

Synthesis of Bis(aziridinyl)phosphinyl-*N*-hydroxyurethane Derivatives as Antineoplastic Agents†

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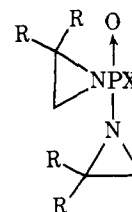
Several new "dual antagonists" were synthesized in which the 2,2-dimethyl (or ring C unsubstituted) aziridine phosphinyl function is linked to *N*-hydroxyurethane rather than the urethane moiety. Three of the new compounds showed very high activities against leukemia L1210 in mice.

Bis(2,2-dimethylaziridinyl)phosphinylurethane (1, AB-132), one of a series of "dual antagonists" synthesized and studied in our laboratory,¹⁻⁸ has been undergoing extensive clinical trials as an anticancer agent at the Roswell Park Memorial Institute.⁹⁻¹⁸ It appears that this drug potentiates the antitumor effects of X-irradiation clinically¹⁴⁻²⁰ as well as in animal experiments.^{20,21} In man, relatively small doses of X-irradiation produced short-term remission of solid tumors when administered with this drug.¹⁴⁻¹⁷ Furthermore, a recently concluded study of the treatment of bronchogenic carcinoma with this compound in combination with radiotherapy showed some encouraging long-term therapeutic effects.^{18,19} In view of these results, it appears of interest to establish the mechanism and structure-activity relationships relevant to the radiation-potentiating effect of this drug.

The structure of compound 1 contains two chemotherapeutically active moieties: the bis(2,2-dimethyl-1-aziridinyl)phosphinyl group, *i.e.*, a bifunctional alkylating moiety, and urethane; these are linked to each other by a P-N bond. In contrast to its ring-C-unsubstituted analog 2 (AB-100), the 2,2-dimethyl-substituted aziridine rings of 1 are rapidly hydrolyzed *via* carbonium ion mechanism,^{2,5} and the 2-amino-*tert*-butyl alcohol moieties formed were found to undergo P-N → P-O rearrangements, with simultaneous release of urethane.^{7,8} It has been reported by Boyland and Nery that urethane undergoes metabolic hydroxylation to *N*-hydroxyurethane (which they believed to be responsible for the carcinogenic effect of urethane).²² Since *N*-hydroxyurethane appears to be similar to *N*-hydroxyurea in its inhibitory action upon DNA biosynthesis,²³ and since the latter compound has been reported to be synergistic with X-irradiation in the clinical treatment of cancer patients,²⁴⁻²⁶ it is conceivable that the radiation-potentiating action of 1 may be due to its release of urethane which, in the presence of X-irradiation, could proceed through an "activated" form, probably similar to *N*-hydroxyurethane.

To test this hypothesis, we synthesized a new series of "dual antagonists" (3-6) in which the bifunctional alkylating moieties were linked to *N*-hydroxyurethane rather than urethane. Presumably, these compounds would release on hydrolysis directly *N*-hydroxyurethane rather than urethane and, therefore, may prove to be more effective antitumor agents when compared to the previously studied series in the absence of X-irradiation.

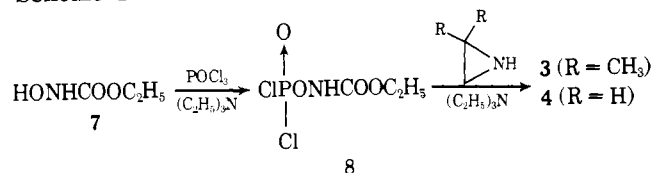
Chemistry. From a structural point of view, the hydroxyurethane moiety can be linked to a bis(1-aziridinyl)phosphinyl group either through a P-O or through a P-N bond. The hydroxyl group of *N*-hydroxyurethane (7) is more reactive than the amide group toward acylation²⁷ as well as alkylation.²⁸ Reaction of 7 with 1 mol equiv of



- 1, R = CH₃; X = NHCO₂C₂H₅ (AB-132)
- 2, R = H; X = NHCO₂C₂H₅ (AB-100)
- 3, R = CH₃; X = ONHCO₂C₂H₅ (AB-182)
- 4, R = H; X = ONHCO₂C₂H₅ (AB-180)
- 5, R = CH₃; X = N(OCOCH₃)CO₂C₂H₅ (AB-183)
- 6, R = H; X = N(OCOCH₃)CO₂C₂H₅ (AB-181)

POCl₃ (see Scheme I) gave the very unstable *O*-phosphorodichloridate 8 which was further allowed to react *in situ* with 2,2-dimethylaziridine, or aziridine, to give the desired products 3 and 4, respectively. Both reaction steps had to be conducted at very low temperatures (below -40°) and even then some polymerization occurred. For the separation of polymeric material, the only purification procedure that could be employed and gave satisfactory results was solvent extraction under special precautionary conditions (see Experimental Section); various chromatographic techniques were tried but invariably led to further polymerization. Both 3 and 4 are colorless liquids; both had the expected ir and nmr spectra which showed no impurities, but only 4 could be processed to elemental analysis without partial decomposition during handling. Samples of both 3 and 4 can be stored in the desiccator (CaCl₂) at 0-5° for several months without observable change (nmr) but compound 3 polymerizes on standing at room temperature for 1-3 days.

Scheme I



In order to link the *N*-hydroxyurethane moiety to the alkylating function through a P-N bond, it is necessary first to block the hydroxyl group. Thus, the known *O*-acetyl-*N*-hydroxyurethane²⁷ (9) could be reacted with POCl₃ (see Scheme II) to give the *N*-phosphorodichloridate 10 which (in contrast to 8) was quite stable and could be purified by vacuum distillation. Reaction of 10 with 2,2-dimethylaziridine or aziridine gave 5 and 6, respectively. These compounds are relatively stable and were readily obtained in high yields and analytical purity. However,

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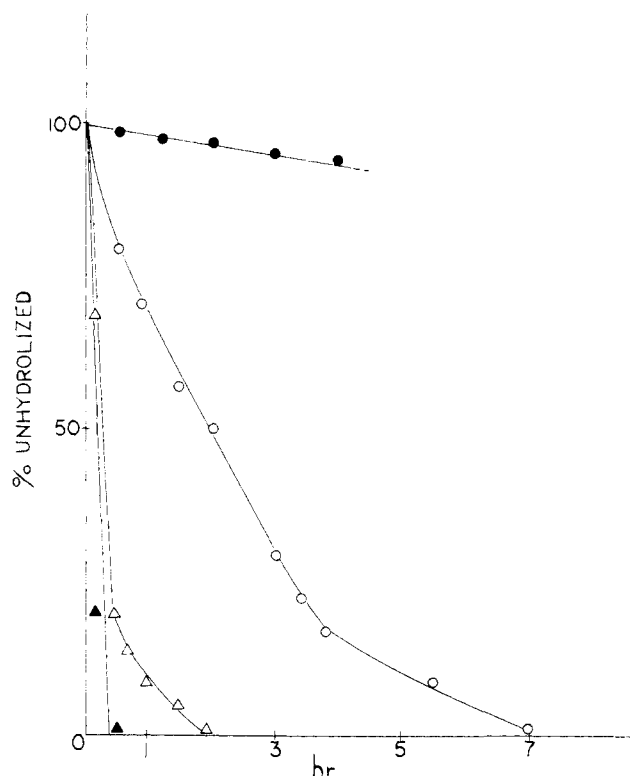
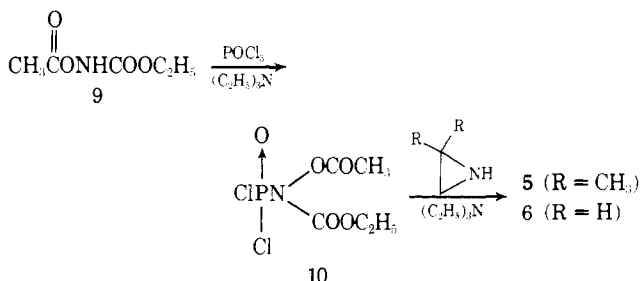


Figure 1. Rate of hydrolysis of compounds 3 (\blacktriangle), 4 (\bullet), 5 (\triangle), and 6 (\circ) in D_2O at 37° .

Scheme II



upon removal of the acetyl group (under the mildest conditions, see Experimental Section) the free *N*-hydroxy derivatives instantly polymerized with cleavage of the aziridine rings (nmr). The same end result was obtained when *O*-benzyl-*N*-hydroxyurethane²⁸ (11) was used as the starting material (see Scheme III) and converted in an analogous manner to the corresponding *O*-benzyl-*N*-phosphinyl derivative 12; upon removal of the benzyl group under hydrogenation conditions which normally preserve the aziridine rings, ring cleavage and polymerization occurred. [*p*-Nitrophenyl bis(2,2-dimethyl-1-aziridinyl)phosphinate could be reduced under these conditions to the corresponding *p*-amino derivative: Patello, Chmielewicz, and Bardos, unpublished results.] Therefore, it was concluded that the free (deblocked) *N*-hydroxyl group promotes the ring-opening reactions of the neighboring aziridine moieties.

The rates of hydrolysis (see Figure 1) of compounds 3-6 were studied by observance of the nmr spectral changes⁵ upon incubation of their solutions in D_2O at 37° for various lengths of time. Compounds 3 and 5 are hydrolyzed even more rapidly than other 2,2-dimethylaziridine derivatives,^{5,8} while 4 is comparable to its urethane analog 2 (AB-100)⁵ in being hydrolyzed at a much slower rate than the corresponding 2,2-dimethylaziridine. In contrast, the rate of hydrolysis of 6 is significantly faster than those of

other ring-C-unsubstituted aziridines (e.g., 2 or 4); the shift of the nmr peak corresponding to the acetyl protons (δ 2.21) indicates that the acetyl group is rapidly hydrolyzed, and this may promote the ring-opening hydrolysis of the aziridine rings (see above). All four compounds (3-6) yield *N*-hydroxyurethane as one of their hydrolysis products which can be extracted from the aqueous mixture with ether in pure form. However, only 23-42% of the theoretical amount of *N*-hydroxyurethane can be recovered in this manner even after complete hydrolysis.

Biological Results. The results of the biological testing of compounds 3-6 for acute toxicity and antitumor activity against leukemia L1210 in mice are summarized in Table I. These results show that compounds 3, 4, and 6 have outstanding chemotherapeutic activity in this assay system when given in a single dose 24 hr after tumor inoculation. It is of particular interest that these excellent antitumor effects were accomplished without significant weight loss or other observable toxicity.

Both 3 and 4 are much more effective agents against this tumor system than their urethane analogs AB-132 and AB-100, respectively. Compound 6 is also more effective than other ring-C-unsubstituted aziridine type alkylating agents (AB-100, TEPA, etc.), while 5 is relatively inactive compared to some other 2,2-dimethylaziridines (e.g., AB-163).⁶

It is apparent that the antitumor effects of these compounds cannot be correlated with their rates of hydrolysis; in the latter respect, the two highly effective agents, 3 and 4, represent the two extremes (see Figure 1). Neither does the relative amount of *N*-hydroxyurethane released (i.e., recovered from the hydrolysates) at the optimal dose level offer any clue with respect to the comparative biological activities of these agents, since the relatively ineffective compound 5 at 450 mg/kg dose releases about 30 times more hydroxyurethane than does compound 6 at its most effective dose of 13.5 mg/kg (see Table I). Thus, the relative biological activities of this particular series of antitumor agents cannot be satisfactorily explained either on the basis of general mechanistic considerations based purely on the rates of hydrolysis or, alternatively, on the basis of typical synergism between the alkylating and hydroxyurethane moieties.^{1,6} It is possible that the biological activity of these agents is enhanced by the formation of reactive intermediate rearrangement products involving the alkylating function as well as the bound (unrecovered, see above) portion of the *N*-hydroxyurethane moiety. Such

Scheme III

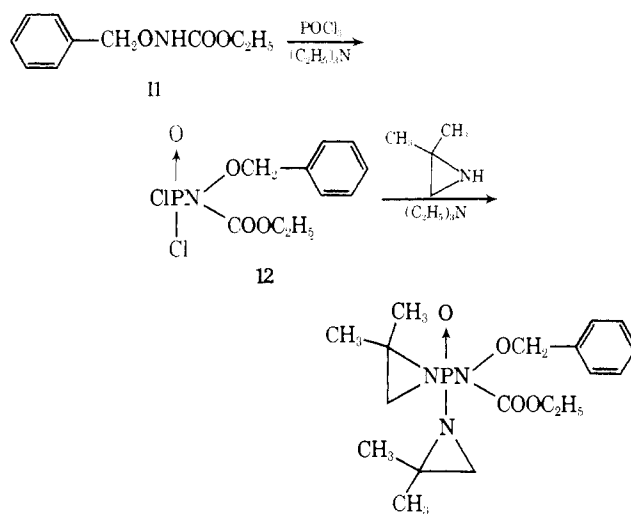


Table I. Activity of Compounds 3-6 in L1210 Leukemia

Compd	Toxicity			Tumor challenge and result						
	LD ₁₀ , mg/kg	LD ₅₀ , mg/kg	Dose, ^a mg/kg	A ^c (10 ⁴ cells)	B ^d	A ^c (10 ⁵ cells)	B ^d	A ^c (10 ⁶ cells)	B ^d	% wt change ^b
3	250	270	100	10.3 + 0.2	0	26.3 + 10.6 (1)	223	14.0 + 0.7	93	
			150	60.3 + 15.1 (4)	467	26.0 + 10.6 (1)	220	16.9 + 2.4	133	+11
			225	90.1 + 9.9 (7)	748	59.6 + 15.3 (4)	634	28.5 + 10.3 (1)	293	0
4	48	54	20	60.3 + 15.1 (4)	467	16.9 + 1.1	108	16.1 + 1.2	122	+3
			30	70.0 + 14.7 (5)	559	42.3 + 13.1 (2)	420	29.8 + 10.3 (1)	310	-1
			45	65.8 + 16.7 (5)	519	82.5 + 12.3 (6)	915	10.8 + 2.4	48	-3
5	410	560	200	12.6 + 0.4	19	11.1 + 0.5	37	9.8 + 0.7	35	
			300	22.4 + 11.1 (1)	111	13.7 + 0.7	68	10.5 + 0.6	59	
			450	15.5 + 2.5	46	15.5 + 0.6	91	11.7 + 1.7	61	+1
6	10	11	6	57.8 + 16.0 (4)	425	13.4 + 1.3	57	12.8 + 0.9	70	
			9	47.1 + 15.6 (3)	328	46.1 + 15.8 (3)	443	16.1 + 0.7	115	+9
			13.5	79.6 + 13.4 (6)	624	57.0 + 16.3 (4)	571	25.0 + 10.9 (1)	233	-13
Controls				10.6 + 0.8	0	8.1 + 0.1	0	7.3 + 0.3	0	
				11.0 + 0.2	0	8.5 + 0.3	0	7.5 + 0.2	0	

^aDrug given as a single ip injection 24 hr after tumor inoculation. ^bAt day 16. ^cColumn A: life span (days) + standard error of a group of eight mice. Number of 100-day survivors in parentheses. ^dColumn B: increased life span (%) over controls. The first control applies to compounds 3-5, the second to compound 6.

intermediates may have alkylating as well as phosphorylating activities.⁸ However, further studies are required to substantiate this hypothesis before proposing specific structures for any possible intermediate rearrangement products.

Experimental Section

Ir and nmr of all compounds were recorded and used for confirmation of structure. The ir spectra were taken in NaCl disk in a Beckman IR-8; nmr spectra were recorded on Varian-60 in CDCl₃ using TMS as standard. Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga., and Galbraith Lab., Inc., Knoxville, Tenn.

***O*-(Dichlorophosphinyl)-*N*-hydroxyurethane (8).** To a three-neck round-bottom flask (250 ml, equipped with thermometer, pressure-equalizing addition funnel, and drying tube) was introduced a solution of POCl₃ (8.754 g, 57 mmol), in 150 ml of dry THF, under cooling at -50 to -60° (Dry Ice-acetone bath). A solution of *N*-hydroxyurethane (6.0 g, 57 mmol) and triethylamine (5.778 g, 57 mmol) in 50 ml of THF was added dropwise, keeping the reaction mixture below -40°. After addition was completed, the reaction mixture was stirred for 2 hr. The precipitated triethylamine hydrochloride (7.0 g) was collected by filtration and the filtrate was immediately used for the next reaction step.

The dichloridate is quite unstable. After standing at room temperature for several hours, evolution of HCl gas could be observed and polymerization occurred.

***O*-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-*N*-hydroxyurethane (3).** To a three-neck round-bottom flask (500 ml, equipped with thermometer, pressure-equalizing addition funnel, and drying tube) was introduced a solution of 2,2-dimethylaziridine (8.122 g, 114 mmol) and triethylamine (11.556 g, 114 mmol) in 150 ml of THF. The mixture was cooled on a Dry Ice-acetone bath (-35 to -45°). A solution of 8 (57 mmol) was added dropwise. The reaction mixture was kept below -35°. After addition was completed, the reaction mixture was stirred at this temperature for 2 hr. The precipitated triethylamine hydrochloride was separated by filtration and the solution was evaporated. The liquid residue was dissolved in 100 ml of ether and separated from undissolved viscous residue by decantation, followed by filtration in a polyethylene glove chamber containing CaCl₂. The solution was concentrated, and after evaporation of residual traces of solvent *in vacuo* for 3 days (at 5-7°), 5.78 g (35%) of pure product (3) was obtained as a colorless liquid: nmr δ 1.21 (t, 3 H), 1.39 (s, 12 H), 2.09 (d, *J* = 13 Hz, 4 H), 4.07 (q, 2 H); ir (neat) 3200 (NH), 1710 (C=O), 1375, 1380 [C(CH₃)₂], 1260 (P=O), 960 cm⁻¹ (P-N).

***O*-[Bis(1-aziridinyl)phosphinyl]-*N*-hydroxyurethane (4).** The procedure described for the preparation of 3 was employed, but 2,2-

dimethylaziridine was replaced by aziridine. The liquid residue was dissolved in 100 ml of CHCl₃ and then added 10 ml of ether. The solution was chilled in refrigerator overnight and separated from undissolved polymeric residue by decantation, followed by filtration (as in the case of 3). The solution was evaporated and dried *in vacuo* to obtain 7.62 g (56%) of pure liquid product (4): nmr δ 1.25 (t, 3 H), 2.19 (d, *J* = 14 Hz, 8 H), 4.14 (q, 2 H); ir (neat) 3200 (NH), 1718 (C=O), 1262 (P=O), 930 cm⁻¹ (P-N). *Anal.* (C₇H₁₄N₃O₄P) C, H, N; calcd, 17.87; found, 17.05.

***N*-(Dichlorophosphinyl)-*O*-acetyl-*N*-hydroxyurethane (10).** A solution of POCl₃ (13.16 g, 86 mmol) in 150 ml of ether cooled on an ice bath (0-5°) was treated slowly with a solution of *O*-acetyl-*N*-hydroxyurethane²⁷ (11.48 g, 78 mmol) and triethylamine (8.683 g, 85.8 mmol) in 50 ml of ether. After the addition was completed, the reaction mixture was stirred at 5-7° for 20 hr. The precipitated triethylamine hydrochloride was collected by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was distilled *in vacuo* and the fraction distilling at 92° (0.5 mm) was collected: yield 11.88 g (57%); nmr δ 1.36 (t, 3 H), 2.28 (s, 3 H), 4.42 (q, 2 H). This compound is highly hygroscopic and was used immediately for the next reaction step.

***N*-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-*O*-acetyl-*N*-hydroxyurethane (5).** The procedure is the same as described in the synthesis of 3, except that the reaction was conducted at -15 to -10°, and ether was used as solvent. After addition, the reaction mixture was stirred at this temperature for 4 hr and then warmed to room temperature. From 6.5 g (24.6 mmol) of 10, a total of 7.09 g (21.2 mmol, 86%) of 5 was obtained as a colorless liquid: nmr δ 1.31 (t, 3 H), 1.45 (s, 12 H), 2.1-2.42 (m, methylene and acetyl protons, 7 H), 4.30 (q, 2 H); ir (neat) 1800 [NO-C(=O)CH₃], 1740 [C(=O)OC₂H₅], 1280 (P=O), 965 cm⁻¹ (P-N). *Anal.* (C₁₃H₂₄N₃O₅P) C, H, N.

***N*-[Bis(1-aziridinyl)phosphinyl]-*O*-acetyl-*N*-hydroxyurethane (6).** The procedure is the same as described in synthesis of 5, but 2,2-dimethylaziridine was replaced by aziridine. From 5.0 g (19 mmol) of 10, a total of 4.1 g (78%) of the pure product (6) was obtained as a colorless liquid: nmr δ 1.36 (t, 3 H), 2.21 (s, 3 H), 2.41 (d, *J* = 16 Hz, 8 H), and 4.30 (q, 2 H); ir (neat) 1800 [NO-C(=O)CCH₃], 1735 [C(=O)OC₂H₅], 1285 (P=O), 935 cm⁻¹ (P-N). *Anal.* (C₉H₁₆N₃O₅P) C, H, N.

Attempted Deacetylation of 5. To a solution (10 ml) of NH₃ in absolute ethanol or ether (prepared by bubbling NH₃ into ethanol or ether at 0° for 5 min) was added a solution of 5 (1.0 g) in 5 ml of absolute ethanol. The solution was stirred at room temperature for 3 hr and then evaporated *in vacuo* at room temperature to dryness. The residue was mixed with 20 ml of ether and stirred for 20 min. A small amount of white solid was removed by filtration. The ethanol solution contained a very viscous material. The nmr spectrum showed that aziridine ring was opened and polymerized. This product could not be purified by column chroma-

Table II. Recovery of *N*-Hydroxyurethane from Hydrolysates

Compd	Amt dissolved, g (mmol)	H ₂ O, ml	Time, days	% recovery
3	2.0 (6.9)	30	3	25
4	1.5 (6.4)	30	14	23
5	1.6 (5)	20	3	42
6	1.2 (4.4)	20	3	26
Control (7)	0.526 (5)	20	3	92

topography or distillation. The result was the same when 1 equiv of morpholine was used for deacetylation.

N-(Dichlorophosphinyl)-*O*-benzyl-*N*-hydroxyurethane (12). The procedure is the same as described in the preparation of 10, except that an equivalent amount of the POCl₃, triethylamine, and *O*-benzyl-*N*-hydroxyurethane²⁸ (11) was used and that the product was not purified by distillation but used immediately for the next reaction step.

N-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-*O*-benzyl-*N*-hydroxyurethane (13). The procedure is the same as described for synthesis of 5. From 25 mmol of 11, an overall yield of 7.2 g (74%) of 13 (crude) was obtained. This crude product was chromatographed on neutral silica gel (300 g) in the cold room (3–7°) with chloroform as the eluent; yield, 1.13 g of pure product; colorless liquid; nmr δ 1.30 (t, 3 H), 1.42 (s, 12 H), 2.31 (d, $J = 14$ Hz, 4 H), 4.27 (q, 2 H), 5.09 (s, 2 H), and 7.37 (br, 5 H); ir (neat) 1725 (C=O), 1275 (P=O), 964 cm⁻¹ (P-N). *Anal.* (C₁₈H₂₈N₃O₄P) C, H, N.

Attempted Debenzylation of 13 by Hydrogenation. Compound 13 (300 mg) was dissolved in THF (20 ml) and 100 mg of Pd/C (10%) was added as catalyst. The mixture was treated with H₂ at 50 psi for 18 hr. The solution was filtered, and the filtrate was evaporated. The nmr spectra showed that the aziridine rings were opened and polymerized. When the reaction time was shortened from 18 to 3 hr, only starting material was recovered.

Hydrolysis Studies. (a) The rates of hydrolysis (see Figure 1) were estimated by dissolving the compounds in D₂O and measuring the per cent decrease of the nmr absorption peaks corresponding to the aziridine methylene protons (δ 2.1–2.4) after incubation at 37° for various lengths of time.

(b) The determination of the hydrolysis product was done in the following manner. Each compound was mixed with deionized water and stirred at 37° (see Table II). The solution was extracted with ether (3 × 30), then lyophilized, and again extracted with 30 ml of ether.

The combined ether extracts were dried over MgSO₄, and the solvent was removed under reduced pressure. The nmr and ir spectra of the liquid residue were found to be identical with those of *N*-hydroxyurethane. The “% recovery” data given in Table II are based on the theoretical amount of *N*-hydroxyurethane present in the solution in bound and/or free form.

Biological Testing. For LD₁₀ and LD₅₀ determinations, incremental logarithmic doses each 1.15 times the starting dose were administered ip to BDF₁ female mice which were individually weighed for calculation of dose. Animals were then observed 50 days for deaths. The results were plotted on a logarithmic probability graph. Results were read from the best line of fit. Animals receiving lethal doses usually die in 5–7 days; however, for compound 5 one-half of the animals died acutely.

Antitumor activity was then tested in L1210 leukemia, the standard animal tumor screening system. This tumor is carried in DBA/2 mice by weekly ip passage of 10⁵ cells. The test animals, BDF₁ female mice, each received 10⁴, 10⁵, or 10⁶ tumor cells ip and were observed for life span.

Drugs were dissolved in normal saline and were injected intraperitoneally on day 1, 24 hr after tumor inoculation. Mean survival time was calculated according to NIH Protocol 11²⁹ and is given in Table I.

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References

- (1) T. J. Bardos, Z. B. Papanastassiou, A. Segaloff, and J. L. Ambrus, *Nature (London)*, **183**, 399 (1959).
- (2) T. J. Bardos, *Biochem. Pharmacol.*, **11**, 256 (1962).
- (3) Z. B. Papanastassiou and T. J. Bardos, *J. Med. Pharm. Chem.*, **5**, 1000 (1962).
- (4) T. J. Bardos, A. K. Barua, Z. F. Chmielewicz, G. E. Crevar, J. P. Dailey, S. Divald, and Z. B. Papanastassiou, *J. Pharm. Sci.*, **54**, 187 (1965).
- (5) T. J. Bardos, Z. F. Chmielewicz, and K. Navada, *J. Pharm. Sci.*, **54**, 399 (1965).
- (6) T. J. Bardos, Z. F. Chmielewicz, and P. Hebborn, *Ann. N. Y. Acad. Sci.*, **163**, 1006 (1969).
- (7) C. K. Navada, Z. F. Chmielewicz, and T. J. Bardos, *J. Pharm. Sci.*, **59**, 1079 (1970).
- (8) D. Lalka and T. J. Bardos, *Biochem. Pharmacol.*, in press.
- (9) C. A. Ross, J. E. Sokal, J. L. Ambrus, D. V. Razis, and T. J. Bardos, *Cancer Chemother. Rep.*, **3**, 263 (1961).
- (10) A. L. Watne, G. E. Moore, C. A. Ross, and J. L. Ambrus, *Cancer Chemother. Rep.*, **16**, 421 (1962).
- (11) C. A. Ross, J. L. Ambrus, J. E. Sokal, H. A. Velasco, L. Stutzman, and D. V. Razis, *Cancer Chemother. Rep.*, **18**, 27 (1962).
- (12) L. Stutzman, D. V. Razis, J. E. Sokal, J. L. Ambrus, and C. A. Ross, *Cancer Chemother. Rep.*, **18**, 31 (1962).
- (13) B. D. Delta, C. M. Ambrus, J. L. Ambrus, and L. Stutzman, *Cancer Chemother. Rep.*, **18**, 37 (1962).
- (14) C. A. Ross, H. A. Velasco, J. E. Sokal, J. L. Ambrus, L. Stutzman, J. Webster, and T. J. Bardos, *Proc. Amer. Ass. Cancer Res.*, **3**, 355 (1962).
- (15) A. L. Watne, M. A. Bozzini, F. A. Mukhtar, F. C. Marchetta, and G. E. Moore, *Cancer Chemother. Rep.*, **25**, 101 (1962).
- (16) L. Castro and M. M. Hreshchyshyn, *Obstet. Gynecol.*, **23**, 551 (1964).
- (17) H. A. Velasco, C. A. Ross, J. H. Webster, J. E. Sokal, L. Stutzman, and J. L. Ambrus, *Cancer (Philadelphia)*, **17**, 841 (1964).
- (18) J. H. Webster, J. Sokal, C. A. Ross, H. A. Velasco, J. L. Ambrus, and L. Stutzman, *Cancer Bull.*, **22**, 59 (1970).
- (19) T. J. Bardos, J. L. Ambrus, and C. M. Ambrus, *J. Surg. Oncol.*, **3**, 431 (1971).
- (20) W. Regelson, O. Pierucci, J. Hananian, M. Bozzini, C. M. Ambrus, T. J. Bardos, and J. L. Ambrus, *J. Med.*, in press.
- (21) W. Regelson and O. Pierucci, *Radiat. Res.*, **22**, 368 (1964).
- (22) E. Boyland and R. Nery, *Biochem. J.*, **94**, 188 (1965).
- (23) C. W. Young, G. Schochetman, S. Hodas, and E. Balis, *Cancer Res.*, **27**, 535 (1967).
- (24) G. J. Richards and R. G. Chambers, *Amer. J. Roentgenol.*, **105**, 555 (1969).
- (25) H. J. Lerner, G. L. Beckloff, H. Lipshutz, R. Campbell, and D. Ritchie, *Plast. Reconstr. Surg.*, **40**, 233 (1967).
- (26) M. M. Hreshchyshyn, *Cancer Chemother. Rep.*, **52**, 601 (1968).
- (27) E. Boyland and R. Nery, *Analyst*, **89**, 520 (1964).
- (28) B. J. Ludwig, F. Dursch, M. Auerbach, K. Tomeczek, and F. M. Berger, *J. Med. Chem.*, **10**, 556 (1967).
- (29) *Cancer Chemother. Rep., Part 3*, **3** (2), 47 (1972).