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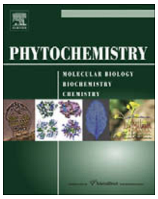

# Absolute configuration of tropane alkaloids from *Schizanthus* species by vibrational circular dichroism

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ABSTRACT

The absolute configuration (AC) of 6β-hydroxy-3α-senecioyloxytropane (**1**), 3α-hydroxy-6β-tigloyloxytropane (**2**), 3α-hydroxy-6β-senecioyloxytropane (**3**), and 3α-hydroxy-6β-angeloyloxytropane (**4**) was assigned as (1*R*,3*R*,5*S*,6*R*) using density functional theory (DFT) calculations at the B3LYP/DGDZVP level of theory in combination with experimental vibrational circular dichroism (VCD) measurements and comparison with the spectra of similar tropanes. The AC of **1** followed from a sample isolated from *Schizanthus grahamii*, while those of the mixture of **2** and **3**, isolated from the same source, were determined by comparing the VCD measurement to a weighted calculation of the individual VCD spectra according to a 69:31 ratio of **2**:**3** determined by $^1$H NMR signal integration. In turn, *Schizanthus pinnatus* provided a 7:3 mixture of **1**:**4** whose AC was determined using the experimental VCD absorptions in the 1150–950 cm$^{-1}$ spectral region which were compared with those observed for **1**–**3** and with those described for other 3α,6β-tropanediol derivatives.

## 1. Introduction

Tropane alkaloids, a class of compounds possessing the 8-azabicyclo[3.2.1]octane skeleton, mainly occur in the Solanaceae, Erythroxylaceae, and Convolvulaceae plant families (Griffin and Lin, 2000). The plants containing these alkaloids have been used in traditional medicine for centuries and some of these alkaloids have important pharmacological activities as anesthetics, against cardiovascular disorders, renal failure and antidote to snakebites (Lounasmaa and Tamminen, 1993; Fodor and Dharanipragada, 1994; Gringauz, 1997; Silva et al., 2001; Muñoz et al., 2006 and therein). Although Index Kewensis lists 72 samples for the genus *Schizanthus* (Solanaceae), after evaluating synonyms it seems to have 31 species indigenous to the southern part of the Andian chain of mountains, mainly in Chile (Griffin and Lin, 2000) with one representative, *Schizanthus grahamii* Gill., whose dispersal area reaches Argentina (Coccuci, 1989). The chemical study of these plants has attracted much attention because they contain a wide range of tropane-derived alkaloids, such as hydroxytropanes esterified with different isomeric residues like angelic, tiglic, or senecioic acids, or with cinnamic, mesaconic, and itaconic acids (San Martín et al., 1980, 1987; Gambaro et al., 1983; De la Fuente et al., 1988; Muñoz et al., 1991) found in alkaloids with a single tropane skeleton, while belladonnine and truxilline (Moore et al., 1996; Dembitsky, 2008) contain two tropane moieties due to their dimeric nature. One alkaloid, grahamine, possesses a complex, trimeric structure containing three 3,7-dihydroxytropane ring systems (Hartmann et al., 1990).

Since tropane alkaloids appear in their host plants as complex mixtures, considerable efforts have been devoted recently using different analytical techniques for the separation and identification of these molecules. These include capillary electrophoresis with UV detection and coupling to MS with electrospray ionization (ESI) (Humam et al., 2005), very fast GC and capillary LC–MS (Bieri et al., 2006a,b) and LC–UV detection–multiple mass spectrometry and LC–NMR (Zanolari et al., 2003). Most tropane alkaloids in the *Schizanthus* genus are derived from 3α,6β-tropanediol (**5**) with various acyl residues, a situation that generates difficulty to ascertain the correct enantiomer (1*R*,3*R*,5*S*,6*R* or 1*S*,3*S*,5*R*,6*S*) since only a few molecules have known absolute configuration (AC) (Muñoz et al., 2010). Recent determinations include the AC assignment of both diasteroisomers of 6β-hydroxyhyoscyamine (Muñoz et al., 2006), and of semisynthetic (−)-(1*S*,3*S*,5*R*,6*S*)-3α,6β-diacetyloxytropane (Muñoz et al., 2010) using vibrational circular dichroism (VCD) in combination with density functional theory (DFT) calculations, as well as the study of (1*R*,3*R*,5*S*,6*R*)-*trans*-3-hydroxysenecioyloxy-6-senecioyloxytropane which was ascertained

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(Humam et al., 2008) using electronic circular dichroism, also in combination with DFT calculations.

In this paper, the AC of 3α,6β-tropanediol monoesters **1**–**4** (Fig. 1) determined by applying the VCD methodology are reported. The AC determination of **1** follows from a pure sample isolated from *S. grahamii*, while in the case of **2** and **3** it was made from VCD spectral measurements of a **2**:**3** mixture isolated from the same species, and the calculation of the individual VCD spectra of both molecules which were weighted according to the 69:31 (**2**:**3**) ratio determined from $^1$H NMR signal integration. The AC assignment of **4** was made after comparison of absorptions in the 1150–950 cm$^{-1}$ VCD spectral region of a 7:3 mixture of **1**:**4** isolated from *Schizanthus pinnatus* Ruiz & Pav. with those observed for **1**–**3** and from the VCD literature data (Muñoz et al., 2010).

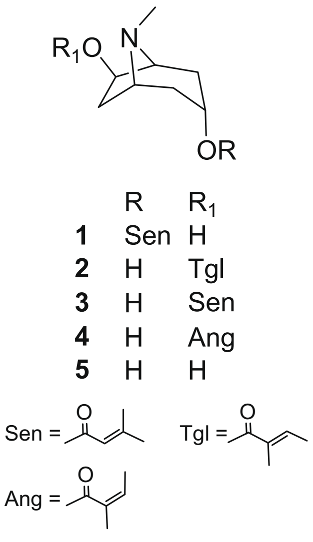
## 2. Results and discussion

Conformational analysis of (1*R*,3*R*,5*S*,6*R*)-6β-hydroxy-3α-senecioyloxytropane (**1**) was initiated following the Monte Carlo protocol (Chang et al., 1989) at the MMFF94 level of theory (Halgren, 1996a,b,c,d; Halgren and Nachbar, 1996) as implemented in the PC Spartan'04 program, considering *axial* or *equatorial* orientations for the *N*-Me group which gave 16 and 22 minimum energy conformers, respectively. These structures were optimized using density functional theory (DFT) (Perdew, 1986) at the B3LYP/6-31G(d) level. The two different sets of conformers were then mixed, providing 10 conformers, when using a 2.5 kcal/mol cutoff, from which only two have the *axial N*-Me group, while eight have the *equatorial N*-Me group, accounting for 72.8% and 27.2% of the total conformational population, respectively (Table 1). These conformers were re-optimized, and the IR and VCD frequencies calculated using the B3LYP functional and the DGDZVP basis set (Burgueño-Tapia and Joseph-Nathan, 2008). The use of this B3LYP/DGDZVP combination of functional and basis set requires less computing time than the 6-31G(d) basis set, while producing very similar results, as is evident in published data (Cerda-García-Rojas et al., 2007, 2008). This situation seems to be associated with the fact that DGauss basis sets, such as DGDZVP, are optimized for DFT methods (Godbout et al., 1992), and are demonstrated as an accurate and efficient approach for the study of molecular geometries, vibrational properties, and reaction energies (Andzelm and Wimmer, 1992). Computer time could become crucial when studying larger molecules, as was done for stypotriol triacetate (Muñoz et al., 2009), a $C_{33}H_{46}O_7$ compound with 300 electrons which required almost 1100 h of computer time when using the B3LYP/DGDZVP level of theory.

**Table 1**
B3LYP calculated relative energies (kcal/mol) and conformational population (%) for the most stable conformers of (1*R*,3*R*,5*S*,6*R*)-**1**.

| Conf [a] | $\Delta E_{6\text{-}31G(d)}$ [b] | % [c] | $\Delta E_{DGDZVP}$ [d] | % [e] |
|---|---|---|---|---|
| **1a** | 0.00 | 36.4 | 0.00 | 36.0 |
| **1b** | 0.01 | 36.4 | 0.00 | 36.0 |
| **1c** | 1.22 | 4.9 | 1.26 | 4.4 |
| **1d** | 1.26 | 4.4 | 1.26 | 4.4 |
| **1e** | 1.30 | 4.2 | 1.22 | 4.6 |
| **1f** | 1.30 | 4.2 | 1.26 | 4.4 |
| **1g** | 1.31 | 4.0 | 1.22 | 4.6 |
| **1h** | 1.37 | 3.8 | 1.29 | 4.1 |
| **1i** | 2.16 | 1.0 | 2.25 | 0.8 |
| **1j** | 2.34 | 0.7 | 2.33 | 0.7 |

[a] Conformers **1a** and **1b** have *axial N*-Me while **1c**–**1j** have *equatorial N*-Me orientation.
[b] Relative to **1a** with $E_{6\text{-}31G(d)} = -494752.48$ kcal/mol.
[c] From Spartan'04 energies according to $\Delta G = -RT \ln K$.
[d] Relative to **1a** with $E_{DGDZVP} = -494813.22$ kcal/mol.
[E] From Gaussian 03W energies according to $\Delta G = -RT \ln K$.



| | R | R₁ |
|---|---|---|
| **1** | Sen | H |
| **2** | H | Tgl |
| **3** | H | Sen |
| **4** | H | Ang |
| **5** | H | H |

**Fig. 1.** Molecular structure of 3α,6β-tropanediols monoesteres **1**–**4** and diol **5**.

After B3LYP/DGDZVP optimization of **1**, eight relevant conformers were observed accounting for 98.5% of the conformational map (Fig. 2). Two of these conformers, with an *axial N*-Me orientation, show hydrogen bonding between the C6 hydroxyl group and the nitrogen atom and contribute to 72.0% of the conformational population. The carbonyl and double bond atoms of the senecioyl ester residue in both conformers are coplanar, differing only in the C2–C3–O–C1′ torsion angle values, which are of −156.6 and −82.5 degrees for **1a** and **1b** (Fig. 2), respectively. The calculated VCD spectra of these eight conformers of **1** were combined in a single weighted plot (Fig. 3) according to the Boltzmann conformational population derived from the relative free energy values of their optimized structures and compared with the experimental VCD spectrum showing a good concordance as can be seen in Fig. 3.

The AC assignment of alkaloids **2** and **3** follows from a 69:31 mixture of **2**:**3**. For this purpose, the conformational analysis of each alkaloid, using the same methodology as for **1**, was undertaken. After B3LYP/DGDZVP reoptimization, 12 conformers were selected for (1*R*,3*R*,5*S*,6*R*)-**2** in a 2.5 kcal/mol cutoff contributing with 96.4% of the total conformational population (Table 2), from which eight conformers correspond to the *equatorial* (82.4%) *N*-Me group orientation and four to the *axial* (14.0%) *N*-Me group orientation. The VCD curve (trace c, Fig. 4) was generated according to the Boltzmann conformational population derived from the DGDZVP free energy values. By analogy, conformational analysis and VCD calculations were made for (1*R*,3*R*,5*S*,6*R*)-**3** yielding nine low energy conformers corresponding to 99.4% of the total conformational population (Table 3) from which five with an *equatorial N*-Me group account for 85.8% and four with the *axial N*-Me group account for 13.6%. The corresponding VCD spectrum of (1*R*,3*R*,5*S*,6*R*)-**3** (trace d, Fig. 4), shows similarity to the spectrum of (1*R*,3*R*,5*S*,6*R*)-**2** (trace c, Fig. 4), since the ester groups are achiral and have no relevant contributions to the VCD spectrum. The final calculated VCD curve for the **2**:**3** mixture (trace b, Fig. 4) was obtained by a combination of the individual VCD spectra in a single weighted plot according to the 69:31 ratio of **2**:**3** obtained from the $^1$H NMR signal integration analysis and is compared (trace a, Fig. 4) to the experimental VCD spectrum, thereby establishing the absolute configuration of both molecules.

In a previous paper (Muñoz et al., 2010), two specific distinctive regions in the VCD spectra of 3α,6β-tropanediol derivatives were highlighted, one between 1300 and 1200 cm$^{-1}$ that drastically changes with the tropane moiety conformation, and the other one between 1150 and 950 cm$^{-1}$ showing little variation with dif-