

An Efficient 4 β -Hydroxylation of Steroidal 5-en-3 β -ols and 1,4-Conjugation of Steroidal 4-en-3-ones Using SeO₂ Oxidation

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Received September 14, 2008, Accepted November 18, 2008

Key Words: Allylic hydroxylation, Steroidal 5-en-3 β -ols, 1,4-Conjugated oxidation, Steroidal 4-en-3-ones

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.

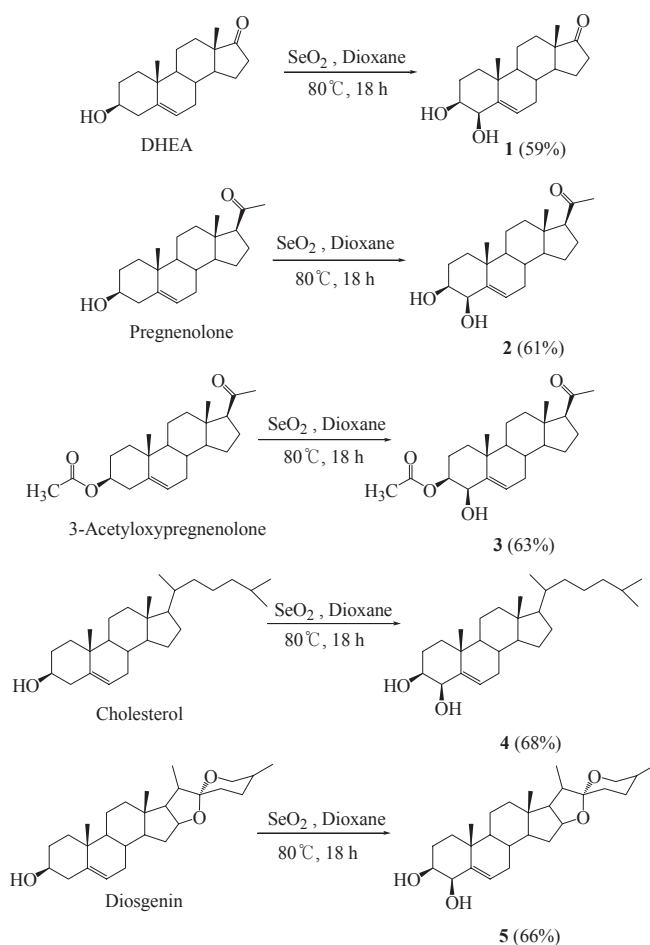
Allylic oxidation of steroidal 5-en-3 β -ols using Cr(VI) reagents, 25% Na₂Cr₂O₇ in acetic acid was reported to yield steroidal 4-en-6 β -ol-3-ones¹ and using pyridinium dichromate in dimethylformamide² were reported to form the corresponding steroidal 4-ene-3,6-diones, respectively. And oxidations of allylic steroidal 5-en-3 β -ols using the Collins reagent in methylene chloride³ 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-3 β -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 6 β -hydroxy derivatives of progesterone and testosterone as steroidal 3-en-4-one by SeO₂ mediated oxidation. 3 β -Benzoyloxy-5 α -cholest-8(14)-en-15-one was reacted with SeO₂ to form 3 β -hydroxy-5 α -cholest-8(14),16-dien-15-one as conjugated products.⁹

Herein, we describe the selective allylic oxidation of steroidal 5-en-3 β -ols and expanded conjugation of steroidal 4-en-3-ones using SeO₂ oxidation in dioxane and a trace of H₂O at 80°C for 18 hours, respectively.

The steroidal 5-en-3 β -ols were reacted with SeO₂ to produce 4 β -hydroxylated derivatives to be oxidized in 4-position of two allylic positions (H-4 and H-6), stereoselectively. (Scheme 1) The structure of 3 β ,4 β -dihydroxy-5-androsten-17-one (**1**) was identified by a new doublet signal corresponding to H-4 in the ¹H-NMR spectrum at 4.16 ppm (*J* = 2.8 Hz). In ¹³C 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. 4 β -Hydroxylation of steroidal 5-en-3 β -ols by using SeO₂.

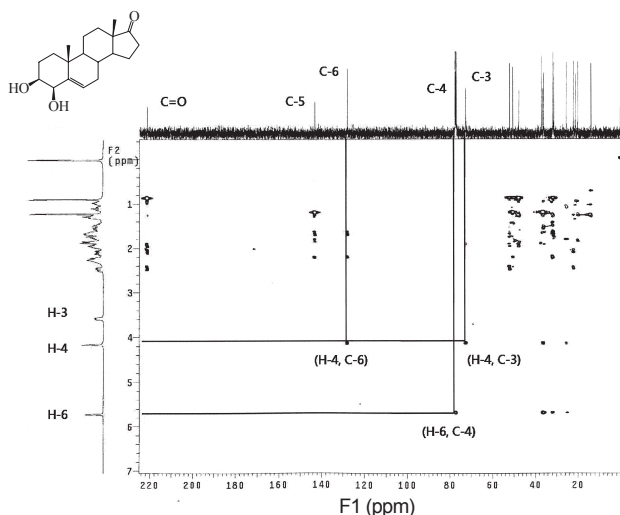


Figure 1. HMBC spectrum of 3 β ,4 β -dihydroxy-5-androsten-17-one (**1**) (CDCl₃, 400 MHz).

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-3 α (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 $(\text{M}-\text{H}_2\text{O})^+$ and $(\text{M}-2\text{H}_2\text{O})^+$ at 286 and 268.

The 4 β -hydroxylation mechanism of steroidal 5-en-3 β -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 3 β ,4 β -dihydroxypregnenolone (**2**) and 3 β -acetoxy-4 β -hydroxypregnenolone (**3**) was established by a new doublet signals in the ^1H 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 3 β ,4 β -dihydroxycholest-5-ene (**4**) and 3 β ,4 β -dihydroxyspirost-5-ene (**5**) was also obtained as a new doublet signal in the ^1H NMR spectrum at 4.13 and at 4.14 ppm, respectively.

Steroidal 4-en-3-ones (4-androstene-3,17-dione (**6**), 4-pre-

gnene-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 3 β -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 ^1H 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 6 β -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-

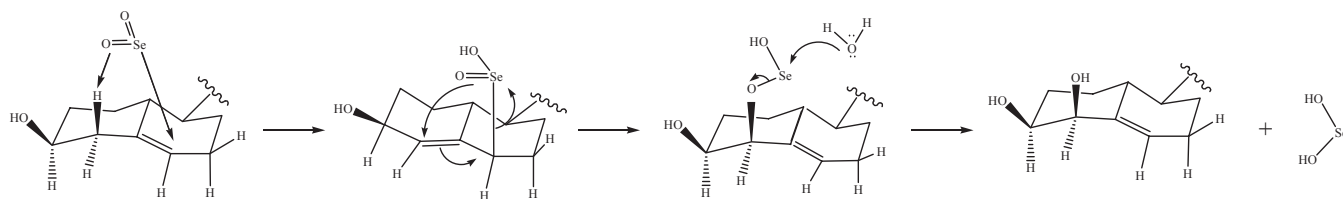
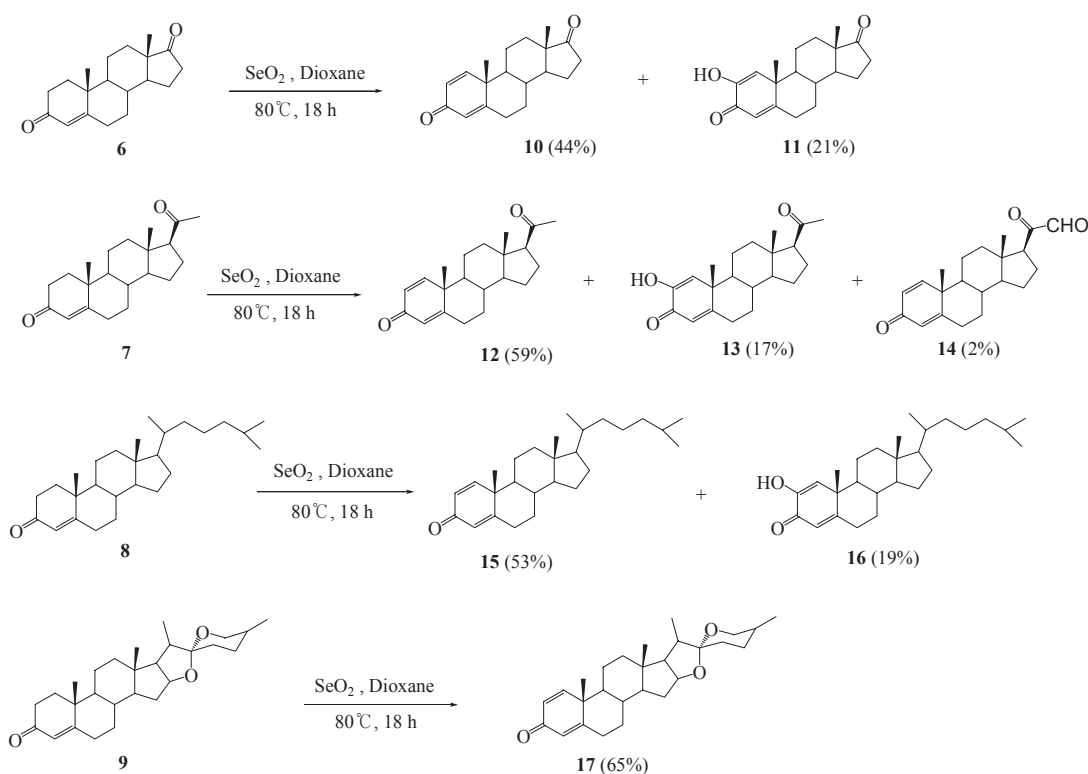


Figure 2. Possible mechanism for 4 β -hydroxylation of steroidal 5-en-3 β -ols by using SeO_2 .



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

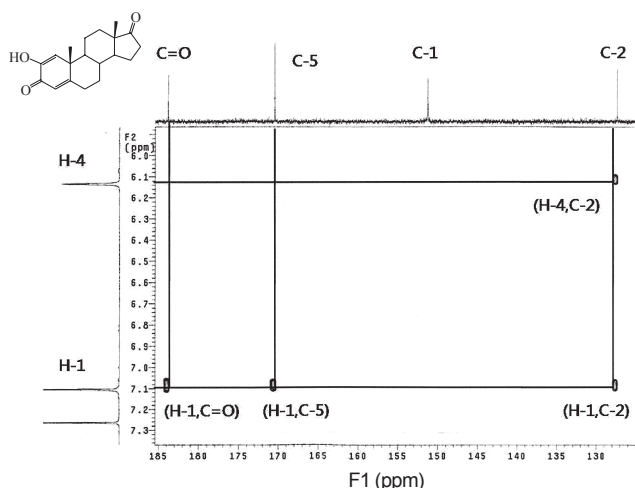


Figure 3. HMBC spectrum of 2 β -hydroxy-1,4-androstadiene-3,17-dione (**11**) (CDCl₃, 400 MHz).

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 SeO₂ 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 SeO₂ 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 SeO₂ 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 SeO₂ 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 ¹³C NMR assignments were performed by 1D-NOESY, HMBC, HMQC spectrum. TLC analyses were carried out on precoated silica gel 60 F₂₅₄ 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 (SeO₂) and DHEA, diosgenin, cholesterol, pregnenolone and progesterone were purchased from Aldrich.

General procedure for synthesis of steroidal 4 β -hydroxy-5-en-3 β -ols (1-5) using SeO₂ oxidation. To a solution of steroidal 5-en-3 β -ols (500 mg, 1 eq) in dioxane (10 mL) and H₂O (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 H₂O. The organic layer was extracted, dried with anhydrous MgSO₄ 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**).

3 β ,4 β -Dihydroxy-5-androsten-17-one (1). Yield : 59% (310 mg), mp : 198-200 °C, IR (cm⁻¹): 3330, 2947, 1742, ¹H-NMR (400 MHz, CDCl₃) 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), ¹³C-NMR (100 MHz, CDCl₃) 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-H₂O)⁺, 268 (M-2H₂O)⁺.

3 β ,4 β -Dihydroxy-5-pregnen-20-one (2). Yield : 61% (320 mg), mp: 180-182 °C, IR (cm⁻¹): 3421, 2938, 1682, ¹H-NMR (400 MHz, CDCl₃) 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, COCH₃), 1.19 (3H, s, H-19), 0.64 (3H, s, H-18), ¹³C-NMR (100 MHz, CDCl₃) 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-H₂O)⁺, 296 (M-2H₂O)⁺.

3 β -Acetoxy-4 β -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, CDCl₃) 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, OCOCH₃), 2.11 (3H, s, COCH₃), 1.22 (3H, s, H-19), 0.64 (3H, s, H-18), ¹³C-NMR (100 MHz, CDCl₃) δ : 209.6 (C-20), 170.2 (OCOCH₃), 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-H₂O)⁺, 338 (M-2H₂O)⁺.

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

3 β ,4 β -Dihydroxy-5-spirostene (5). Yield : 66% (340 mg), mp : 171-173 °C, IR (cm⁻¹): 3390, 2930, ¹H-NMR (400 MHz, CDCl₃) 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), ¹³C-NMR (100 MHz, CDCl₃) 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-H₂O)⁺, 394 (M-2H₂O)⁺.

General procedure for synthesis of steroidal 4-en-3-one using Oppenauer oxidation. To a solution of steroidal 5-en-3 β -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 H₂O. The organic layer was extracted, dried with anhydrous MgSO₄ 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 SeO₂ oxidation. To a solution of steroidal 4-en-3-one (200 mg, 1 eq.) in dioxane (5 mL) and H₂O (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, ¹H-NMR (400 MHz, CDCl₃) 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), ¹³C-NMR (100 MHz, CDCl₃) 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, ¹H-NMR (400 MHz, CDCl₃) δ: 7.10 (1H, s, H-1), 6.13 (1H, s, H-4), 2.10 (3H, s, COCH₃), 1.17 (3H, s, H-19), 0.67 (3H, s, H-18), ¹³C-NMR (100 MHz, CDCl₃) 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, ¹H-NMR (400 MHz, CDCl₃) 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), ¹³C-NMR (100 MHz, CDCl₃) 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, ¹H-NMR (400 MHz, CDCl₃) 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), ¹³C-NMR (100 MHz, CDCl₃) 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, ¹H-NMR (400 MHz, CDCl₃) 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), ¹³C-NMR (100 MHz, CDCl₃) 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)⁺.

Acknowledgments. Financial support from Catholic University of Daegu is gratefully acknowledged.

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