J. Am. Chem. Soc. **1987**, *109*, 6449-6453

nance of Leu12 could be due to binding of $[Cr(CN)_6]^{3-}$ at this site. No inhibition with redox inactive $[Mo(CN)_8]^{4-}$ is observed, however, and there is no extensive association and/or no electron transfer from this site.

The slight broadening of the 2,6H resonance of Phe82 is possibly due to binding at the His59/Lys60 site or a site involving Lys22 and Lys95. Unlike higher plant PCu^{I} there is no observable specific broadening of His87 resonances with up to a 6-fold excess of $[Cr(CN)_6]^{3-}$, although small downfield shifts are observed. The higher rate constants for *A. variabilis* PCu^{II} with $[Fe(CN)_6]^{3-}$ are consistent with a positively charged locality having an influence on the reaction.

To summarize, the reactivity of *A. variabilis* PCu^{I} appears to be similar to that of higher plant PCu^{I} with regard to active site effects, but with a less clearcut picture with regard to the identity of binding sites. This and other studies on the reactions of plastocyanin with inorganic complexes emphasize the following: (i) charge and its distribution on the protein are important; (ii) for any one complex association can occur at more than one site; (iii) not all of these sites need contribute to electron transfer, since there may be preferred highly directional routes for electron transfer; and (iv) association (K) and electron transfer (k_{et}) can both have a controlling influence on a particular reaction. Physiologically relevant protein redox partners are expected to have greater specificity in their binding than that exhibited by inorganic complexes.

Acknowledgment. We thank the U.K. Science and Engineering Research Council for postdoctoral (M.J.S.) and postgraduate (J.D.S.-D.) support and Johnson Matthey Research and SERC for the award of a CASE studentship (M.P.J.) and for the loan of Pt Compounds. This work was also supported by the National Institutes of Health (Grant No. GM36643) to P.E.W.

Registry No. $[Co(phen)_3]^{3+}$, 18581-79-8; $[Co(dipic)_2]^{-}$, 71605-21-5; $[Co(C_2O_4)_3]^{3-}$, 15053-34-6; $[Fe(CN)_6]^{3-}$, 13408-62-3; $[Cr(CN)_6]^{3-}$, 14875-14-0; $[Co(Phen)_3]^{2+}$, 16788-34-4; $[Fe(CN)_6]^{4-}$, 13408-63-4.

Sulfur Does Not Form Double Bonds in Phosphorothioate Anions

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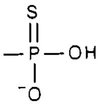
Abstract: Crystal structure, NMR, IR and pK_a measurements have recently been used (P. A. Frey and R. D. Sammons, *Science* (*Washington, D.C.*) **1985**, *228*, 541) to challenge the longstanding assumptions concerning the distribution of charge and bond orders in phosphorothioate anions. Results from ab initio electronic structure calculations are employed to critically evaluate their hypothesis. The claim of appreciable phosphorus–oxygen double bond character along with a correpsonding absence of phosphorus–sulfur double bonds is confirmed, but a systematic variation in the ratio of sulfur–phosphorus to oxygen–phosphorus multiple bonding with anionic charge is also obtained. Thus, singly charged anions are mesomeric and progress to a triply charged anion that possesses a sulfur–phosphorus single bond and a sulfur with unit negative charge.

I. Introduction

Sulfur substitution for oxygen in nucleotides makes possible the generation of substrate analogues with a chiral phosphate, and for several reasons it has become a favored approach in the stereochemical study of nucleotide–enzyme interactions. Thus, (1) these substrate analogues are generally easy to synthesize, and their configurations are easy to determine;[1] (2) since Mg^{2+} coordinates predominantly to the phosphate oxygen while Cd^{2+} to sulfur, a single isomer of a chiral nucleotide phosphorothioate can produce both of the screw sense isomers by choosing the proper cations,[2,3] (3) individual steps in a reaction sequence can frequently be identified because sulfur analogues have relatively slow rates in enzymatic reactions. The recent article by Frey and Sammons,[3] which challenges the conventional structural representation of phosphorothioate anions, is therefore of widespread and fundamental importance to the use and interpretation of its results. They question the localization of charge on oxygen and the assignment of a phosphorus–sulfur double bond in the conventional structural formula



$$\begin{array}{ccc} & S & \\ & \| & \\ {}^{-}O- & P & -OH \\ & | & \\ & {}^{-}O & \end{array}$$

suggesting instead a single-bonded sulfur bearing a negative charge and a PO Lewis bond order greater than one. Their conclusions for this example and related anions are based on a review of bond length data from X-ray crystallography and electron diffraction, ^{18}O and ^{31}P NMR chemical shifts, the pH dependence of ^{17}O NMR chemical shifts, the vibrational spectra of thiophosphate di- and trianions, and pK_a values of phosphoric and thiophosphoric acids.

The Frey and Sammons work calls attention to two longstanding basic questions concerning the differences between first and second row elements: (1) Which of the congeners, sulfur or oxygen, has the greater ability to stabilize negative charge in an anion? (2) Does a second row atom, like phosphorus, prefer to form a double bond with a first row atom, like oxygen, or rather with a second row atom like sulfur? In this article we have studied the compounds $(OH)_3PS$, H_3PO_4, $H_2PO_3S^-$, $H_2PO_4^-$, HPO_3S^{2-}, HPO_4^{2-}, PO_3S^{3-}, and PO_4^{3-} to address these two questions and to test the conclusions of Frey and Sammons. Ab initio electronic structure calculations are employed as data for our investigation (results with use of an experimentally calibrated semiempirical scheme are also included). Measured bond lengths are critically examined,

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[1] Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204.

[2] Pecoraro, V. L.; Hermes, J. D.; Cleland, W. W. *Biochem.* **1984**, *23*, 5262.

[3] Frey, P. A.; Sammons, R. D. *Science* (*Washington, D. C.*) **1985**, *228*, 541.

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Table I. Comparison of 3-21G* and 3-21+G* Bases for Selected Bond Lengths (Å)

anions	method	P–O	P–O*	P–S*	E_T (au)
HPO_3S^{2-}	3-21G*	1.67	1.50	2.08	−958.69464
	3-21+G*	1.69	1.51	2.08	−958.70802
	exp[a]	1.62	1.53	1.97	
HPO_4^{2-}	3-21G*	1.72	1.51		−637.42719
	3-21+G*	1.71	1.53		−637.57103
	exp	1.62	1.53		
PO_3S^{2-}	3-21G*		1.53	2.29	−957.72906
	3-21+G*		1.55	2.21	−959.83104
	exp		1.52	1.99	
PO_4^{3-}	3-21G*		1.56		−636.39530
	3-21+G*		1.58		−636.65573
	6-31G*		1.57		−639.70781
	exp		1.56		

[a] Experimental data references given in Table II.

Table II. Computed and Measured Bond Lengths (Å)

species	methods	P–O	P–O*[i]	P–S
$(HO)_3PS$[a]	MNDO	1.60		1.91
	3-21G*	1.57		1.92
H_3PO_4	MNDO	1.60	1.50	
	3-21G*	1.57	1.45	
	exp[b]	1.57	1.52	
$H_2PO_3S^-$	MNDO	1.63	1.51	1.92
	3-21G*	1.62	1.48	1.97
	exp[c]	1.57	1.48	1.95
$H_2PO_4^-$	MNDO	1.64	1.51	
	3-21G*	1.63	1.48	
	exp[d]	1.58	1.51	
HPO_3S^{2-}	MNDO	1.67	1.53	1.96
	3-21G*	1.67	1.50	2.08
	exp[e]	1.62	1.53	1.97
HPO_4^{2-}	MNDO	1.69	1.54	
	3-21G*	1.72[j]	1.51	
	exp[f]	1.62	1.53	
PO_3S^{3-}	MNDO		1.55	2.04
	3-21G*		1.53	2.29
	exp[g]		1.52	1.99
PO_4^{3-}	MNDO		1.58	
	3-21G*		1.56	
	exp[h]		1.56	

[a] No crystal structure available. [b] Furberg, S. *Acta Chem. Scand.* **1955**, *9*, 1557. [c] Saeger, W.; Eckstein, F. *J. Am. Chem. Soc.* **1970**, *92*, 4712. Mikolajczky, M.; Witczak, M.; Wieczorek, M.; Bokij, N. G.; Struchkov, Y. T. *J. Chem. Soc., Perkin Trans. I* **1976**, *4*, 371. [d] Bacon, D. E.; Peasl, R. S. *Proc. Roy. Soc., London* **1953**, *A220*, 397. [e] PO bond length taken from HPO_4^{2-}; PS bond length assumed to be average of those in $H_2PO_3S^-$ and PO_3S^{3-}. [f] Baur, W. H.; Khan, A. A. *Acta Crystallogr., Sec. B: Struct. Crystallogr. Cryst. Chem.* **1970**, *B26*, 1584. [g] Goldstein, B. M. *Acta Crystallogr. Sect. B: Struct. Crystallogr. Cryst. Chem.* **1982**, *B38*, 1116. [h] Frazer B. C.; Pepinsky, R. *Acta Crystallogr.* **1953**, *6*, 273. Monney, R. C. L. *Acta Crystallogr.* **1956**, *9*, 728. [i] The stars on P–O* and P–S* indicate that O and S are bound only to P. All of the calculated bond lengths are those resulting from complete geometry optimization of each species. [j] Optimization at 6-31G* was carried out for HPO_4^{2-} as well and a value of 1.728Å obtained, thus supporting the interpretation given in the text concerning the difference between computed and experimental values.

Table III. Reference Bond Lengths[a] (Å)

bond	P–O	P=O	P–S	P=S
length	1.56	1.45	2.10	1.92

[a] Corbridge, D. E. C. *Studies in Inorganic Chemistry 6, Phosphorus*, 3rd ed.; Elsevier: Amsterdam, 1985; pp 38–39.

proton affinites determined, and atomic charges and Lewis bond orders assigned. Frey and Sammons concluded that all three phosphorothioate anions, $H_2PO_3S^-$, HPO_3S^{2-}, and PO_3S^{3-}, contained a singly charged sulfur atom and a PS single bond. The computational approach employed here allows a more quantitative discrimination between the three anions, and we find a sulfur charge and a PS Lewis bond order very close to the Frey and Sammons hypothesis for the triply charged anion but a successive change with anionic charge leading to a sulfur charge of $-{}^3/_5$ to $-{}^1/_2$ and a PS bond order of ${}^2/_5$ to ${}^1/_2$ for the singly charged anion. The motivation for studying these compounds has been their biochemical application, but a number of these compounds are of general interest to chemistry and our calculations represent the first attempt to make a quantitative and systematic analysis of P, O, and S containing anions.

II. Computational Methods

Geometry optimized ab initio electronic structure calculations (inclusion of all electrons and use of the nonrelativistic Born–Oppenheimer Hamiltonian) within the framework of the molecular orbital approximation were found adequate to reproduce the trends in the experimental data. Basis sets with separate variation coefficients for inner and outer parts of the valence orbitals and d-orbital polarization functions for second row atoms, P and S, were employed (3-21G* and 3-21 + G*[4]). Calculations were carried out by using the program GAUSSIAN 82.[5] One rationalizaiton for the inclusion of d-functions is the improved agreement with experimental geometries when comparison is made with the best available previous calculations (4-31G basis) for several phosphates.[6] Because most of the systems of interest are anions, the addition of diffuse functions to the basis is also indicated and has been shown to be successful with first row anions.[7] Thus we compared the 3-21G* and the 3-21 + G* bases for four of the most highly charged species (Table I). Diffuse functions bettered the geometry prediction in only one of the eight bond lengths tested (by 0.08 Å in P-S* of PO_3S^{3-}) and were not noticeably superior to 3-21G* itself in discriminating between the bond types and trends that are of interest here. Therefore 3-21G* results were used in our analysis. Geometries from the semiempirical scheme MNDO[8] were utilized for initial guesses in the ab initio calculations. Test of MNDO's ability to produce reasonable geometries is likewise worthwhile because of its potential applicability to large molecules beyond the reach of ab initio methods. From the comparisons in Table II it is apparent that MNDO could be a useful scheme.

Atomic charges have been computed form the Mulliken population method[9] and from the fitting of atomic point charges to electrostatic potentials calculated from the wave functions.[10] This latter method is finding increasing applications, and an efficient computational procedure for implementing it has recently been developed in our research group.[11] The Lewis bond orders needed for constructing structural formulas are obtained from the simple relationship between bond order and the formal charge on an atom in a Lewis dot structure. As given in freshman chemistry texts,[12] its definition is as follows: formal charge, q, = group number − (bond order + number of unshared electrons), and therefore the O* bond order = $2 - |q|$. The formal charges are then compared with charges obtained from quantum mechanical calculations on the phosphate anions where high symmetry permits an immediate assignment of formal charges to the terminal oxygen atoms, O*. A nearly linear correlation is found between the computed and the formal charges on O* (a least-squares fit yields formal charge = 0.696 times the Mulliken formal charge; formal charge = 0.601 times the electrostatic potential derived charge). By using these scaling rules, formal charges and

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(4) (a) Pietro, W. J.; Francl, M. M.; Hehre, W. J.; DeFrees, D. J.; Pople, J. A.; Binkley, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 5039. (b) Clark, T.; Chandrasekhar, J.; Spitznagel, G. W.; Schleyer, P. v. R. *J. Comput. Chem.* **1983**, *4*, 294.

(5) Pople, J. A. Release H, GAUSSIAN 82, Carnegie-Mellon University, G82 written by Binkley, J. S.; Ragavachari, K.; DeFrees, D. J.; Schlegel, H. B.; Whiteside, R. A.; Fluder, G.; Frish, M. J.; Seeger, R.; and Pople, J. A.

(6) Hayes, D. M.; Kollman, P. A.; Rothenberg, S. *J. Am. Chem. Soc.* **1977**, *99*, 2150. Hayes, D. M.; Kenyon, G. L.; Kollman, P. A. *J. Am. Chem. Soc.* **1978**, *100*, 4331.

(7) There are very few studies on anions involving second row atoms. Reasonable trends for first row atoms have been obtained with split valence bases, 4-31G and 3-21G. (a) Radom, L. *Aust. J. Chem.* **1976**, *29*, 1635. (b) Edgecombe, K. E.; Boyd, R. J. *Can. J. Chem.* **1983**, *61*, 45, and **1984**, *62*, 2881. The latter authors find that 3-21+G yields quantitative agreement with experiments for anions of first row atoms. We used 3-21+G* and 3-21G* because second row atoms are involved.

(8) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899, 4907.

(9) Mulliken, R. S. *J. Chem. Phys.* **1955**, *23*, 1832.

(10) See: e.g., Momany, F. A. *J. Phys. Chem.* **1978**, *82*, 592. Cox, S. R.; Williams, D. E. *J. Comput. Chem.* **1981**, *2*, 304.

(11) Chirlian, L. E.; Francl, M. M. *QCPE* Indiana University submitted. The program is available on request.

(12) E.g., Mortimer, C. E. *Chemistry*, 5th ed.; Wadsworth, Inc.: Belmont, CA, 1983; Chapter 6.

Table IV. Mulliken Atomic Charges (3-21G* Basis Set)

species	geometry	P	O	O*[a]	S*
$(OH)_3PS$	opt.[b]	1.44	−0.72		−0.64
H_3PO_4[c]	opt.	1.56	−0.74	−0.68	
$H_2PO_3S^-$	opt.	1.31	−0.75	−0.70	−0.87
$H_2PO_4^-$	opt.	1.38	−0.78	−0.78	
HPO_3S^{2-}	opt.	1.29	−0.84	−0.81	−1.18
	exp	1.25	−0.78	−0.86	−1.09
HPO_4^{2-}	opt.	1.30	−0.83	−0.91	
	exp	1.29	−0.80	−0.94	
PO_3S^{3-}	opt.	1.28		−0.93	−1.49
	exp	1.23		−0.96	−1.35
PO_4^{3-}	opt.	1.23		−1.06	

[a] Stars on O and S mean these atoms are attached to P only. [b] Where bond lengths agree with crystal structure values to within 0.05 Å, only the optimized geometry is used, otherwise values for both geometries are given. [c] The optimized P–O* in H_3PO_4 is 0.07 Å longer than the crystal value, but the optimized value agrees with the reference length in Table III.

therefore bond orders, $2 - |q|$, are obtained for O* and S* in the phosphorothioates. This simple method of computing bond orders makes it possible to directly address the problem of the phosphorus–sulfur double bond assignment described in the Introduction[13] and to make connection with the experimental data assembled by Frey and Sammons.[3]

III. Results and Discussion

A. Geometry. The comparison betweeen the gas-phase 3-21G* calculations and the crystal structure data in Table II shows good overall agreement, but there are two special features that need explantion. First, the ab initio calculations yield slightly shorter P–O* separations than the crystals. This is because crystal structures have strong hydrogen bonds to O* which causes charge transfer away from O* and lengthens the P–O* bond (O* and S* refer to oxygen and sulfur atoms bound only to phosphorus). Second, the P–O and P–S* bond lengths calculated for the gas-phase ions are longer than those in the crystal because the cationic counter ions in the crystals produce bond lengths more nearly like those in neutral molecules. (One might anticipate that in solution, where the typical biochemistry experiments using these substrate analogues are carried out, bond lengths would be between the gas-phase calculations and solid-state experiments.)

In order to obtain structural formula information from the measured and calculated bond lengths in Table II we need reference values for PO and PS single and double bonds. Fortunately, a recent and authoritative compilation has been made by Corbridge[14] and is given as Table III. It is clear that one reason why incorrect structural formulas for phosphorothioate anions have been perpetuated in the literature is that inappropriate reference lengths have frequently been assumed (e.g., several authors[15] have used 1.51 Å for the P–O single bond).

From Table II and III we can draw two simple conclusions: (1) P–O* is not a single bond but lies in the mid-range between a single and double bond, increasing its length with increasing anionic charge. (2) P–S* is not a double bond but rather between a single and double bond (increasing its bond length with increasing anionic charge) becoming close to a single bond for the −3 anion.

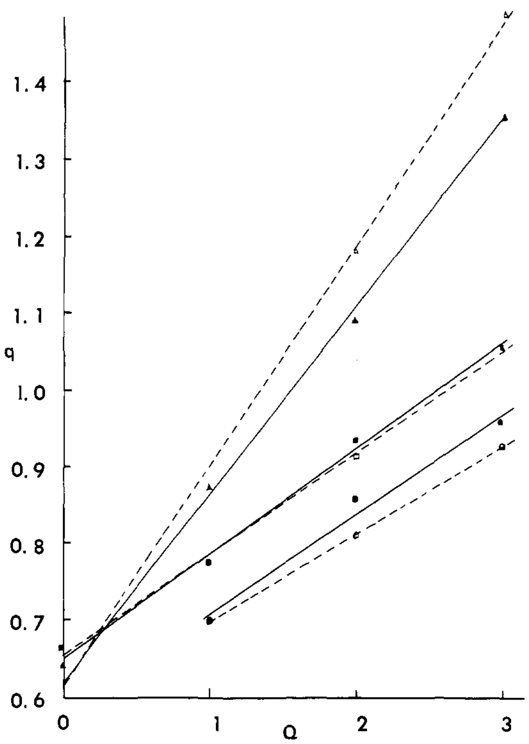

Figure 1. Mulliken atomic charge versus anionic charge wth 3-21G* basis. Solid lines employ experimental geometries, dashed lines, calculated geometries: ▲, △ for S*, ■, □ for O* in phosphates, ●, ○ for O* in phosphorothioates.

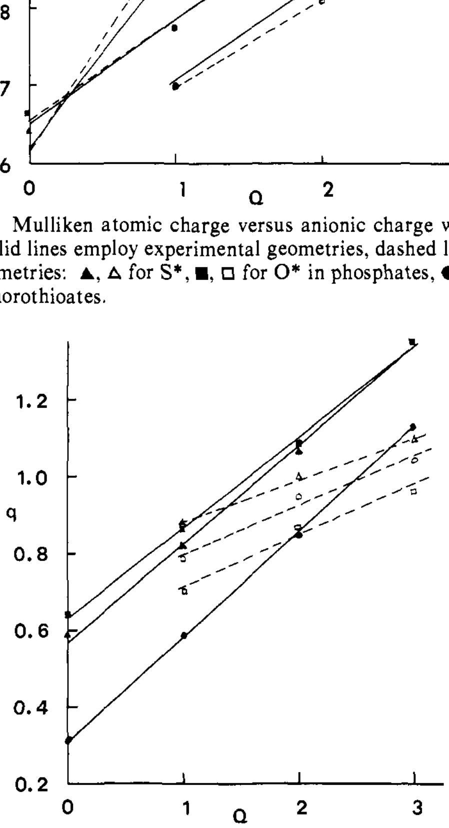

Figure 2. Mulliken atomic charge of S* and O* in phosphorothioates with different basis sets (experimental geometries used). Solid lines for S*, dashed for O*: △, 3-21G basis; □, 3-21G* basis; ○, 3-21+G* basis.

B. Atomic Charges. Table IV lists the Mulliken population analysis charges at optimized geometries by using the 3-21G* basis. As noted above, when the anions are highly charged there is some difference between the gas-phase calculated geometries and crystal structure measurements, and for these cases charges at calculated and measured geometries are both reported. In Figure 1 these charges are plotted versus the anionic charge to determine the relationship between charges on O* and S*. This brings out three aspects of their relationship and one other important point. The latter is the close parallelism between the solid and dashed lines showing that optimized and experimental geometries are giving the same information. The three parts of the O* versus S* relation are as follows: (1) For the anions, S* always bears a greater charge than O*. (2) The differential increase in S* charge is always greater than the differential increase in O* charge when the charge on the species itself is increased. The slope of the S* line (0.26) is twice that of the O* line (0.125).

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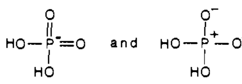
[13] Phosphorus is assumed to maintain five bonds in all of its anions, four fixed σ bonds plus the one π bond whose bond orders are assigned by $\sigma + \pi = 1 + (1 - |q|)$. (Thus resonance structures like those below



$$\mathrm{HO{-}\underset{HO}{\underset{|}{\overset{O}{\overset{\|}{P^-}}}}{=}O} \quad \text{and} \quad \mathrm{HO{-}\underset{HO}{\underset{|}{\overset{O^-}{\overset{|}{P^+}}}}{-}O^-}$$

are assumed to have negligible weight and P is neutral.) Using the computed Lewis bond orders given in Table VII it is immediately verifiable that for each anion they sum to five within a few percent error, thereby providing an internal check on the linear relationship between bond order and the quantum mechanically calculated charges and an independent check on the extrapolation from phosphorates to phosphorothioates.

[14] Corbridge, D. E. C. *Studies in Inorganic Chemistry 6, Phosphorus*, 3rd ed.; Elsevier: Amsterdam, 1985; pp 38–39.

[15] Saenger, W.; Eckstein, F. *J. Am. Chem. Soc.* **1970**, *92*, 4712.

Table V. Atomic Charges[a] of S* and O*

	O*		S*	
species	elec pot.	Mulliken	elec pot.	Mulliken
$(OH)_3PS$			−0.64	−0.64
			−0.74	−0.59
				−0.32
$H_2PO_3S^-$	−0.83	−0.70	−0.93	−0.87
	−0.97	−0.88	−1.01	−0.82
		−0.79		−0.59
HPO_3S^{2-}	−1.03	−0.86	−1.16	−1.09
	−1.14	−1.00	−1.25	−1.07
		−0.96		−0.85
PO_3S^{3-}	−1.18	−0.96	−1.44	−1.35
	−1.29	−1.10	−1.53	−1.35
		−1.04		−1.13

[a] 3-21G* top entry, 3-21G middle, 3-21+G* bottom entry. All charges determined at experimental geometries. Asterisks on O and S mean that atom is bound to P only.

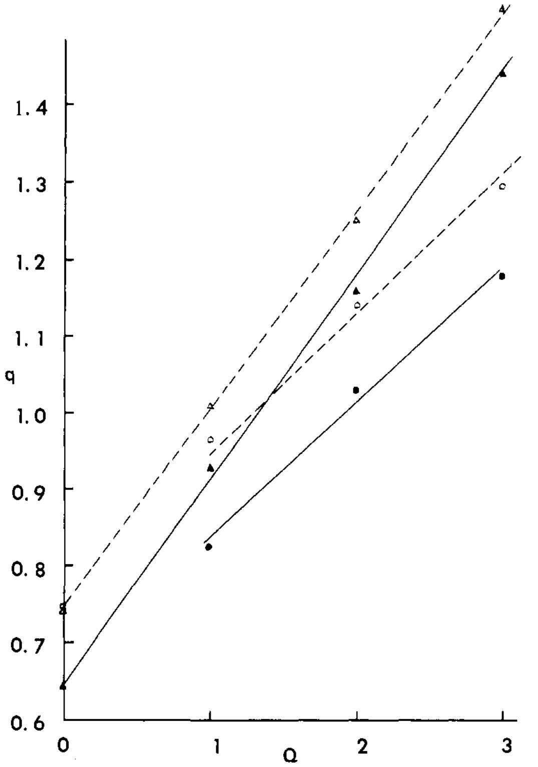


Figure 3. Electrostatic potential derived atomic charges of S* and O* in phosphorothioates. Solid lines correspond to 3-21G*, dashed to 3-21G: ▲, △ for S*; ●, ○ for O*.

(3) O* charge in phosphates is always greater than O* charge in phosphorothioates (by approximately 0.085e), i.e., introduction of sulfur reduces the charge on O*. Because oxygen has a considerably greater electronegativity than sulfur, (1) and (2) are initially surprising.[16] However, the electron affinity[17] of sulfur is 47.7 kcal/mol compared to 33.8 kcal/mol for oyxgen, and the radius ratio[18] (both ionic and covalent) is 1.3–1.4. Figure 2, Mulliken atomic charge calculated from different basis sets (data in Table V), illustrates the basis set dependence of the Mulliken population analysis (the relative magnitude of charge on S* and O* sometimes reverses). However, the important Figure 1 result showing the slope of the S* line to be approximately double that for O* is preserved. To obviate the problem with the basis dependence of Mulliken charges[19] we also calculated the atomic charges from the molecular electrostatic potentials. Figure 3 (data in Table V) compares Mulliken to electrostatic potential derived charges and demonstrates that the slope and magnitude rule, deduced from the Mulliken charges plotted in Figure 1, is also true for electrostatic potential derived charges. (It is not possible to use the 3-21 + G* basis set to derive charges from electrostatic potentials because the diffuse orbitals frequently have their radial maxima on adjacent atoms. A similar effect is the main cause for error in Figure 2: the diffuse orbital for sulfur has its radial maximum approximately at the phosphorus nucleus, and the coefficient of this orbital thus reflects a positively charged environment rather than the true negative charge buildup on S*.)

VI. Proton Affinities[a] (kcal/mol)

reaction	PA
$H_2PO_3S^- + H^+ \rightarrow H_3PO_3S$	342.12
$H_2PO_3S^- + H^+ \rightarrow (HO)_2(SH)PO$	329.15
$HPO_3S^{2-} + H^+ \rightarrow H_2PO_3S^-$	467.72
$HPO_3S^{2-} + H^+ \rightarrow (HO)(SH)PO_2^-$	452.71
$PO_3S^{3-} + H^+ \rightarrow HPO_3S^{2-}$	605.90
$PO_3S^{3-} + H^+ \rightarrow (HS)PO_3^{2-}$	561.11[b]
$H_2PO_4^- + H^+ \rightarrow H_3PO_4$	356.60
$HPO_4^{2-} + H^+ \rightarrow H_2PO_4^-$	496.15
$PO_4^{3-} + H^+ \rightarrow HPO_4^{2-}$	647.52

[a] Proton affinity = E_T(protonated) − E_T(unprotonated). Zero-point corrections (not included) are expected to be small (Edgecomb, K. E.; Boyd, R. J. *Can. J. Chem.* **1984**, *62*, 2887). [b] Minimum for $(HS)PO_3^{2-}$ not obtained by r(P–S) = 2.36 Å, and value tabulated is therefore an upper bound. All proton affinities computed with 3-21G* basis at optimized geometries.

Table VII. Lewis Bond Orders and Formal Atomic Charges[a]

	$H_2PO_3S^-$		HPO_3S^{2-}		PO_3S^{3-}	
property	Mulliken	elec pot.	Mulliken	elec pot.	Mulliken	elec pot.
O* formal atomic charge	−0.49	−0.50	−0.60	−0.62	−0.67	−0.71
P–O* bond order	1.51	1.50	1.40	1.38	1.33	1.29
S* formal atomic charge	−0.61	−0.56	−0.76	−0.70	−0.94	−0.87
P–S* bond order	1.39	1.44	1.24	1.30	1.06	1.13

[a] Bond orders obtained from scaled Mulliken and electrostatic potential atomic charges, q, by the formula $2 - |q|$. Conventional formal charge = 0.696 times Mulliken charge (scale factor obtained by least-squares fit). Corresponding scale factor for electrostatic potential derived charges is 0.601. All computed charges from 3-21G* basis at experimental geometries.

C. Proton Affinity and Acid Strength. In the previous sections we demonstrated that negative charge prefers to go to sulfur rather than oxygen as protons are removed. However, in the usual discussions of acid–base chemistry one is interested in protonation and pK_a values, and therefore it is important to determine whether addition of a proton to phosphorothioate anions will occur on sulfur or oxygen. The calculated proton affinities given in the top part of Table VI answers this question. In all three cases protonation of oxygen is favored over sulfur (i.e., a larger proton affinity is obtained for oxygen protonation in each case). There are two reasons for this result: (1) the O–H bond strength is 102 kcal/mol while that of S–H is 82,[20] and (2) the charge density on oxygen is greater than that of sulfur by a factor varying from 1.7–2.3

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(16) Politzer, P.; Reggio, P. H. *J. Am. Chem. Soc.* **1972**, *94*, 8308.

(17) Weast, R. C. *CRC Handbook of Chemistry and Physics*, 65th ed.; CRC Press Inc.: New York, 1984–1985; p E62.

(18) Huheey, J. E. *Inorganic Chemistry*, 2nd ed.; Harper & Row: New York, 1978; 232.

(19) Another known shortcoming of Mulliken charges is their frequent erratic behavior for phosphorus and this shows up as well in phosphorothioates. Thus, Mulliken charges on phosphorus decrease as anionic charge increases rather than the chemically expected increase given by electrostatic potential derived charges.

(20) Weast, R. C. *CRC Handbook of Chemistry and Physics*, 65th ed.; CRC Press Inc.: New York, 1984–1985; p F174.

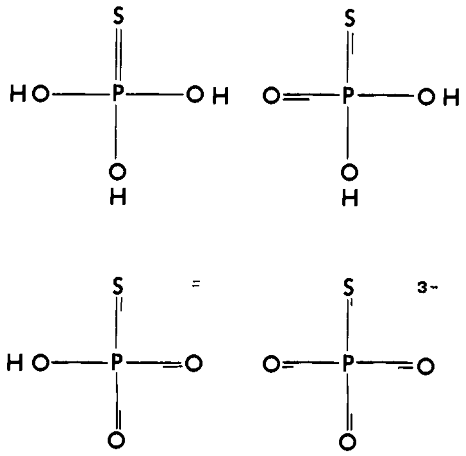

Figure 4. Calculated structural formulas for phosphorothioates.

(depending on the anion in question and whether one assumes an ionic or covalent radius).

The question of phosphorothioate acid strength is also a significant issue. The computed proton affinities for the phosphates (bottom section of Table VI) compared to the oxygen proton affinities in the top section of Table VI show phosphorothioates to be the stronger acids (i.e., they more easily release a proton and therefore have lower proton affinities). This result is in accord with pK_a measurements.[3] Moreover, the difference in acidity becomes larger for greater anionic charge. From Table VI the proton affinity differences for the pairs $H_2PO_3S^-/H_2PO_4^-$, HPO_3S^{2-}/HPO_4^{2-}, and PO_3S^{3-}/PO_4^{3-}, are 14.5, 28.4, and 41.6 kcal/mol, respectively, again in agreement with the corresponding pK_a changes.

D. Lewis Bond Orders and Structural Formulas. Table VII lists the bond orders assigned from Mulliken and electrostatic potential derived charges according to the prescription given in *Computational Methods.* For most practical purposes the two sets of numbers are the same. Lewis bond orders enable us to draw structural formulas and these are shown in Figure 4. It should be noted that in *neutral* phosphorothioates with highly electronegative ligands, e.g., F_3PS, FPS, and $(OH)_3PS$, the traditionally expected PS double bond will manifest itself, and thus we include $(OH)_3PS$ as a reference species in Figure 4. For the anions, -1 to -3, we have decreasing multiple bond character varying from approximately 1.5 to 1, respectively.

IV. Summary

(1) Table VII and Figure 4 give the formal atomic charges and Lewis bond orders for phosphorothioate anions that our results suggest as replacement for the conventional formulas. This agrees with that of Frey and Sammons for the triply charged anion but deviates from their hypothesis for the singly and doubly charged anions.

(2) Three rules for charges on sulfur compared to oxygen were found for phosphorothioate anions: (a) the sulfur charge is greater than that on oxygen, (b) the slope of the line respresenting sulfur charge versus anion charge is approximately twice that for oxygen, (c) replacing an oxygen by sulfur in a phosphate reduces the charge on the remaining oxygens.

(3) Protonation of phosphorothioates occur preferentially on oxygen rather than sulfur, phosphorothioates are stronger acids (and have lower proton affinities) than phosphates, and the difference in their acid strengths increases with increasing anionic charge.

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Note Added in Proof. An important paper by M.-D. Tsai et al. (*Biochemistry* **1986**, *25*, 3435) has been called to our attention by Prof. Fritz Eckstein. Tsai et al. have studied the infrared spectrum of chiral thiophosphastidylcholine and found that the singly charged thiophosphate anion in 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine exists in a mesomeric form—exactly the charge and bond order pattern we have determined by calculation and shown in Figure 4. Because this disagrees with the Frey and Sammons hypotheses for the singly charged anion, Tsai et al. concluded that chiral thiophospholipids in membranes are modified by environmental effects, such as neighboring quarternary ammonium groups. Our results allow one to conclude that it is unnecessary to postulate environmental interactions. A similar judgment applies to recent work by Eckstein and Kennard (*J. Mol. Biol.*, private communication, in press).

J. R. P. Arnold and C. Lowe (*J. Chem. Soc., Chem. Commun.* **1986**, 1487) have measured the ^{18}O isotope shift in the ^{31}P NMR signal of triply charged inorganic thiophosphates and find a charge and bond order pattern agreeing exactly with that shown in Figure 4.