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(−)-*epi*-Presilphiperfolan-1-ol, a new triquinane sesquiterpene from the essential oil of *Anemia tomentosa* var. *anthriscifolia* (Pteridophyta)

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ABSTRACT

(−)-*epi*-Presilphiperfolan-1-ol, (**1**), a new triquinane sesquiterpene, was isolated from the essential oil of *Anemia tomentosa* var. *anthriscifolia* by column chromatography. Its structure elucidation was accomplished by extensive 1D- and 2D-NMR analyses, as well as by GC–MS, chiral bidimensional GC, dehydration reactions, and a comparative (GIAO/DFT) theoretical study of the ^{13}C NMR chemical shifts of **1** and its known isomers (presilphiperfolan-1-ol (**1a**), presilphiperfolan-8-ol (**3**), and presilphiperfolan-9-ol (**4**)).

The family Anemiaceae (family of the ferns) comprises only one genus (*Anemia*, including *Mohria*) with more than 100 terrestrial species, distributed primarily in the New World, but with a few species in Africa, India, and islands of the Indian Ocean.[1] The central and southeast regions of Brazil are centers of diversity for the genus, and thus, many species are endemic to them.[2] The species *Anemia tomentosa*, is popularly known in Brazil as 'espiga-de-ferrugem', and its leaves are used to treat bronchitis, as a contraceptive, and as an antithermal agent.[3] The leaves of *Anemia tomentosa* var. *tomentosa* are used in the District of Santa María and Province of Córdoba, Argentina as a digestive aid, expectorant, and antigripal.[4] There are four recognized varieties of *Anemia tomentosa*, including *Anemia tomentosa* (Savigny) Swartz var. *anthriscifolia* (Schrader) Mickel, the predominant variety, which occurs in rocky regions and has very aromatic leaves.[5]

The essential oil from the plants of this species, collected in Cordoba, Argentina, was previously studied by Juliani and co-workers, and α-bisabolol resulted to be its major constituent.[5] On the other hand, studies conducted by Santos and co-workers revealed sesquiterpene isoafricanol as the major constituent of the oil obtained from plants of the same species collected at the city of São Gonçalo, Rio de Janeiro State, Brazil.[6]

In this work, we report the isolation of a new triquinane sesquiterpene, (−)-*epi*-presilphiperfolan-1-ol (**1**) (Fig. 1), from the essential oil obtained from two populations of *A. tomentosa* var. *anthriscifolia* collected at two different rocky hillsides in Brazil: Bom Jesus do Itabapoana, Rio de Janeiro State, and the city of Vila Velha, Espírito Santo State. Voucher specimens are deposited at the herbarium of the Botanical Garden of Rio de Janeiro under the numbers RB438910 and RB438912, respectively. The essential oils from the fresh aerial parts were obtained by hydrodistillation in a Clevenger-type apparatus for 2 h, yielding 0.6% and 0.3% of essential oil, for the plants collected at Rio de Janeiro and Espírito Santo, respectively. The essential oils obtained from the two different plant populations showed basically the same chemical composition, with the marked presence of triquinane-type sesquiterpenes. GC–MS analysis of both essential oils showed that sesquiterpene alcohol, **1**, (molecular ion peak at *m/z* 222) is the main constituent (at 31.2% and 30.6%, respectively). The structure could not be identified by comparison with the literature mass spectral data[7] nor by comparison with the reference mass spectra from Wiley 6th Ed library.[8] In order to investigate the structure of this compound the essential oils of *A. tomentosa* var. *anthriscifolia* were fractionated by silica-gel column chromatography eluted with hexane and mixtures of hexane and ethyl acetate to afford 18 fractions (A-1 to A-18). Fraction A-9, eluted with hexane–ethyl acetate (95:5, v/v), afforded **1**.

The ^{1}H NMR spectrum of **1** showed signals for three methyl groups at δ_H 0.80, 0.93, and 1.32, which appeared as sharp singlets, besides one doublet at δ_H 0.89, which was attributed to another methyl group (Table 1). The remaining protons were partially overlapping in the 1.4–2.1 ppm range. The ^{13}C NMR spectrum of **1** displayed 15 signals, revealing the presence of one low field oxygenated carbon at δ_C 84.3 (tertiary alcohol). A combined investigation of the information from ^{1}H and ^{13}C NMR spectra with

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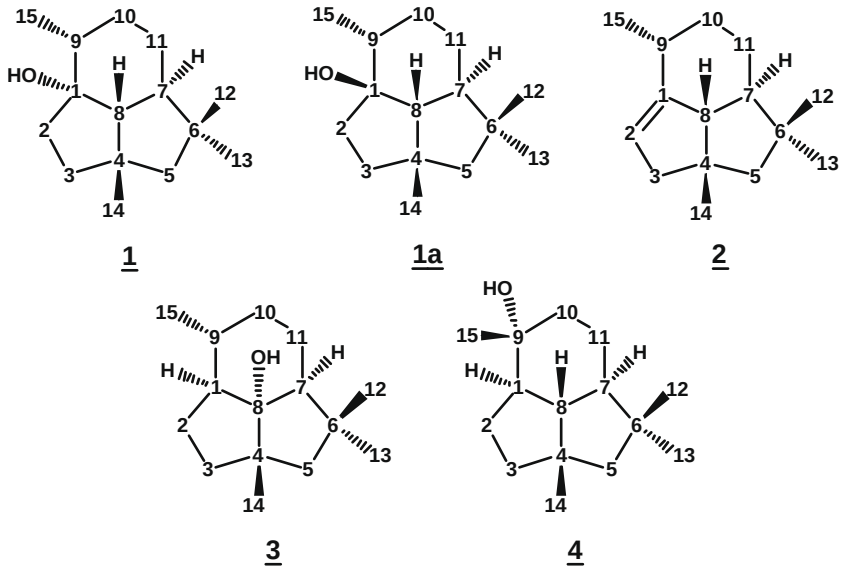

Figure 1. Structures of *epi*-presilphiperfolan-1-ol [**1**], presilphiperfolan-1-ol [**1a**], presilphiperfol-1-ene [**2**], presilphiperfolan-8-ol [**3**], and presilphiperfolan-9-ol [**4**].

Table 1
^{1}H and ^{13}C NMR data of **1** and **1a** (δ in ppm, J in Hz, TMS as internal standard)

C	**1a**		**1**			
	C_6D_6		C_6D_6		HMBC (in $CDCl_3$)	
	δ_C	δ_H	δ_C	δ_H	nH ($^{2}J_{C-H}$)	nH ($^{3}J_{C-H}$)
1	89.0 (s)		84.3 (s)		2, 8	15
2	39.7 (t)	1.62–1.72 (m)	39.5 (t)	1.61–1.72 (m)		8
		1.77–1.92 (m)[a]		1.79–1.87 (m)[a]		
3	42.6 (t)	1.53–1.62 (m)[a]	42.4 (t)	1.54–1.61 (m)[a]	2	5, 8, 14
		1.77–1.92 (m)[a]		1.79–1.87 (m)[a]		
4	46.2 (s)		46.0 (s)		5, 8, 14	2
5	59.4 (t)	1.50 (s)	59.2 (t)	1.50 (s)		8, 12, 13,14
6	41.0 (s)		40.2 (s)		5, 12, 13	
7	51.7 (d)	1.08–1.17 (m)[a]	51.5 (d)	1.09–1.16 (m)[a]	8	5, 12, 13
8	63.9 (d)	1.53–1.62 (m)[a]	63.7 (d)	1.54–1.61(m)[a]		2, 5, 14
9	36.9 (d)	1.53–1.62 (m)[a]	36.7 (d)	1.54–1.61(m)[a]	15	2
10	29.9 (t)	1.20–1.33 (m)[a]	29.7 (t)	1.24–1.34 (m)[a]		15
		1.39–1.45 (m)		1.39–1.45 (m)		
11	20.3 (t)	1.08–1.17 (m)[a]	20.1 (t)	1.09–1.16 (m)[a]		8
		1.20–1.33 (m)[a]		1.24–1.34 (m)[a]		
12	22.3 (q)	0.80 (s)	22.1 (q)	0.80 (s)		5, 13
13	28.7 (q)	0.94 (d, 7 Hz)	28.5 (q)	0.93 (s)		5, 12
14	31.4 (q)	1.32 (s)	31.1 (q)	1.32 (s)		5, 8
15	15.7 (q)	0.89 (d, 7 Hz)	15.5 (q)	0.89 (d, 7 Hz)		

[a] Signals overlapped; recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C).

those of the mass spectra and elementary analysis (found: C, 80.96; H, 11.05, calcd: C, 81.02; H, 11.79) led us to infer a tertiary alcohol structure with three degrees of unsaturation for **1**. A literature search indicated that the sesquiterpene alcohol presilphiperfolan-1-ol, **1a**, which was first isolated and described from *Conocephalum conicum*,[9] had exactly the same set of ^{13}C resonance values and carbon multiplicities (given by HSQC-ED spectrum) as observed for **1**, except for the chemical shift of the tertiary carbon bearing the hydroxyl group, which was observed at δ_C 89.0 ($\Delta\delta$ = 4.7 ppm downfield), indicating that **1** and **1a** were not the same substance. In this way, we conducted some experiments to elucidate the structure of **1**. Considering that the location of the tertiary hydroxyl group at C-8 (which was also consistent with carbon multiplicity) was ruled out by the comparison of its ^{13}C NMR data,[10] we worked with the remaining possibilities of its location which was on C-7 or C-1-*epi*.

Unfortunately, the tertiary nature of the carbinol carbons made the determination of its relative configurations very difficult by traditional NMR techniques (e.g., NOE experiments). However, a 2D-HMBC experiment provided strong evidence for the epimeric hypothesis. The HMBC spectrum of **1** revealed a correlation peak between proton H-15 of the methyl group at δ_H 0.89 (d, J = 7 Hz) and the tertiary hydroxyl carbon at δ_C 84.3, providing evidence for hydroxyl location at C-1. Other HMBC signals confirmed the proposed structure (Table 1 and Fig. 2).

The dehydration reaction product of **1** could represent further evidence for the hydroxyl group position. For verification, compound **1** was submitted to dehydration conditions to give presilphiperfol-1-ene, **2** (Fig. 1). The ^{1}H NMR spectrum of **2** showed the signal for the hydrogen of the double bond at δ_H 4.82, as well as the same MS fragmentation pattern reported for **2**.

Strong evidence of the enantiomeric purity of **1** was obtained by the measure of its optical rotation ($[\alpha]_D^{25}$ −66.0, *c* 0.015), and by the presence of one peak on the bidimensional chiral GC analysis using a derivatized β-cyclodextrin (2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin in PS 086 13%)[11] as the stationary phase.

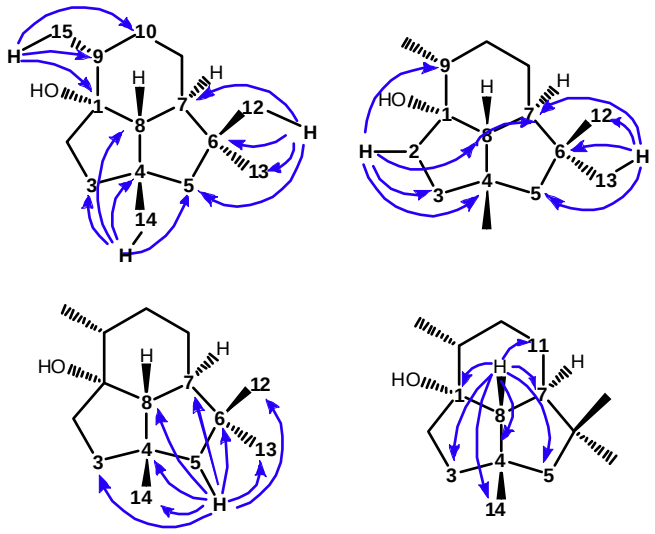

Figure 2. HMBC coupling correlations of **1**.

Table 2
Comparison of experimental and calculated (B3LYP-GIAO/cc-pVDZ//B3LYP/cc-pVDZ) ^{13}C NMR chemical shifts of the carbinol carbon of presilphiperfolanol isomers

Tricyclic sesquiterpenes	^{13}C chemical shifts (in ppm)		
	δ_{exp}	δ_{cal}	$\Delta\delta^{b}$
Presilphiperfolan-8-ol, **3**	96.2[10]	99.4	+3.2
Presilphiperfolan-9-ol, **4**	76.0[13]	77.7	+1.7
Presilphiperfolan-1-ol, **1a**	89.1[9]	88.1	−1.0
epi-Presilphiperfolan-1-ol, **1**	84.3[a]	83.3	−1.0

[a] Present work.
[b] Difference between experimental and calculated chemical shifts.

Additionally, the registered ^{1}H NMR spectrum of **1** with the addition of ca. 20% of the europium salt tris[3-(heptafluoropropylhydroxymethylene)-D-camphorato] europium(III)[12] did not split the methyl signals into two sets of signals, corroborating the enantiomeric purity of **1**.

One last and additional experiment to support the epimeric hypothesis was carried out by a theoretical study focusing on the determination of the ^{13}C NMR chemical shift of the carbinol carbons of **1** and **1a**, seeking any significant difference between these isomers. The following procedures were carried out: (1) construction of the conformationally rigid structures of presilphiperfolan-1-ol, **1a**, and its stereoisomers *epi*-presilphiperfolan-1-ol, **1**, presilphiperfolan-8-ol, **3**, and presilphiperfolan-9-ol,[13] **4,** (Fig. 1) from planar (2D) chemical structures via the 3D Structure Optimization command of ACD's CHEMSKETCH 11.0 program,[14] and structure optimizations were performed without any geometry restrictions; (2) conformational analyses for rotation along (C)C–O(H) bond of all the isomers; (3) optimizations of all the minimum structures found in conformational analysis, without any geometry restrictions; (4) ^{13}C chemical shift calculations, using the Gauge Including Atomic Orbital (GIAO) method[15,16] in all previously optimized conformational minima. The predicted ^{13}C NMR chemical shifts are derived from equation $\delta = \sigma_0 - \sigma$, where δ is the calculated chemical shift, σ is the calculated absolute shielding, and σ_0 is the calculated absolute shielding of the standard (TMS in the case of ^{13}C NMR chemical shifts); (5) determination of the mean value of the carbinol carbon ^{13}C NMR chemical shift for each stereoisomer considering the Boltzmann distribution of all conformers; and (6) comparison of the theoretical ^{13}C chemical shifts with experimental data. All calculations were performed through Density Functional Theory (DFT) model using B3LYP Beckés three-parameter hybrid functional, using the LYP correlation function[17,18] with cc-pVDZ Dunning's correlation consistent basis set,[19] with the GAUSSIAN03 series of programs.[20] The results of our calculations (Table 2) show good correlation between calculated and experimental carbinol ^{13}C chemical shifts for the known isomers of presilphiperfolanols (**1a**, **3**, and **4**; Fig. 1).

In view of the experimental data presented here and the quite perfect agreement between calculated and experimental chemical shifts for **1** ($\delta_{C_{calc.}}$ 83.3 ppm, $\delta_{C_{exp}}$ 84.3 ppm), we propose structure **1** (Fig. 1) for *epi*-presilphiperfolan-1-ol. Its absolute configuration, however, remains to be determined.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.06.046.

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