Journal of the Korean Magnetic Resonance Society **2021**, *25*, 24-32
DOI http://dx.doi.org/10.6564/JKMRS.2021.25.2.024

Structure determination of two new compounds isolated from a marine sponge *Haliclona*(*Gellius*) sp.

Kyung Lee[1], Yun Na Kim[2], Eun Ju Jeong[2,*]

[1]Department of Oceanography, Kunsan National University, Gunsan 54150, Korea
[2]Department of Plant & Biomaterials Science, Gyeongsang National University, Jinju 52725, Korea

Received June 9, 2021; Revised June 19, 2021; Accepted June 19, 2021

Abstract Two new sesterterpenes, including a known sesterterpene, were isolated from the marine sponge *Haliclona* sp. collected in the Gageo island, Korea. One of the new sesterterpenes (**1**) was an unusual compound possessing a spiroketal moiety and the other (**2**) represented a four ring-fused skeleton. The planar structure of compound **1** was identical to gombaspiroketals A and B isolated from the marine sponge *Clathria gombawuiensis*, but the configuration for the two chiral centers was different each other. On the other hand, the skeletal structure of compound **2** was similar to that of phorone A isolated from *Phorbas* sp. and a compound from *C. gombawuiensis*, except for one configuration at C-8. However, in comparing the ^{1}H and ^{13}C NMR spectral data, the proton and carbon chemical shifts for the three compounds were almost consistent. The NOESY spectrum revealed that the C-8 configuration of **2** was reversed to that of the two reported compounds. The configuration for compound **2** was supported by quantum mechanical calculation for the carbon chemical shifts and DP4+ probability for the protons and carbons of **2**.

Keywords Sesterterpene, *Haliclona* sp., Spiroketal, DFT method, 1D and 2D NMR

Introduction

Marine sponges provided structually diverse sesterterpenes exhibiting significant biological activities.[1] Compounds such as halisulfates, cheilanthane, and scalarane posdessed characteristic carbon skeletons and exhibited strong cytotoxic, antimicrobial, and enzyme inhibitory activities.[2-4] Recently new sesterterpenes, phorbaketals and phorbasones, with noble carbon frameworks were isolated from the Korean marine sponge *Phorbas* sp.[5-6] During our continuing search for bioactivbe compounds from Korean sponges, sestertepenes with new carbon skeletons were also isolated from the marine sponge *Haliclona* sp. These compounds were earlier reported as gomaspirketal and prhorone A by other research groups.[7-9] However, the compounds isolated in this study were identified as new stereoisomers or derivatives of the reported compounds. In this paper, we report the isolation and structure determination of two compounds isolated from marine sponge *Haliclona* sp. Specifically, we modified the stereochemistry of C-8 in phorone A and similar compound. This result was obtained through the interpretation of NOESY NMR spectrum and quantum chemical calculations.

Experimental Methods

General Experimental - All NMR spectra were measured on a Varian VNMRS 500 spectrometer

* Correspondence to: **Eun Ju Jeong,** Department of Plant & Biomaterials Science, Gyeongsang National University, Jinju 52725, Korea Tel: +82-55-772-3224; E-mail: jeong.ej@gnu.ac.kr

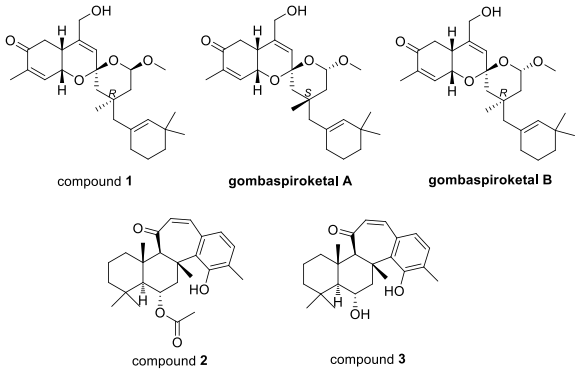


Figure 1. Structures of compounds isolated from a ma rine sponge, Halichlona sp.

using CD_3OD for all compounds. High resolution mass spectra were acquired using an ABSCIEX X500R ESIQTOF instrument. HPLC was performed using a Varian Prostar system with a 355 refractive index (RI) detector and Agilent 1200 Chemstation with DAD detector.

Material - The marine sponge *Haliclona* sp. was collected from Gageo island, Korea in 2010. A voucher (10G-19) has been deposited at the Marine Bioresources Bank, Hannam University.

Extraction and Isolation - The freeze-dried *Haliclona* sp. (4.0 kg) were extracted twice with 100% methanol for overnight in 25ºC. The solvent was removed *in vacuo* and yielded a methanolic extract (737.4 g). The methanolic extract was partitioned into methylene chloride and water for the removal of salt. The organic layer was then repartitioned into hexane and 15% aqueous methanol. The methanol soluble fraction was subjected to reversed silica gel flash column chromatography eluting solvents of decreasing polarity ($MeOH:H_2O$ = 50:50, 60:40, 70:30, 80:20, 90:10, 100:0) to give six fractions (MR1-MR6). Among these, MR5 was further subjected to Sephadex LH20 column chromatography using 100% methanol to give four sub-fractions (MR5-M1-M4). Compound **1** was isolated from subfraction MR5-M3 by HPLC (column: YMC SIL, eluting solvents: 40% ethyl acetate and 60% hexane, detector: RI, flow rate:1.5 ml/min) at a retension time of 28 min. Compounds **2** and **3** were isolated from MR5-M4 (8.0 mg) with HPLC (eluting solvents: 80% methanol and 20% H_2O, column:YMC-H80) at a retention time of 36 min and 29 min, respectively.

Results and Discussion

Three sesterterpenes were isolated from 10% aqueous methanol fraction of *Haliclona* sp. extract (Figure 1). 1D and 2D NMR spectra led to elucidate the structure for the three compounds. Compound **1** had the molecular formula $C_{26}H_{38}O_5$ on the basis of the molecular ion $[M+H]^+$ at *m/z* 399.2552 in the HRFABMS. The IR and UV spectra displayed the absortion peaks at 1682 cm^{-1} and 227.9 nm, suggesting the presence of α, β-unsaturated carbonyl moiety. The detailed structure of **1** was determined by the interpretation of the 1D and 2D NMR spectra. From the ^{13}C and HSQC NMR spectra, **1** was revealed to be composed of five methyls, 8 methylenes, 6 methines and 7 quaternary carbons. Among these, 6 olefinic carbons and 3 oxygen-bearing carbon, 2 ketal or acetal carbons were deduced from the carbon chemical shifts. Next, partial units given as bold lines in Figure 2 were established by the vicinal proton couplings in the COSY spectrum: from H-2 to H-6, between H-15 and H-16, from H-21 to H-23. Following this, the connection with the nonprotonated carbons was conducted by the HMBC data shown as arrows in Figure 2. The four singlet methyl protons in **1** displayed the obvious HMBC correlations with to the neighboring carbons. Based on this information, the HMBC cross peaks between H-6 and the carbonyl carbon at δ_C 200.8 revealed a 2-methylcyclohexenone ring. Furthermore, the extensive HMBC correlations with the three methylene protons (H-9, H-12, and H-17) led to link all partial structures. Specifically, the protons at δ_H 5.59, 1.56, 1.72, 4.54 and 4.83 were correlated to a ketal carbon at δ_C 98.2 in the HMBC spectrum, which formed a spiroketal ring. Finally, a methoxy proton at δ_H 3.45 wad connected to a acetal carbon at δ_C 101.0 by the HMBC correlation. The determined planar structure of **1** was consistent with gombaspiroketals A and B in a literature search. However, the chemical shifts and coupling constants in the ^1H NMR spectra were slightly different for the three compounds, suggesting different stereoisomers. When compared with the ^1H NMR spectra for the three compounds, H-12, H-14, H-15, H-16 and H-17 for each compound have conspicuous differences in

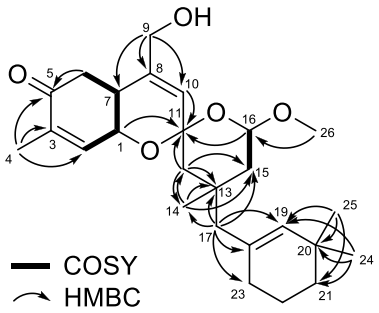


Figure 2. COSY and HMBC correations of **1**.

the chemical shifts. Furthermore, compound **1** showd different proton coupling constants in H-15 and H-16, compared with gombaspiroketals. The spectral data for the protons and carbons of **1** was listed in Table 1. This observation suggested the stereochemistry on the two chiral centers (C-13 and C-16) to be different each other. The stereochemistry of **1** was determined by NOESY experiment and the key NOESY configurations were depicted in Figure 3. Of these, the NOE correlation between H-1 and the methoxy (H-26) was important for determining the stereochemistry of **1**. This correlation, which was not observed in gombaspiroketals A and B, allowed H-1 and the methoxy group to be arranged in the same direction, while H-16 to be configured a *gauch* with H-15a and H-16b. The latter configuration supported the intermediate coupling constants between H-15a and H-16, and H-15b and H-16, which differed from the large and small coupling constants between H-15 and H-16 in gomaspiroketals A and B.

On the other hand, the NOE correlations of H-14/H-12a, H-14/H-15a, H-17/H-12b, H-17/H-15b indicated that the methyl group attached at C-13 is positioned backward and assigned the configuration of C-13 as *R*-from. Accordingly, compound **1** was determined as a new sesterterpene with 13(*R*),16(*R*)-spiroketal moiety. By comparison, gombaspiroketal A and B were assigned as 13(*S*),16(*S*) and 13(*R*),16(*S*), respectively. The compounds with the unusal spiroketal moety have been reported from the marine sponges *Phorbas* sp., *Monanchora* sp., and *Hamigera* sp.[10-11]

Compound **2**, isolated from the same fraction as compound **1**, was yellow amorphous solid and

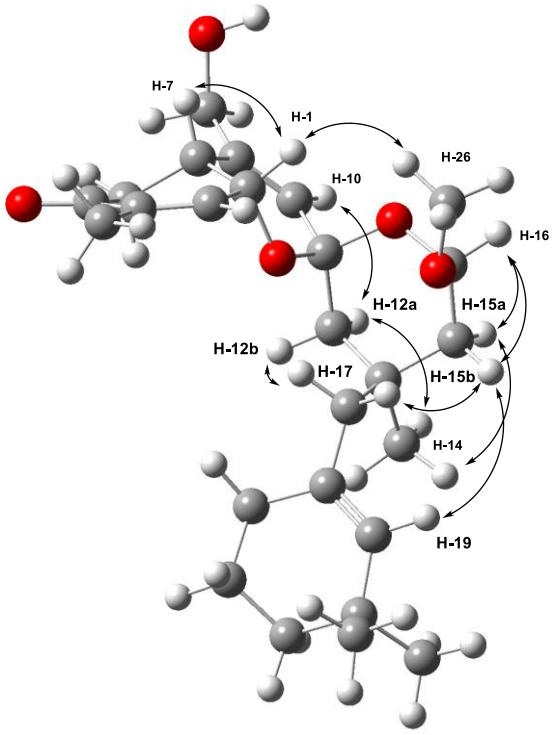


Figure 3. Key NOESY correlations of **1.**

Its formula was deduced to be $C_{27}H_{26}O_4$ on the basis of the pseudo molecular ion $[M + H]^+$ at *m/z* 425.2671 in the HRESIMS and the ^{13}C NMR specrum. Together with the molecular formula, the seven upfield- shifted singlet methyl protons in the ^1H NMR spectrum were characteristic of the properties of terpenoid. Compound **2** had a relatively low number of protons relative to the carbon signals. The spectral data for the protons and carbons of **2** was listed in Table 2. Similar to that of compound **1**, the planar structure of **2** was established by the extensive HMBC correlations on the basis of the proton couplings in the COSY spectrum (Figure 4). In particular, the linkage of consecultive nonprotonated carbons corresponding to C-14,C-19,C-18, and C17 was demonstrated by the HMBC correlations of the two protons in the benzene ring, along with the HMBC correlations with singlet methyl protons at H-22, -24, and -25. Furthermore, a downfield-shifted proton at δ_H 5.14 was correlated to a carbonyl carbon at δ_C 172.7 in the HMBC spectrum, indicating a ester bond. The position of the ketone unit at C-11 was apparently determined by two HMBC correlations of H-9

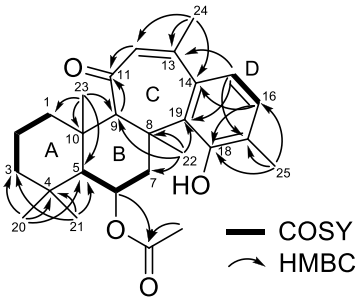


Figure 4. COSY and HMBC correlations of **2**.

and H-11 with the carbon at δ_C 203.1, constructing a seven-membered C ring. The determined structure of **2** was very similar to that of phorone A isolated from the marine sponge *Phorbas* sp.. However, an acetyl group is present at C-6 and the position of a hydroxy group is attached at C-18 instead of at C-16 in phorone A. The relative configuration for **2** was established by analysis of the proton coupling constants and NOESY spectrum. Initially, based on the NOE observation between the two methyl protons at H-21 and H-23, the NOE cross peaks of H-5/H-9, H-6/H-20, H-6/H-21 showed that rings A and B in the decalin unit of **2** was *trans* form (Figure 5). Next, the position of the methyl group (H-22) attached at C-8 was located on the same side of the methyl group of H-23 from the NOE correlations of H-22/H-23, H-7a/H-22 and H-7b/H-22. This observation indicated that rings B and C was also configured to be *trans* and ring B was occurred in the form of a boat conformation. The conformation of ring B was supported by intermediate coupling constants of the two protons at C-7 (J = 16.6, 2.5 Hz for H-7a and J = 16.6, 4.2 Hz for H-7b), which were not configured to be *anti* with H-6. On the other hand, compound **3** isolated in this study was identified to be identical as **2** except for the acetyl group. The chemical structure of compounds **2** and **3** were similar to phorone A reported in the literature. One major difference was the configuration of C-8, of which compounds **2** and **3** was assigned as *R* form, while that of phorone A was given as *S* form. Moreover, compound **3** obtained this study differed from compound **3** reported by shin *et al*. with respect to the configuration for C-8, despite all ^{1}H and ^{13}C NMR data being almost identical. The stereochmistry of C-8 between compounds isolated in this study and those reported in the literature was inconsistent.

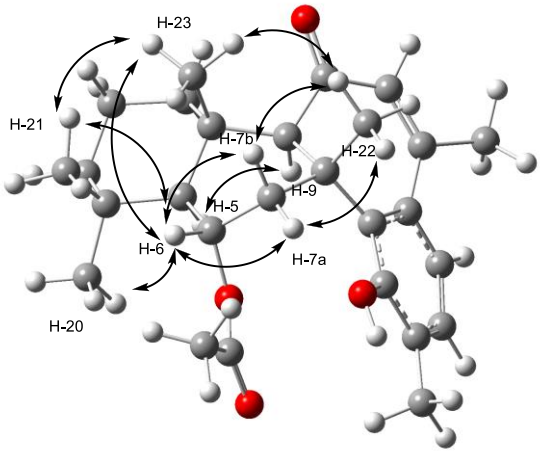


Figure 5. Key NOE correlations of **2**.

In order to support the stereochemistry of compound **2**, the carbon chemical shifts for (8*R*)-configured and (8*S*)-configured compounds were quantum chemically calculated by DFT method. Cacluation of NMR spectra for the two isomers was performed by using MPW1PW91/6-311G(d,p)//B3LYP/6-311G(d,p) model, based on the geometries determined by NOE correlations (Figure 6).[12] Calculated carbon NMR chemical shifts for (8*R*)-configured compound, corresponding to compound **2**, provided good agreement with experimental values (RMS value = 2.93). Additionally the two isomers were compared by using the DP4+ probability mothods,[13] and also in this case, (8*R*)-configured compound appeared to be be most likely as shown in Table 3. Thus, compounds **2**, **3** and phorone A was configured as *trans* between rings A and B, and rings B and C. ^{1}H NMR and ^{13}C NMR spectra of **2** and **3**, and ^{1}H NMR spectrum of **3** were given in Figures 7 and 8.

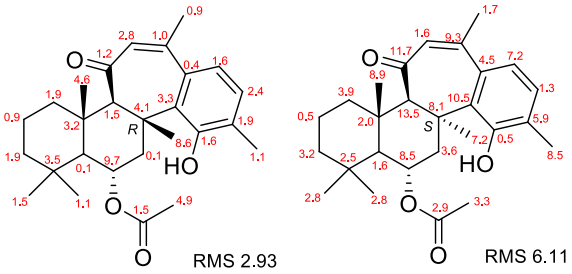
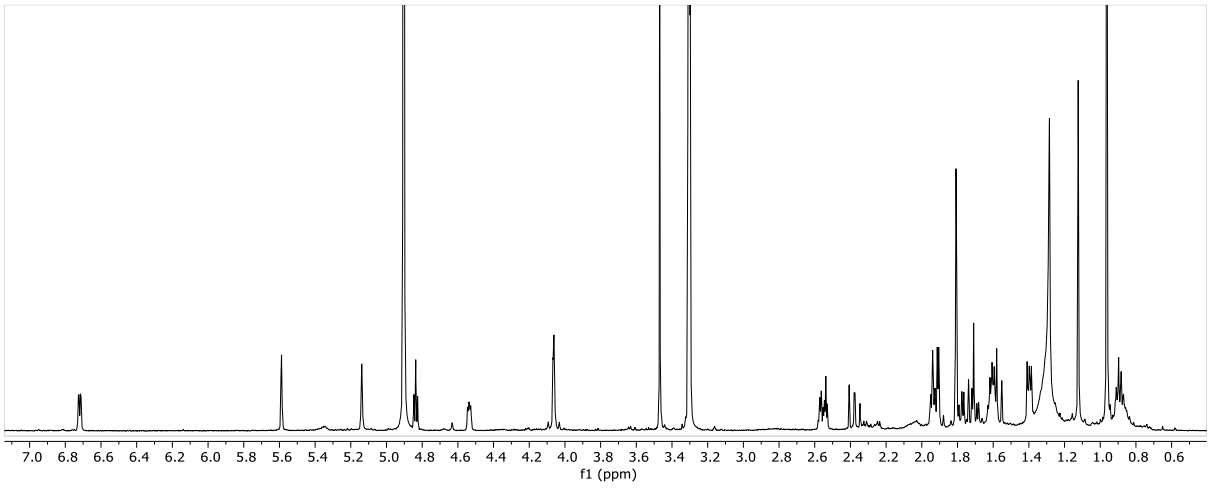


Figure 6. Difference of calculated and experimental carbon chemical shifts in **2** (RMS: root mean square).

(A)



(B)

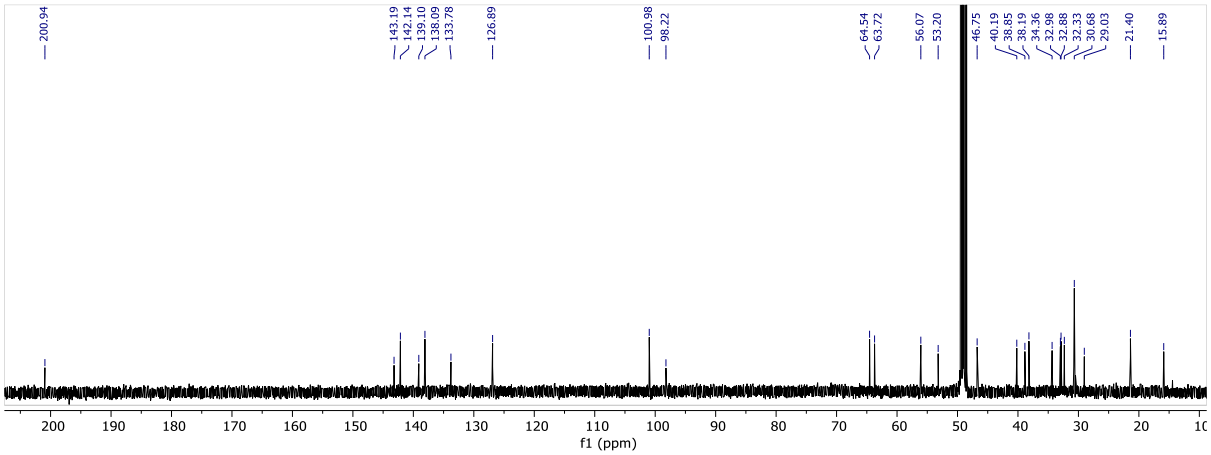


Figure 7. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of compound **1**.

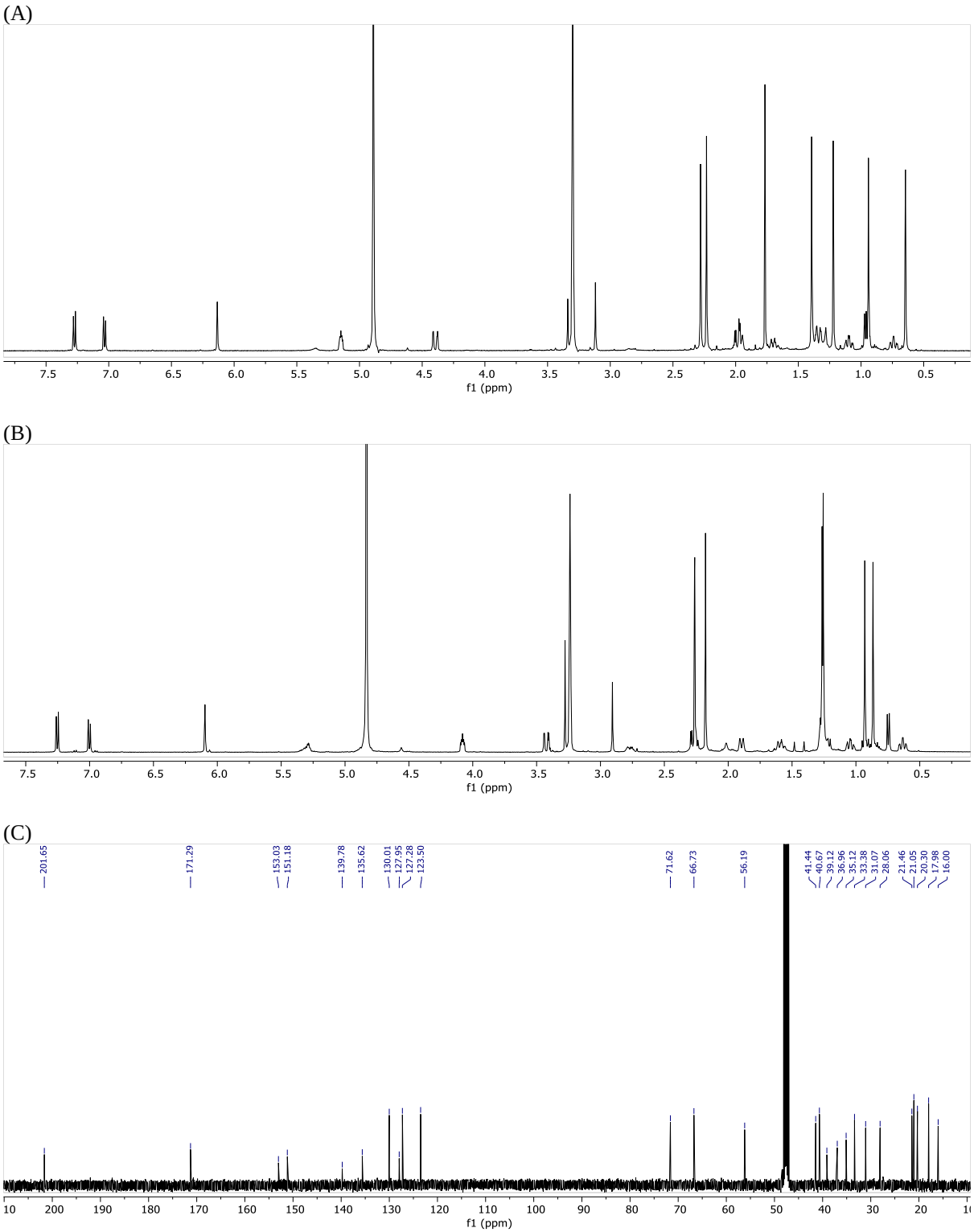


Figure 8. (A) ^{1}H NMR and (C) ^{13}C NMR spectra of compound **2** and (B) ^{1}H NMR spectrum of compound **3**.

Table 1. ^{1}H and ^{13}C NMR spectral data for compound **1** in CD_3OD recorded at 500 MHz and 125 MHz

		1	**Gombaspiroketal A** *	**Gombaspiroketal B** *
No.	δ_C	δ_H	δ_H	δ_H
1	64.5	4.53, dd(5.4, 3.4)	4.52, dd(5.4, 3.5)	4.65, br d(3.7)
2	142.1	6.72, dq (5.4, 1.4)	6.80, dq(5.4, 1.3)	6.79, br d(3.7)
3	139.1			
4	15.9	1.80, br s	1.81, s	1.75, br s
5	200.9			
6a	38.9	2.55, dd(16.4, 3.9)	2.57, dd(16.0, 4.3)	2.71, dd(16.0, 7.7)
6b		2.37, dd(16.4, 13.9)	2.39, dd(16.0, 14.0)	2.63. dd(16.0, 5.1)
7	34.4	2.56, m	2.58, ddd(14.0, 4.3, 3.5)	2.88, m
8	143.2			
9a	63.7	4.08, d(14.4)	4.06, s	4.07, d(13.8)
9b		4.04, d(14.4)		3.99, d(13.8)
10	126.9	5.58, br s	5.59, s	5.56, s
11	98.2			
12a	46.8	1.72, d(14.2)	1.53, d(13.1)	1.82, d(14.2)
12b		1.56, d(14.2)	1.49, d(13.1)	1.28, d(14.2)
13	32.3			
14	29.0	1.12, s	1.16, s	0.90, s
15a	40.2	1.78, dd(13.7, 5.6)	1.56, dd(13.2, 2.1)	1.80, dd(13.4, 2.0)
15b		1.70, dd(13.7, 5.1)	1.30, dd(13.2, 10.0)	1.12, dd(13.4, 10.1)
16	101.0	4.83, dd(5.6, 5.1)	4.92, dd(10.0, 2.1)	5.05, dd(10.1, 2.1)
17a	53.2	1.92, d(13.2)	1.83, s	2.51, d(13.5)
17b		1.89, d(13.2)		2.16, d(13.5)
18	133.8			
19	138.1	5.13, s	5.12, s	5.18, s
20	32.9			
21	38.2	1.40, m	1.38, m	1.41, m
22	21.4	1.60, m	1.61, m; 1.58, m	1.61, m; 1.58, m
23	33.0	1.93, t(5.8)	1.91, dd(11.9, 5.9)	1.97, dd(12.7, 6.3)
24	30.7	0.96, s	0.95, s	0.96, s
25	30.7	0.96, s	0.94, s	0.95, s
26	56.1	3.46, s	3.47, s	3.44, s

*Data from reference

Table 2. ^{1}H and ^{13}C NMR spectral data for compounds 2 and 3 in CD_3OD recorded at 500 MHz and 125 MHz

	2		**3**	
No.	δ_C	δ_H	δ_C	δ_H
1a	42.1	1.96, m	42.6	1.96, ddd(12.5, 3.1, 3.1)
1b		0.74, td(13.0, 3.4)		0.68, ddd(12.5, 12.5, 3.2)
2a	19.4	1.70, dt(13.6, 3.2)	19.4	1.65, m
2b		1.37, m		1.65, m
3a	42.9	1.34, m	43.7	1.31, m
3b		1.10, td(13.7, 3.9)		1.09, ddd(13.0, 13.0, 3.2)
4	34.8		34.7	
5	57.9	0.96, d(6.6)	59.9	0.80, d(8.0)
6	73.1	5.15, m	69.1	4.14, ddd(8.0, 4.7, 3.1)
7a	36.5	4.39, dd(16.6, 2.5)	42.8	3.48, dd(16.6, 3.1)
7b		1.99, dd(16.6, 4.2)		2.32, dd(16.6, 4.7)
8	40.6		40.0	
9	78.2	3.12, s	69.9	2.96, s
10	38.4		38.5	
11	203.1		203.8	
12	131.4	6.13, s	132.3	6.16, s
13	152.6		151.4	
14	137.0		136.3	
15	124.9	7.27, d(8.1)	126.3	7.31, d(8.0)
16	128.7	7.03, d(8.1)	128.8	7.06, d(8.0)
17	129.4		134.1	
18	154.4		152.6	
19	141.2		142.8	
20	32.5	0.65, s	34.4	0.91, s
21	22.5	0.94, s	22.9	0.99, s
22	26.0	1.22, s	26.0	1.31, s
23	19.7	1.39, s	19.7	1.33, s
24	29.3	2.28, s	29.3	2.32, s
25	17.4	2.23, s	18.0	2.24, s
C=O	172.7			
CH_3	21.7	1.77, s		

Table 3. DP4+ probability for two isolmers (8*R* and 8*S*)

	(8*R*) isomer	(8*S*) isomer
DP4+ (^{1}H data)	99.99%	0.01%
DP4+ (^{13}C data)	100%	0%
DP4+ (all Data)	100%	0%

Acknowledgement

This work was supported by the research invigoration program of 2020 Gyeongsang National University.

References

1. A. R. Carroll, B. R. Copp, R. A. Davis, P. A. Keyzers, M. R. Prinsep, *Nat. Prod. Rep*., **37**, 175 (2020)
2. L. M. Stephenson, D. E. Smith, S. P. Current, *J. Org. Chem.*, **37**, 4171 (1982)
3. M. S. Buchanan, A. Edser, G. King, J. Whitmore, R. J. Quinn, *J. Nat. Prod.*, **64**, 300 (2001)
4. L. V. Manes, S. Naylor, P. Crews, G. J. Bakus, *J. Org. Chem*., **40**, 284 (1985)
5. J.-R. Rho, B. S. Hwang, C. J. Sim, S. Joung, H.-Y. Lee, H.-J. Kim, *Org. Lett.*, **11**, 5590 (2009)
6. J.-R. Rho, B. S. Hwang, S. Joung, M. R. Byun, J. -H. Hong, H.-Y. Lee, *Org. Lett.*, **13**, 884 (2011)
7. J.-K. Woo, C.-K. Kim, S.-H. Kim, H. Kim, D.-C. Oh, K-.B. Oh, J. Shin, *Org. Lett.*, **16**, 2826 (2014)
8. W. Wang, Y. Lee, T. G. Lee, B. Mun, A. G. Giri, J. Lee, H. Kim, D. H. Hahn, I. Yang, J. Chin, H. Choi, S.-J. Nam, H. Kang, *Org. Lett.*, **14**, 4486 (2012)
9. J.-K. Woo, C.-K. Kim, C.-H. Ahn, D.-C. Oh, K.-B. Oh, J. Shin, *J. Mat. Prod*., **78**, 218 (2015)
10. W. Wang, B. Mun, Y. Lee, M. V. Reddy, Y. Park, J. Lee, H. Kim, D. Hahn, J. Chin, M. Ekins, S.-J. Nam, H. Kang, *J. Nat. Prod.*, **76**, 170 (2013)
11. R. Forestieri, C. E. Merchant, N. J. de Voogd, T. Matainaho, T. J. Kieffer, R. J. Anderson, *Org. Lett*., **11**, 5166 (2010)
12. W. Hehre, P. Klunzinger, B. Deppmeier, A. Driessen, N. Uchida, M. Hashimoto, E. Fukushi, Y. Takata, *J. Nat. Prod.*, **82**, 2299 (2019)
13. N. Grimblat, M. M. Zanardi, A. M. Sarotti, *J. Org. Chem*., **80**, 12526 (2015)