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Note

Crystalline cellobiono-1,5-lactone: its preparation and growth-inhibitory activity in the *Avena* coleoptile section test

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There has been an increasing interest in aldonolactones since it was observed that they inhibit enzymes that catalyze the hydrolysis of specific glycosides. Recently, 32 aldonolactones were screened for their growth-inhibitory activity in the standard *Avena* coleoptile section test; the results indicated that aldonolactones could be used as plant-growth inhibitors[1]. Cellobiono-1,5-lactone was not among those lactones tested, because our investigations on the purity and structure of the crystalline material had not then been completed.

Cellobiono-1,5-lactone is of special interest because of the possible effect that it might be expected to exert upon enzyme systems involving such substrates as cellulose and other D-glucosides[2–5]. Cellobionic acid and a number of its salts have been prepared in amorphous condition[6–8]. Cellobionic salts in aqueous, acidic solution are readily hydrolyzed, and equilibrate to give a mixture of the acid and the lactone; such an equilibrium mixture has often been used as the source of the lactone for biochemical studies[2,4,5].

Following a method[9] found successful for the preparation of other acids and their lactones, cellobiose was oxidized with bromine water in the presence of cadmium carbonate. Initially, neither the acid nor the lactone crystallized from the syrupy product. Neutralization of the product with dicyclohexylamine produced analytically pure, but amorphous, dicyclohexylammonium cellobionate; neutralization with cadmium carbonate yielded the cadmium salt, which could readily be converted into the free acid with hydrogen sulfide. It was from the syrup obtained by concentrating the aqueous solution of this acid that the first crystals† of cellobiono-1,5-lactone were isolated. The crystalline lactone could be recrystallized from concentrated, aqueous

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†By the procedure he used to prepare maltobionolactone[10], S. K. Dutta[11] has also prepared crystalline cellobiono-1,5-lactone.

solution. Its i.r absorption spectrum (Nujol) showed a band at 1721 cm^{-1} characteristic of a lactone. Thin-layer chromatography (t.l.c.) on silica gel in four anhydrous solvent-systems showed only one component, which gave a positive reaction for a lactone. Gas–liquid chromatography (g.l.c.) after per(trimethylsilyl)ation indicated a purity of at least 94%. Reduction of the lactone with sodium borohydride gave cellobiitol. In water, the lactone mutarotates toward a negative value, the final specific rotation (+0.8 to +1.0°) being identical to that observed by Levene and Wolfrom[7], who started with the acid and noted a mutarotation toward a more positive value. The equilibrium value corresponds to a mixture of 89% of the acid with 11% of the lactone.

The growth-inhibitory activity of cellobiono-1,5-lactone was tested in the standard *Avena* coleoptile section test: 50% inhibition of growth was obtained at a concentration of ~0.6mM (see Table I), regardless of whether the test had been performed in 2% sucrose solution or in the presence of the growth hormone indoleacetic acid (IAA). Comparison of these data with those obtained in a parallel way with a number of other aldonolactones[1] showed the inhibitory activity of cellobiono-1,5-lactone to be in the range of the activities of D-galactono-1,5-lactone, D-glucuronolactone, and D-galacturonolactone, the most potent inhibitors tested.

TABLE I

GROWTH-INHIBITORY ACTIVITY OF CELLOBIONO-1,5-LACTONE IN THE *Avena* COLEOPTILE SECTION TEST

Concentration (mM)	*Inhibition*[a] (%)	
	in 2 μM IAA	*in 2% sucrose*
0.2	6	2
0.4	12	10
0.5	36	28
0.6	58	50
0.7	67	62
0.8	78	70
1.0	92	86
1.2	94	94
1.4	98	98

[a]Growth inhibition was calculated as the percentage of the control in which coleoptile sections grew 5.1 mm in length.

EXPERIMENTAL

General methods. — G.l.c. was performed with a Hewlett–Packard Research chromatograph, Model No. 5750, with nitrogen as the carrier gas, a column (1.8 m × 100 mm o.d.) of 3.8% of SE-30 on Diaport S (60–80 mesh), and a flame-ionization detector. Samples were per(trimethylsilyl)ated with Tri-sil Z (Pierce Chemical Corp.) and passed through the column at 220°. Specific rotations were measured with a Perkin–Elmer 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer 137 i.r.

spectrometer. T.l.c. was conducted on Silica Gel GF (250 μm; Analtech, Inc.) with the following solvent-systems: (*A*) acetone, (*B*) 1:1 dichloromethane–2-methoxyethanol, (*C*) *N*,*N*-dimethylformamide, and (*D*) 5:1 dichloromethane–*N*,*N*-dimethylformamide. Detection sprays employed, in addition to 10% sulfuric acid, were 0.1% dichlorophenolindophenol sodium salt in 90% ethanol[12] (for acids), and hydroxylamine–ferric chloride reagent[12] (for lactones).

Bioassay. — Oat seeds (*Avena sativa*, var. Golden Rain) were germinated in the dark at 25–26°. For assays, coleoptiles (2.8–3.0 cm) were cut, 3 mm below the tip, to a length of 5 mm. Twenty such sections were incubated in the dark for 24 h at 25° with various concentrations of lactone in 2% sucrose solution (10 ml) or in 2μM IAA (10 ml). The sections were then measured under a dissection microscope, and the retarded growth of treated coleoptiles was calculated as a percentage of the growth of the control.

Cellobiono-1,5-lactone. — To a solution of cellobiose (15.0 g, 44 mmoles) in water (557 ml) was added cadmium carbonate (23.4 g, 130 mmoles); then bromine (3.13 ml, 12.5 mmoles) was added, and the mixture was stirred in a stoppered flask for 25 h at room temperature. The suspension was filtered through a thin bed of decolorizing carbon overlaid with Filter-Cel (Celite analytical Filter-aid, Johns–Manville Products), and the clear, colorless filtrate (if colored, the excess of bromine was removed by aeration) was stirred with silver carbonate (15 g) for 20 min. The suspension was filtered (bed of decolorizing carbon overlaid with Filter-Cel), the filtrate was treated with an excess of hydrogen sulfide, the yellow, flocculent precipitate resulting was removed (bed of decolorizing carbon–Filter-Cel), and the filtrate was freed of residual hydrogen sulfide by aeration, and refiltered. The filtrate was evaporated *in vacuo* (35° bath) to a heavy syrup (15 g), which was cooled to room temperature and nucleated*. *tert*-Butyl alcohol (10 ml) was stirred in, and crystallization was allowed to progress at room temperature. After 3 days, the product was removed by filtration, washed with absolute alcohol, and dried *in vacuo*; wt. 8.0 g (54%), m.p. 194–195°. Recrystallized from concentrated aqueous solution, and washed with alcohol, the cellobiono-1,5-lactone had m.p. 204–205°, $[\alpha]_D^{20}$ +35.6 (extrapolated) → +33.2 (5 min) → +27.4 (20 min) → +0.8° (8 and 24 h, equil.) (*c* 1.0, water); ν_{max}^{Nujol} 1721 cm^{-1} (lactone). A fresh solution in *N*,*N*-dimethylformamide gave only one spot (lactone) with a number of anhydrous, solvent systems: *A*, R_F 0.65, *B*, 0.25, *C*, 0.85, and *D*, 0.40. Use of the same solvent-systems for t.l.c. of a fresh, aqueous solution of the lactone revealed the development of a second spot (acid) of low mobility, corresponding to that of cellobionic acid.

Anal. Calc. for $C_{12}H_{20}O_{11}$: C, 42.35, H, 5.92. Found: C, 42.12, H, 6.10.

Attempts to prepare the crystalline phenylhydrazide[13] from the lactone were unsuccessful**.

*Crystals were first observed in a sample of the syrupy lactone that had been stored for several weeks at room temperature.

**For a previous unsuccessful attempt, see ref. 14.

Reduction of cellobiono-1,5-lactone. — Cellobiono-1,5-lactone (250 mg, 0.73 mmole) was added in small portions to a stirred, aqueous solution (15 ml) of sodium borohydride (215 mg, 5.4 mmoles). After 1 h, acetone (2 ml) was added, followed by Amberlite IR-120 (H^+) ion-exchange resin (15 ml); the suspension was filtered, and the filtrate was evaporated *in vacuo* to a syrup (250 mg). Treatment with methanol in the usual way (to remove boric acid) gave a syrup that crystallized[15] from 96% ethanol (56 mg). After recrystallization from 96% ethanol, elementary analysis of the sample gave values corresponding to those calculated for $C_{12}H_{24}O_{11}$; its t.l.c. behavior, i.r. spectrum, and optical rotation were indistinguishable from those of cellobiitol prepared by the reduction of cellobiose; a mixture m.p. was undepressed (143–144°)[15].

Dicyclohexylammonium cellobionate. — To a solution of syrupy cellobiono-lactone (12.3 g, 36 mmoles) in water (11 ml) was added dicyclohexylamine (11 ml, 90 mmoles), and the mixture was stirred overnight at room temperature. Acetone was then added until a hard, gummy material was precipitated. The liquid was decanted, the gum was dissolved in isopropyl alcohol (12 ml), and the solution was kept for several days at −5°. The partially solid salt was filtered off and washed with isopropyl alcohol; it became a colorless syrup on standing at room temperature; $[\alpha]_D^{20}$ +24.9° (*c* 4.2, water). For analysis, a sample was dried under diminished pressure at room temperature.

Anal. Calc. for $C_{24}H_{45}NO_{12}$: C, 53.42; H, 8.41; N, 2.60. Found: C, 53.65; H, 8.25; N, 2.32.

Cadmium cellobionate. — A solution of syrupy cellobiono-1,5-lactone (5.1 g, 1.5 mmoles) in water (20 ml) was mixed with cadmium carbonate (130 mg, 0.8 mmole), and the suspension was stirred overnight. Cadmium hydroxide (11 g) was then added (pH of suspension, 7.3), and the suspension was stirred for 1 h, and filtered through a layer of carbon; the filtrate was evaporated *in vacuo*, yielding a colorless syrup that crystallized when mixed with methanol (10 ml). After filtration, and washing with methanol, the crystalline material weighed 4.5 g.

Anal. Calc. for $C_{24}H_{42}CdO_{24}\cdot 5H_2O$: C, 31.43; H, 5.72. Found: C, 31.65; H, 5.43.

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