Kazan Federal University
Zavoiskii Physical-Technical Institute
Russian Academy of Sciences
Bruker Ltd (Moscow)
"Dynasty" Foundation
Russian Foundation for Basic Reserch

ACTUAL PROBLEMS OF MAGNETIC RESONANCE AND ITS APPLICATION

XV International Youth Scientific School

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KAZAN

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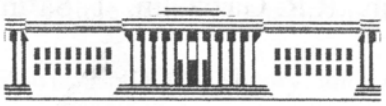

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Spatial structure of felodipine in DMSO-d6 solution by 1-D NOE and 2-D NOESY NMR spectroscopy

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Intriduction

The accurate determination of interproton distance in relatively small, flexible molecules by 1D and 2D NOE NMR spectroscopy is the subject of increasing interest in recent years [1–3] The results of such investigations can be applied to establishing conformational details for biologically active molecules in solutions. It is well known that polymorphism of drug compounds affect the biological activity and plays an important role in the production of pharmaceuticals. In turn, the properties of polymorphs due to the moleculare structure of compounds and their ability to exist in different conformational forms in the solvent form which recrystallization occurs. Therefore, the search for new polymorphic forms of drug closely associated with the study of the conformational state of biological active molecules in solutions.

Felodipine is a calcium channel blocker and widely used in the treatment of hypertension. Conformational flexibility of felodipine is determined by two dihedral angles (c3-c4-c1'-c2') defines the rotation of 2,4 dichlorophenyl group around c1'-c4 bond and the second(c3a-O-c3b-c3c) the rotation of ethyl group around O-3b (fig.1). Teberikidis and Sigalas in 2007 published the result of a theoretical study of felodipine using a hybrid density functional method B3LYP [4]. They located six conformers with very close energies (within 4,2 kJ / mol), starting from number 1(the global minimum) up to number 6 (with a maximum energy). It is important for the purposes of further discussion, that in the conformers 1,2,5 chlorine atoms lying towards dihydropyridine, whereas in the conformers 3,4,6 in the opposite direction.

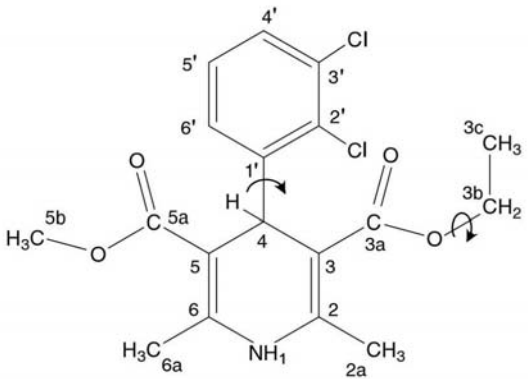

Fig.1. Structure and nomenclature of felodipine

Theory

NOE spectroscopy is a powerful tool of studying the spatial structure and conformations of molecules in solutions. The method of estimating internuclear distances for various pairs of protons in a molecule is based on the existence of a strong dependence of the cross-relaxation rate constant σ_{ij} on the distance r_{ij} between the interacting nuclear spins. Normally, such a dependence is easily approximated by the formula $\sigma_{ij} \sim 1/r_{ij}^6$ and then internuclear distances are obtained according to the formula:

$$r_{ij} = r_0 \left(\frac{\sigma_0}{\sigma_{ij}} \right)^{\frac{1}{6}}, \quad (1)$$

where the quantities r_0, σ_0 refer to a pair of protons chosen for calibration the distance r_0 is supposed to be known from some independent source (e.g. quantum chemical calculations). However, for small, conformationally flexible molecules, of great importance is the problem of more accurate determination of internuclear distances in order to obtain information of relative population of various conformers. This goal may be obtained both by a more accurate interpretation of the 2D NOESY experiment and by taking account of the anisotropic molecular rotation.

Experimental

All NMR experiments were performed on a "Bruker Avance II-500" NMR spectrometer equipped with a 5 mm probe using standart the Bruker TOPSPIN Software. Temperature control was performed using a Bruker variable temperature (BVT-2000) unit in combination with a Bruker cooling unit (BCU-05) to provide chilled air. Experiments were performed at 298 K without spinning. ^{1}H NMR(500 MHz) spectra were recorded using 90° pulses and relaxation delay of 1 s, with a spectral width of 12.02 ppm, 128 scans. 13C NMR spectra were recorded using 45° pulse sequence with power-gated decoupling for suppression of the NOE effect.

The ^{13}C NMR spectra were acquired with relaxation delay 1s, 236 ppm spectral width, 1024 scans. Total correlation spectroscopy (TOCSY), one dimension gradient homonuclear selective total correlation spectroscopy (1D ge-TOCSY), heteronuclear multiple-bond correlation (HMBC)[5] and gradient heteronuclear single-quantum coherence (ge-HSQC), have been used to assign the complex ^{1}H and ^{13}C NMR spectra**.**

Two-dimensional nuclear Overhauser effect spectroscopy (2D ge-NOESY) experiments were performed with pulsed filtered gradient techniques. The spectra were recorded in a phase-sensitive mode with 2048 points in the F2-direction and 512 points in the F1-direction. Mixing time values, were 0.30, 0.50, 0.70 and 0.90 s.The spectra were acquired with 24 scans and 2 s relaxation delay.

Selective One-dimensional nuclear Overhauser effect spectroscopy (ge-1D NOESY) experiments were carried out by using the double-pulse field gradient spin-echo NOE (1D-DPFGSENOE) NOE pulse sequence. The 1D NOESY spectra were acquired with 64 k data points, 160 scans, 12 ppm spectral width, 2 s relaxation delay, acquisition time of 5.45 s, mixing times of 0.3, 0.5, 0.7 and 0.9 s. The pulse programs for all NMR experiments were taken from the Bruker software library.

The list of atomic coordinates for the various conformations of felodipine from supplementary data [4] has enable us to calculate the effective distances required for comparison with experiment (calculated distances in tables 1-4. Effective distances show in tables as a calibration distance, chosen as such because of their equality for all conformations of felodipine. It should be noted that the distance between aromatic protons can not be taken for the calibration because of artifacts observed in the NOE intensities [2].

Results of 2D NOE experiments are presented in table 1. It is obvious that the calculated values of the distances for a conformation 1,2,5 are very close match to experimental data, while for the conformations 3,4,6 agreement with experiment is very poor. The experimental value of rij for the pair of protons 4-6' lies within interval 2.12 Å (conformations 1,2,5) – 3,73 Å (conformations 3,4,6), while the difference between 2.21 A

and 2.12 A goes beyond the experimental error (~ 3%). Really 2D NOE experiments show that at the concentration investigated conformations 1,2,5 prevail although there are of some conformations 3,4,6.

In favor of the existence of a significant amount conformations of 3,4,6 show the result of 1D NOE experiment: the closeness of experimental and calculated values of r_{ij} for protons NH-6'(Table 2).

Table 1. 2D NOESY

Atomic group	Experimental distance Å	Calculated distances Å					
		Conf 1	Conf 2	Conf 3	Conf 4	Conf 5	Conf 6
3b-3c	calibration	2,69	2,69	2,69	2,69	2,69	2,69
4-6'	2,21	2,12	2,12	3,73	3,73	2,12	3,73

Table 2. 1D NOE NH proton selective

Atomic group	Experimental distance Å	Calculated distances Å					
		Conf 1	Conf 2	Conf 3	Conf 4	Conf 5	Conf 6
NH-2a	calibration	2.48	2.48	2.48	2.48	2.48	2.48
NH-6a	2.50 ±0.08	2.48	2.48	2.48	2.48	2.48	2.48
NH-6'	3.44±0.06	-	-	3.50	3.48	-	3.37

Recently Butts et al [6] gave a convincing example of the fundamental possibility of quantitative determination of conformations of flexible molecules by NOE. This success is due to the presence in a molecule arugosin C just five short interproton distances (<3 Å), which can be determined with high accuray. Unfortunately, in our case, for each of conformations we have only one distance less 4 Å (if not take into account 3,92 Å for conf. 2 in table 4). It is well known that errors in measuring distances by NOE in this range (>4 Å) are greatly increased, mainly due to spin diffusion. Therefore the problem of the suppression of spin diffusion in NOE-measurements of interproton distances for small molecules remains very important.

Thus, the result of 1D and 2D NOE studies of felodipine in DMSO solution 0,08 g/l show the dominant presence of felodipine conformations in which chlorine atoms lying towards dihydropyridine. However, the existence of a of some amount of conformations in which chlorine atoms lying in the opposite direction clearly reveals. We were not able to make quantitative evaluation of populations of the felodipine conformations in DMSO because of insufficient accuracy in the range of relativity large distances (~4 Å). In such circumstances, we see two ways to improve the accuracy of interproton distance determination of interproton distances, concerted T-ROESY/NOESY experiments or suppression of spin diffusion. Well try to use both approaches and analyze their capabilities in our next work.

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