Steroids. Part 32.[1] Configurational Analysis of 16-Methyltestosterone Derivatives

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The four possible isomers of 16-methylandrost-5-ene-3β,17-diol (**1**)—(**4**) have been converted into the corresponding 17-hydroxy-16-methylandrost-4-en-3-ones (**5**)—(**8**). The steric structures of the resulting epimers have been determined by ^{13}C and ^{1}H n.m.r. spectroscopy; thereby the exact configurational correlation of several 17-hydroxy-16-methylandrost-4-en-3-ones, reported in the literature, have been established.

The presence of a C-16 alkyl group in steroids often enhances, sometimes significantly, the biological properties of the parent compound.[2-7] The site of this group unequivocally follows from the method of synthesis, but the literature reveals uncertainties in deciding the steric position of the alkyl group.

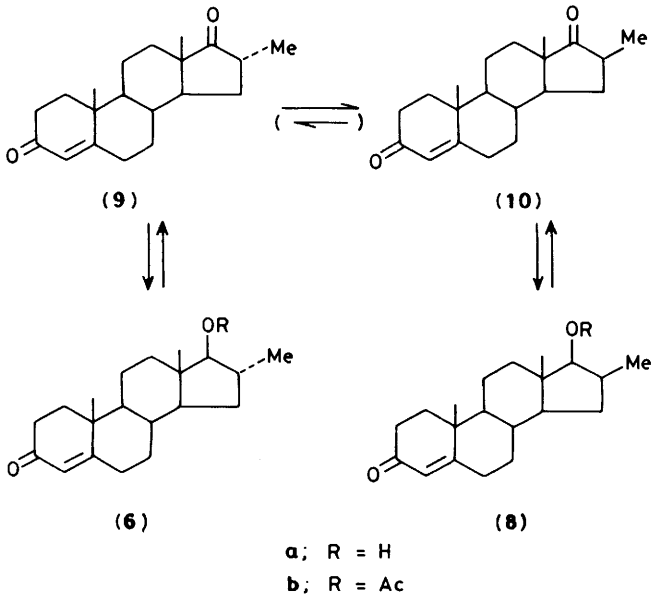
Thus, 17-hydroxy-16-methylandrost-4-en-3-one (**8b**) was prepared[8] *via* a two-step hydrogenation, subsequent selective acetylation, and Oppenauer oxidation from 3β-acetoxy-16-methyleneandrost-5-en-17-one; both the C-16 and C-17 functional groups were assigned uncertain configurations in this work. Using the same starting material, 16-methylandrost-4-ene-3,17-dione (**10**) was synthesized; selective reduction of this gave (**8a**).[9] The latter compound was also prepared[10] by the MnO_2 oxidation of 16-methylandrost-4-ene-3,17-diol, a reduction product of (**10**). The stereospecific synthesis of (**9**) was achieved by oxidative cleavage of the side chain of 16α-methylcortexolone;[11] either acid- or alkaline-induced isomerization of (**9**) furnished (**10**). By reduction of (**9**) or (**10**), followed by selective oxidation of the 3-hydroxy group, compounds (**6a**) and (**8a**) were prepared. At the same time, the stereospecific syntheses of the 16α- and 16β-methylandrost-5-ene-3β,17β-diols (**2a**) and (**4a**) were also effected from 3β-hydroxy-16α- and 16β-methylpregn-5-en-20-one.[12] Oxidation of (**2a**) gave (**9**), and reduction of the pyrrolidine-enamine of the latter yielded (**6a**). After protection of the 17-hydroxy group in (**4a**), it could be oxidized to (**8a**).

The above syntheses of the 17β-hydroxy-16-methylandrost-4-en-3-one epimers (**6a**), (**8a**) were achieved by reduction at C-17 of 16-methyl-17-oxo steroids. As suggested in earlier reports, both the acid and alkaline isomerizations of these compounds should give the thermodynamically more stable 16β-methyl-17-ketones[11-13] (Scheme 1).

Recently it has been shown[14] that both the acid and alkaline isomerizations of 3β-hydroxy-16-methylandrost-5-en-17-one give a *ca.* 20:80 equilibrium mixture of the 16α- and 16β-oxo steroids. Thus the stereochemical homogeneity of the 17-hydroxy-16-methyl epimers obtained by the reduction of 16α- and 16β-methyl-17-oxo steroids is strongly in doubt, because of the possible equilibrium isomerization of the starting compounds.

Our aim was, therefore, to synthesize the four possible isomers of 17-hydroxy-16-methylandrost-4-en-3-one (**5**)—(**8**) from derivatives of proven configurations, instead of using the conventional method of reducing the C-17 ketones. In this way, comparison is possible with the epimers (**6**) and (**8**) prepared earlier by a different route, and the series of isomers can be completed with the steric structures of (**5**) and (**7**).

Recently we reported[1] the conversions of 16-hydroxymethyl-



Scheme 1.

androst-5-ene-3β,17-diols of established configuration into the corresponding 16-methylandrost-5-ene-3β,17-diols (**1a**)—(**4a**). The diacetates of these isomers (**1b**)—(**4b**) were then selectively deacetylated; Oppenauer oxidation of the resulting 16-methylandrost-5-ene-3β,17-diol gave the 16-methyltestosterone isomers (**5b**)—(**8b**). Since the oxidation does not affect the C-16 and C-17 chiral centres, configurations of the products and starting compounds are identical (Scheme 2).

Jones oxidation of the 17-hydroxy-16-methylandrost-4-en-3-one isomers (**6a**) and (**8a**) furnished the stereohomogenous 16-methylandrost-4-ene-3,17-diones (**9**) and (**10**). These were isomerized in acid and in alkaline media, as given in the literature.[11] Comparison of the configuration-characteristic signals of the ^{13}C n.m.r. spectra showed that, irrespective of the starting epimer, the equilibrium mixture contained (**9**) and (**10**) in 21:79 ratio after a reaction time of 48 h (see Figure). This result is in agreement with the behaviour of 3β-hydroxy-16-methylandrost-5-en-17-one.[14]

Steric interaction between the functional groups in the isomers examined (**5b**)—(**8b**), (**9**), and (**10**) gives rise to characteristic shifts in the ^{13}C n.m.r. spectra (Table 1). The

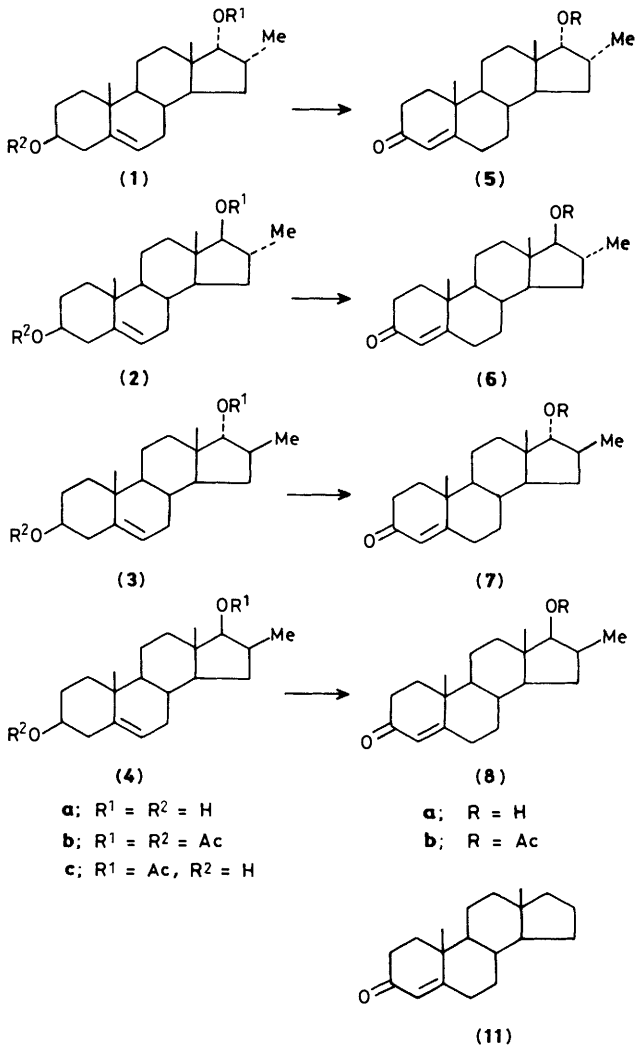
signals were assigned using the off-resonance technique and our own earlier measurments,[15] as well as literature data.[1,16]

Configurational analysis of the C-17 substituent in (**5b**)—(**8b**) was based on the ^{13}C n.m.r. shifts of the δ-carbon atoms (C-12 and C-18). A 17α-substituent causes an upfield shift of 5 p.p.m. for the C-12 signal as a result of δ-interaction. A 17β-substituent similarly gives rise to an upfield shift of 3—4 p.p.m. for the C-18 signal. The *cis*-configuration of the C-16 and C-17 groups is indicated by the strong interaction between them; thus there is an upfield shift of 5 p.p.m. for the C-17 signal compared with that found for the *trans* arrangement. The δ-interaction arising from a *cis* C-17 substituent with a C-16 methyl group and causing an upfield shift of 4 p.p.m. may be used as additional evidence.

Assignments of the configurations of the functional groups in the 16-methyltestosterone isomers (**5b**)—(**8b**) can be based on the following characteristic ^{13}C n.m.r. shifts (*ca.* ±5 p.p.m.).

Substituent	Shift (p.p.m.)
17α	δ(C-18) 16.5; δ(C-12) 31.8
17β	δ(C-18) 12.6; δ(C-12) 37.0
16,17-*cis*	δ(C-17) 83.0; δ(16-Me) 16.8
16,17-*trans*	δ(C-17) 88.3; δ(16-Me) 20.5

The ^{13}C spectra of the two isomers of 16-methylandrost-4-ene-3,17-dione (**9**) and (**10**) are closely similar. A comparison of them with the spectrum of androst-4-en-3-one (**11**), unsubstituted in ring D, reveals that the presence of the 17-oxo group gives rise to considerable δ-interaction with C-12, and to a lesser extent with C-18. In contrast with the shift of 8 p.p.m. for the C-12 signal, the upfield shift for C-18 is *ca.* 3 p.p.m. The signal for the α-methyl adjacent to the 17-oxo group [in (**9**)] shows a *ca.* 4 p.p.m. upfield shift compared with that for the 16β-methyl in (**10**). The characteristic ^{13}C n.m.r. chemical shifts for the 16-methylandrost-4-ene-3,17-dione epimers (**9**) and (**10**) are



Scheme 2.

Table 1. ^{13}C N.m.r. chemical shifts of testosterone derivatives δ/p.p.m.

Carbon no.	Compound (**5b**)	(**6b**)	(**7b**)	(**8b**)	(**9**)	(**10**)	(**11**)
1	35.6	35.6	35.4	35.6	35.7	35.6	35.7
2	33.6	33.8	33.5	33.8	33.9	33.8	33.9
3	199.5	199.2	199.2	199.0	198.9	198.8	199.2
4	123.8	123.8	123.8	123.8	124.0	123.9	123.7
5	171.2	170.8	170.9	170.7	170.2	170.2	171.3
6	32.8	32.7	32.8	32.7	32.6	32.5	32.9
7	32.1	31.4	32.1	31.7	31.6	31.6	32.4
8	35.6	35.2	35.6	34.8	35.0	34.7	35.9
9	53.5	53.7	53.3	53.7	53.9	53.9	54.0
10	38.6	38.5	38.6	38.5	38.6	38.6	38.7
11	20.3	20.4	20.2	20.3	20.2	20.2	20.4
12	31.6	36.7	32.0	37.4	30.7	30.9	38.5
13	45.6	44.0	44.3	42.9	48.0	47.7	40.6
14	48.6	48.7	50.9	49.3	48.2	49.4	54.1
15	33.9	32.2	34.5	34.1	30.1	30.1	25.4
16	33.7	35.2	40.7	32.8	39.1	43.6	21.0
17	82.9	88.7	88.0	83.2	221.9	222.0	40.2
18	15.6	12.6	17.4	13.2	14.3	14.0	17.4
19	17.4	17.3	17.2	17.3	17.4	17.3	17.4
16-Me	16.9	20.2	20.8	16.8	16.6	16.8	
17-OAc	170.8	171.0	170.6	170.8			
	20.9	21.1	21.2	20.8			

Table 2. ^{1}H n.m.r. data of compounds (**5**)—(**8**)

	Chemical shifts δ/p.p.m. 4-H	17-H	16-Me	18-Me	19-Me	17-OH	17-OAc	Coupling constants J/Hz $J_{16,17}$	$J_{16,Me}$
(**5b**)	5.70	4.82	0.92	0.86	1.2		20.5	6.0	7.0
(**6b**)	5.75	4.47	1.05	0.85	1.2		2.08	7.0	6.5
(**7b**)	5.75	4.47	1.19	0.87	1.2		2.05	1.0	6.5
(**8b**)	5.70	4.55	0.92	0.87	1.2		2.08	10.0	6.5
(**5a**)	5.73	3.54	1.02	0.80	1.2	1.6		5.5	7.0
(**6a**)	5.75	3.15	1.10	0.82	1.2	1.7		7.0	6.5
(**7a**)	5.75	3.35	1.18	0.79	1.2	1.6		1.0	7.0
(**8a**)	5.73	3.50	1.00	0.80	1.2	1.6		10.0	6.5

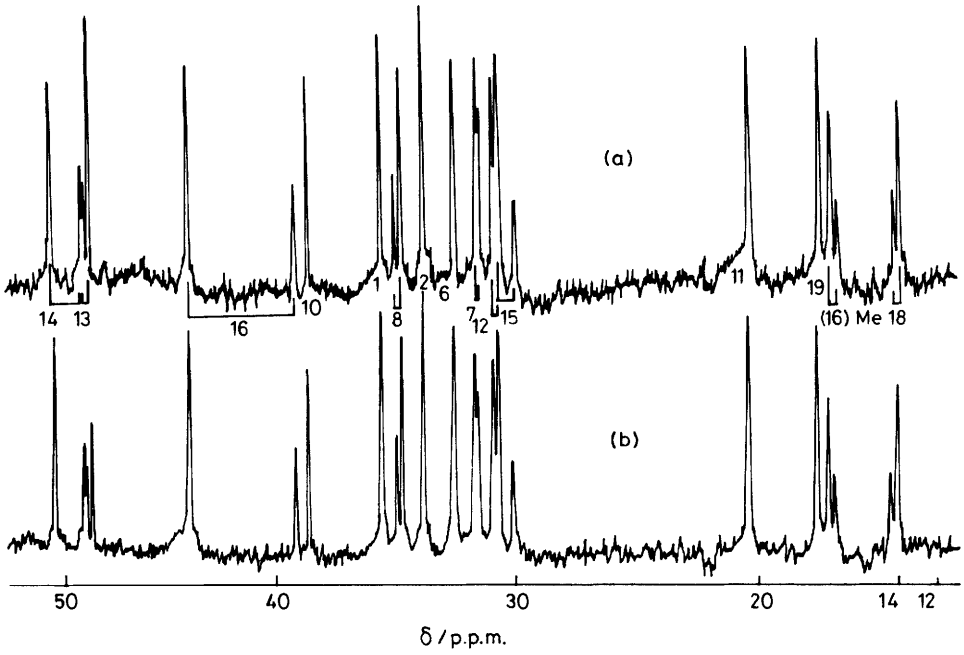


Figure. Isomerization products of the 16-methylandrost-4-ene-3,17-diones: (a) in acidic media and (b) in alkaline media. Characteristic parts of the ^{13}C n.m.r. spectra of the equilibrium mixtures (**9**):(**10**) ≈ (20:80) in $CHCl_3$

16α-methyl-17-ketone δ(C-16), 39.1 p.p.m., and 16β-methyl-17-ketone δ(C-16), 43.6 p.p.m.

The ^1H n.m.r. data show that the configuration of the 16-methyl isomers (**5b**)—(**8b**) is characterized by the $J_{16.17}$ coupling constants (Table 2); the following order is found:

$$J_{16\alpha H.17\beta H} < J_{16\beta H.17\beta H} < J_{16\beta H.17\alpha H} < J_{16\alpha H.17\alpha H}$$
(**7b**) < 1.0 Hz (**5b**) 6.0 Hz (**6b**) 7.0 Hz (**8b**) 10.0 Hz

This finding agrees with that observed for the four possible isomers of 16,17-disubstituted compounds having an oestrane [17] or an androstane [1] skeleton. It appears that the characteristic coupling constants of the 16- 17-isomers are not significantly affected by the AB ring system of the sterane skeleton.

Experimental

M.p.s were determined with a Kofler hot-stage apparatus and are uncorrected. Physical properties and analytical data of the compounds are listed in Table 3. Specific rotations were measured with a Polamat-A polarimeter in chloroform (c = 1). T.l.c. data: Kieselgel-G (Merck) (0.5 mm); methanol–benzene (5:95) as developing solvent; detection by u.v. (365 nm) after spraying with 50% phosphoric acid and heating at 100—120 °C for 15 min. Column chromatography: Al_2O_3 (Brockmann, activity III-IV) 50 g; column: 25 × 2 cm. The ^{13}C n.m.r. spectra were recorded in $CDCl_3$ with a JEOL PS 100/PFT instrument coupled to a Nicolet (NIC 1085) computer, using the broad band proton decoupling technique, at 25.15 MHz, and flip angle 45°; impulse distance 3 s; sweep width, 5—6 kHz; digital resolution: 1.2—1.5 Hz. ^1H N.m.r. spectra were obtained in $CDCl_3$ on a JEOL C-60 HL 60 MHz instrument using $SiMe_4$ as internal standard. Chemical shifts are given in δ.

17-Acetoxy-16-methylandrost-5-en-3β-ol (**1c**)—(**4c**). *General Procedure.*—Compound (**1b**), (**2b**), (**3b**), or (**4b**) [1] (1.95 g, 5 mmol) was dissolved in methanol (100 ml), cooled to 0 °C and a solution of KOH (1.40 g, 2.5 mmol) in methanol (50 ml) was added; progress of the selective hydrolysis was monitored by t.l.c. After 24 h at 0 °C the solution was poured onto ice (500 g), acidified with dilute hydrochloric acid and the precipitate filtered off and washed thoroughly with water. It was dissolved in chloroform and subjected to column chromatography in benzene–chloroform (3:1). The product obtained on evaporation of the combined fractions was crystallized from a mixture of acetone and water.

17-Acetoxy-16-methylandrost-4-en-3-one (**5b**)—(**8b**) (*16-Methyltestosterone 17-Acetate*): *General Procedure.*—A mixture of cyclohexanone (50 ml) and toluene (200 ml) was heated until 100 ml of toluene had distilled off. Aluminium isopropoxide (2 g, 10 mmol) and (**1c**), (**2c**), (**3c**), or (**4c**) (0.860 g, 2.5 mmol) were then added and the solution was refluxed for 5 h. The mixture was then diluted with ice (250 g), acidified with dilute sulphuric acid, and evaporated under reduced pressure. The oily residue was extracted with benzene, washed with water until neutral, concentrated, and subjected to column chromatography. Contaminants were removed with light petroleum, and the product was eluted with benzene–light petroleum (1:1). The combined fractions were evaporated to dryness and crystallized from aqueous methanol.

17-Hydroxy-16-methylandrost-4-en-3-one (**5a**)—(**8a**) (*16-Methyltestosterone*): *General Procedure.*—Compound (**5b**), (**6b**), (**7b**), or (**8b**) (3.45 g, 10 mmol) was dissolved in methanol (100 ml) and KOH (1 g, 2 mmol) was added. After 24 h of room temperature the mixture was neutralized with dilute hydrochloric acid and diluted with water. The precipitate was then filtered off, washed with water until neutral, and crystallized from aqueous acetone.

16-Methylandrost-4-ene-3,17-dione (**9**) *and* (**10**): *General Procedure.*—A solution of compound (**6a**) or (**8a**) (3.0 g, 10 mmol) in acetone (15 ml) was oxidized with Jones reagent (4 ml, 10 mmol), with cooling in ice, for 1 h. The mixture was diluted with

Table 3.

No.	Formula (Mol. wt.)	M.p. (°C) (lit.)	$[\alpha]_D$	R_F	Analysis (%) Calc. (Found) C	H	Yield (%)
(1c)	$C_{22}H_{34}O_3$ (346.51)	173—175	−69	0.65	76.26 (76.4)	9.89 (9.8)	62
(2c)	$C_{22}H_{34}O_3$ (346.51)	148—150	−119	0.60	76.26 (76.2)	9.89 (9.8)	65
(3c)	$C_{22}H_{34}O_3$ (346.51)	159—161	−57	0.55	76.26 (76.2)	9.89 (9.95)	63
(4c)	$C_{22}H_{34}O_3$ (346.51)	162—164	−40	0.60	76.26 (76.4)	9.89 (9.8)	65
(5a)	$C_{20}H_{31}O_2$ (303.46)	208—210	+29	0.65	79.16 (79.3)	10.29 (10.25)	95
(5b)	$C_{22}H_{33}O_3$ (345.50)	89—92	+59	0.75	76.48 (76.35)	9.62 (9.75)	72
(6a)	$C_{20}H_{31}O_2$ (303.46)	158—160 (154—156)[a] (155—157)[b]	+79	0.50	79.16 (79.3)	10.29 (10.2)	92
(6b)	$C_{22}H_{33}O_3$ (345.50)	153—154	+39	0.70	76.48 (76.35)	9.62 (9.8)	70
(7a)	$C_{20}H_{31}O_2$ (303.46)	187—189	+92	0.45	76.16 (79.35)	10.29 (10.15)	94
(7b)	$C_{22}H_{33}O_3$ (345.50)	158—160	+111	0.70	76.48 (76.6)	9.62 (9.5)	68
(8a)	$C_{20}H_{31}O_2$ (303.46)	185—187 (177—180)[a] (183—184)[b]	+101	0.50	76.16 (79.0)	10.29 (10.35)	92
(8b)	$C_{22}H_{33}O_3$ (345.50)	164—165	+120	0.70	76.48 (76.55)	9.62 (9.75)	72
(9)	$C_{20}H_{28}O_2$ (300.44)	134—136	+167	0.70	79.96 (79.8)	9.39 (9.45)	96
(10)	$C_{20}H_{28}O_2$ (300.44)	178—180	+181	0.70	79.96 (79.75)	9.39 (9.3)	94

[a] Ref. 11. [b] Ref. 12.

ice–water and the crystalline precipitate was filtered off, washed until neutral, and recrystallized from aqueous methanol.

Androst-4-en-3-one (**11**). As suggested by Butenandt *et al.*,[18] this was prepared from androst-5-en-3β-ol; it had m.p. 106 °C, $[\alpha]_D$ +110° (lit.,[18] m.p. 104—105 °C, $[\alpha]_D$ +110°).

Equilibration.—(1) Compound (**9**) or (**10**) (0.3 g, 1 mmol) was dissolved in 2% KOH in methanol (5 ml) and after 48 h at room temperature was diluted with water. The precipitate was filtered off, washed, and dissolved in deuteriochloroform. The composition of the equilibrium mixture was determined by ^{13}C n.m.r.

(2) Compound (**9**) or (**10**) (0.3 g, 1 mmol) was dissolved in acetic acid (5 ml) containing 1% hydrochloric acid. After 48 h at room temperature the mixture was treated as described above and the equilibrium concentration determined.

Acknowledgements

The authors thank the chemical works of Gedeon Richter Ltd., Budapest and the Hungarian Ministry of Culture (Grant 90/1981) for supporting this research.

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Received 25*th July* 1984; *Paper* 4/1296