DELAMINOMYCINS, NOVEL NONPEPTIDE EXTRACELLULAR MATRIX RECEPTOR ANTAGONIST AND A NEW CLASS OF POTENT IMMUNOMODULATOR

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION OF DELAMINOMYCIN A

MITSUHIRO UENO, TETSUYA SOMENO, RYUICHI SAWA†, HIRONOBU IINUMA†, HIROSHI NAGANAWA†, MASAAKI ISHIZUKA* and TOMIO TAKEUCHI

Institute for Chemotherapy, Microbial Chemistry Research Foundation, 18-24 Aza-Motono, Miyamoto, Numazu-shi, Shizuoka 410-03, Japan
†Institute of Microbial Chemistry, Microbial Chemistry Research Foundation, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication December 3, 1992)

Delaminomycins, novel extracellular matrix receptor antagonists, have been isolated from a culture broth of *Streptomyces albulus* MJ202-72F3. The structure of delaminomycin A was determined 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]-5-hydroxypyrrolidine-2,4-dione by analyses of spectral properties and chemical studies.

In the course of screening for inhibitors of cell adhesion to fibronectin, laminin and collagen type IV components of the extracellular matrix, we found new nonpeptide antibiotics, delaminomycins, in the cultured mycelium of *Streptomyces albulus* MJ202-72F3. In the preceding paper[1], the taxonomy of the producing strain and production, isolation, purification and biological activities of delaminomycins were reported.

In this paper, we report the physico-chemical properties and structural elucidation of delaminomycin A.

Results and Discussion

Physico-chemical Properties of Delaminomycin A (1)

The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was determined to be $C_{29}H_{43}NO_6$ by FAB-MS, ^{13}C NMR and elemental analysis. The infrared spectrum of delaminomycin A showed absorption at 3400 (NH, OH), 2980~2860 (CH), 1660, 1590 cm^{-1} (C=O, C=C). The ultraviolet spectrum of **1** indicated the presence of α,β-unsaturated ketone. **1** is soluble in MeOH and DMSO, but insoluble in *n*-hexane, EtOAc and water.

Structural Elucidation of Delaminomycin A

The molecular formula of **1** was found to be $C_{29}H_{43}NO_6$. All 29 carbons containing minor concomitant signals due to the presence of the possible equilibrium structures were visible in ^{13}C NMR spectrum of **1** (Table 2). In the DEPT spectra of **1**, 5 quaternary carbons, 15 methines, 4 methylenes and 5 methyls were shown.

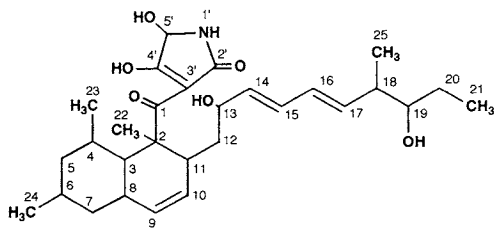
Treatment of **1** with 1N HCl-MeOH (1:3, v/v) gave **2** (Fig. 1). Based on the FAB-MS data and elemental analysis, the molecular formula of **2** was established to be $C_{29}H_{41}NO_5$, suggesting the dehydrated

Table 1. Physico-chemical properties of delaminomycin A (**1**) and its derivative (**2**).

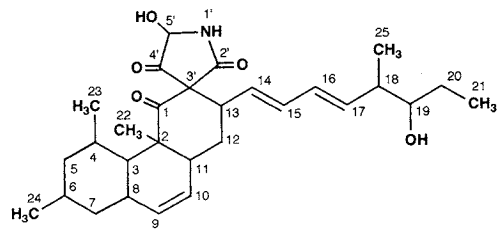
Compound	**1**	**2**
Appearance	White powder	White powder
FAB-MS *m/z*	500 $(M-H)^-$	482 $(M-H)^-$
Molecular weight	501	483
Molecular formula	$C_{29}H_{43}NO_6$	$C_{29}H_{41}NO_5$
Elemental analysis calcd. for	$C_{29}H_{43}NO_6 \cdot H_2O$	$C_{29}H_{41}NO_5$
Calcd:	C 67.03, H 8.73, N 2.70, O 21.55	C 72.02, H 8.54, N 2.90, O 16.54
Found:	C 67.37, H 8.79, N 2.61, O 21.22	C 71.51, H 8.52, N 3.25, O 16.63
UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$) nm	232 (728), 288 (208)	231 (614)
IR ν_{max}^{KBr} cm^{-1}	3400, 2950, 2900, 1660, 1590, 1430, 1100, 950	3350, 2950, 2900, 1790, 1700, 1680, 1450, 1250, 1080, 990
Solubility: Soluble	MeOH, DMSO	$CHCl_3$, EtOAc, MeOH, DMSO
Insoluble	*n*-Hexane, EtOAc, H_2O	*n*-Hexane, H_2O
Color reaction: Positive	Vanillin-H_2SO_4	Vanillin-H_2SO_4
Negative	Ninhydrin, Dragendorff	Ninhydrin, Dragendorff
Silica gel TLC: Rf value 1)	0.49	0.84
2)	0	0.67

1) On Merck Kieselgel 60 F_{254} Art. 5554 (2-PrOH - NH_4OH - H_2O = 9 : 1 : 2).
2) $CHCl_3$ - MeOH - NH_4OH = 40 : 10 : 1.

Fig. 1. Structures of delaminomycin A (**1**) and its derivative (**2**).



1
Delaminomycin A



2

compound of **1** (Table 1). There exists no significant change in the ^{13}C NMR spectra between **1** and **2** except for the chemical shifts of C-3′ and C-13 signals (Tables 2 and 3). Numbering depicted in the structures is used to facilitate discussion of this paper as shown in Fig. 1. The structural studies were carried out first for **2**, because ^1H NMR and ^{13}C NMR spectra of **2** in $CDCl_3$ or CD_3OD were simple and fully analyzable.

The structure of **1** was subsequently determined by comparing their spectral data with those of **2**.

The partial structure of a long side chain attached to C-11 of a dehydro decalin ring was confirmed by analyses of ^1H-^1H COSY and ^{13}C-^1H COSY experiments as shown in Fig. 2. The presence of decalin ring was established by the heteronuclear multiple bond correlation (HMBC)[2] spectrum of **2** as follows. As shown in Fig. 3, olefinic proton of 9-CH (δ_H 5.42) showed couplings to C-7 and C-8. Methyl protons of 24-CH_3 (δ_H 0.89) were coupled to C-5, C-6 and C-7. Methyl protons of 22-CH_3 (δ_H 1.08) and 23-CH_3 (δ_H 0.73) showed couplings to C-11, C-2, C-3 and C-3, C-4, C-5, respectively.

Furthermore, the methyl protons of 22-CH_3 (δ_H 1.08) showed coupling to a carbonyl carbon (C-1, δ_C 214.0), indicating the presence of carbonyl group situated adjacent to C-2 quaternary carbon on the decalin ring.

Table 2. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR spectral data for **1** in methanol-d_4.

Carbon No.	Major carbon	Minor carbon	Major proton	Minor proton	Carbon No.	Major carbon	Minor carbon	Major proton	Minor proton
1	204.8	s			15	131.0	130.9 d	6.13	5.89
2	51.5	51.3 s			16	131.6	d	5.79	
3	45.5	d	1.90		17	138.1	137.5 d	5.57	5.51
4	40.5	39.8 d	1.30	1.28	18	44.3	44.2 d	2.17	
5	48.3	48.1 t	1.71,	1.73,	19	78.1	77.9 d	3.22	
			1.11	1.41	20	28.3	28.1 t	1.48,	
6	35.7	35.2 d	1.63	1.62				1.28	
7	44.3	44.0 t	1.81,		21	11.0	10.8 q	0.91	
			0.91		22	15.4	15.2 q	1.50	1.46
8	43.5	43.0 d	1.81		23	24.3	q	0.84	
9	130.8	d	5.39		24	22.9	q	0.92	
10	129.1	129.0 d	5.80		25	16.5	16.2 q	0.99	0.97
11	37.2	36.8 d	3.27	3.15	1′-NH				
12	43.6	43.3 t	1.78,	1.50,	2′	181.5	180.1 s		
			1.06	0.95	3′	101.3	100.9 s		
13	71.6	71.4 d	4.03	3.94	4′	192.9	192.5 s		
14	137.1	136.6 d	5.48	5.31	5′	79.7	79.6 d	4.82	

Major and minor signals may be interchanged.

Table 3. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR spectral data for **2** in methanol-d_4.

Carbon No.	δ_C	δ_H		Carbon No.	δ_C	δ_H	
1	214.0 s			16	130.9 d	5.93 dd (11.0, 15.0)	
2	55.3 s			17	138.6 d	5.58 dd (8.0, 15.0)	
3	44.9 d	2.43 dd (10.0, 10.0)[c]		18	44.2 d	2.18 m	
4	37.1 d	1.51[a] m[b]		19	77.8 d	3.24 m	
5	48.4 t	1.55[a] m[b]	0.91 m[b]	20	28.2 t	1.48[a] m	1.30 m
6	34.5 d	1.57[a] m[b]		21	10.8 q	0.93 dd (7.0, 7.0)	
7	43.9 t	1.74[a] m[b]	0.96 m[b]	22	17.7 q	1.08 s	
8	41.9 d	1.82 m		23	19.4 q	0.73 d (6.8)	
9	131.5 d	5.42[a] m[b]		24	22.6 q	0.89 d (6.4)	
10	128.5 d	5.64 m		25	16.1 q	0.99 d (6.6)	
11	47.6 d	2.02 m		1′	NH		
12	33.8 t	2.49 ddd (13.3, 13.3, 13.3)	1.70[a] m[b]	2′	170.3 s		
13	45.0 d	2.92 ddd (4.0, 7.8, 13.3)		3′	73.6 s		
14	129.9 d	5.39[a] m[b]		4′	207.1 s		
15	134.9 d	6.04 dd (11.0, 15.0)		5′	80.9 d	5.28 s	

[a] Signals may be interchanged.
[b] Overlapping signals.
[c] *J*-Values are in parentheses (Hz).

These results established the partial structure representing C-1 to C-24 of **2** (Fig. 1).

One diene system was established to be 14*E*,16*E* by $J_{14,15}=15.0$ Hz, $J_{16,17}=15.0$ Hz. Additionally, a large coupling constant ($J=10.0$ Hz) between 3-H and 8-H indicated a *trans* junction for the decalin ring.

From results of ^{13}C NMR, FAB-MS and elemental analysis, the presence of >C=O, –CO–NH–, >CH–OH and one quaternary carbon were indicated as the remaining functions of **2**.

Since the chemical formula of **2** ($C_{29}H_{41}NO_5$) has ten unsaturation number, the remaining unit should form a five membered ring. Furthermore, **2** was predicted to contain one more ring system. Thus, the presence of spiro structure was suggested in **2**. The final problem is, therefore, to decide the positions of

Fig. 2. Partial structures obtained from ^1H-^1H COSY spectrum for **2** in methanol-d_4.

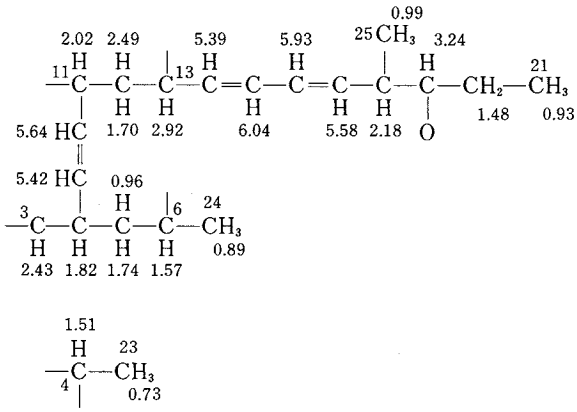


Fig. 3. Proton-carbon correlation of **2** by HMBC in methanol-d_4.

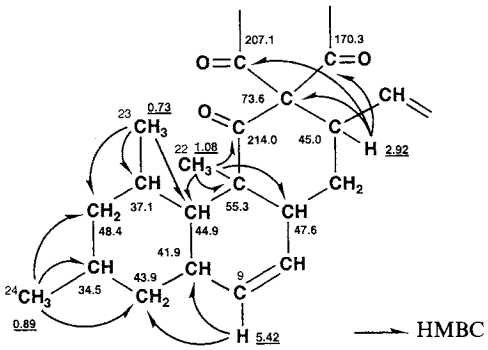
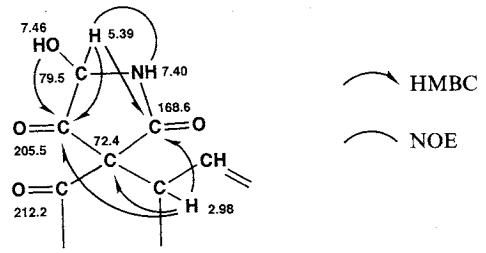


Fig. 4. Proton-carbon correlation of **2** by HMBC and NOE in $CDCl_3$.



the above described groups in the molecule of **2**. By the HMBC experiment for **2**, ^1H-^{13}C long range couplings were observed in CD_3OD between C-13 methine proton (δ_H 2.92) and three quaternary carbons (C-3′, δ_C 73.6; C-2′, δ_C 170.3; C-4′, δ_C 207.1). The remaining >CH–OH and –NH– must be inserted between C-2′ and C-4′ resulting in the formation of a five membered ring. The proposed structure was further confirmed by the HMBC and NOE experiments for **2** in $CDCl_3$ as shown in Fig. 4.

From the above mentioned results, the structure of **2** was determined as shown in Fig. 1. ^1H and ^{13}C NMR assignments for **2** are summarized in Table 3.

The structural elucidation of **1** was then carried out by comparing the spectral data with those of **2**. The ^{13}C NMR spectrum of **1** revealed the presence of twenty nine carbons in **1** as well as in **2**. The ^{13}C NMR spectrum of **1** closely resembled that of **2** except for the chemical shifts of C-13 and C-3′. The DEPT spectra of both compounds revealed the same multiplicities for all carbons. The unsaturation numbers of **2** and **1** are calculated to be ten and nine, respectively, based on the molecular formulae. Taking into consideration of these facts, we predicted the spectral change was caused by the ring transformation from **1** to **2**.

Although the common partial structure shown in Fig. 5 was determined by the ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC spectra of **1**, the chemical shift of C-13 (δ_C 71.6) was significantly different from that

Fig. 5. ^1H-^1H COSY experiment (bold lines) and HMBC (arrow) for **1** in methanol-d_4.

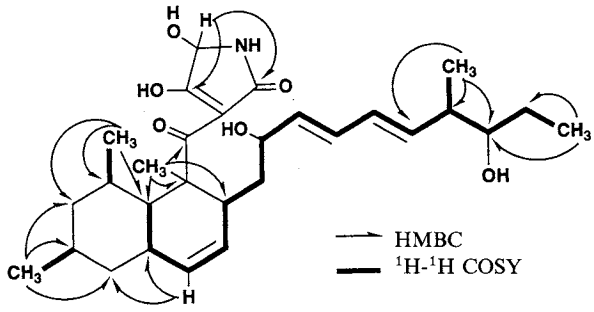
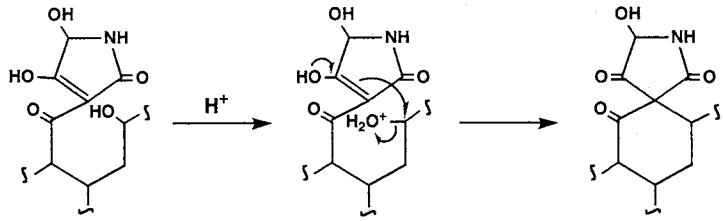


Fig. 6. Spiro ring formation of **2** from **1**.



1 **2**

of **2** (δ_C 45.0). This low field shift indicates that the C-13 methine carbon bears a hydroxyl group. Furthermore, C-3′ quaternary carbon at δ 73.6 observed in **2** moved to the low field at δ 101.3 in **1**, indicating the change of C-3′ quaternary carbon from sp^3 to sp^2.

From these information, the structure of **1** was determined as shown in Fig. 1. Assignments of signals for **1** are summarized in Table 2. The characteristic ultraviolet absorption of **1** supported the presence of tetramic acid moiety. The spiro compound **2** was reasonably ascribed by ring closure of **1** with diluted HCl-MeOH as shown in Fig. 6. **1** possesses an acyl tetramic acid moiety, although C-5′ is occupied with hydroxy group. So far as we know, **1** is the first reported compound consisting of a hydroxylated tetramic acid moiety[3~5].

^1H NMR and ^{13}C NMR spectra of **1** in CD_3OD were complicated due to the equilibrium structures caused by keto-enol tautomerism and labile hemiaminal moiety. Although the possible equilibrium structures are considered in **1**, the proportion of enol form shown in Fig. 1 is high on the basis of NMR studies.

Reduction of **2** with $NaBH_4$ gave tetrahydro derivatives as epimeric mixtures (MW 487). On the other hand, **1** remained to be unreduced, also suggesting the enol form resulted in a strong hydrogen bond between the 4′-hydroxy proton and the carbonyl group at C-1.

1 has a characteristic five membered alkanol amine moiety and showed strong antagonistic activity on cells binding to fibronectin and/or laminin components of extracellular matrix. The clarification of stereochemistry is now in progress.

Experimental

General

NMR spectra were recorded on a JEOL JNM-GX400 NMR spectrometer and mass spectra were

measured using a JEOL JMS-SX102 spectrometer. UV spectra were recorded on a Hitachi 228A spectrometer and IR spectra on a Hitachi 260-10 spectrometer.

Treatment of **1** with HCl-MeOH

To a solution of **1** (220 mg) in MeOH (3 ml), 1 N HCl (1 ml) was added and allowed to stand for 17 hours. The crude product was subjected to preparative HPLC (YMC-Pack A-043, silica gel, 20×250 mm, Yamamura Chem. Co., Ltd.) using a gradient system from solution A to solution B (solution A: *n*-hexane, solution B: $CHCl_3$-MeOH=2:1). Fractions containing **2** were combined and concentrated under reduced pressure and then injected to a preparative HPLC (YMC-Pack SH-343, ODS, 20×250 mm, Yamamura Chem. Co., Ltd.) and eluted with a linear gradient from 80% MeOH to MeOH. The fractions containing **2** were combined and concentrated under reduced pressure. The residue was loaded onto a Sephadex LH-20 column (22×53 mm) and eluted with MeOH. The fractions containing **2** were combined and concentrated to give 56.5 mg of a white powder in 25.7% yield. UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$); 231 nm (614). IR ν_{max}^{KBr}; 1790, 1700 and 1680 cm^{-1}. FAB-MS *m/z*; 482 $(M-H)^-$.

Reduction of **2** with Sodium Borohydride

A solution of **2** (15 mg) in MeOH was hydrogenated with sodium borohydride (3 mg) at room temperature for 28 hours. The reaction mixture was evaporated to dryness. The crude material was purified by a preparative HPLC (YMC-Pack SH-343, ODS, 20×250 mm, Yamamura Chem. Co., Ltd.) using a linear gradient from 80% MeOH to MeOH. The fractions which showed a single spot of vanillin-H_2SO_4 with Rf 0.44 on a silica gel TLC plate ($CHCl_3$-MeOH-NH_4OH=40:10:1) were combined and concentrated. The residue was subjected to Sephadex LH-20 chromatography (22×53 mm) using MeOH as an eluent. The vanillin-H_2SO_4 positive fractions were combined and concentrated to give 8.6 mg of a white powder (yield 57.3%). UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$); 236 nm (728). IR ν_{max}^{KBr}; 1680 cm^{-1}. FAB-MS *m/z*; 488 $(M+H)^+$ and 486 $(M-H)^-$.

References

1) Ueno, M.; M. Amemiya, M. Osono, N. Kinoshita, T. Ikeda, H. Iinuma, M. Hamada, M. Ishizuka, T. Takeuchi: Delaminomycins, novel nonpeptide extracellular matrix receptor antagonists and a new class of immunomodulator. I. Taxonomy, fermentation, isolation and biological activity. J. Antibiotics 46: 719~727, 1993
2) Bax, A. & M. F. Summers: ^1H and ^{13}C assignments from sensitivity-enhanced defection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986
3) Sato, Y.; S. Miyadoh, Y. Takeuchi, T. Mikawa, N. Yoshikawa & H. Ohkishi: Vermisporin, a new antibiotic active against anaerobic bacteria. Program and Abstracts of 30th Intersci. Conf. on Antimicrob. Agents Chemother. No. 433, Atlanta, 1990
4) Hayakawa, Y.; N. Kanamaru, N. Morisaki, K. Furihata & H. Seto: Lydicamycin, a new antibiotic of novel skeletal type. II. Physico-chemical properties and structure elucidation. J. Antibiotics 44: 288~292, 1991
5) 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