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Note

A convenient procedure for producing gram-quantities of brucine L-guluronate and brucine D-mannuronate*

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The preparation of brucine L-guluronate from L-guluronic acid block-polymer[1] is tedious. We now describe a simple method for preparing this important acid in gram-quantities.

Acetone-precipitation of the total mixture[2] of brucine salts prepared from three alginates, namely, Algal SS/DJ, Manujel DJ, and an intact uronide prepared by selective precipitation from Manujel DJ, provided an excellent method for the fractionation of L-guluronic and D-mannuronic acids. In the present study, the best yields of L-guluronic acid (~4%) resulted from the intact uronide[3], although other alginates, relatively rich in L-guluronic acid, could be used equally or more advantageously.

The steps involved in the method are (*a*) hydrolysis of the alginate, (*b*) isolation of the total brucine salt *via* acetone-precipitation or direct drying[2], (*c*) precipitation of the brucine salt mixture with acetone, at room temperature, to give brucine L-guluronate, and (*d*) isolation of brucine D-mannuronate by cooling the supernatant fluid.

Removal of brucine from the crystalline brucine L-guluronate hemihydrate yielded free L-guluronic acid, which was readily converted into the sodium salt {monohydrate, $[\alpha]_D$ +48° (water)}; sodium L-guluronate showed no definite melting-point. Because of its greater stability, the sodium salt is more convenient than the brucine salt for storage of L-guluronic acid.

EXPERIMENTAL

The general experimental methods have been described previously[1].

Preparation and fractionation of brucine L-*guluronate and* D-*mannuronate from alginates.* — Solutions (4%) of alginate samples in 72% sulphuric acid were kept for 17 h at 5° and then in M acid for 6 h at 100°. The barium salts recovered following

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neutralization, filtration, and concentration to dryness were converted (following deionization) into the brucine salts as described previously[1]. To a 5–8% solution of the brucine salt in water was added acetone (4 vol.). The precipitated brucine L-guluronate was collected by centrifugation, washed with water–acetone (1:4) and acetone, and dried. The supernatant solution was kept overnight at 5 to −10°, to give brucine D-mannuronate, which was filtered off, washed with cold water–acetone (1:4) and acetone, and dried to a powder. Further fractionation of the residual material produced impure fractions.

The fractionated brucine salts were essentially pure by paper electrophoresis[4], and crystallized readily from aqueous ethanol[1]. The yields were as follows.

Sample	*Sample size (g)*	*Yield of barium salt (g)*	*Yield of brucine salt (g)*	*Yield of brucine salts on acetone precipitation (g)*	
				GulA	*ManA*
Algal SS/DJ	10	3.6	4.3[a]	0.68 (0.28)[c]	0.65 (0.48)
Manujel DJ	10	4.6	4.8[a]	1.20 (0.85)	0.70 (0.48)
Intact uronide (*ex* Manujel DJ)	10	4.0	6.4[b]	1.60 (1.23)	0.48 (0.34)

[a]Isolated by precipitation[2] with an excess of acetone. [b]Isolated by direct drying[2]. [c]Yield of crystalline salt in parentheses.

Preparation of sodium L-*guluronate.* — Brucine L-guluronate (2.0 g) was dissolved in water (120 ml) by warming, Rexyn-101(H^+) resin (40 ml) was added, and the mixture was stirred magnetically for 2 h. Filtration through pulp, evaporation to dryness, and three co-distillations of ethanol from the residue yielded a fluffy, white solid (0.62 g), which was dried *in vacuo* over anhydrous calcium chloride.

To a solution of the resulting acid (0.61 g, 95%) in water (10 ml) was added sodium hydrogencarbonate (0.26 g), in portions, with stirring. The solution was concentrated (to 5–8 ml) and ethanol was added dropwise, to slight turbidity. The solution was decolorized with charcoal, filtered, and kept at room temperature, in a desiccator, over anhydrous calcium chloride. Ethanol was added from time to time, to replace losses due to evaporation; the solution was maintained in the desiccator for 2 months and then started to crystallize. More ethanol was added as the crystallization proceeded, until a total volume of 15–20 ml was reached. The crystals were collected, washed with ethanol–water (3:1) and then ethanol, and dried *in vacuo* over calcium chloride, to yield the hydrated sodium salt as white needles (0.46 g), which had no definite m.p., but showed mutarotation, $[\alpha]_D^{23}$ +31 (3 min)→+48° (16 min, equil.) (*c* 2, water).

Anal. Calc. for $C_6H_9NaO_7 \cdot H_2O$: C, 30.78; H, 4.75; Na, 9.82. Found: C, 30.38; H, 5.08; Na, 9.91.

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