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TWO NEW INDOLE ALKALOIDS FROM ASPIDOSPERMA SUBINCANUM AND GEISSOSPERMUM VELLOSII

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Abstract – Two new indole alkaloids, subincanadine G (**1**) and pausperadine A (**2**), were isolated from the barks of *Aspidosperma subincanum* Mart and *Geissospermum vellosii* Allemão, respectively. Subincanadine G (**1**) has 1-azoniatricyclo[4.3.3.0^{1,5}]undecane moiety, while pausperadine A (**2**) contains an *N*-oxide moiety. The gross structures and relative stereochemistry of **1** and **2** were elucidated by spectroscopic data.

Brazilian medicinal plants have proven to be a rich source of compounds which might be useful for the development of new pharmaceutical agents.[1] In our search for structurally unique and biogenetically interesting compounds from Brazilian medicinal plants, we previously isolated nitrogen-containing clerodane diterpenoids,[2,3] cembrane diterpenoids with an eight-membered lactone ring,[4] and diarylheptanoids containing a tetrahydrofuran ring,[5] and polycyclic indole alkaloids.[6]

Recently, two new indole alkaloids, subincanadine G (**1**) and pausperadine A (**2**), were isolated from the barks of *Aspidosperma subincanum* Mart and *Geissospermum vellosii* Allemão, respectively. Subincanadine G (**1**) has 1-azoniatricyclo[4.3.3.0^{1,5}]undecane moiety, while pausteradine A (**2**) contains

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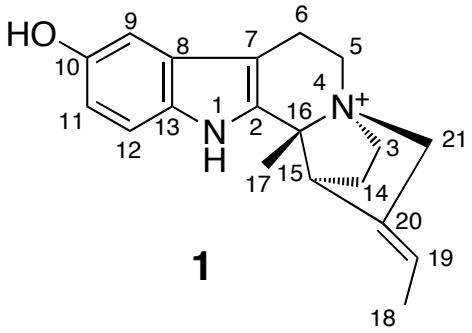
This paper is dedicated to the memory of the Emeritus Professor Kenji Koga of Tokyo University.

an *N*-oxide moiety. The structures and relative stereochemistry of **1** and **2** were elucidated by spectroscopic data and chemical correlations. This paper describes the isolation and structure elucidation of **1** and **2**.

The barks of *Aspidosperma subincanum* Mart were extracted with MeOH and the *n*-BuOH-soluble materials were subjected to silica gel column chromatographies ($CHCl_3$-*n*-BuOH-AcOH-H_2O, 1.5:6:1:1 and then $CHCl_3$-MeOH, 4:1) followed by C_{18} HPLC (CH_3CN-H_2O, 30:70 containing 0.1% TFA) to afford subincanadine G (**1**, 0.002 %).



1

The molecular formula, $C_{19}H_{23}N_2O$, of subincanadine G (**1**) was established by HRFABMS [*m/z* 295.1808 $(M)^+$, Δ -0.2 mmu]. The structure of **1** was elucidated by ^1H and ^{13}C NMR spectral data (Table 1) and 2D NMR spectral correlations (^1H-^1H COSY, HMQC, and HMBC) (Figure 1). The ^{13}C NMR spectral data revealed signals due to ten sp^2 carbons, one sp^3 quaternary carbon, five sp^3 methylene carbons, one sp^3 methine carbon, and two methyl carbons. The carbon chemical shifts of C-3 (δ 58.63), C-5 (δ 46.57), C-16 (δ 77.92), and C-21 (δ 64.21) suggested that these carbons were attached to a quaternary nitrogen atom. The ^1H-^1H COSY (Figure 1) spectrum revealed connectivities of C-3 to C-14, C-14 to C-15, C-11 to C-12, and C-18 to C-19. HMBC correlations (Figure 1) of H-1 to C-2 (δ 132.32), C-7 (δ 103.46), C-8 (127.62), and C-13 (δ 131.45) and H-9 to C-10, C11(δ113.51), and C-13 indicated the presence of an indole ring (C-2, C-7 ~ C-13, and N-1). The ^{13}C NMR chemical shift of C-10 (δ 152.88) suggested that a hydroxyl group was attached to C-10. Thus, the gross structure of subincanadine G was assigned as **1**. The relative stereochemistry of **1** was elucidated by NOESY spectral correlations as shown in Figure 2. NOESY spectral correlations of H_3-17 to H-5b and H-21b and H-3a to H-5a indicated a *β*-orientation of the methyl group (C-17). Geometry of the trisubstituted olefin at C-19 and C-20 was elucidated to be *E* from NOESY spectral correlations of H-19 to H_2-21 (Figure 2). Therefore, subincanadine G was concluded to be **1**.

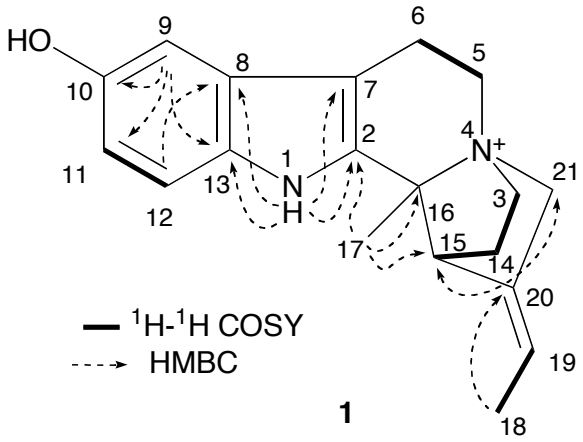


Figure 1. Selected 2D NMR spectral correlations for subincanadine G (**1**)

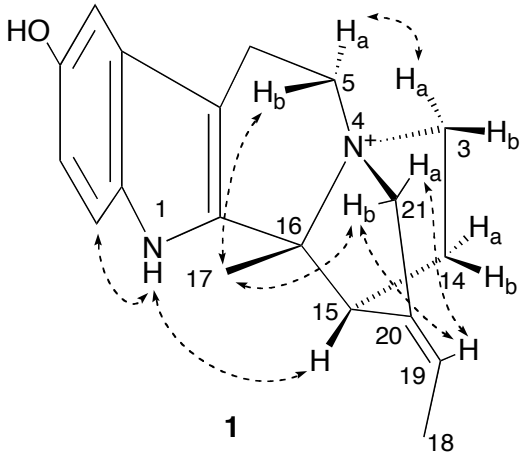


Figure 2. Selected NOESY spectral correlations and relative stereochemistry for subincanadine G (**1**). Dotted arrows denote NOESY spectral correlations.

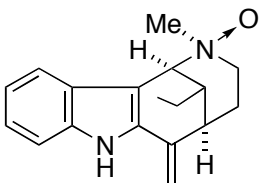
Table 1. ^{1}H and ^{13}C NMR Spectral Data of Subincanadine G (**1**) in Pyridine-d_5

position	^{1}H[a]	^{13}C[a]	H coupled with C [b]
1	12.78 (s)		
2		132.32	H-1, H-17
3a	3.69 (m)	58.63	
3b	3.52 (m)		
4			
5a	4.04 (m)	46.57	
5b	3.78 (m)		

6a	3.08 (m)	18.14	
6b	3.00 (m)		
7		103.46	H-1
8		127.62	H-1, H-12
9	7.36 (s)	103.54	
10		152.88	H-9, H-12
11	7.29 (d, 8.4)	113.51	H-9
12	7.73 (d, 8.4)	113.88	
13		131.45	H-1, H-9, H-11
14a	1.84 (m)	26.30	
14b	1.52 (m)		
15	4.40 (m)	44.89	H-17
16		77.92	H-17
17	1.76 (3H, s)	20.75	
18	1.63 (3H, d, 5.5)	14.22	
19	5.41 (m)	120.95	H-18
20		133.12	H-18
21a	4.35 (d, 12.0)	64.21	H-15
21b	4.21 (d, 12.0)		

[a] δ in ppm. [b] HMBC correlations.

The barks of *Geissospermum vellosii* Allemão were extracted with MeOH and the MeOH extracts were partitioned between hexane and 90% MeOH. The aqueous MeOH layer was partitioned between EtOAc and 1M NaCl, and the aqueous layer was extracted with *n*-BuOH. EtOAc-soluble portions were purified by silica gel column chromatography ($CHCl_3$-MeOH, 4:1) followed by C_{18} HPLC (CH_3CN-H_2O, 35:65 containing 0.1% TFA) to give pausperadine (**2**).



2

The molecular formula, $C_{18}H_{22}N_2O$, of pausperadine (**2**) was established by HRESIMS [*m/z* 283.1819

[M+H]$^+$, Δ +0.8 mmu]. ^{1}H and ^{13}C NMR spectral data (Table 2) and the ^{1}H-^{1}H COSY (Figure 3) spectrum indicated that **2** was an alkaloid related to uleine[7a-d]. ^{1}H-^{1}H COSY spectral correlations revealed connectivities of C-9 to C-12, C-3 to C-14, C-14 to C-15, C-15 to C-20, C-18 to C-20 and C-20 to C-21. The connectivity of C-16 to C-17 was deduced from HMBC spectral correlations of H-17 to C-2, C-15, and C-16.

The relative stereochemistry of **2** was elucidated by NOESY spectral correlations as shown in Figure 4. NOESY spectral correlations of H-20 to H-14a and H-19b to H-21 indicated an α-orientation of the ethyl group (C-20). An α-orientation of the methyl group at N-4 was deduced from NOESY correlations of NC$\underline{H}_3$ to H-21. Thus, the relative stereochemistry of pausperadine was assigned as **2**.

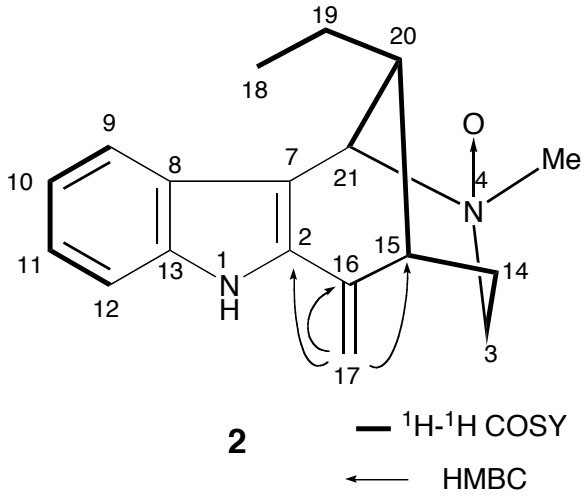


Figure 3. Selected 2D NMR spectral correlations for pausperadine A (**2**)

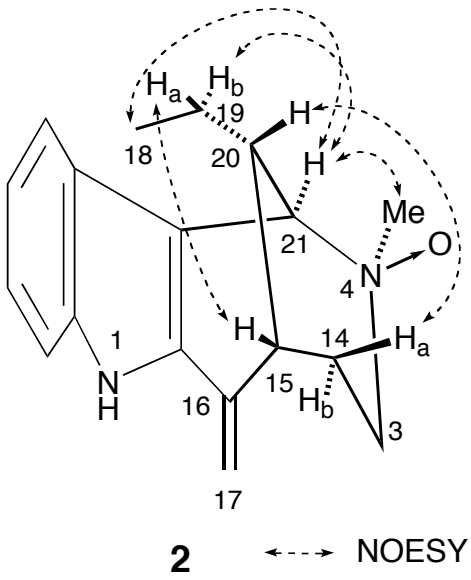


Figure 4. Selected NOESY spectral correlations and relative stereochemistry for pausperadine A (**2**). Dotted arrows denote NOESY correlations.

Sbincanadine G (**1**) and Pausperadine A (**2**) did not show cytotoxicity (IC_{50} > 10 μg/mL) against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro*.[8]

Table 2. ^{1}H and ^{13}C NMR Spectral Data of Pausperadine A (**2**) in CD_3OD

position	$^{1}H^{c}$	$^{13}C^{c}$
1		
2		139.61
3	3.45 (2H, m)	74.03
4		
7		113.77
8		129.07
9	7.73 (d, 7.7)	119.93
10	7.21 (t, 7.7)	123.08
11	7.28 (t, 7.7)	125.62
12	7.48 (d, 7.7)	114.58
13		137.42
14a	2.58 (m)	30.83
14b	1.92 (br d, 14.0)	
15	2.98 (m)	38.55
16		140.17
17a	5.91 (s)	103.87
17b	5.40 (s)	
18	0.94 (3H, t, 6.2)	12.31
19a	1.22 (m)	25.66
19b	1.14 (m)	
20	2.92 (m)	40.55
21	5.16 (br s)	59.27
NMe	3.41 (3H, s)	57.13

EXPERIMENTAL

IR and UV spectra were recorded on a JASCO FT/IR-5300 and a Shimadzu UV-1600PC spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells for $CDCl_3$ or MeOH-d_4 (Shigemi Co., Ltd). The 7.19 and 135.5 ppm resonances of residual pyridine-d_5 were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. The 3.35 and 49.5 ppm resonances of residual MeOH-d_4 were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. FAB MS spectra were obtained on a JEOL HX-110 spectrometer. ESI MS spectra were obtained on a JEOL JMS-SX102A spectrometer.

Plant Material. The barks of *Aspidosperma subincanum* Mart ("Pau-pereira-do-mato", Apocynaceae) and *Geissospermum vellosii* Allemão were purchased in São Paulo, Brazil in March, 2000. The plants were identified by Dr. G. Hashimoto (Centro de Pesquisas de História Natural, São Paulo, Brasil). Each voucher specimen has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Separation. The barks of *Aspidosperma subincanum* (100 g) were extracted with MeOH (300 mL x 3) at rt for 3 days. The MeOH extracts (20 g) were partitioned between hexane (250 mL x 3) and 90% MeOH (250 mL). The aqueous MeOH layer was partitioned between EtOAc (250 mL x 3) and 1M NaCl (250 mL), and then the aqueous layer was extracted with *n*-BuOH (250 mL x 3). A portion (1.0 g) of the *n*-BuOH-soluble materials (12 g) was subjected to silica gel columns ($CHCl_3$-*n*-BuOH-AcOH-H_2O, 1.5:6:1:1 and then $CHCl_3$-MeOH, 4:1) followed by C_{18} HPLC (YMC-Pack ODS-AQ, YMC Co., Ltd., 1 x 25 cm; flow rate: 2.5 mL/min; eluent: MeCN-H_2O, 30:70 containing 0.1% TFA; UV detection at 281 mm) to afford subincanadine G (**1**, t_R 12.4 min, 1.6 mg, 0.002 %). The barks of *Geissospermum vellosii* (2.4 kg) were extracted with MeOH (3.2 L x 3) at rt for 3 days. A part of the MeOH extracts (120 g) was partitioned between hexane (1 L x 3) and 90% MeOH (1 L). The aqueous MeOH layer was partitioned between EtOAc (1 L x 3) and 1M NaCl (1 L), and then the aqueous layer was extracted with *n*-BuOH (1 L x 3). A portion (1.0 g) of the EtOAc-soluble materials (15 g) was subjected to a silica gel column ($CHCl_3$-MeOH, 4:1) followed by C_{18} HPLC (YMC-Pack ODS-AQ, YMC Co., Ltd., 1 x 25 cm; flow rate: 2.5 mL/min; eluent: MeCN-H_2O, 35:65 containing 0.1% TFA; UV detection at 281 mm) to afford pausperadine (**2**, t_R 21.7 min, 1.8 mg, 7.5×10^{-6} %).

Subincanadine G (1): A colorless amorphous solid; $[\alpha]^{23}_D$ -32° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 315 (sh 2.98) and 273 (3.27) nm; IR (KBr) ν_{max} 3425 and 2925 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m/z* 295 [M^+]; HRFABMS *m/z* 295.1808 [M^+] (calcd for $C_{19}H_{23}N_2O$, 295.1810).

Pausperadine A (2): A colorless amorphous solid; $[\alpha]^{22}_{D}$ +2.3° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.03), 242 (sh 3.65), and 307 (3.65) nm; IR (KBr) ν_{max} 3425 and 2925 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS *m/z* 283 $(M+H)^+$; HRESIMS *m/z* 283.1819 $(M+H)^+$ (calcd for $C_{18}H_{23}N_2O$, 283.1811).

ACKNOWLEDGEMENTS

The plants were identified by Dr. G. Hashimoto (Centro de Pesquisas de História Natural, São Paulo, Brasil), and each voucher specimen has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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