MAGNETIC RESONANCE IN CHEMISTRY, VOL. 25, 730-733 (1987)

Ring A Conformation in Steroids

2*—NMR Study of C-2 Monomethyl- and Dimethyl-Substituted 5α-Androstan-3-ones

Kirk Marat†
Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

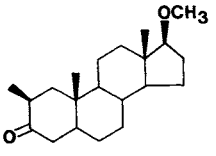
John F. Templeton, R. K. Gupta and V. P. Sashi Kumar
Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Ring A proton shifts and couplings and ^{13}C shifts of all carbons are given for 17β-acetoxy-2α-methyl- and 17β-methoxy-2β-methyl-5α-androstan-3-one and 2,2-dimethyl-17β-methoxy-5α-androstan-3-one. It was concluded that the 2α-methyl derivative exists with ring A in a regular chair conformation while the 2β-methyl derivative exists with ring A in an inverted boat conformation with C-2 and C-5 at the bow/stern positions. The data for 2,2-dimethyl-17β-methoxy-5α-androstan-3-one suggest an equilibrium of these two conformers.

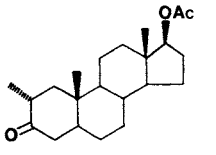
KEY WORDS ^{1}H NMR ^{13}C NMR monomethyl/dimethyl 5α-androstan-3-ones Steroid Ring A conformation

INTRODUCTION

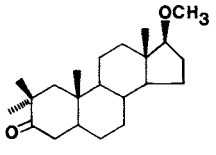
While preparing a series of 2-substituted steroids related to testosterone, we observed that 17β-methoxy-2β-methyl-5α-androstan-3-one (**1**) had a ^{1}H 19-CH_3 shift at 0.29 ppm higher field than the similar 17β-acetoxy-2α-methyl-5α-androstan-3-one (**2**) and 0.25 ppm higher than the corresponding 2,2-dimethyl compound **3**. Since



1



2



3

the shifts of **2** and **3** are close to the expected[1] value for a substituted 5α-androstan-3-one, we concluded that the unusual shift of **1** was probably due to a conformational change in ring A. The effect of C-2 substitution on ring A conformation is of interest because substitution at the C-2 position has been found to have a marked effect on the ratio of anabolic to androgenic activity of these steroids.[2]

Previous CD work on C-2 methyl-substituted 3-oxo-5α-steroids[3] has been inconclusive, and no x-ray data appear to be available. Unsubstituted 3-oxo-5α-steroids generally crystallize with ring A in a regular chair conformation.[4] 17β-Iodoacetoxy-4,4-dimethyl-5α-androstan-3-one and 17β-iodoacetoxy-4,4-dimethyl-19-nor-5α-androstan-3-one have a normal chair for ring A[5,6] while 6α-hydroxy-4,4-dimethyl-5α-androstan-3-one has a boat conformation with C-3 and C-10 in the bow/stern positions.[7] The results of MM1 and MM2 force field (molecular mechanics) calculations on 4,4-dimethyl-5α-androstan-3-one derivatives and related steroids are in general agreement with the x-ray data.[8-10] In 4-en-3-one steroids, ring A is flexible, and differences are often observed between the solution and the crystal conformations.[11-13]

Recent advances in instrumentation and methodology[14-16] have made possible the analysis of NMR spectra of complex organic molecules such as steroids. Since vicinal H-C-C-H couplings[17,18] and geminal H-C-H couplings adjacent to a π system[19,20] are stereospecific, it is possible to determine ring conformation with reasonable certainty if sufficient couplings are known. This may be augmented by the use of stereospecific four-bond couplings and by nuclear Overhauser enhancement (NOE) measurements.[11,21] The NMR method is unique in that it can provide reliable conformational information in solution.

While the unsubstituted 17β-hydroxy-19-nor-5α-pregn-20-yn-3-one and its 5β epimer have been studied at 500 MHz,[22] there appear to be no reliable ring A NMR data for C-2 substituted 3-oxo steroids. In view of the lack of conformational information on these compounds, we decided to study the ring A conformation of the C-2 methylated derivatives **1-3** using a careful analysis of the ^{1}H NMR spectrum aided by NOE measurements.

The difference in C-17 substituents is unlikely to have a significant effect on ring A conformation in solution. We have previously shown that C-17 substitution has no effect on the ring A spectral parameters in a series of 4-en-3-one steroids;[13] the long-range conformational transmission sometimes observed in these compounds

* For Part I, see reference 13.
† Author to whom correspondence should be addressed.

0749-1581/87/080730-04$05.00

Received 22 September 1986
Accepted (revised) 5 January 1987

in the solid state is more likely to be a crystal packing effect.

SPECTRAL ANALYSIS

Proton spectra were analysed in a manner similar to that described previously for 4-en-3-one steroids.[13] Homonuclear correlation spectroscopy (COSY)[23] was used to identify the coupling patterns, with cross-sections used to give approximations to the spectral parameters. Difference-double-resonance (DDR)[24] from 19-CH_3 was used to isolate H-1α for **2** and **3** and H-1β for **1**. The spectra were then analysed with the PANIC[25] program on an ASPECT 3000 computer. The quaternary centre at C-10 and the carbonyl at C-3 conveniently isolate the H-1α, H-1β, H-2, 2-CH_3 spin system from the rest of the molecule. All lines of observable intensity could be assigned and all parameters were iterated. The H-4α, H-4β, H-5 spin system, however, is not isolated from the rest of the molecule. H-5 is coupled to H-6α and H-6β which are, in turn, coupled to H-7α and H-7β. It was therefore not possible to assign transitions to H-5 or to iterate on its chemical shift. This does not create a major difficulty, however, since the COSY spectrum shows H-6α and H-6β to be well removed from H-5 and each other. Simplifying the analysis this way results in accurate values for δH-4α and δH-4β and the couplings involving H-4α, H-4β and H-5, but results in an imprecise value for δH-5. The H-5 shift reported in Table 1 was obtained from the COSY spectrum. Simulations including H-6α and H-6β with shifts and couplings obtained from the COSY spectrum confirmed that neglecting H-6α and H-6β had no effect on the spectrum of H-4α and H-4β. RMS errors were less than 0.1 Hz for all spectra; standard errors for the coupling constants were 0.02–0.07 Hz. The results of the analysis of **1** are shown in Fig. 1. The ring A proton shifts and couplings for **1–3** are reported in Table 1.

Additional evidence for the conformation of **1** was obtained from 2D NOE studies.[26,27] While 1D NOE difference experiments[11,24] are generally preferred, they were not possible owing to the crowded nature of the spectrum.

Table 1. Ring A ^1H shifts (ppm) and coupling constants (Hz) for compounds 1–3

	1	2	3
δ1(α)	1.318	1.060	1.365
δ1(β)	1.987	2.046	1.852
δ2(α)	2.594	—	—
δ(2β)	—	2.456	—
δ(2α-CH_3)	—	1.002	1.164
δ(2β-CH_3)	1.038	—	1.072
δ(4α)	2.248	2.084	2.123
δ(4β)	2.093	2.312	2.434
δ(5)	1.96[a]	1.49[a]	1.62[a]
^2J(1α, 1α)	−13.63	−13.50	−14.00
^3J(1α, 2α)	7.38	—	—
^3J(1α, 2β)	—	12.88	—
^3J(1β, 2α)	10.58	—	—
^3J(1β, 2β)	—	5.97	—
^3J(2, CH_3)	6.96	6.55	—
^2J(4α, 4β)	−18.04	−14.22	−15.94
^3J(4α, 5)	6.10	3.64	4.35
^3J(4β, 5)	12.38	13.80	13.59
δ(19-CH_3)	0.764	1.070	1.004

[a] Not iterated; shift obtained from COSY spectrum.

Heteronuclear correlation spectra[28] of **2** and **3** and comparison with the previously reported spectra of 5α-androstan-3-one[29] and 17β-acetoxy-5α-androstan-3-one[30] were used to assign the ^{13}C spectra.

RESULTS AND DISCUSSION

Geminal couplings are known to have a substantial π contribution when adjacent to a carbonyl.[19,20] This effect is greatest, i.e. $^2J(gem)$ is most negative, when the C=O bond bisects the H—C—H angle. Such is the case in **1**, where $^2J(4\alpha, 4\beta)$ is −18.04 Hz, implying a C-3—C-4 torsion angle of *ca* 0°. In comparison, for a chair conformation this angle is 50–60° with an expected $^2J(4\alpha, 4\beta)$ of *ca* −14 Hz. In **1**, $^3J(4\beta, 5)$ is 12.38 Hz while $^3J(4\alpha, 5)$ is 6.10 Hz, implying a C-4—C-5 torsion angle of 50–60°. $^3J(1\beta, 2\alpha)$ and $^3J(1\alpha, 2\alpha)$ are 10.58 and 7.38 Hz, respectively, implying a torsion angle along the C-2—C-3 bond

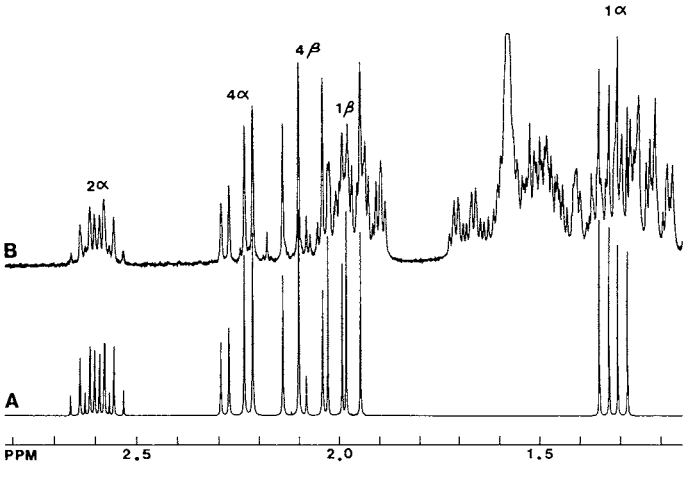


Figure 1. (A) Calculated and (B) experimental spectra of **1** in $CDCl_3$ at 300 K. 2β-CH_3 and H-5 were included in the calculations but are not shown in this plot.

in **1** of 30–50°. The only conformation consistent with these observations is an inverted boat with C-2 and C-5 at the bow/stern positions, as shown in Fig. 2A. The apparently low value for the C-2—C-3 torsion angle may indicate a slight distortion of the ring or it may be the result of an equilibrium between the inverted boat and a regular chair geometry.[13] In the latter case the pseudo-diaxial $^3J(1\beta, 2\alpha)$ of the boat conformation would be averaged with a diequatorial $^3J(1\beta, 2\alpha)$ in the chair conformation. This would explain the low $^3J(1\beta, 2\alpha)$ value and the apparently low C-1—C-2 torsion angle. Further evidence for this conformation comes from the NOE observed between H-5 and H-2α (Fig. 3). This NOE would not be expected in a chair conformation. Other NOEs were observed between H-1α and H-1β, H-2α and 2β-CH_3, H-4α and H-4β and between 19-CH_3 and H-1β. The NOE between 19-CH_3 and H-1β was obscured in the contour plot of the NOESY-TPPI experiment (Fig. 3) by tailing from 19-CH_3 and 18-CH_3, but could be seen in an F1 cross-section at the F2 frequency of 19-CH_3. A 0.7 Hz four-bond coupling was observed between H-1β and 19-CH_3. Since four-bond couplings of this magnitude are often observed between 19-CH_3 and H-1α in a chair conformation (a W-rule coupling), this coupling is surprising, and may be the result of a proximate or through-space[31] coupling mechanism. The closest approach distance between H-1β and the protons of 19-CH_3 is estimated from Dreiding models to be *ca* 1.8 Å. The inverted boat conformation places the protons of 19-CH_3 on the nodal cone between the shielding and deshielding regions of the C-3 carbonyl rather than in the deshielding region, as is the case in the chair conformation,[32] thus explaining our original observation of the high-field 19-CH_3 shift in **1**. The reason for this conformational change is, no doubt, the steric interaction between 2β-CH_3 and 19-CH_3.

The 2α-methyl compound **2** has a more normal value for $^2J(4\alpha, 4\beta)$ of −14.22 Hz, implying a C-3—C-4 torsion angle of *ca* 60°. The vicinal couplings are also typical of a cyclohexanone ring in a chair conformation, with H-1α, H-2β, H-4β and H-5 axial. A 0.4 Hz W-rule coupling was observed between 19-CH_3 and H-1α.

In the dimethyl compound **3**, $^2J(4\alpha, 4\beta)$ lies almost mid-way between the values observed for **1** and **2**. The same is true for $^3J(4\alpha, 5)$ and $^3J(4\beta, 5)$. Unfortunately, the addition of a second methyl at C-2 eliminates the stereospecific couplings between the 1- and 2-positions. A DDR[24] experiment confirmed the coupling of 19-CH_3 to both H-1α (*ca* 0.4 Hz) and H-1β (*ca* 0.2 Hz). These data are consistent with a *ca* 60:40 mixture of normal chair and inverted boat conformers co-existing in rapid equilibrium. Evidently, the steric repulsion between 2α-CH_3 and H-5 must be comparable to that between 2β-CH_3 and 19-CH_3.

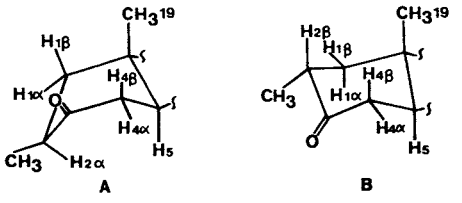


Figure 2. Ring A conformations of (A) **1** and (B) **2**.

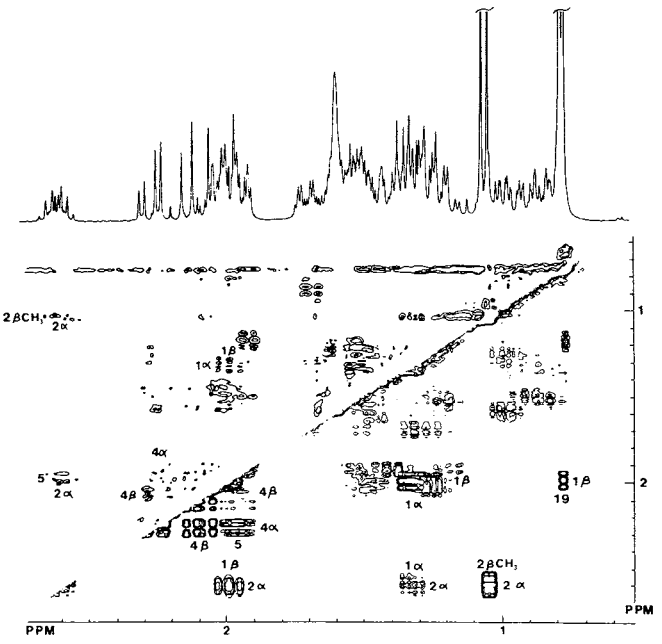


Figure 3. Above diagonal, phase-sensitive NOESY spectrum with TPPI of **1** showing positive contours only; below diagonal, COSY-90 spectrum.

The ^{13}C shifts of **1–3** are reported in Table 2. The carbons of the steroid skeleton have shifts consistent with those reported for 5α-androstan-3-one[29] and 17β-acetoxy-5α-androstan-3-one.[30] The shifts of the C-2 methyl groups of **1** and **2** are 32.9 and 14.5 ppm, respectively. A shift difference this large is unusual considering that both are equatorial to the steroid plane. The C-2 methyl shifts in **3** are between those of **1** and **2**, consistent with a rapid equilibrium.

Table 2. ^{13}C shifts for compounds 1–3

Carbon	1	2	3
1	48.6	48.5	54.4
2	41.2	41.1	44.7
3	209.5	212.9	216.5
4	44.8	44.7	41.8
5	48.2	47.9	46.0
6	28.6	28.6	28.4
7	30.7	31.2	31.2
8	35.2	35.0	34.8
9	54.0	53.0	55.7
10	36.6	36.5	36.5
11	21.2	21.1	21.1
12	37.8	36.8	38.0
13	42.7	42.6	43.0
14	51.2	50.6	51.0
15	23.3	23.5	23.3
16	27.7	27.5	27.7
17	90.7	82.7	90.8
18	11.6	12.1	11.7
19	15.6	12.4	14.1
17-CH_3COO	—	21.1	—
17-CH_3COO	—	171.1	—
17-CH_3O	57.9	—	57.8
2α-CH_3	—	14.5	28.4
2β-CH_3	32.9	—	28.6

EXPERIMENTAL

All spectra were recorded on a Bruker AM300 spectrometer at 300 MHz. Proton samples were 25 mM in $CDCl_3$ and were degassed by bubbling nitrogen through the solution. Moderate resolution enhancement was used when necessary. COSY[23] spectra were recorded with a spectral width of 1200 Hz and a time domain matrix size of 1024×256. Zero filling in F1 yielded a 512×512 matrix after transformation. 2D NOE experiments were performed both with a conventional NOESY[26] experiment and with a phase-sensitive experiment using time-proportional phase increments (TPPI).[27] We have found the latter experiment useful for separating NOE effects from artifacts. Positive NOEs have opposite phase to negative NOEs and the diagonal, while artifacts resulting from scalar coupling have mixed phase. All reported NOEs were observed in both experiments. A mixing period of 1 s was used with a random variation of 20 ms to suppress correlation due to scalar coupling. Matrix dimensions were the same as the COSY experiment. Difference-double-resonance (DDR) experiments[24] were performed with a digital resolution of 0.04 Hz per point or better. Frequency list cycling was used to distribute the effects of long-term changes in spectrometer conditions equally among all spectra.

^{13}C samples were 100 mM in $CDCl_3$ for **2** and **3** and 25 mM in $CDCl_3$ for **1**. Heteronuclear shift correlation spectra[28] of **2** and **3** were recorded with an F2 (^{13}C) width of 5000 Hz over 2048 time domain data points and an F1 (^1H) width of 800 Hz. Zero filling of T1 from 256 to 512 gave a 1024×512 matrix after transformation. The amount of **1** available was insufficient for heteronuclear shift correlation.

17β-Acetoxy-2α-methyl-5α-androstan-3-one (**2**), m.p. 158–160 °C (lit.[33] m.p. 160–163 °C), was prepared by hydrolysis (KOH/MeOH) and acetylation (Ac_2O/pyridine) of the 17β-propionate. The synthesis of **1** and **3** will be reported elsewhere.[34]

Acknowledgements

The authors are grateful to the Medical Research Council of Canada for financial support. The AM300 spectrometer was funded by the Natural Scienes and Engineering Research Council of Canada and by additional funding from the Manitoba Health Research Council and the University of Manitoba Research Board. We are grateful to the Lilly Research Laboratories, Indiana, USA, for a sample of 2α-methyl-17β-propionoxy-5α-androstan-3-one (dromostanolone propionate).

REFERENCES

1. N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry*. Holden-Day, San Francisco (1964).
2. A. Zaffaroni, *Acta Endocrinol., Suppl.* **50**, 139 (1960).
3. D. A. Lightner and F. P. C. Eng, *Steroids* **35**, 189 (1980).
4. W. L. Duax and D. A. Norton (Eds), *Atlas of Steroid Structure*, Vol. 1. Plenum, New York (1975).
5. G. Ferguson, E. W. Macaulay, J. M. Robertson, J. M. Midgley, W. B. Whalley and B. A. Lodge, *J. Chem. Soc., Perkin Trans. 2* 1170 (1980).
6. W. B. Whalley, G. Ferguson and M. A. Khan, *J. Chem. Soc., Perkin Trans. 2* 1183 (1980).
7. J. M. Midgley, J. E. Parkin and W. B. Whalley, *J. Chem. Soc., Perkin Trans. 1* 834 (1977).
8. N. L. Allinger, U. Burkert and W. H. DeCamp, *Tetrahedron* **33**, 1891 (1977).
9. U. Burkert and N. L. Allinger, *Tetrahedron* **34**, 807 (1978).
10. D. A. Dougherty, K. Mislow, J. W. Huffman and J. Jacobus, *J. Org. Chem.* **44**, 1585 (1979).
11. M. W. Barrett, R. D. Farrant, D. N. Kirk, J. D. Mersh, J. K. M. Sanders and W. L. Duax, *J. Chem. Soc., Perkin Trans. 2* 105 (1982).
12. M. M. Bhadbhade and V. Venkatesan, *Acta Crystallogr., Sect. C* **40**, 1905 (1984).
13. K. Marat, J. F. Templeton and V. P. Sashi Kumar, *Magn. Reson. Chem.* **25**, 25 (1987).
14. L. D. Hall and J. K. M. Sanders, *J. Am. Chem. Soc.* **102**, 5703 (1980).
15. L. D. Hall and J. K. M. Sanders, *J. Org. Chem.* **46**, 1132 (1981), and references cited therein.
16. H. J. Schneider, U. Bucheit, N. Becker, G. Schmidt and U. Siehl, *J. Am. Chem. Soc.* **107**, 7027 (1985).
17. M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).
18. C. A. G. Haasnoot, F. A. A. M. DeLeeuw and C. Altona, *Tetrahedron* **36**, 2783 (1980).
19. M. Barfield and D. M. Grant, *J. Am. Chem. Soc.* **85**, 1899 (1963).
20. T. C. Wong and G. R. Clark, *J. Chem. Soc., Chem. Commun.* 1518 (1984).
21. J. H. Noggle and R. E. Schirmer, *The Nuclear Overhauser Effect—Chemical Applicatons*. Academic Press, New York (1971).
22. A. G. J. Sedee, G. M. J. Beijersbergen von Henegouwen, W. Guijt and C. A. G. Haasnoot, *J. Org. Chem.* **50**, 4182 (1985).
23. W. P. Aue, E. Bartholdi and R. R. Ernst, *J. Chem. Phys.* **64**, 2229 (1976).
24. J. K. M. Sanders and J. D. Mersh, *Prog. Nucl. Magn. Reson. Spectrosc.*, edited by J. W. Emsley, J. Feeney and L. H. Sutcliffe, Pergamon Press, Oxford, **15**, 353 (1982).
25. *PANIC Program Version 840419.0*. Bruker–Spectrospin, Fallanden, Switzerland.
26. A. Kumar, R. R. Ernst and K. Wüthrich, *Biochem. Biophys. Res. Commun.* **95**, 1 (1980).
27. G. Bodenhausen, H. Kogler and R. R. Ernst, *J. Magn. Reson.* **58**, 370 (1984).
28. A. Bax and G. Morris, *J. Magn. Reson.* **42**, 501 (1981).
29. H. Eggert and C. Djerassi, *J. Org. Chem.* **38**, 3788 (1973).
30. J. W. Blunt and J. B. Stothers, *Org. Magn. Reson.* **9**, 439 (1977).
31. J. Hilton and L. H. Sutcliffe, *Prog. Nucl. Magn. Reson. Spectrosc.*, edited by J. W. Emsley, J. Feeney and L. H. Sutcliffe, Pergamon Press, Oxford, **10**, 27 (1975).
32. R. K. Harris, *Nuclear Magnetic Resonance Spectroscopy*. Pitman, London (1983).
33. R. Mauli, H. J. Ringold and C. Djerassi, *J. Am. Chem. Soc.* **82**, 5494 (1960).
34. J. F. Templeton, V. P. Sashi Kumar, R. K. Gupta and A. M. Friesen, Steroids, in press.