

Two new indole alkaloids, 2-(3,3-dimethylprop-1-ene)-costaclavine and 2-(3,3-dimethylprop-1-ene)-epicostaclavine, from the marine-derived fungus *Aspergillus fumigatus*

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Abstract Two new indole alkaloids, 2-(3,3-dimethylprop-1-ene)-costaclavine (**1**) and 2-(3,3-dimethylprop-1-ene)-epicostaclavine (**2**), together with the known compounds costaclavine (**3**), fumgaclavine A (**4**) and C (**5**), were isolated from the marine-derived fungus *Aspergillus fumigatus*. The planar structures of the two new compounds were elucidated on the basis of chemical and physicochemical evidence including MS, UV, IR and NMR spectra. Their stereochemistry was studied by NOESY, ^1H – ^1H coupling constant and CD spectra. The compounds **1**, **2**, **3** and **5** showed weak cytotoxicity against a mouse leukemia cell line (P388).

Keywords *Aspergillus fumigatus* · Indole alkaloids · 2-(3,3-Dimethylprop-1-ene)-costaclavine · 2-(3,3-Dimethylprop-1-ene)-epicostaclavine · Stereochemistry

Introduction

The marine environment is now recognized as an important resource of unusual and biologically active secondary metabolites [1]. Marine fungi are interesting sources of natural products. To avoid depletion of marine resources and to enable access to large quantities of interesting

compounds, there has been a particular interest in marine microorganisms which are cultivable [2]. During our study of the symbiotic relationship between zoanthus and its related microorganisms, a fungus was isolated from the inside of a body of zoanthus and identified as *Aspergillus fumigatus*. Even though this genus of fungus has been studied for over 50 years, it continues to provide new bioactive metabolites [3–9]. During our study on its secondary metabolites, two new cytotoxic indole alkaloids, 2-(3,3-dimethylprop-1-ene)-costaclavine (**1**) and 2-(3,3-dimethylprop-1-ene)-epicostaclavine (**2**), together with the known compounds costaclavine (**3**) [3], fumgaclavine A (**4**) [7] and C (**5**) [8] (Fig. 1), were isolated. Here, we report the isolation, structure elucidation, and cytotoxic activity of two new costaclavines.

2-(3,3-Dimethylprop-1-ene)-costaclavine (**1**) was isolated as a pale yellow oil. The molecular formula of **1** was established as $\text{C}_{21}\text{H}_{28}\text{N}_2$ (9 unsaturations) by HRFABMS and ^{13}C NMR. The IR absorption spectrum of **1** showed the characteristic bands of the amino group (3299 cm^{-1}) and indole (1673 cm^{-1}) functionalities. The ^{13}C NMR spectrum showed all the expected 21 signals, which could be assigned to four methyl groups including one methyl group attached to nitrogen ($\delta_{\text{H}} = 3.02\text{ ppm}$ and $\delta_{\text{C}} = 42.3\text{ ppm}$, respectively), four methylene groups with one olefinic methylene ($\delta_{\text{H}} = 5.12, 5.15\text{ ppm}$ and $\delta_{\text{C}} = 112.2\text{ ppm}$, respectively), and seven methine groups (Table 1). The planar structure of **1** was determined by detailed analyses of 2-D NMR spectra including COSY and HMBC. The ^1H – ^1H COSY spectrum of **1** showed the connectivity of carbons 4-5-10-9-8(-18)-7 and 12-13-14, and the COSY correlation between H-22 and H-23 was also detected. The analysis of HMBC data of **1** connected all carbons, assigning the skeletal structure (Fig. 2). Positions of amino groups were determined by HMBC correlations from the

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amino proton to C-2 and C-15, and from the *N*-bearing methyl ($\delta_C = 42.3$) to C-5 and C-7. The general features of UV, IR and NMR spectra of **1** closely resembled those of compound **3** [3]. The NMR signals at C-2 were changed

from *sp*²-methine [7.04, d, *J* = 1.4 Hz, 120.4 (C-2)] in **3** to *sp*²-quaternary carbon [137.9 (C-2)] in **1** and a 1,3-dimethylprop-1-ene group was connected to C-2. Detailed analyses of the ¹H and ¹³C NMR spectra of **1**, including the results from DEPT, HSQC and HMBC experiments, suggest that **1** is a 2-(1,3-dimethylprop-1-ene) derivative compound of **3**. On the basis of all the evidence, we elucidated that the structure of **1** is 2-(1,3-dimethylprop-1-ene)-6,8-dimethylergoline.

The stereochemistry for **1** was elucidated using NOESY, ¹H–¹H coupling constant and CD spectra. The NOESY data of **1** showed correlations between H_β-9 and H-12, and did not give a correlation between H_α-9 and H-12, thus it can be confirmed that H_β-9 and H_α-9 are on different sides in the compound **1**. At the same time, the NOESY signals between 18-Me and H-10 and H_β-9, between H_β-9 and H-10, and between H-5 and H-10, suggested that H-5,

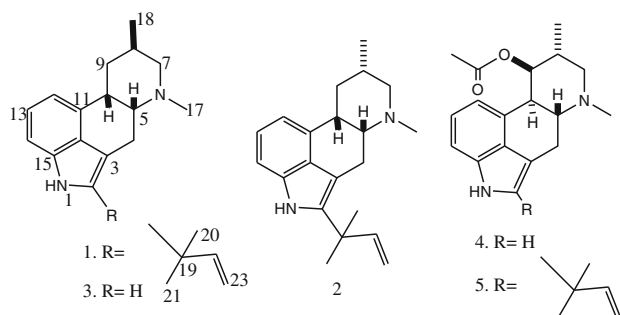


Fig. 1 The structures of compounds from the metabolite

Table 1 NMR spectroscopic data for compounds **1–3**

No.	2-(Dimethylprop-1-ene)-costaclavine ^a (1)		Costaclavine ^c (3)		2-(Dimethylprop-1-ene)-epicostaclavine ^a (2)	
	δ_C , mult	δ_H (<i>J</i> in Hz) ^b	δ_C , mult	δ_H (<i>J</i> in Hz) ^b	δ_C , mult	δ_H (<i>J</i> in Hz) ^b
1-NH		7.87, s		7.87, s		7.88, s
2	137.8 qC		120.4 CH	7.04, d (1.4)	137.9 qC	
3	102.9 qC		108.2 qC		102.9 qC	
4 α	25.1 CH ₂	3.30, dd (11.0, 14.6)	25.6 CH ₂	3.00, ddd (1.7, 11.5, 14.6)	25.5 CH ₂	3.21, dd (11.8, 7.8)
4 β		3.60, dd (3.6, 14.6)		3.70, dd (4.6, 14.2)		3.60, dd (5.8, 7.8)
5	69.0 CH	3.02, ddd (3.7, 5.4, 11.0)	69.9 CH	3.31, m	69.0 CH	3.01, ddd (4.8, 5.8, 11.8)
7 α	61.6 CH ₂	3.02, dd (3.7, 16.7)	62.9 CH ₂	3.44, d (3.9)	63.3 CH ₂	2.48, d (5.8)
7 β		3.78, d (16.4)		3.51, ddd (12.7, 1.7)		3.69, dd (5.8, 11.6)
8	26.9 CH	2.45, ddd (3.7, 5.5, 7.4)	28.2 CH	2.50, m	28.4 CH	2.48, ddd (5.9, 11.6, 13.7)
9 α	32.1 CH ₂	1.89, ddd (5.5, 11.8, 13.4)	32.8 CH ₂	1.91, ddd (4.9, 13.6, 13.6)	34.9 CH ₂	1.26, ddd (9.9, 11.6, 13.8)
9 β		2.59, dd (5.4, 13.4)		2.63, ddd (1.6, 2.2, 13.6)		2.80, dd (3.9, 11.8)
10	33.4 CH	3.69, ddd (5.4, 11.8, 11.8)	35.6 CH	3.39, dq (3.4, 9.3)	38.3 CH	3.50, ddd (4.1, 9.9, 11.6)
11	128.8 qC		130.5 qC		128.8 qC	
12	113.4 CH	6.87, d (6.9)	114.1 CH	6.90, d (7.4)	113.5 CH	6.89, d (6.9)
13	122.4 CH	7.12, dd (8.0, 6.9)	124.0 CH	7.11, dd (7.3, 8.3)	122.5 CH	7.13, dd (8.0, 7.0)
14	108.8 CH	7.16, d (8.1)	110.7 CH	7.21, dd (8.3, 0.8)	108.8 CH	7.12, d (7.8)
15	131.8 qC		135.2 qC		131.9 qC	
16	126.6 qC		126.9, qC		126.6 qC	
17	42.3 CH ₃	3.02, s	42.5 CH ₃	3.07, s	41.7 CH ₃	3.01, s
18	16.7 CH ₃	1.40, d (7.3)	17.4 CH ₃	1.15, d (6.6)	18.7 CH ₃	1.10, d (5.9)
19	39.0 qC				39.0 qC	
20	27.2 CH ₃	1.51, s			27.3 CH ₃	1.50, s
21	27.4 CH ₃	1.51, s			27.4 CH ₃	1.50, s
22	145.1 CH	6.06, dd (10.9, 17.8)			145.2 CH	6.06, dd (10.6, 16.5)
23	112.2 CH ₂	5.12, d (17.8) 5.15, d (10.8)			112.3 CH ₂	5.09, d (16.5) 5.13, d (10.6)

^a ¹H and ¹³C NMR recorded in CDCl₃ at 400 and 100 MHz, respectively

^b The coupling constants were calculated by ¹H NMR and DQF-COSY Spectra recorded in CDCl₃ at 500 MHz

^c ¹H and ¹³C NMR recorded in MeOD-*d*₃ at 400 and 100 MHz, respectively

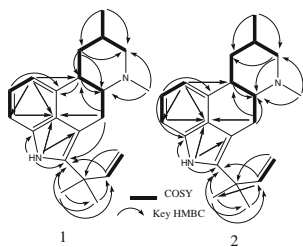


Fig. 2 COSY and HMBC spectral analysis of **1** and **2**

18-Me, H $_{\beta}$ -9 and H-10 are on the same side. The NOESY signals between H $_{\alpha}$ -9 and H-8 and H $_{\alpha}$ -7, and between H $_{\alpha}$ -7 and H-8 and H $_{\alpha}$ -9, showed that H $_{\alpha}$ -7, H-8 and H $_{\alpha}$ -9 are on the other side. A *cis* relationship of both protons at C-5 and C-10 was further supported by comparing the coupling constant between H-5 and H-10 in **1** ($J_{H5-H10} = 5.4$ Hz and $J_{H10-H5} = 5.4$ Hz) with values reported for the *cis* stereoisomers of costaclavine ($J = 3.1$ Hz) and epicostaclavine ($J = 3.9$ Hz) and the *trans* stereoisomers of festuclavine ($J = 9.5$ Hz) [10].

Based on the CD spectra, the compound **1** showed negative cotton effect at 204 (−3.1) and 216 (−5.7) and positive cotton effect at 235 (+1.9), 273 (+1.0) and 294 (+0.8). By comparison of the CD data between **3** and **1**, the CD spectrum of **1** was similar to that of **3** which showed negative cotton effect at 208 (−3.4) and 214 (−3.3) and positive cotton effect at 229 (+1.6), 273 (+0.6) and 294 (+0.5). It can be deduced that both compounds shared the same configuration. Based on the evidence described above, the stereostructure of **1** was determined to be (5*R*,8*R*,10*S*)-2-(1,3-dimethylprop-1-ene)-6,8-dimethylergoline.

2-(3,3-Dimethylprop-1-ene)-epicostaclavine (**2**) was a pale yellow oil which was isolated from the mycelium extract. A molecular formula of C₂₁H₂₈N₂ gave nine degrees of unsaturation, which was established by HRFABMS and ¹³C NMR methods. The IR absorption spectrum of **2** showed the characteristic bands of the amino groups (3319 cm^{−1}) and indole (1674 cm^{−1}) functionalities. The ¹H NMR and ¹³C NMR data of **2** were similar to those of compound **1** except for signals of positions 7, 8, 9, 10 and 18. The planar structure of **2** was established by analysis of 2-D NMR spectra including COSY and HMBC. Although the ¹H and ¹³C chemical shift of positions 7, 8, 9, 10 and 18 were different from those of compound **1**, it still has the same planar structure as **1** (Fig. 2). On the basis of all the foregoing evidence, the structure of **2** was suggested as 2-(1,3-dimethylprop-1-ene)-6,8-dimethylergoline.

The NOESY data of **2** showed correlations between H $_{\beta}$ -9 and H-12, and did not give a correlation between H $_{\alpha}$ -9 and H-12, thus it can be confirmed that H $_{\beta}$ -9 and H $_{\alpha}$ -9 are on opposite sides in compound **2**. At the same time, the NOESY signals between H-8 and H-5, H-10 and H $_{\beta}$ -9,

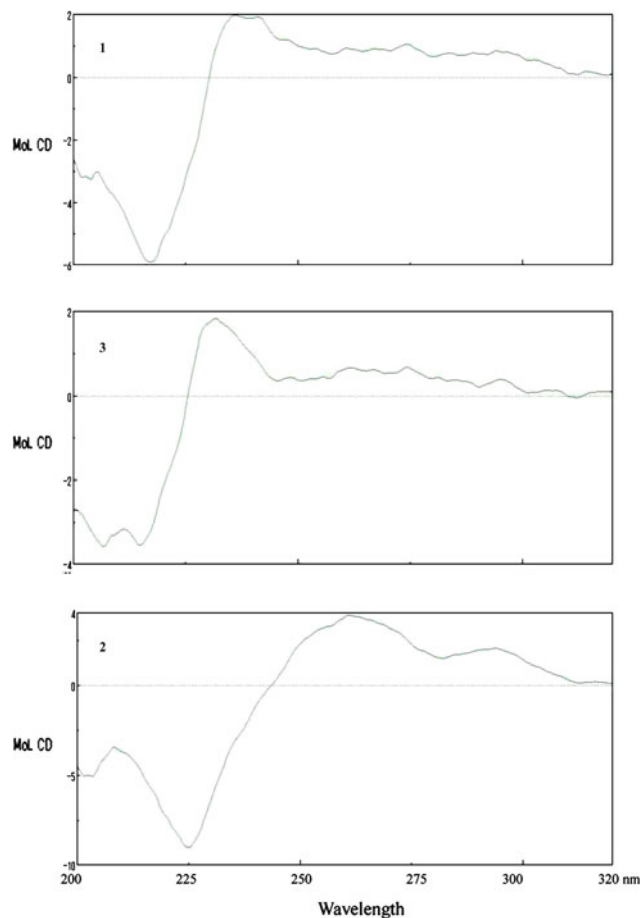


Fig. 3 The CD spectra of compounds **1**, **3** and **2**

H $_{\beta}$ -9 and H-10, and H-5 and H-10, suggested that H-5, H-8, H $_{\beta}$ -9 and H-10 are on the same side. The NOESY signals between H $_{\alpha}$ -9 and 18-Me showed that 18-Me and H $_{\alpha}$ -9 are on the other side. Using the same method as compound **1**, a *cis* relationship of both protons at C-5 and C-10 was further confirmed by comparing the coupling constant between H-5 and H-10.

Based on the optical rotation of compounds **2** and **1**, it is difficult to judge the configuration at C-5, C-8 and C-10. However, the chemical shift of C-5 in **2** [3.01, ddd, $J = 4.8, 5.8, 11.8$ Hz, 69.0] is similar to **1** [3.02, ddd, $J = 3.7, 5.4, 11.0$ Hz, 69.0]. This suggested that the two compounds have the same configuration in C-5. Furthermore, by comparison of the CD data between **2** and **1**, the CD spectrum of **2** was different to that of **1** and showed negative cotton effect at 208 (−3.4) and 224 (−8.9), and positive cotton effect at 260 (+3.7) and 293 (+2.0), indicating that the two compounds had a different configuration in C-8 (Fig. 3). Based on the evidence described above, the stereostructure of **2** was determined to be (5*R*,8*S*,10*S*)-2-(1,3-dimethylprop-1-ene)-6,8-dimethylergoline (**2**).

The known compounds **3**, **4** and **5** were identified by spectroscopic analysis (^1H and ^{13}C NMR, LRFABMS, and $[a]_D$) and comparison to literature data [3, 7, 8].

The compounds of **1**, **2**, **3** and **5** showed weak cytotoxicity against mouse leukemia cell line (P388) with IC_{50} of 64.9, 259.7, 333.3 and 218.8 μM , respectively; **4** did not show this bioactivity.

Experimental section

General experimental procedures

Optical rotation was determined on a JASCO model P-1030 polarimeter. CD spectra were taken on a JASCO J-820 spectropolarimeter. UV/visible spectra were measured on a JASCO V-550 UV/vis spectrometer. IR spectra were recorded on a JASCO FT-IR model 420 spectrometer. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a JEOL ECX400 NMR spectrometer, using solvent peaks as reference standard. ^1H (500 MHz) and DQF COSY spectra were obtained on a JEOL ECA 500 NMR. MS spectra were obtained on a JEOL JMS-700P FAB MS spectrometer.

Fungal isolation and culture

The fungal strain was isolated from a zoanthid, *Zoanthus* sp., collected in Ayamaru Cape, Amami Island, Kagoshima Prefecture, Japan, in April 2003. This microorganism was identified as *Aspergillus fumigatus* by partial sequence analysis of both of the 28S rDNA-D1/D2 and the ITS-5.8S rDNA, followed by GenBank/DDBCJ/EMBL, which resulted in a closest match to *Aspergillus fumigatus* (100%), as judged by the fungal classification company Techno Suruga Co., Ltd. A voucher specimen is deposited at Tokyo University with the code ZTF001. The fungus was cultured (16 L) for 2 weeks (static) at 37°C in Malt medium consisting of soluble starch (0.4%), yeast extract (0.4%), malt extract (1.0%) and distilled water (100%).

Extraction and isolation

The mycelium and broth were separated by filtration using gauze. The mycelium was extracted with acetone–MeOH (v/v 1/1) to afford mycelium extract (0.57 g), which was subjected to silica gel flash chromatography. Elution was performed with *n*-hexane–EtOAc (stepwise, 0–100% EtOAc) to yield six fractions. Fraction 6 was separated into two fractions by GL-pak Carbograph using MeOH–EtOAc (v/v 1/1) and MeOH, respectively. The fraction 6.1

was further separated by PTLC on CH_2Cl_2 –MeOH (10–1) with 0.1% TFA to afford four fractions. Fractions 1–3 were identified as crude compounds **5**, **1**, and **2**, respectively, which were further purified by HPLC (YMC, ODS-C₁₈) utilizing a 30-min gradient program of 50–100% MeOH in H₂O to furnish **5** (30.0 mg), **1** (4.1 mg) and **2** (3.3 mg), respectively. Fraction 4 was purified by HPLC (YMC, ODS-C₁₈) utilizing a 30-min gradient program of 50–75% MeOH in H₂O to furnish **3** (1.9 mg) and **4** (2.7 mg).

Cytotoxicity test against P388 cells [11]

Mouse leukemia cell line P388 cells were grown and maintained in culture medium RPMI1640 (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 5% penicillin/streptomycin solution (Sigma) at 37°C in a humidified 5% CO₂ incubator (Yamato, IT43) using EasyFlasks (Nunc). To maintain exponential growth, cells were subcultured every 96 h. P388 cells subcultured three times were used for the cytotoxicity test. Compounds were dissolved in methanol to obtain a concentration of 1 mg/mL. The solutions were diluted in the medium at concentrations ranging from 1 mg/mL to 1 $\mu\text{g/mL}$. The cell suspension (10,000 cells) was then blended in a 96-well plate (Nunc), each experimental group consisting of 5 wells. After 96 h culture in the incubator, 50 mL MTT solution was added to each well. After 4 h incubation, the 96-well plate was centrifuged and the supernatant discarded. Then, 100 mL DMSO was added to each well, and the absorbance of each well was measured at 570 nm using a micro-plate reader (Bio-Rad, Model 450).

2-(3,3-Dimethylprop-1-ene)-costaclavine (1) was obtained as a pale yellow oil: $[\alpha]_D^{25}$ -21 (*c* 0.29, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 204 (–3.1), 216 (–5.7), 235 (+1.9), 273 (+1.0), 294 (+0.8) nm; UV (MeOH) λ_{max} ($\log \epsilon$) 231 (4.2), 281 (4.0) nm; IR (film) λ_{max} 3299, 2976, 1673, 1474, 1407, 1201, 1192, 1137 cm^{-1} ; ^1H and ^{13}C NMR, see Table 1; FAB MS m/z 308 $[\text{M}]^+$, 309 $[\text{M}+\text{H}]^+$; HRFAB MS m/z 309.2327 (calcd. for C₂₁H₂₉N₂, 309.2331, Δmmu 0.4).

2-(3,3-Dimethylprop-1-ene)-epicostaclavine (2) was obtained as a pale yellow oil: $[\alpha]_D^{25}$ -13 (*c* 0.34, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 208 (–3.4), 224 (–8.9), 260 (+3.7), 293 (+2.0) nm; UV (MeOH) λ_{max} ($\log \epsilon$) 212 (4.2), 226 (4.4), 281 (4.0) nm; IR (film) λ_{max} 3319, 2975, 1674, 1464, 1202, 1189, 1139 cm^{-1} ; ^1H and ^{13}C NMR, see Table 1; FAB MS m/z 309 $[\text{M}+\text{H}]^+$; HRFAB MS m/z 309.2328 (calcd. for C₂₁H₂₉N₂, 309.2331, Δmmu 0.4).

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