Note

The isolation of 3-deoxy-D-*erythro*-hexos-2-ulose from beef livers*

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(Received November 2nd, 1971; accepted in revised form, December 30th, 1971)

An α-ketoaldehyde isolated as a bis(2,4-dinitophenylhydrazone) from beef livers treated with arsenic(III) oxide[1] was identified as the crystalline derivative of 3-deoxy-D-*erythro*-hexos-2-ulose[2]. The same osazone had been earlier isolated from soy beans[3].

The presence of at least eighteen dicarbonyl compounds, including methylglyoxal and 3-deoxy-D-*erythro*-hexos-2-ulose[4], in calf and rabbit livers was recently reported; they were isolated as their bis(2,4-dinitrophenylhydrazones).

The chemical synthesis of 3-deoxy-D-*erythro*-hexos-2-ulose from glycosylamines[3,5,6], *N*,*N*-bis(1-deoxy-D-fructos-1-yl)glycine[7], 3-*O*-methyl-D-glucose[8], and D-glucose 3-phosphate[10] has been reported. A convenient general method for synthesis of 3-deoxyaldos-2-uloses, through their bis(benzoylhydrazones) and *p*-substituted bis(benzoylhydrazones), was reported recently[11].

The rapid *post mortem* formation of 3-deoxy-D-*erythro*-hexos-2-ulose in mouse livers was shown to be dependent on the glycogen concentration[12] and the availability of amino acids[13].

Although 3-deoxy-D-*erythro*-hexos-2-ulose is inactive against neoplasias *in vivo* and *in vitro*[14], the compound serves as a substrate for α-ketoaldehyde dehydrogenase[15], an enzyme[16] that bypasses the glyoxalase system[17] in the metabolism of α-ketoaldehydes. The 3-deoxy-D-hexos-2-ulose is not accepted by the glyoxalases[15,18]. Evidence indicates that these enzymic activities may play a regulatory role in cell division and cancerous transformation[19]. The 3-deoxy-D-*erythro*-hexos-2-ulose can be used in assaying the enzymic pattern in tissue without the interference of other enzymes.

The results show that 3-deoxy-D-*erythro*-hexos-2-ulose, present in relatively high concentration in arsenic(III) oxide-treated, beef-liver extract, can be isolated successfully in pure form without an intermediary osazone step. The method involves the fractionation of the extract by charcoal–Celite column chromatography, followed by cellulose-column chromatography after the removal of ionic species with Biodemin-rolite resin.

*This research was supported by a grant from The Christine and Alfred Sonntag Foundation for Cancer Research, Elgin, Illinois.

The isolation and identification of the other three carbonyl compounds present in the eluate from the charcoal is under investigation.

EXPERIMENTAL

General. — I.r. spectra were recorded with a Perkin–Elmer Model 257 i.r. spectrophotometer. Optical rotations were measured in 1-dm tubes with a Perkin–Elmer Model 141 polarimeter. The melting point was determined with a Thomas–Kofler micro hot-stage and is uncorrected. Elementary analyses were performed with a Perkin–Elmer Model 240 analyzer. Concentrations were effected *in vacuo*.

Liver extraction. — Beef liver (2 kg) was extracted with aqueous methanol and treated with lead tetraacetate and arsenic(III) oxide as described before[1]. The arsenic-treated, alcoholic solution was concentrated to a thick syrup. The residue was suspended in water (60 ml), and the insoluble precipitate was filtered off and washed with a small volume of ice–water. The pH of the combined filtrates was adjusted to 6.0 with sodium hydrogen carbonate and an equal volume of methanol was added. The precipitate formed was removed by filtration, washed with 50% aqueous methanol, and the filtrate was concentrated to a thick syrup. The resultant syrup (20 g) was used for column chromatography*.

Fractionation of the extract on a charcoal–Celite column. — The liver extract, dissolved in 10 mM formic acid in water, was poured into a charcoal–Celite column prepared by suspending equal amounts of neutral Norit A charcoal and Celite (Johns–Mansville) in 10 mM formic acid in water[20]. About 25 g of the charcoal–Celite mixture was used for each gram of syrupy liver-extract.

The column was eluted first with 10 mM formic acid until aliquots (30 ml) of eluted fractions gave no reaction with phenol–sulfuric acid[21] and showed no precipitation of arsenic or lead with H_2S. The assay for carbonyl derivatives with 2,4-dinitrophenylhydrazine[22] at room temperature remained negative, indicating that the 3-deoxy-D-*erythro*-hexos-2-ulose was not eluted with this solvent. (The combined and concentrated fractions revealed by t.l.c. the presence of glucose and amino acids after the eluted arsenic and lead had been removed with H_2S. These fractions were discarded).

The column then was eluted with an exponential methanolic gradient containing 10 mM formic acid, the rate of which was calculated according to Alm *et al.*[23]. Aliquots eluted with the methanolic gradient were tested as already described.

*The arsenic(III) oxide could be removed before column chromatography as follows: The thick syrup, dissolved in water, was treated with H_2S gas at room temperature for 3–4 min. (Immediately a voluminous yellow precipitate appeared.) The excess H_2S was removed by brief evaporation. The residue was diluted with two volumes of methanol and 0.5–1.0 g of neutral Norit-A charcoal was added. The mixture was stirred at room temperature for a few min. The solids were removed by filtration and washed with 60–70% *v/v* aqueous methanol until the filtrate became negative to the phenol–sulfuric acid test. The filtrate was concentrated to a thick syrup, und used for column chromatography. This pretreatment considerably shortened the column elution-time with water as no H_2S precipitable materials were found in the fractions eluted. (See Fig. 1, where pretreatment was not used.)

When the methanol concentration of the effluent became about 50%, the phenol–sulfuric acid and the 2,4-dinitrophenylhydrazine tests became positive, indicating the presence of carbonyl derivatives in the eluate. The samples also gave a slight precipitate with H_2S, indicating that some of the arsenic was held back by the carbonyl derivatives on the charcoal. Elution with the solvent was continued until the tests became negative, and the combined fractions were kept under a layer of toluene. A typical elution pattern of the liver extract is shown in Fig. 1.

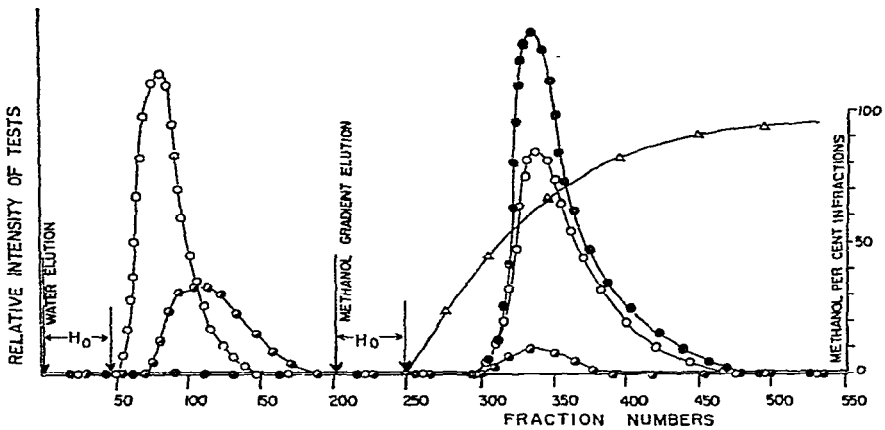

Fig. 1. Typical elution pattern from a charcoal–Celite column (column size, 100-cm height, 5-cm diameter; bed volume, 1962 ml; hold up volume, 1373 ml; volume of fractions collected, 30 ml; Amount of liver used, 2000 g; ○—○ phenol–sulfuric acid test; ◑—◑ H_2S *precipitation test*; ●—● 2,4-dinitrophenylhydrazine test; △—△ calculated methanol concentration (%) at column effluent).

Purification of the eluate. — The pooled methanolic eluate containing the dicarbonyl derivative and a trace of arsenic(III) oxide was concentrated to about 200 ml. A precipitate (colloidal Celite) was removed by Millipore (0.45 μm) filtration.

T.l.c. of the concentrate showed the presence of 3-deoxy-D-*erythro*-hexos-2-ulose, together with other carbonyl compounds and an amino acid (presumably leucine), after the arsenic(III) oxide had been removed by the method as described in the footnote.

The amino acid was removed, on slow stirring, from an aqueous solution (150 ml) of the H_2S-treated syrupy residue, by use of a mixed-bed Biodeminrolit resin containing indicator (The Permutit Co., London, England) in the H^+ and CO_3^{2-} phase respectively. The filtered resin was washed with water until no carbohydrate was detectable in the filtrate by the phenol–sulfuric acid test. The deionized, colorless filtrate was concentrated to a thick syrup; yield 5.0 g (wet wt.). T.l.c. showed the presence of 3-deoxy-D-*erythro*-hexos-2-ulose, together with three more carbonyl compounds, but amino acids and arsenic(III) oxide were absent.

*Isolation and identification of 3-deoxy-*D-erythro-*hexos-2-ulose.* — The syrupy product, dissolved in a solvent mixture (ethanol–ethyl acetate–cyclohexane–water,

50:30:10:10 *v/v*) was applied to a cellulose column (Cellex N-1, Bio-Rad; 100 × 5 cm) and eluted with the same solvent. The fractions emerging shortly after the solvent front were combined and evaporated to a syrup. The syrupy 3-deoxy-D-*erythro*-hexos-2-ulose gave an extremely hygroscopic froth on drying at 20° over P_2O_5; yield 1.96 g, $[\alpha]_D^{25}$ $-2.5 \rightarrow +1.5°$ (*c* 6, water).

Anal. Calc. for $C_6H_{10}O_5$: C, 44.45; H, 6.22; O, 49.34. Found: C, 44.42; H, 6.18; O, 49.40.

The froth, unless kept desiccated, formed a hard, glassy syrup within a few days. The product was indistinguishable, by t.l.c. (silica gel) and by i.r. spectrum, from an authentic sample.

The red, crystalline osazone prepared by treating the syrupy 3-deoxy-D-*erythro*-hexos-2-ulose with 2,4-dinitrophenylhydrazine at room temperature gave an i.r. spectrum closely resembling that of an authentic sample of the osazone[2]; m.p. 264–266° $[\alpha]_D^{25}$ +85.9° (*c* 0.1, methyl sulfoxide).

Anal. Calc. for $C_{18}H_{20}N_8O_{12}$: C, 40.00; H, 3.73; N, 20.73; O, 35.52. Found: C, 40.18; H, 3.53; N, 20.60; O, 35.70.

ACKNOWLEDGMENT

My thanks are due to Dr. Gabor Fodor for providing an authentic sample of chemically synthesized 3-deoxy-D-*erythro*-hexos-2-ulose.

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