7.7 Hz, NH); ^{13}C NMR δ 60.3, 69.9, 74.9, 83.4, 86.1, 111.8, 155.8, 158.6, and 159.3. Anal. ($C_9H_{13}N_5O_6$) C, H, N.

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Synthesis and Biological Properties of Actinomycin D Chromophoric Analogues Substituted at Carbon 7 with Aziridine and Cyclopropyl Functions

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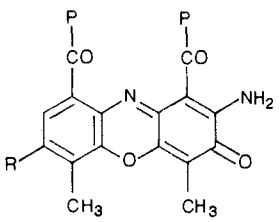
The growing importance of functionalized tricyclic rings, e.g., cyclopropyl and aziridine, in numerous organic biomolecules led us to develop syntheses of novel actinomycin D (AMD) analogues substituted with aziridine and cyclopropyl functions. Reaction of 7-hydroxyactinomycin D with 1-aziridineethyl iodide and bromomethylcyclopropane afforded the desired 7-[2-(1-aziridinyl)ethoxy] and cyclopropylmethoxy analogues, respectively. Calf thymus DNA binding of these analogues was comparable to that of AMD as examined by UV-vis difference spectral measurements, CD techniques, and relaxation of supercoiled closed circular SV40 DNA, indicating an intercalative mode of binding to the DNA duplex. Thermal denaturation of DNA experiments employing higher temperatures than room temperature exhibit a thermal lability of the DNA analogue complexes, suggestive of a probable covalent bond formation with DNA bases. The analogues were found to be $^1/_4$-$^1/_{40}$ as cytotoxic to human lymphoblastic CCRF-CEM leukemia and B_{16} melanoma cells in vitro as AMD, with ID_{50} values in the nanomolar concentration range.

Actinomycin D (AMD, **1b**, Chart I) is a clinically useful chemotherapeutic agent in the treatment of a limited number of tumors.[1,2] It binds to double-helical DNA of the cell by intercalation of its chromophore between guanosine-cytosine sequences with the peptide portion lying in the minor groove of the DNA helix as confirmed by spectroscopic and hydrodynamic studies of this complex.[3-5] This drug-DNA interaction is believed responsible for its selective inhibition of DNA-primed RNA synthesis.[6,7]

Actinomycin D is also known to cause chromosomal damage and appears to require active cell processes for this to occur. To explain this, an alternative mechanism involving enzymatic reduction of the quinone imine structure of AMD to a free-radical intermediate has been proposed.[8,9] These free radicals are presumed to be the critical activated form of the antibiotic to cause intracellular DNA damage and cell death.

In an earlier paper we reported the synthesis of 7-(2,3-epoxypropoxy)actinomycin D (**3b**), which appeared to retain the DNA binding property and to enhance the in vivo tumor-inhibiting activity of AMD.[10] In addition, other functionalized aziridines, namely AZQ and mitomycin C, are reported to act as tumor-inhibiting alkylating agents.[11] In light of these observations, we prepared the corresponding aziridine analogue (**4b**). During attempted preparation of the aziridine (**4b**) by an alternate route, we isolated the linear 2-aminopropoxy compound (**6b**).[12]

Chart I[a]



1, R = H
2, R = OH
3, R = $OCH_2CH(O)CH_2$
4, R = $OCH_2CH(NH)CH_2$
5, R = $OCH_2CH(CH_2)CH_2$
6, R = $OCH_2CH(NH_2)CH_3$
7, R = $OCH_2CH_2N(CH_2)CH_2$
8, R = $OCH_2CH_2NHCH_2CH_2OH$

[a] **a**, P = $N(C_2H_5)_2$. **b**, P = Thr-D-Val-Pro-Sar-MeVal (cyclic, O)

Although calf thymus DNA binding of actinomycin D analogues **4b** and **6b** was comparable to AMD, these were comparatively less cytotoxic to human lymphoblastic CCRF-CEM leukemia and B_{16} cells in vitro. As an extension of this work, we now report our results on cyclopropylmethoxy-substituted derivative (**5b**). We surmised that a cyclopropane ring containing a highly strained covalent bond would be prone to cleavage to an ion-pair intermediate, which would release strain.[13] Ring cleavage of cyclopropane rings by nucleophiles has long been known.[14] The cyclopropane ring could cleave to singlet diradical transition and zwitterion transition state in ep-

(1) Farber, S. *JAMA, J. Am. Med. Assoc.* **1966**, *198*, 826.
(2) Lewis, J. L., Jr. *Cancer*, **1972**, *30*, 1517.
(3) Müller, W.; Crothers, D. M. *J. Mol. Biol.* **1968**, *35*, 251.
(4) Sobell, H. M.; Jain, S. C. *J. Mol. Biol.* **1972**, *68*, 21.
(5) Sobell, H. M. *Prog. Nucleic Acid Res.* **1973**, *13*, 153.
(6) Goldberg, I. H.; Rabinowitz, M.; Reich, E. *Proc. Natl. Acad. Sci. U.S.A.* **1962**, *48*, 2094.
(7) Goldberg, I. H.; Rabinowitz, M.; Reich, E. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *49*, 226.
(8) Nakazawa, H.; Chou, Feng-te E.; Andrews, P. A.; Bachur, N. R. *J. Org. Chem.* **1981**, *46*, 1493.
(9) Bachur, N. R.; Gee, M. V.; Friedman, R. D. *Cancer Res.* **1982**, *42*, 1078.
(10) Sengupta, S. K.; Anderson, J. E.; Kelley, C. *J. Med. Chem.* **1982**, *25*, 1214.
(11) Kinoshita, S.; Uzu, K.; Takahashi, T. *J. Med. Chem.* **1971**, *14*, 109; **1982**, *25*, 1214.
(12) Sehgal, R. K.; Almassian, B.; Rosenbaum, D. P.; Zadrozny, R.; Sengupta, S. K. *J. Med. Chem.* **1987**, *30*, 1626.
(13) Cram, D. J.; Ratajczak, A. *J. Am. Chem. Soc.* **1968**, *90*, 2198.
(14) Yankee, E. W.; Spencer, B.; Howe, N. E.; Cram, D. J. *J. Am. Chem. Soc.* **1973**, *95*, 4220.

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Table I. Bathochromic Shifts in the Absorption Maxima on Binding to DNA

drug	λ_{max}, nm: drugs	drugs + DNA[a]	concn mmol
AMD (**1b**)	440	476–478	0.015
4b	450	502–504	0.011[b]
5b	466	502–504	0.013
6b	458–460	502–504	0.013[b]
7b	457	496–497	0.011
8b	453	489–491	0.015

[a] DNA concentration, 0.26 in mmol. [b] Data reported in an earlier paper (see ref 12).

imerization reactions of cyclopropanes[15] and thus act as an alkylating species for interaction with nucleophilic bases in DNA. Recently, Hurley et al. reported an antitumor-antibiotic (CC-1065) that contains a strained cyclopropyl ring and binds to DNA in nonintercalative fashion along the minor groove and forms an irreversible, covalent adduct.[22] In the present paper, we report the synthesis, DNA-binding property, and growth-inhibitory activity of 7-[(2-aziridinyl)methoxy]- and 7-[(2-cyclopropyl)methoxy]-substituted analogues of actinomycin D (**4b** and **5b**, respectively).

We also report on the preparation of an additional analogue, 7-[2-(1-aziridinyl)ethoxy]-substituted actinomycin D (**7b**) and attempt to correlate its activity with those of **3b–6b**.

Chemistry. The syntheses of the model **5a** and the corresponding actinomycin D derivative **5b** were readily accomplished by reaction of the respective 7-hydroxy compound with bromomethylcyclopropane in dry acetone in the presence of finely powdered anhydrous potassium carbonate at room temperature, and the products were characterized by spectral and analytical data.

A similar reaction of the model compound **2a** and the corresponding actinomycin D compound **2b** with 1-aziridineethyl iodide afforded 7-[2-(1-aziridinyl)ethoxy] analogue **7** and also ring-opened compounds **8**, respectively. For synthesis of 1-aziridineeethyl iodide, 1-aziridineethanol was reacted with trifluoromethanesulfonyl chloride in the presence of sodium hydride in tetrahydrofuran to afford the corresponding triflate and the latter was displaced by iodide (potassium iodide in isopropyl alcohol) to the iodo analogue.

Biophysical Experiments. DNA Binding by Spectral Shift. The analogues' binding to DNA was examined by spectral shift of the absorption maxima summarized in Table I. On complexing with calf-thymus DNA, the long-wavelength absorption maximum of the analogoues underwent simultaneous bathochromic and hypochromic shift. The difference spectra of free and DNA-bound actinomycin analogues were obtained by subtracting from the spectrum of a solution containing actinomycin analogue bound to DNA a spectrum of a reference solution containing the same concentration of free actinomycin analogue. The characteristic hypochromic and bathochromic troughs and peaks in the difference UV spectra of AMD and analogues (Figure 2, supplementary material) show positive and negative maxima indicative of binding to DNA by intercalation.[18] From the $-\Delta A$ value in the difference spectra, which is a measure of the drug's binding affinity to DNA, it is evident that the relative strength of intercalative association of these agents is in the order AMD (**1b**) > (**8b**) ≅ (**7b**) > (**5b**) at 20 °C in 0.01 M phosphate (pH 7.0) buffer in 30 min.

Table II. Elevation of T_m Values of DNA (ΔT_m) on Complexation with Actinomycin D Analogues

drugs	ΔT_m,[a] C	drugs	ΔT_m,[a] C
AMD (**1b**)	8.0 ± 0.1	**6b**	4.8 ± 0.1[b]
4b	2.8 ± 1.1[b]	**7b**	1.8 ± 1.1[c]
5b	−0.6 ± 1.2[c]	**8b**	1.9 ± 0.1

[a] ΔT_m = T_m of DNA–drug complex minus T_m of DNA. Concentration of drug, 2.5×10^{-5} M; of DNA, 5.0×10^{-5} M; in 0.01 M phosphate buffer (pH 7). [b] Data reported in ref 12. [c] Broad ΔT_m values suggest thermal lability of the complex, which is typical of covalently bound drug–DNA adducts.

Table III. In Vitro Antitumor Activity

drug	ID_{50}[a]: CCRF-CEM cells[b]	B_{16} melanoma cells[c]
AMD (**1b**)	13.0[d]	3.3[d]
4b	76.0	110.0
5b	55.0	38.0
6b	60.0	55.0
7b	300.0	139.8
8b	500.0	*e*

[a] Concentration of compound in nanograms/milliliter required for 50% inhibition at 48 h of cells in suspension culture. Compounds were dissolved in Me_2SO–saline; the final growth medium contained less than 1% Me_2SO. [b] Human leukemic lymphoblastic cells. [c] Murine melanoma cell line. [d] These values are an average of three experiments. The standard deviation for all experiments ranged between 8% and 10% of the mean value. [e] Not determined.

By Thermal Denaturation of DNA. These experiments were carried out according to the procedures described earlier.[16] The ΔT_m values are a measure of stabilization of the DNA helical structure as a consequence of drug binding. The ΔT_m data for new analogues bound to the double helices of DNA represent weaker or different modes of association with the DNA helix as compared to AMD (Table II). As a rule, these results are not in conformity with the UV–vis difference spectral data; they indicate that **8b** and **6b** appear to bind to DNA with some definite affinity but less strongly than AMD. In contrast, other drug–DNA complexes of **5b** and **7b** appear to be labilized as a consequence of thermal treatment, which is reminiscent of the behavior of DNA-bound alkylating agents.[10,22]

Relaxation of ccc-SV 40 DNA Determined by Agarose Gel Electrophoresis.[17] Analogues **4b–8b** were found to relax supercoiling of closed-circular DNA in a progressive dose-dependent manner, as additional evidence for intercalation (Figure 1, supplementary material).

Circular Dichroic Spectral Results. The circular dichroic spectra indicate marked resemblance in the electronic nature of their chromophores and in their peptide conformations upon binding to DNA (Figures 3 and 4, supplementary material).

In Vitro Tumor Growth Inhibitory Activity. The analogues were assayed for growth inhibitory activity against human lymphoblastic leukemic cells (nCCRF-CEM) in continuous spinner culture and also monolayer

(15) Chmurny, A. B.; Cram, D. J. *J. Am. Chem. Soc.* **1973**, *95*, 4237.
(16) Sengupta, S. K.; Anderson, J. E.; Kogan, Y.; Trites, D. H.; Beltz, W. R.; Madhavarao, M. S. *J. Med. Chem.* **1981**, *24*, 1052.
(17) Keller, W. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4876.
(18) Sengupta, S. K.; Schaer, D. *Biochim. Biophys. Acta* **1978**, *521*, 89.
(19) Foley, G. E.; Lazarus, H. *Biochem. Pharmacol.* **1967**, *16*, 659.
(20) Ross, W. E.; Bradley, M. O. *Biochim. Biophys. Acta* **1981**, *654*, 129.
(21) Pan, S.; Iracki, T.; Bachur, N. R. *Mol. Pharmacol.* **1986**, *29*, 622.
(22) Hurley, L. H.; Needham-VanDevanter, D. R. In *Mechanism of DNA Damage and Repair; Implications of Carcinogenesis and Risk Assessment*; Simic, M.; Grossman, L., Upton, A. C., Eds.; Plenum: New York, 1986; pp 203–210.

culture of B_{16} melanoma cells, both in the log phase growth (Table III).[19] These assays are sensitive to actinomycin and analogues and indicate their relative cytotoxicity. Actinomycin analogues **4b–6b** are moderately cytotoxic as compared to the extreme cytotoxicity of AMD. Analogue **8b** is found least active in these systems.

Discussion

Actinomycin D is an extremely potent drug and is known to be acutely toxic to its host; the present objective is to reduce its host toxicity and broaden its antitumor activity. Tricyclic ring-substituted analogues **4b**, **5b**, and **7b** intercalate into DNA with a weaker affinity and are less cytotoxic than AMD against the cell lines studied (CEM and B_{16}). Their peptide lactones show similar conformational and circular dichroic effects on interaction with DNA. It is likely that these analogues interact both by intercalation and covalent bond formation with DNA as has been observed in the case of the epoxy compound **3b**, which is also reported to destabilize the DNA helix at temperatures higher than 37 °C and to show a smaller degree of change in ΔT_m value compared to AMD.[10] Of these analogues, **7b** carries a longer substituent chain, i.e., ethoxyaziridine at C-7, which might not be of optimum length[10,16] for its interaction with DNA nucleophiles, and therefore, the ID_{50} values of this agent are not as promising as one would expect. Cyclopropylmethoxy compound **5b** shows a negative ΔT_m value and exhibits better activity than **7b** or **8b**, which may be due to DNA adduct formation by **5b** via radical or carbonium ion intermediate. Therefore, further work is needed to understand the mechanism of cytotoxicity of these agents.

In tumor cells, in vitro mitomycin C generates extensive double-strand breaks, which is attributed largely to the alkylating activity of its aziridine moiety.[21] Mitomycin C is more effective than AMD against L1210 leukemia and P388/ADR in vivo.[21] To enhance the in vivo activity of actinomycin, the new analogues **4b** and **5b**, which bind to DNA not only by intercalation but probably also via alkylation, may be the desired agents. These analogues might prove active against tumors, like P388/ADR, which are not sensitive to actinomycin and other intercalating drugs but are sensitive to alkylating agents.

Experimental Section

The IR spectra were taken with a Perkin-Elmer Model 457A grating spectrophotometer in potassium bromide pellets unless otherwise noted, UV spectra were measured with a Gilford Model 250 spectrophotometer, and NMR spectra were determined on a Varian A-60 or 300-MHz spectrometer in deuteriochloroform with tetramethylsilane as internal standard. Analytical TLC's were done on 5 × 20 cm precoated glass plates with a 0.25-mm layer of silica gel 25 (Macherey-Nagel) with chloroform/acetone (4:1) as the developing agent.

Preparative thin-layer chromatography was performed on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF-254 (E. Merck, Darmstadt, Germany). The compounds were detected by visual examination under UV light (254 nm). Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are specified by symbols of the elements, analytical results obtained for these elements were within ±0.4% of theoretical values. Samples containing acetone of crystallization were dried at 80–100 °C under vacuum (0.4 mm) prior to determination of NMR spectra and biological assay works.

DNA-binding experiments were carried out in 0.01 M phosphate buffer (pH 7) containing EDTA (10^{-5} M). Calf-thymus DNA was purchased from Sigma Chemical Co., St. Louis, MO. Spectral studies involving shift of the absorption maxima on complexing with DNA were performed in a Gilford 250 spectrophotometer in a quartz cell, which, with the addition of a base-line reference compensator (Analog Multiplexer 6064) and thermoprogrammer, auto four cell programmer, and thermoelectric cell holder 2577, was used to obtain thermal denaturation curves. CD spectra were obtained on a Cary 61 spectropolarimeter.

For unwinding studies with covalently closed circular DNA, SV40 DNA (0.235 μg, BRL, Bethesda, MD) was dissolved in 10 mM Trizma base (pH 7.4) buffer containing 50 mM KCl, 50 mM NaCl, 5 mM $MgCl_2$, and 0.1 mM EDTA and incubated with appropriate volumes of drug solution for 60 min at 37 °C. After the addition of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol in 30% glycerol), each sample was added to 1% agarose gel with Tris–borate EDTA buffer (pH 8). Electrophoresis was performed at 35 V overnight. The DNA was visualized by staining the gel with ethidium bromide (0.5 μg/mL) for 30 min and destaining with distilled water. The results of these unwinding experiments prove that, like AMD, analogues **3b**, **4b**, and **5b** relax the supercoiled DNA, indicative of the drugs' intercalation into DNA (Figure 1, supplementary material).

2-Amino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-7-(cyclopropylmethoxy)-3*H*-phenoxazin-3-one (5a). A solution of the 7-hydroxy compound **2a** (56.75 mg, 0.125 mmol) in dry acetone (60 mL) was allowed to react with bromomethylcyclopropane (0.5 mL) and finely powdered anhydrous potassium carbonate (50 mg) in a nitrogen atmosphere at room temperature for 48 h. The bluish color of the reaction mixture turned to red-brown when the reaction was completed. The reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated. The residue was partially purified by silica gel (60 g) column chromatography. Initial elution with chloroform (150 mL) afforded unreacted bromomethylcyclopropane along with impurities. Subsequent elution with 1:1 acetone/chloroform (125 mL) afforded the desired product (**5a**) along with impurities. After removal of the solvent, the product was purified by preparative thin-layer chromatography (silica gel, 1:8 acetone/chloroform), yielding **5a** (14 mg, 22%): R_f 0.66 (R_f of **2a** 0.36); UV λ_{max} ($CHCl_3$) inf 290, 458 nm, (ε 39281); IR 3300, 3440 cm^{-1} (NH_2); NMR δ 6.76 (s, 8-H, 1 H), 3.90 (d, OCH_2, 2 H), 3.02–3.66 (m, $N(CH_2CH_3)_2$, 8 H], 2.2 (s, 4-CH_3, 3 H), 2.34 (s, 6-CH_3, 3 H), 0.80–1.66 [m, $N(CH_2CH_3)_2$, 12 H, cyclopropyl, 1 H], 0.62 (m, cyclopropyl, 2 H), 0.36 (m, cyclopropyl, 2 H). Anal. ($C_{28}H_{36}N_4O_5 \cdot CH_3COCH_3 \cdot H_2O$) C, H, N.

7-(Cyclopropylmethoxy)actinomycin D (5b). A solution of the 7-hydroxyactinomycin D (**2b**) (50 mg, 0.0375 mmol) in dry acetone (60 mL) was allowed to react with bromomethylcyclopropane (0.75 mL) and finely powdered anhydrous potassium carbonate (75 mg) in a nitrogen atmosphere at room temperature in the dark for 48 h. Workup as in the case of the model derivative **5a** afforded **5b** (6 mg, 12%): R_f 0.36; UV λ_{max} ($CHCl_3$) 458 nm, (ε 13355); NMR ($CDCl_3$) δ (*J*) 8.18 (6.0), 8.06 (6.7) [NHC=O(Val)], 7.66 (6.2), 7.35 (6.4) [NHC=O(Thr)], 7.25 (br, $ArNH_2$), 7.2 (s, Ar C8-H), 6.02 (7.5), 5.95 (7.5) [α-CH(MeVal)], 5.25 (2.5), 5.15 (5.0) [β-CH(Thr)], 4.85 (6.0), 4.78 (5.0) [α-CH(Thr)], 2.43 (s, 6-CH_3), 2.25 (s, 4-CH_3), 0.41 and 0.76 (m, cyclopropyl, 2 H), 1.13 (m, cyclopropyl, 1 H), 3.96 (d, OCH_2, 2 H).

2-(1-Aziridinyl)ethyl Trifluoromethanesulfonate (1-Aziridineethyl Triflate). Sodium hydride (300 mg, 80% dispersion in mineral oil) in small portions was added slowly to a solution of 1-aziridineethanol (0.87 g, 10 mmol) in anhydrous tetrahydrofuran (25 mL) in an atmosphere of nitrogen cooled to −80 °C in a methanol–dry ice bath over a period of 30 min. Trifluoromethanesulfonyl chloride (1.68 g, 10 mmol) was then added in small portions, and the mixture was stirred cold for 4 h and then at room temperature overnight. The mixture was decomposed with $NaHCO_3$ solution (2%, 50 mL), and the aqueous layer was extracted with ethyl acetate (6 × 30 mL). Ethyl acetate extract was dried (Na_2SO_4), and after evaporation of the solvent, the residue was purified by distillation under vacuum, bp 48–53 °C (1–2 mm) (1.43 g, 65%): NMR δ 5.53 (t, OCH_2, 2 H), 3.94 (t, NCH_2, 2 H), 1.94 (m, aziridine ring protons, 4 H).

2-Amino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-7-[2-(1-aziridinyl)ethoxy]-3*H*-phenoxazin-3-one (7a). To a solution of the aziridineethyl triflate (1.095 g, 5 mmol) in isopropyl alcohol (25 mL) was added powdered anhydrous potassium iodide (0.91 g, 5.5 mmol), and the mixture heated in an atmosphere of nitrogen for 8 h at a bath temperature of 64 °C. Isopropyl alcohol was distilled under diminished pressure, the residue was treated with water (15 mL), the product was extracted with ethyl acetate (6 × 25 mL), the extract was dried (Na_2SO_4), and solvent was

removed under reduced pressure. The residue was added to a solution of the 7-hydroxy compound (**2a**) (113.5 mg, 0.25 mmol) in dry acetone (50 mL) containing powdered anhydrous potassium carbonate (50 mg) and was stirred in a nitrogen atmosphere at 37 °C for 48 h. The reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated. The residue was purified from excess of the iodo compound by being passed through a short column of silica gel and elution with chloroform. The column was then extracted with 1:1 acetone/chloroform, and after removal of the solvent, the residue was separated into components by preparative thin-layer chromatography (silica gel, 1:8 acetone/chloroform) to afford **7a** (25 mg, 19%), R_f 0.43 and **8a** (43 mg, 32%), R_f 0.24.

7a: UV λ_{max} ($CHCl_3$) inf 290, 450 nm (ϵ 29 500); IR 3320, 3440 cm^{-1} (NH_2); NMR δ 6.73 (s, 8-H, 1 H), 5.42 (s, NH_2, 2 H), 4.06 (m, OCH_2, 2 H), 2.96–3.83 [m, $N(CH_2CH_3)_2$ and NCH_2CH_2O, 10 H], 2.23 (s, 6-CH_3, 3 H), 2.05 (s, 4-CH_3, 3 H), 0.65–1.67 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. ($C_{28}H_{37}N_5O_5 \cdot 2CH_3COCH_3 \cdot H_2O$) C, H, N.

8a: UV λ_{max} ($CHCl_3$) inf 290, 453 (ϵ 26 900); IR 3340, 3440 (br, OH, NH_2); NMR δ 6.83 (s, 8-H, 1 H), 5.43 (br s, NH_2, 2 H), 4.8 (m, NH, 1 H), 4.12 (t, OCH_2, 2 H), 2.90-3.96 [m, $N(CH_2CH_3)_2$, CH_2NCH_2, CH_2OH, 14 H], 2.32 (s, 6-CH_3, 3 H), 2.20 (s, 4-CH_3, 3 H), 0.71–1.53 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. $C_{28}H_{39}N_5O_6 \cdot 3CH_3COCH_3$) C, H, N.

7-[2-(1-Aziridinyl)ethoxy]actinomycin D (7b). A solution of the 7-hydroxyactinomycin D (**2b**) (40 mg, 0.03 mmol) in dry acetone (60 mL) was allowed to react with 1-aziridineethyl iodide (prepared from 1.095 g of 1-aziridine triflate) and finely powdered anhydrous potassium carbonate (50 mg) in a nitrogen atmosphere at room temperature in the dark for 24 h. The brown reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated, chromatographed on a silica gel column (30 g), and eluted with chloroform (150 mL) to remove excess of the iodo compound. Elution with 2:1 chloroform/acetone (140 mL) afforded **7b** along with impurities. Final elution by 1:1 acetone/chloroform afforded **8b** (5 mg, 10%): R_f 0.24; UV λ_{max} ($CHCl_3$) 453 (ϵ 11 150). Compound **7b** was purified by preparative thin-layer chromatography to afford a single yellow band (6 mg, 14%): R_f 0.36; UV λ_{max} ($CHCl_3$) 457 nm (ϵ 11 500).

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Registry No. 2a, 57270-61-8; **2b**, 21478-73-9; **4b**, 109122-96-5; **5a**, 112599-09-4; **5b**, 112599-10-7; **6b**, 109123-01-5; **7a**, 112599-11-8; **7b**, 112599-12-9; **8a**, 112599-13-0; **8b**, 112599-14-1; bromomethylcyclopropane, 7051-34-5; 2-(1-aziridinyl)ethanol, 1072-52-2; 2-(1-aziridinyl)ethyl trifluoromethanesulfonate, 112599-15-2; 1-(1-aziridinyl)-2-iodoethane, 45378-69-6.

Supplementary Material Available: A discussion of the agarose gel electrophoresis of ccc-SV 40 DNA–analogue complexes (Figure 1), Figure 2 illustrating the UV–vis difference absorption spectra on binding to DNA, and Figures 3 and 4, illustrating and discussing CD spectra of free drugs and CD spectra of drug–DNA complexes, respectively (7 pages). Ordering information is given on any current masthead page.

Studies on Scavengers of Active Oxygen Species. 1. Synthesis and Biological Activity of 2-*O*-Alkylascorbic Acids

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A novel series of 2-*O*-alkylascorbic acids (**5a–u**) was synthesized, and their scavenging activities against active oxygen species as well as their suppressive effects on the arrhythmias in rat heart ischemia–reperfusion models were evaluated. Some 2-*O*-alkylascorbic acids (**5e–l**) exhibited potent inhibiting activities against lipid peroxidation in rat brain homogenates and in alleviating effects in the ischemia–reperfusion models. Studies on the structure–activity relationship demonstrated that a free 3-enolic hydroxyl group and the longer alkyl chains substituted on the 2-hydroxyl group of ascorbic acid were beneficial for the biological and pharmacological activities. 2-*O*-Octadecylascorbic acid (**5k**, CV-3611), one of the most potent and promising compounds, markedly inhibited lipid peroxidation ($IC_{50} = 4.3 \times 10^{-6}$ M) and alleviated myocardial lesions induced by ischemia–reperfusion at an oral dose of 1 mg/kg in rats.

Recently, an increasing number of reports[1] describe the roles of active oxygen species (AOS) in the development or exacerbation of various kinds of diseases: heart attack, stroke, emphysema, rheumatism, inflammation, and cancer.[1,2] AOS, including superoxide (O_2^-), hydrogen peroxide, the hydroxyl radical, and the ferryl radical, are considered to be generated by, or formed subsequent to, reduction of molecular oxygen in living organisms.[2,3] The hydroxyl radical and the ferryl radical, a complex of oxygen radical and iron ion, are the most reactive and are thought to be the major species responsible for oxidative injury of enzymes, lipid membranes, and DNA in living cells and tissues.[4] Although the mechanisms of the oxidative injury are not well understood, AOS may bring about membrane perturbation through the lipid peroxidation of cellular and microsomal membranes to affect calcium influx, phospholipase activation,[5] and release of lysosomal enzymes and chemical mediators.[4] Prostaglandins and leukotrienes in the arachidonate pathway linked with lipid peroxidation may amplify the oxidative damage.[6]

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(1) (a) Hammond, B.; Kontos, H. A.; Hess, M. L. *Can. J. Physiol. Pharmacol.* **1985**, *63*, 173. (b) Fridovich, I. *Annu. Rev. Pharmacol. Toxicol.* **1983**, *23*, 239. (c) Burton, K. P.; McCord, J. M.; Ghai, G. *Am. J. Physiol.* **1984**, *246*, H776.
(2) McCord, J. M. *N. Engl. J. Med.* **1985**, *312*, 159.
(3) Clark, R. A.; Leidal, K. G.; Pearson, D. W.; Nauseef, W. M. *J. Biol. Chem.* **1987**, *262*, 4065.
(4) (a) Halliwell, B.; Gutteridge, J. M. *Trends Biochem. Sci. (Pers. Ed.)* **1986**, *11*, 372. (b) Youngman, R. Y. *Ibid.* **1984**, *9*, 280.
(5) (a) Bromberg, Y.; Pick, E. *J. Biol. Chem.* **1985**, *260*, 13539. (b) Beckman, J. K.; Borowitz, S. M.; Burr, I. M. *Ibid.* **1987**, *262*, 1479.
(6) Lands, W. E.; M., Kulmacz, R. J.; Marshall, P. J. *Free Radicals in Biology*; Academic: Orlando, 1984; Vol. VI, p 39.

0022-2623/88/1831-0793$01.50/0