

SYNTHESIS AND CHARACTERIZATION OF D-XYLOFURANOSE-5-PHOSPHATE¹

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ABSTRACT

Phosphorylation of 1,2-O-isopropylidene-D-xylose with diphenylphosphorochloridate yielded crystalline 1,2-O-isopropylidene-D-xylofuranose-5-diphenylphosphate. Subsequent hydrogenolysis in glacial acetic acid over Adams' catalyst quantitativelyremoved phenylgroups asshown by infrared analysis. Mild hydrolysis in acetic acid for two hours at 80°C. removed the isopropylidene grouping, and D-xylose-5-phosphate was isolated as an amorphous barium salt. A yield of 81% of theoretical was obtained from 1,2-O-isopropylidene-D-xylose, or an over-all yield of 72% from xylose. The product was characterized through its amorphous barium, disodium, and dipotassium salts, and its crystalline dibrucine and distrychnine salts.

One of the earliest observations on phosphoryl group migration in sugar phosphates was that of Levene and Raymond (10). In attempts to prepare xylose-3-phosphoric acid (8, 9, 10, 11) as a basis for testing Robinson's suggestion that xylose was the pentose component of nucleic acid (13), 5-acetyl-, 5-benzoyl-, and 5-benzyloxycarbonyl-1,2-O-isopropylidene-D-xylose were separately phosphorylated with phosphorus oxychloride (10, 11). After removal of the acyl and isopropylidene groups by hydrolysis, the only product obtained was D-xylose-5-phosphate. Migration of the phosphoryl group from carbon-3 to carbon-5 at some stage of the synthesis was explained by postulating the formation of a cyclic intermediate (11). The possible significance of this exchange reaction in biological systems, as well as fundamental interest in the mechanism, have indicated the need for more complete investigation. In the present communication a revised synthesis for the preparation of D-xylose-5-phosphate is reported.

The only previously reported synthesis of D-xylose-5-phosphate was that of Levene and Raymond (10, 11). Phosphorylation of 1,2-O-isopropylidene-Dxylose (20 gm.) with phosphorus oxychloride in dry pyridine at -30° C., followed by drastic hydrolysis in 2 N sulphuric acid at 80°C. for two hours yielded 5.5 gm. of barium D-xylose-5-phosphate. This represented 14.3% of the theoretical yield from 1,2-O-isopropylidene-D-xylose, or approximately 12% from xylose. In the present study substitution of diphenylphosphorochloridate as the phosphorylating agent in dry 2,6-lutidine followed by hydrogenolysis and mild hydrolysis in acetic acid has increased the yield from 1,2-O-isopropylidene-D-xylose to 81%. The over-all yield of barium-Dxylose-5-phosphate from xylose in a typical synthesis was 72%.

The phosphorylation product, 1,2-O-isopropylidene-D-xylose-5-diphenylphosphate, may be obtained pure by recrystallization from carbon tetrachloride. Its elementary analysis conforms to the theoretical, and it has an extremely sharp melting point (within 0.2°). Infrared analysis of finely ground crystals

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in a nujol mull shows characteristic phenyl absorption bands and a strong hydroxyl peak. The sharp melting point suggests the absence of isomeric phosphates and the greater reactivity of the primary hydroxyl indicates preferential phosphorylation in the 5-position. The assumption that phenyl groups would prevent double phosphorylation through hindrance is borne out by the elementary and infrared analyses.

If the phosphorylated product is homogeneous, acid conditions during hydrogenolysis and hydrolysis would favor retention of the phosphoryl group in position 5. The final product in the form of its barium salt has no inorganic or acid-labile phosphate, and it consumes 3.0 moles of oxidant when reacted with sodium periodate at pH 4.7. Barium, phosphorus, and pentose are close to theoretical for a monophosphorylated compound. However, when subjected to periodate oxidation either in 0.05 N sulphuric acid or at pH 4.7, samples prepared by different methods release from 3 to 7% of formaldehyde. The amount of formaldehyde released may be correlated with the pH during acetic acid hydrolysis. No exact estimate of the purity of the product may be made at present, although the samples used for characterization probably contain 97% p-xylose-5-phosphate.

Crystalline derivatives of D-xylose-5-phosphate have not previously been reported. Levene and Raymond (10) performed an elementary analysis on the barium salt and determined specific rotations for the barium and disodium salts. In the present study attempts to prepare crystalline metal salts were unsuccessful. Dilithium, disodium, dipotassium, as well as the barium salts precipitated from water and ethanol in amorphous form. In order to place identification on a crystalline basis the dibrucine and distrychnine salts were prepared and their constants determined.

EXPERIMENTAL

Preparation of 1,2-O-Isopropylidene-D-xylose and Diphenylphosphorochloridate

Pure 1,2;3,5-di-O-isopropylidene-D-xylose (115 gm.) was prepared from pure xylose in 87% yield (6). Hydrolysis of this product gave 1,2-O-isopropylidene-D-xylose in 99% yield (12). By molecular distillation this compound was obtained as colorless crystals with $[\alpha]_D^{26} = -19.3$ (c = 5.70 in water) (14). Diphenylphosphorochloridate in essentially pure form (212 gm.) was prepared from 1.1 moles of freshly distilled phosphorus oxychloride and 2 moles of redistilled phenol (79% yield) (3, 4).

Phosphorylation of 1,2-O-Isopropylidene-D-xylose

Crystalline 1,2-O-isopropylidene-D-xylose (5.25 gm.) was mixed with reagent benzene and the benzene distilled to remove traces of water. After removal of the benzene freshly distilled anhydrous 2,6-lutidine (60 gm.) (2) was added, the mixture shaken until solution was complete, and cooled to -20° C. with dry ice. Dry diphenylphosphorochloridate (15 gm.) was added dropwise over a period of 10 min. from a separatory funnel fitted with a drying tube. After standing for two days at 5°C. the reaction mixture was filtered, and the crystalline 2,6-lutidine-hydrochloride washed several times with cold 2,6-lutidine. After evaporation of the filtrate to a thick sirup water was added

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and the distillation to dryness repeated to remove 2,6-lutidine. The resulting sirupy residue was dissolved in 100 ml. of chloroform, washed nine times with 100-ml. portions of water, and the washings discarded. The chloroform solution was evaporated to a sirup *in vacuo* at 35°C., the last traces of chloroform being removed by azeotropic distillation after addition of ethanol. Colorless needles were obtained by crystallization from ether. (Yield 10.8 gm., 93% of theoretical.) After it was recrystallized from carbon tetrachloride, washed with petroleum ether, and dried *in vacuo* at 50°C., the pure 1,2-O-isopropylidene-D-xylose-5-diphenylphosphate had m.p. 102.2°-102.4°: $[\alpha]_D^{20} = +10.8$ (c = 2.21 in chloroform). (Found: C, 56.10; H, 5.38. Calc. for $C_{20}H_{23}O_8P$: C, 56.84; H, 5.49.) The compound was soluble in chloroform and acetone, slightly soluble in ether, ethanol, and carbon tetrachloride, and insoluble in water and petroleum ether.

Hydrogenolysis and Hydrolysis of 1,2-O-Isopropylidene-D-xylose-5-diphenylphosphate

Low pressure hydrogenation of the crystalline phosphorylation product (2.0 gm.) over 0.20 gm. of Adams' catalyst (1) in 60 ml. of glacial acetic acid was complete in 115 min. The solution was filtered and the filtrate, after adjustment to pH 1.5 ± 0.2 with water, was hydrolyzed at $80 \pm 2^{\circ}$ C. for two hours. Acetic acid was removed by vacuum distillation, the sirup dissolved in 90 ml. of water, and 0.3 N barium hydroxide added until the pH reached 8.5. Removal of inorganic phosphate was effected by centrifuging the slightly turbid aqueous solution before the addition of four volumes of ethanol. After standing at 5°C. for two hours the colorless barium salts were separated by centrifugation and washed with 75% ethanol and ether before drying *in vacuo* over anhydrone. (Yield, 1.33 gm.) Concentration of the supernatant liquid to 20 ml. on a Craig evaporator at 30°C., readjustment of the pH to 8.5 with saturated aqueous barium hydroxide, and reprecipitation with four volumes of ethanol recovered 0.245 gm. for a total yield of 1.575 gm. (90% of theoretical).

Characterization of D-Xylose-5-phosphate

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Data obtained by analysis of the barium salt isolated from the reaction mixture agreed with theoretical values calculated for a monophosphorylated pentose. (Found: Ba, 37.51; P, 8.54; pentose 40.2. Calc. for $C_5H_9O_8PBa$: Ba, 37.59; P, 8.58; pentose 41.0.) Pentose was estimated by the modified method of Cohn and Volkin (5). A series of analyses at different time intervals showed maximum color development with D-xylose-5-phosphate in 15 min. so that the heating period was reduced from 20 min. to 15 min.

Free xylose-5-phosphoric acid had $[\alpha]_D^{20} = +25.0$ (c = 2.00 in water) and the specific rotation remained unchanged for two days. The disodium salt, prepared by addition of a stoichiometric quantity of dilute sodium carbonate solution to the barium salt, had an initial $[\alpha]_D^{20}$ of +10.0 (c = 2.00 in water, pH 7.2) which changed with time in a levorotatory direction to $+7.0^{\circ}$, $+4.4^{\circ}$, $+1.9^{\circ}$, 0.0° , and -1.0° after one two, three, four, and five days respectively. The dipotassium salt had $[\alpha]_D^{20} = +13.4$ (c = 2.09 in water, pH 8.9). In

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half-saturated sodium tetraborate solution the specific rotation of the disodium salt increased gradually in a dextrorotatory direction. After two, three, four, and five days the initial rotation of $[\alpha]_{D}^{20} = +5.5$ (c = 2.00 in water) had changed to $+6.8^{\circ}$, $+8.5^{\circ}$, $+10.1^{\circ}$, and $+10.6^{\circ}$ respectively. Several attempts were made to prepare crystalline metal salts of xylose-5phosphoric acid. Under the conditions explored, the dilithium, disodium, dipotassium, and barium salts were always obtained in amorphous form from either water or ethanol.

Acid-labile phosphate, measurable by King's method (7), was not released by hydrolysis of the barium salt for seven minutes in N hydrochloric acid at 100°C. Oxidation with 0.1 M sodium periodate at pH 4.7 consumed 3.0 moles of oxidant in 58 hr. However oxidation of various samples of the barium salt either with 0.1 M periodic acid in 0.05 N sulphuric acid or with an equivalent concentration of sodium periodate at pH 4.7 released from 3 to 7% formaldehyde, measured colorimetrically using chromotropic acid.

To identify D-xylose-5-phosphate on a crystalline basis, dibrucine and distrychnine salts were prepared and their constants determined. The former was prepared by adding a stoichiometric amount of brucine sulphate heptahydrate in 15 ml. of water to 100 mgm. of barium xylose-5-phosphate dissolved in water. Barium sulphate was removed by filtration and the filtrate evaporated to dryness in vacuo at 35°C. The crude dibrucine salt weighed 262.8 mgm. (90% yield). When recrystallized from ethanol by rapid evaporation, it formed long colorless needles of a hydrate from which water of crystallization was completely removed by heating for two hours in vacuo at 50°C. The degree of hydration of the dibrucine salt varied with the conditions during its formation. On standing in room air overnight the anhydrous salt took up water exactly equivalent to an octahydrate. When equilibrated over a water - sulphuric acid solution (sp. gr. 1.420, relative humidity = 35) in a closed vessel, it combined with 6.5 moles of water. Recrystallization from absolute ethanol gave a hydrate with 3.5 H₂O. Analyses were therefore performed on the anhydrous salt. (Found: P, 3.06; pentose 14.02. Calc. for C₅₁H₆₃N₄O₁₆P: P, 3.04; pentose 14.72.) Pentose was determined by the modified Cohn-Volkin procedure (5) after titration of the dibrucine salt in water to pH 10 with N/10sodium hydroxide. The anhydrous salt had $[\alpha]_{D}^{20} = -37.8$ (c = 2.02 in chloroform) and it decomposed without melting above 150°C.

The distrychnine salt was prepared by the same procedure followed for the dibrucine salt. Recrystallized from ethanol and ether it formed a tetrahydrate on standing in room air. (Found: P, 3.07; pentose 15.15. Calc. for $C_{47}H_{55}$ -N₄O₁₂P.4H₂O: P, 3.20; pentose 15.46.) The pure compound had $[\alpha]_D^{20} = -33.2$ (c = 1.02 in water) and like the dibrucine salt it decomposed above 150°C. without melting.

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