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PRIMEVERULOSE (6-*O*-β-D-XYLOPYRANOSYL-D-FRUCTOSE) AND SOME DERIVATIVES THEREOF

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ABSTRACT

Primeverulose (6-*O*-β-D-xylopyranosyl-D-fructose) has been prepared by the partial rearrangement of primeverose (6-*O*-β-D-xylopyranosyl-D-glucose) with aqueous ammonia followed by separation of the two disaccharides on a cellulose column. The syrupy primeverulose had $[\alpha]_D^{20}$ −27.5° in methanol, and was characterized by its crystalline (2,5-dichlorophenyl)hydrazone. Some derivatives that are common to both primeverose and primeverulose, namely, the phenylosotriazole, phenylosotriazole hexaacetate, and (2,5-dichlorophenyl)osazone, are also described.

INTRODUCTION

Primeverose (6-*O*-β-D-xylopyranosyl-D-glucose) was discovered[1] as a constituent of the phenolic glycosides primeverin and primulaverin in 1912, and later in a number of other glycosides from plant sources. Wallenfels and Lehmann[2] isolated primeverose itself from an aqueous extract of ripe carob beans (St. John's bread; *Ceratonia siliqua* L.), and Begbie and Richtmyer[3] isolated primeverose from an aqueous extract of the dried roots of *Primula officinalis* Jacq. In these last two cases, the possibility of an enzymic hydrolysis of a primeveroside to yield primeverose cannot be excluded.

Inasmuch as D-glucose and D-fructose often occur together in plants, it seemed possible that their 6-*O*-β-D-xylopyranosyl derivatives might also occur together in some plants. To aid in the search for primeverulose (6-*O*-β-D-xylopyranosyl-D-fructose) in plant extracts, we rearranged primeverose to that ketose by mild treatment with aqueous ammonia. The mixture of primeverose and primeverulose was readily separated on a cellulose column, and the primeverulose was obtained in a yield of 12.5% as a chromatographically homogeneous, colorless syrup having $[\alpha]_D^{20}$ −27.5° (in methanol). The sugar was identified as a ketose by its characteristic color-reaction on a paper chromatogram sprayed with the orcinol–hydrochloric acid reagent, and the phenylosazone prepared from it was identical with the phenylosazone prepared

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from primeverose. It was further characterized by its crystalline (2,5-dichlorophenyl)-hydrazone. Also prepared were the phenylosotriazole, the phenylosotriazole hexa-acetate, and the (2,5-dichlorophenyl)osazone, common to both primeverose and primeverulose.

EXPERIMENTAL

Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Melting points were observed in capillary tubes. Paper chromatography was conducted with Whatman No. 1 filter paper by the descending method at room temperature, with 6:4:3 butyl alcohol–pyridine–water.

Primeverulose (6-O-β-D-xylopyranosyl-D-fructose) from primeverose (6-O-β-D-xylopyranosyl-D-glucose). — Primeverose β-heptaacetate was synthesized directly from 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucose (35.44 g, 60 mmoles) and tri-*O*-acetyl-α-D-xylopyranosyl bromide (20.35 g, 60 mmoles) with silver perchlorate (12.50 g, 60 mmoles) and Drierite (7.5 g) in nitromethane (100 ml), according to the general procedure of Bredereck *et al.*[4]. The yield of product, crystallized from ethyl alcohol, was 19.12 g (48%). The melting point was low (200–204°), but was raised by one recrystallization from chloroform–methanol[5] to 213–215°, alone and also when mixed with authentic primeverose heptaacetate*.

Prior to the rearrangement to primeverulose, 20 g of the recrystallized β-hepta-acetate was deacetylated with methanolic sodium methoxide for 3 days at 5°. The resulting solution was made neutral with a little carbon dioxide, and evaporated *in vacuo* to remove methanol and methyl acetate. The syrup, containing about 10.3 g of primeverose, was dissolved in distilled water (200 ml), and the solution was diluted with concentrated aqueous ammonia (50 ml), kept for 20 h at 37°, and evaporated *in vacuo* to a syrup. A solution of the syrup in water was passed down a column of Amberlite MB-3 ion-exchange resin, and the eluate and washings were evaporated to a thick syrup that weighed 9 g. Paper chromatography showed several spots besides those for unchanged primeverose and for the expected primeverulose.

The 9 g of syrup thus obtained was dissolved in a small volume of methanol, and 5.54 g of primeverose was recovered by crystallization. The mother liquor was evaporated to a syrup (2.95 g) that was put on top of a cellulose column (92 × 4 cm) and eluted with aqueous butyl alcohol of successively increased concentration, 15-ml portions of eluate being collected. Butyl alcohol that was one-quarter saturated with water removed substances that had the same mobilities on paper chromatograms as D-xylose (fractions 251–290) and D-glucose (fractions 411–510); half-saturated butyl alcohol eluted the primeverulose (fractions 751–1010); and saturated butyl alcohol eluted the primeverose (fractions 1491–1690).

The primeverulose fractions were combined, and evaporated to give 1.29 g of a colorless syrup (12.5% from primeverose β-heptaacetate), $[\alpha]_D^{20}$ −27.5° (*c* 2.6,

*Kindly supplied by Dr. George H. Coleman (see ref. 5).

methanol). The material gave a single spot on paper chromatograms sprayed with ammoniacal silver nitrate or with orcinol–hydrochloric acid; with the latter spray (2% of orcinol and 3% of concentrated hydrochloric acid in butyl alcohol; heated 3 min at 100–110°), the color developed was orange changing to green, the same as that given by D-fructose alone. The mobility of primeverulose under these conditions was 0.79 relative to D-fructose, 0.91 relative to D-glucose, and 1.52 relative to primeverose; the mobility of primeverose was 0.59 relative to D-glucose.

Gas–liquid chromatography (performed with an F & M Model 5750 Research Gas Chromatograph equipped with a flame-ionization detector; copper column (200 × 0.5 cm) packed with 15% by weight of 2,2-dimethyl-1,3-propanediol (neopentyl glycol) succinate polyester on Gas-Chrom A, 60–80 mesh; column temperature, 200°; flow rate, 120 ml of helium/min) showed only one peak for the per(trimethylsilyl) (TMS) ether of primeverulose. The retention times of the TMS ethers of primeverulose and primeverose, relative to that of α-D-glucose, were 15.2 and 27.9, respectively. The retention times relative to sucrose were 1.59 and 2.94, respectively.

6-O-β-D-Xylopyranosyl-D-arabino-hexulose phenylosazone from primeverulose. — A mixture of 59 mg (189 μmoles) of primeverulose, 1 ml of water, 65 μl (660 μmoles) of phenylhydrazine, and 38 μl (664 μmoles) of glacial acetic acid was heated for 2 h on a steam bath; yellow needles began to separate within 15 min. After being kept overnight at room temperature, the mixture was filtered, and the solid product was washed successively with several portions each of 10% aqueous acetic acid, water, cold ethyl alcohol, and ethyl ether. The phenylosazone weighed 57 mg (62%), and its i.r. spectrum (KBr disk) was identical with that of the phenylosazone prepared similarly from primeverose. The "instantaneous melting point" (the lowest temperature at which the compound, in a capillary tube, decomposed within 5 sec when the tube was plunged into a bath at a preset temperature) was estimated to be about 228°; Goris *et al.*[1] reported the "instantaneous m.p." as 224–226° (Maquenne block). The specific rotation of the phenylosazone prepared from primeverulose was $[\alpha]_D^{20}$ −98.9 (15 min)→ −53.6° (1 week, constant; *c* 1, pyridine); the rotation of the phenylosazone prepared from primeverose was $[\alpha]_D^{20}$ −101.3 (6 min)→ −54.2° (1 week, constant; *c* 1, pyridine). Helferich and Rauch[6] reported that the phenylosazone from primeverose had $[\alpha]_D^{20}$ −109.7° (pyridine).

6-O-β-D-Xylopyranosyl-D-arabino-hexulose phenylosotriazole. — A suspension of 0.93 g (1.9 mmoles) of the phenylosazone prepared from primeverose in a solution of 0.52 g (2.1 mmoles) of copper(II) sulfate pentahydrate in 100 ml of water was boiled for 30 min. The light-brown and bright-red precipitates were filtered off, and the yellowish solution was stirred with 2 g of barium carbonate for 1 h. The mixture was filtered and the solution was then deionized with Amberlite IR-120 and Duolite A-4 ion-exchange resins, and decolorized with a small amount of Darco X carbon. The suspension was filtered, and the filtrate was evaporated *in vacuo* to a foamy syrup (0.57 g, 76%). Crystallization was induced by rubbing the syrup with ethyl acetate. Two recrystallizations from ethyl alcohol yielded 0.34 g

of the phenylosotriazole as clusters of fine needles, m.p. 165–166°, $[\alpha]_D^{20}$ −49.0° (*c* 1, water).

Anal. Calc. for $C_{17}H_{23}N_3O_8$: C, 51.38; H, 5.83; N, 10.57. Found: C, 51.23; H, 5.79; N, 10.74.

6-O-β-D-Xylopyranosyl-D-arabino-*hexulose phenylosotriazole hexaacetate.* — Acetylation of 0.1 g of the phenylosotriazole with 3.5 ml each of acetic anhydride and pyridine for 3 days at room temperature yielded the hexaacetate as a syrup in quantitative yield. Crystals were obtained after several weeks, and these were recrystallized four times from aqueous ethyl alcohol. The final product (fine needles, 86 mg, 53%) had m.p. 91–92° and $[\alpha]_D^{20}$ −50.4° (*c* 1.2, chloroform).

Anal. Calc. for $C_{29}H_{35}N_3O_{14}$: C, 53.62; H, 5.43; N, 6.47. Found: C, 53.74; H, 5.42; N, 6.37.

6-O-β-D-Xylopyranosyl-D-arabino-*hexulose (2,5-dichlorophenyl)hydrazone.* — A solution of 0.25 g (800 μmoles) of primeverulose and 0.35 g (2.0 mmoles) of (2,5-dichlorophenyl)hydrazine in 5 ml of methanol was evaporated on a steam bath until crystallization occurred (30 min). Methanol was added, the solution was evaporated, and the process was repeated (total time, 1.5 h). The dry, crystalline residue was broken up with a spatula, extracted several times with ethyl ether by decantation, and then filtered off and washed further with ethyl ether. The air-dried product (yellow needles) weighed 0.37 g (98%) and melted at ~180° (dec.). The elemental analysis was obtained first on this material.

Anal. Calc. for $C_{17}H_{24}Cl_2N_2O_9$: C, 43.32; H, 5.13; N, 5.94; Cl, 15.05. Found: C, 43.50; H, 5.00; N, 5.88; Cl, 14.83.

It was later found that the product could be recrystallized by dissolving it in warm methanol and adding five volumes of petroleum ether. The m.p. was then ~195° (dec.).

Anal. Calc. for $C_{17}H_{24}Cl_2N_2O_9$: C, 43.32; H, 5.13; Cl, 15.05; N, 5.94. Found: C, 43.47; H, 5.13; Cl, 14.80; N, 6.09.

As the i.r. spectra (Nujol mulls) of the unrecrystallized and the recrystallized samples were identical, and yet the m.p. of each sample remained unchanged after several months, it seems probable that the unrecrystallized sample contained a trace of impurity that accelerated its decomposition. The specific rotation of the recrystallized sample was $[\alpha]_D^{20}$ −34.3 (6 min)→−16.3° (4 weeks, constant; *c* 1, pyridine).

6-O-β-D-Xylopyranosyl-D-arabino-*hexulose (2,5-dichlorophenyl)osazone.* — A mixture of 0.15 g (481 μmoles) of primeverose, 0.34 g (1.92 mmoles) of (2,5-dichlorophenyl)hydrazine, 0.11 ml (1.92 mmoles) of glacial acetic acid, and 10 ml of water was heated for 10 h under a reflux condenser on a steam bath. The mixture was kept overnight at 5°, and then filtered; the solid product was washed several times each with 10% acetic acid, water, cold ethyl alcohol (which removed some dark material), and ethyl ether. The yellow needles of the (2,5-dichlorophenyl)osazone weighed 0.16 g (53%) and melted at 222° (dec.). The rotation was $[\alpha]_D^{20}$ −49.9 (7 min)→−22.4° (2 weeks, constant; *c* 1, pyridine).

Anal. Calc. for $C_{23}H_{26}Cl_4N_4O_8$: C, 43.97; H, 4.17; Cl, 22.57; N, 8.92. Found: C, 43.99; H, 4.29; Cl, 22.20; N, 9.02.

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