180-τ-90 pulse sequences. Twelve τ values in the range 0.01-8 s were used. Raw data were analyzed using a nonlinear, three-parameter, exponential fitting program on the Aspect 3000 computer on the spectrometer. Reported T_1's represent an average of two determinations and have an estimated error of 5-10%.

Acknowledgment. This work was supported in part by a grant from the Australian Research Council.

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Registry No. Butaclamol hydrochloride, 36504-94-6.

NMR Studies of the Conformational Interconversion of Butaclamol in Solution

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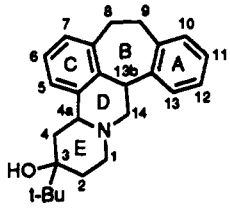
^{1}H NMR experiments at 300 MHz have been carried out to determine the identity and study the interconversion of two conformations of butaclamol in solution. The hydrochloride salt in DMSO exists as an equilibrium mixture of two conformations, which differ in their stereochemistry about the ring junction that contains the single nitrogen atom in butaclamol. The trans form has a relative population of 80% and the cis I form 20%. In $CDCl_3$ only the trans form is observed, while in $CDCl_3$-DMSO mixtures, both forms are detected in a ratio (trans:cis I) that decreases as the percentage of $CDCl_3$ decreases. For the free base in either CD_2Cl_2 or DMSO, only a single set of resonances is observed at room temperature, but as temperature is lowered, peaks from methine protons H4a and H13b near the ring junction broaden and (for samples in CD_2Cl_2) eventually split into two resonances corresponding to the cis and trans forms. It is suggested that nitrogen inversion is the dynamic process responsible for the interconversion of the two forms. Line shape analysis as a function of temperature yielded an energy barrier of 9.6 ± 0.5 kcal/mol for the interconversion, in good agreement with values obtained from saturation transfer experiments. In the hydrochloride salt, the barrier in DMSO was somewhat higher, i.e., 17.3 ± 0.9 kcal/mol, as determined by saturation transfer and variable-temperature measurements.

Introduction

In the preceding paper,[1] a theoretical and NMR analysis of the conformations of butaclamol (1) was reported. This



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compound has been extensively used as a dopamine receptor mapping agent,[2] so a knowledge of its conformational behavior is important for a full understanding of its receptor binding. The theoretical results reported in the previous paper are in general agreement with related studies[3-5] in that four low-energy conformers were identified. NMR data recorded in $CDCl_3$ support the existence of one of these low-energy conformers in solution, but there is apparent conflict with a recent NMR study in DMSO reported by Maryanoff et al.[4] That study (carried out in DMSO because of a reported difficulty in dissolving butaclamol hydrochloride in $CDCl_3$) suggested the presence of two low-energy conformations, one of which did not correspond to any of the previously identified low-energy conformers in three theoretical studies.[1,3,5]

In the present paper, the apparent anomaly regarding the conformation of butaclamol in solution is investigated. It is established that two conformers are indeed present for butaclamol hydrochloride in d_6-DMSO, both of which correspond to low-energy forms previously identified in theoretical studies. The conformational interconversion of butaclamol is analyzed and the role of solvent in stabilizing individual conformers is discussed.

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Results

(a) Comparison of Spectra in DMSO and $CDCl_3$. Spectra of butaclamol hydrochloride and its free base in $CDCl_3$ and d_6-DMSO at 297 K are shown in Figure 1. Major differences in the spectra of the free base relative to those of the hydrochloride include the absence of downfield NH resonances and chemical-shift changes of the methylene and methine protons adjacent to the nitrogen atom. There are a number of other, smaller chemical-shift differences and changes to spin-spin coupling systems in the free base which were previously complicated by the presence of the NH proton in the hydrochloride.

Changing the solvent has little influence on the spectrum of the free base, apart from effects on the OH resonance. However, there are significant differences in the spectrum of the hydrochloride in d_6-DMSO relative to that in $CDCl_3$. The emergence of a second NH resonance for butaclamol hydrochloride in d_6-DMSO, and a doubling or broadening of other resonances, are consistent with the presence of two conformers undergoing chemical exchange in this solvent. The two sets of peaks occur in a ratio of approximately 4:1. Maryanoff et al.[4] previously reported the observation of two NH peaks in an approximate ratio of 4:1. The existence of two conformations can be readily seen in the COSY spectrum in Figure 2, where two sets of peaks are observed for a number of protons. The con-

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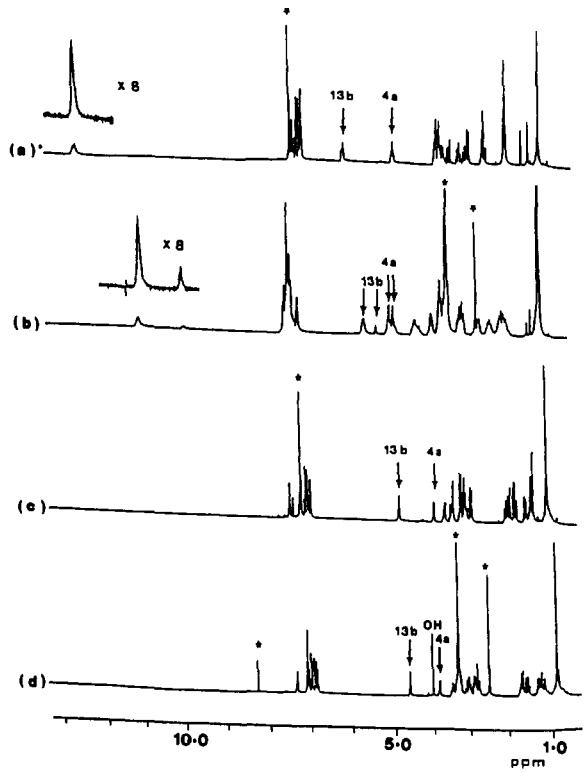

Figure 1. 300-MHz ^{1}H NMR spectra of butaclamol in various solvents: (a) hydrochloride in $CDCl_3$, (b) hydrochloride in d_6-DMSO, (c) free base in $CDCl_3$, (d) free base in d_6-DMSO. The peaks marked with an asterisk correspond to solvent resonances (2.49 ppm = DMSO; 3.30 ppm = H_2O in DMSO; 7.3 ppm = $CDCl_3$; 8.2 ppm = trace of $CHCl_3$ in DMSO). Insets correspond to a vertical scale expansion × 8 in the NH region.

former with the more intense cross-peaks is referred to here as the major form, the other being the minor form.

(b) Identification of Two Conformations of Butaclamol Hydrochloride in DMSO. In an attempt to confirm the existence of an exchange process and to identify the two conformers, a phase-sensitive NOESY spectrum was recorded and is shown in Figure 3. In a phase-sensitive NOESY spectrum for a rapidly tumbling molecule like butaclamol, cross-peaks due to chemical exchange have the same phase as the diagonal, whereas cross-peaks due to direct NOE's have the opposite phase.[6,7] In Figure 3, intense chemical exchange cross-peaks are clearly visible between resonances at 5.11 and 5.42 ppm, and between resonances at 4.65 and 4.80 ppm. The double-quantum-filtered COSY spectrum (Figure 2) revealed that these pairs of resonances, which are in slow exchange on the chemical-shift timescale, are associated with the H13b and H4a protons, respectively. Exchange is also detectable between other sets of protons; for example, in the aromatic region of Figure 3 and for a number of the high-field protons (not shown). Other protons apparently have similar chemical shifts in the two conformers and, because of fast exchange on the chemical-shift time scale, do not lead to two resolved resonances.

Previous studies suggested a number of low-energy conformers for butaclamol with major regions of conformational variability involving the B, D, and E rings.[3-5] Since the major spectral changes shown in Figure 1 involve protons near the NH on the D–E ring junction, it is most likely that the different conformers involve structural

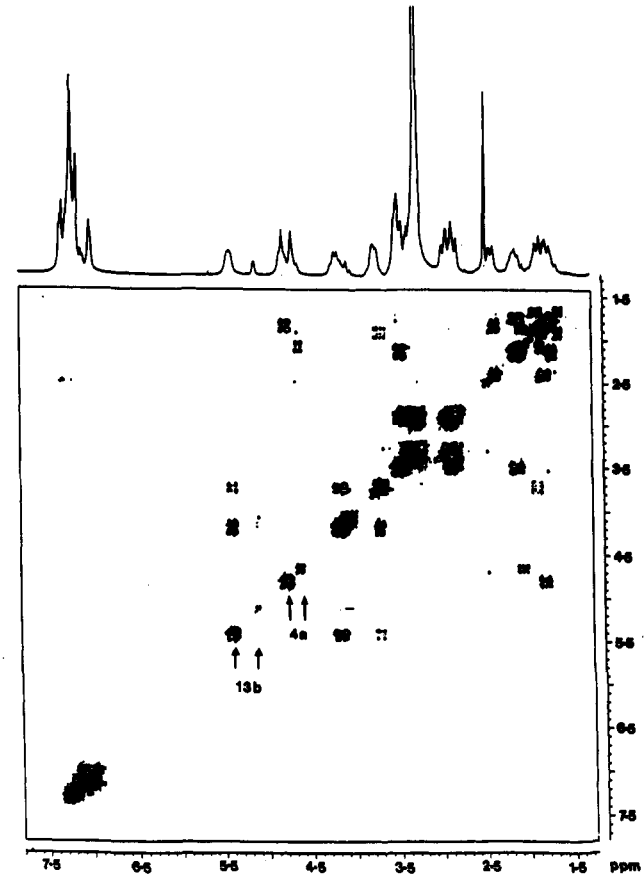

Figure 2. 300-MHz double-quantum-filtered COSY spectrum of butaclamol hydrochloride in d_6-DMSO. Arrows indicate resonances for protons 13b and 4a in the major and minor conformers. The ratio of the two forms is apparent from the relative intensities for H13b in the two forms seen in the one-dimensional spectrum at the top of the figure. The OH proton overlaps with H4a in the minor form, making it difficult to use this latter proton to determine conformer populations.

Table I. ^{1}H NMR Data for Butaclamol Hydrochloride in d_6-DMSO

chemical shift[a] (ppm)	coupling (Hz)	assignment
5.42	12.7, 5.1	H13b major
(5.42)[b]	(12.8, 5.7)[b]	(H13b major)[b]
5.11	5.0	H13b minor
(5.11)[b]	(5.3, 0.0, 0.0)[b]	(H4a minor)[b]
4.80	11.6, 10.5, 2.5	H4a major
(4.80)[b]	(10.7, 9.5, 1.0)[b]	(H4a major)[b]
4.65	12.3, 3.5, 3.5	H4a minor
(4.65)[b]	(11.9, 3.6)[b]	(H13b minor)[b]

[a] Chemical shifts were measured relative to the DMSO peak at 2.49 ppm and expressed relative to TMS. [b] Assignments derived by Maryanoff et al.[4] are given in parentheses. Couplings are accurate to ±0.25 Hz.

modification in the D-E ring region. Previously proposed low-energy forms include those shown in Figure 1 of the preceding article.[1]

Coupling constants measured for the 13b and 4a protons of the major and minor forms are given in Table I and were used to identify the two observed conformers. From a consideration of torsion angles from Dreiding models of the trans, cis I, and cis II conformers (including various chair and boat forms of the E ring in each case) it is a straightforward matter to predict, on the basis of the Karplus equation, the number of large couplings ($^3J > 8$ Hz) which should be observed for H13b and H4a in each of the conformers. For H13b, no such large coupling is

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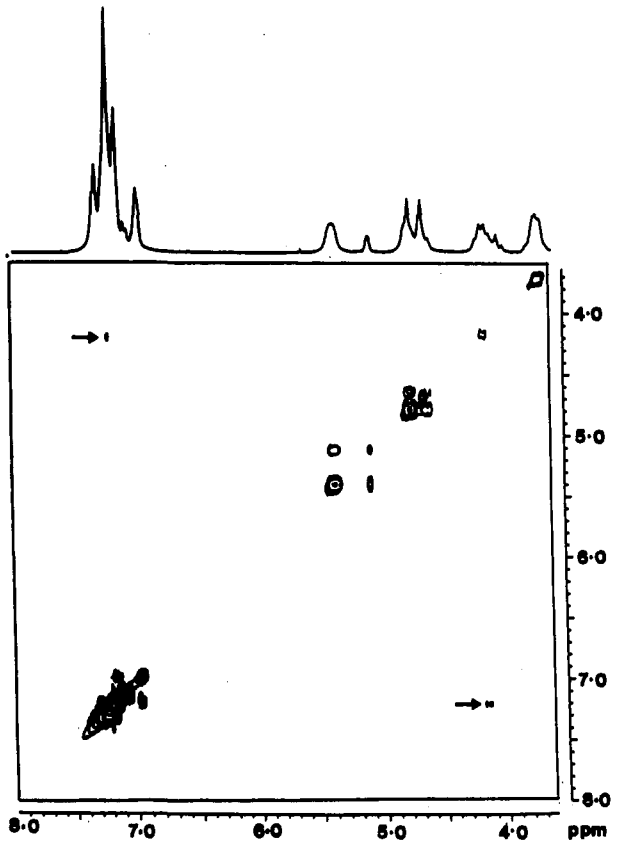

Figure 3. Expanded region of the 300-MHz phase-sensitive NOESY spectrum of butaclamol hydrochloride in d_6-DMSO. The cross-peaks indicated by arrows are of opposite phase to the diagonal and represent an NOE between $H14_{ax}$ and the aromatic proton H13 in the major conformer. All other cross-peaks have the same phase as the diagonal and represent a chemical exchange process.

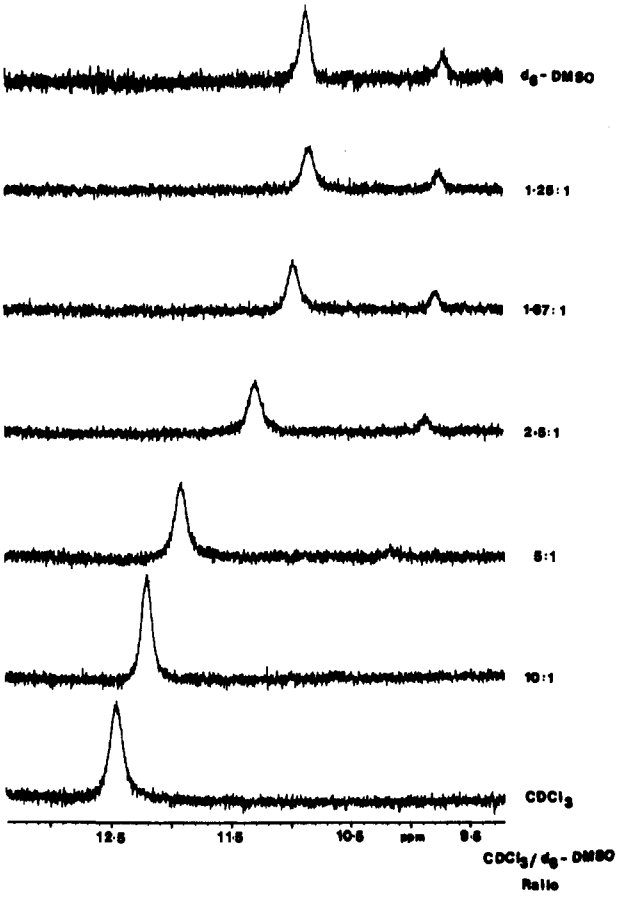

Figure 4. 300-MHz ^1H spectra of the NH region of butaclamol hydrochloride in $CDCl_3/d_6$-DMSO mixtures. Values next to the spectra correspond to volume ratios ($CDCl_3$:d_6-DMSO).

expected in the cis I form; for H4a one large coupling should be present in both cis forms, but two should be present in the trans form. The observed couplings in Table I suggest that the major species present corresponds to the trans form, while the minor form is cis I. (The possibility that the peaks for the minor form are actually due to an averaged set of peaks from both cis forms in rapid equilibrium cannot be totally eliminated, but consideration of coupling constants to H4a and H13b suggest that if this were the case, the relative population of the cis II form would be at least 10-fold less than that of cis I.) Laus et al.[8] previously noted the possibility of two conformations within the seven-membered ring of butaclamol resulting from puckering of the C8/C9 bridge, i.e., conformer A or conformer B. On the basis of experiments in which an NOE is observed between H13b and $H9_{ax}$ but not between H13b and $H8_{ax}$, conformer A has been shown to predominate in both the free base[8] and in the trans form of butaclamol hydrochloride.[1] A similar NOE experiment was carried out for the minor form (cis I) observed in this study, which demonstrated that conformer A is also preferred in this isomer.

(c) Investigation of Possible Exchange Process for Butaclamol Hydrochloride in $CDCl_3$. The presence of a conformational exchange process for butaclamol hydrochloride in d_6-DMSO prompted an investigation to see whether an exchange process might in fact be occurring in chloroform, but at a much faster rate, giving rise to an averaged spectrum. This is one possible explanation for the apparent anomalies relating to the observation of two sets of NH resonances in DMSO, but only a single peak in $CDCl_3$. A variable-temperature study of butaclamol hydrochloride in $CDCl_3$ was thus carried out. However, spectra recorded over a range of temperatures down to 210 K showed no evidence of the presence of more than one conformation in $CDCl_3$. Similarly, spectra recorded in CD_2Cl_2 at temperatures down to 178 K also showed no evidence of multiple conformers.

(d) Solvent Effects on Conformer Stability. The preliminary interpretation of the above observations was, then, that in DMSO the trans and cis I forms are of similar energy and coexist in solution with a moderate barrier to interconversion, whereas in $CDCl_3$ only one form is significantly populated. In order to confirm this and to examine the influence of solvent on the relative stability of conformers, a solvent titration study was performed. Figure 4 shows 300-MHz ^1H spectra of the NH region for butaclamol HCl in $CDCl_3$ with progressive additions of d_6-DMSO. In pure $CDCl_3$, only one NH peak is observed. The appearance of a second NH peak occurs almost immediately after the first addition of d_6-DMSO and steadily increases in intensity with subsequent additions. No coalescence process occurs, consistent with the presence of only one conformer of butaclamol hydrochloride in $CDCl_3$. As noted in the preceding paper, this conformer is that with a trans D–E ring junction and a chair form of ring E.

(e) Conformational Equilibria in Butaclamol Free Base. Following the above examination of the hydrochloride salt in different solvent mixtures, and the finding that the trans form is more strongly preferred in $CDCl_3$ than in DMSO, it was of interest to examine the effect of

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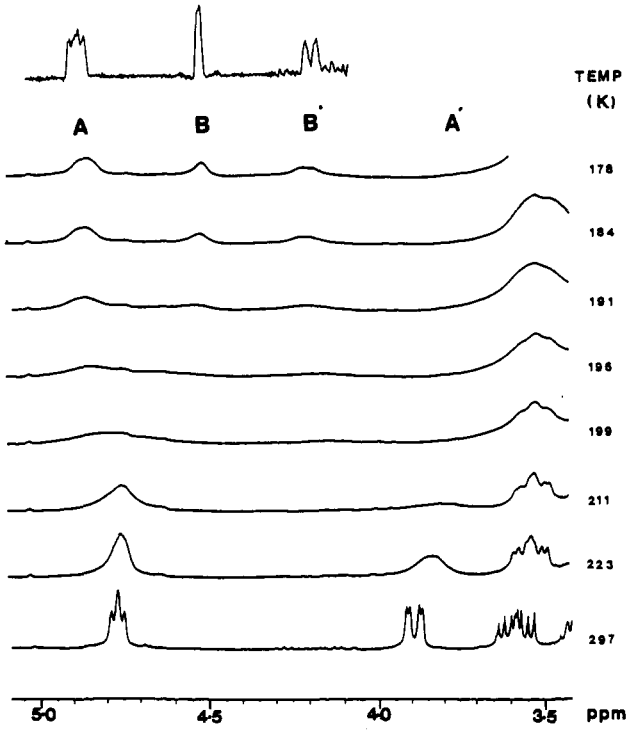

Figure 5. Variable-temperature study of the ^{1}H NMR spectra of free-base butaclamol in CD_2Cl_2. Expanded regions incorporating methine protons H13b and H4a are shown at the indicated temperatures. The peaks labeled A and B are due to H13b in a major and minor conformer observed in slow exchange at 178 K. At temperatures above 211 K they are in fast exchange. Peak B′ is due to H4a in the minor conformer. Peak A′, corresponding to H4a in the major conformer, is obscured by other resonances. Inset corresponds to a 400-MHz resolution enhanced spectrum at 178 K.

deprotonation on conformer stability and interconversion rates. The spectrum of the free base in DMSO in Figure 1d shows no evidence of the presence of multiple conformers, suggesting either that one form is present in large excess (> 20:1 probably represents the detection limit of the minor conformer at the S:N ratio of the recorded spectra) or that the two (or more) forms are in fast exchange on the chemical-shift time scale. A variable-temperature study could potentially have distinguished between these possibilities. However, the high freezing point of DMSO prevented the recording of spectra at low temperature in an attempt to observe separate conformers. Variable-temperature spectra of the free base in other solvents were thus examined. It was of particular interest to examine changes in protons H13b and H4a, since they are adjacent to the key nitrogen atom.

Figure 5 shows results from a variable-temperature study of the free base in CD_2Cl_2 at temperatures ranging from 178 to 297 K. This solvent has a low dielectric constant, similar to that of $CDCl_3$, but was employed in preference to the latter because of its lower freezing point. Both methine protons of interest are doublets of doublets (H13b ~4.78 ppm, H4a ~3.90 ppm), although in the case of H13b the couplings are such that the peak envelope takes on the appearance of a triplet at room temperature. A decrease in temperature resulted in broadening of the peaks until a coalescence temperature of approximately 200 K was reached. Further cooling resulted in the appearance of new peaks due to the presence of two conformers in slow exchange: the two downfield peaks, labeled A and B, are due to H13b in the major and minor conformers, respectively, while the peak labeled B′ at 4.20 ppm is due to H4a in the minor conformer; H4a (A′) in the major conformer is further upfield and obscured by other resonances.

Table II. ^{1}H NMR Data for Butaclamol Free Base in CD_2Cl_2 at 178 K

chemical shift[a] (ppm)	coupling (Hz)	assignment
4.86	9.2, 6.6	H13b major
4.52	2.9, 2.9	H13b minor
4.21	11.8, 2.4	H4a minor
–[b]	–[b]	H4a major

[a] Chemical shifts were measured relative to the CD_2Cl_2 peak at 5.32 ppm and are expressed relative to TMS. [b] Peak occurs at approximately 3.5 ppm but is obscured by other resonances. Couplings are quoted to an accuracy of ±0.25 Hz.

The two conformers occur in a population ratio of approximately 2:1. Coupling constants for the two conformers at 178 K were measured from a corresponding 400-MHz spectrum recorded with resolution enhancement and are shown in Table II. Although even at 178 K the peaks for H4a and H13b are relatively broad, the coupling constants in Table II are sufficiently well defined to confirm that the major conformer is the trans form and the minor the cis I form, as applied in the hydrochloride salt.

(f) Measurement of Barriers to Nitrogen Inversion. The process responsible for fast exchange between the two forms at higher temperatures is inversion of the nitrogen atom. An estimate of the barrier for this process in CD_2Cl_2 was obtained by determining the rate of interconversion, k, at the coalescence temperature via eq 1,[9] where $\delta\nu$ is the

$$k = \pi\delta\nu/\sqrt{2} \quad (1)$$

chemical-shift difference of the two conformers. Substitution of eq 1 into the Eyring equation (2)[9] gives an estimate of the barrier, ΔG, which was found to be 9.6 ± 0.2 kcal/mol at T_c, the coalescence temperature of 200 ± 5 K.

$$\Delta G^\circ = 19.14T_c(10.32 - \log(k/T_c)) \quad (2)$$

The rate constant, k, used above is based on the assumption of equally populated exchange sites. For nonequal populations, as in this case, a correction factor can be applied to eq 1[10] and separate rates can be derived for forward and reverse reactions in the exchange process. The unequal population ratio is 0.65:0.35 (obtained from integration of the spectrum recorded at 178 K, Figure 5), and substitution of appropriate expressions for the forward and reverse rate constants (112 and 207 s^{-1}, respectively) into the Eyring equation yielded energy barriers within the already specified error limits.

An alternative technique for estimating exchange rates of dynamic systems is the saturation transfer method.[11] This is based on the fact that for a system in exchange it is possible for saturation (and NOE's) to be transferred from one species to another via chemical exchange, as indicated by

$$A \underset{k_B}{\overset{k_A}{\rightleftharpoons}} B$$

Saturation of a peak from species A (trans conformer) results in a change of intensity of the peak from the same proton in species B (cis I conformer) relative to the equilibrium intensity in the absence of saturation, according to eq 3.

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(10) Shanan-Atidi, H.; Bar-Eli, K. H. *J. Phys. Chem.* **1970**, *74*, 961.
(11) Sanders, J. K. M.; Mersh, J. D. *Progr. NMR Spectrosc.* **1984**, *15*, 353.

$$I_B/I_{B0} = (1 + k_A T_{1B}[A]/[B])^{-1} \quad (3)$$

Here, I_{B0} and I_B are, respectively, peak intensities in species B at equilibrium and during irradiation of the corresponding peak in species A. For the current system the peak intensities for H13b in the major conformer were measured following irradiation of H13b in the minor conformer, together with the appropriate T_1 value (0.33 ± 0.03 s), to yield k_A. Similarly, irradiation of the major conformer was used to determine k_B. At 178 K, the rates were found to be 4.0 and 7.1 s^{-1}, respectively. The equilibrium constant ($K = k_A/k_B$) derived from these values is in excellent agreement with that based on the relative intensities of peaks from the two observed conformers.

Substitution of the above rate constants into eq 2 led to calculated barriers for the forward and reverse reactions of 9.8 ± 0.9 and 9.5 ± 0.9 kcal/mol, respectively, in agreement with the values obtained from the dynamic NMR experiments.

Saturation transfer experiments were also employed to determine the energy barriers for interconversion of the two conformers of butaclamol hydrochloride in d_6-DMSO. They were performed at 300 K on signals from the H13b protons. Both ^1H T_1 values were 0.33 ± 0.03 s. Substitution of these values and the relative peak intensities into eq 3 yielded rate constants of 0.85 and 2.3 s^{-1} for k_A and k_B, respectively. As was the case for the free base, these values are in good agreement with the approximate 4:1 ratio predicted from the equilibrium constant (as obtained from peak integrals of the trans:cis I forms in Figure 1). Substitution of the rates into eq 2 yielded an average barrier of 17.2 ± 0.9 kcal/mol. Confirmation of this result was obtained from variable-temperature dynamic experiments where substitution of the rate at the coalescence temperature (377 ± 5 K) into eq 2 yielded an energy barrier of 17.3 ± 0.5 kcal/mol.

Discussion

In a theoretical and NMR study of butaclamol hydrochloride in $CDCl_3$ reported in the preceding paper,[1] we found that a trans conformation was adopted for the D–E ring junction. This contrasts with a previous study[4] which suggested that in DMSO two isomeric species of butaclamol hydrochloride were in slow exchange. These isomers were attributed to the trans and cis II[12] conformers of the ring junction. The finding of cis II as a preferred conformer conflicts with our theoretical studies[1] and with those of Froimowitz and co-workers,[3,5] where it was shown that this conformation exhibited a substantially higher energy than the trans or cis I conformers. The current study was undertaken to resolve these anomalies and to identify the conformations of the D–E ring junction under a variety of solvent conditions and protonation states.

The current experiments do confirm the existence of two conformers in DMSO, in agreement with Maryanoff et al.'s study.[4] However, the phase-sensitive NOESY spectrum in Figure 3 shows that the two resonances in conformational exchange are different from those quoted by Maryanoff et al. where, apparently, the resonance at 5.11 ppm was misassigned as proton 4a in the minor conformer instead of H13b. In the present work definitive assignments were made from a combination of DQF-COSY, NOESY, and coupling constant information.

Two alternatives have been considered here to explain the observation of a single set of resonances for butaclamol hydrochloride in $CDCl_3$, despite the fact that two sets of resonances are observed in DMSO. The first is that there may be fast exchange between various conformers in $CDCl_3$, and the second is that the solvent may play a significant role in stabilizing one particular conformer in $CDCl_3$.

The first explanation is considered unlikely on the basis of variable-temperature experiments in $CDCl_3$, which produced no evidence of "freezing out" of separate conformers down to 210 K. Neither did further experiments in CD_2Cl_2 down to 178 K show the presence of separate conformers. The variable-temperature experiments thus showed that in $CDCl_3$ (and CD_2Cl_2), either only one conformer (the trans form) exists (the relative population of a second conformer would have to be less than 5% at the S:N of the acquired spectra) or the barrier to interconversion between two possible conformers is less than the lower limit detectable in dynamic NMR studies (approximately 5 kcal/mol). The latter would seem unlikely, given that the barrier in DMSO was found to be approximately 17 kcal/mol.

A second, definitive experiment designed to distinguish between the two alternatives above was a titration experiment where aliquots of DMSO were added to the $CDCl_3$ solution of butaclamol hydrochloride. If the single sets of resonances observed in $CDCl_3$ was due to fast exchange in this solvent, then since two separate conformers are observed in DMSO, it should have been possible to generate an intermediate solvent mixture in which the exchange regime changes from fast to slow on the chemical-shift time scale. By contrast, if the single set of peaks in $CDCl_3$ was due to stabilization by the solvent, the titration experiment should have produced two sets of peaks of varying relative intensity as the solvent mixture is varied.

The absence of a coalescence phenomenon in this experiment, and the variation in intensity ratio with solvent mixture, leads to the conclusion that in $CDCl_3$, butaclamol hydrochloride adopts a single conformation, while in DMSO two unequally populated conformations exist.

Analysis of coupling constants showed that the two conformers in DMSO are trans and cis I. Interconversion between these forms requires inversion of the ring nitrogen, and it is likely that this process occurs via the free base, as shown below.

$$B_TH^+ \underset{}{\overset{\text{deprotonation}}{\rightleftharpoons}} B_T + H^+ \overset{\text{nitrogen inversion}}{\rightleftharpoons} B_C + H^+ \overset{\text{protonation}}{\rightleftharpoons} B_CH^+$$

Here, B_TH^+ represents butaclamol hydrochloride in the trans form, with B_T being the corresponding free base. Similar definitions apply to the cis form.

Nitrogen inversion was indeed observed for the free base in a variable-temperature experiment done in CD_2Cl_2. Here, a single averaged resonance is observed for H13b at room temperature but two conformers are seen at 178 K. Analysis of the coupling constants in the low-temperature spectra confirmed the existence of trans and cis I conformers as the major and minor forms, respectively. Some of the couplings in the individual conformers for the free base (Table II) are slightly different from the corresponding values in the hydrochloride salt (Table I). This is probably due to the different charge and hybridization of the nitrogen causing small variations in the torsion angle within the D ring.

The corresponding low-temperature measurements could not be made in DMSO because of its high freezing point, but all the evidence suggests that the room-tem-

(12) Cis II was designated cis A in ref 4a. It should be noted that in a subsequent correction to ref 4a, the authors pointed out some doubt in the precise nature of the cis form.[4b]

Table III. Chemical Shifts and Coupling Constants for Butaclamol Free Base in CD_2Cl_2 and d_6-DMSO at 297 K

proton	chemical shift[a] (ppm)	coupling constants (Hz)
13b		
CD_2Cl_2	4.78	5.9, 5.9
d_6-DMSO	4.67	5.5, 5.5
4a		
CD_2Cl_2	3.90	11.4, 3.2
d_6-DMSO	3.87	11.0, 2.8

[a] Chemical shifts measured relative to solvent peaks CD_2Cl_2 (5.32 ppm) and d_6-DMSO (2.49 ppm) and expressed relative to TMS.

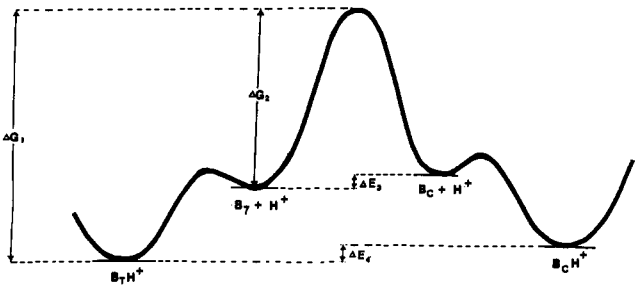

Figure 6. Schematic energy level diagram for interconversion of butaclamol between hydrochloride and free base forms. B_TH^+ corresponds to the hydrochloride in the trans form and B_T to the free base, with similar definitions applying to the cis I form.

perature spectrum represents a fast-exchange averaged case. The ratio of contributing conformers could not be accurately determined, but a comparison of averaged coupling constants in room-temperature spectra in DMSO and CD_2Cl_2 (Table III) provides some guidance. The similarity of values for the two solvents suggests that the population ratio in DMSO is similar to that in CD_2Cl_2 (ie, 2:1 for trans:cis I). There thus appears to be little differential stabilization of the trans form in CD_2Cl_2 relative to DMSO for the free base. This contrasts with the case for the hydrochloride, where the trans:cis I preference went from 4:1 in DMSO to greater than 20:1 in $CDCl_3$.

Having established the nature of the conformational isomers present and their dependence on solvent and protonation conditions, it is useful to summarize the energetics of their interconversion. Figure 6 is a schematic representation of the energy states of the conformers of butaclamol hydrochloride and its free base. The barrier ΔG_1 was determined to be 17.3 kcal/mol from measurements in DMSO, while the observed population ratio of 4:1 (trans:cis) in that solvent corresponds to a value for ΔE_4 of $\simeq$0.84 kcal/mol. This latter energy difference increases in $CDCl_3$, since no population of a cis I form could be detected, but it was not possible to determine whether this was due to stabilization of the B_TH^+ form or destabilization of B_CH^+ with a change of solvent.

The barrier ΔG_2 was determined to be 9.6 kcal/mol in CD_2Cl_2, ΔE_3 being approximately 0.24 kcal/mol (corresponding to a 2:1 ratio of cis:trans forms).

In conclusion, these NMR experiments for butaclamol hydrochloride and free base in $CDCl_3$ and d_6-DMSO show that solvent conditions and the protonation state of the drug have an important influence on the conformations and barriers to interconversion. These factors will play a key role in determining the biological activity of butaclamol. While neither DMSO nor $CDCl_3$ can be considered as biological media, they cover a range of polarities (as exhibited by their dielectric constants of 4.8 and 46.7, respectively) and are thus useful in indicating how the conformational behavior of butaclamol may vary as it moves from an aqueous environment in plasma to more hydrophobic regions in membranes and at receptor binding sites. The energy differences and barriers between trans and cis forms determined in this study are low and could readily be overcome by favorable energetic interactions with functional groups at receptor sites.[13] Thus, in the case of butaclamol it may be inappropriate to refer to *the* biologically active form, and it is possible that different forms (i.e., trans or cis) are populated at different receptor sites, particularly since butaclamol has been shown to bind to various receptor types.[14] While butaclamol is generally considered to exert its antipsychotic effects by binding to dopamine D_2 sites, it has affinity for other receptors (e.g., serotonergic) in different brain regions.[14] This may indicate that interactions via alternative conformations are responsible for some of its reported side effects.[15,16]

Some support for a proposal that it is not possible to uniquely define a single, biologically active form comes from the fact that the reported pK_a of butaclamol is close to 7.0.[17] In principle, then, significant populations of protonated and neutral species will be present at physiological pH. As the results of this study show that in the neutral species, interconversion between cis and trans forms occurs with a barrier of less than 10 kcal/mol, and that this could be overcome by functional group interactions during the binding process,[13] again it may be possible that different forms bind at different receptor sites.

Experimental Section

(+)-Butaclamol hydrochloride was purchased from Research Biochemicals and used without further purification. Butaclamol free base was obtained from the treatment of the hydrochloride salt with 2 M NaOH and extraction with chloroform. ^{1}H NMR spectra were recorded at 300 MHz at various temperatures on a Bruker AM 300 wide-bore spectrometer. Measurements were carried out for solutions of butaclamol hydrochloride in $CDCl_3$ (99.8%, Cambridge Isotope Laboratories) and d_6-DMSO (99.8%, Prolabo). For the free base, CD_2Cl_2 (99.65%, Aldrich) and d_6-DMSO were employed. In all cases 5-mm sample tubes were used. In a number of cases where additional resolution and sensitivity were required, spectra were recorded at 400 MHz on a Varian VXR-400 spectrometer.

Typical conditions for one-dimensional ^{1}H NMR spectra included a repetition delay of 2 s, a pulse flip angle of 45°, spectral width of 3000 Hz, and 16 K data acquisition points, zero-filled to 32 K. Saturation transfer experiments were carried out at various temperatures by acquiring one free induction decay (FID) where low-power, selective irradiation on the peak of interest was applied for at least 3 s prior to data acquisition, and a second FID where the decoupler was set in a control region of the spectrum containing no peaks. The extent of saturation transfer was obtained from the ratio of peak areas in the irradiated and control spectra. Peak areas were analyzed by cutting and weighing peaks and by using an off-line computer routine (Autosketch, Autodesk Australasia Pty Ltd.) on an IBM personal computer. Both methods were found to give equivalent results and were superior to the integration routine supplied with the spectrometer.

Two-dimensional phase-sensitive NOESY[18] and COSY[19] spectra were obtained using repetition times of 1.5 s with minimum spectral widths for the region of interest. A mixing time of 500

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ms was used in the NOESY spectrum. Spectra were acquired using 2048 × 512 real data points. Prior to Fourier transformation in the t_2 dimension, the data matrix was multiplied by a sine-bell function and phase-shifted by 22.5° and 45° for the NOESY and COSY spectra, respectively. The data in the t_1 dimension were multiplied by a sine-bell function, phase-shifted by 45° and 90°, respectively, and zero-filled to 1024 complex data points.

^1H T_1's were measured using the fast inversion recovery FT technique (FIRFT),[20] with a recovery delay of 1.5 s between 180°–τ–90° pulse sequences. Ten τ values were used, ranging from 0.02 to 6 s. Raw data were analyzed using a three-parameter exponential fitting program on the Aspect 3000 computer supplied with the spectrometer. T_1 values cited are an average from at least two experiments. The experimental error is less than ±10%.

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Electron-Deficient Isoalloxazines: Model Systems for Disulfide Prodrug Formation

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Drugs which contain a thiol functionality may be enzymatically or nonenzymatically oxidized to reactive metabolites, some of which cause adverse reactions. The synthesis of disulfide prodrugs to obviate unwanted drug side effects requires the development of novel catalysts. Herein, we describe the synthesis, structure–activity relationship, and mechanism investigations of the oxidation of model thiophenols with isoalloxazine disulfide formation catalysts. *m*-Nitrothiophenol (**31**) reacts with the electron-deficient 8-cyano-*N*-3-(mercaptoalkyl)-10-phenylisoalloxazines (**5–8**) and non-electron-deficient *N*-3-(mercaptoalkyl)-10-methylisoalloxazines (**1–4**) to produce *m*-nitrothiophenol disulfide. *m*-Nitrothiophenol (**31**) reacts with electron-deficient 8-cyano-10-phenyl-3-isoalloxazinepentanoic acid (**10**) or 10-methyl-3-isoalloxazinepentanoic acid (**9**) to form *m*-nitrothiophenol disulfide at a reduced rate, or not at all, respectively. Of the substituted isoalloxazines studied, electron-deficient isoalloxazines containing an *N*-3-mercaptoalkyl side chain were most efficient at catalyzing *m*-nitrothiophenol disulfide formation. Non-electron-deficient isoalloxazines without an *N*-3-alkyl mercaptan side chain (**9**) did not catalyze *m*-nitrothiophenol oxidation. Electron-deficient isoalloxazines without *N*-3-alkyl mercaptan side chains catalyzed *m*-nitrothiophenol oxidation at $^1/_{20}$ the rate for isoalloxazine **5**. From kinetic and product studies, the differences in catalytic activity of **1–10** were judged to be due to changes in the chemical properties of the isoalloxazines and the ability to stabilize intramolecular thiol attack on the C(4a)–N(5) bond of the isoalloxazine. Electron-deficient isoalloxazines may be useful catalysts for the syntheses of disulfide prodrugs.

Introduction

The therapeutic and toxic effects of many thiol-containing drugs including captopril (antihypertensive), penicillamine (antirheumatoid agent), 6-mercaptopurine and 6-thioguanine (cytotoxic agents), *N*-acetylcysteine (mucolytic agent), methimazole, carbimazole, and propylthiouracil (antithyroid agents), and spironolactonethiol (antihypertensive) are dependent upon the chemical reactivity of the mercaptan functional group.[1] Metabolism of thiol-containing drugs is generally extensive, and in many cases, the major metabolite is the disulfide or drug–cysteine disulfide. Extensive covalent binding and reversible disulfide bond formation with proteins in tissue has been observed and in some cases drug side effects may stem from disruption of disulfide bonds in enzymes or by formation of immunogenic drug–protein conjugates. Although a clear relation among covalent binding, disulfide bond formation, and adverse drug reactions has yet to be established, thiol-containing-drug action and toxicity may be influenced by factors which influence the metabolic formation and degradation of disulfides including NADPH levels, the glutathione:oxidized glutathione ratio, and the prooxidant stress status of the cell.[2]

One general approach to the delivery of thiol-containing drugs is to administer a disulfide prodrug. Disulfide prodrugs have been shown to be effectively taken up into cells and reduced to active parent drug within the cell.[3] Development of catalytic agents designed to produce disulfide-containing chemicals and drugs constitutes an important goal of bioorganic chemistry. There are few examples of enzymes that catalyze disulfide synthesis.[2] Glutathione, the predominant cellular thiol/disulfide couple is modulated by glutathione reductase, an enzyme which regulates the glutathione:glutathione disulfide ratio to greatly favor reduced glutathione.[2] Disulfide reductases including glutathione reductase,[4] lipoamide dehydrogenase,[3] and thioredoxin reductase[6] each contain a flavin (FAD) at the active site. Unlike disulfide reduction, the reduction of flavoenzymes by mercaptans (to produce disulfides) has been established in only a few cases.[7] For the few enzymes studied, the mechanism appears to involve nucleophilic attack of a thiol at the C-4a position of the flavin followed by breakdown of the C-4a adduct by displacement of the flavin by thiol[8] or other active site ligands.[9] A number of isoalloxazines have been developed

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