Echinophyllins C−F, New Nitrogen-Containing Clerodane Diterpenoids from *Echinodorus macrophyllus*

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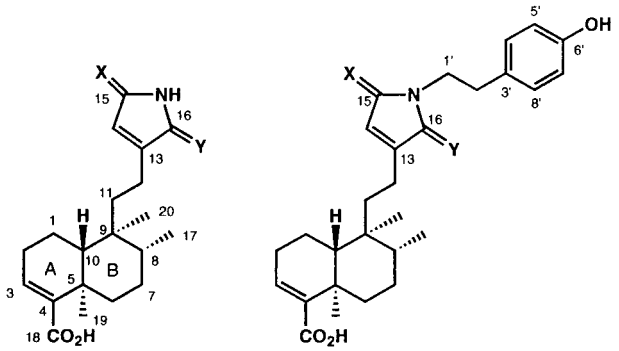
Four new nitrogen-containing clerodane diterpenoids, echinophyllins C−F (**1**−**4**), were isolated from the leaves of the Brazilian medicinal plant *Echinodorus macrophyllus* ("Chapéu-de-couro"), and their structures and relative stereochemistry were elucidated from their spectroscopic data. Compounds **1**−**4** possess an α,β-unsaturated γ-lactam ring consisting of a clerodane diterpene unit and an amine moiety.

In our search for structurally unique compounds from Brazilian medicinal plants,[1] echinophyllins A (**5**) and B (**6**), with an α,β-unsaturated γ-lactam ring consisting of a clerodane skeleton and an amine moiety, have been isolated from the leaves of *Echinodorus macrophyllus*.[2] This plant is known in Brazil as "Chapéu-de-couro" and used to treat difficulties in urination, hepatitis, and rheumatism. Further investigation on extracts of the leaves of this plant have led to the isolation of four new nitrogen-containing diterpenoids with a clerodane skeleton, echinophyllins C−F (**1**−**4**). In this paper we describe the isolation and structure elucidation of **1**−**4**.

The leaves of the Brazilian medicinal plant *Echinodorus macrophyllus* (Kunth) Micheli (Alismataceae) were extracted with MeOH. The MeOH extracts were partitioned between hexane and 90% aqueous MeOH, and then the MeOH-layer was partitioned with EtOAc and H_2O. The H_2O layer was extracted with *n*-BuOH. The EtOAc-soluble portions were subjected to passage over a Si gel column ($CHCl_3$−MeOH, 96:4) and then over a reversed-phase column (MeOH−H_2O, 70:30 → MeOH) to afford an alkaloidal fraction, which was purified by Si gel column chromatography (hexane−acetone, 1:1→1:5) to give echinophyllins A (**5**) and B (**6**) and another alkaloidal fraction. The latter alkaloidal fraction was purified by reversed-phase HPLC (CH_3CN−H_2O, 60:40 or CH_3CN−H_2O, 50:50 containing 0.1% TFA) to afford echinophyllins C (**1**, 0.0085%), D (**2**, 0.0061%), E (**3**, 0.0069%), and F (**4**, 0.0037%), together with known clerodane diterpenes, clerodermic acid[3] (0.0061%) and patagonic acid[4,5] (0.0056%). A further known compound, chichoric acid dimethyl ether,[6] was obtained from the *n*-BuOH-soluble portions.



1 X = H_2, Y = O
4 X = O, Y = H_2

2 X = O, Y = H_2
5 X = H_2, Y = O

3 X = O, Y = H_2
6 X = H_2, Y = O

The molecular formula, $C_{20}H_{29}NO_3$, of echinophyllin C (**1**) was established by HRFABMS [*m*/*z* 332.2238 $(M + H)^+$, Δ +1.2 mmu]. The IR spectrum implied the presence of hydroxy (3423 cm^{-1}) and unsaturated carbonyl (1680 cm^{-1}) groups. The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data (Table 1) aided with 2D NMR experiments (^1H−^1H COSY, HMQC, and HMBC). The ^{13}C NMR data indicated that the molecule possessed one unsaturated carboxylic carbon, one unsaturated lactam carbonyl, two trisubstituted olefins, two sp^3 quaternary carbons, seven methylenes, two methines, and three methyl groups. The ^1H−^1H COSY spectrum revealed connectivities (Figure 1) of C-1 to C-3 and C-10, C-6 to C-8 and C-17, C-11 to C-12, and C-14 to C-15. HMBC correlations (Figure 1) of H_3-19 to C-4, C-5, C-6, and C-10 and of H-10 to C-5 revealed the presence of a cyclohexene ring (ring A), in which a carboxyl group and Me-19 were attached to C-4 and C-5, respectively. The presence of a cyclohexane ring (ring B) with Me-17 at C-8 and Me-20 at C-9 was elucidated by HMBC correlations of H_3-20 to C-8, C-9, and C-10; of H_3-17 to C-9; and of H-10 to C-9. The HMBC correlation of H_3-20 to C-11 allowed the connectivity between C-9 and C-11 to be established. Cross-peaks of H-14 to C-16 (δ_C 177.7), H_2-15 (δ_H 3.91, br s) to C-13 (δ_C 147.4) and C-16, and H_2-12 to C-13 and the chemical shift of C-15 (δ_C 48.6) revealed the presence of an α,β-unsaturated γ-lactam ring

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Table 1. ^1H and ^{13}C NMR Data of Echinophyllins C (**1**), D (**2**), E (**3**), and F (**4**) in CD_3OD

	1		**2**		**3**		**4**	
position	$^1H^a$	$^{13}C^a$	$^1H^a$	$^{13}C^a$	$^1H^a$	$^{13}C^a$	$^1H^a$	$^{13}C^a$
1 (a)	1.49 (m)	19.5	1.63 (m)	18.7	1.63 (m)	19.3	1.55 (m)	18.7
(b)			2.26 (m)					
2	2.23 (m)	28.7	2.17 (m)	28.5	2.25 (m)	28.8	2.28 (m)	28.5
3	6.43 (brs)	134.9	6.43 (brs)	130.5	6.70 (brs)	139.1	6.50 (brs)	136.5
4		141.7		148.0		142.9		145.0
5		39.4		37.7		38.3		37.6
6 (a)	2.93 (dd, 3.0, 10.0)	38.5	2.28 (m)	37.6	2.41 (m)	37.9	2.40 (m)	37.5
(b)	1.20 (m)		1.20 (m)		1.15 (m)		1.21 (m)	
7 (a)	1.51 (m)	28.0	1.47 (m)	27.7	1.47 (m)	28.8	1.56 (m)	28.0
(b)	1.41 (m)		1.43 (m)		1.45 (m)		1.45 (m)	
8	1.59 (m)	38.2	1.49 (m)	37.4	1.55 (m)	35.4	1.59 (m)	37.2
9		40.7		39.9		38.4		40.0
10	1.32 (m)	48.8	1.39 (m)	47.7	1.38 (m)	48.9	1.40 (m)	48.1
11 (a)	1.66 (m)	38.4	1.59 (m)	38.5	1.78 (m)	38.2	1.73 (m)	38.7
(b)			1.32 (m)		1.50 (m)		1.60 (m)	
12 (a)	2.17 (m)	20.7	2.07 (m)	21.4	2.28 (m)	21.9	2.38 (m)	24.1
(b)	2.07 (m)		2.03 (m)		2.24 (m)		2.27 (m)	
13		147.4		148.0		142.8		146.0
14	6.87 (s)	140.4	5.77 (s)	121.1	5.85 (s)	122.0	5.72 (s)	121.0
15	3.91 (brs)	48.6		174.2		172.1		177.6
16		177.7	3.78 (s)	56.8	3.87 (s)	49.8	3.91 (s)	51.9
17	0.84 (m)	17.2	0.81 (m)	16.4	0.88 (d, 6.0)	17.0	0.75 (d, 6.0)	16.4
18		174.9		173.0		172.1		171.8
19	1.28 (s)	22.1	1.29 (s)	23.9	1.30 (s)	19.3	1.18 (s)	21.2
20	0.78 (s)	19.5	0.78 (s)	20.0	0.83 (s)	17.1	0.72 (s)	18.8
1′			3.60 (t, 7.1)	45.1	3.60 (m)	40.9		
2′			2.71 (t, 7.1)	34.9	1.65 (m)	29.2		
3′				130.8	1.65 (m)	29.2		
4′			6.56 (d, 8.3)	116.2	3.47 (m)	38.4		
5′			7.00 (d, 8.3)	130.7		165.1		
6′				157.3	6.48 (d, 15.0)	117.3		
7′			7.00 (d, 8.3)	130.7	7.49 (d, 15.0)	140.3		
8′			6.56 (d, 8.3)	116.2		125.5		
9′					7.16 (s)	112.4		
10′						145.3		
11′						146.3		
12′					6.84 (d, 7.0)	112.4		
13′					7.07 (d, 7.0)	122.0		
MeO-10′					3.93 (s)	57.2		

[a] δ in ppm.

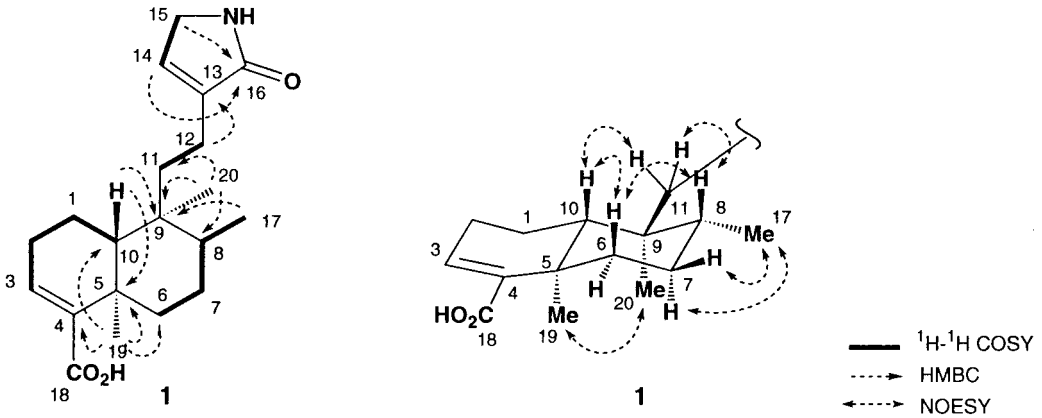

Figure 1. Selected $^1H-^1H$ COSY, HMBC, and NOESY correlations for echinophyllin C (**1**).

(C-13 to C-15, C-16, and N-15) connected between C-12 and C-13. Thus, the planar structure of echinophyllin C could be determined. NOESY correlations (Figure 1) of H_3-19 to H_3-20, H_3-17 to H-7a and H-7b, H-6b to H-8 and H-10, H-8 to H-11b, and H-10 to H-11a indicated a chair conformation of ring B, a trans relationship between rings A and B, α-orientations of Me-17, Me-19, and Me-20, and a *β*-orientation of H-10. Therefore, the relative stereochemistry of echinophyllin C was assigned as **1**.

Echinophyllin D (**2**) showed a pseudomolecular ion peak at *m/z* 452 $(M + H)^+$ in the FABMS. HRFABMS analysis revealed the molecular formula to be $C_{28}H_{37}NO_4$ [*m/z* 452.2770 $(M + H)^+$, Δ −3.1 mmu], which was the same as that of echinophyllin A (**5**). The ^1H and ^{13}C NMR spectra of **2** were very similar to those of echinophyllin A (**5**) except for an α,*β*-unsaturated *γ*-lactam ring (C-13−C-15, C-16, and N-15). The $^1H-^1H$ COSY spectrum revealed connectivities of C-1 to C-3 and C-10, C-6 to C-8 and C-17, C-11 to C-12, C-1′ to C-2′, C-4′ to C-5′, and C-7′ to C-8′ (Figure 2). Cross-peaks of H_2-16 to C-13 (δ_C 148.0) and C-15 (δ_C 174.2), H-14 to C-15 and the chemical shift of C-16 (δ_C 56.8) indicated a *γ*-lactam ring (C-13 to C-15, C-16, and N-15) to be present. Proton signals at δ_H 3.60 (2H, t, J = 7.1 Hz, H_2-1′), 2.71 (2H, t, J = 7.1 Hz, H_2-2′), 6.56 (2H, d, J = 8.3

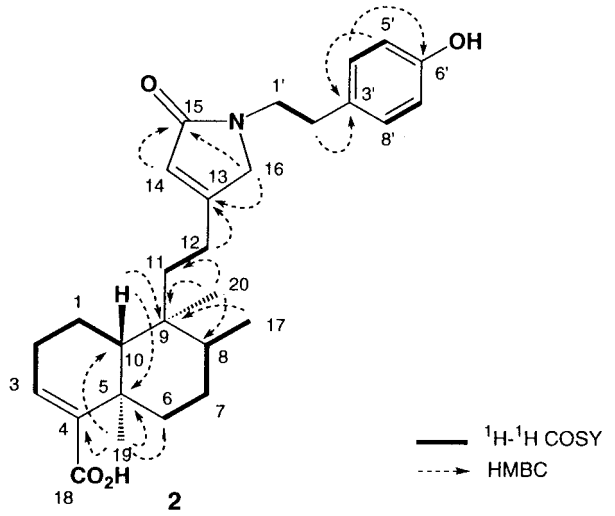

Figure 2. Selected $^1H-^1H$ COSY and HMBC correlations for echinophyllin D (**2**).

Hz, H-4′ and H-8′), and 7.00 (2H, d, J = 8.3 Hz, H-5′ and H-7′), a phenol carbon signal at δ_C 157.3 (C-6′), and HMBC correlations of H_2-2′ to C-3′, H-5′ to C-3′, and H-4′ to C-6′ revealed the presence of a tyramine moiety (C-1′−C-8′, N-15, and 6′-OH). A NOESY experiment, performed on echinophyllin D in the same manner as described for **1**, enabled the relative stereochemistry of this compound was assigned as **2**.

Echinophyllin E (**3**) showed the pseudomolecular ion peak at m/z 579 $(M + H)^+$ in the FABMS. HRFABMS analysis revealed the molecular formula to be $C_{34}H_{46}N_2O_6$ [m/z 579.3413 $(M + H)^+$, Δ −3.6 mmu]. The ^1H and ^{13}C NMR spectra of **3** were very similar to those of echinophyllin B (**6**) except for an α,β-unsaturated γ-lactam ring (C-13−C-15, C-16, and N-15). $^1H-^1H$ COSY connectivities of C-1′ to C-4′ and N-4′ and proton signals at δ_H 3.93 (3H, s, MeO-10′), 6.84 (1H, d, J = 7.0 Hz, H-12′), 7.16 (1H, s, H-9′), 7.07 (1H, d, J = 7.0 Hz, H-13′), 6.48 (1H, d, J = 15.0 Hz, H-6′), and 7.49 (1H, d, J = 15.0 Hz, H-7′; trans-oriented) revealed the presence of an *N*-feruloylputrescine moiety. Proton signals at δ_H 5.85 (1H, s, H-14) and 3.87 (2H, s, H_2-16) and carbon signals at δ_C 142.8 (C-13), 122.0 (C-14), 172.1 (C-15), and 49.8 (C-16) indicated the presence of β-substituted α,β-unsaturated γ-lactam ring (C-13 to C-15, C-16, and N-15). Thus, the structure of echinophyllin E was elucidated as **3**. The relative stereochemistry of the clerodane diterpene moiety of **3** was assigned in the same manner as echinophyllin B (**6**) by NOESY correlations.

The molecular formula, $C_{20}H_{29}NO_3$, of echinophyllin F (**4**) was established by HRFABMS [m/z 332.2217 $(M + H)^+$, Δ −0.9 mmu], which was the same as that of echinophyllin C (**1**). The IR spectrum implied the presence of hydroxy group (3423 cm^{-1}) and α,β-unsaturated γ-lactam ring (1678 cm^{-1}). The ^1H and ^{13}C NMR spectra of **4** were very similar to those of echinophyllin C (**1**) except for the signals of the α,β-unsaturated γ-lactam ring (C-13−C-15, C-16, and N-15). Proton signals at δ_H 5.72 (1H, s, H-14) and 3.91 (2H, s, H_2-16), carbon signals at δ_C 146.0 (C-13), 121.0 (C-14), 177.6 (C-15), and 51.9 (C-16); and HMBC correlations of H-14 to C-12, C-13, C-15, and C-16 and H_2-16 to C-13, C-14, and C-15 indicated the presence of β-substituted α,β-unsaturated γ-lactam ring (C-13 to C-15, C-16, and N-15) connected between C-12 and C-13 (see Figure 3). Thus, the structure of echinophyllin F was elucidated as **4**, with the relative stereochemistry determined from NOESY correlations, which were almost the same as those observed for echinophyllin C (**1**).

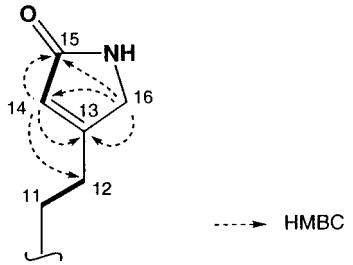

Figure 3. Selected HMBC correlations for echinophyllin F (**4**).

Echinophyllins C−F (**1**−**4**) are new nitrogen-containing diterpenoids with an α,β-unsaturated γ-lactam ring consisting of a clerodane skeleton and an amine moiety, which have been isolated from the Brazilian medicinal plant *E. macrophyllus*. Biogenetically, echinophyllins C (**1**) and F (**4**) may be derived from patagonic acid[4,5] or clerodermic acid[3], respectively, and ammonia, while echinophyllins D (**2**) and E (**3**) may be generated from the clerodermic acid[3] and a tyramine or *N*-feruloylputrescine, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were determined on JASCO P-1030 polarimeter. UV and IR spectra were obtained on JASCO Ubest-35 and JASCO FT/IR-230 spectrometers, respectively. The 3.35 and 49.8 ppm resonances of residual MeOH-d_4 were used as internal references for ^1H and ^{13}C NMR spectra, respectively. FABMS were obtained using glycerol as a matrix.

Plant Material. The leaves of *E. macrophyllus* ("Chapéu-de-couro") were purchased in São Paulo, Brazil, in October 1998. The plant was identified by Dr. T. Nakasumi (Instituto de Pesouisas de Plantas Medicinais do Brasil), and a voucher specimen has been deposited at Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Separation. The leaves (100 g) were extracted with MeOH (500 mL × 3), and the extracts were partitioned between hexane (50 mL × 3) and 90% aqueous MeOH (50 mL). The MeOH layer was partitioned with EtOAc (50 mL × 3) and H_2O (50 mL), and then the H_2O layer was extracted with *n*-BuOH. The EtOAc-soluble portions (1.0 g) were subjected to a Si gel column chromatography ($CHCl_3$−MeOH, 96:4) to afford fractions **a** (188 mg) and **b** (280 mg). Fraction **a** was separated by C_{18} column chromatography (MeOH−H_2O, 80:20) and by C_{18} reversed-phase HPLC (Develosil ODS−HG-5, Nomura Co. Ltd, 1.0 × 25 cm: flow rate 2.5 mL/min; UV detection at 210 nm; eluent MeOH−H_2O, 70:30) to afford clerodermic acid[3] (2.8 mg) and patagonic acid[4,5] (1.1 mg). Fraction **b** was subjected to passage over a C_{18} column (MeOH−H_2O, 70:30→MeOH) to give an alkaloidal fraction (25.3 mg), which was purified by Si gel column chromatography (hexane−acetone, 1:1→1:5) to yield echinophyllins A[2] (**5**, 7.3 mg) and B[2] (**6**, 4.1 mg) and fractions **c** (13 mg) and **d** (17 mg). Fraction **c** was purified by a reversed-phase HPLC (Develosil ODS−HG-5, Nomura Co. Ltd.; 10 × 250 mm, flow rate 2.5 mL/min, eluent CH_3CN-H_2O, 60:40, UV detection at 254 nm) to afford echinophyllins C (**1**, 2.1 mg, t_R 8.0 min), D (**2**, 1.5 mg, t_R 10.0 min), and F (**3**, 1.9 mg, t_R 7.4 min), while fraction **d** was purified by a reversed-phase HPLC (Mightysil RP_{18}, Kanto Chemical Co. Inc.; 10 × 250 mm, flow rate 2.5 mL/min, eluent CH_3CN-H_2O containing 0.1% TFA, 50:50, UV detection at 254 nm) to afford echinophyllin E (**3**, 1.7 mg, t_R 14.4 min). A part (80 mg) of the *n*-BuOH-soluble portion (1.56 g) was subjected to passage over a C_{18} column (MeOH−H_2O, 50:50) to afford chichoric acid dimethyl ether[6] (12 mg).

Echinophyllin C (1): colorless amorphous solid; $[\alpha]^{23}_D$ −25.9° (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (3.85) nm; IR (KBr) ν_{max} 3423 and 1680 cm^{-1}; ^1H and ^{13}C NMR (Table 1);

FABMS *m/z* 332 $[M + H]^+$; HRFABMS *m/z* 332.2238 $[M + H]^+$ (calcd for $C_{20}H_{30}NO_3$, 332.2226).

Echinophyllin D (2): colorless amorphous solid; $[\alpha]^{23}_D$ −23.6° (*c* 0.30, EtOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.03) and 280 (3.20) nm; IR (KBr) ν_{max} 3430 and 1659 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 452 $[M + H]^+$; HRFABMS *m/z* 452.2770 $[M + H]^+$ (calcd for $C_{28}H_{38}NO_4$, 452.2801).

Echinophyllin E (3): colorless amorphous solid; $[\alpha]^{23}_D$ −62.7° (*c* 0.30, EtOH); UV (EtOH) λ_{max} (log ϵ) 210 (4.02), 291 (3.45), and 316 (3.45) nm; IR (KBr) ν_{max} 3427, 1703, and 1633 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 579 $[M + H]^+$; HRFABMS *m/z* 579.3413 $[M + H]^+$ (calcd for $C_{34}H_{47}N_2O_6$, 579.3449).

Echinophyllin F (4): colorless amorphous solid; $[\alpha]^{23}_D$ −34.5° (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.01) nm; IR (KBr) ν_{max} 3423 and 1678 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 332 $[M + H]^+$; HRFABMS *m/z* 332.2217 $[M + H]^+$ (calcd for $C_{20}H_{30}NO_3$, 332.2226).

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