The Journal of Antibiotics (2009) 62, 601–603
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NOTE

Novel siderophore, JBIR-16, isolated from *Nocardia tenerifensis* NBRC 101015

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The Journal of Antibiotics (2009) **62,** 601–603; doi:10.1038/ja.2009.84; published online 21 August 2009

Keywords: *Nocardia tenerifensis*; siderophore; heterobactin; JBIR-16

Nocardia is the causative microorganism of a human infection called nocardiosis. It is also known to produce a variety of compounds with antitumor,[1,2] antimicrobial[3,4] and immunosuppressive activity.[5,6] To take up iron, which is an essential element for all organisms, into cells under iron-deficient conditions, microorganisms produce iron chelators, namely, siderophores. Five types of siderophores have been isolated: hydroxamates, catecholates, salicylates, nitrosophenols and carboxylates.[7] Some siderophores, such as brasilibactin A,[8] asterobactin,[9] nocobactin NA[10] and nocardamine,[11] are produced by *Nocardia* spp. In the course of our screening program for biologically active compounds of microbial origin, we isolated a novel heterobactin[12] analog, JBIR-16 (**1**), containing hydroxamate and catecholate, from the culture broth of *Nocardia tenerifensis* NBRC 101015 (Figure 1). In this paper, we report the production, isolation, structural determination, and briefly the biological activity of **1**.

The producing strain *N. tenerifensis* NBRC 101015 was cultured on a rotary shaker for 6 days in a 50-ml Erlenmeyer flask containing 10 ml of Brain Heart Infusion broth (Difco Lab., Detroit, MI, USA), including 2% glucose as a seed culture. The seed culture was transferred into 500-ml Erlenmeyer flasks containing 100 ml of a producing medium consisting of a twice concentrated nutrient broth (Difco Lab.), including 1% glucose and 1% glycerol, and incubated on a rotary shaker at 250 r.p.m. for 6 days.

After centrifugation of the culture broth, the supernatant (5 l) was applied on a HP-20 column (Mitsubishi Chemical, Tokyo, Japan), the column was washed with water and then eluted with 100% MeOH to obtain the fraction containing **1** (833 mg). The R_f value of **1** showed 0.6 on a TLC analysis using chloroform–MeOH (10:1) as mobile phase. The eluate was evaporated to dryness, applied on a normal-phase medium-pressure liquid chromatography column (Purif-Pack SI-60, Moritex, Tokyo, Japan) and eluted with a chloroform–MeOH gradient (0–80% MeOH). The main fraction (43.8 mg) was finally separated by preparative HPLC using an XBridge Prep C_{18} column (5 μm OBD, 20 i.d. ×150 mm, Waters, Milford, MA, USA: mobile phase; 35% aqueous MeOH, flow rate; 10 ml min^{-1}, detection; 254 nm) to obtain **1** (3.6 mg; retention time 22 min).

Compound **1** was obtained as a white powder ($[\alpha]_D^{25}$ −9.3, *c* 0.1, in MeOH, UV (MeOH) λ_{max} (ε) 205 (50 800), 249 nm (15 085). The IR spectrum of **1** revealed the characteristic absorptions of amide carbonyl (ν_{max} 1643 cm^{-1}) and amide N–H (ν_{max} 1542 cm^{-1}) groups. The HR electron spray ionization-MS of **1** resulted in the $(M+H)^+$ ion at *m/z* 574.2149, consistent with a molecular formula of $C_{26}H_{32}N_5O_{10}$ (calculated for $C_{26}H_{32}N_5O_{10}$, 574.2133).

Although the partial and putative structure of **1** was elucidated by the analyses of a series of NMR techniques, such as constant time heteronuclear multibond correlation[13] and double-quantum filtered (DQF)–COSY, their connectivities could not be confirmed because of the lack of signals of exchangeable protons. Therefore, the structure of **1** was determined by spectral analyses of a pentamethylated derivative **2**, which was prepared by the treatment of **1** with methyl iodide. Compound **2** gave the $(M+H)^+$ ion at *m/z* 644 in the positive mode. In addition, ^1H and ^{13}C chemical shifts of these methyl residues (Table 1) indicated the presence of five hydroxyl groups in **2**. The direct C–H connectivity was established by heteronuclear single quantum coherence (see Table 1). The analyses of DQF–COSY and heteronuclear multibond correlation spectra of **2** established five substructures as follows: In the DQF spectrum, the sequence from methylene proton 1-H_2 (δ_H 3.52, 3.60) to an amide proton 4-NH (δ_H 7.00) through methylene protons 2-H_2 (δ_H 1.95, 2.01), 3-H_2 (δ_H 1.63, 2.37) and an α-methine proton 4-H (δ_H 4.30, δ_C 51.5)

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Received 9 June 2009; revised 28 July 2009; accepted 30 July 2009; published online 21 August 2009

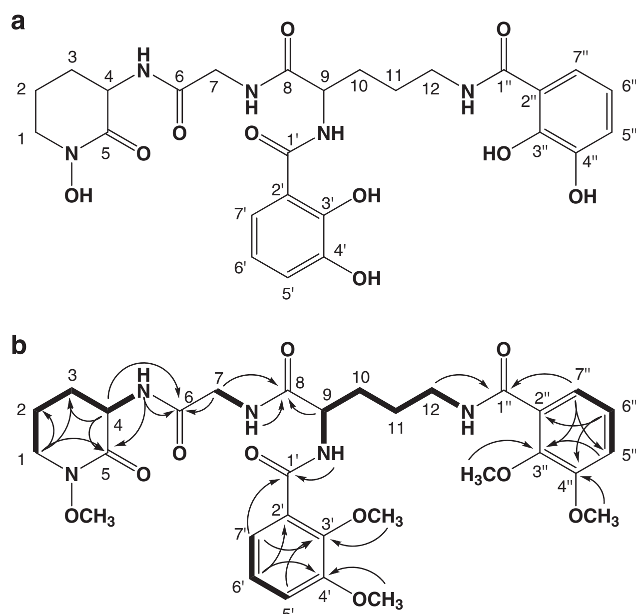

Figure 1 Structure of **1** (**a**), and ^{1}H–^{1}H (bold lines) and main ^{1}H–^{13}C (arrows) correlations in the 2D NMR of **2** (**b**).

Table 1 ^{1}H- and ^{13}C-NMR data of 1 and 2

1			***2***		
Position	δ_C	δ_H	*Position*	δ_C	δ_H
1	52.5	3.58 (m)	1	49.7	3.52 (m)
		3.64 (m)			3.60 (m)
			1-NOCH$_3$	61.3	3.68 (s)
2	21.7	1.94 (m)	2	21.1	1.95 (m)
		2.02 (m)			2.01 (m)
3	28.6	1.82 (m)	3	27.8	1.63 (m)
		2.03 (m)			2.37 (m)
4	51.4	4.46 (dd, 8.3, 5.6)	4	51.5	4.30 (m)
			4-NH		7.00 (d, 6.4)
5	167.2		5	167.1	
6	171.6		6	169.2	
7	43.6	3.90 (d, 8.8)	7	43.5	3.92 (dd, 16.9, 5.6)
					4.08 (dd, 16.9, 6.1)
			7-NH		7.31 (t, 5.8, 5.8)
8	174.0		8	172.5	
9	55.4	4.54 (dd, 10.3, 5.1)	9	53.2	4.84 (m)
			9-NH		8.66 (d, 6.9)
10	30.2	1.90 (m)	10	30.3	1.83 (m)
		2.03 (m)			2.06 (m)
11	27.0	1.78 (m)	11	26.6	1.77 (m)
12	40.0	3.45 (m)	12	38.8	3.40 (m)
					3.80 (m)
			12-NH		8.13 (t, 5.9, 5.9)
1′	171.5		1′	166.0[a]	
2′	117.2		2′	127.0	
3′	150.9		3′	147.7	
			3′-OCH$_3$	61.6	3.90 (s)
4′	147.5		4′	152.8	
			4′-OCH$_3$	56.3	3.88 (s)
5′	119.4	6.91 (d, 7.3)	5′	115.6	7.03 (dd, 8.1, 1.5)
6′	119.1	6.68 (t, 7.8, 7.8)	6′	124.6	7.13 (t, 8.1, 8.1)
7′	118.7	7.21 (d, 8.5)	7′	122.9	7.63 (dd, 8.0, 1.5)
1″	171.6		1″	166.0[a]	
2″	116.7		2″	126.1	
3″	150.7		3″	148.1	
			3″-OCH$_3$	61.9	3.96 (s)
4″	147.6		4″	152.8	
			4″-OCH$_3$	56.4	3.88 (s)
5″	119.4	6.91 (d, 6.8)	5″	116.2	7.05 (dd, 8.8, 1.71)
6″	119.1	6.68 (t, 8.1, 8.1)	6″	124.6	7.13 (t, 8.1, 8.1)
7″	120.0	7.35 (d, 8.1)	7″	123.0	7.63 (dd, 8.0, 1.5)

The ^{13}C (125 Hz) and ^{1}H (500 Hz) NMR spectra were taken on an NMR system 500 NB CL (Varian, Palo Alto, CA, USA) in CD$_3$OD, and the solvent peak was used as an internal standard (δ_C 49.0 p.p.m., δ_H 3.30 p.p.m.).
[a]These assignments are exchangeable.

was observed. ^{1}H–^{13}C long-range couplings in heteronuclear multi-bond correlation spectrum from 1-H$_2$ and 4-H to a carbonyl carbon C-5 (δ_C 167.1), together with a ^{13}C chemical shift of C-1 (δ_C 49.7), revealed a 3-amino piperizin-2-one moiety. The proton spin coupling between an amide proton 7-NH (δ_H 7.31) and methylene proton 7-H$_2$ (δ_H 3.91, 4.08), which in turn ^{1}H–^{13}C long-range coupled to an amide carbonyl carbon C-6 (δ_C 169.2), established a glycine residue. The sequence from an amide proton 9-NH (δ_H 8.66) to another amide proton 12-NH (δ_H 8.13) through an α-methine proton 9-H (δ_H 4.82, δ_C 53.2), methylene protons 10-H$_2$ (δ_H 1.85, 2.06), 11-H$_2$ (δ_H 1.77) and 12-H$_2$ (δ_H 3.40, 3.80), and an ^{1}H–^{13}C long-range coupling from the α-methine proton 9-H to an amide carbonyl carbon C-8 (δ_C 172.5), revealed an ornithine moiety. An aromatic proton 5′-H (δ_H 7.03) was *ortho-* and *meta-* coupled to the aromatic protons 6′-H (δ_H 7.13) and 7′-H (δ_H 7.63), respectively, supporting the presence of a 1,2,3-trisubstituted benzene ring. In addition, ^{1}H–^{13}C long-range couplings from the methoxyl protons 3′-OCH$_3$ (δ_H 3.90) and 5′-H to an oxygenated aromatic quaternary carbon C-3′ (δ_C 147.7), from the methoxyl protons 4′-OCH$_3$ (δ_H 3.88) and 6′-H to an oxygenated aromatic quaternary carbon C-4′ (δ_C 152.8), and 7′-H to a carbonyl carbon C-1′ (δ_C 166.0) were observed. These results established a 2,3-dimethoxybenzoate moiety as shown in Figure 1. In the same manner, another 2,3-dimethoxybenzoate moiety was determined.

The connection of these substructures was elucidated by ^{1}H–^{13}C long-range couplings from 4-NH to C-6, from 7-NH to C-8, from 9-NH to C-1′ and from 12-H to C-1″ (δ_C 166.0), as shown in Figure 1b. In addition to the molecular formula of **2**, no cross signal was observed with the remaining methoxyl group 1-NOCH$_3$ (δ_H 3.68, δ_C 61.3), indicating that this methoxyl group is attached to the nitrogen of the piperidin-2-one moiety. Thus, the structure of **1** was established as shown in Figure 1a. A compound related with **1** is heterobactin A,[12] in which one 2,3-dihydrobenzoate moiety in **1** is replaced by a benzoxazole residue. In addition, rhodobactin,[14] which possesses both hydroxamate and biscatecholate moieties, has been reported as a siderophore. To our knowledge, this is the first report with regard to secondary metabolites from *N. tenerifensis*.

Compound **1** showed a red color in 10% aqueous FeCl$_3$ and the ESI–MS of the complex resulted in the $(M+Fe+H)^+$ ion at *m/z* 627 in the positive mode. The formation of **1**–iron complex was also confirmed by the changes in UV spectrum (absorption maxima at 205 and 249 nm were changed to 245 and 364 nm, respectively). However, **2** did not form a complex with ferric ion. The hydroxamate involved in trichostatin A is known to chelate metal ions.[15] Mycobactin[16] and exochelin[16] have been reported as siderophores that lack catechol units. In contrast, azotochelin,[17] which possesses 2,3-dihydrobenzoate moieties but not hydroxamate functional groups, has

also been reported to show siderophore activity. Thus, both the hydoxamate and catechol functional groups in **1** are considered to have a significant role in chelating properties. The lack of the siderophore activity of **2**, by which hydroxamate and catechols are methylated, also supports the role of these functional groups. Further biological activities are under investigation.

ACKNOWLEDGEMENTS

This work was supported by a grant from the New Energy and Industrial Technology Department Organization (NEDO) of Japan.

1 Tanaka, Y., Gräfe, U., Yazawa, K., Mikami, Y. & Ritzau, M. Nocardicyclins A and B: new anthracycline antibiotics produced by *Nocardia pseudobrasiliensis*. *J. Antibiot.* **50,** 822–827 (1997).
2 Tanaka, Y., Gräfe, U., Yazawa, K. & Mikami, Y. Production of nocardicyclins by clinical isolates of *Nocardia pseudobrasiliensis* and *in vivo* antitumor activity of the antibiotic. *J. Antibiot.* **5l,** 589–591 (1998).
3 Mikami, Y. *et al*. A new antifungal macrolide component, Brasilicardin B produced by *Nocardia brasiliensis*. *J. Antibiot.* **53,** 70–74 (2000).
4 Mukai, A. *et al.* Transvalencin Z, a new antimicrobial compound with salicylic acid residue from *Nocardia transvalensis* IFM 10065. *J. Antibiot.* **59,** 366–369 (2006).
5 Shigemori, H. *et al.* Brasilicardin A. A novel tricyclic metabolite with potent immunosuppressive activity from actinomycete *Nocardia brasiliensis*. *J. Org. Chem.* **63,** 6900–6904 (1998).
6 Mikami, Y. *et al.* A new antifungal macrolide component, Brasilicardin B produced by *Nocardia brasiliensis*. *J. Antibiot.* **53,** 70–74 (2000).
7 Drechsel, H. & Jung, G. Peptide siderophore. *J. Peptide Sci.* **4,** 147–181 (1998).
8 Tsuda, M. *et al.* Brasilibactin A, a cytotoxic compound from actinomycete *Nocardia brasiliensis*. *J. Nat. Prod.* **68,** 462–464 (2005).
9 Nemoto, A. *et al.* Asterobactin, a new siderophore group antibiotic from *Nocardia asteroides*. *J. Antibiot.* **55,** 593–597 (2002).
10 Ratledge, C. & Snow, G. A. Isolation and structure of nocobactin NA, a lipid-soluble iron-binding compound from *Nocardia asteroides*. *Biochem. J.* **139,** 407–413 (1974).
11 DeBoer, C. & Dietz, A. The description and antibiotic production of *Streptomyces hygroscopicus* var. *Geldanus*. *J. Antibiot.* **29,** 1182–1191 (1976).
12 Carran, C. J., Jordan, M., Drechsel, H., Schmid, D. G. & Winkelmann, G. Heterobactins. A new class of siderophores from *Rhodococcus erythropolis* IGTS8 containing both hydroxamate and catecholate donor groups. *Biometals* **14,** 119–125 (2001).
13 Furihata, K. & Seto, H. Constant time HMBC (CT-HMBC), a new HMBC technique useful for improving separation of cross peaks. *Tetrahedron Lett.* **39,** 7337–7340 (1998).
14 Dhungana, S. *et al.* Purification and characterization of rhodobactin: a mixed ligand siderophore from Rhodococcus rhodochrous strain OFS. *Biometals.* **20,** 853–867 (2007).
15 Finnin, MS. *et al.* Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* **401,** 188–193 (1999).
16 Ratledge, C. Iron, mycobacteria and tuberculosis. *Tuberculosis* **84,** 110–130 (2004).
17 Corbin, J. L. & Bulen, W. A. The isolation and identification of 2,3-dihydroxybenzoic acid and 2-N,6-N-di-92,3-dihydroxybenzoyl)-L-lysine formed by iron-deficient Azotobacter vinelandii. *Biochemistry* **8,** 77–762 (1969).