Enantiomeric 1,2,3-Trithiane-Containing Alkaloids and Two New 1,3-Dithiane Alkaloids from New Zealand Ascidians

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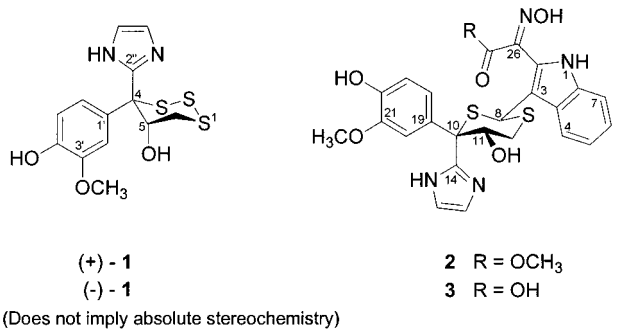
Enantiomeric marine natural products are uncommon, and it is rare to find enantiomers with two or more chiral centers. Although such compounds are usually terpene-derived and typically reported from sponges,[1−4] an ascidian-derived alkaloid enantiomeric pair, segolines B and C, have been recently reported.[5]

As part of our ongoing search for new bioactive compounds from New Zealand ascidians,[6] we have found extracts of the delicate pink stalked ascidian, *Hypsistozoa fasmeriana* (Michaelsen, 1924) (order Aplousobranchia, family Holozoidae)[7] to exhibit a wide range of biological activities. Bioassay-directed fractionation of extracts of specimens collected at Tutukaka, North Island, New Zealand, afforded the new (−) enantiomer of *trans*-5-hydroxy-4-(4′-hydroxy-3′-methoxyphenyl)-4-(2″-imidazolyl)-1,2,3-trithiane (**1**).[8] A second collection of *H. fasmeriana* made at Leigh Harbor, Northland, afforded (−)-**1** and two novel dithiane alkaloids **2** and **3**.

All spectroscopic data observed for (−)-**1** were identical to the literature values originally reported for (+)-**1** with the exception of the chiroptical properties, which were equal in magnitude and opposite in sign at every wavelength measured (see the Experimental Section).[9] To further investigate the enantiomeric nature of (−)-**1** and (+)-**1**, cyclodextrins (CD) were explored as chiral NMR shift reagents.[10,11] NMR titrations of (+)-**1** with α-, *β*-, and *γ*-CD indicated that *β*-CD was the optimal host for **1** and that maximal upfield shifts of the CD protons H-3 and H-5 were observed when the ratio of *β*-CD to **1** reached 2:1.[11] Host−guest binding was confirmed by the observation of ROESY NMR (τ_{mix} 300 ms) correlations between H-3 of *β*-CD and H-4″/5″, H-5′, and H-6′ of **1**. These results combined with the observation of only a single set of resonances for each of *β*-CD and **1** in the mixture indicated that the host and guest were in rapid exchange on the NMR time scale. A racemic mixture of **1** was then prepared by combining both enantiomers of **1**, and as expected, the ^{1}H NMR spectrum of this mixture was indistinguishable from the spectra observed for both pure enantiomers. However, when *β*-CD was added to the racemic mixture, all resonances (with the exception of **1** H-5) doubled, consistent with the formation of diastereomeric host−guest complexes (see the Supporting Material).



(+) - **1**
(-) - **1**
(Does not imply absolute stereochemistry)

2 R = OCH_3
3 R = OH

A molecular formula of $C_{26}H_{24}N_4O_6S_2$ for **2** was established by HRFAB mass spectrometry. Several features of the ^{1}H NMR spectrum of **2** were similar to **1**, including the presence of 3-methoxy-4-hydroxyphenyl- and 2-substituted imidazole rings and a $CH_2CH(OH)$ spin system. Connections between these three fragments were established by HSQC and HMBC NMR experiments allowing construction of the C-10 to C-25 portion of **2**. In addition to these fragments, NMR data were observed consistent with the presence of a 2,3-disubstituted indole ring (NH-1 to C-7), an isolated methine (C-8, δ_H 5.85, δ_C 42.8), and a carboxymethyl ester fragment (δ_H 4.02, δ_C 54.5 and 162.0, ν 1745 cm^{-1}). HMBC correlations observed for H-8 (δ 5.85) allowed connection of C-8 to indole C-3 (δ 97.2). Chemical shift considerations required C-8 (δ 42.8), C-10 (δ 59.2), and C-12 (δ 36.0) to be sulfur substituted, while long-range ^{1}H−^{13}C correlations from H-8 to C-10 and C-12 established the presence of a 2,4,4,5-tetrasubstituted 1,3-dithiane ring. This left the carboxymethyl ester and a molecular fragment of CNOH unaccounted for, which must be present at the indole C-2 position. A weak $^4J_{CH}$ HMBC NMR correlation from the methyl ester

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Table 1. ^{1}H and ^{13}C NMR Data (CD_3OD) for Fasmerianamines A (2) and B (3)

	2		**3**	
atom	^{1}H (δ, mult, J)	^{13}C	^{1}H (δ, mult, J)	^{13}C
1	11.56 (s)[a]		11.53 (s)[a]	
2		131.3 (1, 8)[b,c]		133.1 (1, 8)
3		97.2 (1, 4, 8)		94.8 (1, 4, 8)
3a		126.1 (1, 5, 7)		126.2 (1, 5)
4	7.85 (dd, 7.1, 1.2)	120.3 (6)	7.76 (dd, 6.5, 1.9)	119.7 (6)
5	7.10 (ddd, 7.2, 7.2, 1.1)	121.1 (7)	7.08 (ddd, 7.2, 7.2, 1.4)	121.1[c]
6	7.14 (ddd, 7.2, 7.2, 1.3)	123.3 (4)	7.10 (m)	122.7 (4)
7	7.38 (dd, 7.2, 1.0)	112.6 (5)	7.34 (d, 7.2)	112.5 (5)
7a		134.7 (1, 4, 6)		134.3 (1, 4, 6)
8	5.85 (s)	42.8 (12)	5.87 (s)	42.3 (12)
10		59.2 (8, 11, 12, 20, 24)		59.3 (11, 12, 20, 24)
11	5.07 (dd, 11.2, 3.3)	75.3 (12)	5.10 (dd, 11.1, 3.1)	75.3 (12)
12α	2.98 (dd, 14.5, 3.3)	36.0 (8, 11)	2.95 (dd, 14.5, 3.1)	36.0 (11)
β	3.16 (dd, 14.5, 11.2)		3.16 (dd, 14.5, 11.1)	
14		147.9 (11, 16, 17)		148.0 (11, 16, 17)
16	7.69 (s)	121.4 (17)	7.69 (s)	121.4 (17)
17	7.69 (s)	121.4 (16)	7.69 (s)	121.4 (16)
19		131.4 (11, 23)[c]		131.3 (11, 23)
20	6.90 (d, 2.3)	111.7 (24)	6.94 (d, 2.3)	111.7 (24)
21		149.4 (20, 23, 25)		149.4 (20, 23, 25)
22		148.6 (20, 23, 24)		148.5 (20, 23, 24)
23	6.71 (d, 8.5)	116.4	6.69 (d, 8.4)	116.2
24	6.20 (dd, 8.5, 2.3)	121.0 (20)	6.16 (dd, 8.4, 2.3)	121.1 (20)[c]
25	3.86 (s)	56.6	3.85 (s)	56.8
26		156.0 (28)		155.3
27		162.0 (28)		161.2
28	4.02 (s)	54.5	13.10 (br s)[a]	

[a] DMSO-d_6. Broad singlets were also observed at δ 12.19, 10.93, and 9.25 for **2** and at δ 10.93 and 9.25 for **3**. Due to their line widths the signals could not be assigned to specific protons in the molecules. [b] Numbers in parentheses are protons to which the carbon correlates in HMBC NMR experiments ($^xJ_{CH}$ = 8.3 Hz). [c] Assignments may be interchanged.

protons (δ 4.02) to an sp^2-hybridized carbon at δ 156.0 established the presence of an unusual oximo acetate functional group. ROE correlations between H-11 (δ 5.07) and H-20 (δ 6.90), H-24 (δ 6.20) and H-12α (δ 2.98), and H-8 (δ 5.85) and H-12β (δ 3.16) secured the relative stereochemistry and the structure of **2** as (8α,10β,11β)-methyl-2-[3-[11-hydroxy-10-(22-hydroxy-21-methoxyphenyl)-10-(18*H*-imidazol-14-yl)-9,13-dithian-8-yl]-1*H*-indol-2-yl]-2-oximoacetate.

Fasmerianamine B (**3**) was isolated as a 1:2 mixture with **2**. The NMR data observed for **3** were almost identical to those observed for **2**, with the greatest differences being an absence of the methyl singlet at δ 4.02 and the appearance of a broad exchangeable resonance at δ 13.10 suggesting the presence of a free carboxylic acid in place of the methyl ester. This was confirmed by the molecular formula ($C_{24}H_{22}N_4O_6S_2$) determined by HRFABMS and by the observation of strong IR absorptions at 3200 cm^{-1} (br) and 1685 cm^{-1}.

In our HPLC screening of New Zealand ascidians (N = 130), we have detected **1** in three different species. The (+)-enantiomer has been identified in *Aplidium* sp. D (order Aplousobranchia, family Polyclinidae),[8] collected at Kaikoura, South Island, NZ, an unidentified *Aplidium* species from Stewart Island, NZ,[12] and *Distaplia stylifera* (order Aplousobranchia, family Holozoidae), collected at Cape Reinga, Northland, NZ. To date, however, the (−)-enantiomer of **1** has only been detected in *H. fasmeriana*. Given the close phylogeny of ascidians of the genera *Hypsistozoa* and *Distaplia* (both family Holozoidae), it is interesting to speculate on the biosynthetic divergence that is capable of producing enantiomers of alkaloids containing two stereogenic centers. One possible biogenesis of **1** is via enantiomeric 5-oxo analogues, which then undergo reduction directed by the neighboring imidazole group at C-4.

Both enantiomers of **1** exhibited identical biological activities in a range of assays, including modest cytotoxicity (P388 IC_{50} 21.6 μM) and antimicrobial (*Bacillus subtilis*, *Candida albicans*) properties (4 mm zone of inhibition at 120 μg/disk). Compound (+)-**1** was found to be inactive at the NCI (panel average values of GI_{50} 32 μM, TGI 63 μM, LC_{50} 100 μM), did not inhibit the cell cycle checkpoint enzyme cdc2/cyclin B kinase (at 100 μM), did not induce thiol-generated DNA strand scission,[13,14] and was inactive (10% inhibition at 12.5 μg/mL) against *Mycobacterium tuberculosis* H_{37}Rv. Alkaloids **2** and **3** were inactive in all assays.

Experimental Section

Collection, Extraction, and Isolation Procedures. Specimens of *H. fasmeriana* were collected by scuba at Leigh Harbor and Tutukaka and kept frozen until use.[7] Specimens of *D. stylifera* were collected by scuba at Cape Reinga, Northland, NZ, and kept frozen until use.[7] Voucher specimens are retained at the University of Auckland, Department of Chemistry (*H. fasmeriana*, 99LH3-1), and at the NIWA Museum, Wellington (*D. stylifera*, 99MNP0266). *Aplidium* sp. D was collected at Kaikoura, South Island, NZ, and kept frozen until use. The freeze-dried (4.55 g) specimens of *H. fasmeriana* collected at Tutukaka were extracted with MeOH followed by CH_2Cl_2. The crude extract was partitioned between water and CH_2Cl_2 and the aqueous fraction was subjected to C18 flash chromatography (0.1% aqueous TFA through to MeOH) with the compound of interest eluting at 25% MeOH. This compound was further purified by semipreparative C18 HPLC (4 mL/min, 0.05%

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aqueous TFA+MeOH (6:4)) yielding (−)-**1** as the TFA salt (8 mg, 0.18% dry wt). Freeze-dried specimens of *Aplidium* sp. D (17.79 g) were worked up in the same manner yielding 45.3 mg (0.25% dry wt) of (+)-**1** as the TFA salt. Specimens of *H. fasmeriana* collected from Leigh Harbor were freeze-dried (3.50 g) and extracted with MeOH and CH_2Cl_2. The crude extract was partitioned between water and CH_2Cl_2, and the aqueous fraction was subjected to repeated C18 flash chromatography (0.1% aqueous TFA through to MeOH) eluting fasmerianamine A (**2**) (0.97 mg, 0.03% dry wt) and fasmerianamine B (**3**) (2.4 mg, 0.07% dry wt) as a 2:1 mixture of **2** and **3**.

(−)-1: $[\alpha]^{20}$ (λ) −250 (365 nm), −33 (546), −26 (578), −26 (Na D line) (c = 0.1, MeOH); MS (DEI) m/z 342 (1), 310 (4), 246 (8); HREIMS calcd for $C_{13}H_{14}N_2O_3S_3$ 342.0167, found 342.0146; IR (film) ν_{max} 3400, 1673, 1597, 1522, 1426, 1201, 1134 cm^{-1}; UV (MeOH + TFA) λ_{max} (log ϵ) 285 (3.34) nm; ^1H NMR (CD_3OD, 400 MHz) δ 7.54 (2H, s, H-4″, H-5″), 6.97 (1H, d, J = 2.3 Hz, H-2′), 6.79 (1H, d, J = 8.4 Hz, H-5′), 6.62 (1H, d, J = 2.3, 8.4 Hz, H-6′), 5.26 (1H, t, J = 7.2 Hz, H-5), 3.83 (3H, s, 3H-7′), 3.70 (1H, dd, J = 7.2, 11.0 Hz, H-6β), 2.94 (1H, dd, J = 7.2, 11.0 Hz, H-6α); ^{13}C NMR (CD_3OD, 100 MHz) δ 149.6 (C-3′), 149.1 (C-4′), 147.8 (C-2"), 127.4 (C-1′), 121.7 (C-6′), 120.9 (C-4″, C-5″), 116.8 (C-5′), 111.7 (C-2′), 84.2 (C-5), 67.9 (C-4), 56.6 (C-7′), 39.8 (C-6).

(+)-1: $[\alpha]^{20}$ (λ) +260 (365 nm), +34 (546), +28 (578), +26 (Na D line) (c = 0.1, MeOH); FTIR, UV, MS, ^1H NMR, and ^{13}C NMR data were identical to those of (−)-**1**.

Fasmerianamine A (2): brown gum; $[\alpha]^{20}_D$ = −17.5 (c = 0.4, MeOH); MS (FAB) m/z 541 (MH^+, 3); HRFABMS calcd for $C_{26}H_{25}N_4O_6S_2$ 541.1216, found 541.1224; IR ν_{max} (film) 3305, 1745 (sh), 1677, 1432, 1202, 1136 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 206 (4.13), 283 (3.35), 311 (3.18) nm; UV (MeOH + TFA) λ_{max} (log ϵ) 207 (4.20), 285 (3.36), 311 (3.18) nm; UV (MeOH + KOH) λ_{max} (log ϵ) 208 (4.42), 253 (3.65), 342 (3.25) nm; ^1H NMR (CD_3OD) see Table 1; ^{13}C NMR (CD_3OD) see Table 1.

Fasmerianamine B (3): brown gum; MS (FAB) m/z 527 (MH^+, 0.5); HRFABMS calcd for $C_{24}H_{23}N_4O_6S_2$ 527.1055, found 527.1059; IR ν_{max} (film) 3188 (br), 1685 cm^{-1}; ^1H NMR (CD_3OD) see Table 1; ^{13}C NMR (CD_3OD) see Table 1.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of (−)-**1**, ^1H NMR spectrum of *rac*-**1** + β-CD, ^1H spectrum of **2**, and ^1H and ^{13}C NMR spectra of **3** as a 1:2 mixture with **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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