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Gombaspiroketals A–C, Sesterterpenes from the Sponge *Clathria gombawuiensis*

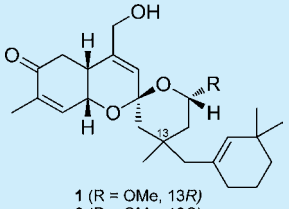
Jung-Kyun Woo,[†] Chang-Kwon Kim,[†] Seong-Hwan Kim,[†] Heegyu Kim,[‡] Dong-Chan Oh,[†] Ki-Bong Oh,*[,‡] and Jongheon Shin*[,†]

[†]Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-742, Korea

[‡]Department of Agricultural Biotechnology, College of Agriculture & Life Science, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-921, Korea

Supporting Information

ABSTRACT: Gombaspiroketals A–C (**1**–**3**), tetracyclic sesterterpenes of a novel skeletal class, were isolated from the Korean marine sponge *Clathria gombawuiensis*. On the basis of the combined spectroscopic analyses, the structures of these compounds were determined to be highly rearranged sesterterpene spiroketal methoxyacetals (**1** and **2**) and a corresponding hemiacetal (**3**). The relative and absolute configurations were assigned by NOESY analysis and ECD calculations, respectively. These compounds exhibited moderate cytotoxicities and antibacterial activities.



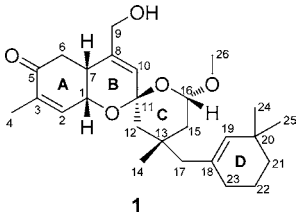
1 (R = OMe, 13*R*)
2 (R = OMe, 13*S*)
3 (R = OH, 13*R*)

Gombaspiroketal A-C (**1**-**3**)

Frequent encounters and structural diversity of sesterterpenes are the most distinctive features that distinguish sponge metabolites from those of terrestrial and other marine organisms.[1] Several compounds, including halisulfates, scalaranes, and suvanines, possess notable carbon skeletons, and exhibit significant antimicrobial, cytotoxic, and/or enzyme inhibitory activities.[1−4]

During the course of our search for bioactive compounds from Korean water sponges, we recently reported gombamide A, a modified cyclic thiopeptide from the organic extract of *Clathria gombawuiensis*.[5] In addition, the ^{1}H NMR and bioactivity test results showed the presence of terpenoids in the moderately polar chromatographic fraction of the same extract. On the basis of spectroscopic analyses, we report here the structure determination of gombaspiroketals A–C (**1**–**3**), which are sesterterpenes of a new skeletal class. These compounds exhibited moderate cytotoxicities and antibacterial activities as well as weak inhibition of Na^+/K^+–ATPase and isocitrate lyase (ICL).

The molecular formula of gombaspiroketal A (**1**) was deduced to be $C_{26}H_{38}O_5$ from HRFABMS analysis. The sesterterpene nature of this compound was apparent from its ^{13}C NMR data, in which signals of 25 carbons and a methoxy carbon at δ_C 56.6 were clearly observed. A conspicuous signal was that of a carbonyl carbon at δ_C 200.7, which was interpreted as a conjugated ketone by the absorption band at 1675 cm^{-1} and the absorption maximum at 228 nm in the IR and UV spectra, respectively. Additionally, six olefinic carbons (3 × C and 3 × CH) at δ_C 144–126 and four oxygen-bearing carbons (1 × C, 2 × CH and 1 × CH_2) at δ_C 100–63 were observed (Table 1). Among the latter carbons, the downfield shifts and multiplicities of two carbons at δ_C 99.3 (C) and 98.2 (CH) were indicative of a ketal and an acetal, respectively, which suggests the highly oxygenated nature of this compound. The remaining 14 carbons were also categorized by their multiplicities (2 × C, 1 × CH, 7 × CH_2, and 4 × CH_3). These ^{13}C NMR data, in conjunction with the eight degrees of unsaturation in the molecular formula, revealed that **1** was a tetracyclic compound.



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Given this information, the structure of compound **1** was determined through a combination of 2-D NMR analyses (Figure 1). Proton COSY and HSQC data revealed the presence of three spin systems. Among these, a methylene and three methine protons, including the olefinic proton at δ_H 6.80, showed several carbon–proton long-range correlations with the carbonyl and a quaternary olefinic carbon at δ_C 200.7 and 139.4, respectively, in the HMBC data.

Additionally, correlations between the vinyl methyl protons at δ_H 1.81 and carbonyl and olefinic carbons were observed, indicating a 2-methylcyclohexenone moiety (C-1–C-7). This partial structure was extended to include an oxygenated vinyl group (C-8–C-10) by the HMBC correlations between the H-

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Table 1. ^{1}H and ^{13}C NMR Assignments for Gombaspiroketals A–C (1–3) in MeOH-d_4[a]

	1		2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	64.3	4.52, dd (5.4, 3.5)	69.5	4.65, br d (3.7)	64.1	4.52, dd (5.1, 3.2)
2	141.7	6.80, dq (5.4, 1.3)	145.5	6.79, br d (3.7)	141.8	6.75, dq (5.1, 1.2)
3	139.4		137.4		139.3	
4	15.9	1.81, s	15.6	1.75, br s	15.9	1.81, s
5	200.7		200.1		200.8	
6a	38.8	2.57, dd (16.0, 4.3)	39.7	2.71, dd (16.0, 7.7)	38.8	2.57, dd (16.1, 4.2)
6b		2.39, dd (16.0, 14.0)		2.63, dd (16.0, 5.1)		2.39, dd (16.1, 13.9)
7	34.4	2.58, ddd (14.0, 4.3, 3.5)	35.8	2.88, m	34.4	2.55, ddd (13.9, 4.2, 3.2)
8	143.7		140.8		143.5	
9a	63.7	4.06, s, 2H	63.6	4.07, dd, (13.8)	63.7	4.05, s, 2H
9b				3.99, d, (13.8)		
10	126.2	5.59, s	127.2	5.56, s	126.4	5.58, s
11	99.3		99.9		99.5	
12a	46.0	1.53, d (13.1)	45.1	1.82, d (14.2)	45.8	1.51, d (13.8)
12b		1.49, d (13.1)		1.28, d (14.2)		1.47, d (13.8)
13	35.2		34.7		35.4	
14	25.9	1.16, s	31.2	0.90, s	25.7	1.15, s
15a	43.3	1.56, dd (13.2, 2.1)	42.3	1.80, dd (13.4, 2.0)	45.1	1.57, dd (13.0, 2.1)
15b		1.30, dd (13.2, 10.0)		1.12, dd (13.4, 10.1)		1.30, dd (13.0, 10.0)
16	98.2	4.92, dd (10.0, 2.1)	98.2	5.05, dd (10.1, 2.1)	90.4	5.24, dd (10.0, 2.1)
17a	55.4	1.83, s, 2H	47.1	2.51, d (13.5)	55.5	1.82, s, 2H
17b				2.16, d (13.5)		
18	133.0		134.4		133.1	
19	138.2	5.12, s	138.0	5.18, s	138.2	5.12, s
20	32.9		32.9		32.9	
21	38.2	1.38, m, 2H	38.2	1.41, m, 2H	38.2	1.39, m, 2H
22a	21.3	1.61, m	21.4	1.61, m	21.4	1.61, m
22b		1.58, m		1.58, m		1.58, m
23	33.0	1.91, dd, 2H (11.9, 5.9)	33.1	1.97, dd, 2H (12.7, 6.3)	33.0	1.92, dd, 2H (11.6, 5.3)
24	30.7	0.95, s	30.8	0.96, s	30.9	0.96, s
25	30.6	0.94, s	30.7	0.95, s	30.7	0.95, s
26	56.6	3.47, s	56.6	3.44, s		

[a] ^{1}H and ^{13}C NMR were measured at 600 and 150 MHz, respectively.

6, H-7 and oxymethylene protons at δ_H 4.06 (2 H, s) and the trisubstituted double bonded carbons at δ_C 143.7 and 126.2. Further extension of this moiety to include a ketal and a methylene was also established by several HMBC correlations between the H-10 olefinic proton at δ_H 5.59 and carbons at δ_C 99.3 (C-11) and 46.0 (C-12).

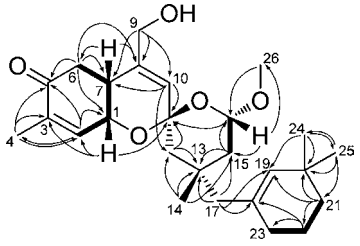

Figure 1. Key correlations of COSY (bold line) and HMBC (arrow) experiments for compound **1**.

Meanwhile, the COSY data also revealed the presence of a linear trimethylene motif (C-21–C-23) whose connection with a geminal dimethyl group was established by several HMBC correlations between the protons and carbons in these groups (Figure 1). Additional HMBC correlations of H-21 and H-22 with the olefinic carbons at δ_C 133.0 and 138.2 indicated a dimethylcyclohexene moiety (C-18–C-25), which was extended to include a methylene group by correlations with the protons at δ_H 1.83 (2 H, s). The connection between this cyclohexene and the earlier established ketone-containing moiety was also indicated by the HMBC data, in which key correlations were observed between an isolated methyl proton at δ_H 1.16 (3 H, s, H-14) with the C-12, C-15 and a quaternary carbon at δ_C 35.2 (C-13).

The remaining proton spin system defined by the COSY analysis was an isolated system consisting of an acetal proton at δ_H 4.92 (1 H, dd, J = 10.0, 2.1 Hz) and methylene protons at 1.56 (1 H, dd, J = 13.2, 2.1 Hz) and 1.30 (1 H, dd, J = 13.2, 10.0 Hz). The connection of this moiety at the C-13 quaternary carbon was deduced from the HMBC correlation of the C-15 methylene carbon at δ_C 43.3 with the H-14. The presence of a methoxy group at the C-16 acetal center was also shown by mutual HMBC correlations between these groups. Similarly, the linkage between the C-16 acetal and C-11 ketal, which created a tetrahydropyran, was demonstrated by the correlation at H-16/C-11 (Figure 1).

The molecular formula of **1** had one remaining degree of unsaturation, which resulted from an oxycyclic moiety involving C-1, C-9, and C-11. With several HMBC correlations for these oxygenated centers, a 3-D model study confirmed a linkage between C-1 and C-11, thus defining a dihydropyran moiety,

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Table 2. Results of Bioactivity Tests[a]

	LC_{50} (μM)		MIC (μg/mL)						IC_{50} (μM)	
			Gram(+) bacteria			Gram(−) bacteria				
compound	A549	K562	A	B	C	D	E	F	Na^+/K^+−ATPase	ICL
1	1.45	0.77	25.0	6.25	12.5	12.5	6.25	>100	10.9	57.4
2	2.02	1.87	>100	ND[c]	ND[c]	ND[c]	50.0	ND[c]	77.9	>100
3	0.85	4.65	25.0	6.25	25.0	25.0	12.5	>100	18.7	66.3
doxorubicin	0.79	0.70								
ampicillin			0.39	0.39	0.39	0.78	0.39	3.12		
ouabain									3.37	
3-NP[b]										2.54

[a]A: *Staphylococcus aureus* (ATCC 6538p), B: *Bacillus subtilis* (ATCC 6633), C: *Kocuria rhizophila* (NBRC 12708), D: *Salmonella enterica* (ATCC 14028), E: *Proteus hauseri* (NBRC 3851), F: *Escherichia coli* (ATCC 35270). [b]3-Nitropropionic acid. [c]ND: not determined.

and the rigid vinyl methylene at C-11 was attached to a hydroxyl group. Thus, the planar structure of gombaspiroketal A (**1**) was determined to be a tetracyclic sesterterpene of a new skeletal class. A literature study revealed that the bicyclic ketal moiety of **1** had a resemblance to phorbaketals and alotaketals from the sponges *Phorbas* sp., *Monanchora* sp., and *Hamigera* sp.[6−8] However, to the best of our knowledge, the highly rearranged carbon skeleton of the C-13−C-25 portion of this compound is unprecedented.

Gombaspiroketal A (**1**) possesses five asymmetric carbon centers: C-1, C-7, C-11, C-13, and C-16. The relative configurations at these centers were determined by proton−proton coupling constants and NOESY analysis (Figure 2). The small vicinal coupling constant (J = 3.5 Hz) and strong NOESY cross-peaks at H-1/H-6a (δ_H 2.57), H-1/H-7, H-6a/H-7, and H-6b (δ_H 2.39)/H-9 revealed a *syn* orientation for H-1 and H-7 and a *cis* A/B ring junction. Similarly, a strong cross peak at H-14/H-16, supported by those at H-12a (δ_H 1.53)/H-14, H-14/H-15a (δ_H 1.56), and H-15b (δ_H 1.30)/H-17, revealed a 1,3-diaxial orientation of these protons on the C-ring. Finally, based on a series of cross-peaks at H-1/H-16, H-1/H-26, and H-10/H-12b (δ_H 1.49), the spiroketal was found to have the C-12 and 11−16 ether oxygen at α and β orientation to the B ring, respectively. Thus, the relative configurations were assigned as 1*R**, 7*R**, 11*R**, 13*S**, and 16*S** for **1**.

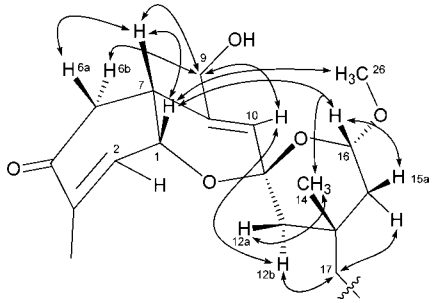

Figure 2. Key correlations of NOESY (arrow) experiment for compound **1**.

The absolute configuration of **1** was determined by comparing its CD spectrum and the ECD spectra of two possible enantiomers, which were calculated using time-dependent density-functional theory (TD-DFT) at the B3LYP/def2-TZVPP//B3LYP/def-SV(P) level for all atoms. As shown in Figure 3, the ECD spectra of (1*R*,7*R*,11*R*,13*S*,16*S*)-**1** was in accordance with the experimental CD spectra of **1**. The ECD spectra and the CD spectra of **1** displayed diagnostic negative and positive cotton effects at around 245 and 340 nm, respectively. In addition, this interpretation agreed with the absolute configurations of the cyclohexenone and dihydropyran moieties obtained by X-ray diffraction analysis of ansellone A from the sponge *Phorbas* sp.[9] As a result, gombaspiroketal A (**1**) was determined to be a sesterterpene spiroketal-methoxyacetal with a novel skeletal structure.

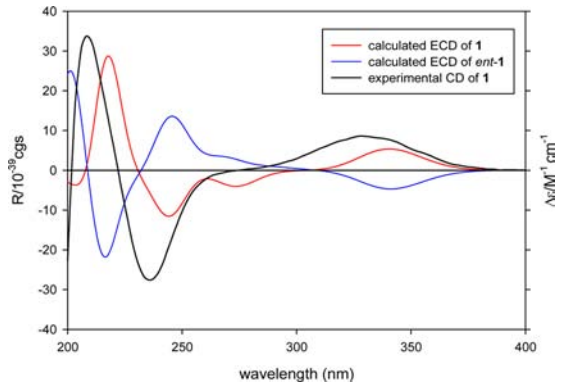

Figure 3. Experimental CD spectra of 1 (black), calculated ECD spectra of 1 (red), and *ent*-1 (blue).

The molecular formula of gombaspiroketal B (**2**) was deduced to be $C_{26}H_{38}O_5$, which is identical to that of **1**, by HRFABMS analysis. The ^{13}C and ^1H NMR data of this compound were also similar to those of **1**. Detailed examination of these NMR data revealed that the signals of the protons and carbons at C-12, C-14, and C-15, which are adjacent to the C-13 asymmetric center, had shifted significantly, while the other asymmetric centers shifted negligibly (Table 1). As the combined 2-D NMR analyses of **2** showed similar proton−proton and carbon−proton correlations as **1**, these NMR spectroscopic changes as well as the opposite sign of the specific rotation indicated the opposite configuration at C-13 compared to **1**. Furthermore, the CD curves of **2** showed variations from **1** and **3**. This interpretation was supported by the NOESY data, as cross-peaks were found at H-14/H-12b (δ_H 1.28), H-14/H-15b (δ_H 1.12), H-17/H-15a (δ_H 1.80), H-17/H-16, and H-17/H-12a (δ_H 1.82). The reversed C-13 configuration based on NOE data also coincided with the downfield shift of the C-14 methyl carbon at δ_C 31.2, which had an equatorial orientation on the C ring.[10] Thus, the structure of

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gombaspiroketal B (**2**) was determined to be the 13*R* diastereomer of **1**.

Gombaspiroketal C (**3**) was isolated as an amorphous solid and was found to have a molecular formula of $C_{25}H_{36}O_5$ by HRFABMS analysis. The ^1H and ^{13}C NMR data of this compound were very similar to those of **1**; however, the signals for the C-26 methoxy group were not present, and the signals of the neighboring C-16 acetal center had substantially shifted. Thus, the 16-methoxy acetal was replaced by the corresponding hemiacetal, which was confirmed by combined 2-D NMR analyses. Therefore, gombaspiroketal C (**3**) was determined to be a hemiacetal derivative of **1**.

The most noticeable difference in this skeleton compared to cyclic sesterterpenes from common biosynthetic pathways is the migration of the dimethylcyclohexenyl methylene moiety (C-17−C-25) from C-16 to C-13. This 1,3-migration may induce the attachment of an oxygen at C-16 to accommodate carbocation that may also result in the formation of a spiroketal moiety at C-11. To the best of our knowledge, this type of 1,3-migration is unprecedented among sponge sesterterpenes.

Sesterterpenes derived from marine sponges exhibit diverse and potent bioactivities. From our experiments, compounds **1**−**3** showed cytotoxicities in the K562 and A549 cell-lines that were comparable to those of doxorubicin (Table 2). Compounds **1** and **3** also displayed moderate antibacterial activities while their diastereomer **2** was virtually inactive. The same trend was also observed for inhibition of the enzymes Na^+/K^+−ATPase and isocitrate lyase (ICL), which can be attributed to the 3-dimensional structure of spiroketal.

■ ASSOCIATED CONTENT

Supporting Information

^1H, ^{13}C NMR and 2D NMR spectra of compounds **1**−**3** as well as ECD and CD data of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: ohkibong@snu.ac.kr.
*E-mail: shinj@snu.ac.kr.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160−258 and earlier reports in the series.
(2) Kernan, M. R.; Faulkner, D. J. *J. Org. Chem.* **1988**, *53*, 4574−4578.
(3) Stephenson, L. M.; Smith, D. E.; Current, S. P. *J. Org. Chem.* **1982**, *47*, 4171−4173.
(4) Manes, L. V.; Naylor, S.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1985**, *40*, 284−286.
(5) Woo, J.-K.; Jeon, J.-e.; Kim, C.-K.; Sim, C. J.; Oh, D.-C.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2013**, *76*, 1380−1383.
(6) Rho, J.-R.; Hwang, B. S.; Sim, C. J.; Joung, S.; Lee, H.-Y.; Kim, H.-J. *Org. Lett.* **2009**, *11*, 5590−5593.
(7) Wang, W.; Mun, B.; Lee, Y.; Reddy, M. V.; Park, Y.; Lee, J.; Kim, H.; Hahn, D.; Chin, J.; Ekins, M.; Nam, S.-J.; Kang, H. *J. Nat. Prod.* **2013**, *76*, 170−177.
(8) Forestieri, R.; Merchant, C. E.; de Voogd, N. J.; Matainaho, T.; Kieffer, T. J.; Andersen, R. J. *Org. Lett.* **2009**, *11*, 5166−5169.
(9) Daoust, J.; Fontana, A.; Merchant, C. E.; de Voogd, N. J.; Patrick, B. O.; Kieffer, T. J.; Andersen, R. J. *Org. Lett.* **2010**, *12*, 3208−3211.
(10) Crews, P.; Bescansa, P. *J. Nat. Prod.* **1986**, *49*, 1041−1052.