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A convenient synthesis of the side chain of loteprednol etabonate—An ocular soft corticosteroid from 20-oxopregnanes using metal-mediated halogenation as a key reaction

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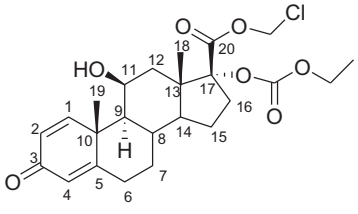
ABSTRACT

A facile synthesis of the side chain of loteprednol etabonate, namely, chloromethyl-17α-[(ethoxycarbonyl))oxy]-11β-hydro of loteprednol etabonate, viz., chloromethyl-17α-[(ethoxycarbonyl))oxy]-11xy-3-oxoandrosta-1,4-diene-17β-carboxylate – an ocular soft corticosteroid, has been described starting from a 20-oxopregnane, namely, 3β-acetoxy-pregn-5(6),16(17)-diene-20-one (16-dehydropregnenolone acetate, i.e., 16-DPA) using our recently developed metal-mediated halogenation as a key reaction.

Corticosteroids have demonstrated excellent anti-inflammatory activity in clinical practice. However, their therapeutic effects are often accompanied by toxicity [1]. Hence, the search for improved topical corticosteroids with less toxicity has been a major goal in pharmaceutical research and development in recent years. Bodor and his coworkers [2–4] introduced the concept of 'soft drug' by means of designing pharmaceutical agents of reduced toxicity with structural modification to achieve a satisfactory therapeutic index [5–10]. 'Loteprednol etabonate', namely, chloromethyl 17α-[(ethoxycarbonyl))oxy]-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylate **1**, which has recently been marketed as an ocular anti-inflammatory agent, is a soft corticosteroid developed on the concept of Bodor and his group [5–7]. The synthesis of a soft drug is achieved by starting with a known inactive metabolite of a known drug. The inactive metabolite is then modified to an active form that after having achieved its therapeutic role, undergoes a predictable and controllable one- or two-step transformation in vivo back to the inactive metabolite via known process of enzymatic deactivation [11,12]. Loteprednol etabonate **1** is one of the first-generation cortienic acid-based soft corticosteroids to get approved by Food and Drug Administration (FDA) for use in all inflammatory and allergy-related ophthalmic disorders [4].

In loteprednol etabonate, a metabolically labile ester function occupies a 17β-position, while a stable carbonate group occupies the 17α-position. The ester is hydrolysed to an inactive carboxylic acid, Δ^1-cortienic acid etabonate, and then into Δ^1-cortienic acid in biological systems [9–14]. As a result of the predictable conversion of loteprednol etabonate into an inactive metabolite in the eye following topical administration, this corticosteroid has a low propensity for undesirable toxicity while possessing increased anti-inflammatory activity. In fact, this drug has been found to be 1.5 times more potent than the parent anti-inflammatory agent dexamethasone. Clinical trials on these drugs are also going on for a safer treatment of gastrointestinal inflammation [13].

In continuation of our work on steroid transformations [15–21], we recently introduced a potential method of metal-mediated halogenation of 20-oxopregnane, namely, 16-DPA and its relatives using the system MnO_2–TMSCl/AcCl–AcOH giving an almost



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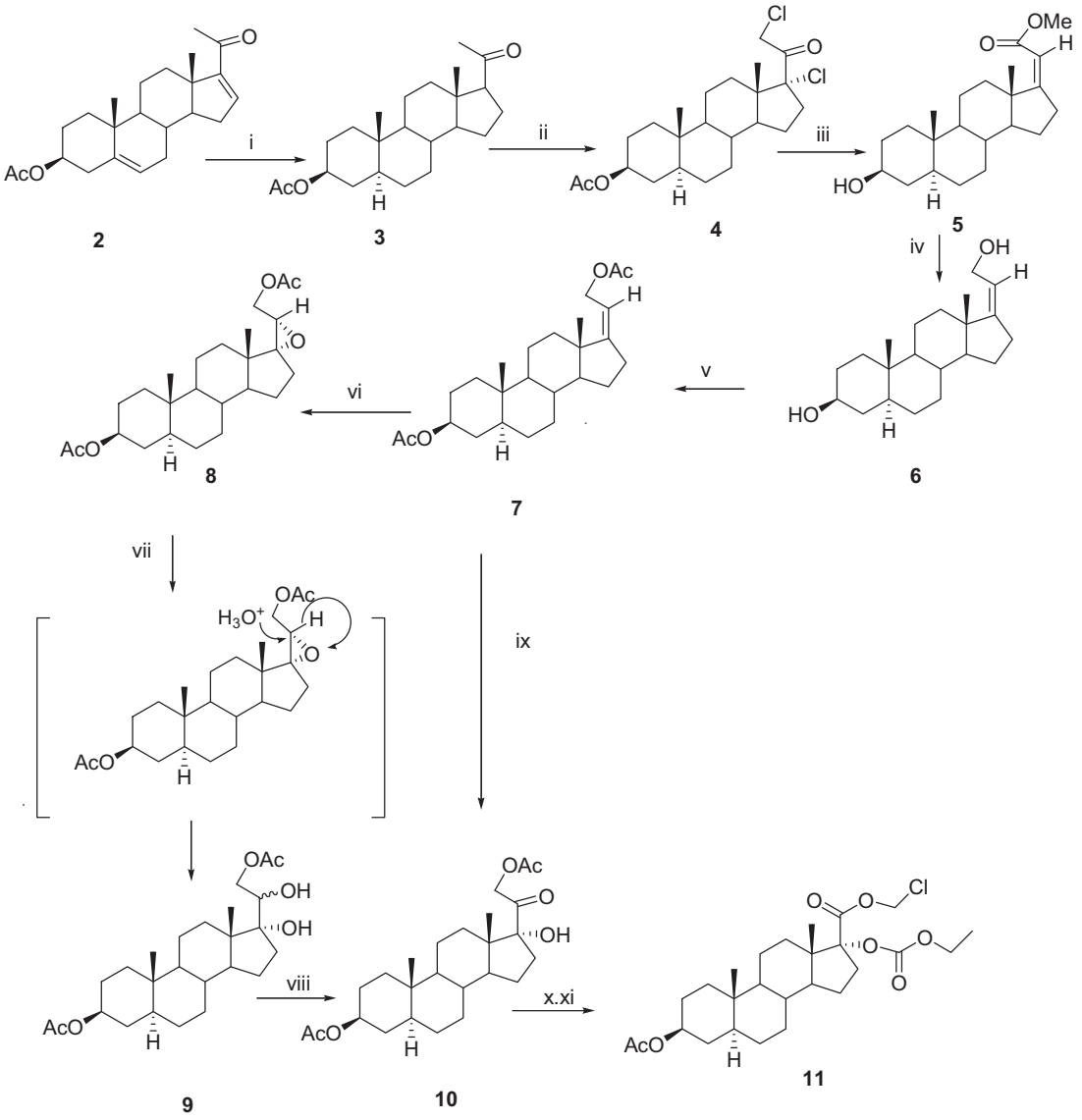


Scheme 1. Reagents and conditions: (i) H_2, Pd–C, 95%, (ii) MnO_2–TMSCl/AcCl–AcOH, 81%, (iii) 3% KOH, MeOH–H_2O, 75%, (iv) $LiAlH_4$, THF, 88%, (v) CAN, AcOH, 75%, (vi) *m*-CPBA, $CHCl_3$, 62%, (vii) H_2SO_4, acetone–H_2O, 48%, (viii) Jones reagent, 57%, (ix) OsO_4–H_2O_2, rt., 50%, (x) $NaIO_4$, Ethyl chloroformate, 70% and (xi) chloromethyl iodide, 75%.

quantitative yield of 17α, 21-dichloro 20-oxo-pregnanes. In the present work, using this halogenation as a key reaction, a short and convenient method for the construction of the side chain of loteprednol etabonate **1** has been developed from 20-oxopregnane, namely, 3β-acetoxy-pregn-5(6),16(17)-diene-20-one (16-DPA) **2**, which is readily available in the laboratory and therefore, had been taken as the model starting material (Scheme 1) [18].

16-DPA **2** was hydrogenated by Pd/C to form 3β-acetoxy-5α-pregnan-20-one **3** in quantitative yield and was subjected to chlorination using our reagent system MnO_2–TMSCl/AcCl–AcOH to give 17α, 21-dichloro-3β-acetoxy-5α-pregnan-20-one **4** in very high yield.

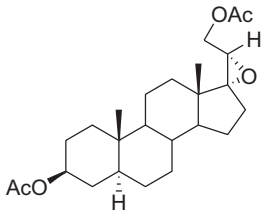
Favorskii rearrangement of the compound **4**, under mild basic condition with 3% KOH in methanol–water (85:15) followed by concomitant deacetylation of 3β-acetoxy group furnished $\Delta^{17(20)}$-20-methoxycarbonyl derivative, namely, pregn-17(20)-ene-3β-ol-21-oic acid methyl ester **5** also in almost quantitative yield [15,16].

Favorskii rearrangement product **5** was then subjected to metal-mediated reduction by using lithium aluminium hydride ($LiAlH_4$) at room temperature in ether or THF which furnished the alcohol derivative, pregn-17(20)-ene-3β, 21-diol **6** in high yield. The compound **6** was acetylated by the newly reported method [19] in acetic acid in the presence of ceric ammonium nitrate (CAN) to obtain the acetylated product, 3β, 21-diacetoxy-pregn-17(20)-ene **7** in quantitative yield.

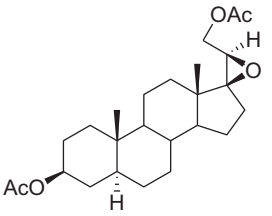
Compound **7** was then epoxidised with *m*-chloroperbenzoic acid (*m*-CPBA) which, after purification by preparative thin layer chromatography (TLC) furnished two isomeric epoxides, 3β, 21-diacetoxy-17α,20α-epoxy-5α-pregnane **8a** and 3β,21-diacetoxy-17β,20β-epoxy-5α-pregnane **8b**. The mass spectrum of both the products showed a molecular ion peak at 418 (M^+) and in ^1H NMR spectrum, both showed a multiplet at near 3.0 ppm for C-20 proton under the epoxide ring.

The major epoxide **8a** was assigned to have a 17α-orientation because of its favourable formation due to the steric factor [22–24] associated with the steroid molecule and

hence the minor epoxide **8b** was assigned to have a 17β-orientation.



8a



8b

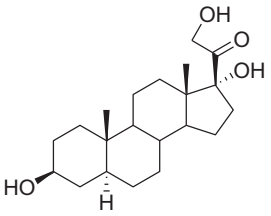
Acid-induced epoxide ring opening reaction was carried out on the major epoxide **8a** to get the desired compound **9** with a 17α-hydroxy group. Although perchloric acid is widely used for opening up of an epoxide ring, the use of this acid led to almost complete hydrolysis of C-3 and C-21 acetate groups of the compound **8a** giving poor yield of the desired diol 3β, 21-diacetoxy-5α-pregnan-17α, 20-diol **9**, even when the acid was used in low concentration.

The use of sulphuric acid in aqueous acetone was used as a reagent of choice because of the fact that it does not affect hydrolysis of the acetate groups present in a steroid molecule [25] which is desirable in this case for the future operation and also would furnish the diol **9** having the requisite 17α-orientation of the hydroxyl group from the α-epoxide **8a**, irrespective of *cis* or *trans* opening due to preferential nucleophilic attack on its C-20 carbon atom as depicted in Scheme 1. Thus, when this epoxide was treated with a small amount of concentrated sulphuric acid in aqueous acetone, it yielded the desired compound, 3β,21-diacetoxy-5α-pregnan-17α,20-diol **9** in good yield. The mass spectrum of the product after purification exhibited molecular ion peak at 436(M^+). IR spectrum confirmed the presence of both hydroxyl (3400 cm^{-1}) and acetate groups (1730 cm^{-1}) in compound **9**.

Compound **9** was then oxidised by Jones reagent at low temperature to furnish 20-oxo compound 3β,21-diacetoxy-5α-pregnan-17α-ol-20-one **10**, which is the immediate precursor for constructing the side chain of loteprednol etabonate **1**. The mass spectrum of the compound exhibited molecular ion peak at 434(M^+). The IR spectrum displayed a band at 1710 cm^{-1} besides displaying acetate bands at 1730 cm^{-1}.

17α-Hydroxy hydrocortisone derivative **10** was also directly obtained from the olefin **7** by the oxidative hydroxylation reaction with hydrogen peroxide in the presence of a small amount of osmium tetroxide. Several workers have reported [26–31] the synthesis of 17α,20α-dihydroxy steroid derivatives and 17α-hydroxy hydrocortisone using osmium tetroxide alone or in combination with various oxidants such as phenyl iodoacetate, H_2O_2, NMO, $K_3[Fe(CN)_6]$. It has been established that 17α-hydroxyl configuration is a consequence of the rearward attack upon the 17–20 double bond (α-face) of the steroid molecule and further by the virtue of the *cis* addition of osmium tetroxide. A similar behaviour of osmium tetroxide was reported by Woodward and Brutcher [27] in the hydroxylation of steroid olefins wherein it led to the formation of *cis*-diol by direct attack from the most accessible α-face of the rigid steroid molecule. Recently Jonsson et al. [32] reported *cis*-hydroxylation of *trans*-5-decene using OsO_4–NMM with the aid of a triple catalytic system using H_2O_2 as the terminal oxidant to suppress over-oxidation, namely, oxidative hydroxylation as has been found to occur in the case of steroidal olefins as mentioned above. The product obtained from **9** by its oxidation and that obtained by oxidative hydroxylation of **7** were found to be identical in all respects (superimposable IR, NMR, and Mass spectra) including their TLC behaviour showing that both the products were the same compound **10**.

To confirm its structure further, compound **10** was hydrolysed with 3% KOH in MeOH–H_2O which furnished a polar compound 5α-pregnan-3β, 17α, 21-triol-20-one **12.** The compound exhibited the molecular ion peak at 350(M^+). The IR spectrum exhibited a broad band at 3300–3400 cm^{-1} for the three hydroxyl groups and another at 1710 cm^{-1} for the C-20 carbonyl group present in the molecule.



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Conversion of compound **10** to get the requisite side chain of loteprednol etabonate **1** is well known [6], which involves the periodic acid oxidation *cum* degradation with sodium metaperiodate to cortienic acid derivative followed by carboxylation of 17α-hydroxy group with ethyl chloroformate and the esterification of 17β-carboxylic acid with chloromethyl iodide. These steps provide the desired 17β-ester functionality and 17α-carbonate functionality present in loteprednol etabonate and related drugs.

Thus the present methodology paves a useful way to construct the side chain of loteprednol etabonate – an ocular soft corticosteroid directly from 20-oxopregnanes *via* its C-21 functionalisation in a much simpler and easier way with this newly developed metal-mediated halogenation technique which avoids harsh and tedious reaction conditions [33–36] associated with this conversion. It is pertinent to note that C-21 functionalised 20-oxopregnanes are important and useful intermediates for synthesising corticosteroids and many other recently developed steroidal drugs including anti-inflammatory agents, hormones, oral contraceptives, and anabolic steroids [33,37–40]

1. Experimental

1.1. General methods

Melting points were determined with an electrothermal melting point apparatus and are uncorrected. All the chemicals used were of reagent grade of E. Merck and were used without further purification. The progress of each of the reactions was monitored on TLC using silica gel (E. Merck) and the plates were activated at 120 °C before use. IR spectra were recorded with a Perkin–Elmer model 2000 series FT-IR spectrometer (Perkin Elmer Limited, Beacons Field Budes, UK) for solutions in chloroform. Infrared absorbance is reported in reciprocal centimetre (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX (300 MHz) spectrometer (Fallanden, Switzerland) using $CDCl_3$ or DMSO-d_6 as solvent with tetramethylsilane (TMS) as internal standard on ppm scale (δ). Multiplicity of the resonance peaks is indicated as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), and multiplet (m). Mass spectrometric analysis was performed by positive mode electrospray ionisation with a Bruker Esquire3000 LC-MS instrument (Bruker Daltonics, Germany). Elemental analysis was carried out in Varian CHN Analyzer (Perkin - Elmer US Instrument Division, Norwalk, USA).

1.2. 3β-Acetoxy-5α-pregnan-20-one 3

Two grams of 16-DPA **2** in 100 ml of ethanol was hydrogenated at 45 psi using 500 mg of 5% Pd/C for a period of 10 h. The reaction mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which was purified by column chromatography over silica gel using petroleum

ether and ethyl acetate as the eluent to furnish 3β-acetoxy-5α-pregnan-20-one **3** in pure form.

Yield: 1.9 g (95%); Mp: 172 °C; IR (cm^{-1}): 1735, 1700, 1450, 1200; ^1H NMR ($CDCl_3$): 0.9 (s, 3H, Me), δ 1.0 (s, 3H, Me), 0.9–1.9 (m, 23H, –CH and $–CH_2$), 2.0 (s, 3H, OAc), 2.2 (s, 3H, COMe), 4.3 (m, 1H, H-3); ^{13}C NMR: δ 34.2(C-1), 35.1(C-2), 78.6(C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 55.4(C-14), 34.7(C-15), 22.9(C-16), 69.5(C-17), 16.7(C-18), 15.2(C-19), 172.9(C–$OCOCH_3$), 212.5(C-20), 21.3(C-21); mass spectrum (mz^{-1}): 360 (M^+), 318, 300, 298, 257; Anal. Calcd. for C_{23} H_{36} O_3: C,76.66; H, 8.91; found: C,76.41; H,9.03.

*1.2.1. 17α,21-Dichloro-3β-acetoxy-5α-pregnan-20-one **4***

To a solution of 200 mg of 3β-acetoxy-5α-pregnan-20-one **3** in 10 ml of glacial acetic acid was added excess of MnO_2 (170 mg) and then 1 ml of AcCl or 4 ml of TMSCl. The reaction mixture was kept overnight at room temperature. Then, the reaction mixture was poured into cold water and extracted with petroleum ether. The organic extract after drying over anhydrous sodium sulphate was evaporated under reduced pressure to furnish a residue which was purified by preparative TLC (EtOAc:petroleum ether: 1:12,v/v) to get 17α,21-dichloro-3β-acetoxy-5α-pregnan-20-one **4** as solid.

Yield: 190 mg (81%); MP: 160 °C; IR (cm^{-1}): 1730, 1710, 1445, 1200; ^1H NMR ($CDCl_3$): 0.9 (s, 3H, Me), δ 1.0 (s, 3H, Me), 0.9–2.0 (m, 22H, –CH and $–CH_2$), 2.1 (s, 3H, OAc), 4.2 (d, J = 1.5 Hz, 2H, H-21), 4.5 (m, 1H, H-3); ^{13}C NMR: δ 34.2(C-1), 35.1(C-2), 78.6(C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 55.4(C-14), 34.7(C-15), 22.9(C-16), 89.7 (C-17), 16.7(C-18), 15.2(C-19), 171.2(C-$OCOCH_3$), 192.6 (C-20), 50.2(C-21); mass spectrum (mz^{-1}): 428 (M^+), 430 (M^++2), 432 (M^++4), 392, 368, 332; Anal. Calcd. for C_{23} H_{34} O_3Cl_2: C,64.49; H,7.94; found: C,64.46; H,7.89.

*1.2.2. Pregn-17(20)-ene-3β-ol-21-oic acid methyl ester **5***

Two-hundred milligrams of the compound 17α,21-dichloro-3β-acetoxy-5α-pregnan-20-one **4** was allowed to stir with 3% KOH in MeOH–H_2O (85:15) at room temperature for a period of 3 h. The reaction was monitored on TLC. When TLC indicated that the starting material had all been used up, the reaction mixture was poured into cold water and was acidified with citric acid. It was then extracted with CH_2Cl_2 (3 × 150 ml). The organic extract was dried over anhydrous Na_2SO_4 and was evaporated under reduced pressure. The residue so obtained was purified by preparative TLC (EtOAc:petroleum ether: 1:5, v/v) to get the pure pregn-17(20)-ene-3β-ol-21-oic acid methyl ester **5** in the form of a gum.

Yield: 120 mg (75%); IR (cm^{-1}): 3400, 1730, 1250; ^1H NMR ($CDCl_3$): δ 0.8 (s, 3H, Me), 1.0 (s, 3H, Me), 0.9–2.1 (m, 23H, –CH and $–CH_2$), 2.4 (m, 1H, 3β-OH), 3.4 (s, 3H, OMe), 3.6 (m, 1H, H-3), 5.3 (s, 1H, H-20); ^{13}C NMR: δ 34.2(C-1), 35.1(C-2), 67.6 (C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 55.4 (C-14), 32.7(C-15), 36.6(C-16), 164.8 (C-17), 16.7(C-18), 15.2(C-19), 114.8C-20), 167.9(C-21), 52.7(C–OCH_3); Mass spectrum (mz^{-1}): 346 (M^+), 328, 288, 270; Anal. Calcd. for $C_{22}H_{34}$ O_3: C,76.30; H,9.83; found: C,76.21; H,9.76.

*1.2.3. Pregn-17(20)-ene-3β,21-diol **6***

Five-hundred milligrams of substrate **5** was dissolved in 25 ml of anhydrous THF. The reaction was allowed to stir at room temperature and to it was added 300 mg $LiAlH_4$. The mixture was allowed to stir for 4 h and was monitored on TLC. When TLC indicated completion of the reaction, the reaction mixture was filtered and the filtrate was poured into cold water and was extracted with CH_2Cl_2. The organic extract after drying over anhydrous Na_2SO_4 was distilled under reduced pressure to get a residue. The residue was purified by preparative TLC, which furnished a solid product pregn-17(20)-ene-3β,21-diol **6**.

Yield: 400 mg (88%); M.P: 181 °C; IR (cm^{-1}): 3300, 1350, 1250; ^1H NMR (DMSO-d_6): 0.9 (s, 3H, Me), δ 1.0 (s, 3H, Me), 0.9–2.1 (m, 22H, –CH and $–CH_2$), 3.5 (m, 3H, H-3 and H-21), 3.8–4.1 (m, 2H, 3β- and 21-OH), 5.3 (m, 1H, H-21); ^{13}C NMR: δ 34.2(C-1), 35.1(C-2), 67.6 (C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9),35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 55.4(C-14), 32.7(C-15), 36.6(C-16), 152.3(C-17), 16.7(C-18), 15.2(C-19), 114.9(C-20), 64.6(C-21); mass spectrum (mz^{-1}): 318 (M^+), 300, 283, 253; Anal. Calcd. for C_{21} H_{34} O_2: C,79.25; H,10.69; found: C,79.18; H,10.03.

*1.2.4. 3β, 21-Diacetoxy-pregn-17(20)-ene **7***

To a solution of 500 mg of pregn-17(20)-ene-3β, 21-diol **6** in 20 ml of glacial AcOH was added 50 mg of CAN. The reaction mixture was heated in the range 50–60 °C and was monitored on TLC. After completion of the reaction, the reaction mixture was poured into cold water and was extracted with petroleum ether. The organic extract was dried over anhydrous Na_2SO_4 and was distilled under reduced pressure to get a residue which was purified by preparative TLC (EtOAc:petroleum ether: 1:10) to furnish 3β,21-diacetoxy-pregn-17(20)-ene **7** in pure form as a solid.

Mp: 154 °C; yield: 450 mg (75%); IR (cm^{-1}): 1725 (broad), 1350, 1250; ^1H NMR ($CDCl_3$): δ 0.9 (s, 3H, Me), 1.1 (s, 3H, Me), 0.8–1.9 (m, 22H, –CH and $–CH_2$), 2.0 (bs, 6H, OAc), 4.3–4.5 (m, 3H, H-3 and H-21), 5.3 (m, 1H, H-20); ^{13}C NMR: δ 34.2(C-1), 35.1(C-2), 72.4 (C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6),32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 53.6(C-14), 32.7(C-15), 36.6(C-16), 152.3(C-17), 16.7(C-18), 15.2(C-19), 114.9(C-20), 71.7(C-21), 173.1(C-$OCOCH_3$); mass spectrum (mz^{-1}): 402 (M^+), 360, 342, 284; Anal. Calcd. for C_{25} H_{38} O_4: C,74.63; H,9.45; found: C,74.51; H,9.39.

*1.2.5. 3β, 21-Diacetoxy-17α, 20α-epoxy-5α-pregnane **8a** and 3β, 21-diacetoxy-17β, 20β-epoxy-5α-pregnane **8b***

To a solution of 500 mg of 3β,21-diacetoxy-pregn-17(20)-ene **7** in 20 ml of chloroform added 150 mg of m-CPBA. The reaction mixture was kept overnight at room temperature. On the following day, TLC indicated the formation of two polar products. The reaction mixture was then poured into cold water and was extracted with dichloromethane. The organic extract was washed first with an aqueous solution of sodium meta-bisulphite and then with a 5% NaOH aqueous solution. Finally the organic extract after drying over anhydrous Na_2SO_4 was distilled under reduced pressure to furnish a residue which was purified by preparative TLC (EtOAc:petroleum ether: 1:1) to give **8a** as the major and **8b** as the minor products.

Yield: **8a**: 320 mg (62%); IR (cm^{-1}): 1730 (broad), 1350, 1260; ^1H NMR ($CDCl_3$): δ 0.9 (s, 3H, Me), 1.0 (s, 3H, Me), 0.91-1.9 (m, 22H, –CH and $–CH_2$), 2.1 (bs, 6H, OAc), 3.1 (m, 1H, H-20), 4.3–4.5 (m, 3H, H-3 and H-21)); ^{13}C NMR ($CDCl_3$): δ 34.2(C-1), 35.1(C-2), 72.4 (C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 53.6(C-14), 32.7(C-15), 36.6(C-16), 74.3(C-17), 16.7(C-18), 15.2(C-19), 57.2(C-20), 72.4(C-21), 173.1(C–$OCOCH_3$); mass spectrum (mz^{-1}): 418 (M^+), 376, 258, 200,184; Anal. Calcd. for C_{25} H_{38} O_5: C,71.77; H,9.09; found: C,71.72; H,9.00.

Yield: **8b**: 100 mg (20%); IR (cm^{-1}): 1735 (broad), 1340, 1250; ^1H NMR ($CDCl_3$): δ 0.9 (s, 3H, Me), 1.0 (s, 3H, Me), 0.9–1.9 (m, 22H, –CH and $–CH_2$), 2.0 (bs,6H, OAc), 3.3 (m, 1H, H-20), 4.4–4.6 (m, 3H, H-3 and H-21); ^{13}C NMR ($CDCl_3$): δ34.2(C-1), 35.1(C-2), 72.4 (C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 53.6(C-14), 32.7(C-15), 36.6(C-16), 74.3(C-17),16.7(C-18), 15.2(C-19), 57.2(C-20), 72.4 (C-21), 173.1(C–$OCOCH_3$); mass spectrum (mz^{-1}): 418 (M^+), 376, 258, 200,184; Anal. Calcd. for C_{25} H_{38} O_5: C,71.77; H,9.09; found: C,71.67; H,8.94.

*1.2.6. 3β, 21-Diacetoxy-5α-pregnan-17α, 20-diol **9***

To a solution of 200 mg of 3β, 21-diacetoxy-17α, 20α-epoxy-5α-pregnane **8a** in 10 ml of acetone were added 2 ml of water and 4 drops of concentrated H_2SO_4. The resulting solution was allowed to stand at room temperature for 24 h. The reaction mixture was poured into cold water and was extracted with dichloromethane. The extract after drying over anhydrous sodium sulphate was distilled under reduced pressure to get a residue which was purified by preparative TLC (EtOAc:petroleum ether: 1:1) to furnish 3β, 21-diacetoxy-5α-pregnan-17α, 20-diol **9**.

Yield: 110 mg (48%); IR (cm^{-1}): 3400, 1735 (broad), 1345, 1250; ^1H NMR (DMSO-d_6): δ 0.9 (s, 3H, Me), 1.1 (s, 3H, Me), 0.9–1.9 (m, 24H, –CH and $–CH_2$), 2.1 (bs, 6H, OAc), 3.4 (m, 1H, H-20), 3.9 (m, 2H, 17α- and 20-OH), 4.4–4.6 (m, 3H, H-3 and H-21); ^{13}C NMR: δ 34.2(C-1), 35.1(C-2), 72.4 (C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 53.6(C-14), 32.7(C-15), 36.6(C-16), 85.2(C-17), 16.7(C-18), 15.2(C-19), 79.1(C-20), 69.2(C-21), 173.1(C-$OCOCH_3$); Mass spectrum (mz^{-1}): 436 (M^+), 418, 376, 300; Anal. Calcd. for C_{25} H_{40} O_6: C,68.81; H,9.17; found: C,68.76; H,9.09.

*1.2.7. 3β, 21-Diacetoxy-5α-pregnan-17α-ol-20-one **10***

*1.2.7.1. Reaction A: from oxidation of compound **9**.* A solution of 200 mg of 3β, 21-diacetoxy-5α-pregnan-17α, 20-diol **9** in 20 ml of dry acetone (distilled over $KMnO_4$) was cooled to 0–10 °C in an ice bath and to it was added 5 drops of Jones reagent and kept under stirring for a period of 1 h. The reaction was monitored on TLC. When TLC indicated the completion of the reaction, 3 ml of methanol was added and poured into cold water. The reaction mixture was extracted with CH_2Cl_2. The organic extract after drying over anhydrous Na_2SO_4 was distilled under reduced pressure to get a residue which was purified by preparative TLC (EtOAc:petroleum ether: 1:5) to furnish the compound 3β, 21-diacetoxy-5α-pregnan-17α-ol-20-one **10** in pure form.

Yield: 115 mg (57%); IR (cm^{-1}): 3400, 1730 (broad), 1710, 1250; ^1H NMR ($CDCl_3$); δ 0.8 (s, 3H, Me), 1.1 (s, 3H, Me), 0.9–1.9 (m, 23H, –CH and $–CH_2$), 2.0 (bs, 6H, OAc), 2.4 (bs, 17α-OH), 4.3–4.5 (m, 3H, H-3 and H-21); ^{13}C NMR ($CDCl_3$): δ 34.2(C-1), 35.1(C-2), 73.8(C-3), 39.5(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 53.6(C-14), 32.7(C-15), 36.6(C-16), 97.7(C-17), 16.7(C-18), 15.2(C-19), 212.1(C-20), 72.4(C-21), 173.1(C–$OCOCH_3$); Mass spectrum (mz^{-1}): 434 (M^+), 416, 374, 356, 298; Anal. Calcd. for C_{25} H_{38} O_6:C,69.12; H,8.76; found: C,69.02; H,8.68.

1.2.8. Reaction B: from oxidative hydroxylation of compound 7

To a solution of 402 mg (1 mmol) of the olefin **7** in acetone (5 ml) and H_2O (15 ml) at room temperature was added OsO_4 (375 μl, 2.5 wt%, 0.03 mmol) followed by H_2O_2 (310 μl, 30% aq., 3 mmol). The reaction mixture was stirred for a period of 20 h at room temperature and then quenched by the addition of $Na_2S_2O_4$ (150 mg) and magnesium silicate (300 mg). The reaction mixture was further stirred for another 3 h and diluted with ethyl acetate (50 ml) and then filtered through a pad of celite and the celite bed was thoroughly washed with ethyl acetate. After that, solvent was removed under reduced pressure to get a residue which was purified by preparative TLC using ethyl acetate and hexane as the eluents.

Yield: 220 mg (50%); IR, NMR, mass, and TLC are superimposable with those of the compound 3β,21-diacetoxy-5α-pregnan-17α-ol-20-one **10**. Anal. Calcd. for C_{25} H_{38} O_6: C,69.12; H,8.76; found: C,69.01; H,8.69.

1.2.9. 5α-Pregnan-3β, 17α, 21-triol-20-one 12

200 mg of 3β, 21-diacetoxy-5α-pregnan-17α-ol-20-one **10** was hydrolysed with 50 ml of 3% KOH in MeOH–water. The reaction mixture was allowed to stir for a period of 2 h and was monitored on TLC. After completion of the reaction, the reaction mixture was poured into cold water and was acidified with an aqueous solution of citric acid. It was then extracted with CH_2Cl_2. The organic extract after drying over anhydrous Na_2SO_4 was distilled under reduced pressure to get a residue which was purified by preparative TLC to furnish 5α-pregnan-3β,17α,21-triol-20-one **12**

Yield: 125 mg (78%); IR (cm^{-1}): 3300–3400, 1710, 1250; ^1H NMR (DMSO-d_6): δ 0.9 (s, 3H, Me), 1.0 (s, 3H, Me), 0.9–1.8 (m, 22H, –CH and $–CH_2$), 3.4 (m, 3H, H-3 and H-21), 4.2–4.4 (m, 3H, 3β, 17α- and 21-OH); ^{13}C NMR: δ 34.2(C-1), 35.1(C-2), 70.6(C-3), 39.2(C-4), 41.9(C-5), 28.1(C-6), 32.0(C-7), 29.0(C-8), 46.2(C-9), 35.4(C-10), 20.9(C-11), 39.5(C-12), 40.5(C-13), 53.6(C-14), 27.2(C-15), 68.1(C-16), 101.8(C-17), 16.7(C-18), 15.2(C-19), 213.6(C-20), 70.6(C-21); mass spectrum (mz^{-1}): 350 (M^+), 332, 314, 296; Anal. Calcd. for C_{21} H_{34} O_4: C,72.00; H,9.71; found: C,71.89; H,9.61.

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