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NOTE

Two new sarpagine-type indole alkaloids and antimalarial activity of 16-demethoxycarbonylvoacamine from *Tabernaemontana macrocarpa* Jack

Puteri Amelia[1,2] · Alfarius Eko Nugroho[1] · Yusuke Hirasawa[1] · Toshio Kaneda[1] · Takahiro Tougan[3] · Toshihiro Horii[3] · Hiroshi Morita[1]

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Abstract
Two new sarpagine-type indole alkaloids (**1** and **2**), together with five known alkaloids; 12-methoxy-4-methylvoachalotine (**3**), 16-demethoxycarbonylvoacamine (**4**), isositsirikine (**5**), affinisine (**6**), affinine (**7**), were isolated from the bark of *Tabernaemontana macrocarpa* Jack. The structures of these alkaloids were determined based on spectroscopic data, chemical correlation, and comparison with the literature. 16-Demethoxycarbonylvoacamine (**4**) showed antiplasmodial activities against *Plasmodium falciparum* 3D7 and cytotoxic activities against human cell line, HepG2 cells.

Keywords Antimalarial activity · Indole alkaloids · *Tabernaemontana macrocarpa* Jack

Introduction

The family Apocynaceae have been known to produce various type of alkaloids with medicinal properties [1–3]. In the course of our continuing search for bioactive natural products from tropical plants, we have reported alkaloids from the Apocynaceae family and their bioactivity, including antimalarial activity [4–16]. *Tabernaemontana* is a large genus which belongs to the Apocynaceae family and is well known for its alkaloid content. This genus, comprising approximately 100 species, is distributed in the tropical and some subtropical regions [17–19]. In Borneo, Indonesia, the exudate from the bark of *Tabernaemontana macrocarpa* Jack has been used traditionally to cure dental disorders, herpes, and eczema. Phytochemical analysis of the stem of this plant revealed the presence of alkaloids, flavonoids, terpenoids, and tannins [20]. In this paper, we reported the isolation and structure elucidation of two new monoterpene indole alkaloids (**1** and **2**) from the bark of *T. macrocarpa* Jack, together with five known alkaloid compounds and their antimalarial activities.

Results and discussions

Structure elucidation of *1* and *2*

Compound **1** was obtained as an optically active brownish amorphous solid, $[\alpha]_D^{25}$ −45 (*c* 1.0, MeOH). The IR spectrum showed two important absorptions at 3382 and 1678 cm^{-1} for hydroxyl and carbonyl groups, respectively, while the UV absorption bands at λ_{max} 225 and 271 nm indicated an indole chromophore. The electrospray ionisation mass spectrometry (ESIMS, pos.) of **1** showed a molecular ion peak at *m/z* 397, and the molecular formula of **1** was established as $C_{23}H_{29}N_2O_4$ from high-resolution ESIMS (HRESIMS). Analysis of the ^1H NMR data (Table 1) suggested the presence of one substituted indole moiety from three aromatic resonances at δ_H 6.77 (1H, d, $J = 8.0$ Hz), 7.02 (1H, t,

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✉ Hiroshi Morita
moritah@hoshi.ac.jp

[1] Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan

[2] Department of Pharmacy, Faculty of Medical Sciences, Universitas Islam Negeri (UIN) Syarif Hidayatullah, Jakarta 15412, Indonesia

[3] Department of Molecular Protozoology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Table 1 ^{1}H and ^{13}C NMR data of **1** and **2** in CD_3OD

Position	**1**[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
2		133.3		133.9[c]
3	5.04 (1H, br d, 10.4)	59.4	4.99 (1H, br d, 10.4)	60.6[c]
5	5.01 (1H, br d, 6.1)	65.8	4.39 (1H, br d, 4.8)	66.0
6a	3.79 (1H, br d, 18.0)	20.1	2.93 (1H, dd, 17.6, 4.8)	25.2
6b	3.25 (1H, dd, 18.0, 6.1)		3.27 (1H, br d, 17.6)	
7		103.4		102.2
8		129.0		129.1
9	7.11 (1H, d, 8.0)	112.6	7.09 (1H, d, 8.0)	112.3
10	7.02 (1H, t, 8.0)	121.8	7.02 (1H, t, 8.0)	121.8
11	6.77 (1H, d 8.0)	105.2	6.75 (1H, d, 8.0)	105.0
12		149.3		149.3[c]
13		128.3		129.0
14a	2.06 (1H, br d, 13.2)	29.4	2.17 (1H, br d, 13.7)	32.5
14b	2.49 (1H, dd, 13.2, 10.4)		2.51 (1H, dd, 13.7, 10.4)	
15	3.35 (1H, br s)	31.1	3.52 (1H, br d, 6.0)	29.5
16		49.9	2.48 (1H, d, 6.0)	50.8[c]
17a	3.58 (1H, d, 10.4)	63.8		
17b	3.72 (1H, d, 10.4)			
18	1.72 (3H, d, 6.0)	12.9	1.71 (3H, d, 6.0)	13.1
19	5.50 (1H, q, 6.0)	120.9	5.52 (1H, q, 6.0)	121.4
20		128.7		129.1
21a	4.29 (1H, d, 16.0)	65.9	4.24 (1H, d, 15.6)	66.2
21b	4.39 (1H, d, 16.0)		4.32 (1H, d, 15.6)	
22		175.1		176.4[c]
N1-Me	3.97 (3H, s)	33.1	3.97 (3H, s)	33.3
N4-Me	3.19 (3H, s)	50.1	3.10 (3H, s)	49.6
OMe-12	3.94 (3H, s)	56.0	3.94 (3H, s)	56.0

[a] ^{1}H NMR spectra were measured on a 400-MHz spectrometer, while ^{13}C NMR spectra were measured on a 100-MHz spectrometer

[b] ^{1}H NMR spectra were measured on a 600-MHz spectrometer, while ^{13}C NMR spectra were measured on a 150-MHz spectrometer

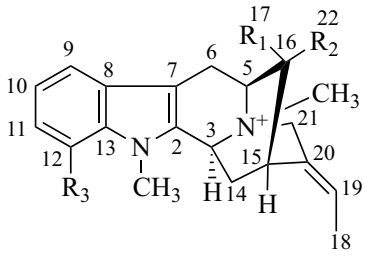
[c] The chemical shift was deduced from two-dimensional NMR

$J = 8.0$ Hz), and 7.11 (1H, d, $J = 8.0$ Hz), three *N*-methyl or methoxy (δ_H 3.97, 3.19 and, 3.94), and one olefinic proton (δ_H 5.50 1H, q, $J = 6.0$ Hz). The ^{13}C NMR data revealed 23 resonances, comprising 7 sp^2 quarternary carbons, 4 sp^2 methines, 4 methyls, 4 sp^3 methylenes, 3 sp^3 methines, and 1 sp^3 quarternary carbon (Fig. 1).

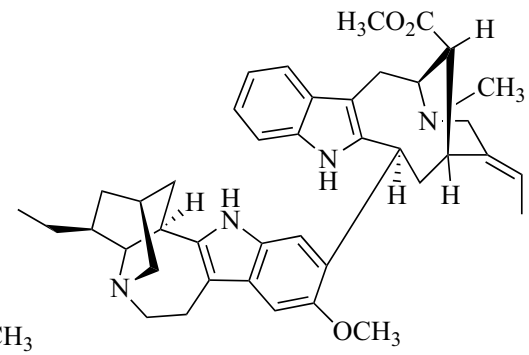
The structure of **1** was deduced from extensive analysis of the two-dimensional (2D) NMR data, including the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single–quantum correlation (HSQC), and heteronuclear multiple–bond correlation (HMBC). In particular, the HMBC cross-peaks of H_2-17 (δ_H 3.72 and δ_H 3.58) to C-22 (Fig. 2) provides additional support for the presence of a carboxyl moiety at C-22. Furthermore, the HMBC correlations of methyl signals at δ_H 3.94 to C-12, δ_H 3.97 to C-2 and C-13, and δ_H 3.19 to C-3, C-5, and C-21 revealed the presence of a methoxy group at C-12 and two *N*-methyl groups. Moreover, an ethylidene side-chain at C-20 was confirmed by an HMBC cross-peak from H_3-18 (δ_H 1.72) to C-20, and H-19 (δ_H 5.50) to C-15 and C-21 (Fig. 2).

The relative configuration of **1** was elucidated by nuclear Overhauser effect spectroscopy (NOESY) correlation to be similar to that of 12-methoxy-4-methylvoachalotine (MMV) (**3**) [21]. First, H-3 and H-5/*N*4-CH_3 were assigned to be α-axially oriented from the NOESY correlations of H-3 and H-5/*N*4-CH_3 while CH_2-17 were deduced to possess β-orientation from the NOESY correlations of H-17a/H-14a. Furthermore, the α configuration of H-15 and the *E* configuration of the ethylidene group were confirmed by the NOE correlation of H-15 to H_3-18 and H-14b (Fig. 3).

Fig. 1 Structures of **1**–**7**



	R_1	R_2	R_3
1	CH_2OH	COOH	OCH_3
2	H	COOH	OCH_3
3	CH_2OH	CO_2CH_3	OCH_3
6	H	CH_2OH	H



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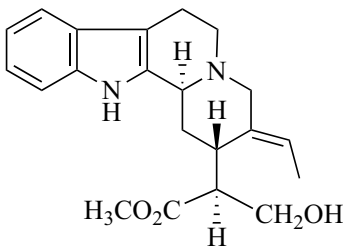

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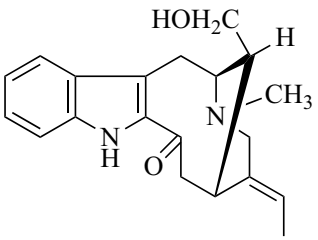


7

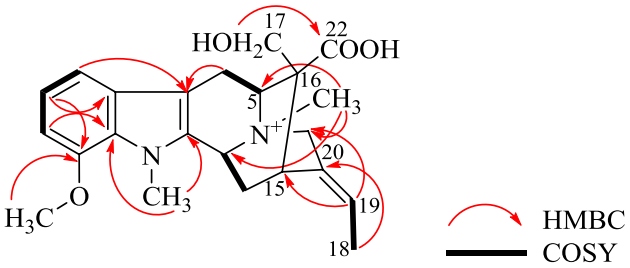
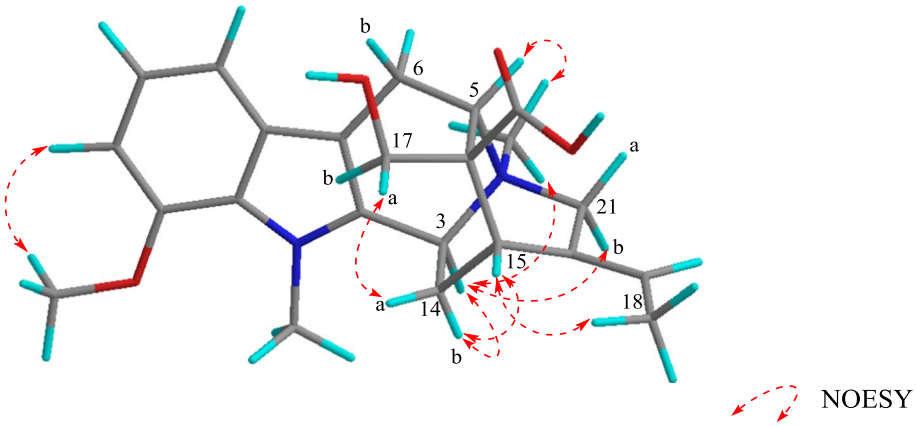


Fig. 2 Selected 2D NMR correlations for **1**

Further analysis of ^{1}H and ^{13}C NMR showed that **1** is very similar to MMV, except for the signal of the methyl ester at C-22 in MMV which was not observed in **1**. Thus, **1** was assumed to be a 22-demethyl derivative of MMV. Finally, stereochemistry of **1** was confirmed by a methyl esterification reaction of **1** using trimethylsilyldiazomethane ($TMSCHN_2$) to form a product with identical spectroscopic data to that of MMV.

Compound **2** was isolated as an optically active brownish amorphous solid, $[\alpha]_D^{25}$ −5 (*c* 1.0, MeOH). The UV spectrum showed two absorption bands at λ_{max} 225 and

Fig. 3 Selected NOESY correlations for **1**



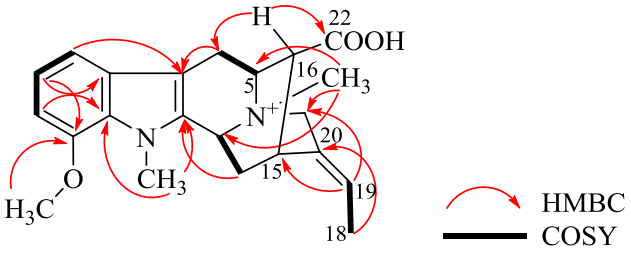


Fig. 4 Selected 2D NMR correlations for **2**

280 nm, characteristic of an indole chromophore. The IR absorptions bands at 3383 and 1685 cm^{-1} resulted from the hydroxyl and carbonyl groups, respectively. The ESIMS spectrum displayed a molecular ion peak at *m/z* 367, and the molecular formula of **2** was established as $C_{22}H_{27}N_2O_3$ from HRESIMS.

The NMR spectra of **2** and **1** exhibited similar resonances, differing by the presence of a doublet signal of methine (δ_H 2.48) in **2** instead of a methyl alcohol signal at δ_H 3.58 and δ_H 3.72 in **1** linkage at C-22, indicating the structure of **2** as a 22-deethyl fuchsiaefoline [22] (Fig. 4). The relative configuration of **2** was assigned by analyses of the ^1H–^1H coupling constant data and the NOESY correlations similar to **1**. In particular, the observed H-16/H-6b NOE indicated that the configuration of C-16 is *R**.

Antimalarial activity

The two new compounds **1** and **2**, together with five known compounds; 12-methoxy-4-methylvoachalotine (**3**), 16-demethoxycarbonylvoacamine (**4**) [23], isositsirikine (**5**) [24], affinisine (**6**), and affinine (**7**) [25], were tested for their antimalarial activity against *Plasmodium falciparum* 3D7 strain. The results showed only the dimeric alkaloid, 16-demethoxycarbonylvoacamine (**4**), possessed moderate in vitro antimalarial activity [the half-maximal (50%) inhibitory concentration (IC_{50}) = 28.8 µM], while the others did not show activity even at 50 µM. Furthermore, the cytotoxic activity was evaluated using a human cell line, HepG2 cells. The cytotoxic activity of **4** was low [the half-maximal (50%) cytotoxic concentration (CC_{50}) = > 50 µM for HepG2 cells].

Experimental section

General experimental procedures Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer, and IR spectra on a JASCO FT/IR-4100 spectrophotometer. HRESIMS was conducted on a LTQ Orbitrap XL (Thermo Scientific). ^1H and 2D NMR spectra were measured on 600-MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150-MHz spectrometer. The residual solvent peaks were used as internal standards (δ_H 3.31 and δ_C 49.0 for CD_3OD). Standard pulse sequences were used for the 2D NMR experiments. Merck silica gel 60 (40–63 µm), amino silica, and HP-20 were used for the column chromatography, and the separations were monitored by Merck silica gel 60 F254, or Merck amino silica gel 60 F254 thin-layer chromatography (TLC) plates.

Plant materials The barks of *T. macrocarpa* Jack were collected in August 2017 from the Centre for Plant Conservation Botanic Gardens, Bogor, Indonesia. Authentication and identification of plant was carried out at the Centre for Plant Conservation Botanic Gardens, Bogor, Indonesia.

Extraction and isolation The barks of *T. macrocarpa* Jack (550 g) were extracted with MeOH, and part of the extract (8 g) was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted at pH 9 with saturated Na_2CO_3 and extracted with $CHCl_3$ to give $CHCl_3$ fraction (1.5 g), followed by partition with butanol to give butanol fraction (3.7 g). The $CHCl_3$ fraction was subjected to CC over silica gel and eluted with hexane/ethyl acetate (49:1 to 1:1, v/v), followed by $CHCl_3$/methanol (49:1 to 100%) to give 16 fractions. Fraction 9 (149 mg) was further separated using Sephadex LH-20 with $CHCl_3$/MeOH (1:1, v/v) to afford 16-demethoxycarbonylvoacamine (**4**, 3.4 mg, 0.0425%), isositsirikine (**5**, 0.6 mg, 0.0075%), affinisine (**6**, 3.1 mg, 0.0388%), and affinine (**7**, 13.0 mg, 0.1625%). Fraction 11 (160 mg) was purified by amino silica CC and eluted with $CHCl_3$/MeOH (1:1, v/v), which yielded 12-methoxy-4-methylvoachalotine (**3**, 151.0 mg, 1.8875%). The butanol fraction was subjected to Diaion HP-20 CC with $CHCl_3$/MeOH (20-100%, v/v) to afford 10 fractions. Fraction 6 was further purified by silica gel CC with $CHCl_3$/MeOH (49:1 to 1:1), affording 20 fractions. Fraction 15 was then successively purified using Sephadex LH-20 with $CHCl_3$/MeOH (1:1, v/v) and high-performance liquid chromatography (HPLC; Cholester 10 × 250 mm, 50% MeOH at 2.4 mL/min, UV detection at 254 nm) to yield **1** (6.3 mg, 0.0788%, t_R 11.3 min) and **2** (3.1 mg, 0.0388%, t_R 23.2 min).

Methyl esterification reaction The preparation of methyl esters from carboxylic acids can be achieved with $TMSCHN_2$ in methanolic benzene. Compound **1** (0.1 mg) was dissolved in MeOH (100 µl), and $TMSCHN_2$ (50 µl) was added. The reaction was easily monitored by the disappearance of the yellow color of $TMSCHN_2$ to form a product with identical spectroscopic data to that of MMV.

Parasite strain and culture P. falciparum laboratory strain 3D7 was obtained from Prof. Masatsugu Kimura (Osaka City University, Osaka, Japan). For the assessment of antimalarial activity of the compounds in vitro, the parasites were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 0.5 g/L L-glutamine, 5.96 g/L HEPES, 2 g/L sodium bicarbonate ($NaHCO_3$), 50 mg/L hypoxanthine, 10 mg/L gentamicin,

10% heat-inactivated human serum, and red blood cells (RBCs) at a 3% hematocrit in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2 at 37 °C as previously described [26]. Ring-form parasites were collected using the sorbitol synchronization technique [27]. Briefly, the cultured parasites were collected by centrifugation at 840 *g* for 5 min at room temperature, suspended in a fivefold volume of 5% D-sorbitol (Nacalai Tesque, Kyoto, Japan) for 10 min at room temperature, and then they were washed twice with RPMI 1640 medium to remove the D-sorbitol. The utilization of blood samples of healthy Japanese volunteers for the parasite culture was approved by the Institutional Review Committee of the Research Institute for Microbial Diseases (RIMD), Osaka University (approval number: 22-3).

Antimalarial activity Ring-form-synchronized parasites were cultured with compounds **1**–**7** at sequentially decreasing concentrations (50, 15, 5, 1.5, 0.5, 0.15, 0.05, and 0.015 μM) for 48 h for the flow cytometric analysis using an automated hematology analyzer, XN-30. The XN-30 analyzer was equipped with a prototype algorithm for cultured falciparum parasites [prototype; software version: 01-03, (build 16)] and used specific reagents (CELLPACK DCL, SULFOLYSER, Lysercell M, and Fluorocell M; Sysmex, Kobe, Japan) [28]. Approximately 100 μL of the culture suspension diluted with 100 μL phosphate-buffered saline was added to a BD Microtainer MAP Microtube for Automated Process K_2 EDTA 1.0-mg tube (Becton–Dickinson and Co., Franklin Lakes, NJ, USA) and loaded onto the XN-30 analyzer with an auto-sampler as described in the instrument manual (Sysmex). The parasitemia (MI-RBC %) was automatically reported [28]. Then 0.5% DMSO alone or containing 5 μM artemisinin was used as the negative and positive controls, respectively. The growth inhibition (GI) rate was calculated from the MI-RBC % according to the following equation:

$$\mathrm{GI}(\%) = 100 - (\text{test sample} - \text{positive control})/(\text{negative control} - \text{positive control}) \times 100.$$

The IC_{50} was calculated from GI (%) using GraphPad Prism version 5.0 (GraphPad Prism Software, San Diego, CA, USA) [29].

Compound **1** (22-demethyl MMV): brownish amorphous solid. $[\alpha]_D^{25}$: −45 (*c* 1.00, MeOH). IR V_{max} (KBr): 3382 and 1678 cm^{-1}. UV/Vis λ_{max} (MeOH) (log ε) 225 (4.30), 271 (3.34) nm. CD (MeOH) λ_{max} ($\Delta\varepsilon$) 228 (−10.4) and 293 (−1.02) nm. ^1H and ^{13}C NMR (CD_3OD): Table 1. MS (ESI): *m/z*: 397 $[M]^+$. HRESIMS *m/z*: 397.2126 $[M]^+$ (calcd. for $C_{23}H_{29}N_2O_4$, 397.2122).

Compound **2** (22-deethyl fuchsiaefoline): brownish amorphous solid. $[\alpha]_D^{25}$: −5 (*c* 1.00, MeOH). IR V_{max} (KBr): 3383 and 1685 cm^{-1}. UV/Vis λ_{max} (MeOH) (log ε) 225 (3.91), 280 (2.91) nm. CD (MeOH) λ_{max} ($\Delta\varepsilon$) 227 (−3.78) and 291 (−0.81) nm. ^1H and ^{13}C NMR (CD_3OD): Table 1. MS (ESI): *m/z*: 367 $[M]^+$. HRESIMS *m/z*: 367.2143 $[M]^+$ (calcd. for $C_{22}H_{27}N_2O_3$, 367.2152).

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