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NOTES

A New Macrolide Antibiotic with Antitumor Activity Produced by *Streptomyces* sp. CS, a Commensal Microbe of *Maytenus hookeri*

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Maytansinoids are compounds having strong cytotoxic and antineoplastic activities[1,2], and are products of a bacterium (*Actinosynnema pretiosum*)[3], mosses[4,5] and three closely related plant families, Celastraceae, Rhamnaceae and Euphorbiaceae[1,2]. It was speculated that maytansinoids produced by endophytic microorganism were accountable for the occurrence of those in higher plants. However, up to date, no literatures were reported about the isolation of endophytes from maytansine-containing plants. Moreover, endophytes occurring in higher plants are important sources of natural products with pharmaceutical potential[6,7]. New experimental models are currently tested in which the cytotoxic activity of maytansine is employed to develop new drugs[8,9]. Therefore, the isolation of maytansine-producing endophytic microorganisms from plants is of significance in the aspects of plant-microbe interactions (coevolution) and new drug discovery.

In the course of our search for maytansine-producing endophytic microorgaisms from the plant *Maytenus hookeri*[10], we found that the extract of the culture material of *Streptomyces* sp. CS (one of the commensal microorganisms) showed potent antifungal activity against *Penicillium avellaneum* UC-4376 by diffusion assay on agar plate as described[11]. Antifungal activity-guided isolation afforded a new macrolide antibiotic **1**, named 24-demethyl-bafilomycin C_1 (Figure 1)[12], based on NMR experiments and HRFABMS data.

Compound **1**, $[\alpha]_D^{28}$ −29.5 (*c*, 20, MeOH), was determined to have the molecular formula $C_{38}H_{58}O_{12}$ based on the negative HRFABMS peak at *m/z* 705.3833 (calcd: 705.3850), and showed the presence of carbonyl groups (1690.7 and 1714.4 cm^{-1}) in its IR spectrum. Inspection of the NMR data (proton, carbon, DEPT, HMQC and HMBC) revealed a bafilomycin-type 16-membered macrolide (Table

Fig. 1. Structural fragments and the conformation of the pyranose ring of **1**, and the selected ^1H-^1H COSY (↔) and HMBC (H→C) correlations.

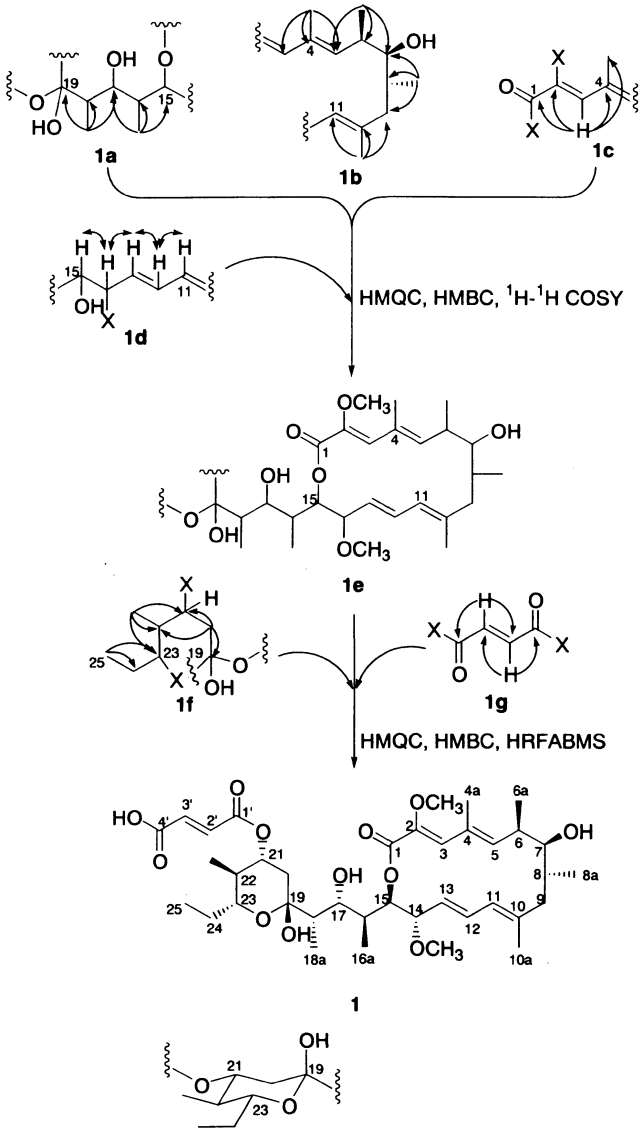

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Table 1. The ^{1}H, ^{13}C NMR assignments and ^{1}H-^{13}C long-range correlations (HMBC) for **1**[a].

No	^{13}C	^{1}H[b]	HMBC
1	167.3s	/	/
2	141.2s	/	/
3	133.6d	6.68 (s)	C-1, C-2, C-4a, C-5,
4	133.0s	/	/
5	142.8d	5.78 (br d, 9.2)	C-4, C-6, C-4a, C-7,
6	36.6d	2.56 (br f, 7.2)	C-5, C-6a
7	81.2d	3.31 (dd, 2.1, 7.7)	C-5, C-6, C-8, C-8a
8	40.0d	1.96 (m)	[c]
9	41.2t	1.93 (m), 2.16 (m)	C-7, C-10
10	143.0s	/	/
11	125.2d	5.83 (br d, 10.4)	C-10a
12	133.0d	6.54 (dd, 10.8, 14.8)	C-10, C-14
13	127.1d	5.19 (dd, 9.2, 14.8)	C-14, C-15
14	82.2d	3.92 (t, 8.8)	C-12, C-15, C-16
15	76.7d	4.97 (br d, 8.4)	C-1, C-14, C-16, C-17, C-16a
16	37.3d	2.16 (m)	C-14, C-17, C-16a
17	70.6d	4.15 (dd, 2.0, 10.4)	C-18, C-18a
18	41.7d	1.80 (dq, 2.0, 7.6)	C-19, C-18a
19	99.0s	/	/
20	39.8t	1.35 (m), 2.43 (dd, 4.8, 11.2)	C-19, C-21, C-22
21	75.1d	5.12 (ddd, 4.8, 10.8, 10.8)	C-1', C-22a
22	40.8d	1.50 (m)	[c]
23	73.5d	3.70 (d, 11.6)	[c]
24	25.6t	1.70 (m), 1.30 (m)	C-22, C-25
25	10.3q	0.90 (t, 7.6, 3H)	C-23, C-24
MeO-2	59.9q	3.64 (s, 3H)	C-2
4a	14.0q	1.99 (s, 3H)	C-3, C-4, C-5
6a	17.3q	1.08 (d, 7.2, 3H)	C-5, C-6, C-7
8a	21.6q	0.95 (d, 6.0, 3H)	C-7, C-8, C-9
10a	20.1q	1.94 (s, 3H)	C-9, C-10, C-11
MeO-14	55.5q	3.25 (s, 3H)	C-14
16a	9.7q	0.85 (d, 6.4)	C-15, C-16, C-17
18a	7.1q	1.05 (d, 7.2)	C-17, C-18, C-19,
22a	12.8q	0.87 (d, 6.8)	C-21, C-22, C-23
1'	164.2s	/	/
2'	132.8d	6.90 (br d, 16.0)	C-1', C-3'
3'	135.4d	6.94 (br d, 16.0)	C-2', C-4'
4'	168.7s	/	/

[a] ^{1}H, ^{13}C NMR and HMBC spectra were obtained at 400MHz, 100MHz and 400MHz, respectively, and recorded in $CDCl_3$ at room temperature.

[b] Coupling constants are presented in Hertz. Unless otherwise indicated, all proton signals integrate to 1H.

[c] Correlations were not recognized because the proton signals overlapped severely.

1)[13]. The ^{13}C NMR and DEPT spectra of **1** showed thirty-eight carbon signals for eight methyl, two methoxy, three methylene, eighteen methine, and seven quaternary carbon atoms including three carbonyl carbons. The ^{1}H NMR spectra of **1** indicated eight methyls including five doublets, two singlets and one triplet, and the ^{13}C NMR spectra give evidence for five methylene carbons in the upfield region (36.6~41.7 ppm), indicating the presence of at least one ethyl group.

The structure of fragment **1a** (Figure 1) was determined based on the ^{1}H-^{13}C long-range correlations of the methyl protons at δ 0.85 (H-16a) with the carbons at δ 76.7 (C-15), 37.3 (C-16) and 70.6 (C-17), and the methyl protons at δ 1.05 (H-18a) with the carbons at δ 70.6 (C-17), 41.7 (C-18) and 99.0 (C-19). The fragment **1b** was recognized by the ^{1}H-^{13}C long-range correlations of the methyl protons at δ 1.99 (H-4a), 1.08 (H-6a), 0.95 (H-8a), 1.94 (H-10a) with the corresponding carbons as well. The proton at δ 6.68

(H-3) showed the ^{1}H-^{13}C long-range correlations with the carbons at δ 167.3 (C-1), 141.2 (C-2), 14.0 (C-4a) and 142.8 (C-5), indicating the presence of fragment **1c**. In the ^{1}H-^{1}H COSY spectra, the proton at δ 6.54 (H-12) showed correlations with the protons at δ 5.83 (H-11) and 5.19 (H-13) and the proton at δ 3.92 (H-14) showed correlations with the proton at δ 5.19 (H-13) and 4.97 (H-15), which revealed the structure of fragment **1d**. Meanwhile, the linkages of the fragments **1a**, **1b**, **1c** and **1d** were revealed by the HMBC experiments. The ^{1}H-^{13}C long-range correlation of the proton at δ 4.97 (H-15) with the carbon at δ 167.3 (C-1) indicated the linkage between C-1 and C-15 *via* oxygen, which determined a 16-member lactone ring. The protons of two methoxy groups at δ 3.64 and 3.25 were connected with the carbons at δ 141.2 (C-2) and 82.8 (C-14), respectively, based on their ^{1}H-^{13}C long-range correlations. Therefore, the structure of the fragment **1e** (Figure 1) was determined. According to the ^{1}H-^{13}C long-range correlations between the protons at δ 0.87 (H-22a), 0.90 (t, J=7.6, H-25), 1.35 and 2.43 (1H each, H-20) and the corresponding carbons (Table 1), the fragment **1f** was formed. The fragment **1g** was determined to be a *trans*-substituted double bond flanked by two carboxyl groups on the basis of two coupled doublets at δ 6.93 (H-2′) and 6.94 (br d, J=16 Hz, H-3′) in the ^{1}H NMR spectra of **1**, and the HMBC experiments. The negative HRFABMS fragment ion at *m/z* 115.0031 gave the molecular constituents $C_4H_3O_4$* (calcd: 115.0031), further confirming the structure of the fragment **1g**. The proton at δ 5.12 (H-21) showed the ^{1}H-^{13}C long-range correlations with C-1′ (δ 164.2), indicating the linkage of fragments **1f** and **1g** *via* oxygen. All oxygen-substitutions were assigned based ^{13}C NMR (Table 1) and HRFABMS data. The coupling constants 9.2, 2.1, 7.7, 9.2, 8.8, <2.0, 10.4 and 2.0 Hz observed between H-5-H-6, H-6-H-7, H-7-H-8, H-13-H-14, H-14-H-15, H-16-H-17 and H-17-H-18, respectively, were similar to those reported for L-681,110A_1 (bafilomycin C_1)[12] and hygrolidin[14,15]. The distinct doublet-triplets of the proton at δ 5.12 (ddd, 4.8, 10.8, 10.8, H-21) indicated its axial orientation, and that the proton at δ 1.50 (m, H-22) was at axial as well (Figure 1). The coupling constant of the proton at δ 3.70 (d, 11.6, H-23) suggested its orientation to be axial. Therefore, the orientations of the substituents at C-21, C-22 and C-23 on the pyranose ring were determined to be equatorial. The hydroxy at C-19 was established to be axial based the chemical shift of the C-19 (δ 99.0), which was similar to that of bafilomycin A_1[16]. The NMR data and optical rotation observed for **1** are consistent with those of bafilomycin C_1[12], indicating that **1** could have the same absolute configuration[17]. Therefore, compound **1** was determined to be 24-demethyl-bafilomycin C_1[12].

Table 2. The inhibitory rate of **1** against tumor cell lines P388 and A-549.

Concentrations (M)	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Cell line	P388				
Inhibitory rate (%)	97.9	88.8	82.7	81.1	78.1
Cell line	A-549				
Inhibitory rate (%)	96.9	93.1	88.8	90.9	89.4

A series of bioactivities were reported about this family of 16-membered macrolide polyketides, such as bafilomycins–antibiotics against Gram-positive, fungi, yeasts[12], and the inhibitors of the Na^+, K^+-ATPase[13], and hygrolidins–antifungal agents against *Valsa ceratosperma*[18] and antitumor agents against various cell lines[19]. In our work, compound **1** exhibited strong inhibitory activity against *Penicillium avellaneum* UC-4376 with the minimal inhibitory amount at 10 μg/disc, and cytotoxic activity against tumor cell lines P388 and A-549 by the methods SRB (sulforhodamine B) and MTT (microculture tetrozolium), respectively, as well (Table 2).

Acknowledgments

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References

1) Kupchan, S. M.; Y. Komoda, A. R. Branfman, A. T. Sneden, W. A. Court, G. J. Thomas, H. J. P. Hintz, R. M. Smith, A. Karim, G. A. Howie, A. K. Verma, Y. Nagao, R. G. Dailey, Jr., V. A. Zimmerly & W. C. Sumner, Jr.: The maytansinoids, isolation, structural elucidation, and chemical interrelation of novel ansa macrolides. J. Org. Chem. 42: 2349~2357, 1977
2) Reider, P. J. & D. M. Roland: "Maytansinoids" *In* "The Alkaloids", Vol. XXIIII, pp. 71~156, Academic Press Inc., 1984
3) I-Egashide, E.; M. Asai, K. Ootsu, S. Tanida, Y. Kozai, T. Hasegawa, T. Kishi, Y. Sugino & M. Yoneda: Ansamitocin, a group of novel maytansinoid antibiotics

with antitumor properties from *Nocardia*. Nature 270: 17~22, 1977

4) SUWANBORIRUX, K.; C.-J. CHANG, R. W. SPIUT & J. M. CASSADY: Ansamitocin P-3, a maytansinoid, from *Claopodium crispifolium* and *Anomodon attenuatus* or associated actinomycetes. Experientia 46: 117~120, 1990
5) SAKAI, K.; T. ICHIKAWA, K. YAMADA, M. YAMASHITA, M. TANIMOTO, A. HIKITA, Y. IJUIN & K. KONDO: Antitumor Principles in Mosses: The first isolation and identification of maytansinoids, including a novel 15-methoxyansamitocin P-3. J. Nat. Prod. 51: 845~850, 1988
6) STROBEL, G. A. & D. M. LONG: Endophytic microbes embody pharmaceutical potential. ASM News (5): 263~268, 1998
7) TAN, R.-X. & W.-X. ZOU: Endophytes: a rich source of functional metabolites. Nat. Prod. Rep. 18: 448~459, 2001
8) COGHLAN, A.: Antibodies deliver killer blow to cancer. New Scientist 20, 1996
9) LIU, C.-N.; B. M. TADAYONI, L. A. BOURRET, K. M. MATTOCKS, S. M. DERR, W. C. WIDDISON, N. L. KEDERSHA, P. D. ARINIELLO, V. S. GOLDMACHER, J. M. LAMBERT, W. A. BLÄTTLER & R. V. J. CHARI: Eradication of large colon tumor xenografts by targeted delivery of maytansinoids. Proc. Natl. Acad. Sci. USA 93: 8618~8623, 1996
10) ZHOU, Y.-L.; L.-Y. HUANG, Q.-R. ZHOU, C.-X. JIANG, J.-G. HE, C.-M. LI, C. WANG & B.-J. LI: Studies on the bioactive components of *Maytenus hookeri* I. Acta Chim. Sin. 39: 427~431, 1981 (in Chinese)
11) ESPINEL-INGROFF, A.; T. WHITE, M. A. PFALLER: Antifungal Agents and Susceptibility Tests. *In*: Manual of Clinical Microbiology, 7th Edn. (MURRAY P. R. *Ed.*). pp. 1640~52. American Society for Microbiology, Washington DC, 1999
12) HENSENS, O. D.; R. L. MONAGHAN, L. HUANG & G. A. ALBERS-SCHONBERG: Structure of the sodium and potassium ion activated adenosinetriphosphatase inhibitor L-680, 110. J. Am. Chem. Soc. 105: 3672~3679, 1983
13) WERNER, G.; H. HAGENMAIER, H. DRAUTZ, A. BAUMGARTNER & H. ZÄHNER: Metabolic products of microorganisms. 224[†] Bafilomycins, a new group of macrolide antibiotics: production, isolation, chemical structure and biological activity. J. Antibiotics 37: 110~117, 1984
14) COREY, E. J. & J. W. PONDER: Stereochemistry of the hygrolidins. Tetrahedron Lett. 25: 4325~4328, 1984
15) SETO, H.; H. AKAO, K. FURIHATA & N. OTAKE: The structure of a new antibiotic, hygrolidin. Tetrahedron Lett. 23: 2667~2670, 1982
16) WERNER, C.; H. HAGENMAIER, K. ALBERT, H. KOHLSHORN & H. DRAUTZ: The structure of the bafilomycins, a new group of macrolide antibioctics. Tetrahedron Lett. 24: 5193~5196, 1983
17) O'SHEA, M. G.; R. W. RICKARDS, J. M. ROTHSCHILD & E. LACEY: Absolute configurations of macrolide antibiotics of the bafilomycin and leucanicidin groups. J. Antibiotics 50: 1073~1077, 1997
18) SETO, H.; I. TAJIMA, H. AKAO, K. FURIHATA & N. OTAKE: The isolation and structures of hygrolidin amide and defumarylhygrolidin. J. Antibiotics 37: 610~613, 1984
19) KAWADA, M.; I. USAMI, S. OHBA, T. SOMENO, J.-W. KIM, Y. HAYAKAWA, K. NOSE & M. ISHIZUKA: Hygrlidin induces p21 expression and abrogates cell cycle progression at G1 and S phases. Biochem. Biophysical Res. Commun. 298: 178~183, 2002