141. *Lactones of Glucosaccharic Acid. Part I. 2 : 5-Dimethyl Δ^4-Glucosaccharo-3 : 6-lactone 1-Methyl Ester, an Analogue of Ascorbic Acid.*

By F. Smith.

Methylation of glucosaccharo-1 : 5-3 : 6-dilactone (IV) either with Purdie's reagents or with diazomethane effects isomerisation and gives crystalline 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VII). This has also been obtained from glucosaccharo-3 : 6-lactone (III), silver saccharate (V), and glucurone (VI) (Smith, *J. Soc. Chem. Ind.*, 1938, **57**, 450).

The structure of (VII) has been proved by the observation that upon ozonisation it yields oxalic acid and the monomethyl threuronic acid (VIII), the latter being characterised by conversion successively into hydroxymethoxythreosuccinic acid (IX) and the corresponding diamide (X).

Hydrogenation of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VII) and the corresponding acid (XIII) has been carried out, giving 2 : 5-dimethyl 4-deoxyglucosaccharo-3 : 6-lactone 1-methyl ester (XIX) and 2 : 5-dimethyl 4-deoxyglucosaccharo-3 : 6-lactone (XIV) respectively. The diamide (XX) of 2 : 5-dimethyl 4-deoxyglucosaccharic acid was found to give a negative Weerman test for α-hydroxy-amides, thus proving the presence of a 3 : 6-lactone ring in (VII).

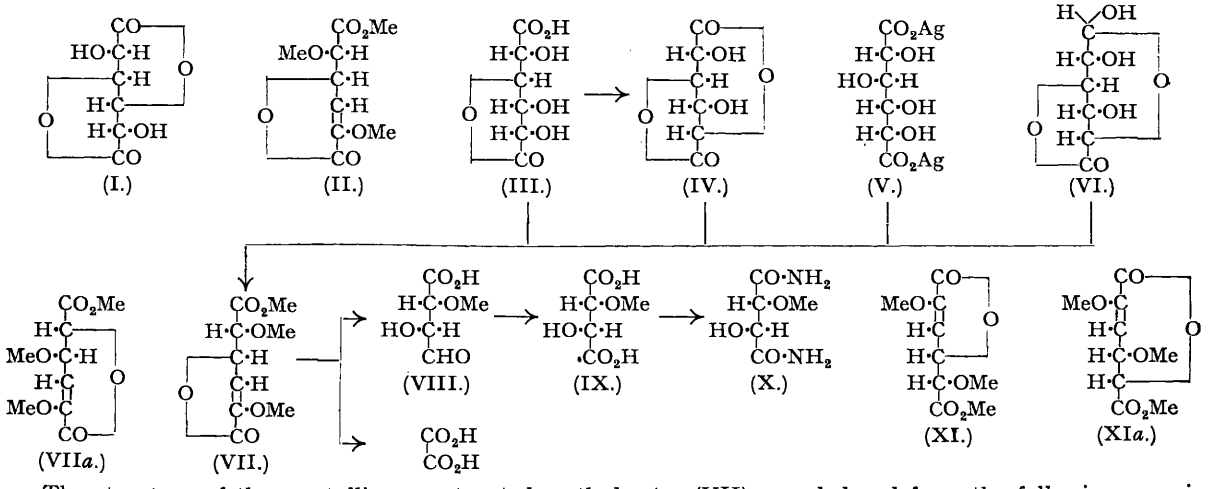
It is suggested that the unsaturated methyl ester (VII) is related to the crystalline substance, obtainable from glucosaccharo-1 : 5-3 : 6-dilactone by the agency of alkaline reagents, which is responsible for the remarkable reducing properties displayed by the dilactone of saccharic acid in alkaline solution or in acid solution after pre-treatment with alkaline reagents.

Methylation of mannosaccharo-1 : 4-3 : 6-dilactone (I) either with silver oxide and methyl iodide or with diazomethane causes molecular isomerisation and there is produced an analogue of ascorbic acid, namely, 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (II), the structure of which has been elucidated (Haworth, Heslop, Salt, and Smith, this vol., p. 217).

Extension of the investigations to glucosaccharodilactone (IV), first prepared by Rehorst and Scholz (*Ber.*, 1936, **69**, 524) from an impure form of glucosaccharo-3 : 6-lactone (III) (Sohst and Tollens, *Annalen*, 1888, **245**, 1, 19), has shown that (IV) behaves similarly to mannosaccharodilactone (I), resembling it in that both acid groups are internally esterified and in the important respect that it displays reducing properties in alkaline solution, or in acid solution after previous treatment with alkaline reagents. It was expected that these two dilactones would resemble one another in their general reactions, and it seemed highly probable that the novel explanation of the unusual reducing action exhibited by (I) would also serve to explain the reducing properties of (IV). Furthermore, it seemed that the isomerisation of the dilactones (I) and (IV) with alkali, resulting in the development of reducing activity, was bound up with the isomerisation of the dilactones brought about by reagents used in methylation. It was important therefore to examine the nature of the product obtained by methylation of (IV).

Glucosaccharodilactone (IV) behaves similarly to (I) when treated either with Purdie's reagents or with

diazomethane, since it affords a crystalline *compound* to which the structure (VII) has been ascribed. This substance, which may be regarded as arising by simultaneous isomerisation and methylation of the dilactone (IV), can also be obtained by the action of silver oxide and methyl iodide upon glucosaccharo-3 : 6-lactone (III), silver saccharate (V), and glucurone (VI). It is also produced from (III) and saccharic acid by the agency of diazomethane (cf. Schmidt, Dippold, and Zeiser, *Ber.*, 1937, **70**, 2402), and, as will be shown in later communications, (VII) is also derivable from glucosaccharo-1 : 4-lactone, from its 6-methyl ester, from glucosaccharo-3 : 6-lactone 1-methyl ester, and from a new dilactone of saccharic acid prepared from glucosaccharo-1 : 4-lactone.



The structure of the crystalline unsaturated methyl ester (VII) was deduced from the following experimental facts. The substance had a formula $C_9H_{12}O_6$ and it contained three methoxyl groups. Its unsaturated

FIG. 1.

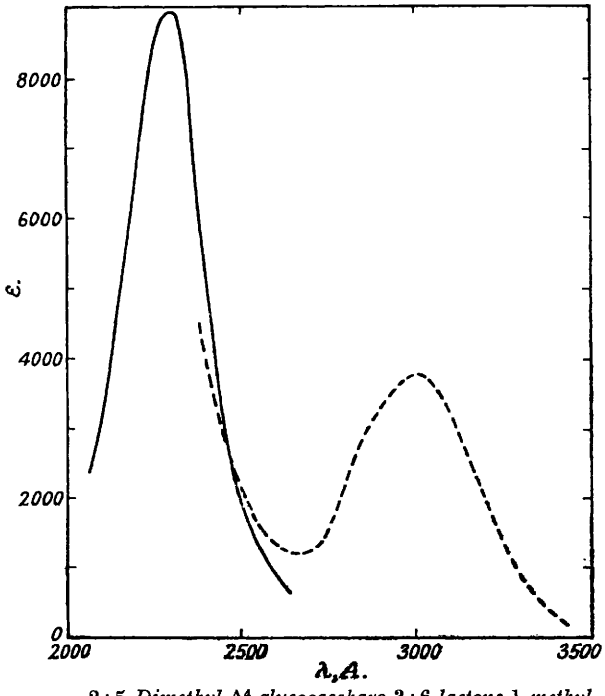
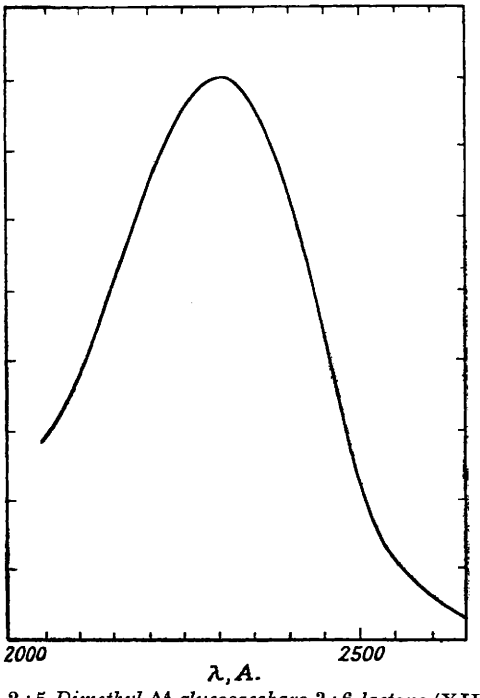


FIG. 2.



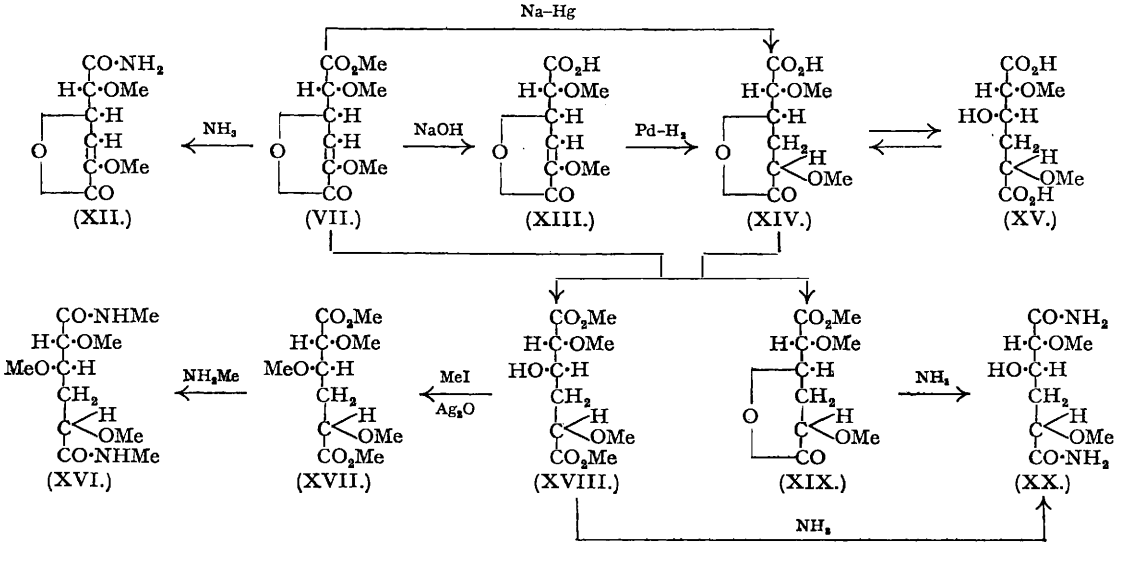
——— 2 : 5-*Dimethyl* Δ^4-*glucosaccharo*-3 : 6-*lactone* 1-*methyl ester* (VII) *in water* (3·3 *mg.*/100 *c.c.*).
------ (VII) *in dilute sodium hydroxide* (10 *mg.*/100 *c.c.*).

2 : 5-*Dimethyl* Δ^4-*glucosaccharo*-3 : 6-*lactone* (XIII) *in water* (3·3 *mg.*/100 *c.c.*).

character was shown by a selective absorption band at λ 2290 A. (see Fig. 1) and by its immediate reaction with chlorine, bromine, and alkaline potassium permanganate. In alkaline solution it reacted with three atomic proportions of iodine. It thus very closely resembled 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (II) (Haworth, Heslop, Salt, and Smith, *loc. cit.*). An aqueous solution of the substance

reacted neutral to litmus, but upon warming it combined with 2 equivs. of sodium hydroxide, and in the process 1 mol. of methyl alcohol was eliminated. Hence, the molecule must contain two potentially acidic groups one of which is probably a carbomethoxy-group, and in consequence the other two methyl residues must be etheric in character. Since the substance (VII) reacted with approximately two atomic proportions of chlorine, the presence of one double bond was indicated; this was proved later (see below) by the fact that hydrogenation of (VII) saturates the double bond, producing a dihydro-derivative. Ozonisation of (VII) proceeded smoothly in glacial acetic acid, giving an ozonide which upon decomposition with water afforded oxalic acid and the aldehydic acid (VIII). The latter reduced Fehling's solution actively upon gentle warming but did not restore the colour to Schiff's reagent. The formation of oxalic acid (a 2-carbon fragment) and the aldehydic acid (a 4-carbon fragment) proved that the double bond must be located either between C_2 and C_3 or between C_4 and C_5. Furthermore, the formation of a new carboxyl group and an aldehydic group by the decomposition of the ozonide indicates that the two carbon atoms linked by the double bond must have attached to them a hydrogen atom and a methoxyl group; the system ·CH:C(OMe)· which affords the ·CHO and the new CO_2H group must therefore be present in (VII). Moreover, since oxalic acid and not glyoxylic acid is produced by ozonolysis, it is clear that the methoxy-group, attached to one of the carbon atoms engaged by the ethylenic link, must be joined to that carbon atom adjacent to a terminal CO group which is potentially a carboxyl group as in the system ·CH:C(OMe)·CO·. This grouping also explains the formation of the 4-carbon fragment aldehydic acid. It is plain therefore that the methoxy-group in the system ·CH:C(OMe)·CO· must be attached either to C_3 or to C_5. Oxidation of the aldehydic acid (VIII) with bromine gave hydroxymethoxythreosuccinic acid (IX), identified as its diamide (X). The existence of a hydroxyl group in this 4-carbon fragment reveals the presence of a ring system in the original unsaturated methyl ester (VII). The isolation and characterisation of the acid (IX) as a derivative of threose establishes the configuration of the groups attached to two of the carbon atoms and at the same time accounts for the third methyl group, the other two having already been located, one in the carbomethoxy-group and the other either at C_2 or at C_5 in the grouping ·CH:C(OMe)·CO·. Since the hydroxymethoxysuccinic acid proved to be a derivative of threo- and not of erythro-succinic acid, it is evident that the lactone ring of (VII) must engage C_6 and not C_1, because if the lactone ring did engage C_1 and a hydroxyl group either at C_4 or at C_5 as in (XI) and (XI*a*) then ozonisation followed by oxidation would afford a hydroxymethoxy-derivative of erythro- and not threo-succinic acid (see Haworth, Heslop, Salt, and Smith, *loc. cit.*).

These experimental facts can be explained by the two formulæ (VII) and (VII*a*) and no others, but the evidence at this stage did not allow a choice to be made between these two. The Weerman test (*Rec. Trav. chim.*, 1917, **36**, 16), which has repeatedly proved of high diagnostic value when performed correctly (Ault, Haworth, and Hirst, J., 1934, 1722; cf. Micheel, *Z. physiol. Chem.*, 1933, **218**, 280), was applied in this case to decide which of (VII) and (VII*a*) was correct. This test, which detects a free α-hydroxy-group in an amide without fail (Haworth, Peat, and Whetstone, J., 1938, 1975), can be applied to di- as well as to mono-amides (Smith, J., 1939, 1724; Luckett and Smith, J., 1940, 1106). It will be seen that if formula (VII) is correct, then opening of the lactone ring would liberate a hydroxyl group in the β-position, whereas in the case of formula (VII*a*) the hydroxy-group set free upon the formation of a diamide would be in the α-position and so would be detected by the Weerman test. Treatment of the unsaturated methyl ester with ammonia gave, however, a mono- and not a di-amide, and since this *monoamide* has a strong absorption band at λ 2300 A., characteristic of the original unsaturated ester, it was still believed to possess the unsaturated lactone ring system as in (XII). Therefore the Weerman test could not be applied to this monoamide (XII).



The correct solution to the problem, however, arose during the course of the following series of experiments. Treatment of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone methyl ester (VII) with a warm solution of sodium hydroxide effects saponification and hydrolysis and there is produced mainly the disodium salt of 2 : 5-dimethyl Δ^4-glucosaccharic acid (see Experimental), which upon acidification affords initially the corresponding dibasic acid. The latter undergoes lactonisation in acidified aqueous solution even at room temperature, giving 2 : 5-*dimethyl* Δ^4-*glucosaccharolactone* (XIII), a crystalline substance which reacts strongly acid to Congo-red paper; it is structurally related to the original unsaturated ester and, like the latter, shows a strong selective absorption band at λ 2290 A. (Fig. 2). The lactone (XIII) reacts directly at room temperature with 1 equiv. of sodium hydroxide, giving a monosodium salt which shows a band at λ 2290 A. and therefore still contains the unsaturated lactone ring system. The monosodium salt is thus analogous to the monoamide (XII). Upon warming with excess of sodium hydroxide, the lactone (XIII) reacts with 2 equivs. of alkali, giving a salt which shows no absorption of ultra-violet light. This observation would appear to indicate that the 5-atom lactone ring as well as the unsaturated grouping is necessary for selective absorption of light (cf. Herbert, Hirst, Percival, Reynolds, and Smith, J., 1933, 1270; Haworth, Hirst, and Smith, J., 1934, 1556). Hydrogenation of the acid lactone (XIII) by means of palladium–charcoal gave crystalline 2 : 5-*dimethyl* 4-*deoxyglucosaccharolactone* (XIV); this was also obtained by reduction of the methyl ester (VII) with sodium amalgam, followed by treatment with dilute sulphuric acid. Esterification of the saturated acid lactone (XIV) with methyl-alcoholic hydrogen chloride yielded the corresponding crystalline *methyl* ester (XIX) (m. p. 77°) together with the open-chain *dimethyl* ester (XVIII) (m. p. 89°). These methyl esters, also obtainable from the unsaturated methyl ester (VII) by hydrogenation in the presence of either a palladium–charcoal catalyst or Raney nickel, are stereochemically related, because upon treatment with ammonia both esters give the same *diamide* (XX) (cf. Smith, *J. Soc. Chem. Ind.*, 1938, **57**, 449). Application of the Weerman test to this diamide gave a negative result, thus proving that the hydroxyl group liberated by amide formation was at C_3 and not at C_2. That a hydroxy-group was really present in the diamide (XX) was shown by the fact that methylation of the dimethyl ester (XVIII) with Purdie's reagents affords methyl 2 : 3 : 5-trimethyl 4-deoxyglucosaccharate (XVII), identified by its transformation into a crystalline *bis-methylamide* (XVI). Confirmation of the structure (VII) and not (VII*a*) for the unsaturated ester is therefore provided.

Further evidence in favour of the presence of a γ-lactone ring in 2 : 5-dimethyl Δ^4-glucosaccharolactone (VII) is forthcoming from the fact that the deoxy-lactone (XIV), having $[\alpha]_D$ + 101° in water, can be readily converted by means of alkali followed by acid into the open-chain dibasic acid, 2 : 5-*dimethyl* 4-*deoxyglucosaccharic acid* (XV), having $[\alpha]_D$ − 22° in water. Thus it is seen that the opening of the lactone ring is accompanied by a rotational change in the negative sense. According to Hudson's rule, this indicates that the lactone ring engages a hydroxy-group on the left-hand side of the carbon chain when the latter is written out according to the Fischer convention (as in the formula above). This will apply only if a 3 : 6-lactone ring obtains as in formula (VII); with a structure (VII*a*) which has a 2 : 6-lactone ring, the opening of the corresponding deoxy-compound would be expected to be accompanied by a rotational change towards a more positive value (cf. Schmidt, Dippold, and Zeiser, *loc. cit.*).

Saturation of the double bond of the 2 : 5-dimethyl Δ^4-glucosaccharolactone methyl ester (VII) and the corresponding acid lactone (XIII) introduces further dissymmetry into the molecule, *viz.*, at C_5, but only one compound was characterised and in this the exact stereochemical arrangement of the substituents at C_5 is as yet unknown.

The investigation upon mannosaccharodilactone and the work now described were undertaken primarily in order to determine why the dilactones of manno- and gluco-saccharic acid display striking reducing properties in alkaline solution, or in acid or neutral solution after pre-treatment with alkaline reagents. As a result of the investigations upon mannosaccharodilactone it was tentatively concluded that the compound produced from it by the agency of alkaline reagents, which shows such strong reducing properties, was related structurally to 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (II).

In this connection it is significant that when glucosaccharo-1 : 5-3 : 6-dilactone (IV) is treated with alkaline reagents, there is produced a highly reactive substance (m. p. 159°, $[\alpha]_D$ + 39° in water) isomeric with the dilactone. This crystalline unmethylated substance bears a very close relationship to 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (VII) : its aqueous solutions exhibit a band at λ 2290 A., show a strongly acid reaction to Congo-red paper, and react immediately with chlorine, bromine, and potassium permanganate; they also reduce hot Fehling's solution and react with iodine in alkaline solution. These facts suggested that the compound (m. p. 159°) is structurally related to (VII) and that it may indeed be the unmethylated form of (VII); the chemistry of this isomeric form of the glucosaccharodilactone will constitute the subject of a later communication.

Experimental.

Preparation of 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester* (VII).—(a) *By the action of diazomethane.* (1) Upon glucosaccharo-1 : 5-3 : 6-dilactone. A solution of the dilactone (1 g.), prepared from glucosaccharo-3 : 6-lactone by Rehorst and Scholz's method (*loc. cit.*), in dry methyl alcohol (10 c.c.) was cooled to − 5°, and an excess of an ice-cold ethereal solution of diazomethane added. There was a brisk evolution of nitrogen. After being kept for 12 hours at − 5°, the solvent and the excess of the diazomethane (indicated by the permanent yellow colour) were removed by distillation under diminished pressure. The syrupy product was redissolved in dry methyl alcohol (10 c.c.), and the treatment with ethereal diazomethane repeated. After several hours at − 5° there separated gradually a

crystalline deposit of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester. After 12 hours the crystals were filtered off and purified by recrystallisation from ethyl alcohol–ether, m. p. 89°. A further small quantity was obtained from the diazomethane mother-liquors (yield 0·5 g.).

(2) Upon glucosaccharo-3 : 6-lactone. Crude glucosaccharo-3 : 6-lactone (Kiliani, *Ber.*, 1925, **58**, 2344) was purified by crystallisation from dry ether, but owing to its low solubility a continuous-extraction method was employed; the material extracted in the initial stages was syrupy and failed to crystallise, consisting apparently of glucosaccharo-1 : 4-lactone to which reference will be made in a later communication. Subsequent material extracted, however, readily crystallised, and this product proved to be almost pure glucosaccharo-3 : 6-lactone, m. p. 143—144°, $[\alpha]_D^{18°}$ +42° (initial value in water; *c*, 1·0) (Kiliani, *loc. cit.*).

After two treatments with diazomethane in the manner described above for glucosaccharo-1 : 5-3 : 6-dilactone, the glucosaccharolactone (0·8 g.) yielded 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester, m. p. 89° (0·8 g.) (after one crystallisation from ethyl alcohol–ether).

(b) *By the action of Purdie's reagents.* (1) Upon glucosaccharo-1 : 5-3 : 6-dilactone. The crystalline dilactone (1 g.) was methylated with silver oxide and methyl iodide during 8 hours, sufficient dry acetone being added initially to dissolve the dilactone. The partially methylated material was isolated by means of methyl alcohol and treated a second time with Purdie's reagents, no acetone being now added since the product was soluble in methyl iodide. The syrup thus produced was dissolved in hot ether; on standing, 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester separated, m. p. 89° (yield 0·3 g.).

(2) Upon glucosaccharo-3 : 6-lactone. Finely ground saccharolactone (5 g.) was boiled with silver oxide (20 g.) and methyl iodide (30 c.c.) in the presence of dry acetone (5 c.c.). After two more treatments under these conditions, followed by two methylations with silver oxide and methyl iodide alone, the syrupy product appeared to be completely methylated (methoxyl estimation carried out after each methylation). Distillation of the product gave a colourless liquid, b. p. (bath temp.) 180°/0·02 mm., $n_D^{17°}$ 1·4660, which crystallised spontaneously. After crystallisation from ethyl alcohol–ether, the 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester had m. p. 89° (yield 3 g.).

(3) Upon silver saccharate. A solution of glucosaccharo-3 : 6-lactone (1·92 g.) in water (30 c.c.) was treated with N-sodium hydroxide (10·5 c.c.) at room temperature for 15 minutes. A solution of silver nitrate (4·25 g.) in water (20 c.c.) was added with shaking. The white flocculent precipitate gradually became crystalline, and after 1 hour it was filtered off, washed with a small amount of ice-cold water, and dried in a vacuum (yield 1·8 g.) (Found: Ag, 50·5. Calc. for $C_6H_8O_8Ag_2$: Ag, 51·0%). The powdered silver saccharate (1·7 g.) was boiled with methyl iodide (10 c.c.) in the presence of dry methyl alcohol (1 c.c.) for 2 hours, the separation of silver iodide then appearing to be complete. More methyl iodide (10 c.c.) was added, and the methylation continued in the presence of silver oxide (10 g.) in the usual manner. The product was isolated by means of methyl alcohol and methylated completely by two treatments with silver oxide and methyl iodide (no solvent other than methyl iodide was required). The syrup obtained was dissolved in hot ether and nucleated with a crystal of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester. After being kept for 12 hours at −5°, the crystalline deposit was separated by decantation; one crystallisation from ethyl alcohol–ether gave 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester, m. p. 89° (yield 0·2 g.).

(4) Upon glucurone. Finely powdered glucurone (0·2 g.) was subjected to four methylations with Purdie's reagents, the first being conducted in the presence of dry methyl alcohol (0·5 c.c.) to dissolve the glucurone. The crude, syrupy material isolated by means of methyl alcohol was distilled, giving (i) a mobile liquid, b. p. (bath temp.) 120°/0·03 mm., $n_D^{19°}$ 1·4900, and (ii) a viscous, pale yellow liquid, b. p. (bath temp.) 180°/0·03 mm., which crystallised spontaneously. Removal of adhering syrup from the crystals by trituration with ether–light petroleum, followed by crystallisation from ethyl alcohol–ether, gave 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester, m.p. 89° (yield 60 mg.). This compound was first obtained by Pryde and Williams (*Biochem. J.*, 1933, **27**, 1205) by methylation of glucurone and was termed by them trimethyl glucuralone.

Properties of 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester* (VII).—This *compound*, prepared by any of the methods described above, is a colourless crystalline substance, m. p. 89°, $[\alpha]_D^{19°}$ +98° in water (*c*, 0·7), $[\alpha]_D^{17°}$ +84° in methyl alcohol (*c*, 0·7). It is soluble in water, methyl alcohol, ethyl alcohol, and acetone, much less soluble in ether, and insoluble in light petroleum (Found: C, 50·1; H, 5·7; OMe, 43·1. $C_9H_{12}O_6$ requires C, 50·0; H, 5·6; OMe, 43·1%). An aqueous solution of this ester reacts neutral to litmus paper and it does not reduce boiling Fehling's solution. The unsaturated character of the substance is shown by the fact that it reacts immediately with alkaline potassium permanganate; it reacts slowly with chlorine in aqueous solution (19·7 mg. reacted by direct titration with 7·1 c.c. of 0·02N-chlorine water during 2 hours. Calc. for the addition of 2 Cl: 9·1 c.c.). In a second experiment an aqueous solution of the ester (VII) was allowed to react at room temperature for 15 mins. with an excess of chlorine water. An excess of potassium iodide was added, and the iodine liberated titrated with 0·01N-sodium thiosulphate (20·18 mg. of ester reacted with 11·42 c.c. of 0·02N-chlorine water. Calc. for the addition of 2 Cl: 9·3 c.c.). Bromine water is decolorised, but iodine in potassium iodide is scarcely affected by the ester.

Aqueous solutions of the ester (VII) display strong selective absorption with the head of the band at 2290 A. (ε *ca.* 8500; *c*, 3·3 mg. per 100 c.c.), and upon addition of sodium hydroxide this band disappears, being replaced by one at 3010 A. (ε, *ca.* 3500; *c*, 10 mg. per 100 c.c.) (see Fig. 1).

2 : 5-Dimethyl Δ^4-glucossaccharo-3 : 6-lactone 1-methyl ester is titrated by sodium hydroxide as a γ-lactone; it reacts with 2 equivs. of sodium hydroxide when kept at room temperature for 5—6 hours with excess of the reagent (13·2 mg. reacted with 6·03 c.c. of 0·02N-NaOH, whence equiv. = 109. $C_9H_{12}O_6$ requires equiv., 108). The reaction with sodium hydroxide proceeds to completion in 30 mins. at 50° (14·8 mg. required 6·75 c.c. of 0·02N-sodium hydroxide; equiv. = 109·5). A freshly prepared solution of the ester (VII) in 0·1N-sodium hydroxide shows $[\alpha]_D$ +6° and no change in rotation takes place on warming or keeping the solution. When this alkaline solution, containing the disodium salt, is acidified with a slight excess of sulphuric acid, mutarotation takes place, and the equilibrium value of $[\alpha]_D^{18°}$ +56° is reached in 2—3 hours.

The presence of one ester methoxy-group in the lactone ester was established as follows. A weighed amount of the substance (20—30 mg.) was warmed with 0·3N-barium hydroxide (3 c.c.) for 1½ hours at 55°. The solution was neutralised with carbon dioxide and evaporated to dryness under slightly diminished pressure. These operations were conducted in a Zeisel apparatus, and the methoxyl content of the residue determined in the usual way (Found: residual OMe, 28·3. Calc.: OMe, 28·7%, after the loss of one ester methoxyl group).

The lactone methyl ester (VII) reacts during 30 mins. at room temperature with *ca.* 3 atomic proportions of iodine in alkaline solution. Some iodoform is produced in this reaction (10·84 mg. treated with 20 c.c. of 0·02N-iodine and 12 c.c. of 0·1N-sodium hydroxide, the solution being acidified with dilute sulphuric acid and the excess of the iodine titrated with 0·02N-sodium thiosulphate). A blank experiment was performed at the same time under the same conditions. Iodine equivalent to 7·53 c.c. of 0·02N-sodium thiosulphate was found to have been used up, *i.e.*, 3·005 g.-atoms per g.-mol. Another experiment showed 2·800 g.-atoms.

Treatment of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester with methyl-alcoholic ammonia at −5° for 24 hours. gave a crystalline *monoamide* (XII). Evaporation of the excess of the solvent in a vacuum over calcium

chloride, followed by crystallisation from ethyl alcohol, gave needles, m. p. 193°, $[\alpha]_D^{18°}$ +75° in water (*c*, 0·3). In aqueous solution this amide has an intense band at 2300 A. (ε, *ca.* 7500; *c*, 5 mg. per 100 c.c.), disappearing upon addition of sodium hydroxide. Such facts suggest that this is a monoamide in which the unsaturated 3 : 6-lactone ring is still intact (Found: C, 48·0; H, 5·7; OMe, 30·5; N, 6·8. $C_8H_{11}O_5N$ requires C, 47·8; H, 5·5; OMe, 30·8; N, 7·0%).

Ozonisation of 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester*.—A solution of the lactonic ester (1·94 g.) in glacial acetic acid (30 c.c.) was treated with a stream of ozonised oxygen at room temperature for 5 hours, the rotation changing from $[\alpha]_D$ +55° (initial value) to $[\alpha]_D$ +14° (constant value). The solution was then diluted with an equal volume of water and freed from solvent by evaporation under diminished pressure. The colourless syrup thus produced evidently contained some undecomposed ozonide because a portion remained insoluble in water. Simultaneous addition and distillation of water under reduced pressure was therefore continued until the syrupy product became completely soluble in water. The syrup obtained consisted of a mixture of 2-hydroxy-3-methoxy-*l*-threuronic acid (VIII) and oxalic acid. The presence of the former was detected by the fact that the syrup reduced Fehling's solution actively on warming, and oxalic acid was readily detected by the formation of calcium oxalate. No glyoxylic acid could be detected (tested with albumin and sulphuric acid).

Oxidation of 2-*Hydroxy*-3-*methoxy*-l-*threuronic Acid to Hydroxymethoxythreosuccinic Acid* (IX).—The syrupy product obtained from the previous experiment was dissolved in water (30 c.c.) and neutralised by warming with barium carbonate. The precipitate, consisting of barium oxalate and the excess of barium carbonate, was filtered off and washed with water. The amount of oxalic acid in the precipitate was determined by dissolving it in hot sulphuric acid and titrating it with 0·1N-potassium permanganate solution (yield of oxalic acid, 70%). The combined mixture of filtrate and washings, which still reduced Fehling's solution strongly on warming, was treated with bromine (1 c.c.) for 2 days at room temperature. The solution, now non-reducing to Fehling's solution, was freed from the excess of bromine by aeration, and neutralised with silver oxide. The silver bromide was filtered off, and silver ions in solution were precipitated as sulphide by passing hydrogen sulphide. Removal of the silver sulphide by filtration, followed by removal of the solvent under diminished pressure, gave a pale yellow, glassy product which was the acid barium salt of hydroxymethoxythreosuccinic acid. This salt was acid to Congo-red paper and gave a positive test for barium. It did not reduce Fehling's solution even on prolonged boiling.

Dimethyl Hydroxymethoxythreosuccinate.—The foregoing acid barium salt was esterified by boiling for 8 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was cooled, neutralised with silver carbonate, filtered after the addition of a little charcoal, and then freed from solvent by evaporation under slightly diminished pressure. Distillation of the syrupy product gave methyl hydroxymethoxythreosuccinate, a colourless, mobile liquid (0·55 g.), b. p. (bath temp.) 110°/0·01 mm., $n_D^{18°}$ 1·4410, $[\alpha]_D^{19°}$ +48·5° in methyl alcohol (*c*, 0·8) (Found: equiv., by hydrolysis with hot 0·02N-sodium hydroxide, 99; OMe, 48·5. Calc. for $C_7H_{12}O_6$: equiv., 96; OMe, 48·5%).

Treatment of this threosuccinate with methyl-alcoholic ammonia for 2 days at −5° readily gave the corresponding diamide. Removal of the solvent in a vacuum over anhydrous calcium chloride, followed by recrystallisation from methyl alcohol, gave an almost quantitative yield of hydroxymethoxythreosuccinamide (X), m. p. and mixed m. p. 201° (decomp.), $[\alpha]_D^{20°}$ +115° in water (*c*, 0·5), $[\alpha]_D^{22°}$ +140° in methyl alcohol (*c*, 0·4) (Found: C, 37·1; H, 6·2; OMe, 18·8; N, 17·3. Calc. for $C_5H_{10}O_4N_2$: C, 37·0; H, 6·2; OMe, 19·1; N, 17·3%).

2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* (XIII).—A solution of the 1-methyl ester (VII) (0·5 g.) in 0·1N-sodium hydroxide (52 c.c.) was warmed for 1½ hours at 55°, then treated with 0·1N-sulphuric acid (51·8 c.c.) and evaporated to dryness under diminished pressure at 40°. Several extractions of the solid residue with acetone gave, upon removal of the solvent, crystalline 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone, m. p. 168°, $[\alpha]_D^{16°}$ +73·5° in water (*c*, 0·7) (constant for 3 days). The compound reacted acid to litmus and Congo-red paper; it did not reduce boiling Fehling's solution (Found: C, 47·35; H, 5·0; OMe, 30·8. Calc. for $C_8H_{10}O_6$: C, 47·5; H, 5·0; OMe, 30·7%) (see Schmidt, Dippold, and Zeiser, *loc. cit.*).

The free carboxyl group in this lactonic acid can be titrated directly with sodium hydroxide at room temperature, 1 equiv. being required (14·27 mg. required 3·55 c.c. of 0·02N-sodium hydroxide for neutralisation; whence equiv., 200. $C_8H_{10}O_6$ requires equiv., 202). Upon heating for 1 hour at 50° 2 equivs. of sodium hydroxide are required for neutralisation (14·27 mg. required 7·0 c.c. of 0·02N-sodium hydroxide; whence equiv., 102. $C_8H_{10}O_6$ requires equiv., 101).

The unsaturated character of (XIII) is shown by the fact that it reacted with chlorine (1 mol. reacted by direct titration with 1·5 atoms during 3 hours), decolorised bromine, and also decolorised an alkaline solution of potassium permanganate. It shows an intense absorption band at 2290 A. (ε, *ca.* 8000; *c*, 3·3 mg. per 100 c.c.) (Fig. 2). The addition of 1 equiv. of sodium hydroxide does not affect the absorption appreciably, since the alkali reacts with the carboxyl group while the unsaturated furone ring remains unaffected; this is also supported by the specific rotation of the monosodium salt, $[\alpha]_D$ +64°. After keeping with slight excess of sodium hydroxide, the rotation of the unsaturated acid falls gradually to a constant value of $[\alpha]_D$ +2·5° (*ca.*) and simultaneously the intensity of the band at λ 2290 A. decreases until, at the equilibrium point, when only the disodium salt is present in solution, the solution shows no selective absorption. Acidification liberates the dibasic acid, which undergoes fairly rapid mutarotation, with the formation of the original 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone: $[\alpha]_D$ ±0° (initial value of free dibasic acid in solution); +47·5° (after 17 mins.); +66° (52 mins.); +74° (112 mins.) (constant value). Concurrently with this mutarotation the band at λ 2300 A. appears, and at the equilibrium point the intensity of the band is equal to that of the original value of the 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone.

In alkaline solution this lactone, like the ester, reacts with approximately 3 atomic proportions of iodine (10·53 mg. reacted with 7·05 c.c. of 0·02N-iodine in alkaline solution, *i.e.*, 1 mol. reacts with 2·690 atoms); 3 minutes after the mixing, a small precipitate of iodoform was formed.

Regeneration of the 1-*Methyl Ester* (VII) *from the Lactone* (XIII).—(*a*) *With Purdie's reagents*. One treatment of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone (145 mg.) with silver oxide (0·5 g.) and methyl iodide (5 c.c.) in the presence of methyl alcohol (0·1 c.c.) during 8 hours regenerated the ester in almost quantitative yield, m. p. and mixed m. p. 89° (after isolation by means of acetone followed by recrystallisation from ethyl alcohol–ether).

(*b*) *With acid methyl alcohol*. The lactone (100 mg.) was boiled for 6 hours with 2% methyl-alcoholic hydrogen chloride (10 c.c.). Neutralisation of the mineral acid with silver carbonate, followed by removal of the solvent under slightly diminished pressure, gave the 1-methyl ester (90 mg.), m. p. and mixed m. p. 89° (after one crystallisation from ethyl alcohol–ether).

Hydrogenation of 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* (XIII).—A solution of the lactone (1·0 g.) in dry methyl alcohol (30 c.c.) was shaken for 1 day at room temperature in hydrogen at *ca.* 1·5 atm. in the presence of a palladium–charcoal catalyst. The solution, which was then transparent to ultra-violet light, was filtered and evaporated to dryness. In this manner there was obtained a colourless, viscous syrup which reacted acid to Congo-red paper and showed no selective absorption band (tested at 20 mg. per 100 c.c.). The product crystallised spontaneously and, after removal of adhering syrup from the crystals by trituration with ether–light petroleum followed by recrystallisation

from acetone, the 2 : 5-*dimethyl* 4-*deoxyglucosaccharo*-3 : 6-*lactone* (XIV) (*ca.* 0·1 g.) had m. p. 130°, $[\alpha]_D^{18°}$ +72° in methyl alcohol (*c*, 0·9), $[\alpha]_D$ +101° initial value in water (*c*, 2·0); +90·5° (after 15 hours); +53·5° (74 hours); +22·5° (267 hours) (Found: C, 46·9; H, 6·0; OMe, 30·3; by titration at room temp., equiv., 198; by titration hot, equiv., 98. $C_8H_{12}O_6$ requires C, 47·1; H, 5·9; OMe, 30·4%; equiv., monobasic, 204; equiv., dibasic, 102).

The syrup obtained from the mother-liquors after the separation of the lactone (XIV) was esterified by boiling for 8 hours with 1·2% methyl-alcoholic hydrogen chloride (50 c.c.). Neutralisation of the solution with silver carbonate, followed by removal of the solvent under reduced pressure, gave a colourless liquid (0·89 g.), b. p. (bath temp.) 155—160°/0·02 mm., which crystallised immediately. Several crystallisations from ethyl alcohol–ether yielded *methyl* 2 : 5-*dimethyl* 4-*deoxyglucosaccharate* (XVIII) as colourless plates, m. p. 89° [depressed by admixture with the 1-methyl ester (VII)]; $[\alpha]_D^{18°}$ −13° initial value in water (*c*, 1·0); −2° (after 14 hours); ±0° (46 hours); +5° (264 hours). The compound did not titrate as a lactone, but upon heating to 50°, 8·72 mg. reacted with 3·75 c.c. 0·02N-sodium hydroxide corresponding to equiv., 116 (Calc.: 125) [Found: C, 48·15; H, 7·35; OMe, 49·2; OMe, 23·8 (after hydrolysis with barium hydroxide). $C_{10}H_{18}O_7$ requires C, 48·0; H, 7·3; OMe, 49·6; residual OMe after saponification, 24·8%].

Fractional crystallisation of the crystalline residue obtained from the mother-liquors, after the compound (XVIII) had been separated, gave further crops of crystals which formed needles, m. p. 77°, from ether–alcohol. This compound, more soluble than (XVIII) in ether–alcohol, separated well from ethyl alcohol–ether–light petroleum, and had m. p. 77°. It proved to be 2 : 5-*dimethyl* 4-*deoxyglucosaccharo*-3 : 6-*lactone* 1-*methyl ester* (XIX) and had $[\alpha]_D^{17°}$ +117° in water (*c*, 0·9) (no mutarotation) (Found: C, 49·3; H, 6·4; OMe, 42·6. $C_9H_{14}O_6$ requires C, 49·5; H, 6·5; OMe, 42·6%).

Hydrogenation of 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester* (VII).—(*a*) A solution of the ester (1·2 g.) in dry methyl alcohol (50 c.c.) was shaken at room temperature in hydrogen at approx. 1·5 atm. in the presence of a palladium–charcoal catalyst. The rapid absorption of hydrogen appeared to be complete within 10 minutes, for after a further 2 hours' shaking very little more had been absorbed and the solution showed no selective absorption (tested at 24 mg. per 100 c.c.). The catalyst was filtered off, washed with methyl alcohol, and the solution evaporated to a crystalline residue. Crystallisation from acetone–ether gave mainly methyl 2 : 5-dimethyl 4-deoxyglucosaccharate (XVIII), m. p. 89° alone or in admixture with that obtained by hydrogenation of the lactone (XIII); $[\alpha]_D^{21°}$ −13·5° in water (*c*, 1·2) (Found: OMe, 49·6%).

From the mother-liquors a crystalline residue, consisting of a mixture of (XVIII) (m. p. 89°) and (XIX) (m. p. 77°) was obtained. Two crystallisations from water completely separated these two compounds, the former being much more soluble than the latter. One further crystallisation of the needles which had separated from aqueous solution yielded the ester of m. p. 77°, $[\alpha]_D^{18°}$ +117° in water (*c*, 0·8) (Found: OMe, 42·6%).

(*b*) A solution of the lactone ester (VII) (0·5 g.) in water (50 c.c.) was vigorously shaken in hydrogen at approx. 1·5 atm. in the presence of a Raney nickel catalyst. The uptake of hydrogen was very rapid, and after 45 minutes the solution was filtered, evaporated to dryness under diminished pressure, and the residue purified by extraction with acetone. The crystalline product, m. p. 67°, $[\alpha]_D^{18°}$ +180° in water (*c*, 2·8), was a mixture of the same two esters as were formed in (*a*), above. The product was therefore hydrolysed by heating for 30 mins. at 60° with 2 equivs. of 0·1N-sodium hydroxide. Addition of 2 equivs. of 0·1N-sulphuric acid, followed by evaporation of the solution to dryness under reduced pressure and extraction of the residue with acetone, gave a crystalline substance, m. p. 142°, composed of a mixture of 2 : 5-dimethyl 4-deoxyglucosaccharo-3 : 6-lactone (XIV) and 2 : 5-dimethyl 4-deoxyglucosaccharic acid (XV). It was heated to 5° above its m. p. in an oil-bath for 10 mins. and then allowed to cool. Recrystallisation from water yielded 2 : 5-dimethyl 4-deoxyglucosaccharo-3 : 6-lactone (XIV), m. p. and mixed m. p. (see below) 129°, $[\alpha]_D^{21°}$ +97° in water (*c*, 1·5) (Found: C, 47·2; H, 5·8; OMe, 30·5. Calc. for $C_8H_{12}O_6$: C, 47·1; H, 5·9; OMe, 30·4%).

Reduction of 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester* (VII) *with Sodium Amalgam.*—A solution of the ester (0·7 g.) in water (60 c.c.) was vigorously stirred and treated with small portions of 2·5% sodium amalgam. From time to time the excess of sodium hydroxide was neutralised by careful addition of 4N-hydrochloric acid. After the addition of 140 g. of amalgam (during *ca.* 8 hours), the solution had no selective absorption in the ultra-violet. After separation of the mercury, the solution was acidified with N-sulphuric acid, concentrated under diminished pressure to eliminate carbon dioxide, and neutralised with N-sodium hydroxide. The free organic acid was liberated from its disodium salt by addition of 0·088N-hydrochloric acid (70·0 c.c. Calc.: 73·7 c.c.). The solution was evaporated to dryness under reduced pressure, and the organic acid extracted with acetone. Removal of the solvent under diminished pressure gave a pale yellow syrup (0·65 g.) which crystallised immediately. Recrystallisation from ethyl acetate gave 2 : 5-dimethyl 4-deoxyglucosaccharo-3 : 6-lactone (XIV), m. p. 130° alone or in admixture with that previously obtained, $[\alpha]_D^{20°}$ +102° in water (*c*, 1·0) (Found: OMe, 30·3%).

Esterification of 2 : 5-*Dimethyl* 4-*Deoxyglucosaccharo*- 3 : 6-*lactone.*—The pure crystalline acid lactone (m. p. 130°, 0·3 g.) was boiled for 8 hours with 2% methyl-alcoholic hydrogen chloride (40 c.c.). Neutralisation of the solution with silver carbonate, followed by removal of the solvent, gave methyl 2 : 5-dimethyl 4-deoxyglucosaccharate (XVIII), m. p. and mixed m. p. 89°.

Treatment of Methyl 2 : 5-*Dimethyl* 4-*Deoxyglucosaccharate* (XVIII) *with Ammonia.*—The ester (XVIII) (210 mg.) was treated with methyl-alcoholic ammonia for 15 hours at −5°. The crystals which had separated from the solution were filtered off and washed with ethyl alcohol and ether. After recrystallisation from water the *diamide* (XX) of 2 : 5-*dimethyl* 4-*deoxyglucosaccharic acid* had m. p. 270° (decomp.) (Found: C, 43·7; H, 7·4; OMe, 28·3; N, 12·8. $C_8H_{16}O_5N_2$ requires C, 43·6; H, 7·35; OMe, 28·2; N, 12·7%). When tested under carefully controlled conditions (see p. 512), this diamide gave a negative Weerman test for α-hydroxy-amides. In a control experiment, carried out simultaneously, the diamide of 2 : 3 : 4-trimethyl mucic acid gave, upon treatment with 1·5N-sodium hypochlorite, sodium *iso*cyanate, detected by its transformation into hydrazodicarbonamide upon the addition of semicarbazide.

Evaporation of the solvent from the methyl-alcoholic ammonia solution from which the diamide (XX) had been separated gave a *monoamide*, m. p. 144° (after one crystallisation from ethyl alcohol and one from ethyl alcohol–ether), $[\alpha]_D^{18°}$ −20° in water (*c*, 1·4) (Found: C, 46·1; H, 7·3; OMe, 40·3; N, 5·9. $C_9H_{17}O_6N$ requires C, 45·9; H, 7·3; OMe, 39·6; N, 6·0%).

Treatment of 2 : 5-*Dimethyl* 4-*Deoxyglucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester* (XIX) *with Ammonia.*—The ester (50 mg.; m. p. 77°) was treated for 2 days at −5° with methyl-alcoholic ammonia. The crystals formed were separated by decantation and recrystallised from water, affording the above diamide (XX), m. p. 268° (decomp.) alone or in admixture with that recorded above.

Treatment of Methyl 2 : 5-*Dimethyl* 4-*Deoxyglucosaccharate with Methylamine.*—With methylamine in methyl alcohol the ester (m. p. 89°) gave a *bismethylamide* which readily separated from the methyl-alcoholic methylamine solution; m. p. 221° after crystallisation from ethyl alcohol, $[\alpha]_D^{18°}$ −13·5° in water (*c*, 2·5) (Found: C, 48·6; H, 8·4; OMe, 24·8; N, 11·7. $C_{10}H_{20}O_5N_2$ requires C, 48·4; H, 8·15; OMe, 25·0; N, 11·3%).

Methyl 2 : 3 : 5-*Trimethyl* 4-*Deoxyglucosaccharate* (XVII).—Four treatments of methyl 2 : 5-dimethyl 4-deoxyglucosaccharate (100 mg.) with silver oxide and methyl iodide gave *methyl* 2 : 3 : 5-*trimethyl* 4-*deoxyglucosaccharate* (isolated

after each methylation by means of acetone) as a colourless liquid, b. p. 130°/0·05 mm., $n_D^{23°}$ 1·4406, $[\alpha]_D^{22°}$ −8° in water (*c*, 2·3) (Found: OMe, 56·1; and after hydrolysis with alkali, OMe, 27·0. $C_{11}H_{20}O_7$ requires OMe, 58·7, 35·2%, respectively). Treatment of this methyl ester (XVII) with methylamine in methyl alcohol yielded the *bis-methylamide* (XVI) of 2 : 3 : 5-trimethyl 4-deoxyglucosaccharic acid, m. p. 168° (after crystallisation from acetone), $[\alpha]_D^{20°}$ −10·5° in water (*c*, 3·3) (Found: C, 50·3; H, 8·5; N, 10·4; OMe, 35·5. $C_{11}H_{22}O_5N_2$ requires C, 50·4; H, 8·5; N, 10·7%; OMe, 35·5).

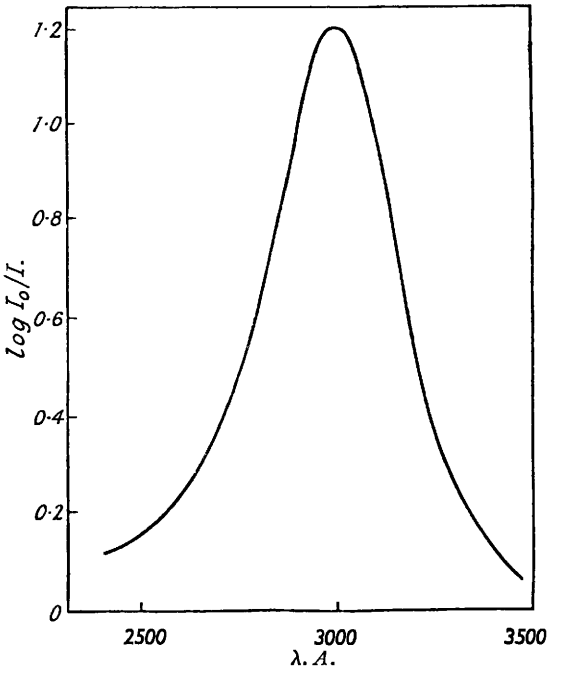
2 : 5-*Dimethyl* 4-*Deoxyglucosaccharic Acid* (XV).—A solution of crystalline methyl 2 : 5-dimethyl 4-deoxyglucosaccharate (0·35 g.) in water (5 c.c.) was heated with N-sodium hydroxide (5 c.c.) for one hour at 55°. The solution was then treated with 0·1N-sulphuric acid (49 c.c.) and evaporated to dryness under reduced pressure. Extraction of the dry crystalline residue with acetone, followed by filtration and removal of the solvent, gave the *acid* (XV), m. p. 161°, $[\alpha]_D^{18°}$ −22° initial value in water (*c*, 1·3), changing in 14 weeks to ±0° (after recrystallisation from acetone or acetone–ether). This reacted acid to Congo-red paper and could be titrated directly with sodium hydroxide solution (Found: C, 43·1; H, 6·3; OMe, 28·2; equiv., 108. $C_8H_{14}O_7$ requires C, 43·25; H, 6·4; OMe, 28·2%; equiv., 111).

The acid (XV) readily lost 1 mol. of water when heated in a capillary tube above its m. p., effervescing and then crystallising upon cooling to form the lactone (XIV), m. p. 130°. The same transformation is readily accomplished by sublimation in a vacuum (0·01 mm.) at 165°, the sublimate being pure 2 : 5-dimethyl 4-deoxyglucosaccharo-3 : 6-lactone, m. p. 131° alone or in admixture with a specimen obtained as on p. 516; $[\alpha]_D^{18°}$ +105° (initial value in water; *c*, 0·7) (Found: OMe, 30·5%).

The Effect of Sodium Hydroxide upon 2 : 5-*Dimethyl* Δ^4-*Glucosaccharo*-3 : 6-*lactone* 1-*Methyl Ester* (VII).—As reported on p. 513, this ester is saponified at 55° to give the free lactone (XIII). Both the ester and the lactone are unsaturated and exhibit intense selective absorption at λ 2300 A. (Figs. 1 and 2). In alkaline solution the lactonic acid is transparent to ultra-violet light, whereas (see p. 514) its ester has a strong band at λ 3010 A. (ε, *ca.* 3500) in alkaline solution (Fig. 1) and a band at λ 2900 A. remains after the alkaline solution has been heated. This curious observation has not yet been studied fully, but the experimental facts would appear to indicate that when 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester is treated with alkaline reagents there is formed, in addition to the main product (the sodium salt of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone), a new compound characterised by a selective absorption band at λ 3010 A. in alkaline solution. Some support for this theory was obtained as follows.

A solution of the lactone ester (VII) (102 mg.) in 0·1N-sodium hydroxide (11·3 c.c.) (solution showed a band at λ 3010 A.) was warmed for 1 hour at 60°, then treated with 0·1N-sulphuric acid (11·2 c.c.) and evaporated to dryness under diminished pressure. Extraction of the dry residue with acetone–ethyl alcohol gave a crude crystalline acid product, showing a band at λ 2900 A. in dilute sodium hydroxide solution (ε, *ca.* 3500; *c*, 10 mg. per 100 c.c.), from which pure 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone could be separated, m. p. and mixed m. p. 168° (no band in sodium hydroxide solution).

FIG. 3.



From a number of preparations of this lactone the yellowish, partly crystalline residues from the mother-liquors were combined. Distillation of these in a vacuum (200—230°/0·03 mm.) gave a pale yellow crystalline sublimate. Three crystallisations from water yielded pale yellow crystals, m. p. 266° (decomp.) (Found: OMe, 30·7. $C_8H_{10}O_6$ requires OMe, 30·65%), which reacted acid to litmus paper. This crystalline compound exhibits a strong selective absorption band at λ 3010 A. (ε, *ca.* 24,000; *c*, 1 mg. per 100 c.c. in 50% aqueous ethyl alcohol), moving on addition of sodium hydroxide to λ 2920 A. (ε, *ca.* 24,000; *c*, as before) (see Fig. 3). The analytical and absorption data suggest that this *compound* is formed from 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone by introduction of a double bond into the molecule between C_2 and C_3, thus giving a conjugated system of four double bonds.

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