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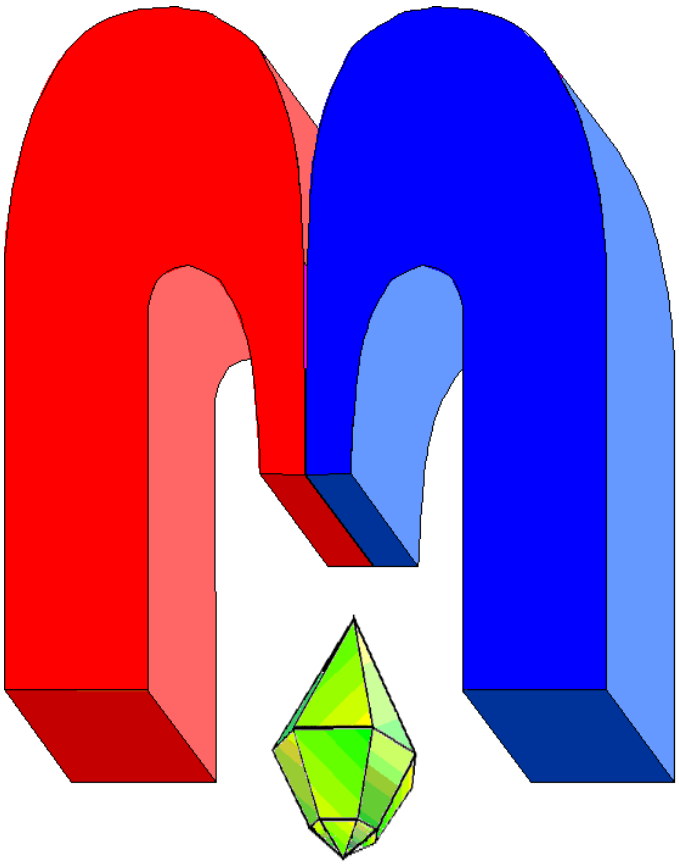

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* In Kazan University the Electron Paramagnetic Resonance (EPR) was discovered by Zavoisky E.K. in 1944.

Obtaining spatial structure of cyclosporine (CsA) in chloroform using 2D NMR

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In this work the cyclic peptide cyclosporine was investigated. Signal assignment was made according to 2D NMR spectra. Using NOESY spectra and computer simulation the spatial structure was also obtained.

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1. Introduction

We have chosen cyclosporine (CsA) as an object for our research (CsA is one of the possible modifications of cyclosporine, for simplicity we will name it further simply as cyclosporine). Cyclosporine is a cyclic peptide which is used as immunosuppressive medication in transplantation. In cells, cyclosporine binds with the protein cyclophilin and, as a result, stops the reaction chain that is responsible for the immune response [1-3]. This peptide is poorly dissolved in water (23 μM at 25°C), but in organic solvents its solubility is high [4]. Chloroform was used as a starting point in our studies, which we suppose to develop further by employing residual dipolar couplings measured in chloroform-based media. On the other hand, it is an example of an apolar solvent. Comparison of the cyclosporine's structure and behavior in such a medium with the situation in different polar media (organic solvents mixed with water, micellar solutions) is of interest. The concentration of our sample was 17 mM.

The aim of our work was to analyze 1D and 2D NMR spectra, and to obtain spatial structure of cyclosporine using the structure calculation. We intend to obtain spatial parameters with high precision.

2. Experimental results and discussion

Schematic structure of cyclosporine molecule is shown in Fig. 1.

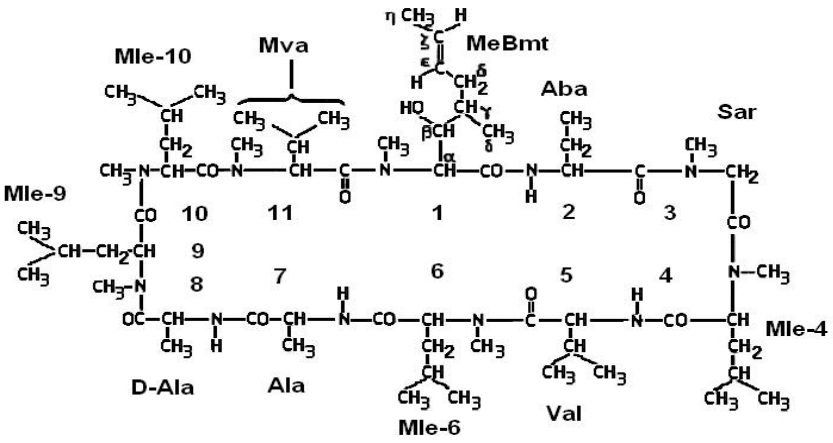

Figure 1. Schematic structure of cyclosporine (Bmt-Aba-Sar-Mle-Val-Mle-Ala-Dal-Mle-Mle-Val).

Let's consider main spectra that were used for the assignment of NMR signals.

At first, we used selective TOCSY spectra. Amide protons were excited, and as a result, we observed individual subspectra of the corresponding amino acids. Besides, we recorded 2D TOCSY which yielded information about all signals of a certain amino acid. Due to this fact, it was used as the main method for assignment.

We obtained signals of carbons with the help of HSQC spectra and measured chemical shifts of attached protons. That method helped us in making assignment.

Also we employed an important experiment called HMBC. A peak of an N-methyl group within the *i*-th residue, lying in the region of carbonyl resonances (chemical shift range $\delta(^{13}C) = 170\ldots174$ ppm), points to the CO group of the residue in position $(i-1)$. Each cross-section at the chemical shift of an Hα atom contains two signals of carbonyl groups: that of the residue with concerned Hα atom and of the preceding one. Moreover, if we connect (Hα,CO)-signals with each other and obtain the cycle, we can prove that the assignment is correct. This is illustrated in Fig. 2.

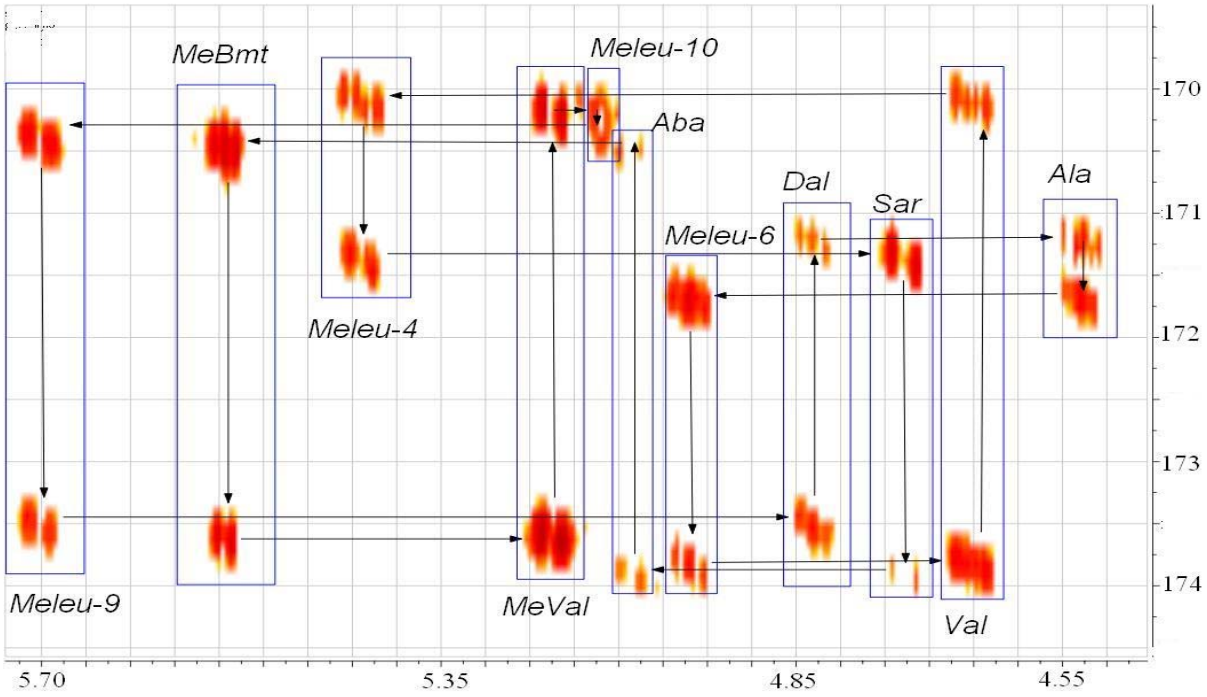

Figure 2. HMBC (^{1}H 500.13 MHz, ^{13}C 125.76 MHz) spectrum of cyclosporine in chloroform at $T = 293$ K. Region of correlations between C = O and NCH_3 groups is shown.

Having made the analysis of 1D and 2D NMR spectra, we obtained the chemical shifts of all protons, which you can see in Tab. 1.

At the next step we had to obtain the distances between protons. For this purpose, we recorded a set of NOESY spectra with different mixing times. Cross-peaks were integrated, and obtained intensities were normalized by the diagonal peaks. It was plotted as a function of the mixing time. The slopes of obtained curves gave the cross-relaxation rates. Then we used correlation $\sigma_{pq} = \sigma_{ij}^0 \left(\frac{r_{pq}}{r_{ij}^0} \right)^{-6}$, where r_{ij}^0 is the reference distance between two nonequivalent α-protons of Sar assumed to be 1.75 Å. After this calculation, interproton distances were obtained. They are listed in Tab. 2. below.

Table 1. Proton chemical shifts.

Amino acid residue				Chemical shifts, ppm			
MeBmt	NCH_3 3.52	α CH 5.5	β CH 3.81	γ CH 1.62	δ CH_2, CH_3 0.71, 2.02	ε CH 5.34	ζ CH, CH_3 4.02, 1.03
Aba	NH 8.0	α CH 5.03	β CH_2 1.61, 1.64	γ CH_3 0.87			
Sar	NCH_3 3.41	α CH 4.73, 3.2					
Mle4	NCH_3 3.12	α CH 5.35	β CH_2 1.97	γ CH 1.64	δ CH_3 0.87, 0.85		
Val	NH 7.49	α CH 4.66	β CH 2.43	γ CH_3 1.08, 0.89			
Mle6	NCH_3 3.26	α CH 4.97	β CH_2 2.07	γ CH 1.4	δ CH_3 1.04, 1.01		
Ala	NH 7.67	α CH 4.54	β CH_3 1.36				
Dal	NH 7.18	α CH 4.84	β CH_3 1.26				
Mle9	NCH_3 3.12	α CH 5.71	β CH_2 2.14	γ CH 1.24	δ CH_3 0.96, 0.88		
Mle10	NCH_3 2.71	α CH 5.08	β CH_2 2.07, 1.28	γ CH 1.28	δ CH_3 0.95, 0.88		
Mva	NCH_3 2.7	α CH 5.13	β CH 2.15	γ CH_3 0.81, 0.98			

Table 2. Distance restraints, derived from the NOESY spectra.

Res name	Atom name	Res name	Atom name	Dist, Å
Aba	Ha	Aba	NH	3.2 ± 0.2
Aba	Hb	Aba	Ha	2.53 ± 0.15
Ala	Ha	Ala	NH	2.52 ± 0.14
Bmt	Ha	Aba	NH	2.1 ± 0.1
Bmt	Hb	Bmt	Ha	2.1 ± 0.1
Bmt	Hd	Aba	Hb2	2.31 ± 0.12
Dal	Ha	Dal	NH	2.99 ± 0.24
Dal	Hb	Dal	Ha	1.92 ± 0.09
Mle10	Ha	Mle9	Ha	1.9 ± 0.3
Mle10	Hb	Mle10	Ha	2.43 ± 0.16
Mle10	Hg	Mle10	Ha	2.6 ± 0.2
Mle10	Hg	Mle10	Hb	1.54 ± 0.07
Mle4	Ha	Val	Hb	2.47 ± 0.13
Mle4	Hb	Mle4	Ha	2.36 ± 0.17
Mle4	Hg	Mle4	Ha	1.9 ± 0.2
Mle4	Hg	Val	Hb	1.63 ± 0.07
Mle6	Ha	Ala	NH	1.8 ± 0.4
Mle6	Hb	Mle6	Ha	2.47 ± 0.12
Mle6	Hg	Mle6	Ha	2.38 ± 0.08
Mle6	Hg	Ala	Ha	1.97 ± 0.09
Mle6	Hg	Mle6	Hb	1.87 ± 0.08
Mle9	Hb	Mle9	Ha	2.85 ± 0.39
Mle9	Hg	Mle9	Ha	2.0 ± 0.1
Mva	Hb	Mva	Ha	2.49 ± 0.13
Mva	Hg1	Mva	Hb	2.0 ± 0.1
Mva	Hg2	Mva	Hb	2.33 ± 0.15
Sar	Ha2	Sar	Ha1	1.8 ± 0.1
Val	Ha	Val	NH	2.08 ± 0.11
Val	Hb	Val	Ha	2.59 ± 0.21
Val	Hg1	Val	Hb	2.21 ± 0.11
Val	Hg2	Val	Hb	2.5 ± 0.2

Knowledge of chemical shifts and distances between protons allowed us to use structure calculation and to get the structure of the cyclosporine molecule, which we are interested in. For this purpose, we used DYNAMO package [5], which is widely used for structural researches.

Obtained spatial structure of cyclosporine with RMSD = 1.4 ± 0.4 Å for backbone can be seen in Fig. 3.

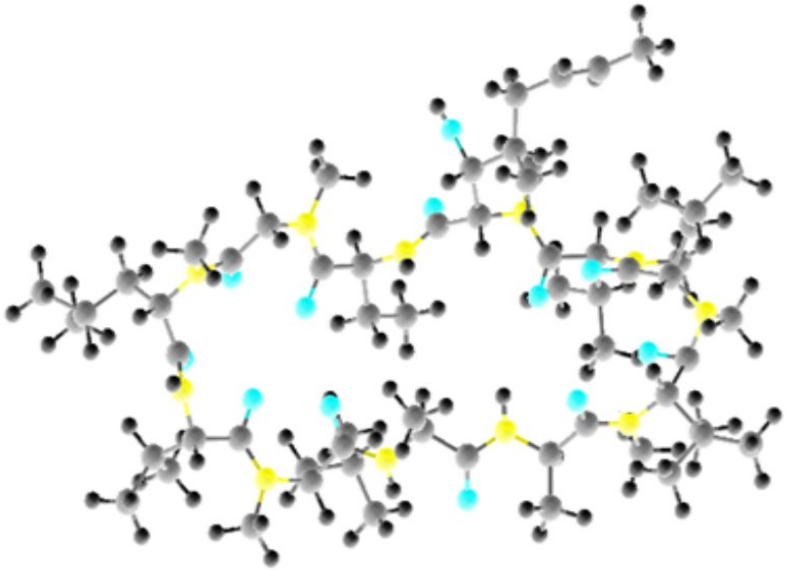

Figure 3. Averaged spatial structure of cyclosporine in chloroform after final energy minimization.

As can be seen, there are only few interresidual NOESY cross-peaks. The reason for this is that the cyclosporine molecule adopts an elongated form, where residues are situated like in a beta-strand or a random coil. These structures are known to produce little or no interresidual NOEs. Only in the loop regions we can seen close contacts between nonadjacent amino acids. We can thus find out the loops, but cannot obtain a single best structure by means of molecular dynamics. That's the explanation for relatively high RMSD of the ten selected backbones (Fig. 4).

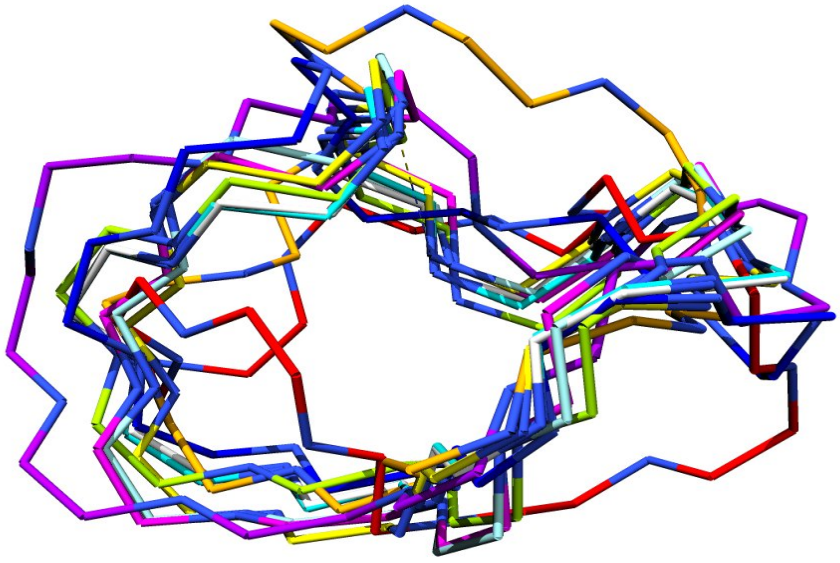

Figure 4. Backbones of 10 lowest energy structures.

3. Summary

According to obtained spectra and signal assignment we got the spatial structure of cyclosporine in chloroform and will use this information in our further research.

References

1. Hsu V.L., Heald S.L., Harding M.W., Handschumacher R.E., Armitage I.M. *Biochem. Pharm*. **40**, 131 (1990)
2. Weber C., Wider G., Freyberg B., Traber R., Braun W., Widmer H., Wuthrich K. *Biochemistry* **30**, 6563 (1991)
3. Fesik S.W., Gampe R.T., Eaton H.L., Gemmecker G., Olejniczak E.T., Neri P., Holzman T.F., Egan D.A., Edalji R., Simmer R., Helfrich R., Hochlowski J., Jackson M. *Biochemistry* **30**, 6574 (1991)
4. Yingqing R., Luwei Z., Qing X., Yalkowsky S.H. *AAPS PharmSciTech* **2**, 23 (2001)
5. Delaglio F., Grzesiek S., Vuister G., Zhu G., Pfeifer J., Bax A. *J. Biomol. NMR* **6**, 277 (1995)