Synthesis of Potential Dual Antagonists III

Ring-Substituted Ethyl [Bis(1-aziridinyl)phosphinyl]carbamates

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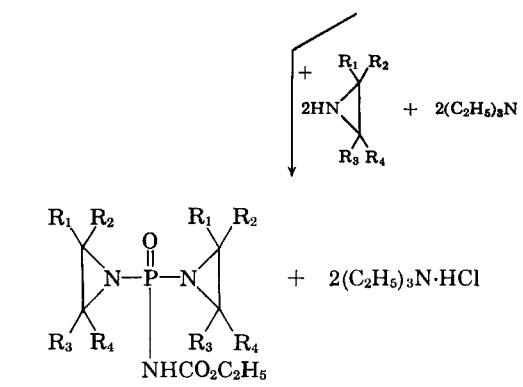
The syntheses of a series of ring-substituted ethyl [bis(1-aziridinyl)phosphinyl]-carbamates are reported. Several of these compounds showed significant tumor inhibitory activities in experimental animals, and one of them, ethyl [bis(2,2-dimethyl-1-aziridinyl)phosphinyl]carbamate (AB-132), is currently under clinical investigation as an experimental antineoplastic agent.

THE SYNTHESIS of a series of bis(1-aziridinyl)-phosphinyl carbamates was previously reported (2, 3). These compounds, termed dual antagonists showed considerable inhibitory activity against a broad spectrum of experimental tumors in rodents (4); two members of this series, the ethyl carbamate, AB-100 (I),[1] and the corresponding benzyl carbamate, AB-103[2] have undergone extensive clinical trial in several hundred cancer patients (5–8). Both of these compounds contain a highly active alkylating function and, therefore, can be applied only at relatively low dosage levels in man. This circumstance may limit, to some extent, the synergistic contribution of the urethan moiety to the total therapeutic effect of these drugs.

With the purpose of utilizing the theoretical advantages of the dual antagonism concept (9) at its optimal effectiveness, the authors sought to decrease the activity of the alkylating portion of the molecule by substituting alkyl groups at one or both carbon atoms of the aziridine ring. Such bulky substituents usually increase the stability (*i.e.*, diminish the reactivity) of small rings by steric hindrance. The synthesis of the new series of ring-substituted bis(1-aziridinyl)-phosphinyl carbamates reported in this paper was undertaken in the hope that the expected lower chemical reactivity of these compounds would result in lower toxicity which would permit them to be administered at higher dose levels, thus rendering the urethan portion of the molecule more effective.

Compounds II–VI were synthesized according to the general method of Papanastassiou and Bardos (3), from dichloroisocyanatophosphine oxide, through the intermediate dichlorophosphinylurethan as shown in Scheme I.



I, $R_1 = R_2 = R_3 = R_4 = H$ (AB-100)(3)
II, $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$ (AB-143)
III, $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$ (AB-132)
IV, $R_1 = R_3 = CH_3$; $R_2 = R_4 = H$ (AB-144)
V, $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$ (AB-140)
VI, $R_1 = C_2H_5$; $R_2 = R_3 = R_4 = H$ (AB-142)

Scheme I

Most of the substituted aziridines used in the second reaction step were prepared by modifications of Wenker's method for the synthesis of (unsubstituted) ethylenimine (10). Probably due to the greater basicity of these alkyl-substituted ethylenimines, their reactions with dichlorophosphinylurethan often led to the formation of by-products resulting from breakdown of the carbamate group. These side reactions were particularly disturbing in the case of the mono-methyl and mono-ethyl derivatives and made the synthesis and purification of II and VI extremely difficult. The relatively greater symmetry and stability of the molecule in the case of III, IV, and V permitted the separation of these compounds by crystallization. Each of the compounds presented different preparative problems, and the details given in the

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[1] Urodepa; trademarked as Avinar by Armour Pharmaceutical Co., Kankakee, Ill.

[2] Benzodepa; trademarked as Dualar by Armour Pharmaceutical Co., Kankakee, Ill.

experimental section (results of many trials and errors) are necessary for reproduction of the reported yields and purities.

In the case of II and VI, the possible presence of diastereoisomers may have added to the difficulties encountered in their purification. These compounds, as well as V, were synthesized with use of the *dl* forms of the corresponding alkyl-substituted ethylenimines. Since two molecules of ethylenimine react with each molecule of dichlorophosphinylurethan, one may expect the formation of two diastereoisomers (*dl* and *meso*). Although II and VI are liquids, they both appear to be chromatographically pure, and V is a crystalline solid having a sharp melting point. Neither of these compounds exhibit optical rotation, and their NMR spectra do not reveal the presence of more than one isomeric form. This problem does not arise, of course, in the case of III and IV which were synthesized with the optically inactive 2,2-dimethylaziridine and the *meso* form of 2,3-dimethylaziridine, respectively.

The infrared spectra of these compounds show the expected similarities as well as differences due to the various alkyl substitutions of the aziridine rings. Band assignments are given in the experimental part, with the exception of the 9–11 μ region which shows the most characteristic differences in the absorption patterns for each of the individual compounds. The authors believe that some of the strong absorption bands found in this region correspond to ring-deformation frequencies which are strongly influenced by the weight distribution of the alkyl substituents.

The NMR spectra (in $CDCl_3$) of I–VI all contain the quartet corresponding to the methylene protons of the carbamate group (5.80 τ) and (in some cases partially masked by the ring-methyl protons) the triplet of the carbamate methyl protons (8.70 τ). In addition, the unsubstituted compound, I, shows a doublet corresponding to its eight ring protons split by the β-phosphorus atom (7.67 τ, J_{PH} = 14 c.p.s.). All bands due to ring protons in the NMR spectra of II–VI are similarly split by the phosphorus which, however, has no effect on the protons of the ring substituents. Detailed spectral assignments for these compounds are given in the experimental part.

Biological Activities.[3]—All ring-substituted derivatives were found to be significantly less toxic in mice than the parent compound, I, but they showed also considerably lower activities against various transplanted rodent tumors. Compounds II and IV exhibited about one-third of the toxicity and one-fifth of the antitumor activity of I, which they qualitatively resembled in their pharmacologic properties and antitumor spectra. On the other hand, III was tolerated at 10–20 times higher dosage levels than I in the various animal species and appeared to have a better therapeutic index. More significantly, this compound showed striking differences (in comparison to I) in its pharmacologic effects; there was a remarkable lack of hemopoietic toxicity at the tumor inhibitory doses, and at the toxic dose levels, CNS toxicity appeared to be the cause of death in the experimental animals. This type of toxicity occurred at much lower dosage levels in the case of V which, therefore, could not be effectively tested as an antitumor agent.

Due to its interesting properties, III (AB-132) was selected for clinical trial against various forms of human neoplasias. Results of some of the clinical studies are reported in several recent publications (11–17).

EXPERIMENTAL[4]

Ethyl [Bis(2-methyl-1-aziridinyl)phosphinyl]carbamate (II).—Dichloroisocyanatophosphine oxide (3), 80 Gm. (0.5 mole) was reacted with ethyl alcohol, 23 Gm. (0.5 mole). The resulting dichlorophosphinylurethan was reacted subsequently with 2-methylaziridine, 57 Gm. (1.0 mole), and triethylamine, 101 Gm. (1.0 mole), in the manner described below for the synthesis of III, except that the reaction mixture was kept under dry nitrogen atmosphere. The precipitated triethylamine hydrochloride was separated by filtration, and the solution was concentrated in the flash evaporator under reduced pressure, at 32–34°, carefully excluding moisture throughout this process. The crude product was obtained in the residue in the form of a viscous liquid; attempts to crystallize it from various solvents failed. Yield: 120 Gm. (98%).

Twenty grams of the liquid residue was dissolved in a minimum amount of petroleum ether (b.p. 30–60°) and chromatographed on a column prepared with 400 Gm. of neutral alumina (Brockmann Grade I).[5] The entire system was kept under dry nitrogen atmosphere, using a pressure-equalized separator for the input of eluents. Carefully dried organic solvents were used. The column was eluted successively with (*a*) benzene; (*b*) benzene–ether, 1:1; (*c*) benzene–chloroform, 3:1; (*d*) benzene–chloroform, 2:1; (*e*) benzene–chloroform, 1:1; (*f*) benzene–chloroform, 1:2; (*g*) benzene–chloroform, 1:3; (*h*) benzene–methanol–chloroform, 1:1:2, and (*i*) benzene–methanol, 3:1. A total of 32 fractions was collected, concentrated on the flash evaporator, and their I.R. spectra examined. The first 13 fractions, obtained with eluents (*a*) through (*d*),

[3] Drs. J. L. Ambrus and N. Back, personal communication.

[4] Melting points were taken on a Drechsel melting point apparatus and are uncorrected. Microanalyses by Dr. H. Galbraith, Galbraith Laboratories, Knoxville, Tenn. The infrared spectra were recorded on a Perkin-Elmer model 237 (Infracord). The NMR spectra were determined on a Varian Associates model A-60 spectrometer in $CDCl_3$ solution with tetramethylsilane as internal standard.

[5] Bio-Rad Act. Gr. I from California Corporation for Biochemical Research, Los Angeles, Calif.

thowed a carbonyl absorption band at 5.8 μ, corsesponding to the carbamate group, but the absorprion bands characteristic of the ethylenimine were absent. The next eight fractions, obtained with eluents (*e*) and (*f*) showed a curious double peak in the carbonyl region, at 5.6 and 5.8 μ. Fractions 21–31, obtained with eluents (*g*) and (*h*), showed the expected infrared absorption bands for both carbamate and ethylenimine, but also some overtones in the far-infrared region (at 13.3 μ) which could not be interpreted readily. Finally, fraction 32, obtained with eluent (*i*), gave an infrared spectrum consistent with the structure of II.

Fractions 22–31 were combined and concentrated, yielding an oily residue, 8.1 Gm. which, by the titrimetric assay of Allen and Seaman (18), gave 87.1% of the theoretical ethylenimine content. The residue of fraction 32, also a viscous liquid, 7.0 Gm., gave an ethylenimine assay corresponding to 100 ± 2% of the calculated value. A sample of this liquid substance was submitted for elemental analysis.

Anal.—Calcd. for $C_9H_{18}N_3O_3P$ (247.18): C, 43.72; H, 7.28; N, 17.00; P, 12.55. Found: C, 43.43; H, 7.54; N, 17.15; P, 12.71.

Infrared absorption bands $\lambda_{max.}^{CHCl_3}$ (μ): 2.93 (N—H); 3.33 (s), 3.40 (w) (C—H); 5.78 (s) (C=O); 6.96 (s) (sh at 6.82 and 6.88)(C—CH_3; CH_2); 7.13 (m); 7.22 (w); 7.30 (w) (CH_3); 7.66 (m) (P=O); 7.95–8.45 (vs) (C—O, C—N); 8.65 (m) (C—CH_3); 9.6 (s); 10.03 (s); 10.98 (s); 11.45 (s); 12.0 (m); additional bands in liquid film: 12.9 (ethylenimine), 13.9 (P—N).

NMR absorption bands: 8.65 τ (J = 4 c.p.s.) doublet (ring-CH_3); 7.10–8.25 τ, overlapping multiplets for three different types of ring-hydrogens (*cis*, *trans*, and adjacent to CH_3) all split by phosphorus. Absorption peaks due to urethan, see above.

Ethyl [Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-carbamate (III).—Dry toluene (1000 ml.) was placed into a 2-L., 3-necked round-bottom flask equipped with a mechanical stirrer, addition funnel, and thermometer, and protected from moisture by a calcium chloride tube. About 50 ml. of the toluene was distilled off to insure dryness, and after cooling to room temperature, 100 Gm. (0.625 mole) of dichloroisocyanatophosphine oxide was added. The mixture subsequently was cooled to about 3°, and under continuous stirring and cooling, a solution of 27.3 Gm. (0.625 mole) absolute ethyl alcohol in 100 ml. of dry toluene was added in the course of 1 hr. The mixture then was allowed to warm to room temperature and concentrated to a volume of 500 ml. in the flash evaporator (bath temperature about 40°).

This toluene solution of ethyl dichlorophosphinylcarbamate was added, in the course of 1 hr., to another, similarly equipped 2-L. flask which contained a cooled (3°) solution of 131.3 Gm. (1.30 moles) of triethylamine and 92.3 Gm. (1.30 moles) of 2,2-dimethylaziridine in 1000 ml. of dry toluene. During the addition, and then for an additional hour, the mixture was stirred continuously and cooled at 3°; thereafter, it was allowed to warm to room temperature, stirred for another hour, and filtered. The precipitate contained nearly the theoretical amount of pure triethylamine hydrochloride. The filtrate was concentrated to dryness, and the oily residue was dissolved in about 500 ml. of dry, peroxide-free ether. The solution was treated with decolorizing charcoal and, after filtering, allowed to cool in a freezer (−27°) overnight. A crystalline product was obtained; this was recrystallized several times from ether until its m.p. reached 57–58°. Additional product was obtained by concentrating the mother liquors to a small volume, cooling at −27°, separating the crystalline precipitate, and recrystallizing it from ether. Yield: 108 Gm. (40%).

Anal.—Calcd. for $C_{11}H_{22}N_3O_3P$: C, 47.99; H, 8.00; N, 15.26; P, 11.25. Found: C, 48.33; H, 8.14; N, 14.87; P, 10.91.

Ethylenimine assay by the thiosulfate method of Allen and Seaman (18) gave only 51–53% of the theoretical value (calculated for two aziridine groups and a mol. wt. of 275). This agrees with the result obtained by this method for *tris*(2,2-di-methyl-1-aziridinyl)phosphine oxide (18).

Infrared absorption bands $\lambda_{max.}^{CHCl_3}$ (μ): 2.93 (w) (N—H); 3.35 (m) (C—H); 5.78 (s) (C=O); 6.95 (s) [sh at 6.75; 6.82 (m)] (C—CH_3, CH_2); 7.20 (m); 7.26 (m) (C—CH_3); 7.45 (m); 7.65 (m) (P=O); 8.1–8.45 (s) (C—O, C—N); 8.78 (s) [C(CH_3)$_2$]; 9.0 (m); 9.42 (w); 10.36 (s); 11.10 (w); 11.82 (s).

NMR absorption bands: 8.56 τ singlet (ring-CH_3); 7.67 τ (J_{PH} = 16 c.p.s.) doublet (ring-hydrogens). Signals for urethan protons, see above.

Ethyl [Bis(2,3-cis-dimethyl-1-aziridinyl)-phosphinyl]carbamate (IV).—3-Amino-2-butanol[6] (a mixture of the DL-*threo* and DL-*erythro* stereoisomers) was cyclized through the amine sulfate (10,19,20) to give a mixture of stereoisomeric 2,3-dimethylaziridines. The *meso*-isomer, b.p. 81–83° [lit. (20) 82.5–82.9°/747 mm.], was separated by fractional distillation through a spinning band column. The reaction of *meso*(*cis*)-2,3-dimethylaziridin with dichlorophosphinylurethan was carried out in a manner similar to that for the preparation of III, but under nitrogen atmosphere. After removal of the triethylamine hydrochloride by filtration, the filtrate was concentrated on the flash evaporator, and a viscous residue was obtained. This was dissolved in dry ether, and dry petroleum ether was added until the solution became slightly turbid. On concentration and cooling, a crystalline material separated after standing a few hours at 3°. After two recrystallizations from ether–petroleum ether mixture, a sharp melting point was obtained. Yield: (pure compound) 30% of the theoretical (m.p. 88–89°). Ethylenimine assay (18) 97.1% 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, 47.59; H, 8.01; N, 15.36.

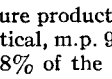
Infrared absorption bands $\lambda_{max.}^{CHCl_3}$ (μ): 2.93 (w) (N—H); 3.34 (s), 3.42 (w) (C—H); 5.76 (s) (C=O); 6.95 (s) (sh at 6.85, 7.08) (C—CH_3, CH_2); 7.26 (m) (C—CH_3); 7.65 (m) (P=O); 7.9 (m); 8.10–8.50 (m) (C—O, C—N); 9.28 (m); 9.42 (w); 10.36 (s); 11.10 (w); 11.82 (s).

NMR absorption bands: 8.78 τ doublet (J = 5 c.p.s.) for the CH_3 protons and a split doublet 7.24 (J_H = 3.5 and J_{PH} = 15 c.p.s.) for the ring protons. Urethan signals as above.

[6] The authors are grateful to Mr. R. DiGiacomo, Commercial Solvents Corp., New York, N. Y., for his gift of the amino alcohols used in this work.

Ethyl [Bis(2,2,3-trimethyl-1-aziridinyl)phosphinyl]carbamate (V).—2,2,3-Trimethylaziridine was first synthesized by Jones (21), according to the general method of Wenker (10); however, no details were given and the reported yield was very low (19%). By applying modifications analogous to those proposed by Leighton *et al.* (19) for the synthesis of ethylenimine by Wenker's method, the yield was increased to 65% in the following manner. To 103 Gm. (1 mole) of 3-amino-3-methyl-2-butanol, in 200 ml. of water, was added, under shaking, 98 Gm. (53 ml.) concentrated sulfuric acid in 190 ml. of water. The mixture was distilled under nitrogen at atmospheric pressure and until the temperature of the residue reached 115°, then distillation was continued at 25–30 mm. vacuum to a temperature of 150–180° until crystallization (solidification) of the residue began. After cooling, the residue was triturated with 500 ml. of hot 95% alcohol and then again cooled to room temperature. The crystalline residue was filtered, washed with alcohol, and dried. Yield: 143 Gm. of 3-amino-3-methyl-2-butyl sulfate. To this salt, 900 ml. of 40% sodium hydroxide solution was added, and the mixture was subjected to distillation. About 400 ml. of distillate was collected. From this, upon saturation with solid KOH, an organic layer was separated; this was dried and distilled at 84–85° (760 mm.), yielding the colorless, liquid trimethylaziridine (55 Gm.). Compound V was synthesized as described for III except that the 2,2,3-trimethylaziridine was used in the second reaction step. After filtration of the triethylamine hydrochloride and concentration of filtrate to dryness, the residue was dissolved in ether and crystallized at −27°. After recrystallization from ether, the pure product was obtained in a yield of 25% of theoretical, m.p. 91–92°. Titratable ethylenimine (18): 48% of the total ethylenimine content.

Anal.—Calcd. for $C_{13}H_{26}N_3O_3P$: C, 51.48; H, 8.58; N, 13.86. Found: C, 51.34; H, 8.60; N, 13.45.

Infrared absorption bands $\lambda_{max.}^{CHCl_3}$ (μ): 2.93 (w) (N—H); 3.33 (s), 3.40 (m) (C—H); 5.78 (s) (C=O); 6.96 (s) (sh at 6.82, 7.08) (C—CH_3, CH_2); 7.26 (m) (C—CH_3); 7.65 (m) (P=O); 8.1–8.45 (s) (C—O, C—N); 8.85 (s) (); 9.25 (m); 9.75 (s); 10.50 (w); 12.15 (m).

NMR absorption bands: 8.84 τ singlet (2-*trans* CH_3 protons), 8.77 τ singlet (2-*cis*-CH_3 protons), 8.76 doublet (J_H = 10 c.p.s.; 3-CH_3 protons); 7.2 τ broad signal for ring hydrogen. Urethan proton signals as above.

Ethyl [Bis(2-ethyl-1-aziridinyl)phosphinyl]carbamate (VI).—The synthesis of this compound was accomplished in the manner described for preparation of II, except that instead of 2-methylaziridine, 2-ethylaziridine was used in the second reaction step. 2-Ethylaziridine was prepared from 2-amino 1-butanol by the general method of Wenker (10), with modifications analogous to those proposed by Leighton *et al.* (19); it was obtained in 65% yield; b.p. (760 mm.) 87–88°; refractive index n_D^{25} 1.416 [identical with data reported by Jones (21)]. Purification of VI by chromatography on alumina was conducted in the manner described for II using the same sequence of eluents. After collection of several impure fractions (which showed similar characteristic I.R. double peaks at 5.6 and 5.8 μ as the benzene eluates obtained in the chromatographic purification of II), VI was obtained upon concentration of the benzene–chloroform eluate fractions, in the form of a clear, colorless oil. Ethylenimine assay (18): 89% of the theoretical.

Anal.—Calcd. for $C_{11}H_{22}N_3O_3P$: C, 47.99; H, 8.00; N, 15.26. Found: C, 46.34; H, 8.02; N, 14.92. All attempts at further purification resulted in decomposition (loss of carbonyl band in infrared spectrum) and/or polymerization of the compound.

Infrared absorption bands $\lambda_{max.}^{CHCl_3}$ (μ): 2.93 (w) (N—H); 3.33 (s), 3.39 (w), 3.46 (w) (C—H); 5.78 (s) (C=O); 6.83 (s) (sh at 6.75, 6.95) (C—CH_3, CH_2); 7.13 (m) (NH); 7.22 (w) (C—CH_3); 7.65 (m) (P=O); 8.00–8.45 (s) (C—O, C—N); 9.1 (w); 9.38 (m); 9.70 (w); 10.35–10.55 (s); 11.08 (w); 11.75 (m).

NMR absorption bands: 8.99 τ triplet (CH_3 protons of side-chain ethyl); signals for CH_2 and ring-protons in 7.1–8.5 τ region.

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