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Sucutiniranes A and B, new cassane-type diterpenes from *Bowdichia nitida*

Yosuke Matsuno [a], Jun Deguchi [a], Yusuke Hirasawa [a], Kunio Ohyama [b], Hiroo Toyoda [b], Chieko Hirobe [c], Wiwied Ekasari [d], Aty Widyawaruyanti [d], Noor Cholies Zaini [d], Hiroshi Morita [a,*]

[a] *Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan*
[b] *School of Pharmacy, Tokyo University of Pharmacy & Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan*
[c] *Seisen University, Higashi Gotanda 3-16-21, Shinagawa-ku, Tokyo 141, Japan*
[d] *Airlangga University, Jalan Dharmawangsa Dalam, Surabaaya 60286, Indonesia*

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ABSTRACT

Two new cassane-type diterpenes, sucutiniranes A (**1**) and B (**2**), have been isolated from the seeds of *Bowdichia nitida* together with 6α-acetoxyvouacapane (**3**) and 6α,7β-diacetoxyvouacapane (**4**), and the structures of **1** and **2** were elucidated by using 2D NMR data and chemical correlations. Sucutinirane A (**1**) and **3** showed a moderate cytotoxicity against human colon carcinoma COLO201 cells, and 6α,7β-diacetoxyvouacapane (**4**) showed in vitro antiplasmodial activity against parasite *Plasmodium falciparum* 3D7.

Bowdichia nitida Spruce ex Benth., common name 'sucupira', is distributed in the Brazilian Amazon, and the seeds of this plant are used for rheumatic, antipyretic, and gouty agents.[1] So far, alkaloids, triterpenes, isoflavonoids, benzofuranes, and benzopyranes have been isolated from the genus *Bowdichia*.[2–4]

Our efforts on identifying new natural products from the seeds of *B. nitida* resulted in the isolation of two new cassane-type diterpenes, sucutiniranes A (**1**) and B (**2**). This Letter describes the structure elucidation of **1** and **2** on the basis of spectroscopic data and chemical correlations as well as cytotoxicity against human colon carcinoma COLO201 cells and antiplasmodial activity.

*Structures of sucutiniranes A (**1**) and B (**2**).* The seeds of *Bowdichia nitida* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. EtOAc-soluble materials were subjected to a silica gel column (hexane/EtOAc and $CHCl_3$/MeOH) and an ODS column (MeOH/H_2O) followed by HPLC (MeOH/H_2O) to afford sucutiniranes A (**1**, 0.0002% yield) and B (**2**, 0.00006%) together with 6α-acetoxyvouacapane (**3**, 0.02%)[5] and 6α,7β-diacetoxyvouacapane (**4**, 0.0008%).[6]

Sucutinirane A {**1**, $[\alpha]_D^{22}$ −24 (*c*, 1.0, $CHCl_3$)} was revealed to have the molecular formula $C_{22}H_{32}O_5$, by HRESITOFMS [*m*/*z* 399.2142 $(M+Na)^+$, Δ −0.5 mmu]. IR absorptions implied the presence of hydroxyl (3480 cm^{-1}) and carbonyl (1740 cm^{-1}) groups. The ^1H and ^{13}C NMR data (Table 1) suggested the presence of two carbonyl carbons, one sp^2 methine, one sp^2 quaternary carbon, five sp^3 methylenes, five sp^3 methines, three sp^3 quaternary carbons, and five methyl groups. The presence of the α, β-unsaturated γ-lactone moiety was substantiated by the signals of one sp^2 methine (δ_C 114.2), one sp^2 quaternary carbon (δ_C 175.0), one sp^3 quaternary carbon with two oxygen atoms (δ_C 107.6), and one carbonyl carbon (δ_C 173.4).

Partial structures **a** (C-1 to C-3) and **b** (C-5 to C-9, C-11, C-14, and C-17) were deduced from a detailed analysis of 2D NMR data of **1** (Fig. 1). The HMBC cross-peaks of H_3-19 to C-3, C-4, C-5, and C-18 indicated the connection among C-3, C-5, C-18, and C-19 through C-4. HMBC correlations for H_3-20 to C-1, C-5, C-9, and C-10 indicated connection among C-1, C-5, C-9, and C-20 through C-10. On the other hand, HMBC correlations for H-14 to C-12 and C-15, H_3-17 to C-13, and H-15 to C-12 and C-16 supported the location of the methyl group at C-14, and the α, β-unsaturated γ-lactone moiety at C-12 and C-13. Furthermore, the presence of an acetoxy group at C-6 was elucidated by the HMBC correlation for H-6 and H_3-22 to C-21. Thus, the gross structure of sucutinirane A was assigned to be **1** with a cassane-type skeleton[7] with the methyl group at C-14 and the α, β-unsaturated γ-lactone moiety at C-12 and C-13. The existence of cassane butenolides is rare as compared to that of cassane furanoditerpenes.

To assign the relative stereochemistry at the hemiketal C-12 position, **1** was acetylated with acetic anhydride in pyridine at

* Corresponding author. Tel./fax: +81 354985778.
E-mail address: moritah@hoshi.ac.jp (H. Morita).

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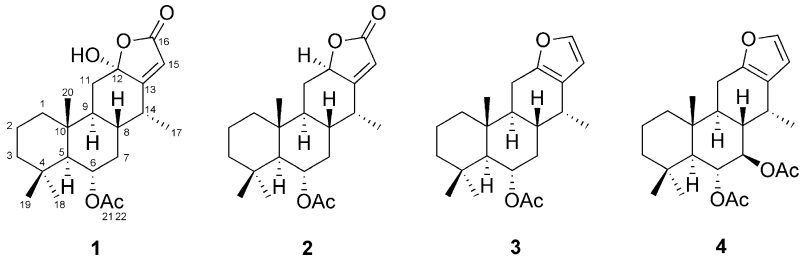


Table 1
^{1}H [δ_H (J, Hz)] and ^{13}C [δ_C] NMR Data of sucutiniranes A (**1**) and B (**2**)

	1[a]		**2**[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
1a	1.10 (1H, m)	40.7	1.02 (1H, m)	39.7
1b	1.74 (1H, m)		1.71 (1H, m)	
2a	1.48 (1H, m)	19.5	1.47 (1H, m)	18.4
2b	1.59 (1H, m)		1.52 (1H, m)	
3a	1.29 (1H, m)	44.6	1.24 (1H, m)	43.4
3b	1.38 (1H, m)		1.39 (1H, m)	
4		34.1		33.1
5	1.31 (1H, d, 11.0)	58.4	1.22 (1H, m)	57.2
6	5.12 (1H, ddd, 11.0, 11.0, 4.1)	73.5	5.07 (1H, ddd, 11.1, 11.1, 4.3)	72.0
7a	1.49 (1H, m)	38.0	1.41 (1H, m)	36.7
7b	1.84 (1H, m)		1.88 (1H, m)	
8	1.84 (1H, m)	40.9	1.82 (1H, m)	38.7
9	1.54 (1H, m)	45.6	1.25 (1H, m)	44.4
10		39.5		38.7
11a	1.25 (1H, m)	38.9	0.99 (1H, m)	33.7
11b	2.38 (1H, dd, 12.9, 3.3)		2.50 (1H, ddd, 11.8, 6.4, 3.0)	
12		107.6	4.84 (1H, dd, 11.4, 6.4)	79.1
13		175.0		176.1
14	2.96 (1H, m)	37.3	2.95 (1H, m)	35.7
15	5.74 (1H, s)	114.2	5.68 (1H, s)	110.9
16		173.4		173.4
17	1.17 (3H, d, 7.3)	12.9	1.08 (3H, d, 7.3)	13.9
18	1.10 (3H, s)	37.2	1.06 (3H, s)	36.5
19	0.91 (3H, s)	23.0	0.89 (3H, s)	22.5
20	0.91 (3H, s)	15.8	0.88 (3H, s)	15.3
21		172.2		170.3
22	2.04 (3H, s)	21.9	2.06 (3H, s)	21.9

[a] In CD_3OD.
[b] In $CDCl_3$.

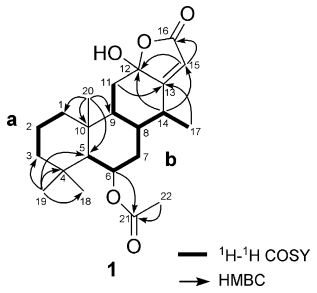


Figure 1. Selected 2D NMR correlations for sucutinirane A (**1**).

room temperature to afford the monoacetylated product **1a**. The relative stereostructure of **1a** as shown in computer-generated 3D drawing (Fig. 2) was deduced from cross-peaks observed in the NOESY spectrum and ^{3}J coupling constants. The NOESY correlation of H_3-17/H_3-24 indicated to be α-orientation for CH_3-17 and CH_3-24. Antiperiplanar conformation between H-5 and H-6 was preferred because of the coupling constant, $^{3}J_{H5/H6}$ = 11.0 Hz. The β-configuration of H-6, CH_3-19, and CH_3-20 was supported by the NOESY cross-peaks among H-6, H_3-19, and H_3-20, while the α-configuration of both H-5 and H-9 was supported by the NOESY cross-peak between H-5 and H-9. Furthermore, oxidation of the furan ring of 6α-acetoxyvouacapane (**3**) with *m*CPBA gave sucutinirane A (**1**) as shown in Scheme 1. Thus, the structure of sucutinirane A including relative stereochemistry was assigned as shown in Figure 2.

Sucutinirane B {**2**, $[\alpha]_D^{22}$ −33 (*c*, 0.2, $CHCl_3$)} was revealed to have the molecular formula $C_{22}H_{32}O_4$, by HRESITOFMS [*m/z* 361.2389 $(M+H)^+$, Δ +2.1 mmu]. IR absorptions implied the presence of carbonyl (1735 cm^{-1}) group. The ^{1}H and ^{13}C NMR data (Table 1), and 2D NMR correlations (Fig. 3) suggested that **2** had the same cassane-type skeleton as that of **1**, except for the presence of an oxymethine at C-12 (δ_H 4.84, δ_C 79.1). The relative stereochemistry of sucutinirane B (**2**) was deduced by NOESY spectrum (Fig. 4) and ^{3}J coupling constants. The configuration of β-oriented H-6, H-8, CH_3-19, and CH_3-20 was supported by the NOESY correlations of H-6/H-8, H-6/H_3-19, and H-8/H_3-20. The coupling constant, $^{3}J_{H5/H6}$ = 11.1 Hz indicated antiperiplanar conformation between H-5 and H-6. The α-configuration of H-5, H-9, H-12, and CH_3-17 was supported by the NOESY cross-peaks of H-5/H-9 and H-12/H-9 and H_3-17. Thus, the structure of **2** was assigned as 12-deoxy-sucutinirane A.

The absolute stereochemistry of sucutiniranes A (**1**) and B (**2**) was deduced by applying CD curves for γ-lactone chromophore.[8] The sign of the CD curve in MeOH [**1**: λ_{max} 222 nm (Δε −0.8) and 244 nm (Δε −0.6), **2**: λ_{max} 218 nm (Δε −1.1)] was negative, indicating that the chirality at C-12 of **1** and **2** was as shown in Figures 3 and 4.

To confirm the proposed structure for **2**, treatment of 6α-acetoxyvouacapane (**3**) with *m*CPBA in the presence of 1 drop 12 N HCl in $CHCl_3$ afforded sucutinirane B (**2**) together with two byproducts, compounds **5** and **6**, which was elucidated by 2D NMR correlations as shown in Figure 5. Stereochemistry of

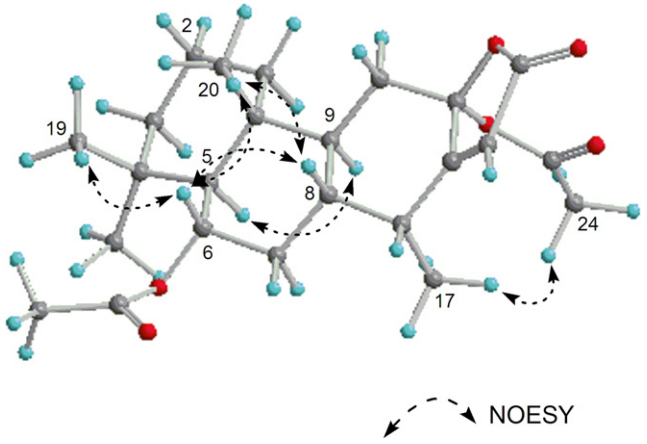
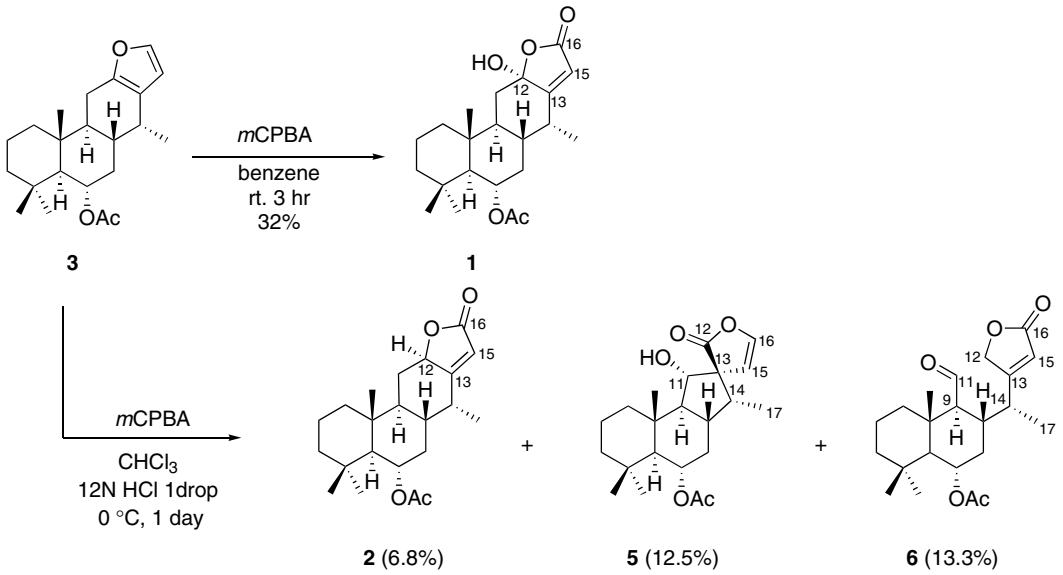


Figure 2. Selected NOESY correlations and relative stereochemistry of compound **1a**.



Scheme 1. Oxidation of 6α-acetoxyvouacapane (**3**) by *mCPBA*.

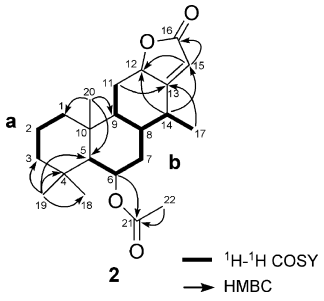


Figure 3. Selected 2D NMR correlations for sucutinirane B (**2**).

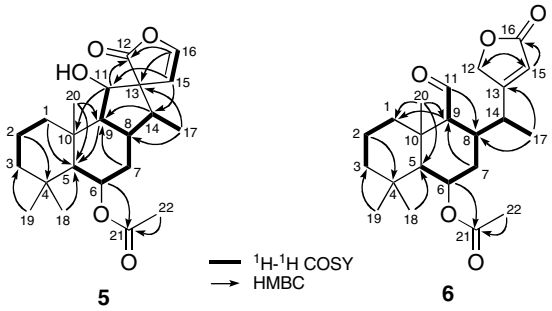


Figure 5. Selected 2D NMR correlations for **5** and **6**.

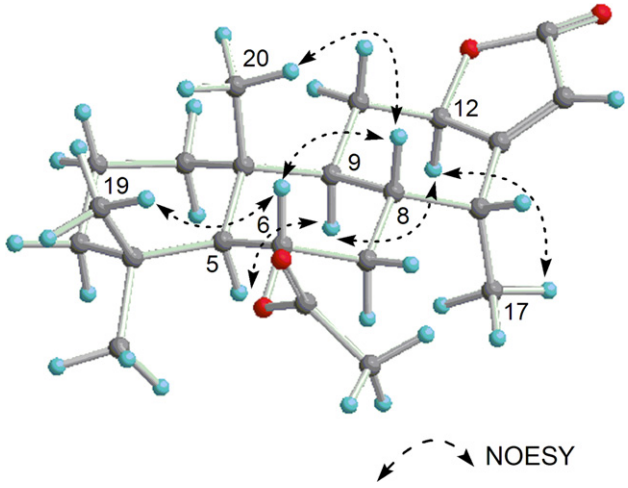


Figure 4. Selected NOESY correlations and relative stereochemistry of sucutinirane B (**2**).

a hydroxyl at C-11 and spiro carbon at C-13 for **5** was assigned by NOESY data of H-8/H-11 and H-15/H_3-17. Compound **5**, which was derived from oxidative intermediate at C-11 followed by epoxidation at Δ^{12} and Pinacol-type rearrangement, possesses a spirojoined β, γ-unsaturated γ-lactone, and cyclopentane bicycles with a hydroxyl group at C-11. On the other hand, compound **6**, which was produced from oxidative intermediate at C-11 followed by epoxidation at Δ^{15} and cleavage between C-11 and C-12 bond accompanied with cleavage of the epoxide, contains an aldehyde moiety at C-11 and an α, β-unsaturated γ-lactone moiety at C-14 (Scheme 1). These structures of **5** and **6** were also supported by HMBC (Fig. 5) and NOESY correlations.

Sucutinirane A (**1**) and 6α-acetoxyvouacapane (**3**) showed a moderate cytotoxicity against human colon carcinoma COLO201 cells with IC_{50} 37.3 and 86.6 μg/mL, respectively, while sucutinirane B (**2**), 6α,7β-diacetoxyvouacapane (**4**), and compounds **5** and **6** were inactive (IC_{50} > 100 μg/mL).

Each compound was also tested for its ability to inhibit *Plasmodium falciparum* growth.[9] 6α,7β-diacetoxyvouacapane (**4**) showed promising in vitro antiplasmodial activity against parasite *P. falciparum* 3D7 (IC_{50} 0.39 μg/mL) and a good selectivity index with regard to the cytotoxicity on COLO201 cells (IC_{50} > 100 μg/mL), whereas other compounds were inactive at a concentration of 1 μg/mL (Chloroqine: IC_{50} 0.006 μg/mL).

Acknowledgments

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