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Effect of the solvent on the conformation of monocrotaline as determined by isotropic and anisotropic NMR parameters

Cleyton Marcos de Melo Sousa[1,2] | **Raquel Brandt Giordani**[3] | **Wamberto Alristenio Moreira de Almeida**[3] | **Christian Griesinger**[4] | **Roberto R. Gil**[5] | **Armando Navarro-Vázquez**[1] | **Fernando Hallwass**[1]

[1] Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil
[2] Instituto Federal de Pernambuco, Caruaru, Pernambuco, Brazil
[3] Laboratório de Farmacognosia, Departamento de Farmácia, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil
[4] Abteilung für NMR-basierte Strukturbiologie, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany
[5] Department of Chemistry, Carnegie Mellon University, Pittsburgh, Pennsylvania

Correspondence
Fernando Hallwass, Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.
Email: hallwass@ufpe.br

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Abstract

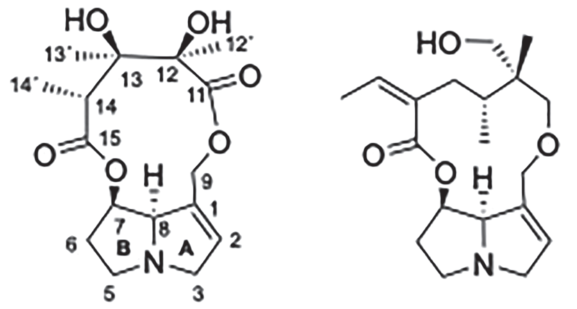
The conformation in solution of monocrotaline, a pyrrolizidine alkaloid presenting an eleven-membered macrocyclic diester ring, has been investigated using a combination of isotropic and anisotropic nuclear magnetic resonance parameters measured in four solvents of different polarity (D_2O, DMSO-d_6, $CDCl_3$, and C_6D_6). Anisotropic nuclear magnetic resonance parameters were measured in different alignment media, based on their compatibility with the solvent of interest: cromoglycate liquid crystal solution was used for D_2O, whereas a poly (methyl methacrylate) polymer gel was chosen for $CDCl_3$ and C_6D_6, and a poly (hydroxyethyl methacrylate) gel for DMSO-d_6. Whereas the pyrrolizidine ring shows an E_6 *exo*-puckered conformation in all of the solvents, the macrocyclic eleven-membered ring adopts different populations of *syn*-parallel and *anti*-parallel relative orientation of the carbonyl groups according to the polarity of the solvent.

KEYWORDS

^1H, ^{13}C, conformation, monocrotaline, NMR, RDC

1 | INTRODUCTION

A common motif in several pyrrolizidine alkaloids is a macrocyclic diester structure either eleven-membered, as in the case of the monocrotaline alkaloid (1), or twelve-membered as in retrorsine (2; Figure 1). The macrocyclic ring may present two main conformations as shown in several X-ray studies.[1] These conformations can be named *anti*-parallel or *syn*-parallel after the relative orientation of the ester carbonyl groups. For instance,



Monocrotaline (1) Retrorsine (2)

FIGURE 1 Chemical structures of monocrotaline (1) and retrorsine (2)

whereas retrorsine (2) [2] and retrorsine hydrobromide[3] present an *anti*-parallel open conformation in solid state, monocrotaline (1) presents a *syn*-parallel conformation.[4] However, very little attention has been devoted to the conformational state of these molecules in solution and, particularly, the solvent dependency of the conformational state. Recently, retrorsine (2) has been the target of several nuclear magnetic resonance (NMR) studies in aligning media, either as a probe molecule for the evaluation of aligning media[5] or in methodological studies for the application of residual chemical shift anisotropies (RCSAs) to structural elucidation.[6–8] The previous residual dipolar coupling (RDC) analysis by Gil-Silva et al. showed that retrorsine preferred in DMSO[5,9] an *anti*-parallel conformation basically superimposable with the X-ray structure.[4]

Here, we analyzed the conformational preference of monocrotaline (1) in solvents of different polarity, using a combination of isotropic ($^{13}C/^{1}H$ chemical shifts, ^{3}J scalar couplings) and anisotropic (RDC) experiments. The results indicate that whereas the pyrrolizidine ring shows a locked E_6 conformation in all solvents, the diester macrocycle conformation is strongly solvent dependent.

2 | METHODOLOGY

2.1 | Plant material

Seeds of *Crotalaria retusa* were collected in Natal, Rio Grande do Norte, Brazil, in March 2013. They were identified by MSc. Alan de Araújo Roque, Federal University of Rio Grande do Norte, and a voucher specimen was deposited at the Herbarium of the Federal University of Rio Grande do Norte, Brazil, under the reference number 16083. The authorization for harvesting the plant material was given by SISBIO (32749-2), and the permission to access the Brazilian genetic patrimony was allowed by CNPq (010142/2012-6).

2.2 | Extraction and isolation

The seeds of *C. retusa* (315 g) were dried in an air circulating oven at 45°C, crushed, and subjected to exhaustive maceration with 96% ethanol at room temperature. The crude extract was filtered, dried under reduced pressure, and solubilized in 10% HCl. The acidic solution (pH = 1) was extracted with $CHCl_3$ (300 ml) to provide Extract A. The aqueous solution was basified to pH = 9 with concentrated NH_4OH and extracted with chloroform (300 ml). The chloroform extract was dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure to give Extract B (0.538 g). Extract B (crude alkaloid fraction) was submitted to recrystallization with methanol to give monocrotaline (1).

2.3 | NMR experiments

2.3.1 | Experiments in D_2O

Isotropic and anisotropic experiments in D_2O were carried out in an Agilent 400 MHz spectrometer operating at 399.7 MHz for ^{1}H, 100.52 MHz for ^{13}C, and 61.3 MHz for ^{2}H, using a 5-mm NMR tube. Isotropic and anisotropic samples were prepared as follows: 15 mg of monocrotaline, in its free base form, were dissolved in the disodium cromoglycate/NaCl/D_2O liquid crystal alignment medium, prepared as described previously.[10] The amount of disodium cromoglycate was adjusted to obtain a fully anisotropic phase in the desired range of temperatures. Anisotropic and isotropic conditions were obtained by placing the temperature of the sample just below (23°C) and above (28°C), respectively, of the liquid crystal clearing point, as monitored by the ^{2}H splitting of the D_2O signal. A 90 Hz deuterium quadrupolar splitting was observed in the anisotropic phase. One-bond proton-carbon ($^{1}D_{CH}$) RDCs in the disodium cromoglycate medium were determined by measuring the total splittings ($^{1}T_{CH}$) at temperatures in the anisotropic and isotropic conditions through a standard F2-coupled heteronuclear single quantum correlation (HSQC) experiment with adiabatic carbon pulses. Spectral widths of 10 kHz in F2 and 4 kHz in F1, 194 ($t1$) × 1,000 ($t2$) complex data points, and eight scans were employed. Proton-proton $^{3}J_{HH}$ vicinal couplings were measured from a ^{1}H spectrum of monocrotaline dissolved in pure D_2O recorded in a 600 MHz Avance III spectrometer.

2.3.2 | Experiments in $CDCl_3$, DMSO-d_6, and C_6D_6

These experiments were performed in a Bruker Avance III 500 NMR spectrometer operating at 500.13 MHz for ^{1}H, 125.77 for ^{13}C, and 76.77 MHz for ^{2}H at 300 K, in a 5-mm NMR tube. For these analyses, solutions of 5.3, 2, and 1 mg of monocrotaline in 0.5 ml of $CDCl_3$, DMSO-d_6, and C_6D_6, respectively, were prepared. Compressible poly (methyl methacrylate; PMMA) gels[11] were let to swell in $CDCl_3$ and C_6D_6 solutions, whereas a poly (hydroxyethyl methacrylate; poly-HEMA)[5] was employed for DMSO-d_6. The degrees of alignment were evaluated through ^{2}H quadrupolar splitting from the deuterated solvent signal. The ^{2}H spectra were recorded with a spectral width of 767.8 Hz and 4,096 complex data points and a single transient.

For the recording of the $^1D_{CH}$ RDCs, $^1T_{CH}$ splittings were acquired using either standard F2-coupled HSQC experiments with adiabatic carbon pulses (spectral width of 3 kHz in F2 and 16 kHz in F1, 256 ($t1$) × 1,024 ($t2$) complex data points, relaxation delay of 1 s, eight scans) or bilinear rotation decoupling-filtered *J*-resolved HSQC spectra (spectral width of 3.5 kHz in F2 and 500 Hz in F1, 256 ($t1$) × 799 ($t2$) complex data points, relaxation delay of 1 s, eight scans) for the isotropic (relaxed gel) and anisotropic (compressed gel) conditions. F1-coupled spectra were acquired with at least eight transients. Spectra were apodized with a cosine-squared window function and zero-filled in both dimensions.

1D (^{1}H and ^{13}C) and 2D (COSY, NOESY, HSQC, and HMBC) experiments were carried out in all solvents to guarantee correct assignment of the ^{1}H and ^{13}C signals. Spectra were processed and analyzed using MestreNova software (see supporting information).

All the reagents used were of analytical grade.

2.4 | Molecular modelling

The conformational space of monocrotaline (1) was explored at the Merck Molecular Force Field 94 (MMFF94)[12] level of theory using the Monte Carlo Multiple Minimum algorithm[13] as implemented in MacroModel. [14] Structures were kept in a window of 21 kJ/mol. MMFF94 structures were then refined at the M062X[15]/6 − 31 + G** level of theory using the Gaussian09 “ultrafine” pruned grid. M062X/6 − 31 + G** optimizations were carried out IEFPCM[16] implicit solvation with either chloroform or water parameters. Chemical shielding tensors were then computed at the GIAO// PBE0[17]/6 − 311 + G** level on the M062X geometries using the Gaussian09 software package.[18]

2.5 | NMR data analysis

For analysis of the conformation, we employed the recently described multiparametric CASE-3D approach.[19,20] This method is based on the selection of statically parsimonious conformational models through the Akaike Information Criterion (AIC), [21] defined as AIC $=\chi^2+2(n-1)$, where n is the number of populations in a certain combination of conformers chosen from the whole density functional theory (DFT) set of conformations[19,22–24]. χ^2 is defined as the sum of scaled quadratic residuals for the measured RDCs;

$$\chi^2 = \sum\left(\frac{D_i^{exp}-D_i^{calc}}{\sigma_D}\right)^2,$$

where D_i^{exp} and D_i^{calc} represent experimental and back-calculated $^1D_{CH}$ RDCs. The σ_D terms represent the estimated uncertainty for the different type of parameters. Following previous work,[20] this term was set to 1.2 Hz. CASE-3D fittings were performed using the C++ StereoFitter module. [25]

3 | RESULTS AND DISCUSSION

The conformational space of monocrotaline (1) was initially explored at the molecular mechanics level, using the MMFF94 force field. Geometries in the obtained conformational ensembles were then refined at the DFT M062X/6-31 + G** level, using implicit solvation with either chloroform or water parameters. Nearly, identical structures and close relative energies were obtained for the two ensembles; hence, all NMR fitting computations were performed just using the $CHCl_3$ computed ensemble. In water, pyrrolizidine alkaloids are weak bases with a reported pKa of 7.08 for monocrotaline.[26] Therefore, modelling of the free base also directly applies to our observed NMR data in pure D_2O or cromoglycate solutions.

3.1 | Conformation of the pyrrolizidine ring

Examination of the DFT ensemble shows several possible conformations of the pyrrolizidine B ring. They can be classified[27] as *exo*-puckered (E_6; $^5T_6$) or the *endo*-puckered (E_5; $^6T_5$) and (E_7; $^6T_7$). Computations show a clear preference for the E_6 form, whereas E_5 and E_7 type conformations appear 2.4 and 3.5 kcal/mol energetically higher than E_6 (see supporting information). The E_6 form is the conformation reported in the X-ray structural analysis.[1]

Our experimental data completely agree with the M062X computations and strongly indicate a preference for the E_6 *exo*-puckered mode in solution (Table 1). When looking at the $^3J_{HH}$ couplings of H7 with H8, H6a and H6b, very different patterns should be expected for the three families of conformations. According to Haasnoot-Altona equation[28] predictions,[29] three small-medium couplings of ca. 2.0 to 5.0 Hz to H7 should be expected for E_6-like conformations, whereas the E_5 conformation should show three couplings of ~8.0 Hz. Finally, one large (~10 Hz) and two medium size (~6 Hz) should be observed for an E_7 conformation. Noteworthy, the first pattern clearly better matches the coupling pattern of H7 found in all the solvents employed in this study (Table 2).

Analysis of the ^{13}C NMR data also strongly supports the preference for an E_6 conformation of the B pyrrolizidine ring. ^{13}C isotropic shieldings were computed at the GIAO//PBE0/6-311 + G**//M062X/6-31 + G** level using chloroform parameters. The shieldings were transformed into shifts by computing isotropic shifts for the cyclohexanone molecule and using reported shifts for the cyclohexanone C4 carbon as a reference. The computed shifts for carbons C5 and C6 of monocrotaline in all the conformations were grouped in a histogram (Figure 2). Inspection of this histogram reveals three different shift patterns corresponding to the three families of conformers, as can be seen in Figure 2, which clearly indicates that E_6 conformers have shieldings centered around ~33 and ~54 ppm. These values perfectly match the observed shifts for these carbons in all studied solvents (Table 3).

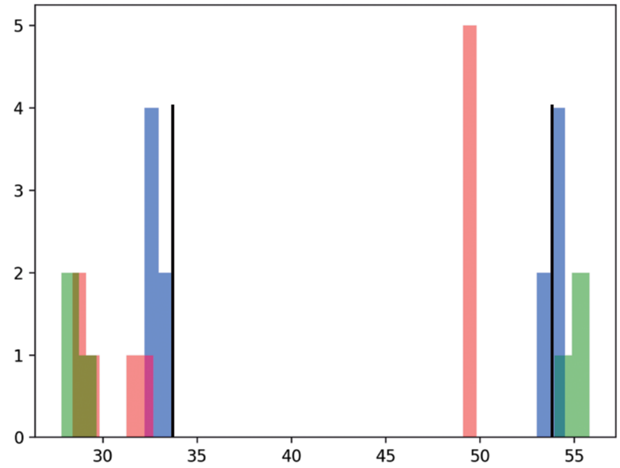


FIGURE 2 Histogram with computed C5 and C6 ^{13}C chemical shifts (ppm) for the E_6, E_5 and E_7 (blue, red, and green, respectively) families of conformations. Experimental shifts in $CDCl_3$ are marked as thing black lines

3.2 | Macrocycle conformation

Conformations of the eleven-membered ring in monocrotaline (1) can be roughly classified as *syn* or *anti* families, according to the relative disposition of the carbonyl groups C11 and C15. Nevertheless, structural variation can be found inside these two families, as reflected in different (C11-C10-O9-C1) Ψ_1 and C15-O16-C7-H7 Ψ_2 dihedral angles (Table 1). The M062X computations prefer an *anti*-parallel disposition (#10, Figure 3, and Table 1) of the two C=O bonds as well as showing an axial-equatorial-equatorial (*ax-eq-eq*) pattern of the Me12′-Me13′-Me14′ methyl groups. A weak hydrogen bond (d = 2.13 Å, α = 101°) between the OH at position C13 and the carbonyl group at C11 can be observed in this form. The second ranked conformation #1 (1.0 kcal/mol over in the D_2O ensemble) presents a *syn* arrangement and an *eq-ax-eq* pattern (Figure 3 and Table 1). Another *anti* form (#6) with a *eq-ax-eq* pattern is placed 1.3 kcal/mol above. Finally, a second *syn* form (#2), where the two carbonyl groups rotate in order to be placed nearly perpendicular to the plane of the molecule, is placed 1.7 kcal/mol above.

RDCs, and particularly one-bond proton-carbon $^1D_{CH}$ RDCs, are an excellent probe of the conformation of heterocyclic rings because they easily probe the orientation of the C—H bonds, or methyl groups, with respect to the plane of the ring.[30–32] $^1D_{CH}$ RDCs were recorded for the different solvents employed here, using a variety of alignment media. Measurements in anisotropic conditions were performed in order to analyze the conformation in different solvents. Table 4 shows the RDC values obtained for monocrotaline for each solvents.

TABLE 1 Relative energy and structural data for the four lowest energy DFT structures of monocrotaline

Conformer	Rel. energy (kcal/mol) ($CHCl_3$/H_2O)	Dipole moment (Debyes)	Relative orientation of carbonyl groups	Pyrrolizidine ring conformation	Ψ_1	Ψ_2
#10	0/0	3.44	*anti*	E_6	81	52
#1	1.4/1.0	5.68	*syn*	E_6	−99	43
#6	1.5/1.3	4.06	*anti*	E_6	71	45
#2	2.0/1.7	5.22	*syn*	E_6	−162	−20

TABLE 2 ^{1}H NMR chemical shift, δ (ppm), and J-coupling (Hz) for monocrotaline (1) in different solvents

Atom	Solvent							
	D_2O		DMSO-d_6		$CDCl_3$		C_6D_6	
	δ ^{1}H	J_{HH}	δ ^{1}H	J_{HH}	δ ^{1}H	J_{HH}	δ ^{1}H	J_{HH}
2	6.04	q $J = 2.0$	6.04	q (broad) $J = 1.9$	6.06	s	5.51	s
3b	3.72	dd $J = 16.8;\ 2.0$	3.41	ddd $J = 16.3;5.7;1.7$	3.57	dd $J = 15.5;3.7$	3.13	dd $J = 16.0;\ 5.8$
3a	3.41	ddd $J = 16.8;\ 5.3;\ 2.0$	3.74	ddt $J = 16.3\ 3.6\ 1.7$	4.14	d $J = 15.5$	3.66	ddt $J = 16.0;\ 3.7;1.7$
5b	2.56	dt $J = 10.0;\ 8.0$	2.55	td $J = 9.7;5.6$	2.74	td $J = 10.6;5.8$	2.38	ddd $J = 11.7;9.0;5.6$
5a	3.04	ddd $J = 10.0;\ 9.0;\ 4.5$	2.99	m	2.48	m	2.97	ddd $J = 8.7;\ 7.4;1.5$
6b	1.94	m	1.81	m	2.18	m	1.76	dddd $J = 13.6;11.7;7.3;\ 4.6$
6a	1.94	m	1.93	ddt $J = 12.8,\ 6.0,\ 3.2$	2.18	m	1.96	ddt $J = 13.6;\ 5.5;1.7$
7	5.00	dt $J = 5.7;\ 3.6$	4.97	td $J = 5.5;2.8$	5.15	t (broad) $J = 3.8$	4.91	td $J = 4.8;\ 1.4$
8	4.32	ddd $J = 5.7;\ 5.3;\ 2.0$	4.26	m	4.65	m (broad)	4.01	m
9b	4.52	d $J = 11.8$	4.42	d $J = 11.4$	4.71	d $J = 12.0$	4.21	ddd $J = 11.8;\ 1.8;\ 0.9$
9a	4.80	d $J = 11.8$	4.56	d $J = 11.4$	4.89	d $J = 12.0$	4.45	d $J = 11.8$
12′	1.37	s	1.30	s	1.45	s	1.46	s
13′	1.26	s	1.18	s	1.35	s	1.52	s
14	2.97	q $J = 7.1$	2.98	q $J = 7.3$	2.82	7.2	2.72	q $J = 7.0$
14′	1.09	d $J = 7.1$	1.07	d $J = 7.3$	1.22	d $J = 7.2$	1.23	d $J = 7.0$

3.3 | Conformational analysis

3.3.1 | Water

$^{1}D_{CH}$ RDCs in water were recorded using the cromoglycate/brine alignment medium.[10] Here, we exploited the low temperature clearing point of the phase[10] to record the data, using a single sample. Isotropic $^{1}J_{CH}$ values were obtained by heating up the sample up to 28°C, whereas the total $^{1}T_{CH}$ splittings were recorded at 23°C where the anisotropic phase was fully formed, as evidenced by a quadrupolar deuterium splitting of 90 Hz in the 1D ^{2}H spectrum. The data were collected acquiring a HSQC spectrum coupled in F2. All $^{1}D_{CH}$ RDCs were measured except for the two methylene protons at C5 likely due to excessive proton-proton splittings. In order to correctly assign the diastereotopic protons, the assignments of protons H3a/H3b and H9a/H9b were permuted during the fitting of RDCs values to structures. (see supporting information). The best fit was obtained for permutation #1, which provided an AIC value of 12.6 (Table S1 from supporting information), for a model consisting of a 69% from the conformation # 1 and 31% from the conformation #4. Both conformations have a *syn*-parallel conformation.

3.3.2 | DMSO

Going down in polarity, the second solvent analyzed was DMSO (dielectric constant of 46.7). For this study, we used a compressible poly-HEMA[5] as alignment

TABLE 3 ^{13}C NMR chemical shifts (ppm) for monocrotaline (1) in different solvents

Atom	Solvent			
	D_2O	DMSO-d_6	$CDCl_3$	C_6D_6
1	132.6	133.2	132.4	133.8
2	133.7	134.8	133.2	134.4
3	60.9	60.8	60.6	62.1
5	53.8	52.8	53.8	54.2
6	33.7	32.4	33.7	34.2
7	74.7	74.1	74.3	75.7
8	76.9	76.6	77.3	77.5
9	60.4	58.4	60.2	60.7
11	173.6	173.7	173.7	174.0
12	78.9	78.3	79.1	79.3
12′	22.1	21.9	22.3	22.4
13	76.7	75.2	76.8	77.5
13′	17.9	17.6	18.0	18.3
14	44.4	41.8	44.4	45.3
14′	13.8	13.7	13.9	14.2
15	173.9	174.6	173.9	174.3

TABLE 4 Experimental RDCs values (Hz) of monocrotaline (1) in different solvents

Atoms	Solvent			
	D_2O ($\Delta\nu_Q$ = 90 Hz)	DMSO-d_6 ($\Delta\nu_Q$ = 7 Hz)	$CDCl_3$ ($\Delta\nu_Q$ = 22 Hz)	C_6D_6 ($\Delta\nu_Q$ = 30 Hz)
C2–H2	4.70	17.4	−6.26	−7.25*
C3–H3a	−19.30	−15.6	−(a)	0.29*
C3–H3b	16.98	−(a)	−(a)	
C5–H5a	(a)	−12.8*	4.78	0.07*
C5–H5b	(a)		(a)	
C6–H6a	−12.80	−7.0 *	−6.69*	−2.07*
C6–H6b	− (a)			
C7–H7	26.00	3.8	−7.57	−15.07*
C8–H8	−0.11	−18.5	−18.67	−19.55*
C9–H9a	20.49	0.5	5.96	−4.07*
C9–H9b	3.69	24.5	−18.74	
C12′–H12′ (CH_3)	−0.70	−0.1	−2.45	−0.34*
C13′–H13′ (CH_3)	−6.11	−1.0	2.14	−0.08*
C14–H14	17.11	9.5	−7.15	−6.51*
C14′–H14′ (CH_3)	−1.94	−4.93	−5.91	3.40*

Note.

*values extracted from the HSQC experiment coupled in F1. (a) very weak signal.

medium. In this medium, we could obtain up to 12 RDCs. Two different values for the diastereotopic protons H9a/H9b were measured in F2-coupled HSQC experiments, resulting in two possible data sets. The best data fit, AIC 7.12, for a conformation population ensemble of #1 (61%) and #2 (39%; Table S2 from supporting information). As in the case of water, both conformations belong to the *syn*-parallel family.

3.3.3 | Chloroform

The third solvent studied was chloroform, which has a dielectric constant of 4.81. In this case, a compressible PMMA gel was employed as aligning medium.[11] It was possible to measure 11 different RDC values in an F2-coupled HSQC experiment. Four possible data sets are generated from diasterotopics protons H5a/H5b and H9a/H9b. The smallest AIC value was 3.68 obtained for a conformation ensemble of 25% of conformation #4 and 75% of conformation # 9, with *syn*-parallel and *anti*-parallel conformation, respectively (Table S3 in the supporting information).

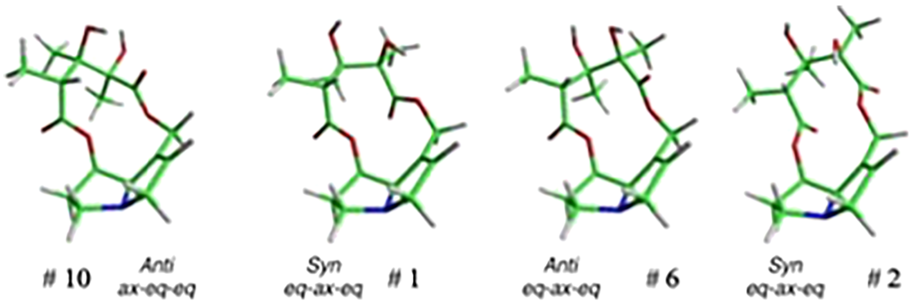


FIGURE 3 Four lowest energy M062X structures of monocrotaline

3.3.4 | Benzene

The last medium studied was benzene, which with a dielectric constant of 2.27 is the least polar solvent in this study. This is the first time compressible PMMA gels swollen in benzene were employed as aligning medium. The best fit provided the following conformational population: 21% of conformer #9, 22% of conformer #10, 11% of conformer #12, 37% of conformer #13, and 9% of conformer #17 (Table S4 in the supporting information). All these conformers share an *anti*-parallel motif in the macrocyclic ring.

The increasing population of the *anti* conformer when decreasing the polarity of the solvent could be explained in simple terms by the change in relative orientation of C=O bond dipoles when going from *syn* to *anti* form. Computed DFT dipoles are more than one Debye smaller in the *anti* form, as can be seen in Table 1. The role of the change in dipolar moment could play a predominant role in the position equilibrium, because strong intermolecular hydrogen bonds seem not to be present in this system according to the computed DFT geometries.

Again, RDCs demonstrated to be a useful tool for probing the solvent dependence of the conformational space of organic compounds, as also recently shown by Martin and coworkers[33] in their analysis of sucrose conformation[34,35] as well as by Farley et al. in cyclic peptide systems. [32]

4 | CONCLUSIONS

Analysis of isotropic and anisotropic NMR data of monocrotaline (1) shows that the conformation of the pyrrolizidine ring is barely influenced by the solvent, being locked in an *exo*-puckered E_6-like conformation. However, the conformation of the eleven-membered macrocycle is strongly dependent on the type of solvent due to the different polarity of the *syn*-parallel and *anti*-parallel conformations. Highly polar solvents, such as water or DMSO, favor the more polar *syn* conformations (See dipole moments in Table 1) as could be expected. The conformational analyses, based on anisotropic RDC data, show that in water or DMSO, the experimental data can be explained in terms of just *syn*-parallel conformations. However, in $CDCl_3$, a mixed model consisting of a 25% of conformations *syn*-parallel and 75% of conformations *anti*-parallel is necessary to explain the observed RDCs. Finally, the data obtained by benzene are best fitted by just using *anti*-parallel conformations. It is clear, therefore, that conformation of pyrrolizidine diester alkaloids can be switched by modifying the polarity of the solvent. Noteworthy, for the first time, compressible PMMA gels swollen in benzene have been applied for the measurement of RDCs.

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ORCID

Armando Navarro-Vázquez https://orcid.org/0000-0003-4364-516X
Fernando Hallwass https://orcid.org/0000-0002-8304-1427

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