

Hemoglobin Cofactors. I. The Crystal Structure of Myoinositol Hexaphosphate Dodecasodium Salt Octatriacontahydrate

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(Received 8 August 1974; accepted 15 April 1975)

The crystal structure of myoinositol hexaphosphate dodecasodium salt has been determined for the octatriacontahydrate form which crystallizes in space group Cc , $a=23.091$ (13), $b=12.203$ (10), $c=22.894$ (14) Å, $\beta=108.30$ (1)°, and $Z=4$. As the crystalline hydrated sodium salt, myoinositol hexaphosphate has the sterically unfavorable (5-axial/1-equatorial) ring conformation. This conformer is stabilized by the mutual repulsion of 1,2-vicinal ionized phosphates, sodium ion bridging of both 1,3-*syn* and 1,2-vicinal phosphates and hydrogen-bonded water molecules. A degree of structural stability is gained from the uncommon mode of sodium coordination to ester oxygens. Three types of polyhedral arrangements are observed for the oxygen atoms coordinated to sodium. These have triangular faces, and examples of face-, edge-, and corner-sharing are found. The phytate molecule assumes an asymmetric conformation with a pseudo-center of symmetry. An empirical stereochemical analysis of the 1,3-*syn* steric effects is qualitatively consistent with observed conformational features. The stereochemical properties of phytate are discussed with regard to its biological roles in enzymatic dephosphorylation and as a structural analogue of polyphosphoinositides.

Introduction

Myoinositol hexaphosphate (phytate) is a major constituent of the phosphate esters of many mature plant seeds and occurs with a pentaphosphate ester of inositol in the blood of some animals (Johnson & Tate, 1969). In embryos and endosperm of seeds, a phytate is commonly associated with ergastic storage protein (Tronier, Ory & Henningsen, 1971) and metal ions, such as potassium and smaller quantities of magnesium and calcium (Eastwood & Laidman, 1971). While several physiological roles have been suggested for phytate in plants (Koller, Mayer, Poljakoft-Mayber & Klein, 1962; Williams, 1970; Biswas & Biswas, 1965; Atkinson & Morton, 1959; Valikhanov, 1969), its function in animal blood seems to be that of a hemoglobin cofactor (Cabrera, Perez & Barrau, 1972; Benesch & Benesch, 1969), analogous to the action of diphosphoglyceric acid (DPG) in the regulation of oxygen transport in human blood (Riggs, 1972; Arnone, 1972; Perutz, 1970). The binding of phytate to human deoxyhemoglobin has been shown to be similar to that of DPG (Arnone & Perutz, 1974). The biochemical activities of phytate depend, among other things, upon the structural and physical properties intrinsic to the phosphate ester structure and the strong chelating behavior of the molecule.

The configuration of phytate was decisively established by nuclear magnetic resonance spectroscopy of the sodium salt (Johnson & Tate, 1969) and preparation of its dodecamethyl ester (Angyal & Russell, 1969). The present study was undertaken to define the three-dimensional structure of the ionized form of phytate and to reveal its manner of coordination to

metal ions. In its crystal structure, phytate was unexpectedly found with its phosphates arranged in the 5-axial/1-equatorial ($5a/1e$) conformation. This structural feature was briefly described in a preliminary report (Blank, Pletcher & Sax, 1971) and it was suggested that ionic forces played a leading role in determining the favored ring conformation. Results from subsequent nuclear magnetic resonance studies by Tate (personal communication) are consistent with the supposition that the phytate ion prefers the ($5a/1e$) conformation and suggest that the protonated conformer is the ($5e/1a$) form.* Furthermore, it has been determined by crystallographic methods that phytate bound to deoxyhemoglobin is in a ($5a/1e$) conformation (Arnone & Perutz, 1974).

Experimental

Sodium phytate (Sigma Chem. Co.) crystallizes from water as a hydrate ($38H_2O$) in the monoclinic space group Cc (Blank, Pletcher & Sax, 1971) with four formula units, $C_6H_{82}Na_{12}O_{62}P_6$, per unit cell. The crystals, of dimensions $0.3 \times 0.2 \times 0.2$ mm, were sealed in glass capillaries for the X-ray diffraction experiments. The cell dimensions, Table 1, were measured on a Picker FACS I system with Cu $K\alpha$ radiation ($\lambda=1.54178$ Å) and were computed by a least-squares method.

A preliminary analysis of this structure (Blank, Pletcher & Sax, 1971) was based on a data set collected as described below, but because of crystal movement,

* The structure of the protonated form of scylloinositol hexaphosphate (to be reported later) has been determined and the molecular conformation is the all-equatorial form.

Table 1. Summary of crystal data

$a = 23.091 (13) \text{ \AA}$
 $b = 12.203 (10)$
 $c = 22.894 (14)$
 $\beta = 108.30 (1)^\circ$
 $D_{\text{obs}} = 1.75 \text{ g cm}^{-3}$ (by flotation in chloroform:dibromomethane)
 $D_{\text{calc}} = 1.744 \text{ g cm}^{-3}$
 $U = 6124.8 \text{ \AA}^3$
 $\mu(\text{Cu } K\alpha) = 36.7 \text{ cm}^{-1}$

Table 2. Atomic positional and thermal parameters for myoinositol hexaphosphate dodecasodium salt octatriacontahydrate

Positional parameters are given as fractions of the lattice translations. Thermal parameters for non-hydrogen atoms are given for the expression: $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$. The isotropic temperature factor from the Wilson plot (2.12 \AA^2) was used for the hydrogen atoms. The estimated standard deviation of the least significant figure is given in parentheses.

(a) Non-hydrogen atoms ($\times 10^4$, except phosphorus $\times 10^5$)

	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
P(1)	1.078(10)	21.961(16)	1.0625(9)	72(4)	211(12)	49(4)	42(5)	26(3)	16(5)
P(2)	-1.5395(9)	57399(17)	4.073(9)	59(6)	447(14)	47(3)	53(6)	19(3)	31(5)
P(3)	-9914(7)	34626(15)	-14415(8)	54(4)	233(12)	45(4)	-25(5)	7(3)	9(5)
F(4)	-946(9)	75931(15)	-474(9)	47(4)	221(12)	46(4)	11(5)	36(3)	45(5)
F(5)	14.663(9)	42592(18)	-4481(10)	43(3)	577(15)	74(4)	80(6)	36(3)	54(5)
F(6)	9841(0)	65740(16)	14877(0)	52(4)	281(13)	42(4)	-17(5)	4(3)	2(5)
O(1)	-48(4)	4531(6)	593(3)	9(2)	25(5)	6(2)	4(2)	3(1)	2(2)
O(2)	-572(3)	5033(6)	91(3)	3(4)	3(5)	7(1)	7(2)	3(1)	3(2)
O(3)	-517(3)	4993(6)	-550(3)	6(1)	25(4)	4(1)	5(2)	2(1)	-3(2)
O(4)	28(3)	5541(6)	-562(4)	8(2)	21(5)	5(2)	3(2)	2(1)	-3(2)
O(5)	648(3)	5238(7)	-421(4)	7(2)	44(6)	6(2)	1(2)	6(1)	2(3)
O(6)	560(3)	5113(7)	604(3)	4(1)	18(3)	4(1)	2(1)	2(1)	2(1)
O(11)	-2(2)	3358(4)	502(2)	9(1)	18(3)	4(1)	2(1)	2(1)	2(1)
O(12)	-387(4)	2824(5)	143(3)	14(2)	44(5)	8(1)	13(2)	9(1)	4(2)
O(13)	-68(2)	1413(4)	727(3)	10(2)	39(4)	7(1)	-2(2)	2(1)	-4(2)
O(14)	771(3)	2509(5)	1416(5)	8(1)	43(4)	10(2)	2(2)	3(2)	3(2)
O(15)	-1121(2)	4633(4)	1.02(2)	5(1)	29(3)	8(1)	-2(2)	2(1)	-3(2)
O(16)	-1617(3)	6499(5)	1.02(3)	13(1)	49(5)	11(1)	11(2)	3(1)	13(2)
O(17)	-1212(3)	5467(5)	1.097(3)	6(1)	46(5)	6(1)	1(2)	2(1)	1(2)
O(18)	-2121(3)	4747(6)	2.61(3)	8(1)	79(6)	9(1)	3(2)	2(1)	1(2)
O(19)	-237(3)	3871(4)	-769(2)	13(1)	17(3)	2(1)	-1(2)	0(1)	0(2)
O(20)	-1439(3)	3586(6)	-1479(2)	8(2)	49(5)	7(1)	2(2)	-1(1)	1(2)
O(21)	-901(3)	2268(5)	-1424(3)	11(1)	23(4)	9(1)	-4(2)	3(1)	0(2)
O(22)	-780(3)	4.051(5)	-1.029(3)	11(1)	44(5)	6(1)	1(2)	5(1)	8(2)
O(23)	-10(2)	6725(4)	-4.86(2)	13(1)	22(3)	6(1)	-1(2)	5(1)	-3(2)
O(24)	299(3)	7191(4)	-143(3)	18(1)	29(4)	13(1)	8(2)	13(1)	1(2)
O(25)	107(2)	8653(4)	-7.20(2)	8(1)	21(3)	9(1)	-4(2)	3(1)	3(2)
O(26)	-773(3)	7599(4)	-11.24(3)	10(1)	30(4)	9(1)	4(2)	1(1)	-4(2)
O(27)	705(3)	1245(5)	-1.63(3)	13(1)	35(4)	10(1)	-3(2)	9(1)	1(2)
O(28)	1170(3)	4389(5)	-13.34(3)	16(2)	64(6)	3(1)	1(2)	5(1)	5(2)
O(29)	1723(3)	3133(6)	-2.43(3)	14(2)	68(6)	16(2)	3(2)	12(1)	12(2)
O(30)	1803(3)	5173(6)	-2.04(3)	19(2)	70(6)	13(2)	-3(3)	14(1)	-6(3)
O(31)	599(6)	6506(6)	8.22(2)	2(1)	28(4)	6(1)	0(2)	0(1)	-3(2)
O(32)	1616(3)	4389(5)	1.527(3)	6(1)	49(5)	10(1)	-4(2)	-1(1)	-2(2)
O(33)	884(3)	7804(5)	1.5(2)	20(4)	61(6)	3(2)	0(2)	1(1)	1(2)
O(34)	753(3)	6.04(5)	1.980(3)	17(2)	43(5)	6(1)	-5(2)	4(1)	6(2)
O(35)	-31(5)	2.677(5)	2.811(4)	22(1)	42(4)	10(1)	0(3)	8(1)	-3(3)
O(36)	-134(3)	3282(6)	-1.75(2)	14(2)	59(6)	23(2)	11(2)	-6(1)	-7(3)
O(37)	11.77(3)	3942(6)	2347(3)	8(1)	31(5)	12(2)	-2(2)	1(1)	0(2)
O(38)	612(4)	-1.22(6)	518(3)	6(1)	47(5)	12(2)	6(2)	0(1)	0(2)
O(39)	45(5)	-337(7)	1750(4)	17(2)	59(6)	13(2)	0(3)	4(1)	-1(3)
O(40)	14.07(3)	221(6)	221(3)	11(2)	46(5)	15(2)	5(2)	0(1)	4(3)
O(41)	-1502(3)	186(8)	11.56(4)	11(2)	96(8)	15(2)	7(3)	3(1)	-9(2)
O(42)	-1.92(3)	336(6)	1210(3)	10(1)	52(5)	14(2)	-9(2)	0(1)	-8(2)
O(43)	-3123(3)	5312(6)	4.83(3)	14(2)	64(6)	18(2)	4(2)	4(1)	1(3)
O(44)	-2389(3)	3447(6)	-775(3)	18(2)	60(6)	15(2)	-9(2)	7(1)	-9(2)
O(45)	-1.83(4)	5900(5)	1.92(3)	13(1)	37(4)	12(1)	3(2)	2(1)	4(2)
O(46)	-655(3)	8330(6)	5.05(3)	8(1)	58(5)	15(2)	-2(2)	2(1)	-4(2)
O(47)	-1183(5)	6099(6)	-2377(3)	9(1)	43(5)	8(2)	2(2)	1(1)	-4(2)
O(48)	-2560(3)	6699(6)	-2363(3)	20(2)	46(5)	10(1)	4(2)	3(1)	1(2)
O(49)	-1186(5)	1.661(6)	-2726(3)	20(2)	48(5)	14(1)	-1(2)	3(1)	-9(2)
O(50)	-1782(3)	7.08(6)	-1.481(3)	11(1)	46(5)	12(1)	-2(2)	0(1)	1(2)
O(51)	-2431(5)	5429(5)	-1.963(3)	14(1)	37(5)	19(2)	5(2)	4(1)	2(2)
O(52)	1375(5)	7952(6)	-2.5(4)	18(2)	47(6)	37(2)	10(2)	0(2)	7(3)
O(53)	-62(4)	10326(7)	-1743(4)	17(2)	37(6)	16(2)	-2(3)	1(2)	-14(3)
O(54)	511(3)	10139(6)	-518(3)	13(2)	41(5)	10(2)	9(2)	4(1)	-4(2)
O(55)	-1404(5)	3277(6)	-2322(3)	13(2)	44(5)	12(2)	2(2)	4(1)	4(2)
O(56)	-1712(5)	769(6)	-920(3)	11(1)	16(2)	14(2)	-2(2)	8(1)	2(3)
O(57)	14.69(4)	9777(7)	-1.204(4)	18(2)	67(6)	14(2)	4(3)	4(2)	2(3)
O(58)	1.00(4)	4750(6)	-1.694(4)	18(2)	50(5)	22(2)	-4(3)	6(1)	-6(3)
O(59)	2317(4)	1.66(8)	84(6)	18(2)	64(6)	11(2)	-3(3)	4(1)	6(3)
O(60)	659(3)	1.645(5)	-560(3)	13(1)	59(5)	15(1)	-8(2)	6(1)	6(2)
O(61)	1.895(5)	6551(6)	-1.207(3)	22(2)	52(5)	15(1)	-9(2)	6(1)	-8(2)
O(62)	2.998(5)	3125(6)	736(4)	10(2)	84(7)	15(2)	-5(3)	1(1)	9(3)
O(63)	3.077(6)	4.76(8)	1.94(3)	9(1)	44(5)	13(1)	-9(2)	2(1)	3(2)
O(64)	1.731(3)	2411(6)	969(9)	13(2)	71(6)	13(1)	1(2)	3(1)	6(2)
O(65)	2358(3)	6431(5)	796(3)	20(2)	50(5)	16(2)	-11(2)	11(1)	-9(2)
O(66)	1133(2)	894(6)	2737(3)	17(1)	51(5)	14(1)	1(2)	6(1)	0(3)
O(67)	-483(2)	7191(6)	11(6)	11(1)	68(5)	23(2)	-7(2)	7(1)	2(2)
O(68)	1.849(2)	5952(5)	3103(3)	7(1)	46(4)	9(1)	3(2)	3(1)	4(2)
O(69)	2576(2)	7298(5)	2366(3)	7(1)	37(4)	14(1)	-7(2)	2(1)	-5(2)
O(70)	1762(3)	923(6)	1.526(3)	9(1)	55(5)	14(1)	-4(2)	3(1)	4(2)
O(71)	2448(3)	4.560(5)	1.94(3)	12(1)	45(5)	14(1)	1(2)	6(1)	4(2)
O(72)	-18(3)	4929(4)	2504(4)	8(1)	47(3)	13(1)	-4(2)	4(1)	0(2)
Na(1)	324(2)	8027(3)	377(2)	18(1)	38(2)	8(1)	3(1)	3(1)	2(1)
Na(2)	2133(2)	774(5)	1642(3)	17(1)	47(3)	10(1)	-6(1)	5(1)	-2(1)
Na(3)	2202(2)	4.089(3)	2902(2)	9(1)	58(5)	11(1)	1(2)	1(1)	2(1)
Na(4)	854(2)	6.021(3)	-1.771(2)	11(1)	42(3)	11(1)	4(1)	5(1)	-2(1)
Na(5)	1.008(2)	9739(3)	-354(2)	13(1)	55(3)	15(1)	-3(1)	5(1)	1(1)
Na(6)	-2302(2)	3.92(5)	-21(2)	11(1)	35(2)	7(1)	5(1)	2(1)	2(1)
Na(7)	-2302(2)	2264(3)	-3286(2)	13(1)	41(3)	11(1)	-1(1)	1(1)	-2(1)
Na(8)	-2210(2)	5923(3)	-2886(2)	6(1)	44(3)	11(1)	2(1)	2(1)	-2(1)
Na(9)	-681(2)	3950(3)	1766(2)	11(1)	47(3)	10(1)	5(1)	5(1)	1(1)
Na(10)	-1408(2)	3.94(5)	-21(2)	12(1)	45(3)	12(1)	-1(1)	4(1)	-1(1)
Na(11)	745(2)	217(3)	2779(2)	12(1)	65(3)	13(1)	-4(1)	4(1)	-1(1)
Na(12)	-761(2)	9856(3)	-2799(2)	11(1)	45(3)	11(1)	2(1)	2(1)	-1(1)

Table 2 (cont.)

(b) Hydrogen atoms ($\times 10^3$)

	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
H(21)	-21(4)	4.69(8)	94(5)	21(4)	49(8)	17(4)	14(4)	14(4)	14(4)
H(22)	-89(4)	513(8)	-28(4)	13(2)	13(2)	5(2)	7(4)	7(4)	7(4)
H(23)	132(4)	512(7)	-7(4)	170(4)	24(8)	-3(4)	101(4)	456(8)	221(4)
H*(232)	101(4)	24(7)	212(4)	201(4)	-24(7)	87(4)	125(4)	124(4)	205(4)
H*(231)	-121(4)	42(7)	96(4)	-215(4)	299(7)	111(4)	-272(4)	526(7)	34(4)
H*(222)	-210(4)	73(7)	-92(4)	-210(4)	274(7)	-221(4)	190(7)	-267(4)	256(4)
H*(221)	-109(4)	54(7)	-210(4)	-158(4)	101(7)	-146(4)	74(7)	-161(4)	30(4)
H*(212)	-221(4)	274(7)	-221(4)	-179(4)	54(7)	-210(4)	172(4)	759(8)	159(4)
H*(211)	-158(4)	101(7)	-146(4)	-221(4)	274(7)	-221(4)	190(7)	-267(4)	256(4)
H*(202)	-81(4)	803(7)	41(4)	-158(4)	101(7)	-146(4)	74(7)	-161(4)	30(4)
H*(201)	-26(4)	1076(7)	-163(4)	-37(4)	981(7)	-59(4)	-100(4)	888(8)	-201(4)
H*(192)	-37(4)	981(7)	-59(4)	-153(4)	939(7)	-211(4)	-121(4)	758(7)	-105(4)
H*(191)	-153(4)	939(7)	-211(4)	131(4)	896(7)	-125(4)	172(4)	769(8)	159(4)
H*(182)	3(5)	297(8)	-179(4)	101(4)	172(7)	-43(4)	205(4)	744(7)	-112(4)
H*(181)	101(4)	172(7)	-43(4)	250(4)	215(7)	53(4)	309(4)	297(7)	74(4)
H*(172)	250(4)	215(7)	53(4)	220(4)	690(7)	211(4)	182(4)	945(7)	180(4)
H*(171)	220(4)	690(7)	211(4)	212(4)	668(7)	84(4)	101(4)	830(7)	278(4)
H*(162)	-73(5)	694(8)	149(5)	-73(5)	694(8)	149(5)	160(4)	606(7)	295(4)
H*(161)	220(4)	690(7)	211(4)	-73(5)	694(8)	149(5)	160(4)	606(7)	295(4)
H*(152)	150(4)	506(8)	174(4)	220(4)	690(7)	211(4)	182(4)	945(7)	180(4)
H*(151)	506(8)	174(4)	220(4)	220(4)	690(7)	211(4)	182(4)	945(7)	180(4)

it contained an unacceptable level of systematic error. All features of the structure analysis previously reported were corroborated by this more accurate determination.

Intensity data were collected on a Picker FACS I automatic diffractometer by $\theta/2\theta$ scans at 2° min^{-1} , using graphite-monochromated $\text{Cu } K\alpha$ radiation. 20 s backgrounds were measured with both crystal and counter stationary at the beginning and end of each scan. Of the 5365 independent reflections recorded for $2\theta \leq 103^\circ$, 438 had intensities less than $2.0\sigma(I)$ as estimated from counting statistics, and were designated unobserved. Three standard reflections were monitored after every 100 intensity measurements. Degradation of the phytate crystal was negligible ($< 0.2\%$) during the data collection. After being corrected for background and the Lorentz-polarization factors, the intensity data were reduced to structure factors (Shiono, 1966a). A Wilson plot (Shiono, 1969) was used to obtain an approximate absolute scale for the data. No corrections were applied for absorption and extinction.

The structure was solved by the symbolic addition procedure for noncentrosymmetric space groups with centered cells (Karle & Hauptman, 1961). The successful set of starting phases which led to the correct structure was: $4, 12, \bar{1}, |E| = 3.31, \varphi = 0; 7, 1, \bar{1}, |E| = 3.25, \varphi = \pi/2; 5, \bar{1}, |E| = 2.88, \varphi = 3\pi/2; \text{ and } 13, \bar{3}, |E| = 2.31, \varphi = \pi$. Ten cycles of reiterative tangent formula refinement (Hall, 1967) were applied to the 369 reflections with $|E| \geq 1.77$. These phases were used in the calculation of an E map (Karle, Hauptman, Karle & Wing, 1958) in which 48 atomic positions were identified. The remainder of the atomic positions, except for the hydrogens, were obtained from subsequent Fourier maps that were phased on the known part of the structure. Three cycles of isotropic block-diagonal least-squares refinement (Shiono, 1970) were followed by several cycles of anisotropic refinement. Difference Fourier maps based on these refined positions revealed the majority of the hydrogen atom positions.

The residual index ($R = \sum |F_{\text{obs}}| - |F_{\text{calc}}| / \sum |F_{\text{obs}}|$) at this time was 0.067. Because of the pseudosymmetry in the molecule and the unusual values for some bond

distances and angles, it was uncertain if the block-diagonal procedure had converged to the correct solution. Limitations on computer time and space precluded treating the entire structure by the full-matrix approach. Overlapping blocks of approximately 230 parameters were refined by full-matrix least squares (Shiono, 1966*b*) until all parameters had been processed twice. The x and z parameters of P(1) were held constant and the anomalous dispersion corrections, $\Delta f'$ and $\Delta f''$ (Templeton, 1968), were applied for the phosphorus, oxygen, and sodium atoms. Only positional parameters of the hydrogen atoms were refined (see Table 2). The data were weighted according to the function suggested by Cruickshank, Pilling, Bujosa, Lovell & Truter (1961); $\omega = [a + b(F_{\text{obs}}) + c(F_{\text{obs}})^2]^{-1}$, $a = 27.0$, $b = 0.1$, and $c = 0.006$. In the last pass, the average shift in the x , y , z parameters was 0.82 times their estimated standard deviations and 0.67 for β_{ij} values. Thirteen parameters exhibited shift/error ratios between 2.5 and 3.0. These were the x parameters of O(W65), O(W33), Na(11), Na(6), O(W57), the z parameters of O(W53), Na(11), O(W41), Na(12), and the β_{ij} 's of Na(1), Na(6) and Na(5). For economic reasons, the refinement was terminated. The final residual index is 0.064 for all reflections, and 0.056 for observed data only. The final atomic parameters and their estimated standard deviations are listed in Table 2.*

* A list of structure factors and an Appendix have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31074 (38 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Results and discussion

Four crystalline hydrates of the dodecasodium salt of myoinositol hexaphosphate have been prepared from aqueous alcohol solutions; see Table 3. Both the crystalline form (*C*), originally obtained by Posternak (1921), and (*A*), are reportedly stable hydrates, while (*B*) is efflorescent. Crystals used in the present study, obtained from an aqueous solution of sodium phytate, lost water of hydration and became highly disordered within a few hours after exposure to air; thus they were necessarily sealed in capillaries during X-ray measurements. These crystals belong to space group *Cc* and are similar to the ones previously reported by Fennessey & Nowacki (1968) to be a stable form. The unit-cell dimensions differ only slightly from the values which they reported. These differences in crystal behavior may in part be attributable to crystal size, since crystals with a smaller surface-to-volume ratio showed an enhanced air stability.

The crystal structure determination of this hydrate confirmed the hexaorthophosphate ester configuration of phytate established by other methods. It demonstrated unequivocally that phytate.12Na⁺ adopts the chair conformation (*5a/1e*) in the solid state, in contrast to inositol which assumes the alternate chair form (*5e/1a*) (Rabinowitz & Kraut, 1964; Lomer, Miller & Beevers, 1963). The inherent properties of the phytate molecule which are largely responsible for this preferred conformation will be discussed in more detail later. The large solvation shell of phytate in this crystal structure suggests that a similar conformation occurs in the solution state. However, because the

Table 3. Crystalline hydrates of the dodecasodium salt of myoinositol hexaphosphate

	Formula	Space group	Z	a (Å)	b (Å)	c (Å)	β (°)	Reference
<i>A</i>	C ₆ H ₆ Na ₁₂ O ₂₄ P ₆ · 33H ₂ O	<i>P</i> 2 ₁ / <i>n</i>	4	14.78	32.14	12.12	102.0	Truter & Tate (1970)
<i>B</i>	C ₆ H ₆ Na ₁₂ O ₂₄ P ₆ · 35H ₂ O	<i>P</i> 2 ₁ / <i>n</i>	4	26.95	20.3	11.77	111	Truter & Tate (1970)
<i>C</i>	C ₆ H ₆ Na ₁₂ O ₂₄ P ₆ · 38H ₂ O	<i>Cc</i>	4	23.065	12.120	22.871	108.21	Fennessey & Nowacki (1968)
				23.091	12.203	22.894	108.30	This work
<i>D</i>	C ₆ H ₆ Na ₁₂ O ₂₄ P ₆ · 44H ₂ O	undetermined						Posternak (1921)

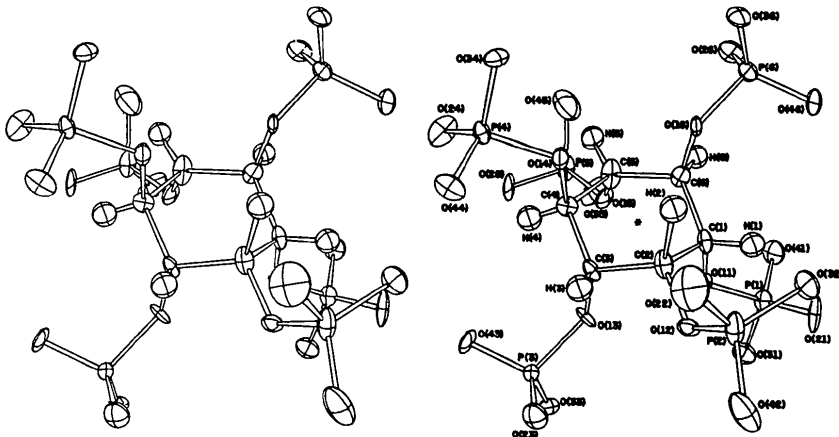


Fig. 1. A stereoscopic view of the phytate molecule (*ORTEP*; Johnson, 1965).

Table 4. Bond distances (Å) and bond angles (°) for phytate

Estimated standard deviations are given in parentheses.

P(1)-O(11)	1.616 (5)	P(5)-O(35)	1.513 (8)
P(1)-O(21)	1.506 (6)	P(5)-O(45)	1.492 (8)
P(1)-O(31)	1.527 (6)	P(6)-O(16)	1.620 (6)
P(1)-O(41)	1.492 (6)	P(6)-O(26)	1.493 (7)
P(2)-O(12)	1.632 (5)	P(6)-O(36)	1.517 (7)
P(2)-O(22)	1.522 (7)	P(6)-O(46)	1.534 (7)
P(2)-O(32)	1.526 (7)	C(2)-C(1)	1.525 (11)
P(2)-O(42)	1.493 (7)	C(3)-C(2)	1.516 (10)
P(3)-O(13)	1.631 (6)	C(4)-C(3)	1.494 (11)
P(3)-O(23)	1.506 (7)	C(5)-C(4)	1.546 (12)
P(3)-O(33)	1.520 (7)	C(6)-C(1)	1.575 (11)
P(3)-O(43)	1.509 (7)	C(6)-C(5)	1.559 (12)
P(4)-O(14)	1.647 (6)	C(1)-O(11)	1.436 (10)
P(4)-O(24)	1.502 (6)	C(2)-O(12)	1.400 (10)
P(4)-O(34)	1.518 (6)	C(3)-O(15)	1.453 (10)
P(4)-O(44)	1.525 (6)	C(4)-O(14)	1.470 (10)
P(5)-O(15)	1.628 (6)	C(5)-O(15)	1.405 (10)
P(5)-O(25)	1.511 (7)	C(6)-O(16)	1.434 (10)
O(11)-P(1)-O(21)	107.7 (3)	O(16)-P(6)-O(26)	108.7 (4)
O(11)-P(1)-O(31)	102.5 (3)	O(16)-P(6)-O(36)	100.8 (3)
O(11)-P(1)-O(41)	107.8 (3)	O(16)-P(6)-O(46)	107.5 (3)
O(21)-P(1)-O(31)	113.4 (3)	O(26)-P(6)-O(36)	115.0 (4)
O(21)-P(1)-O(41)	112.7 (4)	O(26)-P(6)-O(46)	112.3 (4)
O(31)-P(1)-O(41)	112.0 (3)	O(36)-P(6)-O(46)	111.6 (4)
O(12)-P(2)-O(22)	108.6 (3)	P(1)-O(11)-C(1)	121.7 (5)
O(12)-P(2)-O(32)	108.5 (3)	P(2)-O(12)-C(2)	116.9 (5)
O(12)-P(2)-O(42)	102.0 (3)	P(3)-O(13)-C(3)	121.8 (5)
O(22)-P(2)-O(32)	111.2 (4)	P(4)-O(14)-C(4)	121.4 (5)
O(22)-P(2)-O(42)	113.2 (4)	P(5)-O(15)-C(5)	120.7 (5)
O(32)-P(2)-O(42)	112.8 (4)	P(6)-O(16)-C(6)	119.7 (5)
O(13)-P(3)-O(23)	107.8 (3)	C(3)-C(2)-C(1)	115.1 (5)
O(13)-P(3)-O(33)	101.9 (3)	C(4)-C(3)-C(2)	110.2 (6)
O(13)-P(3)-O(43)	108.3 (3)	C(5)-C(6)-C(1)	114.2 (7)
O(23)-P(3)-O(33)	112.7 (4)	C(6)-C(1)-C(2)	108.2 (6)
O(23)-P(3)-O(43)	112.7 (4)	C(6)-C(5)-C(4)	114.1 (7)
O(33)-P(3)-O(43)	112.7 (4)	C(5)-C(4)-C(3)	116.9 (7)
O(14)-P(4)-O(24)	108.1 (3)	O(11)-C(1)-C(2)	112.6 (6)
O(14)-P(4)-O(34)	101.1 (3)	O(11)-C(1)-C(6)	110.7 (6)
O(14)-P(4)-O(44)	106.7 (3)	O(12)-C(2)-C(1)	110.3 (6)
O(24)-P(4)-O(34)	114.9 (3)	O(12)-C(2)-C(3)	111.1 (6)
O(24)-P(4)-O(44)	113.5 (3)	O(13)-C(3)-C(2)	111.6 (6)
O(34)-P(4)-O(44)	111.4 (3)	O(13)-C(3)-C(4)	110.3 (6)
O(15)-P(5)-O(25)	105.2 (4)	O(14)-C(4)-C(3)	108.0 (6)
O(15)-P(5)-O(35)	99.0 (4)	O(14)-C(4)-C(5)	104.1 (6)
O(15)-P(5)-O(45)	112.8 (4)	O(15)-C(5)-C(4)	111.9 (7)
O(25)-P(5)-O(35)	115.0 (4)	O(15)-C(5)-C(6)	106.9 (6)
O(25)-P(5)-O(45)	110.0 (4)	O(16)-C(6)-C(1)	107.1 (6)
O(35)-P(5)-O(45)	114.0 (4)	O(16)-C(6)-C(5)	105.9 (6)

extent of association between phytate and sodium ions in solution is unknown, extrapolation of structural features of the solution state must be done cautiously in the absence of substantiating experimental evidence.

Table 4 contains the values of the bond distances and angles found for the phytate molecule alone. The atomic numbering shown in Fig. 1 is used throughout this discussion. In some instances, the ranges of values for different bond types exceed the commonly observed variations, possibly due to the abnormal bonding modes and steric effects in the structure. However, decreased precision due to systematic errors in the data, such as those caused by absorption, extinction, etc. cannot be discounted.

The average phosphate geometries are within the range of values found for comparable structures, and instances of deviation from the norm can often be attributed to bonding or steric forces. An unusual feature of the structure is the coordination of some of the ester oxygens to sodium ions. For these oxygens, the average ester P-OR bond length, 1.628(3) Å, is nearly statistically different from the similarly coordinated ester linkage found for glyceryl-2-phosphate disodium salt [1.612(8) Å; Haque & Caughlan (1966)]. The ester linkages of phytate lacking such coordination average 1.630(3) Å, comparable to that of glycerol-3-phosphate disodium salt [1.637(7) Å; Fenn & Marshall, 1972]. Examination of the phosphoryl bonds and their geometries with respect to the nature of the immediate solvation components does not reveal a consistent trend to explain the observed bond lengths. Typically, O(26), O(44), O(42) and O(41) all have three close contacts to water molecules, but the bond length of P(4)-O(44) differs significantly from that of the rest. The angular arrangements of the oxygens about the six phosphorus atoms show large deviations, as is normally observed in the flexible phosphate geometry. The local solvation environments are considered to have a significant influence on the angular arrangements of the phosphates. Possible ramifications of these interrelationships are discussed below.

In the crystal structure the phytate molecule with

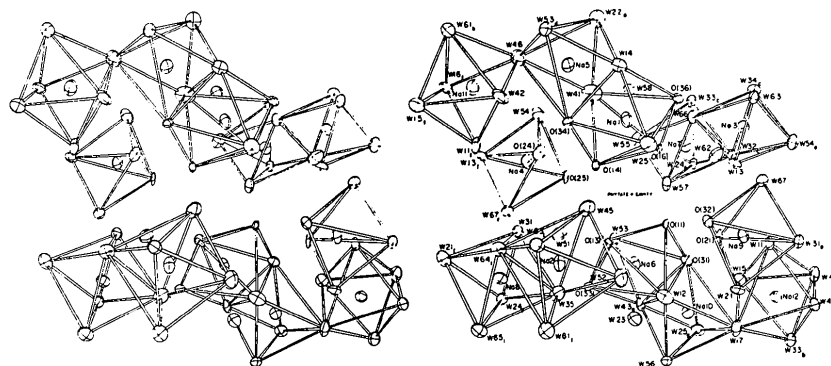


Fig. 2. A stereo drawing showing the solvation components of sodium phytate.38H₂O. Symmetry code: (a) $\frac{1}{2} + x, \frac{1}{2} + y, z$; (b) $x, -y, \frac{1}{2} + z$; (c) $\frac{1}{2} + x, \frac{1}{2} - y, \frac{1}{2} + z$; (d) $x, 1 + y, z$; (e) $x, 1 - y, \frac{1}{2} + z$; (f) $x, 1 - y, -\frac{1}{2} + z$; (g) $x, -1 + y, z$; (h) $\frac{1}{2} + x, -\frac{1}{2} + y, z$; (i) $-\frac{1}{2} + x, \frac{1}{2} + y, z$; (j) $-\frac{1}{2} + x, -\frac{1}{2} + y, z$; (k) $x, 2 - y, -\frac{1}{2} + z$; (l) $-\frac{1}{2} + x, \frac{3}{2} - y, -\frac{1}{2} + z$.

its accompanying solvation shell assumes a structure having a pseudo-center of symmetry at the center of the cyclohexane ring, by making suitable conformational adjustments. With its phosphate equatorial and its hydrogen axial, C(2) has a configurational arrangement opposite to that of C(5). However, P(2) and its phosphoryl oxygens assume a position comparable to P(5) and its phosphoryl oxygens, relative to the pseudo-center, by torsional alterations about its P–O and O–C bonds. The ester oxygen and cyclohexyl hydrogens of C(2) and C(5) are not related by the pseudo-center. The torsional adjustments in these phosphates lead to the close proximity of O(22) and O(45) to their ring hydrogen atoms.

The weighted average C–O bond distance, 1.433(5) Å, agrees with values found in other structures, although the individual deviations from this value were judged statistically insignificant using a σ^2 which is a factor of two larger than that estimated from the

least-squares refinement (Hamilton & Abrahams, 1970). It was noted that the longer bonds are to oxygens coordinated to sodium ions. After adjusting the variances of the C–C bonds in the same fashion, the pseudo-centrosymmetrically related bonds of the

Table 5. *Coordination distances between sodium and oxygen atoms in phytate*

The error in these parameters is probably 0.007–0.008 Å.

	Range (Å)	Average distance (Å)		Range (Å)	Average distance (Å)
Na(1)	2.351–2.832	2.495	Na(2)	2.365–2.635	2.450
Na(3)	2.315–2.505	2.407	Na(4)	2.224–2.460	2.383
Na(5)	2.375–2.593	2.440	Na(6)	2.358–2.718	2.472
Na(7)	2.379–2.599	2.455	Na(8)	2.319–2.491	2.408
Na(9)	2.197–2.490	2.377	Na(10)	2.400–2.557	2.449
Na(11)	2.397–2.546	2.475	Na(12)	2.417–2.518	2.453

Table 6. *The probable hydrogen bonding scheme for phytate oxygen atoms*

Acceptor atom	Hydrogen atom	Donor atom	Angle			Acceptor atom	Hydrogen atom	Donor atom	Angle		
(i)	(j)	(k)	$D(ik)$ Å	$D(ij)$ Å	$(jki)^\circ$	(i)	(j)	(k)	$D(ik)$ Å	$D(ij)$ Å	$(jki)^\circ$
O(21)		O(W11)	2.704			O(34)	H''	O(W43)	2.648	1.88	9
O(31)	H'	O(W14)	2.611	1.63	19	O(34)		O(W42)	3.039		
O(31)		O(W12)	2.999			O(44)	H'	O(W44)	2.707	2.02	37
O(41)		O(W57)	2.722			O(44)	H'	O(W45)	2.750	1.91	8
O(41)	H''	O(W13)	2.684	1.87	13	O(44)		O(W31)	2.716		
O(41)	H'	O(W16)	2.740	2.18	10	O(25)		O(W63)	2.723		
O(22)		O(W45)	2.717			O(25)		O(W51)	2.716		
O(22)	H'	O(W52)	3.021	2.02	4	O(35)		O(W52)	2.068		
O(22)		O(W55)	2.884			O(35)	H'	O(W53)	2.956	2.33	31
O(32)	H'	O(W62)	2.720	2.15	24	O(35)	H'	O(W55)	2.926	2.08	16
O(32)		O(W24)	2.720			O(35)		O(W57)	2.906		
O(42)		O(W21)	2.729			O(45)		O(W58)	2.683		
O(42)	H'	O(W22)	2.612	1.57	13	O(45)	H'	O(W56)	2.974	2.18	18
O(42)		O(W23)	2.755			O(45)		O(W54)	2.842		
O(23)	H''	O(W32)	2.813	2.23	35	O(26)	H'	O(W66)	2.751	1.67	15
O(23)	H'	O(W35)	2.762	1.93	6	O(26)	H'	O(W64)	2.760	1.77	8
O(23)	H'	O(W23)	2.749	1.91	17	O(26)	H'	O(W58)	2.776	2.32	41
O(33)	H'	O(W34)	2.760	2.17	5	O(36)	H'	O(W65)	2.715	1.90	11
O(43)		O(W67)	2.786			O(36)		O(W15)	3.163		
O(43)	H''	O(W31)	2.727	1.82	16	O(46)	H'	O(W13)	2.777	1.93	6
O(43)	H''	O(W51)	3.052	2.23	13	O(46)		O(W67)	2.792		
O(24)		O(W11)	2.765			O(46)	H'	O(W63)	2.987	2.45	23

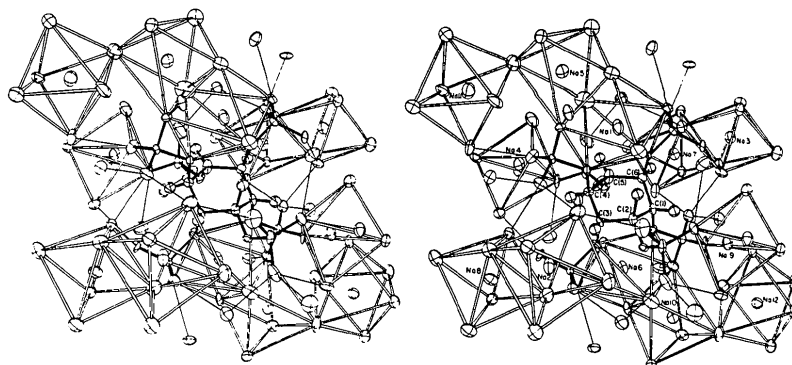


Fig. 3. A stereo drawing showing the phytate molecule with its solvation components (the atomic labeling is the same as that in Figs. 1 and 2).

cyclohexane ring exhibit differences of barely less than $3\cdot0\sigma$ from one another. The weighted average value for these bonds ($1\cdot536(5)$ Å) is normal. If these differences in ring bonds related by molecular pseudo-symmetry are real and not attributable to systematic data errors, they cannot be accounted for by consideration of the molecular properties of phytate alone. Distortion forces may be introduced by solvation components.

The 12 sodium atoms belonging to the phytate solvation sphere, along with their coordination components, exhibit the same pseudo-symmetry found in phytate. They are shown with the water molecules of hydration in Fig. 2. For clarity the phytate molecule has been deleted from the stereographic projection but those phosphate oxygen atoms coordinated to sodium atoms have been included. Each sodium atom is shown with the components of its primary coordination sphere. Coordination distances for these polyhedral arrangements are given in Table 5.

A salient feature of the polyhedral arrangement of the oxygen atoms around the sodium atoms is the presence of three types of coordination: octahedral for sodium atoms 2, 3, 5, 7, 8, 10, 11 and 12; decahedral for 1 and 6; and hexahedral for 4 and 9. The latter co-

ordination was obtained by restricting the acceptable $\text{Na}\cdots\text{O}$ coordination distance to $3\cdot0$ Å or less. If oxygen atoms at distances greater than this value are included, Na(4) and Na(9) would be alternately described as having six-coordinated oxygens in a distorted octahedral arrangement. The average Na–O distance for all polyhedra is $2\cdot431(1)$ Å. The range $2\cdot191$ to $2\cdot899$ Å is somewhat greater than that reported in comparable structures (Haque & Caughlan, 1966; Fenn & Marshall, 1972). The angular geometry is normal for sodiums of octahedral coordination. The polyhedra have triangular faces and examples of all possible polyhedra sharing modes are present (see Fig. 3). Face-sharing occurs between the polyhedra of Na(5) and Na(1). Corner- and edge-sharing can be found between the polyhedra of Na(11) and Na(5), and Na(8) and Na(2), respectively. The other polyhedra in this figure are related to those mentioned by the pseudo-center.

Water molecules O(W51), O(W58), O(W23) and O(W42) uniquely lack coordination to sodium atoms but are held in the structure by hydrogen bonds to phosphate oxygens and other water molecules; see Table 6.

The nature of the sodium–phosphate associations

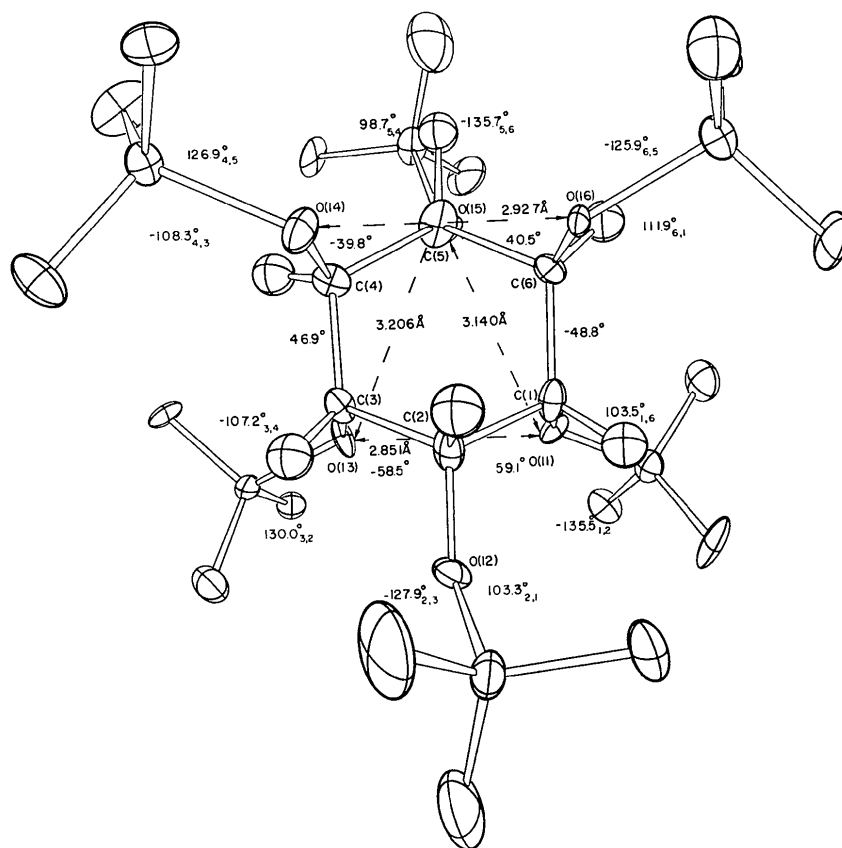


Fig. 4. A view of the phytate molecule showing the distance (Å) between *syn*-1,3-axial oxygens, and selected torsion angles ($^{\circ}$) within the molecule. The torsion angles about the phosphate O–C bonds have the subscripts i, j , where i refers to the phosphorus atom and j designates the terminal carbon atom. The average σ 's for the C–C and O–C torsion angles are $0\cdot9^{\circ}$ and $0\cdot6^{\circ}$, respectively.

can be more readily seen in Fig. 3, which shows phytate with its accompanying hydrogen bonds and coordinated sodium ions. Na(1) and Na(6) have particularly interesting bonding arrangements to the phosphates at C(1), C(3), C(4) and C(6). In these instances, the sodium atoms are bonded to the ester oxygen atoms and one phosphoryl oxygen atom. Bridging the 1,3-*syn*-phosphates in this fashion, the sodium atoms aid in stabilizing an otherwise sterically undesirable conformation. While sodium coordination to the ester oxygen atoms of phosphates is not a common mode of association, similar bonding is exhibited in the solid state structure of glycerol-2-phosphate disodium salt (Haque & Caughlan, 1966) at a distance of 2.541(4) Å. Another mode of sodium bridging between phosphates is through the simultaneous coordination of two phosphoryl oxygen atoms, as shown for Na(4) and Na(9). Interestingly, there is no sodium bridging of phosphates on different phytate ions.

As is usual, the phosphate groups deviate from tetrahedral symmetry. The deviations in phosphates 1, 3, 4 and 6 appear to be correlated with the sodium ion coordination. As mentioned above, each of these phosphates has a sodium ion bridging the ester and a phosphoryl oxygen. The RO-P-O^{δ-} angle for the coordinated oxygens is on the average reduced to 101° from the tetrahedral value. In a compensatory fashion, the O^{δ-}-P-O^{δ-} angles open to an average of 113°. On the other hand, the other mode of sodium bridging occurs at phosphate groups 2 and 5. Specifically, the bridges are O(3,2)···Na(9)···O(2,1) and O(2,4)···Na(4)···O(2,5) where clearly the bridged oxygens belong to different phosphate groups. Nevertheless, sodium ionic forces influence the torsion angle for the P-O-C-C bond sequence in these two cases. For phosphate group 5, the preferred torsion angle places O(45) into steric opposition with the ring hydrogen atom. To relieve some of the strain, the RO-P-O^{δ-} valency angle involving O(45) opens appropriately. The net effect upon the phosphate geometry is comparable to those cases with bridging sodium ions. Since phosphate group 2 is equatorial rather than axial, O(42) is pseudo-centrosymmetrically related to O(45) but without steric interference from the ring hydrogen atom. Despite this difference, the geometry of phosphate group 2 is comparable to that of phosphate group 5. The extent to which packing and ionic forces in the molecule influence the geometries of 2 and 5 is difficult to estimate.

The forces limiting phytate to the (5a/1e) conformation fall into two categories: the intramolecular coulombic and steric interactions, and the ionic and hydrogen-bonding forces of the hydration and coordination components. The coulombic interactions of the vicinal (*e/e*) phosphates are most readily minimized by shifting to the diaxial orientation of the phosphates. While this provides a greater separation between the phosphates, it is accomplished at the ex-

pense of placing each axial ester oxygen in steric opposition with at least one other *syn*-axial oxygen. Such steric repulsion manifests itself by changes in bond angles, torsion angles, and intramolecular distances. The 1,3-*syn*-axial oxygen-to-oxygen distances for phytate are given in Fig. 4. These range from 0.35 to 0.71 Å farther apart than expected (2.5 Å) for the unstrained cyclohexane geometry (Jeffrey & Kim, 1971). For an inositol free from intramolecular hydrogen bonding, 1,3-*syn*-axial oxygens show a separation of 2.96 Å (Jeffrey & Kim, 1971). The increase is attributed to the steric repulsion of the *syn* oxygens. The displacement of O(15) and O(12) from their idealized axial positions is coupled through torsion and bond angle changes to the positions of O(14) and O(16), and O(11) and O(13), respectively. The expected effect is one of additional spreading of the O(14)···O(16) and O(11)···O(13) distances beyond the 2.96 Å value. However, these distances are either equal to or less than 2.96 Å. Since each pair of these 1,3-related oxygens is bridged by a coordinated sodium atom, apparently the cation is able to force the ester oxygens into closer proximity than would be predicted from steric effects alone. No comparable bridging occurs with ester oxygen O(15) which exhibits 1,3-*syn*-axial separations greater than 2.96 Å. This is in part attributable to the effect of the repulsion between O(16) and O(14) on the C(5) position.

Alterations in the shape of the cyclohexane ring and the disposition of the nearest bonded atoms are readily apparent in the two least-squares planes calculations in Table 7. The decrease in ring puckering resulting from *syn*-1,3-axial repulsion places C(2) and C(5) closer to the plane I [unstrained positions are 0.72 Å from this plane; Jeffrey & Kim (1971)]. Plane II corresponds to the theoretical molecular mirror plane of phytate. This calculation reveals that the molecule is an enantiomorphic conformer (Eliel, 1962). Torsional rotations primarily about the O-C bond of P-O-C-C for phosphate groups 2 and 5 are probably largely caused by the Na(9) and the Na(4) bridge between phosphate groups 1 and 2 and 4 and 5, respectively. The rotations break the molecular mirror symmetry. These contacts, O(21)···O(32), 3.792(9) Å, and O(24)···O(25), 3.919(9) Å, are somewhat closer than usual for distances between sodium ligand sites. Since phosphate groups 4 and 5 are *trans* related and phosphate groups 1 and 2 are *cis*, it is not unexpected that phosphate group 5 is displaced more than phosphate group 2 to present an O(24)···O(25) sodium coordination comparable to that of O(21)···O(32). A semiquantitative analysis has been made of the effect produced by *syn*-axial interactions on the conformation of the cyclohexane ring. This analysis is given in detail in the Appendix.*

Irving & Cosgrove (1971) have proposed a model for the mechanism of bacterial phytase action in terms of

* See previous footnote.

Table 7. Least-squares planes through the phytate molecule

The coefficients $\times 10^4$ are given for the equations of the planes having the form $Ax + By + Cz = D$ (referred to the crystallographic axes x, y, z in Å). σ is the root mean square deviation of the atoms forming the plane. The dihedral angle between planes I and II is 92.7° . Displacements from the plane are in $\text{Å} \times 10^3$. Atoms in bold face type are included in the calculation of the least-squares plane.

Plane I: $A=4424, B=8637, C=3683, D=53167; \sigma=0.020$
C(1) -10, C(3) 10, C(4) -10, C(6) 10, C(2) 661, C(5) -492

Plane II: $A=1033, B=1676, C=8984, D=9931; \sigma=0.020$
C(2) -15, C(5) 8, O(12) 10, O(15) -3, P(1) -2694, P(3) 2925, P(2) -363, P(5) 450, P(6) -2943, P(4) 2766, C(1) -1278, C(3) 1283, C(6) -1323, C(4) 1281, O(11) -1339, O(13) 1508, O(16) -1540, O(14) 1384

a three-point attachment of phytate in its (5e/1a) conformer to the enzyme. Some structural properties of phytate found in the crystal structure are not considered in their model. First, phytate might be in transition to, or have attained, the (5a/1e) conformation, since it would be enzyme-bound in an ionized form, and secondly, the initial attack of the enzyme may be on an enantiomeric conformer with a structure similar to that observed in the solid state. The latter point is illustrated for known phytases in Fig. 5. In each instance, the position attacked by the enzyme is that with less steric restrictions relative to the position across the molecular mirror plane. Subsequent hydrolysis to lower phosphates might be governed by the

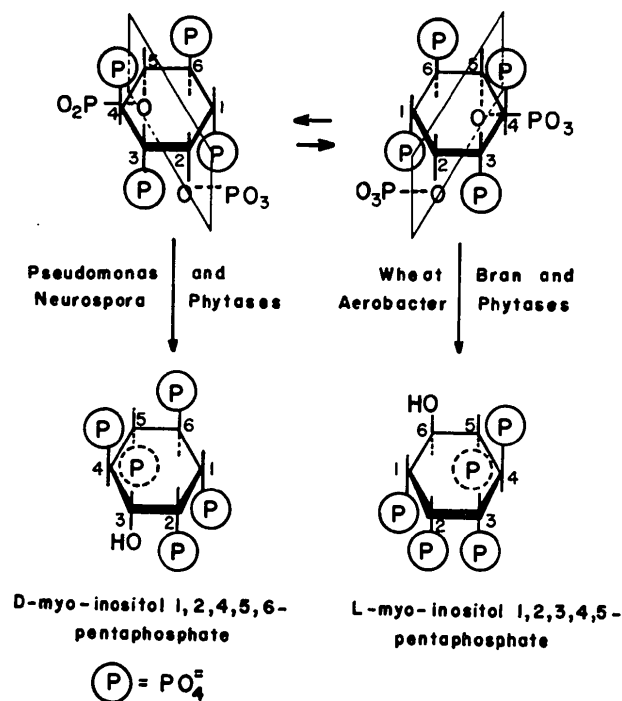


Fig. 5. A schematic representation of the proposed enzymatic hydrolysis of enantiomeric conformers of phytate.

steric accessibility of the phosphates as well as dynamic changes in the phytate conformation.

It is becoming increasingly clear that the mono- and polyphosphoinositides play a special role in synaptic transmission. The turnover of these lipids is specifically controlled by acetylcholine in nervous and other tissue (Hokin & Hokin, 1955, 1958a,b; Hokin, Hokin & Shelp, 1960; Lunt, Canessa & DeRoberts, 1971). The phosphoinositides have been implicated in ion translocation (Durell & Garland, 1969; Durell, Garland & Friedel, 1969) and are essential for the conformational integrity of a proteolipid bearing acetylcholine receptor activity (Hokin, 1969; Hokin & Hokin, 1958b). The dependence of the conformational properties of phytate on the state of phosphate ionization suggests similar behavior for the polyphosphoinositides. Clearly, the extent to which this property contributes to the unique role of these lipids in neural function bears investigation.

This work was supported by the U.S. Public Health Service, National Institutes of Health, Grants GM-01728 and NS-09178.

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Acta Cryst. (1975). **B31**, 2592

Structure and Binding in Molecular Complexes of Cyclic Polyethers.

II.* Hydrogen Bonding and Ion Pairing in a Complex of a Macrocyclic Polydentate Ligand with Butylamine at 120 K

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(Received 3 March 1975; accepted 14 April 1975)

The crystal structure of a 1:1 complex of a macrocyclic polydentate ligand (3,6,9,12,15-pentaoxa-21-carboxybicyclo[15,3,1]heneicosa-1(21),17,19-triene; $C_{17}H_{24}O_7$) with t-butylamine has been determined from three-dimensional intensity data collected on a four-circle diffractometer at 120 K. The crystal is orthorhombic, space group *Pnma*. There are four molecular units in the unit cell of dimensions $a = 17.811$ (3), $b = 13.920$ (2) and $c = 8.763$ (2) Å. The structure was solved by direct methods and refined by the least-squares procedure to a final *R* value 0.038 for 1791 observations above threshold. The ionic constituents of the complex are located on mirror planes. The large host and the smaller guest species are held together by N–H···O hydrogen-bonding and ion-pairing interactions, which also have an effect on the flexible molecular conformation of the substituted polyether moiety. The observed packing arrangement appears to be stabilized by a three-dimensional network of C–H···O interactions. The host–guest complex is characterized by a high degree of symmetry.

Introduction

The present work is part of a study of host–guest compounds that involve synthetic multiheteromacro-

cyclic moieties acting as hosts in reactions of molecular complexation. Recently, problems of design and synthesis of differently shaped host compounds and their properties have been discussed, and a variety of forces potentially available for binding large host molecules to smaller guest species have been outlined (Cram *et al.*, 1975). In the previous publication of this

* Part I: *Acta Cryst.* **B31**, 754–762.

† Contribution No. 3449.