compared with the figures from two control cows receiving exactly the same dietary treatment.

2. The pituitary preparation increased the vitamin A alcohol output in the milk whether greenstuff was present in the diet or not.

3. When there was no greenstuff in the diet the output of vitamin A alcohol in the milk increased and this increase was enhanced in the hormonally treated cows.

4. In the absence of green food there was no vitamin A ester in the blood and yet the output of vitamin A ester in the milk was also increased by the hormone preparation so that the hormonal treatment increased the rate of esterification of vitamin A by the mammary gland.

5. When greenstuff was present in the diet the blood contained vitamin A ester in amounts which were increased by the hormonal treatment.

6. The hormonal treatment increased the vitamin A alcohol in the blood serum whether or not there was greenstuff in the diet.

7. The results lend weight to the hypothesis that, like thyroxine, the hormone preparation accelerated the mobilization of hepatic reserves as vitamin A alcohol into the blood and hence into the milk as alcohol and ester. There was also some evidence that, like thyroxine, the hormone preparation accelerated the transformation of dietary carotene into vitamin A in the gut.

8. The results provided evidence for the hypothesis that vitamin A ester in the milk could, when the diet contained carotene, have either a hepatic or a dietary origin according as it came from the alcohol or the ester of vitamin A circulating in the blood.

The authors wish to thank Miss P. McGuigan and Miss S. McLauchlan for technical assistance and Miss J. Tyers for assistance in the care of the cows. One of us (R. C.) wishes to acknowledge a grant which he received from the Agricultural Research Council during the course of these experiments.

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Transglucosidation in *Penicillium chrysogenum* Q-176

ISOLATION AND IDENTIFICATION OF THE OLIGOSACCHARIDES

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The non-phosphorolytic oligo- and poly-saccharide-synthesizing enzymes have recently been studied in great detail. Amylomaltase (Doudoroff, Hassid, Putman, Potter & Lederberg, 1949; Torriani & Monod, 1949), amylosucrase (Hehre, 1949), cyclodextrinase (Norberg & French, 1950), dextran sucrase and levan sucrase (Hassid & Doudoroff, 1950), dextran dextrinase (Hehre, 1951), *Q*-enzyme (Nussenbaum & Hassid, 1951) and transfructosidase (Edelman & Bacon, 1951) have all been shown to fall in with the general class of 'transglycosidases', the name being suggested by Doudoroff, Barker & Hassid (1947). Yet another transglucosidase, capable of synthesizing branched-chain oligosaccharides from maltose has been reported in *Aspergillus niger* (Pan, Andreasen & Kolachov, 1950),

A. oryzae (Pazur & French, 1951), barley malt and *Bacillus subtilis* (Pan, Nicholson & Kolachov, 1953). Pazur & French (1952) have identified several of the products of synthesis. The presence of a similar enzyme in growing cultures of *Penicillium chrysogenum* Q-176 has been reported by us (Giri, Narasimha Rao, Saroja & Venkataraman, 1953; Giri, Saroja, Venkataraman & Narasimha Rao, 1954). The enzyme has since been prepared from the culture filtrate and the reaction studied in greater detail, resulting in the identification of several of the oligosaccharides synthesized by the enzyme. The results of these investigations are presented in this paper.

METHODS

Organism and growth medium. The enzyme preparations were obtained from 5-day-old culture filtrates of *P. chrysogenum* Q-176 grown as described in a previous publication (Giri *et al.* 1954).

Preparation of the enzyme. At the end of 5 days, the mycelium was removed by filtration and centrifuging and the clear supernatant (pH 6·5) was used as the source of the enzyme. Traces of amylase present were removed by treatment with sweet-potato starch for 30 min. at 0° and filtering. The enzyme was precipitated by the addition of absolute ethanol to 80% final concentration at 0–4°. The precipitate was centrifuged, washed with absolute ethanol and ether and dried over $CaCl_2$ *in vacuo*. The enzyme was obtained as a white hygroscopic amorphous powder.

Preparation of the oligosaccharides. The aqueous solution (25 ml.) of the enzyme (1%, w/v) was incubated with 20% (w/w) maltose (75 ml.) containing 0·2 M Walpole's acetate buffer pH 5·4 (50 ml.) at 25°. Toluene was added to avoid microbial infection. After 72 hr. the enzyme was destroyed by heating the digest to 70° and the qualitative carbohydrate composition of the digest was ascertained by radial paper chromatography (Giri & Nigam, 1953, 1954).

Isolation of the oligosaccharides. The digest was diluted with a synthetic medium for the growth of yeast (Olson & Johnson, 1949) and sterilized by steaming for 30 min. on 3 consecutive days. Glucose and maltose were removed by inoculating aseptically a thick suspension of distiller's yeast in physiological saline, and allowing the fermentation to proceed for 48 hr. Proteins were precipitated by basic lead acetate and the filtrate was fractionated on a column of charcoal (Norit A, Pfanstiehl Chemical Co., U.S.A.) by the elution technique of Whistler & Durso (1950). Fractions I–VII were collected by successive elutions with 0, 2·5, 5, 7·5, 10, 15 and 25% (v/v) aqueous ethanol. The different fractions were concentrated separately and passed successively through columns of ion-exchange resins Amberlite IR-100 and IR-4 B. Each solution was then concentrated to a syrup *in vacuo* and treated repeatedly with absolute methanol, when the sugars were obtained as white amorphous powders.

Hypoiodite oxidation. The molecular weights of the oligosaccharides were determined by hypoiodite oxidation according to the method of Hirst, Hough & Jones (1949), for three different samples of each oligosaccharide.

Periodate oxidation of the oligosaccharides. The sugars (50 mg.) were individually subjected to periodate oxidation in 0·05 M sodium metaperiodate solution. The method followed was essentially that of Halsall, Hirst & Jones (1947). The periodate consumed was determined by an iodimetric procedure, using 0·05 N sodium thiosulphate. In another sample, excess periodate was destroyed by purified ethylene glycol and formic acid was titrated against 0·01 N-NaOH using methyl red as indicator. Formaldehyde was determined by precipitation with dimedon after destroying excess periodate by sodium arsenite (Reeves, 1941).

RESULTS

The qualitative carbohydrate composition of the digest was obtained by spraying the circular paper chromatogram with aniline–diphenylamine–phosphate (Giri & Nigam, 1953, 1954). The products of enzymic reaction revealed themselves as alternately spaced blue and yellowish brown bands. It appears as though in the oligosaccharides having 1:4- and 1:6-linkages, the formation of a blue band is characteristic of the presence of one or more 1:4-linkages in the molecule, whereas the brown bands are those corresponding to oligosaccharides containing 1:6-linkages only. The formation of seven oligosaccharides in addition to glucose and maltose was observed (Fig. 1).

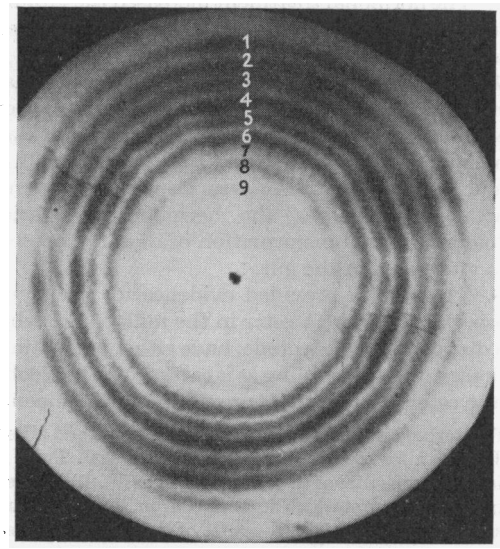

Fig. 1. Circular paper chromatogram showing the formation of oligosaccharides from maltose by the transglucosidase in *P. chrysogenum* Q-176. (1) Glucose; (2) maltose; (3) isomaltose; (4) panose; (5) isomaltotriose; (6) 4-α-isomaltotriosyl-D-glucose; (7) isomaltotetraose; (8) 4-α-isomaltotetraosyl-D-glucose; (9) 4-α-isomaltopentaosyl-D-glucose. Although band no. 9 is not visible in the photograph, it was nevertheless clearly present in the original chromatogram.

Nature of fractions I–VII collected by elution of the carbon column with different concentrations of aqueous ethanol. The first two fractions were free of sugar. The 5% eluate gave the major portion of isomaltose. Isomaltotriose was eluted by 10% and panose by 15% ethanol. These two fractions had to be purified by a second chromatographic separation. The last fraction contained a mixture of higher oligosaccharides.

Characterization of the oligosaccharide fractions. The third fraction was chromatographically pure isomaltose, yielding a white hygroscopic solid not be separated satisfactorily into individual components. A small quantity of a single saccharide (0·1 g.), however, was obtained by controlled elution with 20% ethanol and collection of the middle fraction only, repeating the process three times. This was identified as 4-α-isomaltotriosyl-D-glucose by periodate oxidation. Controlled acid hydrolysis gave glucose, maltose, isomaltose, panose and isomaltotriose. R_F values of these and the other oligosaccharides using radial chromatography on Whatman no. 3 paper with *n*-butanol–pyridine–water (6:4:3, by vol.) solvent and single

Table 1. *Properties of oligosaccharides synthesized from maltose by transglucosidase of* Penicillium chrysogenum Q-176

R_F values determined as in text. The values for glucose and maltose refer to authentic specimens and not to products from the enzyme digest. Calculated values are given in parentheses.

Sugar	R_F value	Mol. wt. (hypoiodite oxidation)	Periodate oxidation values (moles/mole)		
			Periodate consumed	Formic acid liberated	Formaldehyde liberated
Glucose	0·46	179·8	4·9 (5)	4·95 (5)	0·95 (1)
Maltose	0·36	338·8	5·04 (5)	3·18 (3)	1·02 (1)
Isomaltose	0·30	337·1	6·1 (6)	4·89 (5)	<0·05 (0)
Panose	0·23	508·9	7·11 (7)	3·84 (4)	1·04 (1)
Isomaltotriose	0·18	500·8	8·2 (8)	5·94 (6)	<0·05 (0)
4-α-Isomaltotriosyl-D-glucose	0·13	682·2	9·3 (9)	5·2 (5)	0·97 (1)
Isomaltotetraose	0·10	—	—	—	—
4-α-Isomaltotetraosyl-D-glucose	0·07	—	—	—	—
4-α-Isomaltopentaosyl-D-glucose	0·04	—	—	—	—

(*ca.* 2·1 g.), $[\alpha]_D^{25} + 103°$ in water (*c*, 2·281); β-octa-acetate, $[\alpha]_D^{25} + 97°$ in $CHCl_3$ (*c*, 2·0); phenylosazone, m.p. 152°. The β-octa-acetate and phenylosazone were prepared by methods described already (Giri, *et al.* 1954).

Fraction IV removed the last traces of isomaltose along with small quantities of isomaltotriose, and was therefore discarded.

Fraction V was chromatographically homogeneous and gave a single band which had the same R_F value as the isomaltotriose prepared by controlled acid hydrolysis of dextran, by 0·1 M-H_2SO_4 at 100° for 1 hr. On hydrolysis, only glucose was detected in the hydrolysate. The free sugar was a white powder (0·5 g.) having $[\alpha]_D^{25} + 144°$ in water (*c*, 3·75), which value agrees well with the value of Jeanes, Wilham, Jones, Tsuchiya & Rist (1953) for isomaltotriose.

Fraction VI contained panose and was purified by chromatographing twice on carbon. The resulting white powder (0·9 g.) had $[\alpha]_D^{25} + 152°$ in water (*c*, 3·0) (cf. +154°, Pan, Nicholson & Kolachov, 1951). On partial hydrolysis with 0·4 N-H_2SO_4 at 90° for 1 hr. glucose, maltose, isomaltose and the unchanged panose were detected by radial chromatography.

Higher oligosaccharides from fraction VII. These were found in comparatively lower yield and could development are given in Table 1. The other oligosaccharides which could not be isolated in pure form have been assigned tentative structures on the basis of their mobility on paper chromatograms in relation to other known saccharides, prepared by controlled acid hydrolysis of dextran and amylose.

Hypoiodite and periodate oxidation. The molecular weights of the oligosaccharides as determined by hypoiodite oxidation and values for periodate consumption, formic acid and formaldehyde production are given in Table 1.

Other properties. None of these oligosaccharides is attacked by sweet-potato β-amylase or soya-bean β-amylase. They are also non-priming to amylose synthesis by green-gram phosphorylase prepared by two successive precipitations of an aqueous extract of the powder with acetone as described by Ram & Giri (1952). The priming activity was determined by the method of French & Wild (1953*b*). These properties indicate the absence in the saccharides of two or more consecutive 1:4-linkages at the non-reducing end.

DISCUSSION

A close inspection of the R_F values (Table 1) of the oligosaccharides synthesized from maltose by *P. chrysogenum* Q-176 gives an insight to the nature of

several of the higher saccharides. According to French & Wild (1953*a*), the logarithm of the partition function α', defined by $\alpha' = R_F/(1-R_F)$, is a linear function of the molecular size for any particular series of oligosaccharides. In Fig. 2 are plotted the values of log α' against the numbers of hexose units in the oligosaccharides synthesized by the mould. The amylose series of oligosaccharides is given for reference. As a result of enzymic synthesis, the sugars formed fall into two distinct classes, viz. the dextran series (up to isomaltotetraose) and the panose series, the members falling alternately into either series in decreasing order of their R_F values. The sequence, however, fails with the last two members, both of which fall in linearly with the panose series, and may, therefore, be 4-α-isomaltotetraosyl-D-glucose and 4-α-isomaltopentaosyl-D-glucose. The slopes of the straight lines depend upon the nature of the repeating hexose unit, its type of linkage, anomeric form, etc. These must be the same for the two series synthesized, as the two lines are parallel. The difference, therefore, between the mechanism of formation of sugars belonging to the dextran and the panose series is only in the initial acceptor which is glucose for the former and maltose for the latter.

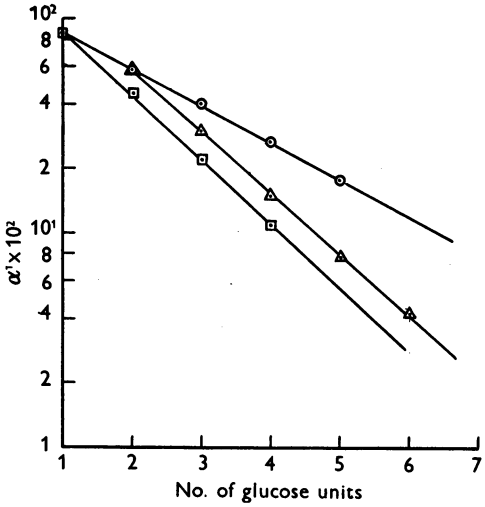

Fig. 2. Paper chromatographic mobilities of oligosaccharides produced by the action of transglucosidase from *P. chrysogenum* Q-176 on maltose. Amylose series included for reference only. See text for definition of α'. △, Panose series; ⊙, amylose series; ⊡, dextran series.

The experimental results obtained by periodate oxidation of the oligosaccharides are in good agreement with the configurations assigned to them, viz. isomaltose, panose, isomaltotriose and 4-α-isomaltotriosyl-D-glucose. The negative test for formaldehyde in the case of isomaltose and isomaltotriose even in an advanced stage of oxidation, and the large proportion of formic acid liberated in these cases in agreement with accepted theoretical values, confirm fully the presence of 1:6-linkages in these compounds. The correspondence of the $[\alpha]_D$ values of compounds 4 and 5 in Table 1 with those of panose and isomaltotriose respectively, points to an α- configuration of the 1:6-linkages in these compounds.

The oxidations of maltose, panose and 4-α-isomaltotriosyl-D-glucose did not terminate during the periods of measurement. The periodate consumption continued at a slower but definite rate with the liberation of formic acid. In these cases, the values quoted in Table 1 correspond to the cessation of the fast primary reaction. The cause of this secondary oxidation may be attributed to a similar mechanism to that proposed by Wolfrom, Thompson, O'Neill & Galkowski (1952) for the anomalous behaviour of maltitol towards periodate, viz. the formation of a reactive substituted malonaldehyde (Huebner, Ames & Bubl, 1946) from the reducing-end group containing the 1:4- linkage. The other saccharides containing only 1:6- linkages soon attain a stable oxidation limit because the reducing-end group is oxidized in these cases to a substituted acetaldehyde, and not a substituted malonaldehyde.

SUMMARY

1. A transglucosidase from *Penicillium chrysogenum* Q-176 catalyses the conversion of maltose into glucose and oligosaccharides.

2. Charcoal chromatography of the oligosaccharide mixture resulted in the separation of four components, identified as isomaltose, isomaltotriose, panose and 4-α-isomaltotriosyl-D-glucose, and a group of higher oligosaccharides, of which isomaltotetraose, 4-α-isomaltotetraosyl- and 4-α-isomaltopentaosyl-D-glucoses are possible constituents.

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The *N*-Terminal Groups of Salmine

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While measuring the absorption spectra in the range 230–450 mμ. of a series of 2:4-dinitrophenyl-amino acids (subsequently referred to as DNP-amino acids), it was found that DNP-L-proline and DNP-L-hydroxyproline had spectra quite unlike those of the derivatives of other amino acids found in proteins. This had been noted independently by Schroeder, Honnen & Green (1953) and by Rao & Sober (1954). To illustrate this, Fig. 1 shows the spectrum of DNP-proline (those of DNP-hydroxy-proline, DNP-proline methyl ester and DNP-sarcosine are similar) and, for comparison, the spectrum of DNP-threonine. Porter & Sanger (1948), using 1-fluoro-2:4-dinitrobenzene, found that proline was the *N*-terminal residue of salmine, but owing to the extreme instability of DNP-proline in hot acid a quantitative estimation was not possible.

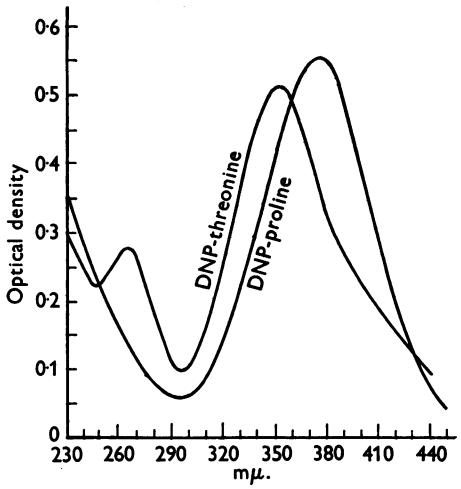

Fig. 1. The absorption spectra of DNP-L-proline and DNP-DL-threonine. Both solutions $31{\cdot}7 \times 10^{-6}$ M in 0·1 N-HCl.

The spectral findings above therefore suggested a new way of proving, from the shape of the spectrum of DNP-salmine, that proline is the *N*-terminal amino acid in the protamine. Moreover, since no hydrolysis is necessary, and as it is reported (Tristram, 1949) that there are no lysine, histidine, tyrosine or cystine residues in salmine, it is reasonable to assume a simple open-chain peptide structure, in which case the spectrophotometric estimation of the proportion of *N*-terminal amino acid should also give the molecular weight.

This paper describes experiments undertaken to throw light on these two problems.

MATERIALS AND METHODS

General. Salmine sulphate from keta salmon (British Drug Houses Ltd.) was used. As far as could be ascertained, the salmine came from mature *Oncorhynchus keta* caught in

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