and low-pH forms of PLP are in excellent agreement with experiment, but the agreement is less satisfactory for PMP. The results reported above predict that for HPH and PMP the absorption spectrum has one major band assigned to the $\pi \rightarrow \pi^*$ excitation, as has long been the accepted interpretation.[6,18,31] However, for LPH the predicted absorption spectrum consists of two intense and two weak transitions which are close enough in energy to form a single absorption band, in agreement with the enzyme solution spectrum.

This method has also afforded an evaluation of the effects of H-bonding interactions on the spectrum of the PLP chromophore by explicitly including selected side chains of active site residues in the calculations. All H-bonding interactions considered here have led to a blue shift in the spectrum, with the single exception of the Asp222 group in LPH. In addition the effects of π-stacking interactions of Trp140 with the PLP or PMP pyridine ring were also found to lead to a blue shift.

The first aim of these studies (see Introduction), i.e., the applicability of the theoretical methods to predict the chromophore's spectrum and account for the effects due to interacting groups of mAspAT, has been satisfactorily met. Some questions remain, in particular regarding the state of protonation which may actually exist under experimental conditions. While these questions cannot be answered solely on the basis of the calculations reported here, the results discussed above lend insight into the effects of the different possible states of protonation on the spectra. The second purpose of this project, i.e., to test the sensitivity of the predicted spectrum to structural changes, has been only partly achieved by the calculations reported here. Studies in progress on the complex of mAspAT with 2-methylaspartate will provide a more rigorous test of this sensitivity.

In assessing the reliability of the calculations it has to be kept in mind that the structural data used here represent a time average of the positions available to the protein atoms in the crystal. The observed spectrum corresponds to a Boltzmann distribution of transitions obtained from distinct structures which sample the accessible points on the energy hypersurface. Thus to obtain a correct prediction of a transition energy, it would be necessary to carry out calculations for all possible geometries and then average over the Boltzmann distribution. Such an approach has been implemented for small molecules embedded in a solvent[34] but, given current computational capabilities, would be extremely expensive for systems of the size and complexity treated in this work. It is therefore gratifying that the predicted spectra agree as well as they do with the experimental ones.

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Conformational Equilibria in Amino Steroids. 2. Energetics of the Chair-Twist-Boat Equilibrium in Ring A of 3α-Hydroxy-2β-(4-morpholinyl)-5α(H)-androstan-17-one

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Abstract: The equilibrium between chair and twist-boat conformations in ring A of the title compound has been studied by variable solvent, high-field ^{1}H and ^{13}C NMR. The distribution between chair and twist-boat forms was estimated from a consideration of vicinal ^{1}H coupling constants within the A ring and also the ^{13}C chemical shift of a "marker group" (10-methyl) that was shown to be sensitive to the conformational change. A unique aspect of the study is that the population analysis was performed in such a way that the results are not dependent on a Karplus equation. The driving force for the transition from a chair conformation to a twist-boat was found to be a fine balance between steric effects, electronic effects, and hydrogen bonding. The energy difference between the chair and twist-boat forms is near zero and ranges from -1.1 to $+1.2$ kcal mol^{-1}, depending on the medium. The intramolecular hydrogen bond contributes approximately 1.5 kcal mol^{-1} to the stability of the twist-boat conformation.

Introduction

An understanding of the preferred stereochemistry of flexible molecules is an essential prerequisite to studies of structure-activity relationships. But, just as there is no guarantee that a molecule has the same structure in solution as was found in a crystal, one cannot always safely assume that a molecule bound to a receptor has the same structure as in solution. Hence there is general interest in determining the structures and distribution between conformers of flexible molecules, particularly when the molecule is a useful drug. Steroids form the building blocks of many important and useful drugs, and it should be recognized that even steroids have flexible regions that are potentially capable of adapting in response to their environment.

We have previously shown that ring A of 3α-hydroxy-2β-(4-morpholinyl)-5α(H)-androstan-17-one (I) is flexible and adopts a chair conformation in DMSO-d_6 solution and a twist-boat conformation in $CDCl_3$.[1] In this article we explore more fully the effect of solvent on the chair-twist-boat equilibrium and show that, in the 16 solvents studied, solutions of I exist as mixtures of chair and twist-boat conformers in a dynamic equilibrium.

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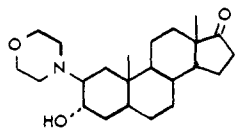
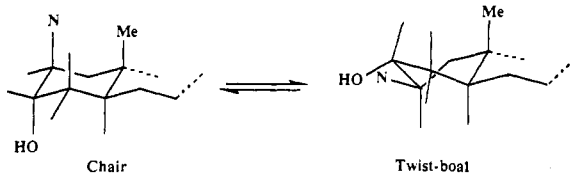


I

The title compound is of particular interest because the vicinal amino alcohol (usually acetylated) is an essential substructure in many neuromuscular blockers,[2] and 3α-hydroxy steroids are neuroactive.[3] Earlier studies have shown that 3α-hydroxy-2β-amino cyclohexanes,[4] decalins,[5] and steroids[5,6] exist in solution as mixtures of the chair and twist-boat forms, but a rational explanation of the conformational change has not been forthcoming. Recent X-ray studies have revealed ring A in a chair form in 3α,12β-dihydroxy-2β-morpholino-5α-pregnan-20-one,[7] and both chair and twist-boat conformations of ring A are known in neuromuscular blockers which contain the 3α-acetyl-2β-piperidino-5α-androstane substructure.[8]



Chair Twist-boat

Several competing effects influence the conformation of ring A in solutions of I. The expected conformation of ring A in an unsubstituted androstane is a chair. In I the chair conformation is destabilized by steric strain between the axial 2β-morpholino and the 10β-methyl groups (labeled C_{19}). The twist-boat conformation is stabilized by an intramolecular hydrogen bond between 2β-N and 3α-OH. Polar solvents will favor the more polar conformation and hydrogen-bonding solvents will interfere with the OH···N hydrogen bond, destabilizing the twist-boat conformation. The steric contributions can be assessed by molecular mechanics calculations and can be studied experimentally in a series of 2β-amines. We also have data for analogues of I that qualitatively show that small amines favor chair conformations and larger amines favor twist-boat conformations.[9] Here we attempt to separate the remaining effects (hydrogen bonding and medium polarity) by undertaking a study of the conformational equilibrium in I in a wide variety of different solvents.

The high-field ^{1}H NMR spectra of steroid hormones have been reviewed,[10] and more recently some interest has been shown in using ^{1}H NMR to establish the conformation of ring A in some 4-en-3-one steroids.[11-13] Literature examples of NMR studies of nonchair conformations of ring B[14] and ring C[15] of steroids can also be found.

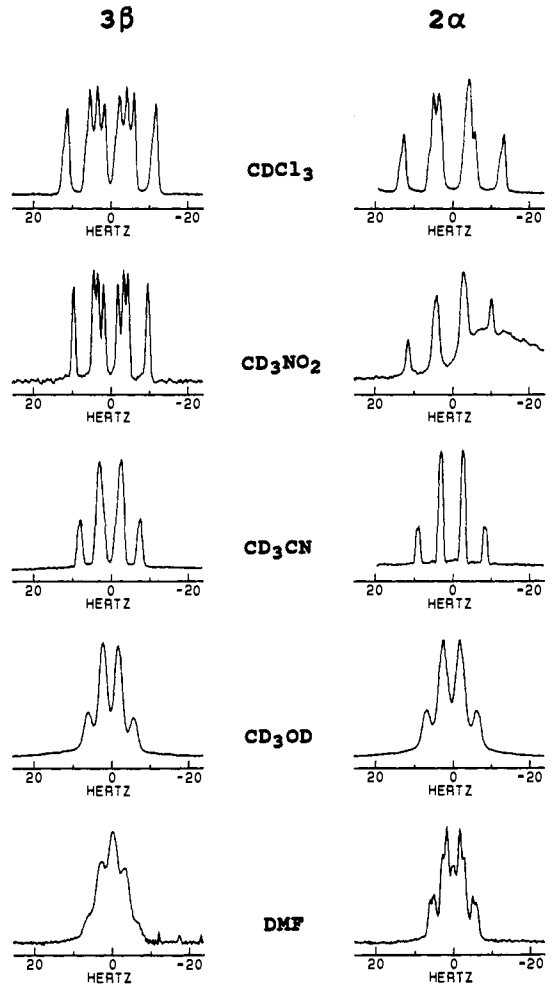


Figure 1. 600-MHz ^{1}H NMR spectra of the 2α and 3β protons of I in several solvents. The signal from 2α overlaps with signals from morpholine protons in the CD_3NO_2 spectrum.

Experimental Section

The synthesis of I from 2α,3α-epoxy-5α-androstan-17-one has been described.[16]

Most of the ^{1}H NMR spectra were obtained at 600.13 MHz. Some data ($CDCl_3$ and THF-d_8 solutions) were collected at 500.14 MHz. The ^{1}H NMR spectra were obtained from 10 mg of I dissolved in 0.75 mL of deuterated solvent (35 mM). Conventional 1D spectra and COSY 45 data were obtained at ambient temperature on a Bruker AM 600 (or AM 500) spectrometer and were downloaded to disc for subsequent remote processing. The 2D data were processed on a Silicon Graphics workstation running NMRi software. The 1D data were processed on an Aspect 3000 computer (in an AM 200) and were fitted with the Bruker spectrum simulation program PANIC. A small quantity of TMS was used as internal reference in the C_5D_5N, THF-d_8, C_6D_6, CD_2Cl_2, and $CDCl_3$ solutions. The other spectra were referenced to residual solvent as follows: DMSO-d_6, 2.49; DMF-d_7, 2.74; CD_3OD, 3.30; acetone-d_6, 2.04; CD_3CN, 1.93; toluene-d_8, 2.03; CD_3NO_2, 4.33.

The ^{13}C NMR spectra were obtained from approximately 0.1 M solutions of I in a variety of deuterated and nondeuterated solvents. Data from solutions in nondeuterated solvents were collected with the sample spinning and the field sweep switched off. The data were obtained on a Bruker AM 200 spectrometer (50.3-MHz ^{13}C frequency) fitted with a 5-mm dual probe. In most cases the ^{13}C spectra were referenced to the solvent signal (see Table IV).

A cross-polarization magic angle spinning ^{13}C NMR spectrum of I was obtained at room temperature on a Bruker MSL-100.

Results

The ^{1}H NMR spectrum of I is remarkably solvent dependent. In all solvents only one species was observed, but the A-ring vicinal couplings varied. This behavior is consistent with two confor-

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Table I. Selected ^1H Shifts and Ring A Coupling Constants for **I** in Various Deuteratd Solvents[a]

shifts (ppm)	DMSO-d_6	DMF-d_7	C_5D_5N	CD_3OD	THF-d_8	acetone-d_6	CD_3CN	toluene-d_8	CD_3NO_2	C_6D_6	CD_2Cl_2	$CDCl_3$
1α	1.21	1.34	1.62	~1.3	1.38	1.39	1.32	1.12	1.44	1.19	1.45	1.46
1β	1.79	1.87	1.88	1.86	1.82	1.82	1.70	1.30	1.67	1.36	1.51	1.50
2α	2.08	2.19	2.53	2.28	2.20	2.27	2.29	2.19	2.46	2.30	2.54	2.58
3β	3.97	4.10	4.43	4.09	4.02	4.08	3.95	3.68	3.95	3.81	3.87	3.89
4α	1.14	1.24	1.60	~1.3	~1.2	1.29	1.27	1.43	1.37	1.57	~1.4	1.51
4β	1.58	1.69	1.93	1.77	~1.7	~1.7	1.66	1.68	1.78	1.80	1.80	1.84
5α	1.63	1.75	2.06	1.67	~1.7	~1.7	1.61	1.40	1.62	1.49	1.58	1.59
19	0.99	1.07	1.11	1.06	1.06	1.06	0.98	0.71	0.99	0.77	0.90	0.90
18	0.74	0.82	0.83	0.85	0.80	0.82	0.81	0.56	0.85	0.64	0.84	0.87
couplings (Hz)	DMSO-d_6	DMF-d_7	C_5D_5N	CD_3OD	THF-d_8	acetone-d_6	CD_3CN	toluene-d_8	CD_3NO_2	C_6D_6	CD_2Cl_2	$CDCl_3$
1α1β	-14.0	-14.2	-14.0	-14.6	-14.1	-14.3	-14.2	-14.1	-14.0	-14.0	-13.9	-14.2
1α2α	4.4	4.7	4.5	~4.5	5.0	5.2	5.8	6.8	6.4	7.1	6.8	7.4
1β2α	2.9	3.2	3.5	4.1	3.9	4.2	5.6	7.1	7.3	6.9	8.9	8.9
1β3β	0.5	0.7	-	0.8	0.9	0.9	0.5	-	-	-	-	-
2α3β	3.1	3.3	3.6	~4	4.0	4.7	5.7	7.0	7.5	7.3	9.3	9.5
2α4α	0.7	1.0	-	0.7	0.9	1.0	0.7	-	-	-	-	-
3β4α	3.4	3.3	3.7		~4		5.0	5.4	5.2	5.2	5.6	5.8
3β4β	2.8	2.7	2.8	3.7	3.3		5.2	6.1	6.4	6.25	7.5	7.8
4α4β	-13.0	-13.6	-12.7	-13.6			-13.5	-14.0	-13.8	-13.8	-13.9	-13.8
4α5α	3.7	4.1	3.7				5.0	5.4	5.4	5.3	6.5	6.8
4β5α	12.7	13.0	12.7	13.2			12.5	12.6	12.2	12.3	12.2	11.9
3β-OH	3.5	3.4	3.4	-	3.4	3.2	-	-	-	-	-	-
ax. CH_3	-	~0.3	-	0.5	-	0.6	0.6	-	0.6	~0.3	0.4	-

[a] A blank space indicates that spectral overlaps and/or strong second-order effects prevented a reliable estimate of the coupling; - indicates the coupling was not resolved.

mations in fast exchange on the NMR time scale, with the position of the equilibrium being determined by properties of the solvent. Figure 1 illustrates the effects on the 3β and 2α protons from five representative solvents. The signals from this pair of protons were well-separated from other peaks in the spectra and are a vivid visual indicator of the state of the equilibrium position. In the chair conformation, 2α and 3β are both equatorial and hence each multiplet is due to three small (<3.5 Hz) vicinal couplings plus a smaller (<1 Hz) *W* coupling. In the twist-boat conformation, 2α and 3β are quasi axial and the multiplet is now produced by three large (ca. 6–10 Hz) vicinal couplings. Figure 1 clearly shows that in DMF-d_7 solution the couplings are small, corresponding to an equilibrium mixture which consists mostly of the chair form. As the solvent is changed to CD_3OD, to CD_3CN, and to CD_3NO_2, both of the multiplets expand (due to increasing vicinal couplings) as the equilibrium shifts away from the chair toward the twist-boat conformation. In the $CDCl_3$ solution, the 3β signal is now an 8-line pattern and the signals are at their widest as the shift to a twist-boat conformation is almost complete.

Table I lists the observed ^1H chemical shifts and couplings of the A-ring protons together with data for the axial methyl groups from **I** in all of the solvents studied. The solvents are listed in a row which reflects the general trend toward more of the twist-boat conformer going from left to right.

Assignment of the $CDCl_3$ and DMSO-d_6 spectra has been described before.[1] The spectrum of the C_6D_6 solution was fully and unequivocally assigned from 2D COSY and NOESY spectra. The spectra of other solutions were assigned with the aid of COSY experiments. Chemical shift correlations with the fully assigned $CDCl_3$, DMSO-d_6, and C_6D_6 solution spectra settled any ambiguities over geminal pairs. At 600 MHz the resolution and dispersion were such that some of the couplings could be measured directly after assignment. However in some spectra, some of the couplings were second order, and in several cases signals from A-ring protons overlap with signals from other parts of the molecule. Hence all of the spectra were simulated with the Bruker computer program PANIC in order to extract accurate coupling constant information. The gaps in Table I correspond to cases where spectral overlaps and/or strong second-order effects prevented a reliable estimate of the coupling. A bar in the table indicates that the coupling was not resolved. Reevaluation of the DMSO-d_6 data has resulted in a slight revision of our earlier quoted figures for couplings in this solvent.[1] The spectra (all solvents) showed that rings B, C, and D were relatively rigid during

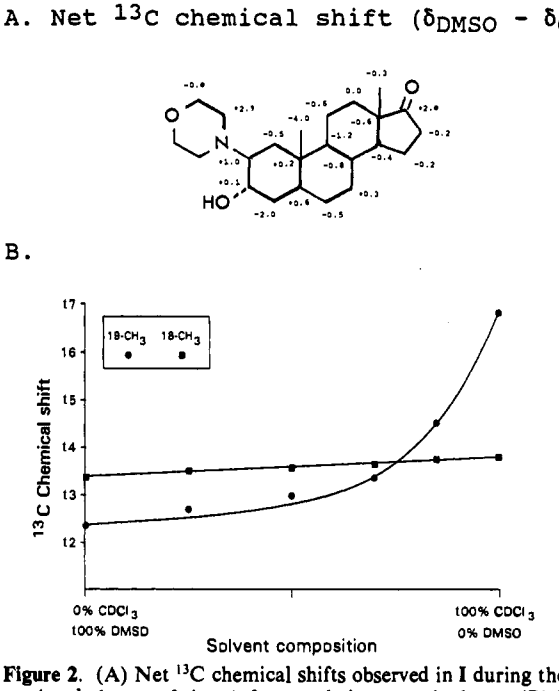

Figure 2. (A) Net ^{13}C chemical shifts observed in **I** during the conformational change of ring A from a chair to a twist-boat. (B) Behavior of the two axial methyl groups during the conformational change from a chair to a twist-boat.

the ring A conformational changes (no significant changes in vicinal couplings), so that only the important A-ring parameters have been determined in detail.

Aspects of the ^{13}C NMR spectra of **I** are illustrated in Figure 2A, which maps the net chemical shift change ($\delta_{DMSO\text{-}d_6} - \delta_{CDCl_3}$) over the steroid skeleton. Figure 2B plots the behavior of the axial methyl carbons in mixed $CDCl_3$/DMSO-d_6 solvent systems. The ^{13}C chemical shifts of the axial methyl carbons for **I** in all solvents are listed in Table IV.

Discussion

^1H NMR Spectra. There is a large body of information in Table I. The chemical shifts are included for completeness, but since nonspecific solvent-induced shifts will obscure any shifts due to

Table II. Composition of the Equilibrium Mixture (Expressed as % Twist-Boat Isomer) as Calculated from the Vicinal Coupling Constants Observed in the 600-MHz ^{1}H NMR Spectra of **I** in Various Deuterated Solvents

	3JMM2 chair (Hz)	DMSO-d_6	DMF-d_7	C_5D_5N	CD_3OD	THF-d_8	acetone-d_6	CD_3CN	toluene-d_8	CD_3NO_2	C_6D_6	CD_2Cl_2	$CDCl_3$	3JMM2 twist-boat (Hz)
1α2α	(3.8)	22	33	26	26	44	52	74	111	96	122	111	133	(6.5)
1β2α	(2.7)	3	7	11	19	16	20	39	59	62	57	84	84	(10.1)
2α3β	(2.6)	7	10	14	20	20	30	44	62	69	66	94	97	(9.7)
3β4α	(3.9)	−26	−22	−7		4		48	56	48	48	63	70	(6.6)
3β4β	(2.2)	9	7	9	22	16		44	57	62	60	78	82	(9.0)
4α5α	(3.0)	22	34	22					75	75	72	109	119	(6.2)
mean		6	11	12	22	20	34	50	70	69	71	90	98	
std dev		18	21	12	3	15	16	14	21	16	26	19	24	

Table III. Composition of the Equilibrium Mixture (Expressed as % Twist-Boat Isomer) and Fitted Best Values of Vicinal Couplings (See Text for Details)

	^3J (fitted) chair (Hz)	DMSO-d_6	DMF-d_7	C_5D_5N	CD_3OD	THF-d_8	acetone-d_6	CD_3CN	toluene-d_8	CD_3NO_2	C_6D_6	CD_2Cl_2	$CDCl_3$	^3J (fitted) twist-boat (Hz)
1α2α	(3.9)	12	19	15	15	27	32	46	70	61	78	71	85	(8.0)
1β2α	(2.0)	11	15	19	27	24	28	46	65	67	62	87	87	(9.9)
2α3β	(2.1)	12	14	18	23	23	31	43	58	64	62	86	88	(10.5)
3β4α	(3.0)	6	9	20		29		63	69	63	63	74	80	(6.5)
3β4β	(1.7)	15	14	15	28	22		49	61	65	63	81	85	(8.9)
4α5α	(3.1)	15	25	15					58	58	55	85	93	(7.1)
mean		12	16	17	23	25	30	49	64	63	64	81	86	
std dev		4	6	2	6	3	2	8	5	3	8	7	4	
ΔG_{294}		+1.16	+0.97	+0.93	+0.71	+0.64	+0.49	+0.02	−0.32	−0.31	−0.32	−0.84	−1.08	

genuine structural changes, we will not attempt to analyze this portion of the table. The lower portion of the table contains the more interesting data which can be related to structure. Long-range splittings of the axial methyl groups were seen in several solvents. Couplings via the *W* pathway were visible in solutions that contained significant quantities of the chair conformation. The most interesting data are those concerning the A-ring vicinal couplings, and clear trends can be seen in these rows of data.

The A-ring vicinal couplings provide a handle on the thermodynamics of the chair–twist-boat equilibrium. For two conformations that are interchanging rapidly, an observed vicinal coupling is simply the weighted average of those in the pure chair and pure twist-boat forms:

$$J_{obsd} = X_c J_c + X_{t\text{-}b} J_{t\text{-}b} \quad (1)$$

where X_c is the mole fraction in the chair conformation, J_c is the vicinal coupling in the chair conformation, and $X_{t\text{-}b}$ and $J_{t\text{-}b}$ are the mole fraction and vicinal coupling in the twist-boat conformation, respectively. Hence, given the limiting values J_c and $J_{t\text{-}b}$, every vicinal coupling in Table I is potentially a measure of the equilibrium constant. However (and this has been the fundamental problem with studies such as this), there appears to be no reliable method to estimate the vicinal couplings in the parent chair and twist-boat forms.

In the following discussion it is convenient to refer to the complete set of vicinal couplings within ring A of the chair conformation and the twist-boat conformation as $\mathbf{J}_C$ and $\mathbf{J}_{T\text{-}B}$, respectively, and individual couplings will be referred to as J_c and $J_{t\text{-}b}$.

Two approaches to estimating $\mathbf{J}_C$ and $\mathbf{J}_{T\text{-}B}$ suggest themselves. First, we might attempt to calculate them. Table II shows the results of the population analysis when $\mathbf{J}_C$ and $\mathbf{J}_{T\text{-}B}$ were calculated from the molecular mechanics generated geometries for the chair and twist-boat forms using the program 3JMM2.[17] The reference values of J_c and $J_{t\text{-}b}$ that were used to solve eq 1 for $X_{t\text{-}b}$ are listed in brackets in the end columns of the table. Note that each experimental value of ^3J from Table I now provides an individual estimate of the mole fraction of the twist-boat conformation in the solution. The 4β5α interaction has been discounted from this analysis because the 4β5α torsion angle is relatively insensitive to the conformational change. Hence, there are now up to six estimates of the chair–twist-boat equilibrium for each solvent. The results are encouraging but the standard deviations are unacceptably large, and some poor estimates of the twist-boat population (e.g., the negative values and values >100%) are clearly the result of poor J_c and $J_{t\text{-}b}$ values.

An alternative and better approach is to iteratively fit values of J_c and $J_{t\text{-}b}$ to eq 1 to find values that minimize the standard deviations of the calculated % twist-boat figure. In other words, we presume that the raw data is good (i.e., not the source of the large errors in Table II) and that there is enough of it that we can calculate conformer ratios *and* the vicinal couplings in the pure chair and twist-boat conformations. The fitting was performed on a spreadsheet which was configured to use the experimental data of Table I and $\mathbf{J}_C$ and $\mathbf{J}_{T\text{-}B}$ to calculate the center field of Table II. In addition, the standard deviation of each vertical column and the mean (i.e., sum/12) standard deviation across the whole table were calculated. The 3JMM2 values of J_c and $J_{t\text{-}b}$ were used as starting values, and they were varied one at a time to reach values that minimized the mean standard deviation of the whole table (i.e., the mean of 12 standard deviations—one for each solvent). The mean standard deviation dropped rapidly during the first iterations to a definite minimum. After six iterations (three at each end of the table), the mean standard deviation had dropped from 17.1 to 4.8 and additional cycles had no significant impact. The result of this procedure is shown in Table III. The arithmetic mean of the columns from Table III was taken to calculate ΔG for the conformational change at 21 °C. In the following discussions, ΔG always refers to the forward reaction: chair → twist-boat.

Note that, as well as providing the sought-after equilibrium information, Table III now gives $\mathbf{J}_C$ and $\mathbf{J}_{T\text{-}B}$ for the pure chair and twist-boat conformers. In usual situations, it is impossible to derive these figures when the rate of interconversion is faster than the NMR time scale. It can be seen that the program 3JMM2 produced a good estimate of ^3J for most of the couplings, but overestimated 1β2α and 3β4α in the chair conformation and underestimated 1α2α and 4α5α in the twist-boat conformation.

^{13}C NMR Spectra. Figure 2 illustrates the changes in ^{13}C chemical shifts that occur when **I** is moved from a DMSO-d_6

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Table IV. Summary of Data from the 50.3-MHz ^{13}C NMR Spectra of I in Various Solvents

solvent	D/H[a]	ϵ[b]	ref[c]	C_{19} (ppm)	C_{18} (ppm)	$C_{19} - C_{18}$ (ppm)	% twist-boat[d]	ΔG_{294}
CP-MAS[e]				13.5	13.5			
DMSO	D	47	39.6	12.52	13.52	−1.00	[12][f]	
DMF	H	37	161.7	12.05	12.88	−0.83	15.3	+1.00
MeOH	D	33	49.0	13.84	14.14	−0.30	25.8	+0.61
acetone	D	21	29.8	14.05	13.92	+0.13	34.4	+0.37
pyridine	H	12	150.0	13.25	13.87	−0.62	19.5	+0.81
THF	H	8	TMS	13.96	13.63	+0.33	38.4	+0.29
CH_3CN	D	37	1.3	14.77	14.16	+0.61	43.9	+0.14
CH_3NO_2	D	36	62.8	16.00	14.30	+1.70	65.6	−0.38
$EtNO_2$	H	28	12.3	15.29	13.91	+1.38	59.2	−0.21
$BrCH_2CH_2F$	H	16	220[g]	15.35	13.40	+1.95	70.6	−0.52
$ClCH_2CH_2Cl$	H	10	TMS	16.04	13.89	+2.15	74.6	−0.64
C_6H_6	D	4–7	128.0	15.18	13.79	+1.39	59.4	−0.21
CH_2Cl_2	H	9	54.2	16.58	13.97	+2.61	83.7	−0.97
CCl_4		2.2	96.7	16.19	14.27	+1.92	70.0	−0.49
$CHCl_3$	D	4.8	77.0	16.49	13.76	+2.73	[86][f]	
	H		77.7	16.99	14.25	+2.74		

[a] Entries in this column indicate the use of deuterated or protonated solvents for the ^{13}C experiments. [b] Solvent dielectric constant.[18] [c] The chemical shift reference for two experiments was internal TMS. All other spectra were referenced to the solvent signal which was assigned to the value indicated here. [d] Proportion of the twist-boat conformation estimated from the chemical shift difference between C_{19} and C_{18} as discussed in the text. [e] Data from a cross-polarization magic angle spinning spectrum of solid I. [f] "Known" reference figures for DMSO-d_6 and $CDCl_3$ solutions are the values taken from the ^1H data. [g] No chemical shift data was found for this solvent so the spectrum was referenced to the carbonyl carbon in I, which was given the arbitary value 220.0 ppm.

environment (88% chair) to a $CDCl_3$ environment (86% twist-boat). The net change for all carbons is recorded on the structure, while the behavior of the axial methyl signals is graphically illustrated with the results of a titration of $CDCl_3$ into a DMSO-d_6 solution. For the purposes of this titration internal TMS was used as a chemical shift reference. ^{13}C chemical shifts are not generally very dependent on solvent, so that any large changes in the spectrum must result from the conformational change. It can be seen (Figure 2A) that with the exception of the C_{17} ketone (which probably is influenced by some specific solvation effects) the signals from carbons in rings B, C, and D do not shift significantly, but there are some noticeable shifts from carbons in or associated with ring A. Most notable is the −4.0 ppm shift of the 10-methyl (C_{19}). This substantial upfield shift must be a direct result of the release of steric pressure between the axial 10-methyl group and the morpholino group and hence is a simple marker of the chair–twist-boat equilibrium.

In Figure 2B the chemical shift of the "reporter" C_{19} is shown in contrast to the "reference" C_{18} in some mixed DMSO-d_6/$CDCl_3$ solutions. This figure suggests that the chemical shift difference between C_{19} and C_{18} might provide another handle on the chair–twist-boat equilibrium. Estimation of the equilibrium from ^{13}C data requires the following assumptions: (1) the observed chemical shift of C_{19} is determined only by the equilibrium and is the weighted average of the shifts found in the pure chair and pure twist-boat forms (fast exchange is also implicitly assumed); (2) the chemical shift of C_{18} is independent of any conformational change in ring A; and (3) any small solvent-induced shifts will be the same for both carbons. The final assumption has the added convenience that it circumvents any difficulties over chemical shift references in different solvents.

Hence, we have an empirical relationship between a readily measured parameter ($\Delta\delta$: the chemical shift difference between C_{19} and C_{18} in any solvent) and the distribution of I between the chair and twist-boat conformations. In practice the observed ^{13}C shifts from $CDCl_3$ and DMSO-d_6 solutions are corrected for the equilibrium concentrations of chair and twist-boat forms found by ^1H NMR to give reference chemical shifts for the pure chair ($\Delta\delta = -1.60$) and twist-boat ($\Delta\delta = +3.43$) forms. The proportion of twist-boat conformation in any solution is now given by the simple formula

$$\%\ \text{twist-boat} = \left(\frac{\Delta\delta + 1.60}{5.03}\right)100 \qquad (2)$$

The results of this treatment are shown in Table IV. The good agreement between the ^1H method and the ^{13}C method for the seven solvents where results from both techniques are available is an indication of the validity of the ^{13}C method.

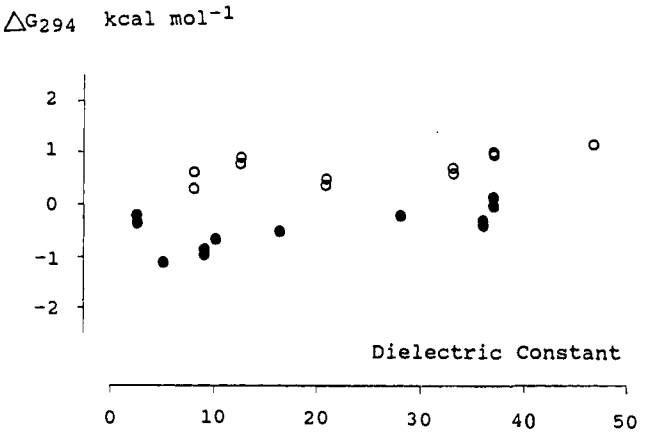

Figure 3. Solvent dependence of the conformational equilibrium. Data are classified for solvents which can (○) and cannot (●) participate in hydrogen bonding. Results from the ^1H-based method are not distinguished from those from the ^{13}C-based method in this plot.

It was of interest to investigate the cross-polarization magic angle spinning ^{13}C spectrum of solid I to obtain information on the conformation of ring A in the crystal lattice. The result is included in Table IV and strongly suggests that in the crystal structure I exists with ring A in the chair conformation. This is in accordance with a recent X-ray determination of an analogous structure, 3α,12β-dihydroxy-2β-morpholino-5α-pregnan-20-one.[7]

Free Energies. Figure 3 shows the relationship between ΔG_{294} for the reaction chair → twist-boat and solvent dielectric constant (data from Tables III and IV). With the exception of the data for benzene, toluene, and carbon tetrachloride solutions, which must be considered as anomalous, the data appear to lie on two separate curves. (The relationship between ΔG and ϵ is not expected to be and is not implied to be linear.) One curve consists of points from solvents that can participate in hydrogen bonding, while the other is from solvents which cannot participate in hydrogen bonding. The curves are parallel and separated by approximately 1.5 kcal mol^{-1}, covering a total range of approximately 2 kcal (i.e., from around +1 to around −1 kcal mol^{-1}).

Hence, the results suggest that the intramolecular hydrogen bond stabilizes the twist-boat conformation by an additional 1.5 kcal mol^{-1}. Conversely, this can be interpreted as the contribution due to solvation of the hydroxyl group by the medium. A conformational change without a hydrogen bond would require about 2 kcal mol^{-1}.

Table V. Calculated Solvation Energy[a] Using the Method of Abraham[19]

conformation	dielectric constant								
	2.2	4.8	8.0	9.0	10.0	16.0	21.0	37.0	47.0
chair[b]	9.48	9.11	8.94	8.91	8.88	8.77	8.71	8.58	8.52
tw-b	9.51	9.16	9.00	8.97	8.95	8.84	8.79	8.67	8.61
ΔG[c]	+0.03	+0.05	+0.06	+0.06	+0.07	+0.07	+0.08	+0.09	+0.09

[a] As defined by Abraham,[19] the solvation energy is the difference between the energy of a molecule in the vapor phase (E^V) and in solution (E^S). [b] Solvation energy (kcal mol^{-1}) averaged over three staggered conformations about the C_3–O bond. [c] ΔG is defined as $E_{\text{twist-boat}} - E_{\text{chair}}$.

Table VI. Effect of Temperature on the ^{13}C NMR Chemical Shifts of the Axial Methyl Groups of **I** in CD_3CN Solution

temp (K)	C_{19} (ppm)	C_{18} (ppm)	% twist-boat	ΔG (kcal mol^{-1})
325	15.282	14.479	47.8	+0.057
292	14.765	14.170	43.6	+0.149
293	14.662	14.099	43.0	+0.164
273	14.359	13.923	40.5	+0.209
253	14.051	13.763	37.5	+0.257
233	13.705	13.588	34.1	+0.305

In an attempt to clarify this result, the method of Abraham[19] has been used to estimate the solvation energy difference over the range of solvents considered experimentally. While it is acknowledged[19] that the method has serious shortcomings when applied to hydrogen-bonding solvents, the results of such calculations could allow a separation of the hydrogen-bonding contribution from the total energy difference.

The results shown in Table V are taken from calculations using the MODELS program[20] using partial charges[21] in place of bond dipoles.[19] In each case only ring A is explicitly considered; the remaining rings are represented by uncharged dummy atoms. We feel justified in using this model as the only other part of the molecule which could interfere with the molecular dipole moment is the carbonyl at position 17. This is too far removed from ring A to be involved in the same solvation shell. Also, test calculations on the complete molecule showed very similar energy differences. It is assumed that rotation about the C_3–O bond is free in the chair case, with the total solvation energy being an average of the three staggered conformations.

It is clear from Table V that there is very little difference between the solvation energies of the chair and twist-boat conformers. Analysis of the different components of the solvation energy showed that this is due to the dipolar and quadrupolar terms giving opposite effects. Although this trend is in the same direction as is found by experiment, it is obvious that the hydrogen bond, either intramolecular or to the solvent, plays a much greater role in mediating the conformational change than does the reaction field surrounding the solvent.

In the non-hydrogen-bonded solvents, it is possible that we are observing a phenomenon not dissimilar to the hydrophobic effect.[22] As the polarity of the solvent increases, solvation of the more hydrophobic twist-boat conformation would result in an overall decrease in entropy. Although no explicit hydrogen-bonding interaction takes place as the conformation changes, the increase in entropy for the solvent immediately surrounding the solute is great enough to counteract the enthalpy increase resulting from the loss of an intramolecular hydrogen bond. This effect is different from that seen in the polar hydrogen-bonding solvents, where both the enthalpy and entropy changes are favorable for the equilibrium moving toward the chair conformer.

Variable-Temperature Data. The CD_3CN solution of **I** ($\Delta G_{298} \approx 0$) was chosen for a study of the temperature dependence (−40 to +72 °C) of the equilibrium process.

From the ^1H spectra (data not shown) it was clear that raising the temperature shifted the equilibrium in favor of the twist-boat conformation (larger vicinal couplings in the 2α and 3β multiplets) and cooling favored the chair conformation (multiplets collapsing). These observations showed that in CD_3CN the chair conformation is the lower energy conformation. Unfortunately, the ^1H data could not be interpreted quantitatively because the A-ring vicinal couplings could not be accurately determined in the low-temperature data. Although the spectra were broadening slightly with decreasing temperature, there was no indication that a coalescence temperature was near. This indicates that the activation energy for the conformational change is very low.

It was possible to use the ^{13}C chemical shifts of the axial methyl groups to obtain quantitative information on the effect of temperature on ΔG. Table VI details the effect of temperature on the chemical shifts of C_{19} and C_{18} from a CD_3CN solution of **I**. These figures were used to calculate the mole fraction of the twist-boat conformation present by the method established in the preceding section. As was predicted by the ^1H data, the proportion of **I** present in the twist-boat form drops as the temperature is decreased. A plot of ΔG versus T was linear (correlation coefficient = 0.996), with slope $-\Delta S = -2.7$ cal mol^{-1} and intercept $\Delta H = +0.93$ kcal mol^{-1}.

Conclusion

In this article, we have described the effects of 16 different solvents on the conformational equilibrium of ring A of 3α-hydroxy-2β-(4-morpholinyl)-5α(H)-androstan-17-one. Variable solvent high-field ^1H NMR combined with molecular modeling proves to be a useful method for dealing with a common (and often intractable) problem—that of determining the populations of different conformations of a flexible molecule. In this case there are two low-energy conformations of **I**, and the problem is overdetermined by six vicinal couplings. The procedure developed to understand the ^1H NMR data leads to a population analysis which is independent of any Karplus equations.

In addition, a purely empirical method of determining the equilibrium was devised, on the basis of the chemical shift difference between C_{19} and C_{18} in the ^{13}C spectrum. The good agreement between measurements of the equilibrium with the ^{13}C method and measurements with the ^1H method was taken as a validation of the ^{13}C method, and hence the simpler ^{13}C method was used to extend the number of solvents studied.

By investigating a large variety of solvents we have been able to separate hydrogen-bonding from solvent dielectric effects. The details of the results defy a complete theoretical explanation, but the results are in line with what might intuitively be expected for this system.

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