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DFT-GIAO ^1H and ^{13}C NMR prediction of chemical shifts for the configurational assignment of 6β -hydroxyhyoscyamine diastereoisomers

Marcelo A. Muñoz^a and Pedro Joseph-Nathan^{b*}

^1H and ^{13}C NMR chemical shift calculations using the density functional theory–gauge including/invariant atomic orbitals (DFT–GIAO) approximation at the B3LYP/6-311G++(d,p) level of theory have been used to assign both natural diastereoisomers of 6β -hydroxyhyoscyamine. The theoretical chemical shifts of the ^1H and ^{13}C atoms in both isomers were calculated using a previously determined conformational distribution, and the theoretical and experimental values were cross-compared. For protons, the obtained average absolute differences and root mean square (rms) errors for each comparison showed that the experimental chemical shifts of dextrorotatory and levorotatory 6β -hydroxyhyoscyamines correlated well with the theoretical values calculated for the (3*R*,6*R*,2'*S*) and (3*S*,6*S*,2'*S*) configurations, respectively, whereas for ^{13}C atoms the calculations were unable to differentiate between isomers. The nature of the relatively large chemical shift differences observed in nuclei that share similar chemical environments between isomers was asserted from the same calculations. It is shown that the anisotropic effect of the phenyl group in the tropic ester moiety, positioned under the tropane ring, has a larger shielding effect over one ring side than over the other one. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: GIAO; ^1H and ^{13}C NMR; tropane; diastereoisomeric differentiation

Introduction

Tropane alkaloids^[1–3] are a class of natural products bearing the 8-methyl-8-azabicyclo[3.2.1]octane that have a wide range of pharmacological activities.^[4] The absolute configurations of the two naturally occurring diastereoisomeric forms of the tropane alkaloid 6β -hydroxyhyoscyamine (3*R*,6*R*,2'*S* **1** and 3*S*,6*S*,2'*S* **2**) have been assigned recently using vibrational circular dichroism (VCD) spectroscopy,^[5] showing that the dextrorotatory form corresponds to structure **1**, whereas the levorotatory form corresponds to structure **2** (Scheme 1). Beyond these findings, some unusual spectroscopic characteristics of these molecules remain unexplained. Despite the clear structural resemblance between them, the ^1H and ^{13}C NMR spectra of these molecules show rather large chemical shift differences of nuclei that share the same chemical environment, when both diastereoisomers^[6] are compared (i.e. the difference between H-6 in **1** and H-6 in **2** is 0.64 ppm whereas that between H-7_{endo} is 0.50 ppm). These observations suggest that if the NMR spectra of these compounds can be accurately predicted, a comparison of calculated and experimental spectra would lead to the assignment of both stereoisomers. Furthermore, the information contained in these calculations would give insights into the nature of the phenomena responsible for these unexpected differences.

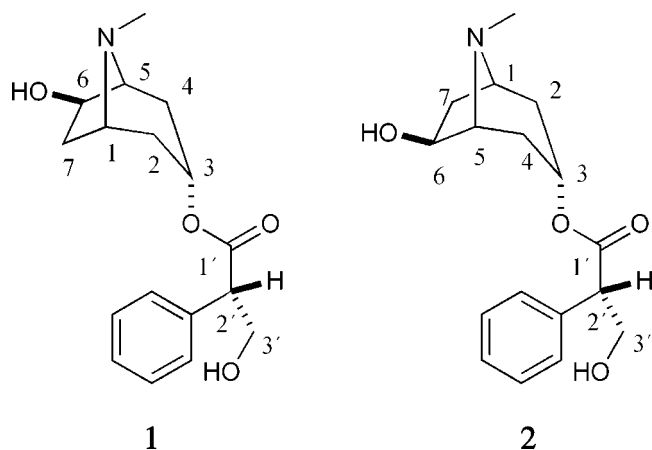
The prediction of NMR chemical shifts has undergone tremendous progresses in recent decades,^[7] allowing its use in a wide variety of fields of chemistry.^[8–10] Some very recent examples show that the use of these techniques has reached the needed accuracy to be of real help to organic chemists,^[11–15] providing an extension of an already indispensable tool, NMR spectrometry.

Although in the natural products field the use of *ab initio* predictions of chemical shifts is currently limited, it has been growing rapidly in the last few years, as exemplified by the successful use of ^{13}C calculated chemical shifts for structure identification, structure reassignment, and confirmation of natural products.^[16–33] Nevertheless, ^1H predicted chemical shifts have been used occasionally for these kinds of molecule.^[34–36] The preferred use of ^{13}C over ^1H predicted chemical shifts lies in the chemical shift range these values can have, the typical ^1H chemical shift range being 0–12 ppm *versus* 0–220 ppm for ^{13}C , thus leading to the assumption that the accuracy of proton chemical shifts would need to be at least one order of magnitude better to obtain similar results as from ^{13}C predictions.^[37] Although this is true for most structural differences, some sources of magnetic unequivalence might have a more obvious effect on ^1H NMR spectra than on ^{13}C shifts. A good example for such a situation is differences induced by the magnetic anisotropy characteristic of aromatic systems.^[38–40] The upfield effect experienced by a proton in the vicinity of the face of a benzene ring is in the same order of magnitude than that experienced by a carbon

* Correspondence to: Pedro Joseph-Nathan, Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, Mexico DF 07000, Mexico.
E-mail: pjoseph@nathan.cinvestav.mx

a Instituto de Química, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

b Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, Mexico DF 07000, Mexico



Scheme 1. Structures of (3*R*,6*R*,2'*S*)-**1** and (3*S*,6*S*,2'*S*)-6β-hydroxyhyoscyamine **2**.

atom, and consequently it will have a more noticeable effect in ^1H NMR spectra than in ^{13}C NMR spectra. A relevant example at this respect is the ^1H NMR spectrum of [8]paracyclophane, where the ring current effect leads the methylene hydrogen atoms at delta position to shift to δ 0.19 in contrast to the equivalent hydrogen atoms in amylbenzene that appear at δ 1.32, whereas the carbon that bear them lacks any noticeable chemical shift effect.^[41]

In the present work, we account on the use of ^1H and ^{13}C NMR chemical shift predictions within the gauge including/invariant atomic orbitals–density functional theory (GIAO–DFT) approximation for the determination of the configuration of both diastereoisomers of 6β-hydroxyhyoscyamine and to explain the differences observed between their NMR spectra. For this purpose, we took advantage of the energetic and geometric data of both naturally occurring diastereoisomers of 6β-hydroxyhyoscyamine as calculated^[5] for the determination of their absolute configuration as 3*R*,6*R*,2'*S* **1** and 3*S*,6*S*,2'*S* **2**, which were used as the starting point of the current work. The corresponding molecular structures were further minimized at a higher level of DFT, and the corresponding chemical shifts were computed and compared with the experimental data which resulted to be in agreement with published values. This was necessary because the identity of **1** and **2** was questionable as a result of confusing published physical data like specific molecular rotations.^[6]

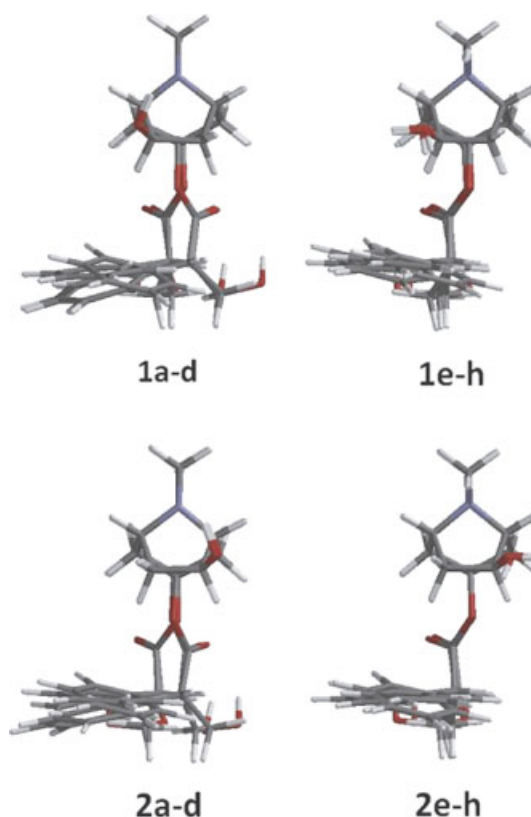


Figure 1. Superposition of the eight lowest energy conformations of **1** and **2**.

Experimental

Samples of **1** and **2** were obtained from (–)-scopolamine using a previously described methodology.^[5] ^1H and ^{13}C NMR measurement were carried out on Varian Mercury spectrometers using CDCl_3 solutions containing TMS as the internal standard. The ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were assigned using two-dimensional (2D) techniques including COSY, HSQC, and HMBC and are in agreement with literature^[6] values.

The previously informed conformational distributions^[5] of **1** and **2** were obtained using systematical conformational searches and conducted independently starting from axial and equatorial *N*-Me group geometries for each diastereoisomer, and considering an initial energy cutoff of 10 kcal/mol above the global minimum. Single point energy calculations at the B3LYP/6-31G(d) level of

Table 1. Calculated relative energies E_{rel} (kcal/mol), abundances (% $_{\text{rel}}$), and selected dihedral angles of the eight most stable conformers of **1**

E_{rel} /dihedral angle	1a	1b	1c	1d	1e	1f	1g	1h
E_{rel} (kcal/mol)	0.00	0.08	0.24	0.25	0.87	0.93	1.12	1.30
% $_{\text{rel}}$	25.66	22.42	17.11	16.83	5.91	5.34	3.88	2.85
C1'–C2'–C3'–O3'	–58.4	56.2	–57.2	57.0	–57.8	–58.4	57.9	54.8
C3'–C2'–C1'–O1'	–152.6	–169.8	–161.7	–174.8	–164.0	–153.4	–176.4	–163.9
C2'–C1'–O1'–C3	–176.3	178.5	–176.7	–178.9	–178.2	–176.3	177.1	–178.3
C1'–O1'–C3–C2	–148.0	–151.7	–89.1	–87.4	–156.5	–153.0	–153.5	–148.4
H1–C1–N–C8	59.4	59.2	59.3	59.1	–41.4	–41.1	–41.3	–40.8
C2'–C3'–O3'–H	56.0	–68.9	57.8	–67.9	–57.8	56.5	–67.0	–69.9
H6–C6–O–H	–174.8	–175.0	–174.8	–175.0	50.6	–52.0	56.0	–53.1
C $_{\text{orto}}$ –C $_{\text{ipso}}$ –C2'–H1'	4.3	–11.2	13.9	–10.2	3.3	3.6	–7.7	–10.4

Table 2. Calculated relative energies E_{rel} (kcal/mol), abundances (% $_{\text{rel}}$), and selected dihedral angles of the eight most stable conformers of **2**

E_{rel} /dihedral angle	2a	2b	2c	2d	2e	2f	2g	2h
E_{rel} (kcal/mol)	0.00	0.23	0.25	0.43	1.19	1.24	1.27	1.42
% $_{\text{rel}}$	30.45	20.66	19.97	14.74	4.09	3.76	3.57	2.76
C1'–C2'–C3'–O3'	–58.4	55.8	–57.6	58.7	–57.4	56.0	–58.5	55.4
C3'–C2'–C1'–O1'	–157.6	–169.0	–163.7	178.2	–166.6	–168.7	–153.5	–166.5
C2'–C1'–O1'–C3	–178.4	178.3	–177.9	–179.9	179.7	178.5	–177.7	179.7
C1'–O1'–C3–C2	83.4	85.4	145.5	144.0	80.2	86.0	85.9	86.5
H1–C1–N–C8	–59.1	–59.1	–58.9	–58.7	41.0	41.0	40.6	40.7
C2'–C3'–O3'–H	58.2	–68.9	59.6	–65.9	61.2	–68.8	56.4	–69.4
H6–C6–O–H	175.8	175.7	175.0	175.2	–62.6	–62.0	56.7	56.6
C $_{\text{orto}}$ –C $_{\text{ipso}}$ –C2'–H1'	2.2	–8.8	7.8	–10.4	4.3	–11.5	4.3	–11.6

Table 3. Weighted absolute shieldings (AS_{W}), theoretical chemical shifts through linear scaling (δ_{LS}), theoretical chemical shifts through TMS subtraction (δ_{TMS}), and experimental chemical shifts (δ_{Exp}) for the hydrogen and carbon atoms of **1** and **2**

		1				2			
Nucleus		AS_{W}	δ_{LS}	δ_{TMS}	δ_{Exp}	AS_{W}	δ_{LS}	δ_{TMS}	δ_{Exp}
^1H	1	28.89	3.15	2.99	3.21	29.07	2.96	2.81	3.11
	2endo	29.83	2.26	2.05	2.13	29.96	2.10	1.92	2.06
	2exo	30.69	1.44	1.19	1.44	30.93	1.16	0.95	1.26
	3	26.88	5.06	5.01	5.01	26.86	5.09	5.03	5.01
	4endo	30.03	2.08	1.86	2.06	29.77	2.28	2.11	2.16
	4exo	30.86	1.29	1.03	1.37	30.53	1.55	1.35	1.62
	5	29.42	2.65	2.46	2.84	29.19	2.84	2.69	2.99
	6	28.50	3.53	3.39	3.72	27.81	4.17	4.07	4.36
	7endo	29.48	2.60	2.40	2.33	30.27	1.80	1.62	1.83
	7exo	30.29	1.83	1.60	1.77	30.59	1.49	1.29	1.62
	2'	28.44	3.58	3.44	3.8	28.45	3.55	3.43	3.78
	3'a	27.94	4.06	3.94	3.8	28.00	3.99	3.88	3.78
	3'b	27.87	4.12	4.01	4.17	27.81	4.17	4.07	4.15
	N–Me	29.28	2.78	2.60	2.46	29.35	2.74	2.54	2.48
^{13}C	1	120.3	58.3	62.2	58.2	120.7	57.9	61.7	58.2
	2	150.9	29.9	31.6	30.3	151.9	29.0	30.6	30.1
	3	108.7	69.1	73.8	67.8	108.1	69.6	74.3	67.6
	4	153.5	27.5	29.0	28.7	153.2	27.8	29.3	28.9
	5	111.2	66.8	71.3	66.7	110.5	67.3	72.0	66.9
	6	102.3	75.1	80.2	75.4	102.0	75.3	80.5	75.5
	7	137.4	42.5	45.1	40.2	137.2	42.6	45.3	39.7
	1'	–1.2	171.0	183.6	171.7	–1.0	170.7	183.5	171.7
	2'	123.6	55.2	58.9	54.5	123.6	55.2	58.9	54.5
	3'	113.3	64.8	69.2	64.1	113.3	64.7	69.1	64.2
	N–Me	146.5	34.0	36.0	36.6	146.9	33.7	35.6	36.5

theory were performed for all molecular mechanics force field (MMFF) conformations derived from the conformational searches and the DFT energies were used in a Boltzmann distribution. The eight relevant conformations, accounting for 99.97% of the conformational distribution in the first 10 kcal/mol, were submitted to geometry optimizations using the DFT B3LYP hybrid functional and the 6-31G(d) basis set, and the magnetic shielding tensors were then calculated with the same functional and the 6-311G++(d,p) basis set, because this basis set has shown to be an excellent compromise between accuracy and computational time for this particular kind of calculations.^[37] Conformational searches and single point energy calculation were made using a Spartan'04 software package,^[42] whereas geometry optimizations

and magnetic shieldings were calculated using a Gaussian 03W software package.^[43]

Results and Discussion

Conformational preferences

The conformational distribution of both diastereoisomers of 6 β -hydroxyhyoscyamine was recently calculated at the B3LYP/6-31G(d) level of theory for the determination of the absolute configuration of these molecules using VCD spectroscopy.^[5] It turned out that the distribution was composed by eight conformers for each stereoisomer (**1a–h** and **2a–h**) accounting for 96.8 and 96.5% in

Table 4. Absolute shieldings of the eight lowest energy conformers of **1**

	Nucleus	1a	1b	1c	1d	1e	1f	1g	1h
¹ H	1	28.88	28.96	28.80	28.92	28.85	28.88	28.90	28.85
	2endo	29.80	29.78	29.79	29.86	29.93	29.91	29.96	29.90
	2exo	30.81	30.80	30.77	30.71	30.32	30.29	30.34	30.33
	3	26.76	26.85	26.98	26.93	27.01	26.92	26.91	26.75
	4endo	30.10	29.96	29.97	29.97	30.12	30.18	29.99	30.09
	4exo	31.20	30.73	30.88	30.96	30.28	30.65	30.13	30.53
	5	29.57	29.41	29.19	29.37	29.31	29.76	29.16	29.68
	6	28.74	29.15	27.59	27.91	29.19	28.46	28.53	28.69
	7endo	29.32	29.64	29.40	29.39	29.64	29.49	29.95	29.61
	7exo	30.29	30.61	30.14	30.22	30.16	29.89	30.33	30.00
	2'	28.62	28.16	28.69	28.25	28.64	28.66	28.35	28.15
	3'a	28.15	27.68	28.04	27.72	28.25	28.23	27.82	27.73
	3'b	27.50	28.31	27.40	28.34	27.54	27.52	28.45	28.33
	N-Me	29.25	29.31	29.29	29.24	29.39	29.23	29.33	29.22
¹³ C	1	122.2	121.2	120.8	120.4	115.2	116.9	115.6	115.8
	2	153.3	153.1	152.9	152.9	140.7	141.2	140.7	141.4
	3	109.8	108.6	108.5	108.4	107.4	108.3	107.1	108.4
	4	155.9	155.2	156.2	156.0	142.7	143.6	142.6	143.0
	5	112.1	112.1	111.0	111.5	109.7	107.1	110.0	106.5
	6	103.2	103.2	102.4	102.6	99.5	99.2	99.4	98.9
	7	137.1	137.3	136.0	135.4	139.5	142.6	139.6	142.9
	1'	-2.3	-0.7	-0.9	-0.7	-0.8	-2.0	-0.3	0.5
	2'	125.3	121.5	125.5	121.7	124.6	125.2	122.2	121.8
	3'	113.9	112.3	114.3	112.2	114.5	114.5	112.6	112.2
	N-Me	148.0	148.3	148.6	148.5	138.4	138.0	138.1	138.1

the first 5 kcal/mol range for **1** and **2**, respectively. The relative energies, abundances, and selected dihedral angles of these conformations are shown in Tables 1 and 2. Moreover, it was established that the conformation preferences of these molecules are ruled mainly by the formation of hydrogen bond interactions between the N atom and the hydroxyl group at C-6, and between the carbonyl group and the hydroxyl group at a C atom. Model superposition of these low energy conformations is shown in Fig. 1 for **1** and **2**, where it can be observed that both molecules show almost identical conformational preferences. In both diastereoisomers, conformations with the phenyl group positioned below the tropane bicycle are preferred, and the orientation of this group is similar regardless the relative position of the tropane hydroxyl group. Considering the preferred disposition of the tropic ester moiety, a hypothesis for the differences in chemical shift of ¹H and ¹³C can be proposed on the basis of the anisotropic magnetic shielding effects characteristic of aromatic compounds, in this case the phenyl group, commonly known as ring current effect. This effect can have different intensities on opposite sides of the tropane ring in each diastereoisomer, causing the observed chemical shift differences.

Correlations between predicted and experimental chemical shifts

The absolute shieldings for protons and carbons of the eight low energy conformers of each stereoisomer were calculated within the GIAO approximation using DFT at the B3LYP/6-311G++(d,p) level of theory. The values obtained for selected hydrogen and carbon atoms of each conformation were then used to obtain weighted average absolute shieldings using the estimated relative abundances (Tables 1 and 2) and are shown in Table 3, whereas a

list of the absolute shieldings calculated for each conformation of **1** is included in Table 4, and of **2** in Table 5. The weighted shieldings were then converted to theoretical chemical shifts using two different procedures. First they were calculated using the linear scaling method^[44] taking into account experimental chemical shifts for the dextrorotatory and levorotatory diastereoisomers. The more straightforward methodology of subtracting the calculated absolute shieldings from theoretical value of TMS was used. As seen in Table 6, the calculations predicted fairly well the experimental differences observed in nuclei sharing the same chemical environment between both diastereoisomers, both qualitatively and quantitatively.

These theoretical chemical shifts calculated for **1** and **2** were then cross-compared with the experimental values of the dextrorotatory and levorotatory stereoisomers obtaining average absolute differences (Δ_{aa}) and root mean square (rms) errors for each comparison, as summarized in Table 7. These indicators allowed to know how well the theoretical values predict the experimental behavior and therefore which calculated spectra correlate best with one of the stereoisomers. For ¹H values, the weighted theoretical chemical shifts calculated for **1** and **2** showed a very good degree of correlation when compared with the experimental values of dextrorotatory and levorotatory 6 β -hydroxyhyoscyamines, respectively, presenting increases in the average absolute difference ranging between 146.5 and 22.3%, and rms errors ranging between 163.8 and 37.3%. Additionally, a better degree of correlation (smaller errors and differences) along with a larger selectivity for the correct isomer (percentage of increase in errors and differences) was achieved using the linear scaling procedure.

Table 5. Absolute shieldings of the eight lowest energy conformers of **2**

	Nucleus	2a	2b	2c	2d	2e	2f	2g	2h
¹ H	1	29.12	29.10	28.95	29.06	29.10	29.03	29.13	29.08
	2endo	29.99	29.92	29.93	29.88	30.10	30.01	30.12	30.04
	2exo	30.98	30.85	31.12	31.12	30.35	30.44	30.70	30.46
	3	26.91	26.88	26.81	26.69	27.07	26.87	26.91	26.86
	4endo	29.72	29.74	29.76	29.80	29.88	29.91	29.91	29.94
	4exo	30.54	30.58	30.63	30.61	30.15	30.14	30.23	30.24
	5	29.19	29.26	29.18	29.11	29.07	28.99	29.35	29.37
	6	27.85	28.09	27.78	27.81	27.42	27.44	27.12	27.41
	7endo	30.70	30.79	29.13	29.61	31.28	30.81	30.49	30.82
	7exo	30.79	30.86	30.26	30.44	30.63	30.55	30.15	30.31
	2'	28.61	28.09	28.71	28.26	28.67	28.18	28.73	28.14
	3'a	28.21	27.69	28.12	27.83	28.28	27.73	28.21	27.69
	3'b	27.43	28.32	27.40	28.38	27.55	28.35	27.48	28.33
	N-Me	29.40	29.35	29.27	29.33	29.26	29.40	29.34	29.39
¹³ C	1	121.4	121.7	121.4	121.8	115.7	115.4	116.2	116.0
	2	153.8	153.3	153.8	153.8	141.1	140.7	141.3	140.9
	3	108.0	108.0	108.0	109.1	106.4	108.1	108.9	108.9
	4	155.0	155.0	155.0	154.3	142.8	142.4	143.0	142.8
	5	110.9	110.8	110.9	110.8	109.7	109.6	106.6	106.2
	6	102.6	102.8	102.6	101.9	98.5	98.6	98.0	98.2
	7	136.6	136.5	136.6	136.4	139.7	139.3	142.8	143.0
	1'	-1.6	0.1	-1.6	-1.5	-0.4	0.4	-1.7	0.3
	2'	124.9	121.7	124.9	121.8	124.7	121.7	125.2	121.6
	3'	114.1	112.5	114.1	112.0	114.6	112.2	114.1	112.0
	N-Me	148.3	148.3	148.3	148.4	138.3	138.1	137.9	138.0

Table 6. Differences in theoretical (through linear scaling ($\Delta\delta_{LS}$), and TMS subtraction ($\Delta\delta_{TMS}$)) and experimental chemical shifts ($\Delta\delta_{exp}$) of nuclei with the same chemical environment between **1** and **2**. The differences were taken subtracting the respective chemical shift of **2** from those of **1**. Only hydrogen and carbon values of the tropane ring are shown

	Nucleus	$\Delta\delta_{LS}$	$\Delta\delta_{TMS}$	$\Delta\delta_{exp}$
¹ H	1	0.17	0.18	0.10
	2endo	0.05	0.13	0.07
	2exo	0.07	0.24	0.18
	4endo	-0.30	-0.25	-0.10
	4exo	-0.43	-0.33	-0.25
	5	-0.23	-0.23	-0.15
	6	-0.55	-0.69	-0.64
	7endo	0.65	0.79	0.50
¹³ C	7exo	0.17	0.31	0.15
	1	0.4	0.4	0.0
	2	0.9	1.0	0.2
	4	-0.3	-0.3	-0.2
	5	-0.6	-0.7	-0.2
	6	-0.2	-0.3	-0.1
	7	-0.2	-0.2	0.5

Although these results that are based on ¹H chemical shifts clearly point out to the confirmation of the configurations previously proposed for these two natural compounds using VCD,^[5] for carbon nuclei the experimentally observed differences in chemical shifts between isomers are too small to allow

differentiation. This is evidenced by the small selectivity towards the configuration of the dextrorotatory experimental chemical shifts, showing increases in average difference and rms errors ranging between 22.9 and 1.8%. Most importantly, the levorotatory experimental chemical shifts correlated better with the incorrect 3*R*,6*R*,2'*S* configuration, showing decreases in average difference and rms errors ranging between 3.9 and 1.2%. This difficulty to differentiate diastereoisomeric species using ¹³C calculated chemical shifts compared with ¹H predictions have been reported earlier for penam β -lactams.^[45]

As mentioned above, chemical shift predictions normally are more accurate for carbon atoms than for hydrogen atoms, although for the herein study molecules the reversed situation became evident. Thus, the current calculated carbon chemical shift values might be of interest for theoretical chemist to improve calculation methodologies.

Chemical shift differences

Nevertheless, the nature of the effects involved in the rather large differences observed between these diastereoisomeric species cannot be asserted from these comparisons, and the initially proposed hypothesis based on the ring current effect of the phenyl group over the tropane bicycle still needs to be tested. At this respect, calculations of the expected influence of the benzene ring over the chemical shifts of tropane ring protons of **1** and **2** were performed for the most stable conformer of each diastereoisomer according to the equation developed by Martin *et al.*^[46] Table 8 summarizes the predicted change in proton chemical shifts for the tropane ring of **1** and **2**, along with their differences between both diastereoisomers. The obtained differences are in qualitative

Table 7. Average absolute differences (Δ_{aa}), root mean square errors (rms), and selectivity percentages ($\%_{sel}$) of correlations between theoretical and experimental chemical shifts of **1** and **2**

Error		Dextrorotatory						Levorotatory					
		3 <i>R</i> ,6 <i>R</i> ,2' <i>S</i>		3 <i>S</i> ,6 <i>S</i> ,2' <i>S</i>		% _{sel}		3 <i>R</i> ,6 <i>R</i> ,2' <i>S</i>		3 <i>S</i> ,6 <i>S</i> ,2' <i>S</i>		% _{sel}	
		δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}
¹ H	Δ_{aa}	0.14	0.20	0.20	0.25	47.3	22.3	0.28	0.28	0.11	0.20	146.5	41.7
	rms	0.17	0.23	0.23	0.33	37.7	39.7	0.36	0.40	0.14	0.23	163.8	71.8
¹³ C	Δ_{aa}	0.9	4.3	1.2	4.4	22.9	1.8	1.0	4.4	1.0	4.4	−1.2	−1.7
	rms	1.2	5.3	1.4	5.4	16.3	1.8	1.3	5.3	1.4	5.4	−3.9	−1.7

Table 8. Calculated chemical shift changes ($\Delta\delta_M$) of selected nuclei in the tropane ring of **1** and **2** induced by the benzene ring, and chemical shift differences calculated from these changes ($\Delta\delta_M$ (**2**−**1**)) and from experimental chemical shifts ($\Delta\delta_{exp}$) of nuclei with the same chemical environment between **1** and **2**

Proton	$\Delta\delta_M$ 1	$\Delta\delta_M$ 2	$\Delta\delta_M$ (2 − 1)	$\Delta\delta_{exp}$
1	0.02	0.24	0.22	0.10
2endo	0.02	0.06	0.03	0.07
2exo	0.03	0.27	0.23	0.18
4endo	0.06	0.02	−0.04	−0.10
4exo	0.32	0.04	−0.29	−0.25
5	0.14	0.02	−0.12	−0.15
6	0.34	0.09	−0.25	−0.64
7endo	0.07	2.51	2.44	0.50
7exo	0.04	0.20	0.16	0.15

Table 9. Calculated shielding tensors (σ_{xx} , σ_{yy} , and σ_{zz}) that contribute to the isotropic shielding and anisotropies (*A*) of selected protons in the tropane ring of the most stable conformer of **1** and **2**

Proton	1				2			
	σ_{xx}	σ_{yy}	σ_{zz}	<i>A</i>	σ_{xx}	σ_{yy}	σ_{zz}	<i>A</i>
1	28.22	31.39	27.17	8.24	26.52	32.35	28.36	7.15
2endo	29.57	29.78	29.95	7.95	33.39	25.54	30.86	7.32
2exo	27.69	35.20	29.55	7.86	29.19	31.06	32.58	6.45
4endo	32.76	26.00	31.35	7.43	28.20	30.88	30.29	7.65
4exo	28.74	32.53	32.23	6.55	27.81	34.56	29.11	7.28
5	27.84	32.35	28.33	5.74	29.89	30.15	27.42	6.63
6	26.30	26.10	33.58	10.20	29.19	23.66	29.60	4.66
7endo	28.90	27.02	32.24	4.73	26.75	28.38	37.77	12.38
7exo	35.16	28.71	26.95	7.94	31.47	32.72	29.64	7.05

agreement with the experimental ones, clearly indicating the existence of the suspected larger overall shielding effect of the benzene ring over one side of the tropane ring (H-4endo, H-4exo, H-5, and H-6 for **1** and H-2endo, H-2exo, H-1, H-7endo, and H-7exo for **2**; left side of the tropane ring for both cases using the representation of Fig. 1) than over the other side. Nevertheless, from the quantitative point of view, large differences between theory and experiment are observed, specifically in the case of the nuclei that are closer to the phenyl group. These differences are better predicted by the direct GIAO calculations, where more conformations and the influence of the substitution on the phenyl group are taken into account.

Furthermore, these direct GIAO calculations can also be used to obtain additional information through the evaluation of the calculated shielding tensors that are unavailable experimentally for isotropic solutions.^[47] Table 9 shows the shielding tensors that contribute to the isotropic shielding and anisotropies of selected protons in the tropane ring of the most stable conformer of **1** and **2**. As can be observed, contributions to the isotropic chemical shifts from the σ_{zz} shielding tensor are larger in nuclei H-4endo, H-4exo, H-5, and H-6 in **1** than for the corresponding nuclei in **2** (with the Z-axis perpendicular to the benzene ring plane). In the same way, nuclei H-2endo, H-2exo, H-1, H-7endo, and H-7exo in **2** also showed larger contributions from the same tensor when compared with the corresponding nuclei in **1**. These theoretical results strongly suggest that this anisotropic effect from the phenyl group is responsible for the observed upfield difference in the chemical shift of nuclei in the left side of the tropane ring (as in Fig. 1) when compared between the 3*R*,6*R*,2'*S* and 3*S*,6*S*,2'*S* isomers. Additionally, the anisotropy of

the calculated isotropic shieldings can confirm these conclusions because a higher level of anisotropy is expected for the nuclei that are more influenced by the benzene ring.^[48] This is confirmed because a larger anisotropy is calculated for H-6 in **1** (10.14 ppm) in comparison with the same proton in **2** (3.90 ppm). Accordingly, H-7endo also presents a larger anisotropy value in **2** (12.87 ppm) when compared with the same nucleus in **1** (4.80 ppm).

Conclusions

The conformational distributions of **1** and **2** showed that the tropane ester moiety is mainly positioned under the tropane bicycle with only small differences between conformations. This conformational preference was in agreement with the proposed hypothesis that explained the observed chemical shift differences between chemically equivalent nuclei on both diastereoisomers in terms of the anisotropic shielding effect of the phenyl group over the tropane bicycle. Both the values of average absolute differences and rms errors showed a significant increase when the theoretical ¹H chemical shifts calculated for the 3*R*,6*R*,2'*S* configuration were compared with the experimental values of the levorotatory form of 6 β -hydroxyhyoscyamine, previously assigned with the 3*S*,6*S*,2'*S* configuration, independent of the methodology used to convert the absolute shieldings into chemical shifts. In the same way, the correlation between the theoretical ¹H chemical shifts calculated for the 3*S*,6*S*,2'*S* configuration and the dextrorotatory form of 6 β -hydroxyhyoscyamine, previously assigned with the 3*R*,6*R*,2'*S* configuration, also showed a clear increment in these two indicators. In the case of ¹³C atoms,

these values showed inconsistencies and small selectivity towards correct and incorrect isomers, which can only be explained by the incapacity of the calculations to differentiate between diastereoisomers.

Additionally, an analysis of the calculated shielding tensors along with the anisotropy of the isotropic chemical shift handed further proof of an increased shielding effect from the phenyl current density on the 3*R*,6*R*,2'*S* isomer than on the 3*S*,6*S*,2'*S* isomer, as the source of the chemical shift difference between diastereoisomers.

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