

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

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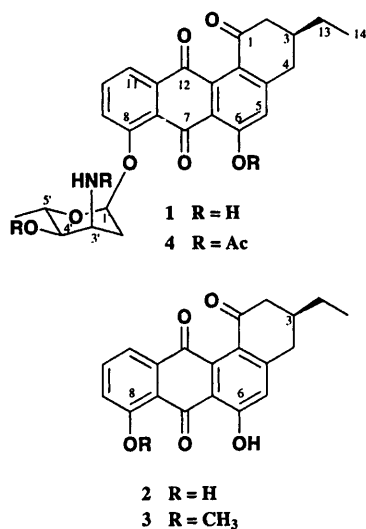
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Three new cytotoxic benz[*a*]anthraquinones with an ethyl group at C-3, brasiliquinones A–C 1–3, have been isolated from the actinomycete *Nocardia brasiliensis* IFM 0089, and their structures elucidated on the basis of spectroscopic data and by chemical 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.



Results and discussion

The brasiliquinones were isolated by HPLC separation of the mycelium extracts. Brasiliquinone A 1 revealed the pseudo-molecular 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, C₂₆H₂₇NO₇, 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 ¹³C NMR data of 1 indicated that the molecule possessed a ketone, two quinone carbonyls, six olefins, five methines, four

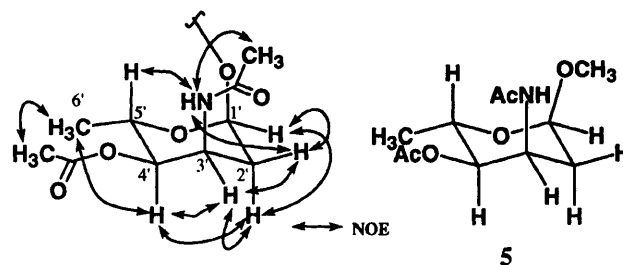


Fig. 1 Relative stereochemistry of the sugar moiety of triacetate 4 of brasiliquinone A 1. J (H, H) in Hz: 1', 2' α >0.1; 1', 2' β 2.0; 2' α , 2' β 12.4; 2' α , 3' 4.3; 2' β , 3' >0.1; 3', 4' 3.8; 4', 5' 10.3; 5', 6' 6.2.

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 Ac₂O 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 ¹³C NMR data of 1 with those of atramycin A⁴ 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*-diacetylristosaminide⁵ 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 B₂⁶ ($[\alpha]_D^{23}$ +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 2 established the molecular formula as C₂₁H₁₈O₅, 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,
2 α	2.63 (dd, 11.5, 15.0)	45.7	4, 13
2 β	2.23 (m)		
3	3.01 (br dd, 4.5, 17.2)	37.5	2, 4 β , 13, 14
4 α	2.70 (br dd, 10.8, 17.2)	36.0	2, 13
4 β			
4a	7.14 (s)	150.6	4, 5
5		127.7	4 β
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	10 α
12		184.3	11
12a		137.7	
12b		133.9	4
13	1.55 (m) ^b	28.9	2 β , 4 β , 14
14	1.01 (t, 7.4) ^f	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 ($[\alpha]_{\text{D}}^{23} + 69$) 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 ristosamine⁸ as an *O*-glycoside moiety.† Most of the angucycline-type antibiotics have a C₁ 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 C₂ 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* (IC₅₀ 0.55 and 0.73 $\mu\text{g ml}^{-1}$, respectively), while brasiliquinones B **2** and C **3** showed cytotoxicity against L1210 cells (IC₅₀ 3.4 $\mu\text{g ml}^{-1}$ each). Compounds **1**, **2** and **3** exhibited antibacterial activity against *Mycobacterium smegmatis* ATCC607 (MIC 12.5, 0.78 and 0.39 $\mu\text{g ml}^{-1}$, respectively), *Micrococcus luteus* IFM2066 (MIC 3.2, 3.2 and 6.3 $\mu\text{g ml}^{-1}$, respectively), *Staphylococcus aureus*

209P (MIC 25 $\mu\text{g ml}^{-1}$, each) and *Staphylococcus aureus* MRSA IFM62971 (MIC 12.5, 25 and 50 $\mu\text{g ml}^{-1}$, respectively), whereas **1**, **2** and **3** showed no antifungal activity against *Aspergillus niger* (MIC > 100 $\mu\text{g ml}^{-1}$ each). Brasiliquinone C **3** showed inhibitory activity against epidermal growth factor (EGF) receptor kinase (IC₅₀ 0.74 $\mu\text{g ml}^{-1}$) and *c-erbB-2* kinase (IC₅₀ 7.3 $\mu\text{g ml}^{-1}$).

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 cm² 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 H₂O 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 C₁₈ HPLC (Capcell Pak C₁₈ SG120, Shiseido, 50 × 250 mm; eluent, CH₃CN–H₂O–CF₃CO₂H, 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; $[\alpha]_{\text{D}}^{25} + 130$ (c 0.30, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 3350, 3150, 1670 and 1630; $\lambda_{\text{max}}(\text{MeOH})/\text{nm}$ 412 (ϵ 7000), 266 (20 000) and 228 (17 000); δ_{H} 1.00 (3 H, t, J 7.4, H₃-14), 1.13 (3 H, d, J 4.0, H₃-6'), 1.50 (2 H, m, H₂-13), 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 C₂₆H₃₀NO₇, 468.2022.

Brasiliquinone B 2. A yellow powder; mp 187–190 °C; $[\alpha]_{\text{D}}^{23} + 51$ (c 0.1, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 3440, 1690, 1675 and 1640; $\lambda_{\text{max}}(\text{MeOH})/\text{nm}$ 428 (ϵ 5000), 267 (22 000) and 228 (23 000); δ_{H} 1.00 (3 H, t, J 7.4, H₃-14), 1.53 (2 H, m, H₂-13), 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

† A few benz[*a*]anthraquinone antibiotics possessing an *O*-glycoside moiety such as atramycins⁴ and landmycins⁹ have been reported.

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 $\text{C}_{20}\text{H}_{16}\text{O}_5$, 336.0997.

Brasiliquinone C 3. A yellow powder; mp 215–217 °C; $[\alpha]_{\text{D}}^{23} + 69$ (c 0.14, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 3440, 1690, 1675 and 1640; $\lambda_{\text{max}}(\text{MeOH})/\text{nm}$ 406 (ϵ 7000), 266 (14 000) and 227 (28 000); δ_{H} 1.00 (3 H, t, J 7.4, H₃-14), 1.51 (2 H, m, H₂-13), 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 $\text{C}_{21}\text{H}_{18}\text{O}_5$, 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 \times 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; $[\alpha]_{\text{D}}^{+90}$ (c 0.22, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 3560, 3350, 1760, 1690, 1660 and 1220; $\lambda_{\text{max}}(\text{MeOH})/\text{nm}$ 356 (ϵ 7000), 266 (20 000) and 228 (17 000); ^1H and ^{13}C NMR (see Table 1); FABMS m/z 594 ($\text{M} + 2 + \text{H}^+$), 592 ($\text{M} + \text{H}^+$), 553 and 369; HRFABMS m/z 592.2153 ($\text{M} + \text{H}^+$). Calc. for $\text{C}_{32}\text{H}_{34}\text{NO}_{10}$, 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 \times 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; $[\alpha]_{\text{D}}^{26} - 136$ (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 ($\text{M} - \text{OCH}_3$)⁺, 185, 153 and 143; HREIMS m/z 214.1085 ($\text{M} - \text{OCH}_3$)⁺. Calc. for $\text{C}_{10}\text{H}_{16}\text{NO}_4$, 214.1079.

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