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Carbohydrate Research 308 (1998) 9–17

Inframolecular acid–base properties of *myo*-inositol 1,2,6-trisphosphate analogues: influence of the hydroxyl groups, phosphate configuration and intracyclic atom substitution

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Received 19 November 1997; accepted 22 February 1998

Abstract

The microscopic acid–base properties of analogues of D-*myo*-inositol 1,2,6-trisphosphate **1** are reported. These analogues differ by the hydroxyl configuration (L-*chiro*-inositol 1,2,3-trisphosphate **2**) or the replacement of the inositol ring by a ring containing an atom of oxygen (1,5-anhydro-D-arabinitol 2,3,4-trisphosphate **3** and 1,5-anhydroxylitol 2,3,4-trisphosphate **4**) or an atom of nitrogen (1,5-anhydro-1,5-imino-D-arabinitol 2,3,4-trisphosphate **5**). In addition, the discussion includes the analogue of **1** where all three hydroxyls were removed (**6**). The studies, performed by potentiometric and ^{31}P-NMR titrations in a 0.2 M KCl medium at 37 °C, allowed the resolution without approximation of the microprotonation scheme of the trifunctional molecules. The constants as well as the chemical shifts of the phosphorus nuclei that characterize each individual phosphate group are analyzed in terms of hydration and hydrogen bonding. Thus, for **2**, the loss of a hydrogen bonding possibility between P-1 and OH-6 leads to an increase of the P-1 basicity with regard to **1**. Also, by comparing **3** and **4** it appears that the lower basicity of P-2 for **4** with regard to **3** may be attributed to a more favourable interaction between P-1 and P-2 in **3** when the two possible chair forms are allowed. Moreover, for **5**, the addition of the first equivalent of protons will protonate only about 75% of the amine, thus indicating that for polyfunctional molecules a given macroscopic protonation constant can hardly be attributed to a specific protonation site.

Keywords: Inositol phosphates; Deoxysugar phosphates; Inframolecular; Protonation; Microconstants; NMR titrations; Hydration

1. Introduction

The *myo*-inositol phosphates (IPs) form a large and steadily growing family of compounds that has received intensive study due to their biological activities in cell signaling. Among them D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3), which links receptor stimulation to intracellular calcium mobilization, is the major representative [1–5].

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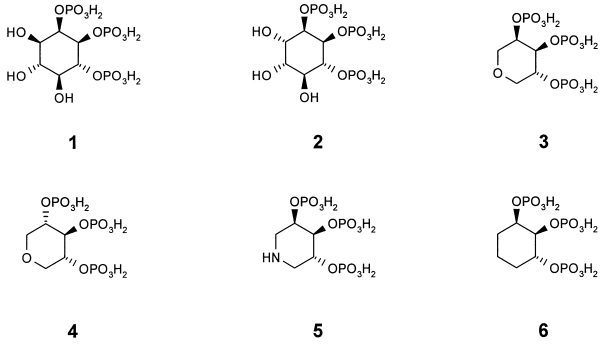
0008-6215/98/$19.00
PII S0008-6215(98)00060-3

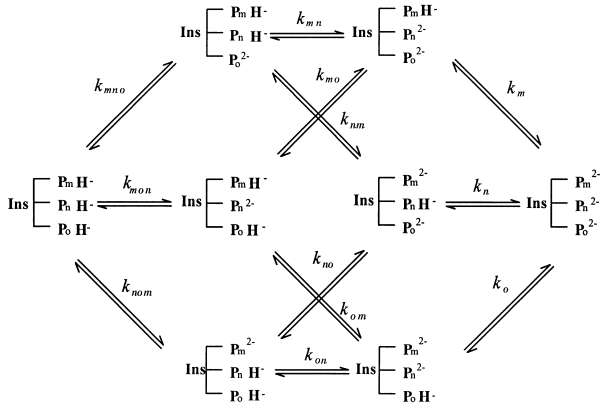
A related regioisomer, D-*myo*-inositol 1,2,6-trisphosphate **1**, that can be produced in large quantities by enzymatic degradation of phytic acid, has shown promising anti-inflammatory and analgesic effects [6–8]. Furthermore, an ester of **1** exhibits antimetastatic effects [9]. Following these observations, a programme was dedicated to the synthesis of analogues of **1** [10–15] in order to enhance its pharmacological activity. A common way of directing the synthesis to more potent compounds is to quantify the correlation between some of their physico-chemical properties and their biological activities (QSAR). Protonation or acid dissociation constants (pK_a) are widely used in QSAR studies as electronic descriptors in order to account for part of the biological activity of the molecule. For monofunctional or polyfunctional groups of well separated basicity these values can be assigned to a given group, and thus satisfactorily describe its ionization state. More often, the ligands contain two or more functional groups of comparable acidity. In this case, the dissociation steps overlap and the thermodynamic quantities characterize the ligand as a whole. Therefore, no information can be drawn from these macroscopic constants about the specific acid–base properties of each individual ionizable group. However, for the understanding, for instance, of the mechanism of binding of a ligand to its receptor there is clearly a need for the knowledge of the charge distribution on the molecule at physiological pH. Such information can be provided by the acid–base properties at an inframolecular level quantified by microconstants which describe the basicity of a given proton-binding site, i.e. of each phosphate group for the IPs, but also the interactions with neighbouring protonating groups.

We now report the study of the microscopic acid–base properties of analogues of **1** differing by the hydroxyl configuration (L-*chiro*-inositol 1,2,3-trisphosphate **2**) or the replacement of the carbon ring backbone by a ring containing an atom of oxygen (1,5-anhydro-D-arabinitol 2,3,4-trisphosphate **3** and 1,5-anhydroxylitol 2,3,4-trisphosphate **4**) or nitrogen (1,5-anhydro-1,5-imino-D-arabinitol 2,3,4-trisphosphate **5**). Furthermore, in the discussion will be included the analogue **6** of **1** previously considered [16] where all three hydroxyls were removed (see structures). For the clarity of the discussion, in the following, the numbering according to the corresponding positions in D-*myo*-inositol trisphosphate will be used to describe compounds **2**–**6**. Thus, they will be called chiro(1,2,6)P_3 **2**, arabino(1,2,6)P_3 **3**, xylo(1,2,6)P_3 **4**, aza(1,2,6)P_3 **5**, deoxy(1,2,6)P_3 **6**. The studies were performed by potentiometric and ^{31}P-NMR titrations in a 0.2 M KCl medium at 37 °C which roughly mimic the physiological ionic strength and temperature.



In addition to the resolution of the microscopic protonation schemes for the above-mentioned compounds, this study also aims at determining the influence of the presence and configuration of the hydroxyl groups of the cyclohexane ring on the acid–base properties of the IPs. This is of general interest in the understanding of the biological mechanism of action of the latter molecules and especially of Ins(1,4,5)P_3, since it has been shown that minor changes affecting the hydroxyls may lead to marked alterations of the biological activity [8,17].

The method of investigation and the calculation for determining the microscopic constants of an inositol trisphosphate, recently described for **1** [18] was applied here to **2**, **3** and **4**. Below is briefly shown how both potentiometric and ^{31}P-NMR titrations lead to the full description of the microprotonation process. Such a process is depicted in Scheme 1 where the subscripts *m*, *n*, and *o* refer to the position of the phosphate on the ring.



Scheme 1.

Assuming that the chemical shift variations of the phosphorus nuclei are primarily governed by the changes in the protonation state of the phosphate group, then the observed chemical shift δ_i^{obs}, corresponds to:

$$\delta_i^{obs} = f_{i,p}\delta_{i,p} + f_{i,d}\delta_{i,d} \quad (1)$$

where $f_{i,p}$ and $f_{i,d}$ are, respectively, the protonated and deprotonated fractions of the phosphate in position i, and $\delta_{i,p}$ and $\delta_{i,d}$ the corresponding chemical shifts. The $\delta_i^{obs} = f(pH)$ curves for **2**–**4** are shown in Figs 2a, 3a and 4a, respectively. Accordingly, $f_{i,p}$ can easily be calculated from the following equation, as illustrated in Figs 2b, 3b and 4b:

$$f_{i,p} = \frac{\delta_i^{obs} - \delta_{i,d}}{\delta_{i,p} - \delta_{i,d}} \quad (2)$$

By summing the individual protonation fractions for a molecule, the mean number of protons bound per molecule of IP can be calculated. On the other hand, $\bar{p}$ can also be obtained from potentiometric data alone using the following:

$$\bar{p} = \frac{C_H - [H^+] + [OH^-]}{C_L} \quad (3)$$

where C_H and C_L correspond to the analytical concentrations of the acid and ligand, respectively. The good superimposition of both ^{31}P-NMR and potentiometric $\bar{p} = f(pH)$ curves (examples of such curves for **2** are shown in Fig. 1) allowed the further interpretation of the δ_i^{obs} in terms of protonation microconstants according to the previous assumption. Thus, $f_{i,p}$ can be expressed as function of the macro- and microprotonation constants and the proton concentration. For instance, the fraction of the protonation in position 1 ($f_{1,p}$) is defined as:

$$f_{1,p} = \frac{\beta_3[H^+]^3 + (k_{12}k_1 + k_{16}k_1)[H^+]^2 + k_1[H^+]}{\beta_3[H^+]^3 + \beta_2[H^+]^2 + \beta_1[H^+] + 1} \quad (4)$$

The microconstants are obtained by fitting the data sets of the curves represented in Figs 2b, 3b and 4b, taking as constant in eq (4) the values of β_1, β_2 and β_3 previously determined by potentiometry under the same conditions. These values refer to the following equilibria: $L^{6-} + yH^+ \Longleftrightarrow H_yL^{(6-y)-}$ with y = *1*, 2 and 3, respectively. For the details of the calculations see ref. [18].

The case of **5** appears more complicated than the others since its nitrogen atom brings an additional functional group to the three phosphates so that 2^4

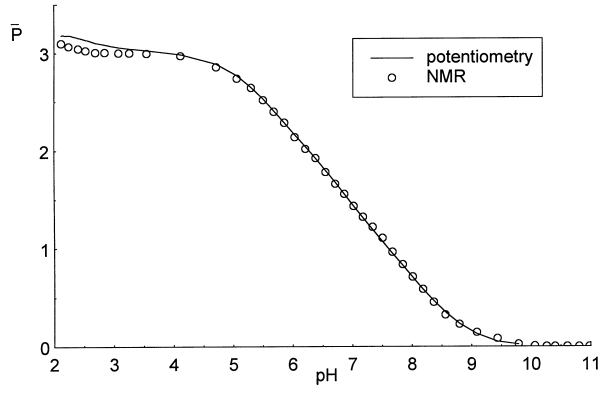


Fig. 1. Mean number of protons bound per ligand ($\bar{p}$) versus pH calculated from potentiometric (solid lines) and ^{31}P-NMR (○) measurements for compound **4**.

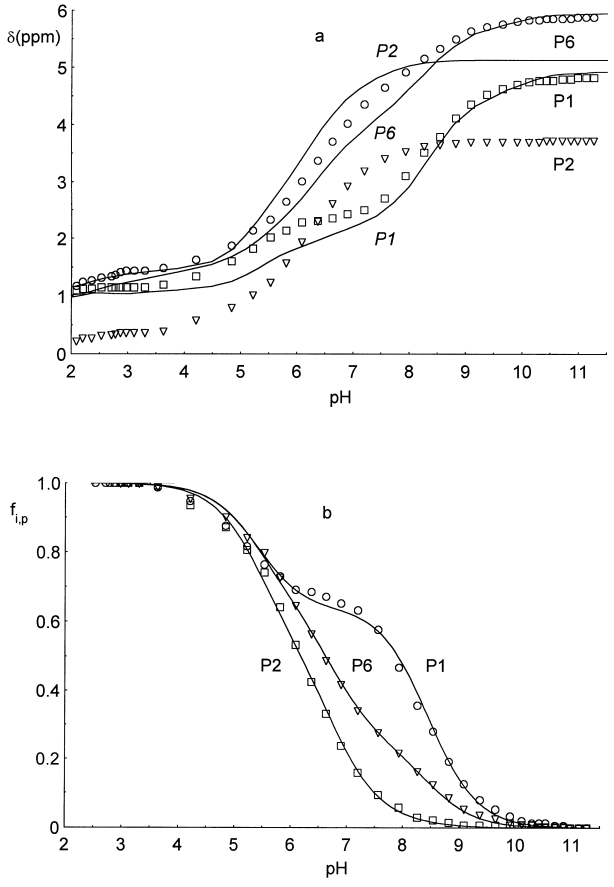


Fig. 2. Chemical shifts δ from ^{31}P-NMR titrations for chiro(1,2,6)P_3 (**2**) (a) and the corresponding protonation fraction curves $f_{i,p}$ (b) as a function of pH in 0.2 M KCl at 37 °C. For purposes of comparison, $\delta_i^{obs} = f(pH)$ for the *myo* equivalent (**1**) are superimposed in (a) (solid line, assignments of the phosphates are shown in italic). The least-squares fit of $f_{i,p}$ versus pH according to eq (4) is shown as asolid line in (b).

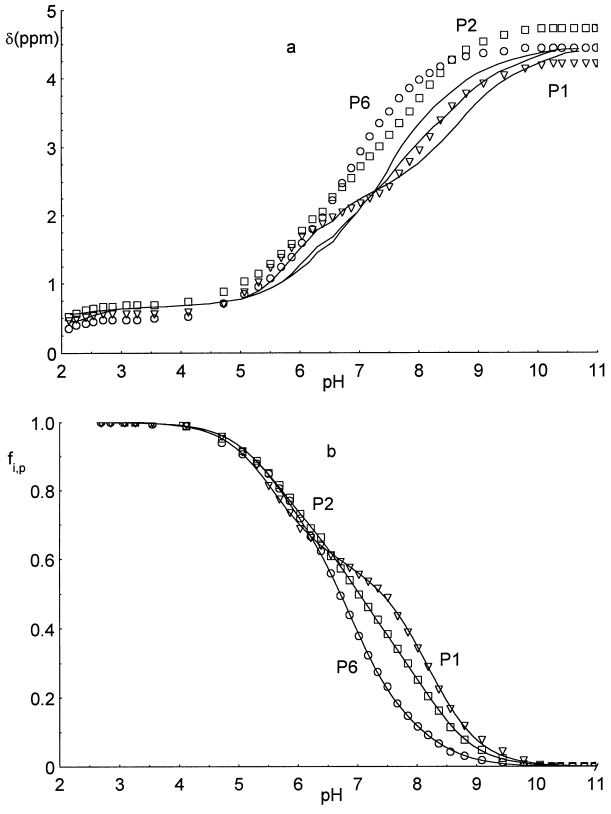


Fig. 3. Chemical shifts δ from ^{31}P-NMR titrations for arabino(1,2,6)P_3 (**3**) (a) and the corresponding protonation fraction curves $f_{i,p}$ (b) as a function of pH in 0.2M KCl at 37 °C. For purposes of comparison the $\delta_i^{obs} = f(pH)$ for deoxy(1,2,6)P_3 (**6**) are superimposed in (a) (solid line). The least-squares fit of $f_{i,p}$ versus pH according to eq (4) is shown as solid line in (b).

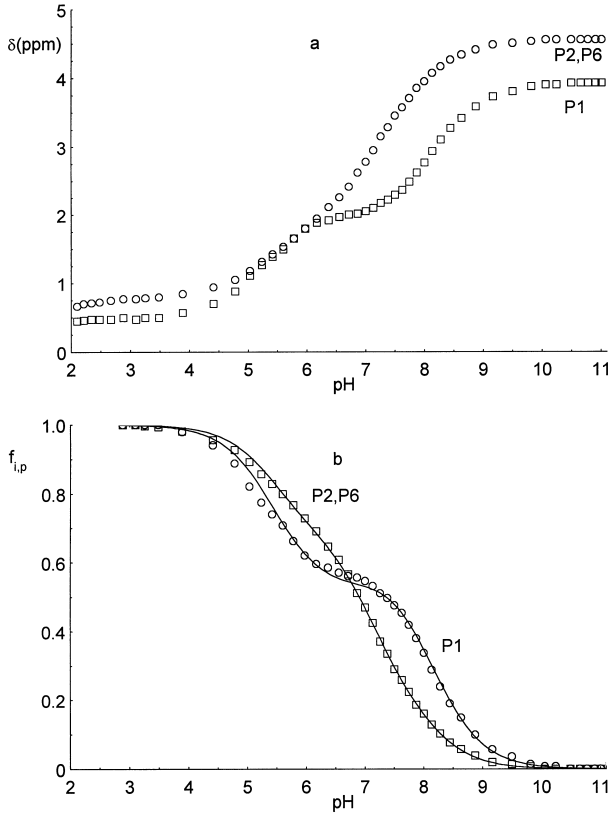


Fig. 4. Chemical shifts δ from ^{31}P-NMR titrations for xylo(1,2,6)P_3 (**4**) (a) and the corresponding protonation fraction curves $f_{i,p}$ (b) as a function of pH in 0.2M KCl at 37 °C. The least-squares fit of $f_{i,p}$ versus pH according to eq (4) is shown as a solid line in (b).

microspecies should be considered. This obviously prevents the determination of the 32 microconstants of the protonation scheme for this tetrafunctional compound. Nevertheless, if we assume that the chemical shift variations of the phosphorus nuclei still describe the variations of the ionisation state of the phosphate groups, then the difference between the potentiometrically and ^{31}P-NMR calculated $\bar{p} = f(pH)$ curves should depict the protonation of the cyclic nitrogen (Fig. 5).

2. Results and discussion

L-Chiro-*inositol 1,2,3-trisphosphate (chiro(1,2,6)P_3* **2***)*.—Fig. 2a and b show the ^{31}P-NMR titration curves of **2** and its corresponding protonation fraction versus pH. As already noted, upon addition of three equivalents of protons, the phosphorus nuclei of all three phosphates undergo a large upfield shift resulting from the protonation of these groups. At a first glance, the general shape of the curves remain the same as for **1** [16,18], being monophasic for P-2, and biphasic to different degrees for P-1 and P-6. However, in contrast to **1**, $\delta_{2,p}$ and $\delta_{2,d}$ of **2** are shifted upfield by 1.02 and 1.56 ppm, respectively, as a consequence of the displacement of OH-3 from a cis to a trans antiperiplanar position with regard to P-2. The change in the orientation of this hydroxyl group will also affect the basicity of phosphate P-2 as can be seen from the microconstants listed in Table 1. While for **1** and **2** $\log k_i$ is constant for P-1 or slightly decreased for P-6, there is a gain of 0.43 log unit for P-2, indicating an increase of the basic character of this group. This can also be illustrated by the concentration ratios of the monoprotonated microspecies which are constant at any pH. For instance, $[IP_1H]/[IP_2H] = k_{21}/k_{12} = 79.3$ for **1** and 26.3 for **2**, and $[IP_6H]/[IP_2H] = k_{26}/k_{62} = 29.6$ for **1** and 7.5 for **2**. The decrease in these ratios of three- or four-fold still emphasizes the consequence of a small structural change on the protonation process of the IPs.

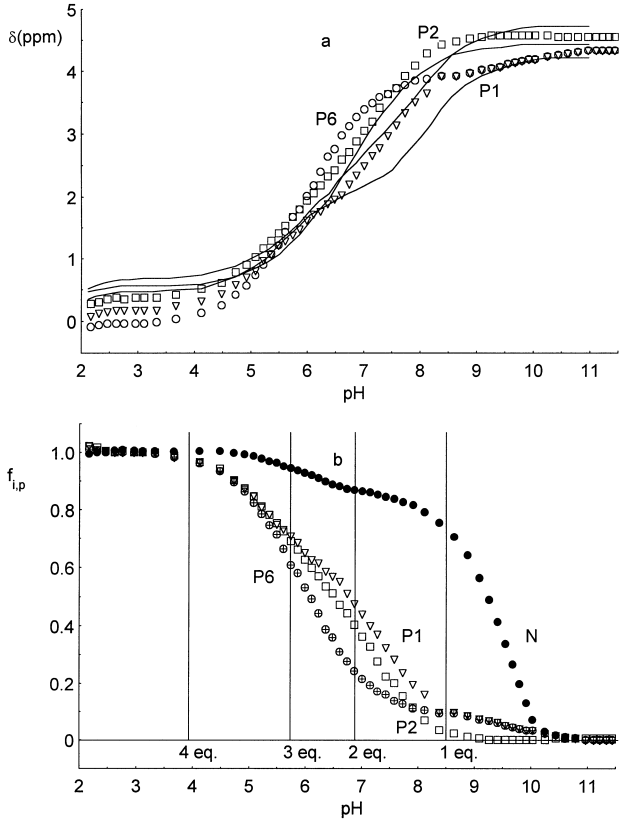


Fig. 5. Chemical shifts δ from ^{31}P-NMR titrations for aza(1,2,6)P_3 (**5**) (a) and the corresponding protonation fraction curves $f_{i,p}$ (b) as a function of pH in 0.2M KCl at 37 °C. For purposes of comparison the $\delta_i^{obs} = f(pH)$ for arabino(1,2,6)P_3 (**3**) are superimposed in (a) (solid line). In (b) the protonation fraction of the nitrogen atom was calculated as indicated in the text. The vertical lines correspond to the theoretical addition of 1–4 equivalents of protons.

1,5-Anhydro-D-*arabinitol 2,3,4-trisphosphate (arabino(1,2,6)P_3* **3***) and 1,5-anhydroxylitol 2,3,4-trisphosphate (xylo(1,2,6)P_3* **4***).*—Compounds **3** and **4** differ by the configuration of P-2, P-2 being, respectively, axial and equatorial. Their titration and protonation fraction curves are displayed in Figs 3 and 4. In comparison with **1** and **2**, these curves look much more tightened and resemble those of **6** [16] (Fig. 3). This indicates that, in the absence of hydroxyls on the ring, the phosphate groups tend to behave similarly. In both cases the P-1 curves are clearly biphasic as a result of the central position of this group, while P-2 and P-6 exhibit a nearly monophasic protonation trend. Only two resonances are observed for **4** since its *meso* structure makes P2 and P-6 equivalent. The $\delta_{i,p}$ values of **3** and **4** also are close to those of **6** whereas the $\delta_{i,d}$ are more scattered and take intermediate values between those of **1** and **6**. Thus, it appears that the heterocyclic oxygen exerts a larger effect on the deprotonated phosphates rather than on the monoprotonated ones. Table 1 contains the macro- and microprotonation constants for **3** and **4**, and, for purposes of comparison, those previously determined for **6** [16]. It can be observed that the replacement of a carbon atom by an oxygen atom markedly decreases the macro- as well as the microconstants. On the other hand, the values of **3** and **4** only slightly differ except for $\log k_2$ which is significantly higher for **3**, indicating a higher basic character for an axial phosphate with regard to an equatorial phosphate. It is also worth noting the low $\log k_2$ value of **1** compared to those of **3** and **4**, whereas $\log k_1$ and $\log k_6$ show exactly the opposite trend.

1,5-Anhydro-1,5-imino-D-*arabinitol 2,3,4-trisphosphate (aza(1,2,6)P_3* **5***).*—Fig. 5a displays the titration curves of **5** and Fig. 5b the corresponding protonation fractions curves along with that calculated for the piperidine nitrogen as described above. Interestingly, with the addition of the first equivalent of protons, only about 75% of the amine will be protonated, the remaining 25% being shared mainly by P-1 (10%) and P-6 (10%). Moreover, the amine will reach its fully protonated state only at pH 5 with the addition of 3.5 equivalents of protons. Thus, this confirms that for polyfunctional molecules a given macroscopic protonation constant can hardly be attributed to a specific protonation site and great caution must be taken if this is done, for instance, in kinetic models. On the titration curves, it can also be seen (Fig. 5a) that the replacement of an oxygen atom by a nitrogen atom decreases the values of $\delta_{i,p}$ by about 0.5 ppm. Furthermore, the curves of **5** are markedly displaced towards lower pH values, thus revealing a less basic character of its phosphates with regard to **3** or **4**. Such a decrease in basicity is also shown by the lower values of $\log K_2$ to $\log K_4$ taken by **5** in comparison with the logarithms of the first three protonation constants of **3** and **4**.

Hydration and hydrogen bonding.—As shown, large differences are observed in both the acid–base properties and δ_i^{obs} values for **1**–**6** although all six compounds carry three vicinal phosphates placed on a six-membered ring. Referring to previous work it can be stated that hydrogen bonding, taken in a large acceptation, as well as hydration will be the main factors responsible for the observed acid–base variations. In order to discuss more thoroughly these variations, it will first be recalled

Table 1
Logarithms of the macro- and microprotonation constants for the studied compounds. The macro constants are stepwise constants which relate to the following equilibria: $H_{y-1}L^{(7-y)-} + H^+ \overset{K_y}{\Longleftrightarrow} H_yL^{(6-y)-}$. $\log k_i$, $\log k_{ii'}$ and $\log k_{ii'i''}$ represent a general designation for, respectively, the logarithms of the first, second and third stepwise microprotonation constants. The uncertainties are estimates of the standard deviation as calculated by Superquad and Enfitter for the macro- and microconstants respectively. The *myo*-inositol numbering is used to describe **3**, **4** and **5**

Ligand	y	$\log K_y$	i	$\log k_i$	ii'	$\log k_{ii'}$	$ii'i''$	$\log k_{ii'i''}$
Ins(1,2,6)P_3 **1**	1	8.44	1	8.28	12	6.10	126	5.84
	2	6.54	2	6.46	16	6.43	162	5.59
	3	5.28	6	8.00	21	8.00	621	5.86
					26	7.91		
					61	6.64		
					62	6.44		
chiro(1,2,6)P_3 **2**	1	8.46 (0.02)	1	8.26 (0.01)	12	6.45 (0.04)	126	5.90 (0.21)
	2	6.78 (0.03)	2	6.89 (0.02)	16	6.51 (0.04)	162	5.83 (0.21)
	3	5.40 (0.02)	6	7.86 (0.01)	21	7.87 (0.03)	621	6.05 (0.21)
					26	6.99 (0.03)		
					61	6.99 (0.01)		
					62	6.77 (0.01)		
arabino(1,2,6)P_3 **3**	1	8.23 (0.02)	1	7.96 (0.01)	12	6.73 (0.01)	126	6.03 (0.03)
	2	6.93 (0.02)	2	7.80 (0.02)	16	6.64 (0.01)	162	6.07 (0.03)
	3	5.57 (0.02)	6	7.38 (0.01)	21	6.90 (0.01)	621	6.03(0.05)
					26	6.90 (0.01)		
					61	7.32 (0.01)		
					62	7.32 (0.01)		
xylo(1,2,6)P_3 **4**	1	8.16 (0.02)	1	7.91 (0.01)	12	6.79 (0.01)	126	
	2	7.13 (0.02)	2	7.46 (0.01)	16	6.79 (0.01)	162	
	3	5.44 (0.02)	6	7.46 (0.01)	21	7.37 (0.01)	621	
					26	7.36 (0.01)		
					61	7.37 (0.01)		
					62	7.36 (0.01)		
aza(1,2,6)P_3 **5**	1	9.47 (0.02)						
	2	7.45 (0.02)						
	3	6.26 (0.02)						
	4	5.14 (0.03)						
deoxy(1,2,6)P_3 **6**	1	8.67	1	8.39	12	7.05	126	6.40
	2	7.30	2	8.26	16	7.05	162	6.39
	3	5.91	6	8.11	21	7.27	621	6.26
					26	7.27		
					61	7.45		
					62	7.45		

how both factors may affect the basicity of the phosphate groups.

Hydration stabilizes the doubly negatively charged $-OPO_3^{2-}$ group relative to the monoprotonated $-OPO_3H^-$ group, thus increasing the acidity of the latter. The basicity of the phosphates may depend upon hydration of nearby groups and, in the case of IPs, upon the presence of OH groups. It has been noted in many cases that a hydroxyl in the vicinity of a phosphate results in a loss of basicity of that group [16,19–23]. Such an effect, which may be transmitted to a different extent either through the molecule or through the surrounding medium, was ascribed to withdrawing effects, hydrogen bonding and solvation. Since we recently showed that inductive effects only play a minor role [24], setting of hydrogen bonds and hydration should mainly account for the observed basicity decrease. Earlier studies [25,26] on amino sugars showed that differences in the orientation of the hydroxyls vicinal to the amino group explain differences in the acid dissociation constants of the ammonium ions. In that case, the relative position of the hydrated OH group(s) with regard to the

ionizable group will determine its hydrophobic environment and thus the effective local dielectric constant. For the IPs as well, the presence of a hydrophilic OH close to a protonated phosphate will increase the local dielectric constant, labilize the mobile proton and contribute to lower the basicity of the phosphate.

The effect of hydrogen bonding on modulating the acid–base properties of the IPs presumably involves a wide variety of hydrogen bonds which compete and interconvert each other in a complex and subtle dynamic process. All H-donor and H-acceptor combinations of both hydroxyls and phosphates must be envisaged in addition to the hydrogen bonds within vicinal diols. Undoubtedly, the relative strength of all these hydrogen bonds will deeply affect the basicity of the phosphate groups. Earlier work on cyclitols clearly showed that *cis* hydroxyls form stronger intramolecular hydrogen bonds than trans hydroxyls. Thus, Huang et al. [27] determined $\Delta G_{cis}-\Delta G_{trans}$, the thermodynamic difference between the intramolecular hydrogen bond strengths of cis and trans vicinal diols in order to account for the selectivity of binding of neutral polyaza receptors towards cyclitols. In chloroform they calculated for two compounds a differential cis/trans strength of 0.29 and 0.58 kcal mol^{-1}. Conformational analysis of inositols by Liang et al. [28] also showed that O···HO hydrogen bonding distances involving two neighbouring equatorial and axial OH groups are shorter than those for equatorial OH groups and are thus energetically favoured. The cis/trans thermodynamics of the interactions between OH and phosphate groups has been less investigated than between vicinal diols. However, a preferential stabilization of OH and OPO_3H_2 in a *cis* stereochemistry over a trans one is likely to occur [28]. Indeed, by optimizing the structure of *scyllo*- and *myo*-inositol-2 phosphoric acids, the expected hydrogen bond lengths are 1.99 and 1.94 Å, respectively. In addition to the mentioned hydrogen bonds, hydrogen bonding of the O–H···O–PO_3^{2-} type as used in evidence in ribonucleoside 3′-ethylphosphate [19] can also be envisaged. Finally, two vicinal phosphate groups may also cooperate to strongly stabilize a proton. In previous studies [18,22] it was clearly shown that two cis phosphates interact less than two trans phosphates. Such an observation differs from the conclusions of Amburgey et al. [29] drawn from modelisation studies of cyclohexane-1,2,4-triol-trisphosphates where the *cis*-1,2 phosphates are of lower energy than the *trans*-1,2-phosphates. However, as stated by these authors, the energy calculations are limited by the absence of full solvent effects, the absence of counterions and variable charge states of the phosphates in the studied pH range.

Modulation of the acid–base properties of the studied compounds by hydrogen bonding and hydration.—As can be observed in Figs 2–5, the titration curves of the inositol phosphates (**1** and **2**) show more scattered $\delta_{i,p}$ and $\delta_{i,d}$ values than those of the deoxy derivatives (**3**–**5**). In addition, the three resonances of the latter are closer together than those of the former. The limit case seems to be **6** (see Fig. 3) where all $\delta_{i,p}$ and $\delta_{i,d}$ are identical and the chemical shifts at any pH differ by less than 1 ppm. This indicates that the hydroxyl groups of the cycle, presumably because of hydrogen bonding, markedly contribute to differentiate the basicity of the phosphates.

Since many types of interaction compete for modulating and determining both the chemical shifts and basicity of the phosphates, only the main changes induced by the structure variations will be discussed at this stage of our investigation. Clearly, there is a large highfield shift by changing the orientation of OH-3 from an equatorial (**1**) to an axial position (**2**). Such a shift may be attributed to the loss of hydrogen bonding possibility between P-2 and OH-3 that can occur in a *cis* stereochemistry as found in the *myo*-inositol configuration but not in a trans antiperiplanar stereochemistry as in the *chiro*-inositol configuration. Therefore, the deprotonated form of **2** may be less stabilized, leading to an increase of P-2 basicity with regard to **1** ($\log k_2(\mathbf{2})-\log k_2(1)=0.43$). In addition, for **2** the dipole of OH-3 being directed down to the plane of the ring, the hydration close to P-2 should be decreased, thus contributing to a P-2 basicity increase. It is also worth noting that $\log k_1$ takes the same values in **1** and **2** as expected for phosphates having the same environment in both compounds whereas $\log k_6$ in **2** is slightly lower than in **1** due to a possible acid strengthening effect on P-6 of the axial OH-3 below the plane of the inositol ring.

In previous work [16,22], it has been demonstrated that the values of the chemical shifts of the monoprotonated and deprotonated phosphates are largely dependent on the presence and configuration of the hydroxyls vicinal to the phosphates. For

6, lacking hydroxyl groups, $\delta_{i,p}$ and $\delta_{i,d}$ take identical values for the three phosphates (see Fig. 3). This has been discussed [16] and attributed to a conformational change towards a twist boat form where all phosphates are more or less equivalent. But it is also possible to suggest that, contrary to the inositol cycle which is undoubtedly in a predominant equatorial chair form [30], the deoxy derivative may adopt the two possible chair forms in rapid interchange, thus leading to mean chemical shift values of both forms. Such an interconversion also observed for highly phosphorylated IPs (IP_5 and IP_6) [30] should minimize the electrostatic repulsion of the anionic phosphates. As far as IP_3 is concerned, such a conformational change may not take place due to the hydrogen bonds set between the hydroxyl groups. The behaviour of **3** and **4** seems to be intermediate between those of the IPs and the deoxy analogue **6**. It is likely that through solvation, the intracylic oxygen introduces a certain degree of rigidity in the ring that would disfavour the predominant axial conformation.

By comparing **3** and **4**, it can be seen that both compounds only differ in their $\log k_2$ values which is 0.36 log unit lower for **4** as compared to **3**. Such a difference in basicity may be attributed to a more favourable interaction between P-1 and P-2 in **3** when the two possible chair forms are allowed. According to the higher $\log k_2$ value of **3**, it is unlikely that P-2 interacts with the intracyclic oxygen. However, this oxygen may operate through a hydration effect which greatly lowers the constants with regard to **6**. It can also be noted that the basicities of P-6 and P-2 are inverted for **3** and **6** in comparison with those of **1** and **2**, leading to closer $\log k_2$ and $\log k_1$ values for the former. This can be interpreted by considering that for the IPs (**1** and **2**), the vicinal phosphates in a trans position better interact to stabilize a proton than those in a *cis* position. However, when a conformational interchange of the ring is allowed as seems to be the case for the deoxy derivatives (**3** and **6**), the interactions of the vicinal phosphate groups are averaged and their basicities become more or less equivalent.

For the aza derivative **5**, the protonation fraction curves are shifted to lower pHs with regard to **3**, but surprisingly their general shape remains the same except at high pH (>8.4) where P-1 and P-6 behave similarly and exhibit a more basic character than P-2. This specific effect on P-1 and P-6 could arise from an interaction of the latter with the lone pair of the intracyclic nitrogen which would increase the apparent basicity of both phosphates. However, such an interaction is likely to occur only if the prevalent axial chair conformation is favoured. Below pH 8.4, the nearly equal decrease in basicity of all three phosphates of **5** compared to **3** should be the result of the additional positive charge on the nitrogen which decreases the overall negative charge density of the molecule. A more pronounced hydration of **3** should also contribute, by increasing the local dielectric constant, to enhance the acidity of the phosphates.

In conclusion, as can be seen in Table 1, few differences are expected in the acid–base behaviour of the studied compounds from consideration of only the macroscopic protonation constants. Therefore, minor changes in the molecular structure appear to only slightly affect the basicity of the phosphate groups. However, the examination of the protonation microconstants clearly shows that the apparent indifference of the overall protonation process towards structural modifications results from the compensation of effects acting on individual groups and having opposite trends. In particular, for IPs and related compounds, hydrogen bonding and solvation effects seem to cooperate in a complex and dynamic process to determine the basicity of a given phosphate group. The respective part of both effects is difficult to ascertain. However, as suggested by Adams and Lerner [31] who studied the effect of stereochemistry on hydroxyl proton chemical shifts and coupling constants in carbohydrates, it is likely that the main effect arises from intramolecular electron distribution, i.e. mainly intramolecular hydrogen bonding.

Due to the considerable biological importance of inositol phosphates, an inframolecular approach such as that presented in this study should be very valuable in explaining part of their mechanism of action.

3. Experimental

Materials.—Compounds **2**–**5** were provided by Perstorp Pharma, Sweden, and used without further purification.

Potentiometric studies and NMR determinations.—Potentiometric and NMR determinations were carried out as previously described [16,18,22]. The experiments were carried out in two steps in which the same initial solution of the ligands at a

concentration of about 3×10^{-3} M is successively subjected to potentiometric and ^{31}P-NMR titrations. The processing of the pH measurements allowed the total concentration of the ligand and the acid as well as the macroscopic protonation constants (by using SUPERQUAD [32]) to be determined. The protonation fraction curves resulting from the $\delta_i^{obs} = f(pH)$ curves were analyzed by non-linear regression with the iterative curve-fitting program 'Enfitter' (Elsevier-Biosoft) in order to yield the microprotonation constants. ^{31}P-NMR spectra were recorded at 81.015 MHz on a Bruker AC200 Fourier transform spectrometer. Chemical shifts were measured relative to an external 85% orthophosphoric acid reference. Field-frequency lock was achieved using 10% ^2H_2O. Phosphorus resonance peaks of **2–5** were assigned by performing 2D ^{31}P–^{1}H chemical shift correlation experiments. The assignment of the proton resonances were done on the basis of the chemical shifts and the coupling patterns confirmed by ^{1}H^{1}H-COSY experiments. Although in certain cases the chemical shifts are close together, the signals are well enough resolved to work out unambiguously the titration curves.

Acknowledgements

The authors express grateful acknowledgement to PERSTORP PHARMA (Sweden) for providing the studied compounds and for support for one of us (K.M.). We wish also to acknowledge the Ligue du Haut-Rhin contre le Cancer for generous financial support.

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