Ab Initio Studies of Lipid Model Species. 2. Conformational Analysis of Inositols

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Abstract: Molecular species containing inositol are among the most ubiquitous naturally occurring compounds. For example they are commonly found in biomembranes as one of the few head groups of phospholipids. To help characterize their structural properties we have studied the conformational preferences of inositol. It has long been held that among the eight possible cis–trans isomers of inositols shown in Figure 1, the all-equatorial (6e) *scyllo*-inositol is intrinsically the most stable. However we report here ab initio calculations using Hartree–Fock and second-order Møller–Plesset perturbation methods and also calculations based on density–functional methods, all of which show that this long held view on conformational analysis of isolated inositol molecules is questionable. We have found that the naturally much more abundant *myo*-inositol, with five equatorial and one axial hydroxyl groups (5e/1a), and the nonnaturally occurring *neo*-inositol with four equatorial and two axial hydroxyls (4e/2a), tend to be slightly lower in energy than *scyllo*-inositol. In terms of the free energy, contributions from nuclear motions also favor *myo*-inositol over *scyllo*-inositol, making the former consistently more stable. Although an axial hydroxyl group introduces strain energy primarily due to 1,4 $O_{axial}\cdots O$ and $O_{axial}\cdots C$ repulsions, it can form more favorable intramolecular hydrogen bonds, which appear to be dominant. The $O_{axial}\cdots H_{axial}C$ interaction also favors the axial hydroxyl orientation. On the other hand, when all intramolecular hydrogen bonds in *scyllo*- and *myo*-inositols are removed by properly orienting OH groups, as may occur in aqueous solution or crystals, *scyllo* becomes lower in energy than *myo*. Calculations were also carried out on phosphoryl inositols, and the results indicate that axial phosphate substitution may be favored as well. These effects were analyzed in terms of the cyclohexane ring structures and compared with experimental results.

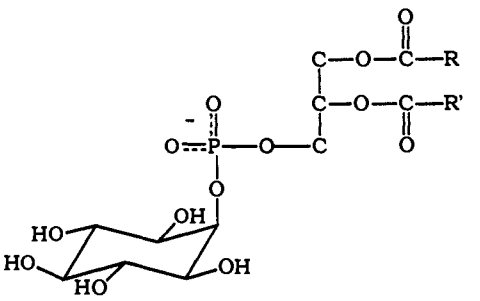
Introduction

Derivatives of inositols, 1,2,3,4,5,6-hexahydroxylcyclohexanes, are widely found in all biological systems, particularly associated with biomembranes.[1] They exist principally as inositol phosphates with one to six phosphates esterified to one inositol. Their biological functions include intracellular communication, phosphate storage and transfer, and they provide the predominant means for covalent anchoring of proteins to membranes.[1] In biomembranes they exist in inositol phosphatides as one of the few head groups forming the structures of naturally occurring phospholipid bilayers (Chart 1 illustrates a phosphatidyl-*myo*-inositol).

As part of a project to study lipid model species in pursuit of a better understanding of biomembranes[2] we have studied the molecular structures, particularly conformational preferences, of inositol. These calculations were carried out also for the purpose of generating better force fields for subsequent phospholipid/biomembrane simulations.[2,3] But in this paper we will focus only on the conformational analysis of inositols and inositol phosphates.

There are eight possible cis–trans isomers with one of them, *dl*, being an enantiomeric pair (Figure 1), making nine distinct

Chart 1



stereoisomers. Among these nine the *scyllo*-, *dl* pair, and *myo*-inositols occur in nature with the *myo*-inositol, which has five equatorial and one axial (5e/1a) hydroxyl groups, being the most abundant.[4,5] We have carried out *ab initio* calculations on, apparently, the five lowest energy inositols: 6e *scyllo*, 5e/1a *myo*, and three 4e/2a isomers: *dl*, *epi*, and *neo*. An important and interesting property of the conformational analysis follows from the following observation: while common knowledge on conformational analysis of cyclohexane derivatives suggests that the all-equatorial (6e) *scyllo*-inositol should be the most stable isomer, it is in fact the 5e/1a *myo* isomer that is naturally the most abundant inositol. In fact, it is *myo*-inositol which is one of the most ubiquitous of naturally occurring compounds and is assumed to be present in nearly all living cells.[4]

The conformational analysis of inositols dates back at least to the 1950s and 1960s because, in addition to their important biological functions, they represent a valuable set of models for

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(1) (a) Stephens, L. R.; Hawkins, P. T.; Stanley, A. F.; Moore, T.; Poyner, D. R.; Morries, P. J.; Hanley, M. R.; Kay, R. R.; Irvine, R. F. *Biochem. J.* **1991**, *275*, 485. (b) Taylor, C. W.; Richardson, A.; *Pharmac. Ther.* **1991**, *51*, 97. (c) Ferguson, M. A. J. *Ann. Rev. Biochem.* **1988**, *57*, 285.

(2) (a) Liang, C.; Ewig, C. S.; Stouch, T. R.; Hagler, A. T. *J. Am. Chem. Soc.* **1993**, *115*, 1537. (b) Stouch, T. R.; Ward, K. B.; Altieri, A.; Hagler, A. T. *J. Comput. Chem.* **1991**, *12*, 1033. (c) Williams, D. E.; Stouch, T. R. *J. Comput. Chem.*, in press. (d) Stouch, T. R.; Williams, D. E. *J. Comput. Chem.*, in press. (e) Stouch, T. R.; Williams, D. E. *J. Comput. Chem.* **1992**, *13*, 622.

(3) (a) Maple, J. R.; Dinur, U.; Hagler, A. T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5350. (b) Dinur, U.; Hagler, A. T. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D. B., Eds.; VCH: New York, 1991; Vol. 2.

(4) (a) Anderson, L. In *The Carbohydrates*; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1972; p 519. (b) Angyal, S. J.; Anderson, L. In *Advances in Carbohydrate Chemistry*; Wolfrom, M. L., Tipson, R. S., Eds.; Academic Press: New York, 1959; p 135.

(5) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. *Conformational Analysis*; Interscience: New York, 1965.

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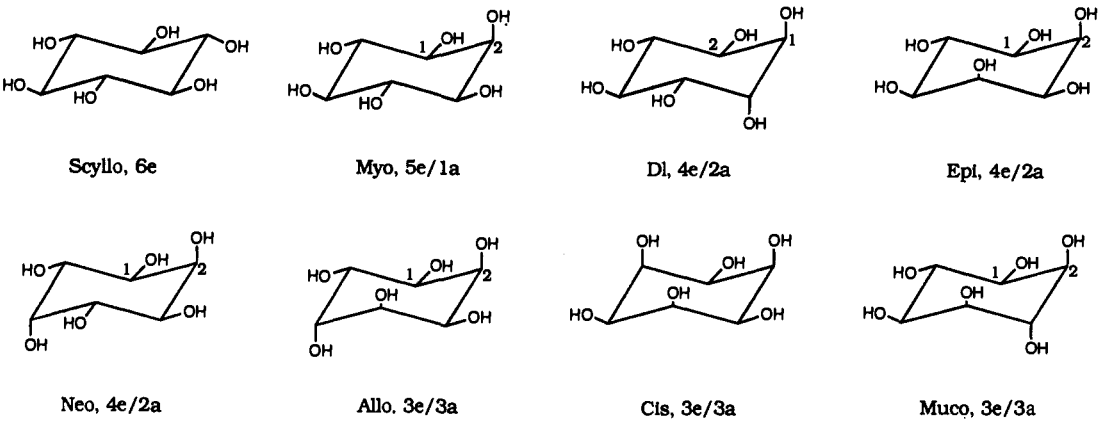


Figure 1. The eight cis–trans isomers of inositol. The numbering of the first two carbons are also given.

investigating general conformational effects in substituted cyclohexanes, and they have therefore received considerable attention.[4,5] It has been widely assumed that axial substituents such as hydroxyl groups, particularly when cumulated on one side of the cyclohexane ring, introduce appreciable additional strain energy.[4-6] Early experiments, based primarily on borate complexation, estimated this extra strain energy to be 0.9 kcal/mol for one axial hydroxyl; i.e., *myo*-inositol should be less stable than *scyllo*-inositol by that much energy.[6] There have also been many other (more indirect) studies on the conformational energies of inositols, all of them seeming to support this general conclusion.[4,5] Thus, nature's preference for *myo*-inositol seems to contradict the conventional wisdom of conformational analysis from an energetic point of view.

Other important experimental studies on inositols include crystal structural determinations for *myo*-[7] and *epi*-inositols[8] and more recently a vibrational spectral study of all the inositols.[9] However to our knowledge no *ab initio* calculation on any of these species has been reported, and therefore this study represents the first attempt.

In this contribution we report gas-phase results obtained employing various *ab initio* theoretical methods to show that, contrary to the traditional view on relative conformational energies of inositols, the 5e/1a *myo*- and 4e/2a *neo*-inositols are *lower* in energy than the 6e *scyllo*-inositol. Contributions from nuclear motions also favor *myo*-inositol over *scyllo*-inositol, making the former consistently more stable. Some of the factors, particularly in terms of the molecular structures, that contribute to these qualitative results are probed. This study also provides valuable information for developing and testing empirical force fields to be used in simulating carbohydrates and phospholipids.

Computational Methods

We first experimented with choice of basis sets for the geometry optimizations. The structures of *scyllo*- and *myo*-inositols were fully optimized using the 6-31G* basis set[10] in conjunction with the Hartree–Fock (HF) approximation. Then diffuse functions[10] were added to the heavy atoms (yielding 6-31+G*), and those structures were reoptimized. No significant changes in the optimized geometries were observed (the most notable change being in C–O torsion, by less than 2°). We further tested the importance of p-polarization functions and/or s-diffuse functions[10] on hydrogens (i.e., the 6-31G**, 6-31+G**, and 6-31++G** basis sets). The results indicated that these additional functions are also not essential for structures (the most significant change being a shortening of OH bonds by about 0.004 Å). Furthermore, Levy and Perdew have shown that for methods such as Hartree–Fock the change in the energy error, upon geometry change, is zero through second order.[11] This means that modest basis sets may be good enough for geometries. Thus, we chose the more economical 6-31G* basis set for further structural determinations. For each of the inositol a/e forms (*scyllo*, *myo*, *dl*, *epi*, and *neo*) we optimized the full molecular structure beginning with a concerted intramolecular hydrogen bonding arrangement of the hydroxyl groups (Figure 2). The harmonic vibrational frequencies at these structures were also determined using the HF/6-31G* method, and they were used in evaluating thermal energy contributions to the free energy.[12]

Subsequent single-point calculations were performed at these optimized structures to determine relative energies. Interestingly, although the 6-31+G* basis set does not appreciably change the 6-31G* predicted geometries, it does change the relative energies. On the other hand, polarization and/or diffuse functions on hydrogens were found to have little effect on energies as well as on geometries (vide supra). Thus, we included only the 6-31+G* in addition to the 6-31G* basis set in our energy evaluations. Electron correlation effects, which are important for systems with intramolecular hydrogen bonding,[13] were considered via the second-order Møller–Plesset perturbation (MP2) method (excitations from core orbitals were neglected).[14]

We also employed methods based on density functional theory (DFT),[15,16] an approach that has recently received increasing attention.[17] Thus, we employed the local density functionals of Vosko, Wilk, and Nusair (VWN) with or without Becke–Perdew (BP) nonlocal corrections in conjunction with the DZVP + Al basis set (for P, 6321/521/1; for C and O, 621/41/1; for H, 41/1).[16] These methods (dubbed VWN/DZVP + Al and BP/DZVP + Al) have been shown to be quite successful in treating a number of hydrogen bonding systems[16] and have the advantage of being able to handle relatively large species. The DZVP basis set in DFT is approximately equivalent to the 6-31G** basis in conjunction with the HF method, but diffuse functions are not as important in DFT as in HF. The Al (auxiliary) basis set was used for exchange-correlation potentials, and a "fine" numerical grid was used in all DFT calculations.[15,16] We estimate that the relative energy error between various molecular structures introduced by numerical integration over this grid is approximately 0.2 kcal/mol for VWN but possibly as high as 0.5 for BP.

Hartree–Fock calculations were performed using the direct method implemented in GAUSSIAN90,[18] while direct MP2 calculations were

(6) Angyal, S. J.; McHugh, D. J. *Chem. & Ind. (London)* **1956**, 1147.
(7) (a) Lomer, T. R.; Miller, A.; Beevers, C. A. *Acta Crystallogr.* **1963**, *16*, 264. (b) Rabinowitz, I. N.; Kraut, J. *Acta Crystallogr.* **1964**, *17*, 159.
(8) Jeffrey, G. A.; Kim, H. S. *Carbohydr. Res.* **1970**, *15*, 310. *Acta Crystallogr.* **1971**, *B27*, 1812.
(9) Williams, R. M.; Atalla, R. H. *J. Phys. Chem.* **1984**, *88*, 508.
(10) (a) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta (Berl.)* **1973**, *28*, 213. (b) Clark, T.; Chandrasekhar, J.; Spitznagel, G. W.; Schleyer, P. v. R. *J. Comput. Chem.* **1983**, *4*, 294.
(11) Levy, M.; Perdew, J. P. *J. Chem. Phys.* **1986**, *84*, 4519.
(12) Ewig, C. S. In *Topics in Chemistry*; Datta, S. N., Ed.; Omega Scientific: New Delhi, 1992; p 65.
(13) Novoa, J. J.; Tarron, B.; Whangbo, M. H.; Williams, J. M. *J. Chem. Phys.* **1991**, *95*, 5179.
(14) Pople, J. A.; Binkley, J. S.; Seeger, R. *Int. J. Quantum Chem. Symp.* **1976**, *10*, 1.
(15) Andzelm, J.; Wimmer, E. *J. Chem. Phys.* **1992**, *96*, 1280 and references therein.
(16) Sim, F.; St-Amant, A.; Papai, I.; Salahub, D. R. *J. Am. Chem. Soc.* **1992**, *114*, 4391 and references therein.
(17) Gill, P. M. W.; Johnson, B. G.; Pople, J. A.; Frisch, M. J. *Chem. Phys. Lett.* **1992**, *197*, 499.
(18) Frisch, M. J.; Head-Gordon, M.; Trucks, G. W.; Foresman, J. B.; Schlegel, H. B.; Raghavachari, K.; Robb, M.; Binkley, J. S.; Gonzalez, C.; Defrees, D. J.; Fox, D. J.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Martin, R. L.; Kahn, L. R.; Stewart, J. J. P.; Topiol, S.; Pople, J. A. GAUSSIAN90 Revision I; Gaussian, Inc.: Pittsburgh, PA, 1990.

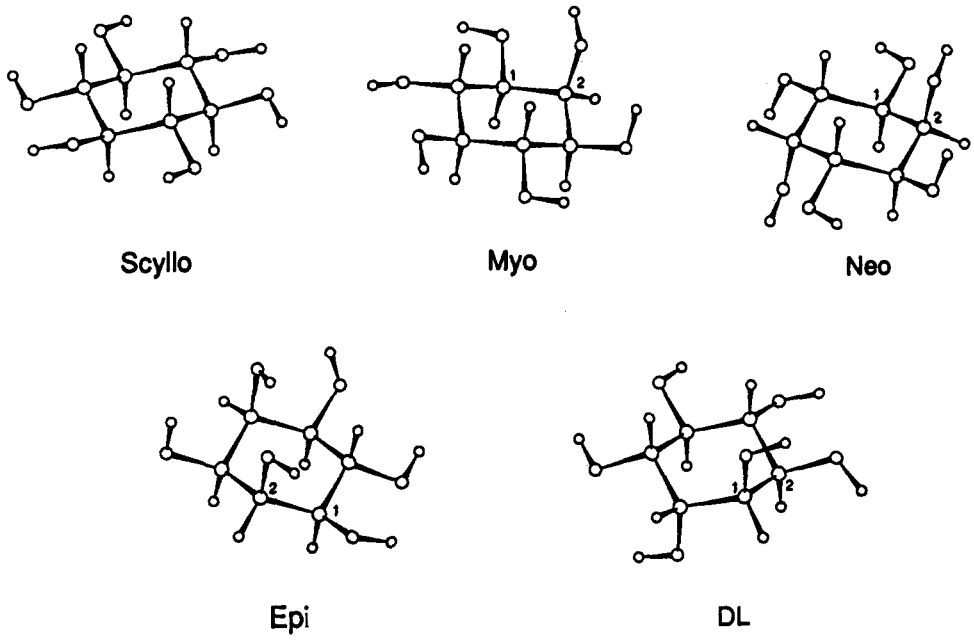


Figure 2. The HF/6-31G* optimized structures for *scyllo*-, *myo*-, *neo*-, *epi*- and *dl*-inositols.

Table 1. Important Torsion Angles of Some High Energy Conformers of *scyllo* and *myo*-Inositols and Their Energies Relative to *scyllo*-Inositol of Table 3[a]

energy	*scyllo* (chair) 7.12		*myo* (chair) 9.26		*myo* (boat) 11.23	
torsion	HC_iOH	$CC_iC_{i+1}C$	HC_iOH	$CC_iC_{i+1}C$	HC_iOH	$CC_iC_{i+1}C$
i =						
1	−72.1	−51.7	−158.3	51.7	−72.8	−54.6
2	−177.4	52.9	176.7	−53.0	−120.1	21.6
3	74.5	−55.5	152.4	55.5	−58.1	37.7
4	−63.0	57.0	−70.1	−57.6	71.3	−68.4
5	65.0	−57.8	63.6	56.2	−55.8	34.8
6	−64.4	55.2	−66.6	−52.6	50.9	24.8

[a] HF/6-31G* results. Energy in kcal/mol, angles in deg. The atom numbering is the same as that shown in Figure 1.

done using TURBOMOLE[19] as well as GAUSSIAN90. DFT calculations were carried out using the deMon program.[20] All calculations were performed on IBM RS/6000 workstations.

Results and Discussion

The optimized inositol structures are shown in Figure 2. In addition to these structures we also considered several other conformations with differing hydroxyl hydrogen orientations. They were all found to be higher in energy and therefore will not be discussed here. Their energetic and important structural information are given in Table 1. In the following we shall first discuss our results for the molecular structures in comparison with available crystal data. We will then examine the computed energies at a temperature of 0 K in the gas phase. The computed Gibbs free energies at finite temperature will be presented next and compared with experiment. The role of hydrogen bonding in inositols will be discussed in some detail. Finally, we describe preliminary results for two inositol phosphates to study the effects of equatorial and axial phosphate substitution on their structures and energies.

A. Structures. Although no symmetry was imposed in optimizing the geometries in Figure 2. *scyllo*- and *neo*-inositols were computed to have S_6 and C_I symmetry, respectively. As one may expect from structures of other carbohydrate compounds such as glucose,[21] the optimized geometries are such that the hydroxyl groups are always oriented to maximize internal hydrogen bonding. (Of course this is not necessarily the case in condensed phases.) For the *scyllo*, *myo*, and *neo* isomers maximum hydrogen bonding is achieved by orienting all OH groups in the same direction, while for the *epi* and *dl* isomers this pattern is interrupted (Figure 2). Since most of the other geometric features are quite normal, the following discussion will focus on the effect of axial substitution, particularly on the cyclohexane ring conformation.

For bond lengths, the C–C bonds in these inositols show no systematic variation with OH positions, and they are relatively short (within 1.520–1.530 Å) compared to those in cyclohexane (1.532 Å, HF/6-31G*). This is due to substitutions by the electronegative hydroxyl groups as predicted by the Bent's rule,[22] which is generally attributed to electron withdrawing reducing the covalent radius of carbon. The axial C–O_a bonds are slightly longer (by 0.003–0.010 Å) than the equatorial C–O_e bonds in *myo*- and *neo*-inositols but not in *epi*-inositol where C–O_a bonds are 0.001–0.015 Å shorter than C–O_e bonds. On average, C–O bonds in the presumably strain-free *scyllo* isomer (1.401 Å) are shorter than those in the others, particularly the *epi*- and *dl*-inositols, in which the longest C–O bonds are 1.413 and 1.417 Å, respectively.

For bond angles, the individual CCC angles do not show correlation with OH a/e positions (Table 2). But the average CCC angles in *myo*- (111.2°), *neo*- (111.7°), *epi*- (111.5°), and *dl*- (111.3°) inositols are 0.4–0.9° larger than those in *scyllo*-inositol (110.8°, Table 2). This suggests that the cyclohexane rings in the former isomers may be slightly flattened (since in the limiting case of a planar cyclohexane ring these angles would be 120°). However the OCC angles are strongly correlated with the orientations of the hydroxyl groups. Thus, in the *scylo*, *myo*, and *neo* isomers an OCC angle on the same side of the OC bond as a hydroxyl group is usually about 4° larger than that on the opposite side (111° vs 107°, see Chart 2 below). This distortion of OCC angles (from a normal tetrahedral angle of 109.5°) may be attributed to intramolecular hydrogen bonding, similar to that

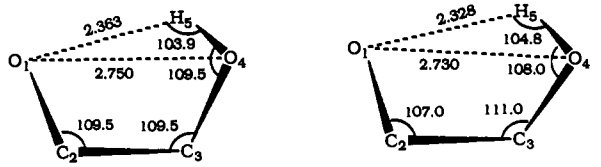
(19) *TURBOMOLE User Guide*, Version 2.1; BIOSYM Technologies, Inc.: San Diego, CA, 1992. For methodology, see: Ahlrichs, R.; Bar, M.; Haser, H.; Horn, H.; Kölmel, C. *Chem. Phys. Lett.* **1989**, *162*, 165.

(20) DeMon, to be distributed by BIOSYM Technologies, Inc.: San Diego, CA. For methodology see refs 15 and 16.

(21) Polavarapu, P. L.; Ewig, C. S. *J. Comput. Chem.* **1992**, *13*, 1255.

(22) Bent, H. A. *J. Chem. Phys.* **1960**, *33*, 1258. *Chem. Rev.* **1961**, *61*, 275.

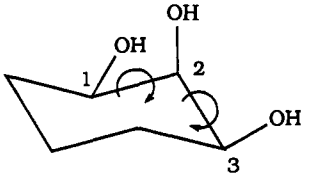
Chart 2



observed in 1,2-ethanediol.[23] Its effect can be illustrated as follows. Using typical bond distances in inositols (C–O, 1.40 Å, C–C, 1.52 Å, O–H, 0.95 Å) and torsional angles of ±56°, when bond angles are 109.5° (Chart 2, left)[24] the O···H and O···O distances are 2.363 and 2.750 Å and the OHO angle is 103.9°. When distorted to OCC angles of 111° and 107° (Chart 2, right), the O···H distance (2.328 Å) becomes shorter and OHO angle (104.8°) larger, thereby more strongly hydrogen bonding. In the mean time, the O···O distance (2.730 Å) is slightly shortened, which should result in stronger repulsion.

The ring torsional angles (Table 2) also seem to be related to OH positions. The CC_1C_2C torsion is usually about 1–3° smaller when C_1 or C_2 has an axial OH group. The average ring torsions in *myo* (55.3°), *neo* (54.0°), *epi* (54.7°), and *dl* (55.2°) are all smaller than that of *scyllo* (56.7°). This indicates that the former four inositols are flattened, reinforcing the observation concerning CCC angles discussed above. This flattening of ring torsions and differences in C–O bonds and CCC angles in *myo*-, *neo*-, *epi*-, and *dl*-inositols as compared to all-equatorial *scyllo*-inositol are all consistent with stereorepulsion involving axial OH group(s).

When compared to available experiments, our computed gas-phase structures for *myo*- and *epi*-inositols are generally in reasonable agreement with their experimental crystal structures.[7,8] The calculated C–C bond lengths are within 0.01 Å of experiment, but the C–O bonds are predicted about 0.03 Å too short, which is typical for this bond by this method because of lack of electron correlation in the Hartree–Fock approximation.[25] Reasonable agreement is also observed between the theoretical and experimental CCC angles (within 2.6°, Table 2) but less so for the OCC angles (differing by about 1–4°) since the hydrogen bonding patterns, which have a large effect on these angles, are very different in the gas phase and the crystal where intermolecular hydrogen bonding dominates.[7,8] For the ring torsional angles, the agreement is poor for *myo*-inositol where the biggest difference is as large as 7.6°. In particular, the crystal structure shows that the torsional angles about the C_1C_2 and C_2C_3 bonds are as large as or larger than the other ring torsions, such as $C_5C_6C_1C_2$. This



trend is the opposite of that observed from the calculated structure (Table 2), which shows the first two torsional angles to be appreciably smaller than all the others. But, as the following and later discussions show, the trend from the calculations agrees

Table 2. Ring Conformations (Bond Angles and Torsional Angles[a]) of *scyllo*-, *myo*-, *neo*-, *epi*-, and *dl*-Inositols

angle	*scyllo*	*myo* (exptl[b])	neo	epi (exptl[c])	*dl*
		Bond Angles			
$C_6C_1C_2$	110.8	111.9 (111.0)	112.2	112.3 (110.8)	111.6
$C_1C_2C_3$		111.6 (109.7)	111.1	111.3 (108.7)	111.5
$C_2C_3C_4$		111.8 (110.6)	111.8	112.0 (114.7)	110.3
$C_3C_4C_5$		111.1 (109.4)		111.0 (110.5)	111.0
$C_4C_5C_6$		110.1 (112.5)		111.7 (110.8)	111.6
$C_5C_6C_1$		110.8 (111.1)		111.7 (109.4)	111.7
av	110.8	111.2 (110.7)	111.7	111.5 (110.8)	111.3
		Torsional Angles			
$C_6C_1C_2C_3$	56.7	52.5 (57)	54.0	55.0 (57)	54.4
$C_1C_2C_3C_4$		−51.9 (−59.5)	−53.8	−54.5 (−53)	−57.0
$C_2C_3C_4C_5$		55.0 (58.5)	54.4	53.8 (52)	57.9
$C_3C_4C_5C_6$		−58.0 (−56)		−53.9 (−54)	−56.4
$C_4C_5C_6C_1$		58.4 (55)		55.2 (60)	53.4
$C_5C_6C_1C_2$		−56.0 (−55)		−55.6 (−62)	−52.3
av	56.7	55.3 (56.8)	54.0	54.7 (56.3)	55.2

[a] Optimized HF/6-31G* results, in deg. Available experimental values are given in parentheses. Note that *scyllo* and *neo* have, respectively, S_6 and C_i symmetry. Only symmetrically unique angles are listed. [b] Rabinowitz, I. N.; Kraut, J. *Acta Crystallogr.* **1964**, *17*, 159. [c] Jeffrey, G. A.; Kim, H. S. *Carbohydr. Res.* **1970**, *15*, 310. *Acta Crystallogr.* **1971**, *B27*, 1812.

with more recent crystal structures of *epi*-inositol and phosphate-substituted *myo*-inositol. The discrepancy between computed and crystal structures for *myo*-inositol may be partially attributed to the experimental uncertainties in this early experimental study and also to crystal forces since comparing the two molecules in an asymmetric unit of the crystal showed considerable differences (e.g., the same CCC angle differs by 1.0–1.8°).[7]

For *epi*-inositol, whose crystal structure has been determined with higher resolution, the agreement between calculation and experiment is better.[8] Although the ring torsions in the crystal showed larger variations (about 10°) than those in our theoretical structure (about 2°), the trend on going about the ring is correctly reproduced (Table 2). The flattening of the cyclohexane ring in *myo*- and *epi*-inositols discussed above is also observed in their crystal structures.

B. Relative Internal Energies. The energetics (not including nuclear motion) for the inositols studied here are reported in Table 3 and show a marked dependence on the theoretical methods. Thus, at the HF/6-31G* level of approximation *neo*-inositol (with four equatorial and two axial hydroxyls) is, surprisingly, the most stable isomer followed by *myo*-inositol (5e/1a) and then by *scyllo*-inositol (6e). But when diffuse functions[10] are added, the relative energies change by as much as 1.6 kcal/mol (for *scyllo* and *neo*) and they lower the energy of *scyllo* more than all others, making it the most stable isomer (HF/6-31+G* results). Inclusion of electron correlation at the MP2 level has even greater effects on the relative energies but with opposite sign: it destabilizes *scyllo* significantly (by as much as 3.8 kcal/mol compared to *neo*), making it the *least* stable one of the five considered here (comparing results from HF and MP2 methods in the 6-31G* basis). When the effects of diffuse functions and electron correlation are considered in a single calculation, the resulting MP2/6-31+G* energies show the following order of decreasing stability: *neo*, *myo*, *epi*, *scyllo*, and *dl*. The large effects of diffuse functions and electron correlation on the relative energies of these inositols are consistent with their degrees of internal hydrogen bonding as will be discussed later.

Since methods based on density functional theory are able to treat, to some extent, electron correlation with large basis sets and have shown promising results in a number of studies,[15-17] we employed the local potential of Vosko, Wilk, and Nusair with or without Becke–Perdew nonlocal corrections in conjunction with the DZVP+Al basis set.[16] As it turns out, while the VWN/DZVP + Al results are close to those from the MP2/6-31G* method, the BP/DZVP + Al relative energies which include

(23) (a) Nagy, P. I.; Dunn, III, W. J.; Alagona, G.; Ghio, C. *J. Am. Chem. Soc.* **1991**, *113*, 6719; **1992**, *114*, 4752. (b) Murcko, M. A.; Dipaola, R. A. *J. Am. Chem. Soc.* **1992**, *114*, 10 010.

(24) One of the referees pointed out that a more appropriate value for a "normal" OCC angle may be 107.3°, which is the experimental OCC angle in ethanol[25] and the 6-31G* calculated OCC angle in the trans conformer of 1,2-ethanediol.[23b] In this case, the O···H and O···O distances in the left figure of Chart 2 would be 2.252 and 2.663 Å, respectively. This may suggest that the O···O repulsion is dominating the interactions between the two hydroxyls. But we note that even in this case it must still be the intramolecular hydrogen bonding which causes one of the two OCC angles change from about 107° to 111° but not the other.

(25) Hehre, W. J.; Radom, R.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley Interscience: New York; 1986.

Table 3. Computed Energies of *scyllo*-Inositol[a] and Energies of Other Inositols[b] Relative to *scyllo*-Inositol

method	6e *scyllo*	5e/1a *myo*	4e/2a *neo*	4e/2a *epi*	4e/2a *dl*
HF/6-31G*	−683.336 41	−0.56	−1.49	0.98	1.17
HF/6-31+G*	−683.363 85	0.32	0.11	2.53	2.31
MP2/6-31G*	−685.177 95	−2.02	−4.35	−2.84	−0.49
MP2/6-31+G*	−685.242 40	−0.73	−1.86	−0.29	1.22
VWN/DZVP+Al	−682.058 83	−1.70	−2.35	−2.76	0.54
BP/DZVP+Al	−687.389 00	−0.86	−0.79	−0.50	1.00

[a] In Hartrees. Note that DFT total energies, such as VWN and BP, cannot be meaningfully compared directly with the HF and MP2 values. [b] In kcal/mol. All calculations performed at the respective HF/6-31G* optimized geometries.

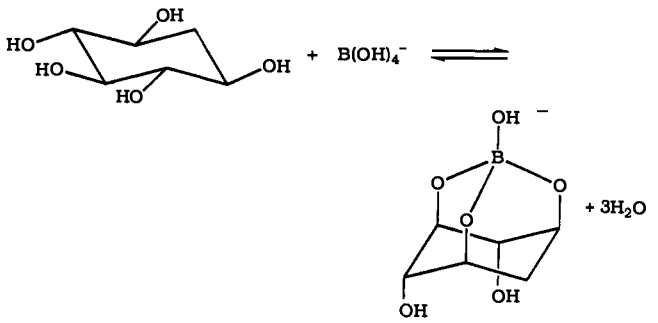
nonlocal corrections are in good agreement with those from the MP2/6-31+G* method. The only significant discrepancy is that the DFT methods underestimate the stability of *neo*-inositol as compared to the MP2 methods (Table 3).

Therefore, all results from our best theoretical methods (MP2/6-31+G* and BP/DZVP+Al) suggest that some inositols with axial hydroxyl(s) may actually be lower in internal energy than the all-equatorial inositol. This is very similar to the situation for glucose where the α-isomer with one axial OH is lower in energy than the all-equatorial β-isomer.[21] But an important difference is that the anomeric effect which is responsible for stability of α-glucose[21] does not exist in any of the inositols. Therefore, there must be other factors governing conformational energies of inositols. But before we probe those factors, we consider contributions from nuclear motions to free energies.

C. Free Energies. To arrive at free energies that may be compared with experimental measurements, we calculated energies (at 298.15 K) arising from nuclear motions using HF/6-31G* molecular structures and harmonic vibrational frequencies. These energies include zero-point vibrational energy (ZPE), thermal energy, and entropy contributions.[12] For the entropy of the *dl* forms we assumed pure enantiomers. As Table 4 shows, although ZPE energies favor the 6e *scyllo* isomer relative to others considered here, the entropy term favors other isomers when the symmetry of the *scyllo* is considered. The net effect of ZPE, thermal energy, and entropy on relative free energies is relatively small. Combining these contributions with our best relative internal energies obtained from MP2/6-31+G* and BP/DZVP+Al energies of Table 3, we arrive at the "final" relative free energies given in the last two rows of Table 4. Both estimates predict that the naturally most abundant 5e/1a *myo*-inositol is more stable than the 6e *scyllo*-inositol. As for the 4e/2a *neo*-inositol the computational results differ significantly but still favor it over *scyllo*-inositol. It should be noted that although *myo*-inositol is the most stable isomer according to both calculations, it is still possible that higher levels of calculations may alter this conclusion, considering the sensitivity of relative energies of these species to theoretical method.

Early experiments[4,5] appeared unequivocal on the conformational energies of inositols. Specifically, the borate complexation experiments, involving a series of quercitols and inositols such as exemplified by Scheme 1 for the reaction of *scyllo*-quercitol with borate,[6] seem to be by far the most often cited ones in this regard. These resulted in a model which indicated that one axial hydroxyl introduces 0.9 kcal/mol in strain energy (since for example the free energy change for the above reaction is −1.0 kcal/mol while that for a similar reaction of *myo*-inositol is −1.9 kcal/mol). Although the reaction shown in Scheme 1 is for *scyllo*-quercitol instead of *scyllo*-inositol, it was used to estimate the free energy difference between *scyllo*- and *myo*-inositols.[6,4b] Hence, it was concluded from the model that the 5e/1a *myo*-inositol is less stable than *scyllo*-inositol by 0.9 kcal/mol, while the 4e/2a *neo*-inositol, with one axial OH on both sides of the ring, is higher by another 0.9 kcal/mol. *epi*-Inositol, having two OH groups on

Scheme 1



the same side of the ring, was predicted to be 2.8 kcal/mol less stable than *scyllo*-inositol. However, these results were deduced from a very simple model considering only axial oxygen–oxygen, axial oxygen–hydrogen, and 1,4 oxygen–oxygen repulsions. Other important effects such as 1,4 oxygen–carbon interactions, changes in bond lengths, angles, and torsional angles as well as the electrostatic interactions were all neglected.[5,6] The neglect of geometry changes may be questionable since geometries, particularly C–O bonds, CCC angles, and ring torsions, do differ significantly among the inositols as discussed above. Also the neglect of key 1,4 interactions is questionable. Specifically, this model neglects contributions from 1,4 O···C and O···O interactions that are very important. For example, since the 1,4 O_a···C distance in *myo*-inositol (2.95 Å) is less than the sum of van der Waal's radii[26] (3.2 Å), while the corresponding O_e···C distance in *scyllo*-inositol (3.75 Å, Table 5) is not, one may expect significant 1,4 O···C repulsion in *myo*-inositol but not in *scyllo*-inositol. Furthermore, the 1,4 O_a···O_e distance in *myo*-inositol (2.72 Å) is also shorter than the 1,4 O_e···O_e distance in *scyllo*-inositol (2.80 Å), suggesting greater 1,4 O···O repulsion in *myo*-inositol. But in the model employed to interpret the borate experiments the 1,4 O···C interaction was neglected and the 1,4 O_a···O_e interaction was not distinguished from the 1,4 O_e···O_e interaction. As a result, the extra strain energy in *myo*-inositol as compared to *scyllo*-inositol was erroneously attributed to the O_a···H_aC interaction that was included in the model.[6] As will be discussed below, the O_a···H_aC interaction should be attractive rather than repulsive.

D. Role of O···HO Hydrogen Bonding. That axial OH substitution may actually be stabilizing rather than destabilizing can be understood from intramolecular hydrogen bonding. It is likely that an axial OH group introduces strain energies as is reflected in larger CCC angles and flattening of cyclohexane rings for inositols with axial OH group(s) (Table 2). But the O···HO hydrogen bonding distances (Table 5) involving axial OH are also shorter than those for equatorial OH and are thus energetically favored. For instance, in *myo*-inositol the two hydrogen bond distances involving axial OH are 2.23, and 2.30 Å, while the O_eH···O_e distances in *scyllo*-inositol are 2.42 Å (Table 5). In *epi*-inositol, the rather strong repulsion between two axial oxygens (2.79 Å apart) may be compensated by strong O_aH···O_a hydrogen bonding with a distance of only 2.01 Å, a typical hydrogen bonding distance. Thus, it is possible for its internal energy to be lower than that of *scyllo*-inositol (Table 3). While six hydrogen bonds are possible for *scyllo*, *myo*, *neo*, and *epi*-inositols (albeit some of them are weak), only five are possible for *dl*-inositol (Figure 2 and Table 5). This may be the reason for its high energy compared to the other four isomers (Table 3).

The existence of (attractive) intramolecular hydrogen bonding between hydroxyl groups on cyclohexane rings is reflected by the "bridging" hydrogens in cyclohexandiol derivatives in nonpolar solvents as seen by ^{1}H NMR and infrared spectra.[27] It is also strongly supported by a recent study of 1,3,5-trideoxy-1,3,5-tris-

(26) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441.
(27) Nader, F. W.; Heinrich, W.; Baar-Schäfer, M.; Hangel, E. *Chem. Ber.* **1985**, *118*, 4314.

Table 4. Thermodynamic Quantities[a] of Inositols Relative to the *scyllo* Isomer

quantities	*myo*	*neo*	*epi*	*dl*
ΔZPE[b]	0.40	0.83	0.85	0.42
ΔE_t[c]	–0.14	–0.28	–0.27	–0.12
$-T\Delta S$[d]	–0.74(0.32)	–0.02(0.63)	–0.40(0.66)	–0.74(0.32)
$\Delta ZPE + \Delta E_t - T\Delta S$	–0.48(0.58)	0.53(1.18)	0.18(1.24)	–0.44(0.62)
$\Delta E(MP2) + \Delta ZPE + \Delta E_t - T\Delta S$	–1.21(–0.15)	–1.33(–0.68)	–0.11(0.95)	0.78(1.84)
$\Delta E(DFT) + \Delta ZPE + \Delta E_t - T\Delta S$	–1.34(–0.28)	–0.26(0.39)	–0.32(0.74)	0.54(1.62)

[a] Calculated at 298.15 K using the HF/6-31G* method, in kcal/mol. E(MP2) and E(DFT) are from MP2/6-31+G* and BP/DZVP+A1 energies, respectively, in Table 3. Values in parentheses neglect the rotational symmetry of the *scyllo* and *neo* forms. [b] For *scyllo*, ZPE = 134.62 kcal/mol. [c] Sum of rotational, translational, and thermal vibrational energies (excluding ZPE), see ref 12. For *scyllo*, E_t = 5.42 kcal/mol. [d] For *scyllo*, TS = 30.52 kcal/mol.

Table 5. The Hydrogen Bonding and Corresponding O···O Distances[a] in Inositols

distance	*scyllo*	*myo*	*neo*	*epi*	*dl*
$O_1\cdots H_2$ ($O_1\cdots O_2$)	2.42 (2.80)	2.30 (2.73)	2.32 (2.74)	2.31 (2.73)	2.36 (2.77)[d]
$O_2\cdots H_3$ ($O_2\cdots O_3$)	2.42 (2.80)	2.23 (2.72)	2.26 (2.74)	2.01 (2.79)[b]	2.46 (2.82)
$O_3\cdots H_4$ ($O_3\cdots O_4$)	2.42 (2.80)	2.44 (2.84)	2.39 (2.83)	2.50 (2.86)[c]	2.41 (2.80)
$O_4\cdots H_5$ ($O_4\cdots O_5$)	2.42 (2.80)	2.41 (2.80)	2.32 (2.74)	2.25 (2.77)	2.37 (2.79)
$O_5\cdots H_6$ ($O_5\cdots O_6$)	2.42 (2.80)	2.40 (2.79)	2.26 (2.74)	2.43 (2.84)	2.26 (2.72)
$O_6\cdots H_1$ ($O_6\cdots O_1$)	2.42 (2.80)	2.30 (2.79)	2.39 (2.83)	2.38 (2.80)	*e*

[a] Calculated from the HF/6-31G* method, in Å. O···O distances in parentheses. [b] Between O_2 and H_4. This is the hydrogen bond between axial hydroxyls. [c] Between H_3 and O_4. H_3 is also in close contact with O_2 with a distance of 2.52 Å ($O_2\cdots O_3$ distance 2.80 Å). [d] Between H_1 and O_2. There is no hydrogen bonding between O_1 and H_2. [e] There is no hydrogen bonding between O_6 and H_1.

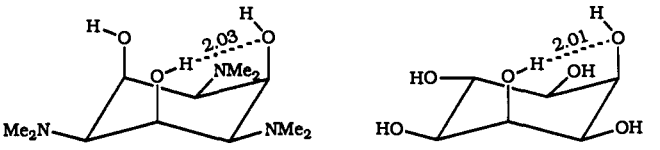
(dimethylamino)-*cis*-inositol (TDCI) which has three axial hydroxyl groups.[28] Its relatively high-resolution (R = 0.045) crystal structure clearly shows a $O_aH\cdots O_a$ hydrogen bond with a distance of 2.03 Å (corresponding to an O···O distance of 2.73 Å), in excellent agreement with the same H-bond in our computed structure for *epi*-inositol (Chart 3). The unusually high solubility of TDCI in nonpolar solvents provides further evidence for intramolecular hydrogen bonding in this molecule.[28]

Hydrogen bonding between equatorial positions was also suggested by an earlier experiment in dilute tetrachloroethylene solutions.[29] In fact, this attraction was estimated to be 0.8 kcal/mol,[29] in direct conflict with estimates from the borate complexation experiments which yielded a *repulsion* of 0.35 kcal/mol for the same interaction.[6] This may reflect the influence of solvent since the latter experiments were carried out in aqueous solutions where intermolecular hydrogen bonding may be more important. It may also be due to the crudeness of the parameters that result from this model, as discussed above.

The kind of intramolecular hydrogen bonding discussed above may also have manifested itself in a recent NMR dynamics study of chair–chair interconversion of *cis*-inositol which has three axial OH groups.[30] It was found that the activation energy of this interconversion (12.7 kcal/mol) is almost twice that of similar interconversion in several dimethylcyclohexyl systems.[31] The authors attributed this difference to the size of hydroxyl groups.[30] But a hydroxyl group should not be bulkier than a methyl group since oxygen (1.5 Å) has smaller van der Waals' radii than carbon (1.7 Å).[26] It seems that a more plausible explanation is the existence of the intramolecular hydrogen bonding in inositol (particularly the fairly strong $O_aH\cdots O_a$ bonding as in *epi*-inositol or TDCI[28] shown in Chart 3 and Scheme 2) that does not exist in dimethyl systems. Apparently, changing from one chair conformer to another has to break the hydrogen bonds in one conformer before new bonds can be formed (Scheme 2) and therefore this process requires a higher activation energy.

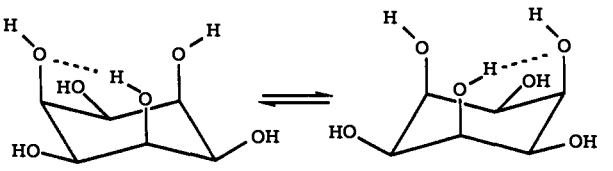
E. Role of O···HC Attraction. Another factor favoring the stability of axial OH substitution is the possible $O_{axial}\cdots H_{axial}C$ attraction as mentioned above. In the borate complexation

Chart 3



TDCI crystal Epi-inositol calculation

Scheme 2



experiments,[6] this interaction was determined to be repulsive (0.45 kcal/mol) and solely responsible for the energy difference between *myo*- and *scyllo*-inositols. This positive axial O···HC oxygen–hydrogen interaction is questionable as one would generally expect interactions between a partial negative charge on the oxygen atom and partial positive charge hydrogen at these relatively long internuclear separations (2.6 Å for O···H and 3.7 Å for O···C) to be attractive. Results from a recent systematic study[13] of the $H_2O\cdots HCH_3$ complex with the O···HC angle being 90° (similar to the arrangement of $O_a\cdots H_aC$ in inositols) are very instructive. Using the MP2 method and large basis sets [6-311G(2d,2p) and 6-311G(2df,2pd)] the O···HC (attractive) interaction energy at similar distances (2.59 and 2.56 Å) are calculated to be about –0.16 kcal/mol (when basis set superposition error is corrected using the countepoise method).[13] When a basis set with near Hartree–Fock limit quality is used, a similar interaction energy (–0.36 kcal/mol) is found but at a slightly longer distance (2.87 Å). These results suggest that the $O_a\cdots H_aC$ interaction in inositols may be slightly attractive. It should be noted that when the O···HC angle is nearly linear the interaction energy is higher and this kind of weak hydrogen bonding has been observed in crystal structures,[32,33] though it is a matter in dispute.[34]

Thus the conformational energies of inositols in the gas phase are governed by weak to modest internal hydrogen bonds as well as nonbonded repulsions (strain energy). The final outcome is

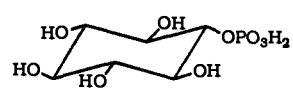
(28) Hegetschweiler, K.; Erni, I.; Schneider, W.; Schmalle, H. *Helv. Chim. Acta* **1990**, *73*, 97.
(29) Pitha, J.; Sicher, J.; Sipos, F.; Michy, M.; Vasickova, S. *Proc. Chem. Soc.* **1963**, 301.
(30) Dill, K.; Huang, L. *Carbohydr. Res.* **1991**, *215*, 351.
(31) Dalling, D. K.; Grant, D. M.; Johnson, L. F. *J. Am. Chem. Soc.* **1971**, *93*, 3678.
(32) Koller, J.; Grdadolnik, J.; Hadzi, D. *J. Mol. Struct.* **1992**, *259*, 199.

a delicate balance of these forces. Since diffuse functions and electron correlation are essential in describing the kind of weak hydrogen bonding such as in O···HC,[13,33] it is understandable that the theoretical results for the relative energies change on going from HF/6-31G* to HF/6-31+G* or to MP2/6-31G* as discussed above. In general larger basis sets tend to reduce the magnitudes of hydrogen bond energies, while electron correlation corrections tend to increase them, as reflected here by the decreased stability of the *myo* form relative to *scyllo* upon going to more diffuse basis sets and its increased stability upon including correlation at the MP2 level of approximation. Despite the variations in computed relative energies, in light of the results for similar interactions studied in a progression of more sophisticated methods, such as for the water/methane dimer cited above, it seems likely that our final computed results are substantially correct.

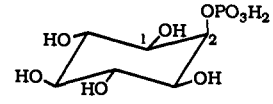
F. Solvent Effects. As is true for other carbohydrate compounds, the structure and properties of inositols may be significantly different in gas and condensed phases, primarily due to the differences in hydrogen bonding between these phases.[21,35] In the gas phase, to which our computed structures correspond, only intramolecular hydrogen bonding is of course possible, and our calculations show that *myo*-inositol may be more stable than *scyllo*-inositol because of more favorable intramolecular hydrogen bonding in the former. On the other hand, in condensed phases such as crystals intermolecular hydrogen bonding may also be important. For example, in crystals of *myo*- and *epi*-inositol intermolecular hydrogen bonding prevails at the expense of intramolecular hydrogen bonding.[7,8] Therefore, the energetics of inositols in condensed phases may be different from that in gas phase. To estimate the effect of intramolecular hydrogen bonding on the relative energy of *scyllo*- and *myo*-inositols, we formed these structures with minimal intramolecular hydrogen bonding by changing the OH orientations in their respective HF/6-31G* optimized structures (Figure 3). Specifically, all HOCH torsions in *scyllo*-inositol were changed into 180°. For *myo*-inositol, the HOC_1H and HOC_3H torsions were changed into 60° and −60°, respectively, and all other HOCH angles into 180°. The energies of these structures were computed using the HF/6-31G*, HF/6-31+G*, and MP2/6-31+G* methods. The results are that *scyllo* is now lower in energy than *myo* by 1.42 (HF/6-31G*), 1.73 (HF/6-31+G*), and 0.49 (MP2/6-31+G*) kcal/mol. This again indicates the importance of intramolecular hydrogen bonding in stabilizing *myo*-inositol relative to *scyllo*-inositol in the gas phase as discussed in previous sections.[36,37]

In solution, intramolecular hydrogen bonding might not be as important as in gas phase due to the presence of intermolecular hydrogen bonding. The reality regarding intramolecular hydrogen bonding is probably somewhere in between that in our optimized structures (where it is optimal) and that in structures where it is minimized as described above. Consequently, the relative energy between *scyllo*- and *myo*-inositols may be somewhere in between 0.73 and −0.49 kcal/mol (MP2/6-31+G* results), depending on the extent of intramolecular hydrogen bonding which in turn depends on the solvent. In dilute solutions with nonpolar solvents, intramolecular hydrogen bonding is likely to dominate and therefore *myo*-inositol would be favored over *scyllo*-inositol. But in aqueous solutions, where the borate experiments were carried out, it is possible that intermolecular hydrogen bonding prevails. In that case *scyllo*-inositol would be more stable than *myo*-inositol as these experiments seem to suggest. Actually, for the closely related glucose molecule a study of the solvent effect[38] indicated that aqueous solutions favor the all-equatorial β-isomer over the α-isomer, presumably because the hydroxyls in the β-isomer are better positioned to form intermolecular hydrogen bonds. Moreover, the observed high activation energy for chair–chair interconversion of *cis*-inositol[30] may again be due in part to intermolecular rather than intramolecular hydrogen bonding since the experiments were performed in aqueous solution (although appreciable intramolecular $O_aH\cdots O_a$ bonding is likely present as well based on the observed crystal structure of TDCI[28]).

G. Phosphoryl Inositols. Since inositols exist in biomembranes primarily as inositol phosphates and phosphatides,[1,4] we studied *scyllo*- and 2-*myo*-inositol phosphoric acid ($C_6H_{13}O_9P$, **1** and **2**) to investigate effects of phosphate substitution. We optimized



1



2

their structures using the HF/6-31G* method (Figure 4). Although we did not try other conformers, we feel that structures shown in Figure 4 are likely the lowest energy structures of the respective molecules since intramolecular hydrogen bonding is optimal in these structures. Table 6 compares the ring and torsion angles for the computed gas-phase and experimental crystal[39] structures of *myo*-inositol-2-phosphoric acid. The agreement is reasonably good. In particular, the variation in the relative magnitudes of ring torsions observed in the crystal is correctly reproduced by the calculations, unlike the situation for the parent molecule. Table 6 also compares cyclohexane ring angles and torsional angles for the parent inositol and phosphate-substituted *scyllo*- and *myo*-inositols. For the *scyllo* isomer, as one might expect from steric repulsion, phosphate substitution slightly enlarges the average ring CCC angles (111.1°) and decreases the mean ring torsion (55.7°) as compared to the parent molecule, thereby flattening the cyclohexane ring. This is particularly true around the site of phosphate substitution (Table 6). Interestingly, according to the HF/6-31G* results phosphate substitution has an opposite effect on the ring conformation of *myo*-inositol: it slightly reduces the CCC angles and enlarges CCCC torsions (Table 6). What is more significant is that the ring torsion angles are more similar (i.e., exhibiting less variation) as compared to the parent molecule. It seems that hydrogen bonds between phosphate and hydroxyls of the ring, seen in Figure 4, are restoring the cyclohexane ring to its "normal" conformation. Comparing crystal structures for the parent[7] and phosphate-substituted[39] *myo*-inositol shows that while the $C_6C_1C_2C_3$ and $C_1C_2C_3C_4$ torsional angles in the parent are among the larger ones, in the substituted molecule they are among the smaller ones as compared to other torsions in the respective structures (note that the axial OH or phosphate is attached to C_2).

Note also that our computed torsional angles for the parent *myo*-inositol agree better with those in the *myo*-inositol phosphoric acid crystal than with the parent *myo*-inositol crystal itself, suggesting again that the latter crystal structure is somewhat less accurate compared to other crystals.

(33) Seiler, P.; Weisman, G. R.; Glendening, E. D.; Weinhold, F.; Johnson, V. B.; Dunitz, J. D. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1175.
(34) Desiraju, G. R. *Acc. Chem. Res.* **1991**, *24*, 290.
(35) Computer Modeling of Carbohydrate Molecules, ACS Symposium Series No. 430, French, A. D., Brady, J. W., Eds.; American Chemical Society: Washington, DC, 1990.
(36) In the MP2/6-31+G* approximation, these structures with minimized intramolecular hydrogen bonding are about 39.5 (for *myo*) and 38.3 (for *scyllo*) kcal/mol higher in energy than are their respective HF/6-31G* optimized structures (with optimal intramolecular hydrogen bonding). Note that these energy differences are not solely due to intramolecular hydrogen bonding.
(37) It should be noted that the hydrogen of the C_2 hydroxyl is only 2.07 Å away from the axial hydrogens on C_4 and C_6.
(38) Ha, S.; Gao, J.; Tidor, B.; Brady, J. W.; Karplus, M. *J. Am. Chem. Soc.* **1991**, *113*, 1553.
(39) Yoo, C. S.; Blank, G.; Pletcher, J.; Sax, M. *Acta Crystallogr.* **1974**, *B30*, 1983.

Table 6. Comparison of Cyclohexane Ring Conformations (Bond Angles and Torsional Angles) for the Parent and Phosphate Substituted *scyllo*- and *myo*-Inositols[a]

	scyllo		*myo*	
angle	parent	subst	parent (exptl[b])	subst (exptl[c])
		Bond Angles		
$C_6C_1C_2$	110.8	110.7	111.9(111.0)	111.4(111.0)
$C_1C_2C_3$		112.8	111.6(109.7)	111.3(110.8)
$C_2C_3C_4$		111.0	111.8(110.6)	111.3(113.0)
$C_3C_4C_5$		110.7	111.1(109.4)	110.8(110.4)
$C_4C_5C_6$		110.2	110.1(112.5)	110.6(111.3)
$C_5C_6C_1$		111.3	110.8(111.1)	110.7(108.5)
av	110.8	111.1	111.2(110.7)	111.0(110.8)
		Torsional Angles		
$C_6C_1C_2C_3$	56.7	53.6	52.5(57)	54.7(55.4)
$C_1C_2C_3C_4$		-52.8	-51.9(-59.5)	-54.3(-51.9)
$C_2C_3C_4C_5$		54.9	55.0(58.5)	55.6(52.6)
$C_3C_4C_5C_6$		-58.1	-58.0(-56)	-57.3(-57.3)
$C_4C_5C_6C_1$		58.4	58.4(55)	57.7(60.8)
$C_5C_6C_1C_2$		-56.0	-56.0(-55)	-56.4(-59.7)
av	56.7	55.7	55.3(56.8)	56.0(56.3)

[a] HF/6-31G* optimized results, in deg. Experimental values are given in parentheses. [b] Rabinowitz, I. N.; Kraut, J. *Acta Crystallography* **1964**, *17*, 159. [c] Yoo, C. S.; Blank, G.; Pletcher, J.; Sax, M. *Acta Crystallogr.* **1974**, *B30*, 1983.

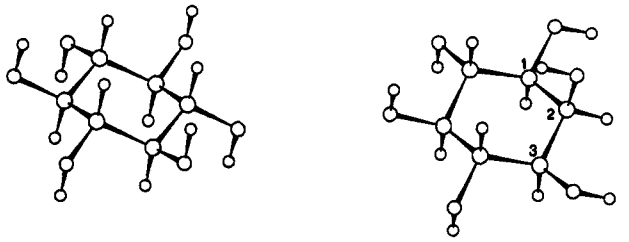


Figure 3. *scyllo*- (Left) and *myo*-inositols generated by changing the OH orientations in their respective HF/6-31G* optimized structures. All HOCH torsions in *scyllo*-inositol are now 180°. For *myo*-inositol, the HOC_1H and HOC_3H torsions are 60° and -60°, respectively, and all other HOCH angles are 180°.

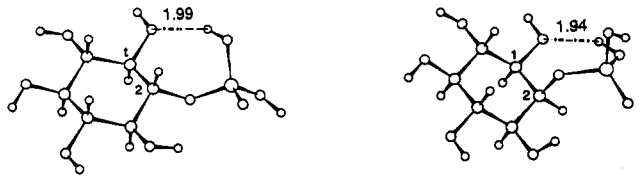


Figure 4. The HF/6-31G* optimized structures for *scyllo*-inositol phosphoric acid (left) and *myo*-inositol-2-phosphoric acid (right). The primary hydrogen bonds between the phosphoric acid and inositol are also shown.

As for relative energies, the *scyllo* isomer is lower in energy than the *myo* by 0.39 kcal/mol at the HF/6-31G* level of theory. While MP2 calculations using the 6-31G* basis would of course be more computationally challenging, DFT calculations are relatively easy (requiring only about 2 h on an IBM RS/6000 550 workstation), demonstrating an advantage of DFT methods.[15-17] Thus, using the HF/6-31G* geometries the VWN/DZVP + Al method favors, instead, the *myo* isomer by 1.48 kcal/mol. Inclusion of nonlocal corrections lowers the difference to 0.75 kcal/mol, still favoring the *myo* isomer. Hence, these theoretical results suggest that axial substitution by a phosphate group may be favored,[40] in contrast to common belief that *myo*-inositol phosphate is a high energy conformation.[1,7] (It should be noted that, for simplicity, we have not included contributions from nuclear motions so as to obtain the free energies in the above comparisons.)

Conclusions

This work suggests that the conformational analysis of inositols remains largely an unresolved issue even 30 years after the borate complexation experiments, which are in large measure the cornerstone of the established view on this subject. The model used in interpreting those experiments now seems too crude since it neglected important contributions from 1,4 O···C interactions, electrostatic/hydrogen bonding interactions, and changes in cyclohexane ring conformation upon axial substitution(s). Intermolecular hydrogen bonding that exists in those aqueous solution experiments may have also significantly altered intrinsic conformation energies of the inositols. On the other hand, accurate theoretical treatment of inositols proves to be quite challenging. Although we have considered basis set and electron correlation effects and employed three different *ab initio* methods (all-electron Hartree–Fock and MP2 as well as density functional), some details, especially the small energy differences (compared to kT at normal temperatures), are clearly not yet converged with respect to the theoretical methods and await further study. But what is clear from this study is that in addition to the well-recognized, unfavorable strain energy introduced by repulsion involving oxygens of axial hydroxyl groups (as is reflected in changes in cyclohexane ring conformations), axial hydroxyl hydrogens are able to form energetically more favorable intramolecular hydrogen bonds, compared to equatorial, leading to enhanced stabilities for axial hydroxyl conformations.

Our best theoretical results for the gas phase (based on MP2/6-31+G* and BP/DZVP+Al energies) suggest that the 5e/1a *myo*-inositol and the 4e/2a *neo*-inositols have lower internal energies as well as free energies (at 298 K) than the all-equatorial *scyllo*-inositol. This is particularly true for the naturally most abundant *myo*-inositol. These results should also apply to inositols in dilute nonpolar solutions where intramolecular hydrogen bonding is likely still dominant. In polar solutions where intramolecular hydrogen bonding is not as important, our preliminary results show that it is possible for *scyllo*-inositol to be more stable than *myo*-inositol. In addition, our results on phosphate-substituted *myo*- and *scyllo*-inositols suggest that in the gas phase they also favor axial substitution.

Due to their considerable biological importance, species containing inositols will likely become the focus of computational studies of their structures and energetics using a number of more approximate methods, such as those based on empirical force fields. The results presented here should be very valuable in developing and testing force fields to be used in carbohydrates and phospholipids simulations, which was one of the major goals of this study.

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(40) We think that the DFT results are more reliable based on the experience on inositols as discussed in subsection II.B.