

The Pressure Dependence of the Rotation of the N-Aralkylpyridinium Moiety in a Synthetic NAD⁺ Model System Studied by High Pressure HRNMR

Johann Hauer, Hans-Dietrich Lüdemann

Institut für Biophysik und Physikalische Biochemie, Universität Regensburg, Postfach 397, D-8400 Regensburg

J. W. Verhoeven

Laboratorium voor Organische Scheikunde der Universiteit van Amsterdam, 1018 WS Amsterdam, Postbus 20241

Z. Naturforsch. **36 c**, 366–368 (1981); received January 30, 1981

High Pressure, NMR, NAD⁺-Model, Activation Volume

The activation volume ΔV^* for the rotation of the N-aralkylpyridinium moiety in a synthetic NAD⁺ model was studied by high pressure HRNMR. From the pressure dependence of the proton spectra a negative ΔV^* of $-3 \text{ cm}^3 \cdot \text{mol}^{-1}$ is derived. It is concluded from this result, that the rotation of the N-aralkylpyridinium ring occurs in a complex geared motion and not in 180°-flips.

Introduction

Synthetic chiral pyridinium nucleotide analogues have been used as stereospecific model systems for asymmetric reductions of organic compounds [1, 2].

From the temperature dependence of HRNMR proton spectra in such compounds Bakker *et al.* [3] could derive the free activation energy for the rotation of the pyridinium moiety around the N-C bond in the N-aralkylpyridinium ion derivative (**1**) (see Fig. 1). It was concluded, that the high ΔG^* of $66 \text{ kJ} \cdot \text{mol}^{-1}$ is caused by the steric interaction between the tert-butyl group and the pyridinium-ring. In the work presented here, high pressure HRNMR was applied in order to study the pressure dependence of this rotation, and to investigate further details of the nature of the transition state. From the pressure dependence of the proton spectra in the exchange broadened region the activation volume ΔV^* defined by

$$\Delta V^* = \frac{\partial \Delta G^*}{\partial p} = -RT \frac{\partial \ln k}{\partial p}$$

can be derived.

Materials and Methods

Compound **1** was prepared according to published procedures [3]. Details of the high pressure

HRNMR cell [4, 5] and the experimental details [6] have been published. The spectra were simulated by the DNMR 5 program [7]. A solution of 3.2% w/w of compound **1** in heavy water (96% deuterated) was used in the studies presented here. The spectra were taken at 100.1 MHz in the FT-mode. 5000 transients were accumulated for each spectrum.

Results and Discussion

Fig. 1 gives a part of the proton spectrum of **1** at 274 and 299 K. Only in the region of maximal chemical exchange broadening between 299 and 302 K is the sensitivity of the spectra to small changes of the rate of rotation k sufficiently pronounced to allow a precise determination of the pressure dependence of k . Only the part of the spectra assigned to α and ε could be simulated with the precision necessary to derive ΔV^* and ΔG^* . This spectrum was treated as an $\alpha_{\text{I}} \varepsilon_{\text{I}} \delta \rightleftharpoons \alpha_{\text{II}} \varepsilon_{\text{II}} \delta$ spin exchange. The modest resolution obtained in the experimental spectra has two origins:

1. In the long accumulation times necessary, a certain deterioration of the magnet field homogeneity cannot be avoided.
2. Deuteriumoxide near room temperature is a fairly viscous solvent, and thus imposes a rather wide natural linewidth (T_2) upon solutes of the size of the compound **1** studied here.

Reprint requests to Prof. Dr. H.-D. Lüdemann.

0341-0382/81/0500-0366 \$ 01.00/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition “no derivative works”). This is to allow reuse in the area of future scientific usage.

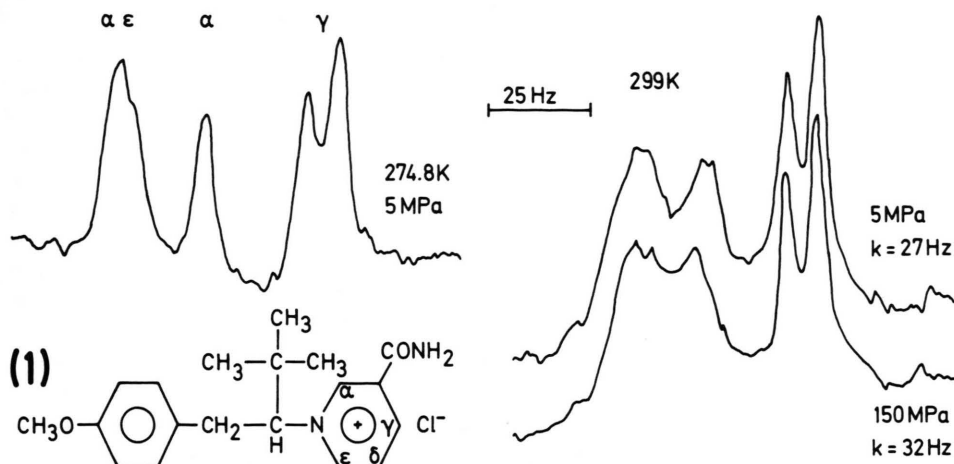


Fig. 1. High pressure proton HRNMR spectra of the pyridinium moiety of the N-aralkylpyridinium ion [1]. Assignments according to [3]. k , rotation rates derived from the simulation.

Table I. Optimal simulation parameters. The left side of the exchange broadened spectra given in Fig. 1 was simulated by introducing a pressure and temperature independent $J_{e\delta} = 7.5$ Hz and incorporation of $J_{e\gamma}$ into the linewidth. The slope of the baseline in these spectra was compensated by application of the base line tilt parameter of the DNMR 5 program.

T [K]	$\Delta v_{\alpha/\text{II}}$ [Hz]	$\Delta v_{\epsilon/\text{II}}$ [Hz]	$\Delta v_{\alpha/\epsilon\text{I}}$ [Hz]	Linewidth I	Linewidth II	population I
299.3	25	40	22	1.5	2.5	0.52
301.5	25	40	22	1.5	2.5	0.52

The simulation parameters are compiled in Table I. No significant pressure dependence of the chemical shift differences Δv_{α} and Δv_{ϵ} is found. Also the equilibrium distribution p of the rotamers is independent of pressure. The free activation energy was found to $\Delta G^*_{\text{MPa}, 300 \text{ K}} = 65 \pm 1 \text{ kJ} \cdot \text{mol}^{-1}$.

Fig. 2. compiles k as function of pressure for the 301.5 K and the 299.3 K isotherms. Both isotherms do have a positive slope. Indicating that pressure accelerates the rotation and that the activation volume is negative. From the isotherms a $\Delta V^* = -3 \pm 1 \text{ cm}^3 \text{ mol}^{-1}$ is calculated by a least squares fit. However this error considers only the statistical deviations of the rotation rates obtained. It needs to be emphasized, that great care was taken not to introduce any artificial pressure dependence into the simulation parameters. Some test simulations were made to check the variation of k when using pressure dependent parameters. Within the range of

parameter values given by extrapolation of the low-exchange limit data these test simulations showed that an additional uncertainty of $\Delta(\Delta V^*) = \pm 1 \text{ cm}^3 \text{ mol}^{-1}$ cannot be avoided. This $\Delta(\Delta V^*)$ does not include the statistical error in calculating ΔV^* from $k(p)$.

A pessimistic estimate including possible hidden systematic errors would give $\Delta V^* = -3 \pm 2 \text{ cm}^3 \text{ mol}^{-1}$. Thus even under the most unfavorable conditions, the negative sign of ΔV^* is firmly established.

For the free rotation in 180° flips of aromatic rings in a peptide G. Wagner [8] derived a positive ΔV^* between $+30$ to $+40 \text{ cm}^3 \text{ mol}^{-1}$, and also for the amide rotation in N,N-dimethylamides, which does occur in 180° -jumps a large positive ΔV^* of $\sim 10 \text{ cm}^3 \text{ mol}^{-1}$ is found [9]. Both ΔV^* can be rationalized in terms of simple steric interactions between the rotating group and the surrounding solvent molecules. Among the few simple molecular rearrangements studied the chair \leftrightarrow chair intercon-

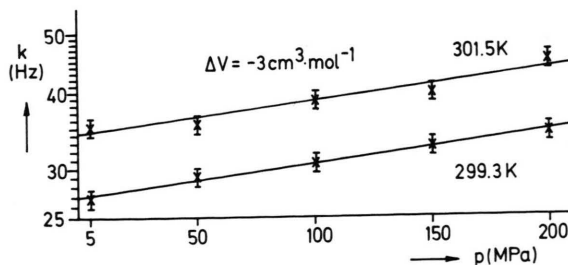


Fig. 2. Rotation rates of the pyridinium moiety around the N-C bond as function of pressure.

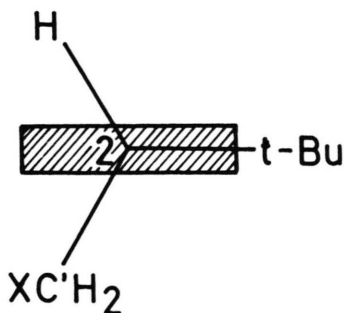


Fig. 3. Transition state for the rotation around N-C. (Shaded area: pyridinium ring X = *p*-CH₃O-phenyl).

version of cyclohexane does have a negative ΔV^* [10]. In this molecule the ring inversion has to pass through a whole series of intermediate transition states and the negative ΔV^* found is reasonably explained by the stronger crowding of the hydrogens in the boat- or twist-conformation leading to a smaller molar volume of these states compared to the chair. Increasing pressure must thus lower ΔG^* and facilitate the ring inversion. Inspection of space

filling models of **1** shows, that in the transition state given schematically in Fig. 3 the *tert*-butyl group and the pyridinium ring do overlap strongly, thus reducing the molar volume of this compound in the transition state. However, these models show also that this overlap leads at most to a reduction of the molar volume by $\sim 5 \text{ cm}^3 \text{ mol}^{-1}$. Compared to the $+30$ to $+40 \text{ cm}^3 \text{ mol}^{-1}$ found for the 180° -flips of the aromatic rings one should still expect a considerable positive ΔV^* , if the rotation would occur in free 180° -flips. It is thus save to conclude, that this rotation is not a free 180° -flip but involves a complex geared rotation around the N-C₁ and C₁-C₂-bond of the ring and the *tert*-butyl group with the rate determining step being only a small change of the torsion angles for both groups. In addition differences of the electrostrictive interaction between solvent and solute might reduce the overall activation volume [11].

Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

- [1] H. J. van Ramesdonk, J. W. Verhoeven, and T. J. de Boer, *Bioorg. Chem.* **6**, 403 (1977).
- [2] A. Ohno, T. Kimura, S. G. Kim, H. Yamamoto, S. Oka, and Y. Ohnishi, *Bioorg. Chem.* **6**, 21 (1977).
- [3] C. J. Bakker, A. J. de Gee, J. W. Verhoeven, and T. J. de Boer, *Recl. Trav. Chim. Pays-Bas* **94**, 61 (1975).
- [4] U. Gaarz and H.-D. Lüdemann, *Ber. Bunsenges. Phys. Chem.* **80**, 607 (1975).
- [5] G. Völkel, E. W. Lang, and H.-D. Lüdemann, *Ber. Bunsenges. Phys. Chem.* **83**, 722 (1979).
- [6] J. Hauer, G. Völkel, and H.-D. Lüdemann, *J. Chem. Res. (S)* **16**, (M) 426, (1980).
- [7] D. S. Stephenson and G. Binsch, *J. Magn. Res.* **32**, 145 (1978).
- [8] G. Wagner, *FEBS Letters* **112**, 280 (1980).
- [9] R. Rauchschwalbe, G. Völkel, E. W. Lang, and H.-D. Lüdemann, *J. Chem. Res. (S)* **448**, (M) 5325 (1978).
- [10] H.-D. Lüdemann, R. Rauchschwalbe, and E. W. Lang, *Angew. Chem. Int. Ed.* **16**, 331 (1977).
- [11] G. Montaudo, G. Kruk, and J. W. Verhoeven, *Tetrahedron Letters*, **1974**, 1841.