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Crystal Structures of Two Salts of *N*-(Phosphonomethyl)glycine and Equilibria with Hydrogen and Bicarbonate Ions

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Received July 22, 1988

Glyphosate (*N*-(phosphonomethyl)glycine, H_3L) is a commercial herbicide in very extensive worldwide use. In part its utility derives from its immobilization upon contact with soil (and presumed complexation to metal ions) and its water solubility in a number of salts. The underlying physical properties that relate to this behavior are examined in this paper. There are three acid/base functional groups of glyphosate (phosphonate, amine, and carboxylate); the neutral molecule (H_3L) is a zwitterion in which a proton transfers from the phosphonic acid to the amine. The protonation constant for the reaction $H_3L + H^+ = H_4L^+$ has been determined from NMR data as $K_{-1} = 0.494$ M^{-1}. The reaction with bicarbonate in solution to form the carbamate, $L^{3-} + HCO_3^- = [L(-H)]CO_2^{4-} (+H_2O)$, is found to have an equilibrium constant of 1.15 M^{-1}. Two glyphosate salts of commercial importance are those of isopropylammonium (IPA) and trimethylsulfonium (TMS). The structures of $[IPA][H_2L]$ and $[TMS][H_2L]$ are found to be very similar, including the conformation of the anion. In the IPA salt there is hydrogen bonding between the cation and anion whereas the TMS salt only has intermolecular hydrogen bonds involving glyphosate anions. The IPA salt, **1**, crystallizes in space group $P2_1$ with $a = 4.8747$ (7) Å, $b = 8.9860$ (5) Å, $c = 11.5338$ (8) Å, and $\beta = 92.97$ (1)°. With $Z = 2$, $d_{calc} = 1.50$ and $d_{obs} = 1.50$ g cm^{-3}. For 667 data with $F^2 > 3\sigma(F^2)$, $R_w = 2.81\%$. The TMS salt, **2**, crystallizes in space group $P2_1/n$ with $a = 10.0547$ (11) Å, $b = 7.4483$ (9) Å, $c = 15.1523$ (15) Å, and $\beta = 108.29$ (1)°. With $Z = 4$, $d_{calc} = 1.51$ and $d_{obs} = 1.51$ g cm^{-3}. For 1132 data with $F^2 > 3\sigma(F^2)$, $R_w = 4.08\%$.

Introduction

N-(Phosphonomethyl)glycine (glyphosate) has become widely used as a commercial herbicide since its discovery in the 1970s.[1] Its commercial success stems in part from its specificity for green plants, its nontoxicity to animals, and its biodegradability. Pure glyphosate is a zwitterionic neutral molecule that is only slightly soluble in water,[1] thereby significantly limiting its range of applications. The salts of the monoanion of glyphosate, however, are readily soluble in water, and the commercial products are prepared as such. While the structure of the zwitterionic free acid, $HO_2CCH_2NH_2^+CH_2PO_3H^-$, has been determined,[2] relatively few simple salts of glyphosate have been structurally characterized. The solid-state structures of the calcium double salt, $Ca(H_2L)_2(H_3L)_2{\cdot}H_2O$,[3] and the relatively insoluble 1:1 calcium salt, Ca(HL), have been reported.[4] The effect of the cation and hydrogen bonding on the solid-state structure and conformation of the molecule will be one of the topics explored in this paper.

Metal ions play a role in the soil immobilization of the herbicide, and therefore the complexation properties of glyphosate have been the subject of particular interest. Most of the solution studies of glyphosate have been pH potentiometric titrations: a study by Wauchope et al.[5] first examined the acid solution equilibria; subsequently, Madsen et al.[6] determined the stability constants of the divalent complexes ML^- ($M = Cu^{2+}$, Zn^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}; L = glyphosate trianion) and redetermined the protonation constants. Glass[7] qualitatively examined the solution complexation with Cu^{2+} but was apparently unaware of the prior studies. More recently, Motekaitis and Martell[8] redetermined the protonation constants of the glyphosate ligand, redetermined the stability constants for the ML^- species, and examined other divalent as well as trivalent metal ion complexes. We have redetermined the calcium ion binding constants using calcium ion specific potentiometry.[4] Although pH potentiometry is the standard technique for complexation studies and is quite powerful, it is limited to determining stoichiometries and equilibrium constants under conditions where hydronium ions are evolved or consumed. Nuclear magnetic resonance (NMR) can be used to complement such studies and reveal structural information, such as protonation sites and geometric details. Recently, an NMR study of the platinum complexes of glyphosate[9] has presented the first information about the solution structure of a glyphosate metal complex.

The presence of carbon dioxide in the atmosphere raises the question of whether carbonate affects the solution chemistry of glyphosate. It is found that the secondary amine reacts with bicarbonate at high pH to form a carbamate species, similar to a number of other carbamates derived from amines or amino acids.[10-16]

We present here an attempt to elucidate some of the structural and chemical properties of this important herbicide. We have prepared single crystals of isopropylammonium (IPA) glyphosate (**1**) and trimethylsulfonium (TMS) glyphosate (**2**) and determined the molecular structures of these compounds by X-ray diffraction. We have also used NMR spectroscopy to examine the solution equilibria of glyphosate as follows: (1) The ^1H, ^{13}C, and ^{31}P chemical shifts have been measured as a function of pH in the presence of TMS^+ and IPA^+, and the protonation sites have been determined directly. (2) The ^{31}P chemical shifts have been used to determine the protonation constant for the formation of H_4L^+, a species that has previously remained unmentioned and yet exists at significant concentrations up to pH = 2. (3) The formation of a carbamate species in the presence of carbonate at high pH has been confirmed, and its formation constant has been determined.

Experimental Section

Materials. All solutions were made with D_2O that was 99.9 atom % D (Cambridge Isotope Laboratories). Glyphosate (Monsanto, 99.9%) and trimethylsulfonium iodide (Aldrich, 98%) were used without further purification. Isopropylamine (Aldrich, 99%) and tributylamine (Aldrich, 99%) were freshly distilled from sodium (under vacuum for the latter).

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0020-1669/89/1328-2052$01.50/0

The pH measurements were made on a Fisher Accumet pH meter (Model 825 MP), which was calibrated in aqueous buffers of pH = 4.01 and 7.00 immediately before use (efficiency 0.99). For the D_2O solutions the meter readings were corrected to pD by using the equation pD = (pH meter reading) + 0.40.[17]

Preparation of Isopropylammonium Glyphosate. *N*-(Phosphonomethyl)glycine (10 g, 59 mmol) was placed into a 100-mL round-bottom flask together with 50 mL of H_2O. Isopropylamine (5.0 mL, 3.5 g, 59 mmol; previously refluxed over Na for 21 h and distilled) was added to the stirred suspension. Upon addition of the amine, the glyphosate solution was warmed to ≃30 °C at which point ≃80% of the glyphosate dissolved. A clear solution was obtained by adding another 0.5 mL of the amine and stirring for 1 h. The product was rotoevaporated to dryness, leaving a clear colorless oil. This oil was recrystallized from methanol/ether by cooling to −26 °C, and the resulting hygroscopic white powder was dried over night at room temperature under vacuum (≃3 g). Anal. Calcd (found): C, 31.58 (30.68); H, 7.51 (7.85); N, 12.10 (11.46). The microanalytical data are consistent with a small amount of water absorption by the salt during sample preparation. This salt dissolves in MeOH and water but only slightly in dioxane or EtOH.

Preparation of Trimethylsulfonium Glyphosate. The 1:1 salt was prepared by the addition of tributylamine (18.5 g; distilled from Na and stored under N_2) and trimethylsulfonium iodide (20.4 g) to glyphosate (16.9 g) and subsequent extraction of tributylammonium iodide into methylene chloride. Since some of the glyphosate did not dissolve (≃5%) upon addition of the amine, the water solution was filtered before extraction. The water layer was initially extracted with 70 mL of methylene chloride and subsequently washed with four 50-mL portions. The water solution was concentrated by rotoevaporation to a partially solidified white oil, which was dried under vacuum for 12 h. The oil was dissolved in ≃30 mL of MeOH, and 2 mL of H_2O was added. This solution was cooled to −26 °C overnight, resulting in the formation of a microcrystalline hygroscopic solid, which was recrystallized as described below and characterized by single-crystal X-ray diffraction.

Crystal Structure Determinations. Suitable crystals of **1** with the formula $[(CH_3)_2CHN^+H_3][HO^-(O)_2PCH_2N^+H_2CH_2COO^-]$ were obtained from a saturated aqueous solution of a 1:1 glyphosate/isopropylamine mixture at room temperature upon addition of some seed crystals. The trimethylsulfonium salt, $[(CH_3)_3S^+][HO^-(O)_2PCH_2N^+H_2CH_2COO^-]$ (**2**), crystallized from a concentrated methanol solution upon cooling to −26 °C for 1 week. Both **1** and **2** are very hygroscopic in the solid state. Suitable crystals were selected in a glovebag filled with nitrogen and placed in thin-walled glass capillaries under nitrogen.

Data Collection for 1. Preliminary X-ray examination of a crystal on a precession camera indicated the symmetry of the crystal lattice (monoclinic) and yielded the preliminary dimensions of the unit cell. The crystal was then transferred to an automated Enraf-Nonius CAD-4 diffractometer. Automatic peak search and indexing procedures gave a monoclinic primitive cell. Final cell parameters were obtained by least-squares refinement of the angular settings of 24 strong reflections in the 2θ range $28.1° \le 2\theta \le 30.7°$. With application of θ–2θ scan techniques and Mo Kα radiation, 810 intensity data $(h,k,\pm l)$ were collected at 25 °C in the 2θ range $3° \le 2\theta \le 45°$.[18] Additional crystal and data collection details are summarized in Table I.

Data Collection for 2. The data collection for **2** was similar to that for **1**, and details are listed in Table I. Precession photographs identified the symmetry of the crystal lattice (monoclinic) and provided preliminary cell constants. Final cell parameters were obtained by least-squares refinement of the angular settings of 25 reflections in the 2θ range $27.4° \le 2\theta \le 28.5°$. Intensity data $(h,k,\pm l)$ were collected at 25 °C in the 2θ range $3° \le 2\theta \le 45°$.[19]

Structure Solution and Refinement for 1. The raw intensity data were converted into structure factor amplitudes and their esd's by correction for scan speed, background, and Lorentz and polarization effects.[20]

Table I. Crystal and Data Collection Details for **1** and **2**[a]

	$[(CH_3)_2CHN^+H_3][HO(O)_2PCH_2N^+H_2CH_2COO^-]$ (**1**)	$[(CH_3)_3S^+][HO^-(O)_2PCH_2N^+H_2CH_2COO^-]$ (**2**)
Crystal Parameters		
a, Å	4.8747 (7)	10.0547 (11)
b, Å	8.9860 (5)	7.4483 (9)
c, Å	11.5338 (8)	15.1523 (15)
β, deg	92.97 (1)	108.29 (1)
V, Å^3	504.5 (1)	1177.4 (3)
space group	$P2_1$ (No. 4)	$P2_1/n$[b]
fw	228.19	245.24
Z	2	4
d_{calc}, g/cm^3	1.50	1.51
d_{obs}, g/cm^3	1.50	1.50
μ_{calc}, g/cm^3	2.65	4.31
cryst size, mm	0.15 × 0.20 × 0.25	0.15 × 0.25 × 0.35
Data Collection Parameters		
radiation; λ, Å	Mo Kα; 0.709 26	
monochromator	highly oriented graphite ($2\theta_m$ = 12.2°)	
detector	cryst scintillation counter	
2θ range, deg	$3 \le 2\theta \le 45$	$3 \le 2\theta \le 45$
reflcns measd	$0 \le h \le 5$	$0 \le h \le 10$
	$0 \le k \le 9$	$0 \le k \le 8$
	$-12 \le l \le 12$	$-16 \le l \le 16$
scan type	θ–2θ	θ–2θ
scan speed (θ), deg/min	0.83–6.7 (variable)	
scan width ($\Delta\theta$), deg	0.65 + 0.35 tan θ	0.67 + 0.35 tan θ
bkgd	measd over 0.25($\Delta\theta$); added to each end of the scan	
aperture to cryst, mm	173	
vert aperture, mm	3.0	
horiz aperture, mm	2.0 + 1.0 tan θ (variable)	
no. of unique data	710	1402
no. of obsd data, $I \ge 3\sigma(I)$	667	1132
Refinement Parameters		
R, %	2.16	2.79
R_w, %	2.81	4.08
R_{all}, %	2.56	4.30
p factor	0.03	0.03
GOF	1.54	2.07
no. of variables	131	131

[a] Estimated standard deviations are given in parentheses in this and all subsequent tables. [b] Nonstandard setting of space group $P2_1/c$ (No. 14).

Inspection of the list of intensity standard reflections revealed that no correction for crystal decomposition was necessary. The azimuthal scan data showed a variation of ±2%, hence no correction for absorption was applied. Systematically absent reflections ($0k0$, $k \ne 2n$) as well as all reflections satisfying the condition $0kl$, $l < 0$, were removed from the data set, leaving 710 unique data. From the systematically absent reflections the space group was determined to be either $P2_1$ (No. 4) or $P2_1/m$ (No. 11). The subsequent structure determination confirmed the initial choice of $P2_1$.

The structure was solved by using MULTAN 11/82[21] and refined via standard least-squares and Fourier techniques. Refinement was carried out in stages by using isotropic and then anisotropic thermal parameters for all non-hydrogen atoms. A difference Fourier map calculated following anisotropic refinement of all non-hydrogen atoms revealed the positions of all hydrogen atoms. However, only atom H1 was included in the least-squares refinement with an isotropic thermal parameter. All other hydrogen atoms were introduced at calculated positions (d(C–H) = 0.95 Å, d(N–H) = 0.87 Å)[22] with isotropic thermal parameters 1.0 Å^2 larger than the B_{eq} of the parent atom and were not refined. A secondary extinction parameter[23] was refined in the final cycles of least squares. Atomic scattering factors for the neutral atoms were used,[24] and all non-hydrogen scattering factors were corrected for both the real and imaginary components of anomalous dispersion.[24]

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(18) The intensities of three standard reflections, (2,5,3), (2,−3,−6), and (0,1,8), were measured every 2 h of X-ray exposure time. Three orientation control reflections were remeasured after every 250 intensity measurements. A new orientation matrix was calculated from an array of 24 reflections if any of the orientation standards was offset from its predicted position by more than 0.1°. Reorientation was necessary once during data collection. Azimuthal scans were recorded for five reflections near χ = 90° at 10° increments of rotation of the crystal about the diffraction vector.
(19) Intensity standard reflections and three orientation check reflections, (5,1,−9), (2,1,−10), and (−4,−1,10), were monitored as in the data collection for **1** with two reorientations necessary during data collection. Azimuthal scans were recorded for four reflections at the end of the data collection.
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(24) Cromer, D. T.; Waber, J. T. *International Tables for X-Ray Crystallography*; The Kynoch Press: Birmingham, England, 1974; Table 2.2B. Cromer, D. T. *Ibid.*, Table 2.3.1.

Table II. Preparation of Glyphosate Solutions

sample no.	mL of A[a]	mL of B[a]	pD[b]	glyphosate:cation expected[c]	glyphosate:cation found[d]
		IPA/Glyphosate Solutions			
1	2.00	0	2.05	1:0	1:0
2	1.60	0.40	2.66	1:0.40	1:0.35
3	1.20	0.80	3.40	1:0.80	1:0.73
4	0.80	1.20	5.51	1:1.20	1:1.09
5	0.40	1.60	6.27	1:1.60	1:1.48
6	0	2.00	9.25	1:2.00	1:1.93
		TMS/Glyphosate Solutions			
1	2.00	0	2.04	1:0	1:0
2	1.601	0.399	2.57	1:0.38	1:0.37
3	1.168	0.832	3.25	1:0.79	1:0.80
4	0.755	1.245	5.29	1:1.18	1:1.27
5	0.305	1.695	6.18	1:1.60	1:1.56
6	0	2.00	6.76	1:1.89	1:1.89
7[e]			9.48		

[a] Volumes were measured by using a 1000-μL "Eppendorf" automatic pipet. [b] pD's were measured on a Fisher Accumet pH meter and pD = (pH meter reading) + 0.40. [c] Glyphosate:IPA ratios are based on expected concentration of stock solutions. The expected ratio for solution B for the IPA salt was determined by the amounts of reagents weighed out, and that for the TMS salt by NMR integration of sample 6. [d] Glyphosate:IPA ratios were determined from NMR integration of each sample. [e] See the text for preparation of this solution. The ratio of TMS:glyphosate could not be determined in this case due to overlapping peaks.

The quantity minimized by the least-squares program was $\sum w(|F_o| - |F_c|)^2$, where w is the weight of a given observation.[25] The final residuals[25] for 131 variables refined against 667 data with $F_o^2 \geq 3\sigma(F_o^2)$ are $R = 2.16\%$, $R_w = 2.81\%$, and GOF = 1.54. The R value for all 710 unique data is 2.56%. Inspection of the residuals ordered in ranges of $(\sin\theta)/\lambda$, $|F_o|$, and parity showed no unusual features or trends. The largest peak in the final difference Fourier map had an electron density of 0.22 e/Å^3 and was located 0.97 Å from the phosphorus atom.

Structure Solution and Refinement for 2. The raw intensities were converted into structure factor amplitudes and their esd's as for **1**, and no correction for decay or absorption (variation of the average curve ±1.6%) was applied. Systematically absent reflections ($h0l$, $l = 2n + 1$; $0k0$, $k = 2n + 1$) uniquely determined the space group to be $P2_1/n$ (nonstandard setting of $P2_1/c$, No. 14). Systematically absent reflections as well as all data satisfying the condition $0kl$, $l < 0$, were removed from the data set to leave 1402 unique data.

Structure solution and refinement was achieved as for **1**. Hydrogen atoms were treated as in the refinement of **1**, with only the positional parameters for H1 (bonded to O1) being refined in the least-squares procedure. The final residuals[25] for 131 variables refined against 1132 data with $F_o^2 \geq 3\sigma(F_o^2)$ are $R = 2.79\%$, $R_w = 4.08\%$, and GOF = 2.07. The R value for all 1402 unique data is 4.30%. A difference Fourier map, based on the final structure model, had a peak maximum of 0.26 e/Å^3, which was located 0.88 Å from the phosphorus atom.

Solution Preparations for the Variable-pH NMR Study. For the isopropylamine solutions, two stock solutions were prepared, one containing the pure acid (0.062 M) and one containing a 2:1 mixture of isopropylamine (0.13 M) and glyphosate (0.062 M). All solutions were prepared in D_2O. Appropriate amounts of these solutions were mixed in order to vary the ratio of IPA to glyphosate from 2:1 to 0:1 (see Table II). For the trimethylsulfonium solutions, the glyphosate solution from above was mixed in various ratios with an approximate 2:1 mixture of trimethylsulfonium ion and glyphosate, as listed in Table II (solutions 1–6). The trimethylsulfonium glyphosate solution was prepared as follows: Glyphosate (0.5275 g, 3.12 mmol) was placed in a 50-mL Erlenmeyer flask with 10 mL of D_2O and 1.16 g (6.25 mmol) of tributylamine. After most of the glyphosate had dissolved, trimethylsulfonium iodide (Aldrich, 98%; 1.302 g, 6.25 mmol) was added and the mixture was transferred to a 60-mL separatory funnel and extracted five times with 25-mL portions of freshly distilled dichloromethane. The D_2O layer was collected and placed on a rotoevaporation apparatus for several minutes (until bubbling stopped) to remove any dichloromethane. This D_2O solution was transferred to a 50-mL volumetric flask and made up to volume by adding D_2O (glyphosate concentration 0.0624 M). The combined dichloromethane layers were dried over $MgSO_4$, and the solvent was removed to give an off-white solid (tributylammonium iodide), which was dried under vacuum to constant weight (1.68 g). ^{1}H NMR ($CDCl_3$): δ 0.97 (triplet, area 3), 1.40 (sextuplet, area 2), 1.82 (pentuplet, area 2), 3.02 (triplet, area 2). The weight suggested that the reaction was 86% complete. Solution 7 was prepared separately in an attempt to attain the same pH as the highest pH IPA solution. A similar extraction procedure was used with a glyphosate:trimethylsulfonium iodide:tributylamine ratio of 1.0:2.6:2.6. The pH meter reading of the resulting glyphosate/TMS solution was only 7.10, indicating that the reaction did not go to completion. In order to raise the pH, a solution of TMS deuteroxide (≃0.5 M) was prepared by the addition of silver oxide to a solution of trimethylsulfonium iodide in deuterium oxide; the resulting yellow precipitate was removed by filtration. The volume of the pH 7.10 TMS/glyphosate solution was reduced under vacuum, the pH was raised with ≃3 drops of the TMS hydroxide solution, acetonitrile was added as an NMR standard (0.1% v/v), and the solution was made up to volume by the addition of D_2O. The pD of this solution was 9.48.

NMR Measurements for the Variable-pH Study. Acetonitrile was used as an internal reference for ^{1}H measurements (δ = 2.069 ppm from TSP). For ^{13}C measurements, the acetonitrile concentration was not sufficient, and dioxane was added to the samples before these measurements (δ = 67.4 ppm from tetramethylsilane). Dioxane is not suitable for ^{1}H measurements because its resonance (3.764 ppm) may interfere with the peak from the methylene group α to the carboxylate in glyphosate. There was no internal reference for ^{31}P measurements, and the chemical shifts were determined by the substitution method, using a 1:1 (v/v) 85% H_3PO_4/D_2O sample as reference.

All measurements were performed on a spectrometer with a superconducting magnet of 4.67 T. At this field the resonance frequencies of ^{1}H, ^{13}C, and ^{31}P nuclei are 201.95, 50.78, and 81.749 MHz, respectively. The samples were contained in high-precision 5-mm-o.d. NMR tubes (Wilmad) that were spun during measurements. The ^{13}C and ^{31}P spectra were proton-decoupled via a broadband decoupler. All measurements were done at room temperature (21 ± 1 °C). For the ^{1}H spectra, four scans were acquired over a sweep width of 2202 Hz (10.9 ppm). The data size is 16K, which gives a resolution of 0.27 Hz/point (0.0013 ppm/point). For the ^{13}C spectra, 1000 scans were acquired over 10 870 Hz (214 ppm) with a data size of 16K and a resolution of 0.75 Hz/point (0.015 ppm/point). For the ^{31}P spectra, eight scans were acquired over 2252 Hz (27.6 ppm) with a data size of 8K and a resolution of 0.55 Hz/point (0.007 ppm/point). The IPA and TMS samples were run successively, to ensure the same conditions of temperature and magnetic homogeneity; the external reference sample was measured first (to set the δ scale) and again after every third measurement of the glyphosate samples. Over the whole series, the magnetic field drift was less than 0.5 Hz (±0.005 ppm).[26]

Preparation of Solutions for H_4L^+/H_3L Determination by ^{31}P NMR Spectroscopy. The following stock solutions were prepared: (A) 1.165 (20) M HCl (from 2.00 M HCl); (B) 0.1165 (17) M HCl (by dilution of solution A); (C) 1.1662 (9) M KCl (from dry KCl; Mallinckrodt AR); (D) 0.1166 (4) M KCl (by dilution of solution C). Solution B was titrated potentiometrically with 0.0967 (1) M KOH, and the result was also used to determine the concentration of solution A. Concentration of solution C was calculated from the weight of KCl (dried overnight at 150 °C and cooled in a desiccator) and also divided by 10 for solution D.

Twelve samples of glyphosate were weighed in 25-mL volumetric flasks; the average weight was 169.25 mg (SD = 0.35 mg; min/max = 168.68/169.87 mg), which gives a glyphosate concentration of 0.04004 (8) M. The flasks were filled with various amounts of HCl and KCl solutions, so as to obtain two series of solutions at either 1 or 0.1 M constant ionic strength:

	sample code					
	A	B	C	D	E	F
mL of KCl	0	4.3	8.6	12.9	17.2	21.5
mL of HCl	21.5	17.2	12.9	8.6	4.3	0

Glyphosate was solubilized on a sonication bath (30–45 min), and the

(25) The reliability factor, R, was defined as $R = \sum||F_o| - |F_c||/\sum|F_o|$ and $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w(|F_o|^2]^{1/2}$, and the standard deviation of an observation of unit weight, GOF, as GOF = $[\sum w(|F_o| - |F_c|)^2/(n_o - n_p)]^{1/2}$, with n_o = number of observations and n_v = number of variable parameters. The weight is given by $w = 1/\sigma^2(|F_o|) = 4(|F_o|)^2/[\sigma^2(I) + (p(|F_o|)^2)^2]$, where p is the factor to lower the weight of intense reflections. Both possible chiral structures for **2** were refined. The correct chirality could be distinguished by its lower R factor.

(26) The magnetic susceptibility of the relatively concentrated reference sample is likely to be different from the susceptibilities of the dilute glyphosate samples; therefore, the absolute chemical shifts measured this way may not be completely comparable.

flasks were made up to volume with H_2O.

NMR Measurements for ^{31}P NMR pK_a Determination. Samples were put in 5-mm-o.d. NMR tubes, together with a sealed capillary containing a 9:1 mixture of D_2O and 85% H_3PO_4. The ^{31}P spectra were obtained at 81.74936 MHz and were proton-decoupled. The temperature was 21.0 (5) °C. Eight scans were acquired over 2252 Hz (27.6 ppm) with a data size of 8K and a resolution of 0.55 Hz/point (0.007 ppm/point). A sample of pure 85% H_3PO_4 containing a capillary of the above D_2O/H_3PO_4 solution was measured first, and the small peak of the dilute solution appears at 0.670 ppm from 85% H_3PO_4. The ^{31}P shifts of the glyphosate samples measured thereafter were corrected accordingly. Note that neither sample 1F nor 2F contains any hydrochloric acid, and they differ only in their ionic strengths. The difference in their chemical shifts [0.003 ppm (0.20 Hz)] is less than the resolution, and thus to a good approximation the data from the two series can be treated together.

Calculations for ^{31}P NMR pK_a Determination. The calculation of the protonation constant of glyphosate had to account for the facts that (a) in addition to $[H_3L]$ and $[H_4L^+]$, the concentration of $[H_2L^-]$ is significant, since the first deprotonation constant is rather low (pK_a = 2.229)[8] and (b) since chemical shifts are obtained not as a function of pH but rather of initial HCl concentration, $[H^+]$ had to be calculated in order to determine the protonation constant K_{-1}. The following equations were used in the calculations (L_T = total ligand concentration, H_T = initial [HCl], $C_2 = [H_2L^-]$, $C_3 = [H_3L]$, $C_4 = [H_4L^+]$, δ_2, δ_3, δ_4 = ^{31}P chemical shifts of H_2L^-, H_3L, and H_4L^+, respectively):

$$[H^+][H_2L^-] = K_1[H_3L] \quad (K_1 = 5.90 \times 10^{-3})^8 \tag{1}$$

$$[H^+][H_3L] = K_{-1}[H_4L^+] \tag{2}$$

$$H_T = [H^+] + [H_4L^+] - [H_2L^-] \tag{3}$$

$$L_T = [H_2L^-] + [H_3L] + [H_4L^+] \tag{4}$$

$$\delta_{calc} = (C_2\delta_2 + C_3\delta_3 + C_4\delta_4)/L_T \tag{5}$$

$$L_T = [H_3L]\left[\frac{K_1}{[H^+]} + 1 + \frac{[H^+]}{K_{-1}}\right] \tag{6}$$

$$[H^+] - H_T = [H_3L]\left[\frac{K_1}{[H^+]} - \frac{[H^+]}{K_{-1}}\right] \tag{7}$$

$$\frac{L_T}{[H^+] - H_T} = \frac{K_1 + [H^+] + [H^+]^2/K_{-1}}{K_1 - [H^+]^2/K_{-1}} \tag{8}$$

$$([H^+] - H_T)(K_1 + [H^+] + [H^+]^2/K_{-1}) - L_T(K_1 - [H^+]^2/K_{-1}) = 0 \tag{9}$$

$$[H^+]^3 + (K_{-1} - H_T + L_T)[H^+]^2 + K_{-1}(K_1 - H_T)[H^+] - K_1K_{-1}(H_T + L_T) = 0 \tag{10}$$

The observed variable is δ_{obs}, and the least-squares program minimized $\sum(\delta_{calc} - \delta_{obs})^2$. In each iterative cycle, the $[H^+]$ values corresponding to each datum were calculated from the cubic eq 10 by using H_T (independent variable), L_T and K_1 (constants), and the current value of K_{-1}. Then the C_2, C_3, and C_4 values at each point were determined, and δ_2, δ_3, and δ_4 were fitted to eq 5. A new value for K_{-1} was derived and a new cycle started. Different values of K_1 and K_{-1} might be used for the set of data at I = 0.1 M and the one at I = 1 M. However, with only six data in each set, covering different regions of the function (i.e. the sets are not overlapping), it seemed prudent to start with only one value of K_{-1} to refine. This was found to be a good approximation.

NMR Investigation of the Carbamate Derivative of Glyphosate. Glyphosate (0.503 g, 2.97 mmol) was dissolved in H_2O, and the pH was raised with 1 M sodium hydroxide to 7–8. Anhydrous sodium carbonate (0.319 g, 3.01 mmol) was added. Upon dissolution the pH was adjusted to 10.0 with 1 M NaOH. This solution was placed on a rotoevaporation apparatus, and the water was removed to give a white solid. This was redissolved quantitatively in a 5-mL volumetric flask with D_2O. To this solution was added 5 μL of dioxane as an NMR standard. The final pH was 9.98 (pD = 10.38). Solutions of pH ≃ 9 and 11 were prepared similarly. For the ^{13}C labeling experiment a D_2O solution of ^{13}C-labeled sodium carbonate was prepared on a vacuum line by condensing 1 L of ^{13}C-labeled CO_2 (Aldrich, 99 atom % ^{13}C) into 40.5 g of a 8.9% sodium hydroxide solution in D_2O. The weight increase (1.75 g, ≃89% of theoretical $^{13}CO_2$ amount) was used to determine the percentage (5.6% w/w) of $^{13}CO_3^{2-}$ in solution. The concentrations and pH values of the NMR samples, as well as acquisition and processing parameters, are given in the figure captions (Figures 10–13). The acquisition and processing parameters for the spectra are also given in the figure captions.

Table III. Final Positional Parameters and Isotropic Thermal Parameters for $[(CH_3)_2CHNH_3][HO_3PCH_2NH_2CH_2COO]$

atom	*x*	*y*	*z*	B,[a] Å^2
P	0.1274 (1)	0.043[b]	0.65822 (5)	1.42 (1)
O1	−0.0802 (4)	−0.0886 (2)	0.6702 (2)	2.26 (4)
O2	0.0507 (4)	0.1433 (2)	0.5590 (2)	2.18 (4)
O3	0.4068 (4)	−0.0265 (2)	0.6582 (2)	2.16 (4)
O4	0.2994 (4)	0.4069 (3)	1.0687 (2)	2.54 (4)
O5	0.5634 (4)	0.2984 (3)	0.9415 (2)	2.42 (4)
N1	0.1864 (4)	0.0709 (2)	0.8988 (2)	1.44 (5)
N2	0.5458 (5)	0.1809 (3)	0.4266 (2)	1.87 (5)
C1	0.1010 (6)	0.1505 (3)	0.7900 (2)	1.74 (6)
C2	0.1705 (5)	0.1681 (4)	1.0028 (2)	1.78 (6)
C3	0.3623 (5)	0.3022 (3)	1.0024 (2)	1.66 (5)
C4	0.4885 (5)	0.0916 (3)	0.3182 (2)	1.66 (5)
C5	0.7286 (6)	0.1023 (4)	0.2413 (3)	2.75 (6)
C6	0.4245 (7)	−0.0665 (4)	0.3497 (3)	2.98 (7)
H1	−0.215 (5)	−0.063 (3)	0.674 (2)	1.9 (6)*
H2	−0.0851	0.1801	0.7950	2.5**
H3	0.2136	0.2363	0.7844	2.5**
H4	0.3549	0.0405	0.8942	2.5**
H5	0.0791	−0.0054	0.9066	2.5**
H6	0.2168	0.1103	1.0699	2.5**
H7	−0.0127	0.2033	1.0061	2.5**
H8	0.4068	0.1743	0.4706	2.5**
H9	0.6924	0.1465	0.4637	2.5**
H10	0.5718	0.2735	0.4083	2.5**
H11	0.3326	0.1304	0.2756	2.5**
H12	0.7618	0.2037	0.2236	4.0**
H13	0.8873	0.0610	0.2805	4.0**
H14	0.6883	0.0487	0.1715	4.0**
H15	0.3883	−0.1229	0.2810	4.0**
H16	0.5771	−0.1083	0.3929	4.0**
H17	0.2678	−0.0683	0.3954	4.0**

[a] Values marked with an asterisk are for atoms refined with isotropic thermal parameters. Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter defined as $^4/_3[a^2B_{11} + b^2B_{22} + c^2B_{33} + ab(\cos\gamma)B_{12} + ac(\cos\beta)B_{13} + bc(\cos\alpha)B_{23}]$. Values marked with two asterisks are for atoms included in the atom set but not refined. [b] The *y* coordinate for P was fixed at *y* = 0.043 to define the origin of the coordinate system used.

Table IV. Intramolecular Distances (Å) and Angles (deg) for $[(CH_3)_2CHNH_3][HO_3PCH_2NH_2CH_2COO]$

	Distances		
P–O1	1.568 (2)	C3–O4	1.260 (3)
P–O2	1.489 (2)	C3–O5	1.236 (3)
P–O3	1.498 (2)	C3–C2	1.525 (4)
P–C1	1.811 (2)	C4–C5	1.507 (4)
N1–C1	1.486 (3)	C4–C6	1.504 (4)
N1–C2	1.489 (3)	O1–H1	0.70 (3)
N2–C4	1.499 (3)		
	Angles		
O1–P–O2	112.76 (10)	N1–C2–C3	113.8 (2)
O1–P–O3	106.05 (11)	C2–C3–O4	114.8 (2)
O1–P–C1	104.72 (11)	C2–C3–O5	119.0 (2)
O2–P–O3	116.36 (10)	O4–C3–O5	126.3 (3)
O2–P–C1	107.24 (11)	N2–C4–C5	109.8 (2)
O3–P–C1	109.08 (11)	N2–C4–C6	109.6 (2)
P–C1–N1	115.0 (2)	C5–C4–C6	112.3 (2)
C1–N1–C2	112.0 (2)	H1–O1–P	112 (3)

Table V. Selected Torsion Angles (deg) for $[(CH_3)_2CHNH_3][HO_3PCH_2NH_2CH_2COO]$

O1–P–C1–N1	63.5	C1–N1–C2–C3	−62.7
O2–P–C1–N1	−176.5	N1–C2–C3–O4	160.3
O3–P–C1–N1	−49.7	N1–C2–C3–O5	−21.4
C2–N1–C1–P	177.5		
O2–P–O1–H1	−54.9	C1–P–O1–H1	61.3
O3–P–O1–H1	176.6		

Results and Discussion

Molecular Structures of 1 and 2. Atomic positional and thermal parameters for **1** are listed in Table III, and the atomic numbering scheme is illustrated in Figure 1 (all structures are ORTEP[20] drawings). Table IV lists intramolecular distances and angles.

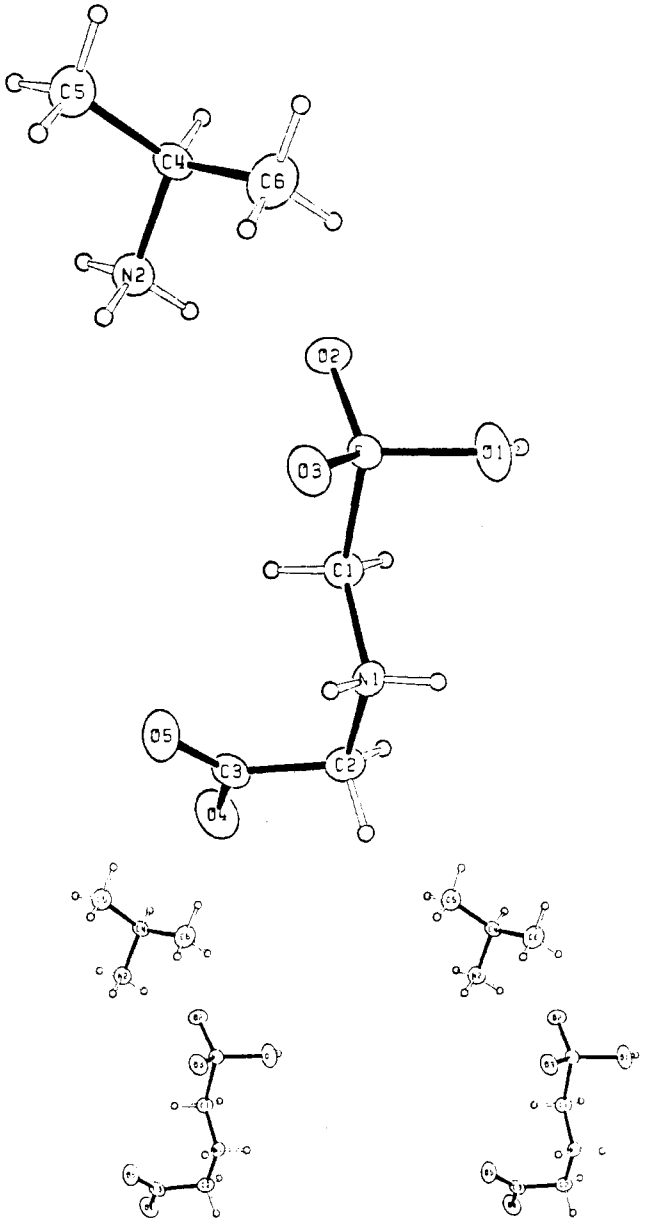

Figure 1. Top: Plot of one molecule of isopropylammonium glyphosate (**1**) identifying the employed numbering scheme. The ellipsoids in this and all other plots are scaled to represent the 50% probability surface. Hydrogen atoms are given as arbitrarily small spheres for clarity. Bottom: Stereoview of one molecule of **1**.

Table VI. Intermolecular Hydrogen-Bond Parameters (Å, deg) for $[(CH_3)_2CHNH_3][HO_3PCH_2NH_2CH_2COO]$

	params		
A-H···B[a]	H···B	A-H-B	A-B
O1-H1···O3[1]	1.87 (3)	167 (3)	2.559 (2)
N1-H4···O4[2]	2.10	157	2.831 (3)
N1-H5···O4[3]	2.04	151	2.915 (3)
N2-H8···O2	2.08	173	2.942 (2)
N2-H9···O2[4]	2.02	160	2.848 (3)
N2-H10···O3[5]	1.96	170	2.819 (3)

[a] Symmetry code: (1) $x-1, y, z$; (2) $-x, 0.5+y, 2-z$; (3) $1-x, 0.5+y, 2-z$; (4) $x-1, y, z$; (5) $1-x, -0.5+y, 1-z$.

Selected torsion angles[26] and parameters of the intermolecular hydrogen bonds are given in Tables V and VI. General temperature factor expressions (B's), root-mean-square amplitudes of thermal vibration and values of F_o and F_c are given in Tables S1–S3, respectively.[27] Figures 1 and 2 show a molecule of **1**, the contents of the unit cell, and the packing arrangement.

Table VII lists positional and thermal parameters for the atoms of **2**. Tables VIII–X contain bond length and angles, torsion angles, and hydrogen bonding parameters, respectively. General temperature factor expressions (B's), root-mean-square amplitudes

(27) Please see paragraph at end of paper regarding supplementary material.

Table VII. Final Positional Parameters and Isotropic Thermal Parameters for $[(CH_3)_3S][HO_3PCH_2NH_2CH_2COO]$

atom	x	y	z	B,[a] Å^2
S	0.18740 (6)	0.25251 (9)	0.14325 (4)	2.68 (1)
P	0.66558 (6)	0.27033 (8)	−0.04926 (4)	1.85 (1)
O1	0.5983 (2)	0.1261 (2)	−0.1259 (1)	2.80 (4)
O2	0.8195 (2)	0.2437 (2)	−0.0159 (1)	2.98 (4)
O3	0.6126 (2)	0.4548 (2)	−0.0810 (1)	2.71 (4)
O4	0.8606 (2)	0.3163 (3)	0.2531 (1)	4.28 (5)
O5	0.7640 (2)	0.2191 (3)	0.3573 (1)	4.58 (5)
N	0.6208 (2)	0.3500 (3)	0.1165 (1)	2.14 (4)
C1	0.5989 (3)	0.2061 (3)	0.0452 (2)	2.56 (6)
C2	0.6153 (3)	0.2895 (4)	0.2088 (2)	2.65 (6)
C3	0.7597 (3)	0.2729 (4)	0.2801 (2)	2.78 (6)
C4	0.0825 (3)	0.4437 (4)	0.1440 (2)	3.13 (6)
C5	0.1589 (3)	0.2214 (4)	0.0224 (2)	3.12 (6)
C6	0.0831 (3)	0.0766 (4)	0.1668 (2)	3.50 (7)
H1	0.515 (3)	0.153 (4)	−0.159 (2)	5.7 (8)*
H2	0.6456	0.1023	0.0739	3.5**
H3	0.5013	0.1851	0.0201	3.5**
H4	0.5563	0.4312	0.0958	3.5**
H5	0.7028	0.3975	0.1244	3.5**
H6	0.5623	0.3740	0.2309	3.5**
H7	0.5706	0.1757	0.2017	3.5**
H8	0.0897	0.4734	0.2063	4.5**
H9	0.1140	0.5421	0.1160	4.5**
H10	−0.0124	0.4180	0.1102	4.5**
H11	0.2102	0.3090	0.0010	4.5**
H12	0.1895	0.1049	0.0121	4.5**
H13	0.0619	0.2336	−0.0104	4.5**
H14	0.1293	−0.0349	0.1681	4.5**
H15	0.0696	0.0974	0.2252	4.5**
H16	−0.0052	0.0742	0.1195	4.5**

[a] Values marked with an asterisk are for atoms refined with isotropic thermal parameters. Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter defined as $^4/_3[a^2B_{11} + b^2B_{22} + c^2B_{33} + ab(\cos\gamma)B_{12} + ac(\cos\beta)B_{13} + bc(\cos\alpha)B_{23}]$. Values marked with two asterisks are for atoms included in the atom set but not refined.

Table VIII. Intramolecular Distances (Å) and Angles (deg) for $[(CH_3)_3S][HO_3PCH_2NH_2CH_2COO]$

Distances			
P-O1	1.573 (1)	C3-O5	1.224 (2)
P-O2	1.483 (1)	C3-C2	1.519 (3)
P-O3	1.494 (1)	S-C4	1.774 (2)
P-C1	1.822 (2)	S-C5	1.777 (2)
N-C1	1.478 (2)	S-C6	1.781 (2)
N-C2	1.486 (2)	O1-H1[a]	0.86 (3)
C3-O4	1.249 (2)		
Angles			
O1-P-O2	108.25 (8)	N-C2-C3	112.59 (15)
O1-P-O3	111.33 (7)	C2-C3-O4	116.0 (2)
O1-P-C1	103.03 (8)	C2-C3-O5	116.5 (2)
O2-P-O3	117.46 (7)	O4-C3-O5	127.5 (2)
O2-P-C1	107.97 (8)	C4-S-C5	101.99 (9)
O3-P-C1	107.79 (8)	C4-S-C6	101.52 (10)
P-C1-N	112.58 (12)	C5-S-C6	101.27 (9)
C1-N-C2	115.46 (14)	H1[a]-O1-P	113 (2)

[a] Refined but not corrected for bond polarization.

Table IX. Selected Torsion Angles (deg) for $[(CH_3)_3S][HO_3PCH_2NH_2CH_2COO]$

O1-P-C1-N	−166.4	C1-N-C2-C3	103.0
O2-P-C1-N	79.2	N-C2-C3-O4	2.0
O3-P-C1-N	−48.6	N-C2-C3-O5	−178.1
C2-N-C1-P	−160.0		
O2-P-O1-H1	−162.0	C1-P-O1-H1	83.8
O3-P-O1-H1	−31.5		

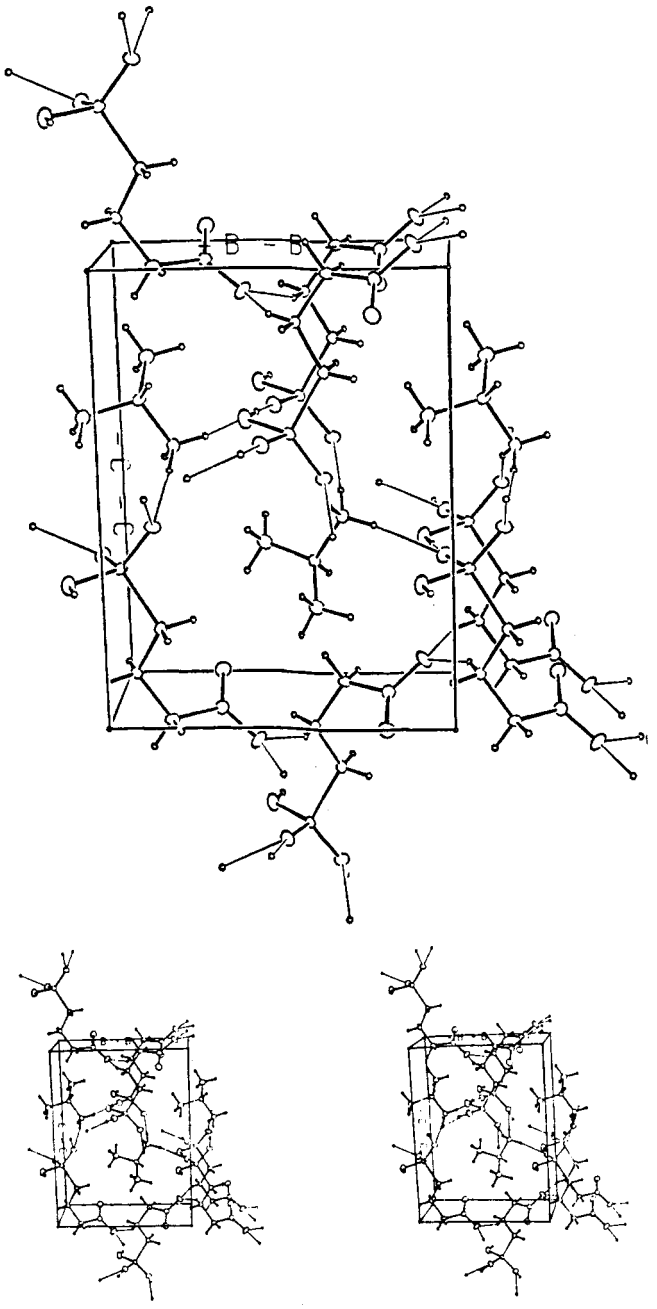

Figure 2. Stereoview of the packing of **1** in the unit cell. Hydrogen bonds are indicated by thin lines.

Table X. Intermolecular Hydrogen-Bond Parameters (Å, deg) for $[(CH_3)_3S][HO_3PCH_2NH_2CH_2COO]$

	params		
A–H···B[a]	H···B	A–H–B	A–B
O1–H1···O4[1]	1.71 (3)	166 (3)	2.551 (2)
N–H4···O3[2]	1.85	156	2.667 (2)
N–H5···O4[b]	2.18	114	2.650 (2)

[a] Symmetry code: (1) $x - 0.5, 0.5 - y, z - 0.5$; (2) $1 - x, 1 - y, -z$. [b] Intramolecular hydrogen bond.

of thermal vibration, and values of F_o and F_c are given in Tables S4–S6, respectively.[27] The labeling diagram and the packing of the molecules in the unit cell are presented in Figures 3 and 4, respectively.

The molecular parameters (bond distances and angles) for the glyphosate anion are remarkably similar in both structures. All P–O, P–C, N–C, and C–C bonds in the anion are identical within three esd's for both salts (Tables IV and VIII). The C–O distances for **1** are about 0.012 Å longer than the same distances for **2** [*d*(C3–O4) = 1.260 (3) Å, *d*(C3–O5) = 1.236 (3) Å in **1**; *d*-

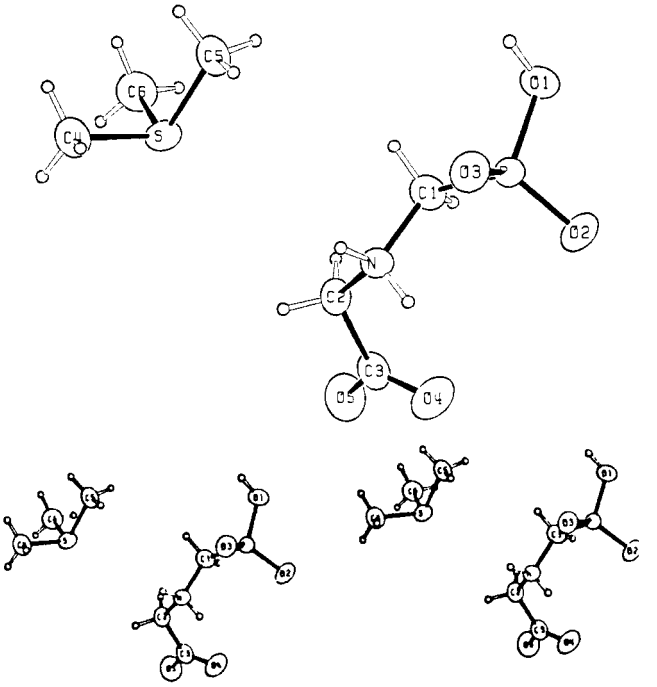

Figure 3. Top: Plot of one molecule of trimethylsulfonium glyphosate (**2**) with the employed numbering scheme. Bottom: Stereoview of one molecule of **2**.

(C3–O4) = 1.249 (2) Å, *d*(C3–O5) = 1.224 (2) Å in **2**], indicative that the carboxylate oxygen atoms in **1** and **2** are involved in different hydrogen bonds. Indeed, O4 in **1** interacts with two of the protic hydrogen atoms on N1 (Table VI), which weakens the C3–O4 and C3–O5 bonds, while atom O4 is involved in a hydrogen bond to the relatively electron-rich hydrogen atom H1 and in a weak intramolecular interaction with H5 (Table X). The bond distances in the cations of **1** and **2** exhibit no unusual features.

The bond angles of the glyphosate anion in **1** and **2** are also very similar. In both anions, the phosphorus atom resides in the center of a distorted tetrahedron, with the angle O2–P–O3 [116.36 (10)° in **1**, 117.46 (7)° in **2**] being the largest and the angle O1–P–C1 [104.72 (11)° in **1**, 103.03 (8)° in **2**] being the smallest. The angles around the central atom of the carboxylate group are also similar with all O–C–C angles smaller than 120° in both salts. The angles H1–O1–P [112 (3)° for **1**, 113 (3)° for **2**] are also identical in both salts. All the C–S–C angles in the $[(CH_3)_3S]^+$ cation are smaller than expected for a regular tetrahedron, due to the space at the sulfur atom lone electron pair. Given the difference in crystal packing (described below), it might be expected that there would be significant differences in the molecular conformations, and resultant torsion angles, of the glyphosate anions in **1** and **2**. However, as Figures 2 and 3 and Tables V and IX show, both anions are formed of zigzag chains of P–C1–N–C2. These four atoms are within 3 and 20° of being coplanar for the IPA and TMS salts, respectively.[28] One methylene proton of C3 is also nearly in this plane, so that the carboxylate is out of this plane (118 and 103°, respectively—recognizing that the anions shown in Figures 2 and 3 are mirror images).

Not only are the molecular parameters of the glyphosate moiety in **1** and **2** almost identical but also they are very similar to the bond distances and angles found in the parent compound glyphosate, $HO_3PCH_2NH_2CH_2COOH$.[2] The major difference between this structure and the two glyphosate salts is found in the bond distances within the carboxylate group, due to its deprotonation. In glyphosate the difference between the C=O and C—OH bond length amounts to 0.104 Å whereas the two C—O distances in each of the salts only differ by 0.01 Å.

Crystal Structures of 1 and 2. The major difference between **1** and **2** is found in their crystal structures, especially in the way

(28) A torsion angle is the angle between two planes defined by the two sets of three adjacent atoms in a four atom chain.

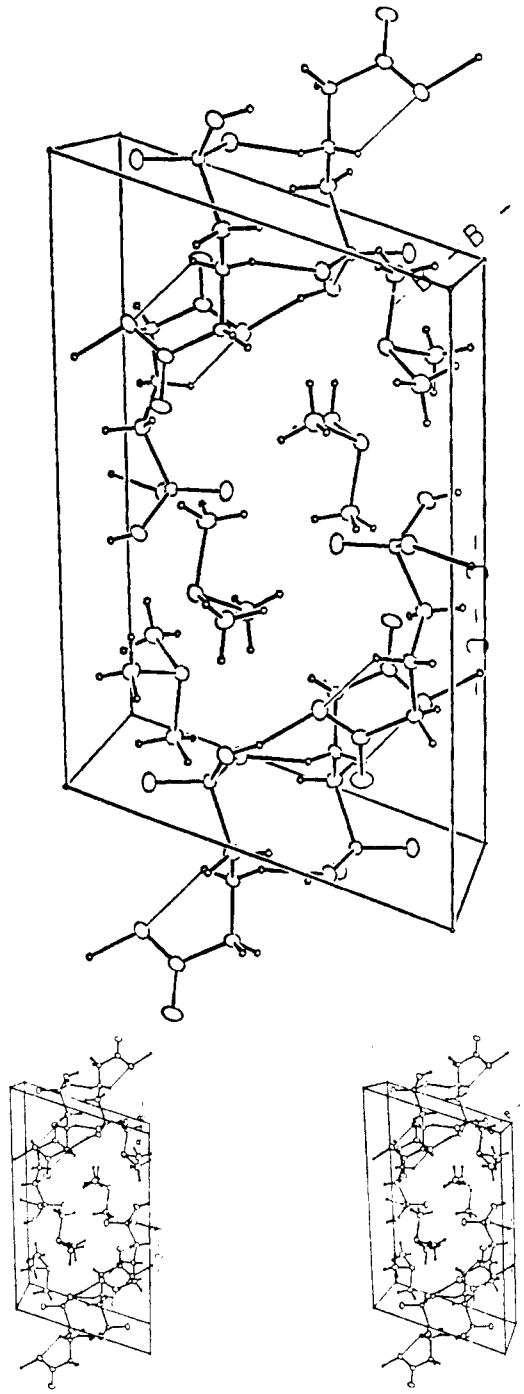

Figure 4. Stereoview of the packing of **2** in the unit cell. Hydrogen bonds are indicated by thin lines.

the molecules form an infinite polymeric network via hydrogen bonds. The isopropylammonium cation of **1** is capable of forming and maintaining various hydrogen bonds to the anion via the three hydrogen atoms at N2. The glyphosate anions themselves are also hydrogen bonded to one another in **1**. However, the cation in **2** cannot maintain any hydrogen bonds, and thus the polymeric crystal structure of this material is made up of hydrogen-bonded glyphosate anions, with isolated discrete cations in the crystal lattice.

Four different hydrogen bonds are found in the crystal lattice of **1** (Table VI). Two of these involve only the glyphosate anion (O4′···H4–N1–H5···O4″, H1′···O3–P–O1–H1···O3″; primed atoms represent symmetry-equivalent positions) and form indefinite polymeric chains propagating through the crystal parallel to the *a* axis. The two other hydrogen bonds involve both the cation and the anion of **1**. One of the two (O2···H8–N2–H9···O2′) forms also polymeric chains parallel to the *a* axis, whereas the other one

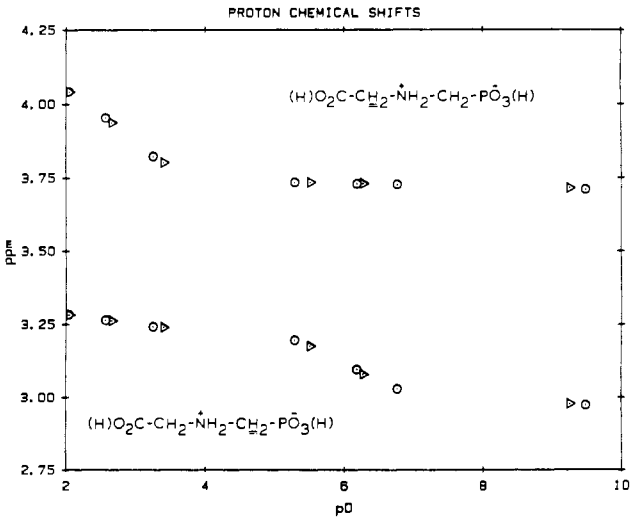

Figure 5. Glyphosate ^1H chemical shifts as a function of pD. Protons giving rise to each data set are underlined (▲ = IPA solutions, ⊙ = TMS solutions).

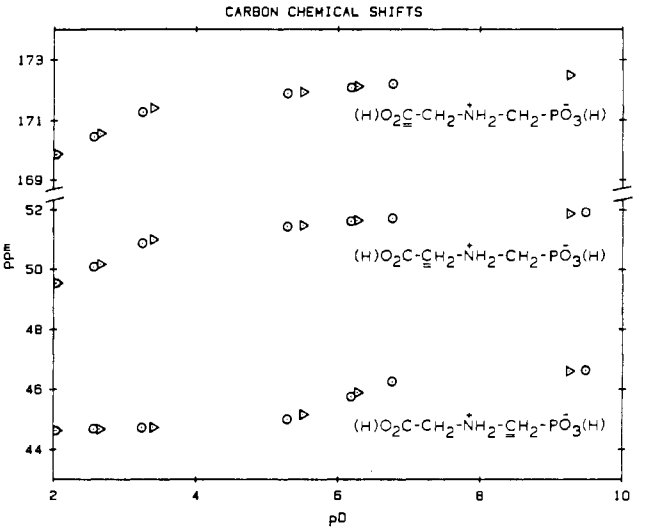

Figure 6. Glyphosate ^{13}C chemical shifts as a function of pD. Carbon atoms giving rise to each data set are underlined (▲ = IPA solutions, ⊙ = TMS solutions).

is terminal, connecting O3 of the anion and H10 of the cation.

Since the cation of **2** cannot participate in any hydrogen bonding, only hydrogen bonds involving the glyphosate anions occur in its crystal structure of **2** (Table X). Of the two types of hydrogen bonds found in **2**, one (O4′···H1–O1–P–C1–N–C2–C3–O4···H1″) produces polymeric chains. Another type (N–H4···O3′) connects only two glyphosate anions related by a center of symmetry. There is also a weak intramolecular interaction between H5 and O4. As for **1**, the hydrogen bonds involving H1 are the shortest and strongest.

pH Dependence of the NMR Spectra of Glyphosate. The ^1H and ^{13}C chemical shifts of the methylene groups of glyphosate are highly dependent on the protonation state of the functional group α to them, and the breaks in the chemical shift curves correspond to the first two pK_a's (2.229 and 5.460);[8] solutions of both salts (isopropylammonium and trimethylsulfonium) were prepared with cation:glyphosate ratios ranging from 2:1 to 0:1. The ^1H chemical shift data are listed in Table XI and plotted in Figure 5 as a function of pD.[29] The ^{13}C results are listed in Table

(29) A result of the incomplete reaction of tributylamine with glyphosate is that the TMS solution contains some TMS iodide, as confirmed by a silver nitrate test. The amount of iodide in solutions 1–6 is significantly less than that in solution 7. The fact that all points lie on the same curve as the IPA solutions confirms that the iodide has no effect and that the chemical shifts are determined solely by the pH.

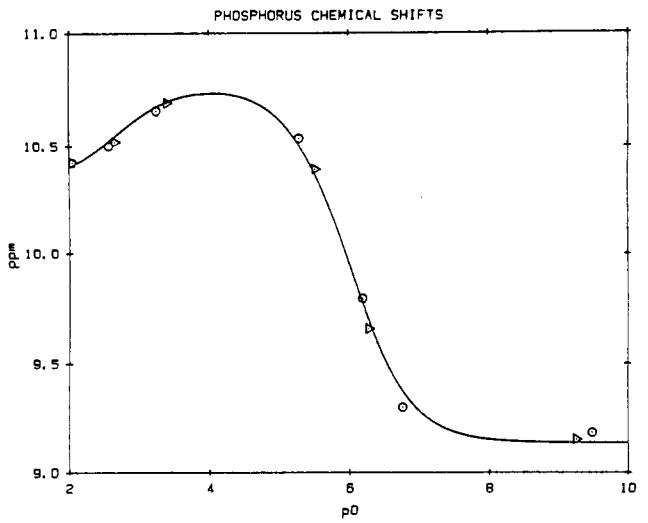

Figure 7. Glyphosate ^{31}P chemical shifts as a function of pD, relative to an external sample of 1:1 (v/v) 85% H_3PO_4/D_2O (▲ = IPA solutions, ⊙ = TMS solutions). The solid curve is a least-squares fit to the data for both salts utilizing the protonation constants of glyphosate (from ref 8) to determine the chemical shifts of the three glyphosate species present. These (and their chemical shifts in ppm) are H_3L (10.32), H_2L^- (10.77), and HL^{2-} (9.13).

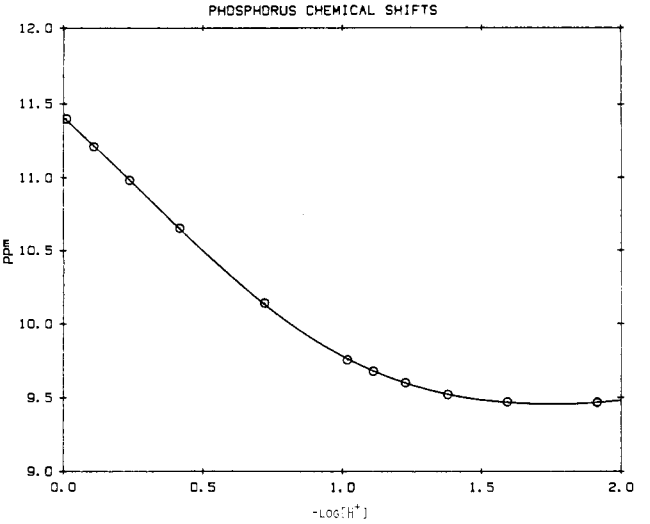

Figure 8. ^{31}P chemical shift data (⊙) and the corresponding calculated curve (—) as a function of −log $[H^+]$.

XII and illustrated in Figure 6. These data directly reveal the protonation sites and are consistent with those reported previously.[9] The marked shift of the carboxylate ^{13}C resonance at pH = 2.3 and the corresponding shift of the methylene ^{13}C resonance adjacent to the carboxylate at this same pH (Figure 6) confirm that the first deprotonation of the neutral acid is from the −COOH group. The corresponding shift of the ^{13}C resonance of the methylene group adjacent to the phosphonate at pH = 5.6 shows that the second deprotonation takes place at the $-PO_3H$ group. The signal from the carbonyl carbon is very weak as compared to those from methylene carbons and in one case could not be distinguished from the noise. In any case, its chemical shift variation with pH is remarkably parallel to that of the methylene carbon α to the carbonyl: the distance between these two peaks, $\Delta\delta$, is almost constant, increasing from 120.32 to 120.62 ppm over the pH range. The coupling constants $^2J_{HP}$ and $^1J_{CP}$ also show a marked dependence on phosphate deprotonation. The other coupling constant $^4J_{CP}$ is too small to be measured accurately, because of a spectral smoothing operation (exponential multiplication) resulting in a 5-Hz line broadening.

The ^{31}P chemical shift data are listed in Table S9[27] and plotted in Figure 7 as a function of pD. The shifts are sensitive to both protonation equilibria. Because of the overlapping equilibria between the neutral acid (H_3L), the monoanion (H_2L^-) and the dianion (HL^{2-}), the resultant chemical shift is a sum of the chemical shifts of each species present, weighted by their relative amounts at each pH. In Figure 7 the solid curve shows the calculated chemical shift for the species H_3L, H_2L^-, and HL^{2-} when the ^{31}P chemical shifts of these species are 10.32, 10.77, and 9.13 ppm, respectively. The acid protonation constants of 2.229 and 5.460[8] were corrected by adding 0.4 and then used to compute the relative concentrations of the glyphosate species at each pD value. The sum of the chemical shifts of these three species, weighted by their relative concentrations, gives the chemical shift plotted as the solid curve in Figure 7. The small *increase* in chemical shift of the ^{31}P resonance in going from H_3L to H_2L^- is ascribed to a change in the intramolecular hydrogen bonding of the glyphosate. This would be expected to be quite different from the largely *inter*molecular hydrogen bonding seen in the solid state.

The NMR results of the Appleton study[9] are compared to ours in Table S10.[27] Apart from the ^{31}P chemical shift, they are in good agreement. The pD dependence curves of ^1H, ^{13}C, and ^{31}P chemical shifts are similar to those reported here between pD = 2 and 10. Appleton et al. also measured beyond these limits, down to below pD = 0 and up to pD ≃ 13.6, but did not determine the protonation constants and the intrinsic δ of each species. The ^{31}P chemical shift of each ligand increases enormously (about 10 ppm) at pH ≃ 11, which is attributed to nitrogen deprotonation.

At the other end of the pH scale (pH = 0.3 in the case of glyphosate; see Figure 8 and the accompanying discussion below) there is an important shift downfield (+3.3 ppm) upon protonation of the neutral ligand to the monocation. It is well established that ^{31}P chemical shifts depend mostly on electronegativity effects, π-electron overlap, and σ-bond angle.[30] In a given class of similar compounds a change in bond angle can produce quite significant changes in chemical shifts (several tens of ppm). The fact that the two most important breaks in the ^{31}P chemical shift curve are in *opposite* directions as pD is increased is probably due to the existence in the 0.3–10.5 pH range of a hydrogen bond between the anionic phosphate end and the cationic amine group that is broken either when the former is protonated or when the latter is deprotonated.

The *smaller* changes in chemical shift occurring at pD = 2 and pD = 5.5 are more difficult to rationalize. As pD is increased, there is first a small shift downfield (≃0.5 ppm) corresponding to deprotonation of the carboxylate and then a slightly larger shift upfield (≃1.5 ppm) corresponding to the second deprotonation of the phosphate. Appleton et al. interpreted this as a deshielding effect of carboxylate deprotonation on the phosphorus nucleus, followed by the shielding effect of phosphate deprotonation. However, structural changes could also contribute to the downfield shift at pD = 2. After deprotonation, the carboxylate end could compete with phosphate for hydrogen bonding to the cationic amine, thus breaking the phosphate–amine five-membered ring.

Determination of the Protonation Constant of Glyphosate. The equilibrium constant for the following reaction was investigated:

$$HOOC-CH_2-NH_2-CH_2-PO_3H + H^+ \rightleftarrows HOOC-CH_2-NH_2-CH_2-PO_3H_2^+$$

The results are listed in Table S11,[27] which includes the values of −log $[H^+]$ determined for each solution and the observed and calculated ^{31}P chemical shifts, which do not differ by more than 0.008 ppm. Figure 8 shows the chemical shift data and the calculated curve as a function of −log $[H^+]$. The fitted parameter values are as follows: K_{-1} = 0.494 (12) M; $\delta(H_2L^-)$ = 9.801 (26) ppm; $\delta(H_3L)$ = 9.230 (10) ppm; $\delta(H_4L^+)$ = 12.492 (28) ppm; R = 0.0496%.[31] The values found for $\delta(H_2L^-)$ and $\delta(H_3L)$ are

(30) Gorenstein, D. G. *Prog. NMR Spectrosc.* **1983**, *16*, 1 and references therein.

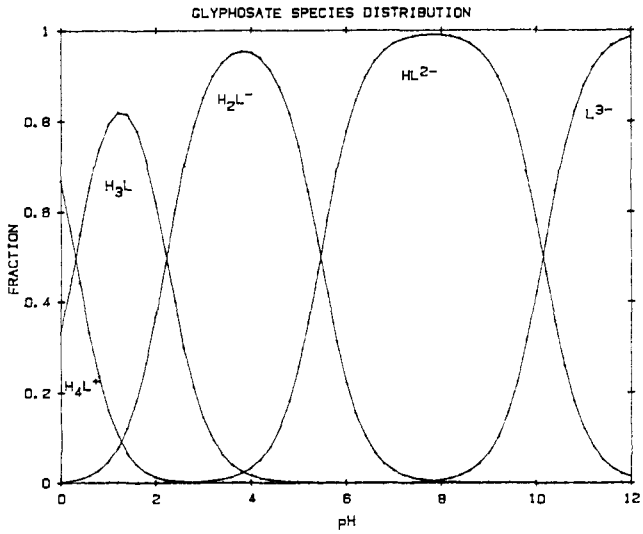

Figure 9. Species distribution diagram calculated by using the new K_{-1} (0.494) from this study and the pK_a's of Motekaitis and Martell[8] (pK_{a_1} = 2.229, pK_{a_2} = 5.460, pK_{a_3} = 10.142).

somewhat different from those determined in IPA or TMS glyphosate solutions (10.77 and 10.32 ppm, respectively), essentially because the latter were in D_2O. However, the same trend is observed: a slight deshielding upon going from H_3L to H_2L^-. The value of K_{-1}, together with the deprotonation constants found by Motekaitis and Martell[8] (pK_{a_1} = 2.229, pK_{a_2} = 5.460, pK_{a_3} = 10.142), was used to generate the species distribution diagram in Figure 9, which shows that the protonated species exists in a significant amount in solution up to pH 2.

NMR Investigation of the Carbamate Derivative of Glyphosate. The existence of a glyphosate carbamate species and an evaluation of the following equilibrium for its formation have been determined:

$$^-OOC-CH_2-NH-CH_2-PO_3^{2-} + HCO_3^- \underset{}{\overset{K_{eq}}{\rightleftharpoons}} {}^-OOC-CH_2-N(COO^-)-CH_2-PO_3^{2-} + H_2O$$

The constant K_{eq} was evaluated by using the relative areas of glyphosate and carbamate peaks. The presence of the carbamate species was confirmed by preparing a sample with ^{13}C-enriched carbonate and observing the otherwise unobserved coupling of ^{13}C to the other nuclei.

Preliminary ^1H measurements of three solutions (0.59 M in glyphosate, 0.60 M in carbonate) at pH = 9, 10, and 11 revealed a new set of resonances in addition to those of glyphosate itself, specifically, a singlet at $\simeq$4.01 ppm and a doublet centered at 3.38 ppm with a coupling constant of 10.2 Hz. These chemical shifts are nearly constant over the pH range ($\Delta \leq 0.01$ ppm), whereas the glyphosate resonances shift more than 0.3 ppm upfield between pH = 9 and 11, because of the deprotonation at the ammonium site. It was thus concluded that the new species did not have a deprotonation step occurring in this pH range. Carbamic acid (H_2NCO_2H) has a pK_a of 6.76,[32] which supports the assumption that the new species is a carbamate derivative.

The integral ratios of the glyphosate peaks to the new species peaks are $\simeq$12 at pH = 9, $\simeq$8 at pH = 10, and >20 at pH = 11. Further study was therefore done near pH = 10. The ^{13}C spectra were not useful because of a poor signal to noise ratio. However the ^{31}P spectra, acquired without proton decoupling,

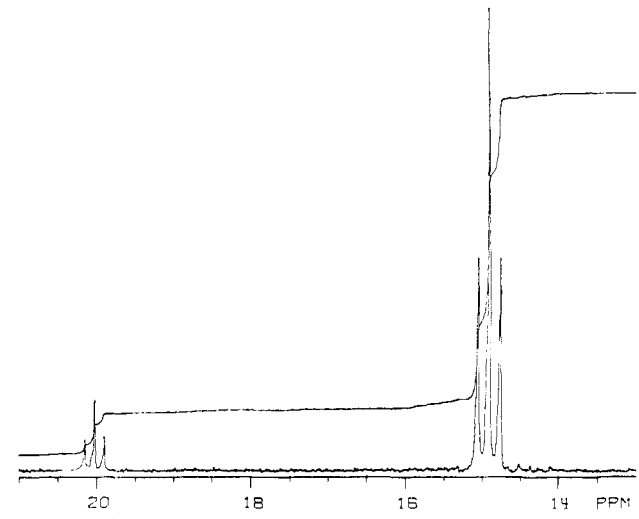

Figure 10. ^{31}P spectrum of a 0.59 M glyphosate and 0.60 M carbonate solution in D_2O at pH = 10 (eight scans; reference 85% H_3PO_4, measured by substitution). The spectral window is 2254 Hz (27.6 ppm), and the data size is 8K (resolution 0.55 Hz/point or 0.007 ppm/point).

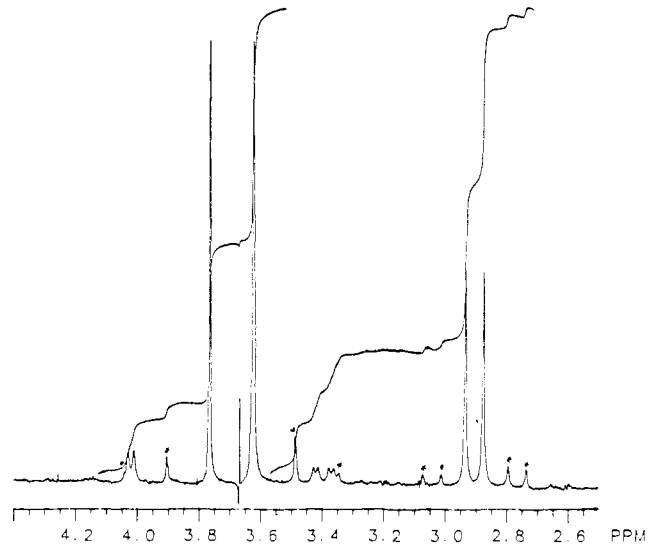

Figure 11. ^1H spectrum of a 0.7 M glyphosate and 0.7 M ^{13}C-labeled carbonate solution in D_2O at pH = 9.7 (four scans; reference internal dioxane (3.764 ppm)). The spectral window is 808 Hz (4.00 ppm), and the data size is 16K (resolution 0.1 Hz/point or 0.0005 ppm/point). Marked peaks (*) are spinning sidebands (28 Hz). Carbamate resonances are found at 4.01 (d) and 3.38 ppm (dd). (The out of phase peak near 3.7 ppm is the position of the rf pulse.)

revealed two triplets (Figure 10), a larger one due to glyphosate ($^2J_{HP}$ = 12.1 Hz) and a smaller one several ppm downfield ($^2J_{HP}$ = 10.1 Hz at pH = 10). Because of the excellent resolution of the two triplets, ^{31}P spectra allow a more accurate integration than the ^1H spectra.

In order to confirm that the new species observed around pH = 10 was a carbamate, ^{13}C-labeled carbonate was used to prepare the samples. Evidence for the carbamate derivative was found by ^{13}C NMR spectroscopy, with the appearance of a strong multiplet at 164.8 ppm, and also in ^1H and ^{31}P spectra, where the carbamate resonances were split by the coupling with the ^{13}C incorporated into the molecule (Figures 11–13).

In the ^1H spectra (Figure 11), carbamate resonances are found at 4.01 ppm (d, $^3J_{HC}$ = 3.8 Hz) and 3.38 ppm (dd, $^2J_{HP}$ = 10.2 Hz, $^3J_{HC}$ = 2.9 Hz). In ^{31}P spectra (undecoupled, Figure 12), the carbamate multiplet is a doublet of triplets ($^3J_{PC}$ = 3.6 Hz, $^2J_{PH}$ = 10.0 Hz). The proton-decoupled carbon spectra show a strong doublet (since the coupling with phosphorus remains) at 164.84, and the ^{13}C spectra acquired without decoupling display a broad multiplet resulting from the couplings with phosphorus

(31) The R factors for the refinement of NMR chemical shifts, δ, parallel those used in the crystallographic least squares: $R = \sum(\delta_{calc} - \delta_{obs})^2 / \sum\delta_{obs}$. This amounts to unit weighting, since we assume essentially constant errors in δ. The error in an observation of unit weight, defined as $[\sum(\delta_{calc} - \delta_{obs})^2/(n_{obs} - n_{var})]^{1/2}$, was 0.0062, where n_{obs} and n_{calc} are the number of observations and variables, respectively.

(32) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1985; Vol. 5, pp 453 and 403.

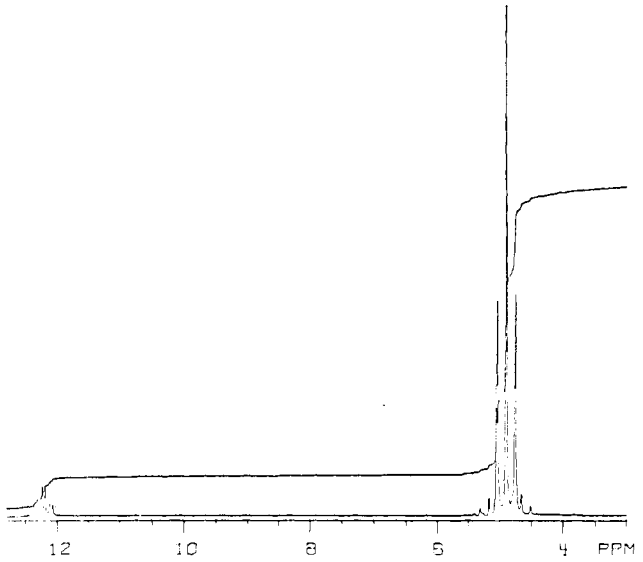

Figure 12. ^{31}P spectrum of a 1.01 M glyphosate and 0.36 M ^{13}C-labeled carbonate in D_2O at pH = 9.5 (40 scans; no reference measured; axis origin arbitrary). The spectral window is 2257 Hz (27.6 ppm), and the data size is 16K (resolution 0.28 Hz/point or 0.003 ppm/point).

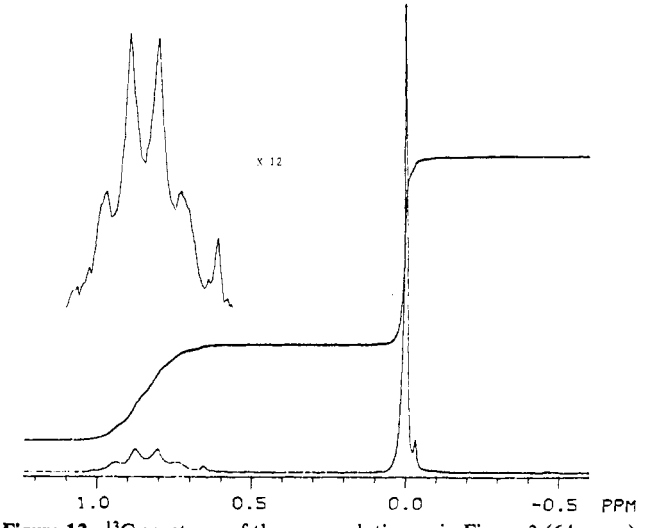

Figure 13. ^{13}C spectrum of the same solution as in Figure 3 (64 scans). The spectral window is 2037 Hz (40.1 ppm), and the data size is 16 K (resolution 0.25 Hz/point or 0.005 ppm/point). The free carbonate singlet used as axis reference has an actual chemical shift of 164.10 ppm (reference dioxane (67.4 ppm)). The small peak on the right side of the multiplet is a spinning sideband. The small peak on the right side of the singlet is thought to be due to an isotope effect: it is also visible in pure ^{13}C-labeled carbonate solution.

and two kinds of protons (Figure 13). Ideally, this multiplet would be a doublet of a triplet of triplets, but the coupling constants are small and comparable (2.9–3.8 Hz); since the peaks have a finite natural line width, the multiplet appears like a broadened sextuplet (Figure 13, inset).

When the integral ratio in Figure 10 and known protonation constants for glyphosate[8] (log $[K_3]$ = 10.142) and carbonate (log $[K_2]$ = 10.00) were used, a value was calculated for the carbamate formation constant in D_2O:

$$K_f = \frac{[LCO_2^{4-}]}{[L^{3-}][HCO_3^-]} = 1.15\ M^{-1}$$

The glyphosate and carbonate protonation constants in D_2O are not known. Exact translation of this into a K_f in H_2O cannot be made. However, the shift in protonation constants in D_2O should be the same and offset each other. Thus, this should be the K_f in H_2O; the value is in the range found for other carbamates.[10-16]

Conclusions

The molecular structures of the IPA and TMS salts of glyphosate are similar to the structure of the free acid, and the relatively minor differences can be ascribed to differences in the hydrogen-bonding networks. Surprisingly, even the anion conformations are essentially the same, although the hydrogen bonding and packing within the two salts are quite different. The results of ^1H, ^{13}C, and ^{31}P NMR spectroscopy individually yield the same conclusion about the chemical shifts of glyphosate solutions: they show a marked dependence on the pH of the solution and the protonation state of the neighboring group, which allows a direct assignment of the protonation sites of glyphosate. The first deprotonation takes place at the carboxylate, the second at the phosphonate, and the third at the amine. The ^{31}P NMR study at low pH has confirmed the presence of H_4L^+ and determined its protonation constant. The existence of the carbamate derivative of glyphosate in concentrated carbonate solutions at high pH has been confirmed by ^{13}C labeling and the corresponding coupling to phosphorus and hydrogen.

Acknowledgment. This research was supported from funds provided by Monsanto Corp.; we thank Drs. John Franz and Dennis Phillion of Monsanto for their cooperation. We also thank Dr. Fred Hollander for experimental assistance in the X-ray diffraction studies (NSF Instrument Grant Nos. CHE-7907027 and CHE-8416692). F.E.H. acknowledges financial support from the German Academic Exchange Service through a NATO postdoctoral fellowship (Aug 1985–April 1987).

Registry No. **1**, 38641-94-0; **2**, 81591-81-3; glyphosate, 1071-83-6.

Supplementary Material Available: Tables S1, S2, S4, S5, and S7–S11, listing general temperature factor expressions (B's) and root-mean-square amplitudes of vibration for **1** and **2**, chemical shifts of IPA and TMS glyphosate solutions for ^1H, ^{13}C, and ^{31}P, NMR parameters of glyphosate in D_2O, and ^{31}P chemical shifts of glyphosate as a function of $[H^+]$ (9 pages); Tables S3 and S6, listing observed and calculated structure factors for **1** and **2** (12 pages). Ordering information is given on any current masthead page.