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Uncialamycin, A New Enediyne Antibiotic

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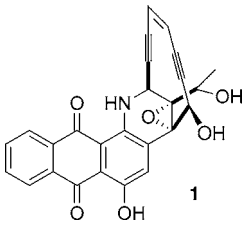
ABSTRACT



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Laboratory cultures of an undescribed streptomycete obtained from the surface of a British Columbia lichen produce uncialamycin (1), a new enediyne antibiotic. The structure of uncialamycin (1) has been elucidated by analysis of spectroscopic data. Uncialamycin (1) exhibits potent in vitro antibacterial activity against Gram-positive and Gram-negative human pathogens, including *Burkholderia cepacia*, a major cause of morbidity and mortality in patients with cystic fibrosis.

The *Burkholderia cepacia* complex (Bcc) is a group of nine species of Gram-negative nonsporulating bacilli that were originally identified as plant pathogens.[1] Bcc have recently emerged as serious opportunistic human pathogens.[2] Lung infections with Bcc in cystic fibrosis patients correlate with poorer prognosis, longer hospital stays, and an increased risk of death. Given the increased morbidity and mortality associated with Bcc infections, especially in children, and the increase in the occurrence of these infections, there is an urgent need to find new antibiotics for treating Bcc lung infections in humans with cystic fibrosis.

As part of a screening program, it was found that crude organic extracts of cultures of a previously undescribed streptomycete showed potent in vitro inhibition of Bcc. Bioassay guided fractionation of the crude extracts led to the identification of the new enediyne antibiotic, uncialamycin (**1**), as the active component. Details of the isolation, structure elucidation, and antimicrobial activity spectrum of uncialamycin are presented below.

The producing strain was extracted from the surface of a lichen *Cladonia uncialis* collected near Pitt River, British Columbia. Characterization by 16S RNA sequencing showed the strain to be related but not identical to *Streptomyces cyanogenus*. Antibiotic activity was assayed by cutting plugs from solid agar cultures of the strain and placing them on lawns of tester strains of bacteria. Good inhibitory activity was detected against Gram-negative (including Bcc) and Gram-positive bacteria, but not against yeasts.

Production cultures of the producing strain were grown as lawns on solid agar medium (ISP4, 16 L) for 14−21 days at 30 °C. The solid agar cultures were extracted repeatedly with EtOAc. Concentration of the combined EtOAc extracts in vacuo gave a gummy residue that was partitioned between EtOAc and H_2O. The EtOAc soluble material was fractionated by sequential application of flash C-18 reversed-phase chromatography (eluent: step gradient from H_2O to MeOH) and reversed-phase HPLC (column-Inertsil ODS-2; eluent: CH_3CN/H_2O 40:60) to give pure uncialamycin (**1**) (∼300

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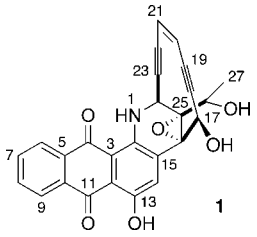
μg) as a bright purple [UV(MeOH): λ_{max}nm (ϵ) 206 (25000), 254 (33000), 280 (shoulder), 320 (shoulder), 539 (9400)] optically active ($[\alpha]_D$ +3300 (*c* 0.005, MeOH)) oil.



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Uncialamycin (**1**) gave a $[M + Na]^+$ ion at *m*/*z* 462.0956 in the HRESIMS appropriate for a molecular formula of $C_{26}H_{17}NO_6$ (calcd for $C_{26}H_{17}NO_6Na$ 462.0954) requiring 19 sites of unsaturation. NMR data for uncialamycin were recorded in DMSO-d_6 at 600 MHz using a cryoprobe. The ^{13}C NMR spectrum (Table 1) showed well-resolved resonances for 26 carbon atoms, and the ^1H NMR spectrum contained resonances integrating for 17 protons, in agreement with the HRMS data. Inspection of the HSQC data revealed that four of the protons (δ 5.39, 6.66, 10.0, and 13.2) were not attached to carbon atoms. Two major fragments A and B (Figure 1) of uncialamycin could be identified from analysis of the COSY, HSQC, and HMBC data obtained for the molecule.

Table 1. ^{13}C and ^1H NMR Assignments for Uncialamycin (**1**). Data were Recorded in DMSO-d_6 at 600 MHz for ^1H

position	δ ^{13}C	δ ^1H (mult., *J* (Hz))
1		10.0 (d, 4.6)
2	143.6	
3	110.4	
4	187.0[a]	
5	134.4[b]	
6	126.1[c]	8.23 (dd, 1.4, 7.6)[c]
7	133.6[d]	7.88 (ddd, 1.4, 7.6, 7.6)[d]
8	134.9[d]	7.94 (ddd, 1.4, 7.6, 7.6)[d]
9	126.6[c]	8.24 (dd, 1.4, 7.6)[c]
10	132.2[b]	
11	182.2[a]	
12	112.7	
13	154.9	
14	129.9	8.51 (s)
15	135.6	
16	63.5	
17	63.0	5.14 (d, 3.3)
18	100.4	
19	89.7	
20	123.4	6.05 (dd, 0.8, 10)
21	124.0	5.97 (ddd, 1.4, 1.5, 10)
22	87.4	
23	98.9	
24	43.2	5.04 (dd, 1.5, 4.6)
25	76.0	
26	63.6	4.31 (qd, 6.0, 6.0)
27	22.1	1.30 (d, 6.0)
13-OH		13.2 (brd.s)
17-OH		6.66 (brd.s)
26-OH		5.39 (d,6.0)

[a–d] May be interchanged.

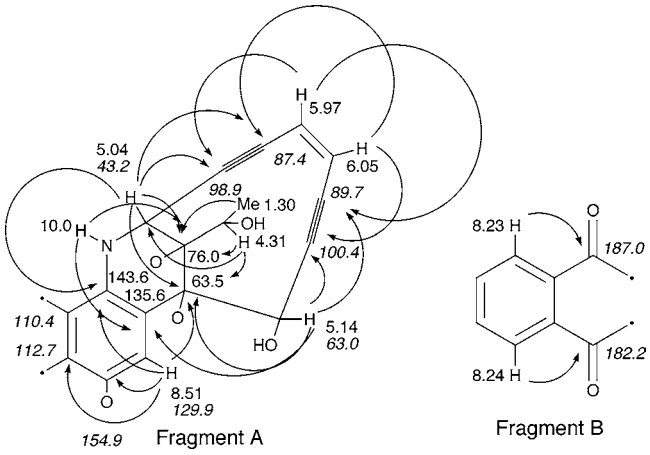


Figure 1. HMBC correlations used to identify two major fragments A and B of uncialamycin (**1**).

A pair of olefinic resonances at δ 5.97 (H-21) and 6.05 (H-20) that were strongly correlated to each other in the COSY spectrum and had a coupling constant of 10 Hz were assigned to a *cis* disubstituted olefin. The upfield olefinic resonance at δ 5.97 (H-21) showed strong HMBC correlations to nonprotonated carbon resonances at δ 89.7 (C-19) and 98.9 (C-23), and the downfield olefinic resonance at δ 6.05 (H-20) showed strong correlations to nonprotonated carbon resonances at δ 87.4 (C-22) and 100.4 (C-18). This suite of HMBC correlations identified an enediyne substructure in **1** (see fragment A in Figure 1). The olefinic resonance at δ 5.97 (H-21) showed a long range COSY correlation to a methine resonance at δ 5.04 (H-24), indicating that the carbon bearing the methine proton (C-24, δ 43.2) was attached to the C-23 alkyne carbon. A COSY correlation observed between the methine (δ 5.04, H-24) and a broad singlet at 10.0, which was not correlated to a carbon in the HSQC spectrum, and the chemical shift of the methine carbon (C-24, δ 43.2) suggested that C-24 had an NH substituent. HMBC correlations observed between the H-24 methine (δ 5.04) and the two alkyne carbon resonances at δ 87.4 (C-22) and 98.9 (C-23) confirmed the attachment of C-24 to the C-23 alkyne carbon.

A methine resonance at δ 5.14 (H-17) showed HMBC correlations to the alkyne carbon resonances at δ 89.7 (C-19) and 100.4 (C-18), which demonstrated that the methine carbon (C-17, δ 63.0) was linked to the second alkyne at C-18. The methine resonance at δ 5.04 (H-24) and the NH resonance at 10.0 both showed HMBC correlations to a deshielded resonance at δ 76.0 (C-25), assigned to a nonprotonated oxygen-bearing carbon, supporting attachment of this carbon (C-25) to C-24. HMBC correlations between a resonance at δ 63.5 (C-16), also assigned to a nonprotonated oxygenated carbon, and both of the proton resonances at δ 5.04 (H-24) and 5.14 (H-17) suggested that the

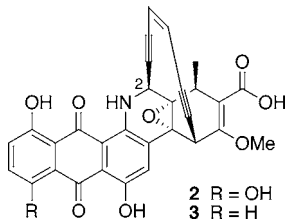
oxygenated carbon (C-16) was joined to both C-25 and C-17 to form a 10-membered ring (C-16−C-25) containing the enediyne substructure. A COSY correlation between the methine resonance at δ 5.14 (H-17) and a broad singlet at 6.66 (17-O*H*) revealed an alcohol functionality attached to the methine carbon.

A methyl doublet at δ 1.30 (Me-27, J = 6 Hz) was correlated in the COSY spectrum to a methine at 4.31 (H-26) that was further correlated to a broad singlet at 5.39 (26-O*H*), assigned to an alcohol. The methyl resonance (δ 1.30, Me-27) showed a HMBC correlation to the carbon resonance at δ 76.0 (C-25), indicating that the hydroxyethyl fragment (C-26 and C-27) was the fourth substituent on the nonprotonated carbon C-25. HMBC correlations observed from the methine at δ 4.31 (H-26) to the carbon resonances at δ 22.1 (C-27), 76.0 (C-25), 43.2 (C-24), and 63.5 (C-16) confirmed the bonds between C-24 and C-25 and between C-25 and C-16.

Both the N*H*-1 proton (δ 10.0) and the H-17 methine (5.14) were correlated to a carbon at δ 135.6 (C-15), and the H-24 methine (δ 5.04) was correlated to a carbon at 143.6 (C-2) in the HMBC spectrum, indicating that the NH and C-16 were vicinal substituents on an olefin or aromatic ring. A deshielded singlet at δ 8.51 (H-14) showed strong HMBC correlations into carbon resonances at δ 63.5 (C-16), 143.6 (C-2), and 112.7 (C-12) and a weak correlation into the carbon resonance at 154.9 (C-13). This set of HMBC correlations confirmed that the NH and C-16 were attached to a benzene ring. On the basis of the assumption that the intense HMBC correlations were through three bonds, these correlations also indicated that the aromatic methine (δ 8.51, H-14) was ortho to C-16 (δ 63.5) and meta to the NH (C-2, δ 143.6). The weak HMBC correlation between δ 8.51 and 154.9 was attributed to a two-bond coupling, placing the carbon at 154.9 (C-13) ortho to the methine carbon (C-14), and its chemical shift required an oxygen substituent.

The second fragment B of uncialamycin contained an isolated ^{1}H spin system comprised of four contiguous aromatic protons (δ 8.23, dd, J = 1.4, 7.6 Hz H-6; 7.88, ddd, 1.4, 7.6, 7.6 Hz H-7; 7.94, ddd, J = 1.4, 7.6, 7.6 Hz H-8; 8.24, dd, J = 1.4, 7.6 Hz H-9). HMBC correlations observed between the proton resonance at δ 8.23 (H-6) and a carbon resonance at 187.0 (C-4) and between the proton resonance at 8.24 (H-8) and a carbon resonance at 182.2 (C-11) suggested that the other two substituents on the benzene ring were quinone carbonyls. Fragments A and B shown in Figure 1 accounted for all of the carbon, hydrogen, and nitrogen atoms in the molecular formula of uncialamycin (**1**), but contained one extra oxygen atom. To complete the quinone and satisfy the remaining aromatic valences in fragment A, the two carbonyl carbons of fragment B (C-4 and C-11) had to be attached to the two substituted aromatic carbons (C-3 and C-12) of fragment A. Finally, it was apparent that the two oxygenated carbons, C-16 and C-25, had to be bridged by an epoxide to account for the number of oxygen atoms and sites of unsaturation required by the molecular formula of **1**. This implied that the C-13 oxygen substituent had to be part of a phenol functionality that would engage in intramolecular hydrogen bonding with the C-11 carbonyl consistent with the observed O*H* chemical shift of δ 13.2.

A strong 1D NOESY correlation observed between δ 4.31 (H-26) and 5.14 (H-17), when 4.31 was selectively irradiated, showed that C-26 and C-17 were *cis* oriented about the C-16/C-25 epoxide and also defined the relative configuration of C-17 as shown. Molecular models indicated that the C-17 to C-23 enediyne containing bridge likely had to be *cis* fused to the piperidine ring. Uncialamycin (**1**) is related to dynemicin A (**2**) and deoxidynemicin A (**3**) isolated from *Micromonospora chersina*.[3] The H-24 resonance in uncialamycin (**1**) has a chemical shift of δ 5.04 and a 4.6 Hz coupling to the N*H*-1 proton, which is nearly identical to the chemical shift (δ 5.05) and coupling (J = 4.3 Hz) of the corresponding methine proton (H-2) in dynemicin A (**2**), in agreement with the assigned C-16/C-24 relative configurations shown in **1**. Comparison of the additional NMR assignments reported for dynemicin A (**2**) and its triacetate derivative (see Supporting Information) provided further strong support for the proposed structure of uncialamycin (**1**). The relative configuration at C-26 in **1** could not be determined from the spectroscopic data, and the small amount of material available to date has thus far precluded chemical degradation efforts to elucidate this final structural feature.



The biogenetic origin of uncialamycin is of interest. On one hand, the obvious structural similarities that exist between uncialamycin (**1**) and dynemicin A (**2**) strongly imply that they arise from the same biosynthetic pathway. On the basis of stable isotope feeding studies, Iwasaki and co-workers proposed that the carbon skeleton of dynemicin A (**2**) arises from coupling of two separate heptaketide chains as shown in Figure 2A.[4] One of the chains forms the anthraquinone fragment, while the other leads to the enediyne fragment. They are linked together through two acetate carbonyl-derived carbons to form the C-8/C-9 bond. Interestingly, the C-30 carboxylic acid carbon in **2** comes from a methyl carbon of acetate.

As shown in Figure 2B, uncialamycin (**1**) might be a degraded analogue of dynemicin A (**2**) in which the C-5, C-6, and C-30 skeletal carbon atoms of a dynemicin-like precursor have been excised. Alternatively, the enediyne fragment of **1** could be formed from an undegraded hexaketide as shown in C, or the entire skeleton of **1** could be

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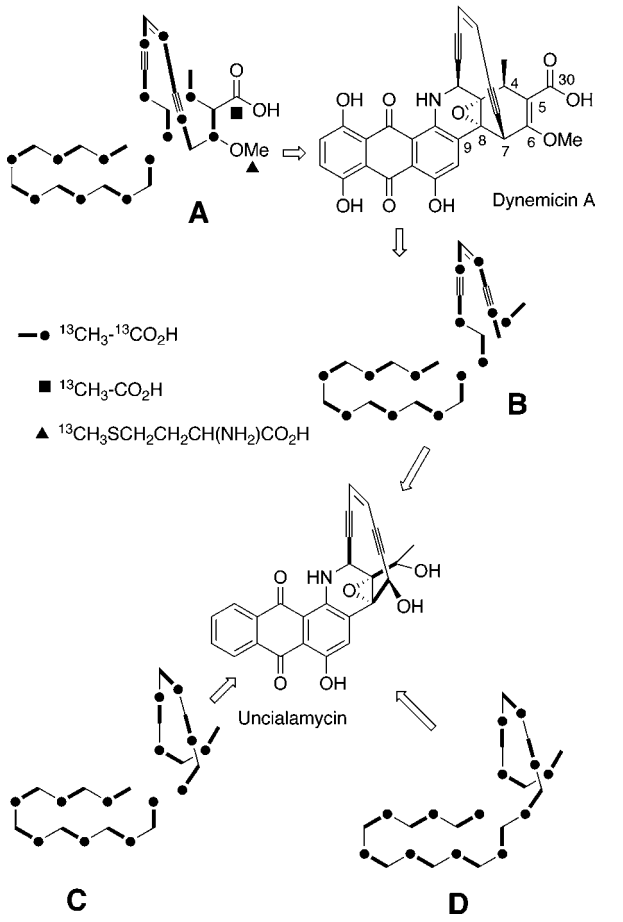

Figure 2. Possible biogenetic origins for uncialamycin (**1**).

formed from a single tridecaketide as shown in the one possible folding pattern D. Biogenesis C is particularly appealing because it retains the two polyketide chain template of dynemicin A biogenesis but does not require removal of a two or three carbon fragment from a dynemicin-like precursor. Stable isotope feeding experiments aimed at identifying the origin of the carbon skeleton in uncialamycin (**1**) are currently underway in our laboratory.

Uncialamycin (**1**) shows potent in vitro antibacterial activity against *Staphylococcus aureus* (MIC 0.0000064 μg/mL), *Escherichia coli* (MIC 0.002 μg/mL), and *Burkholderia cepacia* (MIC 0.001 μg/mL). All enediynes identified to date characteristically act on duplex DNA and cause single- and double-stranded breaks due to the action of benzenoid diradicals formed as a result of Bergmann rearrangement of the antibiotic molecule within the minor groove of the target DNA.[5,6] The identification of uncialamycin as an enediyne led to an examination of its activity as a DNA damaging agent. Initial studies indicate that uncialamycin interacts with plasmid DNA leading to extensive degradation.

The enediynes are potent antitumor agents and have been studied extensively for use in the form of targeted antibody complexes.[7] Although the enediynes have potent antibacterial activity, this class of natural products has not been developed for this purpose. The use of alternative delivery systems, especially in the treatment of serious lung infection, such as cystic fibrosis, has not been explored.

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Supporting Information Available: NMR spectra and NMR assignment comparisons for uncialamycin (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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