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# Strongylodiols A, B and C, new cytotoxic acetylenic alcohols isolated from the Okinawan marine sponge of the genus *Strongylophora* as each enantiomeric mixture with a different ratio

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**Abstract**

Three new cytotoxic long-chain acetylenic alcohols, strongylodiols A, B and C, were isolated from the Okinawan marine sponge of the genus *Strongylophora*. Their gross structures were elucidated based on spectroscopic analysis. During the process for determination of the absolute stereochemistry at C-6 using the modified Mosher's method, these acetylenic alcohols were each found to be an enantiomeric mixture with a different ratio; 91:9 for strongylodiol A, 97:3 for strongylodiol B and 84:16 for strongylodiol C. The *R* configuration for each major enantiomer was established by the modified Mosher's method. Each enantiomer was separated and fully characterized. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* alkynes; diols; enantiomeric purity; cytotoxins; sponges; *Strongylophora*.

A variety of long-chain acetylenic alcohols with remarkable biological activities have been found in marine sponges.[1] Our continuous efforts[2] to find biologically active constituents from Okinawan marine invertebrates resulted in the isolation of three new cytotoxic long-chain acetylenic alcohols, strongylodiols, from the sponge of the genus *Strongylophora* (class Demospongiae, order Haplosclerida, family Petrosiidae). These compounds were found to be the first examples of long-chain acetylenic alcohols, each existing as an enantiomeric mixture

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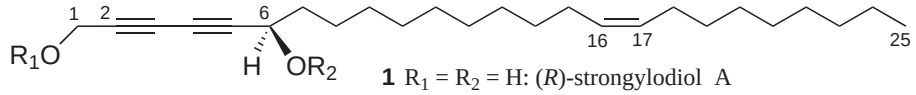
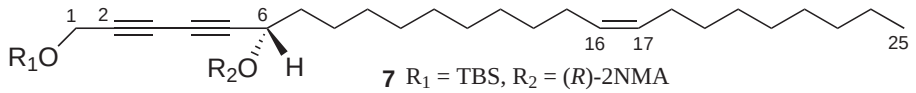
with a different ratio. Although optically active natural products isolated as a racemic mixture have been frequently encountered, those isolated as an enantiomeric mixture with a different ratio are fairly limited.[3]

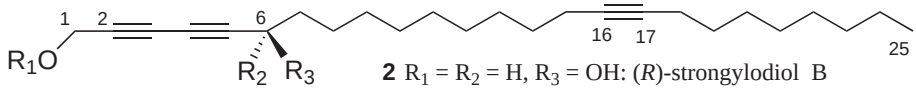
The AcOEt soluble fraction (37.2 g) from the MeOH extract of the sponge[4] (1.93 kg) was chromatographed on a silica gel column. Stepwise elution with hexane, hexane–AcOEt (4:1 and 1:1), AcOEt and MeOH afforded five fractions. The second fraction (eluted with hexane–AcOEt (4:1)) was subjected to further separation by silica gel flash chromatography and repeated reverse-phase HPLC over an ODS column to afford three new acetylenic alcohols, strongylodiols A (0.61% based on the AcOEt soluble fraction), B (0.62%) and C (0.34%).



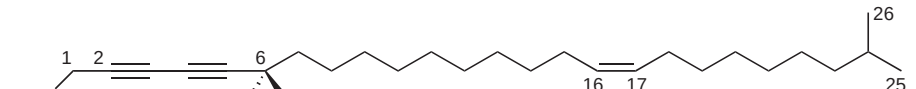

**1** $R_1 = R_2 = H$: (*R*)-strongylodiol A
**4** $R_1 = TBS$, $R_2 = H$
**5** $R_1 = TBS$, $R_2 = (R)$-2NMA
**6** $R_1 = TBS$, $R_2 = (S)$-2NMA
**9** $R_1 = R_2 = (S)$-MTPA




**7** $R_1 = TBS$, $R_2 = (R)$-2NMA
**8** $R_1 = TBS$, $R_2 = (S)$-2NMA
**10** $R_1 = R_2 = (S)$-MTPA
**11** $R_1 = R_2 = H$: (*S*)-strongylodiol A




**2** $R_1 = R_2 = H$, $R_3 = OH$: (*R*)-strongylodiol B
**12** $R_1 = R_3 = H$, $R_2 = OH$: (*S*)-strongylodiol B




**3** $R_1 = R_2 = H$, $R_3 = OH$: (*R*)-strongylodiol C
**13** $R_1 = R_3 = H$, $R_2 = OH$: (*S*)-strongylodiol C

The molecular formula of strongylodiol A was established by HRFABMS to be $C_{25}H_{42}O_2$ (calcd for $[M+H]^+$ 375.3263; obs 375.3269). The IR spectrum showed absorptions due to a hydroxyl group (3330 $cm^{-1}$), triple bond (2255 and 2162 $cm^{-1}$) and double bond (1651 $cm^{-1}$). The $^1H$ and $^{13}C$ NMR spectra[5] showed the presence of a disubstituted double bond [$\delta_H$ 5.34 (2H, m), $\delta_C$ 129.85 (CH), 129.93 (CH)], two triple bonds [$\delta_C$ 68.8 (C), 69.8 (C), 77.5 (C), 80.5 (C)], a primary hydroxyl group [$\delta_H$ 4.34 (2H, s), $\delta_C$ 51.4 ($CH_2$)], a secondary hydroxyl group [$\delta_H$ 4.42 (1H, br dd, $J = 5.9$, 10.2 Hz), $\delta_C$ 62.8 (CH)], and a terminal methyl [$\delta_H$ 0.88 (3H, t, $J = 7.0$ Hz), $\delta_C$ 14.1 ($CH_3$)] together with sixteen methylenes. The characteristic UV absorptions (EtOH) at $\lambda_{max}$ 231 (log $\varepsilon$ 2.68), 243 (log $\varepsilon$ 2.63) and 257 (log $\varepsilon$ 2.41) nm indicated the two triple bonds to be conjugated. After direct C–H bond correlations were made based on the HMQC analysis, the HMBC correlations were analyzed to give three partial structures a, b and c, as shown in Fig. 1. Two methylenic chains should connect between the partial structures a and b, and b and c, respectively, but the numbers of these methylenic chains (namely, the position of the double bond) were not clarified by analysis of the usual NMR and Mass data.

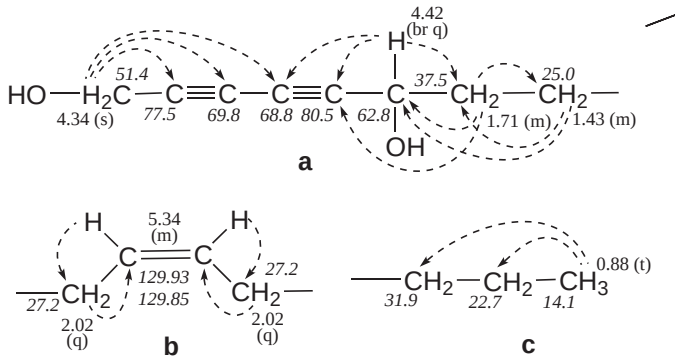



Figure 1. Partial structures, NMR data and HMBC correlations for strongylodiol A

The position of the central double bond of strongylodiol A was determined by collisional activation decomposition in tandem mass spectrometory (MS/MS). In the negative FAB MS/MS spectrum of the pseudo molecular ion at $[M-H]^-$ of $m/z$ 373, a series of charge remote fragmentation ions was observed as shown in Fig. 2 and was consistent with the location of the central double bond between C-16 and 17. The $Z$ configuration of the double bond was determined by comparison of the carbon chemical shift ($\delta_C$ 27.2) for allylic carbons with that in ($Z$)-2-heptene ($\delta_C$ 27.0) and ($E$)-2-heptene ($\delta_C$ 32.8).[6]

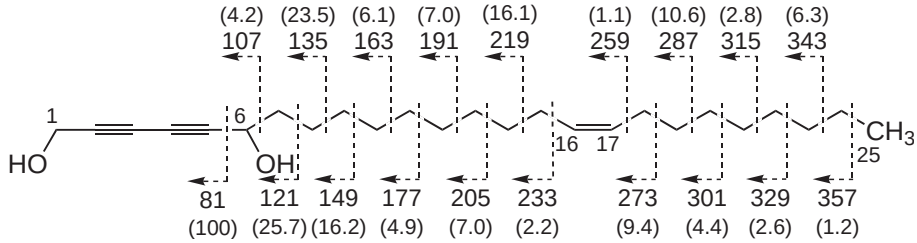



Figure 2. MS/MS data for the $[M-H]^-$ ion ($m/z$ 373) of strongylodiol A (the numerals in parentheses show relative intensities)

The absolute configuration of the chiral center at C-6 bearing a secondary hydroxyl group in strongylodiol A was examined by applying the modified Mosher's method.[7] After protection of the primary hydroxyl group at C-1 by a *tert*-butyldimethylsilyl (TBS) group to give **4**,[8] the secondary hydroxyl group at C-6 was esterified with ($R$)- and ($S$)-methoxy(2-naphthyl)acetic acid (2NMA)[9] to give the ($R$)-2NMA ester (**5**)[10] and ($S$)-2NMA ester (**6**),[10] respectively. The $^1$H NMR spectrum of **5** unexpectedly showed that **5** was accompanied with its diastereomer **7** in an approximate ratio 9:1. The ($S$)-2NMA ester (**6**) was also found to be a diastereomeric mixture of **6** and **8** in a similar ratio. These findings strongly suggested natural strongylodiol A to exist as an enantiomeric mixture with a different ratio. The $\Delta\delta$ values ($\delta_{R\text{-ester}}-\delta_{S\text{-ester}}$) of the corresponding protons at C-1, 7 and 8 between the major diastereomers **5** and **6** were calculated from the $^1$H NMR spectra of each diastereomeric mixture (Fig. 3), indicating the $R$ configuration at C-6 for the major enantiomer of strongylodiol A.

The exact ratios of ($R$)-strongylodiol A (**1**) and ($S$)-strongylodiol A (**11**) were determined by using a diasteromeric mixture of ($S$)-MTPA (methoxytrifluolomethylphenylacetic acid)[7] esters **9** and **10**, prepared by treatment of the natural enantiomeric mixture with ($R$)-MTPA chloride.

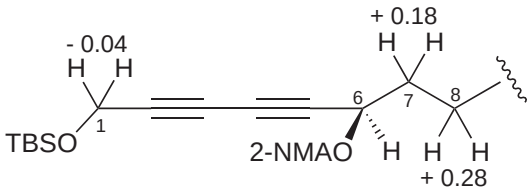



Figure 3. $\Delta\delta$ Values (ppm) for H-1, H-7 and H-8 of strongylodiol A

The reason why MTPA esters were used instead of 2NMA esters was that there was no possibility of epimerization at the chiral center of the reagent during the preparation of the esters. The diastereomeric mixture of **9** and **10** was subjected to a normal phase HPLC equipped with a chromatographic data processor (TOSOH chromatocorder 21) for determining the ratio of diastereomers. The ratio of **9** and **10** was found to be 91:9, thus indicating the ratios of (*R*)-strongylodiol A (**1**) and (*S*)-strongylodiol A (**11**) to be the same. After separation by HPLC, **9** and **10** were independently treated with $K_2CO_3$ in MeOH to give the pure enantiomers, (*R*)-strongylodiol A (**1**) with $[\alpha]_D^{22}$ −7.2° (*c* 1.11, $CHCl_3$) and (*S*)-strongylodiol A (**11**) with $[\alpha]_D^{22}$ +7.5° (*c* 0.067, $CHCl_3$), respectively. The spectral data, except for the optical rotation of **1** and **11**, were identical.

The molecular formula of strongylodiol B was established by HRFABMS to be $C_{25}H_{40}O_2$ (calcd for $[M+Na]^+$ 395.2926; obs 395.2942). The $^1H$ and $^{13}C$ NMR spectra[11] were similar to those of strongylodiol A except for two newly arisen acetylenic carbons [$\delta_C$ 80.2 (C) and 80.3 (C)] instead of the olefinic carbons in strongylodiol A. The position of a new triple bond between C-16 and 17 was determined by the negative FAB MS/MS of the pseudo molecular ion, $[M-H]^-$ of *m*/*z* 371.[12] Strongylodiol B was also found to be an enantiomeric mixture of (*R*)-strongylodiol B (**2**) with $[\alpha]_D^{22}$ −7.1° (*c* 0.42, $CHCl_3$) and (*S*)-strongylodiol B (**12**) with $[\alpha]_D^{22}$ +7.7° (*c* 0.013, $CHCl_3$). The absolute stereochemistry, enantiomeric ratio of **2** and **12** (97:3), separation and characterization were achieved by the same methods as those for strongylodiol A.

The molecular formula of strongylodiol C was determined by HRFABMS to be $C_{26}H_{44}O_2$ (calcd for $[M+H]^+$ 389.3420; obs 389.3395). The $^1H$ and $^{13}C$ NMR spectra[13] were similar to those of strongylodiol A, except for the signals due to an isopropyl group [$\delta_H$ 0.86 (6H, d, $J$=6.6 Hz), 1.51 (1H, nonet, $J$=6.6 Hz), $\delta_C$ 22.6 ($CH_3$×2), 28.0 (CH)] instead of the terminal ethyl group in strongylodiol A, indicating that an additional methyl group attaches at C-24 in strongylodiol C. The position of the double bond between C-16 and 17 was confirmed by the measurement of the negative FAB MS/MS.[14] Strongylodiol C was also found to be an enantiomeric mixture of (*R*)-strongylodiol C (**3**) with $[\alpha]_D^{22}$ −7.5° (*c* 0.093, $CHCl_3$) and (*S*)-strongylodiol C (**13**) with $[\alpha]_D^{22}$ +7.5° (*c* 0.027, $CHCl_3$). The absolute stereochemistry, enantiomeric ratio of **3** and **13** (84:16), separation and characterization were achieved by the same methods as those for strongylodiol A.

Cytotoxic activities for strongylodiols A, B and C against tumor cells (DLD-1 and MOLT-4) and normal cells (IMR-90) were examined, and the result is shown in Table 1 (enantiomeric mixture was used). Significant activity was observed in each compound against MOLT-4 cells.

Strongylodiols are the first long-chain acetylenic compounds isolated from a sponge of the genus *Strongylophora* (family Petrosiidae). Thus far, only meroditerpenoids such as strongylophorines[15] have been recognized as chemical markers for this genus. Long-chain acetylenic compounds are frequently reported from sponges of the order Haplosclerida,[16] and

Table 1
Cytotoxic activities for strongylodiols A, B and C

| Compound | $IC_{50}$ (μg/mL) | | |
|---|---|---|---|
| | MOLT-4[a] | IMR-90[b] | DLD-1[c] |
| Strongylodiol A | 0.35 | 5 | 8 |
| Strongylodiol B | 0.8 | 6.5 | 8.4 |
| Strongylodiol C | 0.85 | 7.5 | 28 |

[a] Human T lymphocyte leukemia.
[b] Human diploid lung fibroblast.
[c] Human colorectal adenocarcinoma.

especially from the genus *Petrosia*, which is considered to be closely related to *Strongylophora* because of the similarity of the spicule complement. Our isolation of hydroxylated long-chain acetylenic compounds from *Strongylophora* with structural similarity to compounds isolated from some *Petrosia* species confirms the close relationships of the two genera.

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4. The sponge was collected on a coral reef off Ishigaki Island (Okinawa Prefecture, Japan) in June 1998 at a depth of 13–15 m. One of the authors, R. W. M. Van Soest, identified the sponge as belonging to the genus *Strongylophora*. A voucher specimen is presently on deposit in the Zoological Museum in University of Amsterdam under the registration number ZMA POR. 14879.
5. $^1$H NMR data (500 MHz, $CDCl_3$, $J$ in Hz) for strongylodiol A; $\delta$ ppm 0.88 (3H, t, $J=7.0$, H-25), 1.25–1.35 (24H, m), 1.43 (2H, m, H-8), 1.71 (2H, m, H-7), 2.02 (4H, m, H-15, 18), 4.34 (2H, s, H-1), 4.42 (1H, br dd, $J=5.9$, 10.2), 5.34 (2H, m, H-16, 17). $^{13}$C NMR data (125 MHz, $CDCl_3$) for strongylodiol A; $\delta$ ppm 14.1 ($CH_3$, C-25), 22.7 ($CH_2$, C-24), 25.0 ($CH_2$, C-8), 27.2 ($CH_2\times2$, C-15, 18), 29.2 ($CH_2$), 29.28 ($CH_2$), 29.30 ($CH_2\times2$), 29.47 ($CH_2$), 29.50 ($CH_2\times3$), 29.7 ($CH_2\times2$), 31.9 ($CH_2$, C-23), 37.5 ($CH_2$, C-7), 51.4 ($CH_2$, C-1), 62.8 (CH, C-6), 68.8 (C, C-4), 69.8 (C, C-3), 77.5 (C, C-2), 80.5 (C, C-5), 129.85 (CH, C-16 or C-17), 129.93 (CH, C-16 or C-17).
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10. The diasteromers **7** and **8** were also contained, respectively.
11. $^{1}$H NMR data (500 MHz, $CDCl_3$, $J$ in Hz) for strongyldiol B; $\delta$ ppm 0.88 (3H, t, $J=6.9$, H-25), 1.25–1.32 (16H, m), 1.36 (4H, m, H-13, 18), 1.43 (2H, m, H-8), 1.47 (4H, m, H-14, 19), 1.71 (2H, m, H-7), 2.13 (4H, t, $J=7.1$, H-15, 18), 4.34 (2H, s, H-1), 4.42 (1H, t, $J=6.6$, H-6). $^{13}$C NMR data (125 MHz, $CDCl_3$) for strongylodiol B; $\delta$ ppm 14.1 ($CH_3$, C-25), 18.7 ($CH_2\times2$, C-15, 18), 22.7 ($CH_2$, C-24), 25.0 ($CH_2$, C-8), 28.83 ($CH_2$, C-13 or 18), 28.86 ($CH_2$, C-13 or 18), 29.10 ($CH_2$), 29.12 ($CH_2$), 29.15 ($CH_2\times3$), 29.21 ($CH_2$), 29.4 ($CH_2\times2$), 31.8 ($CH_2$, C-23), 37.5 ($CH_2$, C-7), 51.4 ($CH_2$, C-1), 62.8 (CH, C-6), 68.8 (C, C-4), 69.8 (C, C-3), 77.5 (C, C-2), 80.2 (C, C-16 or C-17), 80.3 (C, C-16 or C-17), 80.5 (C, C-5).
12. MS/MS data for the $[M-H]^-$ ion ($m/z$ 371) of strongylodiol B: $m/z$ (rel. int.) 355 (1.4), 341 (3.0), 327 (1.6), 313 (1.2), 299 (3.5), 285 (7.7), 271 (2.2), 219 (14.3), 205 (7.8), 191 (6.1), 177 (3.4), 163 (5.6), 149 (17.3), 135 (25.9), 121 (28.0), 107 (5.6), 81 (100).
13. $^{1}$H NMR data (500 MHz, $CDCl_3$, $J$ in Hz) for strongyldiol C; $\delta$ ppm 0.86 (6H, d, $J=6.6$, H-25, 26), 1.15 (2H, br q, $J=6.6$, H-23), 1.25–1.35 (20H, m), 1.44 (2H, m, H-8), 1.51 (1H, nonet, $J=6.6$, H-24), 1.71 (2H, m, H-7), 2.01 (4H, m, H-15, 18), 4.34 (2H, s, H-1), 4.43 (1H, t, $J=6.6$), 5.35 (2H, m, H-16, 17). $^{13}$C NMR data (125 MHz, $CDCl_3$) for strongylodiol C; $\delta$ ppm 22.6 ($CH_3\times2$, C-25, 26), 25.0 ($CH_2$, C-8), 27.2 ($CH_2\times2$, C-15, 18), 27.4 ($CH_2$, C-22), 28.0 (CH, C-24), 29.2 ($CH_2$), 29.29 ($CH_2$), 29.34 ($CH_2$), 29.47 ($CH_2$), 29.50 ($CH_2\times2$), 29.76 ($CH_2\times2$, C-14, 19), 29.81 ($CH_2$, C-21), 37.5 ($CH_2$, C-7), 39.0 ($CH_2$, C-23), 51.5 ($CH_2$, C-1), 62.9 (CH, C-6), 68.8 (C, C-4), 69.8 (C, C-3), 77.5 (C, C-2), 80.6 (C, C-5), 129.86 (CH, C-16 or 17), 129.93 (CH, C-16 or 18).
14. MS/MS data for the $[M-H]^-$ ion ($m/z$ 387) of strongylodiol C: $m/z$ (rel. int.) 343 (3.4), 329 (6.4), 315 (3.2), 301 (7.4), 287 (24.6), 273 (8.0), 259 (3.9), 233 (2.2), 219 (30.4), 205 (21.1), 191 (23.7), 177 (17.0), 163 (19.8), 149 (58.4), 135 (42.0), 121 (37.1), 107 (9.3), 81 (100).
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