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cis-trans-Isomerisation of the Proline-Peptide-Bond in a Cyclic Tetrapeptide Related to Chlamydocin*

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Saturation transfer NMR experiments have been used to study the thermodynamic characteristics of the interconversion of two solute conformations of the chlamydocin derivative **2** in *DMSO-*d_6.

(Keywords: Chlamydocin; Kinetics of conformational changes; Saturation transfer)

Cis-trans-Isomerisierung der Prolin-Peptidbindung in einem Derivat von Chlamydocin

Durch Sättigungstransferexperimente wurde die Kinetik der Isomerisierung mittels NMR-Spektroskopie untersucht. Die thermodynamischen Größen der Reaktion wurden bestimmt.

The threedimensional structure of a peptide or protein can be related to biological functions of this molecule[1]. Therefore the conformational behaviour of small cyclic peptides was the aim of many investigations[2–7]. Chlamydocin (**1**), a cyclic tetrapeptide isolated from culture filtrates of *Diheterospora chlamydosporia*[8], has been studied recently by NMR. It has been shown that chlamydocin and similar cyclic tetrapeptides exist in two conformations in *DMSO-*d_6[5,6,9]. We have studied the conformational behaviour of cyclo[A^{i}bu-L-Phe-D-Pro-L-Ada], **2**, which is closely related to chlamydocin.

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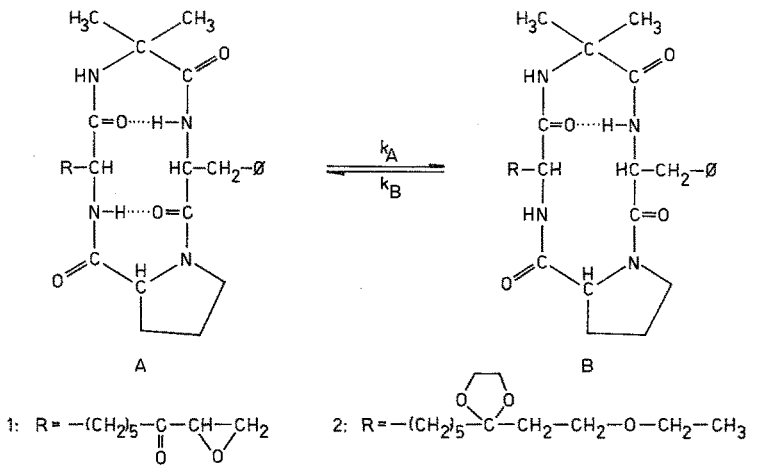

The two conformers of **2** in *DMSO-d_6* have internal hydrogen bonds and interconvert slowly on the NMR time scale. One of these conformers is an all-*trans* conformation with two γ-turns from the amide proton of Phe to the carbonyl oxygen of Ada and from the amide proton of Ada to the carbonyl oxygen of Phe, respectively[5,9]. The second conformer has a *cis* Phe-Pro-amide bond[5,6]. The extreme downfield shift of the amide proton of Ada ($\Delta\delta \sim 2.5$ ppm) in this conformer indicates an exposure of

Table 1. *Chemical shifts* [ppm] *and coupling constants* [Hz] *of the amide protons of the two conformers of* **2** *in DMSO-d_6; peptide backbone angles* Φ_L [deg] *derived from the coupling constants* ($\pm 10\%$); *room temperature*

	A^{i}bu		Phe		Ada	
	A	B	A	B	A	B
δ	8.00	7.94	7.71	6.86	6.98	8.51
^3J (HNCαH)	—	—	11.2	7.6	11.2	8.8

	Ada		Phe	
	A	B	A	B
Φ_L	−120	−80	−120	−80

Ada = ethylene ketal of 2-amino-10-ethoxy-8-oxo-decanoic acid (see formula).

A^{i}bu = aminoisobutyric acid.

For definition of Φ_L see Ref.[10].

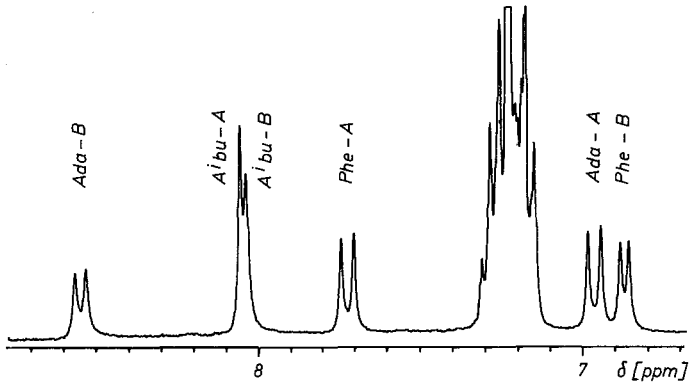

Fig. 1. Downfield part of the 250 MHz-^{1}H-NMR-spectrum of **2** in *DMSO-d*$_6$. Amide resonances are marked according to conformers A and B. $T = 305$ K

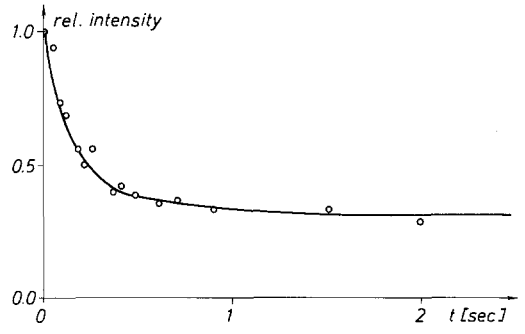

Fig. 2. Time dependence of the intensity of signal Ada-A upon instantaneous saturation of signal Ada-B at 325 K

this proton to the solvent and thus rupture of the γ-turn to the carbonyl oxygen of Phe during the conformational change. This is supported by the values of the coupling constants of the amide protons of Phe and Ada[10,11] (Tab. 1).

We have investigated the kinetics of the isomerisation by NMR double resonance techniques, first employed by *Hofmann* and *Forsén*[12,13]. These experiments have been done in the Pulse-FT-mode with the NH-protons of both isomers (Fig. 1). Instantaneous saturation of the transitions of one NH-proton was transferred to the signal of the corresponding NH-proton in the second isomer via the chemical exchange process, decreasing its intensity. The saturation transfer was

monitored as a function of time (Fig. 2) and yielded the magnetic relaxation times T_{1A} and T_{1B} of the corresponding protons and the values of k_A and k_B of the chemical exchange. Measurements of the rate constants k_A and k_B at various temperatures yielded the thermodynamic parameters of the isomerisation process (Tab. 2).

Table 2. *Kinetic and thermodynamic parameters of the isomerisation* $A \rightleftharpoons B$. We estimate the accuracy of the activation parameters to $\pm 5\%$

T [1]	k_A [2]	k_B [2]	K	ΔG [3]	ΔH [4]	ΔS [5]
295	1.62	1.67	0.97	75		−18
300	2.16	2.27	0.95	124		−18
305	3.08	3.48	0.89	310	−5.2	−18
315	5.24	6.13	0.85	411		−18

T [1]	ΔG_A^* [4]	ΔG_B^* [4]	ΔH_A^* [4]	ΔH_B^* [4]	ΔS_A^* [5]	ΔS_B^* [5]
295	71.0	71.0			−86	−68
300	71.6	71.4			−86	−68
305	71.9	71.6	45.8	51.0	−86	−68
315	73.0	72.5			−86	−68

Units: [1]: K; [2]: s^{-1}; [3]: $J\,mol^{-1}$; [4]: $kJ\,mol^{-1}$; [5]: $J\,K^{-1}\,mol^{-1}$.

ΔG^* of our study corresponds very well with ΔG^* from earlier investigations which revealed values of 65 to 80 $kJ\,mol^{-1}$ for the *cis—trans*-isomerisation of X-Pro-bonds[11,14]. ΔS^* of the conformational change is negative, suggesting a highly ordered transition state. Probably a solvent molecule is bound to the NH-proton of Ada supporting the rupture of the γ-turn. This too will contribute to a negative ΔS^* as well as a high degree of order of the solvent molecules around the activated complex. This indicates the important role of the solvent during conformational changes of cyclic tetrapeptides.

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