Anal. Calcd for $C_{29}H_{23}NO_7$: C, 70.0; H, 4.7; N, 2.8. Found: C, 70.2; H, 4.7; N, 2.9.

Acknowledgment.—Data on the tumor inhibitory activities were obtained by the courtesy of Drs. G. S. Tarnowski, H. C. Reilly, and C. C. Stock of the Division of Experimental Chemotherapy of the Sloan–Kettering Institute. We thank Roche Products Ltd. for the provision of pyridoxine hydrochloride.

Studies on Latent Derivatives of Aminoethanethiols as Potentially Selective Cytoprotectants. V. Syntheses of S-(2-Aminoethyl) Methyl Hydrogen Phosphorothioate[1]

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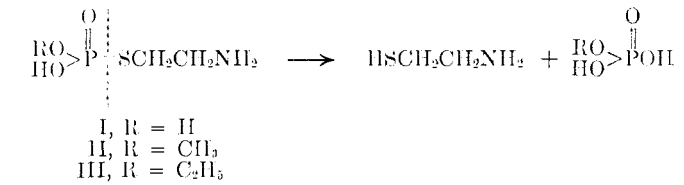
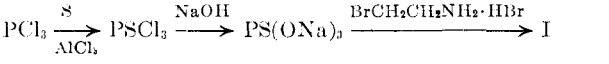
As prototypes of a class of latent cytoprotective agents, we became interested in the synthesis of derivatives of phosphorothioic acid which might release 2-aminoethanethiol *in vivo* as indicated. Should this release occur selectively in normal tissues sensitive to the damaging effects of either administered alkylating



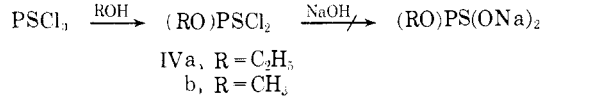
I, R = H
II, R = CH_3
III, R = C_2H_5

agents or radiation and not in a tumor, it would be possible to increase the safely tolerated dose of these therapeutic agents with consequent increase in therapeutic effect in cancer.

The sodium salt of the parent compound cysteamine S-phosphate (I) was prepared by the method of Åkerfeldt,[2] involving the reaction of 2-bromoethylamine hydrobromide with trisodium phosphorothioate, the latter being obtained by the reaction of sulfur with PCl_3 followed by treatment of the product with sodium hydroxide.[3]



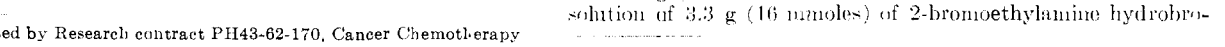
We originally tried to synthesize III by an analogous route. Thiophosphoryl chloride was treated with ethanol to yield O-ethyl phosphorodichloridothioate. Hydrolysis of the latter with sodium hydroxide, however, failed to yield the desired disodium salt (IVa). The O-methyl analog (IVb) had been prepared[4] in 1911



IVa, R = C_2H_5
b, R = CH_3

by the partial hydrolysis of trimethyl phosphorothioate with sodium hydrogen sulfide. However, the procedure was cumbersome and the yields poor. Consequently, it was decided to seek a new route to IVb and hence to the desired II.

We have now succeeded in preparing II by the partial neutralization of trisodium phosphorothioate with hydrochloric acid, followed by methylation with diazomethane. This gave the monomethyl ester (IVb) in good yield. The latter reacted smoothly with 2-bromoethylamine hydrobromide to give S-(2-aminoethyl) methyl hydrogen phosphorothioate (II) as a yellow oil.



The diazomethane reaction is based on an earlier observation[5] that ionizable phosphoric acid groups in the acid form esterify readily and essentially quantitatively with diazomethane, whereas those in the salt form do not react at all. Since one would anticipate that the dianion of phosphorothioic acid would exist mainly in the $(HO)POS^{-}O^{-}$ form, we presumed that reaction with diazomethane would lead primarily to the O-methyl ester (IVb) that was indeed obtained.

Biological Studies.—When injected into Sprague–Dawley rats, cysteamine S-phosphate (I) produced significant concentrations of cysteamine in 13 of the 15 tissues studied, including spleen, brain, pancreas, intestine, thymus, lung, heart, blood, liver, kidney, colon, bone marrow, and tumor and produced insignificant levels in muscle and stomach. The compound was given intravenously at a dose of 300 mg/kg. The levels of cysteamine found in tissues were in the range of 0.5–2.5 μmoles/g of tissue. In contrast, the methyl ester II administered at the same dose level appeared to be essentially uncleaved *in vivo* since it gave undetectable amounts of cysteamine release in all of these tissues except bone marrow which has not been assayed.

Cysteamine was measured in tissue homogenates by a specific spectrophotometric method that was developed[6] for the purpose utilizing the known reaction[7] of aminoethanethiols with 3-fluoropyruvic acid. The reaction product absorbs maximally in the ultraviolet at 300 mμ (ε 5800).

Experimental Section

Disodium O-Methyl Phosphorothioate (IVb).—To 4.5 g (25 mmoles) of trisodium phosphorothioate, prepared according to the procedure of Yasuda and Lambert,[3] was added 25 ml of 1 *N* HCl and to this solution was added absolute methanol until the mixture became cloudy. An ethereal solution of CH_2N_2 was added to the cooled mixture and immediate effervescence occurred. The ether and water were removed under reduced pressure at 30°. The yellow oil which remained was taken up in hot methanol and filtered, and the filtrate was treated with charcoal and then evaporated to dryness. This procedure was repeated. Final removal of methanol and trituration with ether gave a white powder, mp 55.5–57.5°, yield 3.9 g (80%), lit.[4] mp 49°.

S-(2-Aminoethyl) Methyl Hydrogen Phosphorothioate (II).—To 2.39 g (14 mmoles) of IVb in 12 ml of water was added a solution of 3.3 g (16 mmoles) of 2-bromoethylamine hydrobro-

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mide in 10 ml of dimethylformamide and the mixture was stirred for 4 hr. At the end of this time the solvents were removed under reduced pressure at room temperature and the residue was extracted with methanol. Evaporation of the methanol gave a yellowish oil which was treated with ethanol and filtered, and the filtrate was evaporated to give a white powder. This was extracted with chloroform, and the $CHCl_3$ extracts were dried (Na_2SO_4), treated with charcoal, and evaporated to 1.0 g of a yellow oil.

Anal. Calcd for $C_3H_{14}NO_3PS$: C, 21.05; H, 5.85; N, 8.18. Found: C, 21.35; H, 5.67; N, 8.25.

Antiacetylcholinesterase Activity of O,O-Diethyl-S-(acetylphenylurea)dithiophosphoric Acid Esters[1]

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A search for new insecticides led us to synthesize esters of O,O-diethyldithiophosphoric acid by condensation of an alkali metal salt of the acid with chloroacetyl-substituted phenylureas. The insecticidal, rodenticidal, and fungicidal properties of α-chloroacetylphenylureas have been reported by Hoegberg, *et al.*[2a] Introduction of an alkyl chain in the benzene nucleus has been shown to increase lipoid solubility and enhance insecticidal activity.[2b] Furthermore, esters of O,O-dialkyldithiophosphoric acid have been shown to possess insecticidal properties.[3] In the present study antiacetylcholinesterase activity of esters of O,O-diethyl-S-(acetylphenylurea)dithiophosphoric acid was investigated using rat brain homogenate as the source of the enzyme.

Experimental Section[4]

Substituted phenylureas were synthesized by treating a solution of potassium cyanate with aniline, toluidines, and anisidine. α-Chloroacetylphenylureas were prepared according to the method of Jacobs, *et al.*,[5] by refluxing 0.1 mole of the respective phenylurea and 0.11 mole of chloroacetyl chloride in dry benzene for 2–3 hr. On cooling, water was added and the solid mass which separated out was filtered. The α-chloroacetylphenylureas after washing with cold water were recrystallized before further use. O,O-Diethyldithiophosphoric acid was prepared according to the method of Hoegberg, *et al.*[6] Esters of O,O-diethyldithiophosphoric acid were prepared by condensation of α-chloroacetylphenylurea (1 mole) with O,O-diethyldithiophosphoric acid (1 mole) in the presence of anhydrous Na_2CO_3 (1 mole). The mixture was refluxed in dry acetone for 15–18 hr, cooled, and filtered to remove $NaHCO_3$ and NaCl. After distilling the solvent the residue was recrystallized from ether. The characterization of esters of O,O-diethyldithiophosphoric acid was done by their sharp melting points and also by analysis.

Determination of Acetylcholinesterase Activity.—Adult rats weighing approximately 150 g were killed by decapitation. Brains were quickly removed, weighed, and homogenized in ice-cold 0.25 *M* sucrose. The final concentration of the homogenate, without further purification, used throughout these studies was 10% w/v. Acetylcholinesterase activity was determined colorimetrically using acetylthiocholine as substrate.[7]

The inhibitory effect of esters of O,O-diethyldithiophosphoric acid on rat brain acetylcholinesterase during the hydrolysis of acetylthiocholine is shown in Table I. All of the esters inhibited the enzyme activity. Substitution on the phenyl nucleus influenced their enzyme inhibitory properties; the degree of inhibition observed with unsubstituted derivatives was greatly reduced by substituting methyl groups in the *ortho* and *para* position. Substitution of the methyl group in the *meta* position did not alter their inhibitory properties. However, a slight decrease in the inhibition was observed with the methoxy derivative as compared to unsubstituted or *m*-methyl-substituted esters.

TABLE I

ANTIACETYLCHOLINESTERASE ACTIVITY[a] OF ESTERS OF O,O-DIETHYLDITHIOPHOSPHORIC ACID

R	Mp, °C	Yield, %	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found	Antiacetylcholinesterase activity: Inhibition, %	Antiacetylcholinesterase activity: I_{50}, 1×10^{-4} *M*
	84–86	55	$C_{13}H_{19}N_2O_4PS_2$	43.0	42.88	5.2	5.82	...	...	60.5 ± 0.50	2.30
2-CH_3	94–95	70	$C_{14}H_{21}N_2O_4PS_2$	44.6	45.4	5.6	5.49	...	...	22.1 ± 0.32	8.10
3-CH_3	94–96	60	$C_{14}H_{21}N_2O_4PS_2$	44.6	45.1	5.6	5.46	7.4	7.62	60.5 ± 0.50	2.34
4-CH_3	112–115	60	$C_{14}H_{21}N_2O_4PS_2$	44.6	44.8	5.6	6.0	7.4	7.51	18.7 ± 0.97	10.0
3-OCH_3	68–70	60	$C_{14}H_{21}N_2O_5PS_2$	42.8	43.1	5.3	5.6	...	...	50.7 ± 0.84	3.0

[a] Enzyme activity was determined as change in extinction per 100 mg of wet tissue during 10 min of incubation. Each tissue sample was done in triplicate. Suitable controls for tissue and substrate blanks were taken. Per cent inhibition was calculated on the basis of decrease in the enzyme activity using esters at a final concentration of 3×10^{-4} *M*. Mean values with standard error are recorded. I_{50} values indicate the concentration required to produce 50% enzyme inhibition. Eserine was used as a standard acetylcholinesterase inhibitor. The I_{50} value for eserine was 5.49×10^{-7} *M* under identical conditions. The reaction mixture in a volume of 2 ml contained Tris buffer (43.7 m*M*), pH 7.4, NaCl (350 m*M*), acetylthiocholine (1.5 m*M*), and 0.3 ml of 10% brain homogenate.

(1) The authors wish to express their thanks to Drs. M. L. Dhar and N. Nand of Central Drug Research Institute, Lucknow, for providing facilities for microanalysis. Grateful acknowledgment is made to the Council of Scientific and Industrial Research, New Delhi, for providing financial assistance to R. C. Srivastava.

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