# Topoisomerase II-Mediated DNA Cleavage by Adocia- and Xestoquinones from the Philippine Sponge *Xestospongia* sp.

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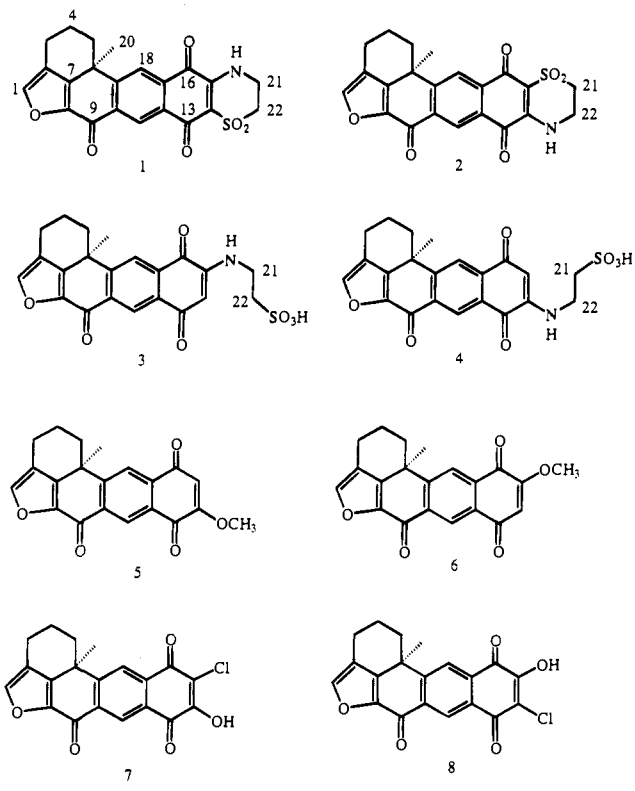
Investigation of an orange *Xestospongia* sp. sponge collected at Cape Bolinao in northern Luzon, Philippines, yielded the known compounds adociaquinones A and B (**1**, **2**) and six new metabolites, secoadociaquinones A and B (**3**, **4**), 14-methoxyxestoquinone (**5**), 15-methoxyxestoquinone (**6**), 15-chloro-14-hydroxyxestoquinone (**7**), and 14-chloro-15-hydroxyxestoquinone (**8**). All compounds showed inhibition of topoisomerase II in catalytic DNA unwinding and/or decatenation assays. Furthermore, adociaquinone B showed activity in a KSDS assay, suggesting it inhibits the enzyme by freezing the enzyme–DNA cleavable complex. Interestingly, adociaquinone B did not displace ethidium bromide from DNA or unwind supercoiled DNA, implying it does not intercalate DNA.

## Introduction

Marine invertebrates are a bountiful source of secondary metabolites with unprecedented molecular structures and biological activities.[1] Collaborative research involving chemists and biologists has yielded many new and exotic natural products that have proven to be useful tools for probing important chemical and pharmacological questions. Previous work from our laboratories has focused on the isolation of novel metabolites from sponges and ascidians, which exhibit cytotoxicity resulting from DNA interactions and/or inhibition of the catalytic activity of the topoisomerase enzymes.[2] The topoisomerases modulate the topological structure of DNA in both eukaryotic and prokaryotic cells and are essential for a variety of cellular processes including replication and transcription.[3,4] To date, two mammalian topoisomerases have been characterized; topoisomerase I and topoisomerase II (α and β).[5] Topoisomerase I utilizes DNA single strand breaks for its passage mechanism while topoisomerase II alters DNA topology by virtue of a DNA double strand break. Elevated levels of topoisomerase II have been detected in tumor cells and coincide with high proliferation,[6] and in retrospect, topoisomerase II has emerged as the molecular target for a number of anticancer agents in clinical use today.[7] We recently reported the characterization of the makaluvamines, a new class of pyrroloiminoquinone alkaloids that inhibit topoisomerase II *in vitro* and show *in vivo* antitumor activity in animals.[8] In our continuing effort to identify new antineoplastic agents, we investigated an orange *Xestospongia* sp. sponge collected at Cape Bolinao in northern Luzon, Philippines. Fractionation of the MeOH extract of the sponge yielded the known compounds adociaquinones A and B (**1**, **2**)[9] and six new metabolites, secoadociaquinones A and B (**3**, **4**), 14-methoxyxestoquinone (**5**), 15-methoxyxestoquinone (**6**), 15-chloro-14-hydroxyxestoquinone (**7**), and 14-chloro-15-hydroxyxestoquinone (**8**). All of these compounds showed inhibitory activity in topoisomerase catalytic enzyme assays,[10] and subsequent biochemical studies confirmed that adociaquinone B inhibits the enzyme by freezing the enzyme–DNA cleavable complex.




## Chemistry

The MeOH extract of the marine sponge *Xestospongia* sp. was subjected to solvent partitioning. The $CHCl_3$ soluble material showed cytotoxicity toward the human colon tumor cell line HCT 116 and enhanced cytotoxicity toward the CHO cell line xrs-6. We have previously reported that the xrs-6 cell line is hypersensitive to agents that produce DNA double strand breaks.[11] The $CHCl_3$ layer was evaporated to dryness and triturated with $CHCl_3$, leaving a yellow precipitate. The yellow precipitate was subjected to Si-gel chromatography

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**Table 1.** $^1$H-NMR 00 MHz) Chemical Shifts for Compounds 1–8[a]

| | compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1**[b] | **2**[b] | **3**[b] | **4**[b] | **5**[c] | **6**[c] | **7**[b] | **8**[b] |
| position | $^1$H δ (mult, *J*) | $^1$H δ (mult, *J*) | $^1$H δ (mult, *J*) | $^1$H δ (mult, *J*) | $^1$H δ (mult. J) | $^1$H δ (mult, *J*) | $^1$H δ (mult, *J*) | $^1$H δ (mult, *J*) |
| 1 | 7.97 (s) | 7.99 (s) | 7.96 (s) | 7.96 (s) | 7.51 (s) | 7.51 (s) | 7.93 (s) | 7.94 (s) |
| 3 | 2.82 (dd, 17.0, 8.5) | 2.83 (dd, 16.5, 7.5) | 2.81 (dd, 17.0, 8.5) | 2.81 (dd, 17,0, 8.5) | 2.85 (dd, 17.0, 8.0) | 2.85 (dd, 17.0, 8.0) | 2.81 (dd, 17.0, 8.0) | 2.81 (dd, 17.0, 8.0) |
| | 2.56 (dd, 17.0, 9.0) | 2.58 (dd, 16.5, 8.5) | 2.57 (m) | 2.57 (m) | 2.62 (dd, 17.0, 9.0) | 2.62 (dd, 17.0, 9.0) | 2.58 (m) | 2.58 (m) |
| 4 | 2.21 (m) | 2.20 (m) | 2.20 (m) | 2.20 (m) | 2.25 (m) | 2.25 (m) | 2.20 (m) | 2.20 (m) |
| | 2.06 (m) | 2.05 (m) | 2.05 (m) | 2.05 (m) | 2.16 (m) | 2.16 (m) | 2.05 (m) | 2.05 (m) |
| 5 | 2.60 (m) | 2.64 (m) | 2.57 (m) | 2.57 (m) | 2.56 (m) | 2.56 (m) | 2.58 (m) | 2.58 (m) |
| | 1.63 (dt, 13.0, 4.5) | 1.62 (dt, 13.0, 4.0) | 1.60 (dt, 13.0, 4.5) | 1.60 (dt, 13.0, 4.5) | 1.73 (dt, 13.0, 4.5) | 1.73 (dt, 13.0, 4.5) | 1.60 (dt, 13.5, 4.5) | 1.60 (dt, 13.5, 4.5) |
| 11 | 8.65 (s) | 8.70 (s) | 8.64 (s) | 8.64 (s) | 9.05 (s) | 9.00 (s) | 8.59 (s) | 8.61 (s) |
| 14 | | | 5.71 (s) | | | 6.22 (s) | | |
| 15 | | | | 5.73 (s) | 6.21 (s) | | | |
| 18 | 8.24 (s) | 8.26 (s) | 8.22 (s) | 8.18 (s) | 8.22 (s) | 8.25 (s) | 8.20 (s) | 8.22 (s) |
| 20 | 1.48 (s) | 1.49 (s) | 1.47 (s) | 1.47 (s) | 1.51 (s) | 1.51 (s) | 1.46 (s) | 1.46 (s) |
| 21 | 3.87 (m) | 3.38 (t, 6.0) | 3.41 (m) | 2.75 (t, 6.5) | | | | |
| 22 | 3.39 (t, 6.0) | 3.86 (m) | 2.75 (t, 6.5) | 3.41 (m) | | | | |
| NH | 9.33 (bs) | 9.23 (bs) | 7.85 (t, 6.0) | 7.94 (t, 5.5) | | | | |
| $OCH_3$ | | | | | 3.92 (s) | 3.92 (s) | | |

[a] Varian Unity 500 spectrometer. [b] δ values are reported in ppm relative to residual undeterated DMSO-$d_6$ (δ 2.49). [c] δ values are reported in ppm relative to residual underterated $CDCl_3$ (δ 7.24).

($CHCl_3$/MeOH/TFA, 190:10:1, 300 mg), which yielded 23 mg of adociaquinone A (**1**) and 106 mg of adociaquinone B (**2**). Chromatography of the $CHCl_3$ fraction on an LH-20 column ($CHCl_3$/MeOH, 6:4, 700 mg) provided mixtures of 14-methoxyxestoquinone (**5**) and 15-methoxyxestoquinone (**6**) (15 mg) and 15-chloro-14-hydroxyxestoquinone (**7**) and 14-chloro-15-hydroxyquinone (**8**) (11 mg). Repeated attempts to separate the isomers were unsuccessful. The MeOH-soluble material from the aqueous fraction was subjected to LH-20 column chromatography (MeOH/$H_2O$/TFA, 80:20:0.5, 100 mg) which afforded 60 mg of a mixture containing secoadociaquinone A (**3**) and secoadociaquinone B (**4**). Five mg of **4** was obtained pure by collecting the last fraction of the LH-20 column chromatography. Repeated attempts to obtain **3** in pure form failed. Detailed analysis of FABMS data (424 $MH^+$; consistent with the molecular formula $C_{22}H_{17}O_6NS$) and $^1$H and $^{13}$C NMR spectra (see Tables 1 and 2) confirmed two of the compounds as the known metabolites adociaquinone A (1) and adociaquinone B (**2**). The $^1$H and $^{13}$C NMR data (Tables 1 and 2) for secoadociaquinone A (**3**) and secoadociaquinone B (**4**) were nearly identical to **1** and **2**, with the exception that the proton resonances for the C-21 and -22 methylenes and the NH were shifted. Also, there are new singlets at δ 5.71 in **3** and δ 5.73 in **4** assigned as H-14 and H-15, respectively. Compound **4** was isolated pure from LH-20 column chromatography; however, **3** remained as a 1:4 mixture with **4** by $^1$H integration. The high-resolution negative ion mass spectrum of this mixture indicated a molecular formula of $C_{22}H_{19}O_7NS$ which differs from **1** and **2** by the addition of an oxygen and two hydrogens. A singlet at δ 5.71 in **3** showed HMBC correlations to C-12 (δ 131.5). This correlation placed the hydrogen at C-14 and the taurine side chain, $-HCH_2CH_2SO_3H$, at C-15. For **4**, a three-bond correlation from δ 5.73 to C-17 (δ 135.2) placed the proton at C-15 and the taurine side chain at C-14. Two additional compounds were isolated from LH-20 column chromatography as inseparable mixtures of regioisomers. The molecular formula for **5** and **6**, $C_{21}H_{16}O_5$, was determined by HREIMS. The $^1$H NMR spectrum revealed the presence of a methoxy group. Their structures were elucidated by comparing NMR data with those of **3** and **4**. Since the mixture contained one major and one minor compound, $^1$H and $^{13}$C assignments were based on the intensities of the signals. The $^1$H NMR spectrum of the mixture revealed doubled signals at (δ 9.05 major, δ 9.00 minor), (δ 8.25 minor, δ 8.21 major), and (δ 6.22 minor, δ 6.21 major). The regiochemistry of the major component (70%), 14-methoxyxestoquinone (**5**), was determined by HMBC correlations. The proton at δ 6.21 showed correlations to C-13, C-14, C-16, and C-17. The correlation to C-17 placed the aromatic proton at C-15 and, therefore, the methoxy at C-14. This regiochemistry is entirely consistent with the model compound 2-methoxynaphthaquinone.[12] The regiochemistry of the minor component (30%) 15-methoxyxestoquinone (**6**) was determined by the key correlation from δ 6.22 to C-12 in the HMBC experiment. The NMR data for **5** were very similar to

**Table 2.** $^{13}$C-NMR (125 MHz) Chemical Shifts for Compounds 1–8[a]

| | compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| carbon | **1**[b] | **2**[b] | **3**[b] | **4**[b] | **5**[c] | **6**[c] | **7**[b] | **8**[b] |
| 1 | 146.2 | 146.2 | 146.0 | 146.0 | 144.9 | 144.9 | 146.1 | 146.1 |
| 2 | 121.6 | 121.6 | 121.6 | 121.6 | 121.5 | 121.4 | 121.6 | 121.6 |
| 3 | 16.3 | 16.2 | 16.3 | 16.3 | 16.9 | 16.9 | 16.2 | 16.2 |
| 4 | 17.8 | 17.8 | 17.9 | 17.9 | 18.4 | 18.4 | 17.8 | 17.8 |
| 5 | 30.3 | 30.3 | 30.4 | 30.4 | 31.2 | 31.2 | 30.1 | 30.2 |
| 6 | 37.2 | 36.8 | 36.8 | 37.1 | 37.4 | 37.2 | 37.0 | 36.9 |
| 7 | 147.7 | 147.9 | 147.8 | 147.7 | 147.2 | 147.2 | 147.7 | 147.8 |
| 8 | 143.0 | 143.1 | 143.1 | 143.0 | 144.0 | 144.1 | 142.9 | 143.0 |
| 9 | 169.3 | 169.4 | 169.7 | 169.5 | 170.3 | 170.3 | 169.1 | 169.3 |
| 10 | 135.9 | 137.9 | 137.3 | 135.5 | 137.6 | 138.3 | 136.3 | 137.1 |
| 11 | 125.4 | 124.7 | 124.3 | 125.0 | 127.3 | 126.7 | 125.1 | 125.0 |
| 12 | 128.6 | 130.9 | 131.5 | 128.9 | 129.5 | 130.5 | 128.3 | 129.9 |
| 13 | 173.7 | 173.7 | 180.3 | 180.3* | 178.9 | 183.6 | 178.4 | 177.1 |
| 14 | 111.7 | 147.1 | 99.8 | 148.6 | 161.0 | 110.6 | 157.0 | 118.2 |
| 15 | 147.2 | 111.3 | 148.5 | 100.0 | 110.1 | 160.5 | 118.2 | 157.0 |
| 16 | 177.9 | 178.3 | 180.9 | 180.4* | 184.4 | 179.9 | 177.3 | 178.9 |
| 17 | 134.4 | 131.8 | 132.1 | 135.2 | 133.5 | 132.5 | 133.2 | 131.7 |
| 18 | 122.9 | 123.4 | 123.0 | 122.5 | 123.0 | 123.4 | 123.3 | 123.4 |
| 19 | 157.3 | 154.6 | 154.1 | 156.8 | 156.7 | 155.6 | 156.4 | 155.3 |
| 20 | 31.8 | 31.6 | 31.8 | 31.8 | 32.5 | 32.5 | 31.6 | 31.6 |
| 21 | 39.4 | 48.2 | 39.0 | 39.0 | | | | |
| 22 | 48.2 | 39.3 | 48.1 | 48.1 | | | | |
| $OCH_3$ | | | | | 56.6 | 56.5 | | |

[a] Varian Unity 500 spectrometer. [b] δ values are reported in ppm referenced to DMSO-$d_6$ (δ 39.9). [c] δ values are reported in ppm referenced to $CDCl_3$ (δ 77.0). * interchangable values.

**Table 3.** Cytotoxicity, Differential Toxicity, and Topoisomerase II Inhibition Assays

| compd | HCT inhibition $IC_{50}$, $\mu M$ | *xrs*-6 inhibition $IC_{50}$, $\mu M$ | DC ratio[a] | topoisomerase II inhibition decat. $IC_{90}$,[b] $\mu M$ | topoisomerase II unwinding $IC_{90}$,[c] $\mu M$ |
|---|---|---|---|---|---|
| 1 | 24 | 78 | 3 | ND | 118 |
| **2** | 21 | 23 | 3 | <11 | 78 |
| **3, 4** | *d* | *e* | 80 | 113 | |
| **4** | *d* | *e* | ND | 113 | |
| **5, 6** | 28 | 4.3 | 3 | ND | 143 |
| **7, 8** | 33 | 27 | 4.5 | 110 | 135 |
| etoposide | 2.5 | 0.14 | 7 | 68 | 85 |
| *m*-AMSA | 6.3 | 0.24 | 4 | 33 | ND |
| mitoxantrone | ND | 0.001 | 9 | 1.1 | 56 |

[a] Differential cytotoxicity (DC) ratio = BR1 $IC_{50}$/*xrs*-6 $IC_{50}$. [b] Concentration at which 90% of monomer-length kDNA production is apparently inhibited, partially purified human ovarian carcinoma topoisomerase II. ND = not determined. [c] Commercially obtained pure *Drosophila* Topoisomerase II. [d] Not toxic at 143 $\mu M$. [e] Not toxic at 287 $\mu M$.

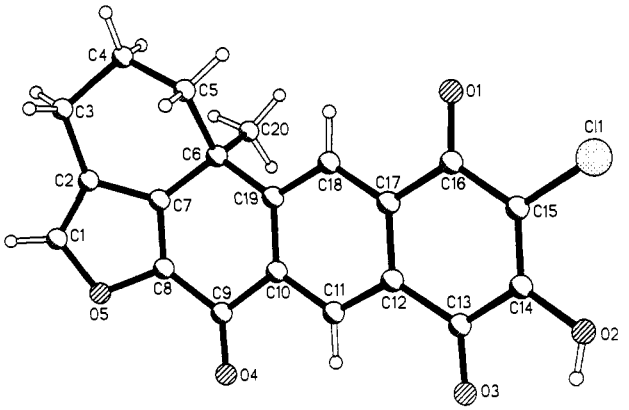


**Figure 1.** Computer-generated perspective drawing of **7**. The absolute configuration, which was not determined in the X-ray experiment, was set to agree with related metabolites already in the literature.

data reported for 14-methoxyhalenaquinone.[13] The final two compounds were assigned structures **7** and **8**. The molecular formula was established as $C_{20}H_{14}O_5Cl$ by HRFABMS in a DTT/DTE matrix. The spectral data was consistent with a pair of regiomers with hydroxyls and chlorine at the C-14 and C-15. A single-crystal X-ray analysis of the major regioisomer revealed 15-chloro-14-hydroxy regiochemistry, structure **7** (see Figure 1). The crystal contained two 15-chloro-14-hydroxyxestoquinone molecules, one methanol, and half of a water molecule per unique part of the cell. The two copies of **7** are identical, and the water molecule is surrounded by six oxygen atoms with $H_2O$- -O distances in the 2.65–2.8 Å range. The methanol molecule forms a hydrogen bond with the $O_2$ hydroxyl group; r($O_2$- - - $O_{1s}$) = 2.61 Å. The structure of the minor regioisomer, 14-chloro-15-hydroxyxestoquinone (**8**), was inferred from the NMR data.

## Results

Adociaquinones A and B and the regioisomer mixtures of methoxyxestoquinones **5** and **6** and chlorohydroxyxestoquinones **7** and **8** showed moderate cytotoxicity toward HCT 116 cells and differential cytotoxicity toward xrs-6 over BR1 with hypersensitivity factors (HF = $IC_{50}$ BR1/$IC_{50}$ xrs-6) of 3, 3, 3, and 4.5, respectively (Table 3). This is suggestive of specific topoisomerase II inhibition, assuming that the compounds do not directly cause DNA double-strand breaks (such breaks were never observed in experiments where adociaquinone B was incubated *in vitro* with DNA, i.e., DNA unwinding experiments). The secoadociaquinones did not exhibit cytotoxicity in either the HCT or CHO assays. With the exception of the secoadociaquinones, cytoxicity and xrs- differential correlate with inhibition of topoisomerase II catalytic activity *in vitro*. We attribute the lack of cytotoxicity of the secoadociaquinones to their polar charged nature and probable inability to penetrate the cell membrane.

The KSDS assay was performed to confirm topoisomerase II cleavable complex formation as the cause of enhanced toxicity toward xrs-6 cells. Adociaquinone B proved potently active in the KSDS assay, giving a signal equivalent to mitoxantrone. For example, mitoxantrone and adociaquinone B give significant precipitable counts at 0.05 $\mu g/mL$. A methotrexate analogue (negative control) gave no counts above background. At higher concentrations the KSDS signal diminished, suggesting a self-limiting effect on cleavable complex formation.

Because we have previously observed similar self-limiting effects on KSDS signal with intercalative compounds (e.g., makaluvamines[8] and mitoxantrone), the ability of adociaquinone B to displace ethidium bromide from DNA was measured. Interestingly, **2** did not displace ethidium bromide from calf thymus DNA. Furthermore, adociaquinone B showed no unwinding effect on supercoiled plasmid DNA[14] at concentrations up to 787 $\mu M$ (data not shown), consistent with the hypothesis that it is not a DNA intercalator.

## Discussion

We have reported the isolation and characterization of the known compounds adociaquinones A and B and six additional members of the adociaquinone-xestoquinone family from the Philippine sponge *Xestospongia* sp. Cell-based and enzyme inhibition assays suggest that the cytotoxicity of these compounds toward human colon tumor cells is mediated by their inhibition of topoisomerase II to produce cleavable complexes. While this general mechanism is common to many useful anticancer drugs, most of them are DNA intercalators (the exception is the epipodophyllotoxins which apparently bind to the complex). These metabolites appear to be similar to the epipodophyllotoxins in that they do not intercalate into DNA. It has recently been reported that the related compounds xestoquinone and halenaquinone inhibit topoisomerase I, but have no effect on topoisomerase II.[15] Interestingly, that report also indicates that the quinones do not intercalate DNA. A preliminary assay indicates that adociaquinone B, secoadociaquinone B, and the chlorohydroxyxestoquinones also inhibit topoisomerase I-mediated relaxation of pBR322 plasmid.

## Experimental Section

Etoposide, *m*-AMSA, ethidium bromide, tris(hydroxymethyl)aminomethane (trizma base), CT DNA, and MTT were purchased from Sigma Chemical Co., St. Louis, MO. All inorganic salts were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. Partially purified human (ovarian carcinoma) topoisomerase IIα was supplied to us by Dr. J. A. Holden of the University of Utah. Mitoxantrone was a gift from Dr. William M. Maiese of Lederle Laboratories.

The $^1H$ and $^{13}C$ NMR spectra were obtained at 500 and 125 MHz, respectively, on a Varian Unity 500 spectrometer. Proton chemical shifts are reported in parts per million relative to residual undeuterated solvent. IR spectra were recorded on a Perkin-Elmer 1600 FT spectrophotometer. UV spectra were obtained in MeOH on a Beckman DU-8 spectrophotometer. High- and low-resolution FAB mass spectral measurements were made on a Finnigan MAT 95 or a VG 7050E spectrometer. Fluorescence measurements were made on an PC1 spectrofluorometer equipped with a thermostated cuvette compartment and interfaced with an IBM computer. For ethidium bromide displacement studies, excitation wavelength was 530 nm while the emission spectra were recorded at a fixed wavelength of 600 nm or from 550 to 700 nm with resolution of 8 nm. All spectra were corrected for background fluorescence.

**Adociaquinone A** (1): yellow solid; IR (film) 3236, 2931, 1689, 1675, 1590, 1343, 1208, 1125 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 272 nm ($\epsilon$ 11 400); $[\alpha]_D$ +31.7° (*c* 4.66, $CH_3CN$).

**Adociaquinone B** (**2**): yellow solid; IR (film) 3260, 1627, 1572, 1548, 1119 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 294 nm ($\epsilon$ 14 400); $[\alpha]_D$ +21.5° (*c* 1.86, $CH_3CN$); LR (+) FABMS (glycerol) *m/z* 424.

**Secoadociaquinone A (3) and B (1/4 mixture)**: orange solid; IR (film) 3330, 2954, 1672, 1595, 1202, 1131, 1043 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 258 nm ($\epsilon$ 11 100); HR (−) FABMS *m/z* 440.0769 $(M - H)^-$, $C_{22}H_{18}O_7NS$ requires 440.0804.

**Secoadociaquinone B** (**4**): orange solid; IR (film) 3330, 2954, 1672, 1595, 1202, 1131, 1043 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 258 nm ($\epsilon$ 11 100); $[\alpha]_D$ +30.3° (*c* 1.12, MeOH); HR (−) FABMS (glycerol) *m/z* 440.0769 $(M - H)^-$, $C_{22}H_{18}O_7NS$ requires 440.0804.

**14-Methoxyxestoquinone** (**5**): yellow solid; IR (film) 2936, 1684, 1672, 1653, 1607, 1458, 1238, 1205 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 272 nm ($\epsilon$ 21 400); $[\alpha]_D$ +13.1° (*c* 3.06, $CHCl_3$); HREIMS *m/z* 348.1013 $(M)^+$, $C_{21}H_{16}O_5$ requires 348.0998.

**15-Methoxyxestoquinone** (**6**): yellow solid; IR (film) 2936, 1684, 1672, 1653, 1607, 1458, 1238, 1205 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 272 nm ($\epsilon$ 21 400); HREIMS *m/z* 348.1013 $(M)^+$, $C_{21}H_{16}O_5$ requires 348.0998.

**15-Chloro-14-hydroxyxestoquinone** (**7**): yellow solid; IR (film) 3318, 2942, 1666, 1443, 1354, 1319, 1290 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 266 nm ($\epsilon$ 76 143); HRFABMS *m/z* 369.0551 $(M + H)^+$, $C_{20}H_{14}ClO_5$ requires 369.0530; $[\alpha]_D$ +29.6° (*c* 2.8, $CHCl_3$).

**14-Chloro-15-hydroxyxestoquinone** (**8**): yellow solid; IR (film) 3318, 2942, 1666, 1443, 1354, 1319, 1290 $cm^{-1}$; UV (methanol) $\lambda_{max}$ 266 nm ($\epsilon$ 76 143); HRFABMS *m/z* 369.0551 $(M + H)^+$, $C_{20}H_{14}ClO_5$ requires 369.0530.

**Single Crystal X-ray Structure Determination of 7.** A small flat needle crystal of 15-chloro-14-hydroxyxestoquionone (**7**) was used for the data collection, which utilized a R3m Seimens four-circle diffractometer and graphite-monochromated Cu Kα radiation. The $P2_1$ cell parameters of $a$ = 41.415(8) Å, $b$ = 7.541 Å, $c$ = 11.722(2) Å, and $\beta$ = 104.3(1)° were obtained by a least-square fit of 25 reflections with $2\theta$ = 25−35°. A total of 2453 reflections were measured using $\omega/2\theta$ scans with variable scan speed (2−29°/min) to a maximum 2= 110°. Three standard reflections were monitored every 97 reflections. No systematic intensity variations were found, and no absorption correction was applied. The structure was solved by direct methods (SHELX-86). Hydrogen atoms were introduced geometrically. The structure was refined on $F^2$ using the full-matrix least-squares technique (SHELX-93) with anisotropic thermal parameters for all non-hydrogen atoms. Final $R_1$ = 6.1% for 1585 $F_o > 4\sigma(F_o)$. No peaks greater than 0.24 e/Å$^3$ were found in the final difference Fourier map.

**Cell Culture.** HCT 116 cells were obtained from the American Type Culture Collection (Rockville, MD). The HCT 116 cells were grown as a monolayer in McCoy's medium containing 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 5 units/mL of nystatin. The CHO cell lines were grown as monolayers in αMEM containing serum and antibiotics as above. All cells were maintained at 37 °C in a humidified 5% $CO_2$ atmosphere. Before drug treatment, cells grown as monolayers were detached with trypsin treatment. Media and antibiotics were obtained from Gibco Laboratories, Grand Island, NY.

**Cytotoxicity Assays.** Cytotoxicity was established in an MTT assay using the human colon tumor cell line HCT 116. Cytotoxicity was also determined against a double strand break repair-deficient Chinese hamster ovary (CHO) cell line xrs-6. We have previously demonstrated that enhanced activity against this cell line relative to a repair competent CHO line BR1 is indicative of agents that cause double strand breaks in DNA or inhibit topoisomerase II by stabilizing the cleavable complex. Cytotoxicity was assessed in a MTT-microtiter plate tetrazolium cytotoxicity assay (MTA). This assay was originally described by Mossman[16] and modified by others.[17] Extracts and purified metabolites were dissolved in 100% DMSO at an initial concentration of 10 mg/mL and serially diluted. The final DMSO concentration in the cell culture wells was 1% or less. HCT 116 and BR1 cells (40 000 cells/well) and xrs-6 cells (60 000 cells/well) were seeded in 200 μL of growth medium in Corning 96-well microtiter plates and allowed to attach for 4 h. Each dose of drug was exposed to four replicate wells on the plate. Each plate also contained eight replicate control wells which were treated with DMSO only. The cell growth in the DMSO wells was used to determine the zero inhibition growth level for each microtiter plate in an experiment. Four hours after seeding, 2 μL of DMSO or 2 μL of DMSO-containing drug was added to the CHO cells. Drug exposure lasted for 18 h, after which the medium was removed and replaced with 200 μL of drug-free medium. HCT 116 cells were treated with 1 μL of drug or DMSO (0.5%) and allowed 3 days incubation. All cultures were refed on the third day of the assay with 100 μL of fresh complete McCoy's medium. This was followed by the addition of 11 μL of MTT solution (5 mg/mL in phosphate buffered saline, pH 7.4) and 4 h further incubation. The MTT is reduced by viable cells to a purple formazan product. The formazan product was solubilized by the addition, to each well, of 100 μL of 0.04 N HCl in 2-propanol. The absorbance at 540 nm for each well was measured using a BIO RAD MP450 plate reader. Average absorbance for each set of quadruplicate drug-treated wells was compared to the average absorbance of the control wells to determine the percent growth inhibition (fractional survival) at any particular drug dose.

**Topoisomerase II Catalytic Assays.** Inhibition of the catalytic activity of topoisomerase II was measured *in vitro* using DNA decatenation and relaxation assays. The decatenation assay measures a compound's ability to inhibit topoisomerase II-catalyzed release of monomer-length DNA from high molecular weight kinetoplast DNA (kDNA). The DNA used in the assay is from the insect trypanosome *Crithidia fasciculata* and consists of highly catenated 2.5 kilobase pair DNA rings. The released monomer-length DNA can be resolved and quantitated on a 1% agarose gel from the fully catenated kDNA, which is too large to penetrate into the gel. Enzyme inhibition is measured by the disappearance of the monomer-length DNA bands as a function of increasing compound concentration. The relaxation assay used supercoiled pBR322 DNA as a substrate. The production of relaxed plasmid species indicates catalytic activity.

**Decatenation of Kinetoplast DNA by Topoisomerase II.** The *in vitro* inhibition of topoisomerase II decatenation of kDNA, as described by Muller,[18] was performed as follows. Each reaction was carried out in a 0.5 mL microcentrifuge tube containing 19.5 μL of $H_2O$, 2.5 μL of 10X buffer (1X buffer contains 50 mM Tris−HCl, pH 8.0, 120 mM KCl, 10 mM $MgCl_2$, 1 mM ATP, 0.5 mM dithiothreitol, and 30 μg of BSA/mL), 1 μL of kDNA (0.2 μg), and 1 μL of DMSO-containing drug. This combination was mixed thoroughly and kept on ice. One unit of partially purified human ovarian carcinoma topoisomerase II was added immediately before incubation in a water bath at 34 °C for 30 min. Following incubation, the decatenation assay was stopped by the addition of 5 μL of stop

buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol) and placed on ice. DNA was electrophoresed on a 1% agarose gel in TAE buffer containing ethidium bromide (0.5 μg/mL). Ultraviolet illumination at 310 nm wavelength allowed the visualization of DNA. The gels were photographed using a Polaroid Land camera.

**Inhibition of Topoisomerase II DNA Relaxation.** The *in vitro* inhibition of topoisomerase II DNA relaxation was performed as above except that 0.5 μg of supercoiled pBR322 was used as substrate. Commercially obtained pure drosophila topoisomerase II (U. S. Biochemical, 3 units per reaction) was used in these experiments. DNA was electrophoresed on a 1% agarose gel in TAE buffer without ethidium bromide. The gel was subsequently stained with ethidium bromide prior to photography.

**Precipitation of Protein Associated DNA (KSDS Assay).** Additional evidence for the production of DNA double-strand breaks via a topoisomerase II cleavable complex mechanism is provided by KSDS protein crosslink experiments. The KSDS assay was performed by the method of Liu and co-workers.[19] BR1 cells ($5 \times 10^4$ cells) were plated into each 25 $cm^2$ culture flask in complete αMEM. The next day the cells were refed with 5 mL of complete McCoy's medium containing 1 μCi/mL of [$^3$H]methylthymidine. The next day, the radioactive medium was removed, and the cells were refed with 5 mL of complete αMEM and allowed to incubate for 1 h. Cells were then treated for 1 h with 0.5% DMSO or an equivalent volume of drug in DMSO. At the end of 1 h the medium was removed and the cells were lysed with SDS solution at 65 °C (1.25% SDS, 5 mM EDTA, 0.4 mg/mL of salmon testis DNA) and transferred to a warm 1.5 mL microfuge tube containing 0.25 mL of a 325 mM KCl solution. This mixture was vortexed at high speed for 20 s and then incubated for 10 min at 65 °C, followed by incubation on ice for 10 min and centrifugation at 5000 rpm for 10 min to pellet precipitated protein. The supernatant was aspirated and the pellet solubilized in 0.8 mL of wash buffer (10 mM Tris, pH 8.0, 100 mM KCl, 1 mM EDTA, and 0.1 mg/mL of salmon testis DNA). The samples were subjected to incubation at 65 °C, on ice, and centrifugation two more times and finally resuspended, transferred to liquid scintillation vials, and counted for radioactivity by standard liquid scintillation counting techniques.

**DNA Intercalation.** An ethidium bromide displacement assay was used to evaluate the relative ability to interact with DNA.[20] When intercalated into DNA, ethidium bromide exhibits dramatically enhanced fluorescence along with a shift of its emission maximum to shorter wavelength. When a competing intercalator displaces ethidium bromide from DNA, its fluorescence decreases. The fluorescence of ethidium bromide in the presence of DNA as a function of increasing concentration of a competing intercalator provides a measure of the relative intercalative ability of that compound. Fluorescence measurements were made on an PC1 spectrofluorometer equipped with a thermostated cuvette compartment and interfaced with an IBM computer. Ethidium bromide displacement fluorescence studies were carried out with excitation at 530 nm and emission at 600 nm (or from 550 to 700 nm with 8 nm resolution). All spectra were corrected for background fluorescence.

**DNA Unwinding.** DNA unwinding studies were performed with the supercoiled plasmid pBR322 to further investigate DNA intercalation. A 0.5 μg portion of supercoiled plasmid (pBR322) DNA was incubated in topoisomerase II reaction buffer (without ATP or enzyme) with various concentrations of adociaquinone B (up to 787 μM-5% DMSO) for 30 min at 37 °C. The DNA was then subjected to agarose gel electrophoresis in 1% agarose, stained with ethidium bromide, and visualized. Intercalation results in unwinding of supercoiled DNA and causes the DNA to migrate more slowly through the agarose matrix.

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**Supporting Information Available:** Tables of crystal data, atomic coordinates, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates (8 pages). Ordering information is given on any current masthead page.

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