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Unciaphenol, an Oxygenated Analogue of the Bergman Cyclization Product of Uncialamycin Exhibits Anti-HIV Activity

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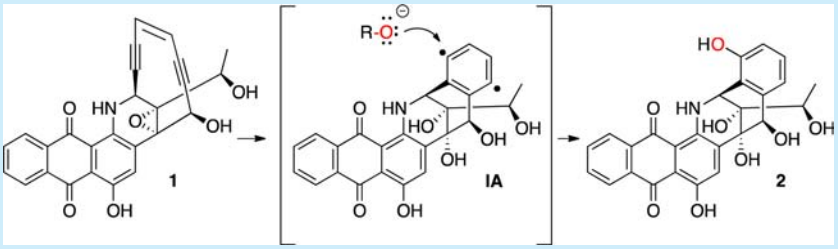
Supporting Information



ABSTRACT: Unciaphenol (**2**), an oxygenated analogue of the Bergman cyclization product of the enediyne uncialamycin (**1**), has been isolated along with **1** from cultures of the actinomycete *Streptomyces uncialis*. It is proposed that the C-22 OH substituent in **2** might arise from the attack of a nucleophilic oxygen species on the *p*-benzyne diradical intermediate **IA** in the Bergman cyclization of **1**. **2** shows in vitro anti-HIV activity against viral strains that are resistant to clinically utilized antiretroviral therapies.

Microbial natural products containing an enediyne substructure exhibit extremely potent cytotoxic and antimicrobial activities that result from their ability to damage chromosomal DNA.[1] The mechanism of DNA damage involves a Bergman cyclization[2] to generate a highly reactive *p*-benzyne diradical that abstracts carbon-bound hydrogen atoms from ribose units of DNA, leading to single-strand or double-strand cleavage. Although more than a dozen enediyne chemotypes have been known for decades,[1] it is rare for their Bergman cyclization products or substituted analogues of their Bergman cyclization products to be reported as natural products either alone or co-occurring with their enediyne precursors.

We previously reported the isolation of the enediyne antibiotic uncialamycin (**1**) from cultures of the actinomycete *Streptomyces uncialis* obtained from the surface of the lichen *Cladonia uncialis* collected near the Pitt River in British Columbia.[3] Further investigation of the *S. uncialis* culture extracts has resulted in the isolation of unciaphenol (**2**), an oxygenated analogue of the Bergman cyclization product of **1**. Product **2** was incorporated into a small library of pure natural products that has been screened for biological activity in a variety of phenotypic and pure molecular target assays, which revealed that unciaphenol has in vitro anti-HIV activity against drug-resistant isolates of the virus. Details of the isolation, structure elucidation, and biological activity of **2**, along with a proposal for its biogenetic origin that involves trapping of a *p*-benzyne diradical with an oxygen nucleophile, are presented below.

Production cultures of *S. uncialis* were grown as lawns on solid agar (ISP4) at 30 °C (Supporting Information).[3,4] The cells and media from the culture were jointly extracted repeatedly with EtOAc. Concentration of the combined EtOAc extracts in vacuo gave a gummy purple residue that was partitioned between EtOAc and H_2O. Fractionation of the EtOAc-soluble material using sequential application of open column step-gradient reversed-phase chromatography, Sephadex LH20 chromatography, and reversed-phase HPLC gave pure **2**.

Unciaphenol **2** was isolated as a dark blue glass that gave a $[M + Na]^+$ ion at m/z 498.1159 in the HRESIMS, appropriate for a molecular formula of $C_{26}H_{21}NO_8$, differing from the molecular formula of **1** by the addition of two molecules of water. The ^1H and ^{13}C NMR spectra recorded for **2** contained resonances that could be directly assigned to the anthraquinone motif (C-2 to C-15) seen previously in **1**. However, the resonances attributed to the enediyne and epoxide fragments in **1** were not present in the NMR spectra of **2**. Instead, a 1,2,3-trisubstituted benzene ring ($\delta_{C/H}$ 154.0, 137.9, 127.8/6.98 t, J = 7.8 Hz, 124.1, 117.8/6.82 d, J

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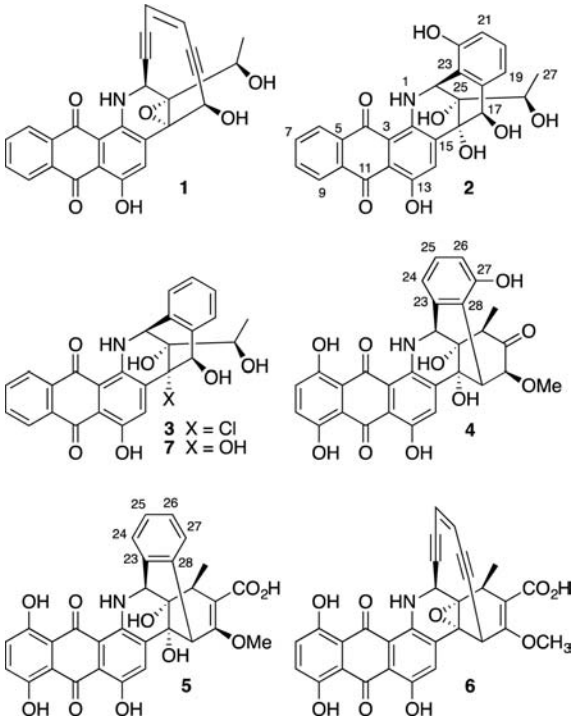
= 7.8 Hz, 113.6/6.67 d, J = 7.8 Hz) and two tertiary alcohols (δ 77.1 and 72.3, both correlated to exchangeable singlet's resonating at δ 5.41 and 4.86 in the gHMBC) were present (see Supporting Information, Table S1).



Analysis of the 1D and 2D NMR data (Figure 1) allowed for the unambiguous assignment of the structure of **2**. It was

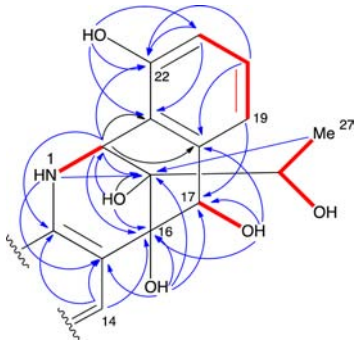


Figure 1. Selected COSY (red) and HMBC (blue) correlations for unciaphenol **2**.

apparent that the epoxide in **1** was replaced by the two tertiary alcohols because, in addition to C-16 (δ 77.1) and C-25 (δ 72.3), the exchangeable resonance assigned to the C-25 OH (δ 4.86) also correlates in the gHMBC to the carbon at δ 50.3 assigned to C-24. Similarly, the 16-OH resonance (δ 5.41) shows gHMBC correlations not only to C-16 and C-25 but also to the aromatic carbon at δ 142.2 assigned to the C-15 of the anthraquinone moiety and to the resonance assigned to the carbinol methine at C-17 (δ 74.4). As in **1**, the Me-27 doublet (δ 1.37) showed a gHMBC correlation to C-25 to establish the carbon−carbon bond between C-25 and C-26. A resonance not seen in the ^{1}H NMR data of **1** was a third deshielded proton singlet (δ 9.77, 22-OH) that did not correlate to a carbon resonance in the gHSQC. This ^{1}H resonance was assigned to a phenol substituent on the 1,2,3-trisubstituted aromatic ring because it showed gHMBC correlations to one protonated and two nonprotonated aromatic ring carbons resonating at δ 113.6 (C-21/H-21: δ 6.67), 154.0 (C-22), and 124.1 (C-23), respectively. A carbon−carbon bond between C-23 and C-24 was established by the observation of gHMBC correlations between the resonance assigned to H-24 at δ 5.28 and both C-22 and C-23 and to the third nonprotonated carbon of the 1,2,3-trisubstituted aromatic ring resonating at δ 137.9. The 17-OH resonance at δ 5.82 showed a gHMBC correlation to the substituted aromatic resonance at δ 137.9 (C-18), and the aromatic H-19 doublet (δ 6.82) showed a gHMBC correlation to C-17, which together established a C-17/C-18 bond that closes the final ring and completes the constitution of **2**.

Nicalaou's group has reported the total synthesis of uncialamycin **1**, which identified its complete absolute configuration and provided material for further chemical and biological studies.[5,6] They found that "uncialamycin proved to be quite stable in the solid phase and in a variety of solvents". However, treating **1** with dry HCl in CH_2Cl_2 at room temperature triggered a Bergman cyclization to give the deep blue chlorohydrin **3** (yield 90%), which has the same skeleton as **2** but lacks the OH substituent at C-22.

With the absolute configuration of **1** established by synthesis,[5,6] the observation of ROESY correlations between the C-16 OH resonance at δ 5.41 and H-14 (δ 7.58), H-17 (δ 4.77), and Me-27 (δ 1.37) confirms the *S* configuration predicted for C-16 that resulted from the expected Bergman cycloaromatization.

Uniciaphenol inhibits in vitro HIV-1 replication (virus strain NL4.3) without concomitant cytotoxicity in CEM-GXR cells, a T-cell line that expresses GFP in response to infection (EC_{50} = 9.9 μM).[7] Similar effects are also observed using recombinant viruses encoding patient-derived sequences that confer resistance to the clinically useful anti-retroviral therapies indinavir (protease inhibitor), efavirenz (non-nucleoside reverse transcriptase inhibitor), or raltegravir (integrase inhibitor),[8] suggesting that uniciaphenol inhibits HIV-1 by a mechanism distinct from these drugs. The measured EC_{50} values for the drug-resistant viruses were as follows: indinavir-resistant, 14.1 μM; efavirenz-resistant, 13.6 μM; and raltegravir-resistant, 6.5 μM.

Uncialamycin and unciaphenol represent a rare example of an enediyne and an analogue of its Bergman cyclization product being isolated simultaneously from the same culture extract. The co-occurrence of **1** and **2** raises the question of whether **2** is an isolation artifact resulting from spontaneous Bergman cyclization of **1** during extraction and purification or is a product of further biosynthetic transformation of **1**. The presence of the phenol OH at C-22 in **2** requires that something other than just simple Bergman cyclization and normal proton abstraction by the *p*-benzyne intermediate has occurred, arguing against the possibility of **2** being an artifact.

Unciaphenol **2** is related to dynemicin O (**4**), an oxygenated and oxidatively decarboxylated analogue of the Bergman cyclization product **5** of dynamycin A (**6**) that has been reported to be a natural product co-occurring with **6**.[9] To the best of our knowledge, **2** and **4** represent the only examples of naturally occurring endiyene Bergman cyclization products, with an oxygen atom added at one of the radical sites in the putative *p*-benzyne diradical intermediate. Two additional examples where modified Bergman cyclization products have been reported as natural products are the sporolides A and B[10] and the cyanosporasides A and B[11] isolated from marine actinomycetes in the genus *Salinispora* by Fenical and co-workers. Fenical

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proposed that these natural products arise from Bergman cyclizations of enediyne precursors that have not yet been isolated as natural products. Interestingly, Fenical's proposal suggests that the Bergman *p*-benzyne diradical intermediates are captured by a chlorine atom, which is a novel mechanism for introducing chlorine into a natural product. Perrin has shown in model systems that bromide, chloride, and acetate ions can act as nucleophiles to trap *p*-benzyne diradicals generated by Bergman cyclizations, providing elegant support for the proposed origin of the sporolides and cyanosporasides.[12,13] It remains uncertain whether the trigger for the Bergman cyclizations and trapping by chloride ions to give the sporolides and cyanosporasides is mediated by an enzyme as part of a biosynthetic sequence or is spontaneous and non-enzymatic.[14] The observation of chlorination in sporolides A and B and the cyanosporasides A and B equally at what would be both Bergman cyclization *p*-benzyne radical sites perhaps argues for non-enzymatic chloride trapping.

The origin of the C-22 OH in **2** and the C-27 OH in **4** is of considerable interest. One possible source of the C-22 OH in **2** and the C-27 OH in **4** would be direct cytochrome *P*450 enzymatic hydroxylation of the benzene rings in the already formed Bergman cyclization products **7** and **5**, respectively, via an arene oxide intermediate.[15,16] Alternatively, since C-22 and C-27 correspond to one of the radical sites in the putative *p*-benzyne intermediates in the Bergman cyclizations of **1** and **6**, respectively, it seems possible that the diradical is trapped by a species R—O$^-$ containing a nucleophilic oxygen atom to generate oxygenation at C-22 in **2** and at C-27 in **4** (illustrated for **2** in Figure 2). Indeed, Perrin showed that acetate ions could add to a *p*-benzyne diradical just like chloride and bromide ions,[13] so there is a precedent for this reaction. The exact nature of the oxygen nucleophile is not clear. It could be acetate,[13] hydroxyl, superoxide,[17] or even an enzyme-bound species. Perrin has provided two mechanistic rationalizations for the new reaction of a *p*-benzyne diradical with a nucleophile, as illustrated in **IA** and **IB** in Figure 2. The first rationalization shown in **IA** features the unusual combination of a two-headed arrow pushing two electrons and a one-headed arrow pushing one electron during the nucleophilic attack, resulting in the formation of a new covalent bond between the aromatic ring and the nucleophilic species and an aromatic carbanion that abstracts a proton from an acidic species in the reaction mixture to generate the substitution product. Perrin's second rationalization illustrated in **IB** suggests that there is an antibond across the ring in the *p*-benzyne diradical and that the attack is an S_N2-like process.[13]

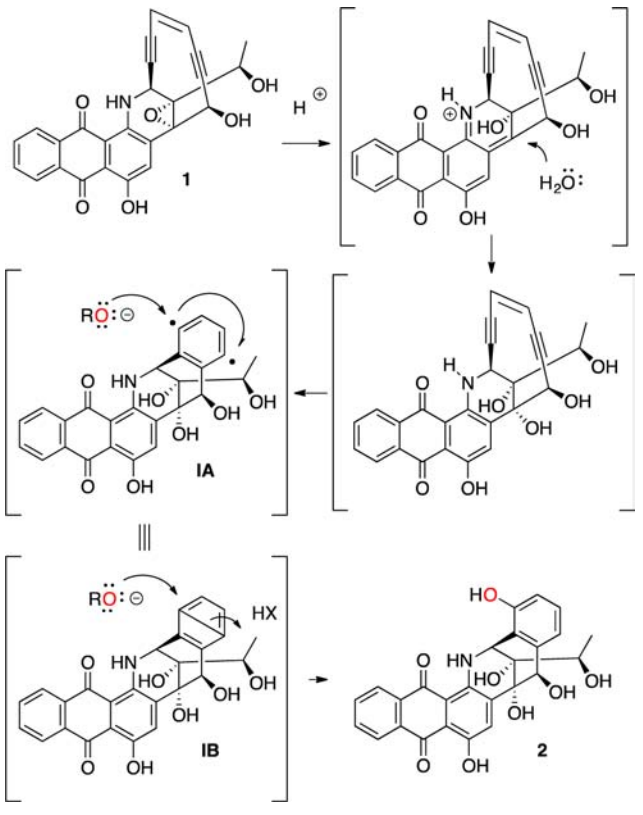

Figure 2. Proposed biogenesis of unciaphenol **2**.

We found no evidence in the culture extracts for the regioisomer of **2** with hydroxylation at C-19, and Iwasaki and co-workers apparently did not find the C-24 OH analogue of **4**,[9] unlike the situation in the sporolides and the cyanosporasides, where the isomers corresponding to chlorination at both *p*-benzyne radical sites were isolated in ratios of ≈1:1.[10,11] Taken together, the location of the hydroxylation at C-22, the absence of the C-19 hydroxylation regioisomer in the culture extract, and the lack of any hydroxylation products being formed in Nicalaou's chemical conversion of **1** to **3**[5,6] are all consistent with an enzyme-assisted oxygenation of the *p*-benzyne diradical intermediate in the Bergman cyclization of **1** proceeding as indicated in Figure 2. Interestingly, the corresponding sequence of events to form **4** involves regioselective trapping of the corresponding alternate *p*-benzyne radical site, again consistent with enzymatic involvement.

Unciaphenol represents a rare example of a naturally occurring endiyene Bergman cyclization product analogue with an oxygen atom added at one of the radical sites in the putative *p*-benzyne diradical intermediate. The origin of the C-22 oxygen atom in **2** is of considerable chemical and biosynthetic interest because the trapping of a *p*-benzyne radical by an oxygen nucleophile, as outlined in Figure 2, would represent a novel mechanism for introducing oxygen atoms into a natural product. Transformation of the enediyne substructure in **1** to a hydroxylated benzene ring in **2** dramatically reduces the cytotoxicity of the scaffold as expected, revealing a mechanistically distinct in vitro anti-HIV activity that is under further investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02664.

1D and 2D NMR data for unciaphenol **2** and experimental details (PDF)

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Notes

The authors declare no competing financial interest.

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