An Efficient 4β-Hydroxylation of Steroidal 5-en-3β-ols and 1,4-Conjugation of Steroidal 4-en-3-ones Using SeO₂ 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% Na₂Cr₂O₇ in acetic acid was reported to yield steroidal 4-en-6β-ol-3-ones⁴ and using pyridinium dichromate in dimethylformamide⁵ 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 chloride⁶ and with Jones reagent in aceton at low temperature afforded steroidal 5-en-3-ones or and 4-en-3-ones.⁷ 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.⁸

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.⁹ The chemical synthesis of 6-hydroxy corticosteroids involving allylic oxidation by selenium dioxide was reported.¹⁰ Strommer et al.¹¹ reported that synthesis of 6β-hydroxy derivatives of progesterone and testosterone as steroidal 3-en-4-one by selenium dioxide mediated oxidation. 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one was reacted with SeO₂ to form 3β-hydroxy-5α-cholest-8(14),16-dien-15-one as conjugated products.¹²

Herein, we describe the selective allylic oxidation of steroidal 5-en-3β-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-3β-ols were reacted with SeO₂ 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-5α-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.** 4β-Hydroxylation of steroidal 5-en-3β-ols by using SeO₂.

**Figure 1.** HMBC spectrum of 3β,4β-dihydroxy-5α-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-3a (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-H₂O)⁺ and (M-2H₂O)⁺ at 286 and 268.

The 4β-hydroxylation mechanism of steroidal 5-en-3-ols by using SeO₂ 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.⁷,¹¹ (Fig. 2)

The structure of 3β,4β-dihydroxy-5-androsten-3-one (2) and 3β-acetoxy-4β-hydroxy-5-androsten-3-one (3) was established by a new doublett 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 3β,4β-dihydroxy-5-androsten-3-one (4) and 3β,4β-dihydroxy-5-androsten-3-one (5) was also obtained as a new doublett signal in the ¹H NMR spectrum at 4.13 and 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 SeO₂ in dioxane and H₂O 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 SeO₂ 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 spectrum. 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 3β-hydroxylated steroidal 4-en-3-ones obtained by Strommer.⁶ 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](image-url)

**Figure 2.** Possible mechanism for 4β-hydroxylation of steroidal 5-en-3β-ols by using SeO₂.

![Scheme 2](image-url)

**Scheme 2.** Dehydrogenation of steroidal 4-en-3-ones by using SeO₂.
between 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-1) 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 a 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 33 and 19% yield. Compound 9 was reacted with SeO₂ to give 1,4-spirostadien-3-one (17) in 63% 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 steroid 4-en-3-ols and dehydrogenation in C1-C2 position of corresponding steroid 4-en-3-one.
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/ether-hexane mixture) to give the pure products (6, 8 and 9).


1. General procedure for the 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 refluxed 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): yield: 44% (87 mg), mp: 142-144°C (141.5-143°C).¹¹ 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), 0.91 (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, 47.9, 46.5, 55.7, 55.2, 22.6, 32.5, 31.6, 22.6, 22.2, 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 12: yield: 17% (36 mg), IR (cm⁻¹): 3348, 2958, 1719, 1640. ¹H-NMR (400 MHz, CDCl₃) δ: 7.00 (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), 0.78 (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).¹⁹ 16: yield: 19% (40 mg), IR (cm⁻¹): 3403, 2951, 1648. ¹H-NMR (400 MHz, CDCl₃) δ: 7.64 (1H, s, H-1), 6.17 (1H, s, H-4), 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), 0.75 (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, 23.8, 21.3, 20.8, 18.9, 18.3, 12.1, GC-Mass (EI) m/z: 598 (M⁺).