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# A CEMBRANE FROM *ECHINODORUS GRANDIFLORUS*

CLARA M. A. TANAKA,†‡ MARIA HELENA SARRAGIOTTO,‡ JULIO ZUKERMAN-SCHPECTOR§ and ANITA J. MARSAIOLI*†

†Instituto de Química, UNICAMP, CP 6154, Campinas 13081-970, SP-Brazil; ‡Departamento de Química, UEM, Maringá-Pr, Brazil; §Instituto de Biologia, UNICAMP-SP-Brazil; ||Departamento de Química, UFSCar, S. Carlos, Brazil

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**Key Word Index**—*Echinodorus grandiflorus*; Alismataceae; leaf petides; echinoic acid; cembrane; X-ray analysis.

**Abstract**—A new cembrane, echinoic acid ((1*E*,3*E*,7*E*,11*E*)-8-carboxyl-1-isopropyl-5,18-dimethylcyclotetradecatetraene), is a major secondary metabolite from *Echinodorus grandiflorus*. Its structure was confirmed by spectral and crystallographic analysis. Copyright © 1997 Elsevier Science Ltd

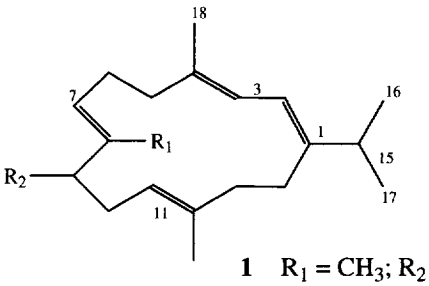
## INTRODUCTION

The genus *Echinodorus* is widespread throughout the American continent from the U.S.A. to Argentina and Chile. They are aquatic plants with little economic relevance. *Echinodorus grandiflorus* ssp. *aureus*, known as *chapéu-de-couro*, 'leather hat', is a herbaceous perennial plant generally found in canals, lake margins and marshes. It is used in folk medicine for diuretic and anti-inflammatory purposes.

Echinodol (**1**), a new cembrane, has been isolated from the aerial parts of this species [1] and its structure established by high-resolution NMR techniques. We report here the isolation of echinoic acid (**2**), a new cembranoid, from the same species.

## RESULTS AND DISCUSSION

The crude ethanol extract of *E. grandiflorus* leaf petioles (5 kg) was partitioned into ethyl acetate. The ethyl acetate soluble fraction (14 g) was submitted to silica gel column chromatography, producing echinoic acid (**2**) as the major constituent. The IR spectrum revealed the presence of a COOH group (3300–2500 and 1720 $cm^{-1}$). Its mass spectrum showed a $[M]^{+\cdot}$ at *m/z* 302, and $^1H$ and $^{13}C$ NMR data (Table 1) suggested the presence of four double bonds (proton resonance, in parentheses) ($\delta$ 118.3 (1H, *d*, 6.04), 121.4 (1H, *dq*, 5.93), 124.4 (1H, *t*, 5.00), 147.3 (1H, *t*, 5.88), 129.4, 134.4, 136.5, 148.1), a carboxyl group at $\delta$ 172.3, an isopropyl group ($\delta$ 22.3 (6H, *d*, 1.05), 34.6 (1H, 2.38) and two methyl groups on a double bond ($\delta$ 16.6 (3H, *bs*, 1.57), 17.4 (3H, *bs*, 1.76)). The remaining




**1** $R_1 = CH_3$; $R_2 = OH$

**2** $R_1 = COOH$; $R_2 = H$

H,C-correlations are given in Table 1. The presence of the dienyl moiety was assigned by comparison with the spectral data for the cembrane echinodol [2]. Long-range C,H-correlations, obtained using an evolution time of $1/J$ seconds ($J = 10$ Hz) and the standard pulse sequence in the Varian Gemini 300 spectrometer for C,H-correlations, indicated that the isopropyl group was connected to the dienyl moiety ($\delta$ 148.1 had a long-range correlation with 1.05 (isopropyl methyl groups)). Further correlations are listed in Table 1.

Structure **2** was obtained essentially by NMR spectroscopy, but the *Z* and *E* configuration of the double bonds was not completely established. Nuclear Overhauser effect (NOE) difference experiments would have provided the necessary evidence, but this macrocycle might have more than one conformation in solution, thus leading to incorrect interpretations. To confirm unambiguously the structure, X-ray analysis was performed (Fig. 1). A needle-shaped crystal of $0.15 \times 0.35$ mm ($C_{20}H_{28}O_2$), obtained by recrystallization from hexane–chloroform, was mounted at random in an automatic CAD-4 Enraf-Nonius diffractometer. Cell dimensions were determined by

*Author to whom correspondence should be addressed.

Table 1. $^1H$ (300 MHz) and $^{13}C$ (75 MHz) chemical shifts of compound **2** obtained by 1D and 2D NMR spectroscopy (7 T $CDCl_3$ solution)

| C | | $^{13}C$ ($\delta$) | $^1H$ ($\delta$), $^nJ_{H,H}$ (Hz) | H–H COSY | C,H long-range correlations (COLOC) |
|---|---|---|---|---|---|
| 1 | C | 148.1 | | | 148.1 (1.05 $H_{16}$ and $H_{17}$, 2.10 $H_{12}$) |
| 2 | CH | 118.3 | 6.04 (*d*, $J = 11.5$) | 6.04 × 5.93 $H_3$ | |
| 3 | CH | 121.4 | 5.93 (*dq*, $J = 11.5, \leqslant 1$) | | 121.4 (1.76 $H_{18}$, 2.25 $H_5$) |
| 4 | C | 134.4 | | | 134.4 (1.76 $H_{18}$, 2.25 $H_5$) |
| 5 | $CH_2$ | 38.5 | 2.26 | 2.26 × 2.76 $H_6$ | 38.5 (5.88 $H_7$, 1.76 $H_{18}$) |
| 6 | $CH_2$ | 26.9 | 2.76 (*dt*, $J = 6.4, 5.5$) | | 26.9 (2.26 $H_5$) |
| 7 | CH | 147.3 | 5.88 (*t*, $J = 6.8$) | 5.88 × 2.76 $H_6$ | 147.3 (2.26 $H_5$) |
| 8 | C | 129.4 | | | 129.4 (2.40 $H_9$, 2.28 $H_{10}$) |
| 9 | $CH_2$ | 33.8 | 2.40 (*m*) | 2.40 × 2.28 $H_{10}$ | 33.8 (2.28 $H_{10}$) |
| 10 | $CH_2$ | 25.4 | 2.28 (overlap of signals) | | 25.4 (2.40 $H_9$) |
| 11 | CH | 124.4 | 5.00 (*br t*, $J = 6.4$) | 5.00 × 2.28 $H_{10}$ | 124.4 (1.57 $H_{20}$, 2.40 $H_9$) |
| 12 | C | 136.5 | | | 136.5 (1.57 $H_{20}$) |
| 13 | $CH_2$ | 39.6 | 2.10 (*br t*, $J = 6.6$) | 2.10 × 2.33 $H_{14}$ | 39.6 (2.33 $H_{14}$) |
| 14 | $CH_2$ | 28.0 | 2.33 (overlap of signals) | | 28.0 (6.04 $H_2$, 2.10 $H_{13}$) |
| 15 | CH | 34.6 | 2.38 (overlap of signals) | | 34.6 (6.04 $H_2$, 1.05 $H_{16}$, $H_{17}$) |
| 16 | $CH_3$ | 22.2 | 1.05 (*d*, $J = 6.4$) | 1.05 × 2.38 $H_{15}$ | |
| 17 | $CH_3$ | 22.2 | 1.05 (*d*, $J = 6.4$) | 1.05 × 2.38 $H_{15}$ | |
| 18 | $CH_3$ | 17.4 | 1.76 (*d*, $J \leqslant 1$) | 1.76 × 5.93 $H_3$ | |
| 19 | C | 172.3 | | | 172.3 (5.88 $H_7$) |
| 20 | $CH_3$ | 16.6 | 1.57 (*d*, $J \leqslant 1$) | 1.57 × 5.00 $H_{11}$ | |

least-squares fitting of the setting angles of 25 reflections ($9 < \theta < 17°$). Intensity measurements were carried out up to $\theta = 25°$, using a $\theta/2\theta$ scan mode and graphite monochromated Mo$K_\alpha$ radiation. The crystal data are: $a = 6.181(1)$ Å, $b = 8.717(1)$ Å, $c = 17.852(2)$ Å, $\alpha = 99.069(9)°$, $\beta = 99.34(1)°$, $\gamma = 90.65(1)°$, $V = 936.5(4)$ Å$^3$, space group $P_1$, $Z = 2$, $d_x = 1.073$ g cm$^{-3}$, 2745 reflections were measured, 2514 unique ($R_m = 0.0148$) of which 1098 with $I > 3*\sigma(I)$ were considered observed. Lorentz and polarization corrections were applied, but not absorption corrections ($\mu$(Mo$K_\alpha$) = 0.626 mm$^{-1}$). The intensities of three standard reflections varied by about 0.8% throughout the experiment. The structure was solved by direct methods [3]. An *E*-map based on the solution with the best combined figure of merit revealed the positions of some non-hydrogen atoms. The rest of the structure was obtained by difference Fourier calculations. The hydrogen atoms were located on stereochemical grounds, except that on the hydroxyl moiety, and included as fixed contributors with an overall isotropic temperature factor which refined to $U_{iso} = 0.128(5)$ Å$^2$; non-hydrogen atoms were refined anisotropically. The refinement [3] was made by minimization of $\Sigma w(|F_o| - |F_c|)^2$ through iterative full-matrix least-squares calculations. Weights $w = 1/[\sigma^2(F_o) + 0.0002F_o^2]$ (where $\sigma^2(F_o)$ is the variance based on counting statistics) were applied. The refinement was conducted until atomic-parameter shifts were smaller than their standard deviations. The

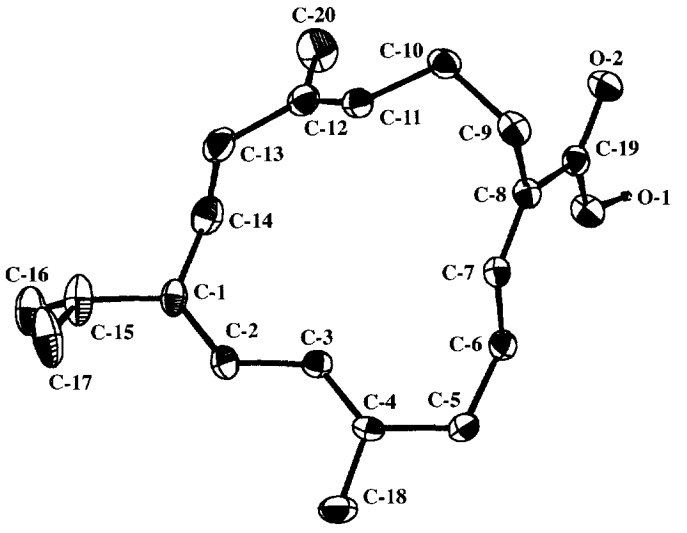



Fig. 1. ORTEP drawing of compound **2** and atom labels.

final unweighted $R$ factor omitting unobserved reflections was 0.0569 and the weighted $R$ factor was 0.0586. The atomic scattering factors used were those included in Sheldrick's program [3]. Lists of atomic fractional coordinates, anisotropic thermal parameters for non-hydrogen atoms, interatomic distances, and angles and structure factors are available upon request. In the crystalline state, the molecules are linked through a hydrogen bond: O-1···O-2$^{i}$ = 2.637, HO-1···O-2$^{i}$ = 1.600(4) Å, O-1–HO-1···O-2$^{i}$ = 161.1(2)° (symmetry operation: $i = 1-x, 1-y, 1-z$).

It was thus confirmed that echinoic acid (**2**) is (1*E*,3*E*,7*E*,11*E*)-8-carboxyl-1-isopropyl-5,18-dimethylcyclotetradecatetraene. It is curious that the cembrane echinodol (**1**) reported previously [2] was not detected in the present work. It should also be mentioned that *E. grandiflorus* collected in January 1995 in Campinas, SP, Brazil, contained neither echinoic acid (**2**) nor echinodol (**1**).

## EXPERIMENTAL

*General.* Mps are uncorr. $^{1}$H NMR spectra were recorded at 300 MHz with $CDCl_3$ as solvent and TMS as int. standard. $^{13}$C NMR spectra were obtained at 75 MHz with $CDCl_3$ (77.0 ppm) as int. standard. The number of hydrogen atoms attached to the carbon atoms were obtained from DEPT-135 spectra: $CH_3$/CH (+), $CH_2$ (−), $C_{quat}$ (absent) and DEPT-90 spectra (CH). 2D NMR spectroscopy was performed with standard COSY (H–H correlation) and HETCOR (C–H correlation) pulse sequences. MS were recorded at 70 eV using the solid probe inlet.

*Plant material and isolation.* Leaf petioles of *E. grandiflorus* (Cham. et Schltdl.) Micheli spp. (Fassett) Haynes & Holm-Ni aureus were collected in January 1994 near the town of Curitiba, Pr, Brazil. A voucher specimen is deposited at the IB/UNICAMP Herbarium under UEC 80748. Leaf petioles (5 kg) subdivided into small portions were extracted with EtOH (10 l) in a blender. The extract was filtered and the solvent reduced to 1 l which was then extracted with EtOAc. Evapn of solvent furnished 14 g of crude extract. Silica gel CC (140 g) of the crude extract eluted with hexane and increasing amounts of EtoAc yielded crude **2** (3 g). Recrystallization from hexane–$CHCl_3$ yielded pure **2**, mp 120° (250 mg). IR (KBr) $\nu_{max}$ cm$^{-1}$ 2928, 1687, 1637. UV (EtOH) $\lambda_{max}$ nm: 250. EI-MS (solid probe) 70 eV *m/z* (rel. int.): 302 (22), 259 (22), 135 (28), 121 (100), 107 (50), 93 (95), 77 (52). $^{1}$H and $^{13}$C NMR: see Table 1. Elemental analysis: $C_{20}H_{30}O_2$ requires C, 79.42; H, 10.00; found C, 79.24; H, 9.82.

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