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Four sesquiterpenes isolated from a Marine Sponge *Topsentia* species

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Abstract Three bicyclic and one monocyclic sesquiterpenoids were isolated from the marine sponge *Topsentia* species. Their planar structures were completely determined from a combination of extensive 1D and 2D NMR experiments, and also the relative stereochemistry on the chiral centers were established by the ROESY experiment. Compound 1 was determined as a new stereoisomer. Furthermore, the NMR spectral data for compounds 2 and 4, of which have not been reported, were listed. Four compounds did not show any cytotoxicity, instead compound 4 exhibited moderate antifungal activity against *Candida albicans.*

Keywords NMR, sesquiterpenoid, sponge *Topsentia* sp., antifungal activity

Introduction

Sesquiterpenoids containing isocyano, isothiocynate, or formamide group have largely been found in sponges of orders Axinellida and Halichondrida and their associated opisthobranch mollusks.[1-4] Interestingly, many of these secondary metabolites exhibited different biological activities.[5-6] Recently, these compounds were reported to exhibit potential nontoxic antifouling ability that inhibits larval settlement of barnacles.7

In a continuing search for bioactive compounds from marine sponges, several sponge extracts were screened for cytotoxicity against K562 cells. Consequently, we encountered strong activity from the Genus *Topsentia* (Order Halichonda, family Halichodriidae) collected at Jeju island, Korea. Followed by an activity-guided fractionation, the extract was separated to afford four nitrogen-containing sesquiterpenes. However, all isolated compounds did not show the initial cytotoxic activity on the extract.

Here, we describe the isolation and the structure determination of the compounds 1 ~ 4.

Experimental Methods

Animal Sample

The specimen of *Topsentia sp.* (Sample No. 05J-4) was collected by hand using SCUBA 25 m depth in June 2005 at Jeju Island, Korea. The sponge is massive, measure 65 mm × 40 mm and 25 mm thick. The surface is rough with projecting spicule. The color in life is orange and gradually changing to ivory in alcohol. In skeleton, megascleres are thick oxeas (680-980 × 18-20 ㎛) and thin oxeas (400-740 × 5-10 ㎛). A voucher specimen (registry No. Spo. 51) is deposited at the Natural History Museum, Hannam University, Korea.

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Extraction and Isolation

The marine sponge was twice extracted with MeOH for 2 days at room temperature. The methanolic extract, showing strong cytotoxicity against K562 cells, was partitioned between Butanol (BuOH) and H_2O solvents and then the organic layer repartitioned between n-hexane and 15% aqueous MeOH for defatting. The MeOH fraction (3.7 g) was subjected on the vacuum column chromatography eluting with seven different solvent mixtures of MeOH and water. Of these, the 10% aqueous MeOH fraction had well splitting NMR signals in the ^{1}H NMR spectrum and cytotoxic activity. This fraction (900 mg) was separated by reversed phase HPLC (YMC ODS-A column, 250mm X 10mm, Varian RI detector) using a solvent system (H2O / MeOH = 20 / 80), and then purified by normal phase HPLC with a solvent system (Hexane / ethyl acetate = 70 / 30) to yield compounds 1 ~ 4.

NMR experiment

The 1D and 2D NMR spectra were obtained on a Varian NMR system working at 500MHz for proton and 125MHz for carbon. The ^{1}H and ^{13}C NMR chemical shifts refer to CD_3OD at 3.30 and 49.0ppm, respectively. For all experiments, the temperature was stabilized at 297 K. The parameters used for 2D NMR spectra were as follows; a gradient COSY spectrum were collected with a spectral width 4,000 Hz in a 512 (t1) X 1024 (t2) matrix applying the pulse gradient of 1 ms duration with a strength of 10 G/m and processed with a sinebell function. The gradient HSQC and HMBC spectra were measured with J_{CH} = 140 Hz and $^nJ_{CH}$ = 7 Hz, respectively, and processed in a 256 (t1) X 1024 (t2) matrix by a linear prediction method for a higher resolution..

Results and Discussion

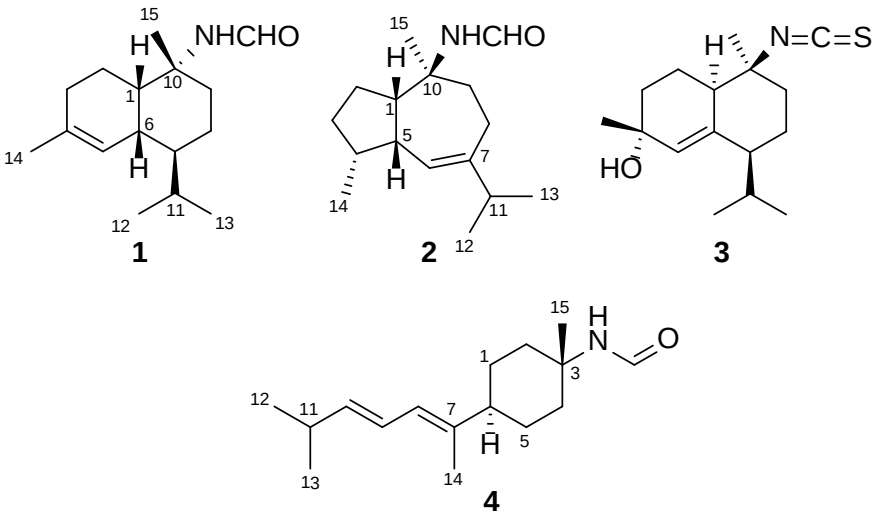

Figure 1. Four compounds isolated from the marine sponge *Topsentia* sp.

The 10% aqueous MeOH fraction of the extract was separated by HPLC to afford four sesquiterpenoids (compounds 1 ~ 4); one new stereoisomer and three known structures. Among them, compounds 2 and 4, which were believed to be a derivative converted from the natural compounds, were isolated as natural products and their NMR experiments were completely conducted in this paper.

Compound 1 was isolated as a colorless oil and its molecular formula was determined as $C_{16}H_{27}NO$ on

the basis of HRFABMS spectrometry ($[M + Na]^+$ ion peak m/z 272.1993, Δ=0.3), consistent with four degrees of unsaturation. The IR spectrum showed a strong absorption band at 3280 and 1685 cm^{-1}, deducing the presence of an amide functionality. The ^1H and ^{13}C NMR spectra presented in Figure 2 included signals corresponding to a trisubstituted double bond, one olefinic methyl, one quaternary methyl, and an isopropyl group. Careful interpretation of 2D NMR spectra revealed the cadinane-type skeleton structure as shown in Figure 1. Furthermore, from the molecular formula, unassigned group could be defined as a formamide, which was connected to the quaternary carbon C-10 by the correlation of the proton at δ_H 7.90 with the carbon at δ_C 57.8 in the HMBC spectrum. Although the cadinane formamides were already reported in other literatures,[7-8] their stereochemistry on chiral centers was not identical. In order to investigate the relative stereochemistry of 1, the ROESY experiment was conducted. The ROE correlations of Me-15 / H-1, Me-15 / H-6, and Me-15 / H-8 allowed the Me-15 to place on axial position to the cis-fused ring system as shown in Figure 4 (a). Another key ROE correlation between H-2 and H-7 supported the cis configuration and enabled to determine the orientation of the propyl group. The carbon chemical shift of Me-15 was corroborated by the fact that is more close to the characteristic value (*ca* 20 ppm) for axial methyls than that (*ca* 30 ppm) for equatorial methyls.[5] Instead, the propyl group was equatorially oriented to the ring system. The stereochemistry on C-1 and C-7 in 1 differed in the reported compounds.[7-8] Accordingly, compound 1 was determined as a new isomer of cadinane formamides.

The molecular formula of compound 2 was the same as that of 1 from the HRESIMS data and the ^{13}C NMR spectrum. Although the IR absorption band and

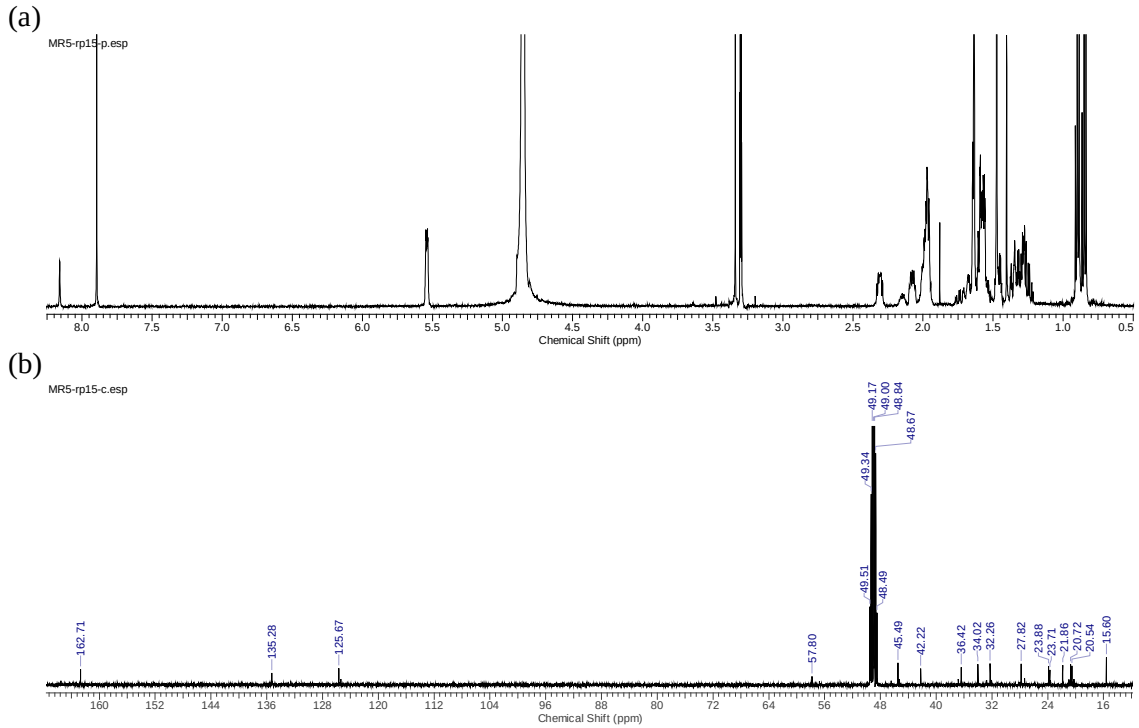

Figure 2. (a) ^1H and (b) ^{13}C NMR spectra of **1**.

a low field proton signal at δ_H 7.96 indicated the presence of an amide group in 2, two ^{1}H NMR spectra showed a big difference. Analysis of 1D and 2D NMR data revealed a guaiene type structure which was a sesquiterpene consisting of five-membered ring fused with seven-membered ring. The coupling of an olefinic proton with the proton at δ_H 2.60 determined the position of a trisubstituted double bond and the formamide group was attached to C-10 as for 1. Therefore, compound 2 was defined to be a guai-6-ene formamide which was characterized by X-ray crystallography.[9] Up to now, the guai-6-ene formamide has been known to be converted from a guai-6-ene cyanide by acid. In our study, this compound was isolated as a natural products without the addition of acid and its structure was established by the NMR method. The stereochemistry of 2 could also be determined by the ROESY spectrum. The extensive ROE correlations of Me-15 / H-1, Me-15 / H-2b, Me-15 / H-9b, Me-14 / H-6 and Me-12 / H-6 enabled us to establish the configuration of 2, which is consistent with the structure displayed by X-ray crystallography.

Compound 3 has the molecular formula $C_{16}H_{25}NOS$,

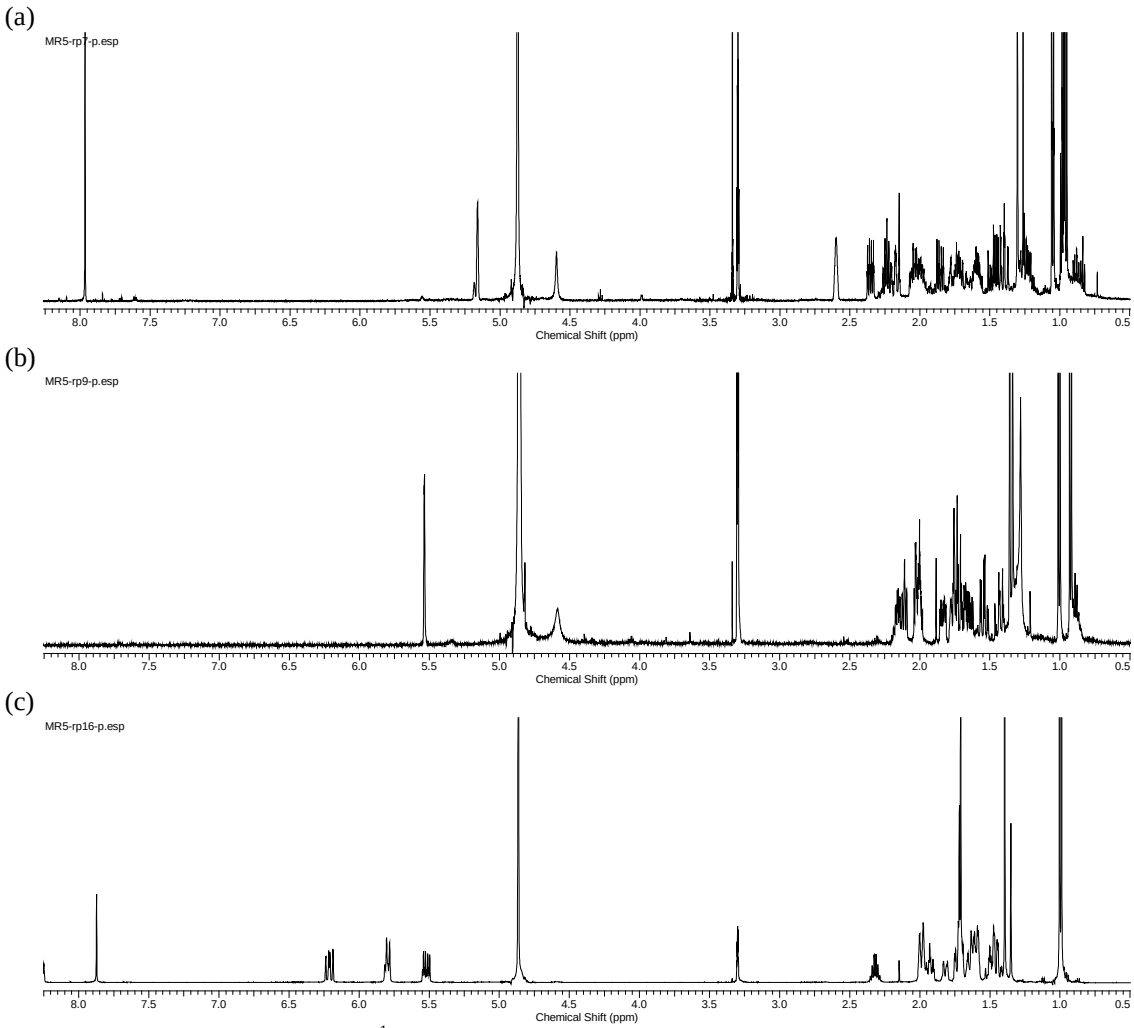

Figure 3. ^{1}H NMR spectra for (a) compound **2**, (b) **3**, and (c) **4**.

determined by HRFABMS. The IR spectrum showed the presence of hydroxyl (3400 cm^{-1}) and isothiocyanate (2120 cm^{-1}) functions. From the 1D and 2D NMR spectra, 3 was elucidated as a cadinane type sesquiterpene containing a trisubstituted double bond at C-5 position. The isothiocyanate group was connected to C-10 by comparison of the carbon chemical shifts for the corresponding structures. Accordingly, 3 was determined as a cadin-5-ene isothiocyanate. The relative stereochemistry of 3 was established by the ROE signals of Me-15 / H-2b, Me-5 / H-1, H-1 / H-3a, H-1 / H-7, and H-2a / Me-4 as shown in Figure 4 (C). The carbon chemical shift of Me-15 was close to *ca* 30 ppm, showing equatorial position to the ring system. The structure of 3 was the same with that isolated from a sponge of the Genus Axinyssa.[10]

The molecular formula of compound 4 was the same with that of 1 and 2. Based on the IR spectrum and a low field proton signal, compound 4 also contained a formamide unit. Furthermore, the well-splitting olefinic proton signals in the ^1H NMR spectrum revealed the existence of a diene group. Inspection of the 2D data led to a bisabolene type monocyclic sesquiterpene. The HMBC correlations of Me-14 with C-6, C-7 and C-8 allowed us to connect a carbon side chain to a cyclohexane ring, and also the HMBC correlation of an aldehydic proton and C-3 led to the linkage of the formamide group. The large coupling constants of three protons in the diene group indicated E, E-configurations and the ROE peak between H-6 and H-2a (H-6 and H-4a) showed that the three protons are oriented to the same direction. On the other hand, Me-15 was correlated to H-2b (H-4b) in the ROESY spectrum, suggesting the opposite direction to H-6. This could be supported by

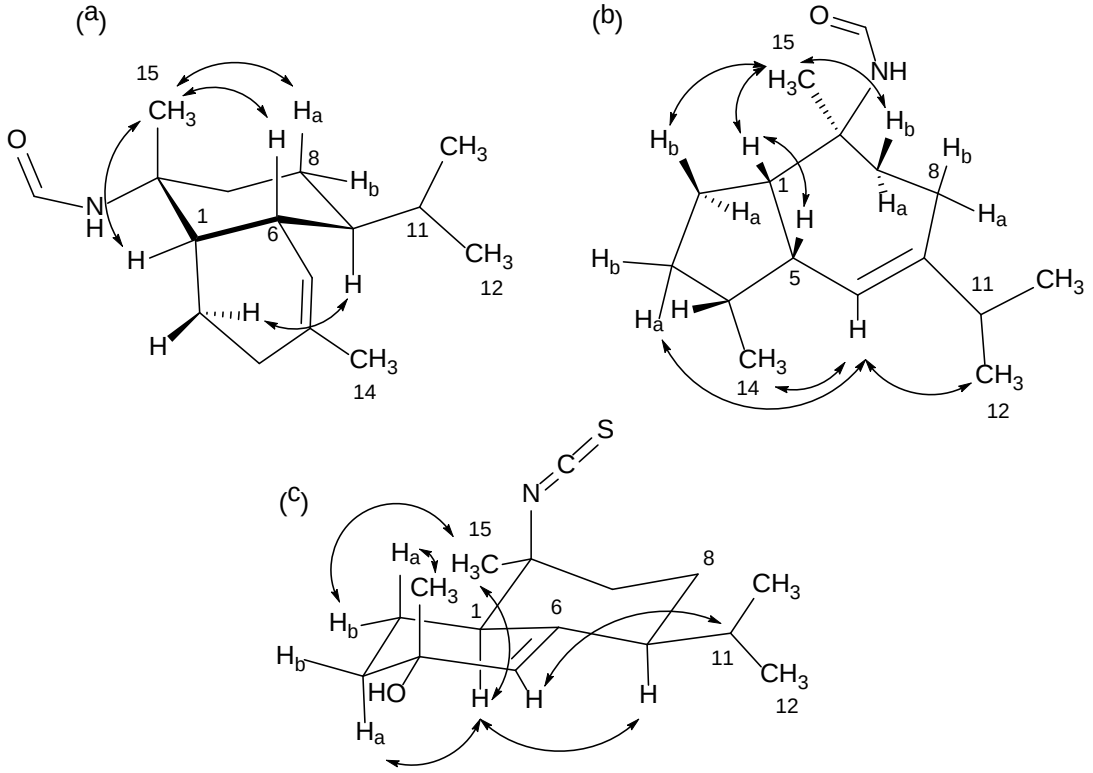

Figure 4. Stereochemistry for compounds **1** ~ **3** (arrows present NOE correlations).

the upfield shifted carbon chemical shift of Me-15; *i.e.* close to *ca* 20 ppm for axial methyls. The structure of compound 4 was reported as Theonellin formamide isolated from the Okinawa sponge *Theonella* cf. *swinhoei* 30 years ago, but unfortunately complete NMR data was not provided.

In this study, we isolated four sesquiterpenes containing formamide or isothiocyanate functionality from a sponge *Topsentia* species and their structures were completely determined by NMR experiments. The crude extract of the sponge showed strong cytotoxicity against K562, but the same measurement for pure compounds showed that all were not active ingredients (LC_{50} > 100 μg/ml). Instead, compound

Table 1. NMR spectral data for compounds **1** and **2** in CD_3OD at 500MHz NMR.

	1		2	
	δ_C	δ_H(*J* in Hz)	δ_C	δ_H(*J* in Hz)
1	42.2, CH	2.31, m	53.8, CH	2.05, m
2	34.0, CH_2	1.57, m	24.3, CH_2	a 1.46, qd (12.0, 5.1); b 1.59, m
3	32.3, CH_2	1.96, m	30.1, CH_2	a 1.22, m; b 1.73, m
4	135.3, C		40.6, CH	1.99, m
5	125.7, CH	5.55, d (5.4)	43.3, CH	2.60, br s
6	36.4, CH	2.07, dt (11.3, 5.4)	119.6, CH	5.16, br s
7	45.5, CH	1.34, m	151.1, C	
8	20.7, CH_2	a 1.26 qd (13.2, 4.9) b 1.45, qd (3.4, 13.2)	25.1, CH_2	a 1.85, ddt (15.4, 7.1, 1.5) b 2.17, dd (15.4, 13.9)
9	34.0, CH_2	1.57, m	32.1, CH_2	a 1.40, dd (14.2, 13.9) b 2.35, ddt (14.2, 7.1, 1.5)
10	57.8, C		60.6, C	
11	27.8, CH	1.97, m	39.4, CH	2.24, hept (6.9)
Me-12	15.6, CH_3	0.84, d (7.3)	21.6, CH_3	0.96, d (6.9)
Me-13	21.9, CH_3	0.89, d (6.9)	21.7, CH_3	0.98, d (6.9)
Me-14	23.7, CH_3	1.64, s	16.4, CH_3	1.05, d (6.9)
Me-15	23.9, CH_3	1.47, s	27.5, CH_3	1.30, s
16	162.7, CH	7.90, s	163.0, CH	7.96, s

Table 2. NMR spectral data for compounds **3** and **4** in CD_3OD at 500MHz NMR.

	3		4	
	δ_C	δ_H(*J* in Hz)	δ_C	δ_H(*J* in Hz)
1	48.0, CH	2.11, t (7.3)	28.4, CH_2	a 1.46, td (12.2, 3.4); b 1.60 td (3.4, 12.2)
2	22.7, CH_2	a 1.65, dddd (13.2, 12.2, 7.3, 3.4) b 2.00, m	37.6, CH_2	a 1.72, m b 1.99, br d (12.2)
3	36.9, CH_2	a 1.54 td (12.2, 3.4); b 1.84, m	54.5, C	
4	69.9, C		37.6, CH_2	a 1.72, m; b 1.99, br d (12.2)
5	131.5, CH	5.54, br s	28.4, CH_2	a 1.46, td (12.2, 3.4); b 1.60 td (3.4, 12.2)
6	138.1, C		47.9, CH	1.93, tt (12.2, 3.4)
7	48.7, CH	1.76, m	141.0, C	
8	23.5, CH_2	a 1.43, m; b 1.73, m	124.8, CH	5.79, d (10.7)
9	41.2, CH_2	a 1.69, m; b 2.03, m	125.0, CH	6.21, dd (15.1, 10.7)
10	67.4, C		140.8, CH	5.50, dd (15.1, 6.8)
11	28.1, CH	2.16, hep d (6.4, 2.9)	32.7, CH	2.32, octet (6.8)
Me-12	18.0, CH_3	0.92 d (6.9)	23.1, CH_3	1.00, d (6.8)
Me-13	22.5, CH_3	1.01, d (6.9)	23.1, CH_3	1.00, d (6.8)
Me-14	28.4, CH_3	1.34, s	15.0, CH_3	1.71, s
Me-15	26.7, CH_3	1.36, s	22.5, CH_3	1.39, s
16	138.1, C		162.9, CH	7.87, s

4 has a moderate antifungal effect against *C.albicans,* compared with that of amphoterocin.

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