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Note

Chromogen formation during the alkaline degradation of hydrocellulose and cellobiose[1]

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Under mild conditions of temperature (below 100°) and alkalinity, hydrocellulose decomposes anaerobically by a stepwise erosion of D-glucose residues at reducing chain-termini by β-alkoxyl elimination (the "peeling" process) with the liberation of saccharinate molecules[2]. The accompanying yellowing of the supernatant solution is well-known, and a colorimetric study of the extracts has revealed that the absorbance values are related rectilinearly to the extent of hydrolytic degrada-

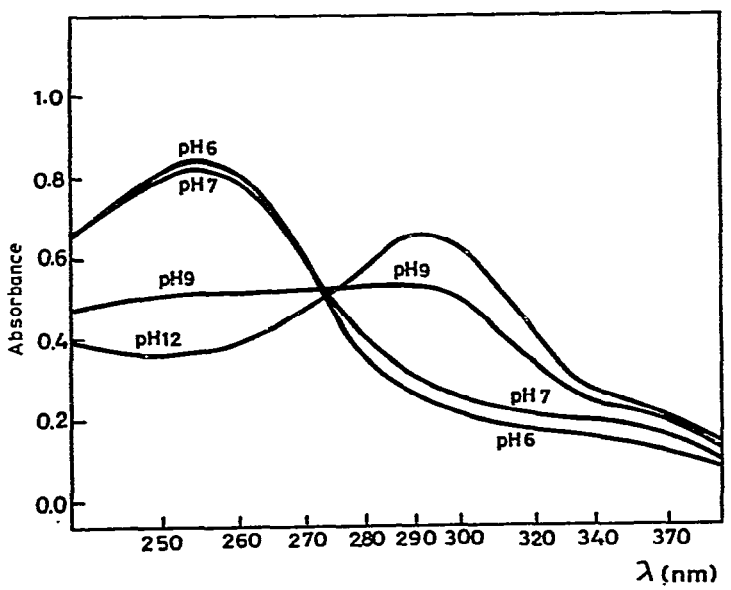

Fig. 1. Alkaline degradation of hydrocellulose; pH-dependence of the u.v. absorption spectrum of the supernatant solution measured against water in the reference beam.

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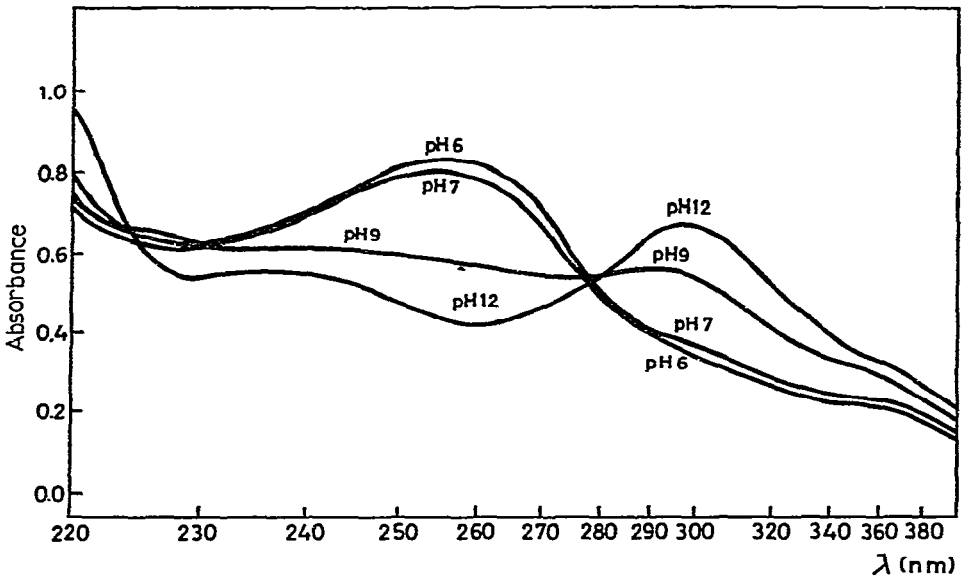


Fig. 2. Alkaline degradation of cellobiose; pH-dependence of the u.v. absorption spectrum measured against an equimolar solution of degraded D-glucose in the reference beam (solutions, 4mM; dilution, 1:20).

tion of the original hydrocellulose[3,4]. We now report on the spectrophotometric properties of the chromophore.

Cotton cellulose that had been hydrolyzed for 6–288 h in 5M hydrochloric acid at 25° served as the substrates in this study. On extracting the hydrocelluloses with boiling 0.6M sodium hydrogen carbonate for 1 h, the supernatant solution became yellow (λ_{max} 290 nm). Acidification caused a blue shift to 256 nm with an accompanying hyperchromic effect that could be reversed by the addition of alkali. The pH-dependence of the u.v. spectrum (Fig. 1) revealed an isosbestic point at 275 nm and permitted the calculation of an apparent pK_a of 8.4–8.6. All of the u.v. activity could be removed by dialysis through a Cellophane membrane, indicating that the chromogen has a low molecular weight.

With cellobiose as the substrate for alkaline degradation, absorption peaks were obtained at 288 and 260 nm, which, on mild acidification, coalesced to a single band at 260 nm that further shifted to 250 nm below pH 3. Alkaline treatment of D-glucose revealed a peak at 260 nm (*cf.* Refs. 1 and 5) that shifted to a shorter wavelength only below pH 3; no isosbestic point was obtained*. These findings indicated that the spectra derived from cellobiose represented a superposition of the

*The degradation of D-glucose in a more strongly alkaline medium (0.63M sodium hydroxide) has been examined by Forsskåhl, Popoff, and Theander [*Carbohydr. Res.*, 48 (1976) 13]. In addition to acidic, aliphatic, degradation products, a series of cyclic enols and phenolic compounds are formed. This complex mixture of chromogens may well account for the absorption at 260 nm observed for degraded D-glucose.

TABLE I

APPARENT MOLAR ABSORPTION COEFFICIENTS[a] (ε') OF ALKALI-DEGRADED CARBOHYDRATES

Substrate	*pH of chromogen solution*	λ_{max} *(nm)*	ε'
Hydrocellulose	1.5	252	945
	7.0	253	1010
	10.5	290	895
Cellobiose–D-glucose[b]	1.5	247	1025
	7.0	259	1050
	10.5	294	820
D-Glucose	1.5	245	1000
	7.0	260	1180
	10.5	261	1050

[a]See text for definition. [b]Difference spectrum with equimolar solutions of alkali-treated D-glucose in the reference beam and alkali-treated cellobiose in the sample beam.

spectra of degraded hydrocellulose and degraded D-glucose. When the spectra of degraded cellobiose were recorded with an equimolar solution of degraded D-glucose in the reference beam (Fig. 2), they were indeed similar to those for the degraded hydrocellulose (Fig. 1) with regard to λ_{max}, isosbestic point, and apparent pK_a.

This qualitative identification is corroborated by the similarity of the molar absorption coefficients (ε). Since most of the decomposed carbohydrates are well-known acids and not chromogenic compounds, a relative measure of ε was obtained by using the absorbance (1-cm cell) of a molar solution of decomposed carbohydrate. This apparent coefficient (ε') was calculated by dividing the absorbance by the weight loss of the hydrocellulose, expressed in base-moles per litre, *viz.* $\varepsilon' = a/c$. The alkaline degradation was continued until the maximal yellow colour was obtained. For the sugar solutions (cellobiose, D-glucose), all of the carbohydrate is then decomposed, and the initial concentration may be used to calculate the apparent molar absorption coefficient. For all of the hydrocelluloses, a maximum value of $\varepsilon' = 895 \pm 91$ (98% confidence limits) was obtained, compared to 820 for the cellobiose–D-glucose difference spectrum (Fig. 2). Under measurement conditions of lower pH, there was a parallel increase of ε' for the hydrocellulose spectra and for the cellobiose–D-glucose difference spectra (Table I).

The β-elimination mechanism for alkaline peeling[2] rationalises these results. The cellobiose molecule fragments into a molecule of D-glucose and a molecule of 4-deoxy-D-*glycero*-2,3-hexodiulose (**1**), which are derived from the non-reducing and reducing residues, respectively. Compound **1** is also the product of the peeling of cellulose chains from the reducing termini[2]. Therefore, chromogens that are formed from each of the two fragments derived from cellobiose should be identical with those formed separately from D-glucose and cellulose.

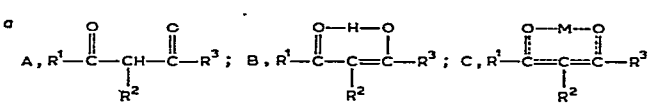
The uniform reversibility of the u.v.-spectral shifts with formation of an isosbestic point seemed to indicate that the alkali-digests of hydrocellulose behave spectrally as a single chromophore. In the previous paper[6] on amylose yellowing, we suggested the formation of a β-hydroxy-α,β-enone chromophore that ionises to the enolate with a pK'_a of 8.5. Since a pure enonic chromogen will have a molar absorption coefficient $>10{,}000$, the observed value for ε' of only 895 (calculated for the total amount of substrate degraded) is consistent with the chromogen's being a minor product of the degradation. An important conclusion is that the chromogen is produced as a result of the peeling process. The initial peeling product **1** has[7] ε 80; therefore, the peeling chromogen must be another compound that appears to be derived from **1**.

An enone system of the type proposed for the chromophore should make a major contribution to the carbonyl region of the i.r. spectrum. A fraction isolated from the alkali-degraded hydrocellulose was found to be essentially homogeneous by paper chromatography, exhibited the u.v.-activity described above, and showed strong i.r. absorption bands at 1720 and 1600 cm^{-1}. The former predominated in acidic solution and is ascribed to an aliphatic β-diketone, whereas the latter predominated in alkaline solution and is ascribed to the tautomeric β-hydroxy-α,β-enone (*A* and *B*, respectively, in Table II). These assignments were discussed in our previous paper[6]. An alternative suggestion that the main contribution to the carbonyl band at 1720 cm^{-1} arises from lactones of carbohydrate acids is inconsistent with the absence of an accompanying C–O stretching absorption at 1370–1250 cm^{-1}, and also inconsistent with the observed shift in alkali to 1600 cm^{-1} without the appearance of an additional band at 1460–1400 cm^{-1} for the symmetrical C=C stretching of a carboxylate. Some fractions of the chromogen exhibited bands assignable to the metal chelate of a β-diketone (*C*, Table II). Addition of ferric chloride to the chromogen solution produced a deep-yellow colour that indicated structure *C*. No coloured product was produced with 2,4-dinitrophenylhydrazine; this result is con-

TABLE II

STRUCTURE OF THE CHROMOPHORE

I.r. absorption (cm^{-1})	*Structure*[a]	*Literature values*[12–14] (cm^{-1})
1730	A	1740–1710
1600	B	1630–1540
1560	C	1560–1550
1410	C	1390–1320

[a] 

sistent with the formation of a pyrazole from a β-diketone. After acetylation, strong i.r. bands were observed at 1230 and 1740 cm^{-1} (ascribed, respectively, to acetate C–O stretch and ester carbonyl), while the u.v. peak at 240 nm indicated a simple conjugated carboxyl; a keto–enol acetate is suggested.

The results obtained herewith agree with those previously reported[6] for chromophore formation during the alkaline decomposition of the α-D-glucans amylose and maltose.

EXPERIMENTAL

Preparation of hydrocelluloses. — Delta-Pine cotton fibre was purified[8,9] by boiling with ethanol, followed by sodium hydroxide (1%), and cut into pieces of 2–3 mm length. Samples of cotton (6 g) were shaken with 5M hydrochloric acid (300 ml) at 25° in the dark. After the required time, the product was washed repeatedly with deionised water to remove acid, and dried over phosphorus pentaoxide *in vacuo*.

Alkaline degradation. — Hydrocellulose (250 mg) was boiled with 0.6M sodium hydrogen carbonate (25 ml) for one or more hours, filtered off, and washed with distilled water several times on the sintered-glass filter. The combined filtrate and washings were quantitatively made up to a suitable volume, and the u.v. spectrum was recorded on a Perkin–Elmer spectrophotometer Model 450 within 15 min of termination of the boiling.

Solutions of sugars (4mM) in sodium hydrogen carbonate (0.6M) were treated similarly for 1 h.

The pK'_a values were calculated[10] from the spectra obtained.

I.r. spectra. — Spectra were obtained for films on sodium chloride with a Perkin–Elmer 257 spectrometer.

Hydrocellulose (288-h hydrolysis, 500 mg) was treated for 6 h with boiling, aqueous sodium hydrogen carbonate (0.6M), and the filtered supernatant solution was neutralised with Amberlite IR-120 (H^+) ion-exchange resin and freeze-dried. Chromatography on a column (45 × 1.6 cm) of silica gel (50 g), with elution by solvents of increasing polarity, yielded a number of u.v.-inactive fractions and a crude u.v.-active fraction that gave i.r. bands at 1720 and 1600 cm^{-1}. Addition of sodium carbonate to the crude u.v.-active fraction gave an augmented band at 1600 cm^{-1} in the i.r. spectrum, but no absorption at 1720 cm^{-1}.

The filtered extract (hydrogen carbonate) of 120-h hydrocellulose was dialysed through a Cellophane membrane, and the dialysate, containing all the u.v. activity, was freeze-dried. An anhydrous ethanol extract yielded an oil (147 mg) containing all the u.v.-activity, ν_{max} 1600 cm^{-1} (v. strong); on addition of conc. hydrochloric acid, 1730 cm^{-1}. Chromatography of a portion (93 mg) of the ethanol extract on a freshly prepared column (45 × 1.6 cm) of silica gel yielded two u.v.-active fractions. The first fraction (9 mg) had ν_{max} 1410 and 1560 cm^{-1} (metal chelate of β-diketone); after treatment with anhydrous formic acid, 1600 cm^{-1} (keto-enol). The second fraction (47 mg), without formic acid treatment, had ν_{max} 1600 cm^{-1}. Paper chro-

matography (ethyl acetate–acetic acid–water, 100:13:10[11]) revealed one main spot (R_F 0.27) for both fractions with a periodate–permanganate spray.

Treatment of the extract with acetic anhydride in pyridine gave a product having ν_{max} 1230 (acetate C–O stretch), 1630 (weak), and 1740 cm^{-1} (ester carbonyl); no absorption remained in the 3300 cm^{-1} (hydroxyl) region, indicating complete acetylation. The product had λ_{max} 240 nm (simple conjugated carbonyl, R.CO.CR′ = COAc.R″).

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