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Note

The oxidation of 3-deoxy-D-*erythro*-hexos-2-ulose ("3-deoxyglucosone") to 3-deoxy-D-*erythro*-2-hexulosonic acid ("2-keto-3-deoxy-D-gluconate") by D-glucose oxidase*

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3-Deoxy-D-*erythro*-2-hexulosonic acid ("2-keto-3-deoxy-D-gluconic acid", **2**) is a bacterial metabolite that may arise from D-gluconic acid[1] and 2-amino-2-deoxy-D-gluconic acid[2] as a result of enzymic action. Preparations of **2** that are described in the literature[1,2] involve the isolation of soluble enzymes that are not commercially available, and, thus, are complicated and time-consuming. A chemical synthesis of **2** that involves the condensation of oxaloacetic acid with D-glyceraldehyde to give the C-4 epimers of **2** has been described[3]. Compound **2** was isolated in 13% yield, after chromatographic resolution of the epimers.

A recent report from this laboratory[4], which described the synthesis of 3-deoxy-D-*erythro*-hexos-2-ulose ("3-deoxy-glucosone"[1]) in pure form, prompted us to investigate its suitability as a precursor to **2**, since oxidation at C-1 of **1** would convert it directly into **2**.

After examining a number of possibilities, it was found that D-glucose oxidase, an inexpensive, commercially available enzyme readily reacted with **1** and converted it cleanly into **2** in good yield. Pure **2** was isolated in 65% yield from the reaction mixture after displacement from an ion-exchange resin. Kinetic studies for **1** as a substrate (using an oxygen electrode) indicated a K_m of 32.4mM and a maximum velocity (V_{max}) of $7.00 \cdot 10^2$ mol of $O_2 \cdot (\text{min} \cdot \text{mg of protein}^{-1})$. Corresponding values for D-glucose as a substrate are 19.0 and $5.43 \cdot 10^4$. Thus, **1** is oxidized by the enzyme at 1/77 the rate. Compound **1** reacts at rates comparable to those reported for D-mannose, D-xylose, and 6-*O*-methyl-D-glucose[5].

The reaction product **2** was shown to be pure and uncontaminated by **1** by

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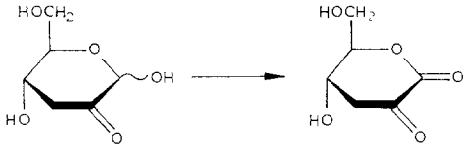
g.l.c. of the per-*O*-(trimethylsilyl)oxime derivative[6], which showed one peak, and, of the per(trimethylsilyl) ether, which showed two peaks, neither of which corresponded to **1**.



1 2

Structural proof for **2** was obtained from the ^{1}H-n.m.r. spectrum (at 60 MHz) of a solution in deuterium oxide, which showed signals (relative to tetramethylsilane) at δ 4.32–3.30 (m, H-4, -5, and -6) and at 2.50–1.57 (m, H-3,3′). The reduction of **2** with sodium borohydride to the corresponding metasaccharinic acids further con-

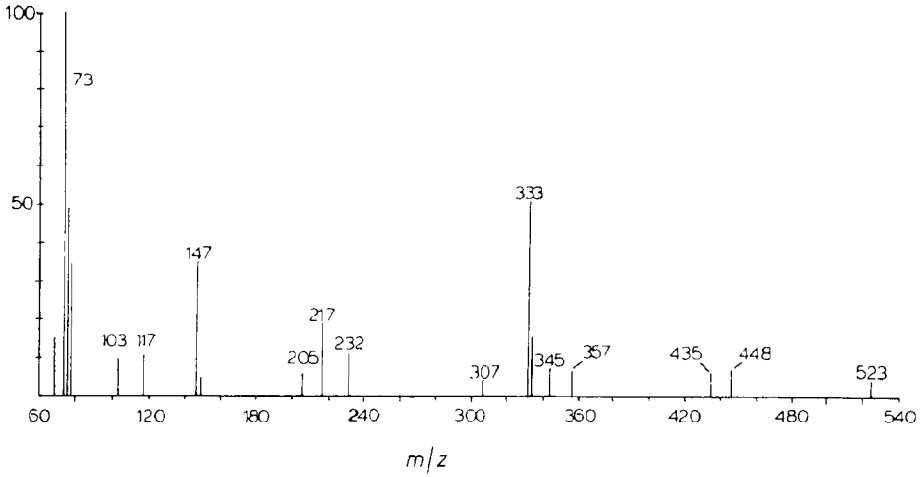
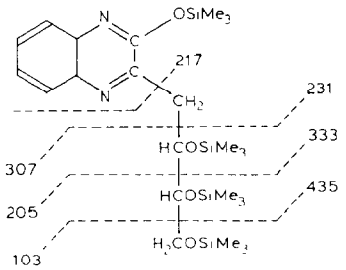


Fig. 1. Mass spectrum of the per-*O*-trimethylsilyl derivative of 3-(D-*erythro*-2,3,4-trihydroxybutyl)-2-quinoxalinol (**3**).



Scheme 1. Structure and fragments obtained by electron impact mass spectrometry of the per-*O*-trimethylsilyl derivative of **3**.

firmed its structure. In addition to the structural studies just discussed, **2** was converted into the crystalline quinoxalinol derivative (**3**). Fig. 1 shows an electron-impact mass spectrum (g.l.c.–m.s.) of the tetrakis(trimethylsilyl) derivative of **3**, and Scheme 1 the peak assignments. Additional physical and spectral properties are reported in the Experimental section.

EXPERIMENTAL

General. — T.l.c. was performed on silica gel plates, and spots were made visible by spraying with 10% ethanolic sulfuric acid, followed by heating for 15 min at 100°. G.l.c. analyses were performed on the trimethylsilyl ethers of the *O*-methyl oxime derivatives, which were prepared as described previously[6], with a Perkin–Elmer Sigma 3 chromatograph equipped with the following nickel columns (3 mm × 1.8 m): (A) 2% GC-SE-30 silicone rubber (Alltech Associates, Inc., Deerfield, IL 60075) on 100–120 mesh Chromosorb W, H.P., and (B) 3% SP-2250 on 80–100 mesh Supelcoport (Supelco Inc., Bellefonte, PA 16825). Nitrogen flow-rates, and detector and injector temperatures were 24 mL/min and 240°, respectively. For mass spectral studies, g.l.c. columns were placed in an F and M chromatograph interfaced with a CEC model 21-110C mass spectrometer. For kinetic studies on D-glucose oxidase, oxygen consumption was measured with a model 53 Biological Oxygen Monitor (Yellow Springs Instrument Co., Yellow Springs, OH 45387) water-jacketed at 20°. Standard 3-deoxy-D-*ribo*-hexono-1,5-lactone ("metasaccharinolactone") was a gift from J. F. Harris (Forest Products Laboratory, Madison, WI 53705). The D-*arabino* isomer was prepared as described previously[7]. Samples of 3-deoxy-D-*erythro*-hexosulose were prepared as described previously[4].

Potassium 3-deoxy-D-erythro-*2-hexulonosate* (**2**). — Into a 100-mL, round-bottomed flask containing lyophilized **1** (162 mg) was added 0.1M potassium phosphate buffer (pH 6.0, 50 mL) and D-glucose:oxygen oxidoreductase, (EC 1.1.3.4) from *Aspergillus niger* (1000 units based on D-glucose; Sigma Chemical Co., St. Louis, MO 63178, Type V). The flask was maintained in an oxygen atmosphere. Progress of the reaction was monitored by t.l.c. using an irrigant composed of 2:1 (v/v) ethyl acetate–methanol (**1**, R_F 0.51; **2**, R_F 0.1). After being kept for 75 h at 23°, (when 15.6% of **1** remained unreacted as determined by g.l.c.), the mixture was passed through columns of Amberlite IR-120 cation-exchange resin (H^+, 30 mL) and Amberlite IRN-78 anion-exchange resin (HCO_3^-, 50 mL) placed in series. The columns were washed with water (1 L), and the anion-exchange resin was eluted with 0.3M formic acid (3 L). After removal of formic acid by evaporation under diminished pressure, the solution of the residue in water was neutralized with 40% potassium hydroxide solution, and lyophilized, to give 140 mg (65% based on consumed **1**). Chromatographic purity was demonstrated by g.l.c. of the per-*O*-trimethylsilyl derivative (T_R 6.6 and 7.0 min) and the per-*O*-trimethylsilyloxime derivative (T_R 8.6 min) on column *B*, programmed from 150° to 220° at a rate of 6°/min with a 2-min initial hold.

Reduction of **2**. — To a solution of **2** (5.0 mg) in water (10 mL) was added sodium borohydride (10 mg), and the mixture kept for 2 h at 23°. The pH was adjusted to 2.5 by addition of Amberlite IR-120 cation-exchange resin (H^+). The suspension was filtered, and the filtrate placed on a column of Amberlite IRN-78 anion-exchange resin (HCO_2^-, 0.8 × 5 cm). The resin was washed with water (25 mL), and the reduction products were eluted with 0.3M formic acid (50 mL). The solution was evaporated under diminished pressure at 20–25°. A solution of the residue in water was transferred to a 3-mL, Teflon-lined, screw-capped vial, and dried with a stream of air. The residue was dissolved in pyridine (0.20 mL), and *N*-trimethylsilylimidazole (0.2 mL) and trifluoro-*N*,*O*-bis(trimethylsilyl)acetamide (0.2 mL) were added. G.l.c. on Column *B* programmed from 150° to 220° at a rate of 6°/min with a 2-min initial hold gave two peaks having retention times T_R 5.3 and 5.5 min identical to those of a mixture of 3-deoxy-D-*arabino*- and 3-deoxy-D-*ribo*-hexonolactones; m.s. of the 3-deoxy-per-*O*-(trimethylsilyl)-D-*arabino*-hexonolactone produced from **2**: *m*/*z* 363(15), 322(2.4), 292(5.2), 273(8.9), 246(11.4), 204(12.3), 147(38.3), 129(39.8), 103(1.8), 77(10.4), 75(12.5), and 73(100). Aside from differences in % of relative abundance, the mass spectrum for the D-*ribo* isomer was identical.

3-(D-erythro-*2,3,4-Trihydroxybutyl*)-*2-quinoxalinol* (**3**). — The potassium salt of **2** (54 mg) was converted into the sodium salt by passage of its solution in water through a column of Dowex 50 ion-exchange resin (Na^+, 200–400 mesh, 0.8 × 5 cm), followed by evaporation to dryness under diminished pressure. A solution of freshly sublimed 1,2-phenylenediamine (30.0 mg) in M acetic acid (5.0 mL) was added, and the mixture stirred for 12 h at 23°. Following evaporation under diminished pressure (35–40°), and transfer of a solution of the residue in water (10 mL) to a 20-mL, screw-capped tube, benzaldehyde (0.2 mL) was added. The tube was vigorously shaken and the solution washed with six 5-mL portions of ether until colorless. The solution was evaporated under diminished pressure, extracted with absolute ethanol, and centrifuged to remove sodium acetate. The yield of crude product, which contained residual sodium acetate, was 55 mg. Crystallization from water gave a material having m.p. 170–171°; ν_{max}^{KBr} 3200 cm^{-1} (C=N, CONH); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 7.80–6.97 (m, aryl), 4.5 (s, OH), 3.63–3.25 (m, H-4, -5, -6,6′), and 3.13–2.79 (m, H-3,3′). Conversion to the per(trimethylsilyl) ether was accomplished by dissolution in anhydrous *N*,*N*-dimethylformamide and addition of trifluoro-*N*,*O*-bis(trimethylsilyl)acetamide; g.l.c. (column *A*, programmed from 200° to 250° at a rate of 6°/min, and a 2-min initial hold): T_R 5.6 min.

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