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Article

Angelocunhol: New Erythroxylane Diterpene and Other Compounds from *Simira sampaioana* (Rubiaceae)

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The first *Simira sampaioana* (Rubiaceae) phytochemical study allowed the isolation and structural determination of a new erythroxylane diterpene named Angelocunhol, 11β,12α-dihydroxy-2,4(18),15-eritroxilatrien-1-one, together with 14 known compounds: simirane B, harman, maxonine, isomalindine, malindine, sitost-4-en-6-ol-3-one, estigmast-4,22-dien-6-ol-3-one, campest-4-en-6-ol-3-one, sitost-4-en-3-one, stigmast-4,22-dien-3-one, campest-4-en-3-one, β-sitosterol, stigmasterol, and stigmast-4,22-dien-3-ol from the wood of a specimen of the species. The structures of these compounds were elucidated on the spectroscopic-data analysis basis, mainly ^{1}H and ^{13}C nuclear magnetic resonance (NMR), including 2D experiments (^{1}H-^{1}H correlation spectroscopy (COSY), nuclear Overhauser spectroscopy (NOESY), heteronuclear multiple-bond correlation-HMBC and heteronuclear single-quantum correlation-HSQC), and high-resolution electrospray mass spectrometry (HRESI-MS).

Keywords: *Simira sampaioana*, Rubiaceae, diterpene, alkaloid, steroid

Introduction

Species from *Simira* genus of Rubiaceae family have been investigated mainly due to the biological activities. Many of these species have been used by diverse communities as coloring producers, antifebrile, tonic and purgative substances, and by the phototoxic activities presented by some of their chemical constituents.[1-4] *Simira sampaioana* (synonyms *Sickingia sampaioana* in the Atlantic Rainforest) is known by its common names "ararіba", "canela-samambaia", "marfim" and "maiate", and economic interest is justified by use as timber and for the afforestation of streets.[1,5] As the first phytochemical study involving *S. sampaioana*, this article describes the isolation and structural characterization of the new diterpene 11β,12α-dihydroxy-2,4(18),15-eritroxilatrien-1-one, named Angelocunhol (**1**), together with 14 known compounds: simirane B (**2**), harman (**3**), maxonine (**4**), isomalindine (**5**), malindine (**6**), sitost-4-en-6-ol-3-one (**7**), estigmast-4,22-dien-6-ol-3-one (**8**), campest-4-en-6-ol-3-one (**9**), sitost-4-en-3-one (**10**), stigmast-4,22-dien-3-one (**11**), campest-4-en-3-one (**12**), β-sitosterol (**13**), stigmasterol (**14**), and stigmast-4,22-dien-3-ol (**15**) (Figure 1) from the wood of the plant. The structures of all compounds were characterized by 1D and 2D nuclear magnetic resonance (NMR) techniques, and also the high-resolution electrospray ionization mass spectrometry (HRESI-MS), infrared spectroscopy (IR) and comparisons with known compounds available in literature data.

Results and Discussion

Angelocunhol (**1**) was obtained as yellow oil. Its IR spectrum exhibited bands at ν_{max} 3362 (broad, ν_{OH}) and 1649 cm^{-1} ($\nu_{C=O}$ of an α,β-unsaturated carbonyl group). The molecular formula was assigned as $C_{20}H_{28}O_3$, based on the *quasi*-molecular peak at *m/z* 339.1934 ($[M + Na]^+$, calcd. for $C_{20}H_{28}O_3Na$, *m/z* 339.1951) revealed by the HRESI-MS (positive mode). This molecular formula suggested a diterpene skeleton (Scheme 1) with seven degrees of unsaturation. Three methyl groups were

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Dedicated to Prof Angelo da Cunha Pinto (*in memoriam*) for his outstanding contributions to the field of Organic Chemistry in Brazil. This article was submitted to the special issue dedicated to Professor Angelo da Cunha Pinto and name Angelocunhol is also additional homage.

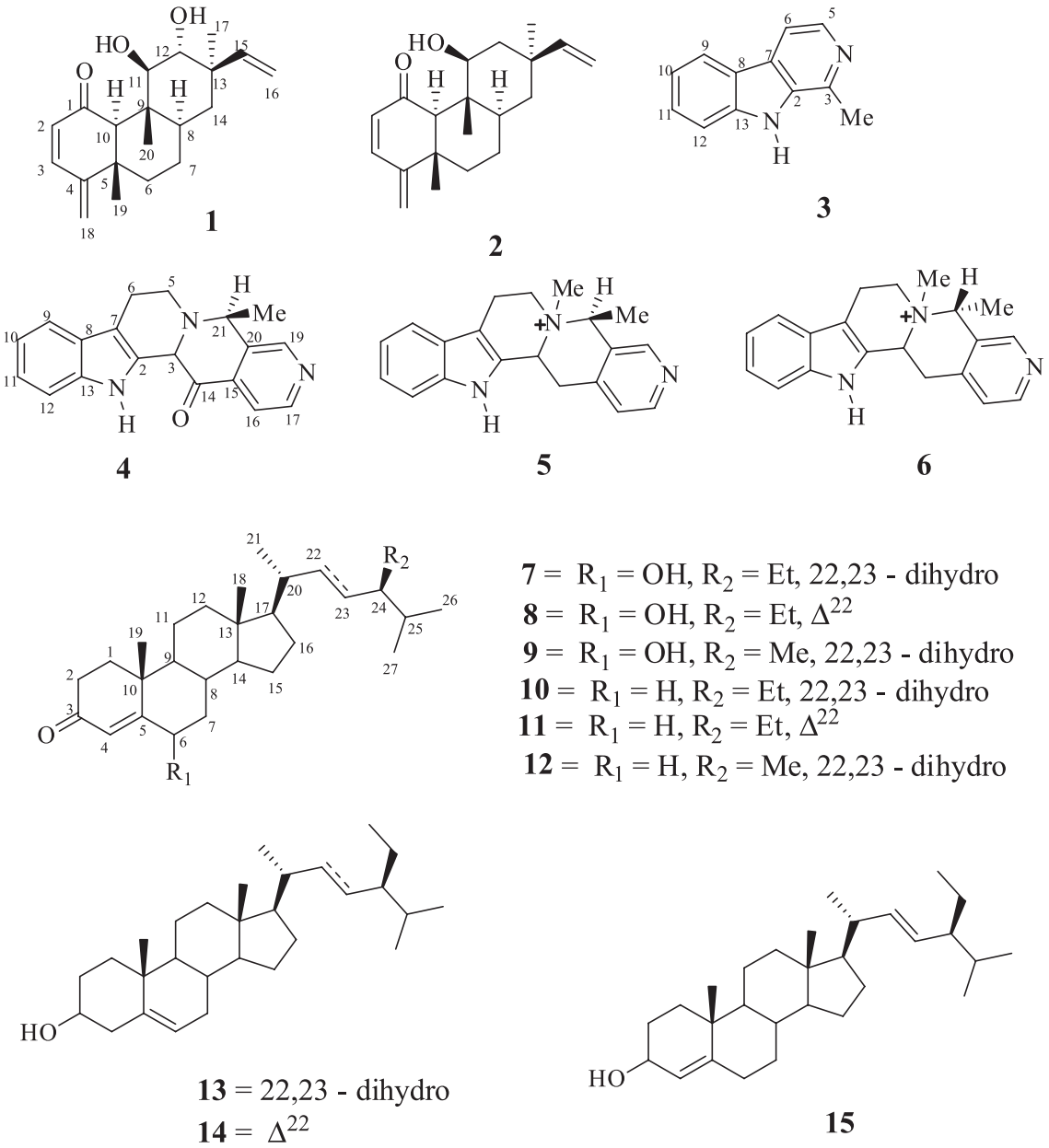


Figure 1. Compounds isolated from *S. sampaioana*.

identified by single signals at δ_H 1.24 (s, 3H-19), 1.21 (s, 3H-20) and 1.09 (s, 3H-17) in the ^{1}H NMR spectrum; the presence of an exocyclic double bond (=CH_2-18) and a vinyl group was deduced by signals at δ_H 5.33 (s, H-18a) and δ_H 5.32 (s, H-18b), indicating an AB system, and δ_H 5.90-5.97 (m, H-15), δ_H 5.08 (dd, *J* 17.5, 1.1 Hz, H-16a) and δ_H 5.03 (d, *J* 10.8, 1.1 Hz, H-16b) compatible with an ABX system of vinyl group. Two doublet signals (*J* 9.8 Hz) corresponding to olefinic hydrogens at δ_H 5.98 and δ_H 6.98 were attributed to H-2 and H-3, respectively. The distortionless enhancement by polarization transfer with retention of quaternaries (DEPTQ) ^{13}C NMR spectrum allowed to recognize signals corresponding to 20 carbon atoms (Table 1): three methylics, five methylenics (including two sp^2 at δ_C 117.6 and 111.3), seven methynics (including three sp^2 at δ_C 147.6, 145.8 and 127.8 and two sp^3 oxygenated at δ_C 79.8 and 76.9) and five quaternary (including one of ketone carbonyl at δ_C 205.9), allowing to establish the expanded molecular formula $C_{20}H_{26}O_3$ and the two hydrogen atoms needed to complete the molecular formula $C_{20}H_{28}O_3$ justified by two hydroxyl groups. These hydroxyl groups were located at CH-11 and CH-12 by the signals at δ_H 3.23 (d, *J* 9.0 Hz, H-11) and 3.52 (d, *J* 9.0 Hz, H-12) revealing vicinal spin-spin interaction each other in the ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear correlations in

the heteronuclear single-quantum correlation (HSQC) ($^1J_{CH}$) spectrum with the ^{13}C signals at δ_C 79.8 (CH-11) and 76.9 (CH-12) and in the heteronuclear multiple-bond correlation (HMBC) these ^{13}C signals showed long-range heteronuclear correlations of the CH-11 (δ_C 79.8) with H-10 (δ_H 2.60, $^3J_{CH}$) and 3H-20 (δ_H 1.21, $^3J_{CH}$) and of the CH-12 (δ_C 76.9) with H-11 (δ_H 3.25, $^2J_{CH}$), H-15 (δ_H 5.97-5.90, $^3J_{CH}$) and 3H-17 (δ_H 1.09, $^3J_{CH}$), summarized in Table 1. The interaction of CH-11 (δ_C 79.8) with the 3H-20 (δ_H 1.21, $^3J_{CH}$) suggested the presence of this methyl group at carbon C-9 (δ_C 42.86), revealing its rearrangement of the carbon C-10 (δ_C 64.45) and signaling for a skeleton. Thus, the hydrogen chemical shifts and hydrogenated carbon atoms were unambiguously assigned by analysis of ^1H-^1H-COSY and HSQC spectra data (Table 1). The HMBC spectrum showed long-range heteronuclear correlations, which were used to confirm the carbon skeleton and localization of the substituents (Table 1). The HMBC spectrum also revealed correlations of hydrogen atoms H-3 (δ_H 6.98) and H-10 (δ_H 2.60) signals with carbon atom C-1 (δ_C 205.3), H-3 (δ_H 6.98) with carbon atom CH_2-18 (δ_C 117.60) and H-10 with carbon atom CH_3-19 (δ_C 24.74), in accordance with a erythroxylane skeleton containing a dienone system involving the carbon atoms C-1 (δ_C 205.3), CH-2 (δ_C 127.84), CH-3 (δ_C 145.79) and the exocyclic double bond CH_2-18 (δ_C 117.60). Thus, these data were used to postulate the structure of a diterpene with erythroxylane skeleton,[6] corroborated by long-range correlations of

Table 1. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data for **1**. Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parenthesis)[a]

	HSQC		HMBC	
	δ_C / ppm	δ_H / ppm	$^2J_{CH}$ / Hz	$^3J_{CH}$ / Hz
C				
1	205.29	–	H-10	H-3
4	154.30	–	H-3; 2H-18	H-2; H-10; 3H-19
5	43.33	–	H-10; 3H-20	H-3; 2H-18
9	42.86	–	H-10	
13	40.69	–	H-12; H-15	2H-16
CH				
2	127.84	5.98 (d, 9.8)		
3	145.79	6.90 (d, 9.8)		
8	40.58	1.45		3H-20
10	64.45	2.60 (s)		H-2; H-11; 3H-19; 3H-20
11	79.83	3.23 (d, 9.0)		H-10; 3H-20
12	76.91	3.52 (d, 9.0)	H-11	H-15; 3H-17
15	147.64	5.94 (m)	2H-16	H-12; H-14a; 3H-17
CH_2				
6	39.15	1.59 (t, 13.2) 1.09		3H-19
7	24.34	1.63 (m), 1.42 (m)		
12	–	–	–	–
14	37.76	1.91 (m), 1.23 (m)		3H-17
16	111.26	5.08 (dd, 17.5, 1.1) 5.03 (dd, 10.8, 1.1)		
18	117.60	5.33 (m), 5.32 (s)		H-3
CH_3				
17	17.25	1.09 (s)		H-12; H-15
19	24.74	1.24 (s)		H-10
20	10.65	1.21 (s)		H-10; H-1

[a]Number of hydrogens bound to carbon atoms deduced by DEPTQ-^{13}C NMR spectrum. Chemical shifts and coupling constants (J) obtained from 1D ^1H NMR spectrum. Superimposed ^1H signals are described without multiplicity and chemical shifts deduced by ^1H-^{13}C COSY $^1J_{CH}$ (HMQC), ^1H-^{13}C COSY-$^nJ_{CH}$ (n = 2 and 3, HMBC) and ^1H-^1H-COSY spectra; HSQC: heteronuclear single-quantum correlation; HMBC: heteronuclear multiple-bond correlation.

CH-11 (δ_C 79.8) with the 3H-20 (δ_H 1.21) and of the CH-12 (δ_C 76.9) with H-11 (δ_H 3.25), H-15 (δ_H 5.97-5.90) and 3H-17 (δ_H 1.09) through the location of hydroxyl groups at 11 and 12 positions (Table 1). Additional heteronuclear long-range couplings are summarized in Table 1.

The relative stereochemistry of **1** (Figure 1) was determined by the relevant hydrogens coupling constants revealed by ^{1}H NMR and ^{1}H-^{1}H-COSY spectra and from the dipolar-dipolar interaction observed in the ^{1}H-^{1}H-NOESY spectrum (Figure 2). The value corresponding to vicinal interaction ($^3J_{H,H}$) between the hydrogens H-11 and H-12 (J 9.0 Hz) is consistent with axial-axial interaction[7] as appear in Figure 1, which also reveals dipolar interaction between H-12 (δ_H 3.52)/3H-20 (δ_H 1.21), H-10 (δ_H 2.60)/H-11 (δ_H 3.23) and H-11 (δ_H 3.23)/3H-17 (δ_H 1.09) revealed by ^{1}H-^{1}H nuclear Overhauser effect spectroscopy (NOESY) spectrum through the cross-peaks assigned to corresponding dipolar interaction (special proximity) shown in Figure 2 (**1a** and **1b**) which indicates the axial positions for these hydrogens. Furthermore, coupling values observed in the ^{1}H NMR spectrum for these hydrogens indicate axial-axial interaction.

Thus, the analysis of the spectral data allowed the structural characterization of this new erythroxylane diterpene 11β,12α-dihydroxy-2,4(18),15-eritroxilatrien-1-one (**1**), named Angelocunhol as a simple tribute to colleague and friend Angelo da Cunha Pinto, excellent researcher who died in November 7, 2015.

The results of the extensive application of 1D and 2D NMR spectral techniques were also used to confirm the structure and to establish the ^{1}H and ^{13}C resonance assignments of **1** (Table 1). Proposed fragmentation mechanisms of this new diterpene **1** (only peaks classified as principals) was summarized in Scheme 1.

The known simirane B (**2**),[6] harman (**3**),[6,8] maxonine (**4**), isomalindine (**5**), malindine (**6**),[9] sitost-4-en-6-ol-3-one (**7**), estigmast-4,22-dien-6-ol-3-one (**8**), campest-4-en-6-ol-3-one (**9**), sitost-4-en-3-one (**10**), stigmast-4,22-dien-3-one (**11**), campest-4-en-3-one (**12**),[10,11] β-sitosterol (**13**), stigmasterol (**14**),[12] and stigmast-4,22-dien-3-ol (**15**) were identified by spectral data, involving mainly 1D and 2D ^{1}H and ^{13}C NMR spectra and comparison with literature values.

Conclusions

A total of fifteen compounds were isolated from *S. sampaioana* (Rubiaceae), among which four alkaloids, nine steroids and two diterpenes. These compounds are in agreement with the secondary metabolites produced by plants of the Rubiaceae family and of the *Simira* genus. It is angelocunhol compound (**1**) unprecedented in the literature, and isomalindine (**5**), malindine (**6**), sitost-4-en-6-ol-3-one (**7**), estigmast-4,22-dien-6-ol-3-one (**8**), campest-4-en-6-ol-3-one (**9**), stigmast-4,22-dien-3-one (**11**), campest-4-en-3-one (**12**) and stigmast-4,22-dien-3-ol (**15**) were reported by first time in the genus. And yet the harman alkaloid (**2**), corroborating the proposition of this alkaloid the taxonomic marker of genus.

Experimental

General experimental procedures

Measure of IR data was on Shimadzu IRAffinity-1. NMR spectra were obtained on Bruker DRX-500 and Avance IIIH (both 500 MHz for ^{1}H and 125 MHz for ^{13}C), with $CDCl_3$ (0.1% tetramethylsilane, TMS) or dimethylsulfoxide (DMSO-d_6) as solvents, used as internal references (δ_H 7.24 and δ_C 70.00). Low-resolution mass analysis was done on Shimadzu GCMS-QP5050A and high-resolution mass spectrometry (HRMS) analysis on Bruker microTOF electrospray-time-of-flight mass spectrometer (ESI-TOF-MS) equipped with an ESI source in the

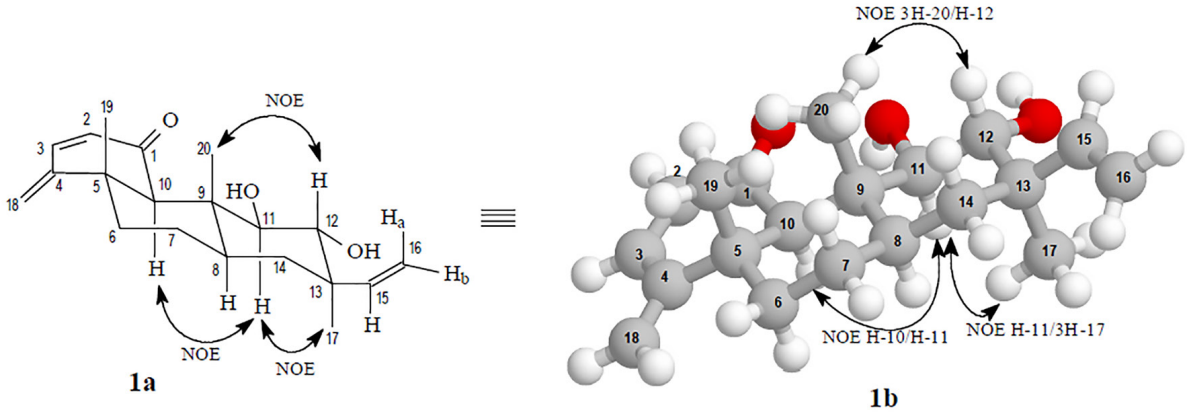
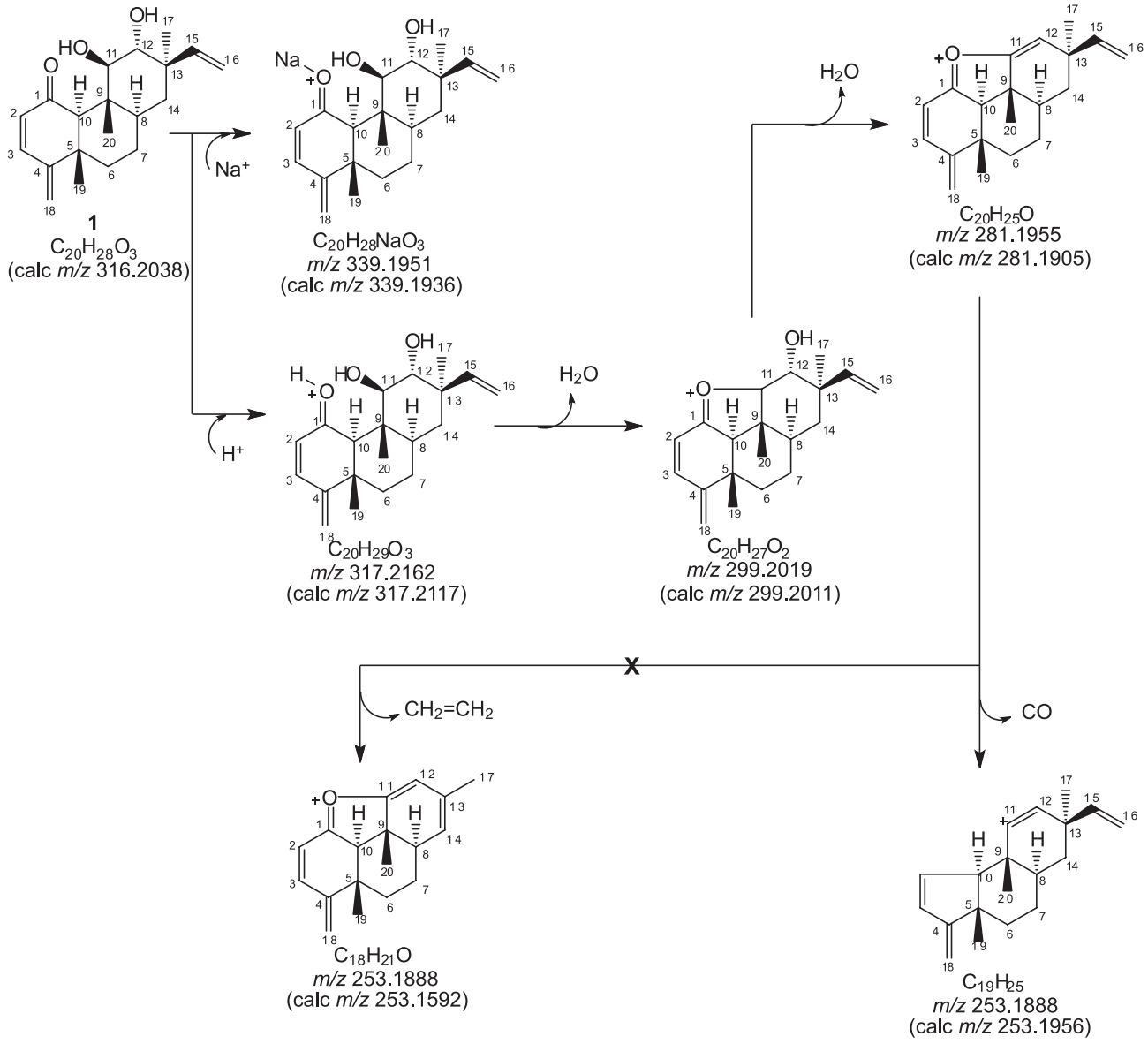


Figure 2. Nuclear Overhauser effect spectroscopy (NOESY) (↔) correlations for compounds **1**.



Scheme 1. Proposed fragmentation mechanisms of **1** (only peaks classified as principals).

positive and negative modes. Column chromatography was conducted using silica gel (Merck). Precoated thin layer chromatography (TLC) sheets (Merck) of silica gel 60 GF254 (0.25 mm) were used, and visualization of plates was carried out using a lamp UV 254 and 356 nm and vanillin (1%) solution in H_2SO_4 (5%).

Plant material

The *S. sampaioana* specimen employed in this study was collected at the Companhia Vale do Rio Doce (CVRD) Atlantic Rainforest, in Linhares city, Espírito Santo State, Brazil, and was identified by Domingos A. Folli. A voucher specimen (CVRD 8796) is deposited at the company's herbarium.

Extraction and isolation

The dried and powdered wood (6 kg) was extracted with Hexane and MeOH at room temperature and providing 0.004 kg of Hexane crude extract and 0.5 kg of MeOH crude extract after solvent evaporation.

The Hexane extract was subjected to column chromatography (CC) (SiO_2, gradient Hex/EtOAc) furnishing eleven fractions. The fraction 9 (333.7 mg) was chromatographed over a silica gel column with a hexane/ethyl acetate (9:1, v/v) to yield pure compound **1** (6.9 mg) besides a solid identified as a mixture of the compounds **7** + **8** + **9** (6.4 mg) by gas chromatography-mass spectrometry (GC-MS) analysis. The fraction 8 (138.5 mg) was chromatographed over a silica gel column with a

hexane/ethyl acetate (9:1, v/v) to yield pure compound **2** (4 mg). The fraction 7 (522.3 mg) was chromatographed on silica gel column using hexane/acetone (9:1, v/v), which furnished a solid identified as a mixture of the compounds **11** + **12** (7.6 mg) by GC-MS analysis. The mixture of the compounds **10** + **15** (55.5 mg) and the mixture of the compounds **13** + **14** (94.4 mg) identified by GC-MS analysis, were isolated from the fraction 5 (515.2 mg) by chromatography using hexane/ethyl acetate (9.5:0.5, v/v).

An aliquot of this extract (2.5 g) was dissolved in a MeOH-H_2O (8:2 (v/v), 100 mL) mixture and partitioned with CH_2Cl_2 (5 × 100 mL), EtOAc (5 × 100 mL) and *n*-BuOH (5 × 100 mL), to give the following fractions: CH_2Cl_2 (1.3 g), EtOAc (0.2 g), *n*-BuOH (0.9 g). The CH_2Cl_2 fraction was subjected to CC (SiO_2, gradient MeOH/CH_2Cl_2) to yield pure compounds **3** (6.4 mg) and **4** (5.4 mg), and the *n*-BuOH fraction was subjected to CC (SiO_2, gradient MeOH/CH_2Cl_2) to yield pure compounds **5** (4 mg) and **6** (7.3 mg).

Supplementary Information

Supplementary information, including ^{1}H NMR, ^{13}C NMR, COSY, NOESY, HSQC, and HMBC spectra, as well as mass spectra and IR (Figures S1-S9), are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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