solidify. It was soluble in chloroform and dioxane, and insoluble in ether, ethyl acetate, and benzene. A cold chloroform solution of this product was neutralized with cold sodium hydroxide (16%). The chloroform layer was separated, and a stream of air was passed through this solution. The solvent was removed and (2-chloroethyl)phenylphosphine oxide (VII), m.p. 92°, was isolated in 72% yield.

Anal. Found: Cl, 28.1.

Phenyldisodiophosphine.—This compound was prepared essentially according to the procedure given by Horner[23,24] with certain modifications. A 500-ml. three-necked flask was fitted with a reflux condenser, high-speed stirrer [rated at a maximum speed of 20,000 r.p.m. (Labline, Inc., Model 1285)] and equal-pressure dropping funnel. The entire system was thoroughly flushed with nitrogen. Into the flask was added 100 ml. of solvent (dioxane or toluene) and 5.1 g. (0.22 g.-atom) of sodium; into the dropping funnel was placed 8.95 g., (0.05 mole) of phenyl phosphonous chloride. The solvent was brought to reflux and the dispersion was prepared by stirring at maximum speed for 1–2 min. Stirring was then discontinued, the heating mantle removed, and 15–20 drops of the chlorophosphine was added immediately. When the initial effervescence had subsided, the stirrer was restarted at a moderate rate and heating was resumed such that a gentle reflux was maintained. After 1–5 min., the dispersion had a definite yellow color and the remaining chlorophosphine, now diluted with 10 ml. of solvent, was added over 45 min. If dioxane was the solvent media, the reaction mixture was boiled under reflux for an additional 4 hr., and a thick suspension of a yellow solid in a red solution was obtained. With toluene as the solvent, the reflux time required for the reaction to go to completion, as shown by the absence of metallic sodium and the formation of a thick, pale green mixture, was 7–9 hr. If no yellow coloration was produced after the initial addition of the chlorophosphine, it was found to be unlikely that the reaction would go to completion. Under such conditions it is essential to redry the solvent and/or redistil the chlorophosphine prior to attempting the reaction again.

Bis(2-*t*-butoxyethyl)phenylphosphine (II) and Mono-2-*t*-butoxyethylphenylphosphine (IX).—The suspension containing phenyldisodiophosphine (0.05 mole) was cooled to room temperature with a water bath and diluted with solvent (50 ml.). A slightly exothermic reaction occurred during the 1-hr. interval required for the addition of 13.7 g. of the chloro ether I (0.1 mole) in 50 ml. of solvent. The water bath was removed and the suspension was stirred for an additional hour at room temperature prior to refluxing the stirred mixture for a further hour. At the end of this interval, the reaction mixture was cooled in an ice bath and 35 ml. of oxygen-free water was added cautiously. The solvent layer was separated, dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. With dioxane as the solvent, distillation of the residue gave two fractions, b.p. 55–70° (0.2 mm.), and 110–115° (0.2 mm.). Redistillation of the higher boiling fraction gave the bis-ether II, b.p. 113–115° (0.2 mm.), in 10–20% yields. The lower boiling fraction was shown to be mono-2-*t*-butoxyethylphenylphosphine (IX) (12–25%). Treatment of this fraction in liquid ammonia with sodium and then the chloro ether I gave the bis-ether II (50%), b.p. 112–115° (0.2 mm.). With toluene as the solvent, only the lower boiling fraction was obtained, b.p. 65–66° (0.15 mm.).

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α-Amidinium Thiosulfates (Bunte Salts) as Antiradiation Drugs

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The synthesis of a number of α-amidinium thiosulfates is described and their protective action against ionizing radiation is reported.

In our search for radiation-protective agents, we turned our attention to the synthesis of functional analogs of cysteamine, $HSCH_2CH_2NH_2$. α-Mercaptoamidines, $HSCHRC(=NH)NH_2$, and their derivatives were considered to fall into this category and their synthesis[2] became the object of our attention.[3] Although α-mercaptoamidines themselves are yet unknown, a number of their thiol esters and Bunte salts have been prepared.[2] Initial biological data revealed that some of the then reported[2] α-amidinium thiosulfates (Bunte salts), $RCH(S_2O_3^-)C(=NH_2^+)NR'R''$ (I) afforded protectivity and hence additional members of this series were synthesized in the hope of learning which molecular modification would bring about increased activity. Biological data of all of the α-amidinium thiosulfates which have been tested to date are compiled in Table I. Several features are apparent from these data. Derivatives of acetamidine (I, R = H), on the whole, showed considerably more activity than their homologs I (R = CH_3 and C_6H_5). On the other hand when the N-alkyl side chain of the acetamidine series is increased (R = R′ = H; R″ from H to *n*-C_5H_{11}), considerable activity is maintained. Although no optimum chain length was determined, the long-chain *n*-nonyl and *n*-decyl derivatives were devoid of activity. The synthesis of additional members of this series is planned in an effort to explore the potential of α-amidinium thiosulfates as radiation protective agents.

The synthesis of the α-amidinium thiosulfates reported here followed essentially that developed previously.[2] α-Halonitriles were converted to the corresponding α-chloramidine hydrochlorides, which were treated with sodium thiosulfate to yield I. In one instance, when a water-soluble product was obtained, the α-amidinium Bunte salt could be isolated when thallous thiosulfate was used instead of the sodium salt. The new members of the series are listed in Table II.

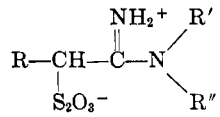
(1) Taken from the M.S. Thesis submitted by Karen Rover Sandberg to the University of Illinois at the Medical Center, Chicago, Ill., June, 1964.

(2) L. Bauer and T. L. Welsh, *J. Org. Chem.*, **27**, 4382 (1962).

(3) During the course of this investigation the synthesis of β-mercaptopropionamidine was reported by T. P. Johnston and A. Gallagher [*J. Org. Chem.*, **28**, 1436 (1963)] and that of β-amidinium thiosulfates, $RC(=NH_2^+)NH(CH_2)_nS_2O_3^-$ (n = 2 and 3) was described by G. Sosnovsky and P. Schneider [*Tetrahedron*, **19**, 1313 (1963)].

TABLE I

PROTECTIVE ACTIVITIES[a] OF α-AMIDINIUM THIOSULFATES



R	R′	R″	Protection against radiation	Drug dose, mg./kg.	Vehicle of administration[b]
H	H	H	None	50 or less	H_2O
H	H	CH_3	Fair	51-150	H_2O
H	H	C_2H_5	Good	51-150	H_2O
H	H	n-C_3H_7	Fair	51-150	CMC/Tw
H	H	i-C_3H_7	None	51-150	H_2O
H	H	n-C_4H_9	Fair	50 or less	CMC/Tw
H	H	i-C_4H_9	Good	51-150	CMC
H	H	n-C_5H_{11}	Fair	50 or less	H_2O
H	H	n-C_9H_{19}	None	50 or less	CMC/Tw
H	H	n-$C_{10}H_{21}$	None	50 or less	CMC/Tw
H	H	$C_6H_5CH_2$	None	50 or less	CMC/Tw
H	H	$C_6H_5(CH_2)_2$	Good	50 or less	CMC
H	H	$C_6H_5(CH_2)_3$	None	50 or less	CMC/Tw
H	H	$C_6H_5(CH_2)_4$	Good	50 or less	CMC/Tw
H	CH_3	CH_3	None	51-150	CMC
H	—$(CH_2)_5$—		None	51-150	H_2O
CH_3	H	H	Good	51-150	H_2O
CH_3	H	n-C_4H_9	None	51-150	CMC/Tw
CH_3	H	$C_6H_5CH_2$	None	50 or less	CMC/Tw
CH_3	H	$C_6H_5(CH_2)_4$	None	50 or less	CMC/Tw
CH_3	CH_3	CH_3	None	50 or less	CMC/Tw
C_6H_5	H	H	None	50 or less	CMC/Tw

[a] The compounds were tested under the auspices of Dr. D. P. Jacobus and Dr. T. R. Sweeney at the Walter Reed Army Institute of Research, Washington, D. C. Compounds were administered intraperitoneally to mice which were tested for 30-day survival against lethal radiation of 1000 r. Complete details are described by L. Field, A. Ferretti, R. Crenshaw, and T. Owen, *J. Med. Chem.*, **7**, 42 (1964). [b] CMC/Tw means that the compound was suspended in a physiological saline solution containing 0.2% methylcellulose (4000 centiposes) and 0.4% Tween 180.

Experimental[4]

Starting Materials.—1-Amino-3-phenylpropane and 1-amino-4-phenylbutane were purchased from Aldrich Chemical Company, Inc., Milwaukee, Wis. Amine hydrochlorides, when not commercially available, were prepared by bubbling dry HCl through an ice-cold solution of the amine in dry ether. Thallous thiosulfate was prepared from thallous formate (obtained from the Ealing Corporation, Cambridge, Mass.) by the method of Lecher and Hardy.[5]

α-Chloropropionamidine Hydrochlorides.—These salts were synthesized by the method described previously.[2] If the reaction mixture became colored quickly, the reaction was carried out at lower temperature for a shorter period. The procedure for the preparation of one of these salts is described in full, the others being made in similar fashion. The reaction times and temperature for the others is indicated in parentheses.

N-(4-Phenylbutyl)-α-chloropropionamidine Hydrochloride.—α-Chloropropionitrile (9.05 g., 0.1 mole) was added dropwise to a stirred solution of 0.01 mole of sodium methoxide in dry methanol (100 ml.) at 25°. One hour after the addition, 1-amino-4-phenylbutane hydrochloride (16.39 g., 0.11 mole) was added and the mixture was stirred for 16 hr. at 25°. A small amount of solid was filtered off and the filtrate was evaporated *in vacuo*. The residue was triturated with ether to give the salt (25.50 g., 90%), m.p. 166–167°.

Anal. Calcd. for $C_{13}H_{20}Cl_2N_2$: N, 10.18. Found: N, 10.30.

N-Methyl-α-propionamidine hydrochloride was obtained in 92% yield (3.5 hr., 25°), m.p. 110–115° dec.

Anal. Calcd. for $C_4H_{10}Cl_2N_2$: N, 17.84. Found: N, 18.00.

N-Ethyl-α-propionamidine hydrochloride was formed in 95% yield (20 hr., 25°), m.p. 152–154° dec.

Anal. Calcd. for $C_5H_{12}Cl_2N_2$: N, 16.38. Found: N, 16.40.

N-Butyl-α-propionamidine hydrochloride was isolated in 89% yield (5 hr., 25°), m.p. 81–84° dec.

Anal. Calcd. for $C_7H_{16}Cl_2N_2$: N, 14.07. Found: N, 13.80.

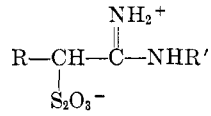
N-Alkyl-α-chloroacetamidine Hydrochlorides.—These salts were all prepared from chloroacetonitrile and the corresponding amine hydrochloride as described above, but could not be induced to crystallize. However, each gum so isolated furnished the corresponding crystalline Bunte salt. The following members were prepared from the requisite amine hydrochlorides, the reaction conditions being shown in parentheses: *n*-propyl (6 hr., 25°); isopropyl (1 hr., 25°; 18 hr., 7°); *n*-butyl (6 hr., 25°); isobutyl (6 hr.; 25°); *n*-nonyl (3 hr., 25°; 18 hr., 7°); *n*-decyl (20 hr., 25°); 3-phenylpropyl (5 hr., 25°); 4-phenylbutyl (1.5 hr., 25°; 18 hr., 7°).

α-Amidinium Thiosulfates.—These were prepared from the corresponding α-chloramidine hydrochlorides whether these were crystalline or not, as described previously.[2] The yield, physical constants, and analyses are compiled in Table II. The yields quoted in Table II are all based on the starting α-chloronitrile, irrespective of whether the intermediate α-chloramidine hydrochloride was crystallized or not. When the product was water soluble, thallous thiosulfate was employed as shown for the ensuing and only example.

S-[1-(N-Ethylcarboxamidino)ethyl]thiosulfuric Acid.—An aqueous solution of N-ethyl-α-chloropropionamidine hydrochloride (7.69 g., 0.045 mole in 100 ml. of water) was added to a hot aqueous solution of thallous thiosulfate (25.1 g., 0.045 mole in 100 ml. of water) and the mixture was heated at the reflux for 1 hr.

TABLE II

α-AMIDINIUM THIOSULFATES (BUNTE SALTS)



R	R′	Crystn. solvent	Yield, %	M.p., °C. dec.	Formula	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	N, % Calcd.	N, % Found	S, % Calcd.	S, % Found
H	n-C_3H_7	Water	46	152–155	$C_5H_{12}N_2O_3S_2$	28.29	28.56	5.70	5.85	13.19	13.04	30.12	30.30
H	i-C_3H_7	Water	20	161–162	$C_5H_{12}N_2O_3S_2$	28.29	28.43	5.70	5.65	13.19	13.10		
H	n-C_4H_9	Ethanol	22	131–133	$C_6H_{14}N_2O_3S_2$	31.84	31.56	6.23	6.38	12.35	12.18		
H	i-C_4H_9	Isobutyl alcohol	38	115–116	$C_6H_{14}N_2O_3S_2$	31.84	31.50	6.23	6.61	12.35	12.12		
H	n-C_9H_{19}	Aqueous methanol	73	133–135	$C_{11}H_{24}N_2O_3S_2$	44.56	44.69	8.16	8.35	9.45	9.33	21.63	21.50
H	n-$C_{10}H_{21}$	Ethanol	37	140–142	$C_{12}H_{26}N_2O_3S_2$	46.45	46.37	8.38	8.54	9.02	9.06	20.60	20.81
H	$C_6H_5(CH_2)_3$	95% ethanol	66	157–159	$C_{11}H_{16}N_2O_3S_2$	45.81	46.10	5.59	5.52	9.71	9.55	22.23	22.17
H	$C_6H_5(CH_2)_4$	95% ethanol	40	126–128	$C_{12}H_{18}N_2O_3S_2$	47.66	47.56	5.99	5.87	9.27	9.12		
CH_3	CH_3	Isopropyl alcohol	19	154–156	$C_4H_{10}N_2O_3S_2$	24.23	24.43	5.08	5.18	14.13	14.06	32.35	32.42
CH_3	C_2H_5	Isobutyl alcohol–ethanol	44	152–154	$C_5H_{12}N_2O_3S_2$	28.29	28.32	5.70	5.65	13.20	13.24	30.28	30.30
CH_3	n-C_4H_9	Aqueous ethanol	28	140–142	$C_7H_{16}N_2O_3S_2$	34.98	35.25	6.71	6.80	11.66	11.52	26.28	26.71
CH_3	$C_6H_5(CH_2)_4$	Water	26	152–155	$C_{13}H_{20}N_2O_3S_2$	49.34	49.54	6.37	6.34	8.85	8.86	20.27	20.23

(4) All melting points are uncorrected. Microanalyses were performed by Dr. Kurt Eder, Geneva, Switzerland, Micro-Tech Laboratories, Skokie, Ill., and those for nitrogen on a Coleman nitrogen analyzer, Model 29.

(5) H. Z. Lecher and E. M. Hardy, *J. Org. Chem.*, **20**, 475 (1955).

After cooling to 20° the precipitate was filtered off and the filtrate was evaporated *in vacuo*. The residue solidified on standing and was purified as indicated in Table II.

Acknowledgment.—The authors wish to thank Drs. D. P. Jacobus, T. R. Sweeney and P. Coad for their advise and helpful suggestions on this project. They are indebted to them for furnishing the testing data reported in Table I. We would also like to acknowledge the generous support by a research contract (DA-49-163-MD-2047) of this Project by the Office of the Surgeon General, United States Army Medical Research and Development Command under whose sponsorship this work was carried out.

Aspects of the Chemical Mechanism of Complex Formation between Acetylcholinesterase and Acetylcholine-Related Compounds[1a,b]

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The mechanism of acetylcholinesterase (AChE) action is briefly reviewed. The stereospecificity of the enzyme and the nature of the binding forces in the enzyme substrate addition complex are discussed. It is recognized that the active surface of AChE is essentially nonpolar in character, a property which allows the operation of hydrophobic forces in the binding of substrates and inhibitors. The positive involvement of van der Waals attractions is shown to be of rare occurrence. The absolute conformation of enzyme-bound acetylcholine (ACh) is deduced through a study of the stereochemistry of the interaction of 1,3-dioxolane analogs (II) with the active surface. This showed that the enzyme displays both absolute and relative stereospecificity towards this series of inhibitors. Optimum affinity is displayed by the L-(+)-*cis*-2-methyl-4-trimethylammoniummethyl-1,3-dioxolane iodide (VI). The mechanism of interaction of the 2-methyl group with the enzyme was elucidated. Structure–activity relationships could be interpreted on the basis of the operation of hydrophobic forces exerted on the 2-substituents and van der Waals attractions in the case of VI. The free energy of binding for the 2-methyl group of the latter was found to be 1.35 kcal., a value which corresponds closely to the one obtained for the free energy of binding of the ester methyl group of ACh. It is concluded that both ACh and VI uniquely form "lock-and-key" type of fits with AChE. Two modes of interactions with AChE are recognized: (a) one of them involves only the operation of hydrophobic forces, thus necessitating an accommodative perturbation of the nonpolar chains on the enzyme; (b) the other calls into play the net contribution of van der Waals forces and is thus conducive to a highly specific perturbation of the protein. On the basis of these considerations, structure–activity relationships at the receptor level are interpreted. The role of the quaternary ion in catalysis by AChE is discussed; the mechanism of binding of quaternary ions is evaluated critically taking into account some recently proposed modifications of the electronic structure of these ions. The hydrophobic nature of such ions is shown to account for their effect on AChE. Finally, the identical stereospecificities of the enzyme and the muscarinic receptor towards the dioxolane series of quaternary salts suggests near identity of these two bioreceptors for ACh.

Over the past 15 years, much has been learned through kinetic studies about the catalytic mechanism of AChE. It is now recognized that the process of hydrolysis of acetylcholine (ACh) involves the formation of an acetyl–enzyme intermediate which undergoes a rate-determining hydrolysis.[2] It is generally agreed also that the enzyme's active surface includes two kinds of sites: esteratic and anionic. Investigations on the influence of pH on catalytic activity has revealed the existence of esteratic groups having pK values of 6.5 and 9.4, and which are involved in both the acetylation and deacetylation steps.[3] These functional groups have been identified tentatively as belonging to an imidazole ring and a tyrosine residue, respectively. The group of pK = 6.5 is apparently prevented from ionizing in the enzyme–substrate addition complex, thus suggesting that it is masked through interaction with the ester function of the substrate.[3] The esteratic site was also shown to be reactive towards phosphoryl, carbamyl, and sulfonyl derivatives which usually bring about profound inhibition of the enzyme.[4] Finally, the remarkable phenomenon of inhibition by excess substrate has been traced to noncompetitive blockade of the deacetylation step by the substrate.[5] Also, it was shown that the acetyl–enzyme intermediate retains the affinity of the free enzyme towards some of the most common competitive inhibitors.[3,5]

The investigations of Wilson and Cabib[2] on the nature of the forces acting in the enzyme–substrate complex have revealed that the interaction of the third methyl group on the nitrogen of ACh with the enzyme is accompanied by a marked decrease in the entropy of the complex, a result suggestive of a profound structural change in the enzyme. In contrast to this third methyl group which does not contribute to affinity for the enzyme, the other two methyl substituents have a marked effect on affinity, each one favoring adsorption by a factor of 7 over the respective desmethyl analogs.[6] The nature of the forces exerted on these methyl substituents have been ascribed to van der Waals attractions.[6,7] This assumption is discussed in

(1) (a) Published as part III of the series "Studies on the Chemical Basis for Cholinomimetic and Cholinolytic Activity." For part II, see B. Belleau and J. Puranen, *J. Med. Chem.*, **6**, 325 (1963). (b) This investigation was supported by the National Research Council of Canada and represents a portion of the thesis submitted by G. Lacasse in partial fulfillment of the requirements for the M.Sc. degree, University of Ottawa.

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(3) R. M. Krupka and K. J. Laidler, *Trans. Faraday Soc.*, **56**, 1477 (1960).

(4) I. B. Wilson in "The Enzymes," P. S. Boyer, H. Lardy, and K. Myrback, Ed., Academic Press Inc., New York, N. Y., 1960, p. 501.

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(6) I. B. Wilson, *J. Biol. Chem.*, **197**, 215 (1952).

(7) S. A. Bernhard, *Discussions Faraday Soc.*, **20**, 267 (1955).