STRUCTURAL REASSIGNMENT OF EPIERYTHRATIDINE, AN ALKALOID FROM *Erythrina fusca*, BASED ON NMR STUDIES AND COMPUTATIONAL METHODS

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(Received: May 9, 2012 - Accepted: July 5, 2012)

ABSTRACT

The diene *Erythrina* alkaloids erysotrine (**1**), erysodine (**2**), erythraline (**3**), erytharbine (**4**), and erysotrine *N*-oxide (**5**), plus the hydroxylated dihydro derivative of **1**, epierythratidine (**6**) were isolated from seeds of *Erythrina fusca* Lour., and their ^{1}H and ^{13}C NMR spectra were completely assigned using 2D experiments (H-H COSY, HMQC, HMBC and H-H NOESY). Our assignments for **1-5** agree well with the literature, but the present work shows that the published interpretation of the spectra of **6** must be revised. A combined study based on NMR data and quantum-mechanical calculations using DFT/GIAO indicate that **6** is the correct structure of epierythratidine.

Keywords: *Erythrina* alkaloids; *Erythrina fusca*; epierythratidine; ^{1}H NMR; ^{13}C NMR; 2D NMR; GIAO/DFT methods

1. INTRODUCTION

The *Erythrina* alkaloids constitute a medium-sized group of natural products sharing a 1,2,3,4,5,6,8,9-octahydroindolo[7*a*,1*a*]isoquinoline skeleton that are limited to the plant genera *Erythrina* (Fabaceae), *Cocculus*, *Hyperbaena* and *Pachygone* (Menispermaceae). The earliest structural studies on these compounds antedated the existence of practical NMR spectrometers and, indeed, the discovery of nuclear magnetic resonance. However, in the 1960's ^{1}H NMR began to be used routinely in the structure elucidation of natural products, and in 1983 a seminal paper was published in which the ^{13}C NMR spectra of 20 *Erythrina* alkaloids were described and interpreted.[1] A 1991 review collected the spectroscopic data of all the members of this family known at that date,[2] including the ^{1}H NMR assignments of 66 of them and the ^{13}C NMR assignments of 27 compounds (which include the 20 ^{13}C NMR interpretations published in 1983). More recent work, based on ^{1}H-^{1}H COSY, NOESY and ^{1}H-^{13}C 2D spectra (e.g. HMQC and HMBC),[3-9] shows broad agreement with the generally accepted ^{13}C NMR patterns of the more extensively studied 1,2,7,8-didehydro 'dienoid' series.

Theoretical methods have been used to calculate the ^{1}H and ^{13}C chemical shifts in several natural products.[10-12] These methods have been very important, together with total synthesis,[13] to determine the structures of unusual natural substances,[14,15] and differentiate diastereoisomers,[16] through calculations of proton-proton and proton-carbon *J* coupling constants as a tool for the assignment of the relative configurations of chiral organic compounds, an approach that agrees very well with the experimental data.[17-18]

Moreover, extensive spectroscopic analyses and quantum mechanical (QM) methods have been used for the reassignment of some structures,[19] and can be very helpful to confirm both rigid and flexible molecular scaffolds.[20,21] On the other hand, this methodology has been used to derive stereostructures by comparing the experimental NMR spectroscopic data with the corresponding results of calculations for all the possible stereoisomers.[22-24]

In this work we report the isolation of erysotrine (**1**), erysodine (**2**), erythraline (**3**), erytharbine (**4**), erysotrine *N*-oxide (**5**) and epierythratidine (**6**) from the seeds of *Erythrina fusca* Lour. The systematic numbering of the fundamental structure of these alkaloids and the commonly used 'erythrinan' numbering are shown in Figure 1. An additional result of this paper is the reassignment of the structure of epierythratidine which had been reported on the sole basis of its one-dimensional ^{1}H and ^{13}C NMR spectra and comparison of its spectroscopic data with those of analogous *Erythrina* alkaloids. In order to complete this limited spectroscopic information, we performed a full assignment of the ^{1}H and ^{13}C NMR spectra of this compound using one- and two-dimensional NMR experiments. This study suggested that the correct structure of epierythratidine is **6** instead of the originally proposed diastereomeric structure. We then used the DFT/GIAO method to find further support for our assignment through the analysis of both stereoisomers at the C2 position. In this case comparison of the experimental and calculated ^{13}C NMR chemical shifts proved once more to be a valuable tool to aid in the elucidation of the correct structure of a natural product.

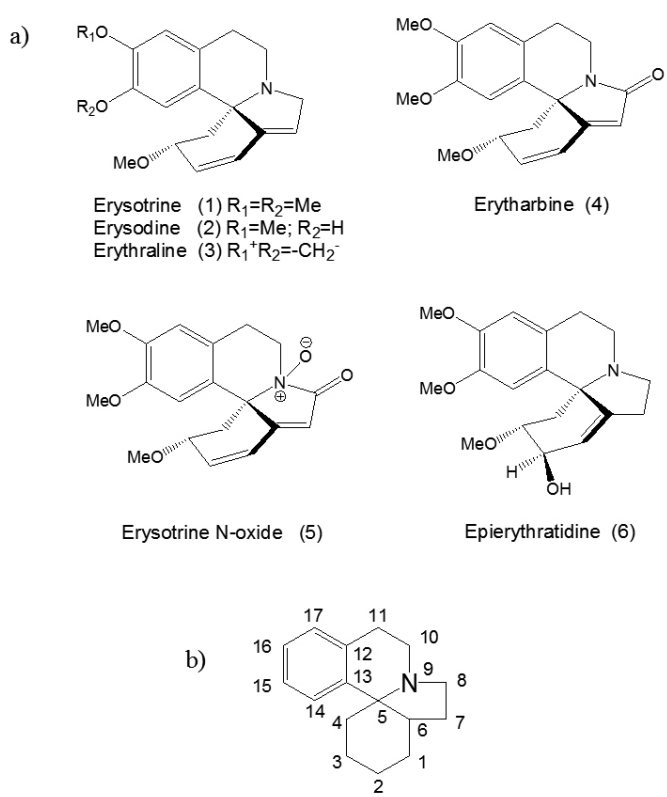

Figure 1. (a) Structures of the alkaloids isolated from seed the *Erythrina fusca* Lour.
(b) Numbering schemes of the *Erythrina alkaloid* skeleton.

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2. RESULTS AND DISCUSSION

A decade ago Calle *et al.* reported the isolation of epierythratidine (**6**) and 8-oxoerysodine from the bark and flowers of *E. fusca*,[25] and previously several other alkaloids had been observed as constituents of the seeds, but without isolating them and only on the basis of a GC/MS study.[26] The isolation of erysotrine (**1**), erysodine (**2**), erythraline (**3**), erytharbine (**4**), erysotrine *N*-oxide (**5**) and epierythratidine (**6**) from the seeds of this species was now carried out following a general protocol for alkaloids. The only noteworthy feature is the relatively high yield (for the usually scanty *Erythrina* alkaloids) and facile crystallization of **6**. All six compounds were identified by comparison of their NMR data with published values which only showed some discrepancies, discussed below, in the case of **6**. A combination of one- and two-dimensional NMR (^{1}H and ^{13}C) methods led to conclusions in complete agreement with the literature assignments for **1-5**,[2] which had generally been based on one-dimensional experiments. In the case of epierythratidine (**6**), however, our results and the published assignments,[1,26] also based on one-dimensional experiments, were at variance. Epierythratidine has a somewhat unusual structure differing from most *Erythrina* alkaloids in having a partially hydrogenated diene system and bearing a hydroxyl group at C-2. Considering this rare hydroxyl substitution on the spiro cyclohexene ring next to a methoxyl group which could lead to some confusion, we confirmed our assignment with further experiments summarized in Table 1.

Table 1. ^{1}H and ^{13}C NMR assignments of epierythratidine (**6**) with ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY correlations.

C No.	^{13}C NMR*[1]	^{13}C NMR*[10]	^{13}C NMR	^{1}H NMR†	^{1}H–^{1}H COSY	HMQC	HMBC	NOESY
1	121.3	121.3	125.3	5.8 (sb, 1H)	H-2, 7	125.3	25.60, 66.99, 80.17	H-2, H-7 (one)
2	81.0	81.5	71.36	4.2 (m, 1H)	H-3	71.36	80.17, 139.3	
3	72.7	72.3	80.17	3.6 (12.0, 4.3, 1H)	H-4	80.17	71.36, 139.3	H-3, H-4 (one)
4	40.1	40.1	37.76	1.75 (12.0, t, 1H) 2.4 (12.0, 4.0 dd, 1H)		37.76	66.99, 71.36, 80.17, 124.4, 139.3	
5	64.5	64.5	66.99					
6	143.8	144.2	139.3					
7	26.7	26.3	25.60	2.36 (m, 1H) 2.54 (m, 1H)		25.60	37.76, 125.8	
8	39.3	39.3	46.74	3.07 (m, 1H) 3.26 (m, 1H)	H-7	46.74	20.85, 66.99	H-7 (both)
10	46.3	46.3	40.09	3.36 (m, 1H) 3.67 (7.0, 2.8 dd, 1H)	H-11	40.09	20.85, 46.74, 123.8, 139.3	H-11 (both), H-8 (both)
11	21.4	21.4	20.85	2.91 (m, 1H) 3.12 (m, 1H)		20.85	20.85, 40.59, 46.74, 112.4	
12	124.9	125	123.8					
13	127.8	128	124.4					
14	111.1	111.1	110.8	6.80 (s, 1H)		106.2	66.99, 110.8, 149.4	H-3
15	147.9	147.9	147.4					
16	146.6	146.5	149.4					
17	111.8	111.8	112.4	6.78 (s, 1H)	H-11	112.4	20.85, 112.4, 123.8, 149.4	H-3′, H-11 (both), H-3′
1′	?	57.1	56.45	3.30 (s, 3H)		56.45	80.17	
2′	56.0	55.9	55.51	3.76 (s, 3H)		55.51	147.4, 110.8	
3′	55.7	55.8	55.14	3.78 (s, 3H)		55.14	149.4, 112.4	

*Values in these columns were taken from the literature.[1, 19]
† d (*J* values, in Hz, in parentheses).

Epierythratidine (**6**) was obtained as an off-white crystalline solid. Its ^{1}H NMR spectrum shows signals at δ 1.75 (t, 1H, *J* = 12.0 Hz) and 2.42 (dd, 1H, *J* = 12.0, 4.0 Hz) suggesting the presence of a methylene group with diastereotopic protons. A heteroatom-bonded methine produces a signal at δ 3.61 (ddd, 1H, *J* = 12.0, 7.0, 4.3 Hz) and, judging from its coupling constants, it seemed likely to be attached to the former methylene. Another proton gives rise to a signal at δ 4.24 (m, 1H) and also appears to be bonded to a heteroatom and, although it does not seem to be coupled to the previously mentioned protons, a ^{1}H-^{1}H COSY experiment showed that all these nuclei are close to each other and also to an sp^2 carbon-bonded proton resonating at δ 5.8 (s, 1H). Further downfield there are signals at δ 2.91 (m, 1H), 3.12 (m, 1H), 3.36 (m, 1H) and 3.67 (dd, 1H, *J* = 7.0, 2.8 Hz) also corresponding to neighboring methylene groups as established by the COSY experiment. Another two adjacent methylene groups give rise to signals at δ 2.36 (m, 1H), 2.54 (m, 1H), 3.07 (m, 1H) and 3.26 (m, 1H). Three methoxyl singlets appear at δ 3.30 (s, 3H), 3.76 (s, 3H) and 3.78 (s, 3H), the first one presumably bonded to an sp^3 carbon atom. Finally, two protons resonate at δ 6.78 (s, 1H) and 6.80 (s, 1H) which, in an *Erythrina* alkaloid, would be expected to reside at the generally free *para* positions (C-14 and C-17) of the aromatic ring.

The ^{13}C NMR spectrum shows the presence of 19 signals, and the APT shows that six of these correspond to quaternary carbons (δ 149.4, 147.8, 139.3, 124.4, 123.8 and 66.99, the latter with sp^3 hybridization and the first two representing oxygen-bonded sp^2 carbons), and five to methines (δ 125.8, 112.4, 110.8, 80.17 and 71.36, the first three involving sp^2 carbons and the latter two with oxygen-bonded sp^3 carbons). Three methoxyl carbons resonate at δ 55.44, 55.51 and 56.45 ppm, with the five remaining signals (δ 46.74, 40.09, 37.76, 25.60, and 20.85) corresponding to methylene groups.

The HMQC experiment shows connectivities between the protons resonating at δ 1.75 and 2.42 with the δ 37.76 carbon nucleus, confirming that both protons are diasterotopic. According to the ^{1}H NMR spectrum, these protons are coupled to the one at δ 3.61, which in turn is connected to the δ 80.17 carbon. The latter nucleus is coupled to the δ 4.24 proton, connected to the δ 71.36 carbon which should bear a hydroxyl group and which is also coupled to the δ 5.80 proton, connected to the δ 125.8 carbon. Other diastereotopic methylene proton pairs resonate at δ 2.91 and 3.12, bonded to the δ 20.85 carbon; at δ 3.36 and 3.67, to the δ 40.09 carbon; δ 2.36 and 2.54

to the δ 25.6 carbon; and δ 3.07 and 3.26 to the δ 46.74 carbon. The presumed aromatic ring protons resonating at δ 6.78 and 6.80 are attached to the δ 112.4 and 110.8 carbon atoms, respectively. The methoxyl correlations are δ 3.30 to 56.45, δ 3.76 to 55.51, and δ 3.78 to 55.14.

Analysis of the HMBC spectrum shows long-range connectivities of the protons resonating at δ 1.75 and δ 2.42 with the δ 66.99, 71.36, 80.17, 124.4 and 138.3 carbons. Similarly, the protons at δ 2.91 and 3.12 are correlated with the δ 20.85, 40.59, 46.74 and 112.4 carbons; the δ 3.36 and 3.67 protons with the δ 37.76, 123.8 and 125.8 carbons; and the δ 3.07 and 3.26 protons with the δ 20.85 and 66.99 carbons. The two protons resonating at δ 6.78/6.80 show connectivity with several carbon nuclei: δ 20.85, 66.99, 110.8, 112.4, 123.8, 147.8 and 149.7. Nevertheless, the most useful correlations for a complete assignment are those of the well identified methoxyl resonances: the protons at δ 3.76 are correlated with the δ 147.4 and 110.8 carbons, δ 3.78 with the δ 149.4 and 112.4 carbons, and the δ 3.30 proton with the δ 80.17 carbon. All the correlations are summarized in Table1.

A NOESY experiment was run to establish the relative stereochemistry of **5**, specifically at C-2 and C-3. As shown by the COSY experiment, C-2 is bonded to a hydroxyl and C-3 to a methoxyl group. The proton resonating at δ 6.80 lies close to the one at δ 3.61, implying that the methoxyl is oriented toward the back of the structure, as shown in Figure 2a. On the other hand, the lone hydroxyl group must face forward as the protons resonating at δ 5.80 and 4.24 lie spatially close to each other, while the one at δ 3.61 does not interact with the former through space. In consequence, the ^{1}H and ^{13}C resonances of epierythratidine (**6**) have been completely and unambiguously assigned.

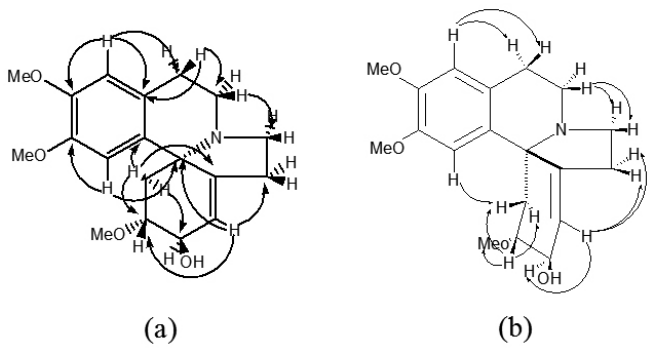

Figure 2. (a) HMBC correlations and (b) NOESY correlations for epierythratidine (**6**).

Our data for erysotrine (**1**), erysodine (**2**), erythraline (**3**), erytharbine (**4**), and erysotrine *N*-oxide (**5**), backed up by two-dimensional experiments, lead to assignments that are in complete agreement with those published in the reviews of Chawla *et al*.[1] and Amer *et al*.,[2] and in most of the later papers, which follow a common pattern. The only assignments we have identified diverging from this scheme are in the previously cited article on *E. fusca* alkaloids by Calle *et al*.,[25] where the ^{13}C NMR assignments of C-1 (135.5 ppm) and C-2 (123.2 ppm) of 8-oxoerysodine, using only one-dimensional spectra, are inverted, and in a paper by Flausino et al.,[27] using ^{1}H-^{1}H COSY, HMQC and HMBC, who arrived at a similar conclusion for 11a-hydroxyerythravine (in pyridine-d_5) and 11a-hydroxyerysotrine (in $CDCl_3$): δ 135.0 and 131.5 (C-1), 124.5 and 125.5 (C-2), plus 124.4 and 123.5 (C-7), respectively. With the possible exception of the 11a-hydroxyerythravine spectrum, due to the specific solvation of the hydroxyl group by pyridine, these assignments are most likely in error. Any future isolation of these compounds should be accompanied by a thorough study of their NMR spectra in order to clear up these points in doubt.

Recently, Bifulco *et al.*[24] developed a new methodology using molecular dynamics (MD) for the search of conformers and QM calculation, which has proved useful to study the conformational change or distortion in polycyclic diasteroisomeric systems. We have used MD using an Amber type force field to search for the differences between the two possible steroisomers of compound **6** (Figure 3). In each case, only a single conformer of the ring system was found. It is worth pointing out that the conformation to the methoxy group in the aromatic ring presents a distribution due to the diehedral torsion.The resulting geometries for both isomers are shown in Figure 4A.

The QM calculation of ^{13}C NMR chemical shifts and proton-proton $J_{H,H}$ coupling constants was done at the mPW1PW91/6-31G(d,p) level of theory from the optimized structures for both minima obtained in the MD, with the same functional and the 6-31G(d) basis set (Figure 4B), because this method minimizes the error in this type of calculation.[21] The NMR results are summarized in Table 2. The differences between theoretical and experimental values $\Delta d = |\delta_{exp}-\delta_{calc}|$, which give a measure of the dispersion between the theoretical and experimental chemical shift values for both isomers, and the MAE = $\sum[|\delta_{Exp}-\delta_{Calc}|]/n$ parameter, that represents the measure of absolute error, have proved very useful for the characterization of stereostructures.[17]

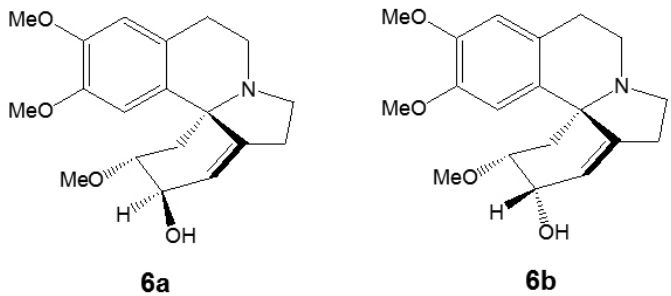

Figure 3. Two alternative structures for epierythratidine **6a** and **6b**

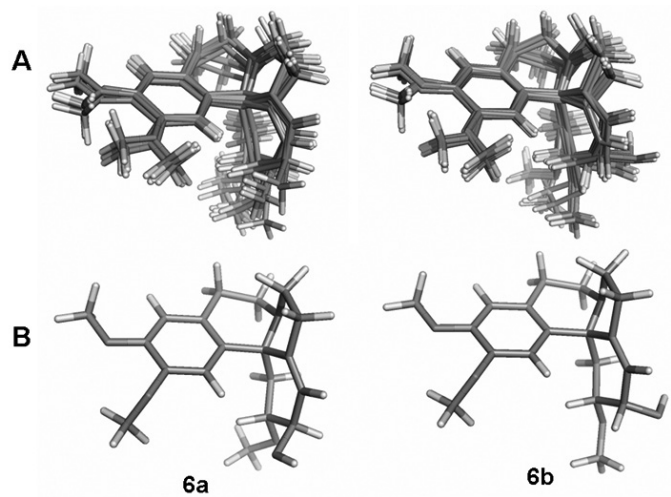

Figure 4. (A) Molecular Dynamics search of conformers (B) optimized geometries for both isomers **6a** and **6b.**

These values show that |Δδ| for C-2 and C-3 are 0.4 and 0.9, respectively, for structure **6a**, much smaller than the increments in these values for structure **6b** (7.2 and 7.3, respectively), indicating that the match with the experimental data for both carbons strongly supports structure **6a**. This result is fundamental for the structure reassignment for compound **6**, considering that the other carbon nuclei present similar |Δδ| values. On the other hand, the differences between the averages of the MAE values for **6a** and **6b** are 3.7 and 5.0, respectively. It should be pointed out that the increment in the MEA is due to the aromatic ring, this is a problem in these measurements since the limit for the error in the method for calculation ^{13}C chemical shift exceeds 100 ppm.[28] However, the values for the non-aromatic rings are in agreement with those obtained from the experimental results.

In summary, the differences reported in the literature for the ^{13}C chemical shift show greater dispersion and inversions in C2 and C3 for both isomers studied. Our results can be judged to be in good agreement with the experimental data for the structural reassignment of compound **6**. On the other hand, due to the fact that the signals for H-1, H-2, H-7, H-8, H-10 and H-11 are multiplets, the coupling constants cannot be easily obtained from the experimental data. However, the coupling constants for protons H-3 and H-4 have been correlated with the calculations which reinforce the comparison with the experimental results.

3. CONCLUSION

From the seeds of *Erythrina fusca* Lour. were isolated and identified by spectroscopic methods the alkaloids erysotrine (**1**), erysodine (**2**), erythraline

(**3**), erytharbine (**4**), erysotrine *N*-oxide (**5**) and epierythratidine (**6**). The structure of compound **6** was reassigned based on experimental and theoretical NMR studies. Our results show that a MD conformational search followed by GIAO/DFT QM calculations on the energy-minimized structures provide an excellent agreement with the experimental ^{13}C chemical shifts and a fair agreement with the experimental $^{3}J_{H,H}$ coupling constants. These values confirm and support the experimentally derived assignments and structure of epierythratidine (**6**).

Table 2. Comparison of calculated vs. experimental ^{13}C NMR chemical shifts and theoretical $^{3}J_{H,H}$ coupling constant values for compounds **6a** and **6b**.

	d $^{13}C_{Exp}$	d $^{13}C_{Calc}$		|Dd|,ppm*		$^{3}J_{H,H}$ (Hz)		
C No.		**6a**	**6b**	**6a**	**6b**		**6a**	**6b**
1	125.3	120.8	121.0	4.5	4.3	$J_{H1,H2}$	4.75	4.60
2	71.36	71.73	64.12	0.4	7.2	$J_{H2,H3}$	4.69	5.77
3	80.17	79.28	72.84	0.9	7.3	$J_{H3,H4a}$ $J_{H3,H4b}$	10.99 4.75	11.33 4.17
4	37.76	39.40	40.33	1.6	2.6			
5	66.99	65.83	65.35	1.2	1.6			
6	139.3	145.1	145.0	5.8	5.8			
7	25.60	29.14	29.58	3.5	4.0	$J_{H7a,H8a}$ $J_{H7b,H8b}$ $J_{H7a,H8b}$	9.46 9.05 1.33	9.76 9.05 1.15
8	46.74	46.57	45.95	0.2	0.8			
10	40.09	40.48	40.88	0.4	0.8	$J_{H10a,H11a}$ $J_{H10b,H11b}$ $J_{H10b,H11a}$	7.22 6.00 10.71	7.37 6.23 10.60
11	20.85	23.32	23.29	2.5	2.4			
12	123.8	127.2	127.3	3.4	3.5			
13	124.4	125.4	125.2	1.1	0.9			
14	110.8	118.8	119.2	8.0	8.4			
15	147.4	141.6	141.3	5.7	6.1			
16	149.4	147.2	147.3	2.1	2.1			
17	112.4	109.9	109.5	2.4	2.9			
1´	56.45	57.67	57.17	1.2	0.7			
2´	55.51	55.32	54.46	0.2	1.0			
3´	55.14	52.59	52.59	2.5	2.6			
MAE*				3.7	5.0			

* $|\Delta d| = |\delta_{exp}-\delta_{calc}|$ and $MAE=\sum[|\delta_{Exp} - \delta_{Calc}|]/n$

4. MATERIAL AND METHODS

4.1. Plant material

Erythrina fusca Lour. seeds were collected on the campus of Tolima University, Ibagué, central Colombia, 4°25' N, 75°12' W, 1,100 m above sea level, by Olimpo García in February 2007. The plant material was identified by Prof. Tirso Medina, and a voucher specimen was deposited in the Herbarium of the Biology Department of Tolima University.

4.2. Extraction and isolation

Dried, ground seeds (3,200 g) of *E. fusca* were defatted with petroleum ether and then exhaustively extracted with methanol at room temperature over a period of 7 days. After concentration under reduced pressure, a viscous yellow liquid (45.5 g) was obtained and taken up with 3 % HCl. The acid solution was extracted several times with $CHCl_3$ and was then made alkaline (pH 9-10) with concentrated aqueous NH_3 and re-extracted with $CHCl_3$ to afford the crude alkaloids (6.8 g). The alkaloid fraction was subjected to silica gel column chromatography eluting with CH_2Cl_2-CH_3OH (95:5 to 80:20), to yield forty-three 200 mL fractions which were pooled according to their TLC patterns, and fractionated again by preparative TLC to afford the pure alkaloids. In this way erysotrine (**1**, 24 mg), erysodine (**2**, 45 mg), erythraline (**3**, 68 mg), erytharbine (**4**, 82 mg), and erysotrine *N*-oxide (**5**, 20 mg) were obtained. One of the later fractions deposited crystals upon concentration. These were washed with cold acetone and recrystallized in MeOH to provide epierythratidine (**6**, 450 mg).

4.3. NMR studies

Compounds **1**, **2**, **3**, **4**, and **5** were dissolved in $CDCl_3$, and **6** in CD_3OD for spectroscopic analysis using a Bruker multidimensional spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) and a Bruker DRX300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C), respectively. TMS was used as an internal standard and the temperature was kept at 298 K for all experiments. Two-dimensional experiments were performed using standard Bruker software for ^1H–^1H COSY (cosygpqf and cosygs), NOESY (noesygpph), HMQC (inv4gpqf and inv4gstp) and HMBC (inv4gplrndqf and inv4gslplrnd).

4.4. Computational details

The conformer search by Molecular Dynamics was performed after minimization of the energy using an Amber model for parameters and force field, with a standard constant temperature velocity−Verlet algorithm. All the structures obtained from MD calculations for each isomer were minimized using the Polak-Ribier conjugate gradient algorithm (PRCG, 100 steps, convergence threshold 0.005 kJ mol^{-1} $Å^{-1}$), implemented in the Gabaedit 2.6 program,[29] leading to the selection of the lowest energy minimum conformer for each diastereoisomer, which were optimized at the mPW1PW91/6-31G(d) level of theory. The ^{13}C NMR chemical shifts and coupling constants were calculated using the GIAO (Gauge Invariant Atomic Orbitals) method,[30] together with solvent effects which were evaluated by performing single-point mPW1PW91/6-31G(d,p) calculations at the gas-phase stationary points using Tomasi's group's polarizable continuum model (PCM).[31,32] The dielectric

constant was set at ε = 32.63 for methanol. Relative chemical shifts were estimated by using the corresponding TMS shielding calculated at the same level of theory and using the PCM method to model the solvent effect. All QM calculations were carried out with the Gaussian 03 suite of programs.[33]

ACKNOWLEDGEMENTS

The authors are pleased to acknowledge financial support from the Millennium Scientific Initiative (Grant P05-001-F). JSD acknowledges a Post-Doctoral Fellowship from a Millennium Nucleus (CILIS), ICM-P10-003-F.

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