Synthesis of Some Quaternary Ammonium Alkylating Agents and Their Effects on Soman-Inhibited Acetylcholinesterase†

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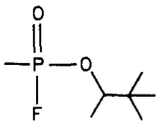
A number of compounds were synthesized and tested for their ability to realkylate the phosphonate anion of "aged", soman-inhibited acetylcholinesterase. None were found able to do so, but two of the compounds in particular, [2-(4-pyridyl)ethyl]diethylmethylammonium iodide (**6**) and its 2-isomer **7**, proved able to slow the rate of aging significantly.

Efforts to reactive acetylcholinesterase (AChE) inhibited by certain organophosphorus nerve agents, notably soman (GD, pinacolyl methylphosphonofluoridate), are complicated by intervention of a phenomenon known as "aging".[1]

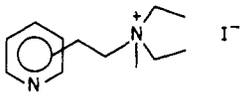


soman

Following phosphonylation by soman of the serine hydroxyl at the active site of the enzyme, aging, which entails loss of the pinacolyl group, takes place rapidly, leaving a phosphonate anion attached to the enzyme. The phosphonate anion is even more resistant to hydrolysis than the neutral ester and cannot be removed by 2-pyridinealdoxime methohalide (2-PAM) or any other available reactivating agent. Loss of the pinacolyl group is indicated to involve an acid-catalyzed process,[2-4] with the protonated imidazole (imidazolium) nucleus of the histidine residue at the enzyme active site quite possibly serving as the proton-donating acid catalyst;[2,5] this picture thus implicates the enzyme itself as the mediator of the process.

Early on, it was suggested that one possible approach to the aging problem would be through the design of agents capable of realkylating the phosphonate anion and reconverting it to a neutral ester group again susceptible to attack by a reactivator. Although early studies carried out with this purpose in mind were disappointing,[6,7] they involved investigation of conditions for realkylating a phosphonate anion that was not attached to the enzyme. It thus appeared worthwhile to see if it might be possible to design enzyme-site-directed agents able to realkylate the phosphonate ion generated in the environment of actual concern, bound to the enzyme in aged, soman-inhibited AChE.

To this end, a number of compounds have been synthesized and tested in vitro. The synthesized compounds all incorporated an alkylating, or "masked" alkylating, function and a quaternary ammonium group, the latter to encourage association with the anionic site of AChE. The intent was to probe structural requirements for activity. As judged by the inability of 2-PAM to reactivate aged, soman-inhibited, rat erythrocyte AChE that had been treated with test compound, none of the candidate agents were able to achieve the desired objective. However, two of the compounds, [2-(4-pyridyl)ethyl]diethylmethylammonium iodide (**6**) and its 2-isomer **7** evidenced the ability to effect a significant reduction in the rate of aging in vitro (Table I). At a concentration of 1 mM in vitro,



6, 4-pyridyl
7, 2-pyridyl

6 reduced the rate of aging of soman-inhibited, rat erythrocyte AChE by a factor of 2. Two other compounds effected a less pronounced, but still significant, reduction in the rate of aging. Schoene[8] had earlier reported a few, unrelated bispyridinium salts that slowed the rate of aging in vitro. One of these, 1,3-bis(3-pyridyl)urea dimethiodide, reduced the rate by a factor of 5 but at a higher concentration (2 mM).

It is not at all clear how these compounds act to cause a slowing of the rate of aging, but it is tempting to speculate that they may have an allosteric effect on the enzyme, which moves the imidazolium ion away from the phosphonyl ester group, thus interfering with the postulated intramolecular acid catalysis of the aging process. None of the compounds slowed the rate at a low enough concentration for therapeutic utility; however, the approach appears worth pursuing since effective retardation of aging in vivo would provide more time for a reactivating agent to carry out its mission.

Chemistry. Test compounds were for the most part synthesized by conventional methods as described in the Experimental Section, but a few points are worth noting. Both **6** and **7** were readily prepared by careful treatment of the corresponding [2-(dimethylamino)ethyl]pyridine base with 1 equiv of methyl iodide, with 2-propanol as the solvent for preparation of **6** and acetonitrile for **7**. However, the two bases showed differences in behavior when efforts were made to prepare the pyridine *N*-methyl iso-

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(1) See Gray, A. P. *Drug Metab. Rev.* **1984**, *15*, 557.
(2) Michel, H. O.; Hackley, B. E.; Berkowitz, L.; List, G.; Hackley, E. B.; Gillilan, W.; Pankau, M. *Arch. Biochem. Biophys.* **1967**, *121*, 29.
(3) Aldridge, W. N. *Croat. Chem. Acta* **1975**, *47*, 225.
(4) Clothier, B.; Johnson, M. K. *Biochem. J.* **1980**, *185*, 739.
(5) Beauregard, G.; Lum, J.; Roufogalis, B. D. *Biochem. Pharmacol.* **1981**, *30*, 2915.
(6) Blumbergs, P., Ash, A. B.; Daniker, F. A.; Stevens, C. L.; Michel, H. O.; Hackley, B. E.; Epstein, J. *J. Org. Chem.* **1969**, *34*, 4065.
(7) Steinberg, G. M.; Lieske, C. N.; Boldt, R.; Goan, J. C.; Podall, H. E. *J. Med. Chem.* **1970**, *13*, 435.
(8) Schoene, K. *Biochim. Biophys. Acta* **1978**, *525*, 468.

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Table I

compd	rat AChE I_{50}, mM		effect on rate of aging rate constant ($\times 10^{-2}$)[a]		
	erythrocyte	brain	control	treated	ratio control/treated[b]
1	0.26	0.26	4.28	3.59	1.19
2	0.06	0.09	6.56	6.72	0.98
3	0.46	0.34	7.62	5.85	1.30*
4	0.83	0.89	6.54	6.38	1.02
5	0.87	0.47	6.49	5.76	1.13
6	0.20	0.20	6.40	3.06	2.09***
7	>1.0	>1.0	7.58	4.72	1.60**
8	0.55	0.50	6.03	5.62	1.07
9	0.10	0.17	7.37	6.61	1.11
10	0.25	0.38	8.83	10.90	0.81
11	0.20	0.20	6.68	5.00	1.34*

[a] Soman-inhibited rat erythrocyte AChE; test compound at 1 mM. First-order rate constant equals slope of regression line times (−2.303). [b] Level of significance was determined by using a T test for the correlation of the two regression lines for each compound:[17] * = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.001$.

Table II. Distribution of Rat Erythrocytes for Experiments on Rate of Aging[a]

tube	vol of cell suspension, mL	soman	compd	2-PAM
1	2.0	−	−	−
2	2.0	−	+	−
3	2.0	−	−	+
4	2.0	−	+	+
5	2.0	+	−	−
6	2.0	+	+	−
7	10.0	+	−	+
8	10.0	+	+	+

[a] Tubes 1–6 are controls; the actual volume required for tubes 7 and 8 is determined by the number of dilutions and time intervals being tested. A minimum volume of 2.0 mL is required for each sample to be assayed for AChE activity. All tubes are processed together through the entire procedure.

mers of **6** and **7**. Repeated attempts were made without success to prepare the pyridine *N*-methyl isomer of **6**, 4-[2-(diethylamino)ethyl]-1-methylpyridinium, in ethanol as solvent and with the more basic amino N blocked with acetic acid or methanesulfonic acid. We were unable to quaternize the pyridine N selectively and, in the presence of acid, observed only formation of what appeared to be a bis-quaternary salt which was unstable and could not be isolated.

On the other hand, although **7** was obtained cleanly in the aprotic solvent acetonitrile, treatment of 2-[2-(diethylamino)ethyl]pyridine with methyl iodide in ethanol, for 2 days at room temperature and with no acid added, gave a low yield of the pyridine *N*-methyl derivative, 2-[2-(diethylamino)ethyl]-1-methylpyridinium iodide in the form of its hydriodide salt (**8**). Neutralization gave the free 2-(diethylamino)-1-methylpyridinium iodide. It is not entirely clear why the 2- but not the 4-substituted pyridine base should afford the pyridine *N*-methylquaternary in a protic solvent, but it would appear that both nitrogens of the 2-isomer must participate in the reaction. For example, both nitrogens in a cisoid conformer of the 2-substituted base may be involved in attack on the methyl group of methyl iodide but the methyl group may end up attached to the pyridine N because of solvation of the amino N. Formation of the hydriodide salt could have been a result of solvolysis of some of the methyl iodide.

Biochemistry

Effect of Compounds on AChE Activity. The effect of each compound on AChE was evaluated by incubating rat erythrocytes or brain homogenates with different concentrations of the compound. The concentration of each compound required to inhibit 50% of AChE activity (I_{50}) in blood and brain is shown in Table I. Among the compounds synthesized, **2**, **6**, **9**, and **11** were the only ones with I_{50} values at or below 0.20 mM. In general, AChE from rat blood and brain were equally sensitive to inhibition by the compounds; however, in the case of **5**, brain AChE appears to be somewhat more sensitive to inhibition than AChE from erythrocytes (Table I).

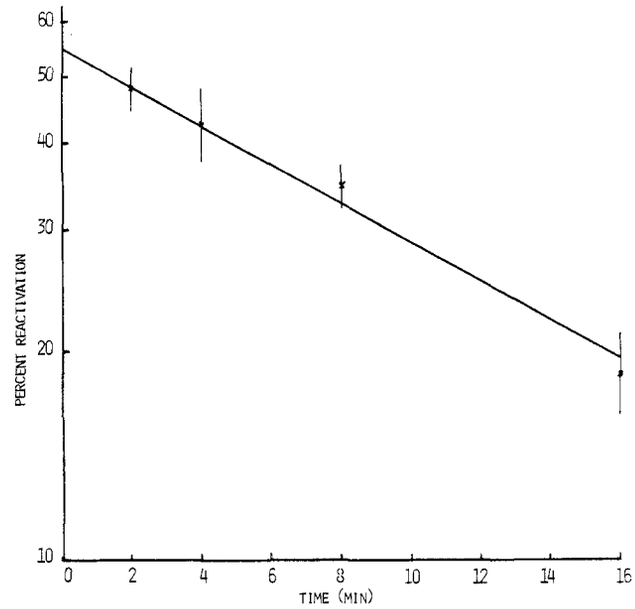


Figure 1. Effect of aging on reactivation of soman-inhibited AChE by 2-PAM. Average of five control experiments showing mean and standard deviation. Aging of soman-treated rat erythrocytes was initiated by the addition of PBS (pH 7.3) at 37 °C. Aliquots were removed at timed intervals and allowed to incubate with 0.1 M 2-PAM to effectively reactivate unaged, soman-inhibited AChE. The *y* axis shows the percentage of AChE reactivated on a logarithmic scale. The line represents a linear regression based on the points shown. The correlation coefficient for the line (0.996) indicates an excellent fit of the data points to a straight line.

Effect of Compounds on the Rate of Aging. Since aged, soman-inhibited enzyme cannot be reactivated by AChE reactivators such as 2-PAM, the inability of 2-PAM to reactivate the dealkylated phosphonyl-AChE can be used to determine the fraction of aged enzyme, and thus to follow the time course of the aging reaction. Aging of soman-inhibited AChE appears to follow first-order kinetics.[15]

The reproducibility of the procedure for estimating the rate of aging is indicated in Figure 1, which shows the decreasing effectiveness of 2-PAM in reactivating AChE

within 16 min of initiation of the aging process. Data represent the mean and standard deviation of control tubes (tube 7, Table II) from five trials. The slope of the line represents the rate of aging. Similar plots were developed to evaluate the rate of aging in the presence of each of the experimental compounds. The ratio (control/compound) of the first-order rate constants (calculated as the slope of each regression line times −2.303) gives a numerical indication of the effect of each compound on the rate of aging (Table I). It is evident that compounds **6** and **7** produced a significant reduction in the rate of aging. The rate of aging was also reduced in the presence of **3** and **11** ($P < 0.05$). The remaining seven compounds had no significant effect on the reaction. Compound **6** was tested three times, and comparable results were obtained in all three trials.

Experimental Section

Chemistry. Solvents were dried or distilled before use. Melting points were obtained on a Mel-temp apparatus (Laboratory Devices) in open capillary tubes and are uncorrected. Infrared spectra (IR) were determined with KBr wafers or on NaCl plates with a Perkin-Elmer 1320 infrared spectrophotometer. The spectra are reported in cm^{-1}. Proton (^1H) nuclear magnetic resonance (NMR) spectra were determined in deuterated chloroform ($CDCl_3$) containing ca. 1% (v/v) tetramethylsilane (Me_4Si) or in deuterium oxide (D_2O) containing ca. 1% (v/v) of 3-(trimethylsilyl)propanesulfonic acid, sodium salt (2,2-dimethyl-2-silapentane-5-sulfonate, DSS), as internal standard with a Varian EM-360 (60 MHz) spectrometer. Chemical shifts are reported in δ units, parts per million (ppm) downfield from Me_4Si. Ultraviolet (UV) and visible spectra were determined on the compounds dissolved in methanol with a Beckman Lambda 3 UV/visible spectrophotometer. Data were obtained over the spectral range 700–200 nm at concentrations of ca. 10^{-5}–10^{-6} M. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. The data were acceptable within ±0.4% of the calculated values. For purity tests, TLC was performed on fluorescent silica gel and/or alumina plates developed in at least two different solvents; only one spot (visualized by UV light and I_2 vapor) was obtained. More accurate purity tests were performed on a Finigan 4000 gas chromatograph/mass spectrometer or Tracor Model 951 high-performance liquid chromatograph.

(3-Chloropropyl)trimethylammonium Bromide (1).[9] Trimethylamine (3.25 g, 5.11 mL, 0.055 mol) in 50 mL of dry benzene at 0 °C was added, dropwise with stirring, to a cold solution of 1-bromo-3-chloropropane (7.87 g, 0.05 mol) in 30 mL of benzene. Addition was complete in 45 min. The mixture was stirred at room temperature for 17 h. The resultant white crystalline precipitate was collected and washed with benzene (2 × 10 mL) to give 2.071 g of product. The filtrate was stirred at room temperature for 3 days to give an additional 3.248 g. The overall yield was 5.32 g (50%). The compound was dried in a vacuum oven at 65 °C for 2 days: mp 210–211 °C (lit.[9] mp 204–206 °C); UV (in methanol) max 205.5 nm; IR (KBr) 3430 (br, weak), 3015, 2965, 1489, 1482, 1423, 1344, 1270, 1048, 973, 959, 928, 780, 748, 713 cm^{-1}; NMR (D_2O/DSS) δ 1.97–2.57 (m, 2 H), 3.13 (s, 9 H, CH_3), 3.33–3.83 (m, 4 H). Anal. ($C_6H_{15}NBrCl$) C, H, N.

1-(6-Bromohexyl)pyridinium Bromide (2). Into a solution of 12.20 g (0.05 mol) of 1,6-dibromohexane in 10 mL of benzene at 0 °C was added, dropwise with stirring, a solution of 3.16 g (0.04 mole) of pyridine in 30 mL of benzene. Addition was complete in 2 h. The mixture was stirred at room temperature for 4 days. A small amount of precipitate (0.728 g) was collected. The filtrate was stirred at room temperature for 4 weeks to give 1.688 g of product (overall yield 18.7%). The product was recrystallized from ethanol/ether to give a white powder: mp 127–128 °C; UV (methanol) λ_{max} 258 nm, 207; IR (KBr) 3010, 2945, 2930, 2860, 1630, 1503, 1490, 1470, 1330, 1300, 1253, 1170, 788, 692, 637 cm^{-1}; NMR (D_2O/DSS) δ 1.20–2.43 (m, 8 H, 4 CH_2), 3.5 (t, J = 6 Hz, 2 H), 4.68 (t, J = 6 Hz, 2 H), 7.93–9.07 (m, 5 H). Anal. ($C_{11}H_{17}NBr_2$) C, H, N.

2-(Chloromethyl)-1-methylpyridinium Methanesulfonate (3). Attempts to prepare the iodide salt by treatment of the base with methyl iodide were unsuccessful, apparently because the iodide anion entered into complicating nucleophilic displacement reactions. 2-Picolyl chloride hydrochloride (5.0 g, 0.0305 mol, Aldrich Chemical Co.) was neutralized with sodium carbonate (1.6 g, 0.015 mol) in ice (30 g). the mixture was stirred and a brown-yellowish liquid (ca. 5 mL) was separated from the ice water solution. The aqueous phase was made alkaline (pH 8) with 0.2 g of sodium carbonate and extracted four times with 10-mL portions of benzene. The separated organic phases were combined, washed with saturated NaCl solution (10 mL), and dried over $MgSO_4$. The colorless benzene solution was treated with methyl methanesulfonate (5.04 g, 0.046 mol, 1.5 equiv), placed in a sealed flask, and stirred at room temperature for 2 weeks. The fine precipitate of colorless crystals was filtered and washed with benzene (2 × 10 mL). The product was recrystallized from ethanol/ether (1:1) to give 2.06 g (28.5%) of colorless, granular crystals: mp 114–116 °C dec; UV (methanol) λ_{max} 267.5 nm, 208.0; IR (KBr) 3470, 3090, 3045, 3005, 1630, 1583, 1520, 1485, 1460, 1420, 1335, 1295, 1280, 1210, 1065, 962, 790, 780, 760, 690, 615 cm^{-1}; NMR (D_2O/DSS) δ 2.80 (s, 3 H, SO_3CH_3), 4.43 (s, 3 H, NCH_3, 5.07 (s, 2 H), 7.82–8.97 (m, 4 H). Anal. ($C_8H_{12}NO_3SCl$) C, H, N.

4-(2-Succinimidoethyl)pyridine Methiodide (4). To a boiling solution of 10.9 g (0.11 mol) of succinimide in 40 mL of isopropyl alcohol containing 10 drops of a 40% methanolic solution of benzyltrimethylammonium hydroxide (Triton-B) was added, dropwise, 10.514 g (0.1 mol) of undistilled 4-vinylpyridine. After being heated for 14 days at reflux, the reaction solution was concentrated to 20 mL and dissolved in 50 mL of water. The aqueous solution was extracted with ether (6 × 50 mL) to remove most of the 4-vinylpyridine and then with ethyl acetate (6 × 50 mL). Both the ether and ethyl acetate solutions were dried ($MgSO_4$) and filtered. The ether solution was concentrated to 60 mL and cooled to −20 °C to give a small amount of product as crystalline needles. This was added to the crude solid product obtained from the ethyl acetate solution and the combined product was recrystallized from ether to give 10.17 g (49.8%) of 4-(succinimidoethyl)pyridine as needle crystals: mp 78–82 °C; IR (KBr) 3188, 3080, 3000, 2960, 1775, 1700, 1605, 1440, 1415, 1370, 1300, 1200, 1160, 1115, 1030, 1010, 1000, 960, 940, 855, 815, 650 cm^{-1}.

A portion of the (succinimidoethyl)pyridine (4.7 g, 0.023 mol) was dissolved in 60 mL of acetone and treated with methyl iodide (9.79 g, 0.069 mol, 3 equiv). After the mixture had been stirred at room temperature for 16 h, the colorless precipitate that had formed was collected. The product was dissolved with heating in ethanol (the solution turned green when heated) and recrystallized. Light green, leaflike crystals were collected. Recrystallization from acetone gave 6.64 g (83.4%) of white crystals: mp 152–154 °C dec; UV (methanol) λ_{max} 257.4 nm, 221.2, 206.3; IR (KBr) 3120, 2960, 2940, 1760, 1705, 1640, 1570, 1520, 1480, 1455, 1400, 1357, 1282, 1258, 1221, 1190, 1160, 1090, 1055, 980, 962, 935, 915, 820, 715, 660 cm^{-1}; NMR (D_2O/DSS) δ 2.83 (s, 4 H, CH_2), 3.25 (t, J = 6 Hz, 2 H), 3.92 (t, J = 6 Hz, 2 H), 4.37 (s, 3 H, NCH_3), 7.98 (d, J = 6 Hz, 2 H), 8.72 (d, J = 6 Hz, 2 H). Anal. ($C_{12}H_{15}N_2O_2I$) C, H, N.

2-(2-Succinimidoethyl)pyridine Methiodide (5). 2-Vinylpyridine (5.257 g, 0.055 mol) was added dropwise to a boiling solution of 5.9 g (0.055 mol) of succinimide in 40 mL of *p*-xylene containing 10 drops of a 40% methanolic solution of benzyltrimethylammonium hydroxide (Triton-B). After being heated for 40 h at reflux, the reaction mixture was cooled in ice, and the crystals were collected. The crystals were decolorized with charcoal in dichloromethane and recrystallized from ethyl acetate and ether to give 5.835 g (57%) of 2-(succinimidoethyl)pyridine as white needles: mp 102–103 °C; IR (KBr) 3460, 3060, 2950, 2880, 1775, 1700, 1590, 1565, 1475, 1455, 1445, 1400, 1360, 1335, 1315, 1300, 1255, 1240, 1150, 1100, 1050, 1030, 995, 950, 850, 805, 775, 755, 660, 640 cm^{-1}; NMR ($CDCl_3$) δ 2.70 (s, 4 H), 3.22 (t, J = 7 Hz, 2 H), 4.00 (t, J = 7 Hz, 2 H), 7.07–7.90 (m, 3 H), 8.53–8.70 (m, 1 H).

2-(Succinimidoethyl)pyridine methiodide showed the following: mp 205–208 °C; UV (methanol) λ_{max} 266.5 nm, 218.5, 205; IR

(9) Gray, A. P.; Archer, W. L.; Spinner, E. E.; Cavallito, C. J. *J. Am. Chem. Soc.* **1955**, *77*, 3805.

(KBr) 3460, 3050, 2970, 2950, 2930, 1770, 1685, 1625, 1520, 1510, 1485, 1400, 1375, 1345, 1320, 1275, 1260, 1210, 1180, 1165, 1110, 1075, 1040, 1010, 820, 780, 660, 635 cm^{-1}; NMR (D_2O/DSS) δ 2.83 (s, 4 H), 3.43 (t, J = 7 Hz, 2 H), 3.97 (t, J = 7 Hz, 2 H), 4.43 (s, 3 H), 7.80–8.13 (m, 2 H), 8.37–8.93 (m, 2 H). Anal. ($C_{12}H_{20}N_2O_2I$) C, H, N.

[2-(4-Pyridyl)ethyl]diethylmethylammonium Iodide (6).[10] A solution of 4-vinylpyridine (10.514 g, 0.1 mol), diethylamine (14.628 g, 0.2 mol), and glacial acetic acid (12.01 g, 0.2 mol) in 2-propanol (50 mL) was stirred and heated at reflux for 7 days. The color of the solution changed from yellow to brown. TLC showed one spot at the origin and a small amount of 4-vinylpyridine at R_f 0.57. The solvent was removed in vacuo and the residue was treated with 90 mL of 10% NaOH to neutralize the acetic acid. The basic solution was extracted with ether (4 × 100 mL). The combined extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was distilled in vacuum (0.8 mm) to give 9.45 g (53.0%) of 4-[2-(diethylamino)ethyl]pyridine: bp 73–76 °C (0.8 mm) [lit.[10] bp 51–53 °C (0.1 mm)]; UV (methanol) λ_{max} 262.5 nm, 250, 220; IR (neat) 3025, 2975, 2810, 1600, 1470, 1415, 1385, 1290, 1220, 1070, 990, 810 cm^{-1}; NMR ($CDCl_3$/Me_4Si) δ 1.0 (t, J = 7 Hz, 6 H, CH_3), 2.50 (q, J = 7 Hz, 4 H, CH_2), 2.67 (s, 4 H), 6.83 (d, J = 4 Hz, 2 H), 8.38 (d, J = 4 Hz, 2 H).

A solution of 4-[2-(diethylamino)ethyl]pyridine (6.5 g, 0.0365 mol) in 2-propanol (30 mL) was treated with methyl iodide (5.18 g, 0.0365 mol). The mixture was stirred at room temperature for 3 days. A yellow crystalline precipitate was collected and washed with fresh 2-propanol to give 5.88 g (50.3%) of product, which was indicated, on the basis of its IR and NMR spectra, to be contaminated by pyridine N-methylated material. The product was decolorized with charcoal and fractionally recrystallized from ethanol to give granular crystals, mp 100–101 °C. The IR spectrum showed a peak at 1600 and no 1640-cm^{-1} absorption. NMR (D_2O/DSS) δ 1.35 (t, J = 7 Hz, 3 H), 1.40 (t, J = 7 Hz, 3 H), 3.11 (s, 3 H), 3.28 (m, 4 H), 3.47 (q, J = 7 Hz, 4 H), 4.67 (s, 1 H, HDO), 7.37 (m, 2 H), 8.40 (m, 2 H). The NMR spectrum (peak at δ 4.67) and the analysis indicated the presence of $^1/_2$ mol of H_2O. Anal. ($C_{12}H_{21}N_2I \cdot 0.5H_2O$) C, H, N.

[2-(2-Pyridyl)ethyl]diethylmethylammonium Iodide (7). 2-[2-(Diethylamino)ethyl]pyridine was obtained in higher yield (87.5%) by the reaction of 2-vinylpyridine (0.2 mol) and glacial acetic acid (0.4 mol) with a large excess of diethylamine (1 mol) as solvent: bp 77 °C (0.9 mm); UV (methanol) λ_{max} 261.5 nm, 210; IR (neat) 3400 (b), 3060, 2965, 2930, 2800, 1590, 1565, 1472, 1434, 1380, 1290, 1148, 1116, 1065, 990, 750 cm^{-1}.

A solution of 2-[2-(diethylamino)ethyl]pyridine (3.2 g, 0.018 mol) in acetonitrile (30 mL) was treated with methyl iodide (2.55 g, 0.018 mol). The mixture was stirred at room temperature for 18 h. The color of the solution turned from light yellow to yellow brown. An oily layer separated when the solution was poured into 250 mL of ether. The ether was decanted, and the oily product was crystallized from 2-propanol/ether to give brown crystals. The crystals were decolorized with charcoal and recrystallized from 2-propanol/ether to give light yellow crystals (4.5 g, 78%): mp 60–61 °C; UV (methanol) λ_{max} 265.5 nm, 259, 253, 220, 205; IR (KBr) 3430, 3080, 3040, 2980, 2945, 1600, 1590, 1570, 1480, 1450, 1438, 1404, 1380, 1350, 1320, 1295, 1255, 1215, 1190, 1163, 1150, 1130, 1115, 1090, 1060, 1048, 1020, 995, 975, 955, 915, 870, 820, 805, 790, 770, 750, 710, 630 cm^{-1}; NMR (D_2O/DSS) δ 1.45 (t, J = 7 Hz, 6 H), 3.33 (s, 3 H), 3.57 (m, 4 H), 3.72 (q, J = 7 Hz, 4 H), 7.0–7.93 (m, 3 H), 8.53 (d, J = 4 Hz, 6 H). Anal. ($C_{12}H_{21}N_2I$) C, H, N.

2-[2-(Diethylamino)ethyl]-1-methylpyridinium Iodide Hydriodide (8). 2-[2-(Diethylamino)ethyl]pyridine (13.308 g, 0.0746 mol) in absolute ethanol (20 mL) was treated with methyl iodide (10.588 g, 0.0746 mol). The mixture was stirred at room temperature for 2 days. A yellow precipitate was collected and recrystallized from ethanol to give yellow granular crystals (3.87 g, 16%) of 8: mp 213–215 °C; UV (methanol) λ_{max} 266 nm, 221; IR (KBr) 3440, 3060, 3020, 2970, 2950, 2930, 2910, 2860, 1623, 1578, 1510, 1480, 1450, 1335, 1290, 1250, 1223, 1200, 1165, 1095, 1055, 1020, 785 cm^{-1}; NMR (D_2O/DSS) δ 1.40 (t, J = 7 Hz, 6 H), 3.45 (q, J = 7 Hz, 4 H), 3.70 (s, 4 H), 4.40 (s, 3 H), 7.90–8.20 (m, 2 H), 8.43–903 (m, 2 H). Anal. ($C_{12}H_{22}N_2I_2$) C, H, N, I.

A solution of 8 in methanol was washed through an Amberlyst A-21 (Rohm and Haas Co., weakly basic anion-exchange resin) column with methanol. The eluent was collected and evaporated to dryness. The residue was crystallized from 2-propanol/ether to give the neutralized quaternary salt, 2-[2-(diethylamino)-ethyl]-1-methylpyridinium iodide: mp 55–58 °C; IR 3460, 3040, 2970, 1630, 1580, 1515, 1470, 1280, 1190, 1050, 770 cm^{-1}; NMR (D_2O/DSS) δ 1.13 (t, 3 H), 1.23 (t, 3 H), 2.40–3.20 (m, 8 H), 4.33 (s, 3 H), 7.80–9.10 (m, 4 H). The IR and NMR spectral data confirmed that the product was not the amino N-methylquaternary salt and were in accord with assignment as the pyridine N-methyl derivative. The same product was obtained by bringing an aqueous solution of 8 to pH 8 with dilute sodium hydroxide.

1-(2-Pyridyl)-4-chloro-3-butanol Methiodide (11). 2-Picoline (1.886 g, 0.020 25 mol) in anhydrous tetrahydrofuran (10 mL) was introduced into a 250-mL three-necked flask fitted with nitrogen inlet tube and addition funnel. The solution was cooled to −30 °C, and n-butyllithium in hexane (12.656 mL, 0.020 25 mol) was added over a 30-min period with stirring. The solution was allowed to stir for an hour while the temperature was allowed to rise to ambient. The solution turned deep red. A solution of epichlorohydrin (1.874 g, 0.020 25 mol) was added over 15 min with vigorous stirring and with the temperature maintained at 25 °C. The mixture was stirred for an additional hour and hydrolyzed with glacial acetic acid (1.16 mL, 0.020 25 mol). Immediately on addition of the glacial acetic acid a copious red precipitate formed. The precipitate dissolved with the addition of water (5 mL) and the reaction mixture was extracted with ether (3 × 15 mL). The combined ether layers were evaporated in vacuo to give 2.719 g (72.3%) of syrup: UV (methanol) λ_{max} 267 nm, 217; IR (neat) 3300 (b), 3080, 3020, 2950, 2880, 1600, 1575, 1480, 1440, 1275, 1165, 1055, 1000, 755, 750 cm^{-1}. The product was dissolved in 30 mL of benzene and treated with methyl iodide (6.236 g. 0.044 mol). Brown needles were collected and recrystallized from ethanol to give 1.525 g (31.8%) of white crystals: mp 117–121 °C dec; IR (KBr) 3340, 3030, 1630, 1580, 1518, 1470, 1455, 1435, 1400, 1275, 1190, 1165, 1100, 1030, 930, 860, 825, 800, 773, 740, 690 cm^{-1}; NMR (D_2O/DSS) δ 2.10 (t, J = 8 Hz, 2 H), 3.23 (t, J = 8 Hz, 2 H), 3.70 (s, 2 H), 4.03 (q, 1 H), 4.30 (s, 3 H), 7.67–8.07 (m, 2 H), 8.30–8.80 (m, 2 H). Anal. ($C_{10}H_{15}NOClI$) C, H, N.

(4-Succinimido-2-oxobutyl)diethylmethylammonium Iodide (9). Succinimide (20.0 g, 0.2 mol) dissolved in hot absolute alcohol (50 mL) was added to a stirred solution of sodium ethoxide (4.6 g of sodium in 50 mL of absolute alcohol). The mixture was stirred for 30 min. 3-Butenyl bromide (27.02 g, 0.2 mol) was added slowly and the mixture was heated at reflux for 6 h. A white precipitate of sodium bromide was filtered off, the filtrate was concentrated in vacuo, and the residue was diluted with 20 mL of water. The organic layer was separated and the aqueous solution was extracted with ether (3 × 50 mL). The combined organic extract was washed with saturated sodium chloride solution (50 mL), dried, and evaporated to dryness in vacuo to give 24.73 g (80.0%) of N-butenylsuccinimide.[11] Distillation afforded a colorless liquid: bp 132–135 °C (16 mm); IR (neat) 3460 (w), 3360 (w), 3080, 2980, 2945, 1770, 1700 (s), 1660, 1570, 1440, 1400, 1360, 1345, 1305, 1295, 1190, 1170, 1130, 1075, 1000, 920, 820, 665 cm^{-1}; NMR ($CDCl_3$) δ 2.33 (q, J = 7 Hz, 2 H), 2.75 (s, 4 H), 3.57 (t, J = 7 Hz, 2 H), 4.83–5.23 (m, 2 H), 5.43–6.13 (m, 1 H).

To a stirred solution of N-butenylsuccinimide (15.03 g, 0.105 mol) in 100 mL of chloroform was added, dropwise over a period of 1 h, a solution of m-chloroperbenzoic acid (22.887 g, 0.115 mol, 1.1 equiv) in 250 mL of chloroform at 10 °C (ice bath). Stirring was continued for 20 h at room temperature. The copious white precipitate of m-chlorobenzoic acid was separated by filtration. Excess peracid in the filtrate was destroyed by addition of 10% sodium sulfite solution until a test with starch–iodide paper was negative. The mixture was washed with 5% sodium bicarbonate solution (3 × 50 mL) and then with saturated sodium chloride solution. The organic layer was dried and evaporated. The residue was distilled to give 15.69 g (88.4%) of epoxybutylsuccinimide: bp 111–115 °C (0.5 mmHg); IR (neat) 3460 (w), 3050, 3000, 2950, 1770, 1700, 1440, 1360, 1345, 1305, 1255, 1190, 1175, 1155, 1125,

(10) Blicke, F. F.; Hughes, J. L. *J. Org. Chem.* **1961**, *26*, 3257.

(11) Clemo, G. R.; Pamage, C. R. *J. Chem. Soc.* **1931**, 49.

900, 860, 820, 750, 665 cm^{-1}; NMR ($CDCl_3$) δ 1.02–1.45 (m, 1 H), 1.55–1.98 (m, 2 H), 2.15–2.52 (m, 2 H), 2.72 (s, 4 H), 3.62 (t, J = 7 Hz, 2 H).

The product (15.69 g, 0.0927 mol) was heated at reflux for 3 days with 3 equiv of diethylamine (21.0 g) (without added solvent). The diethylamine was evaporated in vacuo and the brown residue was distilled to give 20.45 g (91%) of yellow, high-density syrup: bp 184–186 °C (0.6 mm); IR (neat) 3460, 2980, 2950, 2880, 2820, 1770, 1700, 1450, 1410, 1375, 1345, 1300, 1255, 1160, 1130, 1065, 1000, 930, 820, 770, 660 cm^{-1}; NMR ($CDCl_3$) δ 1.02 (t, J = 7 Hz, 6 H), 1.58 (q, J = 7 Hz, 2 H), 2.57 (q, J = 7 Hz, 4 H), 2.73 (s, 4 H), 2.20–2.80 (m, 2 H), 3.40–3.73 (m, 3 H), 3.87 (s, 1 H).

To a stirred solution of *N*-[4-(diethylamino)-3-hydroxybutyl]succinimide (3.6 g, 0.0148 mol) in acetone at 10 °C was added, dropwise, a solution of chromium trioxide (1.63 g, 0.0163 mol, 1.1 equiv) in a mixture of concentrated sulfuric acid (1.82 mL) and water (ca. 2 mL) (Jones reagent[12]). Stirring was continued for 3 h as a dark green solid precipitated and the solution turned from orange to brown. Solid sodium bisulfite was added in small portions until the brown color of chromic acid had disappeared from the upper layer of the two-phase mixture. Saturated sodium carbonate solution was added with stirring to neutralize the acid and raise the pH to 9. The mixture was diluted with water to 100 mL and extracted with dichloromethane (5 × 50 mL). The organic layer was washed with 2 × 50 mL portions of saturated sodium chloride solution and dried over magnesium sulfate. The solvent was evaporated in vacuo to give 2.048 g (56%) of *N*-[4-(diethylamino)-3-oxobutyl]succinimide as a colorless liquid: IR (neat) 2980, 2940, 2820, 1775, 1730 (sh), 1700, 1445, 1410, 1375, 1340, 1300, 1250, 1210, 1180, 1150, 1120, 1075, 1000, 820, 665 cm^{-1}.

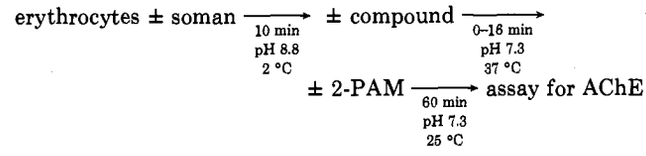
The *N*-[4-(diethylamino)-3-oxobutyl]succinimide (2.048 g, 0.0085 mol) in 20 mL of 2-propanol was treated with 2 equiv of methyl iodide (2.42 g, 0.017 mol). The mixture was stirred at room temperature for 16 h. The collected precipitate was recrystallized from 2-propanol/ether to give 3.15 g (97%) of the methiodide of *N*-[4-(diethylamino)-3-oxobutyl]succinimide as white crystals: mp 119–122 °C; IR (KBr) 3460, 2910, 1770, 1730, 1700, 1485, 1475, 1430, 1400, 1345, 1320, 1250, 1230, 1210, 1180, 1110, 1060, 1045, 1015, 980, 960, 910, 805, 680 cm^{-1}; NMR (D_2O/DSS) δ 1.20 (t, J = 7 Hz, 3 H), 1.30 (t, J = 7 Hz, 3 H), 2.77 (s, 4 H), 2.83 (t, J = 7 Hz, 2 H), 3.13 (s, 3 H), 3.63 (m, 6 H), 4.43 (s, 2 H). Anal. ($C_{13}H_{23}N_2O_3I$) C, H, N.

1,3a,8-Trimethyl-5-(chloroethoxy)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole [*O*-(Chloroethyl)eseroline] Fumarate (10). A solution of 5 g of (−)-eserine (Aldrich) (0.018 mol) in 30 mL of ethanol was placed in a flask and a stream of nitrogen was passed through to expel air. Then 25 mL of a degassed solution of 10% sodium hydroxide was added and the mixture was stirred at room temperature for 4 h under nitrogen. Dilute hydrochloric acid (1 N, 55 mL) was added, and the reaction mixture was poured into a separatory funnel. Ether (250 mL) was added and the aqueous phase was saturated with sodium chloride. The ether layer was separated, washed with saturated sodium chloride solution, dried ($MgSO_4$), and evaporated in vacuo. The brown residue was crystallized from ether/petroleum ether to give 3.87 g (97.6%) of (−)-eseroline as brown crystals: mp 124–125 °C (lit.[13] mp 129 °C); IR (KBr) 3540 (w), 3060, 2960, 2940, 2880, 2860, 1605, 1510, 1490, 1460, 1370, 1320, 1285, 1255, 1220, 1180, 1115, 1060, 1025, 1020, 990, 930, 875, 800, 710, 640 cm^{-1}.

A solution of (−)-eseroline (2.308 g, 0.0106 mol) in absolute ethanol (200 mL) was treated with a solution of sodium ethoxide (0.48 g of sodium in 50 mL of ethanol) under a stream of nitrogen for 15 min. Chloroethyl methanesulfonate (3 equiv, 5.044 g) was added and the mixture was heated at reflux under nitrogen for 3 days. The ethanol was removed in vacuo and the residue was treated with 1 N sodium hydroxide solution (120 mL), saturated with potassium carbonate, and extracted with ether (5 × 150 mL). The ether solution was extracted with 1 N hydrochloric acid solution (150 mL). The aqueous phase was made alkaline with 10% sodium hydroxide solution (100 mL) and extracted with ether (4 × 150 mL). The ether solution was washed with saturated sodium chloride solution (2 × 50 mL), dried ($MgSO_4$), and evaporated in vacuo to give 2.21 g (75%) of *O*-(chloroethyl)eseroline as a brown liquid: IR (neat) 3030, 2970, 2940, 2880, 2700, 1715, 1640, 1600, 1500, 1450, 1430, 1365, 1310, 1285, 1260, 1220, 1180, 1125, 1070, 1040, 1020, 960, 900, 800, 725, 670 cm^{-1}. The crude product (2.21 g, 0.007 87 mol) in ethyl ether (80 mL) was added to a solution of fumaric acid (1.0 g, 1.1 equiv) in ethanol (50 mL) under a nitrogen stream. The mixture was stirred at room temperature for 30 min and the solvent was removed. The brown residue was crystallized from 2-propanol to give 0.85 g (27.2%) of the fumarate salt of the product as white crystals: mp 115–117 °C dec; IR (KBr) 3440 (b), 3010, 2970, 2940, 2580 (b), 1675, 1610, 1500, 1470, 1460, 1440, 1330, 1290, 1220, 1170, 1135, 1125, 1080, 1070, 1040, 1005, 990, 890, 855, 830, 810, 750, 710, 650 cm^{-1}; NMR (D_2O/DSS) δ 1.48 (s, 3 H), 2.37 (t, J = 7 Hz, 2 H), 2.90 (s, 3 H), 2.77–3.0 (m, 2 H), 3.07 (s, 3 H), 3.83 (m, 2 H), 4.23 (m, 2 H), 5.0 (s, 1 H), 6.67 (s, 2 H), 6.57–7.07 (m, 3 H). Anal. ($C_{19}H_{25}N_2O_5Cl$) C, H, N.

Biochemistry. Acetylcholinesterase Assay. AChE activity was measured by using the radiochemical method of Siakotos et al.[14] Enzymatic hydrolysis of the labeled acetylcholine substrate (acetyl-1-[^{14}C]choline iodide) takes place in the presence of erythrocyte lysate or brain homogenate during a 30-min incubation at 37 °C. The reaction is stopped by adding 5 mL of a mixture of dioxane and Amberlite CG-120. Unhydrolyzed acetylcholine is adsorbed on the Amberlite resin and removed by centrifugation; the dioxane supernatant contains the radioactive acetate hydrolysis product which is measured by liquid scintillation spectrometry (Packard TriCarb 300).

Rate of Aging Determinations. The in vitro procedure used to determine the effect of each compound on the rate of aging may be summarized in diagrammatic form as follows:[15,16]



Rat erythrocytes were washed twice in 0.15 M NaCl, 0.167 mM EDTA, and resuspended in 10 vol of 0.01 M sodium borate, 0.15 M sodium chloride, pH 8.8. The suspension was then pipetted into eight tubes as shown in Table II. After the addition of soman to tubes 5–8 (final concentration of 2×10^{-8} M), all samples were centrifuged during a 10-min incubation at 2 °C. The high pH (8.8) and low temperature permit the phosphonylation of AChE by soman but prevent dealkylation (aging). Following centrifugation, the supernatants containing any excess soman were discarded in hazardous waste, and the pellets (erythrocytes) were resuspended in 10 vol of 0.1 M phosphate buffered saline (PBS), pH 7.3, at a temperature of 37 °C, with compound (tubes 2, 4, 6, 8) or without compound (tubes 1, 3, 5, 7) (Table II). Aging begins immediately upon addition of the pH 7.3 buffer. During incubation at 37 °C, 2.0-mL aliquots were removed from tubes 7 and 8 at timed intervals up to 16 min. These aliquots were pipetted directly into equal volumes of 0.2 M 2-PAM, 0.15 M NaCl, pH 7.3, to initiate the reactivation of unaged enzyme. Equal volumes of 2-PAM were added to tubes 3 and 4 and comparable volumes of 0.15 M NaCl (without 2-PAM) were added to tubes 1, 2, 5, and 6 (Table II). Following a 60-min incubation at 25 °C to allow for maximal reactivation of enzyme, samples were centrifuged to recover the erythrocytes. The cells were washed three times with 50 vol of PBS at 4 °C and finally lysed in 20 vol of distilled water at 4 °C. The hemolysates were then assayed for AChE activity.

Collection of Blood and Brain Tissues. Male Sprague–Dawley rats obtained from Charles River Laboratories were al-

(12) Meinwald, J.; Crandall, J.; Hymans, W. E. "Organic Syntheses"; Wiley: New York, 1973; Collect. Vol. V, p 866.
(13) Bartolini, B.; Renzi, G.; Galli, A.; Aiello, P.; Bartolini, R. U.K. Patent GB 2 005 259A, 1979.
(14) Siakotos, A. N.; Filbert, M.; Hester, R. *Biochem. Med.* **1969**, *3*, 1.
(15) Fleisher, J. H.; Harris, L. W.; Murtha, E. F. *J. Pharmacol. Exp. Ther.* **1967**, *156*, 345.
(16) Hobbiger, F. *Br. J. Pharmacol. Chemother.* **1956**, *11*, 295.
(17) Sachs, L. "Applied Statistics—A Handbook of Techniques", 5th ed.; Springer-Verlag: New York, 1982; pp 417, 440–442.

lowed to acclimate to their environment for at least 7 days prior to use. Rats were housed individually in a temperature-controlled animal room maintained on a 12-h light–dark cycle. Laboratory rat chow and water were available at all times. Following decapitation with a guillotine, blood was collected in a beaker containing 0.5 mL of 0.25 M EDTA. Whole brains were immediately excised from the skull and dropped into liquid nitrogen within 1 min after decapitation. Frozen brains were homogenized by pulverization under liquid nitrogen with use of a ceramic mortar and pestle. Samples for the AChE assay were taken after weighed aliquots of the pulverized brain were placed in 10 mM sodium phosphate buffer (pH 7.4) at 4 °C and dispersed with a Polytron homogenizer (Brinkmann Instruments).

Registry No. 1, 6712-43-2; **2**, 62884-14-4; **3**, 93185-37-6; **4**, 93185-38-7; **5**, 93185-39-8; **6**, 93185-40-1; **7**, 6893-34-1; **8**, 7279-54-1; **9**, 93185-41-2; **10**, 93185-43-4; 11, 93185-44-5; 2-PAM, 94-63-3; AChE, 9000-81-1; Me_3N, 75-50-3; $Cl(CH_2)_3Br$, 109-70-6; $Br(CH_2)_6Br$, 629-03-8; $MeSO_3Me$, 66-27-3; Et_2NH, 109-89-7; $CH_2{=}CH(CH_2)_2Br$, 5162-44-7; $MeSO_3(CH_2)_2Cl$, 3570-58-9; CH_3CO_2H, 64-19-7; pyridine, 110-86-1; 2-picolyl chloride hydrochloride, 6959-47-3; 2-picolyl chloride, 4377-33-7; succinimide, 123-56-8; 4-vinylpyridine, 100-43-6; 4-(2-succinimidoethyl)pyridine, 93185-45-6; 2-vinylpyridine, 100-69-6; 2-(2-succinimidoethyl)-pyridine, 74274-11-6; 4-[2-(diethylamino)ethyl]pyridine, 67580-61-4; 2-[2-(diethylamino)ethyl]pyridine, 25877-30-9; 2-[2-(diethylamino)ethyl]-1-methylpyridinium iodide, 93185-46-7; 2-picoline, 109-06-8; epichlorohydrin, 106-89-8; 1-(2-pyridyl)-4-chloro-3-butanol, 93185-47-8; *N*-(3-buten-1-yl)succinimide, 58805-10-0; *N*-(3,4-epoxybutyl)succinimide, 93185-48-9; *N*-[4-(diethylamino)-3-hydroxybutyl]succinimide, 93185-49-0; *N*-[4-(diethylamino)-3-oxobutyl]succinimide, 93222-21-0; (−)-eserine, 57-47-6; (−)-eseroline, 469-22-7; *O*-(chloroethyl)eseroline, 93185-42-3.

Synthesis and Characterization of Selected Heteroarotinoids. Pharmacological Activity as Assessed in Vitamin A Deficient Hamster Tracheal Organ Cultures. Single-Crystal X-ray Diffraction Analysis of 4,4-Dimethylthiochroman-6-yl Methyl Ketone 1,1-Dioxide and Ethyl (*E*)-*p*-[2-(4,4-Dimethylthiochroman-6-yl)propenyl]benzoate

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There is reported the first four members of heteroarotinoids, the names of which are ethyl (*E*)-*p*-[2-(4,4-dimethylthiochroman-6-yl)propenyl]benzoate (**1b**), ethyl (*E*)-*p*-[2-(4,4-dimethylchroman-6-yl)propenyl]benzoate (**1c**), ethyl (*E*)-*p*-[2-(4,4-dimethyl-1-oxothiochroman-6-yl)propenyl]benzoate (**1d**), and (*E*)-*p*-[2-(4,4-dimethylchroman-6-yl)propenyl]benzoic acid (**1e**). IR, ^1H NMR and ^{13}C NMR data have been recorded for each compound and support the structural assignments. To provide a firm basis for comparison purposes of future analogues, an X-ray analysis was performed on a single crystal of ethyl (*E*)-*p*-[2-(4,4-dimethylthiochroman-6-yl)propenyl]benzoate (**1b**) and a precursor 4,4-dimethylthiochroman-6-yl methyl ketone 1,1-dioxide (**18**). These data for the heteroarotinoid **1b** revealed that the two aryl ring systems were nearly perpendicular in each of the two molecules present in the unit cell (86.37° and 84.17°, respectively). The space group for both molecules was $P\bar{1}$ in triclinic systems. Unit cell dimensions (at 15 °C) are as follows: for **1b**, a = 20.568 (6) Å, b = 14.760 (3) Å, c = 7.679 (2) Å, α = 113.33 (2)°, β = 79.45 (2)°, γ = 79.98 (2)°, Z = 4; for **18**, a = 9.292 (5) Å, b = 9.291 (5) Å, c = 7.951 (3) Å, α = 102.16 (3)°, β = 77.49 (3)°, γ = 79.60 (4)°, Z = 2. The sulfur-containing ring is in a distorted half-chair in **1b** and the methyl carbon C(12) is shown to be trans to H(13) at the C(11)–C(13) bond. The biological activity of these arotinoids was determined in the tracheal organ culture assay and compared with *trans*-retinoic acid for ability to reverse keratinization in vitamin A deficient hamsters. The ester **1b** displayed activity about one-half log unit less than that of the reference while **1c** and **1e** had activity nearly one log until less than *trans*-retinoic acid. The sulfoxide was the least active of the heteroretinoids.

Retinoids (vitamin A and derivatives thereof) constitute a group of compounds of enormous current interest.[2] The stimulus for this interest arises from observations that these compounds exhibit some antitumor activity[3] and exert a preventive activity in models of chemical carcinogensis.[2c,3-5] Unfortunately, the use of natural retinoids in cancer chemotherapy has some disadvantages. With the exception of *trans*-retinoic acid, natural and retinoids are stored in the liver, and blood levels of the materials do not increase proportionately even after massive doses.[6] Thus, it is difficult to achieve a good distribution and to deliver a retinoid to specific target sites. In addition, acute toxicity[6] has been associated with high dosages of natural retinoids. This "hypervitaminosis A" limits clinical use of such compounds. Modifications of the basic retinoid structure have been the subject of intensive effort recently.[7]

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(2) (a). Wolf, G. *Nutr. Rev.* **1982**, *40*, 257. (b) Pawson, B. A.; Ehmann, C. W.; Itri, L. M.; Sherman, M. I. *J. Med. Chem.* **1982**, *25*, 1269. (c) Peto, R.; Doll, R.; Buckley, J. D.; Sporn, M. B. *Nature (London)* **1981**, *290*, 201. (d) Peck, G. L. *Gynecol. Oncol.* **1981**, *12*, S331. (e) Sporn, M. B.; Newton, D. L. In "Inhibition of Tumor Induction and Development"; Zedeck, M.; Lipkin, M., Ed.; Plenum Press: New York, 1981; pp 71–100. (f) Newton, D. L.; Henderson, W. R.; Sporn, M. B. "Structure–Activity Relationships of Retinoids"; National Cancer Institute, Revised Edition, February 26, 1980. (g) Sporn, M. B.; Newton, D. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1979**, *38*, 2528. (h) Lasnitzki, J. *Brit. J. Cancer* **1976**, *34*, 239.

0022-2623/85/1828-0116$01.50/0