

Note

Degradation of calcium D-gluconate-2-d with alkaline hydrogen peroxide*

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Of the various methods for shortening the carbon chain of sugars, that of Ruff has been the most widely used^{2–7}. In this process, a soluble salt of an aldonic acid is treated with hydrogen peroxide in the presence of ferric acetate. The ensuing reaction results in decarboxylation of the aldonic acid, with production of the next lower aldose. Many prior workers have assumed that the reaction proceeds by way of the glyc-2-ulosonic acid, which then undergoes decarboxylation^{8,9}.

Experimentally, the glyc-2-ulosonic acid has neither been separated from, nor identified in, the reaction mixture. Although it was reported¹⁰ that, when heated with 12% hydrochloric acid, L-erythro-pentulosonic acid loses carbon dioxide, with production of L-erythrose, the experimental conditions were different from those of the Ruff degradation. Thus, there is no convincing evidence that the degradation does, in fact, proceed through the glyc-2-ulosonic acid.

In view of this situation, and our interest in the degradation of carbohydrates by hydrogen peroxide¹, we have investigated the course of the reaction of calcium D-gluconate-2-d in the Ruff degradation. We postulated that decarboxylation by way of D-arabino-hexulosonic acid would give unlabeled D-arabinose, whereas, if the deuterium remained intact on C-2, the process would afford D-arabinose-1-d. Experimentally, a crystalline D-arabinose was separated, and its structure was determined from a study of its mass spectrum. The per(trimethylsilyl)ated D-arabinose, prepared from calcium D-gluconate-2-d, showed a distinct, molecular-ion peak at m/z 439, and a peak at m/z 424, corresponding to $M^+ - 15$, characteristic of a per(trimethylsilyl)ated aldose derivative¹¹, whereas nondeuterated D-arabinose showed peaks at m/z 438 and 423, respectively.

Production of D-arabinose-1-d in high yield proved that the Ruff degradation

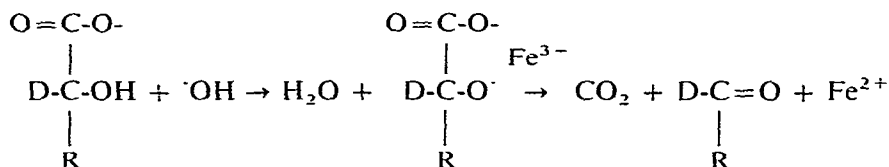
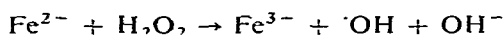
*Reactions of Carbohydrates with Hydroperoxides, Part XV. For Part XIV, see ref. 1.

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of calcium D-gluconate-2-*d* proceeds, in large measure, without intermediate formation of the 2-hexulonic acid.

In accordance with this finding, the overall process may be depicted as follows.



EXPERIMENTAL

General methods. — Evaporations were conducted under diminished pressure with the bath temperature below 40°. Optical rotations were determined with a Perkin-Elmer 141 photoelectric polarimeter. Paper chromatography was performed on Whatman No. 1 paper, with 4:1:1 butanol-ethanol-water. ^{13}C -N.m.r. spectra of the compounds (100–130 mg/mL of D_2O) were recorded with a Bruker-WP-80 instrument, with 1,4-dioxane as the internal standard. In each case, the temperature of the sample was $34 \pm 2^\circ$. The reagents used were of the highest commercial grade. Sodium D-arabino-hexulosonate was prepared by treating commercial calcium D-arabino-hexulosonate (Sigma) with one molecular equivalent of sodium oxalate. The sodium borohydride used was represented as containing 99% of deuterium; the hydrogen peroxide, <0.005% of Fe; and the potassium hydroxide, <0.001% of Fe.

Reaction of sodium D-arabino-hexulosonate with sodium borodeuteride. — A solution of sodium borodeuteride (0.075 g, 1.79 mmol) in water (5 mL) was added, with stirring, to a solution of sodium D-arabino-hexulosonate (0.5 g, 2.3 mmol) in water (10 mL) at 25°. After 1 h, the mixture gave a negative Fehling test. The excess of sodium borohydride was then decomposed with acetic acid (5 mL), and the solution was passed through a column of freshly regenerated Amberlite IR-120- H^+ resin (10 mL). The eluate was evaporated to dryness (8–10 times) with 5-mL portions of methanol, to remove the borate as methyl borate. The syrupy mass was then diluted with water (10 mL), the acid neutralized with a solution of barium hydroxide (0.05M), and the suspension evaporated to dryness. The neutral product was taken up in water, the suspension was filtered, the filtrate was concentrated under an air current to ~3 mL, and ethanol was added to incipient turbidity. After nucleation with several, minute crystals of unlabeled barium D-gluconate, and storage in a refrigerator for 2 days, crystals of barium D-gluconate-2-*d* hydrate separated. The crystals were filtered off, washed several times with 1:1 ethanol-water, and dried under vacuum at room temperature; yield 0.231 g.

Comparison of the ^{13}C -n.m.r. spectrum of the labeled salt with that of unlabeled barium D-gluconate (see Table I) confirmed the presence of deuterium¹²⁻¹⁴

TABLE I

CHEMICAL SHIFTS^a IN THE ¹³C-N.M.R. SPECTRA OF UNLABELED AND LABELED BARIUM AND CALCIUM D-GLUCONATE

Atom	Barium-D-gluconate		Calcium D-gluconate	
	Unlabeled	Labeled	Unlabeled	Labeled
C-1	180.426	180.426	180.353	180.354
C-2	74.549	—	74.402	—
C-3	71.256	71.329	71.109	71.036
C-4	72.719	72.719	72.280	72.280
C-5	71.037	71.037	70.670	70.671
C-6	62.476	62.476	62.402	62.403

^aEmploying 1,4-dioxane as the internal standard.

on C-2, as the spectrum failed to show the C-2 resonance characteristic of the unlabeled salt.

Preparation of calcium D-gluconate-2-d from barium D-gluconate-2-d. — A solution of barium D-gluconate-2-d hydrate (0.676 g, 1.24 mmol) in water (10 mL) was passed through a column of Amberlite IR-120 (H⁺) resin (20 mL). The eluate was concentrated to ~10 mL, calcium carbonate (2 g) was added, and the mixture was boiled for 30 min and then filtered. The filtrate was concentrated to ~5 mL, treated with methanol to incipient opalescence, and stored for 2 days at room temperature. The resulting, crystalline calcium D-gluconate-2-d was separated, washed with ethanol-water, and dried; yield 0.391 g.

Anal. Calc. for (C₆H₁₀DO₇)₂Ca: C, 33.30; H + D, 5.10; Ca, 9.26. Found: C, 33.21; H + D, 5.35; Ca, 8.65.

The ¹³C-n.m.r. spectrum, like that of the barium salt, showed complete absence of the C-2 resonance of the unlabeled salt (see Table I). Other assigned resonances are in substantial agreement with those reported for acyclic carbohydrates^{1,5}.

Preparation of D-arabinose-1-d from calcium D-gluconate-2-d. — A solution of calcium D-gluconate-2-d (0.5 mmol, 0.216 g) in warm water (5 mL) was cooled, and 0.2 mL each of barium acetate (0.92 g/10 mL) and ferrous sulfate (0.92 g/10 mL) were added, followed by 30% hydrogen peroxide (0.1 mL). The solution was then kept in a thermostat at 45°. At three 30-min intervals, 30% hydrogen peroxide (0.1 mL) was added, and, after a fourth such interval, a final addition of 0.05 mL was made. After an additional 30 min, activated charcoal (0.2 g; Norit) was added, and the mixture was heated for 15 min on a steam bath. It was then filtered, and the filtrate was passed through a column containing a 1:1 mixture (50 mL) of Amberlite IR-120 (H⁺) and Duolite A4 (OH⁻) resins. A paper chromatogram showed complete conversion into arabinose. The ion-free eluate was evaporated to a thick syrup, and this was lightly nucleated with unlabeled D-arabinose. After 12 days, the resulting, crystalline D-arabinose-1-d was separated; yield 0.093 g.

Anal. Calc. for $C_5H_9DO_5$: C, 39.74; H + D, 6.60. Found: C, 40.04; H + D, 6.74.

The mass spectrum of per(trimethylsilyl)ated D-arabinose-1-*d* showed molecular-ion peaks at m/z 439 and 424, whereas per(trimethylsilyl)ated, unlabeled D-arabinose showed peaks at m/z 438 and 423. Thus, the deuterium in calcium D-gluconate-2-*d* was retained in the D-arabinose formed by the Ruff degradation.

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