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Note

Crystalline brucine L-guluronate*

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There are many reports in the literature dealing with the characterization of D-mannuronic acid from the hydrolysis of alginates[1–6]. Characterization of L-guluronic acid, the other constituent of alginates, apparently has never been achieved. The purpose of this Note is to report the characterization of L-guluronic acid as a hydrated brucine salt.

A sample of a homopolymeric block-polymer, highly enriched in L-guluronic acid residues, was kindly provided by Dr. C. J. Lawson (Philip Lyle Research Laboratory, Reading, Berks., Great Britain). Acid hydrolysis of the sample yielded a hydrolysate which showed a major and a minor component in paper electrophoresis with a borate–calcium chloride buffer[7]. Recovery of the mixture of barium salts, deionization, and derivatization with brucine yielded the brucine salt, which crystallized from aqueous ethanol as the hemihydrate, m.p. 157–159°, $[\alpha]_D$ −2.5° (water).

Crystalline brucine D-mannuronate, prepared similarly from a commercial alginate sample (Keltone; kindly supplied by Dr. John A. Ziegler, Griffiths Laboratories, Scarborough, Ontario), had m.p. 149–150°, $[\alpha]_D$ −22.5° (water); unlike the previously reported characterization[2], this salt was a monohydrate.

EXPERIMENTAL

Paper electrophoresis was performed on Whatman No. 3MM paper with borate–calcium chloride buffer[7] (pH 9.2), at 800 volts for 4 h. Detection was effected with aniline hydrogen phthalate. Evaporations were carried out at 35° with a rotary evaporator. Optical rotations were measured with a Perkin–Elmer 141 polarimeter.

Acid hydrolysis of L-*guluronic acid block-polymer.* — A solution of the polymer {0.4 g; $[\alpha]_D^{24}$ −141.7° (*c* 0.752, water)} in 72% sulphuric acid (20 ml) was kept for 17 h at 5°. After dilution to 250 ml (*i.e.*, to M sulphuric acid), the solution had $[\alpha]_D^{22}$ −75.1° (*c* 0.162, M H_2SO_4), which changed to a constant value of +27° after heating the solution for 5 h at 100°. The solution was neutralized with barium

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carbonate, filtered, and concentrated to yield a solid (240 mg). A solution of a portion (5 mg) of the product in water (0.3 ml) was deionized with Rexyn-101(H^+) resin, and paper electrophoresis then showed a major (L-guluronic acid) and a minor component (D-mannuronic acid).

Preparation of brucine salt. — A portion (100 mg) of the barium salt was dissolved in water (10 ml), and the solution was deionized with Rexyn-101(H^+) resin. Brucine (240 mg) was added to the solution, and the mixture was heated for 20 min at 100°. The solution was cooled, and extracted three times with equal volumes of chloroform. The aqueous layer was concentrated to 2–3 ml and mixed with a 20–25-fold excess of acetone. The precipitate was removed by centrifugation, washed with acetone, and dried to yield a powder (52 mg), $[\alpha]_D^{28}$ $-3.5°$ (*c* 0.5, water). Paper electrophoresis, following removal of brucine [Rexyn-101(H^+)], showed a major component (M_{GLC} 0.74) and a minor component (M_{GLC} 0.96).

Anal. Calc. for $C_{29}H_{36}N_2O_{11} \cdot H_2O$: C, 57.2; H, 6.2; N, 4.6. Found: C, 57.4; H, 6.4; N, 4.3.

Crystallization of brucine L-*guluronate.* — The remaining brucine salt was suspended in 95% ethanol (4–5 ml) and dissolved by heating on a water bath with dropwise addition of water. The solution was then left at room temperature to crystallize. A second, similar recrystallization gave brucine L-guluronate hemihydrate, m.p. 157–159°, $[\alpha]_D^{29}$ -1.5 (15 min) → -2.5 (90 min) → $-2.5°$ (24 h) (*c* 0.5, water). Paper electrophoresis, following decationization, revealed a single component having an M_{GLC} value identical with that of L-guluronic acid.

Anal. Calc. for $C_{29}H_{36}N_2O_{11} \cdot 0.5\,H_2O$: C, 58.28; H, 6.24; N, 4.69. Found: C, 58.01 and 58.01; H, 6.37 and 6.42; N, 4.52.

Characterization of brucine D-*mannuronate.* — Keltone (400 mg), when hydrolysed under the foregoing conditions, showed $[\alpha]_D^{25}$ -34.4 (initial) → -13.7 (1 h) → $+1.9$ (2 h) → $+11.2$ (3 h) → $+15$ (4 h) → $+18$ (5 h) → $+17.8°$ (6 h) (*c* 0.162, water). The mixture of brucine salts (207 mg), prepared from the barium salt (200 mg), showed $[\alpha]_D^{21}$ $-17.8°$ (*c* 0.5, water). Paper electrophoresis revealed two components, corresponding to D-mannuronic acid (major) and L-guluronic acid (minor).

Anal. Calc. for $C_{29}H_{36}N_2O_{11} \cdot H_2O$: C, 57.2; H, 6.2; N, 4.6. Found: C, 57.0; H, 6.1; N, 4.7.

Four recrystallizations of the salt from aqueous ethanol yielded electrophoretically pure brucine L-mannuronate monohydrate, m.p. 149–150°, $[\alpha]_D^{29}$ -22.2 (4 min) → -22.5 (150 min) → $-22.5°$ (20 h) (*c* 0.55, water); lit.[2] m.p. 147°, $[\alpha]_D^{24}$ $-23.1°$.

Anal. Calc. for $C_{29}H_{36}N_2O_{11} \cdot H_2O$: C, 57.2; H, 6.2; N, 4.6. Found: C, 57.0; H, 6.2; N, 4.5.

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