893. α-1 : 4-*Glucosans. Part VII.* The Enzymic Degradation and Molecular Structure of Amylose.*

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Amylose, prepared in the absence of oxygen, is only partially degraded (70—80%) by purified β-amylase, but is completely degraded by the concurrent action of β-amylase and Z-enzyme. Treatment of amylose with oxygen, either during fractionation or in presence of hot alkali, does not introduce barriers to the action of these enzymes.

β-Amylolysis of potato amylose and a subfraction obtained by aqueous leaching indicates that amylose is heterogeneous.

The specificity of phosphorylase, β-amylase, and barley Z-enzyme is discussed; the last enzyme, which hydrolyses anomalous linkages in amylose, has no action on α-1 : 3-, α-1 : 4-, or α-1 : 6-glucosidic linkages, or on β-linked disaccharides.

Although the main features of the degradation of starch-type polysaccharides by β-amylase have been known for many years, the extent of hydrolysis of amylose and the mode of action (*i.e.*, single- or multi-chain attack) remain uncertain.[1] Recent investigations by Peat,[2] Hopkins,[3] Hassid,[4] and their collaborators have shown that, in contrast to earlier reports, *pure* β-amylase converts only *ca.* 70% of amylose into maltose. This incomplete degradation is not due to retrogradation of the substrate or to inactivation of the enzyme.[2] Since the action of β-amylase ceases when glucosidic linkages other than α-1 : 4 are encountered in the substrate, the amylose samples appear to contain a small number of anomalous linkages. Peat and his co-workers [2] believe that these linkages are structural features of the native amylose; they can be hydrolysed by a specific enzyme (Z-enzyme [5]) which occurs, together with β-amylase, in soya-beans. Unpurified β-amylase preparations

* Part VI, *J.*, 1957, 3432.

[1] For reviews see (*a*) Manners, *Ann. Reports*, 1953, **50**, 288, and (*b*) Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335.

[2] Peat, Pirt, and Whelan, *J.*, 1952, 705, 714.

[3] Hopkins and Bird, *Nature*, 1953, **172**, 492.

[4] Neufeld and Hassid, *Arch. Biochem. Biophys.*, 1955, **59**, 405.

[5] Peat, Thomas, and Whelan, *J.*, 1952, 723.

from this source, therefore, degrade amylose completely. Although the nature of the anomalous linkages is not yet known, Baum, Gilbert, and Scott [6] have suggested that they are introduced into the amylose, by oxidation, during the fractionation of the starch. The specificity of Z-enzyme has not yet been determined, and has been the subject of some controversy.[2-5, 7]

The present communication describes a study of the action of β-amylase, in the presence and absence of Z-enzyme, on highly purified amyloses of high molecular weight. These amyloses, which have a degree of polymerisation ($\overline{D.P.}$) of several thousand glucose residues, were prepared by the fractionation of starch in the presence and in the absence of oxygen. The effect of oxygen and hot alkali on the β-amylolysis limit of certain amyloses has also been examined. In addition, the specificity of barley Z-enzyme has been investigated.

The β-Amylolysis Limit of Amylose.—Purified soya-bean β-amylase was caused to act on several samples of amylose, which had been prepared under neutral conditions in the presence and in the absence of oxygen. The reaction was followed by determination of the liberated maltose rather than by measurement of changes in the iodine-staining power: the latter method is less precise, and gives results which are *ca.* 5% higher than those from reducing-power measurements (see p. 4436 and ref. 2); in addition, the maltose production is independent of the mode of enzyme action (cf. ref. 8). The enzyme preparation, which was free from Z-enzyme [5] and α-amylase, gave 69—86% conversion into maltose with eight amylose samples (see Table 1). Amylose IV had a β-amylolysis limit of 99%. The low β-amylolysis limits were not due to inactivation of the enzyme, since they were unaffected by changes in initial enzyme : substrate ratio, or by addition of fresh enzyme to the digests after 24 hours' incubation.

TABLE 1. *β-Amylolysis limits of amyloses.*

Sample	Conditions of prepn.[a]	$\overline{D.P.}$	β-Amylolysis limit † A	B	C	D	E
I	N_2	3,200	77	92	—	99	98
II	O_2	2,800	75	—	—	98	—
III	O_2	1,600	76	—	—	99	—
IV	N_2	1,800	99	—	—	—	—
V	N_2	2,700	86	—	—	—	—
VI	Air	1,850	73 *	86	72	95	94
VII	Air	*ca.* 1,500	69	83	75	94	94
VIII	N_2	*ca.* 1,500	77	87	74	92	93
IX	N_2	*ca.* 600	77	85	79	99	—

[a] Atmosphere during fractionation (see p. 4435).
* Crystalline sweet-potato β-amylase (free from Z-enzyme) also gave 73% conversion into maltose.
† Soya-bean β-amylase at pH 4·6 on (A) untreated amylose, and (B) amylose treated with oxygen in alkali. Barley β-amylase at pH 3·6 on (C) untreated amylose, and at pH 4·6 on (D) untreated amylose, and (E) amylose treated with oxygen in alkali.

By contrast, barley β-amylase caused 92—99% degradation of the same amylose samples, when incubated at pH 4·6. Although this enzyme preparation was free from α-amylase and maltase, previous studies [9] have indicated that amorphous barley β-amylase shows Z-enzyme activity. The complete degradation of the amylose is therefore due to the combined action of barley Z-enzyme and β-amylase. Since soya-bean Z-enzyme is inactivated at pH 3·6, our barley β-amylase preparation was incubated at this pH with four amylose samples. Only 72—79% conversion into maltose was observed, the detailed results being similar to those obtained with soya-bean β-amylase. Control experiments showed that despite the decreased stability of β-amylase itself at pH 3·6, the digests

[6] Baum, Gilbert, and Scott, *Nature*, 1956, **177**, 889.
[7] Peat and Whelan, *ibid.*, 1953, **172**, 494.
[8] Bourne and Whelan, *ibid.*, 1950, **166**, 258.
[9] Bell and Manners, *J.*, 1952, 3641.

contained sufficient β-amylase to allow the limit to be reached within 6 hr., and that this limit was unchanged when the initial enzyme concentration was increased five-fold.

It appears, therefore, that barley β-amylase alone is unable to degrade amylose completely, and that barley Z-enzyme, like soya-bean Z-enzyme, is acid-labile.

Further evidence for the presence of Z-enzyme in barley was obtained by dividing a digest of amylose I and soya-bean β-amylase and adding equivalent amounts of barley and soya-bean β-amylase severally to the two portions. The addition of soya-bean enzyme had no effect; the barley preparation caused a rapid increase in the maltose content, from 77 to 98%.

The possibility that the Z-linkages may arise from changes in the physical state of the substrate, *i.e.*, "ageing," [10] has also been considered. Incubation of amylose in acetate buffer (pH 4·6) for 48 hr. at room temperature *before* the addition of barley β-amylase resulted in only a small decrease (*ca.* 8%) in β-amylolysis limit. Ageing is not therefore responsible for the resistance of some 20—30% of the amylose to pure β-amylase. Further, when digests containing soya-bean and barley β-amylase were prepared under *identical* conditions, enzyme action was complete within 2—6 hr., *i.e.*, before any appreciable ageing is likely to have taken place. (This result is substantiated by unpublished sedimentation measurements on amylose during β-amylolysis.)

The various amylose samples used in these experiments were prepared either in the presence or in the absence of oxygen, and with the exception of amylose IV (see Table 1), 14—31% of the samples was resistant to pure β-amylase. Probably, therefore, the barriers to β-amylolysis are a structural feature of the native amylose and are not introduced, by oxidation, during the *fractionation* of the starch. However, the possibility of inadvertent modification of the starch during *isolation* cannot be entirely disregarded.

The β-Amylolysis Limit of Amylose treated with Oxygen in Alkali.—Evidence suggesting that oxidation might be the source of the anomalous linkages in amylose was obtained by Gilbert and his co-workers.[6] Amylose, after treatment with oxygen at 95° in both neutral and alkaline solution, was incubated with potato phosphorylase. Only 67—81% degradation occurred whereas, under similar conditions, 90% of the original amylose was degraded (as measured by decrease in iodine-staining power). (It must be noted, however, that phosphorolysis is more accurately assayed by measurement of the production of α-D-glucosyl phosphate.) It was concluded [6] that barriers to phosphorylase action, and by analogy, to β-amylolysis, can be introduced into amylose by molecular oxygen.

We have studied the degradation, by barley β-amylase, of four amylose samples which had been heated at 98° for 20 min. in 0·5M-sodium hydroxide in a stream of oxygen. The results (Table 1) show that this treatment does not alter the percentage conversion into maltose. It follows that, under the conditions employed by Gilbert, molecular oxygen does not introduce barriers to β-amylase or Z-enzyme action.

The above experiments were repeated with pure soya-bean β-amylase, and in all instances, a small *increase* (*ca.* 10%) in β-amylolysis limit was observed. If anomalous linkages had been introduced by oxygen, then the β-amylolysis limit would *decrease*. The increase is attributed to alkaline degradation of the amylose, involving the rupture of non-terminal α-1 : 4-glucosidic linkages and the liberation of new non-reducing end-chains which are susceptible to β-amylase. The alkaline degradation, which is not detected by the combined action of β-amylase and Z-enzyme, may be followed viscometrically. In one experiment a 60% decrease in specific viscosity was observed, indicating that 1—2 α-1 : 4-glucosidic linkages per molecule had been broken. It is clear, however, that the majority of the Z-linkages in the oxygen-treated amylose are intact since the β-amylolysis limit is only 83—92%, and the residual polysaccharide is still stained blue by iodine.

Neufeld and Hassid [4] found that after dissolution of amylose β-limit dextrin in hot alkali (3 min. in 0·19N-potassium hydroxide at 100° *in air*), the polysaccharide had a

[10] Meyer, Bernfeld, Boissonnas, Gürtler, and Noelting, *J. Phys. Coll. Chem.*, 1949, **53**, 319.

β-amylolysis limit of *ca.* 50%. Dissolution of the same dextrin in hot water resulted in an increase of only 5% in β-amylolysis limit. In view of our results, it is probable that alkaline degradation of α-1 : 4-glucosidic linkages rather than Z-linkages is responsible for the increased susceptibility of the limit dextrin to β-amylase.

Comparison of our results with those of Gilbert [6] suggests that β-amylase and phosphorylase differ slightly in specificity. Phosphorolysis of an amylose chain, in which successive α-1 : 4-glucosidic linkages are broken, appears to be arrested when an " oxidised " glucose residue is encountered. In contrast, β-amylase action, involving hydrolysis of alternate glucosidic linkages, is not limited by such residues. Although the nature of the oxidised residue is not known (oxidation at $C_{(3)}$ or $C_{(6)}$ is most likely [1b]), it must differ only slightly from a normal α-1 : 4-linked glucopyranose residue since β-amylase itself has a relatively high degree of specificity.

It will be noted that amylose from two varieties of potato starch, and from wrinkled pea starch, behave in the same way towards β-amylase, Z-enzyme, and oxygen in hot alkali. The above results may, therefore, be typical of the amyloses of plant starches. It is of interest that sago, tapioca, maize, and potato amyloses are similarly degraded by β-amylase and soya-bean Z-enzyme,[2] whilst Neufeld and Hassid [4] have reported that amyloses from seven different sources are incompletely hydrolysed by pure β-amylase.

The Heterogeneity of Potato Amylose.—Several workers have reported that amylose is heterogeneous.[1b] Neufeld and Hassid [4] showed that subfractions of potato and maize amylose had different β-amylolysis limits. Meyer *et al.*[10] have reported $\overline{D.P.}$'s of 200 and 700 for two fractions obtained by aqueous leaching of potato starch, and Hopkins and Bird [3] found that similar fractions had β-amylolysis limits of 80 and 64%.

We found (see Table 2) that aqueous leaching at 70° extracts *ca.* 40% of the amylose from the granule. This fraction (amylose IV) has a $\overline{D.P.}$ of 1800, and it must be linear since it is completely degraded by purified β-amylase. In contrast, amylose I, which represents the whole amylose in the potato starch, has a $\overline{D.P.}$ of 3200 and a β-amylolysis limit of only 77%. It follows that the residual amylose remaining after aqueous leaching has a $\overline{D.P.}$ of 5000—6000 and a β-amylolysis limit of 50—60%. Aqueous extraction of the granules at 100° gives a polysaccharide (amylose V) with properties intermediate between those of amylose I and IV. The presence of the small proportion of anomalous linkages is therefore confined to the amylose fractions of higher molecular weight. Moreover, the anomalous linkages are situated, on the average, near the centre of these molecules, and not near the non-reducing end.

The Specificity of Z-enzyme and the Nature of the Anomalous Linkages.—Peat, Thomas, and Whelan [5] originally isolated Z-enzyme from a soya-bean β-amylase preparation as a stable powder which was free from glycerophosphatase and amylase activity, and with the general properties of a group-specific β-glucosidase. The fact that almond emulsin (a mixture of β-glucosidases) also showed Z-enzyme activity supported this suggestion. However, a later investigation by Neufeld and Hassid [4] showed that the laminarinase, cellobiase, and Z-enzyme activities of almond emulsin were due to distinct enzymes.

In an attempt to determine the specificity of barley Z-enzyme, the action of β-amylase (soya-bean at pH 4·6 or barley at pH 3·6) and that of β-amylase plus Z-enzyme (barley at pH 4·6) on various carbohydrates have been compared. This method of investigation is, however, limited by the availability of suitable substrates, since the action of a carbohydrase is dependent on (*a*) the specificity for the glycosidic linkage, (*b*) the nature of adjacent glycosidic linkages, and (*c*) steric factors arising from the size and shape of the substrate. For example, R-enzyme,[11] which is specific for α-1 : 6-glucosidic linkages, cannot hydrolyse isomaltose, panose, or the α-1 : 6-inter-chain linkages in glycogen and in the interior of amylopectin. The above requirements are satisfied only by the inter-chain linkages in the outer regions of an amylopectin molecule. In the present study,

[11] Hobson, Whelan, and Peat, *J.*, 1951, 1451; Peat, Whelan, Hobson, and Thomas, *J.*, 1954, 4440; Peat, Whelan, and Thomas, *J.*, 1956, 3025.

the fact that Z-enzyme does not hydrolyse a disaccharide containing a particular linkage is not, therefore, considered to be conclusive proof of non-identity with the anomalous linkage.

The possibility that Z-enzyme action could be due to traces of α-amylase has been emphasised by Hopkins and Bird,[3] although Peat *et al.*[5,7] have reported enzymic evidence against this view. However, the constancy of the molecular weight of amylose during barley β-amylolysis [12] shows clearly that random α-amylolytic action is not occurring, and that barley α-amylase and Z-enzyme must be distinct enzymes.

If oxidation of anhydroglucose residues occurred during the isolation of a linear amylose molecule (cf. ref. 6), then the barriers to β-amylase could be " oxidised " residues situated at the non-reducing end or at some position in the chain itself. Such residues could only be removed by Z-enzyme, by the hydrolysis of one of more α-1 : 4-glucosidic linkages, *i.e.*, by an amylase-type action. Since Z-enzyme is not an amylase, it follows that the anomalous linkages cannot involve oxidised glucose residues situated as above.

By similar reasoning, the tentative suggestion [13] that the anomalous linkages are glucose residues attached by a β-linkage to the non-reducing end of a linear α-1 : 4-glucosan is considered to be unlikely.

Recent studies of partial acid hydrolysis [14] have indicated that amylopectin contains a small proportion of α-1 : 3-glucosidic linkages. These linkages may arise through the action of an amylo-1 : 4 ⟶ 1 : 3-transglucosidase acting either with phosphorylase during the synthesis of linear chains, or with Q-enzyme during the amylose ⟶ amylopectin conversion. The presence of an α-1 : 3-glucosidase in Nature has also been reported.[15]

The experiments described below indicate that Z-enzyme is not an α-1 : 3-glucosidase, and that amylose samples I—IX do not contain α-1 : 3-glucosidic linkages. A comparison has been made of the action of β-amylase, in the presence and absence of Z-enzyme, on (*a*) α-dextrins produced by the salivary α-amylolysis of isolichenin [16] and (*b*) a partial acid hydrolysate of isolichenin. This polysaccharide is a linear polymer of D-glucose containing both α-1 : 3- and α-1 : 4-linkages, and should closely satisfy the structural and steric requirements of a substrate for Z-enzyme. Measurements of reducing power showed that no hydrolysis occurred within 48 hr. It is concluded that Z-enzyme has no action on either the non-terminal or the terminal α-1 : 3-glucosidic linkages, which are liberated by the enzymic or chemical degradation of isolichenin.

The barriers to β-amylase action are unlikely to be α-1 : 6-glucosidic linkages, for the following reasons: (*a*) potato amylopectin, on incubation with barley and soya-bean β-amylase, gave similar conversions into maltose (54—56%); (*b*) treatment of amylose IX with isoamylase [17] (which hydrolyses α-1 : 6-inter-chain linkages in glycogen and amylopectin) before addition of β-amylase did not cause a significant increase in β-amylolysis; and (*c*) isomaltose and panose were not hydrolysed by either β-amylase preparation.

The suggestion [5] that amylose contains a small number of β-glucosidic linkages, which are hydrolysed by Z-enzyme, has been considered, although our studies have been limited by the fact that carbohydrates containing *both* α-1 : 4- and β-glucosidic linkages are not available as model substrates. Barley Z-enzyme appears to be distinct from laminarinase and salicinase; whereas Z-enzyme is inactivated at pH 3·6, the laminarinase and salicinase activities are only partly reduced. In addition, the barley preparation had no action on cellobiose or gentiobiose, at pH 4·6, and therefore differs from the soya-bean Z-enzyme preparation.[5]

From the above studies, it is concluded that barley Z-enzyme is not an amylase, and

[12] Cowie, Fleming, Greenwood, and Manners, *Chem. and Ind.*, 1957, 634.
[13] Barker and Bourne, *Quart. Rev.*, 1953, **7**, 82.
[14] Wolfrom and Thompson, *J. Amer. Chem. Soc.*, 1956, **78**, 4116.
[15] Larner and Gillespie, *ibid.*, p. 882.
[16] Chanda, Hirst, and Manners, *J.*, 1957, 1951.
[17] Manners and Khin Maung, *Chem. and Ind.*, 1955, 950.

does not hydrolyse α-1 : 3- or α-1 : 6-glucosidic linkages; these linkages are therefore unlikely to be the barriers to β-amylase action. In addition, although barley Z-enzyme has no action on simple β-glucosides, the possibility that amylose contains a small proportion of β-glucosidic linkages remains.

Experimental

Analytical Methods.—The general methods used were as described in previous papers of this series, except that paper chromatograms were developed with ethyl acetate–pyridine–water (10 : 4 : 3) as solvent.[18] The iodine binding-power of amyloses was measured by the potentiometric-titration method described by Anderson and Greenwood.[19] Limiting viscosity numbers [η] were determined in M-potassium hydroxide and number-average $\overline{D.P.}$ values calculated [20] from $\overline{D.P.} = 7{\cdot}4[\eta]$.

Preparation of Amylose.—The methods of fractionating the potato and wrinkled pea starches are shown in Table 2.

Enzyme Preparations.—β-Amylase was isolated from soya-beans and purified by Peat, Pirt, and Whelan's method [2] in which Z-enzyme is inactivated by a short heat treatment (30 min. at 60° at pH 4·8). The purified solution had an activity of *ca.* 20,000 units/ml. when

TABLE 2. *Preparation of amyloses.*

Sample	Source	Fractionation procedure Method	Ref.	Iodine affinity *
I †	Potato var. Redskin	Thymol and BuOH	20	19·5
II	,, ,,	,, ,,	21	19·5
III	,, ,,	,, ,,	21	19·5
IV ‡	,, ,,	Aq. leaching at 70°	20	19·5
V	,, ,,	Aq. leaching at 98°	20	19·5
VI	,, King Edward	Pyridine	22	19·1
VII	,, ,,	$Al(OH)_3$ and thymol	23	15·8
VIII	,, ,,	,, ,,	23	17·0
IX	Wrinkled pea var. Laxton's Progress	Thymol and BuOH	20	18·5

* Expressed as mg. of iodine bound per 100 mg. of starch.[19]
† Yield approx. 17 g. per 100 g. of starch.
‡ Yield approx. 7 g. per 100 g. of starch.
Samples I, II, IV, and V were recrystallised from butan-1-ol under nitrogen; amylose III was recrystallised in the presence of oxygen.

tested under the conditions suggested by Hobson, Whelan, and Peat.[24] It did not reduce the iodine-staining power of amylopectin β-dextrin and contained only an insignificant trace of maltase. The properties of the barley β-amylase have been described by Liddle and Manners.[25] The stability of this enzyme was investigated by determining the activity after varying periods of incubation at pH 4·6 and 3·6:

Time of incubation (hr.)	0	5	24
Activity units,[24] at pH 4·6	138	123	85
Activity units,[24] at pH 3·6	18	9	0

Isoamylase was isolated from brewer's yeast, as described by Manners and Khin Maung.[17]

Determination of β-Amylolysis Limits.—Digests were normally prepared by incubating, at 35° in the presence of toluene, amylose (20—50 mg.), 0·2M-acetate buffer pH 3·6 or 4·6, β-amylase (*ca.* 50—100 units/mg. of amylose), and distilled water (to a final volume of 50 ml.). Dried amylose samples were moistened with ethanol, dissolved in 0·2N-potassium hydroxide, with shaking (and warming, if necessary), and then neutralised with N-hydrochloric acid (to phenolphthalein). Amylose–butanol complexes were dissolved in distilled water, as required.

[18] Whistler and Hickson, *Analyt. Chem.*, 1955, **27**, 1514.
[19] D. M. W. Anderson and Greenwood, *J.*, 1955, 3016.
[20] Cowie and Greenwood, *J.*, 1957, 2862.
[21] Cowie and Greenwood, unpublished experiments.
[22] Whistler and Hilbert, *J. Amer. Chem. Soc.*, 1945, **67**, 1161; Higginbotham and Morrison, *Shirley Inst. Mem.*, 1948, **22**, 148.
[23] Hobson, Pirt, Whelan, and Peat, *J.*, 1951, 801.
[24] Hobson, Whelan, and Peat, *J.*, 1950, 3566.
[25] Liddle and Manners, *J.*, 1957, 3432.

The amylose concentration was determined, in triplicate, by acid hydrolysis of a portion (1 ml.). of the digest, and estimation of the liberated glucose, as described by Pirt and Whelan.[26] Portions of the digest (1—3 ml.) were removed at intervals for determination of maltose or A.V. (absorption value of the iodine complex at 680 mμ) (see Table 3). The iodine-staining method gave slightly higher β-amylolysis limits. The β-amylolysis limit of potato amylopectin was determined similarly.

TABLE 3.

Digest	Time of incubation (hr.)	1	2	4	7	24	48
Amylose VII, pH 4·6	Conversion into maltose (%)	77	85	89	—	94	94
	Decrease in A.V. (680 mμ) (%)	73	78	91	—	98	—
Amylose VII, pH 3·6	Conversion into maltose (%)	66	69	—	73	75	75
	Decrease in A.V. (680 mμ) (%)	65	74	—	79	80	80

Action of Varying Amounts of Barley and Soya-bean β-Amylase on Amylose.—Amylose I (21·8 mg.) was incubated with barley β-amylase (100 units/mg. of substrate) at pH 4·6; after 24 hours' incubation, a further 100 units of β-amylase/mg. of substrate were added to the remainder of the digest. Results were:

Time of incubation (hr.)	1	2	6	24	48
β-Amylolysis limit (%)	89	95	98	98	98

A similar experiment with amylose I (46·2 mg.) and soya-bean β-amylase (50 units/mg. of substrate) at pH 4·6 gave 77% conversion into maltose after 24 hr. The digest was divided; to portion A, fresh soya-bean β-amylase (50 units/mg. of substrate) was added, and to portion B a similar amount of barley β-amylase. After 24 hours' incubation, the β-amylolysis limits were: A, 77; B, 98%. The residue of portion A gave an intense blue colour with iodine ($\lambda_{max.}$ 660 mμ) whilst that of digest B was achroic.

In a similar experiment with amylose VIII the following results were obtained; β-amylolysis limit (24 hours' incubation with soya-bean enzyme) 77%; after addition of fresh soya-bean β-amylase, 77%; after addition of a similar amount of barley β-amylase, 92%.

The action of barley β-amylase on amylose VIII at pH 3·6 was studied; two digests were prepared containing (*a*) 150 mg. of enzyme (initial activity *ca.* 12,000 units) and (*b*) 30 mg. of enzyme (initial activity *ca.* 2400 units). Portions from digest (*a*) were deproteinised (zinc sulphate–barium hydroxide) before analysis. The β-amylolysis limits were (*a*) 73, (*b*) 75% after 24 and 48 hours' incubation.

Effect of Oxygen on the β-Amylolysis Limit.—Amylose (samples I, VI, VII, VIII, IX) (20—30 mg.), dissolved in 0·5N-sodium hydroxide (10 ml.), was heated in a stream of oxygen at 95° for 20 min. The solution was cooled, neutralised, and incorporated into digests with soya-bean or barley β-amylase. The β-amylolysis limits, determined after 24 hours' incubation, are shown in Table 1, columns B and E. No significant increase was observed when incubation was continued for a further 24 hr. The specific viscosity of amylose VIII fell from 0·40 to 0·16 during the oxygen treatment.

Effect of "Ageing" on the β-Amylolysis Limit.—Amylose I (treated with oxygen and alkali as above) (20·4 mg.) was incubated with barley β-amylase at pH 4·6; after 24 and 48 hr. 97 and 98% conversion into maltose was observed. A similar digest in which 24·5 mg. of amylose and buffer were mixed, at room temperature (17—19°), for 48 hr. before addition of the enzyme was also examined. After a further 24 hr., 92% conversion into maltose had occurred.

The experiment was repeated with amylose VII which has a β-amylolysis limit of 94%. When digests containing 19·6 mg. of amylose dried *in vacuo* at 70°, or 20·7 mg. of air-dried amylose were incubated with buffer for 48 hr. at 18°, the β-amylolysis limits were 86 and 85%. Although no retrogradation of the amyloses was visible, it is probable that, under these extreme conditions, some ageing had occurred, with a small (5—9%) lowering of the β-amylolysis limit.

Action of β-Amylases on Isolichenin Dextrins.—Isolichenin (150 mg.) was incubated with freeze-dried salivary α-amylase [25] (15 mg.) in a 50 ml. digest at 35° for 24 hr. 20 ml. portions were then incubated in 0·2M-acetate buffer (pH 4·6) with soya-bean β-amylase solution (0·1 ml.) or barley β-amylase (25 mg.) in a total volume of 25 ml. In all three digests, the apparent percentage conversion into glucose was 10. In a second experiment, in 0·1M-citrate buffer

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

pH 6·5 (near the pH optimum for soya-bean Z-enzyme [5]), the conversions into glucose were 10, 11, and 9% respectively.

A similar quantity of isolichenin was hydrolysed with 0·5N-sulphuric acid (10 ml.) at 98° for 45 min., to give 32% apparent conversion into glucose. Portions of the neutralised hydrolysate (3 ml.) were incorporated into 10 ml. digests containing 0·2M-acetate buffer (pH 4·6) and β-amylase. After 24 hours' incubation, 32% conversion into glucose was observed with both soya-bean and barley β-amylase.

Action of Isoamylase on Wrinkled-pea Amylose.—Amylose IX (6·9 mg.), 0·2M-acetate buffer pH 5·89 (1 ml.), water (4·5 ml.), and isoamylase (40 mg./ml.; 0·5 ml.) were incubated at 18° for 24 hr. The enzyme was inactivated by heat, the digest cooled, and β-amylase in 0·2M-acetate buffer (pH 4·6) (250 units; 2 ml.) added. The β-amylolysis limit was 77%. In a control experiment without isoamylase, the β-amylolysis limit was 75%.

Action of Barley β-Amylase on Simple Saccharides.—Digests containing gentiobiose, isomaltose, panose, and cellobiose (*ca.* 10 mg.) in buffer pH 4·6 (1 ml.) containing barley β-amylase (10 mg.) were incubated at 35° for 24 hr. Glucose could not be detected (paper chromatography).

Action of Barley β-Amylase Preparation on Laminarin and Salicin.—The laminarinase activity of the enzyme at pH 4·6 and 3·6 was determined by a method due to Anderson and Manners; [27] the activities were 13·5 and 11·1 units respectively. In similar experiments with salicin, the activities at pH 4·6 and 3·6 were 10·1 and 8·8 units respectively.

The authors are grateful to Professor E. L. Hirst, F.R.S., for his interest, to the Rockefeller Foundation for a grant, and to the Department of Scientific and Industrial Research for a maintenance allowance (to I. D. F.).

Department of Chemistry, University of Edinburgh. [*Received, June 12th,* 1957.]

[27] F. B. Anderson and Manners, unpublished experiments.