# Brasilidine A, a New Cytotoxic Isonitrile-Containing Indole Alkaloid from the Actinomycete *Nocardia brasiliensis*

Jun'ichi Kobayashi,*,† Masashi Tsuda,† Akira Nemoto,‡ Yasushi Tanaka,‡ Katsukiyo Yazawa,‡ and Yuzuru Mikami‡

*Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 280, Japan*

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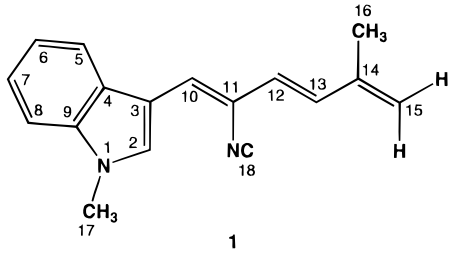
A new cytotoxic indole alkaloid containing an isonitrile group, brasilidine A (**1**), has been isolated from the actinomycete *Nocardia brasiliensis* IFM 0089 and the structure elucidated on the basis of its spectroscopic data.

During our search for bioactive substances from actinomycetes of the genus *Nocardia*, we previously isolated a new 32-membered macrolide possessing immunosuppressive and antifungal activity[1] and three new biogenetically unique benz[*a*]anthraquinones with cytotoxic and antibacterial activity.[2] Recently, a further investigation of extracts of *Nocardia brasiliensis* IFM 0089 has resulted in the isolation of brasilidine A (**1**), a new cytotoxic isonitrile-containing indole alkaloid. Here we describe the isolation and structure elucidation of **1**.




**1**

The mycelium of *N. brasiliensis* IFM 0089 was extracted with MeOH, and the hexane-soluble parts of the EtOAc-soluble materials of the extract were subjected to a silica gel column (hexane/EtOAc) followed by silica gel HPLC (hexane/EtOAc, 30:1) to yield brasilidine A (**1**, 27.3 mg) as a yellowish amorphous solid.

The molecular formula of brasilidine A (**1**) was established to be $C_{17}H_{16}N_2$ by HREIMS, and an IR band at 2105 $cm^{-1}$ indicated the presence of an isonitrile group.[3] UV absorptions [$\lambda_{max}$ ($CH_3CN$) 343 ($\epsilon$ 33 000), 270 (33 000), and 226 nm (78 000)] were characteristic of a 3-alkylindole chromophore.[4] The $^1H$ and $^{13}C$ NMR spectra of **1** showed the presence of signals attributable to an *N*-methyl group, an allylic methyl group, 14 $sp^2$ carbons, of which one was a methylene, eight were methines, and five were quaternary carbons, and a low-field signal [$\delta$ 169.7 (s)], which was assigned to a conjugated isonitrile carbon.[3] Nine out of 11 elements of unsaturation implied by the molecular formula were accounted for. **1** was thus inferred to possess two rings. Connections from C-5 to C-8 and from C-12 to C-13 were revealed by analysis of the $^1H-^1H$ COSY spectrum (Figure 1). The 3-substituted indole ring (N-1−C-9) and

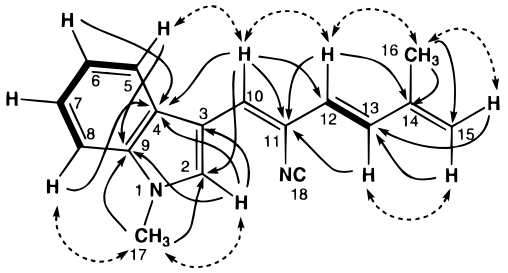



**Figure 1.** 2D NMR data of brasilidine A (**1**) (bold lines, solid arrows, and dotted arrows show cross-peaks observed in $^1H-^1H$ COSY, HMBC, and NOESY spectra, respectively).

a triene unit (C-10−C-16) were assigned by the following HMBC correlations: H-2/C-3, H-2/C-4, H-2/C-9, H-5/C-9, H-6/C-4, H-8/C-4, H-10/C-12, H-12/C-11, H-12/C-14, H-13/C-11, $H_2$-15/C-13, $H_3$-16/C-14, and $H_3$-16/C-15. The methyl group ($\delta_H$ 3.88; $\delta_C$ 33.4) at N-1 was verified by NOE's for H-2/$H_3$-17 and H-8/$H_3$-17 and HMBC correlations for $H_3$-17/C-2 and $H_3$-17/C-9. Connectivity between C-3 and C-10 was deduced from three-bond HMBC correlations for H-10/C-2 and H-10/C-4 and the NOESY cross-peak for H-5/H-10. The $^{13}C-^{14}N$ couplings were observed for C-11 (10.5 Hz) and C-18 (5.4 Hz) in the $^{13}C$ NMR spectrum, indicating that the isonitrile group was attached at C-11. Geometries of the two double bonds at C-10 and C-12 were revealed to be *Z* and *E*, respectively, from the $^1H$ coupling constant ($J_{12,13}$ = 15.3 Hz) as well as the following NOESY cross-peaks; H-5/H-10, H10/H-12, H-12/$H_3$-16, H-13/H-15a, and H-15b/$H_3$-16. Thus, the structure of brasilidine A was assigned to be **1**.

Brasilidine A (**1**) is a new indole alkaloid possessing a unique triene unit conjugated with an isonitrile group from the actinomycete of *N. brasiliensis* IFM 0089, although a few indole alkaloids with an isonitrile group have been reported from cyanophytes[5−7] and bacteria.[8] Brasilidine A (**1**) exhibited cytotoxicity against several tumor cell lines *in vitro* (Table 1). Interestingly, compound **1** was effective against the multidrug-resistant cell lines, P388/ADM ($IC_{50}$, 0.56 $\mu$g/mL) and CHO/MDR ($IC_{50}$, 3.43 $\mu$g/mL), as well as the parent cells. On the other hand, brasilidine A (**1**) showed inhibitory activity against some Gram-positive bacteria and fungi (Table 2), especially against *Aspergillus niger* (MIC, 0.39 $\mu$g/mL) and *Mycobacterium smegmatis* (MIC, 0.78 $\mu$g/mL).

* To whom correspondence should be addressed. Tel: (011)-706-3239. Fax: (011)-706-4985. E-mail: jkobay@pharm.hokudai.ac.jp.
† Hokkaido University.
‡ Chiba University.
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**Table 1.** Cytotoxicity of Brasilidine A (**1**)

| cell line | $IC_{50}$ ($\mu$g/mL) | |
|---|---|---|
| | **1** | adriamycin |
| L1210[a] | 0.25 | 0.09 |
| P388[a] | 0.44 | 0.09 |
| P388/ADR[b] | 0.56 | 0.55 |
| KB[c] | 0.75 | 0.97 |
| CHO[d] | 3.26 | 0.01 |
| CHO/MDR[e] | 3.43 | 0.11 |

[a] Murine leukemia. [b] Adriamycin-resistant P388. [c] Human epidermoid carcinoma. [d] Chinese hamster ovarian. [e] Multidrug-resistant gene 1 (MDR1) transfected CHO.

**Table 2.** Antimicrobial Activity of Brasilidine A (**1**)

| test organisms | MIC ($\mu$g/mL) |
|---|---|
| *Micrococcus luteus* IFM2066 | 3.13 |
| *Staphylococcus aureus* 209P | 100 |
| *Escherichia coli* NIH JC2 | 100 |
| *Bacillus subtilis* PCI189 | 3.13 |
| *Nocardia transvalensis* IFM0333 | 3.13 |
| *N. pseudobrasiliensis* IFM0624 | 3.13 |
| *N. brasiliensis* IFM0236 | 25 |
| *N. otitidiscaviarum* IFM0239 | 6.25 |
| *N. nova* IFM0290 | 1.56 |
| *N. asteroides* IFM0319 | 12.5 |
| *N. farcinica* IFM0284 | 12.5 |
| *Mycobacterium smegmatis* ATCC607 | 0.78 |
| *M. phlei* ATCC11758 | 1.56 |
| *M. flavescens* ATCC14474 | 3.13 |
| *Aspergillus niger* ATCC40606 | 0.39 |
| *Candida albicans* ATCC90028 | 25 |

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

## Experimental Section

**General Experimental Procedures.** These are reported elsewhere.[1]

**Extraction and Isolation.** *N. brasiliensis* strain IFM 0089 was cultivated as previously reported;[9] strain IFM 0089 refers to the strain number at the Research Center for Pathogenic Fungi and Mycotoxicoses, Chiba University. The cultured broth (150 L) was filtered, and the mycelial cake was extracted with MeOH (2 L). The EtOAc-soluble part of this extract was dissolved in hexane (2 L) and filtered to remove hexane-insoluble materials. After evaporation of the solvent, the residue (4.87 g) was subjected to a silica gel column (hexane/EtOAc, 20:1−10:1) to give an antibacterial fraction against *Micrococcus luteus*, which was dissolved in hexane/ether (20:1), and the precipitate was purified by silica gel HPLC (YMC Pack SIL-06, YMC, 20 × 250 mm; eluent, hexane/EtOAc, 30:1; flow rate, 10 mL/min; UV detection, 365 nm) to afford brasilidine A (**1**, 27.3 mg, $t_R$ 18.5 min).

**Brasilidine A (1):** yellowish amorphous solid; IR (film) $\nu_{max}$ 2925, 2955, 2105, 1595, 1520, 1475, 1380, 1245, 1120, 1065, 740 $cm^{-1}$; UV (EtOH) $\lambda_{max}$ 332 ($\epsilon$ 20 000), 275 (35 000), 268 (40 000), 220 (sh), 204 nm (18 000); $^1H$ NMR ($CDCl_3$, 600 MHz) $\delta$ 1.96 (3H, brs, $H_3$-16), 3.88 (3H, s, $H_3$-17), 5.11 (1H, s, H-15b), 5.19 (1H, s, H-15a), 6.25 (1H, d, $J$ = 15.3 Hz, H-12), 6.65 (1H, d, $J$ = 15.3 Hz, H-13), 6.82 (1H, brs, H-10), 7.23 (1H, ddd, $J$ = 1.0, 7.0, 8.0 Hz, H-6), 7.31 (1H, ddd, $J$ = 1.0, 7.1, 8.0 Hz, H-7), 7.37 (1H, brd, $J$ = 8.1 Hz, H-8), 7.69 (1H, brd, $J$ = 8.1 Hz, H-5), 8.06 (1H, s, H-2); $^{13}C$ NMR ($CDCl_3$, 150 MHz) $\delta$ 18.8 (q, C-16), 33.4 (q, C-17), 109.7 (s, C-3), 109.8 (d, C-8), 118.1 (d, C-5), 118.1 (s, C-11), 118.5 (t, C-15), 120.7 (d, C-6), 121.3 (d, C-10), 123.0 (d, C-7), 123.4 (d, C-12), 127.8 (s, C-4), 130.6 (d, C-2), 131.7 (d, C-13), 136.3 (s, C-9), 141.0 (s, C-14), 169.7 (s, C-18); EIMS $m/z$ 248 ($M^+$), 233 $(M - CH_3)^+$; HREIMS $m/z$ 248.1316 ($M^+$), calcd for $C_{17}H_{16}N_2$ 248.1316.

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