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DISCORHABDIN ALKALOIDS FROM THE ANTARCTIC SPONGE *LATRUNCULIA APICALIS*

AIMIN YANG, BILL J. BAKER,*

Department of Chemistry, Florida Institute of Technology, Melbourne, Florida 32901

JULIA GRIMWADE, ALAN LEONARD,

Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901

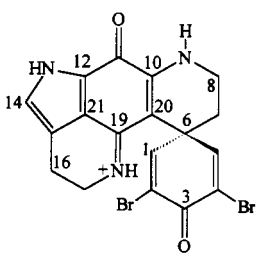
and JAMES B. MCCLINTOCK

Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

ABSTRACT.—An Antarctic collection of *Latrunculia apicalis* contained as its major secondary metabolite the pigment discorhabdin C as well as a previously unreported pigment, discorhabdin G. Discorhabdin alkaloids have a variety of pharmacological and ecological bioactivities, including the mediation of interactions with the potential predator *Perknaster fuscus*, a spongivorous sea star.

Sponges of the genus *Latrunculia* have been the subject of several chemical investigations, leading to the discovery of the ichthyotoxic latrunculins A and B from a Red Sea *L. magnifica* (1), the cytotoxic discorhabdins A–F (e.g., **1**), from several South Pacific *Latrunculia* species including the New Zealand *L.* cf. *bocagei* (2), and *L. brevis* from both New Zealand (3–6) and Australia (7), and terpenoid peroxides from an uncharacterized Australian *Latrunculia* species (8). Discorhabdins A and D have also been isolated from the Japanese sponge *Prianos melanos* (9,10), along with the related prianosin B (11). Discorhabdin A and its probable biosynthetic precursors, the makaluvamines (e.g., **3**), have been reported from the South Pacific sponge *Zyzzya* sp. (12). Our collection of *Latrunculia apicalis* Ridley and Dendy (family Latrunculiidae, order Hadromerida), collected in McMurdo Sound, Antarctica, was found to exhibit significant bioactivity, including activity influencing sea star feeding behavior and antibiotic activity (13–15). In addition to discorhabdin C [**1**], bioactivity in our collections of Antarctic *Latrunculia* can be traced to a new discorhabdin alkaloid, discorhabdin G [**2**].

Sponges were collected using scuba between 6 and 40 m depth from Hut Point, Danger Slopes, and Cape Evans on Ross Island, Antarctica (77° 51.5′ S; 166° 39′ E). Organisms were freeze-dried, then subjected to solvent extraction of increasing polarity: hexane, $CHCl_3$, MeOH, and 70:30 MeOH-H_2O. The $CHCl_3$ and MeOH extracts of *L. apicalis* displayed significant activity in an ecological assay to identify extracts deterrent toward the major sponge predator on the Antarctic benthos, the sea star



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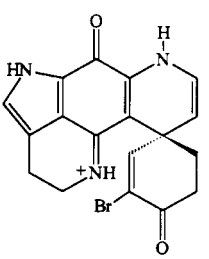

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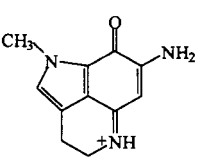

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Perknaster fuscus (14). The MeOH extract was applied to a Sephadex LH-20 column and the first green fraction was collected. Repeated reversed-phase hplc (35% MeOH in 0.05% aqueous trifluoroacetic acid) resulted in purification of a red and a green pigment. The red pigment was identified as discorhabdin C [**1**] by comparison of its ^{1}H- and ^{13}C- (Table 1) nmr data to those reported previously (3). The green pigment could not be correlated to a known discorhabdin alkaloid and was given the designation discorhabdin G [**2**].

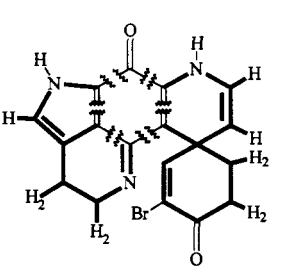
TABLE 1. Comparison of ^{13}C-Nmr Chemical Shifts of **1** and **2**.

Carbon	**1**	**2**
	δ	δ, m
1	152.9	153.1, d
2	124.4	125.3, s
3	172.5	190.3, s
4	124.4	32.8, t
5	152.9	36.4, t
6	46.5	43.0, s
7	35.4	113.2, d
8	39.7	124.7, d
10	153.5	146.7, s
11	167.0	167.5, s
12	124.7	122.9, s
14	127.9	127.4, d
15	121.6	121.0, s
16	19.6	19.3, t
17	45.5	45.9, t
19	155.5	160.0, s
20	92.7	100.7, s
21	125.5	123.7, s

The green pigment was an optically active ([α]D +27.0°) discorhabdin, as evidenced by the similarity of the ^{1}H- and ^{13}C- (Table 1) nmr data to those of **1**. Also supportive of the presence of an iminoquinone ring system was the uv spectrum, which displayed characteristic absorptions, λ max 250 (ε 8100) and 322 (ε 3200), of the cross-conjugated pyrrolo[1,7]phenanthroline chromophore; compare makaluvamine A [**3**], which has only this chromophore, with λ max 242 (ε 24,000) and 348 (ε 15,500).

Ten signals were evident in the MeOH-d_4 ^{1}H-nmr spectrum, including two aromatic methine singlets (δ 7.64 and 7.16), two coupled vinylic methine doublets (δ 6.38 and 5.40), two coupled methylene triplets (δ 3.89 and 2.94), and four mutually coupled high-field multiplets [δ 3.0 (m), 2.68 (dt), 2.55 (ddd), 2.31 (dd)], for a total of twelve non-exchangeable hydrogens. The lsims molecular ion (M^+) was found at *m/z* 384.0357, which provided a molecular formula of $C_{18}H_{15}BrN_3O_2$ (Δ mmu 1.0), suggesting three exchangeable protons were present (**2** was isolated as a TFA salt). In addition to four methines and four methylenes observed in the DEPT spectrum, ten quaternary carbons were evident, including three carbonyls (δ 190.3, 167.5, 160.0), six other sp^2 carbons, and one sp^3 carbon.

Two isolated spin-systems could be constructed from two-dimensional nmr data of discorhabdin G (bold bonds in **4**). The methine proton H-14 (δ 7.16) displayed coupling in the HMBC spectrum to C-15 (δ 121.0) and C-12 (δ 122.9). The HMBC spectrum also showed that C-21 correlated to H_2-16 (δ 2.94) and that H_2-16 further correlated to C-14 (δ 127.4) and C-17 (δ 45.9), of which the latter was supported by ^{1}H-^{1}H vicinyl coupling (J=7.8 Hz) to H_2-17 (δ 3.89). H_2-17 showed further coupling in the HMBC spectrum to C-19 (δ 160.0), the chemical shift of which suggested it was an iminocarbonyl (2). H_2-16 and H_2-17 both correlated to C-15. These correlations are illustrated as the continuous bold bonds on the left side of **4**.



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Further connectivity could be ascertained from the HMBC spectrum, starting with H-1 (δ 7.64) which correlated to carbons C-2, C-3, C-5, and C-20 (δ 125.3, 190.0, 32.8, and 100.7). The methylene proton H-4a (δ 3.0) was partially obscured by the overlapping H_2-16 (δ 2.94) signal, but showed clear HMBC correlation to C-3; ^{1}H-^{1}H geminal coupling (J=17.6 Hz) from H-4a to H-4b was evident in the COSY spectrum. Both H-4a and H-4b displayed ^{1}H-^{1}H vicinyl coupling (J=1.8 and 5 Hz) to H-5a (δ 2.68) and H-5b (δ 2.31), the latter of which shared geminal couplings of 14 Hz. In addition to the aforementioned correlation to H-1, C-20 correlated in the HMBC spectrum to H-7 (δ 5.40), and H-7 showed further coupling to C-6 (δ 43.0) and C-8 (δ 124.7); vicinyl coupling between H-7 and H-8 (J=7.8 Hz) was observed in the COSY spectrum. The remaining correlation defining the right-hand bolded spin-system in **4** was that of H-8 (δ 6.38) to C-10 (δ 146.7).

Compounds **1** and **2** are both antimicrobial agents, with similar activities against both Gram-positive and Gram-negative bacteria (B.J. Baker, A. Yang, J. Grimwade, A.C. Leonard, and J.B. McClintock, unpublished results). In addition, **2** occupies a central position in the chemical ecology of *Latrunculia apicalis*, causing feeding deterrence behavior in the major Antarctic sponge predator, *Perknaster fuscus*, and inhibiting growth in two common water column microorganisms isolated from the surrounding water.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents were distilled from glass. Spectral analyses were performed on an 8.46-T nmr instrument operating at 360 MHz for ^{1}H and 90 MHz for ^{13}C. One-bond heteronuclear ^{1}H-^{13}C connectivities were determined by HMQC; two- and three-bond ^{1}H-^{13}C connectivities were determined by HMBC optimized for 7 Hz couplings; chemical shifts are reported in ppm with the chemical shift of residual solvent nuclides used as internal standards. The ir spectrum was recorded on a Nicolet Magna-IR 550. The uv spectrum was recorded on a Hewlett-Packard 8452A diode-array spectrometer. The lsims spectrum was recorded on a Finnigan MAT-95Q instrument at the University of Florida at a resolution of 3000; the primary ion was cesium at 15 KeV and the matrix was 3-nitrobenzyl alcohol.

ANIMAL MATERIAL.—Sponges were collected using scuba between 6 and 40 m depth from Hut Point, Danger Slopes, and Cape Evans on Ross Island, Antarctica (77° 51.5′ S; 166° 39′ E). A voucher specimen of *Latrunculia apicalis* is on hand at the Department of Biology, University of Alabama at Birmingham.

EXTRACTION AND ISOLATION.—Isolation began with 60 g of freeze-dried *Latrunculia apicalis*, which was extracted sequentially in hexane, $CHCl_3$, MeOH, and MeOH-H_2O (7:3). The MeOH extract (7.8 g, 13% of sponge dry wt) was chromatographed on Sephadex LH-20 in MeOH, and colored bands were collected as fractions. The first band was further chromatographed using reversed-phase hplc (35% MeOH in 0.05% aqueous trifluoroacetic acid) to give 11.5 mg (0.019%) of **1** and 8.0 mg (0.013%) of **2**.

Discorhabdin G [**2**].—[α]D +27.0° (c=0.063, MeOH); uv (MeOH) λ max (ε) 210 (7500), 250 (8100), 322 (3200), 402 (2300), 610 (600) nm; ir (KBr) ν max 3700–2500 (br), 1684, 1619, 1383, 1332, 1210, 1134 cm^{-1}; ^{1}H nmr (MeOH-d_4, 360 MHz) δ 7.64 (1H, s, H-1), 7.16 (1H, s, H-14), 6.38 (1H, d, J=7.8 Hz, H-8), 5.40 (1H, d, J=7.8 Hz, H-7), 3.89 (2H, t, J=7.8 Hz, H_2-17), 3.0 (1H, overlapping m, H-4a), 2.94 (2H, t, J=7.8 Hz, H_2-16), 2.68 (1H, br dt, J=5.1 and 14.1 Hz, H-5a), 2.55 (1H, ddd, J=1.8, 4.32, and 17.6 Hz, H-4b), 2.31 (1H, m, H-5b); ^{13}C-nmr data, see Table 1; lsims m/z [M]$^+$ 384.0357 (Δ mmu 1.0 for $C_{18}H_{15}{}^{79}BrN_3O_2$), [M$^+$+H] 385.0462 (Δ mmu 0.7 for $C_{18}H_{16}{}^{79}BrN_3O_2$).

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LITERATURE CITED

1. Y. Kashman, A. Groweiss, R. Lidor, D. Blasberger, and S. Carmely, *Tetrahedron*, **41**, 1905 (1985).

2. B.R. Copp, K.F. Fulton, N.B. Perry, J.W. Blunt, and M.H.G. Munro, *J. Org. Chem.*, **59**, 8233 (1994).
3. N.B. Perry, J.W. Blunt, J.D. McCombs, and M.H.G. Munro, *J. Org. Chem.*, **51**, 5476 (1986).
4. N.B. Perry, J.W. Blunt, and M.H.G. Munro, *Tetrahedron*, **44**, 1727 (1988).
5. N.B. Perry, J.W. Blunt, M.H.G. Munro, T. Higa, and R. Sakai, *J. Org. Chem.*, **53**, 4127 (1988).
6. J.W. Blunt, M.H.G. Munro, C.N. Battershill, B.R. Copp, J.D. McCombs, N.B. Perry, M. Prinsep, and A.M. Thompson, *New J. Chem.*, **14**, 761 (1990).
7. R.J. Capon and J.K. MacLeod, *Tetrahedron*, **41**, 3391 (1985).
8. R.J. Capon, J.K. MacLeod, and A.C. Willis, *J. Org. Chem.*, **52**, 339 (1987).
9. J. Kobayashi, J. Cheng, M. Ishibashi, H. Nakamura, Y. Ohizumi, Y. Hirata, T. Sasaki, H. Lu, and J. Clardy, *Tetrahedron Lett.*, **28**, 4939 (1987).
10. J. Kobayashi, J. Cheng, S. Yamamura, and M. Ishibashi, *Tetrahedron Lett.*, **32**, 1227 (1991).
11. J. Cheng, Y. Ohizumi, M.R. Wälchli, H. Nakamura, Y. Hirata, T. Sasaki, and J. Kobayashi, *J. Org. Chem.*, **53**, 4621 (1988).
12. D.C. Radisky, E.S. Radisky, L.R. Barrows, B.R. Copp, R.A. Kramer, and C.M. Ireland, *J. Am. Chem. Soc.*, **115**, 1632 (1993).
13. J.B. McClintock and J.J. Gauthier, *Antarctic Sci.*, **4**, 179 (1992).
14. J.B. McClintock, B.J. Baker, M. Slattery, M. Hamann, R. Kopitzke, and J. Heine, *J. Chem. Ecol.*, **20**, 859 (1994).
15. B.J. Baker, B. Kopitzke, M. Hamann, and J.B. McClintock, *Antarctic J. U.S.*, **28**, 132 (1993).

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