CCXXIX.—*The Constitution of Sucrose. Part I. Oxidation of Tetramethyl γ-Fructose.*

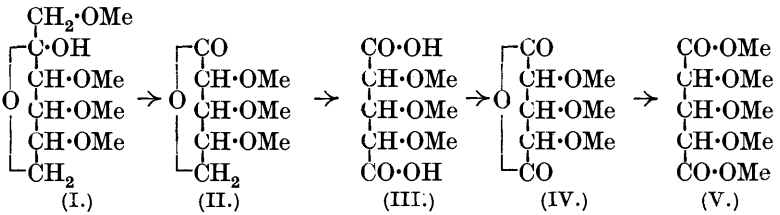
By GEORGE MCOWAN.

IT is now recognised that the fructose component of sucrose is not the ordinary lævorotatory form of the monosaccharide, but is the dextrorotatory variety known as γ-fructose. It follows that the constitution of γ-fructose must be decided before an adequate structural representation of sucrose can be arrived at. Hitherto the evidence bearing on this important question has been obtained almost exclusively by oxidising tetramethyl γ-fructose and identifying the products, this having been provided by Haworth and his pupils in numerous papers.

There is, however, an alternative method of approaching the problem, as it has been shown in the course of investigations conducted in this laboratory that the polysaccharide inulin is also derived from γ-fructose and, in this respect, displays a constitutional property in common with sucrose. This is revealed by the fact that, on hydrolysis, trimethyl inulin yields a trimethyl γ-fructose which is convertible into tetramethyl γ-fructose identical with that obtained from sucrose in a similar series of reactions (Irvine and Steele, J., 1920, **117**, 1474). The natural extension of these researches on inulin by studying the constitution of trimethyl- and tetramethyl γ-fructose has been carried out, but publication of the results has meanwhile been withheld, as they disagreed with those upon which the accepted formula for sucrose is based. In fact, the behaviour, on oxidation, of the methylated γ-fructoses from inulin could in no way be reconciled with Haworth's results and it became necessary to explore the whole situation. Reinvestigation of the

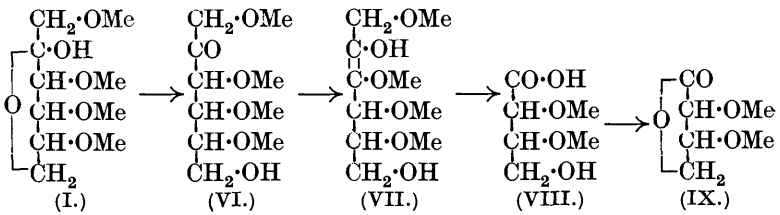
reactions of tetramethyl γ-fructose has convinced us that the structural formula assigned to this sugar, and in consequence to sucrose also, is incorrect.

In the first and second (Haworth and Law, J., 1916, **109**, 1314; Haworth, J., 1920, **117**, 199) of a series of papers on the constitution of sucrose, the γ-fructose residue in the disaccharide was represented as containing an ethylene-oxide ring. This was, no doubt, tentative, but in subsequent investigations (Haworth and Linnell, J., 1923, **123**, 294; Haworth and Mitchell, *ibid.*, p. 301), experimental results were submitted leading to the conclusion " beyond reasonable doubt that the structure of tetramethyl γ-fructose is no longer to be regarded as ethylene-oxide but amylene-oxide in character." This conclusion (expressed in formula I) was reached on the evidence furnished by oxidising tetramethyl γ-fructose, which, it was stated, was converted successively into trimethoxyvalerolactone (II) and trimethoxyglutaric acid, the latter being identified as the anhydride (IV) and the dimethyl ester (V). The sequence of changes, as interpreted by the above workers, is shown below :



(I.) (II.) (III.) (IV.) (V.)

Support for these views was sought (Haworth and Mitchell, *loc. cit.*) in the oxidation of tetramethyl γ-fructose by alkaline potassium permanganate to give dimethoxybutyrolactone (IX), the formation of this product being explained on the assumption that the alkaline reagent induced ketonisation (VI) and enolisation (VII) of the sugar, with the introduction of a double bond between the second and third carbon atoms.

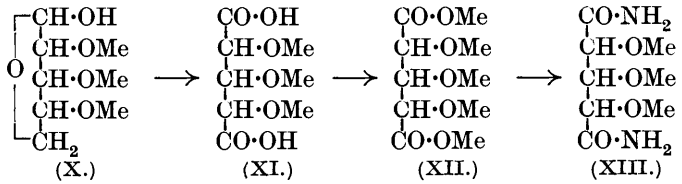


(I.) (VI.) (VII.) (VIII.) (IX.)

This experimental evidence did not appear to us to be convincing. It is obvious that the method used to isolate the lactone (II) was faulty and it is highly improbable that this compound

could have been obtained pure,* as the conditions employed were favourable to esterification of the oxidation acids. In the similar case of oxidising the isomeric form of tetramethyl fructose, where the same methods of isolation were employed (Irvine and Patterson, J., 1922, **121**, 2696), the only pure product obtained was the diethyl ester of dimethoxyhydroxyglutaric acid. Further, the possibility must not be overlooked that the action of alkaline permanganate on compounds such as those now under consideration may give rise to mixtures of oxidation products, and this we have ascertained to be the case.

The results quoted by Haworth and Linnell are, moreover, inconsistent with those obtained in related investigations. For example, the structure of *l*-arabinose has been studied by Hirst and Robertson (J., 1925, **127**, 358), who represent the pentose by formula (X), since the fully methylated sugar was converted into arabotrimethoxyglutaric acid (XI), which was identified through the dimethyl ester (XII) and the diamide (XIII).



(X.) (XI.) (XII.) (XIII.)

This constitution is accepted by Haworth (this vol., p. 93), who nevertheless in the same paper retains the amylene-oxide formula for γ-fructose (p. 98) despite the fact, pointed out by Hirst and Robertson (*loc. cit.*), that the compound indexed as (XII) has practically the same rotation as (V). The compounds ought not to be identical. As (XII) is derived from *l*-arabinose, it should be the enantiomorph of (V), in which the configuration of *d*-glucose is retained. This is a serious discrepancy and, taken in conjunction with the objections already indicated in this paper, leads to the conclusion that the material described by Haworth and Linnell as dimethyl trimethoxyglutarate was in reality a mixture, and there is thus no ground for regarding the other substances analysed by them as having the properties and constitutions attributed to them. The constitution of sucrose is still an open question and the results now contributed show that, in accepting the amylene-oxide structure

* The only solid product isolated by Haworth and Linnell was a small quantity of crystalline matter which separated when their alkylated lactone was kept in a loosely-stoppered tube. This was claimed to be the corresponding acid, but the possibility does not seem to have been followed up of converting the whole of their syrupy product into a definite crystalline compound.

for the fructose residue in the disaccharide, an error has been introduced in the literature of the carbohydrates.

The present investigation was undertaken in the hope of testing the amylene-oxide structure for tetramethyl γ-fructose by reducing the compound claimed to be trimethoxyvalerolactone (II) to the corresponding trimethyl *d*-arabinose and contrasting the product with derivatives of the naturally occurring *l*-series. Although the conditions prescribed by Haworth were carefully followed, we were unable to obtain this trimethoxyvalerolactone and many variations of the conditions led to no better result. The use of inulin as a source of tetramethyl γ-fructose was therefore discarded and recourse was had to sucrose in order to provide an exact control of Haworth's method and to eliminate any adventitious source of error. As described in the experimental part, and using the method described by Haworth (J., 1915, **107**, 12), we found that, after one methylation, the methoxyl content did not reach the value required for hepta-methyl sucrose and the product consisted of a mixture of the hexa- and hepta-methyl derivatives. After two successive methylations, a mixture of octamethyl sucrose and lower methylated products resulted. In view of this experience, it was advisable to take precautions to exclude the possibility that the tetramethyl γ-fructose finally obtained might be contaminated either with trimethyl or tetramethyl glucose. In consequence, only methylated sucrose was submitted to hydrolysis which showed a methoxyl content not higher than that required, and a refractive index not lower than that recorded for a heptamethyl sucrose. In addition, the condition was laid down that, on distillation, the first fraction should conform to both of these requirements.

In the earlier work of Haworth, tetramethyl γ-fructose was separated from trimethyl glucose by distillation, but this is more conveniently effected by extraction of an aqueous solution containing both sugars by means of chloroform. On oxidation of the tetramethyl γ-fructose by means of nitric acid, either alone or followed by treatment with alkaline permanganate, we failed to obtain the compound described by Haworth and Linnell or to confirm the analytical results quoted by them. With nitric acid as the sole oxidising medium, under the conditions described by Haworth and Linnell, a mixture of compounds was formed and this could not be separated completely into its components by distillation. This may be due to a variation in the time of initial heating at 86° to start the reaction, or to a difference in the amount of nitric acid still present when distillation of the products of the oxidation was attempted. The components, however, did not display the properties ascribed to them. All the fractions reduced Fehling's solution

and presumably contained unchanged tetramethyl γ-fructose or alkylated ketonic acids. In the hope of completing the oxidation and isolating trimethoxyglutaric acid, nitric acid was employed at a higher temperature (Hirst and Purves, J., 1923, **123**, 1352). On attempting to isolate the products in the form of the methyl esters a mixture was obtained no component of which had a rotation in agreement with that of the anticipated dimethyl trimethoxyglutarate or yielded a crystalline diamide of the necessary properties. In the alternative process, in which oxidation with nitric acid was followed by treatment with alkaline permanganate, a mixture was again obtained. This consisted for the most part of dimethyl oxalate and a higher-boiling fraction, which did not have the properties of (V) and yielded a crystalline amide which was not the enantiomorph of (XIII).

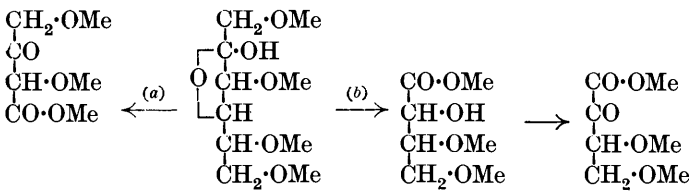
Although the tetramethyl γ-fructose employed above conformed to the standards of the pure compound, we confirmed the fact that the reducing power of the oxidation products was not due to the fortuitous presence of glucose derivatives. First, we prepared tetramethyl γ-fructose from inulin and oxidised it under conditions similar to those described by Haworth, but again obtained products which reduced Fehling's solution. The results of this investigation will be published shortly. Secondly, we utilised the method described by Willstätter (*Ber.*, 1918, **51**, 780) for the estimation of an aldose in admixture with a ketose, based on the oxidation of the former by sodium hypoiodite under specified conditions and the consequent formation of sodium gluconate. It was already known that tetramethyl γ-fructose is not oxidised by bromine (Haworth and Mitchell, *loc. cit.*). The unaffected tetramethyl γ-fructose, after treatment by Willstätter's method, was extracted from the aqueous solution of the sodium salts of the mixed gluconic acids by means of chloroform.

The oxidation of this purified sugar was carried out by the method described by Hirst and Purves (*loc. cit.*), which is known to yield dibasic acids with methylated xylose, arabinose and fructose. The analytical figures showed that the main part of the product was homogeneous, only the first fraction obtained on distillation containing an extraneous compound. Only this portion of the product deposited crystalline material on treatment with ammonia and the crystalline compound was identified as oxamide. Further, the products all reduced Fehling's solution and showed other reactions characteristic of the carbonyl group. With another portion of the purified tetramethyl γ-fructose, oxidation by nitric acid was followed by treatment with alkaline permanganate, with results similar to those already discussed.

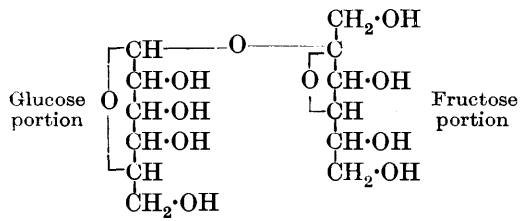
The only difference in the methods applied by us and by Haworth

and Linnell lay in the fact that we used methyl alcohol where they had used ethyl alcohol in removing nitric acid. According to our interpretation of Haworth and Linnell's reaction, these workers should have obtained the ethyl ester of an acid of which we have prepared the methyl ester. The properties and analytical figures of the product obtained by us are in agreement with those required for a methyl ketodimethoxybutyrate, $CO_2Me{\cdot}CO{\cdot}CH(OMe){\cdot}CH_2{\cdot}OMe$ (XIV) or $CO_2Me{\cdot}CH(OMe){\cdot}CO{\cdot}CH_2{\cdot}OMe$ (XV). It is surely no mere coincidence that the analytical figures quoted by Haworth and Linnell for trimethoxyvalerolactone are in accordance with those of the ethyl ester of the same acid. We are unable to account for other results obtained by Haworth and Linnell; on this basis, there is no possibility of the formation of trimethoxyglutaric acid, on the isolation of which the structure of γ-fructose has been based. In place of this, on completion of the oxidation with alkaline permanganate, we invariably obtained dimethyl oxalate, the formation of which from the above compound requires no further explanation, in admixture with other compounds which so far have not been further examined.

While it is with diffidence that yet another formula is proposed for sucrose, the evidence offered renders it imperative to discard the structure which has been assigned to the fructose constituent of the disaccharide. Since it is accepted that the stable form of fructose is butylene-oxidic in character (Irvine and Patterson, *loc. cit.*), it would appear that γ-fructose is possessed of a propylene-oxidic structure. The degradation of the tetramethyl derivative may then occur in either of two ways, (*a*) with fission between carbon atoms 4 and 5 or (*b*) with fission between carbon atoms 2 and 3.



The following formula is tentatively suggested for sucrose :



The structure of the fructose part of the molecule is based on

results contributed here, whilst that of the glucose portion is based on the evidence (this vol., p. 350) supplied by Hirst after oxidation of tetramethyl glucose by nitric acid. With a view to confirming the conclusions reached here and making a more detailed examination of the products obtained on oxidation, further investigations are now in progress.

EXPERIMENTAL.

Methylation of Sucrose.—(1) A solution of 61 g. of sucrose in the minimum amount of water was continuously stirred at 35° while 183 c.c. of methyl sulphate and 165 g. of sodium hydroxide, dissolved in 550 c.c. of water, were simultaneously added during 3 hours. The mixture was then kept at 60° for 30 minutes, at 75° for 30 minutes, and finally at 100° for ½ hour. The methylated sucrose was extracted with chloroform, the chloroform removed by distillation, and the viscous syrup remaining fractionally distilled in a high vacuum. It was collected as follows: 1st Fraction (5 g.), b. p. 175—180°/0·2 mm., n_D 1·4692; 2nd fraction (10 g.), b. p. 181—185°/0·3 mm., n_D 1·4692, OMe 45·24%; 3rd fraction (18·4 g.), b. p. 191—195°/0·26 mm., n_D 1·4698, OMe 41·74%; 4th fraction (16·08 g.), b. p. 200—204°/0·3 mm., n_D 1·4732, OMe 41·42% [Heptamethyl sucrose, $C_{12}H_{15}O_4(OMe)_7$, requires OMe, 49·75%. Hexamethyl sucrose, $C_{12}H_{16}O_5(OMe)_6$, requires OMe, 43·61%].

(2) In other preparations the methylation was carried out as above, with the exception that a second treatment with the methylating reagents was given before the final distillation of the methylated sucrose. The distillate was collected as follows: 1st Fraction (7·3 g.), b. p. 180—183°/0·25 mm., n_D 1·4655; 2nd fraction (5·3 g.), b. p. 184°/0·1 mm., n_D 1·4655, OMe 48·73%; 3rd fraction (30·5 g.), b. p. 183—186°/0·05 mm., n_D 1·4663, OMe 47·24%.

Tetramethyl γ-Fructose.—A solution of 30 g. of methylated sucrose (OMe, 47·24%) in 500 c.c. of hydrochloric acid (0·4%) was heated at 60° for 7 hours, the acid then neutralised with silver carbonate, and the tetramethyl γ-fructose separated from the lower methylated glucose by extraction with chloroform. The pale yellow syrup remaining after removal of the chloroform was twice distilled to remove the last traces of lower methylated products, tetramethyl γ-fructose being obtained as a colourless, mobile syrup showing n_D 1·4550, $[\alpha]_D$ in water + 32·26°, OMe 51·9%. $C_6H_8O_2(OMe)_4$ requires OMe, 52·8%.

Oxidation with nitric acid. A solution of 13·5 g. of tetramethyl γ-fructose in 160 c.c. of nitric acid (*d* 1·2) was kept at 86° for a few minutes until the reaction was in progress, and thereafter at 60° for 20 hours, reaction proceeding very slowly. The nitric acid was removed under diminished pressure at 45°, water (until 2 litres had

collected) and finally methyl alcohol being continuously added. The last traces of acid were removed by maintaining the syrup remaining at 40° for several days and finally at 60—70° in the high vacuum of a mercury pump. The product, an almost colourless syrup, distilled, without leaving a residue, as follows: 1st Fraction (0·6 g.), b. p. 126°/0·6 mm.; 2nd fraction (3·8 g.), b. p. 135—138°/0·5 mm., n_D 1·4540, $[\alpha]_D$ in water + 37·64°, OMe 49·79%; 3rd fraction (3·3 g.), b. p. 130°/0·22 mm., n_D 1·4578, $[\alpha]_D$ in water + 33·98°, OMe 46·07%; 4th fraction (3·6 g.), b. p. 132°/0·22 mm., n_D 1·4590, $[\alpha]_D$ in water + 43·88°, OMe 45·75%.

All the fractions vigorously reduced Fehling's solution and, the oxidation being considered incomplete, the process was repeated. Fractions 2, 3 and 4 were reunited, a total weight of 7 g. was dissolved in 110 c.c. of nitric acid (*d* 1·2), and the temperature maintained at 86° for 6 hours. A vigorous reaction proceeded for 20 minutes. The nitric acid was removed as above, with the exception that, after the water had been replaced by methyl alcohol, methyl alcohol containing hydrogen chloride was added to bring the total volume to 400 c.c. and the content of hydrogen chloride to 1%. The solution was then boiled for 3 hours, neutralised with silver carbonate, and the product isolated by the usual methods. It gave the following fractions: 1st Fraction (0·5 g.), b. p. 130°/0·5 mm., n_D 1·4465; 2nd fraction (1·4 g.), b. p. 140—142°/0·6 mm., n_D 1·4542, $[\alpha]_D$ in methyl alcohol + 25·46° (Found: C, 45·4; H, 6·7; OMe, 24·6%); 3rd fraction (1·4 g.), b. p. 140°/0·6 mm., n_D 1·4598, $[\alpha]_D$ in methyl alcohol + 20·28° (Found: C, 46·8; H, 6·2; OMe, 39·5%); 4th fraction (1·21 g.), b. p. 140—142°/0·6 mm., n_D 1·4630, $[\alpha]_D$ in methyl alcohol + 3·34° (Found: C, 47·1; H, 5·9; OMe, 44·1%).

The product was obviously a complex mixture and, as was seen later, the oxidation had proceeded too far under these conditions. On attempting to prepare crystalline amides by dissolving each of the fractions in methyl alcohol saturated with ammonia, no crystalline products were obtained from (3) and (4), whilst from 1 g. of (2) 0·13 g. of oxamide was obtained.

Oxidation with nitric acid followed by alkaline permanganate. Tetramethyl γ-fructose (11 g.) was oxidised as before with nitric acid at 60° for 20 hours. The acid was then neutralised with 2*N*-potassium hydroxide; thereafter 110 c.c. of 2*N*-potassium hydroxide were added, the temperature was raised to 70°, and potassium permanganate added until a faint pink coloration persisted, 1300 c.c. of a solution containing 31·61 g. of $KMnO_4$ per litre being required. The excess of permanganate was destroyed by a little hydrogen peroxide, the liquid separated from manganese dioxide and evaporated under diminished pressure. To remove the last traces of

water, methyl alcohol was added and removed by distillation and the residual solids were dried for 4 hours at 100°/12 mm. The alkali in them was neutralised with methyl-alcoholic hydrogen chloride, and sufficient of this was then added to bring the acid content to 1%. The mixture was then heated at the boiling point for 3 hours, the liquid removed, and the extraction repeated twice. After neutralisation with silver carbonate, the product was isolated by the usual methods; 3·5 g. only were obtained and this amount could not be increased by repeated extraction of the inorganic residues with further quantities of acid methyl alcohol. The product was partly crystalline and distilled as follows: 1st Fraction (1·1 g.), collected up to 120°/0·5 mm., crystallising spontaneously in the receiver, and identified as dimethyl oxalate; 2nd fraction (1·8 g.), b. p. 130—135°/1·5 mm., n_D 1·4378, $[\alpha]_D$ in water + 32·26° (Found: C, 44·2; H, 6·0; OMe, 50·1%).

On dissolving the second fraction in methyl alcohol saturated with ammonia, crystallisation set in immediately; 0·15 g. was collected after 12 hours, and a further 0·13 g. after the alcohol had been removed at a low temperature. The first crop, when rapidly heated, melted at 297°; when heated in an open test-tube, it partly fused with decomposition and partly yielded a crystalline sublimate. The second crop, dried on tile and without recrystallisation, showed $[\alpha]_D$ in water = + 38·88°, for $c = 0·9$ (Found: OMe, 35·76%). Later work showed the possibility of contamination with oxamide and these figures are quoted with this reservation.

Purification of Tetramethyl γ-Fructose by Willstätter's Method.—70 G. of methylated sucrose, obtained by treating 100 g. of sucrose twice with the methylating reagents as described above, were hydrolysed and the product was isolated by the method already described. No attempt, however, was made to separate octa- from hepta-methyl sucrose, and therefore the final product consisted of a mixture of tetramethyl glucose and tetramethyl fructose.

0·1926 G. was dissolved in the minimum amount of water, 15 c.c. of *N*/10-iodine were added, followed by 30 c.c. of *N*/10-sodium hydroxide. After acidification after 20 minutes, 9·6 c.c. of *N*/10-sodium thiosulphate were required for the back titration. This value was confirmed by subsequent, similar preliminary trials. The syrup therefore contained 25·4% of methylated glucose, the greatest proportion of this being tetramethyl glucose. To 37 g. 1800 c.c. of *N*/10-iodine were added, followed by 3600 c.c. of *N*/10-sodium hydroxide. The unaffected tetramethyl γ-fructose was isolated by extraction with chloroform, and the extract was washed with solutions of potassium iodide and of sodium thiosulphate, and finally with water, and dried over anhydrous sodium sulphate.

After filtration and removal of the chloroform by distillation, the product was isolated by the usual methods; 24 g. of a colourless syrup were obtained, showing n_D 1·4545, $[\alpha]_D$ in water $= +31·6°$ (Found: OMe, 51·9%).

Oxidation with nitric acid. 14 G. of tetramethyl γ-fructose, so isolated, were oxidised with nitric acid (d 1·2) at 86° for 6 hours. The product was esterified by boiling for 3 hours with acid methyl alcohol as has been previously described. It was distilled as follows: 1st Fraction (0·4 g.), b. p. 100°/0·6 mm.; 2nd fraction (3 g.), b. p. 117—120°/0·3 mm., n_D 1·4518 (Found: C, 46·7; H, 6·7; OMe, 48·4%); 3rd fraction (2·6 g.), b. p. 115°/0·18 mm., n_D 1·4542 (Found: C, 47·9; H, 6·9; OMe, 49·0%); 4th fraction (2·8 g.), b. p. 115—117°/0·17 mm., n_D 1·4550 (Found: C, 47·5; H, 6·8; OMe, 48·2%. $C_7H_{12}O_8$ requires C, 47·7; H, 6·8; OMe, 52·3%. The corresponding ethyl ester, $C_8H_{14}O_5$, requires C, 50·5; H, 7·4. Haworth and Linnell found for trimethoxyvalerolactone: C, 50·5; H, 7·26%).

These analytical figures have been repeated and confirmed. Further examination showed that all the fractions reduced Fehling's solution and gave Piloty's test for a keto-group. On attempting to prepare semicarbazones, oils separated which have not yet been further examined.

When the various fractions were treated with ammonia, from fraction (2) only were crystals obtained. These were identified as oxamide, 0·08 g. being obtained from 1·2 g. of syrup. The divergence in the analytical figures of fraction (2) from those of the remainder of the product is therefore to be accounted for by the presence of dimethyl oxalate.

Oxidation with nitric acid followed by alkaline permanganate. The oxidation of 10 g. of tetramethyl γ-fructose, obtained after treatment with sodium hypoiodite, was carried out with nitric acid at 68° for 20 hours (the solution then still reduced Fehling's solution), followed by potassium permanganate as previously described. In all, 900 c.c. of a solution containing 31·61 g. of $KMnO_4$ per litre were necessary. The product was isolated as previously described and was again partly crystalline. On distillation it yielded: 1st Fraction (0·9 g.), crystallising spontaneously and identified as methyl oxalate; 2nd fraction (0·5 g.); 3rd fraction (1·1 g.), b. p. 105°/0·6 mm. On treating this with ammonia, crystals of oxamide were deposited immediately.

The author desires to tender to Principal Sir J. C. Irvine, F.R.S., his warmest thanks for the interest he has taken in this work and for constant criticism and advice.

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