II) and 2×10^{-5} M dansylamide in pH 7.4, 0.1 ionic strength phosphate buffer was placed in the thermostated cell holder of a Perkin-Elmer MPF-44B fluorescence spectrophotometer. The temperature was maintained at 37 °C by using a constant-temperature water circulator. The excitation and emission wavelengths were set at 280 and 460 nm, respectively. Fluorescence intensities were recorded following addition, with stirring, of small, measured aliquots of a solution of the test compound in pH 7.4 buffer. The resulting data were converted to fluorescence intensity vs. compound concentration, corrected for dilution by the titrant, and fitted by nonlinear least squares to a model in which the compound and dansylamide compete for a single binding site on HCA II. The dissociation constant of the dansylamide–HCA II complex, which is needed for these calculations, was found to be 1.98×10^{-6} M under these conditions. It was found in all cases that the data fitted well to a single-site model. There was no evidence for additional, lower-affinity binding sites. All binding determinations were done a minimum of three times.

Acknowledgment. We are indebted to J. M. Sondey and S. R. Michelson for the measurement of I_{50} values, to Y. C. Lee, S. J. Smith, and J. P. Moreau for analytical determinations, and to M. Banker for preparation of this manuscript.

Registry No. 1, 7675-04-9; **2**, 105951-31-3; **2** (acid), 106319-47-5; **2** (acid chloride), 106319-48-6; (±)-**3**, 106319-38-4; ±-**4**, 106400-04-8; (*R*)-**4**, 105951-84-6; (*S*)-**4**, 105951-48-2; **5**, 105951-35-7; (±)-**6α**, 106335-79-9; (±)-**6β**, 106319-49-7; **7**, 105951-67-5; **8**, 105951-71-1; **9**, 21339-38-8; (±)-**10**, 106319-39-5; (±)-**11**, 106319-40-8; (±)-**12**, 106319-41-9; (±)-**12** (sulfonamide), 106319-50-0; (±)-**13**, 106319-42-0; (±)-**13** (MEM ether), 106335-80-2; (*S*,*R*)-**14**, 106319-43-1; (*R*,*R*)-**14**, 101859-94-3; **15**, 105951-32-4; (±)-**16**, 106319-44-2; (±)-**17**, 106319-45-3; **18**, 105951-39-1; **19**, 105951-36-8; (±)-**20**, 106319-46-4; MEMCl, 3970-21-6; (*R*)-$C_6H_5CH(OCH_3)CO_2H$, 3966-32-3; 6,7-dihydro-5*H*-7-oxothieno[3,2-*b*]thiopyran, 7677-33-0; 6,7-dihydro-5*H*-7-oxothieno[3,2-*b*]thiopyran ketal, 106319-51-1; 6,7-dihydro-5*H*-7-oxothieno[3,2-*b*]thiopyran ketal sulfonamide, 106319-52-2; 5*H*-thieno[3,2-*b*]thiopyran, 10558-81-3; carbonic anhydrase, 9001-03-0.

Linear Free Energy Relationships and Cytotoxicities of Para-Substituted 2-Haloethyl Aryl Selenides and Bis(2-chloroethyl) Selenides

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Received July 11, 1986

Examples of a new class of alkylating agents, selenium mustards, were prepared for study of their chemical kinetic properties and cytotoxicities against human lymphoblastoid CCRF-CEM cells. In a series of para-substituted aryl 2-chloroethyl selenides, a linear free energy relationship between the first-order rate constant, k'_{nbp} and σ_p gave a ρ value of −1.3, indicating that formation of a cyclic ethylene selenonium ion is the rate-controlling step for alkylation of 4-(4-nitrobenzyl)pyridine (NBP). Consistent with the ethyleneselenonium ion pathway, rates of solvolyses were extremely sensitive to increasing water content, and a positive correlation was found between reactivity with NBP and nucleophilic selectivity (Swain–Scott *s* constant). The *s* constant, which predicts for variation in intracellular product spread, varied from 0.53 up to 0.95, equal to aliphatic nitrogen mustards. Alkylating activities based on extent of NBP alkylation, however, showed relatively low values, 8–23% of that of mechlorethamine, possibly due to hydrolysis occurring by a separate pathway from nucleophilic substitution. Reactivities and nucleophilic selectivities both showed positive correlations with cytotoxicities, suggesting that the rate and extent of alkylation of relatively strong nucleophilic centers mediate the biologic effects of these compounds. Two bifunctional selenium mustards were substantially more cytotoxic than monofunctional aromatic selenides. No additional cytotoxicity due to the selenium atom was observed, with the exception of diselenide (–SeSe–) compounds. Thus, selenium alkylating agents kinetically and biologically resemble classical, mustard-type alkylating agents.

Discovery of the antitumor properties of mechlorethamine hydrochloride led to the synthesis of thousands[1] of (2-haloethyl)imonium, aziridine, (2-haloethyl)sulfonium, and oxygen analogues by the early 1960s and to the development of alkylating agents as an established class of cancer chemotherapy agents. Classical alkylating agents may be defined as compounds that in protic media undergo aliphatic nucleophilic substitution reactions at saturated, sp^3 carbon bearing an acidic leaving group. Ligand substitution reactions of platinum salts follow a similar nucleophilic reactivity order.[2] Although the synthesis of bis(2-chloroethyl) selenide was first described in 1920,[3] the antitumor potential of 2-haloethyl selenides has not been discussed in the literature until the present paper. This is surprising, given the antitumor activities of selenium antimetabolites,[4] the anticarcinogenic effect of dietary selenium,[5] and the important role of selenium in glutathione metabolism.[6]

Our own interest in 2-haloethyl selenides was prompted by theoretical considerations of alkylating agent nucleophilic selectivity. High nucleophilic selectivity in an alkylating agent, represented by the *s* constant of Swain and Scott,[7] should increase alkylation of the N-7 position of guanine of DNA[8] and other moderately strong intracellular

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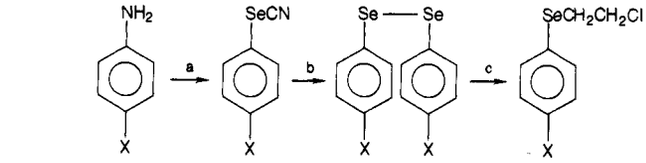
Scheme I[a]



X = H	3a	3b	3c	3
X = OCH_3	5a	5b	5c	5
X = CH_3	6a	6b	6c	6
X = SO_2NH_2	7a	7b	7c	7
X = NO_2	9a	9b	9c	9

[a] a: (i) $NaNO_2$, H^+, (ii) KSeCN. b: KOH/MeOH. c: (i) SO_2Cl_2, (ii) $CH_2{=}CH_2$.

nucleophilic sites. Among structural features of alkylating agents that favor increased nucleophilic selectivity is high polarizability in the leaving group and of other atoms located near the reaction center.[9] Since nitrogen and sulfur atoms formally are the leaving groups in the final alkylation reactions of ethyleneimonium and ethylenesulfonium ions,[10] selenium analogues were logical candidates for study because of the larger, "softer" character of selenium.[11] In a report of *s* constant determination of model and clinical alkylating agents, the presence of an aromatic nucleus at the ethyleneimonium nitrogen also appeared to increase nucleophilic selectivity.[8a] The present investigation of reactivities, nucleophilic selectivities, and cytotoxicities of monofunctional aromatic 2-haloethyl selenides was therefore done with comparison made with bifunctional aliphatic analogues. The 2-haloethyl selenides are found to show a surprisingly wide range of values in these parameters, which show useful correlations with Hammett constants. Some of these compounds are among the most reactive alkylating agents ever described, making them potentially useful by topical application, intraarterial infusion, and intracavitary administration.[10c,12]

Results and Discussion

Chemistry. The bifunctional organoselenium compound $ClCH_2CH_2SeCH_2CH_2Cl$ (1), an analogue of classical sulfur mustard, was prepared by reducing bis(2-chloroethyl) selenide dichloride with sodium metabisulfite.[13] Bis(2-chloroethyl) diselenide (2)[14] was obtained by the reaction of 2-chloroethyl selenocyanate with 0.40 equiv of KOH dissolved in MeOH. Serious decomposition of the diselenide into metallic Se and probably ethylene gas took place on using 1 or more equiv of KOH, although this is a general route to formation of diselenides from corresponding selenocyanates.[15] Some decomposition of the diselenide was found during its purification by silica gel column chromatography, eluting with a mixture of *n*-hexane and chloroform.

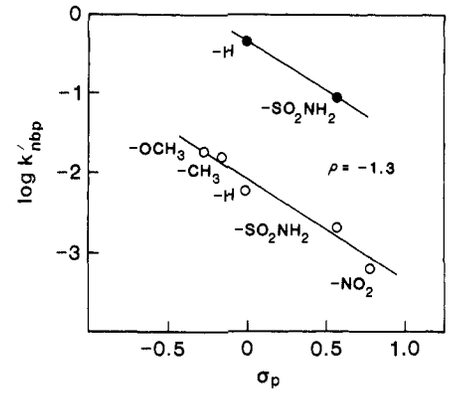


Figure 1. Hammett plot for the alkylation of NBP by para-substituted 2-haloethyl aryl selenides.

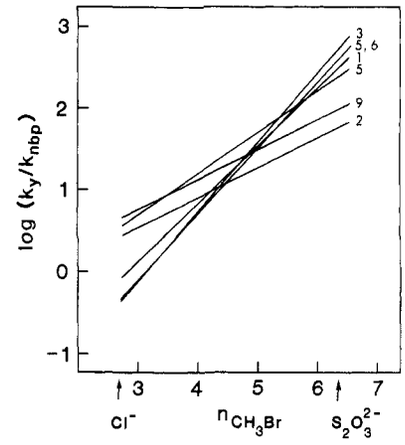


Figure 2. Swain–Scott *s* constants by Cl^- and $S_2O_3^{2-}$ competition for alkylation of NBP by selenium alkylating agents.

The preparation of 2-haloethyl aryl selenides (3–9) was carried out as outlined in Scheme I based on the literature methods.[13] The corresponding bromides 4 (X = H) and 8 (X = SO_2NH_2) were synthesized with Br_2 in place of sulfuryl chloride at step c in Scheme I. In the ^1H NMR spectra of the 2-haloethyl derivatives (1–9) that are compounds of type ZCH_2CH_2Y, AA'BB' splitting patterns with general appearance of symmetry were observed.[16c]

Kinetics. Results of reactivity studies, parameters of nucleophilic selectivity, and cytotoxicities of bifunctional aliphatic selenides (1 and 2) and monofunctional aromatic selenides (3–9) are summarized in Table I. Pseudo-first-order rates of NBP alkylation, k'_{nbp}, were linear in all cases over the first 3 half-lives of reaction, with no evidence of a "shoulder" on semi-log plots of remaining compound vs. time as may occur with some nitrogen mustards, such as chlorambucil.[10b] Rates of solvolysis in the absence of NBP, k'_0, were only 2- to 3-fold slower than k'_{nbp} values. For example, the log k'_0 and log k'_{nbp} values (s^{-1}) of compounds 3 and 9 were −2.580 and −2.208, and −3.650 and −3.197, respectively. These differences are approximately the same order of magnitude as expected on the basis of second-order rate ratios calculated from Swain–Scott *s* and

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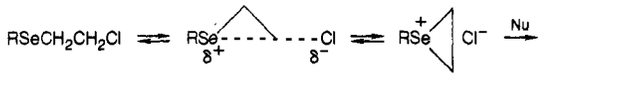
Table I. Chemical Parameters and Cytotoxicities of Para-Substituted Aryl 2-Haloethyl Selenides and Bifunctional Aliphatic 2-Chloroethyl Selenides

compound	σ_p[a]	π[a]	reactivities[b] log k'_{nbp}, s^{-1}	$T_{0.5}$, s	m	nucleophilic selectivities[c] AA, %	log (k_s/k_{nbp})	s	IC_{50},[d] μM
1, $ClCH_2CH_2SeCH_2CH_2Cl$			−1.321	14.5	1.13	22.1	2.65	0.74	0.75
2, $ClCH_2CH_2SeSeCH_2CH_2Cl$			−3.142	960	ND[e]	12.0	1.85	0.33	0.25
$XC_6H_4SeCH_2CH_2Y$									
3, X = H, Y = Cl	0.00	0.00	−2.208	112.5	1.24	23.2	2.93	0.87	8.0
4, X = H, Y = Br	0.00	0.00	−0.335	1.5	ND	10.0	2.89	0.84	15.0
5, X = CH_3O, Y = Cl	−0.27	−0.02	−1.733	37.5	ND	21.5	2.72	0.85	2.7
6, X = CH_3, Y = Cl	−0.17	0.56	−1.801	44.0	1.33	21.0	2.76	0.85	5.0
7, X = NH_2SO_2, Y = Cl	0.57	−1.82	−2.693	341	ND	18.8	2.50	0.53	15.0
8, X = NH_2SO_2, Y = Br	0.57	−1.82	−1.034	7.5	0.40	8.0	2.24	0.61	ND
9, X = NO_2, Y = Cl	0.78	−0.28	−3.197	1092	1.01	16.6	2.09	0.49	8.5

[a] From ref 26. [b] Pseudo-first-order rates of 4-(4-nitrobenzyl)pyridine (NBP) alkylation and half-lives at 37 °C in aqueous acetone (52.2:47.8, v/v) containing 40.6 mM NBP, 17.4 mM Tris-HCl buffer, pH 7.0, and 0.17 mM alkylating agent. Weinstein m constants were calculated from rates of loss of NBP alkylating activity at 52.2% and 70.0% aqueous acetone at 37 °C (in the absence of NBP) relative to the literature value for solvolysis of *tert*-butyl chloride.[19] [c] AA (%) is the alkylating activity as a percentage of the extent of NBP alkylation (absorbance at 560 nm of the final alkyl-NBP product with triethylamine alkalinization) by mechloroethamine hydrochloride (= 100%), correction made for the number of alkylating equivalents/mole. The s constants were obtained by NBP product competition using independent runs with $Na_2S_2O_3$ and NaCl.[8a] [d] Growth inhibition against CCRF-CEM human lymphoblast cells in vitro, average results of duplicate assays. The average SD was 23% of the average value shown. [e] ND, not determined.

n constants, and thus do not suggest significant enhancement of initial rate-controlling ionization by the presence of NBP.

Lindgren reported[17] that formation of an episelenonium ion is very probable as a common intermediate in reactions between bis(2-bromoethyl) selenide and various nucleophiles, such as selenocyanate, iodide, and benzylselenolate. Similarly, a general reaction scheme for solvolysis of the selenides of the present studies (with an exception of the diselenide **2**) can be proposed, analogous to the reactions of nitrogen and sulfur mustards:



$RSeCH_2CH_2Nu$ (1)

The validity of (1) is supported by examination of the effects of electron-donating or -withdrawing para substituents on k'_{nbp} values. Figure 1 shows the negative correlation between σ_p and k'_{nbp}, that has a ρ slope value of −1.31 for the series of 2-chloroethyl aryl selenides and a parallel ρ value of −1.23 for the two 2-bromoethyl analogues (**4** and **8**). While these are lower than the ρ value for analogous 2-bromoethyl aryl sulfides (ρ = −1.9),[18] they are of great enough magnitude to suggest that initial ethyleneselenonium ion generation is rate-controlling for either hydrolysis or NBP alkylation.

Further evidence for the S_N1-type kinetics of the 2-haloethyl selenides is given by the values of m constants, which represent sensitivities of compounds to changing polarity of the solvent. The four 2-chloroethyl compounds (**1**, **3**, **6**, and **9**) were as sensitive (or more so) than published values for *tert*-butyl chloride in aqueous acetone.[23] For the three aryl 2-chloroethyl selenides (**3**, **6**, and **9**), an inverse relationship appears to exist between m and σ_p (eq 7, Table II). Thus, compounds that have a greater tendency to undergo ethyleneselenonium ion formation because of inductive effects that increase the nucleophilicity of the selenium atom may be more susceptible to the effects of solvent participation. Formation of the ethyleneselenonium ion, then, from 2-chloroethyl selenides may be very much analogous to ethylenesulfonium ion formation, which is probably an "internal S_N2" reaction.[10a] The only 2-bromoethyl aryl selenide studied, the sulfonamide **8**, has a low m constant, so that one may assume in the case of this extremely reactive compound that some degree of direct S_N2 displacement by solvent occurs at the terminal carbon with less involvement of a cyclic ethylene intermediate.

Table II. Linear Relationships among Chemical Parameters and Correlates of Cytotoxicity of 2-Chloroethyl Selenides

equation[a]	N	r	p
(1) log k'_{nbp} = −1.31σ_p − 2.09	5	−0.985	0.01
(2) s = −0.42σ_p + 0.80	5	−0.965	0.01
(3) s = 0.052AA − 0.33	7	0.936	0.01
(4) log (k_s/k_{nbp}) = 0.097AA + 0.63	7	0.972	0.001
(5) AA = 4.36 log k'_{nbp} + 29.33	7	0.815	0.02
(6) m = 0.057AA − 0.005	5	0.955	0.02
(7) m = −3.05σ_p + 3.85	3	0.994	0.08
(8) IC_{50} = 0.043$T_{0.5}$ + 1.58	5[b]	0.967	0.01
(9) IC_{50} = 0.24π + 1.82	4[c]	0.997	0.01
(10) IC_{50} = −23.6 s/log k'_{nbp} + 16.4	6	0.850	0.04

[a] Abbreviations used: N, the number of compounds used in linear regression analysis; r, correlation coefficient; p, probability based on t test; k', $T_{0.5}$, m, AA, s, and IC_{50} values from Table I. [b] Excludes results of 2-chloroethyl 4-nitrophenyl selenide (**9**). [c] Excludes results of 2-chloroethyl 4-methoxyphenyl selenide (**5**).

The m constant can be used to estimate rates of hydrolysis under physiologic conditions, i.e., water content approaching 100% of the solvent. At m = 1.0, for a 19% increase in water in 1:1 aqueous acetone at 37 °C there is a 10-fold increase in reaction rate.[19] Thus, the half-life of **I** with m = 1.13, which is 14.5 s in 52.2:47.8 water/acetone, may be less than 0.15 s in 100% water, and the extremely reactive **8** with an m = 0.40 may have a half-life of less than 0.1 s in 100% water. These calculated rates of solvolyses are notably among the fastest reactions ever reported for aliphatic compounds undergoing nucleophilic substitution, including 2-bromoethyl sulfides,[10c,18] and methyl trifluoromethanesulfonate (triflate).[20]

A positive reactivity–selectivity relationship was found among the 2-chloroethyl aryl selenides (eq 5, Table II). An

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increase in nucleophilic selectivity with increasing reactivity obtains with either s or AA, up to a k'_{nbp} of 10^{-2} s^{-1}, where AA is the alkylating activity as a percentage of the extent of NBP alkylation by mechlorethamine hydrochloride (= 100%), corrected for the number of alkylating equivalents per mole. The selectivity values for the 2-bromoethyl compounds (4 and 8), while both lower in the expected direction from the chloro analogues, show a similar trend. Positive reactivity–selectivity relationships have been reported for classical S_N1-type, sterically hindered electrophiles,[21] which may be additional evidence for the importance of the unimolecular pathway in the solvolyses of the present series of selenium mustards.

AA and s are not interchangeable parameters of nucleophilic selectivity, although they correlate (eq 3, Table II), and a general correlation has been suggested.[22] The advantage of use of s constants over AA derives from the lack of effect of extinction coefficient of the alkylated NBP product, the lack of need for exact quantitation of initial alkylating selenide concentration, and the lack of effect of competing hydrolysis.[8a] AA reflects the competition between NBP and water, while s is a measure of competition between moderately strong nucleophiles. Values of s show a surprisingly wide range, perhaps greater than for nitrogen mustards and other model alkylating agents.[7,8a] However, it is difficult to exclude differences in the contribution to increasing s by positive salt effects on solvolysis by decreased nucleophilic selectivity, which could extend the lower limit of s values.[23]

The relatively low and narrow range of AA, in general, of the 2-haloethyl selenides on comparison with their s constants may be consistent with an enhanced tendency of these compounds to hydrolyze, perhaps by attack on incipient cyclic ethyleneselenonium intermediates. Measurement of the extinction coefficient of alkylated NBP would be useful to study this question, as done for simple aliphatic alkylating agents,[22] but the products have proved too unstable to isolate. It should be noted that there exist no published data on this point for any mustard compounds, which apparently generally do not form isolable alkyl-NBP products.

Cytotoxicity Studies. The two bifunctional compounds bis(2-chloroethyl) selenide (1) and bis(2-chloroethyl) diselenide (2) showed dose cytotoxicities against CCRF-CEM human lymphoblasts averaging 10- to 20-fold lower (stronger) than the monofunctional aromatic selenides (3–9). The IC_{50} value of mechlorethamine in control experiments was 0.17 μM, 4-fold greater than that of 1, which is exactly proportional to their relative AA. The bifunctional diselenide 2 was 3-fold more cytotoxic than the corresponding monoselenide 1, an expected result based on the high intrinsic toxicities of diselenides generally.[25] Model diselenides with no alkylating activity (diethyl, dibenzyl, and diphenyl diselenides) showed IC_{50} values against CCRF-CEM cells averaging 4.3 ± 0.5 μM.

As with nitrogen and sulfur mustards, an important determinant of cytotoxicity of the 2-haloethyl selenides is reactivity.[24] Among the 2-chloroethyl aryl compounds, a strong correlation (eq 8, Table II) between solvolytic half-life and IC_{50} value emerged if 2-chloroethyl 4-nitrophenyl selenide (9) was omitted; intracellular reduction of the nitro moiety may have resulted in a more reactive metabolite. There also existed trends toward increased cytotoxicity with nucleophilic selectivity, s values, or AA. When account is made of both the degree of selectivity toward a strong nucleophile and the rate of alkylation, an increased high correlation can be shown for prediction of cytotoxicity using all chloroethyl compounds studied (eq 10, Table II).

An interesting finding was a positive correlation between the hydrophobic parameter, π, of the para substituents[26] and cytotoxicity (eq 9, Table II) of four 2-chloroethyl aryl selenides (3, 6, 7, and 9). However, if the results of the methoxy analogue are included, the correlation is less significant, r = 0.883. Increased hydrophobicity of the diselenide 2 over the monoselenide 1, then, may contribute to its low IC_{50} value.

In conclusion, the kinetic studies provide strong evidence for unimolecular formation of an ethyleneselenonium ion intermediate as the rate-determining step in nucleophilic substitution reactions of the 2-haloethyl selenides, and the in vitro studies demonstrate correlations between cytotoxicities and parameters of alkylation with little or no contribution from the selenium atom, per se (excluding diselenides). Thus, the 2-haloethyl aryl selenides and bis(2-chloroethyl) selenide are true mustards, with considerable potential as antitumor agents. Notable features of these compounds for this purpose are their unusually broad range of reactivities and s constants. With a few exceptions, such as 1, valency restrictions require two selenium centers for cross-linking alkylation. Representative examples of bifunctional compounds and of selenium alkylating agents with slowed reaction rates and exalted nucleophilic selectivities will be reported in subsequent papers.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR4240 spectrophotometer, and ^1H NMR spectra were obtained with a Hitachi Perkin-Elmer high-resolution R24 NMR spectrometer. Absorbancies were measured with a Beckman 25 UV/vis spectrophotometer and mass spectral data were taken with a Hewlett Packard GC/MS 5985A equipped with dual EI/CI. The elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of the theoretical values. Parasubstituted aryl 2-haloethyl selenides (3–6 and 9) were prepared according to the literature methods.[16] Diethyl diselenide,[27] diphenyl diselenide,[28] and dibenzyl diselenide[29] were synthesized in quantitative yields from the corresponding selenocyanates[30] by addition of methanolic KOH at 0 °C.[15]

4-Sulfamoylphenyl Selenocyanate (7b). Sulfanilamide (7a; 40 mmol, 6.89 g) was diazotized with $NaNO_2$ and then reacted with KSeCN as described in the literature.[30] The crystallization of the crude product twice in a mixed solvent of 1,2-dichloroethane and methanol yielded 3.0 g (29%) of a white solid: mp 142–143 °C; IR (KBr) 3360 and 3252 (NH_2), 2153 (SeCN), 1135 and 1170 (SO_2NH_2) cm^{-1}; ^1H NMR (CD_3COCD_3) δ 6.52 (s, 2 H), 7.71 (m, 4 H). Anal. ($C_7H_6N_2O_2SSe$) C, H.

Bis(4-sulfamoylphenyl) Diselenide (7c). Potassium hydroxide (3.03 mmol, 0.17 g) in 10 mL of MeOH was added dropwise to a solution of the selenocyanate 7b (1.72 mmol, 0.45 g) in 5 mL of MeOH in an ice–water bath. A yellow solid immediately precipitated out, following which 1.0 M methanolic HCl

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was added until the pH of the solution became acidic to litmus paper. After 30 min of stirring, the solid was collected and redissolved in THF for removal of NaCl. The THF filtrate was dried over anhydrous sodium sulfate and concentrated in vacuo to give a yellow solid; more of the product was recovered from the methanol filtrate. The combined yield of the diselenide was nearly quantitative: mp 254–255 °C; IR (KBr) 3348 and 3247 (NH_2), 1330 and 1153 (SO_2NH_2) cm^{-1}. Anal. ($C_{12}H_{12}N_2O_4S_2Se_2$) C, H.

2-Chloroethyl 4-Sulfamoylphenyl Selenide (7). To a solution of bis(4-sulfamoylphenyl) diselenide (**7c**; 0.53 mmol, 0.25 g) in 10 mL of THF was slowly added SO_2Cl_2 (0.1 mL, 1.2 mmol) in 2 mL of THF at room temperature. After 10 min of stirring, ethylene gas was bubbled through the burgandy solution until its color changed to pale yellow. The oily residue obtained on removal of the solvent was chromatographed on silica gel and eluted with methylene chloride to give 60 mg (19%) of a white solid: mp 77.5–79.5 °C; IR (KBr) 3348 and 3260 (NH_2), 1340 and 1155 (SO_2NH_2) cm^{-1}; ^1H NMR (CD_3CN) δ 3.19–3.54 (m, 2 H), 3.65–3.98 (m, 2 H), 6.48 (s, 2 H), 7.50–7.92 (m, 4 H). Anal. ($C_8H_{10}NClO_2SSe$) C, H.

2-Bromoethyl 4-Sulfamoylphenyl Selenide (8). Ethylene gas was passed through a solution of 4-sulfamoylbenzeneselenenyl bromide, generated in situ from 0.25 g (0.64 mmol) of bis(4-sulfamoylphenyl) diselenide (**7c**) and 0.102 g (0.64 mmol) of bromine in 20 mL of THF at room temperature, until the color of the solution turned to pale yellow. After removal of the THF, the oily residue was purified by a column chromatograph of silica gel and eluted with methylene chloride to obtain 50 mg (11%) of a white solid: mp 97.5–100 °C; IR (KBr) 3590, 3522, 3350, and 3190 (non-H-bonded and H-bonded NH_2), 1313 and 1159 (SO_2NH_2) cm^{-1}; ^1H NMR (CD_3COCD_3) δ 3.20–3.85 (m, 4 H), 4.52 (s, 2 H), 7.40–7.80 (m, 4 H). Anal. ($C_8H_{10}NBrO_2SSe$) C, H.

Bis(2-chloroethyl) Selenide (1). The selenium mustard was prepared by the Bell and Gibson procedure.[13] Bis(2-chloroethyl) selenide dichloride (2.89 mmol, 0.80 g), which was obtained in a quantitative yield from the reaction of selenium tetrachloride and ethylene gas in benzene, was treated with cold aqueous sodium metabisulfite (2.89 mmol, 0.55 g). The mixture was at once extracted with 15 mL of ether. The etheral solution was washed with water, dried with sodium sulfate, and concentrated in vacuo to give an oily residue. The oil was crystallized in a mixed solvent of *n*-hexane and ether to quantitatively yield a white solid: mp 24 °C (lit.[13] mp 24 °C); ^1H NMR ($CDCl_3$) δ 2.93 (m, 2 H), 3.59 (m, 2 H).

2-Chloroethyl Selenocyanate (2a). 2-Chloroethyl bromide (20.6 mmol, 2.95 g) was refluxed with KSeCN in acetone for 2 h, as described in the literature,[31] to give 1.8 g (57%) of a colorless liquid in the range of 62–70 °C (1 mm) [lit.[31] 74–75 °C (1.5 mm), 91 °C (6 mm)]: IR (neat) 2150 (SeCN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.30 (t, 2 H), 3.91 (t, 2 H).

Bis(2-chloroethyl) Diselenide (2). Methanolic KOH (1.08 mL of 2.2 M) was added dropwise to a solution of 2-chloroethyl selenocyanate (**2a**; 5.93 mmol, 1.0 g) in 8 mL of MeOH at 0 °C. About 10 min of stirring gave emulsion droplets in the reaction mixture. The solution was poured into 70 g of ice–water containing 10 mL of aqueous saturated NaCl, and then the mixture was extracted with chloroform (10 mL × 5). The combined $CHCl_3$ was dried with Na_2SO_4 and concentrated in vacuo to leave an orange residue. The oily residue was dissolved in 14 mL of *n*-hexane and filtered. After removal of the hexane the filtrate gave 0.59 g (70%) of the liquid product:[20] IR (neat) 2910, 1437, 1430, 1273, 1241, 1170, 817, 701, and 608 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.14 (t, 2 H), 3.76 (t, 2 H); mass spectrum, *m/e* 286 (M^+, Se^{80}).

Kinetic Studies. Reactivities. The rate of alkylation of 4-(4-nitrobenzyl)pyridine (NBP), n = 3.6, at 37 °C in aqueous acetone (52.2:47.8, v/v) was done by a modification of the method used for determination of Swain–Scott constants.[8a] To a mixture of 1.0 mL of water, 1.0 mL of freshly prepared 2% (w/v) NBP in acetone, and 0.2 mL of 0.2 M Tris·HCl buffer, pH 7.0, at 37 °C was added 0.1 mL of selenide compound (1.0–10.0 mM) in acetone by rapid injection. At various times after selenide addition, 1.0 mL of triethylamine was added by rapid injection. In all cases, stable chromophores in a single solvent phase resulted, with peaks at 560 nm; readings were taken within 1 min of alkalinization after cooling to 23 °C. Alkylating activity (AA) was determined by following the reaction up to at least 5 and usually 10 half-lives of the reaction, expressed as a percentage of the value found for mechlorethamine with correction made for the number of alkylating equivalents per mole of compound. The observed pseudo-first-order rate constant for NBP alkylation, k'_{nbp}, was determined by linear regression analysis of the logarithm of percent unreacted compound vs. time over the first 2–3 half-lives with an average of six points; all compounds showed high linearity (average correlation coefficient, 0.99).

In order to determine first-order rates of solvolysis of compounds in the absence of NBP, k'_0, and the effect of solvent composition, 0.05 mL of 10 mM selenide was rapidly added to 37 °C mixtures of 1.0 mL water/acetone plus 0.1 mL of 0.2 M Tris·HCl buffer, pH 7.0. Following incubation for varying times, 1.15 mL of water/acetone mixture (adjusted to achieve a final 52.2% water content as in k'_{nbp} determination) containing 17.4 mM Tris-HCl buffer, pH 7.0, and 81.2 mM NBP was added and further incubated for a constant period, usually 5 half-lives. The presence of a chloride salt at this concentration showed no evidence of a common ion effect. Preliminary studies with lithium perchlorate or NaCl addition to reaction mixtures containing **1**, **7**, or **9** have shown significant positive salt effects on hydrolytic rates without evidence found for a common ion effect. The sensitivity of solvolysis to water content was expressed in terms of m constants (see legend, Table I).[19]

Selectivities. Nucleophilic selectivity, which represents the relative preference of an alkylating species for reaction with a strong vs. a weak nucleophile, was determined by a modification of the NBP product competition method.[8a] Conditions were the same as for k'_{nbp} determination, except that various concentrations of $Na_2S_2O_3$ or NaCl were added in with the 1.0 mL of water, and the alkylation reaction was carried out for at last 3 half-lives. A blank without selenide compound was paired with each concentration of competing nucleophile. The ratio of second-order rate constants for alkylation of competing nucleophile (Y) and NBP was calculated from the relationship:

$$\log (k_y/k_{nbp}) = \log \left(\frac{[NBP]}{[Y]} \left[\frac{A_c}{A_0} - 1 \right] \right)$$

where [Y] and [NBP] were the initial concentrations of nucleophiles, and A_0 and A_c the absorbances with and without added competing nucleophile. The s constants were obtained from the relationship:

$$s = \frac{\log (k_s/k_{nbp}) - \log (k_{cl}/k_{nbp})}{6.36 - 2.7}$$

where 6.36 and 2.7 are the n constants for thiosulfate and chloride anions, respectively.[7]

Cytotoxicity Studies of Selenium Mustards 1–9 against CCRF-CEM Cells. Human lymphoblastoid CCRF-CEM cells were seeded at (4.0–5.0) × 10^4 cells/mL in duplicate for each drug concentration in borosilicate test tubes containing Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% dialyzed fetal calf serum, 16 mM HEPES, and 8 mM MOPS buffer, pH 7.2.[32] The test compounds dissolved in Me_2SO were added to the cell cultures, with rapid mixing, with use of a 1:200 dilution to obtain desired drug concentrations (final Me_2SO concentration, 0.5%). Each compound was tested at seven or more different concentrations ranging from 5 × 10^{-4} to 5 × 10^{-10} M by serial dilution, with Me_2SO alone as a control. After 48 h of incubation at 37 °C, the cells were harvested and counted with a Coulter counter.

Acknowledgment. This study was supported in part by NCI Grant 39629. We thank Drs. Joseph Casanova and

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(32) HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid, sodium salts; MOPES, 2-morpholinoethanesulfonic acid, sodium salts.

George A. Olah for helpful suggestions during the preparation of this manuscript.

Registry No. 1, 4730-83-0; **2**, 106471-30-1; **2a**, 106471-37-8; **3**, 50630-24-5; **3a**, 62-53-3; **3b**, 2179-79-5; **3c**, 1666-13-3; **4**, 50630-23-4; **5**, 57878-08-7; **5a**, 104-94-9; **5b**, 32111-94-7; **5c**, 38762-70-8; **6**, 52178-49-1; **6a**, 106-49-0; **6b**, 21856-93-9; **6c**, 21856-94-0; **7**, 106471-31-2; **7a**, 63-74-1; **7b**, 106471-33-4; **7c**, 106471-34-5; **8**, 106471-32-3; **9**, 57878-11-2; **9a**, 100-01-6; **9b**, 19188-18-2; **9c**, 36297-89-9; KSeCN, 3425-46-5; $CH_2{=}CH_2$, 74-85-1; p-$NH_2SO_2C_6H_4SeBr$, 106471-35-6; $(ClCH_2CH_2)_2SeCl_2$, 106471-36-7; $Cl(CH_2)_2Br$, 107-04-0.

Resolved *cis*- and *trans*-2-Amino-5-methoxy-1-methyltetralins: Central Dopamine Receptor Agonists and Antagonists

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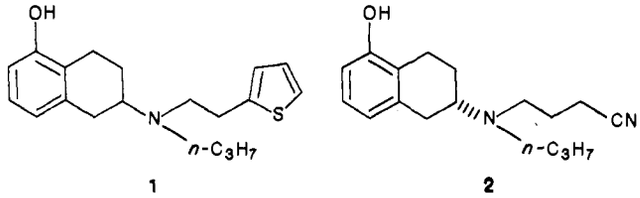
A series of 35 stereochemically well-defined C_1-methyl-substituted derivatives of the potent dopamine (DA) receptor agonist 5-hydroxy-2-(di-*n*-propylamino)tetralin (5-OH-DPAT) have been synthesized. The compounds were tested for central DA receptor agonistic and antagonistic activity, by use of biochemical and behavioral tests in rats. In addition, the compounds were tested for in vivo interactions with 5,6-dihydroxy-2-(di-*n*-propylamino)tetralin (DiPr-5,6-ADTN). On the basis of pharmacological activity profiles, the active compounds have been classified into four groups: (a) classical pre- and postsynaptic DA receptor agonists, (b) DA receptor agonists with preferential action at presynaptic receptors, (c) pre- and postsynaptic DA receptor antagonists, and (d) DA receptor antagonists with preferential action at presynaptic receptors. Results obtained indicate that both 2*R* and 2*S* enantiomers of C_5-oxygenated 2-aminotetralins may be able to bind to DA receptors but that only 2*S* antipodes are able to activate the receptors. O-Methylation of the C_5-oxygenated (1*S*,2*R*)-2-amino-1-methyltetralin derivatives tends to increase their DA receptor antagonistic activity, whereas decrease of the size of the N-substituent(s) from *n*-propyl to ethyl or methyl appears to increase their activity at postsynaptic DA receptors.

Fifteen years after the report of the dopaminergic activity of 5,6-dihydroxy-2-(dimethylamino)tetralin ("M7"),[1] 2-aminotetralin derivatives still continue to attract intense interest.[2] Recently, several laboratories have reported interesting pharmacological properties of novel 2-aminotetralin derivatives, for example, 2-[*N*-*n*-propyl-*N*-(2-thienylethyl)amino]-5-hydroxytetralin[3] (1) and (2*S*)-2-(*N*-*n*-propyl-*N*-(3-cyanopropyl)amino]-5-hydroxytetralin[4] (2), have been reported to be potent dopamine (DA) agonists while (1*S*,2*R*)-5-methoxy-1-methyl-2-(di-*n*-propylamino)-tetralin[5] ((1*S*,2*R*)-18; (+)-UH-232) appears to be a DA antagonist with preferential action on presynaptic DA receptors (DA autoreceptors).



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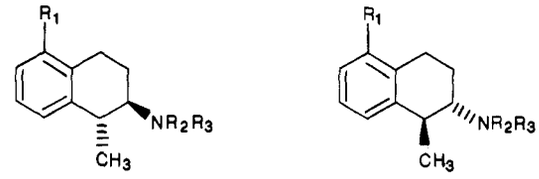
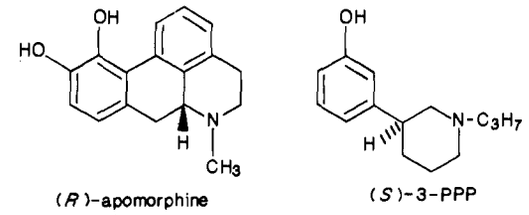
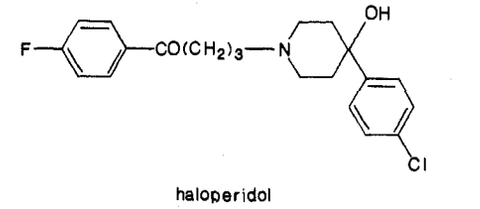
In the present investigation we have synthesized 35 stereochemically well-defined C_1-methyl-substituted 2-aminotetralin derivatives (Schemes I and II). The compounds have been tested for central monoaminergic activity by use of several biochemical and behavioral test methods and can be classified into four groups according to their pharmacological profile: (a) classical pre- and postsynaptic DA receptor agonists (having profiles similar to that of (*R*)-apomorphine),[6] (b) DA receptor agonists with preferential action at presynaptic receptors (having profiles similar to that of (*S*)-3-PPP),[7] (c) pre- and postsynaptic DA receptor antagonists (having profiles similar to that of haloperidol),[8] (d) DA receptor antagonists with preferential action at presynaptic receptors.

Scheme I



	R_1	R_2	R_3
3	OMe	*n*-Pr	H
4	OMe	*n*-Pr	*n*-Pr
5	OH	*n*-Pr	*n*-Pr





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