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BRIEF COMMUNICATION

Aspermicrones A-C, novel dibenzospiroketals from the seaweed-derived endophytic fungus *Aspergillus micronesiensis*

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Abstract

Chemical investigation of the *Kappaphycus alvarezii*-derived endophytic fungus *Aspergillus micronesiensis* lead to the isolation of three novel dibenzospiroketals, aspermicrones A-C (**1**-**3**). Their chemical structures were determined by extensive analysis of HR-ESI-MS and NMR spectral data. The absolute configurations of them were determined by experimental and TD-DFT theoretical calculated circular dichroism spectra. Compound **2** exhibited selective cytotoxic effect toward HepG2 cell line ($IC_{50} = 9.9$ μM). Additionally, both of compounds **2** and **3** displayed anti-microbial activity against *Staphylococcus aureus* (MIC = 123.2 μM for each compound). Compound **1** was inactivity in both cytotoxic and anti-microbial assays.

Seaweed is one of the large and diverse ecosystems, playing an essential role in marine environment. It is mainly involved in global primary production, providing food and shelter for variety of organisms [1]. Additionally, the seaweed surface provides a suitable substratum for the settlement of microorganisms and also secretes various organic substances. These are excellent nutrients for microbial and the marine-derived endophytic fungi which are promising sources of novel interesting bioactive natural products with great pharmacological and agrochemical potentials [2, 3]. *Kappaphycus alvarezii* is an edible red seaweed imported to Vietnam in 1993. Recently, it is grown popular at the sea areas in the Central Vietnam. The *K. alvarezii* is a living environment of numerous endophytic microorganisms as well as the fungus *Aspergillus micronesiensis*. In our ongoing search for structurally novel and bioactive metabolites from marine-derived microorganism, we report herein the isolation, structural elucidation, and biological activity results of three novel dibenzospiroketals **1**–**3** from the culture broth of *K. alvarezii*-derived endophytic fungus *A. micronesiensis*.

Compound **1** was obtained as a yellowish amorphous powder. The molecular formula of **1** was determined as $C_{19}H_{18}O_{10}$ by HR-ESI-MS with the exhibition of quasi-molecular ion peak at *m/z* 405.0833 $[M-H]^-$ (Cacld. for $C_{19}H_{17}O_{10}$, 405.0822). The IR spectrum of **1** exhibited absorption band at 3360 and 1635 cm^{-1}, characteristic for stretching vibration of hydroxy and carbonyl groups, respectively. The ^1H NMR spectrum of **1** exhibited two methyl groups [δ_H 2.13 and 2.03 (3H, each, s)], one methoxy group [δ_H 3.60 (3H, s)], one oxygenated methine [δ_H 5.77 (1H, s)], and one oxygenated methylene [δ_H 5.06 and 5.24 (each, 1H, d, $J = 15.5$ Hz). The ^{13}C-NMR spectra of **1** exhibited the signals of 19 carbon atoms, including two methyl groups (δ_C 10.2 and 12.0), one methoxy group (δ_C 55.9), one oxygenated methylene (δ_C 75.0), one oxygenated methine (δ_C 97.6), and 14 non-protonated carbons confirming by HSQC spectra. Among non-protonated carbons, one ketone group was identified at δ_C 195.4. 12 carbon

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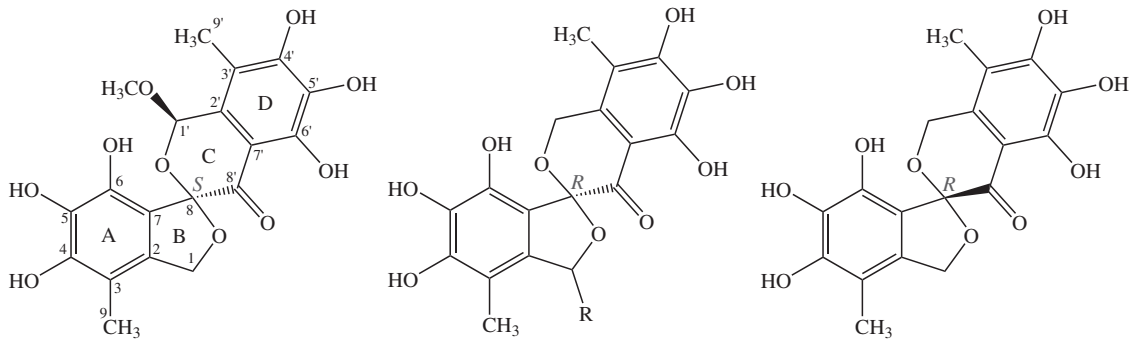

1 **2** R = β-OCH_3 **3** R = α-OCH_3 **4**

Fig. 1 Chemical structures of compounds **1**–**4**

Table 1 NMR data of compounds 1–4

No.	1		2		3		4[#]	
	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	δ_C	δ_H[a,c] (mult., *J* in Hz)
1	75.0, CH_2	5.06 (d, 15.5) 5.24 (d, 15.5)	108.3, CH	6.16 (s)	108.8, CH	6.42 (s)	72.6, CH_2	5.05 (d, 12.0) 4.83 (d, 12.0)
2	133.1, C	–	130.0, C	–	129.8, C	–	131.0, C	–
3	109.7, C	–	112.6, C	–	112.6, C	–	107.6, C	–
4	147.8, C	–	147.8, C	–	147.8, C	–	146.3, C	–
5	132.9, C	–	136.2, C	–	136.2, C	–	132.6, C	–
6	140.5, C	–	140.1, C	–	140.3, C	–	139.7, C	–
7	108.5, C	–	118.6, C	–	118.9, C	–	115.2, C	–
8	109.7, C	–	106.9, C	–	107.1, C	–	107.5, C	–
9	12.0, CH_3	2.03 (s)	10.9, CH_3	2.14 (s)	10.9, CH_3	2.15 (s)	11.9, CH_3	1.98 (s)
1′	97.6, CH	5.77 (s)	62.4, CH_2	4.95 (d, 15.5) 5.18 (d, 15.5)	62.4, CH_2	4.89 (d, 15.5) 5.20 (d, 15.5)	60.4, CH_2	5.00 (d, 15.6) 4.81 (d, 15.6)
2′	131.1, C	–	132.9, C	–	132.9, C	–	131.4, C	–
3′	115.8, C	–	112.5, C	–	112.3, C	–	110.0, C	–
4′	153.8, C	–	153.7, C	–	153.5, C	–	152.5, C	–
5′	134.2, C	–	131.6, C	–	131.6, C	–	130.2, C	–
6′	150.8, C	–	151.3, C	–	151.4, C	–	150.0, C	–
7′	107.3, C	–	107.9, C	–	107.8, C	–	106.2, C	–
8′	195.4, C	–	194.1, C	–	193.5, C	–	192.2, C	–
9′	10.2, CH_3	2.13 (s)	9.9, CH_3	2.06 (s)	9.9, CH_3	2.06 (s)	9.7, CH_3	1.95 (s)
OCH_3	55.9, CH_3	3.60 (s)	53.3, CH_3	3.37 (s)	52.4, CH_3	3.20 (s)		

Measured in [a]CD_3OD, [b]125 MHz, [c]500 MHz, [#]Measured in DMSO-d_6 and previously reported [4]

signals in olefinic chemical shift region corresponded to two benzene rings. An acetal carbon was revealed at δ_C 109.7. The NMR data of **1** were close similarity to those of eleganketal A (**4**), a dibenzospiroketal isolated from the fungus *Spicaria elegan* [4]. The difference between them was the appearance of an oxygenated methine (δ_C 97.6) and a methoxy group (δ_C 55.9) in **1** instead of the oxygenated methylene (δ_C 60.4) in **4** (Fig. 1 and Table 1). In the HSQC spectrum, protons at δ_H 2.13, 2.03, 5.77, 3.60 had cross peaks with carbons at δ_C 10.2, 12.0, 97.6, and 55.9, respectively; protons at δ_H 5.06 and 5.24 had cross peaks with carbon at δ_C 75.0. The HMBC spectrum exhibited the HMBC correlations from H_3-9 (δ_H 2.03) to C-2 (133.1)/C-3 (109.7)/C-4 (147.8), from H_2-1 (δ_H 5.06 and 5.24) to C-2/C-

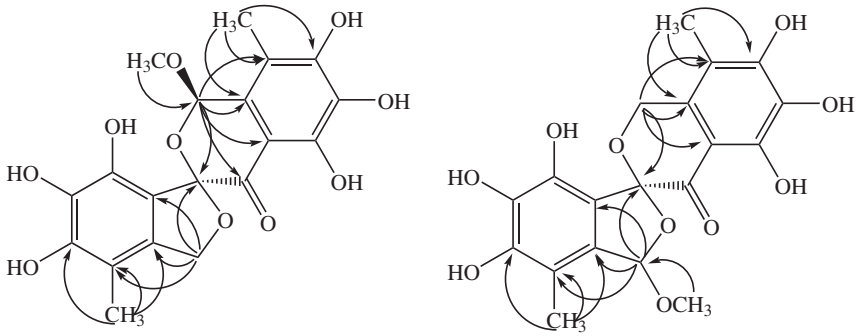

Fig. 2 Key HMBC correlations (H → C) of compounds **1**–**3**

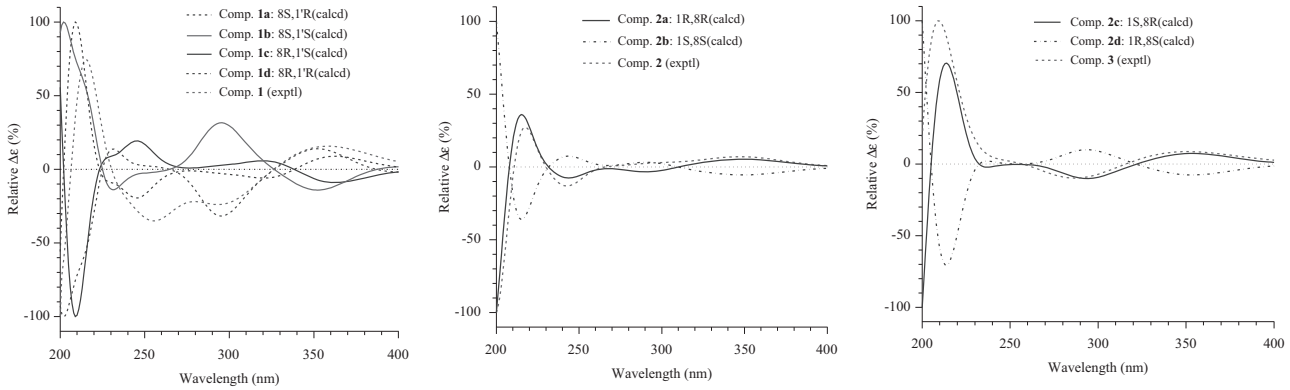

Fig. 3 Experimental ECD spectra of compounds **1**–**3** and TD-DFT calculated ECD spectra of their possible stereoisomers

3/C-7 (δ_C 108.5)/C-8 (δ_C 109.7) confirming the structure of the isobenzofuran system (rings A and B) (Fig. 2). Similarly, the HMBC correlations from H_3-9′ (δ_H 2.13) to C-2′ (δ_C 131.1)/C-3′ (δ_C 115.8)/C-4′ (δ_C 153.8), from H-1′ (δ_H 5.77) to C-2′/C-3′/C-7′ (δ_C 107.3), and four bonding HMBC coupled of H-1′/C-8′ (δ_C 195.4) confirming the structure of the isochroman-4-one system (rings C and D) [4]. The HMBC correlations between H_2-1 (δ_H 5.06, 5.24)/H-1′ (δ_H 5.77) and C-8 (δ_C 109.7) suggested for the connection of isobenzofuran with isochroman-4-one system via the acetal carbon C-8. The methoxy group was attached to C-1′ confirming by the HMBC correlation from methoxy protons (δ_H 3.60) to C-1′ (δ_C 97.6). Due to containing two chiral carbon atoms (C-8 and C-1′), absolute configuration of **1** was attempted to study by ECD spectral analysis. Four possible stereoisomers of **1** including **1a** (8*S*,1′*R*), **1b** (8*S*,1′*S*), **1c** (8*R*,1′*S*), **1d** (8*R*,1′*R*) were subjected to TD-DFT calculation their theoretical ECD spectra [5–7]. The experimental ECD spectrum of **1** showed negative Cotton effects at wavelengths of 255 nm (Δε: −8.63, −34.9% in relative Δε)/293 nm (Δε: −5.89, −23.8 % in relative Δε) and positive Cotton effects at wavelengths of 215 nm (Δε: + 18.45, + 74.5% in relative Δε)/358 nm (Δε: + 3.89, + 15.7% in relative Δε) which were well agreed with the theoretical calculated ECD spectrum of isomer **1a** (8*S*,1′*R*) (Fig. 3). Thus, the structure of compound **1** was established and named as aspermicrone A.

Compound **2** was obtained as a yellowish amorphous powder. The HR-ESI-MS of **2** exhibited a quasi-molecular ion peak at *m/z* 405.0839 $[M-H]^-$ (calcd for $C_{19}H_{17}O_{10}$, 405.0822), suggesting the molecular formula of **2** to be $C_{19}H_{18}O_{10}$. The NMR spectral data of **2** were very similar to those of **1** indicating that these two compounds have the same dibenzospiroketal skeleton structure (Fig. 1). Major difference in the NMR data between compounds **1** and **2** were signals of oxygenated methine and oxygenated methylene groups. The shielded movement of oxygenated methylene carbon signal (δ_C 62.4 in **2** and δ_C 75.0 in **1**) suggested the assignment of oxygenated methylene at C-1′ as that reported in compound **4** [4]. Meanwhile, the deshielded movement of oxygenated methine carbon signal (δ_C 108.3 in **2** and δ_C 97.6 in **1**) expected for the presence of methoxy group at C-1. This deduction was further confirmed by HMBC correlation between methoxy protons (δ_H 3.37) and C-1 (δ_C 108.3). The both absolute configurations at C-1 and C-8 of compound **2** were determined to be *R* by comparison experimental ECD spectrum of **2** [217 nm (Δε: +8.85, +27.3% in relative Δε), 243 nm (Δε: −4.22, −13.0% in relative Δε), 347 nm (Δε: +2.31, +7.1% in relative Δε)] with those theoretical calculation ECD spectra

for its possible stereoisomers (**2a**-**2d**, Fig. 3). Consequently, the structure of **2** was unambiguously established and named as aspermicrone B.

Compound **3** was obtained as yellowish amorphous powder. The molecular formula of **3** was also deduced as $C_{19}H_{18}O_{10}$ by a quasi-molecular ion peak at *m/z* 405.0829 $[M\text{-}H]^-$ in the HR-ESI-MS (calcd for $C_{19}H_{17}O_{10}$, 405.0822). Interestingly, the NMR spectral data of **3** was identical to those of **2** except slight difference in signals of oxygenated methine, ketone, and methoxy group (Table 1). Particularly, the signals of oxygenated methine (C-1) exhibited deshielding trend from $\delta_{H\text{-}1}$ 6.16/$\delta_{C\text{-}1}$ 108.3 in compound **2** to $\delta_{H\text{-}1}$ 6.42/$\delta_{C\text{-}1}$ 108.8 in compound **3**. Meanwhile, the signals of ketone and methoxy groups displayed shielding trend from δ_C 194.1, δ_C 53.3, δ_H 3.37 in compound **2** to corresponding δ_C 193.5, δ_C 52.4, δ_H 3.20 in compound **3**. On the other hand, the HMBC correlations from methoxy at δ_C 3.20 to C-1 (δ_C 108.8), from H-1 (δ_H 6.42) to C-2/C-3/C-7/C-8, as well as from methyl protons at δ_H 2.15 to C-2/C-3/C-4 were observed, confirming the position of methoxy group at C-2 and methyl group at C-3. The HMBC interactions from H-9′ (δ_H 2.06) to C-2′ (δ_C 132.9)/C-3′ (δ_C 112.3)/C-4′ (δ_C 153.5), from H-1′ (δ_H 4.89, 5.20) to C-8 (δ_C 106.9)/C-2′/C-3′/C-7′ (δ_C 107.9) further confirmed the position of methyl group at C-3′. Moreover, NOESY cross peaks from H-1 (δ_H 6.42) to H-9 (δ_H 2.15) and from H-1′ (δ_H 4.89, 5.20) to H-9′ (δ_H 2.06) further indicated the close in proximity of H-1/H_3-9 and H-1′/H-9′, confirming location of methyl groups at C-3 and C-3′. From the above evidence, compound **3** was determined to be a stereoisomer of compound **2**. In the ECD spectrum of **3**, the negative Cotton effect at wavelength of 287 nm (Δε: −1.32, −9.7% in relative Δε) and the positive Cotton effect at 209 nm (Δε: +13.60, +100.0% in relative Δε)/351 nm (Δε: +1.19, +8.7% in relative Δε) which were well agreed with those TD-DFT calculated ECD spectrum of stereoisomer **2c** (Fig. 3). Thus, absolute configurations at C-1 and C-8 of compound **3** were determined to be *S* and *R*, respectively. Compound **3** was also a novel dibenzospiroketal and named as aspermicrone C.

Dibenzospiroketals sharing carbon backbone of compounds **1**-**3** are very rare in the nature. To the best of our knowledge, to date, only one dibenzospiroketal, named eleganketal A (**4**), was isolated from modified culture broth of the fungus *S. elegans* [4]. Compounds **1**–**3** were then evaluated their cytotoxicity against HepG2 and LU-1 cancer cell lines, and Vero normal cell line by SRB assay [8, 9]. Interestingly, compound **2** displayed selective cytotoxic effect against HepG2 cells ($IC_{50} = 9.9$ μM) and did not show cytotoxic activities toward either LU-1 cancer cell or Vero normal cells in our experiments ($IC_{50} > 50$ μM). Compounds **1** and **3** were inactivity ($IC_{50} > 50$ μM). Doxorubicin was used as a positive control with IC_{50} values of 0.53 and 0.57 μM against HepG2 and LU-1 cancer cell lines, respectively. Additionally, compounds **1**–**3** were also evaluated their anti-microbial activity against microorganisms, Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and yeast (*Candida albicans*), using dilution turbidimetric broth method as the standard protocols published by the Clinical and Laboratory Standard Institute [10]. Both of compounds **2** and **3** exhibited a MIC value of 123.2 μM toward Gram-positive *S. aureus*. But they did not inhibit the growth of others tested strains (MIC > 1000 μM). Streptomicin was used as positive control against *S. aureus* bacteria (MIC = 24.75 μM). Compound **1** did not show anti-microbial activity in our conditions (MIC > 1000 μM).

In conclusion, our results indicated that the fungus *A. micronesiensis* produced novel dibenzospiroketals (**1**–**3**). Compounds **2** exhibited selective cytotoxic effect toward HepG2 cell ($IC_{50} = 9.9$ μM). Additionally, among five tested strains, both of compound **2** and **3** displayed anti-microbial activity against *S. aureus* (MIC = 123.2 μM for each compound). The results warned that dibenzospiroketals such as compounds **2** and **3** would be potential anti-microbial agents. Compound **2** would be useful as a selective anti-cancer agent.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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