ORGANIC LETTERS

2009
Vol. 11, No. 3
633−636

Mycangimycin, a Polyene Peroxide from a Mutualist *Streptomyces* sp.

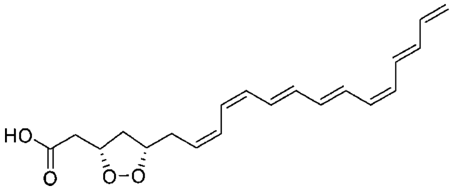
Dong-Chan Oh,[†] Jarrod J. Scott,[‡] Cameron R. Currie,[‡] and Jon Clardy*[,†]

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, and Department of Bacteriology, University of Wisconsin−Madison, Madison, Wisconsin 53706

jon_clardy@hms.harvard.edu

Received November 24, 2008

ABSTRACT



A mutualist actinomycete of the southern pine beetle, *Dendroctonus frontalis*, produces a polyene peroxide with pronounced antifungal activity. Its structure, absolute configuration, and biological activity were determined by spectral analysis, chemical modification followed by the modified Mosher method, and growth inhibitory assays, respectively.

Chemical ecology, the subfield of chemical biology that studies the roles of naturally occurring small molecules in mediating interactons between organisms, has uncovered many biologically active small molecules. One recent study illustrated both the biological complexity and surprising molecules that a detailed analysis of ecological interactions can reveal. The southern pine beetle (*Dendroctonus frontalis*) system, which has been well studied because of the economic losses to conifer production, has emerged as having one of the most complex natural associations with trees, fungi, mites, and beetles.[1] Recently we discovered another symbiont (*Streptomyces* sp. SPB74) that produces a polyene peroxide and demonstrated the ecological role of the bacterium and its polyene peroxide, which we named mycangimycin. Mycangimycin (**1**) selectively inhibits the beetle's fungal antagonist, *Ophiostoma minus*, while the mutualistic fungus *Entomocorticium* sp. is relatively resistant to **1**.[2] Here we report its complete structural determination including absolute configuration along with additional details of its biological activity.

Mycangimycin (**1**)[3] was isolated as a yellow powder, which was analyzed for the molecular formula $C_{20}H_{24}O_4$ by high-resolution mass spectrometry (chemical ionization, $[M + Na]^+$ at m/z 351.1565, calculated 351.1572) and ^1H and ^{13}C NMR spectral data (see Table 1). The ^1H NMR spectrum (in $CD_3OD/CDCl_3$ 5:2) showed 15 olefinic protons from 5.10 to 6.77 ppm, including an unusual terminal olefinic methylene group at 5.23 and 5.10 ppm, two oxygenated methine protons at 4.63 and 4.28 ppm, and six aliphatic protons between 1.97 and 2.89 ppm. The carbon-13 NMR and gradient heteronuclear multiple-quantum correlation (gHMQC) spectra displayed the expected 14 olefinic carbons between 118 and 139 ppm, one carbonyl carbon at 172.8 ppm, two oxygen-bearing carbons at 78.4 and 81.6 ppm, and three aliphatic carbon signals at 32.0, 40.2, and 45.9 ppm. The 14 olefinic carbon resonances were deduced to be seven conjugated double bonds from the UV spectrum (λ_{max} 355,

---

[†] Harvard Medical School.

[‡] Wisconsin−Madison.

(1) (a) Klepzig, K. D.; Moser, J. C.; Lombardero, F. J.; Hofstetter, R. W.; Ayres, M. P. *Symbiosis* **2001**, *30*, 83. (b) Klepzig, K. D.; Six, D. L. *Symbiosis* **2004**, *37*, 189. (c) Klepzig, K. D.; Smalley, E. B.; Raffa, K. F. *J. Chem. Ecol.* **1996**, *22*, 1367.

(2) Scott, J. J.; Oh, D.-C.; Yuceer, M. C.; Klepzig, K. D.; Clardy, J.; Currie, C. R. *Science* **2008**, *322*, 63.

(3) Mycangimycin (**1**): yellow powder; $[\alpha]^{24}_D$ −134 (c 0.033, THF); IR (neat) υ_{max} 3353, 2958, 2884, 1721, 1641, 1438, 1369, 1345, 1191, 1117, 1067, 956, 852 cm^{-1}; UV (THF) λ_{max} (log ε) 338 (4.2), 355 (4.3), 374 (4.4), 395 (4.3) nm; NMR spectral data, see Table 1; HR-CI-TOFMS $[M + Na]^+$ m/z 353.1565 ($C_{20}H_{24}O_4$) calcd $[M + Na]^+$ 353.1572.

10.1021/ol802709x CCC: $40.75

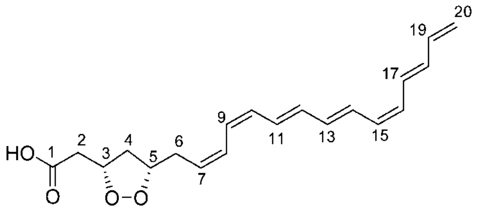
Table 1. NMR Spectral Data for Mycangimycin (**1**) in $CD_3OD/CDCl_3$ (5:2)

C/H	δ_H[a]	mult (J[b])	δ_C[c]	
1			172.8	C
2a	2.66	dd (16.0, 7.5)	40.2	CH_2
2b	2.51	dd (16.0, 6.0)		
3	4.63	m	78.4	CH
4a	2.89	ddd (12.5, 7.5, 7.5)	45.9	CH_2
4b	1.97	m		
5	4.28	m	81.6	CH
6a	2.57	m	32.0	CH_2
6b	2.48	m		
7	5.51	m	128.1	CH
8	6.63	dd (11.0, 11.0)	127.0	CH
9	6.27	dd (12.0, 11.0)	125.4	CH
10	6.13	m	131.2	CH
11	6.77	m	130.3	CH
12	6.75	m	130.1	CH
13	6.16	m	131.1	CH
14	6.49	m	125.8	CH
15	6.52	m	125.6	CH
16	6.08	dd (12.0, 10.0)	130.9	CH
17	6.73	dd (15.0, 12.0)	129.0	CH
18	6.28	dd (15.0, 12.0)	136.0	CH
19	6.43	ddd (15.0, 12.0, 12.0)	138.1	CH
20a	5.23	d (15.0)	118.1	CH_2
20b	5.10	d (12.0)		

[a] 600 MHz. [b] Coupling constants in Hz. [c] 150 MHz

374, and 395 nm). The numbers of the double bonds and the carbonyl carbon account for eight out of nine unsaturation equivalents. Therefore mycangimycin must possess one ring.

Interpretation of the gHMQC spectral data allowed all one-bond proton and carbon correlations to be assigned. Analysis of gradient correlation spectroscopy (gCOSY) and total correlation spectroscopy (TOCSY) revealed the single spin system in this molecule from C-2 to C-20. The gradient heteronuclear multiple bond correlation (gHMBC) NMR spectrum showed the correlations from H-2a (2.66 ppm) and H-2b (2.51 ppm) to C-1, which established the connectivity between the carbonyl carbon (C-1 at 172.8 ppm) and C-2. Other heteronuclear couplings in the gHMBC spectrum confirmed the structure previously connected by $^1H-^1H$ homonuclear correlations in COSY and TOCSY spectra to construct a 3,5-dihydroxy-icosa-7,9,11,13,15,17,19-heptaenoic acid like structure (Figure 1a).



1

Since the molecular formula has 24 protons and only 23 protons were observed in the ^1H NMR spectrum with a CD_3OD and $CDCl_3$ solvent mixture, one proton must be D_2O exchangeable. A broad proton peak was observed in the ^1H NMR spectrum in deuterated dimethylsulfoxide (DMSO-d_6) at 12.40 ppm, indicating a carboxylic acid proton as the exchangeable one. This observation required connecting the two oxygen atoms to make a five-membered peroxide (1,2-dioxolane) ring to complete the planar structure (Figure 1b).

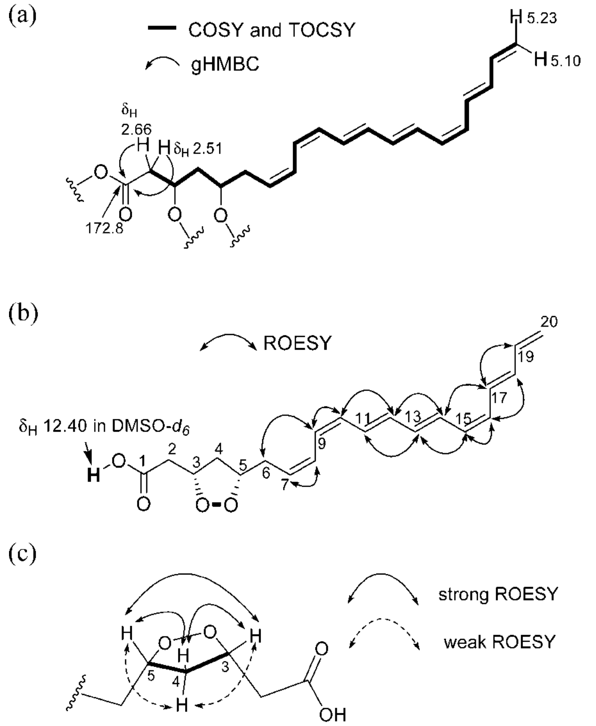
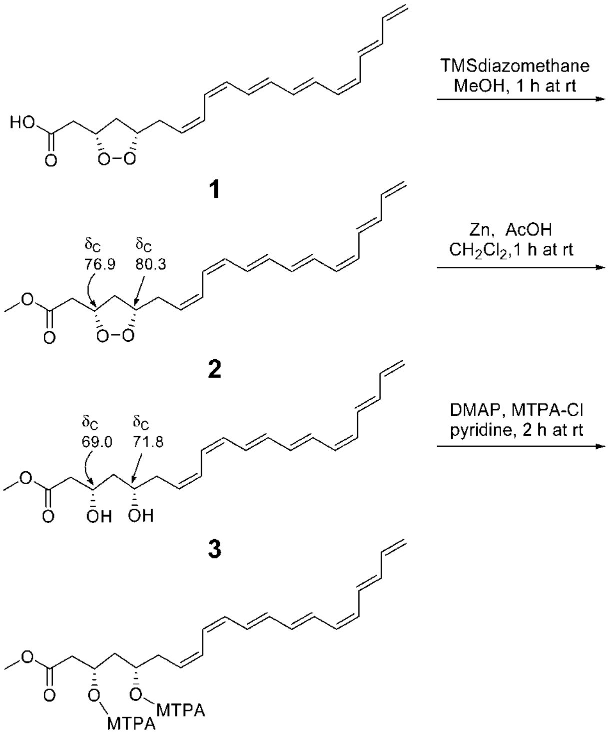


Figure 1. (a) 3,5-Dihydroxy-icosa-7,9,11,13,15,17,19-heptaenoic acid like structure constructed by gCOSY, TOCSY, and gHMBC correlations. (b) Observed D_2O exchangeable proton in DMSO-d_6 and ROESYcorrelations of the conjugated polyene. (c) Key ROESY correlations of the peroxide ring.

The double bond geometries in the conjugated heptaene moiety were determined from the proton−proton coupling constants and rotating-frame Overhauser effect spectroscopy (ROESY) correlations (Figure 1b). The coupling constant (11.0 Hz) between H-7 and H-8 clearly established the geometry as 7*Z*. The 9*Z* geometry was determined from the H-9−H-10 *cis*-coupling constant (12 Hz). A careful analysis of ROESY correlations observed between H-10 and H-12, between H-11 and H-13, between H-12 and H-14, between H-13 and H-15, and between H-14 and H-17 revealed 11*E* and 13*E* geometries. A 15*Z* geometry was assigned from the 10 Hz *cis*-coupling between H-15 and H-16, while the clear *trans*-coupling (15 Hz) between H-17 and H-18 along with the established ROESY coupling of H-17 and H-19 required 17*E*. The relative stereochemistry of the 1,2-dioxolane was deduced from ROESY correlations. The two protons attached to oxygen-bearing carbons at 4.63 (H-3) and 4.28 (H-5) ppm coupled strongly, indicating their *syn*-relationship. H-3 and H-5 showed strong correlations with H-4a at 2.89 ppm and weak correlations with H-4b at 1.97 ppm, also supporting this assignment (Figure 1c).

Scheme 1. Reaction Sequence for Determination of the Absolute Configuration of Mycangimycin



4a (*S*-MTPA) and **4b** (*R*-MTPA)

Mycangimycin (**1**) with its conjugated heptaene, 1,2-dioxolane, and carboxylic acid is a very sensitive molecule. When evaporated to dryness, it decomposes to an almost insoluble material. It behaves poorly on chromatographic resins such as C_{18} and silica. It is also light-sensitive. Establishing its absolute configuration required chemical modifications followed by extensive chromatographic purification.

First, a crude ethyl acetate extract of the bacterial culture was converted to methyl ester (**2**) by trimethylsilyl diazomethane, a sequence that confirmed the carboxylic acid functionality and gave a more soluble product (Scheme 1). The reaction mixture was directly dried with celite, which allowed dry loading on a C_{18} column. The product was then purified by step-gradient water−methanol solvent, and 100% methanol eluted almost pure methyl ester, which was washed with hexane four times to yield pure product **2**. Treatment with metallic zinc and acetic acid opened the peroxide ring to yield a diol (**3**), an important confirmation of the peroxide group. Product **3** was purified from the 80% methanol fraction from a C_{18} column in a similar manner as for the methyl ester (**2**). The formation of **3** was confirmed by ^{13}C chemical shifts of oxygen-bearing carbons (C-3 and C-5) as well as mass spectral data. After reduction, the ^{13}C chemical shifts were 69.0 and 71.8 ppm at C-3 and C-5, respectively, while the peroxide ring shifts were 76.9 and 80.3 ppm for the same carbons. Diol (**3**) was esterified with *R*- and *S*-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride to furnish the corresponding bis-*S*- and bis-*R*-MTPA esters, respectively (**4a** and **4b**). The MTPA esters were purified by reversed-phase HPLC with significant loss of material.

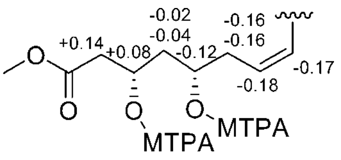


Figure 2. Delta values ($\Delta\delta_{S-R}$) in ppm for bis-*S*- and bis-*R*-MTPA esters (**4a** and **4b**).

The ^{1}H, gCOSY, and TOCSY experiments allowed the assignments of the ^{1}H chemical shifts for **4a** and **4b**. The analysis of $\Delta\delta_{S-R}$ values clearly determined the absolute configurations of C-3 and C-5 of **3** as 3*S* and 5*S* on the basis of the modified Mosher method for *syn*-1,3-diol described by Riguera (Figure 2).[4]

Table 2. Minimum Inhibitory Concentration (MIC) Values of **1** and Amphotericin B against Various Fungal Strains

strain	mycangimycin (MIC μg/mL)	amphotericin B (MIC μg/mL)
C. albicans wild type	0.2	0.4
C. albicans ATCC 10231	0.2	0.4
C. albicans ATCC 200955	0.4	2.5
S. cerevisiae	0.4	0.4
Penicillium sp.	6.2	6.2
O. minus	1.2	2.5
Entomocorticium sp.	19.2	50.0

A few 1,2-dioxolanes have been reported as natural products from the marine sponges *Plakortis* spp.,[5] but none from bacterial sources to the best of our knowledge. The biosynthesis of mycangimycin is not known, but the shifting of all seven double bonds from the usual positions in polyketide synthesis along with the formation of the 1,2-dioxolane ring raise interesting questions.

To fulfill its ecological role, mycangimycin has to inhibit *O. minus* more strongly than it inhibits *Entomocorticium* sp., but what about its ability to inhibit other fungi? As can be seen from Table 2, mycangimycin inhibits *Candida albicans* wild type, *C. albicans* ATCC10231, and amphotericin-resistant *C. albicans* ATCC 200955, and *Saccharomyces cerevisiae* with roughly the same potency it has against *O. minus*.

Mycangimycin's 1,2-dioxolane functionality is similar to pharmacophores with significant antimalarial activity,[6] the

---

(4) Frerie, F.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Org. Chem.* **2005**, *70*, 3778.

(5) (a) Phillipson, D. W.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 7735. (b) Davidson, B. S. *J. Org. Chem.* **1991**, *56*, 6722. (c) Constantino, V.; Fattorusso, E.; Menna, M.; Tagalialatela-Scafati, O. *Curr. Med. Chem.* **2004**, *11*, 1671.

1,2,4-trioxane in artimesinin,[7] and synthetic 1,2,4-trioxolanes.[8] In the antimalarial assay against *Plasmodium falciparum*,[9] mycangimycin had an EC_{50} of 17 ng/mL, comparable to clinical antimalarial drugs such as artemisinin, chloroquine, pyrimethamine, and mefloquine, which have EC_{50}'s in the 10 ng/mL range in the same assay. Mycangimycin's mode(s) of action as an antifungal or antimalarial agent is not known.

These biological assays lead to two important conclusions, one relevant to evolutionary ecology and the second to drug discovery. The *Streptomyces* SPB74 mediated system has evolved so that ascomycetes like *O. minus*, *S. cerevisiae*, and *C. albicans* are especially sensitive and basidiomycetes like *Entomocorticium* sp. are relatively resistant. Since mycangimycin is roughly as potent as amphotericin B, which is a currently prescribed polyene antifungal agent despite its many liabilities, studying insect−bacterial mutualisms like the SPB74-southern pine beetle could be a powerful discovery tool for antifungal, antimicrobial, and other potentially useful compounds. This approach is especially attractive as bacterial−insect mutualisms are clearly widespread.[10]

Acknowledgment. We thank Prof. M. C. Yuceer at Mississippi State University and Dr. K. D. Klepzig at the U.S. Department of Agriculture Forest Service for important observations and the materials. We thank Dr. M. L. Baniecki at Harvard School of Public Health and Joseph Cortese at the Broad Institute for providing the antimalarial data. This work was supported by NIH CA24487 (J.C.).

Supporting Information Available: Experimental section and NMR spectra of **1−4a/4b**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL802709X

---

(6) Tang, Y.; Dong, Y.; Vennerstrom, J. L. *Med. Res. Rev.* **2004**, *24*, 425.

(7) Eckstein-Ludwig, U.; Webb, R. J.; van Goethem, I. D. A.; East, J. M.; Lee, A. G.; Kimura, M.; O'Neill, P. M.; Bray, P. G.; Ward, S. A.; Krishna, S. *Nature* **2003**, *424*, 957.

(8) Vennerstrom, J. L.; Arbe-Barnes, S.; Brun, R.; Charman, S. A.; Chiu, F. C. K.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayam, M.; Tomas, J. S.; Scheurer, C.; Scorneaux, B.; Tang, Y.; Urwyler, H.; Wittlin, S.; Charman, W. N. *Nature* **2004**, *430*, 900.

(9) Baniecki, M. L.; Wirth, D. F.; Clardy, J. *Antimicrob. Agents Chemother.* **2007**, *51*, 716.

(10) (a) Currie, C. R.; Scott, J. A.; Summerbell, R. C.; Malloch, D. *Nature* **1999**, *398*, 701. (b) Kaltenpoth, M.; Göttler, W.; Herzner, G.; Strohm, E. *Curr. Biol.* **2005**, *15*, 475.