

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

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A new triterpene, 1-*epi*-castanopsol, besides eleven known compounds: sitosterol, stigmaterol, campesterol, lupeol, lupenone, simirane B, syringaresinol, scopoletin, isofraxidin, 6,7,8-trimethoxycoumarin and harman, were isolated from the wood of *Simira glaziovii*. The structures of the known compounds were defined by 1D, 2D ^1H , ^{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 ^1H and ^{13}C NMR chemical shift assignments.

Keywords: ^1H 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, stigmaterol, 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 ^1H 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, stigmaterol, 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 ^1H 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 $\text{C}_{30}\text{H}_{50}\text{O}_2$ of 1-*epi*-castanopsol (**6**) was defined by HRMS-ESI ($[\text{M}-\text{H}]^- m/z$ 441.3783 calc for $\text{C}_{30}\text{H}_{49}\text{O}_2$, m/z 441.3732, $\Delta_{m/z}$ 5.1 ppm). The ^1H NMR spectra showed a characteristic profile of a pentacyclic triterpenoid, with six multiplets 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 ^1H - ^1H 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 ^1H 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 $^1J_{\text{CH}}$ of $\delta_{\text{H}}/\delta_{\text{C}}$ 3.27/75.9 and $\delta_{\text{H}}/\delta_{\text{C}}$ 3.43/79.6, observed in HSQC spectrum,

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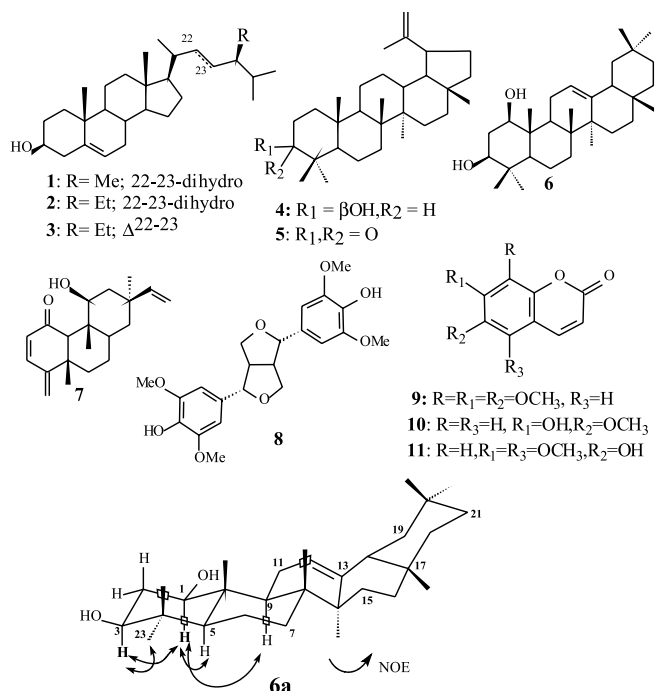


Figure 1. Structures of compounds isolated from *Simira glaziovii*

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 (CH₃-25) and δ_c 15.2 (CH₃-24), 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 ¹H NMR spectrum (Table 1), indicates the relative configuration of 1 β ,3 β -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 1 β ,3 β -dihydroxy groups in the olean-12-ene, with $[\alpha]_D^{25} = +29^\circ$ (*c* 0.0017, CHCl₃), an epimer of castanopsol.⁸

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 H₂SO₄ (5%).

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

Plant material

The trunk of a specimen of *S. glaziovii* (K. Schum.) Steyermark was collected in the Atlantic Rainforest of the Companhia Vale do

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

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

^aCDCl₃, ^bMultiplicity (*J*) in Hertz, δ_H defined by ¹J_{H-C} and ¹H¹H-COSY.

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/H₂O (7:3, v/v) and partitioned in CH₂Cl₂. The residue of the fraction in CH₂Cl₂ (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 ¹H and ¹³C 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)

$[\alpha]_D^{25} +29^\circ$ (c 0.0017, CHCl₃); IR: ν_{\max} (KBr, cm⁻¹): 3437, 2923, 2854, 1639, 1462, 1382, 1099, 1037, 1006. ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (100 MHz CDCl₃): see Table 1; LRMS (EI, 70 eV): m/z (%) = 424.5 [M - H₂O, (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 C₃₀H₅₁O₂, 443.3889); m/z 441.3783 ([M - H]⁺ for C₃₀H₄₉O₂, calc 441.3732); m/z 465.3744 ([M+Na]⁺ for NaC₃₀H₅₀O₂, calc 465.3708).

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