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Abietane Diterpenes from Sagittaria *montevidensis* ssp *montevidensis* Charm. & Schltdl

Vanessa S. C. de O. Radke,[a] Maria do Carmo E. Amaral,[b] Ivânia T. A. Schuquel,[a] Cleuza C. da Silva,[a] Cecília M. A. de Oliveira[c] and Clara M. A. Tanaka*,[a]

[a]Departamento de Química, Universidade Estadual de Maringá, Av. Colombo 5790,
872020-900 Maringá-PR, Brazil

[b]Instituto de Biologia, Universidade Estadual de Campinas, Rua Monteiro Lobato 255,
13083-970 Campinas-SP, Brazil

[c]Instituto de Química, Universidade Federal de Goiás, Campus II, Samambaia,
74000-970 Goiânia-GO, Brazil

Dois novos diterpenos do tipo abietano 3β-hidroxi-9α, 13α-epidioxiabiet-8(14)-eno (**1**) e 3-oxo-9α,13α-epidioxiabiet-8(14)-eno (**2**) foram isolados do extrato bruto metanólico dos pecíolos de *Sagittaria montevidensis* ssp *montevidensis* (Alismataceae). As estruturas de **1** e **2** foram determinadas com base em análises espectrométricas do tipo EM-AR, IV, bem como, RMN de ^{1}H e ^{13}C uni e bi dimensionais.

Two new abietane-type diterpenes, 3β-hydroxy-9α, 13α-epidioxyabiet-8(14)-ene (**1**) and 3-oxo-9α,13α-epidioxyabiet-8(14)-ene (**2**), were isolated from the methanolic crude extract of the petioles of *Sagittaria montevidensis* ssp *montevidensis* (Alismataceae). The structures of **1** and **2** were determined on the basis of spectrometric analyses including HREIMS, IR as well as ^{1}H and ^{13}C 1 and 2D NMR.

Keywords: *Sagittaria montevidensis*, Alismataceae, diterpenes, endoperoxide abietane

Introduction

The family Alismataceae comprises 12 genera and about 75 species of herbaceous aquatic plants and some of these species are used in traditional Chinese medicine. There are only two genera that are naturally found in Brazil: *Echinodorus* and *Sagittaria*. *Sagittaria* species are known to produce biologically active compounds such as clerodane, pimarane, labdane and rosane-type diterpenoids.[1-6] As part of our continuous work on plants from Alismataceae,[7-9] we now describe the isolation and characterization of two new diterpenes abietanes derivatives, 3β-hydroxy-9α, 13α-epidioxyabiet-8(14)-ene (**1**) and 3-oxo-9α, 13α-epidioxyabiet-8(14)-ene (**2**), which were obtained from the crude methanolic extracts of the fresh petioles of *Sagittaria montevidensis* ssp *montevidensis*. The structures of the new metabolites were determined on basis of spectrometric analysis including HREIMS, IR, ^{1}H and ^{13}C 1 and 2D NMR and also by comparison of their NMR data with those of related metabolites.

Results and Discussion

Fresh petioles of *Sagittaria montevidensis* ssp *montevidensis* were extracted with MeOH. After removal of solvent, this extract was partitioned between H_2O and EtOAc. The EtOAc fraction was chromatographed on silica Gel 60 to yield the compounds **1** and **2**.

Compound **1** was isolated as an amorphous white solid, $[\alpha]_D$: +45.7° ($CHCl_3$; c.0.0042). Its IR exhibited absorptions due the hydroxyl (3494 cm^{-1}) and peroxide (1179 cm^{-1}) groups. The HREIMS spectrum of **1** exhibited $[M]^{+\bullet}$ at *m/z* 320.23140, corresponding to the molecular formula $C_{20}H_{32}O_3$ (calculated *m/z* 320.23515).

The structure of **1** was completely assigned by a combination of one and two-dimensional NMR methods. The carbon resonances at δ_C 78.4 (CH), 80.9 (C) and 79.3 (C) in the ^{13}C NMR and DEPT spectra suggested the presence of the oxymethine and endoperoxide groups. Furthermore, the presence of the two sp^2 carbons was inferred from the signals at δ_C 126.9 (CH) and 145.4 (C), confirming a trisubstituted double bond. Six methylene grops were deduced from DEPT

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*e-mail: cmatanaka@uem.br

signals at δ_C 30.3, 27.6, 19.2, 25.1, 22.4 and 26.4; two methine signals at δ_C 43.3 and 32.8; two quaternary carbon signals at δ_C 39.9 and 40.1; and, finally, five methyl groups at δ_C 17.4 (2 Me); 28.2; 15.8 and 19.1. The ^{1}H NMR spectrum of **1** confirmed the presence of an oxymethine hydrogen (δ_H 3.10, dd, *J* 10.8; 4.5 Hz); one olefinic hydrogen (δ_H 6.12, d, *J* 2.7) and an isopropyl group (δ_H 0.93, d, *J* 6.9 Hz). In addition, three intense singlet are also observed at δ_H 0.91, 0.94, 1.06 corresponding to methyl groups.

The combined use of COSY and HMQC on **1**, together with the presence of the major mass spectral fragment at *m/z* 288.23915 [M- O_2]$^{+\bullet}$ allowed us to identify **1** as an endoperoxide abietane-type skeleton. The location of the endoperoxide bridge between the C-9 and C-13 was inferred by comparison of the spectroscopical data of **1** with those of endoperoxide diterpenes.[10-14] The HMBC correlations (Figure 1a) between carbon at δ_C 80.9 (C-9) with the hydrogens at δ_H 1.06 (3H-20), 6.12 (1H-14), 1.96 (1H-11α) and carbon at δ_C 79.3 (C-13) with the hydrogens at δ_H 0.93 (3H-16/3H-17), 6.12 (1H-14) and 1.82 (1H-12b) confirmed these assignments. The β-orientation of the hydroxyl group at C-3 was deduced from the coupling constant (*J* 10.8; 4.5Hz) and NOESY correlations (Figure 2a). In addition, NOESY spectrum showed, apart signals characteristic of the 3β-hydroxy-abietane skeleton, a cross peak between H-20β/H-19β/H-11β suggesting a orientation to endoperoxide group. According to the literature an oxygen on C-9 having a-orientation displaying significant differences on the chemical shifts of C-1, C-5 and C-7 when compared to those the β-orientation due the γ-gauche effect.[11] The above arguments corroborated the proposal structure to **1**.

Compound **2** was obtained as a white solid, $[\alpha]_D$: +5.0^0 ($CHCl_3$; c.0.0015). Its IR spectrum (KBr) exhibited absorptions due to the ketone (1708 cm^{-1}). HREIMS spectrum exhibited the [M]$^{+\bullet}$ at *m/z* 318.21771, corresponding to the molecular formula $C_{20}H_{30}O_3$ (calculated 318.21950). The NMR data of **2** were analogous to those **1** except that the hydroxyl was replaced by one ketone carbonyl group. The carbon resonance at δ_C 215.7 in the ^{13}C NMR and DEPT spectra and the absence of the oxymethyne group at δ_C 78.4 indicated that **2** differs from **1** only in the C-3. The relative stereochemistry was deduced by comparison of its spectroscopic data to those of the compound **1** and from NOE difference spectra. Irradiation of the 3H-20 showed spatial interactions with 3H-19 (0.30%), H-11b (1.30%), H-7β (0.80%), H-1β (1.30%) and H-2β (0.30%) as summarized in Figure 2b. Based on above features, structure **2** is assigned to 3-oxo-9α,13α-epidioxyabiet-8(14)-ene.

Table 1. ^{1}H (300.059 MHz) and ^{13}C (75.458 MHz) NMR spectroscopic data for **1** and **2** ($(CD_3)_2CO$, TMS)

	1		2	
C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)	δ_C
1α	1.90 (td, *J* 13.5; 5.4)	30.3	2.23 (ddd, *J* 13.8; 9.6; 4.5)	30.2
1β	1.33 (dt, *J* 13.5; 3.3)		1.58 (ddd, *J* 13.8; 8.4; 4.5)	
2a	1.54 (m)	27.6	2.33 (ddd, *J* 14.7; 8.4; 4.5)	35.9
2b	1.60 (m)		2.44 (ddd, *J* 14.7; 9.6; 4.5)	
3	3.10 (dd, *J* 10.8; 4.5)	78.4	-	215.7
4	-	39.9	-	47.5
5	1.64 (m)	43.3	2.10 (dd, *J* 9.1; 3.6)	43.8
6	1.70 (m)	19.2	1.76 (m)	18.7
7α	2.44 (m)	25.1	2.56 (m)	24.9
7β	2.57 (m)		2.68 (td, *J* 8.4; 2.7)	
8	-	145.4	-	144.9
9	-	80.9	-	82.9
10	-	40.1	-	39.0
11α	1.96 (m)	22.4	2.04 (m)	22.3
11β	1.44 (m)		1.48 (m)	
12a	1.48 (m)	26.4	1.48 (m)	26.3
12b	1.82 (m)		1.87 (dd, *J* 10.3; 1.8)	
13	-	79.3	-	79.5
14	6.12 (d, *J* 2.7)	126.9	6.18 (d, *J* 2.4)	127.7
15	1.78 (hp, *J* 6.9)	32.8	1.82 (hp, *J* 6.9)	32.8
16	0.93 (d, *J* 6.9)	17.4	0.93 (d, *J* 6.9)	17.3
17	0.93 (d, *J* 6.9)	17.4	0.94 (d, *J* 6.9)	17.4
18	0.91 (s)	15.8	1.08 (s)	21.9
19	0.94 (s)	28.2	0.99 (s)	25.5
20	1.06 (s)	19.1	1.22 (s)	20.0

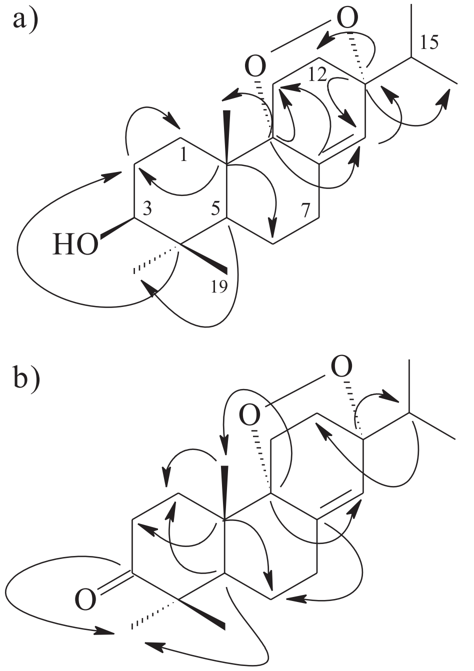

Figure 1. Key HMBC correlations ($^{2,3}J$) observed for compound **1** (a) and **2** (b).

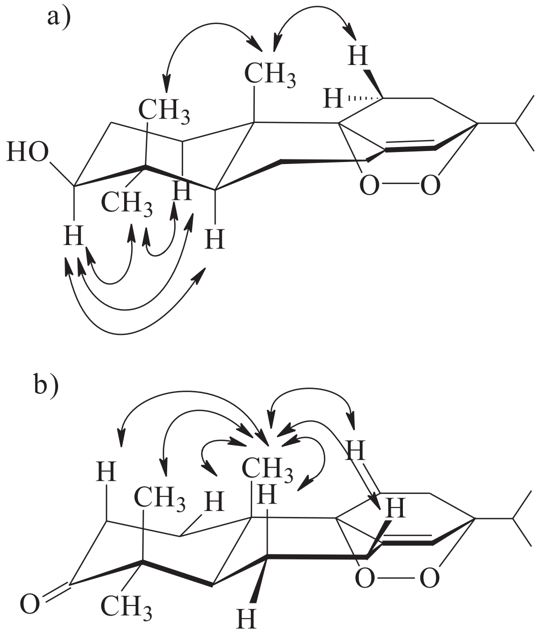

Figure 2. Key NOE correlations observed for compound **1** (a) and **2** (b).

Experimental

General experimental procedures

Optical rotations were measured on an ORD 306 spectropolarimeter J-720 in $CHCl_3$ at 25 °C. IR spectra were measured on a FTIR (KBr) Bomen MB Series spectrometer. ^{1}H, ^{13}C 1 and 2D NMR spectra were taken on a Varian Mercury plus BB spectrometer, operating at 300.059 MHz for ^{1}H and 75.458 MHz for ^{13}C in $(CD_3)_2CO$ solution using TMS as internal standard. HRMS were carried out using a Micromass VG AutoSpec. spectrometer operating at 70 eV.

Plant material

The plant material was collected in Curitiba-Paraná, Brazil and authenticated by Dr. Maria do Carmo Amaral (Instituto de Biologia, Universidade Estadual de Campinas). Voucher specimens (# UEC 115194) were deposited in the Herbarium of the Instituto de Biologia, Universidade Estadual de Campinas.

Extraction and isolation

Fresh petioles of *S. montevidensis ssp montevidensis* were extracted with MeOH. After removal of solvent in *vacuum*, the residue was partitioned between EtOAc and H_2O. The EtOAc (6.0 g) portion was subjected column chromatography on silica gel, eluting with *n*-hexane, *n*-hexane/EtOAc (95:5, 90:10, 80:20, 70:30, 50:50) and MeOH. The fraction eluted with *n*-hexane/EtOAc (80:20) was further purified by column chromatography (CC) over silica gel using *n*-hexane-EtOAc gradient solvent system, to obtain compounds **1** (18.0 mg) and **2** (9.8 mg).

Compound 1

White solid. $[\alpha]_D$: +45.7° ($CHCl_3$; c.0.0042): IR ν_{max}/cm^{-1}: 3494, 3051, 2960, 2932, 2876, 1710, 1658, 1604, 1464, 1360, 1179, 1080, 1027, 1012, 934. For ^{1}H and ^{13}C NMR spectral data, see Table 1. HREIMS *m/z*: 320.23140 $[M]^{+\bullet}$ (Calc. for $C_{20}H_{32}O_3$, 320.23515), 288.23915 (75%), 273.2168 (68%), 68.01355 (100%).

Compound 2

White solid. $[\alpha]_D$: +5.0^0 ($CHCl_3$; c.0.0015). IR ν_{max}/cm^{-1}: 2964, 2934, 2882, 2359, 1708, 1457, 1387, 1191, 1120, 945, 899. For ^{1}H and ^{13}C NMR spectral data, see Table 1. HREIMS *m/z*: 318.21771 $[M]^{+\bullet}$ (Calc. for $C_{20}H_{30}O_3$, 318.21950), 286.22610 (100%), 271.2033 (93%).

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