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HELICASCOLIDES A AND B: NEW LACTONES FROM THE MARINE FUNGUS *HELICASCUS KANALOANUS*

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ABSTRACT.—Two new isomeric δ-lactones **2** and **3** have been isolated from the marine fungus *Helicascus kanaloanus* (ATCC 18591). The structures and relative configurations of these compounds were assigned primarily by nmr studies, and absolute configurations are proposed based on cd data.

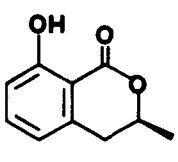
Despite the abundance of biologically active natural products discovered through investigations of terrestrial fungi, reports of marine fungal secondary metabolites have been limited (1–5). Our studies of the Hawaiian mangrove ascomycete *Helicascus kanaloanus* Kohlmeyer (ATCC 18591) have led to the isolation of two new isomeric δ-lactones, and the structures of these compounds are described here.

EtOAc extracts of liquid cultures of *H. kanaloanus* were subjected to cc followed by hplc to afford three major components. One compound was identified as (*S*)-(+)-ochracin [**1**] by comparison of its physical and spectral properties (nmr, ms, ir, [α]D) to literature values (6,7).

Analysis of the second most abundant metabolite by hreims suggested the molecular formula $C_{12}H_{20}O_3$ ($[M]^+$ 212.1418; Δ 0.6 mmu). The nmr and ir spectra indicated the presence of an ester or lactone group, a trisubstituted double bond, one hydroxyl group, two vinylic methyl groups, and two geminal methyl groups attached to a quaternary carbon. The gross structure of the molecule, which we named helicascolide A, was assigned as **2** on the basis of ^{13}C-nmr data and ^{1}H-^{1}H decoupling experiments (Table 1). A series of selective INEPT experiments gave results completely consistent with the proposed structure, and these data were used in making ^{13}C-nmr assignments.

The double-bond geometry and relative stereochemistry were assigned by difference nOe experiments and by analysis of the coupling constants for the ring protons. Irradiation of the signal for H-5 resulted in a 17% enhancement of the olefinic proton signal, suggesting the (*E*)-geometry for the double bond. The coupling constants $J_{H4\text{-}H5}$ and $J_{H3\text{-}H4}$ were measured as 11.2 and 1.7 Hz, respectively, suggesting that H-4 and H-5 are *trans*-diaxial and that H-3 and H-4 must then possess a *cis* relationship. The relative stereochemistry proposed for **2** is based on this observation, because nOe experiments did not provide conclusive supporting evidence. The signal for H-3 was only enhanced by 2% upon irradiation at H-5, and irradiation at H-3 did not measurably affect the signal for H-5.

The hreims spectrum of the third component (helicascolide B) indicated that it is an isomer of helicascolide A. Analysis of the ^{1}H- and ^{13}C-nmr spectra (Table 1) and the results of decoupling and nOe experiments suggested that this component has structure **3**, differing from heliacascolide A only in the configuration at the 3 position. The cou-



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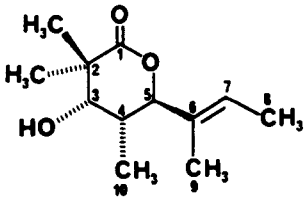

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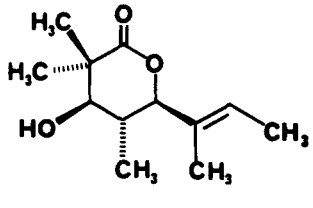

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TABLE 1. ^{1}H- and ^{13}C-Nmr Assignments for Helicascolides A [2] and B [3].[a]

Position	Compound			
	2		3	
	^{1}H	^{13}C	^{1}H	^{13}C
1	—	177.2	—	177.0
2	—	44.1	—	44.5
3	3.53 (dd, 3.7, 1.7)	77.3	3.43 (dd, 10.6, 5.5)	77.4
3-OH	2.08 (d, 3.7)	—	1.80 (d, 5.5)	—
4	2.28 (ddq, 11.2, 6.8, 1.7)	31.2	2.06 (m)	33.8
5	4.66 (d, 11.2)	87.8	4.12 (d, 11.0)	89.0
6	—	131.5	—	131.4
7	5.54 (br q, 5.6)	126.5	5.53 (br q, 6.8)	127.0
8	1.62 (br d, 5.6)	13.2	1.64 (br d, 6.8)	13.3
9	1.60 (br s)	10.3	1.61 (br s)	10.4
10	0.89 (d, 6.8)	13.8	0.93 (d, 6.4)	13.5
11	1.28 (s)	26.5	1.26 (s)	23.8
12	1.32 (s)	22.6	1.36 (s)	20.6

[a]Spectra were recorded at 360 MHz and 90.7 MHz, respectively in $CDCl_3$. Assignments for geminal methyl groups are interchangeable. ^{13}C methyl assignments for **3** were made by analogy to those of **2**.

pling constant between H-3 and H-4 was increased in this case to 10.6 Hz, consistent with a *trans*-diaxial relationship, while the coupling between H-4 and H-5 (11.0 Hz) suggested that their *trans*-diaxial relationship was retained in **3**. NOe experiments in this case suggested a closer spatial relationship between H-3 and H-5. Irradiation of H-5 gave a 7% enhancement for the H-3 resonance and a 16% enhancement for the olefinic proton. Irradiation of the H-3 signal enhanced H-5 by 4%. Thus, the relative stereochemistry and the olefin geometry are proposed as shown in **3**.

The carbonyl absorption frequencies for these molecules (1717 and 1724 cm^{-1} for **2** and **3**, respectively) indicate that they exist predominantly in the half-chair conformation (typically 1730–1750 cm^{-1}) rather than the boat form (1758–1765 cm^{-1}) (8,9). The somewhat low frequencies relative to those of typical δ-lactone half-chair conformations can be accounted for by α-, β-, and/or γ-substituent effects (10,11).

Preference for the half-chair conformation was confirmed by analysis of the cd spectra for **2** and **3**, which also permitted assignment of proposed absolute configurations. The cd curve for **2** displayed a single maximum at 221.5 nm ($[\theta]_{221.5} = +3680$), as expected for a δ-lactone that assumes a half-chair conformation (9,12). Furthermore, based on previous studies of chiral δ-lactones (9,12), the positive sign of the Cotton effect suggests that the dihedral angle C-3–C-2–C-1–O is negative when viewed as a Newman projection along the bond from C-2 to C-1 (i.e., the C-3–C-2 bond must be rotated counterclockwise to bring it into alignment with the C-1–O single bond). Because the ^{1}H-nmr data indicate that the molecule exists predominantly in the more stable half-chair form which places the 5-(1-methyl-1-propenyl) and 4-methyl substituents in pseudo-equatorial positions, the positive Cotton effect suggests that this compound has the absolute stereochemistry shown in structure **2**. The cd curve for **3** is nearly identical to that for **2** (maximum at 221.5 nm; $[\theta]_{221.5} = +3330$). Despite the fact that the stereochemistry at C-3 is different from that in **2**, the conformation placing the substituents at C-4 and C-5 in pseudo-equatorial orientations is again expected to be predominant for **3**. Thus, the configurations at C-4 and C-5 are the same as for **2**, and the stereochemistry for **3** is assigned as shown.

The discovery of these relatively simple, yet previously unreported, compounds from a marine fungus suggests that marine fungi may possess biosynthetic capabilities which differ in some respects from those of terrestrial fungi. Prior to this report, only one novel metabolite from a marine fungus had been described (3). Further studies of marine fungal metabolites are underway in our laboratory.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Cd spectra were recorded in MeOH on an AVIV Model 60DS Spectropolarimeter. Other instrumentation and general experimental procedures employed in this research have been described previously (13).

CULTIVATION.—A culture of *H. kanaloanus* (ATCC 18591) was obtained from the American Type Culture Collection, Rockville, Maryland. Six 2-liter Erlenmeyer flasks, each containing 350 ml of a modified marine medium comprised of Instant Ocean (80% strength), 0.3% yeast extract, 0.3% malt extract, 1.5% D-glucose, and 1.0% soluble starch, were inoculated with several 1-cm^2 plugs taken from 4-day-old Petri dish cultures of *H. kanaloanus*. Flask cultures were incubated at 25–28° and aerated by agitation on an orbital shaker at 200 rpm for 20 days.

ISOLATION AND CHARACTERIZATION OF **1–3**.—The filtered culture broth (2100 ml) was extracted with EtOAc (4 × 500 ml), and the organic phase was dried ($MgSO_4$) and evaporated to afford 1.1 g of a red oil. The crude oil was chromatographed on a column of Sephadex LH-20 (4 × 50 cm) and eluted with CH_2Cl_2-hexane (4:1), collecting 4-ml fractions. Early fractions (13–29) were combined to give 359 mg of a yellow oil that was chromatographed on Si gel (4 × 65-cm column) using a gradient of 0–10% EtOAc in hexane. The major component **1** (60.8 mg) eluted at 1% EtOAc and was identified as (*S*)-ochracin by comparison of spectral data (^{13}C nmr, ^1H nmr, ir, ms, $[\alpha]_D$) to literature values (6,7). Fractions eluting at 2% EtOAc were combined to give 46.3 mg of an oil that was subjected to reversed-phase hplc (C_{18}), affording compounds **2** and **3**. Compound **2** (34.4 mg) was isolated as a white crystalline solid which showed a single peak by hplc [retention time 10.1 min; 5-μ C_{18} column, 250 × 10 mm, MeOH-H_2O (70:30), 2.0 ml/min, 215 nm]. Compound **2** gave the following data: mp 97–98°; $[\alpha]_D$ −25.0° (c = 1.4; $CHCl_3$, 31°); cd (MeOH) $[\theta]_{221.5}$ = +3680; ir ($CHCl_3$) 3636, 3458 (br), 3020, 2982, 1717, 1460, 1398, 1147 cm^{-1}; eims (70 eV) *m/z* $[M]^+$ 212 (2.5% rel. int.), 197 (0.9), 195 (1.8), 156 (11), 154 (6.1), 142 (13), 128 (19), 125 (4.3), 113 (5.2), 96 (100); ^1H and ^{13}C nmr see Table 1; hreims observed *m/z* $[M]^+$ 212.1418, calcd for $C_{12}H_{20}O_3$ *m/z* 212.1412.

Compound **3** (3.7 mg) was isolated as a white crystalline solid with an hplc retention time of 9.1 min under the conditions above. Compound **3** gave the following data: mp 61–62°; $[\alpha]_D$ −27.6 (c = 0.4, $CHCl_3$, 31°); cd (MeOH) $[\theta]_{221.5}$ = +3330; ir ($CHCl_3$) 3626, 3450 (br), 3018, 2981, 2939, 1724, 1470, 1384, 1266 cm^{-1}; eims (70 eV) *m/z* $[M]^+$ 212 (1.5% rel. int.), 197 (0.5), 194 (0.3), 156 (6.0), 128 (8.5), 125 (2.0), 113 (2.2), 96 (48), 85 (100), 70 (91); ^1H and ^{13}C nmr see Table 1; hreims observed *m/z* $[M]^+$ 212.1416, calcd for $C_{12}H_{20}O_3$ *m/z* 212.1412.

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"Herbolaria of Precolumbian Times: Present and Future Perspectives" (Tentative)

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Dr. John Cassady, Dean and Professor, The Ohio State University
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