Nocarasins A−C and Brasiliquinone D, New Metabolites from the Actinomycete *Nocardia brasiliensis*

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Four new metabolites, nocarasins A−C (**1**−**3**) and brasiliquinone D (**4**), have been isolated from the actinomycete *Nocardia brasiliensis* IFM 0667, and the structures were elucidated on the basis of spectroscopic data and chemical means. Nocarasins A−C (**1**−**3**) and brasiliquinone D (**4**) exhibited cytotoxicity and antibacterial activity.

During our search for bioactive substances from pathogenic actinomycetes of the genus *Nocardia*, we previously isolated a cytotoxic anthracycline,[1] a 32-membered macrolide possessing immunosuppressive and antifungal activity,[2] three benz[*a*]anthraquinones with cytotoxic and antibacterial activity,[3] a cytotoxic indole alkaloid,[4] and a tricyclic terpenoid with immunosuppressive and cytotoxic activity.[5] Recently, an investigation of extracts of *Nocardia brasiliensis* IFM 0667 resulted in the isolation of four new metabolites, nocarasins A−C (**1**−**3**) and brasiliquinone D (**4**). Here we describe the isolation and structure elucidation of **1**−**4**.

Results and Discussion

The mycelia of *N. brasiliensis* IFM 0667 were extracted with MeOH, and the Et_2O-soluble materials of the extract were subjected to Si gel column chromatography (hexane−EtOAc and then EtOAc−MeOH), followed by C_{18} HPLC (CH_3CN-H_2O) to yield nocarasins A (**1**, 13 mg), B (**2**, 3 mg), and C (**3**, 5 mg) and brasiliquinone D (**4**, 1 mg), together with known compounds, brasiliquinones A (**6**) and C.[3]

The molecular formula of nocarasin A (**1**), a colorless oil, was revealed to be $C_{17}H_{24}O_2$ by HREIMS [*m*/*z* 260.1787 (M^+), Δ +1.0 mmu]. An IR band at 3370 cm^{-1} was attributed to hydroxyl group(s). UV absorptions [λ_{max} (MeOH) 279 (ϵ 2500) and 226 nm (6000)] and the three aromatic proton signals [H-2, δ_H 6.83 (d, J = 1.5 Hz); H-4, δ_H 6.84 (dd, J = 1.5, 8.0 Hz); H-5, δ_H 7.09 (d, J = 8.0 Hz)] in the ^1H NMR spectrum were characteristic of a 1,2,4-trisubstituted benzene ring. The ^{13}C NMR (Table 1) spectrum, including DEPT experiments, disclosed five sp^2 quaternary carbons, five sp^2 methines, four sp^3 methylenes (one oxygenated), and three methyl signals. The relatively lower-field sp^2 carbon signal at δ_C 154.6 (C-1) and a deuterium-exchangeable proton at δ_H 5.05 (OH-1) were suggestive of the presence of a phenolic hydroxyl group. The chemical shifts of three singlet methyl signals (H_3-14, δ_H 1.63; H_3-15, 1.60; H_3-16, 1.76) indicated that these were vinyl methyl groups. The $^1H-^1H$ COSY spectrum of **1** implied the connctivities of C-2−C-5, C-7−C-8, and C-10−C-12. HMBC correlations from H_3-16 to C-8, C-9, and C-10 revealed connections among C-8, C-10, and C-16 through an sp^2 quaternary carbon of C-9. Two vinyl methyl proton signals (H_3-14 and H_3-15) showed long-range correlations for C-12 and C-13, suggesting that both methyl groups were attached to C-13. The trisubstitued benzene ring was attached to the methylene carbon at C-7 from HMBC correlations from H_2-7 to C-1, C-5, and C-6. The oxymethylene carbon (C-17) and a phenolic hydroxyl group were connected at C-3 and C-1, respectively, judging from HMBC correlations from H_2-17 to C-2, C-3, and C-4, and from H-5 to C-1. The presence of two hydroxyl groups at C-1 and C-17 was established by acetylation of **1**. In the ^1H NMR spectrum of the diacetate (**5**), two methyl signals (δ_H 2.08 and 2.31) of acetyl groups were observed, and the oxymethylene proton signal at C-17 was shifted to the lower field (δ_H 5.22). Thus, the structure of nocarasin A was concluded to be **1**.

Nocarasin B (**2**), a colorless oil, was revealed to possess the molecular formula, $C_{18}H_{24}O_3$, by HREIMS [*m*/*z* 288.1722 (M^+), Δ −0.3 mmu]. The IR spectrum indicated the presence of hydroxyl (3325 cm^{-1}) and ester carbonyl (1725 cm^{-1}) groups. ^1H and ^{13}C NMR data (Table 1) revealed that **2** possessed an ester carbonyl, five sp^2 quaternary carbons, five sp^2 methines, three sp^3 methylenes, one methoxy, and three vinyl methyl groups. Extensive analyses of 2D NMR data implied that the structure of **2** was the same as that of **1** except for the substituent at C-3. The exsitence of the methoxycarbonyl group at C-3 was deduced from HMBC correlations from H-2, H-4, and 17-OCH_3 to C-17. Thus, the structure of nocarasin B was assigned as **2**. This was supported by conversion of **2** into **1** by $LiAlH_4$ reduction.

HREIMS [*m*/*z* 302.1891 (M^+), Δ +0.9 mmu] of nocarasin C (**3**) established the molecular formula as $C_{19}H_{26}O_3$, corresponding to the methyl ether of nocarasin B (**2**). The ^1H and ^{13}C NMR data (Table 1) were analogous to those of **2**. The ^{13}C NMR spectrum contained two methoxy signals [δ_c 55.6 (1-OCH_3) and 52.0 (17-OCH_3)], one of which was not observed for **2**. The HMBC spectrum revealed the H−C long-range correlation from 1-OCH_3 to C-1. Thus, nocarasin C (**3**) was concluded to be 1-*O*-methyl form of nocarasin B (**2**).

Brasiliquinone D {**4**, $[\alpha]^{23}_D$ +87° (*c* 0.40, $CHCl_3$)} was a red amorphous solid, and the molecular formula was elucidated as $C_{28}H_{29}NO_8$ by HRFABMS [*m*/*z* 507.1872 (M^-), Δ −2.1 mmu], corresponding to the monoacetate of brasiliquinone A[3] (**6**). The ^1H and ^{13}C NMR data of **4** were similar to those of **6**. The ^1H NMR spectrum showed an amide (δ_H 8.52) and an acetyl methyl proton signal (δ_H 2.28), sug-

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Table 1. ^{1}H and ^{13}C NMR Data of Nocarasins A (**1**), B (**2**), and C (**3**) in $CDCl_3$

	1			**2**		**3**	
position	δ_C	δ_H (m, Hz)	HMBC (H)	δ_C	δ_H (m, Hz)	δ_C	δ_H (m, Hz)
1	154.6 s		5, 7	154.3 s		157.3 s	
2	114.5 d	6.83 (d, 1.5)	4, 17	122.1 d	7.51 (d, 1.3)	110.9 d	7.49 (d, 1.2)
3	140.5 s		5, 17	132.9 s		135.9 s	
4	119.2 d	6.84 (dd, 1.5, 8.0)	2, 17	116.6 d	7.54 (dd, 1.3, 7.7)	122.1 d	7.58 (dd, 1.2, 7.8)
5	130.1 d	7.09 (d, 8.0)	7	129.8 d	7.17 (d, 7.7)	129.0 d	7.19 (d, 7.8)
6	126.5 s		4, 7	129.4 s		126.8 s	
7	29.4 t	3.36[a] (d, 7.1)	5	29.5 t	3.41[a] (m)	28.4 t	3.36[a] (m)
8	121.7 d	5.32 (t, 7.1)	7, 16	120.8 d	5.31 (br t, 7.0)	121.5 d	5.30 (br t, 7.7)
9	138.4 s		7, 16	139.0 s		137.0 s	
10	39.7 t	2.09[a] (m)	8, 11, 16	39.7 t	2.09[a] (m)	39.8 t	2.06[a] (m)
11	26.5 t	2.12[a] (m)	10	26.5 t	2.12[a] (m)	26.7 t	2.10[a] (m)
12	124.0 d	5.08 (br t, 5.9)	14, 15	123.9 d	5.07 (br t, 5.4)	124.3 d	5.10 (br t, 5.1)
13	131.9 s		14, 15	132.0 s		131.5 s	
14	25.7 q	1.63[b] (s)	15	25.7 q	1.69[b] (s)	25.7 q	1.68[b] (s)
15	17.7 q	1.60[b] (s)	14	17.7 q	1.60[b] (s)	17.7 q	1.60[b] (s)
16	16.2 q	1.76[b] (s)	8	16.2 q	1.75[b] (s)	16.0 q	1.69[b] (s)
17	65.1 t	4.61[a] (s)	2, 4	167.1 s		167.3 s	
1-OH		5.05 (br)			5.59 (br s)		
1-OCH_3				55.6 q	3.89[b] (s)		
17-OCH_3				52.1 q	3.89[b] (s)	52.0 q	3.90[b] (s)

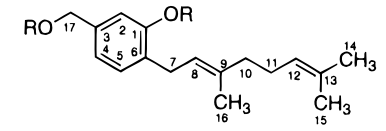
[a] 2H. [b] 3H.

Table 2. Antimicrobial Activity of Nocarasins A−C (**1**−**3**) and Brasiliquinone D (**4**)[a]

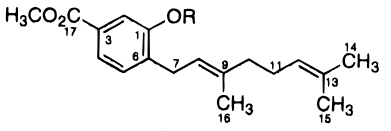
	MIC (μg/mL)				
test organisms	**1**	**2**	**3**	**4**	rifampicin
Nocardia asteroides	3.13	1.56	>25	1.56	50
Gordonia bronchialis	0.39	0.78	25	0.78	0.39
Mycobacterium smegmatis	6.25	6.25	>25	12.5	12.5
Bacillus subtilis PCI189	6.25	1.56	>25	6.25	<0.02
Micrococcus luteus	>25	>25	>25	25	<0.02
Escherichia coli NIHJC2	>25	>25	>25	>25	3.13
Aspergillus niger	25	>25	>25	>25	>100
Candida albicans	>25	>25	>25	>25	>100
Cryptococcus neoformans	>25	>25	>25	>25	>100

[a] Mueller−Hinton broth and Sabouraud dextrose broth were used for bacteria and fungi, respectively.

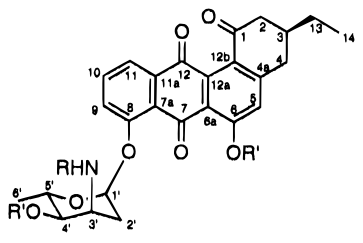
gesting that **4** was the 3′-*N*-acetyl form of brasiliquinone A (**6**). The absolute stereochemistry of **4** was established to be the same as that of **6**, because all spectral data of the diacetate[3] (**7**) of **4** were identical with those of the triacetate of **6**.



1 : R = H
5 : R = Ac



2 : R = H
3 : R = CH_3



4 : R = Ac, R′ = H
6 : R = R′ = H
7 : R = R′ = Ac

Nocarasins A−C (**1**−**3**) are new benzenoid metabolites with a geranyl side chain, while brasiliquinone D (**4**) is a new benz[*a*]anthraquinone with an ethyl group at C-3. Compounds **1**, **2**, and **4** showed inhibitory activity against some Gram-positive bacteria, especially against acid-fast bacteria such as *Nocardia asteroides* (MIC 3.13, 1.56, and 1.56, μg/mL, respectively), *Gordonia bronchialis* (MIC 0.39, 0.78, and 0.78, μg/mL, respectively), and *Mycobacterium smegmatis* (MIC 6.25, 6.25, and 12.5, μg/mL, respectively) (Table 2). On the other hand, there was almost no activity in compound **3**, indicating that the phenolic hydroxyl group at C-1 was important for the activity. Nocarasins A−C (**1**−**3**) exhibited cytotoxicity against HL-60 human promyelocytic leukemia cells in vitro, with IC_{50} values of 0.91, 0.51, and 0.60 μg/mL, respectively, while brasiliquinone D (**4**) showed weak cytotoxicity against murine leukemia L1210 (IC_{50}, 11 μg/mL) and KB human epidermoid carcinoma cells (IC_{50}, 20 μg/mL) in vitro.

Experimental Section

General Methods. The IR and UV spectra were recorded on a JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-500 and JEOL EX-400 spectrometer, respectively. EIMS were recorded on a JEOL DX-303 spectrometer. FABMS were obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix. Antimicrobial activities were determined by the microbroth dilution method using

brain heart infusion (BHI) medium. Cytotoxic activities were determined by the method described.[6] Adriamycin was included as a positive drug control: IC_{50} 0.01 (HL-60), 0.09 (L1210), and 0.97 μg/mL (KB).

Cultivation, Extraction, and Isolation. The voucher specimen (*Nocardia brasiliensis* IFM 0667) was deposited at the Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University. *N. brasiliensis* IFM 0667 strain was cultivated at 32 °C for 4 days in a 15-L jar-fermenter containing nutrient broth medium [glucose (2.0%), glycerol (2.0%), polypeptone (1.0%), and meat extract (0.5%) in H_2O at pH 7.0]. The cultured broth (15 L) was filtered, and the mycelial cake was extracted with MeOH (3 L). The Et_2O-soluble materials of the extract were subjected to Si gel column chromatography (hexane−EtOAc, 40:1, 20:1, and 5:1 and then EtOAc−MeOH, 1:1) and C_{18} HPLC (Soken Pak ODS-W S-15/30, Soken Chemical & Engineering Co., Ltd., 25 × 250 mm; CH_3CN-H_2O, 10:90 to 90:10, 90 min; flow rate 30 mL/min; UV detection at 230 nm) to afford nocarasins A (**1**, 13 mg, t_R 33.5 min, from fraction eluting with hexane−EtOAc, 40:1), B (**2**, 3 mg, t_R 41.4 min, from fraction eluting with hexane−EtOAc, 20:1), and C (**3**, 5 mg, t_R 49.6 min, from fraction eluting with hexane−EtOAc, 5:1) and brasiliquinone D (**4**, 1 mg, t_R 29.5 min, from fraction eluting with EtOAc−MeOH, 1:1).

Nocarasin A (1): a colorless oil; IR (neat) ν_{max} 3370 (br), 2920, and 1620 cm^{-1}; UV (EtOH) λ_{max} 279 (ϵ 2500) and 226 nm (6000); ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 260 (M^+); HREIMS *m/z* 260.1787 (M^+), calcd for $C_{17}H_{24}O_2$, 260.1777.

Nocarasin B (2): a colorless oil; IR (neat) ν_{max} 3325 (br), 2925, and 1725 cm^{-1}, UV (EtOH) λ_{max} 280 (ϵ 3500) and 245 nm (8000); ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 288 (M^+); HREIMS *m/z* 288.1722 (M^+), calcd for $C_{18}H_{24}O_3$, 288.1725.

Nocarasin C (3): a colorless oil; IR (neat) ν_{max} 2930 and 1720 cm^{-1}, UV (EtOH) λ_{max} 279 (ϵ 4000) and 250 nm (10 000); ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 302 (M^+); HREIMS *m/z* 302.1891 (M^+), calcd for $C_{19}H_{26}O_3$, 302.1882.

Brasiliquinone D (4): a red amorphous solid; $[\alpha]^{23}_D$ +87° (*c* 0.40, $CHCl_3$); IR (neat) ν_{max} 3360, 2925, 1680, and 1630 cm^{-1}; UV (EtOH) λ_{max} 413 (ϵ 8300), 268 (34 000), and 215 nm (39 000); ^1H NMR ($CDCl_3$) δ 1.01 (3H, t, J = 7.4 Hz, H_3-14), 1.26 (3H, d, J = 6.1 Hz, H_3-6′), 1.53 (2H, m, H_2-13), 2.18 (1H, m, H-2), 2.24 (1H, br d, J = 15.0 Hz), 2.28 (3H, s, 3′-NAc), 2.34 (1H, dt, J = 15.0, 3.9 Hz, H-2′), 2.55 (1H, dd, J = 11.2, 15.9 Hz), 2.66 (1H, br dd, J = 10.9, 16.6 Hz, H-4), 2.93 (1H, dd, J = 4.7, 15.9 Hz, H-2), 2.97 (1H, br dd, J = 3.6, 16.6 Hz, H-4), 3.60 (1H, dd, J = 3.6, 9.7 Hz, H-4′), 3.86 (1H, dq, J = 9.7, 6.1 Hz, H-5′), 4.68 (1H, m, H-3′), 5.76 (1H, br d, J = 3.0 Hz, H-1′), 7.01 (1H, s, H-5), 7.66 (1H, d, J = 8.3 Hz, H-9), 7.76 (1H, dd, J = 7.5, 8.3 Hz, H-10), 7.84 (1H, d, J = 7.5 Hz, H-11), 8.52 (1H, br d, J = 6.3 Hz, NH-3′), and 12.95 (1H, s, OH-6); ^{13}C NMR ($CDCl_3$) δ 11.1 (q, C-14), 17.4 (q, C-6′), 23.0 (q, 3′-NAc), 28.8 (t, C-13), 33.3 (t, C-2′), 36.5 (t, C-4), 37.0 (d, C-3), 45.7 (t, C-2), 47.2 (d, C-3′), 66.2 (d, C-5′), 74.5 (d, C-4′), 95.8 (d, C-1′), 117.6 (s, C-6a), 119.6 (s, C-7a), 120.3 (d, C-9), 121.2 (d, C-11), 121.4 (d, C-5), 124.2 (s, C-12b), 136.9 (d, C-10), 137.5 (s, C-12a), 137.6 (s, C-11a), 152.9 (s, C-4a), 157.2 (s, C-8), 163.6 (s, C-6), 174.3 (s, 3′-NAc), 183.9 (s, C-12), 189.4 (s, C-7), and 198.0 (s, C-1); FABMS (neg.) *m/z* 507 (M^-); HRFABMS *m/z* 507.1872 (M^-), calcd for $C_{28}H_{29}NO_8$, 507.1893.

Acetylation of Nocarasin A (1). Nocarasin A (**1**, 0.5 mg) was treated with acetic anhydride (100 μL) and pyridine (100 μL) at room temperature for 8 h. The mixture was evaporated in vacuo to afford compound **5** (0.5 mg), a colorless oil: IR (neat) ν_{max} 1760 and 1730 cm^{-1}; UV (EtOH) λ_{max} 280 (ϵ 2700) nm; ^1H NMR δ_H 1.60 (3H, s), 1.676 (3H, s), 1.683 (3H, s), 2.04 (2H, t, J = 7.4 Hz), 2.08 (3H, s), 2.09 (2H, m), 2.31 (3H, s), 3.23 (2H, d, J = 7.2 Hz), 5.06 (2H, s), 5.09 (1H, t, J = 6.5 Hz), 5.22 (1H, t, J = 7.2 Hz), 7.03 (1H, d, J = 1.0 Hz), 7.15 (1H, dd, 1.0, 7.8 Hz), and 7.23 (1H, d, J = 7.8 Hz); EIMS *m/z* 344 (M^+); HREIMS *m/z* 344.1988 (M^+), calcd for $C_{21}H_{28}O_4$, 344.1987.

Reduction of Nocarasin B (2) with $LiAlH_4$. To a solution of nocarasin B (**2**, 1 mg) in ether (200 μL) was added $LiAlH_4$ (2 mg) at 0 °C, and stirring was continued at room temperature for 1 h. After addition of H_2O, the reaction mixture was extracted with Et_2O, and the organic phase was washed with brine, and evaporated in vacuo. The residue was passed through a Sep-Pak silica cartridge (hexane−EtOAc, 2:1) to afford the reduction product of **2** (0.6 mg), of which physicochemical data were identical with those of nocarasin A (**1**).

Acetylation of Brasiliquinone D (4). Brasiliquinone D (**4**, 0.5 mg) was treated with acetic anhydride (100 μL) and pyridine (100 μL) at room temperature for 12 h. The solvent was removed by nitrogen stream to afford the diacetate (**6**) of **4** (0.2 mg), of which physicochemical data were identical with those of the triacetate of brasiliquinone A (**6**).

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