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^{31}P NMR and Potentiometric Studies on the Protonation of Isopropyl Esters of Clodronic Acid

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Protonation constants of four isopropyl esters of clodronic acid were determined by ^{31}P-NMR and potentiometric measurements in aqueous solution at 25°C and $I = 1.0$ (Me_4NCl). The compounds studied were monoisopropyl clodronate trisodium salt, *P,P*-diisopropyl clodronate disodium salt, *P,P'*-diisopropyl clodronate disodium salt and triisopropyl clodronate monosodium salt. All these esters are rather acidic; the first proton dissociates at pH < 1.5. ^{31}P NMR data of monoisopropyl and *P,P*-diisopropyl ester gave evidence of the formation of the cyclic structure in protonation.

Isopropyl esters of (dichloromethylene)bisphosphonic acid (clodronic acid) belong to a group of gem-bisphosphonates of the type P–C–P. They are a class of ligands that are chemically related to, and mimic the physiological behaviour of, pyrophosphates.[1] Because pyrophosphates are hydrolysed in the presence of pyrophosphatase, bisphosphonates are considered with increasing interest for medical applications.[2,3] The first synthetic P–C–P-analogues of pyrophosphate were prepared about 20 years ago, and the number of known gem-phosphonates is now about 3000. There are, however, few data available on the effect of pH on ^{31}P NMR spectra of these compounds. The protonation of these compounds is necessary background information for the study of their properties as ligands to metal ions.

The present paper describes study of the protonation of hydroxy(1-methylethoxy)phosphinodichloromethylphosphonic acid trisodium salt or monoisopropyl clodronate trisodium salt (compound **1**), bis(1-methylethoxy)phosphinodichloromethylphosphonic acid disodium salt or *P,P*-diisopropyl clodronate disodium salt (compound **2**), (dichloromethylene)bisphosphonic acid *P,P'*-bis(1-methylethyl) ester disodium salt or *P,P'*-diisopropyl clodronate disodium salt (compound **3**), bis(1-methylethoxy)phosphinodichloromethylphosphonic acid mono(1-methylethyl) ester monosodium salt or triisopropyl clodronate monosodium salt (compound **4**) in aqueous solution under constant conditions, $I = 1.0$ $(CH_3)_4NCl$ and 25°C by potentiometric titrations and ^{31}P NMR measurements. Recently we reported dynamic thermogravimetric (TG and DTG) and mass-spectrometric (MS) studies on the thermal behaviour of these compounds.[4]

Experimental

Syntheses. Compounds **1–4** were prepared according to procedures described elsewhere.[5]

Reagents. Tetramethylammonium chloride (Fluka, 98%) was purified by dissolving 80 g of the salt in 150 ml of hot methanol (Baker, p.a.). The hot solution was filtered and then 1350 ml of acetone was added by stirring. After cooling, the solution was filtered again, and the salt was washed with 50 ml of acetone (Merck, p.a.) and dried in a vacuum desiccator.[6] Tetramethylammonium hydroxide (Fluka, 98%) was standardised by potentiometric titration with a standard HCl solution (Merck, MOS selectipur).

Tetramethylammonium hydroxide stock solutions were stored in polyethylene bottles equipped with natron lime–calcium chloride drying tubes. The concentration of the HCl solutions was determined by titration of standard solutions of tris(hydroxymethyl)aminomethane with the HCl solutions to be analysed.[7]

Potentiometric measurements. The potentiometric titrations were performed at 25.0 ± 0.1°C. An automatic Radiometer titration system consisting of an ABU 91 autoburette, a VIT 90 video titrator and a HP Think Jet printer was used for the measurements. The indicator electrode was an Orion Research 91-01 glass electrode and the reference electrode either a saturated calomel electrode (compounds **1**, **3** and **4**) or an Orion 90-02 double-junction Ag,AgCl electrode (compound **2**). The outer mantle of the Ag,AgCl electrode was filled with 1.0 M tetramethylammonium chloride solution. The measurements were carried out in a nitrogen (compounds **1**, **3** and **4**) or argon (compound **2**) atmosphere using a

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constant ionic medium of 1.0 M tetramethylammonium chloride. The protonation constants were determined by titration of the clodronate ester solutions with tetramethylammonium hydroxide solution [$I = 1.0$, $(CH_3)_4NCl$]. The total concentration of the esters was varied between 0.007 and 0.016 M.

The electrode system, consisting of the glass and calomel electrodes, was calibrated using buffer solutions of pH 6.98 ± 0.01 and 4.01 ± 0.01 (Merck, 25°C).[8] The free hydrogen ion concentration of the test solutions was obtained from the Nernst equation, eqn. (1), where

$$E = E_0 + 59.16 \log(f_{H^+}[H^+]) + E_j \quad (1)$$

$-\log f_{H^+} = 0.095$ [1.0 M $(CH_3)_4NCl$]. f_{H^+} was determined by measuring the pH of standard HCl solutions [$I = 1.0$, $(CH_3)_4NCl$]. The liquid junction potential, E_j, was determined by titration of 1.0 M $(CH_3)_4NCl$ solution with 0.1 M HCl solution [$I = 1.0$, $(CH_3)_4CCl$]. E_j became significant at pH values lower than 2.8.

The electrode system, consisting of the glass and Ag,AgCl electrodes was calibrated by titration of 0.01 M HCl solution [$I = 1.0$, $(CH_3)_4NCl$)] with 0.1 M $(CH_3)_4NOH$ solution [$I = 1.0$, $(CH_3)_4NCl$] (E_0 determination).[9] The free hydrogen ion concentration was obtained from eqn. (2).

$$E = E_0 + 59.16 \log [H^+] + j[H^+] + (kK'_w)/[H^+] \quad (2)$$

The ionic product of water, K'_w, and the liquid junction potentials j and k in the present medium were determined by titration of 0.1 M HCl solution [$I = 1.0$, $(CH_3)_4NCl$] with 0.1 M Me_4NOH solution [$I = 1.0$, $(CH_3)_4NCl$]. The liquid junction potentials j and k were significant at pH < 2.8 and pH > 12.5, respectively. K'_w, j and k were obtained by Forsgren's method.[10,11]

^{31}P NMR measurements. The measurements were carried out on a Jeol JNM-GX400 FT spectrometer operating at 161.8 MHz for ^{31}P and equipped with a tunable probehead. The sample temperature was 25 ± 1 °C in each case. In order to reach a sufficiently good signal-to-noise ratio, 64 FID signals were accumulated by applying a spectral width of 3 kHz, a pulse width of 26.0 μs (a 90° pulse), a repetition time of 7.3 s and a digital resolution of 0.09 Hz per point. The FIDs were apodized using an exponential function leading to a line-broadening of 2 Hz. Coaxial double-tube systems (outer tube o.d. 10 mm, inner tube o.d. 5 mm) were used. For the compounds **1**, **3** and **4** the locking (D_2O) and reference (H_3PO_4) substances were placed in the annulus and the studied compounds in the inner tube. For compound **2** the order was reversed, and ($P(OH)_4^+ClO_4^-$) was used as a reference substance.[12] Susceptibility corrections to the chemical shifts were neglected, being estimated as small compared with the shift variation with pH.

Many of the ^{31}P NMR spectra of the mono- and trisubstituted clodronates display second-order features. Consequently, the spectra were analyzed with the program LEQUOR modified for a PC.[13] This program is based on the iterative least-squares method and gives the nuclear chemical shifts and spin–spin coupling constants with both their standard deviations and covariances.

The protonation constants were determined by NMR measurements using a batch technique. Test solutions in which the pH was lower than 1.5 were prepared directly in volumetric flasks by addition of a known amount of standard HCl solution and clodronate ester solution. When the pH was higher than 1.5 the test solutions were pipetted from the potentiometric titration cup into an NMR tube. The free hydrogen ion concentration was obtained from eqn. (1) or (2). The total concentrations of the esters in the test solutions were kept constant over the whole pH region studied.

Data treatment. The studied equilibria are written as eqn. (3), and the protonation constants are denoted as

$$pH + rL \rightleftharpoons H_pL_r \quad (3)$$

β_{0pr}. The protonation constants from the potentiometric data were calculated with a modified version of the program SCOGS2.[14,15] The statistical part of SCOGS2 calculates the P value (probability) as well as an agreement index R for each set of constants. At the 95% confidence level the P-value of the model should excede 0.05. The P-value is a measure for the normal distribution of the $(V_i^{calc} - V_i^{obs})$-residuals (V_i^{calc} is the calculated and V_i^{obs} the added titrant volume at point i). The agreement index is calculated from eqn. (4). The best set of constants

$$R = \left[\sum (V_i^{calc} - V_i^{obs})^2 \Big/ \sum (V_i^{obs})^2 \right]^{1/2} \quad (4)$$

gives the smallest R-value.

The protonation constants from the NMR data were calculated with the program SigmaPlot (Sigmaplot Scientific Graphing System Version 4.0, Jandel Corporation 1986–89). It is a nonlinear curve-fitting program which uses the Marquardt–Levenberg algorithm to determine the parameters that minimize the sum of squares of differences between δ_i^{calc} and δ_i^{obs} (δ_i^{calc} is the calculated and δ_i^{obs} the observed chemical shift). The statistical part of the program gives the agreement index Norm for each set of constants. Norm is calculated from eqn. (5). The best set

$$\text{Norm} = \left[\sum (\delta_i^{calc} - \delta_i^{obs})^2 \right]^{1/2} \quad (5)$$

of constants gives the smallest Norm value.

Results and discussion

Potentiometry. The potentiometric data consisted of four or five titrations and 66–215 experimental points for each ester. The treatment of the potentiometric data

was initiated by making Bjerrum plots of $\bar{n}_H$ versus $-\log[H^+]$ (Fig. 1). $\bar{n}_H$ seems to be a function only of $-\log[H^+]$ for the whole pH and C_L region over which the measurements were carried out for each compound, and no significant deviation from the calculated $\bar{n}_H$-curve was observed. This indicates that the values of the protonation constants do not depend upon the concentration of the ligand and that polymerization is negligible. The first deprotonation of the studied esters takes place at pH < 1.5. Thus it is not possible to obtain reliable protonation constants by potentiometric titrations in this pH region. Because the program SCOGS2 gave a *P*-value range 0.035–0.67 and an *R*-index range 0.0006–0.008 for protonation constants calculated from individual titrations, statistically reliable constants were obtained over the pH and C_L region used in the calculations. The proposed protonation constants are given in Table 1.

Monoisopropyl ester of clodronic acid and *P,P*-diisopropyl ester of clodronic acid have two separate protonation regions. At pH < 3.0 the monoisopropyl ester gives off the two most acidic protons, and in the pH region 6–8 the last deprotonation takes place. The ester is completely dissociated at pH > 9. The first proton of the *P,P*-diisopropyl ester dissociates at pH < 1.5 and the last in the pH region 5–7. The ester is completely dissociated at pH 8. The deprotonation of the *P,P'*-diisopropyl ester takes place completely at pH < 3.

^{31}P NMR spectrometry. As examples, the ^{31}P NMR spectra of the studied compounds are shown in Fig. 2–5 at one pH. The assigment of the spectral lines is based on the multiplet structures arising from the indirect ^{31}P–^{31}P and ^{1}H–^{31}P spin–spin couplings, the former varying from ca. 15.6 to 17.9 Hz and the latter from ca. 5.9 to 6.9 Hz. The phosphorus atoms are marked by P_1, P_2 and P_3, referring to the phosphonate groups PO_3Na_2, $PO_3CH(CH_3)_2Na$ and $PO_3(CH(CH_3)_2)_2$, respectively. In Fig. 6 in turn are displayed the ^{31}P chemical shifts as a function of pH. The chemical-shift scale is chosen such that the increasing shift (towards higher frequency) means decreasing nuclear shielding. The ^{31}P-NMR data consisted of one

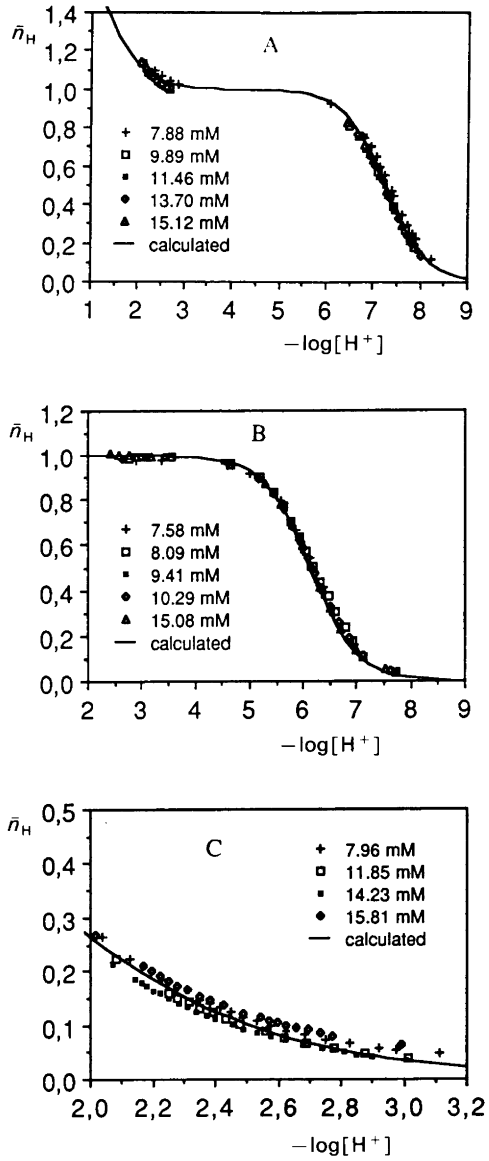


Fig. 1. Experimental and calculated values of $\bar{n}_H$ versus $-\log[H^+]$ of (A) monoisopropyl ester, (B) *P,P*-diisopropyl ester and (C) *P,P'*-diisopropyl ester of clodronic acid.

Table 1. Protonation constants, log β_{0pr} ($\pm 3\sigma$), and acid constants, pK_{ap}, of the present isopropyl esters of clodronic acid at 25°C and $I = 1.0$ (Me_4NCl), determined by potentiometric (pot.) and NMR spectrometric (NMR) methods.

Method	C_L/mM	$-\log[H^+]$	log β_{011}	log β_{021}	log β_{031}	pK_{a1}	pK_{a2}	pK_{a3}
Monoisopropyl ester								
pot.	7.88–15.12	2.1–8.0	7.24 ± 0.01	8.42 ± 0.03			1.18	7.24
NMR	14.67	−0.1–9.2	7.33 ± 0.06	8.73 ± 0.06	8.74 ± 0.5	0.01	1.40	7.33
P,P-*Diisopropyl ester*								
pot.	7.58–15.08	2.8–7.7	6.13 ± 0.03				6.13	
NMR	14.71	−0.1–10.0	6.16 ± 0.04	6.46 ± 0.1		0.30	6.16	
P,P'-*Diisopropyl ester*								
pot.	7.96–15.81	2.1–3.0	1.56 ± 0.02				1.56	
NMR	18.01	0.5–9.7	1.55 ± 0.09				1.55	
Triisopropyl ester								
NMR	17.68	0.0–3.0	0.20 ± 0.05			0.20		

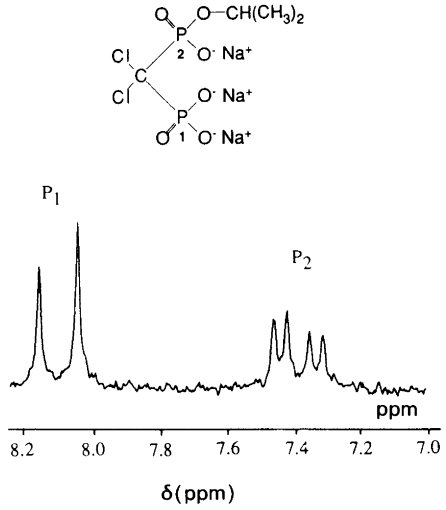


Fig. 2. The ^{31}P-NMR spectrum of monoisopropyl ester of clodronic acid at pH 0.53. The doublet structure at P_1 is due to ^{31}P–^{31}P spin–spin coupling. At P_2 the corresponding doublet is further split into two doublets because of ^{31}P–^{1}H spin–spin coupling. The intensity distribution yields the second-order nature of the spectrum.

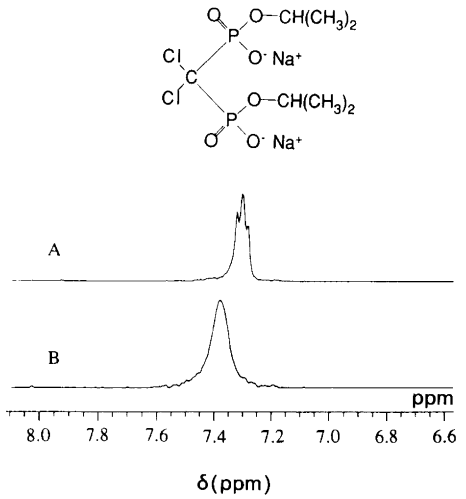


Fig. 4. The ^{31}P-NMR spectrum of *P,P'*-diisopropyl ester of clodronic acid (A) at pH 0.32 and (B) at pH 0.69. Although the phosphorus atoms are chemically equivalent, both the ^{31}P–^{31}P and ^{31}P–^{1}H spin–spin couplings affect the fine structure of the spectrum.

titration and 13–28 experimental points for each ester. The program Sigmaplot gave the Norm value range 0.01–0.14 for each set of constants, indicating that statistically reliable protonation constants were obtained. The proposed protonation constants are given in Table 1.

In the cases of monoisopropyl, *P,P*-diisopropyl and triisopropyl esters the ^{1}H-coupled ^{31}P spectrum gave expected multiplets over the whole pH region which was used in calculations. This means that these esters do not decompose in that pH region by acid- or base-catalysed ester hydrolysis. The experimental ^{1}H-coupled ^{31}P spectrum of *P,P'*-diisopropyl ester showed a broadened singlet in the pH region 0–7.1 and a triplet at pH < 0 or pH > 9.7. Although the phosphorus atoms are chemically

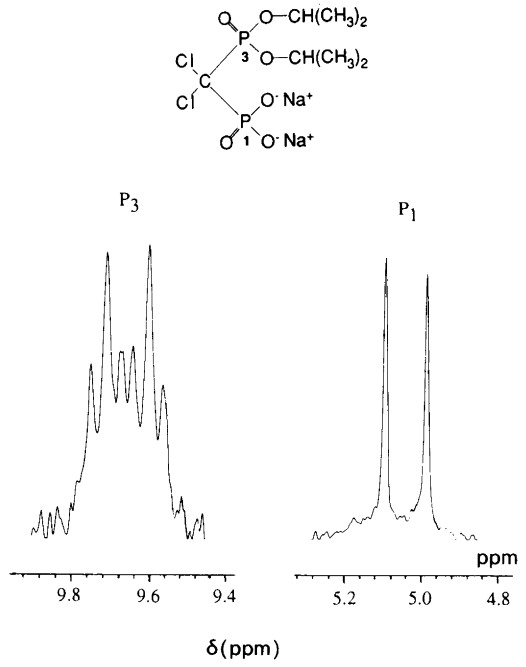


Fig. 3. The ^{31}P-NMR spectrum of *P,P*-diisopropyl ester of clodronic acid at pH 0.1. The doublet (P_1) structure arises from ^{31}P–^{31}P spin–spin coupling and the doublet of triplets (P_3) from ^{31}P–^{31}P and ^{31}P–^{1}H spin–spin couplings.

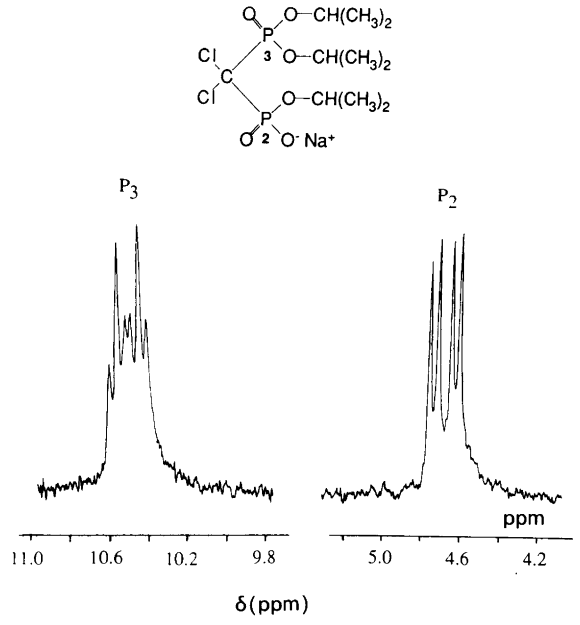


Fig. 5. The ^{31}P-NMR spectrum of triisopropyl ester of clodronic acid at pH 0.0. ^{31}P–^{31}P spin–spin coupling causes the doublet feature of the spectral patterns, whereas the doublet (P_2) and triplet (P_3) structures are due to ^{31}P–^{1}H spin–spin couplings.

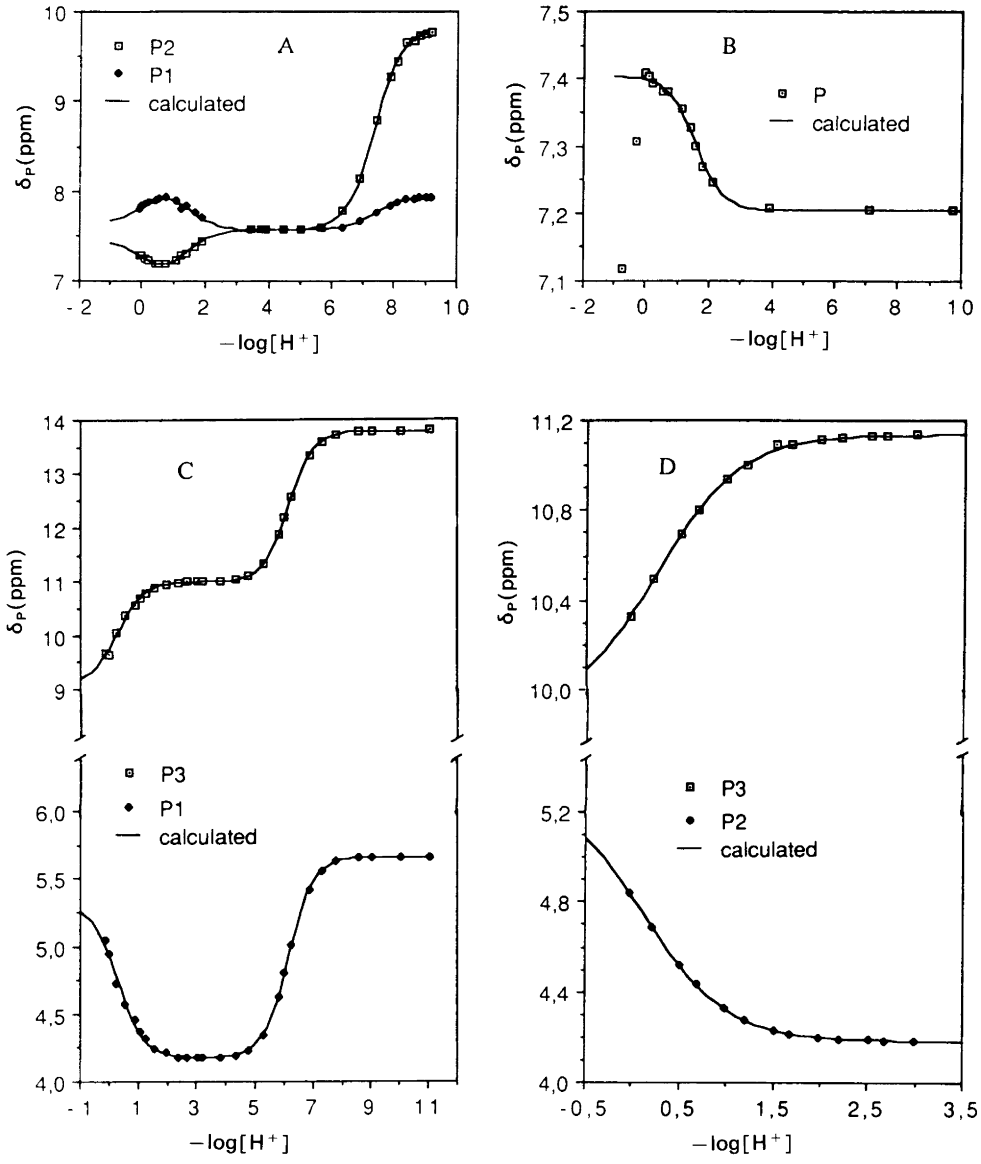


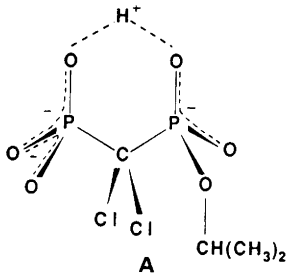
Fig. 6. Experimental and calculated chemical shift versus $-\log[H^+]$ of (A) monoisopropyl ester, (B) *P,P'*-diisopropyl ester, (C) *P,P*-diisopropyl ester and (D) triisopropyl ester of clodronic acid.

equivalent, both the ^{31}P–^{31}P and the ^{31}P–^{1}H spin–spin couplings affect the fine structure of the spectrum. When the theoretical spectra were calculated by the program Lequor assuming that $J_{PP} = 17.5$ Hz, $J_{PH} = 6.0$ Hz (through three bonds), $\Delta\delta_P = 0$ varying the half-width of the peak from 2 to 18 Hz, the calculated spectra showed similar singlets and triplets as the experimental spectra. This means that these peaks are caused by *P,P'*-diisopropyl ester, and acid- or base-catalysed ester hydrolysis does not take place.

^{31}P-NMR studies on series of polymethylenediphosphonic acids showed a strong interaction between the phosphonic acid groups in the P–C–P-type bisphosphonic acids.[16,17] This shielding effect of one phosphorus atom with respect to the other drops off quite rapidly with increasing number of backbone carbon atoms between the two phosphorus atoms, and becomes negligible when there are more than three carbon atoms between the phosphorus atoms (i.e. P–C–C–C–C–P). The similarity of the chemical shifts of the methylenediphosphonic acids to those of their tetraalkyl esters suggests that the electronic environment of the phosphorus atoms in the acids and esters is solely determined by the oxygen atoms. It has also been shown that the chemical shift of the compounds in which phosphorus has four substituents is primarily sensitive to the polarity of the σ-bonds and the total occupation of the dπ-orbitals of the phosphorus.[18] A study on ^{31}P chemical shifts of phosphonate anions and a comparison of these data to quaternary triphenylphosphonium salts reveals that there is a little or no π-character in the P–C bond, and it is therefore reasonable to ascribe essentially all such π-effects to the

P–O bonds of the phosphonate anions. The ^{31}P-NMR study of acid–base equilibria of aminoalkylphosphonic acids showed that the electron-withdrawing effect of the proton through the bonds decreases the electron density around the phosphorus atom of the phosphonate group, where the protonation occurs.[19] Also, the increase of the electron density of that phosphorus atom is assumed to be caused by the formation of a cyclic structure.

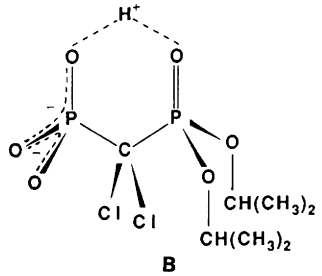
The plot of the ^{31}P chemical shift again the pH for monoisopropyl ester shows three distinct inflections at pH 7.3, 1.4 and 0, corresponding to successive protonation steps. At the first protonation step the ^{31}P the shielding of both P atoms increases. This effect, together with strong binding of the first proton, supports the formation of cyclic structure **A**. At the second protonation step the



A

shielding of the P_2 atom increases and the shielding of the P_1 atom decreases. This is explained by the addition of the second proton to the phosphonate group P_1 by the simultaneous migration of the first proton to the same phosphonate group, leading to rupture of the cyclic structure. At the third protonation step the shielding of the P_2 atom decreases and that of the P_1 atom increases, indicating the addition of the third proton to the phosphonate group P_2. At the second and third protonation steps the decreased shielding of the P atom is assumed to be caused by the electron-withdrawing effect of the proton, and the increased shielding of the neighbouring P arom is assumed to be caused by the shielding effect of one phosphorus atom with respect to the other.

The plot of the ^{31}P chemical shift against pH for *P,P*-diisopropyl ester shows two inflections at pH 6.1 and 0.3, corresponding to successive protonation steps. The change of chemical shift resembles that of the monoisopropyl ester. At the first protonation step the shielding of both P atoms increases. This effect, the strong binding of the first proton and the fact that protonations can take place only to phosphonate group P_1, confirms the formation of cyclic structure **B** at that stage. At the



B

second protonation step the shielding of the P_3 atom increases and that of the P_1 atom decreases. This indicates addition of the second proton to the phosphonate group P_1 by the simultaneous migration of the first proton to the same phosphonate group, leading to rupture of the cyclic structure. At the second protonation step the decreased shielding of the P_1 atom is assumed to be caused by the electron-withdrawing effect of the proton, and increased shielding of the neighbouring P_3 atom is assumed to be caused by the shielding effect of one phosphorus atom with respect to the other.

The plot of the ^{31}P chemical shift against pH for *P,P'*-diisopropyl ester shows an inflection at pH 1.5 corresponding the first protonation step. The decreased shielding of the P atom at the first protonation step is tentatively caused by the electron-withdrawing effect of the proton through consecutive bonds. There is also seen a weak shift to decreasing nuclear shielding in the pH region 0.5–0.7 and a strong shift to increasing shielding at pH < 0. So far we have not found any reliable explanation for these last two effects at pH < 0.5. Thus we omitted this pH region in the calculations.

The plot of ^{31}P chemical shift against pH for triisopropyl ester shows one inflection at pH 0.2. On protonation the shielding of the P_2 atom decreases and the shielding of the P_3 atom increases. These effects, and the fact that protonation can occur only to phosphonate group P_2, confirms the assumption that decreased shielding is caused by the electron-withdrawing effect of the proton through consecutive bonds and that increased shielding is caused by the shielding effect of one phosphorus atom with respect to the other.

Conclusions

As a summary of the present investigation, the ^{31}P-NMR study on acid–base equilibria of isopropylesters of clodronic acid reveals that: (1) At the first step protonation of monoisopropyl and *P,P*-diisopropyl esters a cyclic structure is formed. In the case of *P,P'*-diisopropyl and -triisopropyl esters the formation of a cyclic structure at the first protonation is negligible. (2) An increase in shielding of both P atoms at protonation indicates formation of a cyclic structure. (3) A decrease in shielding of one P atom, coupled with an increase in shielding of the neighbouring P atom at protonation indicates addition of a proton to the phosphonate group at which the P atom shielding decreases. (4) In case (3) the decreased shielding is caused by the electron-withdrawing effect of the proton and the increased shielding is caused by the shielding effect of one P atom with respect to the other. (5) The change of electron density around the P atoms during the protonation of ligand anions cannot be explained solely by the electron-withdrawing effect of the proton. Probably a dπ–pπ interaction in the P–O bonds and a mutual interaction of the P atoms through consecutive bonds or directly through space also has an effect on the electron density around the P atoms.

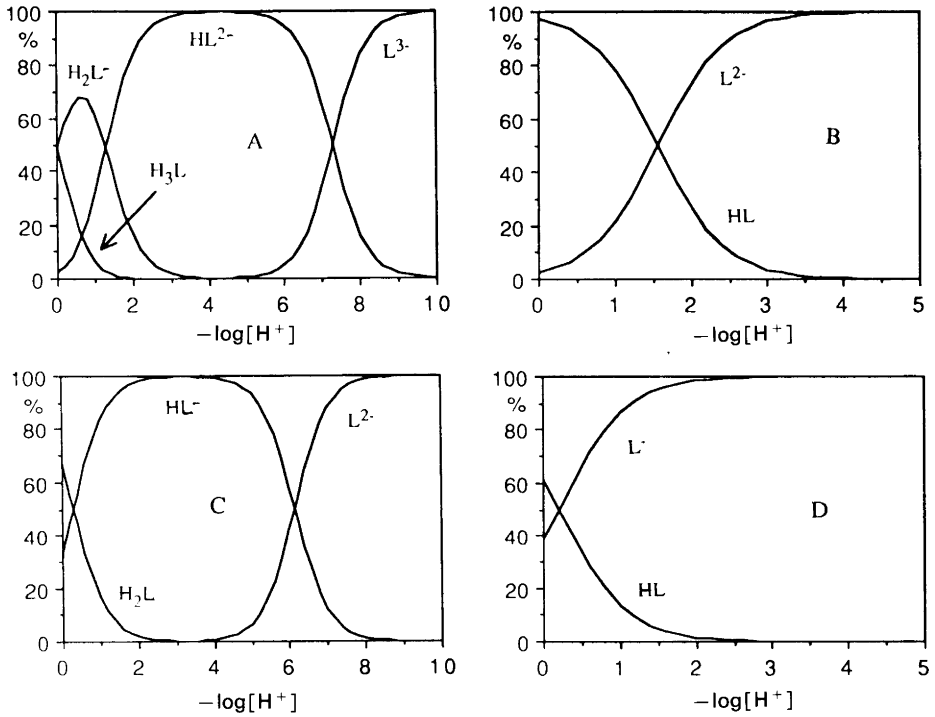


Fig. 7. Distribution of various acid species of (A) monoisopropyl ester, (B) *P,P'*-diisopropyl ester, (C) *P,P*-diisopropyl ester and (D) triisopropyl ester of clodronic acid as a function of $-\log[H^+]$.

According to the distribution diagrams (Fig. 7), the main acid species of the present esters at the pH of the human stomach (pH 1–3) are as follows: H_2L^-, HL^{2-} (monodiisopropyl ester); HL^- (*P,P*-diisopropyl ester); HL^-, L^{2-} (*P,P'*-diisopropyl ester); L^- (triisopropyl ester). The main acid species in the pH of human blood serum (ca. pH 7) are: HL^{2-}, L^{3-} (monodiisopropyl ester); L^{2-} (both diisopropyl esters); L^- (triisopropyl ester).

In the cases of triisopropyl and *P,P*-diisopropyl esters protonations can take place only to the other phosphonate group. This means that the negative charge of the anions is localised at one end of the molecule, and the other end is totally esterified and nonpolar. Thus these two esters can act as washing agents: they can bind to polar and nonpolar surfaces. Because the most acidic proton of the monoisopropyl ester is located on the phosphonate group P_2, both phosphonate groups are negatively charged at the pH of the human stomach and human blood serum. Under these conditions both phosphonate groups of *P,P'*-diisopropyl ester are also negatively charged. Thus monoisopropyl and *P,P'*-diisopropyl esters are polar and can bind only to polar surfaces.

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