DNA Cleavage by 4-Alkynyl-3-methoxy-4-hydroxycyclobutenones

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Summary: 4-Alkynyl-3-methoxy-4-hydroxycyclobutenones, a readily available class of compounds, cleave supercoiled DNA by a mechanism that appears to require at least some involvement of diradical intermediates formed in the ring expansion of the cyclobutenones.

The naturally occurring and synthetic compounds recently reported to cleave DNA by mechanisms involving diradical and/or electrophilic intermediates have stimulated a great deal of interest.[1,2] Reported herein is the design and synthesis of a variety of 4-alkynyl-3-methoxy-4-hydroxycyclobutenones (**3**) and results of DNA cleavage studies.[3] *The data presented favor a mechanism in which DNA damage is induced, at least in part, by the diradicals* **5** *which arise from the cyclobutenones* **3** *via the enynyl*

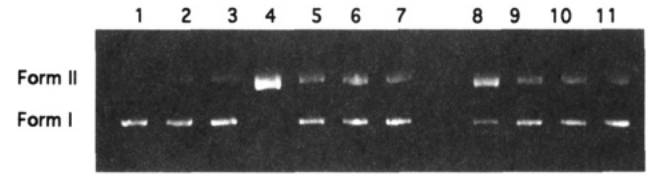

Figure 1. DNA Cleavage by **3a** and **3d** in the presence of the antioxidant 2-*tert*-butyl-4-hydroxyanisole (BHA). ΦX174 form I DNA (57 μM base pair) incubated with **3a** (4.0 mM) and **3d** (2.3 mM) with and without BHA (5, 10, 20 mM) for 19.5 h at 49 °C in a TE buffer (10 mM Tris·HCl, 1.0 mM EDTA, pH, 7.4): lane 1, DNA alone and not incubated; lane 2, DNA incubated; lane 3, DNA and BHA (20 mM); lane 4, DNA and **3d**; lanes 5–7 DNA, **3d** and BHA (5, 10, 20 mM, respectively); lane 8, DNA and **3a**; lanes 9–11 DNA, **3a** and BHA (5, 10, 20 mM, respectively).

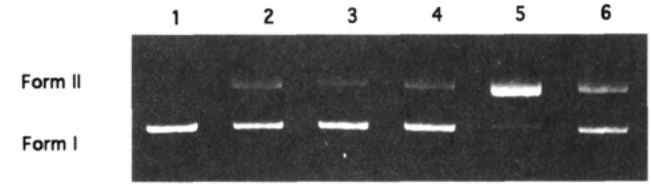

Figure 2. DNA cleavage by the cyclobutenones **3a** and **7** at various temperatures. ΦX174 form I DNA (57 μM base pair) incubated with **3a** (3.9 mM) and **7** (3.9 mM) for 19.5 h at various temperatures in a TE buffer (10 mM Tris·HCl, 1.0 mM EDTA, pH, 7.4): lane 1, DNA at 37 °C; lane 2, DNA and **3a** at 37 °C lane 3, DNA and **7** at 37 °C; lane 4, DNA at 49 °C; lane 5, DNA and **3a** at 49 °C; lane 6, DNA and **7** at 49 °C.

ketene **4**. These proposed intermediates are in agreement with the observation that in the absence of DNA and under aprotic conditions such cyclobutenones readily ring expand to the corresponding quinones; e.g., **3d** rearranges to the **6d** in 71% yield via the sequence **3d** → **4d** → **5d** → **6d** (Scheme 1).[4,5]

The 4-alkynyl-3-methoxy-4-hydroxycyclobutenones **3a–e** (Table 1) were studied as potential DNA damaging agents using covalently closed, supercoiled, double-stranded DNA [phage φX174 (5.4 kb)].[6] Various concentrations of **3** were incubated with the DNA in 10 mM Tris buffer (pH 7.4) with 1.0 mM EDTA.[7] The DNA was then analyzed by gel electrophoresis on 1% Agarose gels. Evidence for single strand breaks was the observation of the conversion of form I to form II DNA. A concentration study using **3d** revealed 12% DNA cleavage at a concentration of 22 μM and 79% at 2.2 mM [densitometry measurements (nicked sample–nicked control)].

Induced DNA damage was particularly apparent for those 4-alkynyl-4-hydroxycyclobutenones showing en-

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(1) For key references to natural products that nick DNA via a diradical mechanism see the following. Esperamicin: (a) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461. (b) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, J.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462. Calicheamicin: (c) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464. (d) 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. (e) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198. (f) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 692. (g) Zein, N.; McGahren, W. J.; Morton, G. O.; Ashcroft, J.; Ellestad, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 6888. Neocarcinostatin: (h) Kappen, L. S.; Goldberg, I. H. *Biochemistry* **1983**, *22*, 4872. (i) Kappen, L. S.; Ellenberger, T. E.; Goldberg, I. H. *Biochemistry* **1987**, *26*, 384. (j) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331. (k) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493. (l) Goldberg, I. H. *Free Radicals Biol. Med.* **1987**, *3*, 41. Dynemicin: (m) Konishi, M.; Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Oki, T.; Kawaguchi, H.; Clardy, J. Poster presented at the 29th Intersci. Conf. on Antimicrobial Agents and Chemotherapy, Houston, TX, Sept. 1989. (n) Konishi, M.; Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Oki, T.; Kawaguchi, H.; Van Duyne, G.; Clardy, J. *J. Antibiot.* **1989**, *92*, 1449. (o) Semmelhack, M. F.; Gallagher, J.; Cohen, D. *Tetrahedron Lett.* **1990**, *31*, 1521.

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(3) The cycbutenones were prepared by standard methods. For examples see: (a) Xu, S.; Yerxa, B. R.; Sullivan, R. W.; Moore, H. W. *Tetrahedron Lett.* **1991**, *32*, 1129. (b) Reed, M. W.; Pollart, D. J.; Perri, S. T.; Foland, L. D.; Moore, H. W. *J. Org. Chem.* **1988**, *53*, 2477. (c) Liebeskind, L. S.; Fengl, R. W.; Wirtz, K. R.; Shawe, T. T. *J. Org. Chem.* **1988**, *53*, 2482.

(4) Foland, L. D.; Karlsson, J. O.; Perri, S. T.; Schwabe, R.; Xu, S. L.; Patil, S.; Moore, H. W. *J. Am. Chem. Soc.* **1989**, *111*, 975.

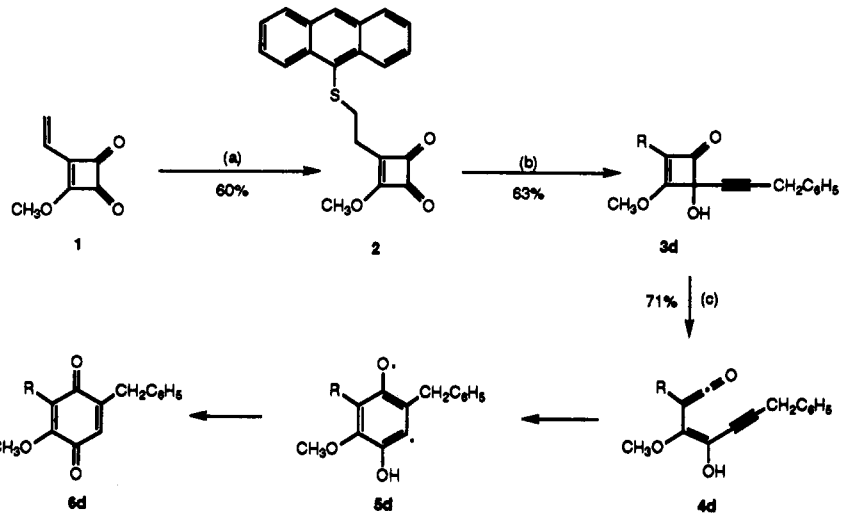
(5) Moore, H. W.; Yerxa, B. *Chemtracts* **1992**, *5*, 273.

(6) Cleavage was also observed using pUC19 (2.8 Kbp) and pHFdx1 (3.2 Kbp) DNA. These are, respectively, commercially available and supplied by Professor Larry W. Vickery (University of California, Irvine). See: Coghlan, V. M.; Vickery, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 835.

(7) Similar results were obtained using sodium hydrogen phosphate as the buffer.

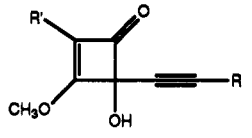
0022-3263/94/1959-2276$04.50/0

Scheme 1[a]



[a] **Reagents**: (a) 9-thioanthracene, THF, 25 °C catalytic amount of triethylamine; (b) $LiCCCH_2C_6H_5$, THF (−78 °C); (c) acetonitrile, 82 °C.

Table 1. Cyclobutenone–DNA Studies[a]



3

	R	R′	$t_{1/2}$, min	DNA Damage
a	$CH_2C_6H_5$	n-C_4H_9	20	++
b	$CH_2C_6H_5$	CH_3O	1270	+
c	C_6H_5	CH_3O		+
d	$CH_2C_6H_5$	CH_2CH_2-S-9-Anth	10	+++
e	$CH_2C_6H_4$-p-$NH(CH_3)_2$	CH_2CH_2-S-2-Py		++

[a] Illustrative examples from a list of more than 20 4-alkynyl-4-hydroxycyclobutenones that were observed to cleave supercoiled DNA (ϕX174).

hanced rates of rearrangements, i.e., those bearing alkyl groups at position 2 as opposed to the corresponding 2-alkoxy analogs.[4] In this regard, the 2-butyl- (**3a**) and 2-[2-(9-anthracenylthio)ethyl]- (**3d**) derivatives effectively damaged DNA at 49 °C. The cyclobutenone **3a** was shown to rearrange to the respective quinone **6a** (66%, acetonitrile, 82°C) and to follow (^{1}H NMR) clean first-order kinetics at three different temperatures over a 20 °C range (55–75 °C, $t_{1/2}$ = 20 min at 75 °C).[8] In comparison, the $t_{1/2}$ for ring expansion of **3b,d** to the corresponding quinones **6b,d** (65–71%) are, respectively, 1270 min (CH_3CN, 82 °C) and 10 min (CH_3CN, 75 °C). The most effective agent is **3d** (54% cleavage) followed by **3a** (40%) and finally by **3b** (21%). That **3d** is the most potent is presumably due to both its facile rearrangement and to the fact that it can intercalate into the DNA polymer, thus increasing the effective concentration of the damaging agent.[9] That **3b** is the least effective is consistent with its slower rate of rearrangement.

In control experiments, under conditions that maximized cleavage by **3a**, little DNA damage was observed for either the quinone **6a** (R = n-C_4H_9) or a reaction mixture arising from a sample of **3a** that had previously been subjected to the cleavage conditions in the absence of DNA. Thus, the agent responsible for the damage is reasonably assumed to be the starting cyclobutenone **3** or a reactive intermediate, e.g., **4** or **5**. Additional data given below favor the diradical **5**.

DNA damage was significantly reduced when the cyclobutenones **3a** and **3d** were incubated with DNA for 19.5 h at 49 °C in the presence of the antioxidant (free-radical scavenger) 2-*tert*-butyl-4-hydroxyanisole (BHA). The data in Figure 1 reveal 27% and 43% reduction in damage by **3a** and **3d**, respectively. The protection of DNA by the antioxidant is consistent with at least some radical contribution to the mechanism of cleavage. In addition, the data illustrate (compare lanes 4 and 8), as noted above, the cyclobutenone bearing an intercalating moiety **3d** to be more effective than **3a** in causing damage.

An additional significant observation is that the cyclobutenone **7** is much less effective in its ability to damage DNA as compared to its 4-hydroxy analog **3a** (14% vs 48% cleavage, respectively) (Figure 2). This is in spite of the fact that **7** rearranges to 4-spiroepoxy-2,5-cyclohexadienone **9** in refluxing toluene *via* an apparent diradical intermediate **8** and the rate of rearrangement is similar to that observed for the 4-hydroxy derivative, i.e., $t_{1/2}$(**7**) = 40 min at 75 °C compared to $t_{1/2}$(**3a**) = 20 min at 75 °C.[4,10]

The following paradigm provides a possible explanation for this difference in DNA damaging abilities between **3a** and **7**; *damage is expected to be less for those compounds that rearrange by a facile intramolecular pathway as opposed to those preceding to products by an intermolecular pathway.* In this regard, the rearrangements of 4-hydroxy-4-alkynylcyclobutenones such as **3a** were previously shown to involve an intermolecular H-atom abstraction from the corresponding diradical intermediate.[11] In contrast, the following data establish an intramolecular pathway for the rearrangement of the 4-alkoxy analogs to the spiroepoxide, e.g., **7** → **9**. Specifically, an equimolar mixture of 4-ethoxy-2,3-dimethoxy-4-(phenylethynyl)cyclobutenone (**12**) and its 4-perdeuterioethoxy analog **14** was subjected to thermolysis in refluxing *p*-xylene (Scheme 2). The spiroepoxides **13** and **15** were

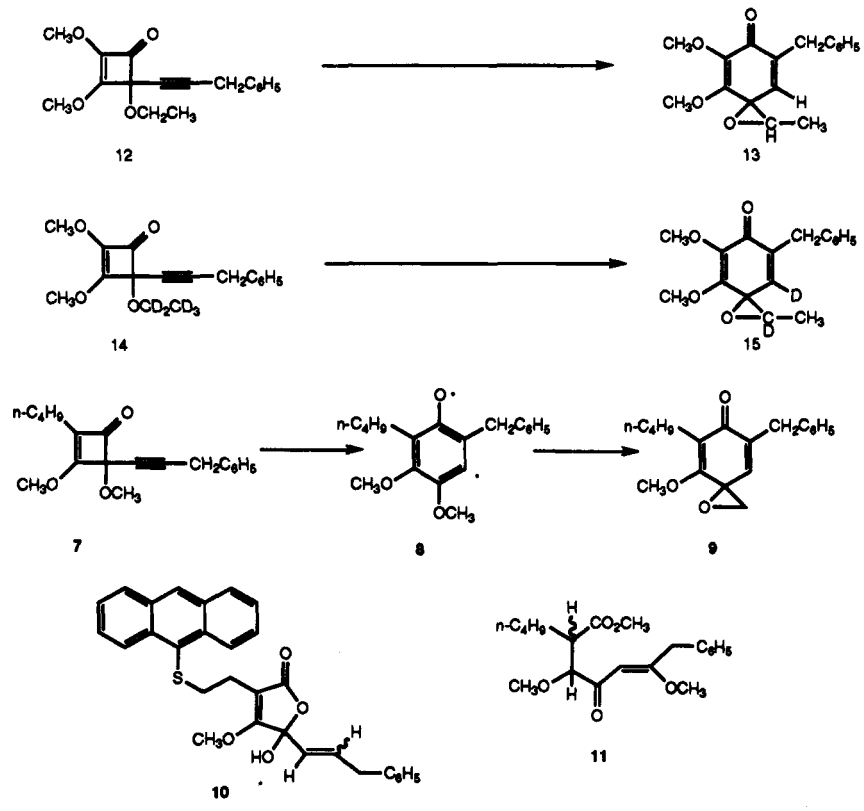
(8) ΔH = 24.1 kcal/mol, ΔS = −4.5 eu, E_a = 24.8 kcal/mol, log A = 12.3, $t_{1/2}$ = 20 min at 75 °C.

(9) Intercalation of **3d** was further probed by incubating it with the DNA in the presence of excess ethidium bromide, an effective competitive intercalation agent. This significantly reduced the amount of DNA damage (35%).

(10) Gayo, L.; Sullivan, R. W.; Moore, H. W. Unpublished data.

(11) Xia, H.; Moore, H. W. *J. Org. Chem.* **1992**, *57*, 3765.

Scheme 2



formed in good yield (72%), and no cross-over products were detected by NMR and high-resolution mass spectrometric analysis.

In spite of the above results, the mechanism of DNA damage warrants further study, due in part, to the fact that product formation is complex under the aqueous conditions employed in the DNA studies. For example, incubation of **3d** in DMF/Tris buffer (1:0.7) gave a complex mixture from which the major product (10% yield) was a mixture of the ketene-derived products, *cis* and *trans* isomers of 3-[2-(9-anthracenylthio)ethyl]-4-methoxy-5-hydroxy-5-(2-phenylethenyl)-5*H*-furanone (**10**). In addition, a trace (1%) of the quinone **6d**, a diradical product, was also isolated. In a related experiment, a 12.6 millimolar methanolic solution of **3a** gave a mixture of the quinone **6a** (R = n-C_4H_9) and the diasteriomeric esters **11** (1:2.6) in a 1:11.5 ratio, respectively, in 76% yield after refluxing for 1.5 h.[12] Thus, products stemming from both intermediates **4** and **5** were realized when the thermolyses were carried out under protic conditions.

(12) The structures of all new compounds were assigned with analytical and spectral data.

(13) Conway, W.; Tarbell, D. S. *J. Am. Chem. Soc.* **1956**, *78*, 2228.

The preparation of **3d** is presented as an illustrative example of the synthesis of all of the 4-alkynyl-4-hydroxycyclobutenones used in this study (Scheme 1). Treatment of 3-ethenyl-4-methoxycyclobutenedione[3a] (**1**) with 9-thioanthracene[13] gave 2-[2-(9-anthracenylthio)-ethyl]-3-methoxycyclobutenedione (**2**) (60%) which led to **3d** (63%) upon reaction with 1-lithio-3-phenylpropyne.

In conclusion, the significant points of this study are the following: (1) 4-alkynyl-4-hydroxycyclobutenones nick supercoiled DNA by a mechanism that involves at least some contribution from diradical intermediates, and (2) since the synthesis of the 4-alkynylcyclobutenones is efficient and general, examples bearing a variety of DNA probes and triggers are potentially available for further studies.

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