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Synthesis of Possible Cancer Chemotherapeutic Compounds Based on Enzyme Approach. IV. Aziridine Derivatives[1]

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Several β-(1-aziridinyl)propionate esters, 1,1′-acylaziridines of dibasic acids, (1-aziridinyl)formates, β-(1-aziridinyl)ethyl esters, and other ethylenimine derivatives have been prepared as possible cancer chemotherapeutic compounds based on enzyme rationale. The acylbisaziridines of dibasic acids are in general unstable. In the preparation of 1,1′-malonyl bisaziridine, only the compound from ring opening, malonic acid bis-(β-chloroethyl)-diamide was obtained. The β-(1-aziridinyl)propionate ester of ethyleneglycol was found to be active in the S-180, CA-755, and L-1210 systems. 1,1′-(2,2-Dimethylpropylene)bis-aziridinyl formate was designed to simulate the well known muscle relaxant, 2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate. Infrared spectra and biological activities of the compounds are discussed.

In order to take advantage of the known difference in esterase activity in normal and neoplastic tissues,[2,3] several series of compounds containing $ClCH_2CH_2S$– groups as the alkylating moiety have been prepared and evaluated as possible cancer chemotherapeutic agents.[4,5] To extend this rationale, many ethylenimine derivatives have also been prepared. This paper reports the synthesis of these compounds together with discussions of some of the interesting observations made in the course of this work.

hydrolyzed by esterase to β-(aziridinyl)alanine, a much less toxic compound. The methyl ester (I) has been prepared by the Michael addition of ethylenimine to the acrylic esters, similar to the method used by Bestian.[6]

The vinyl (II) and allyl (III) esters were made by a slightly modified procedure. Attempts to add a second molecule of ethylenimine to the double bond of the alcohol moiety of these unsaturated esters have thus far failed. When the reaction was carried out in the presence of metallic sodium as a catalyst only a rubbery polymer was obtained. Ethylene glycol di-β-(1-aziridinyl)-propionate (IV) was prepared by the transesterification of methyl β-(1-aziridinyl)propionate and ethylene glycol. Several 1,1′-acylbisaziridines of dibasic acids which are related to these compounds were also prepared (Table II). Although synthesis of these

TABLE I

β-(1-AZIRIDINYL)PROPIONIC ACID ESTERS 

Compound	—R	B.p., °C. (mm.)	Refractive index at n°	Yield, %	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
I	CH_3	63–65 (23)	1.4312^{24}	44.0	$C_6H_{11}NO_2$	55.82	55.96	8.58	8.70	10.84	10.85
II	$CH{=}CH_2$	56–64 (15–18)	1.4490^{22}	52.0	$C_7H_{11}NO_2$	59.59	59.70	7.86	7.80	9.93	10.20
III	$CH_2CH{=}CH_2$	105–106 (30)	1.4482^{23}	76.7	$C_8H_{13}NO_2$	61.91	61.84	8.44	8.50	9.03	9.01
IV	$CH_2CH_2OCOCH_2CH_2N$ 	145–147 (0.2)	1.4660^{23}	38.3	$C_{12}H_{20}N_2O_4$	56.23	56.49	7.87	7.93	10.93	10.89

TABLE II

ACYLBISAZIRIDINES OF DIBASIC ACIDS 

Compound	—R	M.p., °C.	Refractive index at n°	Yield, %	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
VI	$—CH_2—$	unstable, characterized only by its derivative, see Experimental									
VIII	$—CH_2CH_2—$	72–73		20.5	$C_8H_{12}N_2O_2$	57.12	57.20	7.19	7.40	16.66	16.50
IX	$—(CH_2)_8—$		1.4921^{25}	22.0	$C_9H_{14}N_2O_2$	59.32	59.08	7.74	7.90	15.37	15.58

The β-(1-aziridinyl)propionate esters (Table I) contain only one alkylating group. These compounds are

(1) This work was supported by Public Health Service Research Grant CY-2530, from the National Cancer Institute, National Institutes of Health, Bethesda 14, Md.

(2) A. M. Seligman, M. M. Nachlas, L. H. Manheimer, O. M. Friedman, and G. Wolf, *Ann. Surg.*, **130**, 333 (1949).

(3) K. C. Tsou and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 3704 (1954).

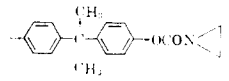
(4) K. C. Tsou, H. C. F. Su, C. Segebarth, and U. Mirarchi, *J. Org. Chem.*, **26**, 4987 (1961).

(5) H. C. F. Su, C. Segebarth, and K. C. Tsou, *ibid.*, **26**, 4990 (1961).

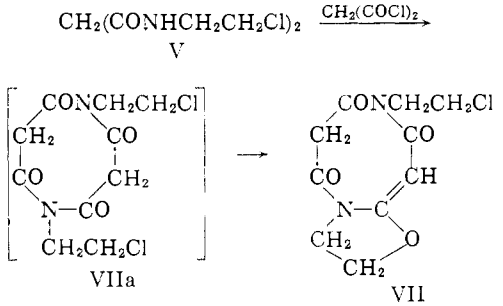
(6) H. Bestian, J. Heyna, A. Bauer, G. Ehlers, B. Hirsekonn, T. Jacobs, W. Noll, W. Weibezahn, and F. Römer, *Ann. Chem.*, **566**, 210 (1950).

Table III

(1-Aziridinyl)formates $\triangleright$NCOOR

Compound		B.p., °C. (mm.)	M.p., °C.	Refractive index at n^{25}	Yield, %	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
X	—$CH_2CH_2OCON\triangleleft$		53		40.0	$C_5H_7N_2O_2$	48.01	47.32	6.04	6.15	14.00	14.30
XI	—$CH_2CH_2OCH_2CH_2OCON\triangleleft$		54.5–55		80.0	$C_{10}H_{16}N_2O_5$	49.17	49.01	6.60	6.72	11.48	11.40
XII	—$CH_2C(CH_3)_2CH_2OCON\triangleleft$			1.4661^{25}	82.6	$C_9H_{16}N_2O_2$	51.53	54.60	7.49	7.40	14.56	14.50
XIII			120.5–121		59.2	$C_{21}H_{24}N_2O_4$	68.81	68.86	6.95	6.19	7.51	7.71
XIV	—$CH_2CH{=}CH_2$	48–51 (2.5)		1.4493^{25}	74.4	$C_6H_9NO_2$	56.68	56.87	7.14	7.08	11.02	10.96

compounds is relatively simple, their isolation and purification were sometimes difficult. From the reaction of malonyl chloride and ethylenimine, in the presence of triethylamine, only the malonic acid bis-N,N′-(β-chloroethyl)diamide (V) was isolated. Evidently the intermediate 1,1′-malonyl bisaziridine (VI) did form, but then underwent a ring opening reaction with HCl. In addition to this substance, another compound (VII) of formula, $C_{10}H_{11}ClN_2O_4$, m.p. 166–167°, was isolated. The assignment of structure VII to this compound remains tentative but there seems little doubt about the stability of such an eight-membered ring. The formation of VII from V can take place *via* the bis-(dimalonyl)-N,N′-(β-chloroethyl)-imide (VIIa) as follows



There are other possibilities, but structure VII has the best support from the infrared spectrum of this compound. A comparison of the infrared spectra of VII and V showed a noticeable reduction in the methylene intensity (3.40 μ) in VII together with the presence of a methine (—CH= C—) band at 5.15 μ, and a cyclic ether band at 8.90 μ. These data led us to assign the bicyclic structure. A scale molecular model (Catalin) was built, which further favored this assignment. In the case of 1,1′-succinyl bisaziridine (VIII) the synthesis was more successful, insofar as we could obtain an analytically and spectrally pure sample. However, after standing at −10° for two days, it polymerized completely. The 1,1′-glutaryl bisaziridine (IX) was obtained as a heavy colorless oil.

The 1-aziridinyl formates (Table III) are slightly more stable than the acylaziridines. Their relation to the urethanes, a known class of cancer chemotherapeutic compounds, makes them an interesting series to compare. They were obtained by a standard procedure of treating the corresponding chloroformates with ethylenimine in the presence of triethylamine. Purification of these compounds was achieved by low temperature crystallization. Most of the 1-aziridinyl formates can be stored under refrigeration for months! They react quite readily with *p*-nitrobenzylpyridine, a reagent which we have used to test other alkylating agents.

Another type of bifunctional ethylenimine compounds that can be hydrolyzed by the esterase are dibasic acid esters of (1-aziridinyl) methanol (XV) and β-(1-aziridinyl)ethanol (XVI). Only the lower members of the aliphatic dicarboxylic acids are of interest because it is generally accepted that separation of the two alkylating groups over too long a chain is undesirable (*e.g.*, methanesulfonates, $CH_3SO_3(CH_2)_nSO_3CH_3$. $n = 6$ is less active than $n = 4$). β-(1-Aziridinyl)-ethanol is readily prepared by reaction of equimolar quantities of ethylenimine and ethylene oxide at 0°. Since our work was completed (1-aziridinyl)methanol has been reported by Kostyanovskii,[7] who prepared it by the reaction of ethylenimine and formaldehyde in ether or benzene solution. When we carried out the reaction with paraformaldehyde the purification was difficult and there was obtained also a high boiling fraction, b.p. 68–68.5° (0.05 mm.). It was identified as bis-(1-aziridinyl)methane (XVII). Because of the instability of the (1-aziridinyl)methanol, only (1-aziridinyl)ethanol was used. Model compounds such as β-(1-aziridinyl)ethyl acetate (XIX), propionate (XX), and butyrate (XXI) were prepared. However, the preparation of the malonate and succinate esters has not been successful. Condensations of glutaraldehyde with ethylenimine gave 1,5-bis-(1-aziridinyl)-1,5-pentanediol (XVIII). The physical constants and analyses of the compounds are summarized in Tables IV and V.

All compounds synthesized were screened for antitumor activity. It suffices to mention here that many of them show some activity in one of the S-180, Ca-755, and L-1210 tumor screening systems. Compound IV was active in all three primary screening systems and Walker 256 (Table VI). Compound XII was prepared because of its structural resemblance to the well known muscle relaxant, 2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate. This empirical structure–activity correlation was indicated by the screening results where the tumor bearing rats treated with this compound appeared more relaxed than rats treated with the other analogs. It does show promising activity in the Dunning Leukemia system (Table VII).

(7) R. G. Kostyanovskii, *Dokl. Akad. Nauk SSSR*, **135**, 853 (1960).

TABLE IV

MISCELLANEOUS BIFUNCTIONAL 1-AZIRIDINYL DERIVATIVES

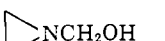
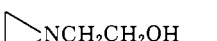
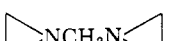
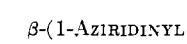
Compound	Structures	B.p., °C. (mm.)	M.p., °C.	Refractive index at n°	Yield, %	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
XV		49–50 (5)		1.4688^{20}	20.5	C_3H_7NO						
XVI		9–71 (16)		1.4531^{25}	21.6	C_4H_9NO	55.14	54.96	10.42	10.42	16.08	15.97
XVII		77–78 (0.1)		1.4896^{21}	11.9	$C_5H_{10}N_2$					28.54	28.32
XVIII			105.5–106		23.1	$C_9H_{18}N_2O_2$	58.02	58.08	9.74	9.66	15.02	15.00

TABLE V

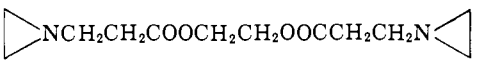
β-(1-AZIRIDINYL)ETHYL ESTERS 

Compound	—R	B.p., °C. (mm.)	Refractive index at n°	Yield, %	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
XIX	CH_3	51–52 (5)	$1.4307^{26.5}$	41.4	$C_6H_{11}NO_2$	55.79	55.84	8.58	8.64	10.85	10.85
XX	C_2H_5	63 (5)	1.4328^{22}	24.4	$C_7H_{13}NO_2$	58.72	58.52	9.15	9.28	9.78	9.82
XXI	$(CH_2)_2CH_3$	70 (4)	1.4355^{22}	44.0	$C_8H_{15}NO_2$	61.12	61.16	9.62	9.61	8.91	8.80

The infrared spectra of the compounds were compared and revealed some useful bands for the characterization of aziridine compounds. Hoffman, Evans, and Glockler[8] have studied the spectra of ethylenimine and N-methylethylenimine and have assigned two bands at 8.25 and 11.5 μ to ring deformation vibrations. These bands were also observed with 1,1′-bisaziridine[9] and were used to confirm the structure of this compound. In the spectra of our aziridine compounds we found these bands to be very inconsistent and unsuitable for characterization of this ring system. A more reliable characterization of aziridine derivatives can be made by the use of the fundamental vibration frequency of the C—H bond in the 3.25 μ region combined with the CH_2 deformation frequency in the 6.75 μ region. An additional band with weak to medium intensity was frequently found in the 6 μ region. Since highly strained rings give rise to CH valency vibrations with higher frequencies and lowered intensities, all our aziridine derivatives absorb without exception in the narrow range of 3.23 to 3.28 μ. This band is of medium to weak intensity but always distinct and sharp. A second absorption in the 3.32 to 3.36 μ range is a weak band and is assigned, in the presence of strong normal strain-free —CH_2—, as the unsaturated aliphatic =CH band. Often it is recognized only as shoulder if not completely masked. Thus, based on the compounds we have examined, the following assignments to aziridine deriv-

TABLE VI

ANTITUMOR ACTIVITY OF ETHYLENE DI-β-(1-AZIRIDINYL)PROPIONATE (IV)[a,b]

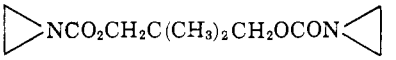


Tumor	Dose, mg./kg.	Survivors	Wt. change, test/control	Tumor wt., (days) test/control	T/C
S-180	27.	3/6	−5.0/−2.5	333/1077	0.31
	27.	4/6	−3.2/−1.6	441/874	0.50
	18.	6/6	−2.4/1.4	225/621	0.36
	13.5	6/6	−4.0/2.9	210/602	0.34
	12.	6/6	−4.0/−2.5	650/1077	0.60
	8.	6/6	−2.8/−2.5	475/1077	0.44
Ca-755	16.	4/10	−3.1/0.0	288/936	Toxic
	8.	8/10	−2.0/0.6	269/777	0.34
L-1210	24.	6/6	−4.3/−0.6	(12.0/7.5)	1.60
	16.	6/6	−3.5/−0.6	(12.0/7.5)	1.60
	10.6	6/6	−2.5/−0.6	(12.7/7.5)	1.69
	7.1	6/6	−1.4/−0.6	(11.3/7.5)	1.50
Wa-256	41.	5/6	3.0/43.0	0.1/5.2	0.00
	20.	6/6	13.0/43.0	1.3/5.2	0.25
	10.	6/6	24.0/43.0	3.3/5.2	0.63
	5.	6/6	37.0/43.0	3.1/5.2	0.59

[a] Screening results obtained through the generous cooperation of the Cancer Chemotherapy National Service Center (CCNSC). [b] The testing procedures for S-180, Ca-755, and L-1210 were described in *Cancer Res.*, **20**, 734 (1960).

TABLE VII

ANTITUMOR ACTIVITY OF 1,1′-(2,2-DIMETHYLPROPYLENE)-BISAZIDIRINYL FORMATE (XII)[a] ON DUNNING LEUKEMIA



Dose, mg./kg.	Survivors	Wt. change, test/control	Survival time, test/control	T/C
30	2/6	−7.0/22.0	17.5/12.6	Toxic
20	2/2	3.0/9.0	19.5/13.5	1.44
15	6/6	12.0/22.0	15.3/12.6	1.22
10	3/4	4.0/13.0	15.6/12.8	1.22
5	2/2	1.0/9.0	18.0/13.5	1.33
2.5	2/2	8.0/9.0	14.5/13.5	1.07

[a] Screening results obtained through the generous cooperation of CCNSC. The testing procedures have been adequately described in *Cancer Res.*, **20**, 734 (1960), and *ibid.*, **22**, 221 (1962).

(8) H. T. Hoffman, G. E. Evans, and G. Glockler, *J. Am. Chem. Soc.*, **73**, 3028 (1951).

(9) A. F. Graefe and R. E. Meyer, *ibid.*, **80**, 3939 (1958).

atives was appropriate: (a) 3.23–3.28 μ (medium intensity, sharp); (b) 3.32–3.36 μ (weak, often as shoulder only); (c) 5.95–6.20 μ (medium weak, of variable shape); (d) 6.80–6.85 μ (medium).

Experimental[10]

Methyl β-(1-Aziridinyl)propionate (I).—Ethylenimine (50 ml.) was added to a solution of methyl acrylate (150 ml.) in 200 ml. of methanol with slight cooling over a 5-min. period. The mixture was then left standing at room temperature for 3 days. After removal of the excess methyl acrylate and unreacted ethylenimine, the ester (29 g.) was obtained by distillation *in vacuo*.

Vinyl β-(1-Aziridinyl)propionate (II).—Ethylenimine (21.5 g., 0.5 mole) in 70 ml. of benzene was added over 1 hr. to a solution of vinyl acrylate (45 g., 0.5 mole) in 250 ml. of benzene, under a nitrogen atmosphere. The mixture was stirred at 25° for 4 hr., followed by the addition of 10 mg. each of sodium methylate and hydroquinone. The solution was then concentrated and distilled *in vacuo* to obtain II.

Allyl β-(1-Aziridinyl)propionate (III).—To allyl acrylate (112 g., 1 mole), ethylenimine [50 ml. (41.6 g., 0.967 mole)] was added portionwise with external cooling. After standing for 2 days at room temperature, the solution was distilled *in vacuo*. The ester III (115.1 g.) was collected at 30 mm.

Ethylene Di-β-(1-aziridinyl)propionate (IV).—Ethylene glycol (15.4 g., 0.25 mole) was mixed with I (64.5 g., 0.5 mole) together with 0.2 g. of sodium methylate and was stirred at room temperature for 3 days. The solution was distilled under slightly reduced pressure to remove the methanol formed; IV (24.5 g.) was obtained by high vacuum distillation.

Attempted Preparation of 1,1′-Malonyl Bisaziridine (VI).—Malonyl chloride (35.25 g., 0.25 mole) in 75 ml. of benzene was added dropwise with stirring to a cooled solution of ethylenimine (22.5 g., 0.525 mole) and triethylamine (80 ml.) in 200 ml. of benzene. After complete addition, the mixture was stirred at 25° for 2.5 hr. and then filtered. The solid was dissolved in a small quantity of water which was then extracted continuously with ether–methylene chloride (6:1) for 4 days. The extract was concentrated and recrystallized from methanol to give 3.3 g. of pale yellow crystals of malonyl N,N′-di-(β-chloroethyl)-amide (V), m.p. 142–143°.

Anal. Calcd. for $C_7H_{12}Cl_2N_2O_2$: C, 37.02; H, 5.33; N, 12.33; Cl, 31.22. Found:[11] C, 37.8; H, 5.4; N, 11.9; Cl, 30.7.

The filtrate was evaporated to dryness. The residue (6 g.), was recrystallized from methanol to furnish yellow crystals, m.p. 166–167° (VII).

Anal. Calcd. for $C_{10}H_{11}ClN_2O_4$: C, 46.43; H, 4.28; N, 10.83; Cl, 13.71. Found: C, 46.9; H, 4.3; N, 10.60; Cl, 13.6.

1,1′-Succinyl Bisaziridine (VIII).—Succinyl chloride (12 g., 0.077 mole) in 200 ml. of benzene was added dropwise to a solution of ethylenimine (10 g., 0.23 mole) and triethylamine (36 ml.) in 20 ml. of benzene at −5 to 0°. After stirring at room temperature overnight, it was filtered to remove the precipitated triethylamine hydrochloride. The filtrate was concentrated *in vacuo* and the residual oil was recrystallized from methanol to yield 2.7 g. of white crystals.

1,1′-Glutaryl Bisaziridine (IX).—A solution of glutaryl chloride (8.45 g., 0.05 mole) in 75 ml. of ether was added to a solution of ethylenimine (4.3 g., 0.1 mole) and triethylamine (10.1 g., 0.1 mole) in 100 ml. of ether at 0°. After stirring at this temperature for 1 hr., it was allowed to stand at 5–10° overnight. After removal of precipitated triethylamine hydrochloride, the solution was concentrated to about 20 ml. in volume. It was cooled to below −50° while a white precipitate separated. It was dried on the porous plate cooled over Dry Ice. The solid was then placed in the beaker (melted again) and dried further over P_2O_5 in a vacuum desiccator at 5–10°. The heavy oil was purified by dissolving in anhydrous ether and filtering to remove the insoluble polymer. The ethereal solution was concentrated to give a heavy colorless oil (2 g.) (XII), $n^{25}D$ 1.4921.

Ethylene Bis(1-aziridinyl)formate (X).[11]—Ethylene bischloroformate (13.75 g., 0.0735 mole) in 20 ml. of benzene was added to a solution of ethylenimine (6.32 g., 0.147 mole) and triethylamine (25 g., 0.247 mole) in 50 ml. of benzene at −10°. After stirring at this temperature for 1.5 hr., the mixture was allowed to warm up to 0° gradually and then filtered. The filtrate was concentrated under reduced pressure, then under high vacuum with slight warming. The residual oil, which solidified on standing, was recrystallized from ethanol to give white crystals.[12]

Diethyleneglycol Bis-(1-aziridinyl)formate (XI).[11]—When diethylene glycol chloroformate was used in place of ethylene bischloroformate in the procedure described as above, XI was obtained as white crystals.

1,1′-(2,2-Dimethylpropylene)bisaziridinyl Formate (XII).—Neopentyl glycol bis-chloroformate (22 g., 0.1 mole) in 100 ml. benzene was added to a stirred solution of ethylenimine (8.6 g., 0.2 mole) and triethylamine (30 ml.) in 200 ml. of benzene at −5 to −10°. After stirring at room temperature for 3 hr., it was filtered. The filtrate was concentrated *in vacuo*. The residual oil was extracted with warm (30–40°) heptane and the heptane extract was cooled at −70°. The white precipitate which formed was collected on a pre-cooled funnel, washed rapidly with cooled (−50°) petroleum ether and transferred to a beaker. It melted into a heavy oil at room temperature. After drying over P_2O_5 *in vacuo*, XII (20 g.) was obtained.

"Bisphenol-A" Bis-(1-aziridinyl)formate (XIII).—2,2-Di-(*p*-hydroxyphenyl)propane bis-chloroformate (16.35 g., 0.046 mole) in 150 ml. benzene was added to an ice-cooled solution of ethylenimine (4.3 g., 0.1 mole) and triethylamine (30 ml.) in 100 ml. of benzene. The mixture was stirred at room temperature for 2 hr. After removal of triethylamine hydrochloride, the filtrate was concentrated to dryness under vacuum. The residue was recrystallized from benzene–methanol to obtain 10 g. of XIII with m.p. 120.5–121° (lit.[12] m.p. 117°).

Allyl 1-Aziridinyl Formate (XIV).—Allyl chloroformate (24.1 g., 0.2 mole) in 100 ml. of benzene was added slowly to a solution of ethylenimine (4.57 g., 0.106 mole) and triethylamine (30 ml.) in 200 ml. of benzene. After standing overnight at 0–5°, the precipitate was removed by filtration. The filtrate was concentrated *in vacuo* to the smallest volume. The residual oil was distilled to yield colorless oily XIV.

β-(1-Aziridinyl)ethanol (XVI).—Ethylene oxide was bubbled into pre-cooled ethylenimine (43 g., 1 mole) until 1 mole (44 g.) of the oxide was absorbed. The mixture was stirred at 0–5° for 2 days. The temperature was then allowed to rise gradually to 20°. The solution was distilled *in vacuo* and after recovery of unreacted ethylenimine, XVI was collected.

(1-Aziridinyl)methanol (XV) and Bis-(1-aziridinyl)methane (XVII).—Ethylenimine (50 g., 1.16 mole) in 130 ml. of ether was added to a suspension of paraformaldehyde (30 g., 0.333 mole) in 100 ml. of ether at 20–25°. The mixture was stirred overnight at this temperature, then concentrated and distilled under vacuum. The product (XV) (15 g.) was obtained at b.p. 49–50° (5 mm.) with $n^{25}D$ 1.4688. It was unstable, and polymerized very readily on standing at 10°. The structure was identified from its infrared spectrum. On further distillation, XVII (11.7 g.) was obtained at b.p. 77–78° (0.1 mm.).

1,5-Bis-(1-aziridinyl)-1,5-pentanediol (XVIII).—Glutaraldehyde (100 ml. of 25% aqueous solution) was saturated with sodium chloride and then extracted twice with 200 ml. portions of ether. The ethereal extract was dried over anhydrous magnesium sulfate overnight. After removal of the solid, the ether solution was cooled to 0–5° and ethylenimine (29.1 g., 0.67 mole) was added. After stirring at this temperature for 30 min., the precipitate was collected, washed thoroughly with ether, and dried *in vacuo* to give 8.25 g. of pure XVIII.

β-(1-Aziridinyl)ethyl Acetate (XIX).—A mixture of XVI (34.8 g., 0.4 mole), ethyl acetate (100 ml.), and sodium methoxide (0.5 g.) was heated on a water bath at 80–90° and distilled at 150–155 mm. to remove excess ethyl acetate and the ethanol which formed. Then the liquid was distilled at 5 mm. to collect a mixture of XIX and unreacted XVI. This distillate mixture was dissolved in 120 ml. of ether and washed 5 times with 40 ml. portions of saturated salt solution, then water. The ether solution was dried and concentrated. The product (XIX) was obtained by distillation *in vacuo*.

(10) Melting points and boiling points are corrected. Analyses by Dr. S. M. Nagy, Microchemical Laboratory, Belmont, Massachusetts.

(11) The assistance of Dr. C. Segebarth in the preparation of these two compounds is acknowledged.

(12) While this manuscript was in preparation, there came to our attention a paper by Y. Iwakura, M. Sakamoto, and H. Yasuda [*Nippon Kagaku Zasshi*, **82**, 606 (1961); *Chem. Abstr.*, **56**, 8534 (1962)] in which they reported the preparation of this compound, m.p. 57–58°.

β-(1-Azirdiinyl)ethyl Propionate (XX).—When methyl propionate was used in place of ethyl acetate in the procedure as described under XIX, XX was obtained.

β-(1-Aziridinyl)ethyl *n*-Butyrate (XXI).—Methyl *n*-butyrate was used instead of ethyl acetate in the synthesis of XIX; the butyrate was obtained as a colorless oil.

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Synthesis of N,N-Bis(2-chloroethyl)-DL-phenylalanine Hydrochloride[1]

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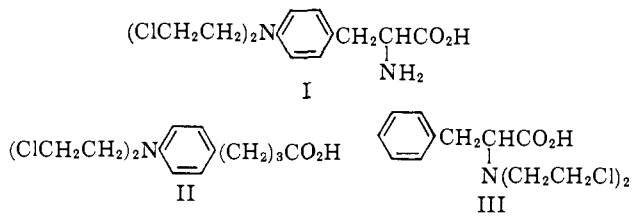
The conversion of methyl DL-phenylalanate (IV) to the α-mustard (III) of DL-phenylalanine is described. Reaction of methyl DL-phenylalanate with ethylene oxide gave 3-benzyl-4-(2-hydroxyethyl)morpholin-2-one. This reacted with ammonia to give 2-[bis(2-hydroxyethyl)amino]-3-phenylpropionamide. Chlorination followed by acid hydrolysis gave the α-mustard (III). Neither the α-mustard nor the morpholine mustard (VII) exhibited significant antitumor activity against Walker 256 Sarcoma, Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210. 2-[Bis(2-chloroethyl)]amino-3-phenylpropionamide was inactive against Walker 256 Sarcoma.

A biological rationale has been proposed[2] to account for the differences in antitumor activity of the clinically interesting nitrogen mustards, *p*-phenylalanine mustard (I)[3] and chlorambucil (II),[4] against a variety of transplanted tumors in mice. Briefly, it was suggested[2] that, in those systems where I was active and II was inactive, the mustard (I) fit an enzyme site normally



occupied by L-phenylalanine primarily by attachment through its amino and its carboxyl groups; on the other hand, in the Walker 256 sarcoma, which is affected by both I and II, only attachment of the alkylating agent through its carboxyl group was required. It was of interest to prepare the related compound III and to compare its antitumor activity with that of I and II as a test of the above hypothesis. However, it is possible that the difference in bulk and basicity of the bis (2-chloroethyl)amino group may obscure these comparisons. The α-mustard III is also of interest for comparison with the α-mustards of glycine and DL-alanine which Izumi[5] prepared and found to be more hydrophilic and less toxic than the simple nitrogen mustards like HN2. The synthesis of III, the first reported α-bis(2-chloroethyl)amino type of mustard of an aromatic amino acid, is the subject of this manuscript.

Reaction of ethylene oxide with methyl DL-phenylalanate (IV) in methanol gave the morpholone (VI) as a liquid that was characterized as the crystalline *p*-nitrobenzoate. Treatment of VI with alcoholic potassium hydroxide afforded the crystalline potassium salt (VIII) that recyclized to the morpholone (VI) on acidification. Ammonolysis of VI in liquid ammonia gave an excellent yield of the crystalline amide IX, which could also be obtained by the reaction of the amide V with ethylene oxide. The action of thionyl chloride on IX afforded the crystalline mustard amide (X) as the hydrochloride. Hydrolysis of X with 6 *N* hydrochloric acid at 85° for 4 hr. gave the hydrochloride of the α-aminomustard (III), which melts over a broad range, in a state of analytical purity. All attempts to recrystallize III failed, perhaps because of its tendency to lose hydrogen chloride. Thus, attempts to dry the hydrochloride of III *in vacuo* at 56° for 45 hours yielded essentially the morpholone (VII) hydrochloride. The free base III could apparently be formed by washing a chloroform solution of the hydrochloride of III with water but it slowly cyclized to the hydrochloride of VII in the chloroform solution. For comparison, the N-hydroxyethyl morpholone (VI) was converted to the morpholone (VII) hydrochloride with thionyl chloride.

A number of unsuccessful routes to III were explored. Thus, the Strecker synthesis utilizing bis(2-chloroethyl)amine, cyanide ion, and the bisulfite addition product of phenylacetaldehyde failed to give the nitrile precursor of III. This method, successful in the glycine and alanine mustard synthesis of Izumi,[5] was unsuccessful when applied to the synthesis of higher homologs here and elsewhere.[6]

Izumi[5] also successfully condensed chloroacetic acid with diethanolamine to yield N,N-bis(2-hydroxyethyl)-glycine, the precursor of glycine mustard. In our hands, the condensation of diethanolamine with the

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