and filtered again, and the filtrate was evaporated to dryness at 40 °C, under reduced pressure to give **3** as an amorphous solid, which was recrystallized from dichloroethane: yield 330 mg (8%); mp 147 °C dec; NMR (Me_2SO-d_6) 5.53 (s, 2 H), 7.0–7.9 (m, 8 H), 8.40 (s, 1 H), 9.29 (s, 1 H). Anal. ($C_{15}H_{12}Cl_4N_4S$) C, H, N.

Biological Methods. Determination of Minimum Inhibitory Concentrations. Minimum inhibitory concentrations (MICs) were determined by agar dilution in Sabouraud dextrose agar in 96-well microtitre plates (Sterilin). Aliquots of drug inocula and medium were added to the wells of the plate with an eight-channel pipet (Titertek). Drugs were dissolved in Me_2SO to give a final concentration of 200 μg/mL. From this stock solution, serial twofold dilutions were made in the wells of the microtitre plate. Final concentrations ranged from 0.8 to 100 μg/mL, and the final concentration of Me_2SO was 1%. For *C. albicans*, the initial inoculum was 10^4 cells/mL. Mycelial inocula were used for dermatophyte species. MICs were read after incubation for 24 h at 37 °C (*C. albicans*) or after 5 days (dermatophytes) at 40 °C. The MIC was taken as the highest dilution of drug at which there was no visible growth. Assays were performed in duplicate.

Determination of the Effect of Compound 1 on the Viability of *Candida albicans*. Aliquots (100 mL) of Sabouraud dextrose broth in conical flasks (250 mL) were inoculated with samples from an overnight culture of *C. albicans* to give an initial population of 10^4 cells/mL. Compound **1**, miconazole, or ketoconazole was added to each flask to give a final concentration of 2×10^{-5} M, 10^{-4} M, and 10^{-3} M. Cultures were incubated at 37 °C on an environmental shaker (L.H. Engineering, Stoke Poges, U.K.), and samples were taken at intervals for determination of viable counts. Diluent was added to each sample prior to culture to ensure adequate dilution of drug carried over. Flasks were prepared in duplicate, and cultures containing no drug were used as negative controls.

Determination of in Vivo Activity against Vaginal Candidiasis in the Rat. Female Wistar rats (6 × 100 g per group) were hysterectomized and ovariectomized. After 3 weeks, and subsequently every 3 days during the experiment, they were injected, subcutaneously, with estradiol diacetate (125 μg) to induce and maintain pseudoestrus. Two days after hormone treatment, estrus was confirmed, and the animals were infected by intravaginal inoculation of 10^7 cells of *C. albicans* on 2 successive days. The infection was allowed to establish for an additional 2 days before treatment commenced. Each animal was considered maximally infected if a colony count of greater than 600 was obtained from 50 μL of vaginal washings at the start of each experiment. Compound 1 was administered orally (by gavage), with ketoconazole at 10 mg/kg used as a positive control. This model was also used to assess the efficacy of compound 1 topically against this infection, with clotrimazole as a positive control. Compound 1 and clotrimazole were each dissolved as 1% solutions [(w/v) in polyethylene glycol 200], and 0.1 mL of these solutions were administered once daily intravaginally for 9 days.

Determination of in Vivo Activity against Systemic Candidal Infection in the Mouse. The mice (10 per group) were infected intravenously with a sublethal dose (50×10^4 cells) of *C. albicans* and treated orally (by gavage) at 0 and 24 h following infection with compound 1 at 20, 10, 5, or 2.5 mg/kg or with ketoconazole at 10 mg/kg. Two days after infection the mice were sacrificed, their kidneys were excised, weighed, and homogenized, and the homogenates were diluted and then inoculated on to glucose–peptone agar. The plates were incubated for 24 h at 37 °C, the resultant colonies were counted, and the numbers were compared with those obtained from infected, untreated controls. The mean yeast count for untreated control mice was 3.5×10^4 per gram per kidney.

Determination of in Vivo Activity against Superficial Ringworm Infections in the Guinea Pig. The shaved backs of female guinea pigs (eight per group) were infected with a suspension of the spores of *Trichophyton mentagrophytes* in saline. The site of inoculation was occluded for 5 days to allow the infection to become established. Each guinea pig before treatment yielded greater than 1000 dermatophyte colonies on culture of skin swabs from the infected area. Treatment was commenced on the 6th day following infection and continued for 8 days. A 1% solution [(w/v) in polyethylene glycol 200] of clotrimazole was used as a positive control for topical application, griseofulvin at a dosage of 60 mg/kg was used as a positive control for oral administration, and the course of disease was followed by cultural studies on skin and hair samples from on and around the sites of infection.

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Registry No. (*E*)-**1**, 80168-44-1; **2**, 27088-04-6; (*Z*)-**3**, 80168-45-2; 2,6-dichlorophenylhydrazine hydrochloride, 50709-36-9.

Synthesis and Antitumor Activity of 2-β-D-Ribofuranosylselenazole-4-carboxamide and Related Derivatives

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Treatment of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1-carbonitrile with hydrogen selenide provided 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonselenoamide (**3**). Compound **3** was treated with ethyl bromopyruvate to provide ethyl 2-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)selenazole-4-carboxylates, which after ammonolysis were converted to 2-β-D-ribofuranosylselenazole-4-carboxamide (**6**) and its α-analogue **7**, respectively. Acetylation of nucleoside **6** provided 2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)selenazole-4-carboxamide, and phosphorylation of **6** provided the corresponding 5′-phosphate **9**. Compounds **6** and **9** were found to be cytotoxic toward P388 and L1210 cells in culture and effective against Lewis lung carcinoma in mice.

Currently available chemotherapeutic agents have shown no major impact on median survival of patients with bronchogenic and lung carcinoma[1] when used individually or in combination; therefore, more effective antitumor agents are required for chemotherapy. Recently, we have reported the synthesis[2] and mechanism of action[2,3] of 2-

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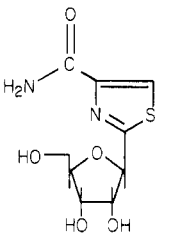
β-D-ribofuranosylthiazole-4-carboxamide (**1**), which has



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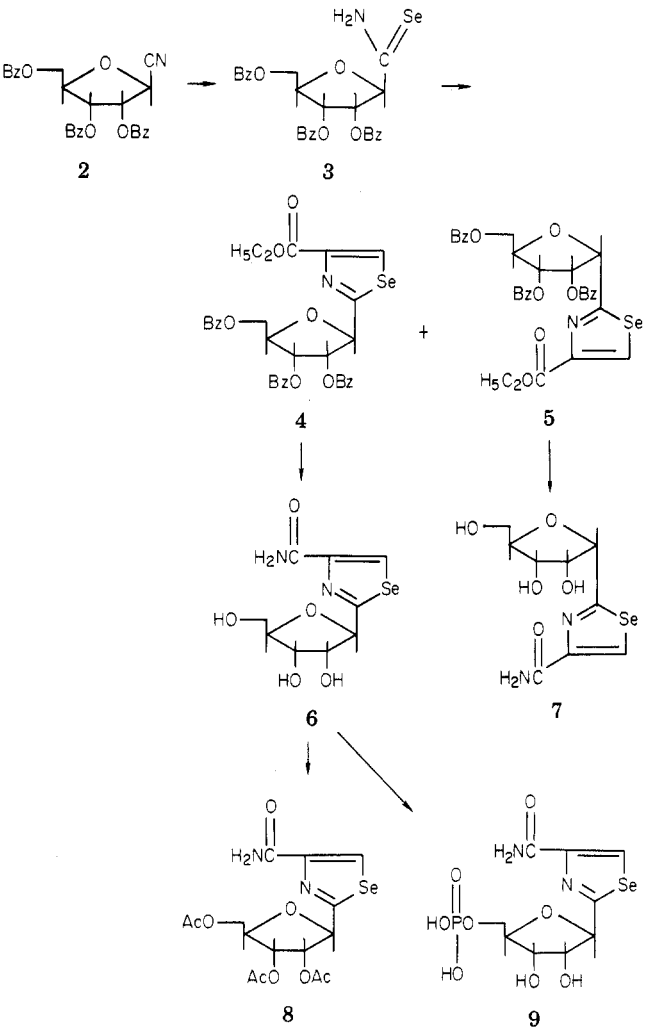
exhibited remarkable activity against Lewis lung carcinoma in mice, producing 10 out of 10 long-term survivors at several dose levels and good activity against L1210 and P-388 murine leukemias in vivo.[4] An understanding of the molecular structure and chemotherapeutic activity relationship[2,5] and the events that led to the discovery of this unique oncolytic nucleoside prompted us to synthesize 2-β-D-ribofuranosylselenazole-4-carboxamide (**6**) and its 5′-phosphate, **9**.

Chemistry. The precursor, 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1-carbonitrile (**2**), was synthesized following the literature procedure,[6] with certain modifications.[7] Treatment of **2** with liquid hydrogen selenide, with 4-(dimethylamino)pyridine as the catalyst, provided 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonselenoamide (**3**) as a foamy material (Scheme I). In analogy to our previous reactions with thioamides,[2] the corresponding selenoamide **3** was treated with ethyl bromopyruvate to provide ethyl 2-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)thiazole-4-carboxylates as a mixture of β (**4**) and α (**5**) anomers, which were readily separated by silica gel column chromatography. The fast moving β anomer and the slow moving α anomer were treated with methanolic ammonia to provide the corresponding 2-β-D-ribofuranosylselenazole-4-carboxamide (**6**) and 2-α-D-ribofuranosylselenazole-4-carboxamide (**7**), respectively. The electron-impact mass spectrum of **6** had a characteristic seven-peak pattern due to the selenium heteroatom and exhibited the molecular ion peak (*m/e* 307) and a fragmentation pattern consistent with structure **6**. Compounds **6** and **7** were also characterized on the basis of elemental analyses. In addition, the selenazole anomers had identical TLC mobilities and very similar IR and ^{1}H NMR properties as compared to those of the corresponding thiazole anomers identified previously.[2,8] In the ^{1}H NMR spectra, the signal for the anomeric proton of **6** appeared at δ 4.88 and was shifted upfield by 0.24 ppm as compared to that of **7**. The relative

Scheme I



chemical shifts observed for the anomeric protons of **6** and **7** were consistent with their assigned β and α configurations, respectively, and the previous observations.[2,9] Compound **6** was also identified as its triacetate, 2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)selenazole-4-carboxamide (**8**), which was obtained via the acetylation of **6** with acetic anhydride with 4-(dimethylamino)pyridine as the catalyst.

Our recent studies[3] indicate that 2-β-D-ribofuranosylthiazole-4-carboxamide (**1**) inhibits inosine monophosphate (IMP) dehydrogenase probably via the anabolism to the corresponding 5′-phosphate, which is a 20 times more potent inhibitor of the enzyme than the parent nucleoside **1**. Due to the close structural similarity, we expected a comparable spectrum of biological activities from the corresponding selenazole analogue **6** and its 5′-phosphate **9**. Phosphorylation of **6** by a usual procedure with phosphoryl chloride and trimethyl phosphate provided only a poor yield of 2-β-D-ribofuranosylselenazole-4-carboxamide 5′-phosphate (**9**). In addition, unidentified side products were also observed, which probably resulted from the reactivity of the $CONH_2$ group toward phosphoryl chloride. Nucleoside **6** was more conveniently phosphorylated with trichloropyrophosphopyridinium chloride,[10] which is generated in situ via the treatment of phosphoryl chloride with

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Table I. Effect of 2-β-D-Ribofuranosylselenazole-4-carboxamide (6) (Experiments 1 and 2) and Its 5'-Phosphate 9 (Experiment 3) on the Life Span of Mice Inoculated Intravenously with Lewis Lung Carcinoma

drug	dose, mg/kg	median life span, days	ILS, %	cures
		Experiment 1		
untreated controls		16.9		0/40
6	200	10.7		0/10
	100	20.3	20	3/10
	50	60.0	255	6/10
	25	60.0	255	7/10
	12	60.0	255	8/10
		Experiment 2		
untreated controls		20.2		0/40
6	24	60	197	9/10
	12	60	197	9/10
	6	60	197	5/10
	3	31	53	0/10
	1.5	22	10	0/10
		Experiment 3		
untreated controls		19.0		0/40
9	75	15.5		1/10
	50	12.5		3/10
	33	12.5		3/10
	20	12.0		2/10
	13	60.0	215	4/10
	9	12.5		1/10

pyridine and water in acetonitrile.

Antitumor Activity. The in vitro antitumor activities of 2-β-D-ribofuranosylselenazole-4-carboxamide (6) and its 5'-phosphate 9 were compared with the corresponding thiazole congeners. Compound 6 exhibited an ID_{50} of 4.0×10^{-7} M for L1210 cells and 3.0×10^{-7} M for P-388 cells and was fivefold more potent than 2-β-D-ribofuranosylthiazole-4-carboxamide (1), which exhibited ID_{50} values of 1.95×10^{-6} and 1.6×10^{-6} M, respectively. 2-β-D-Ribofuranosylselenazole-4-carboxamide 5'-phosphate (9) was as cytotoxic ($ID_{50} = 3.9 \times 10^{-7}$ M) to L1210 cells as the parent nucleoside 6 but was approximately eightfold more potent than 2-β-D-ribofuranosylthiazole-4-carboxamide 5'-phosphate,[11] which exhibited an ID_{50} of 3.2×10^{-6} M.

2-β-D-Ribofuranosylselenazole-4-carboxamide (6) was tested against Lewis lung carcinoma implanted iv in groups of ten $B_6D_2F_1$ mice (40 mice in untreated control groups) 24 h before initiation of therapy,[4,12] and these data are summarized in Table I, experiments 1 and 2. Based on the median survival time of the test animals as compared to that of control animals, compound 6 was found to be an effective antitumor agent. Following daily intraperitoneal (ip) administration of an aqueous solution of 6 on days 1–9 at dose levels of 50, 25, and 12 mg/kg, all of the test animals survived, giving a 255% increase in life span; 6 out of 10, 7 out of 10, and 8 out of 10 cures were achieved, respectively. Compound 6 was also found to be effective at relatively lower doses (Table I, experiment 2); 24, 12, and 6 mg/kg gave 9 out of 10, 9 out of 10, and 5 out of 10 cures, respectively, and a 197% increase in life span. Compound 5, the corresponding 5'-phosphate, was also significantly active, and cures ranging from 1 out of 10 to 4 out of 10 treated mice were obtained at a dose ranging from 9 to 75 mg/kg (Table I, experiment 3). At 13 mg/kg, 215% increase in life span and 4 out of 10 cures were achieved.

(11) 2-β-D-Ribofuranosylthiazole-4-carboxamide 5'-phosphate was synthesized from nucleoside 1 in an analogous manner as described for the synthesis of 9 from 6.

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Experimental Section

The physical properties were determined with the following instruments: melting point, Thomas-Hoover apparatus (uncorrected); IR, Beckman Acculab 2 (KBr); ^1H NMR, Fourier-transform spectrometer (JEOL-FX-900) (resonances reported from the internal Me_4Si standard). The presence of exchangeable protons was confirmed by ^1H NMR spectroscopy in absolute Me_2SO-d_6 by exchange with D_2O followed by reintegration. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Where analyses are indicated by only symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values. Baker analyzed silica gel powder (60–200 mesh) was used for column chromatography, and E. Merck silica gel 60 F-254 precoated TLC sheets (0.20 mm) were used to check the purity of the compounds.

2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonselenoamide (3). A mixture of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1-carbonitrile (2; 10.0 g, 21.2 mmol), 4-(dimethylamino)pyridine (200 mg), and liquid hydrogen selenide (condensed under N_2 atmosphere, 20 mL) was stirred in a sealed bomb at room temperature for 20 h. Hydrogen selenide was allowed to evaporate. The light green-colored residue was dissolved in chloroform (200 mL) and washed successively with water (3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL), and then water again (2 × 50 mL). The chloroform portion was dried ($MgSO_4$) and evaporated under vacuum to provide crude 3 as a foam in almost quantitative yield. The crude product after column chromatography (silica gel; 5% ethyl acetate in chloroform) provided 3 of analytical purity. Compound 3 developed a purple color when the silica gel chromatogram of the product was sprayed with a dilute etaholic solution of 2,3-dichloronaphthoquinone and exposed to ammonia.[2] Anal. ($C_{27}H_{23}NO_7Se$) C, H, N, Se.

Reaction of 2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonselenoamide with Ethyl Bromopyruvate and Synthesis of Ethyl 2-(2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl)selenazole-4-carboxylates (4 and 5). A solution of 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonselenoamide (5.5 g, 10 mmol) in acetonitrile (60 mL) was cooled in ice. Ethyl bromopyruvate (3.0 g) in acetonitrile (20 mL) was added dropwise (10 min). The ice bath was removed, and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated in vacuo, and the residue was triturated with a saturated sodium bicarbonate solution (100 mL) and extracted with ethyl ether (2 × 100 mL). The combined ether portion was washed with water and dried ($MgSO_4$). Ether was evaporated in vacuo, and the residue (syrup) was passed through a silica gel (300 g) column packed in chloroform. Elution with 5% ethyl acetate in chloroform provided the fast moving ethyl 2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-selenazole-4-carboxylate anomer (4; 2.5 g) and the slow moving ethyl 2-(2,3,5-tri-*O*-benzoyl-α-D-ribofuranosyl)selenazole-4-carboxylate anomer (5; 1.0 g), respectively. The TLC mobilities (silica gel; 10% ethyl acetate in chloroform) of these compounds were identical with those of the corresponding thio analogues.[2] Intermediates 4 and 5 were converted, respectively, into 6 and 7, which were fully characterized.

2-β-D-Ribofuranosylselenazole-4-carboxamide (6). Ethyl 2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)selenazole-4-carboxylate (3.2 g, 5 mmol) was dissolved in methanol (100 mL), cooled , and saturated with ammonia (0 °C). The solution was stirred in a pressure bottle at room temperature for 48 h. The solvent was evaporated in vacuo, and the residue was extracted with chloroform (25 mL × 3). The chloroform portion was discarded. The residue was adsorbed in silica gel (10 g) with methanol and applied on a silica gel column (2.8 × 45 cm) packed in ethyl acetate. The column was eluted with solvent E (ethyl acetate–1-propanol–H_2O, 4:1:2; top layer), and the homogeneous fractions (R_f ~0.42, silica gel TLC in solvent E) containing the major product were collected. The solvent was evaporated in vacuo, and the residue was crystallized from 2-propanol: yield 900 mg (60%); mp 131–133 °C. It provided a second crop (200 mg): mp 131–133 °C; mass spectrum, *m/e* 307; ^1H NMR (Me_2SO-d_6) δ 7.4–7.8 (br s, 2, $CONH_2$); ^1H NMR (Me_2SO-d_6–D_2O) δ 4.88 (d, 1, J = 4.7 Hz, $H_{1'}$),

8.84 (s, 1, H_5). Anal. ($C_9H_{12}N_2O_5Se$) C, H, N, Se.

2-α-D-Ribofuranosylselenazole-4-carboxamide (7). Ethyl 2-(2,3,5-tri-*O*-benzoyl-α-D-ribofuranosyl)selenazole-4-carboxylate (800 mg, 1.25 mmol) was dissolved in methanolic ammonia (saturated, 0 °C, 50 mL), and the clear solution was stored in a pressure bottle at room temperature for 48 h. The solvent was evaporated in vacuo, and the residue was partitioned between water (15 mL) and chloroform (30 mL). The water layer was separated and washed with chloroform (30 mL × 2). The water portion was evaporated under vacuum, and the residue was crystallized from ethanol to provide 2-α-D-ribofuranosylselenazole-4-carboxamide: yield 270 mg (70%); mp 204–205 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.4–7.68 (br s, 2, $CONH_2$); ^{1}H NMR (Me_2SO-d_6–D_2O) δ 5.12 (s, 1, J = 2.7 Hz, $H_{1'}$), 8.82 (s, 1, H_5). Anal. ($C_9H_{12}N_2O_5Se$) C, H, N, Se.

2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)selenazole-4-carboxamide (8). A mixture of 2-β-D-ribofuranosylselenazole-4-carboxamide (1.0 g, 3.25 mmol), 4-(dimethylamino)pyridine (catalyst, 80 mg), and acetic anhydride (15 mL) was stirred at room temperature for 3 h. The solvent was evaporated in vacuo and coevaporated with water (10 mL × 2) to provide a white crystalline product, which was triturated with water and collected by filtration. The product was recrystallized from water containing a few drops of ethanol to provide white needles of **8**: yield 1.2 g (85%); mp 117–119 °C. Anal. ($C_{15}H_{18}N_2O_8Se$) C, H, N, Se.

2-β-D-Ribofuranosylselenazole-4-carboxamide 5′-Phosphate (9). Water (151 mg, 8.4 mmol) was added carefully to a solution (maintained at 0 °C by stirring) of phosphoryl chloride (2.0 g, 13.2 mmol), pyridine (1.21 g, 14.4 mmol), and acetonitrile (2.3 g, 56.7 mmol). 2-β-D-Ribofuranosylselenazole-4-carboxamide (921 mg, 3.0 mmol) was added to the solution, and the reaction mixture was stirred for 4 h at 0 °C. A clear solution was obtained, which was poured into ice–water (50 mL), and the pH was adjusted to 2.0 with concentrated sodium hydroxide. The solution was applied to a column of activated charcoal (30 g) and washed thoroughly with water until the eluate was salt free. The column was then eluted with a solution of ethanol–water–concentrated ammonium hydroxide (10:10:1), and the fractions (25 mL each) were collected. The fractions containing pure [TLC, silica gel, acetonitrile–0.1 N ammonium chloride (7:3)] nucleotide were collected and evaporated to dryness under vacuum. The anhydrous residue was dissolved in water and passed through a column of Dowex 50W-X8 (20–50 mesh, H^+ form, 15 mL). The column was washed with water, and the fraction containing the nucleotide **9** was collected. The solution was concentrated to a small volume (5 mL) and passed through a column of Dowex 50W-X8 (20–50 mesh, Na^+ form, 15 mL). The column was washed with water. The fraction containing the nucleotide as the sodium salt was lyophilized. The residue was triturated with ethanol, collected by filtration, and dried (P_2O_5) to provide 580 mg (42%) of 2-β-D-ribofuranosylselenazole-4-carboxamide 5′-phosphate as the monosodium trihydrate in the crystalline form. Anal. ($C_9H_{12}N_2O_8PSeNa{\cdot}3H_2O$) C, H, N, P, Se.

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Registry No. **2**, 23316-67-8; **3**, 83705-10-6; **4**, 83705-11-7; **5**, 83705-12-8; **6**, 83705-13-9; **7**, 83705-14-0; **8**, 83705-15-1; **9**, 83705-16-2; ethyl bromopyruvate, 70-23-5.

DNA Binding Studies of 7-Bulky-Substituted Actinomycin Analogues

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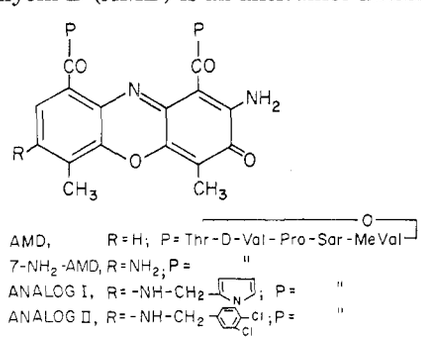
The DNA binding properties of several 7-substituted aralkylaminoactinomycin D analogues have been studied by spectrophotometry, DNA melting temperature studies, DNA–drug dissociation studies, and circular dichroism. Despite the presence of such bulky groups as 2-pyrrolylmethylamino or 3,4-dichlorobenzylamino at the 7 position, these analogues bind to DNA, inhibit RNA synthesis, and exhibit antitumor activity. A model is proposed for the interaction of the pyrrolyl analogue with phosphate groups of the DNA binding site, explaining the increased binding affinity for DNA of this actinomycin D analogue.

Actinomycin D (AMD) is an antitumor antibiotic that



has a 2-aminophenoxazin-3-one chromophore and two cyclic pentapeptide lactones (**1**).[1,2] The biological activity of AMD is believed to be due to its ability to bind to double-stranded DNA and its consequent inhibition of DNA-dependent RNA polymerase.[3] Although AMD possesses curative effects against certain tumors,[4,5] its relatively narrow spectrum of activity in man and its unusual toxicity have prevented its wider chemotherapeutic application. With the hope of overcoming these latter disadvantages, we have been studying chromophore-substituted AMD analogues. It has been shown[6] that substitution at the 7-position of AMD with a nitro, amino, or hydroxy group does not interfere with the DNA binding property or the antitumor activity of the antibiotic. Earlier studies[7] found that substitution of large groups with restricted freedom of rotation, such as an acetamino group,

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