J. Org. Chem. 1993, 58, 6135–6138

Torsional Barriers in α-Keto Amides. Model Studies Related to the Binding Site of FK506

Robert D. Bach,* Iliana Mintcheva,
William J. Kronenberg, and H. Bernhard Schlegel

Department of Chemistry, Wayne State University, Detroit, Michigan 48202

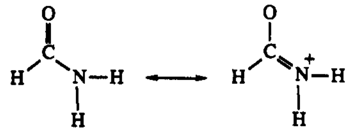
Received March 5, 1993 (Revised Manuscript Received July 29, 1993)

FK506 and rapamycin are immunosuppressive agents that have recently been shown to block T lymphocyte activation.[1a] Both agents have similar structures and appear to bind to the same receptor, FKBP, and the receptor has been shown to be a peptidyl-prolyl cis–trans isomerase. The mechanism for rotamase activity does not appear to involve the formation of a tetrahedral intermediate, but rather involves a twisted amide bond in the transition state.[1b] Since the two carbonyl groups in the α-keto amide functionality in both FK506 and rapamycin exist in an orthogonal orientation in the crystal structure it can potentially serve as a surrogate for a twisted amide and inhibit rotational activity.[2] Consequently, the torsional barriers of the α-keto amide functionality are of relevance to the binding process for these two novel structures.

Recent computational studies on FK506 have provided information about its conformational behavior and hydration process.[3] Considerable flexibility was observed near the amide functional group which is involved in the binding of FK506. *N,N*-Dimethyl-α-ketopropanamide was used as a model system to provide parameters for the torsional barrier between the two carbonyl carbons. Ab initio calculations at the HF/6-31G(d) level on geometries optimized with the HF/3-21G basis set predicted a nonplanar minimum with an O–C–C–O dihedral angle of 134.4° and a very low anti barrier of 0.65 kcal/mol. We now provide an assessment of the steric effects for a methyl group substituent in C–C and C–N torsional barriers in α-keto amides.

We initiated our ab initio molecular orbital studies[4] with an examination of the torsional barriers in the parent structure, α-ketoethanamide (1). α-Ketoethanamide (1) exists as a minimum in the anti conformation with an O–C–C–O dihedral of 180° (**1a**). The syn conformation (0° dihedral angle) exists as a local minimum **1b**, that is 7.2 kcal/mol higher in energy than anti conformer **1a** (Figure 1). The C–C rotational barrier is predicted to be 8.5 kcal/mol and TS-1 has an O–C–C–O dihedral angle of 66.2°. The adjacent NH$_2$ functionality has a modest impact upon this rotational barrier since the C–C torsional barrier in glyoxal (HCOCOH) is 7.5 kcal/mol[6a] with respect to the trans minimum with the DZ basis set and 6.8 kcal/mol at the DZ+P level.[6b]

The C–N torsional barriers in 1 are 19.3 kcal/mol (**TS-2**) from the lower energy anti-conformer **1a** and 13.4 kcal/mol (**TS-3**) from the syn-rotamer **1b**. The OCCO dihedral angles in **TS-2** and **TS-3** are 180.0° and 0.2°, respectively. The magnitude of the C–N rotational barrier in 1 is slightly higher than that in formamide at the HF/6-31G* level (15.7 kcal/mol).[7] The experimental barrier for formamide is 18–19 kcal/mol suggesting that these barriers are calculated satisfactorily at this level of theory. However, better agreement with experiment is observed when polarization functions are also included on the hydrogens (6-31G**)[7b]. Formamide is an interesting case since it has a fairly large barrier to rotation about the C–N bond which has been ascribed historically to double bond character introduced by its zwitterionic resonance structure. However, Wiberg and his co-workers[7] have shown that the oxygen atom does not participate in the C–N bond rotation and its primary function is to provide an adjacent polarized carbonyl group with an electron-deficient carbon atom. The barrier to rotation in formamide is due largely to the amino group being destabilized near the transition state.



It has been established that metal ion binding to the carbonyl oxygen of an amide increases the double bond character of the C–N bond resulting in a higher barrier to rotation.[8] For example, complexation of the C=O oxygen of dimethylformamide with Zn^{2+}, Li^{+}, and Be^{2+} increases its C–N torsional barrier by 4.5, 5.5, and 11.3 kcal/mol, respectively. Complexation of the α-keto amide functionality at an active site should also have an influence upon its torsional barriers. An extreme example of the role of complexation could be approximated by a fully protonated α-keto amide. The global minimum for the simplest protonated α-keto amide has the syn relationship of the carbonyl oxygens **2a**. This isomer is 2.2 kcal/mol lower in energy than the protonated anti-conformer **2b** (Figure 2). The proton affinity of syn conformation **1b** (201.4 kcal/mol) is also 9.9 kcal/mol higher than that of the anti conformation **1a**. The C–C rotational barrier (**TS-4**) is 7.8 kcal/mol. Since this is essentially the same barrier predicted for C–C rotation in **1a**, this suggests that the hydrogen bonding in **2a** is approximately offset by the energy difference between anti- and syn-conformers **1a** and **1b**. As anticipated the C–N rotational barrier for **2a**

---

(1) (a) Schreiber, S. L. *Science* **1991**, *251*, 283 and refs therein. (b) Rosen, M. K.; Standaert, R. F.; Galat, A.; Nakatsuka, M.; Schreiber, S. L. *Science* **1990**, *248*, 863.

(2) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031.

(3) Pranata, J.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 9483.

(4) All structures were fully optimized using an HF/6-31G* basis set unless otherwise specified. Calculations were executed with the Gaussian 92 program system[5a] utilizing gradient optimization[5b] without symmetry constraints. All minima and transition structures were confirmed to have either zero or one imaginary frequency, respectively. All energy differences include zero point energy, calculated at the HF/6-31G* level by an analytical frequency calculation.

(5) (a) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A., GAUSSIAN 92, Gaussian, Inc., Pittsburgh, PA, 1992. (b) Schlegel, H. B. *J. Comput. Chem.* **1982**, *3*, 214.

(6) (a) Dykstra, C. E.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **1975**, *97*, 7210. (b) Osamura, Y.; Schaefer, H. F., III. *J. Chem. Phys.* **1981**, *74*, 4576.

(7) (a) Wiberg, K. B.; Breneman, C. M. *J. Am. Chem. Soc.* **1992**, *114*, 831. (b) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935.

(8) (a) Rode, B. M.; Fussenegger, R. *J. Chem. Soc. Faraday Trans. 2* **1975**, *71*, 1958. (b) Rode, B. M.; Fussenegger, R. *Chem. Phys. Lett.* **1976**, *44*, 95.

0022-3263/93/1958-6135$04.00/0

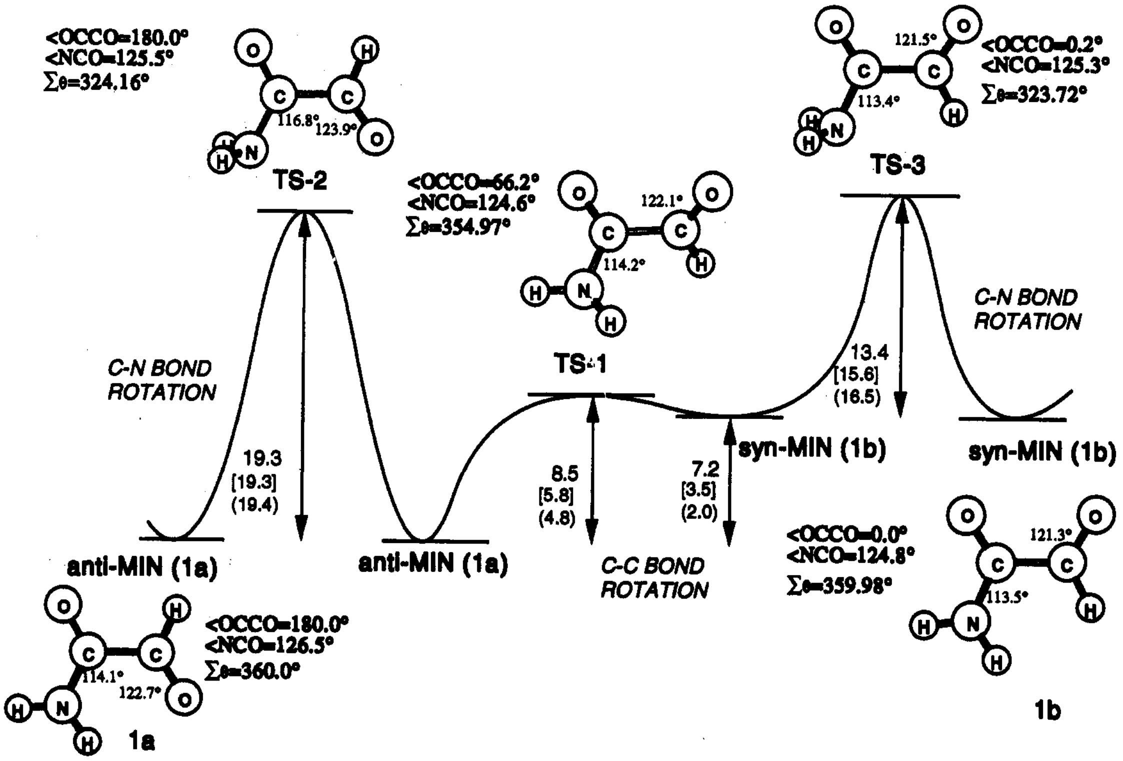


Figure 1. Torsional barriers in kcal/mol for C–C and C–N bond rotation in α-ketoethanamide at the HF/6-31G* level. The values in square brackets represent barriers in $CHCl_3$ and those in parentheses barriers in CH_3CN.

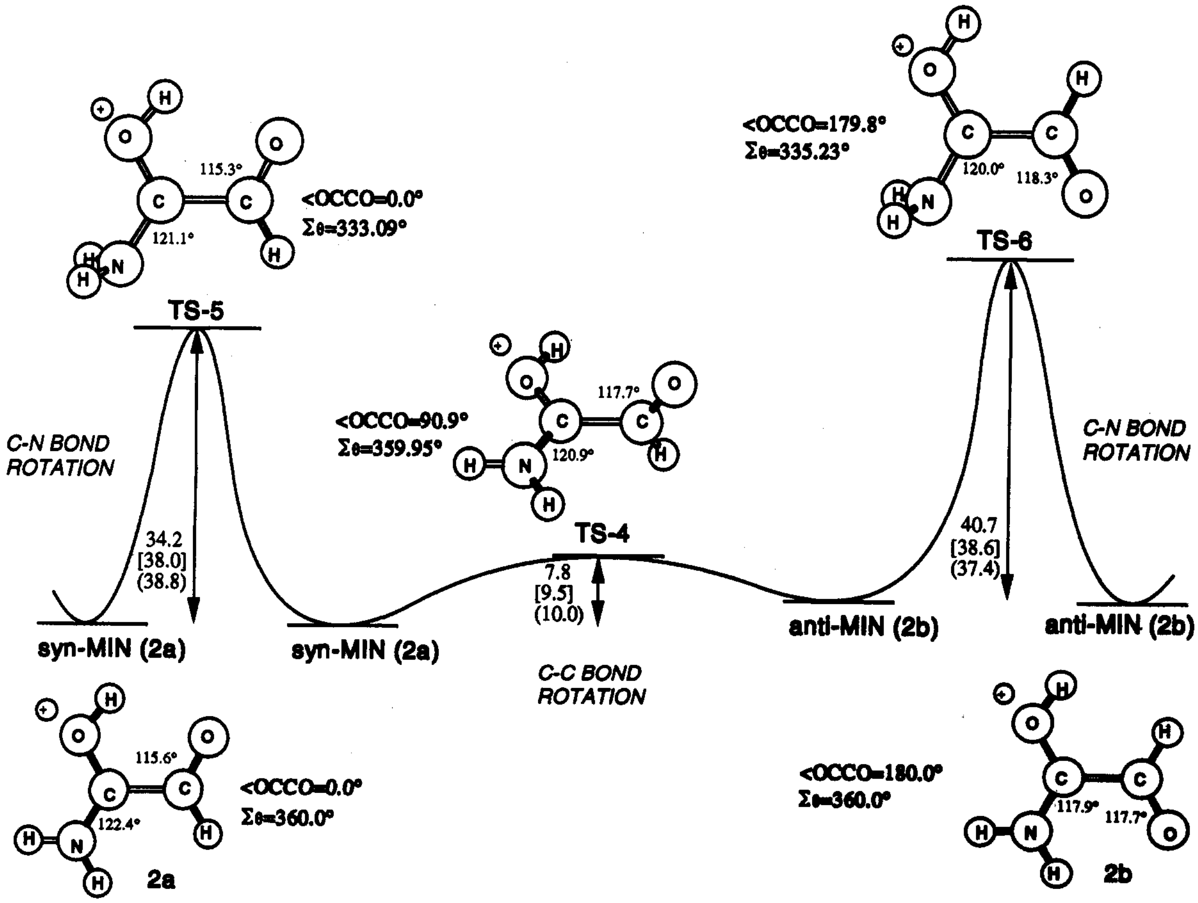


Figure 2. Torsional barriers in kcal/mol for C–C and C–N bond rotation in protonated α-ketoethanamide at the HF/6-31G* level. The values in square brackets represent barriers in $CHCl_3$ and those in parentheses barriers in CH_3CN.

has been significantly increased ($\Delta E^{\ddagger}$ = 34.2 kcal/mol). It is of interest to note that 1, when it is held in a syn conformation 1b, should have a lower C–N rotational barrier due to its higher ground state energy (Figure 1).

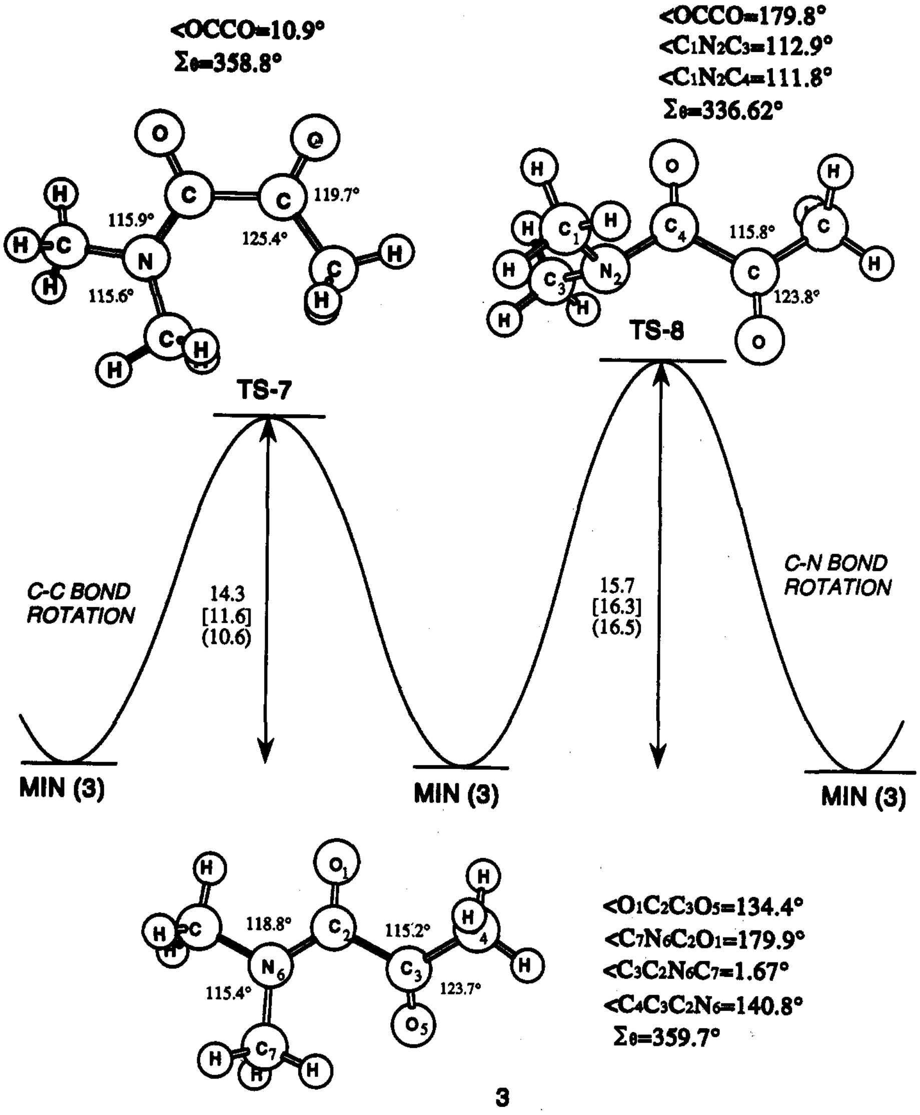


Figure 3. Torsional barriers in kcal/mol for C–C and C–N bond rotation in *N,N*-dimethyl-α-ketopropanamide at the HF/6-31G* level. The values in square brackets represent barriers in $CHCl_3$ and those in parentheses barriers in CH_3CN.

However, complexation of 1 at the active site could substantially increase the C–N rotatinal barrier by increasing the polarization of the carbonyl group and hence C–N double bond character.

The fully substituted α-keto amide 3 exhibits a single minima with an OCCO dihedral angle of 134.4° (Figure 3) reflecting the steric interactions imposed by the methyl substituents on carbon and nitrogen. These data are in excellent accord with ab initio calculations at the HF/6-31G*//HF/3-21G level.[3] The OCCO dihedral angle remains unchanged (134.3°) with full geometry optimization at the HF/6-31G** level. The C–C rotational barrier in 3 (**TS-7**) has increased significantly ($\Delta E^{\ddagger}$ = 14.3 kcal/mol) relative to that predicted (8.5 kcal/mol) for the unsubstituted α-keto amide **1a**. The OCCO dihedral angle in the transition state for this syn rotational barrier is 10.9°. The anti barrier (∠OCCO = 180°) is only 1.54 kcal/mol and this part of the C–C rotational surface (not shown) is fairly shallow since the 90° conformer is only 2.23 kcal/mol higher in energy than global minimum 3. Only when the methyl group begin to interact sterically does the energy rise rapidly. The methyl–methyl interactions in 3 would obviously exclude a significant population of the syn-conformer (∠OCCO = 0°) where the carbonyls are eclipsed since this rotamer would have an energy comparable to that of **TS-7**. The C–N rotational barrier actually exhibits a modest decrease ($\Delta E^{\ddagger}$ = 15.7) relative to unsubstituted α-keto amide 1.

At the TS for C–N bond rotation in an amide the nitrogen prefers to place its lone pair electrons in an orbital with high s-character which requires a pyramidal structure. One measure of the deviation of the nitrogen atom from planarity in the TS is to measure the sum of its three bond angles. The planar structure has Σ_θ = 360.0° while the sum of the three bond angles in a tetrahedral structure has Σ_θ = 328.5°. All of the transition structures for C–N bond rotation have nonplanar nitrogen atoms with Σ_θ varying from 323.7° to 336.6°. As anticipated the nitrogen atoms in 1 and 2 are planar and in 3 essentially planar Σ_θ = 359.7° (Figure 3). The nitrogen atom in the transition state for C–C bond rotation in protonated α-ketoethanamide (**TS-4**) is also planar (Figure 2) while **TS-1** and **TS-7** exhibit a small degree of pyramidality (Σ_θ = 354.97 and 358.8°).

Since we are attempting to model the rotational surface of FK506, a qualitative discussion of the effects of solvation should be included. The most simple of these models is the Onsager treatment[9a,b] used in self-consistent reaction

Table I. Total Energies (au) and C–C and C–N Rotational Barriers (kcal/mol)

compound	HF/3–21 G	HF/6–31 G*	$\Delta E^\ddagger$ HF/6–31 G* in vacuo	$\Delta E,^\ddagger$ HF/6–31 G* in CHCl3[a]	$\Delta E,^\ddagger$ HF/6–31 G* in CH3CN[b]	dipole moment (D)	ZPE HF/6–31 G* (kcal/mol)
anti-MINIMUM **1a**	−280.07531	−281.65769				1.92	37.82
TS-1		−281.64342	8.47 (anti)	5.84 (anti)	4.84 (anti)	4.77	37.33
TS-2	−280.03846	−281.62588	19.29 (anti)	19.34 (anti)	19.35 (anti)	1.77	37.15
TS-3	−280.03702	−281.62373	13.36 (syn)	15.59 (syn)	16.45 (syn)	3.86	37.09
syn-MINIMUM **1b**	−280.06176	−281.64544				5.77	37.36
syn-MINIMUM **2a**		−281.98087				4.61	46.93
TS-4	−281.92363	−281.96745	7.77 (syn)[c]	9.48 (syn)	9.97 (syn)	3.68	46.29
TS-5	−280.33688	−281.92363	34.22 (syn)	38.00 (syn)	38.82 (syn)	2.95	45.24
TS-6	−280.32527	−281.90899	40.73 (anti)	38.58 (anti)	37.38 (anti)	5.10	44.74
anti-MINIMUM **2b**	−280.40002	−281.97684				2.83	46.59
MIN **3**	−396.52443	−398.75501				2.77	94.58
MIN (<OCCO = 90°)	−369.51782	−398.75145	2.23[d]			4.65	
MIN (<OCCO = 180°)	−396.53027	−398.75256	1.54[e]			1.63	
TS-7	−396.50398	−398.73252	14.25	11.62	10.58	6.31	94.71
TS-8	−396.50037	−398.72911	15.74	16.25	16.45	1.17	94.07

[a] Dielectric constant = 4.8. [b] Dielectric constant = 37.5. [c] The values for the protonated compound include the Born charge term. [d] Energy difference (without ZPE) between **3** at its minimum and the dihedral constrained at 90°. [e] Energy difference (without ZPE) between **3** at its minimum and the dihedral constrained at 180°.

field (SCRF) calculations.[9c] Since the Onsager model is purely electrostatic and is governed largely by the dipole moment of the solute, the microscopic structure of the solvent and the role of hydrogen bonding in protic solvent is not taken into account. Hence the absolute magnitudes of $\Delta E^\ddagger$ predicted in condensed phase must be viewed with caution. In order to predict the trends in the rotational barriers with an increase in solvent polarity, we have employed chloroform ($\epsilon = 4.81$) and acetonitrile ($\epsilon = 37.5$) as our solvents.[10] As noted in the figures, with **1a** and **3** the magnitude of the C–N rotational barriers all increase slightly with an increase in solvent polarity while the opposite trend is noted for the C–C rotation barriers. The C–C rotational barrier for protonated α-ketoethanamide **2a** slightly increases in the more polar solvent media. Consistent with these observations the dipole moments of the solute decrease upon C–N bond rotation for the uncharged α-keto amides while the dipole moment increases upon going to the TS for C–C bond rotation. The opposite situation exists for the protonated species.

In summary, the steric interactions between the methyl substituents on *N,N*-dimethyl-α-ketopropanamide (**3**) are largely responsible for its deviation from planarity and for the absence of a syn-conformer as a local minimum. The magnitude of the barrier for C–C bond rotation in **3** and the predicted torsional profile are quite consistent with the observed orthogonal orientation in the crystal structure of FK506. The magnitude of the C–N rotational barrier for model compound **3** suggests that the cis and trans conformations for the amide bond in FK506 can readily interchange at ambient temperature.

Acknowledgment. This work was supported in part by the National Science Foundation (CHE 90-20398) and a NATO Collaborative Research Grant (900707). We are also thankful to the Pittsburgh Supercomputing Center, CRAY Research, and the Ford Motor Co. for generous amounts of computer time.

---

(9) (a) Onsager, L. *J. Am. Chem. Soc.* **1936**, *58*, 1486. (b) Wong, M. W.; Frisch, M. J.; Wiberg, K. B. *J. Am. Chem. Soc.* **1991**, *113*, 4776. The volume of the spherical cavity is obtained by integrating the electron density.

(10) An increase in dielectric constant from 37.5 to 78.5 had virtually no effect upon the rotational barriers.