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NEW INDOLE ALKALOIDS FROM *ALSTONIA MACROPHYLLA*

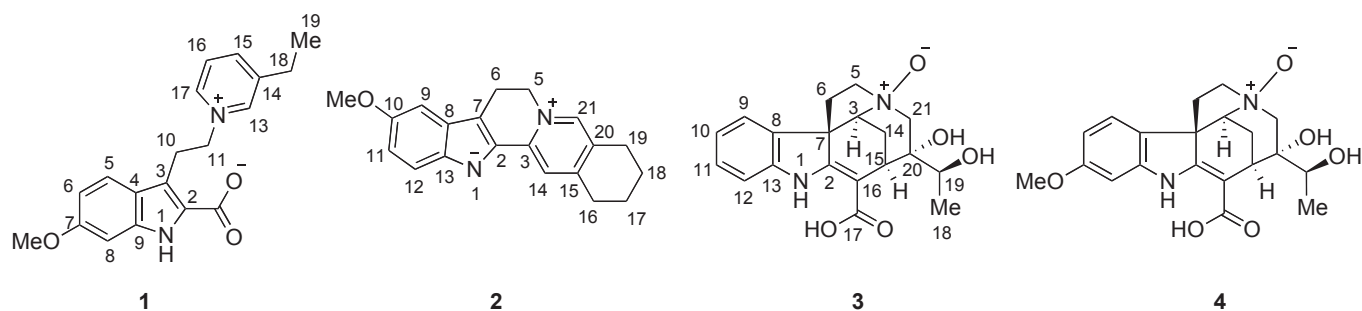
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Abstract – Alkaloidal investigations of *Alstonia macrophylla* led to the isolation of a new 3-alkylpyridinium-indole-2-carboxylate scaffold alkaloid, *N*(1)-demethyl-7-methoxykirydinium A (**1**) and a yohimbane-type alkaloid, 10-methoxydihydrosempervirine (**2**), and two strychnane-type alkaloids, 17-carboxylcompactivervine *N*-oxide (**3**) and 17-carboxylalstovine *N*-oxide (**4**). Their structures were elucidated by NMR spectral analysis using 2D techniques and CD spectra.

Plants of the genus *Alstonia* (Apocynaceae) are widely distributed in tropical regions of Africa and Asia. Several species of *Alstonia* used in traditional medicine throughout Southeast Asia for the treatment of malaria and other ailments including tumours.¹ In the course of our investigation of bioactive indole alkaloids from tropical plants belonging to Apocynaceae, we have reported four new picaline and ajmaline-type indole alkaloids, alstiphyllanines A-D, which showed antiplasmodial activity against *Plasmodium falciparum* and vasorelaxant activity,² and alstiphyllanines E-H, which have biological activities such as vasorelaxant activity and inhibiting sodium glucose cotransporter,³ from *A. macrophylla* collected in Indonesia. In this paper we would like to report the isolation and structure elucidation of new alkaloids **1-4** from *A. macrophylla* collected in Indonesia.

*Dedicated to Professor Ei-ichi Negishi, Purdue University, on the occasion of his 77th birthday.



RESULTS AND DISCUSSION

N(1)-Demethyl-7-methoxyikirydinium A (**1**) showed the pseudomolecular ion peak at m/z 325 ($M+H$)⁺ in ESIMS, and the molecular formula, C₁₉H₂₀N₂O₃, was established by HRESIMS [m/z 325.15862, ($M+H$)⁺ Δ +3.40 mDa]. IR spectrum showed absorption bands (3435 and 1675 cm⁻¹) characteristic of carboxylic acid. The ¹H NMR data (Table 1) showed the presence of a 3-ethylpyridinium, a 1,2,4-trisubstituted benzene, and a 1,2-disubstituted ethane. Diagnostic 2D NMR correlations (Figure 1) indicated an assembly of these structure fragments. The position of a methoxy group was confirmed by an HMBC correlation of *O*-Me to C-7 (δ_C 160.2). The molecular formula of **1** was larger than that of ikirydinium A isolated from *Hunteria umbellata*⁴ by an oxygen atom. Compared with ¹H NMR data of ikirydinium A, the presence of a 3-alkylpyridinium-indole-2-carboxylate backbone without an *N*-methyl group was suggested for **1**.

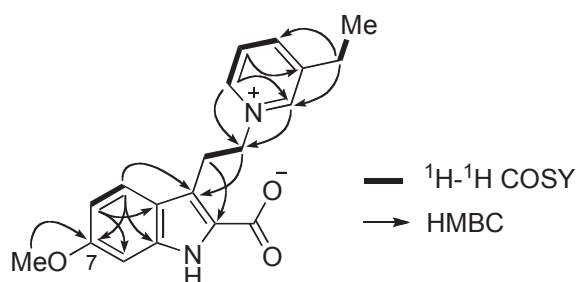


Figure 1. Selected 2D NMR correlations for *N*(1)-demethyl-7-methoxyikirydinium A (**1**)

10-Methoxydihydrosempervirine (**2**) showed the pseudomolecular ion peak at m/z 305 ($M+H$)⁺ in ESIMS, and the molecular formula, C₂₀H₂₀N₂O, was established by HRESIMS [m/z 305.16528, ($M+H$)⁺ Δ +0.44 mDa]. The UV spectrum of **2** was similar to that of sempervirine.⁵ The ¹H NMR spectrum that showed signals corresponding to five aromatic and twelve aliphatic protons were very similar to those of sempervirine that possessed two singlet aromatic protons except for a methoxy group. HMBC cross

peaks indicated the presence of a methoxy group attached to C-10 (δ_C 156.5). The ^1H - ^1H COSY correlations between H₂-5 (δ_H 4.79) to H₂-6 (δ_H 3.37) and H₂-16 (δ_H 3.06) to H₂-19 (δ_H 2.91) indicated the presence of the two sp^3 methylene sequences and HMBC correlations between H₂-5 to C-3 (δ_C 141.7) and C-21 (δ_C 145.1), and H₂-6 to C-2 (δ_C 126.3) and C-8 (δ_C 126.7) suggested **2** was a dihydrosempervirine derivative as shown in Figure 2.

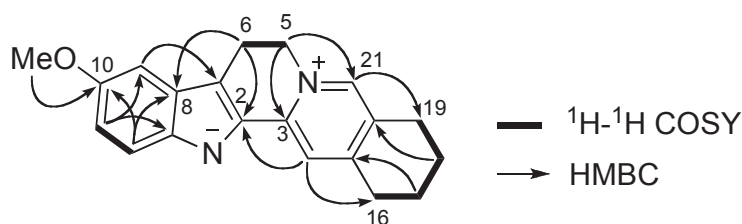


Figure 2. Selected 2D NMR correlations for 10-methoxydihydrosempervirine (**2**)

ESIMS spectra of 17-carboxylcompactivervine *N*-oxide (**3**) and 17-carboxylalstovine *N*-oxide (**4**) showed the pseudomolecular ion peak at m/z 359 ($\text{M}+\text{H}$)⁺ and 389 ($\text{M}+\text{H}$)⁺, respectively, and the molecular formulae were established to be C₁₉H₂₂N₂O₅ and C₂₀H₂₄N₂O₆ by HRESIMS [**3**: m/z 359.16299, ($\text{M}+\text{H}$)⁺ Δ +2.29 mDa; **4**: m/z 389.17386, ($\text{M}+\text{H}$)⁺ Δ +2.60 mDa], respectively. IR spectra of **3** and **4** showed a typical absorption bands (**3**: 3329 and 1614 cm⁻¹; **4**: 3514 and 1612 cm⁻¹) for carboxylic acid. Based on the ^1H and ^{13}C NMR spectra (Tables 1 and 2), signal patterns of **3** and **4** were very similar to those of compactinervine (**5**)⁶ and alstovine (**6**)⁷ except for methyl ester group, respectively, which indicated that **3** and **4** contained a strychnane skeleton. The structures of **3** and **4** were finally established by 2D NMR correlations and, **3** and **4** were indicated the calboxylic acid derivatives of the known alkaloids, **5** and **6**. Low field chemical shifts at C-3, C-5, and C-21 around *N*-4 atom [C-3 (δ_C 62.7 \rightarrow δ_C 75.8), C-5 (δ_C 53.5 \rightarrow δ_C 68.3), and C-21 (δ_C 53.1 \rightarrow δ_C 65.4)] on comparison with those in **5** and [C-3 (δ_C 60.5 \rightarrow δ_C 76.1), C-5 (δ_C 51.5 \rightarrow δ_C 68.8), and C-21 (δ_C 53.8 \rightarrow δ_C 65.4)] on comparison with those in **6**, suggested that **3** and **4** were *N*-oxide form at *N*-4.

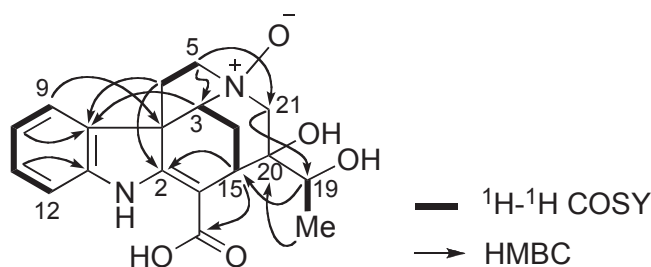


Figure 3. Selected 2D NMR correlations for 17-carboxylcompactivervine *N*-oxide (**3**)

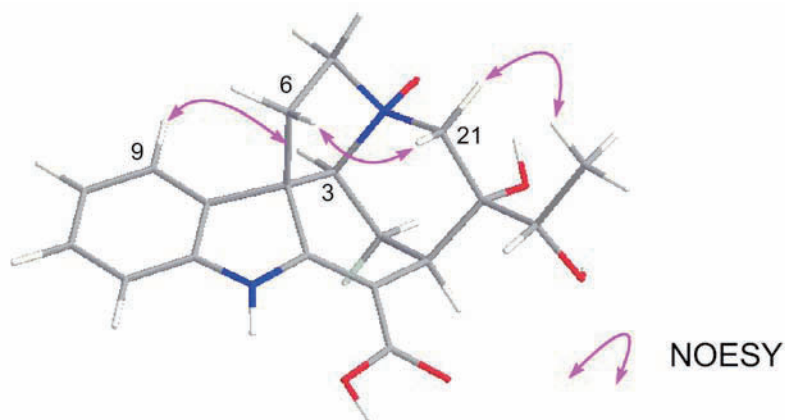


Figure 4. Selected NOESY correlations and relative stereochemistry for 17-carboxylcompactivervine *N*-oxide (**3**)

The relative stereochemistry of **3** and **4** were elucidated by NOESY correlations as shown in computer-generated 3D drawing (Figure 4). The relative configurations of C-19 and C-20 were also deduced by comparison of ^{13}C NMR spectra of **5** and **6**, and of **3** and **4**, which were in excellent agreement with Verpoorte's data⁸ on **5** and Men-Olivier's data⁹ on **6**. The structures of **3** and **4** were confirmed by chemical conversion from compactivervine (**5**) and alstovine (**6**). Oxidation of **5** with *m*CPBA in CHCl_3 gave compactivervine *N*(4)-oxide, which, upon LiOH hydrolysis in aqueous MeOH gave **3**. The similar patterns of Cotton effects in the CD spectra of **3** and **4** [**3**: λ_{max} 203 ($\Delta\epsilon$ +44.6), 239 ($\Delta\epsilon$ +20.8) and 316 ($\Delta\epsilon$ -40.8) nm, **4**: λ_{max} 212 ($\Delta\epsilon$ +32.1), 228 ($\Delta\epsilon$ +27.3) and 310 ($\Delta\epsilon$ -33.5) nm] indicated that the chiral centers have the same absolute configurations as that of 17-carboxyl-*N*(4)-methylechitamide chloride [λ_{max} 206 ($\Delta\epsilon$ +35.7), 239 ($\Delta\epsilon$ +16.6) and 312 ($\Delta\epsilon$ -34.9) nm].¹⁰ Thus, 17-carboxylcompactivervine *N*-oxide and 17-carboxylalstovine *N*-oxide were assigned to be **3** and **4**, respectively.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 automatic digital polarimeter. UV spectra were obtained on an Ultrospec 2100 pro spectrophotometer, CD spectra were measured on a JASCO J-820 spectropolarimeter, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. Positive-mode ESI mass spectra were obtained on a WatersQ-Tof premier spectrometer. High-resolution ESIMS were obtained on a LTQ Orbitrap XL (Thermo Scientific). HPLC was carried out using a JASCO PU-2089 Plus pump equipped with a UV-2075 Plus detector (λ 254 nm) and CAPCELL PAK C-18 MG-II columns (for analytical HPLC, 250 \times 4.6 mm i.d.,

5 μm particle size, and for preparative HPLC, 250 \times 10 mm i.d., 5 μm particle size, Shiseido, Tokyo, Japan). ^1H and ^{13}C NMR spectra were obtained on a Varian INOVA 600 spectrometer using TMS as an internal standard. HSQC experiments were optimized for $^1J_{\text{CH}} = 140$ Hz and HMBC experiments for $^nJ_{\text{CH}} = 8$ Hz.

Table 1. ^1H NMR data [δ_{H} (J, Hz)] of compounds **1-4** in CD_3OD

Position	1	2	3	4
3			4.58 (brs)	4.28 (brs)
5a	7.18 (d, 8.7)	4.79 (t, 7.0)	3.77 (m)	3.60 (m)
5b			3.85 (m)	3.60 (m)
6a	6.63 (d, 8.7, 2.0)	3.37 (t, 7.0)	2.21 (m)	2.04 (m)
6b			2.80 (m)	2.67 (m)
8	6.88 (d, 2.0)			
9		7.11 (d, 2.5)	7.42 (d, 7.6)	7.21 (d, 8.2)
10	3.75 (t, 6.3)		6.94 (t, 7.6)	6.42 (d, 8.2, 1.5)
11	4.87 (t, 6.3)	7.02 (dd, 8.9, 2.5)	7.18 (t, 7.6)	
12		7.38 (d, 8.9)	6.94 (t, 7.6)	6.49 (d, 1.5)
13	8.31 (s)			
14a		7.79 (s)	1.36 (m)	1.32 (m)
14b			3.10 (m)	2.96 (m)
15	8.28 (d, 8.0)		3.30 (m)	3.29 (m)
16	7.79 (dd, 8.0, 5.9)	3.06 (dd, 6.3, 6.3)		
17	8.50 (d, 5.9)	1.93 (m)		
18	2.67 (q, 7.6)	1.93 (m)	1.12 (d, 6.3)	1.13 (d, 6.3)
19	1.10 (t, 7.6)	2.91 (dd, 6.3, 6.3)	3.56 (m)	3.55 (m)
21a		8.50 (s)	3.39 (m)	3.20 (m)
21b			3.56 (m)	3.46 (m)
-OMe	3.80 (s)	3.85 (s)		3.75 (s)

Plant Material. The stems of *Alstonia macrophylla* were collected in Java, Indonesia, in 2007. The botanical identification was made by Ms. Sri Wuryanti, Purwodadi Botanical Garden, Indonesia. A voucher specimen (no. AP070902) has been deposited at Purwodadi Botanical Garden, Pasuruan, Indonesia.

Extraction and Isolation. The stems of *A. macrophylla* (4.7 kg) were crushed and extracted with MeOH. The MeOH extract (20 g) was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 aqueous solution to pH 9 and extracted with CHCl_3 and *n*-BuOH, successively. The CHCl_3 -soluble fraction was purified by a SiO_2 column (NH_3 sat. $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 1:0:0 \rightarrow 5:5:1). The fractions eluted with $\text{CHCl}_3/\text{MeOH}$ (7:3) was purified with ODS HPLC ($\text{MeOH}/\text{H}_2\text{O}/\text{TFA}$, 50:50:0.1) to afford 10-methoxydihydrosempervirine (**2**, 1.0 mg, 0.005%) together with two known alkaloids, compactinervine (**5**) and alstovine (**6**). The *n*-BuOH

-soluble fraction was purified by a HP-20 column (H₂O/MeOH 1:0 → 0:1). The fractions eluted with H₂O/MeOH (60:40) and H₂O/MeOH (50:50) were purified with an ODS column (H₂O/MeOH 1:0 → 0:1), followed by an ODS HPLC (MeOH/H₂O/HCOOH, 30:70:1) to afford *N*(1)-demethyl-7-methoxyikirydinium A (**1**, 0.7 mg, 0.004%), 17-carboxylcompactivervine *N*-oxide (**3**, 1.1 mg, 0.006%), and 17-carboxylalstovine *N*-oxide (**4**, 1.5 mg, 0.008%).

Table 2. ¹³C NMR data [δ_C] of compounds **1-4** in CD₃OD

Position	1	2	3	4
2	127.4	126.3	166.3	164.7
3	116.3	141.7	75.8	76.1
4	123.2			
5	120.6	56.9	68.3	68.8
6	112.9	20.5	40.0	39.6
7	160.2	117.5	53.7	52.8
8	94.9	126.7	134.2	126.7
9	138.3	101.2	121.0	121.5
10	27.6	156.5	122.4	106.8
11	63.6	119.0	130.1	162.4
12		114.4	111.6	98.1
13	145.1	136.5	145.9	147.8
14	146.2	121.1	23.5	23.6
15	145.9	159.6	35.1	35.6
16	128.4	30.6	103.4	100.1
17	143.4	22.5	173.3	175.3
18	26.6	22.5	15.2	15.2
19	14.6	27.0	70.0	70.1
20		135.3	73.9	74.3
21		145.1	65.4	65.4
-OMe	55.9	56.1		55.9
-CO ₂	165.9			

N(1)-Demethyl-7-methoxyikirydinium A (**1**): brown amorphous solid; IR (KBr) ν_{max} 3435 and 1675 cm⁻¹; UV (MeOH) λ_{max} 310 (ϵ 7200), 246*sh* (ϵ 8000), 220 (ϵ 18000), and 201 (ϵ 19000) nm; ¹H and ¹³C NMR, see Tables 1 and 2; HRESIMS [m/z 325.15862, (M+H)⁺, calcd for C₁₉H₂₁N₂O₃, 325.15522].

10-Methoxydihydrosempervirine (**2**): brown amorphous solid; IR (KBr) ν_{max} 1640 cm⁻¹; UV (MeOH) λ_{max} 395 (ϵ 3500), 321 (ϵ 3200), 227 (ϵ 7400), and 206 (ϵ 9500) nm; ¹H and ¹³C NMR, see Tables 1 and 2; HRESIMS (m/z 305.16528 [(M+H)⁺, calcd for C₂₀H₂₁N₂O, 305.16484].

17-Carboxylcompactivervine *N*-oxide (**3**): brown amorphous solid; [α]_D²⁷ -188 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3329 and 1614 cm⁻¹; UV (MeOH) λ_{max} 323 (ϵ 1400), 289 (ϵ 2600), 230*sh* (ϵ 4600), and 204 (ϵ 12000) nm; CD (MeOH) 203 ($\Delta\epsilon$ +44.6), 239 ($\Delta\epsilon$ +20.8) and 316 ($\Delta\epsilon$ -40.8) nm; ¹H and ¹³C NMR, see Tables 1 and 2; HRESIMS (m/z 359.16299 [(M+H)⁺, calcd for C₁₉H₂₃N₂O₅, 359.16070].

17-Carboxylalstovine *N*-oxide (**4**): brown amorphous solid; $[\alpha]_D^{27}$ -211 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3514 and 1612 cm^{-1} ; UV (MeOH) λ_{max} 293 (ϵ 3400), 233*sh* (ϵ 6700), and 204 (ϵ 13000) nm; CD (MeOH) 212 ($\Delta\epsilon$ +32.1), 228 ($\Delta\epsilon$ +27.3) and 310 ($\Delta\epsilon$ -33.5) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS (m/z 389.17386 [(M+H) $^+$], calcd for $\text{C}_{20}\text{H}_{25}\text{N}_2\text{O}_6$, 389.17126].

Chemical Transformation of Compactinervine (**5**) into 17-Carboxylcompactivervine *N*-oxide (**3**).

m-Chloroperoxybenzoic acid (2 equiv.) was added to a stirred solution of compactinervine (**5**) (1.5 mg) in CHCl_3 (0.5 mL) at rt. The mixture was stirred at rt for 3 h. The solution was diluted with CHCl_3 , washed with 20% Na_2CO_3 (aq) and then with H_2O , and concentrated to give a pale yellow oil (1.1 mg), which was used without purification in the next step. The crude *N*-oxide alkaloid was treated with LiOH (5 mg) in an aqueous MeOH (1:1), and the reaction mixture was stirred for 8 h at 60 °C. The resulting mixture was then adjusted to pH = 6 with 2 M HCl (aq) and extracted with *n*-BuOH. The combined organic extracts were evaporated in vacuo. The crude alkaloid was purified by column chromatography (C18 silica gel eluted with 1% aqueous formic acid/MeOH from 90:10 to 0:100) to give the 17-carboxyl-*N*-oxide derivative (0.9 mg), whose spectral data were identical with those of 17-carboxylcompactivervine *N*-oxide (**3**). In the same way, alstovine (**6**) (1.5 mg) was transformed into 17-carboxylalstovine *N*-oxide (**4**) (0.9 mg).

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