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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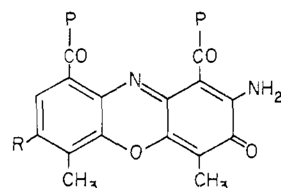
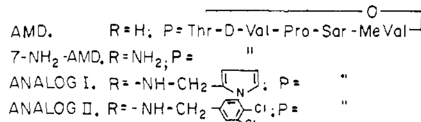
The DNA binding properties of several 7-substituted aralkylaminoactinomycin D analogues have been studied by spectrophotometry, DNA melting temperature studies, DNA-drug dissociation studies, and circular dichroism. Despite the presence of such bulky groups as 2-pyrrolylmethylamino or 3,4-dichlorobenzylamino 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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Table I. DNA Binding Parameters of Actinomycin D and Analogues

compd	ΔT_m [a], °C	K_{app}, [b] $M^{-1} \times 10^6$	rel τ [c]
AMD	8.0	23	100
7-NH_2-AMD	8.0	23	125
analogue I	16.0	92	225
analogue II	7.0	26	60

[a, b] Calf thymus DNA, phosphate buffer (0.01 M, pH 7.0, 20 °C); K_{app} = apparent binding constants (Scatchard plot).[9] [c] τ = time constant of slowest dissociation step of drug from DNA complex with 5% $NaDodSO_4$ in phosphate buffer.

at position 7 resulted in drastically reduced DNA binding affinity and loss of antibiotic activity. However, we have studied a number of 7-substituted-aralkylamino-AMD analogues with rotationally flexible linkages and established that like the parent AMD, these agents bind to DNA, inhibit RNA synthesis, and exhibit comparable or improved in vivo antitumor activity.[8,9] The present paper reports the DNA binding properties of two of these AMD analogues: 7-[[(2-pyrrolyl)methyl]amino]- and 7-[(3,4-dichlorobenzyl)amino]-AMD (analogues I and II, respectively) in comparison with those of AMD and 7-NH_2-AMD. This study was undertaken in order to provide a better understanding of the molecular details of the interaction of these drugs with double-helical DNA.

Spectral Properties. Upon complexation with calf thymus DNA, the long-wavelength chromophore absorption maxima of analogues I and II undergo simultaneous bathochromic and hypochromic shifts: Analogue I (free), ϵ_{510} 20000; analogue I (DNA bound), ϵ_{550} 16300; difference spectrum,[6] $-\Delta\epsilon_{max}$ 5610 at 490 nm. Analogue II (free), ϵ_{510} 17900; analogue II (DNA bound), ϵ_{545} 14100; difference spectrum,[6] $-\Delta\epsilon_{max}$ 4040 at 492 nm. The ϵ values quoted at the respective λ_{max} in phosphate (0.01 M, pH 7.0). [DNA]/[analogue] = 20:1. In the presence of DNA, AMD and 7-NH_2-AMD show similar spectral shifts that are consistent with drug–DNA complex formation via chromophore intercalation.[6]

Thermal Denaturation of DNA. The increases in the thermal denaturation temperature (ΔT_m) of calf thymus DNA in the presence of AMD, 7-NH_2-AMD, and the 7-substituted-AMD analogues I and II are given in Table I.[9] The ΔT_m value observed for analogue II is comparable to those of AMD and 7-NH_2-AMD, indicating comparable DNA binding strengths. On the other hand, analogue I shows a much larger ΔT_m, suggesting that the presence of the 2-pyrrolylmethylamino group at position 7 further enhances the stability of the drug–DNA complex.

Dissociation Studies. The inhibition of RNA polymerase by AMD and its biologically active analogues requires the formation of a stable complex with DNA that does not dissociate quickly. Following the method of Müller and Crothers,[7] we have studied the rates of dissociation for analogues I and II, as well as those of AMD and 7-NH_2-AMD for comparison, by measuring the change in absorbance over time at an appropriate wavelength in the presence of 5% $NaDodSO_4$, which acts to sequester the unbound drug. The results given in Table I indicate that analogue I dissociates significantly more slowly than AMD and 7-NH_2-AMD, whereas analogue II dissociates somewhat more quickly than these two agents.

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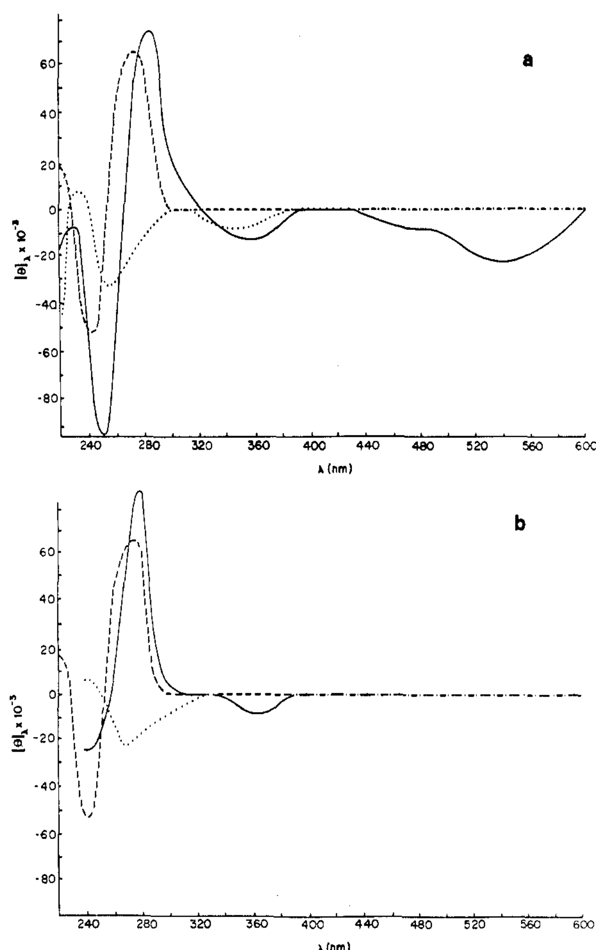

Figure 1. (a) CD spectra of analogue I (···), DNA (- - -), and analogue I and DNA (—) in 0.01 M phosphate buffer, pH 7.0. (b) CD spectra of analogue II (···), DNA (- - -), and analogue II and DNA (—). DNA nucleotide and drug concentrations are 30 and 3 μM, respectively.

CD Spectra. The CD spectra of AMD analogues I and II are quite similar, and in the presence of calf thymus DNA, significant changes in ellipticities take place (Figure 1a,b). For analogue I, the negative chromophore band at 350 nm is enhanced and shifted slightly to longer wavelength, and an additional broad negative band develops in the 400- to 600-nm region. The DNA ellipticities at 240 and 275 nm also undergo changes, intensifying and shifting slightly to longer wavelengths. Analogous changes in CD spectra have been observed for both AMD and 7-NH_2-AMD, indicating that analogue I forms a complex with DNA that is similar to the actinomycin complex.[6] The CD spectrum of analogue II does not show any enhancement of the negative chromophore band at 380 nm upon addition of DNA. However, there is a significant intensification of the DNA band at 275 nm and shift to 280 nm, but little change occurs in the 240-nm band. These results suggest that analogue II binds to DNA in a fashion somewhat different than AMD, 7-NH_2-AMD, or analogue I.

The relative CD spectral changes resulting from drug-DNA complex formation can be seen clearly in the difference CD spectra (Figure 2). In each case a strong positive peak is induced in the region of 275 nm and, except for analogue II, the negative chromophore bands at about 350 nm are enhanced and shifted to longer wavelengths. The negative DNA band at 240 nm undergoes varying changes depending upon the drug. The enhancement is largest for analogue I and 7-NH_2-AMD and minimal for analogue II.

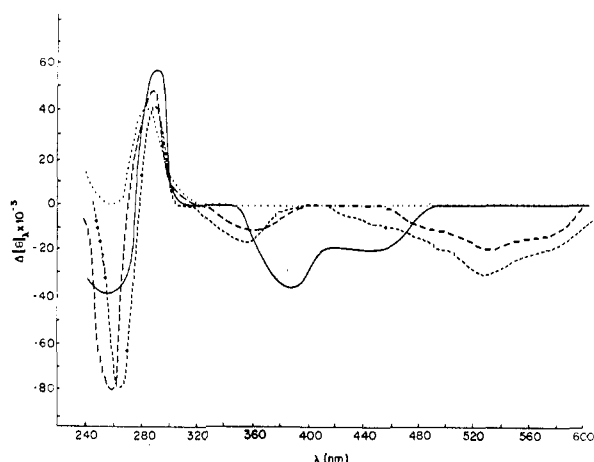

Figure 2. Difference CD spectra of AMD analogues and DNA: AMD (—), 7-NH_2-AMD (- - -), analogue I (– –) and analogue II (···). The difference CD spectrum is obtained by subtracting the CD spectrum of the compound plus that of DNA from the CD spectrum of the DNA–drug complex.

Results and Discussion

On the basis of X-ray crystallographic studies of an actinomycin and deoxyguanosine complex, Sobell and co-workers[10] provided direct evidence for intercalation and supported the mechanism of its binding as proposed by Müller and Crothers.[7] However, it is difficult to visualize how analogue I with a bulky substituent at C-7 of its chromophore can intercalate unless DNA exhibits conformational flexibility. A structural mechanism for intercalation has been proposed that invokes breathing of DNA in solution characterized by alternate disruption and re-formation of hydrogen bonds between base pairs in localized segments of DNA.[11] Fiel and collaborators[12,13] have demonstrated the intercalation of a large porphyrin derivative into DNA and explained it on the basis of such transient deformations. This mechanism can also adequately explain the intercalation of 7-bulky-substituted actinomycin D analogues. It is important to note that analogue I has a planar pyrrolyl substituent attached to the actinomycin chromophore through a flexible $NHCH_2$ linkage that should allow for the formation and aid the stabilization of the intercalated complex with DNA.

Our experimental results (Table I) suggest that analogue I binds to DNA more efficiently and dissociates from the complex at a slower rate than either actinomycin D or 7-aminoactinomycin D. This is in spite of the fact that they all appear to bind by a common mechanism, i.e., intercalation. In order for analogue I to complex with DNA in this manner it would have to approach the minor groove so that the aromatic pyrrolyl substituent is the first portion of the molecule to penetrate between the base pairs, followed by the phenoxazone chromophore. Once the chromophore is fully intercalated and the two cyclopentapeptides are in place in the minor groove, the 2-pyrrolylmethylamino group at position 7 will extend into the major groove of the DNA binding site. Model building studies indicate that in this position it is possible for the pyrrolyl group to bind simultaneously with two adjacent phosphate groups of the DNA backbone. The protonated ring nitrogen can hydrogen bond to one phosphate group, and at the same time the aromatic pyrrolyl group can stack with an adjacent phosphate group. Similar stabilizing interactions involving uridine bases and adjacent phosphate groups have been observed in the anticodon and pseudouridine loop regions of transfer RNA.[14] The stronger binding efficiency (ΔT_m and K_{app} values) and the slower rate of dissociation (τ value, Table I) observed for analogue I are consistent with this proposed model of binding to DNA.

These DNA binding parameters for analogue II, however, are indicative of a somewhat weaker complex formation, and the lack of change in the chromophore ellipticity in the CD spectra of this analogue, which has been discussed earlier, suggests that the mode of interaction of analogue II with DNA is somewhat different from that of analogue I. It may be that the bulkier dichlorophenyl substituent cannot provide the additional stabilizing interactions with DNA that the pyrrolyl group in analogue I can.

Our results demonstrate that the presence of bulky substituents at the N^7 site of 7-aminoactinomycin D does not necessarily prevent strong binding to DNA. The circular dichroic studies suggest that the mode of binding of these 7-substituted actinomycin analogues to DNA depends on the nature of the substituents. It is important to note that an intercalative model of binding of any of these N^7-substituted 7-aminoactinomycin analogues can be rationalized only on the basis that DNA in solution can breathe and allow for rupture of hydrogen bonds between the base pairs in localized segments of DNA to permit entry of the large ligands at the appropriate binding sites.[11-13,15]

Experimental Section

A Gilford Model 250 spectrophotometer was used to record data for the spectral and dissociation (τ values, Table I). The addition of a base-line reference compensator (analog Multiplexer 6064) and 10-cm cell holder was required for the DNA–drug dissociation studies. The circular dichroism spectra were recorded on a Cary 61 spectropolarimeter. Actinomycin D, batch no. NCS #3053, lot L664651-0-10, was provided by Dr. John Douros, Natural Products Branch, National Cancer Institute, Silver Spring, MD. Calf thymus DNA type 1 was purchased from Sigma Chemical Co. All other chemicals were of analytical grade from J. T. Baker Chemical Co.

Unless otherwise stated, all DNA binding experiments were carried out in 0.01 M phosphate buffer (pH 7.0) with purified calf thymus DNA.[6] Spectrophotometric measurements were recorded at 20 °C. Dissociation studies[7] were performed in phosphate buffer at 20 °C in the presence of 5% $NaDodSO_4$ with [DNA(nucleotide)]/[drug] ratios of 20 to 1. Changes in absorbance over time were measured for the drug–DNA complexes at the following wavelengths: AMD, 430 nm; 7-NH_2-AMD, 480 nm; analogue I, 490 nm; analogue II, 492 nm. The circular dichroism spectra were recorded at 20 °C. The θ values, which are direct readings from the recorder, were converted to molar ellipticity values, $[\theta]$, in the standard manner.[6]

Acknowledgment. This investigation was supported by Research Grants CH-34 from the American Cancer

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Registry No. Analogue I, 67230-67-5; analogue II, 84174-12-9; AMD, 50-76-0; 7-NH_2-AMD, 7240-37-1.

Synthesis and Antiarrhythmic Activity of New [(Dialkylamino)alkyl]pyridylacetamides

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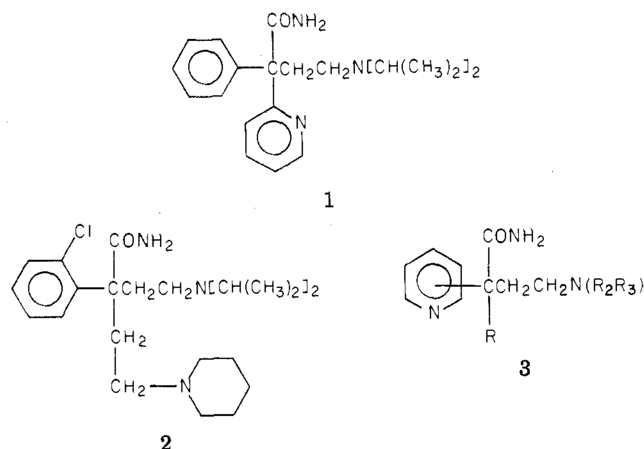
The synthesis of new [(dialkylamino)alkyl]pyridylacetamides is reported. These compounds displayed a potent antiarrhythmic activity, as demonstrated on a model of myocardial infarction in the conscious dog. Structure–activity relationships are discussed within a series of 22 homologues, comparing relative antiarrhythmic properties and cardiac side effects. One of these compounds, **15**, has been selected as a candidate for clinical evaluation in man.

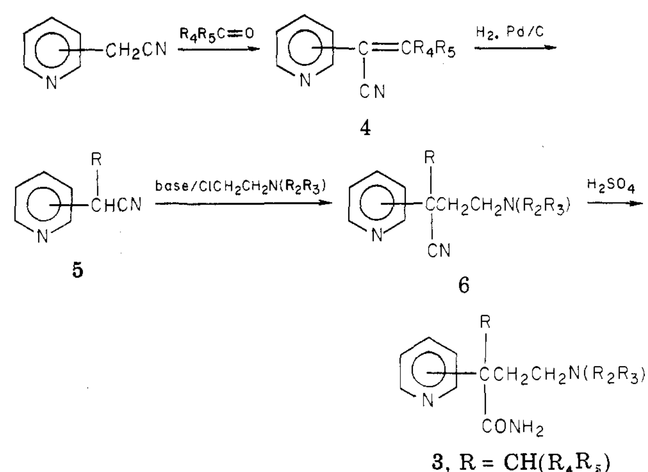
Among derivatives of 2-(2-pyridyl)butyramides, 4-(dialkylamino)-2-phenyl-2-(2-pyridyl)butyramides have been found to possess antiarrhythmic activity. The leading compound in this class, disopyramide (DP, **1**), is active in



the treatment of ventricular dysrhythmias in man.[1] DP gave rise to further developments that led to compounds with activity modulated through variation of amide[2,3] or amine radical.[4] More recently, replacement of one aromatic group in DP by a second (dialkylamino)alkyl group led to active compounds. The most interesting derivative in this later series seems to be 2-(2-chlorophenyl)-2-[2-(diisopropylamino)ethyl]-4-(1-piperidinyl)butanamide (disobutamide, **2**).[5]

With respect to the effect of the phenyl group on the activity of these compounds, it appeared to us that it would be interesting to evaluate the influence of this radical on the antidysrhythmic activity in terms of steric hindrance and/or lipophilicity. This hypothesis led us to study the synthesis and biological properties of a series of 2-alkyl-4-(dialkylamino)-2-pyridylbutyramides, **3**, where the α-phenyl moiety in **1** has been replaced by an alkyl chain.[6]

Scheme I



Chemistry. The preparation of the target compounds was accomplished as follows (Scheme I). The different pyridylacetonitriles were condensed with a ketone or an aldehyde in benzene with piperidine/acetic acid as a catalyst[7] (Table I). When R_4 and R_5 are two different alkyl groups, **4d**, a mixture of the two isomers *E* and *Z* was obtained. Hydrogenation of the double bond with Pd/C in ethanol furnished compounds **5** in near quantitative yields. Compounds **5a** (α-cyclohexyl-2-pyridylacetonitrile) and **5b** [3-phenyl-2-(2-pyridyl)propionitrile] were prepared directly from 2-pyridylacetonitrile by alkylation with bromocyclohexane and benzyl chloride, respectively.[8] Conversion of **5** into **6** was achieved by alkylation with a (dialkylamino)ethyl or (dialkylamino)propyl chloride and sodamide in toluene at reflux temperature or sodium hydride in dimethylformamide at room temperature. Compounds **6** were used without further purification in the last

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