FULL PAPER

New Highly Oxidized Formamidobisabolene-Derived Sesquiterpenes from a Hainan Sponge *Axinyssa variabilis*

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Two new highly oxidized formamidobisabolene sesquiterpenes (**1** and **2**), together with seven known related analogues (**3** – **9**), were isolated from a Hainan sponge *Axinyssa variabilis*. Their structures were determined by extensive 1D- and 2D-NMR and MS spectra analyses, and by comparison of their spectroscopic data with those of the related model compounds. The plausible biosynthetic relationship between compounds **1** – **9** has also been described.

Keywords: Bisabolene, Nitrogenous sesquiterpenes, Hainan sponge, *Axinyssa variabilis*, Biosynthetic pathway.

Introduction

Nitrogenous sesquiterpenes are uncommon natural products found as characteristic metabolites in marine sponges of the order Halichondrida. These sesquiterpenes are usually of bisabolene type with isonitrile, isothiocyanate, formamide, thiocyanate, or amine, and the nitrogenous groups are positioned at C(3) or C(7) [1]. The sponges of the genus *Axinyssa* (order Halichondrida, family Halichodriidae) were rich sources of diverse nitrogenous bisabolenes, which exhibited widespread biological activities such as antimalarial, antifouling, anthelmintic, and antitumor [2][3]. In our previous work [4][5], a series of mono- or dimeric bisabolene-type sesquiterpenes were reported from the Hainan sponge *Axinyssa variabilis*. In the course of searching for more diverse and biological active nitrogenous compounds from marine sponges, we did another collection of the same species of *A. variabilis*, leading to the discovery of two new highly oxidized formamidobisabolene sesquiterpenes (**1** and **2**), and seven known ones (**4** – **9**; *Fig. 1*). Herein, we report the isolation and structure elucidation of the new compounds.

Results and Discussion

Specimens of *A. variabilis* were immediately frozen to −20 °C and stored at that temperature before they were exhaustively extracted by acetone. The Et_2O-soluble fraction of the acetone extract of the sponge was subjected to repeated column chromatography (CC; silica gel, *Sephadex LH-20*, and HPLC) to yield nine formamidobisabolene sesquiterpenes (**1** – **9**; *Fig. 1*). The known compounds were readily identified as axinyssine G (**3**) [6], axinyssine J (**4**) [6], axinyssine K (**5**) [6], axinyssine E (**6**) [6], theonellin formamide (**7**) [7], theonellin isothiocyanate (**8**) [7], and 7α,8α-epoxy theonellin isothiocyanate (**9**) [8], respectively, by comparison of their spectroscopic data with those reported. The observed doubling of many of the NMR signals in the ^{1}H- and ^{13}C-NMR spectra of **6** is due to the occurrence of inseparable *exo* and *endo* isomers of the formamide group, which could be readily recognized by the coupling constant of the aldehydic H-atom with a *doublet* for *exo* ($J \approx 12$ Hz) form and a broad *singlet* for *endo* form. The same phenomenon was also observed in the NMR spectra of the new compounds **1** and **2**, indicating that both of them comprised a formamide moiety.

Compound **1** was obtained as a colorless oil. Its molecular formula, $C_{16}H_{29}NO_4$ was deduced from the HR-ESI-MS (*m*/*z* 322.1982 ($[M + Na]^+$, calc. 322.1994)) combined with the ^{1}H- and ^{13}C-NMR data, indicating three degrees of unsaturation. The characteristic bands at 3273 and 1686 cm^{-1} in the IR spectrum were assigned to a secondary-amide functionality, in agreement with the doubling of most ^{1}H- and ^{13}C-NMR peaks (*Tables 1* and *2*) due to a formamide group as noted above. The ^{13}C-NMR spectrum also provided evidence for one C=C bond, four Me groups, four CH_2 groups, two additional CH groups, and three heteroatom-bearing quaternary sp^3 C-atoms, implying a monocyclic sesquiterpene framework in consideration of the required degrees of unsaturation. These NMR data were strongly reminiscent of those of the co-occurring sesquiterpene axinyssine J (**4**), indicating a bisabolene framework. After detailed comparison of the

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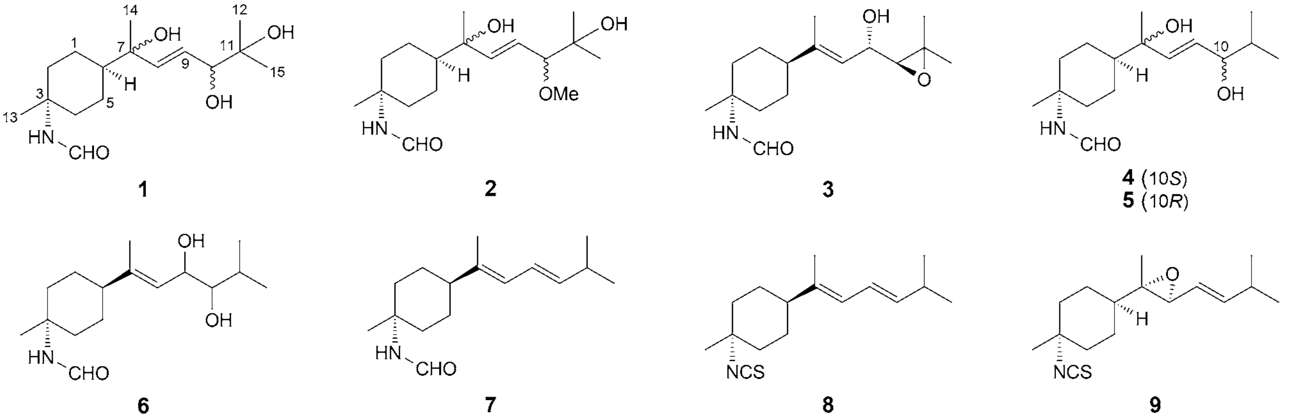

Fig. 1. Structures of compounds **1** – **9**.

Table 1. ^{1}H-NMR data of compounds **1** and **2**[a])

Position	**1**		**2**	
	exo	*endo*	*exo*	*endo*
1/5	1.62 – 1.66 (*m*)	1.41 – 1.43 (*m*)	1.62 – 1.66 (*m*)	1.41 – 1.43 (*m*)
2/4	1.86 – 1.88 (*m*)	1.50 – 1.54 (*m*)	2.05 – 2.08 (*m*)	1.52 – 1.55 (*m*)
6	1.37 – 1.41 (*m*)	1.37 – 1.41 (*m*)	1.37 – 1.41 (*m*)	1.37 – 1.41 (*m*)
8	5.64 (*d*, $J = 15.3$)	5.64 (*d*, $J = 15.3$)	5.77 (*d*, $J = 16.7$)	5.77 (*d*, $J = 16.7$)
9	5.52 – 5.54 (*m*)	5.52 – 5.54 (*m*)	5.57 – 5.58 (*m*)	5.56 – 5.57 (*m*)
10	3.72 (*d*, $J = 6.9$)	3.72 (*d*, $J = 6.9$)	3.52 (*d*, $J = 8.3$)	3.40 (*d*, $J = 8.3$)
12	1.01 (*s*)	1.01 (*s*)	1.14 (*s*)	1.14 (*s*)
13	1.22 (*s*)	1.17 (*s*)	1.38 (*s*)	1.31 (*s*)
14	1.11 (*s*)	1.11 (*s*)	1.36 (*s*)	1.30 (*s*)
15	1.05 (*s*)	1.05 (*s*)	1.32 (*s*)	1.32 (*s*)
NH	–		5.65 (br. *d*, $J = 12.0$)	5.19 (br. *s*)
CHO	8.07 (*s*)	7.77 (*s*)	8.31 (*d*, $J = 12.0$)	8.03 (*d*, $J = 2.1$)
MeO			3.30 (*s*)	3.30 (*s*)

[a]) ^{1}H-NMR Data were measured in $CDCl_3$ on a *Bruker DRX 400* MHz spectrometer; δ in ppm, J in Hz. The assignments were based on DEPT, HSQC, HMBC experiments, and/or by comparison with those of the model compounds.

^{1}H- and ^{13}C-NMR data of both compounds, it is easy to observe that instead of the ^{13}C-NMR signal noted ^{i}Pr group for **4** (δ(C) 33.9), compound **1** displayed the signal for an O-bearing quaternary C-atom (δ(C) 72.4), which suggested the terminal structure moiety Me_2COH. Therefore, the planar structure of **1** was deduced as shown in *Fig. 1*.

The relative configuration at C(3) and C(6) of **1** was proposed to be the same as those of **4**, because the ^{13}C-NMR chemical shifts of the sp^3 C-atoms [6] in the two compounds are almost the same (δ(C) 52.6/53.4, 47.0/47.3 for **1** *vs.* δ(C) 52.6/53.9, 47.1/47.4 for **4**), and their ^{1}H-NMR signals of H–C(6) are the same (δ(H) 1.37 – 1.41 (*m*)). The configuration at C(7) and C(10) was not determined due to the scarcity of the product.

Compound **2** was obtained as a colorless oil. The ESI-MS spectrum of **2** showed a *pseudo*-molecular ion peak at *m*/*z* 336 ($[M + Na]^+$), and its molecular formula was established as $C_{17}H_{31}NO_4$ from the HR-ESI-MS (*m*/*z* 336.2173 ($[M + Na]^+$, calc. 336.2151)). The ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*) spectra of **2** showed great similarities to those of **1**, indicating the presence of a bisabolene skeleton of **2**. The only difference was an additional signal in the NMR spectra of **2** for one MeO group (δ(H) 3.30 (*s*, 1.5 H)/3.26 (*s*, 1.5 H), δ(C) 56.8/56.2), comparing with those of **1**, in agreement with the additional observed molecular weight of 14 mass units. Furthermore, significant ^{1}H,^{13}C-long-range correlations, as shown in *Fig. 2*, allowed to locate the MeO as attached to C(10). Therefore, the constitutional formula of **2** was unambiguously assigned. Based on NMR data comparison and on biogenetic considerations, the relative configuration of C(3) and C(6) of **2** was proposed to be the same as in **1** [8][9].

Although many bisabolene-type sesquiterpenes were reported to have broad bioactivities [10 – 13], the compounds **1** – **9** were found to be inactive against the human lung adenocarcinoma (A549) and the human promyelocytic leukemia (HL-60) cell lines. Further studies should be conducted to the test of other bioactivities for these compounds, such as antifouling or anti-inflammatory properties, *etc.*

Table 2. ^{13}C-NMR data of compounds **1** and **2**[a])

Position	**1**		**2**	
	exo	*endo*	*exo*	*endo*
1/5	22.9	22.8	23.2	22.8
2/4	38.7	36.4	39.3	36.9
3	53.4	52.6	53.8	52.5
6	47.3	47.0	47.4	47.1
7	73.8	73.6	74.5	74.4
8	138.8	138.8	144.0	141.5
9	126.4	126.4	124.8	122.5
10	78.9	78.9	85.4	89.2
11	72.4	72.4	74.4	72.2
12	25.1	25.0	24.3	24.3
13	24.6	24.4	24.4	22.1
14	21.3	21.3	29.7	26.4
15	25.5	25.4	26.1	26.1
CHO	163.1	161.1	162.3	160.2
MeO			56.8	56.8

[a]) ^{13}C-NMR Data were measured in $CDCl_3$ on a *Bruker DRX 400* MHz spectrometer; δ in ppm, J in Hz. The assignments were based on DEPT, HSQC, HMBC experiments, and/or by comparison with those of the model compounds.

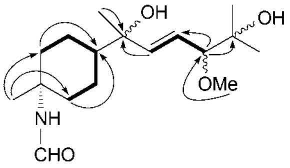

Fig. 2. Selected ^{1}H,^{1}H-COSY (H → C) and HMBC (▬) correlations of **2**.

Compounds **1** – **9**, comprising the same bisabolene skeleton, only differed from each other by the oxidized patterns. Compound **7** could act as the biosynthetic origin of such metabolites, to generate all the other derivatives by oxidation, sulfurization, hydration, *etc*. Such reactions seem to be simple, yet be powerful to result in the diversity of bisabolene-type sesquiterpenes, especially the highly oxidized ones such as the new compounds **1** and **2** (*Scheme*). Their further configuration determination by TDDFT-ECD method or chemical synthesis should be conducted.

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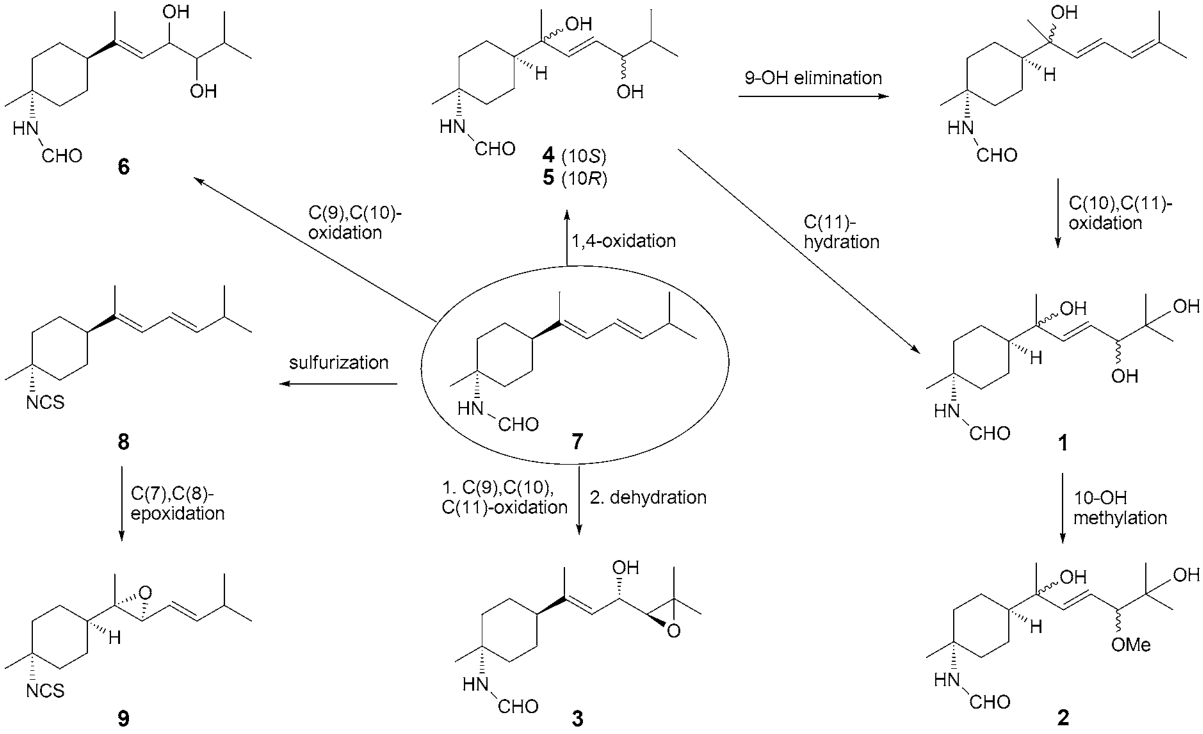
Experimental Part

General

Analytical TLC: precoated silica gel plates (*G60F-254*; *Yan Tai Zi Fu Chemical Group Co.*, Yantai, Shandong,

Scheme. Plausible biosynthetic relationship between the isolated compounds **1** – **9**.



P. R. China). CC: commercial silica gel (SiO_2, 200 – 300 and 400 – 600 mesh; *Qing Dao Hai Yang Chemical Group Co.*, Qingdao, Shandong, P. R. China). Reversed-phase HPLC: *Agilent 1100* series (*Agilent,* Santa Clara, USA) liquid chromatography using a *VWD G1314A* detector (*Agilent,* Santa Clara, USA) at 210 nm and a semiprep. *ODS-HG-5* (5 μm, 10 mm (i.d.) × 25 cm) column was also employed. Optical rotations: *Perkin–Elmer 241MC* polarimeter (Shanghai, P. R. China). NMR Spectra: *Bruker DRX-400* spectrometer (*Bruker Biospin AG*, Fällanden, Switzerland) with the residual $CHCl_3$ (δ(H) 7.26 ppm; δ(C) 77.0 ppm) as an internal standard; δ in ppm, J in Hz. ESI- and HR-ESI-MS: *Q-TOF Micro* LC/MS/MS mass spectrometer; in m/z.

Biological Material. The specimens of *A. variabilis*, identified by Prof. *R.-L. Zhou* of South China Sea Institute of Oceanology Chinese Academy Sciences, were collected off Lingshui Bay, Hainan Province, P. R. China, in November 2003, and were frozen immediately after collection. A voucher sample (LS-333) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The frozen sponge (67 g, dry weight) was extracted with acetone exhaustively at r.t. The acetone extract was concentrated *in vacuo* and the resulting residue was partitioned between Et_2O and H_2O, BuOH and H_2O, resp. The Et_2O-soluble extract (1.35 g) was chromatographed over a SiO_2 column using light petroleum ether (PE) with increasing amount of AcOEt as eluent. The fraction eluted with PE/AcOEt (7:3) was further purified by SiO_2 CC eluting with $CHCl_3$/MeOH (98:2) to give the pure compounds **7** (1.3 mg), **9** (4.7 mg), and **4** (5.4 mg). The fraction eluted with PE/AcOEt (5:5) was further purified by SiO_2 CC eluting with $CHCl_3$/MeOH (95:5) to give the pure compounds **5** (11.3 mg), **2** (1.3 mg), **3** (5.7 mg), and **6** (17.3 mg). The BuOH-soluble extract (2.5 g) was subjected to gel filtration on *Sephadex LH-20* (Pharmacia, USA; MeOH), yielding a mixture that was further purified by RP-HPLC (semiprep. *ODS-HG-5* (5 μm, 250 × 10 mm), MeOH/H_2O (4:6), 2.0 ml/min) to afford pure compounds **1** (0.8 mg) and **8** (4.6 mg).

7,10,11-Trihydroxy-8-en-3-formamidotheonellin (= ***N*-{*trans*-1-Methyl-4-[(3*E*)-2,5,6-trihydroxy-6-methylhept-3-en-2-yl]cyclohexyl}formamide**; **1**). Colorless oil. $[\alpha]_D^{20} = -12$ (c = 0.01, MeOH). ^1H and ^{13}C-NMR data: see *Tables 1* and *2*. HR-ESI-MS: 322.1982 ($[M + Na]^+$, $C_{16}H_{29}NNaO_4^+$, calc. 322.1994).

7,11-Dihydroxy-10-methoxy-8-en-3-formamidotheonellin (= ***N*-{*trans*-4-[(3*E*)-2,6-Dihydroxy-5-methoxy-6-methylhept-3-en-2-yl]-1-methylcyclohexyl}formamide**; **2**). Colorless oil. $[\alpha]_D^{20} = -8$ (c = 0.08, MeOH). ^1H and ^{13}C-NMR data: see *Tables 1* and *2*. HR-ESI-MS: 336.2173 ($[M + Na]^+$, $C_{17}H_{31}NNaO_4^+$, calc. 336.2151).

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