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RESEARCH ARTICLES

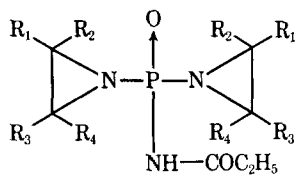
Mechanisms of Reactions of Ring-Substituted Bis(1-aziridinyl)phosphinyl Urethan Antineoplastic Agents

C. K. NAVADA, Z. F. CHMIELEWICZ, and T. J. BARDOS*

Abstract □ Bis(*trans*-2,3-dimethylaziridinyl)phosphinyl urethan (IV) was synthesized and compared with the corresponding *cis*-2,3-dimethyl derivative (III). The comparative alkylating activities and rates of hydrolysis of these two stereoisomeric aziridine derivatives, III and IV, were determined and compared with corresponding data for the monomethyl derivative (V) and two other clinically tested members of this series of antineoplastic agents (dual antagonists), AB-100 (I) and AB-132 (II). The structures of the final hydrolysis products of III, IV, and V were determined and confirmed by direct synthesis. The results indicate that the mechanisms of hydrolysis of III, IV, and V (as that of the unsubstituted aziridine derivative, AB-100) are essentially S_N2, in contrast to the much faster hydrolysis of the 2,2-dimethylaziridine analog, AB-132, which involves a carbonium-ion mechanism. These studies give further support to the hypothesis that the unique pharmacologic properties of AB-132, as compared to other members of this series, may be related to the unique chemical properties of the 2,2-dimethylaziridine moieties.

Keyphrases □ Antineoplastic agents—reaction mechanisms □ Bis(*trans*-2,3-dimethylaziridinyl)phosphinyl urethan—synthesis □ Alkylating activity—*cis*-, *trans*-2,3-dimethylaziridine analogs □ Hydrolysis mechanism—ring-substituted aziridine derivatives □ IR spectrophotometry—identity □ NMR spectroscopy—identity

The synthesis of a series of bis(1-aziridinyl)phosphinyl carbamates, termed "dual antagonists" (I and its analogs containing different carbamate moieties) (1, 2), and their antineoplastic activities in experimental animals (3, 4) and in man (5–9) were previously reported. In an effort to decrease the hematologic toxicity due to the "alkylating" aziridine groups, derivatives were synthesized in which the C-atoms of the aziridine rings were substituted with methyl or ethyl groups (10). One member of this new series, ethyl bis(2,2-dimethyl-1-aziridinyl)phosphinyl carbamate (AB-132, II), has been studied to a considerable extent experimentally (11) as well as clinically (12–17). Its interesting pharmacologic properties [*e.g.*, cholinesterase inhibition (18–20)] and its radiation potentiating effect (21–23) suggested that this compound may act by a different mechanism than the C-unsubstituted aziridine derivatives (24). This conclusion was supported by chemical studies of its hydrolytic and alkylation reactions (11, 25), which indicated that the unique properties of II may be related to the ability of the 2,2-dimethylaziridine group to participate in S_N1 reactions with its substituted carbon (by forming a tertiary carbonium ion) and, alternatively,



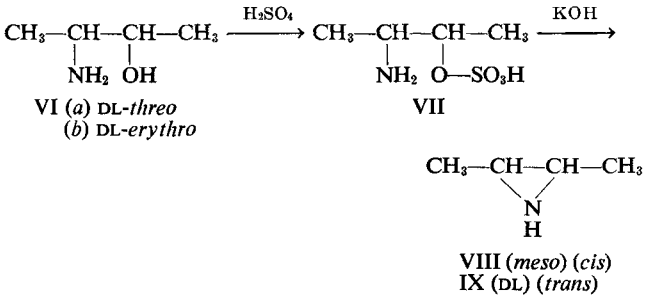
I. $R_1 = R_2 = R_3 = R_4 = H$ (AB-100)
II. $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$ (AB-132)
III. $R_1 = R_3 = CH_3$; $R_2 = R_4 = H$ (*cis*) (AB-144)
IV. $R_1 = R_4 = CH_3$; $R_2 = R_3 = H$ (*trans*) (AB-145)
V. $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$ (AB-143)

in S_N2 reactions with its unsubstituted methylene carbon (25).

In view of the observed profound effect of the 2,2-dimethyl substitution in the aziridine ring on the chemical and pharmacologic properties of II, it was of interest to investigate the chemical reaction mechanisms of the stereoisomeric 2,3-dimethylaziridine derivatives, III and IV (which are position isomers of II), and of the monomethylaziridine derivative, V.

RESULTS AND DISCUSSION

There are three stereoisomeric forms of 2,3-dimethylaziridine: *meso* (*cis*) and L and D (*trans*). The first two of these were synthesized from D- (as well as DL) *threo*-3-amino-2-butanol and L-*erythro*-3-amino-2-butanol, respectively, by Dickey *et al.* (26). By stereospecific syntheses, Dickey *et al.* (26) also established their absolute configurations. In the present work, commercial 3-amino-2-butanol,[1] a mixture of the DL-*threo* and DL-*erythro* isomers (VI), was used as the starting material. Cyclization of the acid sulfates VII gave a mixture of the two optically inactive diastereoisomeric aziridines, VIII and IX. These were separated by fractional distillation, as described in the *Experimental* section, and their NMR spectra were consistent with the configurational assignments of Dickey *et al.* (26) (Scheme I).



Scheme I

The synthesis and physicochemical properties of the ring-substituted bis-aziridinylphosphinyl carbamates III and V were previously reported (10). The *trans*-2,3-dimethyl analog, IV, was synthesized according to the same general procedure, by the reaction of dichlorophosphinyl urethan with DL-*trans*-2,3-dimethylaziridine (IX). Although two diastereoisomers of IV might be expected to arise from this reaction (since two molecules of the racemic aziridine reacted with each molecule of dichlorophosphinyl urethan), only one, sharp melting, crystalline product could be isolated from the reaction mixture by repeated crystallization from *n*-hexane.

The IR spectra of the *cis*- and *trans*-aziridine derivatives, III and IV, are almost superimposable, except in the 700–950 cm.$^{-1}$ region where the differences in the ring-deformation frequencies of the two compounds result in characteristically different absorption patterns. The NMR spectra show the expected differences between the two compounds in the chemical shifts of their ring-CH and -CH_3 protons, corresponding to the differences between the NMR spectra of the aziridines from which they were derived. In the spectra of both III and IV, the multiplets of the ring-CH protons are split by the phosphorus into two symmetrical groups of resonance lines.

The comparative chemical alkylating activities (S_N2-reactivities) of Compounds III, IV, and V were determined according to the previously described method (25, 27) by measuring the rates of alkylation (colored product formation) with γ-(4-nitrobenzyl)-pyridine (NBP) at 80° temperature and standard initial concentrations of the reactants. The initial reaction rates [k'_{80} values (27)] are given in Table I. It is apparent that the *trans*-isomer (IV) is much more reactive than the *cis* (III). This result seems reasonable, considering that in the case of IV the nucleophilic reactant can approach one of the carbon atoms from either side of each aziridine ring; while in the case of III, only one side of each ring is open to nucleophilic attack. Both compounds are considerably less reactive than the "unsubstituted" analog (I), but IV is only slightly less reactive than the monomethyl and gem-dimethyl aziridine derivatives (V and II, respectively) in which one of the aziridine carbons is unsubstituted.

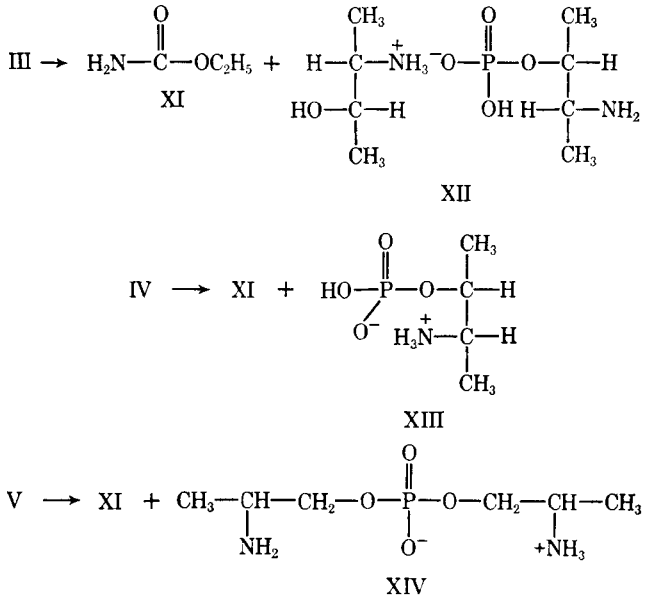
Table I—Comparative Alkylating Activities and Relative Stabilities to Hydrolysis of Ethyl Bis(1-aziridinyl)phosphinyl Carbamates

Compound	Aziridine Ring Substituents	Alkylating Activities,[a] $k'_{80} \times 10^3$ min.$^{-1}$	Half-Lives, hr.,[b] in H_2O at 37°
I	None	3.3[c]	60
II	2,2-CH_3	2.4[c]	2.5
III	*cis*-2,3-CH_3	0.8	125
IV	*trans*-2,3-CH_3	1.9	35
V	2-CH_3	2.0[c]	80

[a] For definition, see *Reference 27.* [b] Based on residual titratable aziridine rings. [c] Data taken from *Reference 25.*

The relative rates of hydrolytic cleavage of the aziridine-rings of Compounds I–V, in distilled water at 37°, can be compared on the basis of the half-life data given in Table I. If the hydrolysis of all compounds was to proceed entirely by an S_N1 mechanism, as was found in the case of the 2,2-dimethyl aziridine analog, AB-132 (II) (11), then the relative rates would depend on the comparative ease with which they are capable of forming a carbonium-ion intermediate. Therefore, the following order of hydrolysis rates would be expected: II > IV ≧ III > V > I. Instead, the rates of hydrolysis show the following sequence: II ≫ IV > I > V > III. Thus, it is evident that the hydrolytic cleavage of the aziridine rings in some of these compounds appears to involve other than S_N1 mechanisms. For example, the 3–4 times faster hydrolysis rate of the *trans*-2,3-dimethylaziridine derivatives IV as compared to the *cis*-isomer III almost parallels their relative S_N2 reactivities in the alkylation of NBP.

Preliminary studies relating to the mechanisms of hydrolysis of I and II were previously reported, indicating that in water solution, I undergoes bimolecular polymerization with its P—N linkages remaining largely intact (25), while the very fast hydrolysis of II proceeds *via* a tertiary carbonium ion and with the cleavage of all three of its P—N bonds (11, 25). To elucidate the mechanisms of hydrolysis of III–V, their final hydrolysis products, obtained after 3 weeks of incubation of each compound in deionized distilled water solution at 37°, were isolated and identified. In all cases, urethan and a phosphate ester were isolated from the hydrolysates as the two major, final hydrolysis products (Scheme II).



Scheme II

[1] Commercial Solvents Corp., New York, N. Y.

The structures of the phosphate esters were identified by elemental analysis, IR and NMR spectra, and alkaline hydrolysis, yielding the known amino alcohols.[2] In addition, most of the phosphate mono- and diesters, salts, and ester-salts that could have resulted from the hydrolysis of Compounds III–V were directly synthesized for comparison with the isolated products (see *Experimental*). The phosphate mono- and diesters were generally prepared by reacting the appropriate aziridine with phosphoric acid. Since the ring-opening reactions of the stereoisomeric 2,3-dimethylaziridines are known to proceed with a single Walden inversion (28), and 2-monosubstituted aziridines are cleaved by strong acids at the unsubstituted 3-position (29), the structures of the phosphate esters were unambiguously defined by their synthesis. However, additional proof of structure was obtained by alkaline hydrolysis to yield the known amino alcohols. The ester-salts were synthesized by reacting the monoesters with one equivalent of the amino alcohol, while the phosphate salts were prepared by combining one equivalent of phosphoric acid with two equivalents of the amino alcohol. If the elemental analysis and melting points did not permit unambiguous identification of the hydrolysis products with the synthetic salts or esters (because of variations in water of crystallization), comparison of the spectra served well for this purpose. In general, the phosphate salts showed much sharper absorption bands in their IR and NMR spectra than the phosphate esters which are zwitterions. Although the positions of the absorption peaks for the phosphate ester and the corresponding salt were similar, it was possible to differentiate between them on the basis of their resolution and splitting patterns. In particular, the splitting by the phosphorus of the CH or CH_2 protons adjacent to the P—O bond in the phosphate esters increased the complexity of the resonance pattern in the 3.5–4.5 p.p.m. region and in the case of XIV gave rise to a well-resolved quartet.

The results show that the *cis*-2,3-dimethylaziridine derivative III yields the phosphate ester-salt of *threo*-3-amino-2-butanol (XII), while the *trans*-2,3-dimethylaziridine analog IV yields the phosphate monoester of *erythro*-3-amino-2-butanol (XIII). If the hydrolysis of the aziridine rings would have proceeded by an S_N1 mechanism, the same carbonium-ion intermediate derived from either III or IV should have given identical mixtures of the phosphates of the two isomeric amino alcohols. Actually, the ring-opening hydrolysis reactions of both III or IV appear to involve in each case a single Walden inversion (*trans*-opening) and, therefore, must be mechanistically S_N2, although they are pseudo-first-order from a kinetic point of view. Furthermore, the structure of the hydrolytic product XIV of the 2-methylaziridine derivative V shows that the ring-opening reaction of this compound occurred at the unsubstituted (less hindered) carbon, thus indicating that the mechanisms of this reaction similarly followed an S_N2 course. The fact that the hydrolysis of all three compounds, III–V, resulted in the formation of a phosphate ester bond, indicates that the ring-opening reaction involves an inter- or intramolecular S_N2 attack of a $P—O^-$ anion on the protonated aziridine ring. This reaction requires either prior or concerted hydrolytic cleavage of a P—N bond, possibly initiated by tautomerization of the O=P—NH— grouping; eventually, all three P—N bonds are cleaved to give the final hydrolysis products.

CONCLUSIONS

On the basis of these results, it is now possible to explain satisfactorily the order of hydrolysis rates for Compounds I–V. Thus, II is the only compound of this series which hydrolyzes by an S_N1 mechanism, and this is consistent with its exceptionally fast rate of hydrolysis. In contrast, the relative rates of hydrolysis of Compounds I, V, and III appear to depend on the comparative steric hindrances of their aziridine moieties to nucleophilic attack. The relative hydrolysis rate of Compound IV is somewhat anomalous and may suggest some smaller contribution of an S_N1 mechanism (which possibility in the case of this compound is not entirely excluded by the structure of the isolated hydrolysis product XIII, since the latter contains only half of the total amount of amino alcohol resulting from the hydrolysis of IV). In any case, the mechanisms of hydrolysis of III–V are largely S_N2 and, therefore, just as the hydrolytic polymerization of the ring-unsubstituted-aziridine derivative I (25), they do not involve carbonium-ion intermediate formation to any substantial degree. The importance of the tertiary carbonium-ion intermediate formed in the hydrolysis of the geminally substituted aziridine derivative II has been previously discussed (11). Other studies on the comparative chemical and biological activities of conventional and "branched-chain" aromatic nitrogen mustards (27) have indicated that the biological activities in a given series of alkylating agents may be related to their different chemical reaction mechanisms (27, 30). On the whole, the results of the present study underline the uniqueness of the 2,2-dimethylaziridine derivative II (AB-132) with respect to its chemical behavior, as compared to other members of this series of alkylating agents.

It should be mentioned that the *in vivo* toxicities and antitumor activities (in several rodent tumor systems)[3] of Compounds I, III–V are qualitatively similar, and that their relative magnitudes closely parallel the relative "S_N2 reactivities" of these compounds with respect to NBP. This is in contrast to the qualitatively and quantitatively different pharmacological activities of AB-132 (II).

EXPERIMENTAL

Comparative alkylating activities and rates of hydrolysis were determined by previously-described procedures (25, 27). The IR spectra were recorded on a Perkin-Elmer model 237 (Infracord), and the NMR spectra were obtained in a Varian Associates model A-60 using deuterium oxide as solvent, except where noted. The melting points were determined on a Mel-Temp melting point apparatus and are uncorrected.

The syntheses of Compounds I–III and V were previously reported (1, 2, 10).

Ethyl [Bis(2,3-*trans*-dimethyl-1-aziridinyl)phosphinyl]carbamate (IV)—Commercially available 3-amino-2-butanol, a mixture of DL-*threo* and DL-*erythro* isomers (VI), was cyclized through its amine sulfate (26) to yield a stereoisomeric mixture of 2,3-dimethylaziridine. The separation of the DL(*trans*)-isomer from the *meso* (*cis*)-isomer, VIII, b.p. 81–83° (771 mm.) [previously employed for the synthesis of III (10)], was accomplished by fractional distillation through a spinning band column, and the fraction, b.p. 75–75.5° (765 mm.) (IX), was collected. NMR spectra (in CCl_4 with tetramethylsilane as internal standard) are as follows: VIII (*cis*): 1.05 (6H, d, J = 6 c.p.s., CH_3), and 2.06 p.p.m. (2H, multiplet, CH); IX (*trans*): 1.12 (6H, d, J = 4 c.p.s., CH_3), and 1.50 p.p.m. (2H, multiplet, CH). The reaction of DL(*trans*)-2,3-dimethylaziridine with dichlorophosphinyl urethan was carried out under nitrogen in the usual manner (10). After filtration, the filtrate was concentrated *in vacuo* at 30° to give an oily residue. Several crystallizations from *n*-hexane gave a pure product, m.p. 103–104°, yield 20%. Ethylenimine assay (31): 102% of the theoretical value (based on two ethylenimine groups and a mol. wt. of 275).

Anal.—Calcd. for $C_{11}H_{22}N_3O_3P$: C, 47.99; H, 8.00; N, 15.26. Found: C, 47.89; H, 7.84; N, 15.42.

IR absorption bands (KBr) ($cm.^{-1}$): 3050, 2960, 1730 (s) (C=O); 1460 (s) sh. 1420, 1440 (m) (C—CH_3, CH_2); 1380 (m) (C—CH_3); 1330 (w) (P=O); 905 (m); 835 (s) (doublet).[4]

NMR (in $CDCl_3$, with tetramethylsilane as internal standard), 1.35 (15H, multiplet, CH_3), 2.42 (4H, multiplet, —CH—N), and 4.15 p.p.m. (2H, quartet, CH_2—O).

Hydrolysis Products—The final hydrolysis products of III–V were obtained by incubating 0.015 mole of each compound with 2.2 moles of deionized distilled water at 37° for 3 weeks. The aqueous suspension was then extracted with ether, and the aqueous layer was lyophilized, washed with ether, and dried. The combined ether extracts, on evaporation of the solvent, yielded in each case a crystalline material which had a melting point and an IR spectrum identical with those of an authentic sample of ethyl

[2] Alkaline hydrolysis of phosphate esters proceeds *via* attack of the hydroxyl ion on the phosphorus (which carries a residual positive charge) and does not involve the asymmetric carbon of the alcohol which is liberated without change in its configuration.

[3] A detailed study on the comparative biological activities of all members of this series of "dual antagonists" in various experimental systems, including a large spectrum of animal tumors, is in preparation and will be published by the cooperating biological investigators.

[4] In the previously reported spectrum of the *cis*-analog III (10), the last three band assignments were erroneously transcribed; this region of the spectrum of III should read: 9.75 μ (1025 $cm.^{-1}$) (s); 9.90 μ (1010 $cm.^{-1}$) (s); 11.00 μ (905 $cm.^{-1}$) (m); and 11.9 μ (835 $cm.^{-1}$) (m).

Table II—Final Hydrolysis Products

	M.p.	Empirical Formula	Analysis, % Calcd.	Found	NMR Spectra δ, p.p.m.
Hydrolysis product of III (XII)	194–195°	$C_8H_{28}N_2O_5P$	C, 37.21 H, 8.92 N, 10.85 P, 12.01	C, 37.08 H, 8.80 N, 11.03 P, 12.17	1.28 (12H, doublet, CH_3) 3.25 (2H, multiplet, CH—N) 3.82 (2H, multiplet, CH—O)
Hydrolysis product of IV (XIII)	275–276°	$C_4H_{12}NO_4P$	C, 28.40 H, 7.10 N, 8.28 P, 18.34	C, 28.53 H, 7.23 N, 8.26 P, 18.12	1.25 (6H, doublet, CH_3) 3.42 (1H, multiplet, CH—N) 4.43 (1H, multiplet, CH—O)
Hydrolysis product of V (XIV)	170–172°	$C_6H_{17}N_2O_4P$	C, 33.96 H, 8.01 N, 13.21 P, 14.62	C, 33.68 H, 7.86 N, 13.13 P, 14.62	1.29 (6H, doublet, CH_3) 2.6–4.2 (6H, multiplet, CH—N, CH_2—O, including overlapping quartet at 3.68)

carbamate. The ether-insoluble products obtained from the aqueous layer were crystallized from methanol–ether. The melting points, elemental analyses, and NMR spectra of these "final hydrolysis products" (XII–XIV) are given in Table II.

Preparation of Phosphate Esters

Phosphate Monoesters—*A. Reaction of Substituted Aziridine with Phosphoric Acid*—To an ice-cooled, stirred solution of the substituted aziridine (0.10 mole) in 10 ml. of water was slowly added an equimolar quantity of 86% phosphoric acid. The resulting solution was stirred at room temperature for 48 hr. and then concentrated to dryness by lyophilization. The products were recrystallized several times from methanol or methanol–ether.

B. Pyrolysis Method—Equimolar quantities of the amino alcohol and phosphoric acid were stirred for 3 hr. at room temperature. The solution was concentrated *in vacuo* at 70°, and the resulting oily residue was heated *in vacuo* (0.8 mm. Hg) for 3 hr. at 160°. The solid residue was recrystallized from methanol or methanol–ether. The product was identical with that obtained by Procedure A, using the corresponding substituted aziridine, by mixed melting point, IR, and NMR.

Phosphate Diesters—The procedure used for the synthesis of the phosphate diesters was essentially similar to that described for the synthesis of the phosphate monoester, Procedure A, except that two equivalents of substituted aziridine (0.2 mole) were reacted with one equivalent (0.1 mole) of phosphoric acid. The products were recrystallized from methanol–ether.

The melting points, elemental analysis, and NMR spectra of the phosphate mono- and diesters are presented in Table III.

Hydrolysis of Phosphate Esters

The phosphate esters were hydrolyzed, to yield the corresponding amino alcohol and sodium phosphate, by refluxing 0.02 mole of the ester for 48 hr. in 100 ml. of 15% sodium hydroxide solution.

Table III—Synthetic Phosphate Esters, Salts, and Half Salts–Half Esters

Compound	M.p.	Empirical Formula	Analysis, % Calcd.	Found	NMR Spectra δ, p.p.m.
(DL-*threo*-Amino-2-butyl) dihydrogen phosphate	86°	$C_4H_{12}NO_4P$	C, 28.40 H, 7.10 N, 8.28	C, 28.25 H, 7.50 N, 7.95	1.26 (6H, doublet, CH_3) 3.24 (1H, multiplet, CH—N) 3.84 (1H, multiplet, CH—O)
Di(DL-*threo*-3-amino-2-butyl) hydrogen phosphate	180–181°	$C_8H_{21}N_2O_4P \cdot H_2O$	C, 37.21 H, 8.92 N, 10.85 P, 12.01	C, 37.26 H, 8.91 N, 11.00 P, 12.15	1.22 (12H, doublet, CH_3) 3.18 (2H, multiplet, CH—N) 3.85 (2H, multiplet, CH—O)
Di-3-(DL-*threo*-2-hydroxy)butylammonium hydrogen phosphate	194–195°	$C_8H_{25}N_2O_6P$	C, 34.78 H, 9.05 N, 10.15	C, 34.79 H, 9.20 N, 10.30	1.36 (12H, doublet, CH_3) 3.23 (2H, multiplet, CH—N) 3.78 (2H, multiplet, CH—O)
3-(DL-*threo*-2-Hydroxy)butylammonium (DL-*threo*-3-amino-2-butyl) hydrogen phosphate	185–187°	$C_8H_{23}N_2O_5P \cdot H_2O$	C, 34.78 H, 9.05 N, 10.15	C, 34.80 H, 9.23 N, 9.99	1.32 (12H, doublet, CH_3) 3.26 (2H, multiplet, CH—N) 3.80 (2H, multiplet, CH—O)
(DL-*erythro*-3-Amino-2-butyl) dihydrogen phosphate	276–278°	$C_4H_{12}NO_4P$	C, 28.40 H, 7.10 N, 8.28 P, 18.34	C, 28.22 H, 7.12 N, 8.26 P, 18.34	1.27 (6H, doublet, CH_3) 3.46 (1H, multiplet, CH—N) 4.42 (1H, multiplet, CH—O)
Di(DL-*erythro*-3-amino-2-butyl) hydrogen phosphate	176–178°	$C_8H_{21}N_2O_4P \cdot 2H_2O$	C, 34.78 H, 9.05 N, 10.15	C, 35.03 H, 9.25 N, 9.88	1.29 (12H, doublet, CH_3) 3.53 (2H, multiplet, CH—N) 4.52 (2H, multiplet, CH—O)
Di(DL-*erythro*-2-hydroxy)butylammonium hydrogen phosphate	199–201°	$C_8H_{25}N_2O_6P$	C, 34.78 H, 9.05 N, 10.15	C, 34.76 H, 9.19 N, 9.89	1.26 and 1.30 (12H, 2 doublets, CH_3) 3.40 (2H, multiplet, CH—N) 4.20 (2H, multiplet, CH—O)
3-(DL-*erythro*-2-Hydroxy)butylammonium (DL-*erythro*-3-amino-2-butyl) hydrogen phosphate	276–277°	$C_8H_{23}N_2O_5P$	C, 37.21 H, 8.91 N, 10.85	C, 36.91 H, 9.08 N, 10.63	1.28 (12H, doublet, CH_3) 3.48 (2H, multiplet, CH—N) 4.25 (2H, multiplet, CH—O)
Di(DL-2-amino-1-propyl) hydrogen phosphate	170–172°	$C_6H_{17}N_2O_4P \cdot CH_3OH$	C, 34.43 H, 8.61 N, 11.48	C, 34.67 H, 8.58 N, 11.58	1.30 (6H, doublet, CH_3) 2.6–4.2 (6H, multiplet, CH—N, CH_2—O)
Di-2-(DL-1-hydroxy)propylammonium hydrogen phosphate	161–163°	$C_6H_{21}N_2O_6P$	C, 29.02 H, 8.46 N, 11.29	C, 28.96 H 8.46 N, 11.38	1.26 (6H, doublet, CH_3) 3.6 (6H, multiplet, CH—N, CH_2O)
Di-1-(DL-2-hydroxy)propylammonium hydrogen phosphate	164–165°	$C_6H_{21}N_2O_6P$	C, 29.02 H, 8.46 N, 11.29	C 28.97 H, 8.58 N, 11.35	1.22 (6H, doublet, CH_3) 3.04 (4H, multiplet, CH_2—N) 4.08 (2H, multiplet, CH—O)

After cooling to room temperature, the solution was saturated with solid sodium hydroxide and extracted with several portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate and, after filtration, the ether was removed *in vacuo*. The oily product was identified by comparison of its IR and NMR spectra with those of an authentic sample of the amino alcohol.

Preparation of Phosphate Salts

To a stirred solution of two equivalents of amino alcohol (0.03 mole) in 20 ml. water was added dropwise one equivalent of phosphoric acid. The reaction mixture was stirred for 1 hr. and then lyophilized. The crude material was recrystallized from methanol. The products were characterized by their melting points, elemental analyses, IR, and NMR spectra (Table III).

Preparation of Phosphate Ester-Salts

To an aqueous solution of the phosphate monoester, prepared as described in Procedure A (0.05 mole in 15 ml. of water), was added 0.05 mole of the corresponding amino alcohol in 10 ml. of water. The mixture was stirred for 24 hr. at room temperature and then lyophilized. The product (95–100% yield) was crystallized from methanol or methanol–ether to yield the phosphate half ester-half salt. Analytical results and spectra are presented in Table III.

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