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Note

The synthesis of 4-deoxy-L-*glycero*-pentulose by biochemical dehydrogenation of 2-deoxy-D-*erythro*-pentitol*

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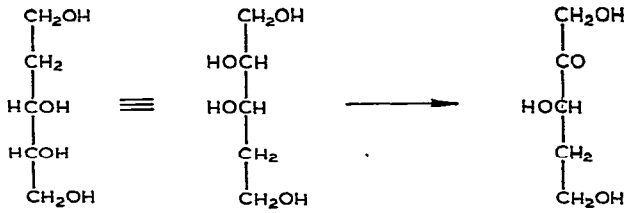
At present, considerable attention is being paid to ketoses, mainly because of their biological activity. They are constituents of some antibiotics[2]; and hydrazones of ketoses inhibit nucleo- and proteo-synthesis in tumorous cells[3].

Continuing our work[4–6] on the synthesis of ketoses and their derivatives, we now describe the synthesis of 4-deoxy-L-*glycero*-2-pentulose (**2**) by biochemical dehydrogenation of 2-deoxy-D-*erythro*-pentitol (**1**). A very convenient method for the preparation of ketoses is the biochemical dehydrogenation of alditols by bacteria; this method may be used to produce not only the ketoses (L-*glycero*-tetrulose[7], D-*threo*-pentulose, and L-*erythro*-pentulose[8]), but their derivatives (5-*S*-ethyl-5-thio-D-*threo*-pentulose[9] and 5-acetamido-5-deoxy-L-*xylo*-hexulose[10]) as well.

The biochemical dehydrogenation of alditols using the bacterial strain *Gluconobacter oxydans* (former names *Acetomonas oxydans*, or *Acetobacter suboxydans*) proceeds according to the Bertrand–Hudson rule[11,12]. It is interesting, however, that, on replacement of one of the hydrogen atoms by a methyl group, this group behaves like a hydrogen atom, and its presence does not affect the course of the dehydrogenation; for example, 1-deoxy-D-galactitol is biochemically dehydrogenated to 1-deoxy-D-*xylo*-3-hexulose[13].

We have prepared the ketose **2** by the biochemical dehydrogenation of the deoxy-alditol **1**. The reaction takes place according to the Bertrand–Hudson rule, and the presence of the deoxy group has no influence on the course of the reaction.

*Ketoses and Their Derivatives, Part III. For Part II, see ref. 1.



1 2

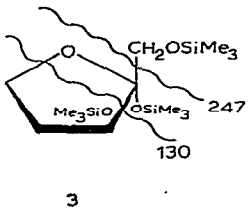
A suitable bacterial strain was chosen among strains that are capable of completely dehydrogenating D-glucitol to L-*xylo*-hexulose. It was found that the growth of bacterial strains associated with the formation of ketose **2** is very slow in media containing the deoxy-alditol **1** as the sole carbon source. However, the addition of small amounts of readily fermentable sugars increases the yield of compound **2**. D-Glucose as a carbon source is particularly convenient, because its metabolites are of an acidic nature, and can be separated from the culture medium on ion-exchange resins. Six different, bacterial strains were tested in order to achieve biochemical dehydrogenation of alditol **1**. By monitoring the reducing sugars during culture, it was found that the dehydrogenation proceeds most efficiently with the strain *Gluconobacter oxydans* CCM 1804; therefore, this organism was employed for large-scale preparation of ketose **2**. The chromatographically pure ketose **2** was isolated as a syrup[14] by cellulose-column chromatography.

Reduction of compound **2** with sodium borohydride afforded a mixture of deoxy-alditol **1** and 2-deoxy-L-*threo*-pentitol, proving that the compound isolated was a ketose.

The position of the carbonyl group was determined by circular dichroism (c.d.)[15]. Comparison of the c.d. spectra of L-*glycero*-tetrulose, D-*threo*-pentulose, L-*erythro*-pentulose, and ketose **2** showed that all of these compounds have the same absolute configuration[15,16], namely *S*, of C-3.

Gas–liquid chromatography of per(trimethylsilyl)ated **2** showed the presence of three Me_3Si derivatives of **2** in the ratios of ~5:3:2 (relative retention times, 0.4, 0.5, and 1, respectively). The compounds of r.r.t. 0.4 and 0.5 produced qualitatively identical mass spectra, and their furanoid structures were deduced from the presence of characteristic ion-peaks, the formation of which is shown for one[17] of the anomers (**3**); thus, the two are Me_3Si derivatives of the anomers of furanoid **2**. The compound having the longest r.r.t. was the Me_3Si derivative (**4**) of the acyclic form of **2**. This followed from the observation that the mass spectrum contained characteristic peaks of ions (**4**), shown together with the origin of the primary ions. The peak at *m/e* 117 proved the presence of the deoxy group at C-4, and, from the peak of $[M - \cdot CH_3 - Me_3SiOH]^+$ at *m/e* 245, the molecular weight was deduced (M = 245 + 15 + 90 = 350).

The phenylosazone prepared from ketose **2** is optically active; this constitutes further proof that the carbonyl group is located at C-2, as the phenylosazone prepared from a 3-ketopentose would be optically inactive.



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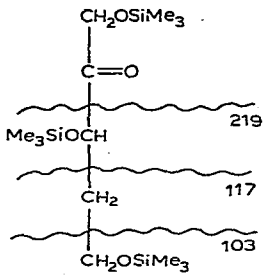

4

EXPERIMENTAL

General. — Melting points were determined on a Kofler micro hot-stage. Solutions were evaporated under diminished pressure at 30–40°. Optical rotations were measured with a Bendix–Ericsson automatic polarimeter. The c.d. spectra were recorded with an ORD/UV-5 JASCO spectropolarimeter, equipped with a c.d. adapter; further details concerning the c.d. spectra of ketose **2** were described in ref. 15.

2-Deoxy-D-*erythro*-pentose was prepared according to ref. 18, and 2-deoxy-L-*threo*-pentose according to ref. 19. The corresponding 2-deoxy-alditols were prepared by sodium borohydride reduction of the 2-deoxy-aldoses.

Descending paper-chromatography was performed on Whatman No. 1 paper with (*a*) 2-butanone saturated with water; (*b*) 6:5:2 (v/v) cyclohexanol–pyridine–water saturated with boric acid and containing phenol (1 g/100 mL); the chromatographic paper was presoaked with 1% aqueous boric acid. Detection was effected with potassium periodate–benzidine.

Preparative chromatography was performed on columns (90 × 4.5 cm) of Whatman cellulose, using solvent (*a*), and on columns (60 × 3 cm) of silica gel (0.04–0.1 mm) with solvent (*c*), namely, 12:1 (v/v) chloroform–methanol.

Mass spectrometry. — Me_3Si derivatives were prepared by the addition of an excess of chlorotrimethylsilane to a solution of a sample (2 mg) in pyridine. The Me_3Si derivatives were analyzed at 110° by using a JGC-20 K gas chromatograph equipped with a column (2 m) packed with 3% of OV-17 on Chromosorb WAW DMCS (80–100 mesh). When working with a f.i.d. detector, nitrogen was used as the carrier gas (inlet pressure, 101.3 kPa). The injection-port temperature was 180°. Helium was the carrier gas during g.l.c.-m.s. analyses, and the other conditions were as just described. Mass spectra (23 eV) were measured at an emission of 300 μA, using a JMS-D 100 spectrometer whose ionization chamber was kept at 180°.

Choice of suitable strains. — Bacterial strains (Czechoslovak Collection of Micro-organisms) used were inoculated on a slanting, agar medium containing D-glucose (5 g), bakers' yeast autolyzate (2.5 g), calcium carbonate (2 g), and agar (2.5 g) in water (100 mL). After 2 days of cultivation at 30° (this temperature was

chosen for all further cultivations), the cultures were re-inoculated on a slanting, agar medium containing D-glucitol (5 g), bakers' yeast autolyzate (2.5 g), and agar (2.5 g) in water (100 mL). After 2 days, the cultures were taken as the inoculum for a medium containing the alditol **1** (2 g), D-glucose (0.07 g), and bakers' yeast autolyzate (3 g) per 100 mL; the cultivation proceeded in Erlenmeyer flasks (5 mL) on a reciprocal shaker. After 2 days of cultivation, reducing sugars were determined according to Schoorl. Results of the analysis (in mL of 0.1N $Na_2S_2O_3$ per mL of the sample): *Acetomonas oxydans* 2370 (3.0), *Acetomonas oxydans* 2356 (1.9), *Gluconobacter oxydans* 1804 (3.2), *Gluconobacter oxydans* 2365 (2.9), *Acetobacter pasteurianus* 1775 (0.2), and *Acetomonas oxydans* 1784 (2.9 mL). The course of the biochemical dehydrogenation was monitored by paper chromatography in solvent (*a*).

Preparative, biochemical dehydrogenation. — *Gluconobacter oxydans* CCM 1804 was twice re-inoculated as described in the preceding section. The biomass obtained from two slanting, agar media was used as an inoculum for a medium (120 mL) containing alditol **1** (2.37 g), D-glucose (0.084 g), and bakers' yeast autolyzate (3.6 g). The medium (120 mL) was added to a 500-mL flask having an elongated neck (13 cm) and a stopper of cotton-wool. After cultivation for 1 day on a reciprocal shaker, the solution contained none of the starting compound, and only the reducing-sugar metabolite was present. The suspension was filtered through an asbestos–cellulose filtration-disk, the filtrate was de-ionized on a column of Wofatit KPS 200 (H^+) cation-exchange resin, the pH was adjusted to 6.9 by addition of Wofatit SBK (OH^-) anion-exchange resin, and the solution was treated with charcoal, filtered, and evaporated.

Preparation of chromatographically pure ketose **2**. — The ketose obtained by biochemical dehydrogenation of alditol **1** was purified on a cellulose column, using solvent (*a*). The effluent containing chromatographically homogeneous ketose **2** was evaporated, and the resulting syrup was dried to constant weight over P_2O_5 to give compound **2** (1.17 g, 50.1%); $[\alpha]_D^{25}$ +10.9→+12.8° (*c* 1.0, water).

Anal. Calc. for $C_5H_{10}O_4$: C, 44.77; H, 7.52. Found: C, 44.83; H, 7.70.

Identification of 2-deoxyalditols. — In the reaction mixture prepared by reduction of ketose **2** with sodium borohydride, the presence of deoxyalditol **1** and 2-deoxy-L-*threo*-pentitol was monitored by paper chromatography using solvent (*b*).

Synthesis of the phenylosazone of compound **2**. — To a mixture of sodium acetate (1 g) and phenylhydrazine hydrochloride (1.3 g) in water (5 mL) was added a solution of ketose **2** (0.4 g) in water (2 mL), and the mixture was kept for 16 h at 20° with occasional shaking. The resulting phenylosazone (0.58 g; 62.3%) was purified on a column of silica gel using solvent (*c*); m.p. 136–138°; $[\alpha]_D^{25}$ +30→+5° (*c* 1.0, methanol).

Anal. Calc. for $C_{17}H_{20}N_4O_2$: C, 65.36; H, 6.45; N, 17.94. Found: C, 65.13; H, 6.59; N, 17.77.

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