



Pergamon

Tetrahedron Letters 41 (2000) 2939–2943

TETRAHEDRON
LETTERS

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

Jun'ichi Kobayashi,^{a,*} Mitsuhiro Sekiguchi,^a Hideyuki Shigemori^a and Ayumi Ohsaki^{b,*}

^aGraduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

^bInstitute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo 101-0062, Japan

Received 11 January 2000; revised 14 February 2000; accepted 17 February 2000

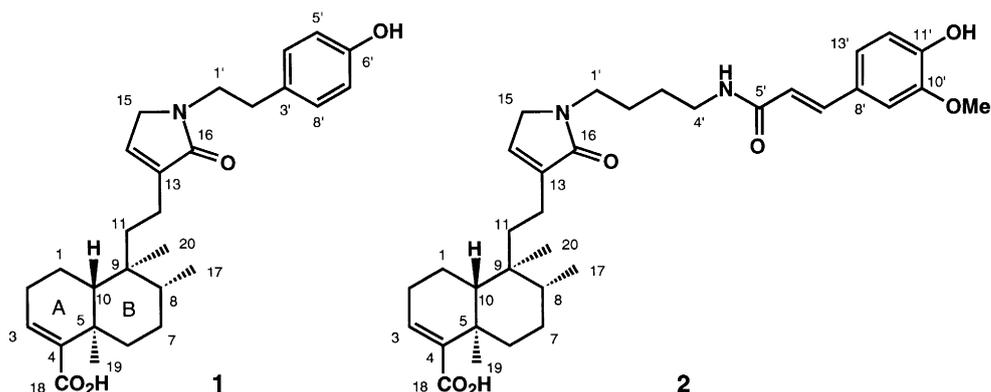
Abstract

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. © 2000 Elsevier Science Ltd. All rights reserved.

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**.

* Corresponding authors. Tel: +81-11-706-4985; fax: +81-11-706-4989; e-mail: jkobay@pharm.hokudai.ac.jp (J. Kobayashi). Tel: +81-3-5280-8153; fax: +81-3-5280-8005; e-mail: a-ohsaki@i-mde.tmd.ac.jp (A. Ohsaki)



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 H₂O. The EtOAc-soluble portions were subjected to a silica gel column (CHCl₃:MeOH, 96:4) and then a reversed-phase column (MeOH:H₂O, 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 acid³ (patagonic acid⁴) (**3**, 2.8 mg, 0.0056%).

The molecular formula, C₂₈H₃₇NO₄, of echinophyllin A (**1**), [α]_D²³ -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 ¹³C NMR data (Table 1) aided with 2D NMR experiments (¹H-¹H COSY, HMQC, and HMBC). The ¹³C 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), H₃-19 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

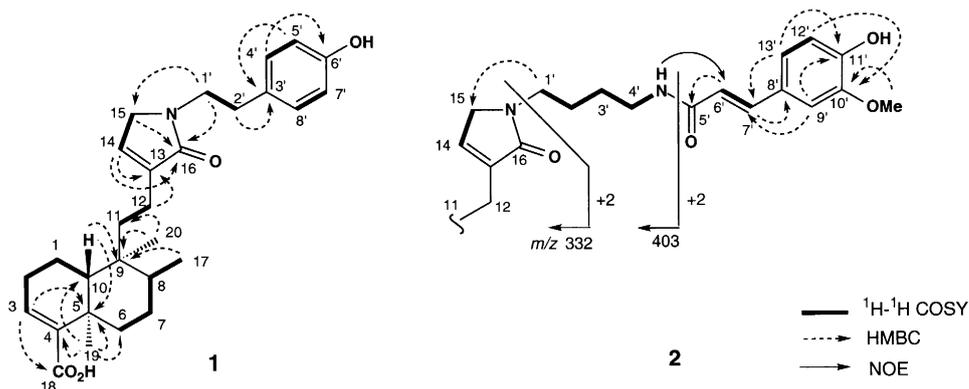


Fig. 1. Selected ¹H-¹H COSY and HMBC correlations for echinophyllins A (**1**) and B (**2**) and NOE and FABMS fragments for **2**

Table 1
¹H and ¹³C NMR data of echinophyllins A (1) and B (2) in CDCl₃

position	¹ H ^a	1 <i>J</i> (Hz)	¹³ C ^a	¹ H ^a	2 <i>J</i> (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 H₃-20 to C-9, H₃-17 to C-9, and H-10 to C-9. The HMBC correlation of H₃-20 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), H₂-15 to C-16, and H₂-12 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, H₂-1'), 2.81 (2H, t, $J=8.0$ Hz, H₂-2'), 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 H₂-2' 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 H₂-1' to C-15 and C-16. Thus, the structure of echinophyllin A was elucidated to be **1**. NOESY correlations (Fig. 2) of H₃-19 to H₃-20, H₃-17 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**.

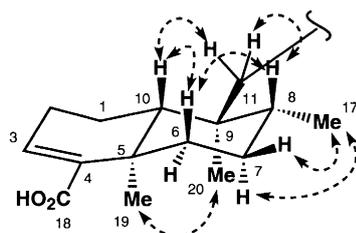
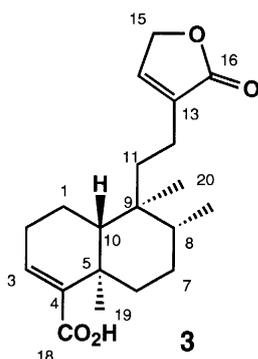


Fig. 2. Selected NOESY correlations of the bicyclic part in echinophyllin A (**1**)



Echinophyllin B (**2**), $[\alpha]_D^{23} -26.3^\circ$ (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 H₃-OMe to C-10' (δ_C 146.6) revealed the presence of a ferulic acid moiety. The HMBC correlation of H₂-1' 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.

Acknowledgements

We thank Dr. T. Nakasumi (Instituto de Pesquisas de Plantas Mediciniais do Brasil) for identification of this plant. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Ohsaki, A.; Takashima, J.; Chiba, N.; Kawamura, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1109–1112.
2. Kobayashi, J.; Sekiguchi, M.; Shigemori, H.; Ohsaki, A. *J. Nat. Prod.* in press.
3. Singh, P.; Jain, S.; Jakupovic, J. *Phytochemistry* **1988**, *27*, 1537–1539.
4. Rivera, A. P.; Faini, F.; Castillo, M. *J. Nat. Prod.* **1988**, *51*, 155–157.
5. Kijjoa, A.; Pinto, M. M. M.; Pinho, P. M. M.; Herz, W. *Phytochemistry* **1993**, *34*, 457–460.