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Note

A polarimetric procedure for determining mannuronic acid/guluronic acid ratios in alginates*

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Alginates find many applications in industry[1] particularly in food The discovery of L-guluronic acid residues[2], in addition to those of β-D-mannuronic acid, in alginates and the observation that the affinity for calcium ions increased in relation to the L-guluronic acid content of the polymer[3] made the ManA/GulA ratio an important factor in the application of these compounds in foods There are several procedures for the determination of ManA/GulA ratios[5–7], following the characterization of brucine L-guluronate[7], we now report a simple polarimetric procedure suitable for routine use

The difference in $[\alpha]_D$ values of the brucine salts of D-mannuronic and L-guluronic acid is sufficient to provide the basis of a polarimetric method for determining the composition of a mixture Such a mixture can be obtained after appropriate acid hydrolysis of alginates

The most suitable conditions of hydrolysis involve treatment first with 72% sulphuric acid for 17 h at 5° and then with M sulphuric acid for 5 h at 100° This treatment is milder than that (80% sulphuric acid, 18 h, 20°, and then M sulphuric acid, 5 h, 100°) used by Haug *et al* [4] The solutions after hydrolysis were pale green and not the usual reddish brown characteristic of degradation of uronic acids Haug *et al* [4] estimated hydrolysis losses of ~35%, with an additional 15% loss on columns during ion-exchange separation no significant, selective loss occurred during neutralization The only loss in our method is that during hydrolysis Since our values are consistently higher than those obtained by the Haug method (Table V), it is estimated that the hydrolysis losses were lower The relative instability of mannuronic and guluronic acids under the present conditions of hydrolysis needs to be established, but a meaningful evaluation should await the availability of a crystalline sample of L-guluronic acid

The recovery of the brucine salts *via* acetone precipitation Table III) produced lower yields and higher ManA/GulA ratios for samples rich in mannuronic acid, and

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*Contribution No 332 of Food Research Institute lower ratios for samples rich in guluronic acid (Table IV) This result is attributed to the preferential precipitation of the brucine salt corresponding to the major uronic acid component in the mixture, and was confirmed by the behaviour on paper electrophoresis of the deionized barium salts in comparison with that of acetone-precipitated, deionized brucine and directly dried, deionized brucine salts The ratios were similar for the barium salts and directly dried brucine salts, but there were wide variations for samples prepared *via* acetone precipitation The latter procedure was therefore abandoned The recovery of the brucine salts by the direct-drying procedure produced higher yields of brucine salts, and ManA/GulA ratios compatible with those obtained by other methods (Table V) However, the presence of a dextrorotatory contaminant such as D-glucose D-xylose, or L-rhamnose, commonly found in alginates, would tend to lower the mannuronic acid content and increase the guluronic acid content Hence, it is necessary that the samples of alginates for analysis be free of such contaminants as far as possible

EXPERIMENTAL

Materials — The alginate samples Keltone and Manucol were generously provided by Dr John A Ziegler (Griffith s Laboratory, Scarborough, Ontario) A second Keltone sample was provided by Dr John Baird (Kelco Company, San Diego, California) Samples of algal SS/DJ, the bacterial S35, and a homopolymeric block polymer of guluronic acid (ex manugel DJ) were gifts from Dr C J Lawson (Philip Lyle Memorial Laboratory, Reading, Berks , England)

Methods — Descending p c was performed on Whatman No 1 paper with the organic phases of *A*, ethyl acetate–pyridine–water (8 2 1), and *B*, 1-butanol–acetic acid–water (4 1 5) Paper electrophoresis was performed on Whatman No 3MM paper with borate/calcium chloride buffer[8] (pH 9 2), at 800 V for 4 h Detection was effected with aniline hydrogen phthalate Hydrolyses of alginate samples (∼5 mg) were conducted with 72% sulphuric acid (0 4 ml) for 17 h at 5° After dilution to M acid (5 ml), the solutions were heated at 100° until the rotation was constant (5–6 h) The hydrolysates were neutralised ($BaCO_3$) and filtered Alginate samples, as 1% aqueous solutions, were purified by centrifugation at 15,000 r p m , and the material in the supernatant was recovered by freeze-drying Moisture contents were determined by drying *in vacuo* to constant weight at 56° Ash contents were determined by the method of Steyermark[9] Uronic acid analyses were carried out[10] by decarboxylation of samples (∼ 10 mg) with 57% HI at 145° for 1 h[11] Concentrations were performed with a rotary evaporator at 35°

Preliminary analysis of alginates — Purified samples of alginates were analysed for moisture ash, uronic anhydride specific optical rotation, and sugar components following hydrolysis The results are summarized in Table I

Determination of ManA/GulA ratios — *Acid hydrolysis* Alginate samples (∼400 mg) were hydrolysed with 72% sulphuric acid (20 ml) for 17 h at 5° Each hydrolysate was made up to 250 ml (*i e* , to M acid) and then kept at 100° The $[\alpha]_D$

value was monitored until constant (5–6 h) The hydrolysates were neutralised ($BaCO_3$), filtered, and concentrated to dryness A solution of the barium salts in water was filtered through pulp and then concentrated to dryness Table II summarizes the data

TABLE I

PRELIMINARY ANALYSIS OF ALGINATE SAMPLES

Sample	$[\alpha]_D$ *(degrees)*	*Moisture* (%)	*Dry-matter basis*		*Hydrolysis products*					
			Ash as sodium (%)	*Uronic anhydride* (%)	*ManA*	*GulA*	*GalA*	*Glc*	*Xyl*	*Rha*
Keltone	−137 7	12 3	11 84	89 29	major	minor	−	−	−	−
Manucol	−131 6	8 73	11 97	82 98[a]	major	minor	+	−	−	−
Algal SS/DJ	−143 8	8 77	11 36	83 02	minor	major	−	−	+	−
Bacterial S35	−100 9	10 95	11 47	63 82[a]	major	minor	−	−	−	−
Guluronic block polymer	−141 7	4 51	11 52	82 85	minor	major	−	−	−	−

[a] Decarboxylation[10] with 19% HCl for 2 h at 145°

TABLE II

RESULTS OF ACID HYDROLYSIS

Sample	*Sample size* (*mg*)	*Hydrolysis*[a] *time* (*h*)	$[\alpha]_D$ (*degrees*)		*Yield of barium salt* (*mg*)
			Initial	*Equil*	
Keltone	410, 412	5	−46 6	+22 4	269 320
Keltone	400, 400	5	−34 4	−18 0	224, 254
Manucol	400, 389	5	−24 7	+21 6	216 202
Algal SS/DJ	405	6	−71 1	+11 0	155
Bacterial S35	404 400	5	−57 7	+15 7	260 220
Guluronic block polymer	403, 405	5	−75 1	−27 0	240, 187

[a] At 100°

Preparation of brucine salts — Solutions of hydrolysed alginate samples (~100 mg) in water (5 ml) were stirred with Rexyn 101 (H^+) resin and then filtered, and the resin was washed with water (7–10 ml) To the combined filtrate and washings (12–15 ml) was added brucine (240 mg), and the solution was heated at 100° for 20 min and then cooled Excess of brucine was removed by extraction with chloroform

(4 × 15 ml), and the aqueous solution was concentrated to 2–3 ml and diluted with acetone (25–30 vol) The precipitate was collected by centrifugation, washed with acetone, and dried for 2–3 h at 63° *in vacuo*

In a second series of experiments, the brucine salts were recovered, following extraction with chloroform, by concentration of the aqueous phase to dryness, with several co-distillations with methanol, and final drying of the residue for 2–3 h at 63° *in vacuo* The elemental analyses for the brucine salts prepared by the two procedures were in excellent agreement with the theoretical value The data are summarized in Table III

Optical rotation data — Carefully dried samples of brucine salts [obtained by acetone precipitation and decolorisation (Norit A, neutral, thoroughly prewashed with 50% ethanol), or by direct drying] as 0 5–1% aqueous solutions were equilibrated for 90 min, and optical rotations were measured with a Perkin–Elmer 141 polarimeter (path-length 100 mm) From the specific optical rotations (±0 2°) (Tables IV and V), the ratios of mannuronic acid and guluronic acid were calculated as follows guluronate (%) = $5[\alpha]_D$ of mixture + 112 5, and mannuronate (%) = 100 − guluronate (%)

TABLE III

YIELDS OF BRUCINE SALTS FOLLOWING PRECIPITATION WITH ACETONE AND DIRECT DRYING[a]

Sample	*Yield of brucine salt (mg)*	
	Acetone precipitation	*Direct drying*
Keltone (Kelco)	60	125
Keltone (Griffith's)	52	132
Manucol	62	136, 140
Algal SS/DJ	23	134
Bacterial S35	31	—
Guluronic block polymer	52	115

[a] The barium salt (100 mg) was used in each case

TABLE IV

OPTICAL ROTATION VALUES AND ManA/GulA RATIOS FOR ACETONE-PRECIPITATED SAMPLES

Origin of brucine salts	$[\alpha]_D$ *(water) (degrees)*	*Temp (degrees)*	*Concentration (%)*	*ManA/GulA ratio*
Keltone (Kelco)	−19 4	25	0 591	5 4
Keltone (Griffith's)	−17 8	21	0 504	3 2
Manucol	−21	26	0 722	12 3
Algal SS/DJ	−2 8	25	0 531	0 015
Bacterial S35	−17 7	26	0 901	3 1
Guluronic block polymer	−3 5	28	0 485	0 05

TABLE V

OPTICAL ROTATION VALUES AND ManA/GulA RATIOS FOR DIRECTLY DRIED SAMPLES

Origin of brucine salts	$[\alpha]_D$ (*water*) (*degrees*)	*Temp* (*degrees*)	*Concentration* (%)	*ManA/GulA ratio*	
				Present method	*Lit*
Keltone (Kelco)	−15 1	28	0 711	1 70	1 8–2 0[a], 1 56[b]
Keltone (Griffith's)	−14 9	28	0 885	1 63	—
Manucol	−15 6 15 6	28	1 346, 1 209	1 89, 1 89	—
Algal SS/DJ	−9 4	28	0 59	0 51	0 39[b]
Guluronic block polymer	−3 44	29	1 25	0 05	0 05[c]

[a]Determined by the Kelco (unpublished) procedure [b]Determined by the Haug procedure[4] [c]Determined at the Philip Lyle Memorial Laboratory

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