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Coibacins A–D, Antileishmanial Marine Cyanobacterial Polyketides with Intriguing Biosynthetic Origins

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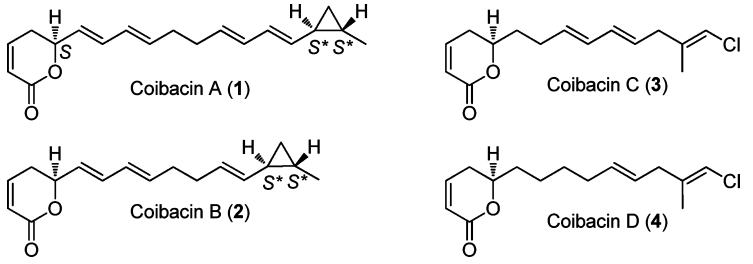
ABSTRACT



Four unsaturated polyketide lactone derivatives, coibacins A–D, were isolated from a Panamanian marine cyanobacterium, cf. *Oscillatoria* sp. The two different types of termini observed in these co-occurring metabolites, either a methyl cyclopropyl ring as seen in curacin A or a methyl vinyl chloride similar to that observed in the jamaicamides, suggest an intriguing flexibility in the "beta branch" forming biosynthetic process. The coibacins possess selective antileishmanial activity as well as potent anti-inflammatory activity.

The secondary metabolites of filamentous marine cyanobacteria show a surprising degree of structural diversity and biological activity.[1] For example, a methyl cyclopropyl ring is found in the curacin series of anticancer metabolites isolated from *Moorea producens* (formerly *Lyngbya majuscula*[2]) (Figure 1, compound **5**),[3] whereas vinyl chloride moieties are present in the jamaicamides (e.g. **6**), isolated from a Jamaican collection of *M. producens*,[4] as well as in most of the more than two dozen malyngamide natural products.[5] Study of the biosynthetic mechanism of formation of these two moieties revealed they are variant products of closely related manifolds, differing only in the

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(1) Tidgewell, K.; Clark, B. R.; Gerwick, W. H. In *Comprehensive Natural Products II Chemistry and Biology*; Mander, L., Lui, H.-W., Eds.; Elsevier: Oxford, UK, 2010; Vol. 2, pp 141–188.

(2) Engene, N.; Rottacker, E. C.; Kastovsky, J.; Byrum, T.; Choi, H.; Ellisman, M. H.; Komárek, J.; Gerwick, W. H. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 1171–1178.

(3) Gerwick, W. H.; Proteau, P. J.; Nagle, D. G.; Hamel, E.; Blokhin, A.; Slate, D. L. *J. Org. Chem.* **1994**, *59*, 1243–1245.

(4) Edwards, D. J.; Marquez, B. L.; Nogle, L. M.; McPhail, K. L.; Goeger, D. E.; Roberts, M. A.; Gerwick, W. H. *Chem. Biol.* **2004**, *11*, 817–833.

(5) Reviewed in: (a) Burja, A. M.; Banaigs, B.; Abou-Mansour, E.; Burgess, J. G.; Wright, P. C. *Tetrahedron* **2001**, *57*, 9347–9377. (b) Van Wagoner, R. M.; Drummong, A. K.; Wright, J. L. C. *Adv. Appl. Microbiol.* **2007**, *61*, 89–217.

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site of proton delivery following enoyl CoA hydratase mediated decarboxylation and, subsequently, enoyl reductase catalyzed hydride addition in the case of curacin A to form the cyclopropyl ring.[6] Previously, these two distinctive structural features have never been reported from a single organism, and their co-occurrence here suggests an intriguing flexibility in the biosynthetic manifold.

Prefractionation of the CH_2Cl_2/MeOH extract of a cf. *Oscillatoria* species collected in the Coiba National Park as part of the Panama International Cooperative Biodiversity Group (ICBG) resulted in several fractions with potent activity to axenic amastigotes of *Leishmania donovani*.[7] Purification using RP-SPE followed by RP HPLC (C_{18}) gave coibacins A−D (**1**−**4**) as the active compounds (Figure 1). ^{1}H NMR analysis indicated that all four compounds contained an α,β-unsaturated δ-lactone. However, metabolites **1** and **2** exhibited signals typical of a methyl-substituted cyclopropyl ring, whereas compounds **3** and **4** showed signals diagnostic for methyl vinyl chloride moieties (Table 1).

High-resolution mass measurement of the sodiated molecular ion for coibacin A (obsd m/z $[M + Na]^+$ 307.1670, calcd m/z 307.1669) yielded a molecular formula of $C_{19}H_{24}O_2$ with 8 degrees of unsaturation. By COSY and HMBC, **1** was determined to possess two conjugated dienes separated by two methylene groups, and by coupling constant analysis (all J values > 12 Hz), the double bonds were entirely *trans* (Table 1). At one end, this linear chain was linked to the unsaturated lactone ring and at the other to the methyl cyclopropyl ring, thus completing the planar structure. The absolute configuration of the α,β-unsaturated lactone ring was determined using circular dichroism. The CD of **1** exhibited a positive Cotton effect at λ 259 nm, which matches well with the *S* configuration of model α,β-unsaturated lactone rings.[8] However, because coibacin A has other UV chromophores adjacent to chiral centers, and these could potentially contribute to the CD, we also meaured the CD of compound **4** (see below) as it lacks any chromophore other than the unsaturated lactone. The CD curves of **1** and **4** were nearly superimposible, thus substantiating our assignment of the configuration of C-5.

The relative configuration of the methyl-substituted cyclopropyl ring of **1** was determined using NOESY correlations and J coupling constant analysis (Table 1). Correlations were observed between the adjacent vinyl proton (H-15, δ 5.15), one of the diastereotopic H_2-17's (δ 0.55), and methine H-18 (δ 0.76). Additional correlations were found between methine H-16 (δ 1.07), the other H-17 (δ 0.49), and H_3-19 (δ 1.05). Taken together with the coupling constants between these protons (Table 1), these data indicated that coibacin A (**1**) possessed a *trans*-configured methyl cyclopropyl ring (*S**,*S**). Unfortunately, because of the low yield of natural product, the absolute configurations at C-16 and C-18 were not assigned.

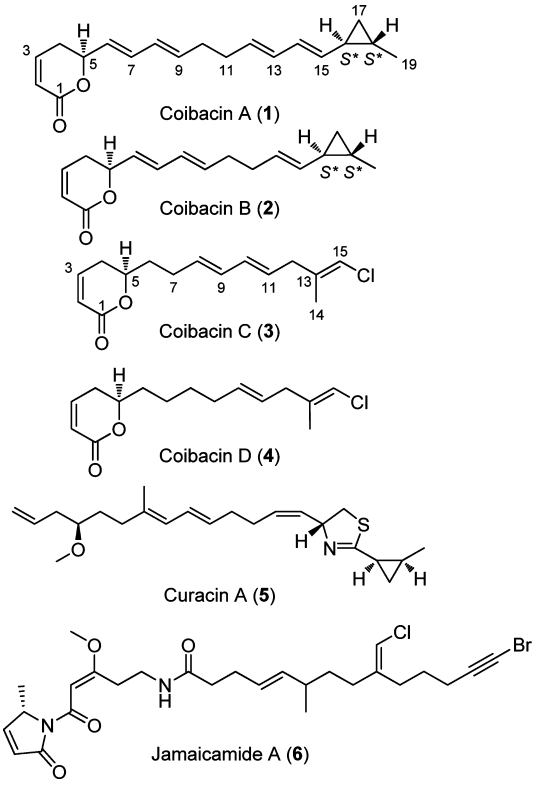

Figure 1. Structures of coibacins A−D (**1**−**4**) with two other marine cyanobacterial metabolites of related biosynthetic origin, curacin A (**5**) and jamaicamide A (**6**).

The unsaturated chain of coibacin B (**2**) was found to be two carbons shorter than in **1** by HRMS and NMR analysis. This deletion was localized to one less olefin in the diene motif adjacent to the methyl cyclopropyl ring. In all other respects, including optical rotation, the spectroscopic data for **2** were essentially identical to those recorded for coibacin A (**1**).

While coibacins C (**3**) and D (**4**) had spectroscopic features similar to coibacins A and B, they were clearly of a different structural series due to their molecular formulas, which both contained one chlorine atom. Coibacin C (**3**), of molecular formula $C_{15}H_{19}ClO_2$ (m/z $[M + Na]^+$ 289.0968), possessed the same α,β-unsaturated-δ-lactone as **1** and **2**, but oxymethine H-5 was approximately 0.5 ppm upfield and by COSY was located adjacent to a methylene rather than an olefin. COSY correlations sequentially extended this spin system to a second methylene (H_2-7), a conjugated diene (*E*,*E* by J values, see Table 1), and a bis-allylic methylene (δ 2.79, H_2-12). HMBC correlations connected the latter doublet to quaternary C-13, methyl C-14, and olefinic C-15 of an adjacent terminal olefin with β-chloro and α-methyl substituents. Comparison of the ^{1}H and ^{13}C NMR shifts for **3** and for model

(6) Gu, L.; Wang, B.; Kulkami, A.; Geders, T. W.; Grindberg, R. V.; Gerwick, L.; Hakansson, K.; Wipf, P.; Smith, J. L.; Gerwick, W. H.; Sherman, D. H. *Nature* **2009**, *459*, 731–735.

(7) Williams, C.; Espinosa, O. A.; Montenegro, H.; Cubilla, L.; Capson, T. L.; Ortega-Barría, E.; Romero, L. I. *J. Microbiol. Methods* **2003**, *55*, 813–816.

(8) Beecham, A. F. *Tetrahedron* **1972**, *28*, 5543–5554.

Table 1. NMR Data for Coibacins A–D (**1**–**4**) in $CDCl_3$

	coibacin A (**1**)		coibacin B (**2**)		coibacin C (**3**)		coibacin D (**4**)	
no.	δ_C,[a] mult	δ_H,[b] mult, *J* (Hz)	δ_C,[a] mult	δ_H[b],mult., *J* (Hz)	δ_C,[a] mult	δ_H,[b] mult, *J* (Hz)	δ_C,[a] mult	δ_H,[b] mult, *J* (Hz)
1	164.0 (C)		164.0 (C)		164.2 (C)		164.5 (C)	
2	121.7 (CH)	6.05,[c] m	121.7 (CH)	6.05,[c] m	121.5 (CH)	6.03, m	121.5 (CH)	6.02, dt, 1.9, 9.7
3	144.5 (CH)	6.88, dt, 4.4, 9.8	144.5 (CH)	6.88, dt, 4.9, 9.8	144.8 (CH)	6.88, dt, 4.9, 9.7	144.9 (CH)	6.88, ddd, 3.6, 5.1, 9.8
4	29.8 (CH_2)	2.45, m	29.8 (CH_2)	2.45, m	29.4 (CH_2)	2.35, m	29.4 (CH_2)	2.33, m
5	77.9 (CH)	4.94, dt, 7.6, 7.8	77.9 (CH)	4.95, m	77.9 (CH)	4.43, m	77.9 (CH)	4.42, m
6	126.7 (CH)	5.64, dd, 6.7, 15.5	126.6 (CH)	5.64, dd, 6.7, 15.3	34.4 (CH_2)	1.73, m	34.7 (CH_2)	1.64, m
						1.92, m		1.79, m
7	133.7 (CH)	6.30, dd, 10.7, 15.7	133.8 (CH)	6.31, dd, 10.5, 15.4	27.8 (CH_2)	1.25,[c] m	24.3 (CH_2)	1.39, m
								1.51, m
8	129.1 (CH)	6.05,[c] m	129.0 (CH)	6.05,[c] m	131.0 (CH)	6.05, m	29.1 (CH_2)	1.39, m
9	136.8 (CH)	5.77, dt, 6.6, 15.2	137.1 (CH)	5.78, dt, 6.6, 15.3	131.6 (CH)	5.57, m	32.2 (CH_2)	2.03, dt, 6.7, 7.0
10	32.6 (CH_2)	2.16, m	32.8 (CH_2)	2.15, m	128.5 (CH)	5.51, m	126.6 (CH)	5.47, dt, 7.3, 15.1
11	32.1 (CH_2)	2.16, m	32.0 (CH_2)	2.07, m	132.3 (CH)	6.05, m	132.8 (CH)	5.34, dt, 6.9, 15.2
12	129.9 (CH)	5.50, dt, 7.3, 14.5	126.4 (CH)	5.43, dt, 6.9, 15.3	40.1 (CH_2)	2.79, d, 7.1	40.2 (CH_2)	2.72, d, 6.8
13	130.8 (CH)	5.97, m	134.3 (CH)	5.00, m	137.4 (C)		137.9 (C)	
14	127.4 (CH)	6.05,[c] m	22.4 (CH)	1.03, m	16.7 (CH_3)	1.78, s	16.6 (CH_3)	1.75, d, 1.2
15	136.3 (CH)	5.15, dd, 9.4, 14.9	14.8 (CH_2)	0.41, ddd, 4.7, 5.6, 8.2	113.1 (CH)	5.82, br s	112.6 (CH)	5.81, q, 1.5, 2.7
				0.48, ddd, 4.5, 4.5, 8.2				
16	22.8 (CH)	1.07, m	14.8 (CH)	0.70, m				
17	15.7 (CH_2)	0.49, ddd, 4.7, 5.3, 8.2	18.5 (CH_3)	1.05, d, 6.2				
		0.55, ddd, 4.7, 4.7, 8.4						
18	15.7 (CH)	0.76, m						
19	18.5 (CH_3)	1.05, d, 6.0						

[a] Measured at 100 MHz. [b] Measured at 400 MHz. [c] Obscured due to overlapping ^1H signals

compounds from the jamaicamide and malyngamide families of metabolites confirmed the identity of this moiety,[4,5] and NOE (H-15 to H_2-12) was used to define the *E*-geometry of this trisubstituted olefin.

Coibacin D (**4**) was of two mass units greater than **3** (*m*/*z* $[M + Na]^+$ 291.1124) and consistent with this was found to lack one of the two olefins forming the conjugated diene in **3**; by COSY this saturation was localized to the C-8/C-9 position. The geometry of the Δ^{10} olefin was *trans* based on a 15.1 Hz vicinal *J* value, and the Δ^{13} olefin was assigned as *E* given very similar ^1H and ^{13}C NMR chemical shifts to those of coibacin C (**3**). Coibacins C and D had optical rotations similar to that of coibacins A and B, and as noted above, coibacin D (**4**) had a CD absorption curve consistent with the *S*-configuration of an α,β-unsaturated-δ-lactone (see the Supporting Information).

Purified coibacins A–D (**1**–**4**) were tested for activity in the Panama ICBG suite of tropical disease assays (Figure S36, Supporting Information). Coibacin A (**1**) presented potent activity against *Leishmania donovani* axenic amastigotes with an IC_{50} value of 2.4 μM, while the other coibacins exhibited slightly less activity in this assay (Table 2). The antileishmanial activity of the coibacins was confirmed using *L. mexicana* axenic amastigotes (Figure S37, Supporting Information); however, when a *L. mexicana* macrophage assay was used they were inactive, perhaps indicating a failure to cross the cell membrane of these latter cell types.

The coibacins were also tested for activity against malaria and Chagas' disease and were inactive (IC_{50} values > 25 μM). In testing against NCI-H460 human lung cancer cells, coibacin D (**4**) was the most cytotoxic, whereas coibacin A (**1**) was least cytotoxic (Table 2). From these data, tentative therapeutic indices against *Leishmania* parasites for **1**–**4** were calculated to be 13.1, 2.4, 1.1, and 1.5, respectively; thus, coibacin A possesses the greatest selectivity and may be a lead for development of an antileishmanial drug candidate.

Table 2. Biological Assay Data for Coibacins A–D (**1**–**4**) in *anti*-Leishmanial, Cytotoxicity, and Nitric Oxide Assays (IC_{50} Values in μM)

	Leishmania donovani axenic amastigotes	cytotoxicity (NCI-H460 cells)	nitric oxide
coibacin A (**1**)	2.4	31.5	20
coibacin B (**2**)	7.2	17.0	5
coibacin C (**3**)	18.7	21.3	11
coibacin D (**4**)	7.8	11.4	21

The coibacins were also tested for anti-inflammatory activity in a cell-based nitric oxide (NO) inhibition assay.[9] In this assay, coibacin B (**2**), was determined to be the most active (Table 2); however, because coibacin A was more abundant, it was further evaluated in gene transcription (TNF-α, IL-6, IL-1b, and iNOS) and protein expression

(9) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. *Anal. Biochem.* **1982**, *126*, 131–138.

studies. Coibacin A (**1**, at 10 μg/mL) was found to significantly reduce gene transcription of all four cytokines tested, with especially notable effects on IL-1β and iNOS (Figure 2). Using ELISA, changes in protein expression for some of these inflammatory cytokines were measured in murine RAW264.7 cells stimulated with lipopolysaccharide (LPS) in the absence or presence of the coibacins. Coibacin A (**1**, at 10 μg/mL) was found to significantly reduce TNF-α and IL-6 secretion (Figure S38 and S40, Supporting Information). Coibacins B−D (**2**−**4**) also affected protein expression of TNF-α and IL-6 albeit with slightly less potency (Figures S39 and S41, Supporting Information).

The isolation of both cyclopropyl ring and methyl vinyl chloride moieties from the same organism suggests intriguing biosynthetic possibilities when compared to the metabolic pathways of curacin A (**5**) and jamaicamide A (**6**). The latter two pathways, originally located in two different source organisms, were found to have high sequence identity for the first three enzymatic steps involved in beta branch formation [HMG Co-A synthase (HCS), halogenase (HAL), and dehydratase (ECH_1)], and these steps are partially responsible for formation of the two different functional groups.[6] There is considerably less sequence identity for the decarboxylase (ECH_2) and the enoyl reductase (ER) enzymes. The isolation of co-occurring metabolites containing these two unique structural moieties from a single organism may indicate variability in the site of proton delivery (α vs γ) following ECH_2-catalyzed decarboxylation (Figure S42, Supporting Information). Subsequently, in the latter case of γ-proton delivery, ER-catalyzed hydride delivery to the β-carbon leads to cyclopropyl ring formation; no further reaction occurs in the former case of α-proton addition. Another structural feature distinguishing the coibacin A/B series from the C/D series is chain length following the β-branch manifold; thus, it is possible that the variable presence of either the cyclopropyl ring or vinyl chloride moieties determines subsequent PKS extension steps.

It should be noted that the cyclopropyl ring of the coibacins A and B are of *trans* stereochemistry, which contrasts with the *cis* cyclopropyl ring of curacin A. Thus, there must be additional, as yet unstudied, points of divergence between these two biosynthetic pathways.

In summary, we report here the isolation and structure elucidation of coibacins A−D (**1**−**4**), polyketide metabolites possessing novel structures with provocative biosynthetic motifs. These cyanobacterial metabolites possess

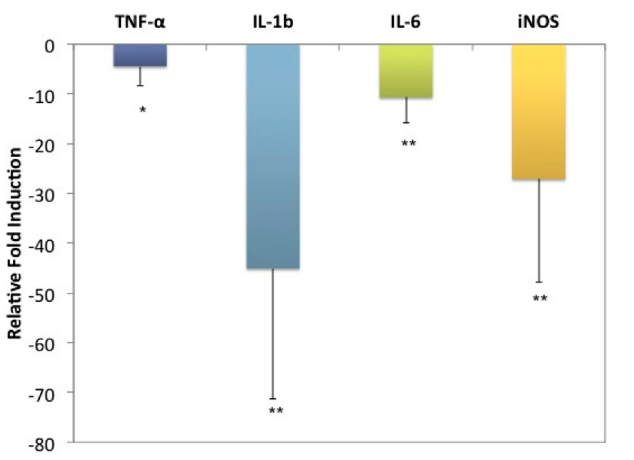

Figure 2. Transcription of pro-inflammatory genes after treatment with coibacin A (**1**) at 10 μg/mL (bars represent the mean ± standard deviation; $N = 3$. *P value < 0.05 compared to LPS treatment alone. **P value < 0.01 compared to LPS treatment alone).

significant antileishmanial and anti-inflammatory activities and, thus, may be useful lead structures for drug development. Additionally, their co-occurrence gives insight into how Nature has developed alternative processing to produce cyclopropyl rings versus vinyl chloride moieties.

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Supporting Information Available. Experimental procedures, full spectroscopic data for new compounds, additional bioassay data, organism photograph, phylogenetic classification, and biosynthetic proposal. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.