Two New Acetylenic Derivatives and a New Meroditerpenoid from a Taiwanese Marine Sponge *Strongylophora durissima*

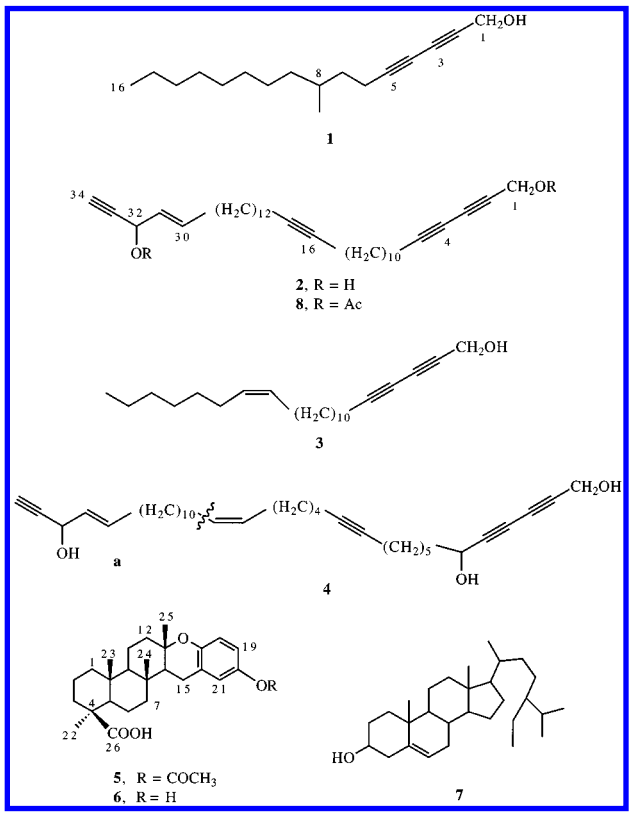
Ya-Ching Shen* and Chaturvedula V. S. Prakash

Institute of Marine Resources, National Sun Yat-sen University, 70 Lien-Hai Road, Kaohsiung 80424, Taiwan, Republic of China

Received May 24, 2000

Further chemical examination of the sponge *Strongylophora durissima* yielded two new acetylenic derivatives, durissimols A (**1**) and B (**2**), and a new meroditerpenoid, strongylophorine-12 (**5**), in addition to the known acetylenic compounds, reneirin-2 (**3**), 18-hydroxyreneirin-2, melyne-C (**4**), duryne, siphanochalynol, and 24-ethylcholesterol (**7**). Among them, durissimol B (**2**) and duryne showed potent cytotoxicity against human gastric tumor (NUGC) cells.

Several acetylenic and polyacetylenic compounds have been isolated from various sponges of the genera *Cribrochalina*,[1] *Petrosia*,[2] *Siphonochalina*,[3] *Reniera*,[4] *Xestospongia*,[5] and *Theonella*.[6] Many of the known polyacetylenes have shown biological activities ranging from antibacterial, fungicidal, and in vitro antitumor properties, to cell division inhibition.[5] In our pursuit of isolating biologically active secondary metabolites from marine sponges,[7−12] we have undertaken an investigation of the chemical constituents of the marine sponge *Strongylophora durissima* Dendy (family Petrosiidae). Its crude extract showed potent ichthyotoxicity, and some of the meroditerpenoids exhibited significant cytotoxicities against mouse leukemia cells (P-338) and human mouth epidermoid carcinoma (KB), lung (A-549), and colon cancer (HT-29) cells.[13] Recently we have reported the isolation of two new meroditerpenoids, strongylophorines-9 and -11, in addition to the known strongylophorines-1, -2, -3, and -8.[12] Our further study of the same sponge furnished the two new acetylenic derivatives, durissimols A (**1**) and B (**2**), and a new meroditerpenoid, strongylophorine-12 (**5**), in addition to the known acetylenic compounds reneirin-2 (**3**),[4] 18-hydroxyreneirin-2,[4] melyne-C (**4**),[5] duryne,[14] siphanochalynol,[3] and 24-ethylcholesterol (**7**).[15] Of the known acetylenic compounds, duryne has been reported as a potent cytotoxic agent inhibiting the growth of a number of tumor cell lines. The structures of the known compounds were determined on the basis of comparison of their spectral data with that reported in the literature. The structures of new compounds **1**, **2**, and **5** are described below.



Compound **1**, obtained as colorless oil, was deduced as $C_{17}H_{28}O$ from HREIMS (*m/z* 248.20993, $[M]^+$) and LREIMS (*m/z* 248, $[M]^+$). Its IR spectrum showed the presence of hydroxyl (3311 cm^{-1}) and acetylenic (2256 cm^{-1}) functionalities in the molecule. The ^1H NMR showed the presence of an oxymethylene singlet at δ 4.32, a triplet at δ 2.28 (J = 7 Hz) for the methylene protons at C-6 adjacent to the triple bond at C-5, and a multiplet at δ 1.55 for another methylene at C-7 similar to the structure from C-1 to C-7 of reneirin-2 (**3**).[4] This was also supported by the ^{13}C NMR values at δ 51.5, 73.4, 70.9, 64.3, and 81.9 for C-1 to C-5, the strong mass fragment at *m/z* 79 (C_5H_3O), and the HMBC correlations (H-1/C-2, C-3; H-6/C-7,C-5, C-4, and H-7/ C-6, C-5). Its ^1H NMR also showed the presence of a doublet for a methyl group at δ 0.84 (J = 7 Hz) and a multiplet for a methine proton at δ 1.35 corresponding to a secondary methyl group, and a methyl triplet at δ 0.88 (J = 7 Hz) along with a multiplet at δ 1.26 for seven methylenes in the *n*-alkyl side chain. The COSY spectrum of **1** showed correlations between the methylene protons H-6, H-7 and the methine proton at δ 1.35 (H-8), suggesting the position of the secondary methyl group at C-8. The position of the secondary methyl group was further established on the basis of the intense fragments at *m/z* 135 ($[M - C_8H_{17}]^+$) and *m/z* 107 ($[M - C_{10}H_{21}]^+$) and from HMBC correlations (H-8/C-17, C-7, C-6; H-7/C-8, and H-6/C-8). The ^{13}C NMR values of durissimol A were assigned for each carbon from the HSQC spectrum and were in good agreement with the structure **1**.

Compound **2**, obtained as pale yellow oil, was assigned the formula $C_{34}H_{52}O_2$ from its CIMS ($[M + H]^+$ at *m/z* 493) and DEPT spectra. Its IR spectrum also showed the presence of hydroxyl (3284 cm^{-1}) and acetylenic (2331 cm^{-1})

* To whom correspondence should be addressed. Tel.: (886) 7-525-2000, ext 5058. Fax: (886) 7-525-5020. E-mail: ycshen@mail.nsysu.edu.tw.

10.1021/np0002583 CCC: $19.00
Published on Web 11/01/2000

Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) Data of Durissimols A (**1**) and B (**2**)

	1		**2**	
position	^{1}H[a]	^{13}C[b]	^{1}H	^{13}C
1	4.32 s	51.5 t	4.31 s	51.0 t
2		73.4 s		73.5 s
3		70.9 s		70.9 s
4		64.3 s		64.4 s
5		81.9 s		81.8 s
6	2.28 t, 7	19.6 t	2.27 t, 7	19.2 t
7	1.55 m	28.1 t	1.55 m	28.8 t
8	1.35 m	37.0 d	1.27 m	29.1 t
9	1.26 m	36.8 t	1.27 m	29.3 t
10	1.26 m	26.4 t	1.27 m	29.4 t
11	1.26 m	29.7 t	1.27 m	29.0 t
12	1.26 m	28.8 t	1.27 m	29.1 t
13	1.26 m	29.1 t	1.27 m	29.4 t
14	1.26 m	31.9 t	1.47 m	28.8 t
15	1.26 m	22.6 t	2.12 t, 7	18.7 t
16	0.88 t, 7	14.1 q		80.2 s
17	0.84 d, 7	19.2 q		80.2 s
18			2.12 t, 7	18.7 t
19			2.12 t, 7	28.8 t
20−27			1.27 br s	29.0−29.4 t
28			1.35 m	28.0 t
29			2.07 q, 7	31.9 t
30			5.90 dt, 15.5, 7	134.6 d
31			5.60 dd, 15.5, 7	128.3 d
32			4.84 d, 6	62.7 d
33				83.3 s
34			2.57 s	73.4 d

[a] δ in ppm and J in Hz. [b] Assignments obtained from HSQC and COSY.

functional groups. Its ^{1}H NMR showed the presence of a methylene singlet at δ 4.31, a triplet at δ 2.27 (J = 7 Hz) for the methylene adjacent to acetylenic carbon, and a multiplet for a methylene at δ 1.55 as in durissimol A, suggesting the same partial structure from C-1 to C-7 in compound **2**. Furthermore, its ^{1}H NMR showed signals for the presence of an acetylenic proton at δ 2.57 (H-34), an oxymethine group at δ 4.84 (H-32, doublet, J = 6 Hz), and two protons in the olefinic region at δ 5.60 (dd, J = 15.5, 7 Hz, H-31) and 5.90 (dt, J = 15.5, 7 Hz, H-30) similar to the partial structure **a** as in melyne-C (**4**).[5] The partial structure **a** was supported by the ^{13}C NMR values assigned from the HSQC spectrum (Table 1). Upon acetylation, compound **2** yielded a diacetate (**8**), which showed a quasi-molecular ion at m/z 577 $[M + H]^+$. By fixing the two partial structures at the two ends of the molecule there were still two degrees of unsaturation to be explained out of a total of nine. In the absence of any additional olefinic protons in the ^{1}H NMR spectrum and from the additional signal at δ 80.2 in the ^{13}C NMR, the two degrees of unsaturation were attributed to the triple bond between C-16 and C-17. This was in agreement with the presence of a triplet at δ 2.12 (J = 7 Hz) in the ^{1}H NMR for the two methylenes adjacent to the central triple bond as in pellynol F.[14] The location of the triple bond at C-16 and C-17 was confirmed by the strong mass fragments at m/z 299, 277, 247, and 329 of compound **8** as shown in Figure 1 and from the HMBC correlations: H-14/C-15, C-16 and H-15/C-14, C-16. COSY correlations (H-6/H-7, H-29/H-30/H-31/H-32) in combination with the HMBC correlations (H-1/C-2, C-3; H-6/C-5, C-7; H-29/C-30; H-30/C-29, C-31, C-32; H-31/C-30, C-32; H-32/ C-31, C-33; and H-34/C-32, C-33) established the structure of **2**.

Compound **5** was obtained as an amorphous powder and its molecular formula was deduced as $C_{28}H_{38}O_5$ from a combination of HREIMS (m/z 454.27192, $[M]^+$) and LREIMS (m/z 454, $[M]^+$). Its ^{1}H NMR spectrum showed

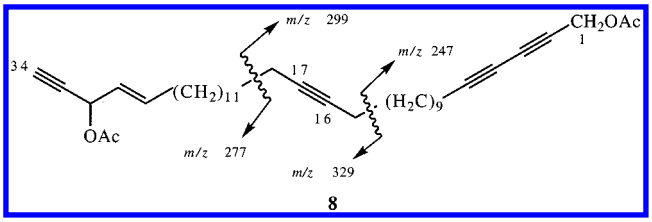


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Figure 1. Selective mass fragmentation of durissimol B diacetate (**8**).

the presence of three protons in the aromatic region (δ 6.78, 6.76, and 6.71), two methylene protons at δ 2.62 (J = 9 Hz, H-15), and four methyl singlets at δ 0.79, 0.91, 1.18 and 1.25, similar to the ^{1}H NMR spectrum of strongylophorine-3 (**6**), except for the presence of an additional methyl singlet at δ 2.26.[13] From the mass spectrum it was observed that compound **5** had 42 mass units greater than strongylophorine-3 (**6**). The observation that there were additional ^{13}C NMR signals at δ 21.0 and 170.1 suggested that compound **5** was the acetate form of strongylophorine-3 (**6**). This was supported by the presence of an acetate carbonyl at 1735 cm^{-1} in the IR spectrum and from the strong mass fragments at m/z 412 and m/z 395 formed by the loss of $-COCH_3$ and $-OCOCH_3$ groups, respectively, from the molecular ion. Strongylophorine-3 (**6**) on acetylation furnished an acetate whose spectral data was same as that of compound **5**, confirming the structure further. Preliminary biological study revealed that durissimol B (**2**) and duryne exhibited potent cytotoxicity against human gastric (NUGC) cells at 10 μM.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. EIMS and CIMS spectra were recorded on a VG Quattro 5022 mass spectrometer. The HREIMS and FABMS data were collected on a JEOL JMS−HX 110 mass spectrometer. The ^{1}H, ^{13}C NMR, DEPT, and COSY are recorded on a Varian FT-500 spectrometer. The HMBC and HSQC spectra were recorded on a Bruker Avance FT-300 spectrometer. The chemical shifts are given in δ (ppm) and coupling constants in Hz.

Animal Material. The sponge *S. durissima* was collected at Lan-Yu, at a depth of 15 m in September 1998. A voucher specimen (LSP-1) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung.

Extraction and Isolation. The fresh sponge (2.65 kg) was macerated in a blender and extracted with acetone (3.5 L × 3). The combined extracts were concentrated under vacuum to give a residue (85 g), which was further partitioned between EtOAc (600 mL) and H_2O (600 mL). The EtOAc-soluble layer was concentrated to a residue (16.8 g), which on chromatography over a Si gel column (400 g) with solvent mixtures of *n*-hexane/$CHCl_3$ and $CHCl_3$/MeOH of increasing polarity provided 7 fractions: A (1.42 g), B (0.82 g), C (0.68 g), D (0.72 g), E (0.66 g), F (0.82 g), and G (0.73 g). Fraction B on further chromatography over a Si gel column (15 g) with *n*-hexane/$CHCl_3$ (80:20) followed by reversed-phase HPLC (UV: 200 nm, LiChrosorb RP-C_{18} column, MeOH) furnished durissimol A (**1**, 4.5 mg), reneirin-2 (**3**, 3.5 mg), and 18-hydroxyreneirin-2 (7.0 mg). Fraction C on further chromatography over Si gel column (10 g) with *n*-hexane/$CHCl_3$ (40:60) gave strongylophorine-12 (**5**, 158 mg). Fraction D on chromatography over Si gel (12 g) with *n*-hexane/$CHCl_3$ (25:75) followed by crystallization with *n*-hexane/$CHCl_3$ (1:1) yielded 24-ethylcholesterol (**7**, 260 mg). Fraction F on further chromatography over Si gel column (12 g) with $CHCl_3$/MeOH (90:10) followed by reversed-phase HPLC (UV: 200 nm, LiChrosorb RP-C_{18} column, 4% aqueous MeOH) yielded durissimol B (**2**, 7.2 mg), duryne (4.8 mg), melyne-C (**4**, 6.5 mg), and siphanochalynol (3.8 mg)

Durissimol A (1): colorless oil: $[\alpha]^{25}_D$ +23.6° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 215 (3.22), 255 (4.13), 268 (3.71), and 284 (3.45); IR ($CHCl_3$) ν_{max} 3311, 2996, 2256, 1548, and 996 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) data, see Table 1; EIMS (70 eV) *m*/*z* 248 (M^+, 0.2), 231 (3), 201 (3), 135 (52), 107 (86), 93 (61), 91 (94), and 79 (100); HREIMS $[M]^+$ *m*/*z* 248.20993 (calcd for $C_{17}H_{28}O$, 248.20955), $[M - H]^+$ *m*/*z* 247.20651 (calcd for $C_{17}H_{27}O$, 247.20619).

Durissimol B (2): pale yellow oil, $[\alpha]^{25}_D$ −10.4° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 216 (3.23), 254 (3.49), 268 (4.04), and 285 (3.17); IR ($CHCl_3$) ν_{max} 3284, 2959, 2331, 1490, 1465, and 965 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) data, see Table 1; CIMS *m*/*z* 493 ($[M + H]^+$, 0.4), 475 (0.5), 391 (1.4), 285 (0.2), 271 (9), 269 (21.6), 257 (25), 243 (100), 225 (21.6), 219 (3), 111 (37.3) and 97 (44.6); EIMS *m*/*z* 287 (0.2), 273 (0.4), 263 (0.8), 249 (1), 243 (2), 229 (1), 221 (3), 219 (2), 209 (5), 205 (2), 195 (8), 183 (11), 169 (13), 157 (17), 145 (24), 131 (31), 117 (28), 105 (40), 95 (55), 91 (77), 81 (100), 67 (78), 55 (70).

Acetylation of Durissimol B (2). Acetylation (Ac_2O/Py, 1:1, 0.2 mL; room temperature) of durissimol B (**2**, 2 mg) and usual workup gave durissimol B diacetate (**8**, 1.4 mg): ^1H NMR ($CDCl_3$) δ 4.74 (2H, s, H-1), 6.01 (1H, m, H-30), 5.57 (1H, dd, J = 7, 15.3 Hz, H-31), 5.85 (1H, d, J = 7.5 Hz, H-32), 2.58 (1H, d, J = 2.1 Hz, H-34), 2.10 (6H, s, $COCH_3$), EIMS *m*/*z* 577 ($[M + H]^+$, 0.1), 503 (0.4), 491 (0.3), 475 (0.7), 449 (0.6), 431 (2.4), 415 (1.2), 403 (0.9), 389 (1), 369 (1), 361 (1), 355 (2), 347 (1.6), 333 (2), 329 (1), 319 (3), 307 (3), 299 (1), 295 (2), 281 (3), 277 (2), 263 (2), 257 (2), 247 (3), 245 (3), 233 (4), 81 (64), 79 (60), 67 (69), 55 (60), 43 (100).

Strongylophorine-12 (5): amorphous powder, $[\alpha]^{25}_D$ −9.4° (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} 229 (3.41), 283 (4.13), and 290 (3.53); IR ($CHCl_3$) ν_{max} 2935, 2869, 1756, 1735, 1718, 1693, 1490, and 1465 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (1H, m, H-1), 1.82 (2H, m, H-1, -6), 1.10 (2H, m, H-2, -11), 1.63 (1H, m, H-2), 0.97 (1H, m, H-3), 2.06 (1H, m, H-3), 1.79 (1H, d, J = 3 Hz), 1.25 (1H, m, H-6), 0.95 (1H, m, H-9), 1.80 (1H, m, H-11), 1.60 (1H, m, H-12), 2.03 (1H, m, H-12), 1.63 (1H, m, H-14), 2.62 (2H, d, J = 9.0 Hz, H-15), 6.76 (1H, d, J = 9.0 Hz, H-18), 6.71 (1H, d, J = 9.0 Hz, H-19), 6.78 (1H, s, H-21), 1.18 (3H, s, H-22), 0.79 (3H, s, H-23), 0.91 (3H, s, H-24), 1.25 (3H, s, H-25), 2.26 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 40.6 (t, C-1), 20.6 (t, C-2), 41.0 (t, C-3), 43.7 (s, C-4), 51.9 (d, C-5), 18.9 (t, C-6), 37.7 (t, C-7), 36.9 (s, C-8), 59.9 (d, C-9), 37.9 (s, C-10), 18.7 (t, C-11), 40.0 (t, C-12), 76.7 (s, C-13), 56.8 (d, C-14), 22.3 (t, C-15), 122.9 (s, C-16), 143.2 (s, C-17), 120.0 (d, C-18), 117.4 (d, C-19), 150.8 (s, C-20), 122.1 (d, C-21), 15.6 (q, C-22), 14.0 (q, C-23), 20.6 (q, C-24), 28.7 (q, C-25), 184.2 (s, C-26), 170.1 (s, OAc), and 21.0 (q, OAc); EIMS (70 eV) *m*/*z* 454 ($[M]^+$, 25), 439 (1), 412 (39), 289 (32), 271 (4), 243 (15), 221 (18), 178 (30), 161 (64), 123 (80); FABMS *m*/*z* 477 ($[M + Na]^+$); HREIMS *m*/*z* 454.27215 (calcd for $C_{28}H_{38}O_5$, 454.27192).

Acetylation of Strongylophorine-3 (6). Acetylation (Ac_2O/Py, 1:1, 2 mL; room temperature) of strongylophrine-3 (**6**, 10 mg) gave a product (8 mg), which was identical in ^1H NMR, EIMS, and R_f value with that of strongylophorine-12 (**5**).

Acknowledgment. This investigation was supported by the National Science Council under grant NSC 89-2811-B-110-0002. The authors thank Professor G. H. Lee, Institute of Oceanology, Academia Sinica, for his identification of sponge *S. durissima*. The National Science and Technology program in Pharmaceutical and Biotechnology is acknowleged for providing the cytotoxicity tests. We also thank Ms. Ho chao Lein and Yu shiu Ching of NSC southern NMR and MS Instrument Center for measurement of NMR and MS spectral data.

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NP0002583