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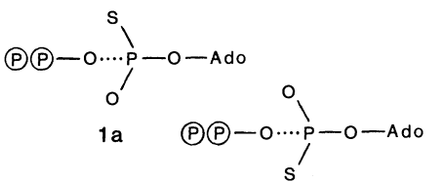
Bond Order and Charge Localization in Nucleoside Phosphorothioates

Perry A. Frey and R. Douglas Sammons

Sulfur-containing analogs of biological phosphoric esters and phosphoanhydrides are widely used as substrate analogs in studies of enzymatic processes. Substitution of sulfur for one of the diastereotopic oxygens at P_α of ATP generates epimers **1a** and **1b** of $ATP_\alpha S$ (*1*),

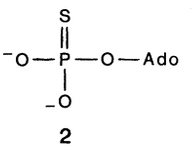


differing in configuration at phosphorus (*2, 3*). Similar substitution at P_β of ATP or the phosphodiester group of 3′,5′-cyclic AMP results in similar epimer pairs (*2, 4–6*). These compounds have been widely used as substrates or substrate analogs to determine the stereochemical course of enzymatic substitution at phosphorus (*7*). Both $ATP_\alpha S$ and $ATP_\beta S$ have been used with divalent metal ions such as Mg^{2+}, Cd^{2+}, and Co^{2+} to determine the coordination structures and stereochemical configurations of active Me · ATP and Me · ADP complexes at the active sites of enzymes (*7–10*).

Epimer pairs such as **1a** and **1b** show varying degrees of stereoselectivity in their interactions with enzymes. In cases in which the chiral phosphorothioate group is the reaction center—that is, a bond in this group is cleaved—enzymes are very highly stereoselective or even stereospecific in their acceptance of only one epimer as a substrate. An interesting specific example of stereoselectivity is the dual interaction of valyl-tRNA synthetase with (R_p)-$ATP_\beta S$ and (S_p)-$ATP_\beta S$. The enzyme utilizes only the (R_p) epimer as an aminoacylation substrate but also catalyzes the interconversion of (S_p)-$ATP_\beta S$ and $ATP_\gamma S$ (*11*). The two processes apparently occur at different sites since neither epimer inhibits the reaction of the other.

Functional selectivity extends to interactions at allosteric sites, as in the activation of cyclic AMP–dependent protein kinases by (S_p)-cyclic AMPS (*12–14*). The (R_p) epimer is bound by the regulatory subunit but does not induce its dissociation from the catalytic subunit. Failure to induce dissociation and activation has been attributed to the absence of an electrostatic or hydrogen bond interaction between the regulatory subunit and the phosphorothioate group in (R_p)-cyclic AMPS. This interaction with cyclic AMP or (S_p)-cyclic AMPS is postulated to touch off a conformational transition in the regulatory subunit and stabilize the new conformation. This presumably weakens subunit interactions between the regulatory and catalytic subunits, leading to their dissociation and the expression of protein kinase activity by the catalytic subunit.

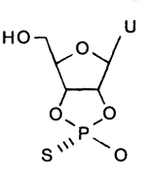
The nature of the differential interactions between a binding site and ligand isomers can give important information about the molecular basis for function when one isomer is functional and the other not. In considering the possibilities for interactions between a nucleotide binding site and epimer pairs such as (R_p)-cyclic AMPS and (S_p)-cyclic AMPS or (R_p)-$ATP_\beta S$ and (S_p)-$ATP_\beta S$, it is essential to know as much as possible about the structures of the phosphorothioate groups. Structural formulas such as **1a** and **1b** for (R_p)-$ATP_\alpha S$ and (S_p)-$ATP_\alpha S$ illustrate only the steric differences between epimers, not the electronic and electrostatic differences. Structural formulas intended to emphasize localization of negative charges and double bonds are frequently shown with a double bond between the phosphorus and sulfur as in AMPS, **2**, below (*7, 9, 15–17*). These structures are intended to indicate that the negative electrostatic charges are localized on oxygen, with little resonance delocalization to sulfur.



2

In this article we review the experimental evidence bearing on the question of charge localization and bond order in phosphorothioate anions and point out that the evidence is not consistent with localization of charge on oxygen or with a bond order of 2 for the P-S bond.

P-O and P-S bond lengths. The crystal and molecular structure of *endo*-2′-3′-cyclic UMPS **3** was published by



3

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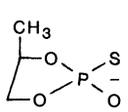
Summary. In the recent literature on nucleoside phosphorothioate anions the structural formulas show a double bond between phosphorus and sulfur and a single bond between phosphorus and oxygen with a negative charge localized on oxygen. However, a review of physical data on these compounds shows the reverse to be the case; that is, in phosphorothioate anions the P-S bond is a single bond with a negative charge localized on sulfur, while the P-O bond order for exocyclic and nonbridging oxygens is greater than 1, approaching 2 in O-alkyl phosphorothioate monoanions and O,O-dialkyl phosphorothioates. The P-O bond orders in phosphorothioate dianions and trianions approach 1½ and 1⅓, respectively, owing to delocalization of negative charge among two or three oxygens. These conclusions are based on bond lengths obtained from x-ray crystallographic data and electron diffraction, the magnitudes of the effects of ^{18}O on the ^{31}P-nuclear magnetic resonance chemical shifts of phosphorus in nucleoside [^{18}O]phosphorothioates, the *p*H-dependence of ^{17}O-NMR chemical shifts in [^{17}O]phosphate and [^{17}O]thiophosphate, the vibrational spectra of thiophosphate di- and trianions, and the pK_a (dissociation constant) values for phosphoric and thiophosphoric acids.

Saenger and Eckstein in 1970 (*18*). In this structure the exocyclic P–O bond length was found to be 1.48 Å and the P–S bond length was found to be 1.95 Å. The *endo*-cyclic P-O bond lengths were found to be 1.58 and 1.61 Å, respectively. The triethylammonium counter cation was located near the exocyclic oxygen of the phosphorothioate group, the triethylammonium N and phosphorothioate O being separated by 2.78 Å, a value consistent with an H-bond distance in which the triethylammonium H is interposed between N and O. Saenger and Eckstein concluded from the bond lengths that the P-O bond order is 1 and the P-S bond order 2; and they cited the proximity of the triethylammonium ion to the exocyclic phosphorothioate oxygen as evidence of the localization of negative charge on oxygen.

In reaching their conclusions, Saenger and Eckstein were guided by compilations of P–O single bond lengths and P=S double bond lengths in diverse molecules, where the P–O single bond was listed as 1.51 ± 0.04 Å. The P-O bond length of 1.48 Å found by Saenger and Eckstein in crystals of **3** was within this range and they, therefore, assigned a bond order of 1. The P-S bond length of 1.95 Å was shorter than the tabulated single bond distances and so was assigned a bond order of 2.

Mikolajczyk *et al.* determined the crystal and molecular structure of 2-hydroxy-4-methyl-2-thio-1,3,2-dioxaphospholane **4** as its imidazolium salt (*19*). In this crystal the P-S bond length was found to be 1.97 Å and the exocyclic P-O bond length 1.49 Å. The endocyclic P-O bond lengths were again 1.58 and 1.63 Å; and the imidazolium ion was hydrogen bonded to the exocyclic oxygen. In the structure of *bis*-cyclohexylammonium 4-carboxy-2-hydroxy-2-thio-1,3,2-dioxaphospholane **5**, the P-S bond length was found to be 1.93 Å and the exocyclic P-O length 1.49 Å (*20*). Thus in three five-member ring thiophosphoric diesters the P-S bond lengths range from 1.93 to 1.97 Å and the exocyclic P-O bond lengths are 1.47 to 1.49 Å.

The P-S bond lengths in crystals of **3**, **4**, and **5** are longer than P=S double bonds and the P-O bonds are shorter than P-O single bonds in five-member ring cyclic thiophosphoryl and phosphoryl chlorides and triesters. The phosphorus-sulfur bond length in 2-chloro-2-thio-1,3,2-dioxophospholane **6** is 1.89 Å (*21*). And the exocyclic P-O bond length in methyl ethylene phosphate **7** is 1.57 Å (*22*), while that in methyl pinacol phosphate is 1.56 Å (*23*). The endocyclic P-O single bonds in these molecules range from 1.57 to 1.59 Å in length. The P-O double bonds in methyl ethylene phosphate **7** and methyl pinacol phosphate, as well as ethylene chlorophosphate (*24*) **8**, are 1.44 Å in length, while the P-S single bonds in ethylene trithiochlorophosphate **9** are 2.12 Å in length (*24*). The P-S bond lengths in crystals of **3**, **4**, and **5** are, therefore, intermediate between the values for single and double bonds in similar molecules; and the exocyclic P-O bonds are nearer the double bond distance of 1.44 Å than to the single bond distances of about 1.57 Å. We conclude that in crystals of **3**, **4**, and **5** the negative charges are delocalized at least equally between sulfur and oxygen and the P-S and P-O bond orders are nearer 1.5 than 2 and 1, respectively.



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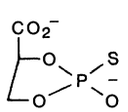

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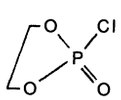

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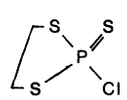
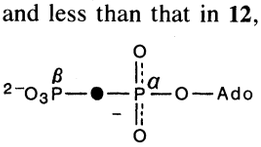


9

In crystals of **3**, **4**, and **5**, the P-S bond lengths could be shorter and the exocyclic P-O bond lengths longer than they would be in aqueous solutions. This is because in each of these crystals the exocyclic phosphorothioate oxygen is hydrogen-bonded to the ammonium countercations, an interaction that can be expected to distort these bond lengths by altering the degree of resonance delocalization that would exist in its absence. Crystal packing interactions are known to distort bond lengths, a case in point being the crystal of **5** in which the two C-O bond lengths in the carboxylate group are 1.23 and 1.28 Å, respectively. In other crystals, carboxylate C-O bond lengths are the same (*25*), reflecting the equivalence of carboxylate oxygens owing to delocalization of the negative charge.

Effects of oxygen-18 on phosphorus nuclear magnetic resonance (NMR) chemical shifts. It is well established that the ^{31}P-NMR chemical shifts for ^{18}O-bonded phosphates are upfield relative to the same ^{16}O-bonded species (*26, 27*). This is known as the ^{18}O-isotope shift, $\Delta\delta_p$, the magnitude of which is proportional to the number of ^{18}O's bonded to the phosphorus and also to the P-O bond order (*28, 29*). The dependence on bond order is especially clear from the linear correlation between the magnitude of $\Delta\delta_p$ and the squared P-O stretching frequencies in methyl-, dimethyl-, and trimethyl phosphates (*30*). Thus the magnitude of $\Delta\delta_p$ in **11** is greater than that in **10** and less than that in **12**, where the P-O bond orders involving ^{18}O in **10**, **11**, and **12** are 1, 1⅓, and 1½, respectively. These differences are useful for distinguishing bridging from nonbridging ^{18}O in phos-



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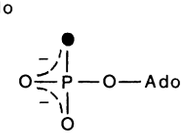

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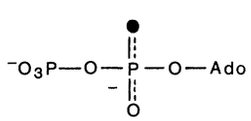
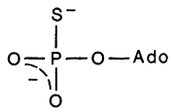


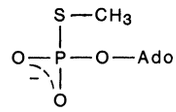
12

phoanhydrides and phosphoric esters.

A collection of data on ^{18}O-isotope shifts for ^{18}O-enriched thiophosphoanhydrides and alkyl phosphorothioates are presented in Table 1. Similar values for most of the species in Table 1 have been reported in other articles. The value for $\Delta\delta_p$ is 0.022 parts per million (ppm) when ^{18}O is attached by a single bond to P in a phosphorothioate, as in [α,β-*bridging*-^{18}O]$ADP_\alpha S$. This value is 0.017 ppm for bridging ^{18}O in phosphates such as **10**. In *S*-carbamoylethyl-[^{18}O]-phosphate the P-O bond order is 1⅓, consistent with the value of 0.027 ppm for the isotope shift. The value of the isotope shift for [^{18}O]AMPS is 0.033 (Table 1). If the P-O bond order were 1 and the P-S bond order 2, as in structure **1**, the value of the ^{18}O-isotope shift should be 0.022 ppm, whereas it is 0.033 ppm corresponding to a bond order of about 1.5. This is consistent with the structural formula **13a** for AMPS. Moreover, this value remains at 0.035 ppm in *S*-methyl-[^{18}O]AMPS **13b**, where S is bridging and the P-O bond order is 1.5 (*31*).

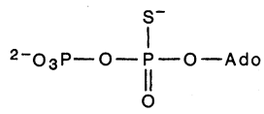


13a



13b

The ^{18}O isotope shift for [α-^{18}O]-$ADP_\alpha S$ is 0.037 ppm, about 1.8 times the value for single-bonded ^{18}O. These values suggest that the structure of $ADP_\alpha S$ is more accurately represented by **14** than by **2**. Ho (*31a*) has obtained similar values for $\Delta\delta_p$ in [α,β-*bridging*-^{18}O]$ATP_\alpha S$ and (S_p)-[β-^{18}O]$ATP_\beta S$ (*31a*).

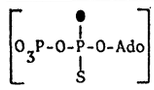
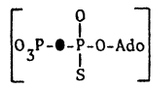
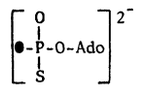
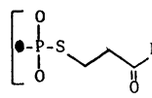


14

Isotope shifts very similar to those in Table 1 have been reported by Webb and Trentham for [β,γ-*bridging*-^{18}O]$ATP_\beta S$ and [β-^{18}O]$ATP_\beta S$ (*32*), and values similar to that for [^{18}O]AMPS have been reported by Gerlt and Wan for uridine 2′-[^{18}O]phosphorothioate and uridine 3′-[^{18}O]phosphorothioate (*33*). Gerlt and Wan also reported a $\Delta\delta_p$ of 0.041 ppm for *endo*-uridine-2′,3′-cyclic-[^{18}O]phosphorothioate, a value that is consistent with a P-O bond order of 2.

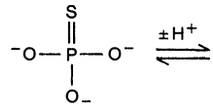
In discussing the different ^{18}O-isotope shifts for [β,γ-*bridging*-^{18}O]$ATP_\beta S$ and [β-^{18}O]$ATP_\beta S$, Cohn referred to singly as compared to doubly bonded ^{18}O (*34*), and in research papers she and co-workers illustrated the structures of $ATP_\beta S$ and $ATP_\alpha S$ with single bonds to sulfur, double bonds to oxygen, and a negative charge on sulfur (*11*). On the basis of the ^{18}O-isotope shifts in Table 1, P-O bond orders involving nonbridging oxygen at P_α of $ADP_\alpha S$ and $ATP_\alpha S$ and P_β of $ATP_\beta S$ are indeed closer to 2 than 1.

Table 1. Relative ^{31}P-NMR chemical shifts for ^{16}O- and ^{18}O-bonded phosphorothioates.

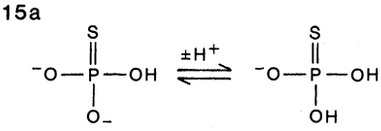
Compound	$\Delta\delta_p$ (ppm)*
	0.037 (P_α)
	0.022 (P_α), 0.017 (P_β)
	0.033
	0.027

*$\Delta\delta_p$ is here defined as the absolute value of the difference between the ^{31}P-NMR chemical shifts for the ^{18}O-enriched phosphorothioates compared with the unenriched compounds. The filled symbols in the structural formulas represent ^{18}O.

Oxygen-17 NMR chemical shifts. While ^{18}O-isotope shifts can be correlated with P-O bond order, they do not necessarily report on charge localization. There is evidence that ^{17}O-NMR chemical shifts for ^{17}O-enriched carboxylic acids, phosphates, and phosphorothioates are sensitive to the electrostatic charge states of ^{17}O. The chemical shift for ^{17}O in formate and C-1 of acetate anions are 23 ppm downfield from those for ^{17}O in the free acids (*35, 36*). Similar data are available for phosphates and phosphorothioates. Gerlt and co-workers measured the ^{17}O-NMR chemical shifts of [^{17}O]phosphate and [^{17}O]thiophosphate as a function of *p*H and observed upfield shifts in the ^{17}O-NMR signals as they titrated the trianions with acid (*37*). They observed a 13 ppm upfield shift in the ^{17}O NMR-signal on going from $P^{17}O_4^{3-}$ to $HP^{17}O_4^{2-}$ and a second 13 ppm upfield shift on going to $H_2P^{17}O_4^-$. The same transitions in the case of [^{17}O]thiophosphate were accompanied by 18.4 ppm upfield shifts in the ^{17}O-NMR signals. Gerlt and co-workers rationalized their observations on the basis of negative charge localization on oxygen resulting from a bond order of 2 for the P-S bond in thiophosphates. They reasoned that the 13 ppm shifts in the case of ^{18}O-phosphate reflected the difference of 1/4 negative charge per oxygen in PO_4^{3-} compared with HPO_4^{2-} and in HPO_4^{2-} compared with $H_2PO_4^-$. The differences of 18.4 ppm in the ^{17}O-NMR signals for the corresponding ^{17}O-thiophosphate species would then reflect a larger difference in charge per oxygen, which they attributed to the presence of a double bond between phosphorus and sulfur. The relevant species are **15a**, **15b**, and **15c**, in which the negative charge difference between **15a** and **15b** is 1/3 per oxygen. The charge difference between **15b** and **15c** is also 1/3 per oxygen.

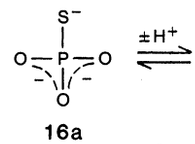


15a

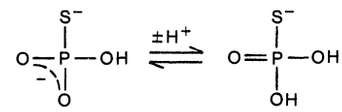


15b **15c**

While the results of ^{17}O-NMR analysis reflect larger differences in negative charge per oxygen among the thiophosphate species than the corresponding species of phosphate, this difference does not necessarily arise from structures **15a**, **15b**, and **15c**. The same difference would arise from structures **16a**, **16b**, and **16c** for these species. In these



16a

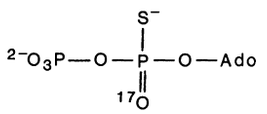


16b **16c**

structures a negative charge is localized on sulfur and the remaining charge delocalized between two or more oxygens. This also results in a charge difference of 1/3 per oxygen on going from **16a** to **16b** or from **16b** to **16c**. Therefore, while the ^{17}O chemical shifts may be correlated with a higher degree of charge localization in the thiophosphates than in the corresponding phosphates, they do not resolve the question of whether negative charge is localized on oxygen or sulfur.

Available data on ^{31}P-^{17}O coupling constants demonstrate a linear correlation with P-O bond orders in phosphates and thiophosphates. Sammons *et al.* noted the linear relationship between the ^{31}P-^{17}O coupling constants and the $^{18}O\Delta\delta_p$ values in 12 compounds (*38*). The $J_{P\text{-}O}$ values for ^{31}P-^{17}O for double bonds range from 154 to 160 hertz in

$(CH_3O)_3P^{17}O$, $Ph_3P^{17}O$, and $(PhO)_3P^{17}O$. The coupling constant for $(PhO)_2P^{17}O_2^-$, with a P-O bond order of 1.5, is 121 hertz. That for [α-$^{17}O_2$]ADP is 123 hertz, also corresponding to the expected bond order of 1.5; but the value for β-cyanoethyl-[α-^{17}O]ADP$_\alpha$S **17** is 147 hertz, corresponding to a bond order of about



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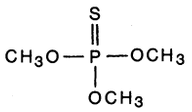
1.8. Gerlt has also reported larger values of $J_{P\text{-}O}$ for $PS^{17}O_3$ than $P^{17}O_4$ (*37*).

Vibrational spectra of phosphorothioates. Steger and Martin measured infrared and Raman spectra of PSO_3^{3-} and $HPSO_3^{2-}$ in aqueous solutions, KBr pellets, and paraffin mulls (*39, 40*). They concluded that P-S bonding is little or not at all involved in resonance effects, whereas the P-O bonds are involved in delocalization of negative charges (*39, 40*). They found the P-S stretching frequency for PSO_3^{3-} and $HPSO_3^{2-}$ to be 436 cm^{-1} and 438 cm^{-1}, respectively, with no sign of the S-H group in $HPSO_3^{2-}$. If delocalization of charge in these species involved P-S bonding, these frequencies would differ. The P-O stretching frequencies differed significantly, however; they were 960 cm^{-1} for PSO_3^{3-} and 1038 cm^{-1} for $HPSO_3^{2-}$, indicating charge delocalization through P-O bonds. Steger and Martin concluded that the structures of PSO_3^{3-} and $HPSO_3^{2-}$ are best represented as **16a** and **16b**, with P-O bond orders of 1⅓ and 1½, respectively. The structures **15a** and **15b** are inconsistent with the higher P-O frequency in the dianion compared with the trianion.

This conclusion was further supported by the values of force constants for the P-S bond in related molecules. Steger and Martin calculated the P-S force constant 2.68 m · dyne/Å for PSO_3^{3-} (*40*) and Goubeau reported 2.86 m · dyne/Å for the same species (*41*). Goubeau also reported a P-S force constant of 4.52 m · dyne/Å for O,O,O-trimethyl phosphorothioate **18**, clearly demonstrating



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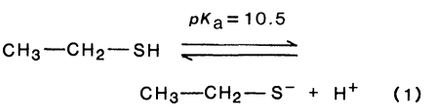
higher P-S bond order in this compound than in the phosphorothioate trianion. Goubeau concluded after a detailed analysis that the P-S bond order even in O,O,O-trimethyl phosphorothioate **18** is less than 2 (*41*). In any case the vibrational spectra are inconsistent with structures **15a**, **15b**, and **15c** but are consistent with **16a**, **16b**, and **16c**.

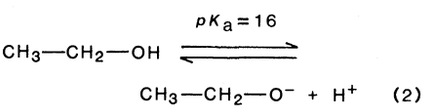
Acid strengths of phosphates and thiophosphates. Phosphoric and thiophosphoric acids are weak tribasic acids. Thiophosphoric acid is somewhat stronger than phosphoric acid, with pK_a values of 1.67, 5.40, and 10.14 (*42*), compared with values of 2.1, 7.2, and 12.3 for phosphoric acid (*43*).

As seen from these values, each species of thiophosphate is stronger as an acid than the corresponding species of phosphate. This relation is inconsistent with the proposed localization of negative charge on oxygen in the mono-, di-, and trianionic forms of thiophosphate. It is, however, consistent with the localization of negative charge on sulfur in these species. These conclusions follow from the known properties of analogous oxygen and sulfur acids and generally accepted theories accounting for the relative strengths of acids.

A major factor that must be taken into account in rationalizing the relative strengths of analogous sulfur and oxyacids is the fact that in aqueous solutions a negative charge localized on sulfur is far less unstable than one localized on oxygen. This is because of the larger size and polarizability of sulfur relative to oxygen, allowing the charge density in a thiolate anion to be less than that in an oxyanion. This contributes to the greater acid strengths of thiols relative to alcohols. Thus the pK_a of ethanethiol, Eq. 1, is 10.5 while that for ethanol (Eq. 2), is 16. This large difference exists despite



$$CH_3—CH_2—SH \xrightleftharpoons{pK_a = 10.5} CH_3—CH_2—S^- + H^+ \quad (1)$$

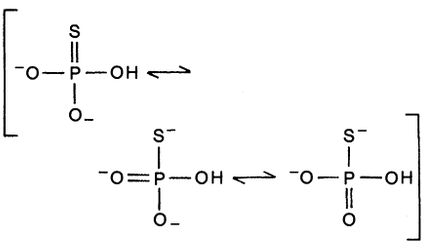


$$CH_3—CH_2—OH \xrightleftharpoons{pK_a = 16} CH_3—CH_2—O^- + H^+ \quad (2)$$

much greater solvation of ethoxide compared with ethanethiolate. Many similar examples can be cited, so ethanethiol and ethanol are not a special case.

Given the above, let us consider the structure of the thiophosphate monanion, shown as **15c**, where the negative charge is localized on oxygen, or **16c**, in which it is localized on sulfur. We expect it to be less stable on oxygen than sulfur. Considering the dianion, the situation is clearer because in **15b** two negative charges are localized on two oxygens, whereas in **16b** one charge is localized on sulfur and the second is delocalized between two oxygens. Similarly, in **15a** three negative charges are localized on three oxygens, whereas in **16a** one charge is localized on sulfur and two are delocalized over three oxygens. It seems clear that structures **16a**, **16b**, and **16c** should be more stable than **15a**, **15b**, and **15c**.

Of course, the true structures of these ions are resonance hybrids; and the foregoing discussion essentially identifies which of the canonical resonance forms are dominant in the hybrids. Considering the thiophosphate dianion the canonical forms are given below. The structure on the left is **15b** and contributes least to the hybrid, since it concentrates charge on oxygen. The two structures at the right are energetically identical degenerative resonance forms that are represented as a hybrid structure in **16b** above. The two



forms contribute much more to the hybrid for two reasons. First, to the extent that charge is localized it is on sulfur rather than oxygen. Second, they are structurally equivalent resonance forms which permit a high degree of charge stabilization through delocalization.

Regardless of resonance effects or the relative stabilities of oxyanions and thiolates, the greater acid strengths of thiophosphates compared with phosphates are inexplicable in terms of inductive effects. Since sulfur is less electronegative than oxygen, its substitution for oxygen should be acid-weakening rather than acid-strengthening.

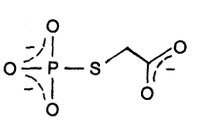
That the differences in acid strength between thiophosphates and analogous phosphates are smaller than those between alcohols and thiols can also be rationalized on the basis of the foregoing considerations. Two factors governing the relative acid strengths of phosphates and thiophosphates are the stabilization of negative charge by localization on sulfur and stabilization by resonance delocalization over two or more oxygens. In thiophosphates, there is less resonance delocalization than in analogous phosphates because of the smaller number of oxygens and the charge density on sulfur. This is an acid-weakening effect. The compensatory acid-strengthening effect is the greater intrinsic stability of thiolate anions relative to oxyanions. The balance of these factors results in slightly greater acid strengths for thiophosphates. The same factors govern the relative acid strengths of acetic and thio-

acetic acids, whose pK_a values are 4.8 and 3.8, respectively (*44*).

Conclusions. The available experimental evidence bearing on the structures of thiophosphate anions is inconsistent with a P-S bond order of 2 and P-O bond order of 1 with charge localization on oxygen. The weight of evidence favors structural formulas **13** and **14** for AMPS and $ADP_\alpha S$ as more representative of the true structure than those such as **1** in which P=S double bonds are shown. The structures of thiophosphate diester monoanions such as **3**, **4**, and **5** may involve similar bond orders and charge distribution in aqueous solutions, although in crystals of their ammonium salts the P-O and P-S bond orders are closer to 1.5.

The localization of charge on sulfur in phosphorothioate anions must be taken into account in rationalizing the interactions of cyclic AMPS, $ADP_\alpha S$, $ATP_\alpha S$, and $ATP_\beta S$ with macromolecules such as enzymes. Similarly, charge localization on sulfur may have significant implications for secondary structure in phosphorothioate analogs of oligonucleotides. It is also a factor contributing to the interactions of $ATP_\alpha S$, $ATP_\beta S$, and related nucleotide analogs with metal ions. Pecoraro *et al.* have measured stability constants for Mg^{2+} and Cd^{2+} complexes of nucleotides and analogous sulfur-containing nucleotides (*45*). The Mg^{2+} is known to coordinate preferentially with oxygen, and Cd^{2+} binds preferentially with sulfur (*8, 10*). The stability constants for Mg^{2+}ATP, Mg^{2+}AT-$P_\alpha S$, Cd^{2+}ATP, and $Cd^{2+}ATP_\alpha S$ among others appear to be more easily correlated on the basis of phosphorothioate structures with charge localization on sulfur than with the traditional structures in which charge is localized on oxygen (*45*).

Ash *et al.* have attempted to rationalize some of the effects on ^{31}P-NMR chemical shifts of substituting S for O in phosphoric esters (*46*). They observed that the phosphorus chemical shift for S-alkyl phosphorothioates such as **19** are 16.4 ppm downfield from the parent



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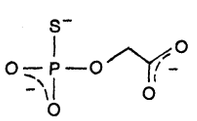

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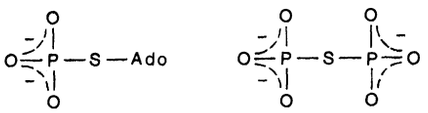
phosphates, while the chemical shifts for the corresponding O-alkyl phosphorothioates such as **20** are 39 to 40 ppm downfield. Ash *et al.* rationalized the relative shifts on the basis of differences in P-S bond orders for bridging and nonbridging S (*46*). It is possible that these P-S bond orders differ within a narrow range near 1 and that the phosphorus chemical shifts are very sensitive to small differences in P-S bond order. However, the effects of S on phosphorus chemical shifts are poorly understood and vary over a wide range. A small collection of S-bridging compounds exhibits a range of downfield phosphorus chemical shifts relative to parent phosphates, from +12.4 ppm for **21** to +20.7 ppm for **22** to +26.4 ppm for **23** (*47, 48*).



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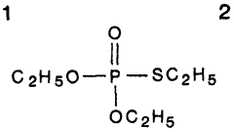

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We emphasize that we have here considered bond order and charge localization in thiophosphate anions, as distinguished from the free acids. The problem of negative charge stabilization does not arise in the free acids, so phenomena such as resonance delocalization and preferential charge localization on sulfur do not come into play. It is conceivable that P-S bond orders may be greater than 1 or even near 2 in the free acids.

References and Notes

1. The abbreviations are: AMPS, adenosine 5′-O-phosphorothioate; $ATP_\alpha S$, adenosine 5′-O-[1-thiotriphosphate]; $ATP_\beta S$, adenosine 5′-O-[2-thiotriphosphate]; $ATP_\gamma S$, adenosine 5′-O-[3-thiotriphosphate]; $ADP_\alpha S$, adenosine 5′-O-[1-thiodiphosphate]; cyclic AMPS, adenosine 3′,5′-cyclic phosphorothioate. The symbols (R_p) and (S_p) refer to the absolute configurations at chiral phosphorus centers.
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