Pergamon

Tetrahedron Letters 40 (1999) 2957–2960

TETRAHEDRON LETTERS

Structure Elucidation of Xanthobaccin A, a New Antibiotic Produced from *Stenotrophomonas* sp. Strain SB-K88

Yasuyuki Hashidoko[1,2)*], Takato Nakayama[1,3)], Yoshihisa Homma[4)] and Satoshi Tahara[1,2)]

1) Applied Bioscience, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan.
2) CREST, Japan Science and Technology Corporation, Honmachi 4-1-8, Kawaguchi 332-0012, Japan.
3) Present Address: National Agriculture Research Center, 3-1-1 Kan-nondai, Tsukuba 305-8666, Japan.
4) Upland Agriculture Research Center, Hokkaido National Agricultural Experiment Station, Memuro-cho, Hokkaido 082-0071, Japan.

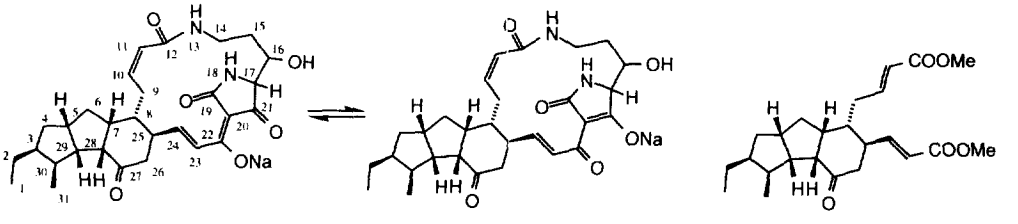
Received 21 November 1998; accepted 12 February 1999

Abstract

The major antibiotic constituent, xanthobaccin A, was isolated from broth cultures of *Stenotrophomonas* sp. strain SB-K88 as a major antifungal substance. The structure elucidation by spectroscopic analyses and chemical conversion revealed it to be a new tetramic acid-containing macrocyclic lactam.

Keywords: pyrrolidinones, antifungal, macrocycles, lactam, bacterium

Stenotrophomonas sp. (formerly classified as *Xanthomonas* sp.) strain SB-K88, which was originally isolated from the fibrous root surface of sugar beet continuously cultivated in a field heavily infested with *Polymyxa betae,* suppressed the rhizomania and seedling damping-off of sugar beet. This bacterium was therefore considered to be a member of the competitive rhizospheric microflora [1]. The preliminary experiments revealed that the bacterium produced a fungitoxic chemical xanthobaccin A (**1**) in liquid cultures and in sugar beet rhizosphere, and **1** showed the minimum inhibitory concentration (MIC) of 1 μg/mL against *Pythium ultimum* [2].



1a-Na salt **1b**-Na salt **2**

Fig. 1 Two tautomeric structures of xanthobaccin A (**1a** and **1b**) sodium salts and its methanolysis product (**2**)

* To whom all correspondence should be addressed. E-mail: yasu-h@abs.agr.hokudai.ac.jp

PII: S0040-4039(99)00336-6

Xanthobaccin A (68 mg) was isolated as a colorless amorphous powder of the sodium salt (**1**) from the culture fluid (15 L) by resin trap (Amberlite XAD-2, Rohm & Haas Co.), moist silica gel (Wakogel C-200, Wako Pure Chemical Industries Co Ltd.) and reversed C-18 silica gel (Cosmosil 75C_{18}-OPN, Nacalai Tesque) column chromatography followed by preparative TLC (Kieselgel 60F_{254}, Merck) in aqueous $CHCl_3$-MeOH. Compound **1** showed UV λmax in MeOH at 239 and 321 nm (ε 18,900 and 9,800, respectively) and a characteristic blue-white fluorescence on thin-layer plates (emission wave length at 428 and 468 nm in MeOH under excitation by 320 nm), obviously due to the presence of a chromophore moiety. The Na-free **1** prepared by passing a small CM-cellulose column (H^+ type) showed a parent ion peak at *m/z* 510 (rel. int. 100 %) in FD-MS. FAB-MS of **1**-Na^+ salt showing a quasi ion peak at *m/z* 509 $[M-H]^-$ by negative ion mode,[1] and reliable FAB-HR-MS (neg.) for the *m/z* 509 (Found 509.2638, Calcd. 509.2652 for $C_{29}H_{37}N_2O_6$) confirmed the formulation of **1**.

Table 1 ^1H and ^{13}C assignments and 2D-NMR correlations of **1**-Na salt and its methanolysis product **2**

	1-Na salt							**2**		
C-number	(δC)	CH(n)	HMQC	HMBC(C→H)	$^1H^1H$-COSY	(δH)	$^1H^1H$-COSY	(δC)	HMQC	$^1H^1H$-COSY
			(C_5D_5N)			(CD_3OD)			($CDCl_3$)	
1	12.7	CH_3	0.81	2	2	0.89	2	12.5	0.87	2
2	26.1	CH_2	0.95	1	1,2b	1.07	1,2b	26.0	1.0	2b,3
			1.5		1,2a,3	1.61	1,2a,3		1.55	2a
3	53.8	CH	1.27	1,4a	4,6b,30	1.41	2b,4,30	53.7	1.4	2a,4,30
4	40.4	CH_2	0.70	6a	4b,5,30	0.89	3,4b,(5)	40.3	0.83	3,4b,5
			1.95		4a,5,30	2.13	3,4a,(5)		2.12	3,4a,5
5	41.1	CH	2.26	29,30	4,6,29	2.43	(4,6a)[a]	41.0	2.37	4,6,8
6	38.8	CH_2	0.9	4a	5,6b,7	1.14	6b,(5),7	39.2	1.07	5,6b,7
			2.0		4,6a	2.16	6a,7		2.20	5,6a,7
7	52.0	CH	1.93	28	6a,8	2.08	6,8,(28)	54.2	1.76	6,8,28
8	44.3	CH	1.44	26	7,9a,25	1.83	7,9a,25	45.4	1.85	7,9,25
9	28.5	CH_2	2.3	10,11	8,9b,10,11*	2.20	8,9b,10,11*	33.2	2.35	7,10,11
			4.19		9a,10	3.57	9a,10		(2H)	
10	138.6	CH	5.81		9,11	6.00**	9,11	144.7	6.84	9,11
11	125.5	CH	6.25		9a*,10	5.83**	9a*,10	123.7	5.83	9,10
12	167.0	C	-	10,11,14a	-	-		166.47	-	
14	37.6	CH_2	3.21	15b	14b,15b,NH	2.80	14b,15	-	-	
			4.05		14a,15b,NH	3.50	14a,15			
15	32.3	CH_2	1.85	17	15b,16	1.36	14,15b,16	-	-	
			2.4		15a,14	1.70	14,15a			
16	71.6	CH	4.86	17	15a,17	3.95	15a,17	-	-	
17	69.1	CH	4.42		16	3.72	16	-	-	
19	180.1	C	-	17	-	-		-	-	
20	102.9	C	-		-	-		-	-	
21	194.5	C	-	17	-	-		-	-	
22	183.3	C	-	23,24	-	-		-	-	
23	143.9	CH	7.01		24,25*	6.38**	24,25	122.5	5.86	24
24	130.4	CH	8.26		23,25	7.45**	23	149.1	6.77	23,25
25	47.4	CH	2.43	23,24	8,23*,24,26	2.40	23,24,26	46.5	2.48	8,24,26
26	47.2	CH_2	2.25	28	25	2.20	25,26b	46.8	2.33	25
			(2H)			2.47	25,26a		(2H)	
27	207.6	C	-	26,28,29	-	-		207.2	-	
28	63.9	CH	2.10	29,30	29	2.43	(7,29)[a]	64.0	2.25	7,29
29	50.9	CH	2.41	5,6b,31	5,28,30	2.40	(5,28),30	50.1	2.3	28,30
30	47.4	CH	0.94	4b,31	3,29,31	1.09	3,29,31	47.7	1.05	3,29,31
31	17.9	CH_3	1.07	29,30	30	1.00	30	17.5	1.01	30
13		NH	9.35		14			($COOCH_3$)		
18		NH	9.15					51.6	3.73	
		OH	6.8					51.7	3.75	

* Allyl coupling.

** Coupling constants of each pair of the olefinic protons (H-10/11 and H-23/24) were both in *J*= 16 Hz.

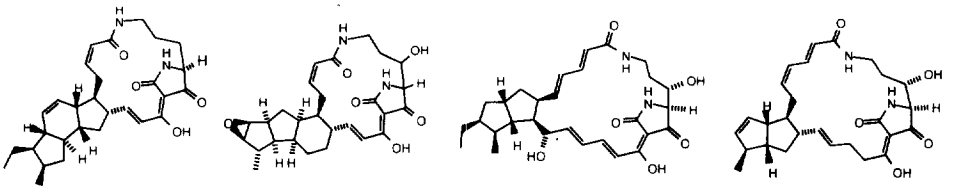
[a] ^1H-NMR spectrum in CD_3OD showed better signal separation but H-5 and H-28 were indistinguishable.

The ^{1}H-NMR (C_5D_5N) of Na salt of **1** displayed two methyl signals at δ_H 0.81 and 1.07, two NH signals at δ_H 9.35 and 9.15, and an OH signal at δ_H *ca.* 6.8. Four olefinic CH signals at δ_H 8.26, 7.01, 6.25 and 5.81 featured two disubstituted double bonds. The complete ^{1}H-NMR assignments were made by the combined use of ^{1}H-^{1}H COSY, HMQC and HMBC spectra (Table 1). The ^{13}C-NMR (C_5D_5N) displayed signals for 29 carbons while the DEPT spectra revealed the presence of 2 methyl, 7 methylene, 14 methine and 6 quaternary carbons in the molecule. The four characteristic olefinic methine signals were shown at δ_C 143.9, 138.6, 130.4 and 125.5.

Two ^{1}H-^{1}H coupling networks were apparent in ^{1}H-^{1}H COSY and TOCSY correlations, and the larger network on the lipophilic subunit was hence identified to have the 5,5,6-tricyclic (tricyclo[7.3.0.0^{2,7}]dodecane) skeleton containing a non-conjugated carbonyl carbon at δ_C 210.6 (C-26). The structure of the lipophilic subunit was further proved by an acid-catalyzed methanolysis of **1** to yield **2** as the major less-polar product.[2] The product **2** showed its molecular ion at *m/z* 402 (100 %) in FI-MS and formulation of $C_{24}H_{34}O_5$ by FI-HR-MS (Found 402.2406, Calcd. 402.2407). In the ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra, proton/carbon chemical shifts of **2** were in accordance with those of the corresponding signals (proton/carbon in CD_3OD/C_5D_5N) of **1** on the lipophilic subunit. The *cis*-olefinic bond in **1** was isomerized to the *trans*-form during the solvolysis (footnote in Table 1).

The other ^{1}H-^{1}H coupling network (▪-CH-CH-CH_2-CH_2-▪) was identified as that of a β-hydroxyornithine unit which formed a tetramic acid ring chromophore between its α-amino carboxylate carbon and one of the carboxylate terminals of the lipophilic subunit. The HMBC correlations between C-12/H-14a and between C-12/H-10, and the ^{1}H-^{1}H COSY correlation between an exchangeable proton at δ_H 9.35/C-14 methylene protons suggested the presence of an α,β-unsaturated amide linkage between these two subunits. A pair of olefinic protons (H-24 and H-23) on the *trans*-C,C-double bond showed HMBC correlations with a carbonyl/enolized carbon at δ_C 183.3 (C-22), which was involved in the chromophoric system. The 3-acylated tetramic acid (4-hydroxy-3-pyrrolin-2-one) ring moiety of **1** is able to show a keto-enol tautomerism (**1a** and **1b**) between C-21 and C-22, but the NMR spectra were of only one tautomeric form of **1**-Na salt. Based on the HMBC shown in Table 1, the C-22 carbon signal at δ_C 183.3 with an upfield shift compared with the C-21 carbon at δ_C 194.5 was most likely enolized. Thus, **1**-Na salt formed the 21-keto-22-enol tautomer (**1a**).

The tetramic acid ring structure of the chromophore was the same as that of ikarugamycin (**3**) [3,4]. The UV λmax (in 0.1 M NaOH/MeOH) of compound **3** was in accordance with that of the sodium salt of **1**, leading to identification of structure **1**. Some other natural products structurally related to **1**, discodermide (**4**) from a marine sponge *Discordermia dissoluta* [5] and alteramide A (**5**) from a bacterium *Alteromonas* sp. separated from another sponge *Halichondria okadai* [6], both of which probably function as defensive chemicals on the sponges, have been reported. Cylindramide (**6**) isolated from *H. cylindrata* has also been speculated to be originated from an associated or symbiotic bacterium of the sponge [7].



ikarugamycin (**3**) discodermide (**4**) alteramide A (**5**) cylindramide (**6**)

Fig. 2 Chemical structures of some reference compounds related to **1**

The relative configuration of the lipophilic subunit of **1** was elucidated by its NOESY correlations shown in Fig. 3. As compounds **5** and **6** have been determined to possess an *erythro*-L-β-hydroxyornithine unit [5,6], the β-hydroxyornithine unit of compound **1** is also likely derived from an L-*erythro*-isomer. The stereochemistry of **1**, especially the stereochemical correlation between the lipophilic and β-hydroxyornithine units is, however, still ambiguous.

Discovery of the tetramic acid-containing macrocyclic lactam from the bacterium comprised of root epiphytic microflora arouses our interest in the ecological, biochemical and genetic aspects of symbiosis between host plants and rhizospheric bacteria in the terrestrial ecosystem, since their symbiotic correlation via a macrocyclic lactam is similar with those between sponges and symbiotic bacteria in the marine ecosystem.

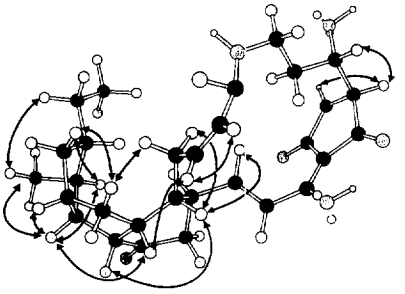


Fig. 3 Some important NOESY correlations of **1** to give its relative configuration

Acknowledgments: The authors thank Mr. K. Watanabe and Dr. E. Fukushi (NMR Laboratory, Faculty of Agriculture, Hokkaido University) for MS and NMR analyses.

REFERENCES

[1] Homma Y, Uchino H, Kanzawa K, Nakayama T, Sayama M. Ann. Phytopathol. Soc. Japan (Jpn). 1993;59:282.
[2] Nakayama T. Chemical study on biological control of sugar beet damping-off disease (Jpn). Hokkaido University, 1996.
[3] Jomon K, Kuroda Y, Ajisaka M, Sakai H. J. Antibiotics 1972;25:271-280.
[4] Ito S, Hirata Y. Bull. Che, Soc. Japan. 1977;50:1813-1820.
[5] Gunasekera SP, Gunasekera M, McCarthy P. J. Org. Chem. 1991;56:4830-4833.
[6] Shigemori H, Bae M-A, Yazawa K, Sasaki T, Kobayashi J. J. Org. Chem. 1992;57:4317-4320.
[7] Kanazawa S, Fusetani N, Matsunaga S. Tetrahedron Lett. 1993;34:1065-1068.

---

1. 3-Nitrobenzylalcohol was used as the matrix for the negative ion mode. The positive ion mode with glycerol showed the quasi ion at *m/z* 533 $[M+Na]^+$.
2. Fine **1**-Na^+ powder (8 mg) dissolved in 4 ml of a 2 M HCl/MeOH solution (12 M HCl/MeOH 1/5, v/v) was heated at 80°C for 2 h. Compound **2** extracted with *n*-hexane from the reaction mixture was purified by TLC to yield 2 mg of colorless plates.