IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(P): 2347-4580; ISSN(E): 2321-8851
Vol. 4, Issue 9, Sep 2016, 117-124

ANTI-BACTERIAL DITERPENES FROM BORNEAN SOFT CORALS *SINULARIA FLEXIBILIS*

TAKASHI KAMADA, WEI-TENG WONG,
CHIN-SOON PHAN & CHARLES SANTHANARAJU VAIRAPPAN
Laboratory of Natural Products Chemistry, Institute for Tropical Biology and Conservation,
University of Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia

ABSTRACT

Two new compounds, (13*Z*,16*E*)-fuscol (**1**) and (13*E*,16*Z*)-fuscol (**2**), along with five known and related metabolites fuscol (**3**), 18-methoxyloba-8,10,13(15),16(17)-tetraene (**4**), isofuscol (**5**), 17,18-epoxyloba-8,10,13(15)-trien-l6-ol (**6**) and loba-8,10,13(15)-triene-16,17,18-triol (**7**) were isolated from the methanolic extract of Bornean soft coral *Sinularia flexibilis* (Dinawan Island). Meanwhile, one new compound, (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-methoxy (**8**) together with a known compound, (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-ol (**9**) were isolated from the methanolic extract of another populations of Bornean *S. flexibilis* from Banggi Island. The structures of these three new molecules were elucidated based on spectroscopic methods (NMR, HR-ESI-MS and IR). In addition, their anti-bacterial activity was evaluated.

KEYWORDS: lobane; diterpenoid; *Sinularia flexibilis*; soft coral; anti-bacterial activity

INTRODUCTION

Soft corals of the genus *Sinularia* have been well recognized as a rich source of sesquiterpenes and diterpenes with unique structural diversity[1-4]. These compounds are believed to play a role in the protection from predators[5]. Among these, lobane diterpenes have been recognized as one of the most prolific sources of the genera *Sinularia*[6,7]. They have been reported to show various biological activities such as cytotoxic, anti-inflammatory and antimicrobial activities[6,8,9]. North Borneo Island has been well-known for its richness of marine biodiversity[10]. In the course of our ongoing research to discover bioactive metabolites from marine organisms, chemical investigation of the soft coral *S. flexibilis* collected from Dinawan Island, Sabah, Malaysia, has led to the isolation of two new diterpenoids (13*Z*,16*E*)-fuscol (**1**) and (13*E*,16*Z*)-fuscol (**2**) together with five known compounds such as fuscol (**3**)[8,11], 18-methoxyloba-8,10,13(15),16(17)-tetraene (**4**)[8], isofuscol (**5**)[4], 17,18-epoxyloba-8,10,13(15)-trien-16-ol (**6**)[12] and loba-8,10,13(15)-triene-16,17,18-triol (**7**)[12] as shown in Figure 1. Meanwhile, *S. flexibilis* collected from Banggi Island afforded one new diterpenoid (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-methoxy (**8**) together with a known compound (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-ol (**9**)[4,13,14]. Compounds **1**-**9** were evaluated their antibacterial activity against four bacterial strains *Escherichia*

coli, *Salmonella thypi*, *Staphylococcus aureus* and *Vibrio cholerae*. Here, we report the isolation, structural elucidation and antibacterial property of these compounds.

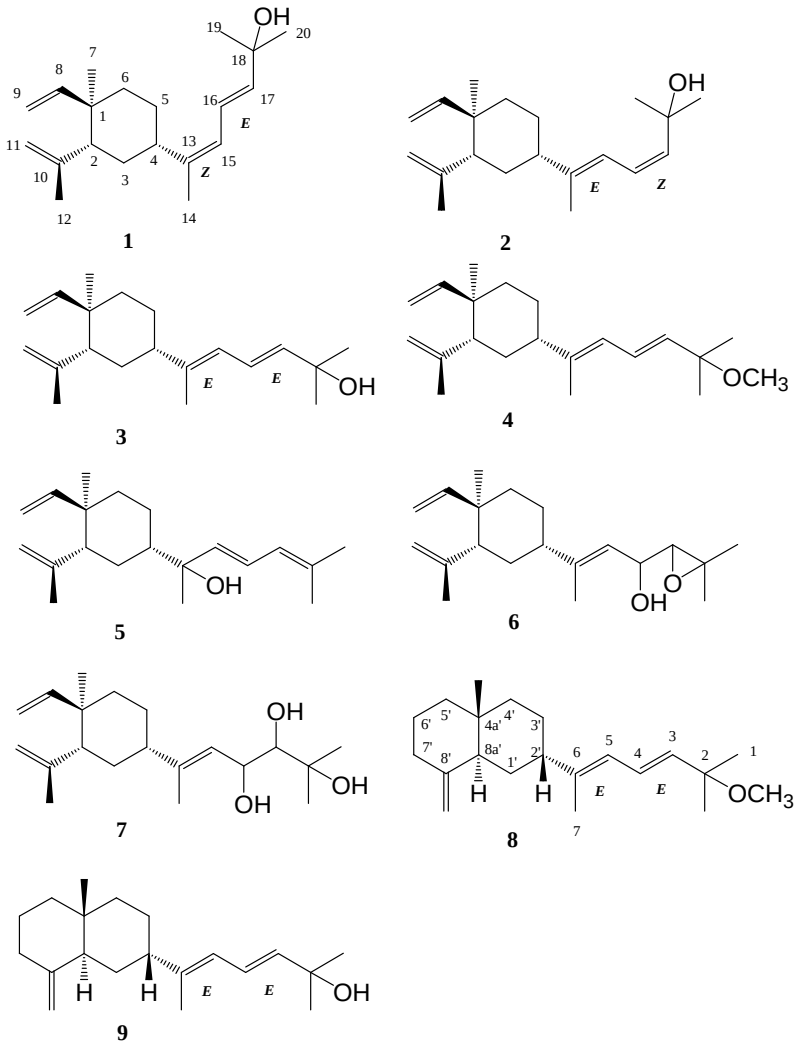

Figure. 1: Chemical structures of **1-9**

METHODS

General Experimental Procedures

Optical rotations were measured on an AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). IR spectra were obtained on a Thermo Nicolet Avatar FTIR spectrophotometer (Thermo, Tokyo, Japan). The NMR spectral data were recorded on JEOL ECA 600 NMR (JEOL, Tokyo, Japan) with tetramethylsilane (TMS) as an internal standard. The HR-ESI-TOFMS data were obtained on a LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Preparative TLC was performed with silica gel plate (Merck, Frankfurt, Germany; Kieselgel 60 F_{254}). Column chromatography (CC) was performed on silica gel (70–230 mesh; Merck, Frankfurt, Germany)..

Plant Material

Specimens of *S. flexibilis* were collected from Dinawan Island (05º50.750'N, 115º59.585'E) and Banggi Island (07º21.711'N, 117º15.223'E), Kota Kinabalu, Sabah, Malaysia in April 2012, respectively. Voucher specimens (BORH37601 and BORH37609) were deposited in the BORNEENSIS Collection of Institute for Tropical Biology and Conservation, University Malaysia Sabah.

Extraction and Isolation

Two populations of the fresh soft coral *S. flexibilis* (300 g) were extracted with MeOH at room temperature (5 liters), respectively. The crude extracts were evaporated under reduced pressure and the residues were partitioned between EtOAc and H_2O. The EtOAc fractions (1.0 g) were chromatographed on a Si gel column using hexane and EtOAc system of increasing polarity as eluent to yield five fractions. First population of *S. flexibilis* fraction 2 (50.0 mg) eluted with hexane/EtOAc (8:2) was subjected to repeated preparative TLC with toluene to yield compounds **1** (2.0 mg), **2** (2.0 mg), **3** (12.0 mg), **4** (13.5 mg) and **5** (3.0 mg). Fraction 4 (50.0 mg) eluted with hexane/EtOAc (1:1) was subjected to repeated preparative TLC with $CHCl_3$ to yield compounds **6** (3.5 mg) and **7** (5.0 mg). Meanwhile, second population of *S. flexibilis* fraction 2 (50.0 mg) eluted with hexane/EtOAc (8:2) was submitted to repeated preparative TLC with toluene to yield compounds **8** (11.0 mg) and **9** (13.0 mg).

- **Compound 1:** A colorless oil; $[\alpha]_D^{28.0}$ +2.0 (*c* = 0.15, $CHCl_3$); IR ν_{max} (cm^{-1}): 3390, 2930, 1738, 1636, 1456 and 890; ^1H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS: *m/z* 271.2413 $[M\text{-}OH]^+$ (calcd for $C_{20}H_{31}$, 271.2420).
- **Compound 2:** A colorless oil; $[\alpha]_D^{28.0}$ +2.2 (*c* = 0.10, $CHCl_3$); IR ν_{max} (cm^{-1}): 3405, 2928, 1658, 1636, 1458 and 907; ^1H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS: *m/z* 271.2422 $[M\text{-}OH]^+$ (calcd for $C_{20}H_{31}$, 271.2420).
- **Compound 8:** A colorless oil; $[\alpha]_D^{28.0}$ +80.0 (*c* = 0.30, $CHCl_3$); IR ν_{max} (cm^{-1}): 2920, 1750, 1445, 1375 and 1225; ^1H and ^{13}C NMR spectral data: see Table 2; HR-ESI-MS: *m/z* 302.2606 $[M+H]^+$ (calcd for $C_{21}H_{35}O$, 302.2610).

Antibacterial Activity

Antibacterial assay was performed using the disc diffusion method against four strains of human pathogenic bacteria: *Escherichia coli* (HP0808), *Salmonella thypi* (HP0809), *Staphylococcus aureus* (HP0810) and *Vibrio cholera* (HP0811) based on methods described by Vairappan *et al*[16].

RESULTS

Compound **1** was obtained as a colorless oil, and its molecular formula was determined to be $C_{20}H_{32}O$ by HR-ESI-MS at *m/z* 271.2413 $[M\text{-}OH]^+$. The broad IR absorption at 3390 cm^{-1} indicated the presence of a hydroxyl group. The NMR spectral data (Table 1) of **1** showed the presence of four olefin groups, including a monosubstituted alkene (δ_C 150.9, d, C-8; 110.6, t, C-9; δ_H 5.84, dd, *J* = 17.2, 11.0 Hz, H-8; 4.92 d, *J* = 17.2 Hz, 4.90 d, *J* = 11.0 Hz, H_2-9), one 1,1-disubstituted olefin (δ_C 148.8, s, C-10; 112.8, t, C-11; δ_H 4.82, br s, 4.59 br s, H_2-11), trisubstituted double bond (δ_C 142.4, s, C-13; 125.1, d, C-15; 20.7, q, C-14; δ_H 5.77, d, *J* = 11.0 Hz, H-15; 1.77, s, H_3-14) and a 1,2-disubstituted double bond (δ_C 122.5, d, C-16; 139.9, d, C-17; δ_H 6.51 dd, *J* = 11.0 and 15.1 Hz, H-16; 5.70, d, *J* = 15.1 Hz, H-17). From the required

five degrees of unsaturation calculated *via* HR-ESI-MS could be attributed to four double bonds and one cyclic system.

Table 1: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1**, **2** and **3** in $CDCl_3$

	1		**2**		**3**	
No.	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1	40.3		40.4		40.4	
2	53.1	2.08 (1H, m)	53.4	2.01 (1H, m)	53.4	2.00 (1H, m)
3	32.4	1.72 (1H, m)	33.4	1.60 (1H, m)	33.3	1.62 (1H, m)
		1.53 (1H, m)		1.51 (1H, m)		1.52 (1H, m)
4	41.0	2.70 (1H, m)	49.0	2.03 (1H, m)	48.3	1.97 (1H, m)
5	26.6	1.59 (1H, m)	27.1	1.55 (1H, m)	27.2	1.56 (1H, m)
		1.39 (1H, m)		1.40-1.50 (1H, m)		1.45 (1H, m)
6	40.3	1.55 (1H, m)	40.5	1.40-1.50 (1H, m)	40.5	1.49 (1H, m)
		1.44 (1H, m)		1.40-1.50 (1H, m)		1.48 (1H, m)
7	17.3	1.02 (3H, s)	17.3	1.01 (3H, s)	17.3	1.00 (3H, s)
8	150.9	5.84 (1H, dd, 17.2, 11.0)	151.0	5.82 (1H, dd, 17.2, 11.0)	150.9	5.81 (1H, dd, 17.2, 11.0)
9	110.6	4.92 (1H, dd, 17.2)	110.6	4.91 (1H, dd, 17.2)	110.6	4.90 (1H, d, 17.2)
		4.90 (1H, dd, 11.0)		4.88 (1H, dd, 11.0)		4.88 (1H, d, 11.0)
10	148.8		148.8		148.3	
11	112.8	4.82 (1H, br s)	112.8	4.81 (1H, br s)	112.8	4.81 (1H, br s)
		4.59 (1H, br s)		4.58 (1H, br s)		4.58 (1H, br s)
12	25.5	1.71 (3H, s)	25.5	1.70 (3H, s)	25.4	1.67 (3H, s)
13	142.4		145.5		144.1	
14	20.7	1.77 (3H, s)	15.0	1.76 (3H, s)	16	1.79 (3H, s)
15	125.1	5.77 (1H, d, 11.0)	119.7	6.54 (1H, d, 11.7)	123	5.86 (1H, d, 11.0)
16	122.5	6.51 (1H, dd, 15.1, 11.0)	125.5	6.17 (1H, t, 11.7)	123.8	6.47 (1H, dd, 15.1, 11.0)
17	139.9	5.70 (1H, d, 15.1)	136.5	5.44 (1H, d, 11.7)	139.9	5.75 (1H, d, 15.1)
18	71.7		72.7		71.6	
19	30.7	1.34 (3H, s)	32.1	1.42 (3H, s)	30.6	1.35 (3H, s)
20	30.4	1.34 (3H, s)	32.1	1.42 (3H, s)	30.6	1.35 (3H, s)

The presence of the vinyl and isopropenyl groups together with a tertiary methyl group (δ_C 17.3, q; δ_H 1.02, s) at C-7 was reminiscent of a 3-isopropenyl-4-methyl-4-vinylcyclohexane-1-yl moiety that was reported in lobane-type diterpenes **3-7**, and this partial structure was confirmed by COSY, HSQC and HMBC spectra. The ^{1}H-^{1}H COSY spectral data suggested one partial structure of correlations corresponding to H-15/H-16/H-17. The large coupling constants value between H-15/H-16 and H-16/H-17 were 11.0 and 15.1 Hz, respectively. These findings suggested a *trans* coupling between H-15 and H-16, and *E*-configuration double bond at C-16/C-17. The NOE correlations between H-4/H-16, H-16/H-19 and H-14//H-15 also strongly suggested these configurations. The hydroxyl group was deduced be attached to a hetero carbon at δ_C 71.7, C-18 based on the downfield of ^{13}C NMR chemical shift and the key HMBC correlations of H_3-19

and H_3-20 to C-17 and C-18. The HMBC correlations of H_3-14 to C-4, C-13 and C-15 has established the connection of (3*E*,5*Z*)-2,6-dimethylheptyl-3,5-dien-2-ol moiety to a cyclic ring at C-4. The conjugation of the diene with the ester function explained the significant differences in the chemical shifts of C-4, C-13, C-14 and C-15, as well as H-4, compared to fuscol (**3**)[8,11].

The relative configuration of the various asymmetric centers were deduced from the comparison with NMR data of diverse lobane congeners (all described lobanes isolated from soft corals have the configurations of 1*R*, 2*R*, 4*S*); the ^{13}C chemical shifts of C-1 to C-12 in all compounds indicated the same relative configuration of the cyclohexane system[4,8,12]. Thus, **1** is reported as (13*Z*,16*E*)-fuscol.

Compound **2** was obtained as a colorless oil, and its molecular formula was determined as $C_{20}H_{32}O$ by the HR-ESI-MS $[M\text{-}OH]^+$ ion at *m/z* 271.2422. The IR spectrum displayed the presence of OH (3405 cm^{-1}) group. Similar significant fragment ions in the mass spectrum, comparable absorption bands in the IR spectra and similar chemical shifts in the ^1H and ^{13}C NMR spectra (Table 1) indicated **1**, **2** and **3** to be stereoisomers. Comparison of 1D NMR data (Table 1) of **2** with those of **1**, revealed the double bond at C-13/C-15 had *E*-configuration instead of *Z*-configuration was deduced from ^{13}C NMR of methyl-bearing olefin at C-14 was δ_C 15.0[15]. Furthermore, it was found H-15 showed NOE correlation to H-4 and H-5, supported the *E*-configuration of double bond at C-13/C-15. In addition, C-16/C-17 had *Z*-configuration instead of *E*-configuration was due to $^3J_{16,17}$ = 11.7 Hz. It was found H-16 showed NOE correlation to H-17, further supported the *Z*-configuration of double bond at C-16/C-17. Hence, **2** is reported as (13*E*,16*Z*)-fuscol.

Compound **8** was obtained as colorless oil and showed a molecular ion peak $[M+H]^+$ at *m/z* 302.2606 in the HR-ESI-MS, consistent with the molecular formula $C_{21}H_{34}O$. The IR absorption at 1375 and 1225 cm^{-1} indicated presence of C-O group. The ^1H and ^{13}C NMR spectra of **8** were comparable to those of (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-ol (**9**)[8,11], except the methylation of the hydroxyl moiety at C-2 in **9**. This was explained by the NMR data (Table 2) of **8** showed an additional signal of methoxy at δ_C 51.0; δ_H 3.18, and the presence of this methoxy was confirmed by the HR-ESI-MS data. This methoxy was attached to C-2 based on its HMBC correlation to C-2. Thus, **8** is reported as (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4 a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-methoxy.

Table 2: ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of **8** and **9** in $CDCl_3$

	8		**9**	
	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1	26.7	1.31 (3H, s)	30.7	1.37 (3H, s)
2	75.8		71.7	
2-CH_3	26.7	1.31 (3H, s)	30.7	1.37 (3H, s)

2-OCH_3	51.0	3.18 (3H, s)		
3	137.3	5.58 (1H, d, *J* = 15.8 Hz)	139.8	5.77 (1H, d, *J* = 15.1 Hz)
4	127.0	6.42 (1H, dd, *J* = 15.8, 10.3 Hz)	124.3	6.52 (1H, dd, *J* = 15.1, 10.3 Hz)
5	124.5	5.95 (1H, d, *J* = 10.3 Hz)	124.3	5.93 (1H, d, *J* = 10.3 Hz)
6	141.1		141.3	
7	17.5	1.76 (3H, s)	17.4	1.76 (3H, s)
2'	40.9	2.45 (1H, m)	40.9	2.45 (1H, m)
3'	23.9	1.85 (1H, m)	23.9	1.84 (1H, m)
		1.79 (1H, m)		1.79 (1H, m)
4'	37.6	1.41 (1H, m)	37.6	1.39 (1H, m)
		1.21 (1H, m)		1.21 (1H, m)
4a'	37.1		37.1	
4a'-CH_3	16.8	0.76 (3H, s)	16.8	0.76 (3H, s)
5'	42.8	1.37 (1H, m)	42.8	1.35 (1H, m)
		1.26 (1H, m)		1.26 (1H, m)
6'	24.2	1.59 (1H, m)	24.2	1.58 (1H, m)
		1.59 (1H, m)		1.58 (1H, m)
7'	37.7	2.27 (1H, m)	37.7	2.27 (1H, m)
		2.00 (1H, m)		2.00 (1H, m)
8'	152.1		152.0	
8'-CH_2	105.8	4.71 (1H, d, *J* = 1.4 Hz)	105.8	4.70 (1H, d, *J* = 1.4 Hz)
		4.44 (1H, d, *J* = 1.4 Hz)		4.43 (1H, d, *J* = 1.4 Hz)
8a'	45.0	1.85 (1H, m)	45.0	1.84 (1H, m)
1'	26.6	1.80 (1H, m)	26.8	1.80 (1H, m)
		1.59 (1H, m)		1.58 (1H, m)

The bioassay findings are given in Table 3. Compound **1**, **2**, **3** and **5** exhibited stronger antibacterial activities (MIC ≤ 25.0 µg/disc) against tested bacteria than the other compounds. Meanwhile, compound **8** and **9** were found inactive. The reason was that these compounds were extremely unstable, probably due to the facile loss of water and subsequent polymerization as has already been described for compound (**9**)[13].

Table 3: The MIC of compounds **1**-**9** against four strains of bacteria

Tested bacteria	Compounds								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
E. coli	12.5	10.0	10.0	50.0	12.5	-	-	-	-
S. thypi	10.0	25.0	10.0	-	10.0	-	-	-	-
S. aureus	25.0	12.5	12.5	-	12.5	-	50.0	-	-
V. cholera	12.5	12.5	10.0	25.0	25.0	-	75.0	-	-

In conclusion, two new compounds, (13*Z*,16*E*)-fuscol (**1**) and (13*E*,16*Z*)-fuscol (**2**), along with five known metabolites fuscol (**3**), 18-methoxyloba-8,10,13(15),16(17)-tetraene (**4**), isofuscol (**5**), 17,18-epoxyloba-8,10,13(15)-trien-l6-ol (**6**) and loba-8,10,13(15)-triene-16,17,18-triol (**7**) were isolated from Bornean *Sinularia flexibilis* (Dinawan Island, Sabah). Meanwhile, a new compound, (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-methoxy (**8**) together with one known compound, (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-ol (**9**) were isolated from *S. flexibilis* (Banggi Island, Sabah). Compound **1**, **2**, **3** and **5** exhibited stronger antibacterial activities (MIC ≤ 25.0 µg/disc) against *E. coli*, *S. thypi*, *S. aureus* and *V. cholera*.

CONCLUSIONS

In conclusion, two new compounds, (13*Z*,16*E*)-fuscol (**1**) and (13*E*,16*Z*)-fuscol (**2**), along with five known metabolites fuscol (**3**), 18-methoxyloba-8,10,13(15),16(17)-tetraene (**4**), isofuscol (**5**), 17,18-epoxyloba-8,10,13(15)-trien-l6-ol (**6**) and loba-8,10,13(15)-triene-16,17,18-triol (**7**) were isolated from Bornean *Sinularia flexibilis* (Dinawan Island, Sabah). Meanwhile, a new compound, (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-methoxy (**8**) together with one known compound, (3*E*,5*E*)-2-methyl-6-[(2'*R**,4a'*S**,8a'*R**)-4a'-methyl-8'-methylene-*trans*-perhydronaphthalen-2'-yl]hepta-3,5-dien-2-ol (**9**) were isolated from *S. flexibilis* (Banggi Island, Sabah). Compound **1**, **2**, **3** and **5** exhibited stronger antibacterial activities (MIC ≤ 25.0 µg/disc) against *Escherichia coli*, *Salmonella thypi*, *Staphylococcus aureus* and *Vibrio cholera*.

ACKNOWLEDGEMENTS AND LEGAL RESPONSIBILITY

This work was supported by the Sabah Biodiversity Centre (SaBC) under Grant No. GL0070.

REFERENCES

1. Kusumi T, Hamada T, Ishitsuka MO, Otani I, Kakisawa H. Elucidation of the relative and absolute stereochemistry of lobatriene, a marine diterpene, by a modified Mosher method. *J. Org. Chem.*1992; 57: 1033-1035.
2. Duh CY, Wang SK, Tseng HK, Sheu JH, Chiang MY. Novel cytotoxic cembranoids from the soft coral *Sinularia flexibilis*. *J. Nat. Prod.* 1998; 61: 844-847.
3. Lin YS, Chen CH, Liaw CC, Chen YC, Kuo YH, Shen YC. Cembrane diterpenoids from the Taiwanese soft coral *Sinularia flexibilis*. *Tetrahedron*. 2009; 65: 9157-9164.
4. Coll JC, Bowden BF, König GM, Braslau R, Price IR. Studies of Australian soft corals. XXXX. The natural products chemistry of Alcyonacean soft corals with special reference to the genus *Lobophytum*. *Bull. Soc. Chem. Belg.* 1986; 95: 815-834.
5. Coll JC. The chemistry and chemical ecology of octocorals (Coelenterata, Anthozoa, Octocorallia). *Chem. Rev.*

1992; 92: 613-631.

6. Chai MC, Wang SK, Dai CF, Duh CY. A cytotoxic lobane diterpene from the Formosan soft coral *Sinularia inelegans*. *J. Nat. Prod.* 2000; 63: 843-844.
7. Hamada T, Kusumi T, Ishitsuka MO, Kakisawa H. Structures and absolute configurations of new lobane diterpenoids from the Okinawan soft coral *Sinularia flexibilis*. *Chem Lett*. 1992; 21: 33-36.
8. Edrada RA, Proksch P, Wray V, Witte L, van Ofwegen L. Four new bioactive lobane diterpenes of the soft coral *Lobophytum pauciflorum* from Mindoro, Philippines. *J. Nat. Prod.* 1998; 61: 358-361.
9. Cheng SY, Chuang CT, Wang SK, Wen ZH, Chiou SF, Hsu CH, Dai CF, Duh CY. Antiviral and anti-inflammatory diterpenoids from the soft coral *Sinularia gyrosa*. *J. Nat. Prod*. 2010; 73: 1184-1187.
10. Ishii T, Zhaoqi Z, Vairappan CS. A new cembrane diterpene from the Bornean soft coral *Nephthea* sp. *Molecules.* 2010; 15: 3857-3862.
11. Govindam SV, Yoshioka Y, Kanamoto A, Fujiwara T, Okamoto T, Ojika M. Cyclolobatriene, a novel prenylated germacrene diterpene, from soft coral *Lobophytum pauciflorum*. *Bioorg. Med. Chem.* 2012; 20: 687-692.
12. Raju BL, Subbaraju GV, Rao ChB, Trimurtulu G. Two new oxygenated lobanes from a soft coral of *Lobophytum* species of the Andaman and Nicobar coasts. *J. Nat. Prod.* 1993; 56: 961-966.
13. Bowden BF, Coll JC, Liyanage N, Mitchell SJ, Stokie GJ, Altena IAV. Studies of Australian soft corals. IV. A novel bicyclic diterpene from *Lobophytum hedleyi*. *Aust. J. Chem.* 1978; 31: 163-170.
14. Matthée GF, König GM, Wright AD. Three new diterpenes from the marine soft coral *Lobophytum crassum*. *J. Nat. Prod.* 1998; 61: 237-240.
15. Couperous PA, Clague ADH, van Dongen JPCM. ^{13}C chemical shifts of some model olefins. *Org. Magn. Reson.* 1976; 8: 426-431.
16. Vairappan CS, Kawamoto T, Miwa H, Suzuki M. Potent antibacterial activity of halogenated compounds against antibiotic-resistant bacteria. *Planta Med.* 2004; 70: 1087-1090.