Pergamon

Tetrahedron Letters 42 (2001) 4179–4181

TETRAHEDRON LETTERS

Micromonospolide A, a new macrolide from *Micromonospora* sp.

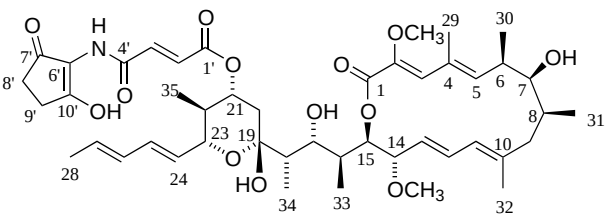
Emi Ohta,[a] Shinji Ohta,[a,*] Natsuki K. Kubota,[a] Makoto Suzuki,[b] Tatsuhiro Ogawa,[b] Akiko Yamasaki[c] and Susumu Ikegami[c,*]

[a]*Instrument Center for Chemical Analysis, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan*
[b]*Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., 3-6-6 Asahi-machi, Machida, Tokyo 194-8533, Japan*
[c]*Department of Applied Biochemistry, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima 739-8528, Japan*

Received 26 March 2001; revised 18 April 2001; accepted 26 April 2001

Abstract—A new macrolide, micromonospolide A, was isolated from an undescribed actinomycete, *Micromonospora* sp., and its structure was elucidated to be a bafilomycin-type macrolide which has a 16-membered lactone ring on the basis of spectroscopic data. Micromonospolide A inhibited gastrulation of starfish embryos at a concentration of 10 ng/ml or greater.

In the course of our search for inhibitors of starfish (*Asterina pectinifera*) embryonic development,[1–8] we found that the *n*-BuOH extract of a new actinomycete of *Micromonospora* sp. showed potent inhibitory activity against gastrulation. Based on the bioassay for inhibition of gastrulation,[9] purification of the crude extract was carried out to afford a new macrolide designated micromonospolide A (**1**). Micromonospolide A (**1**) is a member of macrolides which have a 16-membered lactone ring such as hygrolidins,[10,11] bafilomycins,[12,13] leucanicidins,[14,15] and their related compounds.[16–18] In this paper, we report the isolation and structure elucidation of **1**.



Micromonospolide A (**1**)

The 2-liter broth filtrate of *Micromonospora* sp. was extracted twice with 2 liter of *n*-BuOH. The extract was subjected to chromatography on ODS using MeOH–H_2O (8:2). The bioactive fraction was subsequently chromatographed on ODS (CH_3CN) to afford **1** (57 mg) as a yellow amorphous solid.

Micromonospolide A (**1**), mp 139–143°C (dec.), $[\alpha]_D^{25}$ +14.3° (*c* 0.63, MeOH), was soluble in MeOH and $CHCl_3$ and has a TLC R_f value of 0.43 (ODS; MeOH–H_2O, 9:1). The FABMS showed an $[M+Na]^+$ ion at *m*/*z* 862 in the positive mode and an $[M-H]^-$ ion at *m*/*z* 838 in the negative mode. The molecular formula, $C_{46}H_{65}NO_{13}$, which was determined by HRFAB mass spectrometry (found *m*/*z* 862.4390 $[M+Na]^+$; calcd for $C_{46}H_{65}NNaO_{13}$, 862.4353), requires 15 degrees of unsaturation. The IR [ν_{max}^{KBr} 3400, 1717, 1703, 1688, 1651 (sh), 1618 cm^{-1}] and UV [λ_{max}^{MeOH} 230 (ε 75,800), 240 (sh, 73,300), 252 (61,100), 290 (sh, 24,400), 350 nm (4,700)] spectra showed the presence of OH groups and conjugated carbonyl groups. The ^{13}C NMR spectrum of **1** (Table 1) revealed the presence of 46 carbon atoms. The ^1H NMR, ^1H–^1H COSY and HMQC spectra allowed the presence of the C-5/C-9, C-11/C-18, C-20/C-28 and C-2′/C-3′ units. In the HMBC experiment, correlations were observed from H_3-32 to C-9, C-10, C-11, from H_3-34 to C-17, C-18, C-19, from H-20b to C-19, thereby allowing the connection of these units. Further HMBC correlations from H-3 to C-1, C-2, C-5, C-29, from H-5 to C-3, C-7, C-29, from H_3-29 to C-3, C-4, C-5, from H-15 to C-1 defined the 16-membered macrolide ring (C-1 to C-15) system. The presence of a tetrahydropyran ring (C-19 to C-23) was revealed from the NOESY correlation between OH-19 and H-23 (Fig. 1). The large vicinal ^1H coupling constants associated with H-20b, H-21, H-22 and H-23 ($J_{20b,21}$ = 11.7 Hz, $J_{21,22} = J_{22,23}$ = 10.3 Hz) indicated that the pyran ring

Keywords: biologically active compounds; macrolides; microorganisms; natural products.

* Corresponding authors. Fax: +81-824-24-7486; e-mail: ohta@sci.hiroshima-u.ac.jp

0040-4039/01/$ - see front matter
PII: S0040-4039(01)00683-9

Table 1. NMR data for micromonospolide A (**1**) in $CDCl_3$

C#	δ_C[a]	δ_H	Mult., J (Hz)	C#	δ_C[a]	δ_H	Mult., J (Hz)
1	167.0 s			22	40.9 d	1.52	tq, 10.3, 6.8
2	141.3 s			23	74.6 d	4.15	dd, 10.3, 7.8
3	132.6 d	6.58	s	24	129.4 d	5.40	dd, 15.1, 7.8
4	133.1 s			25	133.0 d	6.12	dd, 15.1, 10.7
5	142.5 d	5.75	d, 8.8	26	130.9 d	5.92	ddd, 15.1, 10.7, 1.5
6	36.6 d	2.51	ddq, 8.8, 7.1, 1.5	27	129.6 d	5.62	dq, 15.1, 6.8
7	81.1 d	3.29	dt, 6.4, 1.5	28	18.0 q	1.72	br d, 3H, 6.8
7-OH		1.62	br	29	14.0 q	1.97	s, 3H
8	40.2 d	1.90	m	30	17.2 q	1.07	d, 3H, 7.1
9a	41.3 t	2.13	br d, 13.7	31	21.7 q	0.93	d, 3H, 6.4
9b		1.94	m	32	20.1 q	1.94	s, 3H
10	143.2 s			33	9.6 q	0.82	d, 3H, 6.8
11	125.2 d	5.81	d, 10.7	34	7.0 q	1.03	d, 3H, 7.1
12	133.0 d	6.51	dd, 15.1, 10.7	35	13.2 q	0.82	d, 3H, 6.8
13	127.1 d	5.15	dd, 15.1, 9.3	1′	164.3 s		
14	81.9 d	3.90	dd, 9.3, 8.8	2′	132.9 d	6.89	d, 15.1
15	76.4 d	4.97	br d, 8.8	3′	133.6 d	7.03	d, 15.1
16	37.3 d	2.12	m	4′	163.4 s		
17	70.2 d	4.07	ddd, 10.3, 3.9, 1.5	6′	114.5 s		
17-OH		4.68	d, 3.9	7′	196.6 s		
18	41.3 d	1.76	br q, 7.1	8′	30.9 t	2.59	br s, 2H
19	99.6 s			9′	30.9 t	2.59	br s, 2H
19-OH		5.80	br s	10′	175.0 s		
20a	39.7 t	2.40	dd, 11.7, 4.9	2-OMe	59.7 q	3.49	s, 3H
20b		1.29	t, 11.7	14-OMe	55.5 q	3.24	s, 3H
21	74.7 d	5.14	ddd, 11.7, 10.3, 4.9				

[a] Multiplicities were determined by DEPT experiments.

exists in a chair conformation, which was also corroborated by the NOESY correlations between H-21 and both OH-19 and H-23. The locations of the methoxyl groups were established on the basis of HMBC correlations from methoxyl protons at δ_H 3.49 (2-OMe) and 3.24 (14-OMe) to C-2 and C-14, respectively. The structure of the remaining $C_9H_8NO_4$ unit was determined to be *N*-(3-hydroxy-2-cyclopentenone-2-yl)fumarylester monoamide by comparison of the ^1H and ^{13}C NMR data for the $C_9H_8NO_4$ unit with those of the known compounds bafilomycins B_1 and B_2.[12] The location of the unit was determined by observation of an HMBC correlation from H-21 to C-1′. The geometry of the trisubstituted olefins was determined to be 2*Z*,4*E*,10*E* by ROE experiments, as shown in Fig. 1. The geometry of the disubstituted olefins was determined to be 12*E*,24*E*,26*E*,2′*E* from the large vicinal ^1H coupling constants ($J_{12,13}=J_{24,25}=J_{26,27}=J_{2',3'}=15.1$ Hz). Comparison of ^1H and ^{13}C chemical shifts and the magnitude of the coupling constants of **1** with those of bafilomycin A_1[19] revealed that the relative stereochemistry of **1** is the same as for bafilomycin A_1, which was supported by the NOESY correlations, as shown in Fig. 1. Consequently, the structure of micromonospolide A was elucidated as **1**.

Micromonospolide A (**1**) has been isolated as a specific inhibitor of starfish (*A. pectinifera*) embryogenesis:

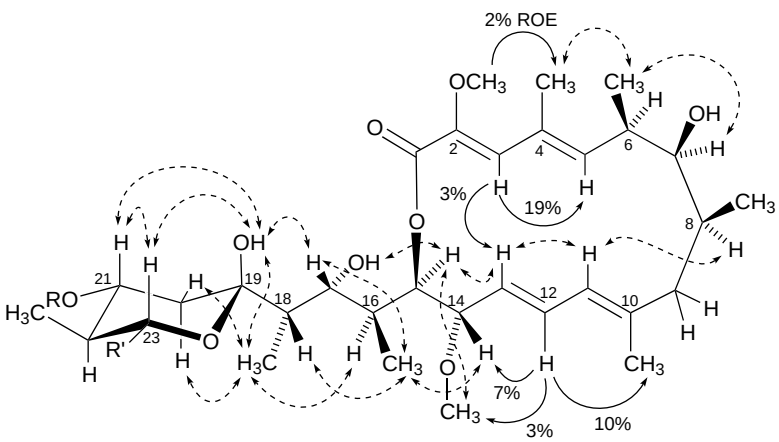


Figure 1. Selected ROEs (→) and NOESY correlations (←----→) of **1**.

when 8-hour-old embryos at the early blastula stage were cultured in the presence of **1** at a concentration of 10 ng/ml or greater, they stopped the progression of embryonic development at the late blastula stage just prior to gastrulation. On the other hand, bafilomycin A_1 did not affect embryogenesis even at 50 ng/ml. Further studies on the structure–activity relationship are in progress.

Acknowledgements

The authors thank Kimika Yamanaka, Toshiko Nakano, and Fumito Koizumi, Kyowa Hakko Kogyo Co., for the help in preparing the fermentation broth, and Hitoshi Fujitaka, Hiroshima University, for NMR measurements. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan.

References

1. Ohta, S.; Okada, H.; Kobayashi, H.; Oclarit, J. M.; Ikegami, S. *Tetrahedron Lett*. **1993**, *34*, 5935–5938.
2. Ohta, S.; Kobayashi, H.; Ikegami, S. *Tetrahedron Lett*. **1994**, *35*, 4579–4580.
3. Ohta, S.; Kobayashi, H.; Ikegami, S. *Biosci. Biotechnol. Biochem*. **1994**, *58*, 1752–1753.
4. Ikegami, S.; Kobayashi, H.; Myotoishi, Y.; Ohta, S.; Kato, K. H. *J. Biol. Chem*. **1994**, *269*, 23262–23267.
5. Ohta, S.; Uno, M.; Yoshimura, M.; Hiraga, Y.; Ikegami, S. *Tetrahedron Lett*. **1996**, *37*, 2265–2266.
6. Uno, M.; Ohta, S.; Ohta, E.; Ikegami, S. *J. Nat. Prod*. **1996**, *59*, 1146–1148.
7. Yanai, M.; Ohta, S.; Ohta, E.; Ikegami, S. *Tetrahedron* **1998**, *54*, 15607–15612.
8. Ohta, S.; Ohta, E.; Ikegami, S. *J. Org. Chem*. **1997**, *62*, 6452–6453.
9. Shimizu, T.; Hamada, K.; Isomura, H.; Myotoishi, Y.; Ikegami, S.; Kaneko, H.; Dan-Sohkawa, M. *FEBS Lett*. **1995**, *369*, 221–224.
10. Seto, H.; Akao, H.; Furihata, K.; Otake, N. *Tetrahedron Lett*. **1982**, *23*, 2667–2670.
11. Seto, H.; Tajima, I.; Akao, H.; Furihata, K.; Otake, N. *J. Antibiot*. **1984**, *37*, 610–613.
12. Werner, G.; Hagenmaier, H.; Albert, K.; Kohlshorn, H. *Tetrahedron Lett*. **1983**, *24*, 5193–5196.
13. Kretschmer, A.; Dorgerloh, M.; Deeg, M.; Hagenmaier, H. *Agric. Biol. Chem*. **1985**, *49*, 2509–2511.
14. Isogai, A.; Sakuda, S.; Matsumoto, S.; Ogura, M.; Furihata, K.; Seto, H.; Suzuki, A. *Agric. Biol. Chem*. **1984**, *48*, 1379–1381.
15. Sakuda, S.; Isogai, A.; Matsumoto, S.; Ogura, M.; Furihata, K.; Seto, H.; Suzuki, A. *Agric. Biol. Chem*. **1987**, *51*, 2841–2842.
16. Goets, M. A.; McCormick, P. A.; Monaghan, R. L.; Ostlind, D. A.; Hensens, O. D.; Liesch, J. M.; Albers-Schonberg, G. *J. Antibiot*. **1985**, *38*, 161–168.
17. Wilton, J. H.; Hokanson, G. C.; French, J. C. *J. Antibiot*. **1985**, *38*, 1449–1452.
18. Meyer, M.; Keller-Schierlein, W.; Drautz, H.; Blank, W.; Zahner, H. *Helv. Chim. Acta* **1985**, *68*, 83–94.
19. Baker, G. H.; Brown, P. J.; Dorgan, R. J. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1073–1079.