Artigo

OTHER COMPOUNDS ISOLATED FROM *Simira glaziovii* AND THE ^{1}H AND ^{13}C NMR CHEMICAL SHIFT ASSIGNMENTS OF NEW 1-*EPI*-CASTANOPSOL[#]

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A new triterpene, 1-*epi*-castanopsol, besides eleven known compounds: sitosterol, stigmasterol, campesterol, lupeol, lupenone, simirane B, syringaresinol, scopoletin, isofraxidin, 6,7,8-trimethoxycoumarin and harman, were isolated from the wood of S*imira glaziovii*. The structures of the known compounds were defined by 1D, 2D ^{1}H, ^{13}C NMR spectra data analyses and comparison with literature data. The detailed spectral data analyses allowed the definition of the structure of the new 1-*epi* isomer of castanopsol and performance of ^{1}H and ^{13}C NMR chemical shift assignments.

Keywords: ^{1}H and ^{13}C-NMR; 1-*epi*-castanopsol; *Simira glaziovii*.

INTRODUCTION

The species of the *Simira* genus (Rubiaceae) have been investigated mainly due to the phototoxic activities of some of their isolated compounds.[1] *Simira glaziovii* is popularly known as "arariba" in the Atlantic Rainforest and is used for public afforestation.[2] In previous work, the isolation and structural identification of a mixture of fatty acids, methyl ester, sitosterol, stigmasterol, stigmastenone, sitostenone glucopiranosylsitosterol, acetyl butirospermol, acetyl euphol, carbohydrate mixture, *trans*-4-hydroxy-3-methoxycinnamate, besides harman and ophiorine B alkaloids isolated from the bark and leaves of *Simira glaziovii* were reported.[3,4] New derivatives of ophiorine B isolated from this specie were also described.[4] The diterpenes simirane A and simirane B, besides other compounds, were isolated from *S. eliezeriana*.[5] The harman alkaloid is considered a chemotaxonomic marker of *Simira* genus.[6,7]

In addition, this paper reports the isolation and structural identification of other compounds, campesterol, lupeol, lupenone, lignan syringaresinol, three coumarins and 1-*epi*-castanopsol, besides the diterpene simirane B, identified by the additional study of *S. glaziovii*. The occurrence of diterpene, lignan, coumarins and the 1-*epi*-castanopsol are described for the first time in *Simira* genus. The detailed analyses of 1D and 2D NMR, including special techniques, allowed performance of unambiguous ^{1}H and ^{13}C NMR data assignments for the new castanopsol epimer.[8]

RESULTS AND DISCUSSION

The chromatographic fractionation of the bark extract of *S. glaziovii* led to the isolation and identification of sitosterol, stigmasterol, campesterol (**1**-**3**), lupeol (**4**), lupenone (**5**), 1-*epi*-castanopsol (**6**), simirane B (**7**), lignan syringaresinol (**8**), three coumarins (**9**-**11**) and harman (**12**) (Figure 1). The compounds **6**-**11** are described for the first time in *Simira* genus. The structures of **1-3**, **4**, **5**, **7**, **8**, **9**, **10**+**11** and **12** were defined by NMR and mass spectra analyses and comparison with literature data.[5,7,9-12] The ^{1}H and ^{13}C NMR spectra, together with GC/MS data analysis, were used to identify the steroids (**1**-**3**), triterpenes (**4**, **5**), and coumarins (**10**-**11**).

The molecular formula $C_{30}H_{50}O_2$ of 1-*epi*-castanopsol (**6**) was defined by HRMS-ESI ([M-H] *m/z* 441.3783 calc for $C_{30}H_{49}O_2$, *m/z* 441.3732, $\Delta_{m/z}$ 5.1 ppm). The ^{1}H NMR spectra showed a characteristic profile of a pentacyclic triterpenoid, with six simplets at δ_H 0.81, 0.85, 0.89x2, 1.01x2, 1.02, and 1.17 attributed to methyl groups; two signals at δ_H 3.27 (1H, *dd*, *J* = 12.4; 5.2 Hz, H-3) and 3.43 (1H, *dd*, *J* = 11.6; 4.8 Hz, H-1) attributed to two hydrogens of oxygenated carbon and a hydrogen of double bond at δ_H 5.17 (1H, *t*, *J* = 7.6 Hz, H-12). The correlations observed in the ^{1}H-^{1}H COSY spectrum enabled construction of the spin system of protons in CH-1 and CH-3. The absorption at 3437 cm^{-1} of the hydroxyl group, and 1639 cm^{-1} of the double bond in the IR spectrum corroborated the ^{1}H and ^{13}C NMR data. The ^{13}C NMR spectrum showed 30 signals. The analyses of ^{13}C-DEPTQ and HSQC NMR experiments allowed the assignment of eight methyl groups at δ_C 11.2, 15.2, 17.1, 23.7, 25.9, 27.9, 28.4 and 33.3, and two signals of double bond at δ_C 122.2 and 144.4, justifying the pentacyclic triterpene, Table 1.[8,9] The signal correlations of $^{1}J_{CH}$ of δ_H/δ_C 3.27/75.9 and δ_H/δ_C 3.43/79.6, observed in HSQC spectrum,

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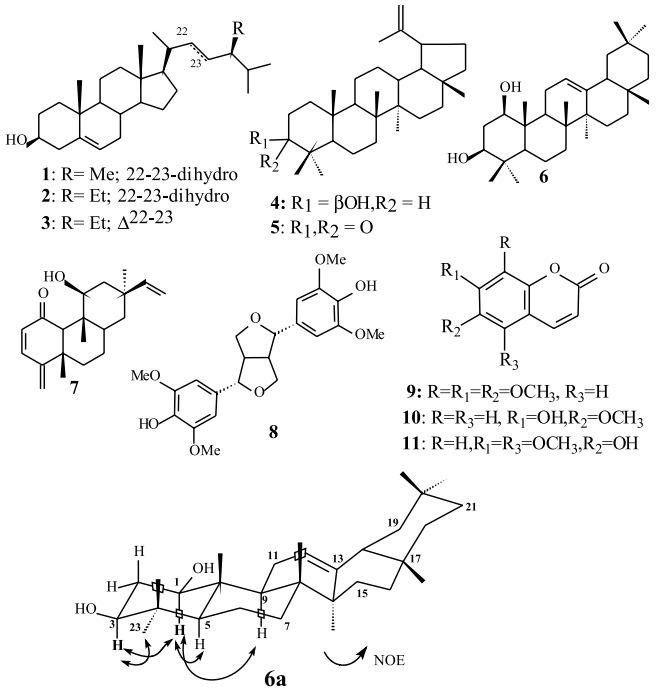

Figure 1. *Structures of compounds isolated from Simira glaziovii*

confirmed the presence of two carbinolic carbons. This proposed presence was supported by the HMBC diagram analyses that showed cross-peaks 3H-23/CH-3, 3H-24/CH-3, 3H-25/CH-1 as well as others listed in Table 1. The shielded signal at δ_C 11.2 (CH_3-25) and δ_C 15.2 (CH_3-24), both due to γ-gauche interaction and the deshielded signal at δ_C 75.9 (CH-3), along with the multiplicity and coupling constant values for H-1 and H-3 observed in ^{1}H NMR spectrum (Table 1), indicates the relative configuration of 1β,3β-dihydroxy groups. The relative configuration was confirmed by NOEDIFF spectra analyses performed by irradiating at H-1, revealing NOE on (H-3, H-5, H-9) and at H-3 observing NOE on (H-1, 3H-23), as depicted in **6a**, Figure 1. Based on the above detailed analyses, all observed carbon and hydrogen chemical shifts of **6** were correlated, including relative stereochemistry of its 1β,3β-dihydroxy groups in the olean-12-ene, with $[\alpha]_D^{25}$ = +29º (*c* 0.0017, $CHCl_3$), an epimer of castanopsol.[8]

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **6**

C	HSQC		HMBC	
	δ_C[a]	δ_H[a,b]	$^2J_{H\to C}$	$^3J_{H\to C}$
1	79.0	3.43 *dd* (12.1, 6.6)		3
2	37.1	1.64(*m*); 1.82(*m*)	1, 3	
3	75.9	3.27 *dd* (12.4, 5.2)		1
4	38.7	-		
5	53.0	0.63 *db* (9.6)		
6	18.1	1.50(*m*); 1.75(*m*)		
7	32.6	1.49(*m*); 1.35(*m*0		
8	43.1	-		
9	48.4	2.12(*m*)		
10	40.3	-		
11	22.7	2.10 (*m*); 2.25(*m*)	12	13
12	122.2	5.23 *t* (7.6)	13	
13	144.4	-		
14	41.5	-		
15	26.2	0.97(*m*);1.18(*m*)		
16	27.4	1.73(*m*)		
17	32.5	-		
18	46.9	1.95(m*)*		
19	46.8	1.00(*m*); 1.65(*m*)		
20	31.1	-		
21	34.7	1.10-1.35(*m*)		
22	37.6	1.17(*m*);1.30(*m*)		
23	27.9	1.01 *s*	4	3, 5, 24
24	15.2	0.81 *s*	4	3, 5, 23
25	11.2	1.02 *s*	10	1, 5, 9
26	17.0	1.01 *s*	8	14, 9, 7
27	25.9	1.17 *s*	14	8, 13, 15
28	28.4	0.85 *s*	17	18, 16, 22
29	33.3	0.89 *s*	20	19, 21, 30
30	23.7	0.89 *s*	20	19, 21, 29

[a]$CDCl_3$, [a]Multiplicity (*J*) in Hertz, δ_H defined by $^1J_{H\text{-}C}$ and ^{1}Hx^{1}H-COSY.

EXPERIMENTAL

General

Optical rotation was recorded on a Perkin-Elmer 343 polarimeter at the sodium-D line. IR data was obtained on a FTIR Vertex 70 Bruker device. A low resolution mass spectrum was produced on a Shimadzu GC-MS-QP2010 Plus, and the HRMS spectrum on a Shimadzu TOF spectrometer equipped with an ESI source in positive and negative modes. Column chromatography (CC) was performed using silica gel (Merck). Pre-coated TLC sheets (Merck or Sorbent) of silica gel 60 GF254 and RP F254 (0.25 mm) were used, and after elution were revealed with vanillin (1%) in H_2SO_4 (5%).

The ^{1}H proton, ^{13}C NMR spectra, DEPTQ, ^{1}H-^{1}H-COSY, HSQC, HMBC, and NOEDIFF experiments, were recorded on a Bruker spectrometer Avance III™ (400 MHz for ^{1}H, and 100 MHz for ^{13}C).

Plant material

The trunk of a specimen of *S. glaziovii* (K. Schum.) Steyermark was collected in the Atlantic Rainforest of the Companhia Vale do Rio Doce (CVRD), in Linhares city, Espírito Santo State, Brazil, and was identified by D. A. Folly. A voucher specimen (CVRD 5004) was deposited at the company's herbarium.

Extraction and isolation

The dried and powdered wood (5.83 kg) was extracted for 72 h with 4.0 L of methanol at room temperature and furnished 430 g of crude MeOH extract after solvent evaporation. This extract was dissolved in MeOH/H_2O (7:3, *v/v*) and partitioned in CH_2Cl_2. The residue of the fraction in CH_2Cl_2 (10.3 g) was submitted to a silica gel column and eluted with a gradient of increasing polarity with hexane/ethyl acetate, furnishing four fractions (FrA-FrD). The fraction FrA (1.6 g) was fractionated on a silica gel column with a gradient of hexane/ethyl acetate as the solvent. A solid (80 mg) was obtained and identified as a mixture of sitosterol (**1**), stigmasterol (**2**) and campesterol (**3**), by ^{1}H and ^{13}C NMR spectra and GC-MS analyses. The same FrA, also yielded two triterpenes, lupeol (**4**, 70

mg) and lupenone (**5**, 20 mg).[9] The FrB (1.20 g) was submitted to a silica gel column and, after elution with gradient of polarity system hexane/acetone, yielded four fractions (FrB1-FrB4). The FrB2 (0.45 g) was fractionated on a flash chromatographic column of silica gel, using the system hexane/acetone (9:1, *v/v*) as mobile phase, resulting in the isolation of 1-*epi*-castanopsol (**6**, 22.5 mg) and simirane B (**7**, 15 mg).[5,8] The fraction FrB3 (0.27 g) was submitted to the same chromatographic procedure as FrB2 and led to isolation of the lignan syringaresinol (**8**, 25 mg) and 6,7,8-trimethoxycoumarin (**9**, 7.0 mg).[10,11] The fraction FrC (1.5 g) was submitted to a silica gel column, using hexane/acetone in gradient of polarity as mobile phase, resulting in the isolation of the coumarin mixture isofraxidin + scopoletin (**10+11**, 8.0 mg) and the chemotaxonomic marker of *Simira* genus, the harman alkaloid (**12**, 10 mg).[6,7,12]

*Compound name: Olean-12-ene-1*β*, 3*β*-diol (**6**)*

$[\alpha]_D^{25}$ +29º (*c* 0.0017, $CHCl_3$); IR: ν_{max} (KBr, cm^{-1}): 3437, 2923, 2854, 1639, 1462, 1382, 1099, 1037, 1006. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz $CDCl_3$): see Table 1; LRMS (EI, 70 eV): *m/z* (%) = 424.5 [M – H_2O, (72)], 218.3 (36), 203.5 (100), 258.0 (18); HR-MS-ESI-MS (positive ion mode) at: *m/z* 443.3835 ($[M + H]^+$, calc. for $C_{30}H_{51}O_2$, 443.3889); *m/z* 441.3783 ($[M - H]^+$ for $C_{30}H_{49}O_2$, calc 441.3732); *m/z* 465.3744 ($[M+Na]^+$ for $NaC_{30}H_{50}O_2$, calc 465.3708).

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