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SYNTHESIS OF 7-DEHYDROCHOLESTEROL THROUGH HEXACARBONYL MOLYBDENUM CATALYZED ELIMINATION REACTION

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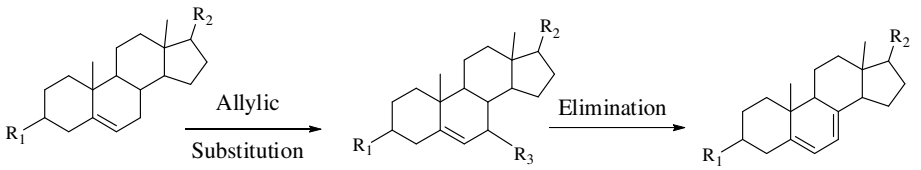
ABSTRACT. The efficiency of hexacarbonyl molybdenum catalyzed elimination reaction of the allylic acetates has been improved by the presence of O,N-bis(trimethylsilyl) acetamide in the reaction medium. The methodology is particularly well employed for the elimination of 7-acetoxycholesterol-3-acetate(cholestrol-3,7-diacetate) for which the resulting product obtained was exclusively 5,7-homoannular diene(7-dehydrocholesterol-3-acetate). Good yield is achieved (up to 70 %) while decreasing the side products formation and reducing the costs as compared to the previously used procedures. Hexacarbonyl molybdenum elimination reaction is greatly influenced by the reaction temperature, at low as well as at high temperature low yield of the homoannular diene product is separated while at moderate conditions of temperature high products formation is observed.

KEY WORDS: Hexacarbonyl molybdenum, Elimination, Deacetoxylation, 7-Dehydrocholesterol, BSA

INTRODUCTION

7-Dehydrocholesterol is an important precursor of vitamin D_3 and its other derivatives [1]. Photoreaction of 7-dehydrocholesterol produces vitamin D_3 [2]. In some cases photo-initiator is required. Our lab has reported an optimized condition for the photo conversion of 7-dehydrosterol derivatives to vitamin D[3]. 7-Dehydrocholesterol has been synthesized throughout the history using different synthetic procedures. Steroidal synthetic route is always a topic of interest for the synthesis of 7-dehydrocholesterol. Allylic oxidation of cholesterol and its derivatives at 7-position and subsequent elimination reaction produces 7-dehydro derivatives (Scheme 1).



R1 = OAc, OH, OBz, O, X etc, R2 = C8O17, O, OH, OAc, R3 = OAc, OH, OBz, O, X

Scheme 1

Allylic bromination of cholesterol at 7-position followed by dehydrobromination produces 7-dehydrocholestrol [4]. The main drawback of this reaction is the formation of side product 4,6-dehydrosterol [5]. This problem is solved to certain extent and the yield of the reaction is improved by adding some intermediate steps [6]. Similarly cholesterol-3-acetate is converted to 7-oxocholesterol-3-acetate following allylic oxidation. 7-Oxocholesterol-3-acetate is reacted

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with tosylhydrazine to produce 7-tosylhydrzone cholesterol-3-acetate and following Bamford-Steven's reaction 7-tosylhydrzone cholesterol-3-acetate is converted to 7-dehydrocholesterol-3-acetate [7, 8]. This route also involves some transition metal catalyzed steps and expansive experimental design. Palladium catalyzed elimination of 7-acetoxycholesterol derivatives to 7-dehydrocholesterol derivatives is the most efficient procedure [9, 10]. But the high prices of palladium catalysts and the complex experimental conditions requirement make it difficult to be applied at commercial scale.

Different procedures have been used for the allylic oxidation of alkene. Some of those procedures have also been applied to steroidal allylic oxidation to synthesize different steroidal oxidized products. Cholesterol is oxidized at allylic position using chromium trioxide and further reduced with sodium borohydride to 7-hydroxycholesterol-3-acetate. 7-Hydroxy-cholesterol-3-acetate esterification produces cholestrol-3,7-diacetate [11]. Choleterol-3,7-diacetate formation is also reported by using electrochemical oxidation of cholesterol [12]. Karash [13, 14] procedure of allylic esterification using copper catalyst has taken much more attention throughout the history for the synthesis of allylic acetates. Different experimental conditions are optimized to make the reaction simple and productive. Peresters are utilized which have been made this reaction more efficient.

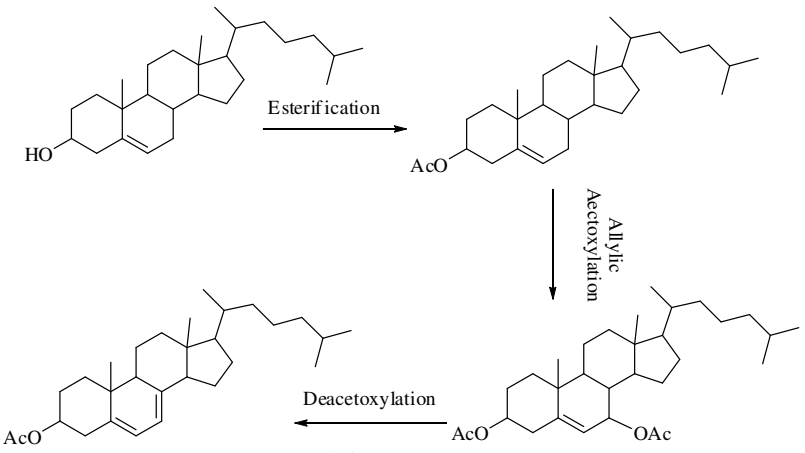
Hexacarbonyl molybdenum and other molybdenum containing catalysts are very important and frequently used for carbon-carbon bond formation at allylic position [15]. Alkene having allylic moiety like acetate, halide, hydroxide, etc. are alkylated by molybdenum catalyst in the presence of a nucleophile [16]. Allyl-molybdenum complex formation of alkene and subsequent attack of nucleophile at allylic position produce allylic alkylated products [17, 18]. Acetates and alcohols conversion to alkene in good yield is another important property molybdenum containing catalyst [19]. When hexacarbonyl molybdenum or other molybdenum containing catalysts are employed in the absence of nucleophile allylic acetates are converted to diene following elimination reaction [20]. In the presence of O,N bis-(trimethylsilyl) acetamide good yield of diene was obtained [21].

Simply we can say that the cheaper and simple copper catalyzed or electrochemically produced allylic acetates are good precursor of diene. Palladium catalyst is a promising system for the conversion of these allylic acetates to diene. But this elimination reaction is disfavored by the high cost of palladium catalyst, requirement of complex reaction conditions and the formation of side products. To minimize the cost and make the reaction specific and productive hexacarbonyl molybdenum is used for the conversion of cholestrol-3,7-diacetate to 7-dehydrocholesterol following elimination reaction.

In this paper we report the detailed synthetic study of cholestrol-3,7-diacetate and further conversion of cholestrol-3,7-diacetate to 7-dehydrocholesterol-3-acetate by following elimination reaction in the presence of hexacarbonyl molybdenum/O,N bis-(trimethylsilyl) acetamide catalytic system. Good yield (70%) of 7-dehydrocholesterol-3-acetate was obtained which is further reduced to 7-dehydrocholesterol.

RESULTS AND DISCUSSION

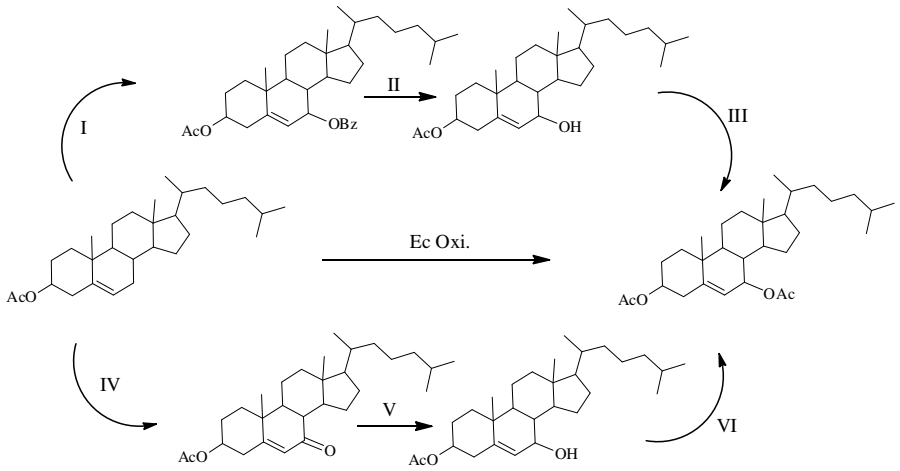
Cholesterol was converted to cholesterol-3-acetate to protect the alcoholic group. Cholesterol-3-acetate was oxidized at allylic position to get cholestrol-3,7-diacetate, which is further converted to 7-dehydrocholesterol-3-acetate by hexacarbonyl molybdenum catalyzed elimination reaction. Scheme 2 shows a simple description of the whole process.



Scheme 2

Acetoxylation of cholesterol-3-acetate at 7-position

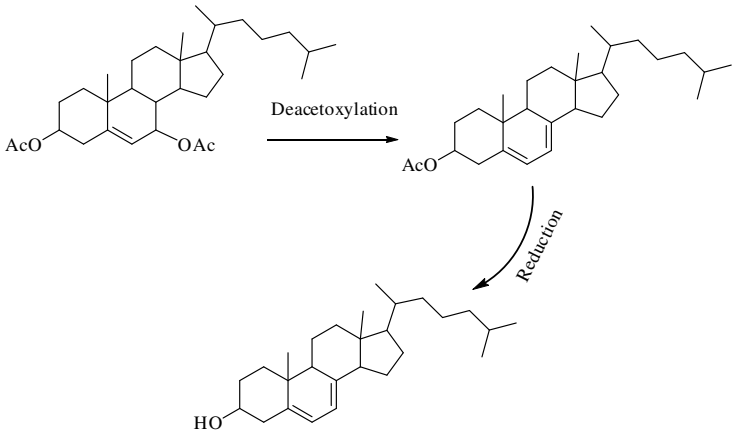
Cholesterol-3,7-diacetate was synthesized by following two main synthetic routes (Scheme 3). Cholesterol-3-acetate allylic benzoyloxylation and further reduction and esterification of to cholestrol-3,7-diacetate as shown by Scheme 3 steps I, II and III. Following this procedure 50% yield was obtained. The second route employed for the synthesis of cholestrol-3,7-diacetate was chromium or cobalt catalyzed allylic oxidation in the presence of ter-butyl hydroperoxide to produce 7-oxocholesterol-3-acetate which is further reduced to 7-hydroxycholesterol-3-acetate and in turn to cholesterol-3,7-diacetate. The final yield of acetate obtained by cobalt and chromium allylic oxidation was almost similar (45%), but due to the hazardous nature of chromium catalyst cobalt allylic oxidation step is frequently used in route 2. A schematic diagram of both routes is represented by Scheme 3.



Scheme 3. I) [22] CuBr *t*-BuOOCOPh, II) [23] $NaBH_4$/methanol, III,VI) acetic anhydride, pyridine overall yield 70%, IV) [24] CrO_3, TBHP, or [25] $Co(Ac)_2$/silica TBHP V) [26] $NaBH_4$, $CeCl_3$ in 30% THF/methanol, Ec Oxi: Electrochemical Oxidation [12].

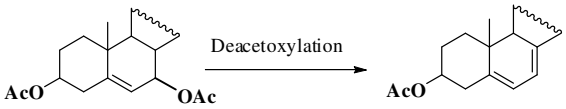
In the above two routes the most productive and less hazardous is route I (copper allylic benzoyloxylation and subsequent reduction and esterification to cholesterol-3,7-diacetate). This route is employed in major in this study, total yield of cholesterol-3,7-diacetate obtained following this route was 50%.

Molybdenum catalyzed deacetoxylation of cholesterol-3,7-diacetate to 7-dehydrocholesterol-3-acetate



Scheme 4. Deacetoxylation: $Mo(CO)_6$, O,N bis-(trimethylsilyl) acetamide, 80 °C, 70% yield. Reduction: $NaBH_4$/methanol.

Molybdenum interaction with alkene double bond bearing allylic moiety is a very important property of molybdenum containing catalysts being utilized in organic synthesis. At high temperature allyl-molybdenum complex is converted to diene in good yield with the abstraction of beta proton. The same conditions are applied to cholestrol-3,7-diacetate which resulted in good yield of the conjugated homoannular diene of cholesterol-3-acetate (7-dehydrocholesterol-3-acetate). Table.1 shows different conditions and yield of this elimination reaction.



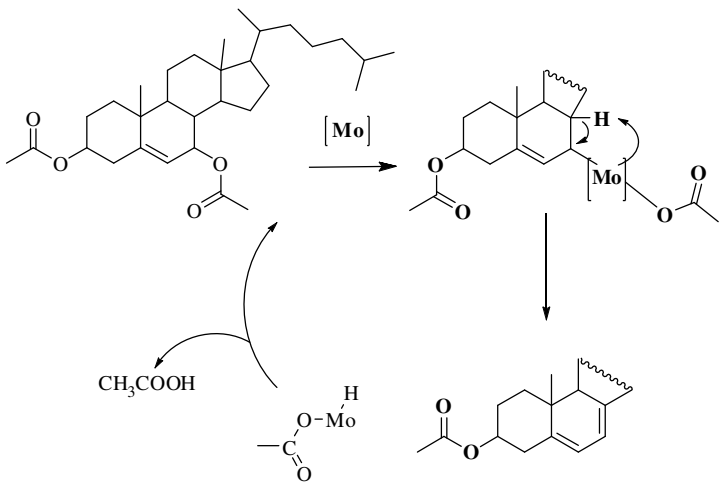
Scheme 5

Table 1. Reaction conditions and percent yield of elimination reaction.

S/No	Cholesterol-3-acetate (mmol)	$Mo(CO)_6$ (mmol)	BSA (mmol)	Time (h)	Temperature (°C)	Yield (%)
1	1.25	0.2	2	8	40	30
2	1.25	0.2	2	8	50	50
3	1.25	0.2	2	8	60	50
4	1.25	0.2	2	8	70	65
5	1.25	0.2	2	8	80	70
6	1.25	0.2	2	8	110	60

Temperature is a very important factor affecting this reaction. At low temperature the yield obtained was low but increasing the temperature increases the diene formation. While increasing the temperature beyond 80 °C the yield of the reaction decreases. At high temperature molybdenum has the ability to eliminate any acetoxyl group attached to carbon [19] in the form of acetic acid molecule. So at high temperature the decrease in diene yield may be due to the removal of acetoxyl group at 3-postion of cholesterol and there may be a triene formation, which is under investigation.

The mechanism of the reaction is the attack of molybdenum on the allylic moiety to make a complex with the allylic position and further beta proton elimination to give diene as a main product.



Scheme 6

No cholesta-4,6-diene-3-acetate product formation is observed by HPLC, which indicated that no double bond migration occurs in this reaction. This suggest the molybdenum complexation with allylic carbon at 7-position with the removal of acetoxyl group and further removal of a proton from 8-postion of cholesterol-3-acetate produces 7-dehydrocholesterol-3-acetate.

CONCLUSIONS

In summary, a selective and productive method for the elimination of allylic acetates of cholesterol is devised. The method afford good yield (70%) of 7-dehydrocholesterol. The reaction expanses are reduced several times because this catalytic procedure is much cheaper as compared to the previously used palladium based catalytic process. No 4,6-heteroannular diene formation is observed which is a drawback of dehydrobromination procedure. In short it is a cheaper method and comparatively pure 7-dehydrocholesterol is obtained by this method. The reaction require moderate conditions of different parameters, furthermore the application of this catalytic system to other cholesterol derivatives, optimization of different parameters like bases (BSA), solvent system and concentrations of different constituents of the reaction medium is under investigation.

EXPERIMENTAL

Analytical grade solvents used were purchased from Beijing Chemical Company (China) and were used without further purification. Hexacarbonyl molybdenum (98%) and tert-butylperoxybenzoate (98%) were purchased from Alfa Acer Chemical Company (USA). O,N-bis(trimethylsilyl) acetamide (95%) and standard 7-dehydrocholesterol (97.5%) containing one mole methanol of crystallization were purchased from Acros Organics (USA). Acetic anhydride was bought from Sinopharm Chemical Reagent Company Ltd (Shanghai, China). For UV analysis Shimadzu UV Spectrophotometer (Japan) was used. IR Spectra were taken by Varion 3200 FT-IR Spectrophotometer (USA). Shimadzu SCL-10A HPLC system with Shimadzu UV detector SPD-10A (Japan) and silica column (Kromasil SiO_2-5μ Φ 4.6*250 nm Dalian Replete Science and Technology Company Ltd (China) was employed for HPLC analysis. Cholestrol-3,7-diacetate was analyzed by HPLC with UV detector at 210 nm wavelength and 7-dehydrocholesterol-3-acetate was quantitatively analyzed by HPLC at 281 nm. The mobile phase used was 10% THF/n-hexane at 1 mL/min flow rate. Shimadzu GCMS QP2010 with Agilent Column DB-5MS (Japan) (30 m*0.250 mm*0.25 um) was used for GC-MS analysis. The injector temperature was 280 ºC, sample was separated in the temperature programming from 50 ºC to 300 ºC at a rate of 20 ºC to 100 ºC hold for one min at 100 ºC, then 40 ºC till 220 ºC and 8 ºC till 300 ºC and hold for 5 min to clean the column. NMR spectra were taken in $CDCl_3$ at 600 MHz on Bruker AV-600 spectrometer (Germany).

Synthesis of cholesterol-3-acetate

5 g of cholesterol was taken in 40 mL of benzene. 10 mL of pyridine and 10 mL of acetic anhydride was added to it and refluxed for 3 h. The mixture was cooled to room temperature and diluted with 30 mL benzene. The reaction was quenched with deionized water and washed several time with 1 M NaOH solution. Then neutralized with 1 M HCl and washed again with deionized water, dried with Na_2SO_4 and vacuum evaporated.

Synthesis of cholesterol-3,7-diacetate

All the following procedures from the cited literature were applied with a little modification.

Allylic benzoyloxylation of cholesterol-3-acetate with copper catalyst in the presence of tertiary butyl perbenzoate. 1 g (2.5 mmol) of cholesterol-3-acetate was taken in 30 mL of dichloromethane. 2 equivalents 5 mmol 0.72 g of CuBr was added to it and stirred for 15 min. 4 equivalents 2.26 mL (2.3 mL 98%), tertiary butyl perbenzoate was added to it drop wise and stirred overnight at 40 °C. The resultant mixture was filtered through a pad of silica, washed several times with brine and deionized water, dried with sodium sulfate and evaporated at low pressure to afford 7-benzoyloxycholesterol-3-acetate.

Conversion of 7-benzoloxycholesterol-3-acetate to 7-hydroxycholesterol-3-acetate. Sodium borohydride (6 equivalents) was suspended in THF. The crude product obtained from the above reaction was added to it and refluxed at 70 ºC with constant stirring. Then methanol (8 mL) was added drop wise during a period of 15 min. Stirring was maintained for one hour. After reaction's completion (checked by TLC), the resulted mixture was cooled to room temperature and quenched with saturated NH_4Cl solution and extracted with dichloromethane. The extract was washed several times with deionized water, dried with Na_2SO_4 and vacuum evaporated to dryness.

Synthesis of 7-oxocholesterol-3-acetate using CrO_3 catalyst. 0.01 equivalents of CrO_3 catalyst was taken in round bottom flask containing 30 mL of dichloromethane and 2 mL of pyridine. 5 equivalents tertiary butyl hydroperoxide was added to it and gentle stirring was begun at room temperature. After a few min 1 g 2.5 mmol of cholesterol-3-acetate was added to it stirring was continued for 24 h at 25 ºC. The reaction mixture was filtered over a pad of silica. The filtrate was washed several times with sodium sulfite solution and deionized water, dried with anhydrous sodium sulfate and evaporated at low pressure to get 7-oxocholesterol-3-acetate.

Synthesis of 7-oxocholesterol-3-acetate using $Co(OAc)_2/SiO_2$ catalyst. In a typical reaction, to a solution of cholesterol-3-acetate 1 g (2.5 mmol) in benzene (20 mL) under nitrogen, 0.01 equivalents cobalt acetate, 1 g of SiO_2 and tert-butyl hydroperoxide 0.5 mL were added. After 24 h under constant stirring at 50 °C, the catalyst was removed by filtration; the filtrate was poured into sodium sulphite solution (10% aq.) and extracted with diethyl ether. Extract was washed with aqueous saturated solution of $NaHCO_3$, water, dried over anhydrous sodium sulfate and evaporated to dryness to give 7-oxocholesterol-3-acetate.

Conversion of synthesis of 7-oxocholesterol-3-acetate to 7-hydroxycholesterol-3-acetate. 1 g of 7-oxocholesterol-3-acetate (obtained from Cr, Co catalyzed allylic oxidation steps) and 1 equivalent of cerium chloride was taken in 30% THF/methanol (30 mL) in a round bottom flask and stirred till complete dissolution of all the solids. 2 equivalents of sodium borohydride in portions was added to it and further stirred for 1 h. 2 mL of acetic acid was added to it and extracted with tetra chloromethane. The solvent was evaporated at low pressure to result 7-hydroxycholesterol-3-acetate.

7-Hydroxycholesterol-3-acetate conversion to cholesterol-3,7-diacetate. 1 g of 7-hydroxycholesterol-3-acetate was taken in 20 mL of benzene. 5 mL of pyridine and 5 mL of acetic anhydride was added to it and refluxed. After 3 h the mixture was cooled to room temperature and diluted with 20 mL benzene. The reaction was quenched with deionized water and washed several time with 1 M NaOH solution. Then neutralized with 1 M HCl and washed again with deionized water, dried with Na_2SO_4 and vacuum evaporated to get solid crude cholesterol-3,7-diacetate product.

Synthesis of 7-dehydrocholesterol (molybdenum catalyzed deacetoxylation reaction)

1.25 mmol 0.5 gram of cholesterol-3,7-diacetate and 2 mmol 0.39 g O,N bis-(trimethylsilyl) acetamide in 30 mL of toluene was heated to 80 °C. 0.2 mmol 0.05 g of hexacarbonyl molybdenum was added to it. After 5 h the reaction mixture was cooled to room temperature, diluted with toluene, washed several time with 1 M sodium hydroxide solution, deionized water and dried with anhydrous sodium sulfate. Low pressure evaporation of the solvent affords yellowish liquid, which is further subjected to sodium borohydride reduction, resulted 7-dehydrocholesterol. Pure product for analysis is obtained by purification of the crude product over silica gel column, using 10% ethyl acetate/n-hexane eluent system. Mass spectrum m/z 426 (M^+), 366(M^+- HOAc), 351, 281, 253, 207, 158, 143, 135, 119.

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