An Efficient 4β-Hydroxylation of Steroidal 5-en-3β-ols and 1,4-Conjugation of Steroidal 4-en-3-ones Using SeO_2 Oxidation

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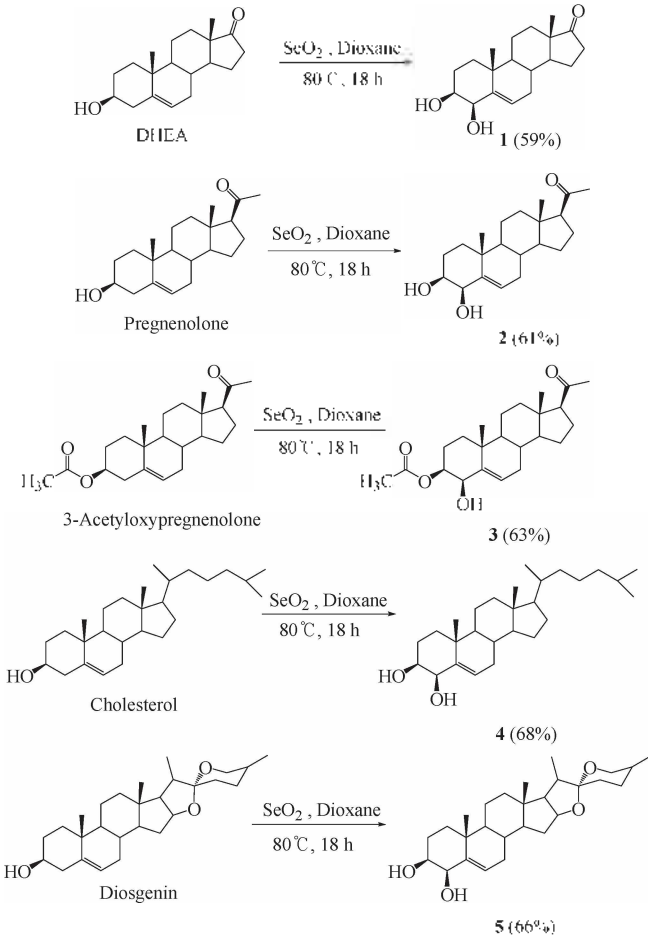
Many of steroidal hormone derivatives were introduced oxygen bearing functionalities in a highly stereoselective and regioselective manner. Because of their biological importance, there has been an immense amount of work on the selective oxidation of steroid hormones.

Allylic oxidation of steroidal 5-en-3β-ols using Cr(VI) reagents, 25% $Na_2Cr_2O_7$ in acetic acid was reported to yield steroidal 4-en-6β-ol-3-ones[1] and using pyridinium dichromate in dimethylformamide[2] were reported to form the corresponding steroidal 4-ene-3,6-diones, respectively. And oxidations of allylic steroidal 5-en-3β-ols using the Collins reagent in methylene chloride[3] and with Jones reagent in acetone at low temperature afforded steroidal 5-en-3-ones or/and 4-en-3-ones.[4] The two-phase oxidation of steroidal 5-en-3β-ols (*via* 5-en-3-ones) into corresponding 4-ene-3,6-diones in diethyl ether with Jones reagent was reported.[5]

Selenium dioxide-mediated oxidation of substituted olefins is regarded as one of the most reliable and predictable methods for introducing a hydroxyl group into allylic position.[6] The chemical synthesis of 6-hydroxycorticosteroids involving allylic oxidation by selenium dioxide was reported.[7] Strommer *et al.*[8] reported that synthesis of 6β-hydroxy derivatives of progesterone and testosterone as steroidal 3-en- 4-one by SeO_2 mediated oxidation. 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one was reacted with SeO_2 to form 3β- hydroxy-5α-cholest-8(14),16-dien-15-one as conjugated products.[9]

Herein, we describe the selective allylic oxidation of steroidal 5-en-3β-ols and expanded conjugation of steroidal 4-en-3-ones using SeO_2 oxidation in dioxane and a trace of H_2O at 80°C for 18 hours, respectively.

The steroidal 5-en-3β-ols were reacted with SeO_2 to produce 4β-hydroxylated derivatives to be oxidized in 4-position of two allylic positions (H-4 and H-6), stereoselectively. (Scheme 1) The structure of 3β,4β-dihydroxy-5-androsten-17-one (**1**) was identified by a new doublet signal corresponding to H-4 in the ^{1}H-NMR spectrum at 4.16 ppm (J = 2.8 Hz). In ^{13}C NMR spectrum of **1**, we also observed two hydroxyl carbon peaks at 77.3 ppm for C-4 and at 72.6 ppm for C-3. The position of hydroxyl group to be introduced was determined by correlation signals of (H-4 and C-3) and (H-4 and C-6) in HMBC spectra. (Fig. 1)



Scheme 1. 4β-Hydroxylation of steroidal 5-en-3β-ols by using SeO_2.

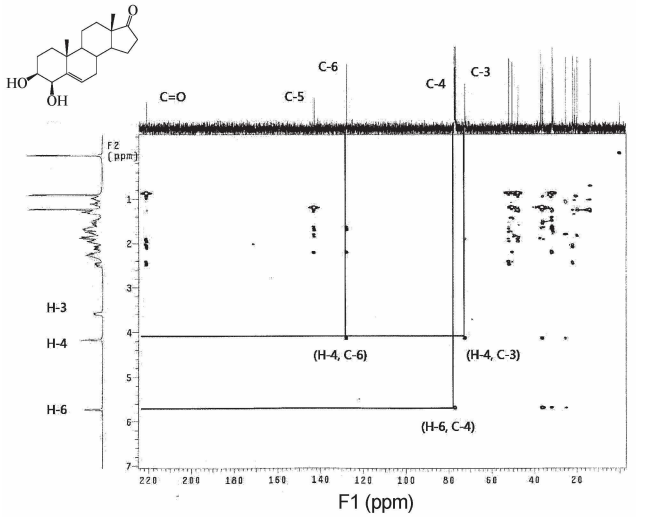


Figure 1. HMBC spectrum of 3β,4β-dihydroxy-5-androsten-17-one (**1**) ($CDCl_3$, 400 MHz).

The β-configuration of 4-hydroxy group was confirmed by irradiation of the H-4 proton (4.16 ppm) which showed an NOE to H-3α (3.55-3.60 ppm) and H-6 (5.72 ppm) in 1D-NOESY spectrum. The IR spectrum showed the absorption band of saturated five membered ring carbonyl and hydroxyl group at 1742 and 3330 cm^{-1} and GC-MS showed the strong signals corresponding to the $(M-H_2O)^+$ and $(M-2H_2O)^-$ at 286 and 268.

The 4β-hydroxylation mechanism of steroidal 5-en-3-ols by using SeO_2 oxidation might be explained that the first step is an ene reaction, transferring the allylic proton to the selenium oxide, and the second step is a [2,3]-sigmatropic reaction.[10,11] (Fig. 2)

The structure of 3β,4β-dihydroxypregnenolone (**2**) and 3β-acetoxy-4β-hydroxypregnenolone (**3**) was established by a new doublet signals in the ^{1}H NMR spectrum at 4.15 ppm (H-4, J = 2.0 Hz) and 4.25 ppm (H-4, J = 2.0 Hz). Similarly, Each H-4 of 3β,4β-dihydroxycholest-5-ene (**4**) and 3β,4β-dihydroxyspirost-5-ene (**5**) was also obtained as a new doublet signal in the ^{1}H NMR spectrum at 4.13 and at 4.14 ppm, respectively.

Steroidal 4-en-3-ones (4-androstene-3,17-dione (**6**), 4-pregnene-3,20-dione (progesterone, **7**), 4-cholesten-3-one (**8**) and 4-spirosten-3-one (**9**)) were reacted with SeO_2 in dioxane and H_2O at 80°C for 18 h. (Scheme 2) Three steroidal 4-en-3-ones were synthesized from 3β-hydroxy-5-androsten-17-one (DHEA), cholesterol and diosgenin by using Oppenauer oxidation, respectively and **7** was purchased from Aldrich. Compound **6** was treated with SeO_2 to give 1,4-androstadiene-3,17-dione (**10**) and 2-hydroxy-1,4-androstadiene-3,17-dione (**11**), in 44 and 21% yield, respectively. The structure of **10** was determined by three double bond protons (H-1, H-2 and H-4) in the ^{1}H NMR spectrum at 7.05, 6.24 and 6.10 ppm. Compound **10** showed the correlation signals between two proton peaks (H-1 and H-4) and C-3 carbon in HMBC spectra. From these spectral data, it was concluded that allylic position (H-6) did not oxidized or dehydrogenated but C1-C2 position was dehydrogenated to form 1,4-conjugated diene-3,17-dione. Therefore, we obtained different result from 6β-hydroxylated steroidal 4-en-3-ones obtained by Strommer.[8] On the other hand, **11** showed two double bond protons at 7.11 (H-1) and 6.13 (H-4) ppm. The position of hydroxyl group to be introduced in **11** was confirmed by HSQC and HMBC spectrum. The HSQC spectrum of **11** exhibited correlation signals be-

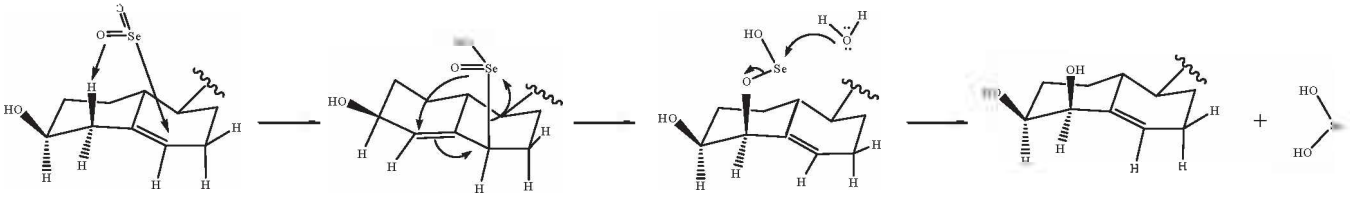
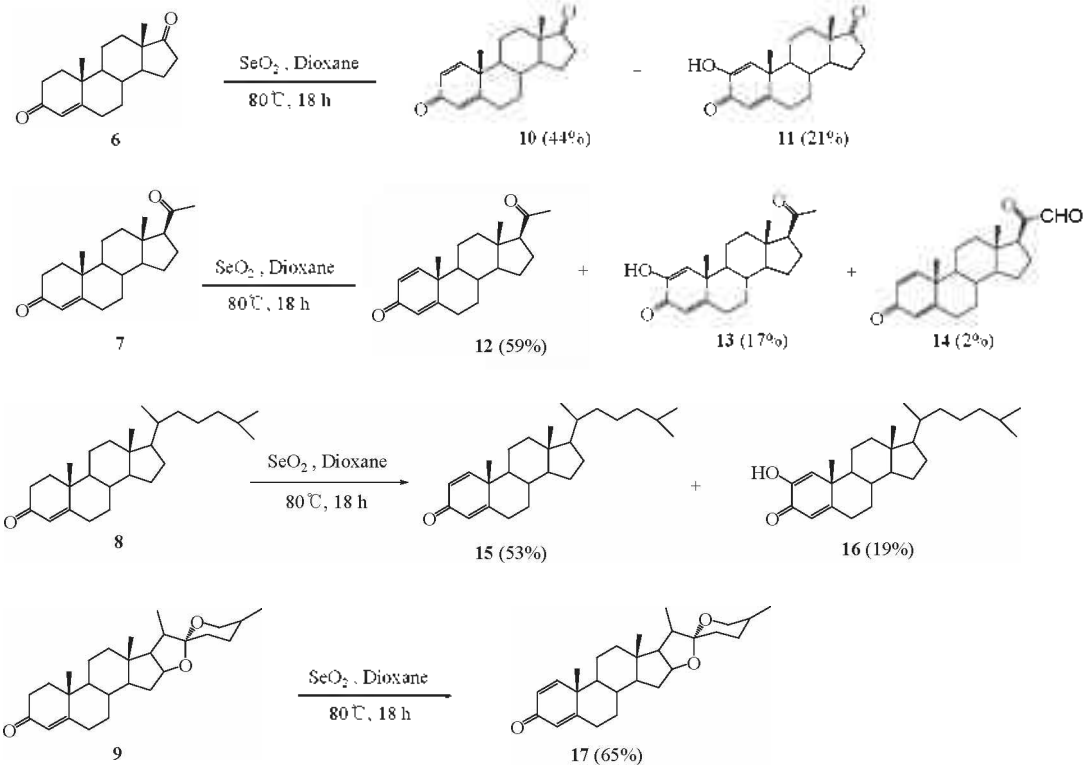


Figure 2. Possible mechanism for 4β-hydroxylation of steroidal 5-en-3β-ols by using SeO_2.



Scheme 2. Dehydrogenation of steroidal 4-en-3-ones by using SeO_2.

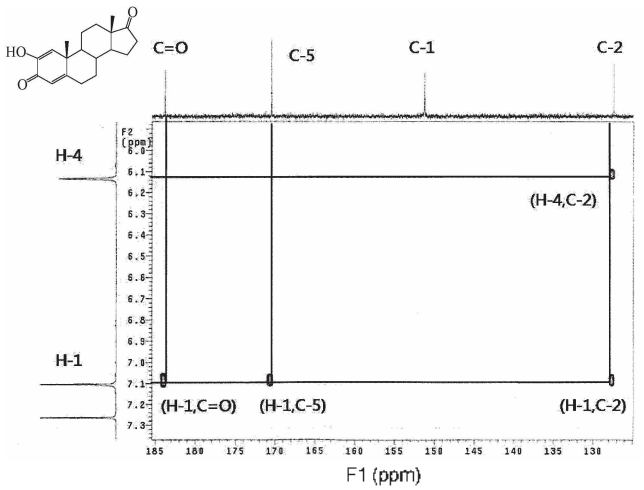


Figure 3. HMBC spectrum of 2β hydroxy 1,4 androstadiene 3,17 dione (**11**) ($CDCl_3$, 400 MHz).

tween carbon peak at 151.5 (C-1) and proton peak at 7.11 (H-1), and between carbon peak at 124.2 (C-4) ppm and proton peak at 6.13 (H-4) ppm, respectively. And we were able to observe the correlation signals between (H-1 and C-5) and (H-1 and C=O) in HMBC spectrum. (Fig. 3) On the basis of these results, the position of hydroxyl group to be introduced was assigned as 2-position.

Compound **7** was reacted with SeO_2 to give 1,4-pregnadiene-3,20-dione (**12**), 2-hydroxy-1,4-pregnadiene-3,20-dione (**13**) and 21-formyl-1,4-pregnadiene-3,20-dione (**14**). The spectral data of **12** and **13** gave the similar pattern with those of **10** and **11**. The structure of **14** was assigned by finding aldehydic proton at 9.24 ppm in ^1H NMR spectrum instead of acetyl hydrogen according to α hydrogen oxidation. Compound **8** was treated with SeO_2 to give 1,4-cholestadien-3-one (**15**) and 2-hydroxy-1,4-cholestadien-3-one (**16**) in 53 and 19% yield. Compound **9** was reacted with SeO_2 to give 1,4-spirostadien-3-one (**17**) in 65% yield. The structures of compounds **12**, **13**, **15**, **16** and **17** were determined by same methods used to identify compounds **10** and **11**, respectively.

In conclusion, these results demonstrate that SeO_2 oxidation enables stereoselective β-hydroxylation in position 4 of certain steroidal 4-en-3-ols and dehydrogenation in C1-C2 position of corresponding steroidal 4-en-3-ones.

Experimental Section

General experimental procedures for melting points, FT-IR spectra, NMR spectra and mass spectrometry have been described previously.[12] ^1H and ^{13}C NMR assignments were performed by 1D-NOESY, HMBC, HMQC spectrum. TLC analyses were carried out on precoated silica gel 60 F_{254} plates (Merck) and substances were visualized by spraying with 5% *p*-anisaldehyde in ethanol followed by heating. TLC solvent systems were ethyl acetate:*n*-hexane mixture (1:1, 1:3, 1:5 and 1:7) or 5% or 10% methanol in dichloromethane. All reactions were performed under nitrogen. Selenium dioxide (SeO_2) and DHEA, diosgenin, cholesterol, pregnenolone and progesterone were purchased from Aldrich.

General procedure for synthesis of steroidal 4β-hydroxy-5-en-3β-ols (1-5) using SeO_2 oxidation. To a solution of steroidal 5-en-3β-ols (500 mg, 1 eq) in dioxane (10 mL) and H_2O (0.05 mL) was added selenium dioxide (1.6 eq.) at room temperature and the reaction mixture was heated at 80 °C for 18 h. Once TLC has confirmed the reaction was complete and the reaction mixture was filtered and evaporated under vacuum to give the crude solid or oily residue, which was dissolved with dichloromethane and H_2O. The organic layer was extracted, dried with anhydrous $MgSO_4$ and filtered, concentrated to brown oily product which was purified by column chromatography (ethyl acetate/*n*-hexane mixture) or MPLC to give the pure products (**1-5**).

3β,4β-Dihydroxy-5-androsten-17-one (1). Yield : 59% (310 mg), mp : 198-200 °C, IR (cm^{-1}): 3330, 2947, 1742, ^1H-NMR (400 MHz, $CDCl_3$) δ : 5.72 (1H, dd, J = 2.0, 5.0 Hz, H-6), 4.16 (1H, d, J = 2.8 Hz, H-4), 3.55-3.60 (1H, m, H-3), 1.21 (3H, s, H-19), 0.89 (3H, s, H-18), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 221.2 (C-17), 143.1 (C-5), 128.1 (C-6), 77.3 (C-4), 72.6 (C-3), 52.1, 50.5, 47.8, 37.1, 36.3, 36.0, 31.7, 31.6, 31.2, 25.5, 22.1, 21.2, 20.1, 13.8, GC-Mass (EI) m/z : 304 $(M)^+$, 286 $(M-H_2O)^+$, 268 $(M-2H_2O)^+$.

3β,4β-Dihydroxy-5-pregnen-20-one (2). Yield : 61% (320 mg), mp: 180-182 °C, IR (cm^{-1}): 3421, 2938, 1682, ^1H- NMR (400 MHz, $CDCl_3$) δ : 5.67 (1H, t, J = 2.8 Hz, H-6), 4.15 (1H, d, J = 3.2 Hz, H-4), 3.56-3.60 (1H, m, H-3), 2.13 (3H, s, $COCH_3$), 1.19 (3H, s, H-19), 0.64 (3H, s, H-18), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 209.8 (C-20), 143.0 (C-5), 128.6 (C-6), 77.4 (C-4), 72.6 (C-3), 63.8, 57.3, 50.2, 44.2, 39.0, 37.1, 36.2, 32.1, 32.0, 21.7, 25.6, 24.7, 23.0, 21.2, 20.8, 13.4, GC-Mass (EI) m/z : 332 $(M)^+$, 314 $(M-H_2O)^+$, 296 $(M-2H_2O)^+$.

3β-Acetoxy-4β-hydroxy-5-pregnen-20-one (3). Yield: 63% (330 mg), mp : 134-136 °C, IR (cm^{-1}) : 3448, 2935, 1737, 1719, ^1H-NMR (400 MHz, $CDCl_3$) δ : 5.70 (1H, t, J = 3.2 Hz, H-6), 4.70-4.75 (1H, m, H-3), 4.25 (1H, d, J = 2.0 Hz, H-4), 2.13 (3H, s, $OCOCH_3$), 2.11 (3H, s, $COCH_3$), 1.22 (3H, s, H-19), 0.64 (3H, s, H-18), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 209.6 (C-20), 170.2 ($OCOCH_3$), 141.5 (C-5), 129.2 (C-6), 75.5 (C-4), 63.6 (C-3), 57.0, 50.1, 44.0, 38.7, 37.0, 36.2, 31.9, 31.7, 31.6, 24.4, 22.8, 21.7, 21.4, 21.0, 20.5, 14.1, 13.2, GC-Mass (EI) m/z: 374 $(M)^+$, 356 $(M-H_2O)^+$, 338 $(M-2H_2O)^+$.

3β,4β-Dihydroxy-5-cholestene (4). Yield: 68% (350 mg), mp : 172-174 °C, (168-172 °C).[13]

3β,4β-Dihydroxy-5-spirostene (5). Yield : 66% (340 mg), mp : 171-173 °C, IR (cm^{-1}): 3390, 2930, ^1H-NMR (400 MHz, $CDCl_3$) δ : 5.64 (1H, s, H-6), 4.41 (1H, dd, J = 6.8, 7.2 Hz, H-16), 4.14 (1H, d, J = 2.6 Hz, H-4), 3.55 (1H, m, H-3), 3.47 (1H, d, J = 9.2 Hz, H-26a), 3.38 (1H, t, J = 10.8 Hz, H-26b), 1.21 (3H, s, H-19), 0.98 (3H, d, J = 6.8 Hz, H-21), 0.80 (3H, s, H-18), 0.79 (3H, d, J = 2.8 Hz, H-27), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 142.9 (C-5), 128.4 (C-6), 109.3 (C-22), 80.8 (C-16), 77.2 (C-4), 72.5 (C-3), 66.9 (C-26), 62.1, 56.7, 50.1, 41.6, 40.3, 39.7, 36.9, 36.2, 32.2, 31.8, 31.4, 30.3, 28.8, 25.4, 21.0, 20.3, 17.1, 16.3, 14.5, GC-Mass (EI) m/z: 430 $(M)^+$, 412 $(M-H_2O)^+$, 394 $(M-2H_2O)^+$.

General procedure for synthesis of steroidal 4-en-3-one using Oppenauer oxidation. To a solution of steroidal 5-en-3β-ol (500 mg, 1 eq.) in cyclohexanone was added aluminum iso-

propoxide (2 eq.) at room temperature and the reaction mixture was refluxed to convert the light orange suspension. The excess of cyclohexanone was distilled off and the residue was dissolved with dichloromethane and H_2O. The organic layer was extracted, dried with anhydrous $MgSO_4$ and filtered, concentrated to brown oily product which was purified by column chromatography or MPLC (ethyl acetate/*n*-hexane mixture) to give the pure products (**6**, **8** and **9**).

Androstene-3,17-dione (**6**): yield: 70% (700 mg), mp: 168-170°C (170-173°C).[14] 4-cholesten-3-one (**8**): yield : 74% (360 mg), mp : 80-82°C, (79-81°C),[15] 4-spirosten-3-one (**9**): yield: 69% (360 mg), mp: 145-147 °C. (149-151°C).[16]

General procedure for synthesis of steroidal 1,4-diene-3, 17-diones and 2-hydroxy-1,4-diene-3,17-diones using SeO_2 oxidation. To a solution of steroidal 4-en-3-one (200 mg, 1 eq.) in dioxane (5 mL) and H_2O (0.05 mL) was added selenium dioxide (1.6 eq.) at room temperature and the reaction mixture was reacted at 80°C for 18 h. The following procedure was performed as the same method described in synthesis of compounds (**1-5**) to synthesize compounds (**10-17**).

1,4-Androstadiene-3,17-dione (10) and 2-hydroxy-1,4-androstadiene-3,17-dione (11). 10: yield: 44% (87 mg), mp: 142-144 °C (141.5-143 °C)[14]. **11**: yield: 21% (44 mg), IR (cm^{-1}): 3422, 2960, 1750, 1649, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.11 (1H, s, H-1), 6.13 (1H, s, H-4), 1.19 (3H, s, H-19), 0.90 (3H, s, H-18), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 219.7 (C-17), 184.0 (C-3), 170.6 (C-5), 151.5 (C-1), 127.6 (C-2), 122.5 (C-4), 53.5, 50.9, 47.8, 46.5, 35.7, 35.2, 32.6, 32.5, 31.6, 22.6, 22.0, 18.9, 13.9, GC-Mass (EI) m/z : 300 $(M)^+$.

1,4-Pregnadiene-3,20-dione (12), 2-hydroxy-1,4-pregnadiene-3,20-dione (13) and 21-formyl-1,4-pregnadiene-3,20-dione (14). 12: yield: 59% (117 mg), mp: 149-152 °C (150-152 °C).[17] **13**: yield: 17% (36 mg), IR (cm^{-1}): 3348, 2958, 1719, 1640, ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 7.10 (1H, s, H-1), 6.13 (1H, s, H-4), 2.10 (3H, s, $COCH_3$), 1.17 (3H, s, H-19), 0.67 (3H, s, H-18),^{13}C-NMR (100 MHz, $CDCl_3$) δ: 208.9 (C-20), 189.1 (C-3), 171.3 (C-5), 152.1 (C-1), 127.5 (C-2), 122.4 (C-4), 63.8, 56.1, 53.3, 46.6, 46.2, 44.4, 38.5, 35.6, 33.6, 32.7, 24.7, 23.3, 23.2, 19.0, 13.6, GC-Mass (EI) m/z: 328 $(M)^+$. **14**: yield : 2 % (4 mg), IR (cm^{-1}): 2963, 1722, 1650, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.24 (1H, s, 21-CHO), 7.04 (1H, d, J = 10.0 Hz, H-1), 6.25 (1H, dd, J = 1.8, 10.4 Hz, H-2), 6.09 (1H, s, H-4), 1.23 (3H, s, H-19), 0.74 (3H, s, H-18),^{13}C-NMR (100 MHz, $CDCl_3$) δ: 200.0 (C-20), 189.0 (CHO), 186.3 (C-3), 168.7 (C-5), 155.5 (C-1), 127.6 (C-2), 124.0 (C-4), 57.4, 55.8, 54.4, 52.2, 45.9, 43.4, 38.5, 35.6, 35.5, 33.5, 32.7, 24.6, 22.7, 18.7, 13.9, GC-Mass (EI) m/z: 326 $(M)^+$.

1,4-Cholestadien-3-one (15) and 2-hydroxy-1,4-cholestadien-3-one (16). 15: yield: 53 % (105 mg), mp: 113-115°C (111-112°C),[18] **16**: yield: 19 % (40 mg), IR (cm^{-1}): 3403, 2951, 1648, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.04 (1H, s, H-1), 6.17 (1H, s, H-4), 1.18 (3H, s, H-19), 0.93 (3H, d, J = 6.2 Hz, H-21), 0.88 (3H, d, J = 6.6 Hz, H-27), 0.86 (3H, d, J = 6.6 Hz, H-26), 0.65 (3H, s, H-18), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 180.5 (C-3), 169.0 (C-5), 149.8 (C-1), 129.3 (C-2), 123.6 (C-4), 57.1, 56.3, 50.4, 42.5, 40.0, 39.7, 37.3, 36.4, 36.2, 36.0, 33.8, 29.4, 28.2, 24.5, 24.0, 23.0, 22.8, 21.3, 20.8, 18.9, 18.3, 12.1, GC-Mass (EI) m/z: 398 $(M)^+$.

1,4-Spirostadien-3-one (17). Yield: 65% (130 mg), mp: 157-159 °C. IR (cm^{-1}): 2945, 1650. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.03 (1H, d, J = 10.4 Hz, H-1), 6.28 (1H, dd, J = 1.4, 10.4 Hz, H-2), 6.07 (1H, s, H-4), 3.45-3.49 (1H, m, H-26a), 3.33-3.39 (1H, m, H-26b), 1.25 (3H, s, H-19), 0.97 (3H, d, J = 6.8 Hz, H-21), 0.85 (3H, s, H-18), 0.79 (3H, d, J = 6.0 Hz, H-27), ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 185.9 (C-3), 169.0 (C-5), 155.7 (C-1), 126.7 (C-2), 122.5 (C-4), 109.3 (C-22), 80.5 (C-16), 73.4 (C-26), 69.5, 62.0, 55.2, 52.4, 41.7, 40.7, 39.5, 35.2, 33.7, 32.8, 31.9, 31.3, 30.3, 28.8, 22.7, 17.1, 16.5, 14,5, GC-Mass (EI) m/z : 410 $(M)^+$.

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