



# A theoretical study of intra-molecular vibrational effects on fractionation factors for molecules containing intra-molecular low-barrier hydrogen bonds

Travis S. Humble, Christopher J. Halkides, Jason D. Keltner, Michael Messina \*

*Department of Chemistry, University of North Carolina at Wilmington, Wilmington, NC 28405, USA*

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## Abstract

The fractionation factor is defined as the equilibrium constant for the reaction;  $R-H + DOH \rightleftharpoons R-D + HOH$ . Of interest are values of fractionation factors that obtain for reactions where reactants and/or products form intra-molecular low-barrier hydrogen bonds. Experimentally measured isotopic fractionation factors are usually interpreted via a one-dimensional potential energy surface along the intrinsic proton hydrogen bond coordinate. Here we show that coupling the intrinsic proton coordinate to an intra-molecular vibrational mode has large effects on the values of isotopic fractionation factors and offer a picture for the fractionation factor for low-barrier hydrogen bonds based on the one-dimensional potential of mean force on the intrinsic proton coordinate. © 1998 Elsevier Science B.V. All rights reserved.

## 1. Introduction

Recent experimental results of several workers has led to the conclusion that short, strong hydrogen bonds can stabilize transition states of enzyme-catalyzed biochemical reactions [1–4]. Evidence for such hydrogen bonding include low values of isotopic fractionation factors. For example, low values of fractionation factors were measured for enzyme groups involved in acid–base catalysis for pyruvate carboxylase [5] and biotin carboxylase [6]. These low values of fractionation factors has been attributed to the formation of low-barrier hydrogen bonds (LBHBs) [1,3,7]. These LBHBs occur when an H atom has the possibility to tunnel between a donor

and an acceptor site through a small energy barrier. The experimental results described above give strong evidence for inter-molecular LBHBs, i.e. a shared proton between donor and acceptor sites on different molecules. In this Letter we will focus on intra-molecular LBHBs, i.e. the proton is shared between donor and acceptor sites in the same molecule because these systems are easier to study and interpret both experimentally and theoretically.

Conclusive experimental evidence of intra-molecular LBHBs yielding low values of isotopic fractionation factors was obtained from measurements of fractionation factors of two similar molecules, maleate and acetic acid. A low value of the fractionation factor  $\varphi = 0.77$  was obtained for the maleate ion, while acetic acid had a value near unity [8,9]. The maleate ion has the ability to form an intra-molecular LBHB while acetic acid can not, and this

\* Corresponding author. Fax: +1-910-962-3013;  
e-mail: messinam@uncwil.edu

is thought to explain the low value of  $\varphi$  for the maleate ion. The first explanation for small values of  $\varphi$  being due to LBHBs was given by Kreevoy and Liang [7]. Kreevoy and Liang treated the important proton coordinate involved in the LBHB as a single-dimensional double-well potential. Kreevoy and Liang computed fractionation factors for several different double-well potentials and found that low fractionation factors arise when the barrier between the two minima in the double well is of low energy [7].

The simple one-dimensional picture of Kreevoy and Liang, although giving an aesthetically pleasing qualitative explanation for the low values of isotopic fractionation factors in LBHBs, cannot be completely correct. First, there must be an effect due to intra-molecular vibrational modes on the fractionation factor, and second there must be solvent effects on the H atom, neither of which is taken into account in the Kreevoy and Liang model. In this Letter we will focus on the effect on intra-molecular vibrational modes on the fractionation factor.

For ease of discussion consider the protonated 1,8-bis(dimethylamino)naphthalene molecule in Fig. 1. Here the H atom is involved in an intra-molecular LBHB between the two N moieties. Experimentally measured values of the isotopic fractionation factor for this molecule is only  $\sim 75\%$  for similar non-hydrogen bonded amines [10]. Also indicated in Fig. 1 is an intra-molecular vibration that can change the distance between the two N atoms. During the course of the vibration indicated, the distance between the donor and acceptor sites (the two N atoms) is modulated. If the distance between the two N atoms is very large, then there would be no intra-molecular H bond and the barrier in the double-well potential would approach infinity yielding two separated wells. If the distance between the two N atoms is small

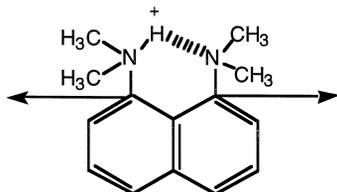


Fig. 1. The 1,8-bis(dimethylamino)naphthalene molecule with an indicated intra-molecular vibration that changes distance between the donor and acceptor sites of the proton.

then there is a possibility of intra-molecular H bonding and the potential energy surface becomes a double well. If the distance between the two N atoms is small enough then the barrier disappears completely and the H atom can freely move between the two N atoms, yielding a single-well potential energy surface. Since the topology of the potential energy surface greatly effects the values of the isotopic fractionation factors, the only rigorous way to quantitatively compute the isotopic fractionation factor for this LBHB coupled to an intra-molecular vibration is to compute the proper quantum statistical mechanical partition functions of the two-dimensional system. Results presented in the next section, yields direct evidence of this statement.

This Letter is organized as follows. In Section 2 we present the theory for computing fractionation factors for molecules containing a LBHB that is directly coupled to an intra-molecular vibrational coordinate. In Section 3 we present numerical results that show intra-molecular vibrational modes can have quite large effects on the value of fractionation factors for molecules containing LBHBs. Finally in Section 4 we discuss the results and conclude.

## 2. Theory

Consider the equilibrium shown below between a protonated and molecule that has an intra-molecular LBHB,



The fractionation factor is defined as the equilibrium constant for the reaction and can be written as the ratio of concentrations,

$$\varphi = \frac{[\text{MD}][\text{HOH}]}{[\text{MH}][\text{DOH}]}, \quad (2)$$

where [MD] and [MH] are the concentrations of the deuterated and protonated compound, respectively. According to equilibrium statistical mechanics the fractionation factor can also be written as the following ratio of partition functions for products and reactants as,

$$\varphi = \frac{q_{\text{MD}} q_{\text{HOH}}}{q_{\text{MH}} q_{\text{DOH}}}, \quad (3)$$

where  $q_j$  denotes the partition function of species  $j$ . Since the ratio of the HOH and DOH partition functions are the same, irrespective of the reaction in which they are participants, we will focus on the reduced fractionation factor defined below,

$$\varphi_R = \frac{q_{MD}}{q_{MH}}. \quad (4)$$

The reduced fractionation factor at temperature  $T$  can be computed by constructing the eigenvalue spectrum of MD and MH, and then composing the partition functions as the proper Boltzmann sums over these eigenenergies. Kreevoy and Liang computed the fractionation factor by considering a single-dimensional potential energy surface along the proton LBHB coordinate,  $\xi$  [7].

We want to explicitly include an intra-molecular vibrational coordinate that is directly coupled to the proton LBHB coordinate  $\xi$ . Physically, this coordinate can represent the symmetric stretch vibration of the two C atoms in a molecule such as 1,8-bis(dimethylamino)naphthalene indicated in Fig. 1. Vibration along this coordinate modulates the distance between the two N atoms during the course of oscillation. First we construct a model potential energy surface for the bare proton coordinate,  $\xi$ , that has the properties: (1) the barrier gets larger for large separation of the two double-well minima; and (2) the barrier gets smaller for small separation of the two double-well minima, i.e. behavior that was described in Section 1. This potential has the functional form

$$V_\xi(\xi) = \frac{V_L(\xi) + V_R(\xi)}{2} - \frac{1}{2} \sqrt{[V_L(\xi) - V_R(\xi)]^2 + 4g^2}, \quad (5a)$$

where

$$V_R(\xi) = \frac{\kappa}{2} (\xi - \xi_0)^2 \quad (5b)$$

and

$$V_L(\xi) = \frac{\kappa}{2} (\xi + \xi_0)^2. \quad (5c)$$

The parameter  $\xi_0$  can be thought of as the one half the separation between the donor and acceptor sites for the LBHB, or  $d = 2\xi_0$ , where  $d$  is the separation

between the donor and acceptor sites. The barrier height  $\Delta E$ , the well frequencies  $\omega$ , and the separation between the double-well minima,  $\Delta x$ , for this potential are given by the following,

$$\Delta E = \frac{\kappa}{2} \xi_0^2 - g + \frac{g^2}{2\kappa\xi_0^2}, \quad (6a)$$

$$\omega = \sqrt{\frac{\kappa}{m} - \frac{g^2}{\kappa m \xi_0^4}}, \quad (6b)$$

$$\Delta x = 2\sqrt{\xi_0^2 - \frac{g^2}{\kappa^2 \xi_0^2}}, \quad (6c)$$

where  $m$  is the mass of the proton (or deuteron). Thus a given barrier height and well frequency can be chosen by suitable choices of parameters  $\xi_0$ ,  $g$ , and  $\kappa$ . As can be seen from Eqs. 6 the barrier height  $\Delta E$  does indeed increase for large donor and acceptor site separation,  $d = 2\xi_0$ , and decrease for small well separation. We now couple the proton coordinate,  $\xi$  to an intra-molecular vibrational coordinate  $y$ . The intra-molecular vibration is modeled as a harmonic oscillator,

$$V_y(y) = \frac{M\omega_y^2}{2} (y - y_0)^2, \quad (7)$$

where  $M$ ,  $\omega_y$  and  $y_0$  are the reduced mass, frequency and equilibrium position associated with the intra-molecular vibration, respectively. We then couple the intra-molecular vibrational coordinate  $y$  to the well minima,  $\xi_0$  as follows

$$\xi_0 = a + by. \quad (8)$$

Eqs. 5, 7 and 8 define the two-dimensional potential,  $V(\xi, y)$  given by

$$V(\xi, y) = V_\xi(\xi, y) + V_y(y). \quad (9)$$

Note that the potential  $V_\xi$  is now a function of both  $\xi$  and  $y$  since the parameter  $\xi_0$  is explicitly dependent on coordinate  $y$  through Eq. (8). According to Eqs. (8) and (6a), large positive values of intra-molecular vibrational coordinate  $y$  increases  $d$ , the distance between the donor and acceptor sites, and the value of the barrier height, and when the value of  $y$  is large and negative the distance between the sites is decreased along with the barrier height. The parameter  $b$  in Eq. (8) is the coupling of the intra-molecular

vibrational coordinate and the proton coordinate, if  $b = 0$  there is no coupling between the two degrees of freedom.

We now must compute the partition functions for MD and MH to form the reduced fractionation factor given in Eq. (4). The proper prescription for the computation of these partition functions must include both coordinates  $\xi$  and  $y$ . It was shown in a previous work that this quantum mechanical partition function can be computed via [11]

$$q = \int d\xi \int dy e^{-\beta V(\xi, y)} W(\xi, y), \quad (10)$$

where  $V(\xi, y)$ , in this case given by Eq. (9), is the full two-dimensional potential energy surface,  $\beta$  is the inverse temperature ( $1/kT$ ), and  $W(\xi, y)$  is a quantum weight factor that accounts for zero-point energies, and the proton tunneling. It was also shown previously that Eq. (10) is very accurate and can be computed extremely efficiently [11]. This approach also allows for computation of quantum partition functions for system that contain many degrees of freedom such as the coupling of the proton LBHB coordinate to many intra-molecular vibrational modes and thus has advantage over conventional basis set calculations.

As shown by Kreevoy and Liang a single-dimensional picture for the fractionation factor can be extremely useful. Here we describe the correct single-dimensional picture for the fractionation factor when the intrinsic proton coordinate  $\xi$  is coupled to an intra-molecular vibration. We rewrite Eq. (10) as follows

$$q = \int d\xi F(\xi), \quad (11a)$$

where

$$F(\xi) = \int dy e^{-\beta V(\xi, y)} W(\xi, y). \quad (11b)$$

If we rewrite the single-dimensional function  $F(\xi)$  as

$$\ln[F(\xi)] = -\beta u(\xi), \quad (12)$$

the partition function as defined in Eq. (11a) can be considered as arising from the single-dimensional potential  $u(\xi)$ ,

$$q = \int d\xi e^{-\beta u(\xi)}. \quad (13)$$

The single-dimensional potential  $u(\xi)$  is then the potential of mean force on the intrinsic proton LBHB

coordinate,  $\xi$ , due to the intra-molecular vibrational mode,  $y$ . This potential is defined through Eqs. (11b) and (12). Eq. (13) shows that if there is to be a simple one-dimensional picture for the fractionation factor for systems containing LBHBs that are coupled to intra-molecular vibrational modes it should be the single-dimensional potential of mean force as defined through Eqs. (11b) and (12).

### 3. Numerical results

We now present results of the effect of the intra-molecular vibrational coordinate on the reduced fractionation factor. We first explore the properties of the potential energy for the bare proton LBHB coordinate defined in Eqs. 5. More specifically we want to plot the isotopic fractionation factor as a function of the distance between the donor and acceptor sites,  $d = 2\xi_0$ . To do this we pick value of  $g = 4.5 \times 10^4 \text{ cm}^{-1}$ , and  $\kappa = 467.5 \text{ J m}^{-2}$ . These parameters yield a barrier height,  $\Delta E = 3000 \text{ cm}^{-1}$ , well frequency,  $\omega = 2000 \text{ cm}^{-1}$ , when the distance between the two wells is equal to 100 pm (i.e., when  $\xi_0 = 50 \text{ pm}$ ). The parameter  $\xi_0$  is scanned from the value of 30 to 70 pm and for each value of  $\xi_0$  the reduced fractionation factor is computed via Eqs. (4) and (10). Fig. 2a shows the reduced fractionation factor plotted as a function of the distance between the donor and acceptor sites,  $d = 2\xi_0$ . Fig. 2b shows the single-dimensional potential energy surface for the proton LBHB coordinate for three values of this distance, the potential at well separation  $d = 100 \text{ pm}$  (solid line and solid circles), distance of  $d = 90 \text{ pm}$  (short dashed line and open squares), and a distance of 60 pm (long dashed line and open circles). The expected behavior of the  $\varphi_R$  with respect to the donor and acceptor distance is captured by the potential energy surface. At a separation of donor and acceptor  $d = 100 \text{ pm}$  there is a large barrier of  $\Delta E = 3000 \text{ cm}^{-1}$ , and a large difference in the zero-point energies between D and H yielding a large value of  $\varphi_R$ . As the separation between the donor and acceptor sites gets smaller the barrier goes down as does the difference between the zero-point energies between D and H yielding smaller values for  $\varphi_R$ . When the distance between the donor and acceptor sites becomes small enough, in this case  $\sim 90 \text{ pm}$ , the

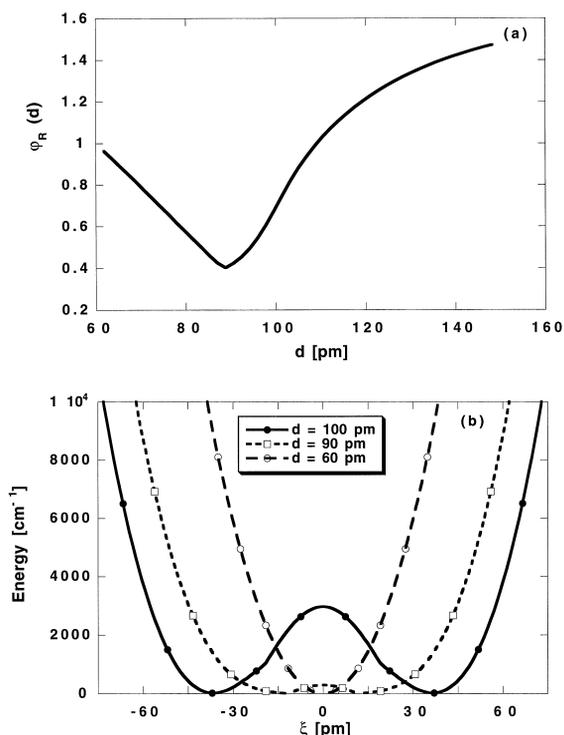


Fig. 2. (a) Plot of the reduced fractionation factor as a function of the distance between the donor and acceptor sites of the proton. The reduced fractionation factors have been normalized by the value at  $d = 60$  pm. (b) Plot of the intrinsic proton LBHB potential energy as a function of three distances between the donor and acceptor sites of the proton.

barrier disappears and the difference between the zero-point energies of D and H is at a minimum translating into the minimum value of  $\varphi_R$ . Finally as the donor–acceptor distance is further decreased the potential becomes a single-well with increasing curvature. Here the zero-point energy differences between D and H starts to increase again as does the values of the fractionation factor. These results show that the one-dimensional bare proton LBHB potential energy surface yields reasonable behavior for the  $\varphi_R$ s when the distance between the donor and acceptor sites,  $d = 2\xi_0$ , is treated as a parameter.

We now consider how the  $\varphi_R$ s behave when the distance between the donor and acceptor sites is modulated via an intra-molecular vibration. We define a ratio,  $R$ , that is the quotient of the reduced fractionation factor with the intra-molecular vibration turned on, the value of  $b$  in Eq. (8) is non-zero,

and with the intra-molecular vibration turned off,  $b = 0$  in Eq. (8),

$$R(b) = \frac{\varphi_R(b)}{\varphi_R(b=0)}. \quad (14)$$

We choose a system that has an intrinsic barrier height of  $3000 \text{ cm}^{-1}$ , a well frequency of  $2000 \text{ cm}^{-1}$ , and donor–acceptor site separation of 100 pm. The values of the parameters  $g$ ,  $\kappa$ , and  $\xi_0$  are as above. We now couple this proton coordinate to the intra-molecular vibration and compute the ratio  $R(b)$  defined in Eq. (14). The results are shown in Fig. 3 for various values of the intra-molecular vibrational coupling parameter  $b$ . The solid line is the ratio when the intra-molecular vibrational frequency is  $2000 \text{ cm}^{-1}$ . As shown in Fig. 3 the ratio  $R(b)$  is nearly unity for all values of  $b$ , the value of the

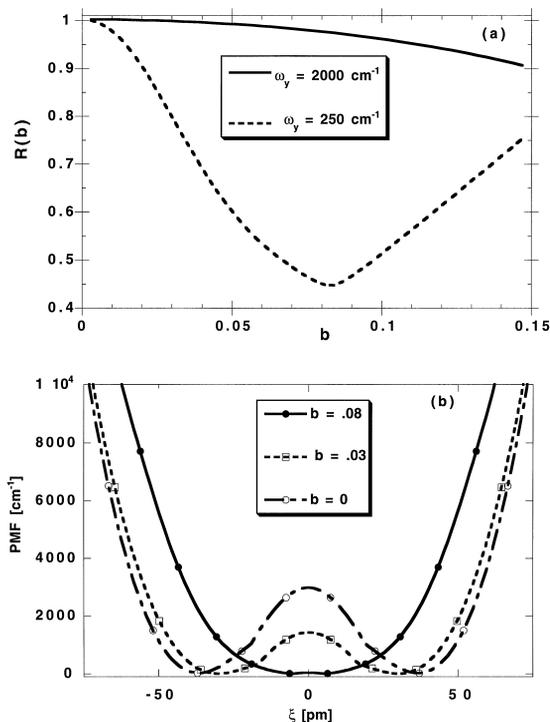


Fig. 3. (a) Plot of the ratio  $R(b)$  vs.  $b$ , the ratio of reduced fractionation factor with  $b = 0$  (no intra-molecular vibrational coupling) to the value of the reduced fractionation factor when  $b$  is non-zero, solid line is for an intra-molecular vibrational mode frequency of  $\omega_y = 2000 \text{ cm}^{-1}$  and dashed line is for  $\omega_y = 250 \text{ cm}^{-1}$ ; (b) the PIMFs along the LBHB proton coordinate for three different values of the intra-molecular vibrational coordinate,  $b$ .

intra-molecular vibrational coupling, presented. This means that this intra-molecular vibration has a negligible effect on the fractionation factor. The dashed line in Fig. 3 shows the ratio  $R(b)$  for an intra-molecular vibration that has a frequency of  $250\text{ cm}^{-1}$ . In this case the  $R(b)$  can go as low as  $R = 0.40$ , showing that this intra-molecular vibration can have a large effect on the value of the reduced isotopic fractionation factor. The dashed line also demonstrates that coupling the intrinsic proton LBHB coordinate to a low-frequency intra-molecular vibration decreases the value of the fractionation factor. These results make sense because the RMS amplitude of the intra-molecular motion scales as  $\omega^{-1}$ , thus for large intra-molecular vibrational frequencies the displacement between the donor and acceptor sites should be negligible leading to small effects on the reduced fractionation factors. On the other hand, there is appreciable motion between the donor and acceptor sites in the molecule for low-frequency intra-molecular vibrational motion leading to large effects on the reduced fractionation factors.

To further the argument that intra-molecular vibrational motion can have a large effect on values of isotopic fractionation factors we must demonstrate that molecules with intra-molecular LBHBs can have low-frequency intra-molecular vibrational modes that couple directly to the proton LBHB coordinate. The intra-molecular vibrational mode in 1,8-bis(dimethylamino)naphthalene indicated in Fig. 1 that is coupled directly to the proton LBHB coordinate was determined by to have a vibrational frequency of  $200\text{ cm}^{-1}$  from mid-level ab-initio electronic structure calculations. Thus this particular molecule contains both a LBHB and a low-frequency intra-molecular vibration that couples directly to the proton LBHB coordinate. We do not expect this molecule to be an isolated example.

To gain a simple one-dimensional picture as to why low-frequency intra-molecular vibrations lower values of the fractionation factor we look to the potential of mean force along the intrinsic proton LBHB coordinate defined through Eqs. (11b) and (12). Again, this PMF is defined as the full two-dimensional potential with the intra-molecular vibrational mode integrated out, thus yielding an effective single-dimensional potential for the proton LBHB coordinate. For the model described above we show

this PMF in Fig. 3b as a function of proton transfer coordinate  $\xi$ . The dotted-dashed line connecting open circles is the original potential with no intra-molecular coupling, the dashed line with open squares shows the PMF, when the intra-molecular vibrational coupling parameter  $b = 0.03$ , and the solid line with solid circles shows the PMF when  $b = 0.08$ . As shown in Fig. 3b low-frequency intra-molecular vibrations have the effect of making the barriers in LBHBs even lower, thus leading to further reduced values of isotopic fractionation factors.

Some intra-molecular motions such as the intra-molecular vibration considered here do not lead to potential energy surfaces that are symmetric about the coordinate, in this case  $y$ . For small  $y$  the energy of the system is lower since the barrier is lower and it is these configurations that are favorably weighted in the potential of mean force, leading, on average, to a smaller effective barrier in the PMF than in the intrinsic proton coordinate. Other motions such as torsional motions that bring planar donor and acceptor sites into non-planar configurations should be expected to be symmetric about the torsional angle. Non-planar configurations of donor and acceptor should lead to larger barriers than planar configurations, and hence coupling the intrinsic proton coordinate to these torsional librations should lead to PMFs that have larger barriers than the barrier between the intrinsic proton coordinate.

#### 4. Conclusions

In this Letter we have shown that a low-frequency intra-molecular vibrational mode that is coupled to a proton LBHB coordinate can have large effects on values of isotopic fractionation factors. We have shown that the intra-molecular vibrational mode makes the barrier along the intrinsic proton LBHB coordinate lower. We have also shown that the proper single-dimensional interpretation of fractionation factors involves the potential of mean force along the intrinsic proton LBHB coordinate, rather than the intrinsic proton LBHB coordinate itself. Interpretation of low values of fractionation factors as arising from molecules containing intrinsic LBHBs must be made very carefully since what is measured by the fractionation factor is not the energy barrier along

the proton LBHB coordinate, but rather the barrier along the PMF. We point out here that in the original work of Kreevoy and Liang the parameters of their single-dimensional potential function were determined to reproduce experimental quantities. In a sense this single-dimensional potential function was a potential of mean-force potential since it included, on the phenomenological level, effects such as intramolecular vibrations and solvent effects.

There are characteristic vibrational spectra of compounds that result from compounds that contain LBHBs coupled to low-frequency vibrational modes. These are Hadzi type II spectra [12] containing Evans window bands [13]. One can compute the vibrational spectra that arise from the two-dimensional potential defined in this paper using standard wave-packet techniques to determine if the computed vibrational spectra show the above-described spectroscopic signatures.

## References

- [1] W.W. Cleland, *Biochemistry* 31 (1992) 317.
- [2] J.A. Gerlt, P.G. Gassman, *Biochemistry* 32 (1993) 11943.
- [3] W.W. Cleland, M.M. Kreevoy, *Science* 264 (1994) 1887.
- [4] Q. Zhao, C. Haghjoo, P. Talalay, A.S. Mildvan, *Proc. Natl. Acad. Sci. USA* 93 (1996) 8220.
- [5] P.V. Atwood, P.A. Tipton, W.W. Cleland, *Biochemistry* 25 (1986) 8197.
- [6] P.A. Tipton, W.W. Cleland, *Biochemistry* 27 (1988) 4325.
- [7] M.M. Kreevoy, T.M. Liang, *J. Am. Chem. Soc.* 102 (1980) 3315.
- [8] R.M. Jarret, M. Saunders, *J. Am. Chem. Soc.* 107 (1985) 2648.
- [9] R.M. Jarret, M. Saunders, *J. Am. Chem. Soc.* 108 (1986) 7549.
- [10] Y. Chiang, A.J. Kresge, R.A. O'Ferrall, *J. Chem. Soc., Perkin Trans. 2* (1980) 1832.
- [11] M. Messina, G.K. Schenter, B.C. Garrett, *J. Chem. Phys.* 98 (1993) 4120.
- [12] D. Hadzi, *Pure Appl. Chem.* 11 (1965) 435.
- [13] J.C. Evans, *Spectrochim. Acta* 16 (1960) 352.