Absolute Configuration and Stereochemical Analysis of 3α,6β-dibenzoyloxytropane. Phytochemistry Lett 5:450

ARTICLE *in* PHYTOCHEMISTRY LETTERS · SEPTEMBER 2012

Impact Factor: 1.45 · DOI: 10.1016/j.phytol.2012.04.003

3 AUTHORS, INCLUDING:

Marcelo A Muñoz
Universidad Austral de Chile
23 PUBLICATIONS **220** CITATIONS
SEE PROFILE

Pedro Joseph-Nathan
Center for Research and Advanced Studies …
253 PUBLICATIONS **2,205** CITATIONS
SEE PROFILE

Phytochemistry Letters 5 (2012) 450–454

Contents lists available at SciVerse ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Absolute configuration and stereochemical analysis of 3α,6β-dibenzoyloxytropane

Marcelo A. Muñoz [a], Martina Martínez [a], Pedro Joseph-Nathan [b,*]

[a] Instituto de Ciencias Químicas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile
[b] Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, México, D.F. 07000, Mexico

ARTICLE INFO

Article history:
Received 24 November 2011
Received in revised form 15 February 2012
Accepted 3 April 2012
Available online 9 May 2012

Keywords:
VCD, HPLC polarimetric detection
Tropane alkaloids
Absolute configuration
3α,6β-Dibenzoyloxytropane

ABSTRACT

Both enantiomers of 3α,6β-dibenzoyloxytropane (**1**) have been prepared from optical active 6β-hydroxyhyoscyamines establishing their absolute configurations as (−)-(3*R*,6*R*) and (+)-(3*S*,6*S*)-dibenzoyloxytropane. Independent stereochemical confirmation was obtained by vibrational circular dichroism measurements, since bands characteristic of (3*R*,6*R*) and (3*S*,6*S*) configurations of tropanediols derivatives were observed. In addition, a chiral HPLC method was developed for determining absolute configurations of tropane-related natural substances at the microgram (μg) level. The complete ^{1}H NMR characterization of the scaffold of **1** is also reported.

1. Introduction

Tropane alkaloids comprise a large group of natural bases found in many species of Convolvulaceae, Erythroxylaceae and Solanaceae plant families which are widely distributed around the world (Lounasmaa and Tamminen, 1993; O'Hagan, 1997, 2000). The medicinal use of these compounds in both traditional and modern medicine is well documented (Christen, 2000), and several pharmacological properties of natural tropane alkaloids, including the reversal of multidrug resistance (Chávez et al., 2002) and the treatment of erectile dysfunction, were reported very recently (Queiroz, 2008).

Nevertheless, a relatively large portion of tropane alkaloids, particularly esters derived from natural (+)-(3*R*,6*R*) and (−)-(3*S*,6*S*)-3α,6β-tropanediol enantiomers, remain with unknown absolute configuration (Lounasmaa and Tamminen, 1993; O'Hagan, 1997, 2000). Although the hydrolysis of these derivatives can unambiguously correlate their absolute configurations to the tropane diols of proven stereochemistry (Muñoz et al., 2006), in most cases the small sample amounts available precludes this chemical procedure. Despite our efforts to clarify these stereochemical relationships (Muñoz and Joseph-Nathan, 2009; Muñoz et al., 2006, 2010), errors related to the absolute configuration of these diols can still be detected in a very recent publication (Humam et al., 2011), in which the levorotatory form is wrongly related to the (3*R*,6*R*) absolute configuration. This (−)-(3*R*,6*R*) relationship was proposed using the lactone rule of Hudson (Fodor et al., 1961), and corrected to (+)-(3*R*,6*R*) by chemical correlation (Fodor and Sóti, 1964, 1965) which was recently confirmed using vibrational circular dichroism (VCD) (Muñoz et al., 2006). Furthermore, the lack of enantiomeric reference compounds or chiroptical properties characteristic of a particular absolute configuration also make it difficult to assign the stereochemistry of the natural substances.

A good example of this situation is natural 3α,6β-dibenzoyloxytropane (**1**), first isolated from the leaves of *Erythroxylum cuneatum* as an optically inactive substance which showed an undepressed mixed melting point of the derived picrate with that of a synthetic racemic sample (El-Imam et al., 1988). Recently the same compound has been isolated from the steams of *Erythroxylum caatingae* (de Oliveira et al., 2011) and, the (3*S*,6*S*) absolute configuration was proposed since the benzoylated compound was isolated together with (3*S*,6*S*)-6β-benzoyloxy-3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)tropane, for which the absolute configuration was proposed from a single crystal X-ray study of the hydrochloride. However since no mention to the use of anomalous dispersion for the absolute configuration assignment nor optical rotation data are reported (de Oliveira et al., 2011), no further confirmation of this proposal can be made.

To overcome these difficulties, different approaches have been used during the last few years to establish the absolute configuration for members of this particular group of tropane

* Corresponding author. Tel.: +52 55 5747 7112; fax: +52 55 5747 7137.
E-mail address: pjoseph@nathan.cinvestav.mx (P. Joseph-Nathan).

1874-3900/$ – see front matter © 2012 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.phytol.2012.04.003

alkaloids, which include the use of ^{1}H NMR chemical shift prediction (Muñoz and Joseph-Nathan, 2009), ^{1}H NMR anisochronicity induced by chiral auxiliary reagents (Humam et al., 2011), electronic circular dichroism (ECD) (Humam et al., 2008) and vibrational circular dichroism (VCD) (Muñoz et al., 2006, 2010; Reina et al., 2010). The later technique has shown rapid applications increase and is now widely used for the stereochemical assignment of natural compounds (He et al., 2011). In addition, in the case of tropane alkaloids we have shown that some VCD bands can directly be used to ascertain their absolute configurations without the need of tedious density functional theory calculations (Muñoz et al., 2006, 2010; Reina et al., 2010).

In continuation to our studies related to the absolute configuration of tropane alkaloids (Muñoz and Joseph-Nathan, 2009; Muñoz et al., 2006, 2010; Reina et al., 2010), herein we present the stereochemical assignment of 3α,6β-dibenzoyloxytropane enantiomers using chemical correlation and VCD. Additionally, a chiral HPLC methodology using both a conventional UV–vis detector and a laser-based polarimetric detector is presented. This chromatographic procedure might allow the absolute configuration assignment of a sample isolated from nature at the μg level. We also report, for the first time, all ^{1}H NMR chemical shift and coupling constant values for the tropane scaffold.

2. Results and discussion

Both enantiomeric forms of the dibenzoylated tropanediol derivative, namely (3*R*,6*R*)-3α,6β-dibenzoiloxytropane [(3*R*,6*R*)-**1**] and (3*S*,6*S*)-3α,6β-dibenzoiloxytropane [(3*S*,6*S*)-**1**], were prepared through benzoylation of (+)-(3*R*,6*R*)-3α,6β-tropanediol and (−)-(3*S*,6*S*)-3α,6β-tropanediol, respectively, which in turn are derived from (+)-(3*R*,6*R*,2′*S*)-6β-hydroxyhyoscyamine (**2**) and (−)-(3*S*,6*S*,2′*S*)-6β-hydroxyhyoscyamine (**3**), respectively. Since the absolute configuration of the natural diastereoisomeric bases **2** and **3** has been established using VCD spectroscopy and DFT calculations (Muñoz et al., 2006), measurement of the corresponding optical rotations for each enantiomer provided the stereochemical assignment as (−)-(3*R*,6*R*)-**1** and (+)-(3*S*,6*S*)-**1**.

Although this procedure unambiguously establishes the absolute configuration of these bases, we have shown that VCD measurements, without computational calculations, can be used directly for the absolute configuration determination of this group of compounds (Muñoz et al., 2006, 2010; Reina et al., 2010). This empirical approach is based on VCD active transitions that appear to be related only to the asymmetric tropanediol scaffold, independent of the presence of type of esters in the C-3 or C-6 positions, as observed for diastereoisomeric 6β-hydroxyhyoscyamines **2** and **3** (Muñoz et al., 2006), 3α,6β-diacetoxytropane (**4**) (Muñoz et al., 2010), and natural 6β-hydroxy-3α-senecioyloxytropane (**5**), 3α-hydroxy-6β-tigloyloxytropane (**6**), 3α-hydroxy-6β-senecioyloxytropane (**7**) and 3α-hydroxy-6β-angeloyloxytropane (**8**) (Reina et al., 2010). These experimental observations are supported by DFT calculations performed for **2**–**4**, which confirmed these bands correspond to vibrational transitions mostly related to the tropane moiety (Muñoz et al., 2006, 2010).

Consequently, the VCD spectra of (3*R*,6*R*)-**1** and (3*S*,6*S*)-**1** were measured in order to verify the presence or absence of these diagnostic bands, and their relationship to the already established absolute configuration. As shown in Fig. 1, the characteristic −/−/+/+ band pattern in the 950–1100 cm^{-1} range observed in other tropane alkaloids having the (3*S*,6*S*) absolute configuration, namely **3** and **4**, is also present in the VCD spectrum of (3*S*,6*S*)-**1**. Accordingly, an antipodal pattern, consistent with the opposite absolute configuration, is observed for (3*R*,6*R*)-**1**, which also is equivalent to the one obtained previously for **2**, and **5**–**8**. The vibrational modes responsible for these bands were detailed in our early VCD study of **2** and **3** (Muñoz et al., 2006), which also revealed the band pattern observed in the 1200–1300 cm^{-1}, although also owing to the tropane scaffold, is unsuitable for absolute configuration diagnosis (Scheme 1).

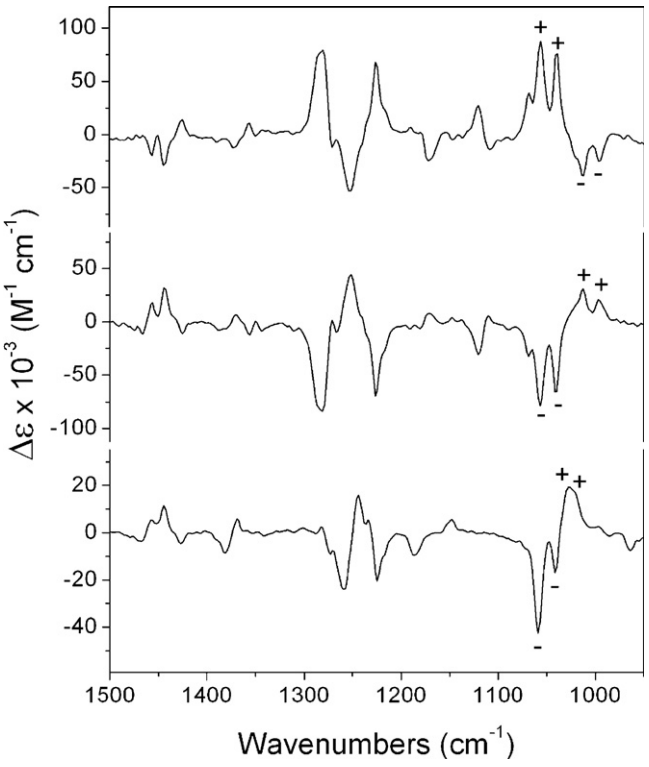
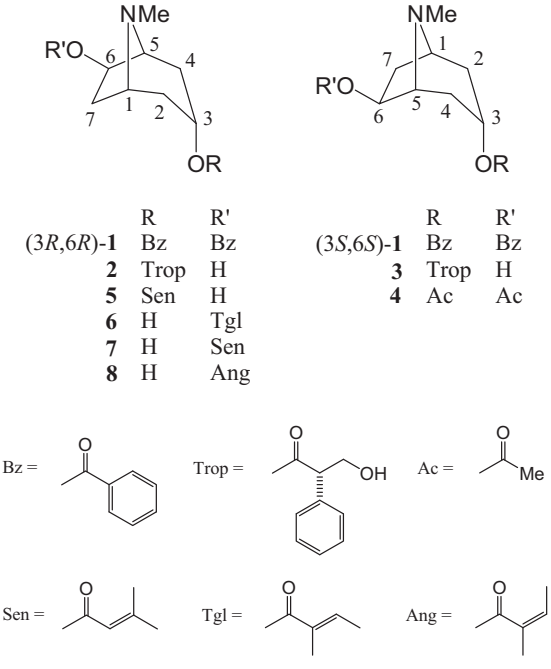


Fig. 1. Experimental VCD spectra of (3*R*,6*R*)-dibenzoate-**1** (top), (3*S*,6*S*)-dibenzoate-**1** (center) and diacetate **4** (bottom). Diagnostic bands in the 950–1100 cm^{-1} range are labeled.



Scheme 1. Structures and carbon atom numbering for tropane alkaloids **1**–**8**.

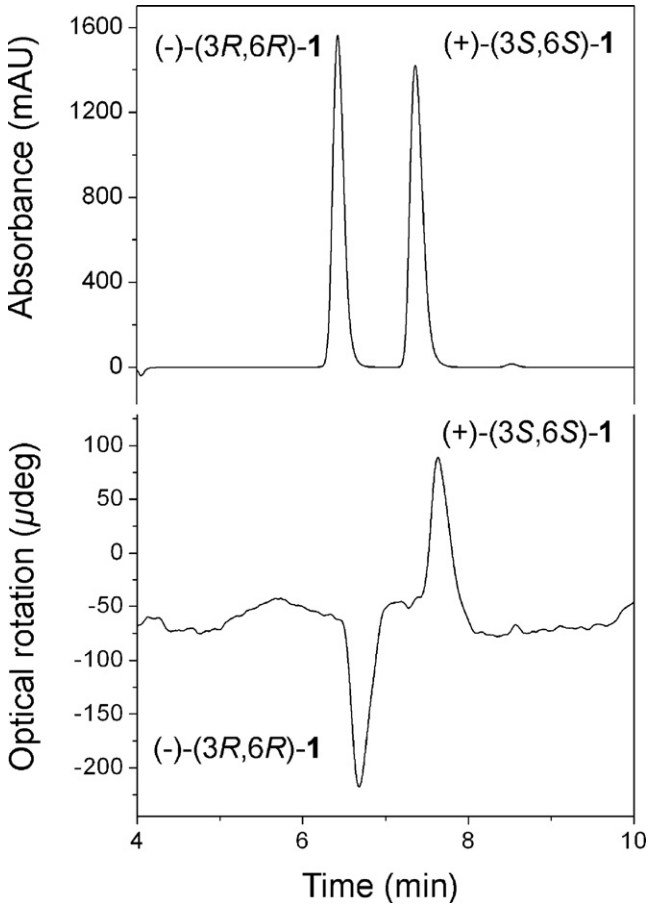


Fig. 2. UV (up) and polarimetric (down) detection of the HPLC chiral separation of racemic **1**, obtained using an 8 μL injection of a 3.6 mg/mL solution of racemic **1**, corresponding to 28.8 μg injected material.

In an independent effort to generate a practical tool to determine the absolute configuration of this alkaloid, a chiral HPLC methodology for resolving racemate **1** was developed. This was done using a commercial amylose-based chiral stationary phase (CSP) under normal phase conditions, a conventional UV–vis detector and a laser-based polarimetric detector, as shown in Fig. 2.

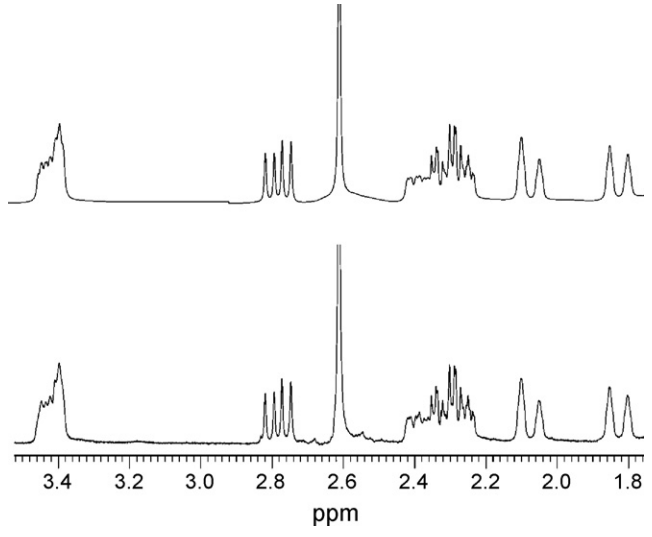


Fig. 3. Comparison of the calculated ^{1}H NMR spin–spin simulation of the tropane scaffold of **1** (top), using PERCH software, and the experimental spectrum (bottom).

Table 1
Aliphatic ^{1}H NMR chemical shifts (ppm), multiplicity and coupling constants (Hz) of **1** obtained through spin–spin simulation using PERCH software.[a]

Atom	δ	Multiplicity	J
H-1	3.435	dddd	J_{7exo} = 7.41, J_{2endo} = 3.59, J_{2exo} = 2.19, J_{7endo} = 0.64
H-2$_{endo}$	2.274	dddd	J_{gem} = −15.22, J_3 = 5.22, J_1 = 3.59, J_{7exo} = 1.37
H-2$_{exo}$	1.829	dddd	J_{gem} = −15.22, J_{4exo} = 2.21, J_1 = 2.19, J_3 = 1.01
H-3	5.354	ddddd	J_{4endo} = 5.39, J_{2endo} = 5.22, J_{4exo} = 1.10, J_5 = 1.02, J_{2exo} = 1.01
H-4$_{endo}$	2.309	ddd	J_{gem} = −15.23, J_3 = 5.39, J_5 = 4.00
H-4$_{exo}$	2.078	dddd	J_{gem} = −15.23, J_5 = 2.38, J_{2exo} = 2.21, J_3 = 1.10
H-5	3.397	ddd	J_{4endo} = 4.00, J_{4exo} = 2.38, J_3 = 1.02
H-6$_{endo}$	5.875	dd	J_{7endo} = 7.55, J_{7exo} = 3.10
H-7$_{endo}$	2.782	ddd	J_{gem} = −14.12, J_{6endo} = 7.55, J_1 = 0.64
H-7$_{exo}$	2.381	dddd	J_{gem} = −14.12, J_1 = 7.41, J_{6endo} = 3.10, J_{2endo} = 1.37

[a] For the remaining atoms see Section 4.

In the shown case, injection of 8 μL of a 3.6 mg/mL solution of racemic **1**, corresponds to 28.8 μg injected material. The use of the same chromatographic conditions for each independently prepared enantiomer confirmed the stereochemistry of both peaks in the racemic mixture. Additionally through the use of a complementary chiral detection, this identity can easily be confirmed and related to a particular absolute configuration. Accordingly, this procedure will evidence the presence of a single isomer or an optically inactive racemic mixture of **1**, as suggested for *E. caatingae* (de Oliveira et al., 2011) and *E. cuneatum* (El-Imam et al., 1988), respectively. In the former case, independent confirmation of the proposed (3*S*,6*S*) absolute configuration will be possible, since in-line assessment of optical activity under the described conditions would unequivocally lead to a particular absolute configuration. The use of this type of detection is important in chiral separations since inversion of enantiomer elution order is fairly common, precluding the use of retention times as a diagnostic tool even in the presence of the racemic mixture.

In an effort to better understand the molecular conformation of the tropane scaffold in **1**, the ^{1}H NMR spectrum was compared in detail with a calculated spectrum obtained by Total-Line-Shape-Fitting as implemented in the PERCH software package (Fig. 3). This software has been successfully used for the study of natural products (Inui et al., 2010; Molina-Salinas et al., 2011; Scher et al., 2010). While the J_{gem} values for the hydrogen atoms at the C2 and C4 methylene groups are identical, the J_{gem} value for the C7 methylene group is smaller, in agreement with its location on a five-membered ring. Of further relevance is the presence of three long-range W-type coupling constants for H_{2endo}–H_{7exo}, for H_{2exo}–H_{4exo}, and for H_3–H_5, as well as the absence of the H_5–H_{6endo} vicinal coupling for which the corresponding hydrogen atoms show a dihedral angle very close to 90°. The complete hydrogen–hydrogen coupling constant set of values for the tropane atoms is given in Table 1 and allows a detailed conformational evaluation of the molecular scaffold in solution.

3. Conclusion

The absolute configuration of both enantiomers of 3α,6β-dibenzoyloxytropane is established for the first time as (−)-(3*R*,6*R*)-**1** and (+)-(3*S*,6*S*)-**1** through chemical correlation with (+)-(3*R*,6*R*,2′*S*)-6β-hydroxyhyoscyamine (**2**) and (−)-(3*S*,6*S*,2′*S*)-6β-hydroxyhyoscyamine (**3**), respectively. Both synthetic enantiomers showed characteristic VCD band patterns in the 950–1100 cm^{-1} range in agreement with previous observations for other tropanediol derivatives, further confirming that these bands are of diagnostic value.

A chiral HPLC methodology based on commercial amylose derived CSP was able to resolve racemate **1**, permitting the stereochemical identification of both chromatographic peaks through retention time comparison with those of (3*R*,6*R*)-**1** and (3*S*,6*S*)-**1**, while the use of chiral laser-based polarimetric detection in series with conventional UV detection permitted chiroptical characterization of both chromatographic peaks, which in turn should allow absolute configuration determination of natural substances at the μg level.

The ^{1}H NMR assignment of all atoms owning to the tropane scaffold is given for the first time showing three long-range W-type interactions and the absence of one vicinal coupling constant. This detailed ^{1}H NMR chemical shifts and coupling constants assignment will be of use in studies to assess the solution state conformation of related tropane alkaloids.

4. Experimental

4.1. General experimental procedures

IR and VCD measurements were performed on a BioTools Chiral*IR* FT-VCD spectrophotometer equipped with dual photoelastic modulation and a long-term detector using samples of 3.0 mg in 150 μL in 100% atom-D $CDCl_3$ placed in a BaF_2 cell with a pathlength of 100 μm for which data were acquired at a resolution of 4 cm^{-1} during 20 h. NMR measurements, including gCOSY, gHSQC, and gHMBC experiments, were performed at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian Mercury 300 spectrometer from $CDCl_3$ solutions using TMS as internal standard. Optical rotations were determined in $CHCl_3$ on a Perkin-Elmer 341 polarimeter.

4.2. Benzoylation of 3α,6β-tropanediols

Solutions of racemic and optically active diols (10 mg) in 5 mL of CH_3CN were treated with excess benzoyl chloride (0.5 mL) under reflux for 3 h. The crude reaction mixture, after CH_3CN evaporation, was dissolved in CH_2Cl_2 (10 mL) and washed (3 × 10 mL) with aqueous NH_4OH (pH 4). After drying over anhydrous Na_2SO_4 and filtering, the solvent was evaporated to yield a yellowish oil which was purified by semi-preparative HPLC (0.017% diethylamine in MeOH over Zorbax Eclipse XDB-C18, 9.4 mm × 250 mm, 5 μm) with yields between 62 and 83%. Samples showed IR ($CDCl_3$) ν_{max} 1713, 1450, 1315, 1279 and 1115 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.11, 8.04, 7.59, 7.57, 7.50, 7.45 and 2.61 ppm, for the remaining atoms see the Table 1. ^{13}C NMR chemical shifts were in accordance with published data (de Oliveira et al., 2011). (3*R*,6*R*)-**1** showed $[\alpha]_{589}$ −25.2, $[\alpha]_{578}$ −28.6, $[\alpha]_{546}$ −33.3, $[\alpha]_{436}$ −68.6 (c 0.21, $CHCl_3$), while (3*S*,6*S*)-**1** showed $[\alpha]_{589}$ +26.2, $[\alpha]_{578}$ +27.2, $[\alpha]_{546}$ +31.0, $[\alpha]_{436}$ +53.3 (c 0.40, $CHCl_3$).

4.3. Chiral HPLC resolution of 1

HPLC resolution of racemic **1** was achieved using a CHIRALPAK AD-H (250 mm × 4.5 mm, 5 μm) column and an *n*-hexane/isopropyl alcohol (9:1) mixture, containing 0.1% of diethylamine, as the mobile phase. The flow was set to 1 mL/min and the column was kept at 25.0 °C in a column oven. UV detection was performed at 230 nm while polarimetric detection was done using an Advanced Laser Polarimeter (ALP) equipped with a laser diode emitter at 670 nm. Fig. 2 shows the results of an 8 μL injection of a 3.6 mg/mL solution of racemic **1**, corresponding to 28.8 μg injected material.

4.4. ^{1}H NMR spin–spin simulation of 1

Simulation of the ^{1}H NMR spectrum of **1** was performed using PERCH software (PERCH Solutions Ltd., Kuopio, Finland). The ^{1}H NMR experimental data at 300 MHz were edited in the preparation module (PAC) of the PERCH shell and the global minimum energy conformer, obtained using the MMXX Monte Carlo search routine in the Spartan'04 W package (Wavefunction, Irvine, CA, USA), was imported to the editor structure module for prediction of the theoretical NMR parameters. The calculated spectrum was adjusted to the experimental spectrum by changing chemical shift values and then submitted to iteration using the Total-Line-Shape-Fitting mode from the PERH iterator initially for not overlapped signals and finally for all signals in the 1.5–6.5 ppm region. The root-mean-square deviation (rmsd) was 0.070 Hz after 30 iteration cycles.

Acknowledgment

Authors acknowledge financial support from Universidad Austral de Chile (project DID-UACH S-2009-14), CONICYT-Chile (project FONDECYT 11090083) and CONACYT-Mexico (grant 152994).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2012.04.003.

References

Chávez, D., Cui, B., Chai, H.-B., García, R., Mejía, M., Norman, R., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M., Kinghorn, A.D., 2002. Reversal of multidrug resistance by tropane alkaloids from the stems of *Erythroxylum rotundifolium*. J. Nat. Prod. 65, 606–610.

Christen, P., 2000. Tropane alkaloids: old drugs used in modern medicine. In: Atta-ur-Rahman (Eds.), Studies in Natural Products Chemistry, vol. 22. Elsevier Science, Amsterdam, pp. 717–749.

de Oliveira, S.L., Tavares, J.F., Castello Branco, M.V.S., Lucena, H.F.S., Barbosa-Filho, J.M., de, F., Agra, M., do Nascimento, S.C., dos, S., Aguiar, J., da Silva, T.G., de Simone, C.A., de Araújo-Júnior, J.X., da Silva, M.S., 2011. Tropane alkaloids from *Erythroxylum caatingae* PLOWMAN. Chem. Biodivers. 8, 155–165.

El-Imam, Y.M.A., Evans, W.C., Grout, R.J., 1988. Alkaloids of *Erythroxylum cuneatum*, *E. Ecarinatum* and *E. australe*. Phytochemistry 27, 2181–2184.

Fodor, G., Sóti, F., 1964. Correlation of valeroidine with S(−)methoxysuccinic acid and of mono- and di-tigloyltropane-3,6-diol with its R(+)antimer. Tetrahedron Lett. 5, 1917–1921.

Fodor, G., Sóti, F., 1965. The stereochemistry of the tropane alkaloids. Part XVII. Correlation of valeroidine with S(−)-methoxysuccinic acid and of mono- and di-tigloyltropane-3,6-diol with its R(+)-antimer. J. Chem. Soc. 6830–6833.

Fodor, G., Vincze, I.W., Tóth, J., 1961. The stereochemistry of the tropane alkaloids. Part XIII. The absolute configuration and a simplified syntheses of valeroidine. J. Chem. Soc. 3219–3221.

He, Y., Wang, B., Dukor, R.K., Nafie, L.A., 2011. Determination of absolute configuration of chiral molecules using vibrational optical activity: a review. Appl. Spectrosc. 65, 699–723.

Humam, M., Christen, P., Muñoz, O., Hostettmann, K., Jeannerat, D., 2008. Absolute configuration of tropane alkaloids bearing two α,β-unsaturated ester functions using electronic CD spectroscopy: application to (*RR*)-*trans*-3-hydroxysenecioyloxy-6-senecioyloxytropane. Chirality 20, 20–25.

Humam, M., Shoul, T., Jeannerat, D., Muñoz, O., Christen, P., 2011. Chirality and numbering of substituted tropane alkaloids. Molecules 16, 7199–7209.

Inui, T., Yuehong, W., Nikolic, D., Smith, D.C., Franzblau, S.G., Pauli, G.F., 2010. Sesquiterpenes from *Oplopanax horridus*. J. Nat. Prod. 73, 563–567.

Lounasmaa, M., Tamminen, T., 1993. The tropane alkaloids. In: Cordell, G.A. (Ed.), The Alkaloids, vol. 44. Academic Press, New York, pp. 1–114.

Molina-Salinas, G.M., Rivas-Galindo, V.M., Said-Fernández, S., Lankin, D.C., Muñoz, M.A., Joseph-Nathan, P., Pauli, G.F., Waksman, N., 2011. Stereochemical analysis of leubethanol, an anti-TB-active serrulatane, from *Leucophyllum frutescens*. J. Nat. Prod. 74, 1842–1850.

Muñoz, M.A., Joseph-Nathan, P., 2009. DFT-GIAO ^{1}H and ^{13}C NMR prediction of chemical shifts for the configurational assignment of 6β-hydroxyhyoscyamine diastereoisomers. Magn. Reson. Chem. 47, 578–584.

Muñoz, M.A., Muñoz, O., Joseph-Nathan, P., 2006. Absolute configuration of natural diastereoisomers of 6β-hydroxyhyoscyamine by vibrational circular dichroism. J. Nat. Prod. 69, 1335–1340.

Muñoz, M.A., Muñoz, O., Joseph-Nathan, P., 2010. Absolute configuration determination and conformational analysis of (−)-(3S,6S)-3α,6β-diacetoxytropane using vibrational circular dichroism and DFT techniques. Chirality 22, 234–241.

O'Hagan, D., 1997. Pyrrole, pyrrolidine, pyridine, piperidine, azepine and tropane alkaloids. Nat. Prod. Rep. 14, 637–651.

O'Hagan, D., 2000. Pyrrole, pyrrolidine, pyridine, piperidine and tropane alkaloids. Nat. Prod. Rep. 17, 435–446.

Queiroz, E.F., 2008. Pharmaceutical composition, pharmaceutical product, obtaining processes of pharmaceutical compounds and use of such compounds for treating erectile dysfunction. In: PCT Int. Appl. WO-2008098329-A2.

Reina, M., Burgueño-Tapia, L., Bucio, M.A., Joseph-Nathan, P., 2010. Absolute configuration of tropane alkaloids from *Schizanthus* species by vibrational circular dichroism. Phytochemistry 71, 810–815.

Scher, J.M., Schinkovitz, A., Zapp, J., Wang, Y., Franzblau, S.G., Becker, H., Lankin, D.C., Pauli, G.F., 2010. Structure and anti-TB activity of Trachylobanes from the liverwort *Jungermannia exsertifolia* ssp. *cordifolia*. J. Nat. Prod. 73, 656–663.