Absolute Configuration Determination and Conformational Analysis of (−)-(3*S*,6*S*)-3α,6β-Diacetoxytropane Using Vibrational Circular Dichroism and DFT Techniques

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ABSTRACT The absolute configuration of semisynthetic (−)-3α,6β-acetoxytropane **1**, prepared from (−)-6β-hydroxyhyoscyamine **2**, has been determined using vibrational circular dichroism (VCD) spectroscopy. The vibrational spectra (IR and VCD) were calculated using DFT at the B3LYP/DGDZVP level of theory for the eight more stable conformers which account for 99.97% of the total relative abundance in the first 10 kcal/mol range. The calculated VCD spectra of all considered conformations showed two distinctive spectral ranges, one between 1300 and 1200 cm^{-1}, and the other one in the 1150–950 cm^{-1} region. When compared with the experimental VCD spectrum, the first spectral region confirmed the calculated conformational preferences, whereas the second region showed little change with conformation, thus allowing the determination of the absolute configuration of **1** as (3*S*,6*S*)-3α,6β-diacetoxytropane. Also, the bands in the second region showed similarities between **1** and **2** in both the experimental and calculated VCD spectra, suggesting that these bands are mainly related to the absolute configuration of the rigid tropane ring system, since they show conformational independency, no variations with the nature of the substituent, and are composed by closely related vibrational modes. *Chirality 22:234–241, 2010.*

KEY WORDS: VCD; conformational distribution; absolute configuration; tropane alkaloids

INTRODUCTION

Tropane alkaloids constitute a well-known group of natural products, mostly found in the Solanaceae, Erythroxilae, and Convolvulaceae botanic families,[1–3] which are responsible for a wide spectrum of interesting pharmacological activities ranging from antispasmodic, antisecretory, and mydriatic effects,[4] to the reversing of multidrug resistance of oral epidermoid carcinoma cells.[5] Most of these molecules exhibit chiroptical properties that arise from asymmetric centers found in the tropane ring, or at substituents attached to the ring system. Specifically, tropane alkaloids derived from 3α,6β-tropanediol, like (−)-3α,6β-diacetoxytropane **1**, can be found either as (3*R*,6*R*) or (3*S*,6*S*) enantiomeric species. Nevertheless, the use a 6β-7β nomenclature, used in some articles and reviews to distinguish both stereoisomers, has added some confusion to the knowledge of the absolute configuration of these molecules.[1] Although more than 50 alkaloids of this type have been found in vegetative material, only a few of them have known absolute configuration.[1] Between them are both diastereoisomers of 6β-hydroxyhyoscyamine (3*S*,6*S*,2′*S* **2** and 3*R*,6*R*,2′*S* **3**), for which the absolute configuration was established using vibrational circular dischroism (VCD).[6] In addition, the recent use of electronic circular dichroism (ECD) has been successfully applied for the determination of the absolute configuration of (3*R*,6*R*)-*trans*-3-hydroxysenecioyloxy-6-senecioyloxytropane isolated from the aerial parts of *Schizanthus tricolor*.[7] This methodology required the calculation of 30 transition states for each of 36 molecular geometries of a model structure in order to obtain the CD spectra of individual geometries. In contrast, simpler 3α,6β-diacetoxytropane **1** (Scheme 1), previously detected in transformed root cultures of *Datura stramonium*, *D. fastuosa*, *D. quercifolia*, *D. ferox,* and *D. sanguinea*, which lacks an UV chromophore, remains with unknown absolute configuration.[8]

The use of optical rotation (OR), electronic circular dichroism (ECD), and vibrational circular dichroism (VCD), in combination with quantum mechanics calcula-

Additional Supporting Information may be found in the online version of this article.

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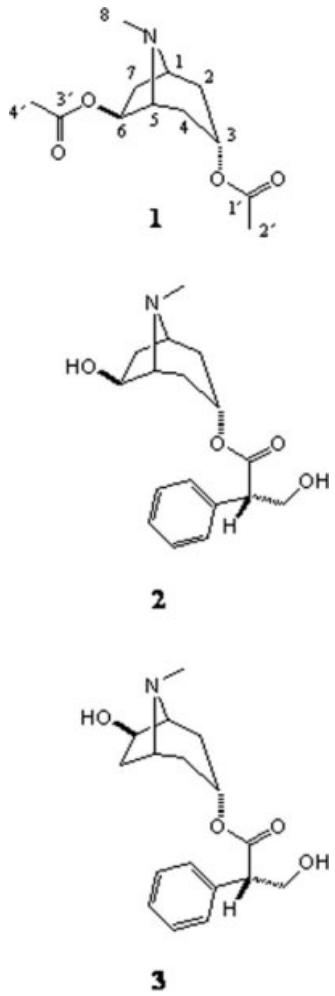

Scheme 1. Molecular structures. 190 × 254 mm (72 × 72 DPI).

tions, has proven to be useful in the task to ascertain the absolute configuration of chiral molecules.[9,10] Nevertheless, both OR and ECD present some disadvantages as compared with the use of VCD. Even when OR is a widely available technique, it is essentially a one-parameter measurement methodology that gives little room to assess the reliability of calculated results. Likewise, a typical ECD spectrum only shows a few bands that can be correlated with theory. Additionally, the use of ECD calculations is dependent upon the presence of a chromophoric group in the studied molecule in order to have a nontransparent ECD spectrum. In contrast, a typical VCD spectrum presents a considerable number of bands that allow a good comparison between experimental and theoretical spectra, thus providing good assessment of the quality of the calculation, and eventually permitting the selection of bands which give specific molecular information.

In addition, the calculations needed in any of these methodologies differ greatly in the level of complexity. In the case of ECD, the electronic transitions observed experimentally require an accurate calculation of the wavefunction in the ground state and all excited states that can contribute to the ECD spectrum, with the consequent huge cost of computational time and resources. In contrast, since VCD spectroscopy arises from vibrational transitions that occur mainly in the ground electronic state, the required calculations are much simpler to perform.

Moreover, the sensibility of VCD data to minor molecular changes provides the extra means to know the conformational distribution of a molecule. Furthermore, the recent simultaneous use of all these techniques for the determination of the absolute configuration of natural products has shown that VCD is the most reliable of these methodologies.[10]

In this article we present the conformational analysis and absolute configuration determination of (−)-3α,6β-diacetoxytropane **1** using a combination of VCD spectroscopy, molecular mechanics, and DFT calculations. Compound **1** was prepared from (−)-6β-hydroxyhyoscyamine **2**, which in turn was studied using VCD, and has been assigned the (3*S*,6*S*,3′*S*) absolute configuration.[6] Our results will be of great importance for future absolute configuration determinations of natural occurring 3α,6β-diacyloxytropane derivatives of unknown absolute configuration, while giving key information to the solution state conformational behavior of these molecules of pharmacological interest.

EXPERIMENTAL

General Experimental Procedures

VCD measurements were performed on a BOMEM-Bio-Tools dualPEM Chiral*IR* FT-VCD spectrophotometer. A sample of 5 mg was dissolved in 200 μl of $CDCl_3$, placed in a BaF_2 cell with a pathlength of 100 μm, and data were acquired at a resolution of 4 cm^{-1} during 4 h. ^1H and ^{13}C NMR measurement were done on Varian Mercury spectrometers using $CDCl_3$ solutions containing TMS as internal standard. Optical rotations $[\alpha]_D$ were measured using a Perkin-Elmer 341 polarimeter at 25°C.

Computational Methods

Conformational searches were started using a systematic search considering an initial energy cutoff of 10 kcal/mol above the global minimum. The searches were conducted independently starting from axial and equatorial *N*-Me group geometries, and performing single point energy calculations at the B3LYP/6-31G(d) level of theory for all MMFF conformations derived from the conformational searches. The different sets of conformers derived from the two *N*-Me group orientations of **1** were mixed, and the DFT energies used in a Boltzmann distribution. Only the eight relevant conformations, accounting 99.97% in the first 10 kcal/mol range, were then submitted to geometry reoptimizations, followed by vibrational calculations using the DFT B3LYP hybrid functional and the DGDZVP basis set. The use of this combination of basis set and functional has shown to require less computing time than the 6-31G(d) basis set while producing very similar results, as is evident in figures of recently published work[11–13] as well as in Figure 1 of the Supporting Information. This situation, as recently highlighted,[12] seems to be associated to the fact that DGauss basis sets, such as DGDZVP, are optimized for DFT methods. Conformational searches and

single point energy calculation were made using the Spartan′04 software package,[14] whereas geometry reoptimizations and vibrational spectra were calculated using the Gaussian 03W software package.[15] Typical calculations required between 20 and 25 h of computational time per conformer when using a desktop personal computer (PC) with 2 Gb RAM operated at 3 GHz. In our previous study on tropane alkaloids[6] we have already shown that for **2** and **3** the B3LYP/6-31G(d) calculations required some 30 to 40 h per conformer, while simply going to the higher B3LYP/6-311G++(d) level of theory consumed in average some 200 h of computing time per conformer, making this an impractical procedure. Calculated dipole and rotational strengths were converted to molecular absorptivities (M^{-1} cm^{-1}), the frequencies were scaled using an anharmonicity factor of 0.97, and plotted as Lorentzian bands with half-widths of 6 cm^{-1}. Tabulated theoretical vibrational frequencies, rotational strengths, and dipole strengths were obtained from the calculations using GaussView software and the frequencies were scaled by 0.97. Experimental vibration frequencies, rotational strengths, and dipole strengths were obtained from experimental IR and VCD spectra by Lorentzian fitting using the PeakFit software.[16]

Preparation of 1

A solution of 205 mg of (−)-(3*S*,6*S*)-6β-hydroxyhyoscyamine **2**[6] and 500 mg of $Ba(OH)_2$ in 50 ml of deionized water was heated under reflux for 4 h. The mixture was carefully acidified with aqueous 0.1 M H_2SO_4, centrifuged, and filtrated to remove the formed $BaSO_4$. Extraction with ethyl ether (20 times, 5 ml) and evaporation of the organic layer lead to the isolation of tropic acid, while addition of 500 mg of $BaCO_3$ to the aqueous phase and stirring for 12 h, followed by evaporation under reduced pressure gave a white residue. The ethanol soluble part of the later residue was separated by filtration and evaporated to yield 62 mg of (−)-(3*S*,6*S*)-3α,6β-tropanediol, $[\alpha]_D$ −9 (c 0.81, EtOH) whose ^1H and ^{13}C NMR data were identical to those reported.[17] Acetylation of the tropanediol (10 mg) by refluxing with an excess acetic anhydride (10 ml, 4 h) gave (−)-(3*S*,6*S*)-3α,6β-diacetoxytropane, $[\alpha]_D$ −9 (c 0.85, EtOH), υ_{max} ($CHCl_3$) 1724 cm^{-1} (C=O), 1252 cm^{-1} (C—O); δ_H (300 MHz, $CDCl_3$) 5.42 (dd, 1H), 5.01 (t, 1H), 3.30 (br t, 1H), 3.14 (br s, 1H), 2.51 (m, 1H), 2.49 (s, 3H), 2.08-2.17 (m, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.77 (d, 1H), 1.46 (d, 1H); δ_C (75 MHz, $CDCl_3$) 171.0, 170.1, 79.0, 67.0, 64.9, 58.9, 38.2, 36.3, 32.4, 30.8, 21.6, 21.4.

RESULTS AND DISCUSSION

The initial conformational distribution of **1**, obtained from MMFF94 systematic conformational searches, allowed the selection of all relevant conformers for single-point energy calculations at the B3LYP/6-31G(d) level of theory. Further geometry optimization of these selected conformations at the B3LYP/DGDZVP, finally lead to eight conformers arising from the combination of three different conformational variables (Table 1 and Fig. 1). The most important of these features is the *N*-Me group orientation, which can be axial or equatorial in relation to the six member ring formed by the C1,N,C5,C4,C3,C2 atoms. There has been a number of studies regarding the *N*-Me orientation of tropane alkaloids in solution, specially (−)-scopolamine, showing that both dispositions can be present, depending on the specific structural characteristics of the molecule and the solvent that can stabilize one form or another.[18–21] In the case of **1**, the conformational distribution can be divided in two groups, **1a–d** and **1e–h**, with equatorial and axial *N*-Me orientations, respectively, showing that approximately 82% of the conformational preference has the equatorial *N*-Me orientation. This preference can be easily explained considering axial–axial interactions that occur when the *N*-Me disposition is also axial, showing a distance of 2.3 Å between the *N*-methyl hydrogens and H2ax and/or H4ax. This distance increases to 2.5 Å between the *N*-Me hydrogens and H7ax and O, causing a decrease in free energy of 0.90 kcal/mol on going from **1a** to **1e**. Similar conclusions have been suggested for (−)-scopolamine using NMR studies,[21] in which the acetyl substitution present in **1** is replaced with an oxirane ring with the same orientation. In contrast, in the case of (3*S*,6*S*)-6β-hydroxyhyoscyamine **2** and its (3*R*,6*R*)-isomer **3** the axial *N*-Me orientation predominates[6] in the 82–86% range because of the presence of a hydrogen bond between the hydroxyl group and the nitrogen atom.

Two of the different dispositions that are possible for both acetyl groups are also present in the conformational distribution. The acetyl group attached to C6 of the lowest energy conformations of **1** shows C—O—C6—H6 dihedral angles of 41.3° (C=O bond oriented outsides the molecule) and −38.3° (C=O bond oriented to the inside of the molecule). The first of these dihedral angles is the more stable of the two orientations, showing a free energy difference of 0.31 kcal/mol between them (**1a** vs. **1d**), and is present in 62.2% (**1a** + **1b** + **1e** + **1f**) of the relative abundance. Moreover, the C3 acetyl group of the lowest energy conformations of **1** shows C—O—C3—H3 dihedral angles of 31.8° (C=O bond oriented towards the substituted side of the tropane ring) and −32.5° (C=O

TABLE 1. Calculated relative energies (kcal/mol), relative free energies and abundances (%) of the eight more stable conformers of 1 using systematic search, energy single point and geometry optimization calculations at the MMFF, B3LYP/6-31G(d) and B3LYP/DGDZVP level of theory, respectively

Conf.	E_{MMFF}[a]	E_{SP}[a]	$\%_{SP}$	ΔG_{OPT}[a]	$\%_{OPT}$[b]
1a	0.00	0.00	27.20	0.00	25.99
1b	0.12	0.16	26.48	0.04	24.29
1c	0.93	0.45	12.77	0.28	16.20
1d	0.94	0.55	10.80	0.31	15.40
1e	2.75	0.78	7.30	0.90	5.69
1f	2.88	0.72	8.08	0.92	5.50
1g	3.99	1.11	4.14	1.17	3.61
1h	4.00	1.27	3.20	1.22	3.32

Conformers are ordered according to their relative abundance.
[a]Relative to the lowest energy conformer.
[b]Calculated using the optimized free energies of the relevant conformers.

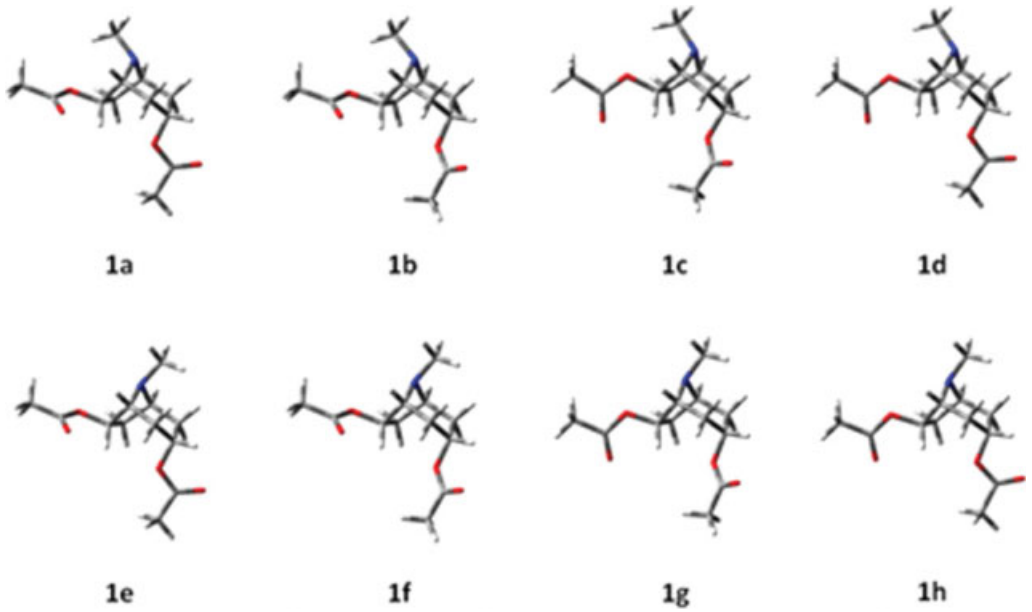

Fig. 1. Optimized structures of the eight more stable conformers of (3*S*,6*S*)-3α,6β-diacetoxytropane 1 at the B3LYP/DGDZVP level of theory. For relative free energies and abundances see Table 1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

bond oriented toward the nonsubstituted side of the tropane ring). These possible orientations are almost isoenergetic, showing an approximate difference of 0.03 kcal/mol between them (**1a** vs. **1b**, and **1e** vs. **1f**).

When the calculated VCD spectra of the lower energy conformers of **1**, using the B3LYP/DGDZVP level of theory, are compared (equatorial *N*-Me, **1a–1d** in Fig. 2, and axial *N*-Me, **1e–1h** in Fig. 3), with the experimental VCD spectrum, it is evident that several strong bands change significantly with conformation, while others remain almost invariant. To further understand the molecular behavior, the bands in the experimental vibrational spectra (Fig. 4, VCD an IR) were numerically labeled. The calculated VCD spectra show two distinctive regions in

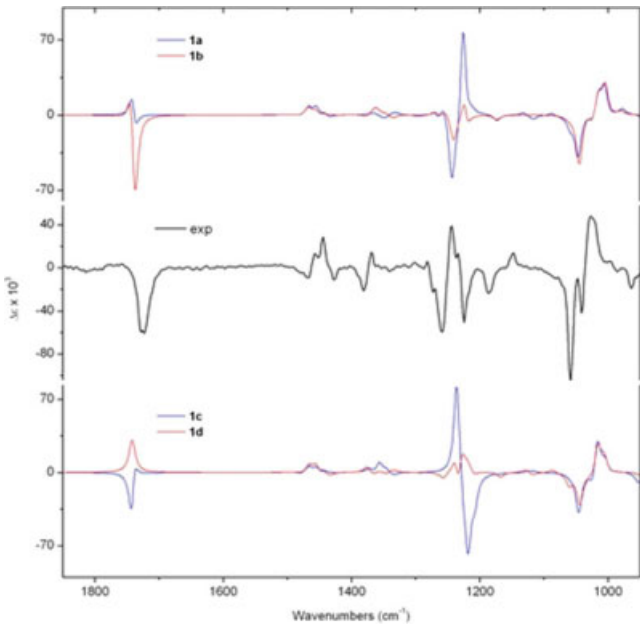

Fig. 2. Comparison of the experimental spectrum and the calculated VCD spectra for the first four (**1a-1d**, equatorial *N*-Me) lower energy conformers of (3*S*,6*S*)-3α,6β-diacetoxytropane 1 at the B3LYP/DGDZVP level of theory. Calculated wavenumbers are scaled by a factor of 0.97. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

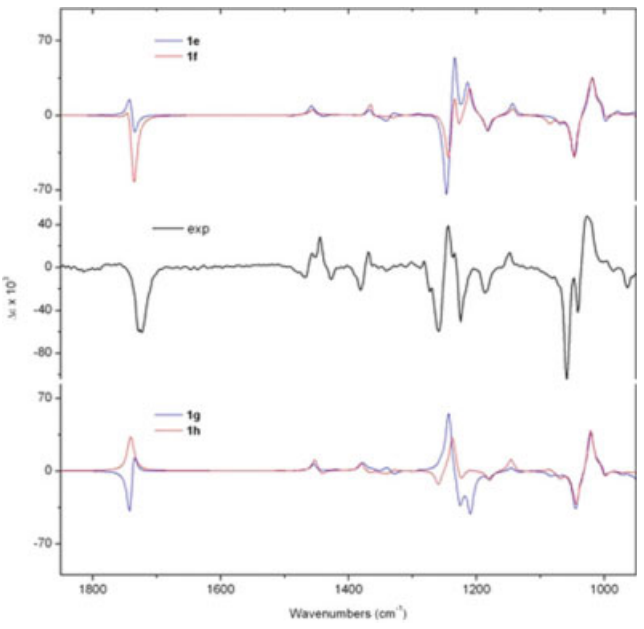

Fig. 3. Comparison of the experimental spectrum and the calculated VCD spectra for the second four (**1e-1h**, axial *N*-Me) lower energy conformers of (3*S*,6*S*)-3α,6β-diacetoxytropane 1 at the B3LYP/DGDZVP level of theory. Calculated frequencies are scaled by a factor of 0.97. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

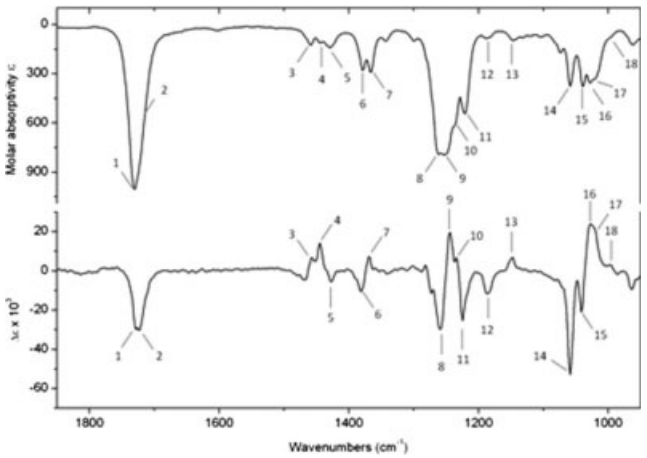

Fig. 4. Experimental IR (top) and VCD spectra (bottom) of (3*S*,6*S*)-3α,6β-diacetoxytropane 1. Selected bands are labeled numerically. For a list of wavenumbers and intensities see Table 2.

the low-energy portion. One between 1150 and 950 cm^{-1} that shows little variation with the conformation, and the other between 1300 and 1200 cm^{-1} that strongly depends on the different conformational characteristics of the molecule. The later spectral range can be used to extract information about the conformational preference of this molecule in solution. As observed in Figure 2, the two lowest energy conformers of each of the two *N*-Me group orientations (**1a** and **1b**; **1e** and **1f**) show two main bands in this range, one with negative intensity and the other one with positive intensity, on going from higher to lower wavenumbers. From the three conformational variables present, these four low energy conformers show the more stable C=O bond orientation of the acetyl group attached to C6. Moreover, the other four low energy conformers either do not present these bands or show bands with inverse intensity. When observed in the same spectral range, the experimental VCD spectrum of **1** presents the same bands (Bands 8 and 9, Fig. 4) attributable to the lowest energy disposition of the C6 acetyl group, strongly suggesting that, as calculated from the conformational searches, this orientation is the more stable one, and thus the preferred one in the solution state.

Furthermore, the Boltzmann weighted vibrational spectra depicted in Figure 5, in which the bands were also labeled, show four main bands in the 1150 to 950 cm^{-1} range (Bands 14–17), two with negative intensity, and other two with positive intensity, on going from higher to lower wavenumbers, constituted by four vibrational modes at 1047, 1024, 1015, and 1006 cm^{-1} as shown in Figure 6 for **1a**. The experimental VCD spectrum of **1** (see Fig. 4) shows these bands in the same spectral region, with the same band labeling, also two of them with negative intensity and the other two with positive intensity collapsed to form a broader band. In fact, all bands show similarities between the experimental and the weighed vibrational spectra over the entire range, as seen in Table 2. The importance of the overall spectral similarity and the great dependency of some vibrational bands with molecular conformation also makes it important that stereochemical conclusions should not be reached on the basis of conformationally labile bands. Therefore, particularly interesting are those spectral regions showing conformational independency which provide the reliability needed to confirm the absolute configuration of **1** as (3*S*,6*S*)-3α,6β-diacetoxytropane. Additionally, the same similarities can be observed

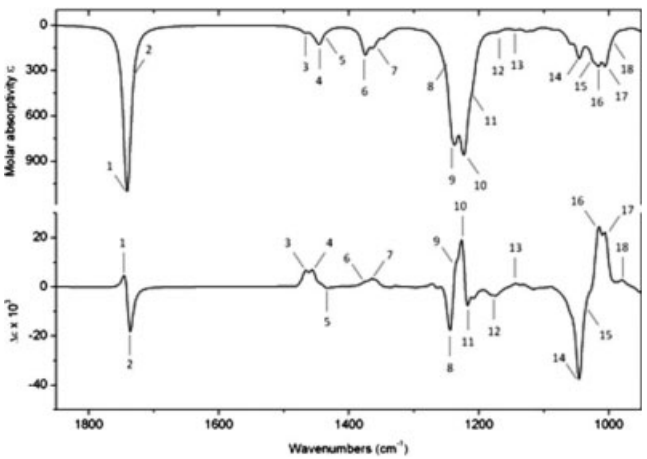

Fig. 5. Boltzman weighted IR (top) and VCD (bottom) spectra of (3*S*,6*S*)-3α,6β-diacetoxytropane 1 calculated at the B3LYP/DGDZVP level of theory. Selected bands are labeled numerically. Wave numbers are scaled by a factor of 0.97. For a list of wave numbers and intensities see Table 2.

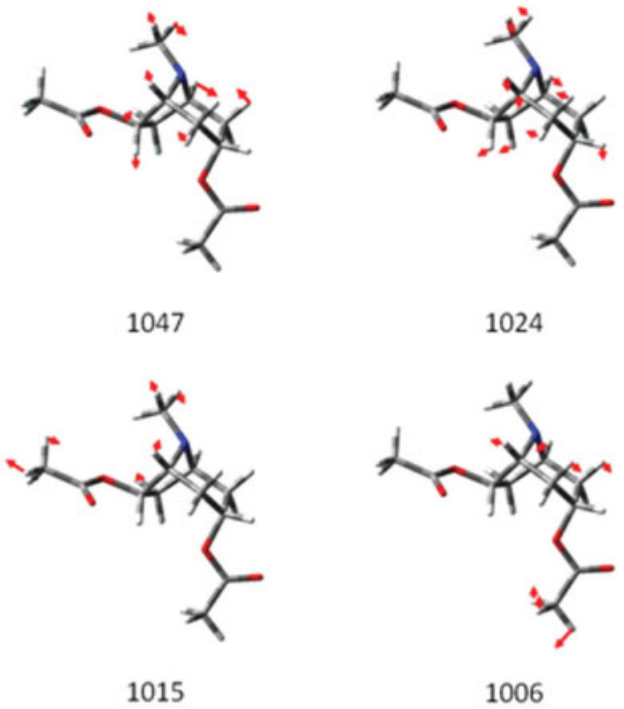

Fig. 6. Relative amplitudes of selected vibrational modes (scaled values in cm^{-1} under each structure) for the lowest energy conformer of 1. Note the arrows pointing to the atoms of interest for the particular transition. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Wavenumbers (ν in cm^{-1}) and intensities (ε and $\Delta\varepsilon \times 10^3$) of selected bands of the experimental and theoretical IR and VCD spectra of 1

	Experimental				Theoretical			
	IR		VCD		IR		VCD	
Band	ν (cm^{-1})	ε	ν (cm^{-1})	$\Delta\varepsilon \times 10^3$	ν (cm^{-1})[a]	ε	ν (cm^{-1})[a]	$\Delta\varepsilon \times 10^3$
1	1730	1004	1729	−60.5	1741	1104	1746	4.7
2	1718	702	1721	−66.4	1736	767	1736	−18.2
3	1460	124	1457	14.6	1466	54	1466	6.7
4	1443	113	1445	31.6	1446	128	1456	6.9
5	1427	141	1427	−14.1	1437	62	1433	−0.5
6	1380	281	1381	−27.8	1375	198	1373	2.3
7	1366	290	1369	16.5	1364	153	1364	3.3
8	1263	792	1259	−81.4	1249	372	1244	−17.6
9	1250	790	1244	48.9	1237	793	1236	8.7
10	1237	620	1234	16.1	1223	860	1227	19.2
11	1221	545	1224	−61.6	1211	455	1217	−7.6
12	1185	86	1186	−31.7	1174	48	1174	−3.6
13	1147	98	1148	17.7	1144	33	1144	1.3
14	1059	377	1059	−106.1	1046	220	1047	−37.6
15	1039	380	1041	−37.4	1025	234	1024	−1.4
16	1026	358	1026	47.7	1016	274	1015	24.5
17	1015	303	1020	41.8	1006	275	1006	22.2
18	998	80	999	6.2	979	3	979	2.8

[a]Wavenumbers are scaled using an anharmonicity factor of 0.97.

when comparing the theoretical and experimental VCD spectra of **1** (Fig. 7 and 8) with those of (3*S*,6*S*,2′*S*)- **2** and (3*R*,6*R*,2′*S*)-6β-hydroxyhyoscyamine **3** calculated at the B3LYP/6-31G(d) level of theory. The lower wavenumber experimental VCD spectral range of **2** shows three bands that present similar wavenumbners, signs, and intensities, while **3** presents the same bands but with opposite intensities as observed in Table 3. As reported earlier,[6] these bands are related to vibrations mostly originated in the rigid tropane ring system, and have the same vibrational movements as the bands shown in the VCD spectrum of **1**. Of these two main bands, one band having negative intensity at 1047 and 1051 cm^{-1} for **1** and **2** respectively, are mainly due to a coupled interaction between the

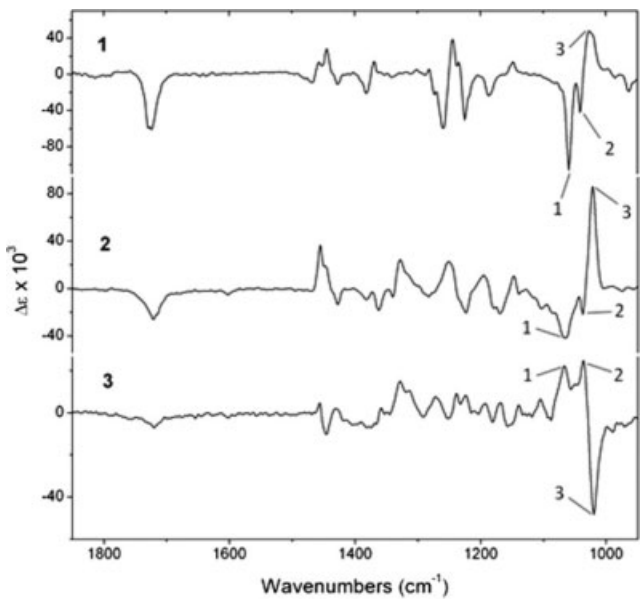

Fig. 7. Experimental VCD spectra of (3*S*,6*S*)-3α,6β-diacetoxytropane 1, (3*S*,6*S*)-6β-hydroxyhyoscyamine 2, and (3*R*,6*R*)-6β-hydroxyhyoscyamine 3.

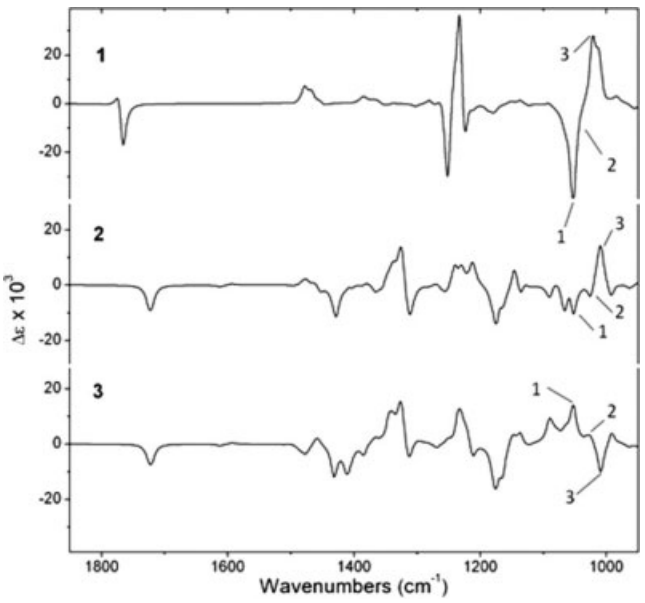

Fig. 8. Calculated Boltzmann weighted VCD spectra of (3*S*,6*S*)-3α,6β-diacetoxytropane 1, (3*S*,6*S*)-6β-hydroxyhyoscyamine 2, and (3*R*,6*R*)-6β-hydroxyhyoscyamine 3 at the B3LYP/6-31G(d) level of theory.

TABLE 3. Wavenumbers (ν in cm^{-1}), intensities ($\Delta\varepsilon \times 10^3$) and vibrational modes of intense bands in the 1150-950 cm^{-1} region of the VCD spectra of 1-3

		Experimental		Theoretical		Vibrational modes[b]
	Band[a]	ν (cm^{-1})	$\Delta\varepsilon \times 10^3$	ν (cm^{-1})	$\Delta\varepsilon \times 10^3$	as, asymmetric stretching; ss, symmetric stretching
1	1 (14)	1059	−106.1	1047	−40.4	(as) O-C6-C7 and (as) N-C1-C2
	2 (15)	1041	−37.4	1024	−16.3	(as) N-C1-C2, (as) N-C5-C4 and (ss) C1-N-C5
	3 (16)	1026	47.7	1015	27.9	(as) C1-N-C5, (as) N-C1-C2, (as) N-C5-C4, and (as) O-C6-C7
2	1 (14)	1065	−41.6	1051	−10.4	(as) O-C6-C7 and (as) N-C1-C2
	2 (15)	1038	−21.8	1025	−4.1	(as) N-C1-C2, (as) N-C5-C4 and (ss) C1-N-C5
	3 (16)	1022	86.3	1009	14.2	(as) C1-N-C5, (as) N-C1-C2, (as) N-C5-C4, and (as) O-C6-C7
3	1 (14)	1067	22.4	1051	14.1	(as) O-C6-C7 and (as) N-C1-C2
	2 (15)	1037	24.9	1026	3.3	(as) N-C1-C2, (as) N-C5-C4 and (ss) C1-N-C5
	3 (16)	1020	−48.4	1010	−10.0	(as) C1-N-C5, (as) N-C1-C2, (as) N-C5-C4, and (as) O-C6-C7

[a]Numeric assignation used in Figures 7 and 8, and assignation (in parenthesis) used in Figures 4 and 5.
[b]Vibrational mode for the most stable conformer.

asymmetric stretching vibrations of O—C6—C7 and of N—C1—C2. The second band, with positive intensity at 1015 and 1009 cm^{-1} for **1** and **2**, respectively, arises manly from the asymmetric stretching vibrations of C1—N—C5, N—C1—C2, N—C5—C4, and O—C6—C7.

Observation of the high-energy portion of the VCD spectra in the 1700–1800 cm^{-1} region, which correspond to the carbonyl stretching mode, reveals that for **1** there is a single experimental absorption band for both carbonyl groups, while in most of the calculated spectra, as seen in the two top and the two bottom traces of both Figures 2 and 3, corresponding to the eight lower energy conformers of **1**, there are two distinctive carbonyl bands which are highly conformational dependent. In the case of **2** and **3** the carbonyl absorption is because of the chiral tropic ester residue and is also highly conformational dependent as can be seen in the individual traces of the eight low energy conformers of each molecule published in Figures 3 and 4 of our previous work.[6] From these observations it follows that the carbonyl region of the VCD spectra of **1-3** is of very limited use. In addition it is known that artifacts are much larger in the carbonyl region and reliable VCD spectra can only be obtained when the racemate is also available to subtract these artifacts.[22] The carbonyl vibrations are known to be influenced by hydrogen bonding solute–solvent complexes as shown in a detailed study of pulegone[23] that evidenced association between its carbonyl group and chloroform. From a practical point of view, it is relevant to derive absolute configuration conclusions from robust VCD signals, which are defined as those that are not too much influenced by association of the studied molecule and the solvent.[24]

Although it has been discussed that there is a risk in establishing relations between spectral patterns and absolute configuration in families of closely structurally related molecules, this has been a widely used practice in ECD, OR, and optical rotatory dispersion (ORD) studies. At this respect it is important to observe the existence of bands that are mostly, or even exclusively, related to a particular absolute configuration. These observations are valuable since they provide a better understanding of the vibrational behavior of specific atoms in a given family of natural products, in particular for those having a conformational rigid scaffold.

CONCLUSIONS

The conformational analysis of (3*S*,6*S*)-3α,6β-diacetoxytropane **1** showed that the equatorial *N*-Me group orientation was the most stable disposition, accounting for 82% of the total relative abundance. In the same way, the C6 acetyl group turned out to be more stable when the C=O bond was oriented outsides the molecule, while both orientations of the C3 acetyl group showed to be almost isoenergetic. Comparison of the theoretical and experimental vibrational spectra (IR and VCD) confirmed these findings and allowed the determination of the absolute configuration of **1** as (3*S*,6*S*)-3α,6β-diacetoxytropane. Because of the conformational rigidity of the tropane ring system, the observed similarities found for **1** and **2**, in both the experimental and calculated VCD spectra, suggest the existence of several bands that are mainly related to the absolute configuration of the molecular skeleton since they show conformational independence in the eight calculated conformers, show very little variation with the nature of the substituent and are composed by closely related vibrational modes. This is further evidenced by the VCD spectrum of **3**, in which the pertinent conformationaly independent bands show opposite signs as those of **2**, in agreement with the diastereomeric nature of **2** and, and the enantiomeric nature of the tropane ring system of these two molecules.

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