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Brasiliquinones A–C, new cytotoxic benz[*a*]anthraquinones with an ethyl group at C-3 from actinomycete *Nocardia brasiliensis*

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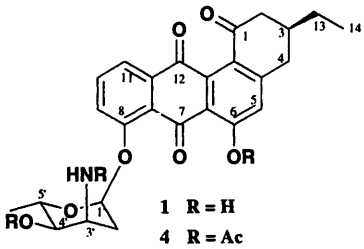
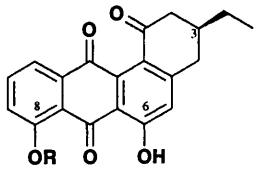
Three new cytotoxic benz[*a*]anthraquinones with an ethyl group at C-3, brasiliquinones A–C 1–3, have been isolated from the actinomycete *Nocardia brasiliensis* IFM 0089, and their structures elucidated on the basis of spectroscopic data and by chemical means. Brasiliquinone A 1 is the first benz[*a*]anthraquinone possessing ristosamine as an *O*-glycoside moiety, while brasiliquinones B 2 and C 3 correspond to the aglycone of 1 and the 8-*O*-methyl derivative of 2, respectively.

During our search for bioactive substances from microorganisms,[1] three new cytotoxic benz[*a*]anthraquinones,[2] brasiliquinones A–C **1–3**, with an ethyl group at C-3 have been isolated from the actinomycete *Nocardia brasiliensis* IFM 0089. Brasiliquinone A **1** is the first benz[*a*]anthraquinone possessing ristosamine as an *O*-glycoside moiety. This paper describes the isolation and structural elucidation of **1–3**.



1 R = H
4 R = Ac



2 R = H
3 R = CH_3

Results and discussion

The brasiliquinones were isolated by HPLC separation of the mycelium extracts. Brasiliquinone A **1** revealed the pseudomolecular ion peak at *m/z* 466 $(M + H)^+$ and that of the reduced form at *m/z* 468 $(M + 2 + H)^+$, which has been often observed for quinones and related compounds,[3] in the FABMS spectrum. The molecular formula, $C_{26}H_{27}NO_7$, was established by HRFABMS [*m/z* 468.2052 $(M + 2 + H)^+$, Δ +3.0 mmu]. The IR spectrum suggested the presence of hydroxy (3450 cm^{-1}) and quinone (1670 and 1630 cm^{-1}) groups. The ^{13}C NMR data of **1** indicated that the molecule possessed a ketone, two quinone carbonyls, six olefins, five methines, four

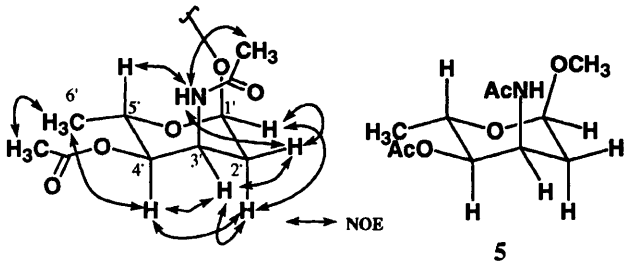


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Fig. 1 Relative stereochemistry of the sugar moiety of triacetate **4** of brasiliquinone A **1**. *J* (H, H) in Hz: 1′, 2′α > 0.1; 1′, 2′β 2.0; 2′α, 2′β 12.4; 2′α, 3′ 4.3; 2′β, 3′ > 0.1; 3′, 4′ 3.8; 4′, 5′ 10.3; 5′, 6′ 6.2.

methylenes and two methyl carbons. Nine of the fourteen unsaturations were accounted for, thus implying that brasiliquinone A **1** consisted of a five ring system. Structure elucidation of **1** was carried out mainly using the triacetyl derivative **4** of **1**, which was obtained by treatment of **1** with Ac_2O and pyridine, since ^1H NMR data of **1** could not be clearly assigned due to broadening of proton resonances. Interpretation of ^1H–^1H COSY, HMQC and HMBC (Table 1) spectra of **4** suggested the presence of three partial structures: C-1–C-6a and C-12b, C-7a–C-12, and a 3-amino-2,3,6-trideoxyhexose moiety (C-1′–C-6′). Two of three acetyl groups were verified by HMBC correlations from 3′-NH and H-4′ to each acetyl carbonyl carbon. On the other hand, the remaining one was attached to the hydroxy group at C-6, since the chemical shift (δ_C 150.3) of C-6 in **4** was observed at higher field than that (δ_C 163.3) of **1**. The HMBC cross-peak for H-1′/C-8 revealed that the amino sugar moiety was connected to a phenolic carbon (δ_C 155.6, C-8) through an *O*-glycoside bond. Although connection of these partial structures to the two remaining carbons (C-7 and C-12a) allowed two possible structures for brasiliquinone A, **1** or its 11*O*-glycosyl isomer, it was assigned as **1** by comparison of ^{13}C NMR data of **1** with those of atramycin A[4] with an *O*-glycoside moiety at C-8. Relative configurations of the amino glycoside moiety were deduced from the proton coupling constants and NOESY data of the triacetate **4** as shown in Fig. 1. Hydrolysis of brasiliquinone A **1** yielded the aglycone **2** (brasiliquinone B) and 1-methyl glycoside, the latter being acetylated to afford methyl *N*,*O*-diacetylristosaminide[5] **5** possessing 1′*S*, 3′*R*, 4′*S* and 5′*S* configurations. The absolute configuration at C-3 of **1** was assigned to be *S* from comparison of the optical rotation of the aglycone **2** ($[\alpha]_D^{23}$ +51) with that of rubiginone B_2[6] ($[\alpha]_D^{23}$ +78) possessing a 3*S*-configuration.[7] Thus the structure of brasiliquinone A was concluded to be **1**.

HREIMS data (*m/z* 350.1175, M^+, Δ +2.1 mmu) of brasiliquinone C **3** established the molecular formula as $C_{21}H_{18}O_5$, corresponding to the methyl ether of brasiliquinone

Table 1 ^1H and ^{13}C NMR data of triacetate **4** of brasiliquinone A **1**[a]

Position	δ_H (m, J in Hz)	δ_C	HMBC (H)
1	2.95 (ddd, 1.4, 4.8, 15.0)	198.2	2,
2α	2.63 (dd, 11.5, 15.0)	45.7	4, 13
2β	2.23 (m)		
3	3.01 (br dd, 4.5, 17.2)	37.5	2, 4β, 13, 14
4α	2.70 (br dd, 10.8, 17.2)	36.0	2,13
4β			
4a	7.14 (s)	150.6	4, 5
5		127.7	4β
6		150.3	5
6a		127.3	5
7		182.0	
7a		122.3	9, 11
8		155.6	10, 1′
9	7.72 (dd, 1.0, 8.4)	120.7	
10	7.69 (dd, 7.6, 8.4)	135.5	
11	7.77 (dd, 1.0, 7.6)	120.8	9
11a		136.9	10α
12		184.3	11
12a		137.7	
12b		133.9	4
13	1.55 (m)[b]	28.9	2β, 4β, 14
14	1.01 (t, 7.4)[c]	11.0	13
1′	5.73 (d, 3.0)	96.4	
2′α	2.18 (m)	33.4	
2′β	2.32 (ddd, 4.0, 4.6, 14.9)		
3′	4.85 (m)	42.8	1′, 2′a, NH-3′
4′	4.67 (dd, 3.9, 10.4)	72.8	2′a, 3′, 5′, 6′
5′	4.06 (dq, 10.4, 6.2)	63.3	1′, 4′, 6′
6′	1.16 (d, 6.2)	17.4	
6-OAc	2.37 (s)	21.1	
		169.0	
NH-3′	8.34 (d, 8.7)		
3′-NAc	2.26 (s)	23.2	NH-3
		171.2	
4′-OAc	2.02 (s)	20.9	H-4′
		170.3	

[a] In $CDCl_3$. [b] 2 H. [c] 3 H.

B **2**. The NMR spectral data of **3** were analogous to those of **2**. The ^1H NMR spectrum of **3** showed a methoxy signal at C-8 which was not observed for **2**, while the HMBC spectrum of **3** revealed an H–C long-range correlation for 8-Me/C-8. Thus the structure of brasiliquinone C was assigned to be **3**. The optical rotation ($[\alpha]_D^{23}$ +69) of **3** suggested a 3*S*-configuration.

Benz[*a*]anthraquinone antibiotics are classified as angucycline-type antibiotics,[2] most of which possess a *C*-glycoside moiety. Brasiliquinone A **1** is the first example of an angucycline-type antibiotic possessing a rare amino sugar ristosamine[8] as an *O*-glycoside moiety.† Most of the angucycline-type antibiotics have a C_1 unit at C-3, while brasiliquinones A–C (**1**–**3**) are the first angucycline-type antibiotics which possess an ethyl group at C-3. A previous biogenetic study of the angucycline-type antibiotics has suggested that the benz[*a*]-anthraquinone skeleton may be derived from a decaketide metabolite decarboxylating at the carboxy end, and that the methyl group at C-3 may correspond to the methyl end of the decaketide.[10] On the other hand, the presence of a C_2 unit at C-3 for **1**–**3** is unusual, and the origin of the C-14 is an interesting subject to be clarified. Brasiliquinone A **1** exhibited cytotoxicity against L1210 and KB tumour cells *in vitro* (IC_{50} 0.55 and 0.73 μg ml^{-1}, respectively), while brasiliquinones B **2** and C **3** showed cytotoxicity against L1210 cells (IC_{50} 3.4 μg ml^{-1} each). Compounds **1**, **2** and **3** exhibited antibacterial activity against *Mycobacterium smegmatis* ATCC607 (MIC 12.5, 0.78 and 0.39 μg ml^{-1}, respectively), *Micrococcus luteus* IFM2066 (MIC 3.2, 3.2 and 6.3 μg ml^{-1}, respectively), *Staphylococcus aureus* 209P (MIC 25 μg ml^{-1}, each) and *Staphylococcus aureus* MRSA IFM62971 (MIC 12.5, 25 and 50 μg ml^{-1}, respectively), whereas **1**, **2** and **3** showed no antifungal activity against *Aspergillus niger* (MIC > 100 μg ml^{-1} each). Brasiliquinone C **3** showed inhibitory activity against epidermal growth factor (EGF) receptor kinase (IC_{50} 0.74 μg ml^{-1}) and *c-erbB*-2 kinase (IC_{50} 7.3 μg ml^{-1}).

† A few benz[*a*]anthraquinone antibiotics possessing an *O*-glycoside moiety such as atramycins[4] and landmycins[9] have been reported.

Experimental

IR and UV spectra were recorded on JASCO FT/IR-5300 and HASCO Ubest-35 spectrophotometers, respectively. Optical rotations were measured on a JASCO DIP-360 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker AMX-600 and JEOL EX-400 spectrometers, respectively and J values are given in Hz. FAB mass spectra were obtained on a JEOL HX-110 spectrometer using 2-thioglycerol as a matrix. EI mass spectra (EIMS) were recorded on a JEOL DX-303 spectrometer.

Cultivation

The voucher specimen (*Nocardia brasiliensis* IFM 0089) was deposited at the Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University. *N. brasiliensis* IFM 0089 strain was cultivated at 32 °C for 4 days in a 150 l jar-fermenter containing a nutrient broth medium [glucose (2.0%), glycerol (2.0%), polypeptone (1.0%) and meat extract (0.5%) in H_2O at pH 7.0].

Extraction and isolation

The cultured broth (150 l) was filtered and the mycelial cake was extracted with MeOH–acetone (1:1, 2 l). The EtOAc-soluble material of the extract was dissolved in hexane (2 l). The hexane extract was evaporated *in vacuo* and subjected to silica gel column chromatography (hexane–EtOAc, 15:1) and silica gel HPLC (YMC Pack SIL-06, YMC, 20 × 250 mm; eluent, hexane–EtOAc, 8:1; flow-rate, 30 ml min^{-1}; UV detection, 380 nm) to afford brasiliquinones B **2** (1.0 mg, t_R 6.0 min) and C **3** (1.4 mg, t_R 12.6 min). The EtOAc-soluble parts of the hexane-insoluble material of the extract were subjected to silica gel column chromatography (benzene–EtOAc and then acetone). The fraction eluted with acetone was purified using a Sephadex LH-20 column ($CHCl_3$–MeOH, 1:1) and linear-gradient C_{18} HPLC (Capcell Pak C_{18} SG120, Shiseido, 50 × 250 mm; eluent, CH_3CN–H_2O–CF_3CO_2H, 20:80:0.1 to 35:65:0.1, 120 min; flow rate, 20 ml min^{-1}; UV detection, 380 nm) to afford brasiliquinone A **1** (5.0 mg, t_R 18 min).

Brasiliquinone A 1. A red powder; mp 132–135 °C; $[\alpha]_D^{25}$ +130 (*c* 0.30, $CHCl_3$); ν_{max}/cm^{-1} 3350, 3150, 1670 and 1630; λ_{max}(MeOH)/nm 412 (ε 7000), 266 (20 000) and 228 (17 000); δ_H 1.00 (3 H, t, *J* 7.4, H_3-14), 1.13 (3 H, d, *J* 4.0, H_3-6′), 1.50 (2 H, m, H_2-13), 2.14 (1 H, m, H-3), 2.27 (1 H, m, H-2′α), 2.48 (1 H, m, H-2′β), 2.54 (1 H, m, H-2), 2.56 (1 H, m, H-4), 2.84 (1 H, br d, *J* 12.0, H-4), 2.88 (1 H, dd, *J* 3.5 and 13.1, H-2), 3.40 (1 H, m, H-4′), 3.56 (1 H, m, H-3′), 3.80 (1 H, m, H-5′), 5.83 (1 H, br s, H-1′), 6.79 (1 H, s, H-5), 7.59 (1 H, d, *J* 7.0, H-9), 7.73 (1 H, dd, *J* 6.3 and 7.0, H-10) and 7.79 (1 H, d, *J* 6.3, H-11); δ_C 11.1 (q, C-14), 17.9 (q, C-6′), 28.9 (t, C-13), 34.6 (t, C-2′), 36.4 (t, C-4), 37.0 (d, C-3), 45.6 (t, C-2), 47.2 (d, C-3′), 65.6 (d, C-5′), 70.2 (d, C-4′), 95.4 (d, C-1′), 117.4 (s, C-6a), 119.9 (s, C-7a), 120.1 (d, C-9), 121.0 (d, C-5), 121.0 (d, C-11), 128.7 (s, C-12b), 136.4 (d, C-10), 137.1 (s, C-11a), 137.6 (d, C-12a), 152.5 (s, C-4a), 156.5 (s, C-8), 163.3 (s, C-6), 184.0 (s, C-12), 188.4 (s, C-7) and 198.0 (s, C-1); FABMS *m/z* 468 $(M + 2 + H)^+$, 466 $(M + H)^+$ and 355; HRFABMS *m/z* 468.2052 $(M + 2 + H)^+$. Calc. for $C_{26}H_{30}NO_7$, 468.2022.

Brasiliquinone B 2. A yellow powder; mp 187–190 °C; $[\alpha]_D^{23}$ +51 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} 3440, 1690, 1675 and 1640; λ_{max}(MeOH)/nm 428 (ε 5000), 267 (22 000) and 228 (23 000); δ_H 1.00 (3 H, t, *J* 7.4, H_3-14), 1.53 (2 H, m, H_2-13), 2.19 (1 H, m, H-3), 2.49 (1 H, dd, *J* 10.8 and 16.2, H-2), 2.60 (1 H, dd, *J* 10.9 and

15.8, H-4), 2.93 (1 H, dd, *J* 2.7 and 15.8, H-4), 3.00 (1 H, ddd, *J* 1.5, 5.6 and 16.2, H-2), 7.00 (1 H, s, H-5), 7.27 (1 H, dd, *J* 1.5 and 7.6, H-9), 7.66 (1 H, dd, *J* 1.5 and 7.8, H-11), 7.68 (1 H, t, *J* 7.8, H-10), 11.69 (1 H, s, 8-OH) and 12.38 (1 H, s, 6-OH); δ_C 11.1 (q, C-14), 28.8 (t, C-13), 36.5 (d, C-3), 36.7 (t, C-4), 45.5 (t, C-2), 115.1 (s, C-6a), 116.6 (d, C-5), 120.1 (d, C-9), 121.2 (s, C-7a), 124.0 (d, C-11), 128.8 (s, C-12b), 135.4 (s, C-11a), 135.4 (s, C-12a), 133.3 (d, C-10), 153.3 (s, C-4a), 162.5 (s, C-6), 163.5 (s, C-8), 183.8 (s, C-12), 192.6 (s, C-7) and 197.6 (s, C-1); EIMS *m/z* 336 (M^+), 308 and 276; HREIMS *m/z* 336.0988 (M^+). Calc. for $C_{20}H_{16}O_5$, 336.0997.

Brasiliquinone C 3. A yellow powder; mp 215–217 °C; $[\alpha]_D^{23}$ +69 (*c* 0.14, $CHCl_3$); ν_{max}/cm^{-1} 3440, 1690, 1675 and 1640; λ_{max}(MeOH)/nm 406 (ε 7000), 266 (14 000) and 227 (28 000); δ_H 1.00 (3 H, t, *J* 7.4, H_3-14), 1.51 (2 H, m, H_2-13), 2.18 (1 H, m, H-3), 2.46 (1 H, dd, *J* 11.2 and 14.9, H-2), 2.60 (1 H, dd, *J* 11.2 and 16.1, H-4), 2.93 (1 H, dd, *J* 3.6 and 14.9, H-4), 2.96 (1 H, dd, *J* 5.1 and 14.9, H-2), 4.06 (3 H, s, 8-OMe), 6.97 (1 H, s, H-5), 7.31 (1 H, dd, *J* 1.6 and 7.6, H-9), 7.73 (1 H, t, *J* 7.6, H-10), 7.76 (1 H, dd, *J* 1.6 and 7.6, H-11) and 13.04 (1 H, s, 6-OH); δ_C 11.2 (q, C-14), 28.7 (t, C-13), 36.5 (t, C-4), 36.7 (d, C-3), 45.6 (t, C-2), 56.6 (q, 8-OMe), 117.4 (s, C-6a), 117.4 (d, C-9), 120.0 (d, C-11), 121.0 (d, C-5), 121.1 (s, C-7a), 128.8 (s, C-12b), 136.3 (d, C-10), 136.7 (s, C-11a), 137.7 (s, C-12a), 152.2 (s, C-4a), 160.3 (s, C-8), 163.6 (s, C-6), 184.5 (s, C-12), 188.4 (s, C-7) and 198.1 (s, C-1); EIMS *m/z* 350 (M^+), 322 and 294; HREIMS *m/z* 350.1175 (M^+). Calc. for $C_{21}H_{18}O_5$, 350.1154.

Acetylation of brasiliquinone A 1

Brasiliquinone A **1** (3.0 mg) was dissolved in pyridine (0.6 ml) and Ac_2O (0.6 ml) at room temperature and left for 12 h. After the mixture was evaporated, the residue was partitioned between $CHCl_3$ (0.5 ml × 3) and H_2O (1 ml), and the $CHCl_3$ layer was evaporated under reduced pressure to afford brasiliquinone A triacetate **4** (4.0 mg) as a red oil; $[\alpha]_D$ +90 (*c* 0.22, $CHCl_3$); ν_{max}/cm 3560, 3350, 1760, 1690, 1660 and 1220; λ_{max}(MeOH)/nm 356 (ε 7000), 266 (20 000) and 228 (17 000); ^1H and ^{13}C NMR (see Table 1); FABMS *m/z* 594 $(M + 2 + H)^+$, 592 $(M + H)^+$, 553 and 369; HRFABMS *m/z* 592.2153 $(M + H)^+$. Calc. for $C_{32}H_{34}NO_{10}$, 592.2183.

Hydrolysis of brasiliquinone A 1

Brasiliquinone A **1** was treated with 0.5 M HCl–MeOH (1 ml) in a shielded tube at 70 °C for 7 h. After evaporation of the solvent, the residue was partitioned between $CHCl_3$ (0.5 ml × 3) and H_2O (1 ml). The $CHCl_3$ layer was evaporated under reduced pressure to yield brasiliquinone B **2**. The H_2O-soluble materials were treated with Ac_2O (0.2 μl) and pyridine (0.2 μl). After evaporation of the solvent, the residue was purified on a silica gel column (EtOAc) to afford methyl *N*,*O*-diacetylristosaminide **5** as a colourless amorphous solid; $[\alpha]_D^{26}$ −136 (*c* 0.02, $CHCl_3$); δ_H 1.19 (3 H, d, *J* 6.0, H-6), 1.88 (1 H, m, H-2a), 1.99 (3 H, s, 3-NAc), 2.01 (3 H, s, 4-OAc), 2.04 (1 H, m, H-2b), 3.41 (3 H, s, 1-OMe), 3.92 (1 H, m, H-5), 4.54 (1 H, dd, *J* 3.2 and 10.1, H-4), 4.06 (1 H, m, H-3), 4.74 (1 H, d, *J* 3.0, H-1) and 6.83 (1 H, m, 3-NH); EIMS *m/z* 214 $(M - OCH_3)^+$, 185, 153 and 143; HREIMS *m/z* 214.1085 $(M - OCH_3)^+$. Calc. for $C_{10}H_{16}NO_4$, 214.1079.

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