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Note

Preparation of D-apiose phenylosotriazole*

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During studies on the cell-free biosynthesis of D-apiose [3-*C*-(hydroxymethyl)-*aldehydo*-D-*glycero*-tetrose][1], it was necessary to prove the identity of radioactive material having the same R_F as D-apiose. One criterion used was to mix the radioactive material with D-apiose, prepare a crystalline derivative, and determine if the specific activity of the derivative remained constant over several recrystallizations. Several crystalline derivatives of D-apiose have been described[2-4], but because most are colored, their usefulness in scintillation counting is limited. The colorless diisopropylidene acetal[4] has the disadvantage of being volatile[5]. D-Apiose phenylosotriazole [3-*C*-(hydroxymethyl)-*aldehydo*-D-*glycero*-tetrose phenylosotriazole], a new derivative described herein, can be prepared from D-apiose in an overall yield of at least 65% and is ideally suited for this type of experiment since it is easily prepared, is colorless, has desirable crystallization properties, and is readily soluble in solvents normally used in scintillation counting.

EXPERIMENTAL

Analytical. — Melting points were determined with a Kofler micro hot-stage (A. H. Thomas Company, Philadelphia, Pa., U. S. A.) and are uncorrected.

D-*Apiose phenylosazone* [*3-*C*-(hydroxymethyl)*-aldehydo-D-glycero-*tetrose phenylosazone*] (1). — A solution of D-apiose[2,6,7] (0.2575 g, 1.715 mmole) in 20 ml of 5M acetate buffer (pH 4.8) was heated to 80° and phenylhydrazine hydrochloride (2.48 g, 17.15 mmole) in 14 ml of the same buffer at 80° was added all at once. After heating this solution for 1 h at 90–95°, 34 ml of distilled water was added slowly with swirling. The osazone separated initially as a mixture of yellow solid and oil. The oil solidified on cooling. After 2 h at 4°, the precipitate was collected by suction filtration, washed with 3 ml of a 1:1 dilution of the above buffer with water and then with 3 ml of water, both at 4°. The well-drained precipitate was immediately dissolved completely in 6 ml of hot abs. ethanol. Distilled water was added to the hot ethanol solution slowly with stirring, until the concentration of ethanol was 15% by volume. After 3–4 h at 4°, the amorphous precipitate was filtered off, washed with

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1 ml of cold 10% aqueous ethanol, and dried to constant weight *in vacuo* over phosphorus pentaoxide to give 0.4844 g (86%), m.p. 153–157°; lit.[2] for crystalline **1**, m.p. 156–157°.

D-*Apiose phenylosotriazole* (**2**). — A suspension of powdered **1** (0.4844 g, 1.475 mmole) in 37 ml of water was heated to boiling and a solution of cupric sulfate pentahydrate (0.4052 g, 1.623 mmole) in 25 ml of water at 80–90° was added all at once. The suspension was refluxed for 1 h. After cooling and filtering, the solution was extracted continuously with diethyl ether for 10 h. The ether solution was evaporated to about 45 ml, heated with 0.1–0.2 g of Darco G-60 decolorizing carbon for 5 min, and filtered. After further evaporation of the ether solution to about 15 ml, compound **2** crystallized readily, as cream-colored plates, by adding boiling petroleum ether (b.p. 30–60°) to incipient cloudiness and then adding several seed crystals. Seed crystals were obtained by evaporating a small portion of the diethyl ether solution to dryness and scratching the syrup. After 2 h at 4° the crystals were filtered off; yield 0.2664 g (77%), m.p. 94–96°. Compound **2** was redissolved in diethyl ether, treated again with Darco G-60, and crystallized as described above, to give white crystals, m.p. 95–96°. Compound **2** could also be crystallized from diethyl ether alone.

Anal. Calc. for $C_{11}H_{13}N_3O_3$: C, 56.16; H, 5.57; N, 17.86. Found: C, 56.28; H, 5.33; N, 17.96.

Oxidation of **2** *with sodium metaperiodate.* — Periodate oxidation of **2** gave the known 2-phenyl-1,2,3-triazole-4-carboxylic acid[8] and formaldehyde. Compound **2** (0.2352 g, 1.0 mmole) was dissolved in 14 ml of water with agitation. To this solution was added 10 ml of 0.3M sodium metaperiodate (3.0 mmole). The suspension obtained was kept for 1 h at 25° and 2 h at 4°, and then the precipitate was collected on a weighed sintered glass disk by suction filtration and washed with two 5-ml portions of ice-cold water. The filtrate and washings were adjusted to 100 ml and immediately analyzed as described below. After drying to constant weight, the precipitate weighed 0.1669 g (0.88 mole per mole of **2**) and melted at 193.5–194.5°. Recrystallization from 10:1 hot water–abs. ethanol did not alter the m.p.; lit.[8] for 2-phenyl-1,2,3-triazole-4-carboxylic acid, m.p. 191–192°.

Anal. Calc. for $C_9H_7N_3O_2$: C, 57.14; H, 3.73; N, 22.21. Found: C, 57.03; H, 3.67; N, 22.28.

An 0.5-ml aliquot of the filtrate was appropriately diluted and analyzed for formaldehyde by using chromotropic acid[9]. The analysis showed that 1.98 moles of formaldehyde was produced per mole of **2**. This value did not increase over 24 h. A second aliquot (20 ml) was acidified with sulfuric acid and then treated with sodium arsenite. The colorless solution was adjusted to pH 6–7 with sodium hydroxide and then 0.561 g of 5,5-dimethyl-1,3-cyclohexanedione in 80 ml of warm water was added. After 10 min at 90°, 6 ml of glacial acetic acid was added and the solution was kept for 12 h at 4°. The amount of crystalline formal dimethone (m.p. 189–190°) obtained, corresponded to the formation of 1.94 moles of formaldehyde per mole of **2**. Analysis of a third aliquot (3 ml) for unreacted periodate by the iodine–arsenite procedure[10] showed that 1.98 moles of periodate had been consumed per mole of **2** oxidized.

These data are consistent with the structure of **2**.

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