Solution Conformation of Three Steroid 19-Nor-4-en-3-ones determined from Two-dimensional Nuclear Magnetic Resonance Spectroscopy, Coupling Constant Calculations, and Circular Dichroism

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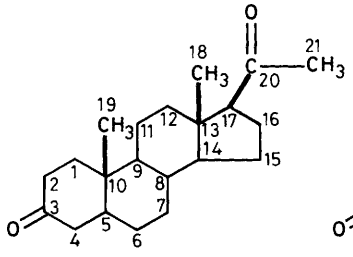
A variety of homo- and hetero-nuclear two-dimensional (2-D) n.m.r. techniques were utilized to make complete assignments of the ^1H and ^{13}C spectra of three 17α-CH_2R-substituted (R = Cl, N_3, or CN) 17β-hydroxyestr-4-en-3-ones. The proton chemical shifts and, particularly, the proton–proton geminal coupling constants were used to show, with the help of molecular orbital calculations, that the normal ring *A* conformation is predominant for all three 19-norsteroid molecules in solution. The relative merits of the various 2-D techniques in the study of steroids are discussed. Chiroptical measurements of the enone $n \longrightarrow \pi^*$ transition supported the conformation assignment derived from n.m.r. spectra. A comparison of n.m.r., *X*-ray crystallographic, and theoretical data indicates an exceptionally high conformational flexibility in the ring *A* region of steroidal 19-nor-4-en-3-ones.

The ring *A* conformation[1] and the conformational flexibility[2] of the steroid backbone have been considered to exert significant influence on receptor binding ability. Therefore, the determination of conformations and conformational flexibility of steroids is of high intrinsic interest.

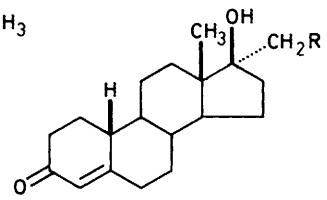
Conformations of steroids in the solid state are usually determined by *X*-ray diffraction. In solution, n.m.r. has been used increasingly for this purpose. Because of crystal packing forces, it has been found in some cases that a given steroid molecule may adopt different conformations in the solid state from those in solution.[3,4]

In the present investigation, the ring *A* conformations of three 17α-CH_2R-substituted 17β-hydroxyestr-4-en-3-ones (**2**)—(**4**) in solution have been studied. These 19-norsteroids display progestational activity[5] as well as a high affinity for the progesterone receptor.[6] Furthermore, the molecular mechanics calculations of Bucourt *et al.*[7] showed that the removal of the 10-methyl group significantly reduces the energy difference between the normal and inverted ring *A* conformations. Therefore, it is of particular interest to compare the ring *A* conformations of these 19-norsteroids in solution with those in the solid state. Progesterone (**1**) has been analysed in the same way for comparison.

Solution ring *A* conformations were determined by a variety of ^1H homonuclear and ^1H–^{13}C heteronuclear two-dimensional (2-D) n.m.r. techniques. The analysis of the ^1H n.m.r. spectra of steroids presents a particular challenge because the chemical shifts of the protons are usually distributed in a rather narrow region and the spins are usually extensively coupled. Before the availability of 2-D n.m.r.,[4,8–14] very little detailed analysis of the ^1H spectra of steroids had been performed. Recent studies utilizing a range of 2-D n.m.r. techniques provide the starting point in establishing a reliable database for the proton n.mr. of steroids. However, during these studies it was observed[12,14] that in many situations some 2-D n.m.r. techniques, such as COSY[15] and homonuclear 2-D *J* spectroscopy,[16] are not very useful for determining assignments. Furthermore, the available data are still too sparse for quantitative evaluation of the shielding and coupling parameters. In the present study, the conformational information was derived from a combination of several 2-D techniques found particularly useful for this purpose. The strategy of ^1H assignment and relative merits of some 2-D experiments for this type of compound are discussed.



(**1**)



(**2**) R = Cl
(**3**) R = N_3
(**4**) R = CN

Experimental

The progesterone (**1**) sample was obtained from Sigma Chemicals and used without further purification. The 19-norsteroids (**2**)—(**4**) were synthesized according to procedures published elsewhere.[5]

N.m.r. Experiments.—The steroid (**1**), (**2**), or (**3**) (*ca.* 150 mg) was dissolved in $CDCl_3$ (0.6 ml) in a 5 mm n.m.r. tube; the same amount of (**4**) was dissolved in $(CD_3)_2CO$. A Nicolet NT-300 spectrometer (300.05 MHz for ^1H and 75.5 MHz for ^{13}C) equipped with 5 mm ^{13}C and ^1H probes was used. A version of

the ^{13}C–^{1}H chemical shift correlation spectroscopy with proton homonuclear decoupling in the F_1 dimension[12,17] was used to generate ^{13}C–^{1}H shift correlations. Typically, 256 × 2 K data blocks were obtained. The proton spectral width was limited to the upfield (0.8—2.4 p.p.m.) region because all downfield protons can be easily assigned. Accumulation time was about 1—1.5 h. The geminal ^{1}H–^{1}H coupling constants were measured *via* a pulse sequence designed to measure $^{2}J_{HH}$ selectively.[18] Only 16 data blocks are usually needed in the t_1 dimension. An experiment therefore took less than half an hour. The $^{1}J_{CH}$ values were measured by using a version of heteronuclear 2-D *J* spectroscopy which suppresses long-range $^{n}J_{CH}$ ($n > 1$) *via* the incorporation of the bilinear rotation pulses[19] in the middle of the evolution period.[20] Thirty-two or sixty-four blocks of data were usually acquired for this experiment. In the heteronuclear 2-D experiments, the ^{13}C and ^{1}H $\pi/2$ pulses were 11 and 30 μs, respectively.

For proton–proton chemical shift correlation, normal COSY,[15] long-range COSY, and double-quantum-filtered COSY (DQF-COSY)[21] experiments were performed. The ^{1}H $\pi/2$ pulse in all these experiments was 8 μs. Usually 256 × 1 K blocks of data were acquired. In the long-range COSY experiment, the last 'read' pulse was set at 45° and the additional delay was 0.3 s. Owing to the limitation imposed by the software, no phase-sensitive 2-D experiment was carried out. Since DQF-COSY requires a 16-step phase cycling,[21] about 2—3 h was required for the data acquisition.

Coupling Constant Calculations.—Proton–proton geminal coupling constants were calculated within the framework of INDO molecular orbital theory using standard parameters.[22] In a first step, a finite perturbation (FP) approach was made for a steroid fragment consisting of rings *A* and *B*. Subsequently, computations were performed for a fragment containing rings *A*, *B*, and *C* by means of the sum-over-states (SOS) method,[23] treating the electron densities at the nuclei as least-squares parameters to fit the results of the FP method. Coulomb integrals are neglected for the calculation of the excitation energies.[24] In general, there exists a good correlation between the results of the FP and SOS procedures.[25]

Circular Dichroism Measurements.—The solutions of steroids (**2**)—(**4**) were prepared by weight, in dimethyl sulphoxide. Circular dichroism spectra were obtained with a Cary 60 spectropolarimeter equipped with a c.d. attachment. The c.d. data are expressed as $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$ in units of l mol^{-1} cm^{-1}, where ε_l and ε_r are dichroitic absorptions for left- and right-polarized light, respectively. The measurements were recorded at room temperature in the wavelength range 260—380 nm by use of quartz couvettes of 1 mm pathlength.

Results and Discussion

For steroids containing few functionalities, such as those in this investigation, the chemical shifts of virtually all protons on the rings occur in a congested range of about 1.5 p.p.m. As a result, one-dimensional spectra do not offer much information for assignment. The starting point for an attack on the problem by 2-D techniques depends on the degree of simplification of the proton spectrum due to functionalities and the magnetic field strength of the n.m.r. spectrometer used. For example, Sedee *et al.*[10] used a 500 MHz spectrometer to obtain complete proton assignment for a 19-norsteroid by employing ^{1}H–^{1}H shift correlation and ^{1}H homonuclear *J*-resolved spectroscopy. The ^{1}H assignment was then used to assign the ^{13}C spectrum. However, this procedure is less desirable with a lower-field (*e.g.* 300 MHz for proton) spectrometer.[11–14] In such a situation, the starting point of the approach is usually the ^{13}C–^{1}H chemical shift correlation.[8,12,14] Since the ^{13}C assignments for steroids, with the exception of a few positions, can be made without much difficulty,[26] the initial assignment of the corresponding protons can, therefore, be made. Subsequently, other 2-D techniques and particularly the ^{1}H–^{1}H shift correlation can be used to eliminate the remaining ambiguities. The results from various 2-D experiments are presented in the following sections.

Table 1. The ^{13}C and ^{1}H chemical shift assignments for the steroids (**1**)—(**4**)[a]

	(1)[b]			(2)			(3)			(4)		
		^{1}H			^{1}H			^{1}H			^{1}H	
	^{13}C	α	β	^{13}C	α	β	^{13}C	α	β	^{13}C	α	β
1	35.68	1.716	2.047	26.30	1.526	2.247	26.27	1.532	2.252	27.27	1.517	2.295
2	33.91	2.337	2.441	36.21	2.222	2.400	36.17	2.228	2.405	36.92	2.250[c]	2.250[c]
3	199.30			199.64			199.81			199.73		
4	123.87	5.730		124.33	5.823		124.26	5.829		123.30	5.722	
5	170.87			166.26			166.51			166.56		
6	32.75	2.280	2.412	35.14	2.266	2.477	35.14	2.259	2.478	35.72	2.317	2.474
7	31.85	1.065	1.870	30.55	1.039	1.849	30.53	1.033	1.842	31.46	1.051	1.869
8	35.50		1.570	40.72		1.461	40.76		1.440	41.73		1.507
9	53.59	0.989		48.99	0.829		49.00	0.824		49.90	0.873	
10	38.53			42.14		2.113	42.15		2.112	42.80		2.212
11	20.98	1.645	1.461	25.72	1.876	1.323	25.72	1.874	1.312	26.53	1.913	1.361
12	38.60	1.456	2.079	31.50	1.279	1.686	31.35	1.237	1.684	32.03	1.272	1.654
13	43.87			46.49			45.75			47.06		
14	55.97	1.181		49.92	1.236		49.71	1.195		49.90	1.340	
15	24.33	1.724	1.267	23.24	1.637	1.394	23.10	1.612	1.360	23.59	1.686	1.398
16	22.78	1.676	2.189	34.26	1.745	2.137	34.08	1.723	2.019	36.65	1.927	2.033
17	63.43	2.548		81.89			83.07			81.72		
18	13.30	0.669		14.35	1.009		13.90	0.962		14.43	0.991	
CH_2X				53.75	3.780		57.88	3.526		28.45	2.655	
					3.609			3.201				

[a] Chemical shifts in p.p.m. downfield from Me_4Si. [b] Determined in ref. 11. [c] These protons are not equivalent. However the difference in δ(^{1}H) is not resolvable (<0.01 p.p.m.).

Table 2. The $^2J_{HH}$ values of the steroids (**1**)—(**4**) (in Hz)

Carbon number	(1)[a]	(2)	(3)	(4)
1	13.4	12.9	12.9	13.4
2	17.0	16.1	16.0	16.3
6	14.4	14.8	15.0	14.5
7	12.7	12.5	12.8	12.5
11	13.9	13.3	13.4	13.3
12	12.3	12.3	12.4	12.5
15	12.0	*b*	10.7	11.8
16	13.7	14.1	14.2	14.4
CH_2X		12.3	10.9	12.2

[a] From ref. 13. [b] Not measurable, probably because of strong coupling of the protons at C-15 with neighbouring protons (ref. 17).

Table 3. Effect of C-10 axial methyl group on proton chemical shifts

Position	(2)	(3)	(4)
1α	0.190	0.184	0.199
1β	−0.200	−0.205	−0.248
2α	0.115	0.109	0.087
2β	0.041	0.036	0.191
6α	0.014	0.021	−0.037
6β	−0.065	−0.066	−0.062
7α	0.026	0.032	0.014
7β	0.021	0.028	0.001
8β	0.109	0.130	0.063
9α	0.160	0.165	0.116
11α	−0.231	−0.229	−0.268
11β	0.138	0.149	0.100

^{13}C–^1H *Chemical Shift Correlation.*—^1H Decoupling in the F_1 dimension in ^{13}C–^1H chemical shift correlation spectroscopy[27] is particularly useful in the case of steroids since each proton multiplet in the steroid usually spans a range of over 30 Hz owing to extensive couplings with neighbouring protons. Unless a sufficiently large number of blocks in the t_1 dimension can be acquired to resolve the coupling patterns (this is frequently limited by the available spectrometer time as well as by disk space), the determination of the ^1H chemical shifts will not be very accurate without decoupling. With proton decoupling in the F_1 dimension, a sharp peak in the proton dimension results, and $\delta(^1H)$ can be determined to within 0.01 p.p.m.[12]

A good example in structures (**2**)—(**4**) is H-8, which is so extensively coupled that a broad and featureless peak was observed in the proton spectrum. The extensive couplings diminish its intensity so much that all cross-peaks due to H-8 were missing from the COSY map. However in the proton-decoupled ^1H–^{13}C chemical shift correlation map, the signal for H-8 in the proton dimension showed a sharp line.

The determination of ^{13}C–^1H shift correlation is rather straightforward. The results (Table 1) were initially based on a tentative ^{13}C assignment and subsequently confirmed by using $^2J_{HH}$ values and ^1H–^1H coupling connectivities. The results for the three 19-norsteroids (**2**)—(**4**) correlate very well with that for (**1**) determined previously.[11] However, some assignments of ^1H resonances to α- and β-protons attached to the same carbon atom may still remain ambiguous. For example, the assignment of chemical shifts of the 1α- and 1β-proton is correct only if the conformation of ring *A* is 'normal,' as in (**1**) (see discussion in later sections). Otherwise, the assignment may be reversed in the 'inverted' ring *A* conformation.

Measurement of $^2J_{HH}$ *Values.*—The selective measurement of $^2J_{HH}$[18] is a rapid method (in terms of both experimental and interpretation time) which provides some useful information that has not been widely recognized.[13] As shown in Table 2, the $^2J_{HH}$ values at various carbon sites are characteristic, usually varying by less than 0.5 Hz, unless there is substitution at the adjacent site or a change of conformation. In particular, carbon atoms α to a double bond, such as C-6, can be easily distinguished from other carbon atoms with similar $\delta(^{13}C)$ values; for example the corresponding $^2J_{HH}$ value for C-6 is usually around 14—15 Hz whereas those of the other carbon atoms are about 12—13 Hz.[13] Only the magnitude of $^2J_{HH}$ was measured by this technique. However, $^2J_{HH}$ values for these compounds are known to be negative.[26] In subsequent discussion of their conformational implications, the sign of the geminal couplings was assumed to be negative. It would be of much more interest to be able to measure $^3J_{HH}$ selectively. However, the method[28] for doing this proved to be laborious and required selective pulses. Two-dimensional *J*-resolved spectroscopy on steroids at this field strength (7.0 T) is usually hampered too much by strong coupling to be of much use.[14] However, $^2J_{HH}$ does provide some information on the conformation of ring *A*.[13] The results will be discussed later.

Table 4. Conformations detected by *X*-ray crystallography (solid-state conformers)

Compound	Ref.	Inverted conf.	Normal conf.
(**2**)	32	1β-Sofa (distorted to 1β,2α-half chair)	1α,2β-Half chair
(**3**)	33	Not found	1α-Sofa and 1α,2β-half chair
(**4**)	34	1β-Sofa	Not found

^1H–^1H *Chemical Shift Correlation: COSY and Double-quantum-filtered COSY.*—As noted in several previous reports,[11,12,14] COSY at moderate magnetic field cannot be used for *a priori* assignment of the ^1H spectrum for steroids with many strongly coupled protons, particularly in the upfield region (0.8—1.8 p.p.m). However, after tentative assignment of the ^1H spectrum through ^{13}C–^1H chemical shift correlation, COSY is helpful in ascertaining whether the ^1H assignment based on ^{13}C assignment is correct. In the present case, for (**2**)—(**4**), several ^{13}C assignments were reversed on the basis of ^1H–^1H connectivities. Usually these reversals involve ^{13}C resonances with $\delta(^{13}C)$ values not more than 2—3 p.p.m. apart. The proton spectrum for (**1**) was assigned from ^{13}C–^1H chemical shift correlation alone.[11] The ^1H–^1H shift correlation experiments performed for (**1**) showed that the previous assignment[11] was correct.

COSY of steroids is usually difficult because the extensive couplings between the spins make the cross-peaks substantially weaker than the diagonal signals. This problem is worsened by the dominating presence of the methyl groups, which are not coupled significantly to other spins. The use of DQF-COSY[21] serves to circumvent these problems partially. Although DQF-COSY does not simplify the rest of the correlation map, it does eliminate the signals of the methyl groups. Furthermore, DQF-COSY reduced the intensities of the diagonal peaks, which, in contrast to COSY, have anti-phase fine structure in the DQF-COSY map.[29] Thus, the effective signal-to-noise of the off-diagonal peaks is improved in DQF-COSY, and the correlation map is more readily interpretable than that obtained from COSY (Figure 1). There is a price to pay for this improvement since DQF-COSY requires a minimum of 16 scans for phase cycling. Therefore for samples of sufficient quantity, in which case fewer scans would have been sufficient for the COSY experiment, a longer acquisition time (*ca.* 2—3 h) is required.

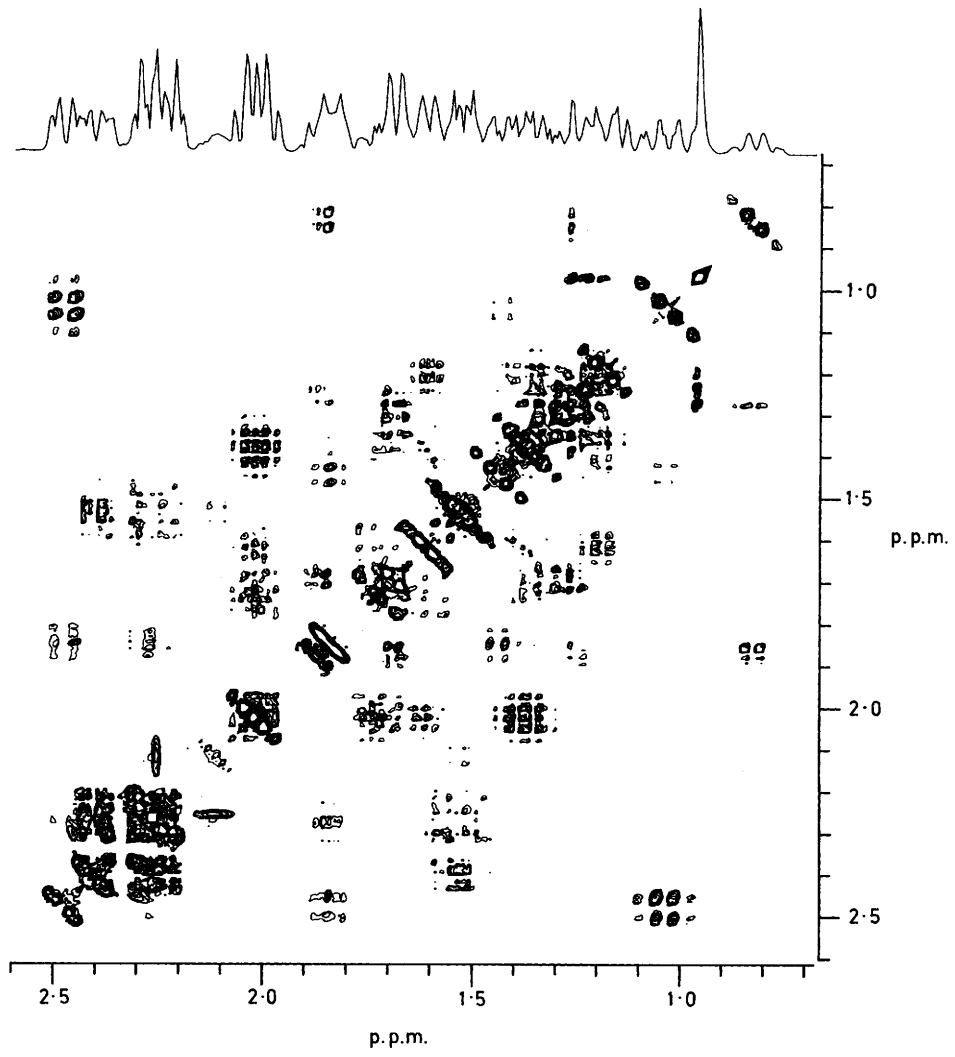


Figure 1. The contour map from the DQF-COSY experiment for (**3**). A 512 × 512 date matrix was acquired. The projected ^{1}H spectrum was displayed above the contour map. In a 1-D spectrum or a projected spectrum from COSY of the same molecule, the signal of the methyl group at 0.962 p.p.m. is at least ten-fold higher

On the other hand, the improvement of DQF-COSY is not due to the increased number of scans. The use of COSY with additional delays to enhance the correlation due to long-range couplings[15] reveals only a limited number of additional cross-peaks, primarily between the methyl group and protons four bonds away.[14] In the present case, its utility is limited.

Effect of Methyl Substitution on the Proton Chemical Shifts.—Comparison of the chemical shifts of the corresponding protons between progesterone (**1**) and the steroids (**2**)—(**4**) reveals the effect of the axial methyl group substitution on position 10. The substitution effects on $\delta(^1H)$, assuming that there is no change in the conformation of ring *A*, are given in Table 3. The direction and magnitude are in general agreement with those observed for cyclohexane and its derivatives.[30]

Conformation of the Ring A *derived from N.m.r. Data.*—The conformation of ring *A* of steroidal 4-en-3-ones is believed to be intimately related to their binding affinity to receptors.[1] Progesterone is known to be in the 'normal' ring *A* conformation.[1,31] However, for 19-nor-4-en-3-ones, the energy difference between the 'normal' and 'inverted' ring *A* conformations is relatively small (*ca.* 4 kJ mol^{-1}).[6,7] Thus, an *X*-ray crystallographic study of (**2**)—(**4**)[32–34] (Table 4) showed that (**3**) has the 'normal' conformation whereas (**4**) assumes the 'inverted' conformation in the crystal state. The steroid (**2**), on the other hand, crystallizes in both conformations. The torsion angle C(1)–C(2)–C(3)–C(4) is usually about 10—15° smaller in the inverted conformation than in the normal conformation.[32–34] It has been demonstrated that, for 17-acetoxy-6α-methylpregn-4-ene-3,20-dione, the ring *A* conformation in the solid state is inverted, but is normal in solution.[3,4] Therefore, the ring *A* conformations of these 19-norsteroids in solution pose interesting questions.

More direct information on the conformation of ring *A* is obtained from the nuclear Overhauser effect (n.O.e.) between the angular 10-methyl group and the protons on the ring *A*[8] and the proton–proton vicinal coupling constants. However, in these 19-norsteroids, there is no methyl group on C-10, thus eliminating the possibility of using the n.O.e. for the purpose. As pointed out in the previous section, homonuclear 2-D *J* spectroscopy was not feasible for these steroids at 300 MHz. As a result, no $^3J_{HH}$ information was obtainable in this study.

The ^{1}H chemical shifts of (**2**)—(**4**) (Table 1) are very similar. In particular, those for (**2**) and (**3**) are virtually identical for protons in rings *A* and *B* (to within 0.01 p.p.m.). This fact

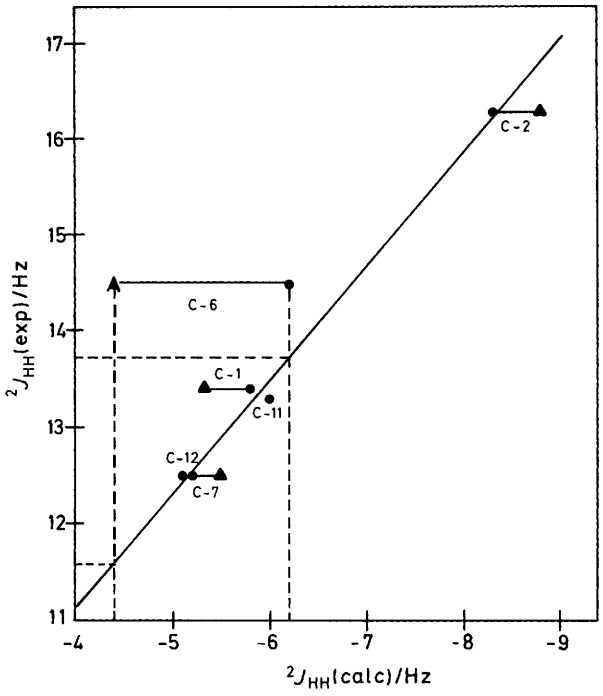


Figure 2. A plot of the magnitudes of the experimentally determined $^2J_{HH}$ values *versus* the calculated $^2J_{HH}$ values for (**4**). The calculated $^2J_{HH}$ values are based on the structure optimized from molecular mechanics calculation for both the normal (●) and inverted (▲) ring *A* conformations. The dotted lines indicate the expected experimental $^2J_{HH}$ at C-6 for the two conformations respectively if the correlation between $^2J_{HH}$(exp.) and $^2J_{HH}$(calc.) (the solid line) is used (see text)

strongly indicates that the ring *A* conformations are identical. The differences between $\delta(^1H)$ of (**4**) and the corresponding values for (**2**) and (**3**) are slightly larger (<0.1 p.p.m.). These differences can be attributed in part to the solvent effect, since $(CD)_3CO$ was used for (**4**) whereas $CDCl_3$ was used for (**2**) and (**3**). One of the most conspicuous differences is in the $\delta(^1H)$ values of protons at C-2. For (**2**) and (**3**) there is a difference of *ca.* 0.18 p.p.m. between protons 2α and 2β. For (**4**), however, the difference is not resolvable from the ^{13}C–^1H chemical shift correlation map, indicating that it is less than 0.01 p.p.m. (Table 1). The smaller chemical shift difference between the 2α- and 2β-proton in (**4**) indicates that the torsion angle C(1)–C(2)–C(3)–C(4) is smaller in (**4**) than in (**2**) and (**3**), *i.e.* protons 2α and 2β are closer to being symmetrically disposed with respect to the C=O bond.[12] This inference is in qualitative agreement with the results on the torsion angles in (**2**)—(**4**) from *X*-ray diffraction studies on the crystals.[32–34] However, this information does not provide a clue to whether ring *A* in (**4**) is 'normal' or 'inverted'. The similarity in the ^1H chemical shifts of (**2**), (**3**), and (**4**) does appear to imply that all three molecules have the same ring *A* conformation, and most probably the normal conformation. This point will be addressed in a later section. However, it should be recognized that such inferences based on $\delta(^1H)$ are far from being conclusive.

The $^2J_{HH}$ values obtained in this work (Table 2) do provide more specific information on the ring *A* conformation. As demonstrated in previous work, [13,35] the $2J_{HH}$ value between protons at C-2, adjacent to the C=O bond, is a good measure of the torsion angle C(1)–C(2)–C(3)–C(4). For the 4-en-3-ones, the empirical equation (1) has been determined,[13] where φ is the

$$^2J_{HH} = -12.6 - 6.0\cos^2\varphi \qquad (1)$$

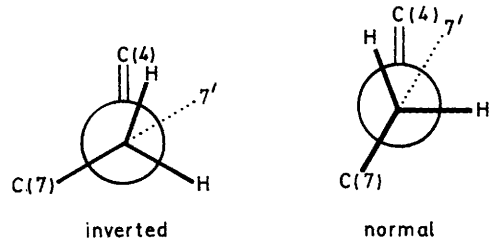


Figure 3. Newman projections for the C(4)C(5)C(6)C(7)7′ segment for the normal (right) and inverted (left) conformations of (**2**)—(**4**). The projections are viewed from C-6 to C-5 along the C(6)–C(5) bond

torsion angle C(1)–C(2)–C(3)–C(4) in this case. The form of this equation has its theoretical justification.[35] On the basis of measured $^2J_{HH}$, the magnitudes of the torsion angle for (**2**)—(**4**) are 40, 41, and 38°, respectively. The difference between these values may not be significant, since the torsion angle cannot be determined to within 2° accuracy. The magnitudes of φ for (**2**) and (**3**) are in good agreement with those obtained for the normal conformation in the solid state from *X*-ray diffraction [40.8 for (**2**) and 39.4 and 30.4° for (**3**)].[32,33] For (**4**), the magnitude of the torsion angle for the inverted conformation observed in the solid state is only 27.8°.[34] This discrepancy may be a result of the fact that, in solution, the molecule has the normal ring *A* conformation which usually has a torsion angle *ca.* 10° larger than that in the corresponding inverted conformation.[32–34] This speculation is supported by INDO-SOS calculation of the geminal coupling constants for these steroid fragments consisting of rings *A*, *B*, and *C* in both normal and inverted ring *A* conformations. Although the calculated magnitudes of $^2J_{HH}$ were found to be far smaller than the experimental values, the calculated $^2J_{HH}$ values for the normal ring *A* conformation did reproduce the trend of variation of the experimental values very well (Figure 2), *i.e.* a straight line relationship could be found between the calculated and experimental $^2J_{HH}$ values. On the other hand, the agreement between the geminal coupling constants calculated for the inverted conformation and the experimental results is far inferior. Using this line of correlation and the calculated values gives $^2J_{HH}$ for protons at C-6 as *ca.* 13.8 Hz for the normal ring *A* conformation and *ca.* 11.7 Hz for the inverted conformation (the dotted lines in Figure 2). Thus, the magnitude of the experimental $^2J_{HH}$ values for protons at C-6 (14.4—15.0 Hz) is much closer to the value corresponding to the normal ring *A* conformation. There is a structural basis for the difference between the expected values of $^2J_{HH}$ in the two conformations. The physical origin of this difference is the same as that demonstrated for $^2J_{HH}$ between the protons at C-2.[13,35] The dependence of the contribution from the C=C π-bond [C(4)–C(5)] to the magnitude of the adjacent (C-6) $^2J_{HH}$ value on the torsion angle is given by equation (2), where φ is the

$$^2J_{HH}\pi = a_0 + a_1\cos^2\varphi \qquad (2)$$

torsion angle along the bond between the carbon atom bearing the protons and that involved in the π-bonding. In this particular case, this angle is represented by the torsion angle C(4)–C(5)–C(6)–7′, where C(6)–7′ is an imaginary bond 180° from C(6)–C(7), and C(6)–7′ bisects the angle $H_α$–C(6)–$H_β$ (Figure 3). From both the molecular mechanics calculation and the *X*-ray structure obtained in the solid state,[6,32–34] it was found that the C(4)–C(5)–C(6)–7′ angle ranges from −43 to −52° for (**2**)—(**4**) in the normal ring *A* conformation, but from −61 to −71° in the inverted conformation (Figure 3). By using these geometrical parameters and equation (2), it can be

Table 5. Results of c.d. measurements

Compound	$\Delta\varepsilon$/l mol^{-1} cm^{-1}	$\lambda_{max.}$/nm
(2)	−1.5	325
(3)	−1.5	326
(4)	−1.4	326

estimated that a change from the normal to the inverted ring *A* conformation would decrease the magnitude of $^2J_{HH}$ at C-6 by *ca.* 3 Hz. Therefore, the relatively large magnitude of $^2J_{HH}$ for protons at C-6 is a strong indication that the normal ring *A* conformation is the predominant conformation for (**2**)—(**4**) in solution. The discrepancy in magnitude between the experimental and the projected theoretical $^2J_{HH}$ values (14.5—15.0 Hz *vs.* 13.8 Hz) may imply that, in solution, the magnitude of the torsion angle C(4)–C(5)–C(6)–7′ is actually less than that determined in the solid state. However, since the calculated value was extrapolated, and the difference is not much greater than experimental uncertainties, little significance should be attached to this discrepancy.

Another piece of information which supports the foregoing conclusion comes from $^1J_{CH}$ measurement. A striking difference in the bond angle C(1)–C(10)–C(9) for (**2**)—(**4**) in the two conformations has been revealed by *X*-ray diffraction and molecular mechanics calculations.[6] For the normal conformation, this angle was found to be 109—111°, values typical of a tetrahedral carbon site; but for the inverted conformation this angle increases to 117—118°. The increase to nearly 120° in the latter conformation means a flattening of the carbon framework at C-10 and a substantial increase of the *p*-character of the C–H bond at C-10. Therefore, it is expected that $^1J_{CH}$ at C-10 would be appreciably smaller than the value of 125 Hz which is characteristic of cyclohexane and an sp^3-hybridized carbon.[36] However, the experimental values for $^1J_{CH}$ at C-10 are 124.4 Hz for (**2**), 124.1 Hz for (**3**), and 126.0 Hz for (**4**). Even though (**4**) was found to be exclusively in the inverted ring *A* conformation in the crystal state, the change in $^1J_{CH}$ at C-10 from that of (**2**) and (**3**) is in fact in the opposite direction from that expected if the molecule had remained in the inverted conformation in solution.

Since molecular mechanics calculations have shown that the absence of the 10-methyl group significantly reduces the energy difference between the normal and the inverted ring *A* conformations, the data observed for solution may reflect averages of the two conformations. If the value of *ca.* 4 kJ mol^{-1} for the energy difference calculated for testosterone[7] is used for the estimation, then there may be up to 20% of the steroid molecules in (**2**)—(**4**) in the inverted conformation. Since $^2J_{HH}$ does not depend on the sign of the torsion angle φ [equation (1)], values of which are opposite for the two conformations, the observed $^2J_{HH}$ value is simply the weighted average of $^2J_{HH}$ for the two conformations. This can be expressed as equation (3),

$$^2J_{HH}{}^{obs} = p_n{}^2J_{HH}{}^n + p_i{}^2J_{HH}{}^i \quad (3)$$

where i and n stand for inverted and normal respectively, and p_i and p_n denote the fractions in each conformation. The $^2J_{HH}$ value in each conformation depends on φ as described in equation (1). However, in the present study, no information on the fractions could be obtained, and no further quantitative treatment is warranted.

C.d. Measurements of the Enone n ⟶ π Band.*—As shown in Table 5, the data for the 4-en-3-one *n* ⟶ π* c.d. bands for the steroids (**2**)—(**4**) are almost identical. The negative sign is consistent with the usually observed normal ring *A* conformation in the case of 10-methyl steroids.[4,37] Although the data are not conclusive, they do suggest that all 19-norsteroids considered exhibit a common (*i.e.* the normal) conformation of ring *A* in solution.

Conclusions

Crystallographic results of a single drug-substance determination are often used as well resolved structural data for discussing structure–activity relationships. In this context, however, it should be recognized that there might be intramolecular conversion phenomena or conformational flexibilities which have a strong influence on the structure in environments other than the crystalline state. Furthermore, the bioactive conformation of a drug molecule may not necessarily be that of the lowest energy. Possible ways in which information about these geometrical variabilities can be obtained are: (i) a systematic analysis of *X*-ray single-crystal structure determinations of a series of similar compounds,[2,38–40] (ii) a calculation of potential energy curves or surfaces of intramolecular rotations,[6,41,42] and (iii) a comparison of conformations adopted in different molecular environments for a given compound (*e.g.* 17-acetoxy-6α-methylpregn-4-ene-3,20-dione in solid state[3] and in solution[4]).

In the present case of the 19-nor-4-en-3-ones (**2**)—(**4**), information from all three methods is available.

X-Ray structural data for only the three steroids under study already shows a variability in ring *A* conformations ranging from 1α-sofa or 1α,2β-half chair to 1β-sofa. In accord with these findings, molecular mechanics calculations reveal that steroidal 19-nor-4-en-3-ones have a small energy difference between the two principal ring *A* conformations in question and the 10-methyl compounds and 4,9-dien-3-ones.[6] The preference for the normal conformation for 19-nor compounds in solution as suggested by the present n.m.r. and c.d. spectra is in agreement with these molecular mechanics results, which reveal that the inverted ring *A* conformation is slightly unfavourable in the Baeyer-strain energy at C-10. Apparently, this conformational energy difference is so small that it can be overcome by crystal-packing forces. Crystal-structure analysis[32,34] has demonstrated that steroids (**2**) and (**4**) can adopt the inverted conformation in a crystal environment, while the normal conformation is assumed in solution.

This conformational flexibility of steroidal 19-nor-4-en-3-ones could be very important in interaction with biopolymers, since it is widely accepted that the ring *A* region of steroid hormones plays an important role in receptor binding phenomena. Conformational inversion should be expected to alter the steroid-backbone hydrophobic bonding capabilities because of modified carbon and hydrogen atomic positions, as well as to influence the hydrogen-bond characteristics involving the oxygen atom at C-3. Therefore, the ability of 19-norsteroids to adopt both principal ring *A* conformations in addition to a certain conformational flexibility within a given ring *A* conformation[2,6] could be favourable for their activity profile. This high degree of conformational variability seems specific to steroidal 19-nor-4-en-3-ones; it was not observed in the case of 4,9-dien-3-ones or 10-methyl-4-en-3-ones.[2,6,43] In this respect, it is noteworthy that Duax *et al.*[1] have proposed that the inverted conformer is the bioactive form of progestational steroids. In contrast to this model, no correlations between the energetic capabilities for stabilizing the inverted conformation and progesterone-receptor binding affinities were found.[6]

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