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# Synthesis of HMG-CoA Reductase Inhibitor Lanost-8-en-3β-ol-7, 11-dione through Oxidation of 3β-acetoxylanost-8-en-7, 11-dione with Pyridinium Chlorochromate

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**Abstract**

Allylic oxidation of 3B-acetoxylanost-8-ene was accomplished with pyridinium chlorochromate (PCC) and chromium trioxide/ acetic acid to form 3B-acetoxylanost-8-en-7,11-dione, which was later reduced to lanost-8-en-3β-ol-7,11-dione. It was found that lanost-8-en-3β-ol-7,11-dione is a significant inhibitor of HMG-CoA reductase (HMGR), a key enzyme in the biosynthesis of cholesterol. A 1.26 μM concentration of lanost-8-en-3β-ol-7,11-dione re resulted in a 50% inhibition of the reductase activity.

**Keywords**: HMG-CoA Reductase, Steroids, Oxysterols, Sterol Synthesis, Cholesterol Biosynthesis, Inhibition, Pyridinium Chlorochromate

## 1. Introduction

As a class of compounds, oxysterols can be defined as sterols bearing second oxygen containing functional group, in addition to that at carbon-3, and having an iso-octyl or modified iso-octyl side chain. A number of oxysterols have been shown to be potent inhibitors of sterol biosynthesis.

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(Accad et al., 1998; Gill et al., 2008; Brown et al., 1974; Kandutsch et al., 1974; Saucier et al., 1987; Saucier et al.,1989; Parish et al., 1988).

The present work concerns the synthesis of lanost-8-en-3B-ol-7,11-dione, a prominent autoxidation (air oxidation) product of naturally occurring 24,25-dihydrolanosterol, and its evaluation as an inhibitor of HMGR activity. Lanost-8-en-3B-ol-7, 11-dione and 24,25-dihydrolanosterol are both found in wool fat (lanolin) and may offer protection via enzyme inhibition. Two methods were used for oxidation, through PCC and chromium trioxide in acetic acid. The chromium trioxide method has been previously reported. A third method has been reported for allylic oxidation of lanosterol compounds, (Shingate et al., 2011) but will not be discussed because the focus of this article is on chromium reagents. Results of these experiments showed that PCC was the better of the two methods for producing a greater percentage of 3B-acetoxylanost-8-en-7, 11-dione, a key intermediate.

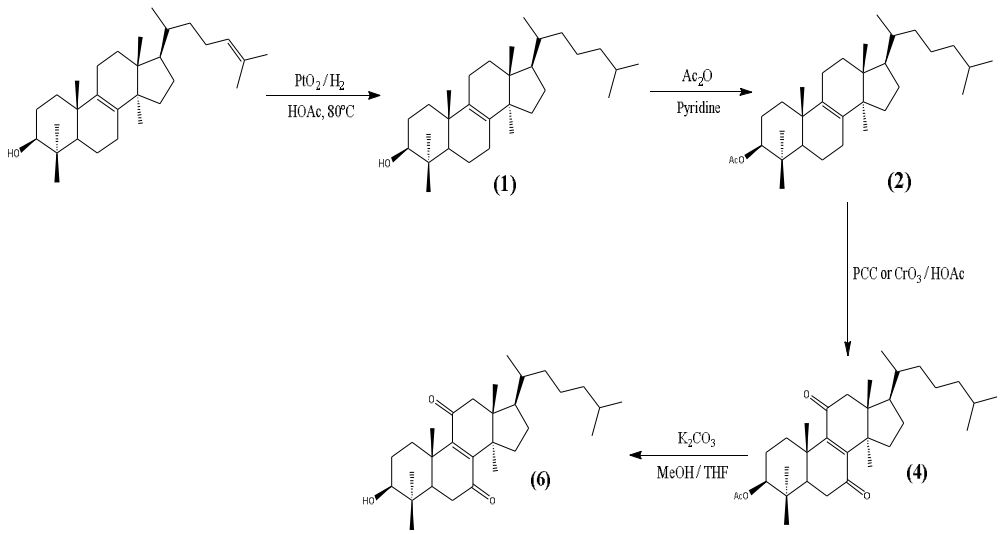


**Figure 1: General Synthetic Scheme**

## Methods

### 24, 25-Dihydrolanosterol (1)

24, 25-dihydrolanosterol was prepared from lanosterol by catalytic hydrogenation. To a solution of 20 g of recrystallized lanosterol in 200 ml of acetic acid, 3.5 g platinum oxide was added. The solution was shaken four hours at 80 $^0$C with hydrogen under a pressure of 55 psi.

After filtering and drying the filtrate, the residue was recrystallized (2x) with acetone/water to yield 17.7 g (88.1%): mp 146-147.5 $^0$C (lit., 148 $^0$C,[23] 146 $^0$C[24] ). $^1$H NMR ($CDCl_3$): 0.69 (s, 3H), 0.81 (s, 3H), 0.86 (s, 3H), 0.88 (m, 6H), 0.98 and 1.00 (s, 6H), 1.25 (s, 3H); $^{13}$C NMR ($CDCl_3$): 79.04 (C-3), 134.50 (C-9), 134.47 (C-8). IR (KBr): 3422, 2950, 1465, 1371, 1028 cm$^{-1}$.

### 3B-Acetoxylanost-8-ene (2)

Acetic acid, 50 ml, was added to 10.0 g (23.3 mmol) of 24,25-dihydrolanosterol in 75.0 ml pyridine and 125 mL acetic anhydride and heated under reflux for 3 hours. The reaction mixture was poured into ice (1500 g) and allowed to stand overnight. Afterword, the product was filtered, washed with water, recrystallized three times from acetone/water and thoroughly dried under vacuum to yield 10.24 g (93.3%): mp 116.5-118 $^0$C (lit., 119 $^0$C[23]). $^1$H NMR ($CDCl_3$): 0.69 (s, 3H), 0.86 (s, 3H), 0.87 (m, 6H), 0.88 (m, 9H), 1.00 (s, 3H), 2.05 (s, 3H), 4.48 (m, 1H); $^{13}$C NMR ($CDCl_3$): 81.01 (C-3), 134.45 (C-9), 134.71 (C-8), 170.77 (C=O). MS: m/z 470. IR (KBr): 2949, 1720, 1371, 1262, 1034 cm$^{-1}$.

**General procedure for allylic oxidation with chromium trioxide/acetic acid**

A mixture of 5 g (10.6 mmol) 3B-acetoxylanost-8-ene in 150 mL acetic acid at 80 $^0$C was added rapidly to a solution of 1.51 g (15.1 mmol) chromium trioxide in 20 mL 90% acetic acid. The solution was kept at 80 $^0$C for 10 minutes and poured into 1 L water where the sterols were extracted thoroughly with ether. The residue was subjected to column chromatography using ether in toluene as the eluting solvents. Elution yielded 3B-acetoxylanost-8-ene (1.38 g, 30%), 3B-acetoxy-9(11)-en-7-one (1.20 g, 26%), 3B-acetoxylanost-8-en-7,11-dione (1.58 g, 30%), and 3B-acetoxylanost-8-en-7-one (0.51 g, 10%) in the respective order.

3B-acetoxy-9(11)-en-7-one (3): mp 150-151 $^0$C (lit., 149-150 $^0$C,[14] 149-151 $^0$C,[25] 150-152 $^0$C[26]). $^1$H NMR ($CDCl_3$): 0.68 (s, 3H), 0.77 (s, 3H), 0.84 (s, 3H), 0.86 (s, 3H), 0.88 (s, 3H), 0.90 (s, 3H), 0.94 (s, 3H), 1.12 (s, 3H), 2.05 (s, 3H), 2.90 (m, 1H), 4.52 (dd, 1H), 5.42 (m, 1H);$^{13}$C NMR ($CDCl_3$): 79.32 (C-3), 150.67 (C-8), 151.72 (C-9), 170.72 (C=O), 201.79 (C-7), 202.37 (C-9). MS: m/z 484. IR (KBr): $_x$1734, 1713, 1671, 1371, 1250 cm$^{-1}$.

3B-acetoxylanost-8-en-7,11-dione (4): mp 157.5-158 $^0$C (lit., 158-159 $^0$C[14]). $^1$H NMR ($CDCl_3$): 0.79 (s, 3H), 0.85 (s, 3H), 0.86 (s, 3H), 0.87 (s, 3H), 0.91 (s, 3H), 0.95 (s, 3H), 1.17 (s, 3H), 1.30 (s, 3H), 2.06 (s, 3H), 4.52 (dd, 1H);$^{13}$C NMR ($CDCl_3$): 79.32 (C-3), 150.67 (C-78), 151.72 (C-9), 170.72 (C=O), 210.79 (C-7), 202.37 (C-9). MS: m/z 498. IR (KBr): 1734, 1713, 1671, 1371, 1250 cm$^{-1}$.

3B-acetoxylanost-8-en-7-one (5): mp 146-148 $^0$C (145-146 $^0$C,[25] 149-151 $^0$C,[27] 151-152 $^0$C[28]). $^1$H NMR ($CDCl_3$): $\delta$ 0.65 (s, 3H), 0.91 (s, 3H), 0.95 (s, 3H), 1.18 (s, 3H), 2.06 (s, 3H), 4.52 (dd, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$ 79.61 (C-3), 139.06 (C-8), 164.6 (C-9), 170.7 (C=O), 198.64 (C=O). MS: m/z 484.

**General procedure for allylic oxidation with pyridinium chlorochromate (PCC)**

To a mixture of 0.5 g (1.06 mmol) 3B-acetoxylanost-8-ene in 25 mL benzene containing molecular sieves, 13.74 g (63.7 mmol) pyridiniumchlorochromate (PCC) was added and stirred gently while refluxing under a nitrogen atmosphere for 24 hours. The benzene solution was decanted, and the remaining contents of the reaction flask were washed with a saturated NaCl solution. After drying, the residue was subjected to column chromatography using a solvent gradient of ether in toluene. Three products were yielded including 3B-acetoxy-8-lanost-9(11)-en-7-one (51.5 mg, 10%), 3β-acetoxylanost-8-en-7, 11-dione (344 mg, 65%), and 3β-acetoxylanost-8-en-7-one (92 mg, 18%) in the respective order. No 3β-acetoxylanost-8-ene was isolated.

**Lanost-8-en-3B-ol-7,11-dione (6)**

A mixture of 2.6 g (21 mmol) 3B-acetoxylanost-8-en-7,11-dione in 130 ml THF and 260 mL ethanol was charged with 26 mL saturated aqueous $K_2CO_3$. The reaction mixture was refluxed for 24 hours under a nitrogen atmosphere. After refluxing, the mixture was poured into 130 mL ice-water.

The precipitate was filtered, dried, and recrystallized from acetone/water to give lanost-8-en-3B-ol-7,11-dione (2.05 g, 86.1%): mp 141-142 $^0$C. $^1$H NMR ($CDCl_3$): 0.80 (s, 3H), 0.85 (s, 3H), 0.86 (s, 3H), 0.87 (s, 3H), 0.89 (s, 3H), 1.02 (s, 3H), 1.20 (s, 3H), 1.30 (s, 3H), 2.87 (t, 1H), 2.90 (t, 1H), 3.27 (m, 1H); $^{13}$C NMR ($CDCl_3$): 77.6 (C-3), 150.65 (C-8), 151.75 (C-9), 202.1 (C-7, C=O), 202.4 (C-11, C=O). MS: m/z 456. IR (KBr): $_x$ 3533, 2932, 2866, 1670 (C=O), 1458, 1380 cm$^{-1}$.

## HMGR Repression Studies

Cell culture studies used a subline of NCTC clone 929 mouse fibroblasts mouse cells, grown in a serum-free medium and the determination of HMG-CoA reductase in cell homogenates was described previously. (Saucier et al., 1987)

## Results and Discussion

Oxidation of 3B-acetoxylanost-8-ene with chromium trioxide in acetic acid yielded 3B-acetoxylanost-8-ene (30%), 3B-acetoxy-9(11)-en-7-one (26%), 3B-acetoxylanost-8-en-7, 11-dione (30%), and 3β-acetoxylanost-8-en-7-one (10%). PCC oxidation of 3β-acetoxylanost-8-ene produced 3β-acetoxy-8-lanost-9(11)-en-7-one (10%), 3β-acetoxylanost-8-en-7, 11-dione (65%), and 3β-acetoxylanost-8-en-7-one (18%). Though both methods produced 3β-acetoxylanost-8-en-7, 11-dione, the PCC method gave a much higher yield. Starting material was also unreacted in the chromium trioxide method. There was, however, a tenth of the substrate used with the PCC method versus the chromium trioxide method.

The concentration of lanost-8-en-3B-ol-7,11-dione in the cell studies gave a 50% repression of HMGR after five hours of incubation. The effect of lanost-8-en-3B-ol-7, 11-dione on the levels of HMGR activity at various concentrations is shown in Figure 2. A 1.26 μM concentration of lanost-8-en-3B-ol-7, 11-dione was required for a 50% inhibition of enzyme activity.

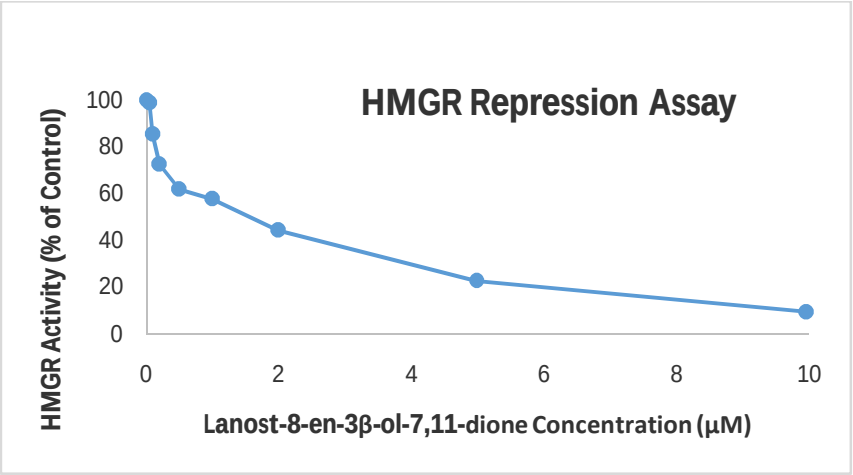


**Figure 2: Effect of 3B-hydroxylanost-8-ene-7,11-dione on the level of HMG-CoA reductase activity in L cells**

## Conclusion

This paper presents a method for the PCC oxidation of 3B-acetoxylanost-8-ene. Allylic oxidation was accomplished with greater yield of 3B-acetoxylanost-8-en-7, 11-dione using PCC rather than chromium trioxide/ acetic acid. The PCC method involved using commercially available lanosterol and ultimately oxidizing it to lanost-8-en-3β-ol-7, 11-dione, which was shown to inhibit HMGR 50% with a 1.26 μM concentration.

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