Crystal Structure and Modelling Studies of *myo*-Inositol 1,2,3-Trisphosphate

Ian D. Spiers,[a] Sally Freeman[a,b] and Carl H. Schwalbe*[a,b]†

[a] *Department of Pharmaceutical and Biological Sciences, Aston University, Aston Triangle, Birmingham, UK B4 7ET*
[b] *Department of Pharmacy, University of Manchester, Oxford Road, Manchester, UK M13 9PL*

The crystal structure of sodium tetra(cyclohexylammonium) *myo*-inositol 1,2,3-trisphosphate is found in the chair form with the three phosphate groups adopting the more stable equatorial-axial-equatorial conformation.

Phytic acid (**1**, *myo*-inositol hexakisphosphate) has potential in the prevention and treatment of a range of cancers, and these properties may be attributed to its ability to bind Fe^{3+} and divalent cations.[1] *myo*-Inositol 1,2,3-trisphosphate **2** has recently been discovered[2] in mammalian cells at concentrations of 1–10 μmol dm^{-3} and hypothesised also to have a role in iron chelation.[3,4] To test this proposal, we have recently described the synthesis and iron binding studies of **2**.[5,6] The results show that **2** resembles phytic acid in its ability to chelate iron and behave as an anti-oxidant,[5,6] and to act as a siderophore by promoting iron-uptake into *Pseudomonas aeruginosa*.[6]

Several other naturally occurring inositol trisphosphates also have interesting biological properties, for example, *myo*-inositol 1,2,6-trisphosphate[7] and the second messenger, *myo*-inositol 1,4,5-trisphosphate.[8] However, X-ray structural information is not available for any inositol trisphosphate; indeed only two crystal structures of *myo*-inositol phosphates have been reported. *myo*-Inositol 2-phosphate monohydrate was found to exist in a slightly distorted chair conformation with the phosphate ester axial and all the hydroxy groups equatorial.[9] Phytic acid, either in the crystalline form as the dodecasodium salt or bound to deoxyhemoglobin, adopts a chair conformation, with five of the phosphate groups (1-, 3-, 4-, 5- and 6-) surprisingly in axial positions.[10,11] In the dodecasodium salt, the phosphate groups were stabilised by bridging sodium ions and hydrogen bonded water molecules which could account for its unusual conformation. NMR studies of this salt form in solution support the 5-axial/1-equatorial structure, but as the free acid, the five phosphate groups adopt equatorial positions, with the conformational change occurring at pH 9.4, triggered by a deprotonation.[12] Raman spectroscopic studies show that the hexacalcium salt adopts the 1-axial/5-equatorial conformation,[12] which is also seen in the crystal structure of hexa(cyclohexylammonium) *myo*-inositol hexasulfate.[13] We were fortunate to obtain crystals of the natural product **2** which were suitable for X-ray crystallography. Of importance to iron binding properties, especially as **1** can adopt different conformations, it was of interest to establish whether the three phosphate groups of **2** adopt either an equatorial–axial–equatorial or an axial–equatorial–axial conformation in the solid state.

The crystal structure of the sodium tetra(cyclohexylammonium) salt‡ of **2** also contains seven molecules of water and a molecule of methanol in the asymmetric unit (Fig. 1).§ The *myo*-inositol ring possesses the expected chair conformation with 1-axial/5-equatorial oxygen positions, in contrast to **1** with its unusual 5-axial/1-equatorial conformation.[10,11] The inositol ring and phosphate groups have bond distances and angles (Table 1) that are consistent with those of *myo*- inositol 2-phosphate.[9] The inositol ring is only slightly distorted from an ideal cyclohexane chair, the presence or absence of phosphate groups making little difference {asymmetry parameters, $\Delta C_2[C(1)–C(6)] = 0.47$, $\Delta C_s[C(3)] = 0.48$}.[17] The ions and solvent molecules are linked by numerous hydrogen bonds. Terminal oxygen atoms of phosphate groups are the best acceptors, receiving up to three hydrogen bonds each. The oxygen atoms of methanol and most water molecules also function as hydrogen bond acceptors. Every inositol hydroxy group donates a hydrogen bond, all four cyclohexylammonium groups donate three hydrogen bonds each, and donation of most water hydrogen atoms is observed. Geometric data for hydrogen bonds involving atoms of compound **2** as both donors and acceptors (Table 2) are in good agreement with surveyed data.[18] The O(15C)–H(15C)···O(14A) intramolecular interaction between phosphate groups is particularly short and strong. One of

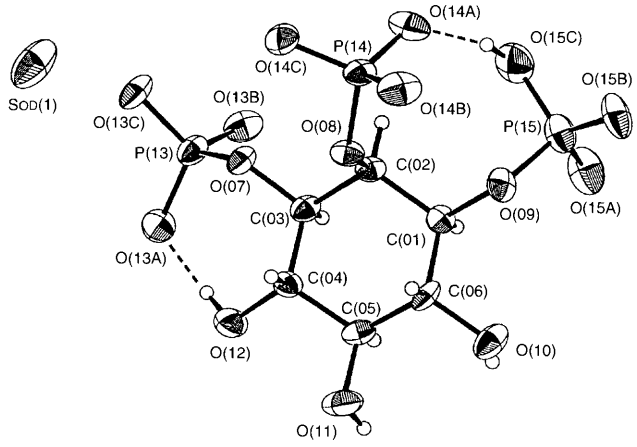
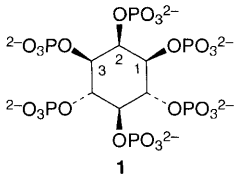


Fig. 1 ORTEP drawing[16] of *myo*-inositol 1,2,3-trisphosphate and sodium cation, showing the labelling scheme for non-H atoms (water, methanol and cyclohexylammonium cations are omitted for clarity). Thermal ellipsoids are drawn at the 50% probability level.

Table 1 Bond lengths (Å) and angles (°) for *myo*-inositol 1,2,3-trisphosphate (e.s.d.'s)

C(01)–C(02)	1.520 (7)	C(01)–C(06)–C(05)	109.3 (4)
C(01)–C(06)	1.528 (7)	C(02)–C(01)–C(06)	112.4 (4)
C(02)–C(03)	1.522 (7)	C(02)–C(03)–C(04)	111.7 (4)
C(03)–C(04)	1.531 (7)	C(03)–C(02)–C(01)	108.9 (4)
C(04)–C(05)	1.505 (7)	C(04)–C(05)–C(06)	112.1 (4)
C(05)–C(06)	1.529 (7)	C(05)–C(04)–C(03)	109.3 (4)
C(01)–O(09)	1.441 (6)	O(07)–C(03)–C(02)	108.7 (4)
C(02)–O(08)	1.437 (6)	O(07)–C(03)–C(04)	112.7 (4)
C(03)–O(07)	1.423 (6)	O(08)–C(02)–C(03)	109.5 (4)
C(04)–O(12)	1.426 (6)	O(08)–C(02)–C(01)	110.5 (4)
C(05)–O(11)	1.412 (7)	O(09)–C(01)–C(02)	110.7 (4)
C(06)–O(10)	1.410 (7)	O(09)–C(01)–C(06)	108.0 (4)
O(07)–P(13)	1.622 (4)	O(10)–C(06)–C(01)	111.0 (5)
O(08)–P(14)	1.619 (4)	O(10)–C(06)–C(05)	111.1 (4)
O(09)–P(15)	1.606 (4)	O(11)–C(05)–C(06)	111.2 (5)
P(13)–O(13A)	1.517 (4)	O(11)–C(05)–C(04)	109.6 (5)
P(13)–O(13B)	1.508 (4)	O(12)–C(04)–C(03)	111.5 (4)
P(13)–O(13C)	1.510 (4)	O(12)–C(04)–C(05)	107.8 (4)
P(14)–O(14A)	1.512 (4)	C(01)–O(09)–P(15)	120.1 (3)
P(14)–O(14B)	1.506 (4)	C(02)–O(08)–P(14)	122.6 (3)
P(14)–O(14C)	1.497 (4)	C(03)–O(07)–P(13)	121.3 (3)
P(15)–O(15A)	1.486 (4)		
P(15)–O(15B)	1.495 (4)		
P(15)–O(15C)	1.556 (5)		



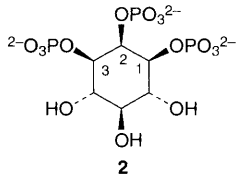


Table 2 Selected hydrogen bonding in *myo*-inositol 1,2,3-trisphosphate (e.s.d.'s)

	d(O···O)/Å	*d*(O–H···O)/Å	*a*(O–H···O)/°	Symm. accep.[a]
O(10)–H(10)···O(13A)	2.766(6)	2.13(7)	161(9)	1 − X, −Y, 1 − Z
O(11)–H(11)···O(13B)	2.754(6)	2.03(6)	178(6)	1 − X, −Y, 1 − Z
O(12)–H(12)[b]···O(13A)	2.685(6)	1.87(9)	175(7)	X, Y, Z
O(15C)–H(15C)···O(14A)	2.553(7)	1.69(7)	160(6)	X, Y, Z

[a] Symmetry operation to be applied to the acceptor atom. [b] Calculated hydrogen position based on electron density and subjected to rotating group refinement.

the inositol hydroxy groups adjacent to phosphate forms an intramolecular hydrogen bond but the other two hydroxy groups prefer intermolecular bonding to phosphate. The sodium cation is surrounded by six oxygen atoms: one from phosphate, the hydroxy O(10), three from water and one from methanol.

The protonation sequences of *myo*-inositol 1,4,5-trisphosphate and related compounds have been analysed by potentiometric and ^{31}P NMR measurements.[19] It was suggested that vicinal phosphate groups repel each other to minimise charge interactions: This brings the phosphate groups into close proximity with neighbouring hydroxy groups, resulting in hydrogen bonding and a reduction in the phosphate basicity. This is verified in the crystal structure of compound **2** by the intramolecular hydrogen bonding between the O(12) hydroxy and O(13A)–P(13) phosphate group (Fig. 1 and Table 2) and by the existence of sodium cation coordination to O(13C)–P(13) even after treatment with Dowex-H$^+$.‡ The first protonation site of fully ionised *myo*-inositol 1,4,5-trisphosphate was estimated to be shared between the phosphate groups at positions 4 and 5.[19] The first protonation site of *myo*-inositol 1,2,6-trisphosphate, with three vicinal phosphate groups, was also suggested to be stabilised by strong hydrogen bonding.[20] The intramolecular hydrogen bond between the neighbouring P(15) and P(14) phosphate groups of compound **2** (Fig. 1) adds support to proton sharing, and accounts for the high sixth p*K*a value of *myo*-inositol 1,2,3-trisphosphate.‡

Optimisation of the two chair conformations of *myo*-inositol 1,2,3-trisphosphate (fully ionised) using semi-empirical molecular orbital calculations with AM1 parameters¶ in MOPAC[21] gave heat of formation values of −173.9 kcal mol^{-1} (1-axial/5-equatorial) and −151.3 kcal mol^{-1} (1 cal = 4.184 J) (5-axial/1-equatorial), which confirms that the equatorial–axial–equatorial orientation of the three phosphate groups is the more stable conformation.

We thank Drs Peter A. Lambert, David R. Poyner and Anthony W. Smith for helpful discussions, the BBSRC for a studentship (I. D. S.) and the Lister Institute for a fellowship (S. F.).

Received, 27th July 1995; Com. 5/05003K

Footnotes

† E-mail: SCHWALCH@pcmail.aston.ac.uk

‡ A solution of the hexasodium salt of **2** was passed down a column of Dowex 50-X8 (H$^+$ form) to give the monosodium salt, attributable to the low p*K*a of the first ionisation: for phytic acid this p*K*a (at C-2) is 1.1.[14] The pH was adjusted to 11 with cyclohexylamine, and acetone was added to crystallise **2**. The P(15) phosphate group is only half ionised due to the high sixth p*K*a value of 9.56.[15] For phytic acid, the highest p*K*a value[14] (at C-3) is 12.0.

§ *Crystal data* for **2**: $(C_6H_{14}N^+)_4Na^+C_6H_{10}O_{15}P_3^{5-}\cdot 6.5H_2O\cdot CH_3OH$, M = 987.41, triclinic, space group $P\bar{1}$, a = 12.355(2), b = 14.803(2), c = 15.101(2) Å, α = 102.90(1), β = 112.57(1), γ = 93.38(1)°, V = 2453.6(6) Å^3, Z = 2, D = 1.336 (calc.), 1.319 (meas.) g cm^{-3}, μ = 0.209 mm^{-1}. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

¶ AM1 calculations were performed through CHEMX with GNORM = 0.5 and CHARGE = −6. Initial structures were adapted from crystal structure geometry.

References

1 A. M. Shamsuddin, *J. Nutr.*, 1995, **125**, 725S.
2 F. M. McConnell, S. B. Shears, P. J. L. Lane, M. S. Scheibel and E. A. Clark, *Biochem. J.*, 1992, **284**, 447.
3 C. J. Barker, P. J. French, A. J. Moore, J. Nilsson, P.-O. Berggren, C. M. Bunce, C. J. Kirk and R. H. Michell, *Biochem. J.*, 1995, **306**, 557.
4 C. J. Barker, J. Wright, C. J. Kirk and R. H. Michell, *Biochem. Soc. Trans.*, 1995, **23**, 169S.
5 I. D. Spiers, S. Freeman, D. R. Poyner and C. H. Schwalbe, *Tetrahedron Lett.*, 1995, **36**, 2125.
6 I. D Spiers, C. J. Barker, S.-K. Chung, Y.-T. Chang, S. Freeman, J. M. Gardiner, P. H. Hirst, P. A. Lambert, R. H. Michell, D. R. Poyner, C. H. Schwalbe, A. W. Smith and K. R. H. Solomons, *Carbohydr. Res.*, in the press.
7 M. Sirén, L. Linné and L. Persson, in *Inositol-Phosphates and Derivatives*, ed. A. B. Reitz, A. C. S. Symposium Series 463, Washington, 1991, ch. 7, 103.
8 D. C. Billington, *The Inositol Phosphates. Chemical Synthesis and Biological Significance*, 1993, VCH.
9 C. S. Yoo, G. Blank, J. Pletcher and M. Sax, *Acta Crystallogr., Sect B*, 1974, **30**, 1983.
10 G. E. Blank, J. Pletcher and M. Sax, *Acta Crystallogr., Sect. B*, 1975, **31**, 2584.
11 A. Arnone and M. F. Perutz, *Nature*, 1974, **249**, 34.
12 L. R. Isbrandt and R. P. Oertel, *J. Am. Chem. Soc.*, 1980, **102**, 3144.
13 G. E. Blank, J. Pletcher and M. Sax, *ACA Summer Meeting Program and Abstracts*, 1976, **4** (2), 74.
14 A. J. R. Costello, T. Glonek and T. C. Myers, *Carbohydr. Res.*, 1976, **46**. 159.
15 L. Schmitt, PhD Thesis, 1993, Université Louis Pasteur de Strasbourg, France.
16 C. K. Johnson, 1976, ORTEP, Report ORNL-5136, Oak Ridge National Laboratory, Tennessee, USA.
17 W. L. Duax and D. A. Norton, *Atlas of Steroid Structure*, Plenum, 1975, pp.16–22.
18 T. Steiner and W. Saenger, *Acta. Crystallogr., Sect. B*, 1992, **48**, 819.
19 L. Schmitt, P. Bortmann, G. Schlewer and B. Spiess, *J. Chem. Soc., Perkin Trans. 2*, 1993, 2257.
20 K. Mernissi-Arifi, C. Wehrer, G. Schlewer and B. Spiess, *J. Inorg. Biochem.*, 1994, **55**, 263.
21 J. J. P. Stewart, *J. Comp. Aided Mol. Design*, 1990, **4**, 1.