Symmetrical Monothiopyrophosphate

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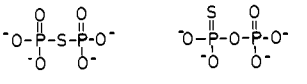
Abstract: Symmetrical monothiopyrophosphate (**1**) has been synthesized by dealkylation of the tetramethyl ester and isolated as partially cleaved barium and lithium salts containing variable amounts of carbonate. The half-life for the hydrolysis of the lithium salt to phosphate and thiophosphate is 4.23 h in pH 10.05 buffer and 0.92 h in pH 9.00 buffer at 25 °C. These values, however, are subject, at the buffer and carbonate concentrations employed, to large negative salt effects. No isomerization of **1** to unsymmetrical monothiopyrophosphate (**2**) has been observed under these conditions. Yeast inorganic pyrophosphatase cleaves **1** at pH 9.95 to phosphate and thiophosphate.

Pyrophosphate salts and their derivatives have long been a subject of chemical and biochemical investigation. Analogues of pyrophosphate (**3a,b**) that contain carbon[1] or nitrogen[2,3] atoms in place of the bridge oxygen are known. Tetraesters of symmetrical monothiopyrophosphate have been prepared by Michalski and his colleagues.[4] Salts of symmetrical monothiopyrophosphate, **1**, however, are unknown and, indeed, are thought to be unstable toward isomerization to their unsymmetrical isomers.[5] We report here the synthesis and chemical properties of the tetralithium salt of **1**.



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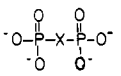


3a, X = CH_2

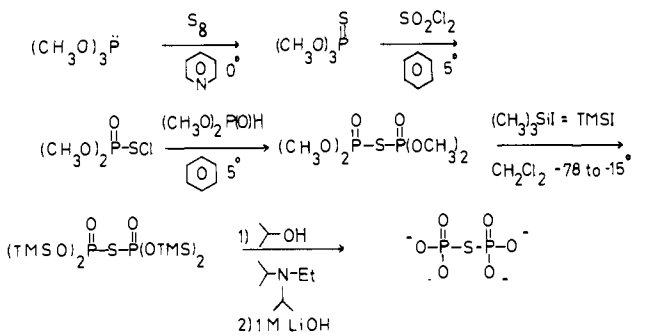
3b, X = NH

The synthesis of tetralithium symmetrical monothiopyrophosphate is outlined in Scheme I. The key to the synthesis is the dealkylation of tetramethyl symmetrical monothiopyrophosphate by using reagents and conditions that do not cause cleavage or isomerization of the labile phosphorus–sulfur–phosphorus bond. This was accomplished by reaction of the tetramethyl ester with trimethylsilyl iodide followed by cleavage of the trimethylsilyl groups using *N*-ethyldiisopropylamine and 2-propanol. The product was dissolved immediately in 1 M lithium hydroxide to give, presumably, the tetraanion, **1**, which, due to the large negative charge at phosphorus, resists nucleophilic attack leading to isomerization to **2** or hydrolysis to phosphate and thiophosphate. This paper presents the data to support this synthetic scheme and a discussion of the chemical and enzymatic properties of **1**.

Experimental Section

Materials and Methods. ^{31}P NMR spectra were recorded at 81 MHz on a Bruker WP200SY spectrometer or at 24 MHz on a Bruker-Physic HX 60 spectrometer, equipped for Fourier transform. Chemical shifts are given relative to 85% phosphoric acid as external standard and reported as positive values for downfield shifts. ^1H NMR spectra were recorded on the same spectrometers at 200 and 60 MHz, respectively, with tetramethylsilane as an internal reference. Infrared spectra were obtained with a Perkin-Elmer 710B infrared spectraphotometer. Elemental analyses were performed by Mikroanalytisches Labor Pascher, Bonn, Federal Republic of Germany.

Scheme I



Benzene and dichloromethane were dried by distilling from calcium hydride and phosphorus pentoxide, respectively, and used immediately following distillation. Pyridine was dried over molecular sieves. Distilled water was used to prepare all aqueous solutions.

Trimethyl phosphite, dimethyl phosphite, and trimethylsilyl iodide (EGA-Chemie), triethyl thiophosphate and trimethylsilyl bromide (Aldrich), diethyl phosphite (Fluka), and dibenzyl phosphite (Schuchardt) were used as supplied. Inorganic pyrophosphatase (yeast, 5 mg/mL, 200 units/mg) and alkaline phosphatase (calf intestine, 3.05 mg/0.61 mL, 7500 U) were purchased from Boehringer Mannheim.

Fractions obtained during column chromatography were assayed for sulfur-containing products by addition of an acid solution of palladium(II) chloride to selected aliquots.[6]

For kinetic experiments, solutions were pipetted into stoppered tubes and stirred magnetically in a water or silicone oil thermostat at the appropriate temperature. For thermolyses, a drying pistol, heated by a surrounding oil bath, was used. The ^{31}P NMR spectra were recorded at 9 °C. Except as otherwise noted, reaction products were identified by ^{31}P NMR spectroscopy in the absence and again in the presence of authentic material. Since the ^{31}P NMR shifts of these compounds are both solvent and pH dependent, this method was necessary to ensure proper identification of reaction products.

Thin-layer chromatography was performed on poly(ethylene imine) cellulose plates in 1 or 0.5 M triethylammonium bicarbonate (TEAB) at 5 °C and proved to be a useful adjunct to ^{31}P NMR spectroscopy for the identification of reaction products. The substance to be chromatographed was dissolved in pH 13 borate buffer or, if already in aqueous solution, titrated to pH 13 before spotting the thin-layer plate. Following development, the plates were dried and then sprayed with either Hanes reagent,[7-9] to identify phosphorus-containing products, or an acid solution of palladium(II) chloride,[6] to identify sulfur-containing products. In 1 M TEAB, a reaction mixture containing phosphate, thiophosphate, diphosphoryl disulfide, symmetrical monothiopyrophosphate, and unsymmetrical monothiopyrophosate gave R_f values of 0.68, 0.48, 0.35, 0.30, and 0.10 respectively. In 0.5 M TEAB, these values were 0.55, 0.28, 0.15, 0.11, and less than 0.05, respectively.

***O,O,O*-Trimethyl phosphorothioate**[10] was synthesized by a procedure analogous to that of Burgers and Eckstein.[11] Trimethyl phosphite (10

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g, 0.08 mol) in 30 mL of dry pyridine was added under nitrogen over 30 min to a suspension of sulfur (2.58 g, 0.08 mol) in 30 mL or pyridine with vigorous stirring at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then for 1 h at room temperature. Evaporation of the pyridine and distillation of the residue gave 8.8 g (70%) of *O,O,O*-trimethyl phosphorothioate as a colorless oil: bp 44–45 °C (5 mm) [lit.[10] 82 °C (20 mm)]; proton-decoupled ^{31}P NMR ($CDCl_3$) δ 73.4 [lit.[12] 73.0]; ^1H NMR ($CDCl_3$) δ 3.79 (d, J = 14 Hz). The compound was approximately 81% pure by ^{31}P NMR and ^1H NMR spectroscopy, the impurity being presumably the thiolate isomer, *O,O*-dimethyl *S*-methyl phosphorothioate. The presence of the thiolate isomer did not appear to cause any problems in subsequent synthetic steps.

Tetralithium Symmetrical Monothiopyrophosphate. It should be pointed out here that tetraalkyl symmetrical monothiopyrophosphates are possibly extremely toxic and care should be taken to avoid inhalation and contact with skin. Tetramethyl symmetrical monothiopyrophosphate was synthesized by a procedure analogous to that previously used by Michalski etal.[4] for the corresponding ethyl ester. Freshly distilled sulfuryl chloride (172 mg, 1.28 mmol) in 6 mL of dry benzene was added under nitrogen over 30 min to *O,O,O*-trimethyl phosphorothioate (200 mg, 1.28 mmol) in 6 mL of benzene with vigorous stirring at 5 °C. The reaction mixture was then stirred for 15 min at 5 °C. The proton-decoupled ^{31}P NMR ($CDCl_3$) spectrum of the reaction mixture showed one major signal at δ 23.82 corresponding presumably to dimethoxyoxophosphoranesulfenyl chloride. Dimethyl phosphite (141 mg, 1.28 mmol) in 6 mL of benzene was then added over 30 min to the reaction mixture with vigorous stirring at 5 °C. After 15 min at 5 °C, the benzene and other volatiles were removed under vacuum at approximately 15 °C over 5–10 min. The proton-decoupled ^{31}P NMR ($CDCl_3$) spectrum of the reaction mixture showed one major signal at δ 19.45 corresponding presumably to tetramethyl symmetrical monothiopyrophosphate. No attempt was made to isolate either of these intermediates since they proved to be very labile and readily decomposed to give complex mixtures of products. The observed ^{31}P NMR shifts, however, are similar to those reported for the corresponding ethyl and isopropyl esters: proton-decoupled ^{31}P NMR (neat)[4] diethoxyoxophosphoranesulfenyl chloride, δ 17.8; tetraethyl symmetrical monothiopyrophosphate, δ 14.3; diisopropoxyoxophosphoranesulfenyl chloride, δ 15.7; tetraisopropyl symmetrical monothiopyrophosphate, δ 12.5.

Trimethylsilyl iodide (1.024 g, 5.12 mmol) in 4 mL of dry dichloromethane was added under nitrogen over 15 min to the crude tetramethyl symmetrical monothiopyrophosphate, synthesized as described above, in 4 mL of dichloromethane with vigorous stirring at −70 °C. The reaction mixture was stirred for 6 min at −15 °C. *N*-Ethyldiisopropylamine (1.324 g, 10.24 mmol) and 2-propanol (0.614 g, 10.24 mmol) in 4 mL of dichloromethane were then added quickly to the reaction mixture with vigorous stirring at −70 °C. The cooling bath was removed, and the solvent and other volatiles were evaporated under vacuum over 5–10 min. The product was dissolved immediately in 5 mL of 1 M lithium hydroxide. The ^{31}P NMR (D_2O, pH 13) spectrum (Figure 1a) shows one major signal at δ 15.9, which shows no coupling to phosphorus or hydrogen atoms. In addition to this product, present in 84 ± 2% yield, phosphate (10 ± 2%) and two unidentified products (less than 4 ± 1%) were observed. In some ^{31}P NMR spectra, variable amounts of thiophosphate and diphosphoryl disulfide were identified. The product mixture was applied to two Dowex AG1-X8 (Serva, 100–200 mesh) columns (2 × 20 cm), each of which had been washed with 200 mL of 1 M hydrochloric acid followed by 200 mL of water and 200 mL of 0.1 M lithium hydroxide. Each column was eluated at a rate of 1.2 mL/min under nitrogen at 5 °C with a linear gradient of 500 mL each of 0.0677 and 0.0687 M lithium bromide in 0.1 M lithium hydroxide. The fractions were tested for sulfur-containing products, and those eluting between 0.0680 and 0.0683 M lithium bromide were immediately frozen in liquid nitrogen and stored at −20 °C: ^{31}P NMR (D_2O pH 13) δ 15.7 (Figure 1b). Addition of six volumes of 2-propanol to the fractions at room temperature gave a precipitate (300–600 mg) which was isolated by centrifugation. The ^{31}P NMR (D_2O, pH 13) spectrum of the precipitate shows the signal at δ 15.7 in addition to signals for phosphate and thiophosphate corresponding to approximately 17% hydrolysis. The product contains variable amounts of lithium carbonate which was identified by ^{13}C NMR spectroscopy. Anal. Calcd for 23.71% $Li_4O_6P_2S$ and 76.29% Li_2CO_3: P, 6.75; S, 3.49; C, 12.40. Calcd (assuming 17% hydrolysis): P, 6.66; S, 3.45; C, 12.40. Found: P, 6.56; S, 3.56; C, 12.05.

If carbonate-free lithium hydroxide (prepared by slowly dissolving lithium metal in freshly distilled water under a vigorous stream of nitrogen) is used in all synthetic and chromatographic steps requiring lithium hydroxide and if care is taken to collect the fractions and to precipitate the product under a stream of nitrogen as well as to carry out the centrifugation in stoppered tubes, a solid (50–60 mg) is obtained which contains at least 42% lithium carbonate and is 50% cleaved as analyzed by ^{31}P NMR spectroscopy. Anal. Calcd for 57.76% $Li_4O_6P_2S$ and 42.24% Li_2CO_3: P, 16.43; S, 8.50; C, 6.87. Calcd (assuming 50% hydrolysis): P, 15.80; S, 8.18; C, 6.87. Found: P, 16.20; S, 8.38; C, 7.29.

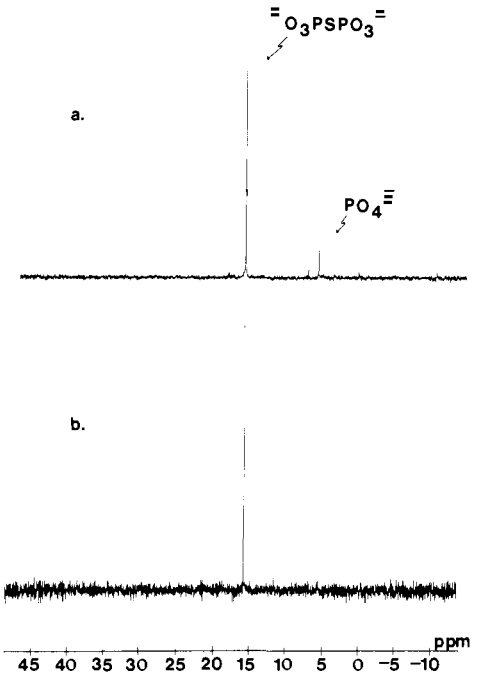

Figure 1. ^{31}P NMR (D_2O, pH 13) spectra of symmetrical monothiopyrophosphate (a) before and (b) after column chromatography.

Dibarium Symmetrical Monothiopyrophosphate. Barium bromide (20 mg) in 1 mL of carbonate-free 0.1 M lithium hydroxide was added at room temperature to each fraction (approximately 7 mL) obtained above. The slightly discolored precipitate was isolated by centrifugation and dried in vacuo over phosphorus pentoxide at room temperature. Anal. Calcd for 85.37% $Ba_2O_6P_2S$ and 14.63% $BaCO_3$: P, 11.38; S, 5.89; C, 0.89. Found: P, 11.40; S, 5.88; C, 0.69. The barium salt was insoluble in basic aqueous solutions.

***O,O,O*-Tribenzyl phosphorothioate** was synthesized from tribenzyl phosphite[13] by a procedure analogous to that of Burgers and Eckstein.[11] Tribenzyl phosphite (2.112 g, 6.81 mmol) and sulfur (0.192 g, 6 mmol) were stirred in 30 mL of dry pyridine under nitrogen for 3.5 h at room temperature. The pyridine was removed under vacuum at approximately 50 °C, followed by addition and evaporation of two 5-mL volumes of toluene. The product was applied to a Kieselgel 60 column (120 g, 3 × 32 cm) and eluted with 1:1 chloroform/petroleum ether (40–60 °C) at the rate of 3–4 drops/s. The fractions were monitored with a UV detector, and the product eluting between 322 and 522 mL was collected. Evaporation of the solvent gave a white solid (1.625 g, 70%): mp 48.5–49.5 °C; proton-decoupled ^{31}P NMR ($CDCl_3$) δ 68.08; ^1H NMR ($CDCl_3$) δ 5.07 (d, J_{H-P} = 10 Hz, 6 H), 7.33 (m, 15 H); IR (KBr) 3040, 2955, 1500, 1455, 1380, 1265, 1220, 990(b), 880, 810, 760, 700 cm^{-1}. The solid was recrystallized from petroleum ether (40–60 °C) and dried in vacuo over phosphorus pentoxide at room temperature. Anal. Calcd for $C_{21}H_{21}O_3PS$: C, 65.61; H, 5.51; P, 8.06; S, 8.34. Found: C, 65.67; H, 5.50; P, 8.20; S, 8.16.

Tetrabenzyl symmetrical monothiopyrophosphate was synthesized analogously to the methyl and ethyl esters. Sulfuryl chloride (35 mg, 0.26 mmol) in 3 mL of dry benzene was added to *O,O,O*-tribenzyl thiophosphate (100 mg, 0.29 mmol) in 3 mL of benzene over 60 min with vigorous stirring at 5 °C under nitrogen. The reaction mixture was then added to dibenzyl phosphite (78 mg, 0.33 mmol) in 3 mL of benzene over 30 min with vigorous stirring at 5 °C under nitrogen. The dibenzyl phosphite contained approximately 13% benzyl alcohol by ^1H NMR analysis, and all efforts to remove this impurity were unsuccessful. The proton-decoupled ^{31}P NMR ($CDCl_3$) spectrum showed one major signal at δ 16.04 corresponding presumably to tetrabenzyl symmetrical mono-

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thiopyrophosphate. In addition, four minor products were observed.

Tetracyclohexylammonium Diphosphoryl Disulfide. The crude tetraamonium salt (550 mg) was synthesized according to the procedure of Ladwig and Thilo.[14] It was applied to a DEAE-Sephadex A-25 column (3 × 30 cm) equilibrated with 0.05 M triethylammonium bicarbonate (TEAB). The column was eluted at 5 °C with a linear gradient of 2.5 L each of 0.2 and 0.4 M TEAB. The fractions were tested for sulfur-containing products, and those eluting between 0.25 and 0.35 M TEAB were combined and concentrated after addition of cyclohexylamine (1 g, 0.01 mol). Following repeated addition and evaporation of methanol, the cyclohexylammonium salt (529 mg) was obtained as a slightly yellow solid: ^{31}P NMR (D_2O, pH 13) δ 16.54 (s). Its structure was confirmed by X-ray crystal structure analysis of crystals obtained from aqueous ethanol.[15]

Unsymmetrical monothiopyrophosphate was synthesized analogously to the procedure of Tridot and Tudo[16] as modified by Dunaway-Mariano.[17] Trilithium thiophosphate hydrate[18] (2.8 g, 0.0121 mol) was converted to the free acid form by passage through a Dowex 50W-X4 (H^+ form) ion-exchange column (3.5 × 33 cm). The solution was titrated to pH 8 with aqueous sodium hydroxide. Water was removed under vacuum and the product (1.6 g) dried overnight over phosphorus pentoxide and then heated for 2 h at 130–140 °C. The ^{31}P NMR spectrum of the products showed approximately 17% of the desired product, unsymmetrical monothiopyrophosphate, in addition to phosphate (18%) and pyrophosphate (62%). The products were applied to a DEAE-Sephadex A-25 column (3 × 30 cm) equilibrated with 0.05 M TEAB. The column was eluted with a linear gradient of 2 L each of 0.2 and 0.4 M TEAB at the rate of 2.5 mL/min, and the fractions were assayed for sulfur-containing products. The product, eluting between 0.30 and 0.33 M TEAB, was isolated by addition of cyclohexylamine to the fractions and evaporations to dryness, followed by repeated addition and evaporation of methanol: ^{31}P NMR (D_2O, pH 13.1) δ 30.83 (d, J = 30 Hz), −5.98 (d, J = 30 Hz).

Kinetic Methods. In kinetic studies, standard solutions of tetralithium symmetrical monothiopyrophosphate containing variable amounts of lithium carbonate were prepared in 500 mM borate buffer, pH 9.4, for the experiments at pH 10.05 and in 500 mM borate buffer, pH 8.6, for the experiments at pH 8.00 or 9.00. The pH was adjusted to the appropriate value after removal by centrifugation of any insoluble material (less than 2%). After the solutions had been heated for appropriate times, aliquots of 300 μL were removed and diluted to 2.5 mL with 50 mM borate buffer, pH 13, containing deuterium oxide and a standard for integration. For the hydrolysis of **1** in solutions containing less than 50 mM lithium carbonate, 5 M sodium hydroxide (100 μL) was added to the ^{31}P NMR sample. The pHs of these ^{31}P NMR samples were 12.9 and 13.6, respectively. Tetrasodium pyrophosphate was used as a standard for integration at pH 10.05 since no pyrophosphate was observed in the hydrolysis mixture under these conditions. At pH 8.00 and 9.00, tetrasodium methylene diphosphonate was used as a standard for integration since some pyrophosphate was observed in the hydrolysis mixture. The pyrophosphate, however, appears to have been formed during addition of small amounts of 1 M hydrochloric acid to adjust the pH of the solution. At pH 9.00, addition of either standard gave the same rates of hydrolysis, indicating that little or no pyrophosphate was formed during the hydrolysis itself. No measureable hydrolysis occurred during the measurement of the ^{31}P NMR spectra at 9 °C.

Total phosphate and thiophosphate concentrations were determined by the method of Chen et al.[19] Under the conditions of the assay, all remaining symmetrical monothiopyrophosphate was cleaved to phosphate and thiophosphate. A linear standard curve was prepared by using equimolar mixtures of phosphate and thiophosphate.

Kinetics. The rates of hydrolysis of tetralithium symmetrical monothiopyrophosphate are presented in Table I. The ^{31}P NMR spectra for a typical determination are shown in Figure 2. First-order half-lives were calculated by the least-squares method: the data yield rate constants of good quality (r 0.93; see Table I).

Reaction of Tetralithium Symmetrical Monothiopyrophosphate with Inorganic Pyrophosphatase. Standard solutions of 9.2 mM tetralithium symmetrical monothiopyrophosphate that contained approximately 300 mM lithium carbonate were prepared in 500 mM borate buffer pH 9.3, containing 100 mM magnesium chloride. The pH was then adjusted to 10.05 with hydrochloric acid. Standard solutions of tetrasodium pyrophosphate decahydrate (10.24 mg/mL, 23 mM) were similarly prepared. Both solutions were cloudy due to the formation of some dimagnesium

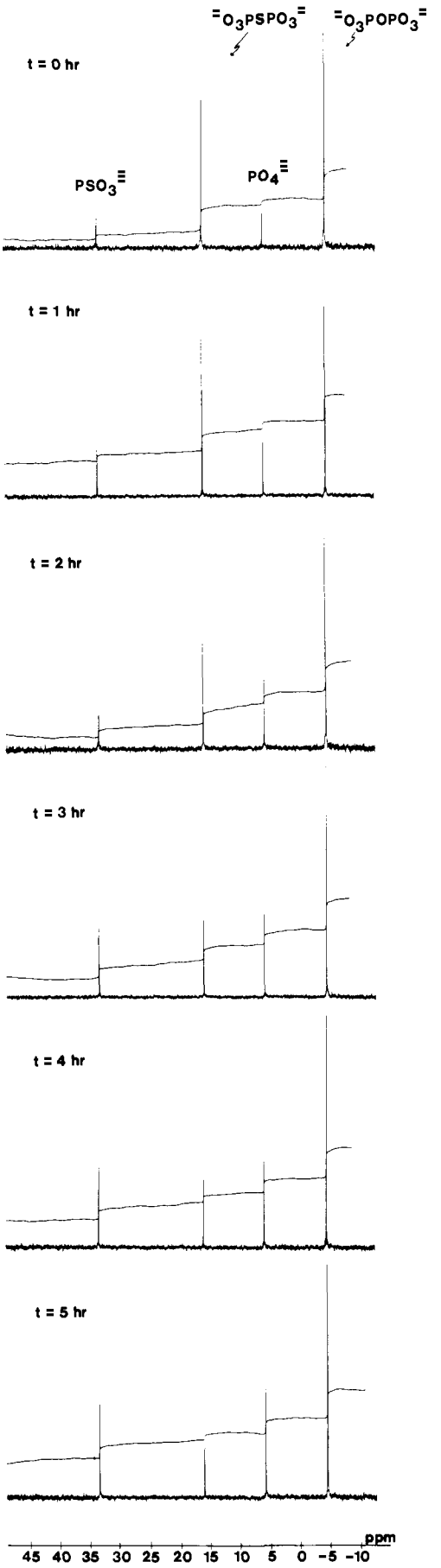

Figure 2. ^{31}P NMR (D_2O, pH 12.9) spectra of the hydrolysis of tetralithium symmetrical monothiopyrophosphate (10.8 mM), containing approximately 90% lithium carbonate, in 500 mM borate buffer, pH 10.05 ± 0.02, at 30.0 ± 0.3 °C (pyrophosphate added as a standard for integration).

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Table I. Rates of Hydrolysis of Tetralithium Symmetrical Monothiopyrophosphate (1) in 500 mM Borate Buffer

[1], mM		$[Li_2CO_3]$, mM		$[Li_2SO_4]$, mM					
I[b]	II[b]	I[b]	II[b]	I[b]	II[b]	T, °C	pH	$t_{1/2}$, h	r
7.8	8.4	314	315			25.0 ± 0.3	10.05 ± 0.02	4.23 ± 0.21[a]	0.963
5.6	10.8	322	307			30.0 ± 0.3	10.05 ± 0.02	2.17 ± 0.11[a]	0.993
17.8	17.8	286	286			35.0 ± 0.3	10.05 ± 0.02	1.33 ± 0.07[a]	0.983
2.6	10.8	331	307			25.0 ± 0.3	9.00 ± 0.02	0.92 ± 0.05[a]	0.973
4.4		325				25.0 ± 0.3	8.00 ± 0.02	0.10 ± 0.01	0.999
3.4	4.4	37	35			25.0 ± 0.3	10.05 ± 0.02	0.92 ± 0.05[a]	0.935
4.4	4.8	41	40	284	315	25.0 ± 0.3	10.05 ± 0.02	4.23 ± 0.21[a]	0.975

[a] Average of two determinations. [b] I and II refer to experimental runs 1 and 2, respectively.

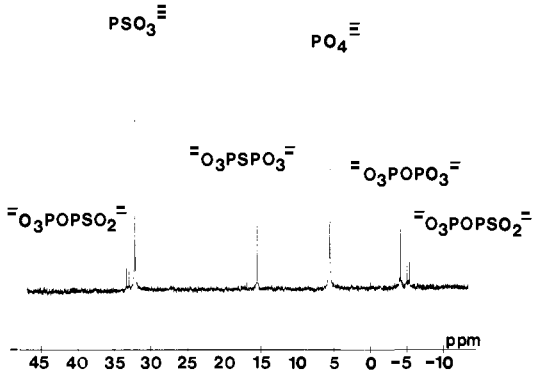


Figure 3. ^{31}P NMR (D_2O, pH 13) spectrum of the thermolysis of tetralithium symmetrical monothiopyrophosphate, containing approximately 90% lithium carbonate, at 50 °C in vacuo for 23 h.

symmetrical monothiopyrophosphate and dimagnesium pyrophosphate, respectively, but were used as such with care being taken to stir the solutions well before removing aliquots for ^{31}P NMR spectroscopy. Inorganic pyrophosphatase (2 μL) was added to 1 mL of each solution to give a final pH of 9.95 ± 0.02, and the solutions were stirred in a water thermostat at 25.0 ± 0.03 °C. Aliqouts (300 μL) were removed at various times and diluted to 2.5 mL with 50 mM borate buffer pH 13, containing deuterium oxide and tetrasodium methylene diphosphonate as a standard for integration. The ^{31}P NMR spectra were then recorded and integrated. After 15 min, approximately 59% of the symmetrical monothiopyrophosphate and 38% of the pyrophosphate were cleaved. After 30 min, the amounts of cleavage were 82% and 64%, respectively. In a second experiment in which 1 μL of enzyme was added and in which the concentration of symmetrical monothiopyrophosphate was 3.8 mM, the amounts of cleavage after 30 min were 31% and 28%, respectively. A control in which the precipitate noted above was removed by centrifugation, the concentration of symmetrical monothiopyrophosphate was 1.6 mM, and 2 μL of enzyme were added showed approximately 79% cleavage after 15 min. At pH 9.90, the half-life for nonenzymatic hydrolysis of symmetrical monothiopyrophosphate is 2.7 h.

Standard solutions of 6 mM tetralithium symmetrical monothiopyrophosphate that contained less than 50 mM lithium carbonate were incubated with 2 μL of inorganic pyrophosphatase under conditions identical with those described above. The symmetrical monothiopyrophosphate showed 64% and 73% cleavage after 15 and 30 min, respectively. Nonenzymatic cleavage in the absence of magnesium chloride accounts for less than half of this cleavage.

Thermolysis of Tetralithium Symmetrical Monothiopyrophosphate. Tetralithium symmetrical monothiopyrophosphate (25 mg), prepared as described above with no precautions to exclude carbonate, was dried for 20 h in vacuo over phosphorus pentoxide at room temperature. It was then heated at 50 °C in vacuo for 23 h. The ^{31}P NMR spectrum (D_2O, pH 13), Figure 3, of the products showed, in addition to 14% remaining symmetrical monothiopyrophosphate, δ 15.6: 59% phosphate and thiophosphate, δ 5.7 and 32.3, respectively; 13% unsymmetrical monothiopyrophosphate, δ 33.2 (d, J = 30 Hz) and −5.1 (d, J = 30 Hz); and 14% pyrophosphate, δ 4.0. These yields were variable, depending probably on the degree of dryness of the sample before thermolysis. A mixture of lithium phosphate and lithium thiophosphate containing lithium carbonate was dried and heated under identical conditions. The ^{31}P NMR spectrum showed only signals for phosphate and thiophosphate. Under these conditions, no monothiopyrophosphate or pyrophosphate were formed.

Results

The synthesis of tetramethyl and tetrabenzyl symmetrical monothiopyrophosphate was carried out essentially as described by Michalski et al.[4] Dealkylation of tetramethyl symmetrical monothiopyrophosphate was achieved by treatment with trimethylsilyl iodide and followed immediately by conversion of the intermediate trimethylsilyl ester to the tetraanion by treatment with *N*-ethyldiisopropylamine and 2-propanol and addition of 1 M lithium hydroxide. The main byproduct of the reaction was phosphate as evidenced by ^{31}P NMR spectroscopy (Figure 1a). The addition of sodium ethoxide in ethanol instead of *N*-ethyldiisopropylamine and 2-propanol, followed by a similar workup, gave nearly identical results, except that small amounts of thiophosphate, pyrophosphate, and unsymmetrical monothiopyrophosphate were also present in the reaction mixture. The side products could be removed by chromatography on an anion exchanger with a gradient of lithium bromide in 0.1 M lithium hydroxide. When tetraethyl symmetrical monothiopyrophosphate was dealkylated in the same manner, the ^{31}P NMR spectrum of the reaction mixture showed a complex mixture of products, but a small amount (approximately 10% yield) of the symmetrical monothiopyrophosphate tetraanion could be identified. Small variations of the time and temperature of the reaction between tetraethyl symmetrical monothiopyrophosphate and trimethylsilyl iodide failed to improve the yield of **1**. Dealkylation of tetramethyl symmetrical monothiopyrophosphate with trimethylsilyl bromide gave only products which resulted from cleavage of the phosphorus–sulfur–phosphorus bond.

Reaction of tetrabenzyl symmetrical monothiopyrophosphate with trimethylsilyl iodide under conditions similar to those described for the methyl and ethyl esters gave complex mixtures of products. The ^{31}P NMR spectra of the reaction mixtures showed signals for a small amount of **1**, in addition to phosphate, thiophosphate, diphosphoryl disulfide, and numerous unidentified products. Due to the presence of benzyl alcohol impurity in the dibenzyl phosphite and the difficulty in removing the benzyl chloride and benzyl iodide, which were formed during the reaction, this route to **1** was not further investigated.

Attempts of dealkylate tetrabenzyl symmetrical monothiopyrophosphate by catalytic hydrogenation were unsuccessful. Moreover, attempts to dealkylate the tetraalkyl esters using sodium iodide in acetone failed. In the former case, no products retaining the phosphorus–sulfur–phosphorus bond were observed in the ^{31}P NMR spectra of the reaction mixtures. In the latter case, only complex mixtures of products were obtained.

The identification and characterization of the tetralithium salt of symmetrical monothiopyrophosphate were complicated by its partial cleavage to phosphate and thiophosphate and the coprecipitation of lithium carbonate during workup. The structural proof is based on ^{31}P NMR spectroscopy, chemical analysis, and its chemical and enzymatic properties.

The ^{31}P NMR spectrum of **1** after chromatographic purification shows only one signal at δ 15.7, which shows coupling neither to phosphorus nor to hydrogen atoms. Compounds containing the phosphorus–sulfur–phosphorus bond are known to have shifts of 10–20 ppm,[4] and, since **1** is symmetric about the central sulfur atom, only one signal, with no observed phosphorus–phosphorus coupling, is expected. Moreover, the absence of phosphorus–hydrogen coupling rules out the possibility of incomplete deesterification. The ^{31}P NMR spectrum shows, in addition, that the compound was less than 5% cleaved to phosphate and thiophosphate at this stage of the isolation (Figure 1b). Elemental

analysis of the tetralithium salt of **1** gives the predicted sulfur to phosphorus ratio of 1:2 and can be accounted for by assuming a mixture of partially hydrolyzed tetralithium symmetrical monothiopyrophosphate and lithium carbonate. The presence of carbonate was confirmed by ^{13}C NMR spectroscopy and is an artifact of the conditions required to isolate **1** since aqueous solutions of lithium hydroxide are known to absorb carbon dioxide from the air.[20] While we were able to minimize the absorption of carbon dioxide, we were not able to eliminate completely the coprecipitation of lithium or barium carbonate during workup. Efforts to precipitate salts of **1** directly from the reaction mixture or to isolate the sodium salt of **1** were unsuccessful, as were all efforts to isolate salts of divalent cations other than barium.

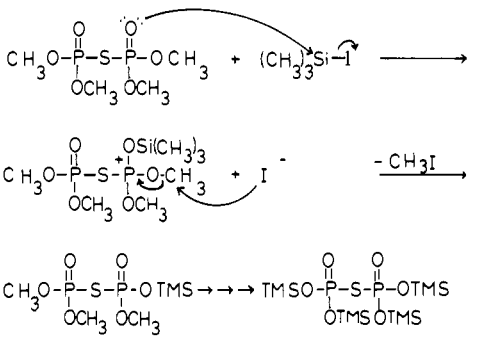
Hydrolytic cleavage of **1** gives phosphate and thiophosphate in equimolar quantities. Moreover, inorganic pyrophosphatase, an enzyme which catalyzes the hydrolysis of the structural analogues of **1**, pyrophosphate[21] and unsymmetrical monothiopyrophosphate,[22] also catalyzes the hydrolysis of **1** to give equimolar amounts of phosphate and thiophosphate. In the absence of magnesium, a required metal cofactor of inorganic pyrophosphatase, the enzyme has no effect on the hydrolysis of **1**. The presence of 100 mM magnesium chloride alone actually slows the rate of nonenzymatic hydrolysis compared to the rates reported in Table I.

Discussion

Michalski and his colleagues reported in 1969 the first definitive syntheses and characterizations of tetraalkyl symmetrical monothiopyrophosphates.[23,24] Although these compounds were very unstable toward rearrangement to their unsymmetrical isomers, Michalski et al. showed, in later work, that this isomerization was subject to nucleophilic catalysis.[4] Indeed, in their pure form, such compounds proved to be significantly more stable to rearrangement or cleavage of the phosphorus–sulfur–phosphorus bond than previously believed to be the case. In this respect, the chemical behavior of the tetraalkyl symmetrical monothiopyrophosphates is similar to that of the tetraalkyl pyrophosphates, which are known to undergo nucleophilic-catalyzed cleavage of the phosphorus–oxygen–phosphorus bond.[25] We reasoned, therefore, that the fully ionized symmetrical monothiopyrophosphate tetraanion should be a stable species since it should resist hydrolysis in analogy to the pyrophosphate tetraanion,[26] assuming that the hydrolytic mechanisms of both species show a similar pH dependence. It should also resist nucleophilic-catalyzed isomerization due to the high negative charge on the phosphoryl moieties. The tetraalkyl symmetrical monothiopyrophosphates appeared to be ideal precursors since they have been well characterized and their chemistry is known. Moreover, several reagents for the dealkylation of phosphorus esters are available. The synthetic problem was reduced, therefore, to the selective cleavage of four alkyl groups in the presence of the labile phosphorus–sulfur–phosphorus bond and the stabilization of symmetrical monothiopyrophosphate as the tetraanion.

Dealkylation of tetramethyl symmetrical monothiopyrophosphate with trimethylsilyl iodide[27-30] followed by cleavage of the trimethylsilyl groups with *N*-ethyldiisopropylamine and 2-propanol proved to be the best route to **1**. The mechanism that has been proposed for the dealkylation of phosphorus esters by trialkylsilyl halides[31] is outlined Scheme II for the dealkylation of the tetramethyl ester of **1**. Nucleophilic attack of the phosphoryl oxygen on silicon gives a phosphonium ion intermediate that is dealkylated in a subsequent step to give the corresponding trimethylsilyl ester. The trimethylsilyl ester can be either hydrolyzed [28,29,32] or, as in this synthesis, cleaved by other nucleophilic reagents.[28,30,33,34] Cleavage of the labile phosphorus–sulfur–phosphorus bond by iodide or other nucleophiles that are present could occur during each of these steps, in particular in the phosphonium ion intermediate. The fact that nucleophilici displacement at ethoxyl carbon occurs approximately 2000 times slower than that at methoxyl carbon[35] may explain the poor yield of **1** from the reaction between tetraethyl symmetrical monothiopyrophosphate and trimethylsilyl iodide. In this case, the phosphonium ion intermediate may have a long enough life under the reaction conditions for competing reactions, in particular cleavage of the phosphorus–sulfur–phosphorus bond, to occur. The milder reaction conditions required to effect dealkylation with trimethylsilyl iodide compared to trimethylsilyl bromide[28,29] may explain the failure to obtain **1** by using the latter reagent.

Scheme II



In some reaction mixtures, diphosphoryl disulfide was a side product. The yield of this compound was increased dramatically when excess trimethylsilyl iodide was used or when the time or temperature of the reaction of tetramethyl symmetrical monothiopyrophosphate with trimethylsilyl iodide was increased. Moreoever, failure to dissolve the reaction products immediately in a high pH aqueous solution, following removal of all volatiles under vacuum, resulted in the appearance of this compound. These results are possibly explained by the presence of iodine as an impurity in the trimethylsilyl iodide or as a side product that is formed during the reaction. Cleavage of the phosphorus–sulfur–phosphorus bond by iodide could yield a phosphoriodidate diester that could react with trimethylsilyl iodide in a subsequent step to give a phosphite triester and iodine. Iodine could then oxidize thiophosphate or its esters to give diphosphoryl disulfide or its esters. The reverse of the first reaction is in fact used to synthesize bis(trimethylsilyl) phosphoriodidate,[36,37] and phosphite was often observed as a side product in the ^{31}P NMR spectra of reaction mixtures in which much diphosphoryl disulfide was present. Moreover, the reaction of iodine with thiophosphate in

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acidic aqueous solution is used to synthesize diphosphoryl disulfide.[14]

It is important to form the tetraanion of symmetrical monothiopyrophosphate immediately after dealkylation. As can be seen from Table I, at pH 8.0 and 25 °C, the half-life of the compound is only a few minutes, but, at pH 10.0, it is increased to several hours. The rates of hydrolysis show a strong temperature and pH dependence in analogy to the hydrolysis show a strong temperature and pH dependence in analogy to the hydrolysis of pyrophosphate.[26] The presence of lithium carbonate (approximately 300 mM) slows the hydrolysis considerably. This appears to be the result of a negative salt effect. This was verified by carrying out the hydrolysis of tetralithium symmetrical monothiopyrophosphate in solutions containing less than 50 mM lithium carbonate in the absence and again in the presence of approximately 300 mM lithium sulfate. Such negative salt effects have been previously observed for the hydrolysis of pyrophosphate.[26]

In aqueous solution at high pH, the rearrangement of **1** to unsymmetrical monothiopyrophosphate was not observed. However, the partially hydrolyzed tetralithium salt of **1** containing lithium carbonate forms a small amount of unsymmetrical monothiopyrophosphate, in addition to pyrophosphate, upon thermolysis at 50 °C. Lithium phosphate and lithium thiophosphate containing lithium carbonate do not form these products under identical conditions. This reaction most likely demonstrates the ability of **1** to phosphorylate thiophosphate and phosphate.

It was of interest to determine whether the symmetrical monothiopyrophosphate would be a substrate for inorganic pyrophosphatase. The unsymmetrical isomer has been reported to be hydrolyzed by this enzyme.[22] Detailed kinetic experiments were not possible due to the instability of the compound, but experiments carried out at one concentration of **1** well above the K_m for pyrophosphate[38] showed **1** to be a substrate. A comparison with pyrophosphate under identical conditions showed the rates of enzymatic hydrolysis for the two compounds to be comparable. Pyrophosphate is also a substrate for alkaline phosphatase.[39] However, an attempt to investigate the substrate properties of symmetrical monothiopyrophosphate for alkaline phosphatase was unsucessful. The rates of hydrolysis at pH 9.3 and 10.0 were essentially identical for experiments carried out in the presence and absence of enzyme.

In summary, we have synthesized the tetralithium and dibarium salts of symmetrical monothiopyrophosphate. Although the compounds appear to be stable as solids, they are hydrolyzed considerably more rapidly than pyrophosphate in aqueous solution.

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Registry No. *O,O,O*-Trimethyl phosphorothioate, 152-18-1; trimethyl phosphite, 121-45-9; tetralithium symmetrical monothiopyrophosphate, 85202-58-0; dimethoxyoxophosphoranesulfenyl chloride, 13894-35-4; tetramethyl symmetrical monothiopyrophosphate, 71861-22-8; dibarium symmetrical monothiopyrophosphate, 85202-59-1; *O,O,O*-tribenzyl phosphorothioate, 81633-39-8; tribenzyl phosphite, 15205-57-9; tetrabenzyl symmetrical monothiopyrophosphate, 85202-60-4; dibenzyl phosphite, 17176-77-1; tetracyclohexylammonium diphosphoryl disulfide, 85202-61-5; unsymmetrical monothiopyrophosphate, 68488-87-9; dimethyl phosphite, 868-85-9.

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Absolute Rate Constants for the Addition of Triethylsilyl Radicals to Various Unsaturated Compounds[1]

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Abstract: The absolute rate constants for the reaction of $Et_3Si\cdot$ radicals with a large number of unsaturated compounds have been measured by laser flash photolysis techniques. The reactivities of C=C double bonds have a wide range, e.g., the rate constants at ca. 300 K are 1.1×10^9, 2.2×10^8, 1.0×10^7, 3.7×10^6, and 9.4×10^5 M^{-1} s^{-1} for acrylonitrile, styrene, tetrachloroethylene, 1-hexene, and cyclohexene, respectively. The range of reactivities for addition to aromatic and heteroaromatic compounds is rather small, the rate constants at ca. 300 K varying from 4.6×10^5 M^{-1} s^{-1} for benzene to 5.0×10^6 M^{-1} s^{-1} for thiophene and α-methylnaphthalene. Acetylenes are slightly less reactive than structurally analogous 1-olefins. For the other types of multiple bonds examined, reactivities decreased in the order isocyanide > nitrone > nitro > isocyanate > nitrile. Arrhenius parameters were determined for a few olefins including ethylene, for which an EPR spectroscopic competition procedure was required. For styrene, $\log (A/(M^{-1} s^{-1})) = 9.35 \pm 0.23$ and $E_a = 1.37 \pm 0.29$ kcal/mol; for ethylene, $\log (A/(M^{-1} s^{-1})) = 8.40 \pm 0.60$ and $E_a = 1.40 \pm 0.80$ kcal/mol.

We have recently shown that the technique of laser flash photolysis can be utilized to measure rate constants for the reactions of trialkylsilyl radicals in solution.[3-5] To date, we have investigated the kinetics for the addition of $Et_3Si\cdot$ radicals to carbonyl compounds[4] and the abstraction of halogens from organic halides.[5] In the present paper we extend these studies to several other important classes of organic substrates. Specifically, we have examined the addition of $Et_3Si\cdot$ to several multiple bonds as well as to aromatic and heteroaromatic substrates.

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