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]

A. K. SEN GUPTA, R. C. SRIVASTAVA, AND SURENDRA S. PARMAR

Department of Chemistry, Lucknow University, and Department of Pharmacology and Therapeutics, K. G. Medical College, Lucknow, India

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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]

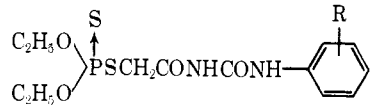
Substituted phenylureas were synthesized by treating a solution of potassium cyanate with aniline, toluidines, and anisidine. α-Chloroacetylphenylureas were prepared according to the method of Jacobs, *et al.*,[5] by refluxing 0.1 mole of the respective phenylurea and 0.11 mole of chloroacetyl chloride 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, %	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.

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