DELAMINOMYCINS, NOVEL EXTRACELLULAR MATRIX RECEPTOR ANTAGONIST

III. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION OF DELAMINOMYCINS B AND C

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As a result of our screening for inhibitors of cell adhesion to fibronectin (FN) and laminin (LM), components of the extracellular matrix (ECM), delaminomycin A (**1**, Fig. 1), a novel compound possessing an acyl tetramic acid moiety[1], has been isolated from the culture broth of *Streptomyces albulus* MJ202-72F3 which was deposited in the Fermentation Research Institute of the Agency of Industrial Science and Technology, Japan, with the accession No. FERM P-12674[2]. The strain also produced some compounds related to delaminomycin A as minor components. In this paper, we report the physico-chemical properties and structural elucidation of two components, delaminomycins B (**2**, Fig. 1) and C (**3**, Fig. 1). These compounds **2** and **3** also inhibited the binding of Con A stimulated-EL4 cells to FN and LM components of ECM[2].

The profile of purification procedures for delaminomycins are shown in Fig. 2. Briefly, **2** and **3** were purified by centrifugal partition chromatography (CPC), preparative reverse phase HPLC and Sephadex LH-20, successively. We isolated 30 mg of **2** and 13 mg of **3** as amorphous colorless powders from 10 liters of cultured broth.

The physico-chemical properties of **2** and **3** are summarized in Table 1.

The ultraviolet spectra of **2** and **3** were almost identical with that of **1** having two absorption maxima at 232 nm and 286~288 nm in methanol, suggesting the presence of α,β-unsaturated ketone. The molecular formulae of **2** and **3** were determined to be $C_{30}H_{45}NO_6$ and $C_{29}H_{43}NO_5$, respectively, by HRFAB-MS and ^{13}C NMR analyses.

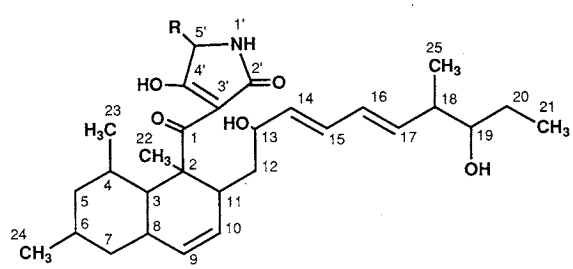
The structure of **1** ($C_{29}H_{43}NO_6$) has been determined by spectroscopic analyses. Complete ^1H and ^{13}C NMR assignments for **1** have been reported[1]. Thus, the structural elucidation of **2** and **3** has been carried out spectroscopically by comparing of the data with those of **1**.

Numbering shown in Fig. 1 is used to facilitate discussion of structure determination.

Combined analyses of ^1H-^1H COSY, ^{13}C-^1H COSY, and heteronuclear multiple bond correlation as well as ^1H NMR and ^{13}C NMR spectra indicated that the carbon skeleton of the three compounds was identical, and that they differed in the pyrrolidine moiety. The characteristic ultraviolet spectra of **2** and **3** suggested the preservation of acyl tetramic acid moiety[3~7].

The ^1H NMR spectrum of **2** indicated the presence of one OCH_3 group on the basis of chemical shift

Fig. 1. Structure of delaminomycins A, B and C.



Delaminomycin A (**1**) R=OH
Delaminomycin B (**2**) R=OCH_3
Delaminomycin C (**3**) R=H

Fig. 2. Purification procedure of delaminomycins A, B and C.

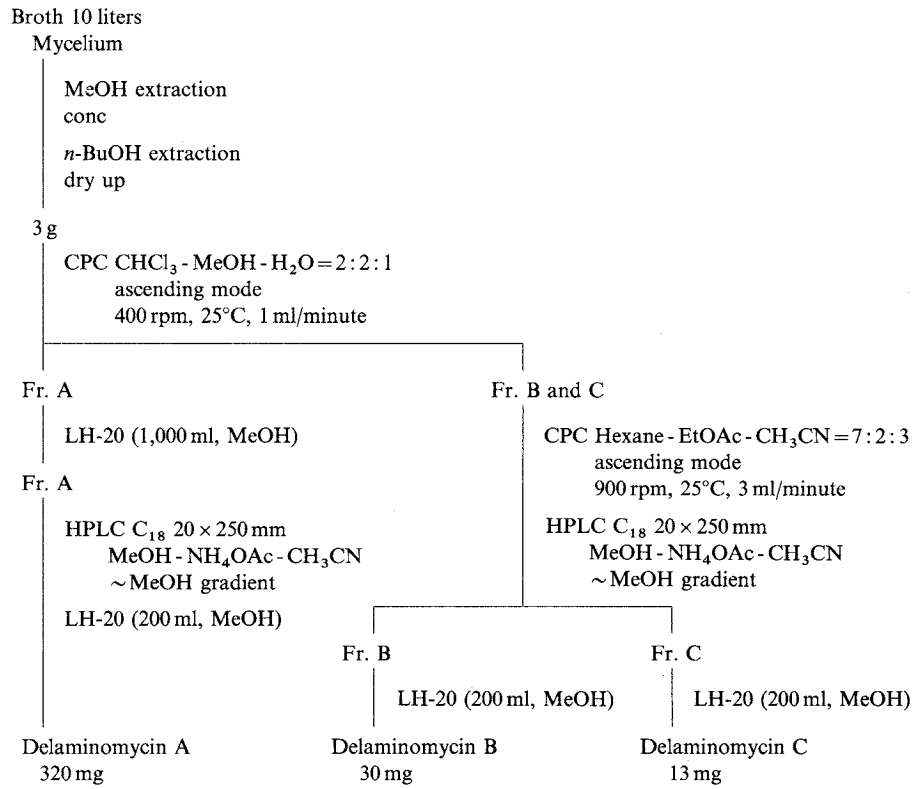


Table 1. Physico-chemical properties of delaminomycins B (**2**) and C (**3**).

Compound	**2**	**3**
Appearance	Colorless powder	Colorless powder
Molecular formula	$C_{30}H_{45}NO_6$	$C_{29}H_{43}NO_5$
Molecular weight	515	485
HRFAB-MS ($(M-H)^-$, m/z)	Calcd for $C_{30}H_{44}NO_6$	Calcd for $C_{29}H_{42}NO_5$
Calcd:	514.3169	484.3063
Found:	514.3163	484.3071
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	232 (563), 288 (138)	232 (530), 286 (144)
IR ν_{max}^{KBr} cm^{-1}	3400, 2950, 2900, 1670, 1600, 1430, 1400, 1100	3400, 2950, 2900, 1650, 1560, 1430, 1400, 900
Solubility: Soluble	MeOH, DMSO	MeOH, DMSO
Insoluble	*n*-Hexane, EtOAc, H_2O	*n*-Hexane, EtOAc, H_2O
Color reaction: Positive	Vanillin - H_2SO_4[b]	Vanillin - H_2SO_4[b]
Negative	Ninhydrin, dragendorff	Ninhydrin, dragendorff
Rf value on silica gel TLC[a]	0.74	0.77

[a] Merck Kieselgel 60 F_{254} Art. 5554: 2-PrOH - NH_4OH - H_2O (9 : 1 : 2).
[b] Purple red.

(δ 3.28) and its intensity (3H) as shown in Table 2. The ^{13}C NMR spectra of **1** and **2** were quite similar to each other except for one methoxy carbon signal at δ_c 52.6 absent in **1** (Table 3). The FAB-MS spectra of **1** and **2** showed a similar pattern with a mass difference of 14 daltons. Other chemical shifts of 29 carbon signals of **1** and **2** were almost identical. Therefore, the difference of structures between **1** and **2** was attributable to the replacement of >CH–OH in **1** with >CH–OCH_3 in **2** at C-5′ on the pyrrolidine

Table 2. ^{1}H NMR chemical shifts of **1**, **2** and **3** in methanol-d_4 (400 MHz).

Proton	Chemical shifts (δ) in ppm		
	1	**2**	**3**
3	1.90 m[a]	1.80 m[a]	1.69 m[a]
4	1.30 m[a]	1.34 m[a]	1.47 m[a]
5	1.11 m[a], 1.71 m[a]	1.02 m[a], 1.64 m[a]	1.12 m[a], 1.69 m[a]
6	1.63 m	1.62 m	1.66 m
7	0.91 m[a], 1.81 m[a]	0.88 m[a], 1.79 m[a]	0.91 m[a], 1.82 m[a]
8	1.81 m[a]	1.77 m[a]	1.86 m[a]
9	5.39 m	5.40 m	5.47 m
10	5.80 m	5.77 m	5.80 m
11	3.27 br	2.99 br	2.82 br
12	1.06 m[a], 1.78 m[a]	1.21 m[a], 1.47 m[a]	1.26 m[a], 1.41 m[a]
13	4.03 m	4.02 m	4.07 m
14	5.48 dd (6.0, 15.2)[b]	5.50 dd (6.2, 15.0)	5.48 dd (6.2, 14.4)
15	6.13 dd (10.0, 15.2)	6.10 dd (10.0, 15.0)	6.02 dd (10.0, 14.4)
16	5.79 m	5.99 dd (10.0, 15.0)	6.01 dd (10.0, 15.0)
17	5.57 dd (8.2, 15.2)	5.57 dd (8.0, 15.0)	5.58 dd (8.0, 15.0)
18	2.17 m	2.17 m	2.18 m
19	3.22 m	3.22 m	3.24 m
20	1.28 m[a], 1.48 m[a]	1.29 m[a], 1.53 m[a]	1.30 m[a], 1.53 m[a]
21	0.91 dd (7.2, 7.2)	0.92 dd (7.0, 7.0)	0.94 dd (6.2, 6.2)
22	1.50 s	1.41 s	1.53 s
23	0.84 d (6.2)	0.72 d (6.2)	0.78 d (6.2)
24	0.92 d (6.0)	0.91 d (6.0)	0.94 d (6.2)
25	0.99 d (6.0)	1.00 d (6.0)	1.01 d (6.4)
5′	4.82 s	4.77 s	3.72 br s
5′-OCH_3		3.28 s	

[a] Overlapping signals.
[b] J-values are in parentheses (Hz).

Table 3. ^{13}C NMR chemical shifts of **1**, **2** and **3** in methanol-d_4 (100 MHz).

Carbon No.	Chemical shifts (δ) in ppm			Carbon No.	Chemical shifts (δ) in ppm		
	1	**2**	**3**		**1**	**2**	**3**
1	204.8 s	204.2 s	203.3 s	16	131.6 d	131.0 d	130.9 d
2	51.5 s	51.6 s	50.5 s	17	138.1 d	137.8 d	138.2 d
3	45.5 d	46.4 d	45.5 d	18	44.3 d	44.3 d	44.3 d
4	40.5 d	38.6 d	38.9 d	19	78.1 d	77.9 d	77.9 d
5	48.3 t	47.8 t	47.6 t	20	28.3 t	28.4 t	28.4 t
6	35.7 d	34.8 d	34.9 d	21	11.0 q	10.7 q	10.7 q
7	44.3 t	44.2 t	43.7 t	22	15.4 q	16.4 q	16.3 q
8	43.5 d	41.9 d	42.3 d	23	24.3 q	23.7 q	23.5 q
9	130.8 d	131.0 d	131.5 d	24	22.9 q	22.8 q	22.8 q
10	129.1 d	128.6 d	128.0 d	25	16.5 q	16.4 q	16.3 q
11	37.2 d	38.6 d	44.3 d	2′	181.5 s	179.6 s	179.8 s
12	43.6 t	43.8 t	44.0 t	3′	101.3 s	104.4 s	103.0 s
13	71.6 d	71.0 d	70.5 d	4′	192.9 s	189.2 s	192.6 s
14	137.1 d	136.7 d	136.4 d	5′	79.7 d	86.1 d	51.1 t
15	131.0 d	130.6 d	130.9 d	5′-OCH_3		52.6 q	

ring as shown in Fig. 1.

The diene system was established to be 14E,16E based on the coupling constants ($J_{14,15}$=15.0 Hz, $J_{16,17}$=15.0 Hz). Thus, the structure of **2** was concluded to be 3-[[2-[(3E,5E)-2,8-dihydroxy-7-methyl-3,5-decadienyl]-1,6,8-trimethyl-1,2,4a,5,6,-

7,8,8a-octahydro-1-naphthyl]carbonyl]-5-methoxy pyrrolidine-2,4-dione as shown in Fig. 1.

On the other hand, comparison of the ^{13}C NMR data of **3** with that of **1** revealed an upfield shift of C-5′ carbon on the pyrrolidine ring from δ79.7 in **1** to δ 51.1 in **3**. By the DEPT experiment, C-5′ carbon in **3** was shown to be a methylene. The remaining 28 carbon signals in the two compounds were almost identical. Accordingly, the difference between the two compounds could be attributable to presence or absence of a hydroxy group at C-5′ position in **3**.

Thus, the structure of **3** was concluded to be 3-[[2-[(3*E*,5*E*)-2,8-dihydroxy-7-methyl-3,5-decadienyl]-1,6,8-trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1-naphthyl]carbonyl]pyrrolidine-2,4-dione as shown in Fig. 1. The ^{13}C chemical shifts for tetramic acid moiety of **3** were in good accordance with those of lydicamycin as follows; C-2′; δ 179.8 vs δ 181.0, C-5′; δ 51.1 vs δ 50.7, C-4′; δ 192.6 vs δ 192.3)[5]. Determination of the stereochemistry of **2** and **3** together with **1** remains to be elucidated.

References

1) UENO, M.; T. SOMENO, R. SAWA, H. IINUMA, H. NAGANAWA, M. ISHIZUKA & T. TAKEUCHI: Delaminomycins, novel nonpeptide extracellular matrix receptor antagonist and a new class of potent immunomodulator. II. Physico-chemical properties and structure elucidation of delaminomycin A. J. Antibiotics 46: 972～978, 1993

2) UENO, M.; M. AMEMIYA, M. OSONO, N. KINOSHITA, T. IKEDA, H. IINUMA, M. HAMADA, M. ISHIZUKA & T. TAKEUCHI: Delaminomycins, novel nonpeptide extracellular matrix receptor antagonist and a new class of immunomodulator. I. Taxonomy, fermentation, isolation and biological activity. J. Antibiotics 46: 719～727, 1993

3) STICKINGS, C. E.: Metabolites of *Alternaria tenuis* Auct.: The structure of tenuazonic acid. Biochem. J. 72: 332～340, 1959

4) HAYAKAWA, Y.; N. KANAMARU, A. SHIMAZU & H. SETO: Lydicamycin, a new antibiotic of a novel skeletal type. I. Taxonomy, fermentation, isolation and biological activity. J. Antibiotics 44: 282～287, 1991

5) HAYAKAWA, Y.; N. KANAMARU, N. MORISAKI, K. FURIHATA & H. SETO: Lydicamycin, a new antibiotic of a novel skeletal type. II. Physico-chemical properties and structure elucidation. J. Antibiotics 44: 288～292, 1991

6) KARWOWSKI, J. P.; M. JACKSON, R. J. THERIAULT, G. J. BARLOW, L. COEN, D. M. HENSEY & P. E. HUMPHREY: Tirandalydigin, a novel tetramic acid of the tirandamycin-streptolydigin type. I. Taxonomy of the producing organism, fermentation and biological activity. J. Antibiotics 45: 1125～1132, 1992

7) KACZKA, E. A.; C. O. GITTERMAN, E. L. DULANEY, M. C. SMITH, D. HENDLIN, H. B. WOODRUFF & K. FOLKERS: Discovery of inhibitory activity of tenuazonic acid for growth of human adenocarcinoma-1. Biochem. Biophys. Res. Commun. 14: 54～57, 1964