Communications to the Editor

DNA Strand Scission by Naturally Occurring 5-Alkylresorcinols

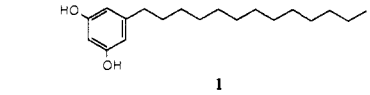
Ralph T. Scannell, John R. Barr, V. S. Murty, K. Sambi Reddy, and Sidney M. Hecht*

Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901

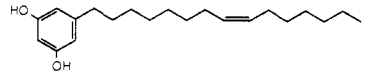
Received December 4, 1987

Recently, considerable effort has been expended on the identification and characterization of molecules that mediate DNA strand scission. Of special interest have been natural products that cleave DNA by novel mechanisms[1] and synthetic derivatives of natural product models that incorporate additional structural elements to facilitate control over some specific aspect of DNA cleavage, e.g., sequence-selective strand scission.[2] Because of their importance in suggesting new mechanistic principles that can be used to mediate DNA cleavage, we have focused on the identification of additional natural products that bind or cleave DNA. Reported herein is a new structural class of DNA cleaving agents that appear to function in a novel fashion.

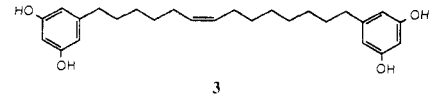
A dichloromethane extract of *Hakea trifurcata* was found to mediate cleavage of ΦX174 DNA in the presence of Cu(II) (Figure 1); bioassay-guided fractionation[3] afforded active constituents **1-3**. Compound **1** had the empirical formula $C_{19}H_{32}O_2$ (*m/z* 292.2418) and a UV spectrum quite similar to that of orcinol.[4] A 5-alkylresorcinol structure was further supported by the IR and ^{1}H NMR spectra, which were strikingly similar to those of orcinol;[5] compound **1** also formed a di-*O*-acetate under



1



2



3

the same conditions (Ac_2O, pyridine, 25 °C, 12 h) employed for known 5-alkylresorcinols.[6] Consistent with the putative 5-alkylresorcinol structure, the ^{13}C NMR spectrum contained resonances (at 100.2, 108.1, 146.2 and 156.4 ppm) corresponding to the aromatic carbons. Because the ^{1}H NMR spectrum of **1** gave no indication of branching within the 5-alkyl substituent, the structure of **1** was assigned as 1,3-dihydroxy-5-tridecylbenzene.[7] The assigned structure was verified by direct comparison of the naturally derived compound with an authentic, synthetic sample.[8]

Compound **2** had the empirical formula $C_{21}H_{34}O_2$ (*m/z* 318.2578). The spectral data for **2** was quite similar to that of **1**, with the exception that the IR contained a strong band at 1620 cm^{-1}, which suggested the presence of an olefin. The ^{13}C NMR spectrum of **2** also contained two additional low field resonances (129.36 and 129.49 ppm) assigned to vinylic carbons; the ^{1}H NMR spectrum contained a two-proton resonance at δ 5.35 (br t, *J* = 7.2 Hz), suggesting the presence of a *cis*-olefin. The position of unsaturation was determined by mass spectrometric analysis of the diacetate.[9] The assigned structure, 1,3-dihydroxy-5-pentadec-*cis*-8′-enylbenzene, was verified by total synthesis.[10]

Compound **3** was found to have M_r 412 by chemical ionization mass spectrometry. UV and ^{1}H NMR spectroscopy indicated that **3** was of the same structural type as **1** and **2**; however, the absence of a (methyl group) resonance at δ 0.92 and formation of a tetra-*O*-acetate (*m/z* 581, chemical ionization mass spectrometry) upon treatment with Ac_2O/pyridine suggested the presence of two

(1) See, e.g.; (a) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740. (b) Chang, C.-H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332. (c) Suzuki, H.; Kirino, Y.; Tanaka, N. *J. Antibiot. (Tokyo)* **1983**, *36*, 583. (d) Aft, R. L.; Mueller, G. C. *J. Biol. Chem.* **1983**, *258*, 12069. (e) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, C. L.; Scahill, T. A. *Science (Washington, D.C.)* **1984**, *266*, 843. (f) Ueda, K.; Morita, J.; Komano, T. *Biochemistry* **1984**, *23*, 1634. (g) Povirk, L. F.; Goldberg, I. H. *Biochemistry* **1984**, *23*, 6304. (h) Eliot, H.; Gianni, L.; Myers, C. *Biochemistry* **1984**, *23*, 928. (i) Kuwahara, J.; Suzuki, T.; Funakoshi, K.; Sugiura, Y. *Biochemistry* **1986**, *25*, 1216. (j) Golid, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462. (k) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466.

(2) (a) Lown, J. W.; Joshua, A. V. *J. Chem. Soc., Chem. Commun.* **1982**, 1298. (b) Schultz, P. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6834. (c) Schultz, P. G.; Dervan, P. B. *J. Am. Chem. Soc.* **1983**, *105*, 7748. (d) Bowler, B. E.; Hollis, L. S.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 6102. (e) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 968. (f) Chu, C. F.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 963. (g) Vlassov, V. V.; Gaidamakov, S. A.; Gorn, V. V.; Grachev, S. A. *FEBS Lett.* **1985**, *182*, 415. (h) Youngquist, R. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 5528. (i) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 8266. (j) Knorre, D. G.; Vlassov, V. V. *Prog. Nucleic Acid Res. Mol. Biol.* **1985**, *32*, 291. (k) Zarytova, V. F.; Kutyavin, I. V.; Sil'nikov, V. N.; Shishkin, G. V. *Bioorg. Khim.* **1986**, *12*, 911. (l) Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1987**, *109*, 1241. (m) Biodot-Forget, M.; Thuong, N. T.; Chassignol, M.; Hélène, C. *C. R. Acad. Sci. Ser 3* **1986**, *302*, 75.

(3) *Hakea trifurcata* (SM.)R.BR. was collected in Western Australia; voucher specimen SPJ-6917 is preserved at the National Herbarium, Washington, D.C. Form I DNA relaxation was assayed as described (Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869).

(4) The UV spectrum of **1** had λ_{max} 280 and 275 nm, and λ_{min} 278 and 246 nm. Orcinol UV spectrum, see: Occolowitz, J. L.; Wright, A. S. *Aust. J. Chem.* **1962**, *15*, 858.

(5) Compound **1**: partial ^{1}H NMR ($CDCl_3$) δ 6.10 (t, 1, *J* = 1.4 Hz) and 6.15 (d, 2, *J* = 1.5 Hz); IR (KBr) ν 3329, 2955, 2914, 2852, 1605, 1512, 1472, 1148, 1001, 987, 943 and 834 cm^{-1}. Orcinol: partial ^{1}H NMR ($CDCl_3$) δ 6.16 (t, 1, *J* = 2.0 Hz) and 6.23 (d, 2, *J* = 2.0 Hz); IR (KBr) 3327, 2953, 2919, 2856, 1632, 1603, 1514, 1475, 1334, 1307, 1147, 1032, 973, 845 and 824 cm^{-1}.

(6) Cannon, J. R.; Chow, P. W.; Fuller, M. W.; Hamilton, B. H.; Metcalf, B. W.; Power, A. J. *Aust. J. Chem.* **1973**, *26*, 2257.

(7) This compound has been identified previously. See: (a) Ritchie, E.; Taylor, W. C.; Vautin, S. T. K. *Aust. J. Chem.* **1965**, *18*, 2015. (b) Shoji, N.; Umeyama, A.; Takemoto, T.; Kobayashi, M.; Ohizumi, Y. *J. Nat. Prod.* **1984**, *47*, 530. (c) Cirigottis, K. A.; Cleaver, L.; Corrie, J. E. T.; Grasby, R. G.; Green, G. H.; Mock, J.; Nimgirawath, S.; Read, R. W.; Ritchie, E.; Taylor, W. C.; Vadasz, A.; Webb, W. R. G. *Aust. J. Chem.* **1974**, *27*, 345.

(8) Condensation of 3,5-dimethoxybenzaldehyde with dodecyl magnesium bromide (Et_2O, 4 h, reflux), followed by dehydration (*p*-$CH_3C_6H_4SO_3H$, C_6H_6), provided 1,3-dimethoxy-5-(tridec-1-enyl)benzene as colorless plates in 68% overall yield, mp 26-28 °C. Successive hydrogenation (10% Pd/C, ethyl acetate, 25 °C, 12 h) and demethylation (BBr_3, CH_2Cl_2, 0 °C → 25 °C, 12 h) gave 5-tridecylresorcinol as colorless microcrystals (from hexane) in 13% yield, mp 78-80 °C. The synthetic and naturally derived materials had identical chromatographic (TLC, HPLC) and spectral (IR, NMR, UV) properties.

(9) Following ozonolysis (O_3, CS_2, -78 °C), the derived aldehydes were analyzed by chemical ionization mass spectrometry (methane, positive ion, direct exposure probe): Barr, J. R.; Scannell, R. T.; Yamaguchi, K.; Hecht, S. M., in preparation.

(10) This compound was synthesized in a fashion similar to that of **1**.[8] Introduction of the double bond was effected by treatment of 5-(7-bromoheptyl)-1,3-dimethoxybenzene with the lithium salt of octyne, followed by hydrogenation of the derived alkyne over 5% Pd/$BaSO_4$ in the presence of quinoline. 1,3-Dihydroxy-5-pentadec-*cis*-8′-enylbenzene has been isolated previously:[7] (a) Morimoto, H.; Kawamatsu, Y.; Sugihara, H. *Chem. Pharm. Bull.* **1968**, *16*, 2282. (b) Tyman, J. H. P.; Tychopoulos, V.; Colenutt, B. A. *J. Chromatogr.* **1981**, *213*, 287.

0002-7863/88/1510-3650$01.50/0

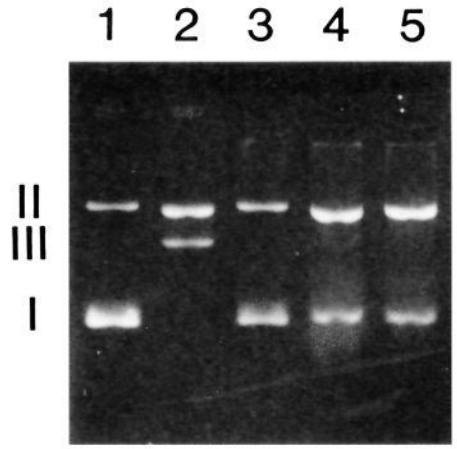


Figure 1. Strand scission of ΦX174 replicative form DNA. DNA samples (180 ng) in 40 μL of H_2O containing 3% dimethoxyethane were treated with 10 μM Fe(II) + 0.1% H_2O_2 (lane 2), 10 μM Cu(II) (lane 3), 20 μg of a CH_2Cl_2 extract of *Hakea trifurcata* + 10 μM Cu(II) (lane 4), 10 μg of extract + 10 μM Cu(II) (lane 5). Lane 1 contained untreated DNA. The reaction mixtures were incubated for 30 min at 25 °C and then analyzed by agarose gel electrophoresis. Form I DNA is supercoiled covalently closed circular DNA; form II DNA is relaxed (nicked) circular DNA; form III DNA is linear duplex DNA.

dihydroxybenzene groups linked through an unbranched alkene. As for **2**, a *cis*-olefin was indicated by ^{1}H NMR (δ 5.33, br t, J = 4.7 Hz); its location was determined by mass spectral analysis of a sample of **3** that had been acetylated and subjected to ozonolysis.[9] On this basis, compound **3** was assigned the structure 1,3-dihydroxy-5-(14′-(3″,5″-dihydroxyphenyl)tetradec-*cis*-6′-enyl)benzene.[11]

DNA cleavage by these 5-alk(en)ylresorcinols was found to be remarkable in a few different ways. First, the compounds exhibited Cu(II)-dependent DNA strand scission in spite of the absence of useful metal ion ligands. Second, although high concentrations of these compounds bound plasmid DNA sufficiently to alter its mobility on agarose gels (Figure 1), none has any functionality capable of mediating association with DNA by a well characterized mechanism (e.g., intercalation, groove binding, or electrostatic interaction[12]).

As regards DNA cleavage, preliminary mechanistic investigations provide some insight. During purification of the 5-alkylresorcinols, it was noted that after some fractionation steps the newly isolated material actually exhibited a *diminished* ability to mediate DNA strand scission. Interestingly, this activity increased upon storage of the sample or by incubation in aerated aqueous solution, especially where the solution was alkaline. It seems possible that DNA cleavage by such compounds may involve initial oxygenation of the benzene nucleus at C-4.[13] 1,3,4-Trihydroxybenzene derivatives produced in this fashion could then chelate Cu(II), providing a complex capable of initiating DNA degradation in the presence of O_2.[14,15] Of possible pertinence to the issue of DNA binding is the observation that DNA cleavage efficiency by 5-alkylresorcinol derivatives was found to be directly proportional to the length of the alkyl substituent. It seems conceivable that upon dissolution in an aqueous solution containing a DNA duplex, the lipophilic moiety of the 5-alkylresorcinols seeks to associate with the least polar component of the duplex, i.e., with the interior.[17]

Acknowledgment. We thank Dr. Jeffrey Ellena for assistance with the 2D NMR measurements. This work was supported by P.H.S. Grant CA 40291, awarded by the National Cancer Institute, D.H.H.S.

(11) This compound has been isolated previously.[7c]

(12) (a) Baguley, B. C. *Mol. Cell. Biochem.* **1982**, *43*, 167. (b) Feigen, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450. (c) Dougherty, G. *Int. J. Biochem.* **1984**, *16*, 1179. (d) Neidle, S.; Abraham, Z. *CRC Crit. Rev. Biochem.* **1984**, *17*, 73.

(13) Certain of these transformations are known to be Cu(II)-catalyzed. See, e.g.: (a) Brackman, W.; Havinga, E. *Recl. Trav. Chim. Pays-Bas.* **1955**, *74*, 937, 1021, 1070, 1100, 1107. (b) Musso, H. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 723.

(14) Although presently unproven experimentally, reductive activation of O_2 by the binary complex could lead to DNA strand scission.[16] Consistent with this scheme, DNA cleavage by 5-alkylresorcinols has been shown to be O_2 dependent (Singh, S., unpublished results).

(15) In fact synthetic 5-alkyl-1,3,4-trihydroxybenzene derivatives were found to cleave DNA at ~100-fold lower concentration than the respective 5-alkylresorcinols.

(16) (a) Wong, A.; Huang, C.-H.; Crooke, S. T. *Biochemistry* **1984**, *23*, 2939. (b) Wong, A.; Huang, C.-H.; Crooke, S. T. *Biochemistry* **1984**, *23*, 2946. (c) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180.

(17) Consistent with this suggestion, we have found that oligonucleotide derivatives containing lipophilic substituents bound with substantially increased affinity to their single-stranded complementary oligonucleotides (Jager, A.; Levy, M. J.; Hecht, S. M., in preparation).

Biosynthesis of Riboflavin. The Structure of the Four-Carbon Precursor

Rainer Volk and Adelbert Bacher*

Lehrstuhl für Organische Chemie und Biochemie
Technische Universität München, Lichtenbergstr. 4
D-8046 Garching, Federal Republic of Germany

Received January 4, 1988

The initial steps in the biosynthesis of riboflavin lead from GTP to the pyrimidine **3** which reacts with a four-carbon moiety under formation of 6,7-dimethyl-8-ribityllumazine (**4**).[1] In vivo studies have shown that the four-carbon moiety originates from the pentose pool by the loss of C-4.[2] In vitro studies became feasible on the basis of the seminal observation by Shavlovsky and his co-workers that cell extracts of the yeast *Candida guilliermondii* catalyze the formation of **4** from **3** in the presence of ribose phosphate.[3] We could show that an intermediary carbohydrate phosphate designated as compound X can be formed from pentose phosphate by the yeast cell extract.[4]

We have purified the enzyme catalyzing the formation of compound X about 1000-fold from the cell extract of *C. guilliermondii*. Whereas crude cell extracts can use several pentose and pentulose phosphates as substrates for the production of compound X, the purified enzyme is limited to ribulose 5-phosphate (**1**) as substrate.

In light of the limited stability of compound X, it was advantageous to study its formation by NMR spectroscopy in the enzyme assay mixture without purification. Five ^{13}C NMR signals designated as a–e were observed after treatment of **1** with the purified enzyme from *C. guilliermondii* (Figure 1, Table I). The proton multiplicities of these signals were determined by DEPT spectroscopy. The ^{13}C NMR signal b at 173 ppm showed evidence for the presence of one proton and was clearly identified as formate by internal standardization with authentic formate. The production of formate by the enzyme-catalyzed reaction was also confirmed by ^{1}H NMR spectroscopy.

Treatment of the reaction mixture with alkaline phosphatase affects predominantly the chemical shifts of signals c and d and is accompanied by the loss of the fine structure of these signals. The dephosphorylated compound was identified as 3,4-dihydroxy-2-butanone on the basis of ^{1}H and ^{13}C NMR spectroscopic comparison with an authentic sample.[5] In experiments

(1) Neuberger, G.; Bacher, A. *Biochem. Biophys. Res. Commun.* **1986**, *139*, 1111.

(2) Bacher, A.; Le Van, Q.; Keller, P. J.; Floss, H. G. *J. Am. Chem. Soc.* **1985**, *107*, 6380.

(3) Logvinenko, E. M.; Shavlovsky, G. M.; Zakalsky, A. E.; Zakhodylo, I. V. *Biokhimiya* **1982**, *47*, 931.

(4) Neuberger, G.; Bacher, A. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 175.

(5) Fischer, H. O. L.; Baer, E.; Pollock, H.; Nidecker, H. *Helv. Chim. Acta* **1937**, *20*, 1213.

0002-7863/88/1510-3651$01.50/0