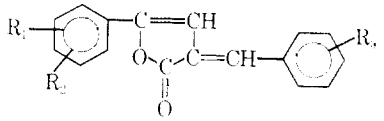
TABLE I



No.	R_1	R_2	R_3	Mp, °C (cor)	Yield, %	Formula	Calcd, % C	Calcd, % H	Calcd, % Cl	Found, % C	Found, % H	Found, % Cl
1	4-Cl	H	*p*-($ClCH_2CH_2$)$_2$N	177–181	61	$C_{21}H_{18}Cl_3NO_2$	59.67	4.29	25.56	59.55	4.37	25.32
2	4-Cl	H	*a*	242–243	73	$C_{16}H_{10}ClNO_2$	67.79	3.56	12.50	67.54	3.73	12.91
3	4-Cl	H	2-Chloro	244–246	85	$C_{17}H_{10}Cl_2O_2$	64.37	3.18	22.35	64.34	3.37	22.53
4	4-Cl	H	4-Methoxy	228–229	77	$C_{18}H_{13}ClO_3$	69.14	4.19	11.34	69.30	4.36	11.65
5	4-Cl	H	2-Acetoxy	192–194	65	$C_{19}H_{13}ClO_4$	66.97	3.84	10.41	66.89	3.88	10.64
6	4-Cl	H	2,3-Dimethoxy	179–180	50	$C_{19}H_{15}ClO_4$	66.57	4.41	10.34	66.42	4.51	10.57
7	4-Cl	H	3-Acetoxy	169–171	48	$C_{19}H_{13}ClO_4$	66.97	3.84	10.41	66.90	3.89	10.75
8	4-Cl	H	*b*	184–188	38	$C_{15}H_9ClO_3$	66.05	3.33	13.00	66.08	3.61	13.42
9	4-Ethoxy	H	*p*-($ClCH_2CH_2$)$_2$N	142–143	64	$C_{23}H_{23}Cl_2NO_3$	63.90	5.36	16.40	63.81	5.49	16.58
10	4-Ethoxy	H	2-Chloro	136–137	87	$C_{19}H_{15}ClO_3$	69.80	4.63	10.85	70.08	4.86	11.02
11	4-Ethoxy	H	*c*	165–167	93	$C_{17}H_{14}O_2S$	68.43	4.73	10.75	68.30	4.76	10.98
12	2-Ethoxy	5-Ethoxy	*p*-($ClCH_2CH_2$)$_2$N	105–107	48	$C_{25}H_{27}Cl_2NO_4$	63.03	5.72	14.89	63.52	5.91	15.25
13	2-Ethoxy	5-Ethoxy	4-Methoxy	175–177	71	$C_{22}H_{22}O_5$	72.11	6.05		72.08	6.24	
14	4-Methoxy	H	2,3-Dimethoxy	165–166	63	$C_{20}H_{18}O_5$	71.01	5.36		71.19	5.48	
15	4-Methoxy	H	4-Dimethylamino	175–177	47	$C_{26}H_{19}NO_3$	74.51	5.95	4.35	74.72	6.11	
16	4-Methoxy	H	3-Ethoxy-4-acetoxy	135–137	55	$C_{22}H_{26}O_6$	68.46	5.30		69.40	5.54	
17	4-Methoxy	H	*c*	175–176	90	$C_{18}H_{12}O_3S$	67.60	4.26	11.27	67.55	4.39	11.52

[a] Phenyl replaced by 3-pyridyl. [b] Phenyl replaced by 2-furyl. [c] Phenyl replaced by 2-thienyl.

Synthesis of 1,1′-Trimethylene-4,4′-Substituted Pyridinium and 1,3′-Halopropyl-4-Substituted Pyridinium Compounds[1]

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Various nucleophilic agents, particularly oximes, have been employed in antagonizing the highly toxic anticholinesterase alkyl phosphates, *e.g.*, chemical warfare agents and insecticides. One of the most effective agents for antagonizing alkyl phosphate intoxication is 1,1′-trimethylenebis(4-aldoximinopyridinium) dibromide.[3,4] The metabolic disposition of this antagonist is being investigated in our laboratory both *in vitro*[5] and *in vivo*.[6] The report herein describes the synthesis of some known or potential biological metabolites of this antagonist and their intermediates, which are being employed to facilitate the investigation of the biochemical transformation of 1,1′-trimethylenebis(4-aldoximinopyridinium) dibromide.

Experimental Section[7]

General.—All reactions were performed under anhydrous conditions and in stoppered flasks protected from light. It should be stressed that the cyano derivatives of types I and II are extremely labile, especially if anhydrous conditions are not carefully maintained. Isonicotinic acid was obtained from Eastman Organic Chemicals. Pyridine-4-aldoxime, 4-cyanopyridine, 1,3-diiodopropane, and 1,3-dibromopropane were purchased from the Aldrich Chemical Co. Care should be exercised in the use of 4-cyanopyridine due to its high vapor pressure and toxic properties. All other reagents and starting materials were of the highest grade and purity commercially available.

The Menschutkin reaction has been employed in the synthesis of these quaternary pyridinium compounds.[8] Minor modification of this reaction was utilized in the synthesis of this antagonist and in the preparation of the compounds presently described. The 4-aldoximino and the 4-cyano derivatives of type I were synthesized by treating the corresponding 1,3-dihalopropane with the substituted pyridine derivative. The 4-pyridone of types I and II was prepared by the alkaline hydrolysis of the corresponding 4-cyano derivative.[9] Bisquaternary derivatives of type II were prepared by treating type I with the appropriate monosubstituted pyridine, with the exception of the biscyano and the pyridone derivatives. The biscyano was prepared by the same procedure as for type I derivatives.

1,3′-Bromopropyl-4-aldoximinopyridinium Bromide (I).—A solution of 12.21 g (0.1 mole) of pyridine-4-aldoxime and 121 g (0.6 mole) of 1,3-dibromopropane in 750 ml of anhydrous nitrobenzene was allowed to stand at room temperature for 90 days. The yellow crystals which formed were collected, washed with ether, and dried *in vacuo*; yield 31.8 g (99%) of product, mp 188–189° dec. Recrystallization from methanol yielded as yellow needles 30.2 g (94%) of the quaternary salt, mp 189–190° dec. Compounds II and III were made with appropriate modification of the general method described above. The results are listed in Table I.

1,3′-Bromopropyl-4-pyridone Picrate (IV).—A solution of 6.35 g (0.021 mole) of 1,3′-bromopropyl-4-cyanopyridinium bromide and 20 ml of 1.0 *N* NaOH in 35 ml of distilled water was allowed to stand at 0–5° for 10 min, was adjusted to pH 9.0 with 1.0 *N* HCl, and was extracted with 1 l. of benzene in a liquid–liquid extractor for 24 hr. The benzene extract was dried ($MgSO_4$) and concentrated *in vacuo*. The yellow oil was crystallized as the picrate salt from benzene.

(1) This work was supported in part by U. S. Public Health Service Grants NB 04541 and MH 10109.

(2) These data are from a thesis to be presented by C. N. Corder in partial fulfillment of the requirements for a Ph.D. degree in pharmacology.

(3) E. J. Poziomek, B. E. Hackley, Jr., and G. M. Steinberg, *J. Org. Chem.*, **23**, 714 (1957).

(4) F. O. Hobbiger, D. G. O'Sullivan, and P. W. Sadler, *Nature*, **182**, 1498 (1958).

(5) C. N. Corder and J. L. Way, submitted for publication.

(6) P. M. Miranda and J. L. Way, submitted for publication.

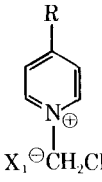
(7) Analyses were performed by Weiler and Strauss, Oxford, England, and by Huffman Laboratories, Inc., Wheatridge, Colo. All melting points were determined with a Thomas–Hoover apparatus and are corrected. Ultraviolet spectra were measured in a Beckman DB and/or Beckman DU spectrophotometer using distilled water as the solvent. Infrared spectra were determined with a Beckman IR-5 spectrophotometer using either KBr pellet, or Nujol mull. Legend for interpretations of principle absorbance bands are: s, strong; m, medium; w, weak. A Cahn Electrobalance was used to weigh spectral samples.

(8) E. N. Shaw, "Pyridine and Its Derivatives, Part II," Monographs from the series "The Chemistry of Heterocyclic Compounds," A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 1961, pp 1–95.

(9) E. M. Kosower, "Molecular Biochemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 179.

TABLE I

TYPE I: 1,3′-HALOPROPYL-4-SUBSTITUTED PYRIDINIUMS

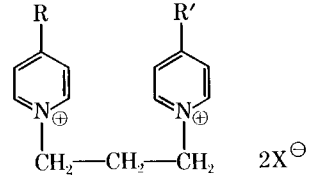


Compd	R	X	X_1^-	Mp, °C dec	Recrystn[a] solvent	Reaction time	Reaction temp, °C	Yield, %	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found	λ_{max}, mμ (log ε)[b]
I	CH=NOH[c]	Br	Br	189–190	A	90 days	20	94	$C_9H_{12}Br_2N_2O$	33.36	33.75	3.73	3.89	8.65	8.36	280 (4.26)
II	CH=NOH[c]	I	I	183–184	A	96 hr	50	70	$C_9H_{12}I_2N_2O$	25.86	26.38	2.89	3.16	6.70	6.72	224 (4.23), 279 (4.24)
III	CN[d]	Br	Br	171–172.5	A, B	40 hr	55	91	$C_9H_{10}Br_2N_2$	35.32	35.59	3.29	3.19	9.16	8.92	228 (4.10), 276 (3.64)
IV	=O[e]	Br	Picrate	165–166	C	10 min	2	42	$C_{14}H_{13}BrN_4O_8$	37.79	37.95	2.94	3.23	12.57	12.26	260 (4.42), 353 (4.12)

[a] A = methanol, B = ether, C = benzene. [b] Water was employed as the solvent. [c] Absorption (cm^{-1}), 980 m ± 20, 1010 s ± 10, 1610 m, 1639 s, and 3375 w were present in all 4-aldoximinopyridinium derivatives. Interpretations were from K. Nakanishi, "Infrared Absorption Spectroscopy—Practical," Holden-Day, Inc., San Francisco, Calif., 1962. [d] KBr pellet (cm^{-1}): 1608 w, 1639 s, 2874 w, and 2941 s. [e] Nujol (cm^{-1}): 1634 s, 2899 s, 3636 s.

TABLE II

TYPE II: 1,1′-TRIMETHYLENE-4,4′-SUBSTITUTED PYRIDINIUMS



Compd	R	R′	X^-	Mp, °C dec	Recrystn[a] solvent	Reaction time, hr	Reaction temp, °C	Yield, %	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found	Oxygen, % Calcd	Oxygen, % Found	λ_{max}, mμ (log ε)
V	CH=NOH	CN[b]	Br	225–226	A, B	65	48	53	$C_{15}H_{16}Br_2N_4O$	42.08	42.31	3.77	4.21	13.09	12.77			226 (4.14), 279 (4.31)
VI	CH=NOH	$CONH_2$[c]	Br	230–231	A	72	70	53	$C_{16}H_{18}Br_2N_4O_2 \cdot H_2O$	38.70	38.53	4.34	4.61	12.05	12.10	10.35	10.46	275 (4.31)
VII[d]	CN	$CONH_2$[e]	Br	260.5–262	C	75	55	25	$C_{15}H_{16}Br_2N_4O$	42.08	42.47	3.77	4.10	13.09	12.25			224 (4.31), 272 (3.88)
VIII[d]	CN[f,g]	CN	Br	276–277	C	96	55	18	$C_{15}H_{14}Br_2N_4$	43.93	43.83	3.44	3.41	13.66	13.37			231 (4.42), 236.5 (4.37), 278 (3.95)
IX	CH=NOH	=O	Picrate	184–186	C	0.167	2	24	$C_{26}H_{21}N_9O_{15}$	43.64	43.50	2.96	3.30	17.62	17.12			260 (4.59), 353 (4.46)

[a] A = methanol, B = ether, C = ethanol. [b] KBr pellet (cm^{-1}): 1610 m, 1639 s, 2976 s, 3344 m. The expected cyano peak at 2250 w was not observed; however, detection of the cyano group was accomplished by alkaline hydrolysis to liberate cyanide ion which was measured by a colorimetric procedure [J. Epstein, *Anal. Chem.*, **19**, 272 (1947)]. [c] KBr pellet (cm^{-1}): 1675 s. [d] Absolute ethanol was employed as the solvent for the reaction mixture. [e] KBr pellet (cm^{-1}): 1253 m, 1689 s, 2232 w, 2985 w, 3012 s, 3236 s. [f] Molar ratio of cyanopyridine–dibromopropane, 4:1. [g] KBr pellet (cm^{-1}): 2232 w, 2825 m, 2907 s, 2967 s, 3077 w.

Trimethylene-1-(4-aldoximinopyridinium)-1′-(4-cyanopyridinium) Dibromide (V).—A solution of 5.76 g (0.018 mole) of 1,3′-bromopropyl-4-aldoximinopyridinium bromide and 11.1 g (0.107 mole) of 4-cyanopyridine in 100 ml of N,N-dimethylformamide was maintained at 65° for 48 hr. The yellow crystals were collected, washed with 2-propanol and ethyl ether; yield 4.06 g (53%) of product, mp 228–229° dec. Recrystallization from methanol–ether yielded yellow-green needles, mp 225–226° dec. Compounds VI–VIII were made with appropriate modification of the general method described above. The results are listed in Table II.

Trimethylene-1-(4-aldoximinopyridinium)-1′-(4-pyridone) Picrate (IX).—A solution of 428 mg (0.001 mole) of trimethylene-1-(4-aldoximinopyridinium)-1′-(4-cyanopyridinium) dibromide in 10 ml of water was mixed with 5 ml of 1.0 *N* NaOH, maintained at 0–5° for 10 min and then adjusted to pH 9.9. The product was isolated from the reaction mixture by column chromatography using a Dowex 50-X12 (Na^+ form) with 0.005 *M* sodium carbonate, pH 9.9, as the eluent, and desalted with charcoal (Barnebey-Cheney RC-2) utilizing ethanol–0.1 *N* HCl (1:1, v/v) as the eluent. The solvent was removed *in vacuo*. The extremely hygroscopic oil was crystallized as the picrate salt from absolute ethanol. Recrystallization from absolute ethanol yielded yellow needles (35 mg, 60%), mp 184–186° dec.

Acknowledgments.—The authors are grateful to Mr. William T. Brady for technical assistance and Dr. J. H. Biel for helpful suggestions. We wish to express especial appreciation to Professor E. M. Kosower and Dr. J. W. Patton for many valuable discussions and helpful advice.

Preparation of 4-Iodoacetamido-1-naphthol as a Histochemical Reagent for Sulfhydryl Groups[1]

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Since the inhibitory action of iodoacetic acid on sulfhydryl groups of protein depends on a stoichiometric reaction, $ICH_2COOH + RSH \rightarrow RSCH_2COOH + HI$, one may anticipate 4-iodoacetamido-1-naphthol (I) to react similarly with sulfhydryl groups in protein to form a thioether which could then couple with diazotized 4-amino-2,5-diethoxybenzanilide (fast blue BBN) to form a blue dye[2] and thereby demonstrate the location of SH groups in *situ*. The dye derived from the parent compound can be easily washed out by organic solvents and does not interfere with the result. The preparation of 4-chloroacetamido-1-naphthol (II) has been reported.[3] A reinvestigation of this sample, softening at 175–180°, mp 199.5–201.5°, as reported by the early workers shows that it is a mixture of II and 4-chloroacetamido-1-naphthyl acetate (III) from infrared spectra. Modified preparations of both II and III are given in this note. The corresponding iodoacetyl derivatives I and IV react with sulfhydryl groups in protein. Owing to the alkali sensitivity of the O-acetyl linkage in IV, it still slowly couples with fast blue BBN at pH 7.5 and is therefore equally satisfactory for our study under appropriate conditions. A preliminary account of histochemical data has been given[4] and its usefulness in demonstrating the bound SH groups in plant tissues has also been reported.[5]

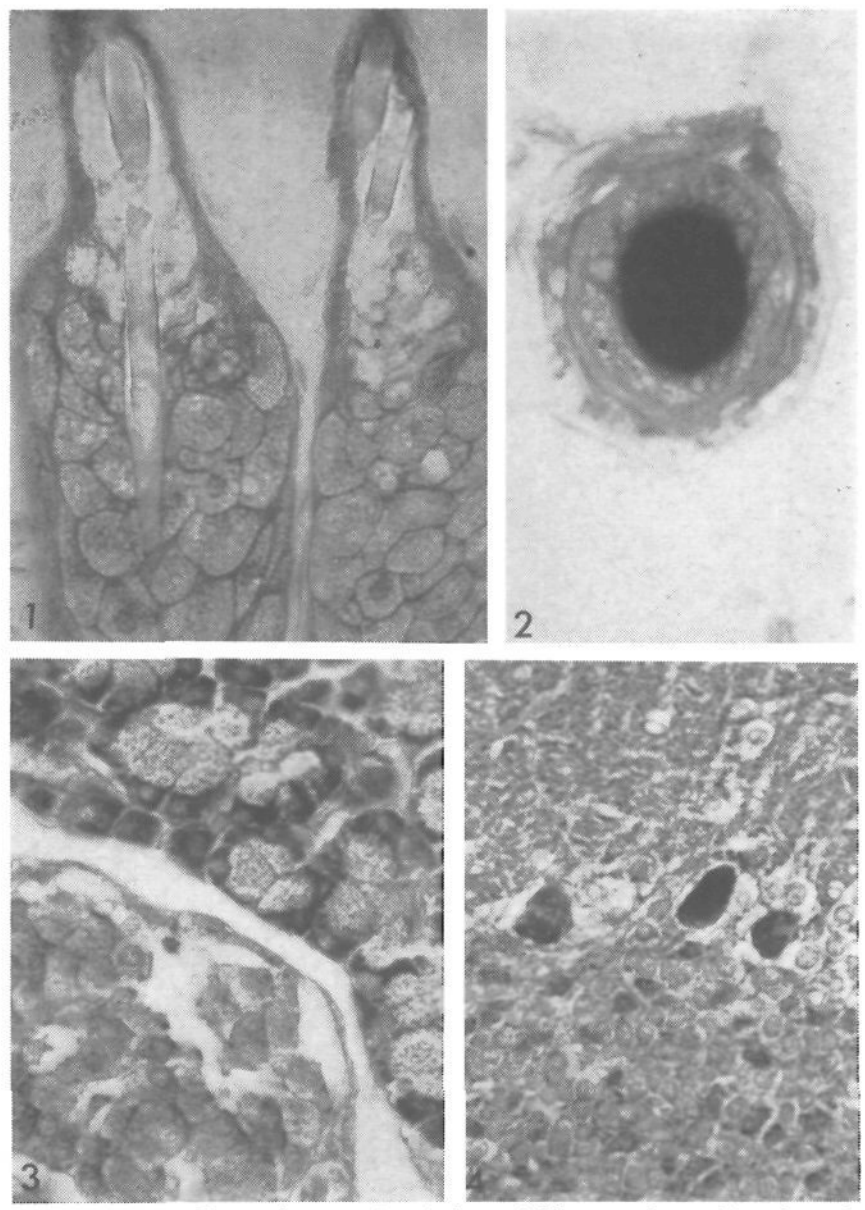

Figure 1.—Rat tissues fixed in trichloracetic acid–ethanol, embedded in paraffin, cut 6 μ thick, and stained for protein-bound sulfhydryl groups with 4-iodoacetamido-1-naphthol and fast blue BBN. 1: The epithelial cells of the sebaceous glands stain prominently. 2: At a deeper level in the dermis, in the region of horny transformation, the hair shaft is stained. 3: The basal and perinuclear region of the pancreatic acinar cells (top) is prominently stained as compared to the very faint stain of cells of the islets of Langerhans (bottom). 4: Purkinje cells of the cerebellum are more prominently stained than cells of the molecular layer (top) and granular layer (bottom).

Experimental Section[6]

4-Chloroacetamido-1-naphthol (II).—4-Amino-1-naphthol hydrochloride (4.9 g) was dissolved in a mixture of 50 ml of glacial acetic acid and 15 ml of dry dioxane, by warming slightly on a steam cone. Then 3.0 g of sodium acetate and chloroacetyl chloride were added in a rapid stream to this solution and the reaction mixture was stirred for 0.5 hr. The excess reagents were hydrolyzed by pouring into 350 ml of ice water with good stirring. The dark purple precipitate was collected, air dried, and recrystallized from ethyl acetate to yield 2.0 g (34%) of shiny purplish small needles, mp 195° dec. Further recrystallization from 95% alcohol and from aqueous methanol gave a sample of mp 196° dec.

Anal. Calcd for $C_{12}H_{12}ClNO_2$: C, 61.2; H, 4.3; N, 5.9. Found: C, 61.1; H, 4.2; N, 5.8.

It is only slightly soluble in $CHCl_3$ and ligroin, gives a positive Beilstein test, and couples readily with diazonium salt in a $NaHCO_3$ suspension.

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(1) This investigation was supported by U. S. Public Health Service Research Grant CA-07339 and CA-02478.

(2) Tyrosine in protein does not form a blue dye with this diazonium salt.

(3) W. A. Jacobs, M. Heidelberger, and I. P. Rolf, *J. Am. Chem. Soc.*, **41**, 458 (1919).

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(6) All melting points are corrected; analyses by Dr. C. K. Fitz, Needham Heights, Needham, Mass. Acknowledgment for histochemical technical assistance is due Mrs. Hannah L. Wasserkrug.