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A SYNTHESIS OF (±)-*myo*-INOSITOL 1-PHOSPHATE

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ABSTRACT

An uncomplicated synthesis of (±)-*myo*-inositol 1-phosphate (**7**) is described. Phosphorylation of (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**2**) with phosphorus oxychloride gave the corresponding 1- and 2- phosphates, isolated as a mixture of their bis(cyclohexylamine) salts. Fractional crystallization of the regenerated free phosphates gave (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol 1-phosphate (**5**), which afforded (±)-*myo*-inositol 1-phosphate (**7**) after removal of the benzyl groups by catalytic hydrogenolysis.

INTRODUCTION

Both of the enantiomers of *myo*-inositol 1-phosphate are naturally occurring compounds; L1-*myo*-inositol 1-phosphate serving as the direct biosynthetic precursor of *myo*-inositol in yeast[1], mold[2], higher plant[3], and mammalian systems[4], and the D stereoisomer is a product of the alkaline hydrolysis of soybean phosphoinositide[5]. The early preparations of racemic *myo*-inositol 1-phosphate were channeled either through *myo*-inositol 2-phosphate or (±)-3,4,5,6-tetra-*O*-acetyl-*myo*-inositol. Treatment of the 2-phosphate with aqueous acid[6], or with *N,N'*-dicyclohexylcarbodiimide and then aqueous acid[5], gave a mixture composed of the 1-phosphate (~80%) and the 2-phosphate (~20%). Phosphorylation of (±)-3,4,5,6-tetra-*O*-acetyl-*myo*-inositol with diphenyl phosphorochloridate, followed by removal of the protecting groups, also gave a mixture composed primarily of the racemic 1-phosphate[5,7].

Recently, Klyaschitskii *et al.*[8a] have reported an alternative route to the title compound. The phosphate was prepared without complication by hydrogenolysis of racemic 2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol 1-(diphenyl phosphate). Furthermore, a parallel synthesis of D1-*myo*-inositol 1-phosphate was successfully routed through the levorotatory pentabenzyl ether. The L-isomer was synthesized by Ballou and Pizer[9] from the enantiomeric, dextrorotatory pentabenzyl ether derived from naturally occurring 1-*O*-α-D-galactopyranosyl-*myo*-inositol (galactinol). More re-

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cently, Gero *et al.*[10] have described an elegant synthesis of this isomer beginning with quebrachitol. Phosphorylation of (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol with phenyl phosphorochloridate[8b] gave a mixture of the corresponding 1- and 2-monophosphates, and the 2,3-cyclic phosphate, all of which were separated with difficulty. A paper by Molotkovsky and Bergelson[11] describes the synthesis of both of the optically active *myo*-inositol 1-phosphates by way of the resolved 2,3,4,5,6-penta-*O*-acetyl-*myo*-inositols.

RESULTS AND DISCUSSION

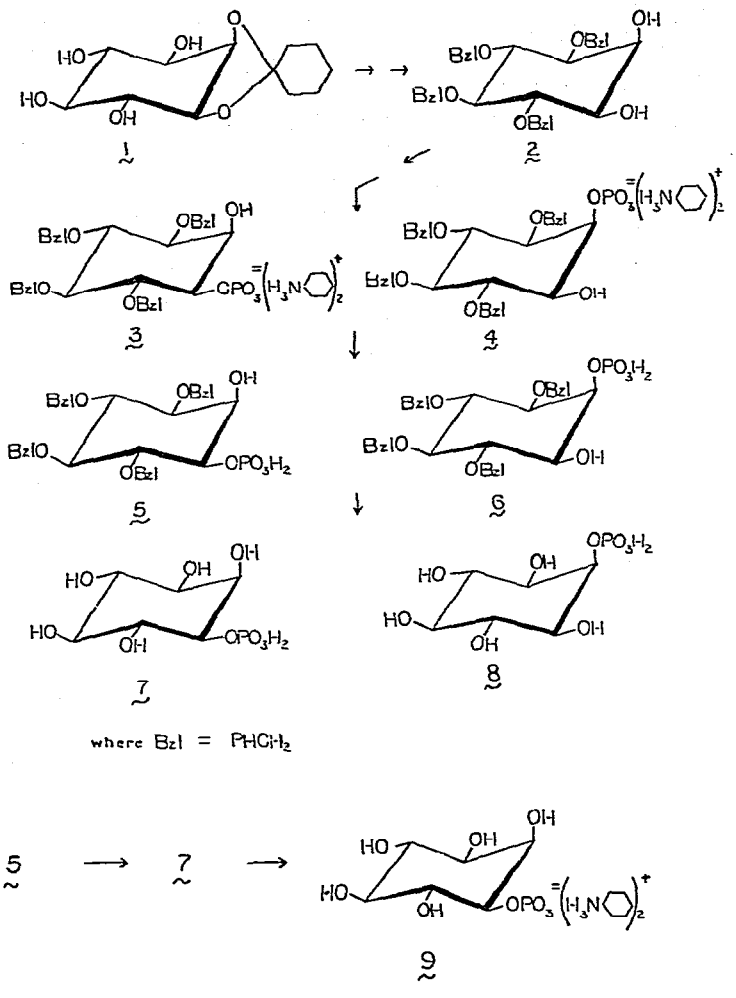
In this paper we describe a new and convenient preparation of (±)-*myo*-inositol 1-phosphate (**7**). Treatment of a chloroform-diluted solution of (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**2**) in pyridine with phosphorus oxychloride gave a crude, crystalline mixture of phosphates, which, even after repeated attempts at fractional recrystallization from a variety of solvents, melted at various temperatures in the range 135–225°. The crude phosphate was readily converted into a crystalline mixture composed of the bis(cyclohexylamine) salts (**3** and **4**) of 3,4,5,6-tetra-*O*-benzyl-*myo*-inositol 1-phosphate (**5**) and 2-phosphate (**6**). The free acids (**5** and **6**) were generated from the salt mixture with an acid-form cation-exchange resin, the benzyl groups removed by catalytic hydrogenolysis, and the final phosphates (**7** and **8**) identified gas chromatographically as their *O*-trimethylsilyl derivatives. As was anticipated, the stereoselectivity of the phosphorylation was reflected in the preponderance of *myo*-inositol 1-phosphate (**7**) (**7**:**8** = 7:2). Attempts to fractionally crystallize one of the isomers from the salt mixture were also without success.

Attention was then focused on the syrupy mixture of free acids obtained by decationizing the salt with resin. Ultimately we found that fractional crystallization of the mixture could be achieved from benzene, giving a first crop of **5** (m.p. 158–162°, 57%) whose purity was 99% or greater. The final conversion of **5** into (±)-*myo*-inositol 1-phosphate (**7**), isolated as its bis(cyclohexylamine) salt (**9**), was then accomplished in high yield (>90%) without complication.

The tetrabenzyl ether (**2**) used in this synthesis was prepared according to the basic procedure described by Angyal and Tate[12] by way of (±)-1,2-*O*-cyclohexylidene-*myo*-inositol (**1**). We had limited success when attempting to prepare **1** from *myo*-inositol by the exact procedure described in ref. 13. In order to obtain yields of **1** comparable to those cited in that paper, the time for the acid-catalyzed hydrolysis of tri-*O*-cyclohexylidene-*myo*-inositol was increased from 15 min to 1 h. Compound **1** was then benzylated and the product, without further purification, was subjected to acid hydrolysis to give **2**.

EXPERIMENTAL

General methods. — Melting points are uncorrected and, unless otherwise specified, were taken on a Fisher–Johns melting-point apparatus. Gas chromatography was conducted at 180° on a Beckman model GC-5 gas chromatograph, fitted



with a flame-ionization detector, with dual 0.25-in. (o.d.) × 6 ft stainless-steel columns filled with 3% SE-30 on Gas Chrom Q, 80–100 mesh (Applied Science Laboratories Inc., State College, Pa.), and helium was the carrier gas.

(±)-*1,2*-O-*Cyclohexylidene*-myo-*inositol* (**1**). — A 2-l flask equipped with a mechanical stirrer, a Dean–Stark trap, and an efficient reflux condenser, was charged with powdered (mortar and pestle) *myo*-inositol (50 g), cyclohexanone (500 ml), and anhydrous benzene (130 ml). The mixture was stirred vigorously and heated to rapid boiling under reflux. After about 15 min, collection of water (~0.5 ml) in the trap had stopped and *p*-toluenesulfonic acid monohydrate (200 mg) was added to the reaction mixture. The water collected at an average rate of about 4 ml/h during 4.5 to 5 h to give a volume of ~14 ml, and then additional acid (200 mg) was added. After a total of 6 h, ~17 ml of water had collected, and the heating was discontinued. The reaction mixture was cooled to room temperature while stirring, kept overnight, and the

supernatant was then decanted from the small amount of remaining undissolved *myo*-inositol. This solid was washed with benzene (50 ml) and the washings and additional benzene (200 ml) were added to the decantate, together with hexane (250 ml), ethanol (65 ml), and (±)-1,2-*O*-cyclohexylidene-*myo*-inositol (**1**) (1 g), in that order. The mixture was stirred for 1 h to precipitate the product and then stored for 24 h at −25°. Triethylamine (1 ml) was added with stirring, the solid was removed by filtration and washed with benzene, and the filtrate and washings were combined and saved. The crude, solid product was boiled with ethanol (1200 ml) containing triethylamine (1 ml), and then filtered off and washed with additional ethanol to give **1** (45.5 g, m.p. 179–180° with a transition at 160–165°; lit.[13] m.p. 181–183° with a transition at 158–160°). The combined ethanolic filtrate and washings were saved for a future extraction.

The original filtrate was stirred for 1 h with ethanol (50 ml), *p*-toluenesulfonic acid monohydrate (3 g), and **1** (1 g). To this hydrolysis mixture, subsequently refrigerated for 24 h at −25°, was added triethylamine (2 ml), and the precipitate, after being extracted with boiling ethanol (used in the extraction of the first crop) containing triethylamine (1 ml), was removed by filtration and washed with ethanol to give additional **1** (16 g, m.p. 179–180°, transition at 158–163°). A third crop of **1** (m.p. 176–178°, transition at 155–160°) was obtained after the combined ethanol extract and washings had been concentrated *in vacuo* and refrigerated overnight (the total yield of **1** was 63.5 g less 2 g for seeding, 85%).

(±)-*3,4,5,6-Tetra*-O-*benzyl*-myo-*inositol* (**2**). — A mixture of (±)-1,2-*O*-cyclohexylidene-*myo*-inositol (**1**, 36.8 g, 0.142 mol), benzyl chloride (407 g, 3.21 mol) and granulated potassium hydroxide (220 g, 3.93 mol, Hooker Chemical Corp., Industrial Chemicals Division, Niagara Falls, N. Y.) was vigorously stirred for 20 h at 90–100°. The cooled mixture was diluted with benzene (400 ml) and washed with several 150-ml portions of water until the aqueous wash was no longer alkaline to litmus. The benzene was removed *in vacuo* and the resulting solution, after dilution with glacial acetic acid (800 ml) and water (160 ml), was kept for 4 h at 90–100°. The acetic acid and water were removed *in vacuo* at 60–70° and the bulk of the high-boiling, organic by-products was removed by distillation at a bath temperature of 70–105° (~ 1 mmHg). The dark-brown, syrupy residue was seeded with crystals of **2**, and the crude solid product was removed by filtration and washed with ligroin. Recrystallization of this material from methanol gave **2**; yield 36 g (42%), m.p. 126–128° (lit.[12] m.p. 114–115° and 127°).

Bis(*cyclohexylammonium*) *3,4,5,6-tetra*-O-*benzyl*-myo-*inositol 1- and 2-phosphate mixture* (**3** *and* **4**). — A solution of **2** (10.8 g, 20 mmol) in chloroform (200 ml), in a flask fitted with a calcium chloride drying tube, was cooled in an ice bath and anhydrous pyridine (50 ml) was added with stirring. Phosphorus oxychloride (10 ml, 109 mmol) in chloroform (25 ml) was added dropwise during 2 h to the stirred solution at 0°, and stirring was continued for an additional 5 h at room temperature. (This procedure is patterned after that[14] employed for preparing testosteronephosphoric acid.) The solution was then poured onto crushed ice (300 ml) and this mixture was

stirred for 1 h to decompose the excess phosphorus oxychloride. The layers were separated and the organic one was washed with 20% aqueous sodium chloride (200 ml) and then with saturated aqueous sodium chloride (200 ml). (Water alone was not used because of excessive formation of emulsion.) The organic layer was dried (magnesium sulfate) and concentrated *in vacuo* at 35° to give a cream-colored paste. A solution of the paste in chloroform (100 ml) and ethanol (100 ml) was stirred with cyclohexylamine (10 ml, 80 mmol) for 1 h under nitrogen. During this time a solid generally precipitated out of solution. The entire mixture was concentrated to a paste *in vacuo* at 35° and residual cyclohexylamine removed *in vacuo* (~1 mm Hg) at room temperature. The resulting solid, after having been stirred with acetone (50 ml) and removed by filtration, was washed thoroughly with more acetone and then ethanol to give a white, crystalline mixture of bis(cyclohexylammonium) 3,4,5,6-tetra-*O*-benzyl-*myo*-inositol 1- and 2-phosphates (**3** and **4**, 8.6 g, 52%, m.p. 218–221°, with softening at 210°.

(±)-*3,4,5,6-Tetra*-O-*benzyl*-myo-*inositol 1-phosphate* (**5**). — A suspension of the crystalline salt mixture (8.6 g, 10.5 mmol), derived from **2**, in glacial acetic acid (100 ml), was stirred for several min with AG 50W-X2 (H^+) resin (75 ml, 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.). Most of the salt dissolved immediately, but the reaction mixture was kept for 1 h with occasional stirring until dissolution was complete. The resin was removed by filtration, washed with glacial acetic acid, and the combined filtrate and washings freeze-dried to give an amorphous, white powder. This material was mixed thoroughly with benzene (30 ml, added in small portions) until the mixture became a translucent, gelatinous paste, which was heated just to boiling in order to dissolve most of the solid. Upon being kept overnight at room temperature the product (**5**) crystallized as white needles (3.7 g, 57%, m.p. 158–162° with softening at 150°). A sample of this material (0.1 g) in glacial acetic acid (20 ml) was subjected to catalytic hydrogenolysis with freshly prepared palladium black (0.06 g) generated from palladium chloride (Engelhard Industries, Inc., Newark, N. J.) in a Parr apparatus under 62 $lb.in^{-2}$ of hydrogen for 72 h. The catalyst was filtered off, washed with glacial acetic acid and then water, and the combined filtrate and washings were freeze-dried. The gummy product, after trimethylsilylation according to the method of Sweeley *et al.*[15], was analyzed gas chromatographically. Upon comparison with authentic samples it was found to be composed of only two components, behaving as *myo*-inositol 1-phosphate (**7**) and *myo*-inositol 2-phosphate (**8**) (**7**:**8**>99:1), having retention times of 20.5 min and 27 min, respectively. The g.l.c. behavior of trimethylsilylated *myo*-inositol 1-phosphate has been reported and its mass spectrum recorded[16]. An analytical sample of **5** was obtained after two recrystallizations from benzene (m.p. 159–161° with softening at 157°). The analytical sample of **5** also contained less than 1% of **6**, as determined gas chromatographically.

Anal. Calc. for $C_{34}H_{37}O_9P$ (620.62): C, 65.80; H, 6.01; P, 4.99. Found: C, 65.56; H, 6.12; P, 5.25.

The filtrate from the original crystallization of the regenerated phosphates was

concentrated *in vacuo* at 35° to a gum that became a paste after being mixed with benzene (2 ml) and seeded with crystals of **5**. The paste was mixed with more benzene (5 ml, added in small portions), heated just to boiling, seeded again with **5**, and allowed to cool. After several days at room temperature, a white, crystalline product precipitated that was removed by filtration and washed with cold benzene (yield 2.15 g, m.p. 145–150°). After a single recrystallization from benzene this product (1.16 g) had m.p. 150–153° and was determined by g.l.c. analysis of the debenzylated material to be composed of **5** enriched with **6** (**5**:**6** ≃ 3:2).

Analysis of the salt derived from **2**. — A sample of the gummy free-acid mixture (0.1 g), generated by resin treatment of the bis(cyclohexylamine) salt as previously described, was debenzylated in acetic acid by catalytic hydrogenolysis with palladium black. A standard g.l.c. analysis of the hydrogenolysis mixture showed that it contained only the isomers **7** and **8** (**7**:**8** ≃ 7:2), having retention times of 20.5 min and 27 min, respectively, which indicates that the 1- and 2-phosphate salts **3** and **4** also co-crystallized in the same ratio.

(±)-*Bis*(*cyclohexylammonium*) myo-*inositol 1-phosphate* (**9**). — Hydrogenolysis of the benzyl groups of (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol 1-phosphate (**5**, 1.5 g) in glacial acetic acid (50 ml) with 10% palladium on carbon in a Parr apparatus (62 lb.in^{-2} of hydrogen) was complete after 72 h. The catalyst, after removal by filtration, was washed with glacial acetic acid (35 ml) and then water (20 ml), and the combined filtrate and washings were freeze-dried. A solution of the slightly yellow, gummy product in water (10 ml) was treated with cyclohexylamine until the pH reached ~9, and the reaction mixture was kept for 30 min under nitrogen. The aqueous solution was then extracted with three 20-ml portions of dichloromethane to remove excess amine, and the water layer freeze-dried to give the crude salt. A solution of the salt in water (4 ml) was diluted to turbidity with acetone (15 ml) and then clarified by the addition of a minimum amount of water. Crystallization was complete after 24 h at −5° and the acetone-washed (±)-bis(cyclohexylammonium) *myo*-inositol 1-phosphate (**9**) was obtained in 91% yield (1.0 g); m.p. 205–207° (dec.), lit.[8a] m.p. 195–201° (dec., determined on a Thomas–Hoover Uni-melt melting point apparatus.) The g.l.c. analysis of the trimethylsilyl derivative of this material showed that it contained less than 1% of the 2-isomer.

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