156. *Lactones of Mannosaccharic Acid. Part III. Isomerisation of Mannosaccharodilactone with Alkali.*

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

The unexpected and hitherto unexplained reducing properties of mannosaccharodilactone (I) in alkaline solution, or in acid solution after pre-treatment with alkaline reagents, are shown to be due to the conversion of the dilactone by alkaline reagents into the *enol* of 5-*keto*-4-*deoxymannosaccharo*-3 : 6-*lactone* (II). This was isolated in the crystalline condition and its structure elucidated; it shows to a marked degree all the reducing properties of mannosaccharodilactone.

Ozonolysis of (II) gave oxalic acid and erythruronic acid (III), the latter being oxidised with bromine to dihydroxyerythrosuccinic acid (*meso*tartaric acid) (IV). Methylation of the enol (II) with diazomethane afforded the 5-*methyl ether* of the *enol* of 5-*keto*-4-*deoxymannosaccharo*-3 : 6-*lactone* 1-*methyl ester* (V), which was converted by the agency of silver oxide and methyl iodide into crystalline 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (VI). The structure of the latter was proved by ozonisation, a process which led to the formation of oxalic acid and 3-methyl *d*-erythruronic acid (VII). Oxidation of (VII) with bromine yielded the known 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid (VIII), characterised as the crystalline bismethylamide (X). A closer examination of the reaction of chlorine with the reducing enol (II) has been made. This reaction is partly one of addition of chlorine to the double bond between C_4 and C_5, as shown by the formation of a crystalline *dichloro-compound* (XVII).

Methylation of mannosaccharo-1 : 4-3 : 6-dilactone (I) either with silver oxide and methyl iodide or with diazomethane has been shown to give 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (VI) (Smith, *J. Soc. Chem. Ind.*, 1938, **57**, 449; Haworth, Heslop, Salt, and Smith, this vol., p. 217). This analogue

Fig. 1

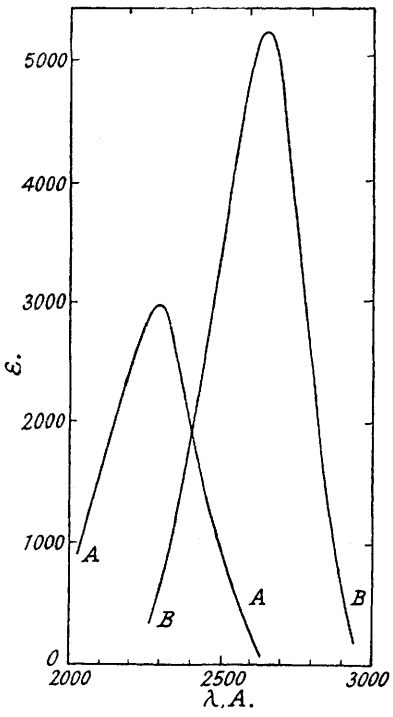


Fig. 2.

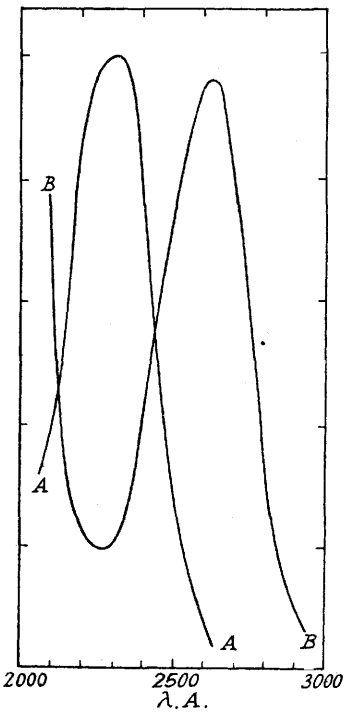
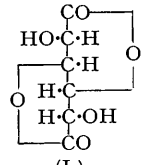


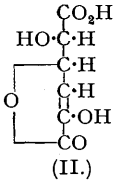
Fig. 1.—A. *Mannosaccharodilactone after treatment with aqueous alkali followed by acid* (10 *mg.* %).
B. *Mannosaccharodilactone in aqueous alkaline solution* (3 *mg.* %).
Fig. 2.—A. *Monohydrate of enol of* 5-*keto*-4-*deoxymannosaccharo*-3 : 6-*lactone* (*II*) *in water* (5 *mg.* %).
B. ,, ,, ,, ,, ,, ,, (*II*) *in aqueous alkali* (5 *mg.* %).

of ascorbic acid was found to display an absorption band at λ 2290 A. (Fig. 4); it reacted with chlorine or bromine, and decolorised alkaline potassium permanganate in the cold. In addition it reacted with iodine in alkaline solution, a process accompanied by the formation of small amounts of iodoform. When mannosaccharodilactone itself is treated with alkaline reagents a substance is produced in solution which resembles in its properties 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (VI). For instance, a solution of the dilactone which has been made alkaline and then acidified shows a band at λ 2290 A. in acid solution moving to λ 2630 A. in alkaline solution (Fig. 1); it reacts with chlorine, bromine, and potassium permanganate. The crystalline dilactone reduces Fehling's solution actively and it reacts with iodine in alkaline solution, giving small amounts of iodoform. These facts led to the suggestion that the reactive substance,

obtained in solution from mannosaccharodilactone by the agency of alkaline reagents, was structurally related to (VI). Such differences as there were between the reactive substance and (VI), *e.g.*, the failure of the latter to reduce Fehling's solution, seemed to be due to the fact that the former was the unmethylated form of (VI).

The proof that this deduction was correct is based upon the following experimental facts. Treatment of mannosaccharodilactone with sodium methoxide in alcohol gave a sodium derivative which reduced Fehling's solution, reacted with alkaline iodine, and showed strong selective absorption with the head of the band at λ 2630 A. An acidified aqueous solution of the sodium derivative showed a band at λ 2290 A., and reacted readily with chlorine, bromine, and potassium permanganate. Addition of methyl-alcoholic hydrogen chloride to the sodium derivative, followed by removal of sodium chloride and solvent, afforded a syrupy product which displayed all the properties of a solution prepared by treatment of the dilactone with alkali followed by acid. If the reactive substance, obtained from the sodium derivative referred to above, is the unmethylated form of 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (VI), then it is clear that the oxidative methods applied to the determination of the structure of the latter should be applicable to the former. This proved to be the case, for when the syrupy reactive substance was subjected to ozonolysis there were formed oxalic acid and an aldehydic acid, erythruronic acid (III). The formation of the extra carboxyl group together with an aldehydic group as a result of ozonisation confirmed the presence of the system ·CH:C(OH)· in the reactive substance. Moreover, the isolation of oxalic acid and the erythruronic acid (III) and the absence of glyoxylic acid proved that the double bond in this system must engage C_4 and C_5, and that the hydroxyl group in it is attached to C_5 and not to C_4, while the hydrogen in the unsaturated system must be linked to C_4, thus $\cdot C_4(H):C_5(OH)\cdot$. If this were not the case and the system was reversed, as in $\cdot C_4(OH):C_5(H)\cdot$, it is clear that ozonisation would give rise to glyoxylic acid and *meso*tartaric acid. The structure of the strongly reducing erythruronic acid (III) was ascertained by its oxidation with bromine to dihydroxyerythrosuccinic acid (*meso*tartaric acid) (IV), recognised in the form of its characteristic crystalline methyl ester and its corresponding diamide.


(I.)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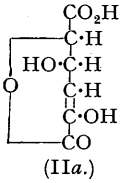
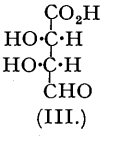

(II*a*.)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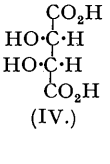
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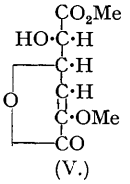
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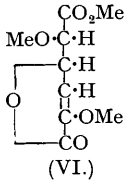
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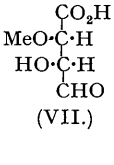
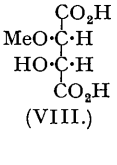

(VII.)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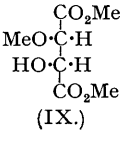
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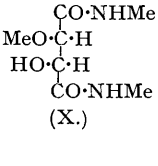
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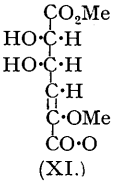
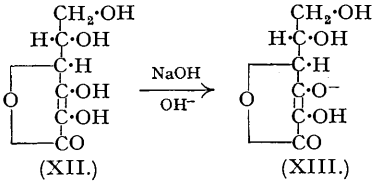
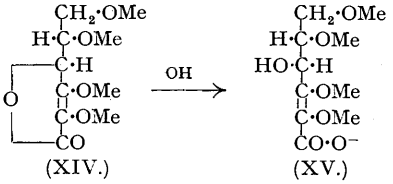

(XI.)


(XII.) (XIII.)


(XIV.) (XV.)

These experimental facts were in accordance with the formula (II), the unmethylated form of (VI), for the reactive substance, but it is clear that the facts could also be explained by the structure (II*a*) which contains the pyrone ring and not the furone ring as in (II).

It became possible, however, to distinguish between these two possibilities when the reactive substance became available in the pure crystalline form. This was achieved by treatment of mannosaccharodilactone in dry methyl alcohol with sodium methoxide, in order to form the sodium derivative of the reactive substance, followed by liberation of the free organic acid by methyl-alcoholic hydrogen chloride. This procedure afforded the crystalline reactive *enol* of *5-keto-4-deoxymannosaccharo-3 : 6-lactone* (an isomeric form of the dilactone) (m. p. 142°, $[\alpha]_D$ — 48°) which readily formed a *monohydrate* (m. p. 82°). This acid substance showed an absorption band at λ 2290 A. (Figs. 2 and 3), moving to λ 2630 A. on addition of sodium hydroxide. In aqueous solution this reactive acid shows slow mutarotation, a change attended by a gradual decrease in the intensity of the band at λ 2290 A.; this observation suggested that a lactone ring was present in the reactive substance and that this ring, containing the unsaturated system, is essential for selective absorption of light. This behaviour is analogous to that of 2 : 3 : 4 : 6-tetramethyl *l*-ascorbic acid (XIV) (Haworth, Hirst, and Smith, J., 1934, 1556) and 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone (Smith, this vol., p. 515), for both these substances show selective absorption in the ultra-violet region of the spectrum, the former at λ 2350 A. and the latter at λ 2290 A., and in alkaline solution, when the rings are opened, no selective absorp-

tion of light is displayed by either substance. The reactive substance (m. p. 142°) (II) reduced Fehling's solution actively, it reacted with chlorine and bromine, and with iodine in alkaline solution to give small amounts of iodoform. A closer examination showed that, whereas the original mannosaccharodilactone reacted with four atomic proportions of iodine in alkaline solution, yet (II) reacted with six atomic proportions under the same conditions, these six probably being consumed in the oxidation of the system $CHR{:}C(OH)R_2$ to $R{\cdot}CO_2H + R_2{\cdot}CO_2H$. This difference in the amount of iodine consumed by the two substances may be due to the fact that alkaline reagents effect only a partial conversion (two-thirds) of the dilactone into the substance (II). It is suggested that a mixture of the sodium derivative of the reactive substance and normal sodium mannosaccharate is formed. Support for this view is found in the fact that, after treatment of mannosaccharodilactone with sodium hydroxide, followed by an equivalent amount of acid, there can be isolated unchanged mannosaccharodilactone. This partial conversion of the dilactone into the isomeric reactive substance is also believed to be responsible for the observation that, although a solution of manno-

FIG. 3.

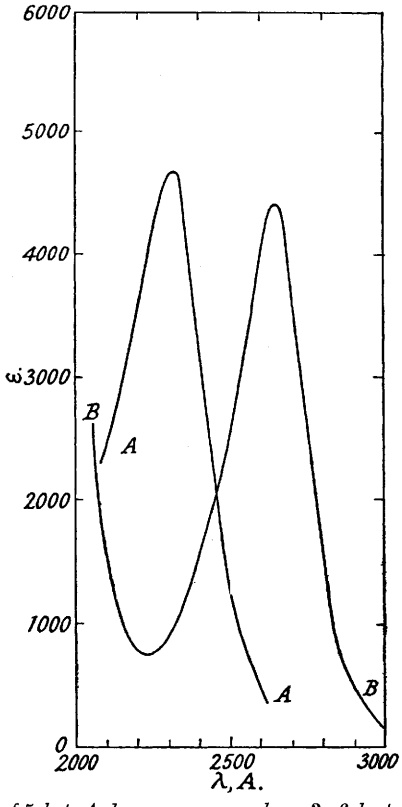


FIG. 4.

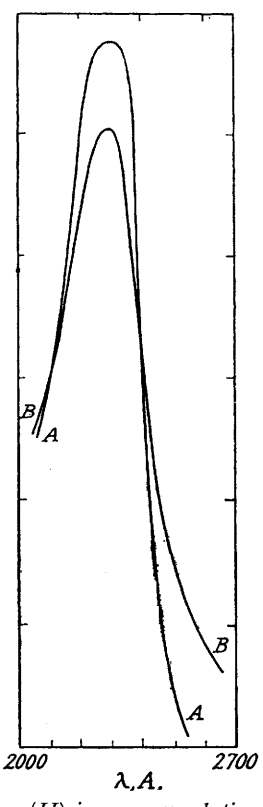


FIG. 3.—A. *The enol of* 5-*keto*-4-*deoxymannosaccharo*-3 : 6-*lactone* (*II*) *in aqueous solution* (5 *mg.* %).
B. ,, ,, ,, ,, ,, (*II*) *in aqueous sodium hydroxide* (5 *mg.* %).
FIG. 4.—A. 2 : 5-*Dimethyl* Δ^4-*mannosaccharo*-3 : 6-*lactone* 1-*methyl ester* (*VI*) *in water* (5 *mg.* %).
B. 5-*Methyt* Δ^4- ,, ,, ,, ,, (*V*) *in water* (5 *mg.* %).

saccharodilactone treated successively with sodium hydroxide and sulphuric acid reacted with two atomic proportions of chlorine, yet the pure crystalline reactive substance (II) accounted for four atomic proportions.

Ozonisation of the crystalline reactive substance (II) (m. p. 142°) gave oxalic acid and the aldehydic acid, erythruronic acid (III), identified by its conversion into dihydroxyerythrosuccinic acid (*meso*tartaric acid) (IV). By these results it became abundantly clear that (II) was identical with the reactive substance present in the syrupy product first examined, and that the reactions already described for the latter were due to the presence of (II) in it. Inspection of the formulæ (II) and (II*a*) revealed that the reactive compound could be represented by either of these, for both explained the experimental results.

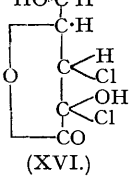
The proof that the reactive crystalline substance has the structure (II) and not (II*a*) was based on the following observations. When (II) was allowed to react with ethereal diazomethane, the introduction of two methoxyl groups was smoothly effected with the formation of a neutral substance (V), the 5-*methyl ether* of the enol of 5-keto-4-deoxymannosaccharo-3 : 6-lactone 1-methyl ester. The latter, like the original substance (II), still exhibited the band at λ 2290 A. (Fig. 4), but unlike (II), which showed a band at λ 2630 A. in alkaline solution, (V) exhibited no selective absorption in alkaline solution; alkaline reagents would therefore appear to open the ring of (V) while they do not affect the ring in the original unmethylated substance (II).

When the enolic hydroxyl at C_5 is free, as in (II), the reactive hydrogen in it shows a tendency to be liberated as a proton, a process leading to the stabilisation of the ring system; thus, in alkaline solution a sodium derivative is formed by loss of a proton at C_5, the unsaturated furone ring responsible for the absorption band remains intact, and hence a band at λ 2630 A. is displayed. In the case of the dimethyl derivative (V) the alkaline reagents cannot affect the enolic hydroxyl at C_5, for this is now blocked, and consequently the furone ring system is unable to stabilise itself by losing a proton from C_5 and opens under the influence of excess of hydroxyl ions, with the formation of a normal open-chain sodium salt which shows no selective absorption band. This rather curious behaviour of the reactive substance is paralleled by that of ascorbic acid (XII), the furone ring of which is also stable to alkali; thus the absorption band of ascorbic acid shown at λ 2450 A. in aqueous solution moves to λ 2650 A. on addition of sodium hydroxide, owing to liberation of a proton from the enolic hydroxyl at C_3 as in (XIII) and the formation of sodium ascorbate in which the unsaturated furone ring system is still present. On the other hand, 2 : 3 : 5 : 6-tetramethyl ascorbic acid (XIV), resembling closely the dimethyl derivative (V), shows a band at λ 2350 A. when the furone ring is intact, whereas upon the addition of sodium hydroxide the ring opens and the selective absorption band disappears.

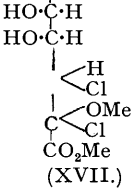
The neutral dimethyl derivative (V) no longer reduced Fehling's solution, showing that the grouping ·CH:C(OH)· (responsible for reducing activity) was no longer present as such but was now in the methylated form ·CH:C(OMe)·. The reactivity of the dimethyl derivative (V) towards chlorine and bromine, associated with the presence of a double bond was, however, still exhibited. Further methylation of (V) with silver oxide and methyl iodide yielded the crystalline trimethyl derivative (VI). The latter showed selective absorption in the ultra-violet region of the spectrum, the head of the band being at λ 2290 A. (Fig. 4); in alkaline solution the furone ring of (VI) opens and the absorption band disappears. The physical properties of this trimethyl derivative (VI) were shown to be identical with those of 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester, previously prepared by simultaneous methylation and isomerisation of mannosaccharo-1 : 4-3 : 6-dilactone (Haworth, Heslop, Salt, and Smith, *loc. cit.*), and the identity of the two was established by ozonolysis, a process which afforded oxalic acid and the 3-monomethyl erythruronic acid (VII). Oxidation of the latter with bromine gave the corresponding dibasic acid, 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid (VIII), which, by means of methyl-alcoholic hydrogen chloride, was smoothly transformed into its methyl ester (IX). This was identified by its conversion into the known crystalline bismethylamide (X) (Haworth, Heslop, Salt, and Smith, *loc. cit.*; Heslop, Salt, and Smith, this vol., p. 225). The presence of the 3 : 6-lactone ring in the trimethyl derivative (VI) and therefore in (II) was thus established. The crystalline unsaturated acid substance (m. p. 142°) obtained by successive treatments of the dilactone with alkali and acid is consequently represented by the furone structure (II) and not by the pyrone structure (II*a*), being therefore the mono-enol of 5-*keto*-4-*deoxymannosaccharo*-3 : 6-*lactone*. This is the substance which is responsible for the unique reducing properties displayed by mannosaccharodilactone.

As would be expected from the close structural relationship between (II) and ascorbic acid (XII), the properties of the two compounds are very similar: both show selective absorption of light, the former having a band at λ 2290 A. and the latter at λ 2450 A., and both behave in the same curious way when treated with sodium hydroxide, (II) losing a proton from the hydroxyl group at C_5, and (XII) one from the hydroxyl group at C_4 to give the ascorbate ion (XIII). In both cases the unsaturated ring system appears to remain unaffected, for in alkaline solution (II) has a band at λ 2630 A. and (XII) one at λ 2650 A. The activity of the enolic hydroxyl groups in these two compounds is also demonstrated by treatment with ethereal diazomethane: (II) gives a 5- and (XII) a 4-methyl ether (the C atom of the $\cdot CH_2 \cdot OH$ group is referred to as C_1) (Haworth, Hirst, and Smith, *loc. cit.*).

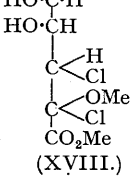
Both (II) and (XII) show strong reducing activity towards Fehling's solution, but the latter reduces silver nitrate in the cold whereas (II) is without action upon this reagent.



(XVI.)



(XVII.)



(XVIII.)

The enol (II) and ascorbic acid react immediately with potassium permanganate in acid solution, with chlorine, and with bromine. In their behaviour towards iodine, however, they differ markedly inasmuch as (XII) reacts quickly with two atomic proportions and (II) reacts with only a small quantity of iodine.

A closer examination of the reaction of the enol (II) and ascorbic acid with chlorine has also established a great difference between the two. Ascorbic acid reacts with chlorine in the same way as it does with iodine, *viz.*, as a reducing agent; two hydroxyl groups appear to be added to the double bond, and two chloride ions are liberated in the process. Moreover, the reaction is reversible, for treatment of the fresh chlorine-oxidised solution with hydrogen sulphide regenerates the ascorbic acid (Herbert, Hirst, Percival, Reynolds, and Smith, J., 1933, 1270). The enol (II), however, consumed four, and not two, atomic proportions of chlorine in the process, which could not be reversed with hydrogen sulphide. Chlorine oxidation of both (II) and (XII)

affords substances which show no selective absorption of light; the latter gives dehydroascorbic acid (2 : 3-diketogulonolactone), probably as a hydrate, as the sole product, whereas the only definite substance isolated from the former was a dichloro-compound (XVI) which probably arises as a result of the addition of chlorine to the double bond of (II). The other chlorine oxidation products were not identified.

The dichloro-compound has been characterised by isolation of the crystalline methylated *dichloro-ester* (XVII), the saturated character of which is shown by the absence of activity towards chlorine and bromine and also by the fact that its aqueous solutions exhibited no selective absorption of light. The crystalline neutral dichloro-compound (XVII) was found to contain three methoxyl groups, two of which were esteric in character since they could be eliminated by warming with barium hydroxide solution. This was confirmed by the isolation of the corresponding dibasic *acid* which contained one ether methoxyl group. The conversion of the dichloro-ester (XVII) into the crystalline monoamide (XVIII), possessing two methoxyl groups, and the fact that this amide showed a positive Weerman test for α-hydroxy-amides, indicated that the ether methoxyl group in the amide (XVIII), and therefore in the ester (XVII), must have been attached either to C_3 or to C_5. The location of this methoxyl group is not certain but it seems improbable that the short time of the reaction of (XVI) with diazomethane would effect the introduction of a methyl group into the secondary alcoholic group at C_3. Since the reaction of the enol (II) with chlorine involves to some extent addition of halogen to the double bond, it is clear why only a portion of the chlorine used is detectable as chloride ion and it also explains why the reaction with iodine proceeds only to a small extent.

Experimental.

Properties of Mannosaccharodilactone.—Aqueous solutions of the dilactone (for preparation, see Haworth, Heslop, Salt, and Smith, this vol., p. 217) show a faint acid reaction to litmus paper; they show no selective absorption (tested at 200 mg. %) but upon addition of sodium hydroxide solution isomerisation takes place with the formation of the enol of 5-keto-4-deoxymannosaccharolactone (II). The presence of the latter is shown by the alkaline solution exhibiting a band at λ 2630 A. which moves to λ 2290 A. upon acidification of the solution (Fig. 1) (Haworth, Heslop, Salt, and Smith, *loc. cit.*). Alkaline solutions of the dilactone reduce Fehling's solution actively and quickly decolorise potassium permanganate. The acidified solutions showing the band at λ 2290 A. react immediately with chlorine water and bromine water, and also decolorise potassium permanganate.

Action of Iodine in Alkaline Solution upon Mannosaccharodilactone.—To a solution of the dilactone (0·1013 g.) in water (20 c.c.), 0·1N-sodium hydroxide (87 c.c.) and 0·1N-iodine (50 c.c.) were added. After 90 minutes the solution was acidified with N-sulphuric acid (10 c.c.), and the excess of the iodine back-titrated with 0·1N-sodium thiosulphate (28·0 c.c.) (a blank experiment was carried out at the same time under the same conditions). In two similar experiments, using 0·1005 g. and 0·1028 g. of dilactone, 21·4 c.c. and 22·3 c.c. of 0·1N-iodine, respectively, were consumed. These results indicate that 1 g.-mol. of the dilactone reacts in alkaline solution with approximately 4 g.-atoms of iodine. Small amounts of iodoform are produced in these oxidations, and the presence of oxalic acid may also be demonstrated (see Haworth, Heslop, Salt, and Smith, *loc. cit.*).

Treatment of Mannosaccharodilactone with Sodium Hydroxide.—A solution of the dilactone (1 g.) in water (18 c.c.), treated with N-sodium hydroxide (23 c.c.), showed the following change in specific rotation: − 55° (initial value); − 41° (after 0·5 hr.); − 18° (1·5 hrs.); − 12·5° (2 hrs.); − 10·5° (3·5 hrs.); − 11·5° (15·5 hrs.); − 13° (20 hrs.); − 13·5° (27 hrs.); − 14° (72 hrs.); − 14·5° (87 hrs.). To this alkaline solution N-sulphuric acid (22·5 c.c.) was added, and the solution, which showed $[\alpha]_D$ +5°, was evaporated to dryness under reduced pressure. Extraction of the residue with methyl alcohol afforded a brownish-yellow syrup which was purified by extraction with ethyl alcohol. On keeping, the syrup crystallised, and after removal of adhering syrup from the crystals by trituration with ethyl alcohol followed by crystallisation from ethyl alcohol, mannosaccharodilactone (0·1 g.) was obtained, m. p. and mixed m. p. 180° (decomp.), $[\alpha]_D^{18°}$ +195° (initial value in water; *c*, 1·3).

Reaction of Mannosaccharodilactone treated successively with Alkali and with Acid.—(*a*) *With potassium permanganate.* A solution of the dilactone (0·108 g.) in water (10 c.c.) was treated with N-sodium hydroxide (3 c.c.) for 3 minutes, followed by N-sulphuric acid (5 c.c.). This acidified solution was titrated with 0·1N-potassium permanganate (8·7 c.c.) until a permanent pink colour persisted. The oxidised solution showed $[\alpha]_{5780}^{18°}$ +83·5° (initial value). In a second experiment the dilactone (0·051 g.), after pretreatment with N-sodium hydroxide followed by acid, required 3·85 c.c. of 0·1N-permanganate.

(*b*) *With chlorine water.* A solution of the dilactone (0·1 g.) in water (5 c.c.) was treated with 0·1N-sodium hydroxide (13 c.c.) for 1 minute and then acidified with 0·1N-sulphuric acid (13·5 c.c.). The solution was titrated with 0·083N-chlorine water (14·1 c.c.), starch–iodide paper being used as indicator (Calc. for two atomic proportions of chlorine: 13·85 c.c.). This chlorine-treated solution, which showed no selective absorption band, had $[\alpha]_{5780}^{12°}$ +84° (initial value); no band appeared upon treatment of the solution with hydrogen sulphide.

A solution of the dilactone (0·077 g.) in water (6·5 c.c.) having $[\alpha]_{5780}^{18°}$ +209°, treated with N-sodium hydroxide (1·5 c.c.), had $[\alpha]_{5780}^{18°}$ −57°. After the addition of N-sulphuric acid (1·5 c.c.), the solution, now containing the enol (II), had $[\alpha]_{5780}^{18°}$ −27°. Chlorine water was added every 5 minutes until a slight excess was present, and the change in specific rotation observed:

Chlorine water added, c.c. ...	1	2	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5	8
$[\alpha]_D$	−19°	−12°	−8°	−3·5°	±0°	+5·5°	+9·5°	+12°	+16°	+21°	+25°	+31°	+36·5°

Chlorine water added, c.c. ...	8·5	9·0	9·5	10	10·5	11	11·5	12	12·5	13	13·5	14
$[\alpha]_D$	+42°	+46°	+49·5°	+53·5°	+57·5°	+61·5°	+65·5°	+70°	+74·5°	+76°	+78°	+79·5°

The chloride ion present in this oxidised solution was precipitated as silver salt by means of silver nitrate solution in the presence of nitric acid, and 75% of the chlorine used was thus recovered.

Treatment of Mannosaccharodilactone with Sodium Methoxide.—The dilactone (5 g.) was dissolved in hot methyl alcohol (400 c.c.) and while still warm the solution was treated with 3 equivs. of sodium methoxide in methyl alcohol (73 c.c.; 1·18N); a pale yellow solid separated. After 3 minutes, a small portion of the solution was centrifuged and the sodium derivative was washed on the centrifuge with methyl alcohol, then with ether, and finally dried in a vacuum (Found: Na, 16·6%). In aqueous acidified solution this sodium derivative showed a selective absorption band at λ 2290 A. (ε, 2000 approx.) moving upon addition of sodium hydroxide to λ 2630 A. (ε, 2500 approx.). An aqueous solution of the sodium derivative had $[\alpha]_D$ + 1° (initial value) (*c*, 2·2); + 4·5° (after 9 hrs.); + 10° (1 day); + 15°

(3 days); + 20° (4 days) (constant for 1 day). The freshly prepared sodium derivative had iodine number, 186. An aqueous solution of the sodium derivative which had undergone complete mutarotation had iodine number, 208.

The main bulk of the solution containing the sodium derivative was treated with methyl alcohol (45 c.c.) containing hydrogen chloride equivalent to the sodium methoxide used in the isomerisation. Removal of the sodium chloride by filtration, and of the solvent by distillation under diminished pressure, gave a colourless syrup. This syrup, which reacted acid to Congo-red, showed in aqueous solution a band at λ 2290 A. (ε, 3500 approx.; *c*, 5 mg. %), moving to λ 2630 A. (ε, 4000 approx.; *c*, 5 mg. %) upon addition of sodium hydroxide. It readily reacted with chlorine and bromine, and decolorised acid potassium permanganate immediately. The syrupy product reduced Fehling's solution actively on warming.

Ozonisation. A solution of the syrup, obtained in the previous experiment, in glacial acetic acid (50 c.c.) was subjected to the action of a stream of ozonised oxygen at room temperature: $[\alpha]_D$ − 17° (initial value); − 14° (after 1 hr.); − 12·5° (2 hrs.); ± 0° (5 hrs.); + 4° (6 hrs.); + 5° (7 hrs.); + 7·5° (7½ hrs.); + 9° (8 hrs.); + 10·5° (9 hrs.). After 6 hrs. the solution ($[\alpha]_D$ + 4°) contained oxalic acid (tested with calcium chloride) and reduced Fehling's solution in the cold. The solution was diluted with water, and freed from acetic acid by distillation under diminished pressure, small amounts of water being added at intervals. The syrupy product thus obtained was acidic; oxalic acid was readily shown to be present in it (calcium chloride test; and formation of phenylhydrazine oxalate, m. p. 178°). The syrupy product reduced Fehling's solution in the cold. No glyoxylic acid could be detected.

Oxidation of Ozonolysis Product with Bromine.—Isolation of dihydroxyerythrosuccinic acid (IV). While still containing oxalic acid and probably some mannosaccharolactone (since the whole of the dilactone is not transformed into the enol), the syrupy product was dissolved in water (20 c.c.) and treated with bromine (1 c.c.) at room temperature. After 12 hours a portion of the solution, when freed from bromine by aeration, still reduced Fehling's solution in the cold. This could not have been due to mannosaccharodilactone, since this does not reduce Fehling's solution in the cold, or to any of the monoenol of 5-keto-4-deoxymannosaccharolactone which had escaped ozonisation, because the solution showed no selective absorption band (tested at 50 mg. %). Hence more bromine (1 c.c.) and barium carbonate (4 g.) were added. After 10 hours, oxidation was complete and the solution was freed from the excess of the bromine by aeration. The solution was neutralised as far as possible by grinding it with the barium carbonate and then finally by careful addition of barium hydroxide (0·3N) until it gave a faint pink colour with phenolphthalein. The white precipitate was filtered off, washed with water, and then triturated with dilute acetic acid to remove barium carbonate. The barium salts of oxalic and dihydroxyerythrosuccinic (*meso*tartaric) acids were filtered off, washed with water, alcohol, and dried in a vacuum at 80°.

All filtrates, obtained in the operations after the addition of the 0·3N-barium hydroxide, were combined (filtrates A) and reserved for further examination (see below).

Esterification. The dry barium salts (6 g.) were boiled for 8 hours with 3% methyl-alcoholic hydrogen chloride (150 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness under slightly reduced pressure. The residue was dissolved in methyl alcohol, and inorganic salts precipitated by addition of excess of ether. Removal of the precipitate, followed by distillation of the solvent, gave a pale yellow liquid which crystallised spontaneously. Trituration with light petroleum, followed by recrystallisation from acetone or acetone–ether, gave methyl dihydroxyerythrosuccinate (*meso*tartrate) (0·52 g.), m. p. and mixed m. p. 114°. The crystals showed no rotation in methyl alcohol (*c*, 1·2) (Found: OMe, 34·8. Calc. for $C_6H_{10}O_6$: OMe, 34·8%).

Removal of the solvent from the mother-liquors after separation of the methyl dihydroxyerythrosuccinate gave a syrup which still contained a considerable amount of this ester, for nucleation readily caused crystallisation. Treatment of this residue with methyl-alcoholic ammonia for 3 days at − 5°, followed by removal of the solvent in a vacuum, gave a crystalline residue (0·6 g.) of dihydroxyerythrosuccinamide, m. p. 184° (decomp.) (after recrystallisation from aqueous alcohol). This amide had no appreciable rotation in water (*c*, 3·3) (Found: C, 32·7; H, 5·4; N, 19·0. Calc. for $C_4H_8O_4N_2$: C, 32·4; H, 5·4; N, 18·9%).

Examination of filtrates (*A*). The combined filtrates (A), see above, were treated with 0·3N-barium hydroxide until no further precipitate was produced. The precipitate was filtered off, suspended in water, and treated with a slight excess of N-sulphuric acid. The excess of acid was removed by addition of a little lead carbonate. The solution was filtered and treated with hydrogen sulphide Removal of the precipitate by filtration and of the solvent by evaporation in a vacuum gave a syrup which crystallised spontaneously. Recrystallisation from ethyl alcohol gave mannosaccharodilactone (140 mg.), m. p. 196° (decomp.), $[\alpha]_D^{20°}$ +201° in water (*c*, 0·6). From the mother-liquor more dilactone (130 mg.) was obtained. Treatment of the syrup obtained from the mother-liquors gave mannosaccharodiamide (100 mg.), m. p. 204° (decomp.).

The Enol of 5-Keto-4-deoxymannosaccharo-3 : 6-lactone (II).—Mannosaccharodilactone (1 g.) was dissolved in the minimum amount of methyl alcohol and treated with 6 equivs. of 0·5N-sodium methoxide in methyl alcohol for 8—10 minutes at room temperature. Methyl-alcoholic hydrogen chloride was then added exactly to neutralise the sodium methoxide used. Removal of the sodium chloride by filtration, and of the solvent by evaporation under reduced pressure, gave a syrup which gradually crystallised. After recrystallisation from ethyl acetate or from ether–ethyl alcohol, the *enol* of *5-keto-4-deoxymannosaccharolactone monohydrate* had m. p. 82°, $[\alpha]_D^{18°}$ −48° (initial value in water; *c*, 1·2); − 34° (after 1 day); − 21·5° (2 days); − 7° (4 days); − 2·5° (5 days); + 2·0° (6 days); + 5° (7 days); + 9·5° (8 days); + 15° (11 days) (constant value). The hydrate immediately reacted with neutral potassium permanganate, bromine water, or chlorine water. It reduced Fehling's solution actively on warming (Found: C, 37·7; H, 5·0. $C_6H_6O_6,H_2O$ requires C, 37·5; H, 4·2%).

A freshly prepared aqueous solution of the enol (m. p. 82°) showed a band at λ 2290 A. (ε, 5000 approx.) (*c*, 5 mg. %), moving to λ 2650 A. (ε, 5000 approx.) on addition of alkali (Fig. 2). An aqueous solution of the substance which had undergone mutarotation still showed a band at λ 2290 A. but the intensity was much reduced. The enol reacted immediately at room temperature with 2 equivs. of sodium hydroxide (8·64 mg. required 8·85 c.c. of 0·01N-alkali for neutralisation, whence equiv., 97·5. $C_6H_6O_6$ requires equiv., 96).

The crystals of hydrated enol were treated with an excess of 0·01162N-iodine (30 c.c.) and N-sodium hydroxide (10 c.c.) at room temperature for 45 minutes. The solution was then acidified with N-sulphuric acid (10·1 c.c.), and excess of iodine titrated with 0·01N-sodium thiosulphate (4·53 mg. reacted with 14·0 c.c. of 0·01N-iodine, *i.e.*, 5·9 g.-atoms per g.-mol.). Small amounts of iodoform were formed.

When the monohydrate (m. p. 82°) was dried for 5 hours in a vacuum at 60° over phosphoric oxide and then for 3 hours at 100°, the water of crystallisation was eliminated and the m. p. rose to 142° (Found: loss, 9·9. $C_6H_6O_6,H_2O$ requires H_2O, 9·4%. Found, for anhydrous substance: C, 40·8; H, 4·0; equiv., 88. $C_6H_6O_6$ requires C, 41·4; H, 3·45; equiv., 87). In aqueous solution the *anhydrous* enol had $[\alpha]_D^{17°}$ −53° initial value (*c*, 1·8); − 43·5° (after 1 day); − 33° (2 days); − 17·5° (4 days); − 9° (5 days); − 2° (6 days); + 5·5° (7 days); + 14° (9 days); + 15·5° (10 days) (constant value). It reduced Fehling's solution actively on heating, decolorised bromine and permanganate, and immediately reacted with chlorine. The anhydrous 5-keto-4-deoxymannosaccharolactone showed a strong band at λ 2290 A. (ε, 4500 approx.; *c*, 5 mg. %), moving upon addition of sodium hydroxide to λ 2650 A. (ε, 4500 approx.; *c*,

5 mg. %) (Fig. 3). As in the case of the monohydrate (m. p. 82°), the mutarotation of the anhydrous material was accompanied by loss of the intensity of the band at λ 2290 A., the $\varepsilon_{max.}$ value at the equilibrium point being 1000 approx. (*c*, 25 mg. %).

In alkaline solution the anhydrous enol reacted with approximately 6 atomic proportions of iodine (6·08 mg. required 17·8 c.c. of 0·01162N-iodine. Calc. for 6 atomic proportions: 18·1 c.c.), a little iodoform being produced.

When titrated directly with chlorine water, with starch–iodide as an external indicator, approximately 4 atomic proportions of chlorine were consumed (11·66 mg. reacted with 9·4 c.c. of 0·0297N-chlorine; *i.e.*, 4·15 g.-atoms of Cl per g.-mol. of $C_6H_6O_6$). The acid was allowed to react for 5 minutes with an excess of chlorine water at room temperature. An excess of potassium iodide was then added to the solution, and the resulting iodine titrated with 0·01N-thiosulphate (11·07 mg. reacted with 8·7 c.c. 0·0297N-chlorine; *i.e.*, 4·05 g.-atoms of Cl per g.-mol. of the enol). Such aqueous solutions of the acid which have been treated with chlorine show no selective absorption.

Contrary to the behaviour with chlorine and bromine, both the enol of 5-keto-4-deoxymannosaccharolactone (m. p. 142°) and its hydrate (m. p. 82°) reacted with only small amounts of iodine.

Ozonisation of the Enol of 5-Keto-4-deoxymannosaccharo-3 : 6-lactone (II).—A solution of the enol (0·55 g.) in glacial acetic acid (25 c.c.) was treated as in previous examples with ozonised oxygen for 5 hours at room temperature. The rotation, initially $[\alpha]_D$ − 60°, had then become constant at + 7°. The solution was diluted with water, and acetic acid distilled off as before (p. 582). The residue was acidic, reduced Fehling's solution readily, and gave a positive oxalate test. A solution of the residue in water (15 c.c.) was neutralised by careful addition of barium hydroxide and treated with bromine (0·2 c.c.) at room temperature for 1 day. The solution was freed from bromine by aeration, neutralised with barium carbonate, and evaporated to dryness under reduced pressure. The dry residue of barium salts was boiled for 7 hours with 2% methyl-alcoholic hydrogen chloride (200 c.c.). Removal of the mineral acid with silver carbonate and of the solvent by distillation gave a syrupy residue which was purified by extraction with acetone–ether. The methyl oxalate, being readily volatile, is eliminated by the time this stage is reached. The syrup, which reacted faintly acid to Congo-red, was treated for 5 minutes in methyl alcohol (3 c.c.) with a slight excess of ethereal diazomethane. Removal of the solvent gave a neutral syrupy product (0·22 g.), which crystallised spontaneously. Recrystallisation from acetone gave methyl dihydroxyerythrosuccinate (*meso*tartrate), m. p. and mixed m. p. 114°. An aqueous solution (*c*, 2·8) of this ester showed no appreciable rotation (Found: C, 40·6; H, 5·65; OMe, 34·3. Calc. for $C_6H_{10}O_6$: C, 40·4; H, 5·6; OMe, 34·8%).

5-Methyl Δ⁴-Mannosaccharo-3 : 6-lactone 1-Methyl Ester (V).—A solution of the hydrate of the enol (II) (25 mg.) in methyl alcohol (2 c.c.) was titrated at 0° with a slight excess of ethereal diazomethane, the presence of the excess being indicated by the persistence of a yellow colour after 10 minutes. Removal of the solvent under reduced pressure gave the *ester* (V) as a syrup which, when purified by extraction with ether, had $[\alpha]_D^{18°}$ −30° in water (*c*, 0·9) (Found: OMe, 29·9. $C_8H_{10}O_6$ requires OMe, 30·7%). An aqueous solution of (V) showed a band at λ 2290 A. (ε, 5000 approx.; *c*, 5 mg. %) (Fig. 4) which disappeared upon addition of sodium hydroxide (tested at 20 mg. %).

Treatment of the anhydrous enol (II) (42 mg.; m. p. 142°) with ethereal diazomethane also gave this ester (V) (Found: OMe, 30·5%) (48 mg.), $[\alpha]_D^{18°}$ − 29·5° in water (*c*, 2·4), which had a band at λ 2290 A. in water (ε, 5000 approx.; *c*, 5 mg. %) (Fig. 4), disappearing upon addition of sodium hydroxide.

2 : 5-Dimethyl Δ⁴-Mannosaccharo-3 : 6-lactone 1-Methyl Ester (VI).—A solution of the enol (II) (0·52 g.) in methyl alcohol (5 c.c.) was allowed to react for 5 minutes with an excess of an ethereal solution of diazomethane. Concentration to dryness gave the syrupy ester (V). This was dissolved in acetone (3—5 c.c.) and allowed to react at 40—45° during 8 hours with silver oxide (5 g.) and methyl iodide (10 c.c.), the silver oxide being added in small portions at intervals of 1 hour. After recovery of the methyl iodide, the residue was exhaustively extracted with acetone and filtered. Removal of solvent from the combined acetone extracts gave a syrup which was subjected to two further treatments with silver oxide and methyl iodide to complete the methylation. Distillation gave 2 : 5-dimethyl Δ⁴-mannosaccharo-3 : 6-lactone 1-methyl ester (VI) (0·28 g.), b. p. (bath temp.) 175°/0·05 mm., $n_D^{20°}$ 1·4720, $[\alpha]_D^{18°}$ −36° in water (*c*, 1·2) (Found: OMe, 45·1. Calc. for $C_9H_{12}O_6$: OMe, 43·1%). Aqueous solutions of (VI) showed a strong band at λ 2290 A. (ε, 5000 approx.; *c*, 5 mg. %) (Fig. 4) which disappeared on addition of sodium hydroxide. On allowing a methyl-alcoholic solution of this syrupy form of (VI) to evaporate, there separated a very small amount of methyl 2 : 5-dimethoxymuconate, m. p. 140° alone or in admixture with a specimen previously prepared by direct methylation of mannosaccharodilactone (Haworth, Heslop, Salt, and Smith, *loc. cit.*). A solution of the crystals (m. p. 140°) in methyl alcohol was optically inactive. The presence of this compound (OMe, 54%) probably accounts for the high methoxyl value of the syrupy lactone ester (VI).

Slow evaporation of an aqueous solution of the syrup yielded crystals, m. p. 58°, $[\alpha]_D^{18°}$ −40° in water (*c*, 3·8) (after recrystallisation from ether). The pure ester (VI) had in aqueous solution a band at λ 2290 A. (ε, 6500 approx.; *c*, 5 mg. %) (Found: C, 49·95; H, 5·6; OMe, 42·7. Calc. for $C_9H_{12}O_6$: C, 50·0; H, 5·6; OMe, 43·1%). This crystalline ester failed to give a crystalline amide when treated with methyl-alcoholic ammonia.

2 : 5-Dimethyl Δ⁴-mannosaccharolactone was prepared by heating the crystalline ester (VI) (20 mg.) with 1 c.c. of N-sodium hydroxide for 30 minutes at 60°. The solution was cooled, treated with 10 c.c. of 0·1N-sulphuric acid, and evaporated to dryness under diminished pressure. Extraction of the residue with ethyl alcohol–ether gave an acidic product which failed to crystallise (Found: OMe, 29·9. $C_8H_{10}O_6$ requires OMe, 30·7%).

Ozonisation of 2 : 5-Dimethyl Δ⁴-Mannosaccharo-3 : 6-lactone 1-Methyl Ester (VI).—A solution of this ester (0·25 g.) in glacial acetic acid (10 c.c.) ($[\alpha]_D^{17°}$ −38·5°) was subjected to the action of a stream of ozonised oxygen for 3 hours at room temperature. The rotation was then constant ($[\alpha]_D^{17°}$ −2°). The solution was diluted with water, and evaporated to dryness under diminished pressure. The syrupy product obtained (0·21 g.) reacted acid to Congo-red; it reduced Fehling's solution actively on warming and gave a positive test for oxalic acid. It contained no glyoxylic acid, and none of this could be detected in the aqueous acetic acid distillate.

A solution of the syrup in water (8 c.c.) was treated with 0·3N-barium hydroxide until it reacted alkaline to phenolphthalein. The barium oxalate was filtered off, washed, and dried (0·1 g.). Evaporation of the filtrate and washings gave the barium salt of 3-methyl *d*-erythruronic acid (VII) as a pale yellow glassy solid (0·34 g.) which readily reduced Fehling's solution.

Formation of 2-Hydroxy-3-methoxy-d-erythrosuccinic Acid.—A solution of the barium salt (0·34 g.) obtained above, in water (10 c.c.), was oxidised with bromine (0·2 c.c.) for 24 hours at room temperature. The excess of bromine was removed by aeration, the solution neutralised with silver carbonate, treated with hydrogen sulphide, again filtered, and evaporated to dryness under reduced pressure. The residue was then boiled for 8 hours with 2% methyl-alcoholic hydrogen chloride (25 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness. The syrupy product, after purification by extraction with ether gave, upon distillation, methyl 3-hydroxy-2-methoxy-*l*-erythrosuccinate (IX) as a colourless, mobile liquid (90 mg.), b. p. (bath temp.) 120°/0·05 mm., $n_D^{17°}$ 1·4414 (Found: OMe, 48·3. Calc. for $C_7H_{12}O_6$: OMe, 48·5%).

When this ester (20 mg.) was treated with methyl-alcoholic methylamine for 24 hours at 0° it gave, after removal of the solvent, the crystalline bismethylamide (X) of 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid, m. p. and mixed

m. p. 135°, $[\alpha]_D^{17°}$ +10·5° in water (*c*, 1·6) (after recrystallisation from ethyl acetate) (Found : OMe, 16·5. Calc. for $C_7H_{14}O_4N_2$: OMe, 16·2%).

The Methyl Ester of the Enol of 3-*Keto*-4-*deoxymannosaccharolactone.*—Mannosaccharodilactone (2 g.) in dry methyl alcohol (10 c.c.) was allowed to react for 3—5 minutes with 3 equivs. of 0·5N-sodium methoxide in methyl alcohol (69 c.c.) at room temperature. An intense yellow colour developed immediately upon addition of the sodium methoxide. The methoxide was then neutralised by the addition of the calculated amount of 5% methyl-alcoholic hydrogen chloride, and the solution filtered. Evaporation of the filtrate at 40° under reduced pressure gave a product which was freed from inorganic impurity by extraction with alcohol containing a little ether. Removal of the solvent yielded a syrup which partly crystallised. The crystals were separated by tiling, and after purification by recrystallisation from ethyl alcohol–ether the *methyl* ester of the enol of 5-keto-4-deoxymannosaccharolactone (200 mg.) had m. p. 111°, $[\alpha]_D^{17°}$ −41·3° initial value in water (*c*, 0·6); − 15° (after 20 hours); + 8° (70 hrs.); constant for further 30 hrs. (Found : OMe, 15·4. $C_7H_8O_6$ requires OMe, 16·5%). Aqueous solutions of this compound showed a selective absorption band at λ 2290 A. (ε, 5000 approx.; *c*, 4 mg. %), moving to λ 2650 A. (ε, 4500 approx.; *c*, 6 mg. %) on addition of sodium hydroxide. Aqueous solutions of the crystals react acid to Congo-red, reduce hot Fehling's solution, and decolorise acid potassium permanganate in the cold. The compound can be titrated directly with approximately 1 equiv. of sodium hydroxide at room temperature (9·502 mg. required 5·4 c.c. of 0·01N-sodium hydroxide. Calc. for 1 equiv. : 5·06 c.c.). On keeping this solution with an excess of 0·01N-sodium hydroxide a further 5·24 c.c. (determined by back titration) were taken up.

When the crystals (m. p. 111°) (9·261 mg.) were treated with 3 c.c. of 0·3N-baryta for 1 hour at 50° the methoxyl group was eliminated, as shown by a nil Zeisel determination, carried out upon the dry residue obtained by evaporation of the solution (neutralised by carbon dioxide) under reduced pressure. This suggested the esteric character of the single methoxyl group.

A solution of the crystals (12·02 mg.) in water (1 c.c.) was treated with 25 c.c. of 0·02N-chlorine water for 5 minutes. Potassium iodide was then added, and the liberated iodine titrated with 0·02N-thiosulphate; 14·5 c.c. of 0·02N-chlorine were used (Calc. for 4Cl : 12·8 c.c.).

Two experiments were carried out in which the crystals (m. p. 111°) were treated with 0·01N-iodine in N-sodium hydroxide (10 c.c.) at room temperature for 45 minutes: 9·872 and 3·899 mg. reacted with 34·0 and 13·6 c.c., respectively, of 0·01N-iodine (Calc. for 6I : 31·5, 12·5 c.c., respectively).

Upon treatment of the ester (m. p. 111°; 25 mg.) in methyl alcohol (3 c.c.) with excess of ethereal diazomethane for 10 minutes at 0°, followed by removal of the solvent, there was obtained a syrup (purified by extraction with ether) which had $[\alpha]_D^{17°}$ −29·5° in water (*c*, 1·0) (Found : OMe, 30·5. $C_8H_{10}O_6$ requires OMe, 30·7%). An aqueous solution of this *dimethyl* derivative had a band at λ 2290 A. (ε, 6000 approx.; *c*, 6 mg. %) which disappeared upon addition of sodium hydroxide. The dimethyl derivative was still unsaturated, for it reacted with chlorine and with bromine. It did not reduce Fehling's solution.

Several attempts to repeat the preparation of the compound, m. p. 111°, failed, the crystalline enol of 5-keto-4-deoxymannosaccharo-3 : 6-lactone being obtained instead. The formation of the compound, m. p. 111°, was probably due to the use of a slight excess of methyl-alcoholic hydrogen chloride which was added to neutralise the sodium methoxide used in the isomerisation of the dilactone.

Treatment of the Enol of 5-*Keto*-4-*deoxymannosaccharolactone* (II) *with Chlorine.*—A solution of the crystalline enol (0·3 g.) in water (10 c.c.) was allowed to react with 0·19N-chlorine water (100 c.c.) for 10 minutes at room temperature. After removal of the excess of the chlorine by aeration, the solution (which now showed no selective absorption) was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure at 40°. The residue was boiled for 7 hours with 2% methyl-alcoholic hydrogen chloride (150 c.c.). Removal of mineral acid with barium carbonate, followed by filtration and elimination of solvent under reduced pressure, gave a syrupy product which reacted acid to Congo-red. A second esterification with 2% methyl-alcoholic hydrogen chloride still failed to effect esterification, and the product reacted acid to Congo-red. The product was therefore dissolved in methyl alcohol (10 c.c.) and treated with a slight excess of an ethereal solution of diazomethane. Immediate concentration of the solution yielded a partly crystalline product which, after recrystallisation from water, had m. p. 127° (0·15 g.), $[\alpha]_D^{20°}$ −13° in ethyl alcohol (*c*, 1·2). This *methyl* 4 : 5-*dichloro*-2 : 3-*dihydroxy*-5-*methoxyadipate* (XVII) did not reduce Fehling's solution or react with bromine. Aqueous solutions of it were transparent to ultra-violet light (Found : C, 35·7; H, 4·2; OMe, 30·0; Cl, 25·5; equiv., by heating at 60° with 0·02N-sodium hydroxide, 146. $C_9H_{14}O_7Cl_2$ requires C, 35·4; H, 4·6; OMe, 30·5; Cl, 23·3%; equiv., 152·5).

Removal of the solvent from the mother-liquors after isolation of the dichloro-ester gave a syrupy product which, although transparent to ultra-violet light, was strongly reducing to Fehling's solution.

The crystalline dichloro-ester (XVII) (50 mg.) (m. p. 127°) was warmed at 60° for 45 minutes with 0·1N-sodium hydroxide (4 c.c.) and then neutralised with 4 c.c. of 0·1N-sulphuric acid. The solution was concentrated to dryness, and the residue extracted with ether–ethyl alcohol. This gave the *dichloro-acid*, a liquid (42 mg.) which reacted strongly acid to Congo-red (Found : OMe, 10·9. $C_7H_{10}O_7Cl_2$ requires OMe, 11·2%).

Treatment of the dichloro-ester (XVII) (40 mg.) with methyl-alcoholic ammonia at − 5° for 3 days, followed by removal of the solvent in a vacuum, gave a crystalline monoamide (XVIII) (25 mg.), m. p. 197°, $[\alpha]_D^{19°}$ −20° in water (*c*, 1·8) (after one crystallisation from acetone and one from water), which showed a positive Weerman reaction.

Three methylations of the dichloro-ester (90 mg.) (m. p. 127°) with silver oxide (2—3 g.) and methyl iodide (3 c.c.), acetone (1 c.c.) being added to aid solution, effected the introduction of one additional methoxyl group, giving *methyl* 4 : 5-*dichloro*-2 (or 3)-*hydroxy*-3 (or 2) : 5-*dimethoxyadipate*, m. p. 116°, $[\alpha]_D^{18°}$ +15° in methyl alcohol (*c*, 1·6) (Found : C, 38·2; H, 4·2; OMe, 39·1; Cl, 22·35. $C_{10}H_{16}O_7Cl_2$ requires C, 37·6; H, 5·0; OMe, 38·9; Cl, 22·3%).

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