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Expeditious synthetic route to B-ring functionalised 2-oxa-steroids: Synthesis of 17-ethylenedioxy-6α-hydroxy-2-oxa-4-androsten-3-one as key synthon

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The easily prepared 17-ethylenedioxy-4-androsten-3,6-dione **8** is transformed to 17-ethylenedioxy-6α-hydroxy-2-oxa-4-androsten-3-one **15** in a few steps with complete stereo- and regio-control. The hydroxylactone **15** provides an easy entry to B-ring functionalised 2-oxa-androgens for evaluation as potential aromatase inhibitors and anabolic agents.

Aromatase is a cytochrome P450 dependent enzyme that catalyses the aromatisation of androgens to estrogens, and hence plays a key role in endocrine physiology and estrogen-dependent diseases[1]. Since estrogens play a significant role in the growth and maintenance of mammary tumours, the inhibition of estrogen levels in the body by aromatase inhibitors is one of the major approaches in the treatment of breast cancer. The synthesis and biochemical evaluation of androst-4-en-3-one **1** derivatives as aromatase inhibitors has provided new leads for breast cancer agents[2]. The substitution of an oxygen atom for the methylene group at C2 will produce steroid hormones in which the A-ring is an α,β-unsaturated δ-lactone such that the position of C3-carbonyl and Δ^4-alkene are unaltered. The synthesis and biological assays of 2-oxasteroid **2** and its derivatives (Figure 1) has yielded active candidates in the category of steroid hormones and drugs [3]. It is well known that the presence of C3-ketone is essential for binding to the receptor site in the parent steroids as well as their lactone analogs. The immense therapeutic application of steroids in the treatment of cancers and tumours as well as their beneficial role in hormone regulation and as anabolic agents is curtailed by the fact that their administration is usually accompanied with adverse and unwanted side effects. For example, use of anabolic male hormone testosterone or its derivatives for muscle development in female patients leads to the appearance of secondary male

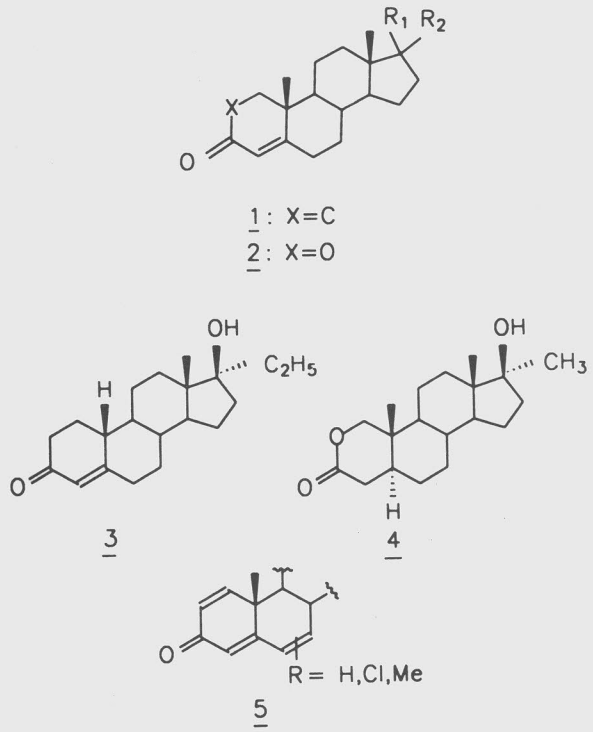


Figure 1—Structures of steroids **1-5**

sex characteristics as a serious side effect. The research from Searle almost 30 years ago demonstrated that separation of activity in these hormones is indeed possible. The introduction of steroids Nilevar® (17α-ethyl-19-nortestosterone **3**)[4] and Anavar® (17β-hydroxy-17-methyl-2-oxa-5α-androstan-3-one **4**)[5] in 1960s provided valuable anabolic agents with negligible androgenic side effects. Subsequently, a large number of research groups focussed on ways to

synthesise the pharmaceutically important 2-oxasteroids from their natural precursors, the most recent contribution being the photosensitised oxidation approach of Frimer and coworkers[6].

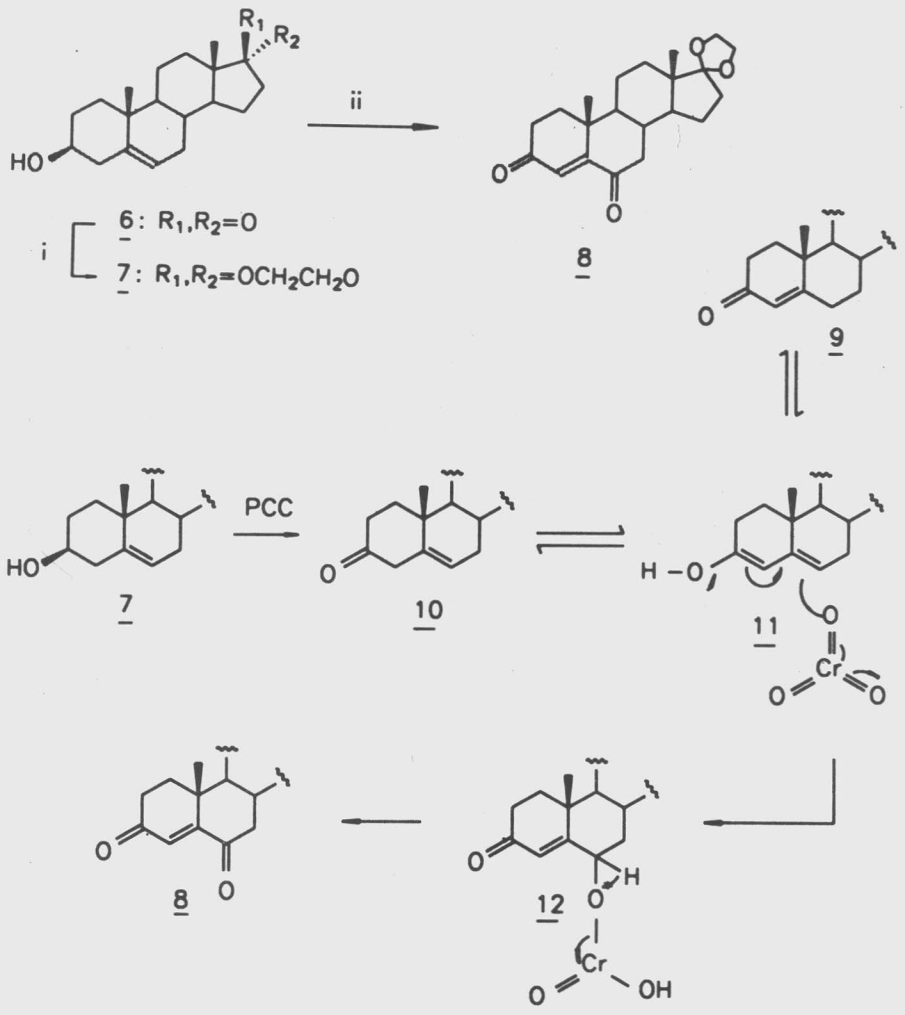
The synthesis of B-ring functionalised 2-oxasteroids proceeds from 3-keto-1,4,6-triene steroids **5** wherein the Δ^1-alkene is oxidatively cleaved and the Δ^6-alkene is utilised for derivatisation.[7,8] This general protocol, which has been widely used, suffers from the following drawbacks: (i) The requisite triene **5** (R=H, Cl or Me) has to be prepared in few steps from the 5-en-3-ol steroid. (ii) Regioselective chemical manipulation of the triene is somewhat difficult and at times mixtures of products are obtained[7]. (iii) The large number of steps and mandatory purifications severely limit the overall efficiency of the protocol. We reasoned that a convenient and flexible method for the synthesis of 2-oxasteroid B-ring derivatives will greatly facilitate the testing of these compounds for further improvement in biological activity. In this background, we report the synthesis of C6 and C7-functionalised 2-oxa-4-androsten-3-one molecules as potential aromatase inhibitors and anabolic agents.

Resuts and Discussion

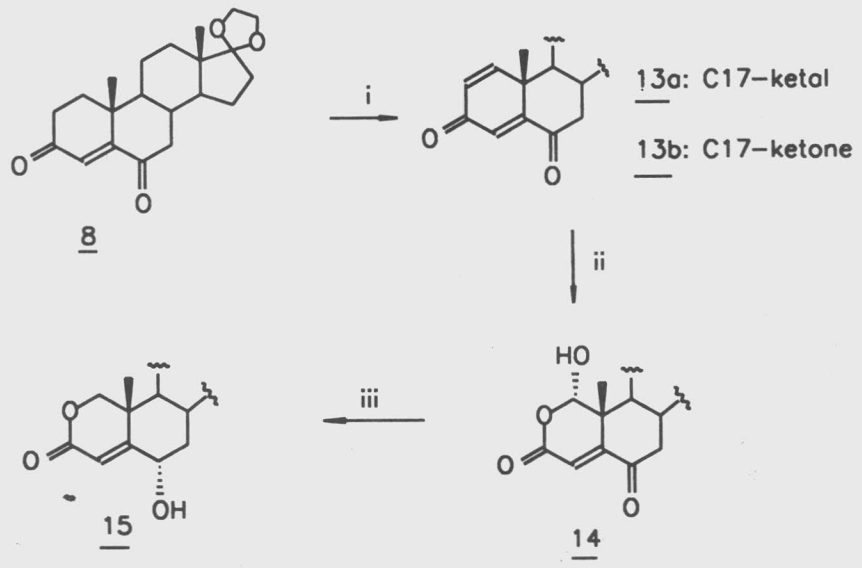
Dehydroisoandrosterone **6** was protected as its C17 dioxolane **7** in a quantitative yield under standard ketalisation conditions. Oxidation of the homoallylic alcohol **7** with PCC afforded the unexpected Δ^4-3,6-dione **8** in which the acid sensitive C17-ketal group was still intact. The generality of this novel transformation of steroidal Δ^5-3-ols **5** to the biologically active Δ^4-3,6-diones **8** under the aegies of mild and versatile PCC oxidant was explored with a few representative substrates.[9] The direct formation of enedione **8** instead of the expected Δ^4-enone **9** is rationalised in Scheme I. Thus, the enol form **11** of Δ^5-enone **10** reacts at C6 vinylogously with Cr=O of PCC reagent to provide the chromate ester **12**. Further oxidation of C6 hydroxy group smoothly provides the Δ^4-3,6-dione **8**.

Adaptation of Frimer's conditions for the photosensitised oxidation of enedione **8** (*t*-BuOK, O_2) led to a mixture of products because of competing enolisation at C2 and C7. Dehydrogenation of 17-ethylenedioxy-4-androsten-3,6-dione **8** with DDQ[8,10] proceeded smoothly and afforded a single regioisomeric product **13** in which the C1-C2 alkene was exclusively formed; no C7-C8 olefin isomer was detected. This was concluded from the ^{1}H NMR spectrum of the crude residue which displayed three coupled olefinic resonances arising from C2, C4 and C1 vinyl protons at δ 6.28, 6.34 and 7.08 (**13a**) and at δ 6.30, 6.40 and 7.08 (**13b**). Formation of Δ^7-olefin would show a singlet in the vinyl proton region which was clearly absent. The regioselectivity in $\Delta^{1,4}$-3,6-dione **13** formation is a consequence of the facile enolisation at C2 and its less crowded steric environment compared to C7. Because of the long reaction period (72 hr) and high temperature (100° C) the product contained C17 ketal **13a** (45%) and the deprotected ketone **13b** (45%), which were easily separated by column chromatography. The concomitant hydrolysis of C17-dioxolane to triketone **13b** was a setback but on the positive side opens the possibility of utilising the 2-oxa congeners as aromatase inhibitors where C17-protection may not be mandatory.

Further elaboration of A-ring dienone to the 2-oxa skeleton was achieved by the selective ozonolysis of the more reactive C1-C2 alkene over C4-C5. When a stream of O_3 was bubbled through a 0.03 *M* solution of dienone **13a** in 1:1 pyridine-CH_2Cl_2 for 15-20 min. at –78° C, the only isolable product after work-up was the desired lactol **14**, produced essentially as a single stereoisomer based on the acetal C1-H singlet at δ 5.60 and its ^{13}C NMR spectrum which displayed signals for 20 carbons[6,8]. Routine $NaBH_4$ reduction of the keto lactol **14** afforded the hydroxy lactone **15** (Scheme II) in 80% yield after purification. Reduction of C6 ketone takes place entirely from the exposed β-face to produce the equatorial alcohol exclusively.[11] In lactone **15** the *AB* CH_2O pattern is centred at δ 4.10 (*J*=12 Hz) and the axial C6-H appears as a multiplet at δ 4.30. The weak coupling[12] of C6β-H to vinylic C4-H at δ 6.12 (*J*=2 Hz) confirms the equatorial orientation of the secondary alcoholic group in **15**. Thus, 17-ethylenedioxy-2-oxa-6α-hydroxy-4-andro-sten-3-one **15** is produced with complete regio- and stereo-control from the easily prepared $\Delta^{1,4}$-3,6-



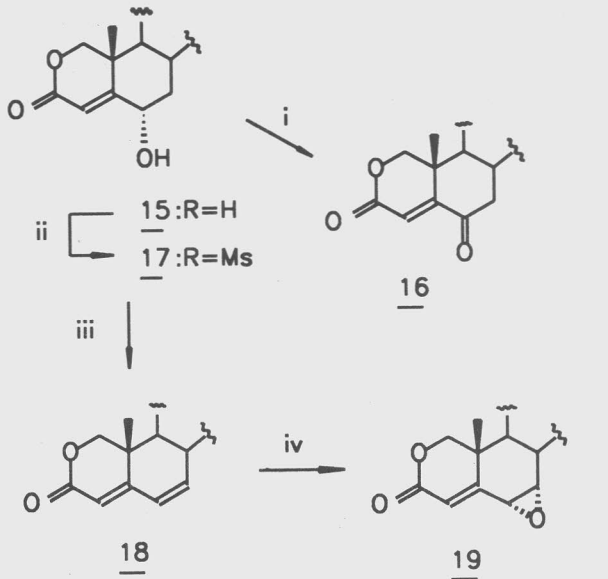
Scheme I–(i) $(CH_2OH)_2$, TsOH, PhMe, reflux 5 hr, 98%; (ii) PCC, CH_2Cl_2, rt, 24 hr, 70%



Scheme II – (i) DDQ, dioxane, reflux, 72 hr, 45%; (ii) O_3, –78° C, 1:1 pyridine/CH_2Cl_2, 15 min., 77%; (iii) $NaBH_4$, 5:1 $CHCl_3/H_2O$, 82%

dione **13a**. The hydroxy lactone **15** lends itself as a key synthon for the synthesis of B-ring functionalised heterosteroids, and this was explored next.

Exposure of secondary alcohol **15** to PCC in CH_2Cl_2 produced 2-oxa-4-androsten-3,6-dione **16** uneventfully. Conversion of **15** to its mesylate **17** (MsCl) and elimination (DBU) to C6-C7 olefin was sluggish because of the equatorial orientation of the hydroxy group. Displacement of the mesylate group with LiBr and *in situ* dehydrobromination proceeded smoothly with Li_2CO_3 in hot DMF to produce the conjugate lactone **18** (Scheme III). Attempts to isolate the intermediate axial bromide were unsuccessful since it underwent spontaneous *anti* elimination. Efforts at inverting the mesylate **17** to obtain the epimeric 6β-alcohol were also in vain. Under basic conditions elimination-type products were produced whereas the neutral protocol of Mitsunobu hydroxy inversion[13] afforded the starting alcohol; no S_N2 displacement-type products were observed. At this juncture we decided to exploit the C6-C7 alkene **18** for further functionalisation of B-ring. Treatment of conjugated lactone **18** with *m*-CPBA (4 equi) in $CHCl_3$ at ambient temperature for 24-36 hr furnished the expected C6-C7α-epoxide **19** (δ 3.30, 3.38) as a single stereoisomer[8]. The reaction was stopped at 70-80% conversion because longer exposure (48 hr) led to bis-epoxidation products. Epoxide **19** marks another crucial synthon in this series. Alcohol **15** and epoxide **19** are versatile precursors for the synthesis of B-ring fluorinated, aminated, and unsaturated 2-oxasteroids as potential antiandrogenic and anabolic agents.



Scheme III– (i) PCC, CH_2Cl_2, rt, 80%; (ii) MsCl, Et_3N, CH_2Cl_2, −5°C, 30 min, 87%; (iii) LiBr, Li_2CO_3, DMF, 100°C, 90%; (iv) *m*-CPBA, $CHCl_3$, rt, 24-36 hr, 70-80%

Conclusions

Our synthetic route to B-ring derivatives of 2-oxasteroids is quite efficient, general in nature, and should be serviceable for the synthesis of diverse analogs. It exploits the C6-ketone as the precursor for functional group manipulation, which is superior and versatile compared to the earlier Δ^6-alkene. The C17-ethylenedioxy protecting group survives most of the chemicals transformations, except the DDQ oxidation. When desired, smooth deketalisation can be effected with 10% aqueous H_2SO_4 in acetone. The screening of these steroidal lactones should reveal correlation of C6- and C7-substitution with biological activity.

Experimental Section

General. IR spectra were recorded on a Jasco 5300 spectrometer. ^1H and ^{13}C NMR (PMR and CMR) spectra were recorded on a Bruker ACF 200 instrument. Elemental analysis was performed on a Perkin-Elmer 240C instrument. LRMS was recorded on a Micromass VG70/70H instrument at IICT, Hyderabad. Ozonolysis was carried out on Welsbach model. SGC refers to silica gel chromatography. Work-up means drying of organic extracts with $MgSO_4$, solvent removal on a rotary evaporator and concentration *in vaccuo*. All reactions were carried out using standard syringe-septum techniques in inert nitrogen atmosphere with magnetic stirring. All reagents and solvents were dried and distilled[14] before use.

17-(Ethylenedioxy)-3β- hydroxy-Δ^5-androstene 7. Dehydroisoandrosterone (2.8 g, 10 mmoles), ethylene glycol (31.6 g, 28 mL, 500 mmoles) and *p*-TsOH (190 mg, 1 mmole) were taken in dry toluene (200 mL) and refluxed for 5 hr with a Dean-Stark apparatus to remove water formed during the reaction. The cooled reaction mixture was immediately washed with saturated $NaHCO_3$ solution and worked-up to afford 3.3 g of pure ketal **7** in 98% yield; IR(KBr): 3472, 2918,

1462 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (3H, s), 1.01 (3H, s), 3.44-3.60 (1H, m), 3.80-3.98 (4H, m), 5.34 (1H, d, *J*=6 Hz); ^{13}C NMR ($CDCl_3$): δ 14.17, 19.39, 20.48, 22.76, 30.58, 31.23, 31.54, 32.17, 34.17, 36.53, 37.28, 42.22, 45.72, 49.98, 50.59, 64.49, 65.11, 71.49, 119.50, 121.30, 140.81.

17-(Ethylenedioxy)-Δ^4-3,6-androstenedione 8. To a suspension of PCC (3.2 g, 15 mmoles) in dry DCM (20 mL) at room temperature was added the ketal-alcohol **7** (1.0 g, 3 mmoles) and the reaction mixture stirred for 24 hr at ambient temperature. The reaction mixture was diluted with ether (50 mL) and filtered through a pad of Celite. Evaporation of the solvent and filtration through silica gel column gave 750 mg (70%) of the diketone **8;** IR(KBr): 3053, 2947, 1684, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, s), 1.15 (3H, s), 2.70 (1H, dd, *J*=12, 3 Hz), 3.80-3.98 (4H, m), 6.14 (1H, s); ^{13}C NMR ($CDCl_3$): δ 13.95, 17.30, 20.11, 22.65, 29.69, 33.69, 34.17, 35.28, 39.50, 45.56, 45.75, 50.19, 50.52, 64.33, 65.02, 118.46, 125.22, 160.54, 198.89, 201.44; Anal. Found: C, 73.20; H, 8.29. Calcd for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19%.

17-(Ethylenedioxy)-$\Delta^{1,4}$-3,6-androstenedione 13a. The enedione **8** (600 mg, 1.7 mmoles) and DDQ (1.5 g, 6.8 mmoles) were taken in dry dioxane (40 mL) and refluxed for 72 hr in inert nitrogen atmosphere. After cooling to room temperature, the precipitated by-product was filtered and the filtrate concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL), washed with 2*N* NaOH (2x10 mL), and worked-up. SGC afforded 200 mg (45%) of the desired dienone **13a** along with the deprotected product **13b** (200 mg, 45%).

Compound 13a: IR(KBr): 2943, 2881, 1699, 1657, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 (3H, s), 1.19 (3H, s), 2.70 (1H, dd, *J*=12, 2 Hz), 3.78-3.94 (4H, m), 6.28 (1H, dd, *J*=9, 2 Hz), 6.34 (1H, d, *J*=2 Hz), 7.08 (1H, d, *J*=9 Hz); ^{13}C NMR ($CDCl_3$): δ 14.24, 18.98, 22.27, 22.42, 29.86, 33.83, 34.64, 44.78, 45.93, 46.18, 48.90, 50.06, 64.54, 65.25, 118.51, 124.88, 128.02, 153.51, 161.41, 185.00, 210,50; Anal. Found: C, 73.41; H, 7.58. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65%.

Compound **13b:** IR(KBr): 2957, 1736, 1660, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.98 (3H, s), 1.26 (3H, s), 2.84 (1H, dd, *J*=12, 2 Hz), 6.30 (1H, dd, *J*=9, 2 Hz), 6.40 (1H, d, *J*=2 Hz), 7.08 (1H, d, *J*=9 Hz); ^{13}C NMR ($CDCl_3$): δ 13.77, 19.06, 21.66, 22.11, 30.90, 33.96, 35.45, 44.60, 45.51, 47.69, 48.96, 51.07, 125.27, 128.28, 153.11, 160.90, 185.13, 200.91, 218.75.

17-(Ethylenedioxy)-1-hydroxy-2-oxa-Δ^4-3, 6-androstenedione 14. The dienone **13a** (60 mg, 0.18 mmole) was dissolved in a 1:1 mixture of DCM/pyridine (4 mL). Ozone was passed through the solution at – 78° C for 20 min. After flushing the excess O_3, the reaction mixture was stirred for 15 min. at room temperature and then diluted with 15 mL $CHCl_3$, washed with 1*M* HCl (3x10 mL) and immediately with satd $NaHCO_3$ (2x10 mL). Work-up and SGC purification afforded 50 mg (77%) of the ketolactol **14**; IR(KBr) : 3375, 3059, 2945, 1732, 1460 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, s), 1.22 (3H, s), 2.74 (1H, dd, *J*=12, 3 Hz), 3.98-3.80 (4H, m), 5.60 (1H, s), 6.45 (1H, s); ^{13}C NMR ($CDCl_3$): δ 14.08, 18.42, 20.09, 22.48, 29.68, 33. 14, 33.78, 42.86 (2C), 45.27, 45.63, 50.20, 64.53, 65.25, 100.69, 118.87(2C), 153.61, 163.31, 198.37.

17-(Ethylenedioxy) -2-oxa-6α-hydroxy-Δ^4-3-androstenone 15. The lactol **14** (50 mg, 0.13 mmole) was dissolved in $CHCl_3$ (10 mL) and $NaBH_4$ (52 mg, 1.3 mmoles) was added. To this was added 2 mL of water followed by 0.2 mL of 1*M* NaOH. The reaction mixture was stirred for 4 hr at room temperature. Dilution with 10 mL $CHCl_3$, work-up and SGC purification furnished 37 mg (82%) of the hydroxy lactone **15**; IR(KBr): 3489, 3057, 2939, 1709 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, s), 1.22 (3H, s), 3.78-3.98 (4H, m), 4.03 (1H, d, *J*=12 Hz), 4.22 (1H, d, *J*=12 Hz), 4.25-4.36 (1H, m), 6.12 (1H, d, *J*=2 Hz); ^{13}C NMR ($CDCl_3$): δ 14.14, 17.28, 21.07, 22.66, 29.98, 33.87, 34.02, 38.39, 39.93, 45.57, 49.25(2C), 64.53, 65.23, 67.59, 77.17, 110.89, 118.89, 164.94, 168.60; Anal. Found: C, 69.23; H, 7.62. Calcd for $C_{20}H_{28}O_5$: C, 68.93; H, 8.10%.

17-(Ethylenedioxy)-2-oxa-Δ^4-3, 6-androstenedione 16. 6-Hydroxylactone **15** (5 mg, 0.014 mmole) was added to a suspension of PCC (20 mg, 0.084 mmole) in 1 mL dry DCM and the mixture stirred for 2 hr at room temperature. Dilution with 5 mL ether, filtration through a pad of Celite, work-up and SGC yielded 4 mg of the pure compound **16**; IR($CHCl_3$): 3059, 2949, 1728, 1705 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (3H, s), 1.24 (3H,

s), 2.75 (1H, dd, *J*=12, 2 Hz), 3.78-3.98 (4H, m), 4.15 (1H, d, *J*=10 Hz), 4.37 (1H, d, *J*=10 Hz), 6.37 (1H, s).

17-(Ethylenedioxy) -2-oxa-Δ^4-6α-mesyloxy-androsten-3-one 17. The hydroxy lactone **15** (15 mg, 0.043 mmole) was taken in 1 mL dry DCM. Et_3N (65.3 mg, 90 μL, 0.6 mmole), followed by methanesulfonyl chloride (50 mg, 34 μL, 0.43 mmole) were added at –5° C and the mixture was stirred for 30 min. The solvent was evaporated and the residue dissolved in 5 mL EtOAc. The organic layer was washed with ice-cold 1*M* HCl (10 mL), then with satd $NaHCO_3$, and worked-up to provide 15 mg (87%) of mesylate **17**; IR($CHCl_3$): 2947, 2891, 1761 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, s), 1.26 (3H, s), 2.44-2.30 (1H, m), 3.12 (3H, s), 3.80-3.98 (4H, m), 4.04 (1H, d, *J*=12 Hz), 4.27 (1H, d, *J*=12 Hz), 5.18-5.30 (1H, m), 6.04 (1H, d, *J*=2 Hz).

17- (Ethylenedioxy) -2-oxa-$\Delta^{4,6}$-androsten-3-one 18. To a solution of mesylate **17** (13 mg, 0.03 mmole) in dry DMF (1 mL), LiBr (26 mg, 0.3 mmole) and Li_2CO_3 (22 mg, 0.3 mmole) were added, and heated at 100° C for 1.5 hr. The cooled reaction mixture was diluted with 10 mL water and extracted with EtOAc (3x10 mL). Work-up and SGC purification gave 4 mg (90%) pure compound **18**; IR($CHCl_3$): 2928, 2878, 1722, 1624 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.93 (3H, s), 1.18 (3H, s), 3.80-4.00 (4H, m), 4.05 (1H, d, *J*=12 Hz), 4.27 (1H, d, *J*=12 Hz), 5.63 (1H, s), 6.16 (2H, s); MS: m/z 330 (M^+), 268, 99, 86.

17-(Ethylenedioxy)-2-oxa-6, 7α-epoxy-Δ^4-androsten-3-one 19. To the diene **18** (5 mg, 0.015 mmole) in dry $CHCl_3$ (1 mL), *m*-chloroperbenzoic acid (10 mg, 0.06 mmole) was added and the mixture stirred for 24 hr at room temperature. The reaction mixture was diluted with 10 mL of $CHCl_3$ and washed with satd sodium bicarbonate solution and brine, dried and evaporated to give **19** ; IR($CHCl_3$): 2926, 2826, 1734 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (3H, s), 1.09 (3H, s), 3.30 (1H, d, *J*=4 Hz), 3.38 (1H, d, *J*=4 Hz), 3.74-3.90 (4H, m), 3.98 (1H, d, *J*=12 Hz), 4.02 (1H, d, *J*=12 Hz), 6.06 (1H, s).

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References

1 Mansuy D, Battioni P & Battioni J P, *Eur J Biochem*, 184, **1989**, 267.

2 (a) Numazawa M & Oshibe M, *J Med Chem*, 37, **1994**, 1312

(b) Numazawa M & Tachibana M, *Steroids*, 59, **1994**, 579.

(c) Darby M V, Lovett J A, Brueggemeier R W, Groziak M P & Counsell R E, *J Med Chem*, 28, **1985**, 803.

3 Zurer P S, *Chem Eng News*, April 30, **1984**, 69.

4 Colton F B, Nysted L N, Riegel B & Raymond A L, *J Am Chem Soc*, 79, **1957**, 1123.

5 (a) Pappo R & Jung C J, *Tetrahedron Lett*, **1962**, 365.

(b) Pappo R, *Inter-Science Chem Rept*, 3, **1969**, 105.

6 (a) Frimer A A, Hameiri-Buch J, Ripshtos S & Gilinsky-Sharon P, *Tetrahedron*, 42, **1986**, 5693.

(b) Frimer A A, Ripstos S, Marks V, Aljadeff G, Hameiri-Buch J & Gilinsky-Sharon P, *Tetrahedron*, 47, **1991**, 8361.

7 Kocor M, Kurek A & Dabrowski J, *Tetrahedron*, 25, **1969**, 4257.

8 shibata K, Takegawa S, Koizumi N, Yamakoshi N & Shimazawa E, *Chem Pharm Bull*, 40, **1992**, 835..

9 Nangia A & Anthony A, *Synth Commun*, 26, **1996**, 225.

10 Kirk D N & Rajagopalan M S, *J Chem Soc Perkin Trans-1*, **1975**, 1860.

11 You Z & Koreeda M, *Tetrahedron Lett*, 34, **1993**, 2745.

12 Jackman L M & Sternhell S, *Applications of nuclear magnetic resonance spectroscopy in organic chemistry*, 2nd Edn (Pergamon Press, Oxford)1972, p. 322.

13 Mitsunobu O, *Synthesis*, **1981**, 1.

14 Perrin D D, Amarego W L F & Perrin D D, in: *Purification of laboratory chemicals*, 2nd Edn (Pergamon Press, Oxford) 1986.