Potential Carcinolytic Agents.[1,2] V. Enamine Mustards

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A number of enamine mustards of the general formula $ZCH{=}C(R)N(CH_2CH_2X)_2$, where Z and R are electron-withdrawing groups or R may be H, and X is Cl, F, or OSO_2CH_3, have been synthesized as possible antitumor agents. These compounds, all deactivated alkylating agents because the electron-withdrawing group(s) decrease the basicity of the nitrogen, were expected to be sensitive to acid hydrolysis and to release a cytotoxic mustard preferentially in the acidic media of the tumor cells. The synthesis, stereochemistry, and stability of the new compounds are discussed. None of the new enamine mustards was more active than bis(2-chloroethyl)amine against transplanted rodent tumors.

In our investigation of potential carcinolytic agents we have focused our attention[2,3] on "deactivated alkylating agents." These are compounds containing a nitrogen mustard moiety, the alkylating power of which has been subdued by attaching to the nitrogen atom of the mustard an electron-withdrawing group. It was therefore hoped that these compounds would display minimal cytotoxicity to normal cells and they would undergo a transformation in the tumor cells that would trigger the release of the active alkylating agent. Compounds falling in this category were first prepared by Everett and Ross.[4] Friedman and Seligman[5] made a deliberate attempt to prepare and test such compounds, and their work was followed by Arnold and Bourseaux's[6] discovery of cyclophosphamide.

In our present investigation, as well as the preceding one,[2] we attempted to prepare deactivated alkylating agents which would be stable in neutral media but would release the cytotoxic mustard in acidic media. In this way, it was thought, the postulated acidity of the tumor cells could be exploited.[7] While this work was in progress, Ross[8] reported the increased antitumor activity of some basic amino acid nitrogen mustard derivatives in glucose-pretreated rats, and Kung, *et al.*,[9] reported the enhancement of the antitumor activity of 5-fluorouracil by glucose. Glucose injections to tumor-bearing animals have been claimed to lower the pH of the tumors.[7]

Enamines are known to be more stable in neutral or alkaline media but are hydrolyzed easily in acids.[10] We have therefore prepared compounds **3** (as shown in Scheme I) in which the basicity of the nitrogen in the nitrogen mustard moiety is diminished by the presence of an electron-withdrawing group Z. The reasons for preparing the fluoro compounds have been discussed in one of our previous publications.[3a]

Methyl and ethyl propiolate and dimethyl and diethyl acetylenedicarboxylate were commercial samples purified by distillation. The remaining esters were prepared from propiolic acid by the method of Ingold,[11] the unsubstituted amides by the method of Murahashi,[12] and the N-propioloylamino acid esters either by the carbodiimide method of Sheehan and Hess[13] or by treating the amino acid ester with propiolic anhydride. The starting material **2**, X = Y = Cl, was obtained by neutralizing its hydrochloride, **2**, X = Y = F, and **2**, X = F, Y = Cl, by the method[14] we have described previously.

The reactions of **1** with **2** were usually exothermic and in the preparation of **3a**, **d**, **h**, **i**, and **j** the presence of a solvent and cooling were found necessary; *e.g.*, in the preparation of **3a** without a solvent the product was contaminated by decomposition and/or polymerization by-products. In the remaining preparations high concentrations of the reactants favored the reaction which in the case of propiolic acid derivatives could be followed by the decrease in the acetylenic peak in the infrared. In the attempted preparation of 2-bis(2-chloroethyl)acrylamide, a mixture of propiolamide and bis(2-chloroethyl)amine exploded when heated to *ca.* 50°. In this reaction and in the reactions between propiolamide or ethyl N-propiolyl-11-aminoundecanoate and bis(2-chloroethyl)amine under various conditions no identifiable products could be isolated. On the other hand, propiolamide reacted with diethanolamine yielding the expected product **3k**, which when treated with methanesulfonyl chloride yielded **3l** by simultaneous dehydration of the amide[15] and esterification (mesylation) of the hydroxyl groups. In general, in the aliphatic series the addition of an amine to the acetylenic bond is influenced by (a) the basicity of the amine, *i.e.*, diethanolamine added to propiolamide but bis(2-chloroethyl)amine did not; (b) the activation of the acetylenic bond, *i.e.*, bis(2-chloroethyl)amine added to the acetylenedicarboxamide but not to propiolamide; and (c) size of ester or substituted amide group, *i.e.*, the reaction of bis(2-chloroethyl)amine

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(2) Paper IV of this series: Z. B. Papanastassiou, R. J. Bruni, E. White, V, and P. L. Levins, *J. Med. Chem.*, **9**, 725 (1966).

(3) (a) Z. B. Papanastassiou, R. J. Bruni, F. P. Fernandes, and P. L. Levins, *ibid.*, **9**, 357 (1966); (b) O. M. Friedman, Z. B. Papanastassiou, R. S. Levi, H. R. Till, Jr., and W. M. Whaley, *ibid.*, **6**, 82 (1963).

(4) J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, 1972 (1949); see also W. C. J. Ross, "Biological Akylating Agents," Butterworth and Co. (Publishers) Ltd., London, 1962.

(5) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655 (1954).

(6) H. Arnold and F. Bourseaux, *Angew. Chem.*, **70**, 539 (1958); H. Arnold, F. Bourseaux, and M. Brock, *Arzneimittel-Forsch.*, **11**, 143 (1961).

(7) For a discussion of the postulated acidity of the tumor cells, see ref 2.

(8) W. C. J. Ross, *Biochem. Pharmacol.*, **8**, 235 (1961).

(9) S. S. Kung, N. D. Goldberg, J. L. Dahl, R. E. Parks, Jr., and B. E. Kline, *Science*, **141**, 627 (1963).

(10) J. Szmuszkovicz, *Advan. Org. Chem.*, **4**, 1 (1963).

(11) E. H. Ingold, *J. Chem. Soc.*, **127**, 1199 (1925).

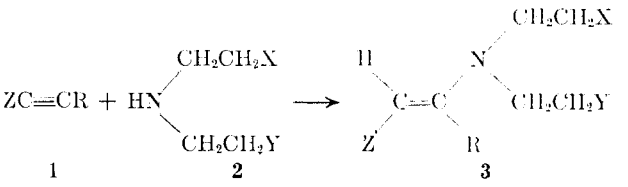
(12) S. Murahashi, *Nippon Kagaku Zasshi*, **77**, 1689 (1956); *Chem. Abstr.*, **53**, 5163 (1959).

(13) J. C. Sheehan and C. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(14) Z. B. Papanastassiou and R. J. Bruni, *J. Org. Chem.*, **29**, 2870 (1964).

(15) P. Oxlex, D. A. Peak, and W. F. Short, *J. Chem. Soc.*, 1618 (1948); C. R. Stephens, E. J. Bianco, and F. J. Pilgrim, *J. Am. Chem. Soc.*, **77**, 1071 (1955).

Scheme I



	Z	R	X	Y
a	$COOCH_3$	H	Cl	Cl
b	$COOCH_3$	H	F	F
c	$COOCH_3$	H	F	Cl
d	$COOC_2H_5$	H	Cl	Cl
e	$COO(CH_2)_3CH_3$	H	Cl	Cl
f	$COO(CH_2)_4CH_3$	H	Cl	Cl
g	$COO(CH_2)_6CH_3$	H	Cl	Cl
h	$COOCH_3$	$COOCH_3$	Cl	Cl
i	$COOC_2H_5$	$COOC_2H_5$	Cl	Cl
j	$CONH_2$	$CONH_2$	Cl	Cl
k	$CONH_2$	H	OH	OH
l	CN	H	Ms	Ms

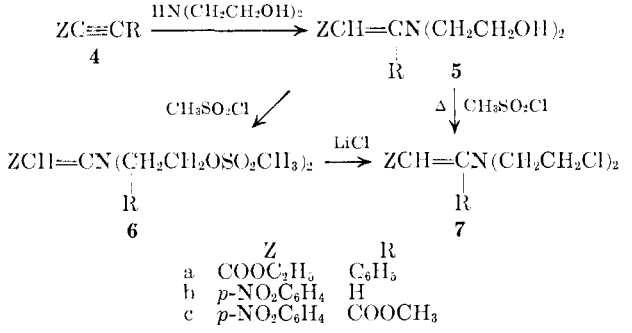
	Z	R	X	Y
m	$CONHCH_2COOC_2H_5$	H	Cl	Cl
n	$CONHCH(CH(CH_3)_2)COOC_2H_5$	H	Cl	Cl
o	$CONHCH(CH_2C_6H_5)COOCH_3$	H	Cl	Cl
p	$CONHCH(CH_2\text{-}C_8H_6N)COOC_2H_5$	H	Cl	Cl

was very exothermic with methyl propiolate but very slow with heptyl propiolate.

The solid products could be isolated by crystallization (for low-melting compounds Dry Ice temperatures were used). The liquid products were purified by distillation, although in the case of the high-boiling compounds high-vacuum, short, preheated Vigreux columns and repeated fast distillations had to be used to avoid excessive decomposition.

Phenylacetylene derivatives **4** were prepared by known methods. *p*-Nitrophenylpropiolic acid, prepared by the method of Perkin and Bellenot,[16] was converted to **4b** by the method of Drewson[17] and to **4c** by refluxing the acid with absolute methanol and H_2SO_4. *p*-Anisylacetylene was prepared by the method of Manchot, *et al.*[18] These compounds did not add bis(2-chloroethyl)amine but those having electron-withdrawing groups reacted with diethanolamine at elevated temperatures. The adducts **5** were transformed to the desired nitrogen mustard derivatives **6** and **7** as shown in Scheme II.

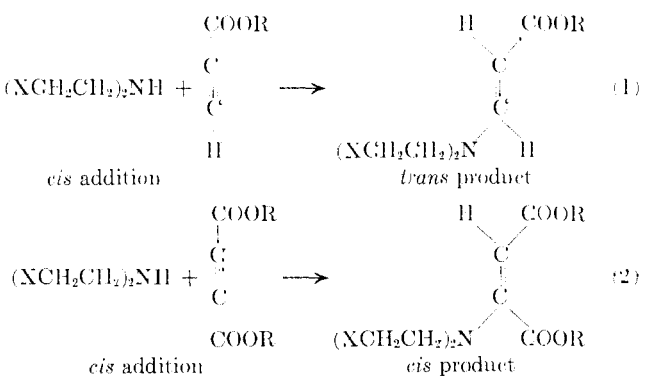
Scheme II



	Z	R
a	$COOC_2H_5$	C_6H_5
b	p-$NO_2C_6H_4$	H
c	p-$NO_2C_6H_4$	$COOCH_3$

The addition of diethanolamine to **4** yielded solid products except in the case of **5a** which was obtained from the reaction mixture as an oil and was used in the next reaction without further purification. No product could be obtained from the reaction of phenylacetylene and *p*-anisylacetylene with diethanolamine. The reaction of **5** with methanesulfonyl chloride leading to **6** was carried out in the presence of pyridine at low temperatures. If the reaction mixture was warmed, the chlorides **7** were obtained.[19] However, purer products were produced if the methanesulfonates **6** were first isolated and then treated with LiCl in dimethylformamide (DMF).

The stereochemistry of the addition of an amine to an acetylenic compound had not been adequately studied at the time we reported our results.[1] As we pointed out at that time, the *trans* products were obtained from the addition of diethanolamine to **4b** in DMF and bis(2-chloroethyl)amine to methyl propiolate in ether, and on the basis of nmr spectra of the products (the coupling constant, J, between olefinic protons is 13–14 cps) we postulated that addition occurred in a concerted *cis* fashion producing a *trans* product in the case of monosubstituted acetylene derivatives (eq 1) and a *cis* product in the case of disubstituted acetylene derivatives (eq 2). Recently Dolfini[20] reported that



the addition of ethylenimine to ethyl propiolate and diethyl acetylenedicarboxylate occurs in a *cis* fashion

(16) W. H. Perkin and G. Bellenot, *J. Chem. Soc.*, **49**, 440 (1886).
(17) V. B. Drewson, *Ann.*, **212**, 150 (1882).
(18) W. Manchot, J. C. Withers, and H. O. Hrogge, *ibid.*, **387**, 257 (1912); E. E. Smissman, R. H. Johnson, A. W. Carlson, and B. F. Aycock, *J. Am. Chem. Soc.*, **78**, 3395 (1956).
(19) R. S. Tipson, *J. Org. Chem.*, **9**, 235 (1944).
(20) J. E. Dolfini, *ibid.*, **30**, 1298 (1965).

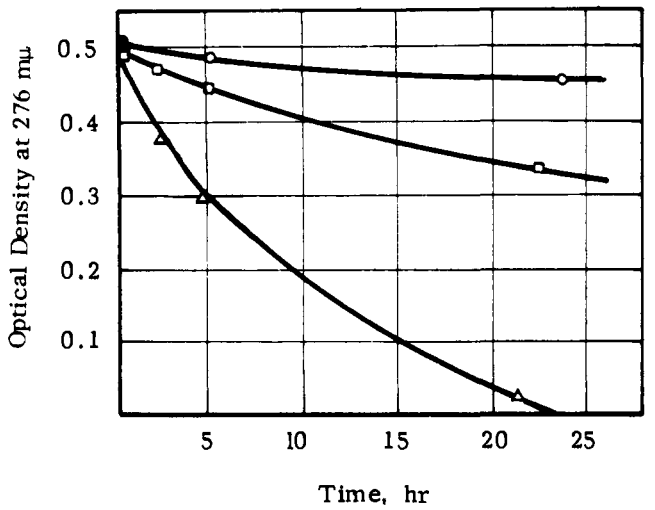


Figure 1.—Stability of 3-[bis(2-chloroethyl)amino]acryloyl-DL-valine ethyl ester (**3n**) (initial concentration 5.6 mg/l.) at pH 6.0 (—Δ—), pH 7.0 (—□—), and pH 7.9 (—○—).

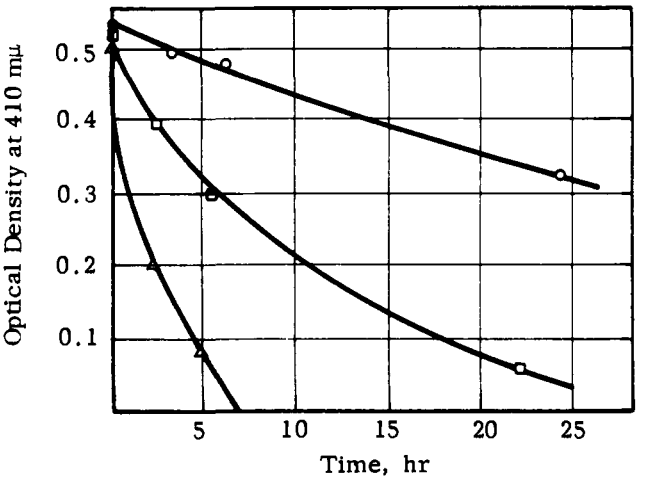
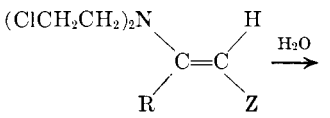


Figure 2.—Stability of β-[bis(2-methanesulfonoxyethyl)-amino]-*p*-nitrostyrene (**6b**) (initial concentration 9.3 mg/l.) at pH 6.0 (—Δ—), pH 7.0 (—□—), and pH 7.9 (—○—).

in aprotic solvents and that mixtures of *cis* and *trans* products were obtained in methanol.

Stability Studies.—The hydrolytic behavior of a representative number of enamine mustards was examined in neutral (pH 7), slightly basic (pH 7.9), and slightly acidic (pH 6) media. The rate of hydrolysis was measured by the decrease of the intensity of the ultraviolet absorption maxima which is due to loss of conjugation according to eq 3.[21]



$$(ClCH_2CH_2)_2NH + RCOCH_2Z \quad (3)$$

All trisubstituted ethylene compounds (when neither R nor Z = H) were stable at the three pH's studied. On the other hand, when R = H the compounds were hydrolyzed, the rate of hydrolysis being higher in acidic than in basic media. Figures 1 and 2 are typical of the hydrolysis profile of an aliphatic and an aromatic enamine mustard.

Antitumor Activity.—As stated in the introduction, the rationale for preparing the enamine mustard is that tumor cells may be more acidic than their normal counterparts and that some of the compounds prepared in this work could exhibit a differential toxicity to normal and tumor cells paralleling the difference in hydrolysis rates in weakly acidic and weakly basic media. This difference might be accentuated by administering glucose to the host. However, in the test systems used by the Cancer Chemotherapy National Service Center[22a] no such relationship can be claimed, as seen from the antitumor activity of these compounds listed in Table I. Most compounds exhibit a borderline activity and only **3a** and **3m** are as active as nor-$HN_2 \cdot HCl$. Both **3a** and **3m** have been shown to hydrolyze at a faster rate at pH 6 than at pH 7.9. Substituting higher alcohols for methyl alcohol in **3a** and other amino acids for glycine in **3m** lowers the *in vivo* activity, suggesting that an increase in lipid solubility in these compounds has an unfavorable effect on the antitumor activity. The fluoro mustards **3b** and **3c** were inactive, a fact that conforms with our previous observations.[2,3a] Compounds **6**, expected to act by a dual mechanism (SN2 or "myleran"-type before splitting and SN1 or nitrogen mustard type after splitting),[2] did not display any significant antitumor effect. The starting materials **4b** and **4c** had an ID_{50} of 7.9 and 2.3 μg/ml, respectively, in the KB tissue culture screening test but were inactive against L1210 and S180.

TABLE I

ANTITUMOR ACTIVITY OF ENAMINE MUSTARDS[a]

Compd	WA256 TI[b]	KB ID_{50}, μg/ml	Dunning leukemia TI	L1210	S180
3a	200/60 = 3	15		I	I
3b		31	I	I	
3c		29	I	I	
3d	100/40 = 2	6			
3e	100/100 = 1				
3f	I[c]	7.6			
3g	I[c]	6.5			
3h	200/200 = 1	54			
3i	200/200 = 1	>100			
3j	I[c]	>100			
3m	>100/25 ≈ 4	16			
3n	>100/75 ≈ 2	>100			
3o		3.5	<1[d]	I	I
3p		20	I	I	I
6a	8/8 = 1	28			
6b	20/20 = 1	>16			
6c	>100/68 ≈ 2	13			
7a	>100/76 ≈ 2				
7b			[e]	I	

[a] The compounds were screened by the Cancer Chemotherapy National Service Center and complete data will be published in a future Cancer Chemotherapy Screening Data Supplement to Cancer Research. For protocols for screening chemical agents against animal tumors and other biological systems, see ref 22a, and for comparison with other known drugs, see ref 22b; WA-256 = Walker 256 adenocarcinoma subcutaneous (rats; given nine injections intraperitoneally, starting one day after tumor implantation; animals sacrificed on tenth day and tumors weighed); I is inactive. [b] Therapeutic index is maximum tolerated dose (LD_{10} in mg/kg)/minimum effective dose (T/C 0.10 in mg/kg). [c] Inactive up to 200 mg/kg without manifestation of toxicity. [d] At 128 mg/kg three out of six rats were "cured." [e] High activity was observed with doses ranging from 4 to 256 mg/kg but results were not reproducible.

(21) C. Moureu and I. Lazennec, *Bull. Soc. Chim. France*, **35**, 1190 (1906).

(22) (a) *Cancer Chemotherapy Rept.*, **25**, 1 (1962); (b) H. E. Skipper and L. H. Schmidt, *ibid.*, **17**, 1 (1962); L. H. Schmidt, R. Fradkin, R. Sullivan, and A. Flowers, *ibid.*, Suppl 2, 1 (1965).

Table II

Aliphatic Enamine Mustards[a]

Compd	Yield, %	Mp or bp (mm), °C	Formula	Calcd, % C	H	Cl	N	Found, % C	H	Cl	N	Reactn solvent	Recrystn solvent	ν_{max}, cm^{-1}	λ_{max}, mμ (ε)	$n^{25}D$ or $[\alpha]^{25}D$, deg (c)
a	53	94–95	$C_8H_{13}Cl_2NO_2$	42.49	5.80	31.36	6.20	42.87	6.11	31.53	6.22	A	E	1680, 1610 K	278 (28,100) N	
b	62	37–40, 101–118 (0.1)	$C_8H_{12}F_2NO_2$	49.75	6.78			49.96	7.03			B	B	1690, 1615 B		1.4992
c	77	131–141 (0.5)	$C_8H_{13}ClFNO_2$	45.83	6.25			45.57	6.26			B	B	1685, 1615 B		1.5230
d	47	134–140 (0.1)	$C_9H_{15}Cl_2NO_2$	45.02	6.30	29.53	5.83	45.19	6.26	29.72	5.87	A	B	1690, 1610 K	276 (24,750) N	1.5360
e	63	30–31	$C_{11}H_{19}Cl_2NO_2$	49.26	7.14			49.54	7.13			A	E	1685, 1610 B		
f	62	26–27	$C_{12}H_{21}Cl_2NO_2$	51.07	7.50	25.13	4.96	51.49	7.61	24.97	5.14	B	E	1685, 1610 B		1.5210
g	33	23–24	$C_{14}H_{25}Cl_2NO_2$	54.19	8.12	22.86	4.52	53.90	7.84	22.79	4.68	B	F	1685, 1610 B		1.5131
h	30	131–135 (0.05)	$C_{10}H_{15}Cl_2NO_4$	42.27	5.32	24.96	4.93	42.70	5.72	24.60	4.96	A	B	1740, 1690, 1585 K	282 (21,100) N	1.5332
i	29	155–158 (0.1)	$C_{12}H_{19}Cl_2NO_4$	46.16	6.14	22.71	4.49	45.95	6.13	22.75	4.68	A	B	1735, 1690, 1580 K	282 (21,700) N	1.5216
j	53	215–217	$C_8H_{12}Cl_2N_3O_2$	37.81	5.16	27.90	16.54	37.70	5.41	27.75	16.35	C	G	1695, 1650, 1570 L	312 (22,100) N	
k	94	107–110	$C_7H_{11}N_2O_3$	48.26	8.10		16.08	47.75	7.70		16.21	C	H	1640, 1540 L		
l	1		$C_9H_{16}N_2O_6S_2$	34.60	5.16			34.49	5.18				K–O	2200, 1625 B		1.5325
m	40	106–108	$C_{11}H_{18}Cl_2N_2O_3$	44.45	6.10	23.86	9.43	44.81	5.94	24.12	9.44	C	I	1740, 1670, 1520, 1600 K	276 (28,300) G	
n	22	98–100	$C_{14}H_{23}Cl_2N_2O_5$	49.56	7.13	20.90	8.26	49.62	7.08	21.24	8.24	C	I–J	1725, 1660, 1590, 1500 K	276 (30,500) G	−22.1 (3.4, J)
o	27	110–111	$C_{17}H_{22}Cl_2N_2O_3$	54.70	5.94	19.00	7.51	55.00	6.18	19.09	7.42	D	K–J	1740, 1660, 1590, 1500 K		+93.0 (5.0, K)
p	33	145–146	$C_{20}H_{25}Cl_2N_3O_3$	56.34	5.91	16.63	9.86	56.63	6.03	16.90	9.67	D	K–J	1730, 1650, 1585, 1520 M	280 (45,000) G	−1.10 (2.7, J)

[a] **A,** ether; B, none; C, DMF; D, CH_2Cl_2; E, hexane; F, heptane; G, CH_3OH; H, acetone; I, CCl_4; J, petroleum ether (bp 30–60°); K, $CHCl_3$; L, KBr; M, Fluorolube and Nujol; N, 50% aqueous ethanol; O, pyridine.

TABLE III
AROMATIC ENAMINE MUSTARDS[a]

Compd	Yield, %	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % Cl	Calcd, % N	Calcd, % S	Found, % C	Found, % H	Found, % Cl	Found, % N	Found, % S	Reactn solvent	Recrystn solvent	ν_{max}, cm^{-1}	λ_{max}, mμ (ε)
5a			$C_{15}H_{21}NO_4$											A			
5b	49	63–65	$C_{12}H_{16}N_2O_4$	57.13	6.39		11.11		57.30	6.45		10.92		B	D	1630, 1580, 1500 I	
5c	35	130–132	$C_{14}H_{18}N_2O_6$	54.19	5.85		9.03		54.21	5.86		9.15		B	D	1680, 1600, 1550, 152) I	
6a	29	87–89	$C_{17}H_{23}NO_3S_2$	46.88	5.79		3.22	14.72	46.88	5.75		3.22	14.56	C	E	1680, 1560, 1490, 1440 I	282 (14,950) E
6b	30	105–108	$C_{14}H_{20}N_2O_8S_2$	41.17	4.94		6.86	15.70	41.28	5.04		6.64	15.74	C	F	1630, 1580, 1500 I	259, 410 (9600, 22,000) E
6c	88	120–121	$C_{16}H_{22}N_2O_{10}S_2$	41.19	4.75		6.01	13.75	40.16	4.49		5.99	13.68	C	F	1700, 1610, 1580, 1530 I	273 (29,800) E
7a	31	52–54	$C_{15}H_{19}Cl_2NO_2$	56.97	6.06	22.42	4.43		56.88	6.25	21.74	4.49		B	G	1680, 1560, 1490, 1430 I	284 (17,800) E
7b	50	84–86	$C_{12}H_{14}Cl_2N_2O_4$	49.84	4.88	24.52	9.69		50.15	5.05	24.74	9.68		B	H	1635, 1585, 1505 I	261, 422 (8300, 24,500) E
7c	27	107–109	$C_{14}H_{16}Cl_2N_2O_4$	48.43	4.65	20.42	8.07		47.71	4.39	20.58	8.01		B	H	1700, 1600, 1575, 1520 J	

[a] A, none; B, DMF; C, $CHCl_3$ + pyridine; D, CH_2Cl_2; E, CH_3OH; F, CCl_4 + CH_2Cl_2; G, petroleum ether; H, CCl_4; I, $CHCl_3$; J Fluorolube and Nujol.

Experimental Section[23]

Esters of Propiolic Acid.—Butyl, pentyl, and heptyl propiolate were prepared by the method of Ingold[11] as follows. To a solution of propiolic acid (1 mole) and the appropriate alcohol (4 moles) was added, with cooling, 2 moles of concentrated H_2SO_4, and the reaction mixture was allowed to stand at room temperature for 2–4 days. It was then poured into ice and the solution was extracted with ether. The ether extract was washed ($NaHCO_3$ solution, H_2O) and dried ($MgSO_4$). The residue obtained after evaporating the solvent was distilled through a short Vigreux column. The yields of the esters were 25–30%; *n*-butyl propiolate (**1e**), bp 62° (19 mm), $n^{25}D$ 1.4220; *n*-pentyl propiolate (**1f**), bp 75–76° (20 mm), $n^{25}D$ 1.4262; and *n*-heptyl propiolate (**1g**), bp 105–106° (17 mm), $n^{25}D$ 1.4340. All esters absorbed in the infrared at 3260 (CH), 2125 (C≡C), 1715 (C=O), and 1230 (COC) cm^{-1}, and they were used in the next step without further identification.

Propiolamide and Acetylenedicarboxamide.—The method of Murahashi[12] was used as follows. Methyl propiolate (or dimethyl acetylenedicarboxylate) was added to excess liquid NH_3 and kept at Dry Ice temperature overnight. The solution was then warmed and the excess NH_3 was evaporated. In the case of acetylenedicarboxamide (**1j**), the residue was triturated with DMF, and the insoluble portion was filtered and washed with fresh DMF and then with $CHCl_3$. Additional amide was obtained by adding $CHCl_3$ to the DMF solution, making a total yield of 53%, mp 192° (lit.[24] mp 190–192° for acetylenedicarboxamide half-hydrate). The impure propiolamide (**1k**) was recrystallized from a large volume of methylene chloride; mp 56–59° (lit.[12] mp 61–62°).

N-Propioloylamino Acid Esters. Method A.—Equimolar quantities of the amino acid ester hydrochloride, N,N-dicyclohexylcarbodiimide, propiolic acid, and triethylamine in CH_2Cl_2 were mixed at ice temperature and then stirred at room temperature for 20 hr. The precipitated dicyclohexylurea was filtered off and the filtrate was washed with 1 *N* HCl, 1 *N* $KHCO_3$, and finally with water. After drying, the CH_2Cl_2 solution was treated with charcoal and concentrated in a rotary evaporator; the residue was then recrystallized.

N-Propioloylglycine ethyl ester (1m), yield 26%, was recrystallized from 6:1 CCl_4–$CHCl_3$ as colorless needles: mp 62–64°; ν^{Nujol} 2110 (C≡C), 1740 (C=O ester), 1620, 1550 (C=O amide) cm^{-1}. *Anal.* Calcd for $C_7H_9NO_3$: C, 54.19; H, 5.84; N, 9.03. Found: C, 54.32; H, 5.73; N, 9.14.

N-Propioloyl-DL-valine ethyl ester (1n), yield 44%, was recrystallized from petroleum ether (bp 30–60°); mp 57–59°; ν^{CHCl_3} 2100 (C≡C), 1730 (C=O ester), 1640, 1520 (C=O amide) cm^{-1}. *Anal.* Calcd for $C_{10}H_{15}NO_3$: C, 60.89; H, 7.65; N, 6.92. Found: C, 61.60; H, 7.75; N, 6.92.

N-Propioloyl-L-tryptophan ethyl ester (1p), yield 48%, was recrystallized from $CHCl_3$–petroleum ether as colorless crystals: mp 94–95°; ν^{CHCl_3} 2120 (C≡C), 1735 (C=O ester), 1660, 1500 (C=O amide) cm^{-1}; $[\alpha]^{26.5}D$ −27.0° (*c* 2.7, methanol). *Anal.* Calcd for $C_{16}H_{16}N_2O_3$: C, 67.59; H, 5.67; N, 9.86. Found: C, 66.90; H, 5.55; N, 9.51.

N-Propioloyl-L-phenylalanine methyl ester (1o), yield 52%, was recrystallized from CCl_4 as yellow crystals: mp 78–79°; $\nu^{CH_2Cl_2}$ 2120 (C≡C), 1745 (C=O ester), 1665 and 1500 (C=O amide) cm^{-1}; $[\alpha]^{26.5}D$ −32.7° (*c* 2.4, methanol). *Anal.* Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.66. Found: C, 66.97; H, 5.70.

Method B.—Triethylamine (2 moles) was added to an equimolar mixture of the amino acid ester hydrochloride and propiolic anhydride (prepared by the method of Straus and Voss[25]) in $CHCl_3$ with cooling and stirring. The resulting solution was allowed to stand at room temperature for 40 hr and then was washed with water, dried, and decolorized with charcoal. The solution was then concentrated to dryness and the residue was recrystallized. **N-Propioloyl-L-tryptophan ethyl ester (1p)**, yield 42%, had properties identical with product prepared by method

(23) All starting materials and solvents were purified before use. Melting points were obtained with a calibrated Mel-Temp apparatus. The spectra were obtained with a Perkin-Elmer 237 spectrophotometer (infrared), a Beckman DK-1A spectrophotometer (ultraviolet), and a Varian Associates A-60 spectrometer (nmr). The optical rotations were obtained with a Zeiss polarimeter (reading accuracy ±0.01°). The microanalyses were performed by Dr. S. M. Nagy of the Massachusetts Institute of Technology.

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A. **Ethyl N-propioloyl-11-aminoundecanoate**, yield 40%, was recrystallized from ethyl ether as needles: mp 68–69°; ν^{CHCl_3} 2120 (C≡C), 1725 (C=O ester), 1660 and 1515 (C=O amide) cm^{-1}. *Anal.* Calcd for $C_{16}H_{27}NO_3$: C, 68.29; H, 9.67. Found: C, 68.26; H, 9.59.

Preparation of Enamine Mustards (3).—Bis(2-chloroethyl)-amine hydrochloride was dissolved in cold water and neutralized with 1 *N* NaOH as fast as possible under constant stirring and cooling. The aqueous mixture was exhaustively but rapidly extracted with ethyl ether, and the ether extract was washed with water and saturated NaCl solution and dried by passing through a Drierite bed. The ether solution was concentrated in the cold and used immediately in the following reaction. It was estimated that about 90% of the free amine was recovered.

Bis(2-fluoroethyl)amine[14] and (2-fluoro-2-chlorodiethyl)-amine[14] were liberated from their hydrochlorides by similar methods.

The following is a general method for the reaction of acetylenic compounds with bis(2-chloroethyl)amine or its fluoro analogs.

A solution of the bis(2-haloethyl)amine **2** in ether or DMF was added to a solution or suspension of the acetylene derivative **1** in the appropriate solvent in the cold. After stirring for 2 hr the reaction mixture was placed in the refrigerator and allowed to stand. The progress of the reaction was examined by withdrawing aliquots from the reaction mixture and determining the infrared spectra. When the reaction was completed, the solvent was evaporated and the residue was recrystallized or purified by distillation. The reaction of **2** with amino acid esters was slow and the reaction mixture was allowed to stand at room temperature for 5–10 days. The properties of the products are listed in Table II.

Methyl *p*-Nitrophenylpropiolate.—*p*-Nitrophenylpropiolic acid was prepared by the method of Perkin and Bellenot[16] from ethyl *p*-nitrocinnamate. It was decarboxylated in 86% yield to *p*-nitrophenylacetylene by the method of Drewson[17] (mp 149–152°, lit.[17] mp 152°) and esterified by heating for 16 hr with methanol and concentrated H_2SO_4 to yield 61% of product, mp 109–111°.

Bis(2-hydroxyethyl)aminostyrene Derivatives (5).—Diethanolamine was added to a solution of an equimolar quantity of the acetylenic compound in DMF (in the case of **5a** no solvent was used). The mixture was heated to 60–80° and the progress of the reaction was followed by examining the infrared spectra of aliquots. The reaction was completed in 1–2 hr. The solvent was removed under very high vacuum in a rotary evaporator and the residue was recrystallized. In the case of ethyl phenylpropiolate, the product was liquid and could not be purified by distillation. Any basic materials were removed by passing the reaction mixture through a bed of Amberlite IRC-50 (weakly acidic ion-exchange resin). The properties of the products are in Table III.

Methanesulfonates (6).—A solution of methanesulfonyl chloride (1 mole) in $CHCl_3$ was added to a solution of 0.5 mole of **5** in $CHCl_3$ and pyridine. The mixture was stored at 0° for 20 hr and it was then washed with water and saturated solution of NaCl and dried ($MgSO_4$). Evaporation of the solvent and recrystallization of the residue yielded the compounds listed in Table III.

Chlorides (7).—A solution of the methanesulfonates **6** and an excess of anhydrous LiCl in DMF was stirred for 20 hr at room temperature. The solvent was evaporated under high vacuum and the residue was triturated with CH_2Cl_2. The CH_2Cl_2 extract was decolorized with charcoal and concentrated to dryness, and the residue was recrystallized. The properties of the products are listed in Table III.

Hydrolysis Studies.—Alcoholic stock solutions of the enamine mustards were mixed with equal volumes of Clark and Lubs buffer solutions (0.1 *M* KH_2PO_4 and 0.1 *N* NaOH solutions) at pH 6, 7, and 7.9. The mixtures were allowed to stand at room temperature and their ultraviolet absorbance was determined at various times. Compounds **3h–j**, **6a** and **c**, and **7a** and **c** were stable in the three pH's examined. Compounds **3a–g**, **m**, **o**, and **p** hydrolyzed with rates very similar to that shown in Figure 1 for **3n**. The hydrolysis rates of **7b** were identical with those of **6b** shown in Figure 2.

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Potential Antitumor Agents. V. Bisquaternary Salts

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A series of bis quaternary ammonium heterocycles has been prepared and evaluated against the L1210 leukemia system.

The observation of Ambrose and co-workers[2,3] that tumor cells apparently have a higher negative surface charge suggests that basic compounds could be concentrated in such cells. Moreover, if these compounds were also cytotoxic it should be possible to demonstrate selective toxicity toward the tumor cells. The antitumor properties of a recently prepared series of bisimidazolines[4,5] convincingly illustrate this point. The remarkable life extension obtained in leukemia with these drugs, coupled with the well-authenticated similarity of pharmacologically active compounds containing either amidinium or quaternary ammonium functions[6,7] prompted us to investigate a series of bisquaternary ammonium heterocyles.

The first demonstration of unequivocal activity against the L1210 mouse leukemia in this laboratory was provided by the bisquinolinium salts I (R′ = H, Table I). While the bismethyl (I, R′ = H; R = CH_3) and bisethyl quaternary salts showed only borderline inhibition against this tumor, the *n*-propyl homolog showed decided inhibition. Maximum activity was reached with the *n*-butyl derivative and dropped off rapidly in the higher homologs, the *n*-hexyl compound being inactive. Our results, with an extensive range of quaternary salts, have led us to the conclusion that there is a marked dependence of biological properties on the relative lipophilic–hydrophilic balance of these compounds. For each structural type it is necessary to construct a homologous series of quaternary salts with a range of lipophilic–hydrophilic properties. Only by selecting the member of each homologous series

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