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Tetrahedron
Tetrahedron 63 (2007) 11108–11113

# New diastereomeric bis-sesquiterpenes from Hainan marine sponges *Axinyssa variabilis* and *Lipastrotethya ana*

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Received 31 May 2007; revised 16 July 2007; accepted 8 August 2007
Available online 14 August 2007

**Abstract**—Three unprecedented diastereoisomeric dimers, *cis*-dimer A (**1**), *cis*-dimer B (**2**) and *trans*-dimer C (**3**), exhibiting a bis-bisabolene skeleton, and a new sesquiterpene, dehydrotheonelline (**4**), their potential precursor, have been isolated from the South China Sea sponges *Axinyssa variabilis* and *Lipastrotethya ana*, along with known related sesquiterpenes. The structure of the novel molecules has been determined by extensive NMR spectral analysis.
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## 1. Introduction

Bisabolene-type sesquiterpenes are a class of biologically active natural products biosynthesized by a diverse range of organisms from both terrestrial and marine habitats. Within the marine environment, numerous bisabolene sesquiterpenes have been reported from sponges,[1,2] molluscs,[3] gorgonians[4] and red algae.[5] In addition, a number of unique functionalized bisabolene compounds such as heterocyclic, nitrogenous, halogenated, polyhydroxylated quinone and acetylated derivatives have significantly broadened the chemical diversity in this distinct group of molecules.

In the course of our ongoing programme focused towards the isolation of biologically active compounds from Chinese marine organisms,[6–8] we carried out a chemical investigation on the sponges *Axinyssa variabilis* and *Lipastrotethya ana*, both collected off the Lingshui Bay, Hainan Province, China. These two sponges were found to possess a similar secondary metabolite pattern characterized by the presence of unprecedented dimeric sesquiterpenes with a bis-bisabolene skeleton. In particular, the diastereomeric dimers *cis*-dimer A (**1**) and *cis*-dimer B (**2**) were isolated from the ether extract of both sponges, while the *trans*-dimer C (**3**) was found only in the ether extract of *L. ana*, along with a new sesquiterpene, dehydrotheonelline (**4**). The known theonelline (**5**)[9] was isolated from both sponges whereas 3-isocyanotheonelline (**6**)[3b,10] was found only in *L. ana*.

## 2. Results and discussion

At SIMM laboratory, the frozen sponge *A. variabilis* (250 g dry weight) was lyophilized and exhaustively extracted with acetone. The $Et_2O$ soluble portion of the acetone extract was concentrated to give a crude extract (3.5 g), which was subjected to separation by silica gel (petroleum ether/acetone gradient). Subsequent purifications on Sephadex LH-20 and reversed-phase HPLC gave *cis*-dimer A (**1**, 5.6 mg), *cis*-dimer B (**2**, 4.4 mg), and theonelline (**5**, 3.4 mg) (Fig. 1).

At ICB laboratory, a small sample of the frozen sponge *L. ana* (3 g dry weight) was extracted with acetone. The $Et_2O$ soluble portion of the acetone extract was concentrated to give a residue (190 mg), which was chromatographed by silica gel column (petroleum ether/$Et_2O$ gradient). Analogously with *A. variabilis*, the $Et_2O$ extract was dominated by the presence of a main apolar UV–visible fraction, which was eluted by petroleum ether. This fraction was subsequently purified by normal-phase HPLC to give an unresolved mixture (1.8 mg) of dimers, *cis*-dimer A (**1**) and *cis*-dimer B (**2**), along with pure *trans*-dimer C (**3**, 0.8 mg). The three sesquiterpene metabolites, dehydrotheonelline (**4**, 0.9 mg), theonelline (**5**, 0.9 mg) and 3-isocyanotheonelline (**6**, 1.0 mg), were also obtained by HPLC purification (Fig. 1).

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doi:10.1016/j.tet.2007.08.028

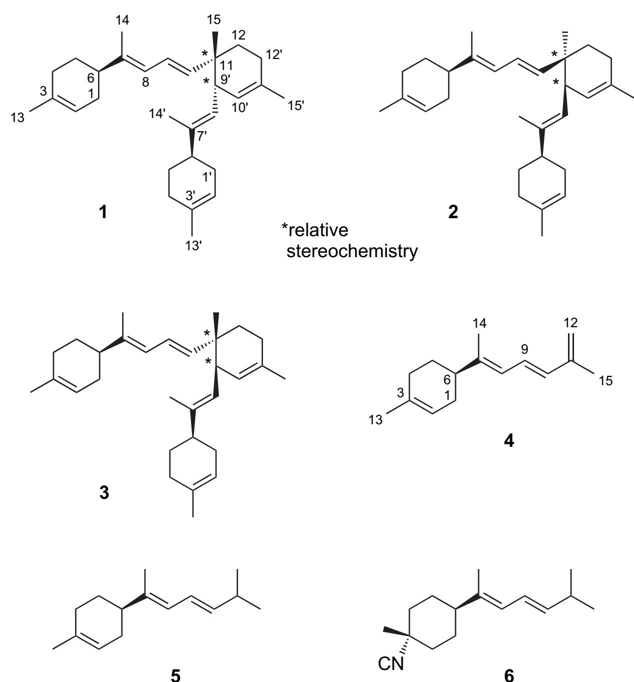



**Figure 1**. Compounds isolated from *A. variabilis* and *L. ana*.

The known molecules **5**[9] and **6**[3b,10] were identified by NMR and mass spectral data. The configuration at C-6 of theonelline (**5**), from both sponges, was assumed to be *R*, the same as that reported in the literature,[9] by comparison of $[\alpha]_D$ value of theonelline samples isolated from both sponges with that reported in the literature.[9] The structures of dimers **1–3** and dehydrotheonelline (**4**), which could be considered as the precursor of **1–3**, were established by extensive NMR analysis, as described below (Fig. 1).

*Cis*-dimer A (**1**) was shown to be a hydrocarbon with molecular formula $C_{30}H_{44}$, which was established by HREIMS analysis on the molecular peak at *m/z* 404, implying nine degrees of unsaturation. The UV spectrum showed an absorption maximum at 225 nm ($\varepsilon$=7426), according to the presence of a trisubstituted conjugated diene chromophore. The $^{13}$C NMR spectrum displayed signals for 30 carbons, which were attributed by DEPT experiment to six methyls, eight methylenes, ten methines (seven of which were sp$^2$ carbons) and six quaternary carbons (five of which sp$^2$). Considering that the $^1$H and $^{13}$C NMR spectra of **1** contained a series of signals almost identical to those recorded for cyclohexene ring of the co-occurring theonelline (**5**),[9] and that the secondary metabolites of sponges of the genus *Axinyssa* are typically sesquiterpenes, it was reasonable to suggest a dimeric sesquiterpenoid structure. The presence of six double bonds in **1** left three sites of unsaturation, which were attributed to a tricyclic skeleton. The $^1$H NMR spectrum showed three broad singlets at $\delta$ 5.41 (H-2), 5.39 (H-2′) and 5.09 (H-10′) that were assigned to olefinic protons on three endocyclic trisubstituted double bonds. Three further olefinic signals at $\delta$ 5.81 (1H, d, $J$=10.8 Hz, H-8), 6.19 (1H, dd, $J$=15.3 and 10.8 Hz, H-9) and 5.71 (1H, d, $J$=15.3 Hz, H-10) were attributed to the protons of a conjugated diene system, the same as theonelline.[9] Three 3H singlets at $\delta$ 1.72 ($H_3$-14), 1.64 ($H_3$-15′) and 1.58 ($H_3$-14′) and a 6H singlet at $\delta$ 1.65 ($H_3$-13 and $H_3$-13′) were assigned to five vinyl methyl groups. Finally, the $^1$H NMR spectrum was completed by signals due to a tertiary methyl group [$\delta$ 1.01 (s, $H_3$-15)], a methine [$\delta$ 2.75 (1H, br d, $J$=9.9 Hz, H-9′)] and an olefinic proton [$\delta$ 4.96 (1H, d, $J$=9.9 Hz, H-8′)]. The $^1$H and $^{13}$C NMR signals of carbons C-1/C-10, C-13 and C-14 (Fig. 2, partial structure **1a**) were almost identical with those of theonelline (**5**), with the exception of the multiplicity of H-10, resonating in **1** as a doublet without any further coupling. The other part of the molecule (Fig. 2, partial structure **1b**) included a C-1′/C-8′ sequence similar to that of theonelline (**5**) and exhibited in addition a second cyclized moiety connected with **1a**. The structures of both portions **1a** and **1b** were confirmed by a series of two-dimensional NMR ($^1$H–$^1$H COSY, HSQC and HMBC) experiments. In particular, the second cyclized portion in **1b** was determined to be a cyclohexene ring containing a bis-allylic proton ($\delta$ 2.75, H-9′), which was coupled with both H-8′ ($\delta$ 4.96) and H-10′ ($\delta$ 5.09), an isolated $A_2B_2$ system [$\delta$ 1.53 (H-12a) and $\delta$ 1.65 (H-12b); $\delta$ 1.91 (H-12′a) and $\delta$ 2.01 (H-12′b)] and a trisubstituted double bond bearing a vinyl methyl ($\delta$ 1.64, $H_3$-15′). Finally, the two partial structures **1a** and **1b** were connected through a quaternary carbon ($\delta_C$ 37.8, C-11) bearing a methyl (C-15) by analysis of the HMBC spectrum of **1** (Fig. 3). Diagnostic correlations between C-10 and both $H_2$-12 and $H_3$-15, and between C-9′ and $H_3$-15, support the proposed structure. All resonances were assigned as reported in Table 1.

The relative stereochemistry of **1** was deduced from the ROESY correlations. The geometry of all three double bonds $\Delta^{7(8)}$, $\Delta^{9(10)}$ and $\Delta^{7'(8')}$ was suggested to be $E$ by the combined analysis of ROESY (H-8/H-10 and H-9/H-14 correlations), the large olefinic proton coupling constant ($J_{H\text{-}9/H\text{-}10}$=15.3 Hz), as well as the shielded carbon resonances of the two vinyl methyls ($\delta_{C\text{-}14}$ 14.8 and $\delta_{C\text{-}14'}$ 14.5). The configuration at C-6 and C-6′ of **1** was proposed to be the same as that at C-6 in **5** due to biogenetical considerations. Furthermore, analysis of the multiplicity of both H-5a [$\delta$ 1.49,

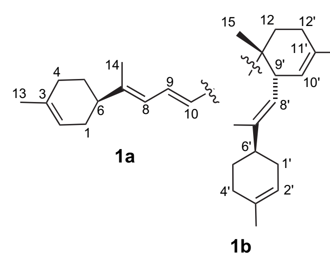



**Figure 2**. Structural fragments of *cis*-dimer A (**1**).

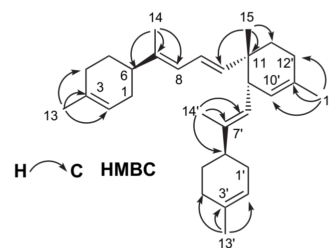



**Figure 3**. Selected key HMBC correlations for *cis*-dimer A (**1**).

**Table 1**. NMR data[a] of *cis*-dimer A (**1**), *cis*-dimer B (**2**)

| Position | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| | δ $^{1}$H[b] m (Hz) | δ $^{13}$C[c] | HMBC[d] | δ $^{1}$H[b] m (Hz) | δ $^{13}$C[c] | HMBC[d] |
| 1 | 2.20 m | 30.6 t | H2, H6 | 2.13 m | 30.5 t | H2, H6 |
| 2 | 5.41 br s | 120.8 d | H1, H$_3$13 | 5.40 br s | 120.9 d | H1, H$_3$13 |
| 3 | — | 133.6 s | H$_3$13, H2, H$_2$3 | — | 133.7 s | H$_3$13, H2, H$_2$3 |
| 4 | 2.20 m, 1.90 m | 30.7 t | H$_3$13, H2 | 2.20 m, 1.90 m | 30.6 t | H$_3$13, H2 |
| 5a | 1.49 dddd (5.9, 11.8, 12.2, 13.9) | 28.0 t | H$_2$4, H6 | 1.66 m, 1.49 dddd (6.0, 11.5, 12.4, 13.9) | 27.8 t | H$_2$4, H6 |
| 5b | 1.66 m | | | | | |
| 6 | 2.15 m | 43.0 d | H$_3$14, H$_2$1, H$_2$5 | 2.15 m | 43.1 d | H$_3$14, H$_2$1, H$_2$5 |
| 7 | — | 140.0 s | H$_2$14, H6, H8 | — | 140.0 s | H$_2$14, H6, H8 |
| 8 | 5.81 d (10.8) | 123.9 d | H$_3$14, H6, H9 | 5.81 d (10.5) | 124.1 d | H$_3$14, H6, H9 |
| 9 | 6.19 dd (10.8, 15.3) | 123.8 d | H8, H10 | 6.18 dd (10.5, 15.6) | 123.9 d | H8, H10 |
| 10 | 5.71 d (15.3) | 138.8 d | H$_3$15, H9, H$_2$12 | 5.71 d (15.6) | 138.8 d | H$_3$15, H9, H$_2$12 |
| 11 | — | 37.8 s | H$_3$15, H10, H$_2$12 | — | 37.9 s | H$_3$15, H10, H$_2$12 |
| 12a | 1.53 dd (4.4, 11.8) | 34.1 t | H$_3$15, H$_2$12′, H10 | 1.50 dd (4.4, 11.6) | 33.9 t | H$_3$15, H$_2$12′, H10 |
| 12b | 1.65 m | | | 1.61 m | | |
| 13 | 1.65 s | 23.5 q | H2, H$_2$4 | 1.66 s | 23.5 q | H2, H$_2$4 |
| 14 | 1.72 s | 14.8 q | H6, H8 | 1.72 s | 14.7 q | H6, H8 |
| 15 | 1.01 s | 25.5 q | H10, H$_2$12 | 1.02 s | 25.6 q | H10, H$_2$12 |
| 1′ | 1.98 m | 30.9 t | H2′, H6′ | 1.98 m | 30.9 t | H2′, H6′ |
| 2′ | 5.39 br s | 121.0 d | H$_3$13′, H$_2$1′, H$_2$4′ | 5.37 br s | 120.9 d | H$_3$13′, H$_2$1′, H$_2$4′ |
| 3′ | — | 133.7 s | H$_3$13′, H2′, H$_2$4′ | — | 133.7 s | H$_3$13′, H2′, H$_2$4′ |
| 4′ | 2.20 m, 1.90 m | 30.7 t | H$_3$13′, H$_2$5′ | 2.20 m, 1.92 m | 30.8 t | H$_3$13′, H$_2$5′ |
| 5′a | 1.75 br ddd (11.2, 12.3, 14.1) | 27.8 t | H$_2$4′, H6′ | 1.75 m | 27.9 t | H$_2$4′, H6′ |
| 5′b | 1.48 m | | | 1.47 m | | |
| 6′ | 2.04 m | 43.0 d | H$_3$14′, H$_2$1′, H$_2$5′ | 2.04 m | 43.0 d | H$_3$14′, H$_2$1′, H$_2$5′ |
| 7′ | — | 139.4 s | H$_3$14′, H6′, H8′ | — | 139.0 s | H$_3$14′, H6′, H8′ |
| 8′ | 4.96 d (9.9) | 125.0 d | H$_3$14′, H9′ | 4.95 d (9.4) | 125.3 d | H$_3$14′, H9′ |
| 9′ | 2.75 br d (9.9) | 44.6 d | H$_3$15, H8′, H10′ | 2.75 br d (9.4) | 44.7 d | H$_3$15, H8′, H10′ |
| 10′ | 5.09 br s | 124.3 d | H$_3$15′, H9′ | 5.09 br s | 124.2 d | H$_3$15′, H9′ |
| 11′ | — | 132.7 s | H$_3$15′, H10′, H$_2$12′ | — | 132.7 s | H$_3$15′, H10′, H$_2$12′ |
| 12′a | 1.91 m | 27.9 t | H$_3$15′, H10′, H$_2$12′ | 1.91 m | 27.9 t | H$_3$15′, H10′, H$_2$12 |
| 12′b | 2.01 m | | | 2.00 m | | |
| 13′ | 1.65 s | 23.5 q | H2′, H$_2$4′ | 1.66 s | 23.5 q | H2′, H$_2$4′ |
| 14′ | 1.58 s | 14.5 q | H6′, H8′ | 1.58 s | 14.3 q | H6′, H8′ |
| 15′ | 1.64 s | 23.4 q | H10′, H$_2$12′ | 1.66 s | 23.4 q | H10′, H$_2$12′ |

[a] Bruker DRX 400 spectrometer in $CDC1_3$, chemical shifts (ppm) referred to $CHC1_3$ (δ 7.26) and to $CDC1_3$ (δ 77.0).
[b] By $^{1}$H–$^{1}$H COSY and HSQC experiments.
[c] By DEPT, HSQC and HMBC ($J$=10 Hz) experiments.
[d] Significant HMBC correlations ($J$=10 Hz).

dddd, $J$=5.9, 11.8, 12.2 and 13.9 Hz] and H-5′a [δ 1.75, br ddd, $J$=11.2, 12.3 and 14.1 Hz] signals indicated that they were axially orientated and coupled with two vicinal axial protons. This implied that both H-6 and H-6′ were axially directed, and consequently the side chain groups at C-6 and C-6′ were equatorial. The relative stereochemistry at the other two chiral centres C-9′ and C-11 was suggested by analysis of the ROESY spectrum of **1**. Diagnostic correlations between H$_3$-15 and H-9′ and between H-10 and H-8′ indicated that H$_3$-15 and H-9′ were *cis* orientated.

The structure of *cis*-dimer A was thereby proposed as **1**, with the configuration at both C-6 and C-6′ the same as theonelline (**5**) and a *cis* relative stereochemistry at C-9′ and C-11.

*Cis*-dimer B (**2**) had the same molecular formula $C_{30}H_{44}$ as *cis*-dimer A (**1**), as determined by HREIMS on the molecular peak at $m/z$ 404. The spectral data of **2** were very similar with those of **1** (Section 3 and Table 1). Detailed analysis of 2D-NMR ($^{1}$H–$^{1}$H COSY, HSQC and HMBC) spectra allowed us to establish for *cis*-dimer B (**2**) the same planar structure as *cis*-dimer A (**1**). Thus **1** and **2** had to be stereoisomers. A detailed analysis of NOESY spectrum of **2** suggested, analogously with **1**, a *cis* relative stereochemistry of the two alkyl chains C-11 and C-9′. In fact, diagnostic correlations were observed between H$_3$-15 and H-9′, H$_3$-15 and H-12β, H-10 and H-8′, indicating that H$_3$-15 and H-9′ had the same α-orientation. Biogenetic considerations led us to assume the same configuration at C-6 and C-6′ as **1**, thus *cis*-dimer B (**2**) differed from *cis*-dimer A (**1**) only in the stereochemistry at both C-11 and C-9′. Finally, the opposite CD profiles of **1** and **2** (Fig. 4) were in agreement with the opposite configuration of the chiral centre C-11 near the dienic chromophore, even though we could not assign the absolute stereochemistry at this centre and consequently at C-9′.

Assuming *R* configuration at C-6 and C-6′, the two dimers differ in their relative stereochemistry at C-9′ and C-11 (*RR* or *SS*). Of course, the suggested stereochemistry at C-9′ and C-11 of compounds **1** and **2** can be inverted. In conclusion if the absolute stereochemistry of dimer **1** is 6*R*,6′*R*,11*R*,9′*R* (as that displayed in **1**), the stereochemistry of **2** will be 6*R*,6′*R*,11*S*,9′*S*. An inversion in stereochemistry at C-11 and C-9′ in **1** leads to assign an *R* stereochemistry at the same chiral centres of **2**.

The molecular formula $C_{30}H_{44}$ of *trans*-dimer C (**3**), isomer of **1** and **2**, was determined by both $^{13}$C NMR and EIMS spectra. NMR spectral data of **3** were very similar with those of **1** and **2** (Table 2), clearly suggesting the presence of the

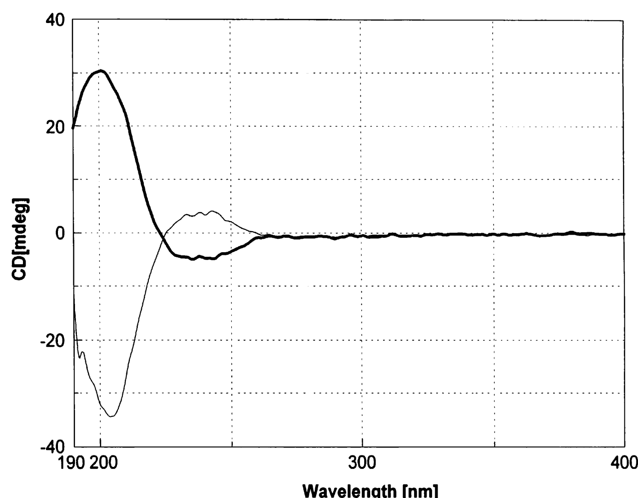



**Figure 4**. Circular dichroism (CD) spectra of *cis*-dimer A (**1**) and *cis*-dimer B (**2**).

same skeleton. Detailed analysis of 2D-NMR spectra of **3** indicated that the difference with the related molecules **1** and **2** was again in the stereochemistry at one or more chiral centres. In particular, the relative stereochemistry at C-11 and C-9′ was determined to be *trans* by diagnostic steric effects observed in a series of NOE difference experiments between $H_3$-15 and H-8′ as well as between H-9′ and H-10. Analogously with **1** and **2** the configuration at C-6 and C-6′ was assumed to be the same as that of theonelline (**5**).

**Table 2**. NMR data[a] of *trans*-dimer C (**3**)

| Position | 3 | | |
|---|---|---|---|
| | $\delta$ $^1$H[b] m, (Hz) | $\delta$ $^{13}$C[c] | HMBC[d] |
| 1 | 2.02 m | 31.0 t | H2, H6 |
| 2 | 5.41 br s | 121.4 d | H1, $H_3$13 |
| 3 | — | 133.5 s | $H_3$13, H2, $H_2$3 |
| 4 | 1.92 m, 1.85 m | 30.9 t | $H_3$13, H2 |
| 5a | 1.70 m, 1.49 m | 28.5 t | $H_2$4, H6 |
| 5b | — | | |
| 6 | 2.11 m | 43.3 d | $H_3$14, $H_2$1, $H_2$5 |
| 7 | — | 140.0 s | $H_2$14, H6, H8 |
| 8 | 6.06 d (10.0) | 124.6 d | $H_3$14, H6, H9 |
| 9 | 6.50 dd (10.0, 15.0) | 123.3 d | H8, H10 |
| 10 | 5.86 d (15.0) | 142.7 d | $H_3$15, H9, $H_2$12 |
| 11 | — | 38.0 s | $H_3$15, H10, $H_2$12 |
| 12a | 1.62 m | 34.0 t | $H_3$15, $H_2$12′, H10 |
| 12b | 1.62 m | | |
| 13 | 1.61 s | 23.6 q | H2, $H_2$4 |
| 14 | 14.8 s | 14.8 q | H6, H8 |
| 15 | 1.11 s | 21.1 q | H10, $H_2$12 |
| 1′ | 2.02 m | 31.0 t | H2′, H6′ |
| 2′ | 5.41 br s | 121.4 d | $H_3$13′, $H_2$1′, $H_2$4′ |
| 3′ | — | 133.5 s | $H_3$13′, H2′, $H_2$4′ |
| 4′ | 1.92 m, 1.85 m | 30.9 t | $H_3$13′, $H_2$5′ |
| 5′a | 1.70 m, 1.49 m | 28.5 t | $H_2$4′, H6′ |
| 5′b | | | |
| 6′ | 2.11 m | 43.3 d | $H_3$14′, $H_2$1′, $H_2$5′ |
| 7′ | — | 139.6 s | $H_3$14′, H6′, H8′ |
| 8′ | 5.21 br d (10.0) | 124.5 d | $H_3$14′, H9′ |
| 9′ | 3.09 br d (10.0) | 43.4 d | $H_3$15, H8′, H10′ |
| 10′ | 5.32 br s | 124.8 d | $H_3$15′, H9′ |
| 11′ | — | 132.2 s | $H_3$15′, H10′, $H_2$12′ |
| 12′a | 1.70 m | 28.0 t | $H_3$15′, H10′, $H_2$12 |
| 12′b | 1.92 m | | |
| 13′ | 1.61 s | 23.6 q | H2′, $H_2$4′ |
| 14′ | 1.62 s | 14.8 q | H6′, H8′ |
| 15′ | 1.62 s | 23.6 q | H10′, $H_2$12′ |

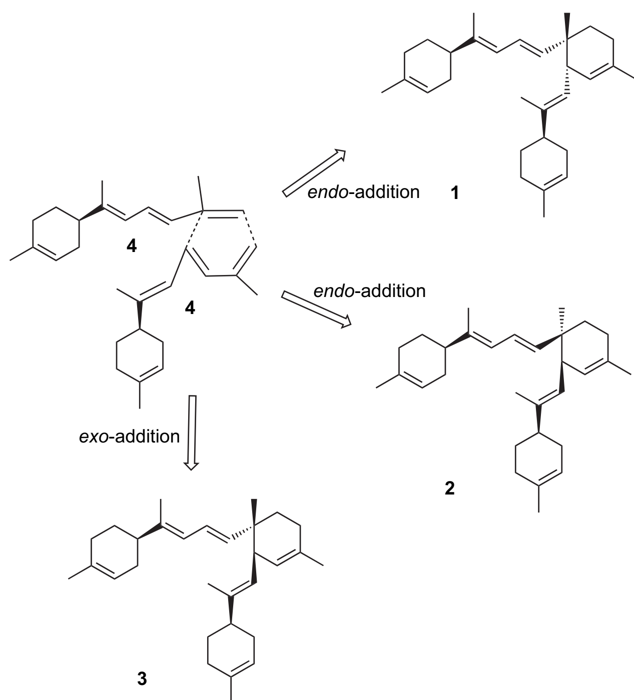
[a] Bruker DRX 600 spectrometer, Bruker DPX 300 spectrometer in $C_6D_6$, chemical shifts (ppm) referred to $C_6H_6$ ($\delta$ 7.15) and to $C_6D_6$ ($\delta$ 128.0).
[b] By $^1$H–$^1$H COSY and HSQC experiments.
[c] By DEPT, HSQC and HMBC ($J$=10 Hz) experiments.
[d] Significant HMBC correlations ($J$=10 Hz).

Dehydrotheonelline (**4**) showed the molecular formula $C_{15}H_{22}$, which was determined by EIMS on the molecular peak at *m/z* 202. The $^1$H NMR spectrum of **4** displayed signals attributed to three vinyl methyls [$\delta$ 1.82 (br s, $H_3$-15), $\delta$ 1.65 (br s, $H_3$-14) and $\delta$ 1.62 (br s, $H_3$-13)] and to an *exo*-methylene group [$\delta$ 5.01 (br s, H-12a) and $\delta$ 4.95 (br s, H-12b)], according to the sesquiterpene nature of the compound. Along with a vinyl sharp multiplet at $\delta$ 5.41 (H-2) due to an isolated double bond, three correlated olefinic signals were also present in the $^1$H NMR spectrum at $\delta$ 6.05 (1H, d, $J$=11 Hz, H-8), $\delta$ 6.36 (1H, br d, $J$=15 Hz, H-10) and $\delta$ 6.56 (1H, dd, $J$=11 and 15 Hz, H-9). Analysis of $^1$H–$^1$H COSY showed a further allylic coupling between H-10 and $H_2$-12 indicating the presence of a triene conjugated system. Accordingly, an absorption maximum at 274 nm ($\varepsilon$=8540) was observed in the UV spectrum of **4**. The *E* geometry of the triene double bonds $\Delta^{7(8)}$ and $\Delta^{9(10)}$ was inferred by the large coupling constant ($J_{H9/H10}$=15 Hz) and by the high field shifted carbon value of the methyl at C-7 ($\delta_C$ 14.9), analogously with theonelline (**5**). The presence of four double bonds was further evidenced by the $^{13}$C NMR spectrum containing eight signals between $\delta$ 143.7 and $\delta$ 115.8, which were attributed to $sp^2$ carbons. The remaining unsaturation degree of those required by the molecular formula of **4** was thus due to a ring. Comparison of both proton and carbon values of **4** with those of theonelline (**5**) showed a close similarity between the two compounds, clearly indicating the same bisabolene skeleton. In particular, compound **4** was the 11,12-dehydro derivative of **5**. All $^1$H and $^{13}$C NMR resonances of **4** were assigned by 2D-NMR experiments ($^1$H–$^1$H COSY, HSQC and HMBC) and were in agreement with the proposed structure. The configuration at C-6 was suggested to be the same as **5** by biogenetical considerations.

The finding of unprecedented bisabolene-based dimers from two different sponges is quite interesting. *A. variabilis* and *L. ana* are members of the order Halichondrida, but belong to two different families, Halichondriidae and Dictyonellidae, respectively. However, some morphological similarities observed in the two sponges may point to a closer relationship between them. The genus *Axinyssa* is not a typical member of Halichondriidae and has been associated with the family Axinellidae in the past. Some members of the family Dictyonellidae have been shown to be polyphyletic in recent studies using molecular sequence data. These facts may indicate that the two sponges producing the same metabolite may in fact be strictly related.

A plausible biosynthetic pathway to *cis*-dimer A (**1**), B (**2**) and *trans*-dimer C (**3**), proposed as shown in Scheme 1, should include an intermolecular [4+2] Diels–Alder cycloaddition involving two molecules of dehydrotheonelline (**4**). *Trans*- and *cis*-dimers should be formed according to *exo*- and *endo*-Diels–Alder coupling, respectively. The co-occurrence of the suggested precursor in the sponges further supported this hypothesis. It remains to clarify if dimers

are biosynthesized in the sponge or they are obtained during the work-up. The comparable amounts of **1** and **2** in *A. variabilis* should support the second hypothesis even though it seems unlikely that two different isolation procedures used for the two sponges could lead to the same artefacts.




**Scheme 1**. Plausible biosynthetic pathway for **1**, **2** and **3**.

Finally, biological properties of dimers were evaluated in the feeding-deterrence test against gold fish *Carassius auratus*, according to the literature.[11] The mixture of *cis*-dimers **1** and **2** and pure *trans*-dimer **3** was active at 50 μg/cm$^2$, suggesting a possible defensive role of these molecules. Dehydrotheonelline (**4**) and theonelline (**5**) were also assayed and resulted to be inactive.

## 3. Experimental

### 3.1. General experimental procedures

At SIMM laboratory: UV spectra were recorded on Varian Cary 300 Bio spectrophotometer; IR spectra were done on Nicolet-Magna FT-IR 750 spectrometer; CD spectra were performed on a Jasco J-810 spectropolarimeter; NMR spectra on Bruker DRX-400 spectrometer; the residual $CDCl_3$ ($\delta_{(H)}$ 7.26 ppm; $\delta_{(C)}$ 77.0 ppm) as an internal standard; $\delta$ in parts per million, $J$ in hertz; mass spectra on Finnigan-MAT-95 mass instrument. Optical rotations were measured on a Perkin–Elmer 241MC polarimeter in $CHCl_3$. Reversed-phase HPLC was performed on an Agilent 1100 series liquid chromatograph equipped with a VWD G1314A detector at 210 nm. A semi-preparative ODS-HG-5 column [5 μm, 10 mm (i.d.)×25 cm] was employed for the purifications. Silica gel (200–300 and 400–600 mesh) and TLC plates (G60 F-254) were purchased from Qing Dao Hai Yang Chemical Group Co. and Yan Tai Zi Fu Chemical Group Co., respectively.

At ICB laboratory: UV spectra were recorded on Beckman Coulter-DU730 spectrophotometer; IR spectra were done on Bio-Rad FTS 135 spectrometer; TLC plates (Merck Silica Gel 60 F254) were used for analytical TLC and Merck Kieselgel 60 was used for preparative column chromatography. HPLC purifications were conducted on a Waters 501 apparatus equipped with a refractometer detector and a direct-phase column Kromasil Silica, 5 μm (250× 4.60 mm, Phenomenex). EIMS were recorded on Thermo-Focus GC (Polaris Q, Ion-Trap). NMR spectra were recorded at NMR Service of Istituto di Chimica Biomolecolare of CNR (Pozzuoli, Italy). 1D and 2D NMR spectra were acquired on a Bruker Avance-DRX600 operating at 600 MHz, in $CDCl_3$ ($\delta$ values reported referred to $CHCl_3$ and $C_6H_6$ at 7.26 and 7.16 ppm, respectively), using an inverse TCI Cryo Probe fitted with a gradient along the $z$-axis. $^{13}C$ NMR were recorded on a Bruker DPX-300 operating at 300 MHz ($\delta$ values are reported with respect to $CDCl_3$ and $C_6D_6$, 77.0 and 128.0 ppm, respectively) using a dual probe. Optical rotations were measured on a Jasco DIP 370 digital polarimeter.

### 3.2. Biological material

Specimens of *A. variabilis* and *L. ana* were collected in February, 2004 by SCUBA divers at a depth of −10 m off Sanya, Hainan Province, China in the South China Sea. Both sponges were identified by one of us (R.v.S.). A voucher specimen of *A. variabilis* is available under number LS-146, registered in the collections of the Zoological Museum of Amsterdam under no. 19128. The sponge is a compressed mass, greyish in alcohol, with an optically smooth but microconulose surface. No apparent oscules. The skeleton is a confused mass of spicules, denser at the surface conules, slightly less so in the choanosome. Spicules are sharp pointed oxeas in a large size range, 330–700×10–24 μm.

A voucher specimen of *L. ana* is available under number LS-102 registered in the collections of the Zoological Museum of Amsterdam under no. 19069. This is a semiglobular mass, pale orange brown in alcohol, with conulose honeycombed surface made up by the choanosomal spicule tracts pushing up the surface membrane. This membrane spans large subdermal cavities between the surface conules. The skeleton consists of ascending spicule columns of 10–20 spicules in diameter (50–250 μm in cross-section), consolidated by some spongin. The columns are separated fairly regularly by collagenous spaces containing few loose spicules, width approximately 300–400 μm. Spicules are fusiform oxeas of rather uniform size, 550–650×15 μm. The identity of this sponge could be confirmed by examination of a fragment of the type (USNM 23094).

### 3.3. Extraction and isolation procedure

*A. variabilis*: at SIMM laboratory, frozen specimens of the sponge (250 g dry weight) were triturated and exhaustively extracted with acetone (1 L×3). The extract was concentrated under vacuum, and the resulting residue (13 g) was extracted with $Et_2O$ (200 mL×3) and BuOH (200 mL×3). The $Et_2O$-soluble portion (3.5 g) was chromatographed on a silica gel column with a stepped gradient from light petroleum ether to acetone. Fractions eluted with petroleum ether was

subjected to Sephadex LH-20 (eluted with $CHCl_3$/MeOH=1:1) to yield 3.4 mg (0.001% dry weight) of theonelline (**5**). Fractions eluted with petroleum ether/acetone (99:1) were purified by reversed-phase HPLC (eluted with MeOH) to afford *cis*-dimers A (**1**, 5.6 mg, 0.002% dry weight) and B (**2**, 4.4 mg, 0.002% dry weight).

*L. ana*: at ICB laboratory, the frozen sponge *L. ana* (3 g dry weight) was immersed in acetone (200 mL×3). After removing the organic solvent at reduced pressure, the aqueous residue was extracted with $Et_2O$ (150 mL×3). The organic portion was concentrated to give 190 mg of a crude extract, which was subjected to silica gel chromatography (petroleum ether/$Et_2O$ gradient). The fraction eluted by petroleum ether was purified by normal-phase HPLC (eluent: *n*-hexane) to give, in order of retention time, theonelline (**5**, 0.9 mg, 0.47% dry weight), dehydrotheonelline (**4**, 0.9 mg, 0.47% dry weight), an unresolved mixture (1.8 mg, 0.95%) of *cis*-dimer A (**1**) and *cis*-dimer B (**2**) (ratio **1/2**, 1:1), pure *trans*-dimer C (**3**, 0.8 mg, 0.42% dry weight) and 3-isocyanotheonelline (**6**, 1.0 mg, 0.53% dry weight).

*Cis*-dimer A (**1**): colourless oil, $R_f$ 0.93 (petroleum ether/diethyl ether 95:5); $[\alpha]_D^{20}$ +72.3 ($CHCl_3$, *c* 0.24); HREIMS *m/z* 404.3434 ($[M]^+$), calcd 404.3443 for $C_{30}H_{44}$; UV $\lambda_{max}$ (MeOH) 225 nm ($\varepsilon$=7426); CD (MeOH) $\Delta\varepsilon_{201}$ +11.8, $\Delta\varepsilon_{235}$ −1.9; IR (liquid film) $\nu$ ($cm^{-1}$) 2960, 2921, 2854, 1436, 1375, 1147, 972, 914, 792; $^1H$ and $^{13}C$ NMR data in Table 1.

*Cis*-dimer B (**2**): colourless oil, $R_f$ 0.93 (light petroleum ether/diethyl ether 95:5); $[\alpha]_D^{20}$ −23.9 ($CHCl_3$, *c* 0.18); HREIMS *m/z* 404.3441 ($[M]^+$), calcd 404.3443 for $C_{30}H_{44}$; UV $\lambda_{max}$ (MeOH) 225 nm ($\varepsilon$=3910); CD (MeOH) $\Delta\varepsilon_{204}$ −12.0, $\Delta\varepsilon_{235}$ +1.3; IR (liquid film) $\nu$ ($cm^{-1}$) 2960, 2921, 2854, 1436, 1375, 1147, 972, 914, 792; $^1H$ and $^{13}C$ NMR data in Table 1.

*Trans*-dimer C (**3**): colourless oil, $R_f$ 0.91 (light petroleum ether/diethyl ether 95:5); $[\alpha]_D^{20}$ +24.0 ($CHCl_3$, *c* 0.08); HREIMS *m/z* 404.3447 ($[M]^+$), calcd 404.3443 for $C_{30}H_{44}$; UV $\lambda_{max}$ (MeOH) 225 nm ($\varepsilon$=6550); IR (liquid film) $\nu$ ($cm^{-1}$) 2955, 2917, 2861, 1448, 1376, 1142, 964, 759; $^1H$ and $^{13}C$ NMR data in Table 2.

Dehydrotheonelline (**4**): colourless oil, $R_f$ 0.95 (light petroleum ether/diethyl ether 95:5); $[\alpha]_D^{20}$ +1.2 ($CHCl_3$, *c* 0.09); HREIMS *m/z* 202.1717 ($[M]^+$), calcd 202.1722 for $C_{15}H_{22}$; UV $\lambda_{max}$ (MeOH) 274 nm ($\varepsilon$=8540); IR (liquid film) $\nu$ ($cm^{-1}$) 3041, 3007, 2963, 2921, 2856, 2834, 1452, 1376, 1147, 960, 880, 780; $^1H$ NMR ($C_6D_6$): $\delta$ 6.56 (1H, dd, *J*=11 and 15 Hz, H-9), $\delta$ 6.36 (1H, d, *J*=15 Hz, H-10), $\delta$ 6.05 (1H, d, *J*=11 Hz, H-8), $\delta$ 5.41 (1H, m, H-2), $\delta$ 5.01 (1H, br s, H-12a), $\delta$ 4.95 (1H, br s, H-12b), $\delta$ 2.12 (1H, m, H-6), $\delta$ 2.01 (2H, m, $H_2$-1), $\delta$ 1.92 (1H, m, H-4a), $\delta$ 1.82 (3H, br s, $H_3$-15), $\delta$ 1.81 (1H, m, H-4b), $\delta$ 1.62 (3H, br s, $H_3$-13), $\delta$ 1.65 (3H, br s, $H_3$-14), $\delta$ 1.46 (2H, m, $H_2$-5). $^{13}C$ NMR ($C_6D_6$): $\delta$ 143.7 (C-7, C), $\delta$ 142.9 (C-11, C), $\delta$ 133.8 (C-3, C), $\delta$ 134.0 (C-10, CH), $\delta$ 126.1 (C-9, CH), $\delta$ 125.0 (C-8, CH), $\delta$ 121.1 (C-2, CH), $\delta$ 115.8 (C-12, $CH_2$), 43.5 (C-6, CH), $\delta$ 30.9 (C-4, $CH_2$), $\delta$ 30.8 (C-1, $CH_2$), $\delta$ 28.1 (C-5, $CH_2$), $\delta$ 23.6 (C-13, $CH_3$), $\delta$ 18.7 (C-15, $CH_3$), $\delta$ 14.9 (C-14, $CH_3$).

Theonelline (**5**): colourless oil, $R_f$ 0.95 (light petroleum ether/diethyl ether 95:5); $[\alpha]_D^{20}$ +3.5 ($CHCl_3$, *c* 0.09) from *L. ana*, $[\alpha]_D^{20}$ +27.0 (*n*-hexane, *c* 0.3) from *A. variabilis*, $[\alpha]_D^{20}$ lit.[9] +23 (*n*-hexane, *c* 0.26); EIMS *m/z* 204 ($[M]^+$); $^1H$ and $^{13}C$ NMR data were identical to literature data.[9]

3-Isocyanotheonelline (**6**): colourless oil, $R_f$ 0.75 (light petroleum ether/diethyl ether 95:5); EIMS *m/z* 231 ($[M]^+$); $^1H$ and $^{13}C$ NMR data were identical to literature data.[3b,10]

### 3.4. Biological assays

At ICB laboratory feeding-deterrence tests against gold fish *C. auratus* were conducted according to the literature procedures.[11] The mixture of *cis*-dimers **1** and **2**, the pure *trans*-dimer **3**, dehydrotheonelline (**4**) and theonelline (**5**) were assayed at 50 μg/$cm^2$ and the activity was shown by mixture of compounds **1**, **2** and **3**.

## Acknowledgements

This research work was financially supported by the National Marine 863 Projects (No. 2006AA09Z447 and 2006AA09Z412), STCSM Projects (No. 054307062 and 06DZ22028) and partly founded by State Key Programme of Basic Research of China (No. 2004CB518905) and by bilateral cooperation programme CNR/CAS: 'Chemical studies on bioactive molecules with biotechnological interest from Chinese marine invertebrates'.

## References and notes

1. Sullivan, B. W.; Faulkner, D. J.; Okamoto, T. K.; Chen, M. H. M.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5134–5136.
2. Li, C. J.; Kelly, M. *J. Nat. Prod.* **1999**, *62*, 1330–1332.
3. (a) Kassuhlke, K. E.; Potts, B. C. M.; Faulkner, D. J. *J. Org. Chem.* **1991**, *56*, 3747–3750; (b) Gulavita, N. K.; de Silva, E. D.; Hagadone, M. R.; Karuso, P.; Scheuer, P. J.; van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5136–5139; (c) Manzo, E.; Ciavatta, M. L.; Gavagnin, M.; Mollo, E.; Guo, Y. W.; Cimino, G. *J. Nat. Prod.* **2004**, *67*, 1701–1704.
4. D'Armas, H. T.; Mootoo, B. S.; Reynold, W. F. *J. Nat. Prod.* **2000**, *63*, 1593–1595.
5. (a) Suzuki, T.; Kikuchi, H.; Kurosawa, E. *Chem. Lett.* **1980**, *10*, 1267–1270; (b) Davyt, D.; Fernandez, R.; Suescun, L.; Mombru', A. W.; Saldana, J.; Dominguez, L.; Fujii, M. T.; Manta, E. *J. Nat. Prod.* **2006**, *69*, 1113–1116.
6. Mao, S.-C.; Guo, Y.-W. *J. Nat. Prod.* **2006**, *69*, 1209–1211.
7. Yan, X.-H.; Lin, L.-P.; Ding, J.; Guo, Y.-W. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2661–2663.
8. Zhang, W.; Gavagnin, M.; Guo, Y.-W.; Mollo, E.; Ghiselin, M.; Cimino, G. *Tetrahedron* **2007**, *63*, 4725–4729.
9. Nakamura, H.; Kobayashi, J. I.; Ohizumi, Y. *Tetrahedron Lett.* **1984**, *25*, 5401–5404.
10. van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5136–5139.
11. Cimino, G.; de Rosa, S.; de Stefano, S.; Sodano, G. *Comp. Biochem. Physiol.* **1982**, *73B*, 471–474.