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CHROMOPHORE FORMATION DURING THE ALKALINE DECOMPOSITION OF AMYLOSE AND MALTOSE[1]

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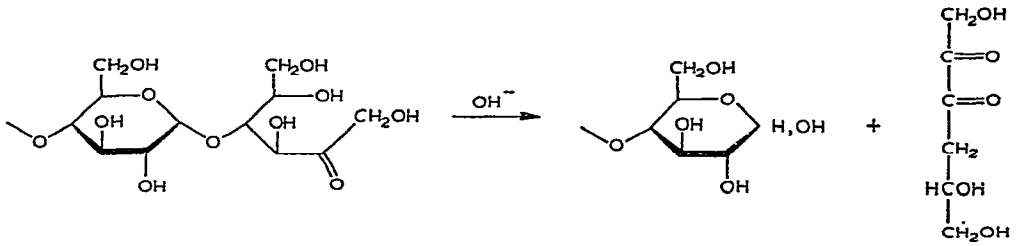
ABSTRACT

Anaerobic digestion of amylose in aqueous, alkaline solution (pH 9.85) yields a chromophore having $\lambda_{max}^{pH\ 11.5}$ 290, $\lambda_{max}^{pH\ 6.70}$ 255 nm, and a pK_a' of 8.5; the absorbance is a linear function of the amount of amylose degraded. Similarly treated D-glucose has $\lambda_{max}^{pH\ 11.5}$ 260 nm. The u.v. spectra of alkali-degraded maltose at various pH values are a summation of the corresponding spectra of degraded amylose and D-glucose. The amylose-derived chromophore is formed *via* 4-deoxy-D-*glycero*-hexo-2,3-diulose. The strong i.r. carbonyl band at 1580 cm^{-1} is attributed to a metal-chelated β-hydroxy-α,β-enone; on removal of the cation, this band is replaced by absorption at 1720 cm^{-1}, indicating conversion into the β-diketone tautomer. It appears that the β-hydroxy-α,β-enone tautomer exists in aqueous solution and is ionised to the enolate form in alkaline conditions.

INTRODUCTION

The formation of D-isosaccharinates, glycolate, and 2-deoxy-D-tetronolactone during the anaerobic degradation of amylose in aqueous alkali is considered to be due to decomposition of 4-deoxy-D-*glycero*-hexo-2,3-diulose (**1**), which is produced by β-elimination in the propagative, stepwise degradation of amylose chains[2]. The other products isolated, namely formate and lactate, could arise by reverse aldol-reaction and cleavage of enediols[2]. Evidence for the formation of enediols has been found in the strong u.v. absorption at 282 nm after the alkaline digestion of amylose[3]. In



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connection with a study of the yellowing of alkali-treated cellulose[4], an investigation of chromophore formation during the alkaline degradation of amylose was undertaken.

RESULTS AND DISCUSSION

In order to avoid oxidative degradation and alkaline hydrolysis of the substrates, the reactions at pH 9.85 were performed anaerobically at 98°. The yellow–brown reaction mixtures obtained on digestion of amylose had no absorption peak in the visible region, but exhibited a strong band at 290 nm. Acidification caused a hypsochromic shift (as previously reported[3]) to 255 nm, with an accompanying hyperchromic effect; these spectral changes could be reversed by the addition of alkali. The pH-dependence of the u.v. absorption (Fig. 1) exhibited an isosbestic point at 278 nm and permitted the calculation of a pK'_a value of 8.5 (Table I).

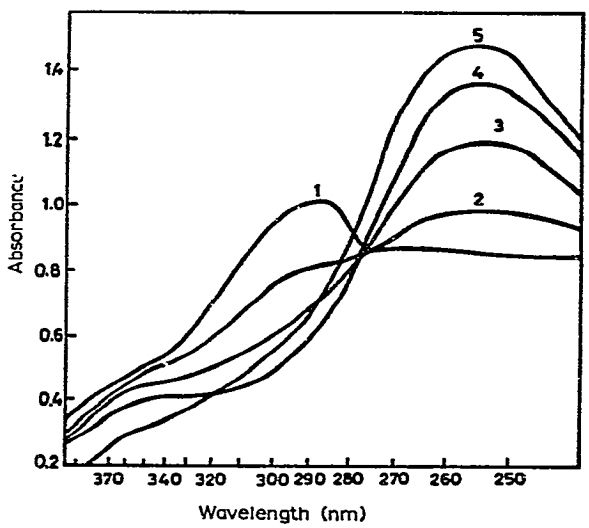


Fig. 1. Spectra of alkali-degraded amylose (1%, pH 9.6, 2 h, dilution × 10) at pH 10 (curve 1), 8.9 (2), 8.2 (3), 7.2 (4), and 1.85 (5).

With maltose as substrate, u.v. absorptions were observed at 290 and 260 nm. After mild acidification, there was a single band at 260 nm, which showed a further hypsochromic shift (Fig. 2) below pH 3. Merck maltose (which contains less dextrin than the BDH product used in the previous experiment) gave the same result.

Alkaline treatment of D-glucose (*cf.* refs. 5 and 6) gave a chromophore having λ_{max} 260 nm, and a reversible hypsochromic and hypochromic shift was obtained only below pH 3 (Fig. 3); no isosbestic point was evident. It appeared that the maltose-derived spectra represented a superposition of those for degraded amylose and degraded D-glucose, and, indeed, the difference spectra of treated maltose against an

equimolar solution of treated D-glucose in the reference beam (Fig. 4) were the same (λ_{max} and isosbestic point) as for the amylose-derived chromophore (Fig. 1); the calculated values for pK'_a also agreed (Table I).

TABLE I

CALCULATED VALUES FOR pK'_a OF ALKALI-TREATED SUBSTRATE

Substrate	*pH*	pK'_a	
		λ = 255 nm	*λ = 290 nm*
Amylose	6.9	8.4	8.2
	7.2	8.3	8.2
	8.2	8.4	8.8
	8.9	8.5	8.8
	9.5	8.8	8.5
	Average	8.5	8.5
Maltose[a]	8.85	8.5[b]	8.7[b]

[a]Spectra measured with an equimolar solution of degraded D-glucose as reference. [b]Average of two determinations.

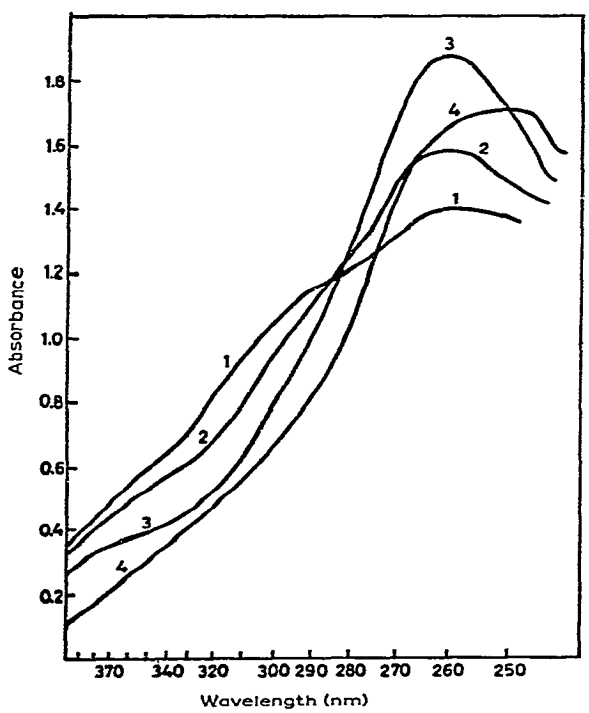


Fig. 2. Spectra of alkali-degraded maltose (4mM, pH 9.8, 2 h, dilution × 5) at pH 11.5 and 9.85 (curve 1), 8.75 (2), 7.15, 6.7 and 5.7 (3), and 2.7 (4).

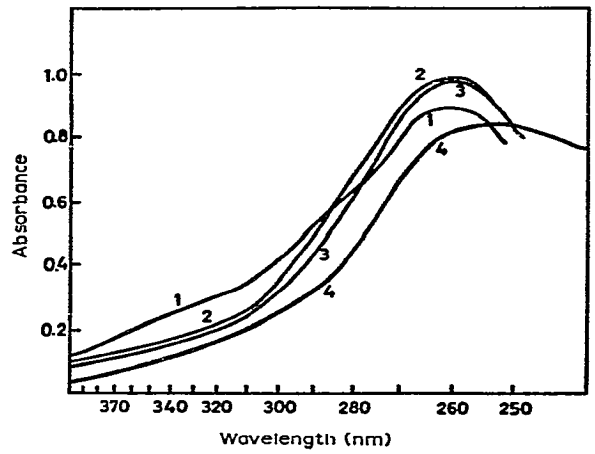


Fig. 3. Spectra of alkali-degraded D-glucose (4mM, pH 9.8, 2 h, dilution × 5) at pH 10.5 and 9.9 (curve 1), 7.2 (2), 6.8 and 5.8 (3), and 2.55 (4).

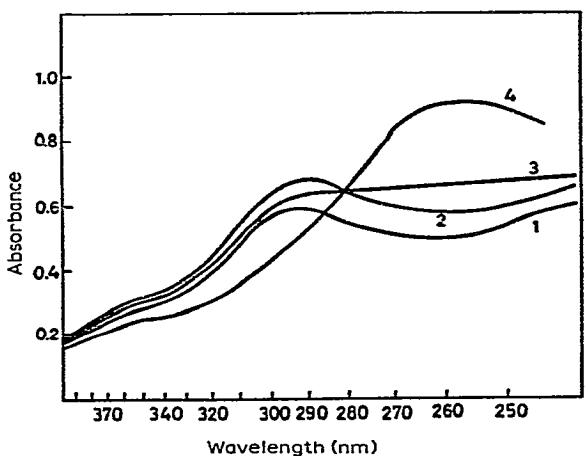


Fig. 4. Spectra of alkali-degraded maltose (4mM, pH 9.8, 2 h, dilution × 5) against reference of alkali-degraded D-glucose (ditto) at pH 11.5 (curve 1), 9.85 (2), 8.85 (3), and 6.65 (4).

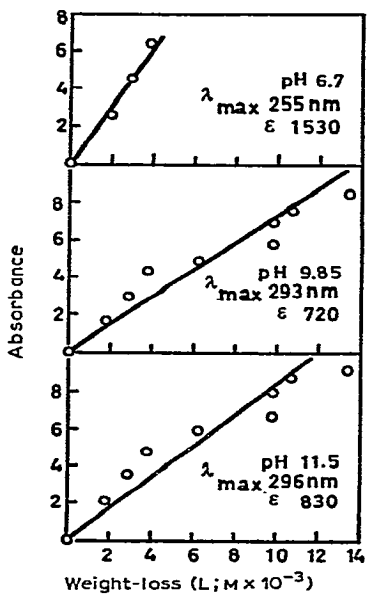
The qualitative similarity between the chromophores derived from amylose and maltose—D-glucose was corroborated by comparison of their molar absorption coefficients (ε) (Table II). The values for amylose were obtained by alkaline treatment for a range of reaction times and by determination of the slopes of the graphs of absorbance (at selected values of pH) plotted against weight-loss (Fig. 5). The molar absorption coefficient (2430) for maltose, against solvent as reference, agrees with the value (2500) calculated from the data of Macmillan and Melvin[3].

TABLE II

MOLAR ABSORPTION COEFFICIENTS (ε^a) OF ALKALI-DEGRADED CARBOHYDRATES

pH	λ_{max} (*nm*)	ε^a		
		Maltose[b]	*Amylose*[c]	D-*Glucose*
2.55	252	—	—	1050
6.7	255	1190	1530	1240
9.85	295	820	720	—
11.5	295	760	830	—

[a]Absorbance in a 1-cm cell of a solution molar in decomposed carbohydrate. [b]Difference spectrum against equimolar, alkali-treated D-glucose as reference solution: average of 5 determinations. [c]From slopes in Fig. 5.



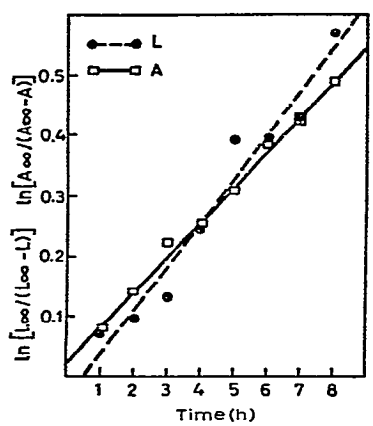


Fig. 5. Absorbance of amylose chromogen as function of weight-loss, $\varepsilon = \Sigma A/\Sigma L$.

Fig. 6. Degradation of 1% amylose solution at pH 9.85. Plot[7] for determination of k_2 from absorbance (A) and weight-loss (L) data.

The linear correlation between absorbance and alkali weight-loss suggested that absorbance measurements may be a useful assay for amylose degradation. This was tested by treating the kinetic data obtained for amylose by the method of Lai and Sarkanen[7] for determining the pseudo-first-order rate constant for termination reactions (k_2), according to the equation:

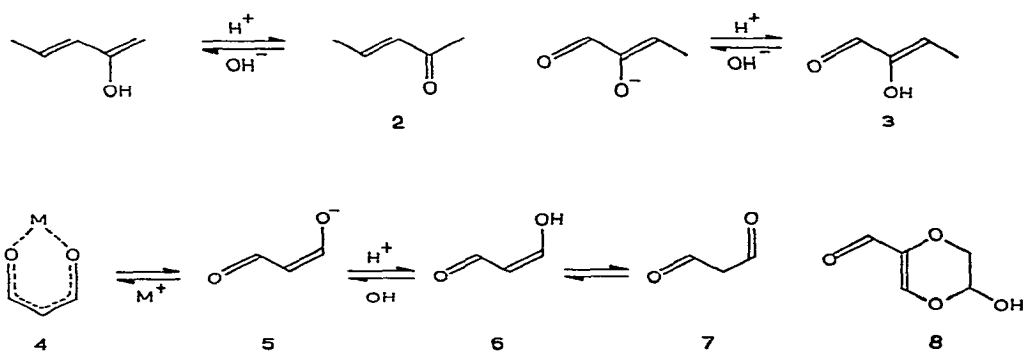
$$k_2 = \ln [L_\infty/(L_\infty - L)]/t,$$

where L_∞ is the fractional weight degraded at infinite time, L is the fractional weight degraded at time t, and t is the reaction time. Fig. 6 illustrates that absorbance gives values of the exponential function that deviate much less from a straight line than those derived from blue-value determinations of amylose concentration; the respective values for the coefficient of variation for k_2 are 3.2 and 9.1%. Furthermore, chromophore absorbance can be measured without the use of standardised reagents, as is necessary in the blue-value method.

An additional methodological advantage of yellowing, as compared with the blue-value method in kinetic studies, is that the photometric readings increase in value as the degradation proceeds, so that initial reaction rates are more reliable.

Resolution of the u.v. spectrum of degraded maltose into those of glucose- and amylose-derived chromophores is in accordance with the β-elimination mechanism of alkali degradation, which requires that each maltose molecule fragments into one molecule each of D-glucose (derived from the non-reducing moiety of maltose) and **1**, the latter product being identical with the *sole* initial product of depolymerisation of amylose chains from the reducing termini. The D-glucose formed from the maltose will then decompose as does an equimolar solution of authentic D-glucose. The results thus indicate that the amylose-derived chromophore is formed as a result of β-elimination and is consequently produced from **1**.

The u.v.-spectral characteristics of the chromophore may be accounted for by an enone structure (**2**), which enolises in alkaline solution (*cf.* Ref. 8). Alternatively, an enonic molecule may contain an auxochromic hydroxyl group that ionises in alkaline solution, so that the structure could be the enol tautomer of an α- or β-dicarbonyl (**3** or **6**, respectively), or the hydroxyl group could be further removed from the enone as in 2,3-dihydro-6-formyl-3-hydroxy-1,4-dioxin[9] (**8**). The hyperchromic effect of acidification of the amylose-derived chromophore distinguishes it from reported enones[8,10] and hydroxyenones[9–11], which, in contrast, undergo a corresponding hypochromic change. The high pK of 8.5 would seem to exclude an α,β-unsaturated carboxylate structure, as well as enols such as ascorbic acid (pK_a 4.15)[12], reductone (pK_a 4.9)[12], tetronic acid (pK_a 3.76)[12], 1,3-cyclohexanedione (pK_a 5.89)[11], and **8** (pK_a 5.6)[9].



A tentative choice between structures such as **2**, **3**, **6**, or **8** was possible using i.r. data. A film cast from ethanol had characteristic absorption maxima at 2830, 1580 (s), 1360 (s), and 780 cm^{-1}. On removal of cations, these bands were replaced by absorptions at 1720 (s) and 1220 cm^{-1}. Reported carbonyl-absorption frequencies for enones[8,13] (**2**), α-diketones[14] (**3**), β-ketoaldehydes (enol form)[14], and the hydroxyenone[9] **8** are, respectively, 1700 and 1600, 1730–1710, 1670–1645, and 1640 cm^{-1}, and their spectra could not be correlated with the spectra obtained. The particularly strong carbonyl band at 1580 cm^{-1} is therefore attributed to a β-hydroxy-α,β-enone system **6**, which is present as a metallic chelate **4** in the ethanolic solution (*cf.* Ref. 15). The radical change in the spectrum on decationisation, especially the shift of the carbonyl band to 1720 cm^{-1}, is taken to indicate tautomerisation to a β-diketone form **7** (*cf.* Ref. 15). It is concluded that, in aqueous solution, the amylose-derived chromophore exists as the β-hydroxy-α,β-enone tautomer (**6**, enolic form of a β-diketone), which is ionised to the enolate **5** on addition of alkali, with a pK'_a of 8.5.

The alkali-induced chromophore discussed here may be related to the chromophores formed from irradiated starches[16–18]. Furthermore, discoloration phenomena, such as the non-enzymatic browning of foodstuffs, the fading of paper, and the yellowing of cotton, may be due in part to alkaline decomposition of starches present.

If the chromophore formed during the alkaline digestion of amylose is indeed derived from the dicarbonyl intermediate **1**, then the same chromophore should be produced by cellulose. A study of the discoloration of alkali-digests of celluloses is described elsewhere[19].

EXPERIMENTAL

D-Glucose was BDH Analar grade. The maltoses used were BDH Laboratory Reagent (1% dextrin) and Merck grade "for biochemical use" (dextrin 0.1%, D-glucose 0.1%). Amylose was a product of AVEBE, Holland, having an intrinsic viscosity of 1.4 dl/g in M potassium hydroxide, and a blue value of 1.35. The inert gas used was Matheson high-purity helium or prepurified nitrogen, and it was filtered through a No. 2 glass sinter before use. A Radiometer Titrator Type TTT lb was used for pH determinations. U.v. spectra were obtained with a Perkin–Elmer 450 Spectrometer, using aqueous solutions in 1-cm cells; the reference used was solvent brought to the same pH as the sample, unless otherwise stated. I.r. spectra were measured on a Perkin–Elmer 257 Spectrometer from films cast from ethanolic solution on sodium chloride. The buffer solution (pH 9.85–9.90) was prepared by mixing M sodium hydroxide (1 vol.) with 5% sodium hydrogen carbonate (4 vol.).

Procedure for anaerobic degradation and u.v. spectrometry. — Solutions of D-glucose and maltose (4mM) were prepared in the buffer. An aliquot (~12 ml) of sugar solution was purged with inert gas, sealed in a glass tube, and immersed in a boiling-water bath (98°). At intervals, the tubes were rapidly cooled and opened, and, after rapid adjustment of the pH of the contents to the desired value with hydro-

chloric acid or aqueous sodium hydroxide, the u.v. spectrum was recorded immediately. Maximal absorbance was attained after incubation for 2 h, which was adopted as the standard reaction time.

Amylose (1 g) was dissolved in M sodium hydroxide (20 ml) under an inert gas and with stirring. Addition of 5% aqueous sodium hydrogen carbonate (80 ml) yielded a 1% solution of amylose in the buffer. The reaction was carried out as described for the sugars. After incubation, the cooled solution was filtered through a No. 2 glass sinter and brought to pH 1.85 with hydrochloric acid; the absorbance was unchanged for 4 h at this pH. Aliquots of this solution were adjusted to the required pH with aqueous sodium hydroxide before spectrometry. The fraction of amylose degraded was obtained from a determination of the blue value[20] of the cooled reaction mixture; this method gives results identical to those obtainable[7] by gravimetric and titrimetric techniques. The λ_{max} of the iodine–amylose complex was constant at 625 nm throughout the decomposition, suggesting that the blue value also remained unchanged (*cf.* Ref. 21).

Values for pK'_a were calculated from the pH dependence of the u.v.-absorption, according to the equation[22]:

$$pK' = pH - \log \frac{(A - A_{AH})}{(A_{A^-} - A)},$$

where A_{AH}, A_{A^-}, and A are the respective absorbances of the acid AH, the ion A^-, and the solution under investigation, all at the same concentration.

I.r. spectra. — Amylose (33 g) was treated with boiling, 5% aqueous sodium hydrogen carbonate (700 ml) for 24 h. After filtration through Whatman No. 3 paper, the cooled reaction mixture (pH 10.75, λ_{max} 293 nm) was adjusted to pH 2.3 with conc. sulphuric acid (11.7 ml). Dissolved gases were then removed by stirring and pressure reduction, and sodium hydroxide (5%) was added to pH 6.1. The solution was concentrated to ~50 ml, filtered, and reconcentrated four times after the addition of ethanol (~200 ml; pH 9.1; *cf.* pure ethanol, pH 9.5). The residue had λ_{max}^{EtOH} 260 nm; ν_{max}^{film} 3370 s (bonded OH), 2930, 2830, 1580 s (C=O), 1410, 1360 s, 1110, 1050, 780, and 665 cm^{-1}. Addition of formic acid to an ethanolic solution of the residue did not change the spectrum. On treatment with Amberlite IR-120(H^+) or MB-3 resins (solution pH 6.2, λ_{max} 260 nm) or with hydrochloric acid, however, the i.r. spectrum changed to 3370 s (bonded OH), 2930, 1720 s (C=O), 1410, 1220, 1110, 1050, and 665 cm^{-1}; the original spectrum was obtained if sodium hydroxide was added to the decationised, ethanolic solution.

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