Conformational study of 3-acety1-2,7,7-trimethyl-4-phenyl-1,4,5,6,7,8-hexahydro-5-quinolone starting from the crystallographics datas

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Received: January 17th, 2000 Acepted: April 27th, 2000

Palabras clave: Estructura cristalina, dihidropiridina, actividad biológica y rayos X
Key words: Crystal structure, dihydropyridine, biological activity and X-ray

RESUMEN. Las 1,4-dihidropiridinas (1,4-DHPs) son compuestos conocidos por su actividad farmacológica como moduladores de los canales del calcio. Las modificaciones químicas que se realizan en el anillo de la 1,4-DHP mediante el cambio de sustituyentes, tienen como objetivo modificar la relación estructura-actividad para lograr una mejor interacción con el receptor. El conocimiento de la conformación estereoquímica requiere el estudio de análogos de las 1,4-DHP. El anillo de ciclohexanona unido al anillo 1,4-DHP, dificulta la entrada de los iones de calcio en el espacio intracelular (agonistas del calcio), el tieno [2,3-b] piridina muestra un efecto antagonista y se conoce de su uso en el tratamiento de la epilepsia, mal de Alzheimer y en la enfermedad de Huntiton. Se reporta la estructura cristalina del 3,5-diacetil-4-fenil-2,6-dimetil-1,4-dihidropiridina; $C_{20}H_{23}NO_2$, miembro de la familia de las 1,4-DHP, perteneciendo a la clase denominada antagonistas del calcio. La estructura cristalina de este compuesto fue refinada para permitir la comparación de las deformaciones del anillo DHP y la conformación del grupo acetil con vistas a describir la importancia de estas sustituciones en la relación estructura-actividad de este tipo de compuesto.

ABSTRACT. 1,4-dihydropyridine (1,4-DHPs) are well known compounds as a consequence of their pharmacological profile as calcium channel modulator. The chemical modifications carried out on the DHP ring such as the presence of different substituents have allowed expansion of the structure activity relationship and afforded some insight into the molecular interactions at the receptor level. The knowledge of stereochemical/conformational requirements for requires the study of other related analogues of the DHP ring. As a fact, cyclohexanone ring fused to 1,4-DHP moiety in striking effect on the entry of calcium ions into the intracellular space. And thieno [2,3-b] pyridines show effect as antagonist and have been also used in treatment of epilepsy; Alzheimer's disease and Huntinton's chorea. The crystal structure of 3,5-diacetyl-4-phenyl-2,6-dimethyl-1,4-dihydropyridine; $C_{20}H_{23}NO_2$, member of the 1,4-DHP class of calcium antagonist, have been determined. The crystal structure of this compound was refined to permit comparison of their DHP ring deformation and acetyl group conformation, molecular features thought to be important in describing the structure-activity relationships for this important class of compound.

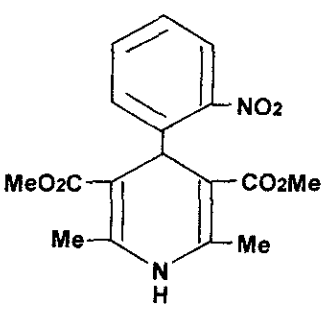
INTRODUCTION

1,4-dihydropyridine (1,4-DHPs) are well-known compounds as a consequence of their pharmacological profile as calcium channel modulator.[1] The chemical modifications carried out on the DHP ring such as the presence of different substituents[2] have allowed expansion of the structure activity relationship and afforded some insight into the molecular interactions at the receptor level. The knowledge of stereochemical-conformational requirements for Goldmad et al.[3] requires the study of other related analogues of the DHP ring.

As a fact, cyclohexanone ring fused to 1,4-DHP moiety in striking effect on the entry of calcium ions into the intracellular space (calcium agonist effect).[4] And thieno [2,3-b] pyridines show effect as antagonists[5] and have been also used in treatment of epilepsy, Alzheimer's disease and Huntington's chorea.[6]

The crystal structure of 3,5-diacetyl -4-phenyl-2,6-dimethyl-1, 4-dihydropyridine, $C_{20}H_{23}NO_2$, member of the 1,4-dihydropyridine class of calcium antagonists, have been refined.

The crystal structure of this compound was refined to permit comparison of their DHP ring deformation and acetyl group conformation, molecular features thought to be important in describing the structure-activity relationships for this important class of compound.



Nifedipine

EXPERIMENTAL METHODS

Synthesis. A mixture of the benzaldehyde (0.01 mol), dicarbonil compound (0.02 mol) (II, 2,3-acetylactone, IV, ethylacetoacetate), ammonium hydroxide, 30 % (2 ml in 20 ml of methanol was heated at reflux over night. The solvent was rotoevapored and the remained solid was recristallized from ethyl ether, as yellow solid in 22 % yield.

The crystal used for the structure determination were obtained by slow evaporation form ethanol. Dimensions (mm): 0.52 x 0.26 x 0.26.

Enraf-Nonius CAD-4 diffractometer with graphite crystal-monochromatized and data collection preceded at 293 K using Mo Kα radiation (λ = 0.71069 Å). Unit cell constants were derivative by least-squares from 25 carefully centered reflections in the range $10 < \theta < 16^{\circ}$. The $\theta/2\theta$ scan mode was used to record the integrate intensities. Peaks were subjected to profile analysis, and any portions of the scan not included in the peaks were used improve background estimates.

The structure was refined used program SHELXL97.[7] They were refined on F^2 by full matrix least-squares, originally with isotropic and later anisotropic temperature factors, from the atomics coordinates reported by Dago, et al.[8] All H-atoms were calculated at the idealized positions based on the molecular geometry C-H bond length of 0.95 Å. They were assigned isotropic temperature factors set at 1.2 time temperature factors of respective factors to which they were bonded. Refinement was continues until all shift\error ratios were < 0.1. Least-squares refinement was performed minimizing the wR value. In the final difference Fourier map, the deepest hole was -0.209 e $Å^{-3}$, and the highest peak was 0.207 e $Å^{-3}$. All program used are part of the SHELXL97. Final atomic parameters are shown in Table 1, and bond lengths and angles in Table 2. Selected torsion angles reported in Table 3.

The space group is, $P2_1/n$ with Z = 4, a = 10.986(2), b = 12.708(3), c = 12.902(3) Å, β = 107.13(3)° and V = 1721.3(7) $Å^3$. Three-dimensional intensity data collection in w:2θ scan mode; total of 5003 reflections, with 208 refined parameters. Data corrected for Lorentz and Polarization effects. Three standard reflections measured every 100 reflections during data collection, (4 -5 -4), (3 -3 -3) and (6 -1 -6), showed no significant change in intensity. Final R = 0.055, wR = 0.107 where $w = 1/[\backslash\sigma^2 (Fo^2) + (0.0495P)^2 + 1.0906P]$ where $P = (Fo^2 + 2Fc^2)/3$.[9–13] Scattering factors taken from *International Tables for X-ray Crystallography (1974)*. The thermal ellipsoids of the molecules with the atomic numbering are shown in Figure 1. Crystal packing is mainly dominated by an extensive hydrogen-bonding scheme (Fig. 2).

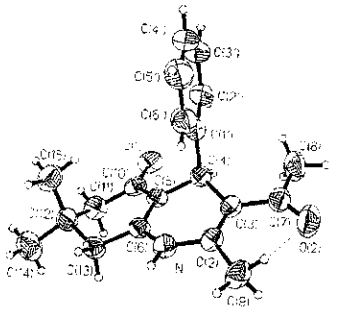


Fig. 1. *Molecular structure and atomic numbering. The thermal ellipsoids are drawn at the 50 % probability level, except for the H atoms (radius 0.05 Å)*

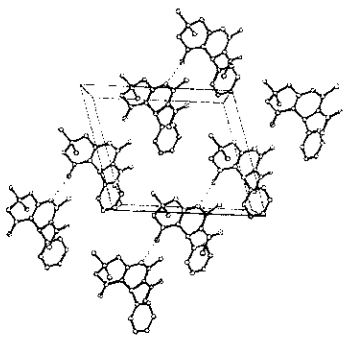


Fig. 2. *Stereoscopic view of the crystal packing. Dashed line indicated the hydrogen-bonds along (010)*

RESULTS AND DISCUSSIONS

Nifedipine [2,6-dimethyl-3,5- dicarbomethoxy-4-(2-nitro-phenyl)-1, 4-dihydropyridine] is a calcium antagonist drug of the 1,4-dihydropyri dine (DHP) type. Compounds of this class are currently being used in the treatment of cardiovascular disorders such as angina and hypertension.[14, 15] Calcium antagonist activity of members of the 1,4-dihydropyridine family is influenced by:

- The presence of the 1,4-dihydropyridine moiety
- Alkyl groups (preferably methyl) substituted at the 2 and 6 positions
- Ester groups at the 3 and 5 positions
- Phenyl substituent at position 4.
- An H atom on N.[14]

The influence of the size of the esterification groups is not fully understood. Variation of the C3 and C5 ester alkyl groups has led to conflicting results. In an early investigation of various DHP derivatives, it was observed that an increase in the bulk of the ester side chain led to an increase in activity.[16]

All of the nifedipine derivatives examined by single-crystal X-ray diffraction[14] exhibit a flattened-boat conformation of the 1,4-dihydropyridine ring with the N atom at the prow and the phenyl ring with in pseudo-axial position at the bow. Structure-activity studies have demonstrated that flattening of the boat conformation correlates with increased activity, presumably due to the concurrent change in position of the phenyl ring. In the majority of the more than 30 crystal structures of members of the nifedipine family, the ester groups are found to be nearly coplanar with the nearest double bond in the DHP ring, with the carbonyl group oriented either cis (sp, synperiplanar) or trans (ap, antiperiplanar) to that bond.[14] In nifedipine itself, the carbonyls of the ester groups are ap and sp and thus point in opposite directions. It is thought that only the sp conformation of the ester group permits hydrogen bonding to the carbonyl O atom as an acceptor atom when the drug binds to its receptor site.[15]

The title compound the substituents groups are: C3 position (sp) acetyl group and 5 and 6 positions dimedone group (Fig. 3).

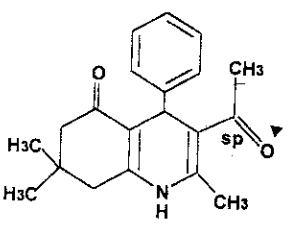


Fig. 3. *1,4-dihydropyridine skeleton with the crystallographic scheme illustrating sp orietation of acetyl group*

Table 1. Final coordinates and equivalent isotropic displacement parameters of the non-hydrogen atoms (e.s.d.'s are in parenthesis)

Label	x/a	y/b	z/c	Ueq
N	1.0493(2)	0.8002(2)	0.5766(1)	0.0469(6)
O1	0.7555(2)	0.8016(1)	0.2316(1)	0.0570(6)
O2	0.8600(2)	1.0575(2)	0.6708(2)	0.0828(8)
C5	0.8932(2)	0.7956(2)	0.4077(2)	0.0380(6)
C6	1.0077(2)	0.7639(2)	0.4725(2)	0.0403(7)
C4	0.8027(2)	0.8568(2)	0.4530(2)	0.0403(7)
C3	0.8751(2)	0.9168(2)	0.5546(2)	0.0417(7)
C2	0.9925(2)	0.8831(2)	0.6133(2)	0.0427(8)
C7	0.8139(2)	1.0075(2)	0.5882(2)	0.0518(10)
C8	0.6866(3)	1.0413(2)	0.5173(3)	0.0788(14)
C9	1.0738(2)	0.9298(2)	0.7168(2)	0.0596(9)
C10	0.8581(2)	0.7716(2)	0.2939(2)	0.0436(8)
C11	0.9523(2)	0.7132(2)	0.2521(2)	0.0562(9)
C12	1.0338(2)	0.6347(2)	0.3319(2)	0.0535(9)
C13	1.0978(2)	0.6943(2)	0.4367(2)	0.0497(8)
C14	1.1357(3)	0.5898(3)	0.2857(3)	0.0833(14)
C15	0.9517(3)	0.5449(2)	0.3523(2)	0.0687(11)
C1'	0.6998(2)	0.7852(2)	0.4713(2)	0.0475(8)
C2'	0.5774(2)	0.7868(2)	0.3996(2)	0.0658(10)
C3'	0.4838(3)	0.7222(3)	0.4155(3)	0.0869(16)
C4'	0.5102(4)	0.6554(3)	0.5012(4)	0.0961(17)
C5'	0.6291(4)	0.6520(2)	0.5730(3)	0.0856(17)
C6'	0.7247(3)	0.7170(2)	0.5580(2)	0.0660(11)

U(eq) = 1/3 of the trace of the orthogonalized U Tensor

Table 2. Bond distances (Å) and selected angles (°) (e.s.d.'s are in parenthesis)

N-C6	1.365(3)	N-C2	1.377(3)	O1-C10	1.236(2)	O2-C7	1.215(3)
C5-C6	1.350(3)	C5-C4	1.508(3)	C5-C10	1.437(3)	C6-C13	1.498(3)
C4-C3	1.523(3)	C4-C1'	1.523(3)	C3-C2	1.358(3)	C3-C7	1.464(4)
C2-C9	1.493(3)	C7-C8	1.492(4)	C10-C11	1.497(4)	C11-C12	1.522(3)
C12-C13	1.529(3)	C12-C14	1.526(4)	C12-C15	1.525(4)	C1'-C2'	1.390(3)
C1'-C6'	1.376(4)	C2'-C3'	1.377(5)	C3'-C4'	1.357(6)	C4'-C5'	1.361(5)
C5'-C6'	1.395(5)						
C6-N-C2	122.8(2)	C6-C5-C4	120.9(2)	C6-C5-C10	119.3(2)		
C4-C5-C10	119.7(2)	N-C6-C5	119.3(2)	N-C6-C13	116.2(2)		
C5-C6-C13	124.4(2)	C5-C4-C3	110.8(2)	C5-C4-C1'	110.8(2)		
C3-C4-C1'	112.9(2)	C4-C3-C2	119.5(2)	C4-C3-C7	118.7(2)		
C2-C3-C7	121.8(2)	N-C2-C3	120.1(2)				

Table 3. Selected torsion angles (°) (e.s.d.'s are in parenthesis)

C2-N-C6-C5	-12.9(3)	C6-N-C2-C3	15.2(3)
C4-C5-C6-N	- 9.3(3)	C6-C5-C4-C3	26.0(3)
C5-C4-C3-C2	-23.5(3)	C4-C3-C2-N	4.8(3)

When the plane of the phenyl ring is perpendicular to the plane of the base of the boat, activity increases.[16] The torsion angle that defines this parameter is C3-C4-C1'-C6'. The deviation from perfect bisection of the phenyl ring with respect to the DHP ring can be expressed as the difference between the torsion angle C3-C4-C1'-C6' and the ideal value of 60°. The compound exhibit the least deviation from the ideal value [11.11°], compared with other values the 14.8 and 21.4°, respectively[18] to phenyl ring to become more perpendicular.

Other parameter is the sumatoria (sum.) of the absolute values of the internal torsion angles of the DHP ring is a measure of its planarity. The title compound exhibit a sum. of 91.97° [NC(2)C(3)C(4): -4.94; C(2) C(3)C(4)C(5): 23.57; C(3)C(4) C(5) C(6): –23.06; C(4)C(5)C(6)N: 9.33; C(2)NC(6)C(5): 12.93 C(6) NC(2) C(3): -15.14]. For parents compounds with higher activity the sum of the absolute values of the internal torsion angles are: 103.4, 100.7 and 73.7° respectively.[18]

The 4-aryl substituent occupies a pseudoaxial position, orthogonal to the plane of the dihydropyridine ring (88.2°). This pseudoaxial position of the 4-aryl ring, which is reported to be essential for pharmacological activity.[19] The dihydropyridine ring of the compound in the solid state is a flattened "boat" (Fig. 4)

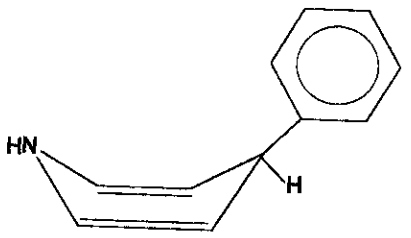


Fig.4. *Definition of "boat" conformation. Substituents on the dihydropyridine ring omitted for clarity*

as in other 4-aryl-1,4-dihydropyridine X-ray crystal structures,- the greatest ring distortions always occur at the nitrogen (N) and the tetrahedral carbon (C4). Both atoms are displaced in the same direction from the ring and form the apexes of a boat-type conformation. The degree of the ring distortions at N and C4 is directly reflected in the magnitude of the torsion angles about the ring bonds emanating from these two atoms.

Table 4. Intermolecular and intramolecular hydrogens bonds (e.s.d.'s are in parenthesis)

D-H···A	D-H (Å)	H···A (Å)	D···A (Å)	D-H···A (°)
C9-H9B...O2	0.969(3)	2.346(2)	2.771(3)	105.8(2)
N-H...O1*	0.858(2)	2.008(2)	2.852(2)	168.0(1)

*x + 1/2,–y + 1/2 + 1, + z + 1/2
D Donor; A Acceptor

In this crystal the pyridine nitrogen is involved in a strong intermolecular hydrogen bond with the N hydrogen: [N-H···O1: 2.852(2) Å (x + .5,-y + .5 + 1, + z + .5)] and intramolecular hydrogen bond between Methyl C9 atom and O2 acethyl group atom [C9-H9b···O2: 2.771(3) Å] (Table 4).

Characteristics of the 1,4-DHP receptor

The conformational characteristics may play an important role, which could have a direct bearing on the modes of ligand-receptor binding to the various gating states of Ca^{2+} L-type channel. It was noted that the degree of ring pucker markedly influences the angles of tilt of the aryl ring as sensed by the direction of its flagpole.

Review of the crystallographic environments of these structures revealed that whereas the minimum surface contacts defining the van der Waals envelope were remarkably similar for all compounds reported, the directionalities of hydrogen bonds experienced by these molecules varied, even within grouping with common ester group orientations. In all structures reported the DHP amine group was found to form a well-defined hydrogen bond in which the proton acceptors for the sp^2 hybridized DHP amine were colinearly directed along the line of sight of the N-H bond and clustered in a small volumen of space.

The above observations indicated that the 1,4-DHP receptor probably recognizes nifedipine antagonists by means of hydrophobic cleft that fits the phenyl ring and upper face of the aryl-DHP van der Waals envelope. This template would properly orient the molecule to form hydrogen bonds to peptide donor and acceptor groups located around the edge of this recognition surface. Give that the aryl ring would be immobilized, it was shown that the degree of DHP pucker would directly affect the angle at which the DHP amine group could form a hydrogen bond (Fig. 5) to its bidding site in the receptor surface. The more active nifedipine analogues, which have flatter DHP rings, would have the DHP amine proton positioned to form a hydrogen bond with the amine acceptor group in the receptor cavity. Weakly active analogues would not have the amine proton pointing in this exact same direction, but aproxymately 2.0 Å higher in the receptor cavity, as a consequence of the greater degree of DHP ring puckering. This requirement for the DHP amine to form hydrogen bonds with the receptor is consistent with SAR studies[18] which indicate that the N-H group is crucial for calcium channel activity, and the replacement of the amine proton by an alkyl group or its removal by oxidation of the 1,4-DHP to a pyridine ring abolishes activity.[16]

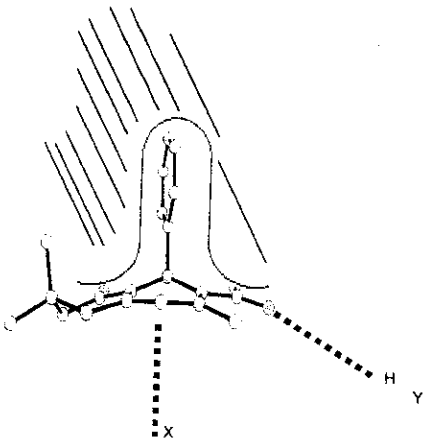


Fig. 5. *Illustrated the mode of ligand binding to the DHP receptor surface. Caudal view depicting the hydrophobic cleft fitting the aryl ring and upper surface of the DHP ring. Hydrogen bond donors to the oxygen atom sp orientation of the acetyl group. The positions of hydrogen bond acceptor for the DHP amine proton are clustered and represented by dashed lines, X and H'Y' represented parts of the receptor*

CONCLUSIONS

The present diffraction study indicates that large differences in the bulk sizes of the substituents may not select a specific *sp, ap* conformation and predispose the molecule for receptor binding. The cis isomerization of the acetyl group does not appear to markedly perturb the degree of puckering of the DHP ring, which suggests that tissue selectivities are largely determined by the nature of the substituents groups.

A receptor binding model has been described, which may, in part, explain the basis of tissue selectivity for the 1,4-DHP Ca^{2+} channel antagonist.

Calcium entry blocker activity of the compound has been evaluated. The study showed an inhibitory effect on KCl-induced contraction of rabbit aortic rings with similar IC_{50s}. The compound was less potent than nifedipine (Table V). The compound showed clear negative inotropic effects with IC_{50s} in the micromolar range, one order of magnitude less potent than nifedipine. The present results indicate that the compound possess Ca-antagonist activity in vascular and cardiac muscles. Others crystallographic supplementary materials were deposited in the X-ray Laboratory of the National Center for Scientific Research, Havana, Cuba.

Table 5. IC_{50} (in μmoles/L) values for nifedipine and 3,5-diacetyl-4-phenyl-2,6-dimethyl-1,4-dihydropyridine, $C_{20}H_{23}NO_2$ for the relaxant effects in myocardium (IC_{50} myoc.) and in vascular smooth muscle (IC_{50} aorta). Vascular selectivity index (VSI)* is give for each compound

Compound	IC_{50}(myoc.)	IC_{50}(aorta)	VSI
Nifedipine	0.17	0.13	1.3
$C_{20}H_{23}NO_2$	4.34	1.05	4.13

*VSI IC_{50}(myoc.)/IC_{50}(aorta)

ACKNOWLEDGEMENTS

The authors thank the Third World Academy of Sciences for financial support through the TWAS South-South research program.

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