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A SYNTHESIS OF 2-DEOXY-D-*arabino*-HEXITOL AND ITS OXIDATION TO 5-DEOXY-D-*threo*-HEXULOSE ("5-DEOXY-D-FRUCTOSE") USING IMMOBILIZED CELLS OF *Gluconobacter oxydans*

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ABSTRACT

2-Deoxy-D-*arabino*-hexitol (**6**) was obtained by borohydride reduction of 2-deoxy-D-*arabino*-hexose (**5**). The synthesis of **5**, starting from 2,3:4,5-di-*O*-isopropylidene-D-arabinitol (**1**), was achieved by a one-carbon chain-elongation involving formylation of the Grignard reagent derived from 1-bromo-1-deoxy-2,3:4,5-di-*O*-isopropylidene-D-arabinitol (**2**), using lithium formate, followed by hydrolytic removal of the isopropylidene groups. Immobilized cells of *Gluconobacter oxydans* (ATCC 15178) selectively oxidized **6** to give 5-deoxy-D-*threo*-hexulose (**7**) in 65% yield (~9% overall yield from **1**).

INTRODUCTION

2-Deoxy-D-*arabino*-hexose ("2-deoxy-D-glucose", **5**) is a naturally occurring carbohydrate, the ^{14}C-labeled analog of which has been widely used[1] for the quantitative study of various aspects of the physiological transport and regional metabolic rates of D-glucose under normal and pathological conditions; several syntheses of **5** have been reported[2–5] in the literature.

2-Deoxy-D-*arabino*-hexitol (**6**) was first synthesized by Bergmann *et al.*[6] starting from D-glucal, and also has been isolated[7] in ~5% yield from the mixture of products from the electro-reduction of D-glucose under conditions of mild alkalinity. When subjected to fermentation[8] by a suspension of cells of *Gluconobacter oxydans* sub-sp. *suboxydans*, **6** was oxidized efficiently to 5-deoxy-D-*threo*-hexulose (**7**). A chemical synthesis of **7**, involving six steps starting from D-fructose, has been reported[9] recently; a key step was the reaction of 2,3-*O*-isopropylidene-β-D-fructopyranose with sulfuryl chloride to give exclusively a 5-chloro-5-deoxy derivative. In the course of that investigation, Szarek and his co-workers[9]

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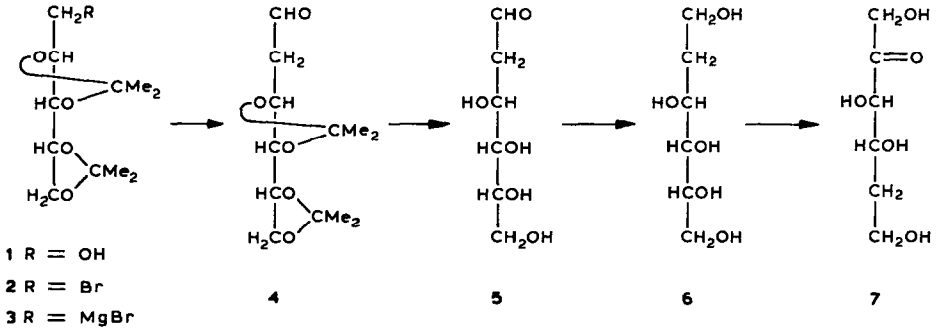
established that **7** is "much sweeter than L-sorbose and nearly as sweet as D-fructose", and consequently, is a potentially useful sweetening agent. This article reports the development of an alternative synthesis of **7** involving both a one-carbon chain-elongation by means of the formylation of a Grignard reagent (see **3**) using lithium formate, and the selective oxidation of **6** by immobilized cells of *Gluconobacter oxydans* (ATCC 15178).



RESULTS AND DISCUSSION

The bromination of 2,3:4,5-di-*O*-isopropylidene-D-arabinitol[10] (**1**) using triphenylphosphine, tribromoimidazole, and imidazole in toluene[11] produced a mixture of compounds from which **2** was isolated in 87% yield as a light-yellow syrup, by distillation under diminished pressure. The Grignard reagent (**3**) was prepared by the action of magnesium on **2** in dry tetrahydrofuran (THF). When the substrate (**2**) was almost completely consumed, the one-carbon chain-extension was effected under anhydrous conditions by the formylation[12] of **3** with an equimolar quantity of lithium formate. The formylation reaction afforded a mixture which was indicated by thin-layer chromatography (t.l.c.) to contain three compounds. The target compound, namely 2-deoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hexose[4,5] (**4**), was isolated in 27% yield by column chromatography of the product mixture. The reaction of **4** with 1:1 (v/v) 31% formic acid–ethanol at 90° achieved the hydrolysis of the isopropylidene groups and gave a colorless oil (95% yield, calculated as **5**) which co-chromatographed with authentic 5-deoxy-D-*arabino*-hexose (R_F 0.45) in t.l.c. using 2:6:2 (v/v) ethyl acetate–2-propanol–water; the treatment of this oil with hot acetone afforded crystalline **5** (yield 65%). Reduction of **5** with sodium borohydride gave 2-deoxy-D-*arabino*-hexitol (**6**) in 95% yield. Immobilized cells of *Gluconobacter oxydans* selectively oxidized **6** to produce **7**, which was isolated in 65% yield from the fermentation mixture.

Immobilization of the cells of *Gluconobacter oxydans* was performed with low-melting agarose gel which was liquefied in warm water, cooled to 30°, mixed thoroughly with a suspension of the bacterial cells, and then regelatinized by cooling. The gel mass, containing the entrapped cells, was coarsely comminuted

(3–5 mm^3) and utilized as a suspension in an aqueous solution of **6**.

Three strains of *Gluconobacter oxydans,* namely ATCC 15178, NRC 17004, and NRRL B-72, were investigated. Each of *G. oxydans* ATCC 15178 and NRC 17004 effected the complete conversion of a 2% (w/v) solution of **6** into product in 12–24 h, whereas the NRRL B-72 strain required approximately twice as long. Although the fermentation of **6** by immobilized cells of *G. oxydans* NRC 17004 was complete in ~12 h, it afforded **7** in only 22% yield; the major portion of the fermentation product was insoluble in methanol and failed to give a color reaction with *N*-(1-naphthyl)-1,2-ethanediamine[13]. In contrast, strain ATCC 15178 afforded a fermentation product from which crystalline **7** was isolated in 65% yield. The growth medium used to produce the required biomass of *G. oxydans* for use in the fermentation of **6** influenced significantly the rate of production of the bacteria—the MG medium required 44–48 h of incubation whereas the YG medium produced the same quantity of cells in 20–24 h—but had no effect on the ability of the cells to effect the selective oxidation.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 241 automatic polarimeter in a 0.1-dm cell at 26 ±3°. I.r. spectra were recorded with a Perkin–Elmer 598 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-n.m.r.) spectra were recorded with a Bruker AM-400 (400 MHz) spectrometer for solutions in chloroform-*d* with tetramethylsilane (Me_4Si) as the internal standard, unless otherwise indicated. Chemical shifts (δ) are given downfield from the signal of Me_4Si.

Solvents were evaporated under diminished pressure at <40°. Analytical t.l.c. was performed using glass plates precoated with Merck silica gel 60F-254 as the adsorbent (layer thickness 0.25 mm). The developed plates were air dried, sprayed with a solution of cerium sulfate (1%) and molybdic acid (1.5%) in 10% aqueous sulfuric acid, and heated at 150°. Column chromatography was performed on Merck silica gel 60 (70–230 mesh). H.p.l.c. was performed with a Varian 5000 liquid chromatograph, using an INTERACTION CHO 620 column eluted with water at 90° and a flow rate of 0.5 mL min^{-1}, equipped with a Varian CDS 401 data station.

Bacterial strains. — *Gluconobacter oxydans* strains were obtained from the American Type Collection[14], Rockville, MD, (ATCC 15178)*, the Northern Regional Research Laboratory, Peoria, IL, (NRRL B-72), and the National Research Council of Canada, Ottawa, ON, (NRC 17004). Subcultures of these microorganisms were maintained by biweekly transfer on YG medium (see next) at room temperature.

*This strain has been called *Acetobacter roseus* (see ref. 14).

Microbiological media and culture conditions. — The YG medium contained (w/v) Difco yeast extract (0.5%) supplemented with D-glucitol (2.5%) and adjusted to pH 6.8. The minimal medium (MG) contained (g L^{-1}): Na_2HPO_4, 3; KH_2PO_4, 2; $MgSO_4 \cdot 7\ H_2O$, 0.5; $(NH_4)_2SO_4$, 5; D-glucitol, 25, and was supplemented with 1 $mL.L^{-1}$ of a trace-element solution described by Whittenbury *et al.*[15] but excluding the ethylenedinitrilo(tetraacetic acid) and copper(II) sulfate which it normally contains.

The cultures were incubated at 30° in 50-mL aliquots of MG medium supplemented with 0.05% KH_2PO_4, using 250-mL Erlenmeyer flasks shaken at 175 c.p.m. for 20–48 h. The inoculum [4% (v/v)] was grown, under the same conditions, in YG medium.

Preparation of washed-cell suspensions. — Cells from 50-mL aliquots of the cultures grown as just described were collected by centrifugation at 17000*g* for 15 min, washed twice with sterile, distilled water, and resuspended in 5 mL of sterile, distilled water.

Immobilization of Gluconobacter *cells.* — Low-temperature gel agarose (0.1 g; Bethesda Research Laboratories, Gaithersburg, MD) was suspended in distilled water (15 mL) and heated until the agarose dissolved completely. The solution was cooled to 30° and mixed with 5 mL of a washed-cell suspension. The suspension was placed in a refrigerator for 20 min to set the gel, which was then removed, comminuted to a uniform particle size (3–5 mm^3), and washed with 50 mL of sterile, distilled water.

*1-Bromo-1-deoxy-2,3:4,5-di-*O*-isopropylidene-*D*-arabinitol* (**2**). — A mixture of **1** (ref. 10, 1.7 g, 7.32 mmol), triphenylphosphine (3.83 g, 7.28 mmol), and imidazole (0.49 g, 7.28 mmol) in toluene (70 mL) was stirred for 1 h at 70° and then for 2 h at 95°. Tribromoimidazole[16] (1.0 g, 3.6 mmol) was added and stirring was continued for 2 h at 95°. The mixture was cooled to room temperature, an equal volume of saturated aqueous sodium hydrogencarbonate was added, and the mixture was stirred for 5 min. Iodine was added in small portions until the organic layer acquired a permanent coloration; the mixture was stirred for 10 min, and a saturated, aqueous solution of sodium thiosulfate was added until the iodine color was eliminated. The toluene was removed by evaporation and the residual aqueous solution was extracted with dichloromethane (~200 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated to afford a yellow syrup from which **2** (1.89 g, 87.5%) was isolated by distillation (b 105° at 427 Pa) as a light-yellow oil, $[\alpha]_D$ +61.2° (*c* 1, chloroform), ν_{max}^{film} 3000, 1370, 1200, 1050, 760, and 670 cm^{-1}; ^{1}H-n.m.r. δ 4.17–4.09 (m, 2 H), 4.09–4.02 (m, 1 H), 3.78–3.67 (m, 2 H), 3.55–3.49 (m, 1 H), 1.45 (s, 3 H, Me), 1.43 (s, 3 H, Me), 1.40 (s, Me), and 1.35 (s, 3 H, Me).

*2-Deoxy-*D*-arabino-hexose* (**5**). — Magnesium (144 mg, 6 mmol) was added to a solution of **2** (1.002 g, 3.4 mmol) in dry THF (40 mL) under hydrogen; a small crystal of iodine was added to initiate the reaction. The mixture was stirred for 3 h, at which point the consumption of **2** was almost complete; lithium formate (312 mg, 6 mmol) was added and the mixture was stirred for 3.5 h at reflux temperature. The

solvent was evaporated and ethyl ether (25 mL) was added to the residue. The mixture was treated with 0.1M hydrochloric acid (20 mL) at 0° and the organic phase was then separated, washed with water, and dried ($MgSO_4$). Evaporation of the solvent yielded a brown residue which was shown by t.l.c. [98:2 (v/v) chloroform–methanol] to consist of 3 components of which that having R_F 0.63 was preponderant. This major component was isolated by column chromatography [99:1 (v/v) chloroform–methanol] as a colorless oil and identified as 2-deoxy-3,4:5,6-di-*O*-isopropylidene-D-*arabino*-hexose (**4**, 223 mg, 27%), $[\alpha]_D$ −27.0° (*c* 0.6, chloroform). The i.r. and ^{1}H-n.m.r. spectral data are consistent with those reported by Shiue *et al.*[4].

A solution of **4** (210 mg) in 1:1 (v/v) 31% formic acid–ethanol (10 mL) was heated for 10 min at 90°. The mixture was cooled to room temperature, and the solvent evaporated to give a colorless oil (134 mg, 95% calculated as **5**) which, upon treatment with hot acetone, afforded **5** (87 mg, 65%) as white needles which co-chromatographed with an authentic sample of 2-deoxy-D-*arabino*-hexose (h.p.l.c. retention time: 10.4 min), m.p. 140–144° (lit.[2] m.p. 142–144°).

2-Deoxy-D-arabino-*hexitol* (**6**). — A solution of sodium borohydride (16 mg, 0.6 mmol) in water (10 mL) was combined with a stirred solution of **5** (80 mg, 0.49 mmol) in water (10 mL). After 30 min, IRA-120 (H^+) ion-exchange resin was added, stirred for 2 h, removed by filtration, and the filtrate evaporated to yield **6** (76 mg, 95%) as an amorphous powder which co-chromatographed with an authentic sample of 2-deoxy-D-*arabino*-hexitol (h.p.l.c. retention time: 13.0 min).

5-Deoxy-D-threo-*hexulose* (**7**). — The gel-entrapped cells of *G. oxydans* (ATCC 15178) were suspended in a filter-sterilized solution of **6** (400 mg, 2.4 mmol) in water (20 mL) and shaken in a 250-mL Erlenmeyer flask at 175 r.p.m. at 30°. The progress of the fermentation was monitored by t.l.c. [7:1:0.2 (v/v) ethyl acetate–methanol–water[9]] using a solution of *N*-(1-naphthyl)-1,2-ethanediamine dihydrogen chloride (0.2%) and sulfuric acid (3%) in methanol to detect the product; the fermentation was judged to be complete after 24 h. The gel-entrapped bacterial cells were removed by centrifugation at 3000*g* for 5 min and the residue was washed once with water (20 mL); the combined supernatant solutions were evaporated and the residue extracted with hot methanol (20 mL). The methanol extract was filtered and the filtrate evaporated to afford **7** (260 mg, 65%), which was recrystallized from methanol–acetone; m.p. 109–111°, $[\alpha]_D$ −67.3° (*c* 0.7, H_2O) {lit.[8] m.p. 110°, $[\alpha]_D$ −67.0° (*c* 1.0, H_2O)}. The i.r. and n.m.r. data were in close agreement with those of Martin *et al.*[9].

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