Amidepsines, Inhibitors of Diacylglycerol Acyltransferase Produced by *Humicola* sp. FO-2942

II. Structure Elucidation of Amidepsines A, B and C

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Structures of amidepsines A, B, C and D, diacylglycerol acyltransferase (DGAT) inhibitors, were determined by spectroscopic studies including various NMR measurements. They were elucidated as 2-hydroxy-4-[[2-hydroxy-4-[(2,4-dimethoxy-6-methylbenzoyl)oxy]6-methylbenzoyl]-oxy]-6-methylbenzoic acid *N*-alanine amide for amidepsine A, 2-hydroxy-4-[[2-hydroxy-4-[(2-hydroxy-4-methoxy-6-methylbenzoyl)oxy]6-methylbenzoyl]oxy]-6-methylbenzoic acid *N*-alanine amide for amidepsine B and 2-hydroxy-4-[[2-hydroxy-4-[(2-hydroxy-4-methoxy-6-methylbenzoyl)oxy]6-methylbenzoyl]oxy]-6-methylbenzoic acid *N*-valine amide for amidepsine C. Amidepsine D was identified with 2,4-di-*O*-methylgryphoric acid.

Amidepsines A to D were isolated from the culture broth of *Humicola* sp. FO-2942 as inhibitors of diacylglycerol acyltransferase (DGAT)[1]. Amidepsine D was identified with 2,4-di-*O*-methylgyrophoric acid, which was originally isolated as lichen tridepside[2]. We will report herein the structure elucidation of amidepsines A, B and C. They are fungal metabolites of a novel type having a tridepside linked with an amino acid.

Materials and Methods

Materials

Amidepsines were isolated from the culture broth of *Humicola* sp. FO-2942 as described in the preceding paper[1].

General Experimental Procedures

UV spectra were recorded on a Shimadzu UV-200S spectrophotometer. IR spectra were recorded on a Horiba FT-210 infrared spectrometer. Melting points were measured with a Yanaco micro melting point apparatus. Optical rotations were obtained with a JASCO DIP-370 digital polarimeter. EI-MS spectra were recorded on a JEOL JMS-D 100 mass spectrometer at 20 eV. FAB-MS spectra were recorded on a JMS-DX300 mass spectrometer. The various NMR spectra were obtained on a Varian XL-400 spectrometer.

Results

Physico-chemical Properties of Amidepsines

Physico-chemical properties of amidepsines A, B, C and D are summarized in Table 1. They showed similar absorption maxima at 209~218, 254~269 and 282~306 nm in the UV spectra (Fig. 1), suggesting the presence of the same chromophore in their structures. Amidepsines A, B and C had optical rotations, but amidepsine D had no optical rotation, suggesting to be an achiral structure only for amidepsine D.

Structure of Amidepsine A

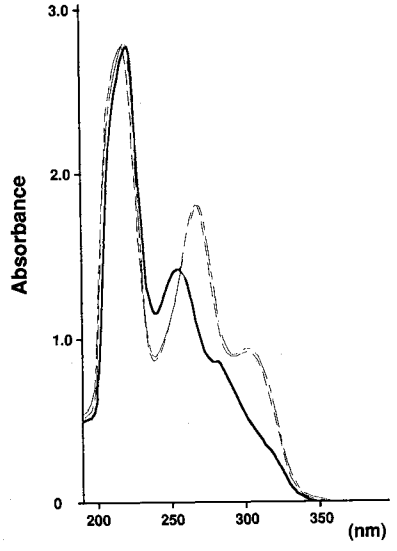
The molecular formula of amidepsine A was determined to be $C_{29}H_{29}NO_{11}$ on the basis of HRFAB-MS measurement (*m/z*, found 566.1674, calcd 566.1662 for $C_{29}H_{28}NO_{11}$ $(M-1)^+$). The ^{13}C NMR spectrum (DMSO-d_6) showed 29 resolved peaks (Fig. 2 and Table 2), which were classified into four $-CH_3$, one $-CH-$, two $-O-CH_3-$, six $-CH=$, and 16 quaternary carbons by analysis of the DEPT spectra. The ^1H NMR spectrum displayed 29 proton signals (Fig. 3 and Table 2). Two downfield singlet protons (δ 9.82 and 12.0~12.4) suggested the presence of hydrogen bonded OH protons. The results supported the molecular formula. The connectivity of proton and carbon atoms was confirmed by the HMQC spectrum (Table 2).

Analyses of ^1H-^1H COSY spectrum and ^{13}C-^1H long-range couplings of ^2J and ^3J observed in the HMBC spectrum revealed the four partial structures I~IV as shown in Fig. 4. 1) ^1H-^1H COSY couplings between NH-27 (δ 8.48) and H-25 (δ 4.34), and between H-25 and CH_3-26 (δ 1.30) showed the sequence of

Table 1. Physico-chemical properties of amidepsines A, B, C and D.

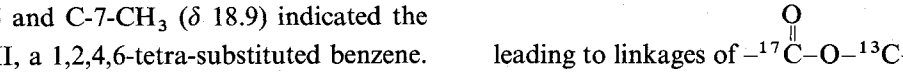
	Amidepsine A	Amidepsine B	Amidepsine C	Amidepsine D
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder	White powder
Molecular formula	$C_{29}H_{29}O_{11}N$	$C_{28}H_{27}O_{11}N$	$C_{30}H_{31}O_{11}N$	$C_{26}H_{24}O_{10}$
Molecular weight	567	553	581	496
FAB-MS (*m/z*)				
Positive	568 $[M+H]^+$	554 $[M+H]^+$	582 $[M+H]^+$	497 $[M+H]^+$
	590 $[M+Na]^+$	576 $[M+Na]^+$	604 $[M+Na]^+$	519 $[M+Na]^+$
Negative	566 $[M-H]^-$	552 $[M-H]^-$	580 $[M-H]^-$	495 $[M-H]^-$
HRFAB-MS (*m/z*) (negative)				
MF-H	$C_{29}H_{28}O_{11}N$	$C_{28}H_{26}O_{11}N$	$C_{30}H_{30}O_{11}N$	$C_{26}H_{23}O_{10}$
Calcd:	566.1662	552.1506	580.1819	495.1291
Found:	566.1674	552.1529	580.1822	495.1294
$[\alpha]_D^{25}$ (*c* 0.1, MeOH)	- 10 °	- 16 °	- 94°	0°
UV λ_{max}^{EtOH} nm (ε)	218 (35,000)	216 (34,000)	209 (37,400)	217 (30,700)
	254 (18,000)	266 (22,160)	269 (24,200)	250 (17,700)
	282 (10,600)	305 (11,100)	306 (12,300)	294 (8,900)
IR υ_{max}^{KBr} (cm^{-1})	1660, 1583, 1506	1656, 1583, 1400	1670, 1612, 1583	1668, 1606, 1419
	1410, 1246, 1136	1248, 1163, 1144	1313, 1248, 1142	1246, 1200, 1140

Fig. 1. UV spectra of amidepsines A, B and C (in EtOH).
A: ——, B: – – –, C: — — —.



NH–CH–CH_3. The long-range couplings from H_3-26 to C-24 (δ 174.2) and C-25 (δ 47.7), from NH-27 to C-1 (δ 166.5), C-25 and CH_3-26 (δ 16.9), and from H-25 to C-24 and CH_3-26 gave the partial structure IV, which contained an alanine moiety. 2) The long-range couplings from C-7-CH_3 (δ 2.24) to C-2 (δ 123.6), C-6 (δ 113.3) and C-7 (δ 137.6), from H-4 (δ 6.56) to C-2, C-3 (δ 155.0), C-5 (δ 150.5) and C-6, and from H-6 (δ 6.52) to C-2, C-4 (δ 106.4), C-5 and C-7-CH_3 (δ 18.9) indicated the partial structure III, a 1,2,4,6-tetra-substituted benzene. 3) The long-range couplings from 11-OH (δ 10.47) to C-10 (δ 118.5), from C-15-CH_3 (δ 2.35) to C-10, C-14 (δ 114.0) and C-15 (δ 137.86), from H-12 (δ 6.65) to C-10, C-11 (δ 156.1), C-13 (δ 152.1) and C-14, and from H-14 (δ 6.63) to C-10, C-12 (δ 107.0), C-13 and C-15-CH_3 (δ 19.2) indicated the partial structure II, another 1,2,4,6-tetra-substituted benzene. And 4) the long-range couplings from C-19-OCH_3 (δ 3.79) to C-19 (δ 158.2), from C-21-OCH_3 (δ 3.83) to C-21 (δ 161.7), from C-23-CH_3 (δ 2.32) to C-18 (δ 114.5), C-22 (δ 107.2) and C-23 (δ 137.90), from H-20 (δ 6.53) to C-18, C-19, C-21 and C-22, and from H-22 (δ 6.50) to C-18, C-20 (δ 96.4), C-21 and C-23-CH_3 (δ 19.4) indicated the partial structure I, a third 1,2,4,6-tetra-substituted benzene. Taking all data into consideration, the eight partial structures were confirmed (Fig. 4), which satisfied the molecular formula.

The sequence of the partial structures I ~ VIII (Fig. 4) was deduced as follows; first, the two hydroxy groups VII and VIII should be attached to C-3 in III and C-24 in IV, respectively, because of their chemical shifts and the additivity rule of substituents. Second, the ^{13}C chemical shifts of C-18 (δ 114.5) in I, C-10 (δ 118.5) in II and C-2 (δ 123.5) in III indicated that they are linked directly to carbons, that is, C-1 in IV, C-9 in V and C-17 in VI. It was plausible that C-18 is bound to C-17 (or C-9) to form a I-IV (or V) linkage, C-10 is to C-9 (or C-17) to form a II-V (or IV) linkage and C-2 is to C-1 to form a III-IV linkage. Finally, the ^{13}C chemical shifts of the two carbonyl carbons C-9 (δ 165.8) and C-17 (δ 165.6) indicated that they have a neighboring carbon,



leading to linkages of $-^{17}\overset{O}{\overset{\|}{C}}-O-^{13}C-$ and $-^{9}\overset{O}{\overset{\|}{C}}-O-^{5}C-$. Thus, the carbon alignment was finalized as I-VI (or V)-II-V (or VI)-III-IV.

The deduced structure was secured by further experiments. The NOEs were observed between 11-OH

Fig. 2. ^{13}C NMR spectrum of amidepsine A (100 MHz, DMSO-d_6).

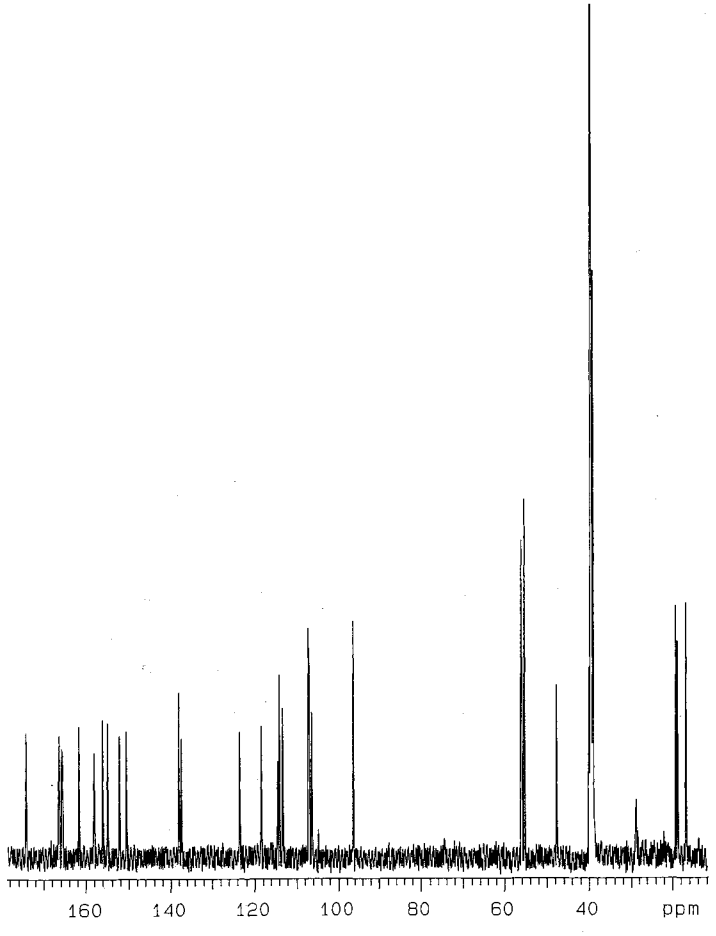
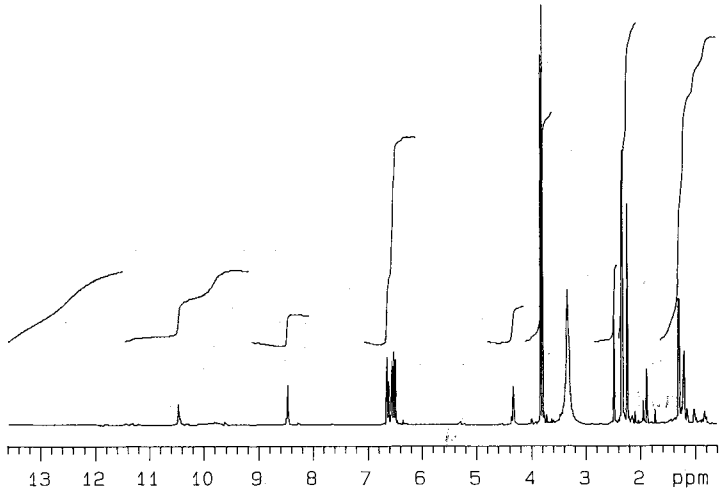


Fig. 3. ^{1}H NMR spectrum of amidepsine A (400 MHz, DMSO-d_6).



and both sp^2 methine protons H-4 and H-12, between H-14 and both methyl protons C-15-CH_3 and C-23-CH_3, and between H-4 and C-15-CH_3 (Fig. 5). Furthermore, the fragment ion peaks (Fig. 6) observed in FAB-MS spectrum supported the structure.

Taken together, the structure of amidepsine A was elucidated as 2-hydroxy-4-[[2-hydroxy-4-[(2,4-dimethoxy-6-methylbenzoyl)oxy]6-methylbenzoyl]oxy]-6-methylbenzoic acid *N*-alanine amide (Fig. 7).

Structures of Amidepsines B, C and D

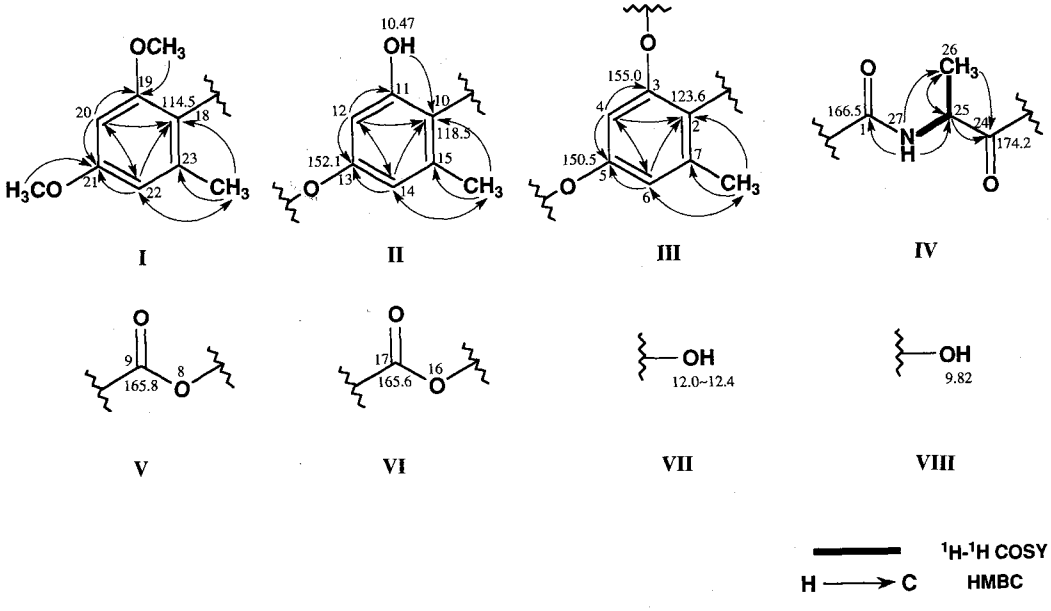
Comparison of the spectral data between amidepsines

Table 2. ^{1}H and ^{13}C NMR chemical shifts of amidepsines A, B, C and D.

Carbon No.	Amidepsine A		Amidepsine B		Amidepsine C		Amidepsine D	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-1	166.5		166.5		166.9 g)		170.4	
C-2	123.6		123.6		123.8		117.3	
C-3	155.0		155.0		155.2		158.8	
C-3-OH		12.0~12.4 (1H, brs)		11.9~12.1 (1H, brs)		10.51 (1H, brs)		ND i)
C-4	106.4	6.56 (1H, d, J=2.2 Hz)	106.4	6.56 (1H, d, J=2.2 Hz)	106.5	6.53 (1H, d, J=2.2 Hz)	107.1	6.61 (1H, d, J=2.2 Hz)
C-5	150.5		150.5		150.4		152.2	
C-6	113.3	6.52 (1H, d, J=2.2 Hz)	113.3	6.53 (1H, d, J=2.2 Hz)	113.2	6.51 (1H, d, J=2.2 Hz)	114.3	6.59 (1H, d, J=2.2 Hz)
C-7	137.6		137.8 e)		137.4 h)		139.5	
C-7-CH3	18.9	2.24 (3H, s)	18.9	2.24 (3H, s)	19.0	2.21 (3H, s)	20.9	2.363 (3H, s)
C-9	165.8 c)		166.7 f)		166.7 g)		165.6	
C-10	118.5		118.4		118.4		118.2	
C-11	156.1		156.1		156.1		156.3	
C-11-OH		10.47 (1H, s)		13.8 (1H, s)		10.40 (1H, s)		10.49 (1H, s)
C-12	107.0	6.65 (1H, d, J=2.0 Hz)	107.1	6.67 (1H, d, J=2.0 Hz)	107.0	6.66 (1H, d, J=2.0 Hz)	107.0	6.65 (1H, d, J=2.0 Hz)
C-13	152.1		152.0		152.0		152.1	
C-14	114.0	6.63 (1H, d, J=2.0 Hz)	114.1	6.65 (1H, d, J=2.0 Hz)	114.1	6.65 (1H, d, J=2.0 Hz)	114.0	6.63 (1H, d, J=2.0 Hz)
C-15	137.86 d)		137.6 e)		137.8 h)		137.9	
C-15-CH3	19.2	2.35 (3H, s)	19.2	2.35 (3H, s)	19.2	2.34 (3H, s)	19.3	2.358 (3H, s)
C-17	165.6 c)		165.9 f)		165.9 g)		165.6	
C-18	114.5		110.8		110.8		114.5	
C-19	158.2		159.1		159.1		158.2	
C-19-OCH3	55.4	3.79 (3H, s)					56.1	3.83 (3H, s)
C-19-OH				14.8 (1H, s)		11.8~11.9 (1H, brs)		
C-20	96.4	6.53 (1H, d, J=2.2 Hz)	99.0	6.36 (1H, d, J=2.2 Hz)	99.0	6.35(1H, d, J=2.2 Hz)	96.4	6.53 (1H, d, J=2.2 Hz)
C-21	161.7		162.1		162.1		161.7	
C-21-OCH3	56.1	3.83 (3H, s)	55.2	3.74 (3H, s)	55.2	3.74 (3H, s)	55.4	3.79 (3H, s)
C-22	107.2	6.50 (1H, d, J=2.2 Hz)	108.0	6.39 (1H, d, J=2.2 Hz)	108.0	6.38 (1H, d, J=2.2 Hz)	107.2	6.50 (1H, d, J=2.2 Hz)
C-23	137.90 d)		139.6		139.6		138.1	
C-23-CH3	19.4	2.32 (3H, s)	20.8	2.38 (3H, s)	20.8	2.36 (3H, s)	19.4	2.32 (3H, s)
C-24	174.2		174.2		173.1			
C-24-OH		9.82 (1H, brs)		9.8~9.9 (1H, brs)		9.82 (1H, brs)		
C-25	47.7	4.34 (1H, dq, J=7.0, 7.0 Hz)	47.7	4.34 (1H, dq, J=7.0, 7.0 Hz)	57.8	4.25 (1H, d, J=7.0 Hz)		
C-26	16.9	1.30 (3H, d, J=7.0 Hz)	16.9	1.30 (3H, d, J=7.0 Hz)	29.6	2.10 (1H, m)		
C-26-CH3					18.3	0.91 (3H, d, J=7.0 Hz)		
					19.3	0.94 (3H, d, J=7.0 Hz)		
27-NH		8.48 (1H, d, J=7.0 Hz)		8.47 (1H, d, J=7.0 Hz)		8.30 (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, d, e, f, g, h) Assignments bearing the same superscript are interchangeable. i) ND; not detected.

Fig. 4. ^{1}H-^{1}H COSY, HMBC and HMQC experiments of amidepsine A.



A and B indicated that a CH_2 unit is lacking in amidepsine B from the molecular formula (Table 1), and that the C-19-OH (δ 14.8) proton was observed for amidepsine B in place of the corresponding methoxy residue for amidepsine A from the NMR spectra (Table 2). The structure was confirmed by ^{1}H-^{1}H COSY and HMBC experiments as shown in Fig. 8, and was further confirmed by NOE and FAB-MS experiments (data not shown). Thus, amidepsine B is 2-hydroxy-4-[[2-hydroxy-4-[(2-hydroxy-4-methoxy-6-methylbenzoyl)oxy]6-

Fig. 5. NOE experiments of amidepsine A.

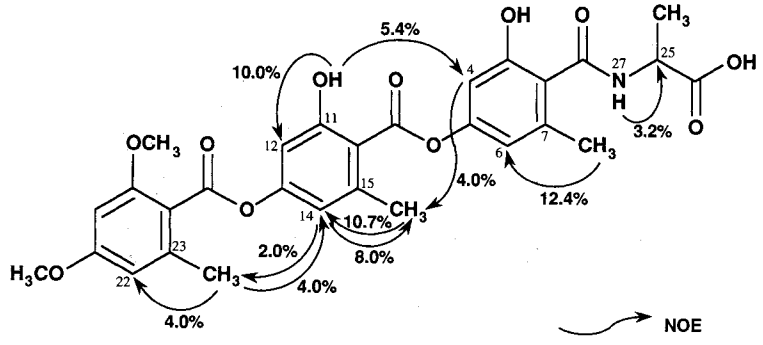


Fig. 6. FAB-MS analysis of amidepsine A.

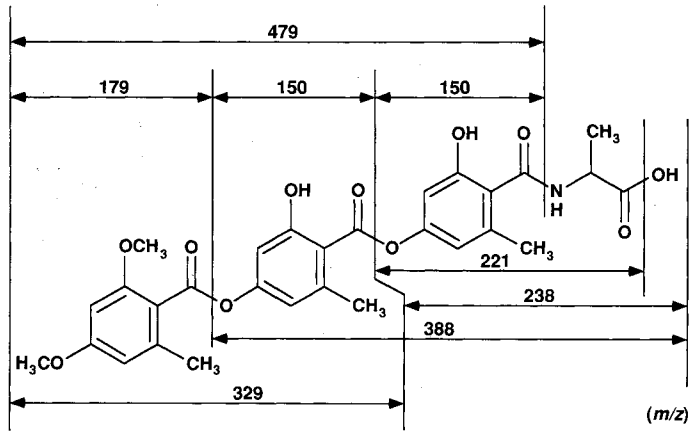
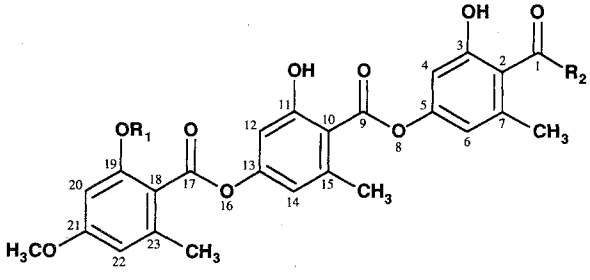


Fig. 7. Structures of amidepsines A, B, C and D (2,4-di-*O*-methylgyrophoric acid).



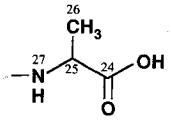
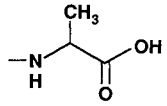
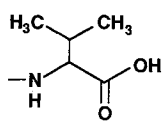
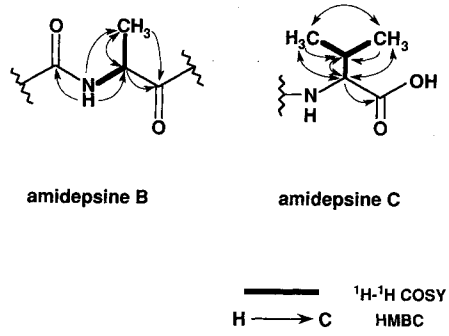
	$-R_1$	$-R_2$
Amidepsine A	$-CH_3$	
Amidepsine B	$-H$	
Amidepsine C	$-H$	
Amidepsine D	$-CH_3$	$-OH$

Fig. 8. ^1H-^1H COSY and HMBC experiments of amidepsines B and C.



methylbenzoyl]oxy]-6-methylbenzoic acid *N*-alanine amide as shown in Fig. 7.

The ^{13}C NMR spectrum (DMSO-d_6) of amidepsine C (Table 2) was similar to that of amidepsine B. The difference was found to lie in the amino acid moiety, Amidepsine C has a valine residue as evidenced by the dimethyl C-26-CH_3 (δ 18.3 and 19.3) and the methine C-26 (δ 29.6) carbons which were observed in the spectrum of amidepsine C. The structure was confirmed

by ^{1}H-^{1}H COSY and HMBC experiments (Fig. 8), and further confirmed by NOE and FAB-MS experiments (data not shown). Thus, amidepsine C was determined with the structure of 2-hydroxy-4-[[2-hydroxy-4-[(2-hydroxy-4-methoxy-6-methylbenzoyl)oxy]6-methylbenzoyl]oxy]-6-methylbenzoic acid *N*-valine amide (Fig. 7).

Comparison of the spectral data between amidepsines A and D indicated that an alanine moiety is lacking in amidepsine D from the molecular formula (Table 1). The structure was confirmed by NMR (Table 2) and FAB-MS experiments (data not shown). Taken together, amidepsine D is 2-hydroxy-4-[[2-hydroxy-4-[(2-hydroxy-4-methoxy-6-methylbenzoyl)oxy]6-methylbenzoyl]oxy]-6-methylbenzoic acid, which is identical with 2,4-di-*O*-methylgyrophoric acid (Fig. 7) as reported previously[2].

Discussion

From the structural analyses described in this paper, amidepsines are a novel group of fungal tridepsides combining an amino acid. Accordingly, they possess one chiral center at the amino acid moiety. To determine the stereochemistry, amidepsines were hydrolyzed with 6 N HCl containing 1% phenol at 115°C for 20 hours. Preliminary analyses of the hydrolysates by HPLC using a chiral column suggested that amidepsines A and B comprised a 3 : 2 mixture of L and D alanines, and that amidepsine C comprised a 3 : 2 mixture of L and D valines (data not shown). Further experiment is necessary to demonstrate amidepsines as enantiomixtures. However, natural products, actinomycete diolmycin A1 (discovered as an anticoccidial agent)[4] and limpet limatulone[5], in fact, were reported to be produced as enantiomixtures. If it is the case for amidepsines, it would be interesting to test which antipode shows the biological activity.

Acknowledgment

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References

1) TOMODA, H.; M. ITO, N. TABATA, R. MASUMA, Y. YAMAGUCHI & S. ŌMURA: Amidepsines, inhibitors of diacylglycerol acyltransferase produced by *Humicola* sp. I. Production, isolation and biological properties. J. Antibiotics 48: 937~941, 1995

2) ELIX, J. A.; V. K. JAYANTHI & C. C. LEZNOFF: 2,4-Di-*O*-methylgyrophoric acid and 2,4,5-tri-*O*-methylhiascic acid. New tridepsides from *Parmelia damaziana*. Aust. J. Chem. 34: 1757~1761, 1981

3) FRESENIUS, W.; J. F. K. HUBER, E. PUNGOR, G. A. RECHNITZ, W. SIMON & TH. S. WEST: *In* Tables of spectral data for structure determination of organic compounds. 2nd English ed. C120~125, Springer-Verlag, Berlin Heidelberg, 1989

4) SUNAZUKA, T.; N. TABATA, T. NAGAMITSU, H. TOMODA, S. ŌMURA & A. B. SMITH III: Asymmetric synthesis of the anticoccidial antibiotic diolmycin A1. Determination of absolute stereochemistry. Tetrahedron Lett. 34: 6659~6660, 1993

5) TAKIKAWA, H.; K. MORI & M. KIDO: Similarly, natural limatulone is produced as a mixture of (+), (−), M. 34th Symposium on the Chemistry of Natural Products, Symposium Papers; Tokyo, 1992; p 707