fitted with a septum, and toluene (25 mL, freshly distilled from sodium hydride and purged with nitrogen) was added via a Leur needle. Isopropenyl acetate (2 mL) and 1 equiv (0.290 mL, 1.00 mmol) of tributyltin methoxide were added via a gas-tight syringe. The solution was stirred in an oil bath under N_2 and at 100 °C. After 4 h and 7 h, additional aliquots (1 mL and 0.15 mL, respectively) of isopropenyl acetate and tributyltin methoxide were added. After 10 h, the solution was cooled, taken up in hexane (50 mL), and extracted first with 5% aqueous solution of Na_2EDTA (15 mL) and then with H_2O (10 mL). The aqueous layers were back extracted with hexane (20 mL). The combined organic extracts were dried (Na_2SO_4), concentrated, and purified by flash chromatography with 1:1 ether/hexane as the eluting solvent. The desired acetonylated product was isolated as a low-melting solid (0.354 g, 0.532 mmol, 53%): ^{13}C NMR ($CDCl_3$) δ -5.4, -4.9, -4.7, -4.3, 17.9, 18.1, 18.5, 25.7, 25.8, 26.1, 29.5, 54.6, 62.3, 71.6, 75.6, 84.8, 88.6, 118.6, 139.6, 150.4, 155.5, 159.0, 204.3; ^1H NMR ($CDCl_3$) δ -0.3 to 0.12 (m, 18 H), 0.91 (m, 27 H), 2.19 (s, 3 H), 3.90-4.09 (m, 3 H), 3.85 (s, 2 H), 4.29 (t, 1 H), 4.61 (t, 1 H), 5.64 (br s, 2 H), 5.95 (d, 1 H), 8.13 (s, 1 H); UV (EtOH) λ_{max} 262 (ϵ 1.3 × 10^3), 300 nm (sh, 2.5 × 10^2); mass spectrum, m/z (relative intensity) 666 (M^+, 2.0), 665 (2.3), 650 (4.0), 608 (100), 552 (3.1), 476 (7.2), 462 (12.3), 436 (3.1), 417 (1.7), 348 (21.8), 306 (3.3), 285 (3.5), 261 (9.6), 248 (8.1), 231 (11.0), 220 (10.3), 211 (9.9), 192 (30.1).

The desilylation procedure of the protected 2-acetonyladenosine (0.514 g, 0.773 mmol) was carried out as described for **7**. 2-Acetonyladenosine (**18**) was obtained as a white crystalline hygroscopic compound in 75% yield (0.187 g, 0.580 mmol) after purification by HPLC: mp 113-115 °C; ^{13}C NMR (Me_2SO-d_6) δ 29.1, 57.7, 61.4, 70.5, 73.2, 85.7, 88.4, 117.3, 140.5, 148.3, 154.9, 157.8, 209.8; ^1H NMR (Me_2SO-d_6) δ 2.15 (s, 3 H), 3.61 (br s, 1 H), 3.77 (s, 2 H), 3.97 (m, 1 H), 4.15 (m, 1 H), 4.60 (m, 1 H), 5.23 (s), 5.43 (br s, 3 H), 5.78 (d), 5.84 (d, 1 H), 8.31 (s, 1 H); UV (EtOH) λ_{max} 262.5 (ϵ 12050), 300 nm (4650); FAB HRMS obsd (M^+ + H) 324.1299, calcd for $C_{13}H_{17}N_5O_5$ 324.1308.

Enzymatic Deamination Studies. All assays with adenosine deaminase (Type I from calf intestinal mucosa, Sigma) were followed spectrophotometrically at 25 °C by using a Gilford Response UV-visible spectrometer. Adenosine was used as the standard. Solutions of substrates of appropriate concentrations in 0.05 M phosphate buffer (pH 7.40) were used, and deamination reactions were initiated by addition of the enzyme. For example, the conversion of **18** to **7** could be monitored quantitatively by the change in the major absorption band in the UV spectrum (263 nm → 250 nm). Details of the procedure for the assays have been previously described by us.[29]

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The Reduction of Steroid 2α-Fluoro 4-En-3-ones

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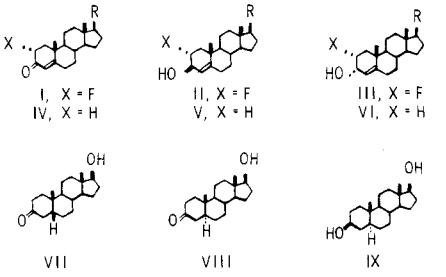
Reduction of testosterone with potassium tri-(*R,S*)-*sec*-butylborohydride gives predominantly the allylic 3β-alcohol, while 2α-fluorotestosterone is converted solely to 2α-fluoro-4-androstene-3α,17β-diol, and 2α-fluoro-4-androstene-3,17-dione to 2α-fluoro-3α-hydroxy-4-androsten-17-one. Reduction of testosterone with (*R,R*)- or (*S,S*)-Rh-DIOP and dihydrosilanes give predominantly allylic alcohols, while with the same catalysts and monohydrosilanes no allylic alcohols are found, the 4-double bond being instead reduced. The chirality of the DIOP reagents contributes only to a minor extent to stereoselectivity of 3-ketone reduction.

Introduction

As a test of the existence of a 2β-hydroxylation step in the enzymic conversion of testosterone to estrogens, we examined the aromatization of 2α-fluorotestosterone (I, R = β-OH) by placental microsomes.[1] Early products of the microsomal transformation appeared to be 2α-fluoro-allylic alcohols which were not found at later time periods; they are probably transformed back to 2α-fluoro 4-en-3-ones and then to the 2-fluoroestrogens. In order to characterize these allylic alcohols, it was necessary to obtain authentic samples of 2α-fluoroandrost-4-ene-3β,17β-diol (II, R = β-OH), its 3α-epimer (III, R = β-OH), and 2α-fluoro-3α-hydroxy-4-androsten-17-one (II, R = O). The previous preparation[2] of the diols II and III (R = β-OH), distinguished only by their melting points, had been effected by reduction of 2α-fluorotestosterone with NADH in presence of a mixture of enzymes obtained as the supernatant on disruption of cells of testosterone-induced *Pseudomonas testosteroni*. The present paper describes easy routes to the 2α-fluoro-4-androstene-3,17β-diols (II, R = β-OH, and III, R = β-OH) and reports a selective reduction of 2α-fluoro-4-androstene-3,17-dione (I, R = O) to 2α-fluoro-3α-hydroxy-4-androsten-17-one (III, R = O), an important early product in the aromatization incubations. Corresponding reduction of testosterone is also reported.



Chiral reducing agents of the dihydrosilane–rhodium–(*R,R*)-DIOP and dihydrosilane–rhodium–(*S,S*)-DIOP type converted testosterone to the 3-hydroxy 4-ene system

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Table I. Reductions of the 4-En-3-one Group

substrate	reagent	reactn time	product ratios: II, R = β-OH	III, R = β-OH	III, R = O	% conversion
I, R = β-OH	K-Selectride	1 h	0	100		20
	K-Selectride	5 h	0	100		66
	K-Selectride	72 h	0	100		100
	$LiAl(O\text{-}t\text{-}Bu)_3H$	1 h	90	10		100
	$NaBH_4$	30 min	62	38		90
I, R = O	K-Selectride	84 h	0	0	100	100

substrate	reagent	catalyst	reactn time, h	product ratios: V, R = β-OH	VI, R = β-OH	VII	VIII	IX	% conversion
IV, R = β-OH	K-Selectride		84	88	12				100
	Et_2SiH_2	Rh-(*R*,*R*)-(+)-DIOP	24	36	22	5	3	34	56
	Ph_2SiH_2	Rh-(*R*,*R*)-(+)-DIOP	24	45	39	8	8	0	63
	Ph_3SiH	Rh/(*R*,*R*)-(+)-DIOP	24	0	0	64	36	0	22
	$(EtO)_3SiH$	Rh-(*R*,*R*)-(+)-DIOP	24	0	0	64	36	0	14
	Et_2SiH_2	Rh-(*S*,*S*)-(−)-DIOP	24	23	25	8	8	36	56
	Ph_2SiH_2	Rh-(*S*,*S*)-(−)-DIOP	24	50	32	8	10	0	65
	Ph_3SiH	Rh-(*S*,*S*)-(−)-DIOP	24	0	0	60	40	0	10
	$(EtO)_3SiH$	Rh-(*S*,*S*)-(−)-DIOP	24	0	0	64	36	0	14

with only minor differences in the ratio of 3-epimers. Reduction by monohydrosilanes, however, took a different path and gave saturated 3-alcohols.

Results and Discussion

The preparation of 2α-fluoro-4-androstene-3β,17β-diol (II, R = β-OH) as the major product was readily accomplished by reduction of 2α-fluorotestosterone (I, R = β-OH) with lithium tri-*tert*-butoxyaluminohydride. As was found with the unsubstituted 4-en-3-one group,[3] 1,4-reduction did not occur. Reduction of the 2α-fluorotestosterone (I, R = β-OH) with sodium borohydride gave a mixture of the 3β- and 3α-allylic alcohols II (R = β-OH) and III (R = β-OH) in which the former predominated; they were separated by high-performance liquid chromatography (HPLC).

A more efficient method of preparation of the 2α-fluoro 4-en-3α-ol system III was sought. Methods involving acid epimerization of the 3β-hydroxy 4-ene system,[4] though feasible, are unlikely to give a high yield and are complicated by formation of the 3,5-diene. Although tris(triphenylphosphine)chlororhodium/phosphorous acid/isopropyl alcohol reduction[5] would give initially the desired 3α-allylic alcohol, the reaction conditions are sufficiently acid to cause further equilibration at the 3-position[4] and dehydration.

A report[6] that potassium tri-(*RS*)-*sec*-butylborohydride (K-Selectride, Aldrich) is capable of reducing saturated 3-ketones to the axial alcohol lead us to examine the reduction of 2α-fluorotestosterone (I, R = β-OH) with this reagent. An excellent yield of essentially pure 3α-allylic alcohol III (R = β-OH) was obtained (Table I). Fortunately, the oxidative workup described by Contreras and Mendoza[6] and Fortunato and Ganem[7] was found to be unnecessary. The low temperature used to improve stereoselectivity of reduction of saturated 3-ketones to the axial alcohols[8] was not necessary in reduction of 2α-fluorotestosterone. A parallel reduction of testosterone showed less stereoselectivity and gave predominantly the opposite stereoisomer, 4-androstene-3β,17β-diol (V, R = β-OH). Reduction of testosterone by sodium borohydride with or without added cerium trichloride[9] gave a similar (1:9) ratio of 3α- to 3β-allylic alcohols.

The presence of the 2α-fluoro substituent apparently causes reduction of the 4-en-3-one group to the pseudoaxial alcohol, a behavior more like that of a saturated 3-ketone than of a conjugated enone. A similar effect of 2α-fluoro substitution has been observed in an enzymic transformation.[10]

As judged by both HPLC and by thin-layer chromatography (TLC) the 2α-fluoro-3β-hydroxy-4-androstene grouping (II) is less polar than the corresponding 3α-alcohol (III). This unexpected result is perhaps due to formation of an intramolecular hydrogen bond of 3β-hydroxyl to fluorine, although to a lesser extent the same inverted order of polarity is seen in androst-4-ene-3β,17β-diol (V) and its 3-epimer (VI).[11] There was, therefore, some doubt as to the assignment of configuration at the 3-position. Ringold et al.[2] had assigned the 3α-configuration (III, R = β-OH) to the higher melting (mp 238 °C) 2α-fluoroallylic alcohol, presumably on the basis of its being the minor allylic alcohol product of borohydride reduction of 2α-fluorotestosterone. They did not, however, describe or discuss the relative polarities of the 3-epimers. Their assignment of configuration is supported by our finding that the lower melting (mp 180–181 °C), less polar allylic alcohol is that produced in good yield on reduction of 2α-fluorotestosterone on incubation with NADH and the *S*,*S*-isozyme of horse liver alcohol dehydrogenase, a 3β-hydroxysteroid dehydrogenase. Reduction of testosterone to the allylic alcohol was also effected by this enzyme, although at equilibrium the allylic alcohol is present in much lower quantity than is the case in the 2α-fluoro series. This result corresponds to that obtained by Ringold et al.[10] with a 3α-hydroxysteroid dehydrogenase; the presence of the 2α-fluorine substituent favors formation of the 3-allylic alcohol and relatively destabilizes the conjugated 4-en-3-one.

Further support for the assignment of 3-configurations is given by the nuclear magnetic resonance (NMR) spectra

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of the 2α-fluoro-3-allylic alcohols II and III when compared to the unsubstituted allylic alcohols V and VI. In the absence of 2-fluorine, the vinyl 4-H of the 3α-allylic alcohol VI is coupled (doublet, J = 5.4 Hz) to the 3β-H. In the 3β-allylic alcohol (V), no such coupling of the 4-H to the 3α-H is observed at 80 MHz. In the presence of 2α-fluorine, in addition to the long-range coupling between fluorine and the 4-H there is observed 3-H,4-H coupling in the more polar alcohol, to which we assign the 3α-hydroxy structure III (R = β-OH) and in the corresponding 17-ketone III (R = O) but not in the less polar alcohol II (R = β-OH).

Selective reduction of 2α-fluoro-4-androstene-3,17-dione (I, R = O) at the 3- but not the 17-position with potassium tri-(*R,S*)-*sec*-butylborohydride was readily achieved, giving cleanly 2α-fluoro-3α-hydroxy-4-androsten-17-one (III, R = O).

Since there is still no good route to the synthesis of the unsubstituted 3α-hydroxy-4-androstene system[11] and since a reasonable degree of success had been achieved in controlling the direction of reduction of 17-ketosteroids by use of hydrosilanes with chiral rhodium–DIOP catalysts,[12] we examined their role in the reduction of testosterone. As can be seen from Table I, the rhodium *R,R*- and the *S,S*-chiral DIOP catalysts with two different dihydrosilanes gave mixtures of the 3β- and 3α-allylic alcohols together with some reduction of the double bond; the results show little synthetic utility. The slow reduction of testosterone by monohydrosilanes and the rhodium DIOP catalyst of either chirality, however, occurred entirely at the double bond (Table I), giving a mixture of A/B cis (VII) and A/B trans products (VIII and IX). Similar 1,2-reduction by dihydrosilanes vs 1,4-reduction by monohydrosilanes has been observed with an achiral rhodium catalyst.[13]

Experimental Section

Melting points were measured on a Köfler apparatus and are uncorrected. NMR spectra were determined at 80 MHz with a Brukker WP 80 on solutions in deuteriochloroform with tetramethylsilane as internal standard. Products were separated by HPLC using a Waters 244 liquid chromatograph with a R401 differential refractometer and a Model 450 variable wavelength detector with a Whatman Partisil silica 10/25 L column. The TLC R_f values reported are on silica GF Analtech Inc. Newark, DE, with solvent system ether/toluene (2:1) in which 4-androstene-3,17-dione standard had R_f 0.51 and testosterone 0.37. Mass spectra were recorded with a Varian MAT311A. K-Selectride was purchased from the Aldrich Chemical Co., the (1,4-cyclooctadiene)rhodium(I) chloride and (*R,R*)- and (*S,S*)-DIOP reagents were from Strem Chem. Co., Newburyport, MA. 2α-Fluorotestosterone was a gift from the Cancer Chemotherapy National Service Center, Bethesda, MD.

Borohydride Reduction of 2α-Fluorotestosterone (I, R = β-OH). 2α-Fluorotestosterone (R_f 0.45, 50 mg) in ethanol was treated with excess $NaBH_4$ in ethanol at room temperature for 30 min. Water was added and the product extracted with ethyl acetate and washed thrice with water. Evaporation of the solvent gave a crystalline solid, separated by HPLC to give 2α-fluoro-4-androsten-3β,17β-diol (II, R = β-OH) as the major product, prisms from methanol: mp 173–177 °C (lit.[5] mp 180–181 °C); R_f 0.55; NMR (ppm) 18-H_3 0.76 (s), 19-H_3 1.11 (s), 4-H 5.13 (d, J = 7.3 Hz, coupled to 2α-F), 2β-H 4.54 (d, J = 44 Hz of multiplets), 3α-H 4.17 (m), 17α-H 3.63 (t, J = 8.2 Hz). The minor product, 2α-fluoro-4-androstene-3α,17β-diol (III, R = β-OH), was obtained as needles from methanol: mp 231–235 °C (lit.[5] mp 238 °C); R_f 0.38; NMR (ppm) 18-H_3 0.77 (s), 19-H_3 1.12 (s), 4-H 5.41 (apparent t, J = 6 Hz due to approximately equal coupling to 2α-F and 3β-H), 2β-H 4.73 (d, J = 48 Hz of multiplets), 3β-H 4.28 (m), 17-H 3.64 (t, J = 8.5 Hz).

K-Selectride Reduction, General. The steroid (20 μmol) in dry tetrahydrofuran under argon was stirred at room temperature. K-Selectride (20 μmol of the 0.3 M solution in tetrahydrofuran) was added. After the time noted in the Table I, water was added, and the steroids were isolated by ethyl acetate/water partition and washing with water. The ratio of products was determined by HPLC, using ether–hexane (3:1) (testosterone series) or ether–toluene (2:1) (2α-fluorotestosterone series).

Selective Reduction of 2α-Fluoro-4-androstene-3,17-dione (I, R = O). The steroid (R_f 0.58, 30 mg. 0.10 mmol) in tetrahydrofuran (10 mL) with K-Selectride (0.60 mL, 0.30 mmol) under argon was left for 84 h, then was extracted as before, and crystallized from methanol to give 2α-fluoro-3α-hydroxy-4-androsten-17-one (III, R = O), 23 mg, recrystallized from methylene chloride–hexane and ether–hexane to constant mp 164–166 °C: R_f 0.55; NMR (ppm) 18-H_3 0.891 (s), 19-H_3 1.125 (s), 2β-H 4.77 (d, J = 48 Hz of multiplets), 3β-H 4.33 (m), 4-H 4.45 (apparent t, J = 6 Hz due to approximately equal coupling to 3β-H and 2α-F); mass spectrum, M^+ 306, prominent fragments at m/z 291 (M – CH_3), 288 (M – H_2O), 286 (M – HF), 273 (M – H_2O – CH_3), 271 (M – CH_3 – HF), 268 (M – H_2O – HF), 216, and 150.

Reduction of 2α-Fluorotestosterone (I, R = β-OH) and of Testosterone (IV, R = β-OH) with Horse Liver Alcohol Dehydrogenase *SS*-Isozyme. The enzyme was purified from horse liver cytosol by ammonium sulfate fractionation followed by successive chromatography on 2-(diethylamino)ethyl cellulose and carboxymethyl cellulose.

To the enzyme (1 mg) in 6.0 mL of 0.1 M sodium phosphate buffer pH 7.0 containing NADH (0.72 mg) was added 2α-fluorotestosterone (0.10 mg) or testosterone (0.10 mg) in *tert*-butyl alcohol (100 μL), and the solutions were incubated at 37 °C. Control incubations with the same ingredients but in which the enzyme had been inactivated by heating to 95 °C for 5 min were also carried out. After 22.5 h of incubation the steroid products were extracted with methylene chloride, the organic layer was washed with water and evaporated, and the products were examined. TLC showed the presence only of starting material in the heat inactivated incubations and of both starting material and the 3β-allylic alcohols in the incubations containing active enzyme. The allylic alcohols are distinctive in that they give colors immediately on spraying with ethanol–sulfuric acid (1:1), the 2α-fluoroallylic alcohols II and III (R = OH) being brilliant blue. No 3α-allylic alcohols were formed in these incubations. HPLC (hexane–isopropyl alcohol; 7:3) with refractive index quantitation showed the formation of 2α-fluoro-4-androstene-3β,17β-diol (II, R = β-OH) in 70% yield from 2α-fluorotestosterone and androst-4-ene-3β,17β-diol (V, R = β-OH) in 2% yield from testosterone.

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Registry No. **I** (R = β-OH), 1649-24-7; **I** (R = O), 107655-32-3; **II** (R = β-OH), 969-82-4; **III** (R = β-OH), 1852-54-6; **III** (R = O), 107534-86-1; **IV** (R = β-OH), 58-22-0; **V** (R = β-OH), 1156-92-9; **VI** (R = β-OH), 1852-61-5; **VII**, 571-22-2; **VIII**, 521-18-6; **IX**, 571-20-0.

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