Natural Products

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Anthracimycin, a Potent Anthrax Antibiotic from a Marine-Derived Actinomycete**

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The human infectious disease anthrax is caused by the spore-forming, Gram-positive bacterium *Bacillus anthracis*. The disease, which is most common in those that handle infected farm animals, has also been used as a bioterrorism weapon. In 2001, just a week after the World Trade Center attacks, anthrax was deliberately spread through the US postal system by sending letters with powdered *B. anthracis* spores. This caused 22 cases of anthrax infection and ultimately claimed five lives.[1] Depending upon the method of exposure (inhalation of spores and direct bacterial contact, among others) *B. anthracis* infections can require prolonged treatment often for six months with a variety of antibiotics. The pulmonary form of anthrax is considered a medical emergency that may require continuous intravenous therapy with potent antibiotics. In the event of a bioterrorism attack, individuals exposed to *B. anthracis* will be given antibiotics prior to the onset of the illness. A vaccine has been developed but is not yet available to the general public.[2] Given the severity of this disease, and the fact that it can be spread by aerosol dispersal, the development of effective new antibiotics remains a high priority.

As a resource for developing microbial antibiotics, we have focused our efforts on marine microorganisms, particularly those that are in the deep oceans. Examination of a *Streptomyces* species (our strain CNH365), isolated from near-shore marine sediments found near Santa Barbara, CA, showed that culture extracts possessed significant activity against *B. anthracis* and methicillin-resistant *Staphylococcus aureus* in broth dilution assays. Subsequent fractionation of the extract following antibacterial activities yielded a pure antibiotic, anthracimycin (**1**). Anthracimycin was isolated as a white solid with the molecular formula $C_{25}H_{32}O_4$, determined by HR-ESI-MS analysis. The ^{13}C NMR spectrum of **1** (Table 1) displayed what appeared to be two ketone functionalities, one ester or lactone group ($\delta_C = 194.1$, 190.9, and 168.9), nine methine carbon signals in the aromatic/olefinic region ($\delta_C = 140$–103), and an oxymethine group ($\delta_C = 70.0$). Analysis of gCOSY NMR data for the olefinic protons at $\delta_H = 6.5$–5.4 revealed the presence of a conjugated

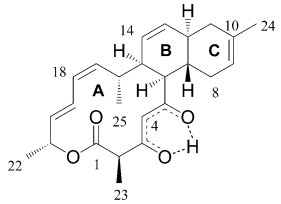

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Table 1: NMR Assignments for Anthracimycin (**1**) in $CDCl_3$.[a]

No.	δ_H (J in Hz)	δ_C	HMBC
1		168.9	
2	3.53, q, (7.0)	49.2	C-3,23
3		190.9	
4	5.96, s	103.0	C-2, 3, 5, 6
5		194.1	
6	2.58, dd (11.8, 6.7)	52.6	C-4, 5, 12, 15, 16
7	1.98, ddd (11.8, 12.5, 14.5)	37.4	C-9
8α	2.39, ddd (16.0, 4.5, 4.5)		
8β	1.52, ddd (4.5, 10.5, 16.0)	31.4	C-10
9	5.36, br d (4.5)	121.0	C-7, 11, 24
10		134.0	
11α	2.02, dd (16.5, 4.0)	37.5	C-9, 24
11β	1.82, dd (16.5, 10.3)		
12	2.64[b]	33.0	C-6, 10, 14
13	5.71, d (10.0)	133.0	C-15
14	5.53, dd (10.0, 5.0)	124.9	C-12, 15
15	2.60[b]	46.0	C-6, 13, 14, 16, 17
16	1.93, m	32.8	C-6, 15, 17, 18, 25
17	5.40, dd (10.5, 9.8)	139.1	C-15, 16, 19
18	5.87, dd (10.5, 11.0)	126.1	C-16, 19, 20
19	6.45, dd (15.2, 11.0)	123.7	C-17, 18, 20, 21
20	5.56, dd (15.2, 2.4)	131.7	C-18, 19, 21, 22
21	5.57, dq (2.4, 6.5)	70.0	C-1, 19, 20, 22
22	1.33, d (6.5)	21.0	C-20, 21
23	1.39, d (7.0)	11.8	C-1, 2, 3
24	1.67, s	23.5	C-9, 10, 11
25	0.94, d (7.0)	16.4	C-16, 17

[a] 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR spectra. [b] The coupling constant could not be measured because of overlapping signals.

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diene, an isolated *Z* olefin and two trisubstituted double bonds.

These spectroscopic data, taking into consideration the ten degrees of unsaturation from the molecular formula, revealed that anthracimycin was a tricarbocyclic metabolite. Clean separation of the signals in the ^{1}H NMR spectrum allowed identification of the main structural fragments by analysis of strong vicinal and weaker long-range correlations in the gCOSY NMR experiment. All gCOSY derived connectivities within the structural fragments were also supported by gHMBC data (Table 1). The interpretation of gCOSY, gHSQC, and gHMBC NMR data allowed a 14-membered macrolide ring containing an enolized β-diketone and a lactone to be identified and confirmed. The methyl proton signal at $\delta_H = 1.39$ (CH_3-23) exhibited long-range correlations with two carbonyl carbon signals $\delta_C = 168.9$ (C-1) and 190.9 (C-3), and a methine carbon signal $\delta_C = 49.2$ (CH-2). Additionally, H-21 ($\delta_H = 5.57$) correlated to the lactone carbonyl (C-1, $\delta_C = 168.9$), confirming the lactone macrolide linkage.

Anthracimycin (**1**) possesses asymmetric carbon centers at C-2, C-6, C-7, C-12, C-15, C-16, and C-21, the relative configurations of which were assigned on the basis of ROESY experiments and proton coupling constant analysis. Interpretation of 2D ROESY NMR cross peaks for H-12/H-8α and H-12/H-6, allowed these axial protons to be identified on the bottom face of the bicyclic system, whereas correlations of H-25/H-7, H-25/H-8β, and H-25/H-11β oriented these protons on the top face. These results coincided well with the coupling constants between key protons ($J_{6,7} = 11.8$ Hz, $J_{6,15} = 6.7$ Hz, $J_{7,8\beta} = 10.5$ Hz, $J_{7,12} = 14.5$ Hz, $J_{11\beta,12} = 10.3$ Hz), and allowed the assignment of a *cis* orientation for the A/B ring juncture and a *trans* orientation for the B/C ring juncture. Analysis of ^{1}H NMR data, showed a *cis* double bond at the C-17–C-18 position, and a *trans* double bond at C-19–C-20. These assignments were made based upon analysis of the vicinal coupling constants of 10.5 Hz and 15.2 Hz, respectively. These assignments were also supported by an observed NOE correlation between H-16 and H-19. NMR data, however, could not be used to define the relative configurations of the adjacent C-2 and C-21 methyl groups.

The full structure and absolute configurations at all asymmetric carbon centers of **1** were ultimately assigned based upon X-ray crystallographic data. X-ray-quality crystals were obtained from slow recrystallization of anthracimycin from CH_2Cl_2/MeOH/Et_2O solutions. The X-ray crystallography experiment showed the absolute configurations of the stereocenters in **1** to be 2*R*, 6*R*, 7*S*, 12*S*, 15*R*, 16*R*, and 21*R* (Figure 1 and Supporting Information). In addition, the X-ray experiment indicated significant disorder in the location of the enol proton of the enolized β-diketone. Thus, while NMR data have not confirmed the keto–enol tautomerization, X-ray data showed the proton to be virtually equidistant between each oxygen atom, thus suggesting a rapid keto–enol tautomerization.

The structure of anthracimycin is composed of a rare combination of 14-, 6-, and 6-membered rings of an apparent polyketide origin. A search of the literature showed no publications on this structure. However, a patent from 2011

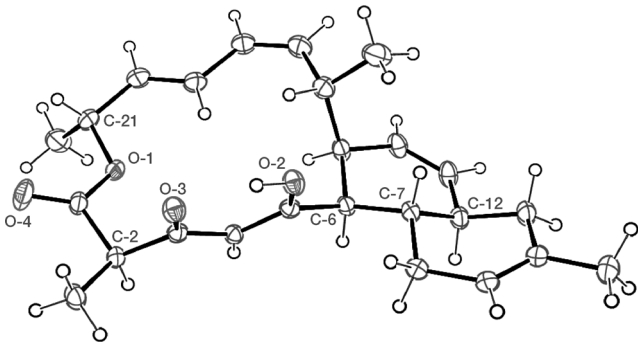
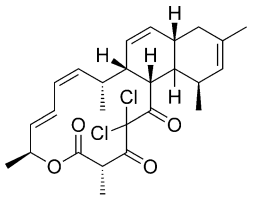


Figure 1. X-ray structure of anthracimycin illustrating its absolute configuration.

shows the planar structure of a compound, similar or possibly identical to **1**, devoid of stereochemical details and without illustration of the keto–enol tautomerization.[3] Because the full stereostructure of this compound was not defined and not peer reviewed, we cannot confidently compare these molecules.

While this class of compounds is extremely rare, a tricyclic metabolite with a similar carbon skeleton is found in chlorotonil (**2**), a rare metabolite produced by the unrelated



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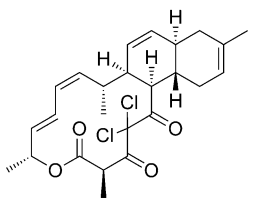

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myxobacterium *Sorangium cellulosum*.[4] Chlorotonil differs from **1** by the presence of an additional methyl group at C-8, a gem-dichloro functionality at C-4, and is enantiomeric at most of the relevant stereocenters. The synthesis of this unique metabolite was also reported[5] but no mention of its bioactivity has been made.

Anthracimycin is a potent antibiotic against *Bacillus anthracis* (strain UM23C1-1) showing a minimum inhibitory concentration (MIC) of 0.031 μg mL^{-1} in a broth microdilution assay.[6] The antimicrobial spectrum of anthracimycin also includes Gram-positive genera such as staphylococci, enterococci, and streptococci, but it either lacked activity or was weakly active against the Gram-negative species tested (Table 2). While the mechanism of action remains to be fully defined, it has been observed that **1** inhibits DNA/RNA synthesis in metabolic labeling experiments.[7] Follow-up gel-based studies demonstrated that the inhibition of nucleic acid synthesis likely does not occur because of intercalation into the DNA.[7] Despite a large MIC shift in the presence of mouse serum, early in vivo results from MRSA infected CD1 mice showed that **1** provided significant protection (90% survival) at 10 mg kg^{-1}.[7] A more thorough examination of the efficacy of **1** in whole animal models will clearly be important.[8]

Table 2: In vitro antibacterial activities of **1** and **3** against Gram-positive and Gram-negative pathogenic bacteria.

Pathogen	Strain	**1**, MIC [$\mu g\,mL^{-1}$]	**3**, MIC [$\mu g\,mL^{-1}$]
B. anthracis	UM23C1-1	0.03125	0.0625
S. aureus	ATCC 13709	0.0625	0.125
E. faecalis	ATCC 29212	0.125	0.5
S. pneumoniae	ATCC 51916	0.25	0.25
E. coli	MCR106 *imp*	>128	16
E. coli	MG1655 *tolC*	>128	>128
H. influenzae	ATCC 31517	>256	32
H. influenzae	ATCC 31517 KO	4	4
Burkholderia thailandensis	E264 KO	>256	32
Pseudomonas aeruginosa	PAO1 KO	>256	32

To determine the impact of chlorination on the bioactivity, as found in **2**, we prepared the analogous dichloro-anthracimycin derivative **3** by treatment of **1** with *N*-chlorosuccinimide in dichloromethane (see Supporting Information). Comparative bioassays conducted with **1** and **3** showed that the dichloro derivative was still significantly active, but had lost about half of its potency against *B. anthracis*. Interestingly, the potency of **3** was identical to **1** against *Streptococcus pneumoniae*, and was more active against the Gram-negative pathogens *Haemophilus influenzae*, *Burkholderia thailandensis*, *E. coli*, and *Pseudomonas aeruginosa*, perhaps indicating a greater ability to penetrate the Gram-negative cell wall.

Anthracimycin is a structurally unique compound that does not compare with any reported antibacterial natural products. Although somewhat similar in structure to chlorotonil (**2**), anthracimycin is a potent antibacterial metabolite with potential for the treatment of Gram-positive pathogens such as *Bacillus anthracis* and methicillin-resistant *Staphylococcus aureus* (MRSA). This compound shows promising preliminary activity in vivo (against MRSA)[7] and the dichloro derivative **3** has surprising activity against problematic Gram-negative pathogens, which suggests that there could be significant use for this new structural class of antibacterial molecules.

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[1] As defined by the US Center for Disease Control, see: http://www.bt.cdc.gov/agent/anthrax/needtoknow.asp.
[2] See Medicine Net website: http://www.medicinenet.com/anthrax.
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[6] Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—ninth edition, M07A9. Wayne, PA, CLSI, **2012**.
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[8] Comprehensive in vivo experiments, currently in progress, will be published elsewhere.

