STUDIES ON THE SYNTHESIS OF B-RING

6-AZASTEROIDS

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information

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Jyothi Swaroop Gandikota

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STUDIES ON THE SYNTHESIS OF B-RING 6-AZASTEROIDS

Jyothi Swaroop Gandikota

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in the Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
August 7, 2006

STUDIES ON THE SYNTHESIS OF B-RING

6-AZASTEROIDS

Jyothi Swaroop Gandikota

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Signature of Author

_____
Date of Graduation

VITA

Jyothi Swaroop Gandikota, son of Vijay Kumar and Seetha, was born on April 17, 1981, in Kakinada, Andhra Pradesh, India. He graduated with a Bachelor of Science degree in Pharmaceutical Sciences in 2002 from Kakatiya University, India. From Jan 2003 to June 2003, he worked for Pfizer India. He entered the Graduate School at Auburn University, Auburn, Alabama in August, 2003.

THESIS ABSTRACT

STUDIES ON THE SYNTHESIS OF B-RING

6-AZASTEROIDS

Jyothi Swaroop Gandikota

Master of Science, August 7, 2006
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55 Typed Pages

Directed by Edward J. Parish

Azasteroids, which carry a nitrogen in the C-6 position of the steroid nucleus are still mysterious and very few synthesized, were found to possess antimicrobial and antifungal properties apart from their pharmacodynamic actions. The literature reports for synthesizing these compounds is also very limited. We developed different approaches and modified, streamlined procedures for the synthesis of these compounds. The target molecules had different side chains (R= H, Me, Et) at the 6^{th} position and were synthesized using ozone and other oxidizing agents. The oxidative cleavage of the B-ring was the key step to insert a nitrogen atom.

ACKNOWLEDGEMENTS

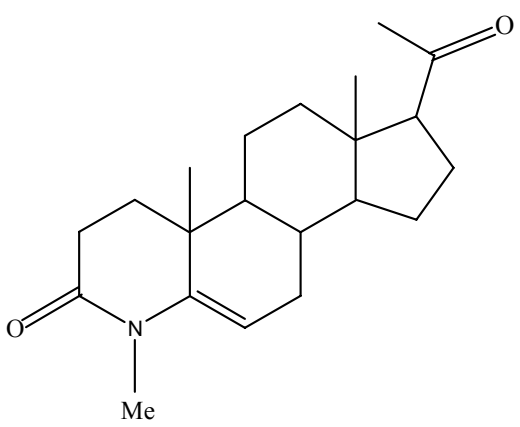
I would like to express my heartlfelt appreciation to my advisor Dr. Edward J. Parish. Not only he has given me invaluable academic guidance but also gave me a lot of moral support throughout my stay in Auburn. I thank all my committee members for their time spent on reading this thesis. I thank the faculty in the Department of Chemistry and Biochemistry for their teaching. Last but not the least, I would like to thank my family and friends for their support during this work.

TABLE OF CONTENTS

LIST OF SCHEMES

INTRODUCTION

Heterosteroids have drawn people attention because of their wide variety of biological properties. Some of these include cytotoxicity, atherogenicity, mutagenicity, hypocholes, terolemic and effects on special enzymes. The inhibition of steroids in mammals has been well established[2]. The well known contraceptive pills and other steroid drugs resulted from this physiological activity of heterosteroids, especially azasteroids, due to their binding with steroid receptors and with target cell determinants, thereby blocking the binding of the actual steroids. Also, the heterosteroids have been found to inhibit the enzymes of steroids metabolism and have been used as contraceptives[6] or antifertility agents[7] such as androgen antagonists, estrogenic and anti-estrogenic compounds. These compounds function also as anti-adrenocorticoids, anti-minerallocorticoids, catatoxic, anabolic, anti-lipidemic neuromuscular blocking, local anesthetic and anti-neoplastic agents. A compound with significant anti-androgenic activity is 6α-bromo-17β-hydroxy-17 α-methyl-4-oxa-5 α-androstan-3-one(BOMT), which competes for the 5 α dihydrotestosterone binding sites in androgen dependent tissues[5]. 4-methyl aza-5-pregenen-3,20-dione(28) possesses mild androgenic activity[17].



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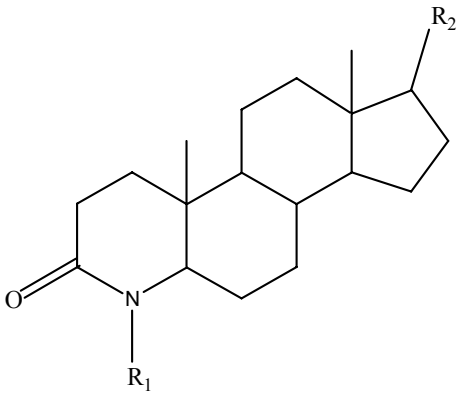


(31)

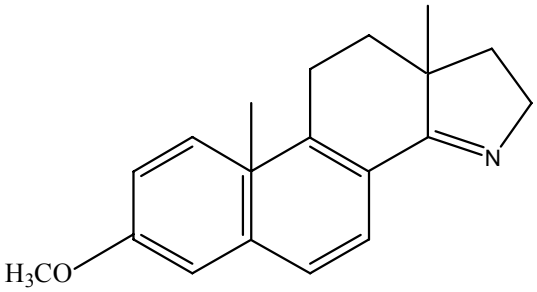
A series of A ring azasteroids(31)[38] has been prepared and tested for inhibition of rat prostatic steroid 5 α-reductase in vitro. 16-azasterone-3-methyl–ether possessed anti-estrogenic properties. The lactam 17β-hydroxy-4-(2-hydroxy ethyl)- 17α-methyl-4-aza-5-androsten-3-one[24] and certain 2,3-diaza-4-methyl-1-one steroids also have some anti-estrogenic activity[25]. 2 azaestratrienes were hormonally inactive but showed moderate hypocholesteromic activity in vivo[26]. 16-aza-estrone-methyl ether, a very weak estrogen, exhibited hypocholesteromic activity activity in adult male rats.

Great interest in azasteroids has been increasing because of their potential value as pharmacodynamic and chemotherapeutic agents[27]. 4–Azasteroids with anti-microbial, hypotensive, anti-inflammatory and hypocholesteromic activities have been prepared by Doorenbos[27].

Azasteroids are well known for their potential pesticidal, chemotherapeutic and pharmacodynamic activity[2]. Use of these compounds as anti-inflammatory, antimicrobial,

hypercholesteromic, antifertility, hypotensive agents has been reported. Tapan has done some research about the inhibition of nematode and other pests[2]. The azasteroids can interfere with steroid metabolism, growth and development in invertebrates. Those results may lead to the development of new anthelmintic agents.

Many azasteroids have shown antibacterial and antifungal activities. Some of these compounds have antiprotozoal and antiviral activities. Thiosemicarbazones of testosterone and progesterone were active[2] as antibacterials Antifungal drug ketoconazole inhibits the production of steroid hormones, including both cortisol and testosterone[8]. Some steroids related synthetic hetero analogues possessing nitrogen in the D-ring are antimicrobial agents(29),(30)[9] and inhibit the growth of bacillus subtilis and Escherichia coli at the concentration as low as 10-5M.



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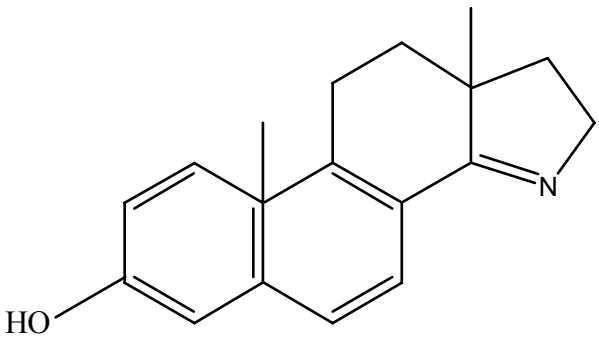

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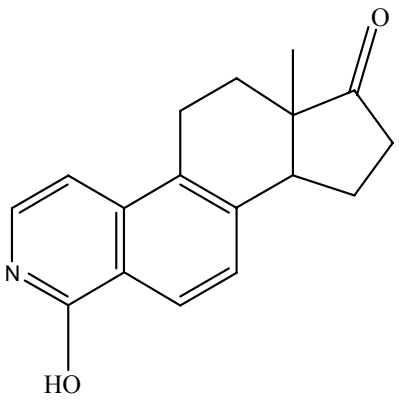
Azasteroids that have N in different positions have been done in different ways, position 3(17), position 4(18), position 6(19) and position 8(20)[27,28,29,30,31].



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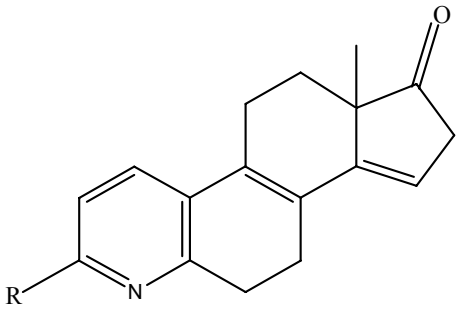
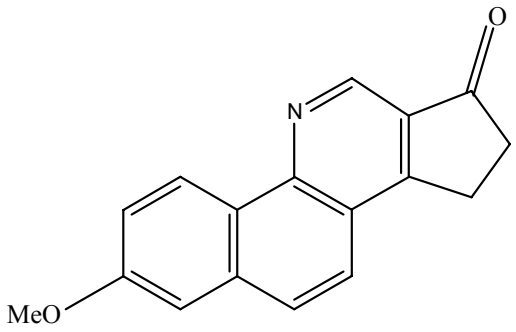


(18)

(a) R = OEt
(b) R = H



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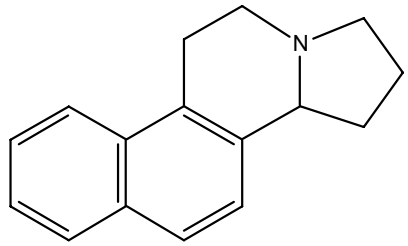

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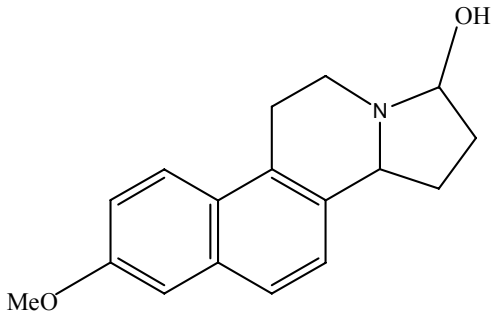

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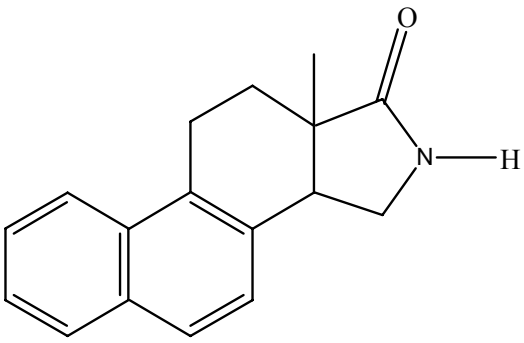

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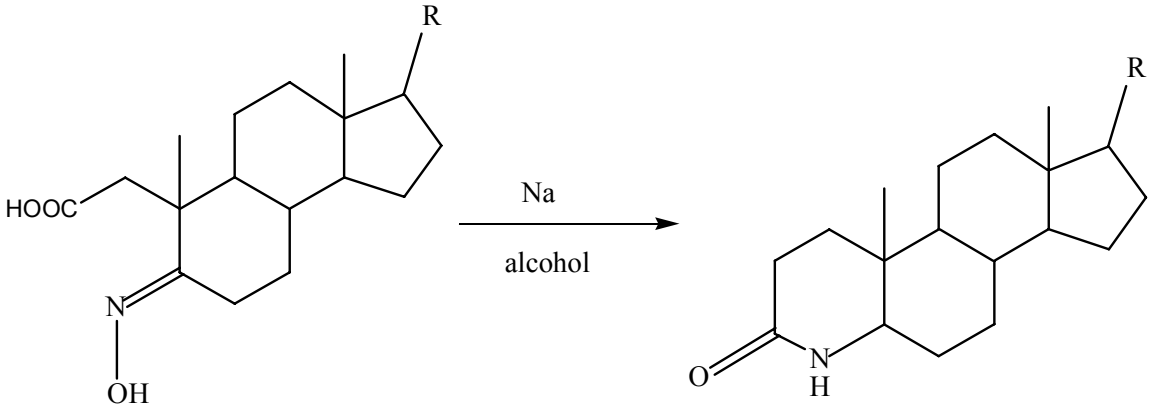

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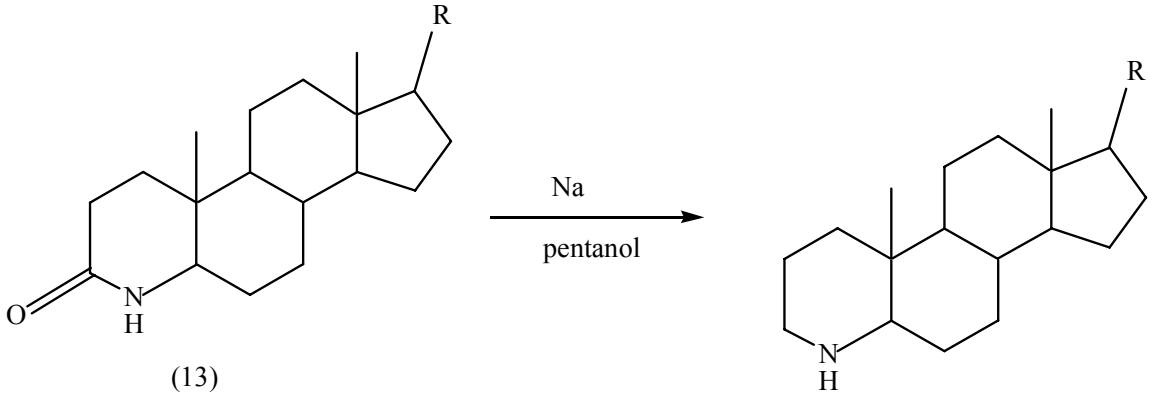

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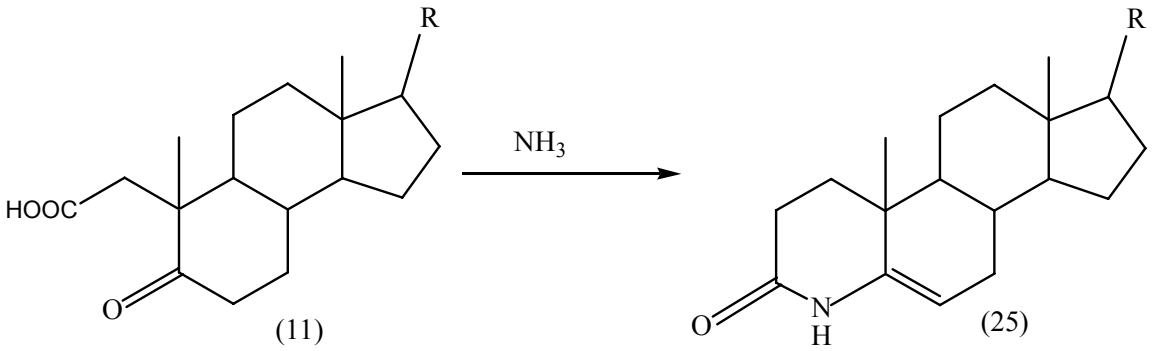

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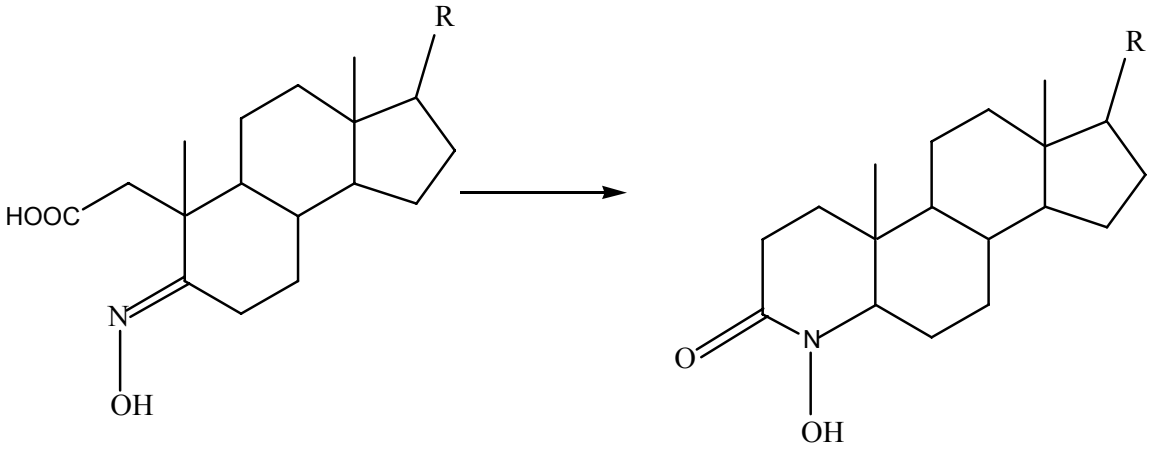

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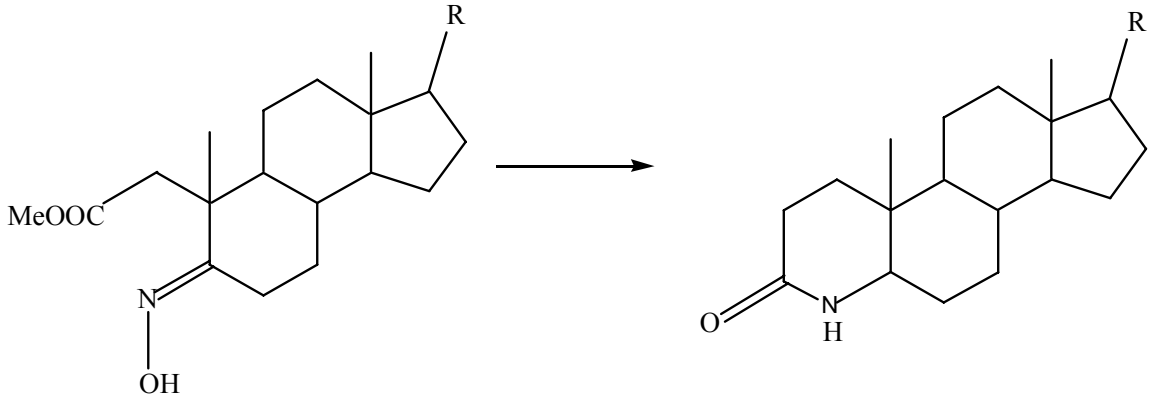

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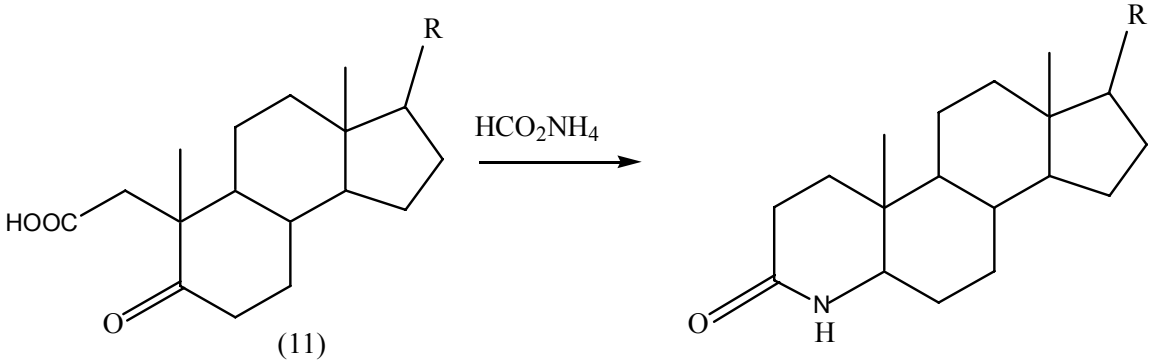
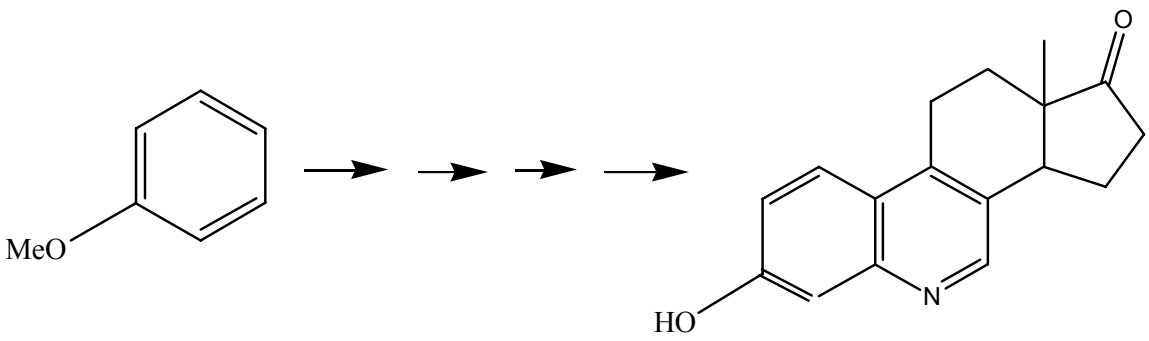


(11) (13)

In 1938, Bolt[36] prepared the lactam(13) by reduction of the ketoxime(12) with sodium and alcohol followed by acidification of the alkaline reduction mixture.

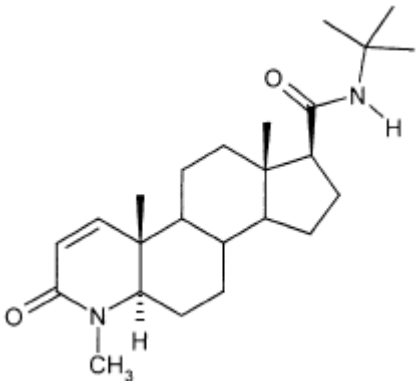
He reduced his lactam to secondary base(14) with sodium and pentanol.

Mckenna and Tulley have improved this procedure by using lithium aluminum hydride. In 1959, Doorenbos reported the synthesis of 4-aza-5-cholestan-3-one from 3,5-seco-4-norcholestan-5-on-3-oic acid by reaction with ammonia at 140 C. Wildi described a similar preparation for this compound. In 1960 Edward and Morand described the hydrogenation of the oxime to the keto-acid(12)[30] with platinum in acetic acid to give the related hydroxyl amino acid which cyclised spontaneously to the monohydrate of N-hydroxy -4-aza-5-cholestan-3-one(26), formation of the n-hydroxy lactam prevents further hydrogenation, but use of the oxime of the keto avoids this difficulty and affords 4-aza-5-cholestan-3-one(13)[32]. Edward and Morand also made(13) 4-aza-5-cholestan-3-one using ammonia formate reacted with 5-oxo-3,5- seco- a- norcholestan-3-oic acid in nitrobenzene at 180-200 C[41] . In 1963, Burckhalter and Watanabe first prepared the 6-azaquilenin using a 15-step synthesis form m-anisidine. In1985, Gumulka made 11-azasteroid(37) using ozonolysis then oxidation with hydrogen peroxide to open the C-ring of 5α- androst-9,11-ene-3,17-dione[44](32). The product is a mixture of keto aldehyde and keto acid. Oxidation of the keto aldehyde using Jones reagent gave keto acid through acid azide(34), isocyanate(35), imine(36) and final product(37).

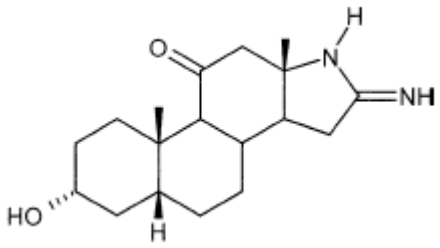

MeO
HO
N
O

BIOLOGICAL SIGNIFICANCE OF AZASTEROIDS

Cholesterol, when replaced with a heteroatom in any of the carbon atoms of its nucleus shows significant biological properties, specially azasteroids. 5α- reductase inhibitors like finasteride, GABA receptor antagonists like RU-5135 , and neuromuscular blocking agents like pancuronium and chandonium are some very good examples exhibiting these properties.



FINASTERIDE

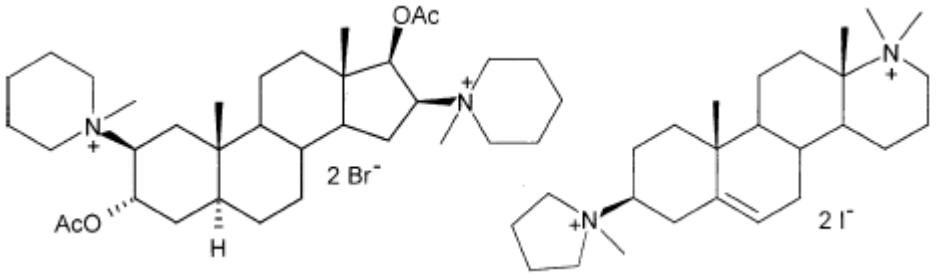


RU-5135

Burbiel et. al in 2003 discussed about the antifungal properties of azasteroids and also reported the effect of nitrogen substitution in different positions of the cholesterol nucleus.

1,2 and 3- Azasteroids : Doorenbos etal reported that 2-methyl-2-aza-5-cholestane and

3-methyl-3-aza-5-cholestane show bactericidal effects on *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albicans* as compared to 4-methyl-4-aza-5-cholestane.



PANCURONIUM CHANDONIUM

4-Azasteroids: Their significant effect on the enzyme 5α-reductase by potentially inhibiting it, and its use in the treatment of benign prostatic hyperplasia made 4-azasteroids an interesting set of compounds for investigation. For more than a decade Doorenbos etal investigated 4-azasteroids for their antimicrobial effects. Although, the biological activity of 4-azasteroids are dependent on the side chain(lipophilicity etc.) , the site of action of these compounds is yet to be known. .

5-,6-, and 7-azasteroids: 6α- and 6β-amino-5α-cholestan-3β-ol are two extranuclear azasteroids found to possess anti-mycotic activity.

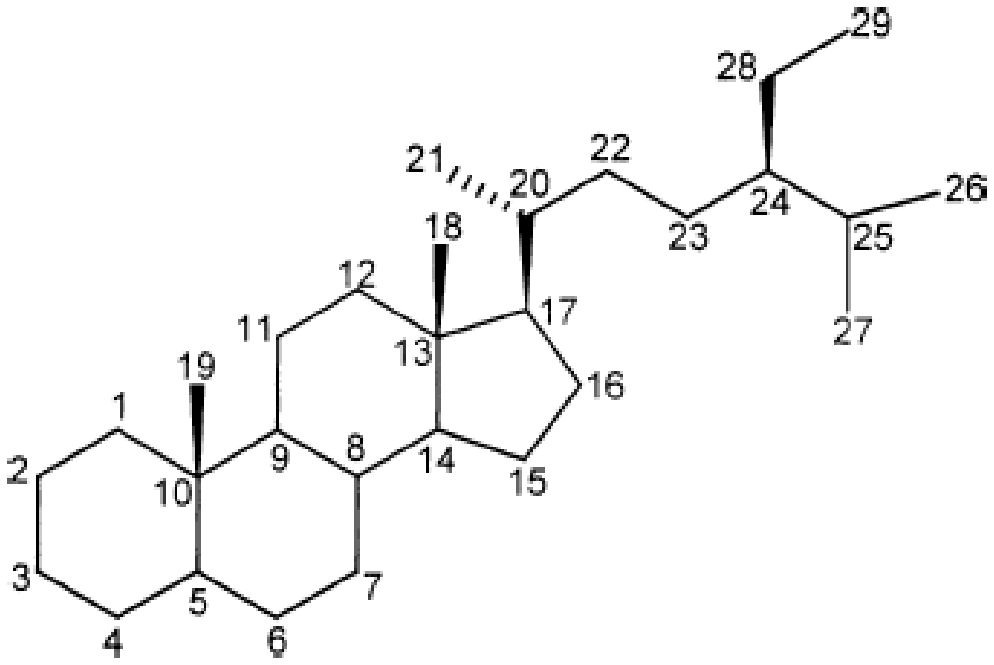
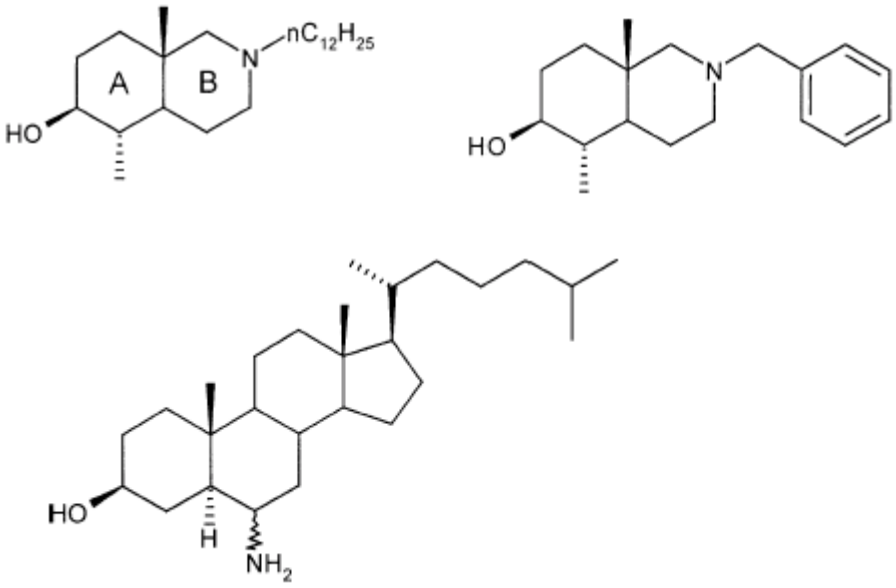


Fig. 32

STEROID NUMBERING



8-azasteroids: Taton etal worked on these compounds and tested a wide variety of N-substituted derivatives on the enzymes and whole cell systems. They concluded in their work that the compounds with long aliphatic side chain and benzyl substituents were found to be biologically active compared to the unsubstituted ring system which is 100 fold less reactive.

9-azasteroids: Zipplies etal has done some work on 9-azasteroids, how ever very little is known about them.

10-, 11-, 12-, 13, 14- azasteroids : No significant biological activity was found for these compounds.

15- azasteroids: These azasteroids exhibit microcidal properties and have been of great interest, there were many publications highlighting their biological activity.

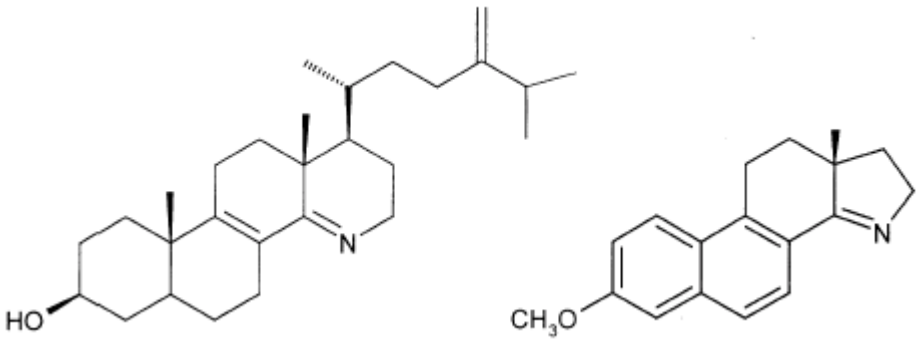
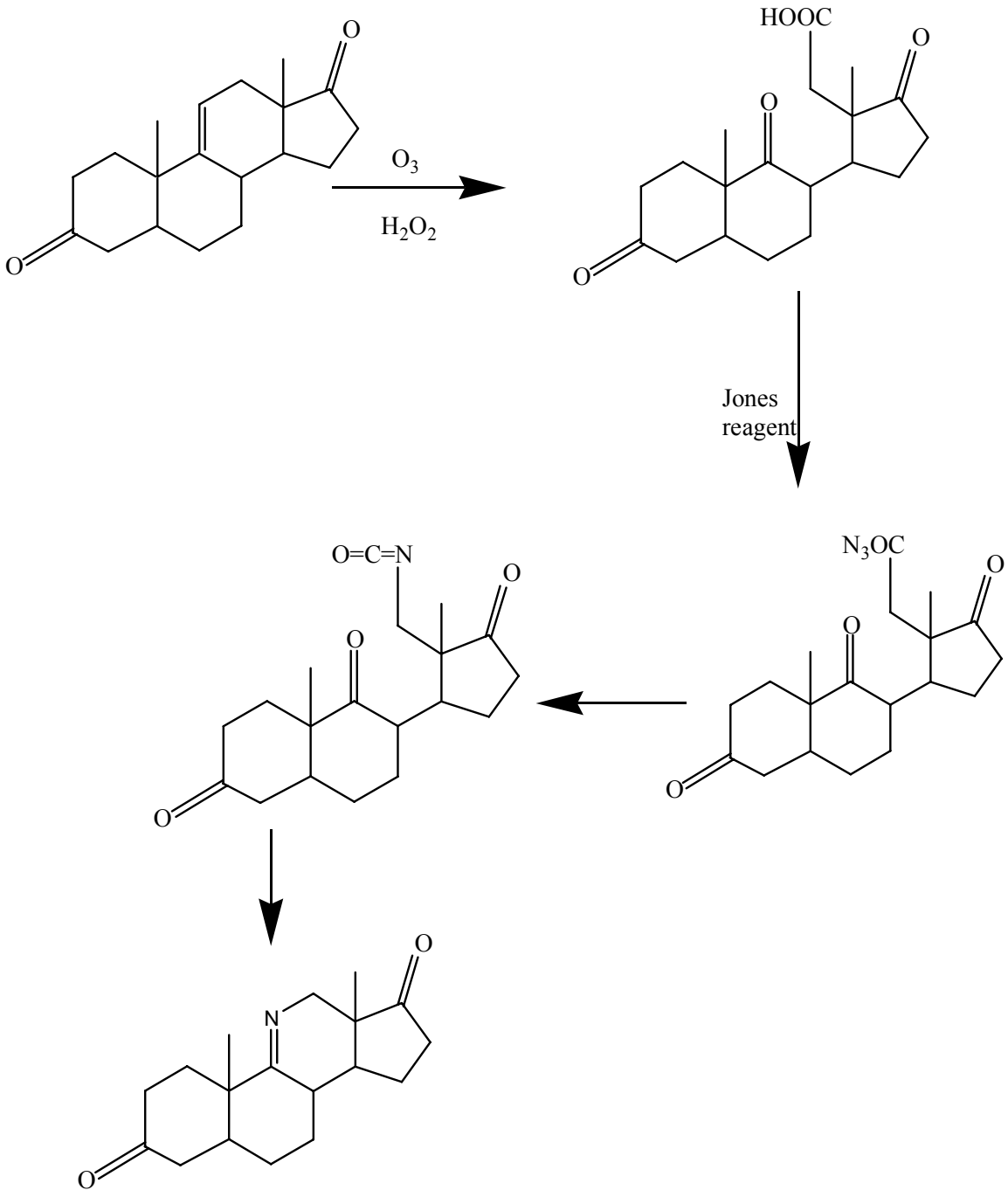
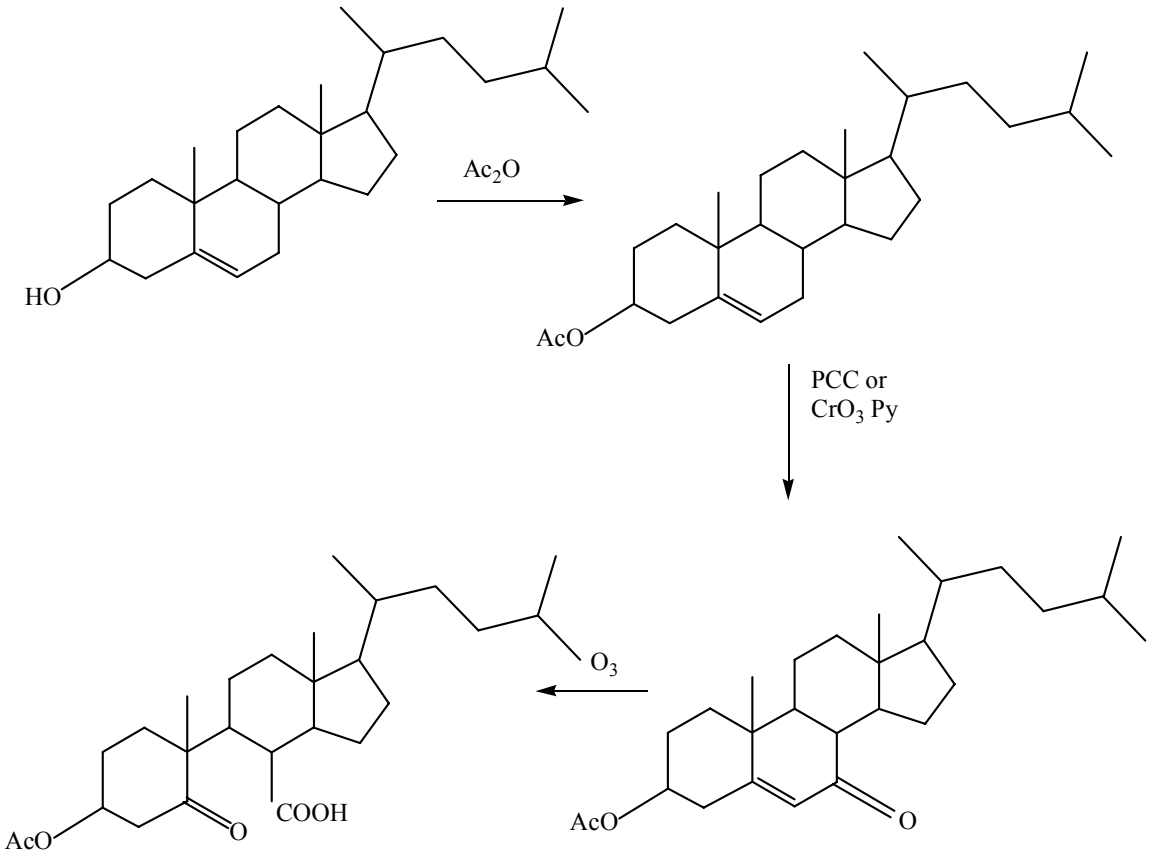


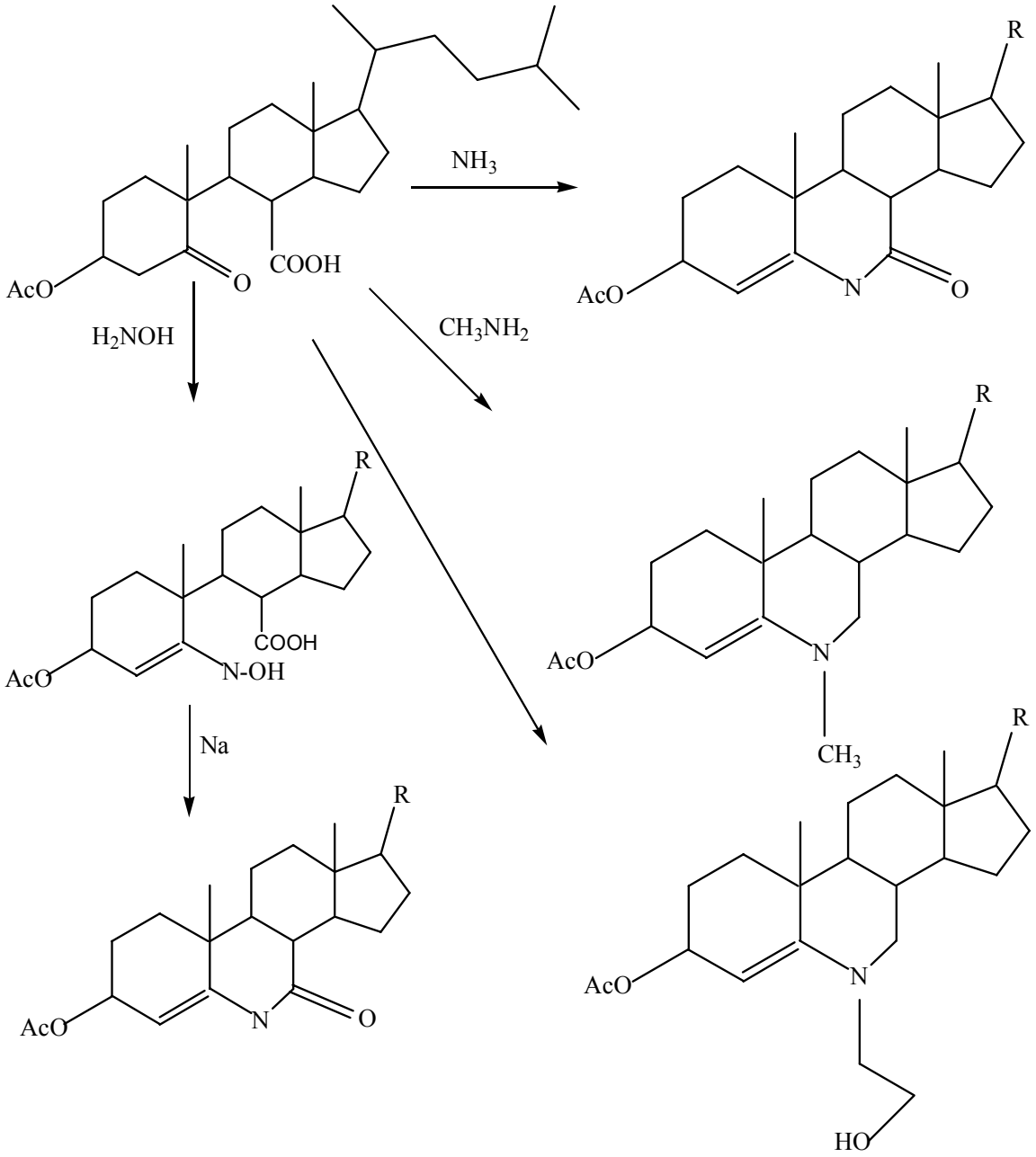
Fig 33



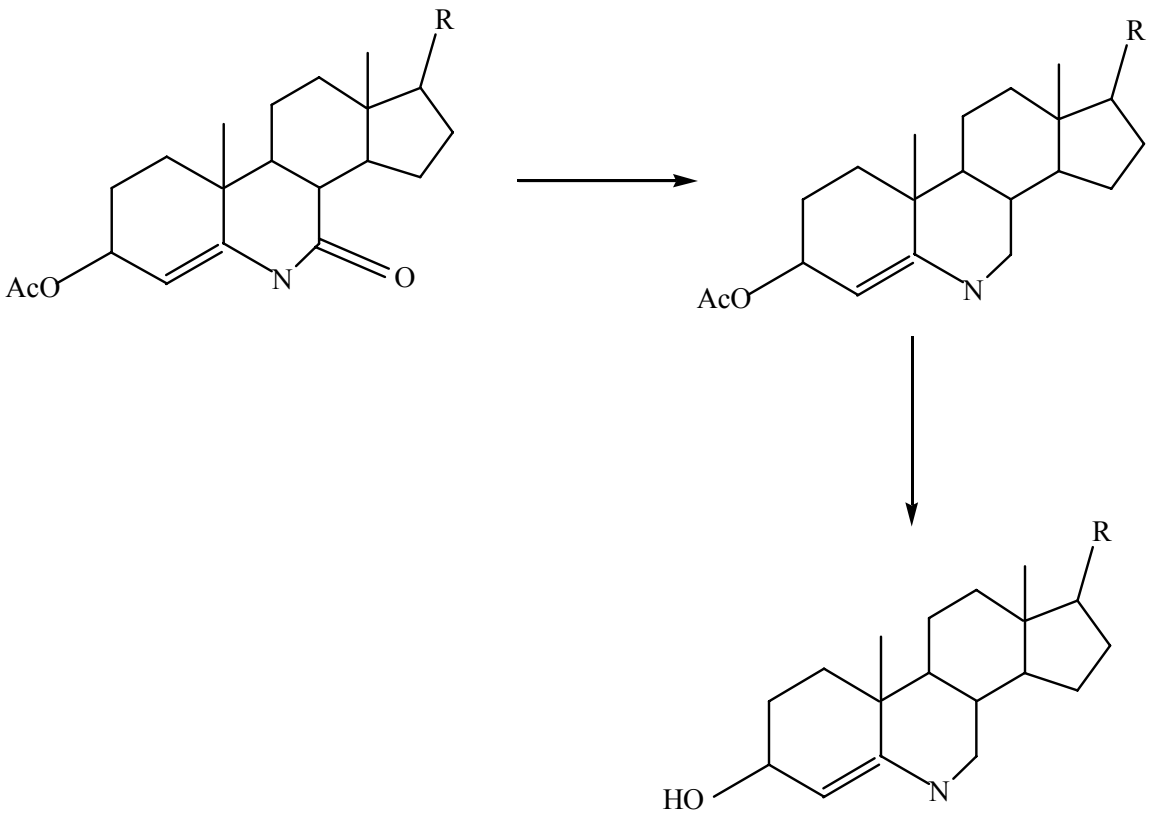
Scheme 1



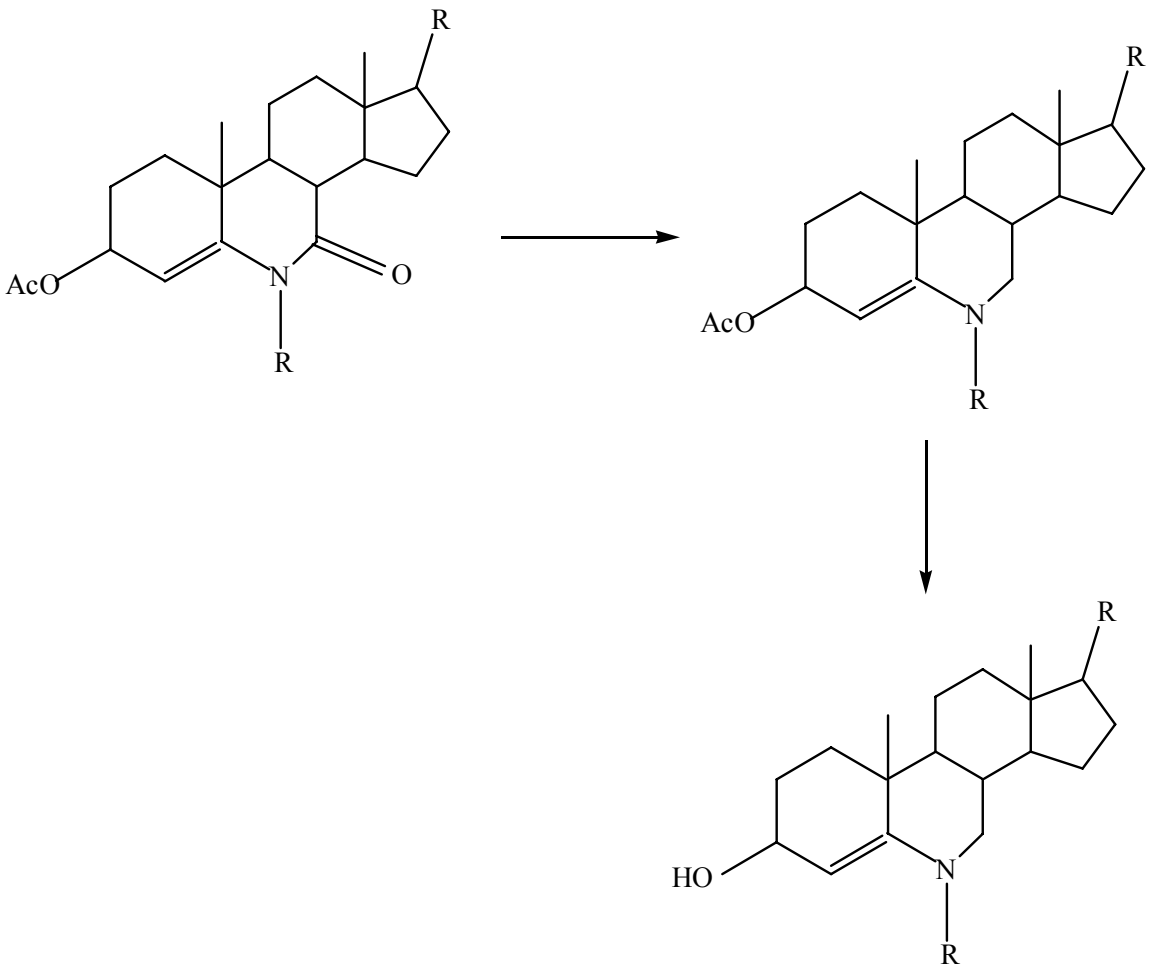
Scheme 2



Scheme 3



Scheme 4



Scheme 5

EXPERIMENTAL SECTION

ANALYTICAL EQUIPMENT :

MELTING POINT APPARATUS: MP was obtained using an electrothermal apparatus and was reported in a range.

NUCLEAR MAGNETIC RESONANCE : NMR spectra were obtained using Avance 400 NMR spectrometer. The solvent used was deuterated chloroform $CDCl_3$ which served the purpose of being the internal standard. The final results were concluded using ppm(parts per million).

MASS SPECTROMETRY: MS was done using Finnegan 3300 magnetic mass spectrometer.

INFRARED SPECTRA : IR was done using IBM Instruments Inc. Model 931 spectrometer. The results obtained were in frequency/ cm.

SEPERATION : The product and reactant separation was done using TLC (Thin Layer Chromatography). UNIPLATE silica gel plates manufactured by ANALTECH, DE were used for this purpose. Solvent used was 50% toluene in ether. The spots were visualized using Molybdic acid Spray.

MOLYBDIC ACID SPRAY COMPOSITION:

20 g of ammonium molybdate tetrahydrate was dissolved in 25 ml of sulfuric acid and heated till it dissolved. Stirring the solute aided dissolution. The final volume was made upto 400 ml using water.

COLUMN CHROMATOGRAPHY: This is the most important analytical technique that we used for the separation of the final reactants and products. Silica gel column was used and the compounds were eluted using the solvents in different compositions.

100% toluene.

90% toluene in ether.

80% toluene in ether.

70% toluene in ether.

60% toluene in ether.

50% toluene in ether.

The solutions that are eluted were evaporated using a ROTAVAP and the solids were analyzed using the techniques mentioned above. The toluene solution was collected after condensation and reused again.

CHEMICALS AND REAGENTS USED:

SIGMA ALDRICH :

Pyridine

Acetic anhydride

Benzoyl Chloride

Chromium trioxide

Glacial acetic acid

Potassium Permanganate

Sodium Periodate

2-methyl – propanol (t-butanol)

Sodium Carbonate

Acetone

Pyrazole

Magnesium Sulfate

Chloroform

Acetone

Ethyl ether (ether)

METHOD TO CLEAVE THE CYCLIC RINGS IN CHOLESTEROL

Cholesterol which is obtained commercially is the best source of preparing the acetate derivative. We start from cholesterol, first to protect the –OH group. The procedure that is followed is cholesterol is dissolved in pyridine, added acetic anhydride and refluxed for 2 hrs to get cholesteryl acetate as white solid in 90% yield. Then, it is oxidized at C-7 position by using PCC or chromium trioxide, this step gave 7-keto cholesteryl acetate in 80% yield. Next step is to open the B-ring of the cholesterol by ozonolysis. The ozonolysis method was limited to the small scale reaction to avoid the accumulation of the dangerous ozonide or the hydroperoxide intermediate. These are hazardous if not

completely destroyed during the reaction. Cella, has devised a convenient two phase method using hydroperoxide anions as oxidant, used as a phase transfer catalyst to transfer the oxidant from aqueous phase to organic phase. This procedure prevents the build up pf hazardous ozonide during the reaction. Then, acidify the aqueous phase to get the product. However, since this procedure uses NaOH in aqueous phase, this can endanger the reaction. Tapan Kanti Majumdar described a similar method he used in his thesis for masters degree to open the A ring of the cholesterol.

PREPARATION OF CHOLESTERYL ACETATE:

100g of cholesterol (MW=386, 259 m mol) was dissolved in 500 ml of pyridine and 250 ml of acetic anhydride in a 2L flask and was refluxed for 2 hrs, it was then poured into ice and allowed to stand for 2 hrs. The ppt was filtered and washed with water. It was then recrystalised using acetone-water and air dried for 24 hrs, later dried in a vacuum desiccators. (99.87g, 233 m mol, white solid, 90%)

MP 112-114 ^{o}C Literature MP 112-115^{o}C

IR 2940, 1720, 1470, 1370, 1240, 1030, 950, 900, 800, 740, 700 cm^{-1}

^{1}H NMR : $CDCl_3$, 0.7, 0.9, 1.0, 0.6-2.2, 2.0, 2.3, 4.6, 5.4

MS: 428(0%) 368(100%) 147(34%) 145(32%) 107(36%) 105(35%) 91(32%) 81(47%)

MW 328

PREPARATION OF 7-KETO-3β-CHOLESTERYL ACETATE:

(a)Pyrazole(10.4g, 153 m mol) was dissolved in 200 ml of dichloromethane containing 1g of 3A molecular sieves. The solution was cooled down to -20^{o}C and 15.3g (153 m

mol) chromium trioxide was added. After stirring at -20^{o}C for 0.5hrs, cholesteryl acetate 6.0g(14m mol) was added. The mixture was seated for 24hrs at -10^{o}C in a freezer. The mixture was then poured into saturated NaCl solution containing 5% HCl and the mixture was thoroughly extracted with chloroform. The resulting extracts were dried over magnesium sulfate, filtered and evaporated to dryness under reduced pressure to give a residue. The purified product was recrystallized from acetone water to give 5.01g of white crystals(81%).

^{1}H NMR: $CDCl_3$, 0.7, 0.8, 0.9, 0.7-2.7, 2.1, 4.7, 5.7

MW: 442.34

M.P: 160-161^{o}C

M.S: 442(0%) 382(15%) 187(21%) 174(100%) 161(32%) 91(30%) 55(35%) 43(93%)

IR : 2950, 2866, 2340, 1730, 1672, 1462, 1370, 1245, 1033950, 635

(b) 10.0g of cholesteryl acetate(23,36 m mol) was dissolved in 500 ml benzene containing 1.5g 3A type molecular sieve in a 1000 ml flask. 13g PCC(Pyridinium chlorochromate 600 m mol) was added and the reaction mixture was stirred and later gently refluxed under a nitrogen atmosphere for 72hrs. The benzene solution was decanted and the remaining contents of the reaction flask were washed several times with ether. The combined extracts were washed with saturated NaCl solution and dried over anhydrous sodium sulfate. The residue was subjected to column chromatography using 5-. 20% ether in toluene. Recrystallization using acetone water gave purified material.(8.06g, 18.24 m mol, 78.1%)

PREPARATION OF 3β-ACETOXY-5-KETO, 7-SECO-6-NORCHOLESTAN-7-OIC-ACID:

A solution of 10.0g(22.62 m mol) 7-keto-cholesteryl acetate in 100 ml chloroform and 3.0g 50% hydrogen peroxide, 2.0g of sodium hydroxide, 0.2g of adogen(methyl trialkyl C8-C10) ammonium chloride phase transfer catalyst was added. Ozone was passed from a Welsbach ozonizer through the reaction mixture for 6 hrs at -5 – 0^{o}C in an ice salt bath. The reaction mixture was stirred for 4hrs at 0^{o}C, separated the aqueous layer, diluted with 50ml cold water, then acidified with cold hydrochloric acid in presence of 200ml ether. The ether was separated and washed with saturated solution of sodium chloride, dried over anhydrous sodium sulfate and concentrated at reduced 40^{o}C. Column chromatography($MeOH/CH_2Cl_2$), recrystalization from ether-pentane gave 5.6g of white crystals(54%).

M.P: 156.0-157.0^{o}C

IR: 1729, 1709, 1246 cm^{-1}

MW : 462

PREPARATION OF 5-KETO-5,7-SECO-6-NOR-3-CHOLESTEN-7-OIC-ACID:

A solution of 2.0g(4.33 m mol) 3β-acetoxy-5-keto, 7-seco-6-norcholestan-7-oic-acid and 2.0g sodium hydroxide in methanol-water solution , refluxed for 0.5hrs, cooled down then added hydrochloric acid, the acid recovered was white solid (1.74g, 100%)

M.P: 164.5-165.5^{o}C

IR: 2960, 2860, 1725, 1701, 1679, 1470, 1390, 1200, 1150, 1002 cm^{-1}

MW: 402

^{1}H NMR : 0.7-2.4, 592, 6.80.

PREPARATION OF 6-AZA-2,4-CHOLESTANDIEN-7-ONE:

1.00 g of 5-keto-5,7-seco-6-nor-3-cholesten-7-oic-acid (2.49 m mol) was dissolved in 20 ml of absolute ethanol that was pre- saturated with dry methyl amine gas at 0^{o}C in an ice-salt bath. The solution was in a 22 ml copper bomb, capped tightly and heated in an oven for 3 days. It was then cooled down for 12 hrs in air, then evaporated the solvent, separated the product by using column chromatography(1% triethyl amine in ethyl acetate and hexane). Pure product was obtained as yellow solid.

^{1}H NMR: 7.76, 7.35, 7.30, 3.52, 0.82-1.88

MS: 397(29%) 382(10%) 148(16%) 134(27%) 122(29%) 107(33%) 81(43%) 69(37%) 57(62%) 43(100%) 29(30%) 15(19%)

IR: 2960, 1665, 1635, 1470, 1304, 1133, 1060cm^{-1}

M.P100^{o}C

PREPARATION OF 6-(β-HYDROXY ETHYL)-6-AZA-CHOLESTANE-DIEN-7-ONE:

A mixture of 5-keto-5,7-seco-6-nor-3-cholestan-7-oic acid (1.00g, 249 m mol) and 5.0 ml of ethanol amine was refluxed after 4hrs. After cooling down to room temperature, water was added and benzene was used to extract the mixture. 1N HCl and water was used to wash the benzene and dried over sodium sulfate. TLC showed a very complicated reaction mixture, very difficult to separate.

MS: 429(6%) 400(4%) 373(31%) 372(100%) 167(18%) 149(56%) 85(43%) 71(61%) 57(88%) 43(89%)

PREPARATION OF 5-KETO-5,7-SECO-6-NOR-3-CHOLESTEN-7-OIC-ACID OXIME:

A mixture of 5-keto-5,7-seco-6-nor-3-cholesten-7-oic-acid (1.00g, 249 m mol) and 1.0g of NH_2 -HCl in 30 ml pyridine was heated under reflux for 1 hr, then poured into ice and seated in refrigerator for 2hrs, solid was filtered and further purified by column (1:1 ethyl acetate/hexane).

MW : 417, white solid, 0.74g(1.77 m mol) (71%)

^{1}H NMR: 0.6-2.4

IR: 2960, 1599, 1457, 1375, 1301, 1261, 1020, 813cm^{-1}

MS: 417(5%) 400(13%) 399(11%) 398(12%) 386(35%) 385(43%) 384(100%) 383(72%) 382(38%) 368(25%) 191(7%) 165(9%) 149(19%) 139(13%) 125(18%) 125(18%) 111(13%)

PREPARATION OF 6-AZA-CHOLESTEN-7-ONE:

0.2g of 5-keto-5,7-seco-6-nor-3-cholesten-7-oic-acid oxime in 10 ml ethanol was taken and added 0.2g of sodium, refluxed under nitrogen for 1hr, cooled and added water, stirred, extract with ethyl acetate.

MW: 385

MS: 385(12%) 368(3%) 354(1%) 162(7%) 135(12%) 121(9%) 107(16%) 95(19%) 81(21%) 69(20%) 55(30%) 44(63%)

^{1}H NMR: 0.7-243, 6.26, 6.65

IR: 2960, 2870, 1700,1450, 1366, 1205, 960 cm^{-1}

PREPARATION OF 5-KETO-5,7-SECO-6-NOR-3-CHOLESTEN-7-OIC-ACID ESTER:

5-keto-5,7-seco-6-nor-3-cholesten-7-oic-acid (1.0g) was taken in a culture tube, add boron trifluoride in MeOH (BF_3 in MeOH), heat in boiling water bath for 3 minutes, cool, add 3ml of hexane, separate layers, evaporate solvent, the residue contains methyl ester.

MW: 416

MS: 416(0.2%) 385(0.68%) 368(0.19%) 174(1.87%) 142(2.2%) 121(3%) 110(100%) 95(11%) 81(7%) 68(23%) 43(12%) ^{1}H NMR: 0.67, 0.85-2.33, 3.6, 5.82, 6.80

GENERAL PROCEDURE FOR THE PREPARATION OF STEROIDAL BENZOATES

The synthesis of intermediate was done using ozone initially, but we carried out an experiment where the intermediate can be synthesized without using ozone. We used oxidizing agents like potassium permanganate, sodium periodate in solvent t-butanol.

The C-3 position of the cholesterol was protected using a benzoate.

This procedure is generally used for the preparation of the benzoate esters at the 3rd position of the carbon atom in the cholesterol.

Cholesterol is taken in a round bottom flask and anhydrous pyridine is added to it.

Benzoyl chloride is used to esterify the 3rd position, which is added finally to the reaction and the reactants are refluxed for 12hrs. The resulting reaction mixture is poured in ice-

water and allowed to solidify. The solid is filtered and allowed to dry for about 24 hrs and further dried to remove trace amounts of water in a desiccator.

The solid is then recrystalised using acetone water.

For the cleavage of the steroidal B ring, we needed an oxidizing agent and found that the best suitable was chromium trioxide in presence of glacial acetic acid. The obtained benzoate derivative was dissolved in glacial acetic acid and chromium trioxide was dissolved in glacial acetic acid in separate flasks. The reactants from both the containers are mixed together and stirred using a magnetic stirrer continuously for 24 hrs. The obtained mixture was poured in ice water and then allowed to solidify. The solid is then recrystalised using acetone- water.

PREPARATION OF 3β-CHOLESTERYL BENZOATE:

Cholesterol (100g; 129 m mol) was placed in a round bottomed flask and dissolved in 400ml pyridine. 200ml of benzoyl chloride was added to the mixture and the reactants were heated at $90^{o}C$ for 6 hrs. The heating was done using reflux condenser. The mixture is then poured into ice water and allowed to solidify. The solid was filtered using a funnel under vacuum and allowed to dry for 24 hrs. The remaining traces of water were removed using a vacuum desiccator.

It was then recrystalised using chloroform

The product weighed 70g (70%)

M.P: 148-$150^{o}C$

IR : 1110, 1276cm^{-1}

^{1}H NMR : 0.69, 1.01, 4.87, 5.43

PREPARATION OF 7-KETO-3β-CHOLESTERYL BENZOATE:

Cholesteryl benzoate (5.4g, 5.50ml) was dissolved in 420 ml of glacial acetic acid. A solution of 4.2g(21.0m mol) of chromium trioxide was dissolved in 42ml of water and 210 ml of glacial acetic acid was added to it. The mixture was stirred at room temperature overnight for 24hrs. The product was then poured in ice water and allowed to solidify. The resulting solid was filtered through buchner funnel and later left for 24hrs to dry. Traces of water were removed by vacuum desiccator and the resulting powder was recrystalised using acetone-water.(3.2g; 60% yield)

M.P: 157-159^{o}C

IR: 1735, 1685, 1670, 1245, 1039 cm^{-1}

^{1}H NMR: 0.67, 1.22, 4.91, 5.73, 7.71

MS: 490, 368, 353, 121, 105

PREPARATION OF THE INTERMEDIATE (3β-HYDROXY-7-KETO-8-CHOLESTANOIC ACID):

The intermediate product synthesis was crucial and we have done it using two methods.

The first method involved the use of ozone and the later method was the usage of strong oxidizing agents like potassium permanganate and sodium periodate in the presence of t-butanol.

A solution of 10.0g(22.62 m mol) 7-keto-cholesteryl acetate in 100 ml chloroform and 3.0g 50% hydrogen peroxide, 2.0g of sodium hydroxide, 0.2g of adogen(methyl trialkyl C8-C10) ammonium chloride phase transfer catalyst was added. Ozone was passed from a Welsbach ozonizer through the reaction mixture for 6 hrs at -5 – 0^{o}C in an ice salt bath.

The reaction mixture was stirred for 4hrs at $0^{o}C$, separated the aqueous layer, diluted with 50ml cold water, then acidified with cold hydrochloric acid in oresence of 200ml ether. The ether was separated and washed with saturated solution of sodium chloride, dried over anhydrous sodium sulfate and concentrated at reduced $40^{o}C$. Column chromatography($MeOH/CH_2Cl_2$), recrystalization from ether-pentane gave 5.6g of white crystals(54%).

M.P: 156.0-157.0^{o}C

IR: 1729, 1709, 1246 cm^{-1}

MW : 462

Another method that we have developed in Dr.Parish's lab was

7-keto-3β-cholesteryl benzoate(0.5g) was taken in a round bottomed flask and dissolved in 30ml of 7.5% sodium carbonate solution. 5g of sodium periodate was added to the solution along with 35 mg of potassium permanganate and 25 ml of t-butanol.

The reactant mixture was heated on steam for more than 12 hrs and 2-3 drops of conc HCl was added along with 100ml water to the resulting solution, extracted with ether and the ether was evaporated using nitrogen gas or rotavap.

The product was then dissolved in toluene and separated by column chromatography using different concentrations of toluene and ether.

ATTEMPTED RECRYSTALLIZATION OF 3β-HYDROXY-7-KETO-8-CHLOESTANOIC ACID:

The resulting solid was sticky in nature and was a tough task to isolate the powder. We attempted to recrystalize using acetone-water. The compound was dissolved in a minimal amount of acetone and small quantities of water are added to the solution by heating it on steam. The crystals obtained were very small and not significant.

So, we recrystalized using another method.

RECRYSTALLIZATION OF 3β-HYDROXY-7-KETO-8-CHLOESTANOIC ACID:

The solid was dissolved in methanol and heated on steam until it dissolved. Very little quantities of water was added and allowed to cool, then cooled in an ice bath until crystals are obtained.

PREPARATION OF 6-AZA-3β-HYDROXY-CHOLESTANE-DIEN-7-ONE:

3β-hydroxy-7-keto-8-cholestanoic acid(0.5g) was taken and dissolved in 13ml of absolute ethanol. Dry ammonia gas was pumped at 3-4^{o}C, the ammonia gas was generated by heating a solution of ammonium hydroxide in to the solution.

The reactants were taken in a copper bomb of 22ml capacity and heated in an oven at 150^{o}C for 2 days. The bomb was cooled to room temperature, and then in a freezer for 12hrs. The ethanol was evaporated on a rotavap or by pumping nitrogen gas

(0.33g)

PREPARATION OF 6-AZA-METHYL-3β-HYDROXY-CHOLESTANE-DIEN-7-ONE:

3β-hydroxy-7-keto-8-cholestanoic acid(0.5g) dissolved in 13ml of absolute ethanol and 5ml of methyl amine (33% methyl amine in absolute ethanol) was added at 3-4^{o}C. The reactants were taken in a copper bomb of 22ml capacity and heated in oven at 150^{o}C for 2 days. The bomb was cooled to room temperature and then put in a freezer for 12hrs. The ethanol was evaporated using a rotavap or by pumping nitrogen gas.

(0.25g)

PREPARATION OF 6-AZA-ETHYL-3β-HYDROXY-CHOLESTANE-DIEN-7-ONE:

3β-hydroxy-7-keto-8-cholestanoic acid(0.5g) dissolved in 13ml of absolute ethanol and 5ml of ethyl amine (2M ethyl amine in absolute methanol) was added at 3-4^{o}C. The reactants were taken in a copper bomb of 22ml capacity and heated in oven at 150^{o}C for 2 days. The bomb was cooled to room temperature and then put in a freezer for 12hrs. The methanol was evaporated using a rotavap or by pumping nitrogen gas.

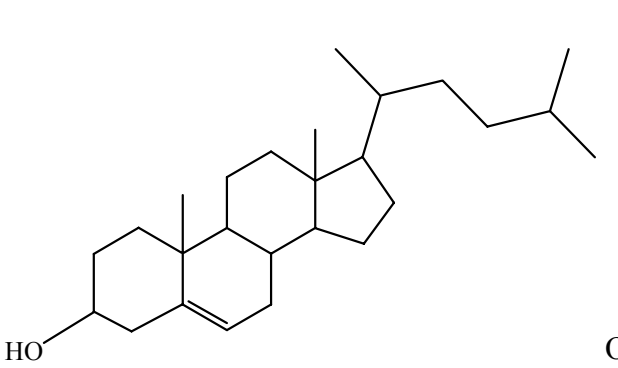
(0.25g)

RESULTS AND DISCUSSION

Literature reports of steroid derivatives with one or more atoms of the ring system replaced by nitrogen are very limited. Generally, oxidative cleavage of the cyclopentanoperhydrophenanthrene ring is needed prior to introducing the nitrogen atom. The oxidative cleavage can be achieved by a number of ethylenic bond cleavage reagents like $RuO_4/NaIO_4$, $KMnO_4/NaIO_4$, $RuO_4/NaOCl$ and ozone[10,11,12]. Windaus has achieved opening ring A by oxidizing cholest-4-en-3-one with chromic acid[10], but the isolation of the oxidation product was difficult and the yields were low. Among the various oxidative methods, ozonolysis is the most convenient, efficient and reliable method to prepare the 3-keto acid. Turner ozonolyzed cholest-4-en-3-one and developed an improved method of isolating the product[10]. Reichstein used the same method to ozonlyze progesterone[11], George ozonolyzed the testosterone acetate[13].

We start from Cholesterol, first to protect the –OH group, dissolved in pyridine, added acetic anhydride and refluxed 2hrs to get cholesteryl acetate(1) as white solid in 90% yield. Then oxidized at C-7 position by using PCC or chromium trioxide, this step gave 7-ketocholesteryl acetate(2) in 80% yield. Both steps have been developed[1,3] in Dr.Parish's lab, next step is to open the B-ring of cholesterol by ozonolysis. The ozonolysis method was limited to small scale reaction to avoid the accumulation of large amounts of the dangerous ozonide or hydroperoxide intermediate[12]. Those are hazardous

if not been completely destroyed during the reaction. Cella has devised a convenient two phase method using hydrperoxide anions as oxidant[12], using a phase-transfer catalyst to transfer the oxidant from aqueous phase to the organic phase. This procedure prevents the



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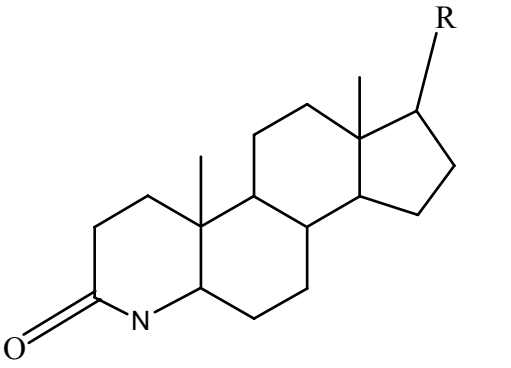

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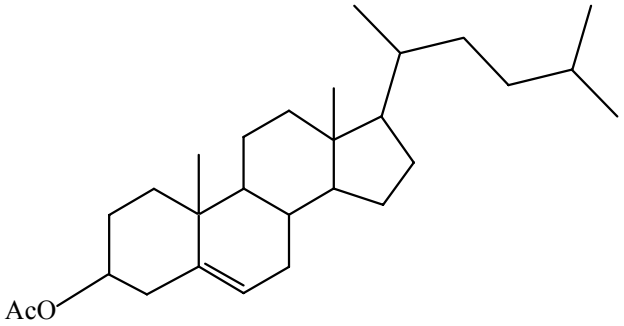

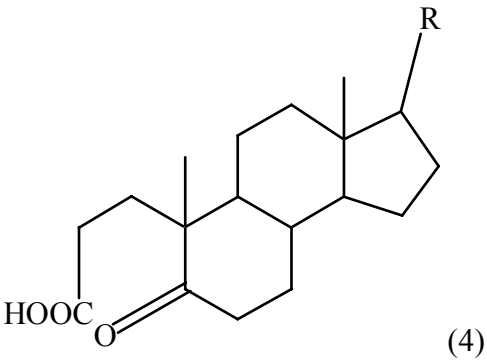

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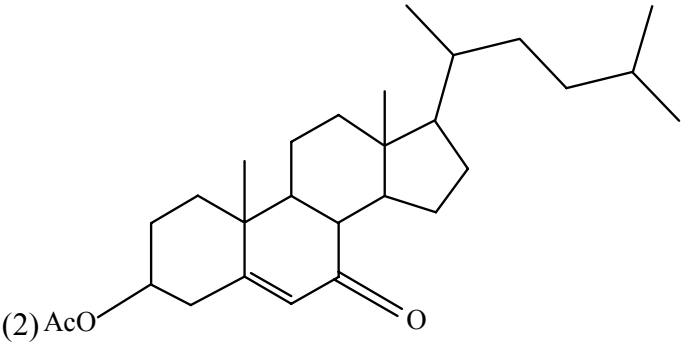

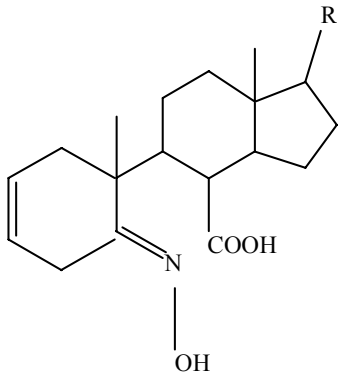

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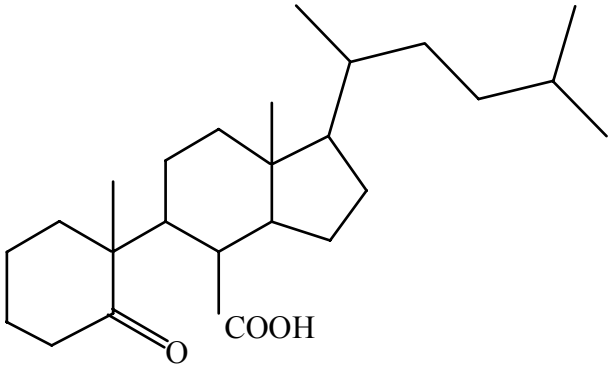

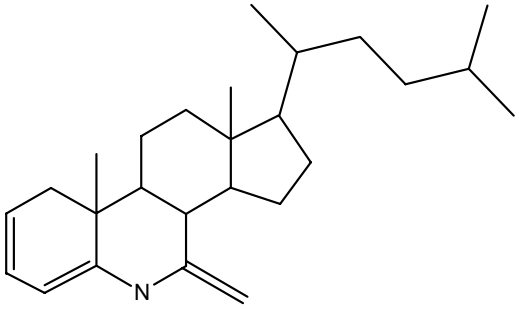

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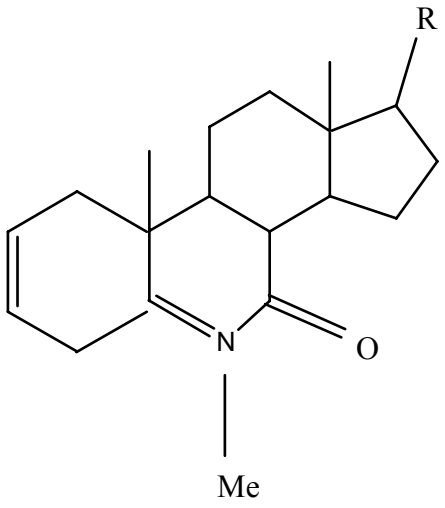
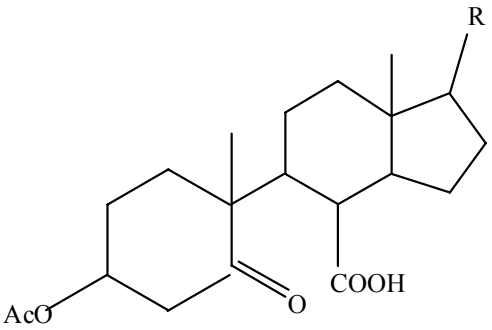




(3)

building up of ozonide and removes the keto-acid product from the organic phase during the reaction. Then, acidify the aqueous phase to get the product. However, since this procedure uses NaOH in aqueous phase, can endanger the acetate group during the reaction.

Tapan Kanti Majumdar described a similar method he used in his thesis for master's degree to open the A-ring of the cholesterol gave compound(11).

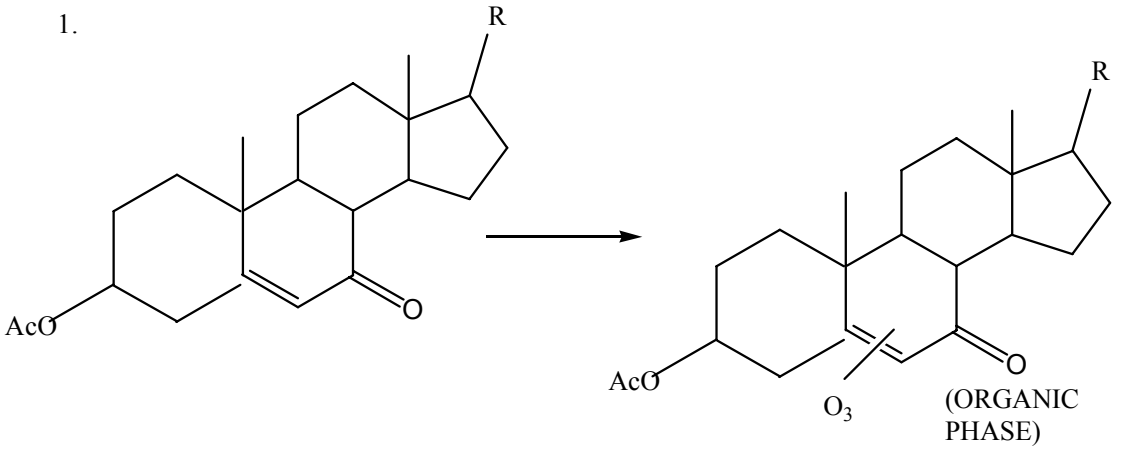
So, we used the same method to treat (2) hoping that we can get (3), we dissolved 7-ketocholesteryl acetate in chloroform., added sodium hydroxide, hydrogen peroxide, phase transfer catalyst, passed ozone at -5-0^{o}C for 6 hrs, then treated with hydrochloric

acid, we got (3) and (4) as mixture of white solid. They are difficult to separate, so we have converted the mixture into (4) by treating it with NaOH.

This reaction formed an intermediate (10), then through a number of steps gave us (3) and (4).

Possible mechanism of ozonolysis is :

1.

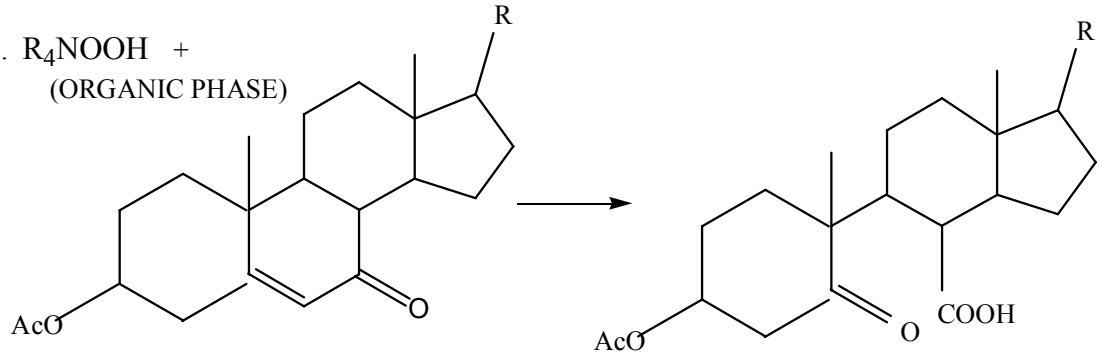


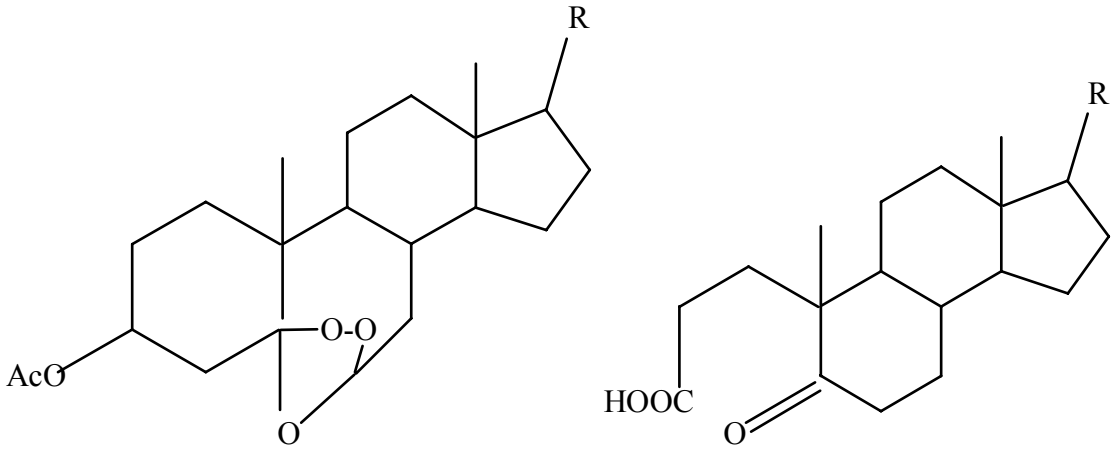
2. H_2O_2 + NaOH $\rightleftharpoons$ HOONa + H_2O (AQUEOUS PHASE)

3. HOONa + R_4NX $\rightleftharpoons$ R_4NOOH + NaX (AQUEOUS PHASE)

4. R_4NOOH (AQUEOUS) $\rightleftharpoons$ R_4NOOH (ORGANIC PHASE)

5. R_4NOOH + (ORGANIC PHASE)

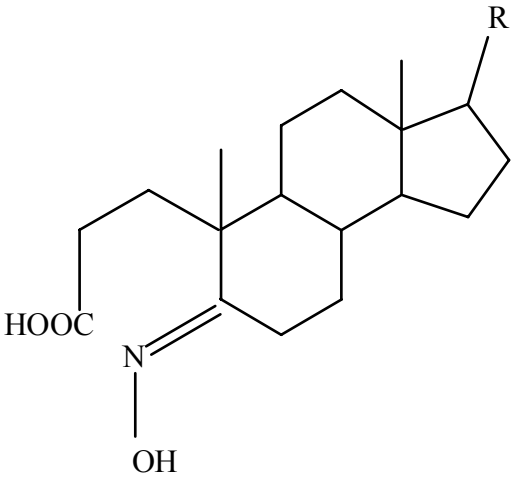




After the ring cleavage of the azasteroid was achieved, the keto acid can be used to synthesize 7-keto-6-azasteroids. Wildi reported a method for the preparation of such compounds as 4-aza-pregnen-3,20-dione[15] and 17α-acetoxy-4-aza-testen-3-one by heating the unsubstituted lactam of steroids with compounds of nitrogen such as ammonia, methylamine, n-propylamine at 200^{o}C.

Woodward[11] also prepared some substituted 4-azasteroids auch as 4-aza-4-benzyl-5-cholest-3-one by heating 5-oxo-3,5-seco-4-norcholestan-3-oic acid in excess of benzylamine at 185^{o}C. Later, Doorenbos reported the synthesis of 4-aza-5-cholesten-3-one[15,16,17], 4-aza-5-pregnen-3,20-dione, 4-aza-4-methyl-5-cholesten-3-one and 4-aza-4-methyl-5-pregnen-3,20-dione. Tapan described the reaction he used to convert (11) to

(13).



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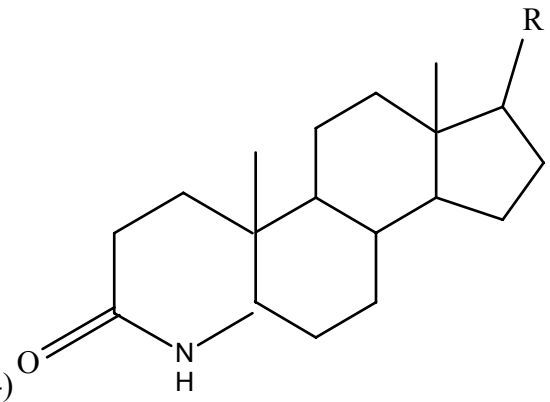
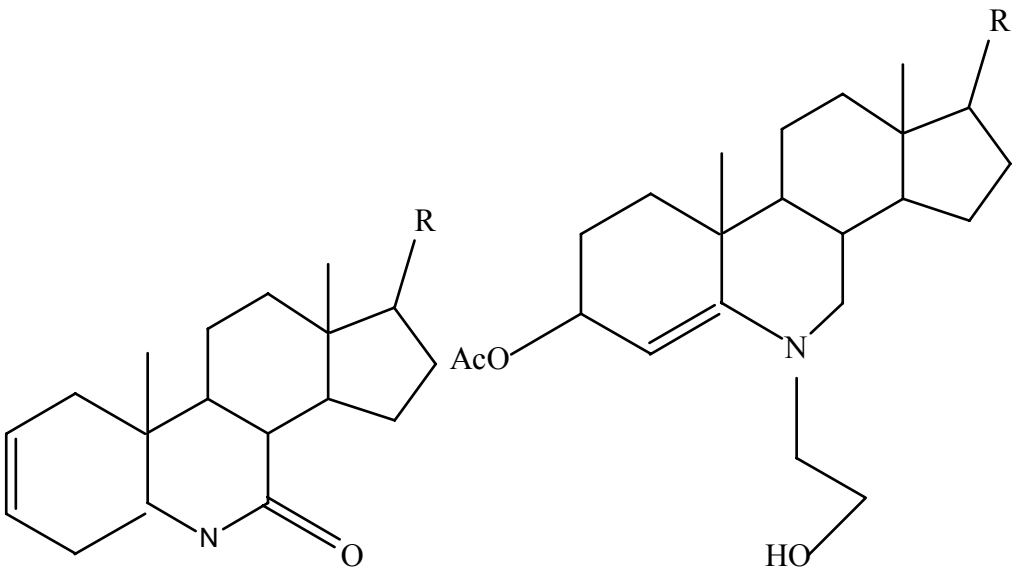
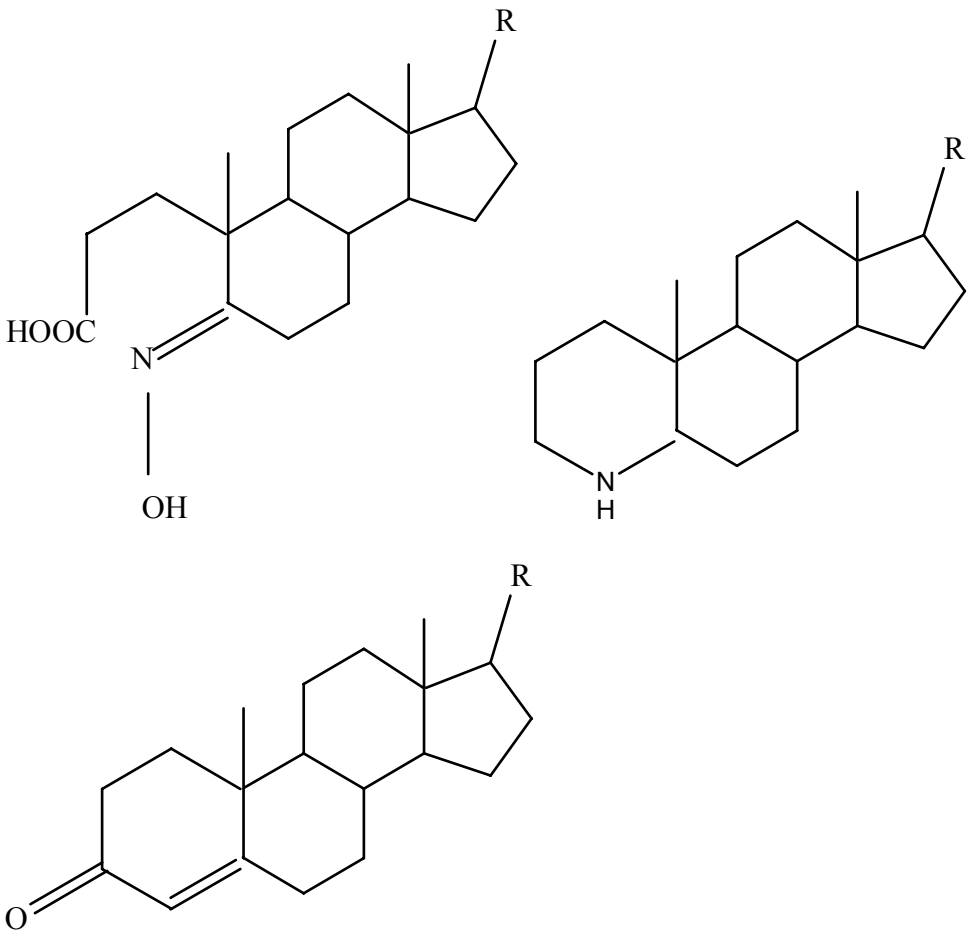


We planned to use Tapan's method to convert (4) to different compounds (5), (6). This reaction involved ammonia gas, at the reaction temperature 150^{o}C, to keep the ammonia gas we needed a reaction container that can stand heating and high pressure. We used a copper bomb which has a capacity of 22ml for this purpose. Ethanol was used to dissolve the starting material and ammonia gas was passed through it for a few minutes sealed the bomb tightly and heated in an oven at 150^{o}C for 48 hrs. The bomb was taken out cooled to room temperature and put aside for 6 hrs then put in a freezer. The excess ethanol and ammonia gas were evaporated using rotavap or nitrogen gas.



We also prepared the oxime of the keto-acid by reaction of the keto-acid with hydroxyl amine hydrochloride. This oxime can be used as intermediate to prepare other azasteroids such as (9). Shoppe described the preparation of testosterone-3-oxime, cholest-4-en-3-oxime and progesterone-3-oxime. The product of this reaction can be a mixture of syn and anti isomers in varying proportions. It is difficult to separate them completely by chromatography because of their close retention times. These isomers change from one to another in different proportions in different solvent systems.

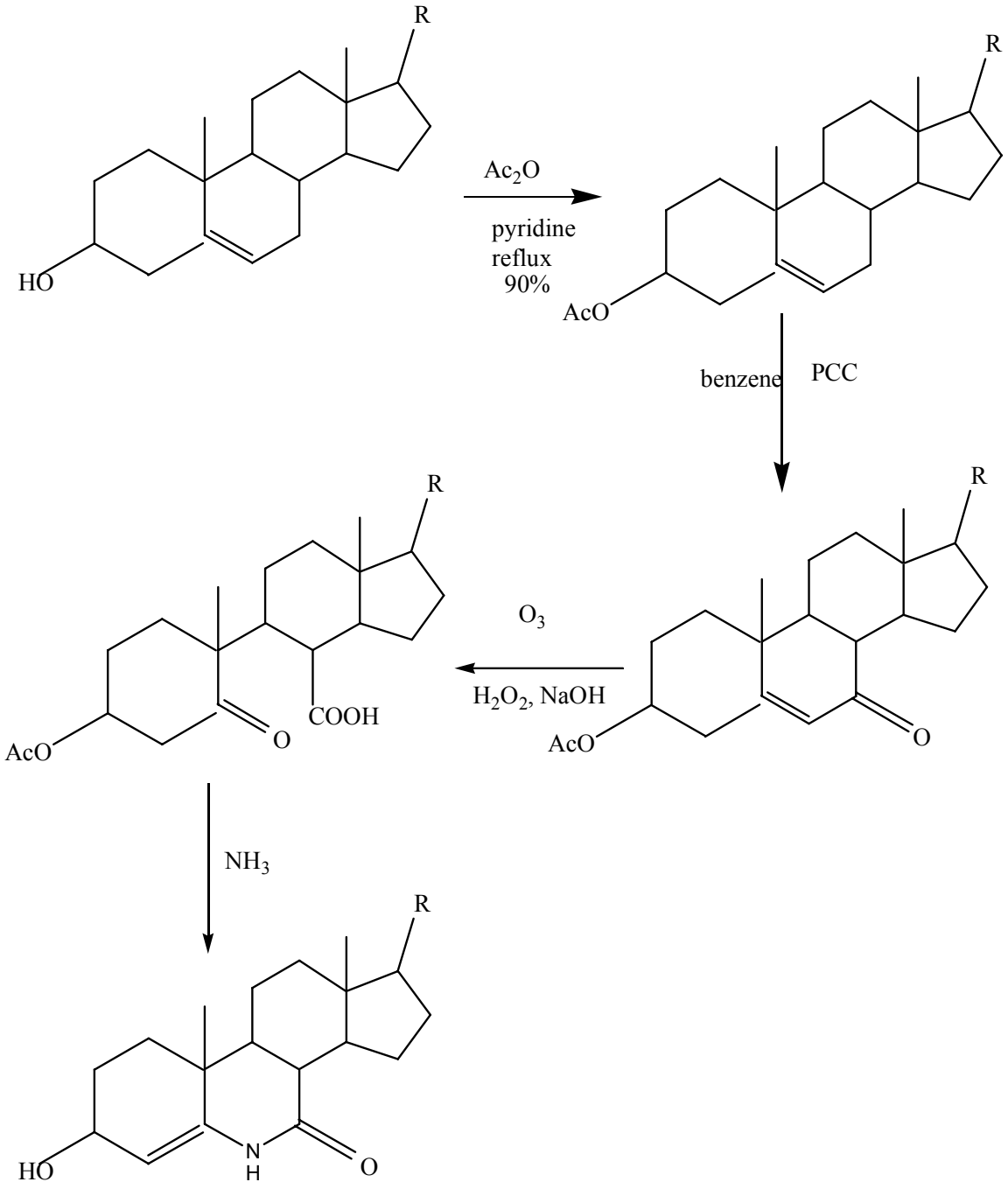


The intermediate we synthesized using the oxidizing agents potassium permanganate and sodium carbonate was showing clearly a cleavage in the B-ring. The benzoate group was deprotected to –OH group in that process. The Thin layer chromatography gave the primary information. The carboxylic acid spots on the TLC plate stayed at the origin when spotted with respect to the starting material. The solvent system we used to analyze the functional group was 1:1 toluene and ether. The reason was that the spots were clearly visible for an aprotic solvent toluene in presence of the slightly polar ether. The plate was then sprayed with molybdic acid spray and heated on a hot plate for visualizing the spots.

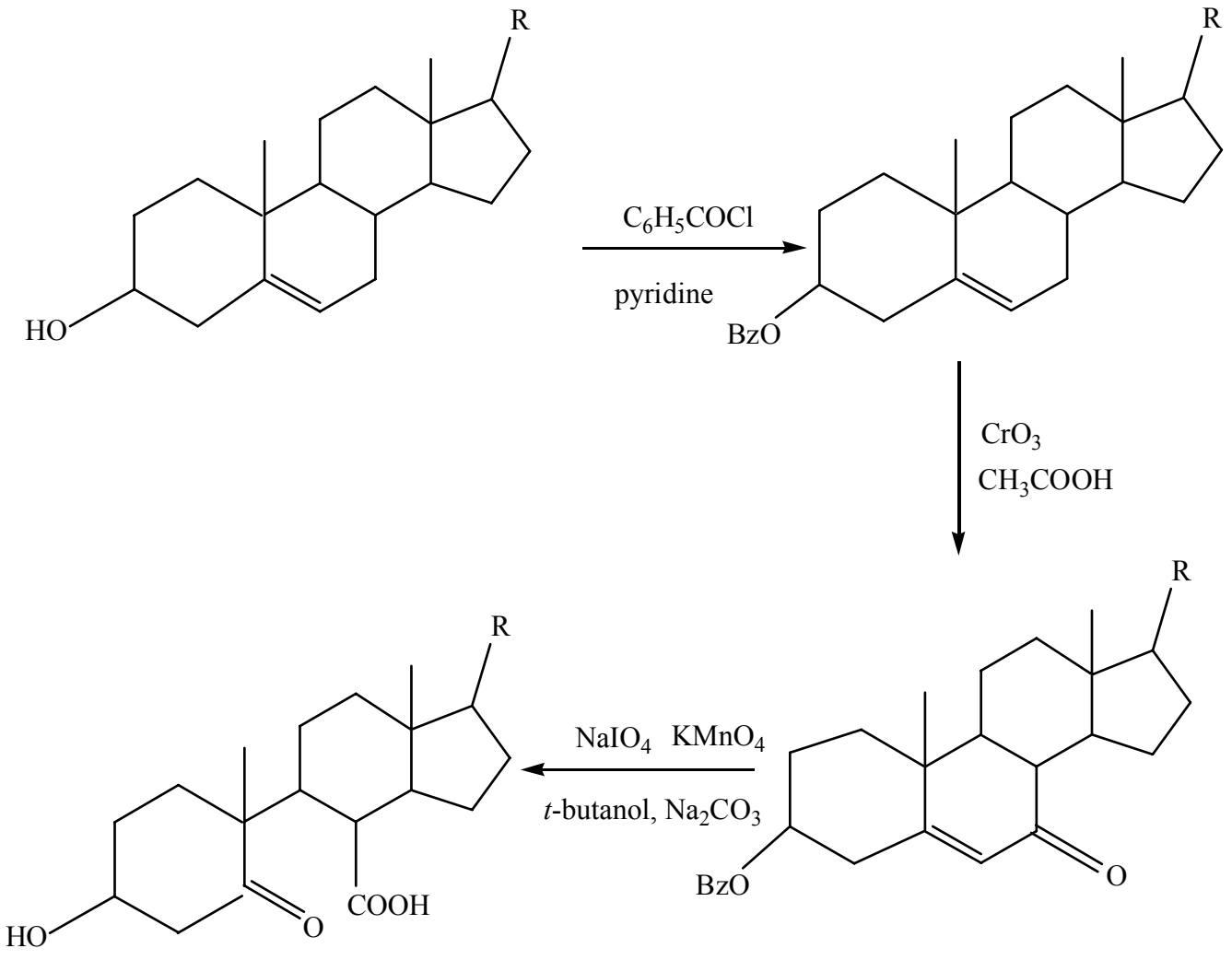
Finally it was separated using column chromatography with silics gel. The solvents were added in proportions starting with 100% toluene and then 10% ether in toluene, 20% ether in toluene upto 50% ether in toluene. The purpose of doing this was for a better separation of the side products from the desired keto-acid. The solvent was evaporated using a rotavap and the yellow colored compound was collected and analyzed using proton NMR, mass spectrometry, IR etc.

The synthesis of azasteroid was an easy process but time consuming. The keto-acid was taken in a bomb of 22ml capacity. Thanks to Dr. Thomas Albert Schmitt for providing it, the keto-acid was heated in an oven at 150^{o}C in ethanol for 2 days. Extra caution had to be taken as ethanol boils at 78^{o}C and there was high pressure building up at 150^{o}C. The reactants were cooled down to room temperature and then placed in a freezer. The excess solvent was evaporated using dry nitrogen gas or rotavap. Initially as the source of amine we wanted to use the dry methylamine an ethylamine gas (99%), but keeping in view of the cost and ease, we used the solutions of methylamine and ethylamine dissolved in ethanol and methanol respectively. The final desired azasteroid was determined using the primary information we obtained from TLC plate again and the spots were staying at the origin similar to the keto-acid. For confirmation, 2-3 drops of triethylamine was added to the TLC tank which had 1:1 toluene and ether, and the spots moved up in the presence of triethyamine. The collected azasteroid was purified using column chromatography. The solvents added were toluene with increasing polarity in presence of ether and triethylamine. Order of adding the solvent was about 200ml of 100% toluene to remove the side products and unreacted starting material, later followed by addition of 2% triethyamine and 10% ether upto 50% ether in toluene. The azasteroid

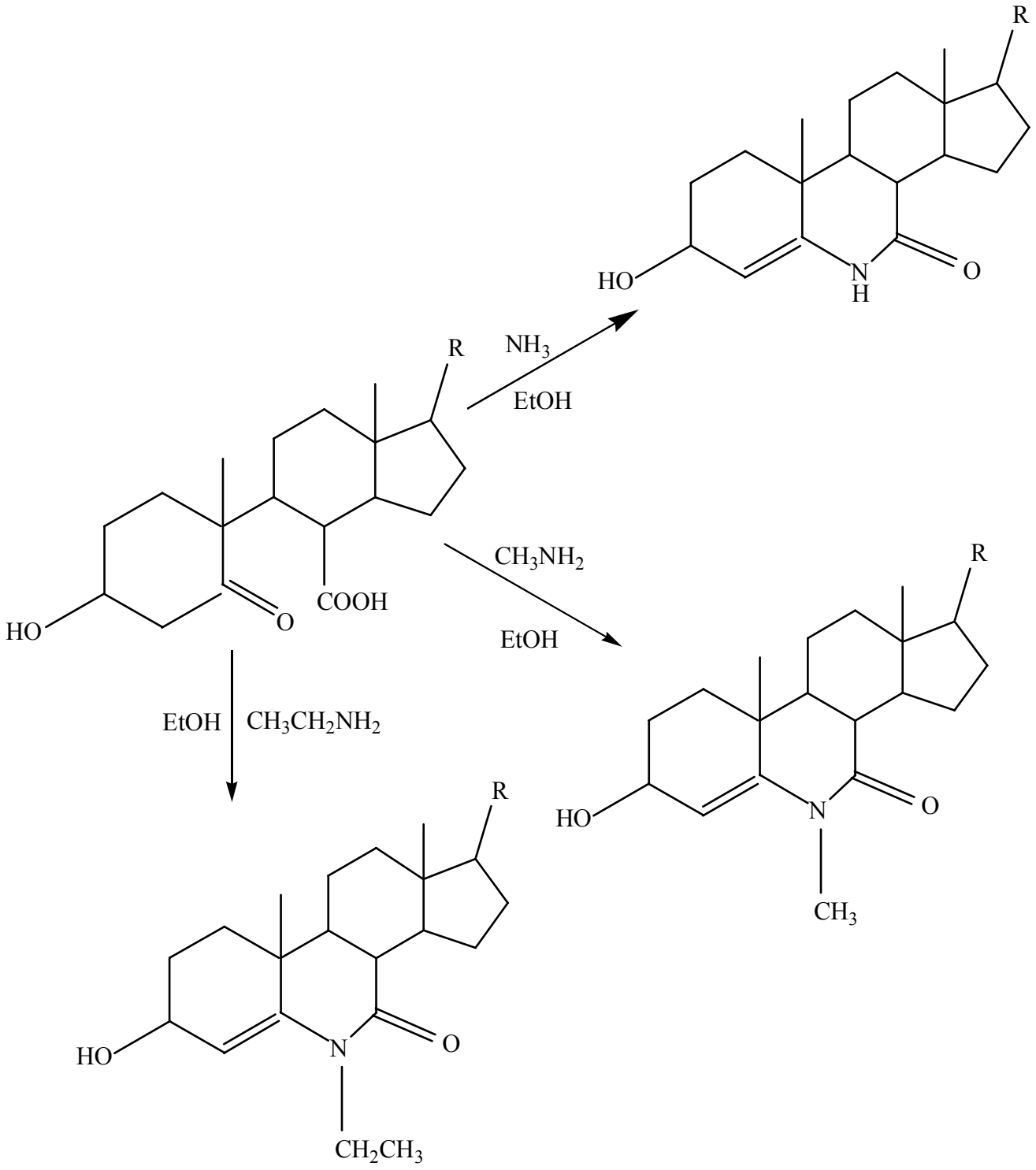
was determined using the TLC plate. The tubes were collected, and solvent was evaporated using rotavap to give the orange- red colored azasteroid.



SCHEME 6


R
HO
C6H5COCl
pyridine
R
BzO
CrO3
CH3COOH
R
BzO
O
NaIO4 KMnO4
t-butanol, Na2CO3
R
HO
O
COOH

SCHEME 7


R
HO
N
H
O
NH3
EtOH
R
HO
COOH
O
CH3NH2
EtOH
R
HO
N
O
CH3
EtOH
CH3CH2NH2
R
HO
N
O
CH2CH3

SCHEME 8

REFERENCES

1. Parish, E.J; Chitrakorn,S; and Todd, K.L; Oppi Briefs, Vol 17,3 (1985)
2. Majumdar, T.K; Thesis for masters degree, Auburn, AL.(1986)
3. Parish, E.J; Wei, T.Y and Livant, P; Lipids Vol 22, 760, 10 (1987)
4. Doorenbos N.J; Huang, C.L; Journal of Organic Chemistry, 26, 4106 (1961)
5. Mangan, F.R; Mainwaring, W.I.P; Steroids, 20, 3, 331.
6. Shroff, A.P; Harper,C.H; Allen, G.O; J.Med.Chem,16, 2, 113.
7. Karkkain, J; Ohisalo, J.J and Lukkain, T, Steroids, 12,5,511.
8. Feldman, D; Tokes, L.G; Stathis, P.A; Miller,S.C; Proc.Natl.Acad.Sci, USA, 81,15,4722(1984)
9. Chesnut, R.W; Durham, N.N; Brown, R.A; Mawdsley, E.A; Berlin, K.D; Steroids,27,4,525(1976)
10. Bolt,C.C; Rec. Trav.Chem, 940(1951)
11. Reichtein, T; Fuchs,G; Helv.Chim.Acta, 23,676(1940)
12. Doorenbos, N.J; Wu, M; Steroids, 212, 1965
13. Doorenbos, N.J; Huang, C.L; Tamorria, C.R; Wu, M.T; J.Org.Chem,26,4548(1961)
14. Doorenbos, N.J; Tamorria, C.R; J.Pharm.Sci, 53, 1473(1965)
15. Shoppe C.W, Kruger,G; J.Am.Chem.Soc, 3641(1962)
16. Shoppe C.W, Kruger,G; Mirrington,R.N; J.Am.Chem.Soc,1055(1962

17. Shoppe, C.W; Ruth,E.L; Newman, B.C; J. Am.Chem.Soc,3388(1964)

18. Hara,S; Oka,K Ike, Y; Chem.Ind, 832(1967)

19. Oka, K; Hara,S; Chem.Ind, 911(1968)

20. Brooks, C.J.W; Smith, A.G; J.Chromatography, 112, 499(1975)

21. Piatak, D.M; Dorfman, R.I; Tibbetts,D; Caspi, E; J.Med.Chem

22. Chorvat, R.J, Pappo, R. J.Org.Chem, 17,2864(1976)

23. Morgan, L.R, Chem.Ind, 293,1963

24. Sluyter,M.A.T; Pandit, U.K, Tetrahedron Letters, 87(1966)

25. Torgov, I.V; Akademial Kiad, 235, (1965)

26. Meyers, A.I; Munoz, G.G; Sobotka, W; Baburao, K; Tetrahedron Letters, 255(1965)

27. Kessar, S.V; Singh, I; Kumar, A, Tetrahedron Letters, 2207(1965)

28. Schleigh, W.R; Catala, A; Popp, F.D; J.H.cyclic.Chem, 379(1965)

29. Jones, E.R.H; British Patent, Chem Abstr, 64, 142(1966)

30. Bachmann, W.E; Ramirez, F; J.Am.Chem.Soc, 72,2527(1950)

31. Bolt, C.C; Rec.Trav.Chim,57,905(1938)

32. Mckenna,J; Tulley, A. J.Am.Chem.Soc; 945(1960)

33. Doorenbos N.J; Huang, C.L; Abstract, 135th National ACS Meeting, 30, 1959.

34. Wildi, R.S. U.S.Patent, 2,897,202(1959)

35. Organon, N.V; Dutch,P; Chem.Zentr, 2829(1940)

36. Edward ; Morand; Canada.J.Chem, (1960)

37. Turner, J.Am.Chem.Soc, 72,583(1950)

38. Rasmusson, G.H; Reynolds, G.F; J.Med.Chem, 27,1690(1984)

39. Gumulka, M; Can.J.Chem, 63, 766(1985)