N^7-Substituted 7-Aminoactinomycin D Analogues. Synthesis and Biological Properties

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A series of N^7-substituted 7-aminoactinomycin D analogues with alkyl, aralkyl, and heteroaralkyl substituents was synthesized and their biological properties were studied. All of these analogues proved to be 22- to 28-fold less toxic than actinomycin D when tested against human lymphoblastic leukemia cells (CCRF-CEM) in vitro. Against the P388 mouse leukemia in vivo, most of the analogues had activity comparable to actinomycin D and one was significantly more active. The results show that substitutions of this kind do not interfere with the antitumor activity of actinomycin D and may be useful for the design of modified actinomycin D analogues with greater selectivity.

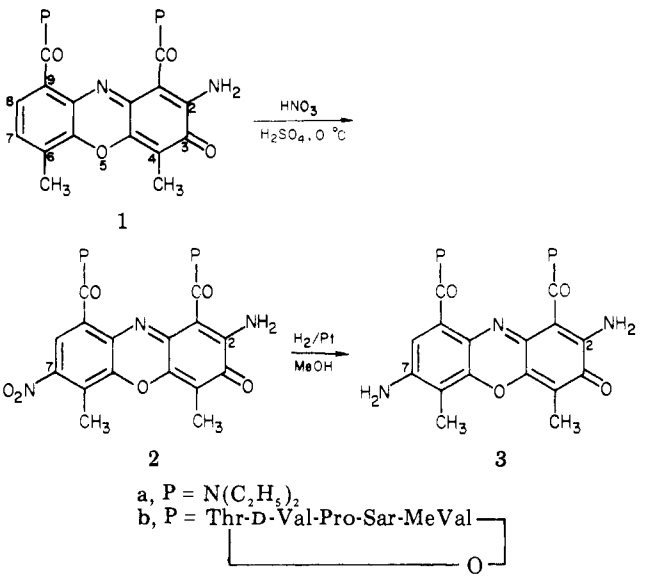
Actinomycin D (AMD, **1b**, Scheme I) is one of a group of antibiotics containing two pentapeptide lactone rings at positions 1 and 9 of the chromophore phenoxazinone ring.[1-3] It has been used clinically as a chemotherapeutic agent for some time, with excellent results in the treatment of Wilms' tumor[4] and gestational choriocarcinoma.[5] In addition, AMD is widely used in studies related to RNA metabolism, due to its highly specific inhibition of DNA-dependent RNA synthesis.[6-8] It is known to bind to double-helical DNA by intercalation of the chromophore and through hydrogen bonding and hydrophobic interactions of the peptide lactone moieties.[9-11] The nature of the binding of AMD to DNA has been established by several means (spectral shift,[9,12-15] viscosity changes,[9,16] thermal denaturation of DNA,[15,16] and others[9,16-18]), and its interaction specifically with the deoxyguanosine and pdG-dC dinucleotide is indicated by X-ray crystallography, spectrophotometry, and NMR studies.[19-22] As an extension of these studies, the overall picture of the mechanism of action of AMD is proposed as its binding to intracellular DNA and thus inhibiting DNA-dependent RNA synthesis.[23,24]

Work has been done by a number of investigators on the chemistry, biological activity, binding specificity, experimental pharmacology, and clinical pharmacology[1,25] of the actinomycins, particularly in the laboratories of Brockmann[26] and Katz[27] (reviewed by Meienhofer and Atherton[28] and by Hollstein[29]). These studies indicate that the intact peptide lactones and the presence of the 2-amino, 3-oxo, and 4- and 6-methyl groups of the chromophore are necessary for optimal antibiotic activity. More recent studies[30,31] have cast some doubt about the absolute requirement of the 2-amino group for biological activity and DNA-binding properties. Bulky substituents in the 7 position of the actinomycins have been claimed to interfere seriously with the DNA-binding properties.[9]

In attempts to increase the activity of AMD against human neoplasms and also to reduce its host toxicity,[28] several analogues have been synthesized[28,29] and their biological activity has been assayed.[32,33] Recently, we showed that 7-nitro-AMD (**2b**) and 7-amino-AMD (**3b**) had DNA-binding properties comparable to AMD.[14,16] These compounds were active against human lymphoblastic leukemia cells (CCRF-CEM) in vitro, and their activity against four transplantable mouse tumors (P388, L1210, and P1534 leukemias and Ridgway osteogenic sarcoma) in vivo was comparable to AMD.[14,34] We also showed that **3b** may be useful for chromosome banding studies because of its fluorescence.[35]

In this report we describe the synthesis and antitumor activity of several N^7-substituted 7-aminoactinomycin D analogues. The DNA-binding properties of these compounds will be reported in a separate communication.[36]

Scheme I



Chemistry. 7-Aminoactinomycin D (**3b**, Scheme I) was prepared by a modification of our previously reported procedure[34] to give this compound in better yield. The substituted analogues listed in Table I were prepared from **3b** by a simple two-step procedure without the isolation of intermediates. A variety of aldehydes, RCHO, when refluxed with **3b** in dry benzene, in the presence of molecular sieves (4Å), generated the intermediate imines (**4**, Scheme II) which were reduced in situ by the addition of dimethylamine–borane[37,38] to give the desired products in good yields.[39] This procedure worked well for aliphatic, aromatic, and heteroaromatic aldehydes and was first worked out on the model compound **3a**. Model alkylated compounds **5a**, **8a**, and **9a** were prepared by this procedure. All of the compounds reported here were characterized by TLC, UV and NMR spectra, and elemental analyses.

Spectral Properties. NMR. The structures of the model substituted compounds **5a**, **8a**, and **9a** could be easily verified by their NMR spectra (see the Experimental Section). The 2-NH_2 protons in these compounds do not differ appreciably in chemical shift from those of the parent compound **3a**.[34] The 7-NH_2 protons of **3a** are absent in these spectra and a single proton (7-NH–) appears upfield. The NMR spectra of compounds **5b–13b** are extremely complicated and are not reported here.

Visible Absorption. Comparison of the visible absorption spectra of the model substituted compounds to those of the corresponding AMD analogues provides evidence for the similarity of structures in both series. The long wavelength absorption maximum for model com-

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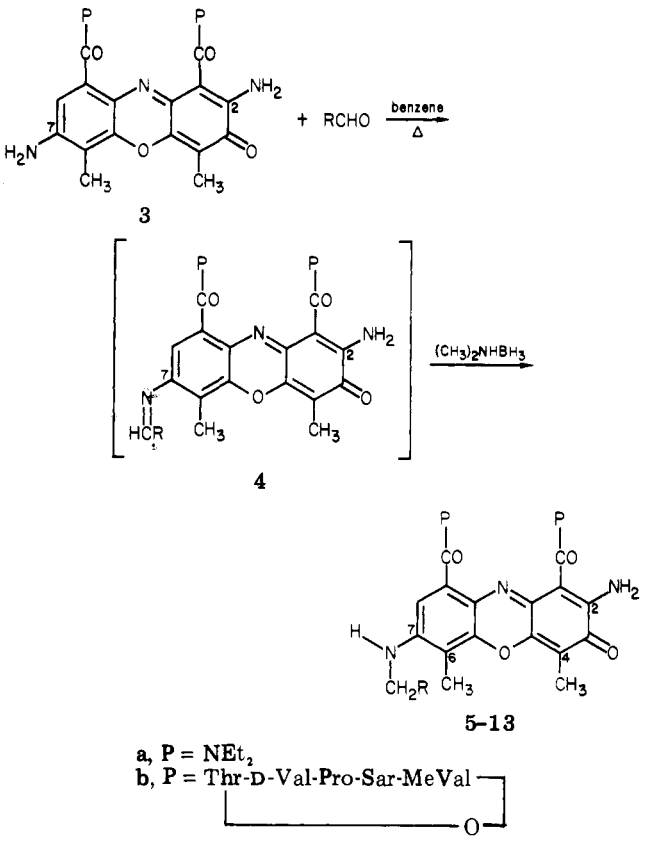
Table I. Reaction Conditions and Physicochemical Properties of N^7-Substituted 7-Aminoactinomycin Analogues[a]

compd	R	time of reflux, min	yield, %	mol formula	R_f	mp, °C	$[\alpha]^{20}_{644}$, deg (c, $CHCl_3$)	$\lambda_{max}^{CHCl_3}$ (ϵ)
5b	phenyl[b]			$C_{69}H_{93}N_{13}O_{16}\cdot 2H_2O$	0.61	212-215		519 (19900)
6b	3',4'-dichlorophenyl	30	79	$C_{69}H_{91}N_{13}O_{16}Cl_2\cdot 2H_2O$	0.58	244-246	−288 ± 12 (0.10)	505 (19900)
7b	*m*-phenoxyphenyl	90	66	$C_{75}H_{97}N_{13}O_{17}\cdot 3H_2O$	0.54	256-258	−313 ± 10 (0.10)	502 (25300)
8b	*p*-carboxyphenyl	30	79	$C_{70}H_{93}N_{13}O_{18}\cdot 2H_2O$	0.55	256-258 dec	−306 ± 15 (0.10)	516 (16900)
9b	*n*-heptyl	60	82	$C_{70}H_{97}N_{13}O_{16}\cdot 2H_2O$	0.67	232-234	−318 ± 22 (0.08)	534 (26900)
10b	2'-furyl	60	80	$C_{67}H_{97}N_{13}O_{17}\cdot 2H_2O$	0.66	236-238	−345 ± 12 (0.10)	484 (18000)
11b	2'-pyridyl	60	69	$C_{68}H_{92}N_{14}C_{16}\cdot 2H_2O$	0.54	228-230		475 (19900)
12b	*p*-trifluoromethylphenyl	45	79	$C_{70}H_{92}N_{13}O_{16}F_3\cdot 2H_2O$	0.54			510 (16900)
13b	2'-pyrrolyl	60	68	$C_{67}H_{92}N_{14}O_{16}\cdot 2H_2O$	0.57	240-242	−303 ± 20 (0.09)	508 (15400)

[a] In each preparation 0.02 mmol (25.4 mg) of 7-aminoactinomycin D (3b) was refluxed with 0.2 mmol of the corresponding aldehyde (RCHO), followed by the addition of 0.6 mmol (35 mg) of dimethylaminoborane except for the case of 7b where the addition of the aldehyde and the reducing agent was repeated once for the completion of the reaction. [b] See ref 38.

Scheme II



pound **3a** is at 490 nm. In the spectra of the substituted model compounds **5a**, **8a**, and **9a** there is a bathochromic shift to 512, 510, and 524 nm, respectively. Similarly, in the corresponding **b** series of compounds there is a bathochromic shift from 501 nm for **3b** to 519, 516, and 534 nm, respectively.

Specific Rotation. Additional evidence that the **b** series of compounds are 7-N-substituted rather than 2-N-substituted is obtained from the specific rotations of the compounds. The values (Table I) are very close to the values for AMD (−300 ± 20°) and for 7-amino-AMD (−348 ± 18°) under the same conditions, as determined in this laboratory. A bulky substituent in the 2 position of AMD might be expected to change the specific rotation drastically due to its effect upon the conformations of the peptide lactones.[41] Meienhofer[31] has shown this to be the case with N^2-(γ-hydroxypropyl)-AMD [$[\alpha]^{25}_D$ −221.8° (CH_3OH)] when compared with AMD [$[\alpha]^{20}_D$ −327° (CH_3OH)].[28]

Biological Evaluation. The analogues **5b**–**13b** were assayed for in vitro growth-inhibitory activity against human lymphoblastic leukemia cells (CCRF-CEM),[42] and the results are reported in Table II. This assay is highly sensitive for AMD and its analogues, and it provides relative cytotoxicity values for these agents. The analogues were about 22–28-fold less cytotoxic than AMD and three- to fourfold less cytotoxic than 7-amino-AMD.

These analogues were also tested for antitumor activity against P388 lymphocytic leukemia in BDF_1 hybrid mice (Table II). This test system has been used extensively in the screening of natural products, especially antibiotics, because it is more sensitive to these agents than is L1210 leukemia.[43,44] The compounds were administered over a range of dosages including a toxic dose, but only the optimal nontoxic dose is listed. In this system most of the analogues have activity comparable to AMD, although at higher dosage levels. One of these compounds, **6b**, is

Table II. In Vivo and in Vitro Antitumor Activity of N7-Substituted 7-Aminoactinomycin D Analogues

compd	in vivo (P388)[a] optimal dose, μg/kg	% ILS[b]	in vitro (CCRF-CEM),[c] ID_{50}, ng/mL
5b	800	+108	250
6b	500	+192	220
7b	225	+130	280
8b	500	+123	260
9b	1500[d]	+73	270
10b	200	+90	260
11b	450	+145	260
12b	500	+100	260
13b	200	+90	260
7-amino-AMD (3b)	150	+164	85
AMD (1b)	75	+122	10

[a] P388 leukemia (10^6 cells) was implanted ip in male BDF_1 mice (seven per group). Compounds were administered in saline solution for four successive days starting 1 day after tumor implantation (qd 1-4). [b] % ILS = 100 × (T/C − 1) where T and C are median survival times of treated and control animals. [c] Human lymphoblastic leukemia cells in suspension culture. [d] Highest nontoxic dose tested.

significantly superior than AMD in this tumor line.

Conclusions

In this report we have shown that AMD analogues with bulky substituents in the 7 position possess antitumor activity in most cases comparable to AMD and in some cases better than AMD. We believe that AMD analogues with broader selectivity and improved antitumor activity can be developed through this approach. In separate communications we will report data which show that these same analogues bind efficiently to double-helical DNA.[36,45] The mode of action of AMD and its analogues in biological systems is still not fully understood, but cellular uptake and retention, drug distribution, and metabolism are important factors. The possibility that the antitumor effectiveness of AMD and its analogues is also associated with sites of action other than inhibition of RNA synthesis cannot be ruled out.[46]

Experimental Section

Actinomycin D, batch no. 3008-30B, was kindly provided by Lederle Laboratories Division, American Cyanamid Co. Quantitative UV spectra were measured on a Cary Model 11 spectrophotometer, and NMR spectra were obtained on a Varian T-60A instrument using Me_4Si as internal standard. Specific rotations were measured on a JASCO-5 ORD/CD spectropolarimeter. Thin-layer chromatography was performed on Analtech prescored silica gel plates using MeOH–EtOAc (1:9) as the developing solvent, and column chromatography was done using silica gel powder (Baker no. 3405, 60–200 mesh). Melting points were measured on a Thomas Model 40 micro hot-stage and are corrected. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn., and by Schwartzkopf Laboratories, New York, N.Y. Where analyses are indicated only by a symbol of the elements, analytical results obtained for those elements were within ±0.5% of the theoretical values.

7-Nitro-AMD (2b). This procedure is superior to that previously reported from this laboratory.[34] Actinomycin D (200 mg, 0.16 mmol) was placed in a polyethylene bottle (30-mL capacity) and was treated with 3.73 mL of cold (0 °C) concentrated sulfuric acid. The capped bottle was shaken in a cold room (4 °C) on a linear vibrator for a few minutes to dissolve the AMD. The solution was treated with 1.0 mL of the nitration mixture (0.1 mL of fuming nitric acid in 4 mL of concentrated sulfuric acid) and then shaken for an additional 20 min. The reaction mixture was poured onto a stirred mixture of 40 mL of $CHCl_3$ and 120 g of ice. The $CHCl_3$ layer was separated and washed with 3 × 15 mL of saturated aqueous $NaHCO_3$ and then with 20 mL of saturated NaCl. Evaporation of the solvent left a solid which was chromatographed on silica gel using MeOH–EtOAc (1:9) as eluent. The fractions showing the desired R_f value (0.38) were combined and evaporated to leave a solid which was recrystallized from EtOAc–hexane to give 142 mg (70%) of pure **2b**: mp 252–253 °C; UV ($CHCl_3$) 314 nm (ε 8060), 438 (23 900), 445 (24 300). Anal. ($C_{62}H_{85}N_{13}O_{18}$) C, H, N.

7-Amino-AMD (3b). This procedure is superior to that previously reported from this laboratory.[34] A mixture of **2b** (200 mg, 0.15 mmol), MeOH (150 mL), and PtO_2 (30 mg) was hydrogenated in a Parr apparatus at 40 psi for 3 h. The mixture was filtered through Celite filter-aid, and the filter pad was washed with MeOH (20 mL). Air was then bubbled through the filtrates for 30 min to complete the oxidation to the phenoxazinone. The resulting solution was evaporated and the residue was recrystallized from hot EtOAc–hexane to give 154 mg (77%) of pure **3b** as purple crystals: mp 252–253 °C; R_f 0.51. Anal. ($C_{62}H_{87}N_{13}O_{16}$) C, H, N.

General Procedure for the Alkylation of 7-Amino-AMD (3b) and the Model Compound (3a). A typical procedure is described for the preparation of 7-*n*-octylamino-AMD (**9b**). A mixture of **3b** (25.4 mg, 0.02 mmol), *n*-octanal (25.6 mg, 2 mmol), benzene (5 mL), and molecular sieves (100 mg, 4 Å) was refluxed for 1 h to complete the formation of the intermediate imine. The mixture was cooled to room temperature and dimethylamine–borane (35 mg, 6 mmol) was added. After stirring for 30 min, TLC indicated that the reaction was over. (This procedure may have to be repeated if TLC shows any unreacted **3b**.) The mixture was diluted with $CHCl_3$ (20 mL) and filtered to remove the sieves, and the solution was washed with saturated aqueous $NaHCO_3$ and then with saturated NaCl. The solvents were evaporated and the residue was recrystallized from hot EtOAc–hexane to give the product as pink crystals.

The experimental details and physicochemical properties for compounds **5b–13b** are listed in Table I. Model compounds **5a**, **8a**, and **9a** were also prepared by the general procedure and physical data for these compounds are listed below.

2-Amino-7-benzylamino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-3*H*-phenoxazin-3-one (5a). The yield of this compound was 76%: mp 140–150 °C; NMR ($CDCl_3$) τ 2.66 (s, C_6H_5), 3.41 (s, 8-H), 4.83 (s, 2-NH_2), 5.48 (m, 3 H, $C_6H_5CH_2$ and 7-NH), 6.1–7.0 (m, 8 H, NCH_2CH_3), 7.68 (s, 6-CH_3), 7.75 (s, 4-CH_3), 8.9–9.2 (m, 12 H, NCH_2CH_3); NMR (Me_2SO-d_6) τ 2.65 (s, C_6H_5), 3.65 (s, 8-H), 4.2 (s, 2-NH_2), 5.5 (m, 3 H, $C_6H_5CH_2$ and 7-NH), 6.4–6.8 (m, 8 H, NCH_2CH_3), 7.7 (s, 6-CH_3), 7.85 (s, 4-CH_3), 8.8–9.2 (m, 12 H, NCH_2CH_3); UV ($CHCl_3$) 512 nm (ε 36 200).

2-Amino-7-*p*-carboxybenzylamino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-3*H*-phenoxazin-3-one (8a). The yield of this compound was 79%: mp 181 °C dec; NMR (Me_2SO-d_6) τ 2.05 (d, 2 H, C_6H_4), 2.5 (d, 2 H, C_6H_4), 3.65 (s, 8-H), 4.2 (s, 2-NH_2), 5.3–5.6 (m, 3 H, $C_6H_4CH_2$ and 7-NH), 6.1–7.0 (m, 8 H, NCH_2CH_3), 7.74 (s, 6-CH_3), 7.85 (s, 4-CH_3), 8.8–9.4 (m, 12 H, NCH_2CH_3); UV ($CHCl_3$) 510 nm (ε 24 320). Anal. ($C_{32}H_{37}N_5O_6$) C, H, N.

2-Amino-7-octylamino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-3*H*-phenoxazin-3-one (9a). The yield of this compound was 78%: mp 134–135 °C; NMR ($CDCl_3$) τ 3.4 (s, 8-H), 4.8 (s, 2-NH_2), 6.15–7.05 (m, 11 H, 7-NH, 7-NCH_2, and NCH_2CH_3), 7.78 (s, 6 H, 6-CH_3 and 4-CH_3), 8.5–9.1 [m, 27 H, $CH_3(CH_2)_6$ and NCH_2CH_3]; UV ($CHCl_3$) 524 nm (ε 35 100). Anal. ($C_{32}H_{47}N_5O_4$) C, H, N.

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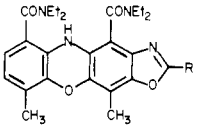
(39) We have shown recently[40] that certain aldehydes can react with **1a** under extensive reflux to form oxazolo derivatives with the general structure below. No evidence for the formation of similar derivatives was found in the present work, under the reaction conditions used.



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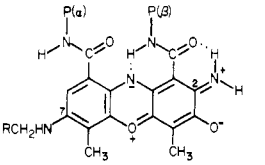
(41) A hydrogen-bonded resonance structure for **5b–13b** is indicated below, on the basis of a similar hydrogen-bonded structure for actinomycins which has been suggested by Müller and Crothers[9] and discussed by Mosher[30] et al. Bulky substituents on the 2-nitrogen might be expected to interfere markedly with the hydrogen bonding between the 2-amine hydrogen and the carbonyl group linking the β peptide to the chromophore, thus affecting the conformation and the spatial configuration of the peptide lactones.[28]



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