Discorhabdins I and L, Cytotoxic Alkaloids from the Sponge *Latrunculia brevis*

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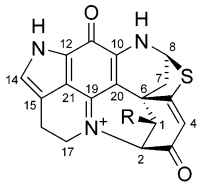
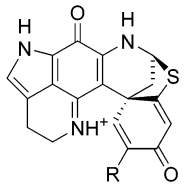
Two cytotoxic alkaloids, discorhabdins L (**1**) and I (**2**), were isolated from *Latrunculia brevis* and their structures assigned on the basis of detailed spectroscopic analysis and comparison with the known metabolites discorhabdins R (**5**), D (**6**), and B (**4**). Compounds **1** and **2** showed strong in vitro cytotoxicity against a panel of 14 tumor cell lines.

Marine sponges continue to be a rich source of novel secondary metabolites with a wide range of biological activities.[1] Bioactive metabolites containing a pyrroloiminoquinone moiety are widely distributed in sponges of the genera *Latrunculia*, *Batzella*, *Prianos*, *Zyzzya*, *Histodermella*, and *Negombata*.[2] Since discorhabdin C was described in 1986 from a sponge of the genus *Latrunculia*, several groups have published on the isolation of other members of this class of compounds (discorhabdins A−R).[2−12] We wish to report here the isolation of two members of this family, discorhabdins L (**1**) and I (**2**), from *Latrunculia brevis* Ridley & Dendy (family Latrunculiidae, order Hadromerida) in the course of our screening program for new anticancer agents. Both compounds were presented at the 1996 ASP meeting by Munro and co-workers.[8] However, the final report on their isolation and structural elucidation was never published.

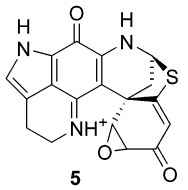
The extract in 2-propanol of a *L. brevis* specimen collected by trawling in Argentina demonstrated growth inhibitory properties against several tumoral cell lines. This extract was subjected to reversed-phase chromatography using MeOH−H_2O as the eluent and then subjected to RP-18 semipreparative HPLC (CH_3CN−H_2O + 0.1% TFA) to yield the TFA salts of discorhabdins L (**1**) and I (**2**) together with those of the known compounds discorhabdins A (**3**) and B (**4**).



1 R=OH
6 R=H



2 R=H
4 R=Br



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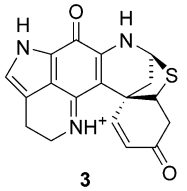

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HRFABMS ([M]$^+$ *m/z* 352.0752) and ^{13}C NMR spectra supported a molecular formula of $C_{18}H_{14}N_3O_3S$ for compound **1**. NMR resonances were consistent with a discorhabdin-like compound. The close similarity between the ^{1}H and ^{13}C NMR spectra in CD_3OD (Table 1) for compound **1** and discorhabdin R (**5**) made us think in the first instance that we had isolated the same compound as the Capon group.[2] However, minor differences observed in the spectra for both compounds prompted us to carry out 2D NMR experiments. The observation of clear HMBC correlations between H-2 and C-17 and C-19 and between H-17a (4.01 ppm) and C-2 (Table 1) revealed the existence of a ring closure between C-2 and N-18, confirming thus the structural difference from discorhabdin R. NOESY cross-peaks between H-2 and both H-17 protons also supported the proposed ring closure in compound **1**. Signals observed at 4.63 and 68.5 ppm in the ^{1}H and ^{13}C NMR spectra, respectively, accounted for the presence of a secondary hydroxyl group in the molecule. This group was placed at C-1 on the basis of a correlation between the signal at 4.63 ppm and the H-2 signal (4.14 ppm) in the COSY spectrum and HMBC correlations between the proton signal at 4.63 ppm and carbons C-2, C-3, C-5, and C-7.

The stereochemistry at C-1 was proposed on the basis of NOE intensities between H-1 and both H-7 protons. Thus irradiation of H-1 caused an enhancement (1.0%) of the signal at 2.57 ppm, whereas no enhancement of the signal at 2.95 ppm was observed. The signal at 2.57 ppm was assigned to the α proton at C-7 on the basis of the existence of a long-range coupling to H-4 observed in the COSY spectrum. This coupling indicates a planar zigzag arrangement of the molecule as in the case of discorhabdin D (**6**).[4] The stereochemistry at C-2 was proposed on the basis of the observation of a NOE enhancement (2.68%) of the signal for H-2 when irradiating H-1. The small coupling observed in the COSY spectrum between H-2 and H-4 also supports the proposed stereochemistry at this center. Despite the presence of four chiral centers in the molecule, compound **1** showed an α_D value of 0° as in the case of discorhabdin D.[4] We therefore concluded that discorhabdin L (**1**) has the structure of 1*R*-hydroxydiscorhabdin D.

Compound **2** has a molecular formula of $C_{18}H_{14}N_3O_3S$ as can be deduced from its ^{13}C NMR and mass spectra. Spectroscopic comparisons between this compound and discorhabdin B (**4**)[5] suggested a common structure for both compounds from C-3 to C-21. The major difference in the ^{1}H NMR spectrum recorded in CD_3OD between **2** and **4** was the absence of the singlet at 7.87 ppm in **4** and the presence of two new signals with a vicinal coupling (d, 7.42 ppm and dd, 6.66 ppm). This feature can be attributed to the absence in **2** of the bromine at C-2 present in the

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Table 1. NMR Data for Discorhabdin L (**1**), in CD_3OD

no.	^{13}C δ	^{1}H δ [m, J (Hz)]	COSY	gHMBC	NOESY
1	68.5	4.63 (d, 3.6)	H-2	C-2, C-3, C-5, C-7	H-2, H-7β, H-7α
2	67.8	4.14 (d, 3.6)	H-1, H-4	C-1, C-3, C-6, C-17, C-19	H-1, H-17a, H-17b
3	184.9				
4	114.0	6.12 (s)	H-2, H-7α	C-2, C-5, C-6, C-20	H-2
5	171.5				
6	48.6				
7β	37.4	2.95 (dd, 12.0, 3.6)	H-7α, H-8	C-6, C-8, C-20	H-2, H-7α, H-8
7α		2.57 (br d, 12.0)	H-4, H-7β, H-8	C-5, C-6, C-8, C-20	H-2, H-7β, H-8
8	63.7	5.58 (dd, 3.6, 1.4)	H-7α, H-7β	C-5, C-6, C-10	H-7α, H-7β
10	148.5				
11	167.4				
12	125.5				
14	127.2	7.10 (s)		C-11, C-12, C-15, C-21	H-16a, H-16b
15	119.2				
16a	20.6	3.18 (dddd, 16.7, 13.0, 7.4, 1.0)	H-16b, H-17a, H-17b	C-14, C-15, C-17, C-21	H-16b, H-17a, H-17b
16b		3.05 (ddd, 16.7, 6.9, 3.0)	H-16a, H-17a, H-17b	C-15, C-17, C-21	H-16a, H-17a, H-17b
17a	52.8	4.01 (ddd, 14.2, 7.4, 3.0)	H-16a, H-16b, H-17b	C-2, C-15, C-16, C-19	H-2, H-16a, H-16b, H-17b
17b		3.90 (ddd, 14.2, 13.0, 6.9)	H-16a, H-16b, H-17a	C-15, C-16, C-19	H-2, H-16a, H-16b, H-17a
19	150.3				
20	101.9				
21	122.7				

structure of **4**. This fact was also confirmed by the absence of dual isotope peaks in the protonated molecular ion. The proposed structure for **2** was confirmed by 2D NMR spectroscopy. Compound **2** therefore has the structure of 1-debromodiscorhabdin B.

Cytotoxicity assays were performed for compounds **1** and **2** against a panel of 14 different tumor cell lines. Both compounds demonstrated activity in the submicromolar range in most of the lines assayed. The best results in terms of potency were obtained against the HT-29 colon cell line with GI_{50} values of 0.12 and 0.35 μM for compounds **1** and **2**, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured on a Büchi 535 apparatus and are uncorrected. Optical rotations were determined on a Jasco P-1020 polarimeter. UV spectra were obtained with a Perkin-Elmer Lambda 15 UV/vis spectrophotometer. IR spectra were determined using a Perkin-Elmer 881 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian "Unity 500" spectrometer at 500 and 125 MHz, respectively. Chemical shifts are reported in ppm using residual CD_3OD (δ 3.30 for ^{1}H and 49.0 for ^{13}C) as internal reference. FABMS were performed on a VGAutoSpec spectrometer employing a *m*-NBA matrix. ESIMS were recorded using an Agilent 1100 Series LC/MSD spectrometer.

Animal Material. *Latrunculia brevis* Ridley and Dendy (1886) was collected in May 2001 by trawling (72−76 m depth) from Tierra del Fuego, Patagonia, Argentina (49°18.1′ S, 65°33.9′ W). The material was taxonomically identified by Dr. Iosune Uriz (Blanes, Spain). A voucher specimen (ORMA000826) is deposited at PharmaMar.

Extraction and Isolation. The frozen sponge (25 g) was diced and extracted with 2-propanol (3 × 300 mL). The combined extracts were concentrated to yield a crude of 745 mg. This material was subjected to VLC on Lichroprep RP-18 with a stepped gradient from H_2O to MeOH. Compounds **1** (12.4 mg) and **2** (3.2 mg) were isolated as their TFA salts from fractions eluting with H_2O−MeOH, 1:1, by semipreparative HPLC (SymmetryPrep C-18, 7.8 × 150 mm, gradient H_2O−MeCN + 0.1% TFA from 10 to 33% MeCN in 15 min, UV detection at 254 nm). TFA salts of discorhabdins A (**3**) (8.7 mg) and B (**4**) (50.1 mg) were also isolated from these fractions.

Discorhabdin L (1): green solid; mp >250 °C (blackened at 160 °C); $[\alpha]^{25}_D$ 0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 268 (3.75), 310 (sh), 352 (3.59), 550 (2.65) nm; IR (KBr) ν_{max} 3400−2300, 1674, 1619, 1562, 1525, 1434, 1412, 1202, 1131 cm^{-1}; ^{1}H (500 MHz) and ^{13}C NMR (125 MHz), see Table 1; ESIMS *m*/*z* 352 $[M]^+$ HRFABMS *m*/*z* 352.0752 $[M]^+$ (calcd for $C_{18}H_{14}N_3O_3S$ 352.0756).

Discorhabdin I (2): green solid; mp >250 °C (blackened at 193 °C); $[\alpha]^{25}_D$ −562.8° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 309 (3.72), 374 (sh), 556 (2.81) nm; IR (KBr) ν_{max} 3600−2800, 1639, 1525, 1437, 1412, 1203, 1143 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.42 (1H, d, J = 9.5 Hz, H-1), 7.20 (1H, s, H-14), 6.66 (1H, dd, J = 9.5, 1.5 Hz, H-2), 6.16 (1H, d, J = 1.5 Hz, H-4), 5.58 (1H, dd, J = 4.0, 1.5 Hz, H-8), 3.94 (1H, ddd, J = 14.5, 6.5, 6.5 Hz, H-17), 3.82 (1H, ddd, J = 14.5, 10.0, 7.5 Hz, H-17), 2.87−2.90 (2H, m, H-16), 2.80 (1H, brd, J = 12.0 Hz, H-7), 2.46 (1H, dd, J = 12.0 and 4.0 Hz, H-7); ^{13}C NMR (CD_3OD, 125 MHz) δ 183.6 (C, C-3), 173.3 (C, C-5), 166.3 (C, C-11), 156.9 (C, C-19) 153.2 (C, C-10), 147.6 (CH, C-1), 133.9 (CH, C-2), 127.7 (CH, C-14), 125.5 (C, C-12), 124.3 (C, C-21), 121.9 (C, C-15), 120.6 (CH, C-4), 98.4 (C, C-20), 61.9 (CH, C-8), 50.4 (C, C-6), 45.8 (CH_2, C-17), 43.5 (CH_2, C-7), 19.1 (CH_2, C-16); ESIMS *m*/*z* 336 $[M + H]^+$; HRFABMS *m*/*z* 336.0801 $[M + H]^+$ (calcd for $C_{18}H_{14}N_3O_2S$ 336.0807).

Biological Activity. A colorimetric type of assay using sulforhodamine B reaction has been adapted for a quantitative measurement of cell growth and viability following the technique described in the literature.[13] The in vitro activity of the compounds was evaluated against a panel of 14 tumor cell lines, including prostate (DU-145 and LN-caP), ovary (SK-OV-3, IGROV, and IGROV-ET), breast (SK-BR3), melanoma (SK-MEL-28), endothelio (HMEC1), NSCL (A549), leukemia (K-562), pancreas (PANC1), and colon (HT29, LOVO, and LOVO-DOX).

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References and Notes

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48, and previous papers in this series.
(2) Ford, J.; Capon, R. J. *J. Nat. Prod.* **2000**, *63*, 1527−1528, and references therein.
(3) Perry, N. B.; Blunt, J. W.; McCombs, J. D.; Munro, M. H. G. *J. Org. Chem.* **1986**, *51*, 5476−5478.
(4) Perry, N. B.; Blunt, J. W.; Munro, M. H. G.; Higa, T.; Sakai, R. *J. Org. Chem.* **1988**, *53*, 4127−4128.
(5) Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron* **1988**, *44*, 1727−1734.
(6) Copp, B. R.; Fulton, K. F.; Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *J. Org. Chem.* **1994**, *59*, 8233−8238.
(7) Blunt, J. W.; Munro, M. H. G.; Batterhill, C. N.; Copp, B. R.; McCombs, J. D.; Perry, N. B.; Prinsep, M.; Thompson, A. M. *New J. Chem.* **1990**, *14*, 761−781.

(8) Perry, N. B.; McCombs, J. D.; Copp, B. R.; Rea, J.; Lill, R.; Major, D.; Andrew, C.; Fulton, K.; Linton, M.; Bringans, S.; Blunt, J.; Munro, M. The 37th Annual Meeting of the American Society of Pharmacognosy, University of California, Santa Cruz, CA, July 27−31, 1996; p 28.
(9) Kobayashi, J.; Cheng, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Lu, H.; Clardy, J. *Tetrahedron Lett.* **1987**, *28*, 4939−4942.
(10) Yang, A.; Baker, B. J.; Grimwade, J.; Leonard, A.; McClintock, J. B. *J. Nat. Prod.* **1995**, *58*, 1596−1599.
(11) Gunasekera, S. P.; McCarthy, P. J.; Longley, R. E.; Pomponi, S. A.; Wright, A. E.; Lobkovsky, E.; Clardy, J. *J. Nat. Prod.* **1999**, *62*, 173−175.
(12) Dijoux, M. G.; Gamble, W. R.; Hallock, Y. F.; Cardellina, J. H., II; van Soest, R.; Boyd, M. R. *J. Nat. Prod.* **1999**, *62*, 636−637.
(13) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

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