Carbohydrate Research, 27 (1973) 341–352

MILD OXIDATION OF STARCHES WITH AQUEOUS BROMINE

PART I* KINETICS AND PRODUCT ANALYSIS

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(Received September 24th, 1971, accepted for publication in revised form, October 12th 1972)

ABSTRACT

Starch suspensions have been treated with dilute, aqueous bromine at 30° in the pH range 6–8, no adsorption of oxidant occurred The oxidation kinetics were first-order in bromine and in accordance with the rate law $-d[\text{bromine}]/dt = k[\text{starch}][\text{bromine}]$, except for a minor, initial rapid-phase in the oxidation of cereal starches, which is attributed to an enhanced reactivity of the granule surface The apparent first-order rate-constants were $2.0–2.8 \times 10^{-3}$ min^{-1}, except for retrograded amylose oxidised at pH 8 when the value was 5.6×10^{-3} min^{-1} The i r spectra of the products indicated the presence of carboxylate and aldehyde groups The functional group contents were determined quantitatively Oxidation of the amylose at pH 6–7 introduced carbonyl groups, whereas at pH 8 carbonyl and carboxylate were found in equal amounts For waxy-maize starch oxidised at pH 6–8, the carbonyl content was twice that of carboxylate Acid hydrolysis of the product obtained by oxidation of amylose proceeded at pH 8 according to first-order kinetics Chromatographic analysis of the anionic components of the hydrolysate indicated the presence of D-glucurono-6,3-lactone, D-gluconic acid, and an unidentified acidic ketose

INTRODUCTION

The industrial utility of hypochlorite-oxidised starch has engendered interest in the action of bromine on starch The oxidation of solutions of amylopectin[2] and of native-starch granules[3] proceeds most rapidly in a mildly alkaline medium and is much faster than the reaction with hypochlorite The heterogeneous reaction has[3] an activation energy of 10–22 kcal/mole After hydrolysing bromine-oxidised starch, Farley and Hixon[4] isolated D-glucuronic acid Doane and Whistler[2], however, did not detect this product but showed that oxidation involves scission between C-2 and C-3 with the formation of glyoxylate and D-erythronate residues In an acidic medium, starch granules adsorb bromine[5] and undergo hydrolysis[6] and oxidation[5]. At pH 3, amylose in solution complexes with bromine and is precipitated by Br_3^-,

*For Part II, see Ref 13, a preliminary communication was presented at the 39th Meeting of the Israel Chemical Society, Jeursalem, 1969[1]

no oxidation was observed[5] The present report deals with the heterogeneous reaction of starches with aqueous bromine at pH 6–8, conditions that have been proposed[7] for desizing cotton

RESULTS AND DISCUSSION

An initial bromine concentration of 2 5mM was routinely taken, and, under the conditions maintained (temperature 30° and pH 6, 7, or 8), the mole fraction of the HBrO species exceeds 0 7 throughout the course of the reaction, reaching 0 95 at pH 7 The remainder of the bromine is mainly Br_2 at pH 6 and BrO^- at pH 8, whereas equal amounts of these two species are present at pH 7

No oxidant was adsorbed from bromine water by the amylose or by waxy-maize and wheat starches at pH values between 6 and 8 At pH 3, however, granular starches and dissolved amylose *do* complex bromine[5] The initial mole fractions for the Br_3^-

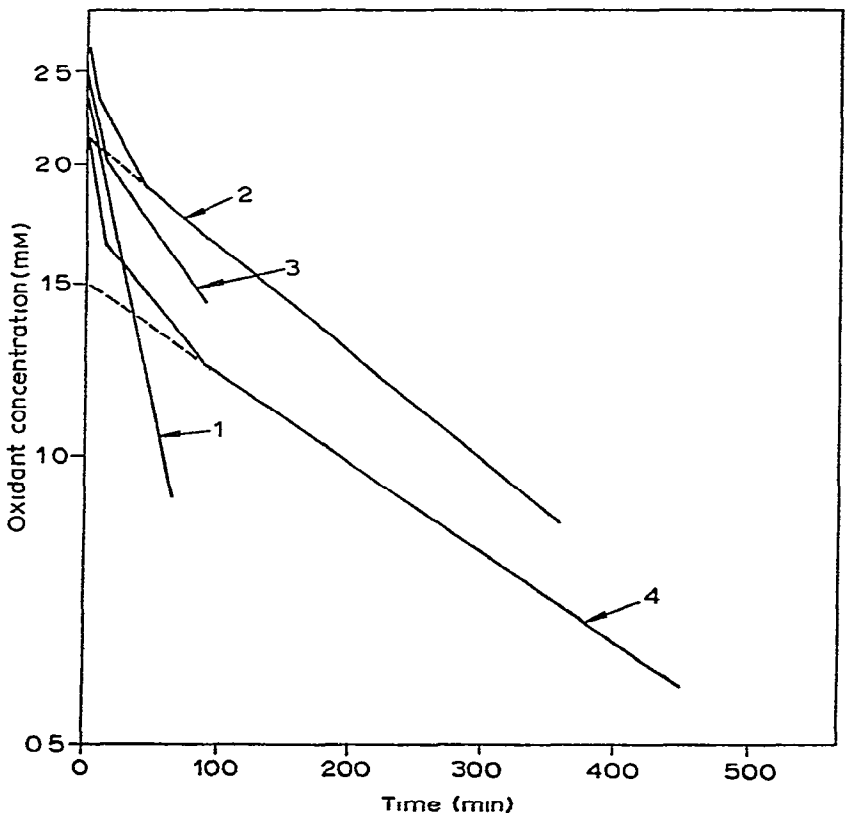

Fig 1. Oxidation of amylopectin with aqueous bromine at pH 8 Curve 1, solution Curve 2, suspension. Curve 3, suspension, after pretreatment with water at pH 8 for 1 h, followed by centrifugation Curve 4, suspension after pretreatment with water at pH 8 for 3 h and direct addition of bromine

and Br_2 species, respectively, were 0 63 and 0 37 at pH 3, and 0 0049 and 0 14 at pH 6. This indicates that granular starch may complex bromine as Br_3^- at pH 3, as has been proved[5] for the *precipitation* of amylose from aqueous solution by bromine

TABLE I

OXIDATION OF STARCHES AT pH 8[a]

Substrate			*Kinetic data*	
Origin	*Form*	*Amylopectin content (%)*	$k_1' \times 10^3$ (min^{-1})	*Rapid-phase consumption (mmole/l)*
Potato	Retrograded amylose	0	5 65 ±0 76[b]	0
Potato	Native granules	80	2 83	0
Maize	Native granules	45	2 81	0 33
Wheat	Native granules	80	2 83	0 37
Maize	Native granules	100	2 49 ±0 19[c]	0 48

[a]Starch (5 4 g/l) oxidised with aqueous bromine (2 5mM) [b]Least-squares treatment of all experimental points from three runs [c]Maximum deviation from average of three runs

The oxidation of native granules of waxy-maize starch has an initial, fast phase during which 25% of the oxidant is consumed, and thereafter the reaction proceeds in accordance with apparent first-order kinetics (Fig 1, curve 2) This diphasic behaviour is also exhibited by wheat and Amylon starches, and the three reaction rates are similar (Table I) The cereal starches conforming to this kinetic pattern contain 45–100% of amylopectin Both native potato-starch (80% of amylopectin) and retrograded amylose particles, however, conform to apparent first-order kinetics from the commencement of the oxidation Therefore, the rapid, initial phase characteristic of the cereal starches is not due to the branched molecular structure of the amylopectin component The enhanced reaction rate for amylose, as compared with the granular starches (Table I), occurs only at the higher pH values (Table II), and it is probably due to differences in supramolecular order Waxy-maize and wheat granules do not swell as a result of bromine oxidation[3] Variations in particle structure and compactness will affect swelling and complex formation in alkali, so that amylose exhibits enhanced reactivity at pH 7–8

The oxidation rate of waxy-maize starch at pH 8 varies with altered reactant concentrations (Table III), in accordance with a second-order empirical rate-law

$$-\mathrm{d}\,[\text{bromine}]/\mathrm{d}t = k_2\,[\text{starch}]\,[\text{bromine}] = k_1'\,[\text{bromine}]$$

Oxidant consumption during the initial, fast phase is independent of pH (Table II) but is directly proportional to initial reactant concentrations (Table III) When waxy-maize starch was pre-incubated for 3 h (routine reaction time) at pH 8 before addition of the bromine, the diphasic mode of oxidant consumption was retained (Fig 1, curve 4) and is therefore not a result of initial hydration or swelling

TABLE II

EFFECT OF pH ON OXIDATION KINETICS[a]

pH	*Potato amylose*[b]		*Waxy-maize starch*[c]	
	$k_1' \times 10^3$ (min^{-1})	*Rapid-phase consumption (mmole/l)*	$k_1' \times 10^3$ (min^{-1})	*Rapid-phase consumption (mmole/l)*
6	2 16 ±0 14	0	1 95 ±0 30	0 48 ±0 03
7	4 53 ±0 17	0	2 18 ±0 20[d]	0 47 ±0 03[d]
8	5 65 ±0 76	0	2 49 ±0 19[d]	0 48 ±0 03[d]

[a]Starch (5 4 g/l) oxidised with aqueous bromine (2 5mM) (experiments in triplicate) [b]Least-squares treatment of all experimental points [c]Data with maximum deviation from average of all runs [d]Duplicate results

TABLE III

OXIDATIONS OF WAXY-MAIZE STARCH AT pH 8

No of experiments	*Starch concentration (g/l)*	*Initial oxidant concentration (mM)*	$k_1' \times 10^3$ (min^{-1})[a]	*Rapid-phase consumption (mmole/l)*[a]
2	2 75	2 53–2 67	1 09 ±0 03	0 27 ±0 06
3	5 40	2 45–2 72	2 49 ±0 19	0 48 ±0 03
1	10 8	2 41	6 25	0 94
2	5 40	5 38–5 47	3 03 ±0 18	1 00 ±0 03
1	5 49[b]	2 12	2 18	0 62
2	5 31[c]	2 56–2 57	4 26 ±0 22	0 45
2	5 40[d]	2 54–2 57	13 1 ±1 0	0

[a]Data with maximum deviation from average of all runs [b]Pretreatment with water for 3 h [c]Pretreatment with water for 1 h and centrifugation before addition of bromine [d]Dissolved amylopectin

of the substrate An initial, rapid phase was also observed when oxidation was commenced after pre-incubation of the starch for 1 h (rapid-phase time), centrifugation from the aqueous medium, and washing with water (Fig 1, curve 3) This result eliminates the possibility that a starch fraction, *e g* of low molecular weight, is dissolved and rapidly oxidised in solution Dissolved waxy-maize starch consumes bromine in accordance with first-order kinetics from the commencement of the reaction, and the rate is enhanced five-fold (Fig 1, curve 1) The reduced reactivity of starch in heterogeneous, as against homogeneous, systems is due largely to severe intermolecular hydrogen-bonding that restricts molecular freedom[8] In some instances, however, molecular chains on the granule surface may be partially free of these constraints and sufficiently hydrated to approach the reactivity of dissolved molecules Accordingly, the first-order plot of cereal starch oxidation may be resolved into an initial phase due to homogeneous-like kinetics superimposed on the major heterogeneous reaction The particular oxidant–substrate ratios used will be critical in revealing this phenomenon

The I R spectra of unoxidised amylose and amylopectin (waxy-maize starch) between 1600 and 1800 cm^{-1} showed absorption only at 1640–1650 cm^{-1} (H_2O) After oxidation of amylose at pH 8, bands at 1625 (RCO_2^-) and 1730 cm^{-1} (C=O) were also observed, the latter being an aldehyde function since it largely disappeared following treatment[9] with bisulphite (*cf* the absorption of dialdehyde starch[10,11] and cellulose[12] at 1730–1736 cm^{-1}) Hitherto, aldehyde groups have not been reported in bromine-oxidised starch and its homologues They may be located at C-6 where a carboxylic function is found[4] after severe oxidation After reduction of pH 8-oxidised amylose with borohydride, only an H_2O band at 1640 cm^{-1} was found, but it masked a RCO_2^- absorption since a RCO_2H shoulder at 1740 cm^{-1} was found after treatment with acid The oxidised amylopectins absorbed weakly for C=O at 1740 cm^{-1} The RCO_2^- band was at 1595 and 1610 cm^{-1} for the amylopectins oxidised at pH 7 and 8, respectively, the former value being due to stronger hydrogen-bonding Treatment with acid replaced these bands by RCO_2H absorption at 1730 cm^{-1}

TABLE IV

FUNCTIONAL GROUPS IN OXIDISED[a] AMYLOSE

pH	*Oxidant consumption*	*Carboxyl content*	*Carbonyl content*
Amylose batch No 1			
Untreated	0	10 0	10 0
6	46 1	12 6	33 3
6	46 1	13 0	40 8
6	41 0	10 7	34 5
7	46 1	18 1	35 7
7	38 9	10 6	32 2
7	40 0	10 6	30 3
8	47 6	22 7	24 4
8	48 8	23 0	22 7
8	47 6	20 2	23 0
8	71 4[b]	21 7	39 1
Amylose batch No 2			
Untreated	0	<0 4	11 0
Untreated after borohydride reduction		—	6 5
8	62 5	19 6	34 5
8 after borohydride reduction		—	7 2

[a]Oxidations performed at 30° with starch (5 4 g/l) and 2 5mM bromine (13 3 D-glucose residues/mole of oxidant) Results expressed in mequiv per 162 g of starch [b]Initial oxidant concentration, 5 0mM

Quantitative determinations of the functional groups (Table IV) revealed that the unoxidised amyloses possess one C=O group/100 D-glucose residues, the $\overline{dp}_n$ being[13] ~1000 After reduction with borohydride, the C=O content was halved, indicating that the high values are not an artifact Oxidation of amylose at pH 6–7 introduced mainly C=O groups, whereas both C=O and RCO_2H were formed in similar amounts at pH 8 and to the same total functional-group level as at lower pH

An increased oxidant consumption at pH 8 enhanced the C=O content The oxidation pattern for amylopectin (Table V) was less variable with pH The C=O content was twice that of RCO_2H, and, as with amylose, less C=O was formed at pH 8 than at lower alkalinity At an increased amylopectin concentration, the functional group contents were proportionately lower, indicating that the reactivity of oxidisable sites is similar These oxidised starches have[13] $\overline{d\ p}_n$ values of 300–400, so that most of the functional groups in oxidised amylose are not located at chain termini

TABLE V

FUNCTIONAL GROUPS IN OXIDISED[a] AMYLOPECTIN

pH	*Oxidant consumption*	*Carboxyl content*	*Carbonyl content*
6	35 7	20	27 8
6	37 0	12	25 6
7	40	11 6	26 4
7	43 5	11 0	27 1
8	44 2	11 3	19 6
8	39 1	12 5	20
8[b]	20	7 6	14 1
8[b]	21 4	7 2	14 3
8[b]	21 7	6 8	14 3

[a]Oxidations and units as in Table IV Untreated material and control samples at each pH value had carboxyl and carbonyl contents of <0 4 mmole/162 g of starch [b]Starch concentration, 11 g/l

TABLE VI

SULPHURIC ACID HYDROLYSIS

Substrate concentration (mg/ml)[c]	*Acid concentration* (M)	*Bath temperature (degrees)*	$k_1 \times 10^3$ (min^{-1})[b]	*Max product measured*		
				Time (min)	*Concentration (mg/ml)*[d]	*Hydrolysis ended*
D-*Glucose*						
19 7	0 5	102[a]	—	0	19 7	—
				130	19 6	—
				190	20 0	—
Amylose						
18 0	0 5	98	—	145	11 3	Yes
Oxyamylose						
87 1	0 25	91–93	2 37	176	30 3	No
43 6	0 5	95 ±0 5	3 79	231	27 8	No
17 4	0 5	95 ±0 5	3 84	285	13 9	No
17 4	0 5	98	—	330	13 9	Yes
17 4	0 5	102[a]	3 35	397	14 9	No
17 4	1 0	102[a]	3 31	413	16 2	No
17 4	1 5	102[a]	6 87	261	16 1	Yes
17 7	2 9	102[a]	—	<83	17 0	Yes

[a]Refluxing solution [b]Calculation from graph of $\log(c-x)$ against time [c]By weight [d]Reducing substances in terms of D-glucose residues

Preliminary to analysing the hydrolysis products of the mildly oxidised starch, it was shown that the use of Amberlite IRA-401(HCO_3^-) resin permitted the quantitative removal of D-glucurono-6,3-lactone from solution and the recovery (50%) of D-glucuronic acid and its lactone after treatment for 2 h with boiling 0 5M sulphuric acid with a 50-fold excess of D-glucose

Experiments with amylose oxidised at pH 8 (Table VI, concentration, 17 4 mg/ml) revealed that hydrolysis was incomplete in 0 5M acid Hydrolysis of unoxidised amylose at the lower acid concentration also left a solid residue (34%), the properties of which (Table VII) indicated considerable degradation. The pseudo-first-order rate constants (k_1' in Table VI) indicated that hydrolysis was approximately first order with respect to oxidised amylose The value of k_1' was unaltered on increasing the acidity from M to 2M, but it doubled in 3M acid, considerable binding of acid to starch[14] in the more-dilute media may cause a decrease in hydrogen-ion activity

TABLE VII

PROPERTIES OF ACID-TREATED AMYLOSE

		Insoluble residue	*Untreated amylose*
Viscosity in M KOH	$[\eta]$ (dl/g)	0 45	1 40
	k'	2 53	0 35
Visible spectrum of iodine complex	λ_{max} (nm)	580	660
	transmittance	0 81	0 40
Infrared spectrum (Nujol mull)			Identical

The anionic components of a hydrolysate of amylose oxidised at pH 8 were examined chromatographically The major component was characterised as an acidic ketose and an α-dicarbonyl compound It was not detected after D-glucuronic acid had been boiled in 1 5M sulphuric acid (*cf* Ref 15) A ketone function at C-2 or C-3 has been postulated[2] in the intermediate leading to oxidative scission of the C-2–C-3 bond, and its incorporation into an enolic dicarbonyl structure may account for the acidic property Smaller amounts of D-gluconic acid and D-glucurono-6,3-lactone were also indicated No glyoxylic acid or erythronolactone was detected

The presence of D-glucurono-6,3-lactone was expected from the action of aqueous bromine on starch granules[4], α-D-glucosides[16], and D-gluconic acid[17] Vigorous bromine oxidation of dissolved amylopectin, however, yields glyoxylate and erythronate, but no D-glucuronate[2]. In a B-amylose helix with 4 D-glucose residues per turn, the hydroxyl groups are orientated away from the helix axis and will be involved in intermolecular hydrogen-bonding[18] This may render HO-6 more reactive towards bromine than HO-2 and HO-3 Such a situation would account for uronate formation during heterogeneous oxidation, in contrast to the relatively enhanced reactivity at HO-2 and HO-3 of amylopectin in aqueous solution

EXPERIMENTAL

Potato starch and retrograded amylose were products of AVEBE, Holland The specifications of the amylose[19] were as follows[20] ash <0 5% ($MgSO_4$), phosphorus <0 001%, protein <0 1%, lipids <0 1%, iodine-binding capacity 18 5–19 5%, particle size 5–15 μm The intrinsic viscosity in M potassium hydroxide was 139 ml/g The waxy-maize and wheat starches used have been described previously[21] The high-amylose maize starch was National Starch and Chemical Corporation Amylon with an iodine-binding capacity of 11% and a protein content of ~0 5% (reported by the supplier) The bromine stock solution (~0 1M, pH 3 8) was stored in a brown-glass bottle at 2° Titrations, before use, with thiosulphate and with arsenite–iodine gave identical results, showing the absence of bromate Water was glass-distilled from potassium permanganate Automatic control of pH was achieved by addition of M sodium hydroxide with a Radiometer Titrator Type TTT 1b Reaction vessels were thermostatted in the dark in a water bath maintained at 30 ±0 5°

Adsorption determination — The titrimetric method described previously[5] was used, except that potassium bromide and phosphate buffer were omitted The mixture contained bromine (2 5mM) and starch (34mM with reference to D-glucose residues) and was stirred magnetically Arsenite titration was used

No adsorption occurred at pH values of 6, 7, or 8, so that changes in oxidant titre during the reaction are due entirely to oxidant consumption

Oxidation procedure — Water (~750 ml) and bromine stock solution (25 ml, ~2 5 mmoles) were introduced into a 1-litre round-bottom flask equipped with a mechanical glass-stirrer and with a Radiometer combined glass-calomel electrode and delivery tube for automatic addition of alkali The pH was adjusted, and 10-ml aliquots were titrated against thiosulphate and arsenite (0 02N standard solutions), giving results identical to the value calculated from the volume of stock solution taken Starch (5 000 g, dry basis) was added with water to make the volume up to 925 ml, giving a starch concentration of 34mM with reference to D-glucose residues

Aliquots (10 ml) were titrated directly with arsenite since gravitational filtration at pH 6 and 7 led to loss of bromine Titration with thiosulphate was not possible, since amylose suspensions gave a stable colour with the iodine liberated and the end-point was not detected However, arsenite titration measures total bromine consumption, part of which may be due to bromate formation Bromate was determined on aliquots (25 ml, filtered through Whatman glass-paper) by titration with thiosulphate and arsenite No bromate was found at pH 6 and 7, but, at pH 8, 4 5% of the bromine was converted into bromate at 50% oxidant consumption Oxidations lasted 3 h and were generally performed in triplicate

Blank runs (without starch) established that bromine solutions were stable under the conditions employed, except for a 3% conversion into bromate towards the end of 3 h at pH 8

Product treatment — The suspension was centrifuged immediately after 50% oxidant consumption, and washed with water until free from bromine and then twice

with methanol by re-suspension and centrifugation The product was air-dried at ambient temperature Control samples of starch were prepared by aqueous treatment in the absence of bromine The bisulphite-addition compound was prepared by shaking the oxidised starch (~200 mg) with saturated, aqueous sodium hydrogen sulphite (30 ml) for 18 h After filtration, the product was thoroughly washed with water and methanol, and then dried in a vacuum desiccator over phosphorus pentaoxide Borohydride reduction of samples was performed by magnetically stirring starch (5 g, dry basis) with water (400 ml) containing sodium borohydride (100 mg) for 24 h at room temperature After thorough washing with water followed by methanol, the material was dried in a vacuum desiccator over phosphorus pentaoxide

Determination of spectra — The i r spectra were obtained with a Perkin–Elmer Model 257 spectrometer Samples were dried for 2 h at ~95° *in vacuo* over phosphorus pentaoxide Pellets, containing a 2-mg sample in 200 mg of potassium bromide, gave unsatisfactory spectra Nujol mulls were therefore used throughout Spectra of approximately the same intensity and background were obtained by varying the mull consistency.

The visible spectrum of the iodine complexes was obtained as follows Starch (15 mg) was dissolved in M potassium hydroxide (5 ml) and the solution was made up to 25 ml An aliquot (5 ml) was neutralised with sulphuric acid (0 5M) and diluted to ~200 ml An iodine solution (0 4 g/l in 0 1M potassium iodide, 2 5 ml) was added and the mixture was made up to 250 ml The spectrum was obtained on a Perkin–Elmer Model 450 spectrophotometer, using 1-cm cells, against an iodine reference solution

Measurement of functional group contents — Carboxyl and carbonyl contents were determined by the copper acetate[22] and cyanohydrin[23] techniques, respectively No results are given for "control" samples of amylose, *i e* treated with water in the absence of bromine (Table IV), since the values obtained were much lower than for the untreated amylose The horny nature of the treated amylose probably prevented reaction, so that the low values would be an artifact

Acid hydrolysis kinetics — The substrate was added to magnetically stirred sulphuric acid at the temperature of hydrolysis Aliquots (0 5 ml) were quenched by neutralisation in alkali and filtered Reducing sugar in the filtrate was determined by the Somogyi–Nelson technique[24], using a Hilger Spekker-type colorimeter with No 4 filter (520 nm) Undissolved residues were separated from the acid solution by centrifugation, washed with water (until free from acid) and methanol, and then dried in thin layers over calcium chloride in a vacuum desiccator

Chromatography — T l c was performed on 0 3-mm layers of Camag silica gel DF-5 or cellulose powder DF-0, and paper chromatography on Whatman No 1 Irrigants used (proportions by volume) were (**1**) 1-butanol–acetic acid–water[25] (2 1 1), (**2**) acetone–water (9 1), (**3**) ethyl acetate–acetic acid–formic acid–water[26] (18 3 1 14), (**4**) water–acetic acid–1-butanol[27] (5 1 4)

Saponification of D-*glucurono-6,3-lactone* — D-Glucurono-6,3-lactone (20μM, 20 ml) was stirred with Amberlite IRA-401 (HCO_3^-) resin (4 4 mequiv) at ambient

temperature T l c (irrigant **4** on cellulose) showed a gradual disappearance of the lactone from solution, but no other compound was detected After 23 h, chromatography of an aliquot (6 μl) revealed no material responding to the permanganate–periodate spray[28] Since the minimum amount of lactone detectable was 7 nmoles, at least 97% had been saponified and absorbed by the resin

Separation of D-*glucuronate from* D-*glucose after acid treatment* — A solution of D-glucurono-6,3-lactone (0 5 mmole), D-glucuronic acid (0 5 mmole), and D-glucose (50 mmoles) in 0 5M sulphuric acid (100 ml) was boiled for 2 h Sulphuric acid was neutralised with barium carbonate, and a portion (62 ml) of the filtered solution was decationised with Amberlite IR-120(H^+) resin (1 g, 4 mequiv) to a constant pH of 2 1 The supernatant was concentrated and then shaken for 16 h with Amberlite IRA-401(HCO_3^-) resin (5 1 mequiv) After elution with water until free from sugar, the resin was swirled with acetic acid (0 3 ml) and a little water for several minutes and returned with the supernatant to the column Elution was performed with M acetic acid (200 ml), and the effluent was concentrated with added water several times T l c (cellulose, irrigant and detection as above) enabled a semi-quantitative comparison with known amounts of D-glucurono-6,3-lactone and D-glucuronic acid, and indicated a 50% recovery

Anionic components of a hydrolysate of amylose oxidised at pH 8 — Oxidised amylose (10 g, dry weight) was boiled in sulphuric acid (1 5M, 500 ml) for 265 min with magnetic stirring After being neutralised, concentrated, and decationised as above, the solution was shaken with Amberlite IRA-401(HCO_3^-) resin (17 5 mequiv) for 44 h After elution of reducing sugars as described above, the resin was stirred with formic acid (0 7 ml) for 1 h and then eluted with 0 1M formic acid (400 ml) The effluent was freeze-dried

T l c (irrigant **1**, silica gel) of the concentrate separated an unidentified component (R_F 0 65) and four components of R_F 0 61, 0 51, 0 43, and 0 35 that were assigned, respectively, to D-glucurono-6,3-lactone, D-glucose, maltose, and D-gluconic acid, on the basis of a comparison with runs using irrigants **2** and **3** and detection with u v illumination and specific sprays It was established that oxalic, D-glucaric, tartaric, and glyoxylic acids, and D-fructose, D-arabinose, and D-erythrose were absent, but the presence of D-glyceric, pyruvic, and lactic acids, and glyoxal was not definitely excluded Two-dimensional t l c (silica gel, irrigant **2** followed by **3**, aniline–diphenylamine spray[29]) confirmed the presence of D-glucurono-6,3-lactone which was also revealed by hydroxylamine–ferric nitrate sprays[30] The unidentified component (irrigant **1**, R_F 0 65) was resolved into two spots in irrigant **3** The more-mobile material (R_F 0 90) fluoresced under 350-nm illumination and was revealed by aniline hydrogen phthalate[31], while the second material (R_F 0 84) strongly absorbed 254-nm illumination and was revealed[31] as an acidic ketose by Bromocresol Green at pH 7 9, aniline–diphenylamine[29], aniline hydrogen phthalate[31], and three ketose-specific reagents[31] (anthrone, naphthoresorcinol–trichloroacetic acid, and urea–hydrochloric acid)

Paper chromatography with irrigant **3** permitted a good separation of marker

erythronolactone from glucurono-6,3-lactone and glucono-1,5-lactone, but erythronolactone was not detected in the hydrolysate The acidic ketose reacted with *o*-phenylenediamine spray to give a green-fluorescing spot with 354-nm illumination, indicating an α-dicarbonyl compound

A solution of D-glucuronic acid (194 mg) in 1 5M sulphuric acid (100 ml) was boiled for 4 5 h and worked up as described for the oxidised-amylose hydrolysate T l c (silica gel, irrigant 3) revealed D-glucurono-6,3-lactone, but no acidic ketose

Unoxidised amylose was hydrolysed and treated as above, t l c revealed only D-glucose

ACKNOWLEDGMENTS

This work forms part of Project No UR-A10-(10)-51 performed for the Agricultural Research Service, United States Department of Agriculture. A Basch is thanked for obtaining and interpreting the i r spectra, and Professor M Lewin for his interest and encouragement AVEBE, Holland, and National Starch and Chemical Corporation, New York, are thanked for their generous gifts of starch samples

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