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NOTE

New chaetoglobosin derivatives, MBJ-0038, MBJ-0039 and MBJ-0040, isolated from the fungus *Chaetomium* sp. f24230

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We screened a library constructed by the advanced compound-identification system based on the accumulated HPLC-MS profiling data combined with strain information designated as 'MBJ's special selection' for bioactive substances, and have isolated five novel eremophilane sesquiterpenoids MBJ-0009 and -0010 from the saprobic fungus *Nectria* sp. and MBJ-0011, -0012 and -0013 from the endophytic fungus *Apiognomonia* sp.[1,2] Further screening for cytotoxic compounds led to the identification of MBJ-0038 (**1**), -0039 (**2**) and -0040 (**3**), together with the known compounds chaetoglobosins C and F[3,4] from *Chaetomium* sp. f24230. Here, we describe the fermentation, isolation, structure elucidation, and, in brief, the cytotoxic activity of **1**–**3**.

Chaetomium sp. f24230 was isolated from a soil sample collected in the Okinawa Prefecture, Japan. The strain was cultivated in 250-ml Erlenmeyer flasks, each containing 25 ml of a seed medium consisting of 2% potato starch (Tobu Tokachi Nosan Kako Agricultural Cooperative Association, Hokkaido, Japan), 1% glucose (Junsei Chemical, Tokyo, Japan), 2% soybean powder (Honen SoyPro, J-Oil Mills, Tokyo, Japan), 0.1% KH_2PO_4 and 0.05% $MgSO_4 \cdot 7H_2O$. The flasks were shaken on a rotary shaker (220 r.p.m.) at 25 °C for 3 days. Aliquots (0.5 ml) of the broth were transferred to 500-ml Erlenmeyer flasks containing 50 ml of a production medium consisting of 2% potato starch (Tobu Tokachi Nosan Kako Agricultural Cooperative Association), 1% glucose (Junsei Chemical), 2% soybean powder (Honen SoyPro, J-Oil Mills), 0.1% KH_2PO_4 and 0.05% $MgSO_4 \cdot 7H_2O$, and were cultured on a rotary shaker (220 r.p.m.) at 25 °C for 4 days.

The whole-culture broth (2 l) was extracted with an equal volume of *n*-BuOH and, following concentration, was successively partitioned between ethyl acetate (350 ml × 3) and water (350 ml). The ethyl acetate extract (4.56 g) was subjected to silica gel medium-pressure liquid chromatography (MPLC; Purif-Pack SI-30, Shoko Scientific Co., Yokohama, Japan) with a gradient system of *n*-hexane–ethyl acetate (0–25% ethyl acetate) followed by $CHCl_3$–methanol (MeOH) with stepwise increases from 0 to 100% MeOH with monitoring by UV at 254 nm. The active 5% MeOH fraction (190.1 mg) was separated by gel filtration on a Sephadex LH-20 column (1:1, $CHCl_3$/MeOH, GE Healthcare BioSciences AB, Uppsala, Sweden) followed by reversed phase-MPLC (Purif-Pack ODS-30; 70–100% aq. MeOH with 10% stepwise increments in the MeOH concentration) purification to yield **3** (90% MeOH, 6.1 mg). The active 10% MeOH fraction (563.9 mg) was subjected to Sephadex LH-20 column chromatography (1:1, $CHCl_3$/MeOH) to yield a crude material containing **2** (98.9 mg). This material was purified using ODS MPLC (Purif-Pack ODS-30; 50–100% aq. MeOH, 10% stepwise) to yield **2** (80% MeOH, 12.3 mg). Another active fraction from the 10% MeOH eluate (1.70 g) was further purified using an ODS MPLC (Purif-Pack ODS-100; 20–100% aq. MeOH in 20% stepwise increments), and an 80% MeOH eluate (940 mg) was re-chromatographed using an ODS MPLC (Purif-Pack ODS-30; 50–100% aq. MeOH, 10% stepwise) to yield crude **1** (80% MeOH, 119.9 mg). Compound **1** (55.8 mg) was finally purified using Sephadex LH-20 column chromatography (1:1, $CHCl_3$/MeOH).

MBJ-0038 (**1**) was isolated as a colorless amorphous powder: $[\alpha]^{25}_{D}$ –66 (MeOH; *c* 0.7); UV λ_{max} (ε) in MeOH: 281 (7700) nm; IR (ATR) ν_{max}: 3400 (hydroxy) and 1689 (carbonyl) cm^{-1}. The molecular formula of **1** was established as $C_{41}H_{44}N_2O_9$ by HR-ESI-MS; (*m/z* 709.3122 $[M+H]^+$, calcd. for $C_{41}H_{45}N_2O_9$ *m/z* 709.3125). The ^{13}C and ^{1}H NMR data for **1** are listed in Table 1. Structural information on **1** was obtained by a series of 2D NMR analyses such as double quantum-filtered COSY (DQF-COSY), HSQC and constant-time (CT)-HMBC.[5]

An indole moiety in **1** was identified based on ^{1}H couplings from a doublet aromatic proton H-4′ (δ_H 7.53) through a doublet of doublets aromatic protons H-5′ (δ_H 7.03) and H-6′ (δ_H 7.10) to a doublet aromatic proton H-7′ (δ_H 7.34), and by following ^{1}H–^{13}C

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Table 1 ^{13}C and ^{1}H NMR spectroscopic data for MBJ-0038 (1), MBJ-0039 (2) and MBJ-0040 (3).

	1		*2*		*3*	
Position	δ_C	δ_H, *multiplicity (J in Hz)*	δ_C	δ_H, *multiplicity (J in Hz)*	δ_C	δ_H, *multiplicity (J in Hz)*
1	176.6		178.3		178.6	
3	54.9	3.85, ddd (2.4, 5.4, 7.8)	55.2	3.87, ddd (2.4, 6.0, 9.0)	56.9	3.41, m
4	52.8[a]	2.64, dd (2.4, 5.4)	52.6	2.63, dd (2.4, 5.4)	55.2	2.62,dd (3.5, 3.5)
5	37.9	1.75, m	37.8	1.74, ovl[b]	36.6	2.53, m
6	58.9		58.9		141.7	
7	63.3	2.72, d (5.4)	63.1	2.73, d (6.0)	126.8	5.36, br s
8	52.8[a]	2.30, dd (5.4, 9.6)	52.3	2.31, dd (6.0, 9.6)	49.9	3.03, br d (10.0)
9	64.8		65.1		67.7	
10	35.7	3.06, dd (5.4, 14.4); 2.85, dd (7.8, 14.4)	35.9	3.08, dd (6.0, 13.8); 2.79, dd (9.0, 13.8)	36.0	2.98, ovl[b], 2.98, ovl[b]
11	12.8	0.62, d (7.2)	12.9	0.61, d (7.2)	14.2	0.94, d (7.0)
12	19.8	1.18, s	19.8	1.20, s	20.3	1.74, s
13	127.6	6.04, ddd (1.8, 9.6, 15.0)	127.9	6.02, ddd (1.2, 9.6, 15.0)	130.5	5.87, dd (10.0, 15.0)
14	135.9	5.15, ddd (3.6, 10.8, 15.0)	135.4	5.18, ddd (3.6, 10.8, 15.0)	132.8	5.06, ddd (2.5, 10.5, 15.0)
15	42.3	2.14, dd (2.4, 13.8); 1.72, ovl[b]	42.2	2.17, br d (13.2); 1.78, ovl[b]	41.9	2.20, br d (10.5); 1.77, ovl[b]
16	33.6	2.42, m	33.6	2.43, m	33.6	2.43, m
17	137.5	4.89, d (9.6)	138.5	4.90, d (10.2)	140.0	4.96, d (10.0)
18	131.8		132.2		131.6	
19	82.2	3.96, s	81.8	3.59, s	81.5	3.34, s
20	209.1		209.1		209.2	
21	51.2	4.34, dd (3.6, 5.4)	52.1	4.13, dd (4.8, 4.8)	53.0	3.94, dd (5.0, 5.0)
22	82.1	5.78, d (5.4)	83.4	5.63, d (4.8)	83.3	5.59, d (5.0)
22a	121.6		133.9		133.8	
23	138.8		112.7		112.5	
24	133.8		144.8		144.8	
25	145.0		134.6		134.6	
26	111.3		137.3		137.1	
26a	135.1		122.5		122.6	
27	82.7	5.81, s	82.1	5.97, s	82.2	5.79, s
28	58.2	3.68, d (3.6)	59.4	3.62, d (4.8)	58.4	3.71, d (5.0)
29	209.0		208.2		208.9	
30	21.5	0.89, d (6.6)	21.3	0.89, d (6.6)	21.2	0.88, d (6.5)
31	11.8	1.55, s	11.7	1.44, s	11.4	1.36, s
32	12.7	2.28, s	12.3	2.00, s	12.5	2.01, s
2′	124.6	7.07, s	124.7	7.10, s	124.8	7.13, s
3′	111.2		111.0		111.2	
3a′	128.7		128.7		128.7	
4′	119.0	7.53, d (8.4)	119.0	7.53, d (8.4)	119.0	7.52, d (7.5)
5′	120.1	7.03, dd (8.4, 8.4)	120.1	7.05, dd (8.4, 8.4)	120.0	7.03, dd (7.5, 7.5)
6′	122.6	7.10, dd (8.4, 8.4)	122.6	7.11, dd (8.4, 8.4)	122.5	7.09, dd (7.5, 7.5)
7′	112.5	7.34, d (8.4)	112.5	7.35, d (8.4)	112.6	7.33, d (7.5)
7a′	138.2		138.2		138.3	

Measured on a 600 NB CL NMR spectrometer (Varian, Palo Alto, CA, USA) at 600 MHz for ^{1}H and 150 MHz for ^{13}C (**1** and **2**) or a 500 NB CL NMR spectrometer (Varian) at 500 MHz for ^{1}H and 125 MHz for ^{13}C (**3**), with the residual solvent peak as the internal standard (3.31 p.p.m. for ^{1}H and 49.0 p.p.m. for ^{13}C in CD_3OD).
[a]Interchangeable.
[b]Overlapped with other signals.

long-range couplings from a singlet aromatic proton H-2′ (δ_H 7.07) to aromatic quaternary carbon C-3′ (δ_C 111.2), C-3a′ (δ_C 128.7) and C-7a′ (δ_C 138.2); from H-4′ to C-3′ and C-7a′; from H-5′ to C-3a′; from H-6′ to C-7a′; and from H-7′ to C-3a′, together with their typical ^{13}C NMR chemical shifts (Figure 1c).

The DQF-COSY spectrum revealed the following proton spin networks, from a methyl proton H_3-11 (δ_H 0.62) through methine protons H-5 (δ_H 1.75), H-4 (δ_H 2.64) and H-3 (δ_H 3.85) to methylene protons H_2-10 (δ_H 3.06 and 2.85); from a methine proton H-7 (δ_H 2.72) through a methine proton H-8 (δ_H 2.30), olefinic methine protons H-13 (δ_H 6.04) and H-14 (δ_H 5.15), methylene protons H_2-15 (δ_H 2.14 and 1.72) and a methine proton H-16 (δ_H 2.42) to an olefinic proton H-17 (δ_H 4.89); and from a methine proton H-22 (δ_H 5.78) to a methine proton H-28 (δ_H 3.68) through a methine proton H-21 (δ_H 4.34). The presence of a six-membered ring (C-4 to C-9) was identified from HMBC correlations from a singlet methyl proton H_3-12 (δ_H 1.18) to a methine carbon C-5 (δ_C 37.9), a quaternary carbon C-6 (δ_C 58.9) and a methine carbon C-7 (δ_C 63.3); and from the methine proton H-8 to a methine carbon C-4 (δ_C 52.8) and a quaternary carbon C-9 (δ_C 64.8). The high-field shift for the C-6 signal supported the presence of an epoxide ring between C-6 and C-7. HMBC correlations from H-4, H-8 and the nitrogen-

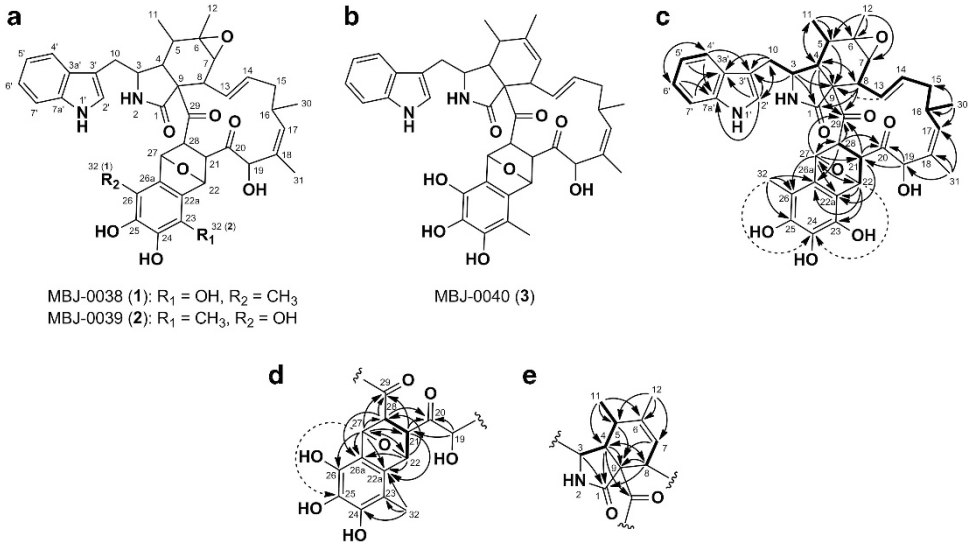

Figure 1 (**a**) Structures of **1** and **2**. (**b**) Structure of **3**. (**c**) NMR analysis of **1**. COSY: bold line; HMBC (^{1}H–^{13}C): arrows (solid, strong; dashed, weak). (**d**) Partial structure of **2**. COSY: bold line; HMBC (^{1}H–^{13}C): arrows (solid, strong; dashed, weak). (**e**) Partial structure of **3**. COSY: bold line; HMBC (^{1}H–^{13}C): arrows.

substituted methine proton H-3 (δ_C 54.9) to a carbonyl carbon C-1 (δ_C 176.6) suggested the presence of a γ-lactam ring moiety, as shown in Figure 1c. Furthermore, the presence of a 13-membered carbon ring structure was revealed by following ^{1}H–^{13}C long-range couplings, from a methyl proton H_3-30 (δ_H 0.89), which exhibited ^{1}H spin coupling to H-16, to an olefinic methine carbon C-17 (δ_C 137.5); from a methyl proton H_3-31 (δ_H 1.55) to C-17, an olefinic quaternary carbon C-18 (δ_C 131.8) and an oxymethine carbon C-19 (δ_C 82.2); from an oxymethine proton H-19 (δ_H 3.96) together with H-21 and H-22 to a ketone carbonyl carbon C-20 (δ_C 209.1); and from H-4, H-8, H-21 and H-28 to another ketone carbonyl carbon C-29 (δ_C 209.0). The direct connectivity between C-10 and C-3′ was evident from HMBC correlations from H_2-10 to C-2′ (δ_C 124.6), C-3′ and C-3a′. Thus, a chaetoglobosin skeleton was established. The structure of the remaining part of the molecule was determined as follows. The structure of a tetrahydrofuran ring moiety was determined from long-range couplings from an oxymethine proton H-27 (δ_H 5.81, δ_C 82.7) to methine carbons C-21 (δ_C 51.2), C-28 (δ_C 58.2) and an oxymethine carbon C-22 (δ_C 82.1). HMBC correlations from an allylic methyl proton H_3-32 (δ_H 2.28) to aromatic quaternary carbons C-25 (δ_C 145.0), C-26 (δ_C 111.3), C-26a (δ_C 135.1) and additionally to C-24 (δ_C 133.8) weakly; from H-27 to an aromatic quaternary carbon C-22a (δ_C 121.6) and C-26; from H-22 to aromatic quaternary carbons C-22a, C-23 (δ_C 138.8), C-24 and C-26a; and from H-28 to C-26a indicated the presence of an 8-methyl-1,2,3,4-tetrahydro-1,4-epoxynaphthalene-5,6,7-triol substructure. The proton chemical shift values at H-22 and H-27, together with low-field shifted ^{13}C chemical shift values at C-22 and C-27 because of the presence of an ether bond agreed well with the corresponding proton chemical shifts of 1,4-epoxy-1,2,3,4-tetrahydronaphthalene-*endo*,*cis*-2,3-dicarboxylic acid (δ_H 5.48 and 5.44, in dimethyl sulfoxide-d_6).[6] A NOESY correlation between H-27 and H_3-32 also supported these assignments. On the basis of the chemical shifts of C-23, C-24 and C-25, and the index of hydrogen deficiency deduced from the molecular formula, all three of these carbons were attached to phenolic hydroxy groups. An *E* geometry for the double bond at C-13–C-14 was deduced from a large coupling constant between H-13 and H-14 (15.0 Hz). The geometry of the position at C-17 was assigned for *E* based on the high-field shift for C-31 signal (δ_C 11.8) owing to a γ-effect. Therefore, the gross structure of **1** was identified to be that shown in Figure 1c.

MBJ-0039 (**2**) was obtained as a colorless amorphous powder: $[\alpha]^{24}_D$ –115 (MeOH; *c* 0.6). The UV absorption maxima in MeOH at 281 nm (ε 6300) and the IR spectrum (ν_{max}: 3400 and 1689 cm^{-1}) of **2** resembled those of **1**. Furthermore, the molecular formula of **2** ($C_{41}H_{44}N_2O_9$) established based on HR-ESI-MS data (*m/z* 709.2923 $[M+H]^+$) was the same as that of **1**. Thus, **2** was considered to be a structural isomer of **1**. The ^{1}H and ^{13}C NMR spectroscopic data of **2** were very similar to those of **1** (Table 1). Detailed analyses of the 2D NMR data revealed a chaetoglobosin skeleton identical to **1**, including the exact assignments for the oxymethine protons H-22 (δ_H 5.63, δ_C 83.4) and H-27 (δ_H 5.97, δ_C 82.1). The structure of the 8-methyl-1,2,3,4-tetrahydro-1,4-epoxynaphthalene-5,6,7-triol moiety of **2** differed from that in **1**. Observation of a NOESY correlation between an oxymethine proton H-22 and an allylic methyl proton H_3-32 (δ_H 2.00) suggested that **2** had an inverted arrangement of **1** for this region. In addition, COSY and HMBC data supported these assignments as shown in Figure 1d. Thus, the structure determination of **2** was accomplished as shown in Figure 1a.

MBJ-0040 (**3**) features the following properties: $[\alpha]^{24}_D$ –110 (MeOH; *c* 0.3); UV λ_{max} (ε) in MeOH: 281 (6000) nm; HR-ESI-MS: *m/z* 691.2986 $[M-H]^-$, calcd. for $C_{41}H_{43}N_2O_8$ *m/z* 691.3019; and IR absorption (ν_{max}) 3400 and 1689 cm^{-1}. Analysis of NMR spectra revealed that the partial structure of **3** was the same as that of **2**, including the geometries of 13*E* and 17*E*, with the exception of the cyclohexane ring moiety (C-4 to C-9) as described below. The DQF-COSY spectrum showed sequences from a methyl proton H_3-11 (δ_H 0.94) through methine protons H-5 (δ_H 2.53) and H-4 (δ_H 2.62) to a nitrogen-substituted methine proton H-3 (δ_H 3.41) and from an olefinic methine proton H-7 (δ_H 5.36, δ_C 126.8) to a methine proton H-8 (δ_H 3.03). Furthermore, the CT-HMBC spectrum showed ^{1}H–^{13}C long-range correlations from H_3-11 to methine carbons C-4 (δ_C 55.2), C-5 (δ_C 36.6) and an olefinic quaternary carbon C-6 (δ_C 141.7); from an allylic methyl proton H_3-12 (δ_H 1.74) to C-5, C-6 and an olefinic methine carbon C-7; and from H-5 and H-7 to a quaternary carbon C-9 (δ_C 67.7), which revealed a

1,6-dimethylcyclohex-1-ene moiety (Figure 1e). Thus, the gross structure of **3** was determined as shown in Figure 1b.

The cytotoxic activities of novel compounds **1–3** and chaetoglobosins C and F against human ovarian adenocarcinoma SKOV-3 cells were tested using the WST-8 ((2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) colorimetric assay (Cell Counting Kit; Dojindo, Kumamoto, Japan). After 72 h of treatment, all compounds exhibited moderate cytotoxic activity against SKOV-3 cells (IC_{50}; **1**: 14 μM, **2**: 11 μM, **3**: 14 μM, and chaetoglobosins C and F: 8 μM and 2 μM, respectively).

Although a number of chaetoglobosin derivatives have been isolated from fungi,[3,4,7] **1–3** are the first samples of chaetoglobosins containing an 8-methyl-1,2,3,4-tetrahydro-1,4-epoxynaphthalene-5,6,7-triol moiety.

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