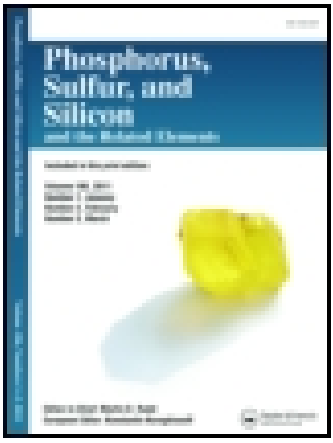

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MONITORING THE RATE OF HYDROLYSIS OF AMINOPHOSPHONIC ACID ESTERS BY UV-VIS- AND NMR-SPECTROSCOPY

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Two aminophosphonic acid dimethylesters **1** and **3** bearing COOH functions attached to UV-VIS absorbing groups were hydrolysed by aqueous NaOH to yield the corresponding monomethylesters **2** and **4**. The rates of hydrolysis were monitored by UV-VIS methods. The type of reaction observed is of pseudo first order with rate constants k_1[l $mol^{-1}s^{-1}$] of $1.92 \cdot 10^{-6}$ (**1**, 25°C), $4.96 \cdot 10^{-5}$(**1**, 50°C) and $4.63 \cdot 10^{-5}$(**3**, 50°C). Hydrolysis is sufficiently slow to enable the practically undisturbed determination of dissociation constants for 1-(4-carboxyphenyl)-1-(N-t. butyl)-aminomethylphosphonic acid dimethylester (**1**) and 1-(4-benzoic-acid)-1-(N-4-phenyl-azo-phenylene)-aminomethylphosphonic acid dimethyl ester (**3**).

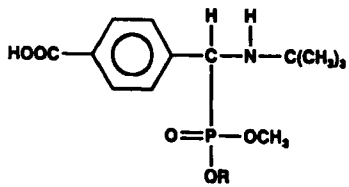
Keywords: Aminophosphonic Acid Dimethyl- and Monomethylester; Hydrolysis; Kinetics; UV/VIS; NMR

INTRODUCTION

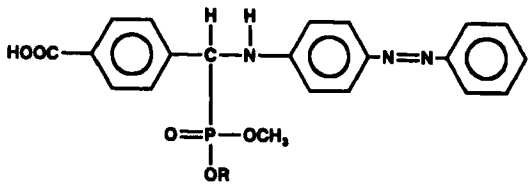
Aminophosphonic acid derivatives have attracted widespread practical interests, e.g. for possessing interesting biological and agrochemical properties and being used as suitable sequestering and complexing agents for many metals including calcium[1]. Up to now, the most widely used calcium complexing agents belong to the class of polycarboxylic molecules, or to organic bisphosphonate compounds, analogues of the inorganic pyrophosphates, in which the oxygen linking the two phosphorus atoms has been

* Correspondence author.

replaced by a carbon atom. The clinical interest for such bisphosphonates is connected with their potential use against several pathological conditions involving irregularities in calcium metabolism (e.g. osteoporosis, osteolysis, bone cancer)[2] or involving calcium deposition (e.g. arteriosclerosis, arthritis, kidney and renal calculus)[3,4]. However, side-effects and the lack of selectivity militate against their diffuse use[2]. In order to produce and test phosphonates with modified molecular structures but able to act as strong complexing agents for biorelevant metals, we recently reported on the synthesis of dimethyl esters of 1-amino-1-arylmethyl phosphonic acid containing free carboxylic groups[5]. Those compounds are soluble both in polar organic solvents ($CHCl_3$, CH_3CN, DMSO, alcohols) as well as in water or in slightly alkaline solutions, rendering them attractive for biological applications. In order to propose such compounds as complexing agents it is necessary to determine the corresponding stability and dissociation constants by varying the pH of the solution. Bearing in mind that alkyl esters of the phosphonates might be easily cleaved to monoester in alkaline solutions it was imperative to determine the rate of ester hydrolysis of such substrates.



1, 2



3, 4

SCHEME 1 Compounds **1, 3**: R=CH_3; **2, 4**: R=H

As model systems to establish the experimental and computing procedures compounds **1** and **3** were selected for the following reasons: the rate of ester hydrolysis can be easily monitored by UV-VIS-spectroscopy due

to the presence of the benzoic moieties in **1** and **3** resp. and the additional contribution of the phenyl-azo-chromophore in **3**. In addition practical considerations hold: The monoesters **2** and **4** originating from **1** and **3** are good candidates for showing peculiar complexing properties towards biorelevant cations, and thus vehiculating them through biological fluids.

Preliminary ^{31}P NMR purity checks showed, that ca. 30 min after dissolution of phosphonates **1** and **3** in KOH/D_2O two distinct resonance groups with different intensities were observed showing an intensity ratio given in **Figure 1** below:

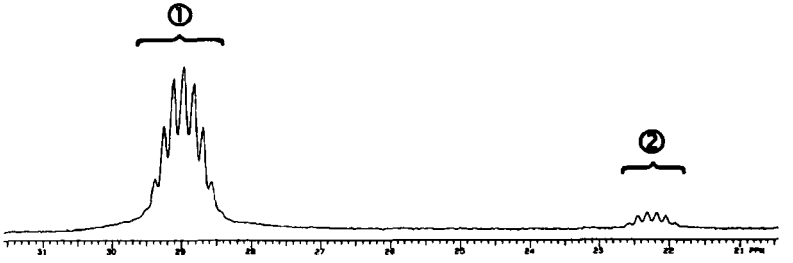


FIGURE 1 81 MHz ^{31}P-NMR-spectrum showing the resonance signals for the educt **1** and the product **2** ca. 30 min. after dissolving **1** in KOH/D_2O

The dimethylester **1** is attributed to the stronger signal ① at $\delta_P = 29.04$ ppm (m, $^3J_{PH} = 11.0$ Hz) while the monomethylester **2** resonates with lower intensity at ② with $\delta_P = 22.28$ ppm (q, $^3J_{PH} = 10.8$ Hz). Corresponding data for **3** and **4** are: $\delta_P = 24.97$ ppm (m, $^3J_{PH} = 10.3$ Hz) and $\delta_P = 19.01$ ppm (q, $^3J_{PH} = 10.2$ Hz) resp.. The intensity ratio for signals **1** and **2** in 81 MHz ^{31}P-NMR-spectra decays with time elapsed after dissolution indicating a slow hydrolysis in alkaline solutions.

Since our primary interest concerned the determination of dissociation constants for **1** and **3** by titration of those acidic compounds vs. NaOH it was imperative to separate the specific effects from esterolytic and protolytic equilibria, henceforth to determine the rate of hydrolysis prior to titration studies.

Some comments on the kinetics of hydrolysis

In principle the hydrolysis of an ester is described by a second order kinetic reaction. e.g:

$$R'P(O)(OR)_2 + HO^- \rightarrow R'P(O)(OR)O^- + ROH \qquad (1)$$

following the general equations:

$$A + B \rightarrow C + D \qquad (2)$$

$$-\frac{dc_A}{dt} = k_2 \cdot c_A \cdot c_B \qquad (3)$$

By using a sufficiently large excess of NaOH the hydrolysis is effectively converted into a pseudo first order reaction following:

$$-\frac{dc_A}{dt} = k_1 \cdot c_A \qquad (4)$$

In experiments described below the ratio of NaOH : ester **1** is set to 10:1.

In order to monitor the reaction by UV-VIS-spectroscopy eq. (4) is converted into eq. (5) correlating the concentration of the substrate with the corresponding absorbance $A_{\lambda,t}$ via Beer's law [6,7]:

$$\frac{d\mathbf{A}_{\lambda,t}}{dt} = \frac{k_1}{q} \cdot (\mathbf{A}_{\lambda,\infty} - \mathbf{A}_{\lambda,t}) \cdot (\mathbf{A}^1_{\lambda,\infty} - \mathbf{A}_{\lambda,\infty}) \qquad (5)$$

$A_{\lambda,t}$ absorbance, measured at wavelength λ at time **t**

$A_{\lambda,\infty}$ absorbance, measured at wavelength λ at the end of reaction for $t \rightarrow \infty$

$\mathbf{A}^1_{\lambda,\infty}$ $(C^0_B \cdot \varepsilon_{\lambda,C} - (C^0_B - C^0_A) \cdot \varepsilon_{\lambda,A}) \cdot d$

q $(\varepsilon_{\lambda,C} - \varepsilon_{\lambda,B} - \varepsilon_{\lambda,A}) \cdot d \left[\frac{dm^3}{mol}\right]$

c^0_X starting concentration of the compound X

$\varepsilon_{\lambda,X}$ extinction coefficient of the compound X at the wavelength $\lambda \left[\frac{dm^3}{cm \cdot mol}\right]$

d length of cell [cm]

Integration of equation (5) lead to equation (6), which was used to calculate the results given in this paper.

$$\ln\left(\frac{\mathbf{A}^1_{\lambda,\infty} - \mathbf{A}_{\lambda,t}}{\mathbf{A}_{\lambda,\infty} - \mathbf{A}_{\lambda,t}}\right) = (\mathbf{A}^1_{\lambda,\infty} - \mathbf{A}_{\lambda,\infty}) \cdot \frac{k_1}{q} \cdot t \qquad (6)$$

In order to eliminate errors from superimposing spectra of di- and monomethylesters difference spectroscopy was introduced. The absorbance due to the dimethylester (first spectrum, prior to hydrolysis) was subtracted from all individual spectra obtained throughout the kinetic series. This method ensures, that the difference spectra reflect indeed the formation of the monoester.

The pseudo-three-dimensional overlay-diagrams in **Figure 2** and **Figure 3** below, which were produced by PHOTO_T [8–11], clearly demonstrate the change in absorbance due to the formation of the monoesters.

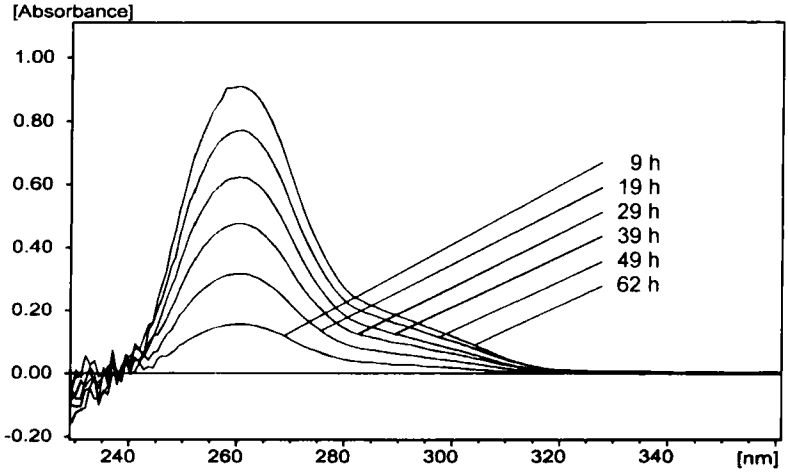


FIGURE 2 PHOTO_T-overlay diagram for the time dependent hydrolysis of **1** leading to the monomethylester **2**. Temperature: 25°C

The total absorbance is due solely to the formation of the monoester. This is clearly established by monitoring the absorbance vs. time as shown in **Figure 4**.

RESULTS

For the pseudo first order reactions inspected above the Swinbourne formalism [12]:

$$\mathbf{A}_{\lambda,t+\Delta t} = \mathbf{A}_{\lambda,\infty} \cdot (1 - e^{-k_1 \Delta t}) + \mathbf{A}_{\lambda,t} \cdot e^{-k_1 \Delta t} \qquad (7)$$

was used to calculate the kinetic data shown in **Tables I to VI**.

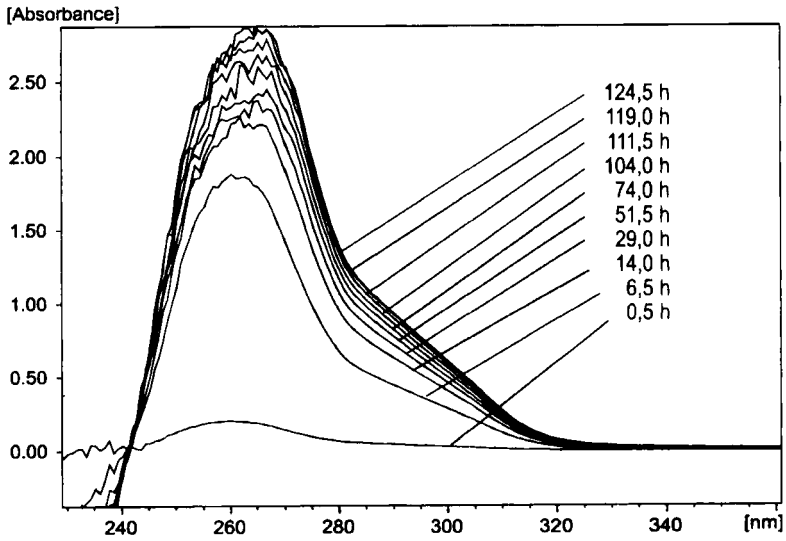


FIGURE 3 PHOTO_T overlay diagram for the time dependent hydrolysis of **1** leading to the monomethylester **2**. Temperature: 50°C

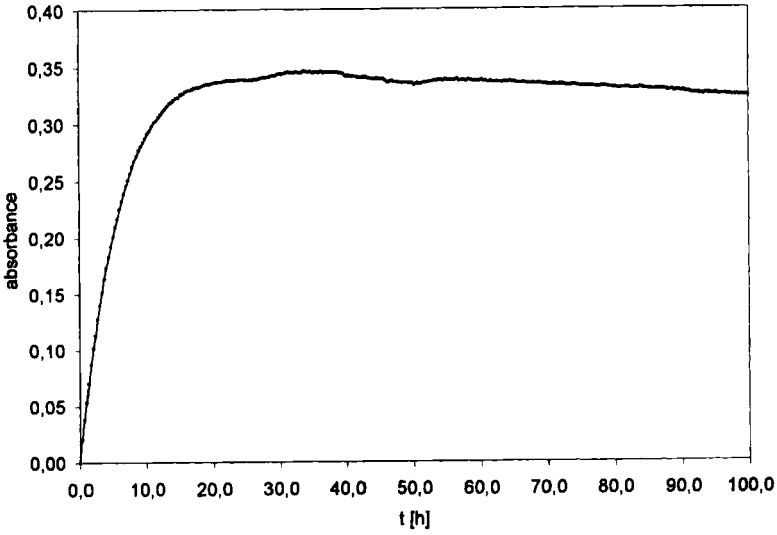


FIGURE 4 Single wavelength scan at 330 nm for the hydrolysis of **3** to **4** at 50°C

TABLE I Results for compound **1**. Temperature: 25°C evaluated according to Swinbourne

λ [nm]:	**266.121**	**271.174**	**281.617**	**286.174**	**296.184**
gradient $S=e^{-k_1\Delta t}$	0.9973	0.9975	0.9976	0.9983	0.9978
axis segment x = 0	0.0072	0.0053	0.0023	0.0014	0.0138
R^2	1.0000	1.0000	1.0000	0.9999	0.9967
$A_{\lambda,\infty}$	2.6673	2.1239	0.9571	2.0580	3 2775
k_1 [l mol^{-1} h^{-1}]	$8.1\ 10^{-3}$	$7.5\ 10^{-3}$	$7.3\ 10^{-3}$	$5.1\ 10^{-3}$	$6.6\ 10^{-3}$
k_1 [l mol^{-1} s^{-1}]	$2.24\ 10^{-6}$	$2.07\ 10^{-6}$	$2.02\ 10^{-6}$	$1.42\ 10^{-6}$	$1.84\ 10^{-6}$
average k_1	$6.9\ 10^{-3}$ l mol^{-1} h^{-1}			$1.92\ 10^{-6}$ l mol^{-1}s^{-1}	
standard deviation σ_{n-1} of k_1	$\pm 1.1\ 10^{-4}$ l mol^{-1} h^{-1}			$\pm 3.14\ 10^{-7}$ l mol^{-1} s^{-1}	

TABLE II Results for compound **1**. Temperature: 50°C evaluated according to Swinbourne

λ [nm]:	**266.121**	**271.174**	**281.617**	**286.174**	**296.184**
gradient: $S=e^{-k_1\Delta t}$	0.9416	0.9418	0.9419	0.9425	0.9437
axis segment x = 0:	0.1860	0.1604	0.0714	0.0544	0.0350
R^2	0.9870	0.9972	0.9999	1.0000	0.9999
$A_{\lambda,\infty}$	2.1043	2.3523	1.2284	0.9463	0.6225
k_1 [l mol^{-1} h^{-1}]	$18.07\ 10^{-2}$	$18.01\ 10^{-2}$	$17.97\ 10^{-2}$	$17.77\ 10^{-2}$	$17.40\ 10^{-2}$
k_1 [l mol^{-1} s^{-1}]	$5.02\ 10^{-5}$	$5.00\ 10^{-5}$	$4.99\ 10^{-5}$	$4.94\ 10^{-5}$	$4.83\ 10^{-5}$
average k_1	$17.84\ 10^{-2}$ l mol^{-1}h^{-1}			$4.96\ 10^{-5}$ l mol^{-1}s^{-1}	
standard deviation σ_{n-1} of k_1	$\pm 2.7\ 10^{-3}$ l mol^{-1} h^{-1}			$\pm 7.55\ 10^{-7}$ l mol^{-1} s^{-1}	

It is sufficient to study the hydrolysis of **3** at one temperature only, here 50°C, and to stop monitoring after 30 h, where the dimethylester **2** is quantitatively converted into the monoester **4**. Reaction rate constants [l mol^{-1}h^{-1}] are found within a narrow expectation range: k_1 of **1** (0.1784, 50°C) is very close to k_1 of **3** (0.1665, 50°C). Corresponding data for constants [l mol^{-1} s^{-1}] are: $1.92{\cdot}10^{-6}$ (**1**, 25°C) $4.96{\cdot}10^{-5}$ (**1**, 50°C) and $4.63{\cdot}10^{-5}$ (**3**, 50°C). These results indicate, that the kinetic processes might not depend significantly on the substituent **R** attached to the NH group in the ArCH(NHR)P(O)$(OR)_2$-structure of aminophosphonates like **1** or **3** resp..

TABLE III Results for compound **3**. Temperature: 50°C evaluated according to Swinbourne

λ [nm]:	**317.848**	**321.178**	**327.836**	**330.332**	**331.996**	**485.109**	**488.402**	**495.810**
gradient: $S=e^{-k_1 \Delta l}$	0.9685	0.9393	0.9378	0.9404	0.9413	0.9412	0.9507	0.9496
axis segment x = 0:	0.0271	0.0500	0.0495	0.0473	0.0478	0.0534	0.0474	0.0408
R^2	0.9951	0.9979	0.9996	0.9998	0.9999	0.9999	0.9992	0.9996
$A_{\lambda,\infty}$	0.8619	0.8239	0.7960	0.7941	0.8138	0.9077	0.9602	0.8082
k_1 [l mol^{-1} h^{-1}]	9.60 10^{-2}	18.79 10^{-2}	19 28 10^{-2}	18.11 10^{-2}	18.16 10^{-2}	18 20 10^{-2}	15 20 10^{-2}	15.54 10^{-2}
k_1 [l mol^{-1} s^{-1}]	2.67 10^{-5}	5.22 10^{-5}	5.36 10^{-5}	5.12 10^{-5}	5.04 10^{-5}	5.06 10^{-5}	4 22 10^{-5}	4.32 10^{-5}
average k_1			16.65 10^{-2} l mol^{-1}h^{-1}			4.63 10^{-5} l mol^{-1}s^{-1}		
standard deviation σ_{n-1} of k_1			± 3.22 10^{-2} l mol^{-1} h^{-1}			± 8.9 10^{-6} l mol^{-1} s^{-1}		

TABLE IV Results for compound **1**. Temperature. 25°C evaluated according to the integrated time law

λ [nm]:	**266.121**	**271.174**	**281.617**	**286.174**	**296.184**
gradient $S=e^{-k_1\Delta t}$	0.0072	0.0071	0.0067	0.0065	0.0062
axis segment x = 0	0.7065	0.7055	0.7075	0.7099	0. 7078
R^2	0.9996	0.9993	0.9989	0.9968	0.9947
A:B	5.09	5.08	5.10	5.1 3	5.10
k_1 [l mol^{-1} h^{-1}]	$7.20\ 10^{-3}$	$7.15\ 10^{-3}$	$6.69\ 10^{-3}$	$6.50\ 10^{-3}$	$6.23\ 10^{-3}$
k_1 [l mol^{-1} s^{-1}]	$2.00\ 10^{-6}$	$1.99\ 10^{-6}$	$1.86\ 10^{-6}$	$1.80\ 10^{-6}$	$1.73\ 10^{-6}$
average k_1	$6.75\ 10^{-3}$ l $mol^{-1}h^{-1}$			$1.88\ 10^{-6}$ l $mol^{-1}s^{-1}$	
standard deviation σ_{n-1} of k_1	$\pm 4.17\ 10^{-4}$ l mol^{-1} h^{-1}			$\pm 1.16\ 10^{-7}$ l mol^{-1} s^{-1}	

TABLE V Results for compound **1**. Temperature: 50°C evaluated according to the integrated time law

λ [nm]:	**266.121**	**271.174**	**281.617**	**286.174**	**296.184**
gradient $S=e^{-k_1\Delta t}$	0.0651	0.0534	0.0464	0.0454	0.0438
axis segment x = 0	0.7199	0.8031	0.8038	0. 7824	0. 7686
R^2	0.9990	0.9998	0.9999	0.9999	1.0000
A:B	5.25	6.36	6.37	6.06	5.87
k_1 [l mol^{-1} h^{-1}]	$6.51\ 10^{-2}$	$5.34\ 10^{-2}$	$4.64\ 10^{-2}$	$4.54\ 10^{-2}$	$4.38\ 10^{-2}$
k_1 [l mol^{-1} s^{-1}]	$1.81\ 10^{-5}$	$1.48\ 10^{-5}$	$1.29\ 10^{-5}$	$1.26\ 10^{-5}$	$1.22\ 10^{-5}$
average k_1	$5.08\ 10^{-2}$ l $mol^{-1}h^{-1}$			$8.79\ 10^{-5}$ l $mol^{-1}s^{-1}$	
standard deviation σ_{n-1} of k_1	$\pm 1.41\ 10^{-3}$ l mol^{-1} h^{-1}			$\pm 2.11\ 10^{-7}$ l mol^{-1} s^{-1}	

From the kinetic data reported here it seem justified to conclude, that the rate of hydrolysis of phosphonate diesters into monoesters is low enough in alkaline solutions, at ambient temperatures, in order to allow accurate determinations of dissociation constants for **1** and **3** without the concomitant reaction to the corresponding monoesters. The dissociation constants were determined from solutions of **1** and **3** resp. in dioxane/water (1:1 v/v) by titration vs. NaOH (in dioxane/water, 1:1 v/v) at 25°C. Corresponding pK_a-data are calculated using the program ITERAX[13]: **1**: $pK_a = 5.77$, **3**: $pK_a = 6.01$. Those numerical values are characteristic for the protolytic equilibria of aromatic carboxylic acids.

TABLE VI Results for compound **3**. Temperature. 50°C evaluated according to the integrated time law

λ [nm]:	**317.848**	**321.1 78**	**327.836**	**330.332**	**331.996**	**485.109**	**488.402**	**495.810**
gradient $S=e^{-k_1\Delta l}$	0.6628	0.6838	0.6997	0.6990	0.7018	0.7184	0.7212	0.7266
axis segment x = 0	0.0809	0.0753	0.0631	0.0603	0.0591	0.0425	0.0425	0.0393
R^2	0.9982	0.9981	0.9995	0.9997	0.9998	0.9912	0.9977	0.9954
A:B	4.60	4.83	5.01	5.00	5.03	5.23	5.26	5.33
k_1 [l mol^{-1} h^{-1}]	8.09 10^{-2}	7.53 10^{-2}	6.31 10^{-2}	6.03 10^{-2}	5.91 10^{-2}	4.25 10^{-2}	4.25 10^{-2}	3.93 10^{-2}
k_1 [l mol^{-1} s^{-1}]	2.25 10^{-5}	2.09 10^{-5}	1.75 10^{-5}	1.67 10^{-5}	1.64 10^{-5}	1.18 10^{-5}	1.18 10^{-5}	1.09 10^{-5}
average k_1			5.79 10^{-2} l $mol^{-1}h^{-1}$			1.61 10^{-5} l $mol^{-1}s^{-1}$		
standard deviation σ_{n-1} of k_1			± 1.55 10^{-2} l mol^{-1} h^{-1}			± 4.31 10^{-6} l mol^{-1} s^{-1}		

Comparative studies using $^{31}P\{^1H\}$-NMR

At this stage of our studies we were tempted to look into the kinetics of esterolytic reactions using proton decoupled phosphorus NMR spectra because the resonance signals are very well separated from each other. To 111.6 mg of compound **1** in 0.3 ml dioxane / water (1:1 v/v) were added 1.7 ml of a 0.09925 m solution of NaOH in dioxane/water (1:1 v/v). In intervals of 30 min repeated $^{31}P\{^1H\}$-NMR spectra were recorded at ambient temperature and optimised integrals were calculated for both signals of the educt **1** (at $\delta p = 29.6$ ppm) and the product **2** (at $\delta p = 22.9$ ppm) as shown in Figures 5 and 6:

Kinetic evaluation of the NMR-intensities following the Swinbourne method

$$I_{t+\Delta t} = I_0 \cdot (1 - e^{-k_1 \Delta t}) + I_t \cdot e^{-k_1 \Delta t} \qquad (8)$$

yielded: $k_1 = 0206$ [l mol^{-1} h^{-1}] or $k_1 = 5.72\ 10^{-5}$ [l mol^{-1} s^{-1}] resp.. The rate constant obtained by the NMR-method (at ambient temperature) is not fully consistent with UVNIS-results (25°C) but comes very close to the rate constant determined at 50°C, because of the intrinsic warming up of the NMR probe by the decoupling technique.

Experimental

Synthesis

Amines, aldehydes, dimethyl- and diethylphosphite, as well as solvents and all other chemicals used were high purity commercial products from Aldrich. All syntheses were performed under a dry N_2 atmosphere. The Schiff base precursors were all prepared in high yield according to the procedure previously described[14]. Compounds **1** and **3** were synthesised by reaction between the Schiff bases and dimethylphosphite according to the following general procedure: to a stirred solution of the Schiff base precursor (0.1 mol) in a mixture of EtOH/dioxane (50 ml) were added dropwise 14 ml (0.15 mol) of $HP(O)(OMe)_2$ and a catalytic amount of NaH. After the addition was completed, the mixture was stirred for a few hours. The solvent was then evaporated and the solid formed was filtered off.

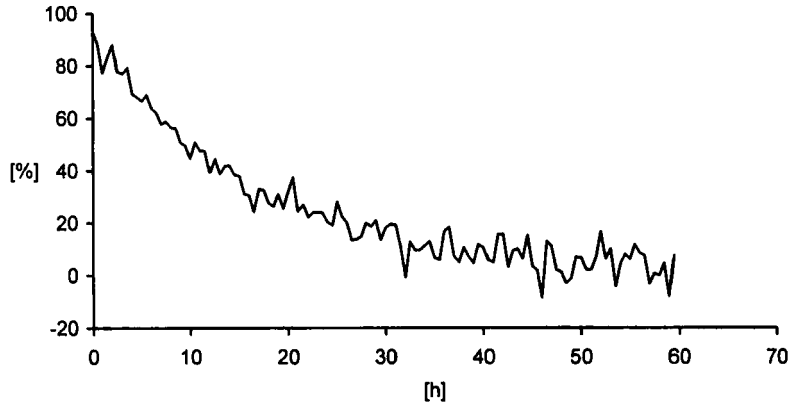


FIGURE 5 Time dependend intensity of the decreasing signal of **1** at δp = 29.6 ppm

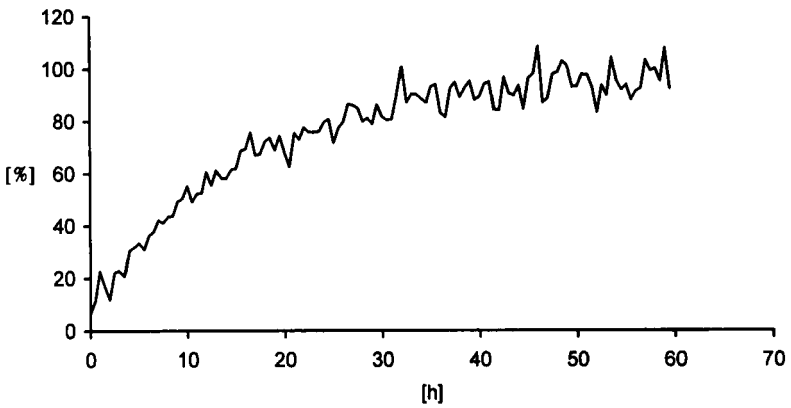


FIGURE 6 Time dependend intensity of the increasing signal of **2** at δp = 22.9 ppm

Compound **1**: white solid, recrystallised from dioxane/ethylacetate; m.p. 198–199°C; yield 81%. ^{1}H-NMR δ ($CDCl_3$, TMS): 1.00 (s, 9H, CH_3); 3.55 (d, $^3J_{PH}$ = 10.6 Hz, 3H, OCH_3); 3.82 (d, $^3J_{PH}$ = 10.4 Hz, 3H, OCH_3); 4.30 ($^2J_{PH}$ = −26.2 Hz, 1H, CH); 7.56 (dd, 2H, ArylH) and 8.05 (d, $^3J_{HH}$ = 8 Hz, 2H, ArylH).

Compound **3**: red-orange solid, recrystallised from dioxane; m.p. 205–207°C; yield 63%. ^{1}H-NMR δ ($CDCl_3$, TMS): 3.55 (d, $^3J_{PH}$ = 10.8 Hz, 3H, OCH_3); 3.84 (d, $^3J_{PH}$ = 10.8 Hz, 3H, OCH_3); 5.00 ($^2J_{PH}$ = –24.8 Hz, 1H, CH); 5.83 (br. s, J_{NH} = 1 Hz, 1H, NH); 6.72 (d, $^3J_{HH}$ = 8.8 Hz, 2H, ArylH); 7.45 (m, 3H, ArylH); 7.63 (m, 2H, ArylH); 7.78 (m, 4H, ArylH) and 8.12 (d, $^3J_{HH}$ = 8.8 Hz, 2H, ArylH). Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected.

NMR

^{1}H-NMR spectra were recorded in $CDCl_3$ with Me_4Si as an internal standard using a Bruker AC-200 instrument operating at 200 MHz. For kinetical studies ^{31}P-NMR-spectra were integrated using the two characteristic signals for **1** and **2** respectively.

UV-VIS

The instrumentation is based upon principles described previously under [8–11] using the titration system TPC2000 from Schott Geräte GmbH, Hofheim, Germany, interfaced with a UV-VIS-spectrometer MCS 320 and an immersion sonde TSB from Zeiss, Oberkochem. Germany. Further materials: IBM-compatible computer, EPSON-FX85-compatible printer, motor burette T100 (Schott)), interface TR250 (Schott) and a stirring motor TM125 (Schott). A home made titration vessel was used. The temperature in the system was kept constant (±0.1°C) by means of a thermostat. All components and procedures were controlled by the computer program TR600 (SCHOTT).

Sample preparation for UV-VIS

0.05 mol of **1** are dissolved in 90 ml bi-dist. water. 10 ml of a 1.0 m solution of NaCl are added as ion buffer. 0.05 mol of **3** is dissolved in a mixture of dioxane-water (1:1 v/v) to make up a total volume of 100 ml.

Monitoring UV-VIS

In intervals of 30 (for **1**) or 20 (for **3**) minutes during a period of 100 hours spectra were recorded automatically. After the complete dissolution of **1** or **3** the first spectrum is used as reference for difference spectroscopy (see text). Then the alkaline reagent is added: for **1**: 2.50 ml, for **3**: 5.00 ml of a 0.1000 m solution of NaOH in water.

Summary

The hydrolysis of dimethylesters derived from aminophosphonic acids bearing a carboxylic group is slow and does not interfere with the determination of dissociation constants via potentiometric titrations.

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