Inhibition of *myo*-Inositol 1-Phosphatase by *cis*-1,2,3-Cyclohexanetriol 1-Phosphate, a Molecular Simplification of the Natural Substrate

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INTRODUCTION

myo-Inositol 1-phosphatase (mIPase), an enzyme involved in the phosphatidylinositol signal transduction pathway, is known to be inhibited by lithium at concentrations comparable to those used therapeutically (1). This enzyme converts both isomers of *myo*-inositol 1-phosphate (1) to *myo*-inositol and plays a role in the formation of phosphatidylinositol (Scheme I). Disruption of *myo*-inositol metabolism may lead to a decline in membrane phosphoinositide levels and may lead to a decrease in the sensitivity of receptor mechanisms requiring inositol phospholipids (2–4). Furthermore, Berridge *et al.* (5) have shown that the action of lithium *in vivo* may be rather selective. Lithium appears to be maximally effective against those cells whose phosphatidylinositol-linked receptors are being abnormally stimulated. Lithium could therefore preferentially affect those receptor pathways that are abnormally active and normalize the neuronal pathways that are responsible for both mania and depression (5,6). These actions would be confined primarily to the central nervous system, since peripheral cells can readily obtain *myo*-inositol from the blood. Due to its high polarity, plasma inositol cannot penetrate the blood–brain barrier and cannot be utilized by central cells (7).

If the recently proposed theory for lithium's mechanism of action is correct, then it should be possible to develop alternatives to lithium in the treatment of manic-depressive disorder. Substrate-based inhibitors of mIPase should mimic the therapeutic actions of lithium; however, toxicities and contraindications of lithium that are independent of phosphoinositide metabolism should not be exhibited by these compounds. In order to design effective inhibitors of this enzyme, it is important to establish which of the five hydroxy groups present in the natural substrate are essential for substrate recognition. Hallcher and Sherman (1) have reported that α-glycerophosphate (2) and β-glycerophosphate (3) can act as substrates for mIPase isolated from testes, thus indicating that substrate recognition can occur with as few as two hydroxy groups in addition to the labile phosphate group. Additionally, work by Baker and associates (8) has suggested that different roles may be assigned to different hydroxy groups. These authors propose that the 2-hydroxy group of 1 is essential for binding and that the 6-hydroxy group is required for the hydrolytic mechanism.

To investigate further the roles of the hydroxy groups of 1, we have designed a series of isomeric 1,2,3-cyclohexanetriol monophosphates. α-Glycerophosphate can be superimposed with either carbons 1, 2, and 3 or carbons 1, 5, and 6 of *myo*-inositol 1-phosphate, while β-glycerophosphate can be superimposed with carbons 1, 2, and 6. The cyclohexanetriol monophosphates corresponding to these three possibilities conformationally restrict the flexibility of α- and β-glycerophosphate and should provide some information regarding the role of these hydroxy groups in the binding and hydrolysis of *myo*-inositol 1-phosphate by mIPase. In this preliminary paper, we report the synthesis of the all-*cis* isomer of 1,2,3-cyclohexanetriol 1-phosphate (4) as well as its effects on mIPase.

MATERIALS AND METHODS

Chemistry

Melting points were determined in capillary tubes on a Thomas–Hoover apparatus and are uncorrected. ^{1}H-NMR spectra were routinely recorded on a Varian EM 360 (60-MHz) or a Bruker WH-300 (300-MHz) spectrometer. Chemical shifts are expressed as parts per million (δ) downfield from the internal standard tetramethylsilane (TMS). Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, and Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of calculated values. IR spectra were obtained on a Perkin–Elmer 1430 infrared spectrometer and were run as KBr pellets except for compound **8**, which was run as a neat liquid. Boiling points are uncorrected. TLC was performed on silica gel plates (silica gel 60 F_{254}) or cellulose plates (20 μ*M* F_{254}). All chemicals were obtained from Aldrich Chemical Co. and used without purification.

cis-*2,3-Dihydroxycyclohexanone (6).* To a mixture of 50 ml of THF and 50 ml of water were added 2-cyclohexen-1-one (5; 4.8 ml, 50 mmol), 7.5 g of $KClO_3$, and 1 ml of OsO_4 (4% in water). The mixture was stirred at room temperature for 72 hr and then filtered. THF was removed via rotavapor apparatus, and the residue was washed once with CCl_4. The aqueous phase was then extracted with 3 × 50 ml CH_2Cl_2. The combined CH_2Cl_2 layers were dried over $MgSO_4$, and the solvent was removed. The product was recrystallized from methanol to provide white crystals (4.5 g, 70%); mp 78–80°C; ^{1}H-NMR ($CDCl_3$) δ 4.40 (m, 1 H, C_3-H), 4.17 (d, J = 4 Hz, 1 H, C_2-H), 3.2 (s, 2 H, C_2 & C_3-OH, exchanged with D_2O), 1.9–2.7 (m, 6 H, CH_2); IR (KBr, cm^{-1}) 3410 and 3470 (OH), 1720 (C=O).

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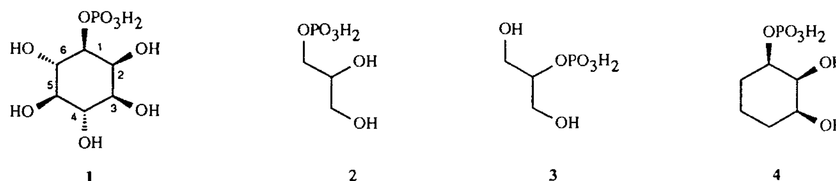


Scheme I

cis-*2,3-O-Isopropylidenecyclohexanone (7).* To a solution of acetone (25 ml) and 2,2-dimethoxypropane (25 ml) was added **6** (2.6 g, 20 mmol). A catalytic amount of *p*-toluenesulfonic acid (50 mg, 0.26 mmol) was added, and the solution was stirred at room temperature overnight. The solution was neutralized with 10% NaOH and filtered if necessary. The solvent was removed, and the residue was dissolved in 50 ml CH_2Cl_2 and successively washed with 3 × 50 ml water, 50 ml brine, and dried over $MgSO_4$. The CH_2Cl_2 was removed, and the residue was recrystallized from ether to afford white needles (3.14 g, 92%); mp 75–76°C. ^{1}H-NMR ($CDCl_3$) δ 4.75 (m, 1 H, C_3-H), 4.3 (d, J = 5 Hz, 1 H, C_2-H), 1.75–2.6 (m, 6 H, CH_2), 1.45 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3); IR (KBr, cm^{-1}) 1720 (C=O), 1360 and 1375 [$C(CH_3)_2$].

cis-*2,3-O-Isopropylidenecyclohexanol (8).* $NaBH_4$ (0.83 g, 22 mmol) was slowly added to a cooled (ice bath) solution of 7 (3.4 g, 20 mmol) in methanol (50 ml). The whole mixture was stirred at 4°C for 1 hr, then 50 ml of water was added. The methanol was removed, and the aqueous layer was extracted with 3 × 50 ml CH_2Cl_2. The combined CH_2Cl_2 layers were dried over $MgSO_4$, and the solvent was removed. The residue was distilled to yield a clear liquid (3.3 g, 95%); bp 108–110°C (25 mm Hg). ^{1}H-NMR ($CDCl_3$) δ 4.23 (m, 2 H, C_2-H and C_3-H), 3.78 (m, 1 H, C_1-H), 2.90 (s, 1 H, C_1-OH, exchanged with D_2O), 1.71 (m, 4 H, CH_2), 1.61 (m, 1 H, CH_2), 1.53 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3), 1.23 (m, 1 H, CH_2); IR (neat, cm^{-1}) 3200–3600 (br, OH), 1360 and 1375 [$C(CH_3)_2$]; *Anal.* ($C_9H_{16}O_3$) C,H.

cis-*2,3-O-Isopropylidenecyclohexanol-1-phosphate (9).* A solution of **8** (1.7 g, 10 mmol) in 30 ml CH_2Cl_2 and 2.0 ml Et_3N (15 mmol) was placed in a three-necked round-bottom flask connected with a N_2 inlet and was cooled to 0°C. The mixture was stirred for 5 min and a solution of $POCl_3$ (1.5 ml, 15 mmol) in 20 ml CH_2Cl_2 was added dropwise. The whole mixture was stirred at 0°C for 1 hr and then overnight at room temperature. Water (50 ml) was added, and the solution was stirred for another 15 min. The CH_2Cl_2 phase was washed with 5 × 50 ml 10% HCl, 2 × 50 ml water, 50 ml brine, and was filtered over $MgSO_4$. The solvent was removed, and the reddish liquid was used in the next step without further purification.

cis-*1,2,3-Cyclohexanetriol-1-phosphate (4).* Compound **9** was added to a mixture of 15 ml water and 15 ml HOAc. The mixture was refluxed at 80°C for 1 hr, and then the solvent was removed. The residue was recrystallized from ethanol to give a white powder (0.6 g, 29% from **8**); m.p. 160–162°C. ^{1}H-NMR (D_2O) δ 4.0 (m, 2 H, C_2-H and C_3-H), 3.0 (m, 1 H, C_1-H), 1.4–1.8 (m, 6 H, CH_2); IR (KBr, cm^{-1}) 3100–3500 (br, OH), 1210 (P=O), 1020 (P-O-C); *Anal.* ($C_6H_{13}O_6P$) C,H,P.

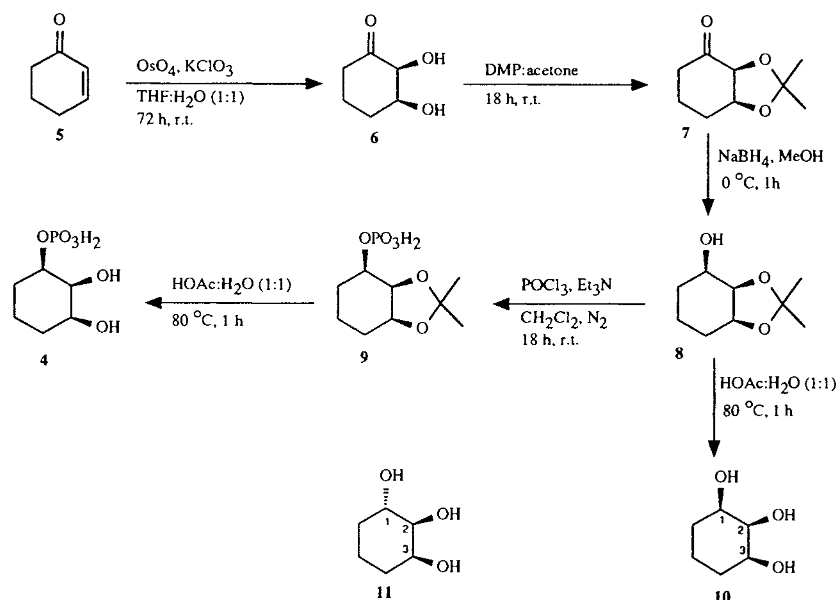
1α,2α,3α-Cyclohexanetriol (10). Compound **8** (0.87 g, 5 mmol) was treated in the same manner as described above for compound **9**. The residue was recrystallised from ethanol and produced a white powder (0.42 g, 63%); m.p. 145–146°C [lit., 146–148°C (9)]. ^{1}H-NMR (D_2O) δ 3.7 (t, J = 2.5 Hz, 1 H, C_2-H), 3.5 (m, 2 H, C_1-H and C_3-H), 1.2–1.7 (m, 6 H, CH_2); ^{13}C-NMR (D_2O) 21.84 (C_5), 29.27 (C_4 and C_6), 73.32 (C_1 and C_3), 75.60 (C_2); IR (KBr, cm^{-1}) 3250–3400 (br, OH); *Anal.* ($C_6H_9O_3$) C,H.

Biology

myo-Inositol 1-phosphatase was purified from bovine brain, and enzyme activity was determined by measuring the release of inorganic phosphate according to the methods of Meek *et al.* (10). The specific activity of the enzyme preparation was 1.6 units/mg with β-glycerophosphate as the substrate. Initial rate data in the presence and absence of the inhibitor were subjected to Hanes analysis for the determination of K_i (11).

RESULTS AND DISCUSSION

The synthesis of the all-*cis* target compound was accomplished as outlined in Scheme II. Commercially available 2-cyclohexenone was treated with a catalytic amount of osmium tetroxide in the presence of potassium chlorate to afford the *syn*-dihydroxylated compound **6**. This diol was then protected using an equimolar mixture of acetone and dimethoxypropane. The ketone in the resulting acetonide (**7**) was then reduced with sodium borohydride to provide alcohol **8** in a 95% yield. Apparently, the *cis* acetonide present in compound **7** provides sufficient steric hindrance that hydride attack occurs exclusively from the opposite side to provide an all-*cis* product. Dumortier *et al.* (12) have previously reported the synthesis of compounds **6**, **7**, and **8** using similar conditions; however, in their communication, they did not report any detailed experimental methods or physical characteristics for these compounds. This information is included here in the above section. Once we had purified compound **8**, it was critical that we verified the stereochemical relationships among the three oxygen functionalities. We verified this relationship by converting acetonide **8** to its corresponding 1,2,3-cyclohexanetriol (**10**). The three possible diastereomers of 1,2,3-cyclohexanetriol have been characterized previously (9) and possess sufficiently distinct melting points and 60-MHz NMR splitting patterns to allow unambiguous identification. The compound we obtained had a melting point of 145–146°C (lit., 148°C) and had an NMR splitting pattern identical to that reported for the all-*cis* isomer of 1,2,3-cyclohexanetriol. The key feature in this NMR spectrum was the presence of a triplet (J = 2.5 Hz) at 3.75 ppm. This triplet corresponds to the C_2 proton and arises from its axial-equatorial coupling with the equivalent C_1 and



Scheme II

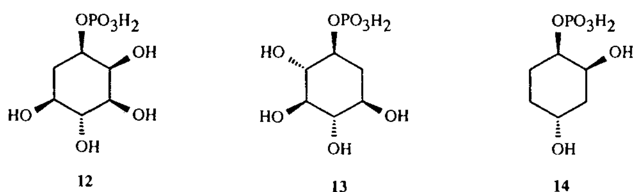
C_3 protons. 1α,2β,3β-Cyclohexanetriol (**11**), the only other diastereomer which could result from the reduction of **7** following acetonide cleavage, has a melting point of 125°C and a considerably more complex NMR splitting pattern (9).

1α,2β,3β-Cyclohexanetriol, unlike **10**, is not symmetrical and the C_1 and C_3 protons are not equivalent, thus causing noticeable differences in their respective NMR spectra. Additionally, ^{13}C-NMR of our compound revealed only four signals. Such a spectrum could be produced only by a symmetrical structure such as **10**. Compound **11**, being unsymmetrical, would have given six signals. Once we had established that compound **8** possessed the desired stereochemistry, the synthesis of our target compound was completed using a two-step process. Phosphorylation of alcohol **8** was accomplished using phosphorous oxychloride and triethylamine under a nitrogen atmosphere; however, the resulting phosphate (**9**) proved to be unstable. This intermediate was therefore not isolated but was immediately treated with a 1:1 mixture of acetic acid and water to produce the desired compound (**4**).

Once synthesized, compound **4** was evaluated for its ability to act as either a substrate or an inhibitor of mIPase using previously published methods (10). Hanes analysis (11) of the initial rare data revealed **4** to be a competitive inhibitor, with a K_i of 0.56 mM. The competitive nature of the inhibition was indicated by the parallel curves which were obtained in a standard Hanes plot. In contrast, **4** was unable to function as a substrate for mIPase at concentrations up to 5 mM.

From both a synthetic and a drug design standpoint, it is important to determine which of the five hydroxyl groups in *myo*-inositol 1-phosphate are essential for substrate recognition. Once the key hydroxy groups and their required stereochemical relationships have been determined, this information can be used to develop a model of substrate binding and to design substrate-based inhibitors of mIPase which are stereochemically less complex than the natural substrates. In addition to being synthetically more accessible, these compounds would be less polar by virtue of a reduced number of hydroxy functionalities. The latter point is especially crucial considering that mIPase inhibition must occur centrally if it is to be of any value in treating manic-depressive disorder (5).

Compound **4** can be considered either as a rigid analogue of α-glycerophosphate (**2**) or as a molecular simplification of *myo*-inositol 1-phosphate (**1**). The biological results seen with this compound are consistent with and complement earlier reports by Baker and his associates (8,13). These investigators reported that 6-deoxyinositol 1-phosphate (**12**) was not hydrolyzed by mIPase but instead functioned as a competitive inhibitor of the enzyme (IC_{50} = 70 μM) (Scheme III). Additionally, they found that 2-deoxyinositol 1-phosphate (**13**) was a weak substrate for the enzyme. They therefore proposed that the 2-hydroxy group was necessary for strong binding to the enzyme and that the 6-hydroxy group was essential for the hydrolytic mechanism (8). The competitive inhibition shown by compound **4** supports this hypothesis. Considering this compound as a molecular simplification of the natural substrate, it can be seen that it possesses the 2-hydroxy group necessary for binding but lacks the 6-hydroxy group necessary for phosphate hydrolysis. The approximately 10-fold lower potency of this compound compared to **12** can obviously be attributed to the 4- and 5-hydroxy groups present in **12** but absent in the current target compound. It appears that most of this activity loss is due to the absence of the 4-hydroxy group. In a paper which appeared while the current work was in progress,



Scheme III

Baker *et al.* (13) reported the synthesis and activity of (1β)-phosphoryloxy-(2β,4α)-dihydroxycyclohexane (14). This compound proved to be a potent inhibitor of mIPase, possessing an IC_{50} value of 7 μ*m*. The design of 14 was based on a superposition of D- and L-*myo*-inositol 1-phosphate and a hypothesis that the 4-hydroxy group is much more important to the binding of these substrates than either the 3- or the 5-hydroxy group. The activity of compound 4 reported here, coupled with the previously reported activity of compound 14, definitely supports the notion that the 4-hydroxy group is much more important to binding than is the 3-hydroxy group.

In summary, *cis*-1,2,3-cyclohexanetriol 1-phosphate (4) is a weak inhibitor of *myo*-inositol 1-phosphatase. Its activity in conjunction with the activity of previously reported compounds should aid in the development of a model for substrate and inhibitor binding to this important enzyme.

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REFERENCES

1. L. M. Hallcher and W. R. Sherman. The effects of lithium ion and other agents on the activity of *myo*-inositol-1-phosphatase from bovine brain. *J. Biol. Chem.* 255:10896–10901 (1980).
2. M. J. Berridge. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* 220:345–360 (1984).
3. S. K. Fisher and B. W. Agranoff. Receptor activation and inositol lipid hydrolysis in neural tissues. *J. Neurochem.* 48:999–1017 (1987).
4. M. J. Berridge and R. F. Irvine. Inositol phosphates and cell signalling. *Nature* 341:197–205 (1989).
5. M. J. Berridge, P. Downes, and M. R. Hanley. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* 206:587–595 (1982).
6. P. F. Worley, W. A. Heller, S. H. Snyder, and J. M. Baraban. Lithium blocks a phosphoinositide-mediated cholinergic response in hippocampal slices. *Science* 239:1428–1429 (1988).
7. R. U. Margolis, R. Press, N. Altszuler, and M. A. Stewart. Inositol production by the brain in normal and alloxan-diabetic dogs. *Brain Res.* 28:535–539 (1971).
8. R. Baker, J. J. Kulagowski, D. C. Billington, P. D. Leeson, I. C. Lennon, and N. Liverton. Synthesis of 2- and 6-deoxy-inositol 1-phosphate and the role of the adjacent hydroxy groups in the mechanism of inositol monophosphatase. *J. Chem. Soc. Chem. Commun.* 1383–1385 (1989).
9. G. E. McCasland, M. O. Naumann, and L. J. Durham. Alicyclic carbohydrates. XXX. Synthesis of the remaining cyclohexanetriols. Nuclear magnetic resonance studies on the nine isomers. *J. Org. Chem.* 31:3079–3089 (1966).
10. J. L. Meek, T. J. Rice, and E. Anton. Rapid purification of inositol monophosphate phosphatase from beef brain. *Biochem. Biophys. Res. Commun.* 156:143–148 (1988).
11. I. H. Segel. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems.* John Wiley & Sons, New York, 1975.
12. L. Dumortier, J. Van der Eycken, and M. Vandewalle. The synthesis of chiral isopropylidene derivatives of 1,2,3-cyclohexanetriols by enzymatic differentiation. *Tetrahedron Lett.* 30:3201–3204 (1989).
13. R. Baker, P. D. Leeson, N. J. Liverton, and J. J. Kulagowski. Identification of (1*S*)-phosphoryloxy-(2*R*,4*S*)-dihydroxycyclohexane as a potent inhibitor of inositol monophosphatase. *J. Chem. Soc. Chem. Commun.* 462–464 (1990).