Synthesis and Biological Properties of N^2-Substituted Spin-Labled Analogues of Actinomycin D

Birandra K. Sinha,* Michael G. Cox, Colin F. Chignell,

Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

and Richard L. Cysyk

Laboratory of Chemical Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.
Received January 31, 1979

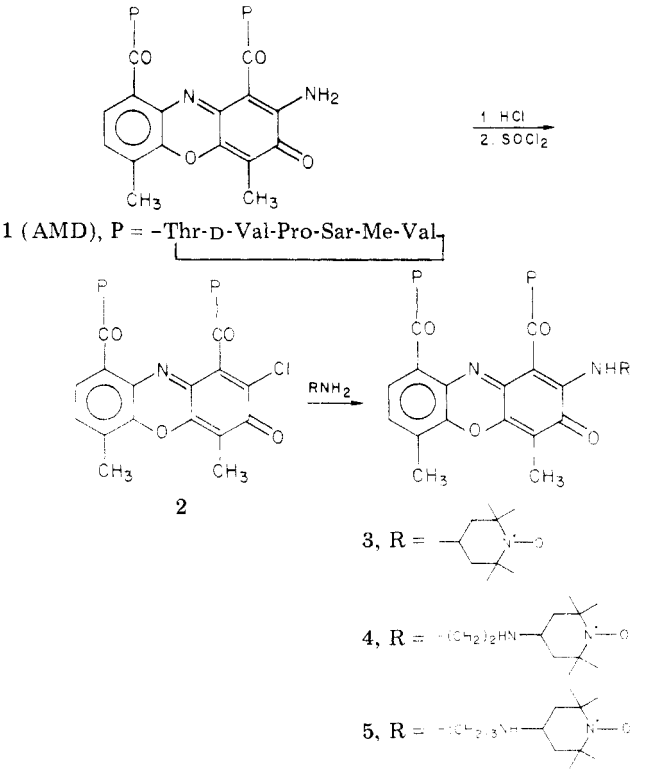
We have synthesized N^2-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)]actinomycin D and the related 1,2-diaminoethane and 1,3-diaminopropane derivatives and evaluated their biological properties. Binding studies with the spin-labeled actinomycin D analogues and DNA were carried out by using circular dichroism, electron spin resonance, and thermal denaturation. These studies have suggested that the derivatives bind to DNA and that their DNA-binding modes are similar but not identical. Spin-labeled actinomycin D derivatives were less potent in inhibiting *Escherichia coli* DNA-dependent RNA polymerase reaction than actinomycin D and were less toxic to L1210 cells in vitro than the parent compound. Spin-labeled actinomycin D derivatives were more common than the parent compounds against P-388 leukemia cells in vitro with little or no toxicity.

Actinomycin D (AMD, Scheme I), a cyclic polypeptide antibiotic, has been used extensively as a molecular probe in studies related to RNA metabolism due to its specific inhibition of RNA synthesis.[1-3] In addition, the related 7-amino derivative has been used as a fluorescent probe in chromosome-banding studies.[4] AMD has been used clinically as a chemotherapeutic agent against Wilms' tumor[5] and gestational choriocarcinoma.[6] The mechanism of action of AMD results from its ability to interact with double-stranded DNA. Using X-ray crystallographic techniques, Sobell[7] has confirmed the earlier findings of Krugh[8] that the phenoxazone ring of AMD is intercalated preferentially between pdG-dC base pairs while the peptide subunits lie in the narrow groove of the DNA helix and interacts with deoxyguanosine residues on the opposite chains through specific hydrogen bonds. The overall result of this AMD–DNA interaction is the inhibition of DNA-dependent RNA synthesis.[9,10]

In order to increase the therapeutic effectiveness while simultaneously decreasing the high toxicity associated with AMD treatment, a large number of AMD derivatives have been prepared and evaluated for their biological activities.[11-14] Structure–activity studies have revealed that the intact peptide lactones and the 2-amino, 3-oxo, and 4- and 6-methyl groups of the phenoxazone ring are necessary for activity. However, Moore et al.[12] and Mosher et al.[13] have recently shown that, although the 2-amino group is required for optimal antibiotic activity, it is not essential for the antitumor activities of the actinomycins. In order to utilize spin-labeled drug analogues as molecular probes for studying drug–receptor interactions, we have synthesized and reported the biological properties of a number of spin-labeled drugs containing stable nitroxide free radicals.[15-17] We have shown in our earlier papers that spin-labeled analogues of 9-aminoacridine bind to nucleic acids by intercalation[18] and recently we have used them to study binding of histones to DNA.[19] In this paper, we report the synthesis and biological properties of some spin-labeled analogues of actinomycin D. The rationale for the synthesis of these compounds was twofold: (a) to use these derivatives as base-specific probes for nucleic acids and their biologically important complexes and (b) to utilize the reported[20,21] antitumor properties of the nitroxide free radicals in order to enhance the therapeutic effectiveness of AMD.

Chemistry. The spin-labeled AMD derivaties **3–5** were prepared from the intermediate 2-chloroactinomycin D (**2**)

Scheme I

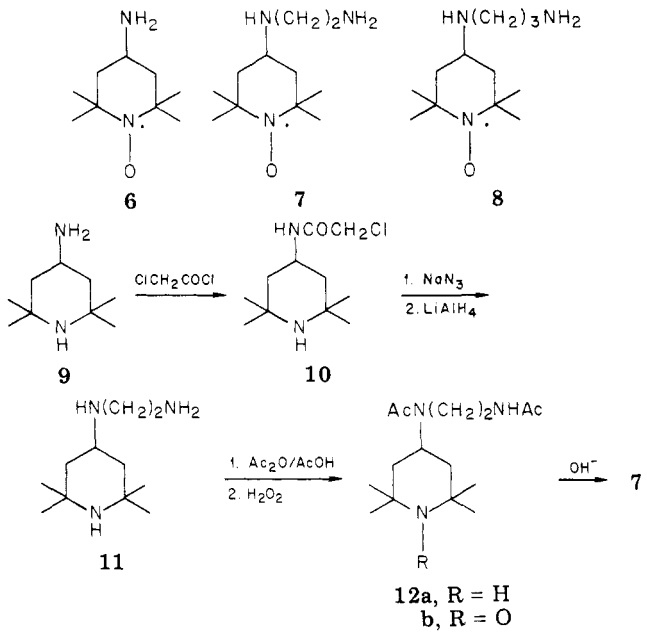


by reaction with the appropriate amines according to the method of Moore et al.[12] The intermediate **2** was prepared according to the reported procedures.[12,13] The product **2** had an R_f value (0.46) in EtOAc–acetone (2:1) similar to that reported by Mosher et al.[13] The spin-labeled amine **8** was prepared as described earlier.[17] Synthesis of amine **7** (Scheme II) involved reaction of chloroacetyl chloride with **9** followed by a nucleophilic displacement of Cl^- with N_3^- as shown. Reduction of the azide with lithium aluminum hydride then afforded triamine **11** in good yield. Reaction of **11** with Ac_2O, followed by H_2O_2 oxidation, afforded the spin-labeled diacetate **12b**. Alkaline hydrolysis gave the desired **7**.

Binding Studies with DNA. (a) Circular Dichroism Spectra. Circular dichroism spectra have been used extensively as an indicator of the peptide conformation in AMD derivatives.[13,22,23] The negative band

0022-2623/79/1822-1051$01.00/0

Scheme II



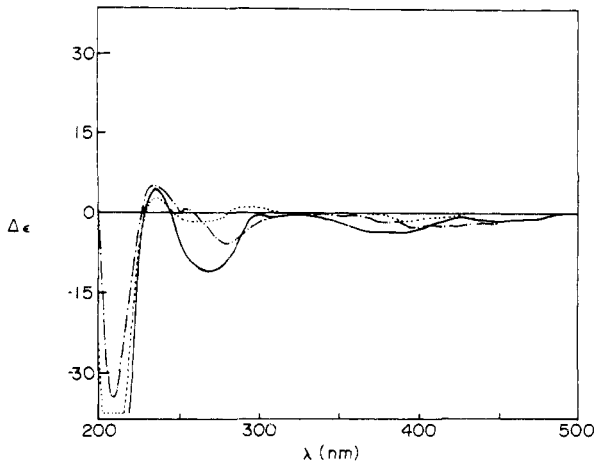


Figure 1. The circular dichroism spectra of spin-labeled AMD in 5 mM phosphate buffer (pH 7.4). The circular dichroism spectra were recorded in a 1.0-cm cell containing 2.0 mL of solutions: **3** (- - -), **5** (···), and AMD (—). The concentration of drug in each case was 1.0×10^{-5} M.

centered around 205–210 nm in AMD is believed[23] to be associated with the peptide conformation. The CD spectra of **3** and **5** together with AMD are shown in Figure 1. The CD spectrum of **4** is almost identical with that of **3** and is not shown. The CD spectra of these analogues are similar, but not identical, with that of AMD in the far-UV region (205–210 nm). However, these spectra are different in the regions 250–300 and 350–500 nm and are shifted to higher wavelengths. These differences may be due to alternations in the special relationship between the peptide side chains and the phenoxazone chromophore induced by the introduction of bulky piperidinyloxy moieties in the N^2 position. The absorption spectra (Figure 2) of these analogues are similar to that of AMD in the far-UV regions but differ somewhat in the visible regions where they are slightly shifted to the higher wavelengths, except in **3** which is shifted to lower wavelengths. Addition of calf thymus DNA (nucleotide/drug = 12:1) resulted in bathochromic shifts. While significantly large bathochromic shifts (3–18 nm) resulted with **5** and AMD upon the addition of DNA, relatively small shifts (1 nm) were observed for analogues **3** and **4**. The CD spectra of **5** and

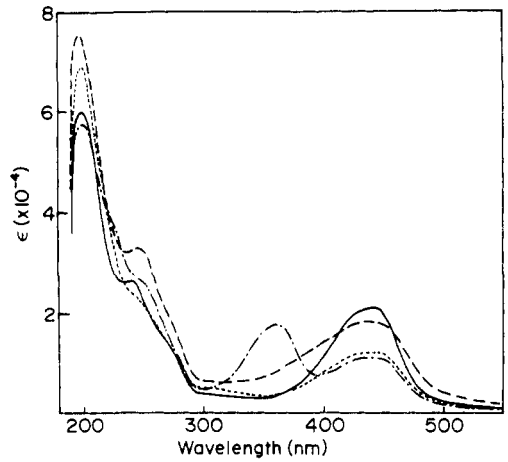


Figure 2. The absorption spectra of AMD and its spin-labeled derivatives in 5 mM phosphate buffer (pH 7.4): **3** (- - -), **4** (···), **5** (···), and AMD (—). The concentration of drug in each case was 1.0×10^{-5} M.

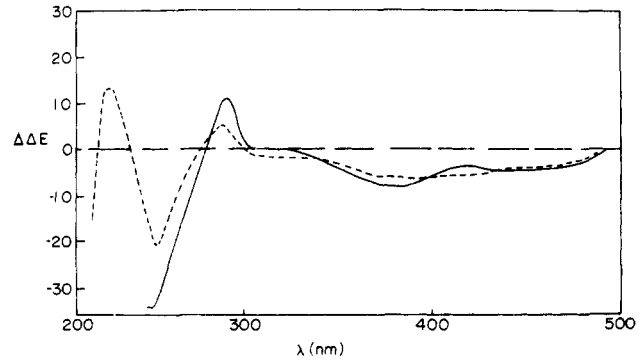


Figure 3. The difference CD spectra of DNA–drug complex in 5 mM phosphate buffer (pH 7.4). The spectra were recorded in a 1.0-cm cell containing 2.0 mL of solution. The calf thymus DNA (50 μg/mL) was added directly to the drug solution and the mixture was incubated for 30 min at room temperature (20–22 °C). The CD spectra of DNA and the free drugs were subtracted from that of DNA–drug complex by computer: AMD (—), **3** (- - -). The concentration of the drug in each case was 1×10^{-5} M.

Table I. T_m Determination[a]

	T_m, °C	ΔT_m, °C
DNA	66.0	
DNA + **3**	67.7	1.7 ± 1.0
DNA + **4**	68.8	2.8 ± 0.5
DNA + **5**	69.5	3.5 ± 0.5
DNA + AMD	74.7	8.7 ± 0.5

[a] The drugs were used at a concentration of 1×10^{-5} M in a solution containing 30 μg/mL of calf thymus DNA in 5 mM phosphate buffer at pH 7.4. The melting temperature was determined at 260 nm by means of a Gilford 250 recording spectrophotometer with a thermoprogrammer 2527 programmed for a temperature rise of 1.0 °C/min. The data represent the average of three experiments.

AMD in the presence of DNA are shown in Figure 3. In each case, a strong positive peak was induced in the 275-nm regions. There is some enhancement in the intensity of the negative band above 350 nm with **5** indicating the participation of the chromophore in DNA binding. In contrast, the addition of DNA to **3** and **4** produced no significant change in the CD spectra, suggesting that the chromophores of **3** and **4** do not participate to any appreciable degree in DNA binding, probably due to steric effects.

(b) Thermal Denaturation Studies. Thermal denaturation studies were carried out according to previously

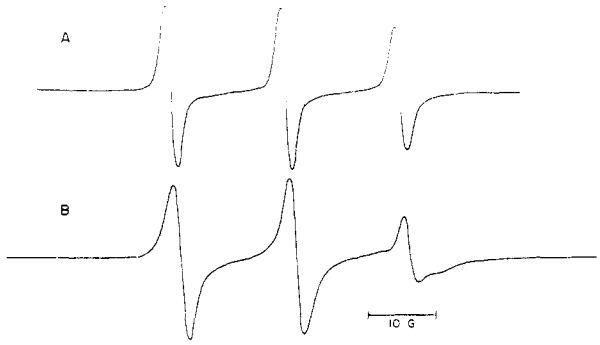


Figure 4. The electron spin resonance spectrum of **5** (1×10^{-5} M) in the absence (A) and presence (B) of calf thymus DNA (2 mg/mL) in 5 mM phosphate buffer (pH 7.4).

Table II. Inhibition of *E. coli* DNA-Dependent RNA Polymerase by Spin-Labeled Actinomycin D[a]

drug concn, μg/mL	AMD		3		4		5	
	CT[b]	ML[c]	CT[b]	ML[c]	CT[b]	ML[c]	CT[b]	ML[c]
20	100	99	78	77	88	91	79	78
10	96	99	62	57	81	78	62	52
5	93	95	41	33	61	56	49	28
2.5	89	92	37	21	43	42	32	22
1.0	79	72						
0.5	61	54						
0.25	62	44						
0.1	30	16						
0.05	17	7						

[a] All assays were run in triplicate. The results represent the average of two separate experiments which did not differ by more than 5%. [b] Calf thymus DNA as primer. [c] *Micrococus luteus* DNA as primer.

published methods,[24-26] and the T_m values (the temperature at which 50% hyperchromicity at 260 nm is induced by heat denaturation of native DNA) are presented in Table I. The data indicate that compounds **3–5** bind to and stabilize the DNA helix toward thermal denaturation. The data also indicate that **5** is more effective than **3** in stabilizing DNA toward thermal denaturation, indicating a stronger DNA binding for **5**. These derivatives, however, are not as effective as AMD, indicating a much weaker DNA binding of **3–5**, possibly due to steric interference from the bulky side-chain substituents.

(c) Electron-Spin Resonance. The ESR spectra of **3–5** in dilute solution consisted of three sharp lines with a splitting of 17.0 G between adjacent lines (Figure 4A). The three-line spectrum results from the anisotropic hyperfine interaction between the unpaired electron and the nuclear spin of the nitrogen atom of the nitroxide radical. In the presence of calf thymus DNA, the ESR spectra of **3** and **4** remained unchanged, indicating no interaction between DNA and the chromophore carrying the nitroxide radical. However, the ESR spectrum of **5** in the presence of calf thymus DNA became broad and asymmetric and was characteristic of a weakly immobilized nitroxide radical (Figure 4B) in which the nitroxide radical is undergoing rapid motion about its X axis. This weakly immobilized spectrum arises from the interaction of the chromophore of **5** with DNA. The ESR data confirm our CD results that the chromophores of **3** and **4** do not participate in binding with DNA.

Biological Studies. (a) DNA-Dependent RNA Polymerase Studies. In vitro inhibition of *E. coli* DNA-dependent RNA polymerase (Table II) using calf thymus DNA and *Micrococcus luteus* DNA as primers with **3–5** and AMD were carried out according to the published procedures.[27-29] The data indicated that AMD (concentration of drug for 50% inhibition = IC_{50} = 0.2 μg/mL) is a very strong inhibitor of this reaction, whereas the spin-labeled AMD analogues (**3–5**) (IC_{50} = 4–7 μg/mL) inhibit this reaction only at higher concentrations and are essentially inactive at lower concentrations. The strong inhibition of the polymerase reaction by AMD may be due to the intercalation of the chromophore into the DNA base pairs in addition to obstruction at the minor groove by the cyclic peptide.

Table III. Cytotoxicity of N^2-Substituted Spin-Labeled Actinomycin D Analogues against L1210 Leukemia Cells in Vitro

drug concn, μg/mL	% inhibn of growth[a]			
	3	4	5	AMD
10	98	99	98	98
5	96	98	69	99
1	57	71	45	97
0.5	39	62	33	99
0.1	21	41	16	78
0.05	10	15	0	78
0.01	0	0	0	57
0.001	0	0	0	51

[a] Each value is the average of three separate experiments, each employing two bottles; two determinations were made on each bottle. Cytotoxicity was determined after 48 h of incubation.

Table IV. In Vivo Antitumor Activities of N^2-Substituted Spin-Labeled Actinomycin D Derivatives against P-388 Leukemia[a]

drug	dose,[b] mg/kg	toxicity[c]	MST,[d] days	% T/C[e]
untreated			10.5	
3	6	+	29	276
	4		26	248
	2.7		19	181
	1.8		20	190
	1.2		17	162
	0.8		17	162
4	6	++	8	76
	4	+	25	238
	2.7		19	181
	1.8		17	162
	1.2		16	152
	0.8		19	181
5	6	+	29	276
	4		18	171
	2.7		18	171
	1.8		19	181
	1.2		15	143
	0.8		15	143
AMD	0.6	++	5	48
	0.4	++	8	76
	0.27	++	10	95
	0.18	+	18	171
	0.12		19	181
	0.08		15	143

[a] 10^6 P-388 cells implanted intraperitoneally on day 0 into groups of eight CDF_1 male mice. [b] Drugs were administered intraperitoneally on days 1, 5, and 9. [c] ++, drug-induced deaths prior to day 9; +, drug-induced weight loss (>10%) determined on day 9. [d] Median survival time. [e] % T/C = (treated survival/control) × 100.

(b) Cytotoxicity of Spin-Labeled AMD against L1210 Cells in Vitro. Cytotoxicity studies were carried out using leukemia L1210 cells (10^5 cells/mL) grown in a static culture RPMI no. 1630 media supplemented with fetal calf serum according to the published procedure.[30] The data are presented in Table III. While the spin-labeled AMD analogues were found to be toxic only at high concentrations, AMD was extremely toxic to these cells

at all concentrations tested.

In Vivo Antitumor Activity. The antitumor activities (Table IV) of the spin-labeled AMD analogues (**3–5**) were measured in lymphocytic leukemia P-388 by standard protocols[31] on a QD 1, 5, 9 schedule. Compounds are considered active if they give reproducible T/C activity[31] values in the P-388 leukemia system equal to or greater than 125%, where T/C represents the ratio of the mean or median survival times of the treated animals to those of the control animals expressed as a percentage. All T/C results reported have been confirmed in a minimum of one additional experiment. All the spin-labeled derivatives displayed antitumor activity that was considerably higher than AMD. At optimal doses (doses that did not result in death but did produce weight loss), derivatives **3–5** were clearly more active than AMD. Optimal doses for these derivatives were nearly 50-fold higher than the optimal for AMD. At doses producing no weight loss, **3** was the most active of the four compounds tested, **4** and **5** being comparable in activity to AMD. Of the derivatives, **4** produced toxicity (weight loss and death) at lower doses than either **3** or **5**.

Conclusion

The electron spin resonance and circular dichroism studies with spin-labeled AMD indicate that the phenoxazone rings of **3** and **4** are not intercalated into double-stranded DNA, possibly due to steric effects; however, intercalation may be involved in the binding of **5**. The peptide conformations in **3–5** are similar, but not identical, with AMD. T_m measurements confirm the ESR and CD data and indicate a stronger binding of **5** to DNA as compared to **3** and **4**. DNA-dependent RNA polymerase studies using either calf thymus DNA or *Micrococcus luteus* DNA as a template indicated similar activities for **3–5** (4–7 μg/mL for IC_{50}), whereas AMD (IC_{50} = 0.2 μg/mL) was considerably more active, suggesting a stronger complex formation with double-stranded DNA. Antitumor activity data against P-388 indicate that **3–5** are more active than AMD. At doses producing no toxicity, **4** and **5** exhibited antitumor activity comparable to that of AMD, while **3** was considerably more active.

The weak binding of **3–5** with double-stranded DNA and their poor activities with DNA-dependent RNA polymerase are consistent with the theory that bulky substitutions in the 2 position greatly reduce intercalative binding. However, the high antitumor activities with little or no toxicity exhibited by **3–5** are somewhat puzzling. The answers may then lie with the nature and conformations of the DNA–drug complexes, relative rates of dissociation, and the specific interactions of the peptides with the DNA grooves. In addition, in vivo metabolism of these compounds to some active derivative may also be important. Bachur et al.[32] have recently shown that AMD is metabolized to a free radical which augments oxygen consumption with the formation of superoxide. The biological activity of AMD may then be related to the superoxide formation. It is tempting to suggest that the increased antitumor effectiveness of derivatives **3–5** may also be due to the in vivo generation of superoxide.

Experimental Section

Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. All mass spectra were taken on an MS 902 (AEI). All elemental analyses were within ±0.4% of the theoretical values, except where indicated. Solvent systems used for thin-layer chromatography were (A) *s*-BuOH–HCOOH–H_2O (75:13.5:11.5) and (B) EtOAc–acetone (2:1). All CD spectra were taken on a Jasco J-40 spectropolarimeter interfaced with a Nicolet computer. The absorption spectra were recorded on a Beckman Acta II spectrophotometer. All ESR spectra were recorded on a Varian E-109. Samples for ESR measurement were introduced into a flat cell in an E-238 TM_{110} cavity. Spectra were recorded at 20 mW, operating at 9.4 MHz. Thermal denaturation studies were carried out at 260 nm by means of a Gilford 250 recording spectrophotometer with a thermoprogrammer 2527 programmed for a temperature rise of 1.0 °C/min. Calf thymus DNA, *Micrococcus luteus* DNA, *E. coli* RNA polymerase, and the nucleoside triphosphate were purchased from Sigma Chemical Co. The [8-^{14}C]ATP (50 mCi/mM) was obtained from New England Nuclear.

***N*-[4-(2,2,6,6-Tetramethyl-1-piperdinyloxy)-1,3-diaminopropane (8)** was prepared according to the published method.[17]

***N*-[4-(2,2,6,6-Tetramethyl-1-piperidinyloxy)]-1,2-diaminoethane (7)** was prepared from **9** as shown in Scheme II. To an ice-chilled solution of **9** (15.6 g, 0.1 mol) in 100 mL of dry $CHCl_3$, 11.3 g (0.1 mol) of chloroacetyl chloride was added dropwise with stirring. After the addition was complete, the reaction mixture was allowed to stand at 4.0 °C for 18 h and extracted with dilute HCl. The acid solution was made basic, and the precipitate was filtered, washed well with H_2O, and dried. This afforded 20.0 g (86%) of **10** as a white crystalline solid, mp 118–120 °C.

To the crude **10** (16.4 g, 0.07 mol) dissolved in 100 mL of DMF, 16 g (0.24 mol) of NaN_3 was added and the reaction mixture was stirred for 18 h at room temperature. The mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed well with H_2O, dried (Na_2SO_4), and filtered. Removal of $CHCl_3$ under reduced pressure afforded 16.0 g (98%) of the desired azide as a colorless liquid: IR ($CHCl_3$) 2210 (N_3), 1665 cm^{-1} (>C=O).

The azide (16.0 g, 0.067 mol) dissolved in 100 mL of dry THF was added dropwise with stirring to a suspension of 10.0 g (0.263 mol) of LAH in 100 mL of dry THF. After the addition was complete, the reaction mixture was refluxed for 48 h and cooled. The excess LAH was decomposed with dilute NaOH, and the THF solution was collected by filtration and dried (Na_2SO_4). Filtration and removal of THF under reduced pressure afforded 9.2 g (69%) of **11** as a colorless liquid: IR (neat) showed no bands at 2110 or 1665 cm^{-1}. The chemical ionization (CH_4) mass spectrum confirmed the molecular ion to be 199 for **11**.

To the triamine **11** (5.5 g, 0.0276 mol) dissolved in 6 mL of glacial acetic acid, 6.0 g (0.059 mol) of Ac_2O was added. The reaction mixture, which turned red, was heated to 100 °C for 2 h, and the excess Ac_2O and AcOH were removed under reduced pressure. The semisolid was dissolved in a small amount of H_2O, neutralized with solid Na_2CO_3, and extracted well with $CHCl_3$. Removal of $CHCl_3$ under reduced pressure afforded a red liquid which showed traces of triacetate upon mass spectral analysis. Purification was achieved by dissolving the liquid in $CHCl_3$ and extracting with dilute HCl. The acid solution was made basic with dilute NaOH and extracted with $CHCl_3$. The $CHCl_3$ solution was decolorized with neutral charcoal and filtered. Workup, followed by crystallization of the semisolid with $CHCl_3$–petroleum ether, afforded 2.6 g (46%) of a light brown solid; mp 107–109 °C; IR ($CHCl_3$) 1640, 1620, 1550 cm^{-1}; MS (16 eV) consistent with the diacetate **12a**, *m/e* (relative intensity) 283 (M^+, 5), 268 (M^+ – 15, 40), 124 [M^+ – (30 + 86 + 43), 100].

To the diacetate **12a** (3.5 g, 0.012 mol) suspended in 20 mL of H_2O, 1.5 g of disodium ethylenediaminetetraacetate, 1.5 g of sodium tungstate, and 20 mL of 30% H_2O_2 were added. The mixture, which turned orange, was stirred overnight at room temperature. The orange solution was saturated with solid NaCl and extracted well with $CHCl_3$. The $CHCl_3$ was dried (Na_2SO_4), filtered, and removed under reduced pressure affording 2.0 g (55%) of a red liquid which solidified upon cooling. Crystallization from $CHCl_3$–hexane gave a red-orange solid: mp 145–148 °C; MS (16 eV) consistent for **12b**, *m/e* (relative intensity) 299 (MH^+, 57), 298 (M^+, 36), 284 (MH^+ – 15, 18), 268 (M^+ – 30, 28).

A suspension of **12b** (1.5 g, 0.005 mol) in 25% aqueous KOH was refluxed for 18 h and cooled. The aqueous solution was saturated with NaCl and extracted with Et_2O. Removal of Et_2O under reduced pressure afforded 1.0 g (98%) of red oil, which solidified upon cooling: IR (neat) 3350, 3300, 1590 cm^{-1} ($-NH_2$); MS (10 eV) consistent for **7**, *m/e* (relative intensity) 215 (MH^+, 18), 214 (M^+, 8), 184 (M^+ – 30, 50), 169 (100), 154 (22), 141 (30), 140 (28).

N^2-[4-(2,2,6,6-Tetramethyl-1-piperidinyloxy)]actinomycin D (3). A General Method. To 200 mg of the 2-deamino-2-chloroactinomycin D (**2**) [prepared according to the published methods,[12,13] R_f 0.46 (B)] dissolved in 20 mL of dry benzene, 150 mg (5 equiv) of 4-amino-2,2,6,6-tetramethyl-1-piperdinyloxy (**6**) in 10 mL of benzene was added. The reaction mixture was stirred at room temperature for 84 h (at this time all of **2** had reacted). The organic solvent was removed under reduced pressure and the residue crystallized from benzene–hexane. Further purification was achieved by LH-20 column chromatography using 95% methanol as eluant, affording 100 mg (40%) of a red solid: mp 240–244 °C dec; TLC (solvent system) R_f 0.84, and homogeneous; UV λ_{max} (CH_3OH) 245 nm (ϵ 37 250), 435 (15 950), 455 (15 550); UV λ_{max} (5 mM phosphate, pH 7.4), 198 nm (ϵ 72 430), 248 (32 170), 433–434 (17 470). Anal. ($C_{71}H_{102}N_{13}O_{17}\cdot 5CH_3OH$) C, N; H: calcd, 7.78; found, 7.21.

N^2-[2-[[4-(2,2,6,6-Tetramethyl-1-piperidinyloxy)]-amino]ethyl]actinomycin D (4). This compound was prepared from **2** and the diamine spin-label **7** in 27% yield: mp 232–235 °C dec, TLC (solvent system B) R_f 0.54; UV λ_{max} (CH_3OH) 245 nm (ϵ 23 818), 435 (10 890); UV λ_{max} (5 mM phosphate, pH 7.7) 199 nm (ϵ 67 230), 240 (24 050), 442 (11 820). Anal. ($C_{73}H_{107}N_{14}O_{17}\cdot 6H_2O$) C, N; H: calcd, 7.68; found, 7.00.

N^2-[3-[[4-(2,2,6,6-Tetramethyl-1-piperidinyloxy)]-amino]propyl]actinomycin D (5). This was similarly prepared from the intermediate **2** and the diamine spin-label **8** in 31% yield: mp 211–213 °C dec; TLC (solvent system B) R_f 0.51; UV λ_{max} (CH_3OH) 245 nm (ϵ 28 750), 358–359 (18 430), 430–432 (10 350); UV λ_{max} (5 mM phosphate, 7.4) 199 nm (ϵ 57 810), 243 (27 850), 360–362 (17 670), 443 (10 450). Anal. ($C_{74}H_{109}N_{14}O_{17}\cdot 6H_2O$) C, H, N.

Acknowledgment. The authors thankfully acknowledge the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, for a supply of actinomycin D. The authors thank Miss Paula Parisius of the Microanalytical Laboratory, National Institute of Arthritis, Metabolism, and Digestive Disease, National Institute of Health, for performing the microanalyses. The technical assistance of Ms. Ovella Ayers in performing cytotoxicity studies is gratefully acknowledged.

References and Notes

(1) W. Kersten, H. Kersten, and W. Szybalski, *Biochemistry*, **5**, 236 (1966).
(2) I. H. Goldberg, M. Rabinowitz, and E. Reich, *Science*, **136**, 315 (1962).
(3) J. E. Kay and H. L. Cooper, *Biochem. Biophys. Res. Commun.*, **35**, 526 (1969).
(4) E. J. Modest and S. K. Sengupta in "Chromosome Identification Technique and Application in Biology and Medicine", T. Casperson and L. Zech, Eds., Academic Press, New York, 1973, pp 327–333.
(5) S. Farber, *J. Am. Med. Assoc.*, **198**, 826 (1966).
(6) J. L. Lewis, Jr., *Cancer*, **30**, 1517 (1972).
(7) H. M. Sobell, *Cancer Chemother. Rep.*, **58**, 101 (1974).
(8) T. R. Krugh, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1911 (1972).
(9) I. H. Goldberg, M. Rabinowitz, and E. Reich, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 2094 (1962).
(10) I. H. Goldberg, M. Rabinowitz, and E. Reich, *Proc. Natl. Acad. Sci. U.S.A.*, **49**, 226 (1963).
(11) H. Brockmann and H. Lackner, *Naturwissenschaften*, **57**, 384 (1964).
(12) S. Moore, M. Kondo, M. Copeland, J. Meinhofer, and R. K. Johnson, *J. Med. Chem.*, **18**, 1098 (1975).
(13) C. W. Mosher, K. F. Kuhlmann, D. G. Kleid, and D. W. Henry, *J. Med. Chem.*, **20**, 1055 (1977).
(14) M. S. Madhavarao, M. Chaykovsky, and S. K. Sengupta, *J. Med. Chem.*, **21**, 958 (1978).
(15) B. K. Sinha and C. F. Chignell, *J. Med. Chem.*, **18**, 669 (1975).
(16) C. F. Chignell, D. K. Starkweather, and B. K. Sinha, *J. Biol. Chem.*, **250**, 5622 (1975).
(17) B. K. Sinha, R. L. Cysyk, D. B. Millar, and C. F. Chignell, *J. Med. Chem.*, **19**, 994 (1976).
(18) B. K. Sinha and C. F. Chignell, *Life Sci.*, **17**, 1829 (1975).
(19) B. K. Sinha and C. F. Chignell, unpublished results.
(20) M. Klimek, *Nature (London)*, **209**, 1256 (1966).
(21) N. M. Emanuel, N. P. Konovola, and R. F. Djachkovskaya, *Cancer Treat. Rep.*, **60**, 1605 (1976).
(22) C. W. Mosher and L. Goodman, *J. Org. Chem.*, **37**, 2928 (1972).
(23) H. Ziffer, K. Yamaoka, and A. B. Mauger, *Biochemistry*, **7**, 996 (1968).
(24) J. A. Allison, R. L. O'Brien, and F. E. Hahn, *Science*, **149**, 1111 (1965).
(25) F. E. Hahn, R. L. O'Brien, J. Ciak, J. A. Allison, and J. G. Olenick, *Mil. Med. Suppl.*, **131**, 1071 (1966).
(26) E. Marquez, J. Cranston, R. W. Rudon, and J. H. Burckhalter, *J. Med. Chem.*, **17**, 856 (1974).
(27) F. J. Bollum, *Proced. Nucleic Acid Res.*, **1**, 296 (1966).
(28) B. K. Sinha, R. Sato, R. P. Phillen, and R. L. Cysyk, *J. Med. Chem.*, **20**, 1528 (1977).
(29) B. K. Sinha and R. Sato, *J. Pharm. Sci.*, **67**, 407 (1978).
(30) G. E. Moore, A. A. Sandberge, and K. Vlrich, *J. Natl. Cancer Inst.*, **36**, (1966).
(31) R. I. Green, N. H. Greenberg, M. M. MacDonald, A. M. Schumaker, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**(2) 1 (1972).
(32) N. R. Bachur, M. V. Gee, and S. L. Gordon, *Proc. Am. Assoc. Cancer Res.*, **19**, 75 (1978).

Antifungal Agents. 5.[1] Chemical Modification of Antibiotics from *Polyangium cellulosum* var. *fulvum*. Alcohol, Ketone, Aldehyde, and Oxime Analogues of Ambruticin

David T. Connor* and Maximillian von Strandtmann[2]

Warner-Lambert/Parke-Davis, Pharmaceutical Research Division, Ann Arbor, Michigan 48106. *Received January 12, 1979*

Alcohol, ketone, aldehyde, and oxime analogues of ambruticin (**1**) were prepared. The analogues were tested against *Histoplasma capsulatum*, *Microsporum fulvum*, *Candida albicans*, and *Streptococcus pyogenes*. Structure–activity relationships are described. Increasing the bulk of substituent at C_1 and C_5 reduces antifungal activity.

In part 4 of this series,[1] we described the structure–activity relationship of esters and amides derived from the potent antifungal antibiotic ambruticin (**1**).[3] Ambruticin was isolated from the fermentation broth of *Polyangium cellulosum* var. *fulvum*. The present paper describes the synthesis and biological activity of alcohols, ketones, al-

0022-2623/79/1822-1055$01.00/0