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New Hopane Triterpene from *Eleocharis sellowiana* (Cyperaceae)

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Do extrato hexânico de *Eleocharis sellowiana* foram isolados o novo triterpeno 3α-hidroxi-13α,17α,21β-hopano-15,19-diona e hexadecanoato de *E*-fitila, caracterizados por dados de RMN e EM.

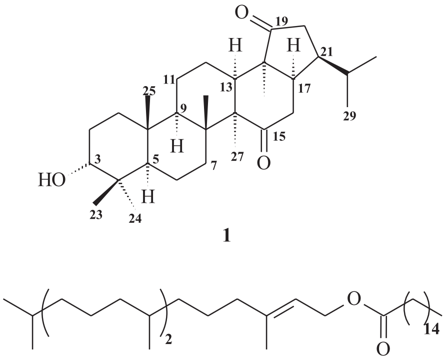
A new triterpene named 3α-hydroxy-13α,17α,21β-hopan-15,19-dione and *E*-phytyl hexadecanoate were isolated from the hexanic extract of *Eleocharis sellowiana*. NMR and MS experiments determined the molecular structures.

Keywords: *Eleocharis*, Cyperaceae, hopane-like triterpene

Introduction

The genus *Eleocharis* R. Br. (Cyperaceae, Cyperoideae, Scirpeae) includes about 200 species, occurring in wet environments like swamps, lakes and rivers margins. Their aerial parts are formed by simple, not ramified stalks that end in a spiciform inflorescence formed by numerous very inconspicuous flowers. Their subterraneous parts are formed by roots and stem (called rhizome or stolon, depending on its form). *E. sellowiana* Kunth occurs in Mexico, Colombia, Paraguay and Brazil. This species is widely distributed in São Paulo State.[1] As many other plants from wet environments like swamps, lakes and rivers margins, *Eleocharis* species are subject of only a few studies. According to the literature[2] only *E. dulcis* Trin., *E. coloradoensis* (Britt.) Gilly, *E. acuta* R. Br., *E. microcarpa* Torr. and *E. smallii* L. had been subjected to phytochemical analysis before.

In continuation of our phytochemical analysis of *Eleocharis* genus,[3] the hexanic extracts of *E. sellowiana* (subterraneous and aerial parts) were subjected to a detailed chromatographic analysis resulting in the isolation of a new pentacyclic triterpene named 3α-hydroxy-13α,17α,21β-hopan-15,19-dione (**1**) and *E*-phytyl hexadecanoate (**2**) (Figure 1).



1

2

Figure 1. Compounds isolated from *E. sellowiana*.

Results and Discussion

Compound **1** was deduced as having an elemental formula of $C_{30}H_{48}O_3$ by HREI-MS (observed $M^{+\bullet}$= 456.3607; required $M^{+\bullet}$= 456.3604). The presence of five signals as singlets (δ_H 0.85, δ_H 0.89, δ_H 0.94, δ_H 0.97 and δ_H 1.15) and two signals as doublets (δ_H 0.86, d, *J* 7.0Hz, CH_3-29, and δ_H 1.00, d, *J* 7.0Hz, CH_3-30) attributed to eight methyl groups in its ^{1}H NMR spectrum (Table 1) suggested a hopane-like or a lupane-like triterpene skeleton. The broad singlet at δ_H 3.44 (br*s*, H-3) together with the chemical shift of C-3 at δ_C 75.9 (CH) in its ^{13}C NMR spectrum (Table 2) suggested the presence of

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Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) and observed correlation in HSQC (vicinal C-H) and in HMBC (long-range C-H) spectra ($CDCl_3$, 11 Tesla) of **1**

H (δ)	C (δ, J^1)	C (δ, J^n)
0.85 (s, H-26)	16.8 (C-26)	55.2 (C-14), 50.4 (C-9), 44.7 (C-8), 34.1 (C-7)
0.85 (s, H-24)	22.0 (C-24)	75.9 (C-3), 48.8 (C-5), 37.4 (C-4), 28.2 (C-23)
0.86 (d, *J* 7.0 Hz, H-29)	20.3 (C-29)	48.9 (C-21), 28.9 (C-22), 22.4 (C-30)
0.89 (s, H-25)	16.3 (C-25)	50.4 (C-9), 37.8 (C-10), 33.7 (C-1)
0.94 (s, H-28)	16.9 (C-28)	224.1 (C-19), 52.1 (C-18), 50.4 (C-17)
0.97 (s, H-23)	28.2 (C-23)	75.9 (C-3), 48.8 (C-5), 37.4 (C-4), 22.0 (C-24)
1.00 (d, *J* 7.0 Hz, H-30)	22.4 (C-30)	48.9 (C-21), 28.9 (C-22), 20.3 (C-29)
1.15 (s, H-27)	18.1 (C-27)	215.7 (C-15), 55.2 (C-14), 44.7 (C-8)
1.76 (oct, *J* 6.0 Hz, H-22)	28.9 (C-22)	20.3 (C-29), 22.4 (C-30), 48.9 (C-21)
1.87 (m, H-9)	50.4 (C-9)	44.7 (C-8), 33.7 (C-1)
1.89 (m, H-17)	50.4 (C-17)	37.2 (C-20), 16.9 (C-28)
1.91 (q, *J* 6.0Hz, H-21)	48.9 (C-21)	52.1 (C-18), 22.4 (C-30), 20.3 (C-29)
2.18 (m, H-20)	37.2 (C-20)	224.1 (C-19), 50.4 (C-17)
2.26 (m, H-20)	37.2 (C-20)	224.1 (C-19), 48.9 (C-21)
2.55 (m, H-16)	37.9 (C-16)	215.7 (C-15)
3.44 (brs, H-3β)	75.9 (C-3)	33.7 (C-1), 48.8 (C-5)

a 3α-OH group[4] (Table 2) while the signals in at δ_C 215.7 (C-15) and δ_C 224.1 (C-19) showed correlation with the methyl hydrogens at CH_3-27 e CH_3-28 in the HMBC NMR experiment (Table 1). These correlations indicated that both carbonyl carbons should not be present at C-ring. Analyzing the other correlations obtained in the HMBC NMR experiment, it was possible to determine the presence of the carbonyls at C-15 (D-ring) and at C-19 (E-ring) (Figure 2). The relative configuration at C/D and at D/E rings fusions was deduced through the valuable information about correlations among the hydrogen spin systems at carbons C-16, C-20 and C-30 furnished by the 1D-TOCSY NMR experiment (Figure 3). Accordingly, when hydrogens H-20 were irradiated, it was possible to identify the hydrogens H-21 (q, *J* 6.0Hz), H-22 (oct, *J* 6.0 Hz), H-29 (s) and H-30 (s). On the other hand, when the hydrogens H-16 were irradiated, only the hydrogen H-17 was observed. The absence of polarization transference between H-17 and H-21 points out to a very small coupling constant between these hydrogens and consequently to a *cis*-D/E rings fusion with a β-isopropyl group. The C/D rings fusion should be a *cis*-fusion with the hydrogen H-13 in a α-position. Through the corresponding molecular model it is possible to see that the methyl group CH_3-26 is under the C-19 carbonyl protection cone, which explains the lower chemical shift of CH_3-26 (δ_H 0.85) hydrogens when compared to those related for the 17α,21β-hopane (δ_H 0.95).[5] In its NOESY-1D spectrum, it was observed that when CH_3-27 (δ_H 1.15) was irradiated increments were observed in H-7a (δ_H 1.72, 1.78%), H-9a (δ_H 1.90, 0.95%) and H-12a (δ_H 1.52, 2.13%) but not in CH_3-28 (δ_H 0.94). On the other hand, when CH_3-28 (δ_H 0.94) was irradiated increments were just observed in H-22 (δ_H 1.76, 1.66%), corroborating with a *cis*-C/D rings fusion. In its HR-EIMS spectrum, there are some important fragment ions[6] that corroborated with the hydroxyl group at C-3 (*m/z* 207, 16%, **1a** and *m/z* 189, 100%, **1b**) and with carbonyl groups location at D- and E-rings (*m/z* 318, 85%, **1c**; *m/z* 300, 24%, **1d**; *m/z* 303, 20%, **1e**; *m/z* 277, 32%, **1f**) (Figure 4).

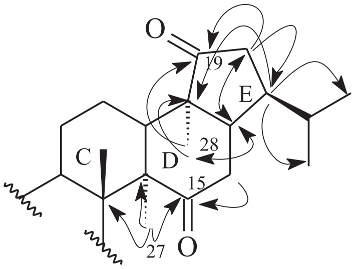


Figure 2. Observed correlation in HMBC (long-range C-H) spectra of **1**.

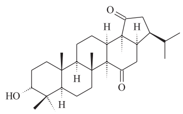
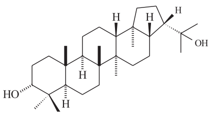
Compound **2** ($C_{28}H_{48}O$), isolated from the hexanic extract of *E. sellowiana* (aerial part), was identified as *E*-phytyl hexadecanoate by comparison of their ^{1}H and ^{13}C NMR and MS data with those previously published.[7] The occurrence of compound **2** in the genus *Eleocharis* is described here for the first time.

Experimental

General

Thin layer chromatography (TLC): Silica gel *60* F_{254} Al sheets (*Merck*); detection at 254 and 365 nm and with anisaldehyde in acidic ethanol solution,[8] CC = column chromatography. ^{1}H, ^{13}C NMR and 2D experiments *Varian Inova-500* (Palo Alto, CA, USA) spectrometer at 11 tesla. Chemical shifts of the compounds were recorded in $CDCl_3$ solutions and were quoted relative to TMS for ^{1}H NMR (δ 0.0) and to $CDCl_3$ (δ 77.0) for ^{13}C NMR. Atributions: The triterpene numering (see Figure 1) is used in the results

Table 2. ^{13}C NMR data of **1** and **3**

C	**1** δ_C ^{a}	**3** δ_C ^{b}
		
CH_2-1	33.7	33.2
CH_2-2	25.3	25.3
CH-3	75.9	76.2
C-4	37.4	37.5
CH-5	48.8	48.8
CH_2-6	18.5	18.3
CH_2-7	34.1	33.2
C-8	44.7	41.8
CH-9	50.4	50.0
C-10	37.8	37.2
CH_2-11	21.9	20.9
CH_2-12	24.5	24.1
CH-13	50.4	49.9
C-14	55.2	41.9
C-15	215.7	34.7
CH_2-16	37.9	21.9
CH-17	50.4	53.9
C-18	52.1	44.1
C-19	224.1	41.3
CH_2-20	37.2	26.6
CH-21	48.9	51.1
CH-22	28.9	73.9
CH_3-23	28.2	28.3
CH_3-24	22.0	22.1
CH_3-25	16.3	15.7
CH_3-26	16.8	17.0
CH_3-27	18.1	17.1
CH_3-28	16.9	16.2
CH_3-29	20.3	28.7
CH_3-30	22.4	30.9

^{a}125 MHz, $CDCl_3$; ^{b}Tanaka and Matsunaga, reference 4.

and discussion and for spectroscopic data. HREIMS experiment: *VG Auto Spec 10000 Micromass* (Manchester, UK) instrument with an ionizing potencial of 70 eV, *m/z* (rel. intensity in%), direct probe.

Plant material

Samples of *E. sellowiana* were collected in Campinas, Brazil, and identified by two of the authors (A. D. F. and M. C. E. A.). Voucher specimens (A. D. Faria *et al.* 1000 - *E. sellowiana*) have been deposited in the Herbarium of the Botany Department of the Biology Institute of Unicamp (UEC), Campinas-SP, Brazil.

Extraction

The collected plant (Es = *E. sellowiana*) was separated into aerial (270.0g, EsA) and subterraneous (428.0g, EsS) parts. Extracts of fresh subterraneous part were obtained by maceration with ethanol. The resultant extracts were combined, concentrated and diluted with H_2O to get an aqueous EtOH solution, which was partitioned with hexane (EsSH, 358.4 mg) and $CHCl_3$ (EsSC, 1303.4 mg); the respective aq. EtOH layers were then named EsSHE (3010.0 mg). The air-dried milled aerial part (named EsA, 95.8g) was successively extracted in a Soxhlet apparatus with hexane (EsAH, 2249.3 mg), CH_2Cl_2 (EsAD, 447.9 mg) and MeOH (EsAM, 3052.0 mg).

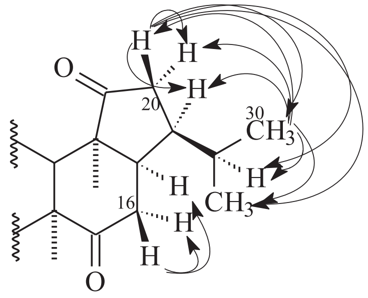
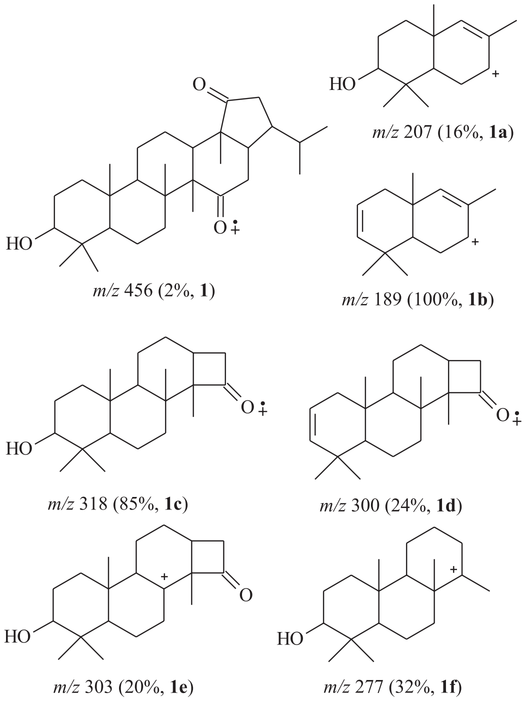


Figure 3. Observed correlation in 1D-TOCSY experiment of **1**.

Isolation

Part of the hexanic extract EsSH (300 mg) was subjected to *flash* CC over silica gel,[8] eluted first with $CHCl_3$-Hex (4:1). The eluent polarity was increased by the gradual addition of chloroform and then methanol until reaching 100% of methanol, furnishing 159 fractions (15



m/z 207 (16%, **1a**)

m/z 456 (2%, **1**)

m/z 189 (100%, **1b**)

m/z 318 (85%, **1c**)

m/z 300 (24%, **1d**)

m/z 303 (20%, **1e**)

m/z 277 (32%, **1f**)

Figure 4. Principal fragments observed in MS spectra of **1**.

mL), which were reduced to 21 groups after TLC. Group 3 (29.4 mg, fractions 7 and 8), after successive preparative TLC run with $CHCl_3$-MeOH (40:1) and with Et_2O-Hex (2:1), afforded **1** (4.0 mg).

*3α-hydroxy-13α,17α,21β-hopan-15,19-dione (**1**)*

It was obtained as a colorless oil; ^1H NMR ($CDCl_3$, 500MHz): Table 2; ^{13}C NMR ($CDCl_3$, 125MHz): Table 3; HREI-MS (70eV), *m/z* (%): 456.3607 (2), 441.3680 (9), 318.2508 (85), 303.2231 (20), 300.2370 (24), 277.2109 (32), 233.1865 (12), 207.1671 (16), 189.1581 (100), 177.1566 (7), 175.1408 (38), 152.1162 (10), 139.1056 (17), 137.1275 (9), 135.1118 (62), 121.0952 (52), 109.0966 (41), 107.0806 (56), 97.0590 (84), 95.0801 (63), 93.0646 (48).

After solvent evaporation, part of the hexanic extract EsAH (467.0 mg) was fractionated by successive preparative TLC run with CH_2Cl_2-MeOH-H_2O (85:15:1). These furnished seven fractions, which were numbered according to their decreasing polarities. Part of the seventh fraction (174.1 mg) was fractionated by successive preparative TLC run with CH_2Cl_2-Hex (9:1). These eleven fractions were numbered according to their decreasing polarities. The fraction 7.11 was purified by preparative TLC continuously run with Hex-Et_2O (39:1) during 100 min to afford **2** (4.0 mg) which was analyzed by its NMR and MS spectra data.

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Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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