The Constitution of a Modified Starch from Malted Barley.

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The starch from malted barley has been isolated and shown to contain 26·0% of amylose. Methylation showed that the amylose consisted of unbranched chains containing an average of *ca.* 330 glucose residues. Methylation end-group assay, supported by β-amylolysis and periodate oxidation, indicated that the amylopectin contained the normal type of branched structure, but with one non-reducing terminal group per 18 ± 1 glucose residues. It is concluded that during the malting of barley the amylopectin has undergone enzymic attack causing shortening of the outer chains but with retention of the branched structure, while the amylose component has been relatively little degraded.

Structural investigations have recently been carried out on starches from a variety of sources. In this paper we report an examination of the starch from malted barley (a mixture of Plumage Archer and Spratt Archer varieties), carried out to determine the changes in structure of the starch during the malting process. The isolation was achieved without the use of reagents likely to cause degradation. The starch had an amylose content of 26·0%, a value significantly higher than that of the starch from the corresponding sample of barley and of the barley starch examined by McWilliam and Percival (*J.*, 1951, 2259). The most satisfactory separation of malted-barley amylopectin was achieved by using 20% aqueous pyridine saturated with butanol as the amylose precipitant (Higginbotham and Morrison, *Shirley Inst. Mem.*, 1948, **22**, 148). The amylopectin so obtained had an amylose content of not more than 0·5%. The amylose was isolated by fractionation of the starch with 15% aqueous pyridine (Whistler and Hilbert, *J. Amer. Chem. Soc.*, 1945, **67**, 1161), followed by successive reprecipitations as the butanol complex (Higginbotham and Morrison, *loc. cit.*).

Methylated amylopectin was prepared by standard methods and quantitative paper chromatography (Hirst, Hough, and Jones, *J.*, 1949, 928) of a small-scale hydrolysate showed the presence of tetra- (5·5%), tri- (86·1%), and di-*O*-methylglucose (8·4%). The mixture of methylated sugars obtained on hydrolysis of the methylated polysaccharide on a larger scale was fractionated by partition chromatography on cellulose, to give 2 : 3 : 4 : 6-tetra- (5·7%), 2 : 3 : 6-tri- (87·4%), and 2 : 3-di-*O*-methyl-D-glucose (4·3%), isolated as crystalline substances or as crystalline derivatives. In addition there was obtained a small fraction (2·6%) containing a mixture of 2 : 6- and 3 : 6-di-*O*-methylglucose. The quantity of tetra-*O*-methylglucose corresponded to the presence of one non-reducing terminal group per 18—19 glucose residues. It is doubtful whether the mixture of 2 : 6- and 3 : 6-di-*O*-methylglucose is of structural significance, since they may have arisen from incomplete methylation of the polysaccharide and by demethylation during hydrolysis. The quantity of 2 : 3-di-*O*-methyl-D-glucose isolated indicates that the majority, at least, of the interchain linkages were through position 6. The yield of formic acid from periodate oxidation of the amylopectin corresponded to one non-reducing group per 18 ± 1 glucose residues, a value in good agreement with that from the methylation data. Estimation of the glucose produced on hydrolysis of the periodate-oxidised polysaccharide indicated that 0·63% of the glucose residues in the amylopectin were unattacked by periodate. It follows that at least 88% of the linkages between unit chains were 1 : 6-linkages.

Further evidence concerning the structure of the amylopectin was obtained from its degradation by β-amylase, when in two experiments 44% and 47% of the molecule was converted into maltose. This value for the β-amylolysis of malt amylopectin is significantly lower than the values (52—62%) normally found for amylopectins (see Manners, *Quart. Rev.*, 1955, **9**, 73). As β-amylase only attacks the exterior chains of amylopectins, the enzymic degradation stopping at one or two glucose units from the inter-chain linkage,

it can be calculated that the β-limit dextrin had an average chain length of 9—10. A value of 8—9 residues for the chain length of the limit dextrin was obtained on periodate oxidation of the polysaccharide isolated after β-amylolysis. This is in close agreement with the values previously found for amylopectin β-limit dextrins (cf. Halsall, Hirst, Hough, and Jones, *J.*, 1949, 3200).

The malted-barley amylose was methylated, initially with diazomethane and subsequently with the usual reagents, and hydrolysis of the methylated polysaccharide gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (0·32%), 2 : 3 : 6-tri-*O*-methyl-D-glucose (98·0%), and a mixture of di-*O*-methylglucoses (1·6%). It is doubtful if the dimethyl sugars are of structural significance. The quantity of tetra-*O*-methylglucose corresponded to the presence of one non-reducing terminal group per 310 ± 20 glucose residues. A value of 330—345 for the average chain length of the amylose obtained from periodate oxidation experiments was in reasonable agreement with that from the methylation data. The malt amylose, therefore, consisted of an unbranched chain of *ca.* 330 glucose residues. It is interesting that this amylose is completely converted into maltose by β-amylase (the experiment was kindly carried out by Dr. D. J. Manners), and, therefore, does not contain the barrier to complete β-amylolysis found in some amyloses (Peat, Thomas, and Whelan, *J.*, 1952, 722).

Investigations were also carried out on the starch from the same sample of barley which had been used to prepare the malt. This barley starch had an amylose content of 22·6% and the amylopectin component had an average chain length of 26 glucose residues. β-Amylolysis of the amylopectin gave a 59% conversion into maltose, from which an average chain length of 10 can be calculated for the limit dextrin. A value of 9—10 glucose residues was obtained on periodate oxidation of the polysaccharide isolated after β-amylolysis of the barley amylopectin.

From these investigations the main structural features of the starch from malted barley emerge. The amylopectin has the type of branched structure that is now well established for this starch component. The average chain length (18 ± 1), however, is significantly shorter than that normally found for amylopectins and, in particular, is shorter than that found for the corresponding barley amylopectin (26 ± 1), a value in agreement with that previously determined by McWilliam and Percival (*loc. cit.*). It is apparent, therefore, that the exterior chains of the amylopectin have been partially removed by enzymic action during the malting of the barley. The chromatographic identification of glucose and maltose in the aqueous-ethanolic extract of the malted barley suggests that the action has been that of β-amylase in conjunction with maltase. On the other hand the isolation of a methylated amylopectin of high molecular weight indicates that there has been no appreciable α-amylolytic action causing scission of the interior chains during malting. An alternative interpretation that the amylopectin component is the product of complete breakdown and resynthesis is less likely in view of the similarity between the β-limit dextrins of the barley and the malted-barley starch. The apparent increase in the amylose content of the malt starch is fully accounted for by the loss of *ca.* 25% of the amylopectin component by shortening of the exterior chains, and it appears that the amylose has undergone relatively little degradation during malting, the polysaccharide isolated after fractionation being of a similar size to the barley amylose examined by McWilliam and Percival (*loc. cit.*). It is to be remembered, however, that the amylose examined may not be representative of all the amylose present in the malt starch, since only 55% of the total amount of this component present in the malt was isolated. Nevertheless any shorter-chain amyloses, which escaped precipitation during the fractionation, were of large enough molecular size to be estimated as amylose in the potentiometric iodine titration.

The general pattern of changes undergone by the starch in the malting process has now been established, but many problems remain unsolved. For instance, the present results do not show whether the degradation of the outer chains of the amylopectin has been completely random, or whether in parts of the macromolecule enzymic action has been carried to one or two glucose units from the inter-chain linkage (as in β-amylolysis) while in other parts of the molecule no degradation has occurred. Although relatively little breakdown of the amylose component has so far been detected, a detailed comparison of the

linear fractions from barley and from malted barley would be of value, since considerable changes in this component could occur before the achroic limit was reached.

EXPERIMENTAL

The following solvents were used to separate the sugars and their derivatives: (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3; top layer), (B) butan-1-ol–ethanol–water (4 : 1 : 5; top layer), and (C) benzene–ethanol–water (169 : 47 : 15; top layer). Blue values were determined by Hassid and McCready's method (*J. Amer. Chem. Soc.*, 1943, **65**, 1154) as modified by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924). Potentiometric determinations of amylose were kindly carried out by Mr. D. M. W. Anderson and Dr. C. T. Greenwood (see Anderson and Greenwood, *J.*, in the press).

Isolation of Starch from Malted Barley.—Malted barley (mixture of Plumage Archer and Spratt Archer varieties; 1·5 kg.) was lightly pounded to split open the husk, most of the husk was blown off with a jet of air, and the starch granules were separated from further fibrous material by passage through a suitable sieve. The starchy material was extracted for 3 hr. with boiling aqueous ethanol to inactivate enzymes and to remove soluble sugars. The ethanolic extract was concentrated, and chromatographic examination of the resulting syrup showed the presence of glucose, fructose, arabinose, and maltose. The inactivated malt was dried, and ground in a " Raymond " laboratory mill, and a suspension in ethanol was passed through a 40-mesh sieve to remove the residual fibre. The separated starch granules were stirred with cold water for 3 hr. to remove water-soluble carbohydrates, the aqueous extract was decanted, and the crude starch was dried with ethanol and ether. After exhaustive extraction with boiling 1 : 19 aqueous methanol to remove fats, crude starch (400 g.) was isolated. Examination under the microscope showed the starch granules to be ovoid to spherical in shape, and many of them appeared to be slightly ruptured.

Purification of Starch.—(a) *Treatment with butanol and toluene.* Crude starch (120 g.) was stirred overnight in 0·1% aqueous sodium hydrogen sulphite (5 l.). Removal of the solid at the centrifuge gave a lower layer containing the heavier starch granules, and a pale brown protein-rich upper layer. The upper layer was removed as completely as possible, the starch layer was subjected to the same treatment three more times, and the starch was freeze-dried. As the starch still contained protein (2·5%), a sample (4 g.) was stirred in water (200 c.c.), and the suspension was shaken with butanol before being allowed to settle. The brown precipitate, obtained at the butanol–water interface was removed, and the procedure was repeated once with butanol and three times with toluene. Starch A (2·3 g.) was isolated by freeze-drying (Found: N, 0·05%).

(b) *Extraction with chloral hydrate.* Crude starch (20 g.) was extracted by the method of Meyer and Bernfeld (*Helv. Chim. Acta*, 1940, **23**, 875) to give starch B (14·3 g.) (Found: N, 0·03%). The starch had a blue value of 0·368.

Examination of Starches.—Starch A had $[\alpha]_D^{18}$ +154° (*c*, 0·9 in N-NaOH) and $[\alpha]_D^{18}$ +178° (*c*, 0·7 in 30% $HClO_4$). Potentiometric titration showed the presence of 26·0% of amylose. Periodate oxidation of a sample of the starch as described by Brown, Halsall, Hirst, and Jones (*J.*, 1948, 27) gave formic acid corresponding to one terminal non-reducing group per 25 glucose residues. The value corresponded to a chain length of 19 glucose residues in the amylopectin fraction.

Starch B had $[\alpha]_D^{16}$ +159° (*c*, 1·1 in N-NaOH) and $[\alpha]_D^{16}$ +182° (*c*, 0·78 in 30% $HClO_4$). Hydrolysis of a sample gave only glucose (97·6%). Potentiometric titration showed the presence of 27·8% of amylose.

Fractionation of Starch A.—Starch A (12 g.) was fractionated by Higginbotham and Morrison's method (*Shirley Inst. Mem.*, 1948, **22**, 148) with 4 : 1 aqueous pyridine and butanol. The amylose complex was separated, washed with water saturated with butanol, dispersed in water, and freeze-dried, to give an amylose-rich fraction (3·8 g.). The amylopectin was precipitated by pouring the supernatant liquor from the fractionation into ethanol (2 vols.), and the precipitate was dispersed in water and freeze-dried to give amylopectin A (5·4 g.). The amylose-rich fraction was dispersed in water, refractionated seven times with butanol (Higginbotham and Morrison, *loc. cit.*), and isolated as before, to give amylose A (1·1 g.).

Examination of Starch Fractions.—*Amylopectin A.* This fraction had $[\alpha]_D^{18}$ +147° (*c*, 0·5 in N-NaOH) and $[\alpha]_D^{18}$ +166° (*c*, 0·5 in 30% $HClO_4$) (Found: N, 0·05%). Potentiometric titration showed the presence of 3·9% of amylose. Periodate oxidation of a sample gave formic acid corresponding to one terminal non-reducing group per 18 glucose residues.

Amylose A. This fraction had $[\alpha]_D^{18}$ +148° (*c*, 0·5 in N-NaOH) and $[\alpha]_D^{18}$ +198° (*c*, 0·7 in 30% $HClO_4$) (Found: N, 0·02%). Potentiometric titration showed that the amylose had a maximum iodine-binding power of 18·3%.

Large-scale Fractionation of Malted Barley Starch.—Preliminary fractionation of samples of the crude malted-barley starch showed that an amylose fraction with the highest blue value was obtained by fractionation of the starch with 85 : 15 water–pyridine (Whistler and Hilbert, *J. Amer. Chem. Soc.*, 1945, **67**, 1161), and that an amylopectin fraction of low amylose content was obtained by fractionation using 4 : 1 aqueous pyridine saturated with butanol (Higginbotham and Morrison, *loc. cit.*).

Crude malted-barley starch (30 g.) was suspended in cold water (200 c.c.), and the suspension was added slowly with vigorous stirring to water (2 l.) and pyridine (450 c.c.) at 90°. The solution was maintained at this temperature for 2·5 hr. to ensure maximum dispersion of starch, cooled to 70°, centrifuged at 2000 r.p.m. to remove insoluble fibrous material, and heated again to 90°. The solution was transferred to a Dewar flask and allowed to cool slowly for 60 hr., during which the amylose–pyridine complex separated. The products from four fractionations were combined and refractionated with water saturated with butanol (twice at an amylose concentration of 0·5%, six times at 0·2%, and four times at 0·1% concentration). The final amylose–butanol complex was dispersed in water (150 c.c.) and freeze-dried, to give amylose B (11·1 g.; this represents *ca.* 55% of the amylose originally present in the crude starch).

Crude malted-barley starch (60 g.) was suspended in water (200 c.c.), and the suspension was slowly added with vigorous stirring to water (1800 c.c.) and pyridine (400 c.c.) at 90°. The paste was stirred at this temperature for 3 hr., subjected to 5 minutes' high-speed stirring in an "Ato-Mix" disperser, cooled to 70°, and centrifuged to remove fibrous material. The paste was heated again to 90°, butanol (*ca.* 400 c.c.) was added slowly to saturate the solution, and the solution was transferred to a Dewar flask and allowed to cool slowly for 60 hr. The amylose complex was separated at the centrifuge and the supernatant liquid was concentrated under reduced pressure to 1500 c.c. and poured into ethanol (4500 c.c.). The precipitated polysaccharide was washed several times with water saturated with butanol, dispersed in water, and freeze-dried. Two such fractionations gave amylopectin B (41 g.).

Examination of malted-barley amylopectin.

Amylopectin B had $[\alpha]_D^{16}$ +149° (*c*, 1·1 in N-NaOH) and $[\alpha]_D^{16}$ +170° (*c*, 0·72 in 30% $HClO_4$). Hydrolysis of a sample gave only glucose (96·0%). The amylopectin had a blue value of 0·085, and potentiometric titration showed the presence of 0·5% of amylose (average of two determinations).

Methylation of the Amylopectin.—Amylopectin (24 g.) was methylated twelve times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature, and the product (24·4 g.; OMe, 43·0%) was fractionated in boiling chloroform–light petroleum (b. p. 60—80°) mixtures, to give a main fraction {20·7 g.; $[\alpha]_D^{15}$ +200° (*c*, 0·5 in $CHCl_3$); OMe, 43·2%}. Part of this fraction (6·0 g.) was methylated twice more with methyl iodide and silver oxide, to give methylated amylopectin {5·2 g.; $[\alpha]_D^{15}$ +200° (*c*, 0·5 in $CHCl_3$); OMe, 43·5%}, used in subsequent experiments. The methylated amylopectin had $\eta_{sp.}/c$, 2·21 (*c*, 0·4 in *m*-cresol), corresponding to an apparent *M* 320,000 (see Hirst and Young, *J.*, 1939, 1475).

Hydrolysis of Methylated Amylopectin and Separation of Methylated Sugars.—A sample of the methylated amylopectin (50 mg.) was hydrolysed successively with methanolic and aqueous hydrogen chloride, and, after neutralisation with silver carbonate, the hydrolysate was examined on the chromatogram. Quantitative estimation (Hirst, Hough, and Jones, *loc. cit.*) revealed the presence of 2 : 3 : 4 : 6-tetra-*O*-methyl- (R_G 1·0, 5·5%), 2 : 3 : 6-tri-*O*-methyl- (R_G 0·84, 86·1%), di-*O*-methyl- (R_G 0·65, 0·58, 8·4%), and traces of mono-*O*-methyl-glucose and glucose. This result indicated the presence of one non-reducing terminal group per 18—19 glucose residues.

Methylated amylopectin (3·32 g.) was hydrolysed successively with methanolic hydrogen chloride (200 c.c.; 1%) for 5 hr. and with hydrochloric acid (300 c.c.; 0·5N) for 5 hr. The hydrolysate was neutralised with silver carbonate and de-ionised with Amberlite resins IR-100 and IR-4B, and the solution was concentrated to a syrup (3·35 g.). The syrup was fractionated on cellulose (64·3 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol partly saturated with water, as eluants to give four fractions.

Fraction 1. The syrup (329 mg.) was examined on the chromatogram and showed only 2 : 3 : 4 : 6-tetra-*O*-methylglucose, but hypoiodite oxidation indicated only 49% of aldohexose.

A sample (10 mg.) was rehydrolysed and chromatographic examination of the hydrolysate showed that 2 : 3 : 6-tri-*O*-methylglucose was also present. The syrup (310 mg.) was rehydrolysed with hydrochloric acid (20 c.c.; 1%) on the water-bath for 5 hr. After neutralisation with silver carbonate, the resulting syrup (290 mg.) was fractionated on filter sheets, using solvent C, to give fractions 1*a* (105 mg.) and 1*b* (112 mg.). Fraction 1*a* crystallised and after two recrystallisations from light petroleum (b. p. 40—60°) had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 86—88° and $[\alpha]_D^{18}$ +98° → +83° (equil.) (*c*, 0·4 in H_2O) (Found : C, 51·2; H, 8·5; OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: C, 51·2; H, 8·5; OMe, 52·5%). The derived 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine had m. p. and mixed m. p. 136—138° (Found : N, 4·3; OMe, 39·4. Calc. for $C_{16}H_{25}O_5N$: N, 4·5; OMe, 39·9%). Fraction 1*b* crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{18}$ +88° → +68° (equil.) (*c*, 0·4 in H_2O) and $[\alpha]_D^{18}$ +67° → −35° (10 hr., const.) (*c*, 0·4 in methanolic 2% hydrogen chloride) (Found : OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). From the above results the amount of tetra-*O*-methyl-D-glucose was calculated to be 160 ± 8 mg., corresponding to one non-reducing terminal group per 18—19 glucose residues.

Fraction 2. The syrup (2·31 g.) crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{18}$ +90° → +66° (equil.) (*c*, 1·0 in H_2O) and $[\alpha]_D^{18}$ +67° → −34° (10 hr., const.) (*c*, 1·0 in methanolic 2% hydrogen chloride) (Found : OMe, 41·5. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 3. Chromatographic examination of the syrup (160 mg.) showed the presence of 2 : 3 : 6-tri-*O*-methyl- and 2 : 3-di-*O*-methyl-glucose. Separation on filter sheets with solvent B gave fractions 3*a* (28 mg.) and 3*b* (95 mg.). Fraction 3*a* was identified as 2 : 3 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 114—116°. Fraction 3*b* was a syrup which did not crystallise, but was chromatographically homogeneous (R_G 0·64 in solvent B) and had $[\alpha]_D^{18}$ +106° → +68° (equil.) (*c*, 0·4 in H_2O) (Found : OMe, 29·2. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). The sugar was identified as 2 : 3-di-*O*-methyl-D-glucose by conversion into 2 : 3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. 160—162° (Found : OMe, 19·2. Calc. for $C_{14}H_{22}O_6N_2$: OMe, 19·7%).

Fraction 4. The syrup (70 mg.), which did not crystallise, had $[\alpha]_D^{18}$ +74° → +58° (equil.) (*c*, 0·5 in H_2O) and $[\alpha]_D^{18}$ +60° → −10° (8 hr., const.) (*c*, 0·5 in methanolic hydrogen chloride) (Found : OMe, 29·0. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). Chromatographic examination suggested the presence of 2 : 6- and/or 3 : 6-di-*O*-methyl-D-glucose (R_G 0·58 in solvent B). The syrup (35 mg.) was converted into the methyl glycosides and treated with sodium metaperiodate by Bell's method (*J.*, 1948, 992). 0·53 Mole of periodate was consumed per $C_9H_{18}O_6$ unit, indicating the presence of 53% of 2 : 6-di-*O*-methyl-D-glucose in the fraction. After destruction of excess of periodate, the chloroform-soluble extract was hydrolysed and chromatographic examination showed a single sugar (R_G 0·59 in solvent B) corresponding to 3 : 6-di-*O*-methyl-D-glucose. Hypoiodite oxidation showed 14·5 mg. of the sugar to be present, corresponding to 41% of the fraction.

Determination of Glucose Residues in the Amylopectin unattacked by Periodate.—Amylopectin (0·985 g.) was dissolved in water (80 ml.) containing potassium chloride (5 g.), and 0·3M-sodium metaperiodate (30 ml.) was added. The mixture was shaken in the dark for 10 days. After removal of periodate and other ions the oxidised polysaccharide was dissolved in 0·2N-sodium hydroxide (30 ml.), and sodium borohydride (0·25 g.) was added. After being kept overnight the solution was neutralised with acetic acid, dialysed, and taken to dryness. The resulting polyol was hydrolysed and the glucose formed was estimated by quantitative paper chromatography. The quantity of glucose found (7·3 mg.) showed that 0·63% of the glucose residues in the amylopectin were unattacked by periodate.

β-Amylolysis of the Amylopectin.—Amylopectin (24·3 mg.) was incubated at 73° with 0·2M-acetate buffer (pH 4·6; 10 c.c.), distilled water (39 c.c.), and crystalline sweet-potato β-amylase solution (1 c.c.) (kindly supplied by Dr. D. J. Manners). Aliquot portions were removed at intervals for determination of reducing power (as maltose) by means of the Shaffer–Somogyi reagent 60 as modified by Hanes and Cattle (*Proc. Roy. Soc.*, 1938, *B*, **125**, 387). After 4 hr. the conversion into maltose was complete at 44%.

Isolation of Malted-barley Amylopectin β-Limit Dextrin.—Amylopectin (0·624 g.) was dissolved in water (305 c.c.), and 0·2M-acetate buffer (pH 4·6; 100 c.c.) and β-amylase solution [25 c.c.; prepared from barley flour by Northcote's method (*Biochem. J.*, 1953, **53**, 348)] were added. After incubation for 48 hr. at 37° the conversion into maltose was complete at 47%. The solution was boiled for 10 min. to inactivate the enzyme, and coagulated protein was removed

by filtration. The solution was dialysed for 2 days, and the dextrin was precipitated with ethanol, redissolved in water (50 c.c.), and freeze-dried (yield, 0·274 g.) (Found: N, 0·9%). The dextrin (0·244 g.) was dispersed in water (100 c.c.) and shaken for 1 hr. with butanol (100 c.c.). The brown layer formed at the interface was removed, and the dextrin was precipitated with ethanol, dispersed in water, and freeze-dried (yield, 0·208 g.) (Found: N, 0·05%). The dextrin had $[\alpha]_D^{18}$ +149° (*c*, 0·5 in N-NaOH). Periodate oxidation of a sample gave formic acid corresponding to one non-reducing group per 8—9 glucose residues.

Examination of malted-barley amylose.

Amylose B had $[\alpha]_D^{16}$ +200° (*c*, 0·5 in H_2O), $[\alpha]_D^{15}$ +143° (*c*, 0·5 in N-NaOH), and $[\alpha]_D^{15}$ +205° (*c*, 0·5 in 30% $HClO_4$), and a blue value of 1·25. Potentiometric titration showed that the amylose had a maximum iodine-binding power of 19·2%. Hydrolysis of a sample gave only glucose (95·0%).

Periodate oxidation of the amylose gave one mol. of formic acid per 110—115 glucose residues. If one assumes the release of two mols. of formic acid from the reducing end-group and one mol. from the non-reducing end-group, this value corresponded to a chain length of 330—345 glucose residues. Hydrolysis of the periodate-oxidised amylose showed that there were no unattacked glucose residues.

Methylation of the Amylose.—Malt amylose (7·5 g.) was partly methylated by being kept at 0° in an ethereal solution of diazomethane (cf. Hough and Jones, *Chem. and Ind.*, 1952, 380). When the ethereal solution became colourless it was removed by decantation and fresh ethereal diazomethane was added. After 34 weeks the partly methylated amylose was isolated (Found: OMe, 20·2%). The polysaccharide was then methylated five times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature, and the product (7·56 g.; OMe, 44·2%) was fractionated in boiling chloroform–light petroleum (b. p. 60—80°) mixtures, to give a main fraction (6·17 g.), $[\alpha]_D^{17}$ +206° (*c*, 1·0 in $CHCl_3$), $\eta_{sp.}/c$, 0·82 (*c*, 0·4 in *m*-cresol) (OMe, 44·8%).

Hydrolysis of Methylated Amylose and Separation of Methylated Sugars.—A sample of the methylated amylose (50 mg.) was hydrolysed and chromatographic examination of the hydrolysate showed the presence of 2 : 3 : 6-tri-*O*-methylglucose together with small quantities of di-*O*-methylglucose. The major part of the methylated amylose (4·25 g.) was hydrolysed as described for methylated amylopectin, and the resulting mixture of sugars (4·21 g.) was fractionated on cellulose in the usual way to give three fractions.

Fraction 1. Chromatographic examination of the syrup (27 mg.) showed only 2 : 3 : 4 : 6-tetra-*O*-methylglucose, but hypoiodite oxidation indicated only 47% of aldohexose. A sample (1 mg.) was rehydrolysed and chromatographic examination of the hydrolysate showed that 2 : 3 : 6-tri-*O*-methylglucose was also present. The syrup (22 mg.) was rehydrolysed with 1% hydrochloric acid (10 c.c.) on the water-bath for 5 hr. After neutralisation with silver carbonate, the resulting syrup (21 mg.) was fractionated on filter sheets with solvent C, to give fractions 1*a* (10·5 mg.) and 1*b* (9 mg.). Fraction 1*a* crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 78—80°. Fraction 1*b* which did not crystallise, had R_G 0·82 in solvent B, corresponding to 2 : 3 : 6-tri-*O*-methyl-D-glucose, but was not examined further. From the above results the amount of tetra-*O*-methyl-D-glucose was calculated to be 12·8 ± 0·4 mg., corresponding to one non-reducing terminal group per 310 ± 20 glucose residues.

Fraction 2. The syrup (3·58 g.) crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{18}$ +94° → +70° (equil.) (*c*, 1·0 in H_2O) and $[\alpha]_D^{18}$ +63° → −34° (8 hr., const.) (*c*, 0·95 in methanolic 2% hydrogen chloride) (Found: OMe, 41·4. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 3. The syrup (51 mg.), which did not crystallise, had $[\alpha]_D^{18}$ +58° → −11° (8 hr., const.) (*c*, 0·4 in methanolic 2% hydrogen chloride) (Found: OMe, 29·0. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). Chromatographic examination showed the presence of sugars corresponding to 2 : 3-di-*O*-methyl-D-glucose (R_G 0·59 in solvent B) and to 2 : 6- and/or 3 : 6-di-*O*-methyl-D-glucose (R_G 0·53). Quantitative determination (Hirst, Hough, and Jones, *loc. cit.*) indicated the presence of 7·5% of 2 : 3-di-*O*-methyl-D-glucose (4 mg.). Periodate oxidation of the derived methyl glycosides (Bell, *loc. cit.*) showed that 79% of the fraction was 2 : 6-di-*O*-methyl-D-glucose. The periodate-oxidised methyl glycosides were hydrolysed and chromatographic examination of the hydrolysate showed the presence of 2 : 3- and 3 : 6-di-*O*-methylglucose.

Examination of barley starch and barley amylopectin.

In the following experiments the barley used corresponded to that from which the malted barley examined above was derived (*i.e.*, a mixture of Plumage Archer and Spratt Archer varieties).

Isolation and Purification of Barley Starch.—Crude barley starch (52 g.) was prepared in a manner similar to that described for malted barley starch, and the purification was carried out as for malt starch A. The pure barley starch (5 g.) had $[\alpha]_D^{18}$ +154° (*c*, 1·0 in N-NaOH) (Found : N, 0·02%). Potentiometric titration showed the presence of 22·6% of amylose. Periodate oxidation of a sample of the starch gave formic acid corresponding to one terminal non-reducing group per 32 glucose residues. The value corresponded to a chain length of 24 glucose residues in the amylopectin fraction.

Preparation of Barley Amylopectin.—Barley amylopectin was prepared from crude barley starch as described for malt amylopectin B. The amylopectin had $[\alpha]_D^{18}$ +148° (*c*, 1·2 in N-NaOH) (Found : N, 0·05%). The formic acid produced on periodate oxidation corresponded to an average chain length of 26 glucose residues. Potentiometric iodine titration showed the presence of 0·70% of amylose.

β-*Amylolysis of Barley Amylopectin and Isolation of the* β-*Limit Dextrin.*—Barley amylopectin was incubated with barley β-amylase, and after 46 hr. the conversion into maltose was complete at 59%. The β-limit dextrin was isolated as described for the malt dextrin. Barley amylopectin β-limit dextrin had $[\alpha]_D^{18}$ +153° (*c*, 0·5 in N-NaOH) (Found : N, 0·04%). Periodate oxidation of a sample gave formic acid corresponding to an average chain length of 9—10 glucose residues.

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