The Solution Conformation of 17α-Acetoxy-6α-methylprogesterone ('Medroxyprogesterone Acetate'): Use of Circular Dichroism, Nuclear Overhauser Effect Difference and Two-dimensional *J* Spectroscopy

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17α-Acetoxy-6α-methylprogesterone, which is known from *X*-ray crystallographic study to have the inverted (1β,2α) half-chair conformation of ring A in the solid state, exhibits c.d. in solution which is normal for a steroidal 4-en-3-one, but the c.d. in the crystal shows inverted sign at the $n\rightarrow\pi^*$ band. The inference that the solution conformation is of normal type, approximating to the 1α,2β-half-chair, is supported by a ^{1}H n.m.r. study at 400 MHz. Application of n.O.e. difference and two-dimensional *J* techniques permitted a full assignment of all ^{1}H chemical shifts and geminal and vicinal coupling constants. These parameters for the protons at C-1 and -2, together with the n.O.e. observations, establish that the preferred conformation of ring A is normal in a variety of solvents.

X-RAY crystallographic studies [1] have shown that 17α-acetoxy-6α-methylprogesterone ('medroxyprogesterone acetate,' MPA) crystallises with ring A in the inverted (1β,2α) half-chair conformation (Figure 1a), although related compounds [17α-hydroxy-6α-methylprogesterone (Figure 1b),[2] 17α-acetoxyprogesterone,[3] and 6α-methyl-16-dehydroprogesterone [4]] exhibit ring A conformations of 'normal' type (Figure 2), not deviating greatly from

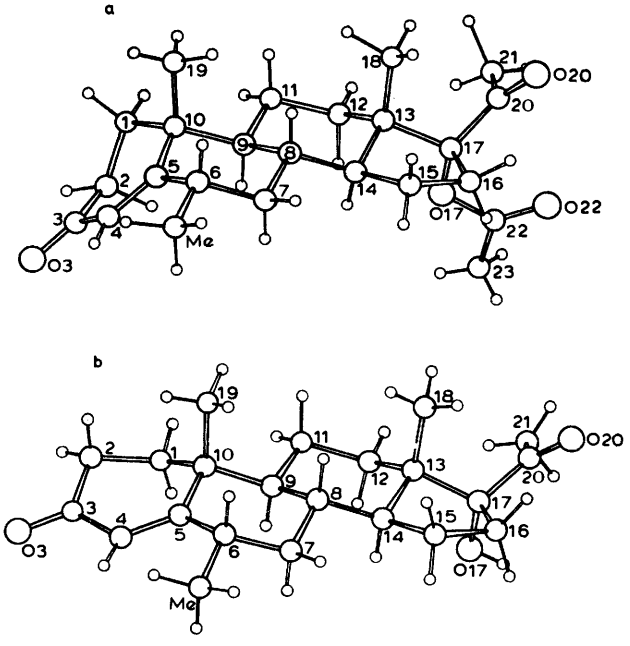

FIGURE 1 Molecular conformations determined by *X*-ray crystallography for: a, MPA, with inverted ring A; b, 17α-hydroxy-6α-methylprogesterone, with normal ring A

the 1β,2α-half-chair. The few other known cases of an inverted conformation of ring A include some 2β-substituted compounds (*e.g.* 2β-acetoxytestosterone 17-chloroacetate [5,6]) where steric effects enforce the conformational change, which persists in solution.[7] Several 19-nor-4,9-dien-3-ones also have inverted rings A in the crystalline state; [8] in such compounds the normal and the inverted half-chair forms coexist in solution, from c.d. evidence.[9]

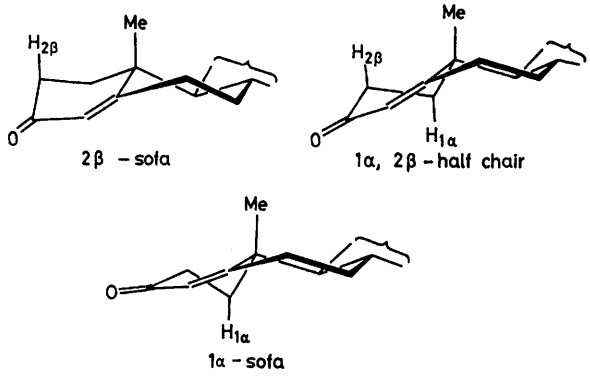

FIGURE 2 Variations on the 'normal' conformation of a steroidal 4-en-3-one

The combined effect of the 17α-acetoxy and 6α-methyl groups in inverting ring A in crystalline MPA has been considered an unusual instance of conformational transmission,[1] and it has been suggested [10] that the ability of this and some other compounds related to progesterone to exist in the abnormal conformation can be correlated with their exceptionally high progestational activity. It was therefore of interest to determine whether the inverted conformation persists in solution.

RESULTS AND DISCUSSION

C.d.—The unusual conformation of MPA in the crystal was reported at the time when we (the Westfield group) were assembling data on the circular dichroism of steroidal 4-en-3-ones,[11] in the hope of deriving new c.d.–structure relationships. We therefore examined the c.d. behaviour of MPA in various solvents, expecting to observe unusual features [9] associated with the presence of the inverted conformation.

The c.d. curves were quite normal, however, from 400 to 200 nm. Like the c.d. curves of most 4-en-3-ones,[11,12] those for MPA, progesterone, and its 17α-hydroxy-, 17α-hydroxy-6α-methyl-, and 17α-acetoxy-derivatives, all showed a negative enone $n\rightarrow\pi^*$ band (>300 nm), and positive bands in the two $\pi\rightarrow\pi^*$ regions (*ca.* 240 and 220—200 nm), as well as the expected positive 20-oxo $n\rightarrow\pi^*$ band (*ca.* 290 nm).[13] Numerical values of Δε for MPA fell within the ranges covered by the other compounds of the series. The c.d. curves were in sharp contrast to those of 2β-acetoxytestosterone 17-chloroacetate and related 2β-substituted 4-en-3-ones, which exhibit positive or bisignate enone $n\rightarrow\pi^*$ and negative $\pi\rightarrow\pi^*$ (240 nm) bands, and have been shown by n.m.r. study [7] to exist in inverted conformation even in solution.

220 nm did ' noise ' levels for crystalline materials sometimes rise to the point where c.d. curves were considered unreliable with regard to the *sign* of the Cotton effect.

Table 1 presents a selection of our results, comparing the c.d. curve in solution with that for the crystalline material. Some apparent inconsistencies in the data were not the only ones found among the c.d. curves of crystalline steroidal enones; the results of a more extensive and detailed study will be presented elsewhere.[16] The results selected here, however, are sufficient to show that c.d. data for MPA, although strongly indicative of a normal conformation in solution, could not be considered to establish this beyond doubt.

N.m.r.—We therefore undertook ^{1}H n.m.r. studies as a means of defining the solution conformation more reliably. Attempts at stereospecific deuteriation at C-1

TABLE 1

C.d. curves: comparisons of solution and crystal c.d.

Compound	Solvent or crystal dispersant	Δε or sign [a] of c.d. maximum, and $\lambda_{max.}$ (nm)			
		Enone $n\rightarrow\pi^*$	20-Oxo $n\rightarrow\pi^*$	$\pi\rightarrow\pi^*$ (1)	$\pi\rightarrow\pi^*$ (2)
Progesterone	MeOH	−1.2 (325)	+4.0 (284)	+8.0 (233sh)	+10.3 (214)
	SE30	(−) (352, 339, 328 [b])	(++) (298)	(+++) (230)	(++) (*ca.* 210)
17α-Hydroxyprogesterone	MeOH	−1.0 (331)	+2.6 (293)	+6.7 (*ca.* 235sh)	+10.3 (218)
	KBr	(−) (346, 332, 320 [b])	(+) (296)	(+) (*ca.* 250sh)	
	SE30	(+) (342, 328, 318 [b])	(+) (282sh)	(++) (268)	
17α-Acetoxyprogesterone	MeOH	−1.7 (321)	+3.3 (287)	+4.6 (245sh)	+9.4 (220—212)
	SE30	(+) (330—310)	(+) (300sh)	(++) (250)	(−) (*ca.* 210)
17α-Hydroxy-6α-methyl-progesterone	MeOH	−1.4 (330)	+2.6 (292)	+6.8 (240sh)	+9.9 (220)
	KBr	(−) (362, 346, 332 [b])	(+) (296)	(++) (258—240)	
17α-Acetoxy-6α-methyl-progesterone (MPA)	MeOH	−1.2 (326)	+3.4 (284)	+6.4 (235sh)	+8.6 (213)
	Dioxan	−1.9 (331)	+2.0 (288)	+6.0 (225)	+6.9 (209)
	KBr	(+) (365, 347, 333 [b])	(++) (290)	(++) (235)	
	SE30	(+) (350—315)	(++) (*ca.* 300)	(+++) (260—250)	(+) (*ca.* 205)

[a] For c.d. of crystals in KBr or SE30, the more (+) or (−) signs the stronger the Cotton effect. [b] Well defined vibrational fine structure.

The obvious inference was that MPA has very predominantly the normal conformation in solution, and that the inversion of ring A in the crystal is an unusual effect of crystal packing forces. We therefore examined the c.d. curves of *crystalline* MPA and some related enones, hoping to establish a clear correlation between the c.d. profile and the inverted conformation of ring A.

Of several methods investigated for obtaining the c.d. spectra of crystalline steroids, two were found to give reasonably reproducible results. These were (a) a disc prepared by grinding and compressing the crystalline steroid with KBr,[14] as for i.r. spectroscopy, and (b) inclusion of the powdered crystal in the silicone polymer SE30, following a published procedure.[15] No attempt was made to obtain a quantitative measure of Cotton effects for the crystalline materials, in view of the practical difficulty in preparing a specimen of reproducible uniformity in the dispersant, and ensuring that the sample was entirely within the light beam of the instrument. C.d. curves from some crystalline enones which were not expected to show any conformational abnormalities had similar profiles to those obtained in solution, even showing matching vibrational fine structure in the $n\rightarrow\pi^*$ region in a number of cases. Only below *ca.*

and -2 by selective homogeneous reduction [17] (with $^2H_2$) of the 1,4-dien-3-one corresponding to MPA were disappointing. The n.m.r. spectrum suggested that deuteriation had occurred without a strong preference for either α- or β-face attack, and we were unable to derive the preferred conformation from the spectrum even with the aid of lanthanide shift reagents.

The problem was eventually solved by a 400 MHz study of MPA, with use of n.O.e. difference, decoupling difference, and two-dimensional (2D) *J* methods [18] to obtain complete resolution of all ^{1}H signals, and all the geminal and vicinal ^{1}H–^{1}H coupling constants, and some long-range couplings. These results show unambiguously that the molecule strongly prefers the normal conformation in all the solvents used.

(a) *Assignment of spectrum.* The 400 MHz spectrum of MPA in either $CDCl_3$ or C_6D_6 showed several well resolved multiplets (Figure 3), but in $CDCl_3$ some of these were clearly second order in appearance, and in each solvent most multiplets overlapped to some extent. Only the protons of the 6α-, 10-, and 13-methyl groups, and 4-H, were trivially assignable by inspection in both solvents, so it was necessary to assign the spectra using a strategy recently proposed by one of us (J. K. M. S.).[18]

TABLE 2

Connectivities established by n.O.e. and decoupling difference spectra [a]

Proton irradiated	Decoupling	+ve n.O.e.	−ve n.O.e.
1β	1α,2α,2β		
2α [b]	1α,1β,2β	1α,1β,2β	
2β [b]	1α,1β,2α	1α,1β,2α	
4	2α,6β		
6α (CH_3)	6β		
7α	6β,7β,8	6α,7β,14	
8	7α,7β,9,14		
9,18 [c]	8,11α,11β,12α	8,11β,12α,12β,14,15β,16β,21	
11β,15β [c]	9,11α,12α,12β,14,15α,16α,16β		
12α	11α,11β,12β		
15α	15β,16α,16β		
16α,21 [c]		12β,14,15α,16β	15β
16β	15α,15β,16α	15β,16α	15α
19		1β,2β,6β,8,11β	2α,7β,11α

[a] In C_6D_6 solution. [b] These n.O.e. results are uncertain since some saturation of the other occurred in each case. [c] These proton signals are almost coincident and were irradiated together.

Scalar coupling connectivities were established by spin decoupling difference spectroscopy, and spatial connectivities (*e.g.* 1,3-diaxial relationships) by n.O.e. difference spectroscopy. N.O.e. difference spectra were particularly valuable for irradiations of the angular methyls (Figure 3) which allowed rapid ' mapping ' of the entire top face of the molecule. Table 2 summarises the connectivities made in these ways, and Table 3 lists the derived chemical shifts. In the course of this work we discovered several significant negative n.O.e.s (Table 2); these will be discussed elsewhere.[19]

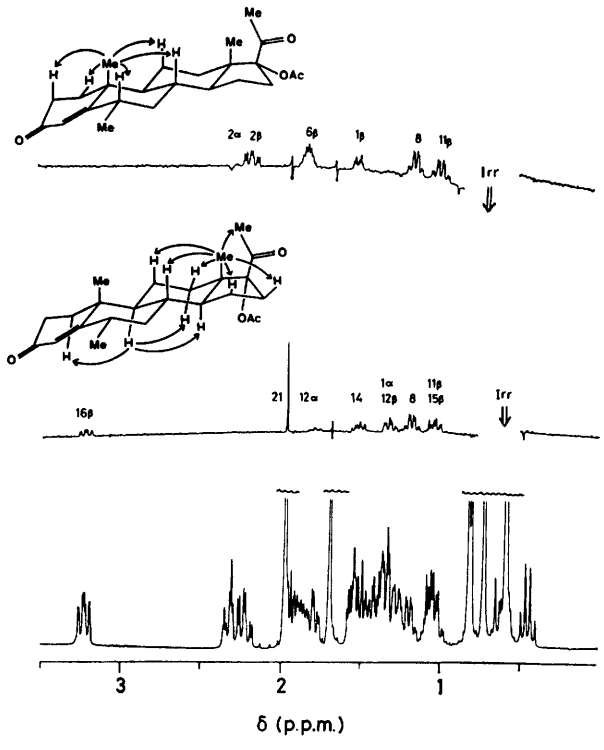

FIGURE 3 Partial 400 MHz ^1H n.m.r. spectra of MPA in C_6D_6 solution. Lowest trace: normal spectrum; centre trace: n.O.e. difference spectrum (×32) from irradiation of 13-Me and 9-H; top trace: n.O.e. difference spectrum from 10-Me irradiation

The 2D J spectrum of MPA in C_6D_6 solution consisted essentially of first-order multiplets (Figure 4) from which accurate values for all the vicinal and geminal couplings could be obtained (Tables 4 and 5). The proton-decoupled spectrum obtained from the 2D J spectrum is shown in Figure 5. Those couplings which could be measured in $CDCl_3$ solution were unchanged from C_6D_6. The 2D J spectrum also confirmed the presence of some notable long-range couplings which were first revealed in decoupling difference spectra. However the absolute value display mode used in 2D spectra exaggerates splittings comparable in size with linewidths,[20] and better values (±0.2 Hz) for these were obtained by a simple spin-echo experiment: [21] $J_{2\alpha,4}$ 0.4 (a ' W-rule ' coupling [22]); $J_{4,6\beta}$ 1.3 (allylic); $J_{12\alpha,18} = J_{1\alpha,19} = 0.4$ Hz.

TABLE 3

Proton chemical shifts for MPA [a]

Proton	δ (p.p.m.) C_6D_6	δ (p.p.m.) $CDCl_3$	Proton	δ (p.p.m.) C_6D_6	δ (p.p.m.) $CDCl_3$
1α	1.27	1.70	11β	0.98	1.42
1β	1.50	2.03	12α	1.75	1.95
2α	2.27	2.35	12β	1.29	1.56
2β	2.17	2.43	14	1.47	1.65 [b]
4	5.91	5.80	15α	1.40	1.65 [b]
6α(CH_3)	0.76	1.07	15β	1.01	1.29
6β	1.82	2.42	16α	1.88	1.65 [b]
7α	0.396	0.88	16β	3.17	2.93
7β	1.34	1.85	17(OAc)	1.64	2.09
8	1.14	1.69	18	0.53	0.67
9	0.53	1.01	19	0.67	1.18
11α	1.22	1.67	21	1.92	2.03

[a] ±0.01 p.p.m.; concentration *ca.* 20mM in the solvent indicated. [b] ±0.05 p.p.m.

Spectra in CD_3OD, [$^2H_6$]DMSO, and aqueous [$^2H_6$]-DMSO were essentially identical to that in $CDCl_3$.

(b) *Conformation of ring* A. The two distinct conformations available to ring A, ignoring minor variations of the types illustrated in Figure 2, are illustrated as P and Q (Figure 6). Identification of P as the dominant (probably >90%) conformer in solution rests on two

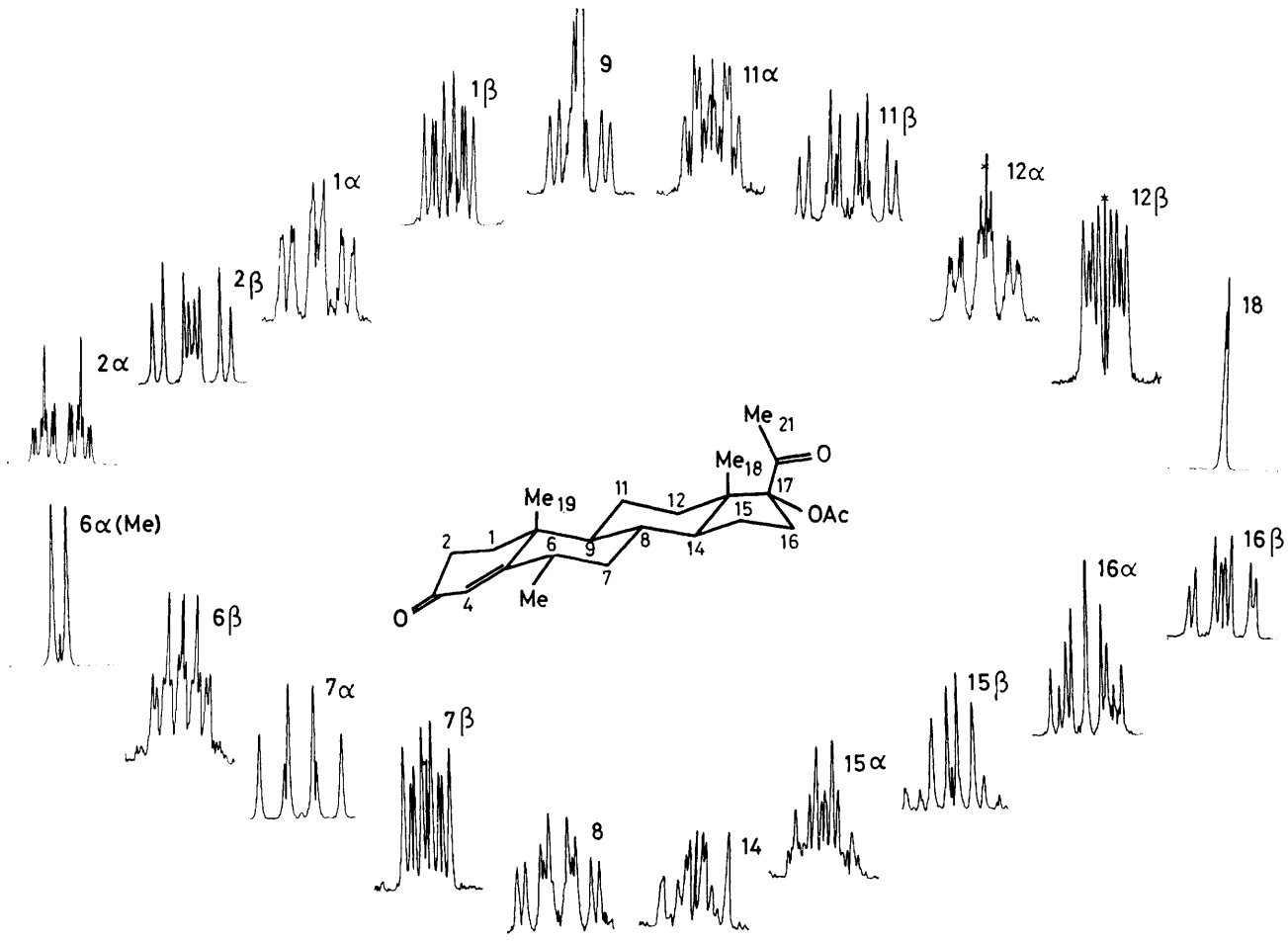

FIGURE 4 Individual proton multiplets (J spectra) of MPA, from the 2D J spectrum (in C_6D_6)

TABLE 4

Geminal and vicinal coupling constants in rings A—C [a,b]

Proton pair	^2J/Hz	^3J/Hz ax-ax	^3J/Hz ax-eq	^3J/Hz eq-eq
1α,1β	13.3			
1α,2α			4.9	
1α,2β		14.2		
1β,2α				3.8
1β,2β			5.0	
2α,2β	16.2			
6β,7α		10.2		
6β,7β			4.9	
7α,7β	12.7			
7α,8β		12.9		
7β,8β			3.5	
8β,9α		10.7		
8β,14α		10.4		
9α,11α			4.3	
9α,11β		12.3		
11α,11β	13.5			
11α,12α			4.6	
11α,12β				2.7
11β,12α		13.5		
11β,12β			4.3	
12α,12β	12.6			

[a] Hz (±0.2), in C_6D_6 solution. [b] $J_{6\alpha\text{-Me},6\beta}$ 6.5 Hz.

independent sets of observations, each quite powerful individually. In conformer P, atoms 1β-, 2β-, 6β-, 8-, and 11β-H are all at similar distances from the 10-methyl group, but 2α- and 1α-H are more distant. In Q, atoms 1α- and 1β-H are almost equidistant from the 10-methyl group and 2β-H is further away. The axial or quasi-axial protons of ring A are 1α- and 2β-H in P but 1β- and 2α-H in Q. N.O.e. difference spectroscopy

TABLE 5

Geminal and vicinal coupling constants in ring D [a]

Proton	14	15β	16α	16β
15α	7.1	12.2	9.3	2.6
15β	12.1		6.7	11.3
16α				16.1

[a] Hz (±0.2) in C_6D_6 solution.

(Figure 3) reveals, on irradiation of the 10-methyl group, one axial and one equatorial proton in ring A, and since the former has a 17 Hz coupling it is undoubtedly 2β-H (large geminal coupling adjacent to carbonyl [23]). The 2β-H n.O.e. is comparable in size to those at 6β-, 8-, and 11β-H. These results are consistent only with P being the dominant conformer.

Coupling constants also strongly support this conformation. The four-bond coupling (*ca.* 0.4 Hz) from 10-methyl to 1α-H is clearly stereospecific: a similar $J_{1\alpha,19}$ coupling is seen in 11β-hydroxyprogesterone,[18b] and

$J_{12\alpha,18}$ of similar magnitudes are found in 1-dehydrotestosterone,[18a] 11β-hydroxyprogesterone,[18b] and MPA. In addition the W-rule coupling of 4- to 2α-H is seen in 11β-hydroxyprogesterone [18b] and is observed for MPA both in $CDCl_3$ and in C_6D_6.

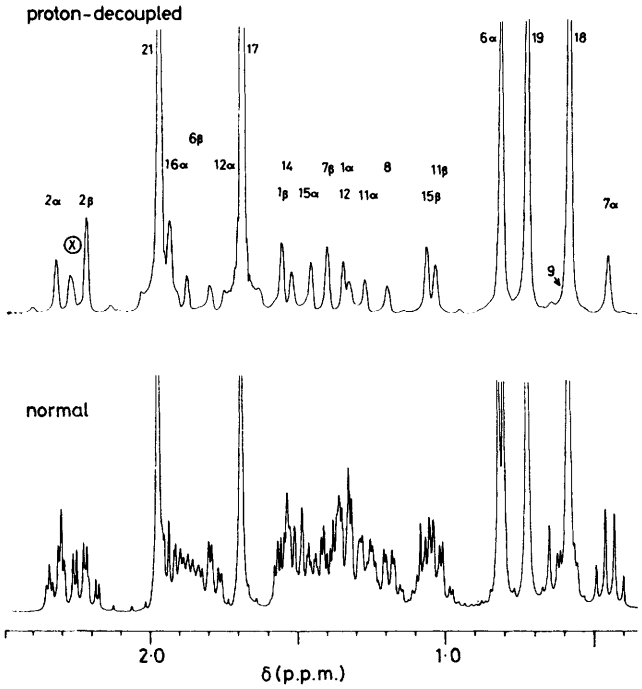

FIGURE 5 Partial normal spectrum (lower) and proton-decoupled spectrum (upper) of MPA in C_6D_6 solution

It is noteworthy that the ring A proton chemical shifts of 11β-hydroxyprogesterone and MPA in $CDCl_3$ are identical to within 0.2 p.p.m. and that neither these parameters nor the couplings are changed significantly in polar solvents. The small value of $J_{6\beta,7\alpha}$ (10.2 *versus* 13.6 Hz in 11β-hydroxyprogesterone) probably indicates

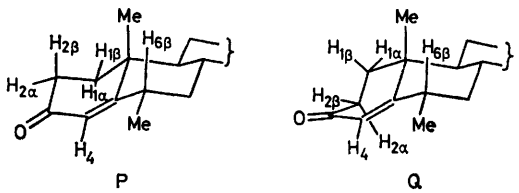

FIGURE 6 Alternative conformations of ring A in MPA: P, preferred in solution; Q, in the crystal

an appreciable distortion (flattening) of ring B, to relieve 4-H–6α-Me compression. The 17β-side-chain is clearly oriented with its methyl group adjacent to 12β-H (n.O.e. results) and the carbonyl oxygen alongside 16β-H (chemical shift), as in the crystal.[1]

There are rather few known cases where the conformation of a steroid in solution differs in type from that observed in the crystal, although quite minor variations in relatively flexible parts of the molecule may be stabilised by crystal packing forces. The A rings of the secosteroid vitamin D and many of its analogues exist in solution as pairs of rapidly equilibrating chair conformers.[24] In some cases the conformers have co-crystallized in a 1 : 1 ratio, [25] but in others, only one of the conformers is observed in the crystal.[26] The pregnan-20-one and cholestane side-chains [5,27,28] and ring A of steroidal 4-en-3-ones [5,10,28,29] provide examples of such minor variations. The 'normal' 4-en-3-ones which have been studied include instances ranging from the '2β-sofa' through the '1α,2β-half chair' to the '1α-sofa' (Figure 2), all of which can be considered as minor variations on the same fundamental conformation of ring A, to accommodate the strains associated with structural changes elsewhere.

The present uncommon instance of a distinct difference between the conformation in the crystal and the preferred conformation in solution suggests that the free energy difference between the two must be unusually small, and raises the question of whether packing forces in the crystal mimic the forces operative at the binding site for progestational activity.

EXPERIMENTAL

C.d. measurements were made at ambient temperature (*ca.* 25 °C) on a Cary 61 instrument purged with oxygen-free nitrogen. Solvents were of spectroscopic grade. Solutions with concentrations in the range 0.2—0.5 mg ml^{-1} were examined in a 10 mm cell for the $n\rightarrow\pi^*$ region, and in a 0.5 mm or 1 mm cell in the $\pi\rightarrow\pi^*$ region. C.d. curves for crystalline samples were obtained by following published procedures (see text).

Solutions of MPA for n.m.r. studies were 0.03—0.1M in the appropriate deuteriated solvent and were examined without degassing.[18a] N.m.r. experiments were carried out at 400 MHz (Bruker WH400) using our previously described automated sequences for the acquisition of spin-decoupling and n.O.e. difference spectra.[18a] Generally 64 transients were acquired for decoupling and 1 024 transients for n.O.e. difference experiments, using 8 K data points over 2 808 Hz.

In the 2D *J* experiment, 4 K points over 2 808 Hz were used in f_2 (the chemical shift dimension) and 256 points over 59 Hz in f_1 (the *J* dimension), giving a digital resolution of 0.23 Hz/point for the latter case. Sine-bell resolution enhancement [18a] was applied in each frequency direction, 48 transients having been acquired for each time interval.

We thank J. M. Bulsing for the spin-echo experiment, and the S.R.C. for a studentship (to J. D. M.), partial funding for the 400 MHz spectrometer, and a grant (to Dr. P. M. Scopes) in support of the c.d. service.

[1/961 *Received*, 15*th June*, 1981]

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