13. A stirred solution of **1a** (1.13 g, 5 mmol) and **13** (0.70 g, 5 mmol) in dry toluene (50 mL) was heated to reflux for 3 days under a gentle stream of N_2 to remove the liberated HCl. After evaporation of the solvent in vacuo, column chromatographic purification on silica gel (CH_2Cl_2) afforded pure product **15** as a white solid (785 mg, 48%): TLC R_f = 0.52 (Et_2O); mp 187–188 °C, resolidify, 211–215 °C dec; ^1H NMR (C_6D_6) δ 2.79 (s, 3 H), 3.07 (s, 3 H), 4.17 (d, J = 9.3 Hz, 1 H, H-9), 4.78 (d, J = 9.3 Hz, 1 H, H-8), 6.32 (t, J = 8.1 Hz, 1 H, Ar), 6.71 (d, J = 8.1 Hz, 2 H, Ar); ^1H NMR (MeOH-d_4/acetone-d_6, 1:1) δ 3.09 (s, 3 H), 3.15 (s, 3 H), 4.94 (d, J = 9.25 Hz, 1 H, H-9), 6.12 (d, J = 9.25 Hz, 1 H, H-8), 7.51 (s, 3 H, Ar); EIMS (20 eV) m/z (rel intensity) 83 (52), 140 (base), 292 (44), 327 (M^+, 5). Anal. Calcd for $C_{13}H_{11}N_3O_3Cl_2$: C, 47.58; H, 3.38; N, 12.80. Found: C, 47.33; H, 3.29; N, 12.75.

15 from 13 and 17. A solution of **13** (420 mg, 3 mmol) and **17** (565 mg, 3 mmol) in dry toluene (30 mL) was stirred at room temperature for 3 days during which time crystalline product **15** appeared in the reaction mixture. The solid was separated by filtration, washed with toluene (1 mL), and dried in vacuo to give the pure product **15** (290 mg). Additional product (152 mg) was obtained from the filtrates and washings in a manner similar to that used for the preparation of **15** from **1a** and **13**. Overall yield of **15** was 442 mg (45%).

Transformation of 15 to 14. To a stirred solution of **15** (100 mg, 0.3 mmol) in THF (8 mL) was added triethylamine (50 mg, 0.5 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 day, and the low-boiling materials were removed under reduced pressure to give **14** (100 mg, 100%).

1,3-Dimethyl-5-(2,6-dichlorobenzoyl)uracil Oxime (16). A stirred solution of **1a** (2.25 g, 10 mmol) and **13** (1.40 g, 10 mmol) in reagent-grade toluene (50 mL) was heated to reflux for 3 days. After evaporation of the solvent in vacuo, the solid residue was washed with Et_2O (3 × 10 mL). Recrystallization from toluene gave **16** as white flakes (2.23 g, 68%): TLC R_f = 0.50 (Et_2O); mp 212–214 °C dec; ^1H NMR (DMSO-d_6) δ 3.08 (s, 3 H), 3.41 (s, 3 H), 7.14–7.44 (m, 3 H, Ar), 8.09 (s, 1 H, H-6), 11.54 (s, 1 H, oxime proton); EIMS (20 eV) m/z (rel intensity) 292 (base), 294 (44), 327 (M^+, 6), 329 (4). Anal. Calcd for $C_{13}H_{11}N_3O_3Cl_2$: C, 47.58; H, 3.38; N, 12.80. Found: C, 47.68; H, 3.41; N, 12.77.

Transformation of 14 to 16. To a stirred suspension of **14** (100 mg, 0.3 mmol) in 50% aqueous ethanol (20 mL) was added concd HCl (0.5 mL), and the reaction mixture was heated to 50–60 °C for 10 h. TLC analysis showed complete conversion of **14** to **16**.

Transformation of 15 to 16. **15** (330 mg, 1 mmol) was suspended in toluene (200 mL) and heated to reflux under a gentle stream of HCl, during which time **15** was cleanly converted to **16** (TLC analysis). Evaporation of the solvent after 10 h gave a white solid (330 mg, 100%), which was identical to **16** in all respects (TLC, mp, and MS).

***N*-(2,6-Dichlorophenyl)-1,3-dimethyl-5-uracilcarboxamide (18).** To a stirred solution of **14** (200 mg, 0.61 mmol) in dry Et_2O (10 mL) was added, drop-by-drop, $SOCl_2$ (0.30 mL, 4.0 mmol) over 10 min at 0 °C. The mixture was stirred at room temperature for 10 h. The reaction mixture was poured into cold water (50 mL) and extracted with EtOAc (2 × 50 mL). The organic layers were combined and washed with water (50 mL), dried with $MgSO_4$, and evaporated to dryness in vacuo. Column chromatography of the residue on silica gel (Et_2O) afforded pure product as a white solid (141 mg, 71%): TLC R_f = 0.41 (Et_2O); mp 262–264 °C dec; ^1H NMR (DMSO-d_6) δ 3.29 (s, 3 H), 3.48 (s, 3 H), 7.30–7.65 (m, 3 H, aromatic), 8.72 (s, 1 H, H-6), 10.61 (brs, 1 H, NH); EIMS (20 eV) m/z (rel intensity) 43 (29), 167 (91), 292 (base), 328 (M^+ + 1, 1). Anal. Calcd for $C_{13}H_{11}N_3O_3Cl_2$: C, 47.58; H, 3.38; N, 12.80. Found: C, 47.83; H, 3.49; N, 12.75.

***N*-(2,6-Dichlorobenzoyl)-1,3-dimethyl-5-aminouracil (19).** To a stirred solution of **16** (150 mg, 0.45 mmol) in dry Et_2O (10 mL) was added, drop-by-drop, $SOCl_2$ (0.22 mL, 3.0 mmol) over 10 min at 0 °C. The mixture was stirred at room temperature for 10 h. Product **19** was separated from the reaction mixture in a manner similar to that used for the preparation of **18**, as a white solid (143 mg, 95%): TLC R_f = 0.60 (Et_2O); mp 211–212 °C dec; ^1H NMR (DMSO-d_6) δ 3.24 (s, 3 H), 3.39 (s, 3 H), 7.35–7.60 (m, 3 H, Ar), 8.48 (s, 1 H, H-6), 10.21 (br s, 1 H, NH); EIMS (20 eV) m/z (rel intensity) 173 (base), 175 (70), 327 (M^+, 55), 329 (36). Anal. Calcd for $C_{13}H_{11}N_3O_3Cl_2$: C, 47.58; H, 3.38; N, 12.80. Found: C, 47.69; H, 3.50; N, 12.63.

1,3-Dimethyl-5-(4-chlorobenzoyl)uracil Oxime (20). Compound **20** was prepared in a manner similar to that used for the preparation of **14**. **20**: white solid (0.60 g, 20%); TLC R_f = 0.46 (Et_2O); mp 245–246 °C dec; ^1H NMR (DMSO-d_6) δ 3.18 (s, 3 H), 3.35 (s, 3 H), 7.40–7.60 (m, 4 H, Ar), 7.84 (s, 1 H, H-6), 11.66 (s, 1 H, oxime proton); EIMS (70 eV) m/z (rel intensity) 44 (base), 140 (15), 276 (14), 292 (14), 293 (M^+, 14), 294 (7), 295 (M^+ + 2, 5). Anal. Calcd for $C_{13}H_{12}N_3O_3Cl$: C, 53.16; H, 4.12; N, 14.31. Found: C, 53.15; H, 4.12; N, 14.15.

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Nucleophilic Addition of 2′-Deoxynucleosides to the *o*-Quinone Methides 10-(Acetyloxy)- and 10-Methoxy-3,4-dihydro-9(2*H*)-anthracenone

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In an effort to understand the chemistry of quinone methides, two simple, *o*-quinone methides 10-(acetyloxy)- and 10-methoxy-3,4-dihydro-9(2*H*)-anthracenone (**3** and **4**) have been constructed and their reactions with 2′-deoxyguanosine and 2′-deoxyadenosine investigated. The quinone methides were stirred with 1.2 equiv of nucleoside in H_2O/CH_3CN to afford products of N(6) alkylation with deoxyadenosine (**3**, 38%; **4**, 16% yield) and N(2) alkylation with deoxyguanosine (**3**, 27%; **4**, 5% yield).

Introduction

Quinone methides have been proposed as intermediates in biosynthesis[1] and in the chemistry of quinonoid antitumor compounds.[2] For example, the anthracycline antitumor antibiotics, a class of complex natural products, are thought to derive at least some of their biological ac-

(1) For leading references to the proposed intermediacy of quinone methides in biosynthesis see: (a) Angle, S. R.; Turnbull, K. D. *J. Am. Chem. Soc.* **1990**, *112*, 3698. (b) Saul, S. J.; Sugumaran, M. *FEBS Lett.* **1991**, *279*, 145. (c) Sugumaran, M.; Semensi, V. *J. Biol. Chem.* **1991**, *266*, 6073. (d) Gottlieb, O. R. *Fortsch. Chem. Org. Naturst.* **1978**, *35*, 1.

(2) General reviews and leading references: (a) Moore, H. W. *Science* **1977**, *197*, 527. (b) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249. (c) Abdella, B. R. J.; Fisher, J. F. *EHP, Environ. Health Perspect.* **1985**, *64*, 3. (d) Powis, G. *Pharmacol. Ther.* **1987**, *35*, 57. (e) Lin, A. J.; Sartorelli, A. C. *J. Med. Chem.* **1976**, *19*, 1336. (f) Lin, T.-S.; Antonini, I.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1984**, *27*, 813. (g) Guadiano, G.; Koch, T. D. *Chem. Res. Toxicol.* **1991**, *4*, 2.

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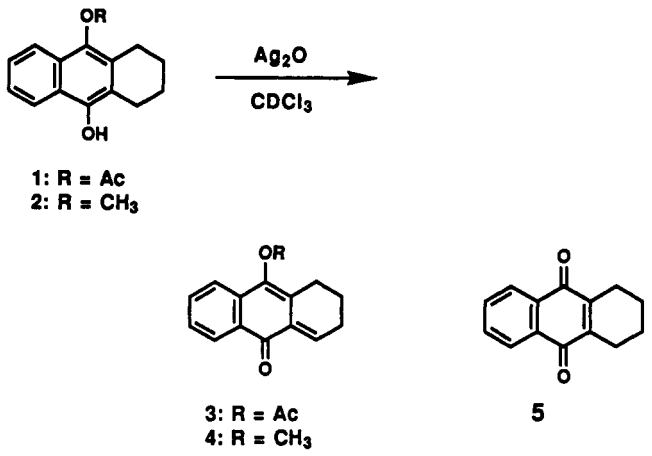
Scheme I. Synthesis of Quinone Methides 3 and 4



tivity via quinone methide formation followed by alkylation of some critical biomolecule such as DNA.[2-4] Due to the instability of the quinone methides derived from the antracyclines and other quinonoid natural products, a thorough investigation of the chemistry of these intermediates has been impossible.[2-4] Thus, a detailed chemical study requires the synthesis of quinone methides that lack some of the complicating functionality found in quinonoid natural products. As part of a continuing effort to understand the chemistry of quinone methides,[5] we have constructed two simple quinone methides and studied their chemistry with deoxynucleosides.

The goal of this study is to better define the chemistry of quinone methides with nucleosides under conditions where other nucleophiles such as water can compete for the quinone methide. In addition to obtaining a better understanding of the chemistry of quinone methides, this research may lead to methods for the selective modification of DNA.[6]

Results and Discussion

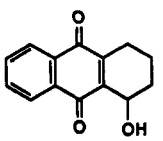
We have previously reported that quinone methide **3** afforded a 1:1 adduct with a protected adenosine derivative.[5] We report herein further studies with this quinone methide and a revision of our earlier proposed site of alkylation on adenosine. In addition, the synthesis and chemistry of quinone methide **4**, a closer electronic surrogate for quinone methides derived from quinonoid natural products such as the anthracyclines, is described.

The quinone methides were prepared by oxidation[5,7] of phenols **1** and **2**. Readily available (acetyloxy)phenol **1**[5] was methylated with CH_3I then hydrolyzed to afford methoxyphenol **2**. Phenol **2** was stored as an ethyl acetate solution under nitrogen since it was not stable neat and underwent oxidation to quinone **5** upon exposure to air. As we reported previously, solutions of **3** could be formed from phenol **1** in excellent yield and >90% purity (^1H NMR) via Ag_2O oxidation.[5,7] Methoxyquinone methide **4** is much less stable than (acetyloxy)quinone methide **3** and requires special handling. The oxidation of **2** afforded solutions ($CDCl_3$) of **4** (routinely >60% purity by ^1H NMR), contaminated with varying amounts of quinone **5**.[8] On several occasions, material of >80% purity (^1H NMR) was obtained (Scheme I).

The reaction of quinone methides **3** and **4** with 2'-deoxyadenosine (1.2 equiv, 1:1 H_2O/CH_3CN, 0.05 M, 96 h) afforded adducts **6** and **7** in 38% and 16% yields, respectively. Adducts **6** and **7** are both 1:1 mixtures of diastereomers. The diastereomers of **7** were separated by fractional recrystallization from $CDCl_3$ to afford a single diastereomer, **7a**, analytically pure (mp 124–125 °C). The other diastereomer, **7b**, was obtained as a 4:1 mixture of **7b/7a** from the mother liquor of the recrystallization.

The yields of alkylated nucleosides were modest, but steadily increased as the reaction time increased. An arbitrary 96-h reaction time was chosen for all reactions to allow the efficiency of the alkylations to be compared. ^1H NMR experiments showed that quinone methides **3** and **4** were not stable in aqueous acetonitrile for 96 h, and yet the yield of adducts **6** and **7** increased with reaction time. It seemed likely that quinone methides **3** and **4** react with H_2O to afford water adducts **10** and **11** in a reversible process (Scheme II). Adducts **10** and **11** may then serve as a source of low steady-state concentrations of quinone methides **3** and **4**. To test this notion, quinone methide **3** was subjected to the reaction conditions in the absence of the nucleoside (1:1 H_2O/CH_3CN, 0.05 M) for 30 min. Workup followed by ^1H NMR showed the complete consumption of the quinone methide. The major product was an unstable compound assigned as water adduct **10** on the basis of its ^1H NMR, IR, and MS data. Rapid flash chromatography (silica gel) afforded **10** contaminated with 11% of quinone methide **4** (^1H NMR, $CDCl_3$). Upon standing for 4 h in solution ($CDCl_3$) the amount of quinone methide increased to 23% (^1H NMR). The ^1H NMR spectrum of **10** ($CDCl_3$, contaminated with 11% of **4**) showed a signal for the benzylic methine hydrogen at δ 5.08 (apparent quartet, J = 6.5 Hz) which upon addition of D_2O collapsed to a doublet of doublets (J = 6.1 and 5.9 Hz), indicative of spin–spin coupling to the alcohol hydrogen.

The reaction of quinone methide **3** with deoxyadenosine afforded water adduct **10** and nucleoside adduct **6** in a 1:2.3 ratio (^1H NMR analysis of the crude reaction mixture). The remaining material consisted of decomposition products of the quinone methide, mainly dimer.[5] The reaction of quinone methide **4** with deoxyadenosine afforded adduct **7**, unstable water adduct **10**, quinone **12** (an air oxidation product of **10**), and decomposition products of the quinone methide, mainly quinone **5**, as a 1:1:0.3:2 mixture (^1H NMR analysis of the crude reaction mixture).



12

(3) For leading references on the possible importance of quinone methides in the chemistry of menogaril see: (a) Boldt, M.; Guadiano, G.; Haddadin, M. J.; Koch, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 2283; **1988**, *110*, 3330 and references cited therein. (b) Egholm, M.; Koch, T. H. *Ibid.* **1989**, *111*, 8291.

(4) For leading references on the possible importance of quinone methides in the chemistry of adriamycin and daunomycin see: (a) Kleyer, D. L.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 1105. (b) Kleyer, D. L.; Koch, T. H. *Ibid.* **1984**, *106*, 2380. (c) Olson, J. B.; Koch, T. H. *Ibid.* **1986**, *108*, 756 and references cited therein. (c) Ramakrishnan, K.; Fisher, J. F. *J. Med. Chem.* **1986**, *29*, 1215. (d) Fisher, J. F.; Abdella, B. R. J.; McLane, K. E. *Biochemistry* **1985**, *24*, 3562. (e) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411. (f) Anne, A.; Moiroux, J. *Nouv. J. Chim.* **1985**, *9*, 83. (g) Land, E. J.; Mukherjee, T.; Swallow, A. J.; Bruce, J. M. *Arch. Biochem. Biophys.* **1983**, *225*, 116. (h) Land, E. J.; Mukherjee, T.; Swallow, A. J.; Bruce, J. M. *Br. J. Cancer* **1985**, *51*, 515.

(5) Angle, S. R.; Yang, W. *J. Am. Chem. Soc.* **1990**, *112*, 4524.

(6) For a report of a selective modification of DNA that may proceed through a quinone methide type intermediate see: Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1991**, *113*, 5116.

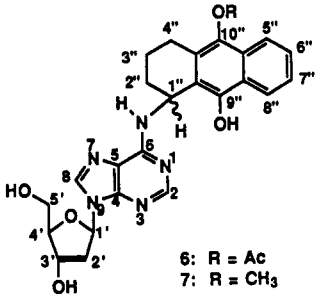
(7) The oxidation conditions are a modification of those reported by: Dyall, L. K.; Winstein, S. *J. Am. Chem. Soc.* **1972**, *94*, 2196. The conditions appear to be quite general for the formation of quinone methides; cf. Angle, S. R.; Turnbull, K. D. *J. Am. Chem. Soc.* **1989**, *111*, 1136.

(8) Quinone **5** is a known compound: Franck, R. W.; Gupta, R. B. *J. Org. Chem.* **1985**, *50*, 4632.

It is interesting to note that diastereomers **7a** and **7b** were both stable when they were resubjected to the reaction conditions or allowed to sit in deuteriochloroform solution at −5 °C for several months. The lack of interconversion of **7a** and **7b** implies that the alkylation of quinone methide **4** is irreversible under the reaction conditions. Adduct **6** was recovered unchanged when resubmitted to the reaction conditions. The reason for the stability of the quinone methide–nucleoside adduct may be due to favorable intramolecular hydrogen bonding interactions.

The site of alkylation of deoxyadenosine is not immediately obvious. Possible alkylation sites include N(1), N(3), N(6), and N(7).[9] In our earlier work,[5] we had proposed the site of alkylation on 2',3'-*O*-isopropylideneadenosine to be N(1), based on the imine stretching band in the infrared spectrum and precedent for N(1) alkylation.[9b] We felt that a more rigorous determination of the site of alkylation was needed. Although nucleoside adduct **7a** is crystalline (fine needles), an X-ray crystal structure was not feasible, due to the lack of suitable crystals. However, this problem has been solved by spectroscopic and chemical methods.[10]

In an effort to determine the alkylation site on adenosine, a detailed ^{1}H NMR study of nucleoside adduct **7a** was undertaken. This study (homonuclear decoupling experiments and double quantum filtered 500-MHz homonuclear COSY, (dq COSY)) has led to the assignment of the site of alkylation as N(6) of adenosine. The ^{1}H NMR spectrum of **7a** showed a signal for the N(6) hydrogen at δ 6.53 (doublet, J = 7.7 Hz) that was exchangeable with D_2O. Irradiation of this signal caused the signal for the benzylic methine hydrogen, H(1″), at δ 5.72 (multiplet) to collapse into a broad singlet. The 500-MHz ^{1}H NMR dq COSY spectrum showed a strong cross-peak for these two hydrogens, indicative of spin-spin coupling and established the connectivity between the N(6) and C(1″).[11]

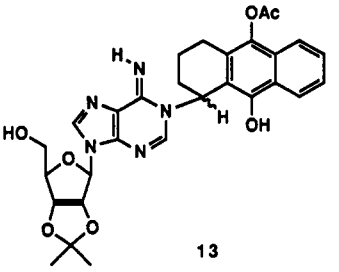


6: R = Ac
7: R = CH_3

The diastereomers of adduct **6** could not be separated by HPLC or fractional recrystallization. ^{1}H NMR studies of **6** as a mixture of diastereomers showed a single broad singlet for the N(6) hydrogen at δ 6.76 that exchanged with D_2O. Irradiation of this signal caused the resonance for the C(1″) hydrogen at δ 5.72 (a single doublet for both diastereomers, J = 8.1 Hz) to collapse to a singlet, again indicative of the connectivity between the N(6) and C(1″) positions.

The UV spectra (λ max, H_2O) of **6** (270 nm), **7a** (264 nm), and **7b** (266 nm) were also consistent with N(6) alkylation. Literature values for the UV spectra of N(1)-methyl- and N(1)-ethyldeoxyadenosine show λ max (H_2O) at 257 and 259 nm respectively.[9c] The UV λ max (H_2O) for N(6)-methyl- and N(6)-ethyldeoxyadenosine are 265 and 267 nm, much closer to those observed for **6**, **7a**, and **7b**.[9c]

With this information in hand, we reexamined the structure proposed for the nucleoside adduct derived from quinone methide **3** and 2′,3′-*O*-isopropylideneadenosine in our earlier work.[5] The structure had been proposed to be **13**, the result of N(1) alkylation; however, the product of N(6) alkylation, **14**, now needed to be considered. The signal for the N(6) hydrogen appeared as a 7.2-Hz doublet at δ 6.50 that exchanged with D_2O. Irradiation of the signal for the C(1″) hydrogen at δ 5.72 (doublet, J = 8.2 Hz) caused the doublet for the N(6) hydrogen to collapse to a singlet, indicative of the spin–spin coupling between the N(6) hydrogen and the C(1″) hydrogen. The spin–spin coupling clearly supports the structure of the adduct as being **14** (N(6)-alkylation) not **13** (N(1)-alkylation) as we had proposed.[5]



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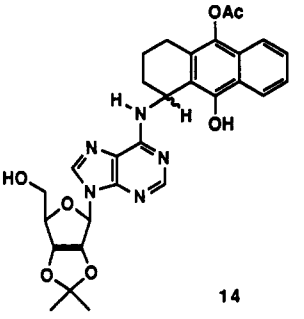


14

The reaction of quinone methides **3** and **4** with 2′-deoxyguanosine (1.2 equiv, 2:1 H_2O/CH_3CN, 0.05 M, 96 h) afforded adducts **8** and **9** in 27% and 5% yield, respectively. The yield of **8** is comparable to that obtained when **3** was reacted with deoxyadenosine. As in that case, the balance of the material consisted of water adduct and quinone methide dimer. The low yield of **9** is due in part to the instability of the compound. Analysis of the ^{1}H NMR spectrum of the crude reaction mixture showed the yield of adduct to be ca. 20%. However, **9** is unstable to chromatography and a considerable amount of material is lost in purification. The balance of the material is derived either from water addition to the quinone methide (**11** and **12**) or from quinone methide decomposition.

The determination of the alkylation site on the guanosine was accomplished by analysis of the 500-MHz ^{1}H NMR spectra of **8** and **9**.[12] The diastereomers of **8** and **9** were inseparable by recrystallization or HPLC. The ^{1}H NMR spectrum of **8** as a mixture of diastereomers showed signals for the N(2) hydrogen (one for each diastereomer) at δ 7.15 (doublet, J = 6.6 Hz) and δ 7.07 (doublet, J = 6.9 Hz) that were exchangeable with D_2O.[13] In a homonuclear

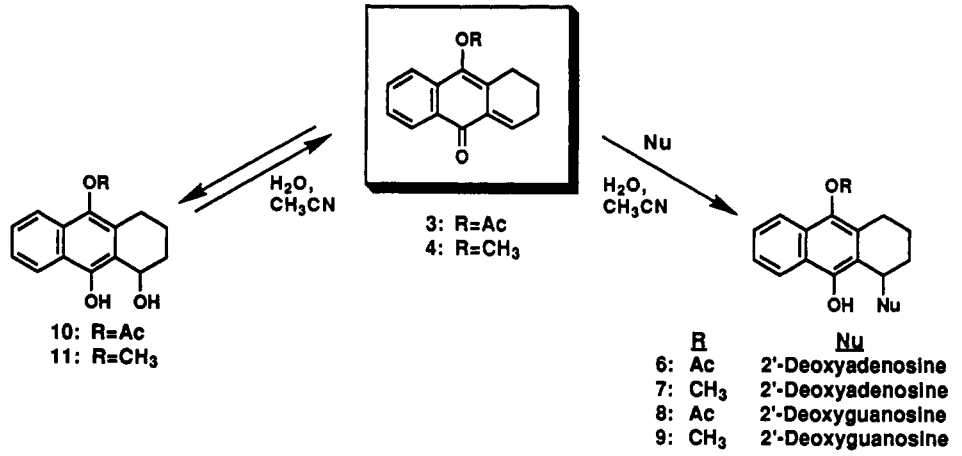
(9) (a) Srivastava, P. C.; Robins, R. K.; Meyer, P. B. Jr. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1988; Vol. 1, pp 113–282. (b) Singer, B. Prog. *Nucleic Acid Res. Mol. Biol.* **1975**, *15*, 219. (c) Singer, B.; Sun, L.; Fraenkel-Conrat, H. *Biochemistry* **1974**, *13*, 1913.

(10) If the adenosine adduct is linked through N(1) of adenosine, Dimroth rearrangement should result in the net interconversion of N(1) and N(6). Thus, one would expect to obtain the N(6) product starting from the N(1) adduct. No Dimroth rearrangement was observed for the adenosine adducts. See pp 243 and 254 of ref 9a for a discussion of, and leading references to, the Dimroth rearrangement.

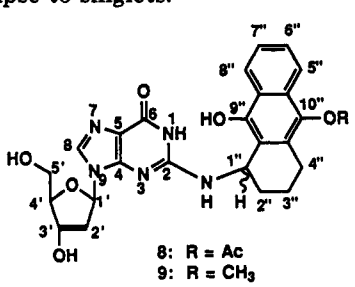
(11) There are several exchangeable hydrogens in **7a**. The assignment of the N(6) hydrogen was made by a careful analysis of the 500-MHz ^{1}H NMR of **7a**. The 3′ hydrogen shows coupling to the C(3′)-OH and the 5′-methylene hydrogens show coupling to the C(5′)-OH, allowing assignment of these exchangeable hydrogens. The remaining exchangeable hydrogen is the phenol C(9″)-OH. This hydrogen was assigned on the basis that the signal for this hydrogen at δ 11.18 disappeared upon oxidation to the corresponding quinone and the NH still remained at roughly the same chemical shift (δ 6.71) and retained the coupling to the C(1″) hydrogen.

(12) Similar spectroscopic evidence has been employed by the Koch group for the assignment of a menogaril–deoxyguanosine adduct structure, see ref 3b.

Scheme II. Reaction of Quinone Methides with Nucleosides



decoupling experiment, irradiation of the signal for the benzylic methine hydrogen H(1″) at δ 5.46 (multiplet, for both diasteromers) caused the two N(2) hydrogen doublets to collapse to singlets. The 500-MHz dq COSY spectrum unambiguously established the assignment of the C1″-H, and thus the connectivity between the N(2) and C(1″) positions. The ^{1}H NMR spectrum of **9** as a mixture of diastereomers was quite similar to that of **8**, showing signals for the N(2) hydrogen (one for each diastereomer) at δ 6.82 (doublet, J = 6.6 Hz) and δ 6.78 (doublet, J = 6.7 Hz) that were exchangeable with D_2O.[13] In a homonuclear decoupling experiment, irradiation of the signal for the benzylic methine hydrogen H(1″) at δ 5.41 (multiplet, for both diastereomers) caused the two N(2) hydrogen doublets to collapse to singlets.



Deoxyguanosine adducts **8** and **9** proved to be much less stable than deoxyadenosine adducts **6** and **7**. Resubmission of acetate **8** to the reaction conditions afforded approximately 10% (^{1}H NMR) of water adduct **10** after 24 h reaction time. The low yield and instability of **9** prevented any further work with this compound.

Conclusion

The results show that the reaction of the quinone methides with 2'-deoxynucleosides in aqueous acetonitrile is a slow reaction that affords stable covalent adducts in modest yield. This study also serves as a model study for quinone methides derived from quinonoid compounds that may derive some of their activity via quinone methide formation.[2] It is indeed possible that these quinone methides alkylate DNA. The results with these simple quinone methides set the stage for the study of quinone methides closely related to the anthracycline antitumor antibiotics. Results of this work will be reported in due course.

(13) The alcohols on the sugar showed coupling to hydrogens on the adjacent carbon(s), allowing them to be assigned. The characteristic chemical shift of the phenol hydrogen allowed its assignment, leaving the N(2) hydrogen as the only remaining exchangeable hydrogen.

Experimental Section[14]

General Information. NMR spectra were recorded on a General Electric QE-300 NMR or a GE GN-500 NMR; shifts reported are relative to internal tetramethylsilane; coupling constants, J, are reported in Hz and refer to apparent peak multiplicities and not true coupling constants. Abbreviations used are as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, p = pentet. Mass spectra were recorded at the UCR-MS facility on a VG-7070EHF or a VG-ZAB1FHF and are reported as percent relative intensity to the parent peak. IR spectra were recorded on a Nicolet-5DX FT-IR. UV spectra were recorded on a Hewlett-Packard 8451A Diode Array Spectrophotometer.[14]

9-Hydroxy-10-methoxy-1,2,3,4-tetrahydroanthracene (2). A suspension of sodium hydride (97%, 60.2 mg, 2.43 mmol) in THF (2 mL) was added to a stirred solution of 10-(acetyloxy)-9-hydroxy-1,2,3,4-tetrahydroanthracene[5] (579 mg, 2.26 mmol) and THF (10 mL). The resulting suspension was stirred for 5 min, then iodomethane (0.2 mL, excess) was added and the reaction was followed by TLC. After an additional 30 min, the reaction mixture was poured into water (10 mL). The aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with brine (2 × 25 mL), dried (Na_2SO_4), concentrated, and chromatographed (9:1 hexane/ethyl acetate) to yield 478 mg (78%) of 10-(acetyloxy)-9-methoxy-1,2,3,4-tetrahydroanthracene as a yellow solid: mp 112.0–113.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (dd, J = 5.5, 3.9 Hz, 1 H, ArH), 7.67 (dd, J = 5.4, 4.0 Hz, 1 H, ArH), 7.44 (m, 2 H, ArH), 3.90 (s, 3 H, OCH_3), 2.97 (bs, 2 H), 2.74 (bs, 2 H), 2.47 (s, 3 H, OAc), 1.83 (bs, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.19, 150.96, 140.15, 127.72, 127.45, 126.66, 126.01, 125.82, 125.36, 121.97, 120.84, 60.90, 24.07, 23.88, 22.27, 20.58; IR (CCl_4) 2938, 2863, 1764, 1595, 1501, 1455, 1360, 1208, 1174, 1054, 925, 889 cm^{-1}; MS (EI, 70 eV) m/z 270 (M^+, 27), 228 (100), 213 (61), 195 (10), 165 (31), 152 (24), 115 (21); HRMS calcd for $C_{17}H_{18}O_3$ 270.1256, found 270.1255. A solution of sodium hydroxide (86 mg in 0.5 mL water, 2.15 mmol, 3.01 equiv) was added to a stirred solution of 10-(acetyloxy)-9-methoxy-1,2,3,4-tetrahydroanthracene (193 mg, 0.715 mmol) and MeOH/THF (1:1, v/v; 6.0 mL). The resulting solution was stirred for 2 min, and then ethyl acetate (25 mL) and $NaHCO_3$ (saturated aqueous, 10 mL) were added. After the solution was stirred for an additional 2 min, the aqueous layer was extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with $NaHCO_3$ (saturated aqueous, 3 × 10 mL) and dried (Na_2SO_4). Since product **2** was unstable in the absence of solvent, it was stored as an ethyl acetate solution and concentrated immediately before use in the next step. Concentration of a similar sample afforded an analytical sample as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 7.7 Hz, 1 H, ArH), 8.02 (d, J = 7.6 Hz, 1 H, ArH), 7.44 (m, 2 H, ArH), 5.14 (s, 1 H, ArO*H*), 3.88 (s, 3 H, OCH_3), 2.96 (t, J = 6.0 Hz, 2 H, $ArCH_2$), 2.78 (t, J = 6.2 Hz, 2

(14) Detailed general experimental protocols have recently been reported, see: ref 5 and Angle, S. R.; Louie, M. S. *J. Org. Chem.* **1991**, *56*, 2853.

H, ArCH_2), 1.78–1.93 (m, 4 H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.45, 144.56, 127.21, 126.32, 125.32, 124.51, 123.15, 121.45, 121.17, 117.60, 60.87, 24.00, 23.30, 22.53, 22.33; IR ($CDCl_3$) 3606, 2940, 1657, 1595, 1454, 1377, 1285, 1059, cm^{-1}; MS (EI, 20 eV) m/z 228 (M^+, 100), 213 (57), 195 (4), 185 (2), 133 (2); HRMS calcd for $C_{15}H_{16}O_2$ 228.1150, found 228.1166.

10-Methoxy-3,4-dihydro-9(2*H*)-anthracenone (4) and 1,2,3,4-Tetrahydroanthraquinone (5). General Procedure for Quinone Methide Formation. Silver(I) oxide (2 equiv) was added to a solution of phenol **2** (1 equiv, 0.35 M $CDCl_3$ solution) in a reaction flask. The resulting suspension was heated in a water bath at 70 °C until the oxidation was complete (15 min, ^{1}H NMR monitoring). The suspension was filtered through glass wool and the residue was rinsed with $CDCl_3$ to give a solution of quinone methide **4** which was concentrated before use in next step. An aliquot of the reaction mixture was concentrated to afford **4** contaminated with 33% of quinone **5** (2:1 mixture of **4**/**5**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, J = 7.8 Hz, 1 H, ArH), 7.59 (apparent d, J = 3.9 Hz, 2 H, ArH), 7.55 (t, J = 4.8 Hz, 1 H, =C*H*CH_2), 7.33 (m, 1 H, ArH), 3.76 (s, 3 H, OCH_3), 2.71 (t, J = 6.4 Hz, 2 H, ArCH_2), 2.50 (q, J = 6.2 Hz, 2 H, =CHCH_2), 1.81 (m, 2 H, CH_2). Chromatography (9:1 hexane/ethyl acetate) of a similar mixture of **4**/**5** afforded **5** as a yellow solid: mp 151–152 °C (lit[8] mp 154–155 °C); ^{1}H NMR (300 Hz, $CDCl_3$) δ 8.05 (dd, J = 5.8, 3.4 Hz, 2 H, ArH), 7.67 (dd, J = 5.7, 3.2 Hz, 2 H, ArH), 2.58 (m, 4 H, C=CCH_2CH_2), 1.73 (m, 4 H, CH_2); ^{13}C NMR (75 Hz, $CDCl_3$) 184.76, 144.69, 133.19, 132.03, 125.98, 23.07, 21.05.

***N*^{6}-[10″-(Acetyloxy)-9″-hydroxy-1″,2″,3″,4″-tetrahydroanthracenyl]-2′-deoxyadenosine (6).** A solution of 2′-deoxyadenosine (65.8 mg, 0.262 mmol, 1.22 equiv) and H_2O/CH_3CN (1:1, v/v; 5 mL) was added to quinone methide **3** (prepared from 54.8 mg, 0.214 mmol, of phenol **1**) in a reaction flask. The resulting homogeneous solution was stirred at room temperature for 4 days. The mixture was then diluted with water (10 mL) and extracted with $CHCl_3$ (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4), concentrated, and chromatographed (1:1 hexane/2-propanol, R_f = 0.23) to afford 41.1 mg (38%) of **6** as a white solid (1:1 mixture of diastereomers by ^{1}H NMR analysis): mp 129.5–131.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 50 °C) δ 11.33 (bs, 1 H, ArO*H*), {8.42 (s), 8.40 (s), 1 H, C2′H)}, {8.34 (d, J = 7.4 Hz), 8.32 (d, J = 6.5 Hz), 1 H, ArH}, 7.59 (bs, 1 H, C7′H), 7.58 (d, J = 7.9 Hz, 1 H, ArH), 7.48–7.37 (m, 2 H, ArH), 6.76 (bs, 1 H, 6-NH), 6.14 (m, 1 H, C1′H), {5.92 (bs), 5.82 (bs), 1 H, 5′-OH}, 5.73 (d, J = 8.1 Hz, 1 H, C1″H), {4.67 (d, J = 4.2 Hz), 4.63 (d, J = 3.8 Hz), 1 H, C3′H}, {4.09 (s), 4.05 (s), 1 H, C4′H}, {3.92 (d, J = 13.0 Hz), 3.87 (d, J = 12.9 Hz), 1 H, C5′H}, 3.71 (m, 1 H, C5′H), 2.96 (m, 2 H), 2.82 (m, 2 H), {2.45 (s), 2.45 (s) 3 H, OAc}, 2.24 (m, 2 H), 2.11 (m, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ {169.97, 169.93}, {153.45, 153.38}, 151.50, {150.03, 149.89}, {147.50, 147.40}, 139.81, 139.42, {136.62, 136.56}, 128.76, 127.13, 125.66, 124.77, 123.38, {120.27, 120.20}, 119.92, 117.13, {89.04, 88.93}, 86.83, {72.39, 72.18}, {62.91, 62.77}, {43.89, 43.77}, {40.47, 40.27}, {28.81, 28.66}, 22.76, 20.57, 16.32; IR ($CDCl_3$) 3613, 3421, 3219, 2942, 2867, 1753, 1625, 1581, 1487, 1373, 1332, 1219, 1106, 1057 cm^{-1}; UV (H_2O) λ_{max} 212, 234, 270 nm; MS (FAB, positive ion, nitrobenzyl alcohol matrix) m/z 506 (MH^+, 63), 505 (M^+, 78), 462 (8), 389 (19), 346 (25), 252 (32), 212 (100); HRMS calcd for $C_{26}H_{27}N_5O_6$ 505.1961, found 505.1951.

***N*^{6}-[9″-Hydroxy-10″-methoxy-1″,2″,3″,4″-tetrahydroanthracenyl]-2′-deoxyadenosine (7).** A solution of 2′-deoxyadenosine (216 mg, 0.858 mmol, 1.2 equiv) and H_2O/CH_3CN (1:1, v/v; 14 mL) was added to quinone methide **4** (prepared from 193 mg, 0.715 mmol, of **2**) in a reaction flask. The resulting solution was stirred at room temperature for 4 days. The mixture was then diluted with H_2O (10 mL) and extracted with $CHCl_3$ (2 × 50 mL). The combined organic extracts were washed with H_2O (2 × 10 mL), dried (Na_2SO_4), concentrated, and chromatographed (1:1 hexane/2-propanol, R_f = 0.28) to afford 53 mg (16%) of **7** as a yellow solid (1:1 mixture of diastereomers by ^{1}H NMR analysis). The two diastereomers were separated by recrystallization from $CDCl_3$ to afford diastereomer **7a** (>15:1 mixture of diastereomers, ^{1}H NMR analysis; crystallized from $CDCl_3$) and the other diastereomer **7b** as a 4:1 mixture of diastereomers (^{1}H NMR analysis; from mother liquor) for analysis. Diastereomer **7a**: white solid; mp 124–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.18 (bs, 1 H, ArO*H*), 8.45 (s, 1 H, C2′H), 8.30 (dd, J = 7.5, 1.0 Hz, 1 H, ArH), 7.95 (dd, J = 7.9, 0.9 Hz, 1 H, ArH), 7.75 (s, 1 H, C7′H), 7.49–7.37 (m, 2 H, ArH), 6.53 (d, J = 7.7 Hz, 1 H, NH), 6.26 (apparent dd, J = 9.6 Hz, 5.5 Hz, 2 H, C1′H, 5′-O*H*), 5.72 (m, 1 H, C1″H), 4.79 (d, J = 4.7 Hz, 1 H, C3′H), 4.19 (s, 1 H, C4′H), 3.93 (dd, J = 12.9, 1.3 Hz, 1 H, C5′H), 3.85 (s, 3 H, $ArOCH_3$), 3.77 (m, 1 H, C5′H), 3.23–2.93 (m, 3 H, C2′1H, C4″2H), 2.33–2.25 (m, 3 H, C2′1H, C2″1H, C3″1H), 2.17–2.09 (m, 3 H, C2″1H, C3″1H, 3′-OH); ^{13}C NMR (75 MHz, CD_3CN) δ 154.77, 152.12, 149.09, 148.64, 146.57, 144.93, 141.41, 128.61, 127.53, 127.11, 125.92, 125.20, 123.91, 121.95, 121.42, 89.83, 87.10, 72.85, 63.43, 61.25, 45.16, 41.07, 29.34, 22.99, 17.61; IR ($CDCl_3$) 3616, 3421, 3208, 2939, 2870, 1664, 1623, 1582, 1525, 1479, 1378, 1331, 1225, 1106, 1066 cm^{-1}; UV (H_2O) λ_{max} 210, 238, 264 nm; MS (FAB, positive ion, nitrobenzyl alcohol matrix) m/z 477 (M^+, 95), 460 (13), 361 (27), 346 (29), 252 (35), 226 (100), 211 (31); HRMS calcd for $C_{25}H_{27}N_5O_5$ 477.2012, found 477.2014.

Diastereomer **7b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.16 (bs, 1 H, ArO*H*), 8.44 (s, 1 H, C2′H), 8.31 (dd, J = 8.1, 0.9 Hz, 1 H, ArH), 7.94 (d, J = 7.6, 1.0 Hz, 1 H, ArH), 7.77 (s, 1 H, C7′H), 7.49–7.36 (m, 2 H, ArH), 6.47 (d, J = 8.2 Hz, 1 H, NH), 6.27 (apparent dd, J = 9.6, 5.4 Hz, 2 H, C1′H and 5′-OH), 5.71 (m, 1 H, C1″H), 4.78 (d, J = 4.9 Hz, 1 H, C3′H), 4.21 (s, 1 H, C4′H), 3.98 (dd, J = 12.9, 1.2 Hz, 1 H, C5′H), 3.85 (s, 3 H, OCH_3), 3.76 (m, 1 H, C5′H), 3.20 (m, 1 H), 3.06–2.92 (m, 2 H), 2.31–2.20 (m, 2 H), 2.11 (bm, 3 H), 2.00 (bs, 1 H, 3′-OH); IR ($CDCl_3$) 3613, 3447, 3210, 2941, 2866, 1664, 1623, 1583, 1525, 1478, 1378, 1331, 1225, 1106 cm^{-1}; UV (H_2O) λ_{max} 210, 236, 266 nm.

***N*^{2}-[10″-(Acetyloxy)-9″-hydroxy-1″,2″,3″,4″-tetrahydroanthracenyl]-2′-deoxyguanosine (8).** A solution of 2′-deoxyguanosine (236.2 mg, 0.827 mmol, 1.2 equiv) and H_2O/CH_3CN (2:1, v/v; 10 mL) was added to quinone methide **3** (prepared from 176.9 mg, 0.691 mmol, of phenol **1**) in a reaction flask. The resulting solution was stirred at room temperature for 4 days. The mixture was then diluted with water (10 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were washed with water (2 × 10 mL), dried (Na_2SO_4), concentrated, and chromatographed (4:1 ethyl acetate/methanol, R_f = 0.13) to afford 95.5 mg (27%) of **8** as a white solid (1:1 mixture of diastereomers by ^{1}H NMR analysis): mp 166–169 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ {10.34 (bs), 10.25 (bs), 10.06 (bs), 9.89 (bs), 2 H, 1-N*H* and ArO*H*}, {8.23 (d, J = 7.6 Hz), 8.21 (d, J = 7.7 Hz), 1 H, ArH}, {7.97 (s), 7.94 (s), 1 H, C8H}, 7.68 (d, J = 8.0 Hz, 1 H, ArH), 7.49 (dd, J = 6.7, 8.4 Hz, 1 H, ArH), 7.44 (dd, J = 6.6, 8.2 Hz, 1 H, ArH), {7.15 (d, J = 6.6 Hz), 7.08 (d, J = 6.9 Hz), 1 H, 2′-NH}, 6.26 (apparent q, J = 7.0 Hz, 1 H, C1′H), 5.45 (m, 1 H, C1″H), 5.35 (bs, 1 H, C3′-O*H*), 4.93 (bs, 1 H, C5′-O*H*), 4.40 (d, J = 2.4 Hz, 1 H, C3′H), 3.85 (m, 1 H, C4′H), 3.66–3.49 (m, 2 H, C5′H), 3.35 (m obscured by H_2O, 2 H, C4″H), 2.75–2.60 (m, 1 H, C2′H), 2.55 (m obscured by solvent, 1 H, C3″H), 2.49 (s, 3 H, OAc), 2.36–2.20 (m, 2 H, C2′H, C3″H), 1.85–1.72 (m, 2 H, C2″H); ^{13}C NMR (75 MHz, CD_3OD) δ {171.62, 171.56}, 159.96, {153.42, 153.40}, 151.99, 151.68, 151.00, 138.28, 138.04, 128.25, 128.07, 125.89, 125.55, {123.56, 123.45}, 121.53, {119.31, 119.17}, 117.69, {88.92, 88.84}, 85.35, {72.53, 72.34}, {63.45, 63.33}, 46.01, {40.91, 40.52}, {30.05, 29.63}, 24.72, 20.51, {18.25, 18.11}; IR (DMSO-d_6) 3511, 3428, 3228, 3054, 2939, 1757, 1733, 1692, 1634, 1602, 1514, 1464, 1365, 1211, 1104, 930, 894 cm^{-1}; UV (H_2O) λ_{max} 210, 236, 258 nm; MS (FAB, positive ion, nitrobenzyl alcohol matrix) m/z 522 (MH^+, 9), 521 (M^+, 8), 500 (7), 384 (11), 341 (14), 312 (14), 290 (61), 255 (14), 212 (48), 174 (77), 152 (100); HRMS calcd for $C_{26}H_{27}N_5O_7$ 521.1910, found 521.1909.

***N*^{2}-[9″-Hydroxy-10″-methoxy-1″,2″,3″,4″-tetrahydroanthracenyl]-2′-deoxyguanosine (9).** A solution of 2′-deoxyguanosine (221.6 mg, 0.777 mmol, 1.2 equiv) and H_2O/CH_3CN (2:1, v/v; 14 mL) was added to quinone methide **4** (prepared from 174.4 mg, 0.646 mmol, of phenol **2**) in a reaction flask. The resulting solution was stirred at room temperature for 4 days. The mixture was then diluted with water (10 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were washed with water (2 × 10 mL), dried (Na_2SO_4), concentrated, and chromatographed (1:1 ethyl acetate/2-propanol, R_f = 0.23) to afford 15.6 mg (5%) of **9** as a white solid (1:1 mixture of diastereomers by ^{1}H NMR analysis): mp 175–178 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ {10.11 (bs), 10.04 (bs), 9.63 (bs), 9.49 (bs), 2 H, 1-N*H* and ArO*H*}, {8.19 (d, J = 8.2 Hz), 8.18 (d, J = 8.2 Hz), 1 H, ArH}, {7.95 (s), 7.94 (s), 1 H, C8H}, 7.92 (d, J = 9.7 Hz, 1 H, ArH), 7.49 (t, J = 7.2 Hz, 1 H, ArH), 7.40 (t, J = 7.3 Hz, 1 H, ArH), {6.82 (d, J = 6.6 Hz), 6.78 (d, J = 6.7 Hz), 1 H, 2′-NH}, 6.25

(apparent q, J = 6.4 Hz, 1 H, C1'H), 5.41 (m, 1 H, C1''H), 5.29 (t, J = 4.6 Hz, 1 H, C3'-O*H*), 4.88 (bs, 1 H, C5'-O*H*), 4.36 (m, 1 H, C3'H), 3.84-3.73 (m, 1 H, C4'H), 3.75 (s, 3 H, OCH_3), 3.60-3.46 (m, 2 H), 3.13-3.07 (m, 1 H), 2.75-2.61 (m, 1 H), 2.34-2.19 (m, 2 H), 1.86-1.71 (m, 2 H); IR (DMSO-d_6) 3504, 3454, 2935, 1731, 1691, 1663, 1602, 1514, 1462, 1366, 1244, 1106, 924 cm^{-1}; UV (H_2O) λ_{max} 208, 238, 256 nm; MS (FAB, positive ion, nitrobenzyl alcohol matrix) m/z 494 (MH^+, 37), 273 (37), 242 (37), 226 (100), 219 (62), 165 (75); HRMS calcd for MH^+, $C_{26}H_{28}N_5O_7$ 494.2040, found 494.2023.

10-(Acetyloxy)-1,9-dihydroxy-1,2,3,4-tetrahydroanthracene (10). A solution of quinone methide **3** (from 88.9 mg, 0.347 mmol, of phenol **1**) and $CDCl_3$ (1 mL) was added to a solution of water (2 mL) and CH_3CN (2 mL). This solution was stirred at room temperature until the reaction was complete (30 min). The reaction mixture was extracted with $CHCl_3$ (2 × 15 mL). The combined organic extracts were dried ($NaSO_4$), concentrated, and chromatographed (4:1 hexane/ethyl acetate) to afford 25.3 mg (27%) of the unstable compound **10** as a yellow oil (9:1 mixture of **10** and **3**): ^1H NMR (300 MHz, $CDCl_3$) δ 8.90 (bs, 1 H, ArO*H*), 8.32 (dd, J = 8.3, 1.2 Hz, 1 H, ArH), 7.61 (d, J = 8.0 Hz, 1 H, ArH), 7.50-7.39 (m, 2 H, ArH), 5.08 (apparent q, J = 6.5 Hz, 1 H, C1-H), 2.80-2.59 (bm, 3 H, C4-2H, C1-OH), 2.44 (s, 3 H, OAc), 2.22 (m, 1 H), 1.94-1.69 (m, 3 H); IR ($CDCl_3$) 3581, 3345, 2946, 2868, 1759, 1662, 1637, 1596, 1576, 1451, 1370, 1213, 1179, 1065 cm^{-1}; UV (H_2O) λ_{max} 208, 238, 264 nm; MS (FAB, positive ion, nitrobenzyl alcohol matrix) m/z 272 (M^+, 14), 255 (65), 228 (11), 212 (100), 197 (9), 165 (10); HRMS calcd for $C_{16}H_{16}O_4$ 272.1049, found 272.1034; (M - OH) calcd for $C_{16}H_{15}O_3$ 255.1021, found 255.1014.

1-Hydroxy-1,2,3,4-tetrahydroanthraquinone (12). Chromatography of high R_f material isolated in the purification of **7** and **9** (9:1 hexane/ethyl acetate) afforded quinone **12** as a pale brown solid: mp 98-99 °C; ^1H NMR (300 Hz, $CDCl_3$) δ 8.05 (m, 2 H, ArH), 7.70 (m, 2 H, ArH), 4.93 (m, 1 H, C1-H), 3.37 (s, 1 H, OH), 2.75-2.65 (m, 1 H, C4-H), 2.52-2.41 (m, 1 H, C4-H), 1.97-1.84 (m, 3 H, CH_2), 1.79-1.69 (m, 1 H, CH_2); ^{13}C NMR (75 Hz, $CDCl_3$) δ 186.28, 185.16, 146.28, 143.25, 133.80, 133.68, 132.01, 131.90, 126.28, 126.16, 62.94, 29.31, 23.55, 17.15; IR ($CDCl_3$) 3583, 2954, 2870, 1662, 1624, 1596, 1420, 1331, 1292, 1251, 1170, 1079, 996 cm^{-1}; MS (EI, 70 eV) m/z 228 (M^+, 100), 210 (17), 200 (37), 181 (21), 173 (62), 115 (35); HRMS calcd for $C_{14}H_{12}O_3$ 228.0786, found 228.0794.

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Supplementary Material Available: ^1H NMR and ^{13}C NMR spectra (19 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Calophycin, a Fungicidal Cyclic Decapeptide from the Terrestrial Blue-Green Alga *Calothrix fusca*

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A broad-spectrum fungicide, calophycin, has been isolated from *Calothrix fusca* EU-10-1, a terrestrial blue-green alga belonging to Nostocaceae, and identified to be a cyclic decapeptide, cyclic (L-Ala-D-Asp-L-Asn-L-Gln-Gly-L-Arg-L-*N*-MeAsn-L-Pro-(2*R*,3*R*,4*S*)-Hamp-L-Val), where Hamp is a (2*R*,3*R*,4*S*)-3-amino-2-hydroxy-4-methylpalmitic acid unit and MeAsn is an *N*-methylasparagine residue. Its total structure, including absolute stereochemistry, was determined by a combination of spectral and chemical studies, including synthesis of the unusual β-amino acid Hamp.

In screening over 1000 strains of laboratory-cultured blue-green algae for fungicidal activity, we have found that extracts of more than 10% of these prokaryotes show activity against one or more of five test organisms, viz., *Aspergillus oryzae*, *Candida albicans*, *Penicillium notatum*, *Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*.[1,2] Nucleosides[3] and macrolides belonging to the scytophycin class[4] have frequently been identified in extracts that exhibit potent, broad-spectrum activity. We report here the isolation and total structure determination of a strongly antifungal cyclic decapeptide, calophycin (1), from *Calothrix fusca* (Kutzing) Bornet & Flahault, strain EU-10-1.[5]

The alga was isolated from a freshwater stream on the island of Oahu and grown in mass culture. Using a bioassay-directed isolation scheme, the extract (70% ethanol) of the lyophilized alga was subjected to repeated reversed-phase chromatography on C-18 and normal-phase chromatography on silica gel to give **1** as an amorphous white solid in 0.18% yield. The FAB mass spectrum indicated that the molecular weight was 1248 Da and detailed analyses of the ^{13}C and ^1H NMR spectra suggested

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(5) In a disc-diffusion soft agar plate assay calophycin at 1.2 μg/disc showed zones of inhibition of 13, 7, 12, 12, and 15 mm against *A. oryzae*, *C. albicans*, *P. notatum*, *S. cerevisiae*, and *T. mentagrophytes*, respectively (zones of inhibition at other doses and comparison with amphotericin B given in supplementary material). MIC values for calophycin against *C. albicans*, *T. mentagrophytes*, and *Aspergillus fumigatus* were found to be 1.25, 2.5, and 1.25 μg/mL, respectively, using Sabouraud dextrose broth as the test medium; by comparison amphotericin B showed MIC values of 0.625 and 1.25 μg/mL against *C. albicans* and *A. fumigatus*, respectively. Calophycin appeared to be moderately cytotoxic (IC_{50} 0.24 μg/mL against the KB cell line, a human nasopharyngeal carcinoma).

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