Germacranolides from *Eremanthus elaeagnus*

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Por extração das folhas de *Eremanthus elaeagnus* foram obtidos quatro germacranólidos, cujas estruturas foram estabelecidas através de espectrometria de RMN, bem como os flavonóides 3-metoxi-quercetina e luteolina.

Extraction of the leaves of *Eremanthus elaeagnus* furnished four germacranolides, whose structure was established by NMR spectrometry, as well as the flavones quercetin 3-methyl ether and luteolin.

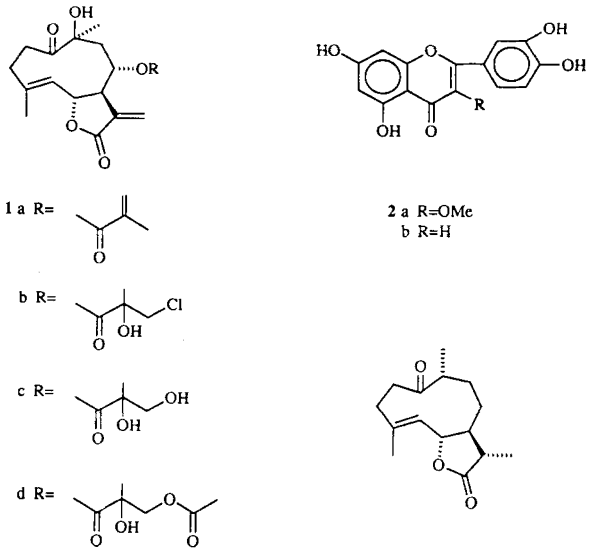
Key words: Eremanthus elaeagnus; *Compositae; Vernonieae; germacranolides.*

Introduction

The Brazilian genus *Eremanthus* (Compositae, Vernonieae) as recently revised by MacLeish[1,2] contains 18 species, eight of which have been studied chemically[3-21]. Particular interest has centered on the schistosomicidal and cytotoxic constituents of the wood of *E. elaeagnus* Sch.Bip[4,7,10] and *E. erythropappa* (DC) MacLeish (syn. *Vanillosmopsis erythropappa* (DC) Sch.-Bip)[5,6,9,19,21], which are sesquiterpene lactones of various types but other *Eremanthus* species also produce similar or identical lactones. In the present article we describe our work on the leaves of *Eremanthus elaeagnus* which have not been studied previously. Isolated were the germacranolides **1**a-d as well as the common flavones **2**a (luteolin) and **2**b (quercetin 3-methyl ether).

The 500 MHz ^{1}H-NMR spectrum (Table 1) of **1**a, $C_{19}H_{24}O_6$, mp (180-182) °C, and selective decoupling permitted deduction of the entire carbon skeleton and stereochemistry. Thus irradiation at the frequencies of H-13a and H-13b whose appearance and chemical shifts (narrowly split doublets at δ 6.21 and 5.67) were characteristic of the γ-hydrogens of an α-methylene-γ-lactone (IR band at 1760 cm^{-1}) simplified the multiplets of H7 at δ 3.27 which was in turn coupled to H-6, a *dd* at 4.60, and H-8, a *ddd* at δ 5.10. Irradiation at the frequency of H-8 simplified not only the signal of H-7 but also the mutually coupled signals of H-9a and H-9b at δ 2.31 and 2.03 which were next to a quaternary center (C-10). Likewise, irradiation at the frequency of H-6 simplified not only the signal of H-7, but also the signal of H-5 at δ 5.17; the latter was allylically coupled to the protons of a vinylic methyl group at δ 1.91 and to the proton of a methylene group (H-3α) at δ 2.84, thus placing the vinylic methyl at C-4. Finally H-3α was not only coupled to its partner 3β, but also the protons of another methylene (H-2α–β) whose chemical shifts (δ 2.73 and δ 2.52 indicated that they were *alpha* to a ketone carbonyl. The presence of the latter was revealed by an IR band at 1698 cm^{-1} and by a signal at δ 215.52 in the ^{13}C-NMR spectrum (Table 2). This necessitated placement of a second methyl group (three proton singlet at δ 1.22) and the tertiary hydroxyl (-OH absorption at δ 3.78), required by the molecular formula, on C-10 and was supported by the ^{13}C-NMR spectrum which in addition to doublets at δ 76.64 and 72.38 (C-6 and C-8) exhibited another signal in the C-O region, a singlet at δ 78.57 (C-10).

Chemical shifts and coupling constants of H-5, H-6, H-7 and H-8 were consonant with those of a 4*E*-germacren-6βH,12-olide containing an α-orientated ester function (IR bond at 1712 cm^{-1}) on C-8; from the mass ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) the ester was obviously a methacrylate.



As for the stereochemistry at C-10 it was originally assumed to be similar to that of other sesquiterpene lactones from *Eremanthus* and related species, i.e. -OH *alpha* if C-10 is depicted in apical form as in formulas **1**a-d. However, while the NOE difference spectrum (Table 3 exhibited the NOE's between H-3α and H-5, H-5 and H-7, H-3β and H-15, H-6 and H-8 and H-6 and H-15 ordinarily displayed by an 8α-acyloxygermacra-1(10),5,11(13)-6βH,12-olide or its equivalents in the usual crown conformation, it also exhibited NOE's between H-15, on the one hand, and H-2β and H-9β on the other and no NOE between the two methyl groups. This requires an arrangement in which the C-10 methyl group is α-orientated and equatorial similar to its orientation in the crow conformation established for the closely related 3 by X-ray analysis[22]. In the conformation adopted by **1**a and its congeners the dihedra angles between H-8 and H-9α,β appear to be somewhat altered from those ordinarily prevailing, thus producing values of $J_{8,9}$ (4 and 3Hz) different from those usually observed (10 and 1 Hz). The same structure, without stereochemistry at C-10, has been ascribed to a non crystalline incompletely characterized lactone from *Vernonia jonesii*[23].

The three other lactones **1**b-**1**d differed from **1**a only in the nature of the C-8 ester side chain whose structures were obvious from the mass, ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2).

Experimental

Extraction of Eremanthus elaeagnus. Leaves of *E. elaeagnus* Sch.Bip. (3.0 kg) were collected in Diamantina, Minas Gerais State, Brasil, in June 1985. The pulverized material was extracted with EtOAc to give 140 g of crude extract which was dissolved in MeOH-H_2O (9:1). After extraction with hexane the aqueous methanol solution was concentrated at reduced pressure and extracted with EtOAc yielding 92.5 g of extract which was chromatographed over 1.5 kg of silica gel, 900 ml fractions being eluted as follows: frs. 1-8 (hexane), 9-12 (hexane-EtOAc, 99:1), 13-16 (hexane-EtOAc, 49:1), 17-19 (hexane-EtOAc, 97:3), 20-23 (hexane-EtOAc, 19:1), 24-30 (hexane-EtOAc, 93:7), 31-35 (hexane-EtOAc, 9:1), 36-45 (hexane-EtOAc, 17:3), 46-52 (hexane-EtOAc, 4:1), 53-60 (hexane-EtOAC, 3:1), 61068 (hexane-EtOAc, 7:3), 69-72 (hexane-EtOAc, 13:7), 73-83 (hexane-EtOAc, 3:2), 84-92 (hexane-EtOAc, 1:1), 93-97 (hexane-EtOAC, 2:3), 980102 (hexane-EtOAc, 1:4), 103-108 (EtOAc), 109-113 (EtOAc-MeOH) and 114-118 (MeOH).

Fr. 11(4.0 g) was rechromatographed over silica gel (100 g). Elution with hexane, EtOAc and EtOH mixtures of increasing polarity afforded 180 mg of a mixture. Further purification by TLC gave 160 mg of pure **1**a. Frs. 15 and 16 were combined (5.9 g) and washed with $CHCl_3$ to give 1.7 g of a yellow mixtures of flavonoids. Pressure chromatography of a 30 mg portion of the mixed flavonoids over a polyamide column and elution with MeOH-EtOAc-HOAc (95:2.5:2.5) gave 12 mg of quercetin 3-methyl ether (**2**a), mp 234°C, identified by MS, UV (MeOH, MeOH-NaOMe, MeOH-$AlCl_3$, MeOH-HCl, MeOH-NaOAc and MeOH-H_2BO_3) and ^{1}H-NMR spectrometry, and 5 mg of luteolin (**2**b) mp. 262°C, identified in the same manner. Concentration of the $CHCl_3$ washings at reduced pressure, chromatography of the residue (4.14 g) over silica gel and elution with hexane, EtOAc and EtOH mixtures of increasing polarity gave 1.2 g of solid which was washed with Et_2O. On being kept in the refrigerator the ether washings deposited 53.6 mg of **1**b.

Dry column chromatography (6 g of silica gel deactivated with 10% H_2O) of fr. 17 (3.10 g) and elution with hexane-EtOAc (2:3) gave 2.11 g of material which on rechromatography over 42.2 g of silica gel and elution with hexane, EtOAc and MeOH mixtures of increasing polarity afforded 52.6 mg of **1**d which after recrystallization from H_2O-MeOH 19:1

Table 1. *^{1}H-NMR spectra of compounds 1a-1d ($CDCl_3$).*

H	1a*	1b**	1c**	1d**
2α	2.52*ddd*(12,4,3.5)	2.3–	2.3–	2.3–
2b	2.73ddd (12,12,3.5)	3.0*m*	3.0*m*	3.0*m*
3α	2.84*brddd*(12,12,3.5)			
3β	2.21*ddd*(12,5,3)			
5	5.17*brdq* (10.5,1.5)	5.15*m*	5.15*m*	5.15*m*
6	4.60*dd* (10.5,9)	4.57*dd*(10.7,10)	4.59*dd*	4.58*dd*
7	3.27*dddd*(10,9,3.5,3)	3.15*dddd* (10.7,6.6,3.3,3)	3.20*dddd*	3.21*dddd*
8	5.10*ddd* (10,4,3)	5.1*m*	5.1*m*	5.1*m*
9α	2.03*dd* (16.5,3)	2.0*m*	2.0*m*	2.0*m*
9β	2.31*dd* (16.5,4)	2.3*m*	2.3*m*	2.3*m*
13a	6.21*d* (3.5)	6.38*d* (3.3)	6.26*d*	6.27*d*
13b	5.67*d*(3)	5.80*d*(3)	5.78*d*	5.84*dd*
14***	1.22*s*	1.28*s*	1.26*s*	1.22
15***	1.91*d*(1.5)	1.91*d* (1.3)	1.90*d*	1.90*d*
3'a	6.22*quint* (1)	4.02*d* (11)	3.95*d* (12)	
3'b	5.69*brs*	3.68*d* (11)	3.57*d* (12)	
4'***	1.99*t* (1)	1.58*s*	1.48*s*	1.50*s*
Ac***		-		2.08*s*
OH	3.78*br*			

* *500 MHz*
** *80 MHz*
*** *Intensity three protons*

Table 2. *^{13}C-NMR spectra of compounds 1a-1d ($CDCl_3$)*

C	1a*	1b*	1c**	1d**
1	215.52s	214.70s	214.27*s*	214.73
2	34.18*t*[a]	34.23*t*[a]	34.20*t*[a]	34.20*t*[a]
3	36.93*t*[a]	35.83*t*[a]	35.89*t*[a]	35.76*t*[a]
4	142.16s	142.06s	142.35s	142.22s
5	124.82*d*	124.72*d*	124.53*d*	124.66*d*
6	76.64*d*	76.12*d*	76.05*d*	76.15*d*
7	47.59*d*	47.34*d*	47.34*d*	47.27*d*
8	72.38*d*	74.69*d*	74.98*d*	74.52*d*
9	35.26*t*[a]	35.77*t*[a]	35.85*t*[a]	35.76*t*[a]
10	78.57*s*	78.73*s*	78.81*s*	75.95*s*
11	136.04*s*[b]	135.95*s*	136.17*s*	135.97*s*
12	169.69*s*	169.30*s*	169.27*s*	169.47*s*
13	123.29*t*	*123.89t*	123.88*t*	123.94*t*
14	29.81*q*	29.31*q*	29.45*q*	29.71*q*
15	18.05*q*	17.51*q*	17.62*q*	17.75*q*
1'	166.26*s*[b]	172.29*s*	174.99*s*	172.78*s*
2'	135.94*s*	74.78*s*	77.45*s*	75.95*s*
3'	126.68*t*	50.68*t*	68.08*t*	68.80*t*
4'	18.05*q*	22.97*q*	21.78*q*	22.30*q*
1"				170.25*s*
2"				20.80*q*

* *at 20 MHz*

** *At 55 MHz*

[a,b] *Assignments in same column with same superscript may be interchanged.*

melted at (152-153) °C. Recrystallization from water of a 300 mg portion of fr. 18 (2.5 g) gave 60 mg of **1c**, mp.(160-162)°C.

(6R, 7R*, 8S*, 10S*)-10-Hydroxy-8-methacryloxy-1-oxogermacra-4,11(13)-dien-6,12-olide* (**1a**). Mp (180-182)°C; MS m/z (rel.int.) 349 ($C_{19}H_{24}O_6^+$+H by intramolecular hydrogen transfer, 30), 331 (6), 262 (100), 244 (47); IR ν cm^{-1} 3440, 1760, 1712, 1698, 1670-1635; ^{1}H-NMR spectrum in Table 1; ^{13}C-NMR spectrum in Table 2.

(6R, 7R*, 8S*, 10S*)-10-Hydroxy-8-(2-methyl-2-hydroxy-3-chloropropanoyloxy)-1-oxo-germacra-4,11(13)-dien-6,12-olide* (**1b**). Mp (163-164)°C; MS m/z (rel.int.) 403 (11) and 401 (35,$C_{19}H_{25}O_7Cl$), 263 (100), 245 (66), 227 (23), 205 (28); IR ν cm^{-1} 3610, 3410, 1760, 1740, 1705, 1665-1625; ^{1}H-NMR spectrum in Table 1; ^{13}C-NMR spectrum in Table 2.

(6R, 7R*, 8S*, 10S*)-10-Hydroxy-8-(2-methyl-2,3-dihydroxy-propanoyloxy)-1-oxogermacra-4,11(13)-dien-6,12-olide* (**1c**). Mp (160-162)°C (from H_2O); MS m/z (rel.int.) 383 ($C_{19}H_{26}O_8^+$+H, by intramolecular hydrogen transfer, 30), 365 (8), 281 (28), 263 (100), 244 (58); IR ν cm^{-1} 3420, 1763, 1722, 1702, 1670-1650; ^{1}H-NMR spectrum in Table 1; ^{13}C-NMR spectrum in Table 2.

(6R, 7R*, 8S*, 10S*)-10-Hydroxy-8-(2-methyl-2-hydroxy-3-acetoxy-propanoyloxy)-1-oxogermacra-4,11 (13)-dien-6,12-olide* (**1d**). Mp (152-153)°C (from H_2O,MeOH 19:1); MS m/z (rel.int.) 425 ($C_{21}H_{28}O_9$, M^++ H by intramolecular hydrogen transfer, 100), 407 (15), 263 (75), 245 (36); IR ν cm^{-1} 3450, 1755, 1735, 1730, 1710, 1670-1650; ^{1}H-NMR spectrum in Table 1; ^{13}C-NMR spectrum in Table 2.

Table 3. *NOE difference spectrum of 1a.*

Irradiated	Observed(%)
H-2β	H-2α(9.1) H-14 (2.7)
H-3α	H-3β (29.3), H-5 (13.1)
H-5	H-3α (5.8), H-7 (14.7)
H-6	H-8 (9.8), H-15 (10.7)
H-7	H-5 (13.3)
H-8	H-6 (6.6), H-9α (1.8), H-9β (2.2)
H-9α	H-8 (3.2), H-9β (12.1)
H-14	H-2β (1.3), H-9β (2.3)

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