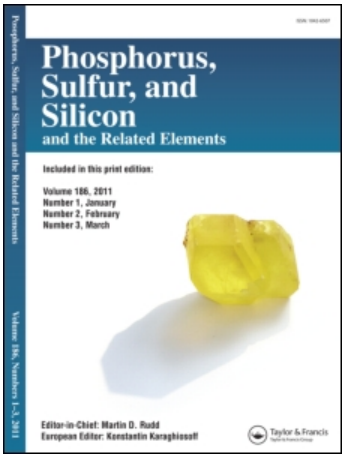

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THE SYNTHESIS OF DISODIUM *O-n*-ALKYLTHIOPHOSPHATES

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THE SYNTHESIS OF DISODIUM *O-n*-ALKYLTHIOPHOSPHATES

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The disodium *O*-alkylthiophosphates were synthesized by reaction of $PSCl_3$ with the corresponding alcohols to the *O*-alkylphosphorodichloridothioates (**1**), which were hydrolyzed in aqueous triethylamin–sodium acetate solution.[1] The *O*-alkylthiophosphates (**2**) were isolated as barium salts and converted to the disodium salts by Na_2SO_4.

Because the barium salts of *O*-ethyl- and *O*-*n*-propyl-thiophosphate were soluble in water, the corresponding dichlorido compounds were hydrolyzed directly to the disodium salts in aqueous sodium hydroxide plus dioxane according to Gray and Hamer.[2]

Further purification was achieved by column chromatography on Sephadex LH20 using water as an eluant. Pure products, which were not contaminated by either inorganic thiophosphate or *O*-alkylphosphates, were thus obtained.

INTRODUCTION

Inorganic thiophosphate and *O*-ethylphosphorothioate have been demonstrated to be interesting tools in biochemical research. Thus, the former has been used to determine disulfide groups,[3] e.g. in lysozyme,[4] and to study phosphate activation in substrate level oxidation.[5] The latter compound was introduced as a substrate to distinguish between acid or alkaline phosphatase.[6]

$$R{-}S{-}S{-}R + S^{32}PO_3^{3-} \longrightarrow R{-}S{-}S{-}{}^{32}PO_3^{2-} + RS^-$$

We have proposed as a working hypothesis for oxidative phosphorylation in mitochondria, that protonized disulfides may be the chemical linkage between proton gradient and phosphate activation[7,8] via a mixed anhydride of sulfenic and phosphoric acid[9] in a hydrophobic environment. Therefore, disodium salts of *O*-alkylthiophosphates of increasing chain length are of interest as potential trapping agents for the proposed sulfenyl group. Only the synthesis of *O*-ethylthiophosphate has been to date described in the literature.[2]

$$[R{-}\overset{\overset{H}{|}}{\underset{\oplus}{S}}{-}S{-}R]X^- \rightleftarrows R{-}SH + RSX$$

$$RSX + H_2PO_4^- \rightleftarrows [R{-}SOPO_3H_2] + X^-$$

DISCUSSION

O-alkylphosphorodichloridothioate (**1**) were prepared conveniently by the method of Pistschimuka.[10,11] However, the hydrolysis of these compounds with aqueous sodium hydroxide plus dioxane, as described for the *O*-ethyl derivative (**1**; $R = C_2H_5$) by Gray and Hamer,[2] did not yield the disodium salts of the *O*-alkylthiophosphates such that they could be purified by simple crystallization with methanol plus acetone. Therefore, the long chain *O*-alkylthiophosphates (**2**) were obtained by mild hydrolysis of the corresponding dichlorido compounds (**1**) in an aqueous solution of equimolar amounts of triethylamin and acetic acid[1] and then precipitated as barium salts by $BaCl_2$. The conversion to the disodium salts (**2**) by less than equimolar amount of Na_2SO_4 did not yield the pure products.

$ROP(S)Cl_2$ (**1**)

$ROP(S)(ONa)_2$ (**2**)

Since the barium salts of the *O*-ethyl- and *O*-*n*-propyl-thiophosphate were found to be soluble in water, the disodium salts of these two compounds could be prepared directly by the procedure of Gray and Hamer.[2]

The disodium salts of the *O*-alkyl-thiophosphates (**2**), obtained by the above hydrolysis procedures,[1,2] contained up to three impurities. Column chromatography on Sephadex LH20 using water as an eluant proved a useful means of separating the desired products from these impurities. Continuous measurement of the refractive index of the column eluant enabled the impurities to be detected, whilst the *O*-alkylthiophosphates were monitored by uv absorption at 254 nm. After freeze drying of the *O*-alkylthiophosphate containing fractions no contaminating *O*-alkylphosphates ROP(O) $(ONa)_2$ or inorganic thiophosphate could be detected by nmr spectroscopy or by thin layer chromatography.

EXPERIMENTAL

Column chromatography (bed dimensions: 2.3 × 150 cm) was carried out using Sephadex LH20 (Pharmacia, Uppsala, Sweden) with water as an eluant. The samples were monitored by an Uvicord II absorptiometer (LKB, Sweden) which recorded uv absorption at 254 nm; simultaneously the refractive index was measured continuously with a Water Differential Refractometer R403. Silica F plates (Merck, Darmstadt) were used for thin layer chromatography (tlc) with the solvent system *n*-propanol/conc. NH_3/H_2O (6:3:1 v/v).[10] Yellow-brown spots were developed by spraying with an acidic solution of $PdCl_2$.[11] The ^{31}P nmr spectra were recorded with a Bruker-Physic HFX60 spectrometer at 24.38 MHz equipped with a Fourier Transform unit Bruker-Data-System B-NC 12 from the Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, Göttingen, Fed. Rep. Germany, by courtesy of Dr. F. Eckstein. Chemical shifts are given in δ units (parts per million) relative to an external standard of 30% aqueous phosphoric acid with positive signs in the downfield direction.

Synthesis of O-Alkylphosphorodichloridothioates

The *O*-alkylphosphorodichloridothioates (**1**) were prepared from the appropriate alcohol by reaction with $PSCl_3$ as described by Martin *et al.*[12] The reaction temperatures were varied according to the alcohols used.

O-Ethyl (**1**; R = C_2H_5) (40%) bp 57–59° (14 mm) [lit.[13] bp 57–59° (14 mm)] (Found: Cl, 39.6. Calc. for $C_2H_5Cl_2OPS$; Cl, 39.61%).

O-*n*-Propyl (**1**; R = *n*-C_3H_7) (49.5%) bp 71–72° (11 mm) [lit.[14] bp 80° (20 mm)] (Found: Cl, 37.33. Calc. for $C_3H_7Cl_2OPS$: Cl, 36.73%).

O-*n*-Pentyl (**1**; R = *n*-C_5H_{11}) (54.7%) bp 51–52° (0.25 mm) [lit.[15] bp 74–75° (1 mm)] (Found: Cl, 31.86. Calc. for $C_3H_7Cl_2OPS$: Cl, 32.07%).

O-*n*-Heptyl (**1**; R = *n*-C_7H_{15}) (54.1%) bp 68–70° (0.2 mm) (Found: C, 37.12; H, 5.87; Cl, 28.15. Calc. for $C_7H_{15}Cl_2OPS$: C, 37.74; H, 6.07; Cl, 28.46%).

O-*n*-Nonyl (**1**; R = *n*-C_9H_{19}) (51%) bp 85–86° (0.1 mm) (Found: C, 40.70; H, 7.07; Cl, 25.39. Calc. for $C_9H_{19}Cl_2OPS$: C, 38.98; H, 6.91; Cl, 25.59%).

Synthesis of Disodium O-Alkylthiophosphates (**2**)

O-Ethyl- and *O*-*n*-propylphosphorodichloridothioate (**1**; R = C_2H_5 and R = *n*-C_3H_7) were hydrolyzed by aqueous sodium hydroxide plus dioxane and the disodium salts of the corresponding *O*-alkylthiophosphate (**2**) were isolated according to Gray and Hamer.[2]

The compounds of longer chain length were obtained by the following procedure: 50 mmoles *O*-alkylphosphorodichloridothioate (**1**) were emulsified in a solution of 0.22 moles sodium acetate in 150 ml water at 0°C. Under vigorous stirring 0.22 moles triethylamin were added dropwise, then the mixture was incubated at below 5°C for 1 hour.[1] After the temperature of the reaction mixture had been allowed to rise to 20°C, it was neutralized with dilute hydrochloric acid and the *O*-alkylthiophosphate precipitated by the addition of 60 mmoles $BaCl_2$ in 100 ml H_2O. The barium salts were collected by filtration, washed with water and acetone, and then resuspended into 50 mls of water. To these suspensions, 48 mmoles of Na_2SO_4 were added, the resultant precipitates were removed and the filtrates freeze dried.

The *O*-alkylthiophosphate were further purified by chromatographing 200 mg portions on Sephadex LH20 using water as an eluant; uv absorption at 254 nm and refractive index were recorded simultaneously (for detail, see Methods). After freeze drying the disodium salts were obtained in high purity.

O-Ethyl (**2**; R = C_2H_5) (86%) (Found: C, 12.90; H, 2.41; Na, 24.26; S, 17.21, Calc. for $C_2H_5Na_2O_3PS$; C, 12.91; H, 2.71; Na, 24.72; S, 17.22%) ^{31}P nmr (D_2O): δ = 42.02 ppm; ^3J(HCOP) = 6.96 Hz.

O-*n*-Propyl (**2**; R = *n*-C_3H_7) (34.6%) (Found: C, 17.91; H, 3.82; Na, 23.19; S, 16.08. Calc. for $C_3H_7Na_2O_3PS$: C, 18.00; H, 3.52; Na, 22.98; S, 16.02%). ^{31}P nmr (D_2O): δ = 42.11 ppm; ^3J(HCOP) = 6.98 Hz.

O-*n*-Pentyl (**2**; R = *n*-C_5H_7) (53.5%) (Found: C, 26.65; H, 4.84; Na, 20.67; S, 14.27. Calc. for $C_5H_7Na_2O_3S$: C, 26.32; H, 4.86; Na, 20.15; S, 14.05%) ^{31}P nmr (D_2O): 42.08 ppm (broad).

O-*n*-Heptyl (**2**; R = *n*-C_7H_{15}) (65%) (Found: C, 32.83; H, 5.93; Na, 18.26; S, 12.61. Calc. for $C_7H_{15}Na_2O_3PS$: C, 32.81; H, 5.90; Na, 17.95; S, 12.51%). ^{31}P nmr (D_2O): δ = 42.08 ppm; ^3J(HCOP) = 6.96 Hz.

O-*n*-Nonyl (**2**; R = *n*-C_9H_{19}) (55%) (Found: C, 37.89; H, 6.91; Na, 16.53; S, 11.00. Calc. for $C_9H_{19}Na_2O_3PS$: C, 38.02; H, 6.73; Na, 16.18; S, 11.27%). ^{31}P nmr (D_2O): δ = 42.05 ppm; ^3J(HCOP) = 6.60 Hz.

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