56. *Lactones of Mannosaccharic Acid. Part I. 2 : 5-Dimethyl Δ⁴-Mannosaccharo-3 : 6-lactone 1-Methyl Ester, an Analogue of Ascorbic Acid.*

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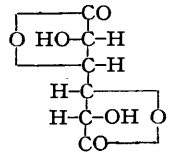
Mannosaccharodilactone, prepared from either mannose or α-methylmannoside by oxidation with nitric acid, reduces Fehling's solution, a property not displayed by simple monolactones of either the γ- or the δ-series. This phenomenon was observed by Kiliani (*Ber.*, 1887, **20**, 2710) and by Fischer (*Ber.*, 1891, **24**, 539), but a satisfactory explanation based on experimental facts has never been put forward. Mannosaccharodilactone only displays reducing properties in alkaline solution or after treatment with alkaline reagents. The substance which is believed to be responsible for these reducing properties shows in alkaline solution an intense selective absorption band at λ 2630 A., moving to λ 2290 A. upon acidification. This paper deals with the methylation of mannosaccharodilactone, a process which appears to isomerise the dilactone in the same way as do alkaline reagents, and which also stabilises the isomeric form of the dilactone as a methyl derivative.

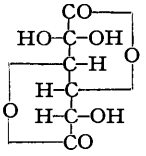
Treatment of mannosaccharodilactone with diazomethane, with silver oxide and methyl iodide, and with diazomethane, followed by silver oxide and methyl iodide, affords mainly 2 : 5-*dimethyl* Δ⁴-*mannosaccharo*-3 : 6-*lactone* 1-*methyl ester* (VI), a substance showing a strong band at λ 2290 A. Methylation of mannosaccharodilactone is not a simple process, because in addition to (VI) there are produced 6-carbomethoxy-3-methoxy-α-pyrone, methyl dimethyl *meso*tartrate, and a trimethyl mannosaccharolactone methyl ester.

Ozonisation of (VI) gives oxalic acid and an aldehydic acid, a monomethyl erythuronic acid (VII). Oxidation of the latter with bromine yields the new 3-*hydroxy*-2-*methoxy*-l-*erythrosuccinic acid* (VIII), the configuration of which has been established; the acid (VIII) was characterised as a crystalline *diamide* and a crystalline *bismethylamide*. These facts prove the structure (VI) assigned to the methylated unsaturated lactone methyl ester. Confirmation of this conclusion followed from the fact that reduction of (VI) gives the 4-*deoxy*-derivative (XIV), the crystalline *diamide* (XV) of which shows a negative Weerman test for α-hydroxy-amides.

It is suggested that the reducing substance produced from mannosaccharodilactone by means of alkaline reagents is structurally related to 2 : 5-dimethyl Δ⁴-mannosaccharo-3 : 6-lactone 1-methyl ester (VI).

One of the characteristic properties of γ- and δ-lactones prepared from sugars by the oxidation of the reducing group is that they exhibit no reducing activity. Indeed, the conversion of a reducing sugar into a lactone is verified by the fact that the solution no longer reduces Fehling's solution. It is therefore not surprising that Kiliani's discovery (*Ber.*, 1887, **20**, 2710) of a sugar acid dilactone, *l*-mannosaccharodilactone, showing reducing properties excited considerable interest. This dilactone reduces Fehling's solution and reacts in alkaline solution with four atomic proportions of iodine. It also reacts with two atomic proportions of sodium hydroxide under conditions suggestive of a dilactone structure, and two alternative formulæ, (I) and (II), were put forward (Kiliani, *loc. cit.*). Formula (I), containing two four-atom lactone rings, is not considered to be a likely possibility owing to the strain inherent in such a structure, but (II), having two five-atom or γ-lactone rings, is much more probable. Formula (III), not mentioned by Kiliani, must also be taken into account; it contains one five-atom and one six-atom lactone ring. The weakly acid character of a freshly prepared aqueous solution of the dilactone and its relatively slow rate of mutarotation favour the structure (II) rather than (III), since the latter, having a δ-lactone ring, would be expected to show relatively rapid mutarotation. It should be borne in mind, however, that, although the rate of mutarotation displayed by a monolactone can be employed in aiding structural studies (Haworth, " The Constitution of the Sugars," 1929), yet it does not follow that the same general rule, relating mutarotation and ring structure, will apply to dilactones. Since this work was


(I.)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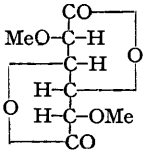
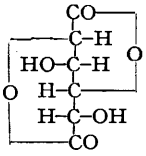

(IIa.)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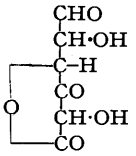
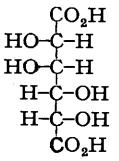

(IV.)

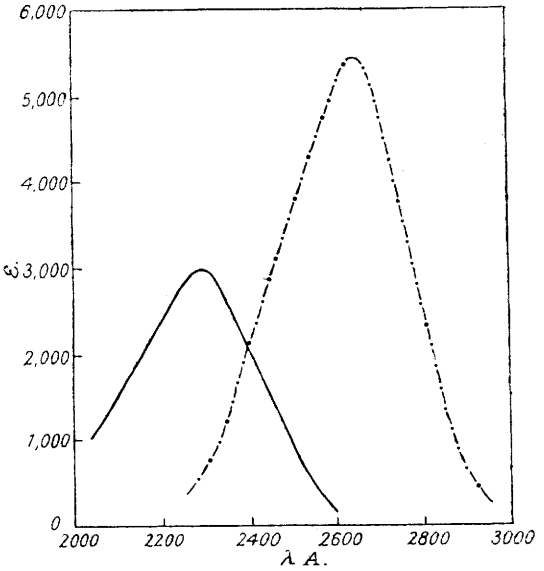

(V.)

completed, it has been shown that mannosaccharodilactone has the structure (II), because upon treatment with moist diazomethane it affords a dimethyl derivative (II*a*), the structure of which follows from the observ-

ation that the diamide derived from it gives no sodium *iso*cyanate when treated with sodium hypochlorite (Schmidt and Kraft, *Ber.*, 1941, **74**, 33). It was recognised by Kiliani that none of these structures explained the unexpected reducing properties of the dilactone, and he suggested that some molecular rearrangement had taken place with the formation of a substance possessing an aldehyde and a keto-group as in (IV). Nevertheless, the ease of formation of a mono- and a di-phenylhydrazide and a diamide from the compound were in accordance with the simple dilactone structure.

The view that the reducing properties were an inherent characteristic of the dilactone itself and not due to some reducing impurity (see Fischer, *Ber.*, 1891, **24**, 539) was substantiated by the production of two series of salts from the dilactone, in one set of which the reducing capacity was retained whereas the other series behaved like the salts of a normal sugar acid in that they showed no reducing activity. Thus, for example, it was demonstrated that when an aqueous solution of the dilactone was allowed to react with an excess of potassium hydroxide, an intense yellow colour developed and the potassium salt isolated from the reaction mixture still reduced Fehling's solution. When, however, the diamide from the dilactone was heated with potassium hydroxide, a different potassium salt was formed which displayed no reducing activity. Moreover, since this non-reducing potassium salt, obtainable also by titration of crystalline mannosaccharic acid (V) with potassium hydroxide (Rehorst, *Ber.*, 1932, **65**, 1475), could not be transformed into the reducing salt except through intermediate conversion into the dilactone, it was evident that the formation in solution of the reducing compound is due to isomerisation taking place in the dilactone itself. This view is supported by the fact that aqueous solutions of the dilactone undergoing mutarotation to the open-chain acid (V) show a gradual decrease in reducing activity, and conversely aqueous solutions of mannosaccharic acid undergo lactonisation upon heating with a consequent increase in reducing power (Rehorst, *loc. cit.*).

FIG. 1.



——•——•—— *Mannosaccharodilactone in aqueous alkaline solution* (*c*, 3 *mg.*/100 *c.c.*).
———— *Mannosaccharodilactone after treatment with aqueous alkali followed by acid* (*c*, 15 *mg.*/100 *c.c.*).

We have obtained some insight into this problem of the reducing activity of mannosaccharodilactone in alkaline solution by the application of spectrophotometry. A freshly prepared aqueous solution of this dilactone shows no selective absorption band in the ultra-violet region of the spectrum, but immediately upon the addition of an excess of sodium hydroxide an intense characteristic band appears at λ 2630 A., accompanied by the development of reducing activity; for instance, such an alkaline solution reduces Fehling's solution, reacts with alkaline iodine to give iodoform, and immediately decolorises potassium permanganate. Furthermore, on acidification of this alkaline solution, the absorption band moves to λ 2290 A. (Fig. 1) and it is noteworthy that the acidified solution still shows reducing activity towards Fehling's solution and neutral potassium permanganate solution. It is evident from these observations that acidification of an alkaline solution of the dilactone gives rise to an active substance in solution, a deduction previously made by Rehorst from his investigations of the reducing activity shown by the sodium and the potassium salt derived from the dilactone. In addition to showing the reducing properties of alkaline solutions of the dilactone, the acidified solution exhibiting a band at λ 2290 A. also behaves as if an unsaturated substance is present, since it reacts quickly with chlorine and with bromine. Treatment of the acidified solution with chlorine causes a profound change in the structure of the reactive isomeric substance, since the acid solution no longer displays selective absorption; this observation suggests, but does not prove, that the substance responsible for the absorption band at λ 2290 A. in acidified solutions is also unsaturated in character and reacts with chlorine. The reaction with chlorine, which will be referred to in a later communication, appears to be complex in nature, for, although the acidified solution after treatment with chlorine is quite transparent to ultra-violet light, it still shows reducing action towards Fehling's solution.

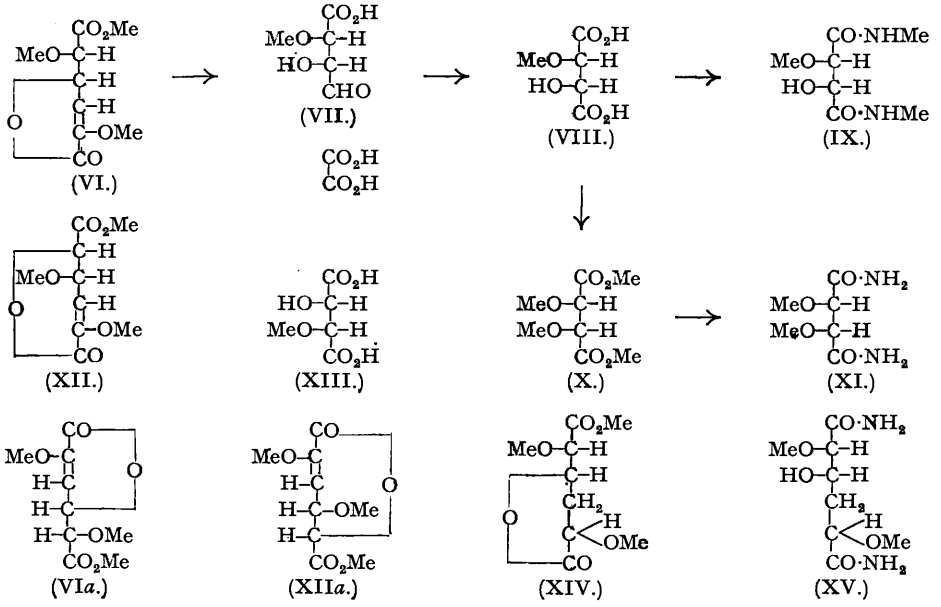
A comparison of these peculiar properties of the active isomeric form of mannosaccharodilactone with those of *l*-ascorbic acid (Herbert, Hirst, Percival, Reynolds, and Smith, J., 1933, 1270) shows that they bear a striking resemblance to each other. For example, ascorbic acid reduces Fehling's solution, decolorises neutral potassium permanganate, and reacts with chlorine and bromine; it also displays a band at λ 2450 A. in acidified aqueous solution, moving to λ 2650 A. on addition of sodium hydroxide; the band at λ 2450 A. disappears upon the addition of a slight excess of chlorine. It will be observed that these properties are very similar to those shown by the active form of mannosaccharodilactone, mentioned previously. Solutions of the active form of this dilactone and of ascorbic acid do, however, differ in some important respects; for instance, the active form of the dilactone requires heating with Fehling's solution before reduction becomes manifest, whereas ascorbic acid reduces Fehling's solution in the cold. Further, ascorbic acid reacts immediately with two atomic pro-

portions of iodine in acid solution, whereas solutions of the active form of mannosaccharodilactone react with only a small amount of iodine. This difference is apparently to be traced to the fact that the reaction of ascorbic acid with chlorine, bromine, and iodine is one of oxidation, whereas that occurring between the active form of mannosaccharodilactone and chlorine or bromine is to some extent one of addition.

Despite these dissimilarities, the question arises as to whether the substance derived from the dilactone by means of alkaline reagents, which exhibits the peculiar reducing properties, possesses, like ascorbic acid, a five-atom lactone ring having an unsaturated conjugated system of double bonds.

The problem was simplified by spectrophotometric measurements which indicated that the peculiar isomerisation of mannosaccharodilactone brought about by the agency of alkaline reagents can also be effected by treatment of the dilactone with diazomethane and with Purdie's reagents (Haworth, *J. Soc. Chem. Ind.*, 1933, 52, 482). Since these two reagents effect not only isomerisation but methylation at the same time, it was decided to determine the structure of the now stabilised isomeric methyl derivative and then proceed to the elucidation of the structure of the more reactive unmethylated active form of mannosaccharodilactone itself.

One treatment of mannosaccharodilactone with diazomethane gave a partly methylated product, but repeated treatment afforded a fully methylated product, and this upon fractional distillation yielded an unsaturated methyl ester (VI). This ester was also produced by the methylation with silver oxide and methyl iodide of the partly methylated material obtained from the dilactone by one treatment with diazomethane, and it resulted also from the direct methylation of mannosaccharodilactone with Purdie's reagents (cf. Schmidt and Kraft, *loc. cit.*).



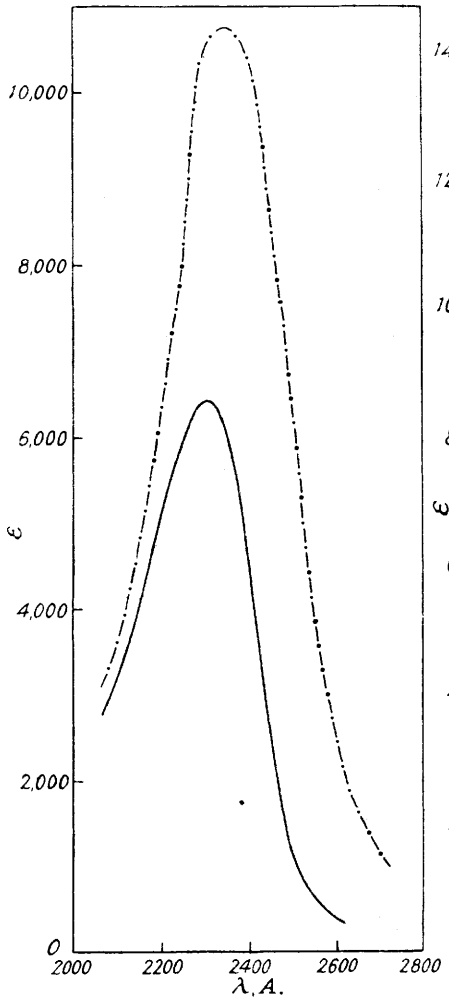
The ester (VI) was non-reducing (to Fehling's solution) but its unsaturated character was shown by its reactivity towards chlorine and bromine. Moreover, an aqueous solution of the ester (VI) exhibited strong selective absorption at λ 2290 A. (see Fig. 2), the band disappearing upon addition of alkali. On acidification of the alkaline solution, this band reappeared, though its intensity was considerably diminished. On keeping the acid solution, however, the intensity gradually increased until it eventually reached that originally shown by the ester. A comparison of the band shown by (VI) with that displayed by 2 : 3 : 5 : 6-tetramethyl *l*-ascorbic acid (Fig. 2) (Haworth, Hirst, and Smith, J., 1934, 1556) suggested that the conjugated system present in the latter was closely related to that in (VI). If this was the case, then the disappearance in alkaline solution of the band at λ 2290 A. is probably due to the opening of the conjugated ring system as in the case of 2 : 3 : 5 : 6-tetramethyl *l*-ascorbic acid, which shows a band at λ 2350 A., disappearing upon addition of alkali. On acidification of the alkaline solution of (VI), an open-chain acid would first be formed, and this would slowly undergo lactonisation with the simultaneous production in solution of the original substance showing selective absorption; the gradual increase in the intensity of the band at λ 2290 A. would therefore be expected.

The ester (VI) was proved to be 2 : 5-*dimethyl* Δ^4-*mannosaccharo*-3 : 6-*lactone* 1-*methyl ester* as a result of the following experimental facts. The substance (VI) had the formula $C_9H_{12}O_6$ and contained three methoxyl groups. Aqueous solutions of (VI) were neutral to litmus and Congo-red paper, but on warming, the substance (VI) reacted with 2 equivs. of sodium hydroxide with the elimination of 1 mol. of methyl alcohol, an observation which showed that two potentially acidic groups were present in (VI) and that one of these (that affording the methyl alcohol upon alkaline hydrolysis) was probably a carbomethoxy-group. The unsaturated nature of the compound was suggested by the fact that it reacted with 2 atomic proportions of chlorine, a process attended by the disappearance of the selective absorption band. The substance (VI) also combined with bromine and

immediately decolorised potassium permanganate in alkaline solution; furthermore, it reacted with 3 atomic proportions of iodine in alkaline solution, with the formation of a small amount of iodoform.

When a solution of the unsaturated ester (VI) in glacial acetic acid was subjected to ozonisation, a considerable change in rotation took place, accompanied by the formation of oxalic acid and the aldehydic acid, monomethyl erythuronic acid (VII). The presence of an aldehydic group in the product after removal of the oxalic acid was shown by its strong reducing action towards Fehling's solution. Since fission of the double bond was accompanied by the formation of an additional carboxyl group and an aldehydic group, as well as the loss of a methoxy-group, it follows that the system $-C(OMe)=CH-$ must have been present in the

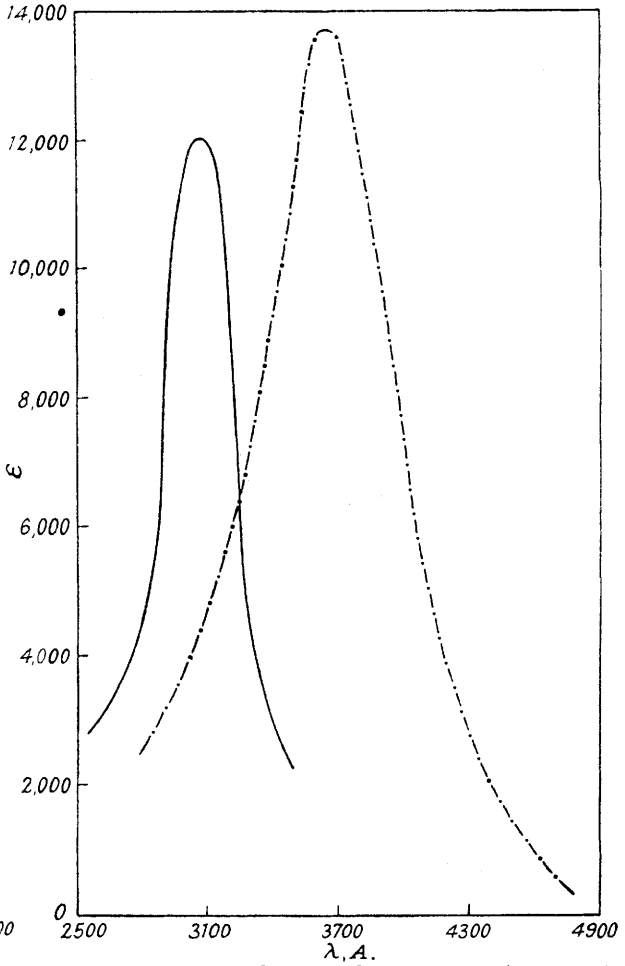
FIG. 2.



———— 2 : 5-*Dimethyl* Δ^4-*mannosaccharo*-3 : 6-*lactone methyl ester in water* (*c*, 5·5 *mg.*/100 *c.c.*).

—·—·—·— 2 : 3 : 5 : 6-*Tetramethyl* l-*ascorbic acid in water* (*c*, 4 *mg.*/100 *c.c.*).

FIG. 3.



———— 6-*Carbomethoxy*-3-*methoxy*-α-*pyrone in water* (*c*, 2 *mg.*/100 *c.c.*).

—·—·—·— 6-*Carbomethoxy*-3-*methoxy*-α-*pyrone in aqueous alkali* (*c*, *mg.*/100 *c.c.*).

unsaturated ester (VI). The production of a two-carbon fragment (oxalic acid) and a four-carbon fragment (VII) indicates that the double bond must be located between C_4 and C_5, and furthermore, since the two-carbon fragment proved to be oxalic acid and not glyoxylic acid, it is evident that the methoxyl residue eliminated as a result of ozonolysis was attached to C_5 and not to C_4; it also follows from these results that the hydrogen atom present in the aldehydic group of the four-carbon fragment was attached to C_4. Oxidation of the aldehydic acid (VII) with bromine gave a *hydroxymethoxysuccinic acid* (VIII), which was characterised, after conversion into the methyl ester, by its transformation into a crystalline *diamide*. The diamide showed no appreciable rotation in aqueous solution, but the molecular dissymmetry of the acid (VIII) was demonstrated by the fact that its methyl ester yielded a crystalline *bismethylamide* (IX) which had a positive rotation. The acid (VIII) was shown to be stereochemically related to 2 : 3-dihydroxyerythrosuccinic acid (*meso*tartaric acid) because methylation of the methyl ester of (VIII) with Purdie's reagents gave methyl dimethoxyerythrosuccinate

(methyl dimethyl *meso*tartrate) (X), identified by its conversion into the known crystalline dimethoxyerythrosuccinamide (inactive dimethoxysuccinamide) (XI) (Haworth and Hirst, J., 1926, 1858). Although these results established the position of the double bond and proved that the acid (VIII) was a monomethyl derivative of dihydroxyerythrosuccinic acid, it was not clear whether this hitherto unknown hydroxymethoxysuccinic acid had the configuration represented by (VIII), *i.e.*, 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, or by the only other possible formulation (XIII), *viz.*, 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid which is the enantiomorph of (VIII); both (VIII) and (XIII) upon methylation would yield the same inactive methyl 2 : 3-dimethoxyerythrosuccinate (X). Hence, in addition to the structure (VI) suggested for the original unsaturated methyl ester, the alternative formulation (XII), in which a δ-lactone ring is present, had to be taken into consideration, because ozonisation of a substance having this formulation (XII), followed by bromine oxidation, would give 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid (XIII), one of the two possible structures for the hydroxymethoxyerythrosuccinic acid. It might be suggested that the formation of oxalic acid and the aldehydic acid (VII) could also be explained by the two formulæ (VI*a*) and (XII*a*), in both of which the double bond is situated between C_2 and C_3, but examination of these two formulations shows that they are identical respectively with the two structures (VI) and (XII) now under consideration. Thus it can be seen that if the hydroxymethoxysuccinic acid derived from the aldehydic acid by bromine oxidation is 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, as in (VIII), then the original unsaturated ester must have the formulation (VI), whereas if the hydroxymethoxysuccinic acid is 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid (XIII), then (XII) is the correct structure of the original unsaturated ester. It is clear therefore that the absolute configuration of the hydroxymethoxysuccinic acid obtained from the aldehydic acid must be ascertained in order to decide which of the two structures (VI) or (XII) is correct.

We have now solved this problem by the synthesis of both the *d*- and the *l*-form of hydroxymethoxyerythrosuccinic acid. One of these, 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, which is the same as 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid, was prepared from *d*-araboascorbic acid by a series of reactions which leaves no doubt about its structure; the enantiomorph, 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid (3-hydroxy-2-methoxy-*d*-erythrosuccinic acid), was obtained by resolution of the racemic mixture of the hydroxymethoxyerythrosuccinic acids prepared from inactive dihydroxyerythrosuccinic acid (*meso*tartaric acid) by methylation with methyl sulphate and sodium hydroxide (see succeeding paper).

The 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, obtained from *d*-araboascorbic acid, gave immediately upon treatment with diazomethane the corresponding methyl ester, which yielded a crystalline bismethylamide identical with that (IX) prepared from the acid (VIII). The latter must therefore be 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, and hence the original unsaturated substance must have the structure shown in (VI) and not that shown in (XII). The 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid prepared from *meso*tartaric acid afforded a bismethylamide which proved to be the enantiomorph of (IX).

Further evidence in support of the structure (VI) assigned to the unsaturated methyl ester was provided by an examination of the deoxy-compound (XIV) obtained from (VI) by hydrogenation. Reduction of (VI) was effected by means of sodium amalgam, and there was obtained a deoxy-acid which upon treatment with boiling methyl-alcoholic hydrogen chloride gave rise to the *deoxy-lactone methyl ester* (XIV). The fact that aqueous and alkaline solutions of this ester showed no selective absorption indicated that saturation of the double bond between C_4 and C_5 had been effected. Saturation of the double bond was also carried out by catalytic hydrogenation. Treatment of the 4-deoxy-ester (XIV) with ammonia in methyl alcohol furnished a crystalline *diamide* (XV) which showed a negative Weerman test for α-hydroxy-amides (*Rec. Trav. chim.* 1917, **36**, 16). Methoxy-groups must therefore be located in positions 2 and 5 of (XV) and therefore of (XIV), and hence it follows that the hydroxy-group set free by opening of the lactone ring during the conversion of the deoxy-lactone methyl ester (XIV) into the diamide (XV) must occupy position 3. The lactone ring in the deoxy-compound (XIV), and therefore in the 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester, must engage carbon atoms C_3 and C_6.

It is noteworthy that saturation of the double bond between C_4 and C_5 in (VI) introduces further dissymmetry into the molecule at C_5, and consequently two 4-deoxy-lactone methyl esters are theoretically possible. Only one crystalline diamide was isolated, however, and in this the spatial arrangement of the hydrogen and the methoxy-group at C_5 still remains to be determined.

From the foregoing it will be seen that there is a close relationship betwen the properties of a solution of 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (VI) and a solution of the " active " form of mannosaccharodilactone prepared from the latter by the agency of alkali, followed by acid; *e.g.*, both solutions show intense bands at λ 2290 A., and both react with chlorine, bromine, alkaline permanganate, and alkaline iodine. In some respects, however, they differ; for example, a solution of the " active " form of the dilactone reduces Fehling's solution, whereas (VI) does not; but such differences as do exist are probably attributable to the fact that (VI) is a methylated compound. Such considerations led us to the view that the substance responsible for the remarkable and unexpected behaviour of mannosaccharodilactone (after treatment with alkaline reagents) has a structure analogous to (VI).

Experimental.

Mannosaccharodilactone.—(a) *From mannose.* A solution of mannose (10 g.) in nitric acid (25 c.c., *d* 1·2) was heated on the water-bath for 48 hours at 55°. The yellow solution was then concentrated slowly in an open dish at 80°. Before darkening of the product occurred and whilst it still contained some solvent (water and nitric acid), the concentration

was completed in a vacuum over potassium hydroxide. The almost colourless crystalline solid was triturated with ethyl alcohol–ether and filtered off. Purification of the compound by recrystallisation from ethyl alcohol gave mannosaccharodilactone as silky plates, m. p. 189°, $[\alpha]_{5780}^{18°}$ +214° (initial value in water; *c*, 1·1) (yield 2·5 g.).

The following modification gave a slightly better yield. The same quantities of mannose and nitric acid were heated on the water-bath for 4 hours at 60°. The temperature was then raised to 85°, and the oxidation continued for a further ½ hour. Removal of the nitric acid by distillation under diminished pressure gave an almost colourless syrup which crystallised spontaneously. The crystals of mannosaccharodilactone were separated by trituration with ethyl alcohol containing a little ether, and recrystallised from ethyl alcohol; m. p. 187° (yield, 3·0 g.).

(b) *From α-methylmannopyranoside.* Oxidation of α-methylmannoside with nitric acid by the above modified method gave a 30% yield of mannosaccharodilactone.

Aqueous solutions of mannosaccharodilactone show no selective absorption band (tested at 200 mg.%) and are unaffected by chlorine, bromine, iodine, and potassium permanganate. Upon addition of excess of N-sodium hydroxide to a solution of the dilactone, a band appears at λ 2630 A. (ε, 5,500; *c*, 3·0 mg.%); acidification of this alkaline solution causes this band to move to λ 2290 A. (ε, 3,000; *c*, 15 mg.%) (see Fig. 1). This acidified solution contains what may be referred to as the active form of mannosaccharodilactone; it reacts immediately with chlorine, bromine, and potassium permanganate. Alkaline solutions of mannosaccharodilactone readily reduce hot Fehling's solution and immediately decolorise potassium permanganate in the cold.

The Action of Alkaline Iodine upon Mannosaccharodilactone.—In accordance with the findings of Kiliani and Rehorst (*locc. cit.*) mannosaccharodilactone was found to react in alkaline solution with approximately 4 atomic proportions of iodine.

A solution of mannosaccharodilactone (0·303 g.) in 0·1N-sodium hydroxide (87 c.c.) was treated with 0·1N-iodine (50 c.c.) at room temperature. After 90 mins., the solution was acidified by addition of N-sulphuric acid (10 c.c.), and the excess of the iodine was titrated with 0·1N-sodium thiosulphate (27·7 c.c.). A blank experiment was carried out at the same time. Hence in alkaline solution 1 mol. of mannosaccharodilactone reacts with 3770 c.c. of N-iodine or approximately 4 atomic proportions. A small amount of iodoform is produced in this alkaline iodine oxidation of mannosaccharodilactone.

Oxalic acid is also formed when alkaline iodine is allowed to react with mannosaccharodilactone. The dilactone (0·1 g.) in water (5 c.c.) was treated with N-sodium hydroxide (5·7 c.c.) and iodine (4·0 c.c. containing 0·4 g. of iodine). After the mixture had stood for 15 mins. at room temperature, it was acidified with glacial acetic acid (2 c.c.) and filtered to remove iodoform. A stream of sulphur dioxide was passed through the solution to decolorise the iodine, and then phenylhydrazine (0·15 g.) was added. Phenylhydrazine oxalate readily separated as large plates, m. p. and mixed m. p. 178°. The complex nature of this alkaline iodine oxidation of mannosaccharodilactone is indicated by the fact that occasionally a yellow phenylhydrazine derivative, m. p. 212°, was obtained (see Rehorst, *Ber.*, 1938, **71**, 923).

The Effect of Chlorine upon the "Active" Form of Mannosaccharodilactone.—A solution of mannosaccharodilactone (0·1 g.) in water (5 c.c.) was treated with 0·1N-sodium hydroxide (13 c.c.) for 1 min. at room temperature. The solution was acidified by addition of 0·1N-sulphuric acid (13·5 c.c.) and, now containing the "active" form of mannosaccharodilactone showing a band at λ 2290 A., it was titrated with 0·085N-chlorine water (13·3 c.c.). The end-point was determined by starch–iodide paper. It may be calculated that 1 mol. of mannosaccharodilactone after the above treatment with alkali followed by acid reacts with 19,700 c.c. of 0·1N-chlorine water, or approximately 2 atomic proportions.

When freshly prepared, the chlorine oxidation solution referred to above shows no selective absorption band and has $[\alpha]_{5780}^{18°}$ +84° (initial value); +78° (after 2½ hours); +65° (8 hours); +45° (22 hours); +35° (30 hours); +25° (40 hours); +15° (60 hours); +12·5° (80 hours); +17° (170 hours); +19° (200 hours); +24° (380 hours, constant value).

The Effect of Potassium Permanganate upon the "Active" Form of Mannosaccharodilactone.—A solution of the dilactone (0·1 g.) in water (10 c.c.) was treated with N-sodium hydroxide (3 c.c.), followed 3 mins. later by N-sulphuric acid (5 c.c.). After the addition of a slight excess of 0·1N-potassium permanganate (6·6 c.c.) (Calc. for 1 atomic proportion of oxygen, 11·6 c.c.) the oxidised solution showed $[\alpha]_{5780}^{18°}$ +87° (initial value).

Both methods of oxidation appear to have the same effect upon solutions containing the "active" form produced from mannosaccharodilactone by the agency of alkaline reagents, and from the nature of the mutarotation of a freshly prepared oxidised solution the active principle would appear to be of the nature of a lactone.

Methylation of Mannosaccharodilactone.—(A) *With silver oxide and methyl iodide.* Finely ground mannosaccharodilactone (4 g.) was boiled with methyl iodide in the presence of silver oxide in the usual manner, the process of methylation being facilitated by addition of just sufficient methyl alcohol to dissolve the dilactone. After the first methylation in this way, the product, isolated by means of methyl alcohol, was soluble in methyl iodide. After three more methylations with Purdie's reagents (no methyl alcohol added) the syrupy product, the methoxyl content of which had reached a maximum, was distilled, giving: Fraction I (0·48 g.), b. p. (bath temp.) 130°/0·01 mm., $n_D^{18°}$ 1·4400, $[\alpha]_D^{18°}$ −9·5° in water (*c*, 0·8); equiv., 123 (Found: OMe, 56·0%). This fraction was mainly methyl dimethyl *meso*tartrate, for upon treatment with methyl-alcoholic ammonia it gave dimethyl *meso*tartramide, m. p. and mixed m. p. 258° (after recrystallisation from hot water) (Found: OMe, 35·2. Calc. for $C_6H_{12}O_4N_2$: OMe, 35·2%). Fraction II (1·3 g.), b. p. (bath temp.) 140°/0·10 mm., $n_D^{19°}$ 1·4645, $[\alpha]_D^{18°}$ −16·5° in water (*c*, 0·7); equiv., 118 (Found: OMe, 47·5, and after saponification by heating with barium hydroxide, OMe, 30·8%). Fraction III (0·6 g.), b. p. (bath temp.) 140—142°/0·01 mm., $n_D^{19°}$ 1·4675, $[\alpha]_D^{18°}$ −15° in water (*c*, 1·0); equiv., 113 (Found: OMe, 46·7, and after saponification with barium hydroxide, OMe, 29·6%). When an aqueous solution of this fraction was treated with a slight excess of sodium hydroxide in the cold, the rotation changed immediately from $[\alpha]_D^{18°}$ −15° to −26°. Fraction IV (1·82 g.), b. p. (bath temp.) 142—145°/0·01 mm., $n_D^{19°}$ 1·4670, $[\alpha]_D^{18°}$ −7° in water (*c*, 0·7); equiv., 108 [Found: OMe, 45·6, and after saponification by heating with barium hydroxide, OMe, 31·0%. $C_9H_{12}O_6$ (2 : 5-*dimethyl* Δ⁴-*mannosaccharolactone methyl ester*) requires OMe, 43·1%; equiv., 108, and after the loss of one ester OMe group by saponification the residual methoxyl value should be 28·7%]. The high methoxyl values of fractions II, III, and IV are due to the presence of some 2 : 3 : 5 (or 2 : 4 : 5)-trimethyl mannosaccharolactone methyl ester, because treatment of small portions of each fraction with methyl-alcoholic ammonia afforded (in small yield) the crystalline *diamide* of 2 : 3 : 5 (or 2 : 4 : 5)-trimethyl mannosaccharic acid, m. p. 258°, $[\alpha]_D^{18°}$ −40° in water (*c*, 1·1). This diamide showed a negative Weerman test for α-hydroxy-amides when the test was performed under the conditions used previously (Smith, J., 1939, 753) (Found: C, 43·2; H, 7·0; N, 11·4; OMe, 37·9. $C_9H_{18}O_6N_2$ requires C, 43·2; H, 7·2; N, 11·2; OMe, 37·2%).

Each of the fractions II, III, and IV, consisting mainly of 2 : 5-dimethyl Δ⁴-mannosaccharo-3 : 6-lactone 1-methyl ester, showed in aqueous solution an intense selective absorption band at λ 2290 A. (ε, 5,000; *c*, 5 mg.%) (see Fig. 2), disappearing on addition of sodium hydroxide, reappearing slowly on acidification.

(B) *With diazomethane.* A solution of mannosaccharodilactone (7 g.) in dry methyl alcohol was cooled in a freezing mixture and treated with excess of an ice-cold ethereal solution of diazomethane for 2 days at −5°. Removal of the solvent under diminished pressure gave a thick syrup, which was non-reducing to Fehling's solution and showed $[\alpha]_D^{18°}$ −24° in 50% aqueous methyl alcohol (*c*, 1·3) (Found: OMe, 36·6%). The syrupy product showed an intense absorption band at λ 2300 A.; ε, 5,000 (*c*, 5 mg.% in water).

After three treatments with ethereal diazomethane, the product had a methoxyl value of 40·5% but it was still insoluble in ether. It was therefore dissolved in a small volume of methyl alcohol and re-treated with ethereal diazomethane for 3 days at −5°. After this fourth treatment the product had $n_D^{20°}$ 1·4795 (Found: OMe, 41·1%). The reaction with ethereal diazomethane was carried out a fifth time for 4 days at −5°. The product was isolated by removal of the solvent and distilled, giving Fraction I (0·3 g.), b. p. (bath temp.) 180°/0·02 mm., $n_D^{18°}$ 1·4934; OMe, 44·3%. The distillate crystallised spontaneously, and after trituration with aqueous ethyl alcohol to remove adhering syrup, followed by recrystallisation from ethyl alcohol, the crystals had m. p. 140° (Found: C, 52·1; H, 6·5; OMe, 49·8. Calc. for $C_{10}H_{14}O_6$: C, 52·2; H, 6·1; OMe, 53·9%). An ethyl-alcoholic solution of the crystals showed no appreciable rotation; they were insoluble in water, and decolorised bromine water. In 50% aqueous ethyl alcohol the crystalline substance showed an intense band at λ 3180 A. (ε, 17,000; *c*, 2 mg.%); no change in the position or intensity of the band occurred on making the solution alkaline. The material was unaffected by treatment with methyl-alcoholic ammonia.

Fraction II (2·5 g.) had b. p. (bath temp.) 180°/0·02 mm., $n_D^{18°}$ 1·4870, $[\alpha]_D^{18°}$ −20° in water (*c*, 1·0); OMe, 43·9, and after saponification with barium hydroxide, OMe, 25·0%, corresponding (by diff.) to an ester methoxyl content of 18·9%.

Fraction III (3·5 g.) had b. p. (bath temp.) 180°/0·02 mm., $n_D^{18°}$ 1·4820, $[\alpha]_D^{18°}$ −18° in water (*c*, 1·2) (Found: OMe, 42·3; and, after saponification, OMe, 24·2%, corresponding to an ester methoxyl content of 18·1%).

Both fractions II and III, consisting mainly of 2 : 5-dimethyl Δ⁴-mannosaccharolactone methyl ester, had an intense band at λ 2290 A. (ε, 6,500; *c*, 5 mg.%). The presence of a small amount of 6-carbomethoxy-3-methoxy-α-pyrone (see below) can sometimes be detected in such fractions of 2 : 5-dimethyl Δ⁴-mannosaccharo-3 : 6-lactone 1-methyl ester by an absorption band at *ca.* λ 3050 A. (ε, 2,000; *c*, 5 mg.%). Aqueous solutions of both fractions reacted with chlorine, decolorised bromine and alkaline potassium permanganate, and reacted with iodine (3 atoms approx.) in alkaline solution.

Further distillation of the residue in the flask after removal of fractions I, II, and III gave a crystalline distillate (0·1 g.), b. p. 180°/0·01 mm., m. p. 212° (after recrystallisation from aqueous ethyl alcohol). This substance was sparingly soluble in cold water and cold alcohol, soluble in acetone, and insoluble in ether and light petroleum. It proved to be identical with 6-carbomethoxy-3-methoxy-α-pyrone, with which it gave no depression of the m. p. (Haworth, Hirst, and Jones, J., 1938, 710; Schmidt, Dippold, and Zeisser, *Ber.*, 1937, **70**, 2402) (Found: C, 52·4, H, 4·1; OMe, 31·5. Calc. for $C_8H_8O_5$: C, 52·2; H, 4·4; OMe, 33·7%). Aqueous solutions of this α-pyrone exhibit a strong band at λ 3070 A. (ε, 12,000; *c*, 2 mg.%), moving to λ 3680 A. (ε, 14,000; *c*, 2 mg.%) upon addition of sodium hydroxide (see Fig. 3).

(C) *With diazomethane followed by Purdie's reagents.* A solution of mannosaccharodilactone (5 g.) in methyl alcohol was treated for 2 days at −5° with an excess of an ethereal solution of diazomethane. The persistent yellow colour of the solution after this time indicated that excess of diazomethane was present. The solution was filtered to remove a small amount of flocculent material, and then evaporated under diminished pressure to give a syrup (5·6 g.) (Found: OMe, 37·0%). The pale yellow syrup had a band at λ 2300 A. (ε, *ca.* 4000; *c*, 5 mg.%).

A solution of this syrup (5·6 g.) in methyl iodide (10 c.c.) was boiled under reflux in the presence of silver oxide, fresh additions of which were made every hour; the reaction mixture was frequently shaken to promote methylation. After removal of the solvent the residue was extracted several times with acetone, filtered, and concentrated to a syrup (Found: OMe, 40·2, after two Purdie treatments; 43·0, after 4. Calc. for $C_9H_{12}O_6$: OMe, 43·1%). The syrupy product was purified by extraction with ether and then distilled, giving Fraction I (0·3 g.), b. p. (bath temp.) 145°/0·04 mm., $n_D^{21°}$ 1·4736; OMe, 44·2%. This pale yellow liquid crystallised spontaneously; after trituration with ethyl alcohol to remove adhering syrup, the crystals had m. p. 140°, and were identical with those obtained by the methylation as in (A), above. Fraction II (3·6 g.), b. p. (bath temp.) 152—158°/0·04 mm., $n_D^{21°}$ 1·4688; OMe, 43·4%; $[\alpha]_D^{18°}$ −25° in water (*c*, 1·0), consisting mainly of 2 : 5-dimethyl Δ⁴-mannosaccharolactone methyl ester, showed in aqueous solution an intense band at λ 2290 A. (ε, 4000; *c*, 5 mg.%).

Ozonisation of 2 : 5-*Dimethyl* Δ⁴-*Mannosaccharo*-3 : 6-*lactone* 1-*Methyl Ester*.—Fractions II, III, and IV of this ester prepared by method (A) were combined, dissolved in glacial acetic acid (20 c.c.), and the solution subjected to the action of a stream of ozonised oxygen at room temperature. Initially, the solution had $[\alpha]_D$ −31° and after 10 hours' ozonolysis it showed $[\alpha]_D$ +8° (constant value). The solution at this stage did not reduce Fehling's solution. Removal of the acetic acid by distillation under diminished pressure gave a syrup, insoluble in water, which was probably the ozonide. Simultaneous addition and distillation of water under reduced pressure decomposed this ozonide, and the syrupy product became readily soluble in water, strongly reducing towards Fehling's solution on gentle warming, and it reacted strongly acid to Congo-red paper; the aqueous distillate contained no glyoxylic acid (tested with albumin and sulphuric acid). Oxalic acid was present in the syrupy product, for when an aqueous solution of a small portion of it was made just alkaline with sodium hydroxide and then acidified with acetic acid it gave a white precipitate of calcium oxalate upon addition of calcium chloride. The syrupy product showed a negative test for glyoxylic acid.

A solution of the syrup (obtained from the ozonolysis as described above) in water (15 c.c.) was neutralised with barium carbonate and filtered to remove the barium oxalate and the excess of the barium carbonate. The filtrate (still strongly reducing to Fehling's solution on slight warming) was then treated with bromine (2 c.c.) at room temperature for 2 days, the solution then no longer reducing Fehling's solution. The excess of the bromine was removed by aeration; the solution was neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and then evaporated to dryness under diminished pressure. The glassy product (an acid barium salt) reacted acid to Congo-red paper and gave a positive test for barium.

This non-reducing acid barium salt of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid was boiled for 8 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness under reduced pressure. Distillation of the syrup thus obtained gave: Fraction I (0·86 g.), b. p. (bath temp.) 105—110°/0·01 mm., $n_D^{17°}$ 1·4440, $[\alpha]_D^{18°}$ −43° in methyl alcohol (*c*, 0·8) [Found: OMe, 51·6%; equiv., 105. $C_7H_{12}O_6$ (*methyl* 3-*hydroxy*-2-*methoxyerythrosuccinate*) requires OMe, 48·5%; equiv., 96]. Fraction II (0·56 g.), b. p. (bath temp.) 125°/0·01 mm., $n_D^{17°}$ 1·4470, $[\alpha]_D^{18°}$ −32·4° in methyl alcohol (*c*, 0·7) (Found: OMe, 54·2%; equiv., 131). Fraction III (0·27 g.), b. p. (bath temp.) 135°/0·01 mm., $n_D^{17°}$ 1·4500, $[\alpha]_D^{18°}$ −21·5° in methyl alcohol (*c*, 0·4) (Found: OMe, 52·0%; equiv., 130. Calc. for $C_{10}H_{16}O_7$ (trimethyl mannosaccharolactone methyl ester): OMe, 50·0%; equiv., 124].

Examination of Fraction I *and Identification of Methyl* 3-*Hydroxy*-2-*methoxy*-l-*erythrosuccinate*.—(a) *Complete methylation of Fraction* I *with Purdie's reagents.* One methylation of Fraction I (120 mg.) with silver oxide and methyl iodide gave methyl dimethoxyerythrosuccinate (methyl dimethyl *meso*tartrate), $n_D^{18°}$ 1·4350. Treatment of this ester with methyl-alcoholic ammonia for 2 days at −5° readily gave the diamide of dimethoxyerythrosuccinic acid (inactive dimethoxysuccinamide), m. p. and mixed m. p. 258° (decomp.) after recrystallisation from water. This amide was optically inactive (Found: C, 40·7; H, 6·3; OMe, 35·3; N, 15·7. Calc. for $C_6H_{12}O_4N_2$: C, 40·9; H, 6·8; OMe, 35·2; N, 15·9%).

(b) *Isolation of* 3-*hydroxy*-2-*methoxy*-l-*erythrosuccinamide.* A solution of Fraction I (250 mg.) in methyl alcohol (3 c.c.), cooled to 0°, was saturated with dry ammonia. After 2 days' keeping at −5°, some crystals of the diamide of 2 : 3 : 5-trimethyl mannosaccharic acid were separated by decantation (m. p. 257°, decomp., after crystallisation from aqueous alcohol). Evaporation of the methyl-alcoholic ammonia solution, decanted from the crystals of the trimethyl mannosaccharamide, gave a crystalline residue which furnished a further small amount of 2 : 3 : 5-trimethyl mannosaccharamide upon crystallisation from aqueous ethyl alcohol. Evaporation of the aqueous ethyl-alcoholic mother-

liquors gave the *amide* of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, m. p. 153° (after recrystallisation from methyl alcohol–ethyl alcohol), showing no appreciable rotation in water (Found: C, 37·1; H, 6·2; OMe, 19·0; N, 17·3. $C_5H_{10}O_4N_2$ requires C, 37·0; H, 6·2; OMe, 19·1; N, 17·3%).

(c) *Isolation of the bismethylamide of 3-hydroxy-2-methoxy-l-erythrosuccinic acid.* Treatment of the remaining portion of Fraction I with methyl-alcoholic methylamine for 2 days at −5°, followed by removal of the solvent, gave a crystalline product in good yield. After recrystallisation from ethyl acetate the *bismethylamide* of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid had m. p. 136°, $[\alpha]_D^{18°}$ +10·7° in water (*c*, 3·2). This bismethylamide proved to be identical with a synthetic specimen of that of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid prepared from *d*-araboascorbic acid (see succeeding paper) (Found: C, 44·3; H, 7·35; OMe, 16·2; N, 14·5. $C_7H_{14}O_4N_2$ requires C, 44·2; H, 7·4; OMe, 16·3; N, 14·7%).

Examination of Fractions II *and* III.—Both these fractions upon treatment with methyl-alcoholic ammonia gave in good yield the *diamide* of 2 : 3 : 5- (or 2 : 4 : 5-)trimethyl mannosaccharic acid, m. p. 258° (decomp.), $[\alpha]_D^{20°}$ −41° in water (*c*, 0·6 after recrystallisation from water) (Found: C, 43·2; H, 7·0; OMe, 37·8; N, 11·05. $C_9H_{16}O_6N_2$ requires C, 43·2; H, 7·3; OMe, 37·2; N, 11·2%). This diamide gave a negative Weerman reaction for α-hydroxy-amides.

In a preliminary experiment, the syrupy acid product obtained from the ozonisation of the syrup containing 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester and some 2 : 3 : 5-trimethyl saccharolactone methyl ester was treated directly with methyl-alcoholic ammonia. Since the ozonolysis product contained oxalic acid, the monomethyl ester of hydroxymethoxy-*l*-erythrosuccinic acid, and trimethyl mannosaccharolactone methyl ester, there were obtained ammonium oxalate, 2 : 3 : 5-trimethyl mannosaccharamide, m. p. 254°, and the *half-amide ammonium* salt of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, m. p. 181° (decomp.) (after crystallisation from methyl alcohol) (Found: C, 33·5; H, 6·8; OMe, 17·6; N, 14·8. $C_5H_{12}O_5N_2$ requires C, 33·3; H, 6·7; OMe, 17·2; N, 15·6%). The last, however, was of no use for identification purposes, since it proved difficult to purify.

Ozonisation of the 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester prepared by method (B). A solution of Fractions I and II (5·7 g.) in glacial acetic acid (30 c.c.) was subjected to ozonolysis for 17 hours at room temperature; $[\alpha]_D$ −41° (initial value); −32° (after ¾ hour); −12° (after 3½ hours); −5° (after 5 hours); −2·5° (after 6½ hours); +2·5° (after 9½ hours; constant value for 7½ hours). The solution was freed from acetic acid by distillation under diminished pressure, and the ozonide was decomposed by simultaneous addition and distillation of water. All traces of acetic acid were removed by evaporation to dryness. The acid product contained oxalic acid and was strongly reducing to Fehling's solution. The residue was dissolved in water (20 c.c.), and the solution neutralised with barium carbonate, and filtered to remove barium oxalate and excess barium carbonate. Evaporation of the filtrate afforded a pale yellow glassy solid which contained barium and reduced Fehling's solution actively on warming. This product was dissolved in water (50 c.c.) and divided into two portions D (20 c.c.) and E (30 c.c.).

Treatment of portion D with bromine. This solution containing the barium salt of the aldehydic acid (2-methyl *l*-erythuronic acid) was oxidised with bromine (1 c.c.) for 2 days at room temperature, the solution then no longer reducing Fehling's solution. The excess of bromine was removed by aeration, the solution neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and then evaporated to dryness under reduced pressure. The dry acid barium salt was boiled for 8 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). Neutralisation of the mineral acid with silver carbonate, followed by removal of the solvent, gave a fairly mobile liquid which distilled giving: Fraction I, methyl 3-hydroxy-2-methoxy-*l*-erythrosuccinate (0·53 g.), b. p. (bath temp.) 110°/0·02 mm., $n_D^{19°}$ 1·4440, $[\alpha]_D^{18°}$ −31° in water (c, 0·8), $[\alpha]_D^{18°}$ −49·5° in methyl alcohol (*c*, 0·5) [Found: OMe, 52·3; OMe, 15·6 (after saponification with barium hydroxide; equiv., 93. Calc. for $C_7H_{12}O_6$: OMe, 50·0%; equiv., 96. After saponification it would have OMe, 16·7%]. Treatment of this fraction with methyl-alcoholic methylamine gave the crystalline bismethylamide of 3-hydroxy-2-methoxyerythrosuccinic acid, m. p. 136°, $[\alpha]_D^{18°}$ +10° in water (*c*, 1·0) (after recrystallisation from ethyl acetate). It gave no depression of the m. p. when mixed with that prepared above. Fraction II (0·3 g.), b. p. (bath temp.) 140—150°/0·02 mm., $n_D^{18°}$ 1·4530. This fraction failed to give a crystalline bismethylamide, but upon treatment with methyl-alcoholic ammonia it yielded the crystalline diamide of trimethyl mannosaccharic acid, m. p. and mixed m. p. with specimen obtained above 259° (decomp.) (after recrystallisation from water).

Treatment of portion E. Examination of the two formulæ (VI) and (XII) proposed originally for the 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester shows that the former gives upon ozonisation the aldehydic acid (VII) (2-methyl *l*-erythuronic acid); upon reduction of its aldehydic group, this acid should give 2-methyl erythronic acid, the amide of which would show a negative Weerman test for α-hydroxy-amides. On the other hand, the substance having formula (XII) would give rise to 3-methyl *l*-erythronic acid, which is an α-hydroxy-amide and would be expected to give a positive Weerman test. With these considerations in mind, portion E (30 c.c.) was subjected to the action of 2½% sodium amalgam (100 g.) during 8 hours with vigorous stirring. A subsequent search for the monomethyl *l*-erythronic acid, however, was unsuccessful.

Hydrogenation of 2 : 5-Dimethyl Δ^4-Mannosaccharo-3 : 6-lactone 1-Methyl Ester.—A solution of this ester [0·5 g. of fraction II prepared by method (C) above] in ethyl alcohol (50 c.c.) was shaken in an atmosphere of hydrogen in the presence of platinum oxide (0·2 g.) during an hour at slightly more than 1 atm. pressure. The absorption of hydrogen then appeared to be complete. The hydrogen was expelled from the reduction vessel with a stream of carbon dioxide. The solution was filtered, and evaporated to dryness under reduced pressure. The syrupy product still had an absorption band in aqueous solution at λ 2290 A., the intensity of which indicated that approximately 75% of the unsaturated compound had been reduced. In order to complete the reduction the syrup was dissolved in ethyl alcohol (60 c.c.) and shaken with hydrogen for 8 hours in the presence of a palladium–charcoal catalyst. This hydrogenation gave a neutral syrup which showed no absorption in aqueous solution (tested at 40 mg./100 c.c.). Distillation of the syrup gave: fraction I (0·1 g.), b. p. (bath temp.) 144°/0·03 mm., $n_D^{20°}$ 1·4400, which yielded the diamide of 2 : 3 : 5-trimethyl mannosaccharic acid, m. p. 257° (decomp.), $[\alpha]_D^{19°}$ −38° in water (*c*, 0·7); fraction II (0·4 g.), b. p. (bath temp.) 160°/0·03 mm., $n_D^{20°}$ 1·4524, $[\alpha]_D^{20°}$ −4° (*ca.*) in water (*c*, 1·0) [Found, by heating with sodium hydroxide: equiv., 113. $C_9H_{14}O_6$ (2 : 5-dimethyl 4-deoxymannosaccharo-3 : 6-lactone 1-methyl ester) requires equiv., 114]. Treatment of this fraction with methyl-alcoholic ammonia for 2 days at −5° gave the *diamide* of 2 : 5-dimethyl 4-deoxy-mannosaccharic acid, which crystallised upon removal of the solvent; m. p. 187°, $[\alpha]_D^{19°}$ −74° in water (*c*, 2·6) (after crystallisation from ethyl alcohol) (Found: C, 42·5; H, 7·3; OMe, 28·15. $C_8H_{16}O_5N_2$ requires C, 42·6; H, 7·3; OMe, 28·2%).

An ice-cold solution of this diamide (20 mg.) in water (0·4 c.c.) was treated with a solution of 1·5N-sodium hypochlorite (0·2 c.c.) for 30 mins. at 0°. Removal of the excess of the sodium hypochlorite was effected by careful addition of a few drops of a fairly concentrated solution of sodium thiosulphate (tested with acidified starch–iodide paper), and this was followed by the addition of solid sodium acetate (0·2 g.) and semicarbazide hydrochloride (0·04 mg.). No hydrazodicarbonamide was obtained. A control experiment carried out under the same conditions with gluconamide (20 mg.) readily gave a positive Weerman test, indicated by the ready formation of hydrazodicarbonamide (m. p. and mixed m. p. 258°) in good yield within a minute of the addition of the semicarbazide hydrochloride.

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