Multidisciplinary Journal of Chemistry
Volume 10 Issue 3, July-September 2022
ISSN: 2995-4398
Impact Factor: 6.22
https://kloverjournals.org/journals/index.php/chem

DISTANT TRANSFORMATIONS: CHROMIC ANHYDRIDES' ROLE IN STEROID SIDE CHAIN MODIFICATION

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Abstract
Natural steroids feature diverse side chains in terms of their structure and composition, presenting unique challenges for organic chemists [3-6]. The modification of existing side chains, even those containing unsaturation, is relatively straightforward compared to the intricate and time-consuming process of constructing new side chains. Many naturally occurring steroids possess saturated side chains devoid of useful functionalities, complicating the chemist's task [1,2]. This study focuses on the intricate field of functionalizing saturated side chains in steroids, with a particular emphasis on cholesterol. Through rigorous chemical synthesis techniques, we aim to address the complexities surrounding this problem and contribute to a deeper understanding of steroid chemistry. Our research seeks to provide valuable insights into the synthesis of functionalized steroids, opening new avenues for drug development, biochemistry, and the pharmaceutical industry.

Keywords: Steroids, Saturated side chains, Functionalization, Chemical synthesis, Cholesterol

1. Introduction

In steroids that occur naturally, side chains vary in their structure and composition [3-6]. Individually, they represent unique challenges to the organic chemist with the task of chemically modifying intact side chains or constructing new ones through multi-step synthesis. Chemical modification of an existing side chain; which may contain unsaturation, is a much easier task than the construction of a new side chain which may be time consuming and require many chemical steps. Many naturally occurring steroids contain side chains that are saturated and are devoid of any functionality which the chemist might use as an intermediate in the modification process. Functionalization of saturated side chains of steroids (eg. cholesterol) by chemical synthesis is a difficult problem for the organic chemist [1,2].

The need to modify the saturated side chain of certain steroids may arise through the study of enzymatic transformations of the side chain and the requirement to have authentic standards for the identification of potential metabolites. Also, the synthesis of biochemical inhibitors would require introducing functionality at selected positions on the side chain. Collectively, the necessity to modify the saturated side chain of steroids has been the driving force for developing facile methods to accomplish this goal. In the past, the side chain of steroids could only be modified by using the unsaturation already available in a naturally occurring steroid. In this way steroids such asdesmosterol(cholesta-5, 24-dien-3□-ol) [7, 9], fucosterol (24□methylenecholest-5-en-3□-ol [8,10], stigmasterol (24□-ethylcholest-5, 2 dien-3□-ol)

[11, 12], and lanosterol (lanosta-8,24-en-3□-ol) [13-16] could be easily converted into side-chain derivatives for various purposes. Unfortunately, steroids such as desmosterol and fucosterol may not be readily available in larger quantities and are costly starting materials.

Another approach is to directlyintroduce side chains, with various functionalgroups, onto an existing steroid nucleus. This approach usually requires a multi-step synthesis and in most cases the final product is produced in low or very moderate overall yields. This area of research continues to be very active and many different investigators have directed their talents to the construction of steroid side chains. These may contain diverse functionality, including unsaturation, which could be further modified in an additional sequence ofreactions. This approach usually involves constructing a side chain by modifying carbon atoms 17-24 [17-35].

A more efficient method to the functionalization of steroid side chains involves the direct introduction of a modification into the side chain in a single chemical reaction. This approach is termed "remote functionalization" and represents a rapidly developing area in steroid chemistry which promises to streamline the synthesis of many difficult to obtain steroids. Through the earlier work of Breslow, methods for the remote functionalization of the steroid nucleus have become well known reactions [36-40]. The functionalization of remote positions on the steroid nucleus and side chain represents some of the most important advances in the steroid field. Remote functionalization of a saturated steroid side chain usually involves oxidation on the tertiary carbon at C-25 to produce a 25-hydroxysterol. In most studies these reactions have been developed in the cholesterol series and the end product of these reactions is cholest-5-en-3□-diol (25-hydroxycholesterol).

The predominant target molecule in many studies has been 25-hydroxycholesterol due to its interesting biological properties and its ability to be dehydrated to desmosterol which can be further chemically modified. 25Hydroxycholesterol is a member of class of sterols known as oxysterols which possess a second oxygen function in addition to that at carbon-3 and are known to have diverse biological activities [41-50]. Some of these include cytotoxicity, atherogenicity, carcinogenicity, mutagenicity, hypocholesterolemia, and various effects on25Hydroxycholesterol is known to be a very potent inhibitor of 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase, a key regulatory enzyme in cholesterol biosynthesis (IC50<l μM) [41,44]. Metabolism in mammalian systems is known to produce side chain oxysterols. Derivatives of cholesterol hydroxylated in the 25- or 26-positions are produced in liver during bile acid synthesis. Another mode of oxysterol biosynthesis has been described which utilizes the isopentenoid pathway to produce side chain derivatives of cholesterol and lanosterol [63]. Such compounds are derived from squalene 2, 3-epoxide by the introduction of a second oxygen function to form. 2, 3; 22, 23-dioxidosqualene prior to cyclization. Thus, this intermediate has been shown to form 24(S), 25-epoxylanosterol, 24(S), 25epoxycholesterol, and 25-hydroxycholesterol in mammalian systems [64-67]. 24(S), 25-epoxycholesterol has been isolated from cultured mouse L cell, Chinese hamster lung fibroblasts, and human liver [61]. These oxygenated side chain derivatives have been shown to be potent inhibitors of HMG-CdAreductase and sterol biosynthesis, and possess a high affinity for the oxysterol binding protein [61, 64-67]. These results add further support to the hypothesis that oxysterols may be natural regulators of cholesterol biosynthesis in mammalian cells [55, 67, 68].

This dioxidosqualene pathway has recently been reviewed [67, 68]. Also, its occurrence in plants, animals, and microorganisms [69, 70] and its evolution in a variety of organisms have been reviewed [71].

2. Synthetic Methods Ofremote Functionalization

Earlier, efforts to obtain 25-hydroxycholesterol directly from cholesterol included attempts to enhance the air or autoxidation process [48]. Fieser examined cholesterol samples of differing only in age and concluded that cholesterol undergoes oxidation to the 3□, 25-diol on storage in the crystalline state inthe presence of air [72]. Bubbling air through a refluxing solution of crystalline cholesterol inbenzene only resulted in air oxidation products other than the 3□, 25-diol.

It was therefore concluded that the observed selectivity of hydroxylation at the C-25 position, in the air oxidation of cholesterol, was a consequence of the regular arrangement of the steroid molecules on the major faces of the plate-like crystals [73]. On this basis a more general hypothesis was advanced that when molecules are observed in a close-packed array the mutual steric effect of one upon the other may hinder reactions at the usual position and thus enhance oxidation of more exposed terminal areas. Using this approach, experiments were conducted in which barium sulfate or neutral alumina bearing absorbed cholesterol was heated in air or oxygen in the presence of an initiation, or irradiated with ultraviolet light. In each of these experiments some3□, 25-diol was formed, but the yields (approx. 3%) were less than those obtained by autoxidation of the crystalline steroid, and the product mixtures were very complex [74]. The poor results of these earlier experiments are not surprising since the C-5 double bond, which is a major site for autoxidation, is not chemically protected and thus contributes tothe low yields of the 3□,25-diol.

Another approach to the direct functionalization of side chains with diatomic oxygen is the use of ozone. Mazur has developed useful procedures for the ozonolysis of steroid substrates adsorbed on dry silica gel to introduce oxygen into unactivated tertiary C-H bonds [75,76]. This method involves preadsorption of substrate on chromatographicgrade silica gel and passing over it ozone at temperatures between 75and -45^{o}C, followed by elution with an appropriate organic solvent. The reactivity of the tertiary C-H bonds towards ozonedepends both on the electronegativity and the steric availability of the carbon atom [75]. Inthis way, the dry ozonation of l□,3□diacetoxy-6,7-dibromo-5□-cholestane led tothe C-25 hydroxylated derivative as the only isolated product (11% conversion and 51% yield) [75].

In a later study, a number of saturated cholestane derivative substituted at positions 5, 6, and 7 which served as protecting groups for ring B double bonds were ozonized on silica gel to produce the C-25hydroxylated products (Figure 1). Also, a
3□-acetoxy-6, 7-dibromo-5□-cholestanewasozonated to its C-25 hydroxy derivative and further dehydrobromination led to the respective 5,7-diene [75]. The protecting groups, for the ring B double bonds, served a dual function. Inaddition to their protecting role, they sterically hinder the approach of ozone to the other tertiary carbon atoms on the steroid nucleus and allow an increasedyield of the C-25 hydroxylatedproduct.

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Impact Factor: 6.22
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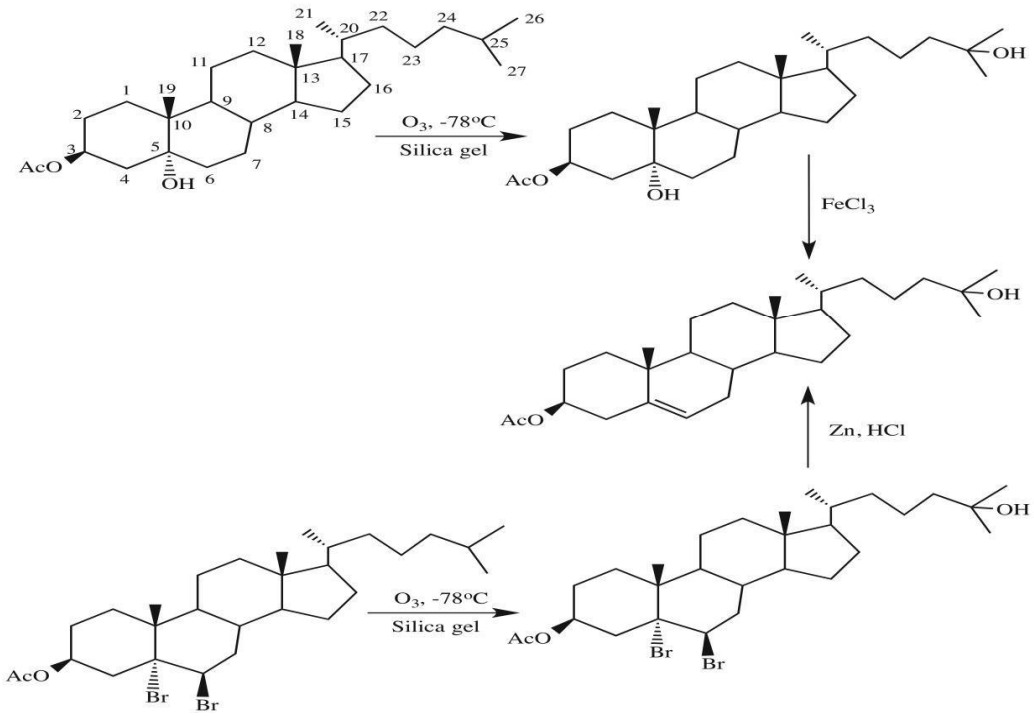
Figure 1



In other studies of side chain remote functionalization, the Gif system has been used to oxidize cholestane derivatives to the corresponding C-20 ketones [7780]. The oxidation of cholest-4-en-3 one by the Gif system to give progesterone has been studied. The optimum temperature was ~20°C and a reaction temperature below 0°C gave 25-hydroxycholest-4-en-3-one as the majorproduct [81]. A long range in tramolecular functionalization of an irradiated 7☐-hypoiodite derivative of cholestane has been reported to yield the C-25 hydroxysteroid as a final product [82]. Another report describes the C-25 hydroxylation of cholesterol by amembrane-spanning Mn(II) porphytin positioned in a synthetic bilayer assembly [83]. This synthetic porphyrin is capable of mimicking the hydroxylation activity of certain cytochrome P450 enzymes. Also, remote functionalizationtoproduce hydroxylation at C-25 has been achieved using dioxiranes. Dioxiranes had previously been described as highly effective reagents in oxyfunctionalization of saturated hydrocarbons and the steroid nucleus [84, 85]. Both dimethyldioxaran and methyltrifluoromethyldioxarane converted3☐-acetody-5☐-cholestane to 3☐-acetolycholest-5☐, 25-diol in a simultaneous double oxy functionalization (Figure 2) [86].

In a related study, the direct and high yield oxyfunctionalization of 5☐cholestan-3-one, 3☐-acetoxy-5☐-cholestane, and 3☐-acetody-5☐,6☐-dibromocholesta new as achieved toproduce the C-25 hydroxy derivative under mild conditions using dimethyldioxirane or its trifluoromethyl analog (Figure 2) [87]. The lattersteroid was cleanly (yield 93%) converted to 3☐-acetoxycholest-5-en-25-ol upon debromination with zinc in acetic acid, thus restoring the $\square^{5,6}$ double bond that had been protected by the dibromide.

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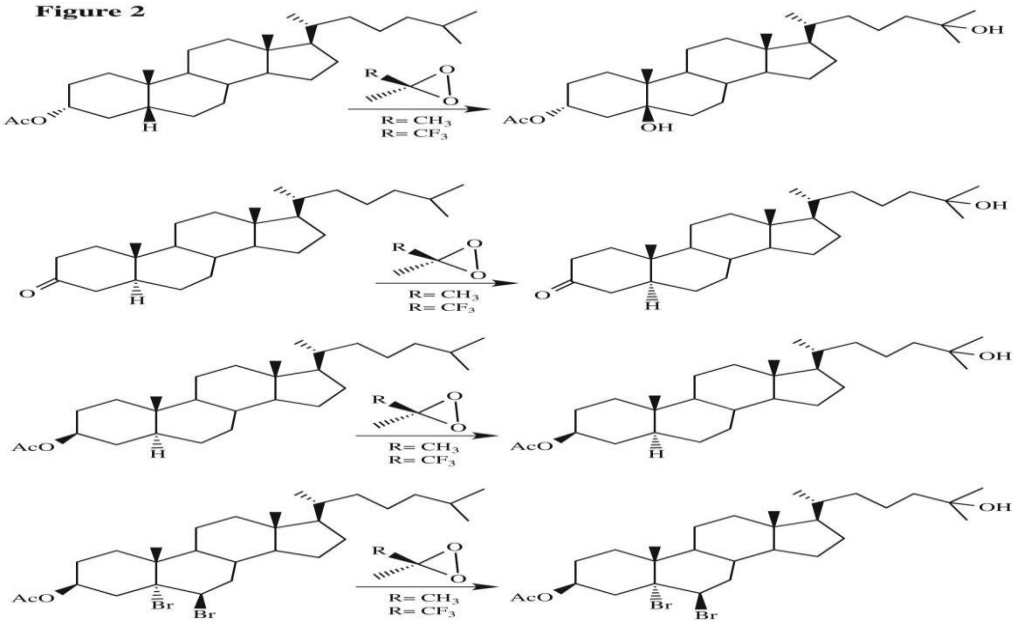
Figure 2



Another successful approach to remote functionalization involves the use of a mixture hydrogen peroxide and trifluoroaceticanhydride (trifluoroperacetic acid) together with sulfuric acid to oxidize and cleave steroid side chains. Deno and Mercer initially developed this method [88, 89] which was also utilized by Takano [90] to oxidize selected steroids to the corresponding C-24 alcohols. In this way, an amide derivative of cholesterol (which was finally converted into the □^{5,6}double bond), cholest-4-en-3-one, and 3-acetoxycholest-5-en-7-one were oxidized to give the C-24 hydroxy derivatives in modest yields (17-19%) (Figure 3).

A significant improvement in this procedure was described by Schroepfer et al. which allowed the isolation of the end product in much higher yields. In this procedure, 3□-acetoxycholest-8(14)-en-15-one was oxidized to a crude mixture of products which was further treated with triethylamine in methanol to provide 3-acetoxy-24-hydroxy-chol-8(14)-en-15-one in 61% yield. [91-94] (Figure 3). In these studies, Schroepfer et al. describes the use of their C-24 alcohol as a key intermediate to modify the steroid side chain.

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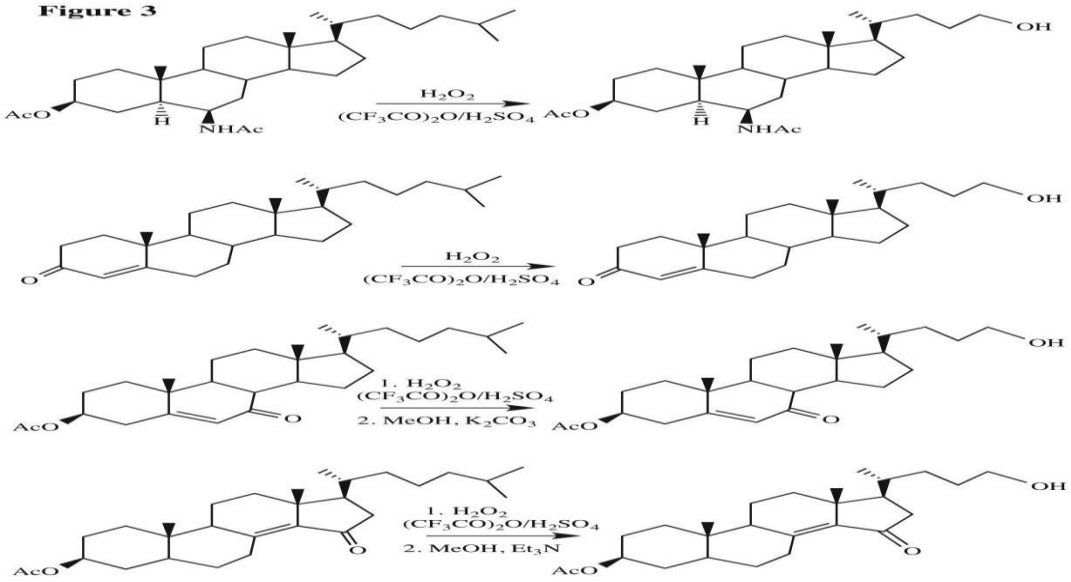
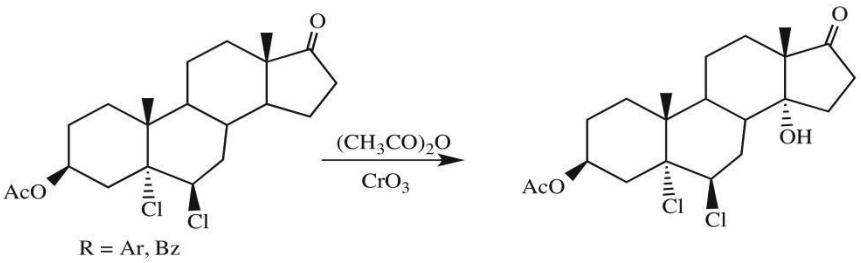
Figure 3



3. Discussion and Results

An alternate approach to remote functionalization, that has been useful forintroducing a 14□-hydroxyl group onto steroids devoid of a side chain, is the use of chromyl acetate (Figure 4) [95-97].

Figure 4



This reagent is made in situ from acetic anhydride and chromium trioxide and was previously used for the oxidation of hydrocarbons [98]. We became interested in this reagent for possible development into a new method for direct hydroxylation at C-25. This procedure possessed the desirable properties of being simple, convenient, and applicable to larger scale reactions. After optimization, we were able to isolate the C-25 hydroxy derivative of 3□-acetoxy- or 3□-benzoyloxy-5□, 6□-dichloro-5□cholestane in yields of 14-18% (Figure 5) [99,100]. The success of this procedure depends on maintaining some starting material during the course of reaction to prevent further oxidation of the initially formed C-25 hydroxylated product. The accessibility of the relatively less hindered C- 25 on the side chain allowed an initial selective oxidation at this position followed by partial oxidation of other tertiary carbons on the steroid nucleus which would result in complex oxidation products. The initial C-25 hydroxylated product was then treated with zinc in acetic acid to remove the 5□,6□-dichloride and restore the □^{5,6} double bond [96] and the ester function at C-3 was removed by mild base hydrolysis [14] to produce 25hydroxycholesterol (Figure 5).

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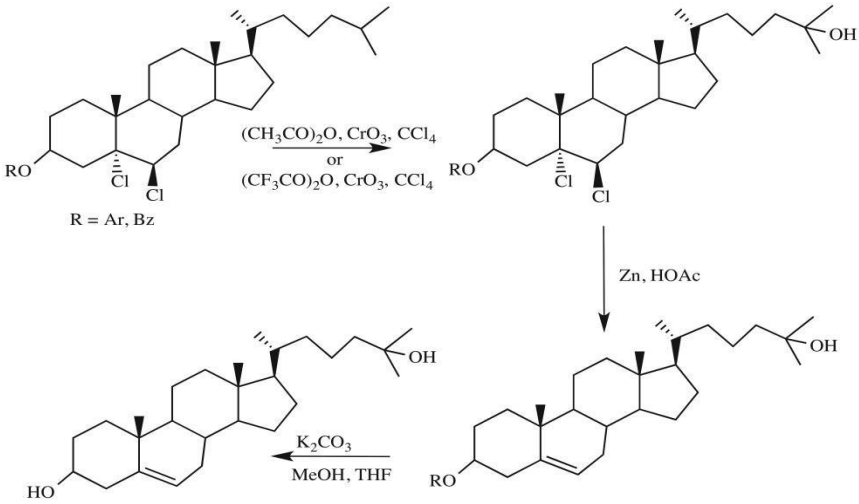
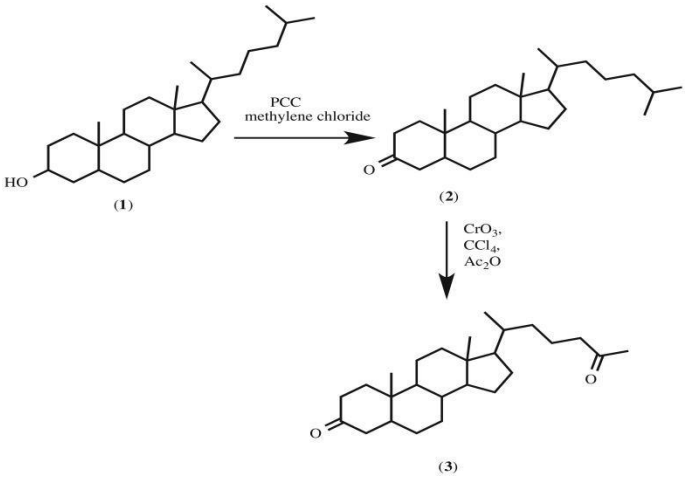
Figure 5



In related experiments, we were able to improve these results through the use of the reagent trifluorochromylacetate which can be prepared in situ from the reaction of trifluoroacetic anhydride and chromium trioxide [101]. Again, this reagent system hasa history of oxidizing hydrocarbons [101]. After optimization and using the same techniques described, we were able to isolate the C-25 alcohol of the previously described starting material in yields of 40-60% (Figure 3) [99,100] Using the chromyl acetate reagent, described in this report, we have applied it to another class of steroids with success.

Figure 6



As shown on Figure 6, we used dihydrocholesterol**1** as a starting material . Compound **1**was treated with pyridiniumchlorochromate in methylene chloride for 1.5 hours. After recrystallization from acetone-water, and purification with column chromatography with toluene as an eluent, dihydrocholestan-3-one (**2**) was obtained in 78% yield. Compound **2** was dissolved in carbon tetrachloride, and was oxidized with chromyl acetate for 30 min. in an ice-bath. Then, the product was purified through column chromatography with toluene, and then with ether in toluene (5-

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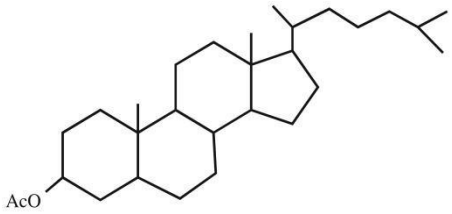
10%) as an eluent, compound (**3**), 27-norcholest-5-en-3□, 25-dione, was obtained in 25% yield. This was confirmed by ^{13}C NMR, C-25 had a chemical shift at 209.2 ppm (209.5 ppm [103]), for C-24 at 44.2 ppm (lit. 44.4 ppm [103]), and for C-26 at 29.8 ppm (lit. 29.8 ppm [103]). Unlike compound (**3**), C-25 of starting material (**2**) had a chemical shift at 27.98 ppm, C-26 at 22.77 ppm, C-27 at 22.53 ppm. Using the same starting material, dihydrocholesterol (**1**), was converted to the 3□-acetate by treating compound **1** with acetic anhydride in pyridine for 3 hours under reflux condition.
After crystallization, dihydrocholesteryl acetate (**4**) was obtained in 75% yield (Figure 7).

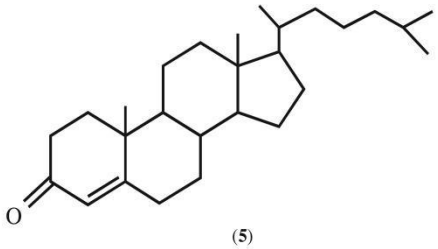
Figure 7



(4)

Compound **4** was oxidized by chromyl acetate for 30 min. in an ice-bath. The product was subjected to TLC analysis and found to be a complex of oxidation products. We tried this reaction with another starting material (**5**) with a keto group at C-3, which was prepared from cholesterol. By treating cholesterol with pyridiniumchlorochromate (PCC) and calcium carbonate in methylene chloride for 0.5 hours, the product (cholest-5-en-3-one) was isomerized in ethanol containing oxalic acid. After recrystallization, cholest-4-en-3-one (**5**) was obtained in 91% yield (Figure 8).

Figure 8



(5)

Compound (**5**) was oxidized with chromyl acetate, and from TLC analysis, many polar products or complex oxidation products were produced. This reaction was not pursued further. The results of those studies have shown that the 3-ketone **2** can be oxidized by chromyl acetate to the C-25 ketone **3**. This is a new remote functionalization reaction in the steroid series. The chemical methods currently available to the organic chemist offer a variety of reagents and techniques for remove functionalization. Each method may offer certain advantages that could be utilized and applied to specific steroidal substrates. In general, methods that are simple, convenient, and produce products inreasonable yields will find more frequent and wide spread use amongresearches. Inour opinion, three methods meet these basic requirements: ozonolysis on dry silica gel, hydrogen peroxide and trifluoroacetic anhydride

with sulfuric acid, and chromyl acetate or trifluorochromyl acetate. These reagents, or their precursors, are commercially available and are convenient to use inthe laboratory and do not require the use of specialized equipment or apparatus which is difficult to acquire. We believe our procedure, which we have described herein, will prove to be a useful method for the remote functionalization at C-25 in steroid side chains and will be an attractive alternative to other known procedures.

4. Experimental

4.1. General Methods

Procedure for recording of melting points (M.P.) and infrared (IR), ^{1}H NMR, and mass (MS) spectra were those used previously [104]. Similarly, details concerning the use of thin-layer (TLC) and column chromatography have been described [105]. Solvent systems for TLC analysis were: 20% ether in toluene (by volumes) in all cases unless stated otherwise.

4.2. Chemical Synthesis

4.2.1. Preparation of Dihydrocholestan-3-one (2)

Dihydrocholesterol (5.0 g; 12.9 mmol; **1**) was dissolved in 150 mL of methylene chloride, 100 mg of molecular sieves (type 4A) was added into the sterol solution. Then, 13.89 g (0.064 mol) of pyrydiniumchlorochromate was added into the sterol solution, and the solution was stirred at room temperature for 1.5 hours. The reaction mixture was filtered through the filtration funnel which was filled with silica gel. The filtrate was evaporated to dryness, and was recrystallized from acetone and water. The crystal was purified through column chromatography (solvent: 0-20% ether in toluene) to give a white crystalline compound (dihyrdocholestan-3-one, **2**, 0.8 g; 85% yield).

M.P.: 128-129^{o}C (Lit. 129-130^{o}C [105]). IR: 1715 cm^{-1} (C=O). 1

H NMR: 0.67 (s, 3H, C-18-CH_3), 1.01 (s, 3H, C-19-CH_3). 13

C NMR: 211 (C-3), 27.98 (C-25), 22.77 (C-26), 22.53 (C-27).

MS: 386 (M; 18%), 371 (M-CH3; 10%), 273 (M-side chain; 2%), 231(100%).

4.2.2. Preparation of 27-Nor-5-cholesten-3□, 25-dione (3)

Chromic anhydride (10.8 g; 108 mmol) was pulverized and added to a solution of acetic anhydride (4.28 mL) in carbon tetrachloride (42.8 mL), and the mixture was stirred at room temperature for 4 hours. A solution of the dihydrocholestan-3-one (1.0 g; 2.6 mmol; **2**) in 14.4 mL carbon tetrachloride was added and stirring was continued for 30 minutes in the ice bath. The mixture was poured into an excess of sodium hydrogen sulphite solution and ice. The products were extracted 3 times with chloroform. Then the extracts were washed with water and saturated salt solution, dried with sodium sulfate anhydrous, and evaporated to dryness. The product was purified through the column chromatography (solvent: 0-30% ether in toluene) to give 27-nor-5-cholest-5-en-3□, 25-dione (**3**, 0.5 g; 50% yield).

M.P.: 84.5-86^{o}C.

IR (KBr):□$_{max}$: 1716, 1442, 1385, 1230 cm^{-1}. 13

C NMR: 211.94 (C-3), 209.2 (C-25), 29.8 (C-26). 1

H NMR: 0.67 (s, 3H, C-18-CH_3), 1.01 (s, 3H, C-19-CH_3), 2.13 (s, 3H, C-26-CH_3).

MS: 386 (M; 5%), 371 (M-CH_3; 3%), 273 (M-side chain; 6%), 231(25%), 43 (100%).

4.2.3. Preparation of Dihydrocholesterylacetate (4)

Dihydrocholesterol (20.0 g; 51.5 mmol; **1**) was placed in a 1 liter round flask and dissolved with 90 mL pyridine. Acetic anhydride (40 mL) was added into the mixture and heated to 35-45^{o}C for 2-3 hours under reflux conditions. The reaction mixture was poured into a beaker of ice-water and allowed to stand for several hours. Using a Buchner funnel, the precipitate was filtered and washed 2-3 times with distilled water. The product was dried to give a white crystalline solid of compound **4** (15.0 g; 34.8 mmol; 68% yield).

M.P.: 109-110^{o}C

IR (KBr): $\square_{max}$: 1738, 1469, 1365, 1238, 1026 cm^{-1}. 13

C NMR: 73.70 (C-3), 28.58 (C-6), 22.52 (C-27), 22.52 (C-26).

MS: 430 (M; 8%), 415 (M-CH_3; 4%), 370 (M-acetic acid; 15%), 355 (M-acetic acidCH_3; 12%), 43(100%).

4.2.3. Preparation of Cholest-4-en-3-one (5)

Anhydrous $CaCO_3$ powder (2.0 g, 19.98 mmol) was added to a solution of cholesterol (1.85 g; 4.79 mmol) in CH_2Cl_2 (160 mL). Pyridiniumchlorochromate (3.5 g; 16.24 mmol) was added and the mixture stirred for 30 min. under nitrogen at room temperature (25^{o}C). A saturated NaCl solution was then added, and the mixture was thoroughly extracted with ether. The resulting extracts were filtered through anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure to give a residue (TLC analysis indicated a product of approx. 98% purity) which was cholest-5-en-3one [106,107]. The residue was dissolved in ethanol containing oxalic acid and isomerized to cholest-4-en-3-one **5** by the method developed by Fieser [108] (**5**; 1.67 g; 91% yield).

M.P.: 80-81^{o}C. (81.0-82.0^{o}C [108]). IR: 1684; 1621; 872 cm^{-1}. 1

H-NMR: 0.72 (s, 3H, C-18-CH_3), 1.20 (s, 3H, C-19-CH_3), 5.69 (s, 1H, C-4-H). 13

C-NMR: 215.80 (C-3); 150.04 (C-4); 120.12 (C-5).

MS: 384 (100, M); 369 (15, M-CH_3); 343 (40); 299 (14); 271 (17, M-CH_3-side chain); 261 (46).

5. Conclusion

The remote functionalization of steroid side chains is a continuing challenge in synthetic organic chemistry. The application of new and known reagents, with a demonstrated ability to oxidize hydrocarbons, has the potential to increase the number of reagents which can be used inthe steroid fields for this purpose.

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