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A Nuclear Magnetic Resonance Spectral Study of 5α,13α-Androstanes[1]

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The chemical shifts of angular methyl protons of 5α,13α-androstane derivatives were measured in deuteriochloroform and the effects of various substituents in ring D on shift values were estimated. The pyridine-induced solvent shifts in 18-proton signal were recognized with the 16- and 17-hydroxylic compounds. Correlation of the coupling constant ($J_{H_{15,16}}$) and conformation of ring D has also been discussed.

It is well known that the chemical shifts of angular methyl protons are characteristic for individual steroid due to the shielding effect of neighboring functional groups. In consequence contribution of the substituent to the signal shift of angular methyl group has been estimated with usual C/D-*trans* steroids.[3] In the previous publication we reported the nuclear magnetic resonance (NMR) spectral study of 5α,14β-androstanes.[4] Further interest in the characteristic feature of C/D-*cis* fusion prompted us to explore the NMR spectra of 13α-steroids. The present paper deals with the chemical shifts of angular methyl protons of 54 kinds of 5α,13α-androstane derivatives having substituents in ring D and the pyridine-induced solvent shifts of the 16- and 17-hydroxylic compounds. In addition correlation of the coupling constant with the conformation of ring D has also been described.

Results and Discussion

The samples measured in this study are 3β-hydroxy-5α,13α-androstanes and their 3-acetates possessing various substituents at C-15, C-16 and C-17. In Table I are summarized the chemical shifts of 18- and 19-protons of these compounds measured in deuteriochloroform. The shift values of angular methyl protons from those of the parent compounds (**1**, **2**), which can be regarded as net contribution of the monosubstituent in ring D, are collected in comparison with 13β,14β- and 13β,14α-series (see Table II). It is evident from the results that the chemical shift of 19-proton is affected by the presence of the functional group in ring D. In particular, Δ^{15}, Δ^{16} and 17-oxo groups exhibit a shielding effect of 0.04—0.06 ppm, suggesting that 19-methyl group may be located in the so-called conical region of C=O or C=C bonds under the anisotropic influence. It is noteworthy that the 16-oxo group does not exert a significant effect on the chemical shift of 19-proton. The present findings are indicative of the specific spatial situation of 19-methyl and these functional groups in 5α,13α-steroids. The substituent effects on the chemical shift of 18-proton are recognized much more distinctly. The presence of a hydroxyl group at 16α exerts a downfield shift of 18-proton signal with

1) This paper constitutes Part XXX of the series entitled "Analytical Chemical Studies on Steroids"; Part XXIX: T. Nambara, Y. Matsuki and T. Chiba, *Chem. Pharm. Bull.* (Tokyo), **17**, 1636 (1969).
2) Location: *Aobayama, Sendai.*
3) *a*) R.F. Zürcher, *Helv. Chim. Acta*, **44**, 1380 (1961); **46**, 2054 (1963); *b*) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **10**, 338 (1962); *c*) N. S. Bhacca and D.H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," 1964, p. 13, Holden-Day, Inc.,; *d*) K. Tori and T. Komeno, *Tetrahedron*, **21**, 309 (1965).
4) T. Nambara, H. Hosoda and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **16**, 374 (1968).

Table I. Chemical Shifts of C-18- and C-19-Protons in 5α,13α-Androstane Derivatives

No.	Compound	Chemical shift (τ) C-18-H	C-19-H
1	5α,13α-Androstan-3β-ol	9.14	9.28
2	5α,13α-Androstan-3β-ol acetate	9.14	9.26
3	5α,13α-Androst-15-en-3β-ol	9.10	9.32
4	5α,13α-Androst-15-en-3β-ol acetate	9.09	9.29
5	5α,13α-Androst-16-en-3β-ol	9.10	9.32
6	5α,13α-Androst-16-en-3β-ol acetate	9.10	9.31
7	5α,13α-Androstane-3β,17β-diol	9.17	9.26
8	5α,13α-Androstane-3β,17β-diol 3-acetate	9.15	9.23
9	5α,13α-Androstane-3β,17β-diol diacetate	9.12	9.23
10	5α,13α-Androstane-3β,17α-diol	9.15	9.26
11	5α,13α-Androstane-3β,17α-diol 3-acetate	9.13	9.23
12	5α,13α-Androstane-3β,17α-diol diacetate	9.07	9.22
13	5α,13α-Androstane-3β,16β-diol	9.17	9.26
14	5α,13α-Androstane-3β,16β-diol diacetate	9.12	9.22
15	5α,13α-Androstane-3β,16α-diol	8.91	9.29
16	5α,13α-Androstane-3β,16α-diol 3-acetate	8.90	9.27
17	5α,13α-Androstane-3β,16α-diol diacetate	8.94	9.25
18	3β-Hydroxy-5α,13α-androstan-17-one	9.02	9.33
19	3β-Hydroxy-5α,13α-androstan-17-one acetate	9.02	9.32
20	3β-Hydroxy-5α,13α-androstan-16-one	8.98	9.27
21	3β-Hydroxy-5α,13α-androstan-16-one acetate	8.98	9.26
22	16β,17β-Epoxy-5α,13α-androstan-3β-ol	9.16	9.28
23	16β,17β-Epoxy-5α,13α-androstan-3β-ol acetate	9.15	9.26
24	16α,17α-Epoxy-5α,13α-androstan-3β-ol	8.89	9.28
25	16α,17α-Epoxy-5α,13α-androstan-3β-ol acetate	8.88	9.26
26	5α,13α-Androst-16-ene-3β,17-diol diacetate	9.02	9.29
27	3β-Hydroxy-16β-bromo-5α,13α-androstan-17-one acetate	8.98	9.29
28	3β-Hydroxy-16α-bromo-5α,13α-androstan-17-one acetate	8.85	9.35
29	3β-Hydroxy-17α-bromo-5α,13α-androstan-16-one acetate	9.03	9.24
30	3β-Hydroxy-15α-bromo-5α,13α-androstan-16-one acetate	8.72	9.25
31	3β,16β-Dihydroxy-5α,13α-androstan-17-one	8.96	9.35
32	3β,16β-Dihydroxy-5α,13α-androstan-17-one 3–acetate	8.86	9.33
33	3β,16β-Dihydroxy-5α,13α-androstan-17-one diacetate	8.96	9.32
34	3β,16α-Dihydroxy-5α,13α-androstan-17-one 3-acetate	8.96	9.33
35	3β,16α-Dihydroxy-5α,13α-androstan-17-one diacetate	8.96	9.34
36	3β,17β-Dihydroxy-5α,13α-androstan-16-one diacetate	8.88	9.25
37	3β,17α-Dihydroxy-5α,13α-androstan–16–one	9.21	9.24
38	3β,17α-Dihydroxy-5α,13α-androstan-16-one 3-acetate	9.21	9.23
39	3β,17α-Dihydroxy-5α,13α-androstan-16-one diacetate	9.10	9.31
40	16β-Bromo-5α,13α-androstane-3β,17β-diol	9.09	9.27
41	16β-Bromo-5α,13α-androstane-3β,17β-diol 3-acetate	9.09	9.25
42	16β-Bromo-5α,13α-androstane-3β,17β-diol diacetate	9.02	9.24
43	16β-Bromo-5α,13α-androstane-3β,17α-diol	9.16	9.24
44	16β-Bromo-5α,13α-androstane-3β,17α-diol 3-acetate	9.15	9.21
45	16β-Bromo-5α,13α-androstane-3β,17α-diol diacetate	9.15	9.18
46	16α-Bromo-5α,13α-androstane-3β,17α-diol 3-acetate	8.95	9.24
47	16α-Bromo-5α,13α-androstane-3β,17α-diol diacetate	8.86	9.25
48	17α-Bromo-5α,13α-androstane-3β,16α-diol	8.93	9.29
49	17α-Bromo-5α,13α-androstane-3β,16α-diol diacetate	8.95	9.28
50	15α-Bromo-5α,13α-androstane-3β,16α-diol	8.79	9.28
51	15α-Bromo-5α,13α-androstane-3β,16α-diol diacetate	8.76	9.27
52	5α,13α-Androstane-3β,16β,17α-triol 3,16-diacetate	9.09	9.24
53	5α,13α-Androstane-3β,16α,17β-triol 3,16-diacetate	9.00	9.26
54	16β,17β-Epoxy-5α,13α-androstane-3β,17α-diol diacetate	9.07	9.26

0.24 ppm due to 1,3-diaxial interaction in contrast to its epimer with a slight upfield shift. As for the 16α-acetate the diamagnetic shift of 18-proton caused by acetylation can be interpreted in terms of the spatial interaction effect.[3b)]

Table II. Effects of Substituents on the Chemical Shifts of C-18- and C-19-Protons

Substituent	Shift value[a)] (ppm)					
	C-18-H			C-19-H		
	13α,14α	13β,14β	13β,14α	13α,14α	13β,14β	13β,14α
Δ^{15}	−0.05			+0.04		
Δ^{16}	−0.04	−0.10	−0.04	+0.05	−0.02	−0.01
16-Oxo	−0.16	−0.19	−0.18	−0.01	−0.03	−0.03
16β-OH	+0.03	−0.01[c)]	−0.19	−0.02	−0.02[c)]	+0.04
16α-OH	−0.24	+0.01	0	+0.01	+0.01	+0.03
16β-OAc	−0.02	−0.03[c)]		−0.04	−0.03[c)]	
16α-OAc	−0.20	0		+0.01	0	
17-Oxo	−0.12	−0.10	−0.17[b)]	+0.06	−0.01	−0.02[b)]
17β-OH	+0.02	−0.04	−0.03[b)]	−0.03	−0.01	0[b)]
17α-OH	0	−0.02	+0.04	−0.03	−0.01	−0.01
17β-OAc	−0.02	+0.04	−0.08[b)]	−0.03	−0.02	0[b)]
17α-OAc	−0.07	0	0	−0.04	−0.01	+0.04
16β,17β-Oxido	+0.02	−0.23	−0.12	0	+0.01	0
16α,17α-Oxido	−0.26	−0.15	−0.03	0	+0.05	0

a) Plus sign represents an upfield shift.
b) Zürcher's value (*Helv. Chim. Acta*, **44**, 1380 (1961); **46**, 2054 (1963))
c) The reported value[4)] should be corrected.

Table III. Pyridine–Induced Solvent Shifts of C-18-Protons

Compound	No.	Chemical shift		Δ[a)]
		τ_{CDCl_3}	$\tau_{pyridine}$	
16β-OH	13	9.17	9.06	−0.11
16α-OH	15	8.91	8.66	−0.25
17β-OH	7	9.17	9.09	−0.08
17α-OH	10	9.15	8.87	−0.28

a) $\Delta = \tau_{pyridine} - \tau_{CDCl_3}$

The chemical shifts of angular methyl groups of 16- and 17-hydroxyl derivatives were then measured in pyridine. It was recently demonstrated that in some hydroxylic compounds the use of pyridine instead of chloroform results in the solvent shift due to the complex formation between pyridine molecule and the polar hydroxyl function. As shown in Table III the pyridine–induced shift of 18-proton is obviously detectable and the result is consistent with the finding of Demarco, *et al.*[5)] They observed that the methyl group occupying position 1,3-diaxial to the hydroxyl function experiences paramagnetic effect of the order of 0.20—0.40 ppm in pyridine relative to chloroform. In 13α-steroids the 16α-hydroxyl group, which is in 1,3-diaxial relation to 18-methyl group, actually exerts a downfield shift of 0.25 ppm. It was also reported that the methyl group vicinally situated to the hydroxyl function is deshielded and the extent of deshielding depends on the magnitude of the dihedral angle between these two groups. In the previous work the authors performed the conformational analysis of ring D with the isomeric 16-deuterio-17-hydroxy compounds on the basis of the

5) P.V. Demarco, E. Farkas, D. Doddrell, B.L. Mylari and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968).

coupling constants ($J_{H16,17}$).[6] The results tell us that the dihedral angle between 18-methyl and 17-hydroxyl groups must be 50—60° in 17α-hydroxylic compound, while 170—180° in its epimer. In actuality the shift value of the former is 0.28 ppm and that of the latter 0.08 ppm, where the magnitude of the solvent effect is in qualitative agreement with that of the dihedral angle. It should be now emphasized that the technique utilizing the solvent effect is extremely useful for the configurational assignment of the epimeric hydroxyl groups in natural and synthetic steroids.

The next argument is focused to the spectral properties of two epimeric 16-acetoxy-17-ketones (**33**,**35**), whose structures have already been established synthetically.[7] The 16α-acetate shows no difference in 18-proton signal from its epimer despite of the existing 1,3-diaxial relationship. This result is not surprising, since the additivity rule of the shift values does not always hold on 18-proton of the steroid polysubstituted in ring D due to the structural and conformational change. However, the magnitude of the coupling constant ($J_{H15,16}$)

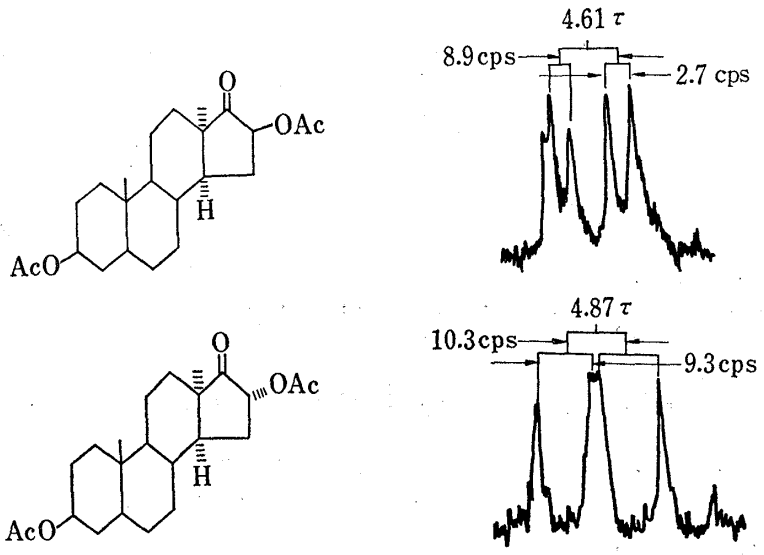
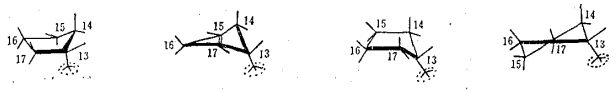


Fig. 1. NMR Spectra of C-16-Protons in Epimeric 16-Acetoxy-17-ketones

Table IV. Coupling Constants ($J_{H15,16}$) Derived from Williamson–Johnson and Abraham Equations[a]

Conformation	Williamson–Johnson		Abraham	
	16α-H	16β-H	16α-H	16β-H
I	6.5	19.9	8.2	20.0
II	9.5	18.0	11.6	19.3
III	14.0	14.0	16.0	16.0
IV	5.3	19.6	6.6	19.0

a) $J_{H15,16}=J_{H15\alpha,16}+J_{H15\beta,16}$ (in cycles per second)



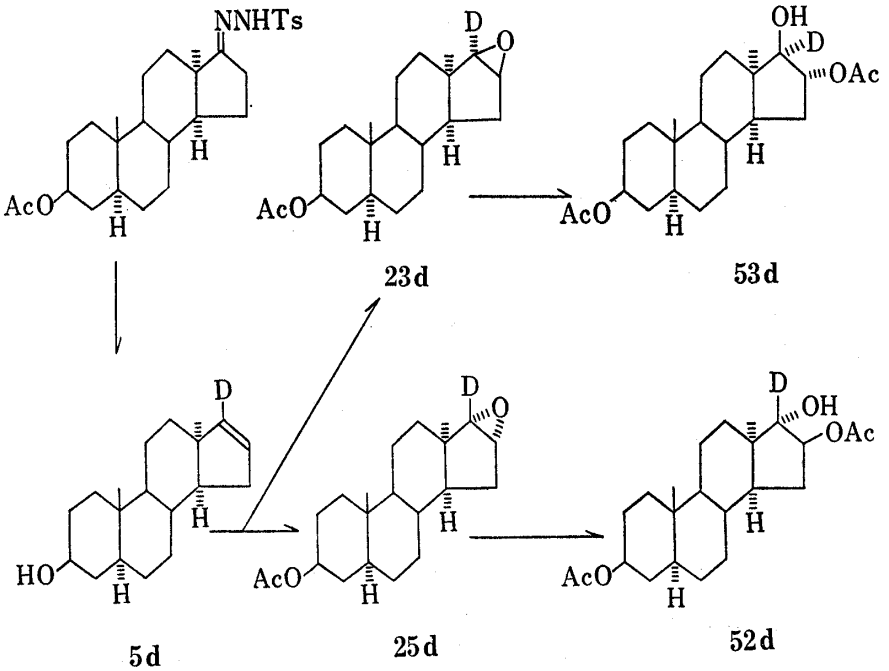
I II III IV

6) T. Nambara, H. Hosoda, M. Usui and J. Fishman, *Chem. Pharm. Bull.* (Tokyo), **16**, 1802 (1968).
7) T. Nambara, H. Hosoda and M. Usui, *Chem. Pharm. Bull.* (Tokyo), **17**, 947 (1969).

Table V. Coupling Constants of 16,17-Disubstituted Derivatives

Substituent C-16 α	Substituent C-16 β	Substituent C-17 α	Substituent C-17 β	Coupling constant[a] $J_{H15,16}$	Coupling constant[a] $J_{H16,17}$
H	Br	D	OH	18.5	
		H	OH		3.4
H	Br	D	OAc	18.5	
		H	OAc		3.8
H	Br	OH	D	16.3	
		OH	H		—
H	Br	OAc	D	15.4	
		OAc	H		8.1
H	OAc	OH	D	13.7	
		OH	H		6.0
H	OAc	OAc	D	13.7	
		OAc	H		—
Br	H	OH	D	14.6	
		OH	H		7.6
Br	H	OAc	D	14.6	
		OAc	H		—
OAc	H	D	OH	17.1	
		H	OH		3.8
OAc	H	D	OAc	16.3	
		H	OAc		—

a) in cycles per second



Ac=CH_3CO-, Ts=p-$CH_3C_6H_4SO_2-$

Chart 1

can serve to distinguish these two epimers. As illustrated in Fig. 1, 16-proton signals of the epimeric α-ketol acetates appear as X portion of the ABX system at 4.61τ (J=11.6 cps) and

4.87τ (J=19.6 cps), respectively. The coupling constants calculated by Williamson–Johnson and Abraham equations[8,9] applying to the dihedral angles for four common conformations are listed in Table IV.[10] The observed values are obviously consistent with the expected, if the ketol acetates may exist in any conformation among four.

It has already been demonstrated that the coupling constant ($J_{H16,17}$) would be a suitable parameter for conformational analysis of the fused cyclopentane ring.[6,11] In some cases, however, the use of the 16,17-disubstituted derivatives appears to be more advantageous in that not only $J_{H16,17}$ but also $J_{H15,16}$ are available. From this point of view the 16,17-bromohydrins, -glycols and their 17-deuterated derivatives were prepared and the coupling constants were measured (see Chart 1, Table V). With the deuterated species the signal of 16α-proton appears as quartet and that of 16β-proton as triplet. Being compared with the coupling constants calculated by Williamson-Johnson and Abraham equations, the observed values are evidently in accord with the expected for conformation III. It is hoped that the data of of the coupling constants will be helpful for structural analysis of the 16- and 17-substituted 13α-steroids.

Experimental

NMR Spectra——Measurements of NMR spectra were carried out by Hitachi Model H-60 spectrometer operated at 60 Mcps with *ca.* 5% solution of the sample. The chemical shifts were obtained using tetramethylsilane as an internal standard. Accuracy of the measurements is within ± 0.02 ppm for chemical shift.

Samples——Almost all the samples except those mentioned below were prepared by the known methods.[6,7,12]

Synthesis[13]

5α,13α-Androstan-3β-ol Acetate (2)——5α,13α-Androstan-3β-ol (**1**) (15 mg) was acetylated with Ac_2O (0.25 ml) and pyridine (0.5 ml) in the usual manner and the crude product was chromatographed on silica gel (2.5 g). Elution with hexane–benzene (1:2) gave **2** (10 mg) as a semicrystalline product. According to TLC the acetylated product was homogeneous. *Rf*:0.44 (benzene), 0.87 (benzene–ether (10:1)), 0.46 (hexane–AcOEt (10:1)). NMR (5% solution in $CDCl_3$) τ: 8.00 (3H, s, CH_3COO–), 5.30 (1H, broad, 3α-H).

5α,13α-Androstane-3β,17β-diol 3-Acetate (8), 5α,13α-Androstane-3β,17α-diol 3-Acetate (11)——To a solution of 3β-hydroxy-5α,13α-androstan-17-one acetate (**19**) (500 mg) in 90% THF was added KBH_4 (600 mg) at 0° and the resulting solution was allowed to stand at room temperature for 10 hr. After usual work-up an oily product obtained was chromatographed on silica gel (20 g). Elution with benzene–ether (100:1) and recrystallization of the eluate from hexane gave **8** (50 mg) as colorless needles. mp 98—100°. $[\alpha]_D^{14}$ −25.0° (c=0.12). *Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.92; H, 9.97. Further elution with benzene–ether (100:1) and recrystallization of the eluate from hexane gave **11** (220 mg) as colorless needles. mp 125—126°. $[\alpha]_D^{14}$ −64.5° (c=0.14). *Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 74.98; H, 9.84.

5α,13α-Androstane-3β,17α-diol Diacetate (12)——Usual acetylation of 5α,13α-androstane-3β,17α-diol (**10**) (40 mg) with Ac_2O (1 ml) and pyridine (2 ml) followed by recrystallization from aq. MeOH gave **12** (40 mg) as colorless needles. mp 132°. $[\alpha]_D^{15}$ −50.0° (c=0.09). *Anal.* Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.47; H, 9.50.

5α,13α-Androstane-3β,16α-diol Diacetate (17)——Usual acetylation of 5α,13α-androstane-3β,16α-diol 3-acetate (**16**) with Ac_2O and pyridine followed by recrystallization from aq. MeOH gave **17** as colorless needles. mp 137—139°. $[\alpha]_D^{19}$ −50.0° (c=0.09). *Anal.* Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.22; H, 9.52.

8) K.L. Williamson and W.S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961); K.L. Williamson, *ibid.*, **85**, 516 (1963).
9) R.J. Abraham and J.S.E. Holker, *J. Chem. Soc.*, **1963**, 806.
10) It was found that Karplus equation (*J. Chem. Phys.*, **30**, 11 (1959); *J. Am. Chem. Soc.*, **85**, 2870 (1963)) would not be applicable for the present system.
11) J. Fishman, *J. Am. Chem. Soc.*, **87**, 3455 (1965); T. Nambara, M. Usui and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **17**, 1611 (1969).
12) T. Nambara, H. Hosoda and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **16**, 1266 (1968); T. Nambara, H. Hosoda and M. Usui, *ibid.*, **17**, 375 (1969); T. Nambara, H. Hosoda and T. Shibata, *ibid.*, in press.
13) All melting points were taken on a micro hot-stage apparatus. Optical rotations were measured in $CHCl_3$ solution.

17-Deuterio-5α,13α-androst-16-en-3β-ol (5d)——To a solution of 3β-acetoxy-5α,13α-androstan-17-one *p*-tosylhydrazone (1.2 g) in THF (50 ml) was added $LiAlH_4$ (2 g) portionwise and the resulting solution was refluxed for 16 hr. The reaction mixture was cautiously treated with D_2O (10 ml), acidified with 10% H_2SO_4 and extracted with ether. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent an oily residue obtained was chromatographed on Al_2O_3 (10 g). Elution with hexane gave **5d** (320 mg) as a crystalline product. According to TLC **5d** proved to be identical with 5α,13α-androst-16-en-3β-ol.[6]

16β,17β-Epoxy-17α-deuterio-5α,13α-androstan-3β-ol Acetate (23d), 16α,17α-Epoxy-17β-deuterio-5α,13α-androstan-3β-ol Acetate (25d)——To a solution of **5d** (320 mg) in $CHCl_3$ (20 ml) was added $C_6H_5CO_3H$–$CHCl_3$ (0.4M, 5 ml), and the resulting solution was allowed to stand at room temperature for 4 hr. The reaction mixture was diluted with ether, washed with 5% $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent an oily residue obtained was treated with Ac_2O (1.5 ml) and pyridine (3 ml). On usual work-up oily product was chromatographed on silica gel (20 g). Elution with benzene and recrystallization of the eluate from aq. MeOH gave **23d** (200 mg) as colorless plates. mp 109—110° (17α-H compound (**23**): reported mp 112—113°).[6] Elution with benzene–ether (10:1) gave **25d** (100 mg) as an oily substance. This product was homogeneous according to TLC and therefore was submitted to further step.

17α-Deuterio-5α,13α-androstane-3β,16α,17β-triol 3,16-Diacetate (53d)——A solution of **23d** (100 mg) in AcOH (3 ml) was refluxed for 30 min. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent an oily residue was submitted to preparative TLC (silica gel H) using benzene–ether (5:1) as developing solvent. Elution of the area corresponding to *Rf* 0.40 and recrystallization of the eluate from acetone–hexane gave **53d** (23 mg) as colorless plates. mp 206—207° (17α-H compound (**53**): reported mp 206—207°).[7]

17α-Deuterio-5α,13α-androstane-3β,16β,17α-triol 3,16-Diacetate (52d)——**25d** (100 mg) was treated with AcOH in the same manner as **23d**, and the crude product was submitted to preparative TLC (silica gel H) using benzene-ether (10:1) as developing solvent. Elution of the area corresponding to *Rf* 0.17 and recrystallization of the eluate from acetone–hexane gave **52d** (35 mg) as colorless needles. mp 171—173° (17α-H compound (**52**): reported mp 175—177°).[6]

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Addendum in Proof: After contribution of this paper the authors have learned publications dealing with the NMR spectral studies on several derivatives of 5α,13α-androstanes (M. Fétizon and J.-C. Gramain, *Bull. Soc. Chim. France*, **1966**, 2289, 3444).