Conformational Equilibria in Amino Steroids. 1. A ^1H and ^{13}C NMR Spectroscopy and Molecular Mechanics Study of 3α-Hydroxy-2β-(4-morpholinyl)-5αH-androstan-17-one

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Abstract: The A ring conformational equilibrium of the title steroid has been investigated using high-field NMR spectroscopy. At 600 MHz the spectral dispersion is such that resolved signals can be seen for most protons. We present a complete analysis of the ^1H and ^{13}C spectra of the title compound in $CDCl_3$ and DMSO solutions and show that the conformation of ring A is determined by the medium: a chair is found in polar solvent, but in an apolar solvent a twist-boat is preferred. The drug design and drug transport implications of this equilibrium are discussed with regard to the neuromuscular blocking agent ORG 9426, whose A ring is modeled here. Molecular mechanics calculations are used to provide reasonable geometries for the two conformations.

Introduction

While the steroidal nucleus is generally associated with drugs having some kind of hormonal activity, the use of non-hormonally active steroids as anaesthetics and neuromuscular blocking agents is now widely documented.[1,2] These apparently novel applications of the steroid nucleus must be attributed in no small part to the clearly defined stereochemistry of the steroid. However, it is also necessary to consider the conformational properties of the steroid and the way in which the A ring substituents may modify the conformational equilibria. In particular, medium-induced effects may play a vital role in determining conformation and properties.

A number of attempts have been made to quantify the conformational equilibria in 3-hydroxy-2-amino steroids, and conformationally locked model decalins and cyclohexanes,[3-5] using IR[3] and NMR[4,5] data. Although these studies show evidence of conformational isomerism at ring A in one medium, they provide no data on the variation of the conformation with respect to solvent composition. Also, due to the nature of the available equipment,[4,5] the authors could only draw limited conclusions from the NMR spectra based upon the line widths of signals from protons α to substituents, or single coupling constant data in a very few cases.[5] It should also be noted that the amino and dimethylamino derivatives considered are of less intrinsic interest as neuromuscular blocking agents than those containing cyclic amines.[2]

From IR data it has been shown that 2β-dimethylamino-cholestan-3α-ol[3] and 3α-hydroxy-2β-amino-5αH-androstanes[6] exist in twist-boat conformations. This unusual conformation is stabilized by a combination of relief of steric strain on the β-face of ring A and formation of a hydrogen bond between the 3α-OH and 2β-N. It would be expected, however, that the A ring conformation of the native androstane skeleton would be a chair.

A number of X-ray diffraction studies of this class of molecules have been carried out[7-9] which, within the constraints of crystal packing considerations, provide direct evidence for both chair and twist-boat conformations. Two of these compounds, pancuronium bromide (3α,17β-diacetoxy-2β,16β-dipiperidino-5α-androstane dimethobromide)[7] and the related 16β monomethobromide (vecuronium bromide),[8] are potent neuromuscular blocking agents. Significantly, these are shown to exist in different conformations in the solid state: pancuronium assumes a twist-boat A ring, but vecuronium exists as a chair. While it is possible to understand the pancuronium structure as resulting from the increased steric crowding due to the quaternized 2β nitrogen, the A ring chair in vecuronium is quite surprising as there would still appear to be a considerable steric interaction between the ring substituent and the 10-methyl group of the steroid. This trend is also followed in a recently published structure containing a morpholino substituent at 2β.[9]

The importance of the ring D substituents in pancuronium and vecuronium has been rationalized by Savage[10] as presenting an acetylcholine-like fragment in a strictly defined geometry at the receptor, providing a complete blockade. As a result of a mistake in the original structure of (+)-tubocurarine, it was thought that a bis-quaternary compound was required, hence two acetylcholine fragments were incorporated in pancuronium. Upon correction,[11] the revised tubocurarine structure suggested that only one quaternary nitrogen was required, leading to the equipotent vecuronium.[12] However, this renders the exact purpose of the A ring substituents unclear, except in the general sense that they bind to some kind of subsidiary binding pocket at the receptor.

More recently, a new neuromuscular blocking agent, ORG 9426,[13] has been developed containing a morpholino substituent at 2β, together with a 3α-hydroxy. This compound, although not as potent as the piperidinyl, vecuronium, shows more rapid onset of action, leading to the postulate that the morpholinyl substituent may confer novel conformational properties on the steroid which improve drug transport properties. For this reason it was decided to investigate the conformational equilibria of amino steroids, using variable solvent NMR techniques to simulate the different media encountered during drug transport.

Analysis of the ^1H NMR spectra of steroids is difficult due to the very small shift range most of the protons fall into; typically, only protons α to substituents can be clearly assigned at 200 MHz. However, some recent studies using two-dimensional techniques, together with the added dispersion at high field, have shown that this problem is now no longer intractable.[14-16] In the case of the

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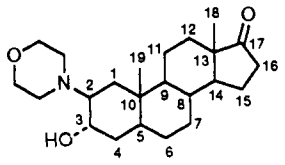
neuromuscular blockers, the problem still remains that the nitrogen substituents at the 2 and 16 positions result in β protons which are nearly identical in chemical shift (a similar situation arises due to the oxygen substituents at 3 and 17), making a full analysis of the ^1H spectrum (and hence the A ring conformation) very difficult. In order to eliminate this problem we have used the title compound I as a model of the A ring conformation of ORG 9426. Calculations show that the substituents on ring D cause very little change in the relative energies of the A ring conformations.[17] The title compound has also been shown to have some activity as an interneuronal blocking agent,[18] as observed by loss of righting reflex in the mouse, indicating that similar in vivo transport problems have been overcome from those experienced by the neuromuscular blocking agent.



I

From an analysis of ^1H spectra, it should be possible to estimate the degree of conformational change in ring A. However, quantitative estimates of this equilibria will be in error when such standard techniques as the reaction field method of Abraham[19] are used because of the possible presence of an intramolecular hydrogen bond in the twist-boat conformation. This would lead to greater stabilization than would be predicted by theory. As an alternative, it should be possible to obtain reasonable geometries from molecular mechanics calculations which can then be used to calculate model coupling constants. Analysis of the observed coupling constants with regard to these model values should provide information regarding the conformational equilibrium. We present here the results of a detailed study of I and show that ring A can adopt a chair or a twist-boat conformation and that the equilibrium between chair and twist-boat forms is in fact mediated by solvent effects.

Experimental Section

The synthesis of I from 2α,3α-epoxy-5α-androstan-17-one has been described.[6]

The 600.13-MHz NMR spectra were obtained from 10 mg of I dissolved in 0.75 mL of DMSO-d_6 and 0.75 mL of $CDCl_3$, respectively (35 mM). Conventional 1D and COSY 45 spectra were obtained at room temperature on a Bruker AM 600 spectrometer using the standard Bruker experiments. The 1D spectra were acquired with a digital resolution of 0.246 Hz/point ($CDCl_3$ solution) and 0.215 Hz/point (DMSO solution), and resolution enhancements of −0.5 Hz line-broadening and 0.2 Hz Gaussian broadening were applied to both sets of data. The $CDCl_3$ spectrum was referenced to a small amount of TMS added directly to the solution. The DMSO spectrum was referenced to the solvent signal DMSO-d_5 at 2.490 ppm (1494.3 Hz).

The ^{13}C experiments (standard 1D experiment, DEPT-135 and ^1H–^{13}C correlation) were performed on a Bruker AM 200 (50.3-MHz ^{13}C frequency), equipped with an Aspect 3000 computer and 5 mm dual $^1H/^{13}C$ probe, and using standard Bruker experiments. A 0.5 M solution was used for the 2D experiment and 0.15 M solutions for other experiments. The ^{13}C spectra were referenced to solvent signals ($CDCl_3$ 77.00 ppm; DMSO 39.50 ppm).

MM2 calculations were performed on a MicroVax 3900 using QCPE program no. 395.[20]

Results

The ^1H spectra of I in DMSO-d_6 and $CDCl_3$ are shown in Figures 1 and 2A, respectively. At 600 Mz the spectra are mostly first order (exceptions are discussed below), and the methine/methylene envelope is sufficiently well resolved as to make a total analysis of the spectrum feasible. In both cases the ^1H spectrum could be almost completely assigned from the COSY spectrum. As the COSY experiment does not always distinguish between geminal protons, additional arguments were made when necessary. The spectra were analyzed as far as was possible by using the Bruker computer program PANIC to produce an iterated fit of a simulated spectrum to the experimental spectrum. The strategy used was to divide the molecule into manageable units of approximately 5–7 spins and to simulate discrete fragments until as many parameters as possible had been fitted. There are 22 protons in the steroid backbone of I (excluding the 2β, 3α substituents and the 18- and 19-methyl groups) and they give rise to 8 geminal and 29 vicinal couplings. In general, for both DMSO-d_6 spectra and $CDCl_3$ spectra, about half a dozen peaks were fully resolved, about ten were partially overlapped with other peaks but with assignable transitions, and the remaining half a dozen peaks were more severely obscured by overlaps with other signals. The analysis (simulation) of a fully resolved multiplet is trivial; its chemical shift and the component couplings can be readily measured to the same accuracy as the line widths. For partially resolved peaks we could still, with varying degrees of success (depending on the extent of the overlap), assign enough transitions to achieve a good simulation, and obtain with confidence both the chemical shift and the contributing couplings. Where the peak was completely obscured we could only estimate the chemical shift from the position of the COSY cross peaks and infer couplings from its resolved neighbors. The ^1H and ^{13}C spectral parameters are presented in Tables I and II. Figure 2B shows the calculated spectrum for the twist-boat conformation using the parameters listed in Table I.

DMSO. The ^1H spectrum of the DMSO-d_6 solution was assigned from the COSY data (not shown) with no ambiguities. W couplings within the A ring distinguished the 1α–1β, and 4α–4β geminal pairs. The B and C rings were assumed to have chair conformations, so assignments of 6α–6β, 7α–7β, 11α–11β, and 12α–12β pairs were made by making a visual inspection of the multiplet (when visible) and/or considering the area of the COSY cross peak to identify the axial proton. Similarly in ring D, the quasiaxial protons 15β and 16α were identified by the presence of an extra, large coupling in the multiplet.

In six of the geminal pairs, the general rule that axial protons appear upfield of equatorial protons was followed. However, this ordering was reversed for the axial protons 4β and 6β. The assignment of 4β was confirmed by the W coupling between 4α and 2α observed in the COSY spectrum. The 4β proton is under the influence of two forces that are known to produce significant downfield shifts—the β face of the A ring is sterically crowded (steric compression shift), and its position relative to 3α-OH (trans, β) is expected to add a further 0.2 ppm downfield shift. The peak assigned to 6β gave rise to COSY cross peaks that were significantly larger than those from the peaks assigned to 6α. This reversal might also be the result of a steric compression shift caused by the general crowding of the β face in the all-chair conformation.

There are three regions in the ^1H spectrum where the signals from individual protons are not well resolved—4α and 6α at 1.14 ppm, 11β and 14α at 1.24 ppm, and 11α and 12β at 1.61 ppm. The signals from 1α, 1β, 4β, 5α, 6β, 8β, 12α, 15α, and 15β are only partially resolved, but it is possible to discern the couplings. Signals from 2α, 3β, 7α, 7β, 9α, 16α, and 16β are fully resolved and hence could be simulated easily. The results of this analysis are recorded in Table I.

The 18- and 19-methyl assignments followed from the assignment of the $CDCl_3$ spectrum (see below).

The ^{13}C spectrum was tentatively assigned by making correlations between a DEPT-135 spectrum and published ^{13}C spectra

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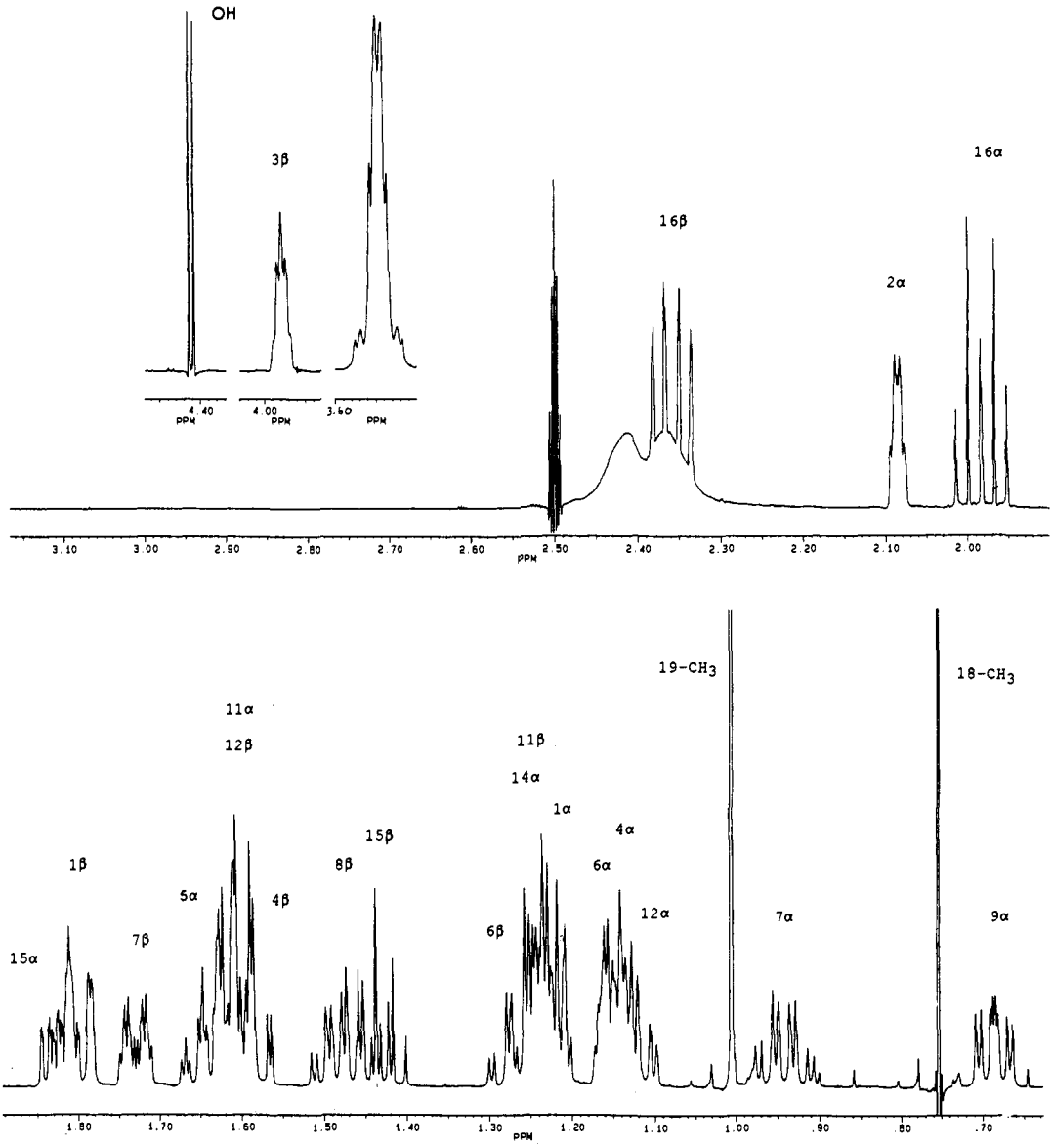

Figure 1. 600-MHz ^{1}H spectrum of I in DMSO-d_6 solution. Nonassigned peaks at 3.6 and 2.4 ppm are due to the morpholino group.

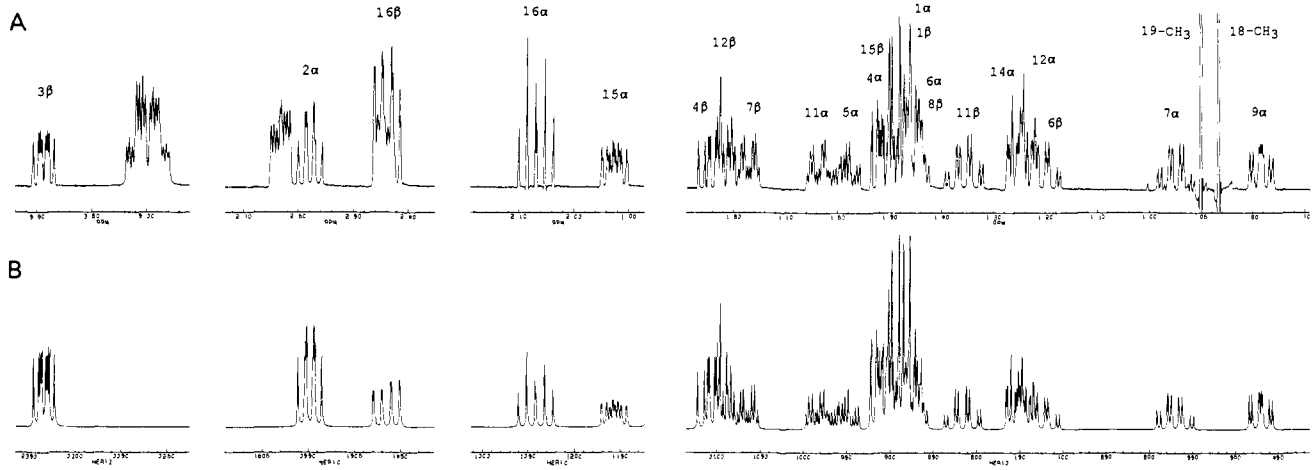

Figure 2. 600-MHz ^{1}H spectrum of I in $CDCl_3$ solution. The experimental spectrum is shown in A, and B shows the calculated spectrum using the parameters listed in Table I. The unlabeled peaks at 3.7, 2.65, and 2.45 are from protons on the morpholino group.

of 5α-androstanes and androstan-17-ones,[21] which have regular conformations (i.e., A, B, and C rings—chair). Any uncertainties from this approach were clarified by following peak positions during a titration of DMSO into a $CDCl_3$ solution (rigorously assigned below). The shifts are unremarkable.

$CDCl_3$. The ^{1}H spectrum of the $CDCl_3$ solution was assigned from its COSY spectrum with the same considerations as for the DMSO spectrum.

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Table I. ^{1}H Shifts and Coupling Constants for I in DMSO and $CDCl_3$

	shift, ppm			J, Hz			shift, ppm			J, Hz	
	DMSO	$CDCl_3$		DMSO	$CDCl_3$		DMSO	$CDCl_3$		DMSO	$CDCl_3$
1α	1.213	1.459	1β	−12.7	−14.2	8β	1.474	1.456	7α	11.1	12.0
			2α	3.1	7.4				7β	3.6	3.5
1β	1.787	1.497	1α	−12.7	−14.2				9α	10.6	11.1
			2α	2.9	8.9				14α	11.2	10.4
			3β	0.3		9α	0.676	0.782	8β	10.6	11.1
2α	2.075	2.579	1α	3.1	7.4				11α	3.8	3.4
			1β	2.9	8.9				11β	12.3	12.6
			3β	3.0	9.5	11α	1.61	1.638	9α	3.8	3.4
			4α	0.4					11β		−13.2
3β	3.969	3.888	1β	0.3					12α	5.9?	4.5
			2α	3.0	9.5				12β		3.4
			4α	3.4	5.8	11β	1.24	1.357	9α	12.3	12.6
			4β	2.5	7.8				11α		−13.2
4α	1.14	1.514	2α	0.4					12α		13.1
			3β	3.4	5.8				12β		3.9
			4β	−13.0	−13.8	12α	1.166	1.247	11α	5.9?	4.5
			5α	3.7	6.8				11β		13.1
4β	1.579	1.839	3β	2.5	7.8				12β		−12.9
			4α	−13.0	−13.8	12β	1.61	1.814	11α		3.4
			5α	12.7	11.9				11β		3.9
5α	1.634	1.585	4α	3.7	6.8				12α		−12.9
			4β	12.7	11.9	14α	1.24	1.251	8β	11.2	10.4
			6α	3.4	3.6				15α	5.8	5.8
			6β	13.1	12.1				15β	12.4	12.4
6α	1.14	1.453	5α	3.4	3.6	15α	1.812	1.926	14α	5.8	5.8
			6β	−13.2	−13.3				15β	−12.4	−12.4
			7α	4.3	4.1				16α	9.0	9.1
			7β	2.8	3.1				16β	0.9	1.1
6β	1.252	1.207	5α	13.1	12.1	15β	1.427	1.501	14α	12.4	12.4
			6α	−13.2	−13.3				15α	−12.4	−12.4
			7α	13.0	13.1				16α	9.1	9.2
			7β	3.7	3.7				16β	8.9	9.1
7α	0.933	0.949	6α	4.3	4.1	16α	1.973	2.068	15α	9.0	9.1
			6β	13.0	13.1				15β	9.1	9.2
			7β	−12.8	−12.9				16β	−19.1	−19.4
			8β	11.1	12.0	16β	2.347	2.439	15α	0.9	1.1
7β	1.720	1.772	6α	2.8	3.1				15β	8.9	9.1
			6β	3.7	3.7				16α	−19.1	−19.4
			7α	−12.8	−12.9	18	0.743	0.866			
			8β	3.6	3.5	19	0.994	0.900			

Table II. ^{13}C Shifts for I in $CDCl_3$ and DMSO (ppm)

	DMSO	$CDCl_3$		DMSO	$CDCl_3$
1	32.0	32.5	12	31.5	31.5
2	66.0	65.0	13	47.2	47.8
3	63.7	63.6	14	50.8	51.2
4	32.1	34.1	15	21.4	21.6
5	39.1	38.5	16	35.4	35.7
6	27.4	27.9	17	219.3	221.1
7	30.7	30.4	18	13.5	13.8
8	34.1	34.9	19	12.5	16.5
9	54.7	55.9	20	51.7	49.0
10	36.1	35.9	21	66.6	67.4
11	19.9	20.5			

Insufficient spectral dispersion was less of a problem in the $CDCl_3$ spectrum, and we were able to completely analyze all of the couplings from the spectrum shown in Figure 2. It can be seen that 2α, 3β, 5α, 7α, 7β, 9α, 11α, 11β, 15α, 16α, and 16β are all fully resolved. There are slight overlaps between 4β and 12β (~1.82 ppm) and between 14α, 12α, and 6β (~1.23 ppm); these overlaps do not prevent an unambiguous analysis of the shifts and couplings present in these multiplets. Signals from six protons (1α, 1β, 4α, 4β, 6α, 8β and 15β) fall into the crowded region from 1.42 to 1.54 ppm (25 resolved transitions), and even this region was uniquely fitted after close scrutiny of the experimental data and trial simulations (see Figure 2B).

The multiplets from the geminal pair 4α and 4β cannot be distinguished on the basis of the data in Figure 1. Both permutations were considered and calculated spectra were iteratively fitted with PANIC. The 4α–4β–5α spin system shows some second-order character, and a significantly better fit was obtained with the peak at 1.839 ppm assigned to 4β. In addition, only this permutation produced vicinal couplings to 3β and 5α which were self-consistent with a twist-boat conformation for ring A. (For example the reversed permutation produced 6.8 Hz for the almost trans 4β–5α interaction.) Similar arguments were used to fix the 1α and 1β chemical shifts.

As an additional check on these important assignments, and to facilitate the analysis of the highly overlapped 1.42 to 1.54 ppm region, we obtained spectra of I in C_6D_6 solution (to be published). In this solvent the twist-boat/chair conformer ratio is 70:30 and the dispersion in the spectrum was much improved. COSY and NOESY experiments confirmed all of the above discussion.

A ^{1}H–^{13}C correlation experiment was necessary in addition to DEPT 135 to make assignments of the ^{13}C spectrum for this nonstandard steroid conformation. An unequivocal assignment of the ^{13}C spectrum was possible by correlating cross peaks in the heteronuclear 2D experiment with ^{1}H shifts that had been fixed by the COSY experiment. This was particularly important for an unambiguous assignment of the closely spaced signals from carbons 6, 7, and 12 as well as 11 and 15.

The axial methyl assignment was made by following the chemical shift changes in a DMSO/$CDCl_3$ titration experiment.

With the exception of some of the couplings from protons neighboring ring C (DMSO solution), the couplings listed in Table I have been fixed from "both directions". The simulated spectral transitions matched the experimental spectra to within at least 0.2 Hz (0.1 Hz for most transitions), i.e. the same as the digital resolution of the spectra, so we believe that these figures for the J values are probably accurate to ±0.3 Hz. The chemical shifts where fitted are ±0.002 ppm, and those not fitted are ±0.01 ppm.

The infrared spectrum of I in CH_2Cl_2 contains a strong, broad

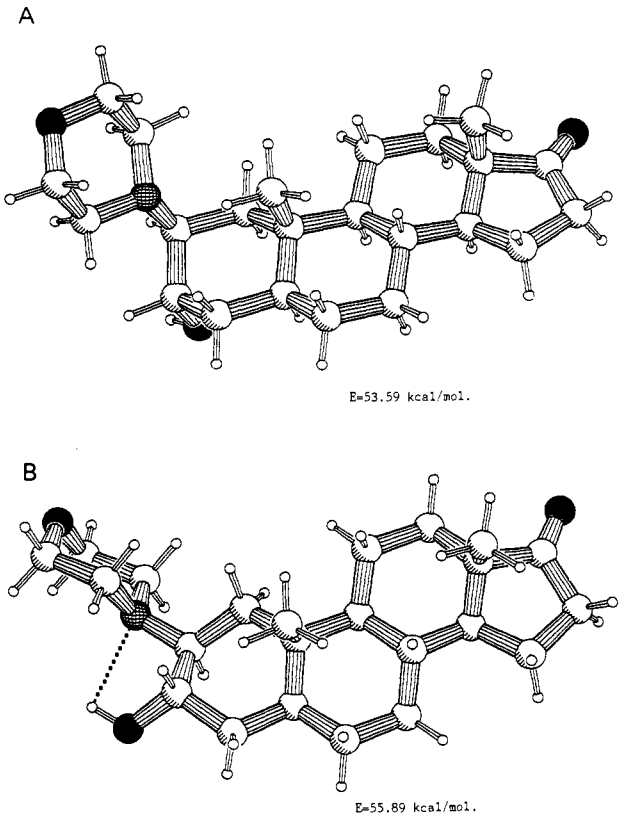

Figure 3. Molecular mechanics geometries and energies of (A) the A ring chair conformation of I and (B) the A ring twist-boat.

absorption at 3445 cm^{-1}. This confirms the presence of a hydrogen bond in nonpolar solvents.[3]

Discussion

The MM2[20] optimized geometries of the two possible ring A conformers are shown in Figure 3. Although it has been postulated[3] that four conformers (chair, twist-boat, α-boat, and β-boat) are possible for these systems, only the chair and twist-boat were stable on minimization. The orientations of the substituents were obtained using the two-angle dihedral drive facility in MM2, showing clearly that there is some stabilization due to the hydrogen bond between the 2β-N and 3α-OH substituents. The small energy difference between the conformers is also an indication of added stabilization of the twist-boat.

It is apparent from the experimental results that there is a conformational difference between the spectra in the two solvents. This is also borne out by spectra acquired in a variety of solvents of differing polarity (to be published) showing dynamically averaged line shapes intermediate between those presented here. Using the above geometries, we have obtained the calculated dipole moments for each conformation using the charge scheme due to Abraham and co-workers.[22] The values are 2.70 and 2.77 D for a chair and twist-boat, respectively. From reaction field considerations[19] it would be expected that the twist-boat would be the preferred conformation in DMSO solution. The solvent continuum approach cannot, however, take into account specific atom–atom solvent–solute interactions, and therefore the effect of hydrogen bonding is poorly treated. A second feature of steroid molecules, and I in particular, is that the most important dipoles can be effectively isolated from each other rendering the concept of solvent screening inadequate. This is illustrated by the pregnan-20-one corresponding to I which shows a dramatic reversal in dipole moments (chair, 3.46 D; twist-boat, 2.59 D); however, the NMR spectra are identical to that of I (unpublished results).

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Table III. Calculated and Experimental ^3J Values (Hz) for the Chair and Twist-Boat Conformations of Ring A

	dihedral	calcd J		obsd ^3J
		a	*b*	
		Chair		
$1\alpha2\alpha$	−51.3	3.1	3.8	3.1
$1\beta2\alpha$	61.0	1.9	2.7	2.9
$2\alpha3\beta$	61.9	5.9	2.6	3.0
$3\beta4\alpha$	54.6	2.0	3.9	3.4
$3\beta4\beta$	−61.5	1.3	2.2	2.5
$4\alpha5\alpha$	59.7	3.9	3.0	3.7
$4\beta5\alpha$	176.5	16.5	12.4	12.7
		Twist-Boat		
$1\alpha2\alpha$	41.6	4.6	6.5	7.4
$1\beta2\alpha$	155.5	13.8	10.1	8.9
$2\alpha3\beta$	180.0	12.0	9.7	9.5
$3\beta4\alpha$	138.5	11.1	6.6	5.8
$3\beta4\beta$	23.2	7.1	9.0	7.8
$4\alpha5\alpha$	42.0	7.3	6.2	6.8
$4\beta5\alpha$	157.1	14.4	11.0	11.9

[a] Calculated with program GANDOUR provided by D. Kelly,[25] University College, Cardiff, 1986. This program solves the equation proposed by Colucci, Jungk, and Gandour.[24] It solves eq 8 of that paper using the substituent constants from Table II of the same paper and a value of 0.04 for all alkyl groups. [b] Calculated with program 3JMM2 provided by C. A. G. Haasnoot. This program solves eq 8 of Haasnoot, de Leeuw, and Altona.[23]

In addition, the solvent cavity is also found to be very insensitive to the conformational change due to the local nature of the equilibrium with respect to the overall molecular volume. Most of the above observations are in line with the already documented limitations of this method.[19]

As the conformations cannot be predicted on the basis of dipole moments, it is necessary to compare the coupling constant data to model conformations. Taking the dihedral angles from the chair and twist-boat conformations, the modified Karplus equations of Haasnoot et al.[23] and Gandour[24] were used to calculate the seven vicinal couplings within ring A. These are compared to the experimental ^3J values, for DMSO and $CDCl_3$ solutions, in Table III. The Gandour method is not strictly applicable to the steroid problem as alkyl groups were not included in the original parameter set. A generic value of 0.04 for all alkyl groups was found to be suitable for most structures but, to our knowledge, this value has never been rigorously defined.[25] These data are included for reference and comparison purposes only. The data in column a were obtained by a more general method, which should give couplings with a root-mean-squared deviation of 0.5 Hz.[23]

An examination of the coupling constants in Table III shows a reasonable degree of consistency between the experimental and calculated values, indicating that in each solvent one conformer is predominant. There is generally good agreement between the chair conformation and the couplings in DMSO. If it is assumed that the spectrum in DMSO results from an A ring chair, with 60° gauche dihedrals, then it would be expected that $J(1\beta2\alpha) > J(1\alpha2\alpha)$ on the basis of the relative orientation of the electronegative nitrogen.[26] The opposite is the case, implying a decrease in dihedral $H(1\alpha)–C_1–C_2–H(2\alpha)$ and an increase in dihedral $H(1\beta)–C_1–C_2–H(2\alpha)$. A similar picture is found for $J(3\beta4\alpha)$ and $J(3\beta4\beta)$. It is evident, therefore, that there is considerable flattening of the chair at positions 2 and 3, due to the steric interaction between the morpholine group and the 10-methyl.

The coupling constants from the $CDCl_3$ spectrum show qualitative agreement with the twist-boat model coupling constants although, here, the agreement is less good than above. The fact that the dihedral angles found for a twist-boat were not well

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Table IV. Calculated and Experimental Coupling Constants for the D Ring of I in $CDCl_3$ and DMSO

	$^3J_{expt}$[a]	$^3J_{expt}$[b]	$^3J_{calc}$[c]
14α-15α	5.8	5.8	5.4
14α-15β	12.4	12.4	11.8
15α-16α	9.0	9.1	11.1
15α-16β	0.9	1.1	1.1
15β-16α	9.1	9.2	7.6
15β-16β	8.9	9.1	11.0

[a] DMSO solution. [b] $CDCl_3$ solution. [c] Calculated using 3JMM2.[23]

represented in the parameterizing data-set of Haasnoot et al.[23] may explain this. The most important 2α-3β coupling is, however, well-reproduced. This is consistent with a predominance (>90%) of the twist-boat conformation in $CDCl_3$.

The remaining coupling constants for the B and C rings are generally in good agreement with the calculated values, with the exception of those to the 8β and 14α protons (data not shown). As these protons are attached to tertiary centers it is possible that additional strain effects, which the Karplus equation does not consider, are coming into play. There is nothing to suggest that the B and C rings deviate from chair conformations. The D ring couplings have been compared to those determined by Schneider et al.[14] for 5α*H*-androstan-17-one. The values are almost identical, indicating that the substituents on the A and D rings do not appear to interact in a way which disrupts the individual conformational equilibria. In Table IV the observed and calculated coupling constants are compared for both solvents. On the basis of these data, and the energy profile for rotation about the C15-C16 bond, it is possible to speculate that there is some interconversion between half-chair and envelope conformations.[14]

The 200-MHz ^1H spectrum of I in $CDCl_3$ (and in mixed $CDCl_3$/DMSO) was also studied from -20 to 50 °C and showed no evidence of any dynamic effects.

Conclusions

In this study we have shown that the ^1H NMR spectra of steroids are amenable to analysis using high-field (600 MHz) instruments, together with 2D experiments to aid with assignment. More importantly, this has allowed the examination of conformational problems.

The solvent-dependent nature of the conformation of I indicates that while steroids offer a rigid stereochemical skeleton upon which to base drug molecules, the conformational changes resulting from the incorporation of certain substituents may have a profound effect on properties. In the case of I it is clearly shown that the molecule assumes a twist-boat ring A in a hydrophobic medium such as $CDCl_3$; in effect the polar group charges are masked via a hydrogen bond. In the much more polar DMSO solution the A ring of the steroid is a chair with the morpholino and hydroxy groups trans-diaxial. The polar groups in this case may be solvated by intermolecular hydrogen bonds with solvent molecules. This is a specific atom-atom solvation effect as opposed to the more general reaction field where solvation is mediated by the solvent dielectric.[19] In future work we will apply molecular dynamics methods to the question of the individual contributions of steric strain and hydrogen bonding to the conformational equilibria.

The medium-dependent behavior of I also has important implications for drug design in that conformational changes in the steroid may facilitate the passage of the molecule through membranes, while a different conformation, and hence different spatial arrangement of substituents, will be assumed at the more polar receptor site.

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Bond Dissociation Energies in DMSO Related to the Gas Phase

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Abstract: Estimates have been made of the homolytic bond dissociation energies (BDEs) for (a) the benzylic or allylic H-C bonds in 14 hydrocarbons, (b) the acidic H-C bonds in 12 hydrocarbons containing one or more heteroatoms, and (c) the H-N bonds in five nitrogen acids as well as thiophenol and phenol. For the 18 compounds where literature gas-phase values were available, agreement to within ±2 kcal/mol was observed for all but three (Ph_3CH, $PhNH_2$, and PhOH). For Ph_3CH and $PhNH_2$, the literature values were shown to be in error. For the BDEs of the acidic H-A bonds in 17 compounds, error limits of ±2 kcal/mol, or better, were established from BDE estimates made for three or more derivatives in which remote substituents were placed on the benzene ring of the parent compound. In all, the BDEs of the acidic H-A bonds of 32 compounds have been established to ±2 kcal/mol or better.

Quantitative measurements in the gas phase by a variety of methods have yielded estimates of homolytic bond dissociation energies (BDEs) that have generally been considered to provide the best measures of relative radical stabilization energies obtained to date.[1] In our laboratory we have developed a method of

$$\Delta BDE(kcal/mol) = 1.37\Delta pK_{HA} + 23.06\Delta E_{OX}(A^-) \quad (1)$$

estimating relative BDEs, e.g. eq 1, for families of acids, HA, by combining equilibrium acidity constants, pK_{HA}, with the oxidation potentials of their conjugate bases, A^-, both measured in DMSO.[2] The method is based on a thermodynamic cycle and is the solution counterpart of one devised by Brauman to estimate BDEs from gas-phase acidities, electron affinities, and ionization potentials.[3]

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