ORIGINAL ARTICLE

Production of a new type of amidepsine with a sugar moiety by static fermentation of *Humicola* sp. FO-2942

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Static fermentation of amidepsine-producing fungus *Humicola* sp. FO-2942 led to the production of six new amidepsines, including a new type of glycosylated congener. Non-glycosylated amidepsine J inhibited both human diacylglycerol acyltransferases 1 (DGAT1) and DGAT2 with the same IC₅₀ value of 40 μM, whereas glycosylated amidepsines F to I showed very weak inhibitory activity against DGAT1 and DGAT2.

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Keywords: amidepsine; diacylglycerol acyltransferase; fungal metabolite; *Humicola* sp.

INTRODUCTION

Diacylglycerol acyltransferase (DGAT) catalyzes the esterification of diacylglycerol with long-chain acyl-CoA to synthesize triacylglycerol. DGAT is actually two isozymes, DGAT1 and DGAT2,^{1,2} and is considered a potential target for the treatment of obesity or metabolic disorder.^{1–3} Accordingly, researchers have focused on the discovery and development of DGAT inhibitors.^{4,5}

Amidepsines, gyropholic acid derivatives produced by *Humicola* sp. FO-2942^{6,7} and *Humicola* sp. FO-5962,⁸ showed inhibitory activity against DGAT. Recently, we completed the total synthesis of amidepsine B (2), which consists of gyropholic acid and alanine moieties, and elucidated its absolute stereochemistry.⁹

To further study the biological characteristics of amidepsines, a large amount of the sample is required, but the original productivity of amidepsine by fermentation of strain FO-2942 was very low (0.1–0.6 μg ml^{−1}); therefore, we started to optimize the fermentation conditions to increase the productivity of amidepsines. We found that the production of amidepsines A (1) to D (3) increased 60- to 480-fold when the strain was cultured under static conditions. Furthermore, six new amidepsine congeners (6–11), including a new type of amidepsines F (6) to I (9) having a sugar moiety (Figure 1), were also isolated from culture broth obtained under this condition. In this study, the fermentation, isolation, structure elucidation and biological properties of new amidepsines are described.

RESULTS

Effects of culture conditions on the production of amidepsines

The efficient culture conditions for amidepsine production were examined. The productivity of amidepsines was monitored by HPLC and compared with that obtained under original condition

a.¹ As shown in Table 1, the production of 1–4 increased 2.6- to 11-fold when fermented under culture condition b. Note that production markedly increased 65- to 480-fold under static culture condition 3. Furthermore, new peaks (6–9, 10 and 11 in Figure 2a), having a UV spectrum similar to that of amidepsines, were found by HPLC analysis (Figure 2a), leading to the isolation of new amidepsines described below.

The time course of amidepsine production under culture condition c is shown in Figure 3. The production of all amidepsines started on day 3. The concentration of 1, the most abundant component, peaked (144 μg ml^{−1}) on day 9. The production of nonglycosylated amidepsines also peaked on day 9, whereas the production of glycosylated 6–9 peaked on day 5. Compound 6 showed the highest concentration (50 μg ml^{−1}) among glycosylated amidepsines.

Isolation of amidepsines F (6) to I (9)

Compounds 6–9 and gyrophoric acid were isolated together with 1–4 from culture broth obtained under condition c. The whole broth (5.0 l) was extracted with an equal volume of ethyl acetate. The extracts were concentrated *in vacuo* to dryness to yield a brown oily material (3.8 g). The material was dissolved in CH₃OH (400 ml) and extracted with hexane. The methanol layer was concentrated *in vacuo* to dryness to yield a yellow powder (1.2 g). The material was dissolved in CHCl₃, applied on a silica gel column (40 g), and eluted stepwise with 100:0, 98:2, 95:5, 9:1, 8:2 and 0:100 (v/v) of CHCl₃–CH₃OH solvents (400 ml for each solvent).

The 9:1 fraction (CHCl₃–CH₃OH), which contained 1–4 and 10–12 (Figure 2b), was concentrated to give a red-brown oily material (340 mg). The oil (50 mg) was finally purified with preparative HPLC (column, PEGASIL ODS (Senshu Scientific, Tokyo, Japan),

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Amidepsine	R ₁	R ₂	R ₃	R ₄
A (1)	-CH ₃	-H	Ala	-CH ₃
B (2)	-H	-H	Ala	-CH ₃
C (3)	-H	-H	Val	-CH ₃
D (4)	-CH ₃	-H	-OH	-CH ₃
E (5)	-CH ₃	-CH ₃	Ala	-CH ₃
F (6)	-CH ₃	Glc	Ala	-CH ₃
G* (7)	-H	Glc	Ala	-CH ₃
H* (8)	-CH ₃	Acglc	Ala	-CH ₃
I* (9)	-H	Acglc	Ala	-CH ₃
J* (10)	-CH ₃	-H	Val	-CH ₃
K* (11)	-H	-H	-OH	-CH ₃
Gyr (12)	-H	-H	-OH	-H

*; New amidepsines described in this study
Glc; D-glucose, Acglc; 2-O-acetyl-D-glucose
Ala; L-alanine, Val; L-valine, Gyr; gyrophoric acid

Figure 1 Structures of amidepsines A (1) to K (11) and gyrophoric acid (12).

Table 1 Effect of culture conditions on the production of amidepsines

Culture condition	Compound ($\mu\text{g ml}^{-1}$)										
	6	7	8	9	1	2	12	10	3	4	11
a	ND	ND	ND	ND	0.3	0.6	ND	ND	0.1	0.3	ND
b	1.2	0.3	3.2	1.1	3.3	1.6	0.7	0.8	0.3	0.8	2.0
c	12	1.9	53	19	144	61	20	24	6.5	50	56

Abbreviation: ND, not detected.

a: Fermentation was carried out in a 50-l jar fermenter.

b: The culture was incubated for 3 days with shaking and then further incubated for 7 days under static conditions.

c: The culture was incubated for 10 days under static conditions.

20×250 mm; solvent, a 60-min linear gradient from 50 to 80% CH₃CN; detection, UV at 210 nm; flow rate, 8.0 ml min⁻¹). Under these conditions, 1–4 and 10–12 were eluted as peaks with retention times of 33, 44, 49, 52, 46, 55 and 45 min, respectively. Each fraction was concentrated *in vacuo* to dryness to give pure 1 (9.4 mg), 2 (4.1 mg), 3 (2.0 mg), 4 (2.0 mg), 10 (3.8 mg), 11 (2.4 mg) and 12 (3.7 mg) as colorless materials.

The CH₃OH fraction containing 6–9 (Figure 2c) was concentrated to yield oily material (147.0 mg), which was subjected to ODS column chromatography (10 g), and eluted stepwise with 5:95, 1:9, 2:8, 1:1 and

0:100 (v/v) of CH₃CN–H₂O solvents (50 ml for each solvent). The 1:1 fraction was concentrated to give a brown material (66.9 mg) that was dissolved in CH₃OH (3.3 ml) and the soluble supernatant was purified by HPLC (column, PEGASIL ODS, 20×250 mm; solvent, 35% CH₃CN; UV at 210 nm; flow rate, 9 ml min⁻¹). Under these conditions, 6–9 were eluted as peaks with retention times of 45, 61, 80 and 113 min, respectively. Each fraction was concentrated *in vacuo* to dryness to yield pure 6 (2.4 mg), 7 (4.2 mg), 8 (4.8 mg) and 9 (5.3 mg) as colorless materials.

Physicochemical properties of amidepsines F (6) to K (11)

Physicochemical properties of 6–11 are summarized in Table 2. They showed similar absorption maxima at 203–213 and 251–281 nm in the UV spectra, suggesting the presence of the same chromophore in their structures. Compounds 6–10 had optical rotations, whereas 11 had no optical rotation, suggesting the achiral structure of 11.

Structures of amidepsines J (10) and K (11)

Comparison of spectral data between 10 and 3 indicated that amidepsine J (C₃₁H₃₃NO₁₁) is larger by a CH₂ unit than 3 (Table 2), and that the methoxy proton (δ 3.83) was observed from the ¹H-NMR spectra for 10 (Table 3). In heteronuclear multiple bond correlation (HMBC) experiments (Figure 4a), long-range coupling was observed from a methoxy proton to C-19 (δ 158.3), indicating that 10 has a methoxy group instead of hydroxyl group C-19 of 3. Thus, 10 is elucidated as 19-methoxy-3 (Figure 1).

Comparison of the spectral data of 4 and 11 indicated that 11 is smaller by a CH₂ unit than 4 (Table 2), and that the 19-OH (δ 10.37) proton was observed for 11 from NMR spectra (Table 3). The structure was confirmed by HMBC experiments, as shown in Figure 4b, in which long-range couplings were observed from 19-OH (δ 10.37) to C-18 (δ 106.7), C-19 (δ 159.1) and C-20 (δ 99.0), indicating that 11 has a hydroxyl group instead of the methoxy group at C-19 of 4. Thus, 11 is elucidated as 19-hydroxy-4 (Figure 1).

Structure elucidation of amidepsines G (7)

The molecular formula of 7 was determined to be C₃₄H₃₇NO₁₆ on the basis of HRFAB-MS measurements (Table 2), indicating that 7 is larger by a C₆H₁₀O₅ unit than 2. Further, 1D NMR spectral data and the physicochemical properties showed that 7 has the same skeleton as 2. The ¹³C NMR spectrum of 7 exhibited six signals of a sugar moiety at δ 62.0, 70.5, 74.0, 78.5, 78.7 and 102.5. These data, together with one anomeric proton signal at δ 5.05 (C-1'), were indicative of the presence of a sugar moiety, which was confirmed by ¹H-¹H COSY and HMBC experiments (Figure 4c). The sugar moiety was assigned as glucose by ³J_{HH} coupling constants between C-1' and C-2' (7.2 Hz), C-2' and C-3' (9.0 Hz), C-3' and C-4' (8.4 Hz), and C-4' and C-5' (9.6 Hz), as shown in Figure 5. The connectivity of the glucose moiety was determined by observing the correlation between the anomeric proton signal (δ 5.05) and the corresponding carbon signal C-11 (δ 156.4) in the HMBC experiment to form a glycoside bond (Figure 4c). Thus, the structure of 7 was elucidated as shown in Figure 1.

Structures of amidepsines F (6), H (8) and I (9)

Comparison of the spectral data between 6 and 7 indicated that 6 is larger by a CH₂ unit than 7 (Table 2), and that the methoxy proton (δ 3.88) was observed for 6 in the ¹H NMR spectra (Table 3). In HMBC experiments (Figure 4e), long-range coupling was observed from the methoxy proton to C-19 (δ 160.2), indicating that 6 has a methoxy group instead of the hydroxy group at C-19 of 7. Thus, the structure of 6 was determined as 19-methoxy-7 (Figure 1).

Figure 2 HPLC profiles of amidepsines. (a) HPLC profile of ethyl acetate extracts of the broth obtained from *Humicola* sp. FO-2942. One μg of ethyl acetate extracts (1 mg ml^{-1} of methanol) was subjected to HPLC under the conditions as described in 'Methods.' (b) Chromatographic profile of 1-4 and 10-12 by analytical HPLC. (c) Chromatographic profile of 6-9 by analytical HPLC.

Figure 3 Time course of amidepsine production under static conditions. (a) Nonglycosylated amidepsines A (1) to D (4), J (10) and K (11) and gyropholic acid (12); (b) Glycosylated amidepsines F (6) to I (9).

The molecular formula of **9** is $\text{C}_{36}\text{H}_{40}\text{O}_{17}\text{N}$, indicating that it is larger by a $\text{C}_2\text{H}_2\text{O}$ unit than **7**. The ^1H -NMR spectrum of **9** was almost identical to that of **7** except for the proton signal H-2' (δ 5.06). In the HMBC spectrum of **9** (Figure 4d), the correlation between H_3 -8' (δ 2.00) and H-2' to C-7' (δ 171.9) indicated that an *O*-acetyl group was connected to the C-2' of **7**. Thus, the structure of **9** was determined as shown in Figure 1.

Comparison of the spectral data between **8** and **9** indicated that **8** is larger by a CH_2 unit than **9** (Table 2) and that the methoxy proton (δ 3.84) was observed for **8** (Table 3). In HMBC experiments (Figure 4f), long-range coupling was observed from methoxy proton to C-19 (δ 160.2), indicating that **8** has a methoxy group instead of the hydroxy group at C-19 of **9**; therefore, the structure of **8** was determined as 19-methoxy-**9** (Figure 1).

Table 2 Physicochemical properties of amidepsines **6** (**6**) to **K** (**11**)

	6	7	8
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder
Molecular formula	C ₃₅ H ₃₉ NO ₁₆	C ₃₄ H ₃₇ NO ₁₆	C ₃₇ H ₄₁ NO ₁₇
Molecular weight	729	715	771
FAB-MS (<i>m/z</i>)			
Positive	730 [M+H] ⁺ 752 [M+Na] ⁺	716 [M+H] ⁺ 738 [M+Na] ⁺	772 [M+H] ⁺ 793 [M+Na] ⁺
Negative	728 [M-H] ⁻	714 [M-H] ⁻	770 [M-H] ⁻
HRFAB-MS (<i>m/z</i>) (<i>positive</i>)			
[M+H] ⁺	C ₃₅ H ₄₀ NO ₁₆	C ₃₄ H ₃₈ NO ₁₆	C ₃₇ H ₄₂ NO ₁₇
Calcd:	730.2347	716.2191	772.2453
Found:	730.2307	716.2146	772.2405
[α] _D ²⁵ (c 0.1, MeOH)	-10°	-13°	-19°
UVλEtOH _{max} nm(ε)	204 (48 000) 281 (5600)	203 (50 500) 270 (15 500)	203 (59 500) 251 (11 700)
IR (KBr) ν _{max} (cm ⁻¹)	3379, 2956, 2927 2858, 1739, 1599 1456, 1257, 1038	3311, 2929, 2860 1738, 1657, 1603 1450, 1254, 1157 1063	3359, 2956, 2927 2858, 1741, 1599 1454, 1254, 1157 1038
Solubility			
Soluble	CH ₃ OH, acetone ethyl acetate CHCl ₃ , CH ₃ CN	CH ₃ OH, acetone ethyl acetate CHCl ₃ , CH ₃ CN	CH ₃ OH, acetone ethyl acetate CHCl ₃ , CH ₃ CN
Insoluble	H ₂ O, n-hexane	H ₂ O, n-hexane	H ₂ O, n-hexane
	9	10	11
Appearance	Pale yellow powder	Pale yellow powder	White powder
Molecular formula	C ₃₆ H ₃₉ NO ₁₇	C ₃₁ H ₃₃ NO ₁₁	C ₂₅ H ₂₂ O ₁₀
Molecular weight	757	595	482
FAB-MS (<i>m/z</i>)			
Positive	758 [M+H] ⁺ 780 [M+Na] ⁺	596 [M+H] ⁺ 618 [M+Na] ⁺	483 [M+H] ⁺ 505 [M+Na] ⁺
Negative	756 [M-H] ⁻	594 [M-H] ⁻	481 [M-H] ⁻
HRFAB-MS (<i>m/z</i>) (<i>positive</i>)			
[M+H] ⁺	C ₃₆ H ₄₀ NO ₁₇	C ₃₁ H ₃₄ NO ₁₁	C ₂₅ H ₂₃ O ₁₀
Calcd:	758.2313	596.2132	483.1291
Found:	758.2296	596.2098	483.1274
(α) _D ²⁵ (c 0.1, MeOH)	-21°	3°	0°
UVλEtOH _{max} nm(ε)	203 (49 400) 270 (14 700)	204 (50 200) 256 (11 500)	213 (38 500) 270 (7900)
IR	3355, 2968, 2931 1745, 1655, 1616 1448, 1252, 1157	3172, 2964, 2929 2862, 1741, 1670 1600, 1456, 1248 1136, 1030	2979, 2935, 2852 1662, 1610, 1581 1421, 1248, 1201 1148
Solubility			
Soluble	CH ₃ OH, acetone ethyl acetate CHCl ₃ , CH ₃ CN	CH ₃ OH, acetone ethyl acetate CHCl ₃ , CH ₃ CN	Dimethyl sulfoxide ethyl acetate
Insoluble	H ₂ O, n-Hexane	H ₂ O, n-Hexane	H ₂ O

Effects of amidepsines on human DGAT1 and DGAT2 activities

Effects of **9–11** on human DGAT1 and DGAT2 activity (IC₅₀ value) are summarized in Table 4. Glycosylated **6–9** showed very weak or almost

no inhibitory activity against human DGAT1 and DGAT2; however, nonglycosylated **10** inhibited both isozymes with the same IC₅₀ value of 40 μM, indicating that the potency is analogous to that of **1**, **2** and **4**.

Table 3 ^1H - and ^{13}C -NMR chemical shifts of amidepsines F (6) to K (11)

Position	6		7	
	δC	δH	δC	δH
2	123.5		123.5	
3	156.5		156.5	
4	107.5	6.65 d (1H, 2.2 Hz)	107.5	6.70 d (1H, 2.2 Hz)
5	153.0		153.0	
6	115.5	6.67 d (1H, 2.2 Hz)	115.5	6.69 d (1H, 2.2 Hz)
7	139.0		140.0	
7-CH ₃	19.4	2.37 s (3H)	19.1	2.39 s (3H)
10	123.0		123.0	
11	156.0		156.4	
12	108.5	7.04 d (1H, 2.0 Hz)	109.0	7.12 d (1H, 2.0 Hz)
13	154.0		153.0	
14	116.0	6.88 d (1H, 2.0 Hz)	118.5	6.92 d (1H, 2.0 Hz)
15	140.0		139.0	
15-CH ₃	19.8	2.41 s (3H)	19.2	2.46 s (3H)
18	108.0		106.2	
19	160.2			
19-OCH ₃	56.4	3.84 s (3H)		
20	100.0	6.47 d (1H, 2.2 Hz)	99.9	6.39 d (1H, 2.2 Hz)
21	163.5		166.1	
21-OCH ₃	55.7	3.89 s (3H)	55.9	3.83 s (3H)
22	111.5	6.50 d (1H, 2.2 Hz)	111.5	6.42 d (1H, 2.2 Hz)
23	144.0		144.0	
23-CH ₃	19.4	2.47 s (3H)	24.0	2.63 s (3H)
24	179.8		176.0	
25	52.0	4.45 dq (1H, 7.0, 7.0 Hz)	49.7	4.60 dq (1H, 7.2, 7.2 Hz)
26	17.0	1.46 d (3H, 7.0 Hz)	17.0	1.49 d (3H, 7.2 Hz)
27-NH		ND		ND
1'	102.5	5.05 d (1H, 7.8 Hz)	102.5	5.05 d (1H, 7.2 Hz)
2'	74.0	3.52 dd (1H, 7.8, 9.0 Hz)	74.0	3.51 dd (1H, 7.2, 9.0 Hz)
3'	70.6	3.40 dd (1H, 8.4, 9.0 Hz)	70.5	3.38 dd (1H, 8.4, 9.0 Hz)
4'	78.7	3.47 dd (1H, 8.4, 8.4 Hz)	78.7	3.47 dd (1H, 8.4, 9.6 Hz)
5'	78.5	3.46 ddd (1H, 2.2, 6.0, 8.4 Hz)	78.5	3.46 ddd (1H, 2.4, 6.0, 9.6 Hz)
6'	62.0	3.70 dd (1H, 6.0, 12.6 Hz)	62.0	3.67 dd (1H, 6.0, 12.6 Hz)
		3.86 dd (1H, 2.2, 12.6 Hz)		3.87 dd (1H, 2.4, 12.6 Hz)
Position	8		9	
	δC	δH	δC	δH
1			170.5	
2	123.5		123.8	
3	156.5		156.7	
4	107.5	6.67 d (1H, 2.2 Hz)	107.7	6.61 d (1H, 2.2 Hz)
5	152.5		152.9	
6	115.5	6.66 d (1H, 2.2 Hz)	115.7	6.68 d (1H, 2.2 Hz)
7	140.0		139.6	
7-CH ₃	19.0	2.37 s (3H)	19.4	2.41 s (3H)
9			166.8	
10	122.5		123.1	
11	156.0		156.2	
12	108.0	7.04 d (1H, 2.0 Hz)	108.5	7.10 d (1H, 2.0 Hz)
13	153.8		153.1	
14	118.5	6.88 d (1H, 2.0 Hz)	118.7	6.91 d (1H, 2.0 Hz)
15	140.0		138.8	
15-CH ₃	19.0	2.41 s (3H)	19.4	2.43 s (3H)
17			166.3	
18	116.0		106.7	
19	160.2		170.8	
19-OCH ₃	56.5	3.88 s (3H)		
20	97.0	6.47 d (1H, 2.2 Hz)	113.9	6.39 d (1H, 2.2 Hz)

Table 3 Continued

Position	8		9	
	δC	δH	δC	δH
21	163.5		166.0	
21-OCH ₃	56.5	3.85 s (3H)	55.9	3.83 s (3H)
22	108.0	6.50 d (1H, 2.2 Hz)	112.0	6.43 d (1H, 2.2 Hz)
23	139.0		144.4	
23-CH ₃	20.0	2.47 s (3H)	24.2	2.63 s (3H)
24	176.0		176.2	
25	49.5	4.61 dq (1H, 7.8, 7.8 Hz)	49.5	4.60 dq (1H, 7.5, 7.5 Hz)
26	17.5	1.46 d (3H, 7.8 Hz)	17.5	1.49 d (3H, 7.5 Hz)
27-NH		8.0 (1H, 7.8 Hz)		7.9 (1H, 7.5 Hz)
1'	100.5	5.19 d (1H, 8.4 Hz)	100.5	5.19 d (1H, 8.4 Hz)
2'	74.6	5.06 dd (1H, 8.4, 9.6 Hz)	74.6	5.06 dd (1H, 8.4, 9.6 Hz)
3'	76.0	3.65 dd (1H, 9.6 Hz)	76.1	3.63 dd (1H, 9.6 Hz)
4'	71.0	3.51 dd (1H, 9.6 Hz)	71.3	3.47 dd (1H, 9.6 Hz)
5'	78.5	3.55 ddd (1H, 1.8, 5.4, 9.6 Hz)	78.5	3.54 ddd (1H, 1.8, 6.0, 9.6 Hz)
6'	62.0	3.75 dd (1H, 5.4, 12.0 Hz)	62.3	3.71 dd (1H, 6.0, 12.0 Hz)
		3.93 dd (1H, 1.8, 12.0 Hz)		3.91 dd (1H, 1.8, 12.0 Hz)
7'	171.5		171.9	
8'	20.5	2.00 s (3H)	21.0	2.00 s (3H)
Position	10		11	
	δC	δH	δC	δH
2	123.9		117.5	
3	155.1		158.5	
3-OH			ND	
4	106.5	6.55 d (1H, 2.2 Hz)	107.2	6.61 d (1H, 2.2 Hz)
5	150.6		152.1	
6	113.3	6.52 d (1H, 2.2 Hz)	114.2	6.59 d (1H, 2.2 Hz)
7	137.5		139.5	
7-CH ₃	19.0	2.21 s (3H)	20.9	2.35 s (3H)
10	118.6		118.0	
11	156.2		156.4	
11-OH				10.47 br. S (1H)
12	107.1	6.65 d (1H, 2.0 Hz)	107.2	6.68 d (1H, 2.0 Hz)
13	152.2		152.1	
14	114.1	6.63 d (1H, 2.0 Hz)	114.5	6.65 d (1H, 2.0 Hz)
15	137.9		138.0	
15-CH ₃	19.0	2.32 s (3H)	19.2	2.35 s (3H)
18	114.5		106.7	
19	158.3		159.1	
19-OH				10.37 br. s (1H)
19-OCH ₃	56.1	3.83 (3H)		
20	96.4	6.53 d (1H, 2.2 Hz)	99.0	6.35 d (1H, 2.2 Hz)
21	161.8		162.2	
21-OCH ₃	55.4	3.80 s (3H)	56.0	3.75 s (3H)
22	107.3	6.50 d (1H, 2.2 Hz)	108.1	6.39 d (1H, 2.2 Hz)
23	137.9		139.5	
23-CH ₃	20.0	2.35 s (3H)	20.9	2.37 s (3H)
25	57.6	4.26br. d (1H, 7.0 Hz)		
26	29.6	2.10 m (1H)		
26-CH ₃	18.2	0.92 d (3H, 7.2 Hz)		
	19.2	0.95 d (3H, 7.2 Hz)		
27-NH		8.3 br. s		

Abbreviation: ND, not detected.

¹H (600 MHz) and ¹³C (125 MHz) NMR spectra were obtained in CD₃OD for **6** to **9** and in DMSO-*d*₆ for amidepsines **10** and **11**. The assignment of ¹³C NMR chemical shifts of **6** to **11** were facilitated by HMQC and HMBC experiments.

Figure 4 ^1H - ^1H COSY and selected heteronuclear multiple bond correlations (HMBC) for amidepsines F (6) to K (11).

Figure 5 Glucopyranosyl moiety in 7.

DISCUSSION

In this study, we found that when *Humicola* sp. FO-2942 was cultured under static conditions, the production of 1–4 increased markedly about 65- to 480-fold. At the same time, new 6–11 were isolated from the culture broth. From the structure elucidation, 6–9 were a new type of amidepsines having a sugar moiety at C-11. Interestingly, amidepsine E (5) produced by *Humicola* sp. FO-5969³ was not detected in the culture of strain FO-2942.

Glycosylated 6–9 were produced only under static conditions and their yields peaked on day 5 after inoculation; however, the production of nonglycosylated amidepsines continued to increase until day 9.

Table 4 Inhibition of DGAT1 and DGAT2 by amidepsines

Compound	$IC_{50}(\mu\text{M})$	
	DGAT1	DGAT2
6	>200	>200
7	>200	>200
8	>200	>200
9	>200	>200
10	40	40
11	>200	>200
12	>100	50
1 ^a	40	70
2 ^a	30	60
3 ^a	170	170
4 ^a	20	30

Abbreviation: DGAT, diacylglycerol acyltransferase.

^aData cited from Inokoshi *et al.*⁴

It is easy to speculate that amidepsine A/B is glycosylated to produce 6/7, and then acetylated at position 2 of the sugar residue to produce 8/9; therefore, glycosylation and acetylation occurred at a specific time (until day 5) after inoculation.

Compounds 1–4 are dual DGAT inhibitors with similar IC_{50} values for DGAT1 and DGAT2.⁷ Nonglycosylated 10 also has a similar characteristic. On the other hand, glycosylated 6–9 showed very weak or almost no activity against the two isozymes, indicating that the presence of a sugar moiety at C-11 is not compatible with DGAT inhibition.

METHODS

Materials

Strain and culture conditions. Fungal strain *Humicola* sp. FO-2942 was used for the production of amidepsines.^{1,2} Strain FO-2942 was grown and maintained on an agar slant containing 0.1% glycerol, 0.08% KH_2PO_4 , 0.02% K_2HPO_4 , 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02% KCl , 0.2% NaNO_3 , 0.02% yeast extract and 1.5% agar (pH 6.0). A loopful of spore of strain FO-2942 was inoculated into seed medium consisting of 2.0% glucose, 0.5% polypeptone, 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2% yeast extract, 0.1% KH_2PO_4 and 0.1% agar (pH 6.0) in a 500 ml Erlenmeyer flask. The inoculated flasks were shaken on a rotary shaker for 4 days at 27 °C to obtain the seed culture, which was transferred to production medium consisting of 2.0% sucrose, 1.0% glucose, 1.0% corn steep liquor, 0.5% meat extract, 0.1% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 ml of trace elements contained in g l^{-1} : 1.0 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.0 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 1.0 $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$, CaCO_3 0.3% and 0.1% agar (pH 6.0) and the fermentation was carried out under the following culture conditions: (a) jar fermentation: the seed culture (500 ml) was transferred to the production medium (30 l) in a 50-l jar fermenter. The fermentation was carried out at 27 °C for 3 days as previously described.¹ (b) Shaking and static culture: the seed culture (1 ml) was transferred into a 500-ml Erlenmeyer flask containing the production medium (100 ml). The first culture was carried out at 27 °C for 3 days on a rotary shaker (200 r.p.m.), and then the second culture was carried out at 27 °C for an additional 7 days under static conditions. (c) Static culture: the seed culture (1 ml) was transferred to the production medium (100 ml) in a 500-ml Erlenmeyer flask. The culture was carried out at 27 °C for 10 days under static conditions. Compounds 6–11 were obtained under culture condition c.

General experimental procedures

Kieselgel 60 (Merck KGaA, Darmstadt, Germany) and SSC-ODS-7515-12 (Senshu Scientific) were used for silica gel and octadecyl silyl (ODS) column

chromatography, respectively. HPLC was carried out using a LaChrom Elite HTA system (Hitachi High-Technologies, Tokyo, Japan). To determine the amount of amidepsine in culture broth, ethyl acetate extracts were dissolved in methanol and analyzed by LC-UV (HP1100 systems; Agilent Technologies, Santa Clara, CA, USA) under the following conditions; Pegasil ODS/5 mm column (4.6×250 mm; Senshu Scientific), a 30-min linear gradient from 5% CH₃CN/0.05% H₃PO₄ to 100% CH₃CN/0.05% H₃PO₄, 0.7 ml min⁻¹, and UV at 210 and 260 nm. Compounds **1–4** and **6–12** were eluted as peaks with retention times of 24.3, 24.8, 26.5, 27.3, 20.3, 20.7, 21.1, 21.6, 25.4, 28.4 and 25.0 min, respectively (Figure 2a).

Ultraviolet spectra were recorded on a spectrophotometer (DU640; Beckman Coulter, Fullerton, CA, USA). IR spectra were recorded on a Fourier transform infrared spectrometer (FT-710; HORIBA, Kyoto, Japan). Optical rotations were measured with a digital polarimeter (DIP-1000; JASCO, Tokyo, Japan). EI-MS spectra and HREI-MS spectra were recorded on a mass spectrometer (JMS-AX505HA; JEOL, Tokyo, Japan). Various NMR spectra were collected with a spectrometer (XL-400; Varian, Palo Alto, CA, USA).

Assay for diacylglycerol acyltransferase

The activity of DGAT1 and DGAT2 was measured in an enzyme assay using microsomal fractions prepared from *Saccharomyces cerevisiae* expressing human DGAT1 and DGAT2, as previously reported.^{4,7}

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