and mixture m.p. with authentic sample, 257°) and α-ethylaminobutyric acid m.p. 248–250° dec. in good yields.

α-[2-(β-Hydroxyethyl)hydrazino]butyric Acid (XVI).—α-Bromobutyric acid (33.4 g., 0.2 mole) was added gradually with cooling to a solution of 60.8 g. (0.8 mole) of β-hydroxyethylhydrazine, b.p. 100–105° (1 mm.) in 100 ml. of water, and the mixture allowed to stand at room temperature for 5 days. The solution was passed through a Duolite C 20 column and eluted as usual. The eluate was concentrated to 200 ml. and passed through an Amberlite IRC 50 column, the effluent evaporated to dryness *in vacuo* and the residue crystallized from 300 ml. of 2-propanol, giving 4.4 g. (13.6%) of XVI, m.p. 133–134° dec.

Reductive Cleavage with Raney Nickel. In Ethanol.—A suspension of 1.9 g. of XVI in 50 ml. of ethanol and 10 g. of Raney nickel catalyst was refluxed for 5 hr. in a reaction flask equipped with an ethanolic HCl trap. The catalyst was filtered, the alcoholic liquors distilled into the ethanolic HCl, and the latter evaporated to dryness. The product (0.8 g.) was crystallized from a mixture of ethanol–ether (1:1), giving ethylaminoethanol hydrochloride, m.p. 225–230°.

Anal. Calcd. for $C_4H_{12}ClNO$: N, 11.24; Cl, 28.51. Found: N, 11.50; Cl, 28.20. The Raney nickel catalyst was combined with the alcohol residue, boiled with 150 ml. of water, and filtered. The green solution was diluted with 300 ml. of water and passed through an Amberlite IRC 50 column, the effluent evaporated to dryness and the residue crystallized from methanol, giving 0.4 g. of N-ethylaminobutyric acid, m.p. 248–252° dec. (sublimation).

Anal. Calcd. for $C_6H_{13}NO_2$: N, 10.68. Found: N, 10.80.

In Water.—XVI (1.6 g.) in 100 ml. of water was hydrogenated in the presence of 5 g. of Raney nickel catalyst at atmospheric pressure. The filtrate was neutralized with 8 ml. of 10% hydrochloric acid, evaporated to dryness, the residue dissolved in 50 ml. of water, filtered, and passed through an Amberlite IRC 50 column. The effluent was passed through a Duolite C 20 column and eluted with 400 ml. of 4% ammonia. The eluate was evaporated to dryness and the residue crystallized from 100 ml. of aqueous ethanol, giving 0.4 g. of α-aminobutyric acid, m.p. 298–300° dec. (sublimation).

Anal. Calcd. for $C_4H_9NO_2$: N, 13.58. Found: N, 13.25.

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Phosphorylated Alkylating Agents Related to DL-Phenylalanine[1]

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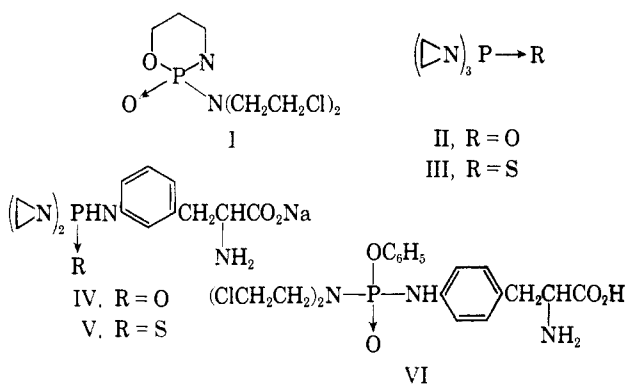
A group of alkylating agents has been prepared which use 3-(*p*-aminophenyl)propionic acid and 3-(*m*- and *p*-aminophenyl)-DL-alanine as the carrier moiety for a number of phosphorylated and thiophosphorylated ethylenimines and bis(2-chloroethyl)amines. Methods were devised for removing the blocking groups used in these syntheses so that the alkylating groups remained intact. Several of the thiophosphorylated ethylenimines were active antitumor agents according to testing results with Walker 256 carcinosarcoma.

A number of phosphorylated alkylating agents, among which are 2-[bis(2-chloroethyl)-amino]-2*H*-1,3,2-oxazaphosphorinane 2-oxide[2] (Cytoxan) (I), tris-(1-aziridinyl)phosphine oxide[3] (TEPA) (II), and tris-(1-aziridinyl)phosphine sulfide[4] (thio-TEPA) (III), have been synthesized and used clinically[5a–c] against cancer. It has been suggested[5d,e] that I and its analogs have latent activity until hydrolyzed by the phosphamidases, present in high concentrations in tumor tissue, to release the cytotoxic nitrogen mustard. A number of modifications of I, II, and III have been synthesized with the phosphorylated alkylating groups attached to various carrier moieties in an attempt to obtain greater selectivity of antitumor action. Because of the importance of nitrogen mustards of the phenylalkanoic acids (*e.g.*, chlorambucil)[6] and of phenylalanine (*e.g.*, sarcolysin),[7] it was considered of interest to use these aromatic moieties as carriers for the phosphorylated alkylating groups. This article describes the preparation and antitumor evaluation of a number of such compounds typified by IV, V, and VI.



Knunyants, *et al.*,[8] have prepared examples of these compounds based on phenylacetic acid and phenylalanine in which certain of the functional groups were left in the blocked state. We were interested in preparing compounds which possessed the free carboxyl and free amino acid functional groups.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

(2) H. Arnold, F. Bourseaux, and N. Brock, *Nature*, **181**, 931 (1958).

(3) H. Bestian, *Ann.*, **566**, 210 (1950).

(4) E. Kuh and D. R. Seeger, U. S. Patent 2,670,347 (1954); *Chem. Abstr.*, **49**, 2481 (1955).

(5) (a) T. B. Haddy, J. A. Whitaker, T. J. Vietti, and H. D. Riley, Jr., *Cancer Chemotherapy Rept.*, **25**, 81 (1962); (b) L. R. Durall, *ibid.*, **8**, 156 (1960); (c) A. L. A. Nasr, *ibid.*, **13**, 185 (1962); (d) O. M. Friedman, E. V. Boger, Grubliauskas, and H. Somer, *J. Med. Chem.*, **6**, 50 (1963); (e) H. Arnold, F. Bourseaux, and M. Brock, *Arzneimittel-Forsch.*, **11**, 143 (1961).

(6) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

(7) L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaia, O. S. Vasina V. I. Trusheikina, and A. M. Novikova, *Lancet*, **269**, 169 (1955).

(8) I. L. Knunyants, O. V. Kil'disherva, N. E. Golubeva, and S. Zurabyan, *Dokl. Akad. Nauk SSSR*, **142**, 370 (1962); *Chem. Abstr.*, **56**, 15604 (1962).

The phosphorylated ethylenimine derivatives of phenylpropionic acid were first examined. The methyl and benzyl esters of *p*-aminophenylpropionic acid (VII and VII) (see Chart I for formulas) were treated successively with phosphoryl chloride and excess ethylenimine to yield the phosphorylated ethylenimine esters, methyl and benzyl 3-{*p*-[bis(aziridinyl)phosphinylamino]phenyl}propionate (IX and X). The experimental modification of replacing a tertiary amine with excess ethylenimine as hydrogen acceptor gave increased yields and purity in our preparation of IX and X, and also in the preparation of the known P,P-bis(1-aziridinyl)-N-(*m*-nitrophenyl)phosphinic amide (XI),[9] which was used as a model.

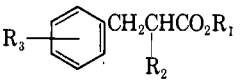
It was anticipated and found that hydrogenolysis of the benzyl group in X simultaneously opened the ethylenimine rings; indeed, the pure acid XII could not be obtained in this manner. The sodium salt (XIII) was prepared in high yield and purity by room temperature hydrolysis of the methyl ester (IX) with one equivalent of base and a small amount of water in alcohol. This salt, unlike the acid, appeared to be stable.

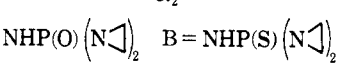
There is evidence that ethylenimines substituted on the nitrogen atom with a phosphorus link are quite sensitive to ring cleavage by acids, as are the unsubstituted or the N-sulfonyl substituted ethylenimines.[10] Gaseous hydrogen chloride bubbled through a solution of the phosphorylated ethylenimines at room temperature gave the corresponding ring-opened products, N,N′-bis(2-chloroethyl)-N″-(*m*-nitrophenyl)phosphoramide (XIV) and benzyl 3-{*p*-[bis(2-chloroethylamino)phosphinylamino]phenyl}propionate (XV). The latter was not isolated, but was immediately hydrogenolyzed to acid XVI. Grechkin[11] obtained similar ring cleavage products upon reaction of equivalent amounts of dialkyl phosphorochloridates and ethylenimine with no added acid acceptor. The cleavage of the N-phosphorylated ethylenimines by acid may explain why some attempts on our part to prepare and react P,P-bis(1-aziridinyl)phosphinic chloride *in situ* with aromatic amines were not successful; some success has been attained with aliphatic amines by Friedman, *et al.*[12]

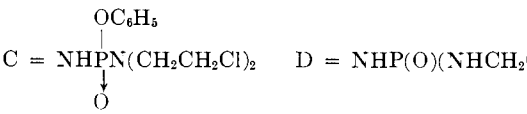
The methods found suitable for the phenylpropionic acid derivatives were used to prepare the phosphorylated ethylenimine derivatives of DL-phenylalanine (XX and XXVIII). The respective starting materials were methyl 3-(*m*-aminophenyl)-N-phthaloyl-DL-alanate (XIX), available from previous work,[13] and benzyl 3-(*p*-aminophenyl)-N-benzyloxycarbonyl-DL-alanate (XXVII). The latter was prepared particularly for the synthesis of the phosphorylated bis(2-chloroethyl)-amino mustards that are discussed below.

Attempts to remove completely the N-phthaloyl and methyl ester groups from XX with 3 moles of base

CHART I

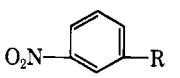






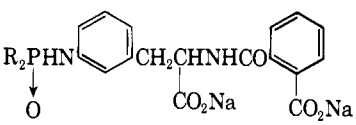
Phth = phthaloyl Z = $CO_2CH_2C_6H_5$

Compound	R_1	R_2	R_3
VII	CH_3	H	*p*-NH_2
VIII	$CH_2C_6H_5$	H	*p*-NH_2
IX	CH_3	H	*p*-A
X	$CH_2C_6H_5$	H	*p*-A
XII	H	H	*p*-A
XIII	Na	H	*p*-A
XV	$CH_2C_6H_5$	H	*p*-D
XVI	H	H	*p*-D
XVII	$CH_2C_6H_5$	H	*p*-B
XVIII	Na	H	*p*-B
XIX	CH_3	NPhth	*m*-NH_2
XX	CH_3	NPhth	*m*-A
IV	Na	NH_2	*m*-A
XXIII	CH_3	NPhth	*m*-B
V	Na	NH_2	*m*-B
XXIV	$CH_2C_6H_5$	NH_2	*p*-NO_2
XXV	$CH_2C_6H_5$	NHZ	*p*-NO_2
XXVI	H	NHZ	*p*-NO_2
XXVII	$CH_2C_6H_5$	NHZ	*p*-NH_2
XXVIII	$CH_2C_6H_5$	NHZ	*p*-A
XXIX	Me	H	*p*-C
XXX	$CH_2C_6H_5$	H	*p*-C
XXXI	H	H	*p*-C
XXXII	$CH_2C_6H_5$	NHZ	*p*-C
VI	H	NH_2	*p*-C



XI, R = A; XIV, R = D; XXXIII, R = C

in alcohol containing some water were unsuccessful; the product obtained was the sodium phthalamate (XXI). Under somewhat more drastic conditions, the ethylenimine rings were opened to give XXII. How-



XXI, R = N◁; XXII, R = $NHCH_2CH_2OH$

ever, the use of 1 mole of hydrazine[14] followed by treatment with 1 mole of base, all at room temperature, gave the sodium phenylalanate (IV).

By replacing the phosphoryl chloride with thiophosphoryl chloride, benzyl 3-(*p*-aminophenyl)propionate (VIII) was converted to the thiophosphoramidic dichloride, which reacted with ethylenimine to give XVII. When the methyl ester (VII) was used, no crystalline product could be isolated. The benzyl ester (XVII) could be hydrolyzed, under the same conditions used for the oxygen analog, to the sodium salt (XVIII). Attempts to acidify XVIII carefully to

(9) A. A. Kropacheva and V. A. Parshina, *Zh. Obshch. Khim.*, **29**, 566 (1959); *Chem. Abstr.*, **54**, 472 (1960).

(10) (a) J. S. Fruton, "Heterocyclic Compounds," Vol. I, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1950; (b) however, Y. Tsuchiki, H. Wada, and Y. Suzuki [Japan Patent 918 (1960); *Chem. Abstr.*, **54**, 19539 (1960)] claimed the preparation of 2-(1-aziridinylmethyl)-3-carboxycyclohexanone by a Mannich reaction.

(11) N. P. Grechkin, *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk*, 538 (1956).

(12) O. M. Friedman, R. S. Levi, Z. B. Papanastassiou, and W. M. Whaley, *J. Med. Chem.*, **6**, 449 (1963).

(13) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(14) F. M. Callahan, G. W. Anderson, R. Paul, and J. E. Zimmerman, *ibid.*, **85**, 201 (1963); E. Taschner, C. Wasielewski, and J. F. Biernat, *Ann. Chem.*, **646**, 119 (1961).

TABLE I

PHOSPHORYLATED ALKYLATING AGENTS

Compd.	Antitumor data[a] WA	SA	CA	LE	M.p., °C.[b]	Cl yield[b]	R_f[c]	Formula	% calcd. over % found C	H	Cl	N	P	S
IV	−				>300	83		$C_{13}H_{28}N_4O_4PNa \cdot 0.5H_2O$	45.7	5.67		16.5	9.07	
									45.5	5.63		16.1	8.73	
V	+				>300 dec.	100		$C_{15}H_{18}N_4O_2PSNa \cdot 0.5H_2O$	43.7	5.35		15.7		8.97
									43.6	5.18		15.1		9.12
VI		−	−	−	153–155[d] (165–175)[d]	(59)[d]	0.85(C)[e] 0.63(B)[e]	$C_{18}H_{22}(Cl_2N_2O_4P \cdot H_2O$	47.7	5.19	14.8	8.79		
									47.7	5.71	14.8	8.79		
IX		−	−	−	94–95[d]	70		$C_{14}H_{20}N_3O_4P$	54.4	6.52		13.6	10.0	
									54.7	6.76		13.6	10.3	
X	−				112.5–113.0[f] (109–110)	(96)	0.83(A) 0.85(B)	$C_{20}H_{24}N_3O_4P$	62.3	6.27		10.9	8.04	
									62.2	6.57		10.7	8.04	
XIII	−				>300	96	0.75(D)	$C_{13}H_{17}N_3Cl_3Na \cdot 0.5H_2O$	47.9	5.56		12.9	9.46	
									47.6	5.40		12.4	9.24	
XIV	−	−	−	−	89.5–90.0[g] (80.5–90.0)	(42)		$C_{16}H_{18}Cl_2N_4O_4P$	35.2	4.13	20.7	16.4	9.33	
									35.7	4.38	21.0	16.2	9.33	
XVI	−				161.5–162[f] (155–157)	81(92)	0.87(B)	$C_{13}H_{20}Cl_2N_3O_4P$	42.1	5.17	19.2		8.42	
									42.1	5.39	19.1		8.10	
XVII	+				84–86[h] (83–85)	(35)	0.90(B)[e] 0.93(E)[e]	$C_{26}H_{24}N_3O_4PS$	60.0	6.03		10.5	7.73	
									60.0	5.89		10.3	7.39	
XVIII	+				270 dec.	96		$C_{13}H_{17}N_3O_2PSNa \cdot H_2O$	44.5	5.15		11.9		9.12
									44.6	5.67		11.1		9.37
XX		−	−	−	92–94[g]	44	0.83(A)	$C_{22}H_{23}N_3O_5P \cdot C_6H_6$	63.1	5.18		10.5	5.82	
									62.7	5.30		10.4	5.92	
XXI	−	−	−	−	275–295 dec.	91		$C_{21}H_{25}N_4O_4P \cdot H_2O$	58.1	4.46		10.8	5.65	
									58.2	4.89		10.5	5.91	
XXIII	−				141.5–142.5[f] (144–145)[i]	(33)[i]		$C_{22}H_{23}N_3O_4PS$	56.2	4.93		11.3		6.81
									56.4	4.92		11.6		6.75
XXVIII					159.5–160.5[j] (164–165)[j]	7(25)[j]	0.86(B)[e]	$C_{28}H_{31}N_4O_5P$	63.0	5.85		10.5	5.80	
									62.9	5.77		10.4	5.74	
XXIX	−	−	−	−	62–64[k]	47	0.66(A)	$C_{20}H_{25}Cl_2N_2O_4P \cdot H_2O$	50.7	5.72	14.9		6.49	
									50.3	5.70	14.6		6.58	
XXX	−				104–105[h] (99–101)	60(69)	0.94(B)[e]	$C_{26}H_{29}Cl_2N_2O_4P$	58.1	5.46	13.2		5.79	
									58.6	5.58	13.1		5.40	
XXXI	−				114–115[l]	70	0.10(B)[e]	$C_{19}H_{23}Cl_2N_2O_4P$	51.2	5.20	15.9		6.95	
									51.1	5.08	15.8		6.71	
XXXII	−	−	−	−	131–132[h]	68	0.89(B)[e]	$C_{30}H_{36}Cl_2N_3O_6P$	59.7	5.27	10.4		1.53	
									59.6	5.31	10.1		1.79	
XXXIII	−	−	−	−	178.0–178.5[g] (172–174)	44 (72)	0.77(B)	$C_{16}H_{18}Cl_2N_2O_4P$	45.9	4.33	16.9		7.40	
									46.1	4.56	16.7		7.40	
XXXIV					172–173 (0.5 mm.)[m] [168–183 (0.5 mm.)][m]	(80)		$C_6H_{13}Cl_2NO_2P$	40.0	4.37	35.5		10.3	
									39.9	4.25	35.5		10.4	
XXXV	−	−	−	−	174–185 (0.5 mm.)	55		$C_{32}H_{38}Cl_3N_4PS$	57.2	4.14	33.6		9.78	
									57.8	4.51	33.3		9.85	

[a] Antitumor screening was performed under the auspices of the Cancer Chemotherapy National Service Center according to its protocol outlined in *Cancer Chemotherapy Repts.*, **25**, 1 (1962). From this source are taken the code designations for the tumor systems: WA for Walker 256 (subcutaneous); SA, Sarcoma 180; CA, Adenocarcinoma 755; LE, Lymphoid Leukemia L1210. A (+) indicates the compound is a confirmed active (CA, SA, LE), or has therapeutic index (T.I.) ≥ 4 (WA), according to the above protocol. [b] Melting points and yields are for analytical samples. Corresponding values in parenthesis are for crude product. [c] Recrystallized from water–ethanol. [d] *Anal.* Found: Cl, 14.5; N, 8.69. [e] Recrystallized from methylene chloride–Skellysolve B. [f] Recrystallized from absolute ethanol. [g] Recrystallized from benzene. [h] Recrystallized from Skellysolve B–ether. [i] *Anal.* Found: N, 11.4. [j] Recrystallized from benzene–ether; product of m.p. 164–165° was analyzed: Found: N, 10.1. [k] Recrystallized from ether and air-dried. Drying *in vacuo*, either at room temperature or above, gave an oil. [l] Recrystallized from water–ethanol. [m] Boiling point. [n] The R_f values are given for the appropriate solvent systems listed in ref. 20. [o] Detected by Clorox® spray.

form the free acid immediately gave a white polymer, insoluble in all organic solvents. In a similar way, treatment of methyl 3-(*m*-aminophenyl)-N-phthaloyl-DL-alanate with thiophosphoryl chloride and ethylenimine gave XXIII. This was separable by crystallization from the large amount of polymer formed in the reaction.[15] Because the yields of XXIII were poorer than those obtained for any of the other phosphorylated or thiophosphorylated ethylenimines, the reaction conditions for preparing XXIII were carefully examined. The thiophosphorylation step was found to be complete after 40 min. at reflux temperature and required a nitrogen atmosphere. The reaction with ethylenimine proceeded best at about 20°; higher and lower temperatures were less satisfactory. Similar to the preparation of IV, room temperature reaction of XXIII with hydrazine, then with base, gave the sodium salt V.

(15) R. Buchner, G. G. Kertesz, and A. F. Jackson [*J. Org. Chem.*, **27**, 1051 (1962)] also noted much polymerization in similar reactions. They could minimize polymerization in some instances by proper choice of solvents.

In preparing phosphorylated nitrogen mustards with DL-phenylalanine as a carrier, the required intermediate was benzyl 3-(*p*-aminophenyl)-N-(benzyloxycarbonyl)-DL-alanate (XXVII). It contained the free aromatic amino and blocking groups that are removable after the alkylating group is attached.[16] In the synthesis of the blocked amine (XXVII), the key step was the successful chemical reduction of the blocked nitro precursor (XXV).

Racemic *p*-nitrophenylalanine[17] was esterified with benzyl alcohol[18] to give benzyl ester XXIV. This was converted conventionally to benzyl N-benzyloxycarbonyl-3-(*p*-nitrophenyl)-DL-alanate (XXV). When the benzyloxycarbonyl acid (XXVI) was prepared first and then esterified, the over-all yields of XXV were poorer. Refluxing nitro ester XXV with zinc dust and am-

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

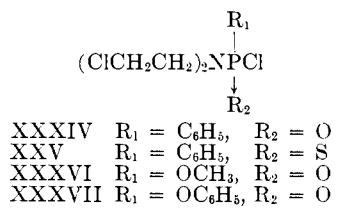
(17) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2109 (1954).

(18) J. E. Skiellis, W. H. McGregor, and F. H. Carpenter, *J. Org. Chem.*, **26**, 1491 (1961).

monium chloride in aqueous methanol gave the amino ester (XXVII) in good yield. The selective reduction of the nitro group in XXV makes an interesting comparison with 2-amino-4-benzyloxyamino-5-nitro-6-methylpyrimidine,[19] where the nitro group could not be reduced without concomitant loss of the benzyloxy group.

Both the methyl and the benzyl esters (VII and VIII), and the benzyl alanate (XXVII) reacted with phenyl N,N-bis(2-chloroethyl)phosphoramidic chloride[16] (XXXVII) to give blocked mustards XXIX, XXX, and XXXII, respectively.

Preparation of other phosphorylated mustards attached to phenylalanine was considered. Two intermediates, N,N-bis(2-chloroethyl)phenylphosphonamidic chloride (XXXIV) and N,N-bis(2-chloroethyl)-phenylthiophosphonamidic chloride (XXXV) were prepared. However, both of these gave unattractive oils when condensed with esters of 3-(*p*-aminophenyl)-propionic acid. Oils were also obtained in similar experiments with methyl N,N-bis(2-chloroethyl)phosphoramidic chloride (XXXVI), which was prepared and used *in situ*.



XXXIV	R_1 = C_6H_5,	R_2 = O
XXV	R_1 = C_6H_5,	R_2 = S
XXXVI	R_1 = OCH_3,	R_2 = O
XXXVII	R_1 = OC_6H_5,	R_2 = O

Antitumor screening of the phosphorylated alkylating agents were performed under the auspices of the C.C.N.S.C. The results are summarized in Table I. The phosphorothioamides V, XVII, and XVIII were active against Walker 256, but the corresponding oxygen analogs were not. Of these, V was the most active and had a therapeutic index of over 20.

Experimental[20]

Benzyl 3-(*p*-Aminophenyl)propionate (VIII) Hydrochloride.—By the method of Shields, *et al.*,[18] the reaction of 33.0 g. (0.20 mole) of 3-(*p*-aminophenyl)propionic acid, 38.0 g. (0.21 mole) of *p*-toluenesulfonic acid monohydrate, and 150 ml. of benzyl alcohol in 500 ml. of carbon tetrachloride for 18 hr. gave the toluenesulfonate salt of the ester which was converted in 86% yield to the benzyl ester hydrochloride, m.p. 130–132°, of purity suitable for use in the next step.

An analytical sample of the benzyl ester hydrochloride, obtained from an earlier run, had m.p. 132–134°; λ_{max}^{Nujol} 3.85, 5.10 (NH_3^+), and 5.71 (C=O) μ; R_f 0.17 in solvent A, R_f 0.85 in B.

Anal. Calcd. for $C_{16}H_{17}NO_2 \cdot HCl$: C, 6.59; H, 6.23; N, 4.81. Found: C, 6.61; H, 6.52; N, 4.90.

Benzyl 3-{*p*-[Bis(1-aziridinyl)phosphinylamino]phenyl}propionate (X).—A mixture of 9.60 g. (32.9 mmoles) of benzyl 2-(*p*-aminophenyl)propionate (VIII) hydrochloride and 50 ml. of phosphoryl chloride was heated at reflux for 30 min. The resultant solution was evaporated to dryness at 40° (1 mm.). The residue was taken up in 150 ml. of methylene chloride and added dropwise over 60 min. to a cooled (−5 to 0°), stirred solution of 50 ml. (0.96 mole) of ethylenimine in 200 ml. of methylene chloride. After the addition was completed, the bath was removed, and the reaction mixture was allowed to stir an additional 60 min. It was washed successively with three 200-ml. portions of water, 200 ml. of 0.01 *N* hydrochloric acid, 100 ml. of saturated aqueous sodium bicarbonate, and 100 ml. of water, and finally dried. Evaporation of the solvent *in vacuo* left 12.2 g. (96%) of white, crystalline X, m.p. 109–110°.

Recrystallization from absolute ethanol gave an analytical sample of X, m.p. 112.5–113.0°; λ_{max}^{Nujol} 3.19 (N–H), 5.81 (C=O), 7.95 (P→O), 10.32, and 10.72 (aziridinyl) μ; for paper chromatography and analytical results see Table I. **CAUTION:** Vigorous acid-catalyzed polymerization of the ethylenimine is possible and the temperature must be carefully watched. For larger scale runs, it is recommended that a tertiary amine such as triethylamine be added as an acid acceptor.

Compounds IX, XI,[9] XX, and XXVIII were prepared similarly. For the preparation of XVII and XXIII, thiophosphoryl chloride was used instead of phosphoryl chloride.

Sodium 3-{*p*-[Bis(1-aziridinyl)phosphinylamino]phenyl}propionate (XIII).—A solution of 0.93 g. (3.0 mmoles) of the methyl ester (IX), 3.0 ml. of *N* sodium methoxide in methanol, 0.10 ml. (5.6 mmoles) of water, and 10 ml. of methanol was kept at room temperature for 3 days and then evaporated to dryness *in vacuo* below 35° (0.1 mm.); with the amino acid derivatives, this temperature is critical. The residue was triturated with hot methylene chloride (in some cases, acetone) and dried *in vacuo* at 56° for 3 hr. to afford 0.91 g. (96%) of XIII (·0.5H_2O) as a white solid, which did not melt below 300°; λ_{max}^{Nujol} 3.01, 3.18 (N–H, H_2O); 6.30, 7.12 (CO_2^-); 7.95, 8.32 (P=O); 12.05 μ. The paper chromatography and analytical results are given in Table I.

An aqueous solution of XIII was treated with a slight excess of acetic acid and the solution was extracted with ethyl acetate to give an oil which had the infrared spectrum expected of acid XII. This oil did not crystallize; after a few days, it was no longer soluble in ethyl acetate, suggesting that polymerization had taken place.

In a similar way, XVIII was prepared from XVII, and an attempt was made to convert the salt to the acid. In this case, acidification immediately gave a white precipitate which was insoluble in water, ethyl acetate, alcohol, and other solvents. From the ethyl acetate, a small amount of solid with a broad melting range was isolated. In a few days, this solid was no longer soluble in ethyl acetate or any other solvent.

N,N′-Bis(2-chloroethyl)-N″-(*m*-nitrophenyl)phosphoramide (XIV).—A solution of 2.00 g. (7.52 mmoles) of bisaziridine XI in 25 ml. of methylene chloride was saturated with anhydrous hydrogen chloride, diluted with 25 ml. of benzene, evaporated *in vacuo* to *ca.* 20 ml., and chilled. The yellow crystals were collected and dried to afford 1.10 g. (42%) of XIV, m.p. 89.5–90.0°. A portion was recrystallized from benzene to afford XIV, m.p. 89.5–90.0°; λ_{max}^{Nujol} 2.95, 3.10, 3.19 (NH); 8.40, 8.52, and 8.67 (P→O) μ; see Table I for analytical data.

3-{*p*-[Bis(2-chloroethylamino)phosphinylamino]phenyl}propionic Acid XVI.—A solution of 2.60 g. of the bisaziridinyl benzyl ester (X) was treated with hydrogen chloride, by the procedure used to prepare XIV to obtain 3.20 g. (102%) of a yellow oil which could not be crystallized. This oil was dissolved in 50 ml. of ethanol and hydrogenated over 1.0 g. of 5% palladium-on-charcoal at 3.3 kg./cm.^{2} for 4 hr., then worked up in the usual way to give 2.30 g. (92%) of acid XVI, m.p. 155–157°. One recrystallization from ethanol–water (1:1) gave an analytical sample as white needles, m.p. 161.5–162°; λ_{max}^{Nujol} 2.98, 3.05 (NH), 3.7–4.2 and 10.4 (COOH), 5.86 (C=O), 7.70, and 7.90 (P=O and CO_2H) μ; for analytical data see Table I.

Sodium 3-{*m*-[Bis(1-aziridinyl)phosphinylamino]phenyl}-DL-alanate (IV).—A solution of 4.54 g. (10 mmoles) of methyl 3-{*m*-[bis(1-aziridinyl)phosphinylamino]phenyl}-N-phthaloyl-DL-alanate (XX), 0.51 g. (10 mmoles) of hydrazine hydrate, and 20 ml. of absolute methanol was stirred at room temperature for 4 days, then evaporated to dryness *in vacuo*. The residue was extracted with 50 ml. of methylene chloride and filtered to remove 1.50 g. (93%) of the hydrazide. The filtrate was evaporated to dryness to leave 2.87 g. (89%) of the methyl alanate as a pale yellow oil. This was dissolved in 25 ml. of methanol, treated with 9.0 ml. (9.0 mmoles) of methanolic *N* sodium methoxide and 0.2 ml. of water, allowed to stand for 20 hr. at room temperature,

(19) E. C. Taylor and J. W. Barton, *J. Org. Chem.* **24**, 127 (1959).

(20) Meeting points, corrected, were determined with the Fisher-Johns apparatus. Anhydrous magnesium sulfate was used as the drying agent. The solvent Skellysolve B is essentially hexane, b.p. 60–68°. Celite is a diatomaceous earth product of Johns-Manville. Paper chromatograms were run by the descending technique on Whatman No. 1 paper and the spots were detected visually under ultraviolet light unless otherwise indicated. The solvent systems used were: A, benzene–methanol–water (2:6:1) on acetylated paper (Schleicher and Schuell No. 2496); B, 1-butanol–water (saturated); C, solvent A with Whatman No. 1 paper; D, 5% disodium hydrogen phosphate, pH 8.9; E, 1-butanol–acetic acid–water (5:2:3); F, 2-propanol–2 *N* hydrochloric acid (65:35).

and evaporated to dryness *in vacuo*. The white residue was triturated with 100 ml. of boiling acetone and dried *in vacuo* to leave 2.52 g. (83%) of analytically pure IV. See Table I for analytical details.

In a similar way, V was prepared from XXIII.

Disodium N-{*m*-([Bis(1-aziridinyl)phosphinyl]amino)-α-carboxyphenethyl}phthalamate (XXI).—A solution of 9.1 g. (20 mmoles) of the phthalimido ester (XX), 45.0 ml. of *N* sodium methoxide in methanol (45 mmoles), 1 ml. of water, and 200 ml. of methanol was stirred at room temperature for 4 days and evaporated to dryness *in vacuo*. The residue was triturated successively with 100 ml. of 95% ethanol and 200 ml. of boiling ethanol and dried *in vacuo* at 100° (0.5 mm.) to afford 9.50 g. (91%) of XXI, m.p. 275–295° dec. (without melting); λ_{max}^{Nujol} 2.98, 3.10 (H_2O and NH), 6.3 (broad) (CO_2^- and amide), 7.10 (CO_2^-), and 10.65 (aziridine) μ; see Table I for analysis.

The aziridinyl rings in the salt XXI were checked qualitatively for stability. After storage for over 6 months, the rings can still be cleaved with anhydrous hydrogen chloride to give a product which, without further purification, analyzed for over 90% of the chlorine content expected of the ring cleavage product (the acid form of XXI where R = $NHCH_2CH_2Cl$).

Disodium N-{*m*-([Bis(2-hydroxyethylamino)phosphinyl]amino)-α-carboxyphenethyl}phthalamate (XXII).—A solution of 6.00 g. (13.2 mmoles) of the phthalimido ester XX and the same proportions of the other reactants used to prepare XXI were heated at reflux for 2 hr. The solution was cooled in ice, diluted with 2 volumes of methylene chloride and filtered. The filtrate was evaporated *in vacuo*, pulverized, triturated with 200 ml. of boiling 95% ethanol, and dried as above to afford 5.60 g. (79%) of XXII, m.p. above 300°; λ_{max}^{Nujol} 9.0 and 9.3 (OH) μ which was different from the spectrum of XXI.

Anal. Calcd. for $C_{21}H_{25}N_4Na_2O_8P \cdot H_2O$: C, 45.4; H, 4.93; N, 10.2; P, 5.60. Found: C, 45.4; H, 5.08; N, 10.2; P, 5.78.

Benzyl 3-(*p*-Nitrophenyl)-DL-alanine (XXIV) *p*-Toluenesulfonate.—By the method of Shields, *et al.*,[18] the reaction of 3-(*p*-nitrophenyl)-DL-alanine with benzyl alcohol gave the product XXIV as the toluenesulfonate salt (70% yield), which affords chromatographically homogeneous free base XXIV and is suitable for the next reaction. Recrystallization of the salt from absolute ethanol gave an analytical sample, m.p. 185.5–186.5°; λ_{max}^{Nujol} 3.6–4.2, 5.00 ($-NH^+$), 5.70 (C=O), 6.60, 7.42 (NO_2), 8.30, and 9.40 (RSO_3^-) μ.

Anal. Calcd. for $C_{16}H_{15}N_2O_4 \cdot HO_3SC_6H_4CH_3$: C, 58.4; H, 5.12; N, 5.93. Found: C, 58.3; H, 5.17; N, 5.87.

Treatment of the toluenesulfonate salt with sodium hydroxide gave free base XXIV, homogenous on paper, R_f 0.14 in A, 0.76 in B, and 0.94 in F.

3-(*p*-Nitrophenyl)-N-benzyloxycarbonyl-DL-alanine (XXVI).—The reaction of 2.0 g. (9.6 mmoles) of 3-(*p*-nitrophenyl)-DL-alanine with 1.71 g. (10.0 mmoles) of benzyloxycarbonyl chloride by the usual method gave, after crystallization from ether–Skellysolve B, 0.45 g. (14%) of white, crystalline product, m.p. 158.5–159.5°; λ_{max}^{Nujol} 3.04 (−NH), 3.65–4.20 (COOH), 5.90 (C=O), 6.55–6.60 (urethane, NO_2), and 7.41 (NO_2) μ; R_f 0.51 in B, 0.55 in D.

Anal. Calcd. for $C_{17}H_{16}N_2O_6$: C, 59.3; H, 4.68; N, 8.13. Found: C, 59.3; H, 4.80; N, 8.22.

Benzyl 3-(*p*-Nitrophenyl)-N-benzyloxycarbonyl-DL-alanate (XXV).—A solution of 30.1 g. (.177 mole) of benzyloxycarbonyl chloride in 80 ml. of ether was added dropwise over 30 min. to a cold (10–15°) stirred solution of 30.0 g. (0.100 mole) of the benzyl alanate XXIV and 38 ml. (0.47 mole) of pyridine in 1.1 l. of ether. The reaction mixture was stirred at room temperature for an additional 30 min., then extracted successively with 800-ml. portions of water, 5% hydrochloric acid, saturated aqueous bicarbonate, and water. The ether layer was dried and evaporated *in vacuo* to leave a yellow oil which crystallized. This was triturated with 50 ml. of 1:1 ether–petroleum ether, b.p. 30–60°, to leave 36.0 g. (83%) of yellow crystals, m.p. 78–86°. Trituration in refluxing ether left 33.0 g. (76%) of XXV, m.p. 96–96.5°; λ_{max}^{Nujol} 3.00 (NH), 5.76 (C=O), 6.47 (NO_2, $CONH_2$), 7.37 (NO_2), 8.21, and 8.29 (ester) μ; R_f 0.05 in A, and 0.91 in B.

Anal. Calcd. for $C_{24}H_{22}N_2O_6$: C, 66.5; H, 5.11; N, 6.46. Found: C, 66.4; H, 5.16; N, 6.37.

Benzyl 3-(*p*-Aminophenyl)-N-benzyloxycarbonyl-DL-alanine (XXVII).—A mixture of 9.30 g. (21.4 mmoles) of the *p*-nitro ester XXV, 2.40 g. of ammonium chloride, 18.5 ml. of water, 190 ml. of methanol, and 14.0 g. (0.214 mole) of zinc dust, added in this order, was stirred and heated at reflux on a steam bath for 70 min. The mixture was cooled in ice, filtered over Celite, and evaporated to dryness *in vacuo*. The solid residue was triturated with 150 ml. of ether and the ether solution was set aside as solution A. The ether-insoluble residue was triturated with 200 ml. of water and dried *in vacuo* to afford 6.66 g. (81%) of amine XXVII, m.p. 129–131°. Recrystallization from absolute ethanol afforded 5.60 g. (69%) of white crystals of XXVII, m.p. 132–133°; λ_{max}^{Nujol} 2.90, 3.02, 3.12 (N–H), 5.78 (C=O, ester), 5.85 (C=O, carbamate), 6.15, 6.22 (aryl, NH_2), 6.50 (amide II), and 8.21 (ester) μ.

Anal. Calcd. for $C_{24}H_{24}N_2O_4$: C, 71.3; H, 5.97; N, 6.93. Found: C, 71.4; H, 5.79; N, 6.84.

The ether solution A was saturated with anhydrous hydrogen chloride to precipitate 1.01 g. (10.8%) of the crystalline hydrochloride of XXVII. A similar material from an earlier run was crystallized from methylene chloride to give XXVII·HCl, m.p. 162–163°; λ_{max}^{Nujol} 3.09 (N–H), 3.90, 5.10 (N^+–H), 5.78, 5.92 (C=O), and 6.50 (amide II) μ; free of absorption at 7.37 (NO_2) μ. It had R_f 0.89 in solvent B.

Anal. Calcd. for $C_{24}H_{24}N_2O_4 \cdot HCl$: C, 65.4; H, 5.72; N, 6.36. Found: C, 65.6; H, 5.89; N, 6.50.

Benzyl 3-{*p*-[([Bis(2-chloroethyl)amino]phenoxyphosphinyl)amino]phenyl}-N-benzyloxycarbonyl-DL-alanate (XXXII).—A mixture of 2.02 g. (5 mmoles) of the blocked *p*-aminophenylalanine (XXVII), 0.79 g. (2.5 mmoles) of phenyl N,N-bis(2-chloroethyl)phosphoramidic chloride (XXXVII), and 10 ml. of chloroform was maintained under a nitrogen atmosphere and heated at 127–130° (bath temperature) until all the chloroform had volatilized and for 60 min. more at this temperature. The gum was dissolved in 25 ml. of methylene chloride, saturated with anhydrous hydrogen chloride, diluted with 3 volumes of ether, and filtered over a Celite pad. The filtrate was evaporated to dryness *in vacuo* and dissolved in 25 ml. of methylene chloride. The solution was washed successively with 50 ml. each of 5% hydrochloride acid and water, dried, and evaporated to dryness *in vacuo* to afford 1.87 g. of gum which was crystallized from 100 ml. of ether to give 1.16 g. (68%) of white crystalline XXXII, m.p. 131–132°; λ_{max}^{Nujol} 3.02, 3.19 (N–H), 5.71, 5.76, 5.85 (C=O of ester, urethane), 6.45 (amide), 7.95 (P→O); 8.15, 8.25, and 8.5 (ester and P=O) μ. See Table I for other data. The use of a tertiary amine as acid acceptor, the use of solvent, longer reaction time, and higher or lower temperatures were less satisfactory.

Compounds XXIX, XXX, and XXXIII were similarly prepared.

3-{*p*-[([Bis(2-chloroethyl)amino]phenoxyphosphinyl)amino]phenyl}-DL-alanine (VI).—A mixture of 0.68 g. (1.00 mmole) of the blocked mustard XXXII, 0.97 g. of 5% palladium-on-charcoal, 1.0 ml. of water, 1.0 ml. of acetic acid, and 25 ml. of 2-methoxyethanol was hydrogenated at 25° and atmospheric pressure overnight (*ca.* 20 hr.). The catalyst was separated and washed with 25 ml. of 2-methoxyethanol. The combined 2-methoxyethanol solutions were evaporated *in vacuo*, two 50-ml. portions of toluene were added, and the evaporation was repeated, finishing at 60° (0.5 mm.). The residue was triturated with 50 ml. of ether, then 25 ml. of water, and dried to leave 0.27 g. (59%) of white, powdery VI, m.p. 165–175°. Recrystallization from water–ethanol gave VI, m.p. 153–155°; λ_{max}^{Nujol} 3.75–4.5, 6.10 (NH_3^+), 6.30 (CO_2^-), and 8.10 (P→O) μ; for analytical details, see Table I.

Compound XXXI was similarly prepared from XXX.

N,N-Bis(2-chloroethyl)phenylphosphonamidic Chloride (XXXIV).—To a mixture of 3.90 g. (20 mmoles) of phenylphosphonic dichloride and 3.57 g. (20 mmoles) of bis(2-chloroethyl)-amine hydrochloride in 50 ml. of benzene at reflux temperature was added, over a 30-min. period, a solution of 4.04 g. (40 mmoles) of anhydrous triethylamine in 25 ml. of benzene. Heating was continued for an additional 5 hr. The hot solution was diluted to turbidity with petroleum ether, b.p. 30–60°, cooled, and filtered. The filtrate was evaporated to dryness *in vacuo* to leave an oil which was distilled through a 5-cm. Vigreux column to give 4.79 g. (80%) of XXXIV, b.p. 168–183° (0.5 mm.); λ_{max}^{liquid} 6.30, 6.75 (C_6H_5), 8.10 (P=O), and 8.95 (P–C_6H_5) μ. A center cut, b.p. 172–173° (0.5 mm.), was analyzed (see Table I).

The same procedure was used to prepare XXXV from phenylphosphonothioic dichloride.

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