BUTTERWORTH HEINEMANN

An approach towards the development of progesterone antagonists: Synthesis of 7α/7β-aryl androstene derivatives

Arvind Singh Negi, Indra Dwivedy, Raja Roy, and Suprabhat Ray

Medicinal Chemistry Division, Central Drug Research Institute, Lucknow, India

*Syntheses of 3β,17β-dihydroxy-7α/7β-(4-hydroxyphenyl)-androst-5-ene 3,17-diacetate (**4** and **5**, R = H) and 3β,17β-dihydroxy-7α/7β-(4-methoxyphenyl)-androst-5-ene 3,17-diacetate (**4** and **5**, R = Me) have been carried out by Friedel-Crafts reaction on 3β,7,17β-trihydroxy-androst-5-ene 3,17-diacetate (**3**, R = H) with phenol and anisole, respectively. Compounds **4** (R = H) and **5** (R = H) have been separated and their stereochemistry assigned on the basis of COSY and NOE experiments.* (*Steroids* **60**:470–472, 1995)

Keywords: progesterone antagonists; 7-aryl androstenes; antifertility; COSY and NOE; Friedel-Crafts reaction

Introduction

In an approach towards the development of progesterone antagonists, a study of appropriately substituted aryl testosterone derivatives at the 7α/7β-position appeared to be of interest. The present study describes the introduction of 7α/7β-(4-hydroxyphenyl) and 7α/7β-(4-methoxyphenyl) groups on to 3β,17β-dihydroxyandrost-5-ene diacetate through Friedel-Crafts reaction on its corresponding 7-ol derivative.

Experimental

All melting points were taken in IEC, precision melting point apparatus using silicon oil. Infrared (IR) spectra (KBr, ν_{max} in cm^{-1}) were recorded on a Perkin-Elmer 157 spectrophotometer. ^1H Nuclear magnetic resonance (NMR), COSY and NOE spectra, in $CDCl_3$, were run on a WM 400 MHz NMR spectrometer using tetramethyl silane 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 (TLC) on silica gel-G. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Microanalysis was estimated on Carlo Ebra Model EA-1108 and Heraeus CHNO rapid instruments.

3β,7,17β-Trihydroxy-androst-5-ene 3,17-diacetate (3, R = H)

To a stirred solution of compound **2** (2.5 g, 0.009 mol) in methanol (15 mL) at 0°C, sodium borohydride (1.0 g, 0.003 mol) was added slowly. The reaction mixture was further stirred for 50 min at 0–6°C and poured onto cold water (100 mL). It was extracted with $CHCl_3$ and the organic layer was washed with water and dried over anhydrous Na_2SO_4 and concentrated to give a solid which was purified on silica gel (washed with 10% $NaHCO_3$ solution) using hexane-ethylacetate as eluant to give compound **3** (R = H) (1.9 g, 75.7%), m.p. 126–128°C (lit.[1] 122–125°C) IR: 3440–3460 (OH), 2930 (CH_2), 1720 ($OCOCH_3$), 1700 ($OCOCH_3$). ^1H NMR: 0.76 (s, 3H, 18 CH_3), 1.00 (s, 3H, 19 CH_3), 2.00 (s, 3H, $OCOCH_3$), 2.02 (s, 3H, $OCOCH_3$), 3.82 (bs, 1H, 7-CH), 4.54–4.70 (m, 2H, 3-CH and 17-CH), 5.36 (s, 1H, 6-CH), MS: m/z 390 (M^+), 372 (M^+-18). Analysis calculated for $C_{23}H_{34}O_5$: C, 70.76; H, 8.72. Found C, 70.82; H, 8.54.

3β,7,17β-Trihydroxyandrost-5-ene 3,17-diacetate, 7-mesylate (3 R = SO_2Me)

To a stirred solution of compound **3** (R = H) (1.2 g, 0.003 mol) in dry pyridine (15 mL) at 0°C, methane sulfonyl chloride (1.5 mL, 0.019 mol) was added dropwise. The reaction mixture was further stirred for 3 h at 0°C and then at room temperature for 4 h. Pyridine was distilled off under high vacuum and the residue was taken up in 200 mL ether, washed well with water, the organic layer dried over anhydrous Na_2SO_4, and the solvent distilled off. The residual crude oil on column chromatography over silica gel using hexane-ethyl acetate as eluant gave the compound (**3**, R = SO_2Me) as an oil. (1.2 g, 83.3%). IR 1350 (S = O), 1720 ($OCOCH_3$), 1730 ($OCOCH_3$), ^1H NMR: 0.76 (s, 3H, 18-CH_3), 0.97 (s, 3H, 19-CH_3), 1.27 (s, 3H, OSO_2CH_3), 2.00 (s, 3H, $OCOCH_3$), 2.02 (s, 3H, $OCOCH_3$), 3.92 (s, 1H, 7-CH), 4.6–4.74 (m, 2H, 3-CH and 17-CH), 5.42 (s, 1H, 6-CH), MS: m/z 468 (M^+). Analysis calculated for $C_{24}H_{36}O_7S$: C, 61.53; H, 7.70. Found: C, 61.91; H, 7.92.

Address reprint requests to Dr. Suprabhat Ray, Assistant Director, Medicinal Chemistry Division, Central Drug Research Institute, Lucknow 226001, India.
Received April 4, 1994; accepted December 7, 1994.

Steroids 60:470–472, 1995

655 Avenue of the Americas, New York, NY 10010

0039-128X/95/$10.00
SSDI 0039-128X(95)00007-D

3β,17β-Dihydroxy-7α/7β-(4-hydroxyphenyl)-androst-5-ene 3,17-diacetate (4 and 5, R = H)

A mixture of compound **3** (R = H) (1.0 g, 0.003 mol) and phenol (1.0 gm, 0.01 mol) was dissolved in 20 mL of dry nitrobenzene-hexane (1:1, v/v) mixture. The reaction mixture was stirred under an inert atmosphere of nitrogen gas, cooled (−20–−30°C), and anhydrous aluminium chloride (5 g, 0.037 mol) was added. After 40 min, when the reaction was complete, dilute HCl was added to decompose the excess of $AlCl_3$. The reaction mixture was extracted with chloroform. 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-ethyl acetate as eluant gave compounds **4** (R = H) and **5** (R = H) in yields of 286 mg (28.0%) and 290 mg (28.3%), respectively.

Compound 4 (R = H)

M.p.: 199–200°C. IR: 3370 (OH), 2920 (CH_2), 1720 ($OCOCH_3$), 1690 ($OCOCH_3$), 1600 (aromatic). ^1H NMR: 0.82 (s, 3H, 18-CH_3), 1.14 (s, 3H, 19-CH_3), 2.02 (s, 3H, -$OCOCH_3$), 2.08 (s, 3H, $OCOCH_3$), 3.20 (dt. 1H, 7-CH, $J_{6,7}$ = 5.5 Hz, $J_{7,8}$ = 5.5 Hz, $J_{7,9}$ = 2.3 Hz), 4.36 (t, 1H, 17-CH, J = 8.2 Hz), 4.72 (quintet, 1H, 3-CH, J = 7.5 Hz), 5.08 (s, 1H, phenolic OH), 5.42 (d, 1H, 6-CH, J = 5.5 Hz), 6.74 (d, 2H, 2 and 6-CH of phenyl ring, J = 8.6 Hz), 7.04 (d, 2H, 3 and 5 CH of phenyl ring, J = 8.6 Hz). Mass: m/z 466 (M^+), $[\alpha]_D$ = −205.2° (c = 1.67, $CHCl_3$). Analysis calculated for $C_{29}H_{38}O_5$ C, 74.68; H, 8.15. Found: C, 74.26, H, 8.23.

Compound 5 (R = H)

M.p.: 125–126°C. IR: 3440–3420 (OH), 2940 (CH_2), 1720 ($OCOCH_3$), 1700 ($OCOCH_3$), 1600, and 1500 (aromatic). ^1H NMR: 0.74 (s, 3H, 18-CH_3), 1.18 (s, 3H, 19-CH_3), 2.02 (s, 3H, $OCOCH_3$), 2.04 (s, 3H, $OCOCH_3$), 2.96 (td, 1H, 7-CH, $J_{6,7}$ = 3.1 Hz, $J_{7,8}$ = 9.4 Hz, $J_{7,9}$ = 2.3 Hz), 4.48 (t, 1H, 17-CH, J = 8.2 Hz) 4.64 (quintet, 1H, 3-CH, J = 7.5 Hz), 4.80 (s, 1H, phenolic OH), 5.10 (bs, 1H, 6-CH), 6.66 (d, 2H, 2 and 6-CH of phenyl ring, J = 8.6 Hz), 6.94 (d, 2H, 3 and 5-CH of phenyl ring, J = 8.6 Hz). Mass: m/z 466 (M^+), $[\alpha]_D$ = +57.5° (c = 1.43, $CHCl_3$). Analysis calculated for $C_{29}H_{38}O_5$ C, 74.68; H, 8.15. Found: C, 74.92; H, 8.66.

Friedel-Crafts reaction with the mesylate (**3**, R = SO_2Me) under above condition gave 31% yield of the mixture of compounds (**4** and **5**, R = H).

3β,17β-Dihydroxy-7α/7β-(4-methoxyphenyl)-androst-5-ene diacetate (4 and 5, R = Me)

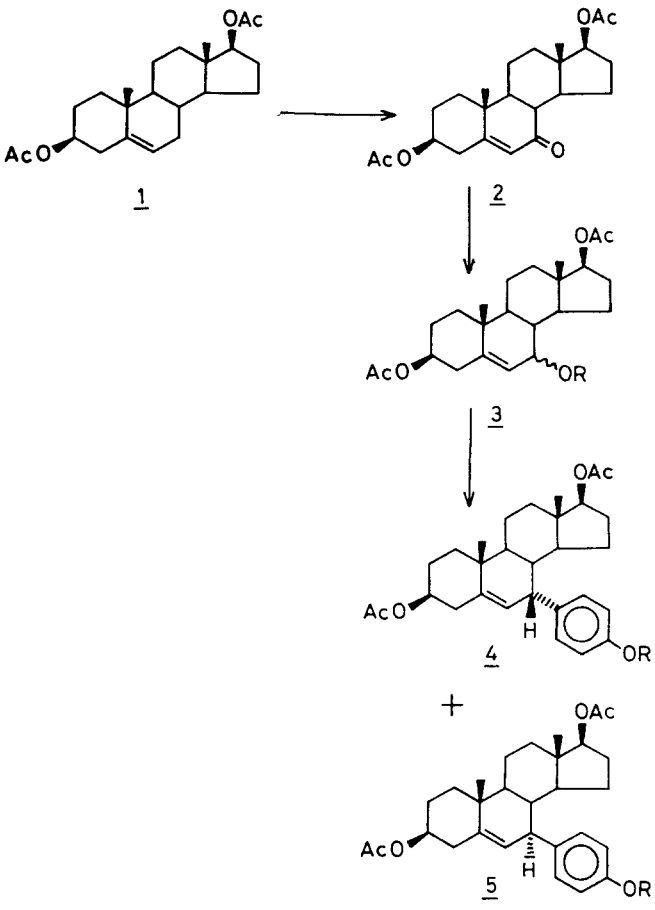
A mixture of compound **3** (R = H) (300 mg, 0.001 mol) and anisole (800 mg, 0.008 mol) was dissolved in 20 mL dry nitrobenzene-hexane (1:1, v/v) mixture. The reaction mixture was stirred under an inert atmosphere of nitrogen gas and cooled (−20–−30°C) and anhydrous $AlCl_3$ (2.4 g, 0.017 mol) was added. After 40 min, when the reaction was complete, excess of $AlCl_3$ was decomposed by adding dilute HCl. The reaction mixture was extracted with chloroform. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated to give an oil. TLC of the crude product on silica gel showed two bands. On column chromatography over silica gel using an increasing proportion of ethylacetate in hexane, the first band obtained appeared to be an inseparable mixture of at least two products which could not be identified. On further elution, the second band obtained was also an inseparable mixture of two products which could be identified as 7α and 7β in a 70:30 ratio on the basis of ^1H NMR. This identity was further proved by the methylation of the compounds **4** (R = H) and **5** (R = H) with diazomethane[2] to compounds **4** (R = Me) and **5** (R = Me), respectively.

Results and discussion

It has been identified that an appropriate substitution at C-11 or C-7 of a progestin leads to progesterone antagonistic activity. A number of 11β-aryl-19-nor-testosterone derivatives have been found to possess potent progesterone antagonistic activity. The promising compounds RU 486, ZK 299, ZK 734 contain a 4-dimethylamino phenyl group as a β-substituent.[3] In 7-substituted compounds, antiprogestational activity has been reported in 7α-methyl substituted compounds.[4] A 7α-methyl substituent was introduced in these testosterone derivatives through copper-catalyzed 1,6-addition of CH_3MgI.[5]

Introduction of a 7-aryl substituent in androstenes[6,7] and in cholesterol series[8] have been recently reported through Grignard reaction on corresponding 7-keto compounds. An attempt to introduce an aryl group through 1,6-addition was unsuccessful.[9] Possibly due to this difficulty in synthesis, to the best of our knowledge, there is no study on appropriately substituted 7α-aryl androstenes as progesterone antagonists.

Synthesis of 7α/7β-androstene derivatives has been carried out as shown in Scheme 1 starting from 3β,17β-dihydroxy-androst-5-ene-7-one diacetate (**2**) which in turn was prepared from 3β,17β-dihydroxy androst-5-ene diacetate (**1**) by its oxidation with 3,5-dimethyl pyrazole-CrO_3 complex.[10] The 7-ketone **2** was reduced with $NaBH_4$ under controlled conditions to give the corresponding 7-ol (**3**, R = H) in which 3- and 17-acetoxy groups were retained. Compound **3** (R = H) was subjected to Friedel-Crafts reaction with phenol in the presence of anhydrous $AlCl_3$ at −20–−30°C in nitrobenzene-hexane to give an isomeric mixture of 3β,17β-dihydroxy-7α/7β-(4-hydroxyphenyl)-androst-5-ene 3,17-diacetate (**4** and **5**, R = H) which could be separated into pure stereoisomers by careful column chromatography over silica gel. The reaction became complicated at higher temperature, possibly due to dehydration of the different hydroxy groups. Friedel-Crafts reaction carried out on the corresponding 7-mesylate derivative (**3**, R = SO_2Me), which in turn was prepared from the 7-hydroxy compound (**3**, R = H) by its treatment with mesylchloride in pyridine, under conditions similar to those described for compounds **4** (R = H) and **5** (R = H), was of no advantage. Yield obtained in this case was rather low (~31%). Compounds **4** (R = H) and **5** (R = H) were screened for antifertility activity in rats following procedures described earlier.[11] Both the compounds were found to be inactive at 10 mg/kg dose. This inactivity of the compounds could be due to the lack of the enone system required for progesterone agonist as well as antagonist activities, and/or to the absence of a basic substituent, as is present in antagonists. Friedel-Crafts reaction of compound **3** (R = H) with anisole under the same experimental conditions as described for compounds **4** (R = H) and **5** (R = H) gave an inseparable mixture of compounds **4** (R = Me) and **5** (R = Me). However, methylation of compounds **4** (R = H) and **5** (R = H) with diazomethane, following standard procedure,[2]



Scheme 1

gave compounds **4** (R = Me) and **5** (R = Me), respectively. ^{1}H NMR of the product mixture (**4** and **5**, R = Me) obtained from anisole was identical to that of individual products **4** (R = Me) and **5** (R = Me), obtained through methylation reaction, merged together.

The stereochemical assignment of the 7-substituent was made on the basis of ^{1}H NMR using COSY and NOE experiments. From the Drieding model of the 7α-phenyl compound **4** (R = H), it could be seen that the dihedral angle (~45°) between 6-H and 7-H is similar to that between 7-H and 8-H, which results in the formation of a triplet pattern. A long-range coupling between 7-H and 9-H, as shown by COSY, results in further splitting of the above triplet into a doublet of a triplet (dt) for the 7-H. However, in the case of 7β-phenyl compound **5** (R = H), significantly different dihedral angles between 6-H and 7-H (J = 3.1 Hz) and 7-H and 8-H (J = 9.4 Hz) and a long-range coupling between 7-H and 9-H (J = 2.3 Hz), results in the formation of a triplet of a doublet (td) for the 7-H, as observed. Drieding model also shows a dihedral angle of ~80° between 6-H and 7-H for the 7β-phenyl compound **5** (R = H), which gives rise to a broad singlet. Whereas for the 7α-phenyl isomer, the observed dihedral angle of ~45° leads to the formation of a doublet (J = 5.5 Hz). These assignments were further confirmed by the NOE difference experiment, in which irradiation of the 7α-H showed no NOE with 8-H, while on irradiation of 7β-H an enhanced peak of 8-H was observed. Thus, compound **4** (R = H) and compound **5** (R = H) have 7β-H and 7α-H stereochemistry, respectively.

Acknowledgment

We are thankful to Dr. Y.K. Hamied, Cipla Ltd., for the generous gift of dehydroepiandrosterone, which was used as starting material.

References

1. Hanson JR, Johnson AW, Kaplan MAC (1978). Abnormal elimination reaction of 3β,17β diacetoxy-5β,6β-epoxy androstan-7β ol. *J Chem Soc Perkin Trans 1* **3**:263–267.
2. In: Practical Organic Chemistry, Vogel (1968). ELBS, London, p. 973.
3. Dwivedy I, Ray S, Grover A (1993). Present status of luteolytic agents in fertility regulation. *Prog Drug Res* **40**:239–267.
4. Ray S, Sharma I (1987). Development of Progesterone antagonists as fertility regulating agents. *Pharmazie* **42**:656–661.
5. Grunwell JF, Benson HD (1976). Antiprogestational agents. The synthesis of 7-alkyl steroidal ketones with antiimplantational and antidecidual activity. *Steroids* **27**:759–771.
6. Li PK, Brueggemeier RW (1990). 7-Substituted 1,4,6-androstatriene-3,17-diones as enzyme-activated irreversible inhibitors of Aromatase. *J Steroid Biochem* **36**:533–539.
7. Grover A, Dwivedy I, Singh AK, Ray S, Singh MM, Kamboj VP (1991). Synthesis and pregnancy-inhibiting activity of 7-substituted androst-5-ene derivatives. *Steroids* **56**:477–480.
8. Bakshi RK, Rasmusson GH (1993). A stereoselective synthesis of 7β-Me cholesterol. *Tetrahedron Lett* **34**:2055–2058.
9. Marshall JA, Heide Roebke (1966). Stereochemical studies on 1,6-additions to 10-methyl-1(9),7-hexal-2-one by Grignard reagents. *J Org Chem* **31**:3109–3113.
10. Takagi H, Komatsu K, Yoshizawa I (1991). Synthesis and mechanism of hydrolysis of estrogen 6-sulphates: Model compounds for demonstrating the carcinogenesis of estrogen. *Steroids* **56**:173–178.
11. Dwivedy I, Singh AK, Singh MM, Ray S (1993). Synthesis of novel 6-aza-B-and 11-aza-C-homoestranes as antifertility agents. *Steroids* **58**:69–73.