[4] a) J. D. Ranford, J. J. Vittal, D. Wu, *Angew. Chem.* **1998**, *110*, 1159–1162; *Angew. Chem. Int. Ed.* **1998**, *37*, 1114–1116; highlight: b) M. J. Zaworotko, *Angew. Chem.* **1998**, *110*, 1269–1171; *Angew. Chem. Int. Ed.* **1998**, *37*, 1211–1213.

[5] See, for example, a) C. Piguet, G. Bernardinelli, G. Hopfgartner, *Chem. Rev.* **1997**, *97*, 2005–2062; b) J. M. Lehn, *Supramolecular Chemistry*, VCH, Weinheim, **1995**.

[6] a) A. Williams, *Chem. Eur. J.* **1997**, *3*, 15–19; b) E. C. Constable in *Comprehensive Supramolecular Chemistry, Vol. 6* (Eds.: J. L. Atwood, J. E. Davies, D. D. MacNicol, F. Vögtle, J. M. Lehn), Pergamon, Oxford, **1996**, p. 213.

[7] a) M. E. Brown, M. D. Hollingsworth, *Nature* **1995**, *376*, 323–326; b) R. Hoss, O. Konig, V. Kramer-Ross, U. Berger, P. Rogin, J. Hulliger, *Angew. Chem.* **1996**, *108*, 1774; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1664.

[8] a) Crystal structure determination of $[\{[Cu(sala)]_2(H_2O)\}_n]$ (**1**). Orthorhombic, space group $P2_12_12_1$, $a = 7.3850(2)$, $b = 10.0669(2)$, $c = 28.6676(8)$ Å, $V = 2131.27(9)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.65$ g cm^{-3}. All hydrogen atoms were located successfully. The positional and common isotropic thermal parameters were refined for the hydrogen atoms in the coordinated water molecule, and riding models were used for the remaining hydrogen atoms. In the final least-squares refinement cycles on $|F|^2$, the model converged at $R_1 = 0.0505$, $wR_2 = 0.0694$, and GOF = 1.039 for 3483 reflections with $F_o > 4\sigma(F_o)$ and 288 parameters, and at $R_1 = 0.1004$ and $wR_2 = 0.0820$ for all 5262 data. The Flack parameter x was refined to $-0.01(2)$. b) General crystallographic details: Data were collected on a Siemens SMART CCD system with graphite-monochromated $Mo_{K\alpha}$ radiation using a sealed tube (2.4 kW) at 23 °C. Absorption corrections were made with the program SADABS (G. M. Sheldrick, Göttingen, **1996**), and the crystallographic software package SHELXTL (SHELXTL Reference Manual, Version 5.03, Wisconsin, **1996**) was used for all calculations. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-133087 (**1**) and -133088 (**3**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[9] L. L. Koh, J. D. Ranford, W. T. Robinson, J. O. Svensson, L. C. Tan, D. Wu, *Inorg. Chem.* **1996**, *35*, 6466–6472.

[10] Analogous Cu^{II} single-crystal X-ray structures for helices with the ligands *N*-(2-hydroxybenzyl)-L-tyrosine, *N*-(2-hydroxybenzyl)glycine, and H_2sala have also been solved. For the latter the compound is the enantiomer of **1**: J. D. Ranford, W. T. Robinson, J. O. Svensson, J. J. Vittal, D. Wu, X. Yang, unpublished results.

[11] Close intermolecular hydrogen-bonding contacts between two [Å] and three centers [°]: H2···O6^{i} 2.01(4), H4A···O7^{i} 2.19(3), H1···O3^{ii} 2.27(4), H4B···O3^{ii} 2.10(5), N2···O6^{i} 2.903(5), O4···O7^{i} 2.898(4), N1···O3^{ii} 2.98(6), O4···O3^{ii} 2.655(4); N2-H2-O6^{i} 161(3), O4-H4A-O7^{i} 161(6), N1-H1-O3^{ii} 156(4), O4-H4B-O3^{ii} 143(5); the N1–H1, N2–H2, O4–H4A, and O4–H4B distances are 0.75(4), 0.93(4), 0.74(3), and 0.74(3) Å, respectively. The H4A-O4-H4B angle is 100(6)°. Operators for generating equivalent atoms: i: $x + \frac{1}{2}, -y + \frac{1}{2}, -z + 1$; ii: $-x + 1, y + \frac{1}{2}, z + \frac{1}{2}$.

[12] See, for example, H. Lodish, D. Baltimore, A. Berk, S. L. Zipursky, P. Matsudaira, J. Darnell, *Molecular Cell Biology*, 3rd ed., Scientific American Books, New York, **1996**.

[13] Thermogravimetric analysis of **1** indicated a weight loss of 3.7% in the temperature range 70–90 °C, corresponding to the loss of one molecule of water per dimer (expected weight loss 3.4%) and the formation of the anhydrous compound **3**. The X-ray powder pattern of this anhydrous material was the same as that of **3**. Species **1** and **3** show the same decomposition pattern, which starts at about 240 °C. The structures of the bulk materials for **1** and **3** were confirmed by matching their X-ray powder patterns with those generated from the corresponding singles. Compound **3** was also prepared independently. The crystal structure was carried out on the single crystal grown during the synthesis (see the Experimental Section).

[14] Crystal structure determination of $[\{Cu(sala)\}_n]$ (**3**): Tetragonal, space group $P4_32_12$, $a = 8.8790(1)$, $c = 24.9879(2)$ Å, $V = 1969.96(4)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.731$ g cm^{-3}. All hydrogen atoms were located successfully, and riding models were used. In the final least-squares refinement cycles on $|F|^2$, the model converged at $R_1 = 0.0226$, $wR_2 = 0.0517$, and GOF = 1.00 for 2371 reflections with $F_o > 4\sigma(F_o)$ and 137 parameters, and at $R_1 = 0.0257$ and $wR_2 = 0.0529$ for all 2526 data. The Flack parameter x was refined to $-0.016(13)$.[8b]

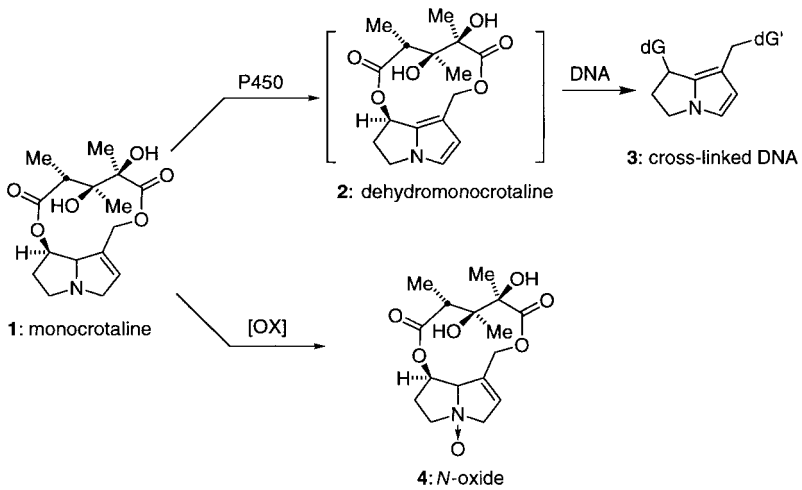
[15] Hydrogen-bonding parameters: H1···O2^{i} 2.03(2) Å, N1···O2^{i} 2.93(2) Å, and N1-H1···O2^{i} 164(2)°. Operator for generating equivalent atoms: i: $x + 0.5, -y + 0.5, -z + 0.25$.

[16] G. R. Desiraju, *Angew. Chem.* **1995**, *107*, 2541–2558; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2311–2327.

Reductive Activation of a Hydroxylamine Hemiacetal Derivative of Dehydromonocrotaline: The First Reductively Activated Pyrrolizidine Alkaloid Capable of Cross-Linking DNA**

Jetze J. Tepe and Robert M. Williams*

Pyrrolizidine alkaloids (PAs), such as monocrotaline (**1**, Scheme 1), are potent hepatotoxins and carcinogens isolated from a wide variety of plants.[1, 2] Owing to their geographical



Scheme 1. Pyrrolizidine alkaloid monocrotaline (**1**) and derivatives **2**–**4**.

[*] Prof. Dr. R. M. Williams, J. J. Tepe
Department of Chemistry
Colorado State University
Fort Collins, CO 80523 (USA)
Fax: (+1) 970-491-5610
E-mail: rmw@chem.colostate.edu

[**] The authors gratefully acknowledge the financial support provided by the National Institutes of Health (grant CA51875). The authors also acknowledge Teri Lansdell and Paul Sebahar for their help with the DNA-cross-linking experiments and HPLC analysis. We would like to thank Prof. Frank R. Stermitz for an improved procedure to prepare dehydromonocrotaline.

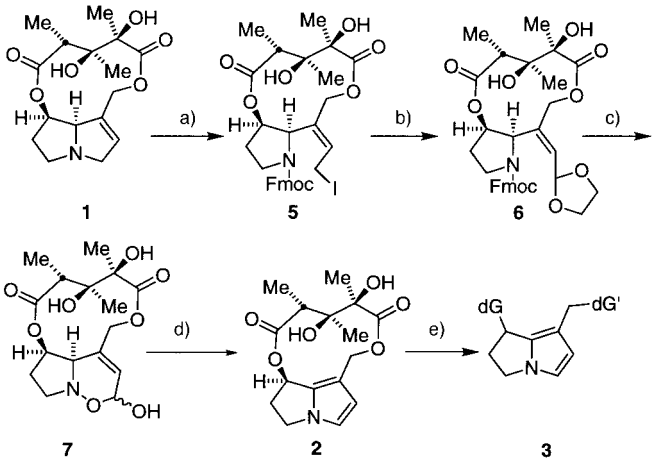
diversity and abundance, the PAs represent a serious health threat to humans and live stock.[2, 3] The biologically active metabolites of these natural products are the corresponding dehydropyrrolizidines (for example, dehydromonocrotaline, **2**), formed upon two-electron oxidation of the 4-azabicyclo[3.3.0]octane ring system by liver cytochrome P450 mixed-function oxidases.[4, 5] These substances have been shown to be potent agents for the interstrand cross-linking of DNA (forming adduct **3**), and it is this biochemical reactivity that is largely responsible for the cytotoxicity that this class of alkaloids displays.[6, 7] Detoxification of the PAs occurs when an alternative N-oxidation reaction occurs (→**4**) with either cytochrome P450 or flavin-containing monooxygenases.[4]

The oxidation of the pyrrolizidine alkaloid ring system results in the electrophilic activation of the C7 and C9 positions, by conjugation with the pyrrole nitrogen lone pair, which are prone to nucleophilic attack by the exocyclic amine group of deoxyguanosine residues in the minor groove of DNA at 5′-CpG-3′ sites.[8, 9]

The potent interstrand DNA – DNA and DNA – protein[10] cross-linking reactions mediated by the pyrrolic PAs have made these compounds of interest as potential antitumor agents. However, the acute hepatotoxicity of these agents has obviated their clinical utility. The hepatotoxicity of these agents is a manifestation of the initial production and release of the reactive DNA-cross-linking species being generated in the liver by the mixed-function cytochrome P450 oxidases. Since the oxidative mode of activation is primarily responsible for the undesirable characteristics of these compounds, it seemed that it would be possible to improve their pharmacological profile by altering the chemical mode of activation. Based on the success of a variety of clinically significant, reductively activated DNA-cross-linking agents, such as mitomycin C, and FR900482 and congeners we anticipated that a reductive mode of activation of the PAs might not only improve the pharmacological profile of these agents, but also induce a similar tumor selectivity that many of the hypoxia-directed agents display.[11, 12] As part of a program aimed at diversifying the chemical and biochemical repertoire of activation of a variety of clinically significant antitumor agents,[13] we describe here the synthesis and DNA-cross-linking reactivity of the first reductively activated pyrrolizidine alkaloid, a progenitor of dehydromonocrotaline.

The synthesis of the reductively activated dehydromonocrotaline derivative **7** is shown in Scheme 2. Commercially available[14] monocrotaline (**1**) was condensed with 9-fluorenylmethyl chloroformate (FmocCl) in the presence of KI in acetonitrile,[15] furnishing the ring-cleaved product **5**. The allylic iodide was oxidized with DMSO and $AgBF_4$ to afford the unsaturated aldehyde in 95% yield. Protection of the aldehyde as the corresponding ethylene glycol acetal **6** followed by removal of the Fmoc moiety with 50% piperidine in THF provide the corresponding free amine. Compound **7**, the hydroxyl amine hemiacetal progenitor of dehydromonocrotaline, was prepared by oxidation of the free amine with *m*-CPBA in a biphasic mixture of CH_2Cl_2 and aqueous saturated $NaHCO_3$ followed by removal of the acetal with 1% HCl.

Similar to the well-studied mode of activation of FR900482



Scheme 2. Synthesis of **7**. a) FmocCl, KI, room temperature, MeCN, 10 h, 30%; b) 1. $AgBF_4$, DMSO, Et_3N, room temperature, 95%; 2. TMSCl, CH_2Cl_2, $HOCH_2CH_2OH$, 95%; c) 1. 50% piperidine, CH_2Cl_2, 96%; 2. *m*-CPBA, CH_2Cl_2, 61%; 3. 1% HCl (aq.), THF, room temperature, 70%; d) Fe^{II}/EDTA, $-2H_2O$; e) DNA. FmocCl = 9-fluorenylmethyl chloroformate, TMS = trimethylsilyl, *m*-CPBA = 3-chloroperoxybenzoic acid, EDTA = ethylenediaminetetraacetic acid.

and congeners, **7** was reductively activated by Fe^{II}/EDTA generated in situ.[8, 16] The HPLC analysis (reverse-phase, C-18 YMC ODS-A, 30% acetonitrile in water at 1.0 mL min^{-1}) of **7** revealed that this substance (10 μL, 10 mM in DMSO, UV: $\lambda_{max} = 212$ nm, retention time 3.8 min) was converted into dehydromonocrotaline (**2**; UV: $\lambda_{max} = 232$ nm, retention time 11.2 min) by the addition of Fe^{II}/EDTA (20 mM, pH 8).

The results of DNA-cross-linking studies with **7** are presented in Figure 1. The DNA interstrand cross-linking ability of **7** was investigated using linear plasmid DNA by denaturing alkaline agarose gel electrophoresis according to

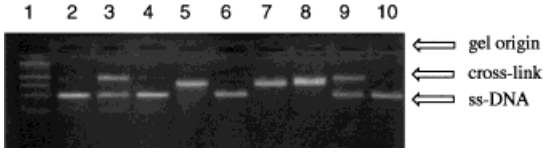


Figure 1. DNA-cross-linking studies of **7** using linear plasmid DNA by denaturing alkaline agarose gel electrophoresis. Conditions: lane 1: 0.5 μg of Lambda Hind III (molecular weight standard); lane 2: 0.5 μg of pBR322 (control); lane 3: 0.5 μg of pBR322 + 10 μM **2** (control); lane 4: 0.5 μg of pBR322 + 1.0 mM FR900482 (control); lane 5: 0.5 μg of pBR322 + 1.0 mM FR900482 + 100 μM Fe^{II}/EDTA; lane 6: 0.5 μg of pBR322 + 1.0 mM **7** (control); lane 7: 0.5 μg of pBR322 + 1.0 mM **7** + 100 μM Fe^{II}/EDTA; lane 8: 0.5 μg of pBR322 + 0.5 mM **7** + 100 μM Fe^{II}/EDTA; lane 9: 0.5 μg of pBR322 + 100 μM **7** + 100 μM Fe^{II}/EDTA; lane 10: 0.5 μg of pBR322 + 10 μM **7** + 100 μM Fe^{II}/EDTA. ss-DNA = single-strand DNA.

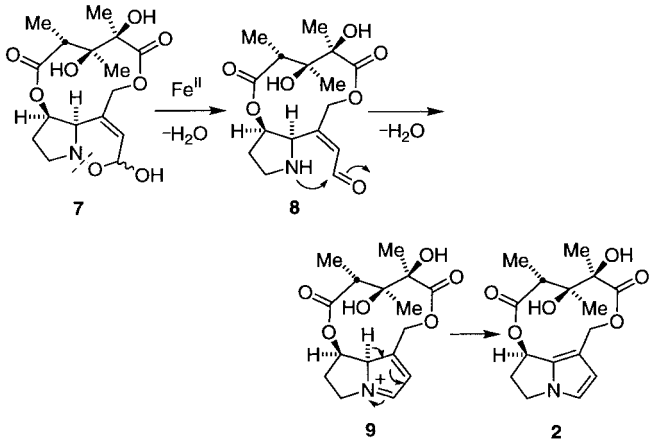
Cech.[17] The duplex DNA substrate employed in this study was pBR322 plasmid DNA, which was linearized by restriction endonuclease digestion with EcoR1. The amount of linearized pBR322 was quantitated by UV analysis at 260 nm, as described by Borer.[18] Compound **7** (solutions of various concentrations were prepared from a 10 mM stock solution made from 4.2 mg of **7** dissolved in 1.2 mL of DMSO) and 0.5 μg of DNA (EcoR1 linearized pBR322) were incubated at 37 °C for 12 h with the reagents described in the legend to

  1433-7851/99/3823-3502 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **1999**, *38*, No. 23

Figure 1 (final volume 10 μL). The Fe^{II}/EDTA was prepared by addition of an equimolar amount of $FeSO_4$ to a solution of EDTA (pH 8). The crude reaction mixture was loaded onto a denaturing 1.2% alkaline agarose gel,[17] and electrophoresis was conducted at 50 V for 3 h (95 mA, 3 W). The gel was stained by the addition of 200 μL of ethidium bromide solution (10 mg mL^{-1}, 100 mM tris(hydroxymethyl)aminomethane (Tris) at pH 7.5, 15 min) and visualized after destaining (50 mM NH_4OAc + 10 mM 2-sulfanylethanol, 1 h) on a UV transilluminator. Lambda Hind III was employed as a molecular weight standard (lane 1).

Control reactions were performed with **2** (10 μM, lane 3) and the known reductively activated antitumor antibiotic FR900482 (1.0 mM, lane 4; and additionally with Fe^{II}/EDTA activation, lane 5).[8, 16] The data in Figure 1 reveal that 100 μM Fe^{II}/EDTA strongly activates compound **7** to form the interstrand-cross-linked DNA at drug concentrations of 100 μM or more (lanes 7–9).

The mechanism of activation of **7** therefore most likely follows the known mechanism of activation of the FR900482 family of compounds: two-electron reductive cleavage of the N–O bond of **7** to form the free amine (**8**, Scheme 3), which cyclizes on the incipient aldehyde moiety and subsequently deprotonates to the highly reactive pyrrolic intermediate **2**.[19, 20]



Scheme 3. Mechanism of reductive formation of **2** from **7**.

These preliminary studies suggest the viability of the hydroxylamine hemiacetal moiety as a potential useful functional group for applications to the reductive activation of DNA-reactive alkaloid progenitors. Since it has been demonstrated that the only other known hydroxylamine hemiketal containing antitumor antibiotic, FR900482 and congeners, can be reductively activated by Fe^{II} salts to cross-link DNA in vitro, and because this reductive activation mechanism has also been implicated for the in vivo activation of these agents,[8, 19, 20] the present system lends support to the notion that the reductively labile hydroxylamine hemiacetal may find other uses in prodrug activation strategies. In addition, these agents may also provide a conceptual framework upon which the design and synthesis of a wide variety of pyrrolic PA prodrugs with potential clinical applications may be examined.

Received: May 3, 1999 [Z13363IE]
German version: *Angew. Chem.* **1999**, *111*, 3711–3713

Keywords: alkaloids · DNA structures · drug research · monocrotaline

[1] A. R. Mattocks, *Chemistry and Toxicology of Pyrrolizidine Alkaloids*, Academic Press, London, **1986**.
[2] L. W. Smith, C. C. J. Culvenor, *J. Nat. Prod*. **1981**, *44*, 129.
[3] "Human Health Implications of Pyrrolizidine Alkaloids and Herbs Containing Them": R. J. Huxtable in *Toxicants of Plant Origin, Vol I: Alkaloids* (Ed.: P. R. Cheeke), CRC Press, Boca Raton, FL, **1989**, pp. 41–86.
[4] a) C. L. Miranda, W. Chung, R. E. Reed, X. Zhao, M. C. Henderson, J.-L. Wang, D. E. Williams, D. R. Buhler, *Biochem. Biophys. Res. Commun.* **1991**, *178*, 546; b) D. E. Williams, R. E. Reed, B. Kedzierski, G. A. Dannan, F. P. Guengerich, D. R. Buhler, *Drug Metab. Dispos.* **1989**, *17*, 58.
[5] C. L. Miranda, R. E. Reed, F. P. Guengerich, D. R. Buhler, *Carcinogenesis* **1991**, *12*, 515.
[6] H. Niwa, T. Ogawa, O. Okamoto, K. Yamada, *Tetrahedron Lett*. **1991**, *32*, 927.
[7] For excellent general reviews pertaining to the biological relevance of DNA interstrand cross-linking, see a) K. W. Kohn in *Topics in Structural & Molecular Biology: 3) Molecular Aspects of Anti-Cancer Drug Action* (Eds.: S. Neidle, M. Waring), VCH, Weinheim, **1994**, p. 315; b) P. D. Lawley, *BioEssays* **1995**, *17*, 561; c) D. J. Paustenbach, B. L. Finley, S. Kacew, *Proc. Soc. Exp. Biol. Med.* **1996**, *211*, 211; d) M. Gniazdowski, C. Cera, *Chem. Rev.* **1996**, *96*, 619; e) J. R. Hincks, H. Y. Kim, H. J. Segall, R. J. Molyneaux, F. R. Stermitz, R. A. Coulombe, Jr., *Toxicol. Appl. Pharmacol*. **1991**, *111*, 90.
[8] S. R. Rajski, R. M. Williams, *Chem. Rev.* **1998**, *98*, 2723.
[9] a) M. F. Weidner, S. T. Sigurdsson, P. B. Hopkins, *Biochemistry* **1990**, *29*, 9225; b) J. Woo, S. T. Sigurdsson, P. B. Hopkins, *J. Am. Chem. Soc.* **1993**, *115*, 3407.
[10] a) H.-Y. Kim, F. R. Stermitz, R. A. Coulombe, Jr., *Carcinogenesis* **1995**, *16*, 2691; b) R. A. Coulombe, G. L. Drew, F. R. Stermitz, *Toxicol. Appl. Pharmacol*. **1999**, *154*, 198.
[11] J. M. Brown, A. Koonng, *J. Natl. Cancer Inst*. **1991**, *83*, 178.
[12] "Hypoxia-Selective Cytotoxins": W. A. Denny in *Cancer Chemotherapeutic Agents* (Ed.: W. O. Foye), ACS Press, Washington, D.C., **1995**, chap. 14, pp. 483–500.
[13] a) J. J. Tepe, R. M. Williams, *J. Am. Chem. Soc*. **1999**, *121*, 2951; b) S. B. Rollins, R. M. Williams, *Tetrahedron Lett.* **1997**, *38*, 4033.
[14] Monocrotaline used in this study was purchased from Aldrich Chemical Co.
[15] J. H. Cooley, E. J. Evain, *Synthesis* **1989**, 1. Compound **5** was obtained as a 1:2 mixture with the alternative regioisomer.[13a]
[16] M. M. Paz, P. B. Hopkins, *Tetrahedron Lett.* **1997**, *38*, 343.
[17] T. R. Cech, *Biochemistry* **1981**, *20*, 1431.
[18] P. N. Borer, *Handbook of Biochemistry and Molecular Biology*, CRC Press, Boca Raton, FL, **1975**.
[19] M. M. Paz, P. B. Hopkins, *J. Am. Chem. Soc.* **1997**, *119*, 5999.
[20] a) R. M. Williams, S. R. Rajski, S. B. Rollins, *Chem. Biol.* **1997**, *4*, 127; b) S. R. Rajski, Dissertation, Colorado State University, **1997**.

  1433-7851/99/3823-3503 $ 17.50+.50/0