

# Acid–Base Equilibria and Thermal Behaviour of Some Hexyl Esters of Clodronic Acid

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Protonation constants of three hexyl esters of clodronic acid were determined by potentiometric measurements in aqueous solution at 25 °C and  $I = 1.0$  (Me<sub>4</sub>NCl). Thermal decomposition of the sodium salts of these esters was interpreted from the TG and DTG curves with help of mass spectrometry. The compounds studied were *p,p*-dihexyl clodronate disodium salt, *p,p'*-dihexyl clodronate disodium salt and trihexyl clodronate monosodium salt. The dimeric species HL<sub>2</sub><sup>3-</sup> was found to form, as well as monomeric species H<sub>p</sub>L of the *p,p'*-dihexyl ester. For the other two acids, the protonation scheme can be expressed exclusively in terms of the species H<sub>p</sub>L ( $p = 1$  or  $2$ ). The TG curves show that *p,p'*-dihexyl and trihexyl esters contain no crystal water, but *p,p*-dihexyl ester contains one water molecule. The thermal decomposition of the *p,p'*-dihexyl and trihexyl esters takes place in two steps, but the *p,p*-dihexyl ester decomposes in four steps. The most stable of the present compounds is the *p,p'*-dihexyl clodronate disodium salt.

Hexyl esters of (dichloromethylene)-bisphosphonic acid (clodronic acid) belong to a group of gem-bisphosphonates which contain a P–C–P bridge. They are structural analogous to P–O–P compounds, the pyrophosphates. Unlike pyrophosphates, bisphosphonates are resistant to enzymatic hydrolysis, and for this reason they are used as therapeutic agents for diseases in the skeletal system and in soft tissue.<sup>1–4</sup> The clinical properties of methylenebisphosphonates can be modified by varying the substituents at the phosphate groups. For this reason the ester derivatives of clodronic acid were synthesised. Because the studied esters are new substances, there are no data available on their acid–base equilibria and thermal behaviour. The protonation of these compounds is necessary background information for the study of their properties as ligands to metal ions. Thermogravimetric curves can be used to control the quality of the sodium salts of these potential pharmaceutical products.

The present paper describes a study of various properties of the *p,p*-dihexyl clodronate trisodium salt, the *p,p'*-dihexyl clodronate disodium salt and the trihexyl clodronate monosodium salt both in the solid state by thermoanalytical methods and mass spectrometry, and in aqueous solutions under constant conditions,  $I = 1.0$  (CH<sub>3</sub>)<sub>4</sub>NCl and 25 °C by potentiometric measurements.

## Experimental

**Syntheses.** The studied esters were prepared by Leiras Oy according to procedures described elsewhere.<sup>4,5</sup>

**Reagents.** Stock solutions of tetramethylammonium chloride, tetramethylammonium hydroxide and HCl were prepared and analysed as described previously.<sup>6</sup>

**Potentiometric measurements.** The potentiometric titrations were performed in a temperate room (25 °C) at 25.0 ± 0.1 °C. A locally constructed titration system consisting of a multichannel high-impedance amplifier, a Hewlett-Packard 3478A multimeter and a Metrohm 665 Dosimat piston burette was used for the measurements. The equipment was controlled with an Amstrad PC 1640 HD 20 computer using the program TIT3.<sup>7</sup> The indicator electrode was an Orion research 91-01 glass electrode, and the reference electrode was an Orion 90-02 double-junction Ag<sub>2</sub>AgCl electrode. The outer mantle of the Ag<sub>2</sub>AgCl electrode was filled with 1.0 M tetramethylammonium chloride solution. The calibration of the electrode system was carried out as described in detail earlier.<sup>6</sup>

The measurements were carried out in a argon atmosphere using a 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

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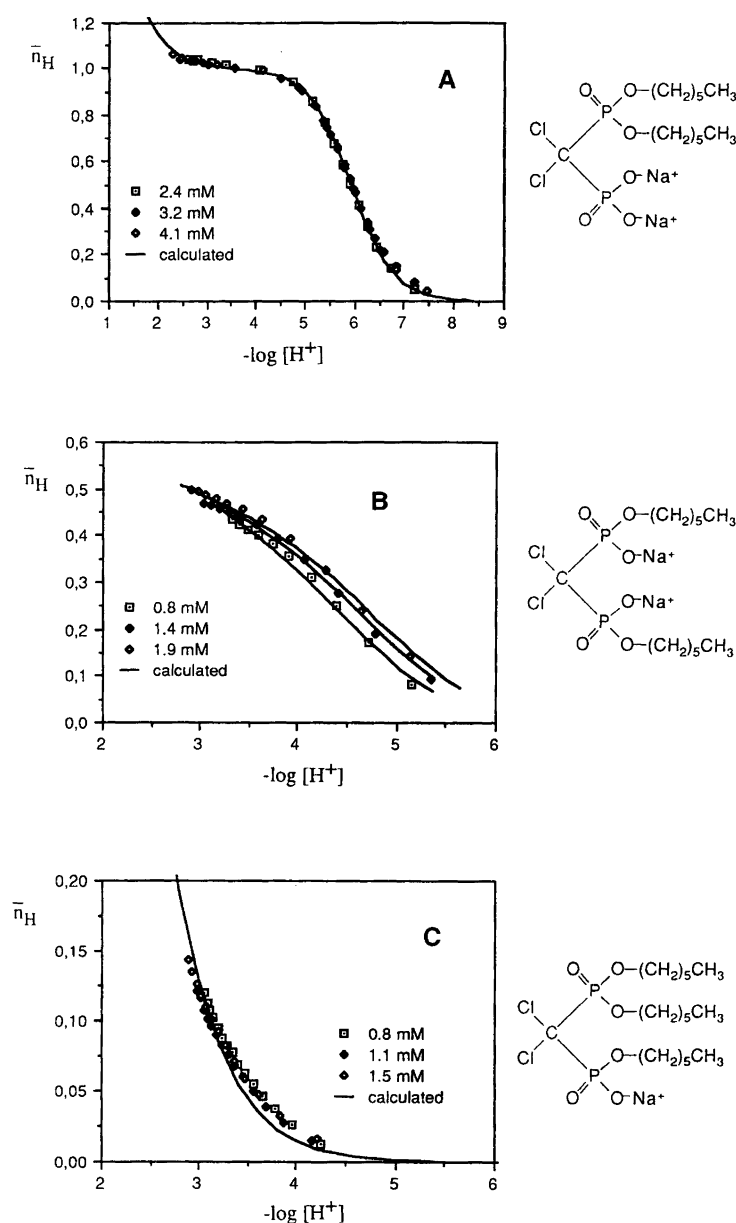


Fig. 1. Experimental and calculated plots of  $\bar{n}_H$  versus  $-\log [H^+]$  of (A) the  $p,p$ -dihexyl ester, (B) the  $p,p'$ -dihexyl ester and (C) the trihexyl ester of clodronic acid.

Table 1. Protonation constants,  $\log \beta_{ppr}$  ( $\pm 3\sigma$ ), and acid constants,  $pK_{ap}$ , of the present hexyl esters of clodronic acid at 25 °C and  $I=1.0$  ( $Me_4BCl$ ), determined by the potentiometric method.

$C_L/mM$	$-\log [H^+]$	$\log \beta_{011}$	$\log \beta_{021}$	$\log \beta_{012}$	$pK_{a1}^a$	$pK_{a2}^a$
$p,p$ -Dihexyl ester 2.4–4.1	2.3–7.2	$5.93 \pm 0.02$	$7.18 \pm 0.02$		$1.25^b$	$5.93^c$
$p,p'$ -Dihexyl ester 0.8–1.9	2.9–5.2	$2.90 \pm 0.09$		$7.41 \pm 0.05$		$2.90^c$
Trihexyl ester 0.9–1.5	2.9–4.2	$2.17 \pm 0.03$			$2.17^d$	

<sup>a</sup> Acid constants,  $pK_a$ , refer to the equilibrium reaction:  $H_pL \rightleftharpoons H_{p-1}L + H^+$ . <sup>b</sup>  $pK_{a1} = \log \beta_{021} - \log \beta_{011}$ . <sup>c</sup>  $pK_{a2} = \log \beta_{011}$ . <sup>d</sup>  $pK_{a1} = \log \beta_{011}$ .

[ $I = 1.0$ ,  $(\text{CH}_3)_4\text{NCl}$ ]. The total concentration of the esters was varied between 0.0009 and 0.004 M.

**Thermogravimetry and mass spectroscopy.** TG curves were recorded on a Mettler TA-3000 thermoanalyzer in the temperature range 25–900 °C. The sample quantity varied between 8 and 10 mg. The dynamic runs were made in air or nitrogen at a flow rate of 200 ml min<sup>-1</sup> and a heating range of 2 °C min<sup>-1</sup>.

The mass spectra of the decomposition products were recorded at a scanning rate of 1 s decade<sup>-1</sup> on a Kratos MS 80 RF mass spectrometer, using the direct inlet technique and heating the sample at a rate of 50 °C min<sup>-1</sup>.

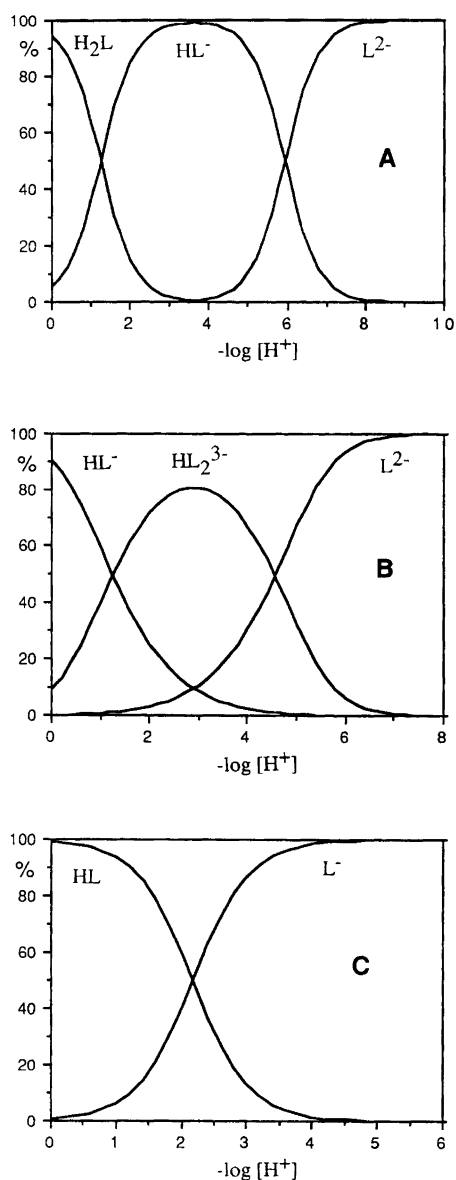


Fig. 2. Distribution of various acid species of (A) the *p,p*-dihexyl ester, (B) the *p,p'*-dihexyl ester ( $C_L = 1.4$  mM) and (C) the trihexyl ester of clodronic acid as a function of  $-\log [\text{H}^+]$ .

**Data treatment.** The studied equilibrium reactions can be written as eqn. (3):



where the protonation constants are denoted as  $\beta_{opr}$ . The mass balance and equilibrium conditions used in our calculations are as given eqns. (4) and (5):

$$C_H = [\text{H}^+] - k_w/[\text{H}^+] + \sum \sum p\beta_{opr}[\text{H}^+]^p[\text{L}]^r \quad (4)$$

$$C_L = [\text{L}] + \sum \sum r\beta_{opr}[\text{H}^+]^p[\text{L}]^r \quad (5)$$

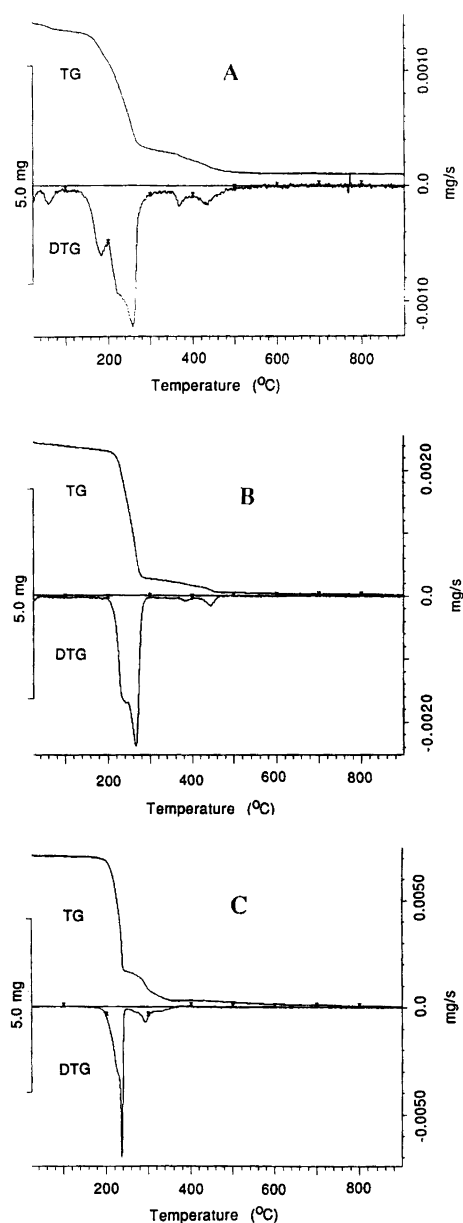


Fig. 3. TG and DTG curves of (A) the *p,p*-dihexyl clodronate disodium salt, (B) the *p,p'*-dihexyl clodronate disodium salt and (C) the trihexyl clodronate monosodium salt in air. The sample weights were 6.055, 6.309 and 6.116 mg for TG runs, respectively. The heating rate was 2 °C min<sup>-1</sup>.

The search for a model ( $pr$ ) and corresponding protonation constants ( $\beta_{opr}$ ) that give the 'best' fit to experimental data was carried out using the modified version of the nonlinear least-squares computer program SCOGS2.<sup>8,9</sup>

The error squares sum  $U = \sum (V_i^{\text{calc}} - V_i^{\text{obs}})^2$  was minimized ( $V_i^{\text{calc}}$  is the calculated and  $V_i^{\text{obs}}$  the added titrant volume at the point  $i$ ). 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 exceed 0.05. The  $P$ -value is a measure of the normal distribution of the  $(V_i^{\text{calc}} - V_i^{\text{obs}})$ -residuals. The agreement index is calculated

from eqn. (6):

$$R = [\sum(V_i^{\text{calc}} - V_i^{\text{obs}})^2 / \sum(V_i^{\text{obs}})^2]^{1/2}. \quad (6)$$

The best set of constants gives the smallest  $R$ -value.  $\bar{n}_H$ , the average number of  $H^+$  bound to each ligand, is calculated from eqn. (7):

$$\bar{n}_H = [C_H - ([H^+] - k_w/[H^+])]/C_L \quad (7)$$

## Results and discussion

**Potentiometry.** The potentiometric data consisted of three titrations and 32–81 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). For the  $p,p$ -dihexyl and trihexyl ester the experimental  $\bar{n}_H$ -curve seems to be a function only of  $-\log [H^+]$  for the whole pH and  $C_L$  region over which the measure-

Table 2. TG data on the decomposition of hexyl clodronate sodium salts.

$T$ -range / $^{\circ}\text{C}$	Weight loss (%)	Calculated weight loss (%)	Leaving group
<i>p,p</i> -Dihexyl ester			
In air flow:			
25–120	3.51	3.78	$\text{H}_2\text{O}$
120–200	11.23	12.99	$\frac{2}{3}\text{C}_4\text{H}_9\text{Cl}$
200–340	34.77	31.88	$\frac{1}{3}\text{C}_4\text{H}_9\text{Cl} + \text{C}_6\text{H}_{13}\text{Cl}$
340–600	8.01	8.42	$\text{C}_2\text{H}_4 + \text{C}$
Residue (%)	42.48	42.93	$\text{Na}_2\text{P}_2\text{O}_6$
In nitrogen flow:			
25–130	3.62	3.78	$\text{H}_2\text{O}$
130–230	14.08	12.99	$\frac{2}{3}\text{C}_4\text{H}_9\text{Cl}$
230–340	33.13	31.88	$\frac{1}{3}\text{C}_4\text{H}_9\text{Cl} + \text{C}_6\text{H}_{13}\text{Cl}$
340–900	7.86	8.42	$\text{C}_2\text{H}_4 + \text{C}$
Residue (%)	41.31	42.93	$\text{Na}_2\text{P}_2\text{O}_6$
<i>p,p'</i> -Dihexyl ester			
In air flow:			
25–300	51.56	52.77	$\text{C}_6\text{H}_{13}\text{Cl} + \text{C}_4\text{H}_9\text{Cl} + \text{C}_2\text{H}_4$
300–500	5.10		
500–900	0.73	6.13	$\text{CO}$
Residue (%)	42.61	41.60	$\text{Na}_2\text{P}_2\text{O}_5$
In nitrogen flow:			
25–320	53.53	52.77	$\text{C}_6\text{H}_{13}\text{Cl} + \text{C}_4\text{H}_9\text{Cl} + \text{C}_2\text{H}_4$
320–900	4.94	6.13	$\text{CO}$
Residue (%)	41.53	41.60	$\text{Na}_2\text{P}_2\text{O}_5$
Trihexyl ester			
In air flow:			
25–250	55.30	54.93	$\text{C}_6\text{H}_{13}\text{Cl} + \text{C}_4\text{H}_9\text{Cl} + \text{C}_2\text{H}_4 + \text{C}_2\text{H}_4$
250–380	13.77	13.31	$\text{C}_4\text{H}_9 + \text{C}$
In nitrogen flow:			
25–275	61.44	60.53	$\text{C}_6\text{H}_{13}\text{Cl} + \text{C}_4\text{H}_9\text{Cl} + \text{C}_2\text{H}_4\text{O} + \text{C}_4\text{H}_9$
275–420	11.04	10.79	$\text{C}_2\text{H}_4\text{O} + \text{C}$
Residue (%)	27.52	28.68	$\text{NaP}_2\text{O}_4$

ments were carried out, 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. For the *p,p'*-dihexyl ester the experimental  $\bar{n}_H$ -curve seems to be a function of  $-\log [H^+]$  and  $C_L$ . This indicates the formation of one or more polymeric species. Computer analyses showed the formation of the dimeric compound  $HL_2^{3-}$  and monomeric  $HL^-$ .

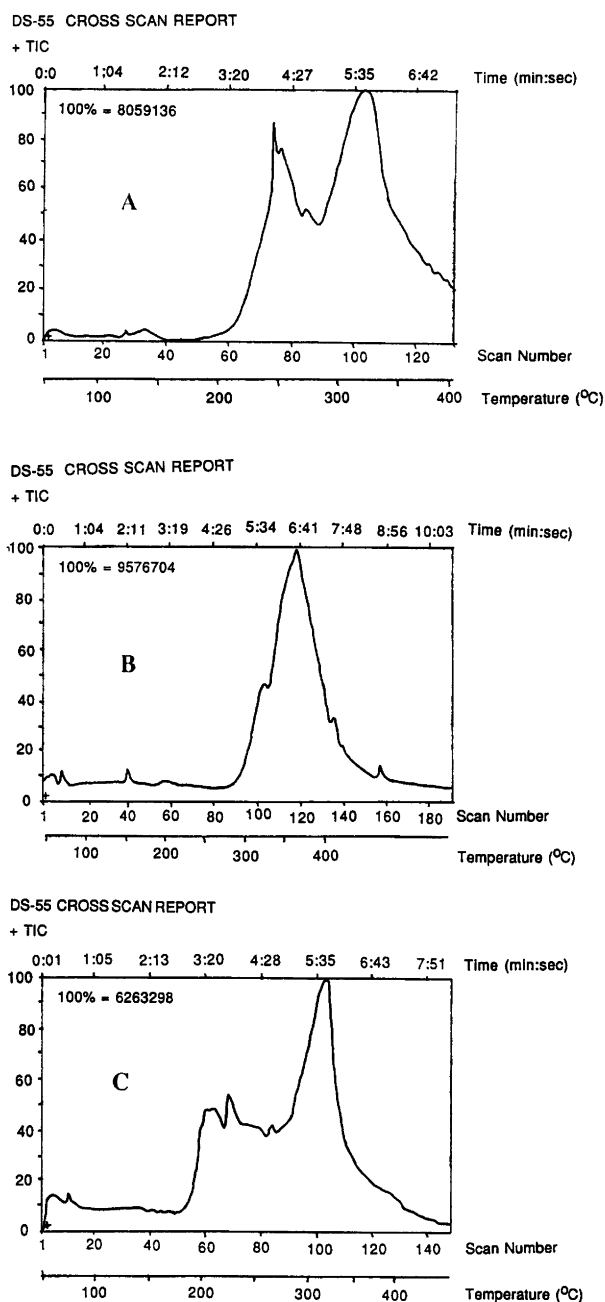


Fig. 4. The total ion chromatograms of (A) the *p,p*-dihexyl clodronate disodium salt, (B) the *p,p'*-dihexyl clodronate disodium salt and (C) the trihexyl clodronate monosodium salt obtained by heating  $50^\circ\text{C min}^{-1}$ .

This ester should be a diprotic acid, but because all the points of the experimental  $\bar{n}_H$ -curve fall between 0 and 1, we did not obtain the first acid constant by potentiometric titrations. Thus the first deprotonation of this ester takes place at  $\text{pH} < 1.0$ . The best set of overall protonation constants,  $\beta_{0pp}$ , obtained with the program SCOGS2 are given in Table 1. Because the program gave a *P*-value range 0.028–0.67 and an *R*-index range 0.0035–0.0086 for protonation constants calculated from individual titrations, statistically reliable constants were obtained over the  $\text{pH}$  and  $C_L$  region used in the calculations.

The *p,p*-dihexyl ester of clodronic acid has two separate protonation regions. At  $\text{pH} < 3.0$  this ester loses its most acidic proton, and in the  $\text{pH}$  region 4.5–8.0 the latter deprotonation takes place. The ester is completely dissociated at  $\text{pH} > 8.5$ . The monomeric species of the *p,p'*-dihexyl ester gives off the latter proton at  $\text{pH} < 5.0$ . In the  $\text{pH}$  region 3–7.5 the dimeric compound  $HL_2^{3-}$  is deprotonated and the ester is completely dissociated at  $\text{pH} > 8.0$ . The deprotonation of the trihexyl ester takes place completely at  $\text{pH} < 4.5$ .

According to the distribution diagrams (Fig. 2) the main acid species of the present esters at the  $\text{pH}$  of the human stomach ( $\text{pH}$  1–3) are as follows:  $H_2L$ ,  $HL^-$  (the *p,p*-dihexyl ester);  $HL^-$ ,  $HL_2^{3-}$  (the *p,p'*-dihexyl ester);  $HL$ ,  $L^-$  (trihexyl). The main acid species at the  $\text{pH}$  of human blood serum (*ca.*  $\text{pH}$  7) are:  $L^{2-}$  (both dihexyl esters);  $L^-$  (trihexyl ester). In the case of the trihexyl and *p,p*-dihexyl esters protonation can take place only to the other phosphonate group. Thus the negative charge of the anions is localised at one end of the molecule, and the other end is totally esterified and nonpolar. The anionic species of these esters at the  $\text{pH}$  of the human stomach and blood serum can bind to polar and nonpolar surfaces. In the  $\text{pH}$  region 1–3 there are also totally protonated neutral species, which most likely bind to only nonpolar surfaces. Both the phosphonate groups of the *p,p'*-dihexyl ester have acidic protons, i.e. the distribution of negative charge of the anion is dependent on  $\text{pH}$ . At the  $\text{pH}$  of human blood serum both phosphonate groups of this ester are negatively charged. The *n*-hexyl tails of the negatively charged phosphonate groups are sufficiently long that the ester can probably bind to polar and nonpolar surfaces. At the  $\text{pH}$  of the human stomach only monomeric  $HL^-$  and dimeric  $HL_2^{3-}$  species exist. Obviously these species can bind to polar and non-polar surfaces.

The structure of  $HL_2^{3-}$  is probably an open dimer, where association takes place through the intermolecular hydrogen bond between the phosphonate groups.<sup>10</sup> According to previous studies of the protonation of isopropyl esters of clodronic acid, we determined the formation of cyclic structure at the first protonation step of the *p,p*-diisopropyl ( $\log \beta_{011} = 6.13$ ) and monoisopropyl ( $\log \beta_{011} = 7.24$ ) esters of clodronic acid.<sup>6</sup> Because the *p,p*-dihexyl ester binds the first proton (Table 1) more strongly than other hexyl esters, this supports the formation of cyclic structure of the type A. In the cases of the

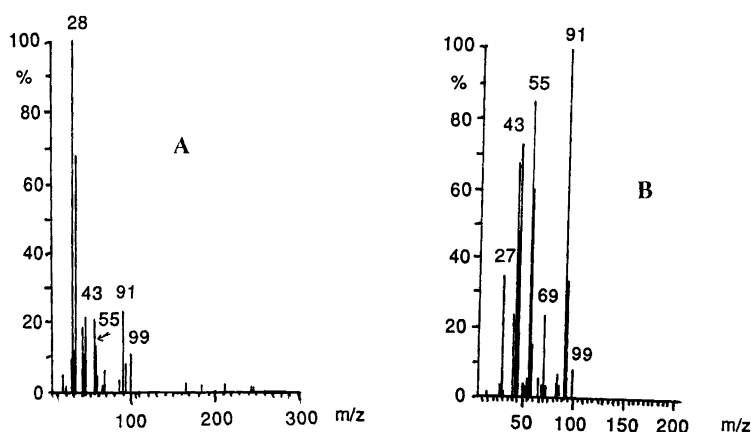


Fig. 5. Mass spectra of the *p,p'*-dihexyl clodronate disodium salt taken from the points of scan numbers (A) 75 and (B) 100 of the total ion chromatogram presented in Fig. 4A.

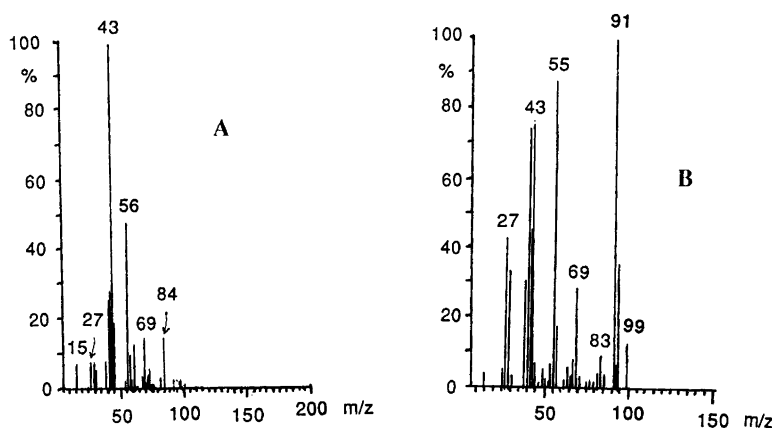


Fig. 6. Mass spectra of the *p,p'*-dihexyl clodronate disodium salt taken from the points of scan numbers (A) 100 and (B) 121 of the total ion chromatogram presented in Fig. 4B.

*p,p'*-dihexyl and trihexyl esters the formation of cyclic structure at the first protonation step is negligible.

**Thermogravimetry and mass spectroscopy.** The thermoanalytical curves in air under normal pressure given in Fig. 3 show that the *p,p'*-dihexyl and trihexyl esters contain no crystal water, but the *p,p*-dihexyl ester contains one water

molecule. The thermal decomposition of the *p,p'*-dihexyl and trihexyl esters takes place in two steps, but the *p,p*-dihexyl ester decomposes in four steps. TG data on the decomposition of the compounds studied are given in Table 2. Thermal decomposition in air and nitrogen was practically the same for each compound with minor exceptions. In each case an amorphous residue was ob-

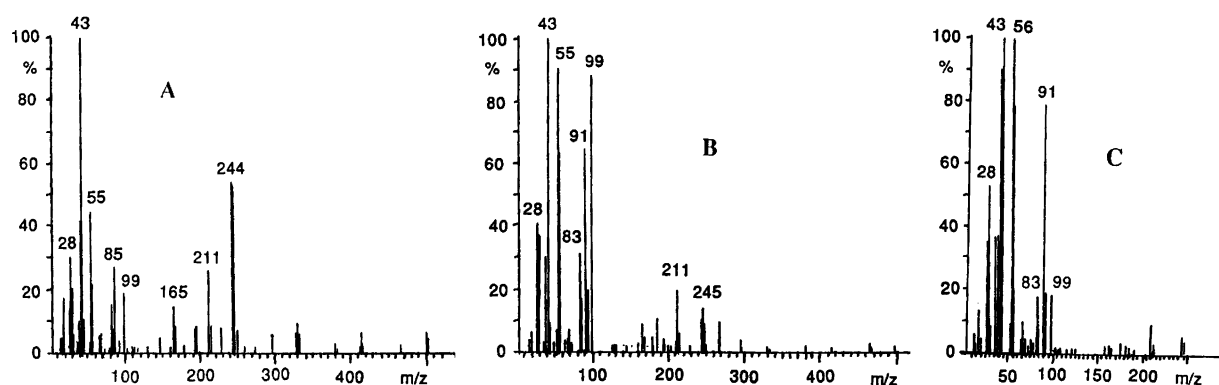


Fig. 7. Mass spectra of the trihexyl clodronate monosodium salt taken from the points of scan numbers (A) 60, (B) 96 and (C) 128 of the total ion chromatogram presented in Fig. 4C.



bond gives the  $C_4H_9$  group. Carbon from the P-C-P group can be released as carbon monoxide or dioxide by reaction with oxygen.

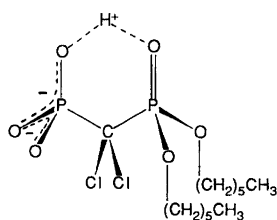
The weight loss at 25–120°C in the TG curves of the *p,p*-dihexyl ester is due to the volatilization of one water molecule. Although the second and third decomposition steps are only partly separated, the weight loss and mass spectra indicate a release of butyl chloride in the second decomposition step at 120–200°C. Then the rest of the butyl chloride, together with hexyl chloride, is released in third step at 200–340°C. The smooth weight loss at 340–600°C is due to volatilization of ethylene together with carbon.

The first decomposition step (200–300°C) of the *p,p'*-dihexyl ester is sharp and corresponds to the volatilization of hexyl chloride, butyl chloride and ethylene. In the last smooth decomposition step (300–900°C) carbon monoxide is released.

Hexyl chloride, butyl chloride, acetaldehyde and ethylene are released simultaneously in the first and very sharp decomposition step (180–250°C) of the trihexyl ester in flowing air. The weight loss of the second step (250–380°C) corresponds to a release of carbon and the  $C_4H_9$  group. In a nitrogen flow the first reaction step (180–275°C) corresponds to volatilization of hexyl chloride, butyl chloride, acetaldehyde and the  $C_4H_9$  group. In the second step (275–420°C) carbon and acetaldehyde are released.

The most stable of the present compounds is the *p,p'*-dihexyl clodronate disodium salt. This can be explained by the symmetric structure of the compound.

The total ion chromatogram obtained by heating the studied esters in the direct inlet probe using a constant heating rate is shown in Fig. 4, and corresponding mass spectra are presented in Figs. 5–7. The total ion chromatogram shows that the *p,p*-dihexyl and trihexyl esters



Cyclic structure A

decompose mainly in two steps and the *p,p'*-dihexyl ester decomposes in one step. The main fragments found were  $C_2H_3^+$  ( $m/z = 27$ ),  $C_2H_4^+$  (28),  $C_3H_5^+$  (41),  $C_3H_7^+$  or  $C_2H_3O^+$  (43),  $C_3H_8^+$  or  $C_2H_4O^+$  (44),  $C_4H_7^+$  (55),  $C_4H_8^+$  (56),  $C_5H_9^+$  (69),  $C_6H_{11}^+$  (83),  $C_6H_{12}^+$  (84),  $C_6H_{13}^+$  (85),  $C_4H_8Cl^+$  (91). The fragments of  $m/z = 69$ , 83, 84, 85 and 91 indicate evaporation of hexyl chloride, and the fragments of  $m/z = 41$ , 43, 44, 55, 56 and 91 could be due to evaporation of butyl chloride.<sup>14,15</sup> Also the evaporation of ethylene, acetaldehyde and butene could produce the peaks  $m/z = 27$ , 27, 43, 44, 55 and 56. The fragmentation routes of the compounds studied are presented in Scheme 1. The interpretations for the main decomposition reactions given in Table 2 are based on the mass spectrometric analyses and amounts of weight loss.

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