

Short Communications

L-ribo-4-Hexulosonic Acid from
Isomerization of
D-Glucuronic Acid

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In an earlier paper¹ it was shown that glucuronic acid in aqueous solution of pH 7 is easily isomerized at 110° and gives rise to two isomeric 5-hexulosonic acids and three hexuronic acids. The reaction products were isolated by anion exchange chromatography. A small amount of an unknown acid which gave a positive carbazole reaction appeared before any of the isolated isomerization products when the elution was carried out in acetic acid. This acid has now been isolated from experiments on a larger scale under the same working conditions and identified.

The acid fraction (denoted by SO) was isolated from runs on preparative columns as an amorphous compound (26 mg from 3 g D-glucuronic acid). A solution of SO in water had the optical rotation $[\alpha]_D^{25}$

$= +32^\circ$ ($c=0.56$). The acid was re-chromatographed on analytical columns in 0.08 M sodium acetate and in 1 M acetic acid, using a three channel analyzer with chromic acid oxidation, periodate oxidation with subsequent determination of formaldehyde, and the color reaction with carbazole.² Only one peak was recorded in each channel.

The carbazole response index was lower than that recorded with 5-hexulosonic and hexuronic acids whereas the response in the periodate-formaldehyde channel was typical of acids containing one primary hydroxyl group and a vicinal secondary hydroxyl group. The D_v values³ given in Table 1 did not agree with those of any acid studied previously. A study of the fully trimethylsilylated trimethylsilyl ester with gas chromatography-mass spectrometry carried out by Göran Petersson showed that the mass spectrum differed significantly from those recorded for hexuronic and 5-hexulosonic acids, but that the molecular weight and the number of hydroxyl groups were the same as with these acids. Reduction with sodium borohydride gave about equal amounts of two hexonic acids which were identified as allonic and gulonic acids by chromatography on anion exchange resins in two media (Fig. 1 and Table 1). Gas chromatography-mass spectrometry of the tri-

Table 1. D_v values of chromatographic bands (s) compared with those of reference acids (r).

Band	Reference	Distribution coefficient, D_v					
		1 M HAc		0.5 M HAc		0.08 M NaAc	
		s	r	s	r	s	r
SO		13.1				14.6	
Reduced SO	Glucuronic		22.7				13.0
	{ Allonic Gulonic			10.1	10.0	9.4	9.5
					14.5	14.6	8.5

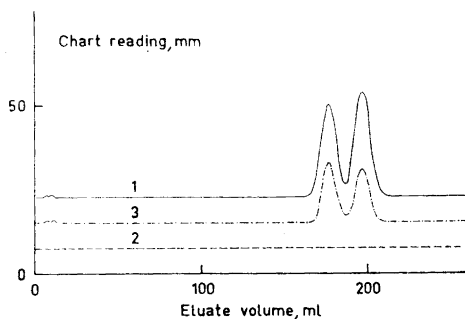


Fig. 1. Separation of acids formed by reduction of acid contained in Band SO.

Resin bed: 6×715 mm, Dowex 1-X8; 17–20 μm .

Eluent: 0.08 M NaAc, pH 5.9

Flow rate: $4.0 \text{ ml} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$.

1, Chromic acid oxidation; 2, Carbazole method; 3, Periodate-formaldehyde method.

methylsilylated 1,4-lactones on a QF-1 column confirmed the identity of the aldonic acids.⁴ The results show that the acid contained in band SO was *ribo*-4-hexulosonic acid not described previously in the literature.

It is reasonable to assume that the absolute configurations at C-2 and C-3 are the same as in the corresponding positions C-5 and C-4 in the starting material, D-glucuronic acid, and that the new acid is *L-ribo*-4-hexulosonic acid. This assumption is corroborated by the fact that no products with inverted configuration in these positions were observed on isomerization of D-glucuronic acid.¹

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Synthesis of ¹⁴C-Pentachlorophenol

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¹⁴C-Pentachlorophenol was synthesized by chlorination of ¹⁴C-phenol. Quinoline was used as catalyst,¹ and the crude 2,3,4,4,5,6-hexachloro-2,5-cyclohexadiene-1-one ("hexachlorophenol") was reduced with zinc and hydrochloric acid.²

The uniformly labelled ¹⁴C-phenol, (0.1 mC, 25.0 mC/mM, The Radiochemical Centre, Amersham, England) was diluted with 20 mg of inactive phenol (analytical grade) dissolved in 0.5 ml of ether, and transferred to a 30 ml Pyrex test tube. The ether was evaporated at room temperature, and 0.4 μl of quinoline, dissolved in carbon tetrachloride (0.15 ml) added. Dry chlorine was led into the solution through a thin polyethylene tube. When the solution was saturated and the air in the tube replaced by chlorine, the tube was sealed and placed in an oven at 120°C. After 24 h the reaction solution was once again treated with chlorine and kept at 120°C for a further 24 h.

The reaction mixture was diluted with 10 ml of ethanol and transferred to a 25 ml Erlenmeyer flask. Granular zinc (1 g) and conc. hydrochloric acid (1 ml) were added. The mixture was kept at 60–70°C for 30 min, and then poured into 100 ml of water, yielding a crystalline precipitate. The crystals were filtered, washed with water and dried. The product was then purified by sublimation (0.001 torr) in a 10 cm glass tube (i.d. 4 mm) at 120°C for 72 h. White, needle-formed crystals were obtained; m.p. 188–189°C (uncorrected); yield 44 %. The specific activity was 1.6 $\mu\text{C}/\text{mg}$.

Isotope dilution analysis was performed with inactive pentachlorophenol (prepared from a commercial product by repeated recrystallization, m.p. 188–190°C, no impurity found by gas chromatography of the methylated compound, see below). By recrystallization from isooctane, constant specific activity was obtained, corresponding to 97 % of the starting mixture.

Two dimensional thin layer radiochromatograms were prepared with about 5 μg of ¹⁴C-pentachlorophenol on a silica gel