36 h. The mixture was allowed to cool to room temperature and poured into 50 mL of ether. The organic phase was washed with two 30-mL portions of water and dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to give 0.63 g (70%) of an oil. Gas chromatographic analysis of the mixture showed that it contained three major components which made up ~65%, ~20%, and ~10% of the mixture and several minor components whose concentrations totaled 5% of the mixture. The three major components were collected separately by preparative gas chromatography. The major product exhibited identical IR and ^{1}H NMR spectral properties with those reported for (-)-α-selinene (**11**).[7,12] It showed $[\alpha]^{25}_D$ -13.1° ($CHCl_3$, 0.5%) [reported[12] $[\alpha]^{25}_D$ -14.5° ($CHCl_3$, 1.0%)]. The second component showed identical spectral properties with those of the cis-fused eudesmane **12**,[11] which we reported previously.[7] It also showed essentially the same spectral properties as those reported for its naturally occurring enantiomer[11] and $[\alpha]^{25}_D$ 102° ($CHCl_3$, 0.7%) [reported[11] for (-)-enantiomer $[\alpha]_D$ 100°]. The third component was not conclusively identified, but its ^{1}H NMR spectrum indicated that four vinyl methyl groups were present. This suggested that it contained an internal conjugated diene system which possibly formed via a 1,5-sigmatropic hydrogen shift in the *Z* diene **10**.

Discorhabdin D, an Antitumor Alkaloid from the Sponges *Latrunculia brevis* and *Prianos* sp.

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Discorhabdins C (**1**), A (**2**), and B (**3**) have been isolated as the major pigments from three sponge species of the genus *Latrunculia* du Bocage (family Latrunculiidae, order Hadromerida) from New Zealand.[1,2] Discorhabdin A (**2**) has also been isolated independently (and named prianosin A) from an Okinawan sponge, *Prianos melanos*[3,4] (family Hymeniacidonidae, order Halichondrida), and its structure and absolute configuration demonstrated by X-ray crystallography. These compounds, representatives of a new class of alkaloid, were strongly cytotoxic and antimicrobial but did not show in vivo antitumor activity. A related compound with significant in vivo antitumor activity has now been found to cooccur with discorhabdin A (**2**) in a New Zealand collection of *L. brevis* Ridley and Dendy. Discorhabdin A (**2**) and this new compound, discorhabdin D (**4**), have also been isolated from a Japanese sponge of the genus *Prianos*.[3,5] The structure of discorhabdin D (**4**) is reported here, based on spectral comparisons with the known discorhabdins.[2] This structure, although based on

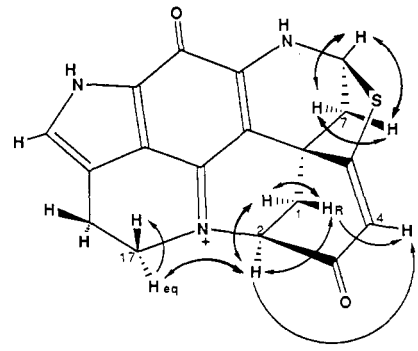
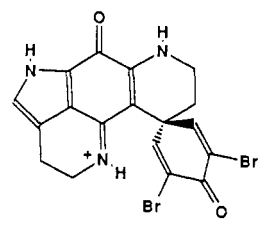
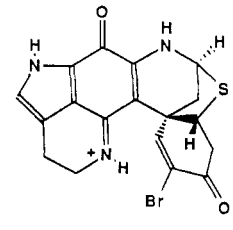


Figure 1. Enhancements observed in difference NOE spectra of discorhabdin D (**4**).

the same ring system as discorhabdin C, possesses two further heterocyclic rings to give a total of seven interlocking rings (four heterocyclic and one spiro) and seven double bonds, which is remarkable in a compound of this molecular weight.



1



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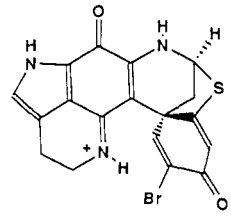

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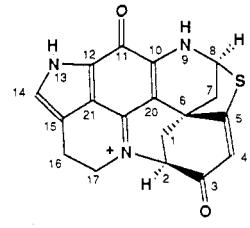

4

Discorhabdin D (**4**) was separated from discorhabdin A (**2**) by either reverse-phase or centrifugal counter current chromatography and characterized as the dark-green hydrochloride. The UV and IR spectra of these two compounds were similar, as were their molecular formulae. High-resolution FABMS established a composition of $C_{18}H_{14}N_3O_2S$ for MH^+ of discorhabdin D (MH^+ of **2** was $C_{18}H_{15}BrN_3O_2S$). This led to the initial hypothesis that the basic C, N, O, and S framework of the new compound was closely related to that in discorhabdin A (**2**), although discorhabdin D (**4**) has one more degree of unsaturation. This similarity was confirmed by homo- and heteronuclear correlation and specific decoupling NMR experiments, which revealed the substructures CCH_2CHNH and $CNH\text{-}CH{=}CCH_2CH_2N$ with chemical shifts (^{1}H and ^{13}C) and coupling constants (J_{HH} and J_{CH}) similar to those of C7 to NH9 and NH13 to C17 respectively in discorhabdin A (**2**) (and B (**3**)).[2]

Since there were the same number of sp^2 carbons in the ^{13}C NMR spectra of discorhabdins A (**2**) and D (**4**) the extra degree of unsaturation in **4** could be ascribed to an additional ring. As there was no signal corresponding to N*H*18 and the C17 signal was some 6 ppm downfield from the C17 signals in discorhabdins A and B (suggesting an additional carbon substituent on N18[6]), it was concluded that this additional ring was formed by a bond between

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N18 and one of the spiro ring atoms. The actual atom in the spiro ring bonded to N18 was identified by difference NOE experiments (Figure 1). The two C17 proton signals could be assigned as pseudo-axial and pseudo-equatorial from their vicinal coupling constants (axial 6.8 and 12.6 Hz, equatorial 3.3 and 7.6 Hz). Low-power cycled irradiation[7] of the pseudo-equatorial proton multiplet gave a significant enhancement (4.5%) of a one-proton triplet at 4.35 ppm (the reverse enhancement, 2%, was also observed). This proton was vicinally coupled to protons of a methylene group, which showed no further coupling, and whose carbon resonated at 30.27 ppm. These observations could only be accommodated in the discorhabdin skeleton by a C2-N18 bond. The remaining proton signal was at 6.07 ppm, similar to that of CH4 of discorhabdin B (**3**). The full structure of discorhabdin D was proposed as **4**.

A Drieding model of this structure showed features in accord with other NMR evidence. The H-C-C-H dihedral angles were consistent with the vicinal couplings observed. Long-range coupling (ca. 1 Hz) was observed between H4 and H2, and between H4 and H7α, shown by the model to be cases of "a planar zig-zag arrangement".[8] Difference NOE spectra with irradiation of the signals of H1R and H2, the protons closest to H4 in the model, showed slight enhancements (0.5 and 0.8%) of the H4 signal (Figure 1).

Discorhabdins A, B, and C are powerful cytotoxins with IC_{50} values against the P388 cell line in the range 0.03-0.01 μg/mL but in the in vivo P388 model were found to be inactive (T/C <120%).[2] Discorhabdin D had a lower in vitro activity against P388 (IC_{50} 6 μg/mL) but in contrast was considered to have significant[9] in vivo P388 activity (T/C 132% at 20 mg/kg). Mode of action and structural modification studies are under way to ascertain the loci of biological activity in this series.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Varian XL300 spectrometer. Chemical shifts are given in ppm on the δ scale, referenced to the solvent peaks: CHD_2OD at 3.30 ppm in CD_3OD, $(CHD_2)_2SO$ at 2.60 ppm, and $(CD_3)_2SO$ at 39.60 ppm in $(CD_3)_2SO$. UV spectra were recorded on a Varian DMS 100 UV/vis spectrometer. [α] measurements were made on a Perkin-Elmer 241 polarimeter. Mass spectra were recorded on VG7070F or VG7070E mass spectrometers. The IR spectrum was recorded on a Pye Unicam SP3-300 spectrometer as a KBr disk.

Isolation from *L. brevis*. Specimens were collected by SCUBA diving at depths of about 30 m from the Sugar Loaf Islands, Taranaki, New Zealand, in March, 1985. Voucher specimens 5NP3-1 and 5NP5-7 have been deposited in the University of Canterbury Marine Collection. The sponges (320 g) were blended and extracted with CH_3OH and CH_3OH/toluene (3:1) to give, after removal of solvents, a green gum (19 g). This was partitioned on a reverse phase (RP) column[10] to give a combined fraction (2.5 g) containing largely discorhabdin A (**2**). Preparative RPLC (Merck Lobar RP-8 column, 310 × 25 mm; 4 mL/min CH_3OH/H_2O (with 0.05% CF_3COOH) (3:7); 254 nm detection) on a subsample (70 mg) gave initially **4** (4 mg) followed by **2** (31 mg). Further preparative RPLC gave pure **4**, which showed a deep green spot at R_f 0.5 on silica gel TLC (Merck DC-Plastikfolien Kieselgel 60 F_{254}, developed with Et_3N/CH_3OH/CH_2Cl_2 (0.1:1:4)). Under these TLC conditions **2** gave a red-green spot at R_f 0.7.

Isolation from *Prianos* sp. Specimens were collected by SCUBA diving from the legs of Aquapolice, a floating building at Ocean EXPO Park, Okinawa in June, 1986. A voucher specimen, RS-39, has been deposited with Dr. Hoshino. The sponge (1.8 kg) was extracted by steeping in acetone (6 L) for 2 days. After removal of the acetone the aqueous suspension was extracted with ethyl acetate (2 × 500 mL) to give, after removal of the solvent, a green oil (4 g). A portion of the oil (910 mg) was separated into 12 fractions by centrifugal counter current chromatography using the system $CHCl_3/CH_3OH/H_2O$ (5:5:6) with the upper phase as the mobile phase. Fractions 7 and 8 (96 mg) were further purified on silica gel ($CHCl_3/CH_3OH$; 2:1) to give **4** as a green solid. Repeated purification of fractions 9-12 (165 mg) on silica gel ($CHCl_3/CH_3OH$; 3:1) gave **2** (40 mg), also as a green solid. **2** and **4** from the *Prianos* sp. were identical with the samples from *L. brevis* by spectral comparison.

Discorhabdin D (4) was characterized as its hydrochloride salt, a deep green solid, mp >360 °C; $[\alpha]_D$ 0°, $[\alpha]_{578}$ -45°, $[\alpha]_{546}$ -160° (*c* 0.15, CH_3OH). HRFABMS: MH^+ found 336.08208, calcd for $C_{18}H_{14}N_3O_2S$ 336.08069. UV (CH_3OH): 248 nm (log ϵ 4.35), 281 (4.15), 320 (3.93), 395 (3.95), 584 (2.84). UV (CH_3OH/KOH): 362 nm (log ϵ 4.49), 290 (4.19), 368 (3.98). IR: 3700-2300, 1650, 1620, 1550, 1525, 1490, 1410, 1310 cm^{-1}. ^{1}H NMR (CD_3OD): δ 7.10 (d, J = 1.0 Hz, H14), 6.07 (t, 0.8, H4), 5.60 (dd, 1.4, 3.4, H8), 4.35 (t, 2.8, H2), 4.0 (ddd, 3.3, 7.6, 14.3, H17α), 3.9 (ddd, 6.8, 12.6, 14.3, H17β), 3.2 (dddd, 1.1, 7.8, 12.5, 16.9, H16α), 3.1 (ddd, 3.2, 7.2, 16.6, H16β), 2.91 (dd, 2.8, 13.5, H1R), 2.80 (dd, 3.6, 11.9, H7β), 2.64 (dd, 1.3, 12.1, H7α), 2.58 (dd, 3.1, 13.3, H1S). ^{1}H NMR ($\{CD_3\}_2SO$): δ 13.45 (s, NH13), 10.8 (s, NH9), 7.37 (s, H14), 6.22 (s, H4), 5.79 (s, H8), 4.47 (s, H2), 4.13 (m, H17α), 3.92 (m, H17β), 3.15 (m, H16), 3.02 (d, 12.8, H1R), 2.80 (d, 12.8, H7β), 2.65 (d, 12.8, H7α), 2.55 (d, 12.8, H1S). ^{13}C NMR ($\{CD_3\}_2SO$): δ 183.08 (s, C3), 173.14 (s, C5), 166.47 (s, C11), 147.84 (s, C10 or C19), 145.90 (s, C19 or C10), 127.00 (d, $^1J_{CH}$ = 190 Hz, C14), 123.69 (s, C12 or C21), 121.50 (s, C21 or C12), 117.71 (s, C15), 112.43 (d, 168, C4), 99.64 (s, C20), 62.79 (d, 169, C8), 62.26 (d, 159, C2), 51.24 (t, 145, C17), 41.19 (s, C6), 38.59 (t, C7), 30.27 (t, 137, C1), 19.49 (t, 134, C16). **4** showed antimicrobial activity (at 30 μg/disk) against *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans*, but not against *Pseudomonas aeruginosa*.

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Titanium-Induced Reductive Elimination of 2-Yne-1,4-diols

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It has been shown that a low-valent titanium surface (Ti(0); Ti (II)) provides a good electron source capable of donating electrons to suitable substrates by a two SET mechanism.[2,3] A good substrate is one with an "electron

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