THE SYNTHESIS OF D-GLYCERALDEHYDE-*3,3-d_2* 3-PHOSPHATE*†

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ABSTRACT

The synthesis of D-glyceraldehyde-*3,3-d_2* 3-phosphate starting from 2-*O*-benzyl-D-arabinose was accomplished by a route that involved the reduction of 2-*O*-benzyl-D-arabinono-1,4-lactone by sodium borodeuteride. The resulting 2-*O*-benzyl-D-arabinitol-*1,1-d_2* was cleaved with sodium metaperiodate to yield 2-O-benzyl-3-*O*-formyl-D-glyceraldehyde-*3,3-d_2*, which was converted into the corresponding diethyl dithioacetal. After removal of the formyl group, the dithioacetal was converted into 2-*O*-benzyl-D-glyceraldehyde-*3,3-d_2* dimethyl acetal. Phosphorylation of the latter with diphenyl phosphorochloridate and removal of the protecting groups afforded D-glyceraldehyde-*3,3-d_2* 3-phosphate. The reaction sequence is also suitable for the synthesis of D-glyceraldehyde-*3-t* 3-phosphate. Use of D-glyceraldehyde-*3,3-d_2* 3-phosphate for the synthesis of D-fructose-*6,6-d_2* 1,6-diphosphate is also described.

INTRODUCTION

Structural studies of complex molecules by nuclear magnetic resonance (n.m.r.) spectroscopy are often aided by the synthesis of specifically deuterated derivatives. In a continuation of our study of D-fructose 1,6-diphosphate by n.m.r. spectroscopy[1], specifically deuterated derivatives were needed in order to simplify the ^1H n.m.r. spectrum of the compound and aid in making assignments for the ^{13}C and ^{31}P n.m.r. spectra. For this purpose, D-fructose 1,6-diphosphate labeled with deuterium at either of the primary carbon atoms would be useful, in that proton coupling to the phosphorus atom at the same carbon atom would be nonexistent in the ^{31}P n.m.r. spectrum, as well as proton coupling to that carbon atom in the ^{13}C n.m.r. spectrum. D-Glyceraldehyde 3-phosphate labeled at C-3 with deuterium was, therefore, syn-

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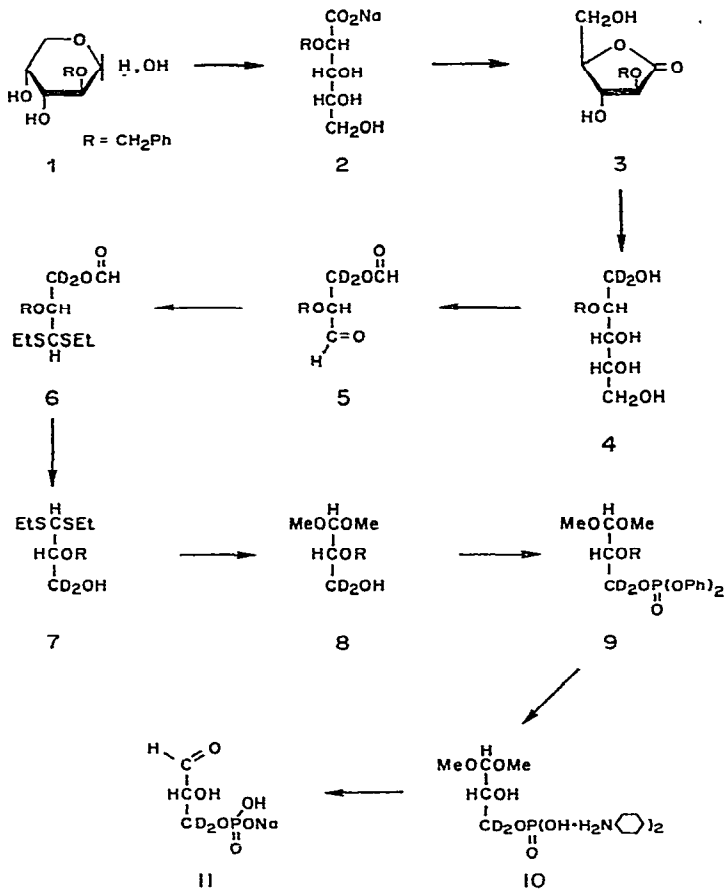
thesized, and converted enzymically into D-fructose-*6,6-d*$_2$ 1,6-disphosphate by aldolase and an equivalent amount of 1,3-dihydroxy-2-propanone 1-phosphate.

RESULTS AND DISCUSSION

The synthesis of D-glyceraldehyde-*3,3-d*$_2$ 3-phosphate was accomplished as shown in Scheme 1. 2-*O*-Benzyl-D-arabinose (**1**) was prepared as described by Wold[2], except that benzyl *β*-D-arabinopyranoside was prepared according to the procedure of McCormick[3]. Oxidation of **1** with sodium hypoiodite[4] yielded sodium 2-*O*-benzyl-



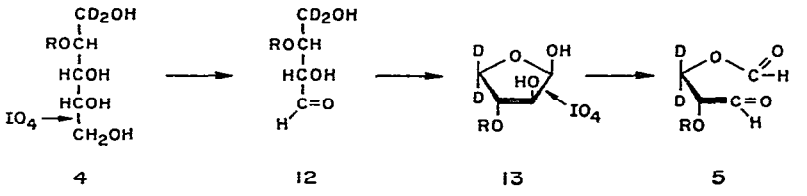
Scheme 1. Synthesis of D-glyceraldehyde-*3,3-d*$_2$ 3-phosphate.

D-arabinonate (**2**), which was converted into crystalline 2-*O*-benzyl-D-arabinono-1,4-lactone (**3**). Investigation of this lactone in methyl sulfoxide-*d*$_6$ by ^{1}H n.m.r. spectroscopy revealed the presence of a total of 14 protons. Evaporation of the sample from D_2O resulted in the loss of 2 protons, indicating the presence of two hydroxyl groups. One of the replaceable protons resonated at τ 4.97 as a triplet (*J* 5.6 Hz), indicating the

presence of a primary hydroxyl group, and the other replaceable proton resonated at τ 4.03 as a doublet (J 3.5 Hz) showing long-range coupling (J 1.5 Hz), indicating the presence of a secondary hydroxyl group[5]. These results indicated that the lactone possesses a five-, not a six-, membered ring. This conclusion was confirmed by the failure of the lactone to consume periodate.

Reduction of lactone **3** with sodium borodeuteride yielded 2-*O*-benzyl-D-arabinitol-*1,1-d*$_2$ (**4**). Periodate oxidation of **4** as described by Wold[2], gave aldehyde **5**, which was immediately converted into the diethyl dithioacetal (**6**). Examination of this compound in chloroform-*d* by ^{1}H n.m.r. spectroscopy revealed the absence of a hydroxyl proton and the presence of a formic ester hydrogen atom resonating at τ 2.17. The material was probably 2-*O*-benzyl-3-*O*-formyl-D-glyceraldehyde-*3,3-d*$_2$ diethyl dithioacetal (**6**), as treatment with sodium methoxide in methanol readily cleaved the formic ester, to produce 2-*O*-benzyl-D-glyceraldehyde-*3,3-d*$_2$ diethyl dithioacetal (**7**).

These results indicated that 2-*O*-benzyl-3-*O*-formyl-D-glyceraldehyde-*3,3-d*$_2$ (**5**), not 2-*O*-benzyl-D-glyceraldehyde-*3,3-d*$_2$ (Ref. 2), is the product of periodate oxidation of 2-*O*-benzyl-D-arabinitol-*1,1-d*$_2$ (**4**). As shown in Scheme 2, this product may arise via a favored attack by periodate at the C-4–C-5 bond of **4**, to yield 3-*O*-benzyl-D-threose-*4,4-d*$_2$ (**12**), which rapidly cyclizes to **13**. The second molecule of periodate cleaves the C-1–C-2 bond of the cyclic threose derivative **13**, to yield 2-*O*-benzyl-3-*O*-formyl-D-glyceraldehyde-*3,3-d*$_2$ (**5**).



Scheme 2. Periodate oxidation of 2-*O*-benzyl-D-arabinitol-*1,1-d*$_2$.

The conversion of 2-*O*-benzyl-D-glyceraldehyde-*3,3-d*$_2$ diethyl dithioacetal (**7**) into D-glyceraldehyde-*3,3-d*$_2$ 3-phosphate (**11**) was performed as described by Ballou and Fischer[6] for the corresponding nondeuterated analogs, except that 2-*O*-benzyl-3-*O*-(diphenylphosphono)-D-glyceraldehyde-*3,3-d*$_2$ dimethyl acetal (**9**) was purified by chromatography on a Florisil column prior to hydrogenation.

The ^{1}H n.m.r. spectrum of D-glyceraldehyde-*3,3-d*$_2$ dimethyl acetal 3-(dicyclohexylammonium phosphate) (**10**) is consistent with the structure assigned. The H-1 resonance (τ 5.48) and that of H-2 (τ 6.18) are coupled only to each other, and thus give rise to equal doublets. In contrast, H-2 of the nondeuterated analog is observed as a complex multiplet superimposed on the H-3 signals at τ 6.25. The fact that H-2 gives a doublet rules out the presence of the 2-phosphate[6], as coupling to the phosphorus atom would give rise to a quartet. Singlets are observed at τ 5.10 and 6.47 due

to the HDO line and the acetal methyl groups, respectively; peaks at τ 6.6–7.2, 7.8–9.0 are due to cyclohexylammonium.

Removal of the acetal methyl groups produced D-glyceraldehyde-*3,3-d*$_2$ 3-phosphate (**11**); this was assayed for base-labile phosphate[7], and enzymically by l-glycerol phosphate dehydrogenase with added triose phosphate isomerase[8]. Both methods of assay gave equivalent concentrations of D-glyceraldehyde 3-phosphate. Under the conditions of the hydrolysis, inorganic phosphate (17%) was produced, and a trace of the dimethyl acetal remained.

When rabbit-muscle aldolase was added to equal proportions of D-glyceraldehyde-*3,3-d*$_2$ 3-phosphate and 1,3-dihydroxy-2-propanone 1-phosphate, D-fructose-*6,6-d*$_2$ 1,6-diphosphate was produced in 93% yield. The deuterated diphosphate was indistinguishable by paper chromatography and enzymic assay[9] from authentic D-fructose 1,6-diphosphate.

The reaction sequence is suitable for the synthesis of D-glyceraldehyde-*3-t* 3-phosphate of very high specific activity; but, if a lower specific activity is desired, 2-*O*-benzyl-D-arabinose (**1**) may be reduced to 2-*O*-benzyl-D-arabinitol-*1-t* with sodium borohydride-*t*, thus bypassing the synthesis and reduction of lactone **3**. The tritium label at C-3 is not exchanged with solvent by aldolase and triose phosphate isomerase, as are the hydrogens at C-1 and C-2, respectively[10]. D-Glyceraldehyde-*3-t* 3-phosphate should, therefore, be useful in studying biochemical reactions in which D-glyceraldehyde 3-phosphate is involved, or is proposed to be involved, as in the biosynthesis[11] of vitamin B_6.

D-Glyceraldehyde-*3,3-d*$_2$ 3-phosphate should prove a useful intermediate in the synthesis of specifically deuterated ketose phosphates, as demonstrated by its conversion into D-fructose-*6,6-d*$_2$ 1,6-diphosphate. The conversion of D-glyceraldehyde-*3,3-d*$_2$ 3-phosphate into 1,3-dihydroxy-2-propanone-*1,1-d*$_2$ 1-phosphate is also readily accomplished by triose phosphate isomerase, as the equilibrium of this enzyme-catalyzed reaction greatly favors formation of the ketone phosphate. 1,3-Dihydroxy-2-propanone-*1,1-d*$_2$ 1-phosphate can be utilized in the aldolase-catalyzed synthesis of other ketose phosphates that have been deuterium-labeled at C-1. The availability of these analogs should greatly assist structural investigations of 2-ketose phosphates by n.m.r. spectroscopy.

EXPERIMENTAL

General. — ^{1}H n.m.r. spectra were recorded with a Varian A-60 Spectrometer at 37 $\pm$1°. Tetramethylsilane was used as the internal reference standard for samples dissolved in chloroform-*d*, and as the external reference for samples dissolved in deuterium oxide.

Phosphoric esters were examined for purity by chromatography on Whatman No. 40 paper in one of two solvent systems: for acid-sensitive phosphates, 3:1:1 (v/v) ethanol–ammonia–water (Solvent 1), and for base-sensitive phosphates, 7:2:5 (v/v) butanol–acetic acid–water (Solvent 2). After being dried, the chromatograms were

developed by spraying with the perchloric acid–molybdate spray of Hanes and Isherwood[12].

Gas-chromatographic analyses were performed on an Aerograph Hy-Fi, Model 600-D gas chromatograph having a flame-ionization detector operated with a hydrogen flow-rate of 30 ml.min^{-1}. A column (4 ft × 0.125 in.) packed with neopentyl glycol sebacate, 10% on 80–100 mesh Gas Chrom Q (Applied Science Laboratories, Inc.), was used with a helium flow-rate of 30 ml.min^{-1}. The column was operated at 200°.

2-O-Benzyl-D-arabinono-1,4-lactone (**3**). — To a solution of 2-*O*-benzyl-D-arabinose[2] (**1**, 10 g) in water (400 ml) were added 20 ml of 0.05M iodine solution (0.05M I_2, 0.25M KI) and 30 ml of 0.1M sodium hydroxide alternately, until 920 ml of the former and 1.4 liters of the latter had been added; the additions required 1.25 h. After an additional 0.25 h at room temperature, the mixture was passed through a column (500 ml) of Dowex 50 (H^+) ion-exchange resin, and the effluent was run into a rapidly stirred, aqueous slurry of silver carbonate (75 g). The suspension was cooled to 4° and filtered through Celite. The filtrate was treated batchwise with 75 ml of Dowex 50 (H^+), adjusted to pH 7.2 with M sodium hydroxide, and evaporated *in vacuo* at 50°; the product (**2**) was dried by repeated addition and evaporation of benzene.

To the dry sodium salt **2** were added dry methanol (180 ml) and concentrated sulfuric acid (3 ml), and the suspension was boiled under reflux for 1.5 h, and cooled. Barium carbonate (50 g) was added and, after vigorous shaking, the mixture was filtered. Evaporation of the filtrate *in vacuo*, and crystallization of the residue from water, yielded 6.45 g (65%) of 2-*O*-benzyl-D-arabinono-1,4-lactone (**3**), m.p. 119–120°, $[\alpha]_{546}$ −20.9° (*c* 2.2, ethanol).

Anal. Calc. for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.35; H, 5.73.

2-O-Benzyl-D-arabinitol-1,1-d_2 (**4**). — A solution of sodium borodeuteride (1.01 g, Alfa Inorganics) in 7 ml of 1 mM sodium hydroxide was cooled and added to a cold solution of **3** (5 g) in water (100 ml). The mixture was stirred for 50 h at 4°, warmed to room temperature, deionized with 60 ml of Dowex 50 (H^+) resin, and filtered. The filtrate was evaporated *in vacuo* at 40° and then 10 portions of dry methanol were added and evaporated off to yield a syrup (5 g). Gas chromatography of the trimethylsilyl derivative revealed a major peak that corresponded to that of per(trimethylsilyl)ated, authentic 2-*O*-benzyl-D-arabinitol, and a trace of material having a retention time of 12.5 min.

2-O-Benzyl-D-glyceraldehyde-3,3-d_2 *dimethyl acetal* (**8**). — A solution of sodium metaperiodate (12.86 g) in 250 ml of water was added to **4** (5 g) in 100 ml of water. After 20 min at room temperature, the solution was extracted several times with ether (1 liter total), and the ether layers were combined, and evaporated *in vacuo* to a syrup which was redissolved in ether (200 ml); the solution was washed twice with water, dried (anhydrous sodium sulfate), and evaporated *in vacuo* to yield a syrup (3.03 g). The free aldehyde was immediately converted into the dithioacetal by dissolving it in 5 ml of ethanethiol and adding 4 ml of concentrated hydrochloric acid.

After occasional shaking for 0.75 h in an ice bath, cold water (30 ml) was added, and the mixture was extracted with several portions of dichloromethane. The extracts were combined, washed twice with cold water, dried (anhydrous sodium sulfate), and evaporated *in vacuo* to afford **6** as a syrup (4.00 g, 62%). Removal of the formyl group by treatment with a dilute solution of sodium methoxide in methanol afforded a quantitative yield of **7**, which was converted into **8** as described by Ballou and Fischer[6]. The ^{1}H n.m.r. spectrum of the product was identical with that of 2-*O*-benzyl-D-glyceraldehyde dimethyl acetal, except for the absence of H-3 signals in the region τ 6.5, and concomitant loss of coupling of those protons to H-2. ^{1}H n.m.r. data: τ 2.71 (multiplet, 5 protons, Ph), 5.35 (singlet, 2 protons, $PhCH_2$), 5.63 (doublet, H-1), 6.53 (doublet, H-2), 6.65 (singlet, 6 protons, CMe_2), and 6.93 (broad singlet, 3-OH).

*2-O-Benzyl-3-O-(diphenylphosphono)-*D*-glyceraldehyde-3,3-d$_2$ dimethyl acetal* (**9**). — Compound **8** (3.0 g) was treated with diphenylphosphorochloridate as described by Ballou and Fischer[6] for the nondeuterated analog, to yield an impure syrup (5.52 g). A portion (4.4 g) of the product was dissolved in benzene (10 ml), and the solution was applied to a column containing 90 g of Florisil (Floridin Company) in benzene. Elution of the column with 1 liter of benzene yielded 900 mg of an ~1:1 mixture of **9** and an unidentified compound (as judged by ^{1}H n.m.r. spectroscopy). The column was then eluted with ether. The solute in the transition eluate (2.25 g) and in the ether eluate (1.14 g) was shown to be **9** by comparison of its ^{1}H n.m.r. spectrum with that of the nondeuterated analog. ^{1}H n.m.r. data: τ 2.75 (multiplet, 15 protons, Ph), 5.36 (singlet, 2 protons, $PhCH_2$), 5.67 (doublet, H-1), 6.34 (quartet, H-2), 6.67, 6.70 (singlets, 3 protons each, CMe_2).

D-Glyceraldehyde-3,3-d$_2$ dimethyl acetal 3-(dicyclohexylammonium phosphate) (**10**). — Compound **9** (1.1 g) was reduced with hydrogen in the presence of palladium and then with hydrogen in the presence of platinum, and the product was neutralized with cyclohexylamine as described by Ballou and Fischer[6]. Recrystallization from 10 ml of hot isopropyl alcohol (10 ml) by the addition of ether (5 ml) afforded pure **10** (680 mg, 69%), m.p. 151–154°, $[\alpha]_{546}$ + 9.3° (*c* 4.0, water). The product migrated with authentic D-glyceraldehyde dimethyl acetal 3-(dicyclohexylammonium phosphate) (R_F 0.55) in Solvent 1. The ^{1}H n.m.r. spectrum of this material was consistent with its being the dicyclohexylammonium salt (**10**) of glyceraldehyde dimethyl acetal 3-phosphate.

D-Glyceraldehyde-3,3-d$_2$ 3-phosphate (**11**). — Compound **10** (408 mg, 980 μmoles) was dissolved in water (15 ml) and the solution was treated batchwise with 5 ml of Dowex 50 (H^+) resin. The resin was removed by filtration, and washed with three 5-ml portions of water, and the filtrate and washings were combined and kept at 37°. After 72 h, the pH of the solution was adjusted to 6, and the volume was lessened to 20 ml by lyophilization. Phosphate analyses[7] indicated the presence of 647 μmoles of base-labile phosphate and 167 μmoles of inorganic phosphate. Enzymic analysis[8] indicated the presence of 636 μmoles of D-glyceraldehyde 3-phosphate. Paper chromatography in Solvent 1 indicated that a trace of the dimethyl acetal remained.

D-*Fructose*-6,6-d_2 *1,6-diphosphate, tetracyclohexylammonium salt.* — D-Glyceraldehyde-*3,3-d*$_2$ 3-phosphate (295 μmoles) was added to 295 μmoles of 1,3-dihydroxy-2-propanone 1-phosphate[1], and the pH was adjusted to 7.5 with M sodium hydroxide. Aldolase (10 mg, specific activity 10 $\mu moles \cdot min^{-1} \cdot mg^{-1}$ of protein) was added, and formation of the diphosphate was monitored by the decrease in base-labile phosphate. After 5 min, 41 μmoles of the initial 590 μmoles of base-labile phosphate were present, indicating a 93% conversion into D-fructose-*6,6-d*$_2$ 1,6-diphosphate. The solution was adjusted to pH 6 and lyophilized; the product was mixed with water, and the suspension was filtered through a Diaflo membrane (to remove protein). The faintly yellow solution was decolorized with Darco X and lyophilized, and the residue was converted into the cyclohexylammonium salt, which was crystallized from water–acetone to yield 478 mg of material. Paper chromatography in Solvent 2 revealed the presence of D-fructose 1,6-diphosphate (R_F 0.15) and a minor component (R_F 0.44). Pure material was obtained by preparative paper-chromatography on Whatman No. 17 paper, with Solvent 2 as the eluant.

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