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Conformations of Anaesthetic Steroids: a ^{1}H and ^{13}C NMR Study of (2β,3α,5α)-2-[(2*R*)-Ethyl-4-morpholinyl]-3-hydroxypregnane-11,20-dione and (2β,3α,5α)-2-[(2*S*)-Ethyl-4-morpholinyl]-3-hydroxypregnane-11,20-dione.

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The completely assigned ^{1}H and ^{13}C NMR spectra of the title diastereoisomers in solutions of $CDCl_3$ and DMSO-d_6 are reported. NOE experiments show that these molecules adopt specific conformations with no rotation about the C-2—N bond in either solvent. In $CDCl_3$, ring A of the steroid is in a twist-boat conformation and an intramolecular hydrogen bond from the 3α-OH to the morpholine N acts as a conformational lock preventing rotation of the morpholine ring. In DMSO-d_6 solutions, ring A of the steroid is in a chair conformation and van der Waals contacts with the 19-methyl group prevent free rotation of the morpholine ring. Molecular mechanics calculations produced four structures that account for the experimental data. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

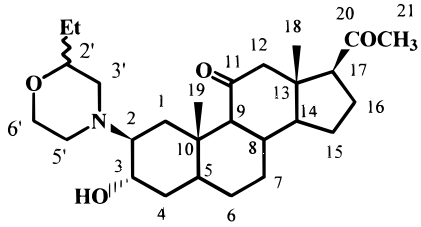
(2β,3α,5α)-2-[(2*R*)- 2-Ethyl-4-morpholinyl]- 3-hydroxypregnane-11,20-dione (**I**) is under investigation as a potential new anaesthetic.[1,2] Its diastereoisomer (2β,3α,5α)-2-[(2*S*)-2-ethyl-4-morpholinyl]-3-hydroxypregnane-11,20-dione (**II**) was also prepared during the drug discovery project. An earlier report[3] from this laboratory discussed the 50 MHz ^{13}C NMR spectra of the morpholine moiety of these compounds in $CDCl_3$ solution and showed how the absolute stereochemistry of the 2-alkylmorpholine could be inferred from a consideration of the chemical shifts of the morpholine carbons C-3′ and C-5′. The 200 MHz ^{1}H spectra were considered to be too poorly resolved to contain useful structural information. Molecular modelling played a vital role in this earlier work and a key finding was that intramolecular hydrogen bonding caused restricted rotation of the morpholine ring about the bond between C-2 and N, resulting in a specific conformation of the morpholine moiety. Here we report the results of high-resolution ^{1}H NMR studies which fully validate the earlier assumptions and present direct NOE evidence for some specific solution conformations. A knowledge of the solution conformations of these molecules is useful for an understanding of the mechanism by which they cause anaesthesia.

RESULTS AND DISCUSSION

Assignments

The $CDCl_3$ solution spectra were assigned from COSY, ^{13}C detected HETCOR, NOESY and HMBC experiments. The chemical shifts of resolved peaks were measured from the 1D spectra and the chemical shifts of overlapped peaks were measured from the HETCOR spectrum. Usually COSY and HETCOR data provide sufficient information to assign confidently proton signals to a particular carbon and NOE data can resolve any ambiguity about α or β positions. HMBC was useful in differentiating C-11 and C-20.

The DMSO-d_6 data were assigned similarly, except that more use was made of the COSY and NOESY data to estimate the shifts of protons in overlapped



I (*R*)

II (*S*)

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regions. Compared with $CDCl_3$, data from DMSO-d_6 solutions generally have poorer resolution (^{1}H) and poorer signal-to-noise ratios (^{13}C). These problems can be alleviated somewhat by warming the solution. It was more difficult (because of congestion) to make the correct stereochemical assignments for the signals from geminal protons on C-4, C-6 and C-7 in DMSO-d_6 solutions. A row from the phase-sensitive NOESY spectrum of **I** containing the cross peaks from H-3β contained equally intense enhancements of signals from protons attached to C-4 at 1.62 and 1.16 ppm. The signal at 1.62 ppm was second order (overlaps with H-5α) and could not be assigned from its shape. The signal at 1.16 ppm resembled a doublet, hence this was assumed to be an equatorial proton and is assigned H-4α. Assignments of 6α/6β and 7α/7β were made so that they are consistent with the $CDCl_3$ data and with previous ^{1}H NMR studies of steroids.[4]

The solvent-induced conformation changes of ring A of 2β-amino-3α-hydroxy steroids have been fully described.[5,6] Compounds **I** and **II** are expected to exist with ring A in a chair conformation in polar solvents (DMSO) and in a twist-boat conformation in non polar solvents ($CHCl_3$).

$CDCl_3$ solutions

The 400 MHz ^{1}H NMR spectrum of **I** and **II** in $CDCl_3$ are shown in Fig. 1 and the complete ^{1}H and ^{13}C assignments are listed in Tables 1 and 2. Some of the ^{13}C NMR data have been discussed in an earlier paper.[3] The present account includes the full ^{13}C assignments, but focuses primarily on the ^{1}H data. All of the noticeable differences between the data for **I** and **II** are in the chemical shifts of morpholine protons. This can be seen most readily in the region 2.0–2.8 ppm, to a lesser extent in the region 3.2–4.0 ppm and also around 1.5 ppm, where the methylene protons of the ethyl substituents appear. The chemical shifts of steroid protons are effectively identical (see below). The room temperature spectra show that ring A of the steroid is almost completely in a twist-boat conformation (pseudo-axial signals from H-2α and H-3β). Signals

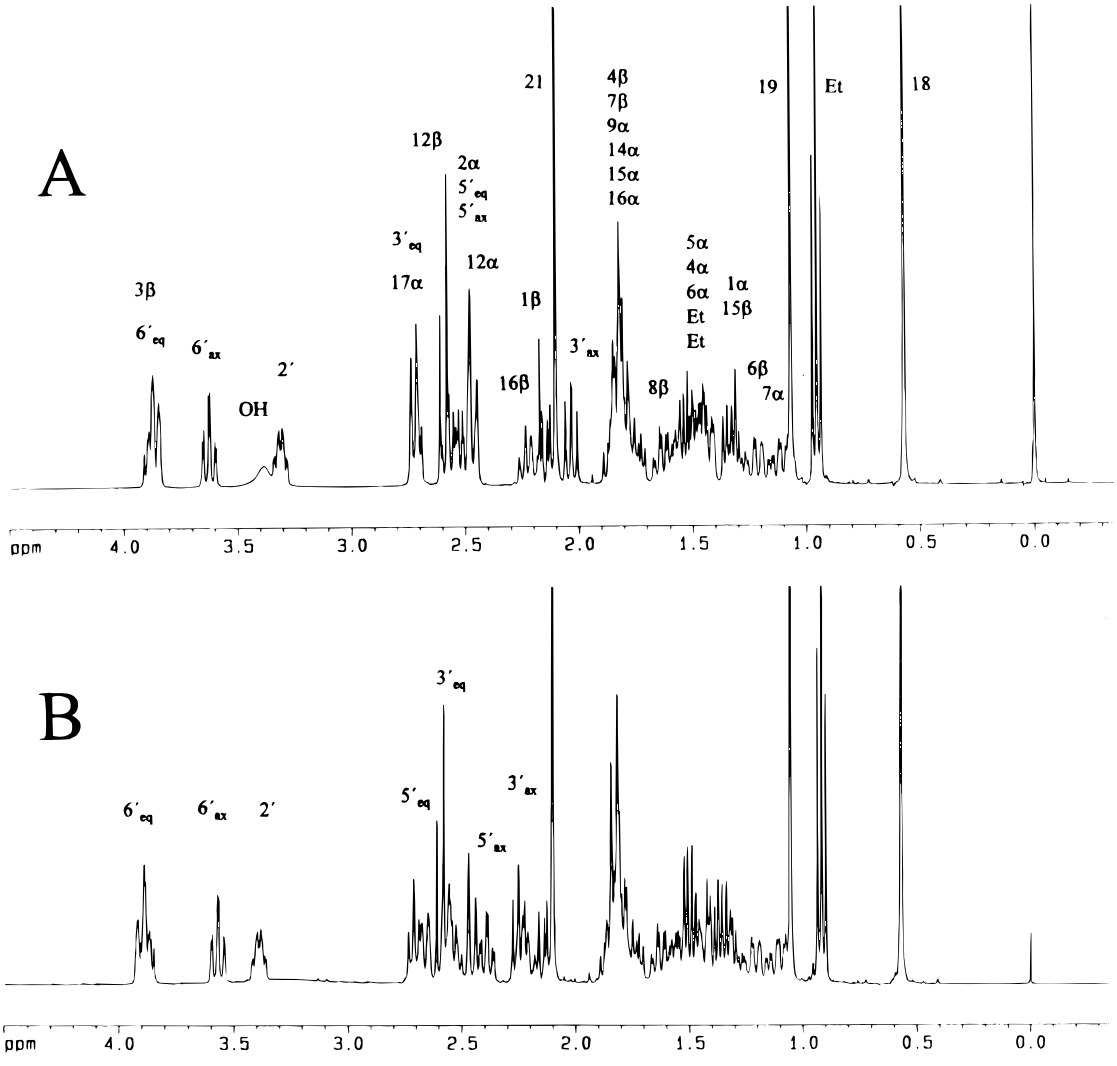

Figure 1. 400 MHz ^{1}H NMR spectra of (A) **I** and (B) **II** in $CDCl_3$ (30 mg) at 23 °C. All signals are assigned on spectrum A. For clarity, only the signals from morpholine ring protons are assigned on B. Signals from singlet methyl protons are truncated at about 50% of peak height.

Table 1. ^{1}H chemical shift data (δ, ppm) for I and II in $CDCl_3$ (23 °C) and DMSO-d_6 (57 °C)[a]

	$CDCl_3$		DMSO-d_6	
	I	II	I	II
1α	1.34	1.34	1.19	1.20
1β	2.14	2.14	2.47	2.44
2α	2.53	2.50	2.06	2.08
3β	3.88	3.87	3.94	3.95
4α	1.48	1.48	1.16	1.16
4β	1.83	1.81	1.62	1.62
5α	1.56	1.56	1.61	1.60
6α	1.42	1.43	~1.1	1.12
6β	1.22	1.23	~1.2	1.24
7α	1.08	1.09	~1.1	1.14
7β	1.79	1.79	~1.7	1.71
8β	1.63	1.62	1.61	1.60
9α	1.83	1.82	1.82	1.83
12α	2.47	2.45	2.61	2.62
12β	2.59	2.59	2.35	2.34
14α	1.74	1.73	1.81	1.81
15α	1.82	1.83	~1.7	1.71
15β	1.30	1.30	1.21	1.21
16α	1.82	1.83	~1.8	1.77
16β	2.22	2.23	2.04	2.02
17α	2.71	2.71	2.78	2.77
18	0.57	0.57	0.44	0.44
19	1.07	1.06	1.13	1.12
21	2.10	2.10	2.03	2.03
2′	3.31	3.38	3.24	3.26
$3'_{eq}$	2.73	2.55	3.18	2.88
$3'_{ax}$	2.03	2.24	1.58	1.65
$5'_{eq}$	2.58	2.65	2.81	3.03
$5'_{ax}$	2.46	2.38	1.93	1.91
$6'_{eq}$	3.86	3.90	3.75	3.80
$6'_{ax}$	3.62	3.56	3.45	3.45
$Et_{(CH_2)}$	1.54	1.51	1.46	1.39
$Et_{(CH_2)}$	1.44	1.37	1.39	1.39
$Et_{(CH_3)}$	0.96	0.92	0.91	0.87

[a] Shifts were read directly from 1D spectra where possible or were measured from the location of cross peaks in 2D experiments.

Table 2. ^{13}C chemical shift data (δ, ppm) for I and II in $CDCl_3$ (23 °C) and DMSO-d_6 (57 °C)

	$CDCl_3$		DMSO-d_6	
	I	II	I	II
1	32.82	32.93	31.15	31.34
2	64.84	64.98	65.77	65.79
3	63.54	63.61	63.64	63.44
4	33.77	33.85	31.90	31.96
5	38.20	38.21	38.55	38.43
6	27.55	27.59	26.76	26.79
7	32.25	32.27	31.90	31.88
8	36.41	36.43	35.39	35.42
9	66.02	66.07	63.55	63.56
10	35.09	35.09	35.20	35.18
11	209.39	209.37	209.78	209.80
12	56.36	56.38	55.61	55.64
13	46.95	46.95	46.29	46.27
14	55.23	55.24	54.12	54.07
15	23.84	23.86	23.13	23.14
16	23.27	23.29	22.80	22.84
17	62.13	62.15	60.86	60.83
18	14.27	14.26	13.75	13.72
19	16.32	16.41	12.07	12.27
20	208.06	208.05	207.61	207.65
21	31.28	31.27	30.61	30.64
2′	77.65	77.69	76.31	76.39
3′	50.54	57.04	55.42	56.36
5′	51.92	45.44	51.41	50.43
6′	67.23	67.10	66.04	65.75
Et	26.64	26.48	26.11	25.98
Et	9.94	9.80	9.52	9.56

from the morpholine moiety are consistent with a chair conformation with an equatorial ethyl group (H-2′ is axial). There is no observable averaging of equatorial with axial protons and therefore the morpholine ring is not subject to a fast ring inversion or nitrogen inversion process. The temperature dependence of the $CDCl_3$ spectra of **I** and **II** was examined to ensure that any possible dynamic effects were considered. Although the chemical shifts of some proton signals were slightly temperature dependent, there was no significant change in any line shapes over the range −25 to 57 °C.

^{1}H–^{1}H NOE experiments reveal dipolar interactions between protons that are unique for each isomer **I** and **II** and provide information about the solution conformation of these molecules. For the purpose of providing clear illustrations, gradient NOE with second selective echo (DPFGSE NOE)[7,8] experiments were performed in addition to phase-sensitive NOESY experiments. Irradiation of 19-CH_3 in a DPFGSE NOE experiment on **I** in $CDCl_3$ produced enhancements [Fig. 2(A)] of H-3β, H-1β, H-4β, H-8β and 18-CH_3 on the steroid and more significantly of H-$3'_{eq}$ on the morpholine ring. This was the only visible enhancement of any morpholine proton and strongly suggests that the morpholine ring is fixed in a specific orientation with respect to the steroid moiety. The conformation derived from modelling is shown in Fig. 3(A) and some morpholine–steroid internuclear distances are listed in Table 3. A similar experiment on **II** in $CDCl_3$ [Figure 2(B)] showed cross peaks between the same steroid protons and also H-$5'_{eq}$

Table 3. Molecular mechanics-derived internuclear distances between some morpholine protons and steroid protons in the minimum energy conformations of I and II in $CDCl_3$ and in DMSO-d_6 solutions

Conformation	Vector	Distance (Å)
I twist-boat	19-CH_3 to H-3′(eq)	3.11
	19-CH_3 to H-3′(ax)	3.59
	Next nearest to 19-CH_3	>5
I chair	H-1β to H-3′(eq)	2.00
	H-1β to H-3′(ax)	2.94
	Next nearest to H-1β	>4.2
II twist-boat	19-CH_3 to H-5′(eq)	3.04
	19-CH_3 to H-5′(ax)	3.75
	Next nearest to 19-CH_3	>5
II chair	H-1β to H-5′(eq)	2.00
	H-1β to H-5′(eq)	2.98
	Next nearest to H-1β	>4.2

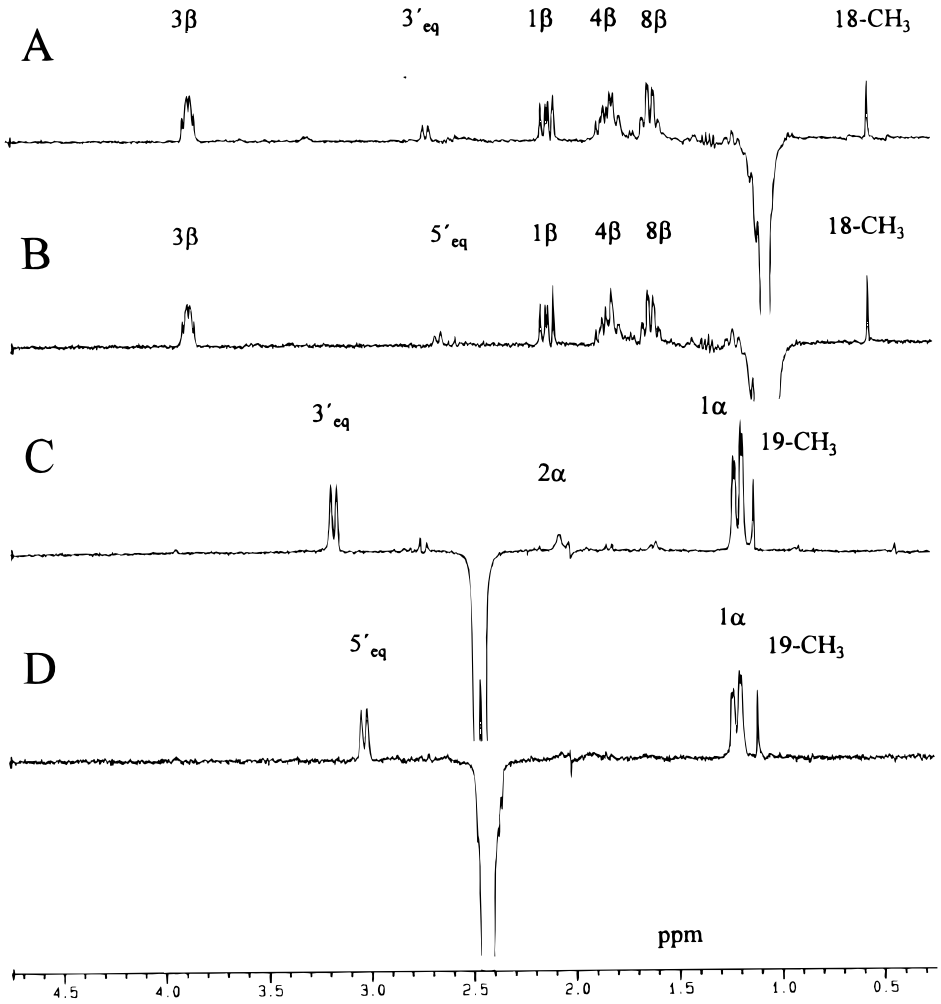

Figure 2. 400 MHz DPFGSE NOE spectra of (A) **I** in $CDCl_3$, irradiated at 19-CH_3, (B) **II** in $CDCl_3$, irradiated at 19-CH_3, (C) **I** in DMSO-d_6, irradiated at H-1β and (D) **II** in DMSO-d_6, irradiated at H-1β.

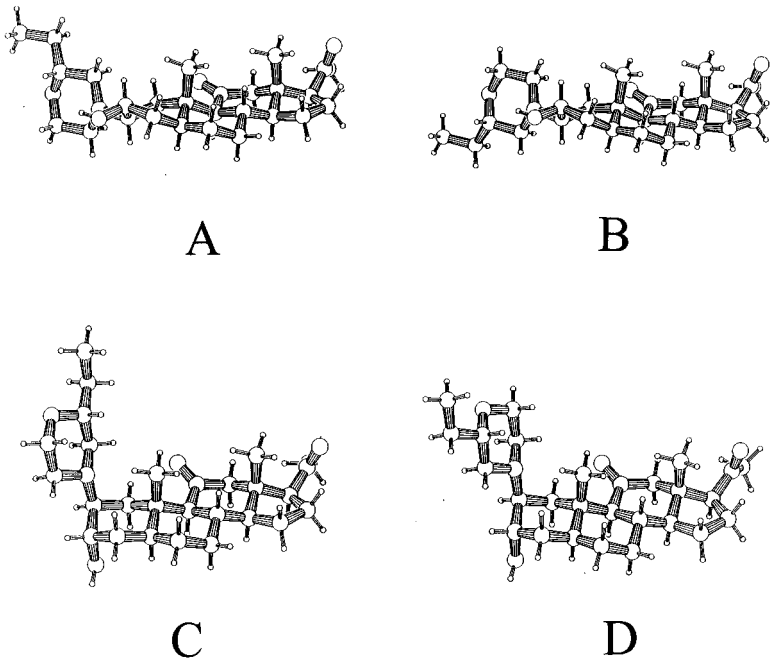

Figure 3. Calculated minimum energy conformations of **I** and **II** in both solvents. (A) **I** in $CDCl_3$; (B) **II** in $CDCl_3$; (C) **I** in DMSO-d_6; (D) **II** in DMSO-d_6.

with no correlations to any other morpholine protons, again suggesting no rotation about the C-2—N bond and a very specific morpholine/steroid geometry. The calculated minimum energy conformation is shown in Figure 3(B) and relevant internuclear distances are listed in Table 3. The calculated conformation satisfactorily accounts for both the observed NOEs between 19-CH_3 and H-3′$_{eq}$ (**I**) and H-5′$_{eq}$ (**II**), and also the complete lack of any other cross peaks between steroid protons and morpholine protons.

DMSO-d_6 solutions

The ^{1}H NMR spectra of **I** and **II** in DMSO-d_6 were investigated over the temperature range 21–97 °C. There was some slight variation of morpholine proton chemical shifts with temperature, but no change in the peak shapes other than the sharpening that accompanies a decrease in solvent viscosity. There was no change (other than narrowing) of the steroid proton signals. These observations suggest that there is no significant dynamic behaviour of the molecules conformation at these temperatures. At 57 °C the spectra were sharp and well resolved (Fig. 4) and this was a convenient temperature at which to perform NMR experiments. The steroid ring A is in a chair conformation (H-2α and H-3β are clearly equatorial). The morpholine ring is in a chair conformation with the ethyl group equatorial (H-2′ is axial) and again there is no evidence for a fast ring inversion process in the morpholine ring.

The chemical shifts of H-4α and H-4β are an exception to the general rule that equatorial protons in six-membered rings appear at lower fields to their axial counterparts.[9] Note also from Table 1 the large chemical shift differences between the pairs of geminal protons on carbons 1, 3′ and 5′. The rule that equatorial protons are generally at lower fields than corresponding axial protons is upheld and the large spread in chemical shifts assists the data analysis. For **I** in DMSO-d_6 the chemical shift difference between H-3′$_{eq}$ and H-3′$_{ax}$ is 1.6 ppm.

In DMSO-d_6 solution, the ^{1}H signal from H-1β is well resolved from other resonances and a DPFGSE NOE experiment at this frequency illustrates very clearly that the morpholine moiety is fixed and adopts a specific orientation relative to the steroid. Figure 2(C) (for **I**) shows the expected intra-steroid NOE cross

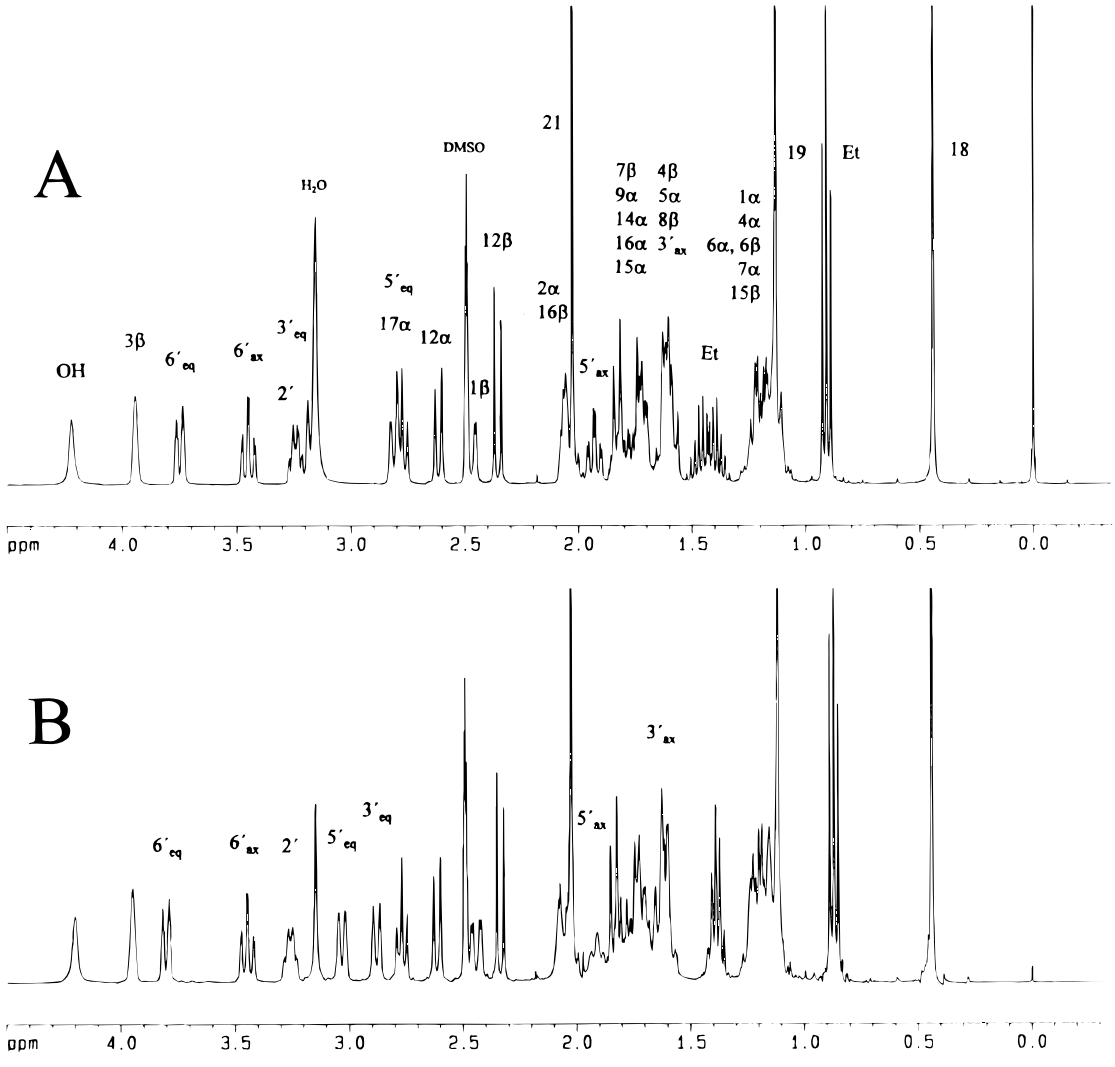

Figure 4. 400 MHz ^{1}H NMR spectra of (A) **I** and (B) **II** in DMSO-d_6 at 57 °C. For clarity all protons are assigned on A and only morpholine ring protons are assigned on B. The signals from singlet methyl protons are truncated at about 50% of true heights.

peaks and also a strong interaction between H-1β and H-3$'_{eq}$. Note the complete absence of signal from any other morpholine proton, even from H-3$'_{ax}$. Figure 2(D) shows the result of an identical experiment on **II** which this time reveals an interaction between H-1β and H-5$'_{eq}$. The output of the molecular modelling, which shows the conformations responsible for these NOE results, are shown in Fig. 3(C) and (D) and internuclear distances are provided in Table 3.

General discussion

The clearly discernible differences between the spectra of **I** and **II** (in either solvent) demonstrate some of the interest as well as the power and usefulness of high-field NMR to structural studies. Having explained the origins of the differences between the spectra we may now consider (qualitatively) some of the other aspects of Figs 1 and 4 (and Table 1). For instance, we have already pointed out that the differences between the spectra of **I** and **II** (in any solvent) are due to changes in the chemical shift of morpholine protons. The steroid proton spectrum is to a good approximation independent of stereochemistry at C-2′ (see below) and the chemical shifts of protons on C-2′ and C-6′ are not very solvent dependent. Hence the major changes between the spectra shown in Fig. 1(A) and (B) and in Fig. 4(A) and (B) are due to chemical shift changes of just four protons (H-3$'_{eq}$, H-3$'_{ax}$, H-5$'_{eq}$ and H-5$'_{ax}$). This result complements the ^{13}C NMR data (discussed in Ref. 2) where only the chemical shifts of C-3′ and C-5′ differ significantly between **I** and **II**.

The major change that we see when we compare the spectrum of **I** (or **II**) in $CDCl_3$ with that in DMSO-d_6 is due to the conformation change of ring A, which is a chair in DMSO-d_6 and a twist-boat in $CDCl_3$. It is not surprising that this major change of conformational geometry results in large changes to ring A ^{1}H chemical shifts and to H-6α/6β of ring B. However we would not expect to see a conformation change in ring A to be reported by protons in ring C, so the reversal of the assignments of H-12α and H-12β as the solvent is changed is surprising. The assignments were not problematic. Clear NOE enhancements were seen between C-18 methyl protons and H-12β in both solvents and H-12α was broadened, as expected from a long-range coupling to C-18 methyl. An explanation for the anomaly is not readily apparent as ring C is restricted to its normal chair conformation and solvent-induced conformational changes in ring A had no significant impact on the chemical shifts of ring C protons in an androstane analogue of **I** and **II**.[5] It seems likely, then, that the reversal may be a consequence of solvation of the C-11 carbonyl group in DMSO-d_6 solution. The solvent dependence of the chemical shifts of the four protons attached to C-3′ and C-5′ is a consequence of the solvent induced conformation change of ring A.

Finally, we consider the effect that a change in stereochemistry at C-2′ has upon the ^{1}H and ^{13}C NMR spectra of the steroid and see (from Tables 1 and 2) that it is negligible except for some small shifts in signals from protons and carbons associated with ring A. The only steroid protons that exhibit a chemical shift difference of more than $\pm$0.02 ppm between **I** and **II** are H-2α in $CDCl_3$ solution (0.03 ppm) and H-1β in DMSO-d_6 solution (0.03 ppm). The picture is very similar with the ^{13}C data where chemical shift differences between **I** and **II** are less than $\pm$0.05 ppm except for C-1, C-2, C-3, C-4 and C-19 in $CDCl_3$ and C-1, C-3, C-5 and C-19 in DMSO-d_6. These findings are consistent with those of earlier NMR studies of steroid diastereoisomers (e.g. cholesterol,[10–12] estrone,[13] estradiol[13] and 5α-dinosterane[14]), where similarly small shifts were reported between diastereomerically related pairs of steroids.

EXPERIMENTAL

The syntheses of **I** and **II** have been reported.[1] All of the data were obtained on a standard Bruker DRX 400 spectrometer, fitted with a 5 mm inverse gradients probe, BGU 2 gradients unit, BGPA 10 power amplifier, operating under XWINNMR 1.0 and using standard Bruker experiments. Data were obtained from non-degassed solutions of 10 mg (^{1}H), and 30 mg (^{13}C), **I** or **II** in 0.7 ml $CDCl_3$ (23 °C) or DMSO-d_6 (57 °C) and a trace of TMS. The ^{1}H data are referenced to the internal TMS. The ^{13}C data were acquired with a sweep width of 30 303 Hz and 32 K data points, giving 0.01 ppm digital resolution, and are referenced to the solvent peak at 77.00 ppm for $CDCl_3$ and 39.60 ppm for DMSO-d_6. A gradient accelerated COSY45 experiment was used with 1 ms gradient pulses set at 10% of maximum power. HETCOR data were acquired with a sweep width typically from 5 to 100 ppm in F_2 and 4 K data points, 128 increments (F_1) and a relaxation delay of 2.0 s. The acquisition time was typically 0.2 s and with 16 scans per increment the total experiment time was around 1.25 h. NOESY data were collected with a 500 ms mixing time and 2.0 s relaxation delay. DPFGSE NOE data were acquired with 20 or 70 ms selective pulses, a 500 ms mixing time and 2.0 s relaxation delay. The gradient pulses G_1, G_2 and G_3 were of 1 ms duration at 30%, 25% and 80% of maximum power, respectively.

All structures were modelled in Chem-X.[15] A 2β-morpholinyl-3α-hydroxy-5α-pregnane-11,20-dione template was constructed in 2D and the 3D structure generated by the Chem-X 2D-3D Builder, with the steroid A ring in a chair conformation. Ethyl groups were added at the appropriate positions with all carbon—carbon bonds *gauche*. Gasteiger charges[16] were calculated and each structure was geometry optimized using the default Chem-X molecular mechanics parameters. Structures with the steroidal A-rings in twist-boat conformations were also generated by manipulation of the A-ring torsion angles in Chem-X. For the twist-boat structures, hydrogen bonds were created between the morpholine nitrogen and the 3α-hydroxyl group, Gasteiger charges were assigned, again followed by geometry optimization. Each structure was conformationally analysed by rotation about the steroid C-2—morpholine N bond. Conformers were generated by rotation of this bond through 360° in 5° steps, with geometry optimization at each step. For the structures with the steroid A-ring in a chair conformation the

global energy minimum was identified and found to be more than 5 kcal mol^{-1} lower in energy than any other minimum in each case. For the twist-boat structures, only one energy minimum was detected in each case. The relevant inter-proton distances were calculated from the global minimum energy conformations. Free rotation of the 19-CH_3 group was assumed and the inter-proton distances for 19-CH_3 hydrogen to morpholine protons are the minimum distance in all cases.

CONCLUSION

We have validated earlier low-field ^{13}C NMR experiments with fully assigned high-field ^{1}H NMR data. Diastereoisomers **I** and **II** give rise to very different spectra and this is simply due to differences in the chemical shifts of just four protons attached to C-3′ and C-5′. These same four protons are also very sensitive to the solvent-dependent conformation change that takes place in ring A. NOE experiments show clearly that the morpholine moiety cannot rotate freely in either $CDCl_3$ or DMSO-d_6 solutions and molecular modelling provides the solution conformations that explain the experimental data.

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