NOTES

Amidepsine E, an Inhibitor of Diacylglycerol Acyltransferase Produced by *Humicola* sp. FO-5969

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As we reported previously[1,2], amidepsines A to D were isolated from the culture broth of *Humicola* sp. FO-2942 as inhibitors of diacylglycerol acyltransferase[3] (acyl-CoA: 1,2-diacyl-*sn*-glycerol *O*-acyltransferase, abbreviated as DGAT) [EC 2.3.1.20]. In the course of our continuous screening, a fungal strain FO-5969 was found to produce different members of amidepsines. Amidepsine A and a new compound named amidepsine E (Fig. 1), were isolated from the culture broth of the strain. Amidepsine E was not detected in the FO-2942 culture broth prepared under the reported condition[1]. In this paper, the taxonomy of the producing strain, fermentation, isolation, biological properties and structure elucidation of amidepsine E are described.

The strain FO-5969 was isolated from a soil sample collected at Asaka, Saitama, Japan. On potato-dextrose agar, corn meal agar, malt extract agar and yeast extract-soluble starch (YpSs) agar, this strain grew rapidly to form white to gray colonies with diameters of 60~90 mm after incubation for 14 days at 25°C. At 37°C, growth is nil. Conidia, abundantly born on the colony surface, were solitary, and were produced directly on the vegetative hyphae or on short branches (Fig. 2). They were smooth, dark brown, unicellular, thin-walled, globose to subglobose, and 10~16 μm in size. The above characteristics indicated that the strain FO-5969 belongs to the genus *Humicola*[4].

A slant culture of the strain FO-5969 grown on YpSs agar was used to inoculate a 50-ml test tube containing 10 ml of the seed medium (glucose 2.0%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, Polypepton 0.5%, KH_2PO_4 0.1% and agar 0.1%, pH 6.0). The test tube was shaken on a reciprocal shaker for 3 days at 27°C. One ml of the seed culture was transferred into 100 ml of the production medium (sucrose 2.0%, glucose 1.0%, corn steep liquor 1.0%, meat extract 0.5%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, trace elements containing in g/liter: $FeSO_4 \cdot 7H_2O$ 1.0, $MnCl_2 \cdot 4H_2O$ 1.0, $ZnSO_4 \cdot 7H_2O$ 1.0, $CuSO_4 \cdot 5H_2O$ 1.0 and $CoCl_2 \cdot 2H_2O$ 1.0 (1 ml), $CaCO_3$ 0.3% and agar 0.1%, pH 6.0) in a 500-ml Erlenmeyer flask. The fermentation was carried out at 27°C under a stationary condition.

To the 13-day old whole broth (1.6 liters) was added 1500 ml of acetone. After the acetone extracts were filtered and concentrated, the resulting aqueous solution was extracted with 1500 ml of ethyl acetate. The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to dryness to yield a brownish oil (570 mg). The material suspended in 30% aq acetonitrile, was subjected to an octadecyl silyl (ODS) column (Senshu SSC-ODS-7515-12, 30 g). The materials were eluted stepwise with CH_3CN-0.05% H_3PO_4 solutions (300 ml each, 3:7, 5:5, 7:3 and 10:0, v/v), and each 30 ml of the eluate was collected. The 18th~19th fractions were combined, concentrated and extracted with ethyl acetate to give after evaporation a yellowish powder (28.0 mg). The resulting active powder was purified by preparative HPLC (YMC-pack D-ODS-5, 20×250 mm; 50% CH_3CN in 0.05% H_3PO_4; UV at 210 nm; 8.0 ml/minute). Under these conditions, amidepsines A and E were eluted as a peak with retention times of 54.0 and 57.0 minutes, respectively, each of which was concentrated and extracted with ethyl acetate to give pure amidepsine A (6.7 mg) as a pale yellow powder and impure amidepsine E (3.0 mg). Amidepsine E was finally purified by preparative HPLC (YMC-pack D-ODS-5, 20×250 mm; 45% CH_3CN in 0.05% H_3PO_4; UV at 210 nm; 9.0 ml/minute). It was eluted with a retention time of 62.0 minutes to give pure amidepsine E (2.9 mg) as a pale yellow powder.

Fig. 1. Structures of amidepsines A and E.

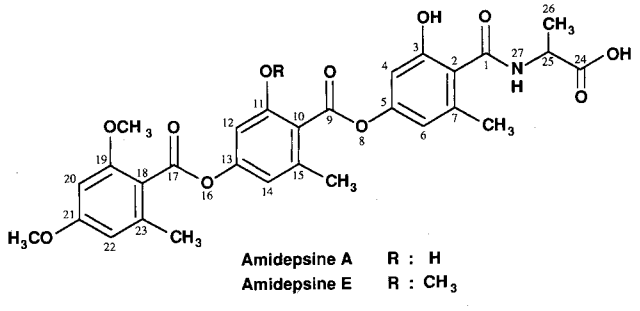


Fig. 2. Photomicrograph of conidia of strain FO-5969.

Bar represents 20 μm.

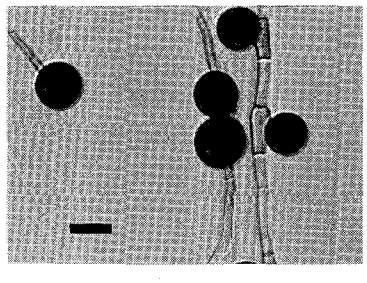
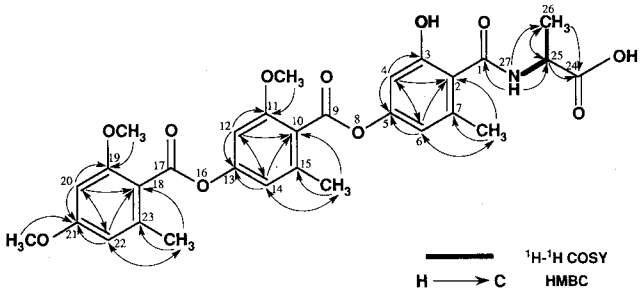


Table 1. Physico-chemical properties of amidepsine E.

Appearance	Pale yellow powder
Molecular weight	581
Molecular formula	$C_{30}H_{31}O_{11}N$
FAB-MS (*m/z*)	
Positive	582 $[M+H]^+$
	604 $[M+Na]^+$
Negative	580 $[M-H]^-$
HRFAB-MS (*m/z*)	
Calcd:	582.1975 (for $C_{30}H_{32}O_{11}N$)
Found:	582.1977
$[\alpha]_D^{28}$ (*c* 0.1, CH_3OH)	−7.0°
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	206 (75,500), 246 (14,000), 281 (10,400)
IR ν_{max}^{KBr} (cm^{-1})	3410, 2927, 1732, 1597, 1462, 1327, 1250, 1139
Melting point	215°C
Solubility	
Soluble:	CH_3OH, $CHCl_3$, CH_3CN, Acetone, C_2H_5OH, Ethyl acetate, DMSO
Insoluble:	H_2O

Fig. 3. ^1H-^1H COSY and HMBC experiments of amidepsine E.



Physico-chemical properties of amidepsine E are summarized in Table 1. The UV spectrum showed maxima at 206 (ε 75,500), 246 (ε 14,000) and 281 nm (ε 10,400), suggesting that amidepsine E has the same chromophore as amidepsine A[2].

The molecular formula of amidepsine E was determined to be $C_{30}H_{31}O_{11}N$ on the basis of high resolution fast atom bombardment mass spectra (HRFAB-MS) measurement (*m/z*, found 582.1977, calcd 582.1975 for $C_{30}H_{32}O_{11}N$ $[M+1]^+$). Comparison of the spectral data with those of amidepsine A indicated that from the molecular formula amidepsine E is larger by a CH_2 unit, and that the O-CH_3 (δ 3.87) proton was observed from the NMR spectra for amidepsine E in place of the corresponding hydroxy residue for amidepsine A[2]. In the ^1H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) experiments the long-range couplings was observed from C-11-OCH_3 (δ 3.87) to C-11 (δ 157.3). Thus, amidepsine E is suggested as 11-methoxy amidepsine A. The structure was confirmed by ^1H-^1H COSY and the HMBC experiments as shown in Fig. 3. Furthermore, the fragment ion peaks

Fig. 4. FAB-MS analysis of amidepsine E.

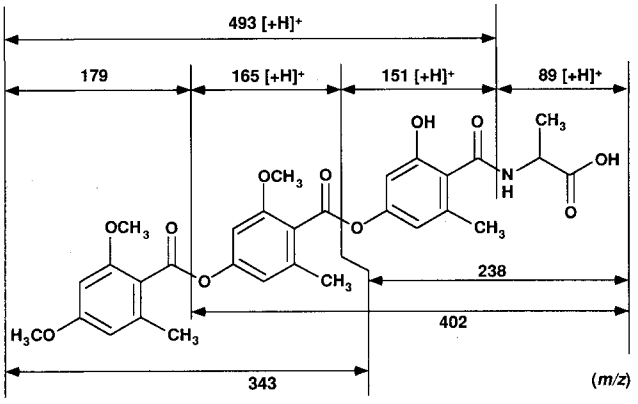


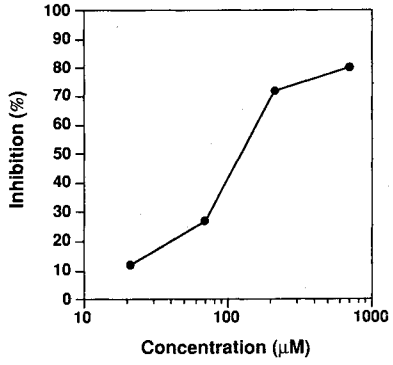
Table 2. ^1H and ^{13}C NMR chemical shifts of amidepsine E.

Carbon No.	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]
C-1	166.5	
C-2	123.7	
C-3	155.2	
C-3-OH		ND[c]
C-4	106.4	6.53 (1H, d, *J*=2.2 Hz)
C-5	150.4	
C-6	113.2	6.52 (1H, d, *J*=2.2 Hz)
C-7	137.6	
C-7-CH_3	18.9	2.24 (3H, s)
C-9	165.6	
C-10	120.1	
C-11	157.3	
C-11-OCH_3	56.5	3.87 (3H, s)
C-12	103.5	6.83 (1H, d, *J*=2.0 Hz)
C-13	152.4	
C-14	115.5	6.80 (1H, d, *J*=2.0 Hz)
C-15	137.3	
C-15-CH_3	18.8	2.37 (3H, s)
C-17	165.4	
C-18	114.3	
C-19	161.7	
C-19-OCH_3	55.4	3.80 (3H, s)
C-20	96.3	6.54 (1H, d, *J*=2.2 Hz)
C-21	158.4	
C-21-OCH_3	56.1	3.84 (3H, s)
C-22	107.2	6.51 (1H, d, *J*=2.2 Hz)
C-23	138.2	
C-23-CH_3	19.6	2.35 (3H, s)
C-24	174.0	
C-24-OH		ND[c]
C-25	47.9	4.31 (1H, dq, *J*=7.0, 7.0 Hz)
C-26	17.0	1.29 (3H, d, *J*=7.0 Hz)
27-NH		8.45 (1H, d, *J*=7.0 Hz)

[a] Chemical shifts are shown with reference to DMSO-d_6 as 39.5 ppm.
[b] Chemical shifts are shown with reference to DMSO-d_6 as 2.48 ppm.
[c] ND; not detected.

(Fig. 4) observed in FAB-MS spectrum supported the structure. Taken together, the structure of amidepsine E was elucidated as 2-hydroxy-4-[[2-methoxy-4-[(2,4-

Fig. 5. DGAT inhibition by amidepsine E in rat liver microsomes.

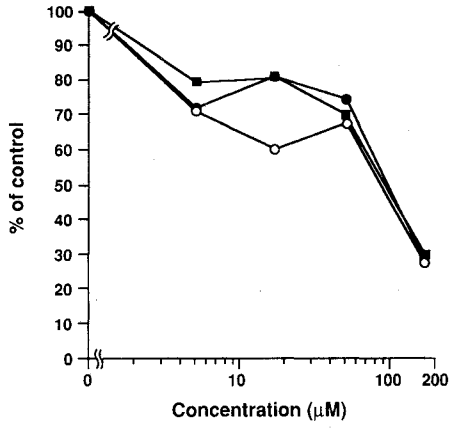


dimethoxy-6-methylbenzoyl)oxy]6-methylbenzoyl]-oxy]-6-methylbenzoic acid *N*-alanine amide (Fig. 1).

Amidepsine E inhibited DGAT activity dose-dependently in the enzyme assay using rat liver microsomes[1] (Fig. 5). Amidepsine E was found to be a very weak DGAT inhibitor with an IC_{50} value of 124 μM in comparison with amidepsine A with an IC_{50} value of 10.2 μM[1]. This indicates that the free hydroxy group at the C-11 position is responsible for eliciting potent DGAT inhibition. To investigate the specificity for DGAT inhibition, effects of amidepsine E was studied on triacylglycerol (TG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) syntheses in intact Raji cells[1] (Fig. 6). Amidepsine E showed no cytotoxic effect on Raji cells with the drug concentrations used. TG synthesis was inhibited dose-dependently with IC_{50} values of 91 μM for amidepsine E. However, the drug also inhibited both PC and PE syntheses to analogous extents, suggesting that it is not specific for DGAT inhibition.

Antimicrobial activity was tested using paper disks. No antimicrobial activity was observed at a concentration of 1 mg/ml (10 μg/paper disk) for amidepsine E against the following microorganisms; *Bacillus subtilis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Candida albicans*, *Saccharomyces sake*, *Pyricularia oryzae*, *Mucor racemosus* and *Aspergillus niger*.

Fig. 6. Effects of amidepsine E on triacylglycerol (TG; —■—), phosphatidylethanolamine (PE; —○—) and phosphatidylcholine (PC; —●—) syntheses in Raji cells.



Acknowledgments

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References

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