485. *The Enzymic Synthesis and Degradation of Starch. Part XXIII.* Structural Requirements of D-Enzyme with Respect to Acceptors.*

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The capacities have been tested of nearly fifty sugars, sugar derivatives, and related compounds to act as acceptors of glycosyl radicals transferred from maltodextrins by the agency of potato D-enzyme. Acceptor activity shows a large measure of specificity. The most effective is D-glucose; only nine other substances showed appreciable activity.

It is shown by the use of methyl α-D-glucopyranoside as acceptor, that it is the $C_{(4)}$-hydroxyl group of the acceptor which receives the transferred glycosyl radical. This reaction has been used for the preparation, pure for the first time, of methyl α-maltoside, α-maltotrioside, and α-maltotetraoside.

IN Part XX [1] we reported the preparation and properties of potato D-enzyme, which catalyses disproportionating reactions between maltodextrins by the simultaneous scission and synthesis of α-1 : 4-linkages. For example, a maltosyl radical can be transferred from one maltotriose molecule to another, giving glucose and maltopentaose. This reaction seems to be freely reversible since it was possible to incorporate [^{14}C]glucose into maltodextrins by incubation with D-enzyme and maltopentaose. The participation of glucose in the reaction can also be recognised by the observation of iodine-staining power. Maltopentaose and higher dextrins are disproportionated to chain products which are sufficiently long to form red iodine complexes; [1] the addition of glucose to such a digest in equilibrium causes the staining-power to disappear, presumably because the longer chains are shortened by transfer of segment to glucose. Similarly, when amylopectin is incubated with D-enzyme in the presence of glucose the staining power diminishes by a much greater amount than it does in its absence. Other substances exhibit this property, *e.g.* methyl α-glucoside, mannose, and xylose, presumably because of similarities between their structures and that of glucose. In an attempt to elucidate the mechanism of D-enzyme action, the activities of 47 sugars, glycosides, and sugar alcohols were examined as acceptors in the D-enzyme catalysed transfer. The method of testing was by iodine-staining after the substance had been added to an equilibriated D-enzyme digest of maltodextrins.

TABLE 1. *D-enzyme disproportionation. Activities of some carbohydrates as acceptors.*

Compounds with acceptor activity: (D-glucose = 100, equimolar basis):

Methyl α-D-glucoside (37), methyl β-D-glucoside (12), D-mannose (13), L-sorbose (10), methyl α-L-sorboside (5), D-xylose (10), maltose (11), sucrose (6), leucrose (34), αα-trehalose (10).

Compounds with no acceptor activity:

(*a*) Isomaltose, maltulose, melezitose; α-D-glucose 1-phosphate.
(*b*) Methyl 2 : 6-di-*O*-methyl-β-D-glucoside; cellobiose, gentiobiose, laminaribiose, melibiose, lactose, raffinose; salicin.
(*c*) Glycerol, DL-erythritol, D-arabitol, D-mannitol, D-sorbitol, *i*-inositol, DL-glyceraldehyde.
(*d*) 3-, 6-, 2 : 3-Di-, 2 : 3 : 6-tri- and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose; 1 : 2-5 : 6-di-*O*-*iso*propylidene-D-glucose; D-glucose 6-phosphate; levoglucosan, 1 : 6-anhydro-α-D-glucofuranose.
(*e*) D-Fructose, D-galactose, D-arabinose, L-arabinose, D-rhamnose, L-fucose.
(*f*) D-Glucosamine hydrochloride, *N*-acetyl D-glucosamine.

The results are shown in Table 1, the relative efficiencies in causing a fall in iodine-staining power being computed on a molar basis. Such comparison assumes that only one hydroxyl group per acceptor molecule participates in the reaction. Having regard to the

* Part XXII, *J.*, 1956, 3025.

[1] Part XX, *J.*, 1956, 44.

structures of the substances showing positive response, this assumption is probably true for all with the possible exception of αα-trehalose. It is considered that any substance causing a fall in iodine-staining power greater than 5% (glucose, 100%) has significant acceptor activity. There were nine such substances, the most efficient being methyl α-D-glucoside. Although the evidence is insufficient to indicate the precise structural features required in an acceptor, attention may be drawn to certain points :

(1) The ideal acceptor appears to be α-D-glucopyranose and any departure from this structure is accompanied by a diminution, or by a complete loss, of acceptor activity. The most potent acceptor, after glucose, is methyl α-D-glucopyranoside (37%). It is suggested that the α-form of free glucose is more effective than the β-form because methyl α-glucoside is a better acceptor than is methyl β-glucoside (12%).

(2) Only a limited number of α-glucosides function as (weak) acceptors. These are maltose (11%), αα-trehalose (10%), and sucrose (6%). The α-glucosides, isomaltose, maltulose, melezitose, and glucose 1-phosphate, display no activity. Leucrose [*O*-α-D-glucopyranosyl-(1 ⟶ 5)-D-fructose] [2] appears to be a good acceptor (34% of the activity of glucose). Our specimen of leucrose was chromatographically pure and no explanation of its relatively high activity can be offered at present.

(3) Of those tested, the only β-glucoside which showed any acceptor activity was methyl β-D-glucoside (12%).

(4) The pyranose ring is an essential part of the acceptor structure. All the sugar alcohols tested, including D-sorbitol, were inactive, as was DL-glyceraldehyde.

(5) Substitution at any of the carbons atoms of glucose, apart from $C_{(1)}$, leads to complete loss of acceptor activity.

(6) Departure from the configuration of D-glucose also leads in most cases to complete loss of activity. The exceptions are D-mannose (13%), D-xylose (10%), and L-sorbose (10%), but even in these cases acceptor activity is seriously impaired. It may be significant to note that D-fructose, which might be considered closer in configuration to D-glucose than is L-sorbose, is devoid of acceptor activity.

There are four ways in which D-enzyme could transfer a portion of a maltodextrin chain to the acceptor. These are illustrated diagrammatically below. The donor substrate is N–R, N containing the non-reducing end and R the reducing end of the maltodextrin, and the acceptor substrate is A. If we consider the case in which A is glucose then there are two ways in which a compound of A and N could be formed. In the first (eqn. 1) the N–R bond is broken and N is joined through its liberated reducing group to the $C_{(4)}$-hydroxyl group of the glucose acceptor. Alternatively (eqn. 2), the glucose acceptor could be joined through its reducing group to $C_{(4)}$ of the non-reducing end glucose unit of the transferred fragment, N. The third and fourth reactions proceed in similar fashion except that the transferred portion of the donor is now the reducing fragment R.

$$N-R+A \rightleftharpoons N-A^{*}+R \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$N-R+A \rightleftharpoons A-N^{*}+R \quad \ldots\ldots\ldots\ldots \quad (2)$$

$$N-R+A \rightleftharpoons R-A^{*}+N \quad \ldots\ldots\ldots\ldots \quad (3)$$

$$N-R+A \rightleftharpoons A-R^{*}+N \quad \ldots\ldots\ldots\ldots \quad (4)$$

(Reducing end of product marked with asterisk.)

In this type of transglycosylation it has been generally assumed that the reaction proceeds according to eqn. (1), and indeed equations (2) and (4) can be eliminated on the basis of our experiments on acceptor specificity which show that transfer to methyl α-glucoside occurs although $C_{(1)}$ is blocked by the methyl group. It seemed likely that the transfer occurred to the $C_{(4)}$-hydroxyl of the methyl glucoside, in which case incubation of a donor

[2] Stodola, Sharpe, and Koepsell, *J. Amer. Chem. Soc.*, 1956, **78**, 2514.

substrate with D-enzyme and methyl α-glucoside should give methyl α-glucosidic derivatives of the maltodextrins. This experiment was carried out on a large scale with amylopectin as donor substrate. The products were fractionated by charcoal–Celite chromatography, and paper chromatography showed each fraction (di-, tri-saccharides, etc.) to consist of a reducing and a non-reducing substance. The reducing substances probably arose from the action of R-enzyme [3] and/or amylase on the linkages of the amylopectin. It is known that our D-enzyme preparations contained R-enzyme and a trace of amylase. Purification of the non-reducing di-, tri-, and tetra-saccharides was achieved by paper chromatography. The products (amorphous) were examined for specific optical rotation, methoxyl content, periodate uptake, and formic acid liberation, as well as for R_f value.

The methoxyl contents and periodate oxidation data (Table 2) agreed well with the values calculated on the assumption that the non-reducing di-, tri-, and tetra-

TABLE 2. *Properties of methyl α-maltoside, α-maltotrioside, and α-maltotetraoside.*

	R_f *		$[\alpha]_D^{18}$ in	MeO (%)		IO_4^- oxidn. Moles consumed		IO_4^- oxidn. Moles $H{\cdot}CO_2H$ produced		Priming
Glycoside	(*a*)	(*b*)	H_2O	Found	Calc.	Found	Calc.	Found	Calc.	activity †
α-Maltoside	0·37	0·19	+180°	8·68	8·75	3·06	3·0	1·02	1·0	0·1 (0)
α-Maltotrioside ...	0·28	0·09	+200	5·89	5·99	3·93	4·0	1·01	1·0	2·3 (2·1)
α-Maltotetraoside...	0·21	0·04	+213	4·58	4·56	5·02	5·0	1·01	1·0	9·0 (9·1)

* (*a*) R_f values in propan-1-ol–ethyl acetate–water (6 : 1 : 3); (*b*) R_f values in butan-1-ol–acetic acid–water (4 : 1 : 5).

† Towards phosphorylase–glucose 1-phosphate. Amounts of inorganic phosphate liberated, expressed in terms of colorimeter units (see Experimental section). Priming activities of equimolar amounts of the corresponding free sugars, measured at the same time, are given in parentheses.

saccharides were methyl α-maltoside, α-maltotrioside, and α-maltotetraoside. When plotted against degree of polymerisation both the molecular optical rotations and the $R_M\{= \log[(1/R_F) - 1]\}$ values showed a linear relation, indicative of uniformity of linkage.[4–7] The high optical rotations show that the polymeric linkages have the α-configuration. The periodate oxidation data eliminate the possibility of 1 : 3- or 1 : 6-linkages, leaving α-1 : 4- and α-1 : 2-linkages as the only possible types. Having regard to the mode of formation of these substances and the fact that they are degraded by starch-metabolising enzymes (see below) there is little doubt that the α-1 : 4-link is the only polymeric bond present. We are led to conclude therefore that these products are of the expected constitution and that D-enzyme action must proceed according to eqn. (1) or (3). Evidence will be presented later that eqn. (1) represents the course of the reaction.

It was of interest to compare the properties of the methyl maltodextrins with those of the free sugars in respect of the actions of various starch-metabolising enzymes. The priming activities towards the phosphorylase–glucose 1-phosphate system are closely similar to those of the corresponding free sugars.[8] That is, methyl α-maltoside had no priming activity, the trioside weak activity, and the tetraoside the same strong activity as maltotetraose (Table 2). β-Amylolysis of methyl α-maltotetraoside yielded maltose and methyl α-maltoside. Under the same conditions of low enzymic activity the methyl maltoside and maltotrioside were not attacked. The behaviour of the glycosides is therefore identical with that of the corresponding free maltodextrins.[4,9] Crystalline salivary

[3] Part XIV, *J.*, 1951, 1451; Part XIX, *J.*, 1954, 4440.
[4] Whelan, Bailey, and Roberts, *J.*, 1953, 1293.
[5] French, *Adv. Carbohydrate Chem.*, 1954, **9**, 149.
[6] Lindberg and McPherson, *Acta Chem. Scand.*, 1954, **8**, 985.
[7] Haq and Whelan, *J.*, 1956, 4543.
[8] Whelan and Bailey, *Biochem. J.*, 1954, **58**, 560.
[9] Whelan and Roberts, *ibid.*, 1954, **58**, 569.

α-amylase slowly attacked the methyl maltotrioside to give glucose and methyl maltoside, but maltose and methyl α-glucoside were not formed. This is in contrast to the action of α-amylase on maltotriose according to the experiments of Pazur and Budovich [10] which show that both linkages in maltotriose can be attacked by α-amylase, the more susceptible link being that which in the methyl maltotrioside is not readily attacked. α-Amylolysis of methyl α-maltotetraoside yielded large amounts of maltose and methyl maltoside together with small amounts of maltotriose and methyl glucoside. In agreement with Pazur [11] we found that maltotetraose yielded maltose as the major product, with small amounts of maltotriose and glucose. Whelan and Roberts in a similar experiment [12] failed to detect the formation of glucose and maltotriose.

Experimental

Measurement of Activity of D-Enzyme.—The measurement made was that of glucose liberated during the action of D-enzyme in maltotriose. The digest contained maltotriose (60 mg.), 0·2M-citrate buffer (pH 7·0; 0·3 ml.), and enzyme in a total of 3 ml. After incubation for 30 min. at 35° the enzyme was inactivated by heat, and the digest transferred to a charcoal–Celite column (1 : 1, w/w; 6·0 × 1·5 cm.) and eluted with water. The first 5 ml. of eluate were discarded. The next 25 ml. were collected, passed through a Seitz filter, and the optical rotation of the eluted glucose measured in a 4-dm. tube. A linear relation was observed between glucose liberated and concentration of enzyme in the range 0—9 mg. of glucose. One unit of enzyme was defined as the amount required to liberate 1 mg. of glucose under these conditions.

Preparation of D-Enzyme.—The method used is based on observations made by the late Mr. G. W. F. Kroll. These are (i) that the addition of a small amount of copper sulphate to the ammonium sulphate used in precipitating D-enzyme from potato juice effectively eliminates phosphorylase activity, and (ii) that repeated precipitation of the D-enzyme fraction with ammonium sulphate largely destroys its Q-enzyme activity.

To charcoal-clarified potato juice [1] (540 ml.) was added copper sulphate (1 g.) and 50% (w/w) ammonium sulphate solution (pH 7·0; 460 ml.) giving a final ammonium salt concentration of 23% (w/v). After the solution had been kept for 24 hr. at 2° the precipitate was removed on the centrifuge, dissolved in 0·01M-citrate buffer (pH 7·0, 250 ml.), and precipitated by the addition of ammonium sulphate to 20% (w/v) concentration. After 24 hr. at 2° the precipitate was removed, redissolved, and re-precipitated twice, as before except that it was kept for only 2 hr. before centrifugation. The final product was taken up in 0·2M-citrate buffer (pH 7·0; 50 ml.) and freeze-dried. The amounts of enzyme obtained after the second, third, and fourth precipitations were 1720, 1370, and 1036 units, respectively, while the specific activities, expressed as units of enzyme/mg. of protein nitrogen were 1·05, 1·78, and 2·33, respectively.

Acceptor Specificity of D-Enzyme.—The donor substrate, a mixture of maltodextrins ranging in degree of polymerisation from about 5 to 10, was prepared as follows. Potato amylose [13] (5·6 g.) was dissolved in warm 0·7N-sodium hydroxide (170 ml.), the solution neutralised to phenolphthalein with 3N-sulphuric acid, and a further 30 ml. of acid added. The solution was heated on a boiling-water bath until a small portion gave no colour with iodine. Then the solution was cooled quickly, neutralised (NaOH), and transferred to a charcoal–Celite column (1 : 1, w/w; 43 × 7·5 cm.), which was irrigated with water (2 l.) and then 20% aqueous ethanol until optically active material was no longer desorbed. Thereafter 50% ethanol was used as the eluant and the optically active eluate so obtained was evaporated to dryness, and inorganic matter largely removed by dissolving the dextrins in 80% aqueous methanol, and filtering and again evaporating the solution, to yield about 1 g. of product.

D-Enzyme (1036 units, see above) was dissolved in 0·2M-citrate buffer (pH 7·0; 50 ml.) and a portion (5 ml.) was incubated at room temperature with the mixed maltodextrins (300 mg.; 10 ml.). Portions (0·2 ml.) of the digest were added to iodine solution (0·005% in 0·05% of potassium iodide solution; 6 ml.) and the absorption value recorded in an E.E.L. colorimeter having 1·3 cm. diameter cells and a no. 404 filter (wavelength of peak transmission, 510 mμ).

[10] Pazur and Budovich, *Science*, 1955, **121**, 702.
[11] Pazur, *J. Biol. Chem.*, 1953, **205**, 75.
[12] Whelan and Roberts, *J.*, 1953, 1298.
[13] Part XIII, *J.*, 1951, 801.

After digestion for 5 hr. the solution had constant absorption value, and portions (0·2 ml.) were added to weighed amounts (approx. 10 mg.) of the acceptor substance in colorimeter tubes, which were kept for 13 hr. at constant temperature before being treated with iodine solution (6 ml.), and the light absorption measured. The values so obtained were compared with that of a digest portion to which no acceptor had been added and the fall in iodine stain, if any, was compared on a molar basis with that induced by glucose, reckoned as 100% (see Table 1). It had been shown that a direct proportionality existed between weight of glucose added and fall in iodine-staining power, within the weight range of sugar concentration employed.

Action of D-Enzyme on Amylopectin and Methyl α-Glucoside.—A solution of potato amylopectin [13] (24 g.) in warm 0·1N-sodium hydroxide (750 ml.) was cooled, neutralised (phenolphthalein) with N-sulphuric acid, and diluted to 1950 ml. Methyl α-glucoside (20 g.) was then added, followed by D-enzyme (prepared as above from 5 kg. of " Majestic " potatoes) in 50 ml. of 0·2M-citrate buffer (pH 7·0). The digest was incubated at 20° with one of similar composition (10 ml.) except for the omission of methyl α-glucoside. Absorption values of iodine-stained portions (0·2 ml.) were measured as before, by use of an E.E.L. colorimeter with an Ilford filter no. 608 (peak transmission, 680 mμ). Expressed as a percentage of the original absorption the values after incubation for 6, 17½, 39, 66, and 90 hr. were, respectively (glucoside digest given first), 84·5, 93; 74·5, 89·5; 64·5, 85·5; 57·5, 82·5; 52·5, 81%. At 90 hr. ethanol (8 l.) was added to the main digest and 1 hr. later the precipitate was removed on the centrifuge and washed with 80% ethanol (2 l.), and the combined supernatant liquid filtered and evaporated to dryness at 40°. The residue was dissolved in water (250 ml.) and a portion examined by paper chromatography in propan-1-ol–ethyl acetate–water (6 : 1 : 3, by vol.). When sprayed with silver nitrate–sodium hydroxide solution [14] there were revealed a series of reducing and a series of non-reducing sugars. The reducing sugars correspond to glucose and the maltodextrins. The reducing power of the solution was equivalent to 416 mg. of glucose (from 24 g. of amylopectin). At this stage we attempted to oxidise the reducing sugars with alkaline iodine by Kline and Acree's method,[15] barium hydroxide being used in place of sodium hydroxide, in the hope that the methanol-insoluble barium salts of the sugar acids could be removed. However, only half the expected amount of iodine was consumed. The pH was then adjusted to 6·0 (N-sulphuric acid) and the solution taken to dryness and extracted with hot methanol (2·5 l.). The evaporated extract was dissolved in water (150 ml.); chromatographic examination showed that reducing sugars were still present. Consequently the solution was adsorbed on charcoal–Celite (1 : 1, w/w; 165 × 6 cm.) and eluted by the gradient method,[16] a reservoir of water (20 l.) above the column being kept at constant level by the addition of 40% ethanol. Fractions (250 ml. each) were collected and their optical rotation measured in a 4-dm. tube. The optical rotation returned to zero after each of the first three peak values was attained. Thereafter the resolution became increasingly less complete. The eluate fractions were combined in 9 batches which displayed the following properties (for the purposes of easier description the substances revealed by paper chromatography are named in terms of their subsequently identified structure): (*a*) 4·2—6·4 l. of eluate in order of collection, 12·1 g. of methyl α-glucoside; (*b*) 9·0—10·0 l., 0·80 g. of methyl α-maltoside + maltose; (*c*) 10·0—12·2 l., 1·81 g. of methyl α-maltoside and a trace of maltose; (*d*) 13·9—17·8 l., 2·10 g. of methyl α-maltotrioside + maltotriose; (*e*) 18·5—20·6 l., 1·10 g. of methyl α-maltotetraoside + maltotetraose; (*f*) 20·9—22·3 l., 0·75 g. of methyl α-maltotetraoside, α-maltopentaoside, and a trace of maltopentaose; (*g*), (*h*), and (*i*), 22·3—28·2 l., total wt. 1·52 g. of methyl α-maltopentaoside, maltopentaose, and higher reducing and non-reducing oligosaccharides. Separation of the non-reducing from reducing sugars was achieved by chromatography of portions of batches (*b*)—(*e*) on Whatman no. 3 paper in butan-1-ol–acetic acid–water (4 : 1 : 5, by vol.). In this way chromatographically pure specimens of methyl glycosides of the di-, tri-, and tetrasaccharides were obtained. Solutions in hot 80% methanol were filtered and taken to dryness, the residues dissolved in water (50 ml.), such solutions treated with Somogyi's deproteinising reagents [17] and freeze-dried, and the products finally dissolved in 50 ml. of water. All measurements of properties of the sugars were made on portions taken from these solutions.

Properties of the Methyl Maltodextrins.—The amounts of dextrin present were estimated by

[14] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[15] Kline and Acree, *Ind. Eng. Chem. Anal. Edn.*, 1930, **2**, 413.
[16] Alm, *Acta Chem. Scand.*, 1952, **6**, 1186.
[17] Somogyi, *J. Biol. Chem.*, 1945, **160**, 69.

acid hydrolysis [18] to glucose. The molecular weights of the dextrins being assumed, the final weights of purified materials were: methyl α-maltoside, 47·5 mg.; methyl α-maltotrioside, 132 mg.; and methyl α-maltotetraoside, 254 mg.

(a) *Reducing power.* When heated with Somogyi reagent [19] for 45 min., portions (5 mg.) of the malto-trioside and -tetraoside developed no detectable reducing power while the methyl maltoside had reducing power equivalent to 1% of that of an equal weight of maltose.

(b) *Methoxyl content.* Volumes of solution containing from 9 to 25 mg. of glycoside were taken to dryness in the reaction vessel of the apparatus described by Milton and Waters.[20] Methoxyl content was then determined as described by Belcher, Fildes, and Nutten [21] (see Table 2).

(c) *Periodate oxidation.* The glycosides were oxidised in 12·5mM-sodium metaperiodate, in the dark at room temperature. Consumption of periodate was measured after 24 hr. and 48 hr. by adding portions of digest to acidified potassium iodide and titrating with 0·05N-thiosulphate. Formation of formic acid was measured at the same times by addition of neutral potassium iodide to portions of digest after removal of periodate with ethylene glycol. 5mN-Thiosulphate was used to titrate the iodine liberated. Oxidation was complete after 24 hr. (Results are given in Table 2.)

(d) *Action of enzymes.* In the test for phosphorylase priming activity the digests contained glucose 1-phosphate (2 ml.; 0·1M; pH 7·07), citrate buffer (1 ml.; pH 7·0), ammonium molybdate (0·1 ml., 8·3%), mercuric chloride [22] (0·1 ml., 0·004%), methyl glycoside (1 μmole), freeze-dried potato phosphorylase [8] (1 ml., 50 mg.), and water to 6 ml. The enzyme solution (at 35°) was added last to the mixture of components maintained at 35°. A primer-free digest was also included. After 30 min., a portion (0·2 ml.) was removed from each digest for measurement of inorganic phosphate by Whelan and Bailey's method.[8] The results given in Table 2 are corrected for the phosphate content of the primer-free digest.

For α-amylolysis portions (20 mg.) each of the trioside, tetraoside, maltotriose, and maltotetraose were incubated separately for 30 min. at 30° in 2 ml. of 0·05M-acetate buffer (pH 7·0) containing 1 drop of a suspension of crystalline human salivary α-amylase,[23] kindly provided by Professor Jytte Muus. The enzyme was inactivated by heat and paper chromatographic examination was carried out in propan-1-ol–ethyl acetate–water as described above.

β-Amylolysis was carried out as for α-amylolysis except that the pH was 4·8. The enzyme was purified soya-bean β-amylase,[24] in amount insufficient to hydrolyse the maltotriose during 30 minutes' incubation (cf. Whelan, Bailey, and Roberts [4]).

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[18] Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224.
[19] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.
[20] Milton and Waters, "Methods of Quantitative Micro-Analysis," Edward Arnold, London, 1949, p. 113.
[21] Belcher, Fildes, and Nutten, *Analyt. Chim. Acta*, 1955, **13**, 16.
[22] Bailey, Thomas, and Whelan, *Biochem. J.*, 1951, **49**, lvi.
[23] Muus, *Compt. rend. Trav. Lab. Carlsberg*, 1953, **28**, 317.
[24] Part XVI, *J.*, 1952, 714.