Naturally Occurring Inhibitors of Topoisomerase I Mediated DNA Relaxation

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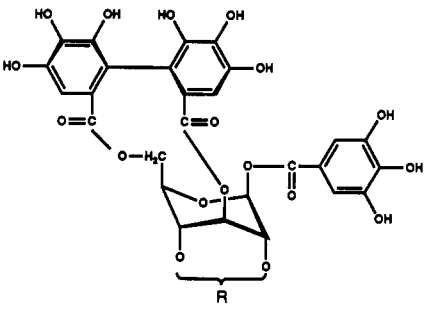
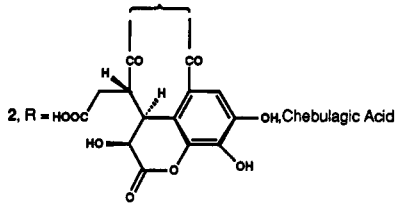
Summary: Bioassay-guided fractionation of a methanol extract of *Erodium stephanianum* found to inhibit DNA topoisomerase I mediated DNA relaxation afforded corilagin (1) and chebulagic acid (2). The latter is the most potent inhibitor of mammalian topoisomerase I yet reported.

DNA topoisomerases alter the topological state of DNA, thereby carrying out a function essential for several cellular processes including DNA replication, transcription, and recombination.[1] There are two types of DNA topoisomerases: type I enzymes change DNA linking number[1a,b,2] by transiently breaking one strand of duplex DNA, while type II enzymes transiently break both strands. Inhibitors of the topoisomerases are of special interest as they may faciliate an understanding of the remarkable process of DNA "strand passage"[1b,3] and also because several topoisomerase II inhibitors have useful antitumor activity.[4]

At present the camptothecins are the only compounds demonstrated to inhibit DNA relaxation by specifically interfering with topoisomerase I function.[5,6] They do so by stabilizing the covalent binary complex formed between topoisomerase I and DNA.[5] Recently, an electrophilic camptothecin analogue was shown to effect alkylation of the protein constituent of the DNA–topoisomerase I binary complex after (initially reversible) binding.[7] Because certain camptothecins have demonstrated antitumor activity in animal tumor models[8] and in clinical trials,[9] we sought to identify additional potent inhibitors of DNA topoisomerase I. Presently we report that both corilagin (1) and chebulagic acid (2) specifically inhibit topoisomerase I mediated DNA relaxation and that chebulagic acid is the most potent inhibitor of the enzyme yet reported.



1, R = H, Corilagin



2, R = HOOC, OH, Chebulagic Acid

A methanol extract of *Erodium stephanianum*[10] was found to inhibit the relaxation of supercoiled plasmid DNA by calf thymus DNA topoisomerase I.[11] Bioassay-guided

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(10) *Erodium stephanianum* was collected in China in 1980; voucher specimen M-4529 is preserved at the National Herbarium, Washington, DC.

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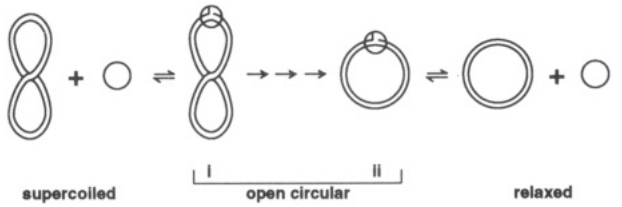
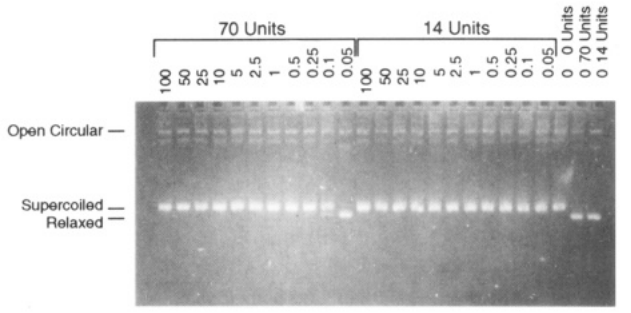


Figure 1. Putative mechanism of relaxation of supercoiled plasmid DNA by topoisomerase I (O). The enzyme becomes covalently attached to the DNA via a 3′-phosphorotyrosine linkage with transient breakage of the phosphate ester backbone of DNA (i). After strand passage is complete (ii), the open circular DNA is resealed with release of the enzyme to afford relaxed duplex DNA.

fractionation on Sephadex LH-20 and C_{18} reversed-phase HPLC[12] afforded two highly active purified principles as off-white solids. Compound **1** was found to have M_r 634 by FAB mass spectrometry. The IR spectrum contained a broad peak at 3600–3100 cm^{-} indicating the presence of OH groups, and a broad carbonyl stretching band at 1720 cm^{-1}. Peracetylation (Ac_2O, pyridine, 25 °C, 12 h) afforded a compound having M_r 1096, consistent with the presence of 11 OH groups. Because the UV spectrum of **1** revealed a characteristic phenolic base shift (220 and 274 nm in CH_3OH; 240 and 326 nm in CH_3OH containing 0.1 N NaOH), the number of phenolic OH groups was established (as 9) by demonstrating that treatment of **1** with diazomethane afforded a compound having M_r 760. The ^1H and ^{13}C NMR spectra were consistent with the presence of one galloyl and one 4,4′,5,5′,6,6′-hexahydroxydiphenoyl ester, as well as a glucose moiety.[13] That compound **1** was identical with corilagin was verified by direct comparison of the ^1H and ^{13}C NMR chemical shifts of the natural product in acetone-d_6 with those of authentic corilagin.[14] The structure of compound **2** (M_r 954) was assigned as that of chebulagic acid[15] on the basis of the ^1H and ^{13}C NMR resonances relative to those reported for chebulagic acid, chebulinic acid, and chebulic acid derivatives.[15,16]

As shown in Figure 1, topoisomerase I effects the relaxation of supercoiled covalently closed circular DNA via putative open circular intermediates (i and ii). The known topoisomerase I inhibitor camptothecin[17] noncovalently



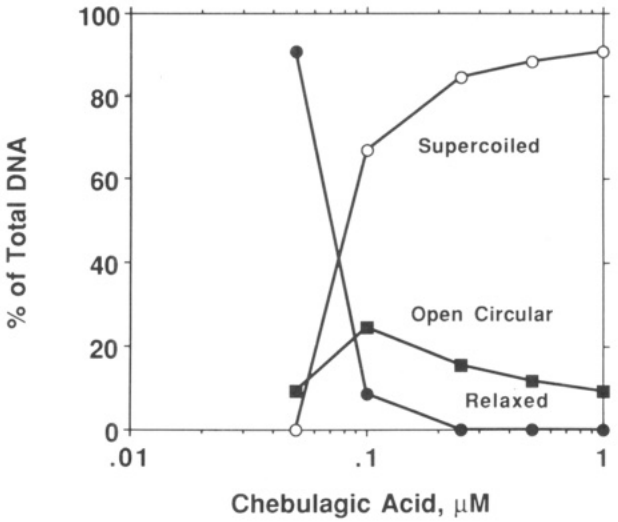


Figure 2. Inhibition of DNA relaxation by chebulagic acid (concentration for each lane in "μM") in the presence of 70 units (concentration dependence depicted graphically) or 14 units of topoisomerase I. The faint band that migrates slightly more rapidly than open circular DNA is believed to correspond to supercoiled, catenated DNA.

binds to and stabilizes the open circular form of DNA,[5,7] but inhibits the rate of relaxation only when the enzyme is present at very low concentration.[5c] In contrast, chebulagic acid inhibited enzyme-mediated DNA relaxation in a concentration dependent fashion even when the enzyme was present at high concentrations (Figure 2).[18] As in the case of camptothecin, however, the inhibitory effect of chebulagic acid was a function of the concentration of topoisomerase I. For example, while the IC_{50} for chebulagic acid was 100 nM in the presence of 70 units of topoisomerase I, complete inhibition of DNA relaxation was obtained at 50 nM chebulagic acid when 14 units of enzyme were present.[19] This suggests strongly that chebulagic acid functions by binding to the enzyme or to the (noncovalent) binary complex between enzyme and DNA, rather than to the DNA substrate alone.[20] The selectivity of chebulagic acid as an inhibitor of DNA topoisomerase I was also studied. When compared at equivalent enzyme concentrations, chebulagic acid was ~40-fold less active as an inhibitor of mammalian DNA topoisomerase II and

(11) In a typical assay DNA (100 ng of supercoiled plasmid pDPT2789 DNA, 6.4 kb), was incubated with 70 units of calf thymus topoisomerase I in 20 μL (total volume) of 40 mM Tris-HCl, pH 7.5, containing 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 μg/mL of bovine serum albumin, and either 2 μg of extract or 0.05–100 μM purified **1** (or **2**). After 30 min at 37 °C, the reaction was stopped (2.5% sodium dodecyl sulfate and 0.75 mg/mL proteinase K, 1 h, 37 °C) and analyzed by electrophoresis on 1% agarose gels containing 1 μg/mL of ethidium bromide.

(12) The Sephadex LH-20 column (2.5 × 50 cm) was washed with CH_3OH; HPLC separation involved isocratic elution of a Beckman Ultrasphere ODS (5 μm) column (10 mm × 25 cm) with 25% methanol in water containing 0.1% trifluoroacetic acid.

(13) ^1H NMR resonances for **1** due to aromatic H's included one 2 H singlet at 7.10 ppm and two 1-H signals at 6.68 and 6.82 ppm; ^{13}C NMR spectroscopy indicated the presence of ester carbonyl carbons at 166.7, 168.5, and 170.1 ppm. Resonances due to the glucose moiety included those at 4.04 (br s), 4.10 (dd, J = 11 Hz), 4.45 (br s), 4.5 (m), 4.82 (br s), 4.95 (dd, J = 11 Hz), and 6.36 (br s) in the ^1H NMR spectrum, and at 62.5, 65.0, 69.5, 71.6, 76.2, and 95.0 ppm in the ^{13}C NMR spectrum.

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(18) Camptothecin was shown to inhibit plasmid DNA relaxation when the topoisomerase I–DNA ratio was 1:55.[5c] The enzyme–DNA ratio in Figure 2 was 15:1 (at 70 units of topoisomerase I).

(19) Corilagin was less potent, having an IC_{50} of 40 μM when 70 units of topoisomerase I were present.

(20) Unlike camptothecin, chebulagic acid and corilagin did not increase the proportion of open circular DNA in a simple, concentration-dependent fashion, indicating that they probably did not act via stabilization of a covalent enzyme–DNA complex. Likewise, the lack of effect of **1** and **2** on the gel mobility of the DNA argues against direct binding of these species to DNA.

~200-fold less active against avian myeloblastosis virus reverse transcriptase.

Chebulagic acid is the most potent inhibitor of mammalian DNA topoisomerase I yet reported.[21] While its molecular mechanism of topoisomerase I inhibition is uncertain at present, chebulagic acid is 10–50-fold more active than camptothecin[5] or 10-hydroxycamptothecin[6d] as an inhibitor of the overall process of DNA relaxation (cf. Figure 1). Chebulagic acid was >800-fold more potent than these camptothecins in diminishing the initial enzyme-mediated DNA nicking reaction.

(21) Both calf thymus and human colon adenocarcinoma (Colo 201) topoisomerase I were inhibited by 1.

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Silacupration of Oxabicyclic Compounds. An Interrupted Ring Opening Reaction

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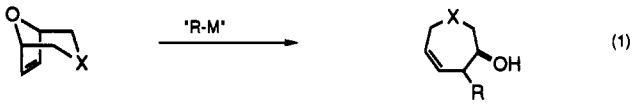
Summary: Oxabicyclo[3.2.1]octenes have been found to undergo silacupration when treated with $(PhMe_2Si)Cu\cdot LiCN$. Ring closure of the cuprate onto a remote carbonyl group at C-3 provides tricyclic compounds. Trapping experiments with a variety of other electrophiles prior to the closure reaction have also been achieved.

We have recently reported the addition/ring-opening reactions of oxabicyclo[3.2.1] and -[2.2.1] compounds with organocuprate and organolithium reagents. Complimentary stereochemistry is observed using the two classes of reagents. Organolithium reagents effect a net S_N2' reaction with retention of stereochemistry (i.e., for X = CHOR', the cis product is obtained) whereas cuprates promote an S_N2' reaction with overall inversion (the trans product is obtained for X = C=O), eq 1.[2,3] We chose to examine the

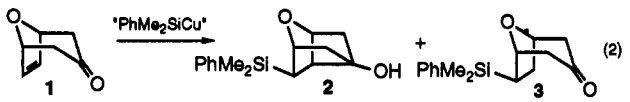


(1)

reactivity of silylcuprates and silylcopper reagents as nucleophiles to promote the ring opening due to the synthetic utility of the allylsilane which would result from ring opening.[4] We report an alternative pathway for this class of cuprates which represents a novel interrupted ring-opening process and demonstrate that silacupration of strained olefins is a facile process.

Silylcuprates and silylcopper reagents have been shown to be reactive nucleophiles toward a variety of electrophilic partners including allylic and propargylic acetates, enones, and ynones.[5] Unactivated acetylenes and allenes also undergo silacupration.[6] To our knowledge, the silacupration of simple unactivated olefins has not been reported.[7]

Treatment of 8-oxabicyclo[3.2.1]oct-6-en-3-one (1, X = C=O) with $(PhMe_2Si)Cu\cdot LiCN$ in THF at 0 °C for 1 h yields a product whose spectra are inconsistent with the expected product, eq 1, R = $PhMe_2Si$. While the product contained a phenyldimethylsilyl group, no olefinic resonances were observed, and the oxabicyclo ring was intact. Furthermore, no carbonyl stretch was observed in the infrared spectrum. We speculated that silacupration of the olefin had occurred followed by ring closure onto the ketone to generate the novel cyclobutanol **2**, eq 2. The



(2)

stereochemistry of the silicon-bearing carbon could not be conclusively determined by ^1H or ^{13}C NMR. Previous studies had established that attack from the endo face was the usual course of events in oxabicyclo[3.2.1] openings with organocuprates.[2] However, examination of molecular models indicated this product would experience severe nonbonded interactions which would disfavor the subsequent closure reaction. X-ray crystallography of the 3,4-dinitrobenzoate ester of **2** confirmed that, in fact, the silyl group had attacked from the exo face of the oxabicyclic system. Since both the stereochemistry of attack and failure to undergo ring opening were novel results in this system, an investigation of the generality of this phenomenon was undertaken.

The presence of copper is essential for controlled reactivity in this transformation since treatment of **1** with

(1) (a) A. P. Sloan Foundation Fellow, 1991-93; NSERC (Canada) University Research Fellow 1987-1992; Bio-Mega Young Investigator 1990-1993. (b) Author to whom inquiries regarding the X-ray structure should be addressed.

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