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THE SOLUTION STRUCTURE OF ALFILERAMINE. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND MOLECULAR MECHANICS STUDY

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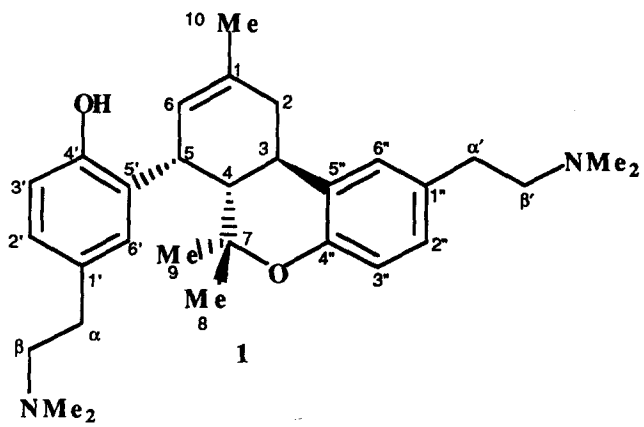
Abstract: ^{1}H nmr spectra show that alfileramine (**1**) exists in solution as an equilibrium mixture of two rotamers around the bond between C-5 and C-5' with a rotation barrier of about 18 Kcal/mol. Molecular mechanics calculations and nOe experiments were used to determine their structure.

Alfileramine (**1**), the first example of bishordeninyl terpene alkaloids, was originally isolated from *Zanthoxylum punctatum* leaves[1] some years ago. Its structure has been deduced from spectroscopic data and X-ray crystallographic studies[2] of its rigid derivative isoalfileramine.



As a part of a research project on metabolites of plants used in folk medicine of Caribbean countries, we isolated a substance from Z. *chiriquinum*[3] leaves which was shown to be homogeneous by hplc and identical to an authentic sample of alfileramine; nevertheless, some features of the nmr spectrum of this compound led us to study its conformational properties. In this paper we describe our results on the solution structure of this alkaloid.

Results and Discussion

Alfileramine shows in the ^1H nmr (250 MHz, $CDCl_3$) spectrum the existence of some duplicate peaks, in particular the doublets at δ 5.80 and 5.40, assigned to H-6, the singlets at δ 1.84 and 1.78, assigned to Me_{10}, the singlets at δ 1.66 and 1.55, assigned to Me_8 and the singlets at δ 0.51 and 0.48, assigned to Me_9.

	$CDCl_3$	Acetone-d_6	MeOH-d_4	DMSO-d_6	Benzene-d_6
$H_{6'}$ y $H_{6''}$	7.10, 7.06	7.17	7.14, 7.03	7.14, 7.00	7.45 and 7.30, 7.21 and 7.18
$H_{2'}$ y $H_{2''}$	6.88	6.85	6.90	6.83	6.92
$H_{3'}$ y $H_{3''}$	6.64, 6.59	6.79, 6.55	6.70, 6.55	6.69, 6.50	7.00, 6.69
H_6	5.80 and 5.40	5.40	5.38	5.32	5.60
H_5	4.33	4.35	4.29	4.16	4.73
H_3	3.30	3.45	3.25	3.29	3.71
H_2	2.80	2.95	2.90	2.86	2.75
$ArCH_2$	2.70	2.70	2.65	2.55	2.75
CH_2N	2.28	2.40	2.65, 2.50	2.38, 2.29	2.46
NMe	2.33, 2.28	2.22, 2.16	2.38, 2.32	2.15, 2.08	2.09, 2.00
H_4	2.20	2.02	2.10	1.99	2.46
H_2	2.10	1.90	1.95	1.85	2.03
Me_{10}	1.84 and 1.78	1.77	1.77	1.74	2.05 and 2.02
Me_8	1.66 and 1.55	1.61	1.58	1.53	1.68 and 1.62
Me_9	0.51 and 0.48	0.44	0.39	0.32	0.89

TABLE 1.- ^1H-nmr of alfileramine.

We have also observed the appearance of doubled peaks in other non polar solvent (C_6D_6) but not in polar ones (MeOH-d_4, DMSO-d_6) (table 1). This fact supported

that alfileramine presented in solution some type of equilibrium phenomenum, heavily affected by a change of solvent.

The population of the two components of the equilibrium in $CDCl_3$ was shown to be 4:1 by ^1H-nmr, and accordingly we measured the coalescence temperature and calculated the free energy of activation of the process using the H-Shanan-Atidi and K.H. Bar-Eli method,[4] which gave a result of T_c=353 K for Me_{10} and Me_8 and T_c=337 K for Me_9, and $\Delta G^{\#}$ of about 18 Kcal/mol.

Two explanations could be advanced at this point to justify the observed equilibrium. First, and in accordance with the pK values for the phenol and amine groups, the coexistence of neutral and zwitterion forms in solution was considered, but this hypothesis was discarded because the NMe and H-3' signals were not sensibly displaced in MeOH-d_4 or DMSO-d_6, solvents that should stabilize the charged form.

Secondly, we explored the existence of a conformational equilibrium. By manipulation of a framework model of alfileramine, it is clear that the rotation around the bond between C-5 and C-5' can be hindered because, at some points of the process, the distance between Me_9 and the phenolic OH or the H-6' is too short.

In order to get an estimate of that rotation barrier that would permit us to compare it with the experimental one, we carried out detailed molecular mechanics calculations (Allinger's MMPI program)[5] for alfileramine. The geometry of the most stable conformer is represented in figure 2, and was localized after optimization of the pseudochair-pseudoboat forms of the aliphatic rings.

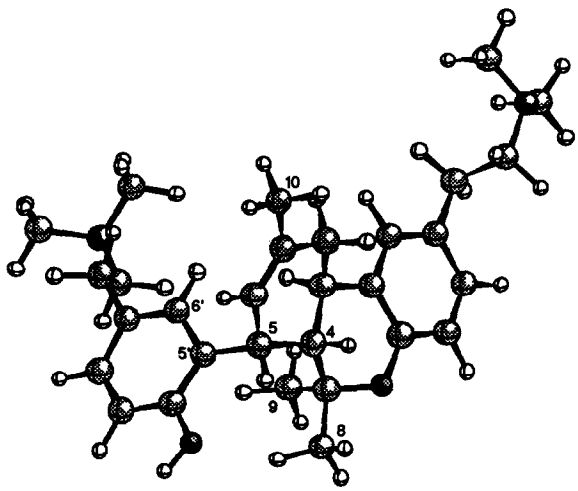


FIGURE 2

In this conformer (conformer 1), the torsion angle between C-6, C-5, C-5', and C-6' is 55.2°, with a calculated energy of 49.79 Kcal/mol. Dihedral drive calculations with 10° increments around bond C-5, C-5' allowed us to detect a second energetic minimum (conformer 2) at a torsion angle of 235.4° (figure 3), and a saddle point at 22.1°.

While conformers 1 and 2 present a half chair structure in their saturated rings, typical of cannabinoids,[6] the saddle point is represented by a much more distorted conformation and accordingly the calculated height barrier was found to be aprox. 19 Kcal/mol.

This result, as all the molecular mechanics data, represents gas phase properties only, but we think that they should resemble very closely the results obtained in non polar solvents such as Cl_3CD and C_6D_6, so that the concordance between the calculated barrier height (19 Kcal/mol) and the experimental one (18 Kcal/mol by nmr) give strong support to our hypothesis that alfileramine presents restricted rotation around the bond C-5, C-5'. A similar barrier has also been found in simpler models.[6]

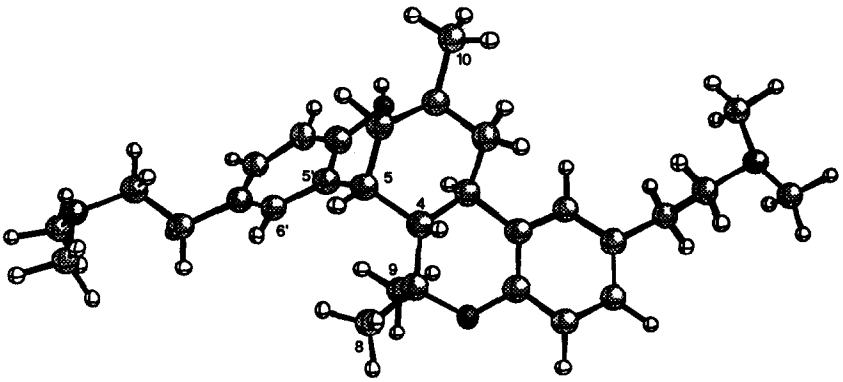


FIGURE 3

The molecular mechanics calculations also showed that the principal source of the high energetic barrier is, as advanced, the interaction between the protons of Me_9 and the phenolic OH. Small non bonded distances between these atoms, as well as unusual valence angles around C-4, C-5 and C-6 are noteworthy (table 2).

As for the differences found in the nmr spectrum of alfileramine in different solvents, we think that in methanol and DMSO, both capable of forming hydrogen bonds with the phenolic OH group, the less stable rotamer is expected to be destabilized due to the greater interaction of the solvated group with the Me_9 (figure 3). This would also explain the presence of a single rotamer and the lack of duplicate nmr signals observed in those solvents.

	1º Minimum	Saddle point	2º Minimum
Relative energy	0.0	19.59	2.25
Dihedral angles			
6-5-5'-6'	+55.2	+22.1	235.4
1-6-5-4	+17.5	+38.4	+20.1
6-5-4-3	-45.9	-63.9	-49.8
5-4-3-2	+58.4	+62.2	+59.3
4-3-2-1	-41.5	-33.1	-37.9
3-2-1-6	+14.1	+3.7	+8.6
2-1-6-5	-1.7	-6.6	-0.5
Valence angles			
3-4-5	112.3	104.1	111.5
4-5-6	111.5	109.6	110.5
5-6-1	123.7	121.2	123.9
Distances			
OH-H_9	4.94	2.52	3.31
OH-H_3	5.25	3.92	3.39
OH-H_5	2.34	2.88	4.75
H_3-$H_{6'}$	2.23	3.67	5.78
H_5-$H_{6'}$	5.37	3.35	3.78

TABLE 2

Finally, as can be observed from table 1, tne ^{1}H-nmr spectrum of alfileramine in C_6D_6 presents two clearly distinguishable signals for H-6' and another two for H-6", these were identified by COSY experiments and we though they could be of great value in order to prove definitively the structure of both rotamers. To this end, we carried out nOe experiments irradiating the signals corresponding to H-6'. The strongest signal (δ 7.45) showed nOe with the multiplet at δ 3.71 (H-3), while the weakest signal (δ 7.30) showed nOe with the broad triplet at δ 4.75 (H-5). These nOe experiments prove the relative positions of these atoms in the two components of the equilibrium, and are coherent with the interatomic distances (2.23 and 3.78 Å

respectively) calculated by molecular mechanics for the two most stable rotamers of alfileramine.

In conclusion, all these facts allow to us to affirm that alfileramine exists in non polar solvents as an equilibrium mixture of two rotamers around bond C-5, C-5', both of them half-chair and easily distinguishable by ^{1}H-nmr. This equilibrium is very sensitive to the solvent and it is interesting to point out that the mono- and di-N-demethyl derivatives of alfileramine isolated from *Z. coriaceum*[3] present restricted rotation too.

Experimental

^{1}H-nmr spectra were recorded at room temperature on a Bruker WM-250 spectrometer operating at 250.13 MHz for proton and at 62.83 MHz for carbon. Chemical shifts given in δ values were measured with respect to Me_4Si, nOe difference experiments were run automatically by a microprogram developed according to Neuhaus et al[7]. Two-dimensional spectra were obtained with the standard Bruker software.

The structure of alfileramine was calculated by means of general valence force field methods using the MMPI computer program. The minimum energy conformer was localized by optimization of different entry geometries corresponding to the chair and boat conformation of the cyclohexene ring.

Physical and spectroscopic data of alfileramine (**1**); m.p. 190-193 °C; $[\alpha]_D$ 0°; υ_{max}(KBr) 3250, 2960, 1620, 1490, 1290, 1250 cm^{-1}; λ_{max} (EtOH): 283, 290 nm; λ_{max} (EtOH + HO^-): 290, 306 nm; δ_H (250 MHz, $CDCl_3$): 7.10 (1H, d, J 2.3 Hz, Ph), 7.06 (1H, d, J 2.3 Hz, Ph), 6.96 (1H, br s, disappears with D_2O), 6.88 (2H, m, Ph), 6.64 (1H, d, J 8.2 Hz, Ph), 6.59 (1H, d, J 8.2 Hz, Ph), 5.80 and 5.40 (1H, two br d, J 5.4 Hz, 6-H), 4.33 (1H, br s, 5-H), 3.30 (1H, m, 3-H), 2.80 (1H, dd, J 5.8 and 16 Hz, 2-H), 2.70 (4H, m, Ph-C*H*$_2$), 2.60 (4H, m, C*H*$_2$-N), 2.33 (6H, s, NMe), 2.28 (6H, s, NMe), 2.20 (1H, dd, J 5.1 and 10.0 Hz, 4-H), 2.10 (1H, m, 2-H), 1.84 and 1.78 (3H, two s, 10-Me), 1.66 and 1.53 (3H, two s, 8-Me), 0.51 and 0.48 (3H, two s, 9-Me);.δ_H (250 MHz, acetone-d_6): 7.17 (2H, br s, Ph), 6.85 (2H, m, Ph), 6.79 (1H, d, J 8.2 Hz, Ph), 6.55 (1H, d, J 8.2 Hz, Ph), 5.40 (1H, d, J 5.4 Hz, 6-H), 4.35 (1H, br s, 5-H), 3.45 (1H, m, 3-H), 2.95 (1H, dd, J 5 and 9 Hz, 2-H), 2.70 (4H, m, Ph-C*H*$_2$), 2.40 (4H, m, C*H*$_2$-N), 2.22 (6H, s, NMe), 2.16 (6H, s, NMe), 2.02 (1H, m, 4-H), 1.90 (1H, m, 2-H), 1.77 (3H, s, 10-Me), 1.61 (3H, s, 8-Me), 0.44 (3H, s, 9-Me); δ_H (250 MHz, methanol-d_4): 7.14 (1H, d, J 1.7 Hz, Ph), 7.03 (1H, d, J 2 Hz, Ph), 6.90 (2H, m, Ph), 6.70 (1H, d, J 7.2 Hz, Ph), 6.55 (1H, d, J 7.2 Hz, Ph), 5.38 (1H, br d, J 5.2 Hz, 6-H), 4.29 (1H, br s, 5-H), 3.25 (1H, m, 3-H), 2.90 (1H, dd, J 4.9 and 10 Hz, 2-H), 2.65 (6H, m), 2.50 (2H, m), 2.38 (6H, s, NMe), 2.32 (6H, s, NMe), 2.10 (1H, dd, J 5.0

and 10 Hz, 4-H), 1.95 (1H, m, 2-H), 1.77 (3H, s, 10-Me), 1.58 (3H, s, 8-Me), 0.39 (3H, s, 9-Me); δ_H (250 MHz, DMSO-d_6): 9.39 (1H, s, OH), 7.14 (1H, d, J 1.8 Hz, Ph), 7.00 (1H, d, J 2 Hz, Ph), 6.83 (2H, dt, J 2.0 and 8.6 Hz, Ph), 6.69 (1H, d, J 7.2 Hz, Ph), 6.50 (1H, d, J 7.2 Hz, Ph), 5.32 (1H, br d, J 6.4 Hz, 6-H), 4.16 (1H, br s, 5-H), 3.29 (1H, m, 3-H), 2.86 (1H, dd, J 6.0 and 16.4 Hz, 2-H), 2.55 (4H, m, Ph-CH_2), 2.38 (2H, m, CH_2-N), 2.29 (2H, m, CH_2-N), 2.15 (6H, s, NMe), 2.08 (6H, s, NMe), 1.99 (1H, dd, J 5.0 and 12.4 Hz, 4-H), 1.85 (1H, m, 2-H), 1.74 (3H, s, 10-Me), 1.53 (3H, s, 8-Me), 0.32 (3H, s, 9-Me); δ_H (250 MHz, benzene-d_6): 7.45 and 7.30 (1H, two d, J=2.1 Hz, Ph), 7.21 and 7.18 (1H, two d, J 2.1 Hz, Ph), 7.00 (1H, d, J 8.2 Hz, Ph), 6.92 (2H, m, Ph), 6.69 (1H, d, J 8.2 Hz, Ph), 5.60 (1H, br d, J 5.4 Hz, 6-H), 4.73 (1H, br s, 5-H), 3.71 (1H, m, 3-H), 2.75 (5H, m, Ph-CH_2 + 2-H), 2.46 (5H, m, CH_2-N + 4-H), 2.09 (6H, s, NMe), 2.05 and 2.02 (3H, two s, 10-Me), 2.03 (1H, m, 2-H), 2.00 (6H, s, NMe), 1.68 and 1.62 (3H, two s, 8-Me), 0.89 (3H, s, 9-Me); δ_C (62 MHz, $CDCl_3$): 152.94 (-C-), 151.25 (-C-), 132.59 (-C-), 131.81 (-C-), 131.28 (CH), 130.48 (-C-), 128.93 (-C-), 127.45 (-C-), 127.35 (CH), 127.25 (CH), 126.92 (CH), 126.01 (CH), 117.22 (CH), 115.52 (CH), 77.44 (CH), 51.74 (CH_2), 48.29 (CH), 45.10 (CH_3), 38.17 (CH_2), 33.44 (CH_2), 33.27 (CH_2), 33.10 (CH), 27.93 (CH_3), 23.33 (CH_3), 20.00 (CH_3); m/z 464 (M^++2, 0.2%), 463 (M^++1, 0.8), 462 (M^+, 3.1), 461 (3.2), 460 (5.0), 405 (1.5), 404 (1.9), 403 (2.0), 402 (0.6), 233 (0.4), 232 (1.8), 231 (0.6), 230 (3.9), 174 (1.3), 173 (1.0), 171 (1.8), 171 (2.9), 58 (100); δ_C (62 MHz, $CDCl_3$): 152.94 (-C-), 151.25 (-C-), 132.59 (-C-), 131.81 (-C-), 131.28 (CH), 130.48 (-C-) 128.93 (-C-), 127.45 (-C-), 127.35 (CH), 127.25 (CH), 126.92 (CH), 126.01 (CH), 117.22 (CH), 115.52 (CH), 77.44 (CH), 51.74 (CH_2), 48.29 (CH), 45.10 (CH_3), 38.17 (CH_2), 33.44 (CH_2), 33.27 (CH_2), 33.10 (CH), 27.93 (CH_3), 23.33 (CH_3), 20.00 (CH_3).

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References

1.- M. A. Caolo and F. R. Stermitz, *Tetrahedron*, **1979**, *35*, 1487.

2.- M. A. Caolo, O. P. Anderson, and F. R. Stermitz, *Tetrahedron* **1979**, *35*, 1493.

3.- M. Marcos, Ph.D. Thesis, Universidad de Santiago de Compostela, **1989**.

4.- H. Shanan-Atidi and K. H. Bar-Eli, *J. Phys. Chem.*, **1970**, *74*, 961.

5.- N. L. Allinger and Y. Y. Yuh, *Quantum Chemistry Program Exchange*, Indiana University: Bloomington, IN, no 365.

6.- V. V. Kane, A. R. Martin, C. Jaime and E. Osawa, *Tetrahedron*, **1984**, *40*, 2919.

7.- D. Neuhaus, R. N. Sheppard, and I. R. C. Bick, *J. Am. Chem Soc.*, **1983**, *105*, 5996.