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Echinophyllins A and B, novel nitrogen-containing clerodane diterpenoids from *Echinodorus macrophyllus*

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Abstract

Two novel nitrogen-containing clerodane diterpenoids, echinophyllins A (**1**) and B (**2**), were isolated from the leaves of the Brazilian medicinal plant *Echinodorus macrophyllus* ('Chapéu-de-couro'), and their structures and relative stereochemistry were elucidated by spectroscopic data. Echinophyllins A (**1**) and B (**2**) possess a unique α,β-unsaturated γ-lactam ring consisting of a clerodane diterpene and a tyramine unit or *N*-feruloyl putrescine unit, respectively.

Keywords: clerodane diterpenoids; plants; natural products; nitrogenous metabolites.

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 bioactive compounds from Brazilian medicinal plants, a new *seco*-labdane-type diterpenoid, chapecoderin A, and two new rearranged labdane-type diterpenoids, chapecoderins B and C, with an α,β-unsaturated γ-lactone ring in the side chain, 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 the extracts of leaves from another collection of this plant led to the isolation of two novel nitrogen-containing clerodane-type diterpenoids, named echinophyllins A (**1**) and B (**2**). In this paper we describe the isolation and structure elucidation of **1** and **2**.

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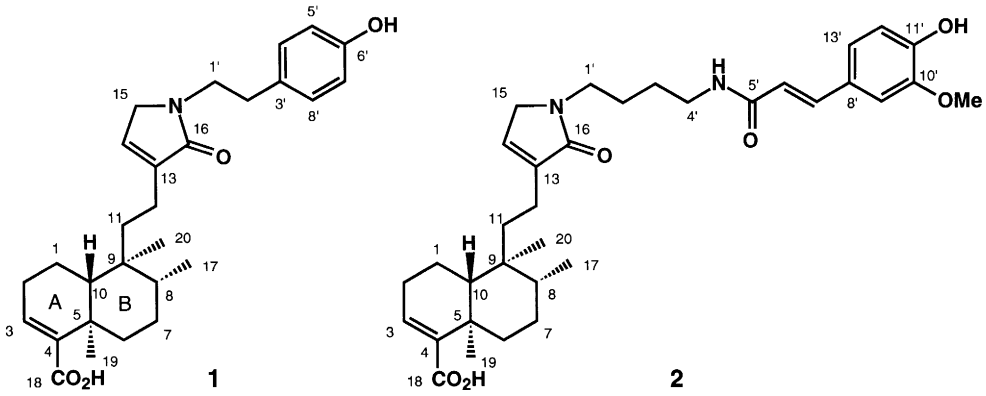


The leaves of the Brazilian medicinal plant *Echinodorus macrophyllus* (Kunth) Micheli (Alismataceae) were extracted with MeOH. The MeOH extracts were partitioned between hexane and 90% aqueous MeOH, and then the MeOH-layer was partitioned with EtOAc and H_2O. The EtOAc-soluble portions were subjected to a silica gel column ($CHCl_3$:MeOH, 96:4) and then a reversed-phase column (MeOH:H_2O, 70:30→MeOH) to afford an alkaloidal fraction, which was purified by a silica gel column (hexane:acetone, 1:1→1:5) to give echinophyllins A (**1**, 7.3 mg, 0.015%) and B (**2**, 4.1 mg, 0.0082%) as colorless amorphous solids together with a known clerodane diterpene, 16-oxo-15,16*H*-hardwickiic acid[3] (patagonic acid[4]) (**3**, 2.8 mg, 0.0056%).

The molecular formula, $C_{28}H_{37}NO_4$, of echinophyllin A (**1**), $[\alpha]_D^{23}$ −53.4° (*c* 0.73, MeOH), was established by HREIMS [*m/z* 451.2696 (M^+), Δ +2.7 mmu]. The IR spectrum implied the presence of hydroxy (3405 cm^{-1}) and unsaturated carbonyl (1666 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 olefin, six aromatic carbons (one of which was bearing an oxygen atom), two sp^3 quaternary carbons, nine methylenes, two methines, and three methyl groups. Since seven out of eleven unsaturations were thus accounted for, it was concluded that **1** contained four rings. The ^1H–^1H COSY spectrum revealed connectivities (Fig. 1) of C-1 to C-3 and C-10, C-6 to C-8 and C-17, C-11 to C-12, C-14 to C-15, C-1′ to C-2′, C-4′ to C-5′, and C-7′ to C-8′. HMBC correlations (Fig. 1) of H-3 to C-5 and C-18 (δ_C 170.8), H_3-19 to C-4, C-5, C-6, and C-10, and H-10 to C-5 revealed the presence of a cyclohexene ring (ring A), in which a

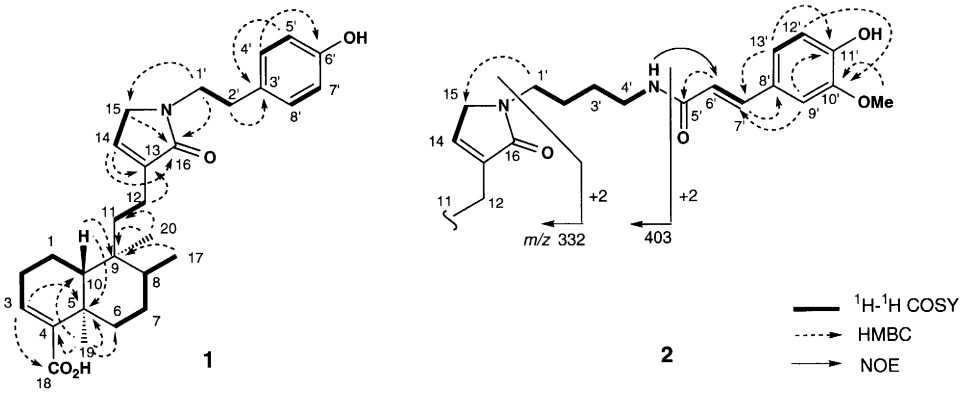


Fig. 1. Selected ^1H–^1H COSY and HMBC correlations for echinophyllins A (**1**) and B (**2**) and NOE and FABMS fragments for **2**

Table 1
^{1}H and ^{13}C NMR data of echinophyllins A (**1**) and B (**2**) in $CDCl_3$

position	**1** ^{1}H[a]	*J*(Hz)	^{13}C[a]	**2** ^{1}H[a]	*J*(Hz)	^{13}C[a]
1 (a)	1.68 m		17.4	1.65 m		17.5
(b)	1.40 m			1.48 m		
2	2.24 m		27.4	2.27 m		27.4
3	6.82 brs		140.0	6.80 brs		139.7
4			141.0			141.3
5			38.0			38.7
6 (a)	2.43 dd	3.0, 10.0	37.6	2.43 m		37.6
(b)	1.13 m			1.18 m		
7 (a)	1.49 m		27.3	1.48 m		27.2
(b)	1.40 m			1.40 m		
8	1.59 m		36.1	1.59 m		36.1
9			38.9			38.8
10	1.36 m		46.7	1.37 m		46.7
11 (a)	1.66 m		38.7	1.66 m		38.8
(b)	1.49 m			1.49 m		
12 (a)	2.17 m		19.3	2.18 m		19.3
(b)	2.07 m			2.07 m		
13			141.0			141.6
14	6.56 brs		133.7	6.67 brs		133.7
15	3.72 brs		51.6	3.89 brs		51.1
16			171.9			172.3
17	0.79 d	7.0	16.0	0.85 d	7.0	16.1
18			170.8			170.2
19	1.23 s		20.6	1.23 s		20.6
20	0.75 s		18.4	0.80 s		18.5
1'	3.67 t	8.0	44.3	3.53 m		41.9
2'	2.81 t	8.0	34.1	1.67 m		26.6
3'			130.0	1.60 m		26.0
4'	6.78 d	10.0	140.0	3.43 m		39.4
5'	7.04 d	10.0	129.6			166.4
6'			154.7	6.37 d	15.0	118.5
7'	7.04 d	10.0	129.6	7.58 d	15.0	141.1
8'	6.78 d	10.0	140.0			127.5
9'				7.00 s		109.8
10'						146.6
11'						147.1
12'				6.90 d	8.0	114.7
13'				7.08 d	8.0	121.9
MeO-10'				3.92 s		56.0

a) in ppm

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-9, H_3-17 to C-9, and H-10 to C-9. The HMBC correlation of H_3-20 to C-11 allowed the connection between C-9 and C-11. Cross-peaks of H-14 to C-13 (δ_C 141.0) and C-16 (δ_C 171.9), H_2-15 to C-16, and H_2-12 to C-13 and the chemical shift of C-15 (δ_C 51.6) revealed the presence of a γ-lactam ring (C-13 to C-15, C-16 and N-15) connected between C-12 and C-13. Proton signals at δ_H 3.67 (2H, t, *J*=8.0 Hz, H_2-1′), 2.81 (2H, t, *J*=8.0 Hz, H_2-2′), 6.78 (2H, d, *J*=10.0 Hz, H-4′ and H-8′), and 7.04 (2H, d, *J*=10.0 Hz, H-5′ and H-7′), a phenol carbon signals at δ_C 154.7 (C-6′), and HMBC correlations of H_2-2′ to C-3′, H-4′ to C-6′, and H-5′ to C-3′ revealed the presence of a tyramine moiety (C-1′ ~C-8′, N-15, and 6′-OH). The connectivity of N-1′ to C-15 and C-16 to form a γ-lactam ring was deduced from HMBC correlations of H_2-1′ to C-15 and C-16. Thus, the structure of echinophyllin A was elucidated to be **1**. NOESY correlations (Fig. 2) 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 β-orientation of H-10. Therefore, the relative stereochemistry of echinophyllin A was assigned as **1**.

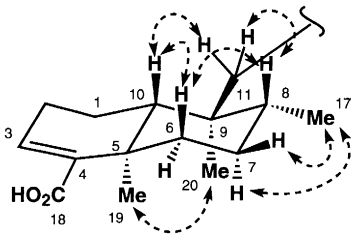
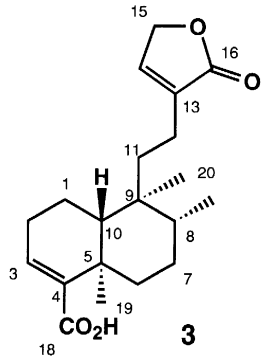


Fig. 2. Selected NOESY correlations of the bicyclic part in echinophyllin A (**1**)



Echinophyllin B (**2**), $[\alpha]_D^{23}$ −26.3° (*c* 0.25, MeOH), 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* 579.3464 $(M+H)^+$, Δ +3.0 mmu]. The ^1H and ^{13}C NMR spectra of a clerodane diterpene moiety containing an α,β-unsaturated γ-lactam ring (C-1 to C-20, and N-15) were similar to those of echinophyllin A (**1**). Analysis of the ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum provided one unsaturated amide carbonyl, one disubstituted olefin, six aromatic carbons (two of which were bearing an oxygen atom), four methylenes (two of which were bearing a nitrogen atom), and one methoxy group other than the clerodane diterpene moiety containing the γ-lactam ring. ^1H–^1H COSY connectivities of C-1′ to C-4′ and N-4′ and the ^{13}C chemical shifts of C-1′ (δ_C 41.9) and C-4′ (δ_C 39.4) indicated the presence of a putrescine (1,4-diaminobutane) moiety. Proton signals at δ_H 3.92 (3H, s, MeO-10′), 6.90 (1H, d, *J*=8.0 Hz, H-12′), 7.00 (1H, s, H-9′), 7.08 (1H, d, *J*=8.0 Hz, H-13′), 6.37 (1H, d, *J*=15.0 Hz, H-6′), and 7.58 (1H, d, *J*=15.0 Hz, H-7′, *trans*-oriented) and HMBC correlations (Fig. 1) of H-6′ to C-5′ (δ_C 166.4), H-7′ to C-8′ (δ_C 127.5), H-9′ and H-13′ to C-7′ and C-11′ (δ_C 147.1), H-12′ and H_3-OMe to C-10′ (δ_C 146.6) revealed the presence of a ferulic acid moiety. The HMBC correlation of H_2-1′ to C-15, the NOE enhancement of H-6′ on irradiation of 4′-NH, and the FABMS fragments at *m/z* 332 and 403 (Fig. 1) indicated that the ferulic acid was connected to the α,β-unsaturated γ-lactam through the putrescine unit. Thus, the structure of echinophyllin B was elucidated to be **2**. The relative stereochemistry of the clerodane diterpene moiety of **2** was assigned as the same as that of **1** (Fig. 2) by NOESY correlations.

Echinophyllins A (**1**) and B (**2**) are novel nitrogen-containing diterpenoids with a unique α,β-unsaturated γ-lactam ring consisting of a clerodane skeleton and a tyramine unit or an *N*-feruloyl putrescine unit, respectively, from the Brazilian medicinal plant *Echinodorus macrophyllus*, although a bis-clerodane imide has been obtained from the higher plant *Polyalthia viridis*.[5] Biogenetically, echinophyllins A (**1**) and B (**2**) may be derived from 16-oxo-15,16*H*-hardwickiic acid (**3**) through coupling with tyramine or *N*-feruloyl putrescine, respectively.

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