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Article

Terpenoids and Steroids from *Trichilia* Species

Mônica T. Pupo[a], M. Angela T. Adorno[b], Paulo C. Vieira*[b], João B. Fernandes[b], M. Fátima das G. F. da Silva[b] and José R. Pirani[c]

[a] *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. Zeferino Vaz, s/n, 14040-903, Ribeirão Preto - SP, Brazil*

[b] *Departamento de Química, Universidade Federal de São Carlos, CP 676, 13565-905, São Carlos - SP, Brazil*

[c] *Instituto de Biociências, Departamento de Botânica, Universidade de São Paulo, São Paulo - SP, Brazil*

O trabalho descreve o isolamento de três novos sesquiterpenóides do extrato metanólico dos galhos de *Trichilia claussenii*: 14-hidroxielemol, germacra-10(14)-en-9,11,15-triol e germacra-3,10(14)-dien-9,11-diol-4-carbaldeído. Também foram isolados os sesquiterpenos β-eudesmol, criptomeridiol e o triterpeno 22,25-diidroxi-9β,19-ciclolanost-23-en-3-ona. Do extrato diclorometânico das folhas de *T. lepidota* foram isolados mistura de hidrocarbonetos ($C_{29}H_{60}$, $C_{31}H_{64}$ e $C_{33}H_{68}$), mistura dos sesquiterpenos epóxido de cariofileno e epóxido de humuleno, espatulenol e os esteróides 24-metileno-12β-hidroxicolesterol, 24-metil-12β-hidroxicolest-4-en-3-ona, 3-palmitato de 24-metil-12β-hidroxicolest-5-eno, mistura de 24-metilenocolesterol, campesterol, estigmasterol e β-sitosterol, além de α-tocoferol e fitol. O extrato metanólico forneceu a *N*-metil-4-hidroxiprolina. As estruturas das substâncias isoladas foram estabelecidas com base em dados espectroscópicos.

The methanol extract of stems of *Trichilia claussenii* yielded three new sesquiterpenoids: 14-hydroxyelemol, germacra-10(14)-en-9,11,15-triol and germacra-3,10(14)-dien-9,11-diol-4-carbaldehyde. Two known sesquiterpenoids β-eudesmol, cryptomeridiol and the triterpenoid 22,25-dihydroxy-9β,19-cyclolanost-23-en-3-one were also isolated. The dichloromethane extract from the leaves of *T. lepidota* afforded a mixture of hydrocarbons ($C_{29}H_{60}$, $C_{31}H_{64}$ and $C_{33}H_{68}$), a mixture of the sesquiterpenes caryophyllene and humulene epoxides, spathulenol, and a series of sterols: 24-methylene-12β-hydroxycholesterol, 24-methyl-12β-hydroxycholest-4-en-3-one, 24-methylene-12β-hydroxycholest-5-ene-3-palmitate; a mixture of 24-methylenecholesterol, campesterol, stigmasterol and β-sitosterol; besides α-tocoferol and phytol. From the methanol extract it was isolated *N*-methyl-4-hydroxyproline. The structures of the isolated compounds were determined on the basis of spectroscopic analysis.

Keywords: *Trichilia claussenii*, *Trichilia lepidota*, Meliaceae, sesquiterpenoids, cycloartane triterpenoid, steroids

Introduction

The Meliaceae family has shown to be of much interest among phytochemists because either it contains plants which produce very complex chemical structures, or because of their biological activity, mainly against insects. As part of our interest in the chemistry of this family, we have already reported the isolation of γ-lactones from the fruits,[1] androstane and pregnane steroids from the steams[2] and cycloartane triterpenoids and a mixture of ω-phenyl alkanoic and alkenoic acids from the leaves[3] of *Trichilia claussenii* collected in Brazil. In continuation of our study of *Trichilia* species, we now report the isolation of five sesquiterpenoids and a cycloartane triterpenoid from the steams of *T. claussenii* and steroids from the leaves of *T. lepidota* Martius ssp *schumanniana* Harms, which has never been investigated before.

Experimental

General experimental procedures

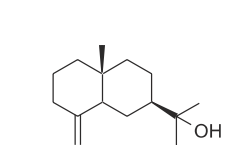
^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.62 MHz, respectively, in a Bruker DRX spectrometer.

* e-mail: paulo@dq.ufscar.br

The spectra were run in $CDCl_3$ with TMS as internal standard, pyridine-d_5 and D_2O. DCCC was performed in an Eyela Droplet Counter Current Chromatograph D.C.C. - 300. The GC-MS analyses were carried out with a Hewlett Packard System 5995 fitted with a dimethylpolysiloxane HP-1 (25 m x 0.20 mm i.d. x 0.30 film tickness) capillary column, with hydrogen as the carrier gas. The temperature was programmed initially at 120 °C for 2 min., then increased with a rate of 8 °C min^{-1} to 280 °C and kept for 20 min. The injection volume was 1 μL in a split mode and temperature of the injector was 280 °C.

Isolation of the constituents from T. claussenii

Stems (2.4 kg) of *T. claussenii* were collected in Rio Claro, SP, Brazil, and a voucher is deposited in the Herbarium of Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP, Brazil. The stems were dried, powdered and extracted with MeOH. The MeOH extract (45.7 g) was suspended in MeOH:H_2O (1:3 v/v) and partitioned with CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2 fraction was concentrated and then partitioned with hexane and MeOH. The MeOH fraction (10.35 g) afforded 9 fractions after dry silica gel column using as eluent hexane:CH_2Cl_2:MeOH (10:10:1 v/v/v). Fr-6 (1.38 g) after flash chromatography (*n*-hexane:CH_2Cl_2:Me_2CO 10:15:1 v/v/v) afforded 14 fractions. Fr-6-7 (73 mg) after flash chromatography (*n*-hexane:EtOAc 9:1 v/v) afforded 10.1 mg of sesquiterpene **1**. Fr-8 after flash chromatography (*n*-hexane:CH_2Cl_2:MeOH 10:10:1 v/v/v, gradient elution) afforded 26 fractions. Column chromatography of Fr-8-11 (89 mg) on silanized (dimethylsilane, 63-200 μm) silica (Me_2CO:H_2O 1:1 v/v) afforded 7 fractions. Fr-8-11-4 after chromatography on silanized silica column (Me_2CO:H_2O 1:1 v/v) yielded 2 fractions. Fr-8-11-4-2 (19.5 mg) was acetylated (Ac_2O-pyridine) and the mixture obtained was purified on flash chromatography (CH_2Cl_2:Me_2CO 19:1 v/v) to yield **3a** (2.5 mg). Fr-8-12 (318 mg) afforded 7 fractions after florisil column (CH_2Cl_2:Me_2CO 19:1 v/v; 9:1 v/v). Column chromatography of Fr-8-12-3 (76 mg) on silanized silica (Me_2CO:H_2O 1:1 v/v, 2:1 v/v) yielded 9 new fractions. Fr-8-12-3-2 (14 mg) after chromatography on silica gel flash column ($CHCl_3$:MeOH 19:1 v/v) afforded 5.5 mg of sesquiterpene **5**. Flash chromatography of Fr-8-12-3-3 (29 mg) ($CHCl_3$:MeOH 19:1 v/v) yielded 14.2 mg of sesquiterpene **3**. Fr-8-12-3-6 (8 mg) after flash chromatography (*n*-hexane:Me_2CO 3:1 v/v) yielded compound **6** (2.9 mg). Column chromatography on silanized silica of Fr-8-12-6 (Me_2CO:H_2O 1:1 v/v) afforded sesquiterpene **2** (6.0 mg). Fr-8-15 (215 mg) afforded, after chromatography on florisil column (CH_2Cl_2:MeOH 19:1 v/v), 6 fractions. 19 mg of Fr-8-15-3 after flash chromatography (CH_2Cl_2:MeOH 23:2 v/v) yielded 11.6 mg of sesquiterpene **4**. Fr-8-15-3 (16 mg) was acetylated



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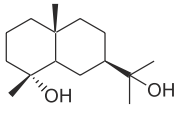

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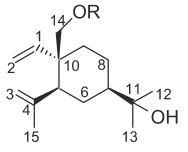
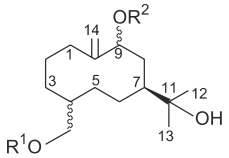
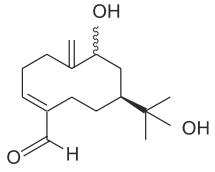


3 R = H
3a R = Ac



4 $R^1 = R^2 = H$
4a $R^1 = R^2 = Ac$
4b $R^1 = Ac, R^2 = H$
4c $R^1 = H, R^2 = Ac$



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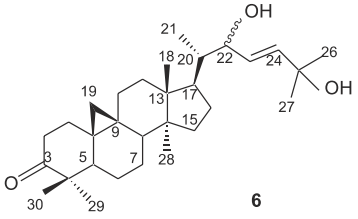

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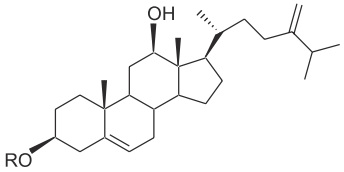
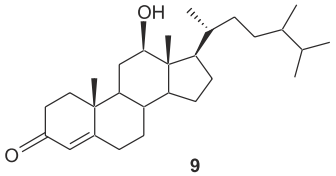


7 R = H
8 R = $CO(CH_2)_{14}CH_3$



9

(Ac_2O-pyridine) and the mixture obtained was purified on silica gel column (CH_2Cl_2:MeOH 19:1 v/v) to yield **4a** (4.7 mg), **4b** (3.4 mg) and **4c** (1.9 mg).

Isolation of the constituents from T. lepidota

Leaves of *T. lepidota* Martius ssp *schumanniana* Harms were collected in Rio de Janeiro, Brazil. The CH_2Cl_2 extract (7.5 g) was chromatographed on silica gel (*n*-hexane, CH_2Cl_2, EtOAc and MeOH). The *n*-hexane fraction (769 mg) afforded a mixture of hydrocarbons. Chromatography of the dichloromethane fraction yielded three fractions. Fr-1 (656 mg), after flash chromatography (*n*-hexane:MeOH 49:1 v/v and *n*-hexane:Me_2CO 99.4:0.6 v/v), was hydrolyzed with KOH (10%) in EtOH followed by chromatography on silica flash (*n*-hexane:Me_2CO 99:1 v/v), to afford 4 mg of phytol. Fr-2 (770 mg), after flash chromatography and then prep-tlc (benzene:ethyl ether 99.6:0.4 v/v), afforded a mixture of sesquiterpenes: caryophyllene and humulene epoxides (7.3 mg) and α-tocopherol (1.3 mg), spathulenol (35.6 mg) and 24-methylen-12β-hydroxycholest-4-en-3-palmitate (3.8 mg) (**8**). Compound **8** was hydrolyzed with KOH (10%) in EtOH to yield **7** and a mixture of lauric, palmitic and stearic acids, which was methylated with diazomethane. Fr-3 (396 mg) was chromatographed on silica gel (*n*-hexane:EtOAc 4:1 v/v) and then on florisil (*n*-hexane:EtOAc 4:1 v/v), to yield 6 mg of a mixture of 24-methylenecholesterol, campesterol, stigmasterol and β-sitosterol. Two fractions eluted with EtOAc were chromatographed on florisil using as eluent CH_2Cl_2 and *n*-hexane:EtOAc (9:1 v/v), to afford **7** (30 mg) and **9** (1.5 mg) that was further purified by recycling-HPLC. The methanol extract was first submitted to a liquid-liquid partition, followed by DCCC of the MeOH fraction (4.4 g) using the mobile and stationary phases obtained from the organic and aqueous layers, respectively, of the mixture $CHCl_3$:MeOH:H_2O (13:7:4 v/v/v), giving 40 fractions of 18 mL each. Column chromatography of fraction 37 on silica gel (CH_2Cl_2:Me_2CO 97:3 v/v) yielded **7** (4.9 mg). Flash chromatography of the *n*-BuOH fraction (500 mg) ($CHCl_3$:MeOH:H_2O 10:10:1 v/v/v) afforded *N*-methyl-4-hydroxyproline (19.2 mg).

Identification of the isolated compounds

14-Hydroxyelemol (**3**): Yellow oil. $[\alpha]_D^{26}$ +4.40° ($CHCl_3$, *c*0.0126). IR ν_{max}/cm^{-1} 3401, 3079, 2967, 2938, 2869, 1639, 1447, 1377, 1033, 910, 757 (KBr). GC-MS: R_t 13.67 min., EIMS *m/z* (rel. int.): 220 [M – H_2O] (1), 201 (3), 189 (24), 159 (8), 147 (23), 133 (23), 119 (25), 105 (35), 91 (47), 79 (49), 59 (100). ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.10 (dd, *J* 17.6, 11.2 Hz, H-1), 5.36 (dd, *J* 17.6, 1.6 Hz, H-2a), 5.20 (dd, *J* 11.2, 1.6 Hz, H-2b), 4.95 (t, *J* 1.6 Hz, H-3a), 4.79 (d, *J* 1.6 Hz, H-3b), 4.29 (d, *J* 10.8 Hz, H-14a), 4.03 (d, *J* 10.8, H-14b), 2.57 (dt, *J* 13.2, 3.2, 3.2 Hz, H-9β), 2.21 (dd, *J* 12.4, 4.0 Hz, H-5), 1.78 (s, Me-15), 1.40 (s, Me-12), 1.39 (s, Me-13). ^{13}C NMR: Table 1.

Germacra-10(14)-en-9,11,15-triol (**4**): Yellow oil. $[\alpha]_D^{26}$ –11.55° (MeOH, *c*0.00308). IR ν_{max}/cm^{-1} 3396, 2928, 2852, 1655, 1461, 1025, 685 (KBr). ^{1}H NMR (pyridine-d_5, 400 MHz) δ 5.66 (br. s, H-14a), 5.27 (br. s, H-14b), 4.59 (br. t, H-9), 3.77 (dd, *J* 10.4, 5.8 Hz, H-15a), 3.66 (dd, *J* 10.4, 6.6 Hz, H-15b), 2.44 (m, H-1), 2.36 (dt, *J* 15.2, 5.2, 5.2 Hz, H-8a), 2.25 (H-4), 2.22 (H-7), 2.19 (H-2a), 1.97 (dt, *J* 15.2, 3.5, 3.5 Hz, H-8b), 1.93 (H-3a), 1.89 (H-2b), 1.73 (H-5a), 1.65 (H-5b), 1.57 (H-6a), 1.45 (H-3b), 1.40 (s, Me-13), 1.30 (s, Me-12), 1.30 (H-6b). ^{13}C NMR: Table 1. HMBC: Table 2.

Germacra-10(14)-en-9,15-diacetate-11-ol (**4a**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.60 (dd, *J* 8.0, 4.0 Hz, H-9), 5.18 (br. s, H-14a), 5.17 (br. s, H-14b), 3.87 (dd, *J* 10.8, 6.4 Hz, H-15a), 3.81 (dd, *J* 10.8, 6.4 Hz, H-15b), 2.05 (s, OCO<u>Me</u>), 2.04 (s, OCO<u>Me</u>), 1.25 (s, Me-13), 1.18 (s, Me-12). ^{13}C NMR: Table 1.

Table 1. ^{13}C NMR data for sesquiterpenes and their acetates (δ, 100 MHz)

C	**3**[a]	**4**[a]	**4a**[b]	**4b**[b]	**4c**[b]	**5**[b]
1	148.0	32.9	33.0	31.6	33.8	35.5
2	113.2	24.2	23.6	22.8	25.0	27.3[c]
3	112.5	33.7	33.1	33.2	34.3	150.0
4	147.7	37.2	33.5	32.3	38.2	142.0
5	53.3	29.5	29.0	29.6	29.8	21.2
6	32.3	28.9	28.3	28.6	28.3	27.4[c]
7	49.8	41.8	42.4	40.2	43.4	40.6
8	23.0	30.7	26.2	30.9	26.2	32.6
9	29.4	72.8	74.2	72.8	74.6	72.8
10	44.6	153.2	146.2	151.7	147.2	148.9
11	71.6	72.2	73.7	73.0	72.7	73.0
12	27.8	30.3	28.1	30.3	28.7	30.6
13	27.4	25.2	26.6	28.2	27.4	24.3
14	61.5	111.2	114.4	113.0	114.6	115.5
15	25.0	67.4	69.2	69.2	67.2	195.2
O<u>C</u>OMe	-	-	171.3	not obs.	-	-
O<u>C</u>OMe	-	-	170.4	-	170.4	-
OCO<u>Me</u>	-	-	21.4	21.0	-	-
OCO<u>Me</u>	-	-	21.0	-	21.2	-

[a] Pyridine-d_5; [b] $CDCl_3$. Multiplicities obtained from DEPT or PENDANT. [c] Signals may be interchangeable.

Germacra-10(14)-en-15-acetate-9,11-diol (**4b**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (br. s, H-14a), 5.15 (br. s, H-14b), 4.31 (m, H-9), 3.87 (dd, *J* 10.8, 6.0 Hz, H-15a), 3.80 (dd, *J* 10.8, 6.4 Hz, H-15b), 2.05 (s, OCO<u>Me</u>), 1.26 (s, Me-13), 1.13 (s, Me-12). ^{13}C NMR: Table 1. *Germacra-10(14)-en-*

9-acetate-11,15-diol (**4c**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.05 (dd, *J* 9.6, 4.4 Hz, H-9), 5.42 (br. s, H-14a), 5.20 (br. s, H-14b), 3.71 (br. s, H-15a), 3.70 (br. s, H-15b), 2.06 (s, OCOMe), 1.40 (s, H-13), 1.37 (s, H-12). ^{13}C NMR: Table 1.

Germacra-3,10(14)-dien-9,11-diol-4-carbaldehyde (**5**): Amorphous solid. $[\alpha]_D^{26}$ –9.97° ($CHCl_3$, *c*0.0065). IR ν_{max}/cm^{-1} 3417, 2966, 2928, 1709, 1684, 1644, 1463, 1377, 756 (KBr). GC-MS: R_t 18.45 min., EIMS *m/z* (rel. int.): 234 [M – H_2O] (3), 219 [M-CH_2O] (6), 216 [M – 2xH_2O] (4), 191 (9), 173 (17), 145 (59), 131 (34), 119 (37), 105 (65), 91 (100), 81 (46), 59 (99). ^{1}H NMR (pyridine-d_5, 400 MHz, δ): 9.38 (d, *J* 1.3 Hz, H-15), 6.47 (dd, *J* 11.7, 4.6 Hz, H-3), 5.12 (br. s, *J* 1.3 Hz, H-14a), 5.06 (br. s, H-14b), 4.26 (t, *J* 3.2 Hz, H-9), 2.80 (ddt, *J* 13.7, 12.0, 12.0, 4.8 Hz, H-2a), 2.58 (H-1a), 2.52 (H-1b), 2.47 (H-5a), 2.42 (H-5b), 2.36 (H-2b), 1.95 (ddd, *J* 16.3, 8.3, 3.2 Hz, H-8a), 1.79 (H-8b), 1.68 (H-6a), 1.42 (H-7), 1.14 (s, Me-12), 1.11 (H-6b), 1.08 (s, Me-13). ^{13}C NMR: Table 1. HMBC: Table 2.

Table 2. HMBC correlations for **4** and **5**

	H	
C	**4**	**5**
1	9, 14a, 14b	2b, 14b
2	-	-
3	15a	1b, 2a, 2b
4	-	2a, 2b, 6a, 15
5	6b, 15a, 15b	15
6	7, 8b	5
7	9, 12, 13	12, 13
8	-	8a, 9
9	7, 14a, 14b	8a, 8b, 9
10	8a, 9	1a, 8a, 9
11	7, 8a, 8b, 12, 13	12, 13
12	13	-
13	12, 13	7
14	9	1a, 9, 14a, 14b
15	15a, 15b	-

22,25-Dihydroxy-9β,19-cyclolanost-23-en-3-one (**6**): Amorphous solid. IR ν_{max}/cm^{-1} 3431, 2924, 2359, 1702, 756, 299 (KBr). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.85 (dd, *J* 15.6, 0.8 Hz, H-24), 5.71 (dd, *J* 15.6, 7.2 Hz, H-23), 4.22 (m, H-22), 2.71 (td, *J* 14.0, 14.0, 6.4 Hz, H-2β), 2.30 (ddd, *J* 14.0, 4.4, 2.4 Hz, H-2α), 1.88 (m, H-1a), 1.53 (m, H-1b), 1.45 (m, H-20), 1.34 (s, Me-26 and Me-27), 1.10 (s, Me-30), 1.05 (s, Me-29), 1.03 (s, Me-18), 0.91 (d, *J* 6.8 Hz, Me-21), 0.86 (s, Me-28), 0.79 (d, *J* 4.4 Hz, H-19a), 0.58 (d, *J* 4.4 Hz, H-19b). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 216.7 (s, C-3), 140.4 (d, C-24), 125.1 (d, C-23), 74.4 (d, C-22), 70.8 (s, C-25), 50.2 (s, C-4), 49.2 (d, C-17), 48.4 (d, C-5), 47.8 (d, C-7), 45.6 (s, C-13), 42.3 (d, C-20), 37.5 (t, C-2), 35.7 (t, C-12), 33.4 (t, C-1), 32.7 (t, C-15), 30.1 (q, C-26), 29.9 (q, C-27), 29.7 (t, C-19), 27.4 (t, C-7), 26.6 (t, C-16), 26.0 (s, C-10), 25.9 (t, C-11), 22.2 (q, C-29), 21.5 (t, C-6), 21.1 (s, C-9), 20.8 (q, C-30), 19.3 (q, C-28), 18.2 (q, C-18), 12.0 (q, C-21). Signal for C-14 was not observed. Multiplicities were obtained from DEPT.

24-methylene-12β-hydroxycholesterol (**7**): Amorphous solid, mp 119-121°. $[\alpha]^{27}_D$ -1.89° (Me_2CO; *c* 0.72); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.1 (s), 140.9 (s), 121.9 (d), 106.3 (t), 79.7 (d), 71.9 (d), 57.5 (d), 54.9 (d), 49.7 (d), 47.9 (s), 42.3 (t), 37.4 (t), 36.8 (s), 34.0 (d), 33.7 (d), 33.6 (d), 32.7 (t), 31.8 (t), 31.5 (t), 30.9 (d), 24.7 (t), 24,0 (t), 22.2 (q), 22.1 (q), 21.8 (q), 19.5 (q), 8.1 (q). The multiplicities were obtained from DEPT. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.30 (br. s, H-6), 4.67 (s, H-28a), 4.61 (s, H-28b), 3.50 (m, H-3), 3.39 (dd, *J* 4.4; 10.8 Hz, H-12).

24-methylene-12β-hydroxycholest-4-en-3-palmitate (**8**): Amorphous solid; EIMS *m/z* (rel. int.): 413 (7), 412 (21), 396 (9), 229 (27), 212 (5), 147 (48), 137 (22), 125 (15), 124 (94), 107 (49), 91 (76); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.38 (br. s, H-6), 4.73 (s, H-28a), 4.69 (dd, H-12), 4.66 (s, H-28b), 4.64-4.58 (m, H-3); IR: $\nu_{max.}$/cm^{-1} 1726 (KBr).

24-methyl-12β-hydroxycholest-4-en-3-one (**9**): Ft-IR: ν_{max}/cm^{-1} 3414, 1685 (film); EIMS *m/z* (rel. int.): 269 (45), 241 (6), 161 (30), 145 (36), 105 (54), 91 (68), 55 (100); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.74 (br. s, H-4), 3.46 (dd, *J* 4.4; 10.8 Hz, H-12).

Results and Discussion

The methanol extract of the stems of *T. claussenii* was submitted to solvent partition followed by several column chromatographies. This procedure led to the isolation and characterization of five sesquiterpenoids: two known eudesmane derivatives, β-eudesmol (**1**), and cryptomeridiol (**2**), one new elemane derivative, 14-hydroxyelemol (**3**), two new germacrane derivatives, germacra-10(14)-en-9,11,15-triol (**4**) and germacra-3,10(14)-dien-9,11-diol-4-carbaldehyde (**5**), and one cycloartane triterpenoid, 22,25-dihydroxy-9β,19-cyclolanost-23-en-3-one (**6**). The structures of the compounds were identified on the basis of their spectral data and comparison with compounds reported in the literature.

The data reported for β-eudesmol[4] and cryptomeridiol[5,6] confirmed the structures for **1** and **2**. Other possible isomers for **2** were ruled out through comparison with authentic compounds reported in the literature.[6,7]

Compound **3** was established from ^{1}H and ^{13}C NMR spectral data. The IR spectrum suggested the presence of hydroxyl groups (3401 cm^{-1}). The ^{13}C NMR spectrum showed 15 carbon signals. The multiplicities of the carbons

determined by PENDANT led to the attribution of 3 C, 3 CH, 6 CH_2 and 3 CH_3. The signals at δ 71.6 (s) and δ 61.5 (d) were attributed to carbinolic carbons, allowing us to propose the molecular formula $C_{15}H_{26}O_2$ for compound **3**. In the MS the parent ion observed, *m/z* 220, does not represent the molecular ion which was expected to be *m/z* 238, due to the loss of one molecule of water. The PENDANT spectrum showed four olefinic carbons at δ 148.0 (d), 147.7 (s), 113.2 (t) and 112.5 (d). The remaining unsaturations suggested that **3** was a monocyclic sesquiterpenoid. The proton signals at δ 1.39 (s) and 1.40 (s) in the ^{1}H NMR spectrum and at δ 27.4 (q), 27.8 (q) and 71.6 (s) in the PENDANT spectrum suggested the presence of a 1-hydroxy-1-methylethyl group, which was confirmed in the mass spectrum by the fragment *m/z* 59. In the ^{1}H-^{1}H COSY experiment of **3**, H-1 (δ 6.10, dd) was coupled to two H-2 (δ 5.20, dd and 5.36, dd). Both H-2 were coupled to each other. These olefinic signals suggested a vinyl group connected to a quaternary carbon. The olefinic protons H-3 (δ 4.95 and 4.79) were not coupled to other protons and the signals for C-3 (δ 112.5, t) and C-4 (δ 147.7, s) suggested an exomethylene group, where a methyl group H-15 (δ 1.78, s) is attached to C-4. These data together with consideration of isoprene rule led us to propose a sesquiterpene elemane derivative for **3**. The ^{1}H NMR spectrum of **3** also showed an AX coupling system (δ 4.29, 4.03, both d, *J* 10.8 Hz) and the ^{13}C NMR showed a carbinolic carbon at δ 61.5 (t), which led us to suggest that methyl group (C-14) was replaced by a hydroxymethylene group. Other carbon data are described in Table 1. The attribution of the carbon signals was based on related elemanes from the literature.[8,9] Acetylation of **3** with acetic anhydride in pyridine provided the monoacetate of the primary alcohol (**3a**). The relative configurations of **3** were proposed on basis on the biogenesis of the elemane derivatives.[10]

Compound **4** was also found to be a monocyclic sesquiterpenoid. The IR spectrum suggested the presence of hydroxyl groups (3396 cm^{-1}). The ^{13} C NMR showed 15 carbon signals. The multiplicities of the carbons determined by PENDANT led to the attribution of 2 C, 3 CH, 8 CH_2 and 2 CH_3, of which 3 were carbinolic carbons: δ 72.8 (d, C-9), 72.2 (s, C-11) and 67.4 (t, C-15). The carbon signals at δ 153.2 (s, C-10) and 111.2 (t, C-14) suggested the presence of an exomethylene group. The molecular formula proposed for **4** was $C_{15}H_{28}O_3$. The ^{1}H NMR spectrum showed the olefinic proton signals at δ 5.66 (br. s, H-14a) and 5.27 (br. s, H-14b). The ^{1}H-^{1}H COSY spectrum showed that both carbinolic protons at δ 3.77 (dd, H-15a) and 3.66 (dd, H-15b) were coupled to each other and coupled to another proton at δ 2.25 (m, H-4). The HMBC experiment showed the correlation of the carbinolic proton at δ 4.59 (br. t, H-9) with the olefinic carbons C-10 and C-14, therefore H-9 should be carbinolic and allylic. The ^{1}H-^{1}H COSY experiment showed that H-9 was coupled with two protons at δ 2.36 (dt, H-8a) and 1.97 (dt, H-8b), which are both coupled to each other and coupled to another proton at δ 2.22 (m, H-7). The HMBC experiment showed that H-9 was also correlated with C-7 (δ 41.8) and C-1 (32.9). The presence of a 1-hydroxy-1-methylethyl group was also characterized by the correlation of the methyl proton signals at δ 1.30 (s, H-12) and 1.40 (s, H-13) with the quaternary carbinolic carbon at δ 72.2 (C-11) in the HMBC experiment and the fragment *m/z* 59 in the mass spectrum. The possible bisabolane structure for compound **4** was ruled out by these correlations in addition to the correlations of C-11 with H-7 and C-7 with methyl protons (H-12 and H-13), which confirmed a germacrane structure for the sesquiterpenoid **4**. The chemical shifts for C-12 and C-13 are very close for compounds **1** ($\Delta\delta$ 0.1), **2** ($\Delta\delta$ 0.6) and **3** ($\Delta\delta$ 0.4), and more distinct for **4** ($\Delta\delta$ 5.1). This difference may be due to the hydrogen bond between the hydroxyl groups at C-9 and C-11, which might fix a preferential conformation. The chemical shifts observed for C-12 and C-13 for acetates **4a** ($\Delta\delta$ 1.5), **4b** ($\Delta\delta$ 2.1), and **4c** ($\Delta\delta$ 1.3) suggested that the hydrogen bond is stronger when hydroxy group at C-9 is not esterified.

The IR spectrum of **5** suggested the presence of hydroxyl (3417 cm^{-1}) and α,β-unsaturated carbonyl (1684 cm^{-1}) groups. The ^{1}H NMR spectrum showed two methyl groups at δ 1.14 (s, H-12) and 1.08 (s, H-13), one carbinolic and allylic proton at δ 4.26 (t, H-9) and three olefinic protons at δ 6.47 (dd, H- 3), 5.12 (br. s, H-14), and 5.06 (br. s, H-14). A signal at δ 9.38 (d, H-15) suggested the presence of an aldehyde group. The ^{13}C NMR spectrum of **5** showed 15 carbon signals. The multiplicities of the carbons determined by DEPT led to the attribution of: 3 C, 4 CH, 6 CH_2 and 2 CH_3. Four olefinic carbons were observed at δ 150.0 (d, C-3), 142.0 (s, C-4), 148.9 (s, C-10), and 115.5 (t, C-14), indicating the presence of two double bonds, including one exomethylene group. The signal at δ 195.2 (d, C-15) confirmed the presence of an aldehyde group. Two carbinolic carbons were observed at δ 72.8 (d, C-9) and 73.0 (s, C-11). The molecular formula proposed for **5** was $C_{15}H_{24}O_3$. In the MS the parent ion observed, *m/z* 234, does not represent the molecular ion which was expected to be *m/z* 252, due to the loss of one molecule of water. Compound **5** was also found to be a germacrane sesquiterpenoid and the spectral data suggested the presence of one 1-hydroxy-1-methylethyl group, similar to the other sesquiterpenoids described before. The chemical shifts for the olefinic proton (δ 6.47) and olefinic

methine carbon (δ 150.0) suggested that this double bond should be conjugated with the aldehyde carbonyl group. The location of the double bond between C-4 and C-5 was ruled out mainly through analysis of the NMR data. The ^{1}H-^{1}H COSY experiment showed that the olefinic proton (δ 6.47, H-3) was coupled to two allylic protons at δ 2.80 (H-2a) and 2.36 (H-2b). These allylic protons are also coupled to another two allylic protons at δ 2.58 (H-1a) and 2.52 (H-1b). These couplings are not possible for the double bond $\Delta^{4,5}$.

The complete assignment of the protons and carbons of the new sesquiterpenoids **4** and **5** was done on basis on HMBC, HMQC and ^{1}H-^{1}H COSY data, Table 1 and 2. A closely related germacrane-type sesquiterpenoid of **5**, 6-hydroxy-5-methylene-8-isopropenylcyclodec-1-enecarbaldehyde, has been recently described and isolated from *Leitneria floridana* (Leitneriaceae).[11]

It is proposed that elemanes, germacranes and eudesmanes might share the same biogenetic pathway, which involves Cope rearrangement of germacranes to elemanes.[10] The Cope rearrangement of germacranolides to elemanolides has been investigated.[12] Eudesmanolides were also produced in this approach. The co-occurrence of elemanes, germacranes and eudesmanes in *T. claussenii* is a good evidence that the biogenetic pathway of these sesquiterpenoids involves a single precursor.

The IR spectrum of **6** suggested the presence of hydroxyl (3431 cm^{-1}) and carbonyl (1702 cm^{-1}) groups. The ^{13}C NMR spectrum showed 30 carbon signals. The multiplicities of the carbons determined by DEPT 135^o led to the attribution of: 7 C, 7 CH, 9 CH_2 and 7 CH_3, which suggested that **6** was a triterpenoid. The ^{1}H NMR spectrum showed an AB coupling system at δ 0.79 and 0.58 (both d, J 4.4 Hz), characteristic for H-19 of the cyclopropane ring of the cycloartane triterpenoids. In the ^{13}C NMR spectrum, the signal at δ 216.7 (s) suggested the presence of a ketonic carbonyl group at C-3. The signals of the α-carbonyl protons were observed at δ 2.30 (ddd, J 2.4, 4.4, 14.0 Hz, H-2α) and 2.71 (dt, J 6.4, 14.0, 14.0 Hz, H-2β). The olefinic proton signals of the side chain were observed at δ 5.85 (dd, H-24) and 5.71 (dd, H-23). The chemical shift of H-22 (δ 4.22) suggested that this proton should be carbinolic and allylic. The ^{1}H-^{1}H COSY experiment showed that H-22 was coupled with two olefinic protons at δ 5.85 and 5.71, and with another proton at δ 1.45 (H-20). In the ^{1}H-^{1}H COSY experiment, the olefinic protons were coupled to each other and to the carbinolic proton. The quaternary carbinolic carbon signal at δ 70.8 led us to propose one hydroxyl group at C-25. A similar compound has been reported as a diglucosylcycloartane showing the same side chain of **6**.[12] We have already reported the isolation and identification of cycloartane triterpenoids from the leaves of *T. claussenii*.[3]

Chromatography of the dichloromethane extract from leaves of *T. lepidota* yielded a mixture of hydrocarbons as the less polar compounds, besides sesquiterpenoids and several steroids. The mixture of hydrocarbons was analyzed through GC/MS, leading to the identification of three main components $C_{29}H_{60}$, $C_{31}H_{64}$ and $C_{33}H_{68}$.

The ^{1}H NMR spectrum of the steroid **7**, 24-methylene-12β-hydroxycholesterol, showed a pair of broad singlets (δ 4.61 and 4.67), characteristics of a double bond in the side chain and signals of carbinolic protons (dd, δ 3.39 and m, δ 3.50); this was confirmed by its ^{13}C NMR spectrum (δ 79.7 and 71.9). These data allowed us to propose the structure of a steroid for this molecule, which contains two hydroxyl groups, one of those located at C-3. The location of the second hydroxyl was based on the ^{13}C NMR spectrum, where a signal at δ 8.1 (Me) was attributed to C-18, requiring the hydroxyl attached to C-12. Moreover, the hydroxyl should also occupy a β position, being *cis* to Me-18. After acetylation, the ^{1}H-^{1}H COSY experiment showed crosspeaks between a doublet (Me, δ 0.89) and a multiplet (δ 1.4), two methyl signals (d, δ 1.03) with an allyl proton (δ 2.34). These observations led us to propose a 24-double bond; the double doublet at δ 3.40 showed a shift to 4.70, with J 6.8 and 11.2 Hz, which confirms the β-configuration of the 12-OH. This was corroborated by the presence of a γ-gauche effect between the hydroxyl group on C-12 and the methyl group (C-18, δ 8.1). The steroid **8** showed to be very closely related to **7**. The main difference observed in the ^{1}H NMR of **8**, when compared to **7**, is the down field shift observed in the signal of H-3 (m, δ 4.61) and an extra signal (br. s, δ 1.26) indicating a linear long methylene chain. Compound **8** was hydrolyzed under basic conditions yielding **7**, besides a mixture of fatty acids, which was methylated with diazomethane and analyzed by GC/MS. The methyl esters of palmitic, lauric and stearic acid where identified in the mixture in a ratio obtained by GC of 8:1:1. The steroid **9** was also very closely related to **7** and **8**; the main difference found in the ^{1}H NMR spectrum was the replacement of the hydroxyl at C-3 by an α,β-unsaturated carbonyl system, like stigmast-4-en-3-one[14]. The IR spectrum confirmed the presence of the carbonyl group (1680 cm^{-1}). Therefore, this compound was identified as 24-methyl-12β-hydroxy-cholest-4-en-3-one (**9**). The other steroids were identified mainly by ^{1}H NMR and GC/MS data, through comparison with the literature[15] as 24-methylenecholesterol, campesterol, stigmasterol and β-sitosterol.

The aminoacid *N*-methyl-4-hydroxyproline was also isolated from *T. lepidota*. Comparison of the data obtained

and the literature[16] showed the identity of both compounds. The sesquiterpenes spathulenol and a mixture of caryophyllene epoxide with humulene epoxide,[17] were also isolated from the same plant.

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