CCCVIII.—*The Constitution of the Disaccharides. Part XV. Sucrose.*

By John Avery, Walter Norman Haworth, and Edmund Langley Hirst.

THE normal variety of *d*-fructose has now been brought within the generalisation that hexoses and pentoses tend to exist ordinarily as amylene-oxide forms (Haworth and Hirst, J., 1926, 1858;

Haworth, Hirst, and Learner, this vol., p. 1040). This development has necessitated the abandonment of the butylene-oxide formula generally current for the normal sugar and latterly supported by the experiments of Irvine and Patterson (J., 1922, **121**, 2696), and has in consequence introduced many uncertainties into the reasoning by which the constitution of the more labile γ-isomeride of fructose was deduced. This consequence was indicated in the papers by two of us referred to above.

It will be seen on reference to the papers by Haworth and Linnell (J., 1923, **123**, 294) and by Haworth and Mitchell (*ibid.*, p. 301) that the inferences drawn from the experimental work therein recorded were based on the appropriation of the butylene-oxide structure for normal fructose. The consideration of a butylene-oxide structure for γ-fructose derivatives was for this reason excluded, the conclusion being drawn that " the choice of a constitution for the tetramethyl γ-fructose derived from methylated sucrose appears to be limited to one of the following forms : ethylene oxide, propylene oxide, amylene oxide." This later work on *n*-fructose has therefore rendered uncertain and insecure the arguments by means of which an amylene-oxide structure for tetramethyl γ-fructose has been deduced, and it was decided to take the present opportunity to review the whole position in the light of the experience gained in determining the constitution of other sugar derivatives. It has become evident in the meantime that, owing to the peculiar nature and extent of the isomerism in the sugar group, it is necessary to withhold the application of structural formulæ unless the substance under investigation has been transformed by well-defined methods into products which can be compared with or further degraded to give simple and crystalline reference compounds of definitely verified structure, such as the simple tartaric and hydroxyglutaric acids. In the earlier experiments (Haworth and Linnell, *loc. cit.*; Haworth and Mitchell, *loc. cit.*) the oxidative degradation products were, with few exceptions, liquid distillates and an extended examination of these substances has now yielded crystalline derivatives of such a nature that the structure of the tetramethyl γ-fructose from which they originated may be clearly deduced.

The experiments now to be described have involved a repetition of the work previously recorded on the oxidation of tetramethyl γ-fructose (I) by nitric acid and by alkaline permanganate, with the result that the accuracy of these experimental data has been substantiated, and proof has been obtained that in almost every case the products described, although liquids, were essentially homogeneous.

Oxidation of tetramethyl γ-fructose with either dilute or concen-

trated nitric acid leads, after esterification with ethyl alcohol, to the isolation of an ethyl ester giving C, 50·4; H, 7·8; OMe, 47·6, which agree reasonably well with either of the formulæ $C_{11}H_{20}O_7$ or $C_8H_{14}O_5$. This ethyl ester reduces Fehling's solution on warming, undergoes profound decomposition in the presence of hot alkali, and contains a reducing hydroxyl group which can be methylated to give a stable, non-reducing substance, the behaviour of which towards alkali is now normal. Owing to this behaviour with alkali, the quantitative hydrolysis of the ethyl ester gave figures which were higher than those calculated for $C_{11}H_{20}O_7$ and agreed with those required by a substance $C_8H_{14}O_5$; but, although the recorded analytical data and the behaviour during titration are in better agreement with the lactone formula $C_8H_{14}O_5$, originally proposed by Haworth and Linnell, yet this compound must now be recognised, for the following reasons, as the ethyl ester, $C_{11}H_{20}O_7$, of the lactol acid $C_9H_{16}O_7$ (II).
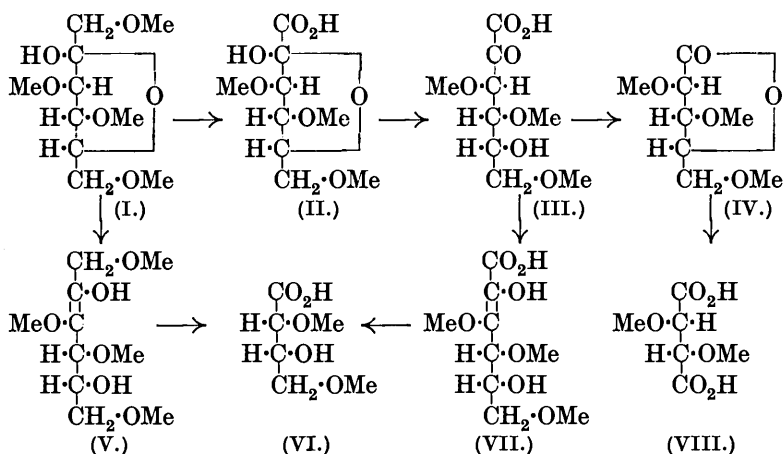
On methylation of this product, the higher homologue was obtained which was characterised by its conversion to a crystalline amide, m. p. 99—100°, identical with that previously described by Haworth, Hirst, and Nicholson (this vol., p. 1517). The relationship between the methyl ester obtained by these authors and the ethyl ester now described is thus demonstrated. The degradation of the lactol acid by means of alkaline permanganate yielded a hydroxymethoxy-acid which was isolated in the form of its methyl ester. In the experimental section the detailed analysis of this is described, and it is shown that the main product is occasionally accompanied by an impurity which substantially enhances the methoxyl and titration values. Specimens of the ester gave analytical figures which corresponded closely with those recorded by Haworth and Linnell

for their final esterified oxidation products, whilst others corresponded more nearly to methyl hydroxydimethoxybutyrate.* By treatment with methyl-alcoholic ammonia, each of them gave, in yield varying from 60—87%, the crystalline amide, m. p. 104—105°, of a hydroxydimethoxybutyric acid (VI), proof being thus afforded that the latter acid was the essential constituent. The same product was also obtained by direct oxidation of tetramethyl γ-fructose with alkaline permanganate (compare Haworth and Mitchell, *loc. cit.*), and the identity of the hydroxydimethoxybutyric acid from both these sources was demonstrated by the conversion of each specimen into the same crystalline amide. Methylation of the ester gave a methyl trimethoxybutyrate, which was characterised through the corresponding crystalline amide, m. p. 58—59°.

Under certain conditions, the above hydroxydimethoxybutyric acid appeared to lose the elements of water and give a hygroscopic anhydride, which has been described previously as a lactone by Irvine and Patterson (J., 1922, **121**, 2699) and by Haworth and Mitchell (*loc. cit.*). Sufficient evidence is not yet available, however, to enable a final choice to be made between the formulation of this anhydride as a lactone or a lactide (see footnote).

The application of the Weerman test (*Rec. trav. chim.*, 1917, **36**, 16) as a method for characterising α-hydroxy-acids by the action of sodium hypochlorite on their amides, indicated that the hydroxydimethoxybutyric acid under examination did not belong to this class, and the attempted oxidation of the free acid by means of nitric acid led to the recovery of the material unchanged, thus proving that the free hydroxyl group did not occupy a terminal γ-position in the chain. These indications pointed provisionally to the recognition of the hydroxydimethoxybutyric acid as a β-hydroxy-acid.

A complete verification of these results was secured, and a simple and direct proof of the structure of these substances and of γ-fructose derivatives was attained, by the degradative oxidation of the lactol acid $C_9H_{16}O_7$ (II) by means of barium permanganate in acid solution. Under conditions which required careful control, one

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* The reason for this variability of composition becomes clear when it is recognised, as will appear later in this paper, that we were handling a β-hydroxy-acid. The parent substance of this product, β-hydroxybutyric acid, has been fully investigated by McKenzie (J., 1902, **81**, 1402), who has observed the readiness with which it passes into an anhydride form and into crotonic acid by loss of water. If in the present case a dimethoxycrotonic acid were formed, this would lead under the conditions employed to methoxymalonic acid, and ultimately to its methyl ester. We are at present studying the various forms of hydroxydimethoxybutyric acid, obtained synthetically from dimethoxyacetoacetic ester.

atomic proportion of oxygen was absorbed for each molecule of lactol acid, and a yield of nearly 50% of crystalline d-trimethyl γ-arabonolactone (IV) was obtained. We were able to recognise this compound with certainty, since we were in possession of the corresponding l-isomeride of the lactone (Baker and Haworth, J., 1925, **127**, 365; Haworth and Nicholson, J., 1926, 1899; Drew, Goodyear, and Haworth, this vol., p. 1244), and the properties of the two specimens are given for comparison :

	d-Trimethyl γ-arabonolactone.	l-Trimethyl γ-arabonolactone.
M. p.	32—33°	33°
$[\alpha]_D$ (initial) in water............	$+44\cdot5°$	$-44\cdot2°$
$[\alpha]_D$ (after 20 days)	$+25\cdot5°$	$-25\cdot2°$

We have confirmed the structure assigned to these lactones, wherein the methyl groups are allocated to the positions 2, 3, 5, by effecting their further oxidation with nitric acid. The d-trimethyl γ-arabonolactone originating from tetramethyl γ-fructose gave l-dimethoxysuccinic acid (VIII), identified as the crystalline methylamide and also as the amide, and these crystalline derivatives were compared directly with specimens which were specially prepared from l-tartaric acid (see later papers by Haworth, Hirst, and Learner and by Haworth and Jones).

The reaction between the lactol acid and acid permanganate is thus typical of the behaviour of either α-ketonic acids or ketol-acids which are potentially α-ketonic in nature (compare Ohle, *Ber.*, 1927, **60**, 1165), leading to the formation of a carboxylic acid containing one carbon atom less in the chain. Since the substance isolated in the present instance has been proved definitely to be $2 : 3 : 5$-d-trimethyl arabonic acid, it follows immediately that the original lactol acid must have contained methyl groups in positions 3, 4, and 6. The oxide linking present in the lactol acid and also in tetramethyl γ-fructose must connect the carbon atoms in positions 2 and 5. Tetramethyl γ-fructose has therefore a γ- or butylene-oxide structure, and the lactol acid, $C_9H_{16}O_7$, is seen to have the constitution provisionally applied to it by Haworth and Hirst (1926). Similarly the hydroxydimethoxybutyric acid (VI) is recognised as $2 : 4$-dimethyl d-erythronic acid.

The recent views we have expressed (Haworth, Hirst, and Nicholson, *loc. cit.*) relating to the structure of tetramethyl γ-fructose, resting on the analogy with other γ-sugars and on the ease of transformation of γ-fructose derivatives into furan bodies (see also Bergmann, J., 1923, **123**, 1277), are thus finally supported by the experimental results herein outlined. It follows that this sugar is now to be recognised as $1 : 3 : 4 : 6$-tetramethyl fructose, and since

this derivative arises from the hydrolysis of methylated sucrose, the constitutional formula (IX), which we have applied in recent papers to sucrose, receives additional support and confirmation.

(IX.)
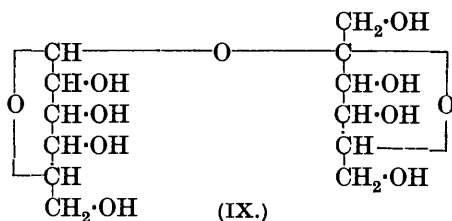
 EXPERIMENTAL.

Oxidation of Tetramethyl γ-Fructose with Nitric Acid (d 1·42). *Isolation of the Ethyl Ester of the Lactol Acid* $C_9H_{16}O_7$ (II).—A mixture of tetramethyl γ-fructose (10 g.) and concentrated nitric acid (70 c.c.; *d* 1·42) was cautiously heated on the water-bath until at 70° a vigorous reaction ensued with evolution of nitrous fumes. The reaction was kept under control by cooling at intervals, and the temperature of the mixture was gradually raised to 93°. The reaction appeared to be complete after $1\frac{1}{2}$ hours. Water was then added to the cooled solution and the nitric acid was almost entirely removed by distillation at 50—60°/13 mm., the usual precautions being observed to avoid undue heating during the concentration. This procedure was many times repeated and finally the product was taken down to a syrup. At this stage, absolute ethyl alcohol was added and distilled from the product to remove water. The syrupy residue was dissolved in 200 c.c. of absolute ethyl alcohol, containing 6 g. of hydrogen chloride, and boiled gently under reflux for 9 hours. Thereafter, the mineral acid was neutralised with silver carbonate, the solution filtered, and the solvent evaporated. The syrupy residue distilled without decomposition :

Fraction 1, at bath temperature up to 125°/0·1 mm.; 2·2 g., $n_D^{16°}$ 1·4500.

Fraction 2, at bath temperature 130—135°/0·1 mm.; 7 g., $n_D^{14°}$ 1·4520.

Fraction 3, at bath temperature 150°/0·1 mm.; 0·5 g., $n_D^{14°}$ 1·4564. Fraction 2, which showed $[\alpha]_D^{24°} + 25·8°$ in water ($c = 2·7$), gave the following analytical data—Found : C, 50·35; H, 7·75; OMe, 47·6. $C_{11}H_{20}O_7$ requires C, 50·0; H, 7·6; OMe, 47·0. $C_8H_{14}O_5$ requires C, 50·5; H, 7·4; OMe, 48·9%. This material reduced Fehling's solution on warming and gave coloured decomposition products when heated with aqueous alkali. As stated on p. 2310, this product consisted essentially of the ethyl ester, $C_{11}H_{20}O_7$, of the

lactol acid, $C_9H_{16}O_7$ (II). *Titration.* 0·269 G. required 13·5 c.c.
$N/10$-sodium hydroxide (on heating for $\frac{1}{2}$ hour at 90°). Calc., for
$C_{11}H_{20}O_7$: 10·2 c.c.; for the lactone, $C_8H_{14}O_5$: 14·2 c.c.

Methylation and Amide Formation.—A specimen of the above
product (Fraction 2) was treated with Purdie's reagents for $5\frac{1}{2}$ hours.
A syrup which remained colourless when heated with aqueous alkali
and which did not reduce Fehling's solution was obtained, distilling
at 12 mm. from a bath heated at 155—160°. This showed $[\alpha]_D^{23°} + 3°$
in water ($c = 1·07$). *Titration.* 0·1048 G. required 4·2 c.c.
$N/10$-sodium hydroxide for hydrolysis and neutralisation.

The methylated product was dissolved in methyl alcohol, saturated
with dry ammonia, and cooled in ice, and the mixture was kept for
4 days. Removal of the solvent in a vacuum gave a crystalline
product (yield above 60%), m. p. 99—100° (from light petroleum);
$[\alpha]_{5461}^{21°} - 83°$ in water ($c = 0·98$). A specimen of the crystalline
amide was mixed with the specimen of the amide $C_{10}H_{19}O_6N$,
obtained by Haworth, Hirst, and Nicholson (this vol., p. 1517)
from the corresponding methyl ester, and the m. p. showed no
depression.

Oxidation with Alkaline Permanganate.—The product (2·5 g.)
isolated from the process of oxidation with nitric acid as described
above (Fraction 2) was treated at 70° with $2N$-potassium hydroxide
(20 c.c.) and with N-potassium permanganate (88 c.c.), the latter
being gradually added with frequent shaking. In working up the
product the alkali was neutralised with mineral acid in presence of
ice, and after removal of the precipitated manganese oxides the
filtrate was made slightly alkaline by means of potassium carbonate
and evaporated (water-bath) to small bulk. Thereafter the whole
of the water was removed in a high vacuum, yielding a white, solid
mass. Preliminary experiments showed that no advantage was to
be gained by extracting the solid with organic solvents. It was
therefore dissolved in water and the organic acid liberated by
acidifying with mineral acid. The water was then removed in a
high vacuum, and the white mass extracted with chloroform. This
extract yielded a syrupy organic acid which was esterified with
methyl-alcoholic hydrogen chloride. The liquid ester distilled
under 0·07 mm. from a bath heated to 100°, and showed $[\alpha]_D^{20°} + 19°$
(in water, $c = 0·96$) and $n_D^{17°}$ 1·4400; yield 0·8 g. (Found : C, 46·3;
H, 7·9; OMe, 49·2. $C_7H_{14}O_5$ requires C, 47·2; H, 7·9; OMe,
52·2%). *Titration.* 0·0946 G. required 5·2 c.c. $N/10$-sodium
hydroxide for hydrolysis and neutralisation (Calc. for $C_7H_{14}O_5$:
5·3 c.c.).

This product has been obtained on several occasions under
similar experimental conditions to those outlined above, and the

physical properties and analyses of these specimens are tabulated below :

(a) $n_D^{17°}$ 1·4400, $[\alpha]^{20°}$ + 19°, C, 46·3; H, 7·9; OMe, 49·2%; (b) $n_D^{10°}$ 1·4410, $[\alpha]_D^{19°}$ + 22·4°, OMe, 54·9%; (c) $n_D^{13·5°}$ 1·4390, OMe, 48·8%; (d) $[\alpha]_D^{20°}$ + 21·8°, C, 46·75; H, 7·8; OMe, 61·2%; (e) n_D 1·4421, $[\alpha]_D$ + 31°, C, 47·8; H, 7·2; OMe, 60%.

The analyses of (a) and (d) were made independently by Fein-Chemie, G.m.b.H., Tübingen. These products correspond with that obtained by Haworth and Linnell (loc. cit., p. 301), who quote C, 48·4; H, 7·2; OMe, 59·7%. It was evident that all these products were mixtures containing a variable quantity of a substance giving high methoxyl and titration values, and this factor is referred to in the footnote on p. 2311. That each of these specimens was essentially, however, methyl hydroxydimethoxybutyrate was demonstrated by treating them with methyl-alcoholic ammonia. This led to the isolation of the crystalline amide described below in yields from 60—87%, the largest yield being obtained from the specimen (a).

Hydroxydimethoxybutyramide was isolated as described above as crystals, m. p. 104—105° (from petroleum); $[\alpha]_{5461}^{19°}$ + 37° in water (c = 1·05), and $[\alpha]_D^{19°}$ + 33° [Found : C, 44·2; H, 8·0; N, 8·7; OMe, 36·2. $C_4H_7O_2N(OMe)_2$ requires C, 44·2; H, 8·0; N, 8·6; OMe, 38·0%].

Oxidation of Tetramethyl γ-Fructose with Alkaline Permanganate.—In general the method described by Haworth and Mitchell (J., 1923, 123, 306) was followed, except that the product was isolated as the methyl ester as described in the earlier paragraph above. The ester distilled from a bath heated to 130° under 13 mm., and showed $n_D^{14°}$ 1·4413. This material corresponded exactly with the specimens (a)—(e) described above, and gave on treatment with methyl-alcoholic ammonia a crystalline amide, m. p. 104—105°, identical in all respects with that described above as hydroxydimethoxybutyramide.

Hydrolysis.—A specimen of the above methyl ester was hydrolysed with dilute hydrochloric acid (5%) during 4½ hours at 80°. After the usual process of isolation, the product was distilled from a bath heated to 125° under 0·04 mm., and was obtained as a colourless, hygroscopic syrup having acid properties, but which did not crystallise on preserving in a vacuum over phosphoric oxide for several days. A further purification was effected by fractional distillation, and the substance remained as a colourless syrup. This appeared to be identical with the product obtained by Haworth and Mitchell (loc. cit.) and Irvine and Patterson (loc. cit., p. 2699), and described as the lactone of hydroxydimethoxybutyric acid.

The observations now to be recorded indicate, however, that the product may have been a lactide of a β-hydroxy-acid.

Experiments on the Determination of the Structure of the Hydroxy-dimethoxybutyric Acid.—(a) *Weerman reaction on the amide.* Weerman's method (*loc. cit.*) for the characterisation of α-hydroxy-acids depends on the interaction of the amides of these acids with sodium hypochlorite, this reagent forming sodium *iso*cyanate with such amides but not with those of other hydroxy-acids. The qualitative test applied for the presence of sodium *iso*cyanate is the addition of hydrazine hydrate, followed by the addition of benzaldehyde, which leads to the isolation of benzalsemicarbazone. An alternative method is the addition of semicarbazide, which in presence of sodium *iso*cyanate forms crystalline hydrazidicarbon-amide. When the test was applied to the hydroxydimethoxy-butyramide which we had prepared during the present work, a negative result was obtained, indicating that this amide was other than an α-hydroxy-compound. Control experiments were carried out with gluconamide, trimethoxyglutaramide and acetamide, and only in the case of gluconamide was a positive result obtained, as should be the case.

(b) *Attempted oxidation with nitric acid.* The above result indicated that the related acid must be either a β- or a γ-hydroxy-acid. In the latter case the hydroxyl group would be in the terminal position as a –CH_2·OH group, which should be characterised by its ease of oxidation to a carboxyl group. When the substance was digested, however, with nitric acid (*d* 1·2) during 6 hours at 70—100°, it gave a product which, on conversion into the methyl ester, was identical with the original material, thus proving that no oxidation had taken place. This conclusion was verified by conversion of this ester into the crystalline hydroxydimethoxy-butyramide, m. p. 104—105°. It became evident, on the basis of these results, that the hydroxydimethoxybutyric acid under examination was probably a β-hydroxy-acid.

(c) *Methylation of the ester.* The specimen (a) described previously, and consisting essentially of methyl hydroxydimethoxy-butyrate, was methylated with Purdie's reagents, and the product on distillation showed $n_D^{12°}$ 1·4282; $[\alpha]_D^{21°}$ + 19° in water (*c* = 1·04). *Titration.* 0·1006 G. required 5·25 c.c. of *N*/10-sodium hydroxide for hydrolysis and neutralisation (Calc. for $C_8H_{16}O_5$: 5·24 c.c.). Found: C, 49·7; H, 6·5; OMe, 62·3. Calc.: C, 50·0; H, 8·3; OMe, 64·6%.

This product was identified as methyl trimethoxybutyrate by conversion into the crystalline amide. The trimethoxybutyramide separated from light petroleum as colourless needles, m. p. 58—59°,

$[\alpha]_D^{18°} + 40\cdot5°$ in water ($c = 1\cdot09$ (Found: C, $47\cdot7$; H, $8\cdot5$; N, $7\cdot7$; OMe, $49\cdot7$. Calc.: C, $47\cdot5$; H, $8\cdot5$; N, $7\cdot9$; OMe, $52\cdot5\%$).

Confirmatory Proof of the Constitution of Tetramethyl γ-Fructose. Isolation of Crystalline d-2 : 3 : 5-*Trimethyl Arabonolactone by Degradative Oxidation of the Lactol Acid,* $C_9H_{16}O_7$.—The ethyl ester, $C_{11}H_{20}O_7$, of a lactol acid, $C_9H_{16}O_7$, was described as Fraction 2 on p. 2313. Preliminary experiments showed that the desired stage of oxidation by means of acidified permanganate was completed when one atomic proportion of oxygen had been absorbed. The details of the procedure are as follows : The material of Fraction 2 ($3\cdot2$ g.) was hydrolysed during 3 hours at 90° with 60 c.c. of $2\cdot5\%$ hydrochloric acid. The solution was neutralised with silver carbonate, and the silver removed from the filtrate with the aid of hydrogen sulphide. The filtrate from the silver sulphide was evaporated, and the free lactol acid, $C_9H_{16}O_7$, was collected as a syrup, which was dissolved in 110 c.c. of water and mixed with 40 c.c. of *N*-sulphuric acid. Thereafter, a solution of barium permanganate (4%) was added very slowly until the required amount had been admitted, the reaction being conducted at room temperature. An excess of barium hydroxide solution was then added, followed by the admission of carbon dioxide to precipitate the excess of barium hydroxide. At this stage, the precipitated salts were removed by filtration, and to the solution, which contained the soluble barium salt of an organic acid, the equivalent of dilute sulphuric acid was added. The filtrate obtained after removal of barium sulphate was now evaporated under diminished pressure, and yielded a syrup which crystallised spontaneously when cold (yield 1 g.). The crystalline compound distilled under 11 mm. from a bath at 145°, and the distillate solidified instantly (yield $0\cdot75$ g.).

Extraction of this crystalline product with light petroleum left a minute quantity of residual oil which darkened in the air. From the solution in light petroleum, needles or shining plates crystallised according to the conditions of cooling (m. p. 32—33°; yield $0\cdot6$ g.); $[\alpha]_D + 44\cdot5°$ in water ($c = 0\cdot712$)—this initial value decreased during 18 days to the constant value $+ 25\cdot5°$ (Found: C, $50\cdot2$; H, $7\cdot7$; OMe, $47\cdot6$. Calc. for $C_8H_{14}O_5$: C, $50\cdot5$; H, $7\cdot4$; OMe, $48\cdot9\%$). *Titration*. $0\cdot0512$ G. required $2\cdot7$ c.c. $N/10$-sodium hydroxide for neutralisation, the substance behaving during titration as a lactone.

This product corresponds to *d*-trimethyl γ-arabonolactone, and in the theoretical section it is shown that the rotation values are equal and opposite to those of the *l*-isomeride, whilst the m. p. is identical.

A similar oxidation was carried out with the corresponding methyl

ester of the lactol acid, $C_{10}H_{18}O_7$ (compare Haworth, Hirst, and Nicholson, *loc. cit.*). In this example, the experiment was modified by omitting isolation of the free acid, inasmuch as control experiments indicated that the alcohol liberated on hydrolysis of the ester was not appreciably oxidised under the experimental conditions employed. The methyl ester (8·9 g.) was heated for $2\frac{1}{2}$ hours at 85° with $N/2$-sulphuric acid (104 c.c.). Barium permanganate equivalent to one atomic proportion of oxygen was then admitted to the cold solution, which had been diluted to a volume of 500 c.c. by the addition of a further 100 c.c. of $N/2$-sulphuric acid and water. The product was isolated in the manner already described, and weighed 4·1 g., of which 3·0 g. were collected as a distillate under 11 mm. from a bath at 145°. This distillate crystallised immediately when cold. A similar portion of an oily by-product was again noticed. The recrystallised d-trimethyl γ-arabonolactone, m. p. 33°, weighed 2·6 g.

It should be added that qualitative tests conducted with the ethyl or methyl esters dissolved in acidified permanganate solution showed that the reaction proceeded slowly until hydrolysis of the ester had taken place. Oxidation then proceeded rapidly at room temperature. The oxidation proceeds smoothly and is accompanied by practically no change in the rotation value of the solution.