Eucapsitrione, an Anti-*Mycobacterium tuberculosis* Anthraquinone Derivative from the Cultured Freshwater Cyanobacterium *Eucapsis* sp.

Megan Sturdy,[†] Aleksej Krunic,[†] Sanghyun Cho,[‡] Scott Franzblau,[‡] and Jimmy Orjala*[,†]

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Room 539, Chicago, Illinois 60612, and the Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Room 412, Chicago, Illinois 60612

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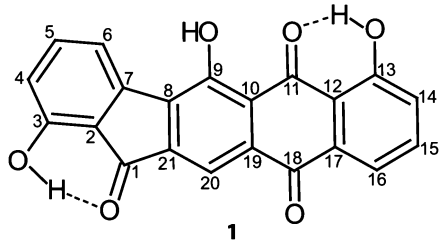
Eucapsitrione (**1**), an anthraquinone derivative with an indeno-anthracene-trione skeleton, was isolated from the cyanobacterium *Eucapsis* sp. (UTEX 1519) by bioassay-guided fractionation. The chemical structure was determined by analyzing MS and 1D and 2D NMR spectroscopic data. Eucapsitrione (**1**) showed anti-*Mycobacterium tuberculosis* activity in the microplate Alamar blue assay and low-oxygen-recovery assay with MIC values of 3.1 and 6.4 μM, respectively.

Cyanobacteria have proven to be a valuable source of novel bioactive agents.[1−3] A plethora of active secondary metabolites have been reported from several species of cyanobacteria.[1−3] There are, however, few reports of chemical investigations of the genus *Eucapsis*, all focused on identifying classes of compounds or proteins.[4−6] Members of the genus *Eucapsis* mostly grow in small colonies consisting of a minimal number of cells.[7] This minimal growth makes it difficult to obtain sufficient biomass for chemical investigations from field-collected material.[8] However, biomass from this genus can also be obtained from laboratory cultures.[4,9] In our ongoing search for anti-*Mycobacterium tuberculosis* compounds from cyanobacteria, the extract from a cultured freshwater *Eucpasis* sp. displayed potent anti-*M. tuberculosis* activity (MIC = 9.7 μg/mL).[9] Bioassay-guided fractionation yielded a novel anthraquinone derivative, eucapsitrione (**1**, MIC = 3.1 μM). Herein, we report the isolation, structure elucidation, and biological activity of eucapsitrione, the first anthraquinone derivative reported from a *Eucapsis* sp. and one of a few anthraquinone derivatives to be reported from cyanobacteria.[10,11]



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Eucapsitrione (**1**) was obtained as a red, amorphous solid. The molecular formula was determined by HRESIMS as $C_{21}H_{10}O_6$ (m/z = 357.04291 $[M - H]^-$), requiring 17 degrees of unsaturation. The ^{1}H NMR spectrum of **1** contained resonances for two phenolic hydroxy hydrogens (δ_H 13.28 and 13.99), as well as six aromatic signals, one of which integrated to two hydrogen atoms (δ_H 7.56) (Table 1), leaving one exchangeable proton. This phenolic proton was observed in a subsequent NMR experiment using dry DMSO (Table 1). The ^{13}C NMR spectrum revealed the presence of all 21 carbon signals suggested by the molecular formula and indicated the polyaromatic character of **1** (Table 1): three conjugated carbonyl moieties (δ_C 187.1, 184.8, 180.2), three aromatic carbons with oxygen substitution (δ_C 161.1, 177.2, 161.9), and 15 low-field carbon signals (seven methines δ_C 121.6, 135.2, 118.3, 122.8, 134.5, 117.3, 116.2 and eight quaternary carbon signals δ_C 137.2, 116.4, 126.3, 124.6, 118.2, 135.1, 130.3, 131.0).

Table 1. NMR Data of Eucapsitrione (**1**) in DMSO-d_6[a]

position	δ_C, mult.	δ_H, mult., J (Hz)	COSY	HMBC[b]
1	187.1, C			
2	137.2, C			
3	161.1, C			
4	121.6, CH	7.12, dd (7.9, 1.2)	H-5	2, 3, 7
5	135.2, CH	7.60, t (7.9)	H-4, H-6	2, 3
6	118.3, CH	7.56, m	H-5	4, 7, 9, 13, 17
7	116.4, C			
8	126.3, C			
9	177.2, C			
10	124.6, C			
11	184.8, C			
12	118.2, C			
13	161.9, C			
14	122.8, CH	7.14, dd (1.2, 7.6)	H-15	13, 15, 16
15	134.5, CH	7.56, m	H-14, H-16	4, 7, 9, 13, 17
16	117.3, CH	7.52,dd (7.6, 1.2)	H-15	12, 14, 18
17	135.1, C			
18	180.2, C			
19	130.3, C			
20	116.2, CH	7.15, s		1, 8, 9, 10, 16, 18, 19, 21
21	131.0, C			
OH at C3		13.28, s		1, 3, 4, 5, 7
OH at C9		6.59, s[c]		
OH at C13		13.99, s		11, 12, 13, 14, 15

[a] Frequency: 226 MHz for ^{13}C and 600 MHz for ^{1}H. [b] HMBC correlations are from proton(s) stated to the indicated carbon(s). [c] Observed in dry DMSO-d_6.

The final structure of **1** was assembled by analysis of 2D NMR data. The presence of three partial structures, 1−3 (Figure 1), was established by interpretation of COSY and HMBC data. The COSY spectrum revealed the presence of two CHCHCH spin systems indicative of two 1,2,3-trisubstituted aromatic rings (C-5 to C-4 and C-6, and C-15 to C-14 and C-16, Figure 1 and Table 1). Partial structure 1 was further deduced by HMBC correlations observed from H-4 to C-2, C-3, and C-7 and from the phenolic proton attached at C-3 to C-4, C-5, and C-7. An additional correlation from this phenolic proton to C-1 indicated a carbonyl moiety attached at C-2, effectively constructing partial structure 1 (Figure 1). Partial structure 2 was established as a pentasubstituted aromatic

* To whom correspondence should be addressed. Tel: 1-312-996-5583. Fax: 1-312-413-4034. E-mail: orjala@uic.edu.
[†] Department of Medicinal Chemistry and Pharmacognosy.
[‡] Institute for Tuberculosis Research.

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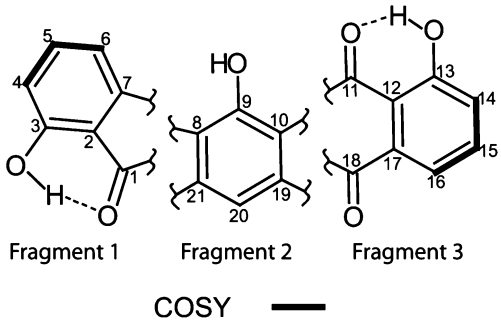

Figure 1. Partial structures 1−3 of eucapsitrione constructed from HMBC and COSY data. The dashed line represents a hydrogen bond.

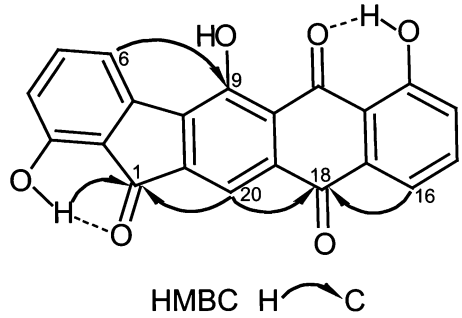

Figure 2. Key HMBC correlations of eucapsitrione (**1**). The dashed line represents a hydrogen bond.

ring by the HMBC correlations from H-20 to C-21, C-8, C-9, C-10, and C-19. The phenolic hydroxy was placed on C-9 to satisfy the carbon chemical shift of δ_C 177.2. The second 1,2,3-trisubstituted aromatic ring observed in the COSY spectrum was part of partial structure 3, which was further constructed by HMBC correlations observed from H-15 to C-13 and C-17 and from the C-13 phenolic proton to C-12, C-13, and C-14. Additional correlations from the C-13 phenolic proton to C-11 and from H-16 to C-18 revealed the presence of two carbonyl moieties, one attached at the C-12 and one attached at the C-17 positions, respectively, thus finalizing fragment 3.

These three partial structures were connected by HMBC correlations as shown in Figure 2. Partial structure 1 was connected to partial structure 2 by the correlations observed from H-6 to C-9 and from H-20 to C-1. Partial structure 2 was in turn connected to partial structure 3 by the correlations observed from H-20 to C-18 and from H-16 to C-18, establishing the C-19 and C-18 connection. The final connection between C-10 and C-11 was deduced by consideration of carbon NMR chemical shifts and the molecular formula. This resulted in the final structure of eucapsitrione (**1**), which satisfied the required 17 degrees of unsaturation.

Eucapsitrione (**1**) possessed an indeno-anthracene-trione skeleton that has previously only been derived from anthraquinone or anthracene derivatives by synthetic methods, and this molecular scaffold has not been reported from a natural source.[12,13] This also represents one of a few reported anthraquinone derivatives from cyanobacteria and the first report from the genus *Eucapsis* sp.[10,11]

Eucapsitrione (**1**) displayed anti-*M. tuberculosis* activity in the microplate Alamar blue assay (MABA) (MIC = 3.1 μM).[14] This assay is representative of rapidly growing *M. tuberculosis*; however, a physiological state of nonreplicating persistence (NRP) is responsible for the antimicrobial tolerance in *M. tuberculosis*.[15] The low-oxygen-recovery assay (LORA) has been developed to mimic this NRP phenotype of the bacterium.[15] Eucapsitrione also displayed anti- *M. tuberculosis* activity in the LORA assay (MIC = 6.4 μM). Eucapsitrione displayed no antimicrobial activity at 55 μM against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Mycobacterium smegmatis*.[16,17] These results suggest eucapsitrione to be a selective agent against *M. tuberculosis*. The cytotoxic effects of eucapsitrione were evaluated using the Vero cell assay, and the IC_{50} value was determined to be >28 μM.[18] In summary, eucapsitrione displayed potent activity in both the MABA and LORA tests with limited cytotoxic effects, and eucapsitrione may be a candidate for further synthetic optimization to increase potency toward *M. tuberculosis*.

Experimental Section

General Experimental Procedures. The UV spectrum was obtained on a Cary 50 Bio UV−visible spectrophotometer. IR spectra were obtained on a Jasco FTIR-410 Fourier transform infrared spectrometer. NMR spectra were obtained on a Bruker Avance DRX600 MHz NMR spectrometer with a 5 mm CPTXI Z-gradient probe and a Bruker AVII900 MHz NMR spectrometer with a 5 mm ATM CPTCI Z-gradient probe, referenced to the corresponding solvent peaks. High-resolution ESI mass spectra were obtained on a Shimadzu LCMS IT-TOF mass spectrometer.

Biological Material. *Eucapsis* sp. was acquired from the Culture Collection of Algae at the University of Texas at Austin (UTEX 1519). The cyanobacterium was grown aerated in a 13 L glass carboy with 12 L of inorganic media (Z45).[19] The culture was illuminated with fluorescent lamps at 1.93 klx with a 18/6 h light/dark cycle and maintained at a constant temperature of 22 °C. The biomass of cyanobacteria was harvest by centrifugation after five weeks and freeze-dried.

Extraction and Isolation. The freeze-dried biomass (3.81 g from 12 L) was extracted by repeated maceration with CH_2Cl_2/MeOH (1:1) to yield 381.8 mg of an extract. The extract showed inhibitory activity against *M. tuberculosis* (MABA MIC 9.7 μg/mL). The extract was fractionated on a Diaion HP20SS column using a step gradient with increasing amounts of 2-propanol in water (0%, 20%, 40%, 60%, 70%, 80%, 90%, and 100% 2-propanol/H_2O) to afford eight fractions. Fraction 3 (24.7 mg) was the most active fraction against *M. tuberculosis* (MABA MIC 5.6 μg/mL) and was further subjected to reversed-phase HPLC (Varian Dynamax C8, 10 μm, 250 × 10 mm, 4 mL/min) with a solvent gradient of MeOH/H_2O (5:95) to 100% MeOH over 35 min to afford four subfractions (SF1−SF4). Subfraction 3 (t_R = 10 min) was further separated using reversed-phase HPLC (Varian Dynamax C8, 10 μm, 250 × 10 mm, 4 mL/min) with a solvent gradient of MeOH/H_2O (30:70) to 100% MeOH over 25 min to afford four fractions (SSF1−SSF4). SSF4 (t_R = 21 min) proved to be eucapsitrione (**1**) (1.1 mg).

Eucapsitrione (1): red, amorphous powder; UV (MeOH) (log ε) λ_{max} (3.34) 488.0, (3.53) 405.1, (3.38) 282.0 nm; IR (neat) ν_{max} 1616, 1559, 1457, 1372, 1331, 1273, 1210, 1152 cm^{-1}; ^1H and ^{13}C (see Table 1); HRESIMS *m/z* 357.04291 $[M - H]^-$ (calcd for $C_{21}H_9O_6$, 357.03992).

Mycobacterium tuberculosis. The inhibitory activity of fractions and compounds against *M. tuberculosis* was determined using the microplate Alamar Blue assay and the low oxygen-recovery assay.[14,15] Virulent H37Rv strain was used in both assays. The MIC value was determined as the lowest drug concentration affecting an inhibition of ≥90%.

Other Organisms. The broth microdilution MIC method was used to test the activity of the compound against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Mycobacterium smegmatis*.[16,17]

Cytotoxicity. The cytotoxicity was evaluated using green monkey kidney cells (Vero).[18] Cell viability was measured using the CellTiter 96 aqueous nonradioactive cell proliferation assay.

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Supporting Information Available: 1D and 2D NMR spectra in DMSO-d_6 of eucapsitrione (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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