption band shown by ethyl glucosaccharate in alkaline solution (Fig. 7) is different from that shown by the other four substances in alkaline solution and, furthermore, acidification of its alkaline solution does not result in the appearance of the band at λ 2290 A. (Fig. 7).

EXPERIMENTAL.

Treatment of Saccharo-1 : 5-3 : 6-dilactone (V) *with Sodium Methoxide.*—The dilactone (5 g.) was dissolved in ethyl alcohol (25 c.c.) and treated with 3 equivs. of sodium methoxide (73 c.c.; 1·18N) for 5 minutes. To 5 c.c. of the solution was added ethyl alcohol (30 c.c.) with stirring, and the sodium derivative thus precipitated was centrifuged off, washed with ethyl alcohol, ether, and dried in a vacuum (Found: Na, 17%). This sodium derivative had $[\alpha]_D^{20°}$ + 10° in 0·2N-sulphuric acid (*c*, 1·5); I.V., 145. It reduced Fehling's solution actively.

To the main bulk of the solution was added methyl-alcoholic hydrogen chloride (57·6 c.c.; 1·44N). The solution was then evaporated to dryness in a vacuum, extracted with ethyl alcohol, and filtered. Removal of the solvent gave a pale yellow syrup which reacted acid to Congo-red paper and reduced Fehling's solution actively on warming. This product immediately decolorised bromine water and potassium permanganate. It showed selective absorption with the head of the band at λ 2290 A. (ε, ca. 3500) (*c*, 5 mg. % in water).

Ozonisation. A solution of the syrupy reducing product in glacial acetic acid (40 c.c.) was subjected to the action of a steady stream of ozonised oxygen at room temperature. After two hours, two drops of the solution were removed, diluted to 1 c.c., and boiled for one minute. This solution gave a positive oxalate test, and reduced Fehling's solution in the cold after 2—3 minutes. Little or no change in rotation was observed during the ozonolysis, which was continued for 9 hours. At this stage oxalic acid could be detected readily, and the solution reduced Fehling's solution in the cold. The main bulk of the solution was diluted with water (50 c.c.) and evaporated under diminished pressure to remove acetic acid, a process facilitated by addition and distillation of further small amounts of water. When freed from acetic acid and water, the product (6·3 g.) reacted acid to Congo-red paper and reduced Fehling's solution in the cold.

A portion of this syrup (5·2 mg.) in water (1 c.c.) was made just alkaline by the addition of 0·1N-sodium hydroxide, and then acidified with dilute acetic acid. Two drops of calcium chloride were added, the mixture warmed, and the precipitated calcium oxalate centrifuged and washed twice with distilled water on the centrifuge. The precipitate was dissolved in 0·1N-sulphuric acid, the solution heated to 70°, and titrated with 0·02N-potassium permanganate (1 c.c. required). This corresponds to 1·1 g. of oxalic acid in the total weight of syrup (6·3 g.).

In alkaline solution the syrup (18·6 mg.) reacted with 5·6 c.c. of 0·02N-iodine, corresponding to the presence of 2·5 g. of threuronic acid in the 6·3 g. of syrup obtained after ozonisation.

Oxidation with bromine. The syrup (6·2 g.) obtained as above was dissolved in water (20 c.c.) and treated with bromine (1·5 c.c.). After standing overnight, a portion of the solution, when freed from excess of bromine by aeration, still reduced Fehling's solution. Barium carbonate (5 g.) and bromine (1 c.c.) were added, and the oxidation allowed to proceed for a further 24 hours; it was then complete, and the excess of the bromine was removed by aeration. The white precipitate (A) formed during the oxidation was filtered off, washed with water, and dried (3·8 g.); this was barium oxalate and corresponded to 1·15 g. of oxalic acid. The filtrate, which was acid and showed $[\alpha]_D^{20°}$ +25°, was neutralised (litmus) by addition of 0·3N-barium hydroxide, whereby there was obtained a precipitate (B) (1·07 g.), which was filtered off, washed with water, alcohol, ether, and dried in a vacuum. To the neutral filtrate (after the separation of B) was added 0·3N-barium hydroxide until the liquid reacted weakly alkaline to phenolphthalein; this afforded precipitate (C) (1·7 g.) which showed a positive test for tartrate when heated with concentrated sulphuric acid and β-naphthol. After removal of (C), addition of 0·3N-barium hydroxide to the liquid until there was no further precipitation, followed by ethyl alcohol (equal in volume to the aqueous solution), gave a precipitate (D) (4·7 g.), which also showed a positive tartrate test. The precipitates (C) and (D) were washed with alcohol, ether, and dried in a vacuum.

Examination of precipitates (B), (C), and (D). These fractions were combined (7 g.) and boiled for 8 hours with 2·5% methyl-alcoholic hydrogen chloride (200 c.c.). The solution was cooled, neutralised with silver carbonate, and an equal volume of ether added to precipitate the dissolved barium chloride, which was filtered off and washed with ether. Removal of the solvent from the combined filtrate and washings gave a liquid which was purified by distillation (1·8 g.), b. p. 180° (bath temp.)/20 mm. The distillate crystallised spontaneously, and after trituration with ether–alcohol the methyl dihydroxythreosuccinate (methyl *d*-tartrate) had m. p. and mixed m. p. 49°, $[\alpha]_D^{19°}$ +19° in water (*c*, 2·4) (Found: OMe, 35·4. Calc. for $C_6H_{10}O_6$: OMe, 34·8%).

Treatment of this ester with methyl-alcoholic ammonia for 2 days at − 5° gave dihydroxythreosuccinamide, which crystallised upon removal of the solvent in a vacuum; m. p. 205—206° (decomp.), $[\alpha]_D^{18°}$ +116·5° in water (*c*, 1·2) (after three crystallisations from aqueous alcohol).

Isolation of 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester* (VIII; R = R′ = Me).—In another experiment the glucosaccharo-1 : 5-3 : 6-dilactone (V) (0·5 g.) was dissolved in ethyl alcohol (10 c.c.) and treated with sodium methoxide (8 c.c.; 1·2N) for 5 minutes at room temperature, followed by methyl-alcoholic hydrogen chloride (4 c.c.; 2·4N). The solution was evaporated to dryness and extracted with ethyl alcohol. Removal of the ethyl alcohol gave a pale yellow, acid syrup, which was subjected to two methylations with silver oxide and methyl iodide, the first requiring a little methyl alcohol to aid complete solution of the product. The methyl ester (VIII; R = R′ = Me) was isolated by means of acetone and dissolved in a small volume of hot ether; on cooling 0·33 g. separated, m. p. and mixed m. p. 88°.

Formation of the Enol of 5-*Keto*-4-*deoxyglucosaccharo*-3 : 6-*lactone* (VIII; R = R′ = H) *by Alkaline Isomerisation.*—(a) *Of glucosaccharo*-1 : 5-3 : 6-*dilactone* (V). To a solution of the dilactone (m. p. 133°, $[\alpha]_D^{18°}$ +166° initial value in water) (1 g.) in dry methyl alcohol (10 c.c.) were added 3 equivs. of methyl-alcoholic sodium methoxide (15 c.c.; 1·2N). After 5 minutes the solution was treated with 3 equivs. of methyl-alcoholic hydrogen chloride (18 c.c.; N), then evaporated to dryness under diminished pressure at 40°, and the residue extracted with ether. Removal of the ether under slightly reduced pressure gave a pale yellow syrup which crystallised slowly on keeping. Trituration with ether–acetone to remove adhering syrup, followed by recrystallisation from ether–acetone, gave the *enol* of 5-*keto*-4-*deoxyglucosaccharo*-3 : 6-*lactone* (72 mg.), m. p. 159°, $[\alpha]_D^{18°}$ +39° in water (*c*, 1·5) (Found: C, 40·5; H, 3·9. $C_6H_8O_6$ requires C, 41·3; H, 3·5%). *Absorption.* A solution of glucosaccharo-1 : 5-3 : 6-dilactone in 50% aqueous ethyl alcohol showed on addition of an excess of N-sodium hydroxide a band at λ 2630 A. (ε, 2500 approx.; *c*, 10 mg. %), and on acidification the band moved to λ 2290 A. (ε, 2200 approx.; *c*, 10 mg. %) (see Fig. 2).

(b) *Of glucosaccharo*-1 : 4-3 : 6-*dilactone* (IV). Glucosaccharo-1 : 4-lactone monohydrate (1 g.) was converted into this lactone by 15 hours' heating in a vacuum at 100° (see Heslop and Smith, *loc. cit.*), and a solution of this dilactone (0·78 g.) in dry methyl alcohol (10 c.c.) was treated with 3 equivs. of sodium methoxide for 4 minutes. Addition of 3 equivs. of hydrogen chloride in methyl alcohol, followed by removal of solvent under reduced pressure, gave a residue from which the above enol was extracted with ether. Evaporation of ether gave a syrup, which crystallised on keeping. Trituration with ethyl alcohol gave the pure enol (0·27 g.), m. p. 159°, $[\alpha]_D^{20°}$ +38° in water (*c*, 2·4). *Absorption.* In aqueous alkaline solution the dilactone (VI) showed a band at λ 2650 A. (ε, 900 approx.; *c*, 19 mg. %), moving on acidification to λ 2290 A. (ε, 1400 approx.; *c*, 19 mg. %) (see Fig. 3). In alcoholic solution the isomerisation of the dilactone to the enol proceeds to a greater extent; *e.g.*, a solution of the dilactone in methyl alcohol after treatment with sodium methoxide shows a band at λ 2630 A. (ε, 4500 approx.; *c*, 5 mg. %).

(c) *Of glucosaccharo*-1 : 4-*lactone* 6-*methyl ester* (II; R = Me). This ester (0·8 g.), treated as described above with 3 equivs. of sodium methoxide for 5 minutes, followed by methyl-alcoholic hydrogen chloride, yielded the same enol (VIII; R = R′ = H) (0·15 g.), m. p. 159°, $[\alpha]_D^{20°}$ +39·5° in water (*c*, 2·0). *Absorption.* To 1·64 mg. of the crystalline ester (II; R = Me) was added 1 drop of 5N-sodium hydroxide, and the volume made up to 4 c.c. This solution showed a band at λ 2650 A. (ε, 1000 approx.; *c*, 41 mg. %), moving to λ 2290 A. upon acidification with 5N-sulphuric acid (see Fig. 5).

(d) *Of glucosaccharo*-3 : 6-*lactone* 1-*methyl ester* (III; R = Me). This ester (1·6 g.), made by the method previously given (Smith, *loc. cit.*), was dissolved in dry methyl alcohol (10 c.c.) and treated with sodium methoxide for 4 minutes, followed by methyl-alcoholic hydrogen chloride in the manner described above. After isolation by extraction with ether–ethyl alcohol, the enol had m. p. 159°, $[\alpha]_D^{19°}$ +39° in water (*c*, 2·9). *Absorption.* Aqueous alkaline solutions of glucosaccharo-3 : 6-lactone 1-methyl ester show a band at λ 2630 A. (ε, 900 approx.; *c*, 60 mg. %), moving upon acidification to λ 2290 A. (ε, 1000 approx.; *c*, 26 mg. %).

The enol of 5-keto-4-deoxyglucosaccharo-3 : 6-lactone reacted acid to Congo-red; it reduced Fehling's solution actively on warming, decolorised potassium permanganate solution immediately, and also decolorised bromine water. Unlike ascorbic acid, the enol does not reduce silver nitrate solution.

In aqueous solution the enol showed a band at λ 2290 A. (ε, 6000; *c*, 5 mg. %), moving on addition of sodium hydroxide to λ 2650 A. (ε, 5000; *c*, 5 mg. %) (Fig. 6). It reacted immediately with 2 equivs. of sodium hydroxide at room temperature (9·81 mg. required 11·02 c.c. of 0·01N-solution, corresponding to equiv., 89. Calc. for $C_6H_6O_6$: equiv., 87). The enol reacted with 6·2 atomic proportions of iodine in alkaline solution (5·85 mg. required 20·8 c.c. of 0·01N-iodine), and in aqueous solution it combined with 4 atomic proportions of chlorine (9·46 mg. required 10·84 c.c. of 0·02N-chlorine water. Calc.: 10·87 c.c.).

Ozonisation of the Enol of 5-Keto-4-deoxyglucosaccharolactone.—A solution of this compound (0·46 g.) in glacial acetic acid (30 c.c.) was subjected to the action of a stream of ozonised oxygen during 9 hours. The solution showed $[\alpha]_D^{18°}$ +33° (initial value); +20° (after 1 hr.); +8° (2 hrs.); +5° (3 hrs.); +3° (4 hrs.); ±0° (5 hrs.; constant value). After addition of water, followed by distillation under reduced pressure to remove acetic acid and water, there was obtained a syrupy product, which reacted acid to Congo-red and reduced Fehling's solution actively; it contained oxalic acid (calcium chloride test). The aqueous acetic acid distillate gave a negative test for glyoxylic acid (tested with albumin and concentrated sulphuric acid).

Oxidation with bromine. A solution of the syrupy product from the ozonisation in water (10 c.c.) was treated with bromine (0·2 c.c.) at room temperature for 14 hours in the presence of a small amount of barium carbonate. The excess of bromine was removed by aeration, and the aqueous acid solution, which did not now reduce Fehling's solution, was neutralised by careful addition of barium hydroxide. The precipitate of barium salts was dried.

Esterification. The dry barium salts (0·42 g.) were boiled for 8 hours with 2% methyl-alcoholic hydrogen chloride (100 c.c.), the solution cooled, neutralised with silver carbonate, filtered, and concentrated in a vacuum. Purification of the resulting liquid by extraction with acetone, followed by removal of the solvent, gave a liquid (0·15 g.), b. p. (bath temp.) 130°/0·04 mm., $n_D^{20°}$ 1·4394, which crystallised spontaneously. After recrystallisation from ether the methyl dihydroxy-*l*-threosuccinate (methyl *d*-tartrate) had m. p. and mixed m. p. 48°, $[\alpha]_D^{19°}$ +19° in water (*c*, 2·5). Treatment of this methyl ester with methyl-alcoholic ammonia for 15 hours gave the corresponding diamide (*d*-tartramide), m. p. 202°, $[\alpha]_D^{18°}$ +105° in water (*c*, 4·0) (Found: C, 32·6; H, 5·55; N, 18·7. Calc. for $C_4H_8O_4N_2$: C, 32·4; H, 5·4; N, 18·9%).

Treatment of the Enol of 5-Keto-4-deoxyglucosaccharo-3 : 6-lactone with Chlorine.—The crystalline enol (0·3 g.), dissolved in water (3 c.c.), was treated for 15 minutes with 0·02N-chlorine water (50 c.c.). The excess of chlorine was removed by aeration, and the solution neutralised with barium carbonate, filtered, and evaporated to dryness in a vacuum. The residue, consisting of the barium salt of an organic acid, was boiled for 8 hours with 2% methyl-alcoholic hydrogen chloride (50 c.c.). The solution was cooled and neutralised (Congo-red) with barium carbonate. After filtration and evaporation under diminished pressure, there was obtained a syrup (0·25 g.) which still reacted acid to Congo-red. This was esterified by treatment with a slight excess of a solution of ethereal diazomethane at 0°. Removal of solvent gave a neutral liquid (0·11 g.), b. p. (bath temp.) 140—150°/0·03 mm., $[\alpha]_D^{15°}$ +42° in water (*c*, 0·8). This product reduced Fehling's solution on boiling (Found: OMe, 28·0. $C_9H_{14}O_7Cl_2$ requires OMe, 30·5%).

Treatment of this syrup (90 mg.) with methyl-alcoholic ammonia for 3 days at − 5° gave a crystalline *amide* believed to be the (C_1) monoamide of methyl 4 : 5-dichloro-2 : 3-dihydroxy-5-methoxyadipate, m. p. 189° (after recrystallisation from acetone) (Found: C, 33·6; H, 4·0; N, 5·4; OMe, 20·6. $C_8H_{13}O_6NCl_2$ requires C, 33·1; H, 4·5; N, 4·8; OMe, 21·4%). This compound is analogous to the one prepared from the enol of 5-keto-4-deoxymannosaccharo-3 : 6-lactone (Heslop and Smith, *loc. cit.*).

5-Methyl Δ⁴-Glucosaccharo-3 : 6-lactone 1-Methyl Ester (VIII; R = Me, R′ = H).—A solution of the enol (0·1 g.) in dry methyl alcohol (5 c.c.) was treated with a slight excess of ethereal diazomethane at 0°, as indicated by a yellow colour in the solution. Removal of the solvent after 5 minutes gave crystalline *5-methyl Δ⁴-glucosaccharo-3 : 6-lactone 1-methyl ester*, m. p. 142°, $[\alpha]_D^{18°}$ +91° in water (*c*, 1·5) (after recrystallisation from ethyl alcohol) (Found: C, 47·8; H, 5·15; OMe, 30·3. $C_8H_{10}O_6$ requires C, 47·5; H, 5·0; OMe, 30·7%). An aqueous solution of this compound showed a band at λ 2290 A. (ε, 9000 approx.; *c*, 4 mg. %) (Fig. 1). On addition of sodium hydroxide this band disappeared. Reacidification with dilute sulphuric acid apparently regenerated the original unsaturated furone structure, for the solution showed a band at λ 2290 A. (ε, 1500 approx.).

This enol was shown to contain one ester methoxyl group as follows. The compound (15·64 mg.) was heated with 0·3N-barium hydroxide (3 c.c.) at 50° for 1 hour. The solution was neutralised (phenolphthalein) by shaking it gently while a stream of carbon dioxide was passed over the surface, then evaporated to dryness under diminished pressure at 50° in a current of dry air. All these operations were carried out in a Zeisel apparatus. A methoxyl estimation on the residue gave OMe, 15·35 (Calc. for loss of one OMe from $C_8H_{10}O_6$: OMe, 15·35%).

2 : 5-Dimethyl Δ⁴-Glucosaccharo-3 : 6-lactone 1-Methyl Ester (VIII; R = R′ = Me).—The foregoing 5-methyl ether (VIII; R = Me, R′ = H) (40 mg.) was given one methylation with silver oxide (1 g.) and methyl iodide (2 c.c.) during 8 hours. Extraction of the product with ethyl alcohol, followed by removal of the solvent, gave the crystalline compound (VIII; R = R′ = Me), m. p. and mixed m. p. 87°, $[\alpha]_D^{17°}$ +92° in water (*c*, 1·1) (after recrystallisation from ethyl alcohol–ether) (Found: C, 50·15; H, 5·6; OMe, 42·0. Calc. for $C_9H_{12}O_6$: C, 50·0; H, 5·6; OMe, 43·1%). An aqueous solution of this substance showed a band at λ 2290 A. (ε, 8500 approx.; *c*, 4 mg. %) (Fig. 1).

Treatment of Ethyl Glucosaccharate with Sodium Hydroxide.—A solution of ethyl glucosaccharate (11·1 mg.) in water (0·2 c.c.) was treated with 1 drop of 5N-sodium hydroxide, the solution brought to the boil, cooled, and diluted to 3 c.c., it then showed bands at λ 2660 A. (ε, 1500 approx.) and at λ 3280 A. (ε, 1000 approx.; *c*, 360 mg. %). Acidification of the alkaline solution caused no change in the position of the bands, but the intensity was reduced to approximately ε, 1300 and ε, 600 for the respective bands (*c*, 360 mg. %) (see Fig. 7).

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[*Received, September 1st*, 1944.]