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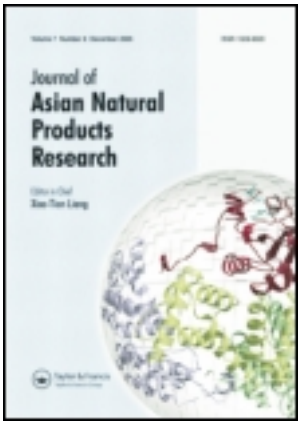

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Trans-dimer D, a novel dimeric sesquiterpene with a bis-bisabolene skeleton from a Hainan sponge *Axinyssa variabilis*

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Trans-dimer D, a novel dimeric sesquiterpene with a bis-bisabolene skeleton from a Hainan sponge *Axinyssa variabilis*

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A novel bisabolene sesquiterpene dimer named *trans*-dimer D (**1**) and its diastereoisomer *trans*-dimer C (**2**) reported in our previous work have been isolated as an inseparable mixture in a ratio of 1.5:1 from the South China Sea sponge *Axinyssa variabilis*. The structure of **1** was determined on the basis of extensive spectroscopic analysis and by comparison of its NMR spectral data with those of structurally related compounds. Compound **1** represents the fourth example of a sesquiterpene dimer with a bis-bisabolene skeleton.

Keywords: sponge; *Axinyssa variabilis*; *trans*-dimer D; bis-bisabolene

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1. Introduction

Marine sponges of the genus *Axinyssa* (order: Halichondrida, family: Halichondriidae) have proved to be a rich source of secondary metabolites with unusual structures as well as interesting biological activities. The metabolite pattern of the *Axinyssa* sponge was extensively characterized by a variety of sesquiterpenes, exhibiting an array of skeletal types [1], and with a few exceptions, the nitrogen-containing group such as an isothiocyanate, formamide, isonitrile, and thiocyanate functionality, as the only hetero function present in the molecule [2–6]. Biological activities such as antihelmintic [7], antimicrobial [7,8], and cytotoxic [9,10] properties have been ascribed to some of these nitrogen-containing sesquiterpenes, although the most significant results have been described in the antifouling [11] and antimalarial [12] areas.

In the course of our ongoing research on the biologically active substances from Chinese marine invertebrates [13–16], we have recently examined the sponge *Axinyssa variabilis*, collected off the Lingshui Bay, Hainan Province, China, resulting in the discovery of two unprecedented *cis*-dimeric sesquiterpenes, *cis*-dimers A and B [17], and two uncommon nitrogenous sesquiterpenes [18]. Our continuing studies on the minor constituents of the same specimen led to the isolation of two additional *trans*-dimeric sesquiterpenes *trans*-dimers D (**1**) and C (**2**) (Figure 1) as an inseparable mixture of diastereoisomers in a ratio of approximately 1.5:1. *Trans*-dimer C (**2**) was obtained from a different isolate of *Lipastrotethya ana* in an earlier work from our laboratory [17]. To the best of our knowledge, *trans*-dimer D represents the fourth example of a sesquiterpene dimer with a bis-bisabolene skeleton.

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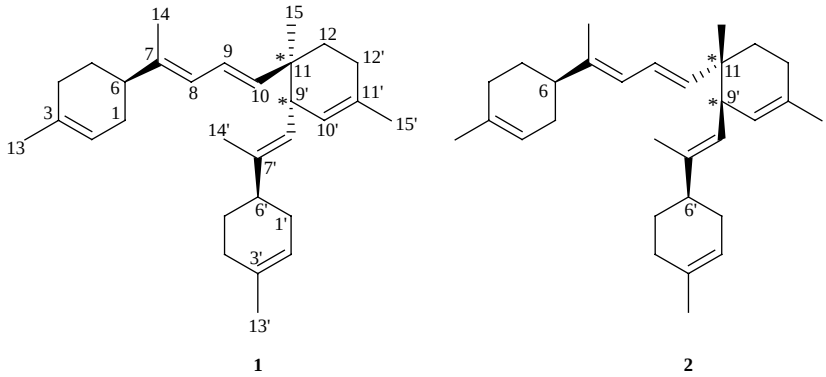

Figure 1. Chemical structures of *trans*-dimers D (**1**) and C (**2**).

Table 1. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of *trans*-dimers D (**1**) and C (**2**) ($CDCl_3$).

	1		**2**	
No.	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)
1	1.90–2.20 (m), 1.90–2.20 (m)	30.5 (t)	1.90–2.20 (m), 1.90–2.20 (m)	30.6 (t)
2	5.40 or 5.39 (br. s)	120.8 (d)	5.40 or 5.39 (br. s)	120.8 (d)
3	–	133.7 (s)	–	133.7 (s)
4	1.90–2.20 (m), 1.90–2.20 (m)	30.6 (t)	1.90–2.20 (m), 1.90–2.20 (m)	30.8 (t)
5	1.49–1.70 (m), 1.49–1.70 (m)	27.9 (t)	1.49–1.70 (m), 1.49–1.70 (m)	28.2 (t)
6	2.15 (m)	43.1 (d)	2.15 (m)	43.2 (d)
7	–	140.3 (s)	–	140.4 (s)
8	5.81 (d, $J = 10.6$)	124.0 (d)	5.81 (d, $J = 10.6$)	124.0 (d)
9	6.21 (dd, $J = 15.5$, 10.6)	122.5 (d)	6.21 (dd, $J = 15.5$, 10.6)	122.5 (d)
10	5.61 (d, $J = 15.5$)	142.5 (d)	5.61 (d, $J = 15.5$)	142.5 (d)
11	–	37.7 (s)	–	37.7 (s)
12	1.48–1.62 (m), 1.48–1.62 (m)	33.5 (t)	1.48–1.62 (m), 1.48–1.62 (m)	33.5 (t)
Me-13	1.65 (s)	23.5 (q)	1.65 (s)	23.5 (q)
Me-14	1.72 (s)	14.8 (q)	1.72 (s)	14.8 (q)
Me-15	0.89 (s)	20.8 (q)	0.90 (s)	20.8 (q)
1′	1.90–2.20 (m), 1.90–2.20 (m)	30.9 (t)	1.90–2.20 (m), 1.90–2.20 (m)	31.1 (t)
2′	5.39 or 5.40 (br s)	121.0 (d)	5.39 or 5.40 (br s)	121.0 (d)
3′	–	133.7 (s)	–	133.7 (s)
4′	1.90–2.20 (m), 1.90–2.20 (m)	30.7 (t)	1.90–2.20 (m), 1.90–2.20 (m)	30.8 (t)
5′	1.49–1.70 (m), 1.49–1.70 (m)	27.9 (t)	1.49–1.70 (m), 1.49–1.70 (m)	27.9 (t)
6′	2.15 (m)	43.1 (d)	2.15 (m)	43.2 (d)
7′	–	139.2 (s)	–	139.7 (s)
8′	4.99 (d, $J = 9.9$)	124.2 (d)	4.99 (d, $J = 9.9$)	124.0 (d)
9′	2.82 (br d, $J = 9.9$)	43.2 (d)	2.82 (br d, $J = 9.9$)	43.2 (d)
10′	5.09 (br s)	124.3 (d)	5.09 (br s)	124.3 (d)
11′	–	132.6 (s)	–	132.5 (s)
12′	2.01 (m), 1.91 (m)	27.8 (t)	2.01 (m), 1.91 (m)	27.8 (t)
Me-13′	1.65 (s)	23.4 (q)	1.65 (s)	23.5 (q)
Me-14′	1.57 (s)	14.6 (q)	1.57 (s)	14.6 (q)
Me-15′	1.65 (s)	23.4 (q)	1.65 (s)	23.5 (q)

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We herewith report the isolation and structural elucidation of this uncommon new compound.

2. Results and discussion

Freshly collected animals were immediately put at −20°C and kept frozen until used. Frozen material was extracted exhaustively with acetone, and the acetone extract was then partitioned between diethyl ether and water. The organic-soluble extract was repeatedly chromatographed by silica gel and Sephadex LH-20 column chromatography (CC) followed by reversed-phase HPLC to afford the mixture of *trans*-dimer D (**1**) and *trans*-dimer C (**2**).

Trans-dimers D (**1**) and C (**2**) were obtained as an inseparable mixture of diastereomeric dimer in a 1.5:1 ratio as determined from the ^{1}H and ^{13}C NMR spectra (Table 1). Exhaustive efforts to separate the mixture using CC and HPLC employing different stationary and mobile phase were unsuccessful in even partially resolving the diastereoisomers. However, the minor diastereoisomer, *trans*-dimer C (**2**), was solely obtained as a pure compound from a different sponge *L. ana* in our earlier work [17]; thus, structure elucidation of the major diastereoisomer *trans*-dimer D (**1**) could be independently performed on the **1/2** mixture.

The NMR data for the minor diastereoisomer, *trans*-dimer C (**2**), were unambiguously assigned in our previous publication, so only the data of the major diastereoisomer *trans*-dimer D (**1**) are described in detail here. *Trans*-dimer D (**1**) was shown to be a hydrocarbon with the molecular formula $C_{30}H_{44}$ on the basis of its HR-EI-MS at *m/z* 404.3445 $[M]^{+}$, an isomer of *trans*-dimer C (**2**) [17], suggesting nine degrees of unsaturation. The presence of a trisubstituted conjugated diene moiety was evident from an UV absorption maximum at 225 nm. The ^{1}H and ^{13}C NMR spectral data of **1** were almost identical to those of **2**. The ^{13}C NMR and DEPT spectra of **1** revealed the presence of 30 carbon signals, including 6 methyl groups, 8 methylene units, 10 methine units (7 sp^2 carbons), and 6 nonprotonated carbons (5 sp^2). Like **2**, the ^{1}H NMR spectrum of **1** also showed three broad singlets at δ_H 5.40 or 5.39 (H-2), 5.39 or 5.40 (H-2′), and 5.09 (H-10′) assignable to olefinic protons on three endocyclic trisubstituted double bonds, three olefinic proton signals at δ_H 5.81 (d, $J = 10.6$ Hz, H-8), 6.21 (dd, $J = 15.5, 10.6$ Hz, H-9), and 5.61 (d, $J = 15.5$ Hz, H-10) attributed to the protons of a typical conjugated diene moiety, and six methyl groups at δ_H 1.72 (3H, s), 1.65 (9H, s), 1.57 (3H, s), and 0.89 (3H, s). In addition, four isolated proton spin–spin systems corresponding to the Me-14/H-8/H-9/H-10, Me-14′/H-8′/H-9′/H-10′/H-12′/H-12, H-1′/H-2′/Me-13′, and H-1/H-2/Me-13 subunits of structure **1** were established on the basis of ^{1}H–^{1}H COSY data and confirmed by HMBC correlations (Figure 2). Consideration of the above observations led to the conclusion that *trans*-dimer D (**1**) has the same planar structure as *trans*-dimer C (**2**). Thus, they had to be stereoisomers.

The relative stereochemistry of **1** was established by a ROESY experiment (Figure 3) running on the **1/2** mixture. The *E* geometry of three double bonds at $\Delta^{7(8)}$, $\Delta^{9(10)}$, and $\Delta^{7'(8')}$ on two alkyl chains, analogous to **2**, was inferred by the high-field-shifted carbon values of two vinyl methyls Me-14 (δ_C 14.8) and Me-14′ (δ_C 14.6) and the large olefinic proton coupling constant ($J_{H\text{-}9/H\text{-}10} = 15.5$ Hz). Biogenetic considerations allowed us to assume the same configuration at C-6 and C-6′ as **2**; thus, the differences between them occurred only in the stereochemistry at the other one or two chiral centers (C-9′ and/or C-11). The relative stereochemistry at C-9′ and C-11 in **1** was determined to be *trans*, analogous to **2**, by significant ROESY correlations between Me-15 and H-8′ and from H-10 to H-9′. These

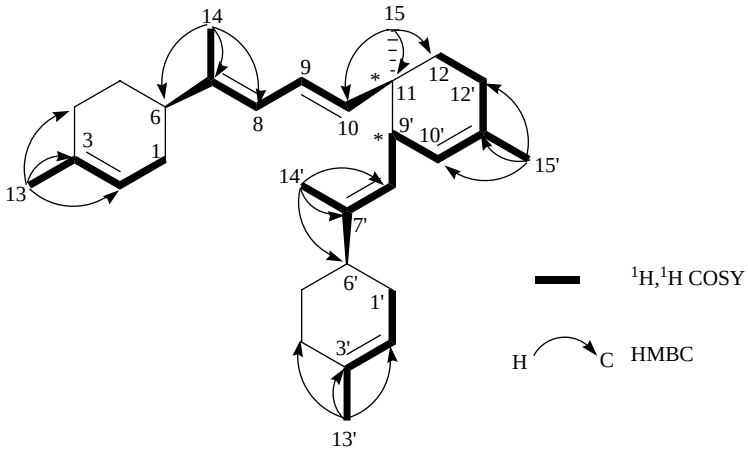

Figure 2. Selected ^{1}H–^{1}H COSY and HMBC correlations for *trans*-dimer D (**1**).

observations upon **1** indicated that Me-15 was α-orientated, while H-9′ was β-orientated. Consequently, the structure of *trans*-dimer D was depicted as **1**, with the configuration at both C-6 and C-6′ the same as the co-occurring sesquiterpene, theonelline [19], and a *trans* relative stereochemistry at C-9′ and C-11.

A similar rationale to *cis*-dimers A and B led to the tentative assignment of the absolute stereochemistry of *trans*-dimers D (**1**) and C (**2**) [17]. Assuming *R* configuration at C-6 and C-6′ the same as theonelline, the two diastereoisomers **1** and **2** differ in their stereochemistry at C-9′ and C-11 (*RR* or *SS*). In conclusion, if the absolute configuration of **2** is tentatively assumed to be 6*R*,6′*R*,11*S*,9′*S* (as that proposed in **2**), the stereochemistry of **1** will be 6*R*,6′*R*,11*R*,9′*R*. Of course, the suggested stereochemistry at C-9′ and C-11 of compounds **1** and **2** can be inverted (6*R*,6′*R*,11*R*,9′*R* for **2**; 6*R*,6′*R*,11*S*,9′*S* for **1**).

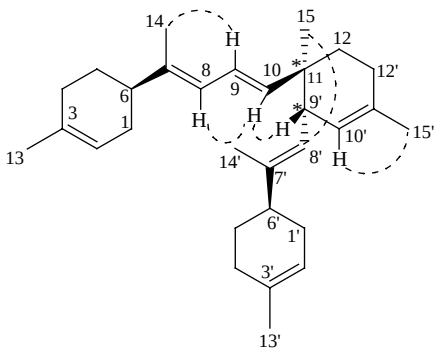

Figure 3. Selected key ROESY correlations for *trans*-dimer D (**1**).

Interestingly, we have heretofore isolated four unprecedented diastereomeric dimers, namely *cis*-dimers A and B as well as *trans*-dimers C (**2**) and D (**1**), with a bis-bisabolene skeleton, from the sponge *A. variabilis*. Among them, to the best of our knowledge, *trans*-dimer D (**1**) represents the fourth example with such a carbon skeleton. It may be worth to point out that the secondary metabolites structurally related to these compounds were not found in the other *Axinyssa* sponges by our group. It raises the necessity to check the correctness of taxonomy of the sponge, as well as to clarify that if these dimers are biosynthesized via enzymatic catalysis in the sponge or they are artifacts formed during the isolation process of biological material.

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3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a PerkinElmer 241MC polarimeter. UV spectra were recorded by a Varian Cary-300 Bio spectrophotometer. IR spectra were recorded using a Nicolet Magna-FT-IR-750 spectrometer. NMR spectra were run in $CDCl_3$ on a Bruker DRX-400 spectrometer at 400 MHz for ^1H and at 100 MHz for ^{13}C with residual $CHCl_3$ (δ_H 7.26, δ_C 77.0) as an internal standard. The HR-EI-MS spectrum was measured using a Finnigan MAT-95 spectrometer. CC was performed with silica gel (200–300 and 400–600 mesh, Qingdao Marine Chemical Company, Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC analysis was carried out on pre-coated TLC plates with silica gel 60 F_{254} (Yan Tai Zi Fu Chemical Group Company, Yantai, China). Detection was achieved by spraying with 10% H_2SO_4 in H_2O followed by heating. Reversed-phase HPLC was performed using an Agilent 1100 chromatography equipped with a VWD-G1314A detector, using a Develosil ODS-HG-5 column [5 μm, 10 mm × 25 cm, Nomura Chemical Co., Ltd., Aichi, Japan].

3.2 Biological material

Specimens of *A. variabilis*, identified by Prof. Rob van Soest of the Zoological Museum, University of Amsterdam, were collected in February 2004 by SCUBA techniques at a depth of − 10 m off Sanya, Hainan Province, China in the South China Sea. A voucher specimen is available for inspection at the Institute of Materia Medica, SIBS-CAS, under registration No. 04LS-146.

3.3 Extraction and isolation

The frozen animals (250 g dry weight) were cut into small pieces and exhaustively extracted with acetone (1 L × 3) at room temperature. The extract was concentrated under vacuum, and the resulting residue was extracted with Et_2O (200 ml × 3) and BuOH (200 ml × 3). The Et_2O-soluble portion (3.5 g) was fractionated by SiO_2 CC (100–200 mesh) eluted with light petroleum ether (PE) with increasing amounts of acetone (100:0 → 0:100) to afford nine fractions (A–I) on the basis of TLC analysis. Fraction B (89 mg) was further subjected to Sephadex LH-20 eluting with PE–$CHCl_3$–MeOH (2:1:1) to yield three fractions (B1–B3). Fraction B2 was purified followed by reversed-phase HPLC using pure MeCN as the mobile phase (4 ml/min) to afford an inseparable mixture of *trans*-dimers D (**1**) and C (**2**) (7 mg, $t_R = 56$ min).

3.3.1 Trans-*dimer D (**1**)*

Colorless oil. R_f 0.93 (light PE/diethyl ether: 95:5). $[\alpha_D^{24}]$ − 15.8 ($c = 0.40$, $CHCl_3$). UV (MeOH) λ_{max} (log ε): 225 (3.86) nm. IR (KBr) ν_{max} (cm^{-1}): 2958, 2916, 2850, 1436, 1375, 1143, 968, 743. ^1H and ^{13}C NMR spectral data ($CDCl_3$): see Table 1. HR-EI-MS: *m/z* 404.3445 $[M]^+$ (calcd for $C_{30}H_{44}$, 404.3443).

Acknowledegments

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