Synthesis of Potential "Dual Antagonists." I. Some Bis(1-aziridinyl)phosphinyl Carbamates[1] and Their Structural Analogs

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The syntheses of three bis(1-aziridinyl)phosphinyl carbamates (including the new experimental cancer drugs, I and III), as well as of some structurally related new carbamates and carbamides, are reported. These compounds were prepared through the intermediate dichloroisocyanatophosphine oxide, or through pyrolysis of I.

Three years ago, in a joint communication[3] with our biological collaborators, we reported on a new series of compounds termed "dual antagonists," which exhibited significant inhibitory activities against a broad spectrum of experimental animal tumors. Since then, two of these compounds, "AB–100" (I)[4] and "AB–103" (III),[5] have been undergoing extensive clinical trial against various forms of neoplastic disease in humans, and they were the subject of recent publications by several groups of investigators.[6–10] The present paper describes the syntheses of these compounds and of some of their chemical analogs.

"Dual antagonists" are designed to incorporate the biologically essential structural features of two different but synergistic inhibitors into a single molecule.[11] Among the compounds reported in the

(1) N-[Bis(ethylenimido)phosphoro]carbamates, according to the nomenclature used in our previous publications and in some clinical papers.

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(3) T. J. Bardos, Z. B. Papanastassiou, Albert Segaloff, and J. L. Ambrus, *Nature*, **183**, 399 (1959).

(4) Uredepa; Avinar;® AB–100.

(5) Benzodepa; Dualar®; AB–103.

(6) J. B. Weeth and A. Segaloff, *Southern Medical Journal*, **54**, 39 (1961).

(7) S. McCraken and J. Wolf, *Cancer Chemotherapy Reports*, **6**, 52 (1960).

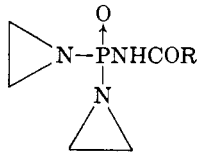
(8) D. M. Hayes, C. L. Spurr, and J. D. Hines, *Am. Assoc. Cancer Research*, Proc., **3**, 233 (1961).

(9) D. V. Razis, J. L. Ambrus, C. A. Ross, L. Stutzman, J. E. Sokal, A. M. Rajeli, and T. J. Bardos, *Cancer*, **14**, 853 (1961).

(10) R. K. Ausman, G. E. Crevar, H. Hagedorn, T. J. Bardos, and J. L. Ambrus, *J. Am. Med. Assoc.*, **178**, 735 (1961).

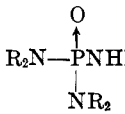
(11) The mode of action of such compounds is the subject of continuing investigations. T. J. Bardos, *Biochem. Pharmacology*, **11**, 256 (1962).

present paper, only I, II, and III satisfy this definition: because (1) they unite the bis(1-aziridinyl)phosphinyl radical, a bifunctional group with known "alkylating" activity, through an amide linkage, to a carbamate, and (2) ethyl carbamate, a known tumor inhibitor, is believed to exhibit synergism in combination with alkylating agents.[12]



I, R = OC_2H_5
II, R = OCH_3
III, R = $OCH_2C_6H_5$
IV, R = NH-*cyclo*-C_6H_{11}
V, R = NHC_6H_5
VI, R = $N(C_2H_5)C_6H_5$

Other compounds reported in this paper were synthesized to test our premise that each synergistic component should be present in the molecule in such form (chemical linkage) that it can exert its biological activity. Compounds IV, V and VI contain the same "alkylating" group as I–III, while the carbamate portions of the latter are replaced by sterically similar but biologically inactive carbamide (ureido) groups. In contrast, Compounds VII and X were designed to represent "non-alkylating" (or "open-chain") analogs of I, in which the carbamate portion and the "central core" of the latter compound remains the same, and only the ethylenimine groups are replaced by diethylamine or dimethylamine, respectively.



VII, R = C_2H_5; R′ = $COOC_2H_5$
VIII, R = C_2H_5; R′ = $CON(C_2H_5)_2$
IX, R = C_2H_5; R′ = H
X, R = CH_3; R′ = $COOC_2H_5$
XI, R = CH_3; R′ = $CON(CH_3)_2$

The key intermediate used in the synthesis of most of these compounds was the dichloroisocyanatophosphine oxide (XII) first described by Kirsanov.[13] This highly reactive compound was obtained by an interesting reaction reported by the Russian author

$$PCl_5 + H_2NCOOC_2H_5 \rightarrow \underset{\text{XII}}{Cl_2P(O)NCO} + C_2H_5Cl + 2HCl \qquad (1)$$

We found that the use of a solvent (ethylene chloride) improved the safety and reproducibility of this reaction. The reaction conditions are quite critical; our modified procedure, described in the experimental part of this paper, was developed on the basis of many trials.

The second step in the synthesis of some of these compounds in-

(12) H. E. Skipper, *Cancer*, **2**, 475 (1949).
(13) A. V. Kirsanov, *J. Gen. Chem. USSR*, **24**, 1031 (1954).

volves the reaction of XII with appropriate alcohols or amines,[14,15] to give the corresponding dichlorophosphinylcarbamates or dichlorophosphinylureas, respectively.

$$Cl_2P(O)NCO + ROH \rightarrow Cl_2P(O)NHCOOR \quad (2a)$$

XIII, R = C_2H_5
XIV, R = CH_3
XV, R = $CH_2C_6H_5$

$$Cl_2P(O)NCO + R_1R_2NH \rightarrow Cl_2P(O)NHCONR_1R_2 \quad (2b)$$

XVI, R_1 = H; R_2 = C_6H_5
XVII, R_1 = C_2H_5; R_2 = C_6H_5

However, in the case of the carbamates, we usually did not isolate these extremely labile intermediates (XIII–XV), but preferred to use them in *statu nascendi*, in toluene or ethylene chloride solution, for the third step of our synthesis, *i.e.*, the reaction with ethylenimine (or with the dialkylamines, see (3b) below).

$$Cl_2P(O)NHCOOR + 2HN\!\!\diagup\!\!\diagdown + 2(C_2H_5)_3N \rightarrow \quad (3a)$$

$$I(II \text{ or } III) + 2(C_2H_5)_3N \cdot HC$$

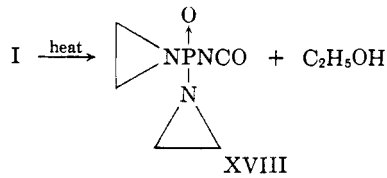
In these reactions, triethylamine was used as the hydrogen chloride acceptor; the triethylamine hydrochloride formed was separated from the desired products by precipitation and solvent extraction techniques described in the experimental section.

Compounds I, II and III are extremely reactive, and they readily polymerize in the presence of trace amounts of water and carbon dioxide; therefore, all manipulations had to be carried out as rapidly as possible and, preferably, under a dry nitrogen atmosphere. If the final product was not obtained immediately in pure form, its purification by recrystallization was usually quite difficult, because the presence of polymeric material hindered crystallization and sometimes, deceptively, increased rather than depressed the melting point of the compound. Furthermore, the rate of polymerization was found to be strongly dependent on the temperature, and even a brief period of heating under presumably dry conditions was found to accelerate this process. Compound III, in dry powder form, was found to be unstable even at room temperature; this compound had to be stored below 0°, preferably in absolute alcohol solution.

On the other hand, compound I could be pyrolyzed, by rapid heating in dry toluene solution, to give the corresponding isocyanate (identified by its characteristic infrared absorption band at 4.45 μ), without appreciable polymerization. This reaction was used for the

(14) A. V. Kirsanov and E. S. Levchenko, *J. Gen. Chem., USSR*, **26**, 2285 (1956).
(15) A. V. Kirsanov and I. V. Zhmurova, *J. Gen. Chem., USSR*, **26**, 2642 (1956).

conversion of I to other carbamates and ureas, as shown in our alternative procedure for the synthesis of III.



XVIII

$$XVIII + C_6H_5CH_2OH \rightarrow III$$

However, the two reactions were carried out more conveniently in one step, by conducting the pyrolysis in the presence of the higher-boiling benzyl alcohol.

Some unexpected difficulties were encountered in the preparation of the "open-chain" analogs of I. The products obtained in the reaction of XIII with the dialkylamines were found to be mixtures of the desired carbamate (VII or X) with the corresponding carbamide (VIII or XI, respectively). Apparently, the very labile ester group of XIII readily ammonolyzes; attempts to prevent this, by varying the reaction conditions, failed.

$$\underset{\text{(XIII)}}{Cl_2P(O)NHCOOC_2H_5} + R_2NH \begin{cases} \nearrow (R_2N)_2P(O)NHCOOC_2H_5 \\ \searrow (R_2N)_2P(O)NHCONR_2 \end{cases} \quad (3b)$$

Only one of the desired carbamates, VII, was separated in pure form from its carbamide mixture, by fractional distillation at 1–2 mm. vacuum; in this process, the carbamate underwent partial pyrolysis to the corresponding isocyanate but was reconstituted by the addition of ethanol to the distillate. When the fractionation was conducted at 5 mm. vacuum (*i.e.*, higher temperature), further degradation with loss of the carbethoxy group occurred, leading to the formation of IX. In the case of the product mixture obtained in the reaction with dimethylamine, fractional distillation proved ineffective; however, the urea derivative XI could be isolated from the mixture by solvent extraction and crystallization techniques.

Results of animal tumor studies on I, II and III have been reported previously.[3] Their carbamide analogs, IV, V, and VI, have been tested to date only against Walker carcinosarcoma 256 in rats, to ascertain their "alkylating agent-type" activities, to which this tumor is particularly sensitive. In this system, all three compounds

showed 80–99% inhibition of tumor growth at 10 mg./kg./day dosage level, *i.e.*, they had the same order of activity as the conventional alkylating agents. Conversely, the "open-chain" analog, VII, was inactive in Walker carcinosarcoma 256, but showed the same activity as urethane against Adenocarcinoma 755 in mice (about 40% inhibition at 250 mg./kg.).[16]

Experimental[17]

Dichloroisocyanatophosphine Oxide (XII).—The original procedure of Kirsanov[13] was modified in the following manner:

A suspension of 208.5 g. (1.0 mole) phosphorus pentachloride in 300 ml. of dry ethylene chloride was placed into a 3-neck round-bottomed flask provided with a glass stopper, a thermometer and an air condenser, the latter connected with a bubble counter containing concentrated sulfuric acid. The mixture was cooled to 0–5°, and a second suspension containing 89 g. (1.0 mole) of urethane in 300 ml. of ethylene chloride was added to the flask in small portions over 5–10 min., under occasional swirling of the reaction mixture. Evolution of gas (ethyl chloride and HCl) started almost immediately, and a solution was obtained. The hydrogen chloride evolved was absorbed in water; aliquots of the hydrochloric acid solution were titrated with 2 *N* NaOH. After the evolution of gas subsided, the mixture was stirred with a magnetic stirrer and slowly warmed until it reached reflux temperature, then refluxing was continued for about 0.5 hr. During warming, the evolution of gases continued, and, after completion of the refluxing period, about 95% of the calculated HCl was determined by titration. The apparatus was then flushed with dry nitrogen, and the reaction flask was cooled to room temperature; it was stoppered and stored in the refrigerator overnight.

The solution was then treated with charcoal and, after removal of the decolorizing agent by filtration, the filtrate was concentrated in the flash evaporator (Buchler, batch model) under reduced pressure (bath temperature 40°). The residue then was distilled through a Vigreux column, b.p. 36–37° (8 mm.), $n^{21}D$ 1.4682 (lit.[13] b.p. 19.7–20° (5 mm.); $n^{15}D$ 1.470). A characteristic sharp infrared absorption band was noted at 4.45 μ (isocyanate). The yield of the distilled product was 120 g. (75%).

Ethyl [Bis(1-aziridinyl)phosphinyl]carbamate (I).—A solution of 13.8 g. (0.3 mole) of absolute ethyl alcohol in 400 ml. of dry toluene was added during 2–2.5 hr. to a cold solution of 48 g. (0.3 mole) of dichloroisocyanatophosphine oxide (XII) in 400 ml. of dry toluene. The temperature during this addition was maintained at *ca.* −10°, and the mixture was vigorously stirred. After the addition was completed, the mixture was slowly warmed to room temperature and stirred for an additional hour. At this point, a sample of the solution showed in its infrared spectrum the disappearance of the isocyanate peak at 4.45 μ and the presence of a strong ester–carbonyl band at 5.78 μ, indicating the formation of dichlorophosphinylurethane (XIII).

The solution was then concentrated to a volume of *ca.* 400 ml. in a flash evap-

(16) Private communication from Dr. J. L. Ambrus, Roswell Park Memorial Institute, Buffalo, New York.

(17) All melting points are uncorrected. Microanalyses by Midwest Microlab., Indianapolis, Ind., and Drs. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England.

orator (Buchler), at a bath temperature of about 40°. The concentrated toluene solution of XIII was added, in the course of 2–2.5 hr., to a cold solution of 25.8 g. (0.6 mole) of ethylenimine and 60.6 g. (0.6 mole) of triethylamine in 500 ml. of toluene. During the addition, the temperature was kept at 0°, and the mixture was stirred vigorously.

After the addition was completed, the mixture was warmed slowly to room temperature and stirred for another hour. The white precipitate, a mixture of I and triethylamine hydrochloride, was then separated by filtration and washed with toluene. (Evaporation to dryness of the toluene filtrate yielded a small quantity of I which was purified by recrystallization from benzene–petroleum ether mixture.) The precipitate was mixed with 300 ml. of warm (40°) benzene and stirred for 15 min. The supernatant solution was decanted, and the solid was reextracted with benzene, in a similar manner, three more times. The combined benzene extracts were filtered and the filtrate was evaporated to dryness. The residue, m.p. 89–90°, gave satisfactory elemental analysis and titration values for I without recrystallization; yield, 46.2 g. (70.2%).

Anal. Calcd. for $C_7H_{14}N_3O_3P$: C, 38.36; H, 6.44; N, 19.17; P, 14.13. Found: C, 38.49; H, 6.58; N, 19.38; P, 14.09. Quantitative determination of ethylenimine groups by the titrimetric method of Allen and Seaman[18] gave 99.2% of the theoretical value, calcd. on the assumption that the mol. wt. is 219.17, and that there are two ethylenimine groups per molecule. Characteristic infrared absorption bands (0.5% KBr) at λ_{max} (microns): 2.95 (N—H); 3.30, 3.50 (C—H); 5.78 (C=O); 6.70, 7.00, 7.20 (CH_2 and CH_3); 7.90 (P=O); 8.10 (C—N); 8.49 (C—O); 10.6 (P—N<|); 12.0(ethylenimine); 14.2 (P—N).

Methyl [Bis(1-aziridinyl)phosphinyl]carbamate (II).—Essentially the same procedure as described for I was employed, using methyl alcohol instead of ethyl alcohol in the reaction with the dichloroisocyanatophosphine oxide (XII). From 24.5 g. (0.15 mole) of XII a total of 23.5 g. (76.5%) of pure II was obtained; m.p. 119–121°; infrared spectrum similar to I. Ethylenimine titration[18] result: 98.8% of theoretical, based on mol. wt. 205.15, and 2 ethylenimine groups per molecule.

Anal. Calcd. for $C_6H_{12}N_3O_3P$: C, 35.12; H, 5.90; N, 20.48; P, 15.10. Found: C, 35.25; H, 5.89; N, 20.6; P, 15.5.

Benzyl [Bis(1-aziridinyl)phosphinyl]carbamate (III).—(a) **From I.**—A solution of 25 g. (0.23 mole) of benzyl alcohol in 1 l. of toluene was heated to boiling, and 21.9 g. (0.1 mole) of I was added to it. The mixture was further boiled for 10 min., and then the contents of the reaction flask were concentrated slowly under vacuum until the temperature of the mixture dropped to 60°. At this point, the residue was filtered from some insoluble material, and the filtrate was concentrated further under reduced pressure (in a flash evaporator) until crystallization began. The oily crystals which formed on concentration were filtered and washed with 150 ml. of benzene–cyclohexane (1:1) mixture. After drying, a white solid was obtained; yield 16 g. (54%), m.p. 133–134°. Ethylenimine titration: 100.9% of theoretical (based on mol. wt. 281.25 and 2 ethylenimine groups per molecule).

Anal. Calcd. for $C_{12}H_{16}N_3O_3P$: C, 51.24; H, 5.73; N, 14.94; P, 11.01. Found: C, 51.54; H, 6.00; N, 15.18; P, 10.86.

(b) **From XII.**—The reaction of dichloroisocyanatophosphine oxide (XII, 32 g., 0.2 mole) with benzyl alcohol (21.6 g., 0.2 mole) and, subsequently, with

(18) E. Allen and W. Seaman, *Anal. Chem.*, **27**, 540 (1955).

ethylenimine (17.2 g., 0.4 mole) and triethylamine (40.4 g., 0.4 mole) was accomplished in the manner described for the preparation of I, except for the fact that, in this case, ethylene chloride was used as the solvent, because of the insolubility of the product in cold toluene. The triethylamine hydrochloride formed was separated by filtration, and the filtrate was evaporated to dryness. The solid residue was triturated with benzene at 60° and the solution obtained was filtered. Addition of cyclohexane to the filtrate precipitated III; yield, 19.0 g. (34%), m.p. 133–134°, identical with product obtained by method (a). Ethylenimine assay was 99.6% of the calculated.

Pyrolysis of I.—Compound I, 1.08 g., was mixed with 75 ml. of dry toluene, and the mixture was boiled for 5 min., then cooled immediately and filtered. The infrared spectrum of the filtrate exhibited a strong absorption peak at 4.45 μ (characteristic of the isocyanate group). This peak was unaltered after 1.5 hr. standing, but disappeared almost completely after 60 hr.

Derivatives of Bis(1-aziridinyl)phosphinyl Urea (IV–VI).—A sample of the pyrolysate (see above) was treated immediately with a solution of cyclohexylamine in toluene. The solution was evaporated to dryness, and the oily residue was recrystallized from a mixture of benzene and cyclohexane. The white solid obtained, m.p. 250–252° dec., had an infrared spectrum compatible with the structure of N-[bis(1-aziridinyl)phosphinyl]-N′-cyclohexylurea (IV). Characteristic λ_{max} (μ): 2.95 (N—H); 3.35 and 3.45 (C—H); 6.00 (C=O); 6.50, 6.90 (CH_2); 7.90 (P=O); 8.35 (C—N), 9.50; 10.5 (P—N⟨); 10.95; 12.15; 13.2, 14.6 (P—N). Ethylenimine titration result: 95.5% of the theoretical.

Anal. Calcd. for $C_{11}H_{21}N_4O_2P$: N, 20.59. Found: N, 20.58; 20.49.

Two other urea derivatives were prepared by treating the corresponding N′-substituted dichlorophosphinylureas with ethylenimine and triethylamine in a manner analogous to the synthesis of I. The intermediates used in these reactions, N-dichlorophosphinyl-N′-phenylurea (XVI) and N-dichlorophosphinyl-N′-ethyl-N′-phenylurea (XVII), respectively, were described by Kirsanov and Levchenko.[14] The products obtained, N-[bis(1-aziridinyl)phosphinyl]-N′-phenylurea (V), m.p. 250° dec., and N-[bis(1-aziridinyl)phosphinyl]-N′-ethyl-N′-phenylurea (VI), m.p. 90–93°, respectively, were identified by their infrared spectra, similar to IV, above. (For VI, λ_{max} (μ); 2.95, 3.35, 6.00, 6.25, 6.70, 6.90, 7.25, 7.90, 8.35, 8.82, 10.6, 12.2, 14.4.) The elemental analyses for C, H, N, and P were not quite satisfactory, but they were all within 1% of the calculated values.

N,N,N′,N′-Tetraethyl-N″-Carbethoxyphosphoramide (VII).—A solution of 29.2 g. (0.4 mole) of diethylamine in 200 ml. of dry toluene was added to a solution of 41.2 g. (0.2 mole) of dichlorophosphinylurethan (XIII) in toluene. The latter was prepared by the reaction of 32 g. (0.2 mole) of dichloroisocyanatophosphine oxide (XII) with ethanol in toluene solution, according to the method described in the preparation of I. During the addition, which was completed in 1.5 hr., the mixture was stirred vigorously and cooled at 0°. Then the solution was allowed to warm to room temperature; the solid formed was separated by filtration and washed with dry acetone. Evaporation of the filtrate to dryness yielded 17 g. of an oily residue. This material was chromatographed through a "Florisil" column using cyclohexane as the solvent; however, no separation was obtained. The filtrate from the column was concentrated to dryness, and the residue was distilled at 1–2 mm. pressure. After a forerun, three fractions were collected between 96° and 110°. All three showed strong absorption bands in their infrared spectra of 4.45 μ (isocyanate) and 5.78 μ (ester carbonyl), and all

except the first fraction also showed a weak absorption band at 6.03 μ (amide carbonyl); the distillation residue exhibited a very strong absorption peak at the latter wave length. Since only the first fraction (b.p. 96–97° (1.25 mm.)) appeared to be free of the "amide" impurity, this material was dissolved in a mixture of 15 ml. of benzene and 4 ml. of absolute ethanol and allowed to react with the alcohol at room temperature for 20 hr. The mixture was then concentrated to dryness. A colorless oil was obtained which had an infrared spectrum compatible with the structure of VII; yield, 3.5 g. (17%), $n^{25}D$ 1.458, d_{25} 1.060.

Anal. Calcd. for $C_{11}H_{26}N_3O_3P$: C, 47.30; H, 9.38; N, 15.04; P, 11.09. Found: C, 46.92; H, 9.60; N, 15.39; P, 11.03.

In one experiment, the reaction mixture was distilled at 5 mm. pressure, and the first distillate (b.p. 130°), on standing, deposited a small quantity of long, white needles. This crystalline material was separated by filtration and washed with petroleum ether. After drying, the compound obtained, m.p. 119–120°, was identified as *N,N,N′,N′-tetraethyl-phosphoramide* (IX), by its infrared spectrum (absence of carbonyl, presence of NH_2 absorption) and by its elemental analysis.

Anal. Calcd. for $C_8H_{22}N_3OP$: C, 46.36; H, 10.70; N, 20.27; P, 14.94. Found: C, 46.41; H, 10.74; N, 19.87; P, 14.28.

Attempted Preparation of N,N,N′,N′-Tetramethyl-N″-carbethoxyphosphoramide (X).—A toluene solution of dichlorophosphinylurethane (XIII) was prepared from 24 g. (0.15 mole) of dichloroisocyanatophosphine oxide (XII) as described in the preparation of I. Infrared spectrum of an aliquot of the solution indicated the presence of an ester carbonyl (5.8 μ) and absence of the isocyanate group (4.45 μ). The solution was cooled again to 0° and a cold solution of 30.3 g. (0.3 mole) triethylamine in 100 ml. of dry toluene was added during 10 min. Then a slow stream of dimethylamine was passed through the mixture for 1 hr. while the temperature was kept at −5°. The mixture was then allowed to warm to room temperature and to stand overnight. The solid formed was collected by filtration, and, after drying *in vacuo*, it was identified as dimethylamine hydrochloride. The filtrate was concentrated to dryness, and the residue was triturated with cyclohexane. A yellow solid was separated which was recrystallized from hot cyclohexane with the addition of charcoal. A white solid (7.5 g.) was obtained, m.p. 107–108°. The infrared spectrum showed the presence of an *amide* carbonyl (6.0 μ) and the absence of ester carbonyl, indicating that, instead of the desired carbamate, a ureide was obtained. The elemental analysis of this compound was in agreement with the molecular formula of N,N,N′,N′-tetramethyl-N″-(dimethylcarbamyl)phosphoramide (XI).

Anal. Calcd. for $C_7H_{19}N_4O_2P$: C, 37.83; H, 8.62; N, 25.21; P, 13.94. Found: C, 38.05; H, 8.83; N, 25.67; P, 14.45.

In another attempt to synthesize X, only one-half of the calculated amount of dimethylamine was used in toluene solution, in an analogous manner as in the preparation of VII. A mixture of carbamate and carbamide was obtained which, however, could not be separated by the vacuum distillation procedure described in the preparation of VII.

Acknowledgment.—The authors wish to express their appreciation to Dr. Z. F. Chmielewicz and Mr. Robert D. Dillard for repeating some of the experiments and checking some of the results reported in this paper.