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Synthesis of *myo*-inositol 1,4,6-trisphosphate, an analogue of *myo*-inositol 1,4,5-trisphosphate

Yutaka Watanabe [a,*], Tomio Ogasawara [a], Shoichiro Ozaki [a], Masato Hirata [b]

[a] *Department of Applied Chemistry, Faculty of Engineering, Ehime University, Matsuyama 790, Japan*
[b] *Department of Biochemistry, Faculty of Dentistry, Kyushu University 61, Fukuoka 812, Japan*

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Abstract

myo-Inositol 1,4,6-trisphosphate, in optically inactive and active forms, was prepared in order to compare its biological activity with that of *myo*-inositol 1,3,4,6-tetrakisphosphate which releases Ca^{2+} from an intracellular store.

1. Introduction

The role of 1D-*myo*-inositol 1,4,5-trisphosphate [D-Ins(1,4,5)P_3] as a second messenger which releases calcium ion from an intracellular calcium store is well established [1]. Disclosure of its structure and activity relationships is very important in order to understand the interaction with its receptor and the metabolic enzymes, Ins(1,4,5)P_3 3-kinase and 5-phosphatase at the molecular level. From this standpoint, some regio- and stereo-isomers have been prepared and evaluated biologically [2]. Recently, *myo*-inositol 1,3,4,6-tetrakisphosphate was discovered in Nature and synthetic material was found to release intracellular calcium ion [3]. We tried to explore the structural similarity between Ins(1,4,5)P_3 and Ins(1,3,4,6)P_4 in order to obtain information on the essential features of the activity of the tetrakisphosphate. Comparison of their structures illustrated below suggested that three phosphate groups of 1D-*myo*-inositol 1,4,6-trisphosphate and 1L-*myo*-inositol 1,3,4-trisphosphate, which are derived by deleting the phosphate function at the 3 and 4 positions, respectively, from the 1,3,4,6-tetrakisphosphate, are conformationally identical with those of D-Ins(1,4,5)P_3. The consideration prompted us to

* Corresponding author.

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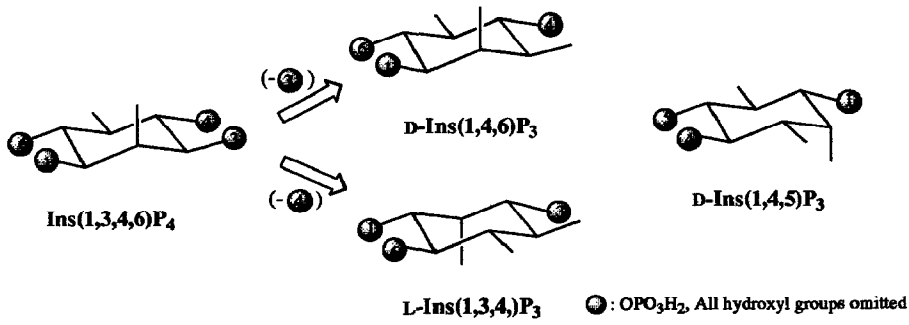


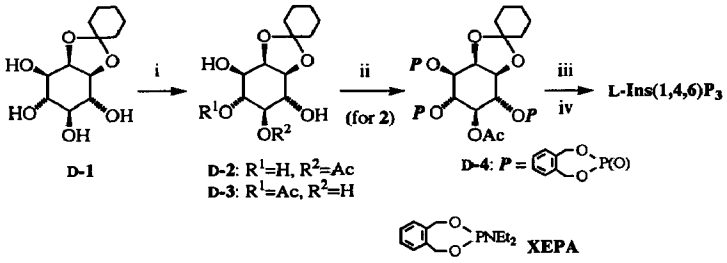
Fig. 1.

prepare DL-, D-, and L-Ins(1,4,6)P_3. We describe here their synthesis and biological activities obtained in preliminary experiments.

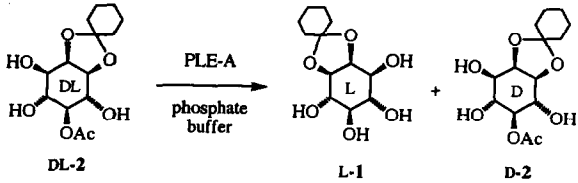
2. Results and discussion

Acetylation of 1,2-*O*-cyclohexylidene-*myo*-inositol (**1**), which was a crucial step for the concise synthesis of Ins(1,4,6)P_3, was achieved practically by the reaction with acetic anhydride in the presence of 4A molecular sieves (MS 4A) in *N*,*N*-dimethylacetamide (DMA) to afford a ca. 1:1 mixture of 4- and 5-mono-*O*-acetyl derivatives **2** and **3** in a reasonable yield (62–83% yields). It should be noted that, while some electrophiles were reported to react predominantly at the 3-position under basic conditions containing amine bases [4], the present acetylation in the presence of triethylamine instead of MS 4A gave a complex mixture. Replacement of the solvent, DMA with *N*,*N*-dimethylformamide also gave mixtures.

The desired 5-*O*-acetyl inositol **2** was readily obtained by recrystallization of the regioisomeric mixture. Phosphorylation (99% yield) of triol **2** using *O*-xylylene *N*,*N*-diethylphosphoramidite (XEPA [5]) followed by deprotection in two steps afforded the final product as the trisodium salt (88% yield) as shown in Scheme 1. In a similar manner, a mixture of regioisomeric acetates, **2** and **3** without separa-



Scheme 1. (i) Ac_2O, MS 4A, DMA, r.t.; (ii) XEPA, 1*H*-tetrazole then mCPBA; (iii) H_2, Pd–C, aq MeOH; (iv) NH_4OH.



Scheme 2.

tion was phosphorylated, and the 4-acetyl and 5-acetyl phosphates were isolated in 38 and 36% overall yields, respectively.

Optically active D- and L-Ins(1,4,6)P_3 were prepared starting respectively from the corresponding 1L-1,2-*O*-cyclohexylidene-*myo*-inositol (**L-1**) and D-5-acetate derivative **D-2** which were obtained by an enzymatic method (Scheme 2). Thus, racemic 5-*O*-acetyl-1,2-*O*-cyclohexylidene-*myo*-inositol (**DL-2**) was subjected to hydrolysis in a phosphate buffer solution containing porcine liver esterase-A (PLE-A) for 12 h at ambient temperature followed by recrystallization of the product and recovered starting material to give **L-1** and **D-2** with high optical purities (96% and 94% ee). The optical purity * of **L-1** was evaluated based on the optical rotation of the tetrol obtained independently in this laboratory †, whose purity was established by its derivatization to 1D-1,4,5,6-tetra-*O*-benzoyl-*myo*-inositol [8] followed by HPLC analysis using Chiralcel OD™ [7,9]. The optical purity of the recovered acetate was determined by comparison of its optical rotation with that of the opposite enantiomer **D-2**.

Optically active cyclohexylidene derivatives were also obtained by the enzymatic acetylation of racemic **1** with lipase CES from *Pseudomonas sp.* (Amano Pharmaceutical Co. Ltd), which afforded almost optically pure **L-1** and the 3-acetate of **D-1** [7,9]. The latter acetate (96% ee) was converted into L-Ins(1,4,6)P_3 via **D-1** which was obtained by ammonolysis of the acetate. This process was more effective than that described above since **D-2** formed in the enzymatic hydrolysis was contaminated with acetyl-migration products and several recrystallizations were required.

D-Ins(1,4,6)P_3 bound to the Ins(1,4,5)P_3 receptor with high affinity [16-fold less potent than Ins(1,4,5)P_3]; however, interestingly, its ability to release Ca^{2+} was much less potent (1/125). Thus, the potency of D-Ins(1,4,6)P_3 with respect to receptor binding was found to be the same as that of Ins(1,3,4,6)P_4. Consequently, the three phosphate groups at D-1, D-4, and D-6 positions in the tetrakisphosphate must be essential for the activity. Racemic Ins(1,4,6)P_3 was also recognized by the Ins(1,4,5)P_3 5-phosphatase of human erythrocyte ghosts with a higher affinity than seen with Ins(1,4,5)P_3, but it was poorly dephosphorylated (relative rate: 1/10),

* Optical rotations of both enantiomers of **1** were reported [6]: $[\alpha]_D^{20}$ −39.4° (*c* 1.53, EtOH) and $[\alpha]_D^{20}$ +42.4° (*c* 0.33, EtOH). However, comparison of these data with ours in the same solvent was impossible because our tetrols were not soluble in EtOH.

† 95% ee of the tetrol showed $[\alpha]_D^{21}$ +35.4° (*c* 1.75, MeOH). From this result [7], optically pure **L-1** is expected to have $[\alpha]_D$ +37.6°.

thereby indicating that Ins(1,4,6)P_3 could function as an inhibitor for Ins(1,4,5)P_3 5-phosphatase. These characteristic features of Ins(1,4,6)P_3 will be utilized as a promising tool for molecular recognition studies. The biological results will be reported elsewhere [10].

3. Experimental

General methods.—Aqueous 85% H_3PO_4 was used as the external reference for the ^{31}P NMR spectra. The ^{13}C and ^{31}P NMR spectra were ^1H-decoupled. IR spectra were recorded with a Hitachi EPI-G3 spectrometer. Flash-column chromatography was performed on Wakogel C-300 and column chromatography on microgranular cellulose powder CC-31 (Whatman BioSystems). Extracts were dried over $MgSO_4$ or Na_2SO_4.

5-O-Acetyl-1,2-O-cyclohexylidene-myo-*inositol* (**2**).—To a solution of racemic 1,2-*O*-cyclohexylidene-*myo*-inositol (1.0 g, 3.84 mmol) in *N*,*N*-dimethylacetamide (30 mL) were added Ac_2O (2.2 mL) and powdered 4A molecular sieves (0.5 g), and the mixture was stirred for 24 h at ambient temperature and then subjected to distillation under reduced pressure (0.1 mmHg), to give an oily residue. It was chromatographed on silica gel (10:5:1 EtOAc–CH_2Cl_2–MeOH) to afford a mixture of 4- and 5-monoacetyl derivatives (0.82 g, 69%) from which the 5-acetate (0.37 g, 32%) was obtained by recrystallization from MeOH and Et_2O; mp 171–173.5°C; ν_{max} (Nujol) 3480, 1710 cm^{-1}. ^1H NMR data (270 MHz, $CDCl_3$–CD_3OD): δ 1.41 (b, 2 H), 1.60 (b, 8 H), 2.14 (s, 3 H), 3.75 (dd, 1 H, *J* 9.65 and 7.33 Hz), 3.80 (c, 2 H), 4.06 (dd, 1 H, *J* 7.02 and 5.49 Hz), 4.43 (dd, 1 H, *J* 5.49 and 2.04 Hz), 4.68 (m, 1 H). Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.62; H, 7.33. Found: C, 55.27; H, 7.30.

L-**2** $[\alpha]_D^{22}$ +30° (*c* 0.9 MeOH); mp 172–174.5°C (from MeOH).

5-O-Acetyl-1,2-O-cyclohexylidene-myo-*inositol 3,4,6-tris(o-xylylene phosphate)* (**4**).—To a solution of **2** (36 mg, 0.119 mmol) and XEPA (102 mg, 0.428 mmol) in CH_2Cl_2 (3 mL) was added 1*H*-tetrazole (50 mg, 0.71 mmol), and the mixture was stirred at room temperature for 30 min. After treatment with H_2O (10 mg, 1.19 mmol) for 10 min, *m*-chloroperoxybenzoic acid (mCPBA) (163 mg, 0.952 mmol) was added at 0°C and then the mixture was stirred at room temperature for an additional 30 min. The CH_2Cl_2 extract was washed successively with aq 10% Na_2SO_3, H_2O, satd aq $NaHCO_3$, and H_2O, and dried. Chromatography on silica gel (8:1 $CHCl_3$–acetone) afforded the phosphate **4** (0.1 g, 99%); mp 139–141°C (from Et_2O–CH_2Cl_2); ν_{max} (Nujol) 1740 cm^{-1}. NMR data ($CDCl_3$): ^1H (270 MHz) δ 1.40 (b, 2 H), 1.69 (b, 8 H), 2.25 (s, 3 H), 4.30 (dd, 1 H, *J* 7.18 and 4.78 Hz), 4.76 (c, 2 H), 4.87–5.66 (m, 15 H), 7.24–7.42 (c, 12 H); ^{31}P (109 MHz) δ −3.04, −1.11, 0.81. Anal. Calcd for $C_{38}H_{43}O_{16}P_3$: C, 53.77; H, 5.12. Found: C, 53.45; H, 5.42.

L-**4**: $[\alpha]_D^{19}$ −29.0° (*c* 0.9, CH_2Cl_2); mp 141–143.5°C (from CH_2Cl_2–Et_2O).

D-**4**: $[\alpha]_D^{20}$ +29.5° (*c* 1.0, CH_2Cl_2); mp 141.5–145.0°C (from CH_2Cl_2–Et_2O).

When a mixture of the 4- and 5-*O*-acetyl derivatives **2** and **3** was phosphorylated, **4** and its regioisomer were isolated in 36 and 38% yields, respectively, based on tetrol **1**, by the same procedure as that described above.

myo-*Inositol 1,4,6-tris(hydrogen phosphate) [Ins(1,4,6)P_3]*.—A mixture of **4** (180 mg, 0.21 mmol), 5% Pd–C (200 mg), and aq 20% MeOH (5 mL) was stirred under H_2 at room temperature for 12 h, then filtered, and concentrated. The residue was treated with 28% NH_4OH overnight and concentrated. Column chromatography on cellulose (4:5:1 28% NH_4OH–PrOH–H_2O), with application of pressure, gave the fully deprotected final product which was then acidified by passing through a column of cation-exchange resin (H^+ form). Evaporation of the eluate with pyridine under reduced pressure followed by passing through a column of cation-exchange resin (Na^+ form) gave 103 mg of DL-Ins(1,4,6)P_3 (88%) which was recrystallized from H_2O–MeOH to give fine crystals (78 mg); mp > 240°C. NMR data (D_2O): ^1H [40 mg in 0.75 mL, pD 5.95, 270 MHz, DOH (δ 4.64) as the reference]: δ 3.42 (t, 1 H, *J* 9.46 Hz), 3.55 (dd, 1 H, *J* 9.46 and 2.44 Hz), 3.93 (dt, 1 H, *J* 9.46 and 2.44 Hz), 4.03 (bt, 1 H), 4.04 (q, 1 H, *J* 9.46 Hz), 4.16 (q, 1 H, *J* 9.46 Hz); ^{13}C [40 mg in 0.75 mL, pD 5.95, 67.8 MHz, dioxane (δ 67.4) as the external reference]: δ 71.37, 72.14, 74.04 (m), 75.81 (dd, *J* 5.50 and 2.44 Hz), 77.50 (d, *J* 6.10 Hz), 77.71 (t, *J* 6.10 Hz); ^{31}P (40 mg in 3.1 mL, pD 6.04, 109 MHz): δ 1.91, 2.06, 3.34. Anal. Calcd for $C_6H_{12}Na_3O_{15}P_3 \cdot 3H_2O$: C, 13.34; H, 3.36. Found: C, 13.18; H, 3.15.

D-Ins(1,4,6)P_3 (Na salt): $[\alpha]_D^{20}$ −8.9° (*c* 0.90, H_2O).

L-Ins(1,4,6)P_3 (Na salt): $[\alpha]_D^{20}$ +9.4° (*c* 0.85, H_2O) for the trisphosphate derived from the PLE-A-catalyzed hydrolysis product; $[\alpha]_D^{20}$ +9.3° (*c* 1.0, H_2O) for the compound derived from the lipase CES-catalyzed acetylation product.

*Enzymatic hydrolysis of (±)-5-O-acetyl-1,2-*O*-cyclohexylidene-*myo*-inositol* (**DL-2**).—Porcine liver esterase A (Amano Pharmaceutical Co., Ltd) (80 mg) was added to the racemic 5-acetate **DL-2** (224 mg, 0.74 mmol) in a phosphoric buffer solution (3 mL), and the mixture was stirred at room temperature for 12 h. After addition of MeOH (ca. 20 mL) and filtration of the resulting precipitate, the filtrate was chromatographed on silica gel (20:10:1 → 20:10:2 EtOAc–CH_2Cl_2–MeOH) to afford 1L-1,2-*O*-cyclohexylidene-*myo*-inositol (**L-1**, 98 mg, 50% yield, 74% ee) which was then recrystallized twice from MeOH, giving **L-1** (85 mg, 96% ee) [11]; $[\alpha]_D^{22}$ +36.0° (*c* 0.95, MeOH); mp 188–189°C. The other product, 1D-5-*O*-acetyl-1,2-*O*-cyclohexylidene-*myo*-inositol (**D-2**; 5–10% yields), was isolated after several recrystallizations from MeOH–Et_2O in order to remove the acetyl-migration product and increase the optical purity; $[\alpha]_D^{20}$ −29.5° (*c* 1.05, MeOH); mp 170–172.5°C (from MeOH–Et_2O).

Each enantiomer was transformed by procedures similar to those described above for racemic compounds where the physical data of optically active derivatives are attached.

Acknowledgments

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