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METABOLITES OF THE NEW CALEDONIAN SPONGE *CLADOCROCE INCURVATA*

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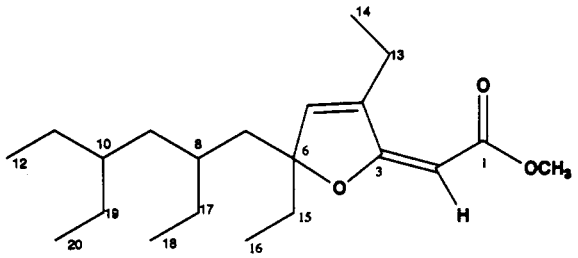
ABSTRACT.—The deep-water New Caledonian sponge *Cladocroce incurvata* contains two "polyketide" metabolites. Cladocrocin A [**1**] appears to be derived from fatty acids with ethyl side chains, thus incorporating butyrate units. Cladocroic acid [**2**] is a straight chain fatty acid which incorporates a terminal enyne functionality and a cyclopropane ring directly attached to the carboxylic acid function. The structures were elucidated by interpretation of spectral data, and the cis stereochemistry of the cyclopropane ring in cladocroic acid [**2**] was derived after the synthesis of *cis*- and *trans*-2,3-methanohexanoic acid models and nmr spectral comparisons.

In the course of our continuing studies on natural products from New Caledonian marine organisms, we undertook an investigation of the extracts of the deep water sponge *Cladocroce incurvata* Lévi, C. & Lévi, P. (family Renieridae) collected south of New Caledonia at ca. 500 m depth. This led to the isolation of two new compounds, cladocrocin A [**1**] and cladocroic acid [**2**], both appearing to be derived biosynthetically from fatty acids via the polyketide pathway.

The sponge was freeze-dried and extracted in a Soxhlet apparatus with *n*-hexane. Mplc of the extract on Si gel followed by reversed-phase hplc provided the pure cladocrocin A [**1**] and cladocroic acid [**2**].

Cladocrocin A [**1**], $[\alpha]_D$ 50.9°, yielded the molecular formula $C_{21}H_{36}O_3$ by hreims. ^{1}H- and ^{13}C-nmr spectra indicated the presence of two trisubstituted double bonds [δ_H 6.20 (br s) and 4.80 (s), δ_C 166.9 (s), 140.3 (s), 140.0 (d), 83.8 (d)] and of a methyl ester group [δ_H 3.68 (3H, s); δ_C 171.5 (s) and 50.5 (q)]. Three of the four required degrees of unsaturation are accounted for in the ^{13}C-nmr data, indicating that cladocrocin A [**1**] must contain one ring. The ^{13}C-nmr spectrum also contained signals for a fully substituted oxygen-bearing carbon at δ_C 98.1 (s) and for five methyl groups at δ_C 8.1, 10.4, 10.5, 10.8, and 12.1.

In the ^{1}H-nmr spectrum, one methyl triplet at δ_H 1.16 (3H, t, J=7 Hz) is coupled to a methylene signal downfield-shifted to δ_H 2.18 (2H, br q, J=7 Hz), thus indicating the presence of one ethyl group on a double bond. The four remaining methyl signals overlap and resonated as an apparent quartet centered at δ_H 0.79 (12H), but when the ^{1}H-nmr spectrum was measured in C_6D_6 four distinct triplet signals at δ 0.68 (3H), 0.81 (6H), 0.89 (3H), 0.91 (3H) were observed, indicating that all the methyl groups belong to ethyl residues. Cladocrocin A [**1**] showed uv absorption at λ max (hexane) 282 nm (ε=8300), suggesting that the olefinic bonds are conjugated to the ester functionality. The unusual olefinic carbon signals at δ 83.8 (d) and 166.9 (s) along with the highfield-shifted olefinic proton at δ 4.80, which is correlated with the signal at δ 83.8 [HMQC experiment (1)] (Table 1) suggested that a double bond is involved in an enol ether function. These signals were similar to the C-2 [84.4 ppm, δ_H 4.81 (s)] and C-3 (166.0 ppm) of the ester **7**, one major metabolite from the EtOH extracts of the sponge *Plakortis halichondrioides* (2). Thus, these data al-



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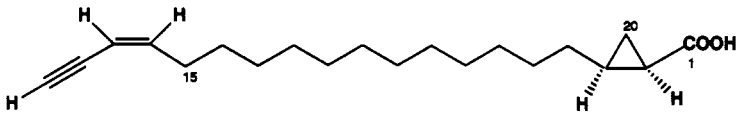

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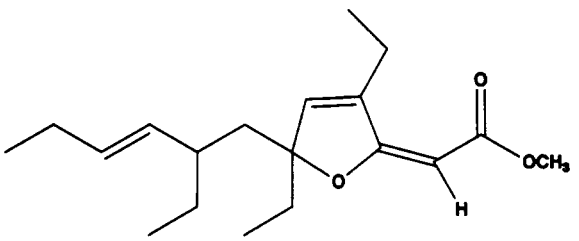

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lowed us to define the furanoid portion of the molecule. The 2*E* geometry in cladocrocin A [**1**] is derived from the chemical shift of the C-2 proton (δ 4.81), which was the same as that of the C-2 proton in ester **7** and was close to the calculated value (δ 4.85) for the 2*E* geometry (3). An HMBC experiment (4,5) (Table 1) supported the furanoid structure of cladocrocin A [**1**] and revealed, interestingly, cross peaks between the methoxy signal at δ 3.68 (s) and the carbon signal observed at δ 166.9 (C-3) and between the signals observed at δ 6.20 (H-5) and 171.5 (C-1). These unusual $^5J_{CH}$ couplings were observed because of the high degree of conjugation of cladocrocin A [**1**]. Futhermore, the HMBC experiment revealed cross peaks between signals observed at δ 1.72 and 1.86 (CH_2-15) and carbon signals at 98.1 (C-6, via $^2J_{CH}$) and at 140.0 (C-5, via $^3J_{CH}$), which confirmed the location of an ethyl residue on the quaternary C-6.

The COSY experiment revealed the presence of a methylene group [δ 1.62 dd (15, 7.5 Hz)–1.72 dd (15, 3.8 Hz)] placed between the quaternary carbon C-6 and a methyne carbon, thus allowing location of the third ethyl residue at C-8. The remaining two ethyl groups can be placed at C-10 by biosynthetic considerations. Accordingly, the structure for the natural ester was assigned as **1**. Related "polyketide" compounds which appear to be derived from fatty acids with ethyl side

TABLE 1. Selected ^{1}H- (500 MHz) and ^{13}C- (125 MHz) nmr Data ($CDCl_3$) of Cladocrocin A [**1**] from COSY, HMQC, and HMBC Experiments.[a]

Position	δ_C	δ_H	HMBC (^{1}H)
1	171.5	—	H-2, H-5
2	83.8	4.80 s	—
3	166.9	—	H_3-21
4	140.3	—	H_2-19
5	140.0	6.20 bs	H-15′, H-15″
6	98.1	—	H-15′, H-15″
7	42.1	1.62 dd (15, 7.5), 1.72 dd (15, 3.8)	—
8	31.8	—	H-7′, H-7″
9	38.6	—	—
10	37.3	1.21 m	—
11	25.3[b]	—	—
12	10.8[c]	0.78[d] t (7.5)	—
13	18.6	2.18 bq (7)	H_3-20
14	12.1	1.16 t (7)	—
15	31.6	1.72 dq (15, 7.5) 1.86 (15, 7.5)	—
16	8.1	0.78[d] t (7)	—
17	25.4[b]	—	—
18	10.4[c]	0.80[d] t (7)	—
19	27.5[b]	—	—
20	10.5[c]	0.80[d] t (7)	—
21	50.5	3.68 s	—

[a]This experiment was optimized for long range couplings with a fixed delay Δ=60 msec. The low pass *J*-filter in the experiment to eliminate responses from direct ($^1J_{C\text{-}H}$) pairs was optimized for 135 Hz. Coupling constants are in parentheses, and the values are given in Hz.

[b,c,d]These values are interchangeable.

chains have been isolated from marine sponges of the genus *Plakortis* (2,6,7) and from *Chondrosia collectrix* (8), a sponge that is very similar in appearance to *Plakortis* species. The first example of these compounds was plakortin, which is a cyclic peroxide (6).

Cladocroic acid [**2**], [α]D 14.3° ($C_{21}H_{34}O_2$, methyl ester) showed spectral data indicating the presence of a long alkyl chain (strong signal at δ_H 1.26) and of a cis enyne terminus. The presence of this end group was derived from uv and ^{1}H- and ^{13}C-nmr spectra: λ max (hexane) 225 nm, δ_H 3.08 (1H, d, J=1.7 Hz, H-19), 5.45 (1H, dd, J=10.2, 1.7 Hz, H-17) and 6.07 (1H, dt, J=10.2, 7.2 Hz, H-16), and δ_C 81.1, 79.0, 107.9, 146.3 (C-19, C-18, C-17, and C-16, respectively) (9). The *Z* configuration for the double bond was inferred from the ^{1}H-nmr coupling constant (10 Hz) of the olefinic protons, and from the diagnostic ^{13}C-nmr resonance of the adjacent vinylic methylene at δ_C 29.7. The ^{13}C-nmr spectrum also contained signals for a carboxylic acid carbonyl at δ_C 178.5 and three carbons at δ_C 23.0 (d), 17.9 (d), 14.4 (t) ascribable to a disubstituted cyclopropane ring. The corresponding cyclopropyl protons in the ^{1}H-nmr spectrum were observed at δ_H 0.95, 1.10, 1.29, and 1.66 (each 1H, m's). The absence of the characteristic triplet signal in the ^{1}H-nmr spectra in the range of 2.2–2.4 ppm, typical of a methylene adjacent to a car-

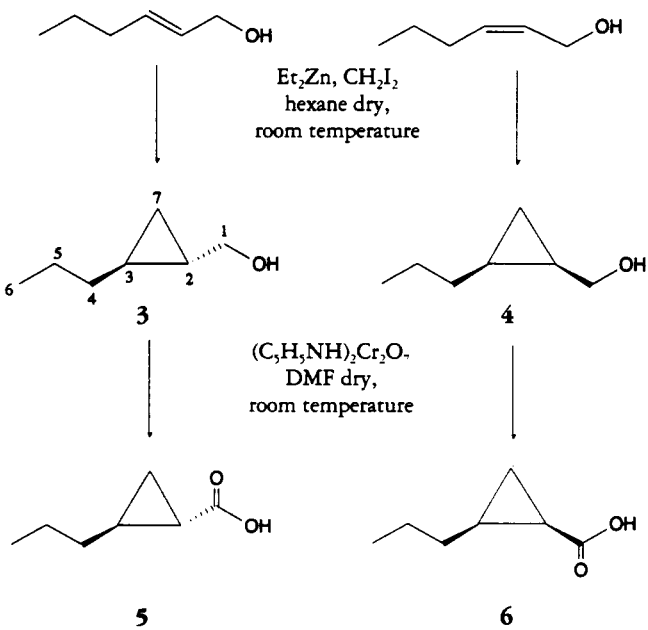
bonyl, suggested that the cyclopropane ring is directly linked to the carboxylic acid function. The presence of a cyclopropyl ^{1}H-nmr signal downfield-shifted to δ 1.66 supported this conclusion.

The remaining feature needed to establish the structure was the stereochemistry of the cyclopropane ring. For this purpose we have carried out the synthesis of both *cis* and *trans*- 2,3-methanohexanoic acids **5** and **6**, from *cis*- and *trans*-2-hexen-1-ol, respectively, via the modified Simmons-Smith cyclopropanation (10) and successive oxidation of cyclopropyl alchols **3** and **4** by pyridinium dichromate in dry DMF (Scheme 1). The synthetic models **5** and **6** can be easily differentiated by ^{1}H and ^{13}C nmr, and comparison of their spectral data with those of cladocroic acid [**2**] clearly indicated the cis configuration of the cyclopropane ring in the natural fatty acid (Table 2).

EXPERIMENTAL

GENERAL METHODS.—Medium pressure liquid chromatography (mplc) was performed on a Buchi 861 apparatus using an SiO_2 (230–400 mesh) column; reversed-phase hplc was performed by using Waters equipment (M 6000 A pump, U6K injector, R 401 refractometer, Whatman M9 10/25 ODS-2 column i.d. 3.9 mm, flow rate 2.0 ml/min); mass spectra were recorded at 70 eV on a Kratos MS 50 mass spectrometer; optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. All nmr measurements were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. The samples of cladocrocin A [**1**] were prepared by dissolving 1.1 mg in 0.4 ml of either $CDCl_3$ or C_6D_6; spectra of cladocroic acid [**2**] were obtained with 13.9 mg dissolved in 0.4 ml of $CDCl_3$.

The 2D homonuclear proton shift correlation (COSY) experiment was measured by employing the conventional pulse sequence. The COSY spectrum was obtained using a data set ($t_1 \times t_2$) of 1024 ×512 points for a spectral width of 3546.1 Hz (relaxation delay 1 sec). The data matrix was processed using an unshifted sine bell window function following transformation to give a magnitude spectrum with symmetrization (digital resolution f_2 3.46 Hz/pt). ^{1}H-detected {^{1}H,^{13}C} shift correlation experiments (at 305 K) utilized a 5-mm probe with a reverse geometry, and the sample was not spun. The ^{1}H-detected homonuclear multiple quantum coherence (HMQC) experiment was performed according to Bax and Subramanian (1), using an initial BIRD pulse to



SCHEME 1

TABLE 2. Selected ^1H and ^{13}C nmr of Cladocroic Acid [**2**] and Synthetic Models **5** and **6**.

Position	Compound					
	2		**6** (cis)		**5** (trans)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	—	178.5	—	179.2	—	180.1
2	1.67 m	17.9	1.67 m	17.9	1.34 m	20.0
3	1.29 m	23.0	1.31 m	22.8	1.22 m	23.7
4	1.54 m	26.9	1.54 m	28.9	1.42 m	35.1
20[a]	0.95 m, 1.10 m	14.4	0.96 m, 1.08 m	14.4	0.77 m, 0.88 m	16.3

[a]The cyclopropyl methylene C-20 in cladocroic acid [**2**] corresponds to methylene C-7 in the synthetic models.

suppress ^1H resonances not coupled to ^{13}C and a GARP sequence for ^{13}C decoupling during the data acquisition. Four hundred experiments of 40 scans each (relaxation delay 1.5 sec, delay after BIRD pulse 0.3 sec, fixed delay 3.3 msec) were acquired in 1K data points. A 45° shifted sine square window function was applied in t_2 dimension and a trapezoidal window (TM_1 0.06 Hz and TM_2 0.7 Hz) in t_1 dimension before Fourier transformation to give a 1024×512 point ($f_2 \times f_1$) matrix. ^1H-detected heteronuclear multiple bond correlation (HMBC) spectroscopy was performed according to Bax and co-workers (4,5). FIDs (256 of 64 scans each) were acquired in 1K data points (relaxation delay 3 sec low pass *J*-filter delay 3.3 msec, long range couplings evolution delay 60 msec). The data processing was identical to that used for the HMQC experiment.

SPONGE COLLECTION AND EXTRACTION.—*Cl. incurvata* was collected south of New Caledonia at ca. 500 m depth by dredging. A voucher sample is kept at the ORSTOM Centre de Nouméa under reference R1381. The sponge was freeze-dried, and the lyophilized material (0.8 kg) was powdered and Soxhlet-extracted using *n*-hexane (3 liters). The extract was taken to dryness to give an oily residue (3 g), which was chromatographed by mplc on an SiO_2 column eluting with *n*-hexane and increasing amounts of EtOAc.

ISOLATION OF CLADOCROCIN A [**1**] AND CLADOCROIC ACID [**2**].—The fraction eluted with 2% of EtOAc was further purified by hplc using 15% H_2O in MeOH as eluent to give cladocrocin A [**1**], 1.1 mg (Rt 13 min). Fractions eluted with 30% of EtOAc gave cladocroic acid [**2**] (13.9 mg) after hplc purification (5% H_2O in MeOH as eluent).

Cladocrocin A [**1**].—Physical data: $[\alpha]_D$ 50.9°; ^1H and ^{13}C nmr see Table 1; hreims *m/z* 336.2670 (required for $C_{21}H_{36}O_3$, 336.2666).

Cladocroic acid [**2**].—Physical data: $[\alpha]_D$ 14.3°; ^1H nmr (500 MHz, $CDCl_3$) 0.92 (1H, m), 1.05 (1H, m, H_2-20), 1.26 (18H, bs), 1.29 (1H, m, H-3), 1.40 (2H, m, H_2-14), 1.54 (2H, q, J=6.5 Hz, H_2-4), 1.66 (1H, m, H-2), 2.32 (2H, bq, J=7.2 Hz, H_2-15), 3.07 (1H, d, J=1.7 Hz, H-19), 5.45 (1H, bd, J=10.2 Hz, H-17), 6.0 (1H, dt, J=10.2, 7.2 Hz); ^{13}C nmr (125 MHz, $CDCl_3$) 178.5 (C-1) 17.9 (C-2), 23.0 (C-3), 26.9 (C-4), 28.7–30.3 (10C, nonvinylic methylenes), 29.7 (C-15), 146.3 (C-16), 107.9 (C-17), 79.0 (C-18), 81.1 (C-19), 14.4 (C-20).

SYNTHESIS OF *trans*-2-*n*-PROPYL-CYCLOPROPANE-CARBOXYLIC ACID [**5**].—Diethylzinc (30 ml of a 1.0 M hexane solution, 30 mmol) was added to a stirred solution of *trans*- 2-hexen-1-ol (1 g, 10 mmol) in dry hexane (30 ml). Then methylene iodide (10 ml, 0.12 mol) was added dropwise under Ar atmosphere during 1 h at 0°. The reaction mixture was stirred at room temperature for 6 h, then slowly poured into 1% aqueous HCl (100 ml) under stirring, and the product was extracted repeatedly with Et_2O. The organic layers were washed with H_2O and dilute aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated under reduced pressure to afford 0.924 g of *trans*-2,3-methanohexan-1-ol [**3**] as colorless oil: ^1H nmr ($CDCl_3$) δ 0.22 (1H, m, H-7), 0.29 (1H, m, H-7), 0.52 (1H, m, H-3), 0.75 (1H, m, H-2), 0.84 (3H, t, J=7 Hz, H_3-6), 1.15 (2H, m, H_2-4), 1.34 (2H, sextet, J=7 Hz, H_2-5), 3.30–3.38 (each 1H, dd's, J=11, 7 Hz, H_2-1).

The alcohol **3** (0.1 g) was oxidized in dry DMF (4 ml) by PDC (1.14 g) overnight at room temperature under stirring. The solution was treated with H_2O (50 ml) and extracted with Et_2O (5×20 ml). The organic layers were dried over $MgSO_4$ and concentrated in vacuo to give 40 mg of **5**: ^1H and ^{13}C nmr see Table 2.

SYNTHESIS OF *cis*-2-*n*-PROPYL-CYCLOPROPANE-

CARBOXYLIC ACID [**6**].—The alcohol **4** (0.84 g) was prepared from *cis*-2-hexen-1-ol (1g, 10 mmol) using the same procedure described for compound **3**. ^{1}H nmr ($CDCl_3$) δ −0.05 (1H, bq, J=5 Hz, H-7′), 0.70 (1H, m, H-7″), 0.88 (1H, m, H-3), 1.08 (1H, m, H-1), 0.91 (3H, t, J=7 Hz, H_3-6), 1.23 (2H, m, H_2-4) 1.43 (2H, sextet, J=7 Hz, H_2-5), 3.59–3.65 (each 1H, dd's, J=11, 7 Hz, H_2-1).

Oxidation of compound **4** (0.1 g) to afford the acid **6** was carried out by using the same procedure described for the preparation of compound **5**, yielding 40 mg of **6**: ^{1}H and ^{13}C nmr data see Table 2.

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