Synthesis of Calicoferol E

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Dedicated to Professor S.V. Kessar on the occasion of his 70th birthday
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Abstract
A concise linear synthesis of 9,10 secosteroid Calicoferol E **6** has been completed. Key features of the process include: (i) the preparation of hitherto unknown 9α-hydroxycholest-1, 4-diene-3-one **5** from commercially available 5α-cholestan-3β-ol using conventional chemistry; (ii) the acid catalyzed rearrangement of **5** to give **6.**

Keywords: 9,10-Secosterol, DDQ dehydrogenation, acid catalyzed aromatization, 3-keto-1,4-diene

Introduction

Aromatic A-ring 9,10-secosteroids such as Calicoferol E and Astrogorgiadiol are naturally occurring biologically active compounds[1]. They have been isolated from a gorgonian of genus *Calicogorgia* and are toxic against brine shrimp larvae. These compounds are less studied vitamin D_3 analogues[2]. Considerable efforts have already been directed towards the preparation of aromatic A ring 9,10-secosteroids either from 3-keto-4-ene and /or 3-keto-1,4-diene steroidal systems. The methods involve the use of techniques such as pyrolysis[3], radical initiated processes[4] and microbial transformations[5]. These procedures however have severe limitations[5,6] towards the manipulation and interconversion of substituents required in a natural product[1]. This problem was indeed solved in a recently reported[6] convergent synthesis of Calicoferol E (6). The key step of this very lengthy synthesis is the radical coupling of des-AB-cholest-8-methyledine-9-one (obtained from vitamin D_3 via Grundmann's ketone) with 2-methyl-4-MEM(methoxyethoxymethyl)ether of benzyl iodide. Overall yield of the reported 14 step synthesis is 6.6%. We report herein a short and convenient synthesis of Calicoferol E using 9α-hydroxycholesta-1,4-diene-3-one. It is essentially based on (i) deprotection of a 1,3-dioxalane derivative to ketone and dehydrogenation of 9α-hydroxy-5α-cholestan-3-one by DDQ[7] and (ii)

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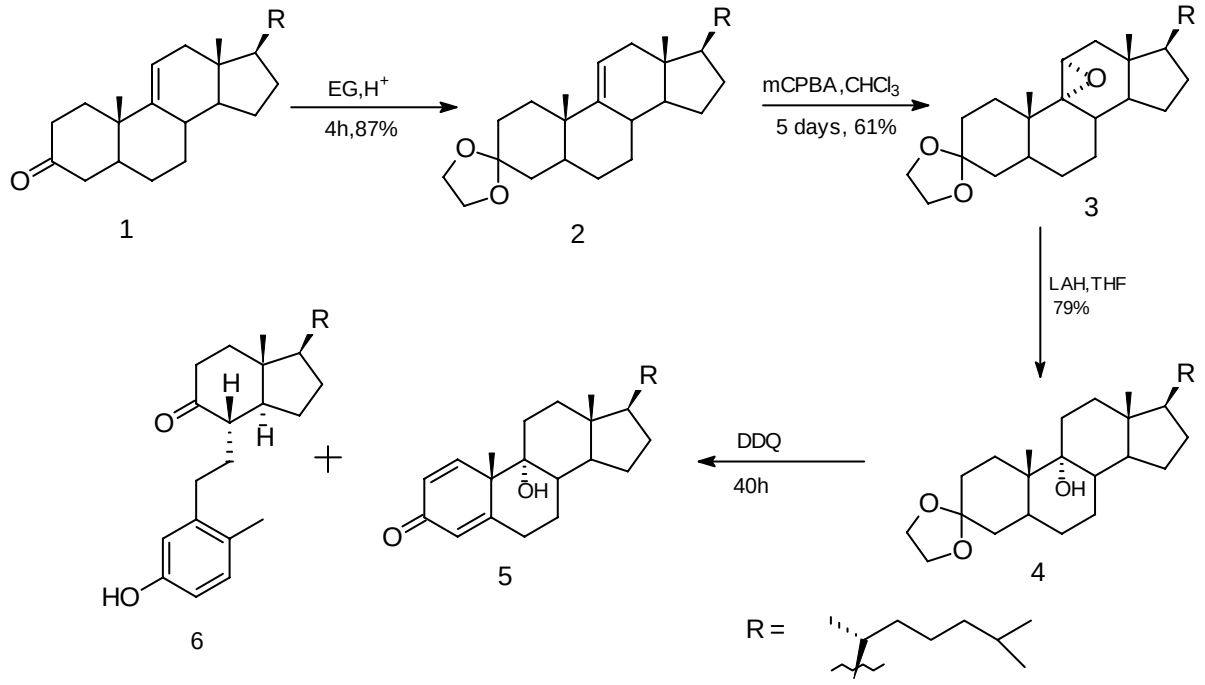
the chemistry of acid catalyzed dienone-phenol rearrangement developed by Caine[8].

Results and Discussion

The four step conversion of cholest-9(11)-ene-3-one **1**[9] to the precursor **5** was carried out using conventional chemistry. The protection of keto function using ethyleneglycol in the presence of p-toluenesulphonic acid gave cyclic ketal in 87% yield. Treatment of **2** with *m*-chloroperoxybenzoic acid in chloroform afforded 9α,11α-epoxide **3** in 61% yield. Reduction of **3** with lithium aluminumhydride in THF furnished 9α-hydroxy-3-ethylenedioxy cholestane **4** (79%).



Scheme 1

The deketalization of 9α-hydroxy-3-ethylenedioxy cholestane **4** was performed using DDQ[4] in catalytic amount. Further oxidation was continued by addition of more DDQ. 9α-hydroxycholestane-1,4-diene-3-one **5** (8%) and Calicoferol E **6** (2%) were isolated along with two minor products. The IR spectrum of **5** showed bands at 3440 cm^{-1} due to –OH and 1660 cm^{-1} due to cross conjugated dienone moiety. Its ^1H NMR showed the angular methyls at δ0.73 (18-Me) and δ1.22 (19-Me), doublet was seen at δ6.02 (J=2Hz) for olefinic proton at C-4. The double doublet was observed at δ6.18-6.22 (J=10Hz, 2Hz) due to olefinic proton at C-2 and another double doublet was seen at δ7.01-7.04 (10Hz, 2Hz) due to olefinic proton at C-1. The mass spectrum showed the molecular ion at m/z 398, (M^+-H_2O) peak at m/z 380 and [M^+-

(H_2O+CH_3)] peak at m/z 365. Calicoferol-E **6** showed IR absorption bands at 3400 cm^{-1} (-OH) and 1712 cm^{-1} (C=O). Its UV spectrum showed the absorption maximum at λ 282 nm characteristic of C=O ($n\pi^*$). The ^{1}H NMR showed a singlet at δ2.25 (3H, methyl group at aromatic ring), two doublets of triplet at δ2.48 and 2.66 (2H, $ArCH_2$-CH_2-), a broad peak at δ4.50 (1H, ArOH), a double doublet at δ6.54-6.58(J=7.97, 2.75 Hz, 1H, C_2H), a doublet at δ6.64-6.65 (J=2.6 Hz, 1H, C_4H) and a doublet at δ6.96-6.99 (J=8.5 Hz, 1H, C_1H). The Mass spectrum exhibited the molecular ion at m/z 398. The above data is in accord with literature[1]. Finally, the structure was confirmed by X-ray crystallography[10] (Figure 1.)

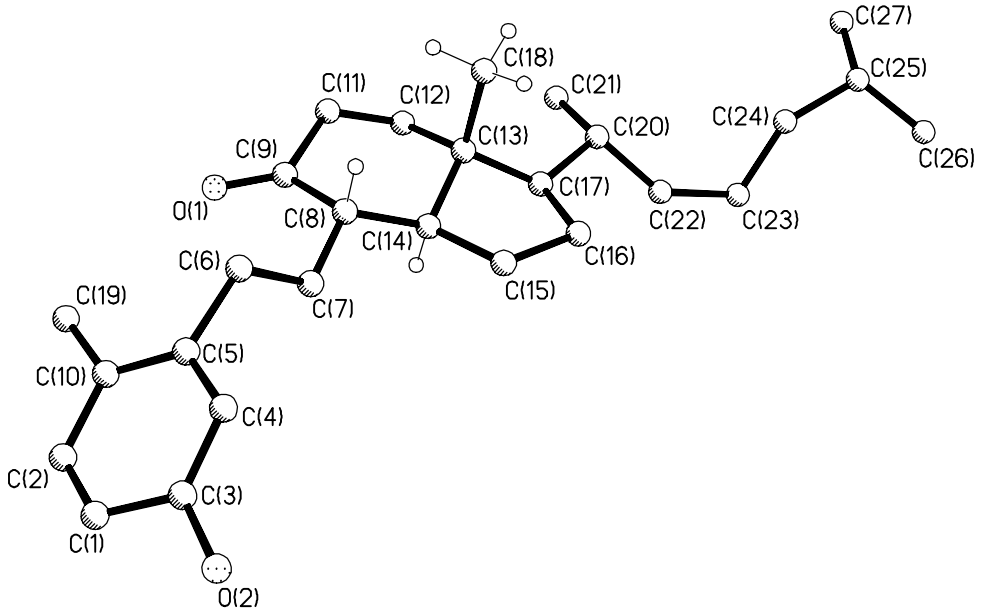
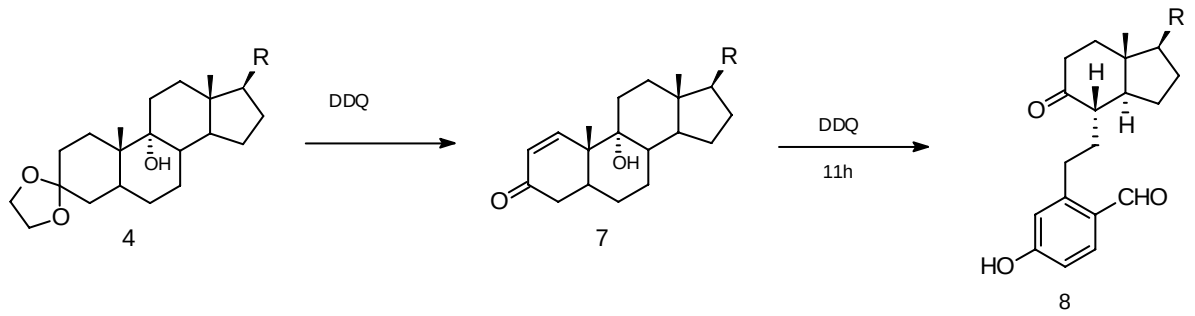


Figure 1. A perspective view of Calicoferol E (**6)** with atom numbering scheme. Hydrogens of C8, C14 and C18 are shown to highlight the stereochemistry at these centers. Other hydrogens are omitted for clarity.

In another experiment the reaction of 9α-hydroxy-3-ethylenedioxy cholestane **4** with DDQ produced 9α-hydroxycholest-1-ene-3-one **7** as the major product (49%). 9α-Hydroxycholest-1-ene-3-one **7** was a white solid (m.p. 120-121°C). Its UV spectrum showed an absorption maximum at λ 226 nm. The ^{1}H NMR displayed a singlet at δ 0.71 (3H, 18-Me), a singlet at δ 1.10 (3H,19-Me), C-1,C-2 protons appeared at δ 5.93, 7.21 (J_{HH} = 10Hz). The ^{13}C NMR showed carbonyl carbon at δ199.9 (C-3), the two olefinic carbons at δ 157.6 (C-2) and 128.9 (C-1) and carbon bearing –OH group at δ 74.9 (C-9). The MS showed the molecular ion at m/z 400, the [(M^+-H_2O+CH_3)] at m/z 367. The HRMS gave the molecular ion exact mass as 400.3336 (calculated 400.3341).

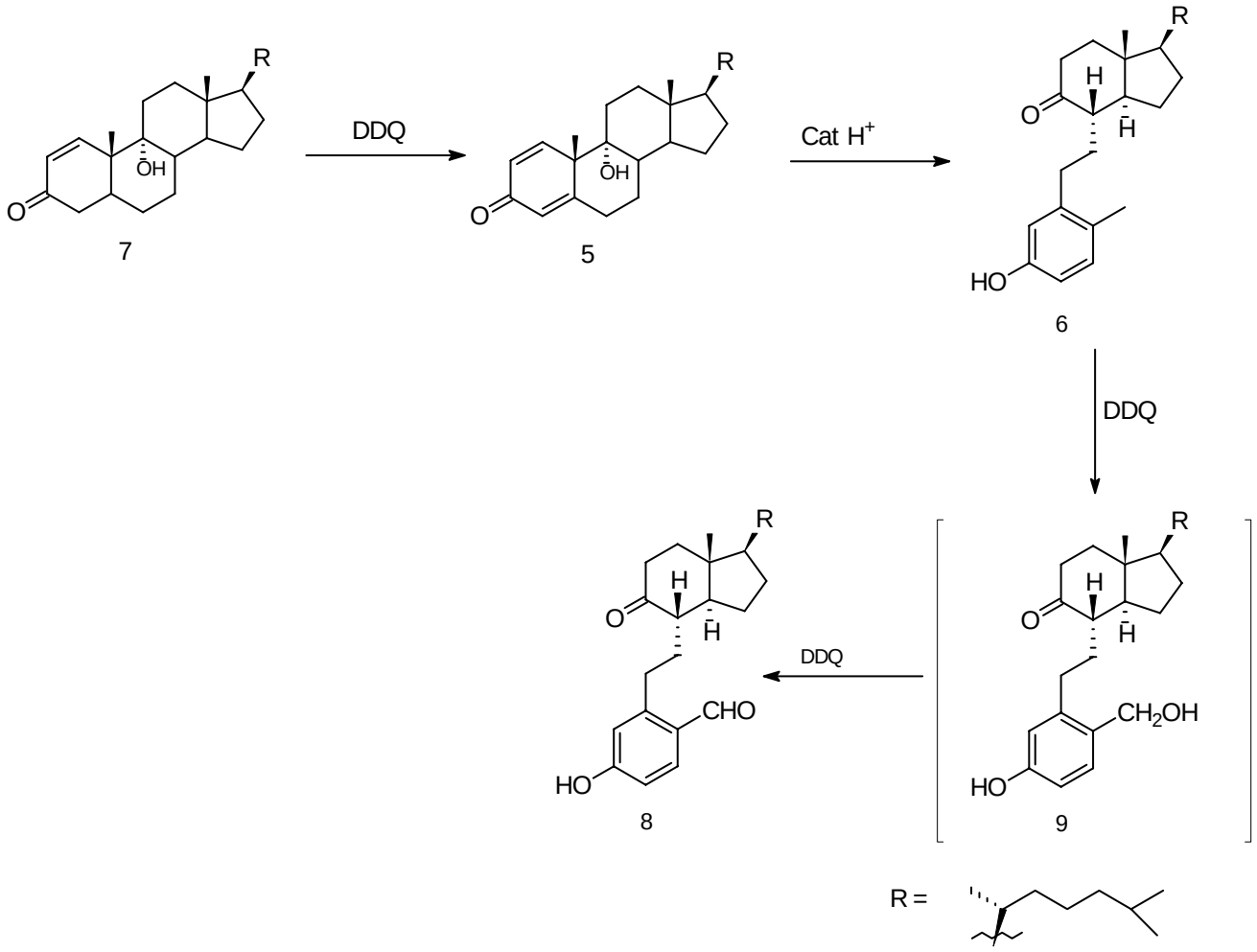


Scheme 2

9α-Hydroxycholest-1-ene-3-one **7** was further subjected to DDQ treatment and a single product **8** was obtained in 86% yield. Its IR spectrum showed bands at 3320 cm^{-1}(-OH), 2730 and 1695 cm^{-1}(-CHO) and 1712 cm^{-1}(C=O). The ^{1}H NMR displayed a doublet of triplet at δ2.92-2.88 (Ar-CH_2-CH_2-), a doublet of triplet at δ 3.05-3.09 (Ar-CH_2-CH_2-), a double doublet at δ 6.72 –6.84 (2H, aromatic), a singlet at δ7.22-7.75 (1H, aromatic) and a singlet at δ10.12 (-CHO). The ^{13}C NMR showed the peaks at δ 214.6 (-CHO), 191.1 (C=O) and 161.2, 148.8, 134.9, 127.2, 117.5, 113.8, 96.2. The mass spectrum showed the molecular ion at m/z 412. Its exact mass from HRMS was 412.2972 (calculated 412.2977). On the basis of forgoing data the structure **8** was assigned to this product. The mechanism for the formation of **8** as well as **6** may be visualized as depicted below Scheme 3. The dehydrogenation of **7** occurs with DDQ to form **5**. Its intermediacy has already been established during the formation of **6** from **4** (Scheme 1).

Calicoferol E (6) undergoes benzylic oxidation at C-19 with DDQ[11] to give phenolic aldehyde **8** via the alcohol **9**.

In summary, we have presented the first linear synthesis of calicoferol E (6), with the use of rearrangement of 9α-hydroxycholest-1,4-diene-3-one to calicoferol E. We believe that it is possibly the first biomimetic construction of this molecule. The low yield of calicoferol E **6** may be attributed to the fact that the treatment with DDQ is responsible for various transformations such as deketalisation, dehydrogenation, oxidation of tertiary alcohol, aromatisation followed by C-C bond cleavage. Besides this, further oxidation of the natural product is detrimental. The attempts to control the over oxidation product(s) are in progress.



Scheme 3

Experimental Section

General Procedures. Melting points were determined in Thomas Hoover apparatus and are uncorrected. IR spectra were recorded with Perkin-Elmer model 1430 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian EM-390 (90MHz) and Brücker ACF300 (300MHz) using TMS as internal standard. ^{13}C NMR spectra were also recorded on ACF300 instrument. Mass spectra were recorded on VG analytical 70-250S machine and the relative intensities of all peaks are given in parentheses, Cholestanol and DDQ were purchased from Fluka (Switzerland) and used as such.

Cholest-9(11)-ene-3-one (1). Cholest-9(11)-ene-3-one **1** can be readily obtained in over all yield of 52% from 5α-Cholestane-3β-ol, followed by Jones oxidation[9,2c], mp. 109-110°C (methanol-ether) (lit[2c] 109-110°C).

3-Ethylenedioxycholest-9(11)-ene (2). A solution of cholest-9(11)-en-3-one **1** (8.34g, 21.71 mmol), ethylene glycol (1.33 mL, 23.88 mmol) and *p*-toluenesulphonic (10 mole%) acid in benzene (80mL) was heated under reflux for 4h using a Dean and Stark water separator . Benzene was removed and the residue was dissolved in ether (250mL). The ether mixture was washed with 5% aq. NaOH(3×100mL), water (4×150mL) and dried. Removal of ether yielded a light yellow coloured solid (8.47g). Purification by flash chromatography afforded a white solid, mp. 99-101°C(8.08g, 87%). IR (CCl_4): 1560cm^{-1} (C=C); ^{1}H NMR (90MHz, CCl_4) δ5.2 (d, 1H, 11-H); 3.8 (s, 4H, -O-CH_2-CH_2-O-); 2.1-0.6 (m, 43H, steroidal envelope); 0.99 (s, 3H, 19-Me), 0.6 (s, 3H, 18-Me), HRMS calculated for $C_{29}H_{48}O_2$ 428.3654 found 428.3664.

9α, 11α-Epoxy-3-ethylenedioxycholestane (3). A solution of 3-ethylenedioxy cholest-9(11)-ene **2** (7.89g, 18.44 mmol) and *m*-chloroperoxybenzoic acid (55%, 11.84g) in chloroform (125mL) was stirred at room temperature for 5 days. The progress and completion of reaction was monitored by TLC. The chloroform mixture was treated with sodium meta bisulphite (2×100 mL). The aqueous layer was extracted with chloroform (2×100mL). The combined chloroform extract was washed with water (4×150mL), aq. Na_2CO_3 (2×150mL) and brine (2×150mL) and dried. Chloroform was distilled off under reduced pressure to give a white solid (7.83g). It was purified by flash chromatography (hexane, ethyl acetate, 97.5:2.5) to obtain a white solid. mp. 144-145°C (5g. 61%). ^{1}H NMR (90MHz, $CDCl_3$) δ 3.79 (s, 4H, -OCH_2-CH_2O-), 2.94 (d, IH, *J*= 6Hz, -CHOC), 0.90(s, 3H, 19-Me), 0.60(s, 3H, 18-Me); LRMS m/z (relative intensity) 444(59), 292(21), 289(19), 231(13), 168(13), 145(11), 133(10), 125(47), 115(40), 107(19), 99(100). HRMS calculated for $C_{29}H_{48}O_3$ 444.3603, found 444.3614.

9α-Hydroxy-3-ethylenedioxycholestane (4). To lithium aluminum hydride (2.56g, 67.62mmol) was added a solution of 9α, 11α-epoxy-cholestan-3-ethylenedioxide **3** (5.0g, 11.27mmol) in dry THF (115mL) under nitrogen atmosphere. The reaction mixture was heated under reflux for 18h, under nitrogen atmosphere. After cooling to room temperature, aq. NaOH solution (1N, 12.5mL) was added slowly, followed by sodium sulphate and the mixture was filtered using a sintered funnel. The filtrate was evaporated to yield a white solid, m.p.130-132°C(5.52g). Recrystallization from ethanol afforded white needles, mp. 147-148°C(4g, 79%). ^{1}H NMR (60MHz, CCl_4), δ 3.89(s, 4H, -O-CH_2-CH_2-O-), 3.68(brs, IH, -OH, exchangeable), 0.93(s, 3H, -CH_3), 0.6 (s, 3H, -CH_3). MS m/z (relative intensity) 446(M^+, 24.5), 428(M^+-H_2O, 16), 155(14), 125(61), 105(12), 99(100). HRMS calculated for $C_{29}H_{50}O_3$ 446.3759, found 446.3756.

Treatment of 9α-hydroxy-3-ethylenedioxy cholestane (4) with DDQ. A solution 9α-hydroxy-3-ethylenedioxy cholestane **4** (0.91g, 2.04mmol), DDQ (0.092g, 0.40mmol) and dioxane (40mL) was heated under reflux for 16h. DDQ (1.38g, 6.12mmol) was again added in three installments at 8h interval, and the reaction mixture was heated under reflux for 40h. Filtration through sintered glass funnel afforded a reddish brown filtrate which was evaporated in vacuum to yield a dark oily residue (0.995g). This was filtered through a small silica gel (40-60μ) pad using hexane/ethyl acetate (80:20) and the filtrate was evaporated under reduced pressure to yield a faint yellow viscous material. This was purified by flash chromatography (hexane, ethyl acetate;

92.5:7.5) to provide four compounds, out of which only two could be identified.

9α-Hydroxycholestan-1,4-dien-3-one (5). 67.8 mg(viscous oil). IR (CH_2Cl_2); 3440, 1660 cm^{-1}; ^1H NMR (300 MHz $CDCl_3$) δ 7.04-7.01 (dd, *J*=10Hz, 2Hz, 1H, 1-H); 6.22-6.18(dd, *J*=10Hz, 2Hz, 1H, 2-H), 6.02 (d, J=2 Hz, 1H, 4-H), 1.22 (s, 3H, 19-CH_3), 0.73(s, 3H, 18-CH_3), 2.5-0.85 (steroidal protons) ^{13}C NMR (75MHz, $CDCl_3$) δ 186.5(C-3), 169.7(C-5), 156.2(C-1), 127.4(C-2), 123.7(C-4), 64.2(C-9); LRMS m/z (relative intensity) 398(M^+,29), 380(64), 365(16), 267(35), 259(17), 247(72), 173(52), 159(51), 147(40), 122(100); HRMS calculated for $C_{27}H_{42}O_2$ 398.3184, found 398.3197.

Calicoferol-E (6). 15.7mg(white solid) mp. 94-95°C (Lit[6] mp. 94-95°C); $[\alpha]_D^{23}$ + 23.6; UV (MeOH) λ_{max} (log ε) 218 (3.95), 282 (3.39) nm; IR(neat) 3400, 1742, 1712, 1462 cm^{-1}; ^1H NMR (300 MHz $CDCl_3$) δ 6.99-6.96(d, *J*=8.5Hz, 1H, 1-H), 6.65-6.64(d, *J*=2.6Hz, 1H, 4-H), 6.58-6.54(dd, *J*=7.97 Hz, 2.75Hz, 1H, 2-H), 4.50(brs 1H,-OH), 2.66 (m, 1H, 6-H), 2.48(m, 1H, 6-H), 2.25(s, 3H, -CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) 213.6 (C-5), 131.1 (C-1), 127.9(C-10), 115.8 (C-4), 112.6 (C-2), 55.33(C-14), 55.12 (C-17), 50.5 (C-8), 42.9 (C-13), 35.9 (C-24), 38.4 (C-11), 38.5(C-12), 35.9 (C-22), 35.7 (C-20), 31.1 (C-6), 29.1(C-16), 28.1(C-25), 27.7(C-7), 25.2(C-15), 23.9(C-23), 22.9(C-26), 22.6(C-27), 18.6(C-21), 18.4(C-19), 11.6(C-18), LRMS m/z relative intensity 398 (M^+, 24) 264 (29), 294 (9), 193(39), 180(6), 157(70), 134(100), 121(48), 109 (9); HRMS calculated for $C_{27}H_{42}O_2$ 398.3185 found 398. 3178.

X-ray Crystallography. Needle shaped single crystals of compound **6** were grown from benzene-dichloromethane (2:1;v/v) and a crystal with dimension 0.27x0.15x0.12 mm was mounted along its largest dimension and used for data collection. The crystals, in general, were weakly diffracting and the peak-width was high, deterring a good intensity data collection. Nevertheless, the molecular features and thus the identity of **6** is very clear from the diffraction experiment. The structure was solved by Direct Methods using SHELX-97[10]package and also refined using the same one. During the refinement, it is observed that the aliphatic side chain was severely disordered with high thermal parameter values and hence were only refined isotropically. Other non-hydrogen atoms were refined anisotropically. The hydrogen atoms, except those of side chain, were included in the ideal positions with fixed isotropic U values and were riding. Crystal data for **6**: $C_{27}H_{42}O_2$, M_r=398.61, Orthorhombic, $P2_12_12_1$, a=8.510(2), b=14.126(2), c=20.938(4)Å, V=2516.9(9) $Å^3$, Z=4, MoKα, λ=0.71073Å, T=293(1) K, GOOF=0.965, R=0.1635, wR=0.3360 for 1179 reflections [F> 4σ(F)].

Treatment of 9α-hydroxy-3-ethylenedioxycholestane (4) with DDQ. To a solution 9α-hydroxy-3-ethylenedioxy cholestane **4** (3.89g, 8.73mmol) in dioxane (175ml) was added DDQ (0.407g, 8.73mmol) and the reaction mixture was heated under reflux for 16h under nitrogen atmosphere. DDQ (6.10g, 26.9mmol) was added in three installments at intervals of 11-12h. The reaction mixture was refluxed under nitrogen atmosphere, the progress of reaction being monitored by TLC and HPLC. The reaction mixture was filtered to remove phenols. Dioxane was evaporated from filtrate under vacuo. The residue was purified by flash chromatography

(hexane: ethylacetate; 9:1) to yield three components including 9α-hydroxycholesta-1-en-3-one **7**. **9α-Hydroxycholestan-1-en-3-one (7).** 1.72 g, mp. 120 -121°C, UV (ethanol) λ_{max} (log ε) 226 nm; IR($CHCl_3$) 3400, 1665 cm^{-1}; ^1H NMR (300 MHz $CDCl_3$) δ 7.22-7.19 (dd, *J*=10Hz, 1H, olefinicH), 5.95-5.91(d, 1H, *J*=10Hz, olefinic H), 1.10 (s, 3H, 19-CH_3),0.71(s, 3H, 18-CH_3), 2.63-0.85 (steroidal protons) ^{13}C NMR (75MHz, $CDCl_3$) δ 199.9(C=O), 157.6(C-2), 128.9(C-1), 74.9(C-9); LRMS m/z (relative intensity) 400(M^+,7), 382(25), 367(12), 269(10), 243(13), 134(100), 122(19); HRMS calculated for $C_{27}H_{44}O_2$ 400.3341, found 400.3336.

Treatment of 9α-hydroxycholest-1-en-3-one (7) with DDQ. A solution of 9α-hydroxycholest-1-en-3-one **7** (0.05 g, 0.12 mmol), DDQ (85mg, 0.37mmol) and dioxane (5mL) was heated under reflux for 11h. The reaction mixture was filtered to remove phenols. The dark red colored filtrate was evaporated and the residue was purified by flash chromatography (hexane: ethylacetate; 9:10) to yield aldehyde **8** (12mg, 86%). IR($CHCl_3$) 3320, 2730, 1712, 1695, 1580,1470 cm^{-1}; ^1H NMR (300 MHz $CDCl_3$) δ10.12(s,1H,-CHO), 7.75-7.72(d, 1H, 1-H), 7.10(s, 1H, -OH, exchangeable), 6.84-6.72(dd, 2H,2-H and 4-H), 3.09-3.05(ddd, 2H, benzylic –CH_2-),2.92-2.88(ddd,1H,Ar-CH_2), 2.53-0.70(m, steroidal envelope). ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.6 (C=O), 191.1(C=O) 161.2, 148.8, 134.9, 127.2, 117.5, 113.9, 96.2; LRMS m/z (relative intensity) 412(2), 394(47), 263(100), 249(9), 193(35), 151(66), 136(23), 134(22), 133(22), 122(8), 121(16), 107(32), 93(31); HRMS calculated for $C_{27}H_{40}O_3$ 412.2977 found 412.2972.

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