Multinuclear NMR Study of Coupled Bond Motions Involving Amide Groups Attached to Iodinated Aromatic Rings

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Abstract: The amide groups of 2,4,6-triiodo-3,5-diacetamidobenzoates are displaced above or below the plane of the aromatic ring by the adjacent, bulky iodine atoms. The NMR spectra of representative compounds at 303 K in dimethyl sulfoxide solution were decomposed into signals from six different molecular conformations. The amide groups in the most stable conformations are *trans* and are either on the same face of the ring (*syn*) or on opposite faces (*anti*). In dimethyl sulfoxide there is essentially no interaction across the ring between the amide groups. The distance is too great for steric interactions, and the solvent screens the carbonyl oxygens from electrostatic interactions with each other. Two-dimensional exchange NMR at 313 K showed that the fastest component of conformational interchange is a jump of a *trans* amide from one face of the aromatic ring to the other. Detailed analysis showed that the jump, which at the end amounts to rotation about the aniline bond alone, must proceed with internal rotation about both the aniline and amide bonds and possibly distortions of bond angles. For a *cis* amide such extensive rotation about the amide bond takes place in conjunction with rotation about the aniline bond that the *cis* amide almost invariably is converted into a *trans* amide as it transfers from one ring face to the other.

Most organic molecules are flexible, continually changing conformation through internal rotations about one or more single bonds. When several conformations are stable, that is, exist at the bottom of energy wells, the energy barriers between the wells are often less than 10 kcal/mol and rarely exceed 25 kcal/mol. The energy differences between the well bottoms are even less, often lower than 2 kcal/mol.[1−3] Energy differences of 2 kcal/mol, though small by ordinary chemical standards, are large enough to ensure population ratios between stable conformations of more than 25:1 at room temperature. When added to the overall energy of a molecule or transition state, conformational energies can have a profound effect on the courses of chemical reactions.[1,4]

Conformational changes rarely involve rotations about one bond alone. The well-known ring inversion of cyclohexane and its derivatives obviously requires coupled rotations around several bonds. Similarly, docking of substrate molecules with enzymes can require conformational changes involving several different amino acid residues, and thus several single bonds. The long-range cooperative motions believed to occur in synthetic polymers in the amorphous phase and in solution must also involve coupled motions around multiple bonds.[5−9]

NMR spectroscopists have studied restricted rotation in amides for almost forty years,[2,10] and several studies have specifically focused on compounds in which rotations around two different bonds may be coupled.[11,12] Other work has concerned molecules in which there might be "geared" motion of different alkyl groups.[13] With the most recent NMR techniques[14] we have been able to expand on these studies and obtain detailed information about the coupling of rotation around the amide and aniline bonds in the sterically crowded molecules **I**−**III**.

Compounds **II** and **III** typify materials exhibiting conformational polymorphism.[15] They form several types of crystal structures, each containing molecules in a different molecular conformation. The information we have obtained about the structure of these materials in solution provides important background data relevant to the molecular structure in the solid state. Studies of the crystalline solids themselves with X-ray diffraction, NMR spectroscopy, and Raman spectroscopy will be described in separate reports.

Experimental Section

Compounds **I** and **II** were prepared by reacting the sodium carboxylate of the corresponding acid with ethyl 2-bromoacetate or 4-methoxybenzyl chloride in dimethylformamide (DMF). Compound **III** was synthesized by sequential alkylation of sodium diatrizoate with 2-methoxyethoxymethyl chloride (1 equiv) and 1-bromohexane (2 equiv) in DMF, separation of two forms using silica gel chromatography, and hydrolysis of each isolated structure with dilute hydrochloric acid. All compounds had ^{1}H NMR spectra, mass spectra, and combustion analyses consistent with the assigned structures and were homogenous by thin layer chromatography (TLC).

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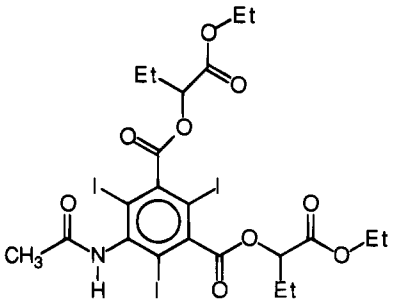

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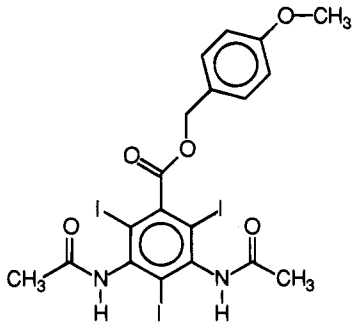
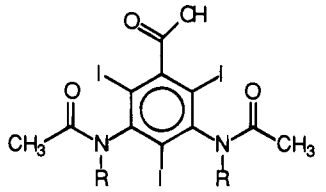


II



III, R = n-hexyl

The samples for ^{13}C and 2D NMR analysis contained 200−300 mg of compound in 0.7 mL of deuterated dimethyl sulfoxide. Samples for ^{1}H NMR alone sometimes contained as little as 5 mg in dimethyl sulfoxide. No concentration effects on the conformational equilibria were detected, but the addition of deuterated chloroform to a dimethyl sulfoxide solution did shift the resonances and change the intensities as described in the results section. The NMR spectra were acquired as noted on Bruker AMX-500, AMX-360, and ARX-300 spectrometers and were referenced to the proton and carbon frequencies of tetramethylsilane indirectly through the frequency of the deuterium lock signal.

Typical proton free-induction-decay signals, generated with 90° pulses and relaxation delays between excitation pulses of 1.5−3.0 s, were digitized into 32K data points and were transformed without exponential broadening into spectra with the same number of points. The typical carbon time-domain signal, generated with 30° pulses and relaxation delays of 0.5 to 1.5 s, was digitized into 32K data points. Exponential filtration equivalent to 1 Hz of line broadening was applied prior to Fourier transformation.

Proton/carbon correlation through long-range coupling was detected with a heteronuclear, multiple-bond correlation (HMBC)[16] experiment with 2K data points in each dimension. Prior to transformation, the signals in the t_2 and t_1 domains were multiplied by sine squared or Gaussian functions to reduce peak overlap.

The spectral width, spectrometer offset, and window functions for the two-dimensional chemical-exchange spectra were carefully chosen to maximize the digital resolution and to minimize peak overlap. The final spectral widths in each dimension were 350 Hz, digitized in 1K points. Vertical slices were used for quantitative measurements so that t_1 noise from only a single diagonal signal affected each slice.

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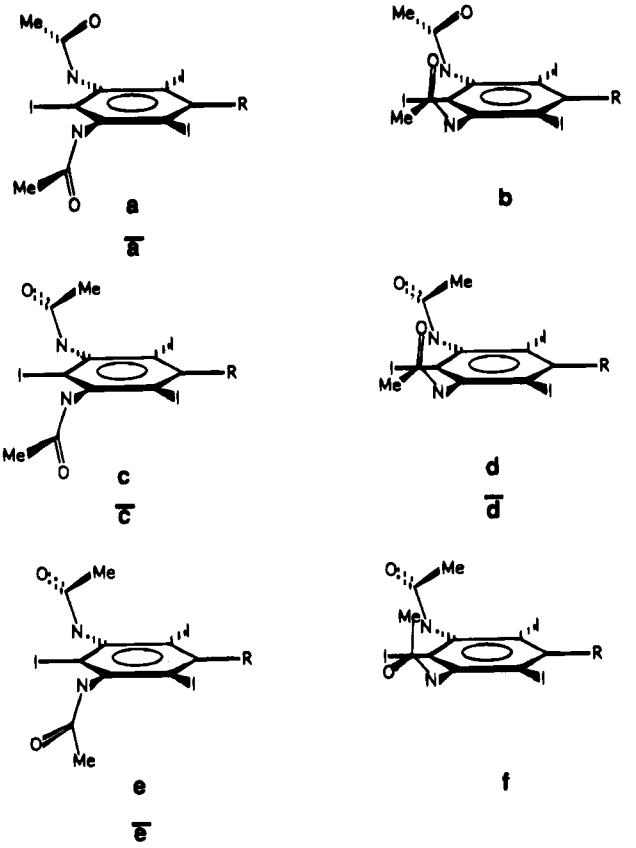

Figure 1. Conformations of II involving the amide groups. The ester group (R) changes conformations rapidly enough that it, at room temperature and above, effectively has cylindrical symmetry. Amide groups on opposite faces of the ring are *anti* to one another; those on the same face are *syn*. In expanded notation, **a** is *anti, trans, trans*, **b** is *syn, trans, trans*, **c** is *anti, trans, cis*, **d** is *syn, trans, cis*, **e** is *anti, cis, cis*, and **f** is *syn, cis, cis*. All conformations but **b** and **f** have nonsuperimposable mirror-images, indicated with a bar over the appropriate letter, for example, **ā**. There are a total of ten different conformations when the mirror-image forms are included, but when the compound is dissolved in an achiral solvent, only six nonequivalent contributions to the NMR spectrum can be distinguished.

Results

Owing to the partial double-bond character of the carbonyl−nitrogen bond, most amides are planar. For secondary amides there are two stable conformations, *cis* and *trans*, typically separated by a free-energy barrier of 15−30 kcal/mol.[2,3]

cis *trans*

In the lowest-energy conformations of **I** to **III**, the bulky halogens force the plane of the *cis* or *trans* amides almost perpendicular to the ring plane.[17−22] Rotations about both the aniline bond, which attaches the amide group to the ring, and the amide bond, which connects the carbonyl carbon to the nitrogen, are restricted and can be observed with NMR spectroscopy. Figure 1 suggests ten stable conformations for

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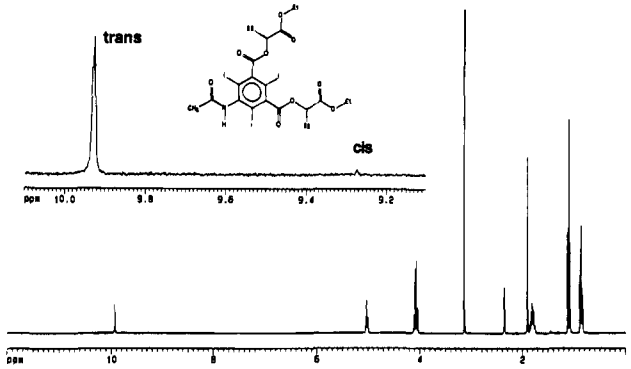

Figure 2. The ^{1}H NMR spectrum of I in DMSO-d_6, recorded at 300 MHz and a temperature of 303 K. The signals from the amide protons, which fall between 9 and 10 ppm, are shown expanded in the inset.

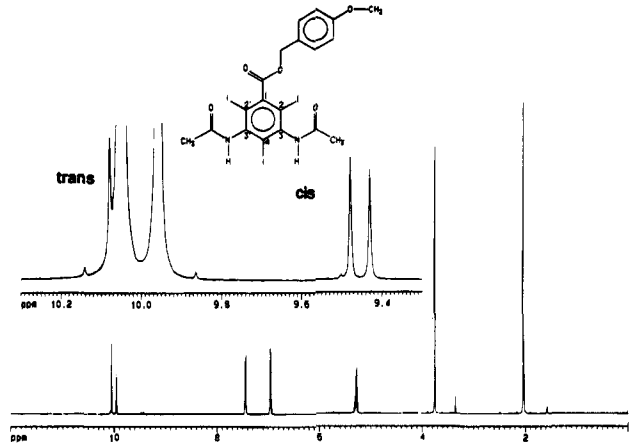

Figure 3. The ^{1}H NMR spectrum of II in DMSO-d_6, recorded at 500 MHz and a temperature of 303 K. The signals of the amide protons, which fall between 9 and 10 ppm, are shown expanded in the inset. Complete assignments for these peaks are indicated on Figure 8.

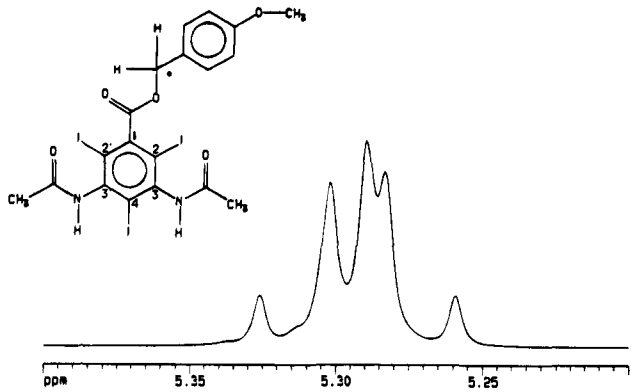

Figure 4. Expanded section containing the signals from the methylene protons of II from the 500 MHz ^{1}H NMR spectrum in Figure 3. The most abundant conformation gives a symmetrical AB quartet with two large peaks at 5.283 and 5.305 ppm and smaller peaks at 5.260 and 5.327 ppm. The other major conformation gives rise to the large singlet at 5.288 ppm. The minor conformations give signals barely visible, around 5.318 and 5.338 ppm.

II and **III**. We label the conformations either by a letter or by indication of both the local conformations of the amide groups and whether those amides are *syn*, that is, on the same face of the ring, or *anti*, on opposite faces of the ring.

Initially we recorded spectra of **II** in both the slow-exchange regime, where the observed spectrum is a superposition of the weighted spectra of each conformation, and in the fast-exchange regime, in which the observed spectrum is a weighted average of the spectra of the individual conformations. Analysis of the spectrum of **I**, which has a single amide group, provided the necessary background information for interpretation of both types of spectra of **II**.

Proton Spectrum of I. The resonance frequency of the amide proton in **I** reflects simply whether the amide is *cis* or *trans*, not how that amide is oriented relative to the ring. Thus the spectrum of **I** serves as a useful reference point from which to begin the analysis of the other compounds. In the spectral region for amide protons, between 10.5 and 9.0 ppm, there is a large signal at 9.92 ppm for **I** and a very small one at 9.27 ppm (Figure 2). Since the *trans* conformation in other acetanilides with large ortho substituents is energetically favorable to the *cis*,[2,18–20,23,24] we assigned the large peak of **I** also to the *trans* amide and the small peak to the *cis* amide.

Proton Spectra of II. As one might expect from the fact that **II** has many more stable conformations than **I**, the ^{1}H NMR spectrum is considerably more complex (Figure 3). There are at least six amide proton signals, suggesting that **II** is distributed in dimethyl sulfoxide over six or more conformations. The intensities of the NMR signals indicate that two of these, those giving the amide signals at 10.04 and 9.95 ppm, are highly favored over the others. Because the largest signals from the amide protons of **II** are at similar frequencies to those of the signals from the *trans* amide of **I** and because there are no major peaks for **II** near 9.3 ppm, where a *cis* amide would absorb, we concluded that the most favorable conformations of **II** contain only *trans* amides. These are **a** (*anti, trans, trans*) and **b** (*syn, trans, trans*).

The two smallest peaks in the expanded spectrum in Figure 3, at 10.14 and 9.86 ppm, are ^{15}N satellites of the most intense signals and can be ignored. However, the small peak at 10.08 ppm is from a *trans* amide in a minor conformation. The two signals at 9.44 and 9.48 ppm are from *cis* amide groups in minor conformations. All three small signals have similar intensities, and we concluded that the *cis* and *trans* amides giving the signals were actually part of the same overall conformations, either **c** (*anti, trans, cis*) or **d** (*syn, trans, cis*). One *trans* signal for these conformations is missing in the NMR spectrum as a consequence of the spectral crowding around the resonances coming from conformations **a** or **b**.

Because *cis* amide groups appear to be highly unfavorable in compounds **I** and **II**, we expected to see only very small single peaks for conformations **e** and **f**, in which both amide groups are *cis*. One of these appears just to the left of the signals for the *cis* amides of **c** and **d**, at 9.49 ppm. The other is probably buried.

Up to this point our analysis did not address whether **a** (along with its energetically equivalent mirror image $\bar{\mathbf{a}}$) or **b** predominates in dimethyl sulfoxide solution. The methylene signals of the benzyl ester, which are shown expanded in Figure 4, allowed discrimination between the alternative possibilities. The major group of peaks is a quartet with peaks at 5.259, 5.283, 5.303, and 5.327 ppm, a pattern characteristic of two methylene protons in nonequivalent environments. The asymmetry of the methylene group in the most abundant conformation requires that the methylene be attached to a group with no local plane of symmetry. The major conformation of **II** is, therefore, the **a**/$\bar{\mathbf{a}}$ pair.

We assigned the singlet at 5.290 ppm to the methylene group of the chiral conformation **b**, the conformation for **II** containing two *trans* amides with a plane of symmetry. The intensities of both the amide and methylene signals showed that the asym-

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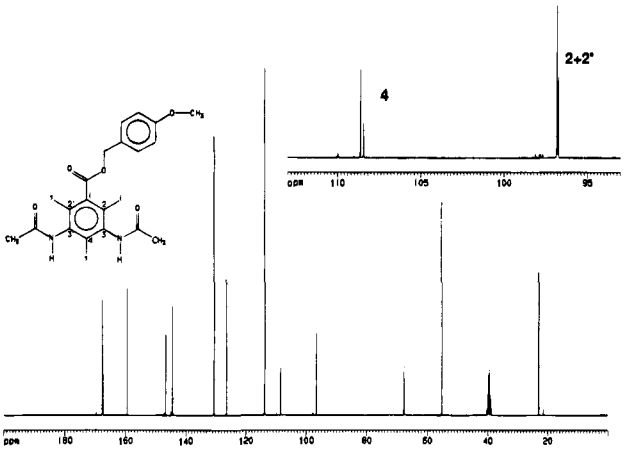

Figure 5. The ^{13}C NMR spectrum of II recorded at 303 K and a frequency of 90.6 MHz. The signals from the iodinated carbons, 2, 2′, and 4, are shown expanded in the inset.

metrical pair of conformations **a** and **ā** is twice as abundant as the chiral conformation **b**.

The addition of deuterated chloroform to the deuterated dimethyl sulfoxide solution of **II** increased the signals of conformation **b** relative to those of conformations **a** and **ā**. On the other hand, the change in solvent left the signals of conformation **c** almost equal in intensity to those of conformation **d**.

Carbon Spectra of II. The carbon spectrum of **II** confirmed the identity of the major conformations. The ^{13}C chemical shifts of the iodinated carbons were particularly diagnostic.

We expected one resonance from each of the conformations of **II** that could be assigned to the iodinated carbon C4. We also predicted there would be only one signal for C2 and C2′ of **a**, which are equivalent because each is adjacent to a *trans* amide. Likewise, C2 and C2′ of **b** were anticipated to contribute to the same resonance. However, we predicted that C2 and C2′ of **c**, and of **d**, might give carbon signals with different chemical shifts, as the two carbons in each conformation are adjacent to amides in different conformations. There would be one C2 signal each for **e** and **f**.

The spectrum in Figure 5 verifies the predictions for the carbon spectrum. At 108.8 and 108.6 ppm are two peaks, the larger of which we assigned to C4 of **a** and the smaller to the C4 of **b**. Similarly there are two peaks with a 2:1 intensity ratio for C2 and C2′ at 106.8 ppm. All of the predicted carbon resonances for conformations **c** and **d** also appear in the spectrum: one each for C4 at 110.0 ppm and two each for C2 and C2′ between 97 and 99 ppm, all of similar intensity. The resonances of conformations **e** and **f**, which are highly disfavored energetically, were not discerned.

Proton−Carbon Correlation Spectroscopy of II. Knowing that the coupling constants for interactions between carbon and protons through two and three bonds typically are between 3 and 6 Hz and fall off rapidly for couplings through additional bonds,[25,26] we predicted that an experiment optimized for couplings of about 6 Hz would detect correlations between each amide proton and C4 plus either C2 and C2′, but not both C2 and C2′. Contrary to expectation, the section of the two-dimensional spectrum in Figure 6 shows correlation peaks for each of the amide protons of **c** and **d** and both C2 and C2′. The relevant signals are especially obvious at the proton frequencies for the *cis* amide protons, between 9.4 and 9.5 ppm. The train

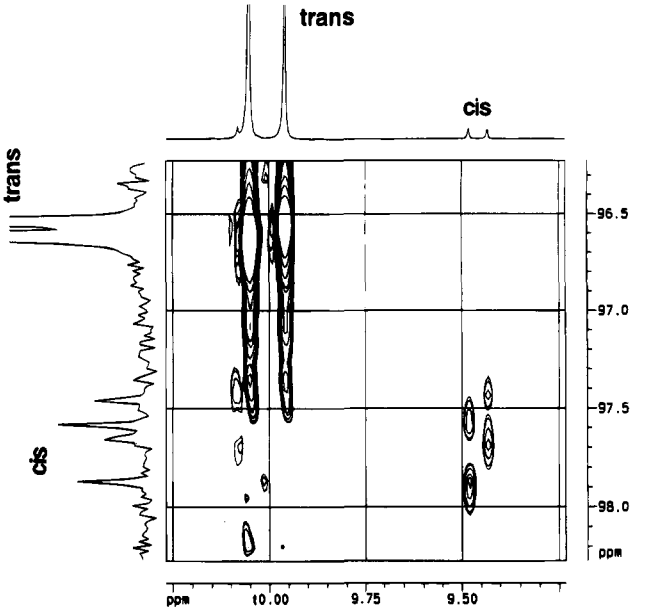

Figure 6. Two-dimensional NMR HMBC spectrum correlating through multiple bonds the ^1H spectrum of the amide protons (shown above) and the low-frequency ^{13}C signals of the iodinated carbons of II (shown at left). The spectrum was recorded at a proton frequency of 500 MHz and a temperature of 303 K. Figure 8 gives full assignments for the proton signals.

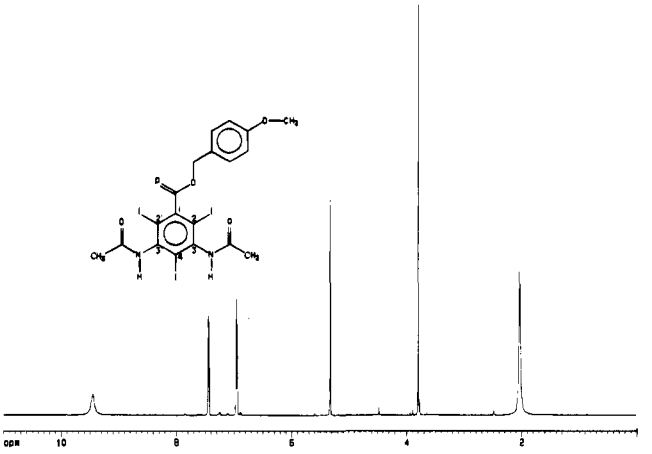

Figure 7. The ^1H NMR spectrum of II in DMSO-d_6, recorded at 500 MHz and a temperature of 413 K. The small signals around 7.0 ppm, at 9.9 ppm, and in other regions of the spectrum persisted when the sample was recooled to room temperature and were assigned to decomposition products.

of t_1 noise from the signal of conformation **a** obscures two of the correlation peaks for the protons of the *trans* amides, but those with proton chemical shifts at 10.08 ppm can be picked out. Their carbon frequencies match those of the cross peaks at 9.44 ppm from *cis* amides, proving that the *trans* peak of highest frequency and the *cis* peak of lowest frequency come from the same conformation. The experimental data do not allow identification of whether this conformation is **c** or **d**.

Variable-Temperature Spectroscopy of II. As expected, the proton NMR spectrum of **II** collapsed into an average of the spectra of all the conformations when the sample temperature was raised from 303 to 413 K (Figure 7). At 413 K the signals of the amide protons, the acetamide methyls, and the ester methylenes all were singlets. However, the spectrum also showed evidence of sample decomposition at high temperature.

Spectra acquired at intermediate temperatures indicated that rotations about the aniline and amide bonds in **II** take place

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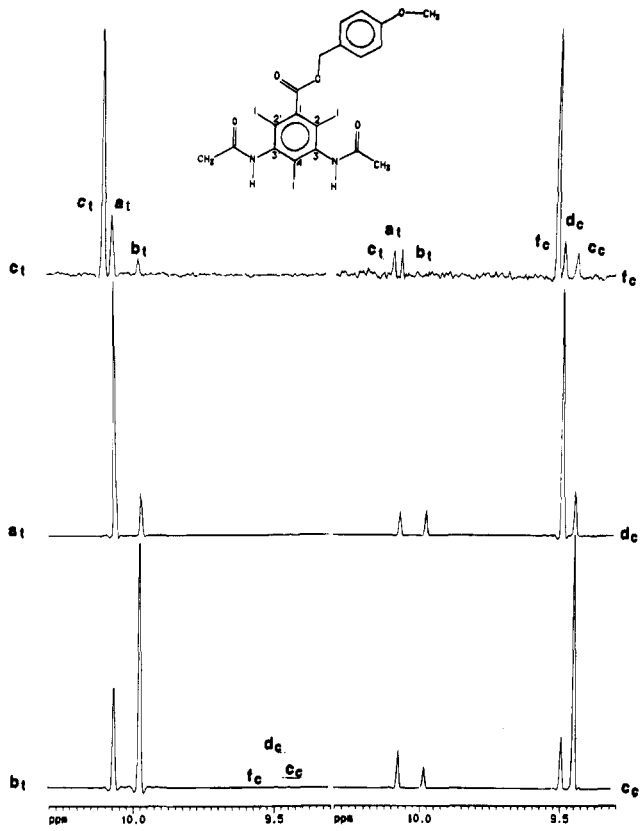

Figure 8. Vertical slices through the two-dimensional, chemical-exchange spectrum of II. Each slice passes directly through one of the six resolvable diagonal peaks for the amide protons. The assignments of each slice are shown to the left and right. The assignments of the individual signals are shown above them. The spectrum was recorded at 313 K and 500 MHz with a mixing time for conformational exchange of 200 ms.

with almost equal facility; if one of the processes were much faster than the other, we should have observed stepwise collapse of the NMR spectrum as the temperature was increased. For faster rotation around the aniline bond, the weak peaks for the *cis* amides would first have been absorbed into those for the *trans* amides, then at higher temperatures there would have been complete collapse as rotation about the aniline bonds became fast on the NMR time scale. In the opposite circumstance, the two large peaks of the *trans* amides would have merged first, then complete collapse would have taken place. In fact, we observed that spectral collapse of all the amide signals took place in the same temperature range.

A full line-shape analysis of the variable-temperature spectra might have provided kinetic parameters for conformational interconversion in **II**.[3] A simpler approach, which avoids the problem of sample degradation at elevated temperature, was the use of 2D exchange NMR.

Two-Dimensional Exchange NMR of II. A two-dimensional exchange spectrum is essentially a chart of transfers of nuclear magnetization from one chemical environment to another during a "mixing period" incorporated into the pulse sequence used to generate the spectrum. Magnetization components that start and end the mixing period in the same chemical environment give the diagonal peaks in the two-dimensional spectrum, those with the same frequency along each axis. Magnetization components that start the mixing period with one chemical environment, but end with another, give the cross peaks. These have two different associated frequencies, one for the starting chemical site and one for the final site. The intensities of the cross peaks, when compared to the intensities of the other cross and diagonal peaks, provide quantitative information about the relative degree to which magnetization transfers from one chemical site into another.[14]

The usual contour plot of the two-dimensional exchange NMR spectrum does not lend itself to quantitative analysis. Much more useful are one-dimensional slices passing through the signals on the spectral diagonal. Figure 8 shows a set of vertical slices from an exchange spectrum for **II** recorded at 313 K with a mixing time of 200 ms. Theory stipulates that either vertical (f_1) or horizontal (f_2) slices are suitable for quantitative analysis because the spectrum should be symmetrical about the diagonal.[14] We chose to use vertical slices to minimize the effect of t_1 noise on the final results.

Each slice corresponds to one of the resolvable signals in the one-dimensional spectrum and, thus, to a particular site in the molecular conformations **a** through **f**. However, we had only identified unambiguously the signals of **a** and **b** from the one-dimensional results. Additional assignments were made in the course of the full analysis of the two-dimensional spectrum according to the internal consistency in the kinetic parameters derived from individual cross peaks. The full set of assignments is shown on the figure. The final assignments do have some uncertainty. Reversal of the assignments for **c** and **d** would have little effect on the internal consistency of the rate parameters derived from the spectrum. Reversal of the assignments for **e** and **f** would lead to internal inconsistencies beyond expectation on the basis of random error.

Each slice from a two-dimensional exchange spectrum taken through one of the diagonal peaks is equivalent to a one-dimensional spectrum measured with selective perturbation at the frequency of that diagonal peak. In an actual one-dimensional experiment the perturbation would be used selectively to create certain nonequilibrium components of nuclear magnetization aligned in the direction of the magnetic field. These components would be converted into observable magnetization with a nonselective 90° radio-frequency pulse after a "mixing period" following the selective pulse. If the mixing period were short, the observed signal would have a frequency characteristic of the perturbed site alone. If the mixing period were long, the observed signals would be those of the perturbed site and any additional sites to which magnetization were transferred.

The mixing period for the two-dimensional spectrum of Figure 8 was 200 ms, short enough to ensure that the largest signal in each slice is that of the diagonal peak. Ideally, quantitative analysis of the data should be done with two-dimensional integration of the diagonal and off-diagonal peaks in the full spectrum. Under the experimental conditions we used, the peak widths are essentially equal, and the peak intensities were suitable for the analysis.

In favorable cases, the full two-dimensional exchange spectrum provides sufficient information for calculation of the exchange matrix **K**, which amounts to a table of the various rate constants governing interconversion of the various chemical species. The exchange matrix is analogous to a simple first-order rate constant. If the concentrations of the interchanging species are arranged as a vector **A**, the time derivative of the concentration vector is related to the concentrations through eq 1[14]

$$\frac{d}{dt}\mathbf{A} = \mathbf{K}\cdot\mathbf{A} \tag{1}$$

which has the formal solution

$$\mathbf{A}(t) = e^{\mathbf{K}t}\mathbf{A}(0) \tag{2}$$

Table 1. Exchange Matrix (**K**) for Conformational Interchanges Resulting from Single-Bond Rotations[a]

a	ā	b	c	c̄	d	d̄	e	ē	f	
a	$-A$	0	k_{ttj}	k_{ct}	0	0	k_{ctj}	0	0	0
ā	0	$-A$	k_{ttj}	0	k_{ct}	k_{ctj}	0	0	0	0
b	k_{ttj}	k_{ttj}	$-2A$	k_{ctj}	k_{ctj}	k_{ct}	k_{ct}	0	0	0
c	k_{tc}	0	k_{tcj}	$-(A+B)$	0	k_{ttj}	k_{ccj}	k_{ct}	0	k_{ctj}
c̄	0	k_{tc}	k_{tcj}	0	$-(A+B)$	k_{ccj}	k_{ttj}	0	k_{ct}	k_{ctj}
d	0	k_{tcj}	k_{tc}	k_{ttj}	k_{ccj}	$-(A+B)$	0	k_{ctj}	0	k_{ct}
d̄	k_{tcj}	0	k_{tc}	k_{ccj}	k_{ttj}	0	$-(A+B)$	0	k_{ctj}	k_{ct}
e	0	0	0	k_{tc}	0	k_{tcj}	0	$-B$	0	k_{ccj}
ē	0	0	0	0	k_{tc}	0	k_{tcj}	0	$-B$	k_{ccj}
f	0	0	0	k_{tcj}	k_{tcj}	k_{tc}	k_{tc}	k_{ccj}	k_{ccj}	$-2B$

[a] k_{tc} = rate constant for *trans* to *cis* amide conversion. k_{ct} = rate constant for *cis* to *trans* amide conversion. k_{ttj} = rate constant for *trans* amide jump past the aromatic ring. k_{ccj} = rate constant for *cis* amide jump past the aromatic ring. k_{tcj} = rate constant for *trans* amide jump past the ring with conversion to *cis* amide. k_{ctj} = rate constant for *cis* amide jump past the ring. $A = (k_{ttj} + k_{ct} + k_{ctj})$. $B = (k_{ccj} + k_{tc} + k_{tcj})$.

for which the exponential of the matrix $\mathbf{K}t$ is defined by an infinite series.

To the extent that individual signals in the NMR spectrum can be identified with the exchanging species represented by **A**, the two-dimensional spectrum provides an experimental measure of $e^{\mathbf{K}t}$, from which the exchange matrix **K** itself theoretically could be determined. In practice, every element of $e^{\mathbf{K}t}$ need be known with high precision if the calculation is to be successful; it is rare that the direct approach works. Generally, peak overlap or low signal intensity prevent the determination of at least one of the matrix elements. Fortunately, for small values of t, all but the first two terms of the series expansion of $e^{\mathbf{K}t}$ can be ignored and $e^{\mathbf{K}t} \approx \mathbf{1} + \mathbf{K}t$, where **1** is a matrix with each element on the diagonal having a value of unity and all other elements having values of zero. For short mixing times the cross peaks of the two-dimensional spectrum effectively measure the off-diagonal elements of **K** itself.

In order to reduce the number of independent pieces of information contained in the full conformational exchange matrix, we assumed that the two amide groups of **II** are dynamically independent. In other words, there are no concerted changes of conformation involving both amides at the same time. We then defined six primitive processes affecting the individual amide groups: forward and backward *cis*−*trans* conversion of an amide that remains on the same face of the ring, forward and backward *cis*−*trans* conversion of an amide that jumps from one face of the ring to the other during the conversion, and jumping of a *cis* or *trans* amide from one face of the ring to the other while it retains its internal conformation. The rate constants that go with these various processes are k_{tc} and k_{ct} for amide conversion without a jump, k_{tcj} and k_{ctj} for amide conversion with a jump, and k_{ccj}, and k_{ttj} for amide jumping with retention of local conformation. Table 1 shows the full conformational exchange matrix for **II**, defined in terms of these rate constants.

In the form given, the exchange matrix in Table 1 is unsuitable for actual analysis of the two-dimensional NMR spectrum. Some of the conformations give two amide NMR signals, and, in an achiral environment, the two enantiomers of the chiral conformations give identical signals. To complete the spectral analysis, we reduced the full matrix to the simpler one in Table 2, in which the columns and rows correspond to actual NMR signals. The labels in Table 2 were modified to indicate both the identity of the molecular conformation and the local conformation of the amide group giving rise to each NMR signal.

Corresponding to Table 2 should be an experimental matrix from which the values of the various rate constants can be found. Unfortunately, the ambiguities in the peak assignments in the one-dimensional spectrum meant that we actually had several potential experimental exchange matrices. However, each of these provided several measures for most of the kinetic parameters, and we could discriminate among the assignments on the basis of the internal consistency of the derived constants. For two of the assignments, the ratios of the individual values for k_{ctj} and k_{ct} were as much as 4:1. However, for the assignments in Figure 8 the ratios were no greater than 2:1, and much less for most pairs of values. We accepted the final assignment as correct, noting that the assignment with **c** and **d** would also be acceptable with regard to the criterion of internal consistency. From high frequency to low, the final assignments for the amide proton signals of the one-dimensional spectrum are $\mathbf{c}_t$, $\mathbf{a}_t$, $\mathbf{b}_t$, $\mathbf{f}_c$, $\mathbf{d}_c$, and $\mathbf{c}_c$.

Table 2. Exchange Table for NMR Resonances[a]

	$\mathbf{c}_t$	$\mathbf{a}_t$	$\mathbf{d}_t$	$\mathbf{b}_t$	$\mathbf{e}_c$	$\mathbf{d}_c$	$\mathbf{f}_c$	$\mathbf{c}_c$
$\mathbf{c}_t$		$k_{tc}/2$	$k_{ttj} + k_{ccj}$	k_{tcj}	$k_{ct}/2$	0	k_{ctj}	0
$\mathbf{a}_t$	k_{ct}		k_{ctj}	$2k_{ttj}$	0	k_{ctj}	0	k_{ct}
$\mathbf{d}_t$	$k_{ttj} + k_{ccj}$	$k_{tcj}/2$		k_{tc}	$k_{ctj}/2$	0	k_{ct}	0
$\mathbf{b}_t$	k_{ctj}	k_{ttj}	k_{ct}		0	k_{ct}	0	k_{ctj}
$\mathbf{e}_c$	k_{tc}	0	k_{tcj}	0		k_{tcj}	$2k_{ccj}$	k_{tc}
$\mathbf{d}_c$	0	$k_{tcj}/2$	0	k_{tc}	$k_{ctj}/2$		k_{ct}	$k_{ttj} + k_{ccj}$
$\mathbf{f}_c$	k_{tcj}	0	k_{tc}	0	k_{ccj}	k_{tc}		k_{tcj}
$\mathbf{c}_c$	0	$k_{tc}/2$	0	k_{tcj}	$k_{ct/2}$	$k_{ttj} + k_{ccj}$	k_{ctj}	

[a] Subscripts in the designations for the conformations indicate *cis* and *trans* amides. k_{tc} = rate constant for *trans* to *cis* amide conversion. k_{ct} = rate constant for *cis* to *trans* amide conversion. k_{ttj} = rate constant for *trans* amide jump past the aromatic ring. k_{ccj} = rate constant for *cis* amide jump past the aromatic ring. k_{tcj} = rate constant for *trans* amide jump past the ring with conversion to *cis* amide. k_{ctj} = rate constant for *cis* amide jump past the ring.

Table 3. Experimental NMR Exchange Table[a]

	$\mathbf{c}_t$	$\mathbf{a}_t$	$\mathbf{d}_t$	$\mathbf{b}_t$	$\mathbf{e}_c$	$\mathbf{d}_c$	$\mathbf{f}_c$	$\mathbf{c}_c$
$\mathbf{c}_t$		0.00		0.00		0.00	0.06	0.00
$\mathbf{a}_t$	(0.18)			(0.29)		(0.06)	(0.07)	(0.10)
$\mathbf{d}_t$	(0.18)			(0.29)		(0.06)	(0.07)	(0.10)
$\mathbf{b}_t$	0.05	0.15				0.08	0.00	0.05
$\mathbf{e}_c$	(0.00)	(0.00)		(0.00)			(0.10)	(0.14)
$\mathbf{d}_c$	(0.00)	(0.00)		(0.00)			(0.10)	(0.14)
$\mathbf{f}_c$	0.00	0.00		0.00		0.00		0.00
$\mathbf{c}_c$	0.00	0.00		0.00		0.13	0.06	

[a] Signals for $\mathbf{a}_t$ and $\mathbf{d}_t$ and for $\mathbf{d}_c$ and $\mathbf{e}_c$ overlap. Items directly above one another in parentheses represent summed intensity.

Table 3 shows the experimental exchange table, and Table 4 gives the values of the primitive rate constants, derived from it with the help of the measured ratio of *trans* to *cis* amides in the one-dimensional spectrum, which was 22. For the accepted peak assignments, k_{ct} is larger than k_{ctj}. For the alternative assignment, in which the peaks of **c** and **d** are reversed, k_{ctj} is larger than k_{ct}.

NMR Results for III. We obtained two stable forms of **III**, **IIIa**, and **IIIb**. In freshly prepared solutions C2 and C2′ of each of these resonated at the same frequencies, 99.07 and 98.85

Table 4. Calculated Rate Constants

	source	range (s^{-1})	av value (s^{-1})	$\Delta G^{\ddagger}$ (kcal/mol)
k_{tc}	k_{ct}		2	17.5
k_{ct}	3 elements	35−50	40	15.6
k_{ttj}	2 elements	73−75	74	15.2
k_{tcj}	k_{ttj} and 2 elements		<10	
k_{tcj}	k_{ttj}		1.5	17.7
k_{ctj}	5 elements	25−30	28	15.8

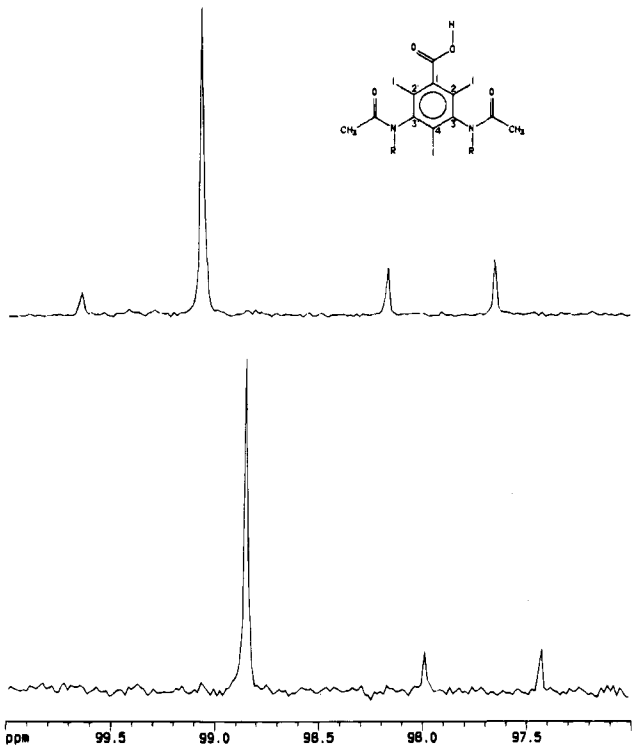

Figure 9. The portions of the ^{13}C spectra coming from the 2 and 2′-carbons of **IIIa** (top) and **IIIb** (bottom) in DMSO-d_6. Prior to acquisition of the spectra, the solutions were allowed to equilibrate at 303 K for about 1 h. Nevertheless, each sample gives rise to a different set of NMR signals, indicating that each contains an independent manifold of conformations and that interchange between the manifolds is extremely slow at room temperature.

ppm, respectively. After the solution of **IIIa** had been equilibrated at 353 K for about an hour, C2 and C2′ gave three additional signals, at 97.66, 98.16, and 99.67 ppm (Figure 9). Carbons C2 and C2′ of a similarly treated sample of **IIIb** gave two additional signals, at 97.42 and 98.00 ppm. After 4 weeks at room temperature each sample gave barely detectable signals corresponding to those of the other compound.

The literature indicates that when both an alkyl and an aromatic group are attached to an acetamide nitrogen, it is the alkyl group that preferentially takes the position *trans* to the acetamide methyl.[24] Thus we assumed that both separated forms of **III** contained only *cis* amides and that the difference between the compounds in the two crystals was that the amides on opposite faces of the ring were *syn* to one another in one case and *anti* in the other. The freshly prepared solutions contained purely conformations **e** or **f**, and there was only one signal for C2 and C2′ for each sample. No attempt was made to differentiate which structure corresponded to which spectrum.

Within an hour at 353 K significant amounts of **c** and **a** developed from **e** as a result of *cis* to *trans* conversion of the amide groups; significant amounts of **d** and **b** developed from **f**. These gave rise to four new signals from C2 and C2′ of each compound. For either compound *cis*−*trans* interconversion, while slower than in **II**, was much faster than *syn*−*anti* interconversion. The two forms apparently reach equilibrium in solution only after a period of months.

Discussion

Conformations of II. The experimental observations for **II** in dimethyl sulfoxide indicate little or no interaction between the amide groups. The apparent two-to-one predominance of conformation **a** over **b** results from the fact that **a** is always accompanied by its mirror image, **ā**, in an achiral environment. Both **a** and **ā** are energetically equivalent and contribute to the same signals of the NMR spectrum, which thus have double the intensity of the signals from the corresponding nuclei in the achiral conformation **b**. It is quite possible that in a chiral solvent all ten conformations of **II** shown in Figure 1 would give resolvable NMR resonances, each of equivalent intensity.

The absence of interaction between the amide groups is specific to dimethyl sulfoxide solutions. In a sample that was swamped with chloroform, conformation **a** predominated strongly. At first glance it might appear simply that the less polar solvent chloroform favors the conformation with the smaller overall dipole moment, **a**. However, chloroform has very little effect on the equilibrium between conformations **c** and **d**, and the difference in dipole moment between **c** and **d** must be similar to that between **a** and **b**. The alternative explanation is that dimethyl sulfoxide strongly solvates the amide groups, effectively screening them electrostatically from each other. The screening is especially important for **a** and **b**, in which the carbonyl oxygens point toward each other over the aromatic ring but play a minor role for conformations **c** and **d**, in which one of the carbonyl oxygens is directed away from the ring center. In either case the amide groups are far enough separated that steric interactions are small.

The partial double-bond character of the amide bond favors a locally planar conformation. A conformation in which the amide plane coincided with that of the aromatic ring would be further favored by delocalization of the lone pair of the nitrogen into the ring. However, in a planar form of **II** the large *ortho* substituents adjacent to the amide group would interact with either the oxygen or methyl of the amide group, depending on whether the amide were in the *cis* or *trans* conformation. The "rule of six" indicates that the sixth atom in a series of bonds is optimally situated for interacting sterically with the first atom.[4] Both the oxygen and the methyl of an acetamide attached to an aromatic ring are at the proper distance from the nearby iodines in the ring for large steric effects.

In conformations **a** or **ā** of **II**, the methylene protons in the ester group are nonequivalent, and the **a**/**ā** pair is the obvious source of the largest NMR signals of **II**. Theoretically, mirror-image conformations in which the amide groups were coplanar with the aromatic ring would also explain the experimental observations but only if one amide were *cis* and the other were *trans*. The argument against this possibility is the expectation that both amides should have the same local conformation in the most favorable structures. Only very strong interactions between the two amide groups could interfere. Because, in dimethyl sulfoxide, interactions between the amide groups are almost certainly small, the conformations of **II** lying at energy minima must be those in which the amide groups have equivalent conformations and are twisted out of the plane of the ring. The coplanar forms must lie at energy maxima rather than minima.

Conformation of the Ester Group. Because the ring iodines of **II** crowd the amide groups severely, we might expect that they would dictate the conformations of the attached ester group similar to the way they control the conformations of the amide

groups, forcing the ester carbonyl out of the ring plane. Indeed, they probably do. However, the barrier to rotation around the bond connecting the ester carbonyl to the ring is much lower than those involving the aniline bonds. At room temperature interchange among the various conformations of the ester group is fast enough to average the NMR spectrum. The ester group acts effectively as if it were cylindrically symmetric, and we have ignored its conformations in our analysis.

Conformations of III. The amide protons in **II** have little steric volume, and in the lowest-energy structures they, rather than the much larger aromatic ring, eclipse the acetamide methyl groups. In a conformation of **III** comparable to that of **II** there would be severe steric interactions between the alkyl on the nitrogen and the acetamide methyl. The most stable conformations of **III** are those in which the hexyl groups, rather than the aromatic ring, are *trans* to the acetamide methyl.[24]

A consequence of the reversal in position of the aromatic rings in the most stable conformations of **II** and **III** is that the NMR patterns of the methyl group are turned around. The largest ^1H signals of **III**, those of the most favorable conformation, resonate at high frequency rather than at low frequency as they do in **II**. Likewise, the ^{13}C patterns of **II** and **III** are reversed.

Conformational Interchange in II. We were successful in analyzing the NMR spectra of **II** with the assumption that there is negligible interaction between the amide groups in the lowest-energy conformations. To be consistent in the analysis, we assumed also that interactions between amides have no effect on the course of conformational interchange in dimethyl sulfoxide. In other words, simultaneous changes of the local conformation of both amides are so slow that they can be ignored. Overall changes in molecular conformation can be broken down into changes involving one or the other of the amide groups alone.

The *syn*−*anti* nomenclature, which describes the relative position of two amide groups, is awkward when applied to the dynamic properties of **II** when there are no interactions between the amide groups. Both *syn* to *anti* and *anti* to *syn* conversions involve the same fundamental process, a jump of an amide from one face of the ring to the other. In defining primitive rate constants, we focused on whether a given amide was in the *cis* or *trans* conformation before a change and whether the change involved a jump from one face of the ring to the other, not on how that amide was oriented with respect to a second one across the aromatic ring.

A jump of a *trans* amide from one face of the ring to the other ultimately looks like a pure rotation around an aniline bond. However, during the course of the process, rotation around the aniline bond has to be accompanied by rotation around the amide bond as well as possible distortion of the amide group through changes in bond angles and bond lengths. Rotation around the amide bond without other changes would bring the amide group and the aromatic ring coplanar at some point, and the carbonyl oxygen would severely interfere with an iodine. Rotation about the aniline with rotation or distortion of the amide bond allows the molecule to sidestep the oxygen/iodine collision. In a sense, the amide carbonyl acts as a turnstile, controlling passage from one face of the ring to the other. When the amide group is planar, the turnstile is closed and top-to-bottom motion is prohibited. As the carbonyl group turns out of the plane or the amide group distorts by expansion of the bond angle, the turnstile opens up and the amide jump takes place.

Only partial opening of the turnstile is required to permit the jump of a *trans* amide. Full opening by rotation about the amide bond would correspond to 90° secondary rotation. However, a *trans* amide, having undergone a 90° internal rotation, could just as readily pass on to a *cis* amide as return to a *trans* amide. The rate of *trans*−*cis* conversion would equal the jumping rate. Instead, Table 4 shows that a *trans* amide jumps to the other face of the ring about 35 times as often as it converts into a *cis* amide. Apparently, just slight rotation about the amide bond, or simply a little bond distortion, is sufficient to allow the amide group passage between the large iodine atoms. After the amide has moved from one ring face to the other, it returns to the favorable planar local conformation.

A *cis* amide follows a different pathway from one ring face to the other. It is a large methyl group of the *cis* amide, rather than an oxygen, that is oriented toward the aromatic ring. Rotation about the aniline bond alone would bring that methyl, rather than the carbonyl oxygen, into conflict with an iodine atom. The coplanar structure of a *cis* amide is even more destabilized than that of a *trans* amide. Much greater rotation about the internal amide bond or internal distortion is required to alleviate the steric problem. Having undergone such a high degree of distortion, a molecule is more likely to continue on to a *trans* conformation rather than return to a *cis* conformation.

In fact, almost every *cis* amide traveling along the potential energy surface toward a *cis'* amide ends up being diverted towards a *trans* amide. Some of amide groups return to the same face of the ring from which they started during the transformation, and others go on to the opposite face. Table 4 shows that the sum of k_{ct} and k_{ctj} is close to k_{ttj}. A conformational change of some type is about as likely to take place for a *cis* as for a *trans* amide. However, the net consequence of a jump from one ring face to the other for a *cis* amide is quite different than that for a *trans* amide.

Conformational Interchange in III. The alkyl groups in **III** have profound effects on the rates at which conformational interchange takes place. In **III** an amide jumps from one face of the ring to the other so slowly that the structures in which the amides are *syn* and *anti* can be isolated as effectively different compounds. The barrier to interconversion is on the order of 30 kcal/mol. *Cis* to *trans* interconversion in **III** takes place much more rapidly, but still on a time scale of hours, rather than fractions of seconds, as in **II**.

The Wider Significance of Coupled Rotations about Single Bonds. Polymer motions in the rubbery state, and in solution, are generally believed to involve cooperative rotations about multiple bonds.[27,28] One type of cooperativity is that which results in simultaneous net rotation around two or more bonds during a change in molecular conformation. The transition from a *cis* to a *trans* amide in **II** accompanied by a jump of the amide from one ring face to the other exemplifies this kind of cooperativity. A second type of cooperativity is that in which rotation around two or more bonds is required during a transition but in which the complete conformational transition ends up with a net change in the rotation angle of one bond alone. This type of cooperativity is illustrated by the transition of a *trans* amide on one face of the ring to the other face. On balance there is net rotation around only the aniline bond even though substantial rotation around the amide bond is required during the transition to make the rotation around the aniline bond possible.

All indications are that most conformational transitions in polymers occur as isolated rotations around individual bonds.[9,27] However, the second type of cooperativity undoubtedly must be important. The results for **I**−**III** help to show how this is possible.

(27) Adolf, D. B.; Ediger, M. D. *Macromolecules* **1991**, *24*, 5834.
(28) Baysal, C.; Erman, B.; Bahar, I. *Macromolecules* **1994**, *27*, 3650.

Most experimental methods detect a distribution of correlation times for polymer motions. It is tempting to identify this distribution of correlation times with a distribution of inverse rate constants for conformational interchanges. In a complicated system in which rotations around many different bonds are possible, there necessarily will be a distribution of inverse rate constants for the whole body of conformational transitions that may involve sums and differences of the primitive inverse rate constants as well as the inverse rate constants themselves. However, Adolf and Ediger have pointed out that a distribution of inverse rate constants differs from the experimental distribution of correlation times.[27] Some experimental measurements, such as nuclear relaxation times, are sensitive to motions within an energy well, in addition to transitions between energy wells. Because each type of motion is affected by cooperativity of bond rotations in a different way, it is understandable that measurements of distributions of correlation times and measurements of distributions of inverse rate constants do not always give the same results.[27]

The molecules of a given protein, unlike those of synthetic polymers, have a uniform conformation. Nevertheless, prediction of that conformation with knowledge of the chemical structure of the protein is one of the great unsolved problems of biochemistry. Prediction of how that conformation might change as the protein interacts with other substances, such as an enzyme substrate, is equally difficult. The results presented here for simple amides provide important clues about how internal rotations about several bonds associated with organic amides are coupled. The information obtained provides a basis for explaining how conformational changes in complex materials may occur.

Conclusions

The enormous steric constraints in compounds **I**−**III** affect both the ground-state conformations and the mechanisms by which interchanges among them take place. Determination of a complete set of dynamic parameters for conformational interchange with two-dimensional NMR methods has provided exceptional insight into the nature of conformational interchanges involving coupled rotations about several bonds. An understanding of the nature of these processes helps to elucidate how dynamic processes affect the properties of large molecules, such as synthetic polymers and biological macromolecules.

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