Examination of the Stereochemistry of Hydrogenolysis of Steroidal C-3 Substituents using H N.M.R. Spectroscopy

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The hydrogenolysis of 3β-*p*-tolylsulphonyloxyandrost-5-en-17-one with lithium aluminium deuteride and of 3β-iodoandrost-5-en-17-one with zinc–[^{2}H]acetic acid has been shown by ^{2}H n.m.r. spectroscopy not to be stereospecific, contrary to earlier reports.

STEREOSPECIFICALLY deuteriated steroids have been widely used in mechanistic, spectroscopic, and biosynthetic studies.[1] Analysis of the C–D i.r. stretching frequencies led [2] to the conclusion that hydrogenolysis of cholesteryl toluene-*p*-sulphonate by lithium aluminium hydride proceeded with retention of configuration. It was also claimed that reduction of 3β-iodocholestane with zinc and [^{2}H]acetic acid proceeded with (>95%) retention of configuration. A similar analysis led to the preparation of stereospecifically 7- [3] and 11-deuteriated steroids.[4] These labelled steroids have played a central role in determining the stereochemistry of enzymatic hydroxylation,[5] in the mechanism of some rearrangement reactions,[6] and more recently in the study of the stereochemistry of tetrahymanol biosynthesis.[7] Since the original studies, a number of nucleophilic substitution reactions of cholesteryl toluene-*p*-sulphonate have been shown [8] to proceed with inversion rather than retention of configuration. In view of the stereospecificity noted in the earlier report, it seemed important to re-investigate the stereochemistry of the hydrogenolysis reaction with the aid of ^{2}H n.m.r. spectroscopy.

The deuteriated steroids were prepared as follows. 3β-*p*-Tolylsulphonyloxyandrost-5-en-17one (1) [9] was reduced with lithium aluminium deuteride. The 3ξ-deuterioandrost-5-ene, which was the minor product, was separated from the 3,5-cyclo-steroid by oxidation of the crude mixture to the 17-ketones and epoxidation with *m*-chloroperbenzoic acid. The cyclo-steroid (4) and the 5,6-epoxide could then be separated chromatographically. The 5,6-epoxide was then reduced with sodium iodide, zinc, and acetic acid [10] to regenerate the 3ξ-deuterioandrost-5-en-17-one (2). The deuterio-steroid with the opposite configuration at C-3 was prepared from testosterone acetate (5). Methanolysis of its enol–trichloroacetate [11,12] gave 17β-acetoxyandrost-5-en-3-one which was reduced with sodium borodeuteride to afford 17β-acetoxy-3α-deuterio-3β-hydroxyandrost-5-ene.[13] The 3α-deuterio-3β-toluene-*p*-sulphonate (6) [14] was hydrogenolysed with lithium aluminium hydride to give a mixture of 3ξ-deuterio-17β-hydroxyandrost-5-ene and 3ξ-deuterio-17β-hydroxy-3,5-cycloandrostane. These were again separated as their 17-ketones [(3) and (8)] *via* the 5,6-epoxide.

3β-Hydroxyandrost-5-en-17-one was converted into the 3β-iodo-steroid (9) with *o*-catechol phosphochloridate and iodine [15] and then reduced with zinc–[^{2}H]acetic acid to a 3ξ-deuterioandrost-5-en-17-one. This product was hydrogenated over palladium–charcoal to afford the 3ξ-deuterio-5α-androstan-17-one. The zinc–acetic acid reduction of the iodo-steroid provides a more convenient route to androst-5-enes than that based on the sodium–ethanol reduction of the chloro-steroid.[16]

TABLE 1

^{2}H N.m.r. signals of the deuteriated steroids (in p.p.m.)

	3β–(*eq*)–^{2}H		3α–(*ax*)–^{2}H	
Androst-5-enes	δ	Rel. int.	δ	Rel. int.
$LiAlD_4$ + (1) [≡ (2)]	1.71	1	1.17	1.65 [a]
$LiAlH_4$ + (6) [≡ (3)]	1.69	1.65	1.15	1 [b,c]
Zn–DOAc + (9)	1.71	1.52	1.17	1
Androstanes				
Zn–DOAc + (9) [d]	1.63	1.42	1.17	1
Zn–DOAc + (10)	1.64	1	1.17	1.27

[a] The 3,5-cyclo-steroid (4) showed resonances at δ 0.97 (rel. int. 1) and 1.8 8(3). [b] The 3,5-cyclo-steroid (8) showed a resonance at δ 0.98. [c] These spectra were measured at 61.4 MHz on a Bruker WH 400 spectrometer. [d] Androstane formed by subsequent catalytic hydrogenation.

3β-Hydroxy-5α-androstan-17-one was converted into the 3α-iodo-5α-androstan-17-one (10) with the *o*-catechol-phosphochloridate–iodine reagent and this was then reduced with zinc–[^{2}H]acetic acid.

The ^{2}H n.m.r. spectra were determined at 30.72 MHz whilst the sites of deuteriation were confirmed by the

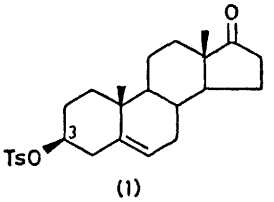
TABLE 2

^{13}C N.m.r. isotope shifts (in p.p.m.)

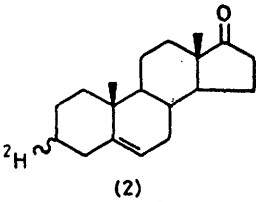
	^{13}C(D) resonance	Δδ	$J(^{13}C–^2H)$ (±1.5 Hz)
3ξ-Deuterioandrost-5-en-17-one (2)	27.5 (4)	0.43	20
3ξ-Deuterio-5α-androstan-17-one (11)	26.5 (3)	0.41	23
6ξ-Deuterio-3,5-cycloandrostan-17-one (4)	28.8 (2)	0.42	18
3β-Deuterio-3,5-cycloandrostan-17-one (8)	22.9 (9)	0.43	24
3α-Deuterio-3β,17β-diacetoxyandrost-5-ene (7)	73.2 (2)	0.55	23

changes in the appropriate resonances in the ^{13}C n.m.r. spectra. Deuterons possess the same chemical shift (in p.p.m.) as the corresponding protons. In particular the 3α-axial deuterons resonate at higher field than the 3β-equatorial isomers.[17] Examination of Table 1 shows that although the hydrogenolysis reactions possess some stereoselectivity, they are by no means as stereospecific

as the earlier analysis would suggest. The hydrogenolysis of the Δ^5-3β-toluene-*p*-sulphonates may comprise three components. First there is a direct nucleophilic displacement leading to inversion of configuration at C-3, secondly a concerted homo-allylic nucleophilic displacement affording the 6β-deuterio-3,5-cyclo-steroid, and thirdly an i-steroid reaction in which the intermediate carbo-cation is quenched by nucleophilic attack from the 6β- or the less-hindered 3β- and 6α-faces. These

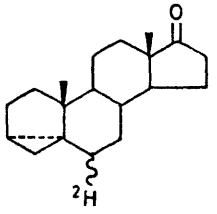


(1)

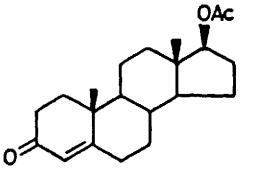


(2)

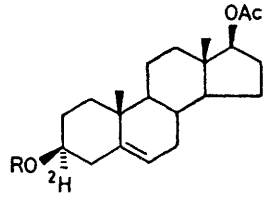
(3) ^{2}H epimeric mixture



(4)

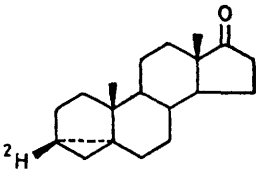


(5)

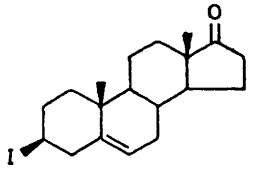


(6) R = Ts

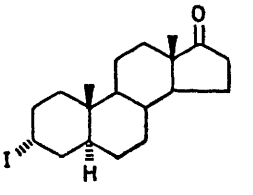
(7) R = Ac



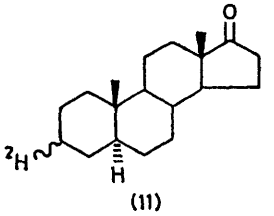
(8)



(9)



(10)



(11)

results suggest that a critical re-evaluation of some features of the earlier evidence for the stereochemistry of enzymatic hydroxylation may be required.

The sample of 3ξ-deuterioandrost-5-en-17-one obtained from the lithium aluminium hydride reduction of the 3α-deuterio-3β-toluene-*p*-sulphonate (6) also contained a ^{2}H n.m.r. signal at δ 1.88. Analysis of the ^{13}C n.m.r. spectrum suggested that this could be assigned to an androst-4-ene impurity. The latter arose through incomplete deconjugation of the testosterone acetate. Lithium aluminium hydride reduction of the minor amount of 17β-acetoxy-3α-deuterio-3β-*p*-tolylsulphonyloxyandrost-4-ene proceeded with inversion of configuration to afford a 3β-deuterioandrost-4-ene which would be concentrated in the olefinic fraction. In the 6-deuterio-cyclopropyl steroids the 6β-(axial) deuteron resonates at lower field than the 6α-(equatorial) deuteron. A similar effect has been observed [18] with the C*H*(OH) resonances of the C-6 alcohols.

Analysis of the ^{13}C n.m.r. spectra of the deuteriated steroids (see Table 2) showed the anticipated [19] isotope shift for the monodeuteriated carbons. The ^{13}C–^{2}H coupling constants were also measured although these are ' average ' values for the mixture of epimers.

EXPERIMENTAL

General experimental details have been described previously.[20] The ^{2}H n.m.r. spectra were determined on Bruker WP 200 and WH 400 spectrometers operating at 30.72 and 61.4 MHz respectively. The samples (*ca.* 30 mg) were dissolved in chloroform (0.4 ml) and 200 scans were accumulated with ^{1}H noise decoupling.

Lithium Aluminium Deuteride Reduction of 3β-p-*Tolylsulphonyloxyandrost-5-en-17-one* (1).—The steroid (1) (2 g) [9] in tetrahydrofuran (30 ml) was treated with lithium aluminium deuteride (0.75 g) under reflux for 5 h. The solution was poured into dil. hydrochloric acid (20 ml) and the steroid extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogencarbonate, dried, and evaporated to afford a gum (2 g). The gum was dissolved in acetone and treated with the 8N chromium trioxide reagent (5 ml) for 30 min. Excess of aqueous sodium sulphite was added and the steroids were extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogencarbonate, dried, and evaporated to afford a gum. The latter was taken up in chloroform and treated with *m*-chloroperbenzoic acid (1 g) at room temperature for 12 h. The solution was then washed with aqueous sodium sulphite and aqueous sodium hydrogencarbonate and dried. The solvent was evaporated off and the residue chromatographed on alumina. Elution with 10% ether–light petroleum afforded 6ξ-deuterio-3,5-cycloandrostan-17-one (4) (840 mg) which crystallized from methanol as needles, m.p. 142—144 °C (lit.,[21] 143—144 °C), $\nu_{max.}$ 2 140 and 1 730 cm^{-1}, δ 0.9 (3 H, s, 18-H) and 0.97 (3 H, s, 19-H). Elution with 15% ether–light petroleum gave 3ξ-deuterio-5α,6α-epoxyandrostan-17-one which crystallized from methanol as needles, m.p. 138—141 °C (lit.,[16] 140—141 °C), $\nu_{max.}$ 2 140 and 1 740 cm^{-1}, δ 0.83 (3 H, s, 18-H), 1.07 (3 H, s, 19-H), and 2.90 (1 H, d, *J* 4 Hz, 6-H).

3ξ-*Deuterioandrost-5-en-17-one* (2).—The foregoing epoxide (70 mg) in acetic acid (10 ml) was treated with zinc powder (100 mg) and sodium iodide (100 mg) at room temperature with vigorous stirring for 2 h. The solution was filtered and the zinc powder was washed with acetone. The combined filtrate and washings were concentrated and the residue taken up in ethyl acetate. The organic phase was washed with water and aqueous sodium hydrogencarbonate, and dried. The solvent was evaporated off to

afford a gum which was purified by preparative layer chromatography on silica in ether–light petroleum (1 : 1) to afford 3ξ-deuterioandrost-5-en-17-one (2) (45 mg) which crystallized from methanol as needles, m.p. 103—107 °C (lit.,[22] 106—107°), $\nu_{max.}$ 2 140 and 1 740 cm^{-1}, δ 0.87 (3 H, s, 18-H), 1.03 (3 H, s, 19-H), 5.27 (1 H, d, *J* 4 Hz, 6-H). 17β-Acetoxy-3α-deuterio-3β-*p*-tolylsulphonyloxyandrost-5-ene [13] (6) (2 g) was reduced with lithium aluminium hydride (2 g) in tetrahydrofuran (30 ml) and the products separated as above to afford 3β-deuterio-3,5-cycloandrostan-17-one (8) (0.95 g) and 3ξ-deuterioandrost-5-en-17-one (70 mg).

3β-*Iodoandrost-5-en-17-one* (9).—3β-Hydroxyandrost-5-en-17-one (5 g) in dry dichloromethane (100 ml) was treated with catechol phosphochloridate (3.5 g) and pyridine (1.5 g) with stirring at room temperature for 30 min. Iodine (7 g) was added and, after a further 2 h, the excess of iodine was destroyed with aqueous sodium sulphite. The organic phase was washed with dilute hydrochloric acid and water, and dried. The solvent was evaporated off and the product passed through a short column of alumina in ether. The solution was concentrated to afford 3β-*iodoandrost-5-en-17-one* (9) (6.5 g) which crystallized from ether as needles, m.p. 174—176 °C, $[\alpha]_D$ +28° (*c* 0.6) (Found: C, 57.2; H, 6.8. $C_{19}H_{27}IO$ requires C, 57.3; H, 6.8%), $\nu_{max.}$ 1 735 cm^{-1}, δ 0.9 (3 H, s, 18-H), 1.1 (3 H, s, 19-H), 4.07 (1 H, m, 3-H), and 5.37 (1 H, d, *J* 4 Hz, 6-H).

3ξ-*Deuterioandrost-5-en-17-one* (2).—The iodo-steroid (9) (5 g) in dry ether (100 ml) was treated with zinc (20 g) and [^{2}H]acetic acid (10 ml) with stirring at room temperature for 10 h. The solution was filtered and diluted with ethyl acetate. The organic phase was washed with water and aqueous sodium hydrogencarbonate, and dried. The solvent was evaporated off to afford 3ξ-deuterioandrost-5-en-17-one (2) (3.1 g) which crystallized from methanol as needles, m.p. 107—108 °C, identical to the material just described.

3ξ-*Deuterio-5α-androstan-17-one* (11).—The olefin (2) (200 mg) in ethyl acetate (10 ml) was stirred with 10% palladium–charcoal under hydrogen at room temperature for 3 h. The solution was filtered and the solvent evaporated off to afford 3ξ-deuterio-5α-androstan-17-one (11) (155 mg) which crystallized from methanol as plates, m.p. 116—118 °C (lit.,[23] 118—122 °C), $\nu_{max.}$ 1 745 cm^{-1}, δ 0.8 (3 H, s, 18-H) and 0.87 (3 H, s, 19-H).

3α-*Iodo-5α-androstan-17-one* (10).—3β-Hydroxy-5α-androstan-17-one (2 g) in dry dichloromethane (50 ml) was treated with catechol phosphochloridate (1.5 g) and pyridine (0.7 ml) at room temperature for 15 min. Iodine (2.5 g) was added and the solution was stirred for 1 h at room temperature. The solution was poured into an excess of aqueous sodium sulphite and the steroid was recovered in ethyl acetate. The organic phase was dried, filtered through a short plug of alumina, and evaporated to afford 3α-*iodo-5α-androstan-17-one* (10) (2.6 g) which crystallized from methanol as cubes, m.p. 124—125 °C, $[\alpha]_D$ +19° (*c* 0.6) (Found: C, 57.4; H, 7.3. $C_{19}H_{29}IO$ requires C, 57.0; H, 7.3%), $\nu_{max.}$ 1 740 cm^{-1}, δ 0.83 (3 H, s, 18-H), 0.87 (3 H, s, 1-H), and 4.93 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3-H).

3ξ-*Deuterio-5α-androstan-17-one* (11).—The foregoing iodo-steroid (500 mg) in dry ether (25 ml) was treated with zinc powder (2 g) and [^{2}H]acetic acid (2 ml) with stirring overnight. The solution was filtered and the filtrate washed with water and aqueous sodium hydrogencarbonate. The solution was dried and the solvent evaporated off to afford 3ξ-deuterio-5α-androstan-17-one (11) (260 mg) which crystallized from methanol as plates, m.p. 116—118 °C, identical to the material obtained above.

We thank Professor L. Crombie F.R.S. for a preliminary ^{2}H n.m.r. spectrum.

[9/1276 *Received*, 10*th July*, 1979]

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