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by Idha Kusumawati

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Voacalgines A–E, new indole alkaloids from *Voacanga grandifolia*

Yusuke Hirasawa [a], Hiroko Arai [a], Abdul Rahman [b], Idha Kusumawati [b], Noor Cholies Zaini [b], Osamu Shirota [c], Hiroshi Morita [a,*]

[a] Faculty of Pharmaceutical Sciences, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan
[b] Faculty of Pharmacy, Airlangga University, Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia
[c] Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, 1314-1 Shido, Sanuki City, Kagawa 769-2193, Japan

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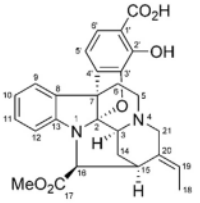
ABSTRACT

Five new indole alkaloids, voacalgines A–E (**1**–**5**) consisting of a *C*-mavacurine type of skeleton with 2,3-dihydroxybenzoate moiety, a macroline-type of skeleton, or a macroline-type of skeleton with C_6 unit, were isolated from the bark of *Voacanga grandifolia*. Their relative structures were determined by means of NMR data. Voacalgine A showed moderate cell growth inhibitory activities against HL-60 and HCT116 cells.

1. Introduction

Voacanga grandifolia (Miq.) Rolfe is a member of the Apocynaceae family distributed in Indonesia and India, and is found mostly in Java.[1] The bark and leaves have been known to produce various skeletal alkaloids such as voacinol,[2] vobtusine,[3] vobtusinelactone,[4] and rhazine.[5] In our search for bioactive alkaloids from tropical plants,[6–14] voacalgines A–E (**1**–**5**), five new indole alkaloids consisting of a *C*-mavacurine[15] type of skeleton with fused benzoic acid moiety, a macroline[16] type of skeleton, or a macroline-type of skeleton with another C_6 unit, have been isolated from the bark of *V. grandifolia*. In this paper, we describe the isolation and structure elucidation of **1**–**5** as well as their in vitro cell growth inhibitory activities against three human cell lines.



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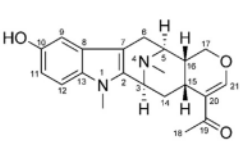

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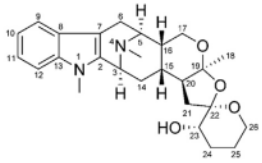

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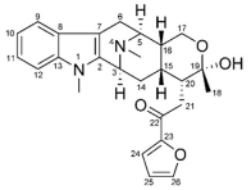

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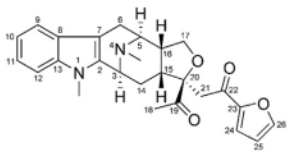

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* Corresponding author. E-mail address: moritah@hoshi.ac.jp (H. Morita).

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2. Results and discussion

2.1. Voacalgine A (1)

Voacalgine A (**1**) showed a molecular formula, $C_{27}H_{26}N_2O_6$, which was determined by HRESITOFMS [*m*/*z* 475.1842 $(M+H)^+$, Δ −2.7 mmu]. IR absorption band was characteristic of carbonyl (1750 and 1670 cm^{-1}) and hydroxyl (3023 cm^{-1}) groups. ^1H and ^{13}C NMR data (Table 1) suggested the presence of two sp^3 quaternary carbons, four sp^3 methylenes, three sp^3 methines, two methyls, seven sp^2 methines, and nine sp^2 quaternary carbons. Among them, two sp^3 methylenes (δ_C 48.8; δ_H 2.83 and 2.83, and δ_C 53.7; δ_H 3.11 and 4.34) and two sp^3 methines (δ_C 52.8; δ_H 3.38, and δ_C 58.7; δ_H 4.67) were attached to the nitrogen atom, and one sp^3 quaternary carbon (δ_C 104.7) was ascribed to that bearing both an oxygen and a nitrogen atoms.

Table 1
^1H (*J*, Hz) and ^{13}C NMR data of voacalgine A (**1**) in CD_3OD at 300 K

Position	δ_H	δ_C
2		104.7
3	3.38 (1H, dd, 2.9, 2.9)	52.8
5	2.83 (2H, m)	48.8
6a	2.32 (1H, ddd, 15.2, 10.6, 6.7)	28.8
6b	2.55 (1H, br d, 15.2)	
7		49.8
8		136.4
9	6.79 (1H, dd, 7.8, 1.8)	123.6
10	6.81 (1H, ddd, 7.8, 7.8, 0.8)	122.0
11	7.10 (1H, ddd, 7.9, 7.8, 1.8)	128.8
12	6.39 (1H, d, 7.9)	111.5
13		146.6
14a	1.82 (1H, ddd, 13.6, 3.4, 3.4)	27.5
14b	2.77 (1H, ddd, 13.6, 3.3, 3.3)	
15	3.55 (1H, m)	32.6
16	4.67 (1H, d, 4.2)	58.7
17		171.3
18	1.65 (3H, dd, 6.8, 2.3)	12.6
19	5.50 (1H, dq, 1.7, 6.8)	122.0
20		135.3
21a	3.11 (1H, d, 12.8)	53.7
21b	4.34 (1H, br d, 12.8)	
1′		108.0
2′		152.9
3′		147.1
4′		128.5
5′	7.30 (1H, d, 8.3)	118.6
6′	7.26 (1H, d, 8.3)	123.1
1′-COOH		170.0
OMe	3.78 (3H, s)	52.9

The gross structure of **1** was deduced from extensive analyses of the two-dimensional NMR data, including the ^1H–^1H COSY, HMQC, and HMBC spectra in CD_3OD (Fig. 1). The ^1H–^1H COSY and HMQC spectra revealed connectivity of five partial structures **a** (C-5–C-6), **b** (C-9–C-12), **c** (C-3, C-14–C-16), **d** (C-18–C-19), and **e** (C-5′–C-6′) as shown in Fig. 1.

HMBC cross-peaks of H_2-5 to C-3 and C-21, and H-3 to C-21 established the connections among C-3, C-5, and C-21 through N-4. The connectivity of partial structures **a**, **c**, and indoline ring (C-2, C-7–C-13 and *N*-1) was revealed by the HMBC correlations of H-9 and H_2-5 to C-7 (δ_C 49.8), and H-6b and H-14a to C-2 (δ_C 104.7). HMBC correlations from H_3-18 to C-20 and H-19 to C-15 and C-21 established the presence of piperidine ring (C-3, C-14–C-15, C-20–C-21, and *N*-4) with ethylidene side chain at C-20. HMBC correlations from H-16 to C-13, and H-16 and methoxy protons to C-17 indicated voacalgine A possessed *C*-mavacurine type skeleton. On the other hand, the presence of 2,3-dihydroxybenzoate including partial structure **e** was presumed from the HMBC correlations from H-5′ to C-1′ and C-3′, and H-6′ to C-2′, C-4′, and carboxyl carbon. In addition, the connectivity between this moiety and indole alkaloid moiety at C-4′ and C-7 was assigned by the HMBC correlations from H-6a to C-4′ and H-5′ to C-7. Moreover, the connectivity between C-3′ (δ_C 147.1) and C-2 through an oxygen atom was elucidated by comparison of chemical shifts with bipleiophylline[17] (δ_C 146.9 and δ_C 103.2, respectively). Thus, the gross structure of voacalgine A (**1**) was assigned to be a new indole alkaloid consisting of a *C*-mavacurine type of skeleton and 2,3-dihydroxybenzoic acid.

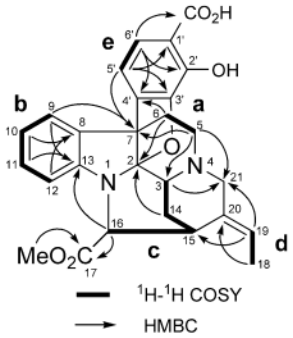

Fig. 1. Selected 2D NMR correlations for voacalgine A (**1**).

The relative stereochemistry of **1** was elucidated by the NOESY correlations. A 3,8-diazatricyclo[6.2.2.$0^{4,9}$]dodecane ring (C-2–C-7, C-14–C-16, C-20, C-21, *N*-1, and *N*-4) strongly required that both H-3 and H-15 were α-orientation, and α-orientation of benzoic acid moiety was supported by the NOESY correlations of H-6b/H-5′ and H-6a/H-21b. An α-configuration of H-16 and *E*-configuration of double bond (C-19–C-20) were elucidated by the correlations of H-14b/H-16 and H-15/H_3-18, respectively (Fig. 2).

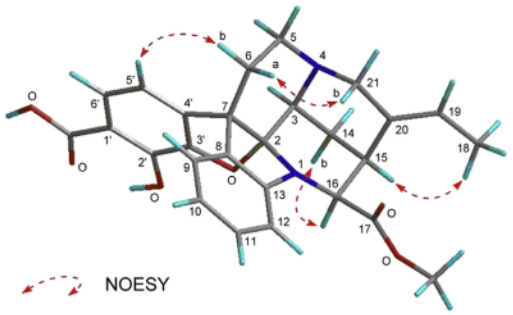

Fig. 2. Selected NOESY correlations for voacalgine A (**1**).

2.2. Voacalgine B (2)

Voacalgine B (**2**) showed a molecular formula, $C_{21}H_{24}N_2O_3$, which was determined by HRESITOFMS [*m*/*z* 353.1861 $(M+H)^+$, Δ +0.1 mmu]. IR absorption band was characteristic of α,β-unsaturated ketone (1650 and 1620 cm^{-1}) and hydroxyl (3300 cm^{-1}) groups. ^1H and ^{13}C NMR data (Table 2) suggested the presence of three sp^3 methylenes, four sp^3 methines, three methyls, four sp^2 methines, and seven sp^2 quaternary carbons. Among them, two sp^3 methines (δ_C 57.5; δ_H 4.97, and δ_C 57.7; δ_H 3.98) and two methyls (δ_C 29.6; δ_H 3.68, and δ_C 40.6; δ_H 2.92) were attached to the nitrogen atom, and one sp^3 methylene (δ_C 65.4; δ_H 4.27 and 4.35), one sp^2 methine (δ_C 160.5; δ_H 7.83), and one sp^2 quaternary carbon (δ_C 152.5) were ascribed to that bearing an oxygen atom.

Table 2
^{1}H (*J*, Hz) and ^{13}C NMR data of voacalgine B (**2**) in CD_3OD at 300 K[a]

Position	δ_H	δ_C
2		128.9
3	4.97 (1H, br s)	57.5
5	3.98 (1H, d, 7.4)	57.7
6a	3.10 (1H, d, 18.0)	24.1
6b	3.49 (1H, dd, 18.0, 7.4)	
7		105.5
8		127.2
9	6.90 (1H, d, 2.3)	103.7
10		152.5
11	6.83 (1H, dd, 8.8, 2.3)	113.7
12	7.29 (1H, d, 8.8)	111.2
13		134.5
14a	1.99 (1H, dd, 11.8, 11.8)	31.4
14b	2.42 (1H, m)	
15	2.68 (1H, dt, 11.8, 6.0)	25.0
16	2.45 (1H, m)	39.0
17a	4.27 (1H, dd, 10.7, 10.7)	65.4
17b	4.35 (1H, dd, 10.7, 2.6)	
18	2.13 (3H, s)	25.0
19		198.3
20		119.5
21	7.83 (1H, s)	160.5
N(1)-Me	3.68 (3H, s)	29.6
N(4)-Me	2.92 (3H, s)	40.6

[a] TFA salt.

The gross structure of **2** was deduced from extensive analyses of the two-dimensional NMR data, including the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra in CD_3OD (Fig. 3). The ^{1}H–^{1}H COSY and HMQC spectra revealed connectivity of three partial structures **a** (C-3, C-14–C-17), **b** (C-5–C-6), and **c** (C-11–C-12) as shown in Fig. 3.

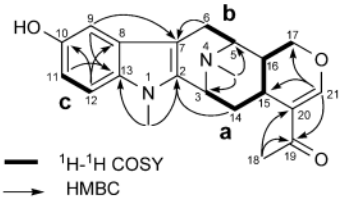

Fig. 3. Selected 2D NMR correlations for voacalgine B (**2**).

The presence of 5-hydroxy-*N*-methylindole ring was deduced by HMBC correlations of *N*(1)-Me to C-2 and C-13, H-9 to C-7 and C-13, H-12 to C-8 and oxygenated C-10, and H-11 to C-13. HMBC cross-peaks of *N*(4)-Me to C-3 and C-5 established the connection between C-3 and C-5 through *N*-4. The connection among indole ring and partial structures **a** and **b** was indicated by HMBC correlations of H-6 to C-7 and H-14 to C-2. HMBC correlations from H_3-18 to C-19 and C-20, and H-21 to C-15, C-17, and C-19 established the presence of 3,4-dihydro-2*H*-pyran ring (C-15–C-17, C-20–C-21 and *O*) with an acetyl group at C-20. Thus, the gross structure of voacalgine B (**2**) was assigned to be a new indole alkaloid with a hydroxyl group at C-10 of alstonerine.[18]

The relative stereochemistry of **2** was elucidated by the NOESY correlations. The correlations of H-14a/H-17a, H-16/H-15 and H-6a indicated the α-orientation of an *N*-methyl group at *N*-4 and the β-configuration of H-15 and H-16. Thus, the relative stereochemistry of **2** was assigned as shown in Fig. 4.

The CD spectrum of **2** showed a similar pattern to that of alstonerine.[8] Thus, the absolute configurations of **2** were elucidated to be 3*S*, 5*S*, 15*R*, and 16*R*.

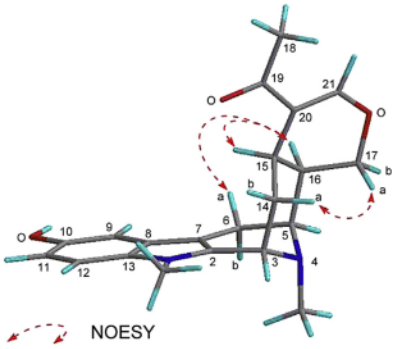

Fig. 4. Selected NOESY correlations for voacalgine B (**2**).

2.3. Voacalgine C (3)

Voacalgine C (**3**) showed a molecular formula, $C_{26}H_{34}N_2O_4$, which was determined by HRESITOFMS [*m*/*z* 439.2618 $(M+H)^+$, Δ +2.1 mmu]. IR absorption band was characteristic of hydroxyl (3400 cm^{-1}) group. ^{1}H and ^{13}C NMR data (Table 3) suggested the presence of seven sp^3 methylenes, six sp^3 methines, two sp^3 quaternary carbons, three methyls, four sp^2 methines, and four sp^2 quaternary carbons. Among them, two sp^3 methines (δ_C 55.5; δ_H 4.44, and δ_C 58.0; δ_H 3.35) and two methyls (δ_C 29.3; δ_H 3.68, and δ_C 41.4; δ_H 2.59) were attached to the nitrogen atom, and an sp^3 methine (δ_C 70.5; δ_H 3.50), two sp^3 methylenes (δ_C 64.0; δ_H 3.82 and 4.00, and δ_C 62.7; δ_H 3.48 and 3.83), and two sp^3 quaternary carbon (δ_C 107.2 and 107.3) were ascribed to that bearing an oxygen atom.

Table 3
^{1}H (*J*, Hz) and ^{13}C NMR data of voacalgine C (**3**) in CD_3OD at 300 K[a]

Position	δ_H	δ_C
2		131.0
3	4.44 (1H, br s)	55.5
5	3.35 (1H, m)	58.0
6a	2.70 (1H, br d, 16.1)	23.8
6b	3.34 (1H, m)	
7		106.9
8		127.2
9	7.46 (1H, d, 7.6)	119.0
10	7.06 (1H, dd, 7.6, 7.4)	120.3
11	7.17 (1H, dd, 7.4, 7.9)	122.7
12	7.36 (1H, d, 7.9)	110.1
13		139.0
14a	1.78 (1H, m)	32.3
14b	2.48 (1H, ddd, 14.7, 14.7, 3.5)	
15	1.78 (1H, m)	27.4
16	2.23 (1H, ddd, 10.0, 5.1, 5.1)	37.7
17a	3.82 (1H, m)	64.0
17b	4.00 (1H, dd, 11.9, 10.0)	
18	1.58 (3H, s)	26.0
19		107.3
20	2.07 (1H, dd, 11.8, 7.6)	44.2
21a	1.78 (1H, m)	38.8
21b	2.02 (1H, dd, 12.3, 12.3)	
22		107.2
23	3.50 (1H, br t, 3.0)	70.5
24a	1.62 (1H, m)	28.2
24b	1.97 (1H, dddd, 12.8, 12.6, 3.0, 3.0)	
25a	1.29 (1H, m)	20.9
25b	1.87 (1H, ddddd, 12.6, 12.6, 12.4, 3.4, 3.4)	
26a	3.48 (1H, m)	62.7
26b	3.83 (1H, m)	
N(1)-Me	3.68 (3H, s)	29.3
N(4)-Me	2.59 (3H, s)	41.4

[a] formic acid salt.

The gross structure of **3** was deduced from extensive analyses of the two-dimensional NMR data, including the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra in CD_3OD (Fig. 5). The ^{1}H–^{1}H COSY and HMQC spectra revealed connectivity of four partial structures **a** (C-3, C-14–C-17, and C-20–C-21), **b** (C-5–C-6), **c** (C-9–C-12), and **d** (C-23–C-26) as shown in Fig. 5.

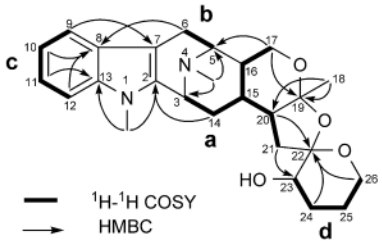

Fig. 5. Selected 2D NMR correlations for voacalgine C (**3**).

By analysis of HMBC spectrum as shown in Fig. 5, three partial structures **a**–**c** composed of a macroline-type skeleton at C-2–C-17. The HMBC correlations for H_3-18 to C-19 (δ_C 107.3) and C-20 (δ_C 44.2), and H_2-17 to C-19 indicated the presence of 2-methyltetrahydropyran ring (C-15–C-20 and *O*). Furthermore, correlations of H-20, H-24a, and H-26a to C-22 (δ_C 107.2) and H-21b to C-23 (δ_C 70.5) suggested the presence of tri-cyclic polyether structure on C-15–C-26. Thus, the gross structure of voacalgine C (**3**) was elucidated to be possessing a macroline-type skeleton with tetrahydropyran-2-spiro-2′-tetrahydrofuran ring as 25-deoxy form of macrodasine E.[19]

The relative stereochemistry of **3** was mainly elucidated by the NOESY correlations. In the 2-methyltetrahydropyran ring (C-15–C-20), the NOESY correlations of H_3-18/H-14b, H-17b, and H-20, and a large ^{3}J coupling constant (10.0 Hz) between H-16 and H-17b suggested that CH_3-18 and H-20 were α-oriented and H-15 and H-16 were β-oriented. The correlations of H-5/H-17a and H-6a/H-16 indicated the α-orientation of an *N*-methyl group at N-4 (Fig. 6).

On the other hand, the NOESY correlations of H-24b/H-26b and large ^{3}J coupling constants between H-24b/H-25b (12.4 Hz) and H-25b/H-26 (12.4 Hz) indicated that the tetrahydropyran ring (C-22–C-26) took chair conformation. And an α-oriented hydroxy group at C-23 was deduced from a small ^{3}J coupling constant (3.0 Hz) between H-23/H-24b. Finally, the relative configuration of a spiro carbon at C-22 was elucidated by the NOESY correlation of H_3-18/H-26b.

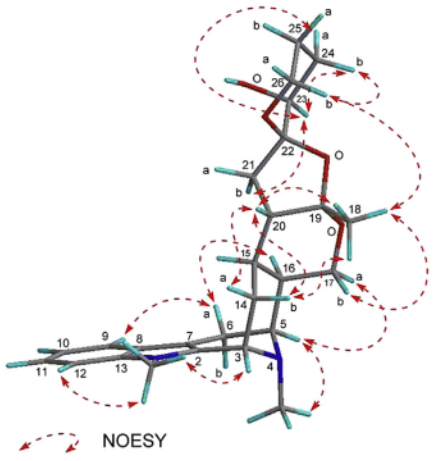

Fig. 6. Selected NOESY correlations for voacalgine C (**3**).

The stable conformer corresponding to the axial orientation of OH-23 was generated after conformational searching by computer modeling (MMFF force field energy minimization) and the result was consistent with the coupling constants of H-23 (br t, 3.0 Hz).

2.4. Voacalgine D (4)

Voacalgine D (**4**) showed a molecular formula, $C_{26}H_{30}N_2O_4$, which was determined by HRESITOFMS [*m*/*z* 435.2299 $(M+H)^+$, Δ +1.6 mmu]. IR absorption band was characteristic of α,β-unsaturated ketone (1670 cm^{-1}) and hydroxyl (3400 cm^{-1}) groups. By analysis of 1D and 2D NMR spectra (Table 4), voacalgine D possessed a macroline-type skeleton as well as voacalgine C.

Table 4
^{1}H (*J*, Hz) and ^{13}C NMR data of voacalgine D (**4**) in CD_3OD at 300 K[a]

Position	δ_H	δ_C
2		132.3
3	4.20 (1H, br s)	55.6
5	3.13 (1H, m)	57.0
6a	2.54 (1H, d, 16.6)	23.6
6b	3.24 (1H, m)	
7		107.1
8		127.3
9	7.38 (1H, d, 7.6)	118.8
10	7.00 (1H, dd, 7.6, 7.4)	119.9
11	7.12 (1H, dd, 7.4, 7.9)	122.1
12	7.24 (1H, d, 7.9)	109.8
13		138.7
14a	1.68 (1H, m)	27.2
14b	2.87 (1H, m)	
15	1.65 (1H, m)	28.5
16	1.97 (1H, ddd, 11.8, 3.4, 3.4)	44.8
17a	3.46 (1H, m)	61.0
17b	4.49 (1H, dd, 11.7, 11.7)	
18	1.32 (3H, s)	28.4
19		98.3
20	2.21 (1H, ddd, 10.6, 5.3, 5.3)	43.6
21a	2.82 (1H, m)	37.9
21b	2.90 (1H, m)	
22	7.09 (1H, br d, 3.6)	190.6
23	6.54 (1H, dd, 3.6, 1.7)	153.9
24	7.66 (1H, dd, 1.7, 0.5)	119.2
25	3.44 (3H, s)	113.5
26	2.42 (3H, s)	148.7
N(1)-Me		29.1
N(4)-Me		41.5

[a] Formic acid salt.

The HMBC correlations from H_3-18 to C-19 (δ_C 98.3) and C-20 (δ_C 43.6), and H-17a to C-15 (δ_C 28.5) and C-19, and H-21a to C-15 indicated the presence of 2-hydroxy-2-methyltetrahydropyran ring (C-15–C-20 and *O*). And, the correlations of H-26/C-23 and C-24, and H-24/C-23 and 26 suggested the presence of a 2-furyl group on C-23–C-26. Furthermore, the connectivity of C-21 and C-23 through C-22 ketone was deduced from the HMBC correlation of H-21a to C-22 and the NOESY correlation of H-24/H-21a. Thus, the gross structure of voacalgine D (**4**) was assigned to be a new indole alkaloid consisting of a macroline-type skeleton with a 2-furyloyl group at C-21 (Fig. 7).

The relative stereochemistry of **4** was elucidated by the NOESY correlations. The correlations of H-14b/H-17b, H-15/H-16, H-16/H-20, and H-6a/H-16 indicated the α-orientation of an *N*-methyl group at *N*-4 and the β-configuration of H-15, H-16, and H-20. The

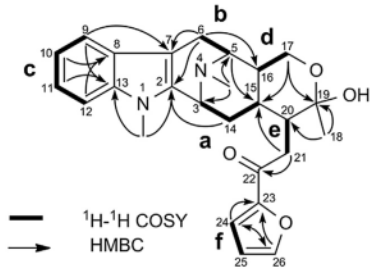

Fig. 7. Selected 2D NMR correlations for voacalgine D (**4**).

configuration of a methyl group at C-19 was assigned as equatorial by the NOESY correlation of H-20/H_3-18 and no correlation of H_3-18/H-17b (Fig. 8).

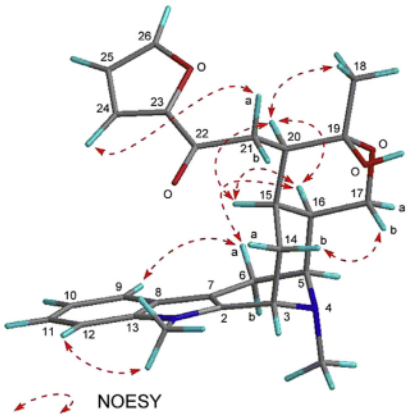

Fig. 8. Selected NOESY correlations for voacalgine D (**4**).

2.5. Voacalgine E (5)

Voacalgine E (**5**) showed molecular formula, $C_{26}H_{28}N_2O_4$, which was determined by HRESITOFMS [*m*/*z* 433.2144 $(M+H)^+$, Δ +1.7 mmu]. IR absorption band was characteristic of ketone (1710 cm^{-1}) and α,β-unsaturated ketone (1670 cm^{-1}) groups. By analysis of 1D and 2D NMR spectra (Table 5), voacalgine E possessed a macroline-type skeleton and a 2-furyloyl group as well as voacalgine D.

The gross structure of **5** was deduced from extensive analyses of the two-dimensional NMR data. The ^{1}H–^{1}H COSY and HMQC spectra revealed connectivity of four partial structures **a** (C-3, C-14–C-17), **b** (C-5–C-6), **c** (C-9–C-12), and **d** (C-24–C-26) as shown in Fig. 9. Since, the left-half of the structure of voacalgine E in Fig. 9 showed similar HMBC correlations as well as voacalgine D (**4**), **5** was presumed to have a macroline-type skeleton. The HMBC correlations of H_2-17 to C-20 (δ_C 92.9), H_3-18 to C-19 (δ_C 214.9) and C-20, and H-15 to C-19 revealed the presence of 2-acetyltetrahydrofuran ring (C-15–C-20). On the other hand, the presence of a 2-furyloyl group was elucidated by the HMBC correlations from H-25 and H-26 to C-23 (δ_C 153.6), and H-24 to C-22 ketone (δ_C 186.6). And the connection of this moiety and C-20 through C-21 methylene was deduced by the HMBC correlations of H_2-21 to C-19, C-20, and C-22. Thus, the gross structure of voacalgine E was assigned to be a new indole alkaloid consisting of a macroline-type skeleton that E-ring is transformed to five-membered ring with a 2-furyloyl group at C-21 and an acetyl group at C-20.

Table 5
^{1}H (*J*, Hz) and ^{13}C NMR data of voacalgine E (**5**) in CD_3OD at 300 K[a]

Position	δ_H	δ_C
2		126.8
3	5.09 (1H, br s)	56.0
5	4.01 (1H, br s)	55.6
6a	3.08 (1H, d, 17.6)	24.0
6b	3.42 (1H, dd, 17.6, 6.0)	
7		105.4
8		126.8
9	7.54 (1H, d, 7.7)	119.5
10	7.13 (1H, dd, 7.7, 7.6)	121.0
11	7.27 (1H, dd, 7.9, 7.6)	123.9
12	7.46 (1H, d, 7.9)	110.6
13		139.6
14a	2.28 (1H, ddd, 12.1, 11.8, 0.8)	27.6
14b	2.40 (1H, br d, 11.8)	
15	2.14 (1H, ddd, 12.1, 7.1, 5.9)	38.0
16	2.74 (1H, ddd, 8.2, 8.2, 7.1)	44.3
17a	4.23 (1H, dd, 9.9, 9.7)	69.4
17b	4.34 (1H, dd, 9.7, 9.4)	
18	2.24 (3H, s)	27.0
19		214.9
20		92.9
21a	3.36 (1H, m)	43.8
21b	3.49 (1H, m)	
22		186.6
23		153.6
24	7.36 (1H, br d, 3.4)	120.0
25	6.64 (1H, dd, 3.4, 1.2)	113.8
26	7.78 (1H, br s)	149.1
N(1)-Me	3.78 (3H, s)	29.7
N(4)-Me	2.94 (3H, s)	40.3

[a] Formic acid salt.

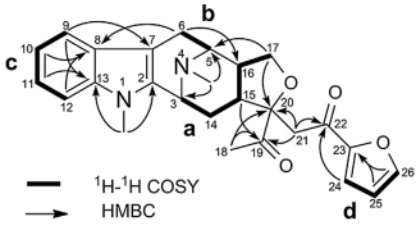

Fig. 9. Selected 2D NMR correlations for voacalgine E (**5**).

The relative stereochemistry of **5** was elucidated by the NOESY correlations. The correlations of H-15/H-16, H-16/H_3-18, and H-6a/H-16 indicated the α-orientation of an *N*-methyl group at *N*-4 and the β-configuration of H-15, H-16, and an acetyl group (Fig. 10).

3. Plausible biogenetic pathway

A plausible biogenetic pathway of voacalgines A, D, and E (**1**, **4**, and **5**) with rare skeletons was proposed as shown in Fig. 11. Voacalgine A (**1**) is the second example combined with *C*-mavacurine type of skeleton (**6**) and 2,3-dihydroxybenzoic acid (**7**). Voacalgines D (**4**) and E (**5**) might be derived from the ring-opened form of alstonerine (**8**)[20] through introduction of C_6 unit[21] to C-20 followed by cyclization.

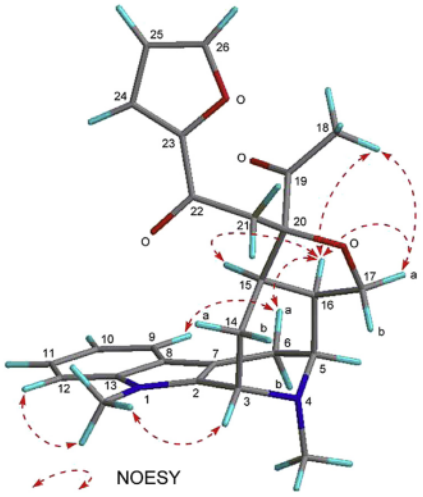

Fig. 10. Selected NOESY correlations for voacalgine E (**5**).

moderate cell growth inhibitory activities against HL-60 and HCT116 cells (IC_{50} for **1**: 12.1 μM for HL-60, and 45.7 μM for HCT116).

5. Experimental section

5.1. General experimental details

5.1.1. General methods. 1D and 2D NMR spectra were recorded on a Bruker AV700 spectrometer, and chemical shifts were referenced to the residual solvent peaks (δ_H 3.31 and δ_C 49.0 for methanol-d_4). Standard pulse sequences were employed for the 2D NMR experiments. $^1H-^1H$ COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1 K data points for each of 256 t_1 increments. NOESY spectra in the phase-sensitive mode were measured with a mixing time of 800 ms. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1 K data points were collected. For HMBC spectra with *Z*-axis PFG, a 50 ms delay time was used for long-range C–H coupling. Zero-filling to 1 K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

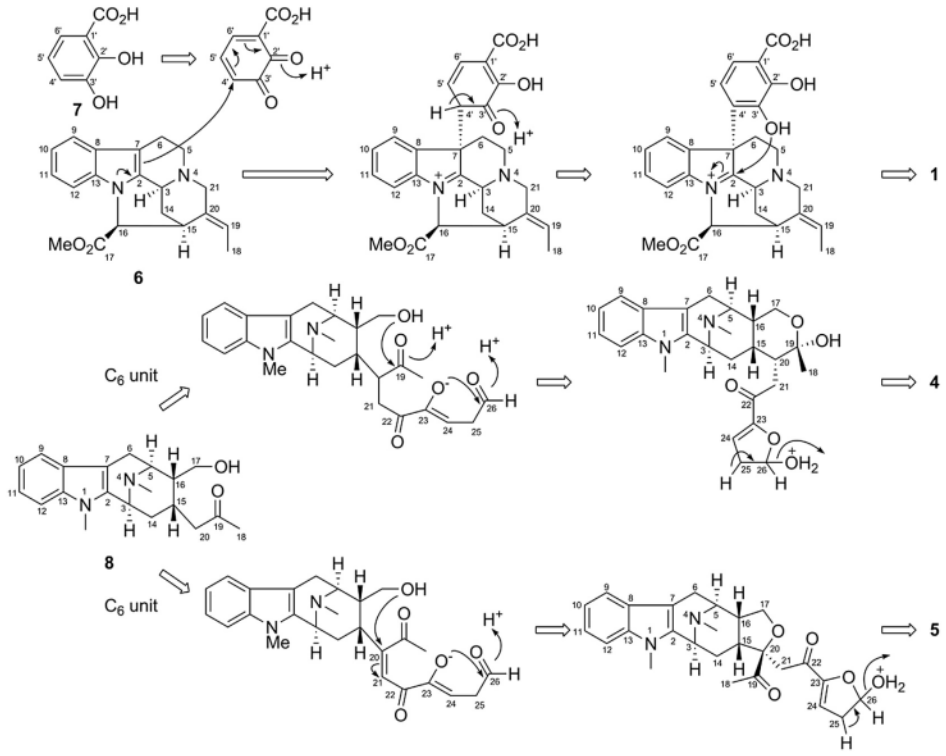

Fig. 11. Plausible biogenetic pathway of voacalgines A (**1**), D (**4**), and E (**5**).

4. Conclusion

In this work, five new indole alkaloids, voacalgines A–E (**1**–**5**) were isolated from the bark of *V. grandifolia*. The structures and stereochemistry of **1**–**5** were elucidated by 2D NMR analysis.

Voacalgines A–E (**1**–**5**) were tested for cytotoxic activity against HL-60, HCT116, and MCF7 cell line. Voacalgine A only showed

5.2. Material

The bark of *V. grandifolia* was collected at Purwodadi Botanical Garden, Indonesia in 2006. The botanical identification was made by Ms. Sri Wuryanti, Purwodadi Botanical Garden, Indonesia. A voucher specimen (no. AP070910) has been deposited in the herbarium at Purwodadi Botanical Garden, Pasuruan, Indonesia.

5.3. Extraction and isolation

The bark of *V. grandifolia* (444 g) was extracted with MeOH, the extract (32 g) was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 (aq) to pH 10 and extracted with $CHCl_3$ to give an alkaloidal fraction (4.3 g). The alkaloidal fraction was subjected to an amino SiO_2 column in hexane/EtOAc (1:0→0:1) and then $CHCl_3$/MeOH (1:0→0:1).

The $CHCl_3$/MeOH (50:1) eluted fraction was chromatographed over a SiO_2 column in $CHCl_3$/MeOH (1:0→0:1) and the $CHCl_3$/MeOH (50:1) eluted fraction was applied to an amino SiO_2 column in $CHCl_3$/MeOH (1:0→0:1) to afford voacalgine A (**1**, 4.0 mg, 0.0009%).

The $CHCl_3$/MeOH (1:0) eluted fraction of the first amino SiO_2 column was chromatographed over a SiO_2 column in $CHCl_3$/MeOH (1:0→0:1) and the $CHCl_3$/MeOH (50:1) eluted fraction was separated by an ODS HPLC (47% MeOH aq with 0.1% TFA, 2.0 mL/min, 254 nm) to afford voacalgine B (**2**, 3.7 mg, 0.0008%, t_R=15 min).

The hexane/EtOAc (2:1) eluted fraction of the first amino SiO_2 column was chromatographed over a SiO_2 column in $CHCl_3$/MeOH (1:0→0:1) and the $CHCl_3$/MeOH (80:1) eluted fraction was separated by an ODS HPLC (42% MeOH aq with 0.1% formic acid, 2.0 mL/min, 254 nm) to afford voacalgine E (**5**, 1.3 mg, 0.0003%, t_R=15 min), whereas the $CHCl_3$/MeOH (50:1) eluted fraction was chromatographed over an amino SiO_2 column in hexane/EtOAc (1:0→0:1) and the hexane/EtOAc (2:1) eluted fraction was separated by an ODS HPLC (40% MeOH aq with 0.1% formic acid, 2.0 mL/min, 254 nm) to afford voacalgine C (**3**, 0.9 mg, 0.0002%, t_R=20 min).

The $CHCl_3$/MeOH (100:1) eluted fraction of the first amino SiO_2 column was separated by an ODS HPLC (48% MeOH aq with 0.1% formic acid, 2.0 mL/min, 254 nm) to afford voacalgine D (**4**, 2.6 mg, 0.0006%, t_R=16 min).

5.4. Characterization of natural products

*5.4.1. Voacalgine A (**1**).* Brown amorphous solid; $[\alpha]_D^{26}$ +110 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3023, 1750, and 1670 cm^{-1}; UV (MeOH) λ_{max} 201 (ε 34,600), 229 (15,600), and 334 (3100) nm; ESIMS *m*/*z* 475 $(M+H)^+$; HRESITOFMS *m*/*z* 475.1842 [$(M+H)^+$, Δ −2.7 mmu, calcd for $C_{27}H_{27}N_2O_6$, 475.1869].

*5.4.2. Voacalgine B (**2**).* Brown amorphous solid; $[\alpha]_D^{27}$ −67 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3300, 1650, and 1620 cm^{-1}; UV (MeOH) λ_{max} 201 (ε 19,000) and 232 (19,900) nm; CD (MeOH) λ_{max} 301 ($\Delta\varepsilon$ −6.63), 259 (+7.68), 229 (−9.35), and 205 (+5.34); ESIMS *m*/*z* 353 $(M+H)^+$; HRESITOFMS *m*/*z* 353.1861 [$(M+H)^+$, Δ +0.1 mmu, calcd for $C_{21}H_{25}N_2O_3$, 353.1860].

*5.4.3. Voacalgine C (**3**).* Brown amorphous solid; $[\alpha]_D^{22}$ −22 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3400 cm^{-1}; UV (MeOH) λ_{max} 201 (ε 12,100), 229 (18,600), and 336 (3800) nm; ESIMS *m*/*z* 439 $(M+H)^+$; HRESITOFMS *m*/*z* 439.2618 [$(M+H)^+$, Δ +2.1 mmu, calcd for $C_{26}H_{35}N_2O_4$, 439.2597].

*5.4.4. Voacalgine D (**4**).* Brown amorphous solid; $[\alpha]_D^{24}$ −6 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3400, 1670, and 1630 cm^{-1}; UV (MeOH) λ_{max} 229 (ε 30,400) and 273 (14,500) nm; ESIMS *m*/*z* 435 $(M+H)^+$; HRESITOFMS *m*/*z* 435.2299 [$(M+H)^+$, Δ +1.6 mmu, calcd for $C_{26}H_{31}N_2O_4$, 435.2234].

*5.4.5. Voacalgine E (**5**).* Brown amorphous solid; $[\alpha]_D^{22}$ −14 (*c* 0.5, MeOH); IR (KBr) ν_{max} 1710 and 1670 cm^{-1}; UV (MeOH) λ_{max} 201 (ε 14,900), 228 (20,400), and 374 (10,300) nm; ESIMS *m*/*z* 433 $(M+H)^+$; HRESITOFMS *m*/*z* 433.2144 [$(M+H)^+$, Δ +1.7 mmu, calcd for $C_{26}H_{29}N_2O_4$, 433.2127].

5.5. Cytotoxicity

HL-60, human promyelocytic leukemia cells were maintained in RPMI-1640 medium; MCF7, human breast adenocarcinoma; and HCT116, human colorectal adenocarcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) medium. Both growth media were supplemented with 10% fetal calf serum and 1% penicillin–streptomycin. The cells (5×10^3 cells/well) were cultured in Nunc disposable 96-well plates containing 90 μL of growth medium per well and were incubated at 37 °C in a humidified incubator of 5% CO_2. Ten microliters of serially diluted samples (50 μM, 25 μM, 12.5 μM, and 6.25 μM) were added to the cultures at 24 h of incubation. After 48 h of incubation with the samples, 15 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) was added to each of the wells. The cultures were incubated for another 3 h before the cells supernatant are removed. After the removal of the cells supernatant, 50 μL of dimethyl sulfoxide (DMSO) was added to each well. The formed formazan crystal was dissolved by re-suspension by pipette. The optical density was measured using a microplate reader (Bio-Rad) at 550 nm with reference wavelength at 700 nm. In all experiments, three replicates were used. Cisplatin was used as positive control (IC_{50}: 0.87 μM for HL-60, 27.7 μM for MCF7, and 16.0 μM for HCT116).

Acknowledgements

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