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Helioxenicins A-C: Diterpenes from the Blue Coral *Heliopora coerulea*

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Abstract: Three new diterpenes, helioxenicins A-C (**2-4**), have been isolated from the blue coral *Heliopora coerulea*. The structures were initially determined from NMR spectroscopic data and confirmed by an X-ray analysis of helioxenicin B.

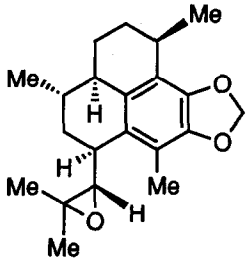
INTRODUCTION

The blue coral *Heliopora coerulea* is the sole extant member of the octocorallian order Coenothecalia, and is unique from other octocorallian corals in that it possesses a solid tubular skeleton composed of aragonite, resembling scleractinian reef-building corals in its gross morphology.[1] It is also a source of highly functionalised diterpenes, exemplified by helioporin A (**1**).[2,3] We have now discovered that other diterpenes are present in the same coral, but belonging to the xenicane class. Such diterpenes have already been found in the alcyonacean genus *Xenia*[4] and in gorgonian species.[5] We describe the isolation and determination of structure of helioxenicins A-C from two samples of *H. coerulea*, one collected in Okinawa (A-C) and the other near Orpheus Island, Great Barrier Reef (A,C).

RESULTS AND DISCUSSION

The extraction of freshly collected *H. coerulea* with methanol or methanol/ dichloromethane gave an oil which by chromatography afforded pure samples of helioxenicins A-C.

Helioxenicin A (**2**) was obtained as a gum. The molecular formula of $C_{26}H_{36}O_{10}$ was established by HR EIMS. The presence of three acetate functions [IR: 1740 cm^{-1}; ^1H NMR in C_6D_6: δ 1.65 (s, 3H), 1.70 (s, 3H), 1.84 (s, 3H); ^{13}C NMR: δ 20.3 q, 20.4 q, 20.8 q, 169.0 s, 169.1 s, 169.9 s] indicated that the rest of the molecule was a diterpene. The presence of four additional sp^2 carbon atoms revealed an exocyclic methylene group (δ 143.5 s, 115.7 t) and a trisubstituted double bond bearing an oxygen atom (δ 142.2 d, 112.2 s). Four additional double bond equivalents were indicative of a tetracyclic structure. Two rings were assigned to disubstituted and trisubstituted epoxide groupings, since four of the remaining seven oxygenated sp^3 carbon atoms had resonances at δ 59.1 d, 62.5 d, 58.0 s and 61.8 d, in the ^{13}C NMR spectrum. The presence of a dioxygenated carbon atom (δ 92.1 d), an oxygen-bearing sp^2 carbon atom (δ 142.2), and the three remaining oxygenated sp^3 carbon atoms (δ 70.2 s, 71.0 d, and 74.1 d) were consonant with an ether-type linkage and an alcohol [IR (CCl_4) 3590, 3500], since the acetates occupy three of these oxygenated sites and eight of the ten oxygen atoms were accounted for by the three acetates plus the two epoxides. Three aliphatic methyl groups [^1H NMR: δ 1.28 (s, 3H), 1.05 (s, 3H), 1.20 (s, 3H); ^{13}C NMR: δ 19.2, 24.2, 32.9], three methylene carbon atoms (δ 27.1, 32.6, 33.1) and two methine carbon signals (δ 31.2, 44.3) completed the spectrum. The ether-type linkage meant that only one carbocyclic ring, namely, the xenicane skeleton[4,5] accommodated all the elements indicated by the spectra. 2D NMR (COSY, CHCOSY) studies revealed the connectivity, and allowed complete assignment of the ^1H and ^{13}C data (Table 1). Some cross peaks: C1 to H3; C7 to H8, H18; C8 to H18; C10 to H19; C11 to H10, H11a; C15 to H16, H17; in long range CHCOSY (J = 10 Hz) facilitated structure elucidation. The full relative stereochemistry was secured by converting helioxenicin A to helioxenicin B, the structure of which was solved by an X-ray crystallographic study.



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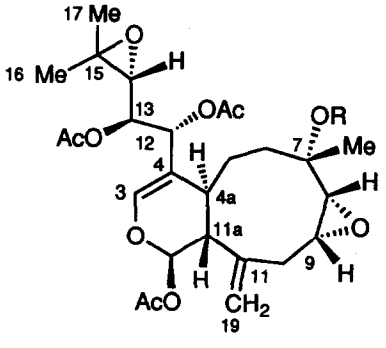


2 R = H, **3** R = Ac,

Helioxenicin B (3) was obtained as a crystalline solid, mp 166-168°C (EtOH). The molecular formula of $C_{28}H_{38}O_{11}$ was established by HR EIMS. The similarity of the ^1H and ^{13}C nmr data (Table 1) to that of **2** immediately suggested that **3** was simply a further acetylated derivative of **2**. This was confirmed by the acetylation of **2**, which gave **3**. The structure and assignments for **3** were established by comparing the spectral data of **3** with those of **2** and confirmed by X-ray analysis of a single crystal (Figure 1).

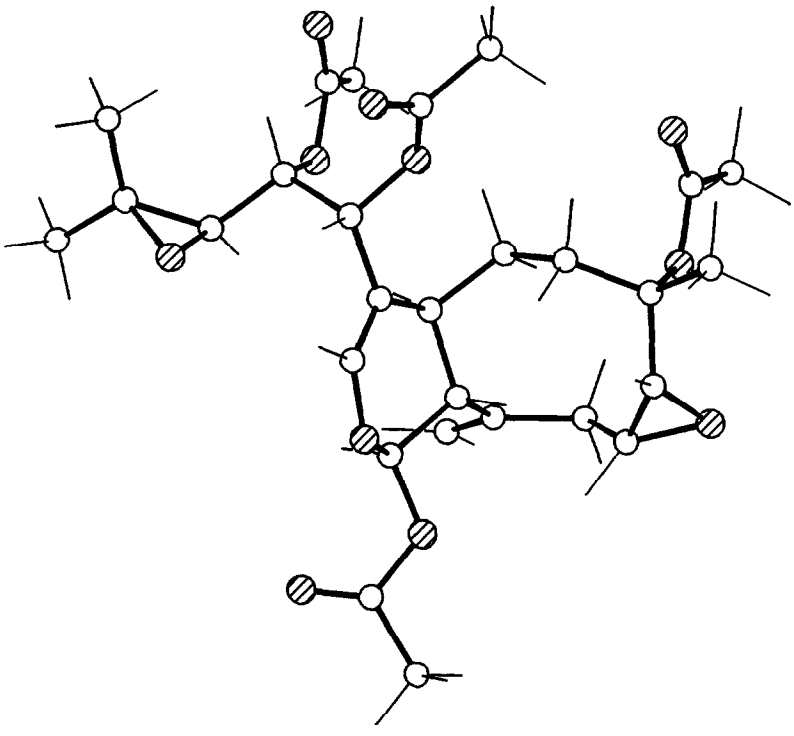


Figure 1. Perspective view of the crystal structure of helioxenicin B. The oxygen atoms are shaded

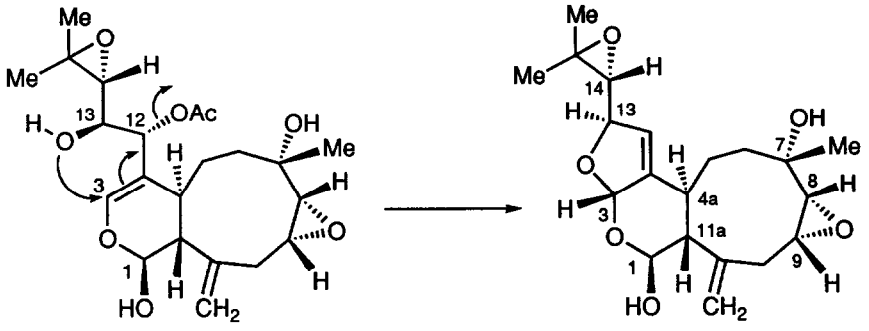
Helioxenicin C (4) was obtained as a crystalline solid, mp 154-156°C (hexane-benzene). The molecular formula of $C_{20}H_{28}O_6$ was established from HR EIMS by measurement of the $(M - H_2O)^{\cdot+}$ peak. The structure and full assignments for helioxenicin C were obtained from 2D NMR experiments, including COSY, CHCOSY, and HMBC. The following long-range ^1H-^{13}C correlations (HMBC, J = 7Hz), were used to derive the structure of the xenicane side chain: H3 to C12, C13; H12 to C3, C13; H14 to C12, C13, C16; H16 to C14, C15, C17; H17 to C14, C15, C16. All the other long-range correlations were consistent with the same ring structure and substitution pattern which characterized the helioxenicin A nucleus. The relative stereochemistry of **4** was initially proposed from the presumed course of its formation from the helioxenicin A analogue **5**. The hydroxyl group at C13 was assumed to have attacked the *re* face of the C3 atom in an SN2' process with displacement of the C12 acetate group.

Table 1. NMR Data for Helioxenicins A (2), B (3), and C (4) in C_6D_6 (500 MHz)

C No.	Helioxenicin A (2) ^{13}C	^1H	Helioxenicin B (3)[7] ^{13}C	^1H	Helioxenicin C (4) ^{13}C[a)]	^1H
1	92.1 d	6.38 d, J = 5.5	92.1 d	6.57 d, J = 5.5	98.0 d	4.98 dd, J = 3.3, 3.3
3	142.2 d	6.73 d, J = 1.5	132.6 d	6.73 s	103.6 d	6.27 s
4	112.2 s		110.9 s		143.8 s	
4a	31.2 d	3.42 br ddd, J = 6.8, 6.8, 6.8	33.8 d	2.87 m	30.0 d	4.08 br dd, J = 10.0, 11.0
5	27.1 t	1.73 m	27.0 t	2.03 m	25.5 t	1.59 m
		2.15 m		2.20 m		1.48 ddd, J = 2.0, 14.7, 14.7
6	33.1 t	1.52 m	31.4 t	1.61 m	34.1 t	1.26 br d, J = 14.7
		1.95 m		2.49 ddd, J = 5.6, 5.6, 15.0		1.84 ddd, J = 2.6, 14.7, 14.7
7	70.2 s		78.6 s		72.1 s	
8	62.5 d	2.28 d, J = 4.0	63.1 d	2.14 d, J = 4.3	61.8 d	2.35 d, J = 4.4
9	59.1 d	2.75 ddd, J = 4.0, 4.0, 11.0	57.7 d	2.73 m	61.0 d	2.89 ddd, J = 4.4, 4.4, 11.4
10	32.6 t	2.56 dd, J = 4.0, 11.0	34.7 t	2.73 m	29.8 t	2.78 dd, J = 4.4, 13.0
		2.85 dd, J = 11.0, 11.0		2.93 br dd, J = 12.0, 12.0		3.07 ddd, J = 2.0, 11.4, 13.0
11	143.5 s		143.6 s		143.5 s	
11a	44.3 d	2.72 dd, J = 5.5, 6.8	40.3 d	2.87 m	57.1 d	2.37 dd, J = 3.3, 11.0
12	74.1 d	5.82 d, J = 7.0	74.5 d	5.70 d, J = 7.3	118.9 d	5.75 br s
13	71.0 d	5.27 dd, J = 7.0, 8.1	71.0 d	5.09 dd, J = 7.3, 7.7	84.7 d	4.76 br d, J = 8.8
14	61.8 d	2.91 d, J = 8.1	61.9 d	2.88 d, J = 7.7	64.9 d	3.01 d, J = 8.8
15	58.0 s		57.7 s		59.4 s	
16	19.2 q	1.28 s	19.2 q	1.20 s	18.6 q	1.17 s
17	24.2 q	1.05 s	24.3 q	1.03 s	24.4 q	1.34 s
18	32.9 q	1.20 s	27.2 q	1.65 s[b)]	32.0 q	1.17 s
19	115.7 t	4.85 s; 5.01 s	113.5 t	4.82 s; 4.93 s	119.0 t	4.93 s; 4.95 s
AcO	169.0 s;		168.7 s;			
	169.1 s;		168.8 s;			
	169.9 s;		169.5 s;			
			169.7 s;			
	20.3 q	1.65 s;	20.4 q	1.65 s;		
	20.4 q	1.70 s;	20.4 q	1.70 s;		
	20.8 q	1.84 s;	20.7 q	1.73 s[b)];		
			22.1 q	1.84 s;		

a) ^{13}C data in $CDCl_3$ solution measured at 75MHz. b) Interchangeable.
Assignments are based on 2D NMR (COSY, CHCOSY) for **2**, and (COSY, CHCOSY, HMBC) for **4**.
Assignments for **3** are by COSY and comparison with **2**.

The relative stereochemistry of H13 and H14 was assigned from the vicinal coupling constant ($J_{13,14}$ = 8.8 Hz) which compares well with that observed for helioxenicin A ($J_{13,14}$ = 8.1 Hz). The vicinal coupling constant displayed by the 8,9-epoxide ring (J = 4.4 Hz) is typical of a *cis*-epoxide; a *trans*-epoxide would give a smaller value, e.g. J = approx. 2.5 Hz.[6] Although not proven, the stereochemistry at C7 was assumed to be the same as that in helioxenicins A and B. Evidence for the *trans* ring fusion of the xenicane ring system was provided by the bridgehead coupling constant ($J_{4a,11a}$ = 11.0 Hz). Since no NOE was observed between H3 and H1, H3 and H4a, or H3 and H13, the β-orientation was assigned to H3. This orientation is consistent with the proposed biogenesis of **4** from **5**.



EXPERIMENTAL SECTION

Extraction and Isolation. A fresh sample (20 kg) of the blue coral *Heliopora coerulea*, collected at -15 m off Komesu, Okinawa in April, 1989, was twice extracted by steeping in MeOH (16 L) for several days. The combined extracts were concentrated, and the resulting aqueous suspension extracted with EtOAc. The organic layer gave 14 g of an oil. The oil was first separated by flash chromatography on silica gel using 1:1 hexane-EtOAc, EtOAc, and 1:1 EtOAc-MeOH. The combined residue (3.61 g) of the third and fourth fractions was further separated on a silica gel flash column using hexane-EtOAc. The fraction (1.87 g) eluted with 1:1 hexane-EtOAc was separated on a Lobar RP-18 column by eluting with 10:1 MeOH-H_2O to give two fractions. The first (930 mg) of them was subjected to repeated HPLC (Hibar Si60, 1:1 hexane-EtOAc) to give helioxenicin B (**3**, 113 mg), helioxenicin A (**2**, 30 mg), and helioxenicin C (**4**, 5.5 mg).

Another sample (718 g) of *H. coerulea*, collected at -6 m from Cattle Bay, Orpheus Island in November, 1989, was exhaustively and successively extracted with CH_2Cl_2 and MeOH to yield a crude extract (2.89 g). Subsequent rapid chromatography on silica gel gave 15 fractions. Further HPLC separation of fractions 10 and 11 (Technoprep 5-20 and Si 100 7 μm columns in tandem, 20:7 petroleum ether-EtOAc) afforded helioxenicin A (**2**, 106 mg) and helioxenicin C (**4**, 24 mg).

Helioxenicin A (2). Gum, $[\alpha]_D^{24}$ -4.2° (*c* 0.60, $CHCl_3$), IR (CCl_4) 3590, 3500, 2980, 1740, 1660, 1380, 1225, 1180, 1030 cm^{-1}; UV (MeOH) λ_{max} 206 nm (ε 5500); ^1H NMR (300 MHz, $CDCl_3$) δ 6.54 (1H, d, *J* = 1.5, H3), 6.10 (1H, d, *J* = 5.6, H1), 5.47 (1H, d, *J* = 6.6, H12), 5.07 (1H, s, H19), 5.04 (1H, s, H19), 4.94 (1H, dd, *J* = 6.6, 8.2, H13), 3.20 (1H, m, H4a), 3.00 (1H, ddd, *J* = 4.8, 4.8, 9.7, H9), 2.74 (1H, d, *J* = 4.8, H8), 2.73 (2H, m, H10), 2.80 (1H, d, *J* = 8.2, H14), 2.66 (1H, dd, *J* = 5.6, 5.6, H11a), 2.05 (3H, s, OAc), 2.04 (3H, s, OAc), 2.01 (3H, s, OAc), 2.00 (1H, m, H5), 1.95 (2H, m, H6), 1.72, (1H, m, H5), 1.38 (3H, s, H18), 1.29 (3H, s, H16), 1.26 (3H, s, H17); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1 s, 169.3 s, 170.0 s (acetates), 142.2 s (C11), 141.8 d (C3), 116.0 t (C19), 111.5 s (C4), 91.7 d (C1), 73.5 d (C12), 70.8 d (C13), 70.2 s (C7), 62.5 d (C8), 61.4 d (C14), 59.1 d (C9), 58.2 s (C15), 43.9 d (C11a), 33.2 q (C18), 32.9 t (C6), 32.3 t (C10), 31.2 d (C4a), 26.4 t (C5), 24.2 q (C17), 20.7 q, 20.9 q, 21.1 q (acetates), 19.1 q (C16); EIMS *m/z* 508 (M^+, 7), 493 (2), 449 (8), 95 (100 rel%); CIMS (NH_3) *m/z* 526 ($M+NH_4^+$, 5), 509 ($M+H^+$, 5), 484 (11), 449 (36), 424 (19), 407 (37), 389 (54), 347 (100 rel%), 329 (86), 289 (83); HR EIMS obsd 508.2306, calcd for $C_{26}H_{36}O_{10}$ 508.2306.

Helioxenicin B (3). mp 166-168°C (EtOH); $[\alpha]_D^{24}$ +34° (*c* 0.22, $CHCl_3$), IR (CCl_4) 2980, 1740, 1670, 1380, 1220, 1175, 1040 cm^{-1}; UV (MeOH) λ_{max} 206 nm (ε 5300); EIMS *m/z* 550 (M^+, 5), 490 (18), 447 (7), 95 (100 rel%); HR EIMS obsd 550.2404, calcd for $C_{28}H_{38}O_{11}$ 550.2411. Crystallographic data: μ = 0.089 mm^{-1}, F(000) = 1176, d_X = 1.24 $g.cm^{-3}$, orthorhombic, $P2_12_12_1$, Z = 4, a = 11.484(1), b = 13.366(1), c = 19.229(3) A°, V = 2951.6(6), a colourless prism (0.22 x 0.26 x 0.30 mm) mounted on a quartz fibre. Cell dimensions and intensities were measured at room temperature on a Nonius CAD4 diffractometer with graphite-monochromated Mo[Kα] radiation (λ = 0.71069 A°), ω-2θ scans, scan width 1.2° + 0.25 tg θ, and scan speed 0.02-0.14°/s. Two reference reflections measured every 45 min showed variations less than 3.0 σ (I). 0 < h < 13; 0 < k < 15; 0 < 1< 22; 2666 measured reflections, 2607 unique reflections of which 2095 were observable (|Fo| > 4 σ (Fo)); R_{int} for equivalent reflections 0.013. Data were corrected for Lorentz and polarisation effects, but not for absorption. The structure was solved by direct methods by using MULTAN 87,[8] all other calculations used the XTAL system[9] and ORTEP[10] programmes. Atomic scattering factors and anomalous dispersion terms were taken from tables.[11] Full-matrix least-squares refinement based on F using a weight of $1/\sigma^2$ (Fo) gave final values of R = 0.062, ωR = 0.045, and S = 2.18 for 352 variables and 2095 contributing reflections. The maximum shift/error on the last cycle was 0.007. Hydrogen atoms were placed in calculated positions and the 39 other atoms were refined with anisotropic displacement parameters. The final difference electron density map showed a maximum of +0.29 and a minimum of -0.34 $eA^{\circ -3}$. Atomic coordinates and displacement parameters, bond lengths, bond angles and torsional angles have been deposited with the *Cambridge Crystallographic Data Centre*, University Chemical Laboratory, 12 Union Road, Cambridge, CB2 1EZ, England.

Helioxenicin C (4). mp 154-156°C (hexane-benzene); $[\alpha]_D^{24}$ -92° (*c* 0.10, $CHCl_3$); IR (KBr) 3480, 2930, 1460, 1385, 1320, 1115, 1080, 1010, 985, 920 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.08 (1H, br s, H3), 5.73 (1H, br s, H12), 5.17 (1H, s, H1), 5.16 (1H, s, H19a), 5.15 (1H, s, H19b), 4.57 (1H, br d, *J* = 8.7 Hz, H13), 3.83 (1H, dd, *J* = 10.7, 10.7 Hz, H4a), 3.05 (1H, ddd, *J* = 4.4, 4.4, 11.4 Hz, H9), 2.85 (1H, m, H10a), 2.76, (1H, m, H8), 2.62 (1H, d, *J* = 8.7 Hz, H14), 2.39 (1H, dd, *J* = 3.3, 10.4 Hz, H11a), 1.75 (1H, m, H5a), 1.39 (3H, s, H16), 1.36 (3H, s, H18), 1.33 (3H, s, H17); CIMS (NH_3) *m/z* 347 (MH^+-H_2O, 100 rel%), 331 (11), 329 (14), 273 (19), 247 (13), 229 (14), 221 (23), 201 (13), 191 (23) 177 (22), 163 (31), 149 (26), 135 (30), 125 (33), 109 (44) ; HR EIMS obsd 346.1779, calcd for $C_{20}H_{28}O_6$-H_2O 346.1780.

Acetylation of Helioxenicin A (2) to give Helioxenicin B (3). Helioxenicin A (2), 5.0 mg, was dissolved in 0.8 ml of dry pyridine and 0.8 ml of acetic anhydride, then 2.5 mg of 4-dimethylaminopyridine was added. The mixture was stirred under a nitrogen atmosphere for 24 h at room temperature. The reaction mixture was partitioned between EtOAc and 1N HCl and the organic layer washed with 1N NaOH and water. After evaporation of the solvent, the mixture was subjected to HPLC (Cosmosil $5C_{18}$-AR, 3:2 MeOH-H_2O) to give 1.3 mg (24%) of helioxenicin B (3) and 2.6 mg (52%) of the starting material. The HPLC, IR and ^1H NMR data of synthetic **3** were identical to those of natural **3**.

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