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Subincanadines A−C, Novel Quaternary Indole Alkaloids from *Aspidosperma subincanum*

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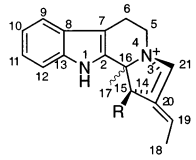
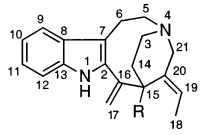
Three novel quaternary indole alkaloids with an unprecedented 1-azoniatricyclo[4.3.3.0^{1,5}]undecane moiety, subincanadines A−C (**1**−**3**), as well as two new indole alkaloids with a 1-azabicyclo[5.2.2]-undecane moiety, subincanadines D (**4**) and E (**5**), and a new indole alkaloid with a 1-azabicyclo-[4.3.1]decane moiety, subincanadine F (**6**), have been isolated from the barks of *Aspidosperma subincanum* Mart, and the structures of **1**−**6** and the stereochemistry of **1**−**3** were elucidated by spectroscopic data and chemical means.

Introduction

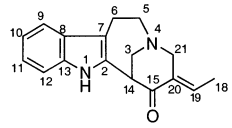
Brazilian medicinal plants have proven to be a rich source of compounds that might be useful for the development of new pharmaceutical agents.[1] In our search for structurally unique and biogenetically interesting compounds from Brazilian medicinal plants, we previously isolated nitrogen-containing clerodane diterpenoids[2] and cembrane diterpenoids with an eight-membered lactone ring[3] from the leaves of *Echinodorus macrophyllus* (Alismataceae) and diarylheptanoids containing a tetrahydrofuran ring[4] from the seeds of *Renealmia exaltata* (Zingiberaceae). Recent investigation of extracts from the barks of the Brazilian medicinal plant *Aspidosperma subincanum* Mart (Brazilian name "Pau-pereira-domato", Apocynaceae) resulted in the isolation of subincanadines A−C (**1**−**3**), three novel quaternary indole alkaloids with an unprecedented 1-azoniatricyclo[4.3.3.0^{1,5}]-undecane moiety, as well as subincanadines D (**4**) and E (**5**), two new indole alkaloids with a 1-azabicyclo[5.2.2]-undecane moiety, and subincanadine F (**6**), a new indole alkaloid with a 1-azabicyclo[4.3.1]decane moiety. This paper describes the isolation and structure elucidation of **1**−**6**.



1: R = OH, C-17 β
2: R = OH, C-17 α
3: R = H, C-17 β



4: R = OH
5: R = H



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Results and Discussion

The barks of *A. subincanum* Mart were extracted with MeOH and the MeOH extracts were partitioned between hexane and 90% MeOH. The aqueous MeOH layer was partitioned between EtOAc and 1 M NaCl, and the aqueous layer was extracted with *n*-BuOH. *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, 36:64 containing 0.1% TFA) to afford subincanadines A (**1**, 0.014%), B (**2**, 0.002%), and C (**3**, 0.002%). EtOAc-soluble portions were purified by silica gel column chromatographies ($CHCl_3$−MeOH, 98:2 and then $CHCl_3$−*n*-BuOH−AcOH−H_2O, 1.5:6:1:1) followed by C_{18} HPLC (CH_3CN−H_2O, 40:60 containing 0.1% TFA) to give subincanadines D (**4**, 0.003%), E (**5**, 0.002%), and F (**6**, 0.002%) together with a known indole alkaloid, apparicine[5] (**7**, 0.001%).

The molecular formula, $C_{19}H_{23}N_2O$, of subincanadine A (**1**) was established by HRESIMS [*m*/*z* 295.1813 (M^+), Δ −0.3 mmu]. The IR spectrum suggested the presence of hydroxyl and/or amino (3424 cm^{-1}) groups, while the UV absorption (289 nm) indicated the presence of an indole chromophore. The gross structure of **1** was deduced from detailed analysis of ^1H and ^{13}C NMR data (Table 1) aided by 2D NMR experiments (^1H−^1H COSY, HMQC, and HMBC). The ^{13}C NMR data indicated that the

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(2) (a)Kobayashi, J.; Sekiguchi, M.; Shigemori, H.; Ohsaki, A. *Tetrahedron Lett.* **2000**, *41*, 2939−2943. (b) Kobayashi, J.; Sekiguchi, M.; Shimamoto, S.; Shigemori, H.; Ohsaki, A. *J. Nat. Prod.* **2000**, *63*, 1576−1579.
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(4) Sekiguchi, M.; Shigemori, H.; Ohsaki, A.; Kobayashi, J. *J. Nat. Prod.* **2002**, *65*, 375−376.
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TABLE 1. ^{1}H and ^{13}C NMR Data of Subincanadine A (1) in DMSO-d_6

position	^{1}H[a]	^{13}C[a]	H coupled with C[b]
1	10.89 (s)		
2		129.85	H-1, H-6a, H-6b, H-17
3a	3.78 (m)	57.56	H-5b, H-21a, H-21b
3b	3.60 (m)		
4			
5a	3.81 (m)	46.72	
5b	3.70 (m)		
6a	3.14 (m)	17.51	H-5b
6b	3.12 (dd, 17.1, 6.7)		
7		104.99	H-1, H-5a, H-6
8		125.52	H-1, H-10, H-12
9	7.44 (d, 8.2)	118.57	H-11
10	7.05 (dd, 8.2, 7.4)	119.33	H-12
11	7.15 (dd, 7.6, 7.4)	122.34	H-9
12	7.48 (d, 7.6)	112.38	H-10
13		137.02	H-1, H-9, H-11
14a	2.05 (ddd, 17.8, 10.9, 3.0)	31.36	H-3a, OH
14b	1.82 (dd, 17.8, 11.2)		
15		84.31	H-14a, H-14b, H-17, H-19, OH
16		74.44	H-3a, H-3b, H-14a, H-17, H-21a
17	1.61 (3H, s)	18.90	
18	1.94 (3H, d, 6.9)	12.33	
19	5.65 (qd, 6.9)	121.43	H-18, H-21a
20		132.68	H-14b, H-18, H-21a, H-21b
21a	4.31 (d, 14.0)	64.17	H-3a, H-3b, H-19
21b	4.22 (d, 14.0)		
OH	7.03 (s)		

[a] δ in ppm. [b] HMBC correlations.

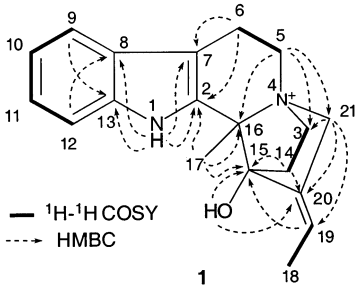


FIGURE 1. Selected 2D NMR correlations for subincanadine A (**1**).

molecule possessed 10 sp^2 carbons, two sp^3 quaternary carbons (one of them bearing an oxygen atom), five sp^3 methylenes, and two methyl groups. The carbon chemical shifts of C-3 (δ 57.56), C-5 (δ 46.72), C-16 (δ 74.44), and C-21 (δ 64.17) suggested that these carbones were attached to a quaternary nitrogen atom. The ^{15}N NMR chemical shift of N-4 (δ 76.12) in DMSO-d_6, which was assigned by the ^{1}H−^{15}N HMBC correlation from H$_3$-17, also supported the presence of the quaternary nitrogen.[6] The ^{1}H−^{1}H COSY (Figure 1) spectrum revealed connectivities of C-3 to C-14, C-5 to C-6, C-9−C-12, and C-18 to C-19. HMBC correlations (Figure 1) of H-1 to C-2(δ 129.85), C-7(δ 104.99), C-8(δ 125.52), and C-13(δ 137.02); H-12 to C-8; and H-9 to C-13 revealed the presence of an indole ring (C-2, C-7−C-13, and N-1). Cross-peaks of H$_2$-6 to C-2 and C-7, H$_3$-17 to C-2 and C-16 (δ_C 74.44), and H-5a to C-16 in the HMBC spectrum implied the presence of a piperidine ring (C-5−C-7, C-2, C-16, and N-4) with a methyl group (C-17) at C-16. The presence of a pyrrolidine ring (C-3, C-14−C-16, and N-4) with a hydroxy group at C-15 was deduced from HMBC correlations of H-5b to C-3, H$_2$-14 to C-15, 15-OH to C-14 and C-15, and H$_3$-17 to C-15. HMBC correlations of H-5b to C-21; H$_2$-14 to C-20; H$_2$-21 to C-3, C-19, and C-20; and H-19 to C-15 indicated the presence of another piperidine ring (C-3, C-14, C-15, C-20, C-21, and N-4) with an ethynyl group (C-18−C-20) at C-20. Thus, the structure of subincanadine A was elucidated to be **1**, a novel quaternary indole alkaloid possessing a 1-azoniatricyclo-[4.3.3.0^{1,5}]undecane.

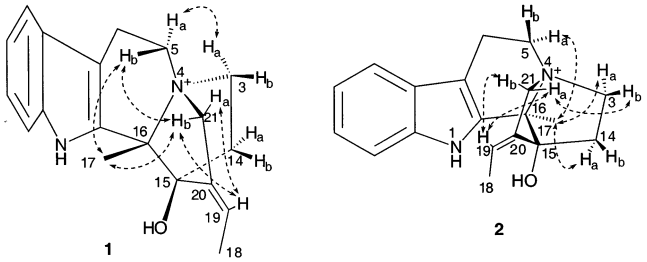
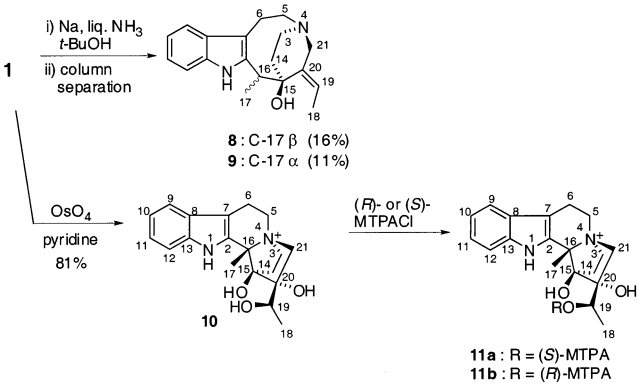


FIGURE 2. Selected NOESY correlations and relative stereochemistry for subincanadines A (**1**) and B (**2**). Dotted arrows denote NOESY correlations.

SCHEME 1



The relative stereochemistry of **1** was elucidated by NOESY correlations as shown in Figure 2. NOESY correlations of H$_3$-17 to H-5b and H-21b, H-3a to H-5a, and H-5b to H-21b indicated both β-orientations of the methyl group (C-17) at C-16 and the hydroxy group at C-15. Geometry of the trisubstituted olefin at C-19 and C-20 was elucidated to be *Z* from NOESY correlations of H-19 to H$_2$-21 (Figure 2).

Birch reduction of **1** with sodium in liquid ammonia afforded compounds **8** (C-17β) and **9** (C-17α), ring-opened products generated through cleavage of the N-4−C-16 bond, in a ratio of 3:2, respectively (Scheme 1), of which the structures were assigned by its 2D NMR data. The backbone structures of **8** and **9** were the same as those of subincanadines D (**4**) and E (**5**), as described later. To determine the absolute stereochemistry at C-15 in **1**, introduction of (a*R*)- and (a*S*)-2-(2′-methoxy-1′-naphthyl)-3,5-dichlorobenzoic acid (MNCB)[7a] to the hydroxy group of C-15 was tried, but its esters were not obtained.[7b] Therefore, the double bond at C-19 and C-20 in **1** was

(6) Pettit, G. R.; Gieschen, D. P.; Pettit, W. E.; Rawson, T. E. *Can. J. Chem.* **1981**, *59*, 216−221.

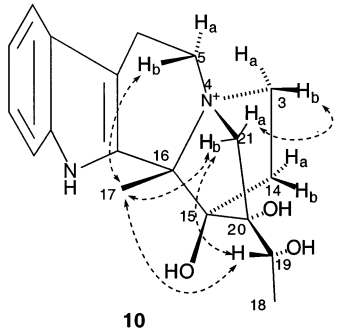


FIGURE 3. Selected NOESY correlations and relative stereochemistry for triol derivative (**10**) of subincanadine A (**1**). Dotted arrows denote NOESY correlations.

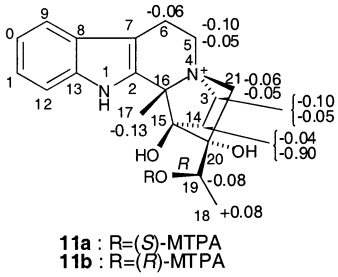


FIGURE 4. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**11a** and **11b**) at C-19 of triol derivative (**10**) of subincanadine A (**1**).

oxidized with OsO_4 to obtain the triol **10** (Scheme 1), of which the structure was elucidated by the spectral data. NOESY correlations of H_3-17 to H-19, H-5b, and H-21b; H-19 to H-21b; and H-3b to H-21a in **10** indicated an α-orientation of the hydroxyl goup at C-20 and an erythro relation between C-19 and C-20 (Figure 3). A modified Mosher's method[8] was applied to elucidate the absolute configuration at C-19. Compound **10** was treated with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) to provide the 19-(*S*)- and -(*R*)-MTPA esters (**11a** and **11b**), respectively. $\Delta\delta$ values ($\delta_S - \delta_R$) obtained from ^{1}H NMR data of **11a** and **11b** are shown in Figure 4, in which the $\Delta\delta$ value for H_3-18 was positive, while negative $\Delta\delta$ values were observed for H_2-3, H_2-5, H_2-6, H_2-14, H_3-17, and H_2-21, thus indicating a 19*R*-configuration. Therefore, the absolute configurations at C-15 and C-16 of subincanadine A (**1**) were assigned as *R* and *S*, respectively.[9]

Subincanadine B (2) showed the molecular ion peak at *m*/*z* 295 (M^+) in the ESIMS. HRESIMS analysis revealed the molecular formula to be $C_{19}H_{23}N_2O$ [*m*/*z* 295.1812 (M^+), Δ −0.4 mmu], which was the same as that

TABLE 2. ^{1}H and ^{13}C NMR Data of Subincanadines B (2) and C (3)

	2[a]		3[b]	
position	^{1}H[c]	^{13}C[c]	^{1}H[c]	^{13}C[c]
1	10.9 (s)		13.33 (s)	
2		131.39		131.32
3a	3.90 (m)	58.67	3.68 (m)	58.81
3b	3.73 (m)		3.51 (m)	
4				
5a	3.82 (m)	46.19	4.01 (dd, 7.3, 11.9)	46.65
5b	3.71 (m)		3.81 (dd, 7.6, 11.9)	
6a	3.11 (m)	17.82	3.15 (m)	18.21
6b	3.11 (m)		3.15 (m)	
7		103.82		103.14
8		125.35		126.63
9	7.43 (d, 7.6)	118.53	7.53 (d, 7.5)	118.75
10	7.02 (dd, 7.8, 7.6)	119.26	7.21 (dd, 7.5, 7.3)	119.99
11	7.11 (dd, 7.8, 7.2)	122.11	7.31 (dd, 7.9, 7.3)	123.04
12	7.44 (d, 7.2)	112.43	7.85 (d, 7.9)	113.11
13		136.81		137.90
14a	2.54 (m)	32.29	1.83 (m)	26.42
14b	2.24 (m)		1.53 (m)	
15		83.74	4.58 (brs)	44.96
16		74.55		78.11
17	1.66 (3H, s)	17.89	1.76 (3H, s)	20.79
18	1.84 (3H, d, 6.9)	11.91	1.67 (3H, d, 6.5)	14.28
19	5.10 (q, 6.9)	116.71	5.41 (q, 6.5)	121.18
20		131.39		133.17
21a	4.19 (d, 14.7)	62.45	4.32 (d, 13.4)	64.21
21b	4.11 (d, 14.7)		4.21 (d, 13.4)	
OH	7.03 (s)			

[a] In DMSO-d_6. [b] In pyridine-d_5. [c] δ in ppm.

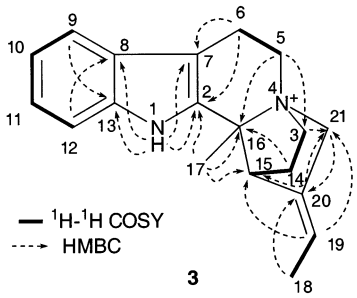


FIGURE 5. Selected 2D NMR correlations for subincanadine C (**3**).

of subincanadine A (**1**). ^{1}H and ^{13}C NMR data (Table 2) of **2** were quite similar to those of **1**. Detailed analysis of 2D NMR data (^{1}H−^{1}H COSY, HMQC, and HMBC) suggested that the gross structure of **2** was the same as that of **1**. An α-orientation of the methyl group at C-16 and an erythro-relationship between C-15 and C-16 were deduced from NOESY correlations of H_3-17 to H-3a, H-5a, and H-14a (Figure 2). A *Z*-configuration of the double bond at C-19 and C-20 was deduced from the NOESY correlations of H-19 to H_2-21 (Figure 2). Thus, subincanadine B (**2**) was assigned as the epimer at C-16 of subincanadine A (**1**).

The molecular formula, $C_{19}H_{23}N_2$, of subincanadine C (**3**) was established by HRESIMS [*m*/*z* 279.1845 (M^+), Δ −1.6 mmu], which corresponded to that of a deoxy form of subincanadine A (**1**). The UV absorption (289 nm) indicated the presence of the indole chromophore. ^{1}H and ^{13}C NMR data (Table 2) and 2D NMR correlations (^{1}H−^{1}H COSY, HMQC, and HMBC) (Figure 5) indicated that **3** was the deoxy form at C-15 of **1**. NOESY correlations of H_3-17 to H-5b and H-21b, and H-5b to H-21b indicated

(7) (a) Fukushi, Y.; Yajima, C.; Mizutani, J. *Tetrahedron Lett*. **1994**, *35*, 9417−9420. (b) The MNCB esters of subincanadine A (**1**) were not obtained, probably due to steric hindrance around the tertiary hydroxy group at C-15.

(8) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(9) (a) The configuration at C-1 of the tetrahydro-β-carboline moiety in many indole alkaloids was discussed on the basis of its CD spectra. Ohmori, O.; Kumazawa, K.; Hohino, H.; Suzuki, T.; Morishima, Y.; Kohno, H.; Kitajima, M.; Kakai, S.; Takayama, H.; Aimi, N. *Tetrahedron Lett*. **1998**, 7737−7740. (b) The configuration at C-16 deduced from the CD spectrum (λ_{ext} nm ($\Delta\epsilon$) in MeOH: 203 (0), 213 (+3.89), 229 (0), 265 (−1.33) 288 (0), 291 (+0.27), 294 (0)) of **1** was coincident with the result obtained from the Mosher's method.

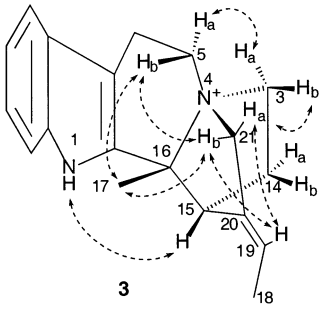


FIGURE 6. Selected NOESY correlations and stereochemistry for subincanadine C (**3**). Dotted arrows denote NOESY correlations.

a β-orientation of the methyl goup at C-16 (Figure 6). A β-orientation of H-15 was deduced from NOESY correlation of H-1 to H-15. The trisubstituted olefin at C-19 and C-20 was elucidated to have the *E*-configuration from NOESY correlations of H-19 to H_2-21.

The molecular formula of subincanadine D (**4**) was revealed to be $C_{19}H_{23}N_2O$ by HRESIMS [*m*/*z* 295.1815 $(M+H)^+$, Δ +0.4 mmu]. UV absorptions (294 and 223 nm) implied the presence of a conjugated indole chromophore. The structure of **4** was deduced from detailed analysis of ^1H and ^{13}C NMR data (Table 3) aided with 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The ^{13}C NMR data indicated that the molecule possessed 12 sp^2 carbons, one sp^3 quaternary carbon, five methylenes, and one methyl group. The ^{13}C NMR chemical shifts of C-3 (δ 45.17), C-5 (δ 56.62), and C-21 (δ 53.06) suggested that these carbones were attached to nitrogen. The chemical shift (δ_C 71.56) of C-15 indicated that oxygen was connected to C-15. $^1H-^1H$ COSY correlations (Figure 7) revealed connectivities of C-3 to C-14, C-5 to C-6, and C-9−C-12. HMBC correlations (Figure 7) of H-1 to C-2, C-7, C-8, and C-13; H-9 to C-13 and C-7; and H-12 to C-8 revealed the presence of an indole ring (C-2, C-7−C-13, and N-1). The presence of a 1-azacyclononane ring (C-5−C-7, C-2, C-15, C-16, C-20, C-21, and N-4) with a hydroxyl group at C-15, an exo-methylene (C-17) at C-16, and an ethynyl group (C-18−C-20) at C-20 was deduced from HMBC correlations of H-3a to C-15, H-6b to C-2 and C-7, H-5b to C-21, H-21b to C-15 and C-19, H_2-17 to C-2 and C-15, H_3-18 to C-20, and H-19 to C-15. The HMBC correlation of H-21b to C-3 implied the presence of a piperidine ring (C-3, C-14, C-15, C-20, C-21, and N-4). Thus, the structure of subincanadine D was elucidated to be **4**, possessing a 1-azabicyclo[5.2.2]undecane moiety. The geometry of the trisubstituted olefin at C-19 and C-20 was elucidated to be *Z* from the NOESY correlation of H-19 to H-21b.

The molecular formula, $C_{19}H_{23}N_2$, of subincanadine E (**5**) was established by HRESIMS [*m*/*z* 279.1840 $(M+H)^+$, Δ −2.1 mmu], which corresponded to that of a deoxy form of subincanadine D (**4**). ^1H and ^{13}C NMR data (Table 3) of **5** were very close to those of **4**, in which the only different point was the presence of a hydrogen (δ_H 4.19, H-15; δ_C 44.28, C-15) in **5** in place of a hydroxyl group at C-15 (δ 71.56) in **4**. 2D NMR data (Figure 8) revealed that subincanadine E (**5**) was the deoxy form at C-15 of **4**.

The molecular formula, $C_{17}H_{18}N_2O$, of subincanadine F (**6**) was established by HRESIMS [*m*/*z* 267.1498 $(M+H)^+$, Δ +0.1 mmu]. The IR spectrum suggested the presence of amino (3422 cm^{-1}) and α,β-unsaturated ketone (1627 cm^{-1}) functionalities. The UV absorption (223 nm) indicated the presence of α,β-unsaturated ketone. The gross structure of **6** was deduced from detailed analysis of ^1H and ^{13}C NMR data (Table 4) aided with 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The $^1H-^1H$ COSY (Figure 9) spectrum revealed connectivities of C-5 to C-6, C-3 to C-14, C-9−C-12, and C-18 to C-19. HMBC correlations (Figure 9) of H-9 to C-13 (δ 137.53) and H-11 to C-8 (δ 128.70) and the carbon chemical shifts of C-2 (δ 132.26) and C-7 (δ 112.52) suggested the presence of an indole ring (C-2, C-7−C-13, and N-1). Cross-peaks of H-21a to C-3 (δ 51.78), C-15 (δ 189.24), C-19 (δ 144.11), and C-20 (δ 128.32), and H-14 and H-19 to C-15 in the HMBC spectrum implied the presence of a piperidine ring (C-3, C-14, C-15, C-20, C-21, and N-4) with a ketone carbonyl group at C-15 and an ethynyl group (C-18 ∼ C-20) at C-20. The presence of a 1-azacycloheptane ring (C-3, C-14, C-2, C-7, C-6, C-5, and N-4) was deduced from HMBC correlations of H-3a to C-2 (δ 132.26) and C-5 (δ 57.38), and H_2-6 to C-2 and C-7 (δ 112.52). Thus, the structure of subincanadine F was elucidated to be **6**, possessing a 1-azabicyclo[4.3.1]decane moiety. The geometry of the trisubstiduted olefin at C-19 and C-20 was elucidated to be *E* from NOESY correlations of H_3-18 to H_2-21 (Figure 9).

Subincanadines A−C (**1**−**3**) are novel pentacyclic quaternary indole alkaloids from *A. subincanum* (Apocynacea), although some quaternary indole alkaloids such as C-alkaloid-O[10] and ophiorrhizine[11] have been isolated from higher plants of the genera *Strychnos* (Loganiaceae) and *Ophiorrhiza* (Rubiaceae), respectively. A plausible biogenetic path of subincanadines A−F (**1**−**6**) is proposed in Figure 10. A stemmadenine[12]-type alkaloid might be a biogenetic precursor of subincanadines D (**4**) and E (**5**), from which subincanadines A−C (**1**−**3**) could be derived through the C−N bond formation between N-4 and C-16, while the biogenetic precursor of subincanadine F (**6**) and apparicine (**7**) also seem to be stemmadenine-type alkaloid.[13]

Subincanadines E (**5**) and F (**6**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50}, **5**, 0.3 μg/mL; **6**, 2.4 μg/mL) and human epidermoid carcinoma KB cells (IC_{50}, **5**, 4.4 μg/mL; **6**, 4.8 μg/mL) in vitro, while subincanadines A−D (**1**−**4**) did not show such activity (IC_{50} > 10 μg/mL).

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer. The 2.49 and 39.0 ppm resonances of residual DMSO-d_6 and the 7.19 and 135.5 ppm resonances of residual pyridine-d_5 were used as internal references for ^1H and ^{13}C NMR spectra, respectively. For the $^1H-^{15}N$ HMBC experiment, 95% formamide in $CDCl_3$ was used for external reference (δ_N 112.4) of ^{15}N NMR.

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TABLE 3. ^{1}H and ^{13}C NMR Data of Subincanadines D (4) and E (5)

	4[a]		5[b]	
position	^{1}H[c]	^{13}C[c]	^{1}H[c]	^{13}C[c]
1	12.15 (s)			
2		135.48		139.87
3a	3.51 (m)	45.17	3.30 (m)	48.67
3b	2.71 (ddd, 15.8, 7.8, 5.5)		3.10 (m)	
4				
5a	3.79 (d, 13.1)	56.62	3.68 (d, 13.4)	60.07
5b	3.47 (m)		3.39 (d, 13.4)	
6a	4.03 (d, 15.9)	20.44	3.88 (d, 14.5)	22.69
6b	3.12 (d, 15.9)		3.12 (dd, 1.4, 14.5)	
7		108.14		110.15
8		127.82		130.87
9	7.62 (d, 7.8)	111.31	7.48 (d, 8.0)	120.06
10	7.29 (dd, 7.8, 7.2)	119.11	7.05 (dd, 8.0, 7.4)	121.72
11	7.36 (dd, 7.9, 7.2)	122.76	7.14 (dd, 8.0, 7.4)	125.17
12	7.60 (d, 7.9)	118.32	7.36 (d, 8.0)	116.25
13		135.98		139.92
14a	2.59 (dd, 14.3, 5.4)	36.42	2.41 (ddt, 7.2, 7.2, 14.4)	28.31
14b	2.00 (dd, 14.3, 5.4)		1.82 (m)	
15		71.56	4.19 (brs)	44.28
16		145.48		144.75
17a	6.59 (s)	117.81	5.59 (s)	123.15
17b	5.92 (s)		5.56 (s)	
18	2.06 (3H, d, 6.3)	14.27	1.83 (3H, d, 6.8)	16.04
19	5.97 (q, 6.3)	131.23	6.08 (q, 6.8)	131.15
20		135.68		133.49
21a	4.62 (d, 15.8)	53.06	4.24 (d, 15.0)	55.72
21b	3.95 (d, 15.8)		3.96 (d, 15.0)	

[a] In pyridine-d_5. [b] In CD_3OD. [c] δ in ppm.

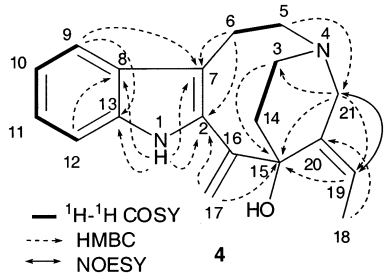

FIGURE 7. Selected 2D NMR correlations for subincanadine D (**4**).

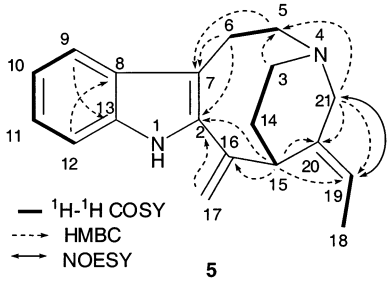

FIGURE 8. Selected 2D NMR correlation for subincanadine E (**5**).

^{1}H−^{15}N HMBC spectra were measured using 50 ms delay for long-range N−H coupling.

Plant Material. The barks of *A. subincanum* Mart ("Paupereira-do-mato", Apocynaceae) were purchased in São Paulo, Brazil, in March 2000. The plant was identified by Dr. G. Hashimoto (Centro de Pesquisas de História Natural, São Paulo, Brasil), and a voucher specimen has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

TABLE 4. ^{1}H and ^{13}C NMR Data of Subincanadine F (6) in CD_3OD

position	^{1}H[a]	^{13}C[a]	H coupled with C[b]
1			
2		132.26	H-3a, H-3b, H-6a, H-6b, H-14
3a	4.22 (dd, 4.8, 17.6)	51.78	H-14, H-21a, H-21b
3b	4.13 (m)		
4			
5a	3.83 (m)	57.38	H-3a, H-6a, H-6b, H-21a, H-21b
5b	3.65 (d, 13.4)		
6a	3.24 (dd, 6.4, 18.0)	20.64	
6b	3.21 (dd, 6.4, 18.0)		
7		112.52	H-5a, H-5b, H-6a, H-6b, H-14
8		128.70	H-10, H-12
9	7.43 (d, 7.9)	118.72	H-11
10	7.01 (dd, 7.9, 7.5)	120.41	H-12
11	7.10 (dd, 8.1, 7.5)	123.28	H-9
12	7.30 (d, 8.1)	112.17	H-10
13		137.53	H-9, H-11
14	4.11 (t, 4.8)	45.43	
15		189.24	H-3, H-14, H-19, H-21a
18	1.89 (d, 7.1)	13.92	
19	7.04 (q, 7.1)	144.11	H-18, H-21a, H-21b
20		128.32	H-18, H-21a, H-21b
21a	4.60 (d, 15.6)	51.68	
21b	4.40 (d, 15.6)		

[a] δ in ppm. [b] HMBC correlations.

Extraction and Separation. The barks of *A. subincanum* (100 g) were extracted with MeOH (300 mL × 3). The MeOH extracts (20 g) were partitioned between hexane (250 mL × 3) and 90% MeOH (250 mL). The aqueous MeOH layer was partitioned between EtOAc (250 mL × 3) and 1 M NaCl (250 mL), and then the aqueous layer was extracted with *n*-BuOH (250 mL × 3). A portion (1.0 g) of the *n*-BuOH-soluble materials (12 g) was subjected to silica gel columns ($CHCl_3$−

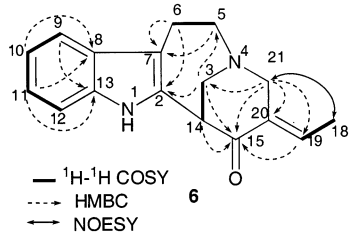

FIGURE 9. Selected 2D NMR correlations for subincanadine F (**6**).

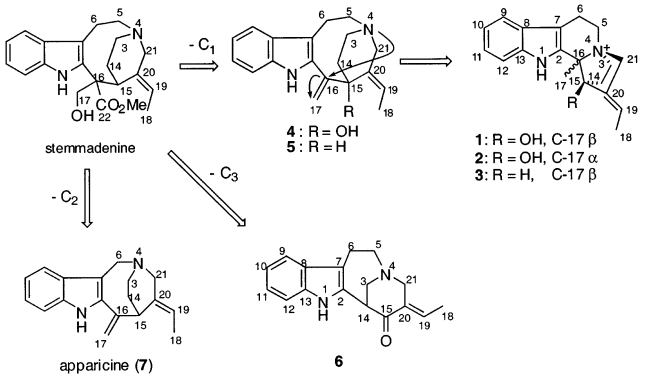

FIGURE 10. Plausible biogenetic path of subincanadines A−F (**1**−**6**).

n-BuOH−AcOH−H_2O, 1.5:6:1:1 and then $CHCl_3$−MeOH, 4:1) followed by C_{18} HPLC (1 × 25 cm; flow rate, 2.5 mL/min; eluent, CH_3CN-H_2O, 36:64 containing 0.1% TFA; UV detection at 281 mm) to afford subincanadines A (**1**, t_R 10.4 min, 13.7 mg, 0.014%), B (**2**, t_R 13.6 min, 2.0 mg, 0.002%), and C (**3**, t_R 16.0 min, 2.3 mg, 0.002%). The EtOAc-soluble portions (1.6 g) were subjected to silica gel columns ($CHCl_3$−MeOH, 98:2 and then $CHCl_3$−*n*-BuOH−AcOH−H_2O, 1.5:6:1:1) followed by C_{18} HPLC (flow rate, 1.0 mL/min; CH_3CN-H_2O, 40:60 containing 0.1% TFA; UV detection at 281 mm) to afford subincanadines D (**4**, t_R 12.8 min, 2.0 mg, 0.002%), E (**5**, t_R 14.8 min, 2.5 mg, 0.003%), and F (**6**, t_R 8.8 min, 2.2 mg, 0.002%), and apparicine (**7**, t_R 12.3 min, 1.1 mg, 0.001%).

Subincanadine A (1): a colorless amorphous solid; $[\alpha]^{23}_D$ −11° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 289 (3.50), 279 (3.64), 271 (3.67), and 225 (4.12) nm; IR (KBr) ν_{max} 3430 and 2925 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m/z* 295 (M^+); HRESIMS *m/z* 295.1813 (M^+) (calcd for $C_{19}H_{23}N_2O$, 295.1816).

Subincanadine B (2): a colorless amorphous solid; $[\alpha]^{23}_D$ +41° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 289 (3.54), 279 (3.64), 273 (3.64), and 223 (4.23) nm; IR (KBr) ν_{max} 3424 and 2925 cm^{-1}; ^1H and ^{13}C NMR (Table 2); ESIMS *m/z* 295 (M^+); HRESIMS *m/z* 295.1812 (M^+) (calcd for $C_{19}H_{23}N_2O$, 295.1816).

Subincanadine C (3): a colorless amorphous solid; $[\alpha]^{23}_D$ +5.0° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 289 (3.45), 279 (3.60), 271 (3.63), and 225 (4.09) nm; IR (KBr) ν_{max} 3430 and 2925 cm^{-1}; ^1H and ^{13}C NMR (Table 2); ESIMS *m/z* 279 (M^+); HRESIMS *m/z* 279.1845 (M^+) (calcd for $C_{19}H_{23}N_2$, 279.1861).

Subincanadine D (4): a colorless amorphous solid; $[\alpha]^{23}_D$ −3.3° (*c* 1. 0, MeOH); UV (MeOH) λ_{max} (log ϵ) 294 (3.58), and 223 (4.00) nm; IR (KBr) ν_{max} 3424 and 2925 cm^{-1}; ^1H and ^{13}C NMR (Table 3); ESIMS *m/z* 295 (M + H)$^+$; HRESIMS *m/z* 295.1815 (M + H)$^+$ (calcd for $C_{19}H_{23}N_2O$, 295.1811).

Subincanadine E (5): a colorless amorphous solid; $[\alpha]^{23}_D$ +39° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 301 (3.80) and 226 (4.01) nm; IR (KBr) ν_{max} 3413 and 2926 cm^{-1}; ^1H and ^{13}C NMR (Table 3); ESIMS *m/z* 279 (M + H)$^+$; HRESIMS *m/z* 279.1840 (M + H)$^+$ (calcd for $C_{19}H_{23}N_2$, 279.1861).

Subincanadine F (6): a yellow amorphous solid; $[\alpha]^{23}_D$ +17.8° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 356 (sh 2.87), 274 (3.50), and 223 (4.12) nm; IR (KBr) ν_{max} 3422, 2925 and 1627 cm^{-1}; ^1H and ^{13}C NMR (Table 4); ESIMS *m/z* 267 (M + H)$^+$; HRESIMS *m/z* 267.1498 (M + H)$^+$ (calcd for $C_{17}H_{19}N_2O$, 267.1497).

Birch Reduction of Subincanadine A (1). A solution of **1** (9.6 mg, 33 μmol) and *t*-BuOH (60 μL) in THF (2 mL) was added to a solution of lithium (81 mg, 3.5 mmol) in liquid ammonia (25 mL). The mixture was stirred at −78 °C for 1 h, and then the mixture was allowed to warm to 0 °C over 2 h under a stream of argon. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with *n*-BuOH. The organic layer was concentrated and purified by silica gel column chromatography ($CHCl_3$−*n*-BuOH−AcOH−H_2O, 1.5:6:1:1) followed by reversed-phase C_{18} HPLC (1 × 25 cm; flow rate, 2.5 mL/min; eluent, CH_3CN-H_2O, 48:52 containing 0.15% TFA; UV detection at 281 mm) to give compoounds **8** (t_R 24 min, 1.6 mg) and **9** (t_R 25.6 min, 1.1 mg). **8**: colorless amorphous solid; $[\alpha]^{23}_D$ −57° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 290 (3.44), 283 (3.47), and 225 (4.07) nm; IR (KBr) ν_{max} 3415 and 2925 cm^{-1};^1H NMR (pyridine-d_5) δ 1.73 (3H, d, *J* = 7.5 Hz, H-17), 2.05 (1H, m, H-14b), 2.16 (3H, d, *J* = 6.8 Hz, H-18), 2.38 (1H, m, H-14a), 2.39 (1H, m, H-3b), 3.15 (1H, m, H-6b), 3.38 (1H, m, H-3a), 3.41 (1H, m, H-5b), 3.55 (1H, m, H-6a), 3.61 (1H, m, H-5a), 3.81 (1H, d, *J* = 14.2 Hz, H-21b), 3.83 (1H, q, 7.5, H-16), 4.52 (1H, d, *J* = 14.2 Hz, H-21a), 5.81 (1H, d, *J* = 6.8 Hz, H-19), 7.26 (1H, t, *J* = 7.4 Hz, H-10), 7.30 (1H, dd, *J* = 7.4 and 6.8 Hz, H-11), 7.56 (1H, d, *J* = 6.8 Hz, H-12), 7.59 (1H, d, *J* = 7.4 Hz, H-9), and 11.98 (1H, m, H-1); EIMS *m/z* 296 (M^+); HREIMS *m/z* 296.1893 (M^+) (calcd for $C_{19}H_{24}N_2O$, 296.1889). **9**: colorless amorphous solid; $[\alpha]^{23}_D$ −36° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 290 (3.39), 283 (3.43), and 226 (4.05) nm; IR (KBr) ν_{max} 3430 and 2925 cm^{-1}; ^1H NMR (CD_3OD) δ 1.63 (1H, m, H-3b), 1.65 (3H, d, *J* = 7.4 Hz, H-17), 1.79 (1H, m, H-14b), 1.92 (1H, m, H-14a), 2.09 (3H, dd, *J* = 7.1 and 2.1 Hz, H-18), 3.08 (1H, m, H-3a), 3.21 (1H, m, H-6b), 3.38 (1H, m, H-5b), 3.56 (1H, m, H-5a), 3.79 (1H, m, H-6a), 3.83 (1H, d, *J* = 15.6 Hz, H-21b), 4.05 (1H, q, *J* = 7.4 Hz, H-16), 4.19 (1H, d, *J* = 15.6 Hz, H-21a), 6.05 (1H, d, *J* = 7.1 Hz, H-19), 7.04 (1H, dd, *J* = 7.8 and 7.1 Hz, H-10), 7.11 (1H, dd, *J* = 8.1 and 7.1 Hz, H-11), 7.41 (1H, d, *J* = 8.1 Hz, H-12), and 7.45 (1H, d, *J* = 7.8 Hz, H-9); EIMS *m/z* 296 (M^+); HREIMS *m/z* 296.1894 (M^+) (calcd for $C_{19}H_{24}N_2O$, 296.1889)

Osmium Oxidation of Subincanadine A (1). To a solution of **1** (0.9 mg, 3 μmol) in pyridine was added OsO_4 (21 μL, 3.2 μmol, 4% in *t*-BuOH) at 0 °C. The mixture was stirred at 0 °C for 12 h and then the reaction was quenched by addition of aqueous $Na_2S_2O_3$. The mixture was extracted with *n*-BuOH and concentrated in vacuo. The residue was purified by C_{18} HPLC (1 × 25 cm; flow rate, 2.5 mL/min; eluent, CH_3CN-H_2O, 34:66 containing 0.15% TFA; UV detection at 281 mm) to afford compound **10** (t_R 9.6 min, 0.8 mg): colorless oil; $[\alpha]^{17}_D$ +11.5°(*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 328 (3.10), 289 (3.27), 282 (3.30), and 221 (3.90) nm; IR (KBr) ν_{max} 3430 and 2925 cm^{-1}; ^1H NMR (CD_3OD) δ 1.40 (3H, d, *J* = 6.0 Hz, H-18), 1.83 (3H, s, H-17), 2.00 (1H, m, H-14b), 2.59 (1H, m, H-14a), 2.66 (1H, m, H-3b), 3.26 (1H, d, *J* = 15.9 Hz, H-6b), 3.47 (1H, d, *J* = 16.1, H-21b), 3.69 (1H, m, H-5b), 3.82 (1H, m, H-5a), 3.82 (1H, d, *J* = 15.9 Hz, H-6a), 3.90 (1H, m, H-3a), 4.22 (1H, d, *J* = 16.1 Hz, H-21a), 4.34 (1H, d, *J* = 6.0 Hz, H-19), 7.08 (1H, t, *J* = 7.4 Hz, H-10), 7.17 (1H, dd, *J* = 7.4 and 6.8 Hz, H-11), 7.42 (1H, d, *J* = 6.8 Hz, H-12), and 7.48 (1H, d, *J* = 7.4 Hz, H-9); FABMS *m/z* 329 (M^+); HRFABMS *m/z* 329.1851 (M^+) (calcd for $C_{19}H_{25}N_2O_3$,329.1865).

(*S*)-MTPA Ester (11a) of 10. To a solution of compound **10** (0.15 mg, 0.46 nnmol) in CH_2Cl_2 were added 4-(dimethylamino)pyridine (20 μg), triethylamine (1 μL), and (*R*)-(−)-MTPACl (0.5 μL) at room temperature, and stirring was contimued for 15 min. *N*,*N*-Dimethyl-1,3-propanediamine (1 μL) was added, and the reaction mixture was stirred for 15 min. The mixture was concentrated, diluted with H_2O (100 μL), extracted with EtOAc (100 μL × 3), and evaporated in vacuo. The residue was purified by C_{18} HPLC (1 × 25 cm; flow

rate, 2.5 mL/min; eluent, CH_3CN-H_2O, 67:33 containing 0.15% TFA; UV detection at 281 mm) to afford compound **11a** (t_R 9.6 min, 0.12 mg, 0.22 nmol, 48%): colorless oil; ^1H NMR ($CDCl_3$) 1.40 (1H, m, H-14a), 1.71 (3H, J = 6.3 Hz, H_3-18), 1.99 (3H, s, H_3-17), 3.10 (1H, m, H-14b), 3.21 (2H, m, H_2-6), 3.45 (3H, m, H-3a, H-5a, H-21a), 3.46 (3H, s, MeO), 3.53 (2H, m, H-3b, H-5b), 3.68 (1H, m, H-21b), 5.70 (1H, m, H-19), 7.15 (1H, t, J = 6.9 Hz, H-10), 7.27 (1H, t, J = 6.9 Hz, H-11), 7.43 (4H, t, m, Ph, H-12), 7.46 (1H, m, H-9), 7.59 (2H, m, Ph), and 9.10 (1H, m, NH); ESIMS m/z 545 (M^+); HRESIMS m/z 545. 2253 (M^+) (calcd for $C_{29}H_{32}N_2O_3F_3$, 545.2263).

***(R)*-MTPA Ester (11b) of 10.** To a solution of compound **10** (0.3 mg, 0.91 nnmol) in pyridine were added 4-(dimethylamino)pyridine (0.5 mg), triethylamine (2 μL), and (*S*)-(+)-MTPACl (1 μL) at room temperature, and stirring was continued for 2 h at 40 °C. *N*,*N*-Dimethyl-1,3-propanediamine (2 μL) was added at room temperature, and the reaction mixture was stirred for 15 min. The mixture was concentrated, diluted with H_2O (100 μL), extracted with EtOAc (100 μL × 3), and evaporated in vacuo. The residue was purified by C_{18} HPLC (1 × 25 cm; flow rate, 2.5 mL/min; eluent, CH_3CN-H_2O, 65:35 containing 0.15% TFA; UV detection at 281 mm) to afford compound **11b** (t_R 10 min, 0.15 mg, 0.28 nmol, 31%): colorless oil; ^1H NMR ($CDCl_3$) 1.44 (1H, m, H-14a), 1.63 (3H, J = 5.3 Hz, H_3-18), 2.12 (3H, s, H_3-17), 3.19 (1H, m, H-14b), 3.27 (2H, m, H_2-6), 3.50 (3H, m, H-3a, H-5a, H-21a), 3.53 (3H, s, MeO), 3.63 (2H, m, H-3b, H-5b), 3.74 (1H, m, H-21b), 5.78 (1H, m, H-19), 7.15 (1H, t, J = 6.9 Hz, H-10), 7.26 (1H, t, J = 6.9 Hz, H-11), 7.42 (4H, t, m, Ph, H-12), 7.46 (1H, m, H-9), 7.54 (2H, m, Ph), and 9.18 (1H, m, NH); ESIMS m/z 545 (M^+); HRESIMS m/z 545. 2265 (M^+) (calcd for $C_{29}H_{32}N_2O_5F_3$, 545.2263).

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Supporting Information Available: 1D and 2D NMR spectra for compound **1** and ^1H and ^{13}C NMR spectra for **2**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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