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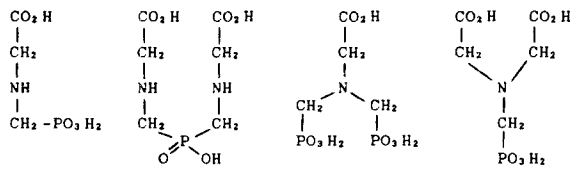
ORGANOPHOSPHORUS HERBICIDES AND PLANT GROWTH REGULATORS PART 1. SYNTHESIS AND PROTONATION BEHAVIOUR OF GLYPHOSATE AND RELATED COMPOUNDS

M .A. DHANSAY, P. W. LINDER AND R. G. TORRINGTON
Department of Chemistry, University of Cape Town, Rondebosch 7700, South Africa
T. A. MODRO
Department of Chemistry, University of Pretoria, Pretoria 0002, South Africa

Ionization constants of *N*-phosphonomethylglycine (glyphosate) and three structurally related plant-growth regulators were determined by potentiometric titration. The effect of pD on the chemical shifts of the ^{1}H and ^{13}C nuclei of the methylene groups and of the ^{31}P nucleus in these substrates was measured by NMR spectroscopy. The results allowed an assignment to be made of a specific site (carboxylic, phosphonic, ammonium) in the molecule to each of the determined pK_a values.

INTRODUCTION

N-Phosphonomethyl (and *N*-phosphinomethyl) derivatives of glycine find wide application as herbicides and stimulators of plant growth processes.[1] *N*-Phosphonomethylglycine (glyphosate, **1**) itself inhibits the enzymatic conversion of shikimic acid to anthranilic acid,[2] and its thiocarbamate[3] or *C*-sulphonamide[4] derivatives show herbicidal activity. The same activity has been found[5] in the aminotriazolinium salts of **1** and of *N*,*N*-bis(phosphonomethyl)glycine (glyphosine, **3**) and *N*-(phosphonomethyl)-*N*-(carboxymethyl)glycine (**4**). Amine salts and esters of di[*N*-(carboxymethyl)aminomethyl]phosphinic acid (**2**) show fungicidal, herbicidal and plant growth-regulating properties.[6]



1 **2** **3** **4**

Compounds **1**–**4** belong to the family of mixed carboxylic–phosphonic amino acids, and can contain up to five acidic centres (CO_2H, PO_3H_2, NH_2^+). Although the pK_a values for some of these substrates have been determined previously, little information is available on the sites and the sequence of the protonation equilibria in these multi-centred systems. In connection with our studies[7] on the metal ion complexation behaviour of biologically active organophosphorus compounds, we decided to determine ionization constants for the acidic groups in **1**–**4** by potentiometric study, and to assign the individual protonation sites by measuring for each substrate the effect of acidity on their NMR spectra. NMR spectroscopy involving chemical shift measurements has been used in protonation studies with a wide variety of weak bases,[8] and offers a number of advantages over spectrophotometric techniques. Compounds **1**–**4** are particularly well suited for NMR study, since the protonation equilibria can be probed by following the chemical shift changes in the ^{1}H, ^{13}C and ^{31}P NMR spectra. The absence of π-bonds in the molecular framework of the compounds studied should minimize the magnetic anisotropy contributions, and the presence of a formal charge(s) developed near the magnetic nuclei should lead to a substantial deshielding effect(s).[9] Appleton *et al.*[10] successfully applied NMR to the study of the acid–base equilibria of aminoalkylphosphonic acids, and observed both large variations in the chemical shifts of the individual nuclei with pD, and well defined inflection points on the titration curves. The same group, in a study of the Pt^{II} complexes of amino- and iminophosphonic acids, determined the plots of δ_P, δ_C and δ_H against pD for substrate **1**, and interpreted their results in terms of the sequential

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deprotonations of the individual acidic centres in **1**.[11] We decide to repeat Appleton's measurements on **1** in order to confirm the applicability of the method to this and the related systems **2**, **3** and **4**.

RESULTS AND DISCUSSION

Substrates **1**–**4** were prepared according to the procedures available in the literature; some modifications were introduced in a few cases to improve the yields and purity of the products. Full details are given under Experimental: the syntheses were based on the following reactions.

N-Phosphonomethylglycine (**1**) was prepared according to equation (1), by addition of diethyl phosphite to the trimer[12] of methyleneaminoacetonitrile (prepared[13] from formaldehyde, NaCN and NH_4Cl), followed by hydrolysis of the nitrile and ester functions[14] and purification of the final product by ion-exchange chromatography.

$$1/3(CH_2{=}NCH_2CN)_3 + (EtO)_2P(O)H \xrightarrow{\text{i, ii}} \mathbf{1} \quad (1)$$

(i) dry HCl, room temp.; (ii) conc. HCl, reflux.

Phosphinic derivative **2** was prepared from *N*-benzylglycine[15] by condensation[16] of its hydrochloride with formaldehyde and hypophosphorous acid according to equation (2), to give the bis(*N*-benzyl) derivative (**2a**). Debenzylation of **2a** to **2** was then achieved by catalytic hydrogenation.[16]

$$2PhCH_2NHCH_2CO_2H + 2CH_2O + H_3PO_2 \rightarrow \underset{\textit{2a}}{[HO_2CCH_2N(CH_2Ph)CH_2]_2PO_2H} \quad (2)$$

Synthesis of glyphosine (**3**) was based on the condensation of glycine with formaldehyde and phosphorous acid in strongly acidic solution [equation (3)].[17]

$$H_2NCH_2CO_2H \xrightarrow{\text{i, ii}} \mathbf{3} \quad (3)$$

(i) $2H_3PO_3$, conc. HCl, reflux; (ii) $4CH_2O$, reflux

The same method, starting with iminodiacetic acid, was used for the preparation of **4**.

Protonation

The potentiometric method used is described under Experimental. The protonation constants found are listed in Table 1, together with the available literature values. With a few exceptions, the values obtained for the dissociation constants agreed well with the data reported previously.

NMR (^1H, ^{13}C, ^{31}P) spectra of substrates **1**–**4** showed only the presence of the signals expected from their structures; all peaks appear as sharp singlets or doublets (due to the coupling to the ^{31}P nucleus). Since the NMR 'titrations' were performed in D_2O, only the signals of the methylene groups appeared in the PMR spectra. The effect of pD was measured using the signals of the CH_2 groups (in the ^1H and ^{13}C NMR spectra) and the ^{31}P signals in the pD range 1–13. Owing to the reduced sensitivity of the carboxylate carbon atoms in the ^{13}C NMR spectra, together with solubility limitations for some of the substrates, we did not include the signals of the carboxylic carbons in our plots. Within this range of acidity, significant changes in the chemical shifts were observed: 0·5–1·1 ppm for ^1H, 3·8–7·5 ppm for ^{13}C and 7–19 ppm for ^{31}P NMR spectra.

Figure 1 shows the effect of pD on the chemical shift of both methylene groups (^1H and ^{13}C atoms) and the phosphorus atom in **1**. The results obtained are in excellent agreement with those reported previously[11] for this substrate. In the vicinity of the first pK_a value

Table 1. Acid dissociation constants and standard deviations (s.d.) in H_2O, $I = 0{\cdot}1$ M $[Na^+][Cl^-]$, 25·0°C[a]

Dissociation constant	Substrate			
	1	**2**	**3**	**4**
pK_1	2·11 (2·32[18])	2·10	1·42 (1·7[17])	1·25 (2·00[19])
S.d.	0·02(6)	0·02(4)	0·01(0)	0·06(0)
pK_2	5·42 (5·86[18])	6·55	2·10 (2·0[17])	2·28 (2·25[19])
S.d.	0·01(1)	0·00(8)	0·00(6)	0·02(1)
pK_3	10·06 (10·86[18])	9·00	5·02 (5·1[17])	5·61 (5·57[19])
S.d.	0·00(6)	0·00(5)	0·00(4)	0·01(3)
pK_4			6·40 (6·45[17])	10·35 (10·76[19])
S.d.			0·00(4)	0·00(8)
pK_5			11·19 (10·98[17])	
S.d.			0·00(3)	

[a] Literature data (where available) are given in parentheses.

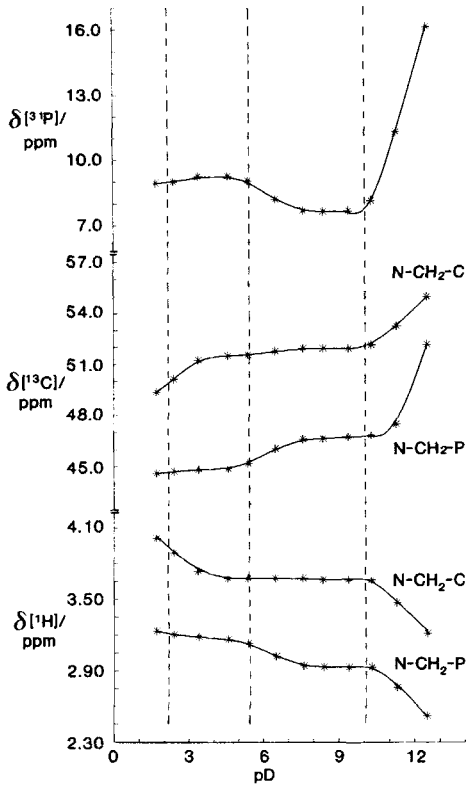


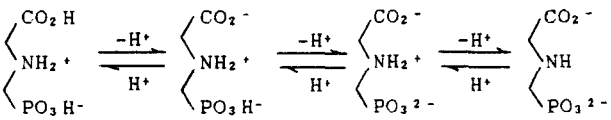
Figure 1. Effect of pD on chemical shift of nuclei in **1**

(2·11), the chemical shift of the ^{1}H and ^{13}C nuclei of the phosphonomethyl CH_2 group and that of ^{31}P remain constant, whereas the ^{1}H and ^{13}C nuclei of the glycine methylene group undergo significant shielding and deshielding, respectively. Deprotonation of acidic functions usually causes downfield shifts of the signals of neighbouring carbon atoms,[20] and these changes have been explained[21] as a result of competition between the deshielding effect of a decrease in excitation energy and the shielding effect of an increase in electron density on deprotonation. The shielding of the ^{1}H nuclei on the first deprotonation is *ca* −0·4 ppm, whereas the deshielding of the methylene carbon is *ca* +3 ppm, in agreement with the corresponding average values of −0·21 and +3·5 ppm observed for the α-methylene groups on ionization of aliphatic carboxylic acids.[22] The observed effects allowed us to conclude that the first deprotonation of **1** occurs at the carboxylic group.

At the second pK_a value (5·42) the singlets of the glycine CH_2 group do not change their chemical shift values, whereas the ^{1}H and ^{13}C nuclei of the CH_2P group are affected, giving rise to a *ca* −0·3 ppm shielding and *ca* 2 ppm deshielding, respectively. This result clearly indicates that the second deprotonation of **1** involves the phosphonic group, which is changing from the mono- to the dianionic form.

The third deprotonation of **1** (pK_a 10·06) involves the nitrogen atom: ^{1}H nuclei of *both* methylene groups undergo significant shielding (−0·4 to −0·5 ppm), and *both* ^{13}C nuclei significant deshielding (+3·5 to +5·5 ppm). The latter shifts can be compared with an average value of *ca* +3 ppm reported[23] for the α-carbon atom on deprotonation of the primary alkylammonium ions.

The effect of pH on the shielding parameters of the ^{31}P nucleus in **1** is more complex. Around the second pK_a value weak shielding (*ca* −1·5 ppm) was observed, whereas near the third ionization strong deshielding (*ca* +8·5 ppm) of the ^{31}P nucleus was obtained. Although the second deprotonation of orthophosphoric acid results in a ^{31}P low-field shift of *ca* 3 ppm,[24] the presence of a highly positive active site in the vicinity of the phosphate group is known[25] to change the $\delta(^{31}P)$ vs pH dependence of this group. We suggest that although the second deprotonation of **1** involves the PO_3H^- group, the effect of the charge is mostly neutralized by strong intramolecular hydrogen bonding of the adjacent NH_2^+ group. Only on the third deprotonation is the 'free' PO_3^{2-} group released, and that has a strong effect on the ^{31}P shielding. The deprotonation behaviour of **1** (represented in its true zwitterionic form) can therefore be illustrated by the equation



1 (4)

For substrate **2**, because of the variation in solubility with acidity, no reliable ^{13}C NMR data could be obtained over the whole range of pD. Figure 2 shows the variations of the ^{1}H and ^{31}P chemical shifts with pD for **2**.

As in **1**, the first ionization (pK_a 2·10) involves one of the carboxylic groups, since it is the glycine, not phosphinic, methylene protons which experience shielding effects (*ca* 0·2 ppm).

When approaching the second ionization (pK_a 6·55), both types of methylene protons (glycine *and* phosphinic) undergo shielding effects, whereas the ^{31}P signal begins to move downfield. This behaviour is consistent with the second deprotonation occurring at one of the nitrogen atoms and affecting the shielding parameters of all the nuclei studied.

The third ionization (pK_a 9·00) involves the deprotonation of the second NH_2^+ group and results in further shielding of the methylene groups, and also in a dramatic (*ca* +19 ppm) deshielding of the ^{31}P atom.

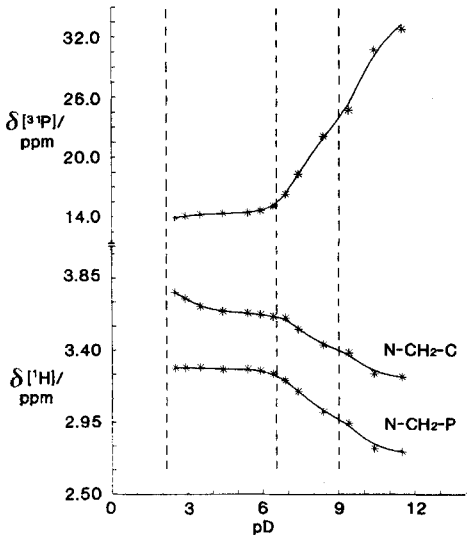
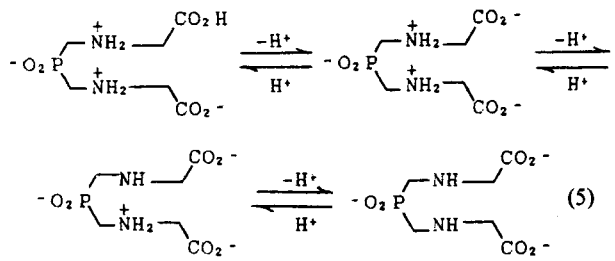


Figure 2. Effect of pD on chemical shift of nuclei in **2**

This latter effect, which necessarily cannot involve the ionization of the POH group (phosphinic substrate), supports our conclusion about the strong deshielding of phosphorus caused by the deprotonation of an adjacent ammonium centre. The deprotonation sequence for **2** is presented in equation (5).



Substrate **3** can undergo five consecutive ionizations, and the effects of pD on the chemical shift of its methylene ^{1}H and ^{13}C nuclei and the ^{31}P atom are shown in Figure 3.

The first deprotonation (pK_a 1·42) involves the non-ionized phosphonic group, resulting in weak shielding and deshielding of the ^{1}H and ^{13}C nuclei of the phosphonomethyl groups. It is interesting that this ionization has no effect on the ^{31}P chemical shift, probably owing to the compensating effects of the intramolecular hydrogen bonding. The next ionization occurs at the carboxyl group, as can be seen from the effect on the chemical shift of the glycine methylene group. The next two ionizations (pK_a 5·02 and 6·40) involve both phosphonic groups; the chemical shifts of the glycine moiety remain constant, but the CH_2 atoms

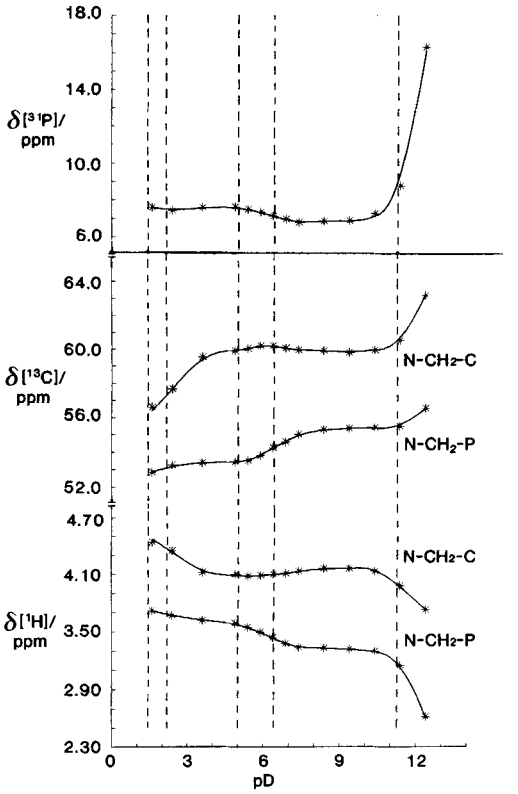
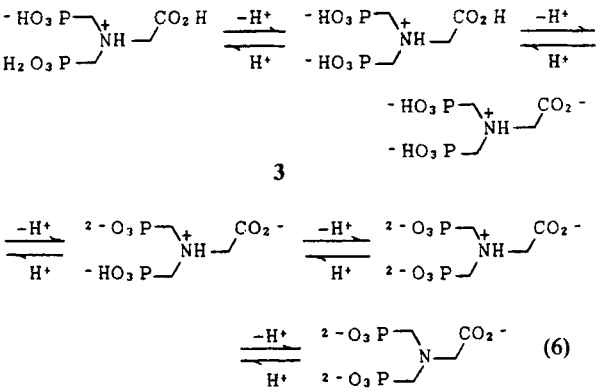
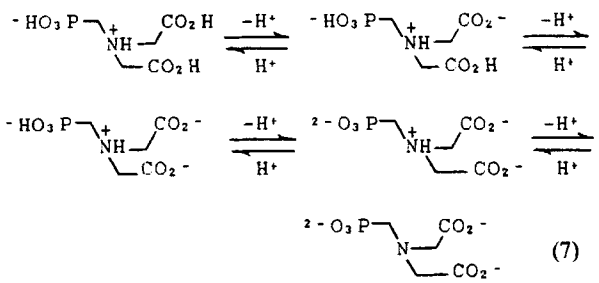


Figure 3. Effect of pD on chemical shift of nuclei in **3**

of the phosphonic part undergo further shielding and deshielding effects (−0·3 and +2 ppm, respectively). The least acidic hydrogen atom of the NH^+ group undergoes ionization at pH 11·9, causing strong shielding (−0·4 and −0·7 ppm) of *all* methylene protons, deshielding (+3 and +1·5 ppm) for *all* methylene carbons and strong deshielding (+10 ppm) of the ^{31}P nuclei. The ionization of **3** is shown in equation (6).



As with **2**, owing to solubility problems with **4**, ^{13}C NMR spectroscopy did not produce reliable results over the whole pD range. Substrate **4** is the strongest acid in the series and sufficient chemical shift data could not be obtained in the vicinity of the first dissociation (Figure 4). The effect of the second deprotonation (pK_a 2·28) on the chemical shift is also not clear; it seems to affect all the nuclei studied only weakly. The effect of the third deprotonation is obvious, however: the chemical shift of the glycine methylene protons remains constant, whereas for the CH_2P group a distinct inflection curve is obtained ($\Delta\delta = -0{\cdot}26$ ppm) with a pK_a value corresponding well with that (5·61) determined potentiometrically. This deprotonation therefore corresponds to the ionization of the monoanionic phosphonic group. The last ionization (pK_a 10·35), as before, affects the chemical shift of all nuclei studied in the expected manner, and corresponds to the deprotonation of the nitrogen atom. The protonation behaviour of **4** is presented in equation (7).



(7)

4

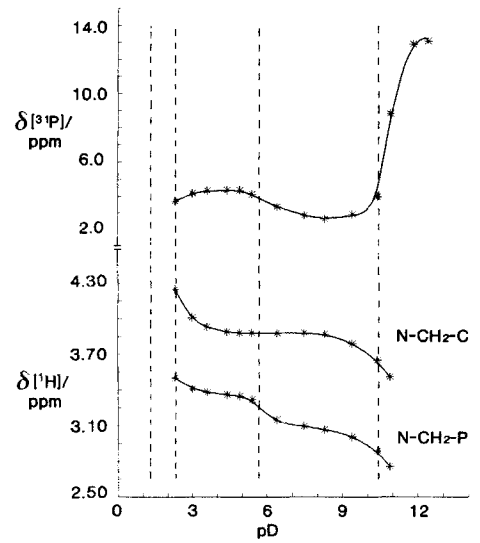


Figure 4. Effect of pD on chemical shift of nuclei in **4**

In conclusion, we confirm that NMR spectroscopy offers a successful method for studies on the protonation behaviour of multibasic systems, and complements well the more quantitative potentiometric data. For such multifunctional, branched systems as the plant growth regulators **1**–**4**, a knowledge of the detailed structure of the ionic form of each substrate at a given pH should be helpful in predicting the complexation properties and selectivity with respect to metal ions present in the investigated system.

EXPERIMENTAL

All solvents and commercially available reagents were purified by conventional methods before use. Deuterium oxide (Merck, min. 99·75%), sodium deuteroxide (Wilmad Glass, 40% in D_2O, min. 99% D) and deuterium chloride (Nuclear Magnetic Resonance Ltd, 20% in D_2O, min. 99% D) were used to prepare solutions for NMR measurements. pD was measured by means of a Radiometer pH Meter 29 pH meter fitted with a Radiometer GK 2401C combination electrode, standardized against a Beckman pH 4.00 buffer solution. Hence pD was given by pD = meter reading + 0·40.[2] The NMR (^1H, ^{13}C, ^{31}P) spectra were recorded on a Varian VXR-200 superconducting FT spectrometer at a probe temperature 23·0 °C, using dioxane (^1H, ^{13}C) or trimethyl phosphate (^{31}P) as internal standards. Elemental analyses (C, H, N) were obtained using a Heraeus Universal combustion analyser.

Preparation of substrates

N-Phosphonomethylglycine (**1**). A 12·2 g (0·176-mol) amount of the trimer of methyleneaminoacetonitrile (prepared according to Ref. 13) was dissolved in 48 ml (0·372 mol) of freshly distilled (b.p. 84–87 °C/12 mm Hg) diethyl phosphite and dry HCl was passed with stirring into the solution for 3 h at room temperature. The resulting precipitate was filtered off, washed with cold diethyl ether, dried and recrystallized from methanol to give the hydrochloride salt of *N*-(diethylphosphonomethyl)aminoacetonitrile, 23·0 g (54%); m.p. 136–138 °C. ^1H NMR (D_2O): δ 1·37 (6H, t, 2 × CH_3), 3·68 (2H, d, J_{HP} 14 Hz, CH_2P), 4·28 (2H, s, CH_2CN), 4·28 (4H, d of q, 2 × CH_2O). Analysis: calculated for $C_7H_{16}N_2O_3PCl$, C 34·60, H 6·60, N 11·55; found, C 34·55, H 6·35, N 11·70%.

A 10 g (0·041-mol) amount of this product was dissolved in 120 ml of concentrated HCl and the solution was heated under reflux for 6 h. The solvent was removed on a rotary evaporator, yielding 10·3 g (97%) of a 1 : 1 mixture (as determined by elemental analysis) of **1**·HCl and ammonium chloride. A 5-g amount of this mixture was dissolved in water (50 ml) and the solution was passed through a cation-exchange column

(Amberlite IR-12H). Two bed volumes of deionized water were used to elute the product. The solvent was removed on a rotary evaporator, yielding **1**, 2·4 g (60%); m.p. (decomp.) 220–225 °C. ^{1}H NMR (D_2O): δ 3·26 (2H, d, J_{HP} 14 Hz, CH_2P), 3·99 (2H, s, CH_2CO_2). Analysis: calculated for $C_3H_8NO_5P$, C 21·30, H 4·70, N 8·20; found, C 21·25, H 4·70, N 8·25%.

Di-[N-(carboxymethyl)aminomethyl]phosphinic acid (**2**). *N*-Benzylglycine (prepared according to Ref. 15) was reacted with hypophosphorus acid and formaldehyde according to Ref. 16, yielding the hydrochloride salt of di[*N*-(carboxymethyl)-*N*-benzylaminomethyl]phosphinic acid, yield 74%; after crystallization (EtOH), 59%; m.p. 200–205 °C (lit.[16] m.p. 211–214 °C). ^{1}H NMR (D_2O): δ 3·50 (4H, d, J_{HP} 11 Hz, 2 × CH_2P), 4·08 (4H, s, 2 × CH_2CO_2), 4·60 (4H, s, 2 × CH_2Ph), 7·54 (10H, s, 2 × Ph). Analysis: calculated for $C_{20}H_{25}N_2O_6P \cdot HCl$, C 52·6, H 5·7, N 6·1; found, C 51·8, H 5·3, N 6·0%. This product was then debenzylated according to the procedure given in Ref. 16, yielding **2** (42%); m.p. (decomp.) 272–278 °C. ^{1}H NMR (D_2O): δ 3·88 (4H, d, J_{HP} 14 Hz, CH_2P), 3·88 (4H, s, CH_2CO_2). Analysis: calculated for $C_6H_{13}N_2O_6P$, C 30·0, H 5·42, N 11·67; found, C 29·75, H 5·50, N 11·50%.

N,N-Bis(phosphonomethyl)glycine (**3**).[17] A 17·2-g (0·20-mol) amount of 97% phosphorous acid was added to the solution of glycine (7·5 g, 0·10 mol) in a mixture of water (15 ml) and concentrated HCl (20 ml) and the solution was heated under reflux. To this refluxing solution, 35·0 ml (0·41 mol) of aqueous formaldehyde (35%) were added dropwise and the refluxing was continued for a further 1 h. The mixture was cooled, filtered and the filtrate was evaporated under reduced pressure, yielding a viscous oil that crystallized after addition of a small volume of ethanol. The crystalline material was filtered off and washed with cold ethanol to give **3**, 26·3 g (66%); m.p. (decomp.) 189–193 °C. ^{1}H NMR (D_2O): δ 3·72 (4H, d, J_{HP} 14 Hz, 2 × CH_2P), 4·44 (2H, s, CH_2CO_2). Analysis: calculated for $C_4H_{11}NO_8P_2$, C 18·25, H 4·20, N 5·30; found, C 18·25, H 4·20, N 5·30%.

N-(Phosphonomethyl)-N-(carboxymethyl)glycine (**4**). This product was prepared similarly to **3**, starting with 20·1 g (0·151 mol) of iminodiacetic acid, 12·4 g (0·147 mol) of 97% phosphorous acid and 26 ml (0·328 mol) of 35% formaldehyde. After the work-up, 24·1 g (72%) of pure **4** were obtained; m.p. (decomp.) 214–218 °C. ^{1}H NMR (D_2O): δ 3·56 (2H, d, J_{HP} 14 Hz, CH_2P), 4·30 (4H, s, 2 × CH_2CO_2). Analysis: calculated for $C_5H_{10}NO_7P$, C 26·43, H 4·40, N 6·17; found, C 26·40, H 4·35, N 6·15%.

Potentiometry

Sodium hydroxide solutions (0·05 M) were freshly prepared at frequent intervals by dilution of the contents of Merck ampoules under nitrogen and standardized against potassium hydrogenphthalate (Merck). Hydrochloric acid (0·01 M) was also prepared by the use of Merck ampoules and standardized against sodium hydroxide.

Boiled-out, glass-distilled water was used to prepare the solutions. Sodium chloride (BDH, Aristar grade) was added during the preparative stage to the above-mentioned sodium hydroxide solutions and hydrochloric acid so as to produce solutions with a total chloride concentration of 0·1 M.

To determine the acid dissociation constants for **1–4**, weighed quantities of the substrates were placed in a Metrohm EA876-20 double-walled titration vessel. Accurately measured volumes of the 0·01 M hydrochloric acid/chloride solution were added so as to produce a substrate concentration in the range 2–12 mM. The titration vessel was fitted with a Metrohm EA109 glass electrode and a Metrohm EA404 calomel electrode containing saturated sodium chloride. The vessel was thermostated by circulating water at 25 °C.

Potentiometric titrations were carried out by delivering the sodium hydroxide/chloride solution from a Radiometer ABU80 Autoburette controlled by a PEP computer, which also recorded the volume delivered and the e.m.f. of the cell as measured by a Radiometer PHM64 pH meter. During the titration a purified nitrogen atmosphere was maintained in the titration vessel. The data obtained were used to calibrate the electrodes and determine the protonation constants simultaneously. This was done by applying the data on the OBJE task of ESTA[27] with the weight at each titration point based on a standard deviation of the titre of 0·005 cm^3 and of the e.m.f. of 0·1 mV.

NMR measurements. Solutions of substrates **1–4** in D_2O (0·03–0·10 M) were prepared and their pD was adjusted by addition of the required quantity of NaOD–D_2O or DCl–D_2O solution. For a given solution, the chemical shifts for individual nuclei remained constant within the solute's concentration range given above. Chemical shifts were measured relative to internal standards. It should be noted that since the pK_a values of dioxane and trimethyl phosphate are of the order of −3 and −4, respectively,[28] any protonation of the standards in the pH range studied is negligible.

ACKNOWLEDGEMENTS

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