VOLUME 120, NUMBER 19
MAY 20, 1998

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Copper-Mediated Nuclease Activity of a Tambjamine Alkaloid

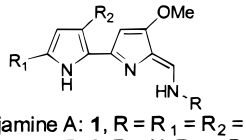
Sumit Borah, Matt S. Melvin, Niels Lindquist,† and Richard A. Manderville*

Contribution from the Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina 27109-7486

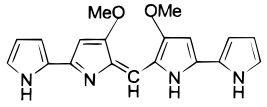
Received August 25, 1997

Abstract: The marine natural product tambjamine E (**5**) has been found to efficiently bind DNA and carry out DNA cleavage in the presence of Cu(II) and molecular oxygen *without addition of an external reducing agent*. DNA cleavage studies utilizing supercoiled plasmid DNA showed that the cleavage is inhibited by the enzyme catalase, which lowers solution concentrations of hydrogen peroxide (H_2O_2), but not superoxide dismutase (SOD), which converts the superoxide radical ($O_2^{\bullet -}$) into H_2O_2. The cleavage is also dependent on salt concentration and is not efficiently inhibited by hydroxyl radical scavengers. Evidence from UV−vis spectroscopy and electrospray mass spectrometry indicates that tambjamine E (**5**) binds Cu(II) to form a dimeric complex with 2:2 stoichiometry. Once bound to Cu(II), the bipyrrole nucleus of **5** is envisioned to reduce Cu(II) → Cu(I), while it is oxidized to a π-radical cation. Evidence in favor of this hypothesis was derived from the finding that generation of the dimeric copper complex of **5** in methanol was followed by dimerization of **5** to yield a tetrapyrrole derivative, (tambjamine E)$_2$. Thus, Ci(I), generated through the intermediacy of the π-radical cation of tambjamine E, is envisioned to react with H_2O_2 to yield a copper−oxo species that initiates DNA cleavage.

The tambjamines are a family of bipyrrole alkaloids derived from bacterial[1] and marine sources.[2] A common structural feature is a 4-methoxy-2,2′-bipyrrole ring system that may contain a bromine atom, and exist as an enamine tautomer, as shown below for tambjamines A−E (**1**−**5**). Related natural polypyrroles include the red-pigmented prodigiosins[3] and the blue-pigmented tetrapyrrole derivative **6**.[2,4] The tambjamines serve as chemical defenses against predators in marine organisms,[2] while sources from *Streptomycete* exhibit promising antitumor activities.[1]



tambjamine A: **1**, R = R$_1$ = R$_2$ = H
tambjamine B: **2**, R = H, R$_1$ = Br, R$_2$ = H
tambjamine C: **3**, R = CH$_2$CHMe$_2$, R$_1$ = R$_2$ = H
tambjamine D: **4**, R = CH$_2$CHMe$_2$, R$_1$ = H, R$_2$ = Br
tambjamine E: **5**, R = CH$_2$CH$_3$, R$_1$ = R$_2$ =H



6

Our interest in the tambjamines stemmed from their potential DNA targeting properties. Indeed, the planar bipyrrole nucleus[1−3] may bind DNA by intercalation while the methoxy group and ring nitrogens provide hydrogen bonding sites that could

† Institute of Marine Sciences, The University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, North Carolina 28557.

(1) (a) Kojiri, K.; Nakajima, S.; Suzuki, H.; Okura, A.; Suda, H. J. *Antibiot.* **1993**, *46*, 1799. (b) Nakajima, S.; Kojiri, K.; Suda, H. J. *Antibiot.* **1993**, *46*, 1894.

(2) (a) Carté, B.; Faulkner, D. J. *J. Org. Chem.* **1983**, *48*, 2314. (b) Paul, V. J.; Lindquist, N.; Fenical, W. *Mar. Ecol. Prog. Ser.* **1990**, *59*, 109. (c) Lindquist, N.; Fenical, W. *Experientia* **1991**, *47*, 504. (d) Blackman, A. J.; Li, C. *Aust. J. Chem.* **1994**, *47*, 1625.

S0002-7863(97)02974-0 CCC: $15.00
Published on Web 04/29/1998

contribute to a groove binding mode,[5] features similar to the bithiazole of bleomycin.[6] Since polypyrroles are easily oxidized,[7] as evidenced by their utility as electrochemical DNA sensors,[8] we also speculated that the tambjamines would show nuclease activity in the presence of a suitable redox-active metal. Such activity could then be used to determine whether the tambjamines interact with DNA in a sequence-specific fashion.

To test our hypotheses, the DNA targeting activities of tambjamine E (**5**), isolated from the marine ascidian *Atapozoa sp.*,[2c] were examined. We report here that **5** binds DNA efficiently and facilitates copper-mediated DNA cleavage in a process that *does not require an external reductant.* This behavior differs from numerous agents that carry out copper-mediated DNA cleavage only in the presence of large quantities of an external reducing agent.[9] Our findings are the first to demonstrate nuclease activity by a tambjamine, and imply that DNA may be a therapeutic locus for this class of antitumor agents.

Results and Discussion

DNA Binding. The ability of tambjamine E (**5**) to bind DNA was determined with UV−visible spectroscopy. As shown in Figure 1, addition of calf thymus DNA caused the absorption for free **5** at 393 nm (λ_{max}) to decrease in intensity and exhibit a 4 nm red shift—spectral characteristics not inconsistent with intercalation of the bipyrrole of **5**.[10] An equilibrium binding constant (K) was derived from a titration with use of 15 μM **5** with calf thymus DNA added in 190 μM base pair aliquots. Scatchard analysis[11] yielded a linear fit (Supporting Information), indicative of DNA binding by a single mode, and provided an equilibrium binding constant of 3×10^5 M^{-1}. Not surprisingly, this value is similar in magnitude for DNA binding by the structurally similar bithiazole of bleomycin.[12]

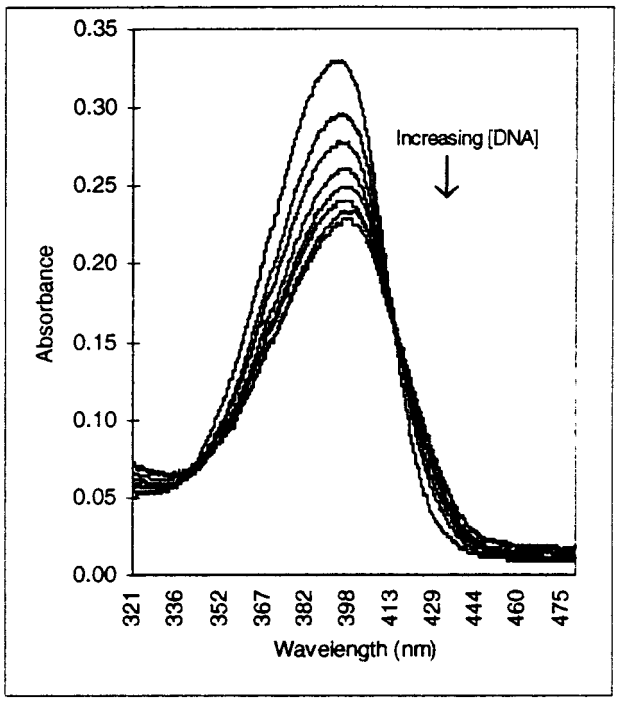


Figure 1. UV−vis titration of tambjamine E (**5**) with calf thymus DNA in 50 mM MOPS buffer (pH 7.4) containing 100 mM NaCl. Tambjamine E was 15 μM; DNA added in 150 μM (base pair) aliquots.

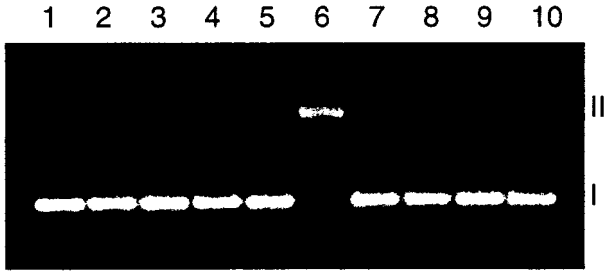


Figure 2. Relaxation of supercoiled DNA by **5** in the presence of metal ions. Reaction mixtures (20 μL total volume) contained 400 ng of supercoiled (Form I) DNA in 10 mM MOPS buffer, pH 7.4, and 100 mM NaCl. Cleavage was carried out at 37 °C for 30 min and then analyzed by agarose gel electrophoresis. Lane 1, DNA alone; lane 2, 50 μM **5**; lane 3, 50 μM Ni(II); lane 4, 50 μM Ni(II) + 50 μM **5**; lane 5, 50 μM Cu(II); lane 6, 50 μM Cu(II) + 50 μM **5**; lane 7, 50 μM Fe(III); lane 8, 50 μM Fe(III) + 50 μM **5**; lane 9, 50 μM Zn(II); lane 10, 50 μM Zn(II) + 50 μM **5**.

DNA Cleavage. The ability of **5** to mediate DNA cleavage was assessed in the presence of Cu(II), Fe(III), Ni(II), and Zn(II) by using supercoiled plasmid DNA and agarose gel electrophoresis. Interestingly, DNA cleavage by **5** was observed only in the presence of Cu(II), as shown in Figure 2 (lane 6). That both **5** and Cu(II) were required for DNA cleavage was demonstrated in lanes 2 and 5. Additional experiments demonstrated a dependence on both the concentration of **5** and Cu(II). Lowering the concentration of either reagent decreased the extent of cleavage, indicating that the reaction is not catalytic (data not shown).

To gain insight into the mechanism of DNA cleavage, the effect of oxygen on the relaxation of supercoiled DNA by the

(3) (a) Wasserman, H. H.; McKeon, J. E.; Smith, L.; Forgione, P. *J. Am. Chem. Soc.* **1960**, *82*, 506. (b) Rapoport, H.; Holden, K. G. *J. Am. Chem. Soc.* **1962**, *84*, 635. (c) Boger, D. L.; Patel, M. *J. Org. Chem.* **1988**, *53*, 1405. (d) Wasserman, H. H.; Lombardo, L. J. *Tetrahedron Lett.* **1989**, *30*, 1725.

(4) Wasserman, H. H.; Friedland, D. J.; Morrison, D. A. *Tetrahedron Lett.* **1968**, 641.

(5) (a) Dervan, P. B. *Science* **1986**, *232*, 464. (b) Wilson, W. D.; Li, Y.; Veal, J. M. *Adv. DNA Sequence Specific Agents* **1992**, *1*, 89. (b) Lown, J. W. *CHEMTRACTS* **1993**, *6*, 205.

(6) (a) Wu, W.; Vanderwall, D. E.; Stubbe, J.; Kozarich, J. W.; Turner, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 10843. (b) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1994**, *116*, 10851. (c) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 7891. (d) Wu, W.; Vanderwall, D. E.; Turner, C. J.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1996**, *118*, 1281. (e) Mao, Q.; Fulmer, P.; Li, W.; DeRose, E. F.; Petering, D. H. *J. Biol. Chem.* **1996**, *271*, 6185. (f) Lui, S. M.; Vanderwall, D. E.; Wu, W.; Tang, X.-J.; Turner, C. J.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1997**, *119*, 9603.

(7) For instance, see: (a) Frommer, J. E. *Acc. Chem. Res.* **1986**, *19*, 2. (b) Diaz, A. F.; Lacroix, J. C. *New J. Chem.* **1988**, *12*, 171. (c) Patil, A. O.; Heeger, A. J.; Wudl, F. *Chem. Rev.* **1988**, *88*, 183.

(8) (a) Livache, T.; Roget, A.; Dejean, E.; Barthet, C.; Bidan, G.; Theoule, R. *Nucleic Acids Res.* **1994**, *22*, 2915. (b) Korri-Youssoufi, H.; Gamier, F.; Srivastava, P.; Godillot, P.; Yassar, A. *J. Am. Chem. Soc.* **1997**, *119*, 7388.

(9) See for example: (a) Ehrenfeld, G. M.; Rodriguez, L. O.; Hecht, S. M.; Chang, C.; Basus, V. J.; Oppenheimer, J. J. *Biochemistry* **1985**, *24*, 81. (b) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. (c) Mack, D. P.; Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 7572. (d) Thederahn, T. B.; Kuwabara, M. D.; Larsen, T. A.; Sigman, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 4941. (e) De Rosch, M. A.; Trogler, W. C. *Inorg. Chem.* **1990**, *29*, 2409. (f) Pamatong, F. V.; Detmer, C. A., III; Bocarsly, J. R. *J. Am. Chem. Soc.* **1996**, *118*, 5339. (g) Routier, S.; Bernier, J.-L.; Waring, M. J.; Colson, P.; Houssier, C.; Bailly, C. *J. Org. Chem.* **1996**, *61*, 2326. (h) Harford, C.; Sarkar, B. *Acc. Chem. Res.* **1997**, *30*, 123.

(10) (a) Waring, M. J. *J. Mol. Biol.* **1970**, *54*, 247. (b) Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Strekowski, L.; Boykin, D. W. *J. Am. Chem. Soc.* **1989**, *111*, 5008. (c) Armitage, B.; Yu, C.; Devafoss, C.; Schuster, G. B. *J. Am. Chem. Soc.* **1994**, *116*, 9847.

(11) (a) Scatchard, G. *Ann. N. Y. Acad. Sci.* **1949**, *51*, 660. (b) Peacocke, A. R.; Skerrett, J. N. H. *Faraday Soc. Trans.* **1956**, *52*, 261.

(12) (a) Chein, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641. (b) Boger, D. L.; Colletti, S. L.; Honda, T.; Menezes, R. F. *J. Am. Chem. Soc.* **1994**, *116*, 5607.

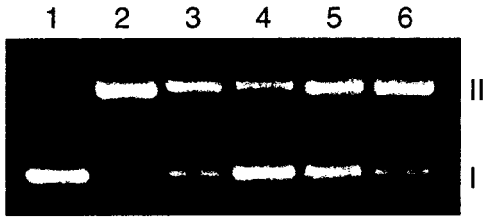


Figure 3. Relaxation of supercoiled DNA by Cu(II)/tambjamine E. Reaction mixtures contained 50 μM Cu(II) and 50 μM **5** and were incubated at 37 °C for 30 min in MOPS buffer (pH 7.4), containing 100 mM NaCl. Lane 1, DNA alone; lane 2, in an ambiently oxygenated atmosphere; lane 3, in an atmosphere previously flushed with argon; lane 4, + 100 mM NaN_3; lane 5, + 1 M *tert*-butyl alcohol; lane 6, + 1 M DMSO.

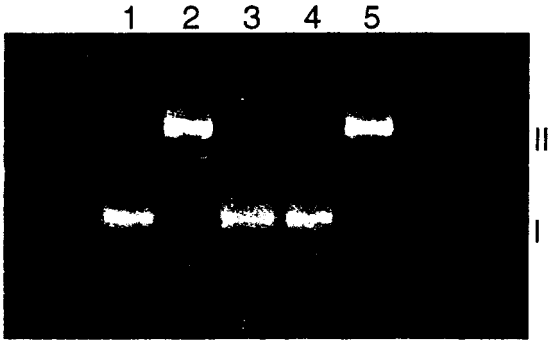


Figure 4. Relaxation of supercoiled DNA by Cu(II)/tambjamine E and the effect of EDTA, catalase, and superoxide dismutase (SOD) on the cleavage reaction. Reaction mixtures (20 μL total volume) contained 400 ng of supercoiled (Form I) DNA in 10 mM MOPS buffer, pH 7.4, and 100 mM NaCl. Cleavage was carried out at 37 °C for 30 min and then analyzed by agarose gel electrophoresis. Lane 1, DNA alone; lane 2, 50 μM **5** + 50 μM Cu(II); lane 3, + 100 mM EDTA, lane 4, + 1000 units/mL catalase; lane 5, + 1000 units/mL SOD.

1:1 Cu(II)/tambjamine E (50 μM) mixture was examined. Figure 3 shows a comparison of the extent of cleavage under normal atmospheric conditions with that under an argon atmosphere, and in the presence of various oxygen scavengers. Comparison of lanes 2 and 3 demonstrates that DNA cleavage by Cu(II)/tambjamine E was diminished in an argon atmosphere. The singlet oxygen scavenger, sodium azide[13] (lane 4), was also much more effective as an inhibitor than the hydroxyl radical scavengers, *tert*-butyl alcohol (lane 5) and DMSO (lane 6).[14] At 50 μM Cu(II)/tambjamine E, addition of NaCl up to 100 mM increased the efficiency of DNA cleavage, while above 250 mM the reaction was inhibited (not shown). Both the inefficiency of DMSO and *tert*-butyl alcohol to quench the reaction (Figure 3) and the extent of cleavage being dependent on the concentration of NaCl argue against participation of a freely diffusible hydroxyl radical.[15]

Additional evidence for oxygen dependence in Cu-mediated DNA cleavage by **5** was obtained from the finding that catalase, an enzyme that disproportionates H_2O_2 to afford $H_2O + O_2$,[16] completely inhibits the cleavage. Figure 4 clearly shows the inhibitory effect of catalase (lane 4) and the metal chelator EDTA (lane 3), while superoxide dismutase, SOD (lane 5), failed to inhibit cleavage. These findings indicate that copper binding by **5** is essential and that H_2O_2, not $O_2^{\bullet-}$, is involved in mediating DNA cleavage.

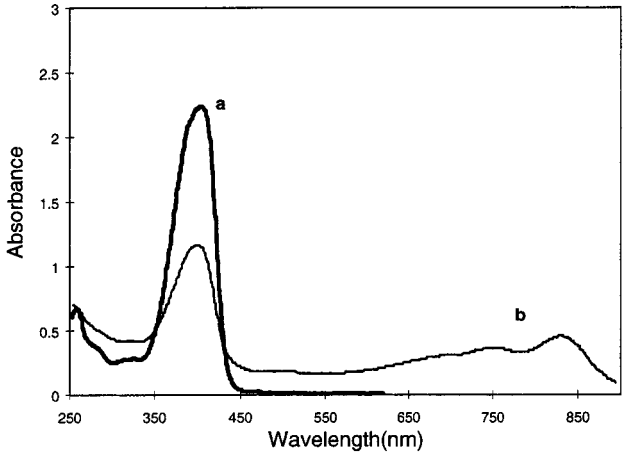


Figure 5. UV−vis absorbance spectra of **5** in methanol (spectrum a) and in the presence of 1 equiv of $Cu(OAc)_2$ (spectrum b).

Reaction of 5 with Copper(II). To ascertain the nature of the copper/tambjamine E interaction, UV−vis spectroscopy and electrospray mass spectrometry were utilized. Figure 5 shows UV−vis spectra for the reaction between **5** and $Cu(OAc)_2$ in methanol at ambient temperature. As shown in spectrum **a** in Figure 5, free tambjamine E shows a strong absorption at 405 nm (ϵ = 24 700 M^{-1} cm^{-1}).[2c] Admixture of $Cu(OAc)_2$ immediately produced a blue-green solution with absorbances at 385, 693, 749, and 828 nm (Figure 5, spectrum **b**). Unexpectedly, analysis of the blue-green solution by electrospray mass spectrometry using position ionization (ES^+) indicated formation of dimeric copper complexes with 2:2 stoichiometry. No evidence for a 1:1 Cu/tambjamine E complex was obtained and three dimeric copper-bound species with *m*/*z* 558, 572, and 588 $[M + H]^+$ were present (Figure 6). The major species with *m*/*z* 558 $[M + H]^+$ corresponds to a complex with molecular formula $Cu_2C_{24}H_{27}N_6O_2$.[17] Collision-induced dissociation of the species with *m*/*z* 572 and 588 $[M + H]^+$ indicated loss of *m*/*z* 14 and 30 mass units, respectively, to afford the major complex with *m*/*z* 558 $[M + H]^+$. This suggests the possibility that solvent (water from the $Cu(OAc)_2$ solution, and methanol), or some other small fragments,[18] was incorporated into the dimeric complex with *m*/*z* 558 to produce the two complexes with *m*/*z* 572 and 588 $[M + H]^+$.

Over 1 h at ambient temperature, the blue-green solution ascribed to the dimeric copper complex turned black. Analysis of the black solution by mass spectrometry (ES^+) showed that a polymeric species with repeating mass units of *m*/*z* 44 (starting at *m*/*z* 405.3 $[M + H]^+$ and ending at *m*/*z* 845.5 $[M + H]^+$) was present (Supporting Information). While the exact nature

(13) (a) Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1989**, *264*, 15435. (b) Li, Y.; Trush, M. A. *Carcinogenesis* **1993**, *14*, 1303.

(14) (a) Aruoma, O. I.; Halliwell, B.; Dizdaroglu, M. *J. Biol. Chem.* **1989**, *264*, 13024. (b) Sagripanti, J.-L.; Kraemer, K. H. *J. Biol. Chem.* **1989**, *264*, 1729.

(15) (a) Lu, M.; Guo, Q.; Wink, D. J.; Kallenbach, N. R. *Nucleic Acids Res.* **1990**, *18*, 3333. (b) Knapp Pogozelski, W.; McNeese, T. J.; Tullius, T. D. *J. Am. Chem. Soc.* **1995**, *117*, 6428.

(16) Walsh, C. *Enzymatic reaction mechanisms*; W. H. Freeman and Co., New York, 1979.

(17) A dinuclear copper(II) complex of a bis-terpyrrole derivative has been reported by Sessler and co-workers (Weghorn, S. J.; Sessler, J. L.; Lynch, V.; Baumann, T. F.; Sibert, J. W. *Inorg. Chem.* **1996**, *35*, 1089.). The complex had two bridging chlorine atoms and its UV−vis spectrum in CH_2Cl_2 showed four major absorptions at 505, 810, 884, and 1024 nm. The UV−vis characteristics of the dimeric copper/tambjamine E complex observed here with bands at 385, 693, 749, and 832 nm are similar to the UV−vis characteristics reported by Sessler and co-workers, if one accounts for the expected red shift of a terpyrrole (porphyrin) compared to a bipyrrole.

(18) Sessler and co-workers have also observed the incorporation of a fragment 14 mass units heavier than that anticipated for copper(II) binding by a tripyrrolic (tripyrrane) ligand (Sessler, J. L.; Gebauer, A.; Král, V.; Lynch, V. *Inorg. Chem.* **1996**, *35*, 6636). In their studies, incorporation of an oxygen atom with loss of two protons occurred during the course of copper chelation by the tripyrrane ligand.

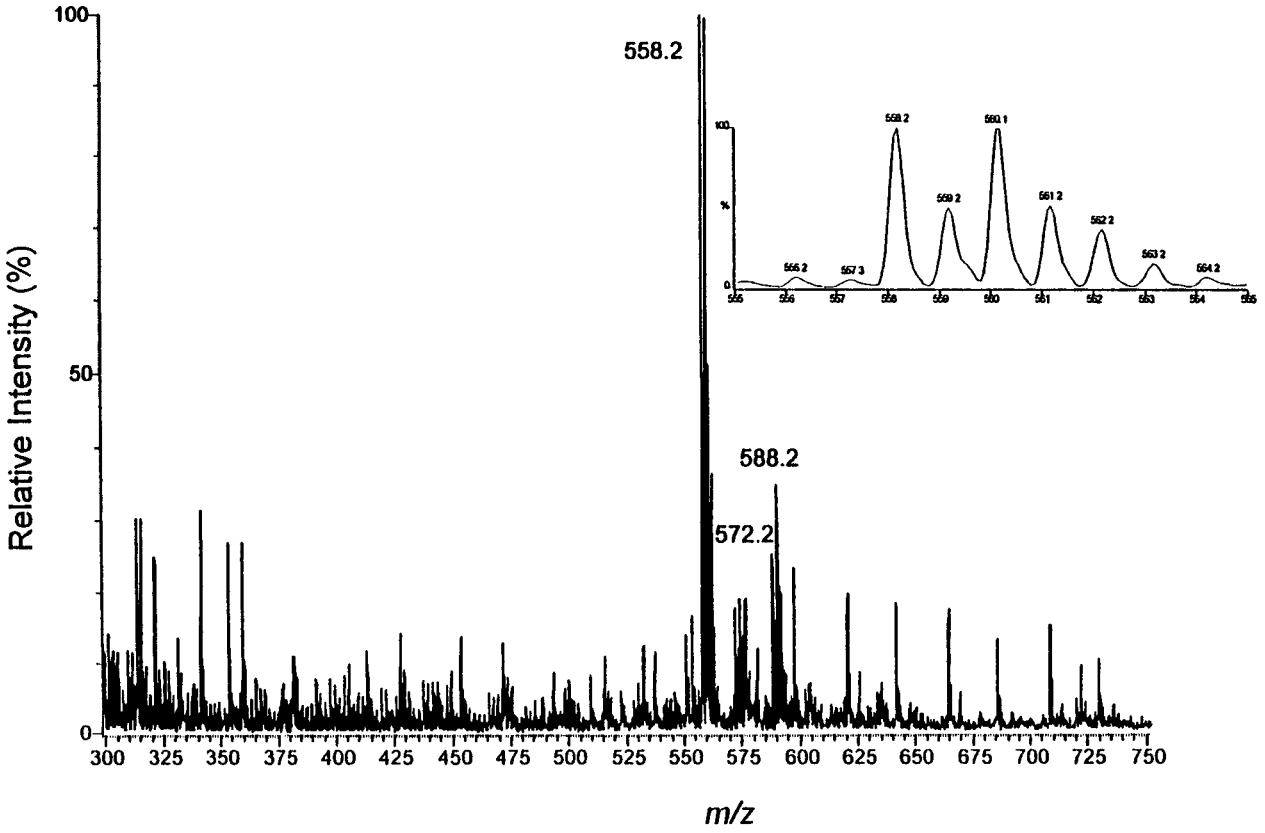


Figure 6. Electrospray mass spectrum of the dimeric (2:2) copper complexes of tambjamine E (*m*/*z* 558, 572, and 588 $[M + H]^+$). The inset shows the isotopic distribution of the peak with *m*/*z* 558.

of the polymer is uncertain, it is informative to note that the major product from the reaction had *m*/*z* 493 $[M + H]^+$. Upon collision-induced dissociation, the ion at *m*/*z* 493 $[M + H]^+$ decomposed to form a species with *m*/*z* 433 $[M + H]^+$, which showed a tandem mass spectrum similar to tambjamine E, **5** (*m*/*z* 218 $[M + H]^+$, Supporting Information). The presence of the compound with *m*/*z* 433 $[M + H]^+$ is consistent with dimerization of tambjamine E (217 + 217 − 2H) to produce a tetrapyrrole derivative, (tambjamine E)$_2$. On the basis of analogy to polymerization of pyrroles by Cu(II) salts,[19] which produces pyrrole black, and dimers, trimers, etc., through π-radical cation coupling,[7,19,20] it is most likely that the unsubstituted α-terminal position of **5** participates in dimer formation.[7,20]

The interaction of **5** with Cu(II) in aqueous buffered media (pH 7.4) was also examined by UV−vis spectroscopy. Figure 7a shows a double-reciprocal (Bernesi−Hildebrand) plot[21] derived from a UV titration of **5** with Cu(OAc)$_2$. From the slope and *y*-intercept in Figure 7a, an apparent equilibrium-binding constant of 925 M^{-1} was obtained. The affinity of **5** for Cu(II) was then re-examined with **5** pre-bound to excess calf thymus DNA. Interestingly, under these conditions the affinity of **5** for Cu(II) increased from that free in solution, and an apparent binding constant of approximately 6.5×10^3 M^{-1} was determined (Figure 7b). It is also informative that the solution with **5** pre-bound to DNA was blue-green after titration with Cu(OAc)$_2$ and showed absorbances at 388, 730, and 840 nm (Supporting Information). These spectral characteristics are similar to the results obtained in methanol (Figure 5), and as our ES^+ data imply (Figure 6), a dimeric copper complex may also form in the presence of DNA.

Having established equilibrium constants for calf thymus DNA binding by **5** ($K_1 = 3 \times 10^5$ M^{-1}), and for Cu(II) binding both free in solution ($K_2 = 925$ M^{-1}) and pre-bound to calf thymus DNA ($K_3 = 6.5 \times 10^3$ M^{-1}), an estimate of the DNA binding affinity for the copper/tambjamine complex was determined to be $K_4 = 2.1 \times 10^6$ M^{-1}, where $K_4 = K_1K_3/K_2$ (Scheme 1). This indicates that Cu-bound **5** binds DNA with greater affinity than free tambjamine E, which is consistent with previous studies on DNA binding by metal complexes.[9,22]

Mechanism of DNA Cleavage. The results described herein have allowed us to propose the mechanism outlined in eq 1a−g for Cu(II)-mediated DNA cleavage by tambjamine E (**5**). According to our UV−vis and mass spectral results, Cu(II) and **5** combine to form a complex with a DNA binding affinity of ca. 2.1×10^6 M^{-1}. While our mass spectral results in methanol suggest that a dimeric 2:2 Cu(II)$_2$/(tambjamine E)$_2$ complex is formed, in the ensuing discussion the nature of the complex is undefined and is depicted as Cu(II)/tambjamine E (eq 1a). The source of electrons for the reduction of Cu(II) → Cu(I) would be the bipyrrole nucleus of **5**. Thus, Cu(I) would be formed reductively as the bipyrrole of tambjamine E is oxidized to π-radical cation to generate Cu(I)/(tambjamine $E^{\bullet+}$) (eq 1b). Since rate constants for dimerization of bipyrrole radical cations in water are around 10^9 L mol^{-1} s^{-1},[20] one fate of the tambjamine E π-radical cation would be dimerization to afford (tambjamine E)$_2$ and 2Cu(I) (eq 1c). Oxidation of Cu(I) by O_2 would yield superoxide ($O_2^{\bullet-}$) and Cu(II) (eq 1d). The liberated $O_2^{\bullet-}$ would then furnish H_2O_2 after disproportionation (eq 1e),

(19) (a) *Pyrroles. Part One: The synthesis and the physical and chemical aspects of the pyrrole ring*; Jones, R. Alan, Ed.; John Wiley & Sons: New York, 1990; p 518. (b) Rapi, S.; Bocchi, V.; Gardini, G. P. *Synth. Meth.* **1988**, *24*, 217.

(20) Guyard, L.; Hapiot, P.; Neta, P. *J. Phys. Chem. B* **1997**, *101*, 5698.

(21) (a) Bernesi, H.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703. (b) Conners, K. A. *Binding constants. The measurement of molecular complex stability*; John Wiley & Sons: New York, 1987; p 152.

(22) Chang, C.-H.; Meares, C. F. *Biochemistry* **1984**, *23*, 2268.

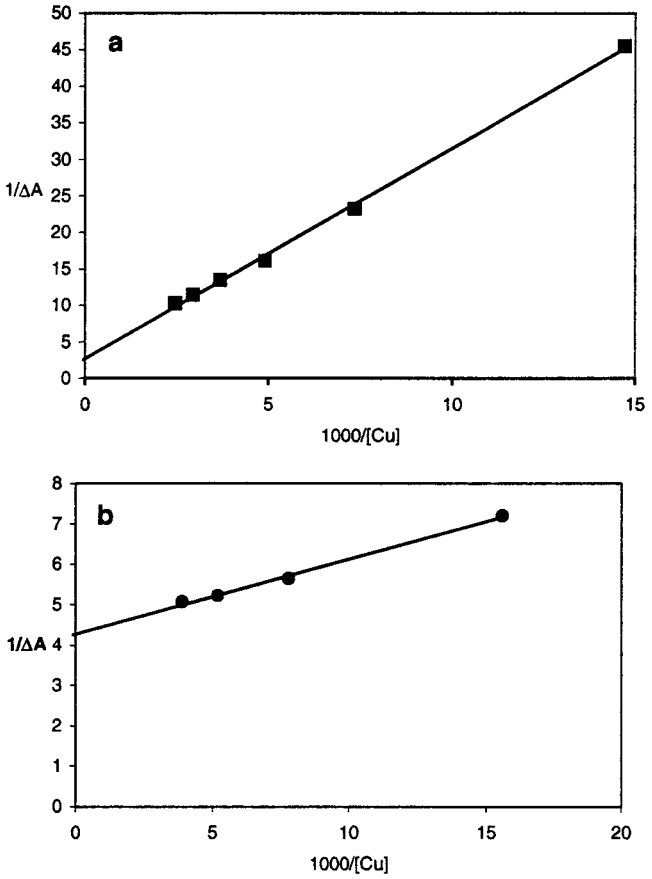
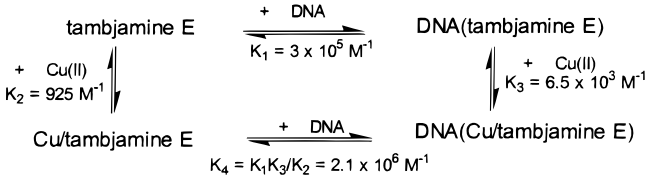


Figure 7. (a) Double-reciprocal (Benesi−Hildebrand) plot derived from a UV−vis titration of **5** (34 μM) with $Cu(OAc)_2$ at 25 °C in 10 mM MOPS buffer, pH 7.4. (b) Plot as in part a, but with **5** (34 μM) pre-bound to calf thymus DNA (100 μM base pair concentration).

Scheme 1. Thermodynamic Cycle for the Interaction of Tambjamine E (**5**) with DNA and Copper



and oxidation of Cu(I) by H_2O_2 would yield a copper−oxo species (eq 1f) that initiates DNA cleavage (eq 1g). This proposal stems from similarities noted in our quenching experiments to that established for thiol-potentiation of the 1,-10-phenanthroline−copper ($(OP)_2Cu^{2+}$) complex, which cleaves DNA through generation of a copper−oxo species.[9d,23]

$$\text{Cu(II)} + \text{tambjamine E} \rightarrow \text{Cu(II)/(tambjamine E)} \quad (1a)$$

$$\text{Cu(II)/(tambjamine E)} \rightarrow \text{Cu(I)/(tambjamine E}^{\bullet+}\text{)} \quad (1b)$$

$$2\text{Cu(I)/(tambjamine E}^{\bullet+}\text{)} \rightarrow 2\text{Cu(I)} + \text{(tambjamine E)}_2 \quad (1c)$$

$$\text{Cu(I)} + O_2 \rightarrow \text{Cu(II)} + O_2^{\bullet-} \quad (1d)$$

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1e)$$

$$\text{Cu(I)} + H_2O_2 \rightarrow \text{copper−oxo species} \quad (1f)$$

$$\text{copper−oxo species} + \text{DNA} \rightarrow \text{DNA cleavage} \quad (1g)$$

Conclusions

On the basis of the results that we have presented, it is clear that the tambjamines possess activities that potentially could be developed as novel DNA probes and DNA targeting anticancer agents. The bipyrrole nucleus binds both DNA and copper effectively. Due to the redox nature of the bipyrrole, these compounds induce DNA cleavage in the presence of copper without activation by an external reductant. This implies that Cu(I) is formed reductively through the concomitant oxidation of the tambjamine to a π-radical cation. While one fate of the proposed π-radical cation could be its dimerization, it is also interesting to speculate that such a species may damage DNA, possibly through electron transfer with the DNA bases. Currently, experiments are underway to determine the exact nature of DNA damage by the copper/tambjamine mixture. Structural features of the dimeric copper tambjamine complex formed prior to initiation of DNA damage are also being pursued.

Experimental Section

Materials and Methods. The sample of tambjamine E (**5**) used in these studies was isolated from organic extracts of the marine ascidian *Atapozoa* sp. (see ref 2c for details and spectral characteristics). Purification by high-pressure liquid chromatography (HPLC) was performed on a Hitachi L-7000 series (L-7100 pump and L-7400 UV detector) with a C-18 column (8:2 methanol/water with 0.1 M ammonium acetate buffer). Tambjamine E (**5**) was obtained as a yellow residue and its identity was verified by ^{1}H NMR and mass spectrometry. HREIMS obsd (M+) *m*/*z* 217.1218; $C_{12}H_{15}N_3O$ requires 217.1215. Concentrations of **5** were obtained from UV−visible measurements in MeOH: 405 (ϵ = 24 700 M^{-1} cm^{-1}), 280 (sh), 257 (5700).

Supercoiled plasmid (Form I) DNA was a gift from Dr. Fred W. Perrino, Department of Biochemistry, Wake Forest University School of Medicine. The plasmid was a derivative of pOXO4 containing the dnaQ gene.[24] The following enzymes and reagents were obtained commercially and used without further purification: sodium azide, copper acetate ($Cu(OAc)_2$), 4-morpholinepropanesulfonic acid (MOPS), ethylenediaminetetraacetic acid (EDTA) (Aldrich) and catalase and SOD (Sigma).

Absorption measurements were made on a Hitachi U-2001 UV−vis spectrophotometer equipped with a thermostated cell compartment. Distilled, deionized water from a Milli-Q system was used for all aqueous solutions and manipulations. Agarose gel electrophoresis was carried out in 40 mM Tris−acetate buffer (pH 8.0), containing 5 mM EDTA. Agarose gel loading buffer: 40 mM Tris−OAc (pH 8.0), 5 mM EDTA, 40% glycerol, 0.3% bromophenol blue.

Mass spectra were acquired with a Micromass Quattro II operating in the positive ionspray mode (ES^+). The system acquired signal over a *m*/*z* range of 200−1000 at 8 s/scan with a 0.06 step. Samples of tambjamine E (**5**) and Cu(II)/(tambjamine E) (0.1 mg/mL) were prepared in methanol and injected via syringe into a fixed loop injector port (100 μL volume) interfaced to the ionspray source. Fragment ion (Tandem or MS/MS) mass spectra were obtained by collision-induced dissociation of precursor ions selected by their *m*/*z* value in the first quadrupole.

DNA Binding. UV−vis titration experiments were carried out at 25 °C with sonicated, phenol-extracted calf thymus DNA (Sigma). The DNA concentration was determined by UV (ϵ_{260} = 12 824 M^{-1} in base pair). Samples were prepared in 50 mM MOPS buffer (pH 7.4)

(23) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180.

(24) Parsonage, D.; Miller, H.; Ross, R. P.; Claiborne, A. *J. Biol. Chem.* **1993**, *268*, 3161.

containing 100 mM NaCl and 1% DMSO. The optical density of the tambjamine E solution (15 μM) at 393 nm was measured initially and after each addition of calf thymus DNA (190 μM base pair aliquots). The fraction of bound drug, α, was determined as described,[11b] and $0.20 \leq \alpha \leq 0.80$ values were used to yield K from the slope of an r/c *vs* r plot, where $r =$ [bound drug]/[DNA] and $c =$ [free drug].

Copper Binding. The copper binding affinity of tambjamine E was determined by UV−vis spectroscopy at 25 °C in 10 mM MOPS buffer, pH 7.4. The optical density of the tambjamine E (**5**) solution (34 μM) at 393 nm was measured initially and after each addition (2 μL) of $Cu(OAc)_2$ (stock solution = 34 mM). The titration was then repeated with **5** pre-bound to calf thymus DNA (100 μM base pair concentration) and the change in absorption at 397 nm for DNA-bound **5** was recorded. Double-reciprocal plots of $1/\Delta A$ (change in absorption) versus 1/[Cu] afforded straight lines from which the association constant (K_{11}) was determined from $K_{11} =$ (y-intercept)/(slope).[21]

Relaxation of Supercoiled DNA by Metal Ions in the Presence of Tambjamine E. Reaction mixtures (20 μL total volume) contained 400 ng of Form I DNA, 50 mM MOPS (pH 7.4), 100 mM NaCl, and 50 μM each of tambjamine E (**5**) and metal ion. The metal salts employed were $Cu(OAc)_2$, $Fe(Cl)_3$, $Zn(OAc)_2$, and $NiCl_2$. Reaction mixtures were incubated at 37 °C for 30 min, then quenched by the addition of 4 μL of loading buffer. Samples were loaded onto a 1% agarose gel containing ethidium bromide (1 μg/mL). The gel was run at 110 V for 2 h and visualized by UV illumination.

Relaxation of Supercoiled DNA by Cu(II)/Tambjamine E. Reaction mixtures (20 μL total volume) contained 400 ng of Form I DNA, 50 mM MOPS (pH 7.4), 100 mM NaCl, 50 μM $Cu(OAc)_2$, and 50 μM tambjamine E (**5**). Reaction mixtures were incubated at 37 °C for 30 min, then quenched by the addition of 4 μL of loading buffer. Samples were loaded onto a 1% agarose gel containing ethidium bromide (1 μg/mL). The gel was run at 110 V for 2 h and visualized by UV illumination. In separate experiments, the quenching effects of sodium azide (NaN_3, 100 mM), DMSO (1 M), *tert*-butyl alcohol (1 M), catalase (1000 units/mL), and SOD (1000 units/mL) were examined. The extent of cleavage in an argon-purged atmosphere was also examined. For this experiment, stock solutions of $Cu(OAc)_2$ and supercoiled plasmid DNA pre-mixed with tambjamine E in septum-capped vials were purged with argon. Injection of 1 equiv of the stock copper solution via syringe into the DNA/tambjamine solution (100 μL total volume, 50 mM MOPS, pH 7.4, 100 mM NaCl) initiated the reaction. The mixture was incubated at 37 °C for 30 min and then analyzed by agarose gel electrophoresis.

Acknowledgment. We thank Dr. Fred W. Perrino (Department of Biochemistry, Wake Forest University School of Medicine) for the sample of plasmid DNA and Dr. Gary Byrd (RJR Tobacco Co) for acquisition of the mass spectral data.

Supporting Information Available: Plot of bound drug (Tambjamine E, **1**) versus [DNA]/[drug] from a UV−visible titration experiment of **1** with calf thymus DNA and Scatchard plot of the UV−visible data; mass spectrum obtained from the reaction of tambjamine E (**5**) with 1 equiv of $Cu(OAc)_2$ in methanol. Tandem mass spectrum of the major product with m/z 493 $(M + H)^+$ highlighting loss of 60 mass units to produce a species with m/z 433 $[M + H]^+$; Uv−vis spectrum obtained after titration of **5** with $Cu(OAc)_2$ (5 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9729746