Synthesis and pregnancy-inhibiting activity of 7-substituted androst-5-ene derivatives

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Synthesis of 7-aryl/allyl-substituted androstene derivatives **3a** *through* **3g** *has been carried out by Grignard reaction on 3β,17β-diacetoxyandrost-5-en-7-one* (**2**) *with aryl/allyl magnesium bromide. Isomeric mixture of products* **3b** *and* **3c/3e** *and* **3f/3h** *was separated by column chromatography. Stereochemical assignment at C-7 has been made on the basis of ^{13}C nuclear magnetic resonance studies and chemical considerations. Compounds* **6a** *and* **6b** *were synthesized by alkylation of compound* **5** *with β-(N,N-diethylamino)ethyl chloride hydrochloride and 1-(2-chloroethyl)pyrrolidine hydrochloride, respectively. Compound* **3g** *(isomeric mixture) prevented pregnancy in 60% of rats at 10 mg/kg daily dose administered orally on days 1 to 7 of pregnancy; however, its only isolable 7β-hydroxy isomer,* **3h**, *was inactive at this dose. (Steroids* **56**:477–480, 1991)

Keywords: steroids; androst-5-ene derivatives; 7-substituted androstenes; antifertility agents; contraceptives; fertility inhibition

Introduction

Relatively recently, antifertility activity has been found in C-11–substituted steroids.[1] In this respect, corresponding C-7–substituted compounds, which have some apparent similarity to C-11 products, have been less studied. Therefore, in the present endeavor, which is reported here, a number of 7-substituted androst-5-ene derivatives have been synthesized and screened for anti-implantation activity[2] in rats.

Experimental

All melting points were taken in IEC, Precision melting point apparatus using silicon oil. Infrared (IR) spectra (KBr or neat, ν_{max} cm^{-1}) were recorded on a Perkin-Elmer 157 spectrophotometer. ^1H Nuclear magnetic resonance (NMR) and ^{13}C NMR spectra in $CDCl_3$/DMSO-d_6 were run on a WM 400 MHz NMR spectrometer using trimethylsilane as the internal standard (chemical shifts in δ ppm). Computerized mass spectra were recorded on a JEOL-D300 mass spectrometer. The homogeneity of the compounds was checked by thin-layer chromatography on silica gel-G. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter.

7-Phenylandrost-5-ene-3β,7ξ,17β-triol (3a)

To a stirred solution of phenyl magnesium bromide (0.0125 mol) in dry tetrahydrofuran (THF), compound **2** (1.0 g, 0.0025 mol) dissolved in dry THF was added; the reaction mixture was stirred for 4 to 5 hours. The reaction mixture was decomposed with NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with water and dried over Na_2SO_4, and ethyl acetate was distilled off. The residue obtained was column chromatographed on silica gel to remove biphenyl and give compound **3a** in a 0.79-g yield (71.1%, oil). IR (neat): 3,600, 3,320 (OH), 2,980 (CH_2), 1,600 and 1,500 (C=C) (aromatic); ^1H NMR ($CDCl_3$) (CFT-20): 0.8 (s, 3H, 18-C*H*$_3$), 1.2 (s, 3H, 19-C*H*$_3$), 3.2 to 3.45 (m, 1H, 3-C*H*), 3.70 (t, J = 5 Hz, 1H, 17-C*H*), 5.2 (s, 1H,

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Table 1 Tentative assignment of ^{13}C NMR: chemical shift data of compounds

Carbon No.	3b	3c	5	3e	3f	3h
18-CH_3	11.13	10.89	11.38	10.85	11.11	10.83
19-CH_3	18.73	18.39	18.58	18.56	17.50	18.42
CH_2	20.28	20.53	20.14	20.40	20.40	19.93
CH_2	26.01	25.46	25.66	26.07	26.10	25.64
CH_2	30.13	29.36	29.87	29.94	29.80	36.64
CH_2	31.16	30.32	31.46	31.39	31.37	39.9
C	31.35	31.25	36.65	36.5	35.80	35.10
CH_2	35.31	35.88	36.93	36.15	36.40	36.64
CH_2	37.03	36.93	35.74	37.04	36.60	36.76
CH_2	41.56	41.63	41.85	41.60	41.60	31.02
CH_2	—	—	—	41.96	46.20	—
10-C	42.06	43.32	42.70	42.90	42.80	42.56
CH	44.35	43.09	44.03	42.63	37.80	43.97
CH	44.71	44.4	44.29	43.9	44.70	44.27
OCH_2	69.87	70.11	—	—	—	—
3-CH	71.24	71.10	70.21	70.20	69.38	70.53
7-C	75.68	73.73	75.74	72.20	70.31	76.56
17-CH	80.47	81.42	79.29	79.70	79.80	79.73
CH_2	—	—	—	116.75	116.75	—
6-CH	113.49	114.86	113.65	130.00	129.20	110.83
CH	127.31	126.54	128.21	135.90	135.50	127.82
CH	127.74	127.03	128.47	139.30	142.20	130.25
CH	128.35	127.38	131.00	—	—	130.25
CH	128.50	127.50	—	—	—	—
CH	130.07	127.50	—	—	—	—
C	135.49	137.17	134.5	—	—	131.09
C	136.90	137.17	138.49	—	—	139.24
C	140.32	140.82	—	—	—	—
C	157.41	157.35	155.50	—	—	148.52
N—CH_3	—	—	—	—	—	39.99

6-C*H*), 7.2 to 7.55 (m, 5H, Ar*H*); MS: (M—H_2O) m/z 364. Analysis calculated for $C_{25}H_{34}O_3$: C, 78.53; H, 8.90. Found: C, 78.30; H, 8.50%.

7α-(4-Benzyloxyphenyl)androst-5-ene-3β,7β,17β-triol **(3b),** *7β-(4-benzyloxyphenyl)androst-5-ene-3β,7α,17β-triol* **(3c),** *and 7α-(4-benzyloxyphenyl)-7β,17β-dihydroxyandrost-5-en-3β-yl acetate* **(3d)**

To a refluxing mixture of *p*-benzyloxy bromobenzene (15.64 g, 0.058 mol) and dry magnesium (1.42 g, 0.058 mol) in dry THF (70 ml), two to three drops of dibromoethane were added to initiate the formation of the Grignard reagent. After 1.5 hours, compound **2** (2.5 g, 0.064 mol) in dry THF (50 ml) was added dropwise. Refluxing was continued for 10 hours. The reaction mixture was decomposed with NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated to give an oil that on careful column chromatography over silica gel using hexane/benzene as eluant gave compounds **3b, 3c,** and **3d** in yields of 1.5 g (48%), 0.2 g (6.37%), and 0.41 g (12.03%), respectively. Compound **3b:** mp 97 to 98 C; IR (KBr): 3,580 (OH), 2,990 (CH_2), 1,600 (aromatic); ^1H NMR ($CDCl_3$): 0.88 (s, 3H, 18-C*H*$_3$), 1.02 (s, 3H, 19-C*H*$_3$), 5.3 (s, 3H, OCH_2Ph), 6.6 to 7.12 (m, 9H, Ar*H*); ^{13}C NMR (see Table 1); MS: m/z 488; $[\alpha]_D^{20}$ − 81° (C = 1, MeOH). Compound **3c:** oil, ^{13}C NMR (see Table 1). MS: m/z 488 $[\alpha]_8^{20}$ + 20° (C = 1, MeOH). Analysis calculated for $C_{32}H_{40}O_4C$: C, 78.68; H, 8.19. Found: C, 78.98; H, 8.02%. Compound **3d:** mp 132 to 33 C; IR (KBr): 3,350 (OH), 2,800 (CH_2 stretching), 1,725 ($OCOCH_3$), 1,620 (aromatic); ^1H NMR ($CDCl_3$): 0.8 (s, 3H, 18-C*H*$_3$), 1.2 (s, 3H, 19-C*H*$_3$), 2.05 (s, 3H, OCOC*H*$_3$), 5.15 (s, 2H, $OCH_2C_6H_5$), 5.25 (s, 1H, 6-C*H*), 7.01 (d, J = 9Hz, 2H, Ar*H*), 7.3 to 7.7 (m, 7H, Ar*H*). Analysis calculated for $C_{34}H_{42}O_5$: C, 76.98; H, 7.92. Found: C, 76.95; H, 8.00%.

7α-Allylandrost-5-ene-3β,7β,17β-triol **(3e)** *and 7β-allylandrost-5-ene-3β,7α,17β-triol* **(3f)**

To a stirred solution of allyl magnesium bromide (0.083 mol) in dry THF (20 ml), a solution of compound **2** (1.0 g, 0.0025 mol) was added; the reaction mixture was stirred for 3 to 4 hours. The reaction mixture was then decomposed with water and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 (anhydrous), and concentrated. The residue obtained was column chromatographed over silica gel to give compounds **3e** (0.25 g, 28.03%) and **3f** (0.45 g, 50.46%). Compound **3e:** mp 143 to 46 C; IR (KBr): 3,500 (OH), 2,950 (CH_2); ^1H NMR (DMSO-d_6): 0.58 (s, 3H, 18-C*H*$_3$), 0.9 (s, 3H, 19-C*H*$_3$), 5.03 (d, J_{trans} = 17 Hz, 1H, C*H*$_2$=CH), 5.075 (d, J_{cis} = 10 Hz, 1H, C*H*$_2$=CH), 5.21 (d, J = 2 Hz allylic coupling, 1H, 6-C*H*), 5.6 to 5.7 (m, 1H, CH_2=C*H*—); MS: (M—H_2O)$^+$ m/z 328 $[\alpha]_D^{20}$ − 39° (C = 1, MeOH). Compound **3f:** mp 112 to 115 C; IR (KBr): 3,500 (OH), 2,950 (CH_2); ^1H NMR (DMSO-d_6): 0.56 (s, 3H, 18-C*H*$_3$), 0.76 (s, 3H, 19-C*H*$_3$), 5.0 (d, J = 17 Hz, 1H, C*H*$_2$=CH), 5.075 (d, J_{cis} = 10 Hz, 1H, C*H*$_2$=CH—), 5.21 (d, J = 2 Hz allylic coupling, 1H, 6-C*H*), 5.6 to 5.7 (m, 1H, CH_2=C*H*—); MS: (M—H_2O)$^+$ m/z 328, $[\alpha]_D^{20}$ + 102° (C = 1, MeOH). Analysis calculated for $C_{22}H_{34}O_3$: C, 76.30; H, 9.83. Found: C, 76.60; H, 9.6%.

7α-(4-N,N-Dimethylaminophenyl)androst-5-ene-3β,7β,17β-triol **(3h)**

To a stirred solution of *N,N*-dimethylaminophenyl magnesium bromide (0.025 mol), compound **2** (1.0 g, 0.0025 mol) dissolved in dry THF (20 ml) was added and the mixture was refluxed for 6 to 7 hours. The reaction mixture was then decomposed with NH_4Cl solution and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 (anhydrous), and concentrated. The residue obtained was rapidly chromatographed through a flash column to give a possibly isomeric mixture of products (**3g**) (mass and analysis same as that of the required material) showing closely running spots on silica gel, yield 0.5 g (47%). This mixture was further chromatographed on neutral alumina to give **3h** as a single spot compound (0.3 g, 56.7%): mp 237 C; IR (neat): 3,400 (OH), 3,000 (CH), 1,620 (C=C); ^1H NMR ($CDCl_3$): 0.76 (s, 3H, 18-C*H*$_3$), 1.16 (s, 3H, 19-C*H*$_3$), 2.94 (s, 6H, $N(CH_3)_2$), 5.14 (s, 1H, 6-C*H*), 6.66 (d, J = 8 Hz, 2H, Ar*H*), 7.26 (d, J = 8 Hz, 2H, Ar*H*); MS, (M—H_2O)$^+$ m/z 407, $[\alpha]_D^{20}$ − 159° (C = 1, MeOH). Analysis calculated for

$C_{27}H_{39}NO_3$: C, 76.23; H, 9.15; N, 3.29. Found: C, 75.98; H, 9.15; N, 2.89%.

3β,7β-Dihydroxy-7α-(4-benzyloxyphenyl)androst-5-en-17-one (4)

To a stirred solution of compound **3b** (0.03 g) in acetone ($KMnO_4$ treated, 10 ml), Jones reagent (0.2 ml) was added under nitrogen atmosphere at 0 C. The reaction mixture was stirred for 5 minutes, poured into water (40 ml), and extracted with ethyl acetate. The organic layer was washed with water and dried over Na_2SO_4 (anhydrous) to give compound **4** (0.02 g, 66.6%) as an oil. IR: 3,500 (OH), 3,000 (CH_2), 1,760 (17-CO), 1,720 ($OCOCH_3$); ^1H NMR ($CDCl_3$ + DMSO-d_6): 0.8 (s, 3H, 18C*H*$_3$), 0.96 (s, 3H, 19-C*H*$_3$), 1.9 (s, 3H, OCOC*H*$_3$), 5.03 (s, 2H, OC*H*$_2$), 5.13 (s, 1H, 6-C*H*), 6.4 to 8.0 (m, 9H, Ar*H*); MS: $(M—H_2O)^+$ m/z 510. Analysis calculated for $C_{34}H_{40}O_5$: C, 77.25; H, 7.57. Found: C, 77.20; H, 7.68%.

7α-(4-Hydroxyphenyl)androst-5-ene-3β,7β,17β-triol (5)

A mixture of compound **3b** (0.1 g, 0.0002 mol), Pd-C (10%, 0.1 g), and a drop of triethylamine in methanol (25 ml) was shaken under 50 psi pressure of hydrogen. The catalyst was filtered off through a pad of Hyflo-supercel. Methanol was distilled off, and the residue obtained was crystallized with benzene/hexane to give compound **5** (0.05 g, 62.4%), mp 241 to 242 C: ^1H NMR ($CDCl_3$): 0.65 (s, 3H, 18-C*H*$_3$), 1.1 (s, 3H, 19-C*H*$_3$), 5.15 (s, 1H, 6-C*H*), 6.67 (d, J = 8 Hz, 2H, Ar*H*), 7.3 (d, J = 8 Hz, 2H, Ar*H*); MS: $(M—H_2O)^+$ m/z 380. Analysis calculated for $C_{25}H_{34}O_4$: C, 75.32; H, 8.54. Found: C, 75.50; H, 8.05%.

7α-(4-(2-N,N-diethylaminoethoxy)phenyl)androst-5-ene-3β,7β,17β-triol (6a)

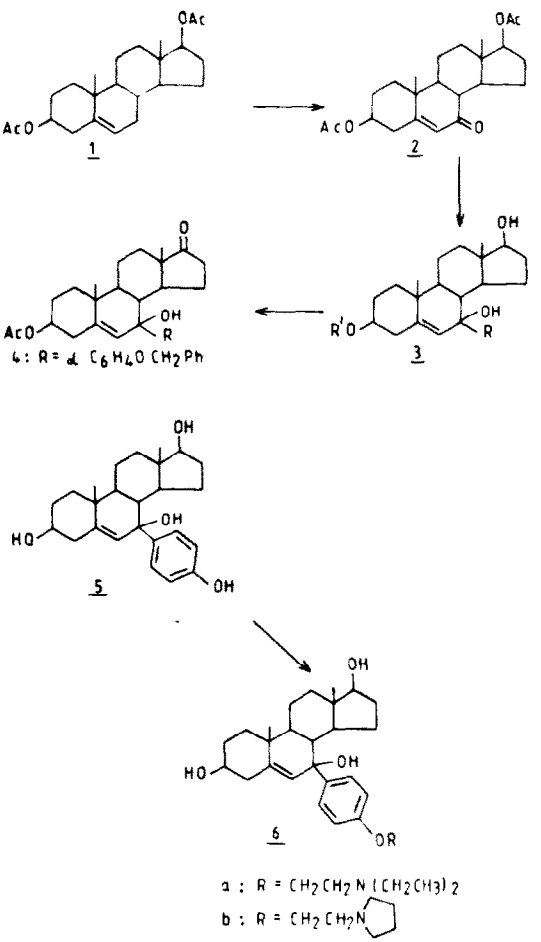
A mixture of compound **5** (0.5 g, 0.0013 mol) anhydrous K_2CO_3 (2.0 g, 0.014 mol) and *N,N*-diethylaminoethyl chloride hydrochloride (0.5 g, 0.0036 mol) in anhydrous acetone (40 ml) was refluxed for 20 hours. The K_2CO_3 was filtered out and acetone was distilled off. The residue was taken up in ethyl acetate and washed with water, then dried over Na_2SO_4 (anhydrous). Ethyl acetate was distilled off to give compound **6a** as an oil (0.35 g, 56.0%): IR (neat): 3,450 (OH), 3,000 (CH_2); ^1H NMR ($CDCl_3$): 0.5 (s, 3H, 18-C*H*$_3$), 0.9 (s, 3H, 19-C*H*$_3$), 3.9 (t, J = 6 Hz, 2H, OC*H*$_2$), 5.0 (s, 1H, 6-C*H*), 6.65 (d, J = 8, Hz, 2H, Ar*H*), 7.2 (d, J = 8 Hz, 2H, Ar*H*); MS: m/z 497. Analysis calculated for $C_{31}H_{47}NO_4$: C, 74.8; H, 9.46; N, 2.83. Found: C, 75.00; H, 9.50; N, 2.93%.

7α-(4-(2-Pyrrolidinoethoxy)phenyl)androst-5-ene-3β,7β,17β-triol (6b)

A mixture of compound **5** (0.6 g, 0.0015 mol), anhydrous K_2CO_3 (1.9 g, 0.0135 mol), 1-(2-chloroethyl)pyrrolidine hydrochloride (1.0 g, 0.006 mol), and dry acetone (100 ml) was refluxed for 10 hours. K_2CO_3 was filtered off, acetone was distilled off, and the residue was diluted with water. The reaction mixture was extracted with ethyl acetate, dried over Na_2SO_4 (anhydrous), and concentrated. The oil thus obtained was filtered through a basic alumina column to give compound **6b** as an oil (0.240 g, 32%): IR (neat): 3,450 (OH), 3,000 (CH_2); ^1H NMR ($CDCl_3$): 0.75 (s, 3H, 18-C*H*$_3$), 1.2 (s, 3H, 19-C*H*$_3$), 3.45 (t, 2H, O—C*H*$_2$), 5.15 (s, 1H, 6-C*H*), 6.75 (d, J = 8 Hz, 2H, Ar*H*); MS: $(M—H_2O)^+$ m/z 477. Analysis calculated for $C_{31}H_{45}O_4N$: C, 75.22; H, 9.09. Found: C, 75.08; H, 8.90%.

Results and discussion

Synthesis of 7-substituted androstene derivatives has been carried out as shown in Scheme 1, starting from 3β,17β-diacetoxyandrost-5-en-7-one (**2**), which in turn was prepared from 3β,17β-diacetoxyandrost-5-ene (**1**) by its oxidation with dipyridine-chromium trioxide



a:	R = C_6H_5(Mixt)-	R^1 = H
b:	R = α-$PhCH_2OC_6H_4$—	R^1 = H
c:	R = β-$PhCH_2OC_6H_4$—	R^1 = H
d:	R = α-$PhCH_2OC_6H_4$—	R^1 = Ac
e:	R = α-CH_2=CH—CH_2—	R^1 = H
f:	R = β-CH_2=CH—CH_2—	R^1 = H
h:	R = α-$(CH_3)_2NC_6H_4$—	R^1 = H

Scheme 1

complex.[3] Grignard reaction of compound **2** with various aryl/allyl magnesium bromides led to the formation of 7α/β-aryl/allylandrost-5-ene 3β,7β/α,17β-triols (**3**). These isomeric mixtures of 7α- and 7β-aryl/allyl products were separated into pure isomeric components by careful column chromatography over silica gel or neutral alumina. However, isomeric separation could not be effected in the case of **3a.** On column chromatography over neutral alumina, isomeric mixture of the *N,N*-dimethyl amino phenyl derivative **3g** gave only the less polar 7β-hydroxy isomer **3h,** while a minor isomer (7α-hydroxy) could not be isolated in the pure form and underwent rapid conversion, possibly to the dehydrated product. Extremely poor yield of these products did not permit their full characterization.

In the Grignard reaction with *p*-benzyloxybromobenzene, partial formation of a monoacetoxy compound **3d** at 3-hydroxy was also observed. The position of the acetyl group was confirmed by its Jones oxidation, which led to the formation of the corresponding 17-ketone compound as confirmed by its IR absorption at 1,750 cm^{-1}.

Debenzylation of compound **3b** to **5** could be effected by its partial hydrogenation in methanol at 40 psi pressure in the presence of 10% Pd-C catalyst. Alkylation of **5** with 2-diethylaminoethyl chloride hydrochloride and 1-(2-chloroethyl)pyrrolidine hydrochloride in a refluxing mixture of anhydrous K_2CO_3 and dry acetone gave corresponding 7α(4-(2-*N,N*-diethyl aminoethoxy)phenyl)-androst-5-ene-3β,7β,17β-triol (**6a**) and 7α-(4-(2-pyrrolidinoethoxy)phenyl)androst-5-ene-3β,7β,17β-triol (**6b**), respectively.

Stereochemical assignment of the C-7 substituent was made on the basis of following considerations.

1. In the Grignard reaction, the approach of the incoming group from the less hindered α side of the molecule in the axial direction and a preferred equatorial disposition of the bulky OMgBr substituent of the intermediate should result in a major isomer having a 7-equatorial (β) hydroxy group.

2. It is known that *trans*-diaxial dehydration is relatively facile. In the case of 7-*p*-benzyloxyphenyl compound **3c,** the minor isomer was found to dehydrate on silica gel column, thus supporting the 7-axial (α) orientation of the hydroxy group.

3. Final support of the stereochemical assignment is on the basis of ^{13}C NMR data of the isomers (see Table 1). A study of the Dreiding model of the 7-allyl isomers **3e** and **3f** suggests that Sp^3 hybridized CH_2 of 7α-allyl would experience two γ-gouche interactions of C-9 and C-14 hydrogen, which would cause appreciable shielding. Accordingly, the isomer that showed upfield shift of 4.3 ppm (46.2 and 41.9 ppm for **3f** and **3e,** respectively) of this CH_2 was assigned 7α-allyl stereochemistry. A shielded CH at 37.8 ppm is likely to be the 8-CH that would experience shielding due to the allyl grouping *cis* to it in the 7β-allyl isomer **3f.** An axial OH is known to shield the carbon atom attached to it. Accordingly, isomers that showed an upfield shift of 2 ppm of the 7-C were assigned 7-axial (α) hydroxy stereochemistry.

Biologic activity

Compounds **3a** to **3c, 3e** to **3h, 5, 6a,** and **6b** were tested[4] for antifertility efficacy in rats. Only compound **3g** was found to prevent pregnancy in 60% of animals at a 10 mg/kg dose in 10 animals when administered orally on days 1 to 7 postcoitus. However, the pure product **3h** (7β-OH isomer) isolated from the isomeric mixture (**3g**) was found to be inactive. Since the other isomer (7α-OH) could not be isolated in the pure form, the reason for the observed activity of fraction **3g** could not be ascertained.

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