Echinodolides A and B, New Cembrane Diterpenoids with an Eight-Membered Lactone Ring from the Leaves of *Echinodorus macrophyllus*

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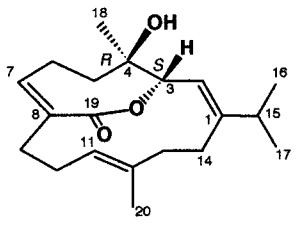
Two new cembrane diterpenoids with an eight-membered lactone ring, echinodolides A (**1**) and B (**2**), were isolated from the leaves of the Brazilian medicinal plant *Echinodorus macrophyllus* ("Chapéu-de-couro"), and the structures and relative stereochemistry were elucidated from their spectroscopic data.

In our continuing search for structurally unique compounds from Brazilian medicinal plants, labdane-derived diterpenoids, chapecoderins A−C,[1] and nitrogen-containing clerodane diterpenoids, echinophyllins A−F,[2,3] have been isolated from the leaves of *Echinodorus macrophyllus*. The leaves of this plant are 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 led to the isolation of two new cembrane diterpenoids with an eight-membered lactone ring, echinodolides A (**1**) and B (**2**). In this paper we describe the isolation and structure elucidation of **1** and **2**.



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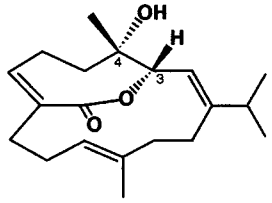

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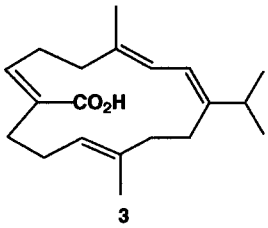

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The leaves of *Echinodorus macrophyllus* (Kunth) Micheli (Alismataceae) were extracted with MeOH. The MeOH extracts were partitioned between hexane and 90% aqueous MeOH, and then the MeOH layer was partitioned with EtOAc and H_2O. The EtOAc-soluble portions were subjected to a silica gel column ($CHCl_3$−MeOH, 9:1) and then a reversed-phase column (ODS, MeOH−H_2O, 4:1) followed by reversed-phase HPLC (ODS, CH_3CN−H_2O, 63:37) to give echinodolides A (**1**, 0.00091%) and B (**2**, 0.00023%) together with a known cembrane diterpene, echinoic acid (**3**).[4,5]

The molecular formula, $C_{20}H_{30}O_3$, of echinodolide A (**1**) was established by HREIMS [*m/z* 318.2194 (M^+), Δ −0.1 mmu]. The IR spectrum implied the presence of hydroxyl (3426 cm^{-1}) and unsaturated ester (1709 cm^{-1}) groups, while the UV absorption at 209 nm also supported that **1** possessed an unsaturated ester group. 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 ester carbonyl, three trisubstituted olefins, one oxygenated quaternary carbon, one oxymethine, six methylenes, one methine, and four methyl groups. The ^1H−^1H COSY spectrum indicated connectivities (Figure 1) of C-2 to C-3, C-5 to C-7, C-9 to C-11, C-13 to C-14, and C-15 to C-16 and C-17. HMBC correlations (Figure 1) of H-3 to C-1 (δ_C 154.5) and C-4 (δ_C 73.7), H-5 to C-4, H-6a and H_2-9a to C-8 (δ_C 129.1), H_2-10 and H-13b to C-12 (δ_C 136.8), and H-14b to C-1 revealed that **1** possessed a 14-membered macrocyclic ring, while an isopropyl group (C-15 to C-16 and C-17) and two methyl groups (C-18 and C-20) were attached to C-1, C-4, and C-12, respectively, from HMBC correlations of H-15, H_3-16, and H_3-17 to C-1, H_3-18 to C-3, C-4, and C-5, and H_3-20 to C-11, C-12, and C-13, thus indicating that compound **1** possessed a cembrane skeleton. A hydroxy group was connected to C-4, judging from the chemical shift (δ_C 73.7) of C-4. HMBC cross-peaks of H-3, H-7, and H-9b to C-19 (δ_C 171.3) suggested the presence of an eight-membered lactone ring consisting of C-3−C-8, C-19, and O-3. The geometries of three trisubstituted olefins at C-1, C-7, and C-11 were elucidated to be *E*, *Z*, and *E*, respectively, from NOESY correlations (Figure 2) of H-2 to H-16, H-3 to H-14b, H-7 to H-9b, and H-10 to H_3-20. Thus, the gross structure of echinodolide A was assigned as **1**. The relative stereochemistry at C-3 and C-4 was elucidated to be *anti* between H-3 and Me-18 by NOESY correlations of H_3-18 to H-2, H-5a, and H-5b, and H-3 to H-6b. The bicyclic ring conformation was also deduced from other NOESY correlations as shown in Figure 2. The absolute configuration of **1** was elucidated by the CD exciton chirality method. NOESY correlations of H_3-18 to H-2, H-5a, and H-5b, and H-3 to H-6b of compound **4**, which was derived from **1** by *p*-bromobenzoylation, indicated that the conformation of the eight-membered lactone ring of **4** was similar to that of **1**. The CD spectrum of **4** showed well-split intense Cotton effects (θ −1300 at 240 nm and θ +1700 at 227 nm), indicating that the projection of the *p*-bromobenzoate at C-4 and the α,β-unsaturated ester at C-3 should be anticlockwise (Figure 3). Therefore, these results indicated the absolute configurations at C-3 and C-4 to be *S* and *R*, respectively.

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Table 1. ^{1}H and ^{13}C NMR Data of Echinodolide A (**1**) in $CDCl_3$

position	^{1}H[a]		J(Hz)	^{13}C[a]		H coupled with C[b]
1				154.5	s	H-3, H-13a, H-13b, H-14, H-15
2	5.40	d	9.8	115.7	d	H-3, H-14b, H-15
3	5.48	d	9.8	78.2	d	H-5b, H-18
4				73.7	s	H-3, H-5b, H-18
5 (a)	1.93	dd	15.1, 13.0	33.5	t	H-3, H-7
(b)	1.61	dd	15.1, 6.8			
6 (a)	2.59	dddd	13.6, 13.0, 6.8, 4.9	24.8	t	H-5b
(b)	2.20	dd	13.6, 4.9			
7	5.99	t	4.9	135.3	d	H-5a, H-5b, H-6a, H-9a, H-9b
8				129.1	s	H-6a, H-9a, H-9b, H-10b
9 (a)	2.92	brd	13.6	37.3	t	H-7, H-10a, H-10b, H-11
(b)	2.18	dd	13.6, 4.9			
10 (a)	2.21	m		24.1	t	H-9b, H-11
(b)	1.99	brd	14.2			
11	5.05	brd	11.3	125.6	d	H-9a, H-10a, H-10b, H-20
12				136.8	s	H-10a, H-10b, H-13b, H-14b, H-20
13 (a)	2.29	m		36.7	t	H-14a, H-14b, H-20
(b)	1.95	dd	16.3, 12.8			
14 (a)	2.57	td	13.0, 4.6	32.3	t	H-13b, H-15
(b)	2.13	dt	13.0, 3.9			
15	2.37	sept	6.7	32.6	d	H-2, H-14a, H-16, H-17
16	1.13	d	6.7	24.1	q	H-15, H-17
17	1.05	d	6.7	23.9	q	H-15, H-16
18	1.23	s		23.9	q	H-3, H-5a
19				171.3	s	H-3, H-7, H-9b
20	1.47	s		20.9	q	H-11, H-13b

[a] δ in ppm. [b]HMBC correlations.

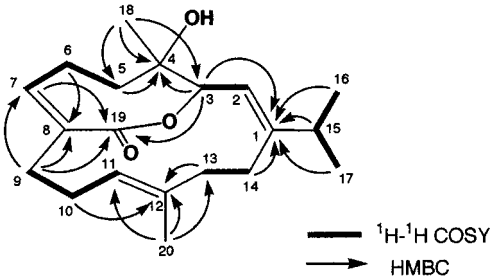


Figure 1. Selected 2D NMR data of echinodolide A (**1**).

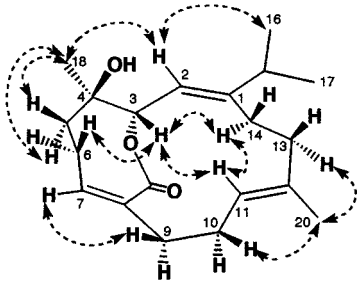
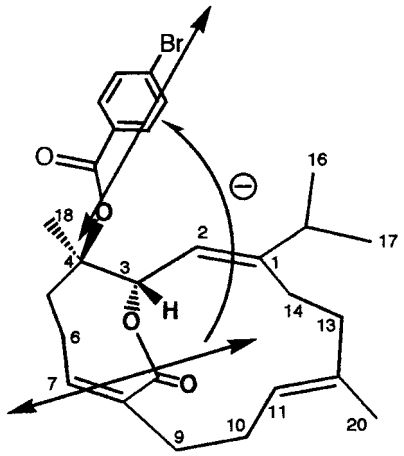


Figure 2. Relative stereochemistry of echinodolide A (**1**). Dotted arrows denote NOESY correlations.

Echinodolide B (**2**) showed the molecular ion peak at *m*/*z* 318 (M^+) in the EIMS. HREIMS analysis revealed the molecular formula to be $C_{20}H_{30}O_3$ [*m*/*z* 318.2209 (M^+), Δ +1.4 mmu], which was the same as that of echinodolide A (**1**). The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The ^{1}H−^{1}H COSY spectrum indicated connectivities of C-2 to C-3, C-5 to C-7, C-9 to C-11, C-13 to C-14, and C-15 to C-16 and C-17. HMBC correlations revealed that **2** possessed a 14-membered macrocyclic ring, while an isopropyl group (C-15 to C-16 and C-17) and two methyl groups (C-18 and C-20) were attached to C-1, C-4, and C-12, respectively. A hydroxy group was connected to C-4, judging from the chemical shift (δ_C 74.8) of C-4. HMBC cross-peaks of H-3, H-7, and H-9b to C-19 (δ_C 171.2) suggested the presence of an eight-membered lactone ring consisting of C-3−C-8, C-19, and O-3. The geometries of



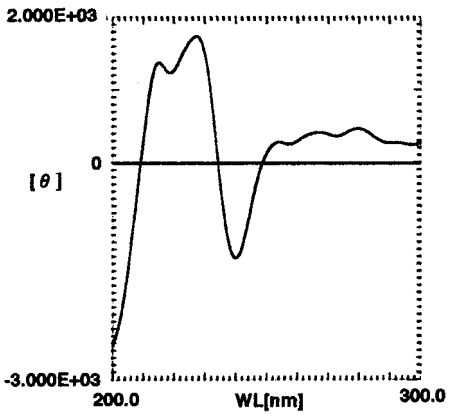


Figure 3. CD spectrum of compound **4**.

three trisubstituted olefins at C-1, C-7, and C-11 were elucidated to be *E*, *Z*, and *E*, respectively, from NOESY correlations (Figure 4) of H-2 to H-16, H-7 to H-9b, and H-11 to H-14b. Thus, the gross structure of echinodolide B

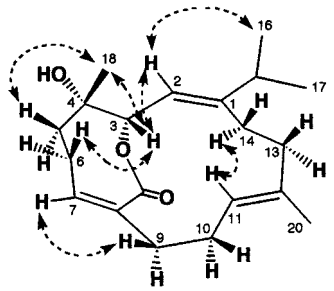


Figure 4. Relative stereochemistry of echinodolide B (**2**). Dotted arrows denote NOESY correlations.

(**2**) was the same as that of **1**. The relative stereochemistry of C-3 and C-4 was elucidated to be *syn* between H-3 and Me-18 by NOESY correlations of H_3-18 to H-3 and H-5b, and H-3 to H-6b. The bicyclic ring conformation was deduced from other NOESY correlations as shown in Figure 4, indicating the bicyclic ring conformation of **2** was very similar to that of **1**. Therefore, compound **2** was assigned to be the C-4 epimer of **1**.

Echinodolides A (**1**) and B (**2**) are unique cembrane diterpenoids with an eight-membered lactone ring from the Brazilian medicinal plant *Echinodorus macrophyllus*, while echinoic acid (**3**) was previously isolated from leaves of *Echinodorus grandiflorus*.[4,5] Although many cembrenoids possessing γ- or δ-lactone rings or a few cembrenoids having a seven-membered lactone ring have been isolated from marine invertebrates,[6,7] compounds **1** and **2** are the first examples of cembrenoids with an eight-membered lactone ring from natural sources. Biogenetically, compounds **1** and **2** may be derived from oxidation of the olefin at C-3 of echinoic acid (**3**) followed by its esterification.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-1030 polarimeter. UV and IR spectra were obtained on JASCO Ubest-35 and JASCO FT/IR-230 spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-500 spectrometer. The 7.26 and 77.0 ppm resonances of residual $CDCl_3$ were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. EI mass spectra were obtained on a JEOL FABmate spectrometer operating at 70 eV.

Plant Material. The leaves of *Echinodorus 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). The EtOAc-soluble portions (1.0 g) were subjected to silica gel column chromatography ($CHCl_3$−MeOH, 9:1), followed by C_{18} column chromatography (ODS, MeOH−H_2O, 4:1) to afford a fraction (20 mg). The fraction was purified 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 CH_3CN−H_2O, 63:37) to yield echinodolides A (**1**, 1.6 mg, t_R 22.0 min) and B (**2**, 0.4 mg, t_R 22.5 min).

Echinodolide A (1): colorless amorphous solid; $[\alpha]^{23}_D$ −6.2° (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.86) nm; IR (KBr) ν_{max} 3426, 1709, and 1648 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); EIMS *m*/*z* 318 [M$^+$]; HREIMS *m*/*z* 318.2194 [M$^+$] (calcd for $C_{20}H_{30}O_3$, 318.2195).

Echinodolide B (2): colorless amorphous solid; $[\alpha]^{23}_D$ +9.7° (*c* 0.42, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.18) nm; IR (KBr) ν_{max} 3434, 1705, and 1634 cm^{-1}; ^{1}H ($CDCl_3$) δ 5.92 (1H, t, J = 5.6 Hz, H-7), 5.59 (1H, d, J = 10.1 Hz, H-2), 5.33 (1H, d, J = 10.1 Hz, H-3), 5.04 (1H, brd, J = 11.9 Hz, H-11), 2.95 (1H, ddd, J = 13.0, 5.2, 3.4 Hz, H-9a), 2.37 (1H, m, H-6a), 2.34 (1H, sept, J = 6.6 Hz, H-15), 2.31 (1H, m, H-14a), 2.29 (1H, dt, J = 12.1, 3.6 Hz, H-13a), 2.22 (1H, m, H-10a), 2.21 (brdd, J = 13.0, 5.2 Hz, H-9b), 2.14 (1H, m, H-14b), 2.12 (1H, m, H-10b), 1.99 (1H, m, H-6b), 1.98 (1H, dd, J = 19.2, 5.2 Hz, H-5a), 1.92 (1H, m, H-13b), 1.70 (1H, m, H-5b), 1.49 (3H, s, H-20), 1.13 (3H, d, J = 6.6 Hz, H-16), 1.05 (3H, d, J = 6.6 Hz, H-17), and 1.02 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 171.2 (s, C-19), 151. 3 (s, C-1), 138.0 (s, C-12), 133.7 (d, C-7), 129.6 (s, C-8), 125.2 (d, C-11), 116.4 (d, C-2), 80.8 (d, C-3), 74.8 (s, C-4), 37.2 (t, C-9), 36.6 (t, C-13), 35.5 (t, C-5), 33.0 (d, C-15), 32.8 (t, C-14), 26.3 (t, C-10), 24.0 (t, C-6), 22.9 (q, C-17), 21.9 (q, C-18), 20.9 (q, C-16), and 19.9 (q, C-20); EIMS *m*/*z* 318 [M$^+$]; HREIMS *m*/*z* 318.2209 [M$^+$] (calcd for $C_{20}H_{30}O_3$, 318.2195); HMBC correlations ($CDCl_3$, C/H) 1/14, 1/16, 2/14, 2/15, 3/5b, 3/18, 4/5b, 4/18, 7/9a, 7/9b, 8/9a, 8/9b, 11/20, 12/20, 13/20, 15/16, 15/17, 16/15, 16/17, 17/15, 17/16, 19/3, 19/7, and 19/9b.

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