125. *The Structure of Isolichenin.*

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Isolichenin is isolated in an improved yield from Iceland moss by a modification of the method of Chanda, Hirst, and Manners, whose report that isolichenin is constituted solely of glucose units united by 1,4- and 1,3-linkages is confirmed by the method of partial acid hydrolysis. It is further shown that these linkages have the α-configuration inasmuch as maltose and nigerose are components of the partial hydrolysate.

There is some lack of regularity in the distribution of the α-1,3- and α-1,4-links in isolichenin although the detection of nigerotriose and maltotriose in the trisaccharide fraction of the acid hydrolysate shows that both types of linkage can occur at least in pairs. No evidence is forthcoming that branching occurs in isolichenin.

It is further established that isolichenin is not identical with nigeran, in which there is an alternation of α-1,4- and α-1,3-links. Nevertheless the ratio of 1,3- to 1,4-links (55 : 45) in isolichenin is not far removed from that in nigeran (approx. 50 : 50).

THE lichenin of Iceland moss (*Cetraria islandica*) is accompanied by isolichenin, and it was early recognised that, while lichenin itself was cellulosic in character, isolichenin partook more of the nature of starch in respect of its iodine-staining power and its susceptibility to diastatic action. Indeed it was originally named " lichen-starch " and was regarded as a food-reserve material.[1] Later investigations [2,3] revealed that lichen-starch was a mixture of polysaccharides and in its acid hydrolysate mannose and galactose were found in addition to glucose. The name isolichenin is now restricted to the iodophilic polyglucose component of this mixture, from which it was separated in a pure state by Chanda, Hirst, and Manners.[4] Our specimen of isolichenin was prepared, in improved yield, by a modification of the method described by these authors, about which we had knowledge before its publication, through the courtesy of Professor E. L. Hirst.

The study reported here of the structure of isolichenin followed as a consequence of the investigation of lichenin [5] and the same method of linkage analysis was adopted, namely, examination of the products of partial acid hydrolysis.

The purified isolichenin (57 g. from 5 kg. of tannin-free moss) gave with iodine a grey-blue colour of low intensity (the blue value [6] was 0·005) and showed the exceptionally high specific rotation in water of $[\alpha]_D$ +272° (lichenin from the same moss had $[\alpha]_D$ +18·4° in 2N-sodium hydroxide [5]). Complete acid-hydrolysis yielded glucose as the sole carbohydrate product.

Partial Acid-hydrolysis.—The Kuhn formula [7] indicates that the maximum yield of di- and tri-saccharide fragments should be attained at an apparent conversion into glucose of about 45%. Accordingly, isolichenin (38 g.) was submitted to hydrolysis with 0·33N-sulphuric acid until the apparent conversion was 43%. The neutralised hydrolysate was fractionated by graded elution with ethanol on a charcoal–Celite column. The sole monosaccharide component of the hydrolysate was glucose.

The disaccharide fraction. Ionophoresis in borate buffer demonstrated the presence of two disaccharides, which were chromatographically separated on charcoal with an ethanol–borate eluant.[8] The first to be desorbed had a relatively high electrophoretic

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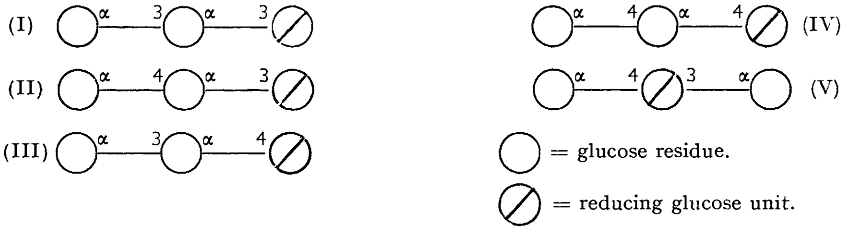
[1] Hönig and Schubert, *Monatsh.*, 1887, **8**, 452.
[2] Karrer and Joos, *Z. physiol. Chem.*, 1924, **141**, 311.
[3] Meyer and Gürtler, *Helv. Chim. Acta*, 1947, **30**, 761.
[4] Chanda, Hirst, and Manners, *J.*, 1957, 1951.
[5] Peat, Whelan, and Roberts, *J.*, 1957, 3916.
[6] Bourne, Haworth, Macey, and Peat, *J.*, 1948, 924.
[7] Kuhn, *Ber.*, 1930, **63**, 1503.
[8] Barker, Bourne, and Theander, *J.*, 1955, 4276.

mobility (M_G 0·55) and was shown by comparison of its properties, and those of its β-octa-acetate, with authentic specimens (Table 1) to be nigerose (3-*O*-α-glucosylglucose). The slower-moving component was proved in the same way to be maltose. The yield of nigerose (3·4 g.) was approximately double that of maltose (1·55 g.). These yields can, of course, not be regarded as more than a rough estimate of the relative proportions of α-1,3- and α-1,4-linkages in isolichenin since the yields of nigerose and maltose will be determined in part by the relative rates of hydrolysis of the α-1,4- and α-1,3-links, respectively. It is improbable that the two disaccharides are formed in these quantities by acid-reversion from glucose. They have been obtained as reversion products in very small amounts but they are accompanied by isomaltose and gentiobiose in substantially greater amounts.[9,10] Neither of these 1,6-linked disaccharides was detected in the isolichenin hydrolysate. It was thus established that in isolichenin the polymeric linkages are of α-1,3- and α-1,4-glucosidic types, and that very probably these are the only forms of glucosidic linkage.

The trisaccharide fraction. Paper chromatography having revealed the presence of at least two reducing trisaccharides with closely similar R_F values, the whole fraction was separated into two subfractions, *A* and *B*, by elution from a charcoal column, initially with ethanol–borate,[8] and subsequently with aqueous ethanol. Each of the fractions moved ionophoretically as a single substance, *A* with high (M_G 0·55) and *B* with low mobility (M_G 0·21).

Since the examination of the disaccharide fraction had shown only maltose and nigerose to be present, it could be inferred that the reducing-end linkage in *A* (high mobility) was of the 1,3-type and in *B* (low mobility) was the 1,4-type. When materials *A* and *B* were partly hydrolysed with acid and examined by ionophoresis the hydrolysate of *A* contained glucose (M_G 1·0) and a substance having M_G 0·21, presumably maltose, as well as the expected nigerose. The hydrolysate of *B* contained glucose and a substance having M_G 0·55, presumably nigerose, as well as the expected maltose. It was thus evident that both reducing trisaccharide subfractions contained the same linkages, *viz.*, an α-1,4- and an α-1,3-link, and differed only in the order and arrangement of these linkages.

If it is assumed that isolichenin has an unbranched structure, and there are some grounds for this, the possible trisaccharides derivable from it would be nigerotriose (I), 3-α-maltosylglucose (II), 4-α-nigerosylglucose (III), and maltotriose (IV).



The reducing end glucose units in (I) and (II) carry free hydroxyl groups at positions 2 and 4 and would therefore form strong borate complexes[11] showing relatively high electrophoretic mobility, whereas (III) and (IV), in which the 4-hydroxyl group is blocked, would form weak borate complexes with low electrophoretic mobility. It follows from the method of separation of the trisaccharides that *A* could be a mixture of (I) and (II), and *B* a mixture of (III) and (IV) if all four possible trisaccharides were present. Although the trisaccharide fractions *A* and *B* moved electrophoretically as if each were a single substance, the following observations made it evident that each was in fact a mixture of trisaccharides. The materials *A* and *B* were separately reduced with sodium borohydride to the corresponding trisaccharide alcohols, *RA* and *RB*. The reducing end glucose unit

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[9] Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309.
[10] Peat, Whelan, Edwards, and Owen, *J.*, 1958, 586.

in each of the four possible trisaccharides (I—IV) would by this procedure be converted into a non-reducing sorbitol unit. These alcohols will be designated (RI—RIV) respectively. The products of partial acid-hydrolysis of the trisaccharide alcohols would consist of reducing disaccharides and non-reducing disaccharide alcohols as well as glucose and sorbitol. (RI) would give nigerose and nigeritol; (RII) maltose and nigeritol; (RIII) nigerose and maltitol; and (RIV) maltose and maltitol. Under the conditions of hydrolysis employed, no assay of the disaccharide alcohols could be made, but it was shown that the alcohol *RA* yielded two reducing disaccharides which had the electrophoretic mobility (M_G) of maltose and nigerose, derived respectively from (RI) and (RII). Two reducing disaccharides, *viz.*, maltose and nigerose, were also obtained by the partial acid hydrolysis of the alcohol *RB* and these must have come from (RIV) and (RIII) respectively. It appears therefore that there exists some degree of lack of regularity in the distribution of α-1,3- and α-1,4-links in isolichenin, inasmuch as the four trisaccharides (I—IV) are integral parts of the chain molecule. It should be pointed out that the " branched " trisaccharide (V) was not detected in the partial hydrolysate of isolichenin and, although this negative evidence is not strong, it is an indication that the isolichenin chains are not branched.

Structure of Isolichenin.—The experiments reported here confirm the observations by Chanda *et al.*[4] that isolichenin is a polymer of D-glucose joined through 1,3-linkages and 1,4-linkages. It is further shown that these linkages are exclusively in the α-configuration and the arrangement of the linkages is to some extent shown by the components of the trisaccharide fraction of the partial hydrolysate. The proof that two trisaccharides containing 1,4- and 1,3-linkages were present means that both linkages occur in the same polymer molecule. The detection of nigerotriose and maltotriose among the trisaccharides means that the two types of linkage can occur at least in pairs. It is not apparent, however, whether any regularity exists in the structure but experiments to be reported later by Goldstein and Whelan show that a regular repeating sequence of linkages may well be present.

The slight action of α- and β-amylase on isolichenin, as shown by loss of iodine staining power, and the slight development of reducing power, could be cited as evidence for the occurrence of sequences of α-1,4-linkages in isolichenin, but an alternative explanation might well be that isolichenin is contaminated by a starch-like substance and it is this which is responsible for these effects. None of the stages of purification of the isolichenin would be expected to remove a starch component but fractionation experiments designed to do this are now being attempted.

These experiments establish that isolichenin is not identical with nigeran, which is also constituted of glucose units joined by α-1,4- and α-1,3-links only, and which shows an optical rotation (+283°) of the same high order as isolichenin.[12-14] Approximately half of the glucosidic links in nigeran are α-1,4- and the pattern is an ordered alternation of α-1,4- and α-1,3-links.

It will be noted that if the ratio of 1,3- to 1,4-links in isolichenin is also 1 : 1, then approximately equal weights of trisaccharides *A* and *B* would have been produced. Actually the weight ratio was 2 : 1, agreeing with the similar weight ratio of the disaccharides, nigerose : maltose.

A more accurate estimate of the ratio of 1,3- to 1,4-links can be obtained by oxidation of the polysaccharide with periodate. Accordingly, isolichenin was treated with unbuffered sodium metaperiodate at room temperature and in the dark. The initial rapid uptake of oxidant was followed by a much slower rate of overoxidation of the malonyl residue at the reducing end of the polysaccharide chain. The amount of periodate consumed at the

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[11] Foster, *J.*, 1953, 983.
[12] Barker, Bourne, and Stacey, *Chem. and Ind.*, 1952, 756.
[13] Barker, Bourne, and Stacey, *J.*, 1953, 3084.
[14] Barker, Bourne, O'Mant, and Stacey, *J.*, 1957, 2448.

point of inflexion in the oxidation curve was taken as a measure of the degree of normal oxidation. A glucose unit linked at position **3** as well as at position 1 is not susceptible to oxidation by periodate so that the uptake of periodate during the initial rapid oxidation must be due to the glucose members linked through $C_{(1)}$ and $C_{(4)}$ and, of course, to the end groups. On this basis, it is calculated that the ratio of 1,3- to 1,4-links in isolichenin is 55 : 45, a result in fair agreement with that found by Chanda *et al.* (60 : 40), also by periodate oxidation.[4] The discrepancy between this result and the weight ratio of nigerose to maltose (2 : 1) may be ascribed, as mentioned above, to a slower hydrolysis of the α-1,3-links and the consequent preferential accumulation of nigerose in the partial acid-hydrolysate.

EXPERIMENTAL

Purification of Isolichenin.—The water-soluble polysaccharides from Iceland moss were extracted by the method of Meyer and Gürtler,[15] which is originally due to Hess and Lauridsen.[16] Tannins were removed from air-dried Iceland moss (1 kg.) by six extractions, each for 24 hr., with cold 2% potassium carbonate solution (17 l.), and the moss was then washed with water until free from alkali.

The tannin-free moss (1 kg.) was extracted with water (6 l.) at 95—98° for 24 hr., and filtered hot through muslin. The residual moss was extracted twice with water (2 × 6 l.) in an autoclave at 15 lb. pressure for 1 hr.; this ensured almost complete extraction of iodine staining material (blue) from the moss. The filtrates were combined (total 18 l.).

The combined extracts from five 1 kg. batches of moss were frozen and thawed three times [17] and the lichenin was removed by filtration and centrifugation. The supernatant liquid was concentrated to a viscous liquid (7 l.) and dialysed in a Cellophane bag (6 cm. in diameter) against water to remove salts and possible saccharides of low molecular weight. The solution of impermeable materials was concentrated to 4 l. and the polysaccharides precipitated by the slow addition of acetone (5 l.). The precipitate, isolichenin A, was collected in a centrifuge, washed with acetone and alcohol, and hardened by grinding under ether (dry wt., 195 g.). The crude isolichenin, dissolved in water (1 l.), was treated with Fehling's solution (1·2 l.) until the supernatant liquid was deep blue. A flocculant precipitate was discarded and the mother-liquor acidified to pH 4·5 with 3N-acetic acid (900 ml.). Sufficient acetone (*ca.* 4 l.) was then added to ensure complete precipitation of the polysaccharide. The centrifuged precipitate, dissolved in water (2 l.), was poured slowly, with stirring, into ethanol (8 l.). The dried precipitate (110 g.) was dissolved in water (1 l.), acidified to pH 3·9 with 3N-acetic acid, and again precipitated by slow addition of acetone (800 ml.). This precipitate (66 g.) was dried and dissolved in water (1 l.), and the viscous solution was poured slowly into ethanol (4 l.). The polysaccharide was thus precipitated as a white fibrous material and was separated by centrifugation, washed, and dried in the usual manner (yield, 57 g.). The specimens showed $[\alpha]_D$ +272° (*c* 0·49 in H_2O) and blue value 0·0054, and gave no ash. In the hydrolysate of a portion (15 mg.) with 0·33N-sulphuric acid (1 ml.) only glucose was detected by paper chromatography.

Hydrolysis.—Isolichenin (38·4 g., based on carbohydrate content [18]) was dissolved in hot water (3733 ml.) and mixed with 5N-sulphuric acid so that the final acid concentration was 0·33N and the polysaccharide concentration about 1%. The solution was heated at 100° and portions (2 ml.) were withdrawn at intervals, neutralised with aqueous sodium hydroxide, and diluted to 25 ml., and the reducing powers (as glucose) of 5 ml. portions were determined with the Somogyi copper reagent.[19]. The hydrolysis was arrested after 75 min. by neutralisation with 3N-sodium hydroxide; the reducing power then corresponded to an apparent conversion into glucose of 42·5%.

Fractionation of the Hydrolysis Products.—The neutralised hydrolysate was concentrated to about 1·8 l., and added to a charcoal–Celite column (5 × 203 cm.). The column was irrigated under pressure, applied by a constant head of eluant some 15 ft. above the top of the

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[15] Meyer and Gürtler, *Helv. Chim. Acta*, 1947, **30**, 751.

[16] Hess and Lauridsen, *Ber.*, 1940, **73**, 115.

[17] Ulander and Tollens, *Ber.*, 1906, **39**, 401.

[18] Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224.

[19] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.

column, first with water and then by graded elution [20] with ethanol, 20% ethanol being allowed to flow into a mixing chamber containing water (15 l.). The optical rotation of each fraction (volume 250 ml.) was measured in a 4 dm. tube. The appropriate fractions were combined and evaporated under diminished pressure at 40°.

Examination of the Hydrolysis Products.—The fractions were combined as indicated and evaporated to dryness *in vacuo*. Concentrations of isolechenin solutions for quantitative analyses were determined by complete hydrolysis to glucose.[18]

The solvent systems used for descending paper chromatography were butan-1-ol–acetic acid–water (4 : 1 : 5, by vol.), and propan-1-ol–ethyl acetate–water (6 : 1 : 3, by vol.). It was usual to examine a sugar fraction in each of these solvents on Whatman no. 54 paper and to locate the component(s) with benzidine–trichloroacetic acid [21] (reducing sugars) and silver nitrate–sodium hydroxide [22] (reducing and non-reducing sugars).

(a) *Monosaccharide.* Paper chromatographic examination of the combined fractions nos. 25—43 showed the presence of a single reducing sugar which migrated with an R_F value corresponding to that of glucose. The sugar, crystallised from methanol, had m. p. 145—146° and $[\alpha]_D$ $+52\cdot5°$ (equilibrium value in H_2O).

(b) *Disaccharides.* A portion of fractions nos. 91—120 was shown by paper chromatography to contain two disaccharides, each migrating with an R_F value close to that of maltose, and ionophoresis in borate buffer [8] (pH 8·7) showed the presence of two components, one with a high and the other with a low ionophoretic mobility.

The disaccharide mixture (about 10 g.) was dissolved in water (about 100 ml.) and adsorbed on a charcoal–Celite column (5 × 75 cm.). The column was eluted by adding a 10% solution of ethanol in water containing also sodium borate (0·572 %) and boric acid (0·247%) (pH 8·7), into a mixing chamber containing 10 l. of aqueous sodium borate (0·572%) and boric acid (0·247%), which led to the column. The optical rotations of the fractions (*ca.* 100 ml.) were measured and the elution curve so obtained showed two elution peaks. The fractions embracing these peaks, namely, nos. 21—54 and nos. 55—90, were combined and brought to pH 5 with dilute sulphuric acid. Each batch was concentrated to small volume and adsorbed on charcoal–Celite columns (4 × 93 cm.). The columns were eluted, first with water to remove borate ions, and then with 7·5% of ethanol in water. Appropriate fractions were combined in each case and evaporated to dryness. Each residue was then extracted with boiling 80% methanol (75 ml.; 2 × 25 ml.), and the combined methanolic extracts were evaporated to dryness.

Paper chromatography showed each fraction to be homogeneous. Ionophoresis in borate buffer of pH 8·7 showed disaccharide (I) to have M_G 0·55 and disaccharide (II) M_G 0·21. In borate buffer [11] of pH 10·0 nigerose has M_G 0·69 and maltose M_G 0·32.

TABLE 1. *Properties of sugars and their derivatives isolated from a partial acid-hydrolysate of isolichenin.*

	Experimental values			Authentic values			
		β-Octa-acetate			β-Octa-acetate		
Sugar	$[\alpha]_D$ (equil. in H_2O)	$[\alpha]_D$ in chloroform	M. p.	$[\alpha]_D$ (equil. in H_2O)	$[\alpha]_D$ in chloroform	M. p.	Ref.
Glucose ...	$+52\cdot5°$	—	—	$+52\cdot7°$	—	—	*a*
Maltose ...	$+135\cdot6$	$+63\cdot9°$	158—159	$+136$	$+62\cdot6°$	159—160	*a*
					$+80\cdot0$	151—153	*b*
Nigerose...	$+138\cdot8$	$+84\cdot9$	149	$+136$	$+75\cdot7$	151—153	*c*
					$+78\cdot0$	155—157	9

(*a*) Bates, "Polarimetry and Saccharimetry," 1942, Nat. Bureau Stand., Washington. (*b*) Wolfrom, *J. Amer. Chem. Soc.*, 1955, **77**, 6403; Matsuda and Aso, *J. Fermentation Technol.* (*Japan*), 1954, **32**, 399. (*c*) Peat, Whelan, and Hinson, *Chem. and Ind.*, 1955, 385.

The β-octa-acetates of the two disaccharides were prepared by reaction with sodium acetate–acetic anhydride,[23] and the properties are reported in Table 1 (Found, for β-nigerose octa-acetate: C, 49·9; H, 5·6. For β-maltose octa-acetate: C, 49·5; H, 5·9. Calc. for $C_{28}H_{38}O_{19}$: C, 49·5; H, 5·9%).

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[20] Alm, Williams, and Tiselius, *Acta Chem. Scand.*, 1952, **6**, 826; Alm, *ibid.*, p. 1186.

[21] Bacon and Edelman, *Biochem. J.*, 1951, **48**, 114.

[22] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 44.

[23] Erwig and Koenigs, *Ber.*, 1889, **22**, 2207.

(c) *Trisaccharides.* The trisaccharide mixture (2·30 g., air-dried) from the isolichenin hydrolysate, dissolved in water (*ca.* 50 ml.) and adsorbed on a charcoal–Celite column (3·8 × 52 cm.), was eluted with ethanol–borate buffer (pH 8·7) by allowing 10% ethanol (in borate buffer, pH 8·7, see above) to flow into a mixing chamber containing borate buffer, pH 8·7 (4 l.). Fractions (100 ml.) were collected, their optical rotations were measured, and the elution curve was plotted. Two definite elution peaks were obtained but these were incompletely separated. Trisaccharide batch A (fractions nos. 25—59) and trisaccharide batch B (fractions nos. 71—94) were separately acidified to pH 5 with dilute sulphuric acid, concentrated to a small volume, and adsorbed on charcoal–Celite (3·8 × 38 cm.). The column was eluted with water until removal of the borate ions was complete and then with 15% ethanol. Fractions (100 ml.) were collected and the respective elution curves plotted. The appropriate fractions were combined in each case and evaporated to dryness. Each residue, A and B, was then extracted with boiling 80% methanol (75 ml., 25 ml.) and the combined methanolic extracts were evaporated to dryness.

Trisaccharide A was shown by ionophoresis in borate buffer (pH 8·7) to contain a single reducing component with M_G 0·55. A portion (15·8 mg.) was partially hydrolysed by being heated in a water-bath with 0·33N-sulphuric acid (1 ml.) for 40 min. Ionophoresis of the hydrolysate in a borate buffer (pH 8·7) revealed the presence of two components: one had M_G 0·21 corresponding to that of a maltose control; the other had M_G 0·55 corresponding to that of a nigerose control. The component with M_G 0·55 was present in appreciably greater amount.

Trisaccharide A (226 mg.) was mixed with sodium borohydride (238 mg.) in water (10 ml.) and kept at room temperature for 24 hr. (cf. ref. 24). The solution was then acidified to pH 5 with 3N-acetic acid. After removal of the borate ions on a small charcoal–Celite column (1·8 × 25 cm.), the trisaccharide alcohol was eluted (25 ml. fractions) with 15% ethanol. Fractions containing the reduced trisaccharide were evaporated to dryness, the residue was extracted with boiling 80% methanol (25 ml., 2 × 20 ml.) and the combined extracts were evaporated to dryness (yield, 205 mg.). Paper chromatography revealed the presence of a non-reducing substance having an R_F value corresponding approximately to that of a trisaccharide.

A portion (15 mg.) of this reduced material *RA* was heated with 0·33N-sulphuric acid (1 ml.) in a boiling-water bath for 40 min. Ionophoretic examination of the cooled hydrolysate showed the presence of glucose and reducing components with M_G 0·21 (corresponding to maltose) and M_G 0·55 (corresponding to nigerose).

Trisaccharide B moved ionophoretically as a single substance with M_G 0·21. Ionophoretic examination of a partial acid-hydrolysate of the mixture revealed the presence of two components, one migrating with a high (0·55) and the other with a low (0·21) mobility. These values corresponded to those for authentic nigerose and maltose respectively.

Trisaccharide B was reduced with sodium borohydride, and the alcohol mixture so obtained was partially hydrolysed in 0·33N-sulphuric acid. Examination of the hydrolysate by ionophoresis revealed components identical with those detected in a partial acid-hydrolysis of trisaccharide A.

Periodate Oxidation of Isolichenin.—Isolichenin (1·123 g.) was dissolved in water (*ca.* 180 ml.), 0·25M-sodium metaperiodate (50 ml.) was added, and the solution diluted to 250 ml. The solution was stored in the dark at 19°. A control solution also was prepared by diluting 0·25M-sodium metaperiodate (50 ml.) to 250 ml.

At intervals, a portion (3 ml.) of the reaction mixture was removed and diluted with water (*ca.* 50 ml.). 10% w/v Potassium iodide solution (3 ml.) and 2N-sulphuric acid (2 ml.) were then added, the mixture was shaken, and the liberated iodine titrated immediately with N/30-sodium thiosulphate. A portion (3 ml.) of the control digest was similarly treated. Results are in Table 2.

TABLE 2.

Time of oxidn. (hr.)	0·33	1·0	1·5	10	21	51	78	117	165	261	286
$NaIO_4$ consumed (mole per $C_6H_{10}O_5$)	0·411	0·421	0·435	0·449	0·453	0·465	0·471	0·485	0·501	0·533	0·543

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[24] Abdel-Ahker, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691; Whelan and Morgan, *Chem. and Ind.*, 1955, 1447.

Action of Salivary α-Amylase on Isolichenin.—Human saliva (25 ml.) was diluted with an equal volume of water, and the mixture centrifuged to remove the mucins. The supernatant liquid was then used as the source of α-amylase.

(i) *Diminution in iodine-staining power.* A digest of isolichenin (437 mg.), 50% human saliva (10 ml.), and 0·2M-citrate buffer (10 ml.) in water (100 ml.) was incubated at 35° and after about 2 hr. more saliva (5 ml.) was added. Aliquot parts (5 ml.) were withdrawn at intervals, mixed with " Spekker " iodine (2 ml.) and three drops of 3N-hydrochloric acid, and diluted with water to 100 ml. The absorption value (at 680 mμ) was measured on a Spekker photoelectric absorptiometer.[6]

Time of digestion	0	2 min.	5 min.	12 min.	25 min.
Absorption value	0·228	0·108	0·100	0·089	0·079
Time of digestion	3 hr.	6 hr.	19 hr.	35 hr.	
Absorption value	0·042	0·035	0·016	0·006	

The measurement at zero time was made on 5 ml. of the original polysaccharide solution and corresponds to a blue value [6] of 0·0052.

(ii) *Increase in reducing power.* A digest of isolichenin (253 mg.), 50% saliva (5 ml.), and 0·2M-citrate buffer, diluted to 50 ml., was incubated at 35° and after about 2 hr. a further 5 ml. of saliva was added to the digest. Aliquot parts (5 ml.) were withdrawn at intervals and the apparent conversion into maltose determined by the Somogyi method: [19]

Time	0	7 min.	15 min.	3·5 hr.	8·5 hr.	24·5 hr.	34·5 hr.	48·5 hr.
Apparent conversion into maltose (%)	1·26	1·36	1·36	2·50	3·30	3·90	3·96	4·26

(iii) *Products of α-amylolysis of isolichenin.* A digest of isolichenin (about 500 mg.), 50% saliva (*ca.* 50 ml.), and 0·2M-citrate buffer (10 ml.) in 100 ml. was incubated at 35° for 48 hr. The enzyme was inactivated by heating it at 100° for 5 min. A portion (about 20 ml.) of the solution was poured into ethanol (4 vol.) to remove protein and salts, and the mixture was centrifuged. The supernatant liquid was evaporated to dryness and analysed by paper chromatography. Maltose, maltotriose, and glucose were detected on the chromatogram, maltose being present in the largest quantity.

Action of Soya-bean β-Amylase on Isolichenin.—A portion (0·5 ml.) of a stock soya-bean β-amylase solution (1 ml., 4000 units [25]) was diluted to 25 ml. with 0·2M-acetate buffer [pH 4·8; prepared by dissolving hydrated sodium acetate (32·64 g.) and glacial acetic acid (9·6 g.) in water and dilution to 2 l.].

(i) *Iodine-staining powder.* An aliquot part (50 ml.) of the isolichenin solution (891 mg./100 ml.) with β-amylase solution (25 ml.), diluted to 100 ml., was incubated at 35°. Aliquot parts (5 ml.) were withdrawn from the digest at intervals and the absorption values determined as described previously.

Time of digestion	0	15 min.	30 min.	45 min.	1·0 hr.
Absorption value	0·257	0·170	0·159	0·163	0·159
Time of digestion	1·25 hr.	1·5 hr.	15 hr.	42 hr.	
Absorption value	0·163	0·170	0·152	0·152	

The value at zero time was measured on the original polysaccharide solution and corresponded to a blue value of 0·0058.

(ii) *Reducing power of the digest.* A reducing-power determination [19] was made on a portion (5 ml.) of the digest after the absorption value had become constant, *i.e.*, after 24 hr. Duplicate samples gave values for the apparent conversion into maltose of 0·45% and 0·58%.

(iii) *Products.* Isolichenin (*ca.* 500 mg.) was digested at 35° with " stock " soya-bean β-amylase (0·3 ml.) in a solution (100 ml.) of pH 4·8. After 48 hr. the enzyme was inactivated at 100° (5 min.). A portion of the cooled solution was then treated as described above and the residue analysed by paper chromatography. Maltose was the only sugar detected on the chromatogram.

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[25] Pirt, Ph.D. Thesis, University of Wales, 1950.