Allylic Oxidation of Steroidal Compounds Using Vanadyl Acetylacetonate and Tert-butyl Hydroperoxide

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
August 1, 2015

Keywords: Steroids, Vanadyl Acetylacetonate, Allylic Oxidation, Steroidal Dimers

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Abstract

Allylic oxidation is an important reaction in steroid chemistry. Previously reported methods have various limits which do not apply when using vanadyl acetylacetonate and tert-butyl hydroperoxide. These limits include costs, need for inert environments, short half-lives of catalysts, over-oxidation, need for protection groups, and low yields. Vanadyl acetylacetonate is inexpensive and shelf stable. Here, we describe the novel use of vanadyl acetylacetonate for oxidation of a variety of Δ^5 steroidal olefins, without protection groups, in ambient atmosphere and at room temperature.

Acknowledgments

The author thanks God, his wife, his family, Dr. Parish, and the faculty and professors at Auburn University for a challenging and enlightening journey.

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List of Abbreviations

acac	Acetylacetonate
DCE	Dichloroethane
DCM	Dichloromethane
DHEA	Dehydroepiandrosterone
NHPI	N-Hydroxyphthalimide
OAc	Acetate
PCC	Pyridinium Chlorochromate
PFC	Pyridinium Fluorochromate
Py	Pyridine
TBAB	Tetrabutylammonium Bromide
TBHP	Tert-Butyl Hydroperoxide
tBu	Tert-Butyl
$VO(acac)_2$	Vanadyl Acetylacetonate

1. Sterols and Steroids

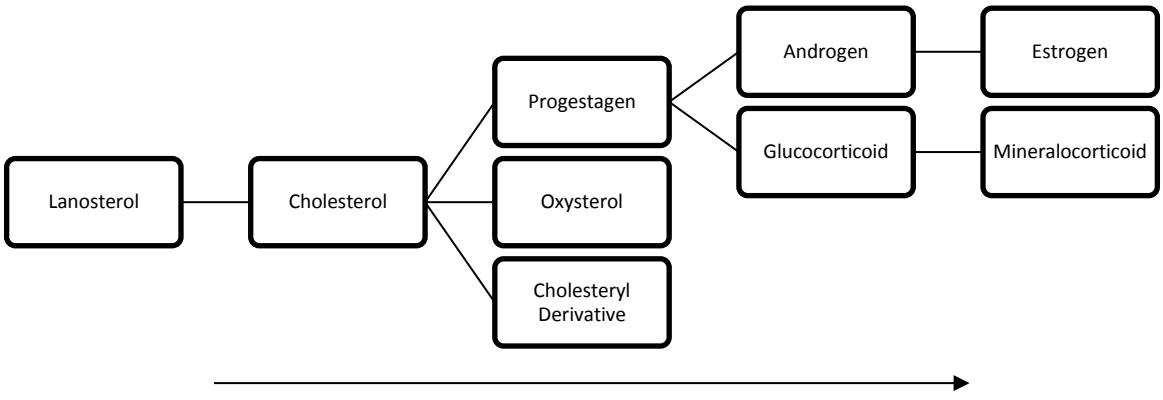
1.1. Identifying Sterols and Steroids

Animals with their adrenal glands removed will die within two weeks, unless given the steroid aldosterone.[1] The mineralocorticoid is but one steroid that plays a major biological function in everyday human life. Everything from male/female maturation, muscle growth, metabolism, sexual attraction, to the body's response to stress is affected by steroids.[1] Even after hundreds of years of research, steroid chemistry is abuzz with new knowledge and the pursuit of synthetic means and new derivatives.[2-4]

The general definition of steroids as defined by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry is (1989), "compounds possessing the skeleton of cyclopenta[α]phenanthrene or a skeleton derived therefrom by one or more bond scissions or ring expansions or contractions. Methyl groups are normally present at C-10 and C-13. An alkyl side chain may also be present at C-17." They also defined sterols as, "steroids carrying a hydroxyl group at C-3 and most of the skeleton of cholestane. Additional carbon atoms may be present in the side chain."[5]

Sterols have more rightly been defined as (1977) “any hydroxylated steroid that retains some or all of the carbon atoms of squalene in its side chain and partitions almost completely into the ether layer when it is shaken with equal volumes of water and ether.”[1] William D. Nes recently defined sterols even better as (2011), “amphipathic compounds that originate in isoprenoid biosynthesis with the main frame composed of a nucleus [the four rings] and a side chain.”[6] The emphasis on the side chain (the alkyl group at C17) is important because sterols and steroids are often differentiated with respect to the side chain. Sterols have a side chain of eight or more carbons and steroids have a side chain of 2 carbons or less, including no side chain. Progestogen, androgen, estrogen, and the andrenocorticoids are often called steroids rather than sterols, as per Nes’ defining of sterols.[6]

Scheme 1.1. General Overview of Sterol and Steroid Biosynthesis



Sterol and steroid chemistry both have as a foundation the study of the compound cholesterol. ("Chole" means bile and "stereos" means solid.)[1] Given that cholesterol is the precursor of steroids, the logic is simple for steroid chemistry. While cholesterol is not the precursor to all sterols, cholesterol is considered the most important of the sterols. Vitamin D, bile acids, and important oxysterols (sterols oxidized by addition of oxygen, not including ester or ether formation though) are derived from it. In addition, cholesterol accounts for about 30% of total lipids in mammalian cell membranes,[7] where it plays an important role in keeping the phospholipid bilayers in a "liquid crystalline ordered" phase (organized and fluid).[8]

1.2. Nomenclature

A basic four ring structure is found in both sterols and steroids. (Figure 1.1) The three cyclohexanyl rings are in the chair configuration and have an A through D standard labeling.[1] (Figure 1.2) Carbon labeling is also standard for both sterols and steroids as can be seen with cholesterol and its distant derivative estrone.[1] (Figure 1.3) Sterols and steroids also have an α and a β side.[1] (Figure 1.4) The methyl groups at C10 and C13, and the C17 side chain are found on the β side. These preferences for the β side are likely a result of enzymatic influence on squalene during cyclization.[1, 6, 9]

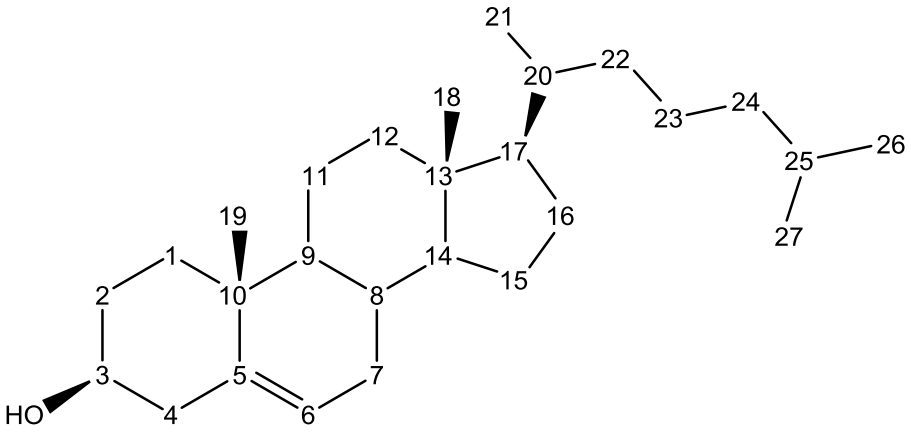


Figure 1.1. The Structure and Carbon Labeling of Cholesterol

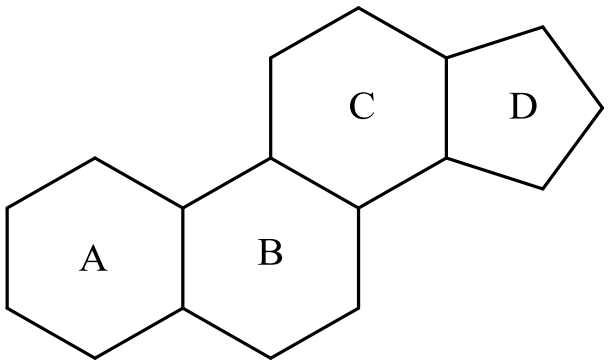


Figure 1.2. Ring Labeling of a Steroid Nucleus

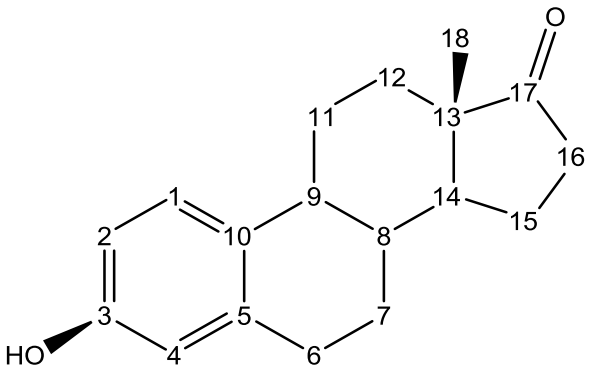


Figure 1.3. Structure and Carbon Labeling of Estrone

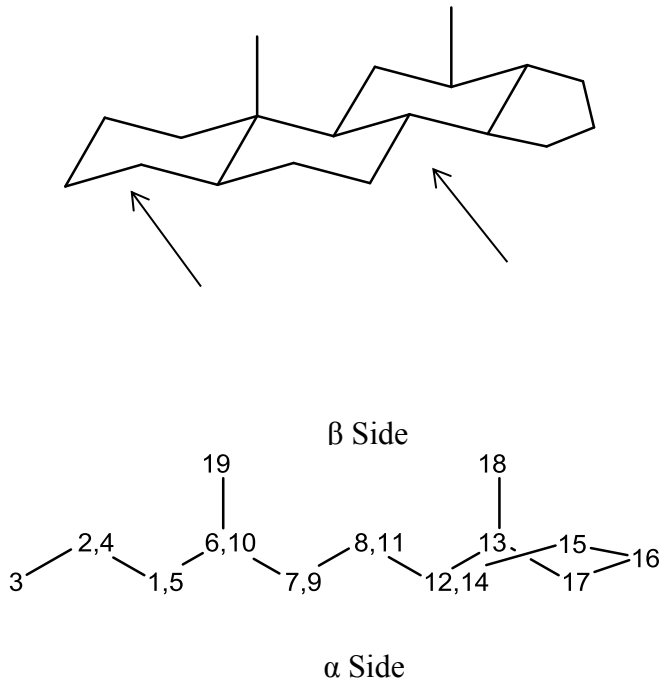


Figure 1.4. The Side View of a Steroid Nucleus

Many steroidal compounds have their own common name. For the many more that do not, they are named by their parent compound. They have in their title, usually where “en” and “an” would be noted, an abbreviated form of the parent compound’s name such as cholest, pregna, andro, estra, and corti. For example 4-cholesten-3-one would mean a cholesterol frame with a double bond at the C4 position and a ketone at the C3 position. Often, the symbol “Δ” is used followed by a number in superscript to represent where the double bond is. For instance, $\Delta^{8(14)}$ would mean there was a double bond between C8 and C14. If no number is in parenthesis, the sequential number is assumed. For instance, Δ^{5} would mean there is a double bond between C5 and C6.

1.3. Reactivity

Ronald Breslow was able to exploit the exposed α side with his famous remote functionalization reaction.[10] Using Bose inversion-esterification[11] (very similar to a Mitsunobu reaction) at the C3 carbon, m-iodobenzoate tethers were used as radical relays to remotely functionalize the α facing C9 carbon with a radical.[12] Breslow's group exploited several other exposed carbons on the α side, but not the β side.[10, 13] The ester tethers would not have worked well on the β side due the C18 and C19 methyl groups. Indeed, carbon and protons on the β are shielded by steric hindrance. This important factor lends to the selectivity of the C7 allylic hydrogen over the C4 allylic hydrogen.[14] Even with estrone acetate, reactivity occurs on the α side.[15]

Steroidal (sterols and steroids) reactivity is also unique because of the rigidness imparted by the four fused rings. The three cyclohexane rings are in chair confirmations,[1] with exclusion to sterols modified by intestinal bacteria,[1] where the carbon bonds are approaching optimal angles close to 109˚ from each adjacent bond. Ring junctions of the cyclohexane rings (C5, C8, C9, C10, C13, and C14) have their R and S configurations locked in by their bonds, resistant of ring flips.

The change from sp^3 to sp^2 hybridization via double bond formation requires the tetrahedral carbon, sp^3, to assume a trigonal planer molecular geometry (sp^2) with the covalent bonds moving near a 120˚ angle from adjacent bonds of the same carbon.[16] Tetrahedral carbon covalently bonded to carbon with changing hybridization are forced into greater angular distortion, causing

what has been labeled “olefinic strain.”[17, 18] In a mono-cyclic compound, this bond angle distortion is inhibited only by the ring strain of that ring.[19] In steroidal compounds, conformational transmission increases ring strain for angular distortion of the carbon bonds.[20] Because of ring strain, the double bond of steroids is Δ^5 rather than Δ^7.[21]

The energy required to overcome conformational transmission ring strain and the steric shielding in steroidal compounds impart stability. A *Journal of Lipid Research* title emphasizes this well: “Surprising unreactivity of cholesterol-5,6-epoxides toward nucleophiles.”[22] Only under catalytic conditions where the authors able to accomplish nucleophilic addition to the epoxide. Even then, only the 5,6-α-epoxy-cholesterol would react. No reactivity was seen with the 5,6-β-epoxy-cholesterol. The reactivity was mainly attributed to a slight conformational change due to the α-epoxy, straining the ring A and B junctions and the steric strain that would result from placing a hydroxyl group on the C5 carbon directly adjacent to the C19 methyl.

Sterol C_8H_{17} side chains are also stable despite their acyclic structure. Steroidal chemists have had to either use strong oxidizing reagents such as chromyl acetates,[23] ozone,[24] hydrogen peroxide/trifluoroacetic acid andhydride/sulfuric acid mix,[25] and dioxiranes[26] to oxidize the side chains or otherwise bond a new side chain to an androgen.[27] Because of the stability of the side chain, radical oxidation of the steroid nucleus is possible without side chain oxidized derivatives.

The ends of steroid nucleuses (C3 and C17) are more likely to undergo oxidation than the side chain. (This reactivity differentiates steroids from sterols, which have a C_8H_{17} aliphatic side chain described in the previous paragraph.) Typically, a hydroxyl or ketone group is found at C3 for most steroidal compounds. Steroids generally have either short side chains (typically two carbon) with a ketone on that side chain or no side chain with only a ketone or hydroxyl group on the C17 carbon. Most oxidation reactions for steroidal compounds require protection of the C3 and C17 hydroxyl groups, usually with esterification.[28]

Δ^5-Steroidal compounds auto-oxidize with molecular oxygen.[29] The oxidation occurs on the allylic C7 carbon. Auto-oxidation of cholesterol is too slow to observe at room temperature.[30] Rates have been reported for cholesterol auto-oxidation at 85˚C[30] and temperatures above 100˚C.[31] (At higher temperatures, the auto-oxidation products of cholesterol also degrade and oxidize.)[31] Steroidal auto-oxidation products are numerous. With cholesterol, 7-ketocholesterol and 7-hydroxycholesterol are the major products. Both are formed initially through an ene reaction with singlet oxygen.[29, 30]

It has been said that, “Placing an oxygen at C-11 presented the greatest single obstacle to synthesizing cortisone.”[32] The breakthrough happened with the development of a microbial conversion that would place a ketone at C11.[32] This oxidation has been mimicked on cholestanol.[33] The mechanism is similar to that of Breslow’s reaction, except that an oxometalloporhinate is used instead of an m-iodobenzene.[33] This method suffers from oxidative degradation of the

catalyst. Breslow reported a similar catalytic fate.[34, 35] Synthetic methods designed to mimic enzyme remote functionalization have only yielded limited success.

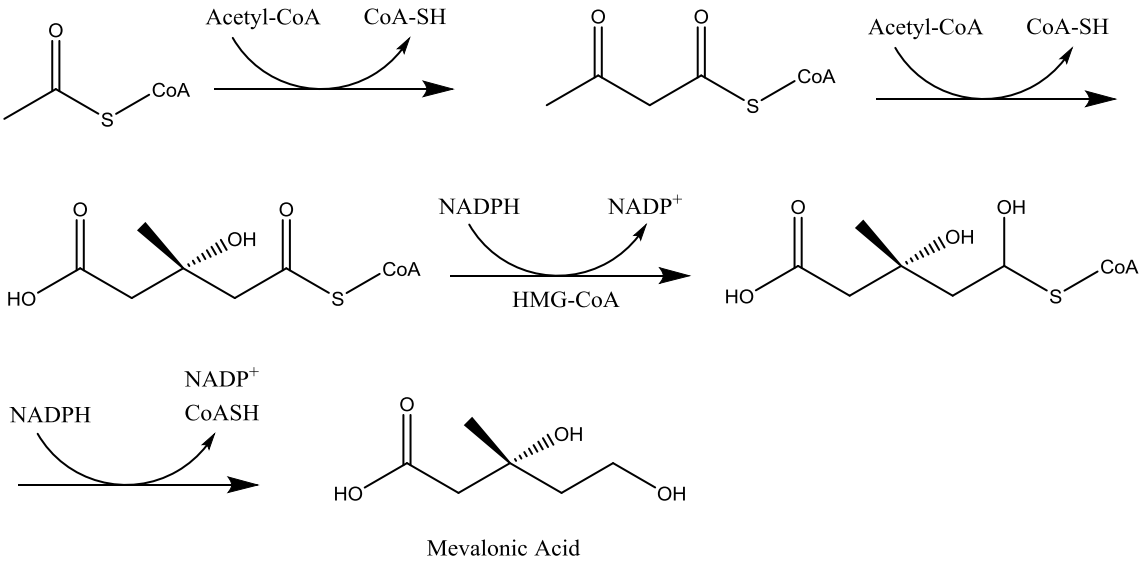
1.4. Biosynthesis

The biosynthesis of cholesterol[1, 6, 9, 36] can be broken down into five phases: synthesis of mevalonic acid, synthesis of the isoprene and terpene units, polymerization of the isoprene monomers, cyclization of squalene, and reduction of lanosterol to cholesterol. In the first phase, acetic acid is used as the building block to form mevalonic acid. (Scheme 1.2) Regulation of this phase is accomplished, but not exclusively, through suppression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA) activity.[37] This happens via regulation of sterol regulatory element-binding proteins (SREBP), which activate transcription of HMG-CoA synthase and reductase.[38-40] Only in sterol depleted cells is SREBP released.[39, 41]

The second phase begins with the mevalonic acid and ends with isopentyl pyrophosphate, which has the resonance form of dimethylallyl pyrophosphate. Because steroidal compounds are polymerized from isoprenes, they are called "isoprenoids."[1] Two isoprenes make a terpene and steroidal compounds are also called "terpenoids."[42] These pyrophosphates are polymerized to form squalene in the third phase. The squalene is then cyclized in the fourth phase via acid catalysis and carbocation rearrangement consisting of several cascading ring closures and methyl and hydride shifts. Of note is the possibility of forming

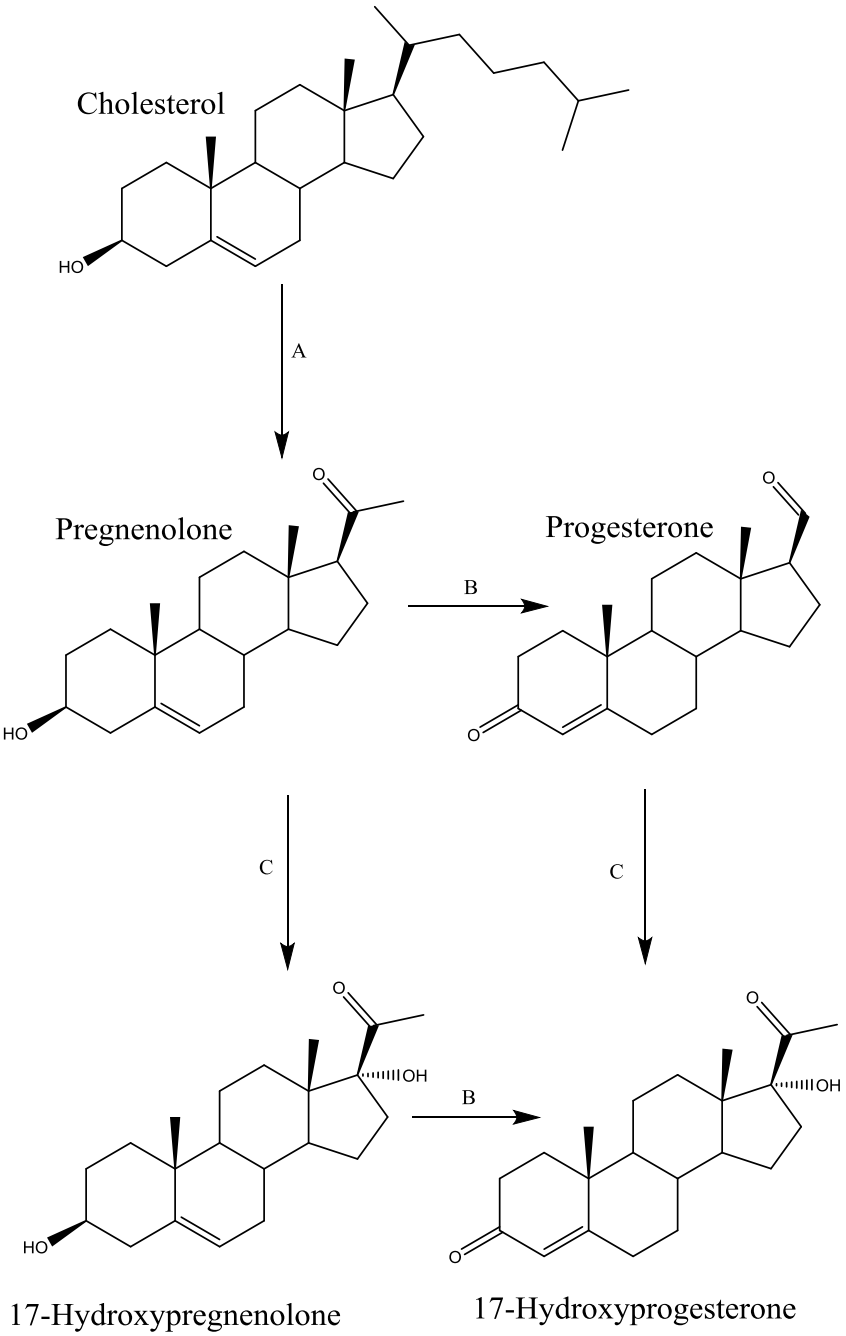
squalene dioxide with an epoxide on both ends of the squalene.[43] This very minor product is believed to lead to a shunt route for formation of side chain oxidized oxysterols. In the fifth phase, the lanosterol is reduced to cholesterol through 19 steps.[36] In parallel, 24,25-oxidolanosterol is converted to 24,25-epoxycholesterol.[43]

Scheme 1.2. Synthesis of Mevalonic Acid

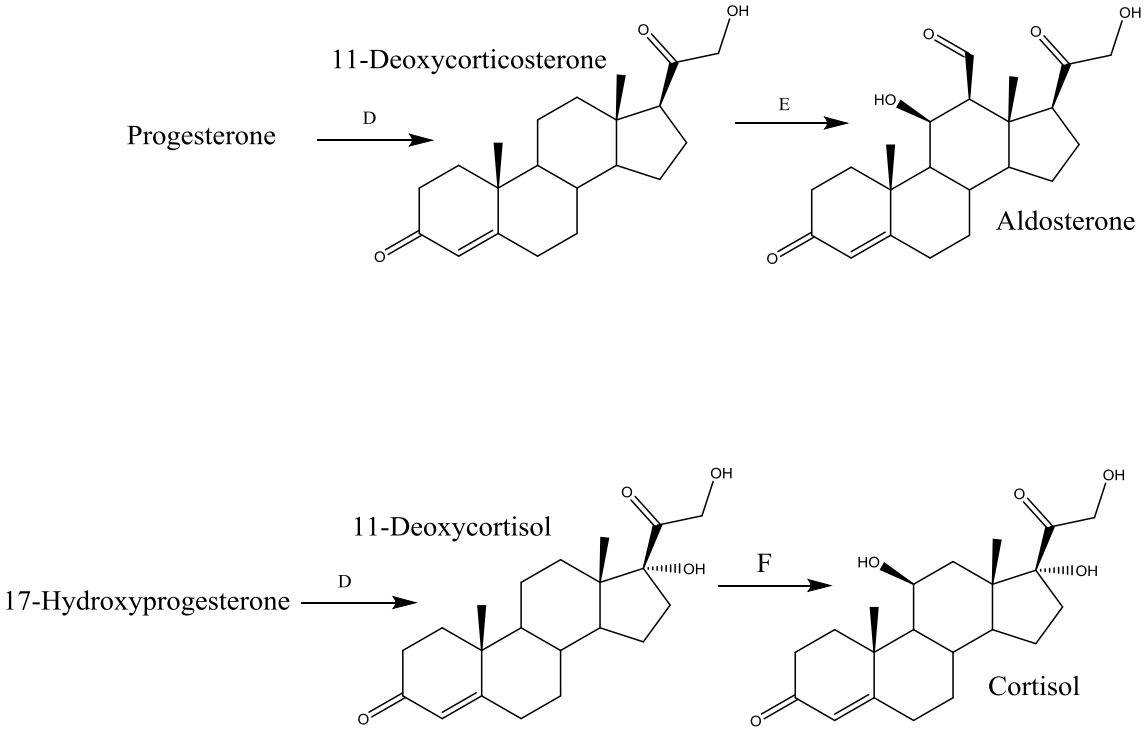


It is important to note that steroids are made from modification and cleavage of cholesterol, which has 27 carbon atoms.[44] Thus, progestogens, the reduced products of cholesterol, have 21 carbon atoms. Glucocorticoids and mineralocorticoids are oxidized derivatives of progestogens and also have 21 carbons. Androgens, the reduced product of progestogens, have only 19 carbons and estrogens, the reduced products of androgens, have only 18 carbon atoms. Thus, androgens are sometimes called “C19” steroids[45-47] and estrogens, “C18” steroids.[48-50]

Scheme 1.3. Biosynthesis of Progestogens.[44] A. Steroidogenic acute regulatory protein, cholesterol side chain enzyme system. B. 3β-Hydroxysteroid dehydrogenase/isomerase type 2. C. 17-Hydroxylase/17,20-layse, cytochrome P450 oxidoreductase (POR).



Scheme 1.4. Biosynthesis of Corticosteroids[44] D. 21-Hydroxylase, POR. E. Aldosterone synthase. F. 11-Hydroxylase



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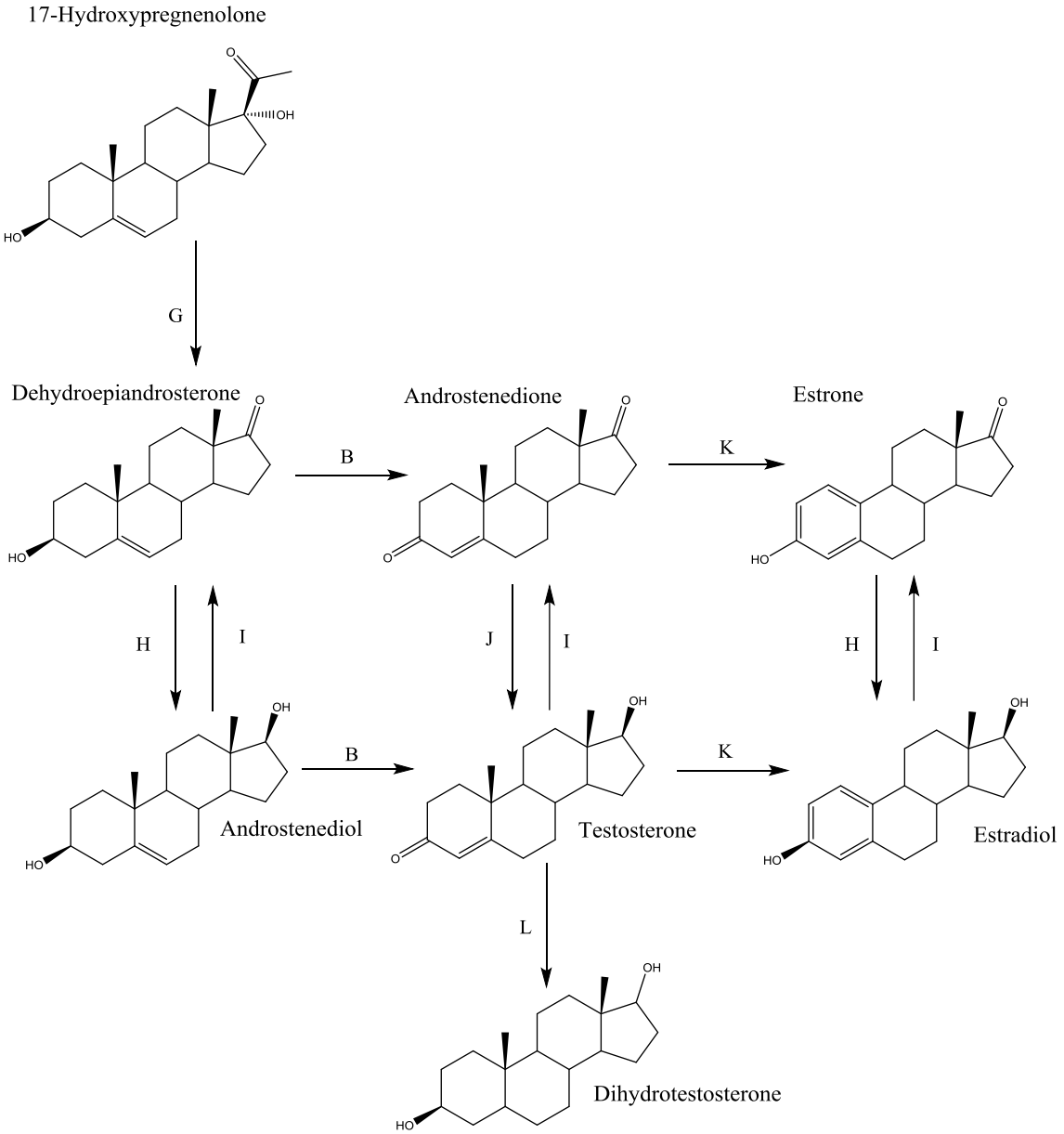
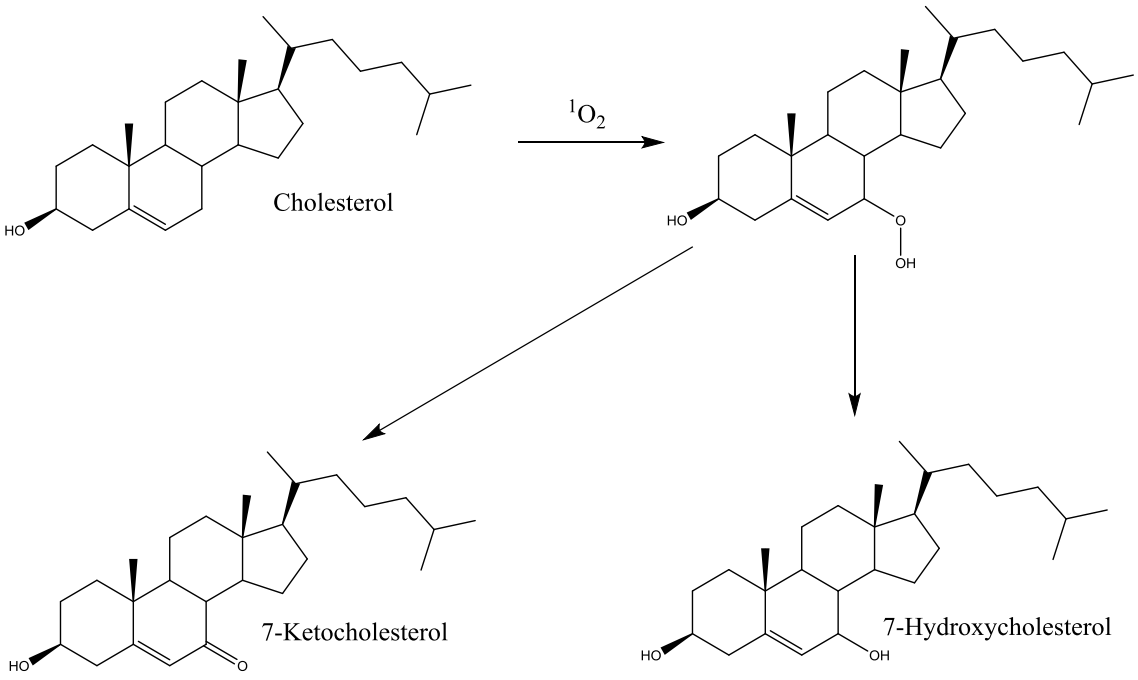
Scheme 1.5. Biosynthesis of Androgens and Estrogens.[44] B. 3β-Hydroxysteroid dehydrogenase/isomerase type 2. G. 17-Hydroxylase/17,20-layse, POR (b5). H. 17β-Hydroxysteroid dehydrogenase/isomerase type 1. I. 17β-Hydroxysteroid dehydrogenase/isomerase type 2. J. 17β-Hydroxysteroid dehydrogenase/isomerase type 3. K. Aromatase, POR. L. 5α-Reductase



1.5. Oxysterols

Sterols that have gained a ketone, epoxide, or hydroxyl functional group in addition to the C3 hydroxyl group are called “oxysterols.”[51] “Oxysteroids” has been a term used to describe oxidized cholesterol-like sterols.[52] The term “oxysterols” does not apply to steroidal compounds that have no hydroxyl group such as esterified cholesterol. The three most popular oxysterols are the auto-oxidation products of cholesterol, which are 7-ketocholesterol, 5,6-epoxycholesterol, and 7-hydroxycholesterol.[29, 53] Albeit, the 24,25-epoxycholesterol, 27-hydroxycholesterol, and 24-hydroxycholesterol are becoming much more well known for their roles as cholesterol homeostatic response “fine tuners”[54] and important intermediates in the regulation of cholesterol in the brain.[55]

Scheme 1.6. Auto-Oxidation of Cholesterol[29, 56]

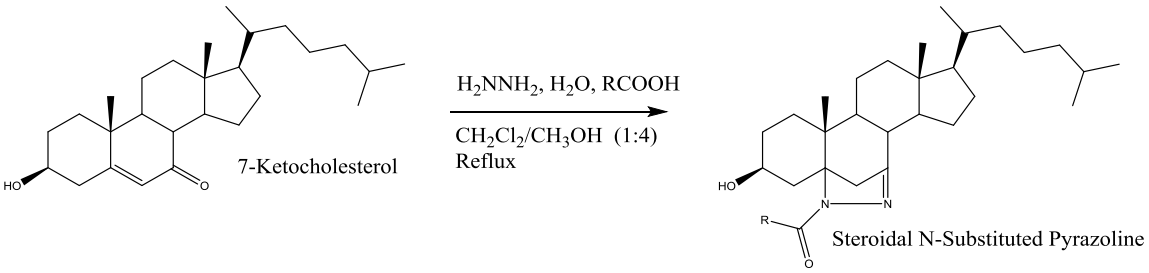


Oxysterols exhibit numerous biological activities.[57] As previously mentioned, 24 and 27-hydroxycholesterol are very important for controlling the concentration of cholesterol in the brain.[55] The “blood-brain barrier” (BBB) prevents cholesterol from entering or exiting the cerebral area; however, 27-hydroxycholesterol can enter the brain through the BBB and 24-hydroxycholesterol can exit into the blood through the BBB. It was found that 7, 20α, 22, and 25 hydroxylated cholesterols and 7-ketocholesterol suppresses HMG-CoA activity in mouse liver cells whereas pure cholesterol did not.[58, 59] Additionally, 24,25-epoxycholesterol has been shown to be a potent ligand for liver X receptors, also regulating the biosynthesis of cholesterol.[57] It is speculated that these oxysterols modulate cholesterol biosynthesis rather than serve as the master controls of it.[60] Oxysterols are also known to be inducers of apoptosis,[61] raising fears amongst food researchers.[62-64] The most infamous of all oxysterols is 5,6-epoxicholesterol.[65] Metabolism of this oxysterol leads to cholestan-3,5,6-triol, which is a suspected carcinogen. Other oxysterol bio-functions include: bile acid and steroid hormone synthesis, pro-inflammatory signaling, and modulation of estrogen receptor function.[57]

An important use for oxysterols that must not be overlooked is their function as synthetic starting material. For example, 7-ketocholesterol can, through a one pot reaction, be converted via imine formation and 1,4 conjugate addition to a pyrazoline derivative.[66] Compounds carrying this pyrazoline moiety are desired for their pharmaceutical value. The vanadium oxidation reaction, described here, can yield 7 keto products from Δ^5 sterols and steroids for the

synthesis of the pyrazoline steroidal derivatives. In addition, formation of an α,β unsaturated ketone on the B ring should lead to the synthesis of more B ring connected steroidal dimers.

Scheme 1.7. 7-Ketocholesterol to Steroidal Pyrazoline[66]



1.6. Oxidized Steroids

Steroids oxidized in manner similar to oxysterols have shown numerous biological functionalities. Perhaps the most famous examples are the glucocorticoids, which are themselves oxidized derivatives of progestogens. Another popular example, for all the wrong reasons, is 7-ketodehydroepiandrosterone.[67] DHEA, the most abundant steroid in humans,[68] is an androgen and the label has made it desirable to athletes. While it is true that DHEA does have androgenic effects, the 7-keto derivative shows minimal androgenic activity.[69] Additionally, it decreases testosterone levels.[70, 71] However, 7-ketodehydroepiandrosterone has been shown to improve the memory of mice[72] and 3-acetyl-7-oxo-DHEA increases the resting metabolism of people on calorie restrictive diets.[73] Some oxidized steroids can even be used to

counter unwanted side effects of other steroids, such as with 7-ketopregnenolone's anti-cortisone properties.[74] The 7-keto oxysterols and steroids are products yielded in our vanadium oxidation reaction described here.

2. Allylic Oxidation of Δ^5 Steroidal Compounds

2.1. Introduction to Steroidal Allylic Oxidation

The oxidation of the B ring in steroidal compounds is of great pharmaceutical interest.[75] Ring B oxidized sterols and steroids have shown anti-cancer activity, particularly cytotoxity toward cancer cells.[76-78] There is an antiestrogen binding site (AEBS) that is a "hetero-oligomeric complex" containing two post lanosterol cholesterogenic enzymes.[78] The AEBS is a B ring oxysterol binding protein. When B ring oxysterols bind to the protein, the substrates of the two enzymes are no longer taken up and begin to accumulate, leading to apoptosis.[78]

A general introduction to sterols and steroids was introduced in the first chapter to elucidate nuances of steroid chemistry. In this chapter, allylic oxidation of Δ^5 steroidal olefins will be focused on, as it is most relevant to our research. Though not all sterols and steroids are Δ^5 olefins, only the Δ^5 moiety and pertaining allylic reactivity will be discussed here exclusively. The Δ^5 steroidal olefins are common, as can be observed in scheme , whereas other steroidal olefins, with the exception of Δ^4 olefins, are unique cases. Discussion of allylic

oxidation of Δ^4 steroidal olefins is also not necessary given the decreased reactivity via the allylic carbon (C6) being located on the β side.

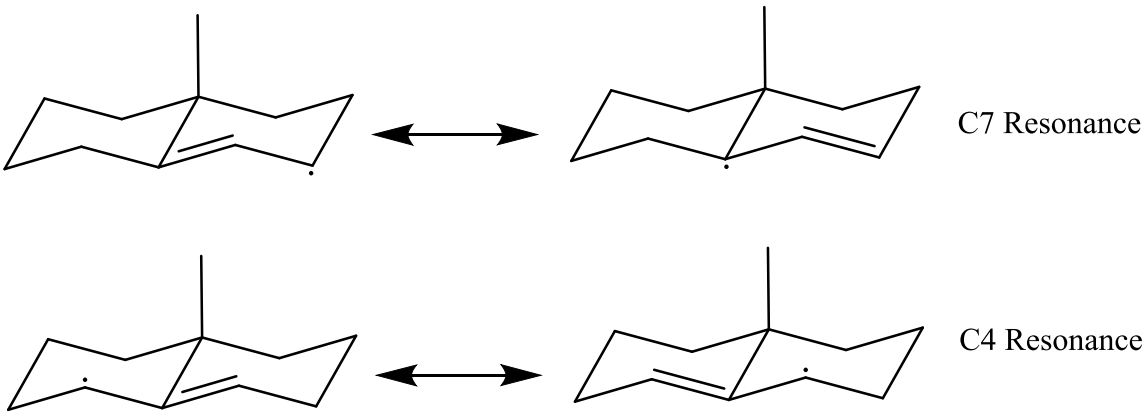
Cholesterol has a double bond between C5 and C6, in the B ring. As the precursor of steroids, many steroids retain the double bond between the C5 and C6 carbons until the steroids are enzymatically isomerized.[44] There are three carbons allylic to the double bond at C4, C7, and C10. (Figure 2.1) The C10 carbon is a quaternary carbon and therefore unreactive. Thus, allylic oxidation occurs only at C4 and C7, albeit not equally.

Allylic carbons C4 and C7 are not equivalent. The C4 carbon is located on the β side with its axial hydrogen extending also in the β direction. On the other hand, the C7 carbon is located on the exposed α side with its axial hydrogen extending further in the α direction. There is also a concave shape to steroidal nucleus (the four rings) upon which the C7 hydrogens protrude from the vertex, placing them further away from intermolecular steric hindrance. Thus, C4 is more sterically hindered than C7.[14]

There is also an energetic advantage for C7 oxidation. Both C4 and C7 are acidic and vulnerable to hydrogen abstraction by virtue of the resonance afforded to them by the adjacent double bond,[79-82] however, resonance originating from C7 is more energetically favored than resonance originating from C4. This is due to the radical or carbocation delocalizing to a tertiary carbon (C5) when C7 is the point of origination.[83] A radical at a C4 delocalizes to the secondary C6 carbon. It has been calculated that radical oxidation at C7 is

favored by -4.65 kcal/mol over C4 on a two ring system containing the A and B ring moiety of cholesterol.[83] (Scheme 2.1)

Scheme 2.1. C4 and C7 Resonance



Thus, Δ^5 steroidal compounds control oxidative selectivity through steric hindrance and electronic effects. With selectivity conquered, the other questions regarding Δ^5 steroidal allylic oxidation pertain to strength of oxidant, C3 hydroxyl group protection, environmental concerns, and side products. To the first question, steroidal compounds are fairly resistant to deprotonation due to the ring strain that is incurred from the sp^3 to sp^2 hybridization change, as previously described. Usually, a radical mechanism is needed, such as with Breslow's remote functionalization reaction.[10] There are several tertiary carbons, one in particular being C25, that can be radically oxidized,[84] leading to undesired side products. The selectivity-reactivity rule is in play and steroidal compounds need oxidants that tip toward the reactive side. Too selective and there is no reaction. Too reactive and oxidation of the side chain can occur concurrent with allylic

oxidation.[85] In the following tables (2.1.-2.6.), DHEA is the second most used steroidal compound for Δ^5 olefin oxidation and a likely reason is that researchers are avoiding potential oxidation with the side chain.

To address the second question, many steroid chemists will esterify the C3 hydroxyl group with acetic anhydride to make cholesteryl acetate. This is evident in that our survey of all of the reported Δ^5 steroidal allylic oxidations that we could find, cholesterol acetate was the most utilized substrate. (Table 2.4) In our lab, we prefer esterification with benzoyl chloride since cholesteryl benzoate products can be more easily recrystallized in acetone and water than the steroidal acetates. This esterification is important because many oxidants and catalysts will convert the C3 hydroxyl group to a ketone.[28]

Recently, in part due to interest in “green” or environmentally benign chemistry, chemists have questioned the ethics of earlier catalysts, particularly those containing chromium.[86] Environmental and health concerns have motivated the search for new oxidants and catalysts.[76] From chromium based catalysts, the next phase in steroidal allylic oxidation manifested through more environmentally friendly metallic catalysts that use tert-butyl hydroperoxide (TBHP) as an oxygen donor. Meanwhile, several methods have been reported to give steroidal oxidation without any metal catalysts using as sodium chlorite[86] and sodium hypochlorite.[86, 87] Additionally, recoverable heterogenous catalysts, clay supported[88] and organometallic polymer catalysts,[89, 90] have been reported to yield allyic oxidation products of steroidal compounds.

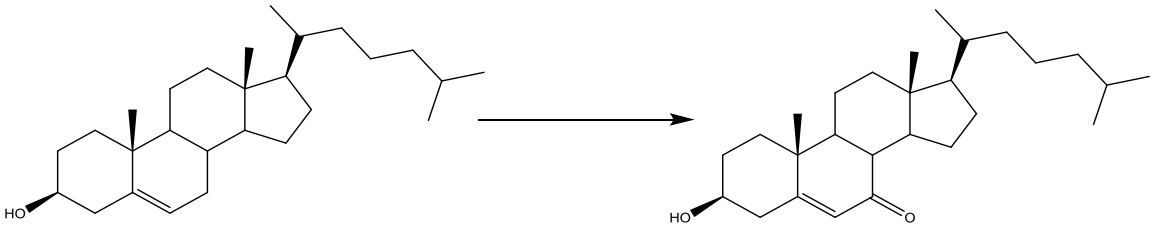
Auto-oxidation exemplifies a major issue inherent with all steroidal allylic oxidation. Though the C7 carbon is oxidized as predicted, the peroxide product can rearrange into several products.[29] For example, a C7 peroxyl group can be converted to an α -7-hydroxy, β-7-hydroxy, 7-keto, or, perhaps 5,6-epoxy oxysterol. An α,β unsaturated ketone would be energetically and sterically favored over an enol in nonpolar solvent and the epoxy requires assistance to form. Thus, 7-ketocholesterol is the major allylic oxidation product, albeit the additional sp^2 carbon likely increases ring strain.

2.2. Compilation of Steroidal Allylic Oxidation Methods

A survey of allylic oxidation reactions of steroids has been compiled to understand the broader picture. The following tables (2.1.-2.6.) are divided by substrates used in our allylic oxidation reaction described here and show the reagents, conditions, dates, and isolated yields reported for various steroidal allylic oxidation reactions. All reagents are listed, with TBHP given a special column. (Note that TBHP is used exclusively, without hydrogen peroxide or di-tert-butyl peroxide.) Caution must be taken when comparing the reported yields. There were various methods used to identify “isolated” yields (using HPLC instead of obtaining mass for example),[87] differing standards on purity of the isolated product (i.e. reporting an isolated yield that is 67% pure),[86] differing sample sizings, and an overall lack of supporting information. Several reported steroidal allylic oxidation reactions that have not been included in the tables

because yields were too low (≤ 3%), such as oxygen irradiation without and without photosensitizer[91] and Gif chemistry.[92]

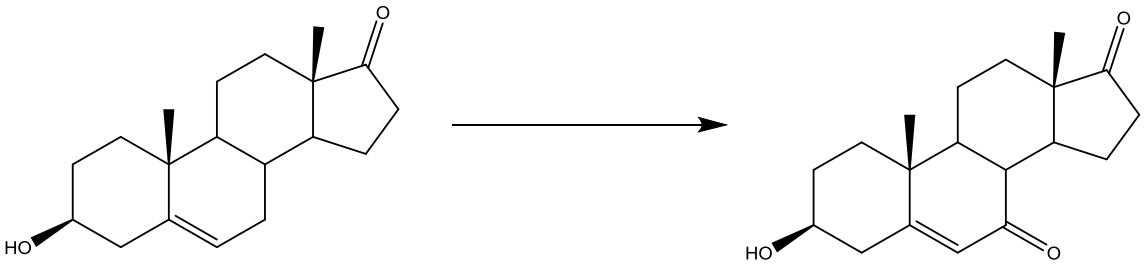
Table 2.1. Cholesterol to 7-Ketocholesterol



Substrate: Cholesterol

Catalysts, Reagents, Solvents, and Conditions	**TBHP used as Oxidant (Yes/No)**	**Date Reported**	**% Yield Reported**
$Rh_2(cap)_4$,DCM (DCE), r.t., 15h (20h)	Yes, (Yes)	2009[93] (2007)[94]	30 (63)
NaOCl, DCE, 4˚C, 10h	Yes	2004[87]	68
CrO_3/NHPI-activated clay, DCM, r.t., 58h	No	2009[88]	52
2-quinoxalinol salen Cu(II) complex catalyst, acetonitrile, 70˚C, 12h	Yes	2010[83]	69
$RuCl_3$, cyclohexane, r.t., 24 h	Yes	1996[95]	51

Table 2.2. DHEA to 7-KetoDHEA



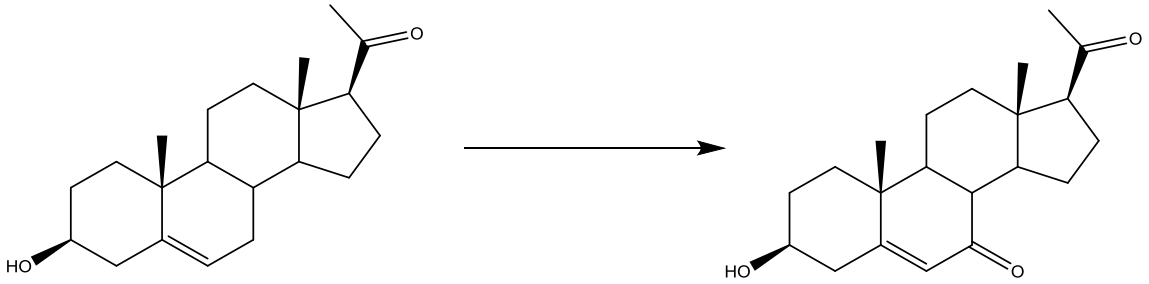
Substrate: DHEA

Catalysts, Reagents, Solvents, and Conditions	TBHP used as Oxidant (Yes/No)	Date Reported	% Yield Reported
$Rh_2(cap)_4$, DCE, 40˚C, 20h	Yes	2007[94]	74
NaOCl, ethyl acetate/tert-butanol (8:2), 4˚C, 10h	Yes	2004[87]	70
CrO_3/NHPI-activated clay, DCM, r.t., 58h	No	2009[88]	67
$BiCl_3$, acetonitrile, 70˚C, 28h	Yes	2005[96]	80
$BiCl_3$/K-10, acetonitrile, 70˚C, 11h	Yes	2005[96]	77
$NaClO_2$, acetonitrile/water (2:1), 50˚C, 20h	Yes	2007[86]	65

Table 2.2. DHEA to 7-KetoDHEA *continued*

Catalysts, Reagents, Solvents, and Conditions	TBHP used as Oxidant (Yes/No)	Date Reported	% Yield Reported
$NaClO_2$/NHPI, acetonitrile/water (2:1), 50˚C, 11h	No	2007[86]	50

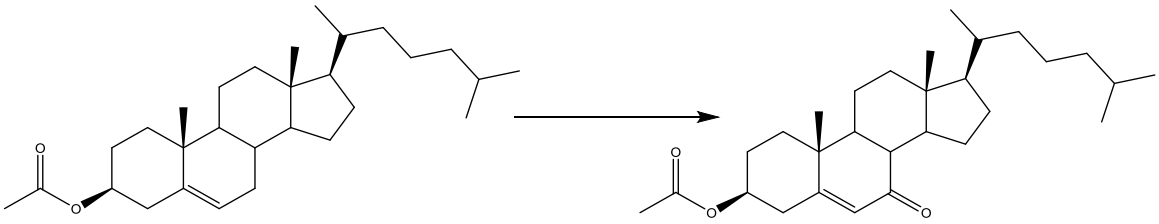
Table 2.3. Pregnenolone to 7-Ketopregnenolone



Substrate: Pregnenolone

Catalysts, Reagents, Solvents, and Conditions	TBHP used as Oxidant (Yes/No)	Date Reported	% Yield Reported
$Rh_2(cap)_4$, DCE, 40˚C, 20h	Yes	2007[94]	40
CrO_3/NHPI-activated clay, DCM, r.t., 58h	No	2009[88]	54
2-quinoxalinol salen Cu(II) complex catalyst, acetonitrile, 70˚C, 12h	Yes	2010[83]	53

Table 2.4. Cholesteryl Acetate to 7-Ketocholesteryl Acetate



Substrate: Cholesteryl Acetate

Catalysts, Reagents, Solvents, and Conditions	**TBHP used as Oxidant (Yes/No)**	**Date Reported**	**% Yield Reported**
$Co(OAc)_2/SiO_2$, benzene, 50˚C, 24 h, N_2	Yes	2001[89]	70
$ZrO_2/SiO_2/Cr(VI)$, benzene, r.t., pH 3	Yes	1999[97]	48
$RuCl_3$, cyclohexane, r.t., 24 h	Yes	1996[95]	51
$Rh_2(cap)_4$, DCE, 40˚C, 20h	Yes	2007[94]	80
$TiO(acac)_2$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	25
$VO(acac)_2$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	26
$Cr(acac)_3$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	52

Table 2.4. Cholesteryl Acetate to 7-Ketocholesteryl Acetate *continued*

Catalysts, Reagents, Solvents, and Conditions	TBHP used as Oxidant (Yes/No)	Date Reported	% Yield Reported
$Mn(acac)_2$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	11
$Mn(acac)_3$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	10
$Fe(acac)_3$, benzene, reflux, 24 h, Ar	Yes	1979[98]	74
$Co(acac)_2$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	12
$Co(acac)_3$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	43
$Ni(acac)_2$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	38
$Cu(acac)_2$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	83
$Ce(acac)_2$, benzene, 80˚C, 24 h, Ar	Yes	1981[98]	24
$Cu(Oac)_2/SiO_2$, benzene, 70˚C, 48 h, N_2	Yes	2002[90]	72

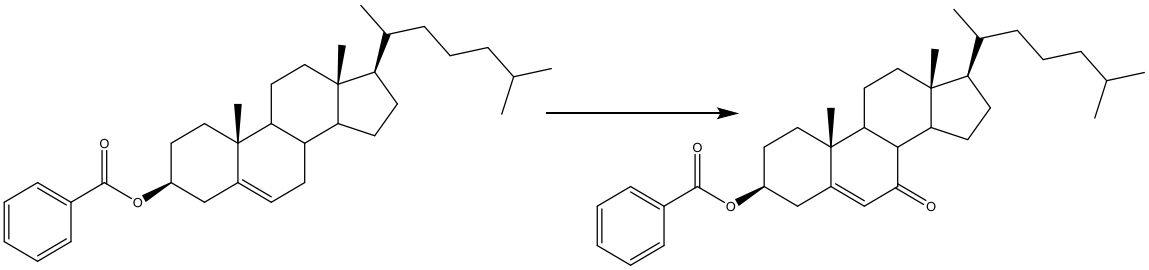
Table 2.4. Cholesteryl Acetate to 7-Ketocholesteryl Acetate *continued*

Catalysts, Reagents, Solvents, and Conditions	**TBHP used as Oxidant (Yes/No)**	**Date Reported**	**% Yield Reported**
CuI, acetonitrile, reflux, 4 h	Yes	2003[99]	79
CuI/TBAB, DCM, reflux, 4h	Yes	2003[99]	76
CrO_3/Py_2, triflourotoluene (DCM), r.t., 31h (24h), N_2	Yes (No)	2006[100] (1969)	76 (74)
PDC, DCM, 40˚C, 50h	Yes	2006[100]	78
PCC, DCM, 40˚C, 66h	Yes	2006[100]	41
CrO_2, acetonitrile/benzene (9:1), reflux, 72h, N_2	No	Not Submitted Yet[101]	48
$Cr(CO)_6$, acetonitrile, reflux, 15h	Yes	1985[102]	80
$Mn_3O(Oac)_9$, ethyl acetate, 40˚C, 48h, N_2	Yes	2006[103]	87

Table 2.4. Cholesteryl Acetate to 7-Ketocholesteryl Acetate *Continued*

Catalysts, Reagents, Solvents, and Conditions	TBHP used as Oxidant (Yes/No)	Date Reported	% Yield Reported
NaOCl, DCE, 4˚C, 10h	No	2004[87]	68
2-quinoxalinol salen Cu(II) complex catalyst, acetonitrile, 70˚C, 12h	Yes	2010[83]	97
$BiCl_3$, acetonitrile, 70˚C, 22h	Yes	2005[96]	82
$NaClO_2$, acetonitrile, 60˚C, 80h	Yes	2007[86]	66
$NaClO_2$/NHPI, 1,4-dioxane/water (3:1), 50˚C, 25h	No	2007[86]	60

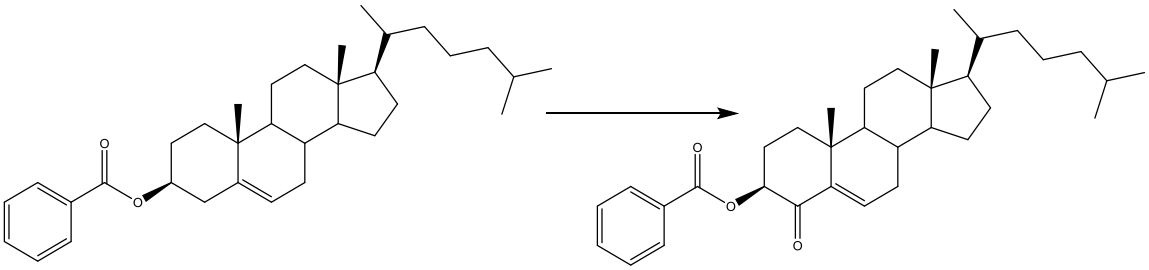
Table 2.5. Cholestryl Benzoate to 7-Ketocholesteryl Benzoate



Substrate: Cholesteryl Benzoate

Catalysts, Reagents, Solvents, and Conditions	**TBHP used as Oxidant (Yes/No)**	**Date Reported**	**% Yield Reported**
PFC, benzene, reflux, 48 h, N2	No	1996[104]	88
CrO_2, acetonitrile/benzene (9:1), reflux, 72h, N_2	No	Not Submitted Yet[101]	52
CrO_3/DMP, DCM, -10 to -20˚C, 4h	No	1978[105]	75
PCC, benzene, refluxed, 24h, N_2	No	1987[106]	87

Table 2.6. Cholesteryl Benzoate to 4-Ketocholesteryl Benzoate



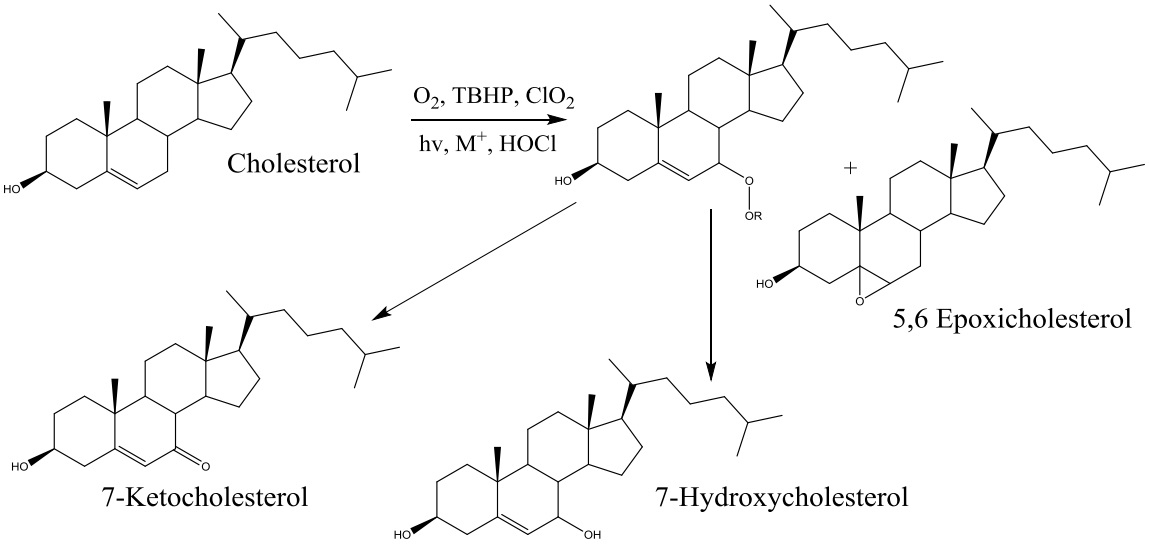
Substrate: Cholesteryl Benzoate

Catalysts, Reagents, Solvents, and Conditions	TBHP used as Oxidant (Yes/No)	Date Reported	% Yield Reported
2-Pyridineseleninic anhydride/iodoxybenzene, benzene,reflux, 10h, N_2	No	1985[107]	62
Perfluorooctylseleninic acid/iodoxybenzene, trifluorotoluene, reflux, "until completion"	No	2004[108]	65

The importance of identifying TBHP usage in allylic oxidation reactions is that those reactions share a similar mechanism. It has been noted, "that different catalysts produce essentially the same mixture of products with the same relative yields suggests that the catalyst is not involved in product-forming steps."[93] Indeed, tert-butoxide and tert-butyl peroxy radicals are formed through degradation of TBHP by catalysts. Those radicals then oxidize steroidal compounds rather than the catalyst.[86, 87, 93, 98, 99, 103, 109]

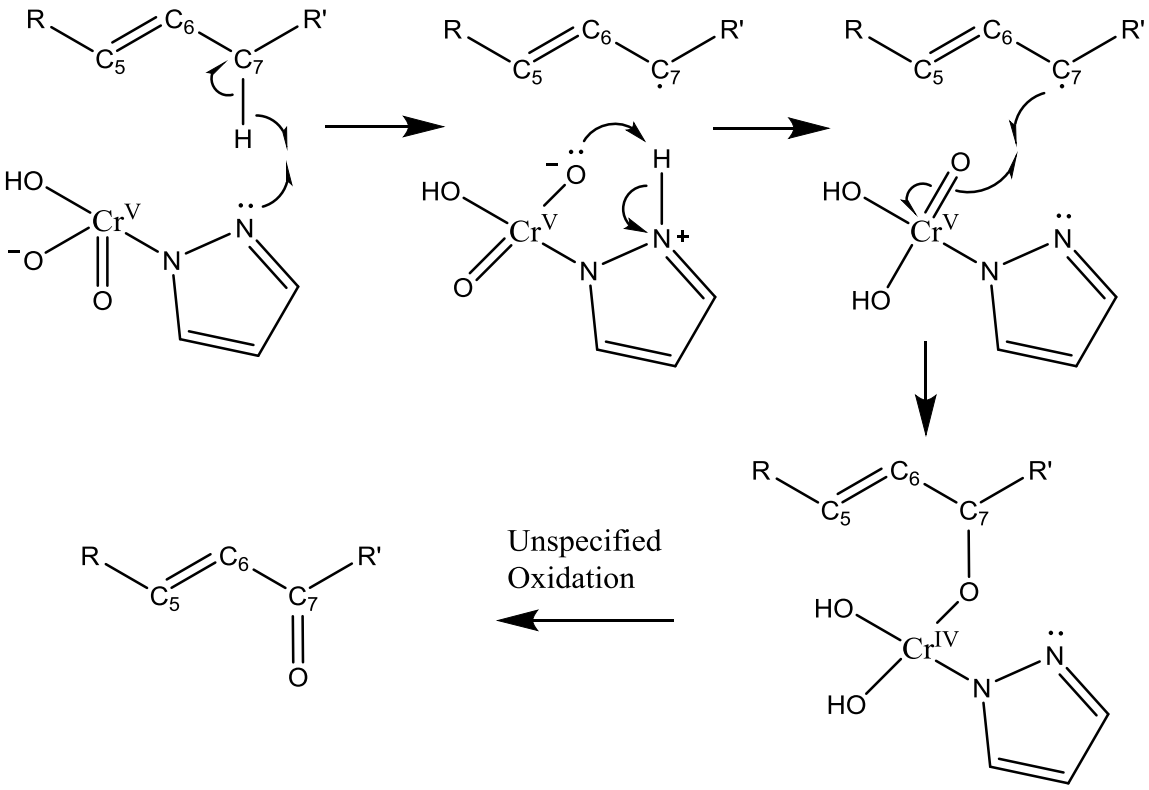
Although there are many reported steroidal allylic oxidation reactions, there are only three mechanisms presented in Tables 2.1.-2.6. The first mechanism is shared by auto-oxidation, TBHP-metal oxidation, and hypochlorite oxidation. A peroxide is formed via singlet oxygen (ene reaction) at the C5 carbon,[91] which rearranges to the C7 position.[29] Likewise, TBHP degradation by metal leads to radicals that form a C7 peroxide, as described in the previous paragraph. Bleach initiates TBHP radicals similar to the metal catalysts.[87] When only sodium chlorite and NHPI are used, NHPI becomes phthalimide N-oxyl (PINO), a radical initiator of molecular oxygen.[86] Those radicals in addition to radicals formed from ClO_2 lead to a C7 peroxide. Thus, these different pathways all go through a C7 steroidal peroxide intermediate. That C7 peroxide degrades to form a ketone or hydroxyl group.[109] 5,6-Epoxycholesterol is often a side product.

Scheme 2.2. TBHP and Singlet Oxygen Oxidation's Shared Mechanism



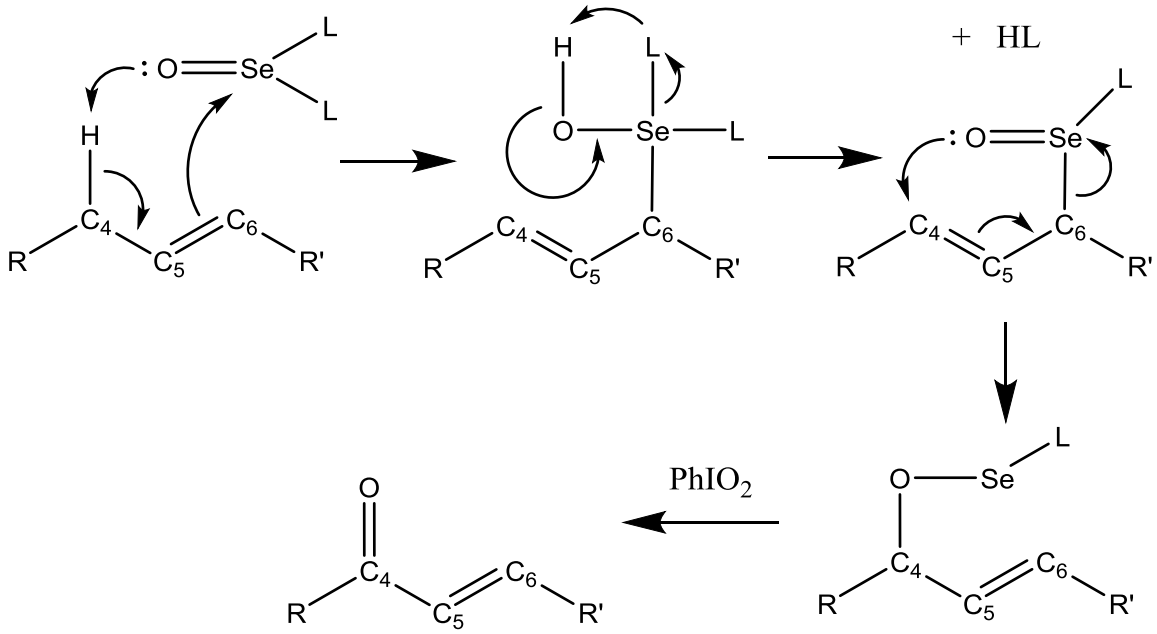
Chromium reagents use another mechanism and they are also oxygen sources.[105] The first step in the mechanism[105] is complexation with chromium and a ligand having a functional group, imine preferably, such as DMP or pyridine. After complexation, the ligand abstracts the C7 hydrogen leaving a resonating steroidal radical. An oxo group on the chromium will terminate the radical, reducing the chromium. The chromium catalyst will then continue oxidation of the steroid in an unidentified manner. It is important to note that the chromium complex may be monomeric.[105]

Scheme 2.3. Suggested Mechanism of Allylic Oxidation by Chromium[105]



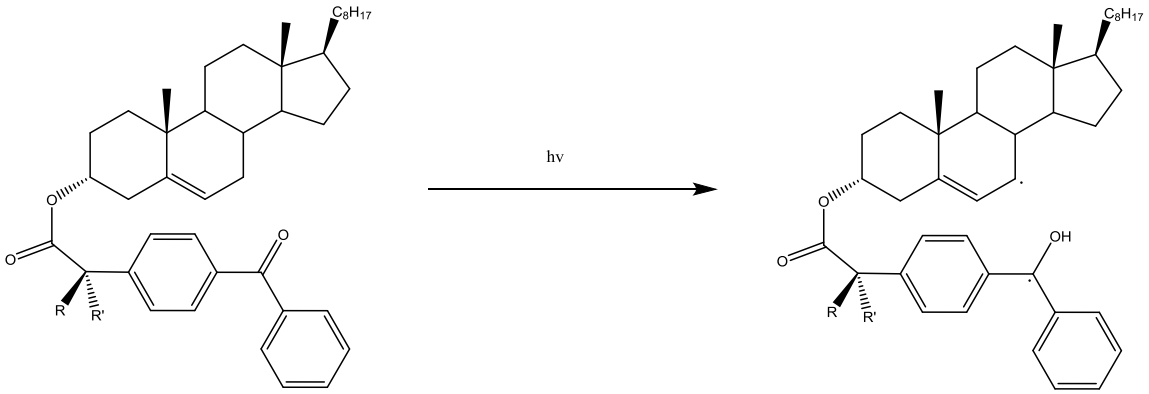
Allylic oxidation by selenium catalysts is peculiar because the end major product is a C4 ketone. The suggested mechanism[107, 108] (Scheme 2.4.) begins with the nucleophilic attack of the steroidal double bond onto the selenium atom. Concurrent, the oxo group on the selenium complex deprotonates the C4 allylic carbon, whose electrons form a double bond from C4 to C5. An ene-like product is formed. This is followed by a 2,3 sigmatropic rearrangement causing the selenium complex to be bound to the steroidal compound through an oxygen ligand. Iodooxybenzene is then used to substitute that oxygen ligand. Subsequent oxidation leads to an α,β unsaturated ketone of the cholesteryl benzoate. It is not clear why the ketone is centered on C4 instead of C7, but this regiochemical selectivity is not unique to cholesteryl benzoate. For example, 1-phenylcyclohexene oxidizes on the more hindered allylic carbon using selenium.[108]

Scheme 2.4. Suggested C4 Allylic Oxidation Mechanism by Selenium[107, 108]



Acetonitrile, benzene, pyridine, DCM, DCE, trifluorotoluene, 1,4-dioxane/water, and cyclohexane were used as solvents. Using laser flash photolysis and benzophenoneaminocholestene, it has been shown that the C7 hydrogens are abstracted at a much greater rate (more than double) in DCM than in acetonitrile, dioxane, and methanol.[110] (Scheme 2.5.) Thus, the least polar solvents appear to work best for allylic oxidation. This is, however, just considering the starting material. After oxidation, some oxy-steroidal compounds, particularly the androgens and progestogens, are not very soluble in non-polar solvents. Some steroidal starting materials do not dissolve into benzene until TBHP is added, which usually contains 30% water.

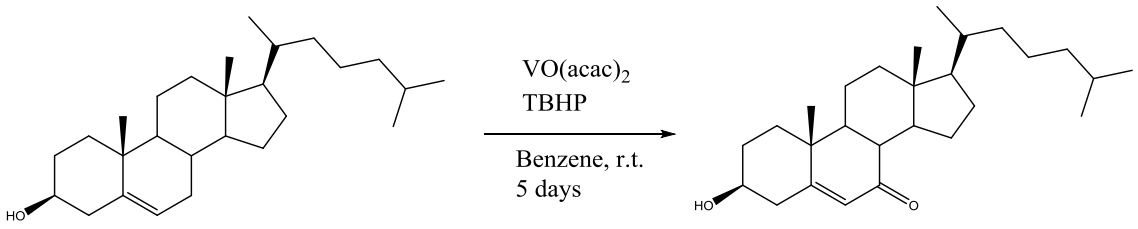
Scheme 2.5. Hydrogen Abstraction in Benzophenoneaminocholestene[110]



3. Vanadium Mediated Allylic Oxidation of Steroidal Olefins and Other Compounds

Amongst the various projects attempted in our lab, allylic oxidation of steroids and sterols using vanadyl acetylacetonate and TBHP proved to be the most successful. Herein, we describe our discovered reaction (Scheme 3.1) and elucidate on its discovery, mechanism, and possible uses outside of steroid chemistry. Additionally, our work on this reaction has been publish in *Steroids* journal (2015, volume 101, 103-109).

Scheme 3.1. Our Vanadium Mediated Allylic Oxidation Reaction



3.1. History of Vanadium and TBHP

Background information of steroids and sterols and specifically Δ^5 allylic oxidation has been described here. These have given a general overview, but to thoroughly understand the thought processes regarding our vanadium catalyzed allylic oxidation reaction, it is important to recognize that vanadium and TBHP have been extensively used as epoxidation reagents. Indeed, our understanding of the vanadium mediated allylic oxidation relies on literature regarding epoxidation. The following is a brief history of TBHP and vanadium.

In 1966, molecular oxygen was still being used to epoxidize olefins via vanadium catalysts.[111] However, this method, amongst several others including chlorohydrin, peracid, and hydrogen peroxide synthetic routes were not industrially efficient enough for commercial use.[112] In 1967, John Kollar patented[113] what would become known as the Halcon (epoxidation) Process, named after Halcon International. The process was at one time the "most important industrial process for the manufacture of propylene oxide" from propylene.[114]

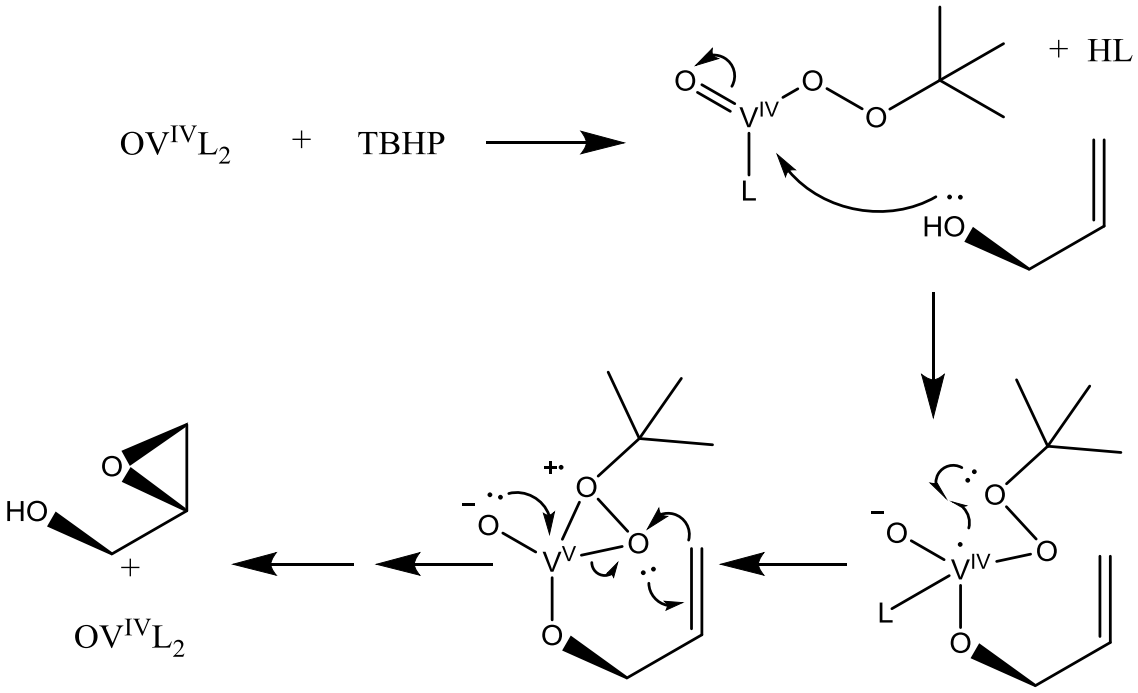
Kollar's inspiration came through an Edwin G. E. Hawkins' article published in 1950.[112] Hawkins' major discovery was that organic peroxides could be used in the oxidation of olefins.[115] The Halcon Process popularized the combination of the metallic catalytic oxidation and use of organic peroxides as oxygen donors. Although Kollar only mentioned the use of vanadium pentoxide

as a catalyst in Hawkin's experiments, he did not list a vanadium catalyst in the patent.[112] However, d^0 metals such as Mo^{VI}, Ti^{IV}, and specifically V^V would become widely utilized catalysts for the Halcon Process.[114] This is not surprising given that researchers at the time where realizing the potential of organic peroxides, particularly TBHP, and metal catalysts. Even before the 1967 patent, an article was published in 1965 regarding vanadium, chromium, and molybdenum catalytic epoxidation of 2,4,4-trimethyl-1-pentene[116] and Kollar, himself, obtained a Belgian patent in 1964 regarding use of these metals.[117]

In 1968, Edwin S. Guild used $VO(acac)_2$ and TBHP to epoxidize cyclohexene and cyclooctene[118] and later proposed that V^V was the reactive species.[117] Karl B. Sharpless had been studying these epoxidation reactions and reported in 1976 (published in 1977) that $VO(acac)_2$ would selectively epoxidize geraniol using TBHP as the oxygen donor.[119] (Scheme 3.2)

Since that time, vanadium and TBHP have been a popular choice for epoxidation.[120-129] However, vanadium was in the early 80's reported to be a less-able catalysts than its molybdenum counterparts (under certain conditions)[98] and lacked the stereoselectivity of titanium tetraisopropoxide paired with either (+) or (-) diethyl tartrate.[130] Nevertheless, the vanadium and TBHP combination is still, largely, being driven by researchers interested in epoxidation and its history and proposed mechanisms are intertwined with epoxidation.

Scheme 3.2. Epoxidation of a Homoallylic Alcohol by Vanadyl Catalysis and TBHP[131]



L = Unspecified ligand.

3.2. A New Use for Vanadium and TBHP

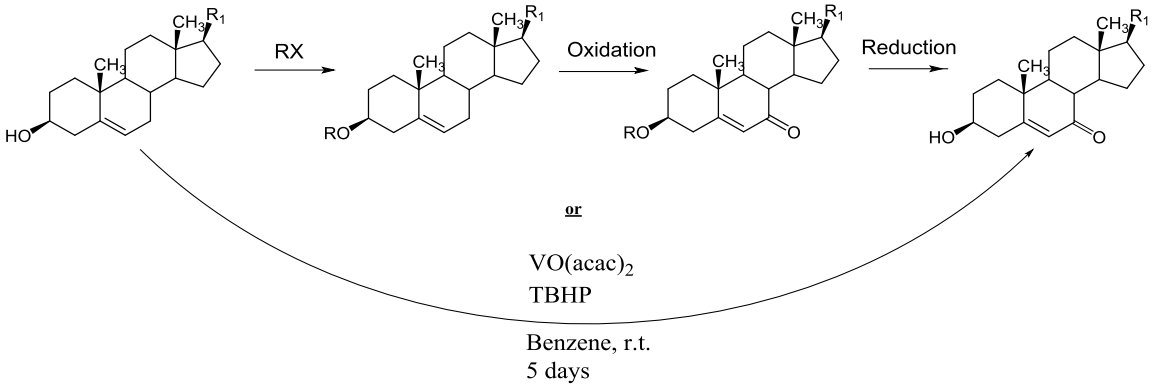
In 2010, vanadyl acetylacetonate and TBHP were reported to have shown greater allylic oxidation than epoxidation.[124] While attempting epoxidation of cyclohexane, the researchers found that the major oxidized product was tert-butyl-2-cyclohexenyl-1-peroxide. The yields were almost twice that of cyclohexene oxide and cyclohexane-1,2-diol. Furthermore, benzylic oxidation was reported by Chuo Chen et al. in 2012 using TBHP and vanadium catalysts.[132] Ketone formation was achieved at the benzylic sites of various

small aromatic compounds. These recent discoveries reveal that vanadium catalysts are capable of producing major products through C-H activation, under mild conditions.

We were unable to discover any publication using vanadium catalyst to make C7 oxysterols. To the best of our knowledge, we are aware of only one other group[133] using vanadium and TBHP for the sole purpose of allylic oxidation. Thus research on allylic oxidation via vanadium and TBHP appears largely ignored. (It should be noted that vanadium catalysts are used to produce maleic anhydride from oxidation of n-butane.[134, 135] While very different than the reaction reported here, the process involves C-H activation using vanadium catalysts.)

There are several inherent benefits to using vanadium. Albeit, vanadium catalysts initially complex with alcohols, the end products are stereochemically the same (i.e. the β-hydroxyl group of the starting material will be a β-hydroxyl group in the end product).[119, 125-127] This is a major advantage in lei of C3 hydroxyl oxidation and completely eliminates the need for hydroxyl group protection. (Scheme 3.3) The commercial availability of vanadium catalysts is also advantageous. Additionally, vanadium catalysts are generally safe, shelf stable, and affordable.

Scheme 3.3. Traditional Allylic Oxidation (top) Versus Vanadium Mediated Method (bottom)

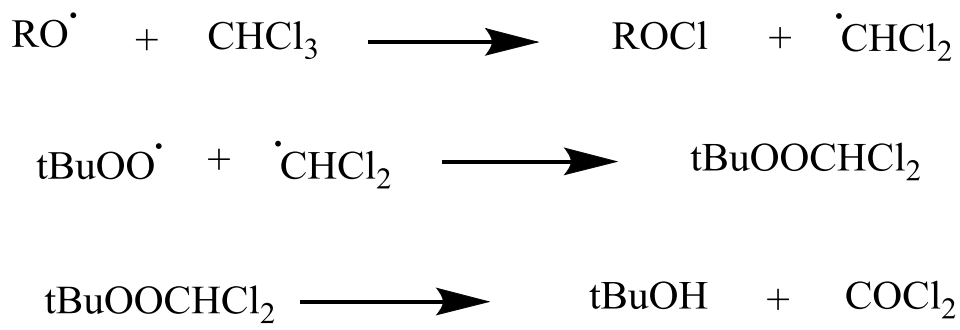


3.3. Developing the Experimental Method

We derived two experimental parameters for our reaction from Chen's 2012 benzyllic oxidation article.[132] First, the reaction takes five days. We have confirmed this by following the reaction with TLC. A possible reason for this reaction not having been reported previously may be related to the low rate of transformation that characterizes the process. Reviewing the Tables 2.1 to 2.6 of the last chapter reveals that five days is longer than all of the steroidal allylic oxidations reported. Second, $VO(acac)_2$ and bis(cyclopentyldienyl)vanadium(IV) dichloride (Cp_2VCl_2) were the best catalysts reported for the benzyllic oxidation reactions.[132] Both of these mediators were tested and produced similar results on cholesterol. However, $VO(acac)_2$ is much cheaper and has, therefore, been used as our primary catalyst.

Sterols and steroids are solids. ("Stereos" means "solid.")[1] They had to be dissolved. We found that cholesterol oxidation occurred in benzene, methanol/benzene (1:4), and chloroform. Due to the possibility of forming phosgene gas,[136] (Scheme 3.4) chloroform was abandoned. Conversion of cholesterol was also reduced with the addition of methanol. Thus, pure benzene was the clear choice of solvent. Several other solvents were tested as well, including tert-butyl alcohol, pyridine, acetonitrile, acetonitrile/chloroform (3:1), and methanol. Acetonitrile is reported in many steroidal allylic oxidations (Tables 2.1 to 2.5), but we encountered significant solubility issues while trying to dissolve cholesterol, leading us to add chloroform (before becoming aware of the potential to produce phosgene). In the 3:1 acetonitrile: chloroform solvent, cholesterol did dissolve, but still did not show any significant reactivity.

Scheme 3.4. Suggested Mechanism for Generation of Phosgene Gas Based on Reference 113



In addition, several additives were tested. Seeing that methanol was detrimental to the conversion of cholesterol in the reaction, we attempted to remove some of the water present in the 70% TBHP injections with molecular sieves. No effect was observed. Besides, the water was actually needed to dissolve some of the substrates. Addition of acetic acid also had no effect in small amounts, but seemed detrimental in higher doses. Toluene was also added in a small amount (1 mL toluene / 24 mL benzene) to the reaction and drastically decreased cholesterol conversion. Research is currently being planned to test other possible additives. Specifically given that cholesterol conversion is near 100%, the search is focused on finding an additive that would promote the steroidal peroxide intermediate to form a ketone and disfavor hydroxyl group formation.

We simplified the reaction by running it at room temperature, negating the need for a heating apparatus. However, the reaction was run at 50˚C to see what would happen. The contents in the reaction flask became a murky and viscous substance. Chen's group also found that their benzylic oxidation was stifled at higher temperatures leading them to assume that the vanadium catalyst was thermally unstable.[132] The large viscosity increase in our reaction is also likely related to the catalyst. In previous work, we have refluxed cholesterol in benzene in the presence of di-tert-butyl peroxide and iron (II) chloride with no viscosity issues.

Type of atmosphere was the next question encountered. We had to determine whether the reaction gave better results in an ambient atmosphere,

exposed to oxygen, or under an inert environment such as a nearly pure nitrogen atmosphere. Molecular oxygen can oxidize radical reagents, forming a new reactive species.[137] Furthermore, metal catalyst can also undergo oxidation in the presence of molecular oxygen.[138] Given that our reaction uses TBHP with a metallic initiator, the presence of oxygen did not seem beneficial at first. Whether running the reaction in ambient or under a nitrogen environment, full conversion of cholesterol was observed via TLC. However, quadrapole time-of-flight (Q-TOF) mass spectroscopy analysis (atmospheric pressure ionization and positive ion modes) of neat solution from those tests showed the ambient atmosphere was preferable. One reason, as shown in Figures 3.1 and 3.2, is that the mass spectrum of the reaction under ambient conditions was much cleaner. In Figure 3.2, which is the mass spectrum of our reaction under a nitrogen atmosphere, there are several unspecified peaks between *m/z* 100 and 175. Those peaks are absent in Figure 3.1, which is the mass spectrum of our reaction in ambient conditions. Our desired product was *m/z* 401.3 in both spectra and the peaks between *m/z* 100 and 175 are side products. Thus, side products are inhibited in the presence of oxygen. (Molecular oxygen is known as a radical polymerization inhibitor[139] and the side products are likely radical side products.) In addition, the *m/z* 401 ion count was much greater for the reaction under ambient atmosphere, indicative of more desired product being formed. Perhaps molecular oxygen is used as an additional oxygen source in the ambient environment.

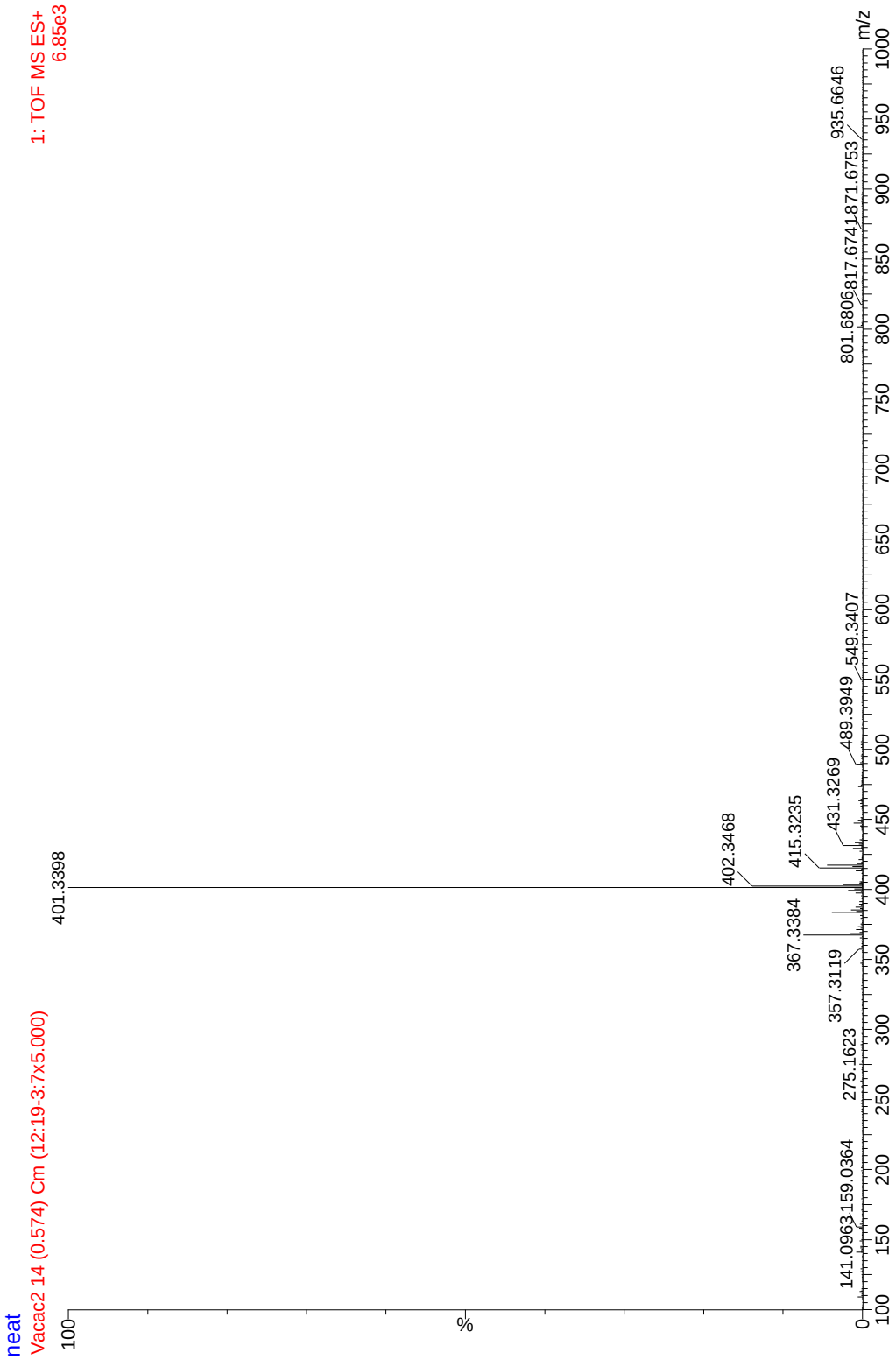


Figure 3.1. Q-TOF Mass Spectrum of Neat Product Solution for Reaction with Ambient Atmosphere

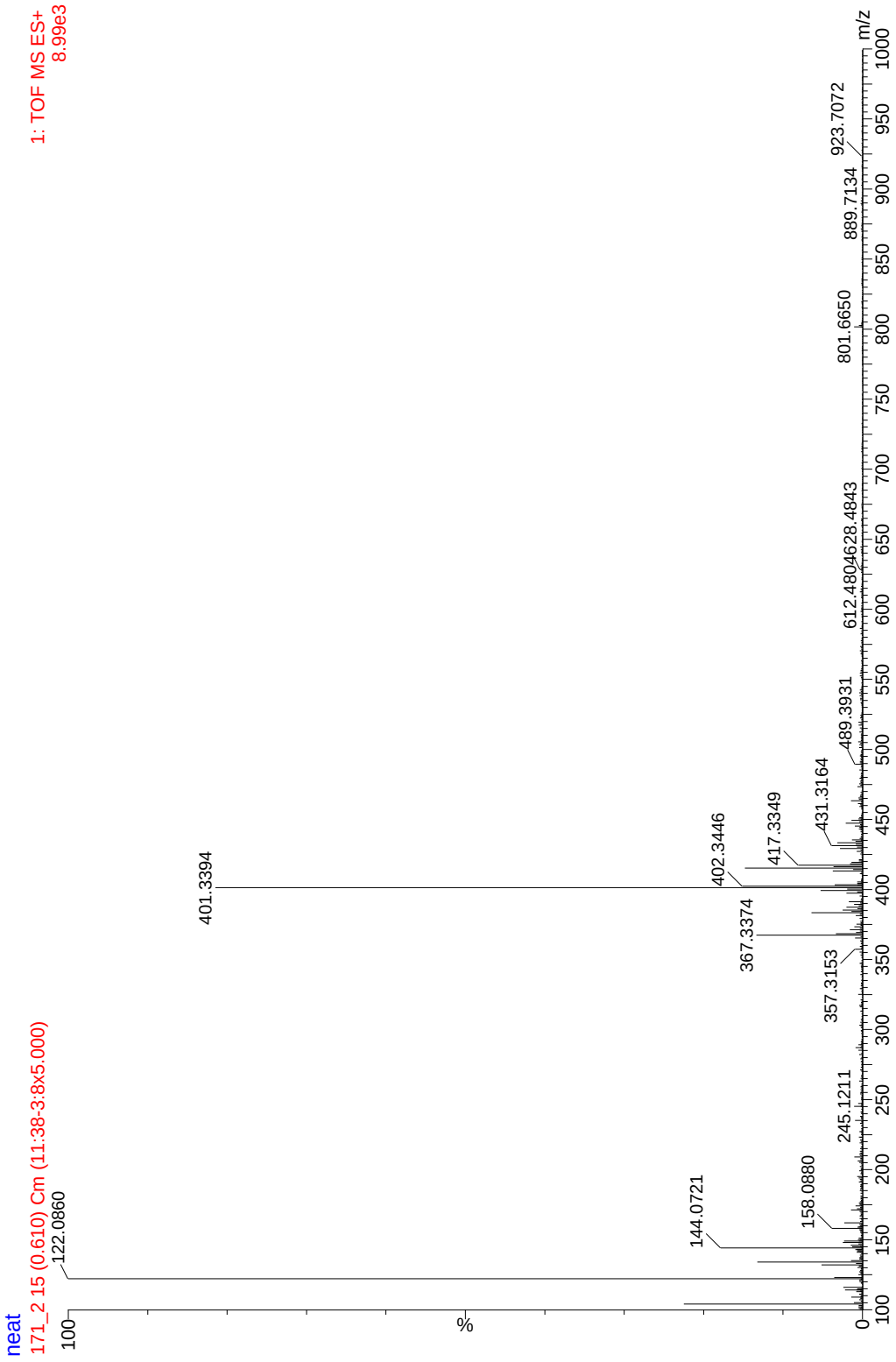


Figure 3.2. Q-TOF Mass Spectrum of Neat Product Solution for Reaction with Nitrogen Atmosphere

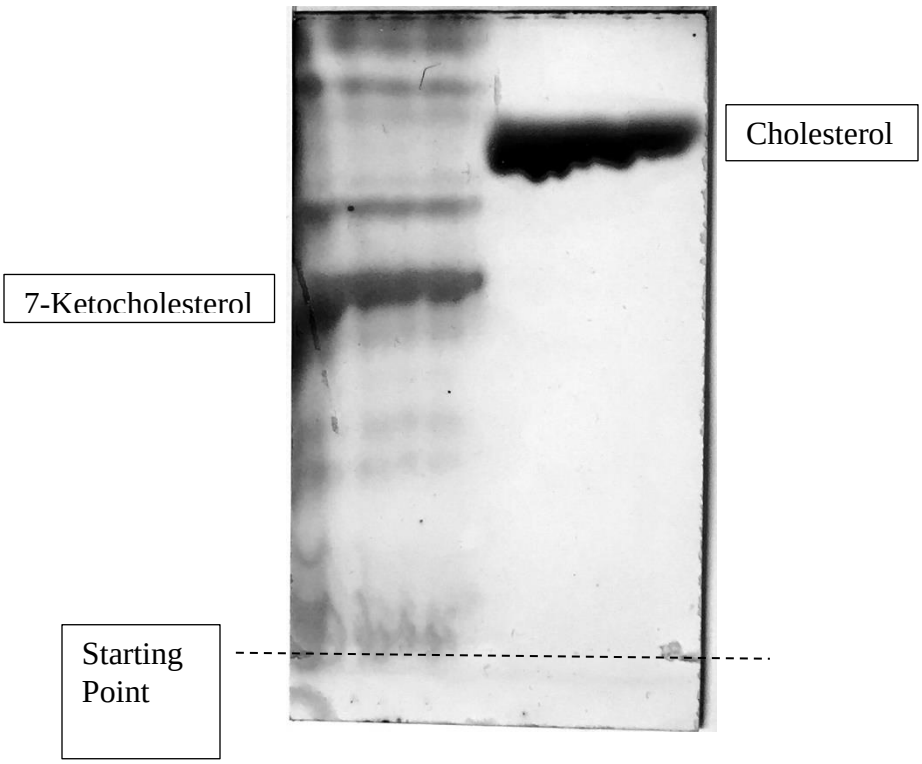


Figure 3.3. TLC Plate of Neat Product. Left on the TLC plate: neat product of our reaction. Right on the TLC plate: cholesterol starting material. Both were eluted at the same time and in the same solvent, 50/50 ethyl acetate/ toluene. (It is just one plate, silica-g.)

Molar equivalence was used to describe reagent and catalyst amounts used in oxidative reactions for a majority of the accounts in Tables 2.1 to 2.6. We initially began our optimization with this mentality, but found it to be very problematic. The most significant issue is that our reaction is more dependent on concentration than on molar equivalence. Nevertheless, we originally zoned in on the optimal amounts of reagents as shown in the Table 3.1.

Table 3.1. Optimization of Reagents

Entry Number	Molar Ratio (Ch: TBHP: V)	TBHP/ V Molar Ratio	% Conversion of Ch	% Yield 7Keto-Ch	TBHP Molarity	V Molarity X 10^{-2}
1	1.00: 9.28: 0:00	n/a	≤1	≤1	0.85	0
2	1:00: 13.5: 0:00	n/a	≤1	≤1	0.70	0
3	1.00: 9.01: 0.86	10.5	0	0	0.47	4.5
4	1.00: 3.00: 0.29	10.5	≤1	≤1	0.16	1.5
5	1.00: 3.00: 0.23	13.1	30	15	0.37	2.9
6	1.00: 3.00: 0.19	15.8	50	30	0.44	2.8
7	1.00: 6.01: 0.15	41.2	30	10	0.31	0.8
8	1.00: 7.15: 0.15	49.0	90	70	0.74	1.3
9	1.00: 9.01: 0.15	63.0	50	30	0.47	0.8
10	1.00: 9.28: 0.15	63.6	100	80	0.85	1.3
11	1.00: 9.28: 0.15	63.6	100	80	0.43	0.7
12	1.00: 8.44: 0.13	63.6	100	80	0.85	0.8
13	1.00: 9.46: 0.15	66.2	100	80	0.86	1.3
14	1.00: 10.7: 0.15	73.5	100	80	0.99	1.3
15	1.00: 12.0: 0.15	82.4	100	80	1.06	1.3
16	1.00: 12.0: 0.15	84.1	90	70	0.62	0.8

Table 3.1. Optimization of Reagents *continued*

Entry Number	Molar Ratio (Ch: TBHP: V)	TBHP/ V Molar Ratio	% Conversion of Ch	% Yield 7Keto-Ch	TBHP Molarity	V Molarity X 10^{-2}
17	1.00: 13.5: 0.15	96.5	100	80	0.70	0.8
18	1.00: 15.0: 0.15	103.0	100	80	1.28	1.2
19	1.00: 14.2: 0.15	103.0	30	10	0.77	0.8
20	1.00: 18.0: 0.15	126.1	100	10	0.93	0.8
21	1.00: 9.28: 0.07	127.0	90	70	0.85	0.7
22	1.00: 18.6: 0.15	127.5	100	80	0.85	0.7
23	1.00: 9.28: 0.01	636.4	80	60	0.85	0.1

Ch= cholesterol. V = vanadyl acetylacetonate. Conversion and yield percentages were estimated from TLC analysis.

Table 3.1 is a survey of some of our tests to find the optimal amounts of reagents, specifically $VO(acac)_2$, TBHP, and starting material (cholesterol in this instance) in benzene solvent with an ambient atmosphere and at room temperature for five days. The three main variables of interest were the TBHP/ $VO(acac)_2$ molar ratio, TBHP molarity, and $VO(acac)_2$ molarity. To understand the results in lei of the many variable changes, three charts of data were made for the conversion percentage of cholesterol versus TBHP molarity (Figure 3.4), $VO(acac)_2$ molarity (Figure 3.5), and TBHP/ $VO(acac)_2$ molar ratio (Scheme 3.6).

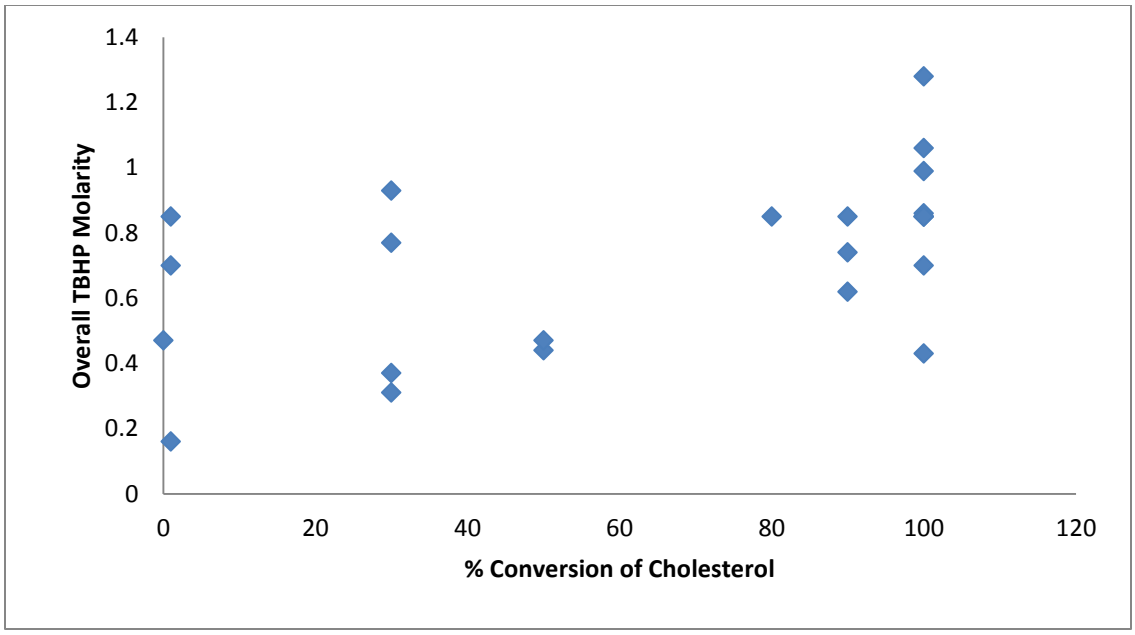


Figure 3.4. Conversion of Cholesterol Percentage versus TBHP Molarity

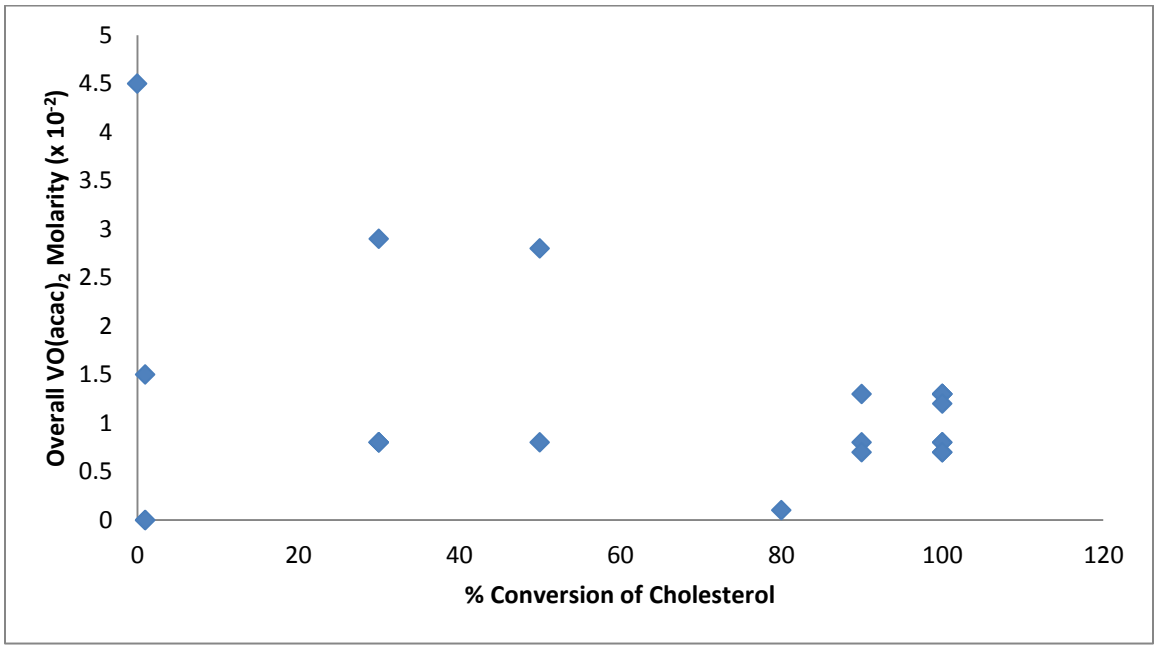


Figure 3.5. Conversion of Cholesterol Percentage versus $VO(acac)_2$ Molarity

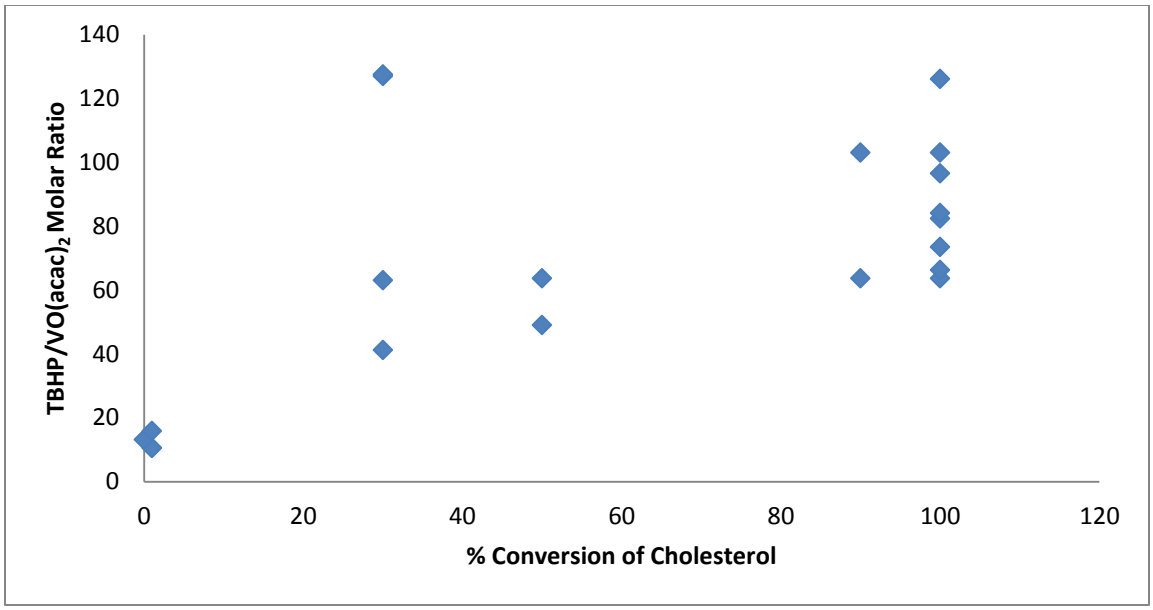


Figure 3.6. Conversion of Cholesterol Percentage versus TBHP/ $VO(acac)_2$ Molar Ratio. Entries 1,2, and 23 are not included in Figure 3.5.

Figure 3.3 shows 100% conversion of cholesterol was achieved at 0.43 to 1.28 M TBHP. Full conversion of cholesterol was also achieved at $VO(acac)_2$ concentrations of 0.7 to 1.3 x 10^{-2} M. In Figure 3.6, 100% conversion was achieved between 63.6 and 126.1 molar ratio of TBHP to $VO(acac)_2$. We determined that entry 10 reagent concentrations were optimal (within both TBHP and $VO(acac)_2$ molarity and molar ratio ranges) based on the 100% conversion and in particular, reproducibility of results.

Our optimization tests centered on cholesterol. It was assumed that higher conversion would lead to higher product yield given that the allylic position of the cholesterol would not be open in 7-ketocholesterol. (As previously discussed, the C3 hydroxyl group and C7 allylic hydrogens are the most susceptible parts of cholesterol and the vanadium does not oxidize the hydroxyl group in this reaction.) Entry 10 was 1 g of cholesterol, 0.1 g $VO(acac)_2$, and

3.09 g of 70% TBHP (30% water) in 25 mL of benzene (0.85 M TBHP and 1.3 x 10^{-2} M). This reaction was scaled up five and ten fold using these optimal quantities and produced similar results.

A high TBHP to $VO(acac)_2$ molar ratio was thought to be important because of the intermolecular competition that would occur for the vanadium. For allylic oxidation to occur, the vanadium must not coordinate with the C3 hydroxyl group or the Δ^5 double bond. TBHP should also substitute the vanadium complex's ligands. Readily present TBHP would compete with the C3 hydroxyl group and the Δ^5 double bond in addition to being available for ligand substitution. Entries 1 through 10 support this assertion. The starting material conversion remained high throughout the following entries with the exception of entry 19, also indicating that our reaction favors high TBHP to $VO(acac)_2$ molar ratios.

Di-tert-butyl peroxide and hydrogen peroxide did not work as oxygen donors for our reaction. Furthermore, these oxidants were not used seen in any of the reactions recorded in Tables 2.1 to 2.6. The mechanistic explanation is that neither oxidants can complex with the vanadium metal, which is the first step in our reaction. Di-tert-butyl peroxide is not a good nucleophile for steric reasons and in "base-free media," neither is hydrogen peroxide.[140]

3.4. Results

Fourteen steroidal compounds were tested under optimal conditions to varying degrees of success. Some steroidal substrates showed no significant

oxidation. (Figure 3.7) Others showed very unselective oxidation. (Figure 3.8) As was hoped, oxidations of the Δ^5 steroidal substrates were very successful. (Scheme 3.5)

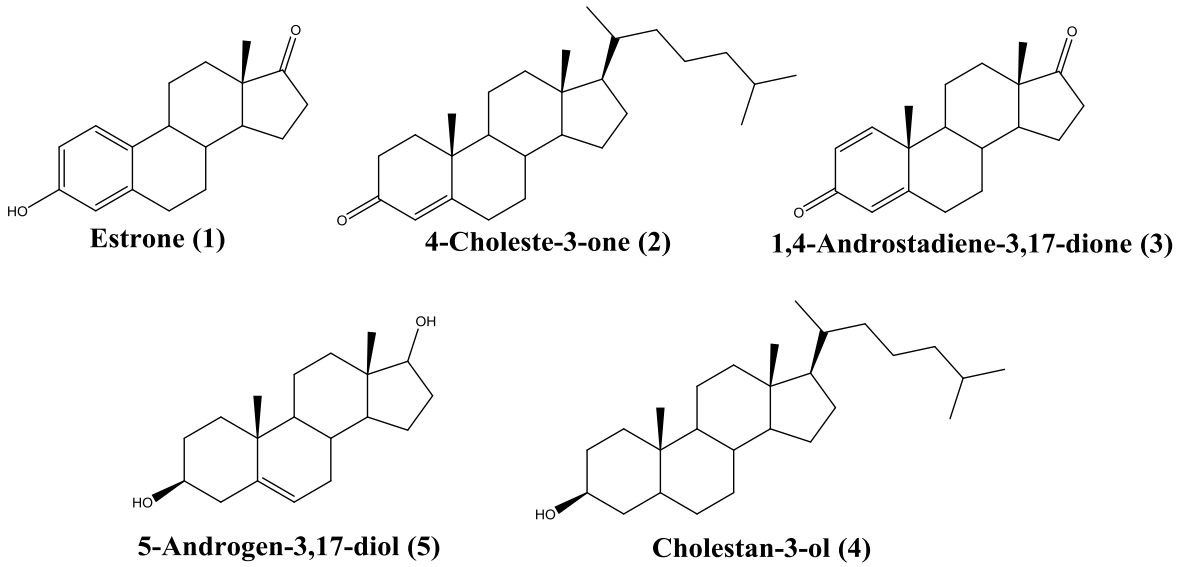


Figure 3.7. Stable Steroidal Substrates

Five of the steroidal compounds showed no significant oxidation. Among them were **(1)** estrone, **(2)** 4-cholesten-3-one , **(3)** 1,4-androstadiene-3,17-dione, **(4)** cholestan-3-ol, and **(5)** 5-androgen-3,17-diol. The benzylic C6 carbon of estrone was not oxidized. Neither did the C6 carbon of compounds (2) and (3). Two possible explanations for C6 stability, as with any steroid, are steric hindrance and ring strain. The C6 axial hydrogen is on the β side and an allylic oxidation of C6 would necessitate a resonance structure across the juncture of two rings. Future computational studies would be useful in identifying the specific cause for C6 stability.

Steroid (5) was the only mono-unsaturated Δ^5 olefin to not oxidize significantly. It was partially oxidized, but not well enough to be considered successful. The insolubility of steroid 5 was assumed to be the major inhibitor of the reaction and lead to the conclusion that any substrate used, had to be soluble for oxidation to occur.

Of the five steroids discussed thus far, cholestan-3-ol was the most important. Because it had no double bond, the C3 hydroxyl group and tertiary carbons were the most susceptible targets. Cholestan-3-ol's lack of oxidation confirmed that the $VO(acac)_2$ and TBHP combination were inert toward cholesterol with the exception of the allylic carbon.

Three of the fourteen substrates had high conversion percentages, but lacked selectivity. Those steroidal compounds included **(6)** 7-dehydrocholesterol, **(7)** 7-cholesten-3-ol, and **(8)** 24,25-dihydrolanosterol. These steroids produced a complex mixture of oxidation products. This occurrence was not unexpected for sterol (7) given that there are three allylic sites, two being tertiary carbons located on the α side. Triterpenoid (8) is sterically hindered on the α side because of the two α-methyl groups at C4 and C14, causing less steric related selectivity of C7 over C11 than would be in a cholesterol nucleus. Given that both are also secondary carbon, we assumed that C7 and C11 would represent two equally viable allylic positions for reactivity in (8). The 5,7-diene (6) has been known for a long time to make numerous oxidation products.[141]

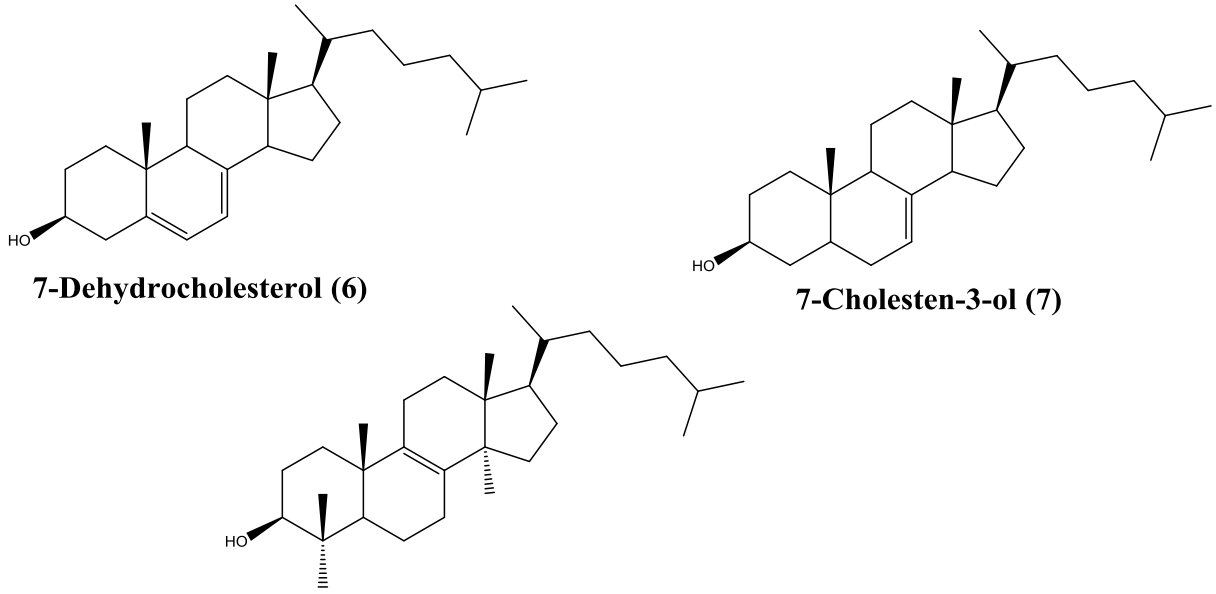


Figure 3.8. Reactive Steroidal Substrates Lacking Selectivity

Several Δ^5 steroidal compounds were oxidized to their 7-keto derivatives. These were **(9)** cholesteryl benzoate, **(10)** cholesteryl acetate, **(11)** cholesterol, **(12)** pregnenolone, **(13)** DHEA, and **(14)** 5-pregnen-3,17-dione. The stereoselectivity of these compounds has already been described in the previous chapter. Results of substrates (9)-(14) are described in Table 3.2.

Scheme 3.5. Reactive and Selective Steroidal Substrates

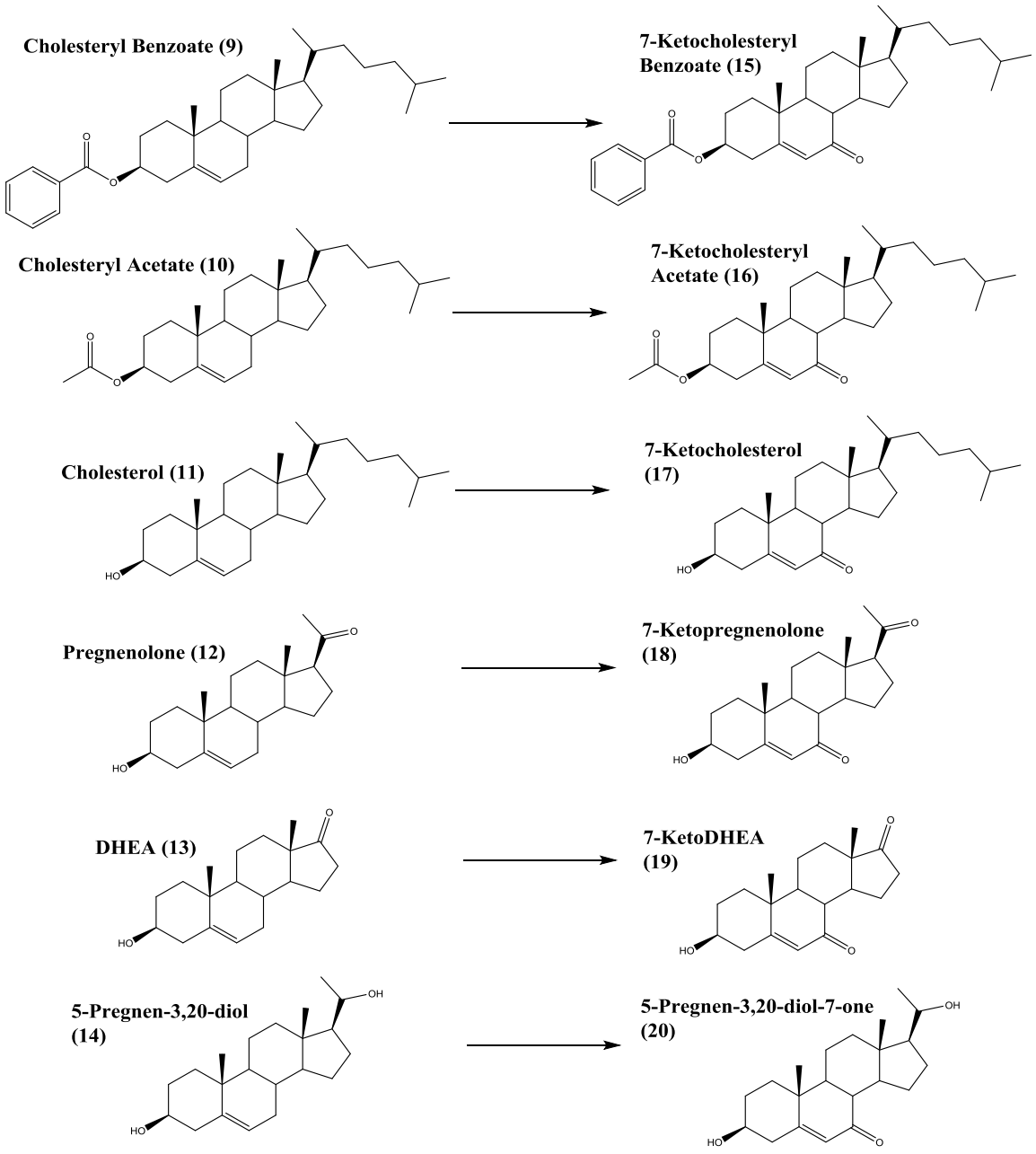
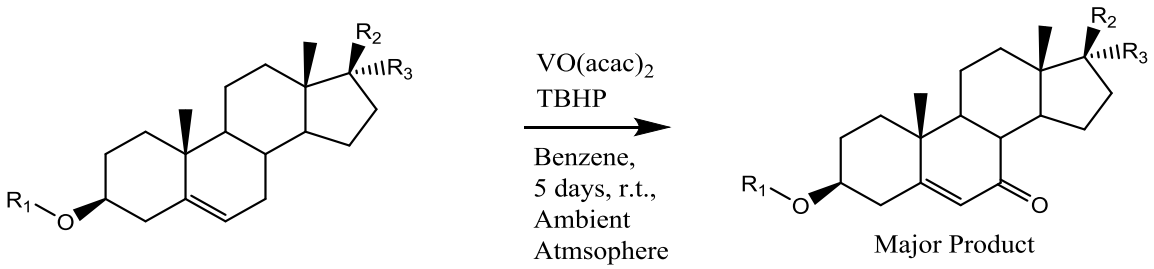
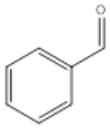


Table 3.2. Results for Allylic Oxidation of Δ^5 Steroidal Olefins (9) – (14)



R_1	R_2	R_3	Substrate	Product	Yield %[a]
	C_8H_{17}	H	(9)	(15)	98
	C_8H_{17}	H	(10)	(16)	83
H	C_8H_{17}	H	(11)	(17)	45
H		H	(12)	(18)	24
H	$R_2 + R_3$	ketone	(13)	(19)	19
H		H	(14)	(20)	26

a. Isolated

Table 3.2. shows that steroidal olefins (9) through (14) were oxidized to their 7-keto products to varying isolated yields. A correlation of the hydrophobic character of the olefin substrates and the isolated yield is present. The greater the hydrophobic character, the greater the isolated yield. This is seen in the decrease of isolated yield between the benzoate (9) to the acetate (10), the ester (10) to the hydroxyl (11), and from the side chain (11) to no side chain (12). The differences between (12) through (14) are likely due to work-up inefficiency. It should also be noted that TLC analysis of neat solutions from the reactions in Table 3.2. confirmed complete or nearly complete conversion of the steroidal substrates during the reactions.

The difference in work-up explains, in part, the greater isolated yields for products (15) and (16). These products were recovered through simple recrystallization after washing the product solution, whereas isolation of the other products required open column chromatography. While this would recover (15) and (16) in greater quantities than the other products, it is not a sufficient explanation beyond that.

3.5. Mechanism

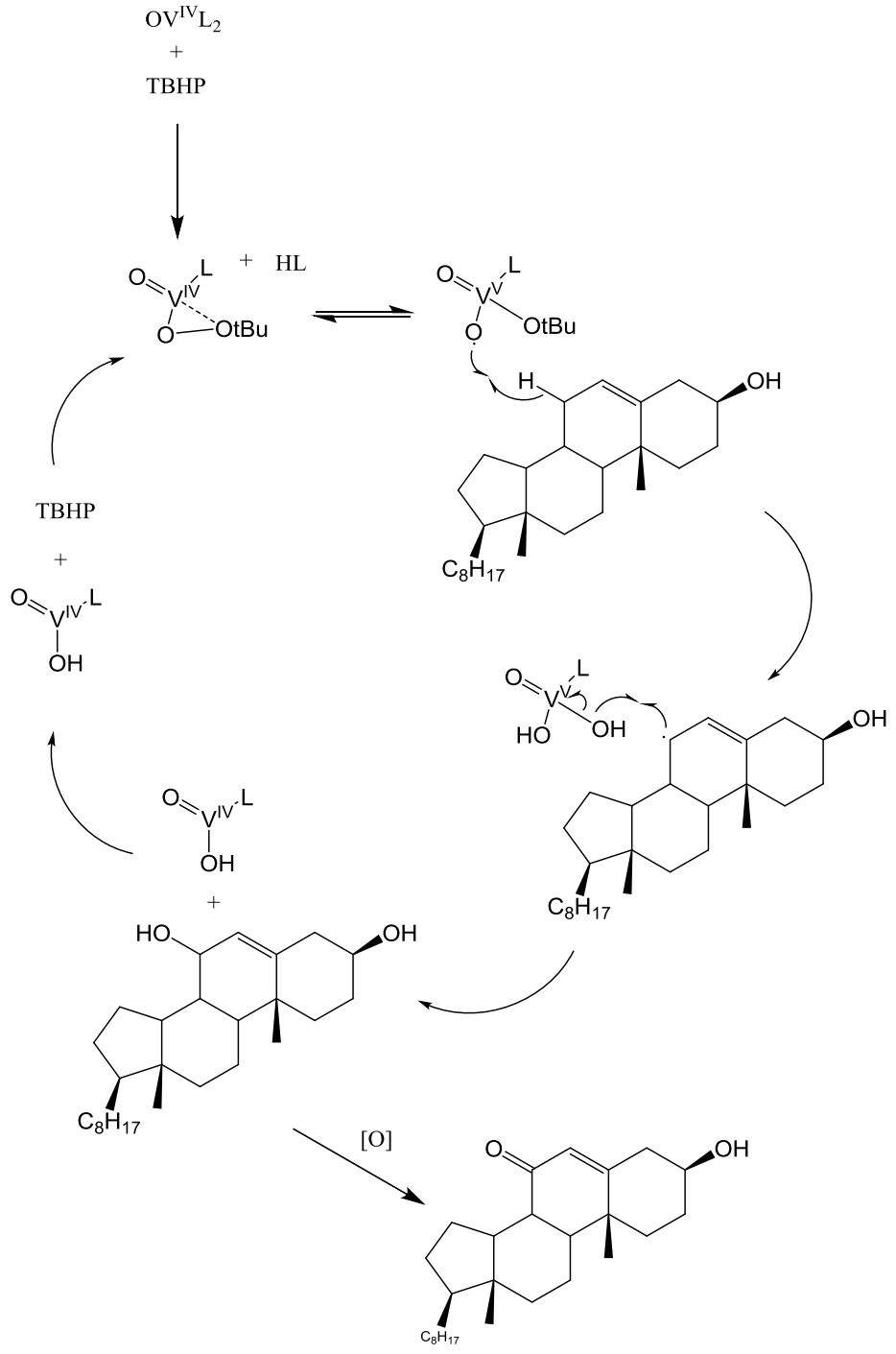
As stated before, we relied largely on research oriented toward epoxidation, in which the TBHP and vanadium combo has been thoroughly studied. Accordingly, C-H activation has been deemed a radical side product of the epoxidation reaction.[98, 129] We agree that a radical mechanism is the primary mechanism, given that allylic oxidation is indicative of radical selectivity.

Deprotonation did not seem likely because there is no literature (Tables 2.1 to 2.6) supporting it.

Nevertheless, three possible mechanisms emerged for our oxidation reaction. Those mechanisms were free radical oxidation, through singlet oxygen formation, and oxidation via a reactive vanadium complex. Because the reaction last over five days, each of these mechanisms had to account for the reactive longevity. This immediately pointed us to oxidation via an active vanadium complex, given that radicals and singlet oxygen do not have long lifespans.

Chen's benzylic oxidation article suggested that benzylic oxidation of small aromatic molecules was the result of a reactive vanadium species.[132] Chen reported that no pseudo-first-order could be established because the vanadium catalyst is actually a pre-catalyst.[132] The proposed catalyst was a vanadium (V) complex resulting from the cleavage of a peroxide ligand. That radical metal complex was responsible for the initial abstraction of the C7 hydrogen. His group asserted that the vanadium (V) species would then donate a hydroxyl radical back to the carbon from which the hydrogen was abstracted. The benzylic alcohol would, in turn, be oxidized to a ketone by an unspecified vanadium complex. A modification of their scheme has been applied to our reaction and is shown in the Scheme 3.6 for the oxidation of cholesterol.

Scheme 3.6. Suggested Mechanism for Reactive Vanadium Complex[132]

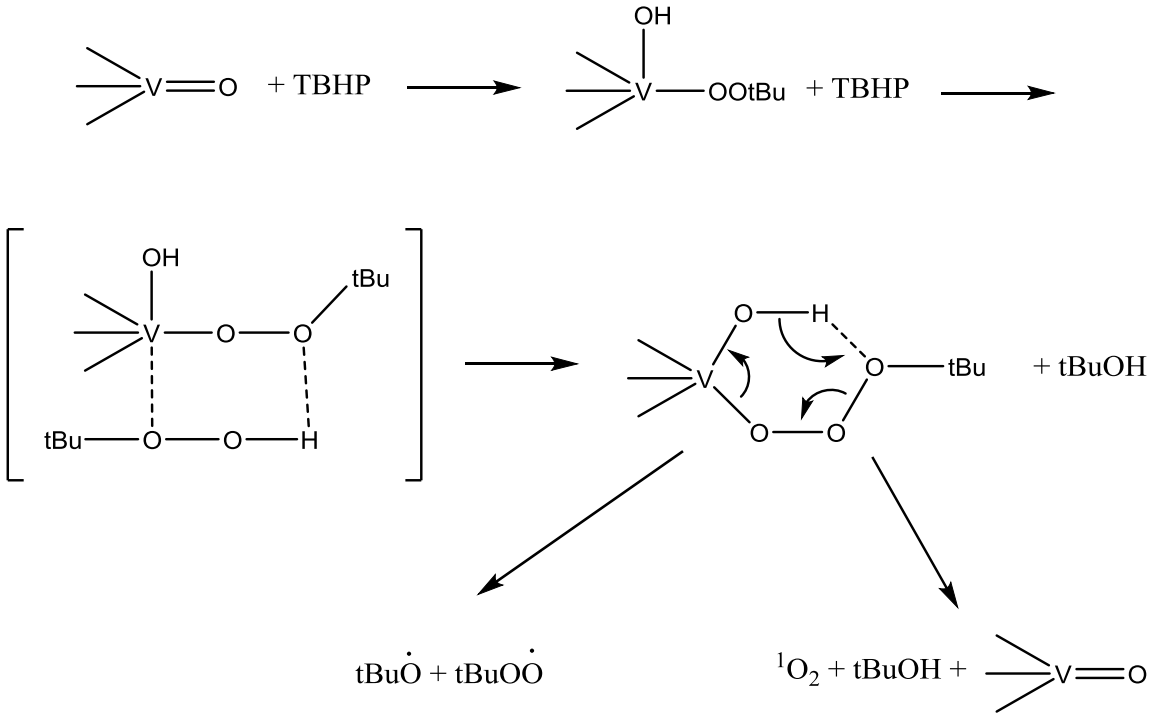


The mechanism begins with the substitution of an acetylacetonate ligand by TBHP. A strained peroxide is formed, to which the metal is complexing with one oxygen atom and interacting with the other. The peroxide is homolytically cleaved forming an oxo radical, which abstracts a hydrogen from the steroidal allylic position. Sequentially, a hydroxyl radical is released from homolytic cleavage of the hydroxyl group and metal bond. The hydroxyl radical then combines with the steroidal radical at the allylic carbon from which the hydrogen was abstracted. One electron returns back to the vanadium making it vanadium (IV). The resulting allylic alcohol is further oxidized in an unspecified manner to an enone. TBHP complexes with the vanadium (IV) species to restart the cycle.

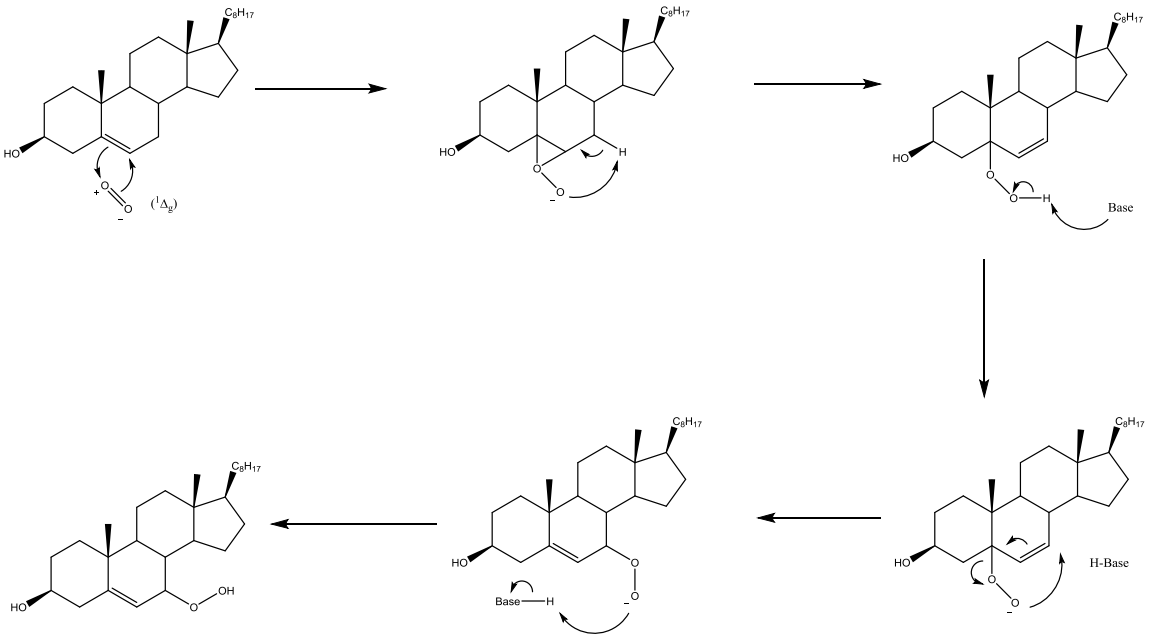
In the reactive vanadium complex mechanism, we found several criteria from literature to be present. First, vanadium (V) is formed during the reaction because the solution turns yellow.[117, 128] Second, we know that the ligands are lost because of the precipitate on the glass of the reaction flask.[142] The precipitate on the glass of the reaction flask dissolves in water overnight and is therefore not steroidal. It has been reported that IR spectroscopy and elemental analysis have identified the precipitate as $HOVO_{2,}$[142] which is not present in Scheme 3.6 Third, the initial vanadium complex has a peroxyl group complexed with it, identified from the red color that appears directly after mixing and disappears after a few hours.[117, 128] However, a major flaw in the reactive vanadium complex scheme is that the vanadium complex is supposed to oxidize the allylic alcohol. As was shown with cholestanol (4), oxidation of alcohol does not occur in our reaction.

The singlet oxygen mechanism did not seem possible at first, considering the short lifespan of singlet oxygen. However, we discovered in the literature a pathway through which singlet oxygen is generated continuously.[142] Singlet oxygen, being the way of cholesterol auto-oxidation, also seemed very logical. Scheme 3.7 shows how singlet oxygen generation may happen and Scheme 3.8 shows how the generated singlet oxygen would lead to the 7-keto product.

Scheme 3.7. Generation of Singlet Oxygen.[108]



Scheme 3.8. Allylic Oxidation Via Singlet Oxygen

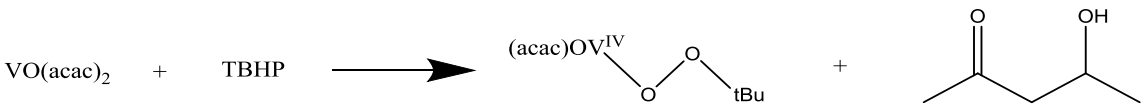


At first, the vanadium catalyst is oxidized with the addition of a peroxyl ligand. TBHP is then used to add an oxygen to the peroxyl group forming an unstable ligand. The complex rearranges back to the original catalysts, ejecting singlet oxygen, tert-butyl alcohol, and radicals in the process. The singlet oxygen then forms a perepoxide[143] steroidal intermediate that leads to ene reaction products. According to this scheme, the ligands are never substituted. In present form, the scheme is therefore wrong because the ligands are substituted as per the precipitate during the reaction.[142]

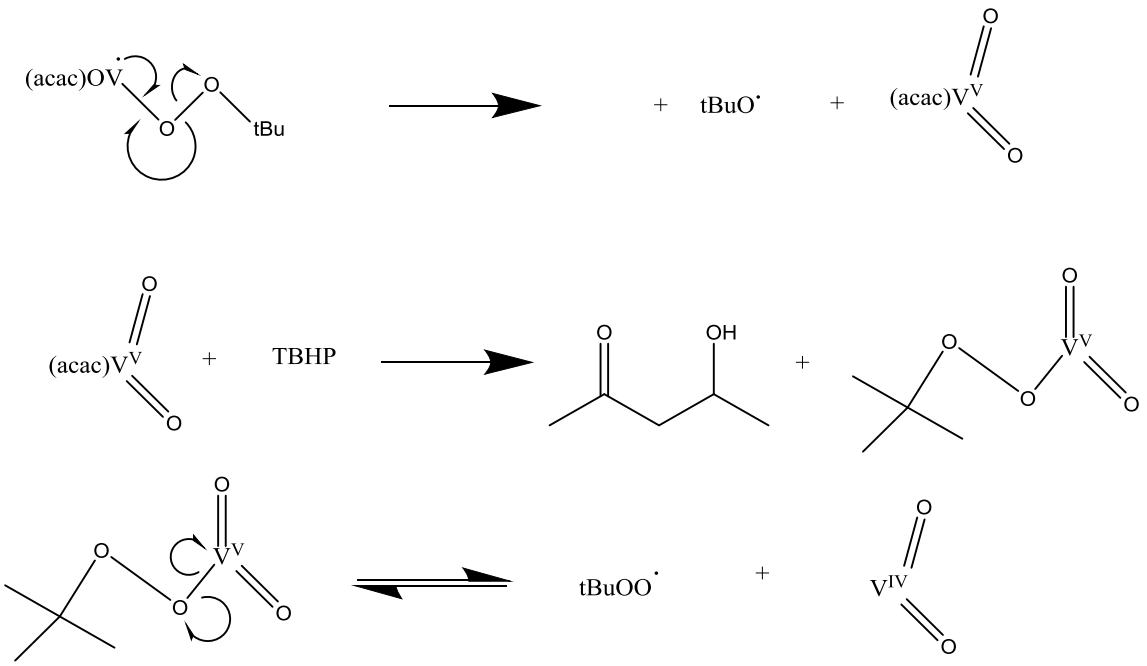
As mentioned prior, literature suggest that the radical mechanism is the pathway through which allylic oxidation occurs during epoxidation reactions using vanadium and TBHP.[98, 129] It was also the mechanism through which several

reported steroidal oxidation reactions occur. [86, 87, 93, 98, 99, 103, 109] The radical mechanism seemed the most logical to us as well, in accordance with our observations. Our proposed mechanism is broken down into three phases, presented in Schemes 3.9, 3.10, and 3.11.

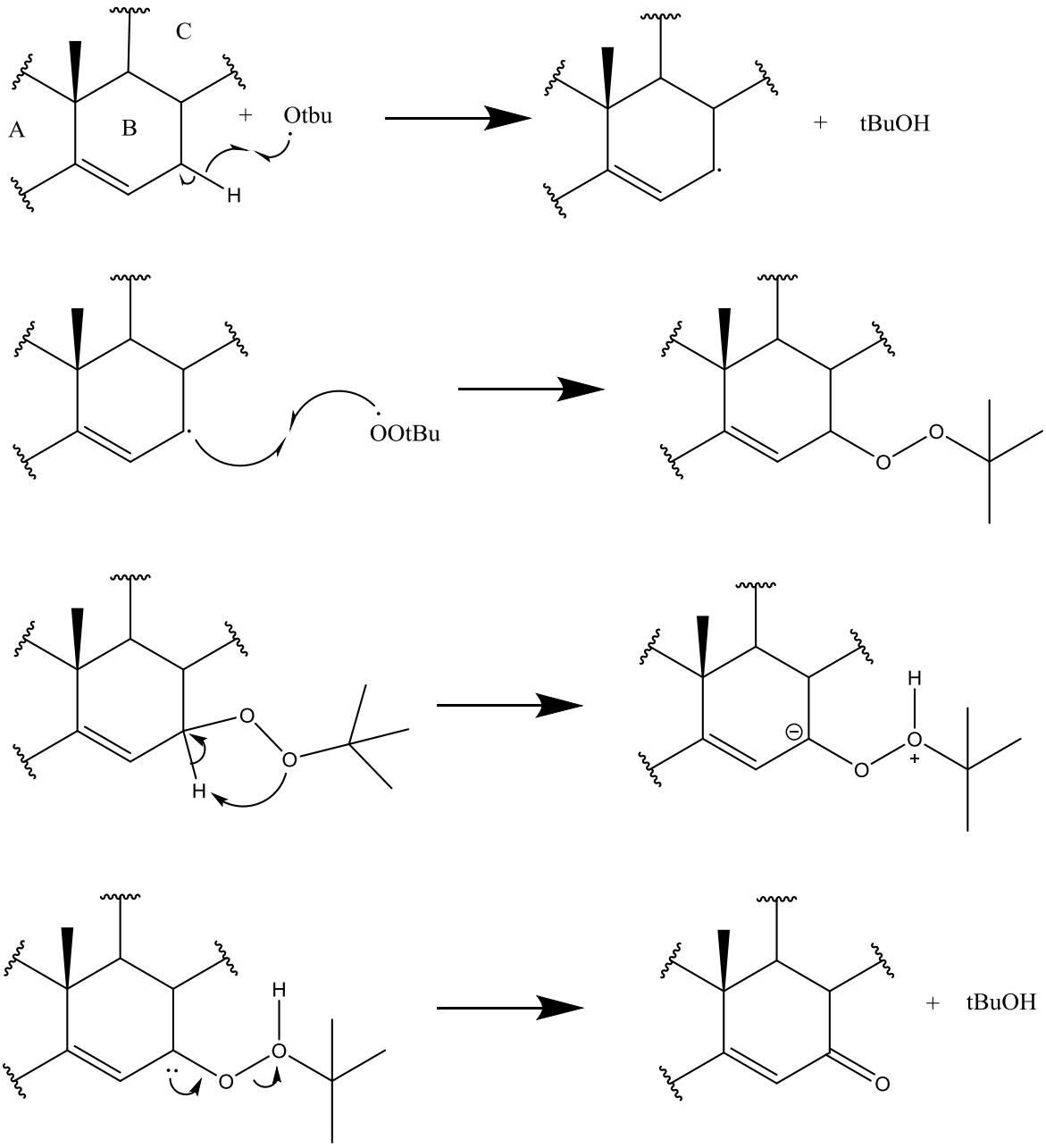
Scheme 3.9. Initial Substitution of the Acetylacetonate Ligand



Scheme 3.10. Formation of Free Radicals



Scheme 3.11. Abstraction, Radical Combination, and Enone Formation



Upon adding TBHP to the reaction mixture, a reddish color is observed, indicating that the vanadium (IV) peroxide complex is formed. [117, 128] (Scheme

3.9) Precipitate quickly forms on the glass of the reaction flask also indicating that the acetylacetonate ligand has been substituted.[142] In Scheme 3.10, the peroxide homolytically cleaves into a tert-butoxide radical and a $V^{V}O_2$(acac) complex.[129] Another TBHP substitutes the remaining acetylacetonate ligand, forming the metal complex, $V^{V}O_2$(OOtBu).[129] This time, with vanadium (VI) being an unstable oxidation state, the peroxide-metal bond is homolytically cleaved instead to form a peroxyl radical and a $V^{IV}O_2$ complex.[129] The vanadium (IV) complex can then be oxidized back into $V^{V}O_2$(OOtBu) with another TBHP molecule, thus repeating the cycle. Given the small molar amount of vanadium, the mediator has to be regenerated. Therefore we have written the liberation of tert-butyl peroxyl radicals as a reversible step. In the third phase (Scheme 3.11), the free radicals begin to interact with the steroidal compound at the allylic position. Through subsequent hydrogen abstraction, radical combination, and rearrangement, the radical mechanism ends with an α,β unsaturated ketone as the major product. We have submitted a possible route for the formation of the enone in Scheme 3.11, but the exact degradation of the steroidal peroxide is a matter for further investigation.

3.6. Other Vanadium Catalysts

If the reaction occurs through no direct contact between the steroidal compound and the vanadium mediator and the ligands are substituted by TBHP, then other vanadium species should be usable. We tested this hypothesis using direct injection quadrupole time-of-flight mass spectroscopy (Q-TOF). This type of mass spectroscopy has been utilized to quantitatively analyze sterol lipids in

plants.[144] The direct injection function for neat product was of great value because isolation of oxysterols can be precarious. Additionally, work-up procedures for each type of vanadium mediated reaction also varied, which would lead to systematic errors in comparisons. It should also be noted that direct injection provides higher analyte signal than UHPLC.

Two significant concerns arise when utilizing the Q-TOF to quantitatively analyze steroidal compounds. First, ionization efficiency (percentage of product ionized) must be the same throughout every test. This was easily solved by using the same product (i.e. differing pKa values can lead to different ionization efficiencies), which was 7-ketocholesterol, and the same solvent systems, which meant similar pH levels. (We used Q-TOF atmospheric pressure ionization, which relies on soft ionization.) The second concern was the need for precise injection. Although the injection was done by instrument, we assumed that there would be some variance in the volume of sample collected by the injection loop for analysis at the microliter level. Therefore, neat solution from each reaction was injected three times for three different analysis of the same reaction to ensure that equal volumes were loaded. We also took note of the total ion counts (TIC), also called total ion currents, to ensure precise volume injections. If the volumes of sample injected are the same, the TIC in each of the analysis of the same reaction will also be nearly the same. Because the TIC were not exact, we used the median of the range of values given by TIC and *m/z* 401 which corresponds to the M + H peak of 7-ketocholesterol. If the TIC values in a set

were arbitrarily too small or high, we extrapolated those values accordingly to make them comparable to other values.

Figure 3.9 shows an example of the chromatograms that would be produced after Q-TOF analysis of a reaction. The top chromatogram shows the intensity of ions that correspond to an *m/z* value of 401.34 +/- 0.5 Daltons. TIC is shown in the bottom chromatogram. The ion counts are noted in both chromatograms for *m/z* 100 to *m/z* 1000.

Table 3.3 displays the Q-TOF raw data of the oxidation reactions using various vanadium compounds. This data was obtained in conjunction with TLC to identify the presence of starting material, cholesterol, which we discovered could also give a 401 peak through interaction with the Q-TOF's . Since our reaction could fully convert the starting material, this was not a significant problem for most of the entries. Those entries accounting reactions that did not achieve full conversion of starting material have been noted. TIC and the *m/z* 401 (+/- 0.5 Dalton) are also listed in Table 3.3. Additionally, we have also listed the percentage of *m/z* 401 ions of the TIC. The average percent *m/z* 401 of TIC values for each reaction set are also listed. These percentages gave us another way of comparing our data, which did not include extrapolation. The median *m/z* 401 and average *m/z* 401/TIC % values are displayed in the Figures 3.10 and 3.11 respectively.

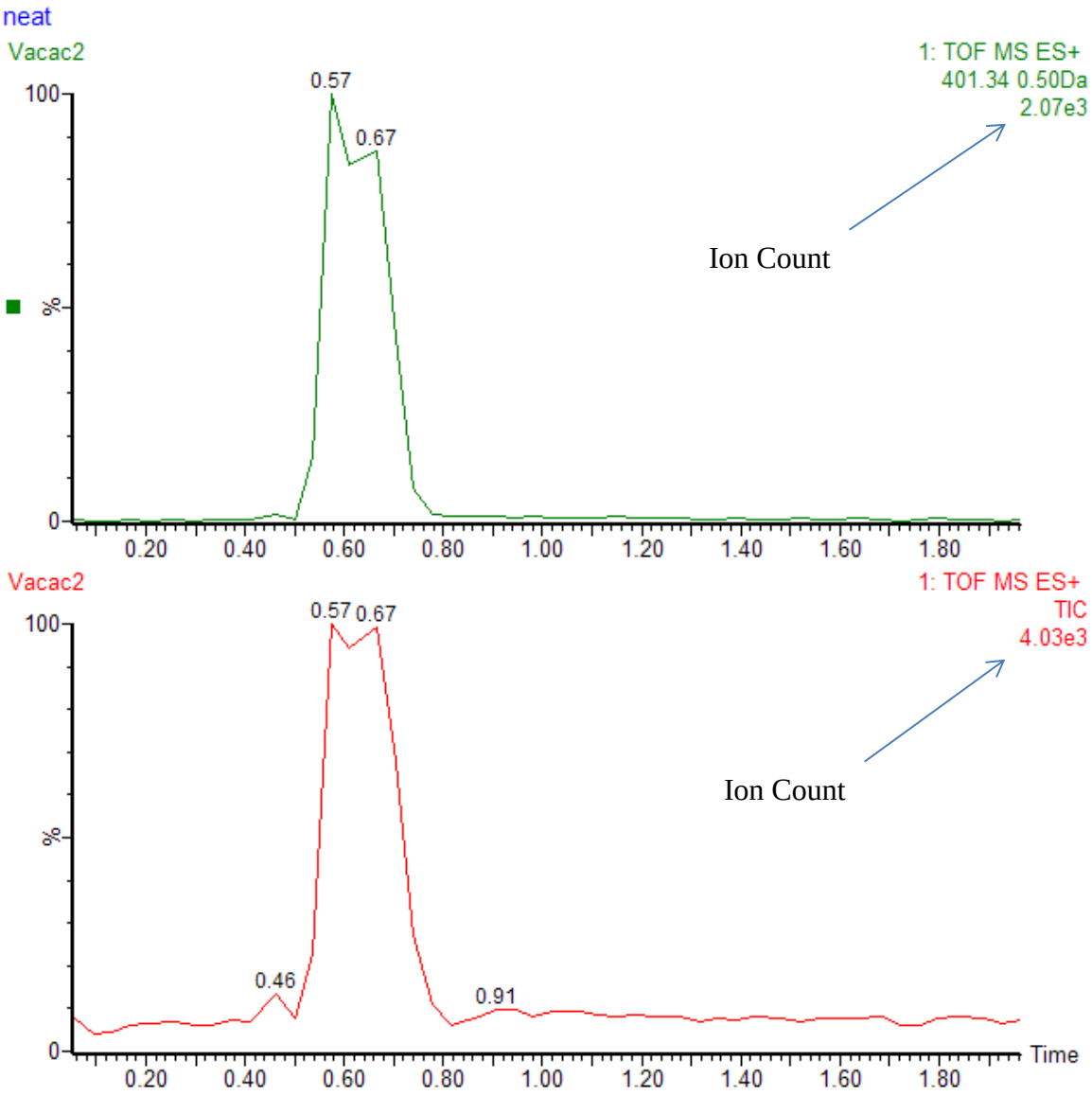


Figure 3.9. Example of Chromatograms Showing TIC and *m/z* 401 Ion Count

Table 3.3. Q-TOF Raw Data

Vanadium Complex Used	TIC ($x10^3$)	*m/z* 401 Ions ($x10^3$)	*m/z* 401/ TIC %	Median *m/z* 401 ($x10^3$)	Avg %	100% Conv.
Bis(cyclopentadienyl)vanadium (IV) Dichloride	6.58	1.76	26.7	2.07	28.1	Yes
	7.30	2.24	30.7			
	8.76	2.37	27.1			
Vanadyl (IV) Acetylacetonate	4.53	2.18	48.1	2.14	47.7	Yes
	4.03	2.07	51.4			
	4.79	2.09	43.6			
Vanadyl (IV) Acetylacetonate, N_2 Atmosphere	4.10	0.861	21.0	1.12	24.9	Yes
	4.75	1.37	28.8			
	2.46	139	5.7			
Vanadium (IV) Oxide Sulfate Hydrate	1.27	0.201	15.8	0.25	16.8	Yes
	1.88	0.288	15.3			
	1.88	0.307	16.3			

Table 3.3. Q-TOF Raw Data *Continued*

Vanadium Complex Used	TIC ($x10^3$)	*m/z* 401 Ions ($x10^3$)	*m/z* 401/ TIC %	Median *m/z* 401 ($x10^3$)	Avg %	100% Conv.
Vanadium (III) Acetylacetonate	1.61	0.229	14.2	0.40	21.7	Yes
	2.00	0.566	28.3			
	1.49	0.335	22.5			
Vanadium (IV) Carbide	9.45	6.04 (3.79)[a]	63.9	3.21	53.5	No
	2.92	1.29 (2.62)[a]	44.2			
	5.93	3.10	52.3			
Vanadium tris(triphenylsiloxy) vanadium (V) Oxide	6.87	4.82	70.2	4.96	72.2	Yes
	11.3	8.39 (5.10)[b]	74.2			
	3.69	1.48	40.1			
Vanadium (V) Oxytripropoxide	4.94	3.12	63.2	3.56	57.4	Yes
	7.61	4.00	52.6			
	6.00	3.38	56.3			

Table 3.3. Q-TOF Raw Data *Continued*

Vanadium Complex Used	TIC ($x10^3$)	*m/z* 401 Ions ($x10^3$)	*m/z* 401/ TIC %	Median *m/z* 401 ($x10^3$)	Avg %	100% Conv.
Vanadyl (IV) Acetylacetonate	21.1	9.97 (2.14)[d]	47	2.17	47.8	
	22.8	11.1 (2.21)[d]	49			
	24.2	10.4 (2.06)[d]	45			
	38.6	19.3 (2.27)[d]	50			
Vanadyl (IV) Acetylacetonate	3.52	0.953 (1.92)[d]	42.3	2.18	47.4	Yes
	2.93	1.35 (2.09)[d]	46.1			
	2.25	1.89 (2.43)[d]	53.7			
No Vanadium	1.10	0.107	9.7	0.20	13.3	No
	1.94	0.253	13			
	1.75	0.301	17.2			
Cholesterol In Benzene Only	91.6	2.88	0.31	1.80	0.53	No
	74.0	0.301	0.41			
	82.3	0.721	0.88			

Table 3.3. Q-TOF Raw Data *Continued*. TIC is total ion count. *m/z* 401 ions is the *m/z* 401 +/- 0.5 Dalton ion count. *m/z* 401/TIC is the percentage of TIC that is *m/z* 401. Median *m/z* 401 is the median of the range of "*m/z* 401 ions" for the set per vanadium complex. Avg % is the average of "*m/z* 401/TIC %" values. 100% Conv. is the listing of whether or not the reaction with the vanadium complex converted cholesterol completely (Yes) or not (No). a. Extrapolated to TIC = 5.93. b. Extrapolated to TIC= 6.87. c. column 401/ column TIC; d. Extrapolated TIC to 4.53. Highlighted values were deemed unusable because they differed greatly from concurrent spectra. All reactions, unless otherwise noted, were done using the optimal molar amounts in 25 mL of benzene under ambient conditions, room temperature, and five days.

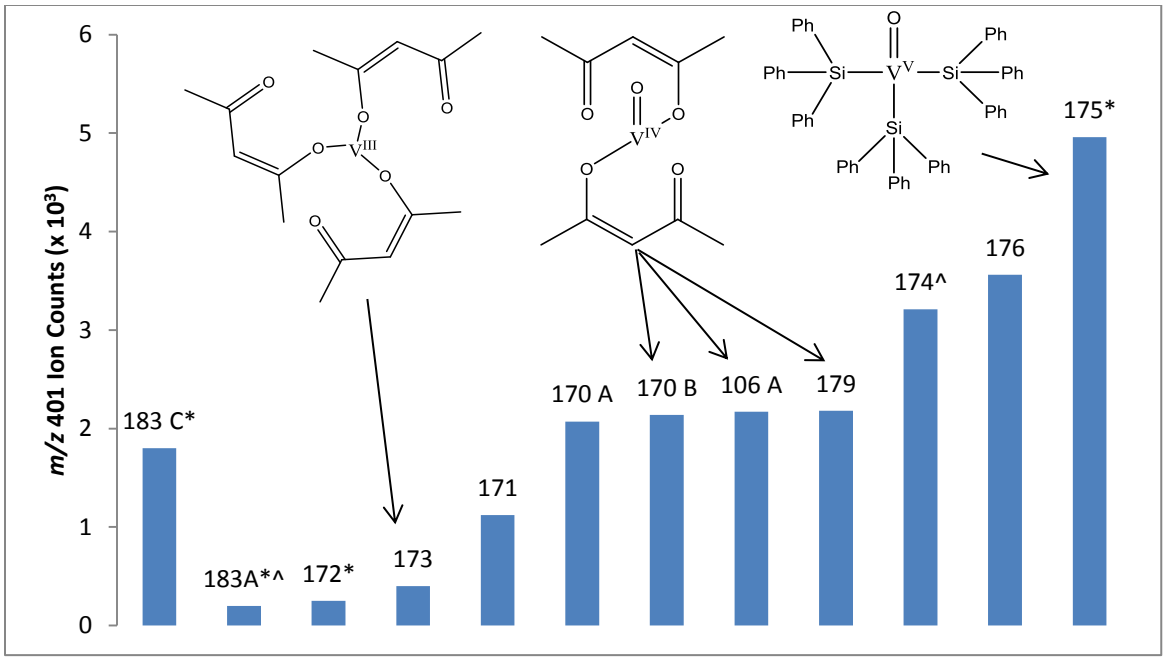


Figure 3.10. Median *m/z* 401 Ion Counts. **183 C**: Cholesterol in benzene only; **183 A**: No vanadium; **172**: Vanadium (IV) Oxide Sulfate Hydrate; **173**: Vanadium (III) Acetylacetonate (structure drawn); **171**: Vanadyl (IV) Acetylacetonate in anaerobic N_2 atmosphere; **170 A**: Bis(cyclopentadienyl)vanadium (IV) Dichloride; **170 B**, **179**, and **106 A**: Vanadyl (IV) Acetylacetonate (structure drawn); **174**: Vanadium (IV) Carbide; **176**: Vanadium (V) Oxytripropoxide; **175**: Vanadium tris(triphenylsiloxy)vanadium (V) Oxide (structure drawn). * indicates no precipitate was observed in the reaction solvent. ^ indicates over-inflated value because of cholesterol contributing to the *m/z* 401 ion count. (Identified by TLC)

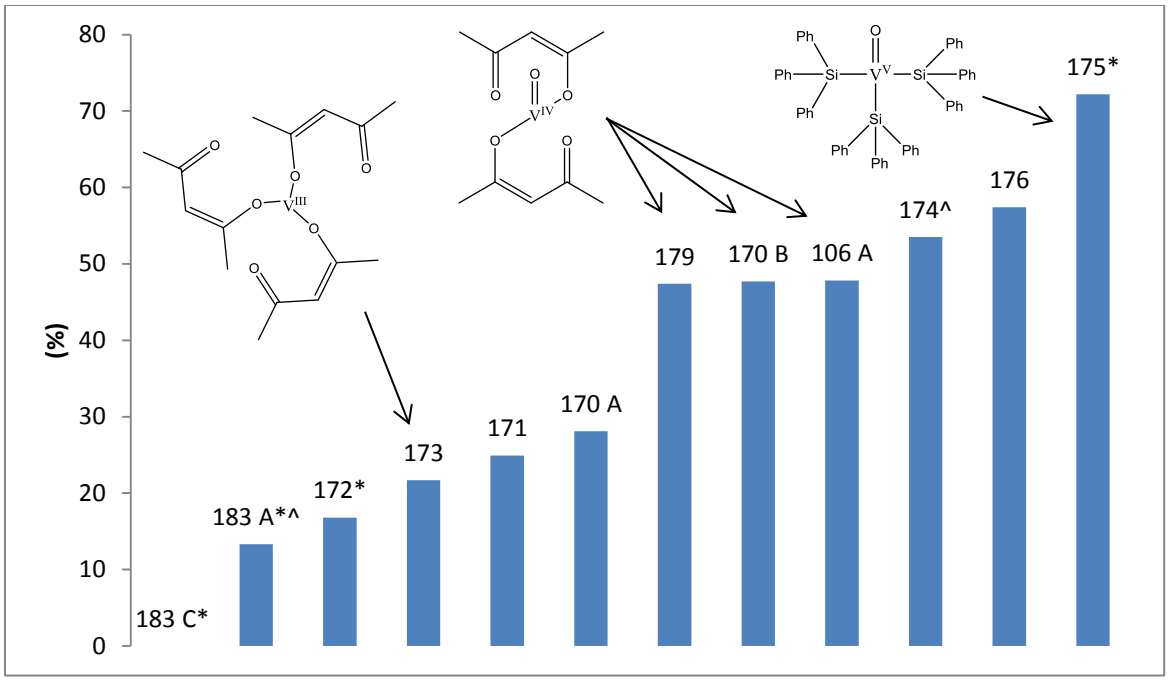


Figure 3.11. Average % (*m/z* 401/TIC). **183 C**: Cholesterol in benzene only; **183 A**: No vanadium; **172**: Vanadium (IV) Oxide Sulfate Hydrate; **173**: Vanadium (III) Acetylacetonate (structure drawn); **171**: Vanadyl (IV) Acetylacetonate in anaerobic N_2 atmosphere; **170 A**: Bis(cyclopentadienyl)vanadium (IV) Dichloride; **170 B**, **179**, and **106 A**: Vanadyl (IV) Acetylacetonate (structure drawn); **174**: Vanadium (IV) Carbide; **176**: Vanadium (V) Oxytripropoxide; **175**: Vanadium tris(triphenylsiloxy)vanadium (V) Oxide (structure drawn). * indicates no precipitate was observed in the reaction solvent.

Both Figures 3.10 and 3.11 gave consistent results with the exception of the cholesterol in benzene data set (which had a different solvent system than the one shared by all of the other tests) and proved that other vanadium complexes could mediate the reaction. The higher oxidation states of vanadium gave both higher overall *m/z* 401 ion counts and *m/z* 401/TIC percentages. (This

was noted and we were able to obtain a 30% isolated yield of 7-ketocholesterol using vanadium tris(triphenylsiloxy)vanadium (V) Oxide with an un-optimized work-up procedure.) Of the vanadium (IV) species tried, $VO(acac)_2$ produced the highest *m/z* 401 ion count. Although higher yields are reported for vanadium (IV) carbide, TLC showed that starting material was still present after its reaction, likely inflating the *m/z* 401 ion count. Because of the incomplete conversion of starting material, the vanadium (IV) carbide reaction was deemed to have a lower un-inflated *m/z* 401 ion count than that of the vanadyl acetylacetonate reactions.

Figures 3.10 and 3.11 also show that vanadyl acetyl acetonate was tried three separate times to form three separate entries. We did this to prove the reproducibility of the analysis method. The vanadyl acetylacetonate tests were separated by weeks, in which Q-TOF maintenance (mechanical, tuning, and calibrating) occurred. As can be seen from Figures 3.10 and 3.11, the vanadyl acetylacetonate reactions produced almost exactly the same results.

3.7. Other Metal Catalysts

Noting that the reaction occurred through no direct contact between the mediator and the substrate, we expanded our research past vanadium. Several metal acetylacetonate complexes including bis(acetylacetonato) dioxomolybdenum (IV), copper (II) acetylacetonate, and iron (III) acetylacetonate were tried. These were tested in the place of the vanadium, using cholesterol as the substrate, and under optimal conditions.

Copper (II) acetylacetonate and iron (III) acetylacetonate gave results that were similar, but different from those obtained with the vanadium catalysts. The conversion of cholesterol in each of these tests was at most, 50%, but the reactions appeared very clean on TLC, only yielding one major product and two minor ones. We isolated the major product, but found through comparison of relative ^{13}C NMR peak sizes in multiple reaction tests, that it consisted of three different products with a similar retention time. The major product (s) had an R_f value greater than 7-ketocholesterol's and we thought it (they) may be an epoxide. Thus, we synthesized 5,6-epoxycholesterol using $KMnO_4$-$CuSO_4$.[145] However, the major product (s) and epoxide did not exhibit the same R_f values.

Bis(acetylacetonato) dioxomolybdenum (IV) gave the same products as the $VO(acac)_2$, albeit with a much lower conversion percentage. Thus, vanadium was shown by TLC to be the best mediator of these five metal acetylacetonates. Although this is merely anecdotal, it is interesting that vanadium and molybdenum seem to share the same reactivity, albeit not the same reaction rates.[117] Perhaps the answer lies in Gould's statements regarding the role of the positive metal center in epoxidation reactions. Boron and titanium (IV) d^0 systems were not effective epoxidation catalysts, despite being able to form peroxide complexes, because they were not strong enough to polarize the oxygen-oxygen to the point of heterolysis and Cr(VI), Se(VI), Mn(VII), and Os(III) were too strong, reducing too easily.[117] However, V(V) and Mo(VI) were between the "extremes."[117] However, Gould also noted that he could not explain why both vanadium and molybdenum were superior to W(VI).[117]

3.8 Other Substrates

The next phase in our research was to take the reaction and try new substrates. Nimbidiol was a possible target. The diterpene is a natural product isolated from the root of Azadirachta indica and is sought for its biological potential.[146] Our interest in the compound was that it had a moiety somewhat like an estrogen with a ketone on the benzylic carbon. This was also the case for inuroyleanol, another natural product gathered from the Himalayas for its medicinal properties.[147, 148]

Majetich was able to add a 7-keto group (or 6-keto depending on how the A ring is assigned) to a similar compound on the way to total synthesis of nimbidiol.[149] (Figure 3.12) However, PCC was used as the oxidant and the two hydroxyl groups had to be protected. The additional two steps were not completely successful at deprotecting the hydroxyl groups, and the final yield was only 23%. This seemed like a good place for vanadium mediated oxidation given that the hydroxyl groups would not need protection, albeit our reaction did not work on estrone. To test our reaction, we chose a cheaper compound, podocarpic acid, with a similar moiety to that of nimbidiol. (Podocarpic acid has been oxidized to the 7-keto product with CrO_3.)[150]

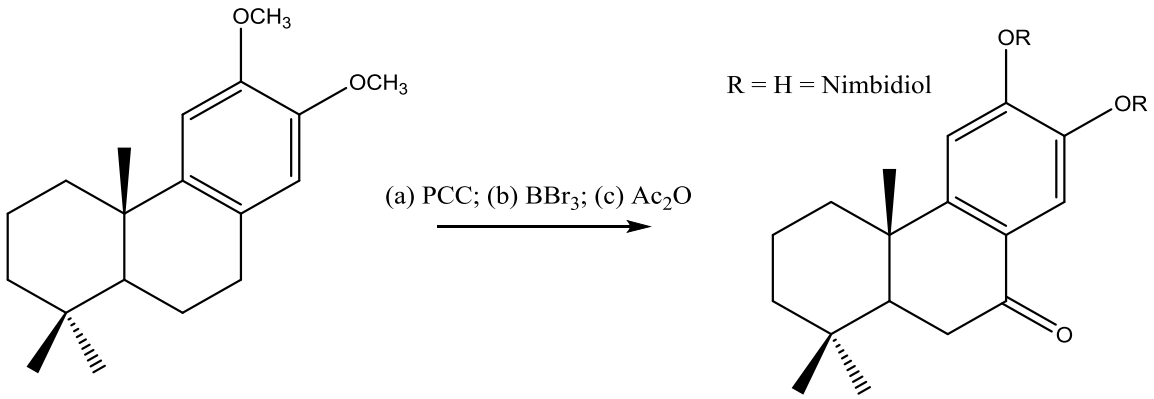


Figure 3.12. Majetich's Synthesis of Nimbidiol[149]

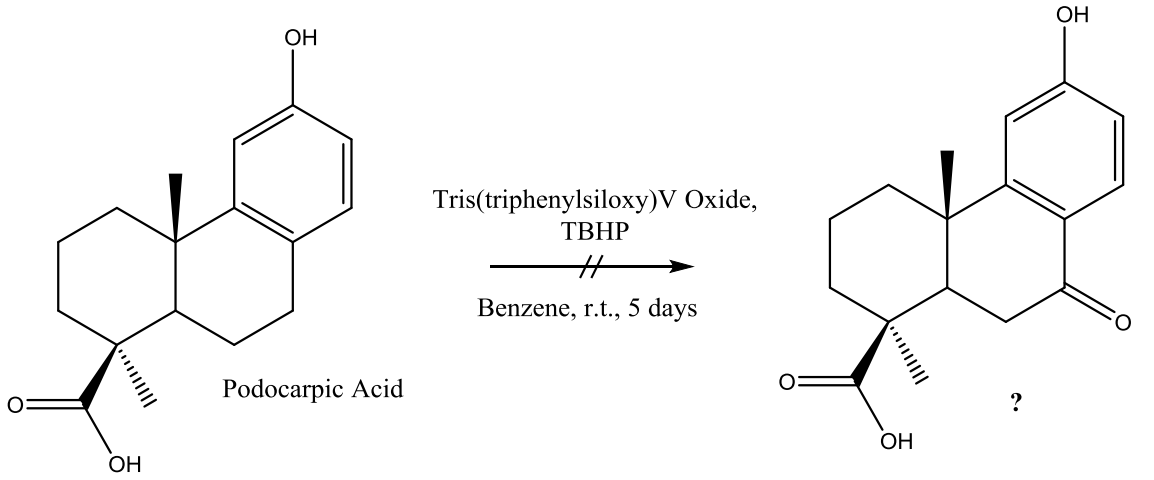


Figure 3.13. Attempted Podocarpic Acid Oxidation to Nimbidiol Moiety

We found some oxidation, but not of the 7-keto variety. Using NMR, two new peaks appeared at δ 79.4 and 77.2. Without more costly research or literature pertaining thereto, it is hard to say were these hydroxyl groups were added to the substrate. We were able to use ChemDraw® NMR prediction software to predict that one may be bound at the tertiary C5 position. Thus, even with podocarpic acid, we were unable to achieve benzylic oxidation of the steroidal ring moiety.

Cyclohexane oxidation was tested next. For these reactions, cyclohexane was the solvent and no benzene was used. Three trials were conducted over five days with cyclohexane at room temperature and in ambient conditions: with the optimal amounts of TBHP and $VO(acac)_2$, with only the optimal amount of $VO(acac)_2$ (no TBHP), and with only the optimal concentration of TBHP (no $VO(acac)_2$). Neat reaction solution from these completed reactions were tested by gas chromatography time-of-flight mass spectroscopy (GCT). When only $VO(acac)_2$ was present, no significant oxidation was observed. The reaction with only TBHP showed nearly the same oxidation as the reaction with both TBHP and $VO(acac)_2$, as per the similar chromatograms. Thus, the only vital factor appeared to be the presence of TBHP and it is evident that cyclohexane does not need a mediator to be oxidized in a concentrated solution of TBHP, nor can $VO(acac)_2$ oxidize alone.

Those chromatograms are displayed in Figures 3.14, 3.15, and 3.16. Figure 3.14 shows a curved baseline, with the initial declining intensity (four to nine minutes) being cyclohexane. Cyclohexane and relevant oxidation products of figures 3.15 and 3.16 have a box drawn around them. Those values are: (≈4.26 min) *m/z* 98, perhaps methyl cyclohexane; (≈6.59 min) *m/z* 98, cychexanone or cyclohex-2-ene-1-ol; (≈11.79 min) *m/z* 166, bycyclohexyl; (≈12.69 min) *m/z* 126, unidentified oxidized cyclohexane product.

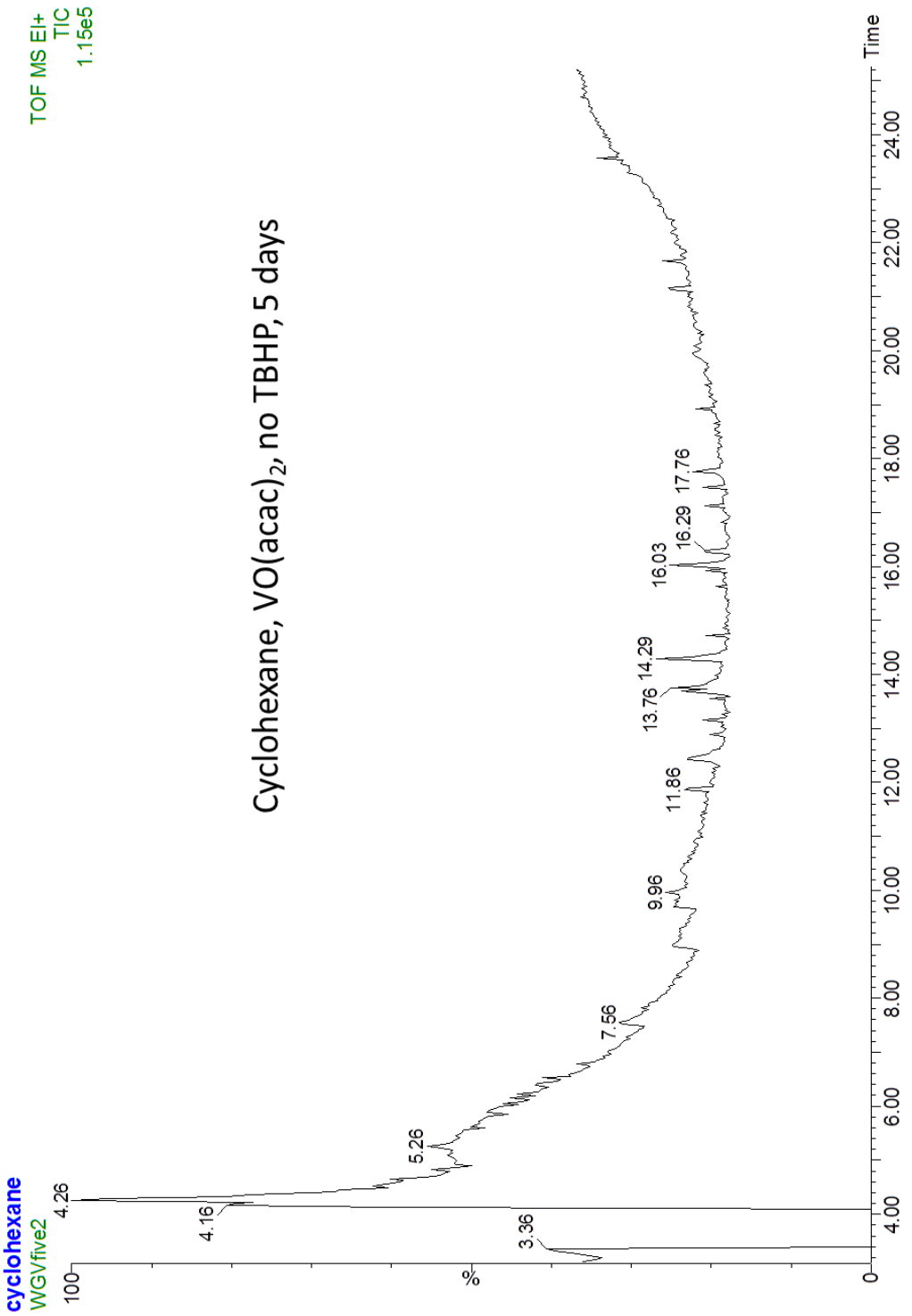


Figure 3.14. GCT Chromatogram of Cyclohexane, $VO(acac)_2$, no TBHP, 5 Days

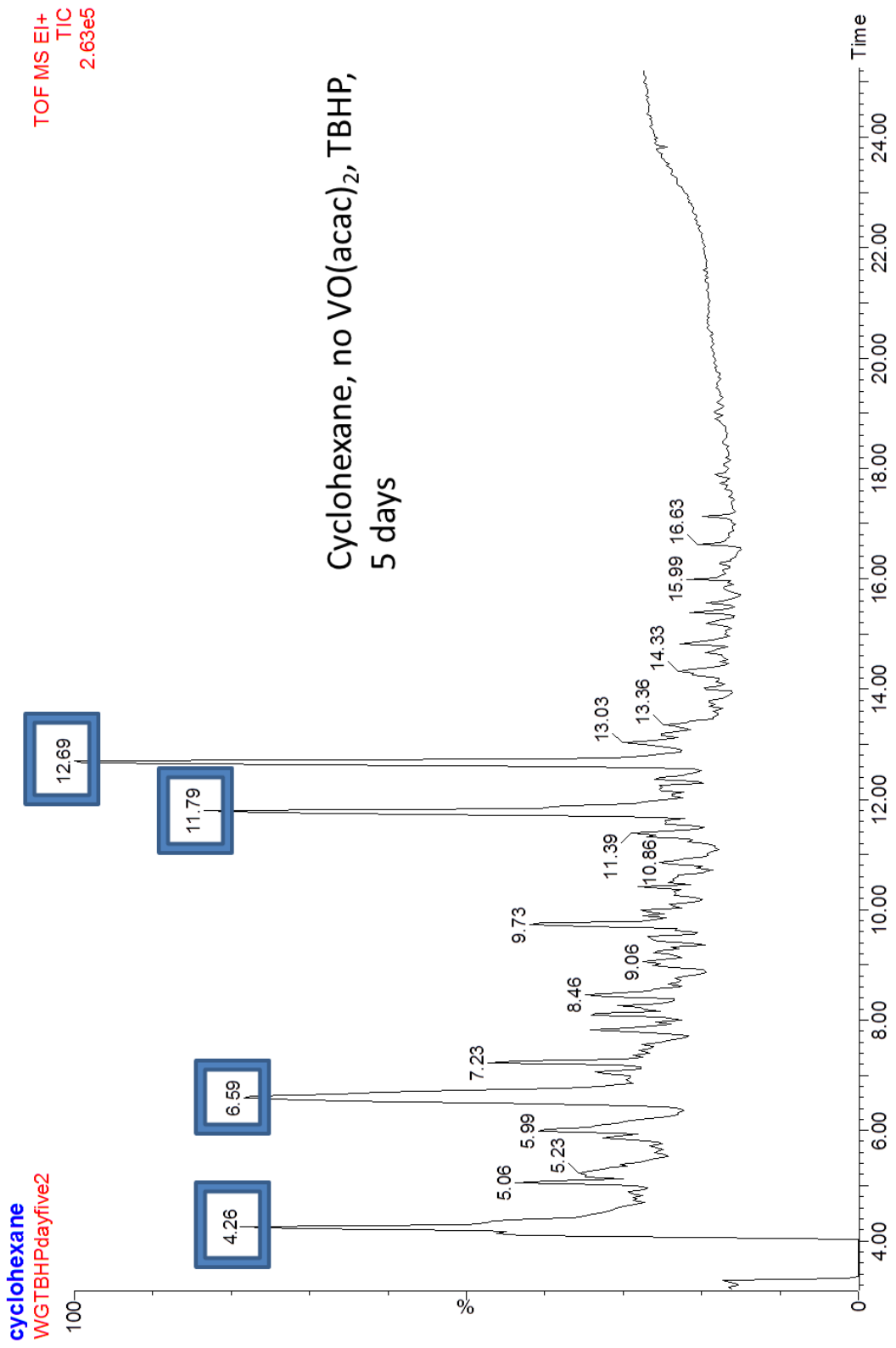


Figure 3.15. GCT Chromatogram of Cyclohexane, no $VO(acac)_2$, TBHP, 5 Days

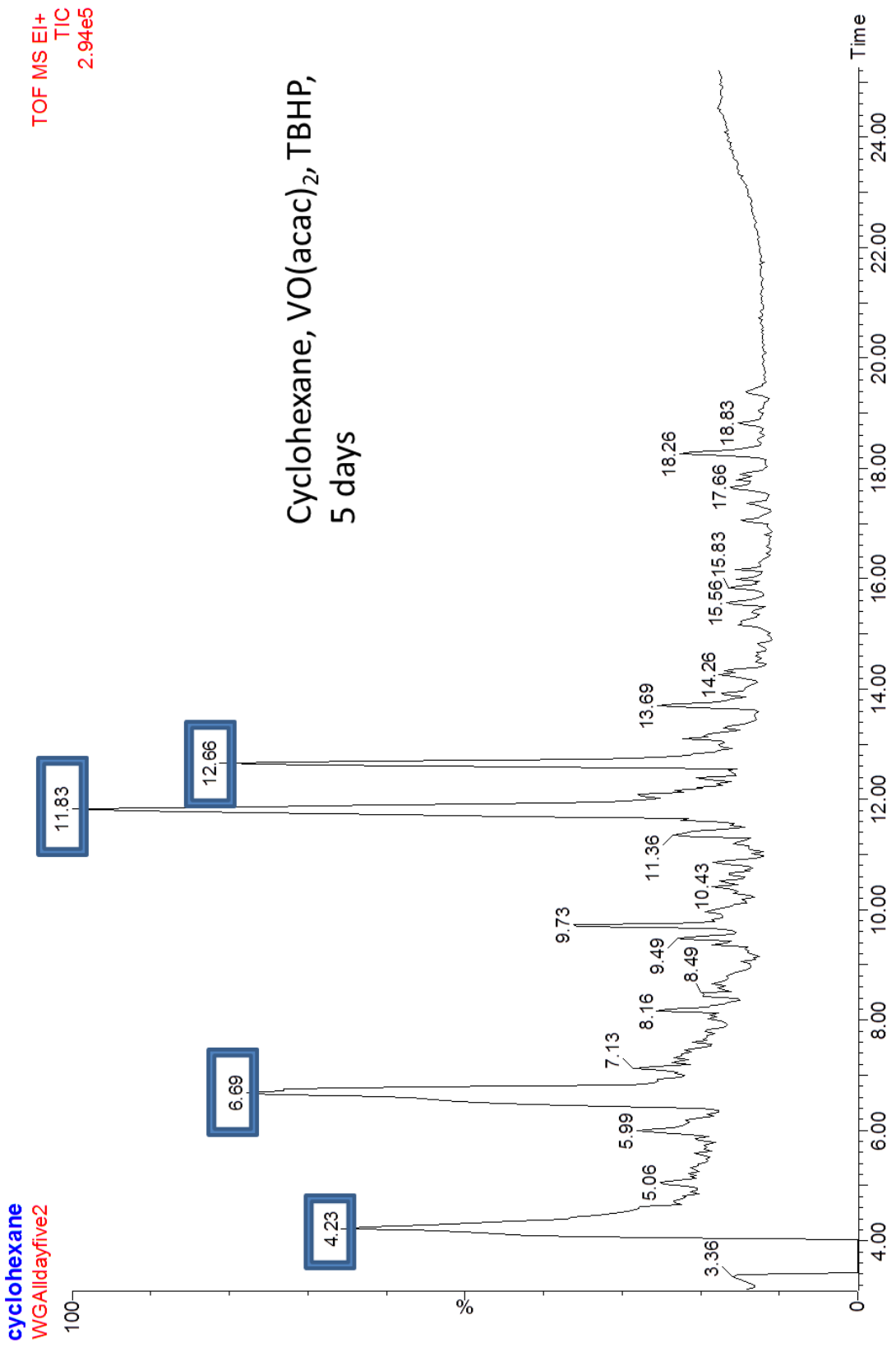


Figure 3.16. GCT Chromatogram of Cyclohexane, $VO(acac)_2$, TBHP, 5 Days

Following the study of cyclohexane oxidation, we tested another small molecule that was tougher to oxidize. We wanted to know if our reaction could oxidize limonene into carvone. (Figure 3.17) Two experiments were conducted with optimal conditions and varying reagents using limonene as the substrate and solvent. One had both the optimal concentrations of $VO(acac)_2$ and TBHP and the other had only TBHP. Indeed, both trials yielded carvone and other oxidation products. From the chromatograms (GCT) in Figure 3.18, the test containing both $VO(acac)_2$ and TBHP yielded more oxidation products than with TBHP only. However the test with only TBHP yielded a higher ion count for carvone, likely because the test with both $VO(acac)_2$ and TBHP oxidized the substrate and carvone.

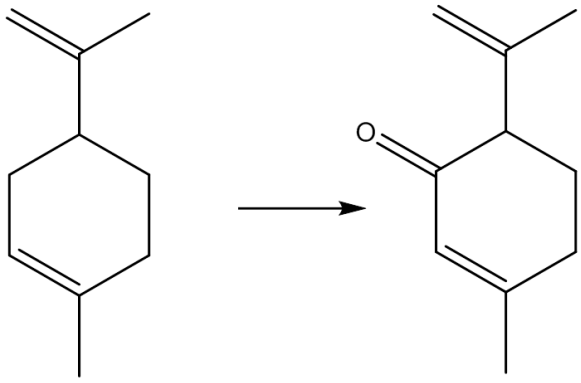


Figure 3.17. Limonene to Carvone

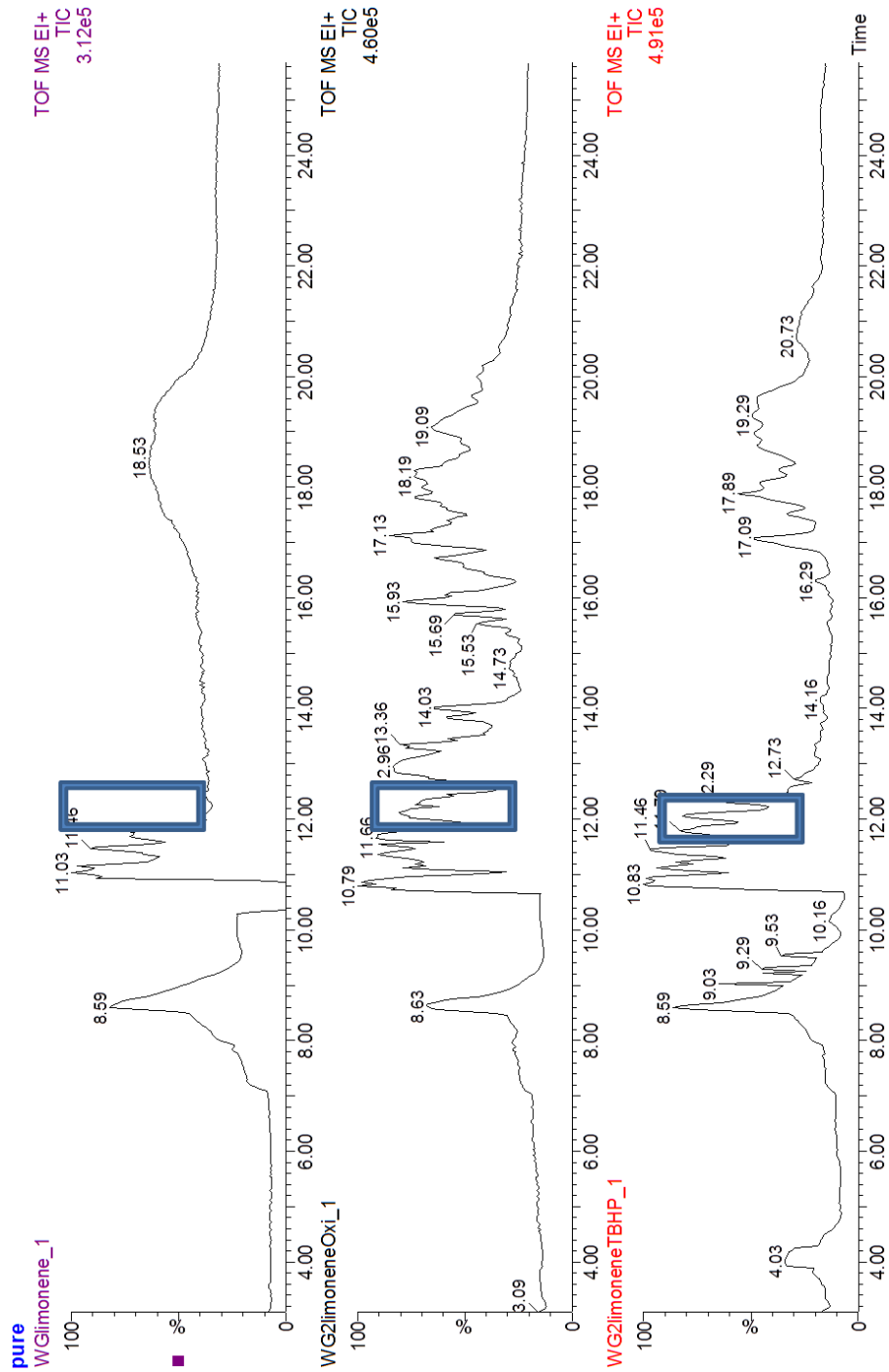


Figure 3.18. GCT Chromatograms of (from left to right) Pure Limonene, Oxidation Using TBHP and $VO(acac)_2$, and Oxidation Using Only TBHP. The drawn box shows where carvone appears. Pure limonene had no carvone.

3.9. 7-Keto Functionality

Phytosterols are thought to lower cholesterol serum levels by competing for absorption with cholesterol and then not being fully absorbed by the body.[151] We pondered whether this same competition could be used to inhibit microbes. Thus, our vanadium catalyzed allylic oxidation reaction was used make 7-keto-β-sitosterol from β-sitosterol. Preliminary tests were then conducted for *E. coli* inhibition with the 7-keto-β-sitosterol.

E. coli colonies were grown in lysogeny broth (LB) solution for twenty four hours. The *E. coli* was then transferred into three flasks and allowed to grow for one more hour. Each of the three flask would then be charged with varying amounts (0, 0.1 mmol, and 1 mmol) of oxyphytosterol. For a period of five hours, the contents were scanned by UV-vis spectrophotometry every hour to monitor growth. That absorbance (600 nm) was plotted in the Figure 3.18.

What can be seen from Figure 3.19 is that the addition of 1 mmol inhibited *E. coli*'s growth. Adding a larger portion of 7-keto-β-sitosterol caused the growth to become even more linear, indicative of growth inhibition. However, after a certain concentration, the oxyphytosterol precipitates out of solution. This is seen in Figure 3.20, where 10 mmol addition inhibited the growth of *E. coli* better than 1 mmol and 30 mmol, which precipitated out of solution.

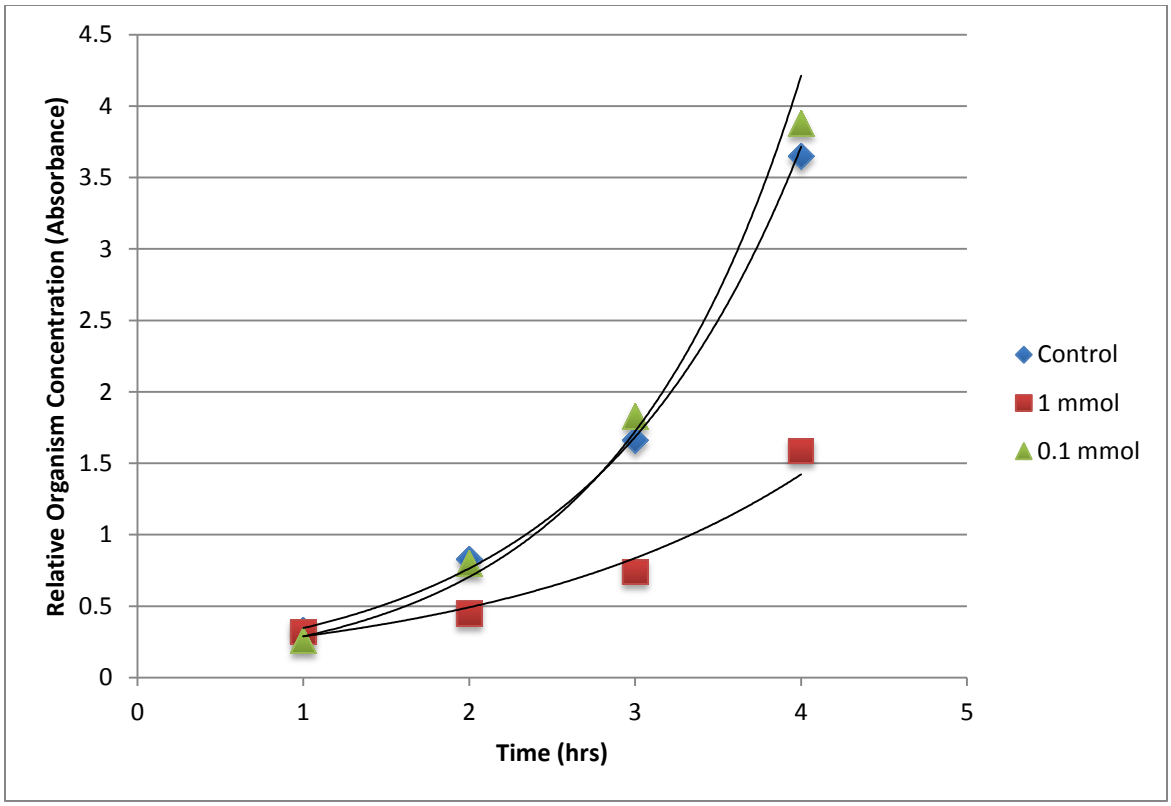


Figure 3.19. Plotted Growth of *E. coli* Bacteria with 1 and 0.1 mmol of Oxyphytosterol

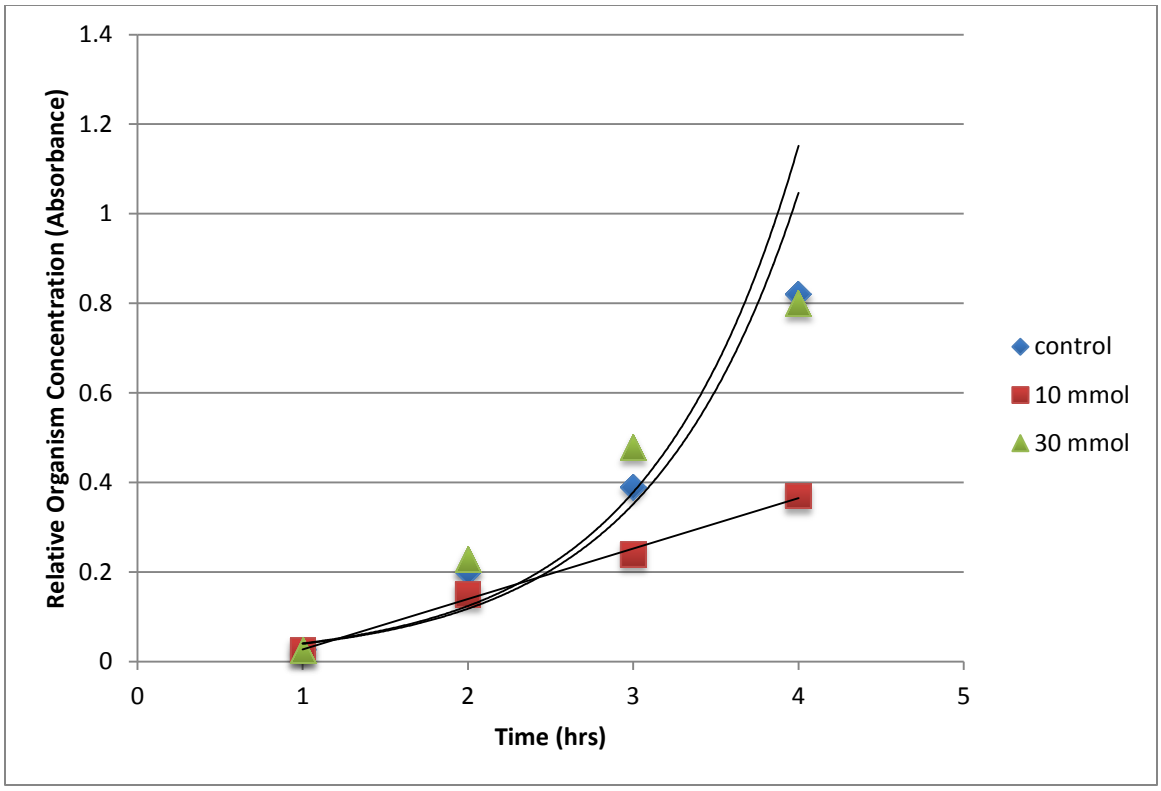


Figure 3.20. Plotted Growth of *E. coli* with 10 and 30 mmol of Oxyphytosterol

4. Dimerization of Steroidal α,β Unsaturated Ketones

4.1. Utility of Steroidal Dimers

The amphipathic structures of steroidal compounds are able to pass through phospholipid layers of cellular membranes unlike many medicinally potent compounds. With this in mind, there has been research to impart steroidal maneuverability to these hindered medicinal compounds, namely by covalently pairing them with steroids.[152, 153]

Steroid dimers have garnered much interest for their drug delivery capabilities. The first “molecular umbrella” was prepared from cholic acid.[152] Molecular umbrellas are essentially surfactants that pass through polar and nonpolar environments while covalently carrying a molecule that would otherwise by hindered from passing through water or lipid walls.[152, 153] Bile acids have been thought to make good molecular umbrellas because they contain both hydrophobic and hydrophilic parts, have low toxicity, and have carboxylic acid groups exploitable for dimerization.[152, 153] In addition to acting as a small transport capsules, it has also been postulated that molecular umbrellas could be used to remove toxic chemicals from cells.[152]

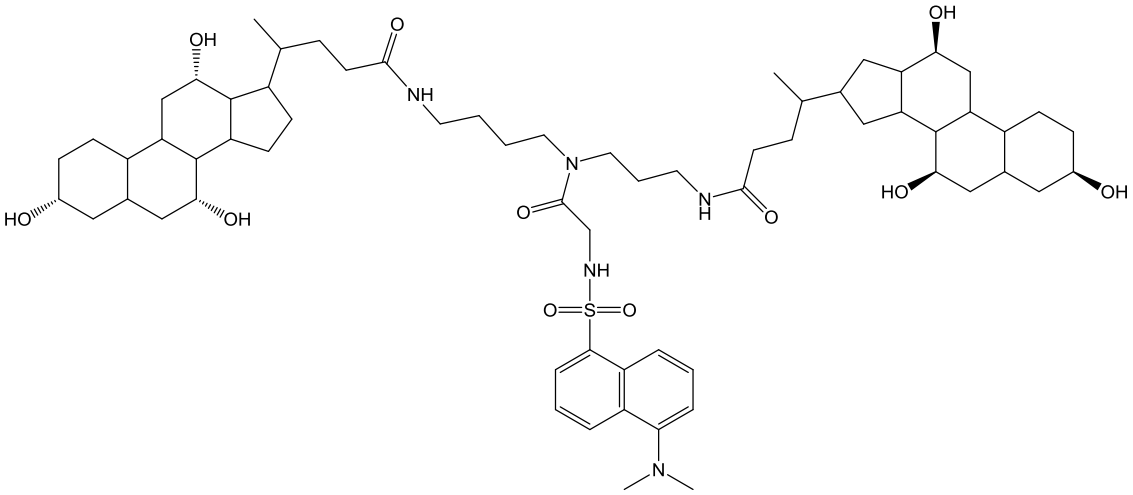


Figure 4.1. Molecular Umbrella Synthesized with Bile Acid[153]

Several dimers have emerged as potential drugs themselves. Namely, cephalostatins,[154] crellastatins,[155] and ritterazines[154] have shown significant cytotoxicity toward cancer cell lines. (These dimers were first discovered in marine organisms and are displayed in Figure 4.2.) It has been said of cephalostatin 1 that it is "one of the most powerful cancer cell growth inhibitors ever tested by the US National Cancer Institute."[152] Steroid dimers have also found their place amongst anti-malarial drugs. Several steroidal dimers containing the tetraoxane moiety have shown anti-malarial activity.[156, 157]

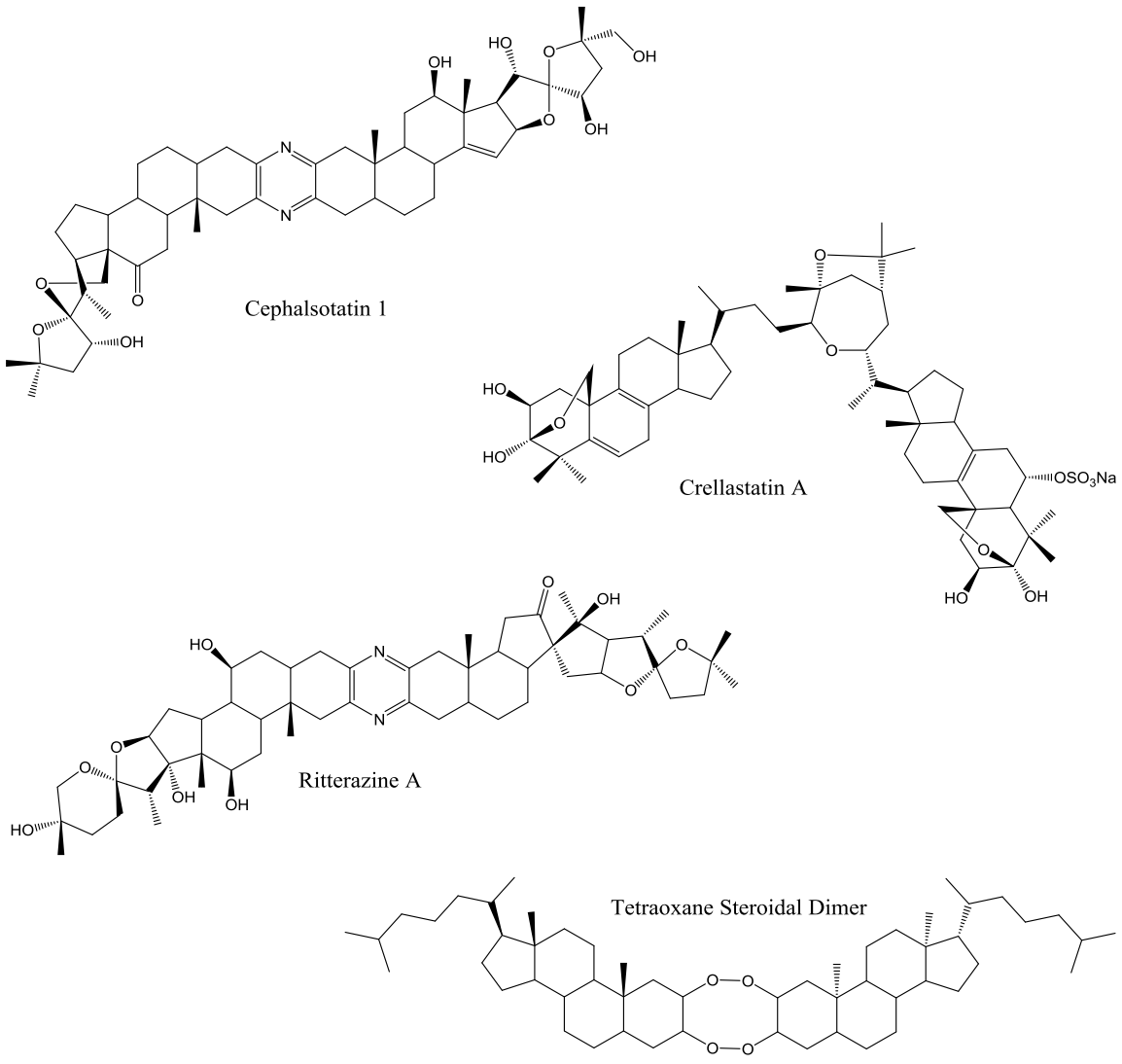
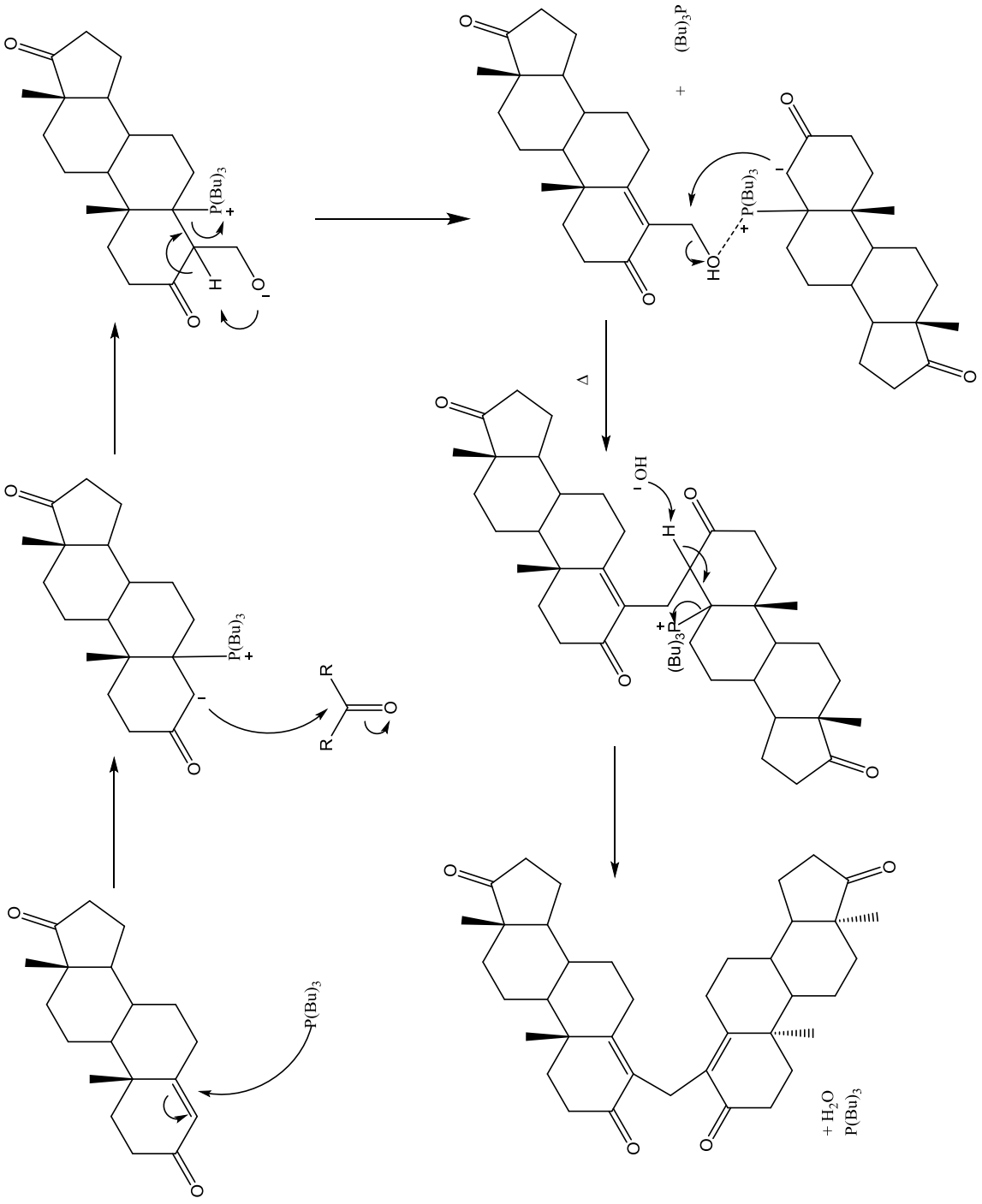


Figure 4.2. Cephalostatin 1,[154] Crellastatin A,[155] Ritterazine A,[154] and a Tetraoxane Steroidal Dimer.[152] These dimers are shown from top to bottom in their respective written order.

Various synthetic methods are utilized to make steroid dimers, including Pinacol, Grignard, McMurray, photochemical, Grubbs catalysts, and substitution reactions.[152] Steroid dimers have been covalently bound through direct linkage and by spacers such as $COCl_2$, $H_2N(CH_2)_nNH_2$, and isophthaloyl dichloride.[152] In general, direct linkage is suitable for connecting A rings, side chains, and the D rings when a long side chain is not present. Direct linkage of B rings is rare[152] and no example of C ring direct linkage has been given. This is expected given the steric shielding of the B and C rings. Additionally, an example of direct linkage with the D rings of steroids containing a long side chain was not observed.

Our lab reported in 2000 an unexpected formation of steroid dimers from Δ^4 steroids androstendione and progesterone in 3.6 and 2.3 percent yields respectively.[158] The dimerization was achieved by reacting the Δ^4 steroids, dissolved in methanol, with a 1.3 equivalent amount of paraformaldehyde and 40-50 µL of tri-butyl phosphine under nitrogen for 24 hours. Afterwards, the mixture was heated on a rotary evaporator under vacuum at 60˚C until dry. Without the heating, no dimerization occurred. The proposed mechanism is shown in Scheme 4.1.[158]

Scheme 4.1. Proposed Mechanism for Dimerization of A Ring α,β Unsaturated Ketones[158]



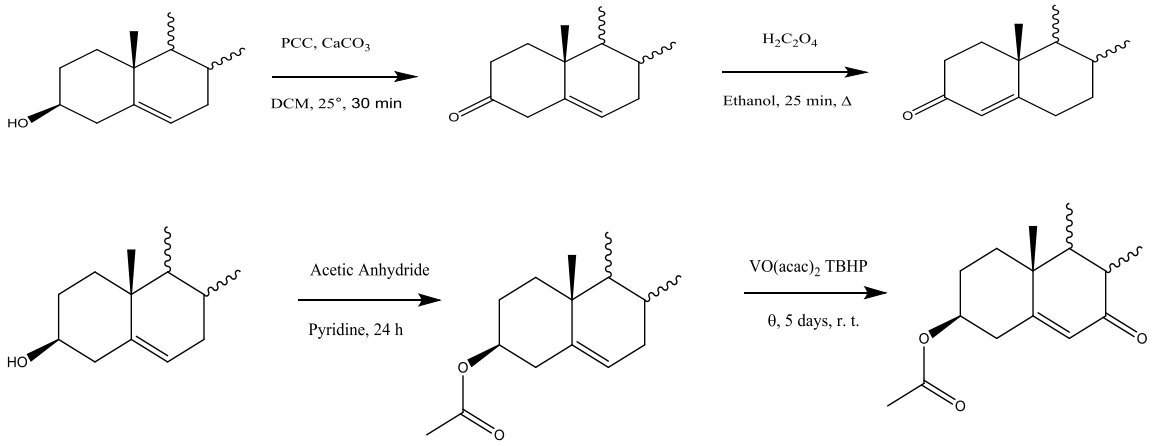
According to Scheme 4.1, tri-butyl phosphine initiates the reaction by 1,4 conjugate addition of the α,β unsaturated ketone. The electrons on the α carbon then attack the carbonyl groups of the paraformaldehyde, which reacts like formaldehyde. A methyl oxide is formed, which then takes the remaining α proton. The subsequent elimination reaction causes the phosphine to leave, however, not all of the steroids are reacted and in some cases steroidal zwitterions still remain. These, when heated, begin to react with the newly formed alcohols. Phosphine initially acted as a Lewis base, but later acts as a Lewis acid, stabilizing the substitution of the alcohol by the α carbon of the zwitterion. This is followed by an elimination reaction resulting in two steroids linked by a single carbon bond on the A rings.

This dimerization reaction became our next research project after the allylic oxidation studies. It was contemplated that if heat could be applied for a longer period of time, more dimer product would form. We tested this hypothesis on androstendione. The reaction was run as reported with two major exceptions. First, when it was time to rotovap the reaction mixture, it was gently refluxed with a steam bath for 24 hours instead. At the reported paraformaldehyde concentration, this made an insoluble orange precipitate. When the reaction was conducted at half the concentration of paraformaldehyde, we obtained a 12% yield.

We wanted to try to make different steroidal dimers as well. Using a two-step synthesis, Δ^5 sterols, particularly cholesterol, could be converted to Δ^4 enones.[159, 160] (Scheme 4.2) Additionally, our vanadium catalyzed allylic

oxidation reaction was used to make 7-keto products of cholesterol, DHEA, and pregnenolone acetates. (Scheme 4.2) Because the reaction was sensitive to hydroxyl groups, we converted those groups to acetates with acetyl anhydride.

Scheme 4.2. Formation of α,β Unsaturated Ketones on the A (top) and B (bottom) Rings



We have tested 4-cholesten-3-one and 7-ketocholesteryl acetate to no avail. No prior success was reported with 4-cholesten-3-one, which would seem to be an obvious substrate. The absence suggests that the dimerization on the cholest compounds did not work. Indeed, a 57% higher yield for dimerization of androstendione (3.6%) than progesterone (2.3%) was reported, albeit the yields for both were small.[158] If the yield was lowered by the presence of the small side chain on progesterone, then it is reasonable to assume that the longer side chain on the cholest compounds is extremely inhibitive.

5. Looking Forward

5.1. Isolation of Oxysterols

The most time consuming and difficult part of the research on allylic oxidation of steroidal compounds was the work up process. A good method isolating 7-keto products was never obtained from searching the literature. Indeed, it may not be possible to get a 99% pure yield of many oxysterols at the laboratory level. We got fairly close, as seen in the Figure 5.1, but our ^{13}C NMR of 7-ketocholesterol from isolated yield is, by far, better than any we had found in the literature. On the industrial level, for perspective purposes, Santa Cruz Biotechnology sells 98% pure 7-ketocholesterol for $200 per 2.5 mg.[161] Sigma Aldrich sells 100 mg of ≥ 90% pure 7-ketocholesterol for $61.50.[162] With as many methods as were listed in Tables 2.1 to 2.5, one may be falsely led into thinking that a simple oxidation product of cholesterol would be much cheaper. That 7-ketocholesterol is so costly is indicative of the difficulty in isolating the oxysterol from other oxysterol byproducts.

Three major problems arose in the work up of 7-keto steroidal compounds. First, the steroidal compounds without an ester at C3 could not be

recrystallized. All of the oxysterols, which were listed in chapter 3, could be dissolved in acetone and precipitated with water as per a normal recrystallization of cholesterol. Even in warm acetonitrile, 7-ketocholesterol could be dissolved and precipitated out by cooling. However, the problem lay in the filtering. Precisely, the oxysterols are more gel like than a granulated powder and will clogged up the filter. (7-Ketocholesterol's appearance when dried is similar to wax, albeit the dried precipitate can be ground into powder.) Perhaps, the additional ketone prevented the oxysterols from stacking, preventing a crystalline phase in solvent. Because of the inability to recrystallize the oxysterols which do not have an ester at C3, the un-esterified oxysterols must be isolated by column.

The second major issue lies within the elution of the oxysterols through the column. Retention times of several oxysterols are very close. Steroidal compounds are large amphipathic molecules before oxidation and addition of one new functional group, particularly a ketone on the B ring, does not change the retention time much. Nevertheless, the best yields came when the products were eluted as quickly as possible while still maintaining signal separations. This was accomplished by using better mobile phases, as opposed to pressuring the column in order to achieve an increased flow rate. When eluted slower, products were lost because as the oxysterols became too dispersed over time and it was harder to detect significant concentrations in the fractions. Many fractions with small concentrations would be simply discarded. Additionally, oxysterol retention

times (ranges of time) tended to overlap during slower elutions. Quickening the elutions sharpened the retention times.

A particularly strange third problem was encountered when using toluene as solvent for the elution. (We used a binary solvent system, originally with ethyl ether in toluene.) After eluting, the toluene could not be removed as seen in Figure 5.2. Several attempts to remove it were tried, including dissolving the product in chloroform and vacuum drying it under high vacuum three consecutive times. Toluene was not removed. The issue may have to do with the surfactant nature of steroids. Their amphipathic structures cause oxysterols to act like cleaning surfactants. It is common to obtain an, otherwise, very pure ^{13}C NMR spectrum with unspecified signals between δ 160 and 200. As can be seen in Figures 5.3 to 5.5, those unspecified signals are not consistently present. They seem to carry impurities with them. Unfortunately, the recrystallization option is not available to fix this issue.

To remedy the toluene problem, we began using hexanes. Additionally, we switched from ethyl ether to ethyl acetate. (The ethyl ether did not always smoothly mix with the toluene and gas pockets would form inside the column.) However, an odd occurrence has been noted while using the hexane/ ethyl acetate solvent. In TLC's, a ring is formed around the 7-ketocholesterol spots. (Figure 5.6) It is as if the oxysterol pushes everything away from it. This becomes a problem when identifying fractions because the ring can mask exactly where the product is.

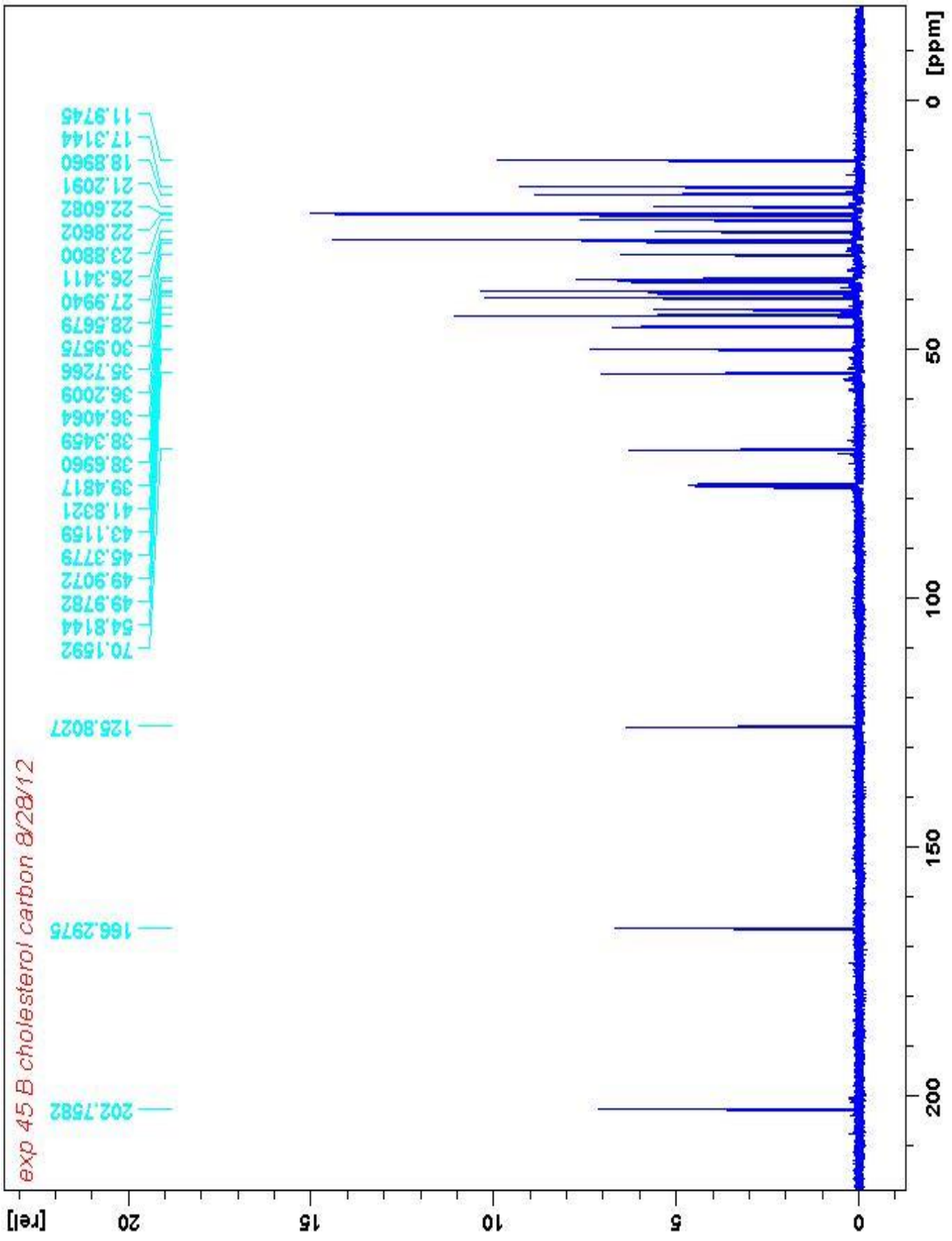


Figure 5.1. ^{13}C NMR of Isolated 7-Ketocholesterol from Our Lab

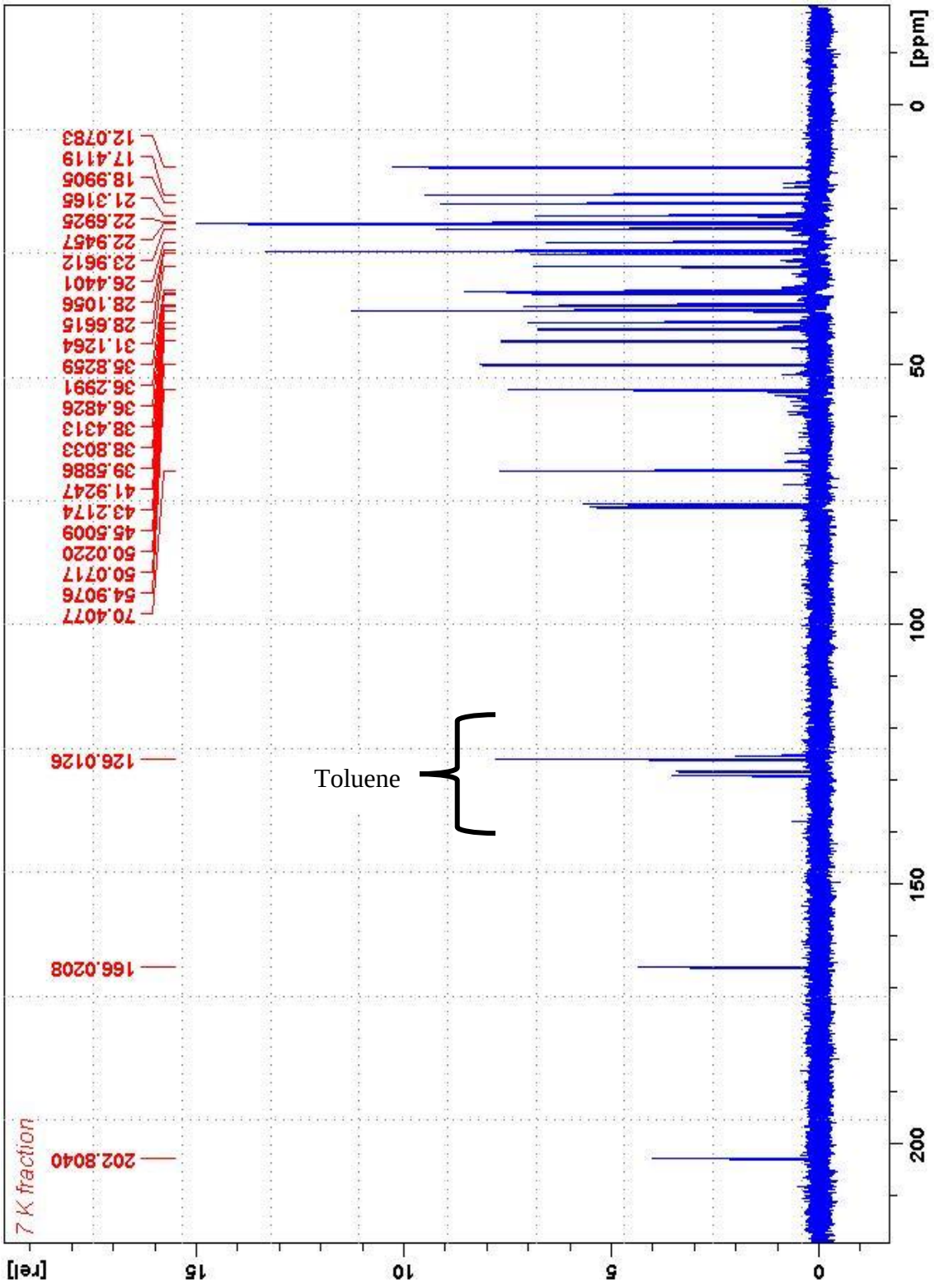


Figure 5.2. 7-Ketocholesterol with Toluene

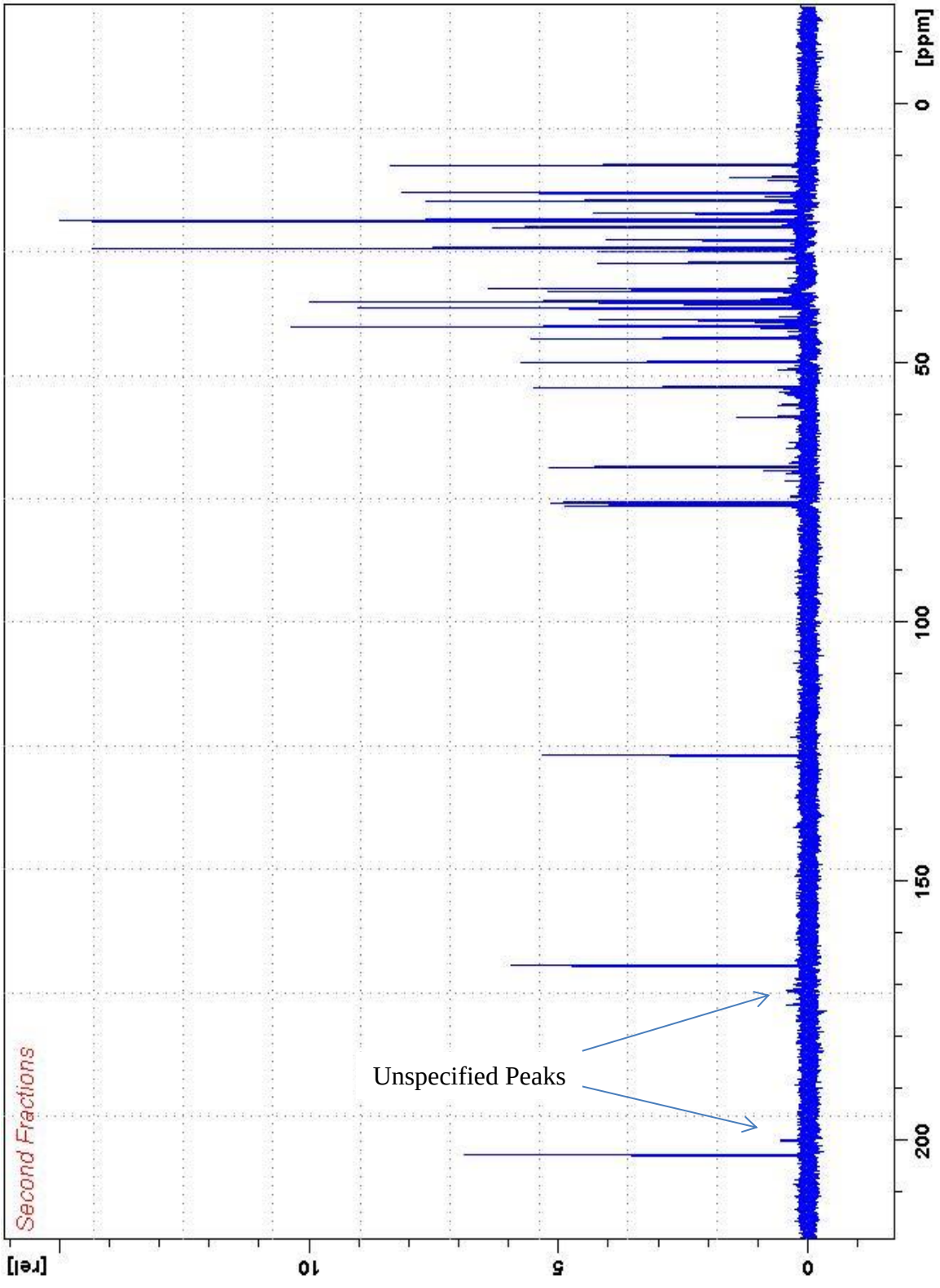


Figure 5.3. 7-Ketocholesterol with Unspecified Peaks

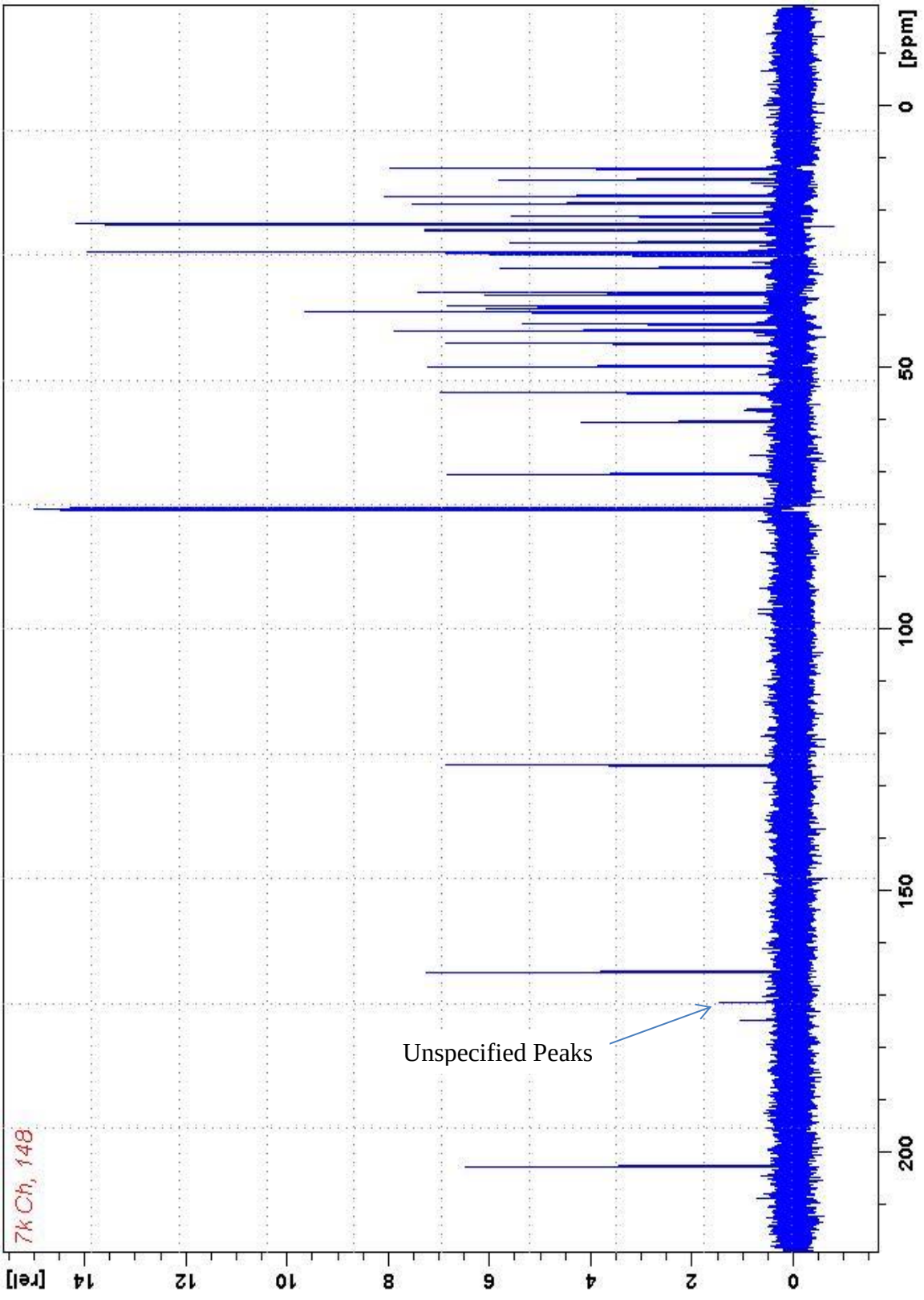


Figure 5.4. 7-Ketocholesterol with Unspecified Peaks

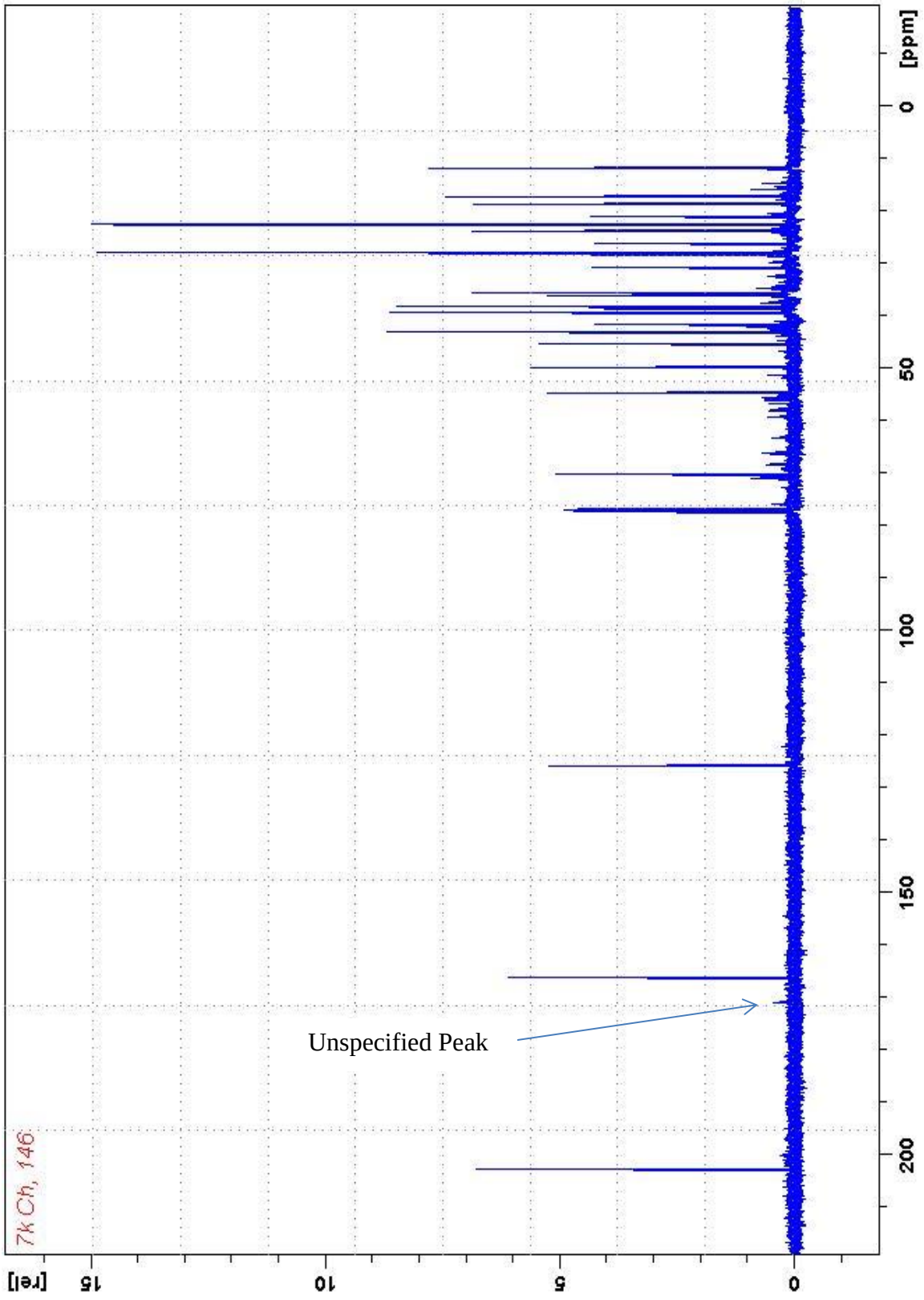


Figure 5.5. 7-Ketocholesterol with Unspecified Peak

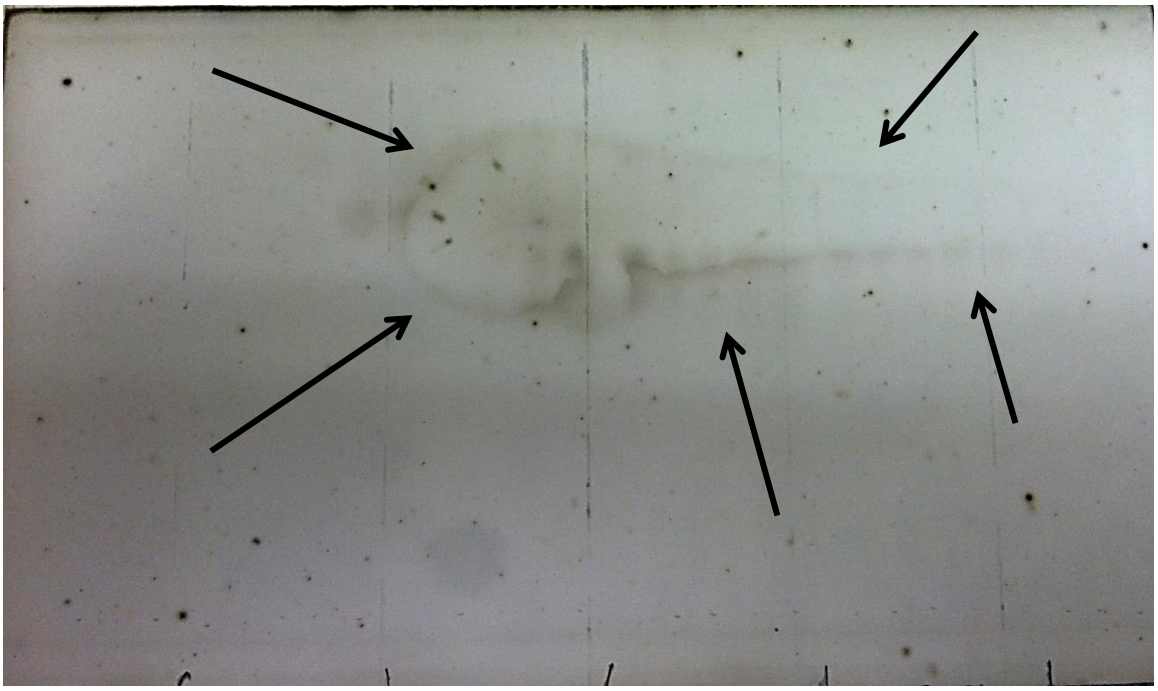


Figure 5.6. TLC of Eluted 7-Ketocholesterol. The 7-ketocholesterol was isolated by column and then run a second time through another column. This is the TLC of fractions collected from the second time through the column. Eluted fractions are marked in the TLC above.

Looking forward, there is a definite need for a better isolation process of 7-keto steroidal products. Future work is focused on enhancing the work up and developing a better isolation process for oxysterols at the laboratory level. Given that our vanadium catalyzed allylic oxidation reaction is fairly inexpensive, we can make plenty of test material, as well. In addition, optimization of steroid dimers will also continue.

6. Experimental

6.1. Optimization of $VO(acac)_2$ Catalyzed Allylic Oxidation

Reaction conditions were optimized using cholesterol as a primary substrate. Conversion of cholesterol was determined by TLC (silica gel on glass plates) and molybdic acid after eluting with 50:50 ethyl acetate and toluene.[163] The optimal conditions were found to be 2.6 mmol cholesterol, 0.38 mmol $VO(acac)_2$, 24 mmol of 70% TBHP (0.85 M overall), and 25 mL of dry benzene at room temperature and ambient atmosphere for 5 days.

6.2. General Procedure For Vanadium Catalysed Allylic Oxidation

Substrate, 2.6 mmols (only 1.3 mmols for 5-androgen-3,17-diol, pregnenolone, DHEA, and 5-pregnen-3,20-diol for lack of solubility), and 0.38 mmols of $VO(acac)_2$ were put into a round bottom flask. The flask was then charged with 25 mL of benzene. After dissolving as much substrate as possible, 24 mmols of TBHP were added. DHEA, 5-androgen-3,17-diol, and 5-pregnen-3,20-diol dissolved fully when the TBHP was added. The reaction flask was loosely capped to evaporation of solvent and the reaction was allowed to run for 5 days.

At the end of 5 days, the benzene solvent was removed by evaporation. Residue was then extracted with deionized (DI) water and ethyl ether into a separatory funnel. (If time is available, the precipitate will dissolve into the aqueous phase overnight.) The ether layer was retained and the ether was evaporated under reduced pressure at 55˚C.

Once the ether was removed, products 7-ketocholesteryl benzoate and 7-ketocholesteryl acetate were dissolved in acetone and recrystallized with DI water. The suspension was chilled for 5 hours with an ice bath. Products 7-ketocholesteryl benzoate and 7-ketocholesteryl acetate were then filtered out, rinsed with DI water, and dried under reduced pressure with P_2O_5. The filtrate from the first recrystallization of 7-ketocholesteryl acetate should be recrystallized again to extract more product. Beyond work-up of 7-ketocholesteryl benzoate and 7-ketocholesteryl acetate, we advise against recrystallization for the following products.

After removing the ether, products 7-ketocholesterol, 5-pregnen-3-ol-7,20-dione, 3-Hydroxyandrost-5-ene-7,17-dione, and 5-Pregnen-3, 20-diol-7-one were separated with column chromatography (silica gel). Product 7-ketocholesterol was extracted with hexane into the column and eluted using a gradient (0 to 40% ethyl acetate in hexane). Also, product 5-pregnen-3-ol-7,20-dione was extracted with 20% ethyl acetate in hexane and eluted with a gradient (20 to 50% ethyl acetate in hexane). Products 3-Hydroxyandrost-5-ene-7,17-dione and 5-Pregnen-3, 20-diol-7-one were extracted into the column with 50:50 ethyl acetate and hexane and were isocratically eluted with 50:50 ethyl acetate and hexane.

Fractions were identified by TLC, collected, and dried under reduced pressure with P_2O_5. (We found that, particularly for elution of 7-ketocholesterol, it is better to get the products through the column as quickly as possible, without losing separation.)

6.3. General Procedure for Use of Other Vanadium Complexes to Produce 7-Ketocholesterol from Cholesterol

For each reaction, 1 gram (2.6 mmol) of Cholesterol, was dissolved in 25 mL of benzene with 0.38 mmols of one of the following vanadium catalysts: bis(cyclopentadienyl)vanadium (IV) dichloride (0.1g), vanadium (IV) oxide sulfate hydrate (0.06g), vanadium (III) acetylacetonate (0.13g), vanadium (IV) carbide (0.02g), tris(triphenylsiloxy)vanadium (V) oxide (0.34g), and vanadium (V) oxytripropoxide (0.09g). To these, 3.09 grams (24 mmol) of 70% TBHP was added. (0.85 M TBHP overall) Results were analyzed through TLC (50:50 ethyl acetate and toluene) and Q-TOF (positive ion mode, atmospheric pressure ionization mode) of neat product.

6.4. General ProcedureUse of Other Metal Acetylacetonates

Under ambient atmosphere and at room temperature, 2.6 mmol cholesterol, 24 mmol 70% TBHP (0.85 M overall), and 25 mL of benzene charged with 0.38 mmol of either bis(acetylacetonato) dioxomolybdenum (IV), copper (III) acetylacetonate, or iron (III) acetylacetonate and allowed to react, loosely capped to prevent evaporation, for 5 days. The neat products of the three tests were then analyzed with TLC (50:50 ethyl acetate and toluene).

6.5. General Procedure for Attempted Oxidation of Podocarpic Acid

Podocarpic Acid, 0.1 g, was mixed with 0.03 g tris(triphenylsiloxy)V Oxide and 0.31 g of 70% TBHP in 2.5 mL of benzene. The reaction was allowed to run for 5 days under ambient atmosphere (loosely capped) and at room temperature. (Tris(triphenylsiloxy)V oxide's ligands are insoluble and sink to the bottom of the flask like sand in liquid.) Neat supernatant was extracted and tested by ^{13}C NMR for an indication that the 7-keto (or 6-keto depending on how the A ring is assigned) product was formed. Additionally, TLC (90:10 hexane/ ethyl acetate) was used, which showed that there was oxidation, but was otherwise not definitive because of low R_f values.

(Tris(triphenylsiloxy)V oxide was used instead of $VO(acac)_2$ because our quantity of podocarpic acid was limited and tris(triphenylsiloxy)V oxide had produced the most 7 ketocholesterol of the vanadium catalysts we tested. Plus, $VO(acac)_2$ had failed to catalyze benzylic oxidation of estrone.)

6.6. General Procedure for Limonene and Cyclohexane Oxidations

For limonene and cyclohexane oxidations, the solvent and substrate were the same. A 50 mL round bottom flask was filled with 25 mL of either cyclohexane or limonene for each test. For cyclohexane tests, three flasks were filled with cyclohexane. One was charged with 0.1g $VO(acac)_2$. Another was charged with 3.09 g of 70% TBHP. The third was charged with both $VO(acac)_2$, 0.1 g, and TBHP, 3.09 g. Each flask was magnetically stirred for 5 days at room temperature under an ambient atmosphere. Both the cyclohexane and limonene

tests were loosely capped to prevent evaporation. At the end of five days, neat supernatant from each test was analyzed by GCT. For Limonene tests, two flasks were filled with limonene. One flask was charged with 3.09 g TBHP. The other flask was filled with both 0.1 g $VO(acac)_2$ and 3.09 g of 70% TBHP. (We already knew that the presence of the vanadium complex, alone, did not lead to oxidation and therefore did not test the limonene with only $VO(acac)_2$.) The two limonene tests were loosely capped to prevent evaporation and were allowed to react for 5 days under ambient atmosphere and at room temperature. At the end of the reaction, neat supernatant was analyzed by GCT.

6.7. General Procedure for Steroidal Dimerization

A round bottom flask was charged with 4 mmol of steroid, 3 mmol of paraformaldehyde, and 30 mL of methanol. The mixture was magnetically stirred for 2 hours. After 2 hours, the atmosphere was purged and filled with argon. Next, 50 µL of 95% tri-n-butyl phosphine was injected into the mixture. The reaction ran overnight with magnetic stirring. Following the overnight reaction, the magnetic stir bar was removed and the contents of the flask were exposed to air. (Solids will form around the stir bar in the next step if it is present.) The mixture in the flask was then refluxed with a gentle steam bath for 24 hours. Following the reflux, the methanol was evaporated off (do not use the rotovap), leaving solids in the flask. Those solids were then dissolved with acetone. (It takes several hours for the solids to dissolve in acetone at room temperature.) Hexane was added to precipitate the product while at room temperature (do not cool to aid precipitation), which was then suction filtered and rinsed with hexane.

(After adding the hexane, do not stir the product back into solution.) The product was dried and then analyzed by NMR.

6.8. Compounds.

6.8.1. Estrone**.** White solid. ^{13}C NMR (150 MHz, $CDCl_3$): δ 153.6, 138.1, 132.2, 126.6, 115.4, 112.9, 50.5, 48.2, 44.0, 38.5, 36.0, 31.6, 29.5, 26.6, 21.6, 14.0. TLC showed one band and spectroscopic data matched expected values.[164]

6.8.2. 4-Cholesten-3-one. White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.0, 172.1, 123.9, 56.2, 56.0, 54.0, 42.5, 39.8, 39.6, 38.8, 38.8, 36.3, 35.9, 35.1, 35.7, 34.1, 33.1, 32.2, 28.3, 28.2, 24.3, 24.0, 23.0, 22.7, 21.2, 18.8, 17.5, 12.1. TLC showed one band and spectroscopic data matched expected values.[164]

6.8.3. 1,4-Androstadiene-3,17-dione. White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 186.4, 168.5, 155.5, 127.9, 124.3, 52.5, 50.6, 47.9, 43.6, 35.8, 35.3, 32.7, 32.5, 31.4, 22.3, 22.1, 18.9, 14.0. TLC showed one band and spectroscopic value matched expected values.[164]

6.8.4. Cholestan-3-ol. White solid. ^{13}C NMR (MHz 100, $CDCl_3$): δ 71.3, 56.5, 56.3, 54.4, 44.9, 42.6, 40.1, 39.5, 38.2, 37.0, 36.2, 35.8, 35.5, 35.5, 32.1, 31.5, 28.8, 28.3, 28.0, 24.2, 23.9, 22.9, 22.6, 21.3, 18.7, 12.3, 12.1. TLC showed one band and spectroscopic data matched expected values.[164]

6.8.5. 5-Androgen-3,17-diol was obtained through reduction of DHEA using Luche reduction.[165] Cerium (III) Choride heptahydrate, 7.5 grams, was dissolved in 100 mL of methanol. DHEA, 4.59 grams, was then added and dissolved. To

the mixture, 1 gram of sodium borohydride was slowly added. The solution was stirred overnight with a magnetic stir bar. DI water was added to the mixture to quench the reaction. The reaction mixture was then poured into a separatory funnel. Ethyl ether was added to the separatory funnel to make a second layer. The ether layer was then washed with DI water and collected. TLC revealed only one band from the ether layer. The ether was evaporated and the residue was used as substrate. White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.9, 121.4, 81.9, 71.7, 51.3, 50.2, 42.3, 37.3, 36.6, 32.0, 31.5, 30.5, 23.5, 20.7, 19.5, 11.0. TLC showed one band.

6.8.6. 7-Dehydrocholesterol. White solid. ^{13}C (100 MHz, $CDCl_3$): δ 141.6, 140.0, 119.8, 116.5, 70.6, 56.1, 54.7, 46.4, 43.1, 41.0, 39.7, 39.4, 38.6, 37.2, 36.4, 36.3, 32.2, 28.3, 28.2, 24.1, 23.0, 22.8, 21.3, 19.1, 16.5, 12.0. TLC showed one band and spectroscopic data matched expected values.[166]

6.8.7. 7-Cholesten-3-ol was obtained through hydrogenation of 7-dehydrocholesterol. 7-Dehydrocholesterol, 15 grams, was dissolved in 235 mL of ethyl acetate and 15 mL of glacial acetic acid (a 95:5 volume ratio respectively). The mixture was heated by steam bath and kept warm throughout the hydrogenation process by heat lamps to keep the 7-dehydrocholesterol soluble. Platinum dioxide, 1.5 grams, was added to the solution. The solution was then pressurized with H_2 gas (55-60 pounds) for 2 days. Upon completion of the 2 days, the solution was filtered while warm to remove the catalyst. The filtrate was then chilled causing 7-cholesten-3-ol to precipitate. After filtering out the precipitate and rinsing it with DI water, the 7-cholesten-3-ol was dissolved in

acetone and recrystallized with DI water. The precipitate was filtered out and rinsed with DI water. 7-Cholesten-3-ol was then dried under reduced pressure. White solid. ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.60, 5.42, 5.18, 3.63, 2.09, 1.81, 1.65, 1.56, 1.37, 1.28, 1.16, 0.92, 0.83, 0.65, 0.58. ^{13}C NMR (400 MHz, $CDCl_3$): δ 139.8, 117.6, 71.3, 56.4, 55.2, 49.6, 43.6, 40.4, 39.8, 39.7, 37.3, 36.4, 36.3, 34.4, 31.6, 29.9, 28.2, 28.2, 24.1, 23.2, 23.0, 22.8, 21.8, 19.1, 13.3, 12.1. ^{1}H NMR showed this starting material to be 74% 7-cholesten-3-ol and 24% 7-dehdrocholesterol. Spectroscopic data matched expected values.[167]

6.8.8. 24,25-Dihydrolanosterol was obtained through hydrogenation of a lanosterol/24,25-dihydrolanosterol (40:60 respectively) mixture that was commercially obtained. The mixture's identity and purity were confirmed by ^{1}H NMR, ^{13}C NMR, and TLC. Lanosterol mixture, 10 grams, was dissolved in 300 mL of glacial acetic acid. To the solution, 1.75 grams of a platinum dioxide and platinum black (46:54 respectively) mixture was added. The lanosterol mixture was warmed by steam bath and kept warm during the hydrogenation process with heat lamps. To the mixture, 55-60 pounds of H_2 gas were applied for 17 hours. The mixture was then filtered while warm to remove the catalyst. Recrystallization occurred by cooling the reaction solution and adding DI water. The precipitate was filtered out and rinsed with water. It was then dissolved in acetone and recrystallized with DI water. The precipitate was filtered out and allowed to dry using P_2O_5 under reduced pressure. White solid. ^{1}H (400 MHz, $CDCl_3$): 3.25, 2.07, 1.72, 1.52, 1.36, 1.27, 1.00, 0.89, 0.82, 0.70, 0.58. ^{13}C (62.5 MHz, $CDCl_3$): 134.4, 130.9, 125.3, 79.0, 50.4, 79.8, 44.5, 38.9, 37.0, 35.6, 31.0,

30.9, 29.7, 28.0, 27.8, 26.5, 25.8, 24.3, 21.0, 19.2, 18.3, 17.7, 15.8, 15.5. TLC showed one band.

6.8.9. Cholesteryl benzoate was obtained by slowly adding 100 mL of benzoyl chloride to 50 grams of cholesterol dissolved in 200 mL of pyridine at 40˚C for 3.5 hours. That mixture was poured into chilled DI water. Cholesteryl benzoate precipitated and was filtered out. It was rinsed with DI water and dried. The precipitate was dissolved in warm chloroform. That solution was put into a freezer for 5 hours, during which time the cholesteryl benzoate precipitated. The precipitate was filtered, rinsed with methanol, and dried. White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 166.2, 139.8, 132.9, 131.0, 129.7, 128.4, 123.0, 74.8, 56.9, 56.3, 50.2, 42.5, 39.8, 38.7, 37.3, 36.2, 32.1, 28.3, 24.2, 23.2, 21.5, 19.6, 12.3. TLC showed one band and spectroscopic data matched expected values.[106]

6.8.10. Cholesteryl acetate was obtained by slowly adding 12 grams of acetic anhydride to 10 grams of cholesterol dissolved in 200 mL of pyridine at 0˚C. The reaction was allowed to warm to room temperature after 3 hours and was left overnight with magnetic stirring. To quench the reaction, DI water was added, also causing the cholesteryl acetate to precipitate. The precipitate was filtered out, rinsed with DI water, and dried. After drying, the solids were dissolved in acetone and recrystallized by adding DI water. The precipitate was filtered out, rinsed with DI water, and dried. White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 170.5, 139.7, 122.7, 74.0, 56.7, 56.1, 50.0, 42.3, 39.5, 36.6, 35.8, 31.9, 31.9, 28.0, 24.3, 22.8, 22.6, 19.3, 18.7, 11.9. TLC showed one band and spectroscopic data matched expected values.[164]

6.8.11. Cholesterol was purified from stock via recrystallization. Stock portions were dissolved in warm acetone and the solutions were allowed to cool to room temperature. Water was then added slowly to the solution until precipitate formed and did not go back into solution. The suspension was kept in an ice bath for at least 3 hours. Precipitate was then suction filtered from the suspension and rinsed with water. The cholesterol was then dried under reduced pressure. White solid. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 140.8, 121.7, 71.7, 56.8, 56.2, 50.2, 42.3, 42.2, 39.8, 39.5, 37.3, 36.5, 36.2, 35.8, 31.9, 31.6, 28.3, 28.0, 24.3, 23.9, 22.8, 22.6, 21.1, 19.4, 18.7, 11.9. TLC showed one band and spectroscopic data matched expected values.[164] R_f= 0.85 (ethyl Acetate/Toluene = 50/50).

6.8.12. Pregnenolone. White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 209.8, 140.9, 121.2, 71.5, 63.7, 56.9, 49.9, 44.0, 42.2, 38.8, 37.3, 36.5, 31.8, 31.7, 31.4, 31.5, 24.5, 22.8, 21.1, 19.4, 13.2. TLC showed one band and spectroscopic data matched expected values.[164]

6.8.13. Dehydroepandrosterone. White solid. ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.2, 120.7, 71.3, 51.7, 50.2, 47.5, 42.1, 37.2, 35.8, 31.5, 21.9, 20.3, 19.4, 13.5. TLC showed one band and spectroscopic data matched expected values.[164]

6.8.14. 5-Pregnen-3,20-diol was obtained through reduction of pregnenolone using Luche reduction.[165] Cerium (III) Choride heptahydrate, 7.5 grams, was dissolved in 100 mL of methanol. Pregnenolone, 5 grams, was then added and dissolved. To the solution, 1 gram of sodium borohydride was added. The

solution was stirred overnight with a magnetic stir bar. DI water was then added to quench the reaction. The reaction mixture was poured into a separatory funnel and ethyl ether was added forming a second layer. The ether layer was washed with DI water and collected. TLC revealed only one band from the ether layer, which was subsequently evaporated leaving 5-pregnen-3,20-diol. White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.28 (s, 1H), 4.64 (s, 1H), 4.12 (m, 1H), 3.50 (m, 1H), 3.27 (m, 1H), 2.52 (s, 1H), 2.14 (m, 3H), 2.00-0.50 (comp, 25H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.8, 121.6, 71.7, 70.6, 70.4, 58.4, 58.4, 56.6, 56.2, 50.1, 50.1, 42.2, 41.6, 39.9, 38.8, 37.3, 36.5, 31.9, 31.7, 31.6, 31.5, 25.8, 25.6, 24.2, 23.5, 20.9, 20.8, 19.4, 12.4, 12.4.

6.8.15. 7-Ketocholesteryl benzoate. White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, 2H, J= 7.3Hz), 7.57 (t, 1H, J= 7.4Hz), 7.46 (t, 3H, J= 7.6Hz), 5.75 (m, 1H), 4.98 (m, 1H), 2.75-0.97 (comp, 28H), 0.94 (d, 3H, J= 6.5Hz), 0.87 (dd, 6H, J=6.7Hz, J= 1.8Hz), 0.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.1, 165.9, 164.0, 133.1, 130.3, 128.4, 127.0, 72.9, 54.8, 50.0, 49.8, 45.5, 43.1, 39.5, 38.4, 37.9, 36.2, 35.7, 28.6, 28.0, 26.3, 23.9, 22.9, 22.6, 18.9, 17.3, 12.0. HRMS (Q-TOF, ESI+) *m/z*: $[M+H]^+$ calculated for $C_{34}H_{49}O_3$ 505.3682; found 383.3228. MS (GCT) *m/z*: 504.4295, 502.0367, 382.3056, 269.2161, 218.9884, 174.1001, 161.1125, 105.0313, 77.0457. (1.28 g, 98%) Spectroscopic data matched expected values.[106]

6.8.16. 7-Ketocholesteryl acetate. White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.66 (s, 1H), 4.74-4.64 (m, 1H), 2.57-0.94 (comp, 32H), 0.90 (d, 3H, J= 6.4Hz), 0.86-0.82 (m, 6H), 0.66 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.9, 170.2,

163.9, 126.7, 72.2, 54.8, 49.9, 49.8, 45.4, 43.1, 39.5, 38.7, 38.3, 37.7, 36.2, 36.0, 35.7, 28.5, 28.0, 27.3, 26.3, 23.8, 22.8, 22.6, 21.3, 21.2, 18.6, 17.2, 12.0. MS (Q-TOF, ESI+) *m/z*: $[M+H]^+$ calculated for $C_{29}H_{47}O_3$ 443.3525; found 885.6874, 443.3505, 383.3316, MS (GCT) *m/z*: 382.1558, 173.9955. (0.96g, 83%) Spectroscopic data matched expected values.[83]

6.8.17. 7-Ketocholesterol**.** White solid. H^1 NMR (400 MHz, $CDCl_3$): δ 5.70 (d, 1H, J= 1.9Hz), 3.74-3.62 (m, 1H), 2.56-0.96 (comp, 30H), 0.93 (d, 3H, J= 6.6Hz), 0.87 (dd, 6H, J= 6.6Hz, J=2.o Hz), 0.69 (s, 3H). C^{13} NMR (100 MHz, $CDCl_3$): δ 202.8, 166.0, 126.0, 70.4, 54.9, 50.1, 50.0, 45.5, 43.2, 41.9, 39.6, 38.8, 38.4, 36.5, 36.3, 35.8, 31.1, 28.7, 28.1, 26.4, 24.0, 23.0, 22.7, 21.3, 19.0, 17.4, 12.1. MS (Q-TOF, ESI+) *m/z*: $[M+H]^+$ calculated for $C_{27}H_{43}O_3$ 401.3420; found 401.3335. MS (GCT) *m/z*: 400.3997, 346.3411, 161.1247, 81.0792. R_f= 0.60 (ethyl acetate/toluene = 50/50). (0.47 g, 45%) Spectroscopic data matched expected values.[83]

6.8.18. 5-Pregnen-3-ol-7,20-dione**.** White solid. ^{1}H NMR (400 MHz, Deuterated DMSO): δ 5.70 (s, 1H), 3.72-3.62 (m, 1H), 2.59-0.50 (comp, 28H). ^{13}C NMR (100 MHz, Deuterated DMSO): δ 210.3, 201.8, 166.0, 126.2, 70.7, 62.5, 50.2, 45.6, 42.1, 38.5, 36.6, 32.0, 26.8, 23.8, 21.3, 17.5, 13.7. MS (Q-TOF, ESI+) *m/z*: $[M+H]^+$ calculated for $C_{29}H_{31}O_3$ 331.2273; found 331.2200. MS (GCT) *m/z*: 329.9635, 245.1098, 161.1338, 91.0515, 79.0365. (0.20 g, 23%) Spectroscopic data matched expected values.[83]

6.8.19. 3-Hydroxyandrost-5-ene-7,17-dione**.** White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (s, 1H), 3.75-3.64 (m, 1H), 2.87-2.77 (m, 1H), 2.60-0.80 (comp, 23H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.1, 166.1, 126.0, 70.3, 50.1, 47.9, 45.7, 44.3, 41.9, 38.4, 36.3, 35.7, 31.1, 30.7, 24.2, 20.6, 17.5, 13.8. MS (Q-TOF, ESI+) *m/z*: $[M+H]^+$ calculated for $C_{19}H_{27}O_3$ 303.1960; found 303.1958. MS (GCT) *m/z*: 301.8061, 161.534, 91.0481, 79.0190. (0.14 g, 19%)

6.8.20. 5-Pregnen-3, 20-diol-7-one. White solid. ^{1}H NMR (400 MHz, Deuterated DMSO): δ 5.58 (s, 1H), 4.90 (d, 1H, J= 4.4Hz), 4.15-4.10 (m, 1H), 3.54-3.25 (m, 2H), 2.49 (m, 5H), 2.42-0.95 (comp, 18H), 0.67 (s, 3H), 0.60 (s, 1H) ^{13}C NMR (100 MHz, Deuterated DMSO): δ 201.7, 167.4, 125.3, 69.5, 68.9, 56.8, 50.1, 45.2, 43.1, 38.5, 36.4, 31.5, 26.9, 24.4, 21.1, 17.4, 12.5. MS (Q-TOF, ESI+) *m/z*: $[M+H]^+$ calculated for $C_{21}H_{33}O_3$ 333.2430; found 333.2350. MS (GCT) *m/z*: 332.1760, 161.0795, 91.0423. (0.11 g, 19%)

6.8.21. 5,6-β-Epoxycholestan-3-ol was obtained by reacting 1 g cholesterol, 6 g $KMnO_4$, 3 g $CuSO_4 \cdot 5H_2O$, 0.4 mL H_2O, and 1.5 mL tert-butanol in 30 ml of DCM. The mixture was refluxed for five minutes and then left at room temperature overnight. The next day, TLC revealed only one band with the expected R_f value, between cholesterol and 7-ketocholesterol R_f values. (This reaction stains glassware.)

6.8.22. 5-Cholesten-3-one. Anhydrous CaCO3, 6 g , was mixed with 5.55 g cholesterol, and 10.5g of PCC in 480 mL of DCM for 30 min. (Do not add NaCl as written in reference 136. The liquid phase will become red and very viscous,

reminiscent of thick spaghetti sauce.) Reaction contents were poured through a gravity filter into a separatory funnel containing DI water. Ethyl Ether was then added to the separatory funnel. After vigorous mixing, only the ether layer was retained. It contained pure 5-cholesten-3-one, confirmed by 13C NMR. (The PCC reaction stains glassware.) ^{13}C NMR (50 MHz, $CDCl_3$): δ 210.4, 138.5, 122.9, 56.6, 56.1, 49.2, 48.4,42.4, 39.7, 39.5, 37.7, 36.9, 36.2, 35.8, 31.9, 31.8, 28.2, 28.0, 24.3, 23.8, 22.8, 22.6, 21.4, 19.2, 18.7, 11.9.

6.8.23. 4-Methylene-androstendione-dimer. ^{13}C NMR (50 MHz, $CDCl_3$): δ 220.3, 198.0, 163.8, 133.0, 54.6, 51.2, 50.8, 47.5, 39.5, 35.7, 35.1, 34.9, 33.8, 31.5, 31.3, 30.6, 27.7, 21.7, 21.4, 20.4, 17.6, 13.7.

6.8.24. 7-Keto-β-Sitosterol was obtained by mixing 5.1 g 90% β-sitosterol, 0.51 g $VO(acac)_2$, and 15.45 g of TBHP in 125 mL of benzene. The reaction was magnetically stirred for 5 days at room temperature and under ambient atmosphere (loosely capped to prevent evaporation). After the reaction, the benzene solvent was evaporated and the reaction material was extracted with DI water and ethyl ether into a separatory funnel. The ether layer was retained and rotovaped at 55˚C. Hexane was then used to extract product from the flask to an open column packed with silica gel. Products were eluted through the column with gradient elution using 0 to 40% ethyl acetate in hexane. Appropriate fractions were collected and dried under reduced pressure. Carbon NMR was used to confirm the identity and proton NMR showed the contents to be 76% 7-keto-β-sitosterol. (Phytosterols are usually in mixtures; the other 24% were also 7-keto products, but had a double bond on the side chain.) ^{13}C NMR (50 MHz,

$CDCl_3$): δ 202.7, 165.5, 126.3, 70.7, 54.9, 50.1, 46.0, 45.6, 43.3, 42.0, 38.5, 36.5, 36.3, 31.4, 29.3, 26.5, 23.2, 21.0, 20.0, 19.2, 19.1, 17.5, 12.2.

7. References

1. Nes, W. R.; McKean, M. L., *Biochemistry of steroids and other isopentenoids*. University Park Press: 1977.

2. Le Bideau, F.; Dagorne, S., *Chemical reviews* **2013,** *113*, 7793-7850.

3. Hanson, J. R., *Nat. Prod. Rep.* **2005,** *22*, 104-110.

4. Hanson, J. R., *Natural product reports* **2010,** *27*, 887-899.

5. Moss, G. P., *Pure and Applied Chemistry* **1989,** *61*, 1783-1822.

6. Nes, W. D., *Chemical Reviews* **2011,** *111*, 6423-6451.

7. Krause, M. R.; Regen, S. L., *Accounts of chemical research* **2014,** *47*, 3512-3521.

8. McMullen, T. P. W.; Lewis, R. N. A. H.; McElhaney, R. N., *Current opinion in colloid & interface science* **2004,** *8*, 459-468.

9. Akoh, C. C.; Min, D. B., *Food Lipids: Chemistry, Nutrition, and Biotechnology, Third Edition*. Taylor & Francis: 2008.

10. Breslow, R.; Corcoran, R. J.; Snider, B. B.; Doll, R. J.; Khanna, P. L.; Kaleya, R., *Journal of the American Chemical Society* **1977,** *99*, 905-915.

11. Bose, A. K.; Lal, B.; Hoffman, W. A.; Manhas, M. S., *Tetrahedron Letters* **1973,** *14*, 1619-1622.

12. Breslow, R.; Corcoran, R. J.; Snider, B. B., *Journal of the American Chemical Society* **1974,** *96*, 6791-6792.

13. Reese, P. B., *Steroids* **2001,** *66*, 481-497.

14. Dauben, W. G.; Lorber, M. E.; Fullerton, D. S., *The Journal of Organic Chemistry* **1969,** *34*, 3587-3592.

15. Bovicelli, P.; Lupattelli, P.; Mincione, E.; Prencipe, T.; Curci, R., *The Journal of Organic Chemistry* **1992,** *57*, 2182-2184.

16. Bent, H. A., *Chemical Reviews* **1961,** *61*, 275-311.

17. Maier, W. F.; Schleyer, P. V. R., *Journal of the American Chemical Society* **1981,** *103*, 1891-1900.

18. Wiberg, K. B., *Angewandte Chemie International Edition in English* **1986,** *25*, 312-322.

19. Dudev, T.; Lim, C., *Journal of the American Chemical Society* **1998,** *120*, 4450-4458.

20. Barton, D. H. R.; Head, A. J.; May, P. J., *Journal of the Chemical Society (Resumed)* **1957**, 935-944.

21. Robinson, M. J. T.; Whalley, W. B., *Tetrahedron* **1963,** *19*, 2123-2133.

22. Paillasse, M. R.; Saffon, N.; Gornitzka, H.; Silvente-Poirot, S.; Poirot, M.; De Medina, P., *Journal of lipid research* **2012,** *53*, 718-725.

23. Parish, E.; Boos, T.; Kaneshiro, E., *Journal of Chemical Research, Synopses* **1999**, 708-709.

24. Cohen, Z.; Mazur, Y., *The Journal of Organic Chemistry* **1979,** *44*, 2318-2320.

25. Manley, R. P.; Curry, K. W.; Deno, N. C.; Meyer, M. D., *The Journal of Organic Chemistry* **1980,** *45*, 4385-4387.

26. Bovicelli, P.; Lupattelli, P.; Mincione, E.; Prencipe, T.; Curci, R., *The Journal of Organic Chemistry* **1992,** *57*, 5052-5054.

27. Liu, D.; Stuhmiller, L. M.; McMorris, T. C., *Journal of the Chemical Society, Perkin Transactions 1* **1988**, 2161-2167.

28. Parish, E. J.; Kizito, S. A.; Qiu, Z., *Lipids* **2004,** *39*, 801-804.

29. Tai, C. Y.; Chen, Y. C.; Chen, B. H., *Journal of Food and Drug Analysis* **1999,** *7*, 243-257.

30. Weiner, N. D.; Noomnont, P.; Felmeister, A., *Journal of lipid research* **1972,** *13*, 253-255.

31. Kim, S. K.; Nawar, W. W., *Lipids* **1993,** *28*, 917-922.

32. Katz, M.; Gans, E. H., *Journal of pharmaceutical sciences* **2008,** *97*, 2936-2947.

33. Kaufman, M. D.; Grieco, P. A.; Bougie, D. W., *Journal of the American Chemical Society* **1993,** *115*, 11648-11649.

34. Breslow, R.; Zhang, X.; Huang, Y., *Journal of the American Chemical Society* **1997,** *119*, 4535-4536.

35. Breslow, R.; Gabriele, B.; Yang, J., *Tetrahedron letters* **1998,** *39*, 2887-2890.

36. Risley, J. M., *Journal of chemical education* **2002,** *79*, 377.

37. Goldstein, J. L.; Brown, M. S., *Nature* **1990,** *343*, 425-430.

38. Shimano, H., *Progress in Lipid Research* **2001,** *40*, 439-452.

39. Brown, M. S.; Goldstein, J. L., *Cell* **1997,** *89*, 331-340.

40. Sato, R., *Archives of Biochemistry and Biophysics* **2010,** *501*, 177-181.

41. Wang, X.; Sato, R.; Brown, M. S.; Hua, X.; Goldstein, J. L., *Cell* **1994,** *77*, 53-62.

42. Nes, W. D.; Zhou, W., Terpenoids: Higher. In *eLS*, John Wiley & Sons, Ltd: 2001.

43. Spencer, T. A., *Accounts of chemical research* **1994,** *27*, 83-90.

44. Ghayee, H. K.; Auchus, R. J., *Reviews in Endocrine and Metabolic Disorders* **2007,** *8*, 289-300.

45. Storbeck, K.-H.; Bloem, L. M.; Africander, D.; Schloms, L.; Swart, P.; Swart, A. C., *Molecular and Cellular Endocrinology* **2013,** *377*, 135-146.

46. Vatalas, I. A.; Dionyssiou-Asteriou, A., *Nutrition, metabolism, and cardiovascular diseases: NMCD* **2001,** *11*, 388-393.

47. Lorence, M. C.; Murry, B. A.; Trant, J. M.; Mason, J. I., *Endocrinology* **1990,** *126*, 2493-2498.

48. Townsley, J. D.; Brodie, H. J., *Biochemistry* **1968,** *7*, 33-40.

49. Oertel, G. W.; Treiber, L., *European Journal of Biochemistry* **1969,** *7*, 234-238.

50. Novak, F.; De Clerck, D.; Paesen, G.; Swevers, L.; De Loof, A., *International journal of invertebrate reproduction and development* **1987,** *11*, 255-263.

51. Björkhem, I.; Diczfalusy, U., *Arteriosclerosis, thrombosis, and vascular biology* **2002,** *22*, 734-742.

52. Javitt, N. B., *Trends in Endocrinology & Metabolism* **2004,** *15*, 393-397.

53. Otaegui-Arrazola, A.; Menéndez-Carreño, M.; Ansorena, D.; Astiasarán, I., *Food and Chemical Toxicology* **2010,** *48*, 3289-3303.

54. Brown, A. J.; Jessup, W., *Molecular aspects of medicine* **2009,** *30*, 111-122.

55. Björkhem, I.; Cedazo-Minguez, A.; Leoni, V.; Meaney, S., *Molecular aspects of medicine* **2009,** *30*, 171-179.

56. Lyons, M. A.; Brown, A. J., *The international journal of biochemistry & cell biology* **1999,** *31*, 369-375.

57. Olkkonen, V. M., *Lipid Insights* **2008,** *2*, 1.

58. Kandutsch, A. A.; Chen, H. W., *Journal of Biological Chemistry* **1973,** *248*, 8408-8417.

59. Kandutsch, A. A.; Chen, H. W., *Journal of Biological Chemistry* **1974,** *249*, 6057-6061.

60. Björkhem, I., *Biochimie* **2013,** *95*, 448-454.

61. Vejux, A.; Lizard, G., *Molecular aspects of medicine* **2009,** *30*, 153-170.

62. Saldanha, T.; Sawaya, A. C. H. F.; Eberlin, M. N.; Bragagnolo, N., *Journal of agricultural and food chemistry* **2006,** *54*, 4107-4113.

63. Boselli, E.; Rodriguez-Estrada, M. T.; Fedrizzi, G.; Caboni, M. F., *Meat science* **2009,** *81*, 224-229.

64. Guardiola, F., *Cholesterol and Phytosterol Oxidation Products: Analysis, Occurrence, and Biological Effects*. Taylor & Francis: 2002.

65. Poirot, M.; Silvente-Poirot, S., *Biochimie* **2013,** *95*, 622-631.

66. Khanam, H.; Mashrai, A.; Sherwani, A.; Owais, M.; Siddiqui, N., *Steroids* **2013,** *78*, 1263-1272.

67. Delbeke, F. T.; Van Eenoo, P.; Van Thuyne, W.; Desmet, N., *The Journal of steroid biochemistry and molecular biology* **2002,** *83*, 245-251.

68. El Kihel, L., *Steroids* **2012,** *77*, 10-26.

69. Mo, Q.; Lu, S.-f.; Simon, N. G., *The Journal of steroid biochemistry and molecular biology* **2006,** *99*, 50-58.

70. Šulcová, J.; Hill, M.; Mašek, Z.; Češka, R.; Nováček, A.; Hampl, R.; Starka, L., *Physiol Res* **2001,** *50*, 9-18.

71. Šulcová, J.; Hampl, R.; Hill, M.; Starka, L.; Nováček, A., *Clinical Chemical Laboratory Medicine* **2005,** *43*, 221-227.

72. Shi, J.; Schulze, S.; Lardy, H. A., *Steroids* **2000,** *65*, 124-129.

73. Zenk, J. L.; Frestedt, J. L.; Kuskowski, M. A., *The Journal of nutritional biochemistry* **2007,** *18*, 629-634.

74. Marshall, C. W.; Ray, R. E.; Laos, I.; Riegel, B., *Journal of the American Chemical Society* **1957,** *79*, 6308-6313.

75. Carvalho, J. F. S.; Silva, M. M. C.; Moreira, J. N.; Simões, S. r.; Sá e Melo, M. L., *Journal of medicinal chemistry* **2010,** *53*, 7632-7638.

76. Salvador, J. A. R.; Silvestre, S. M.; Moreira, V. M., *Current Organic Chemistry* **2006,** *10*, 2227-2257.

77. Parish, E. J.; Chitrakorn, S.; Luu, B.; Schmidt, G.; Ourisson, G., *Steroids* **1989,** *53*, 579-596.

78. De Medina, P.; Paillasse, M. R.; Ségala, G.; Khallouki, F.; Brillouet, S.; Dalenc, F.; Courbon, F.; Record, M.; Poirot, M.; Silvente-Poirot, S., *Chemistry and physics of lipids* **2011,** *164*, 432-437.

79. Szwarc, M., *Chemical Reviews* **1950,** *47*, 75-173.

80. McMillen, D. F.; Golden, D. M., *Annual Review of Physical Chemistry* **1982,** *33*, 493-532.

81. Luo, Y. R., *Handbook of Bond Dissociation Energies in Organic Compounds*. CRC Press: 2002.

82. Hioe, J.; Zipse, H., *Organic & biomolecular chemistry* **2010,** *8*, 3609-3617.

83. Li, Y.; Wu, X.; Lee, T. B.; Isbell, E. K.; Parish, E. J.; Gorden, A. E. V., *The Journal of organic chemistry* **2010,** *75*, 1807-1810.

84. Parish, E.; Aksara, N.; Boos, T.; Kizito, S., *Recent Research and Development in Organic Chemistry* **1998,** *2*, 95-105.

85. Takano, S.; Sato, S.; Ogasawara, K., *Chemistry Letters* **1985**, 1265-1266.

86. Silvestre, S. M.; Salvador, J. A. R., *Tetrahedron* **2007,** *63*, 2439-2445.

87. Marwah, P.; Marwah, A.; Lardy, H. A., *Green Chemistry* **2004,** *6*, 570-577.

88. Liu, J.; Zhu, H.-Y.; Cheng, X.-H., *Synthetic Communications®* **2009,** *39*, 1076-1083.

89. Salvador, J. A. R.; Clark, J. H., *Chemical Communications* **2001**, 33-34.

90. Salvador, J. A. R.; Clark, J. H., *Green Chemistry* **2002,** *4*, 352-356.

91. Kulig, M. J.; Smith, L. L., *The Journal of organic chemistry* **1973,** *38*, 3639-3642.

92. Barton, D. H. R.; Boivin, J.; Hill, C. H., *Journal of the Chemical Society, Perkin Transactions 1* **1986**, 1797-1804.

93. McLaughlin, E. C.; Choi, H.; Wang, K.; Chiou, G.; Doyle, M. P., *The Journal of organic chemistry* **2008,** *74*, 730-738.

94. Choi, H.; Doyle, M. P., *Organic letters* **2007,** *9*, 5349-5352.

95. Miller, R. A.; Li, W.; Humphrey, G. R., *Tetrahedron letters* **1996,** *37*, 3429-3432.

96. Salvador, J. A. R.; Silvestre, S. M., *Tetrahedron letters* **2005,** *46*, 2581-2584.

97. Baptistella, L. H. B.; Sousa, I. M. O.; Gushikem, Y.; Aleixo, A. M., *Tetrahedron Letters* **1999,** *40*, 2695-2698.

98. 木村道也; 武藤俊樹, *Chemical and Pharmaceutical Bulletin* **1981,** *29*, 35-42.

99. Arsenou, E. S.; Koutsourea, A. I.; Fousteris, M. A.; Nikolaropoulos, S. S., *Steroids* **2003,** *68*, 407-414.

100. Fousteris, M. A.; Koutsourea, A. I.; Nikolaropoulos, S. S.; Riahi, A.; Muzart, J., *Journal of Molecular Catalysis A: Chemical* **2006,** *250*, 70-74.

101. Parish, E.; Lu, D., Allylic oxidation of delta 5 steroids with chromium dioxide (Magtrieve^{TM}). In Not submitted yet. ed.

102. Pearson, A. J.; Chen, Y.-S.; Han, G. R.; Hsu, S.-Y.; Ray, T., *Journal of the Chemical Society, Perkin Transactions 1* **1985**, 267-273.

103. Shing, T. K. M.; Yeung, Y.-Y.; Su, P. L., *Organic letters* **2006,** *8*, 3149-3151.

104. Parish, E. J.; Sun, H.; Kizito, S. A., *Journal of chemical research. Synopses* **1996**.

105. Salmond, W. G.; Barta, M. A.; Havens, J. L., *The Journal of Organic Chemistry* **1978,** *43*, 2057-2059.

106. Parish, E. J.; Wei, T.-Y.; Livant, P., *Lipids* **1987,** *22*, 760-763.

107. Barton, D. H. R.; Crich, D., *Tetrahedron* **1985,** *41*, 4359-4364.

108. Crich, D.; Zou, Y., *Organic letters* **2004,** *6*, 775-777.

109. 木村道也; 武藤俊樹, *Chemical & pharmaceutical bulletin* **1979,** *27*, 109-112.

110. Andreu, I.; Palumbo, F.; Tilocca, F.; Morera, I. M.; Boscá, F.; Miranda, M. A., *Organic letters* **2011,** *13*, 4096-4099.

111. Kenneth, A., Oxidation of olefines. In Google Patents: 1966.

112. John, K., Epoxidation process. In Google Patents: 1967.

113. John, K., Process for the co-production of isoprene and oxygenated compounds from isopentane hydroperoxide. In Google Patents: 1967.

114. Mimoun, H.; Mignard, M.; Brechot, P.; Saussine, L., *Journal of the American Chemical Society* **1986,** *108*, 3711-3718.

115. Hawkins, E. G. E., *Journal of the Chemical Society (Resumed)* **1950**, 2169-2173.

116. Indictor, N., *The Journal of Organic Chemistry* **1965,** *30*, 2074-2075.

117. Su, C.-C.; Reed, J. W.; Gould, E. S., *Inorganic Chemistry* **1973,** *12*, 337-342.

118. Gould, E. S.; Hiatt, R. R.; Irwin, K. C., *Journal of the American Chemical Society* **1968,** *90*, 4573-4579.

119. Chong, A. O.; Sharpless, K. B., *The Journal of Organic Chemistry* **1977,** *42*, 1587-1590.

120. Maurya, M. R.; Kumar, M.; Kumar, A.; Pessoa, J. C., *Dalton Transactions* **2008**, 4220-4232.

121. Conte, V.; Floris, B., *Dalton Transactions* **2011,** *40*, 1419-1436.

122. Da Silva, J. A.; da Silva, J. J. R. F.; Pombeiro, A. J. L., *Coordination Chemistry Reviews* **2011,** *255*, 2232-2248.

123. Bhunia, S.; Koner, S., *Journal of Porous Materials* **2011,** *18*, 399-407.

124. Leus, K.; Muylaert, I.; Vandichel, M.; Marin, G. B.; Waroquier, M.; Van Speybroeck, V.; Van Der Voort, P., *Chemical Communications* **2010,** *46*, 5085-5087.

125. Zhang, W.; Yamamoto, H., *Journal of the American Chemical Society* **2007,** *129*, 286-287.

126. Makita, N.; Hoshino, Y.; Yamamoto, H., *Angewandte Chemie International Edition* **2003,** *42*, 941-943.

127. Zhao, K.; Wang, Y.; Billington, D. C., *Tetrahedron: Asymmetry* **2001,** *12*, 1211-1217.

128. Linden, G. L.; Farona, M. F., *Inorganic Chemistry* **1977,** *16*, 3170-3173.

129. Vandichel, M.; Leus, K.; Van Der Voort, P.; Waroquier, M.; Van Speybroeck, V., *Journal of Catalysis* **2012,** *294*, 1-18.

130. Katsuki, T.; Sharpless, K. B., *Journal of the American Chemical Society* **1980,** *102*, 5974-5976.

131. Sharpless, K. B.; Verhoeven, T. R., *Aldrichim. Acta* **1979,** *12*, 63-75.

132. Xia, J.-B.; Cormier, K. W.; Chen, C., *Chemical Science* **2012,** *3*, 2240-2245.

133. Liu, J.; Wang, F.; Ma, Z.; Lin, J.; Gu, Z., *Catalysis Communications* **2011,** *15*, 103-107.

134. Feng, R.-M.; Yang, X.-J.; Ji, W.-J.; Chen, Y.; Au, C.-T., *Journal of Catalysis* **2007,** *246*, 166-176.

135. Shen, S.; Zhou, J.; Zhang, F.; Zhou, L.; Li, R., *Catalysis today* **2002,** *74*, 37-43.

136. Tatarova, L. A.; Trofimova, K. S.; Khaliullin, A. K., *Russian journal of organic chemistry* **2004,** *40*, 1403-1406.

137. .

138. Matyjaszewski, K.; Coca, S.; Gaynor, S. G.; Wei, M.; Woodworth, B. E., *Macromolecules* **1998,** *31*, 5967-5969.

139. Decker, C.; Jenkins, A. D., *Macromolecules* **1985,** *18*, 1241-1244.

140. Sawyer, D. T.; Sobkowiak, A.; Matsushita, T., *Accounts of chemical research* **1996,** *29*, 409-416.

141. Xu, L.; Korade, Z.; Porter, N. A., *Journal of the American Chemical Society* **2010,** *132*, 2222-2232.

142. Stepovik, L. P.; Gulenova, M. V., *Russian Journal of General Chemistry* **2009,** *79*, 1663-1670.

143. Sheppard, A. N.; Acevedo, O., *Journal of the American Chemical Society* **2009,** *131*, 2530-2540.

144. Wewer, V.; Dombrink, I.; vom Dorp, K.; Dörmann, P., *Journal of lipid research* **2011,** *52*, 1039-1054.

145. Parish, E. J.; Li, S., *The Journal of Organic Chemistry* **1996,** *61*, 5665-5666.

146. Majumder, P. L.; Maiti, D. C.; Kraus, W.; Bokel, M., *Phytochemistry* **1987,** *26*, 3021-3023.

147. Bhat, S. V.; Kalyanaraman, P. S.; Kohl, H.; De Souza, N. J.; Fehlhaber, H. W., *Tetrahedron* **1975,** *31*, 1001-1004.

148. Edwards, O. E.; Feniak, G.; Los, M., *Canadian Journal of Chemistry* **1962,** *40*, 1540-1546.

149. Majetich, G.; Liu, S.; Fang, J.; Siesel, D.; Zhang, Y., *The Journal of Organic Chemistry* **1997,** *62*, 6928-6951.

150. McKee, N. A.; Bradford, S.; Parish, D. E.; Neely, W. C.

151. Smet, E. D.; Mensink, R. P.; Plat, J., *Molecular nutrition & food research* **2012,** *56*, 1058-1072.

152. Sarker, S.; Nahar, L., *Steroid Dimers: Chemistry and Applications in Drug Design and Delivery*. Wiley: 2012.

153. Janout, V.; Lanier, M.; Regen, S. L., *Journal of the American Chemical Society* **1996,** *118*, 1573-1574.

154. Moser, B. R., *Journal of natural products* **2008,** *71*, 487-491.

155. D'Auria, M. V.; Giannini, C.; Zampella, A.; Minale, L.; Debitus, C.; Roussakis, C., *The Journal of organic chemistry* **1998,** *63*, 7382-7388.

156. Opsenica, D.; Pocsfalvi, G.; Juranic, Z.; Tinant, B.; Declercq, J.-P.; Kyle, D. E.; Milhous, W. K.; Šolaja, B. A., *Journal of medicinal chemistry* **2000,** *43*, 3274-3282.

157. Todorović, N. M.; Stefanovic, M.; Tinant, B.; Declercq, J.-P.; Makler, M. T.; Šolaja, B. A., *Steroids* **1996,** *61*, 688-696.

158. Rao, Z. Synthesis of Novel Aromatase Inhibitors and New Steroidal Dimers. Auburn University, Auburn, Alabama, 2000.

159. Parish, E. J.; Chitrokorn, S., *Organic Preparations and Procedures International* **1983,** *15*, 365-367.

160. Fieser, L. F., *Organic syntheses* **1955**, 43-43.

161. Biotechnology, S. C. 7-Ketocholesterol (CAS 566-28-9). (June 30),

162. Sigma-Aldrich 5-Cholesten-3β-ol-7-one. (June 30),

163. Knapp Jr, F. F.; Schroepfer Jr, G. J., *Steroids* **1975,** *26*, 339-357.

164. Frenkel, M.; Marsh, K. N., *Spectral Data for Steroids*. Taylor & Francis: 1994.

165. Luche, J.-L.; Rodriguez-Hahn, L.; Crabbé, P., *Journal of the Chemical Society, Chemical Communications* **1978**, 601-602.

166. Dugas, D.; Brunel, J. M., *Journal of Molecular Catalysis A: Chemical* **2006,** *253*, 119-122.

167. Wilson, W. K.; Sumpter, R. M.; Warren, J. J.; Rogers, P. S.; Ruan, B.; Schroepfer, G. J., *Journal of lipid research* **1996,** *37*, 1529-1555.