solving 20 g of polyvinylpyrrolidone and 500 mg hexamethonium chloride in 100 mL of H_2O. *Crinia* angiotensin showed 90.6 ± 4.99% (n = 26, 7 rats) of the pressor activity of [Ile^5]angiotensin II.

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Synthesis of 5′-Thymidinyl Bis(1-aziridinyl)phosphinates as Antineoplastic Agents

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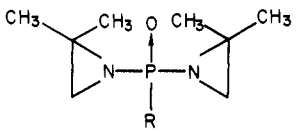
Reaction of 3′-acetylthymidine with phosphorus oxychloride in trimethyl phosphate yielded the phosphorodichloridate **5**, which was subsequently reacted with aziridine or 2,2-dimethylaziridine to give compounds **6** and **7**, respectively. The 2,2-dimethylaziridine derivative **7** was considerably more active than **6** against leukemias L1210 and P-388 in mice but less active than the previously synthesized, simpler phosphinate derivatives **2** and **3**. It appears that the thymidine moiety did not enable these compounds to use the nucleoside transport mechanism of the cells and also failed to increase the selectivity of the 2,2-dimethylaziridine analogues by interference with their binding to cholinesterase. Compound **7** strongly inhibited horse serum cholinesterase, while **6** was inactive.

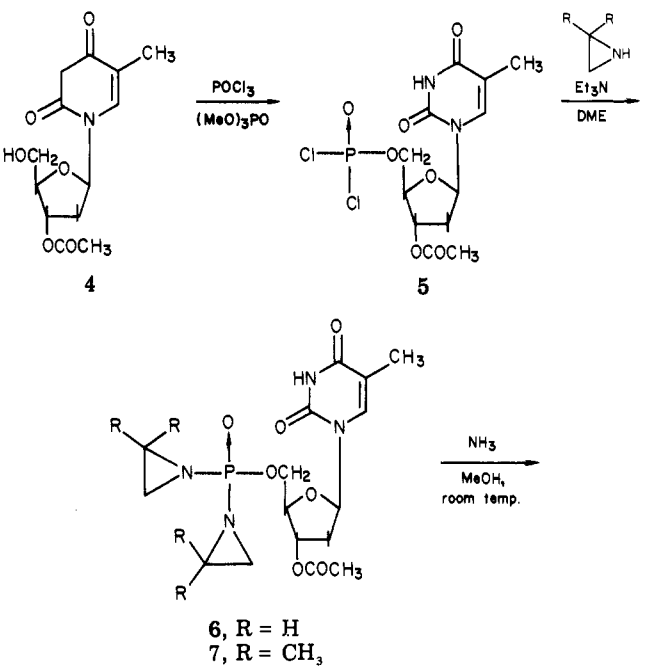
Several compounds termed "dual antagonists"[1] containing the bis(2,2-dimethylaziridinyl)phosphinyl moiety showed significant antitumor activities[2,3] and, in addition, demonstrated some marked radiation potentiating,[4-6] as well as cholinesterase inhibitory,[7] effects which were attributed to the potential phosphorylating activities of their transient hydrolysis products having five-membered oxaphospholidine ring moieties.[8] Characteristically, the 2,2-dimethyl-substituted phosphoraziridines (**1–3**) showed



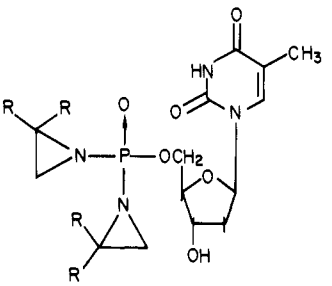
1 (AB-132),[1,2] R = $NHCO_2C_2H_5$
2 (AB-163),[2] R = OC_2H_5
3 (AB-182),[3] R = $ONHCO_2C_2H_5$

relatively little or no hematologic toxicity in animal experiments as well as in the clinical studies;[4-9] instead, gastrointestinal and CNS toxicities related to cholinesterase inhibition appeared to be their dose-limiting side effects.

Scheme I



6, R = H
7, R = CH_3



8, R = H
9, R = CH_3

In an effort to increase the selectivity of action of these agents on the DNA template,[8] we decided to link their common reactive moiety to the 5′ position of thymidine. Compounds **7** and **9** (Scheme I) represent the desired "DNA-targeted" 2,2-dimethylphosphoraziridines, while compounds **6** and **8** are the corresponding ring-C-unsub-

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stituted aziridine derivatives which were expected to possess alkylating, but not phosphorylating, activities and were synthesized mainly for the sake of comparison.

Results and Discussion

Chemistry. The syntheses of compounds **6–9** were accomplished as outlined in Scheme I. 3′-Acetylthymidine (**4**)[10] was converted to the phosphorodichloridate **5**; complete removal of the excess phosphorus oxychloride reagent and of the hydrogen chloride byproduct, under high vacuum, was found to be a critical step in the procedure. Compound **5** was then immediately reacted with the appropriate aziridine in the presence of triethylamine to give the 3′-acetyl-blocked title compounds **6** and **7** which were isolated and purified as described under Experimental Section. Actually, these derivatives were used in most of the studies reported below, because the free 3′-hydroxyl compounds, **8** and **9** (which were obtained by deacetylation with methanolic ammonia at room temperature), proved to be very unstable and difficult to handle.

Cholinesterase Inhibitory Activity. Using our previously described[7] in vitro assay with slight modifications (see Experimental Section), compound **7** at 1.4×10^{-4} M concentration completely inhibited the horse serum cholinesterase almost immediately (2 min contact time or less). Thus, this compound appeared to bind to the enzyme at least as strongly and more rapidly than its ethyl phosphinate analogue, AB-163 (**2**)[7].

Since thymidine itself, at the same molar concentration, had no effect on the enzyme, one must assume that compound **7** is much more rapidly and effectively converted to a hydrolytic intermediate having phosphorylating activity[8] than were the previously studied 2,2-dimethl-substituted phosphoraziridines, including **2**. Our previously proposed mechanism for the formation of a reactive phosphorylating intermediate requires (1) protonation, (2) opening of one of the aziridine rings to give rise to a tertiary carbonium ion, and (3) recyclization of the latter into a five-membered oxaphospholidine ring.[8] The last step could be accelerated intramolecularly by a third group attached to the phosphorus capable of either increasing the polarization of the P→O bond, or, alternatively, undergoing rapid hydrolysis resulting in the formation of a P—O^- anion. It is possible that the hydrolytic transformation of **7** to the oxaphospholidine intermediate is triggered by rapid hydrolysis of the 5′-phosphinate linkage. Incubation of **7** in water at 37 °C indeed resulted in the progressive liberation of thymidine (identified by TLC); however, this hydrolysis reaction required more than 15 min for completion which was probably not fast enough to account for the immediate inhibitory action of **7** in the cholinesterase assay. Therefore, it appears that the vicinity of the uracil nucleus may exert some additional effect on the hydrolytic transformation of the phosphoraziridine moiety. At any rate, the unexpectedly high anticholinesterase activity of **7** negated already at the molecular level one of the purposes of its design, i.e., to increase selectivity by increasing the strength of binding to DNA relative to cholinesterase. In contrast, compound **6** did not show any appreciable cholinesterase inhibitory activity, in accordance with our expectation.

Antitumor Activity. Compounds **6–9** were tested in the screening program of the National Cancer Institute (NCI) against leukemia L1210 mice; only **6** and **7** were tested in the P-388 leukemia screen. In addition, compounds **6** and **7** were also tested by Dr. G. Wampler (Virginia Commonwealth University) against leukemia L1210 at higher dose levels. The results are shown in Table I. Only compound **7** showed dose-dependent activity against leukemia L1210 which was significant at the highest dose tested (500 mg/kg). Against the P-388 leukemia, both **6** and **7** showed activity at 200 mg/kg, but only **7** was significantly active also at the 100 and 50 mg/kg dose levels.

Table I. In Vivo Screening Results against Experimental Leukemias in Mice[a]

compd	NSC no.	screener	dose, mg/kg	survival, % T/C	
				L1210	P-388
6	200726	NCI	200	112	142
			100		124
			50	107	112
			25		104
		W[b]	500	111	
			250	111	
7	200727	NCI	200	123	154
			100	112	141
			50	110	138
			25		110
		W[b]	500	137	
			250	125	
8	200728	NCI	200	94	
			100	100	
			50	107	
9	200729	NCI	200	124	
			100	105	
			50	94	

[a] NCI standard assay systems. [b] Tests by Dr. G. L. Wampler were also conducted according to the NCI standard assay guidelines.

The relatively low antitumor activities of these compounds as compared to those of **1–3** were disappointing. It has been hoped that thymidine would serve as an effective carrier for the phosphoraziridine moieties across the cell membranes and that the overall structural similarity of these derivatives to some nucleotide intermediates and substrates of enzymes involved in DNA metabolism might have a directing influence on the intracellular localization and selectivity of action of these agents. However, some recent studies of Dr. M. Rabinovitz[11] of the National Cancer Institute may help to explain the above results. In his in vitro L1210 system,[12] both compounds **6** and **7** were noncytotoxic at 0.1 mM, but **7** became cytotoxic at a concentration of 0.2 (70% survival) and 0.4 mM (20% survival). At these concentrations the cells could not be protected with high-affinity nucleoside substrates such as adenosine of 6-benzylaminopurine riboside. The failure to obtain protection may indicate that **7** cannot utilize the nucleoside transport system and that its cytotoxicity at relatively high concentrations is associated with passive diffusion of the compound into the cells. In the latter case, the thymidine moiety would be an obviously poor transport carrier and would hinder rather than promote the cellular uptake of these phosphoraziridines.

Based on the above considerations, we are now preparing similar derivatives containing the adenosine moiety, because the purine nucleosides are known to have a much higher affinity for the nucleoside transport system.[11] Alternatively, a carbamate linkage between the nucleosides and the phosphoraziridine moieties may provide more favorable transport properties via diffusion than the simple phosphinate ester linkage and, in addition, may diminish

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the interaction of the thymine and phosphoraziridine moieties. Therefore, the corresponding carbamate derivatives will be synthesized and investigated.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. NMR spectra were obtained on Varian A-60 and T-60 spectrometers in deuterated chloroform solution, using tetramethylsilane as internal standard. Infrared spectra were taken in a Beckman IR-8 spectrometer. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, or by Galbraith Laboratories, Inc., Knoxville, TN.

5′-(Dichlorophosphinyl)-3′-acetylthymidine (5). To a solution of $POCl_3$ (4.904 g, 32 mmol) in $(MeO)_3PO$ (10 mL), cooled in an ice bath, was added 3′-acetylthymidine (**4**;[10] 4.548 g, 16 mmol). The reaction mixture was stirred in a cold room (4–7 °C) for 16 h. The excess $POCl_3$ and the HCl formed were removed under high vacuum (0.5–1 mmHg) at room temperature for 4 h. The resulting solution was immediately used for the next reaction step.

5′-[Bis(1-aziridinyl)phosphinyl]-3′-acetylthymidine (6). To a three-neck round-bottom flask (100 mL, equipped with thermometer, drying tube, and pressure equalized additional funnel) was introduced a solution of aziridine (1.724 g, 50 mmol) and triethylamine (4.048 g, 40 mmol) in 40 mL of 1,2-dimethoxyethane (DME). The container was cooled to 0–5 °C on an ice bath. The solution of **5** (16 mmol) in 10 mL of $(MeO)_3PO$ (see above) was added dropwise. The reaction temperature was maintained below 9 °C. After the addition was completed, the reaction mixture was stirred in a cold room (4–7 °C) for 42 h. The precipitated solids were collected by filtration and washed with DME (20 mL) and then with ether (20 mL). The precipitate was extracted with DME (300 mL), and the extracted solution was concentrated to 10 mL at room temperature under reduced pressure. The residue was chilled overnight in the refrigerator to obtain 2.05 g (overall yield, 31%) of **6** as white crystals: mp 183–185 °C; NMR ($CDCl_3$) δ 7.54 (br, 1 H), 6.30 (q, J = 6 Hz, 1 H), 5.31 (m, 1 H), 4.38 (m, 2 H), 4.15 (m, 1 H), 2.25, 2.07 (d, J = 15 Hz, 13 H, methylene protons of aziridine rings, 2′-CH_2 of thymidine and CH_3 of acetyl), 1.93 (d, J = 1, Hz, CH_3); IR (KBr) 1730 (ester), 1675 (amide), 830 cm^{-1} (P-N); $[\alpha]^{25}_D$ +4.45° (c 1.12, $CHCl_3$). The analytical sample was recrystallized from DME. Anal. ($C_{16}H_{23}N_4O_7P$) C, H, N, P.

5′-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-3′-acetylthymidine (7). This compound was synthesized by the same method as described for the preparation of **6**. From 4.54 g (16 mmol) of compound **4** was obtained 3.4 g (7.2 mmol, 45%) of **7**: mp 171–173 °C; NMR ($CHCl_3$) δ 7.61 (br, 1 H), 6.43 (q, J = 6 Hz, 1 H), 5.35 (m, 1 H), 4.30 (m, 3 H), 2.21 (d, J = 14 Hz, 6 H; methylene protons of aziridines and 2′-CH_2 of thymidine), 2.12 (s, 3 H; acetyl), 1.95 (br, 3 H), 1.46 (s, 12 H; methyl protons of aziridine rings; IR (KBr) 1732 (ester), 1680 (amide), 1390, 1380 [$C(CH_3)_2$], 1270 (P=O), 955 (PN) cm^{-1}; $[\alpha]^{25}_D$ +7.14° (c 0.98, $CHCl_3$). The analytical sample was recrystallized from DME. Anal. ($C_{20}H_{31}N_4O_7P$) C, H, N, P.

5′-[Bis(1-aziridinyl)phosphinyl]thymidine (8). To 15 mL of absolute MeOH saturated with ammonia (10 mL) in an ice bath was added compound **6** (800 mg, 1.9 mmol). The solution was warmed up to room temperature, stirred for 4 h, and then evaporated to dryness. The residue was dissolved in $CHCl_3$ (10 mL), and dry ether (10 mL) was added until precipitation was observed. The solution was chilled in the refrigerator overnight; the precipitate was collected and dried under vacuum to give 0.50 g of **8** (1.3 mmol, 67%) as a foamy solid: mp 61–64 °C; NMR ($CDCl_3$) δ 7.47 (s, 1 H), 6.35 (t, J = 6 Hz, 1 H), 4.0–4.7 (two broad bands, 4 H), 2.32 (d, J = 15 Hz, 8 H, CH_2), 1.90 (s, 3 H, CH_3); IR (KBr) 1675 (CONH), 1266 (P=O), 930 (PN) cm^{-1}. The product was highly hygroscopic and difficult to analyze; therefore, its identification was based on the NMR spectra. The latter showed well-resolved peaks (see above) which integrated satisfactorily in the case of the freshly prepared sample; however, the spectra became blurred after a few days storage (even at refrigeration temperature), indicating polymerization.

5′-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]thymidine (9). Compound **7** was deacetylated in the same manner as described for the preparation of **8**. From 800 mg (1.7 mmol) of compound **7** was obtained 0.433 g (yield 57%) of the deblocked compound **9**: mp 50–53 °C; NMR ($CDCl_3$) δ 7.46 (s, 1 H), 6.36 (t, 1 H), 4–4.6 (br m, 4 H, 3′-CH, 4′-CH, 5′-CH_2), 2.17 (d, J = 14 Hz, 6 H, CH_2), 1.92 (s, 3 H, CH_3), 1.42 (s, 12 H, CH_3); IR (KBr) 1680 (CONH) 1389, 1387 [$C(CH_3)_2$], 1270 (P=O), 960 (PN) cm^{-1}. The compound was highly hygroscopic and difficult to handle. Anal. ($C_{18}H_{29}N_4O_6P \cdot H_2O$) C, H, N.

Assays of Cholinesterase Inhibitory Activity. The in vitro inactivation of horse serum cholinesterase, with procaine hydrochloride as substrate, was measured by a slight modification of the method of Lalka and Bardos.[7] The enzyme solution, 20 mL, 5.0 units/mL in 0.066 M NaH_2PO_4 buffer, pH 7.4, was made 1.4×10^{-4} M with respect to the inhibitor, at time zero, by adding 1.0 mL of a 2.94×10^{-3} M solution of the inhibitor in the same buffer. The resulting solution containing the enzyme and the inhibitor were placed in a 37 °C shaker bath, and 1.5-mL aliquots were withdrawn at various times and cooled to 24 °C in tap water. The substrate solution, 1.5 mL of 5.6×10^{-5} M procaine hydrochloride in the same buffer, was added to each tube as well as to a control containing no inhibitor, and the initial rate of the decrease of absorbance at 300 nm was measured as described previously.[7]

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(Phenylthio)phenylamine Derivatives as Potential Antiinflammatory Compounds

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A series of (phenylthio)phenylamines and related compounds was prepared as potential antiinflammatory agents. Among them, *N*-[(diethylamino)acetyl]-4-[(4-chlorophenyl)thio]phenylamine and *N*-[(diethylamino)acetyl]-2-[(4-chlorophenyl)thio]phenylamine showed good antiinflammatory activity in the carrageenin assay on rats.

In a previous work, we reported the synthesis of 2-aminonicotinic acids, N-substituted by different diphenyl sulfide groups. Among these products, an analogue of compound III (Table I), with X = Cl, Y = H, and R =

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