SYNTHESIS, CHARACTERIZATION AND BIOCIDAL ACTIVITIES OF THE DERIVATIVES OF STEROIDS AND PENTACYCLIC TRITERPENOIDS

A Thesis submitted to the University of North Bengal

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Doctor of Philosophy (Ph.D.) in Chemistry

By

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February, 2015

To my parents...

DECLARATION

I declare that the thesis entitled "SYNTHESIS, CHARACTERIZATION AND BIOCIDAL ACTIVITIES OF THE DERIVATIVES OF STEROIDS AND PENTACYCLIC TRITERPENOIDS" has been prepared by me under the guidance of Dr. Pranab Ghosh, Associate Professor, Department of Chemistry, University of North Bengal, Darjeeling-734013. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

This is to certify that Smt. Antara Sarkar has prepared the thesis entitled "**SYNTHESIS, CHARACTERIZATION AND BIOCIDAL ACTIVITIES OF THE DERIVATIVES OF STEROIDS AND PENTACYCLIC TRITERPENOIDS**" for the award of Ph.D degree of the **University of North Bengal,** under my guidance. She has carried out the work at the Department of Chemistry, University of North Bengal, Darjeeling, West Bengal- 734013.

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Abstract

Chapter I: A general introduction to steroids and pentacyclic triterpenoids

Natural products such as steroids, terpenoids, alkaloids, flavonoids play a major role in drug discovery, and nearly half of all newly introduced drugs into the market in the past two decades are natural products or their direct derivatives. Steroids are able to penetrate cells and bind to nuclear and membrane receptors. To perform some of the fundamental biological functions, the steroid system is naturally selected and this is not only the basis of new discoveries in this field but is also the source of interests of biochemists and endocrinologists. Sterols, oxysterols, secosteroids, bile acids, hormones and saponins are some of the important steroids.

On the other hand, pentacyclic triterpenoids (PTs), the secondary metabolites, are widely distributed in plants and are traditionally used as medicines. A number of natural PTs are potent and unique biologically active molecules. In order to exploit the therapeutic potential of natural PTs and/ or their derivatives, intense pharmacological and mechanistic studies have been carried out. Anti-HIV, Antitumor, antiviral, anti-inflammatory, antidiabetic, antiparasitic, antimicrobial, cardio-hepato- and gastro-protective, analgesic and wound-healing effects are included in these bioactivities. Several PTs are now being marketed as therapeutic agents, and a couple of natural/ synthetic PT derivatives are now under clinical trials. Friedelane, lupane, ursane, oleanane, serratane are some of the important groups of the PT series.

Thus, in this chapter, a general description of the steroid and PT groups of natural products, giving emphasis on their fundamental skeletons, classifications etc. along with their immense importance are covered in brief.

Chapter II: Transformative reactions of steroids using mercuric(II) acetate and *N*-bromosuccinimide

Section A: Action of mercuric(II) acetate on 16-Dehydropregnenolone acetate (16-DPA)

The versatile oxidizing capacity of mercuric acetate led the investigation of its action on 16-dehydropregnenolone acetate (16-DPA), an important steroid moiety. When 16-DPA was treated with mercuric(II) acetate, a cyclic ether derivative was formed. The cyclic ether was actually introduced onto the ring-D of 16-DPA, involving the carbonyl oxygen and the double bond in its α,β-position. Hence, a single-step conversion of exocyclic α,β-conjugated

ketone functionality of a steroid resulted a ring-D-fused four-membered cyclic ether which preserves the main significance of the study.

Section B: Action of *N*-bromosuccinimide (NBS) on cholesterol and β-sitosterol: synthesis of A-ring aromatized steroids in solid phase

A thorough review consequences the importance given towards the selective aromatization of steroids and thus working on the subject produced finally a novel synthesis of A-ring aromatized cholesterol and β-sitosterol *via* a very simple one-pot solid phase reaction using *N*-bromossuccinimide (NBS). Cholesterol was reacted with NBS on solid support to result 1-methyl-19-norcholesta-1,3,5(10)-triene.

The reaction was attempted both on solid support as well as in solution phase. However no aromatization was observed in solution phase.

The reaction condition was optimized thoroughly giving emphasis on reaction temperature, solid phase (along with its amount) and recyclability of the catalyst.

Towards the probable mechanistic investigation it was found that, to have the aromatized product, C-3 containing groups donot act as leaving groups and both the C-4 hydrogens are required.

Applying the same reaction protocol, corresponding aromatized β-sitosterol analogue, 1-methyl-19-norstigmasta-1,3,5(10)-triene was prepared and 4β-hydroxy cholesterol produced 3-keto-4β,5α,6β-trihydroxy derivative.

Chapter III: *p*-TsOH-Mediated green transformations of di- and trihydroxy steroids toward diverse A/B-ring oxo-functionalization and evaluation of their cytotoxicity

Considering the significant usefulness of the ketosteroids, on the perspective of different chemical synthetic approaches and anticipated biological activities (after having a thorough review), the author became interested to undertake the above work. Thus, the present work was designed to utilize some oxysteroid biomolecules as the starting materials such as 4β-hydroxy- and 4β,7α-dihydroxy steroids. 4β-Hydroxy cholesterol is the most abundant cholesterol metabolite in human circulation and the degradation of cholesterol into bile acids involves 7α-hydroxylation as the rate determining step. As a consequence, 4β,7α-dihydroxy cholesterol owes prime attention in the metabolic research. The corresponding analogues of β-sitosterol, the major phytosterol available in nature, were also used as the starting materials. Moreover, considering the present day requirement of green chemistry applications, the

author tried to attempt the transformations on solvent-free solid supports and to our fortune, the results were satisfactory.

At a glance, the present work describes the solid support/*p*-TsOH-mediated oxidative transformations of 4β-hydroxy- and 4β,7α-dihydroxy derivatives into the corresponding oxo-functionalized steroids. Many of the oxo-steroids or keto steroids, furnished in the reactions, were interestingly isomeric through the involvement of the ring-A and -B of the steroid skeleton.

When 4β-hydroxy cholesterol was heated with *p*-TsOH on activated silica, three products isolated were analyzed as cholest-4-en-3-one, cholest-4-ene-3,6-dione and 5α-cholestane-3,6-dione.

The reaction protocol was optimized in detail, generalized through the application on similar different substrates, and efforts toward the mechanistic understanding were also evaluated.

The reaction was generalized by application of the same protocol on 4β-hydroxy β-sitosterol, 4β,7α-dihydroxy cholesterol and 4β,7α-dihydroxy β-sitosterol. 4β-Hydroxy β-sitosterol furnished three products, as was expected- stigmast-4-en-3-one, stigmast-4-en-3,6-dione and 5α-stigmastane-3,6-dione whereas 4β,7α-dihydroxy steroids resulted the corresponding steroidal- 4-ene-6-one; 3,5-dien-7-one; 4,6-dien-7-one; and 4,7-dione.

The anti-proliferative effect of the substrates and product molecules was estimated on protozoan parasite *Leishmania donovani* and the results are discussed. The most effective compound was found to be cholesta-3,5-dien-7-one which inhibited the *L. donovani* AG83 (MHOM/IN/83/AG83) promastigotes by 54% ($P<0.003$).

Chapter IV: Synthesis of A-ring modified friedelane triterpenoids and evaluation of their phytotoxicity

A-ring modified natural and synthetic friedelane triterpenoids along their bioactivities are thoroughly reviewed which, along with the consideration of the accelerating and versatile effect of $BF_3.OEt_2$/ Ac_2O led us finally to result a key-transformative reaction of friedelin to yield friedel-2-ene, friedel-3-enol acetate (the major product) and 2-keto-friedel-3-enol acetate.

Experiments showed $BF_3.OEt_2$ to be the only commonly available Lewis acid which performed the transformation and other common acetylating/ benzoylating agents instead of acetic anhydride were found ineffective.

A probable mechanism was postulated for the formation of the enol acetate and the same reaction protocol was applied on friedelin oxime and 3β-hydroxyfriedelane.

Then, a number of common reaction strategies, e.g., oxidation, reduction, acetylation, oximination, etc., were employed to achieve a library of C-2, C3- and C4-functionalised friedelin/ cerin derivatives. The derivatives were then biologically evaluated as their anticipated phytotoxicity taking *Oriza sativa* as the targeted entity. The sets of the isomeric compounds were very much helpful too to analyze the structure activity relationship studies (SAR) during the evaluation of the phytotoxic effect of the compounds. The phytotoxicity studies revealed the potential activity of the synthesized derivatives in comparison to the starting natural products which, indeed, imply presumably, the scope of the compounds to possess enhanced useful cytotoxicity and to use as agrochemicals.

Preface

The thesis is the compilation of the research work carried out by the author under the supervision of Dr. Pranab Ghosh in the Department of Chemistry, University of North Bengal during the period 2008 to 2015. It comprises the synthesis, characterization and biocidal activities of the derivatives of steroids and pentacyclic triterpenoids.

Natural products play a major role in drug discovery, and nearly half of all newly introduced drugs into the market in the past two decades are natural products or their direct derivatives. And among these natural products, steroids and triterpenoids play an important role in the field of medicinal chemistry. These act both as the raw materials as well as the inspiration for designing most lead discoveries in pharmaceuticals and for drug development. So, there lies the importance of the natural products and research in this field for the improvement of mankind. Therefore, through our research work we have tried to enrich the area of natural products as well as to synthesize and modify some nature-derived organic compounds for future prospectus in medicinal chemistry.

I feel it an imperative to express my sincere gratitude to my supervisor, Dr. Pranab Ghosh, Associate Professor, Department of Chemistry, University of North Bengal, West Bengal for giving me the opportunity to carry out my research work with all the help and advice. His encouragement and guidance has played a pivotal role in providing a good basis for the present thesis.

I would like to thank all the faculty members of the Department of Chemistry, NBU whose valuable suggestions helped me a lot during my research. Special thanks to Dr. Asis Kumar Nanda and Dr. Sajal Das for their help in NMR and Prof. Parag Sinchan Roy for his guidance to carry out FTIR.

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I would also like to thank my father for keeping faith on me, my mother for making me capable of being in this stage of my life, my elder sister and younger brother for being with me whenever I need them, my husband Jayanta and my in-laws for supporting me to complete this work, although I was hardly capable to spent sufficient time with them. I hereby record my appreciation for them, last but not the least.

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Appendix A

Research papers published/ communicated/ under preparation:

1. Mercuric(II) acetate oxidation of steroidal exocyclic α,β-unsaturated ketone: transformation into a cyclic ether. Pranab Ghosh, **Antara Sarkar**, Jayanta Das, *Molbank,* **2009**, *2009(3)*, M616.
2. One-pot solid phase selective aromatization of cholesterol using *N*-bromosuccinimide: an optimized green methodology. Pranab Ghosh, Jayanta Das, **Antara Sarkar**, *Green Chem. Lett. Rev.,* **2012**, *2(5),* 173-177.
3. Oxidation with selenium dioxide: the first report of solvent selective steroidal aromatization, efficient access to 4β,7α-dihydroxy steroids, and syntheses of natural diaromatic ergosterols. Pranab Ghosh, Jayanta Das, **Antara Sarkar**, Seik Weng Ng, Edward R.T. Tiekink, *Tetrahedron*, **2012**, *68(32),* 6485-6491.
4. Solid phase selective oxidation of β-sitosterol and 4β-hydroxy cholesterol by *N*-bromosuccinimide-a green approach. Pranab Ghosh, **Antara Sarkar**, Jayanta Das, *J. Ind. Chem. Soc.* **2014**, *91(12)*, 2255-2257.
5. *p*-TsOH-Mediated green transformations of di- and trihydroxy steroids toward diverse A/B-ring oxo-functionalization. (Manuscript under preparation)
6. $BF_3.OEt_2$-Mediated oxidations toward phytotoxic A-ring modified friedelane triterpenoids. (Manuscript under preparation)

Appendix B

List of research papers presented in National/International Seminar/ Conference/ Workshop.

1. Microwave-assisted one-pot solid phase aromatization of ring-A of cholesterol and its antimicrobial properties (Poster presentation). Jayanta Das, Antara Sarkar and Pranab Ghosh. 12th CRSI National Symposium in Chemistry. Feb. 4-7, 2010, IICT & NIPER, Hyderabad.
2. Concerted oxidation and reduction of 4β–hydroxy steroids (Poster presentation). Jayanta Das, Antara Sarkar and Pranab Ghosh. National Seminar on Frontiers in Chemistry 2011 & Celebration of the International Year of Chemistry 2011. Mar. 14-16, 2011. Department of Chemistry, University of North Bengal.
3. 4β, 7α-Dihydroxy steroids: easy efficient synthesis, and *p*-toluenesulfonic acid induced 'on-silica' rearrangement (Poster presentation). Antara Sarkar, Jayanta Das and Pranab Ghosh. CRSI Eastern Zonal Meeting, 2011 & Celebration of the International Year of Chemistry 2011. Jul. 22-24, 2011. Department of Chemistry, University of North Bengal.
4. Friedelin and its chemical modification: scope and biological evaluation (Oral presentation). Antara Sarkar and Pranab Ghosh. UGC Sponsored National Seminar on Frontier of Chemistry. Nov.15-16, 2011, Department of Chemistry, Gour Mahavidyalaya, Malda.
5. Organic Synthesis on Solid Support: a Green Concept (Oral presentation). Antara Sarkar and Pranab Ghosh. UGC Sponsored Seminar on Green Chemistry for Environmentally Benign Chemical Synthesis. Feb. 14-15, 2012, Department of Chemistry, Raiganj Surendranath Mahavidyalaya & Department of Chemistry, University of North Bengal.
6. A-ring modified bioactive friedelane triterpenoids *via* $BF_3.OEt_2$-mediated oxidative transformation (Poster presentation). Antara Sarkar, Jayanta Das and Pranab Ghosh. 16th CRSI National Symposium in Chemistry (NSC-16). Feb. 7-9, 2014, IIT Bombay, Powai, Mumbai.
7. A-ring modified friedelane triterpenoids: Synthesis and Phytotoxicity (Oral presentation). Antara Sarkar and Pranab Ghosh. National Seminar on Frontiers in Chemistry-2014, March 11-12, 2014, Department of Chemistry, University of North Bengal.

Appendix C

Abbreviations:

AcOH: acetic acid

ADP: adenosine diphosphate

ATP: adenosine triphosphate

BA: betulinic acid

Calcd: calculated

$CDCl_3$: deuteriated chloroform

DCM: dichloromethane

DEPT: Distortionless enhancement by polarization transfer

deriv: derivative

dil: dilute

DMF: dimethyl formamide

DMSO: dimethyl sulphoxide

16-DPA: 16-dehydro pregnenolone acetate

FTIR: Fourier transformed infra-red

h: hours

hydrol: hydrolysis

*m*CPBA: *m*-chloroperbenzoic acid

min: minute

m.p: melting point

NBS: *N*-bromosuccinimide

NMR: nuclear magnetic resonance

OA: oleanolic acid

Pd-C: palladised-charcoal

Pet. ether: petroleum ether

p-TsOH: *p*-tolunesulphonic acid

PT: pentacyclic triterpenoid

RT: room temperature.

TLC: thin layer chromatography

TMS: trimethyl silanne

UA: ursolic acid

UV: ultraviolet

Chapter I

A general introduction to steroids and pentacyclic triterpenoids

I.1 Introduction: Natural products

Natural products are the compounds originated from nature. Plant kingdom or animals are the major sources of these natural products. Steroids, terpenoids, alkaloids, flavonoids are different types of natural products. Natural products play a major role in drug discovery, and nearly half of all newly introduced drugs into the market in the past two decades are natural products or their direct derivatives.[1-2] Among all the natural products, the steroids and terpenoids, specifically pentacyclic triterpenoids, both of which are derived from squalene by various cyclisation or other changes[3] are being concerned here.

I.2 Steroids

Steroids are an important class of natural products. Marine plants and animals are the richest source of steroids displaying interesting biocidal activities.[4] The structure of the steroids are based on the 1,2-cyclopentenophenanthrene (**1**) skeleton with 17 carbon atoms (**Figure 1**).

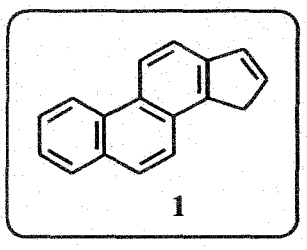

Figure 1. 1,2-Cyclopentenophenanthrene.

Sterols, vitamin D, the bile acids, a number of sex hormones, the adrenal cortex hormones, some carcenogenic hydrocarbons, certain saponins, antibiotics, etc are included in the steroids. Steroids are able to penetrate cells and bind to nuclear and membrane receptors. To perform some of the fundamental biological functions the steroid system is naturally selected and this is not only the basis of new discoveries in this field but is also the source of interests of biochemists and endocrinologists. Some of the very important class of steroids will be discussed herein in this chapter.

I.2.1 Sterols and oxysterols

Sterols occur in animal and plant oils and fats. Sterols contain at least one alcohol group e. g.; cholesterol (**2a**), β-sitosterol (**2b**), ergosterol (**3**) etc. (**Figure 2**). The most abundant sterol in

human and animal tissues is cholesterol, as the formation and function of cellular membranes are not possible without it. It plays a vital role to maintain the overall integrity of eukaryotic cells by giving rise to the requisite structure, fluidity, and organization of the membrane.[5] It was biosynthesized in multistep from lanosterol though none of the intermediate sterols was able to replicate cholesterol's function to order and condense lipid membranes.[6-9]

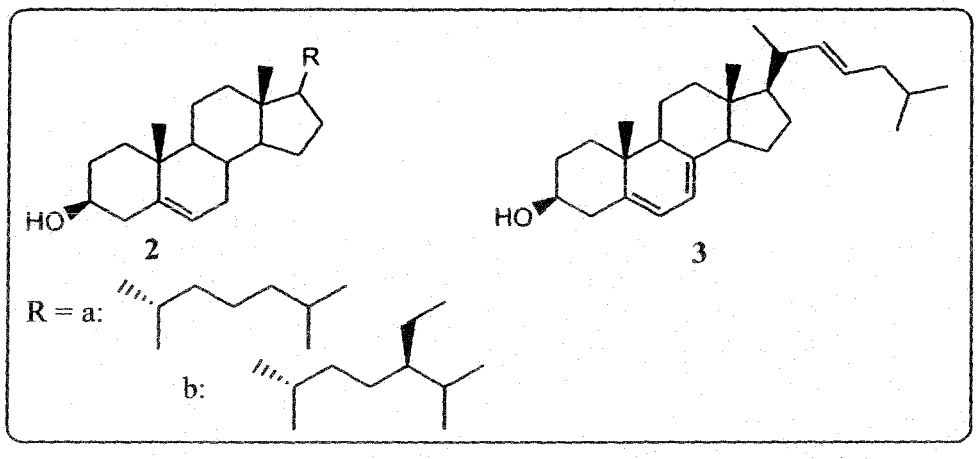

Figure 2. Cholesterol (**2a**), β-sitosterol (**2b**) and ergosterol (**3**).

On the other hand, oxysterols, identified in animal and human tissues,[10-11] are nothing but the partial oxidized products of respective sterols. Oxy-cholesterols occur both during the *in vivo* conversion of cholesterol to bile acids and hormones, and also *in vitro*, when cholesterol is exposed to heat, air, light and oxidizing agent.[11] These oxysterols are biologically very active and effective on cholesterol homeostasis by acting as ligands for certain nuclear receptors,[12] platelet aggregation, sphingolipid metabolism and cytotoxicity.[13] Compare to pure cholesterol, oxysterols are found to be more atherogenic when the experiments were done on animals.[14-16] But when the experiments were carried out on human, due to the toxic effect of oxysterols to arterial endothelium and smooth muscle cells, oxysterols were implicated in the initiation and progression of atherosclerosis.[17-19] Many naturally occuring polyhydroxylated sterols and oxysterols are found to have potent biociadal activities.[11,13,20] Oxysterols produce immunosuppression,[21] enhance colon carcinogenesis,[22] and also promote tissue inflammation and necrosis.[23] Some of them are potent cytotoxic to cacer cells[24-27] and are found in folk

medicines used in cancer treatment.[28-30] Some examples of oxysterols are cholestenone (**4a**), stigmastenone (**4b**), 5α-stigmastane-3,6-dione (**5**), 7-keto cholesterol (**6**), etc (**Figure 3**).

Steroids having cross conjugated dienone system in ring-A are mostly found in marine organism, such as bryozoans,[31] sponge,[32] bacterium[33] and especially in soft coral.[34-39] These particular types of steroids are foud to possess antitumor,[36-37] antioxidant[39] and anti-infective[32] activities.

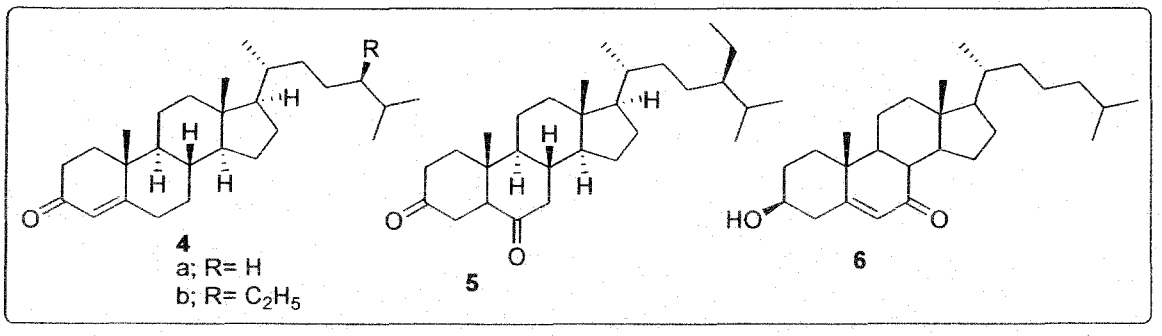

Figure 3. Cholestenone (**4a**), stigmastenone (**4b**), 5α-stigmastane-3,6-dione (**5**) and 7-ketocholesterol (**6**).

I.2.2 Secosteroids

Another type of steroid called secosteroids has attracted considerable interest due to their broad range of biological activities such as vitamins D, which is found in nature. Vitamin D has antiproliferative effects on a large variety of cancer cells. Apart from it secosteroids are found to have cytotoxicity,[40-42] antihistamine[43] and anticancer[44] activity. Some secosteroids were synthesized and their hormonal and antihormonal activity[45-50] was reported. The activities of secosteroids in humans and in animals are the matter of scientific interest and in future these may result novel, pharmaceutically useful compounds. Isogosterones-A (**7a**), Isogosterones-B (**7b**), Isogosterones-C (**7c**) Isogosterones-D (**7d**) are four secosteroids isolated from a Japanese octocoral *Dendronephthya* sp (**Figure 4**). These are found to have potent antifouling effect.[38]

I.2.3 Bile acids

The main component of bile is bile acids (BAs) which are the end product of cholesterol catabolism. Bile acids are group of acidic steroids typically composed of 24 carbons with an A/ B ring junction generally fused in the cis configuration. Bile Acids are amphipathic is nature.

Most of the bile acids are present as sodium salts and they function as emulsifying agents in the intestinal tract, e.g., fats, which are insoluble in water, are rendered soluble, and so may be absorbed in the intestine. So bile acids play a vital role in nutrient metabolism and absorption.[51-52] Most of the bile acids are hydroxy-derivatives of either 5β-cholanic acid (**8**) or 5α-cholanic acid and dehydration followed by reduction of bile acid lead to the corresponding cholanic acid.[48] Bile acids are considered to be signaling molecules for endowing genomic and nongenomic effects.[49] Some of the important natural bile acids are cholic acid (**9**), lithocholic acid (**10**), deoxycholic acid (**11**) etc. (**Figure 5**).

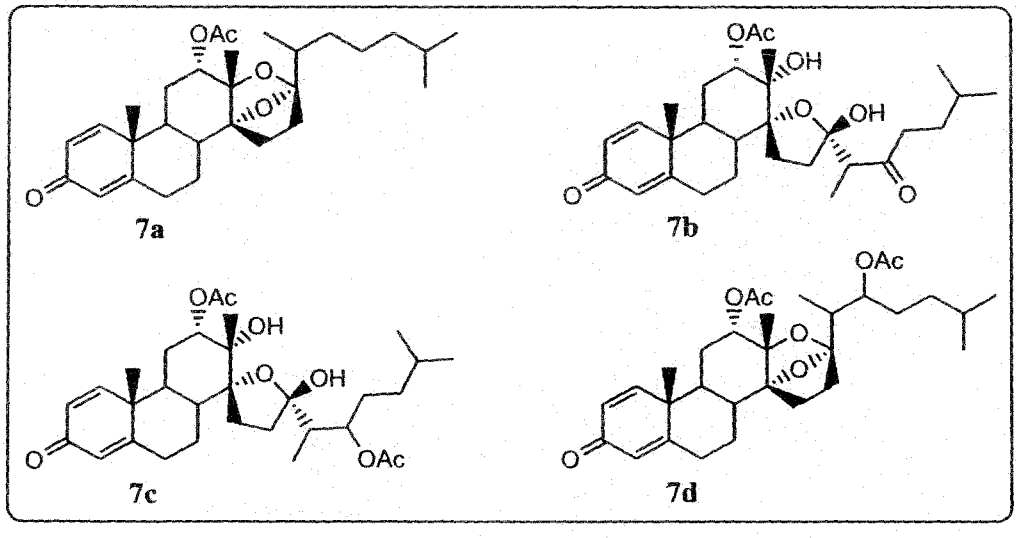

Figure 4. Isogosterones A-D (**7a-7d**).

I.2.4 Hormones

Another important class of steroids are sex hormones and these are produced in the gonads. Their activities are controlled by the hormones produced in the anterior lobe of pituitary gland. Androsterone, testosterone (**12**) of androgens group, oestrone or estrone (**13**), oestriol of oestrogens group and progesterone (**14**) of gestogens group belong to these classes of steroids (**Figure 6**).

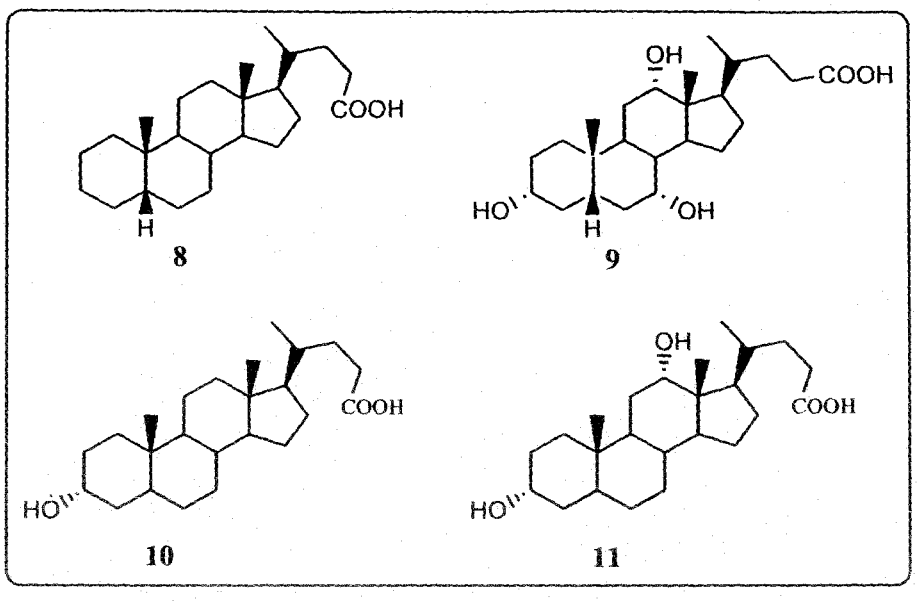

Figure 5. 5β-Cholanic acid (**8**), cholic acid (**9**), lithocolic acid (**10**) and deoxycholic acid (**11**).

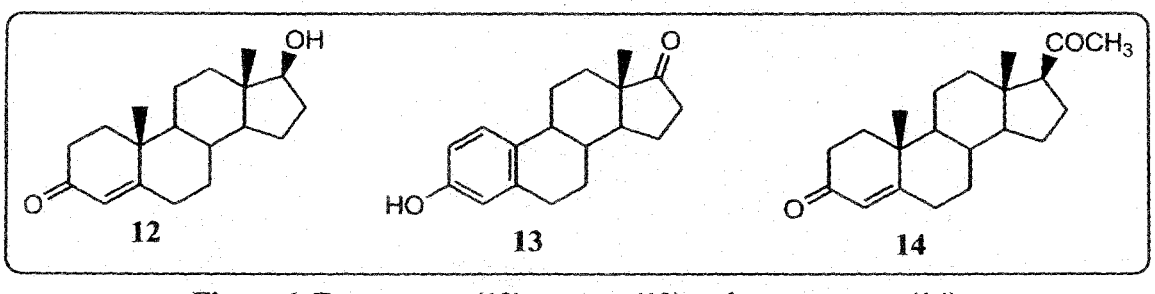

Figure 6. Testosterone (**12**), estrone (**13**) and progesterone (**14**).

One of the important sex hormones is estrogens. Estrogens are required for normal growth and development of tissue but these also help to promote certain tumors like, prostatic hyperplasia, endometrial, prostate, and especially breast cancers[53] and 30-50% of breast cancers are estrogen-dependent in their progression.[54-55] In vivo estrogen biosynthesis is carried out by aromatase which is the cytochrome P-450 enzyme complex. Aromatase converts the aliphatic androgens testosterone and androstenedione to the aromatic estrogens estradiol and estrone respectively and maintain a homeostatic balance between androgen and estrogen.[56-59]Aromatase inhibitors decrease the circulating levels of estrogens in postmanopausal women and thus control

the progression of hormonone sensitive breast cancer.[60-67] Different natural and synthetic aromatase inhibitors are reported by a number of research groups.[68-72]

I.2.5 Saponins

Saponins occur in many plants and are often associated with the cardiotonic glycosides, e.g., digitonin (**15**) has been isolated from various species of *Digitalis*. The aglycon of saponins are called sapogenins are characterized by the presence of a spiroketal side chain. The sapogenin of digitonin is digitogenin. Diosgenin is such a sapogenin which is used as the starting material for the partial synthesis of progesterone. Other examples are tigogenin, sarsasapogenin, digitogenin, diosgenin (**16**), etc. (**Figure 7**)

Saponins have a characteristic property. When intravenous injection of the aqueous solutions of saponins is carried out into animals, haemolysis occurs. These solutions are comparatively harmless if taken orally. Digitonin has a particular characteristic to form molecular complexes with various 3β-hydroxysteroids.[51]

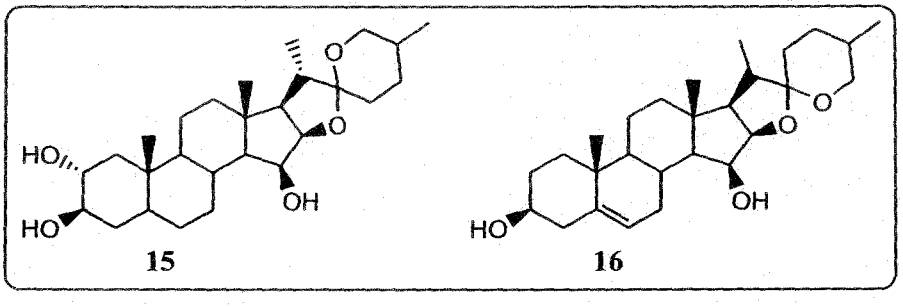

Figure 7: Digitonin (**15**) and diosgenin (**16**).

I.3 Pentacyclic triterpenoids

Pentacyclic triterpenoids, as the name reveals, are consisted of five rings together with some methyl groups in their skeleton. So, basically these are of C_{30}-isoprenoid compounds having few funtional groups in their skeleton.[73] Pentacyclic triterpenoids, the secondary metabolites are widely present in plants and are traditionally used as medicines.[74] Natural PTs are potent and unique biologically active molecules. In order to exploit the therapeutic potential of natural PTs or their derivatives, intense pharmacological and mechanistic studies have been carried out. Antitumor, antiviral, anti-inflammatory, antidiabetic, antiparasitic, antimicrobial, cardio-hepato-

and gastro-protective, analgesic and wound-healing effects are included in these bioactivities. PTs have received the most attention due to their activities as the hepato-protective, anti-HIV, antitumor and anti-inflammatory agent and it is evidenced as several PTs are now being marketed as therapeutic agents, and a couple of synthetic PT derivatives are now under clinical trials. A huge number of research works in this field indicate the increasing interest of pharmacological research into PTs.

I.3.1 Classification of pentacyclic triterpenoids

Pentacyclic triterpenoid group is by far the largest group and is divided into six broad groups, 1) friedelane (**17**), 2) lupane (**18**), 3) ursane (**19**), 4) oleanane (**20**), 5) serratane (**21**) and 6) Ψ-taraxastane (**22**) (**Figure 8**).

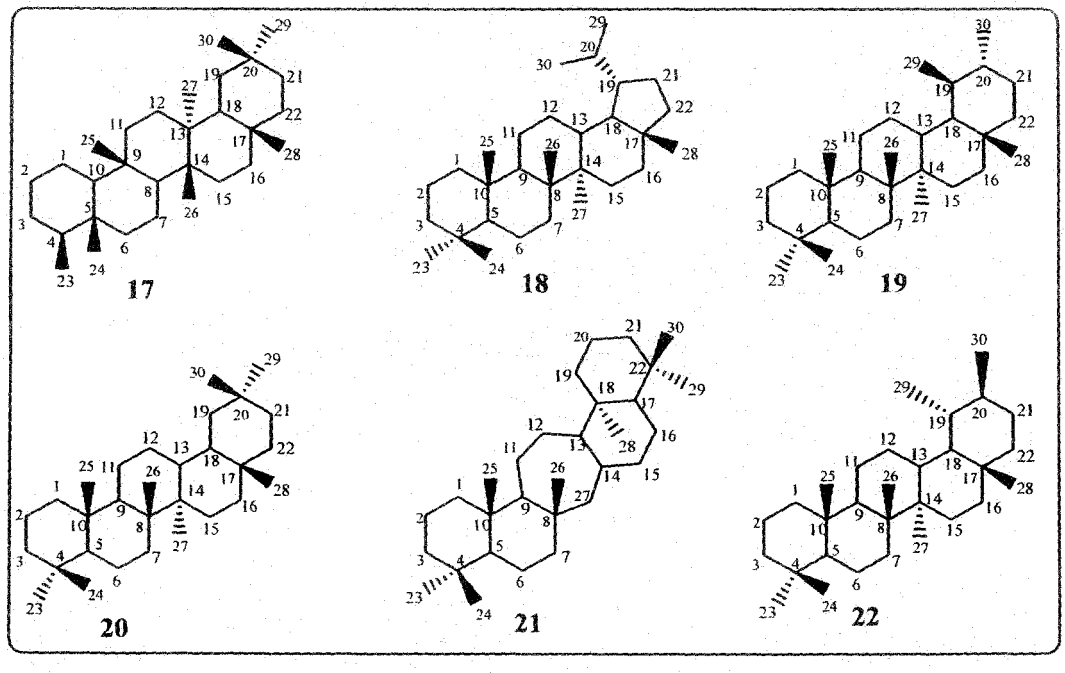

Figure 8. Structural skeletons of different pentacyclic triterpenoids (**17-22**).

I.3.1.1 Friedelane triterpenoids

Friedelane triterpenoids bear a [6-6-6-6-6]-fused pentacyclic skeleton with eight -Me groups at C(4), C(5), C(9), C(13), C(14), C(17), and C(20) (geminal-dimethyl), respectively. In the past 30 years a huge number of friedelanes with different skeleton[75] and potent biocidal activities have

been reported.[76] Some of them being very active are used in pharmacy or in drug development, such as celastrol (**23**) and correolide (**24**) (**Figure 9**).[77]

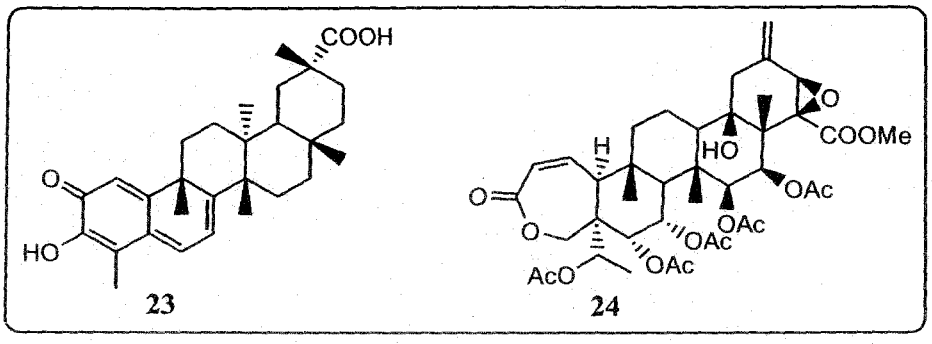

Figure 9. Celastrol (**23**) and correolide (**24**).

Biobased products which are derived from nature contribute in different sectors such as agrifood, forest products, health care, plastics, chemicals, energy and the environment. And the source of the world's most important biobased, nontimber forest products is the cork industry. Friedelin (**25**) and cerin (**26**) are the two most important products obtained from cork smoker washed solids (**Figure 10**). These two friedelane triterpenoids, especially the first one and some of its derivatives are found to possess potent biocidal activities, such as antibacterial, antifungal, antitumor, cytotoxicity,[78-79] phytotoxicity, tripanocidal activity, leishmanicidal activity etc. 3-Oxofriedelan-29-oic acid (**27**), 3-oxofriedelan-28-oic acid (**28**) and 28,29-dihydroxyfriedelan-3-one (**29**) are found to be cytotoxic against A-549 tumor cell while for L-1210 and KB tumor cells only 3-oxofriedelan-28-oic acid (**27**) is effectively cytotoxic (**Figure 11**).[80]

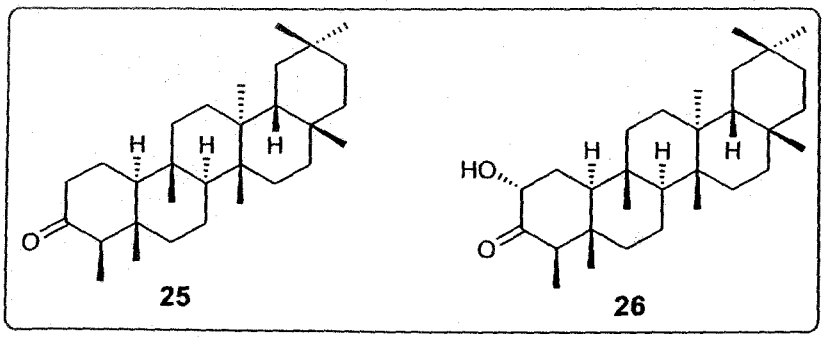

Figure 10. Friedelin (**25**) and cerin (**26**).

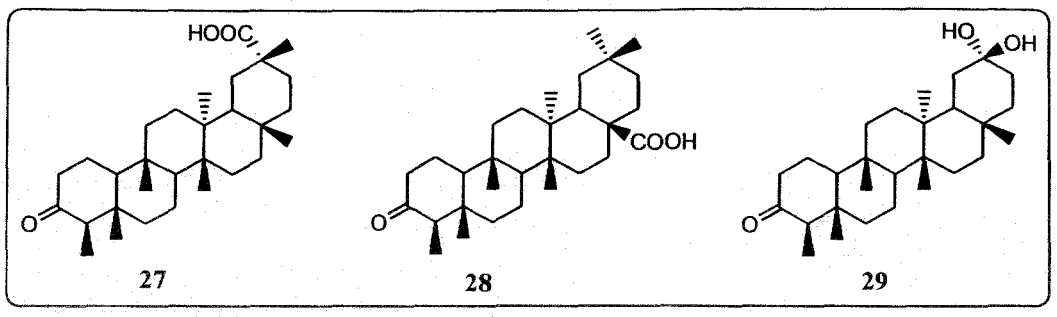

Figure 11. 3-Oxofriedelan-29-oic acid (**27**), 3-oxofriedelan-28-oic acid (**28**) and 28,29-dihydroxy friedelan-3-one (**29**).

Due to the increasing population year by year throughout the world, amount of cultivated land is decreasing and hence the demand of more amount of food production in the available area is increasing and for that a huge amount of pesticides and fertilizers are used which leads to environmental pollution as well as causes some chronic diseases. Natural products are used to control insects since ancient times as these are easily degradable, ecologically safe and have no adverse effects on environment.[81-82] Moreover, these protect plants from diseases.[83-84] Friedelin was tested on two pests and it showed good antifeedent, larvicidal and growth inhibitory activities against both of them. Friedelin had no ecotoxicological effect on freshwater fish. So, friedelin can be used for the development of new pesticidal formulation in order to manage agricultural pests.[85] Friedelin also has diuretic activity.[86] Another friedelane triterpenoid 2-formyl-2,3-secofriedelane-3-oic acid (**30**) was found to be a potent inhibitor of human lymphocyte proliferation and it was found to have no lymphocytotoxicity.[78] Again, 3,4-secofriedelan-3-oic acid (**31**) found in *Maytenus imbricata* (Celastraceae) has been reported to inhibit several photosynthetic activities (**Figure 12**).[87] Zhan et al., in a recent review showed the vast family of friedelane triterpenoids having 'fascinating structure and interesting bioactivities'. This review points out the fact that friedelane triterpenoids can be a 'prime candidates for developing new drugs'.[77]

I.3.1.2 Lupane triterpenoids

Lupane triterpenoids are a [6-6-6-6-5]-fused pentacyclic skeleton with six -Me groups at C(4) (geminal-dimethyl), C(8), C(10), C(14), C(17), and an isopropyl group at C(20). Lupane

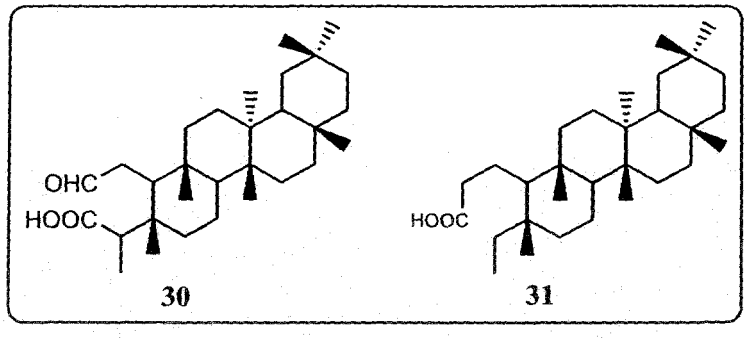

Figure 12. 2-Formyl-2,3-seco friedelan-3-oic acid (**30**) and 3,4-secofriedelan-3-oic acid (**31**).

triterpenes found in *Melilotus messanensis* (Fabaceae) possess potential allelopathic activity.[88] Betulinic acid (BA, **32**) is the most important member of this group (**Figure 13**). It is a naturally occurring plant-derived pentacyclic triterpenoid found in many fruits and vegetables[90-91] and exhibits a wide variety of pharmacological and biochemical effects including anti-inflammatory and anticancer activities[89-91] with low toxicity to normal cells.[92] Besides, BA and its derivatives represent a promosing class of anti-HIV agent.[93-96] The natural compound BA shows potent anticancer through activation of the mitochondrial pathway of apoptosis in cancer cells. BA may also be used in combination protocols to enhance its antitumor activity, e.g., with chemo- or radiotherapy or with the death receptor ligand TRAIL. Because of its relative selectivity cytotoxicity against malignant compared to normal cells, BA is a promising new experimental anticancer agent for the treatment of human cancer.[97] Currently, BA is in second phase of clinical trials for dysplastic nervus treatment and therefore, it is highly potent to be used as clinical practice in future.[98] Among these, many more betulinic acid derivatives were prepared and their biocidal activities were studied.[99-101] Apart from BA other important lupane triterpenoids are lupeol (**33**) and its derivatves. These were found to have antiurolithic effects[102] along with other properties of pharmacological importance like antioxidative,[103] anti-inflammatory,[104] hepatoprotective,[105] and antilipidemic activities.[106,107]

I.3.1.3 Ursane and oleanane triterpenoids

These two groups of triterpenoids are structurally isomeric. Both the groups contain a [6-6-6-6-6] -fused pentacyclic skeleton with eight methyl groups. In ursane PTs, the methyl groups are at

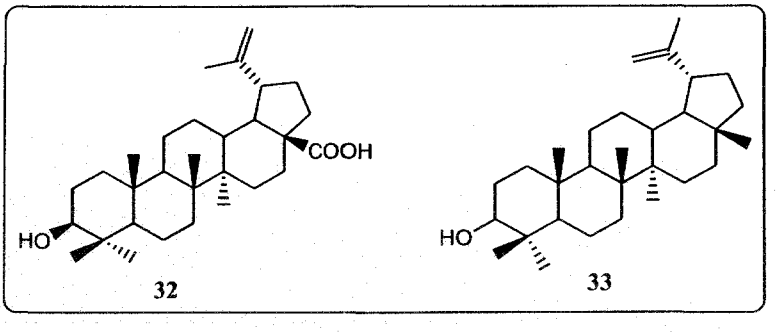

Figure 13. Betulinic acid (**32**) and lupeol (**33**).

C(4) (geminal-dimethyl), C(8), C(10), C(14), C(17), C(20)and C(21) whereas for the oleanane PTs, the only difference is at C(20) where germinal dimethyl groups are present and no methyl group is there in C(21). Ursolic acid (UA, **34**) and oleanolic acid (OA, **35**) are the most important compounds of the respective groups (**Figure 14**). Based on pharmacological tests, oleanolic acid, ursolic acid, their derivatives and structurally related triterpenoids are also found to possess bioactivities such as anti-inflammatory,[108-111] diuretic, anti-tumor,[112-113] hepatoprotective[114-115] and anti-HIV[116-177] activities. Ursolic acid is cytotoxic against A-549, L-1210 and KB tumor cells.[118] In fact, some reviews, covering the last 15 years, have highlighted the broad spectrum of biological activities including phytotoxic[79] and photosynthesis inhibition activities[89] and low toxicities of oleanane and ursane triterpenoids.[119-123]

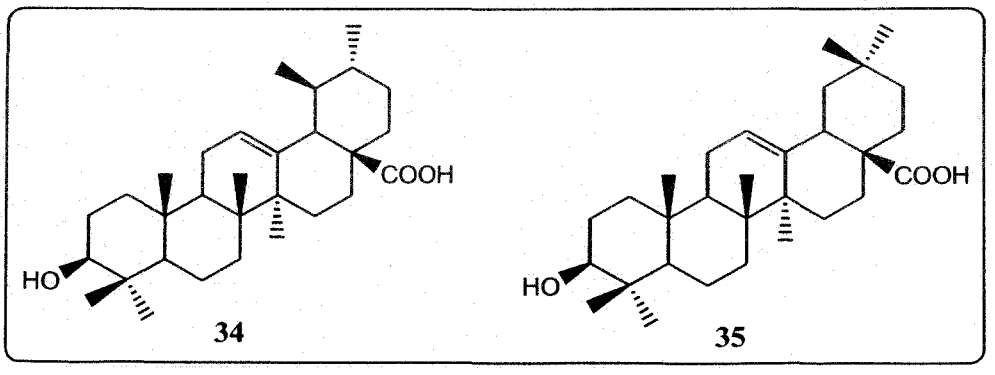

Figure 14. Ursolic acid (**34**) and oleanolic acid (**35**).

I.3.1.4 Serratane and Ψ-taraxastane

Serratane group of triterpenoid is different from the other groups with respect to basic skeleton. It is a [6-6-7-6-6]-fused pentacyclic skeleton with seven -Me groups at C(4) (geminal-dimethyl), C(8), C(10), C(18), and C(22) (geminal-dimethyl). One compound of serratane group, named phlegmanol A (**36**) was reported to be found in *Lycopodium phlegmaria*.[124] On the otherhand Ψ-taraxastane group's skeleton is identical with ursane group except in C-20 and C-21. The stereochemistry of the methyl groups are just the reverse to that of ursane group. Some of the compounds of this group are faradiol, heliantriol B_o, heliantriol C, arnidiol (**37**), all of which are found to be cytotoxic, the last one being most effective (**Figure 15**).[125]

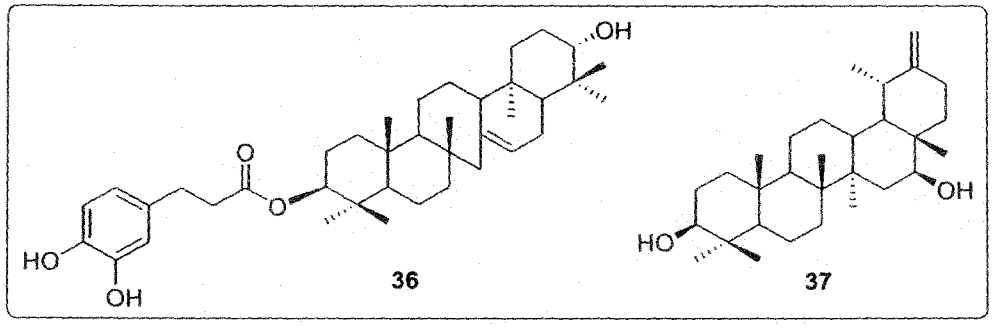

Figure 15. Phlegmanol A (**36**) and arnidiol (**37**).

I.4 Some marketed drugs based on steroids and triterpenoids

Considering the PT- and steroid-based drugs, available in the market, steroids are much more familiar compared to PTs. The broadest spectrum of clinical utility is occupied by steroids as these are globally the most diversified therapeutic class of compounds. Steroids are used in cancer treatment since a long time. To treat the acute leukemias in children a steroidal drug, named, prednisone (**38a**), is used with combination with other antineoplastic agents.[72,126-128] Another drug exemestane (**38b**) is used as aromatase inhibitors to treat postmenopausal breast cancer.[129] Dutasteride (**38c**) is used in clinic to treat androgen-dependent prostate cancer and fluoxymesterone (**38d**) is used for the treatment of advance and metastatic neoplasm of the breast (**Figure 16**).[130-133]

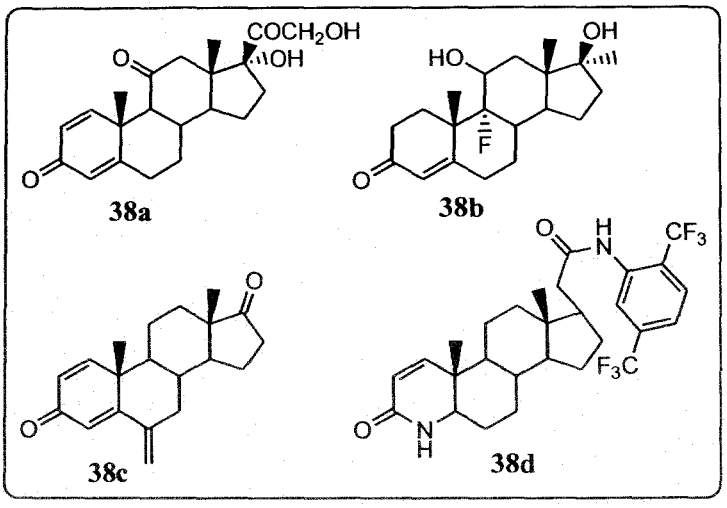

Figure 16. Some steroid-based marketed drugs prednisone (**38a**), exemestane (**38b**), dutasteride (**38c**) and fluoxymesterone (**38d**).

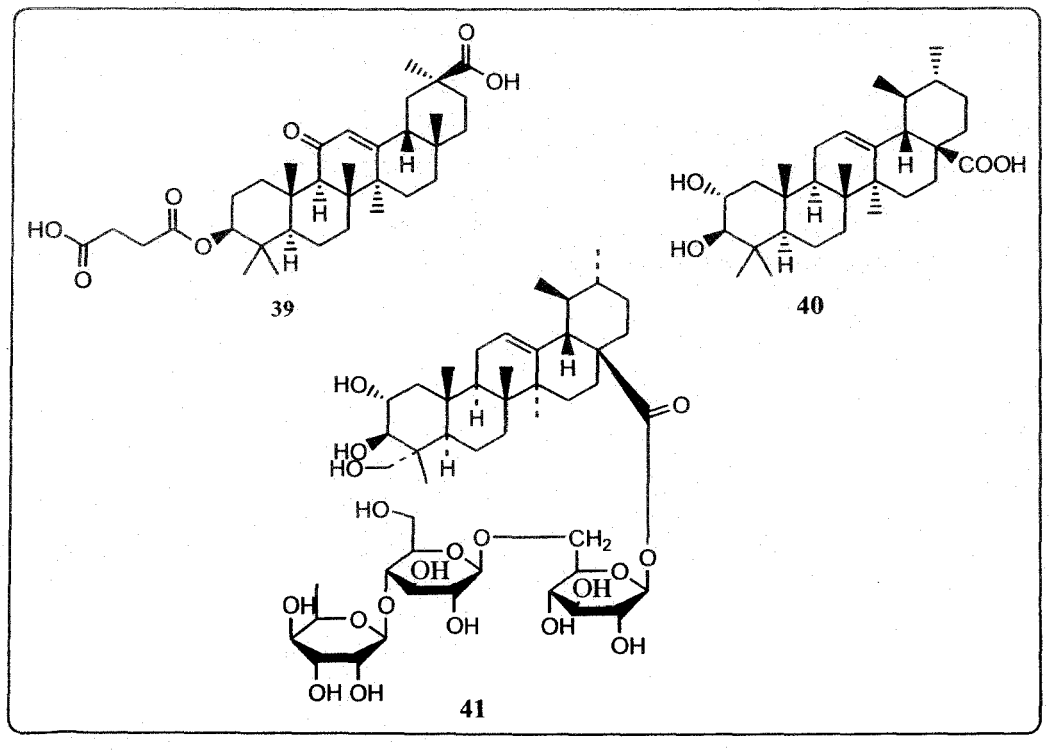

Figure 17. Some pentacyclic triterpenoid-based marketed drugs carbenoxolone (**39**), corosolic acid (**40**) and asiaticoside (**41**).

Some pentacyclic triterpenoid-based drugs are also found in the market such as carbenoxolone (**39**) for oesophageal ulceration and inflammation, corosolic acid (**40**) for diabetes and obesity, asiaticoside (**41**) for wound healing and Perkinson's disease and oleanolic acid (**35**) for lever disease etc. (**Figure 17**)

I.5 Conclusion

Both steroids and triterpenoids play an important role in the field of medicinal chemistry. Some of these compounds are biologically active and many more of the derivatives possess potent biocidal activities. These act both as the raw materials as well as the inspiration for designing most lead discoveries in pharmaceuticals and for drug development. So, there lies the importance of the natural products and research in this field for the improvement of mankind and this is the objective and justification of our research work in the same area.

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Chapter II

Transformative reactions of steroids using mercuric(II) acetate and *N*-bromosuccinimide

This chapter is divided into two sections, **Section A** and **Section B**.

Section A comprises the action of mercuric(II) acetate on 16-dehydropregnenolone acetate (16-DPA) and **Section B** comprises the results of oxidative transformation of cholesterol, β-sitosterol and 4β-hydroxy cholesterol using *N*-bromosuccinimide (NBS) in solid phase.

Section A

Action of mercuric(II) acetate on 16-dehydropregnenolone acetate (16-DPA)

II.A.1 Introduction: A short review on the action of mercuric(II) acetate on steroids

Mercuric acetate is being used in the field of steroid since a long time. Oxidative transformation of steroids using mercuric (II) acetate has been reported earlier by a number of research groups. Windaus and Linsert,[1-7] in the year 1928 reported the same reagent as a dehydrogenating agent in the steroid field and the reagent was found to introduce an ethylenic bond in conjugation with an existing double bond. According to Henbest and Nicholls, a γ,δ-unsaturated alcohol, bicyclo-[2,2,1]-hept-5-en-2α-ylmethanol on treatment with mercuric (II) acetate formed a cyclic ether.[8] A similar formation of a cyclic ether from the γ,δ-unsaturated alcohol, 2,6-dimethylhept-5-en-2-ol was also reported by Brook, Rodgman and Wright in1952.[9]

Dehydroergosterol (**42**) from both of ergosterol (**3**) and isopyrocalciferol (**43**) by mercuric(II) acetate was reported by Müller,[10] whereas pyrocalciferol (**44**) and lumisterol (**45**) on similar treatment gave dehydrolumisterol (**46**) as reported by Heilbron, Spring, and Stewart in the year 1935[11] (**Figure 1**).

In the year 1951, Djerassi et al., reported that Δ^7-allopregnene-3β-20β-diol diacetate (**47**) on treatment with mercuric(II) acetate in chloroform and acetic acid mixture was dehydrogenated to $\Delta^{7,9(11)}$-allopregnadiene-3β,20β-diol diacetate (**48**) (**Figure 2**).[12]

When cortisone (**49**) was synthesized from ergosterol (**3**), diosgenin (**16**) and stigmasterol (**50**),[13-14] the prerequisite $\Delta^{7,9(11)}$-allo steroids were conveniently prepared from Δ^7-allo steroids using mercuric(II) acetate as a reagent though the original procedure for the conversion of $\Delta^{7,22}$-ergostadiene-3β-ol acetate (**51**) to $\Delta^{7,9(11),22}$-ergostatriene-3β-ol acetate (**52**) was used after modification.[15] Again $\Delta^{7,9(11),22}$-ergostatriene-3β-ol acetate (**52**) reacted with mercuric(II) acetate more slowly than **51**, and mercurous(I) acetate and a complex steroidal reaction product containing in part a mercurated steroid derivative were produced (**Figure 3**).[16]

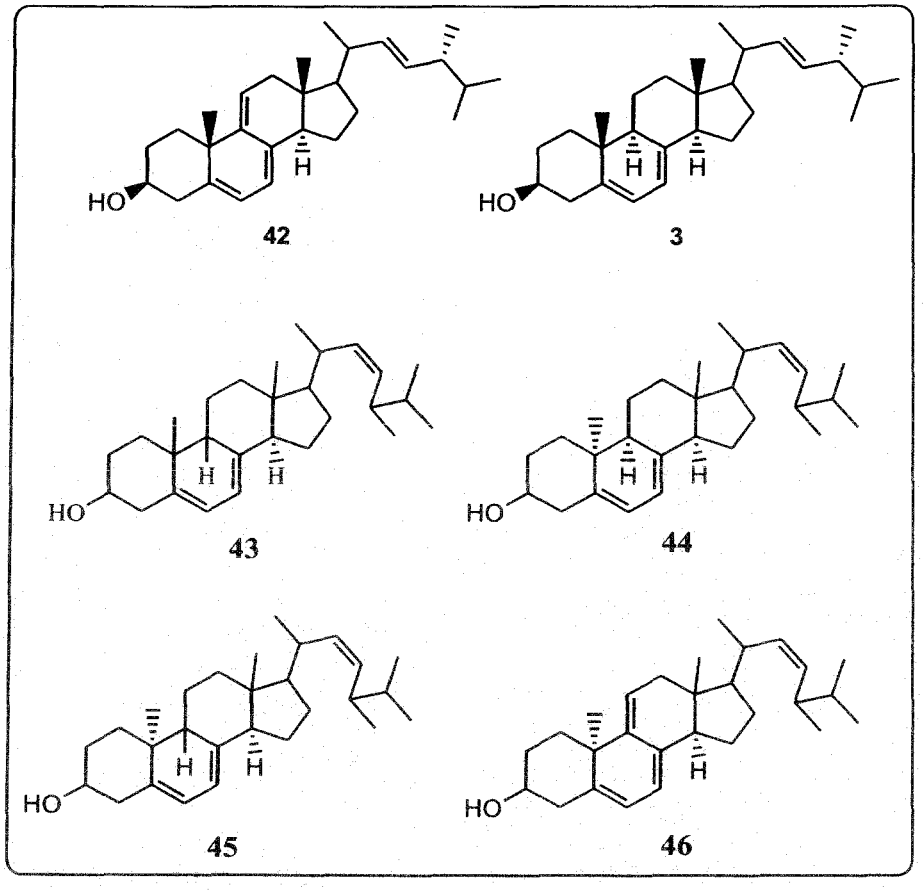


Figure 1. Dehydroergosterol (**42**), ergosterol (**3**), isopyrocalciferol (**43**), pyrocalciferol (**44**), lumisterol (**45**) and dehydrolumisterol (**46**).

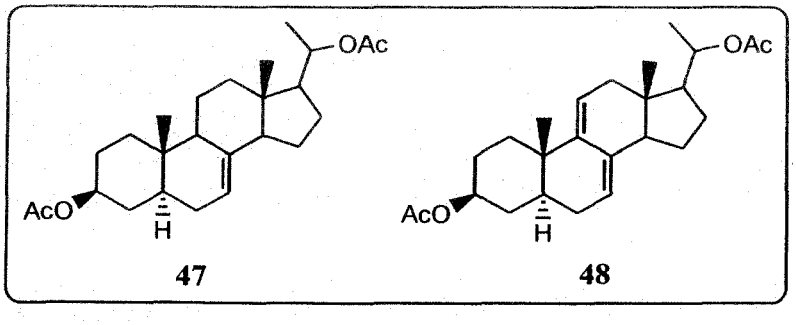


Figure 2. Δ^{7}-Allopregnene-3β,20β-diol diacetate (**47**) and $\Delta^{7,9(11)}$ -allopregnadiene-3β,20β-diol diacetate (**48**).

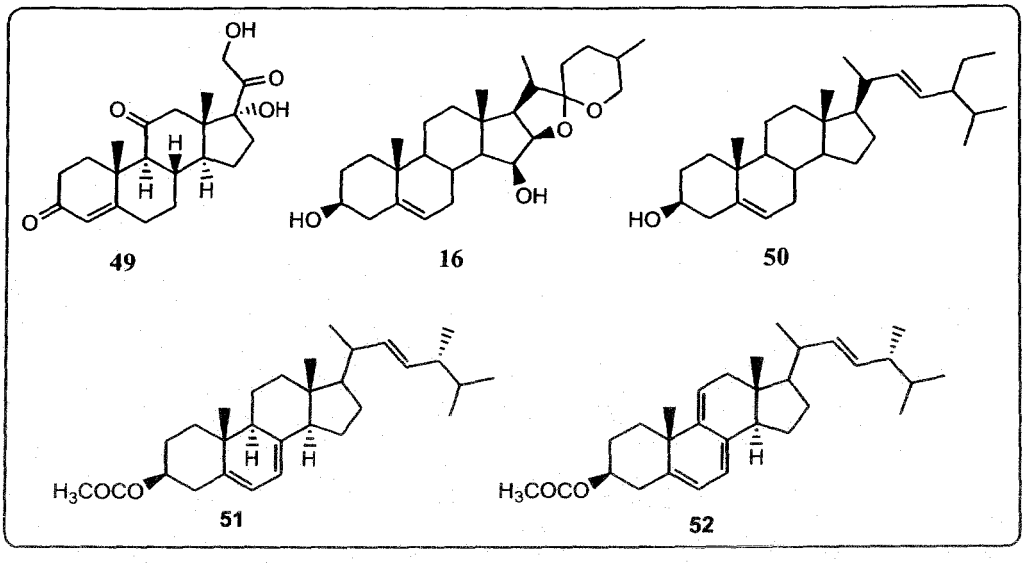


Figure 3. Cortisone (**49**), diosgenin (**16**), stigmasterol (**50**), $\Delta^{7,22}$-ergostadiene-3β-ol acetate (**51**), $\Delta^{7,9(11),22}$-ergostatriene-3β-ol acetate (**52**).

Mercuric(II) acetate in glacial acetic acid was also used to mercurate the steroids as was done by Chin and Warren to prepare 4-mercuri-17β-estradiol (**53**) from 17β-estradiol (**54**)[17] (**Figure 4**), where the mercurated product might be used for affinity labeling studies of estrogen receptors, the steroid moiety guiding the molecule to the binding site and the mercury reacting with a sulfhydryl group adjacent to or within that site.[18]

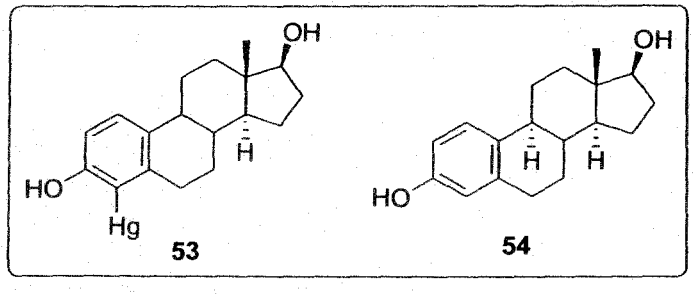


Figure 4. 4-Mercuri-17β-estradiol (**53**) and 17β-estradiol (**54**).

It was also reported that mercuric(II) acetate oxidation of the A-secolanosterol derivative 3-nitrilo-3,4-secolanosta-4(30),8-diene (**55**) produced an aromatic product **56** which caused fragmentation while was further oxidized with the same as it contained a double bond at C-8(9) (**Figure 5**).[19]

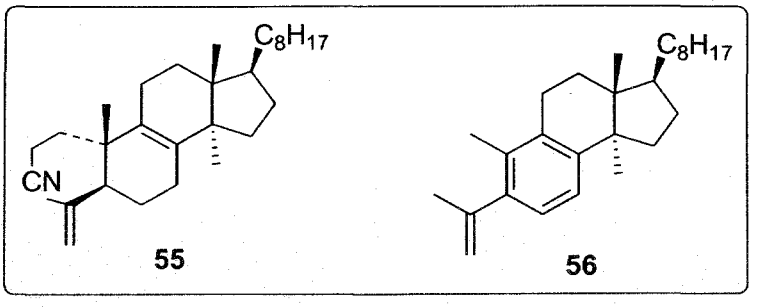


Figure 5. 3-Nitrilo-3,4-secolanosta-4(30),8-diene (**55**) and the aromatic derivative **56**.

Simes et al. also reported that the same reactions with carboxy, amide or methyl carboxy group in the place of cyanide group, of compound **55** were unsuccessful. And they also found that in cases of the compounds like **57** and **58,** aromatization did not occur (**Figure 6**).[19]

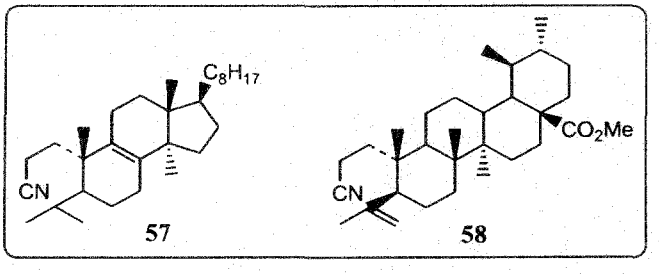


Figure 6. Nitrilo compounds **57** and **58**.

On the other hand, progesterone and its bis-ethylene ketal with mercuric(II) acetate in methanol were found unreactive though the same got oxidized when boiled in acetic acid.[20] Kocor and Gumulka oxidised 17α-methyl-1,4,6-androstatrien-17β-ol-3-one (**59**) with mercuric(II) acetate in acetic acid to obtain 2-acetoxymercuri-17α-methyl-1,4,6-androstatrien-17β-ol-3-one (**60**).[21] But 1,4-androstadiene-3,17-dione (**61**) needed 4-equivalents of excess of mercuric acetate to yield 2-acetoxymercuri derivative **62** though similar attempts with 1,4-androstadien-17β-ol-3-one (**63**), 1,4-pregnadiene-3,20-dione (**64**) and 4,6-pregnadiene-3,20-dione (**65**) were unsuccessful. This revealed that 3-keto steroids having a readily abstractable allylic proton when heated with mercuric(II) acetate in methanol and in acetic acid resulted in the formation of mercurous(I) acetate, presumably, due to oxidation of the steroid. But 3-ketosteroids which did not have this structural feature, though there was a double bond at C-1,

addition of acetoxymercuri ion occured in acetic acid at the α-side of the C-1 double bond. The proton at C-2 was abstracted, and the 2-acetoxymercuri-1-en-3-one was formed (**Figure 7**).[22]

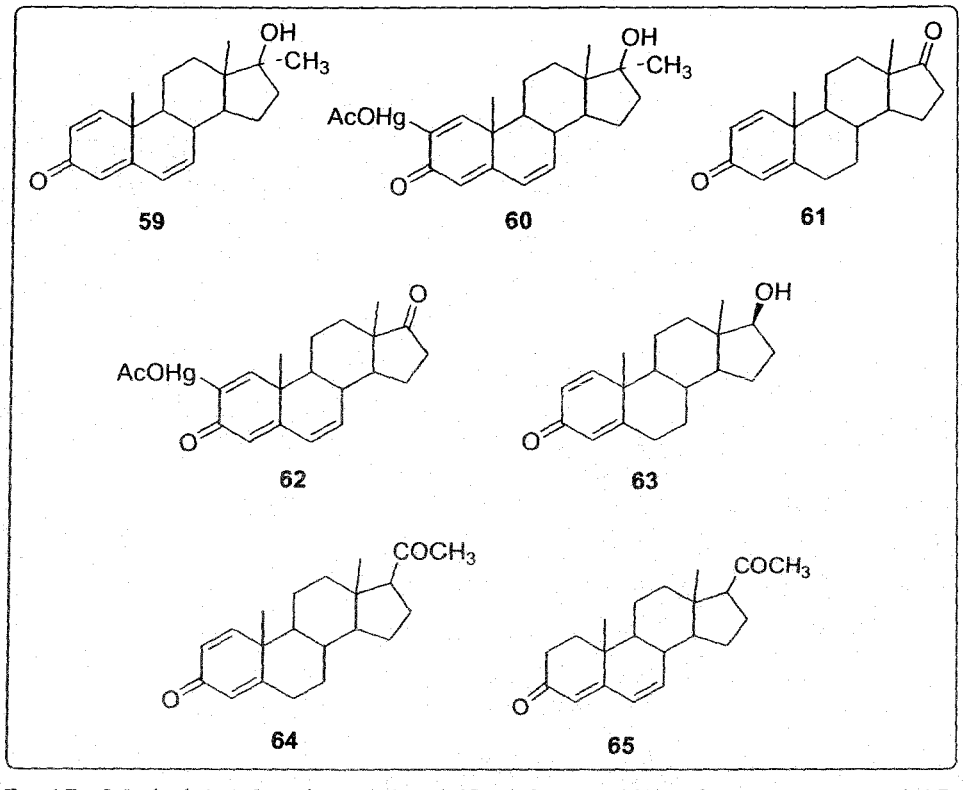


Figure 7. 17α-Methyl-1,4,6-androstatrien-17β-ol-3-one (**59**), 2-acetoxymercuri-17α-methyl-1,4,6-androstatrien-17β-ol-3-one (**60**), 1,4-androstadiene-3,17-dione (**61**), compound **62**, 1,4-androstadien-17β-ol-3-one (**63**), 1,4-pregnadiene-3,20-dione (**64**) and 4,6-pregnadiene-3,20-dione (**65**).

Blossey et al., in the year 1973, reported that certain steroids containing di- and tri-substituted double bonds form α,β-unsaturated ketones when reacted with mercury(II) acetate. When 2 equivalents of mercury(II) acetate was reacted separately with 5α-ergost-14-ene (**66a**), 3β-hydroxy-5α-ergost-14-ene acetate (**66b**), and 3β-hydroxy-5α-cholest-14-ene acetate (**66c**), they isolated 8(14)-en-15-one (**67a**), **67b** and **67c** respectively as the major products instead of getting the expected 16ε-acetoxy-14-ene; although cholest-8(14)-ene (**67d**) was unreactive towards mercuric(II) acetate. On the other hand, 5α-cholest-2-ene (**68**) when refluxed with mercuric(II) acetate in chloroform/acetic acid, produced only 20%, after 66 h of reaction time, 5α-cholest-1-ene-3-one (**69**).[23] Angelastro et al., found that treatment of 3β-[(1,1-

dimethyl)ethyl]siloxyandrost-5-en-17β-ol (**70**) with ethyl vinyl ether in presence of mercuric(II) acetate afforded vinyl ether (**71**) (**Figure 8**).[24a]

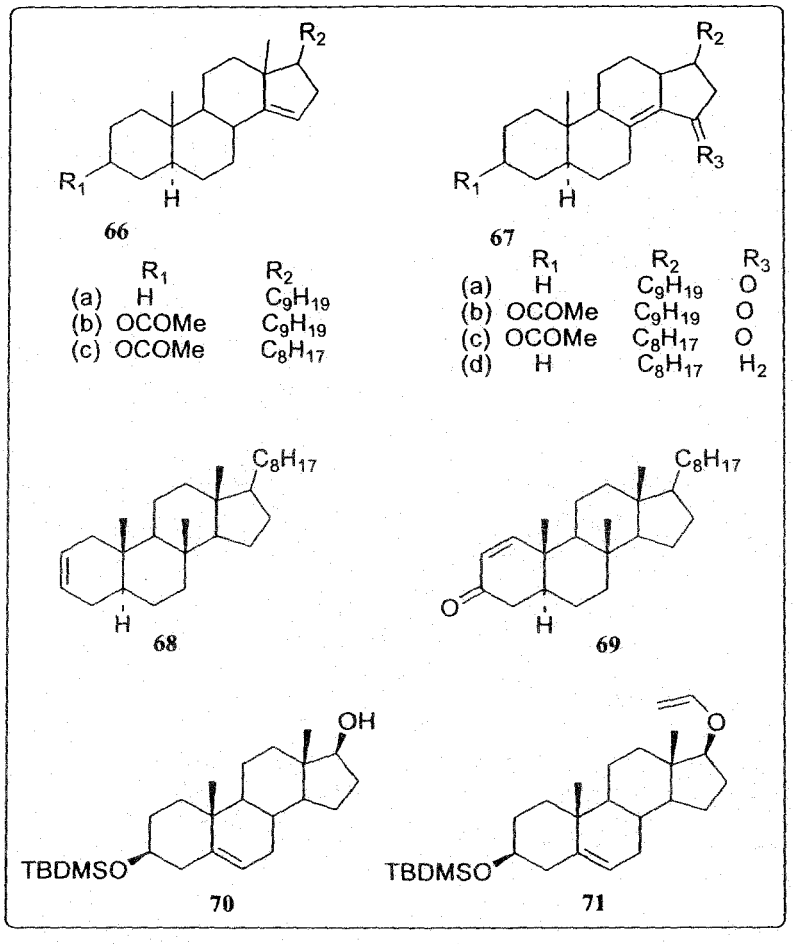


Figure 8. 5α-Ergost-14-ene (**66a**), 3β-hydroxy-5α-ergost-14-ene acetate (**66b**), 3β-hydroxy-5α-cholest-14-ene acetate (**66c**), 8(14)-en-15-one (**67a**), derivatives **67b** and **67c**, cholest-8-(14)-ene (**67d**),5α-cholest-2-ene (**68**), 5α-cholest-1-ene-3-one (**69**), 3β-[(1,1-dimethyl)ethyl]siloxyandrost-5-en-17β-ol (**70**) and *O*-vinyl derivative **71**.

Thus, mercuric(II) acetate is found to be a very useful reagent which can result, within the steroid functionality, a number of essentially important derivatives like, lactones, cyclic ethers, α,β-unsaturated ketones, dehydrogenation, orthomercuration and acetoxymercuration.

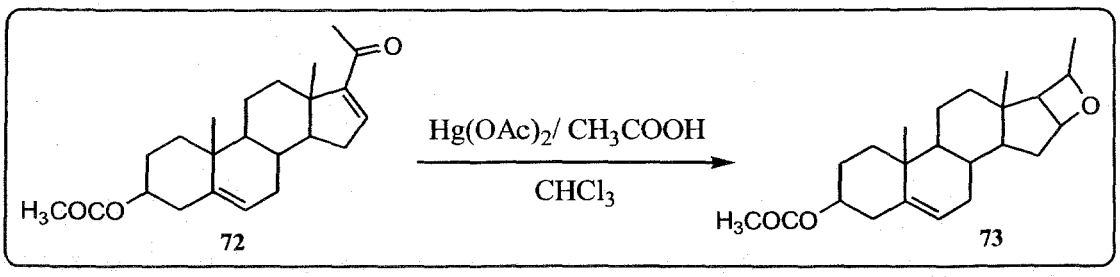
II.A.2 Present work

II.A.2.1 Preparation of 16-dehydropregnenolone acetate (16-DPA, 72) from diosgenin (16)

16-DPA was prepared from a steroidal saponin diosgenin (**16**) obtained by extraction from naturally occurring *Dioscorea Floribunda* (collected from Research and Development Laboratries, Directorate of Sinchona and other medicinal Plants, Mungpoo, Darjeeling, West Bengal, India). Diosgenin was first acetylated by acetic anhydride in xylene in an autoclave. Diosgenin (5g, 0.012 mol) was then charged with acetic anhydride (4 mL, 0.04 mol) and xylene (15 mL) in an autoclave at 250^0C for 10 hours. Then the reaction was stopped and allowed to cool down for 1 hour and the solvent was removed by rotary vacuum evaporator under reduced pressure and pseudo diosgenin diacetate of m.p. 98^0C (lit[24b], 97-98^0C) was obtained. It was then oxidised to diosone by CrO_3. To carry out the oxidation process first a solution of CrO_3, water and glacial acetic acid (0.5 mol + 5mL + 2mL) at (0-5)^{0}C was made. Then it was added dropwise to an ice cold solution of 5g pseudo diosgenin diacetate, 10 mL dichloromethane, 10 mL glacial acetic acid and 2.5 mL water. After completion of addition the temperature of the reaction mixture was allowed to raise upto 15^0C and the mixture was stirred at this temperature for about 25 minutes. Then 0.5 g sodium chloride in 20 mL water and 1 mL methanol solution was added to it and the reaction mixture was stirred for 20 minutes. Extraction of the organic layer followed by coloum chromatography gave the pure diosone. Finally diosone was directly hydrolysed with glacial acetic acid for 2 hours and extraction followed by repeated distillation and finally recrystalisation gave the creamy coloured 16-DPA of m.p. 172^0C (lit[24c],172-173^0C).

II.A.2.2 Result and Discussion

The versatile oxidizing capacity of mercuric(II) acetate encouraged us to carry out the present investigation on 16-dehydropregnenolone acetate (16-DPA, **72**), an important steroid moiety. When 16-DPA was treated with mercuric(II) acetate in chloroform, cyclic ether derivative **73** was formed. The cyclic ether was actually introduced onto the ring-D of 16-DPA, involving the carbonyl oxygen and the double bond in its α,β-position (**Scheme 1**). Hence, a single-step conversion of exocyclic α,β-conjugated ketone functionality of a steroid resulted into a four-membered cyclic ether which may possess potent biocidal activity[24a], preserves the main significance of the study. The structure of the product was confirmed by the spectral data analysis.



Scheme 1. Single step synthesis of steroidal ring-D fused four-membered cyclic ether (**73**)

II.A.3 Experimental

II.A.3.1 General: Melting points were measured in open capillary methods and were uncorrected. The FAB mass spectra were recorded on a Jeol SX102/Da-600 mass spectrometer/Data System using Argon/Xenon as the FAB gas. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avance 300 MHz FT-NMR spectrometer using 5 mm BBO probe. $CDCl_3$ was used as solvent and TMS as internal reference. Data are presented as follows: Chemical shifts–in parts per million on the scale relative to $\delta_{TMS}=0$; coupling constant-*J*/Hz. Elemental analysis was performed using a Vario EL-III elementary analyser. Infrared spectra were recorded on Shimadzu FT-IR 8300 Spectrometer as thin films (KBr) as indicated in the experimental procedures, and at room temperature. Frequencies are given in wave numbers (cm^{-1}). For column chromatography silica gel G, 60-120 mesh was used with petroleum ether/ethyl acetate mixture as the eluent. For thin layer chromatography (TLC), freshly made silica gel plates (using silica gel for TLC + petroleum ether) were used and visualization was achieved by staining with iodine.

II.A.3.2 Reaction Procedure for the synthesis of cyclic ether 73 from 16-DPA (72) and mercuric(II) acetate: 16-DPA (**72**, 0.5 g) was dissolved in chloroform (12.5 mL) and a solution of mercuric(II) acetate (9 g) in hot glacial acetic acid (70 mL) was added. The reaction mixture was maintained at 100 °C in an oil bath for 4 hours. On cooling, the precipitated mercuric(I) acetate was filtered off and the whole filtrate was diluted with water and extracted with chloroform. The chloroform layer was washed well with water and then dried over anhydrous sodium sulphate. Removal of chloroform gave an orange solid which was dissolved in pyridine and H_2S was passed for 2 hours. The black reaction mixture was filtered and pyridine was removed by very dilute HCl and then extracted with ether. The brownish black residue (0.38 g)

so obtained was then chromatographed over silica gel. The product cyclic ether (**73**) was obtained using ethyl acetate and petroleum ether mixture (1:6) as eluent and it was recrystallised from absolute alcohol to get white crystals.

II.A.3.3 Characterization of the product steroidal ring-D fused cyclic ether (73): m.p. 132-134°C (absolute ethanol).^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37 (s, 1H, CH), 4.61 (s, 1H, CH), 3.85-4.20 (m, 2H, CH), 2.95(dd, J = 3.0 Hz and 6.0 Hz,1H, CH), 2.18 (d, J = 6.0 Hz, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.66 (s, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5 (>CO), 139.6 (=C5), 121.9 (=C6H), 73.7 (-O-CH), 71.1 (-O-CH), 70.6 (-C3H), 55.6, 49.6, 47.0, 45.6, 38.5, 37.9, 36.8, 36.5, 33.3, 31.8, 31.5, 31.3, 27.6, 21.4, 20.8, 19.2, 14.2. FTIR (KBr, cm^{-1}): υ 1726, 1696, 1240, 1030 and 668. MS (FAB): m/z = 358 (M^+), 343, 315, 298, 297 (100%). Elemental Analysis: Calculated for $C_{23}H_{34}O_3$ (358.52): C, 77.05%; H, 9.56%; O, 13.39%. Found: C, 77.08%; H, 9.61%; O, 13.45%.

Section B:

Action of *N*-bromosuccinimide (NBS) on cholesterol and β-sitosterol: synthesis of A-ring aromatized steroids in solid phase

II.B.1 Introduction

II.B.1.1 A brief review on the synthesis of A-ring aromatized steroids

Steroids and their derivatives are one of the most important classes of compounds both in terms of chemical and biological consideration.[25,26] Among those, oxidized steroids are very significant and various oxidizing agents were used to carry out the oxidation process. This oxidation sometimes leads to the selective aromatization of the steroids or in some cases oxysteroids were the final product. Though the transformation of steroidal nucleus into its aromatic analogue was first reported by Marker and co-workers[27] but it was Inhoffen and Zuhhdarff who established the fact in the year 1938 by carrying out the transformation of 1,4-cholestadien-3-one to a phenol entity. This rearrangement was reported as an acid-catalysed dienone-phenol rearrangement and the structure of the phenol entity was later on confirmed by the others.[28-35] The same rearrangement along with the aromatic derivative of 1,4-cholestadien-3-one was prepared thermally by Romo and his group.[36,37] Cholesterol (**2a**) was transformed into A-ring aromatized

On the other hand, androsta-1,4,9(11)-trien-3,17-dione (**79**) yielded Δ^9-estrone (**80**) on reflux with zinc in pyridine or ethylene glycol (**Figure 9**).[46]

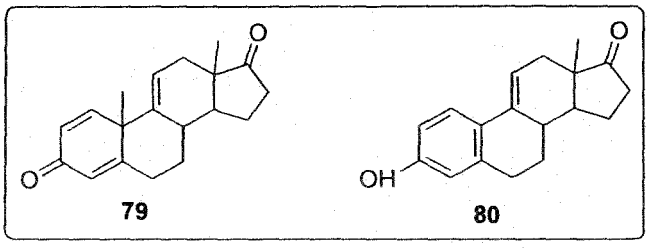
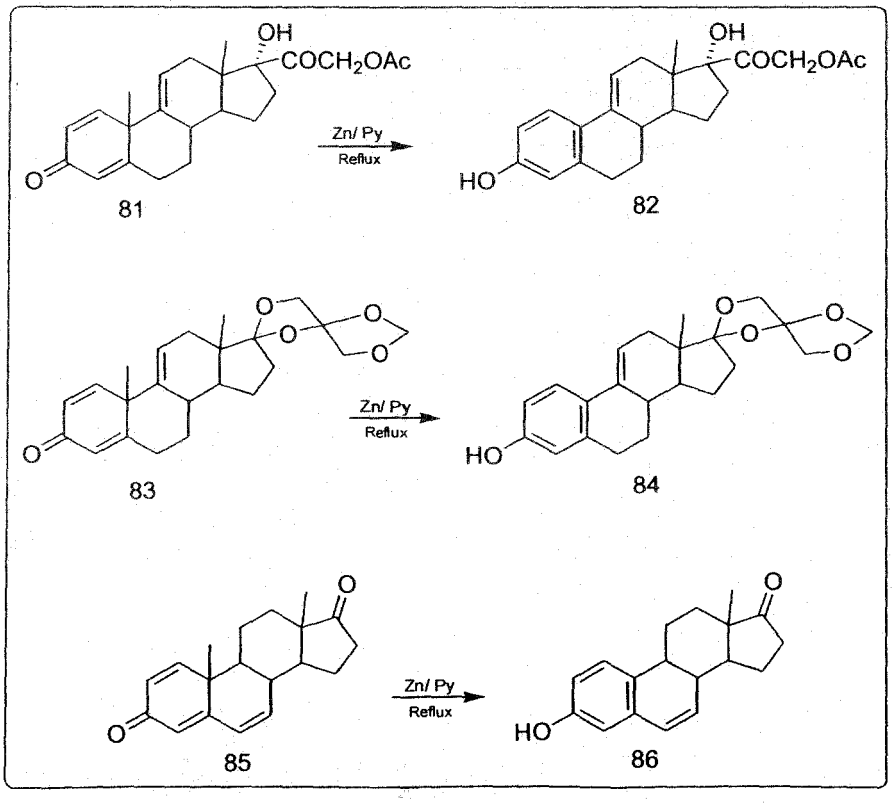


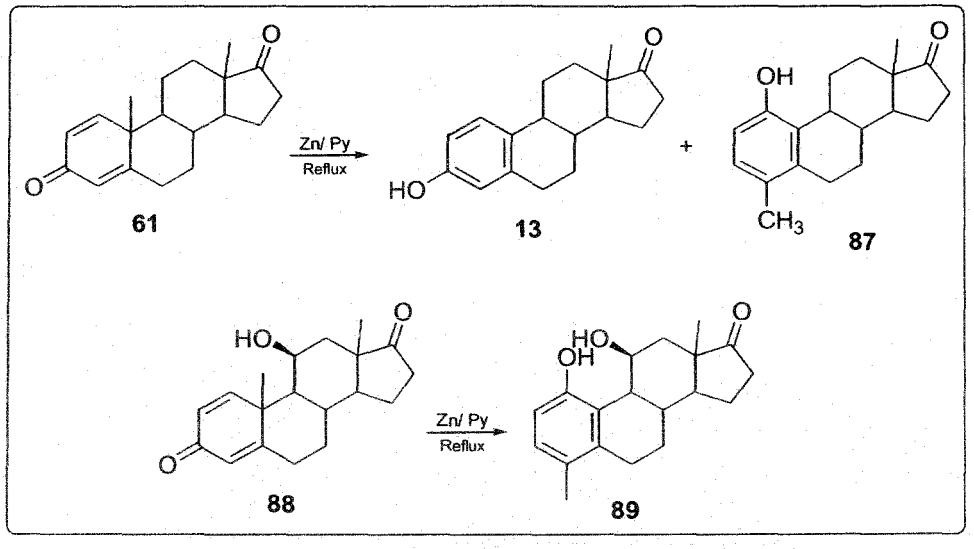
Figure 9. Androstan-1,4,9(11)-trien-3,17-dione (**79**) and Δ^9-estrone (**80**).

As was expected, similar results were shown by compounds **81**, **83** and **85** to form **82**, **84** and **86** respectively (**Scheme 5**).[47-49]



Scheme 5. A-ring aromatization from different steroidal A-ring dienones.

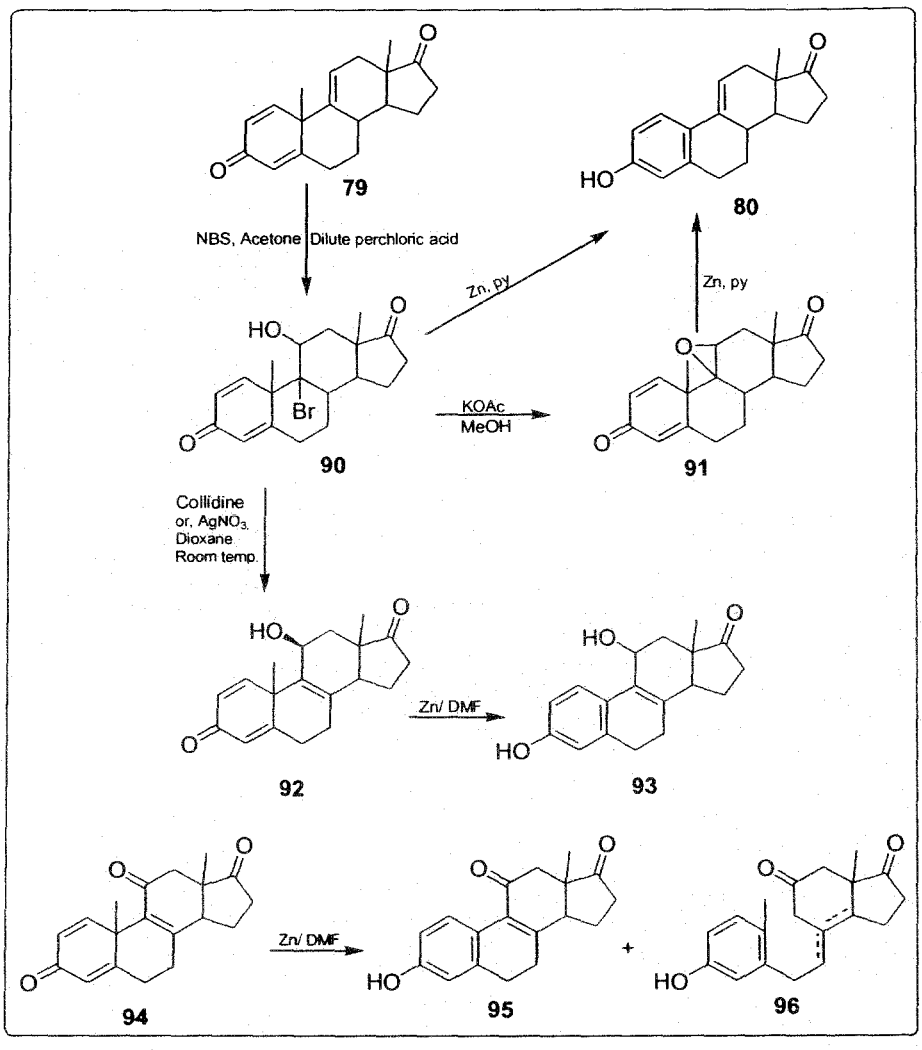
Zinc in pyridine was also found to be effective to form *p*-cresol type rearrangement product **87**, and estrone **(13)** from **61**; or **89** from **88**.[35,50] These transformations indeed described a new type of dienone-phenol rearrangement of cross-conjugated dienone or trienone systems **(Scheme 6)**.[47]



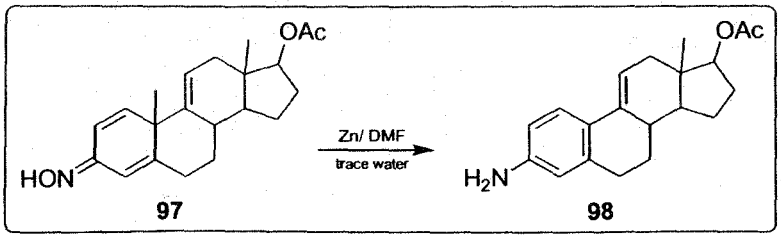
Scheme 6. Dienone-phenol rearrangement of cross-conjugated dienone or trienone systems.

In the year 1963, K. Tsuda, E. Ohki and S. Nozoe reported that when androsta-1,4,9(11)-triene-3,17-dione **(79)** was treated with *N*-bromosuccinimide, a bromohydrin **(90)** was isolated which on treatment with potassium acetate in methanol formed an epoxide **(91)**.[51,52] Both the compounds **90** and **91** produced derivative **80** when they were reacted, separately, with Zn in pyridine. Again **90**, when was reacted with collidine, yielded derivative **92**, which formed readily 3,11β-dihydroxyestra-1,3,5(10),8-tetraen-17-one **(93)**. Again, androsta-1,4,8-triene-3,11,17-trione **(94)** formed **95** and **96** when was treated with Zn in DMF **(Scheme 7)**.[52]

Similar reaction protocol using zinc was also applied to 17-acetoxyandrosta-1,4,9(11)-triene-3-one oxime **(97)** in DMF in presence of a little amount of water to form A-ring aromatized derivative **98 (Scheme 8)**.[53]



Scheme 7. Transformtive reactions of androsta-1,4,9(11)-triene-3,17-dione (**79**) and androsta-1,4,8-triene-3,11,17-trione (**94**) to result A-ring aromatization.



Scheme 8. Conversion of 17-acetoxyandrosta-1,4,9(11)-triene-3-one oxime (**97**) into A-ring aromatized derivative **98**.

Thus, it was observed that $\Delta^{1,4}$-dienone-3-one systems containing an additional double bond at the 6-, 8- or 9(11)- position underwent A-ring aromatization with simultaneous demethylation of the 19-methyl group, but *p*-cresol type of rearrangement product and 9/10-seco-aromatized derivatives were found to be formed when androsta-1,4-diene-3,17-dione and androsta-1,4-diene-3,11,17-trione were the starting compounds respectively.

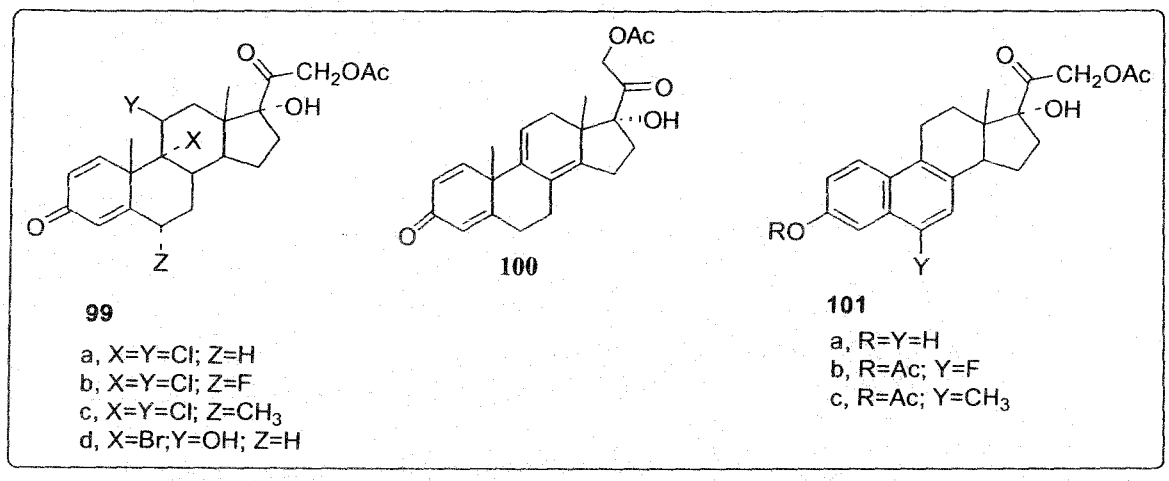
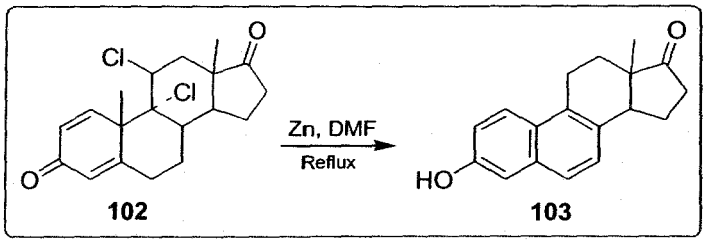


Figure 10. 21-Acetoxy-9α,11β-dichloro-17α-hydroxypregna-1,4-diene-3,20-dione (**99a**) and its derivatives, 21-acetoxy-3,17α-dihydroxy-19-norpregna-1,3,5(10),6,8-pentaen-20-one (**101a**) and its derivatives and 21-acetoxy-17α-hydroxypregna-1,4,8(14),9(11)-tetraene-3,20-dione (**100**).

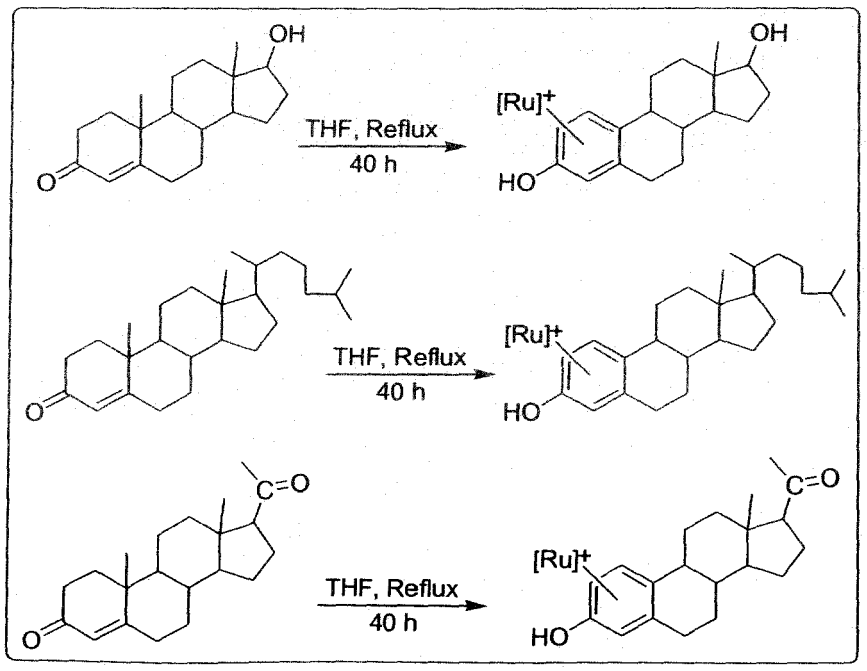
Either ring-A or -B or both ring-A and -B of the steroids were found to be aromatized by using different protocols such as Pd-C catalysis or acid elimination of an allylic hydroxyl group and subsequent rearrangement or pyrolysis by selenium dioxide etc. It is reported that, on refluxing with DMF or pyridine, 21-acetoxy-9α,11β-dichloro-17α-hydroxypregna-1,4-diene-3,20-dione (**99a**) formed 21-acetoxy-17α-hydroxypregna-1,4,8(14),9(11)-tetraene-3,20-dione (**100**) and 21-acetoxy-3,17α-dihydroxy-19-norpregna-1,3,5(10),6,8-pentaen-20-one (**101a**). Varying the substituents of **99a**, as in **99b**, **99c** and **99d**, different products **101b**, **101c** and **101a**, respectively, all consisiting of aromatized AB ring were obtained, though compound **101c** from **99c** was obtained after acetylation and compound **101a** was also obtained from compound **99d** on refluxing with DMF (**Figure 10**). Similar treatment on dienone **102** yielded the diaromatic steroid derivative **103** (**Scheme 9**).[54]



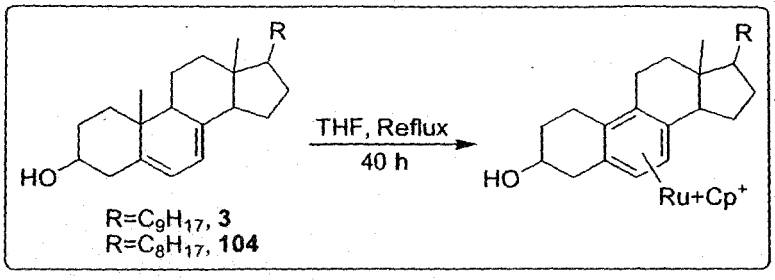
Scheme 9. Transformation of dienone **102** into diaromatic steroid **103**.

The authors in their next communication, reprted that pyridine alone was also effective for aromatization though it took a longer period of time.[55]

When there was a keto group in the A-ring of steroid, it was found to be aromatized by '$(C_5Me_5)Ru^+$', *via* demethylation of the 19-methyl group and/or dehydrogenation reactions.[56,57] '$(C_5Me_5)Ru^+$ was obtained from $[Cp^*Ru(OMe)]_2$ by the treatment of trifluorosulfonic acid (CF_3SO_3H). Some of the reactions are shown in the following scheme (**Scheme 10**). Aromatization was also occurred at B-ring when the same was applied to ergosterol (**3**) or 7-dehydrocholesterol (**104**) (**Scheme 11**).[58]

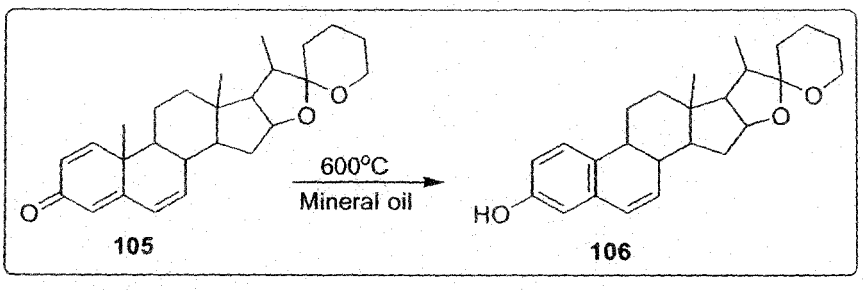


Scheme 10. A-ring aromatization of some steroids by '$(C_5Me_5)Ru^+$'.



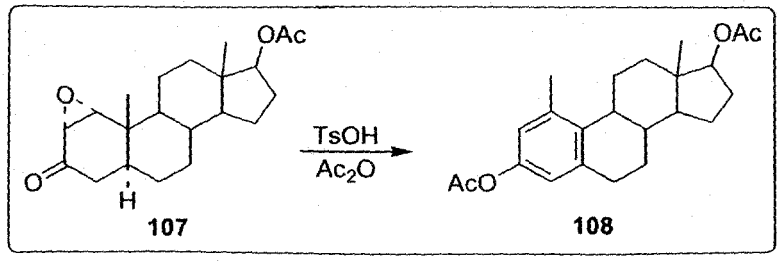
Scheme 11. Transformation of ergosterol (**3**) or 7-dehydrocholesterol (**104**) into B-ring aromatized products.

Aromatization was also carried out on one of the steroidal sapogenin, diosgenin series as was reported by F. Sondheimer and his group where they showed that $\Delta^{1,4,6}$-22a-spirostatriene-3-one (**105**) on vapour phase aromatization at 600^{0}C in mineral oil or in tetralene afforded the corresponding 3-hydroxy-$\Delta^{1,3,5(10),6}$-tetraene (**106**) with the elimination of C-19 methyl group (**Scheme 12**).[59]



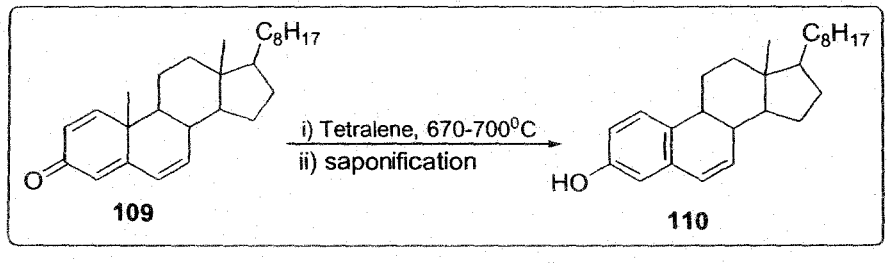
Scheme 12. A-ring aromation of $\Delta^{1,4,6}$-22a-spirostatriene-3-one (**105**).

Epoxy steroids were also found to be aromatized by acid treatment e.g. 17-β-acetoxy-1α,2α-epoxy-5α-androstan-3-one (**107**) formed diacetate of 1-methylestradiol (**108**) (**Scheme 13**).[60]



Scheme 13. Aromatization of ring-A of 17-β-acetoxy-1α,2α-epoxy-5α androstan-3-one (**107**).

Aromatization experiments in the cholesterol series were investigated also by Djerassi and his group.[36-38] They synthesized the aromatic cholesterol compounds following dienone-phenol rearrangement. Initially, 1,4,6-cholestatrien-3-one (**109**) was taken for aromatization reaction and finally compound **110** was obtained (**Scheme 14**).



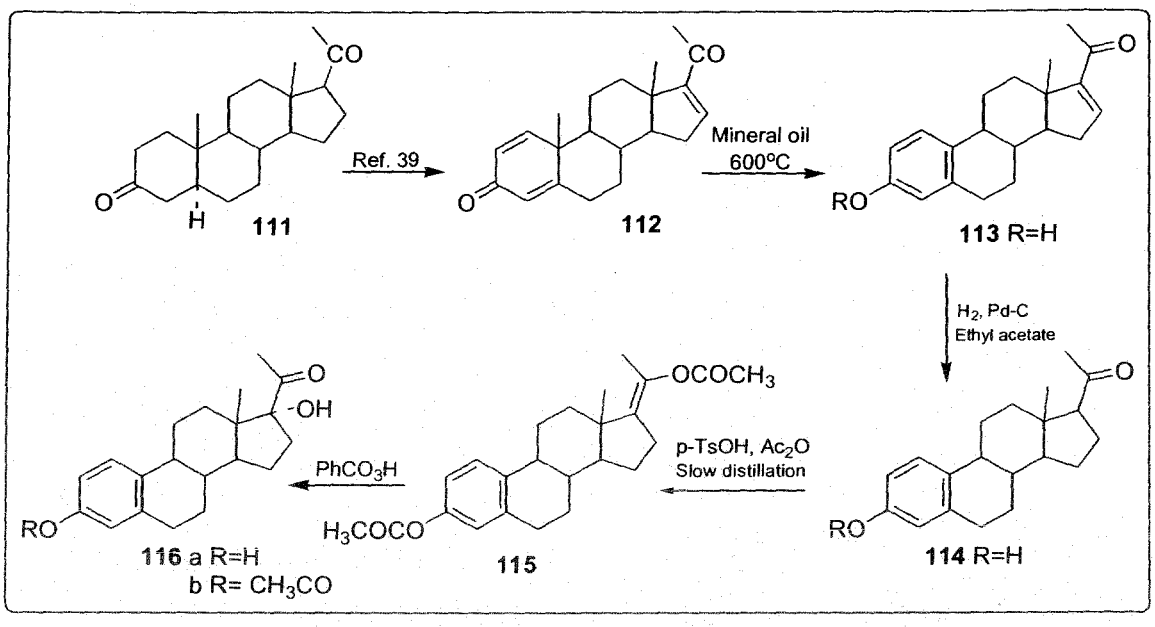
109 **110**

Scheme 14. A-ring aromatized cholesterol from 1,4,6-cholestatrien-3-one (**109**).

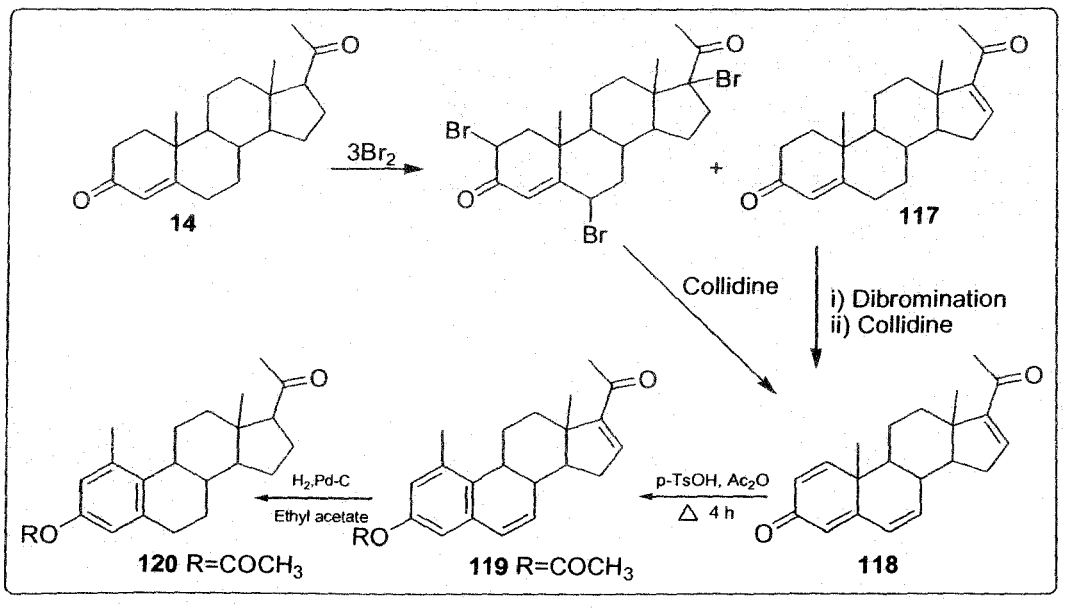
Djerassi et al. prepared[39] 1,4,16-pregnatriene-3,20-dione (**112**) from allopregnane-3,20-dione (**111**) and the reactant **112** was also converted to 3-hydroxy-17-acetyl-1,3,5,16-estratetraene (**113**) by mineral oil vapor phase aromatization. Compound **113** was transformed into the aromatic analogs **114** and **116** of the corpus luteum hormone, progesterone (**14**), and the adrenal hormone, 17β-hydroxyprogesterone. A-ring aromatized **116** was formed from compound **114** *via* **115** (**Scheme 15**).[38]

Progesterone (**14**) or 16-dehydroprogesterone (**117**) on tri- or di-bromination, respectively, followed by collidine dehydrobromination led to 1,4,6,16-pregnatetraene-3,20-dione (**118**), which after dienone-phenol rearrangement or on 4 hours distillation with *p*-tolunesulphonic acid in acetic anhydride formed **119** and this on hydrogenation yielded an aromatic analog **120** of progesterone bearing a methyl group at C-1 (**Scheme 16**).[38]

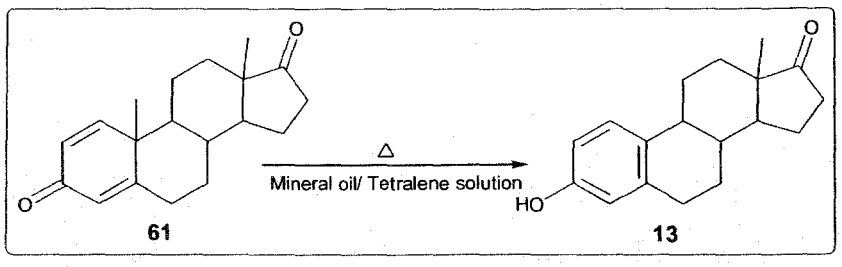
Dryden, Webber and Wieczorek in the year 1964, reported the prepation of estrone (**13**) by the pyrolysis of androsta-1,4-diene-3,17-dione (**61**) in mineral oil-tetralene solution (**Scheme 17**) which was in fact a steroidal aromatization reaction.[61]



Scheme 15. A-ring aromatization of some progesterone derivatives.



Scheme 16. A-ring aromatized products of progesterone (**14**) and 16-dehydroprogesterone (**117**).



Scheme 17. Formation of estrone (**13**) from androsta-1,4-diene-3,17-dione (**61**).

Alvarez and Ruiz reported the aromatization process done in alcohol media[62] on 10β-acetoxyestr-4-ene-3,17-dione (**121**) and 10β-acetoxy-19-norpregna-4-ene-3,20-dione (**122**) or in presence of primary or secondary amine on the former one (**Figure 11**).[63]

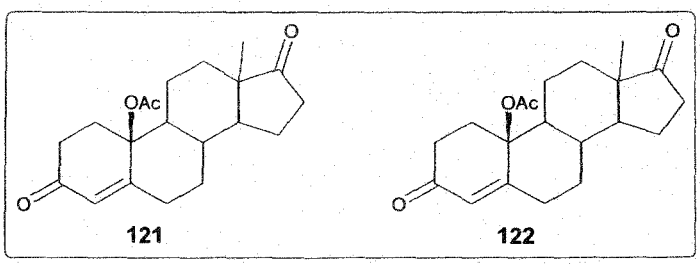


Figure 11. 10β-Acetoxyestr-4-ene-3,17-dione (**121**) and 10β-acetoxy-19-norpregna-4-ene-3,20-dione (**122**).

Not only that, people had also carried out some experiments to prepare aromatized steroids of stomach. R. Y. Kirdani and D. S. Layne studied the rate of aromatization of 10β-hydroxy-19-nortestosterone (**123**) and 17α-ethynyl-10β-hydroxy-19-nortestosterone (**124**) in HCl at 37^0C.[64] Human gut bacteria was found to aromatize the A-ring of 3-oxo-4-cholen-24-oic acid (**125**), 4-androstene-3,17-dione (**126**) and cholesterol (**2a**) (**Figure 12**).[65-66]

Apart from chemical transformations, steroidal hormones were also found to be degraded by microorgsnisms.[67-69] Transformation of 19-hydroxyandrost-3, 17-dione (**127**) to estrone (**13**) by *Pseudomonas sp.*[70] and by *Nocardia restrictus* were reported.[71] 4-Androstene-3-17-dione (**126**) when fermented with a species of *Pseudomonas* isolated from cotton, 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione (**128**) was obtained.[70] *Nocardia restrictus* is such an

organism which degraded androst-4-ene-3,17-dione (**126**) to yield 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione (**128**) (**Figure 12**).[72]

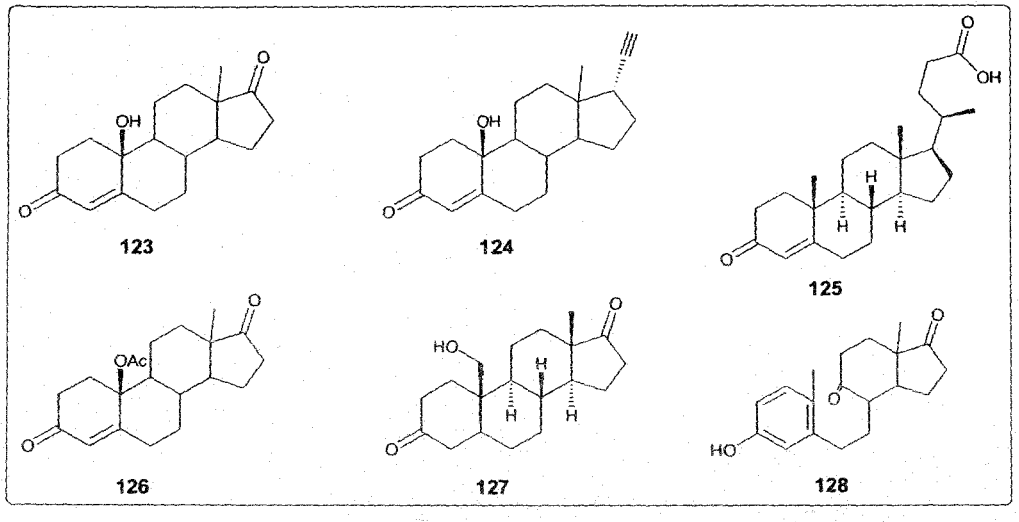
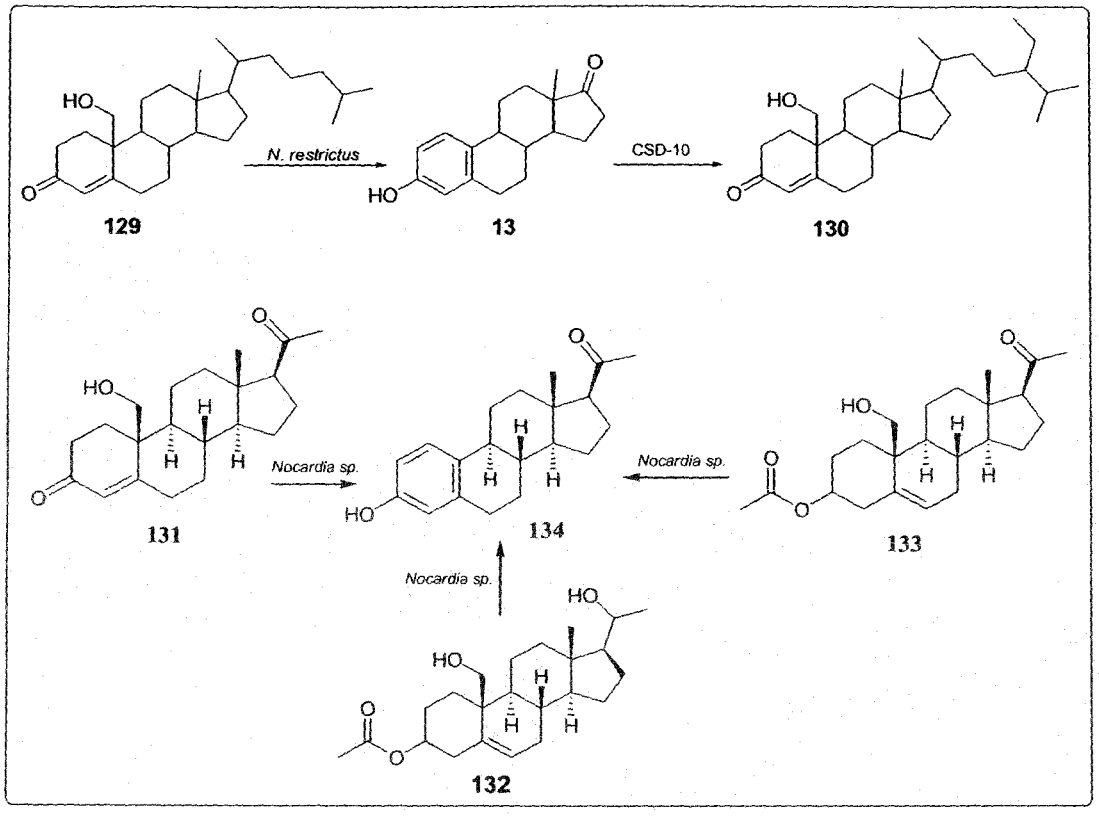


Figure 12. 10β-Hydroxy-19-nortestosterone (**123**), 17α-ethynyl-10β-hydroxy-19-nortestosterone (**124**), 3-oxo-4-cholen-24-oic acid (**125**), 4-androstene-3,17-dione (**126**), 19-hydroxyandrost-3, 17-dione (**127**) and 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione (**128**).

In the year 1965, Charles J. Sih and K. C. Wang reported that **129**, on incubation with *N. restrictus* yieled estrogen (**13**) and the same was obtained when **130** was treated with CSD-10, an organism isolated from soil utilizing cholesterol as a sole carbon source.[73] *Nocardia sp.* was also found to be effective to aromatize 19-hydroxyprogesterone (**131**), 3β,19-dihydroxy-pregn-5-en-20-one 3-acetate (**132**) and pregn-5-ene-3β,19,20β-triol 3-acetate (**133**), into 3-hydroxy-19-norpregna-1,3,5(10)-trien-20-one (**134**), though the side chain was intact. On the contrary, *Septomyxa affinis* aromatized the ring-A and cleaved the side chain of 19-hydroxyprogesterone (**131**) to yield estrone (**13**) (**Scheme 18**).[74]

Hence, in order to aromatize steroidal compounds selectively, people have used several steroidal enones, hydroxyketones, epoxy steroids, dieneones, etc. as the starting materials.



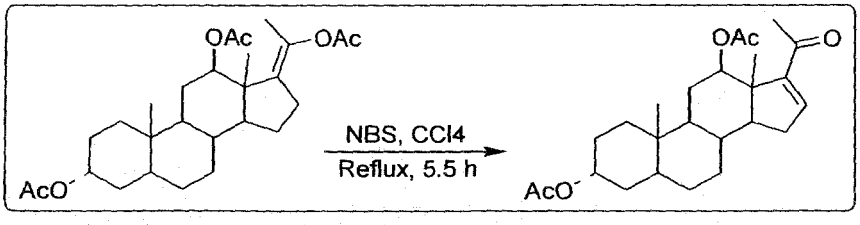
Scheme 18. Steroidal aromatization by microorganisms.

II.B.1.2 A short review on the action of NBS on steroids

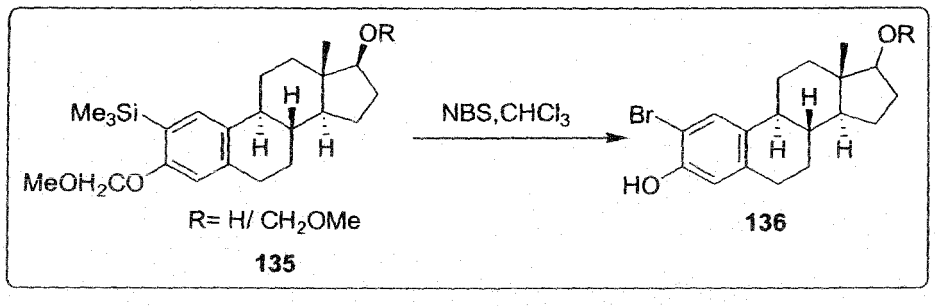
Hanson and Organ reported NBS as a poorer alternative to hydantoin, as mentioned earlier, towards the A-ring aromatization of cholesterol.[40-42] Thus, having the scope of aromatization of steroids using NBS, we reviewed also the various transformative reactions of steroids using NBS.

NBS was used as a good dehydrogenating agent for the enol acetate of 20-keto steroid to produce C16-C17 double bond[75] (**Scheme 19**) and for Δ^7-cholestenyl benzoate to produce $\Delta^{7,9(11)}$ diene.[76]

O-Lithiation of the methoxymethyl ethers of oestradiol (**135**) using *s*-butyl lithium, followed by treatment with trimethylsilyl chloride, yielded the 2-trimethylsilyl derivatives, which were readily converted into 2-bromo-oestradiol (**136**) by NBS in $CHCl_3$ (**Scheme 20**).[77]

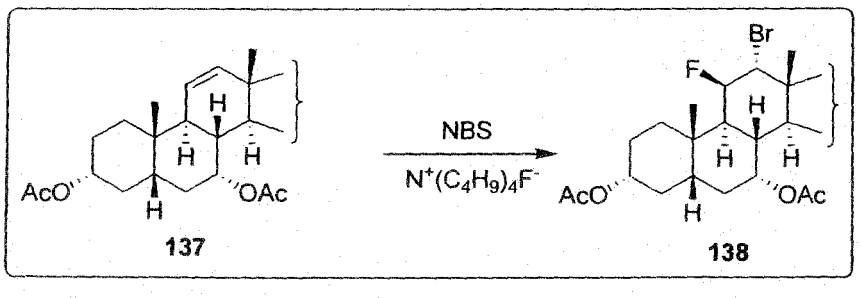


Scheme 19. Dehydrogenation of enol acetate of 20-keto steroid.



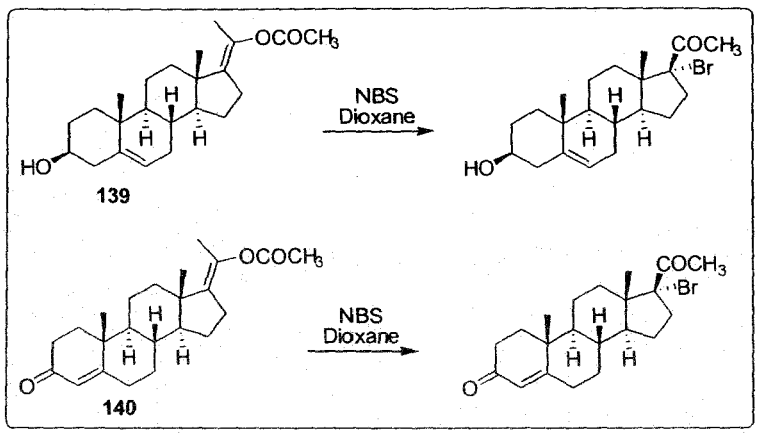
Scheme 20. Transformation of estradiol derivative **135** into 2-bromoestradiol **(136)** by NBS.

N-bromosuccinimide and tetra-*n*-butylammonium fluoride on the steroid derivative **137** resulted the corresponding 11β-fluro-12α-bromoester derivative **(138)** **(Scheme 21)**.[78]



Scheme 21. Conversion of **137** to its bromo-fluoro derivative **(138)**.

17,20-Enol acetate of progesterone **(139)** and pregnenolone **(140)** on NBS treatment afforded the corresponding 17-brominated compounds respectively **(Scheme 22)**.[79]



Scheme 22. Formation of 17-brominated derivatives of 17, 20-enol acetate of progesterone (**139**) and pregnenolone (**140**).

Fischer and Rajagopalan reported the formation of desoxycholic acid etherate (**141**) from cholic acid (**9**) by using NBS in acetic acid-water followed by reduction. NBS also oxidized methyl etiocholate (**142**) to the 7-keto derivative **143** and desoxycholic acid/deoxycholic acid (**11**) to diketo acid (**144**) in aqueous *t*-butanol.[80-83] Similarly, NBS with methyl-3α-acetoxy $\Delta^{7,9(11)}$-choladienate (**145**) in *t*-BuOH/dil. H_2SO_4 at 0^0C furnished methyl-3α-acetoxy-7-keto-$\Delta^{9(11)}$-cholenate (**146**), methyl-3α-acetoxy-11-keto-Δ^8-cholenate (**147**) and methyl-3α-acetoxy-11α-hydroxy-7-keto-Δ^8-cholenate (**148**).[84] When Δ^2-3-acetoxycholestene (**149**) was treated with NBS, Δ^1- and Δ^4-cholesten-3-one (**150** and **4a** respectively), 2-bromocholestan-3-one (**151**) and the unreacted starting material were isolated. With the increase of the reaction time, the amount of 2-bromocholestan-3-one (**151**) was found to increase and that of the Δ^1-cholestene-3-one (**4a**) was decreased. The same reaction with Δ^2-3-acetoxycoprostene (**152**) resulted Δ^4-cholesten-3-one (**150**) and crude Δ^1-coprosten-3-one (**153**) (**Figure 13**).[85]

NBS was also found effective in forming cholestane-3β,5α-diol-6-one (**154**) either from cholesterol (**2a**), cholestane-3β,5α,6β-triol (**155**) or cholesterol α-oxide (**156**) in aqueous acetone, though monobromo- and dibromo-derivatives (**157** and **158** respectively) were also formed at poor amount from the last substrate. Cholesteryl-α-oxide acetate (**159**) was also converted to the corresponding 6-keto compound **154** by *N*-bromosuccinimide (**Scheme 23** and **Scheme 24**).[86]

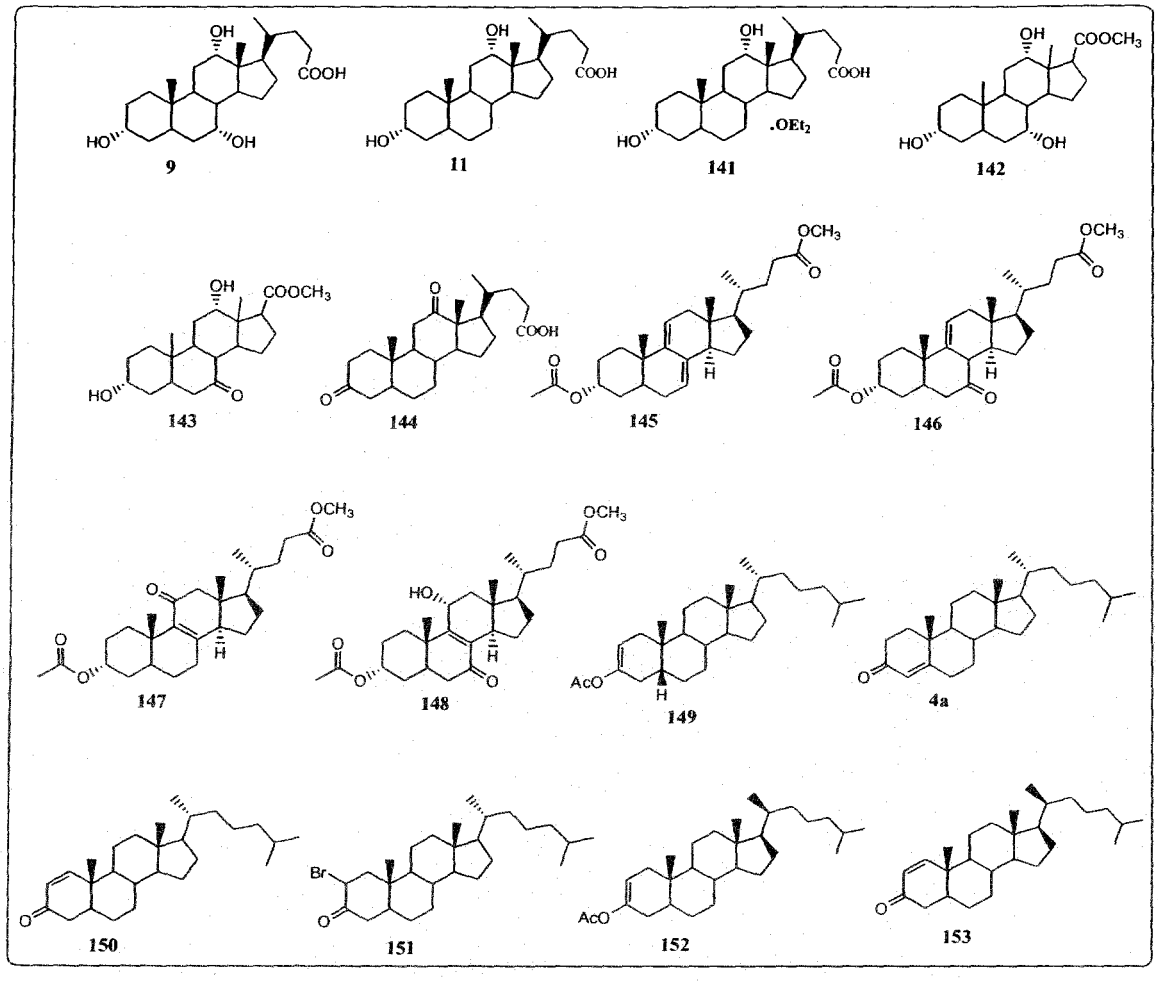
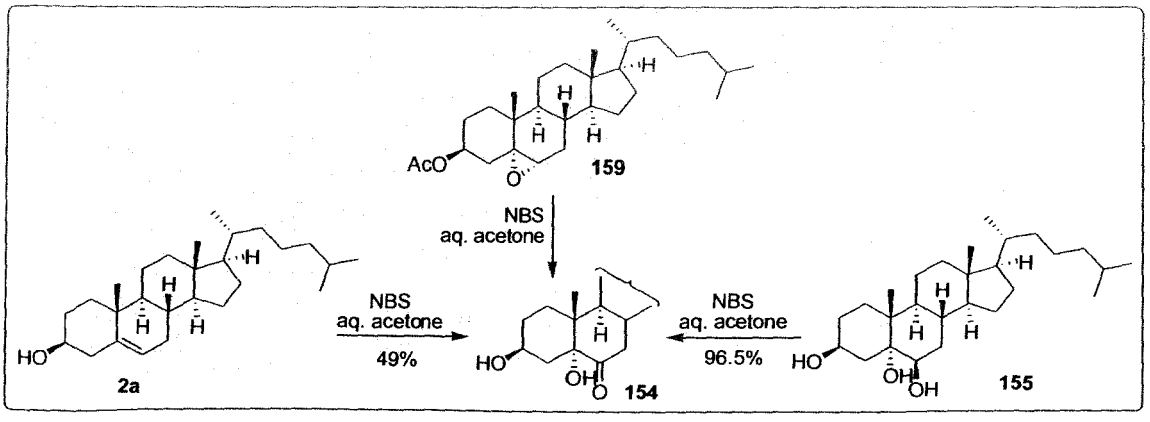
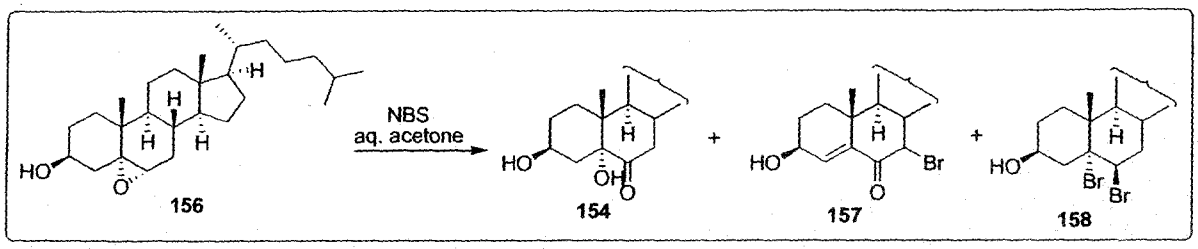


Figure 13. Cholic acid and cholesterol derivatives.

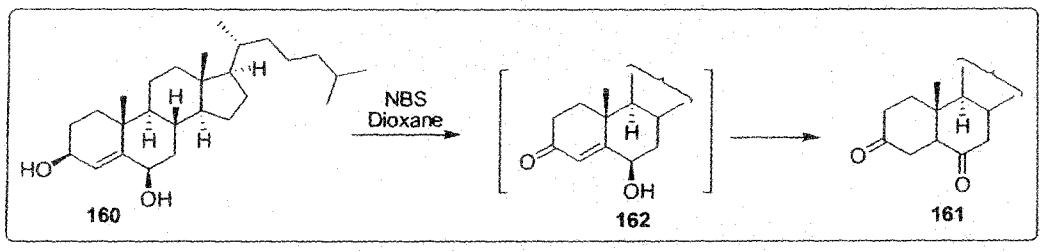


Scheme 23. Formation of cholestane-3β,5α-diol-6-one (**154**) from different substrates by NBS.

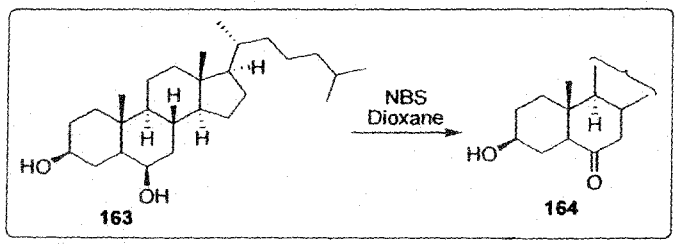


Scheme 24. Formation of cholestane-3β,5α-diol-6-one (**154**) and mono- and dibromo-derivatives (**157** and **158** respectively) from cholesterol α-oxide **156** using NBS.

Cholest-4-ene-3β,6β-diol (**160**) on treatment with NBS formed cholestane-3,6-dione (**161**) *via* 6β-hydroxy cholest-4-en-3-one (**162**) while similar treatment on the corresponding saturated diol (**163**) converted only the 6-hydroxy group into keto group (**164**) (**Scheme 25** and **Scheme 26**).

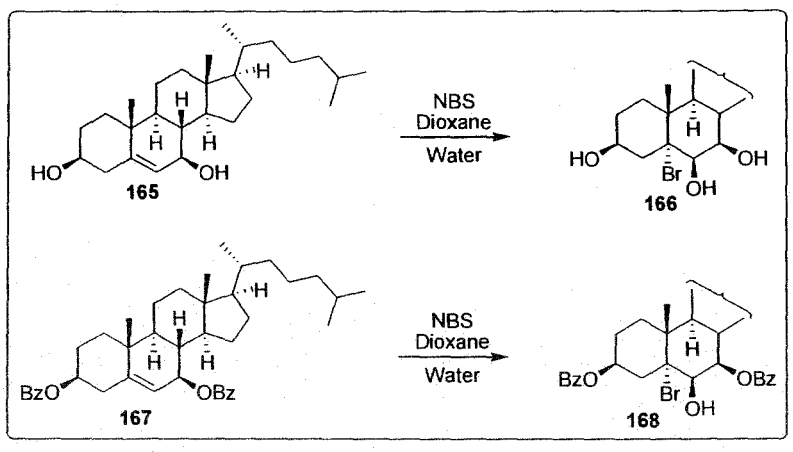


Scheme 25. Conversion of cholest-4-ene-3β,6β-diol (**160**) to cholestane-3,6-dione (**161**).



Scheme 26. Conversion of cholest-3β,6β-diol (**163**) to 3β-hydroxycholestan-6-one (**164**).

But when 7β-hydroxy cholesterol (**165**) was reacted with NBS in aqueous dioxane, none of the hydroxy groups were affected, rather 5α-bromo-cholestane-3β,6β,7β-triol (**166**) was formed. The bromohydrin derivative **168** was obtained by a similar reaction from cholest-5-ene-3β,7β-diol dibenzoate (**167**) (**Scheme 27**).[87]

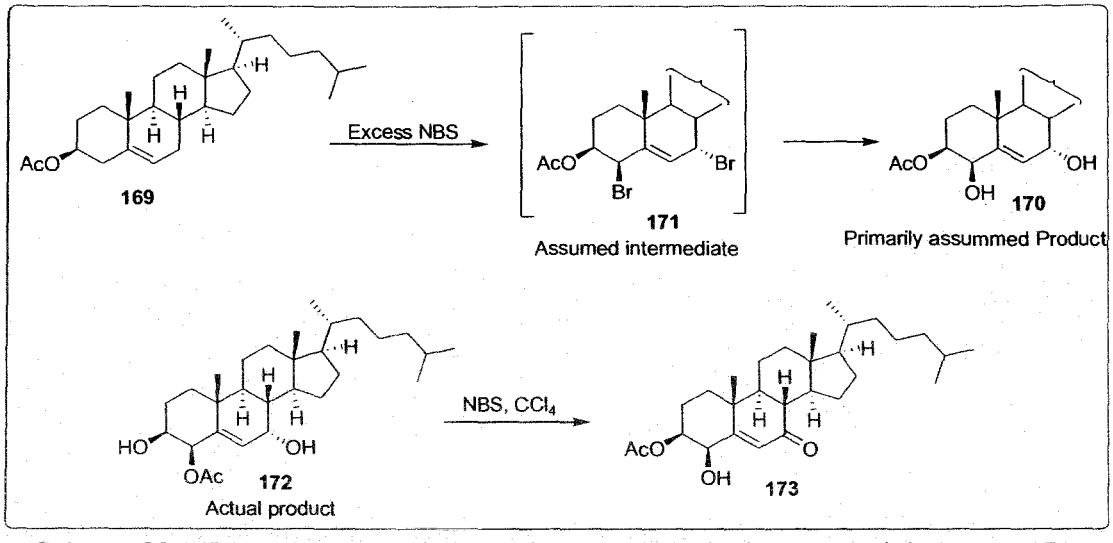


Scheme 27. Action of NBS on 7β-hydroxy cholesterol (**165**) and its dibenzoate derivative (**167**).

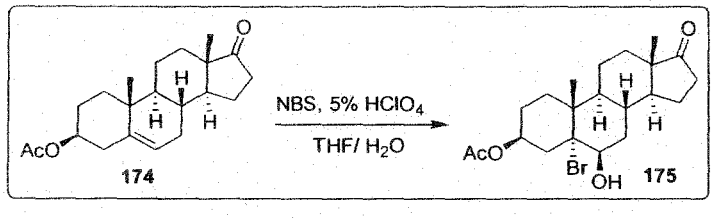
After reflux with *N*-bromosuccinimide in carbon tetrachloride, cholesteryl acetate (**169**) produced a solid primarily assumed to be 3β-actoxy-Δ^5-cholestene-4β,7α-diol (**170**) and the author suggested that it was derived from cholesteryl acetate by α,α′–dibromination (**171**) followed by the replacement of the bromines by hydroxyl group during chromatography.[88-90] NBS was capable to convert the product to the corresponding 7-keto compound (**173**).[91] Later the product 3β-actoxy-Δ^5-cholestene-4β,7α-diol (**170**) was identified as 4β-acetoxy-Δ^5-cholestene-3β,7α-diol (**172**) by R. W. Jailer and his co-workers (**Scheme 28**).[92]

19-Norandrostane, a very important steroid belongs to a class of known androgenic, anabolic, gestagenic agents and ovulation inhibitors.[93] A key intermediate for the synthesis of 19-norandrostane was 3β-acetoxy-5α-bromo-6β-hydroxyandrostan-17-one (**175**) which could be prepared from the reaction of 3β-acetoxy-5-androstene-17-one (**174**) with *N*-bromosuccinimide (**Scheme 29**).[94]

Similarly, 5α-bromocholestane-3β,6β-diol 3-acetate (**176**) was achieved from cholesteryl acetate (**169**) by NBS.[95] NBS in a number of organic solvents was also employed to convert 11β,17α-dihydroxy-21-acyloxy-4-pregnene-3,20-dione (**177**) into 17α-hydroxy-21-acyloxy-4-pregnene-3, 11, 20-trione (**178**) (**Figure 14**).[96] NBS could also play the role of a catalyst for the esterification of tertiary alcohols of cyproterone (CYP, **179**) and medroxyprogesterone (MGP, **181**) to produce 6-chloro-17-hydroxy-1α,2α-methylene-4,6-pregnadien-3,20-dione 17α-acetate (**180**) and 17α-hydroxy-6α-metylpregn-4-ene-3,20-dione acetate respectively (**182**) (**Scheme 30** and **Scheme 31**).[97]



Scheme 28. NBS treatment on cholesteryl acetate (**169**) and cholesteryl derivative **172**.



Scheme 29. Treatment of 3β-acetoxy-5-androstene-17-one (**174**) with NBS.

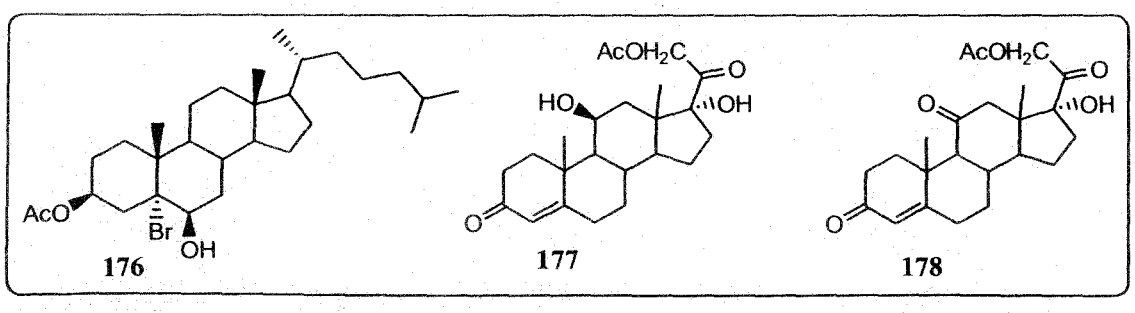
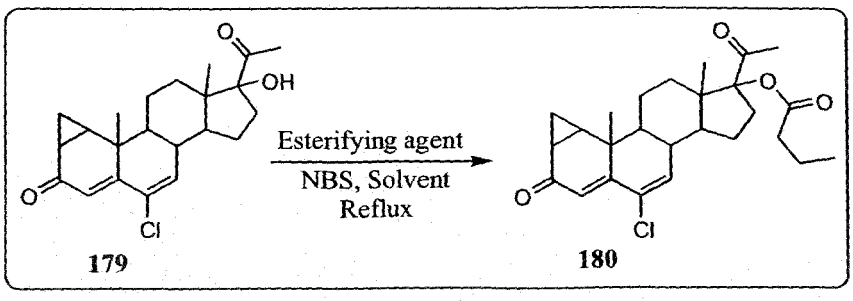
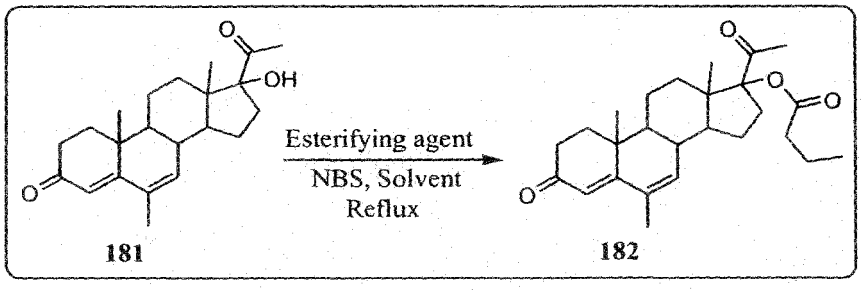


Figure 14. Structures of 5α-bromocholestane-3β,6β-diol 3-acetate (**176**), 11β,17α-dihydroxy-21-acyloxy-4-pregnene-3,20-diones (**177**) and 17α-hydroxy-21-acyloxy-4-pregnene-3,11,20-trione (**178**).



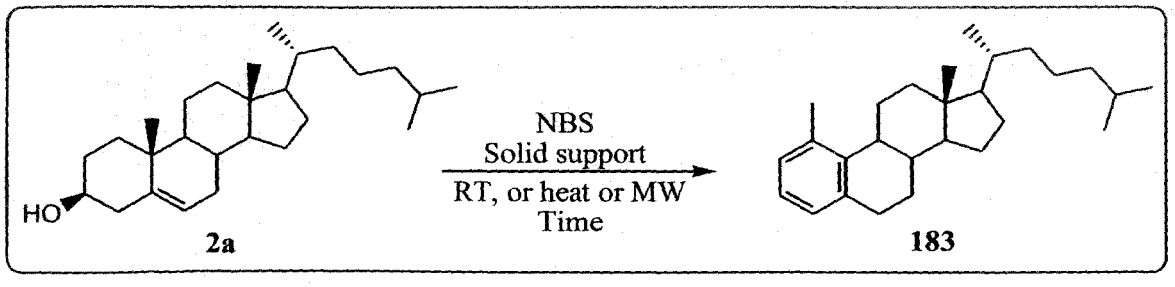
Scheme 30. Acylation of CYP



Scheme 31. Acylation of MGP.

II.B.2 Present Work

As a consequence of the importance given towards the selective aromatization of a steroidal skeleton, we started working in this area and finally could able to report a novel synthesis of A-ring aromatized cholesterol and β-sitosterol *via* a very simple one-pot solid phase reaction using *N*-bromossuccinimide. Cholesterol (**2a**) was reacted with *N*-bromosuccinimide on solid support to result 1-methyl-19-norcholesta-1,3,5(10)-triene (**183**) (**Scheme 32**).



Scheme 32. A-ring aromatization of cholesterol (**2a**) by NBS.

II.B.2.1 Present work: Results and Discussion

A mixture of cholesterol [**(2a)**, 0.38 g, 1.0 mmol] and *N*-bromosuccinimide (0.42g, 5.0 mmol) in activated silica furnished a selective aromatic product 1-methyl-19-norcholesta-1,3,5(10)-triene, **(183)** **(Scheme 32)**. To the best of our knowledge, the isolated compound is being reported for the first time and it is actually isomeric to the compound reported by Hanson and Organ.[40-42]

Compound **183** appeared as pale greenish yellow gum (0.17 g, 0.46 mmol, 46%) with characteristic odour which on standing (about 30 days at room temperature) slowly solidifies. The solidified mass was analysed (TLC and NMR) to be a mixture of compounds which could not be isolated and moreover, it did not contain the aromatized product (**183**) (by comparison with the TLC and NMR spectra of the gummy sample (**183**) and its solidified mass).

The isolated product (**183**, as a gum) was characterized on the basis of UV, IR, ^{1}H, ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The UV spectrum of **2** showed a peak, the pattern of which is similar to the benzenoid derivatives (λ_{max}=277 nm, €=2360). The IR spectrum showed absorptions at ν_{max} 2951, 1462, 738 and 815 cm^{-1} characteristic for an aromatic ring. ^{1}H NMR spectrum showed peaks at δ 2.19 (3H, s, CH_3), 6.97 (1H, d, *J*=7.2 Hz, CH), 7.05 (1H, t, *J*=7.5 Hz, CH) and 7.17 (1H, d, *J*=8.1 Hz, CH) ppm which correspond to the aromatic A-ring of the product. The ^{1}H decoupled ^{13}C NMR spectrum of **183** showed 27 distinct resonances of which 6 belong to the aromatic carbons. The mass spectrum displayed a molecular ion peak (M^+) and base peak at m/z 366 (38%) and 365 (M^+-H) respectively.

The reaction was attempted both on solid support as well as in solution phase. However no aromatization was observed in solution phase.

II.B.2.1.1 Optimization of the reaction conditions

For a preliminary study of optimization we performed the reaction using three different protocols, viz., (i) reaction at room temperature; (ii) by the use of microwave irradiation (taking silica gel 60-120 mesh and F254, both) and (iii) at elevated temperatures (taking only silica gel 60-120 mesh). Although the conversion is found to be rapid both in microwave irradiation and at higher temperature but the yield was not satisfactory following either of the protocols. It was further established that the optimized time (in terms of % yield) at room temperature is 72 h, at thermal condition 5 min and in microwave it was 5-7 min (150W, 100^0C). (**Table 1** and **Table 2**).

Table 1. Optimization of the reaction condition taking cholesterol and NBS[a]- at room temp and MW irradiation.

Silica used[b]	Room temp Yield (%)[c]					MW induced Yield (%)[c]
	12h	24h	48h	72h	120h	5-7 min
60-120 mesh	11	22	25	46	18	30
F254	5	13	13	11	4	27

[a]reactions were performed on 0.25 g, 0.65 mmol substrate.

[b]1 g $mmol^{-1}$ of activated silica gel was used.

[c]yield refers to isolated pure product.

The slow reaction rate of the transformation is quite obvious from the fairly longer optimized reaction time at room temperature. Investigation indicated that beyond 72 h there is a gradual decrease of % yield of the product (**Table 1**). This may be explained on the basis of the observation that beyond 72 h it slowly started solidifying and consequently analysis of the solid mass indicated that it is different from aromatized product **183** and finally identified as a mixture of compounds as stated above.

Table 2. Optimization of the reaction condition[a] taking cholesterol and NBS[b]- at elevated temperatures.

Temp.	Yield (%)[c] in various time intervals				
	1 min	5 min	15 min	30 min	60 min
50^0C	18	36	33	25	21
100^0C	15	35	27	24	19

[a] reactions were performed on 1 g $mmol^{-1}$ of activated silica gel (60-120 mesh).

[b]on 0.25 g, 0.65 mmol substrate.

[c]yield refers to isolated pure product.

Optimization study for the amount of silica support (silica gel 60-120 mesh) for the above aromatization reaction was also performed and it was established that 1 g $mmol^{-1}$ of silica is best suitable for the transformation. (**Table 3**)

Table 3. Optimization for the silica support [a] - taking cholesterol and NBS[b].

Entry	Silica in g $mmol^{-1}$	Isolated yield of **183** (%)
1	0.25	22
2	0.50	37
3	0.75	40
4	1.00	46
5	1.50	46
6	2.00	44

[a]reactions were performed on activated silica gel (60-120 mesh). [b]on 0.25 g, 0.65 mmol substrate and at room temperature (72 h).

Variation of solid supports (**Table 4**) indicated that silica gel 60-120 mesh, (used normally for column chromatography) found to be the most effective support for the transformation. Silica gel F254 gave poor yield, alumina and KF-alumina did not yield the product **183** at all, bentonite and 4Å molecular sieves showed very little transformation. After each and every experiment the unreacted starting material, cholesterol, has been recovered quantitatively. Catalytic role of silica for the above transformation has also been tested (**Table 5**), other solid supports used in the study gave very poor yield of the product.

Table 4. Optimization for the solid support [a] - taking cholesterol and NBS[b].

Solid support	Yield (%)[c]	Recovered Cholesterol (%)[c]
Silica gel (60-120 mesh)	46	35
Silica gel F254	11	87
Alumina neutral	NR[d]	96
KF-Alumina	NR[d]	97
4 A Molecular sieve	9	87
Bentonite	12	85
neat	NR[d]	98

[a]1g $mmol^{-1}$ activated solid support was used. [b]reactions were performed on 0.25 g, 0.65 mmol substrate. [c]yield refers to isolated pure compounds. [d]NR= no reaction observed.

Recyclability of the catalyst was tested by the following way - after the reaction is over, chloroform was added to the reaction mixture and the silica gel was filtered off followed by successive washings with methanol (x 2), acetone (×1), and was activated (150°C/ 1mm Hg, 1 h). The recovered activated silica was then used in a fresh transformation as the supporting medium for the reaction and it was found that upto six consecutive runs, there was no appreciable change in the % yield of the desired product. Thus silica may be considered as a very effective reusable solid support for this specific transformation. **(Table 5)**

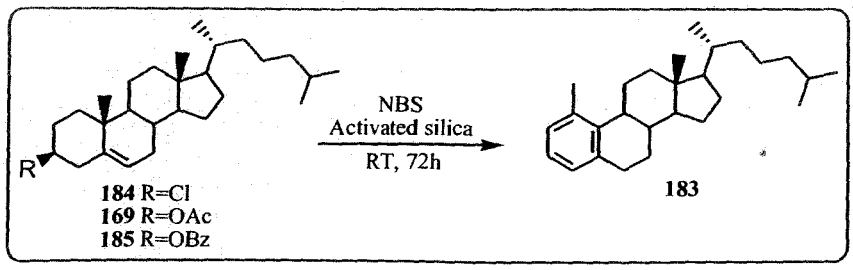
Table 5. Recycling experiment using cholesterol and NBS catalyzed by silica[a].

Run	Isolated yield of **183** (%)
1	43
2	45
3	43
4	42
5	40
6	42

[a]1g $mmol^{-1}$ of silica gel (60-120 mesh) was used.

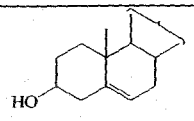
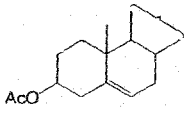
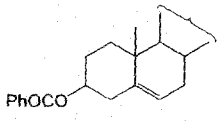
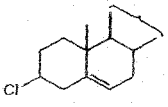
II.B.2.1.2 Mechanistic investigation

In order to find out the probable mechanistic pathway we have carried out the reaction taking different cholesterol derivatives. Replacement of C-3 -OH by -Cl (**184**) showed similar result but introduction of –OAc (**169**) gave better yield compared to cholesterol itself. On the other hand the benzoate derivative (**185**) gave poor yield of the product (**Table 6**). Thus the transformation cannot be correlated with the position of the subsbstituent in ring-A of cholesterol (**Scheme 33**).



Scheme 33. Formation of **183** from cholesterol derivatives.

Table 6. Effect of the 3-substituents on yield of **183**.

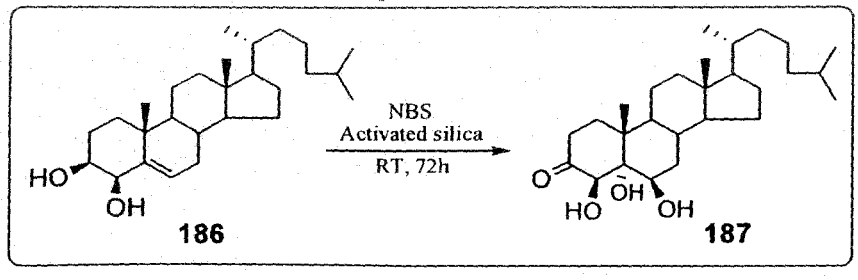
Entry	Substrate[a]	Reaction conditions[b]	Yield (%)[c]
1		RT, 72 h	46
2		RT, 72 h	60
3		RT, 72 h	32
4		RT, 72 h	42

[a] reactions were performed on 1 mmol scale.

[b] 1g activated silica gel (60-120 mesh) was used.

[c] yield refers to isolated pure product.

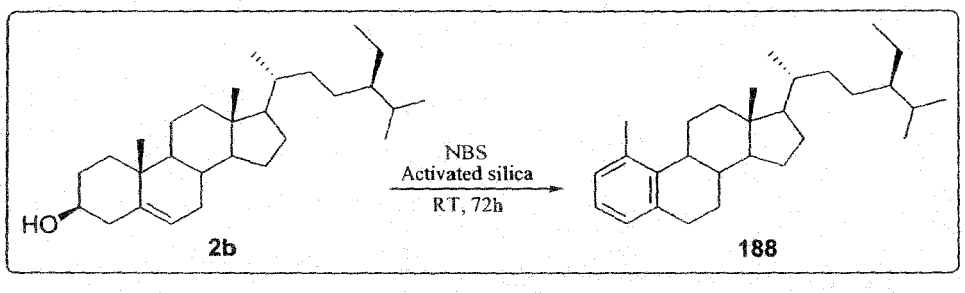
To find out the role of C-4 hydrogens we applied the same protocol on 4β-hydroxy cholesterol (**186**). Surprisingly we did not obtain any A-ring aromatized product rather we got a totally different product (**187**) (**Scheme 34**). Thus, from this investigation it was clear that to have the aromatized product both the C-4 hydrogens were required.



Scheme 34. One-pot synthesis of 3-keto-4β,5α,6β-trihydroxy cholesterol (**187**).

II.B.2.1.3 Application of the reaction protocol on other steroids

In order to generalize the findings, we extended the above reaction on other steroidal systems, such as diosgenin (**16**) and β-sitosterol (**2b**) and, whereas, the corresponding aromatic β-sitosterol analogue was obtained but surprisingly we could not isolate similar aromatic analogue of diosgenin using NBS, although Sondheimer and his group has reported its aromatization *via* another route.[59] The aromatized β-sitosterol analogue, i.e. 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**) was identical to that obtained by the dehydrohalogenation of 3β-chloro-5α-bromo-5α-stigmastan-6β-ol, as was performed by A.A. Akhrem et. al. (**Scheme 35**).[98]



Scheme 35. One-pot synthesis of 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**).

II.B.3 Experimental

II.B.3.1 General: UV spectrum was measured on JASCO V-530 UV/VIS Spectrophotometer. Infrared spectrum was measured in neat with a Shimadzu FT-IR 8300 Spectrometer. The NMR spectra were recorded on a 300 MHz BrukerAvance FT-NMR spectrometer with $CDCl_3$ as solvent and TMS as internal reference, chemical shifts are expressed as d ppm. The DART-MS was recorded on a JEOL-Accu TOF JMST100LC mass spectrometer having a DART (Direct Analysis in Real Time) source. Analytical-TLC was performed with silica gel plates using silica gel G for thin layer chromatography (Merck).

II.B.3.2 Representative reaction for the synthesis of products 183, 187 and 188: Silica gel (60-120 mesh, 1.0 g) was activated (150°C/1mm Hg, 1 h) and mixed with cholesterol (0.38 g, 1.0 mmol) and *N*-bromosuccinimide (recrystalized from hot water to have white crystals, 0.42 g, 5.0 mmol) taking in a dry mortar. The mixture was then pasted well to dust by a pestle, transferred to a round bottom flask and was kept in stirring for 72 h. Next, chloroform (50 mL) was poured, filtered and the filtrate was washed with water (3×30 mL) and dried over Na_2SO_4. The solvent

was evaporated and the residue was purified by column chromatography using petroleum ether as the eluent. Compound **183** appeared as light greenish yellow gum (0.17 g, 0.46 mmol, 46%) with characteristic odour.

II.B.3.3 Product Characterization

II.B.3.3.1 1-Methyl-19-norcholesta-1,3,5(10)-triene (183): Eluent in column chromatography: petroleum ether. Pale yellow gum, Yield: 46%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.85 (3H, s, CH_3), 1.25 (3H, s, CH_3), 2.19 (3H, s, CH_3), 6.97 (1H, d, *J*=7.2 Hz, CH), 7.05 (1H, t, *J*=7.5 Hz, CH), 7.17 (1H, d, *J*=8.1 Hz, CH). ^{13}C NMR (75MHz, $CDCl_3$): d 11.9, 18.6, 19.8, 22.5 and 22.8 ($5CH_3$), 23.8, 23.9, 26.8 and 27.2 ($4CH_2$), 28.0 (CH), 28.3 and 29.7 ($2CH_2$), 35.8 (CH), 36.2 (CH_2), 37.8 (CH), 39.5 and 40.0 ($2CH_2$), 42.6 (C), 44.4, 55.6, 56.3, 123.0, 125.2 and 127.1 (6CH), 135.1, 136.1 and 140.5 (3C). UV (Petroleum ether): λ_{max}=277 nm, €=2360. FTIR (neat, cm^{-1}) ν 2951 (Ar-H str.), 2867 and 2929 (CH_3 str.), 1462 (Arom. ring vib.), 1455 and 1380 (CH_3 def.), 815 and 738 (Arom. H). Anal.Calcd for $C_{27}H_{42}$ (366.34): C, 88.44; H 11.56%. Found: C, 88.51; H 11.47%. DART-MS, m/z (%): 366 (M^+, 38), 365 (100), 364 (17), 363 (32), 361 (9), 353 (4), 351 (4).

II.B.3.3.2 3-Keto-4β,5α,6β-trihydroxy cholesterol (187): Eluent in column chromatography: 25% ethyl acetate in petroleum ether. White solid, Yield: 30%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, *J*=6.3 Hz, 3H), 0.91 (d, *J*=6.3 Hz, 3H) 1.21 (s, 3H), 1.42 (s, 3H), 2.30-2.44 (m, 1H), 5.35 (t, *J*= 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.1, 18.6 and 19.0 ($3CH_3$), 21.5 (CH_2), 22.5 and 22.8 ($2CH_3$), 23.7 and 23.9 (CH_2), 28.0 (CH), 28.1 (CH_2), 30.1 (CH), 30.5, 32.5 and 32.6 ($3CH_2$), 35.7 (CH), 36.1, 39.4 and 39.6 ($3CH_2$), 42.6 and 43.8(2C), 46.2, 55.5, 56.1, 70.4, 71.5 and 78.7 (6CH), 210.7(C=O). IR (KBr, cm^{-1}) ν 3500 (Intramolecular H-bond), 3224 (intermolecular H-bond), 1718 (C=O), 1456 (CH_3, CH_2 def.), 1382 (C-CH_3 def.), 1051-1253, (C-O str.) 713-833. DART-MS, m/z (%): 400, 399 (100%), 398, 397.

II.B.3.3.3 1-Methyl-19-norstigmast-1,3,5(10)-triene (188): Eluent in column chromatography: petroleum ether. Pale yellow gum, Yield: 10%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.82 (d, *J*=6 Hz, 3H), 0.85 (s, 3H), 0.95 (d, *J*=6Hz, 3H), 1.25 (s, 3H),2.21 (s, 3H), 6.99(d, *J*=7.2 Hz, 1H), 7.07(t, *J*=7.5 Hz, 1H), 7.19(d, *J*=7.8 Hz, 1H) ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ 11.9,

18.6, 18.8, 19.1 and 19.8 ($5CH_3$), 23.1 (CH_2), 23.9 (CH_3), 23.9, 26.1, 27.2, 27.8 and 28.3 ($5CH_2$), 29.2 (CH), 29.7 (CH), 33.9 (CH_2), 36.2 (CH), 37.9 (CH_2), 40.1 (CH_2), 42.7 (C), 44.5, 45.9, 55.7, 56.3, 123.1, 125.2 and 127.2 (7 CH), 135.3, 136.3 and 140.7 (3C). FTIR (neat, cm^{-1}) ν 2922 (Arom. H str.), 1377 (CH_3 def.), 721 (Arom. H).

II.C Conclusion: A one-pot green approach has been developed to aromatize A-ring of cholesterol selectively using *N*-bromosuccinimide. The simplicity of the reaction condition and a cost effective reaction protocol is highly encouraging for the environmentally benign chemical transformations. The reaction was found equally effective for the formation of corresponding aromatized β-sitosterol analogue, and application of the reaction condition to 4β-hydroxy cholesterol produced 3-keto-4β,5α,6β-trihydroxy cholesterol as the single product.

II.D Supporting spectra (NMR, IR, Mass):

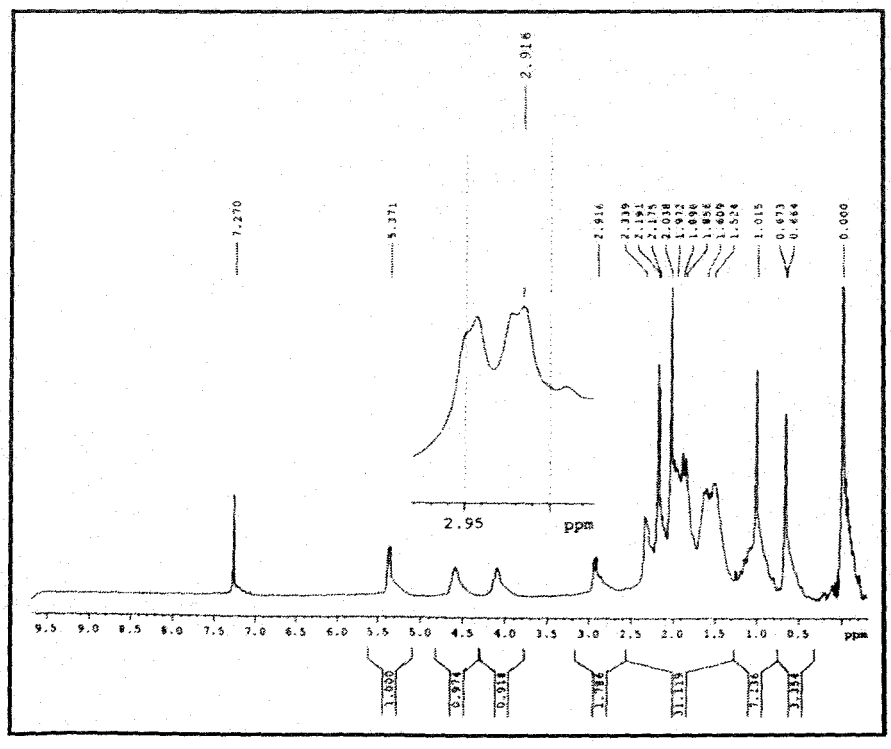


Figure 15. ^1H NMR spectrum of cyclic ether **73**.

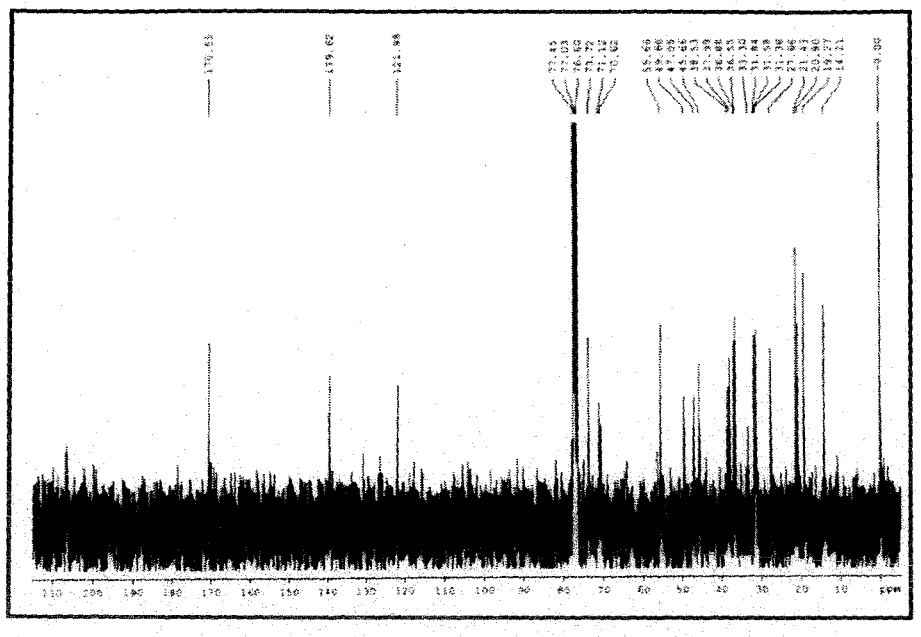


Figure 16. ^{13}C NMR spectrum of cyclic ether **73**.

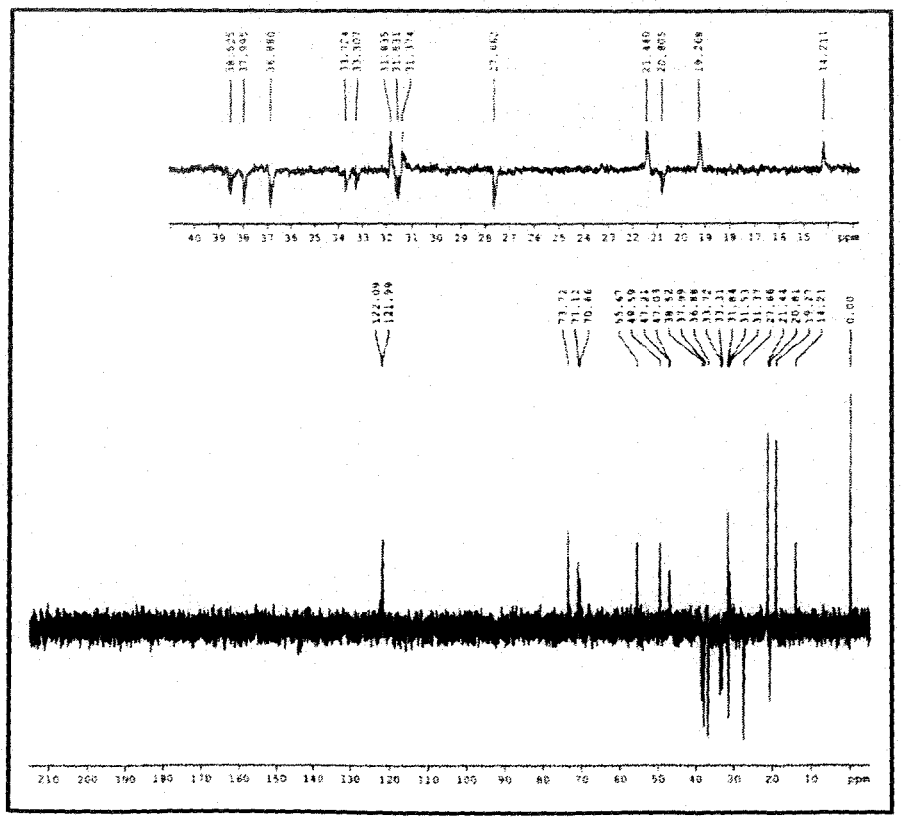


Figure 17. DEPT-135 NMR spectrum of cyclic ether **73**.

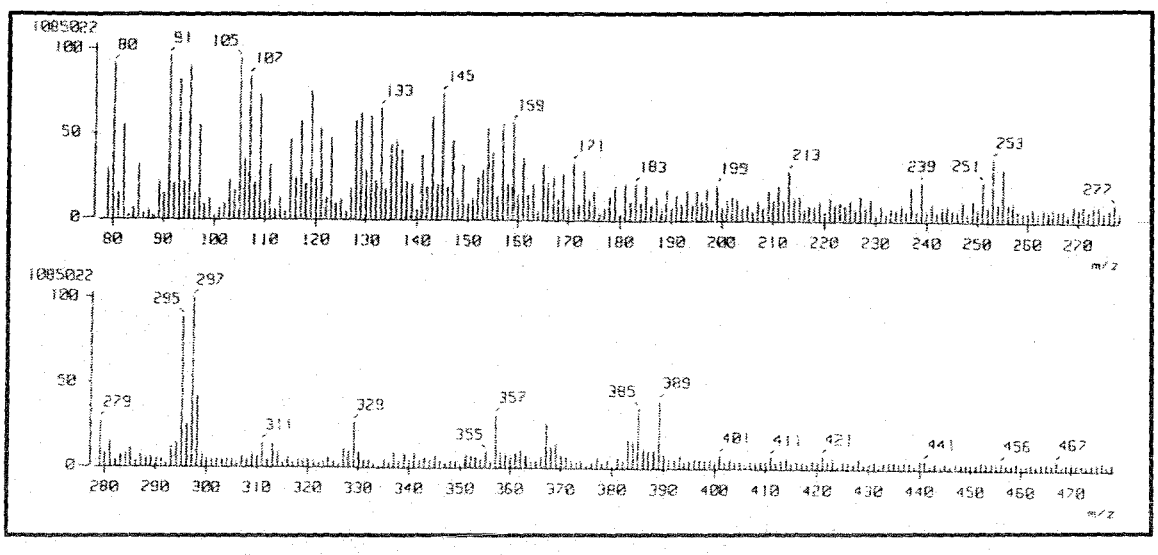


Figure 18. Mass spectrum of cyclic ether **73**.

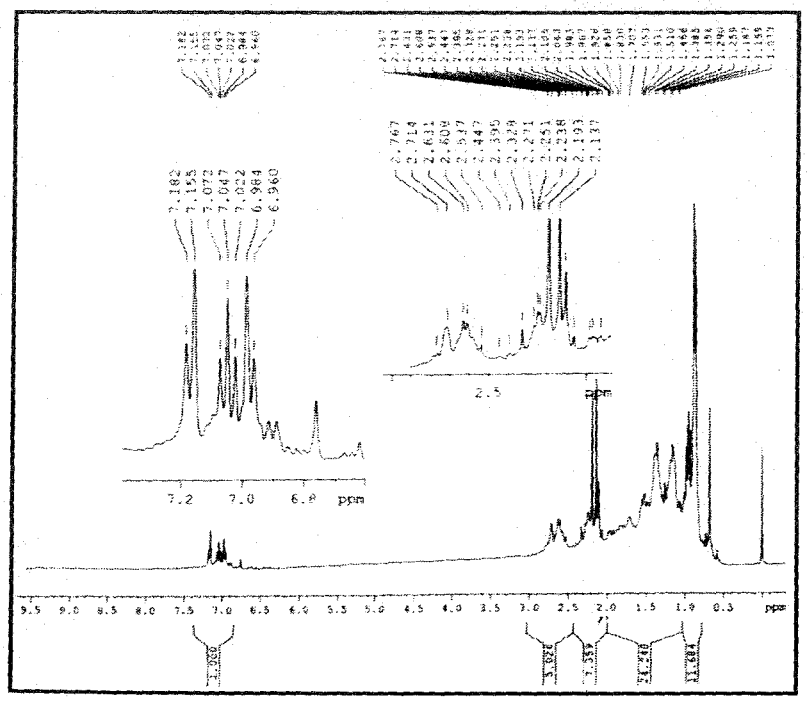


Figure 19. ^{1}H NMR spectrum of 1-methyl-19-norcholesta-1,3,5(10)-triene (**183**).

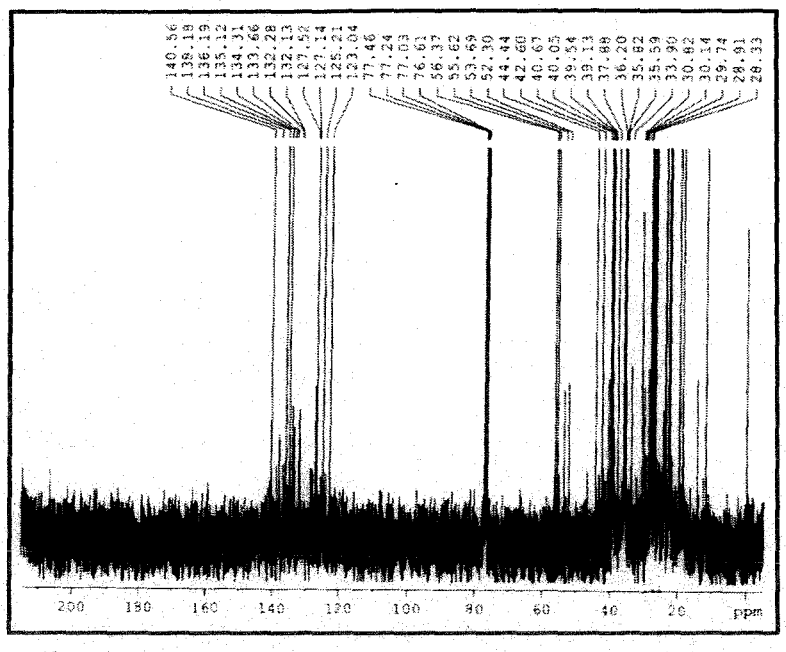


Figure 20. ^{13}C NMR spectrum of 1-methyl-19-norcholesta-1,3,5(10)-triene (**183**).

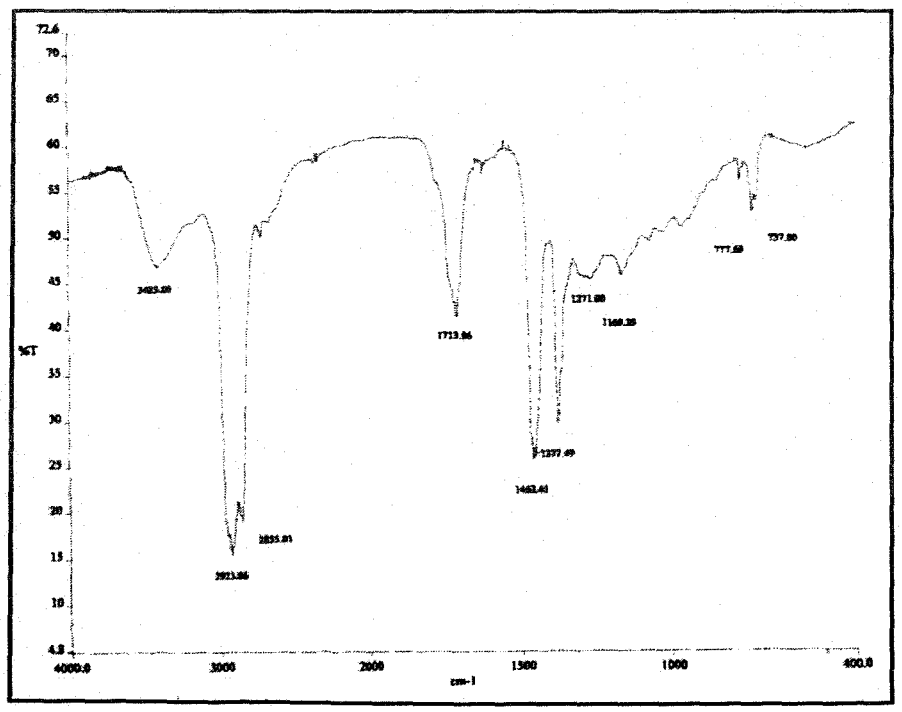


Figure 21. IR spectrum of 1-methyl-19-norcholesta-1,3,5(10)-triene (**183**).

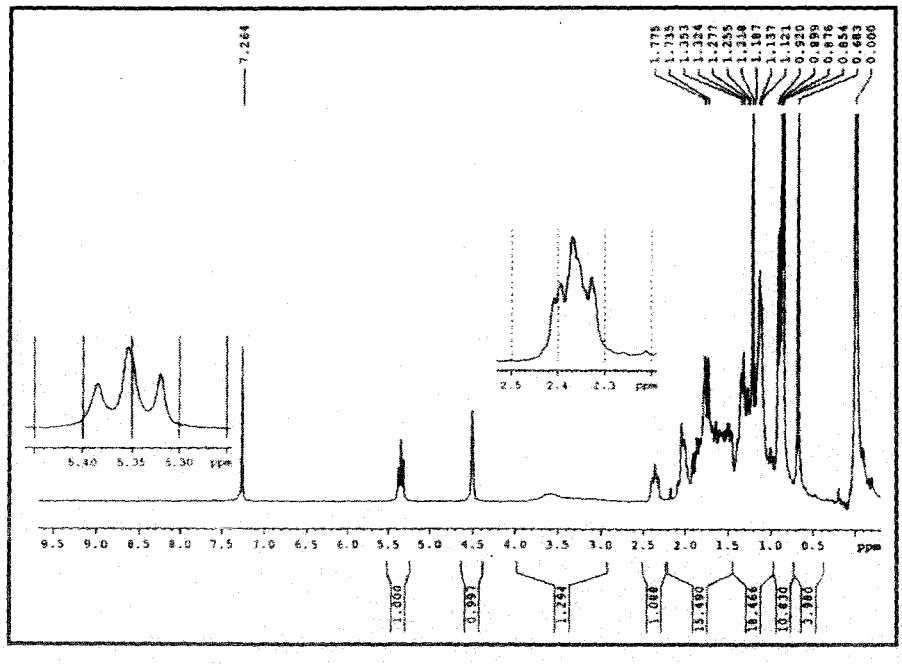


Figure 22. ^{1}H NMR spectrum of 3-keto-4β,5α,6β-trihydroxy cholesterol (**187**).

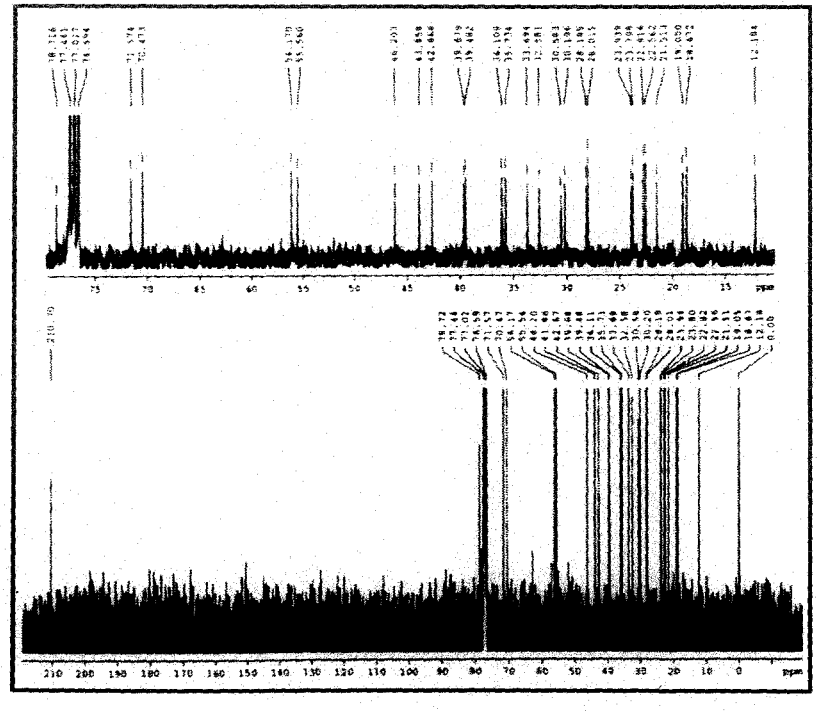


Figure 23. ^{13}C NMR spectrum of 3-keto-4β,5α,6β-trihydroxy cholesterol (**187**).

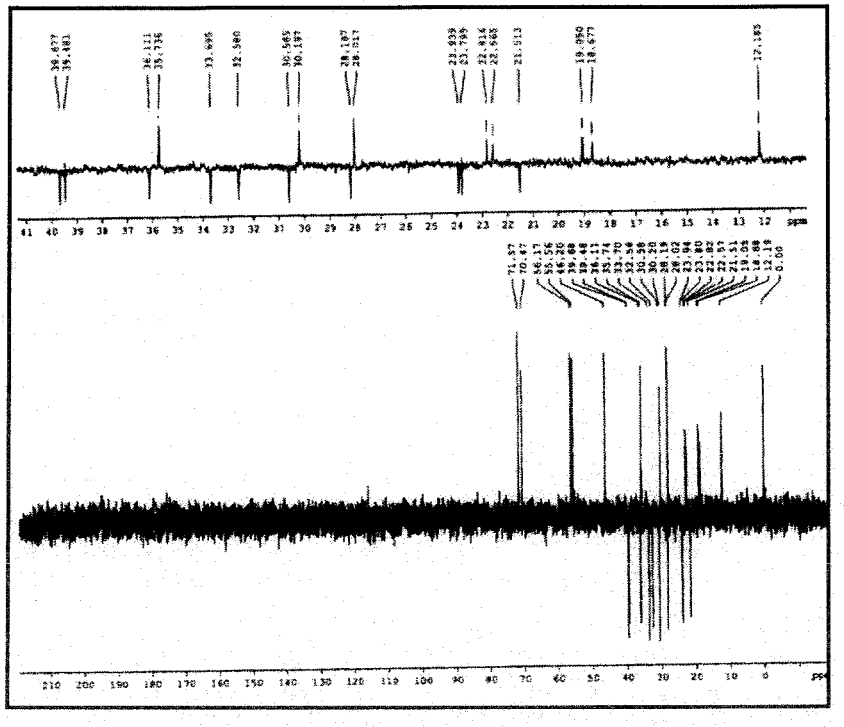


Figure 24. DEPT-135 NMR spectrum of 3-keto-4β,5α,6β-trihydroxy cholesterol (**187**).

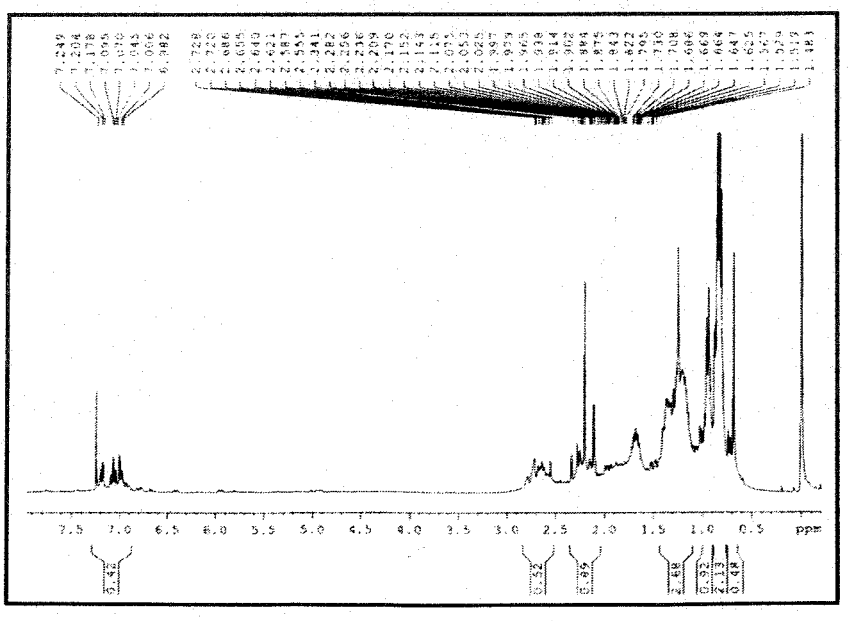


Figure 25. ^{1}H NMR spectrum of 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**).

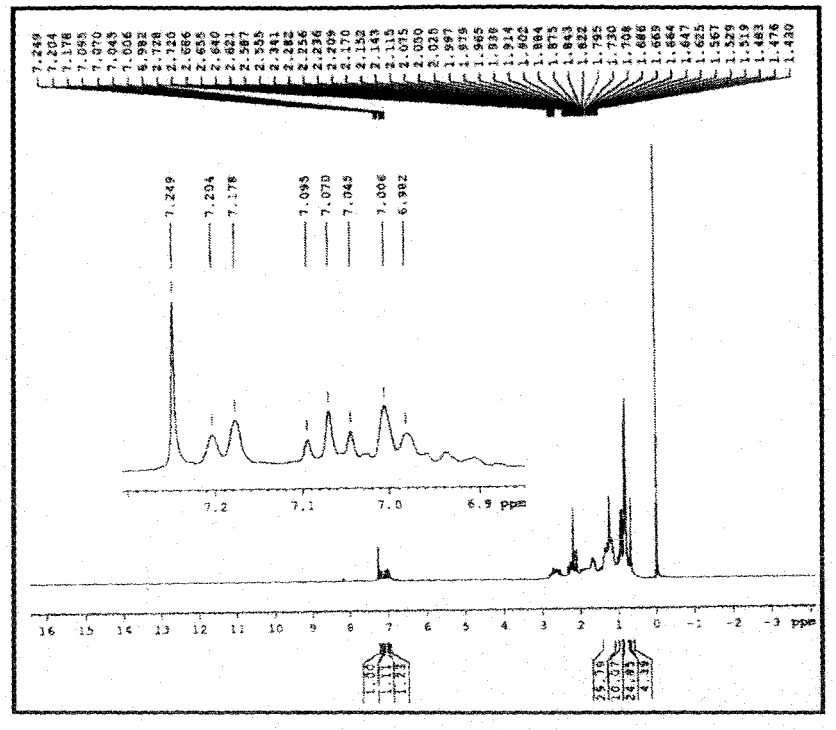


Figure 26. ^{1}H NMR spectrum (with partial expansion) of 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**).

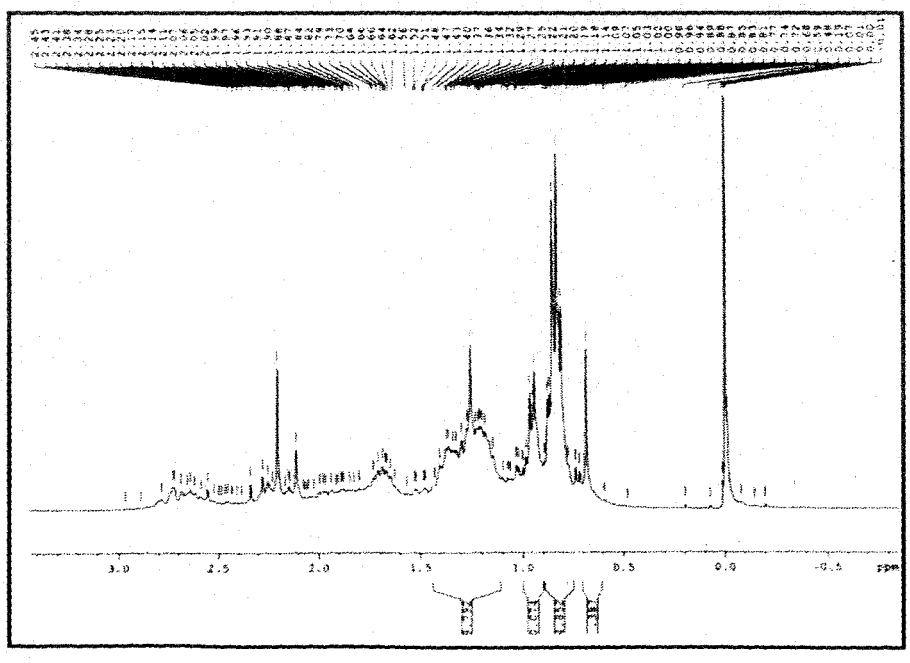


Figure 27. ^{1}H NMR spectrum (partially expanded 1) of 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**).

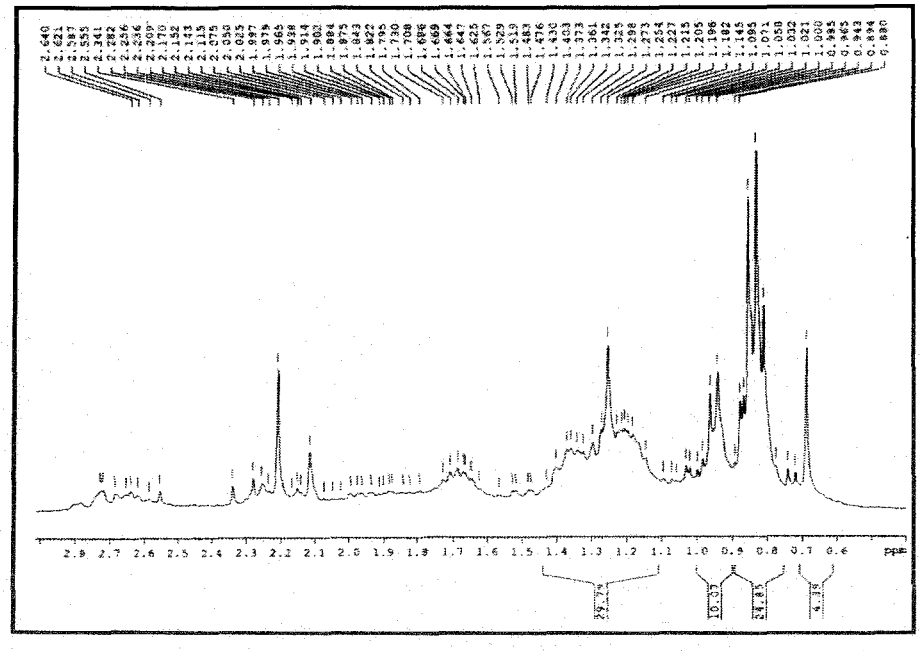


Figure 28. ^{1}H NMR spectrum (partially expanded 2) of 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**).

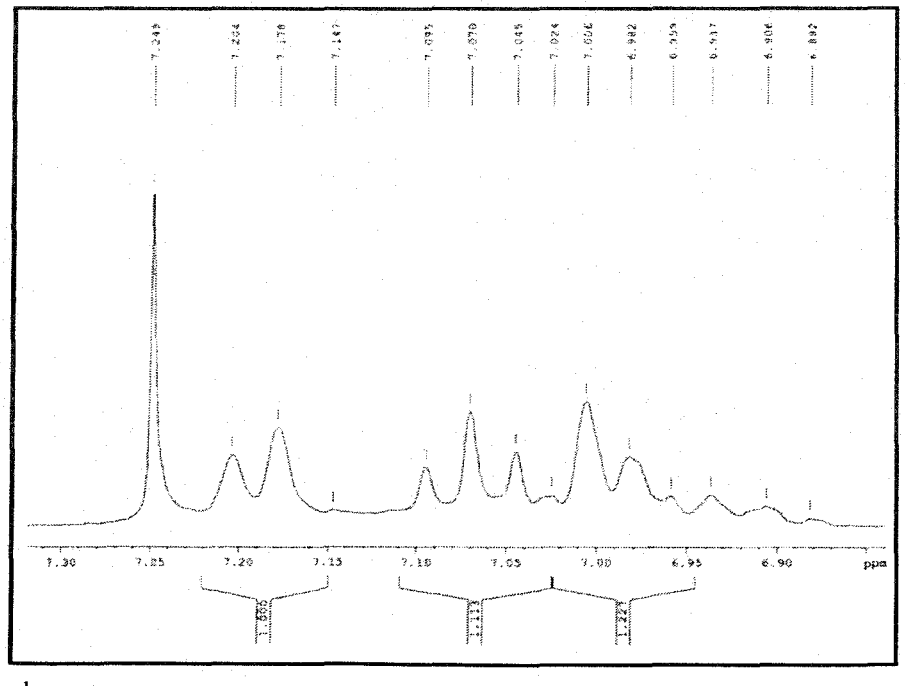


Figure 29. ^{1}H NMR spectrum (partially expanded 3) of 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**).

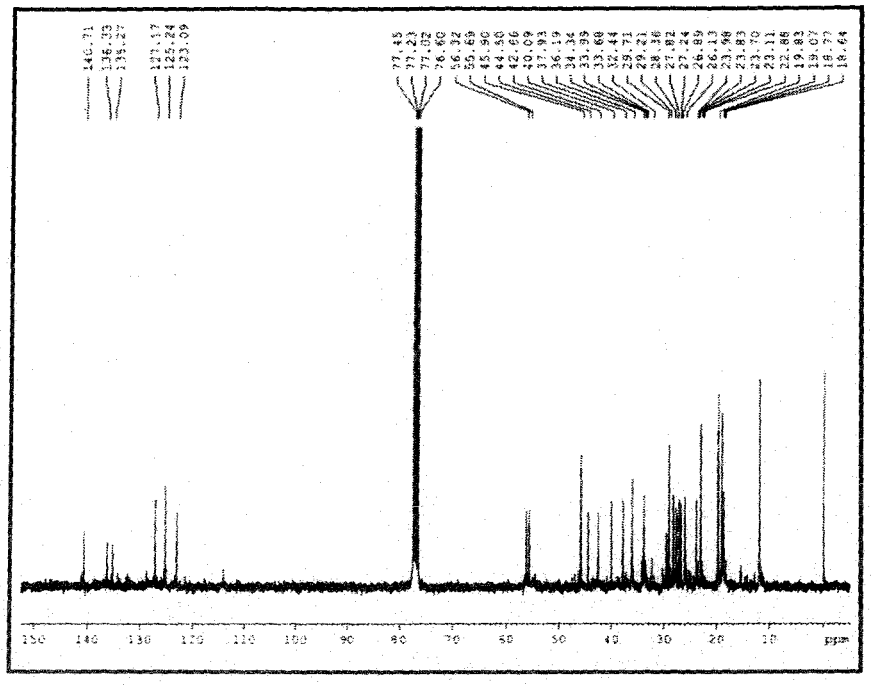


Figure 30. ^{13}C NMR spectrum of 1-methyl-19-norstigmast-1,3,5(10)-triene (**188**).

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Chapter III

p-TsOH-Mediated green transformations of di- and trihydroxy steroids toward diverse A/B-ring oxo-functionalization and evaluation of their cytotoxicity

III.A Introduction: A short review on oxo-steroids or ketosteroids

In general, steroids having different oxygen bearing functionalities, *viz.*, -OR (R= H, alkyl, aryl, acyl etc.) and =O, directly attached to the steroid skeleton are known as oxysterols or oxysteroids, whereas the steroids having one or more oxo- or keto group (=O) are termed as oxosteroids or ketosteroids. These are an important class of steroid compounds both from chemical and biological perspective. Considering the steroidal skeleton it can easily be predicted that there is a huge possibility of formation or presence of different kinds of ketosteroidss in a reaction or in nature respectively either in the form of cholesterol or β-sitosterol derivatives, for cholesterol is the major *zoo*-steroid whereas β-sitosterol is the most abuntdant *phyto*-steroid. Due to the presence of same functional group (the keto group) in different skeletal positions these ketosteroids possess a variety of biological activities depending on the various isomeric options. These factors increased the importance of the study of this group of steroids toward the new chemical syntheses as well as the useful biological applications.

III.A.1 Natural ketosteroids

From the very early stages, nature remains the major source of ketosteroids and especially marine plants and animals are the richest source of them. In the year 1974, Sheikh and Djerassi isolated cholestenone (**4a**) for the first time from sponge *Stelleta clarella*[1] and then it was found in *Patingera magellanica* by another group of workers.[2] Stigmastenone (**4b**) was also found from the latter one though it was first isolated from the bark extract of *Cryptomeria japonica D* along with β-sitosterol, Iguestol, phyllocladan-16α-ol etc.[3] Marine sponge *Geodia cydonium* and demospongia *Cinachyra tarentina* were found to contain many ketosteroids such as cholest-4-en-3,6-dione (**189**), stigmast-4,6-dione (**190**), cholestenone (**4a**) etc.[4,5] Cholestenone (**4a**) was also found to be present in the ketosteroid fraction of the mollusk *Onchidiopsis variegate* along with stigmastenone (**4b**) and cholest-4-en-3,6-dione (**189**).[6] The β-sitosterol analogue of the latter one was isolated from *Sambucus ebulus*[7] and also from the roots of *Echiumvulgare L.*[8] 5α-Cholestane-3,6-dione (**191**) was isolated from *Acantophora spicifera*, a red alga from West coast of India[9] while the corresponding β-sitosterol analogue (**5**) was found in the methanol extract of the aerial parts of *E. lagascae*[10] and from the roots of *Piper nigrum.*[11-12] Cholesta-3,5-diene-7-one (**192**) was found in *Fucus evanescens* though the author believed it as a product of isolation procedure[13] and the stigmasta-3,5-diene-7-one (**193**) was isolated from the

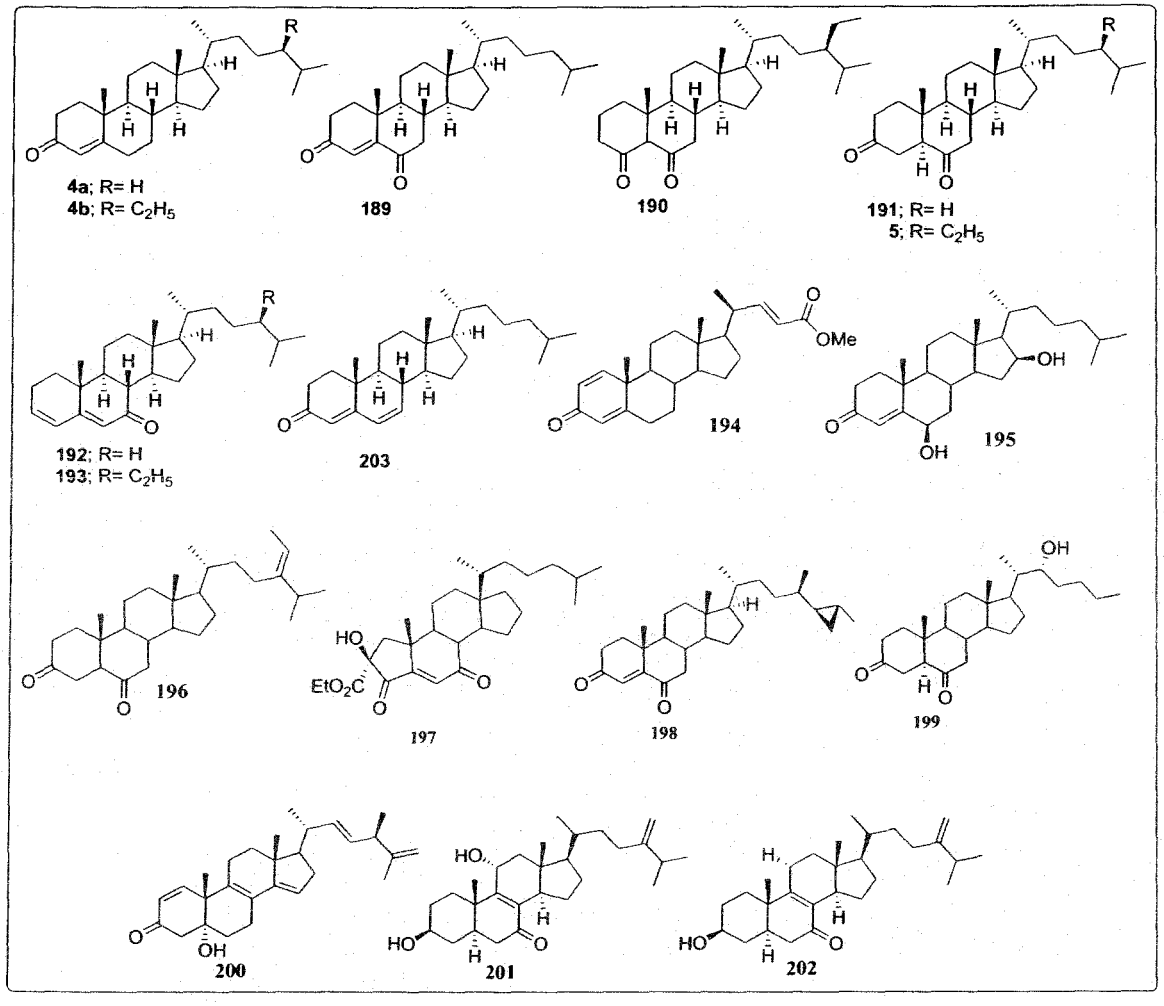


Figure 1. Natural ketosteroids.

ethyl acetate extract of the roots and aerial parts of *Gypsophila trichotoma.*[14] Another ketosteroid methyl (20S, 22E)-3-oxochola-1,4,22-trien-24-oate **(194)** was isolated from *Alcyonium gracillimum* and *Dendronephthya sp.*[15] The extract of *Jania adhaerens* (Al-Shoaiba coast, Red Sea) was reported to have the ketosteroid **195**[16]; another ketosteroid **196** was isolated from *Tydemania expeditionis* (Yellow Sea, China) along with three known sterols,[17] and an A-nor ketosteroid **197** was isolated from marine sponge *Acanthella cavernosa.*[18] Petrosterol-3,6-dione **(198)** from *Ianthella sp.* (Namyet Is., Vietnam),[19] ketosteroid **199** from *Cystoseira myrica,*[20] **200** from Axinella sp.[21] were also isolated. From the marine brown alga *Sargassum kjellmanianum,* an endophytic fungus *Aspergillus ochraceus* EN-31 was isolated. The culture extract of this

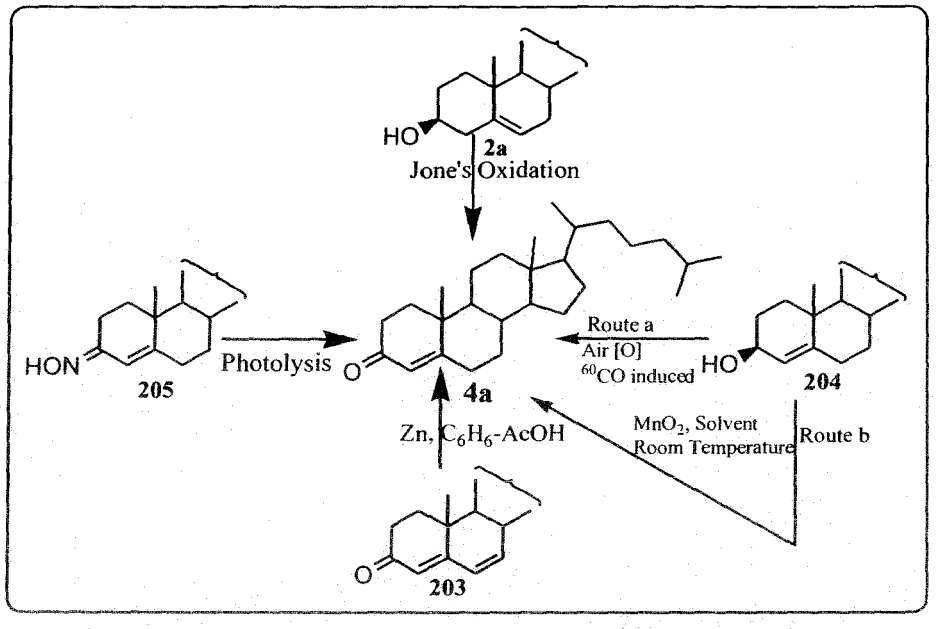
fungus was found to contain two ergosterol based ketosteroids- 3β,11α-dihydroxyergosta-8,24(28)-dien-7-one (**201**) and 3β -hydroxyergosta-8,24(28)-dien-7-one (**202**).[22]

Cholest-4-en-3-one (**4a**) was found in rat serum[23] and in LDL and VLDL of normal human; though for the latter claim there was no strong proof in its favour.[24] Cholesta-3,5-dien-7-one (**192**) is a potentially significant metabolite formed in the liver of rats as was studied in 1954.[25] This compound was proved to be a normal constituent of livers of a group of cockerels.[26] Arshad et al. reported the presence of this compound in plasma of normal and diabetic human subjects.[27] It was also found in native LDL of the normocholesterolemic and hypercholesterolemic monkeys and also in electronegatively charged LDL of hypercholesterolemic cynomologous monkeys.[28] Ryzlak et al. reported the presence of this compound in normal human liver as well as in alcoholic fatty liver, but the level was much higher in latter liver group. On the other hand, it was absent in cirrhotic liver group.[29] In human atherosclerotic lesions though this was found to be present but the level was very low as it was found to be a decomposition product of 7-keto cholesterol.[30] Cholesta-3,5-dien-7-one (**192**) was also present in extraordinary high level in rabbit's plasma.[31-32] Human gallstones and gallbladder bile also were found to contain cholest-4-en-3-one (**4a**), cholest-4,6-diene-3-one (**203**) and cholesta-3,5-dien-7-one (**192**).[33]

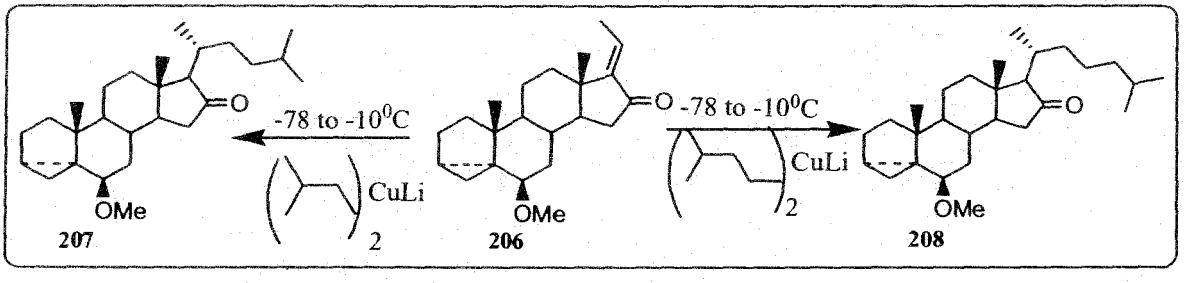
III.A.2 Syntheses of ketosteroids

Among the ketosteroids, cholestenone (**4a**) is the commonest one. The first synthesis of it was the oxidation of cholest-4-en-3β-ol (**204**) by manganese dioxide in a variety of solvents and at room temperature.[34] The same group prepared cholestenone simultaneously by reducing cholest-4,6-dien-3-one (**203**) by zinc in benzene-acetic acid.[35] Air oxidation, induced by ^{60}Co γ-radiation of cholest-4-ene-3β-ol (**204**) and cholesterol (**2a**) resulted cholest-4-en-3-one (**4a**)[36] and cholest-4-ene-3,6-dione (**189**)[37] respectively. Photolysis of cholest-4-en-3one oxime (**205**) in benzene is also reported to produce cholestenone (**4a**).[38]

Natural C-20 configured steroids were synthesized from C-17(20) unsaturated steroids using organocopper reagent. Reaction of the designed substrate **206** with lithium isoamylcuprate and with lithium diisohexylcuprate resulted useful novel ketosteroids **207** and **208** respectively.[39]



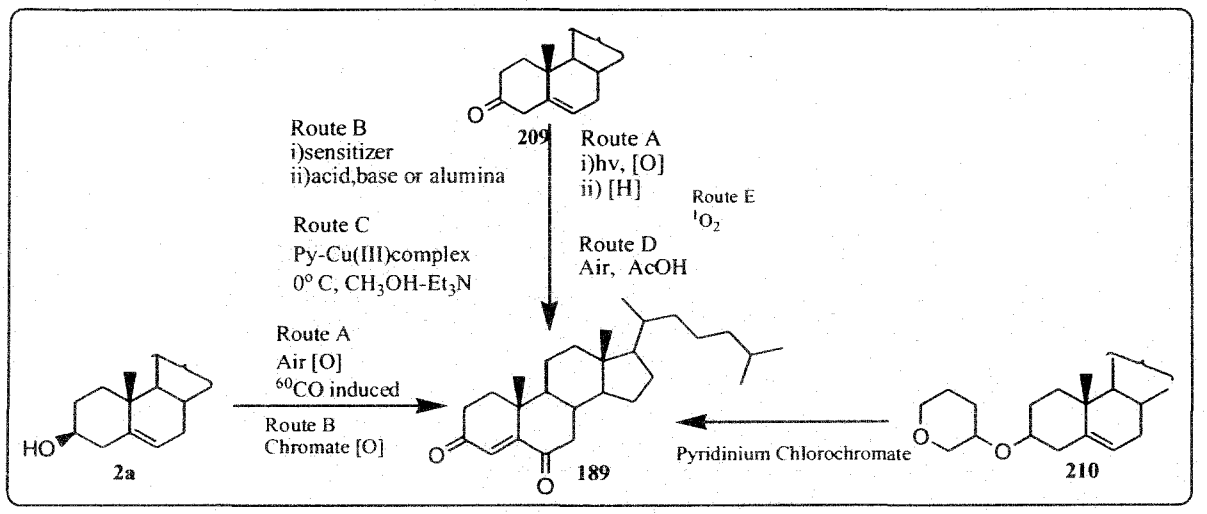
Scheme 1. Laboratory syntheses of cholestenone.



Scheme 2. Syntheses of C-20 natural configured steroids.

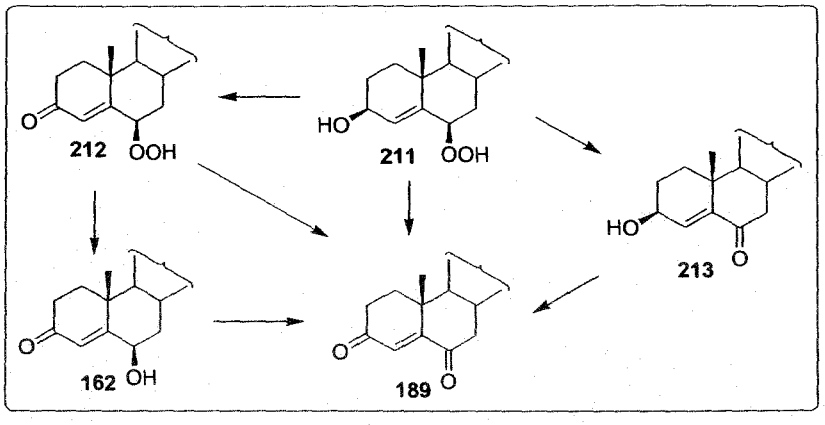
In 1965, Nickon and Mendelson showed that in absence of sensitizer, photooxidation of cholest-5-en-3-one (**209**), followed by reduction resulted cholest-4-ene-3,6-dione (**189**) as a side product while in presence of sensitizer, followed by acid, base or alumina treatment resulted cholesta-4,6-dien-3-one (**203**) and a little amount of cholest-4-ene-3,6-dione (**189**).[40] In both the cases 6α- and 6β-hydroxide were the prime products.[40] Cholest-5-en-3-one (**209**) when treated with pyridinecopper(III) complex at 0°C in methanol-triethylamine, cholest-4-ene-3,6-dione (**189**) was formed.[41-42] It was isolated along with cholest-4-ene-3-one (**4a**) from cholest-5-en-3-one (**209**) when the latter was treated with air in acetic acid.[43] Cholest-5-en-3-one (**209**) also gave cholest-4-ene-3,6-dione (**189**) along with 6α- and 6β-hydroperoxide and hemiperketal by

singlet molecular oxygen.[44] Compound **189** was found to be an important intermediate for the formation of different A-ring modified derivatives.[45-47] Δ^5-3β-Hydroxysterols were also oxidized by chromate to the corresponding Δ^4-3,6-dione.[48] Perish et al., in 1995, synthesised cholest-4-ene-3,6-dione (**203**) from cholest-Δ^5-en-3β-ol-3-tetrahydropyranyl ether (**210**) in non-aqueous media with pyridiniumchlorochromate (PCC).[49]



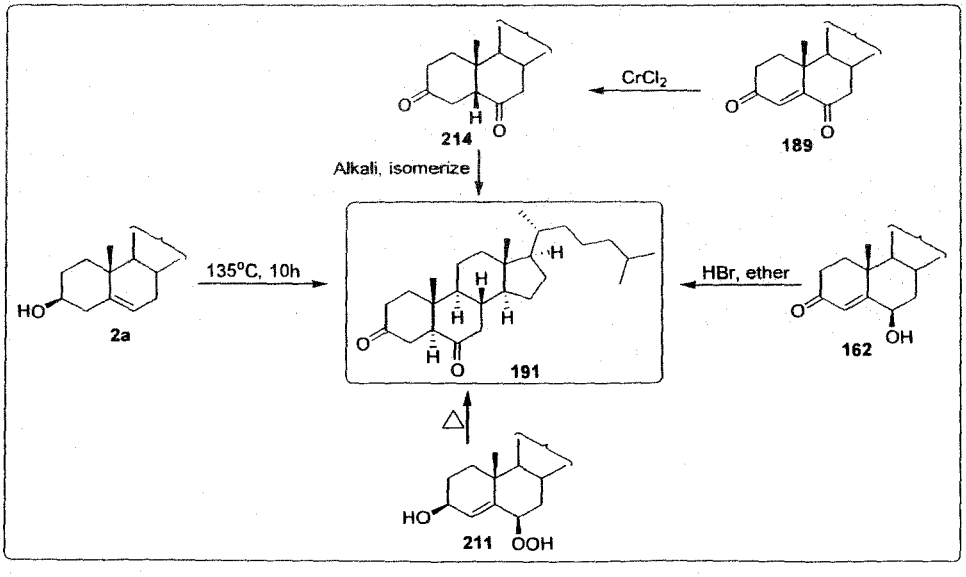
Scheme 3. Laboratory syntheses of cholest-4-ene-3,6-dione.

Cholest-4-ene-3,6-dione (**189**) was also prepared directly from cholesterol by *t*-butyl chromate oxidation in pyridine in 1962.[50] Later on it was again prepared from cholesterol using PCC in dichloromethane at 20°C for 96 hours[51] or by Jones oxidation[52-53] or by sodium dichromate and benzene in acetic acid.[54] It was also found to be the pyrolysis product of 6β-hydroperoxide cholest-4-en-3β-ol (**211**). It was proposed that it might be formed *via* 6β-hydroperoxide cholest-4-en-3-one (**212**) or starting from **211** (to **212**) to 6β-hydroxy cholest-4-ene-3-one (**162**) or from **211** to 3β-hydroxy cholest-4-ene-6-one (**213**).[34] Crystal structure of cholest-4-en-3,6-dione (**189**) was determined by Rajnikant et al., in the year 2001.[55]



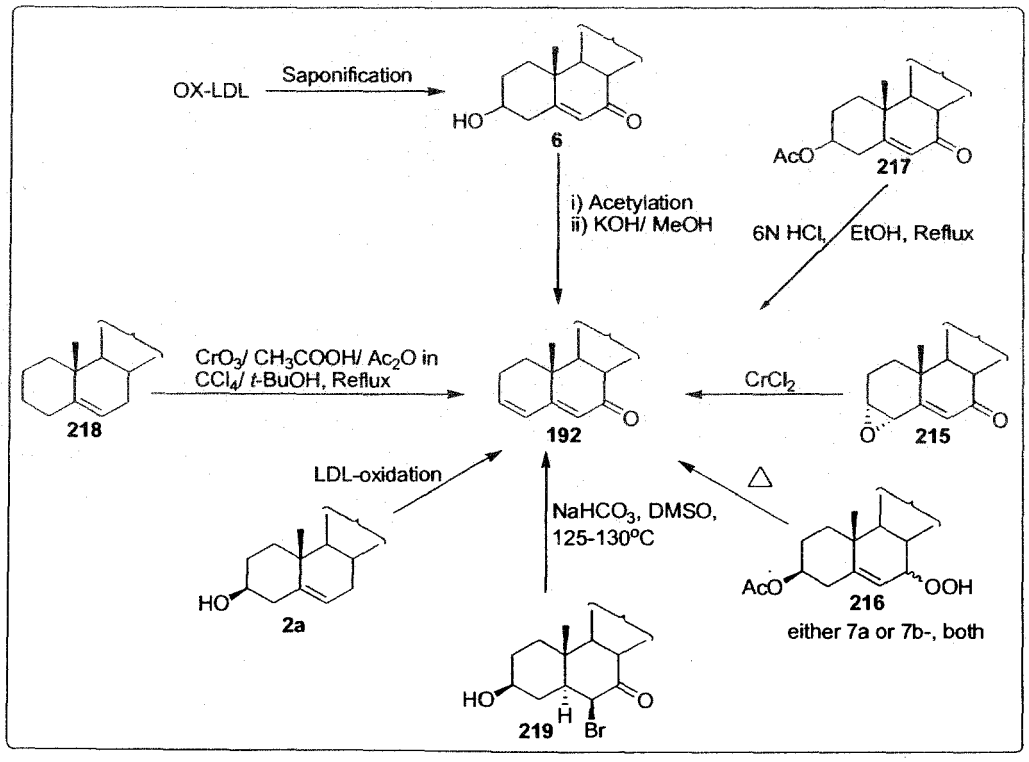
Scheme 4. Probable pyrolytic routes of 6β-hydroperoxy cholest-4-en-3β-ol toward cholest-4-ene-3,6-dione.

Cholest-4-ene-3,6-dione (**189**) was reduced by chromium(III) chloride to 5β-cholestane-3,6-dione (**214**) which was converted to the 5α-analogue (**191**) by alkali isomerization. The same reagent converts 3α,4α-epoxycholest-5-en-7-one (**215**) to cholesta-3,5-diene-7one (**192**). 5α-cholestane-3,6-dione (**191**) was prepared from 6β-hydroxy cholest-4-en-3-one (**162**) using 47% HBr in ether.[56] It was also found to be the pyrolysis product of **211**. It might be formed from **211** to **212** to **162** or from **211** to **213**.[35]



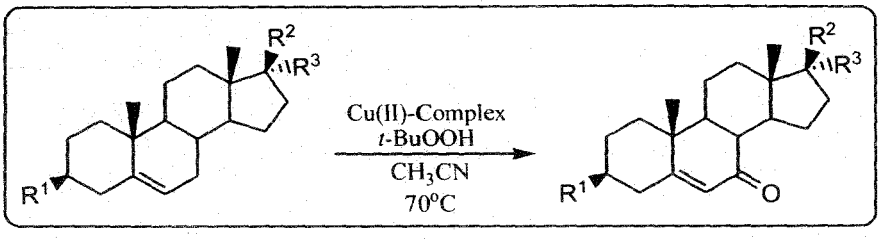
Scheme 5. Laboratory syntheses of 5α-cholestane-3,6-dione.

Cholesta-3,5-dien-7-one (**192**) was found to be a degradation product of 7-ketocholesterol (**6**) upon saponification of Ox-LDL.[57] Cholesta-3,5-dien-7-one was also formed as an artefact during the GC-MS chromatogram of the reaction mixture which was obtained from the electrochemical acetoxylation of cholesterol (**2a**). Probably the dehydration of the two major products 7α- and 7β- acetoxycholesterols in the injector or in the GC-column was the cause of formation of cholesta-3,5-dien-7-one.[58] Compound **192** was found to be formed at a low amount from the thermal decomposition of 7α- and 7β-hydroperoxides of cholesterol acetate (**216**).[59] It was reported that cholesta-3,5-dien-7-one was formed from 7-ketosterol (**6**) due to its base-catalyzed decomposition.[60-61] It was again prepared from 7-ketocholesteryl acetate (**217**) by refluxing with 6(N) HCl in absolute alcohol or methanol.[62] Again, cholest-5-ene (**218**) in CCl_4 when was refluxed with *t*-butyl alcohol, CrO_3, acetic acid and acetic anhydride, compound **192** was found to be formed.[63] 6β-Bromo-5α-cholestan-3β-ol-7-one-3-acetate (**219**) also produced the corresponding 3,5-diene-7-one when was heated with DMSO/ sodium bicarbonate at 125-130°C.[64]



Scheme 6. Laboratory syntheses of cholesta-3,5-dien-7-one.

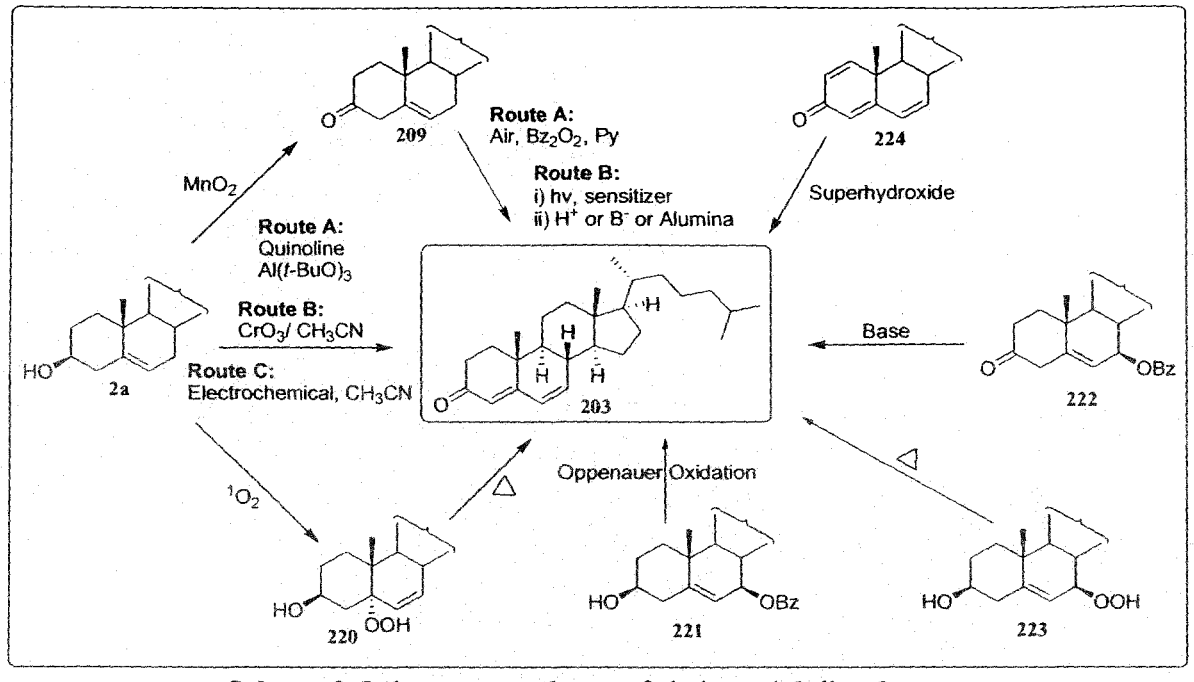
Li et al., in 2010, carried out an allylic oxidation pathway of Δ^5-steroids with *t*-butyl hydroperoxide and 2-quinoxalinol-salen-Cu(II) complex and the protocol was found to be applicable to a variety of Δ^5-steroidal substrates to result the corresponding 5-en-7-ones.[65]



Scheme 7. Allylic oxidation of Δ^5-steroids to result the corresponding enenones.

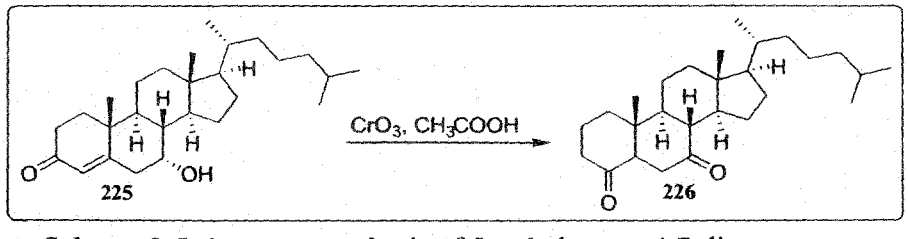
Dane et al. were the first group to synthesize cholesta-4,6-dien-3-one (**203**)[66] and again it was synthesized from cholesterol (**2a**) with quinoline and aluminum *tert*-butoxide by a modifided process.[67-68] 6β-Bromocholestenone,[69] 3-acetoxy-3,5-cholestadiene,[70] cholest-4-ene-3-one[71] and cholesta-4,6-dien-3β-ol[72-73] were also used to synthesize the 4,6-dien-3-one derivative. Singlet molecular oxygen was also able to convert cholesterol (**2a**) into 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (**220**) which on thermal decomposition formed cholesta-4,6-dien-3-one (**203**) along with the corresponding alcohol.[74-76] But the most efficient preparation of this compound was from cholesterol and CrO_3 in acetonitrile.[77] Oppenear oxidation on 3β-hydroxy-7β-benzoxy-5-cholestene (**221**) and mild base treatment on 7β-benzoxy-cholest-4-en-3-one (**222**) resulted to the product **203** separately.[62,78] Electrochemical oxidation of cholesterol in acetonitrile resulted the formation of cholesta-4,6-dien-3-one[79] which was later on modified to prepare the targetted compound at useful synthetic scale by Y.-Y. Hosokawa et al.[80] Manganese dioxide was capable to dehydrogenate cholest-4-en-3-one (**4a**) directly to the dieneone **203** though the authors did not consider it as a preparative method; rather they showed that these steroidal 4,6-dien-3-ones could be obtained directly from corresponding steroidal Δ^5-3β-ols *via* steroidal-5-ene-3-one (**209**) by using the same reagent.[81] Actually this process was similar to that of Wettstein's method but was found, in practice, better in various aspects.[33] It can also be formed by heating cholesterol (**2a**) at 135°C for 10 hours. Cholestenone (**4a**) and cholestane-3,6-dione (**161**) were also formed along with other oxysterols by this process.[81] Thermal

decomposition of cholesterol-7-hydroperoxide (**223**) resulted the formation of cholesta-4,6-dien-3-one along with other products.[82] Cholesta-1,4,6-trien-3-one (**224**) on reduction with super-hydride (12 eqv.) also resulted the corresponding 4,6-dien-3-one.[83]



Scheme 8. Laboratory syntheses of cholesta-4,6-dien-3-one.

As an early report, in 1938, Windaus performed chromium trioxide oxidation of 7β-hydroxycholest-4-en-3-one (**225**) to produce 5α-cholestane-4,7-dione (**226**).[84]



Scheme 9. Laboratory synthesis of 5α-cholestane-4,7-dione.

An steroidal A-ring dienone, **194**, along with its stereoisomer methyl-(20R, 22E)-3-oxochola-1,4,22-trien-24-oate (**227**) were synthesized by Linker et al.[85] In 2007, Anastasia et al. prepared an unusual keto steroid 3β-acetoxy-9α-methoxy-15α-hydroxy-5α-cholest-8(14)-en-7-one (**228**) from 3β-acetoxy-14α,15α-epoxy-5α-cholest-8-en-7-one.[86]

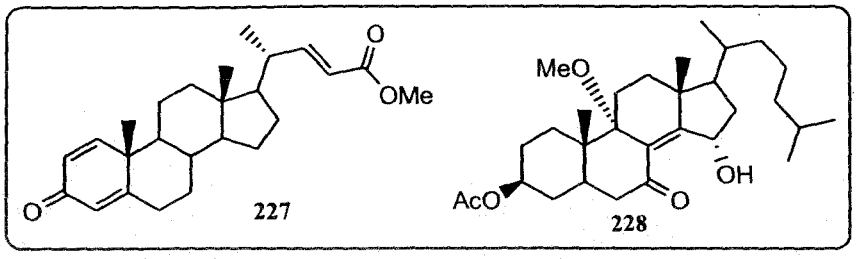
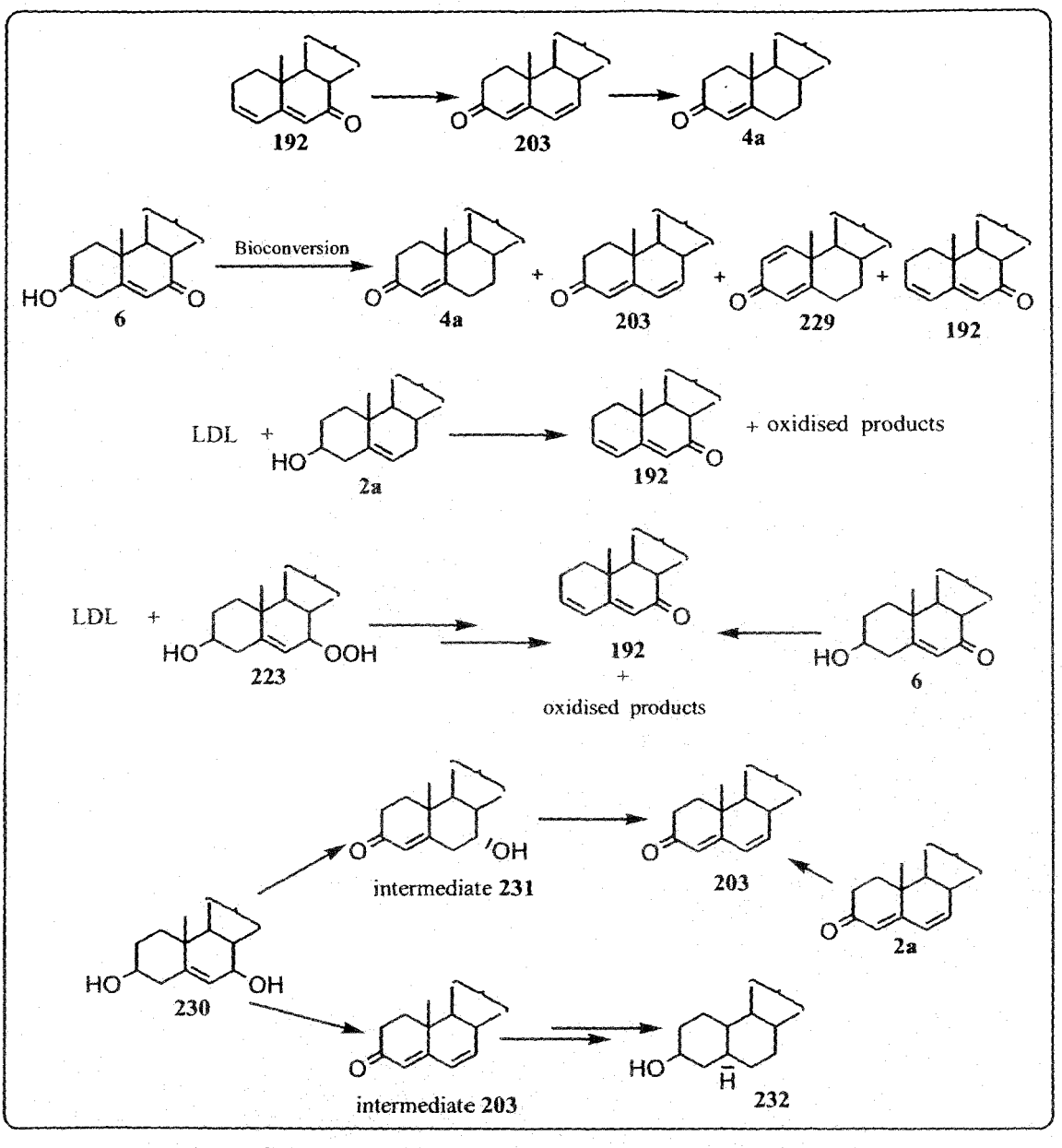


Figure 2. Methyl (20R, 22E)-3-oxochola-1,4,22-trien-24-oate (**227**) and 3β-acetoxy-9α-methoxy-15α-hydroxy-5α-cholest-8(14)-en-7-one (**228**).

III.A.3 Bioconversion

Cholestenone (**4a**) was formed when aortic smooth muscle cells of rabbit were incubated with cholesterol oxidase.[87] When cholesta-3,5-diene-7-one (**192**) was administered to rats it was found to isomerise into cholesta-4,6-dien-3-one (**203**) in rat liver and then subsequently it was reduced to cholestenone (**4a**).[88] From the bioconversion of 7-oxocholesterol (**6**), cholest-4-en-3-one (**4a**), cholesta-1,4-dien-3-one (**229**) and cholest-4,6-dien-3-one (**203**) etc. were obtained. In 1994, H. N. Hodis and his group showed that cholesterol containing LDL, present in monkeys could be oxidized into cholesta-3,5-dien-7-one (**192**) as one of the major products.[89] According to Malavasi et al., concentration of 7-hydroperoxide cholesterol (**223**) in oxidized human LDL was decreased upon time of oxidation and that of **192** along with many other products were increased.[90] According to Adachi et al.,[91] it is also present in erythrocyte membranes of alcoholic patients but not "in significant amounts in control subjects." From the findings of Adachi et al., it was clear that 7-ketocholesterol (**6**), present in opacified cornea of fish was decomposed to cholesta-3,5-dien-7-one (**192**) by the process employed.[92] Cholesta-4,6-diene-3-one is a biologically very important compound and was reported to be the intermediate in the biosynthetic pathway of cholestanol in some lipid-storage diseases.[93-94] Skrede et al. reported that during the conversion of 7α-hydroxycholesterol (**230**) to bile acid, an intermediate 7α-hydroxycholest-4-en-3-one (**231**) was converted to cholesta-4,6-dien-3-one (**203**) when incubated with rat liver microsomes.[95] Compound **203** was also found to be an intermediate during the conversion of 7α-hydroxycholesterol (**230**) to 5α-cholestanol (**232**) in rats and humans.[96] It was also prepared biologically from cholesterol.[97] The bioconversions of the ketosteroids as described above are presented in **Scheme 10**.



Scheme 10. Bioconversion of some ketosteroids.

III.A.4 Biocidal Activities

Several C-17 steroidal carbamates such as **233** is found to inhibit CYP17 of human lyase with IC_{50} value of 11.5μm.[98] Similarly, steroids in the oxazolidonyl and oxazolinyl series **234-237**, showed potent IC_{50} values ranging from 3μm to 59μm against rat testicular C-17, 20-lyase.[99-101]

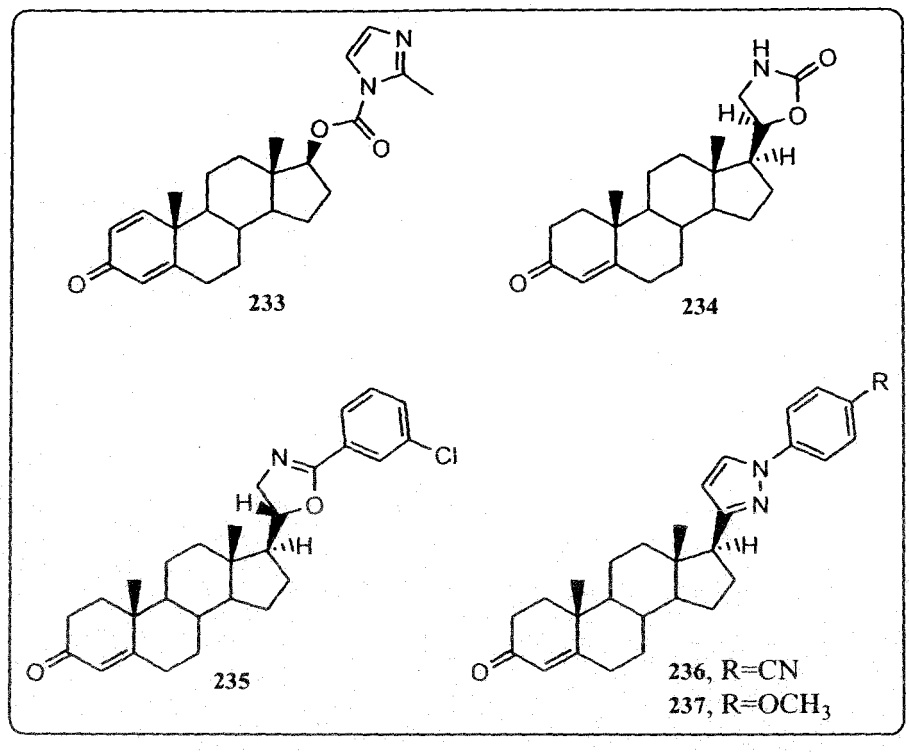


Figure 3. Some CYP17 (**233**) and C17,20-lyase (**234-237**) inhibitors.

Cholest-4-en-3-one (**4a**) was found weakly capable of inhibiting sterol synthesis in cultured mouse cell.[102-103] Compound **4a** was capable of binding to antiestrogen-binding site (AEBS) of chicken liver cytosol[104] to AEBS of the 10,000-*g* supernatant fraction of rat liver[105] and also to the AEBS of sonicated preparations of MCF7 cells (a human breast cancer cell line).[106] It can substantially decrease the body weight gain of rats and markedly increase the adrenal gland weight when it was administered in their diet.[107] It also suppressed the body weight gain of male and female mice,[108] and ketosteroids cholesta-4,6-dien-3-one (**203**) and cholest-4-ene-3,6-dione (**189**) also showed similar activities. Compound **189** was found to have no antimicrobial activity against *S. cerevisiae* (ATCC 28383), *S. aureus (53-154), C. albicans (1180-79), C. albicans(1663-86).*[109] But it showed significant inhibitory effects on sterol synthesis in intact animals.[110] 5α-Cholestane-3,6-dione (**191**) has the potency to inhibit the sterol synthesis in mouse L-cells.[111] Stigmast-4-en-3,6-dione (**238**) inhibited the germination of barley by 40% at a concentration of 3mM and also showed moderate stimulatory effect at concentration below 0.1mM.[112] When the effect of 5α-Stigmastane-3,6-dione (**5**) was tested on the reversal of

multidrug resistance (MDR) in *human MDR1 gene-transfected mouse lymphoma cells*, it was found to be much more active.[113] Cholesta-3,5-dien-7-one (**192**) was an "endogenous inhibitor of aldehyde dehydrogenase." It was a potent inhibitor of hepatic E1 isoenzyme (IC_{50} 5–10 μM) and also of mitocondrial E2 isoenzyme but at higher concentration only. Cholesta-4,6-dien-3-one (**203**) and 7-ketocholesterol (**6**) were inactive to E1 and E2 whereas E3 was unaffected by all the three compounds tested (**192**, **203** and **6**).[114] According to Cao et al.,[115] compound **192** increased "steryl esters" and decreased the level of free desmosterol on the cellular levels. Highly concentrated cholesta-3,5-dien-7-one (**192**) showed slightly stimulatory effects when leveled oleic acid was incorporated into the tryglycerides of human fibroblasts.[116] Selley et al.[117] reported that this oxosterol "potentiated the aggregation of human platelets induced by ADP, thrombin, or collagen." Dietary administration of this compound to cockerels showed no effect on serum cholesterol level, no or little effect on liver weight and increased effect on intestines weight.[118] Cholest-4-en-3-one (**4a**), cholesta-4,6-dien-3-one (**203**) and cholesta-3,5-dien-7-one (**192**), present in human gallstones and gallbladder bile can cause apoptosis in cultured gallbladder epithelial cells when the compounds were uptaken from bile and then were incorporated into the mitochondria. As a result cytochrome-C was released into the cytosol.[119] It has been observered that in certain cases introduction of a keto group at 7-position increased its bioacitivity as Δ^5-7-keto steroids are able to cell replication and hence more toxic to cancerous cells, e.g., 5-androstene-7,17-dione (**239**).[120] Compound **194** was found to have no antifouling activity against barnacle larvae (*Balanus amphitrite*) but it showed lethality to that larvae at a concentration of 100 μg/mL (LD_{100}).[14] Compound **195** was found antigenotoxic ketosteroid[15] and compound **196** showed modest activity against prostate cancer cells.[16] Compound **43** showed moderate antifouling activity towards the barnacle Balanus albicostatus (EC_{50} 8–32 mg/mL).[17] Compound **201** also displayed cytotoxicity against the SMMC-7721 cell line with an IC_{50} value of 28.0 μg/mL[21] and **198** was found to be cytotoxic and caused apoptosis.[18] 9α,11α-Dihydroxyergosta-4,6,8(14),22-tetraen-3-one (**240**) present in myxobacterial strain Sorangiineae SBNa008 was found to be active against human SW480 colon adinocarcinoma cells.[121]

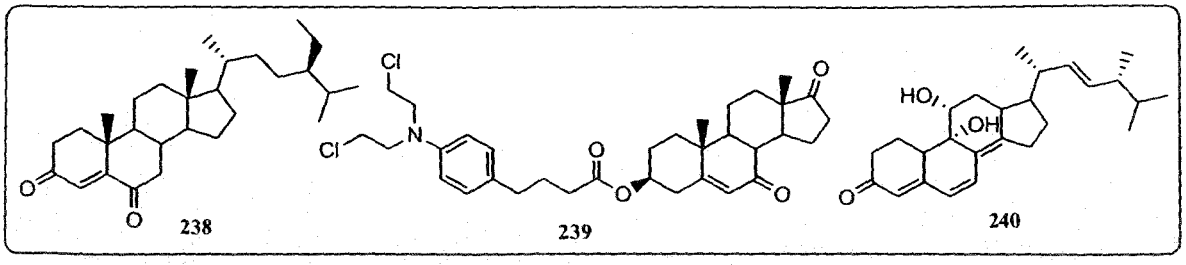


Figure 4. Stigmast-4-en-3,6-dione (**238**), 5-androstene-7,17-dione (**239**) and 9α,11α-dihydroxyergosta-4,6,8(14),22-tetraen-3-one (**240**).

III. B Present work

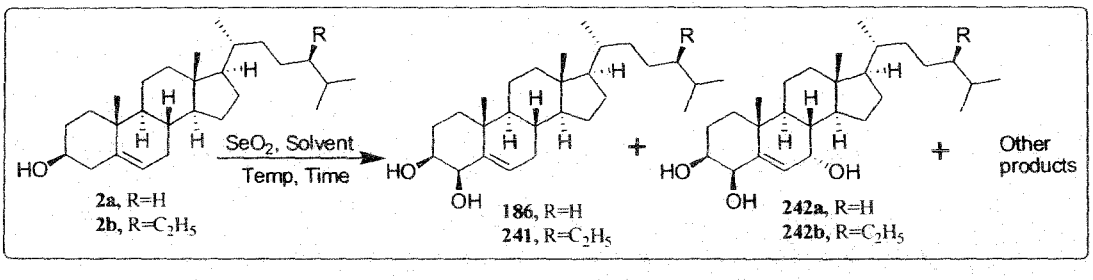
III.B.1 Background and abstract of the work

Considering the significant usefulness of the ketosteroids, on the perspective of different chemical synthetic approaches and biological anticipated activities, the author intended to add a contribution to the concerned steroidal field. And the present work was designed to utilize some oxysteroid biomolecules as the starting materials such as 4β-hydroxy- and 4β,7α-dihydroxy derivatives of cholesterol and β-sitosterol. 4β-Hydroxy cholesterol is the most abundant cholesterol metabolite in human circulation and the degradation of cholesterol into bile acids involves 7α-hydroxylation as the rate determining step. As a consequence, 4β,7α-dihydroxy cholesterol owes prime attention in the metabolic research. The corresponding analogues of β-sitosterol, the major phytosterol available in nature, were also used as the starting materials. Moreover, considering the present day requirement of green chemistry applications, the author tried to attempt the transformations on solvent-free solid supports and to our fortune, the results were satisfactory.

In brief, the present work describes the solid support/*p*-TsOH-mediated oxidative transformations of 4β-hydroxy- and 4β,7α-dihydroxy derivatives into the corresponding oxo-functionalized steroids. Many of the oxo-steroids or keto steroids, furnished in the reactions, were interestingly isomeric through the involvement of the ring-A and B of the steroid skeleton. The reaction protocol was optimized in detail, generalized through the application on comparable different substrates, and efforts toward the mechanistic understanding were also evaluated.

III.B.2 Preparation of the starting materials

The starting materials for the present work, as mentioned above, were- 4β-hydroxy (i.e., diol) (**186** and **241**) and 4β,7α-dihydroxy (i. e., triol) (**242a** and **242b**) derivatives of cholesterol (**2a**) and β-sitosterol (**2b**). These biomolecules were synthesized from the respective parent steroids by the oxidation with selenium dioxide (**Scheme 11**). The triols were also prepared from the diols after the treatment with same reagent. Detailed study of the present section is reported elsewhere.[122]



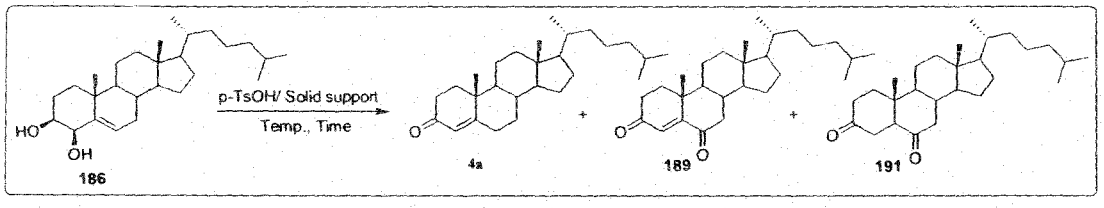
Scheme 11. Preparation of 4β-hydroxy and 4β,7α-dihydroxy derivatives of cholesterol (**2a**) and β-sitosterol (**2b**).

III.B.3 Results and Discussion- *Chemical*

III.B.3.1 Optimization of the reaction conditions

During the test reactions, when 4β-hydroxy cholesterol (**186**) was heated with *p*-TsOH on activated silica, three products were isolated and those were analyzed as cholest-4-en-3-one (**4a**), cholest-4-ene-3,6-dione (**189**) and 5α-cholestane-3,6-dione (**191**) (**Scheme 12**). The reaction was attempted in solution phase also using dichloromethane and ethanol separately as solvents which yielded, at room temperature (5-10 min.), cholest-4-en-3-one (**4a**) only and allowing the reaction for longer time (upto 45 min.) did not yield ene-dione **189** and/ or dione **191** at all (by careful TLC experiments). Thus, it was concluded, presumably, that solid silica directed, somehow, the formation of the products **189** and **191**. Then for the preliminary optimization of the reaction conditions we planned to perform the reaction (with *p*-TsOH primarily at 120 mol%) at three different temperatures, *viz.*, at 60°C, 120°C and 180°C using silica gel 60-120 mesh as the reaction medium (**Table 1**; entries 6-9). The reactions at 120°C and 180°C resulted, in a very short time (5-10 minutes), comparable product distribution; whereas at 60°C the reaction took

longer time (2 h) for completion and moreover, cholest-4-ene-3,6-dione (**189**) and 5α-cholestane-3,6-dione (**191**) were produced at lower yields. As our main focus was to emphasize the formation of compound **189** and **191**, we found it suitable to carry out further optimization reactions at 120°C and 180°C. And considering the anticipated catalytic potentiality of *p*-TsOH,[123-125] the reactions were carried out taking different amount of the reagent, ranging from 30-240 mol%. Analyzing the isolated yields from all the reactions, 60 mol% reagent and 120°C was found to be the optimum reaction condition (**Table 1**, entry 3). Though at 180°C and 60 mol% p-TsOH, the yield of the compounds **189** and **191** was little bit higher, the overall conversion was not satisfactory and hence was not chosen as the optimum reaction temperature.



Scheme 12. Reaction of 4β-hydroxy cholesterol (**186**) with *p*-TsOH in solid support.

Table 1. Optimization of the reaction conditions taking 4β-hydroxy cholesterol (**186**) and *p*-toluenesulfonic acid on activated silica.[a]

Entry	p-TsOH (mol%)	Temperature (°C)	Time (minute)	Yield[b] %		
				4a	**189**	**191**
1	30	120	10	44	06	04
2	30	180	05	40	08	05
3	**60**	**120**	**10**	**55**	**10**	**10**
4	60	180	05	35	12	10
5	60	180	10	27	13	11
6	120	60	120	55	07	06
7	120	120	10	45	08	10
8	120	180	05	41	08	06
9	240	120	10	44	05	<1

[a]All the reactions were performed on 100 mg (0.248 mmol) of substrate and on 4 g $mmol^{-1}$ of activated silica (60-120 mesh). [b]Isolated pure products by column chromatography.

Table 2. Optimization of solid supports taking 4β-hydroxy cholesterol (**186**) and *p*-TsOH.[a]

Entry	Solid support[b]	Yield[c] %		
		4a	**189**	**191**
1	**Silica 60-120[d]**	**55**	**10**	**10**
2	Silica F-254	58	05	05
3	Alumina Neutral	40	03	06
4	Alumina acidic	46	02	03
5	4Å molecular sieves[e]	Traces[f]	NF	NF
6	Montmorillonite KSF	36	07	07
7	Neat	53	NF	NF

[a]All the reactions were performed on 100 mg (0.248 mmol) of substrate (4β-hydroxy cholesterol) and 60 mol% of reagent (*p*-TsOH) and for 10 minutes in 120°C. [b]All preactivated solid supports were used at 60 mol% amount. [c] Isolated pure compounds from column chromatography. [d]Under dinitrogen atmosphere the three products were isolated at 62, 5 and 8% of yield respectively. [e]65% of unreacted substrate was recovered. [f]No product was isolated through column chromatography though TLC pointed its formation.

Table 3. Optimization of the amount of silica 60-120 mesh for the reaction of 4β-hydroxy cholesterol (**186**) with *p*-TsOH.[a]

Entry	Silica (60-120) mesh	Yield %		
		4a	**189**	**191**
1	100 mg (0.5 g $mmol^{-1}$)	47	4	3
2	250 mg (1 g $mmol^{-1}$)	50	5	4
3	500 mg (2 g $mmol^{-1}$)	50	5	7
4	750 mg (3 g $mmol^{-1}$)	52	9	10
5	**1g (4 g $mmol^{-1}$)**	**55**	**10**	**10**
6	1.5g (6 g $mmol^{-1}$)	57	7	10
7	2g (8 $gmmol^{-1}$)	58	7	9

[a]All the reactions were performed on 100 mg (0.248 mmol) of substrate (4β-hydroxy cholesterol) and 60 mol% of reagent (*p*-TsOH) and for 10 minutes in 120°C.

At the optimized reaction condition, various potential and useful solid supports were then employed as the reaction media (**Table 2**). However, first the reaction was tested without any solid supports which furnished only cholestenone **4a** at 53% yield and no **189** and **191** at all (**Table 2**, entry 7). Silica gel 60-120 mesh, usually used for the column chromatography produced better result in comparison to silica gel F-254. Both neutral and acidic alumina gave lower yields of compounds **189** and **191**. Montmorillonite KSF, a well applicable clay in the solid phase organic reactions, produced the expected compounds at lower yields in comparison to silica gel 60-120. Ene-dione **189** and dione **191** were not at all produced from 4Å molecular sieves; only cholestenone **4a** was isolated at trace amounts.

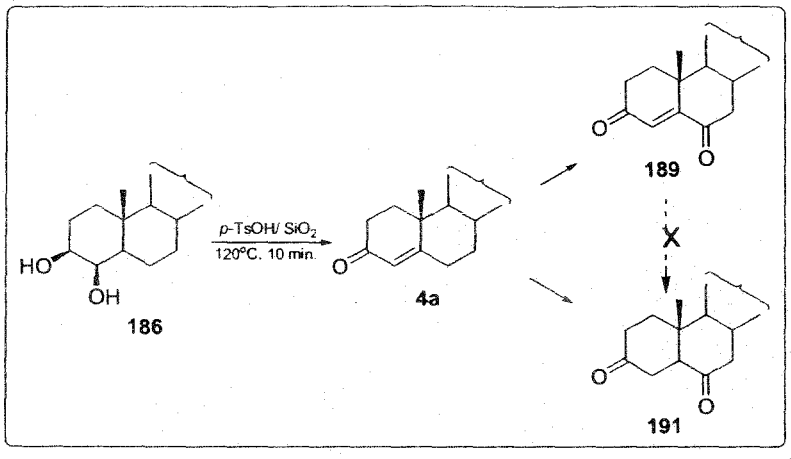
The variation of the amount of silica gel used in the reaction was then performed to observe that whether it can alter the yield distribution. Detailed experiments showed (**Table 3**) that 3g and above of silica gel per mmol of substrate produced comparable yields of the three products. As we already used 4g $mmol^{-1}$ of silica for the previous experiments, the latter experiments were also performed taking the same amount of silica.

III.B.3.2 Mechanistic investigation

After careful examination it was observed that at the same reaction condition silica itself could not react with diol **186**. That *p*-TsOH has its crucial role towards producing **189** and **191** was then well evident from the fact that the reaction employing glacial acetic acid or conc. sulfuric acid on silica produced only cholestenone **4a**. Moreover at the same reaction condition, considerable amount of unreacted diol **186** was found in the reaction mixture (by TLC). Whereas only *p*-TsOH (neat, without SiO_2 or other medium, **Table 2**, entry 7) reacted with the diol at the same reaction condition to produce cholestenone **4a** only at 53% yield, a separate reaction with *p*-TsOH (60 mol%) along with catalytic amount of silica (50 mol %) induced the formation of **189** and **191**(by TLC). These experiments proved a solid support to be an essential medium to achieve the one-pot transformation of the steroidal diol **186** into the enone **4a**, endione **189** and dione **191**.

When cholestenone **4a** itself was heated at 120°C for 10 minute neither **189** nor **191** were found to be produced which led us to discard the possibility of aerial oxidation of **4a** into **189** and/ or **191**.In addition, it is interesting to mention that when we applied the same reaction condition on **189**, no transformation was achieved at all. These results led us to conclude that **189**

and **191** were formed from **4a** following two different pathways (**Scheme 13**).On activated silica, no appreciable amount of transformation was achieved in glacial acetic acid, but in sulfuric acid, only cholestenone **4a** was found to produce along with considerable amount of unreacted diol. (These reactions were performed on small scale, taking 15 mg, 0.037 mmol of diol with 200 mol% of acid and the conclusions were drawn on careful TLC experiments. Again, the reaction with acetic acid was performed at 110°C, instead of 120°C).



Scheme 13. Dienone **189** and dione **191** are formed from **4a** following two different routes.

Yamamoto et al., in their very informative review[126] flourished the concept of 'designer acids' through the combination of a Brønsted acid with a Lewis acid where one can synergistically affect the other. As such acid combinations were found to produce satisfactory results in the solution phases, and there is no report of any such designer acid working on the solid phase, we thought to examine the concept in our solvent-free ketosteroid syntheses reactions. Thus, we tried the transformation of diol **186** accomplished by *p*-TsOH/ SiO_2, separately in presence of $MgCl_2$, $ZnCl_2$ and anhydrous $FeCl_3$. But for the present solid support transformation the designer acids concept was remained uneffective as the reactions conducted along with Lewis acids produced almost comparable yield of the three products to the reactions that did not use Lewis acids (**Table 4**).

III.B.3.3 Application of the reaction protocol on different polyhydroxy steroids.

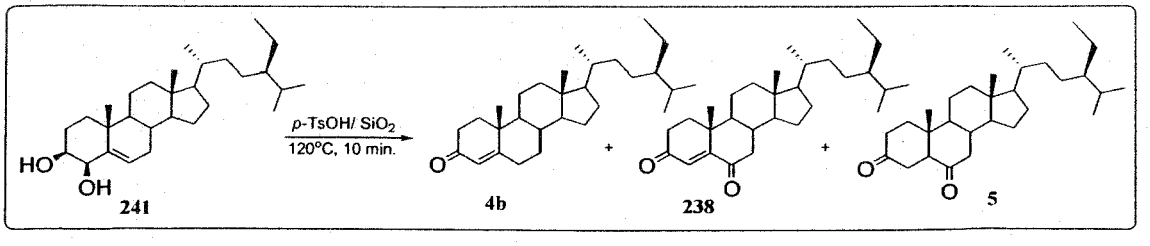
The optimized reaction condition was then applied to other comparable steroidal biomolecules. To generalize the reaction methodology the di-/tri-hydroxy steroids chosen were the 4β-hydroxy derivatives of β-sitosterol (**241**) and 4β,7α-dihydroxy derivatives of cholesterol and β-sitosterol (**242a** and **242b** respectively). We prepared these oxysteroids following the procedures developed by ourselves.[122]

Table 4. Effect of Lewis acids along with *p*-TsOH on the solvent-free transformation of 4β-hydroxy cholesterol.[a]

Entry	Lewis acid[b]	Yield[c] %		
		4a	**189**	**191**
1	$MgCl_2.6H_2O$	48	7	7
2	$ZnCl_2$ (anh.)	55	8	10
3	$FeCl_3$ (anh.)	52	10	7

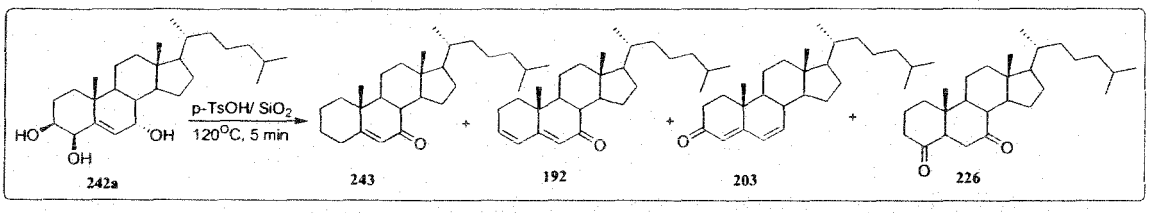
[a]All the reactions were performed on 100 mg (0.248 mmol) of substrate (4β-hydroxy cholesterol) and 60 mol% of reagent (*p*-TsOH) and for 10 minutes in 120°C taking 1g (4g $mmol^{-1}$) of activated silica 60-120 as the solid support. [b]For each reaction equimolar amount of Lewis acid (to *p*-TsOH, i. e., 60 mol%) was employed. [c]Isolated pure products by column chromatography.

III.B.3.3.1 Reaction on 4β-hydroxy β-sitosterol: When 4β-hydroxy β-sitosterol (**241**) was treated with 60 mol% of *p*-TsOH on activated silica 60-120 at 120°C for 10 min, stigmastenone (**4b**, 42%), stigmast-4-en-3,6-dione (**238**, 10%) and 5α-stigmastane-3,6-dione (**5**, 8%) were isolated as pure products after column chromatographic separation (**Scheme 14**).



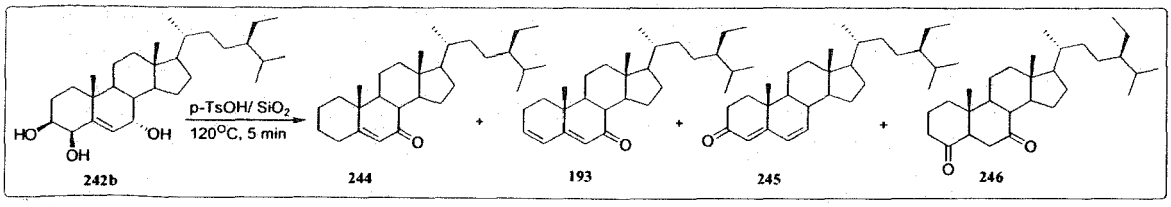
Scheme 14. Reaction of 4β-hydroxy-β-sitosterol (**241**) with *p*-TsOH in solid support.

III.B.3.3.2 Reaction on 4β,7α-dihydroxy cholesterol (242a): The same reaction protocol was applied on 4β,7α-dihydroxy cholesterol (**242a**) at optimized temperature and time. The reaction furnished four different ketosteroids; i) cholest-4-en-6-one (**243**, 8%), ii) cholesta-3,5-dien-7-one (**192**, 13%), iii) cholesta-4,6-dien-3-one (**203**, 17%) and iv) 5α-cholestane-4,7-dione (**226**, 7%). The products are very interesting as these led to some interesting isomeric pairs in the ketocholesterol series, such as **192** and **203** are isomeric to each other, whereas **243** is isomeric to **4a** and **226** to **191** (**Scheme 15**).



Scheme 15. Reaction of 4β,7α-dihydroxy cholesterol (**242a**) with *p*-TsOH in solid support.

III.B.3.3.3 Reaction on 4β,7α-dihydroxy β-sitosterol (242b): Accordingly, when 60 mol% of *p*-TsOH was employed to 4β,7α-dihydroxy β-sitosterol (**242b**) in solid activated silica at 120°C for 10 minute, four keto functionalized derivatives of the phytosteroid were isolated: i) stigmast-4-en-6-one (**244**, 10%), ii) stigmasta-3,5-dien-7-one (**193**, 18%), iii) stigmasta-4,6-dien-3-one (**245**, 17%) and iv) 5α-stigmastane-4,7-dione (**246**) (**Scheme 16**); but compound **246** could not be isolated in pure form. It was obtained as a non-seperable mixture with other enones and dienonones (by NMR). However, like the cholesterol analogues, here also the isolated compounds correspond to some interesting isomeric pairs in the keto-β-sitosterol series, such as **244** is isomeric to **4b**; **193** and **245** are isomeric to each other and **246** is isomeric to compound **5**.



Scheme 16. Reaction of 4β,7α-dihydroxy β-sitosterol (**242b**) with *p*-TsOH in solid support.

III.B.4 Results and Discussion- *Biological*

The effect of some of the synthesized ketosteroids on *Leishmania donovani* promastigotes were evaluated (**Table 5** and **Figure 5**). The compounds screened for the tests were **4a**, **189**, **191**, **4b**, **238**, **5**, **192**, **203**, **226**, **244**, **193**, **186**, **241**, **242a**, **242b** and all were tested at 50 μg/ mL concentration. One set without any compound was considered as control. The most effective compound was found to be cholest-3,5-dien-7-one **(192).** It inhibited the *L. donovani* AG83 (MHOM/IN/83/AG83) promastigotes most efficiently by 54% ($P<0.003$). But surprisingly, its β-sitosterol analogue was less potent (Inhibition: 7%). On the other hand, the synthesized ketosteroids cholestenone (**4a**) and cholesta-4-en-3,6-dione (**189**) were found to have more inhibitory effect (23% and 21% respectively) than the starting material 4β-hydroxy cholesterol (**186**) (inhibition: 9%) although the effect of 5α-cholestane-3,6-dione (**191**) was (inhibition 10%) comparable to **186**. The reverse trend was observed for β-sitosterol series. Here, stigmastenone (**4b**) and stigmasta-4-en-3,6-dione (**238**) were less potent (6% inhibition in both the cases) than 4β-hydroxy β-sitosterol (**241**) (inhibition: 10%) but 5α-stigmasta-3,6-dione (**5**) was more effective (inhibition: 15%). Again, in comparison to the starting material 4β,7α-dihydroxy cholesterol (**242a**) (inhibition: 41%), the synthesized keto steroids cholesta-4,6-dien-3-one (**203**) and 5α-cholestane-4,7-dione (**226**) were less active to inhibit the proliferation of the promastigotes *in vitro* (21% and 10% respectively), but compound **192** still, as described earlier, was the most effective one. In case of β-sitosterol series, 4β,7α-dihydroxy β-sitosterol (**242a**) was more potent (inhibition: 27%) than stigmasta-3,5-dien-7-one (**193**, inhibition: 7%) but less effective than stigmast-4-en-6-one (**244**, inhibition: 30%) to inhibit the tested parasite (**Table 5**).

III.C Experimental

III.C.1 General:

Melting points were measured in open capillary methods and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on BruckerAvance 300MHz FT-NMR spectrometer using 5 mm BBO probe. $CDCl_3$ was used as solvent and TMS as reference material. Data are presented as follows: Chemical shift -in ppm on the scale relative to $\delta_{TMS} = 0$; coupling constant- *J*/Hz. Infrared spectra were recorded either on Shimudzu FT-IR 8300 Spectrometer or Perkin Elmer FT-IR Spectrometer *Spectrum RX 1* as neat or thin films (KBr or Nujol) as indicated in the experimental procedures, and at room temperature. Frequencies are given in wave numbers

(cm^{-1}). For column chromatography silica gel G, 60-120 mesh was used with petroleum ether-ethyl acetate mixture as the eluent. For thin layer chromatography (TLC), freshly made silica gel plates (using silica gel for TLC + petroleum ether) were used and visualization was achieved by staining with iodine.

Table 5. Measurement of survivality/ inhibition for the tested compounds on *Leishmania donovani* promastigotes.

Compounds	**O. D[a]. (M-1[b])**	**Survivality (%)**	**O. D[a]. (M-2[b])**	**Survivality (%)**	**SM[c] (%)**	**SE[d]**	**Survivality (%, Rounded off)**	**Inhibition (%)**
Control	3.117	100	2.697	100	100	0	0	0
4a	2.380	76.35	2.121	78.64	77.50	1.15	78	22
189	2.403	77.09	2.178	80.76	78.93	1.84	79	21
191	2.838	91.04	2.423	89.84	90.44	0.6	90	10
4b	2.765	88.71	2.788	100	94.36	5.65	94	06
238	2.727	87.49	3.227	100	93.75	6.26	94	06
5	2.494	80.01	2.436	90.32	85.17	5.16	85	15
192	1.349	43.28	1.328	49.24	46.26	2.98	46	54
203	2.501	80.24	2.112	78.31	79.26	0.97	79	21
226	2.496	80.07	2.878	100	90.04	9.97	90	10
244	2.114	67.82	1.940	71.93	69.88	2.06	70	30
193	3.174	100	2.308	85.57	92.79	7.22	93	07
186	2.558	82.07	2.682	99.44	90.76	8.69	91	09
241	2.615	83.89	2.609	96.74	90.32	6.43	90	10
242a	1.759	56.43	1.649	61.14	58.79	2.36	59	41
242b	2.159	69.27	2.059	76.34	72.81	3.54	73	27

[a]Measured optical density; [b]1st Measurement (M-1) and 2nd Measurement (M-2); [c]Survivality mean; [d]Survivality error.

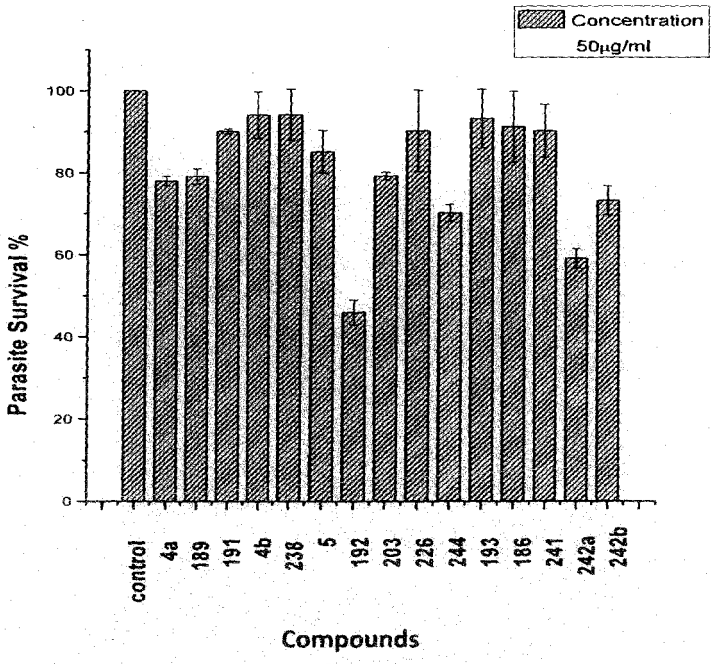


Figure 5. Parasite survivality percentages of different compounds.

III.C.2 Representative reaction for the syntheses of the ketosteroids

4β-Hydroxy cholesterol (100 mg, 0.25 mmol) and a given amount of *p*-toluenesulfonic acid (please follow **Table 1**) were mixed homogenously with the pre-activated (150°C/1 mm Hg, 1h) solid support (1g) in a dry morter to dust by a pestle, transferred into a round bottomed flask (50 mL). The mixture was then heated in an oil bath at defined temperature for specific time. The reaction mixture was cooled, water (10 mL) was poured into it and the organic materials were extracted in diethyl ether, washed with water (3× 15 mL) and the organic extract was dried (Na_2SO_4) and the solvent was removed (vacuum). The residue was then subjected to silica gel column chromatography to separate the products: cholestenone (**4a**, from 5% ethyl acetate/ pet ether, starting fractions), cholest-4-ene-3,6-dione (**189**, from 5% ethyl acetate/ pet ether, latter fractions) and 5α-cholestane-3,6-dione (**191**, from 8% ethyl acetate/ pet ether).

III.C.3. Bioassay: cytotoxicity assay by MTT method using *L. donovani* promastigotes

The anti-proliferative effect of the molecules was estimated on protozoan parasite *Leishmania donovani* as per the guidelines of biosafety committee of West Bengal State University.

Leishmania donovani AG83 (MHOM/IN/83/AG83) was originally obtained from Indian Institute of Chemical Biology, Kolkata, India. The parasites were maintained in animal facility as per the guidelines of institutional animal ethics committee of West Bengal State University, Barasat. Promastigotes were transformed from intracellular amastigotes, acquired from splenic aspirates of infected BALB/c mice in complete M 199 medium (Invitrogen) supplemented with 1% penicillin-streptomycin (Invitrogen) and 10% FCS (GIBCO) at requisite temperature, 22- 24°C for *L. donovani* (AG83,LV9). To estimate the inhibitory effect, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) micro method was used. At first late-log-phase promastigotes (MHOM/IN/83/AG83) were treated with compounds **4a**, **189**, **191**, **4b**, **238**, **5**, **192**, **203**, **226**, **244**, **193**, **186**, **241**, **242a**, **242b**, dissolved in DMSO separately and after 48 hours of incubation these were pellet down. In one set no compound was added to use as a control. Then the cells were resuspended in M 199 media in a 96-well flat-bottom plate (200 µl per well; BD Falcon) and MTT (10 mg/ml, 10 µl per well) was added to each well and the plates were incubated for an additional 4 h at 37°C. Then stock buffer solution was added and the absorbances of the coloured products were measured at 590 nm by spectrophotometer. The experiment was performed in duplicate. Survival percentages of the parasite were finally plotted using origin software.

III.C.4 Product characterization

III.C.4.a Cholestenone (4a): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: trace-58%. Pale yellow crystal. m.f. $C_{27}H_{44}O$. m.p. 78-79°C (methanol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.71 (s, 3H, Me-18), 0.85 (d, *J*=1.5 Hz, 3H, Me-27), 0.88 (d, *J*=1.5 Hz, 3H, Me-26), 0.91 (d, *J*=6.6 Hz, 3H, Me-21), 1.18 (s, 3H, Me-19), 2.03 (dd, *J*=13.2 Hz and 3.3 Hz, 1H, H-2), 2.29-2.44 (m, 1H, H-2), 5.73 (s, 1H, H-4). ^{13}C NMR: (75 MHz, $CDCl_3$): δ 11.89 (CH_3-18), 17.51(CH_3-19), 18.65 (CH_3-21), 21.00 (CH_2-11), 22.56 (CH_3-26), 22.83 (CH_3- 27), 23.79 (CH_2-15), 24.17 (CH_2-23), 28.00 (CH_2-25) 28.17 (CH_2-16), 32.02 (CH_2-22), 32.95 (CH_2-6), 35.58 (CH_2-1), 35.66 (CH_2-2), 35.74 (CH-20), 36.06 (CH-8), 38.58 (CH_2-7), 39.47 (CH_2-24), 39.47 (CH_2-12), 39.59 (C-10), 42.36 (C-13), 53.78 (CH-9), 55.84 (CH-17), 56.06 (CH-14), 123.71 (CH-4), 171.80 (C-5), 199.74 (C-3). FTIR (nujol, cm^{-1}): ν 2345, 1684, 1654, 1618, 1560, 1508, 1377, 1266, 1227, 868.09, 722.

III.C.4.b Cholest-4-en-3,6-dione (189): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: 5-13%. m.f. $C_{27}H_{42}O_2$, pale yellow crystal. m.p. 124-125°C (methanol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (s, 3H, Me-19), 0.86 (s, 3H, Me-27), 0.88 (s, 3H, Me-26), 0.93 (d, *J*=6.6Hz, 3H, Me-21), 1.17 (s, 3H, Me-18), 2.49-2.55 (m, 3H, H-2 and H-7), 2.63 (dd, *J*=15.9Hz and 3.9Hz, 1H, H-2 or H-7), 6.17 (s, 1H, H-4). ^{13}C NMR: (75 MHz, $CDCl_3$): δ 11.89 (CH_3-18), 17.51 (CH_3-19), 18.65 (CH_3-21), 20.88 (CH_2-11), 22.56 (CH_3-26), 22.81 (CH_3- 27), 23.79 (CH_2-15), 23.97 (CH_2-23), 28.01 (CH_2-25) 28.01 (CH_2-16), 33.98 (CH_2-1), 34.22 (CH_2-22), 35.54 (CH_2-2), 35.68 (CH-20), 36.06 (CH-8), 39.14 (CH_2-24), 39.46 (CH_2-12), 39.82 (C-10), 42.54 (C-13), 46.83 (CH_2-7), 50.99 (CH-9), 55.96 (CH-17), 56.56 (CH-14), 125.45 (CH-4), 161.11 (C-5), 199.55 (CH_2-6), 202.38 (C-3). FTIR (nujol, cm^{-1}): ν 2345, 1686, 1654, 1560, 1542, 1508, 1376, 1222. Analysis calcd: C, 81.35; H, 10.62. Found: C, 81.15; H, 10.82.

III.C.4.c 5α-Cholestane-3,6-dione (191): Eluent in column chromatography: 10% ethyl acetate in petroleum ether. Yield: trace-11%. m.f. $C_{27}H_{44}O_2$, pale yellow crystal. m.p. 170-173°C (acetone). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.69 (s, 3H, Me-18), 0.86 (s, 3H, Me-27), 0.88 (s, 3H, Me-26), 0.92 (d, *J*=6.3Hz, 3H, Me-21), 0.96 (s, 3H, Me-19), 2.30-2.50 (m, 5H, H-4, H-5, H-7), 2.54-2.61 (m, 2H, H-2). ^{13}C NMR: (75 MHz, $CDCl_3$): δ 12.01 (CH_3-18), 12.57 (CH_3-19), 18.62 (CH_3-21), 21.66 (CH_2-11), 22.55 (CH_3-26), 22.82 (CH_3- 27), 23.79 (CH_2-15), 23.99 (CH_2-23), 28.00 (CH_2-25) 28.00 (CH_2-16), 35.68 (CH-20), 36.04 (CH_2-22), 37.00 (CH_2-1), 37.40 (CH_2-2), 38.03 (CH-8), 38.08 (CH-4), 39.35 (CH_2-24), 39.44 (CH_2-12), 41.26 (C-10), 42.99 (C-13), 46.63 (CH_2-7), 53.43 (CH-9), 56.07 (CH-17), 56.66 (CH-14), 57.49 (C-5), 209.27 (CH_2-6), 211.44 (C-3). FTIR (nujol, cm^{-1}): ν 1717, 1654, 1560, 1376. Analysis calcd: C, 80.94; H, 11.07. Found: C, 81.15; H, 11.17.

III.C.4.d Stigmastenone (4b): Eluent in column chromatography: 3% ethyl acetate in petroleum ether. Yield: 42%. m.f. $C_{29}H_{48}O$, pale yellow crystal. m.p. 93-94°C (acetone-methanol).^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (s, 3H, Me-18), 0.80 (s, 3H, Me-27), 0.84 (d, J=6.3Hz, 3H, Me-26), 0.87 (s, 3H, Me-29), 0.92 (d, *J*=6.6 Hz, 3H, Me-21),1.18 (s, 3H, Me-19), 2.03 (dd, *J*=19.8 Hz and 3.3Hz, 1H, H-2), 2.29-2.47 (m, 1H, H-2), 5.73 (br s, 1H, H-4). ^{13}C NMR: (75 MHz, $CDCl_3$): δ 12.01 (CH_3-18), 12.01 (CH_3-29), 17.45 (CH_3-26), 18.76 (CH_3-21), 19.01 (CH_3-19), 19.83 (CH_3- 27), 21.11 (CH_2-11), 23.18 (CH_2-28), 24.24 (CH_2-15), 26.28 (CH_2-25) 28.22 (CH_2-16),

29.31 (CH_2-23), 32.14 (CH_2-2), 33.00 (CH_2-6), 34.02 (CH_2-22), 34.02 (CH_2-7), 35.78 (CH_2-1), 35.78 (CH-8), 36.17 (CH-20), 38.67 (C-10), 39.73 (CH_2-12), 42.48 (C-13), 45.96 (CH_2-24), 53.92 (CH-9), 55.98 (CH-17), 56.14 (CH-14), 123.80 (CH-4), 171.56 (C-5), 199.51 (C-3). FTIR (nujol, cm^{-1}): ν 2345, 1683, 1618, 1560, 1542, 1508, 1377, 1267, 1227, 1186, 958, 866, 722.

III.C.4.e Stigmast-4-en-3,6-dione (238): Eluent in column chromatography: 3% ethyl acetate in petroleum ether. Yield: 10%. m.f. $C_{29}H_{46}O_2$, pale yellow crystal. m.p. 151-152°C (chloroform-methanol). ^1H NMR (300 MHz, $CDCl_3$): δ 0.72 (s, 3H, Me-18), 0.81 (s, 3H, Me-27), 0.83 (s, 3H, Me-26), 0.85 (s, 3H, Me-29), 0.94 (d, *J*=6.3Hz, 3H, Me-21), 1.17 (s, 3H, Me-19), 2.04 (d, *J*=3.0Hz, 1H, H-7), 2.47-2.55 (m, 2H, H-2), 2.68 (dd, *J*=15.6Hz and 3.9Hz, 1H, H-7), 6.17 (br s, 1H, H-4). ^{13}C NMR: (75 MHz, $CDCl_3$): δ 11.88 (CH_3-18), 11.96 (CH_3-29), 17.50 (CH_3-19), 18.69 (CH_3-21), 18.99 (CH_3-26), 19.83 (CH_3- 27), 20.85 (CH_2-11), 23.02 (CH_2-28), 23.96 (CH_2-15), 25.95 (CH_2-23), 28.01 (CH_2-16), 29.07 (CH_2-25), 33.79 (CH_2-22), 33.97 (CH_2-2), 34.19 (CH-8), 35.50 (CH_2-1), 36.03 (CH-20), 39.09 (CH_2-12), 39.82 (C-10), 42.51 (C-13), 45.75 (CH_2-24), 46.83 (CH_2-7), 50.94 (CH-9), 55.80 (CH-17), 56.51 (CH-14), 125.44 (CH-4), 161.12 (C-5), 199.61 (C-3), 202.46 (CH_2-6). FTIR (nujol, cm^{-1}): ν 2345, 1686, 1654, 1618, 1560, 1542, 1508, 1376, 1222. Analysis calcd: C, 81.63; H, 10.87. Found: C, 81.41; H, 10.62.

III.C.4.f 5α-Stigmastane-3,6-dione (5): Eluent in column chromatography: 8% ethyl acetate in petroleum ether. Yield: 8%. m.f. $C_{29}H_{48}O_2$, pale yellow crystal. m.p. 192-194°C (acetone). ^1H NMR (300 MHz, $CDCl_3$): δ 0.69 (s, 3H, Me-18), 0.81 (s, 3H, Me-27), 0.83 (s, 3H, Me-29), 0.85 (s, 3H, Me-26), 0.93 (d, *J*=6.3Hz, 3H, Me-21), 0.96 (s, 3H, Me-19), 2.25-2.45 (m, 5H, H-4, H-5, H-7), 2.54-2.61 (m, 2H, H-2). ^{13}C NMR: (75 MHz, $CDCl_3$): δ 11.96 (CH_3-18), 12.01 (CH_3-29), 12.56 (CH_3-19), 18.66 (CH_3-21), 18.99 (CH_3-26), 19.83 (CH_3- 27), 21.66 (CH_2-11), 23.02 (CH_2-28), 24.00 (CH_2-15), 25.96 (CH_2-23), 28.05 (CH_2-16), 29.06 (CH_2-25) 33.78 (CH_2-22), 36.04 (CH-20), 37.00 (CH-4), 37.40 (CH_2-2), 38.03 (CH-8), 38.08 (CH_2-1), 39.34 (CH_2-12), 41.27 (C-10), 42.99 (C-13), 45.75 (CH_2-24), 46.63 (CH_2-7), 53.43 (CH-9), 55.97 (CH-17), 56.58 (CH-14), 57.50 (C-5), 209.28 (CH_2-6), 211.47 (C-3). FTIR (nujol, cm^{-1}): ν 1707, 1685, 1618, 1560, 1542, 1508, 1377, 1260, 1239. Analysis calcd: C, 81.25; H, 11. 29. Found: C, 81.05; H, 11.02.

III.C.4.g Cholest-5-en-7-one (243): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 8%. m.f. $C_{27}H_{44}O$, pale yellow gum. ^1H NMR (300 MHz, $CDCl_3$): δ 0.71 (s, 3H, Me-19), 0.72 (d, *J*=7.2 Hz, 3H, Me-27), 0.82 (d, *J*=6.3 Hz, 3H, Me-26), 0.91(d, *J*=6.6 Hz, 3H, Me-21), 1.13 (s, 3H, Me-18), 3.08 (d, *J*=7.2 Hz, 1H, H-8), 6.07 (s, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.97, 17.15, 18.68, 20.99, 22.56, 22.82, 23.09, 23.83, 24.24, 28.02, 28.21, 31.05, 31.81, 34.68, 35.23, 35.77, 36.15, 37.80, 39.52, 39.72, 42.38, 54.27, 56.03, 56.17, 140.46, 141.13, 193.67. FTIR (nujol, cm^{-1}): ν 1684.

III.C.4.h Cholesta-3,5-dien-7-one (192): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 13%. m.f. $C_{27}H_{42}O$, pale yellow crystal. m.p. 112-114°C (ethanol).^1H NMR (300 MHz, $CDCl_3$): δ 0.69 (s, 3H, Me-18), 0.86 (s, 3H, Me-27), 0.88 (s, 3H, Me-26), 0.92 (d, *J*=6.6 Hz, 3H, Me-21), 1.09 (s, 3H, Me-19), 6.11 (dd, *J*=9.9 Hz and 3.0 Hz, 1H, H-4), 6.80 (q, *J*=2.4 Hz, H-6) (for 19 Me), 6.89 (ddd, *J*=10.2 Hz, 6.3 z and 2.4 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.92, 18.75, 21.04, 21.41, 22.57, 22.82, 23.85, 24.17, 28.03, 28.19, 31.26, 31.93, 35.77, 38.62, 36.19, 39.32, 39.53, 39.58, 42.39, 49.44, 56.15, 56.56, 129.19, 134.81, 141.74, 147.63, 188.48. FTIR (nujol, cm^{-1}): ν 1677, 1635, 1619, 1560, 1542, 1377, 1301, 1265, 1150, 1078, 1021, 960, 891, 849, 815, 723, 670. Analysis calcd: C, 84.75; H, 11.06. Found: C, 84.55; H, 11.22.

III.C.4.i Cholesta-4,6-diene-3-one (203): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: 17%. m.f. $C_{27}H_{42}O$, pale yellow crystal, m.p. 80-81°C (methanol). ^1H NMR (300 MHz, $CDCl_3$): δ 0.76 (s, 3H, Me-18), 0.86 (d, *J*=1.2 Hz, 3H, Me-26), 0.89 (d, *J*=1.2 Hz, 3H, Me-29), 0.93 (d, *J*=6.6 Hz, 3H, Me-21), 1.12 (s, 3H, Me-19), 5.67 (s, 1H, H-4), 6.12 (ddd, *J*= 1.8 Hz, 18 Hz and 9.9 Hz, 2H, H-6 and H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.93, 16.32, 18.70, 20.74, 22.56, 22.80, 23.77, 23.90, 28.03, 28.18, 34.00 (2), 35.81, 36.14, 36.19, 37.83, 39.54, 39.65, 43.49, 50.80, 53.53, 56.14, 123.55, 127.82, 141.56, 164.01, 199.55. FTIR (nujol, cm^{-1}): ν 1688. Analysis calcd: C, 84.75; H, 11.06. Found: C, 84.65; H, 10.92.

III.C.4.j Cholest-4,7-dione (226): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: 7%. m.f. $C_{27}H_{44}O_2$, pale yellow crystal, m.p. 144-146°C (chloroform-methanol). ^1H NMR (300 MHz, $CDCl_3$): δ 0.66 (s, 3H, Me-18), 0.85 (d, *J*=1.2 Hz, 3H, Me-27),

0.87 (d, *J*= 1.2 Hz, 3H, Me-26), 0.91 (d, *J*=6.3 Hz, 3H, Me-21), 1.01 (s, 3H, Me-19), 2.25-2.35 (m, 3H), 2.52-2.54 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.09, 13.21, 18.81, 22.05, 22.80, 22.56, 23.81, 24.89, 28.02, 28.40, 29.71, 35.65, 36.17, 37.17, 38.74, 38.94, 39.49, 40.95, 42.44, 42.79, 48.86, 49.56, 55.12, 55.95, 59.89, 209.58, 212.52. FTIR (nujol, cm^{-1}): ν 1719, 1705, 1653, 1376, 1270, 1154, 751, 721, 667. Analysis calcd: C, 80.94; H, 11.07. Found: C, 85.11; H, 11.02.

III.C.4.k Stigmast-5-en-7-one (244): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 10%. m.f. $C_{29}H_{48}O$, pale yellow gum. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.71 (s, 3H, Me-18), 0.80 (s, 3H, Me-27), 0.84 (d, *J*=6.3 Hz, 3H, Me-26), 0.87(s, 3H, Me-29), 0.92 (d, *J*=6.3 Hz, 3H, Me-21), 1.17 (s, 3H, Me-19), 6.07 (s, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.99, 17.16, 18.75, 19.06, 19.83, 21.00, 23.12, 24.25, 26.15, 28.24, 29.22, 29.71, 31.07, 31.82, 33.94, 34.69, 35.26, 37.81, 36.15, 39.73, 42.40, 45.89, 54.28, 56.04, 140.46, 141.14, 193.66. FTIR (nujol, cm^{-1}): ν 1686. Analysis calcd: C, 84.40; H, 11.72. Found: C, 84.64; H, 11.82.

III.C.4.l Stigmasta-3,5-diene-7-one (193): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 18%. m.f. $C_{29}H_{46}O$, pale yellow solid, m.p. 122°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.70 (s, 3H, Me-18), 0.79 (s, 3H, Me-27), 0.84 (d, *J*=6.6 Hz, 3H, Me-26), 0.88 (s, 3H, Me-29), 0.94 (d, *J*=6.6 Hz, 3H, Me-21), 1.09 (s, 3H, Me-19), 2.06 (dt, *J*=6.0 Hz and 3.3 Hz, 1H, H-2),2. 32 (t, *J*= 5.4 Hz, 1H, H-8), 2.45 (dd, *J*= 19.8 Hz and 6.3 Hz, 1H, H-2), 6.11 (dd, *J*=9.9 Hz and 2.7 Hz, 1H, H-4), 6.80 (q, *J*=2.7 Hz, 1H, H-6) (for 19 Me), 6.88 (ddd, *J*=9.9 Hz, 6.3 Hz and 2.4 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.92, 12.01, 18.81, 19.07, 19.83, 21.04, 21.40, 23.13, 24.18, 26.17, 28.21, 29.22, 31.26, 31.93, 33.98, 36.14, 38.62, 39.32, 39.58, 42.39, 45.90, 49.44, 56.06, 56.56, 129.19, 134.81, 141.74, 147.63, 188.47. FTIR (nujol, cm^{-1}): ν 1670, 1654, 1630, 1617, 1560, 1377, 1274, 1155, 960, 783, 722. Analysis calcd: C, 84.81; H, 11.29. Found: C, 84.65; H, 11.02.

III.C.4.m Stigmasta-4,6-diene-3-one (245): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: 17%. m.f. $C_{29}H_{46}O$, pale yellow crystal, m.p. 66-70°C (methanol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.76 (s, 3H, Me-18), 0.82 (d, *J*=6.3 Hz, 3H, Me-26), 0.87 (d, *J*=7.2 Hz, 3H, Me-29), 0.93 (d, *J*=6.3 Hz, 3H, Me-21), 1.11 (s, 3H, Me-19), 5.66 (s, 1H,

H-4), 6.12 (ddd, *J*= 1.8 Hz, 18 Hz and 9.1 Hz, 2H, H-6 and H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.92, 12.01, 16.32, 18.75, 19.08, 19.83, 20.72, 23.14, 23.77, 27.12, 28.21, 29.26, 29.53, 33.98, 33.98, 36.13, 37.14, 37.81, 39.62, 43.47, 50.77, 45.92, 53.51, 56.02, 123.53, 127.80, 141.61, 164.06, 199.63. FTIR (nujol, cm^{-1}): ν 1687. Analysis calcd: C, 84.81; H, 11.29. Found: C, 84.55; H, 11.09.

III.C.4.n 5α-Stigmastane-4,7-dione (246) (as mixture):): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. From NMR spectral data of the non-seperable mixture of the titled compound with other enones and dienones, only the values correspond to 5α-stigmastane-4,7-dione (obtained with the help of the spectral values of the corresponding pure 5α-cholestane-4,7-dione, **226**) are produced here. The δ values, especially for ^{13}C, as collected from a mixture of a number of peaks, may be little bit different for the actual pure compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.66 (s, 3H, Me-18), 0.84 (d, *J*=6.0 Hz, 3H, Me-27), 0.88 (d, *J*= 2.7 Hz, 3H, Me-26), 0.92 (d, *J*=6.3 Hz, 3H, Me-21), 1.01 (s, 3H, Me-19). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.94, 12.01, 13.20, 14.10, 18.8, 21.83, 22.53, 22.70, 23.15, 24.91, 28.21, 28.42, 36.14, 37.19, 38.75, 38.95, 39.59, 40.94, 42.44, 42.79, 45.45, 48.88, 49.40, 49.60, 55.04, 55.96, 59.91, 209.54, 212.50.

III.D Conclusion:

In summary, the present work describes the solid support/*p*-TsOH-mediated milder oxidation procedure to yield ring-A and/ or ring-B oxo-functionalized steroids. Interesting isomers involving the ring-A and/ or ring-B of the steroidal skeleton have been isolated during the process. When 4β-hydroxy cholesterol was heated with *p*-TsOH on activated silica, three products isolated were analyzed as cholest-4-en-3-one, cholest-4-ene-3,6-dione and 5α-cholestane-3,6-dione.

The reaction protocol was optimized in detail emphasising the substrate/ reagent ratio, temperature, type of solid supports (with amount) etc., generalized through the application on similar different substrates, and efforts toward the mechanistic understanding were also evaluated. The reaction was applied on 4β-hydroxy β-sitosterol, 4β,7α-dihydroxy cholesterol and 4β,7α-dihydroxy β-sitosterol. 4β-Hydroxy β-sitosterol furnished three products, as was expected- stigmast-4-en-3-one, stigmast-4-en-3,6-dione and 5α-stigmastane-3,6-dione whereas

4β,7α-dihydroxy steroids resulted the corresponding steroidal- 4-ene-6-one; 3,5-dien-7-one; 4,6-dien-7-one; and 4,7-dione.

The anti-proliferative effect of the substrates and product molecules was estimated on protozoan parasite *Leishmania donovani* and the results are discussed. The most effective compound was found to be cholesta-3,5-dien-7-one which inhibited the *L. donovani* AG83 (MHOM/IN/83/AG83) promastigotes by 54% ($P<0.003$).

III.E Supporting NMR spectra:

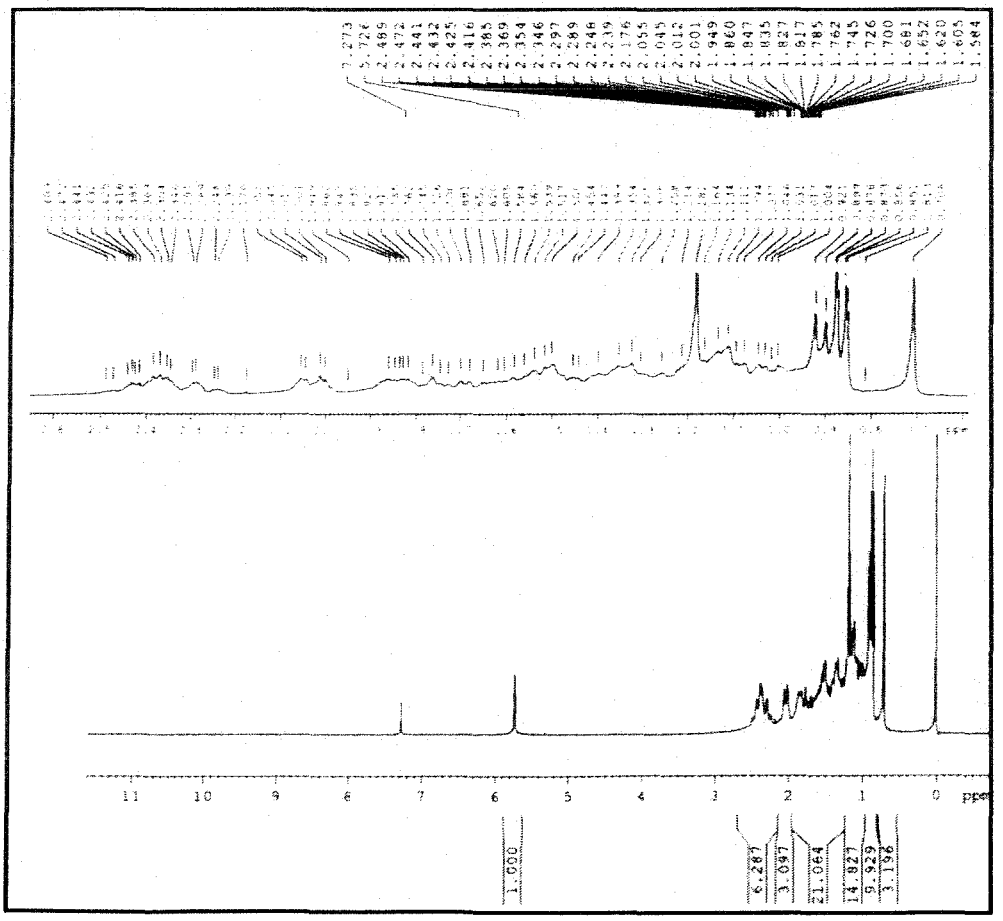


Figure 6. ^{1}H NMR spectrum of cholestenone (**4a**).

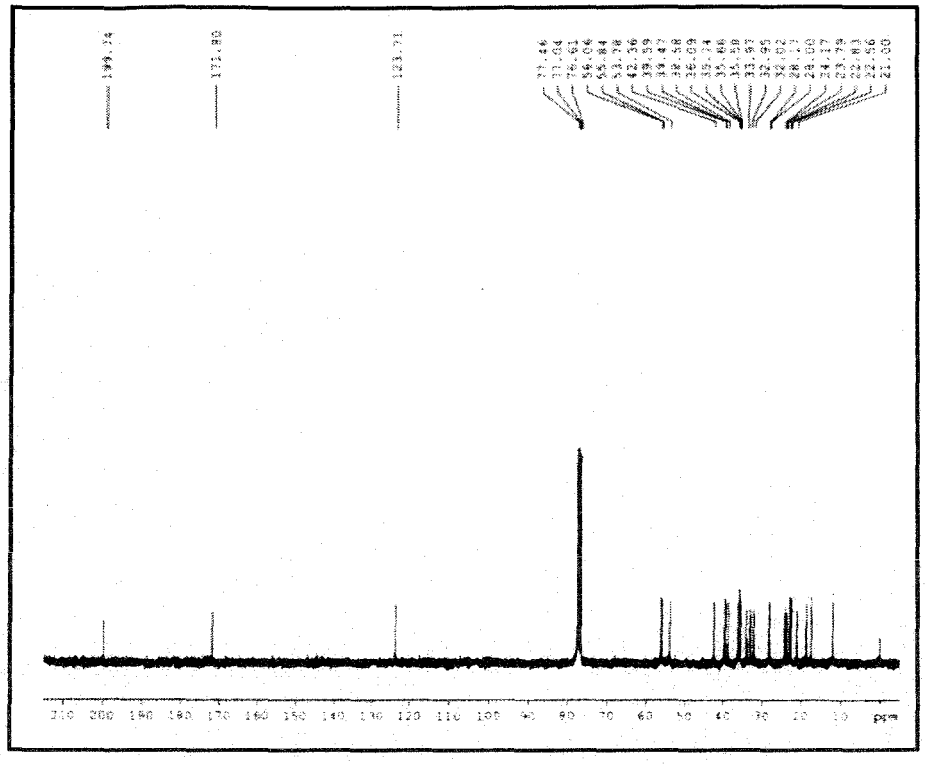


Figure 7. ^{13}C NMR spectrum of cholestenone (**4a**).

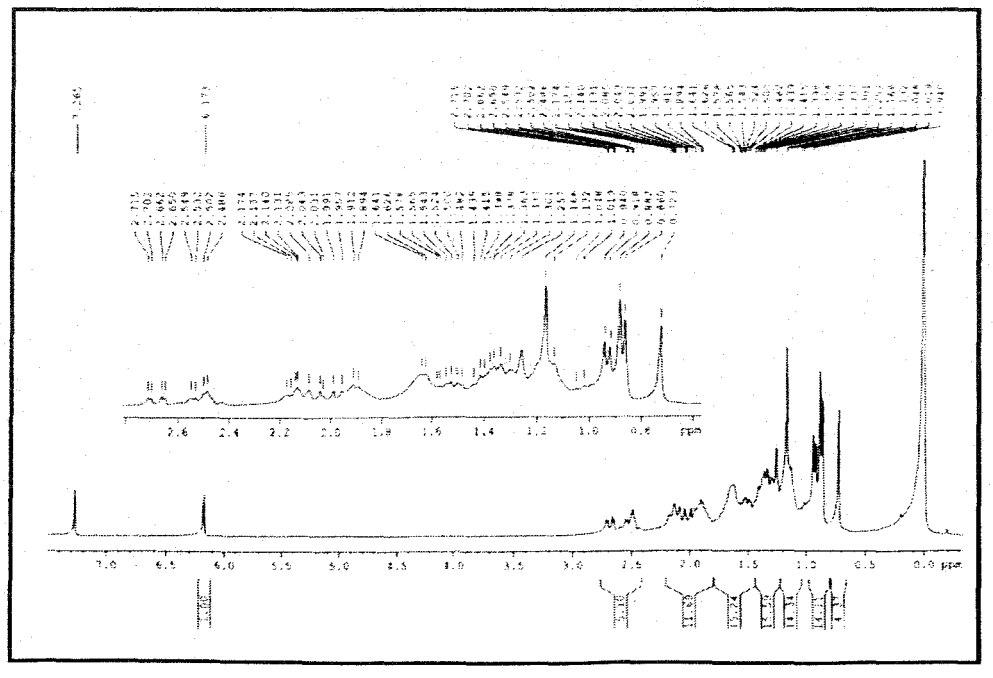


Figure 8. ^{1}H NMR spectrum of cholest-4-ene-3,6-dione (**189**).

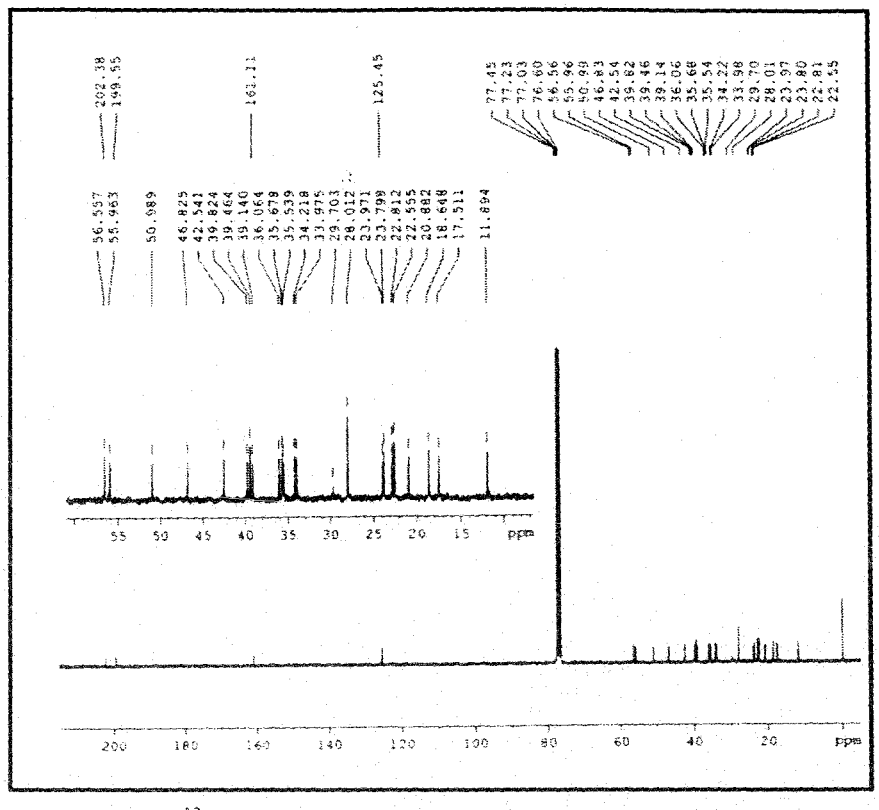


Figure 9. ^{13}C NMR spectrum of cholest-4-ene-3,6-dione (**189**).

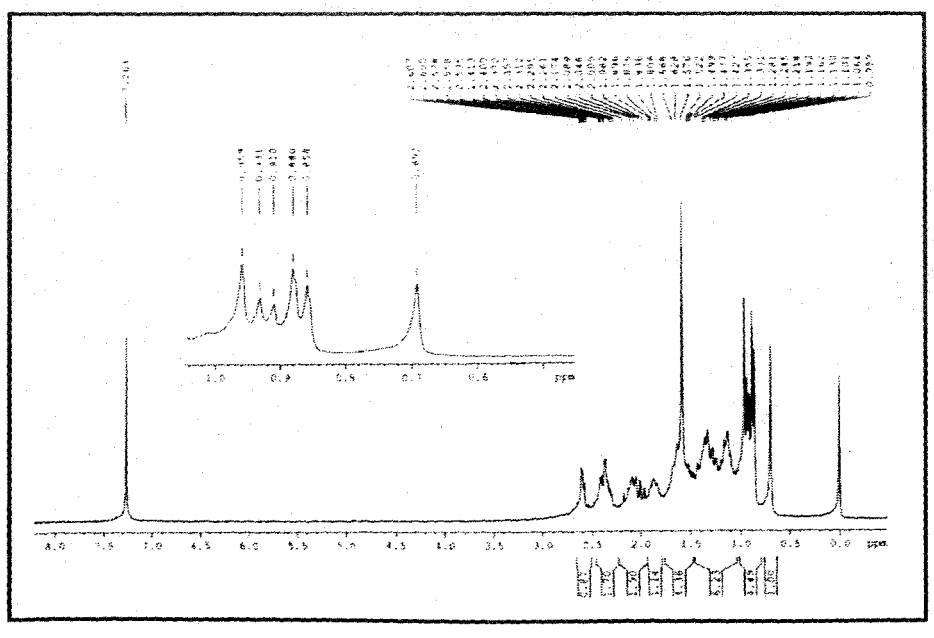


Figure 10. ^{1}H NMR spectrum of 5α-cholestan-3,6-dione (**191**).

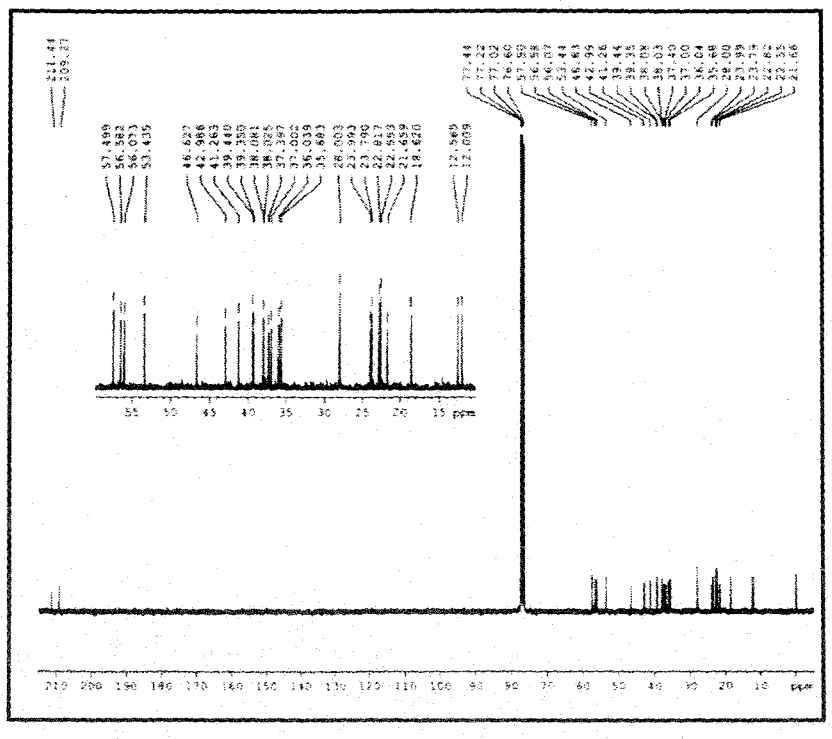


Figure 11. ^{13}C NMR spectrum of 5α-cholestan-3,6-dione (**191**).

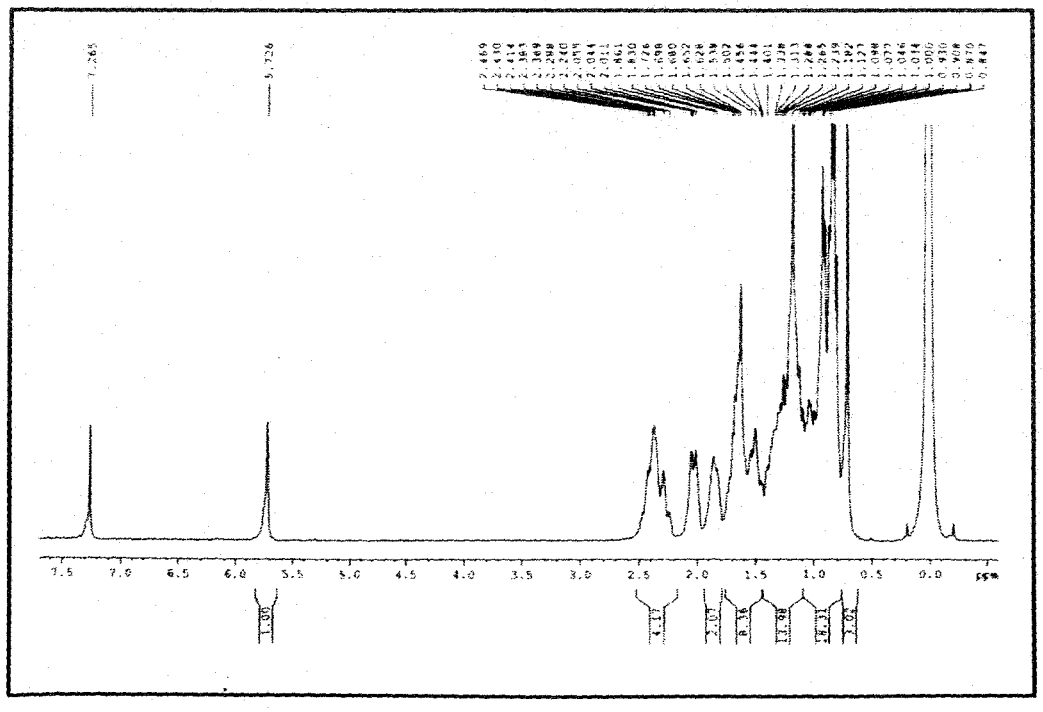


Figure 12. ^{1}H NMR spectrum of stigmastenone (**4b**).

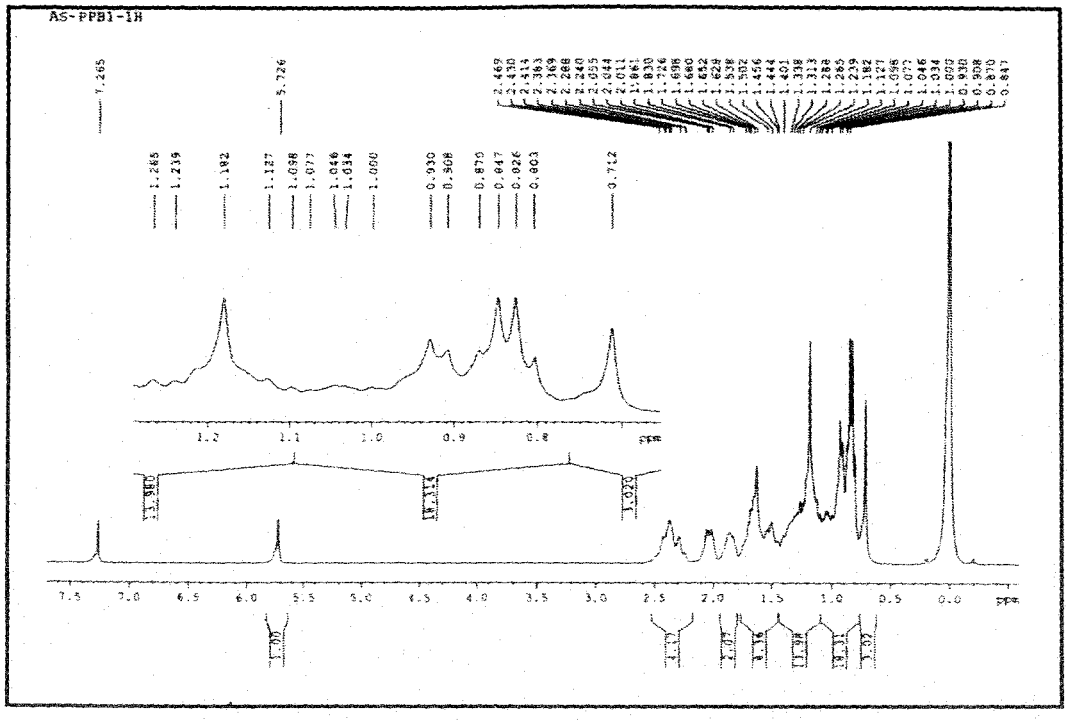


Figure 13. Extended ^{1}H NMR spectrum of stigmastenone (**4b**).

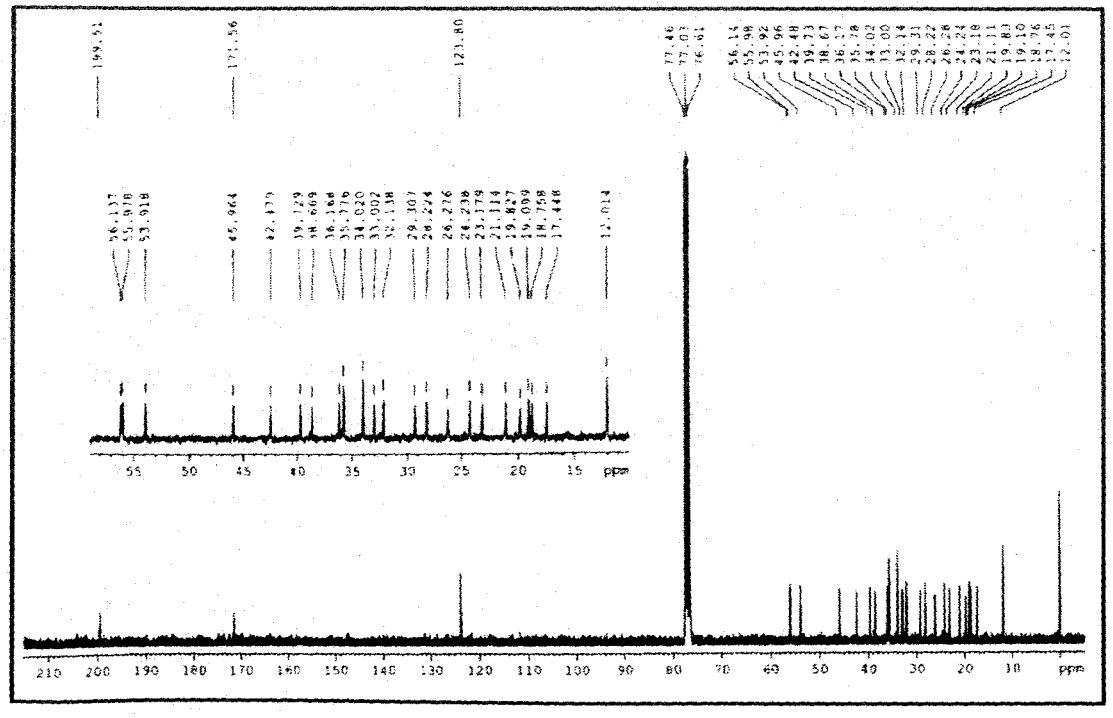


Figure 14. ^{13}C NMR spectrum of stigmastenone (**4b**).

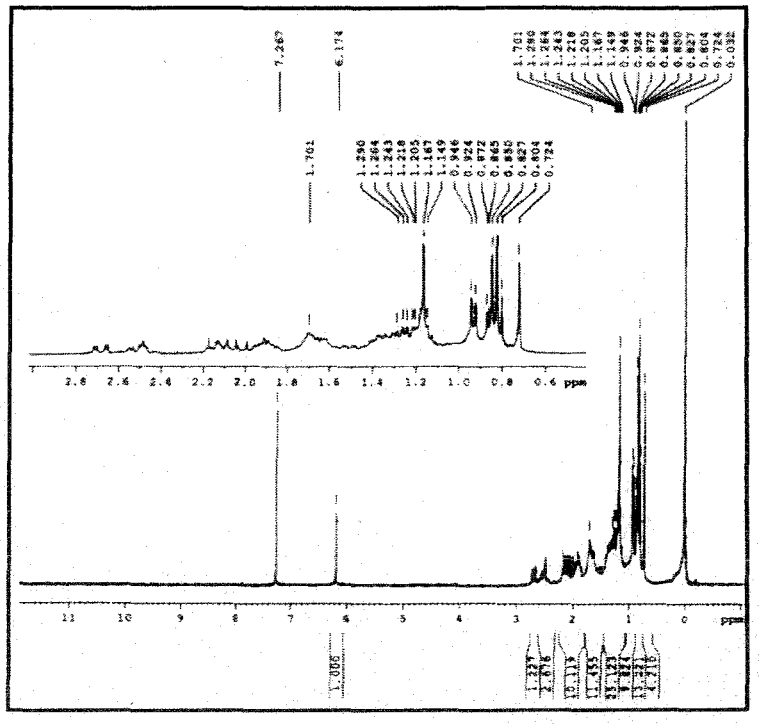


Figure 15. ^{1}H NMR spectrum of stigmast-4-ene-3,6-dione (**238**).

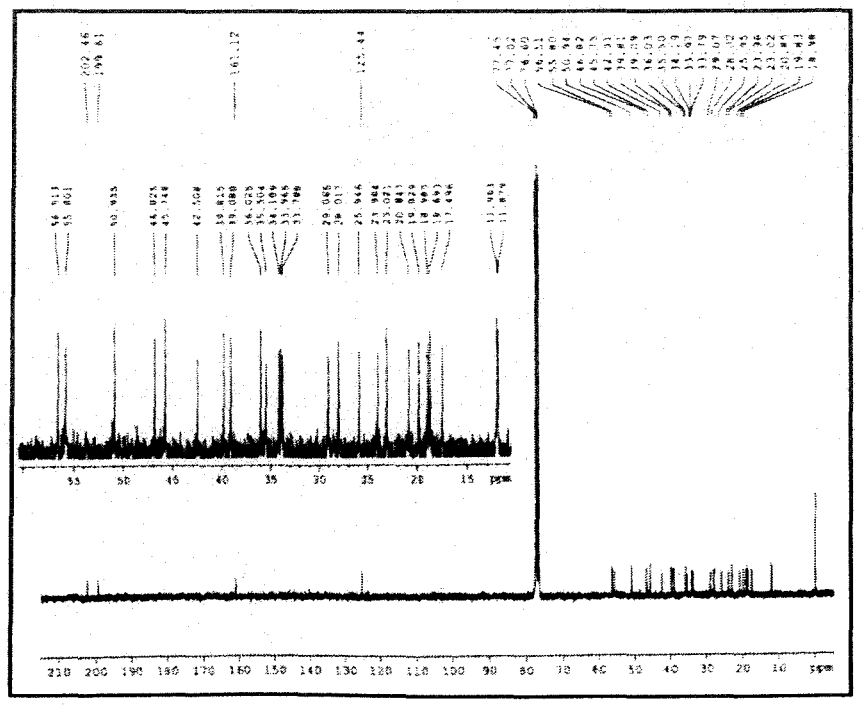


Figure 16. ^{13}C NMR spectrum of stigmast-4-ene-3,6-dione (**238**).

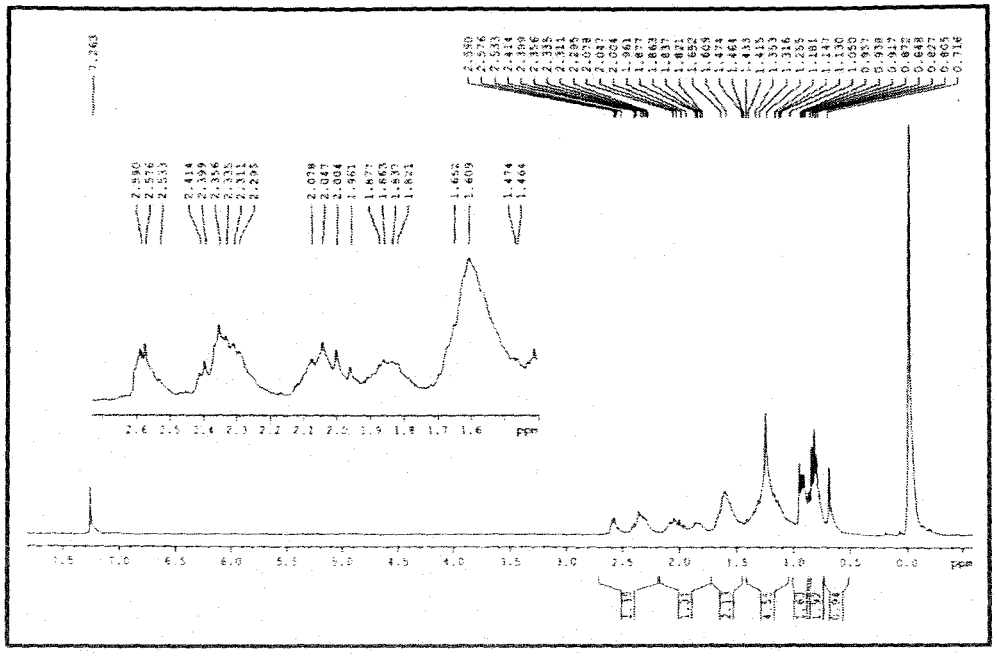


Figure 17. ^{1}H NMR spectrum of 5α-stigmastane-3,6-dione (**5**).

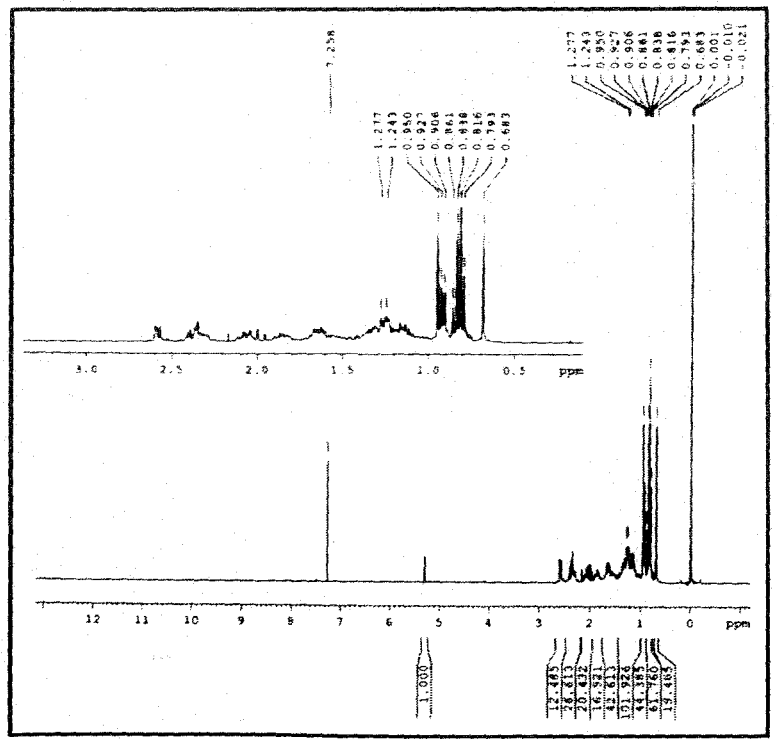


Figure 18. ^{1}H NMR spectrum (expanded) of 5α-stigmastane-3,6-dione (**5**).

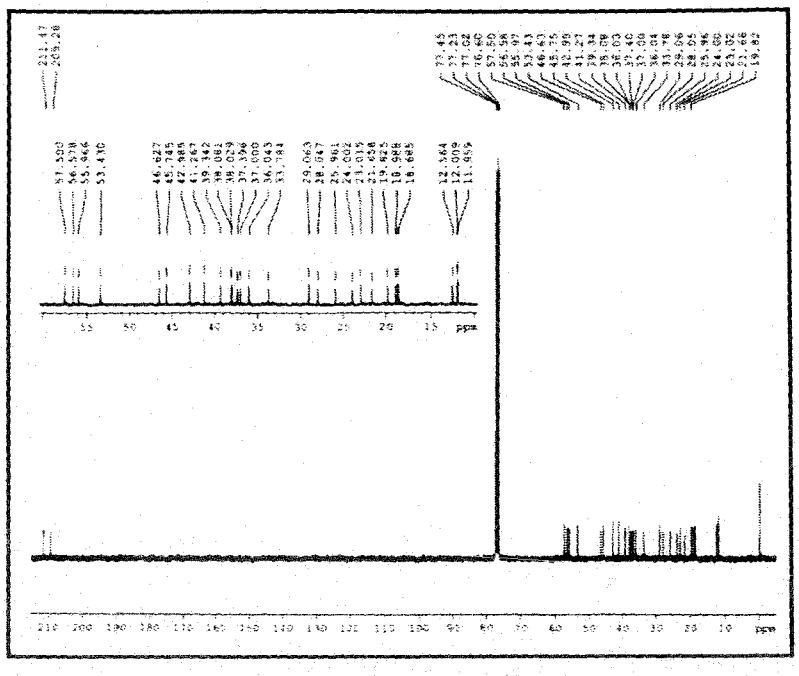


Figure 19. ^{13}C NMR spectrum of 5α-stigmastane-3,6-dione (**5**).

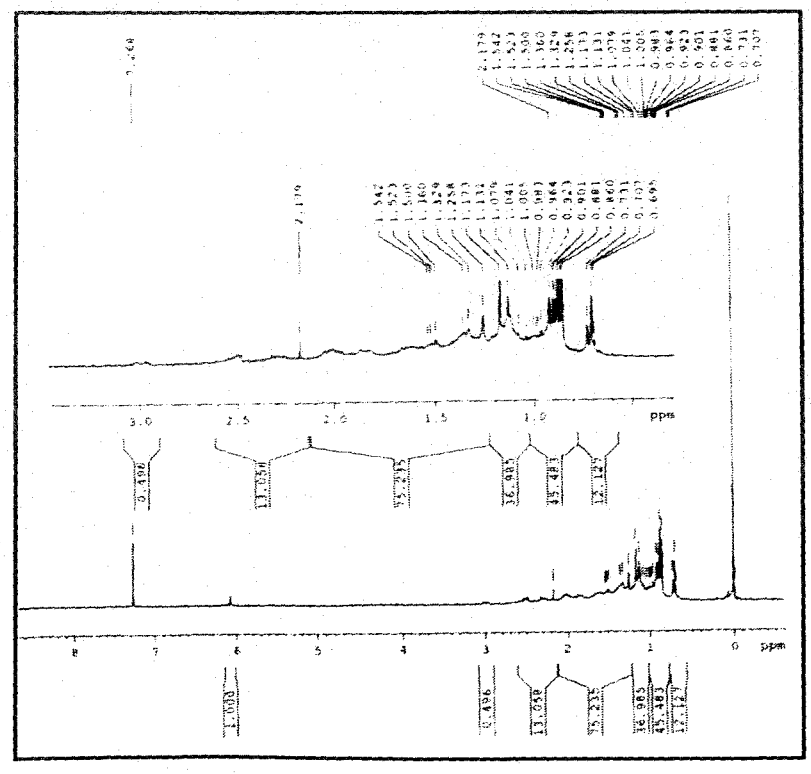


Figure 20. ^{1}H NMR spectrum of cholest-5-en-7-one (**243**).

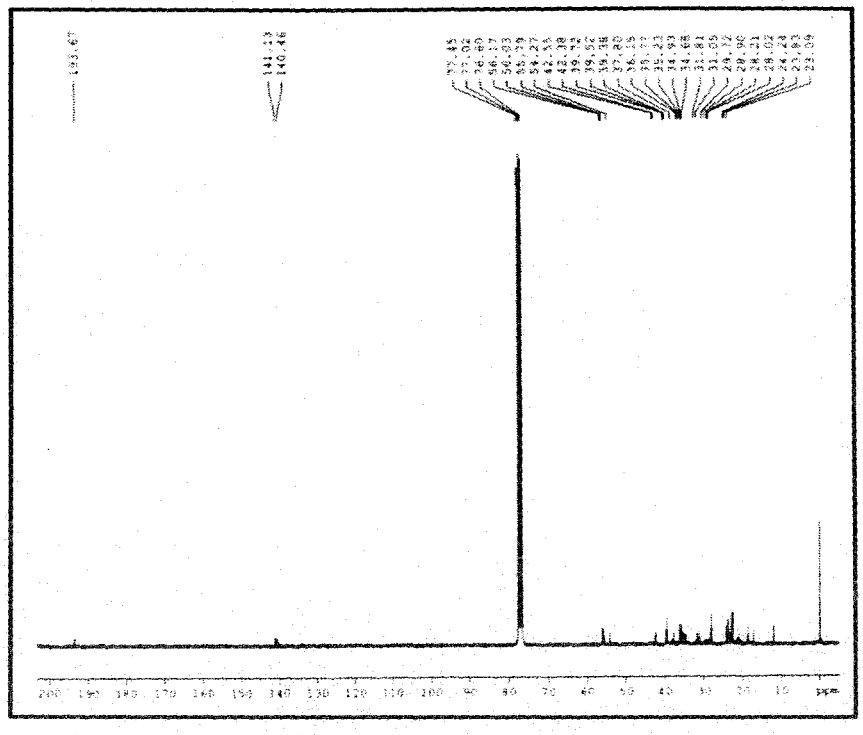


Figure 21. ^{13}C NMR spectrum of cholest-5-en-7-one (**243**).

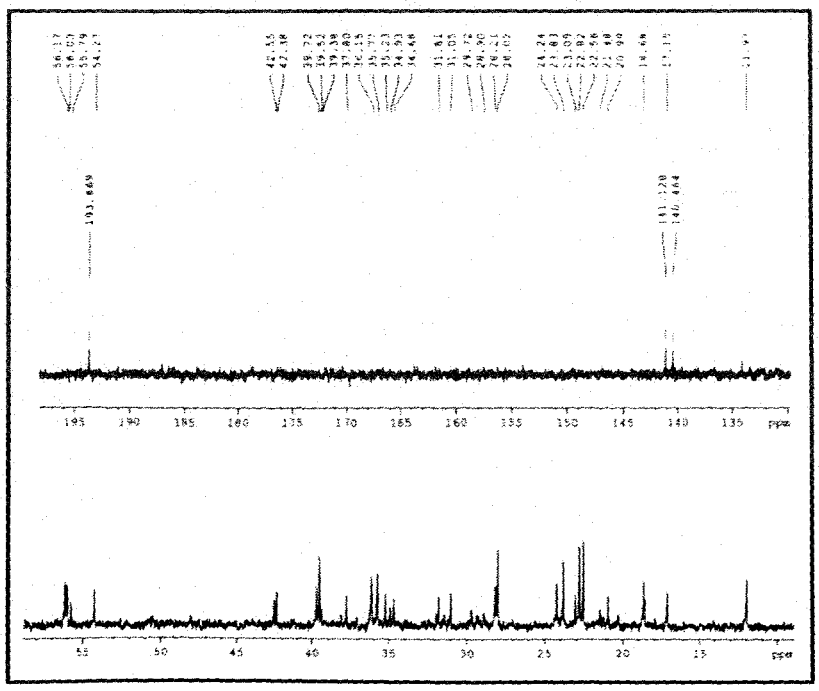


Figure 22. Expanded ^{13}C NMR spectrum of cholest-5-en-7-one (**243**).

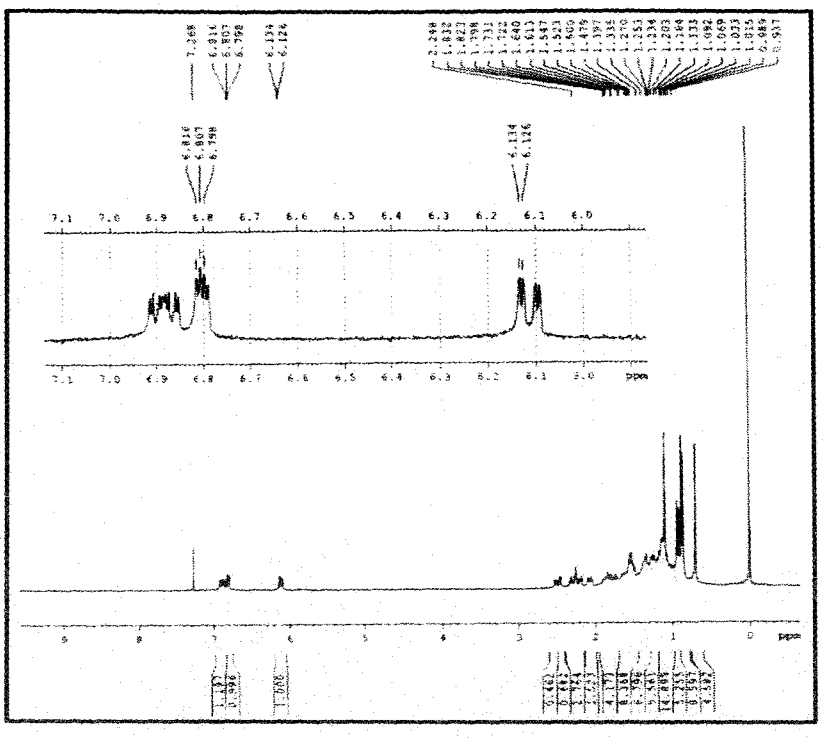


Figure 23. ^{1}H NMR spectrum of cholesta-3,5-diene-7-one (**192**).

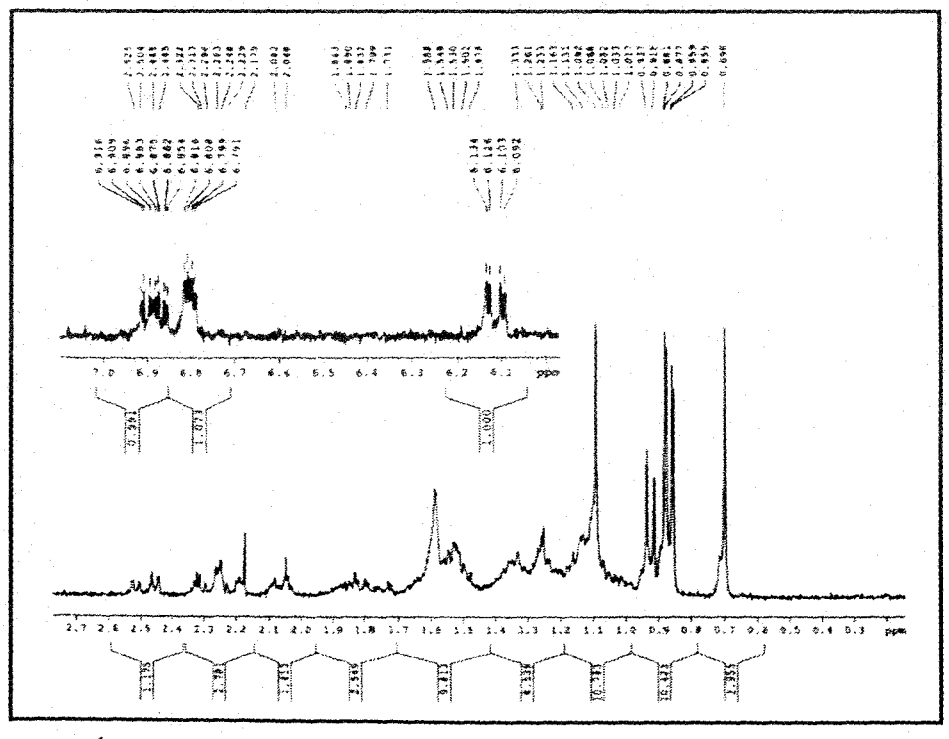


Figure 24. ^{1}H NMR spectrum (partially expanded) of cholesta-3,5-diene-7-one (**192**).

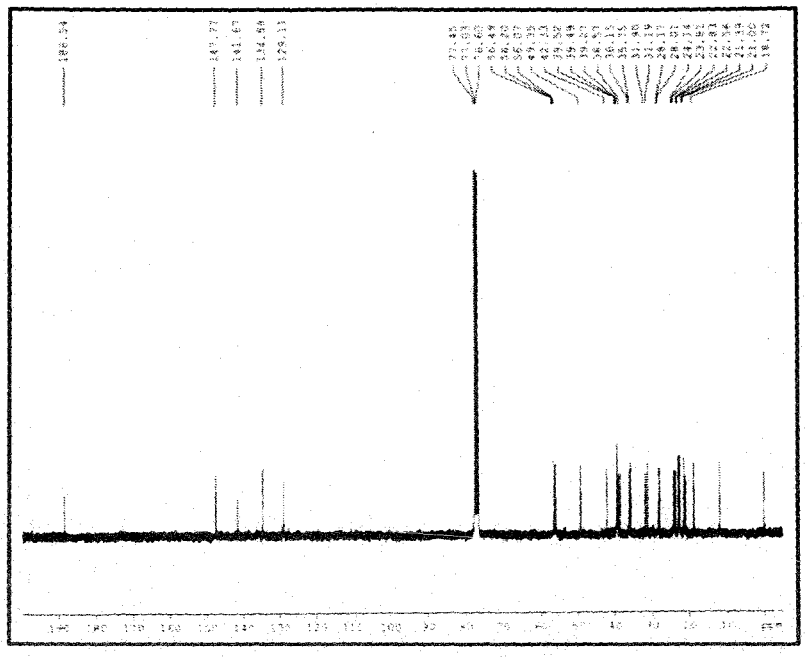


Figure 25. ^{13}C NMR spectrum of cholesta-3,5-dien-7-one (**192**).

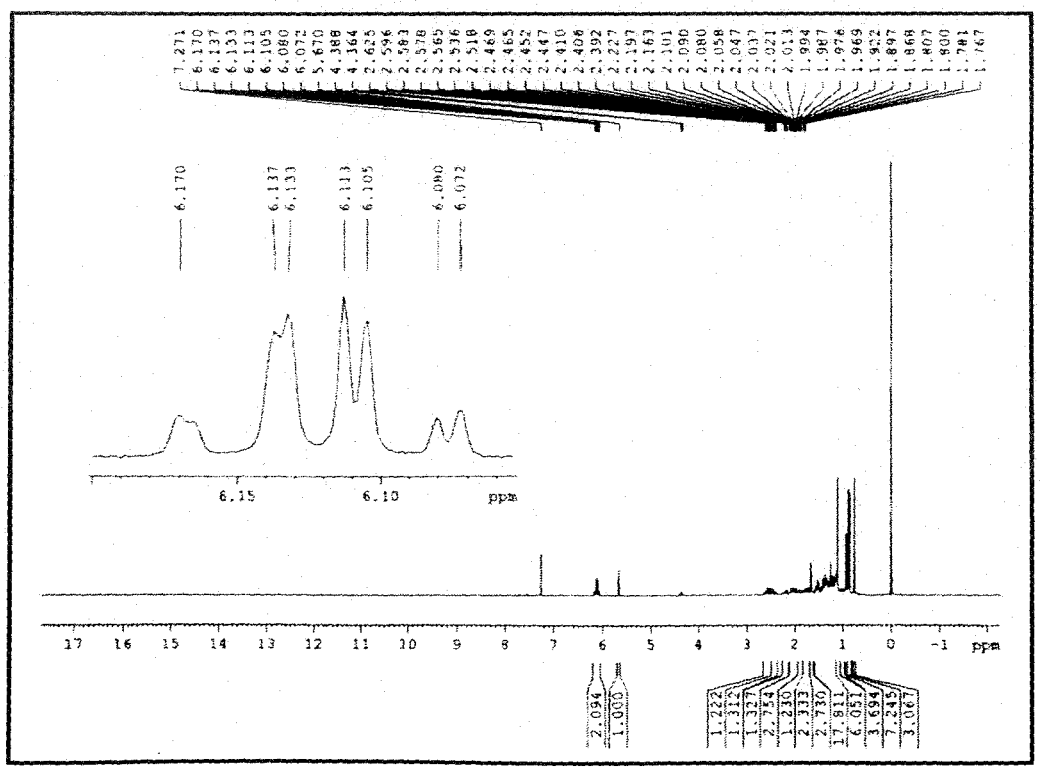


Figure 26. ^{1}H NMR spectrum of cholesta-4,6-diene-3-one (**203**).

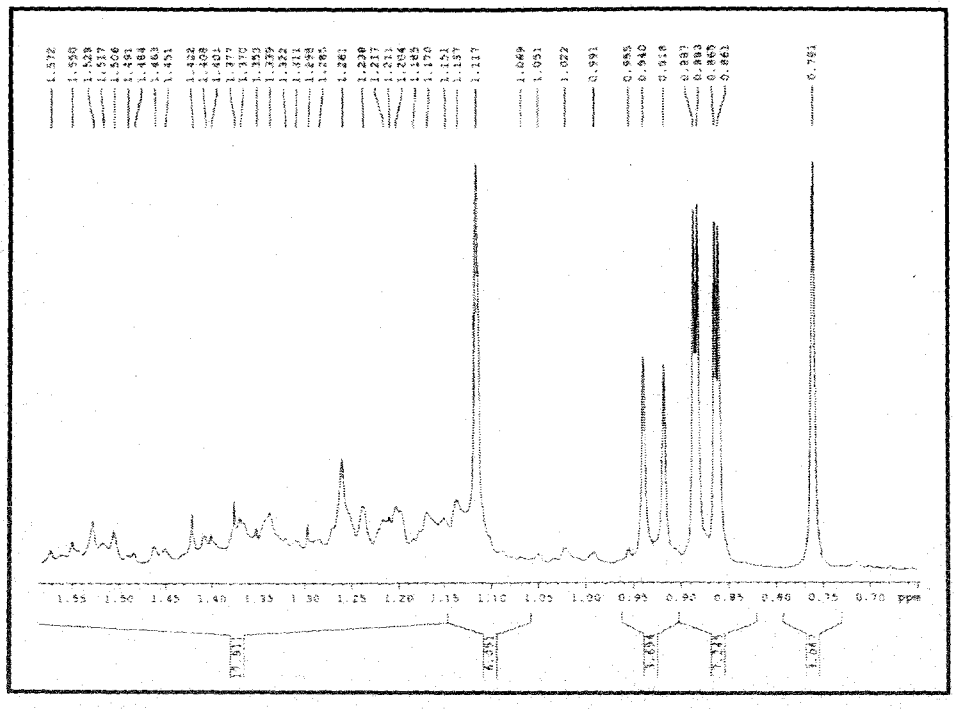


Figure 27. ^{1}H NMR spectrum (expanded methyls) of cholesta-4,6-diene-3-one (**203**).

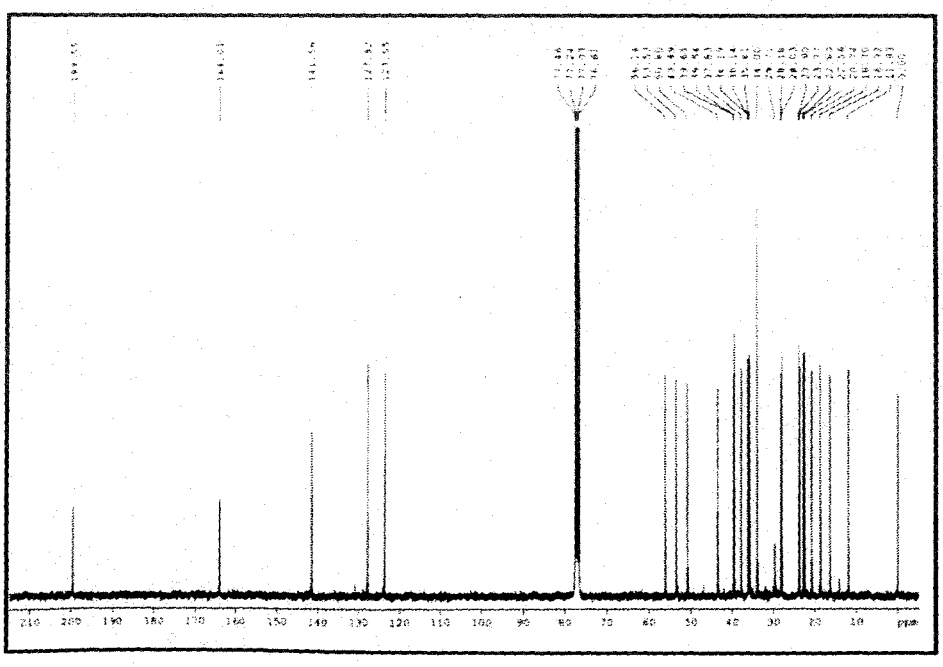


Figure 28. ^{13}C NMR spectrum of cholesta-4,6-diene-3-one (**203**).

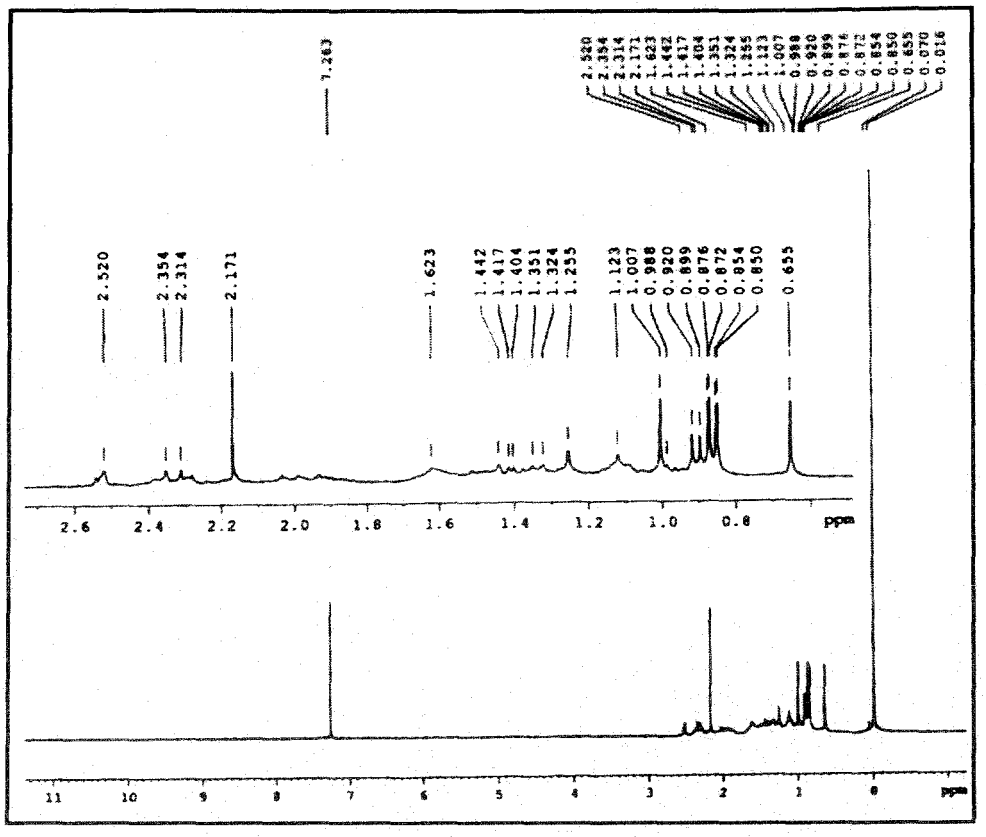


Figure 29. ^{1}H NMR spectrum of cholest-4,7-dione (**226**).

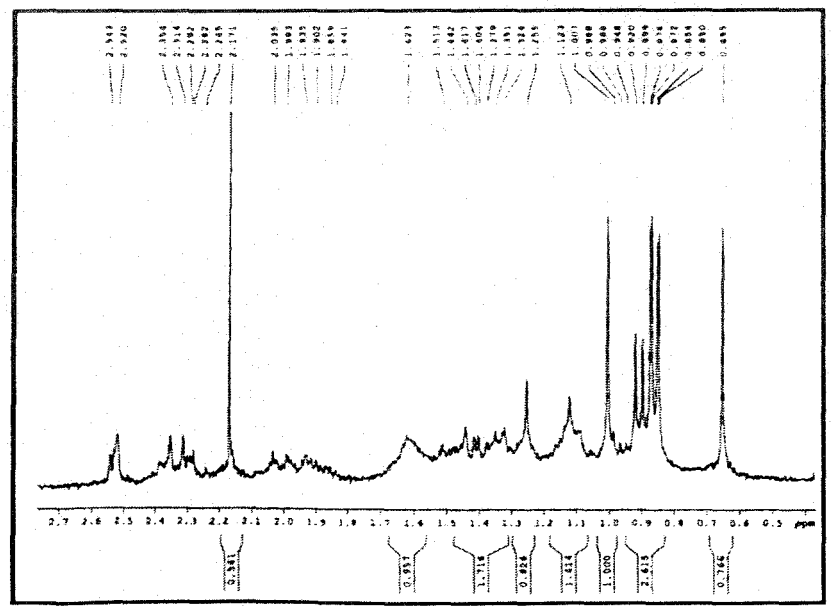


Figure 30. Extended ^{1}H NMR spectrum of cholest-4,7-dione (**226**).

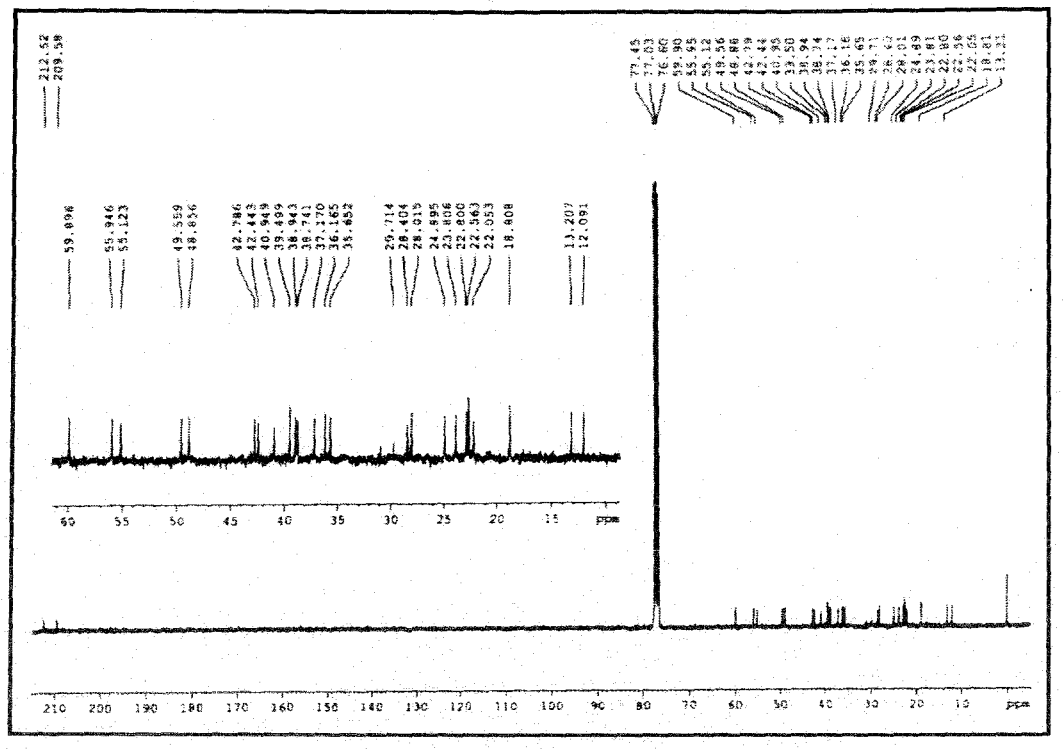


Figure 31. ^{13}C NMR spectrum of cholest-4,7-dione (**226**).

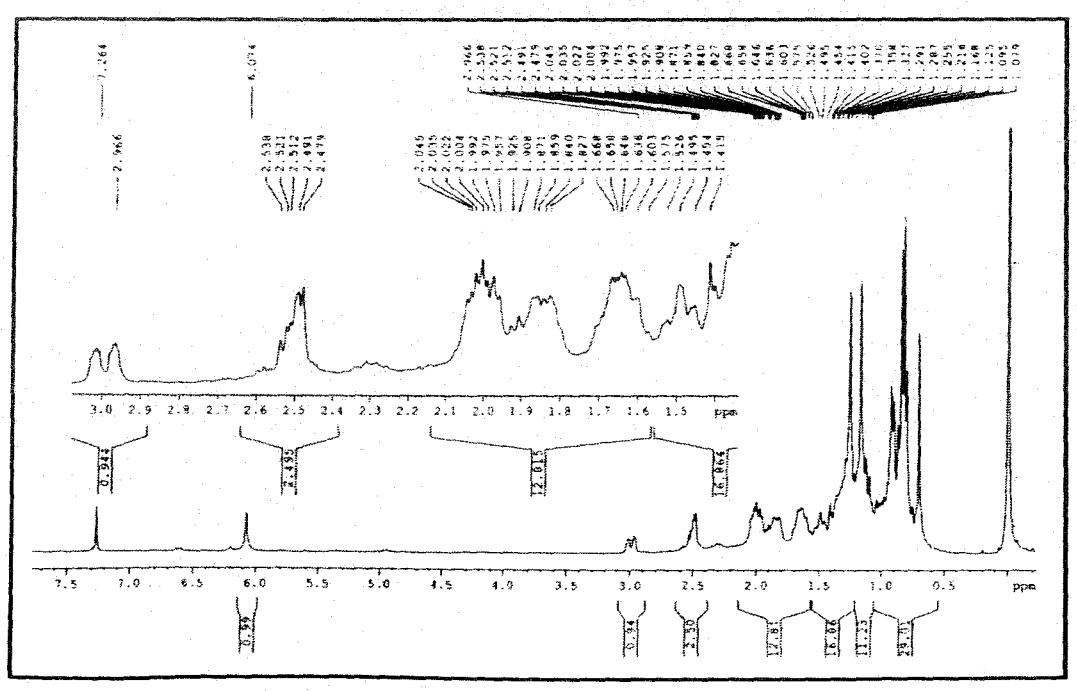


Figure 32. ^{1}H NMR spectrum of stigmast-5-en-7-one (**244**).

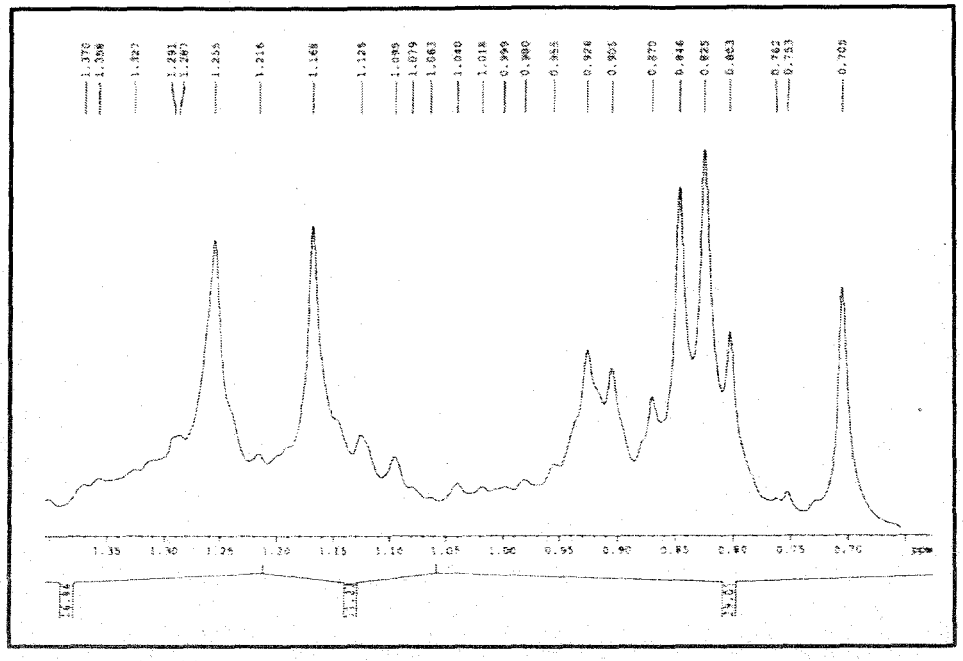


Figure 33. Partially expanded ^{1}H NMR spectrum of stigmast-5-en-7-one (**244**).

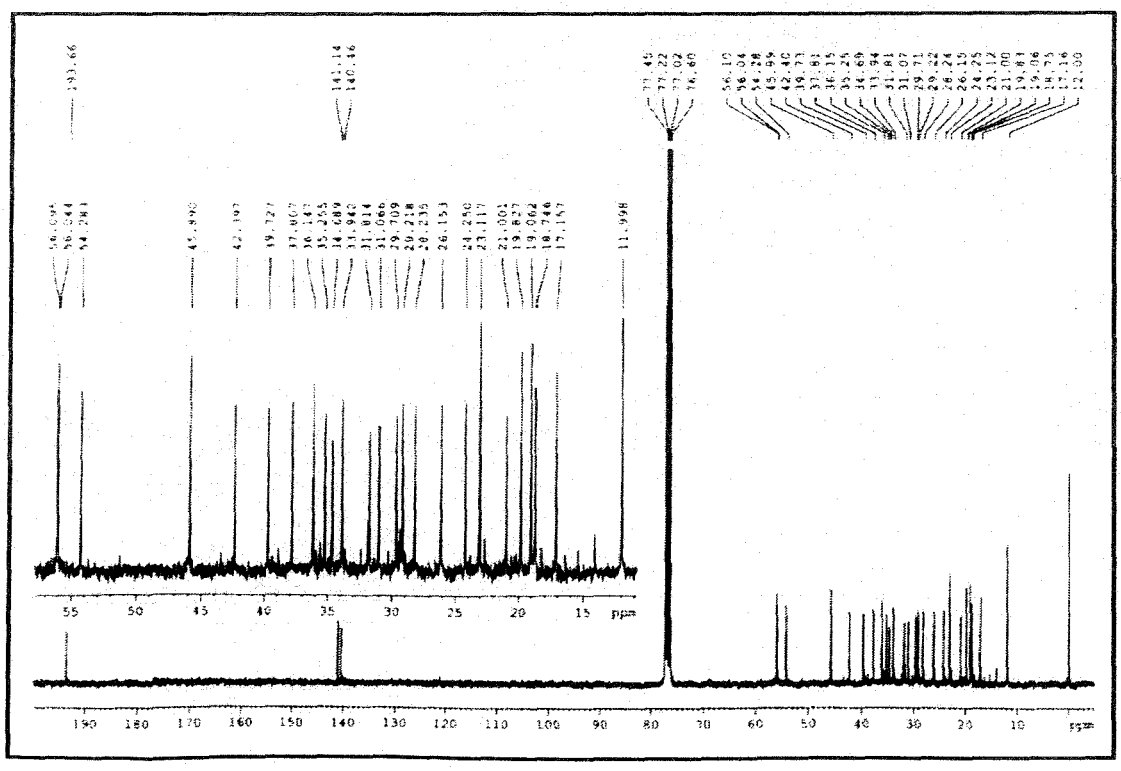


Figure 34. ^{13}C NMR spectrum of stigmast-5-en-7-one (**244**).

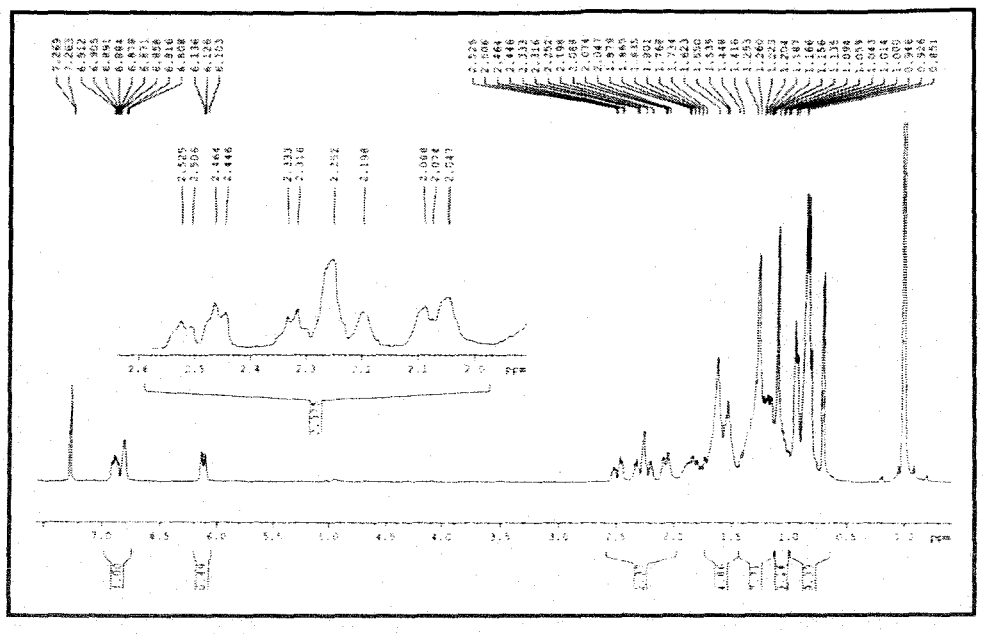


Figure 35. ^{1}H NMR spectrum of stigmasta-3,5-diene-7-one (**193**).

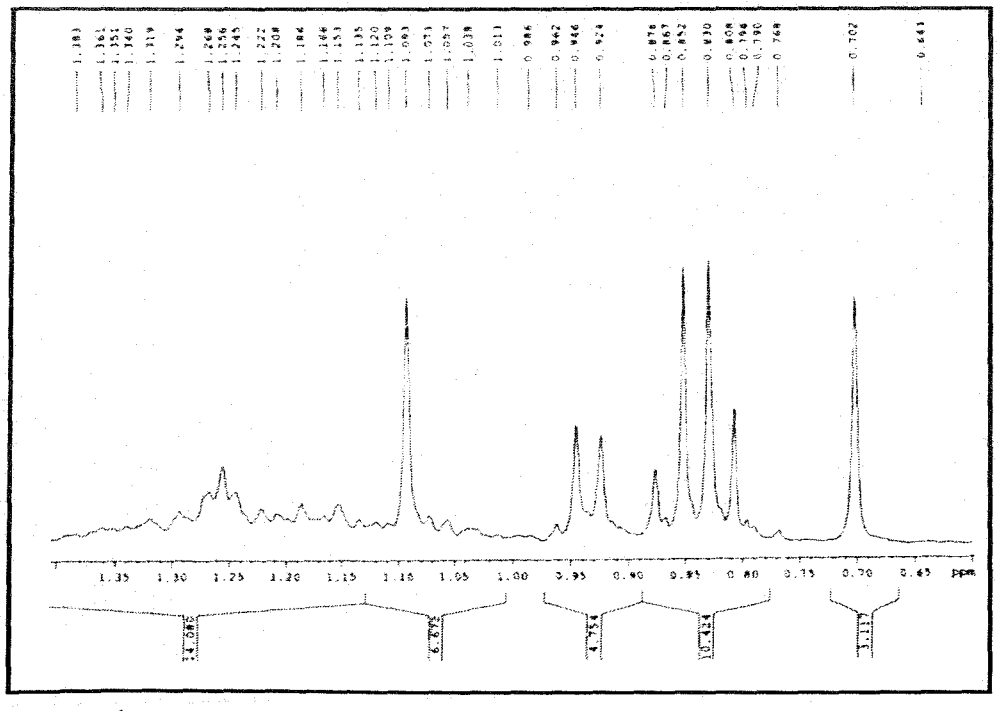


Figure 36. ^{1}H NMR spectrum (expanded methyls) of stigmasta-3,5-diene-7-one (**193**).

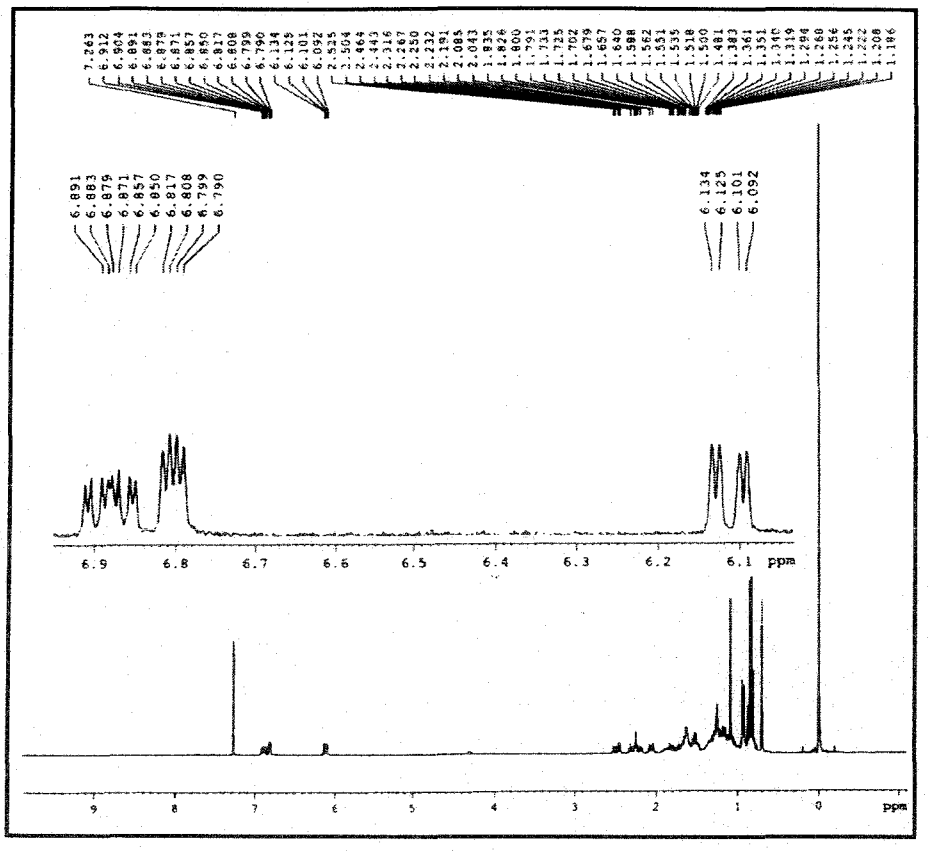


Figure 37. ^{1}H NMR spectrum (partially expanded) of stigmast-3,5-en-7-one (**193**).

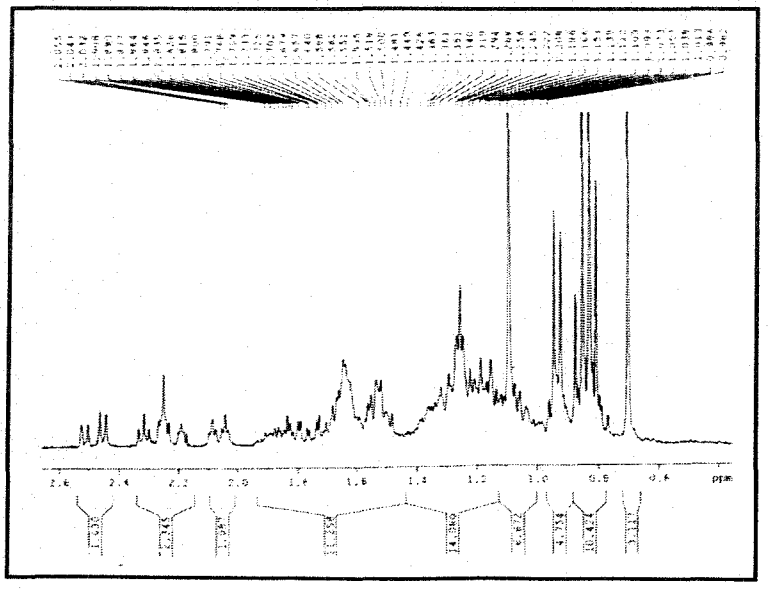


Figure 38. Partial ^{1}H NMR spectrum of stigmasta-3,5-diene-7-one (**193**).

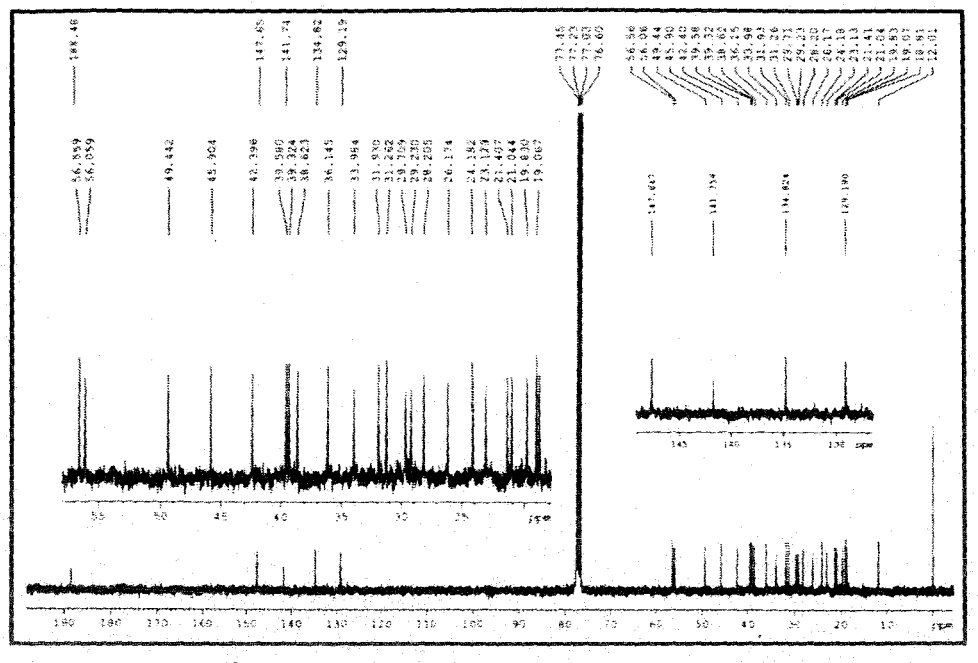


Figure 39. ^{13}C NMR spectrum of stigmasta-3,5-diene-7-one (**193**).

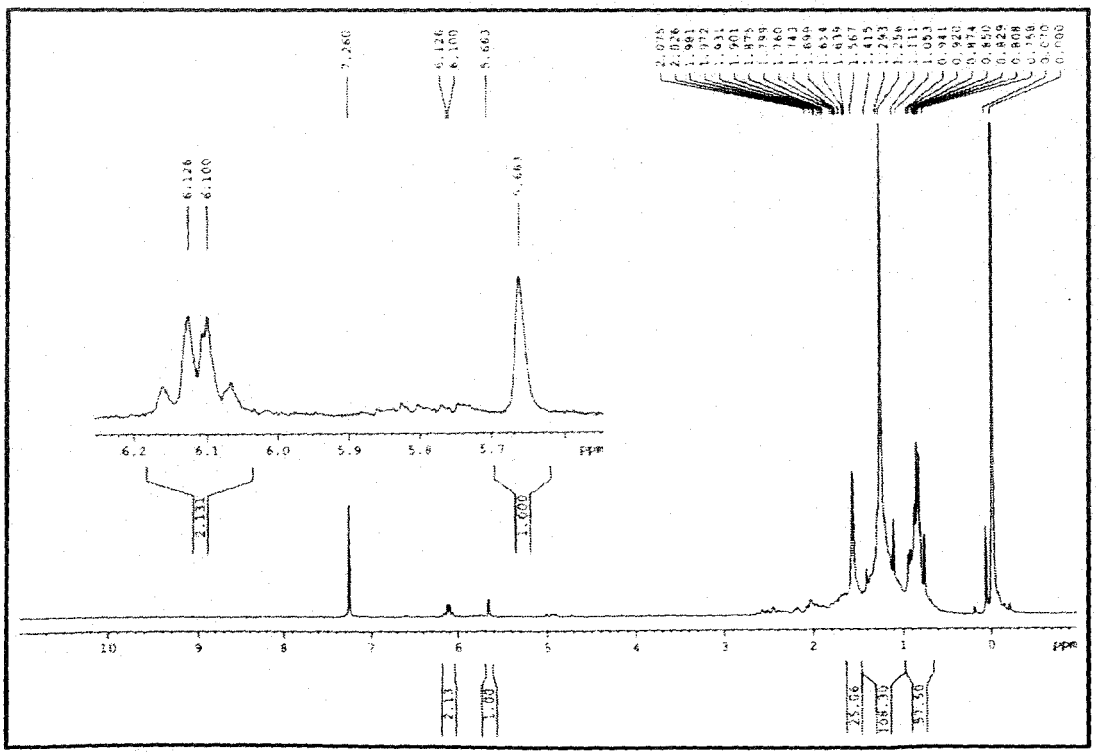


Figure 40. ^{1}H NMR spectrum (with partial expansion) of stigmasta-4,6-diene-3-one (**245**).

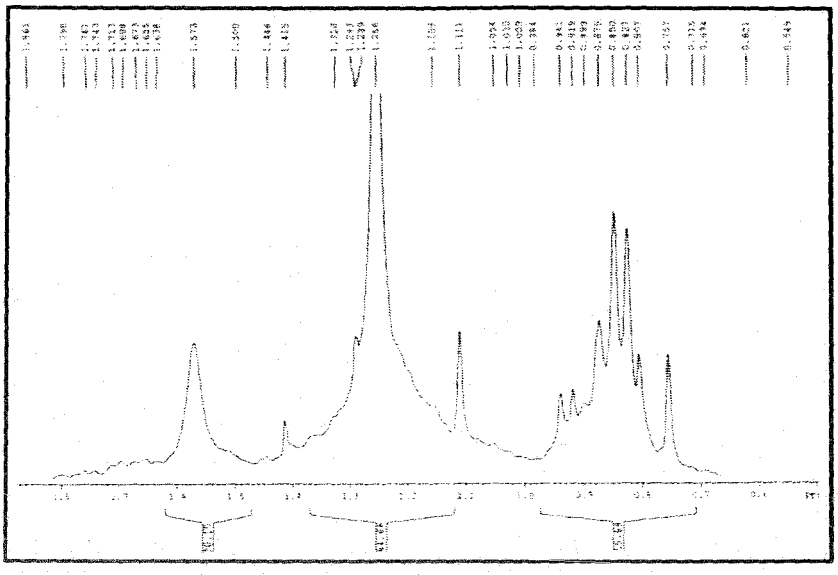


Figure 41. Partial ^{1}H NMR spectrum of stigmasta-4,6-diene-3-one (**245**).

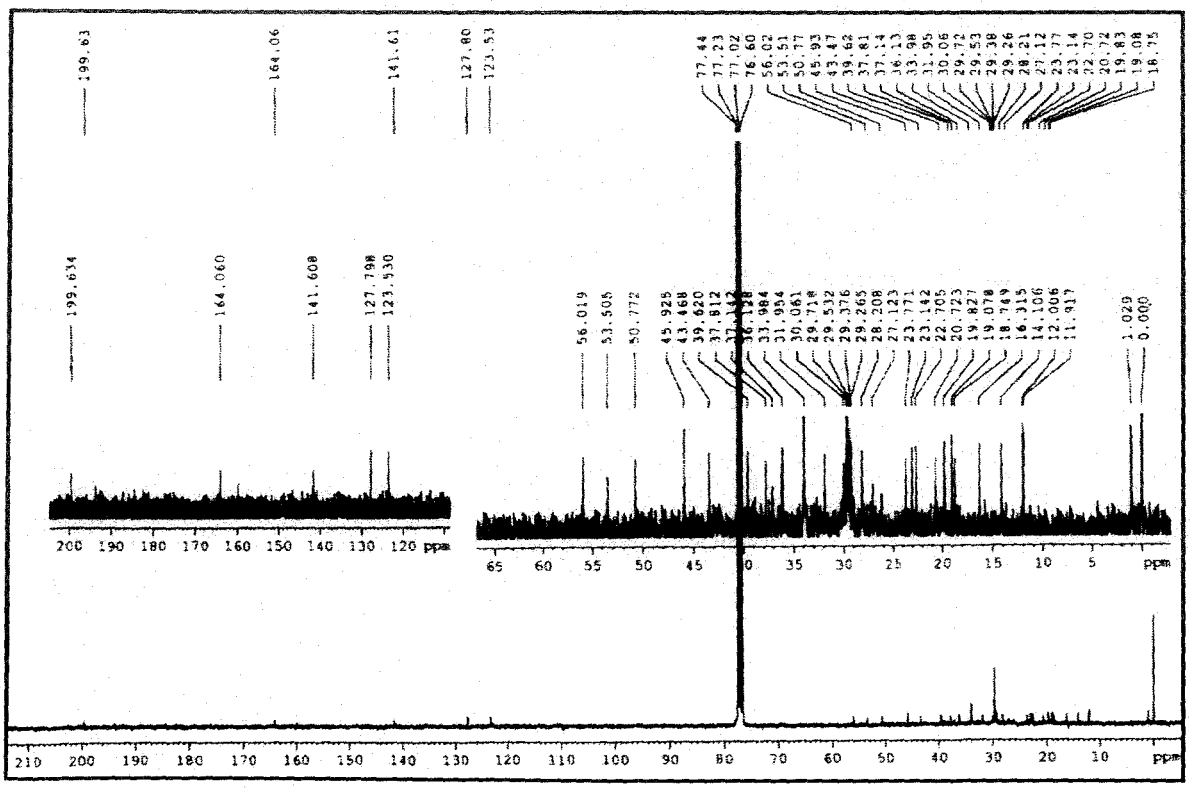


Figure 42. ^{13}C NMR spectrum of stigmasta-4,6-diene-3-one (**245**).

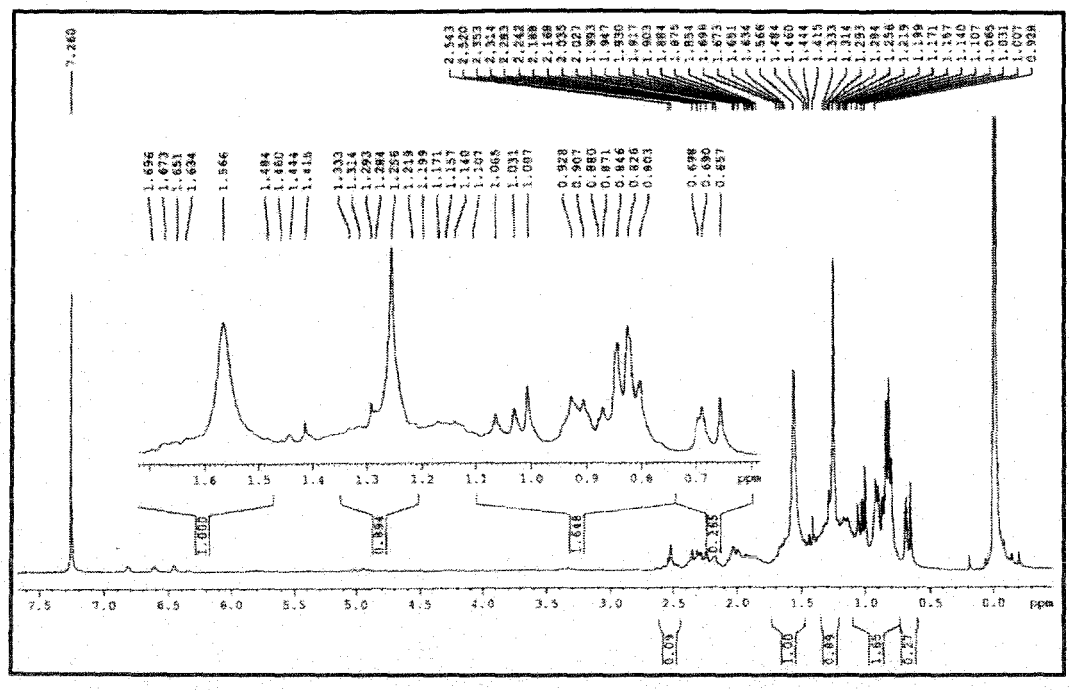


Figure 43. ^{1}H NMR spectrum of 5α-stigmastane-4,7-dione (**246**) (as mixture).

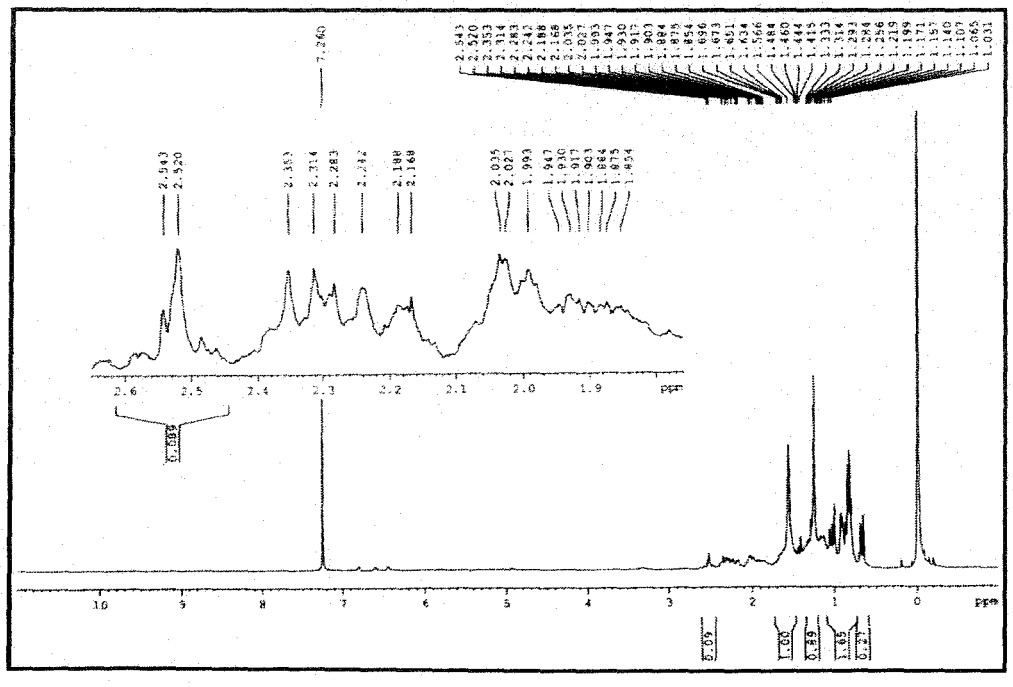


Figure 44. ^{1}H NMR spectrum (with partial expansion) of 5α-stigmastane-4,7-dione (**246**) (as mixture).

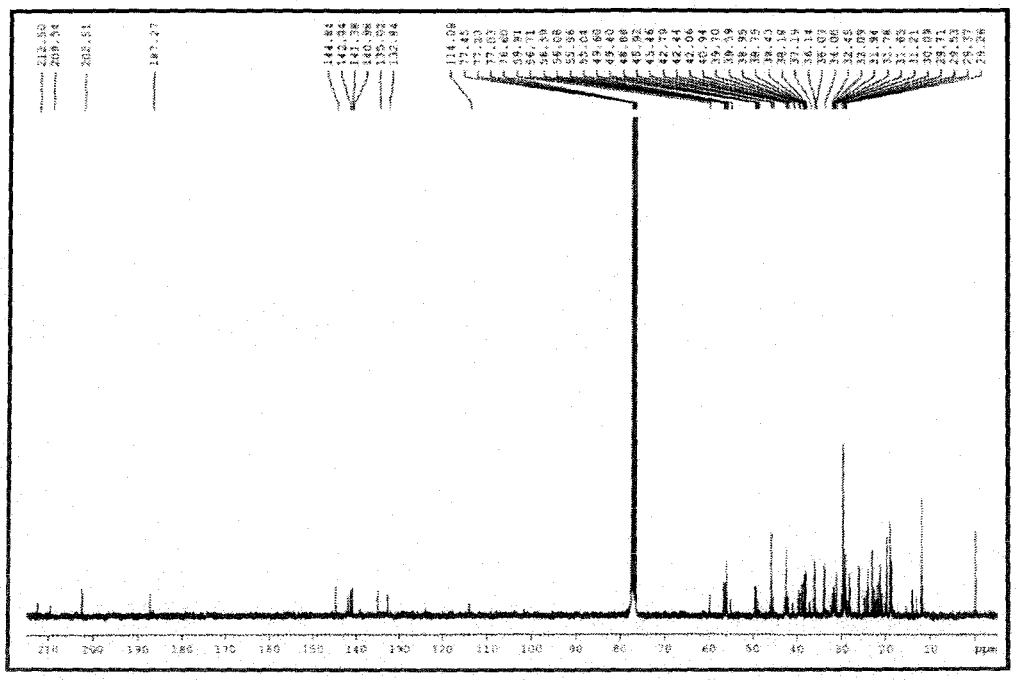


Figure 45. ^{13}C NMR spectrum of 5α-stigmastane-4,7-dione (**246**, as mixture).

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Chapter IV

Synthesis of A-ring modified friedelane triterpenoids and evaluation of their phytotoxicity

IV.A Introduction

IV.A.1 A short review on the bioactive friedelane triterpenoids

Friedelin (**25**), a pentacyclic triterpenoid, extracted from the bark of *Querques Suber*, along with cerin (**26**) as far back as 1807 by Chevreul[1,2] got its name in honour of Friedel who was probably the first one to disclose the presence of a ketone group in friedelin.[3] Istrati and Ostrogovich[4] confirmed that the cork extract contained two products, one of which contained a ketone group as was mentioned also by Friedel. Later, Drake et al., in their series of works established the molecular formula of friedelin and showed that it can be converted to oxime by hydroxyl amine hydrochloride, enol esters and carbonyl derivatives and to the saturated parent hydrocarbon friedelane. This oxime forms oxime acetate when refluxed with acetic anhydride, undergoes Beckmann rearrangement when treated with PCl_5 and reconverts to friedelin on hydrolysis with H_3PO_4 in n-amyl alcohol.[5-9] But it was the success of Corey and Ursprung and Dutler, Jeger and Ruzicka, separately, in 1956 to give the complete structures of friedelin and cerin and then Brownnlie and his collaborators gave their stereochemistry (**Figure 1**).[10-12] This opened up a broad scope of thorough study with friedelin and its derivatives.

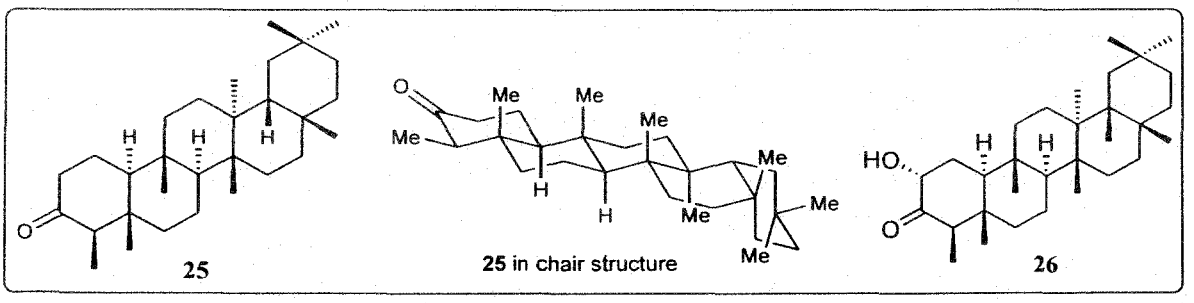


Figure 1. Friedelin (**25**), its chair form and cerin (**26**).

IV.A.1.1 Major natural sources of friedelane triterpenoids and their bioactivity

More than 400 natural friedelane triterpenoids are isolated till date. The families of the plants where friedelanes were found most abundantly are *Celastraceae*, *Hippocrateaceae*, *Euphorbiaceae*, *Flacourtiaceae*, and *Guttiferae* and among them the first two are the richest sources of it. Shoppee and Johnston isolated the same two compounds from the bark of the pimply ash, *Balanops australiana* F. Muell, found mainly in the tropical rain-forests of North Queensland.[13] Some of the triterpenoids are found in leaf and twig parts and others in root parts.

Surprisingly, friedelanes are also present in some fungi.[14-15] Friedelanes do not occur as glycosides in nature though there are reports where other triterpenoids are found in nature as saponins.[16-18] In a recent review Zhan et al., have beautifully summarize the natural sources of friedelane triterpenoids.[19] Very few of those are described herein again in short.

Inner bark extract of *Kokoona zeylanica* Thwaites was found to contain kokoononol which is actually 27-hydroxyfriedelane-3,21-dione (**247**). Kokoondiol and kokoonol, present in the same extract were proved to be 21α,27-dihydroxyfriedelane-3-one (**248**) and 27-hydroxyfriedelane-3-one (**249**) respectively (**Figure 2**).[20]

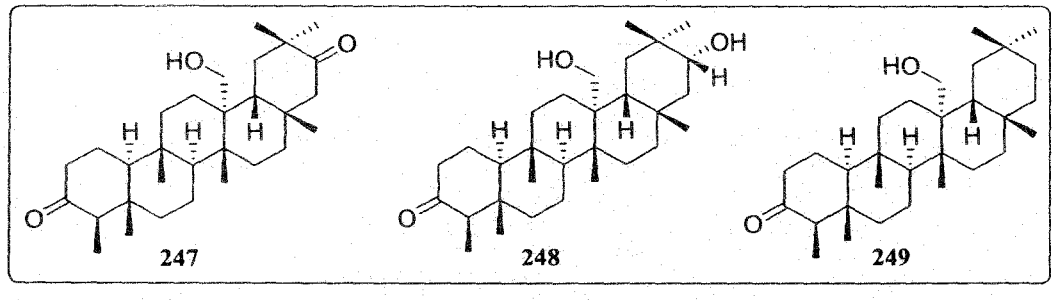


Figure 2. Kokoononol (**247**), kokoondiol (**248**) and kokoonol (**249**).

In 1991, Luis and his group isolated friedoolean triterpenoids from the root extract of *Schaefferia cuneifolia* and it was proved to be 2-oxofriedoolean-3-en-29-oic acid (**250**).[21] The corresponding methyl ester derivative (**251**) was also prepared (**Figure 3**).

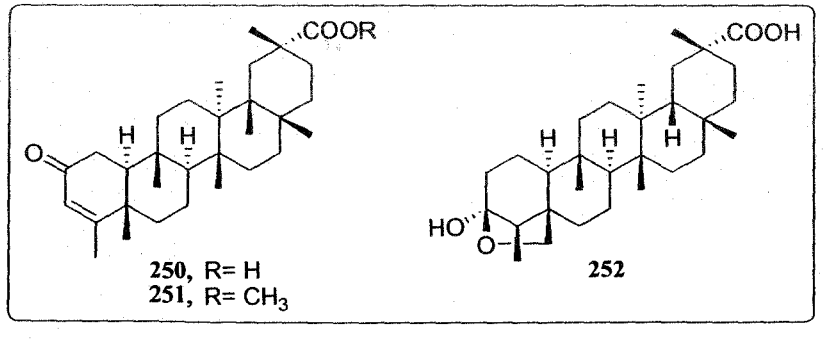


Figure 3. 2-Oxofriedoolean-3-en-29-oic acid (**250**) and its methyl ester derivative (**251**) and salaspermic acid (**252**).

Choloroform extract of *Tripterygium wilfordii* gave salaspermic acid (**252, Figure 3**), a compound having friedelane skeleton. This compound was found to inhibit HIV replication in H9 lymphocytes with an IC_{50} value of 5 μg/mL (10 μM), and it inhibited uninfected H9 cell growth with an IC_{50} of 53 μM.[22]

Stems and bark extract of *Peritassa compta* was found to contain friedelane-3,15-dione (**253**), 15α-hydroxyfriedelin (**254**) and 15α-hydroxyfriedelane-1,3-dione (**255**), along with friedelin (**25**) and friedelane-1,3-dione (**256**) (**Figure 4**).[23]

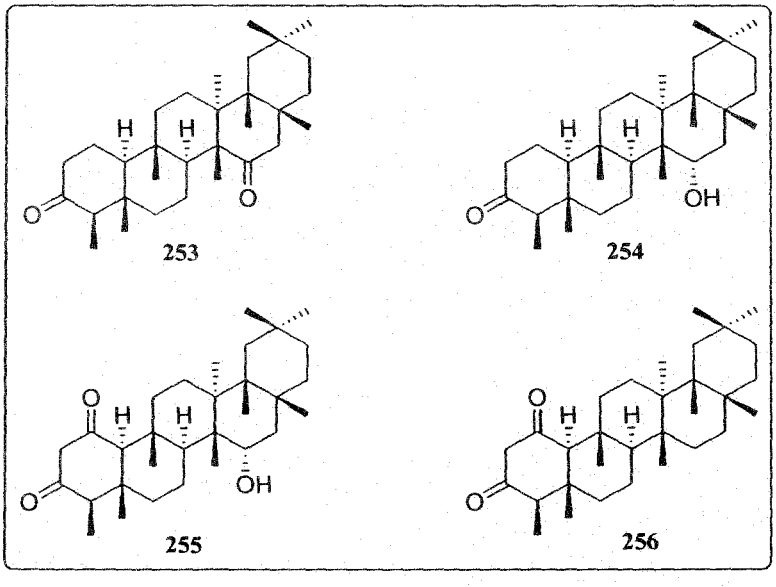


Figure 4. Friedelane-3,15-dione (**253**), 15α-hydroxyfriedelin (**254**), 15α-hydroxyfriedelane-1,3-dione (**255**) and friedelane-1,3-dione (**256**).

Friedelin was found to be a major constituent of *Grapefruit epicuticular* Wax,[24] *A. indica*[25] and also the stem bark exudates of *Maytenus macrocarpa,* though in the latter case friedelane (**17**), 3-oxo-29-hydroxyfriedelane (**257**) and 3-oxofriedelan-25-al (**258**) were also isolated (**Figure 5**).

The biocidal activities of **17** were studied and found to be inactive to the antitumor activity against P-388 lymphoid neoplasm, A-549 human lung carcinoma, HT-29 human colon carcinoma, or MEL-28 human melanoma cell lines, but showed weak activity against aldose reductase.[26]

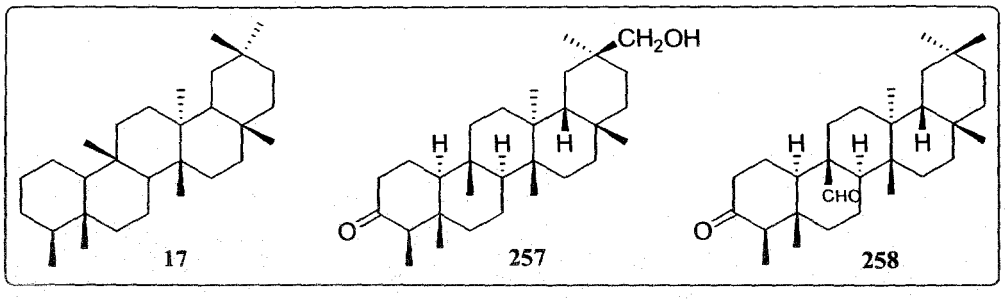


Figure 5. 3-Oxo-29-hydroxyfriedelane (**257**) and 3-oxofriedelan-25-al (**258**).

From the undescribed *Salacia* species from Costa Rica, two epimers, 30-hydroxyfriedelan-3-one-28-al-28(R)-hemiacetal (**259R**) and 28(S)-hemiacetal (**259S**) were found (**Figure 6**).[27]

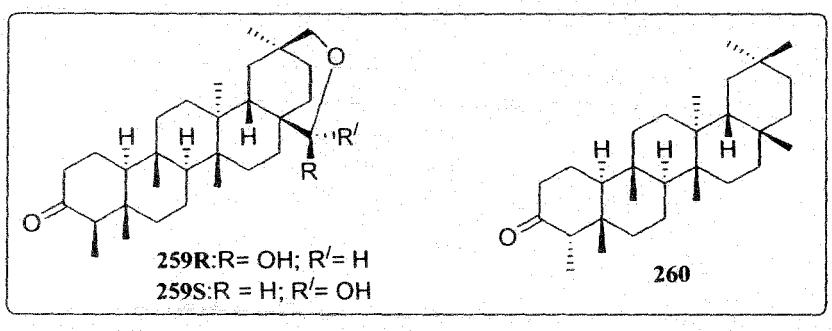


Figure 6. 30-Hydroxyfriedelan-3-one-28-al-28R-hemiacetal (**259R**) and -28S-hemiacetal (**259S**) and 4-*epi*friedelin (260).

The leaves of *Syzygium formosanum* were found to be a good source of 4-*epi*friedelin (**260**) along with 12 other known terpenoids (**Figure 6**).[28]

During phytochemical investigation of *Maytenus truncata* Reiss, 3β-hydroxy friedelane (**261a**) and 3α-hydroxy friedelane (**261b**) were isolated (**Figure 7**).[29]

Maytenus aquifolium and *M. ilicifolium* plant extracts show anti-ulcer activity and are used medicinally in Brazil under the name "espinheirasanta". Lancas et al., showed that friedelin (**25**) and 3β-hydroxy friedelane (**261a**) are markers of this plants.[30-31] Friedelin and quinonemethide were accumulated in the leaves and root bars of *M. aquifolium* and *S. campretris* respectively. From the enzymatic extracts of the leaves it was found that cyclase converted the substrate oxidosqualene to the triterpenes, 3β-hydroxy friedelane (**261a**) and friedelin (**25**). Moreover,

when mevalonolactone was administrated to the leaves of *M. aquifolium* seedlings, radio labelled friedelin was produced in the leaves, twigs and stems, while labelled maytenin and pristimerin were found in the root bark. From this important observation the author concluded that triterpenes once biosynthesized in the leaves were translocated to the root bark and further transformed to the antitumoral quinonemethide triterpenoids.[31-32]

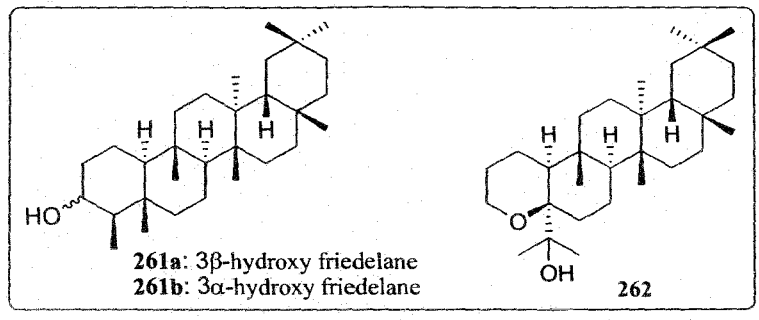


Figure 7. 3β-Hydroxy- and 3α-hydroxy friedelane (**261a** and **261b**, respectively) and terminaline A (**262**).

Investigation of the leaves and barks of the Indian *L. racemosa* collected from Sundarban and Kakinada[32] and the stems of the same collected from the Indian Bhiravapalem Island[33] revealed the presence of friedelin. *Terminalia glaucescens* was also found to contain a secotriterpene terminaline A (**262, Figure 7**) and friedelin.[34]

Faure et al. isolated three new friedelane-type triterpenoids, 3,4-secofriedelane-3,28-dioic acid (**263**), 27-hydroxyacetate canophyllic acid (**264a**) and 3-oxo-27-hydroxyacetate friedelan-28-oic acid (**264b**), from the leaves of *Calophyllum inophyllum* (Clusiaceae) found in French Polynesia (**Figure 8**).[35]

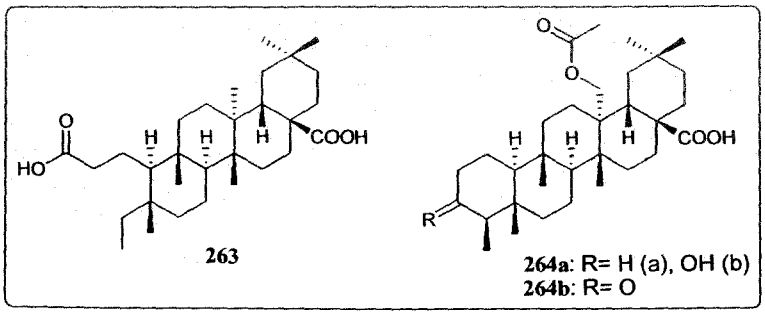


Figure 8. 3,4-Secofriedelane-3,28-dioic acid (**263**), 27-hydroxyacetate canophyllic acid (**264a**) and 3-oxo-27-hydroxyacetate friedelan-28-oic acid (**264b**).

The stem and bark of the Mangrove plant *Hibiscus tiliaceus* was found to be a source of a new friedelane-type triterpene named 27-oic-3-oxo-28-friedelanoic acid (**265**) along with five others friedelane-type triterpenoids (**266a-266e**) (**Figure 9**).[36]

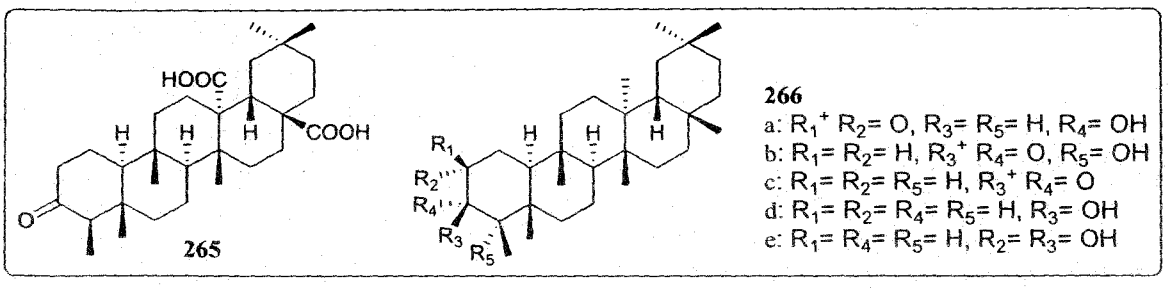


Figure 9. 27-Oic-3-oxo-28-friedelanoic acid (**265**) and five other triterpenoids (**266a-266e**).

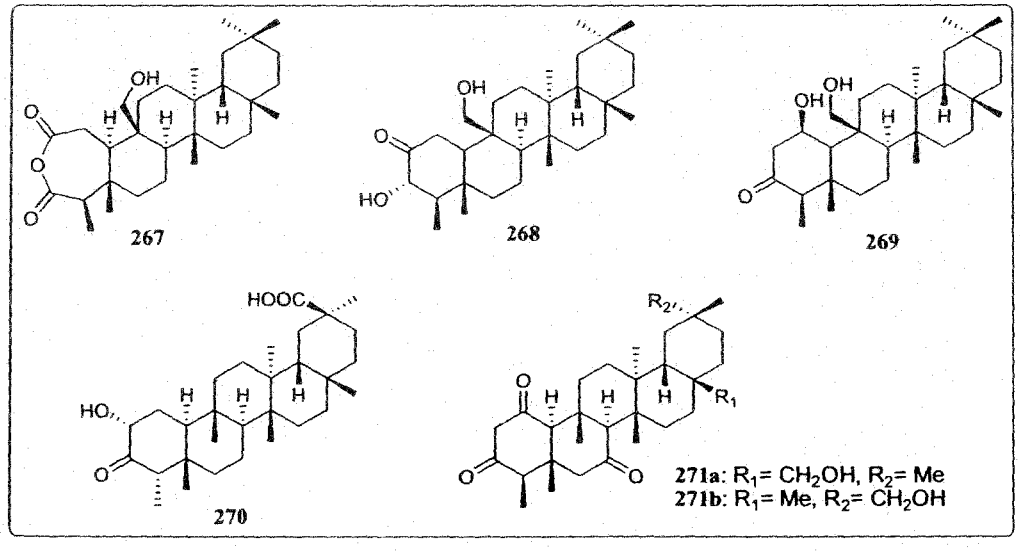
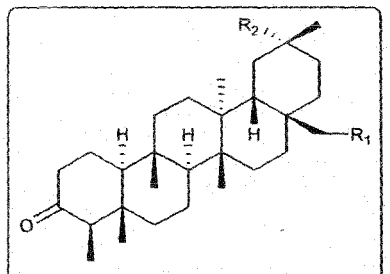


Figure 10. Lobatanhydride (**267**), 3α,25-dihydroxyfriedelan-2-one (**268**), 1β,25-dihydroxyfriedelan-3-one (**269**), 2α-hydroxy-3-oxofriedelan-30-oic acid (**270**), 28-hydroxyfriedelane-1,3-dione (**271a**) and 29-hydroxyfriedelane-1,3-dione (**271b**).

Friedelin (**25**) and 3β-hydroxy friedelane (**261a**) were isolated from *Artocarpus altilis*[37] and friedelin (**25**) along with 4-*epi*friedelin (**260**) were isolated from the leaves of *Salacia chinensis*.[38] The first example of triterpene anhydride is Lobatanhydride (**267**), containing A-ring expanded seven-membered ring. It was isolated from *Crossopetalum lobatum* Lundell along with

3α,25-dihydroxyfriedelan-2-one (**268**) and 1β,25-dihydroxyfriedelan-3-one (**269**), friedelin (**25**), 2α-hydroxy-3-oxofriedelan-30-oic acid (**270**), 28-hydroxyfriedelane-1,3-dione (**271a**), and 29-hydroxyfriedelane-1,3-dione (**271b**) (**Figure 10**).[39]

When the photosynthetic inhibitory activity of β-hydroxy friedelane (**261a**), friedelin (**25**), canophyllol (**272a**), pulpononic acid (**272b**) (**Figure 11**), and 3-oxo-29-hydroxyfriedelane (**257**), all isolated from *Celastrus vulcanicola,* were tested, compound **261a** was found to be an "energy transfer inhibitor, interacting and enhancing the light activated Mg^{2+}-ATPase" and **272a** acted as a Hill reaction inhibitor. The author also showed that in presence of the two compounds photosynthesis activity of chloroplasts was affected.[40]



272a: R_1= OH, R_2= H
272b: R_1= H, R_2= COOH

Figure 11. Canophyllol (**272a**) and pulpononic acid (**272b**).

Leaves of *Garcia pariflora* was a good source of 1,2-dehydro-2,3-secofriedelan-3-oic acid (**273**), 1β-hydroxyfriedelin (**274**), and 3β-hydroxyfriedelan-23-oic acid (**275**) and friedelin-3,4-lactone (**276**) (**Figure 12**).[41] A number of derivatives of **274** were prepared using various oxidation, reduction and esterification strategy (**Scheme 1**). Cytotoxicity of synthetic as well as the natural compounds was tested against human cancer cell lines U251, PC-3, K562, HCT-15, MCF-7 and SKLU-1. Compound **277** (**Figure 12**) was found to be a potent inhibitor of U251 cells.[41]

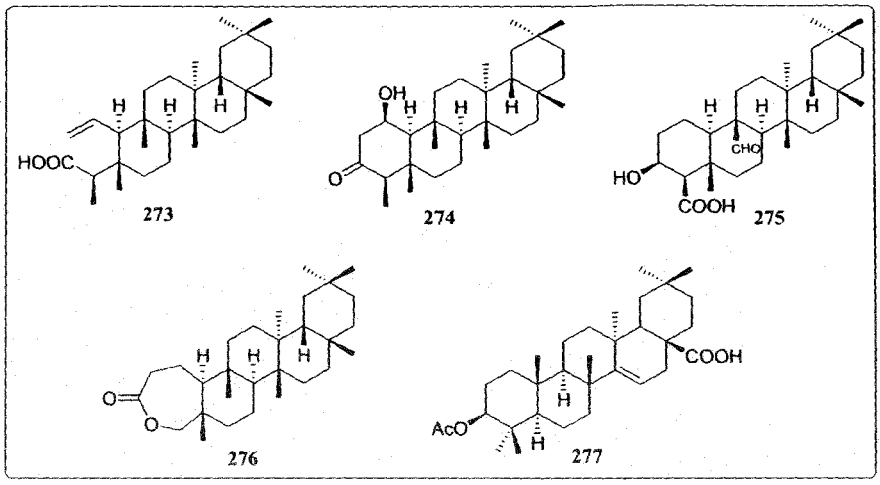
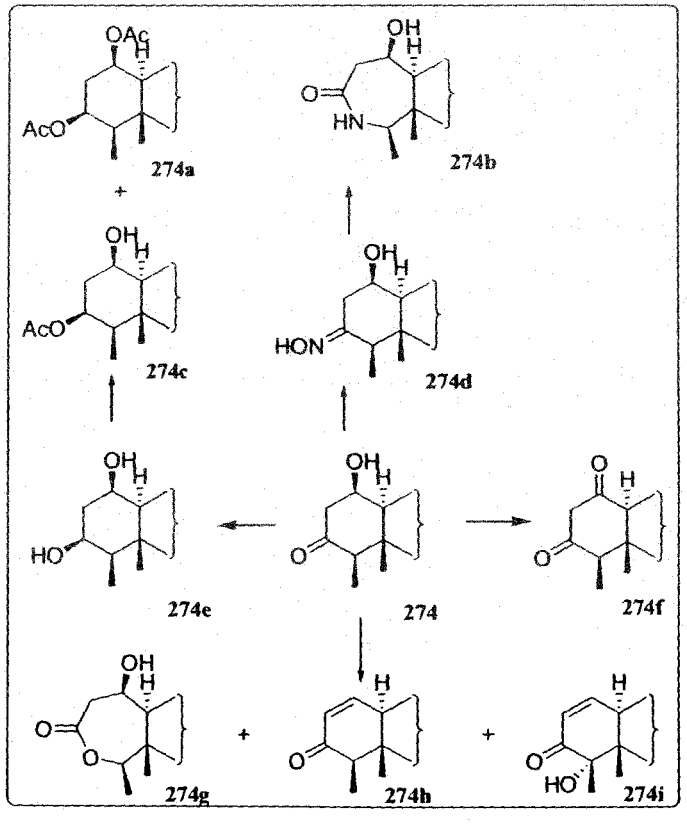


Figure 12. 1,2-Dehydro-2,3-secofriedelan-3-oic acid (**273**), 1β-hydroxyfriedelin (**274**), 3β-hydroxyfriedelan-23-oic acid (**275**), friedelin-3,4-lactone (**276**) and compound **277**.



Scheme 1. Derivatives of **274** [a]

[a] Details of scheme, ref. 41.

Oliveira et al. found *epi*friedelin (**260**) from the foliar epicuticular waxes of the leaves of *A. Esperanzae.*[42,43]

Baskar et al. isolated friedelin (**25**) from the leaves of *Azima tetracantha* and found friedelin to show 75.28% antifeedant, 66% larvicidal and 66.66% pupicidal activities against *H. armigera* and *S. Litura* respectively.[44]

Very recently, Liu and his group found *Malpighia emarginata* branches and roots to contain three novel norfriedelanes (**278-280**) (**Figure 13**), among which norfriedelanes **278** and **280** showed acetylcholine esterase inhibitory effects with the IC_{50} values of 10.3 and 28.7 μM, respectively.[45]

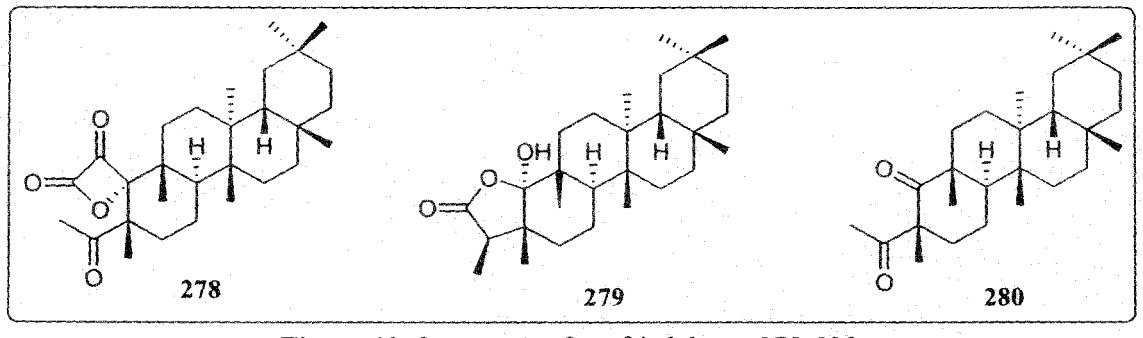


Figure 13. Structures of norfriedelanes **278-280**.

Chinese *Heritiera littoralis* bark was found to contain friedelin (**25**), 29-hydroxy-methyl-friedelin (**257**) and 3,4-seco-friedelan-3-oic acid (**31**) (**Figure 14**).[46]

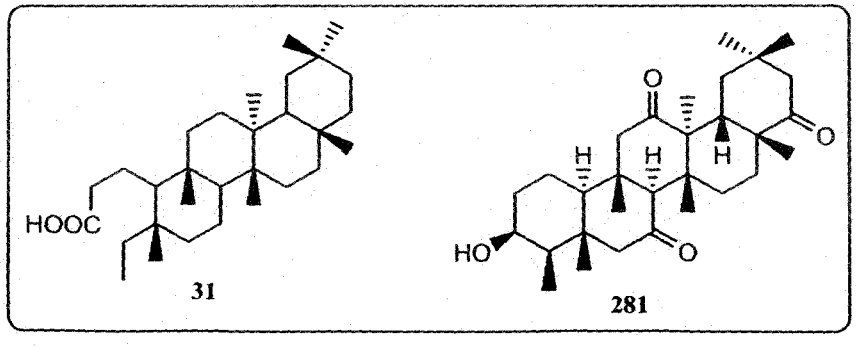


Figure 14. 3,4-Seco-friedelan-3-oic acid (**31**) and 3β-hydroxyfriedelane-7,12,22-trione (**281**).

Polyketo compound 3β-hydroxyfriedelane-7,12,22-trione (**281**), isolated from *Drypetesla ciniata* was found to moderate antimicrobial and antifungal activities (**Figure 14**).[47]

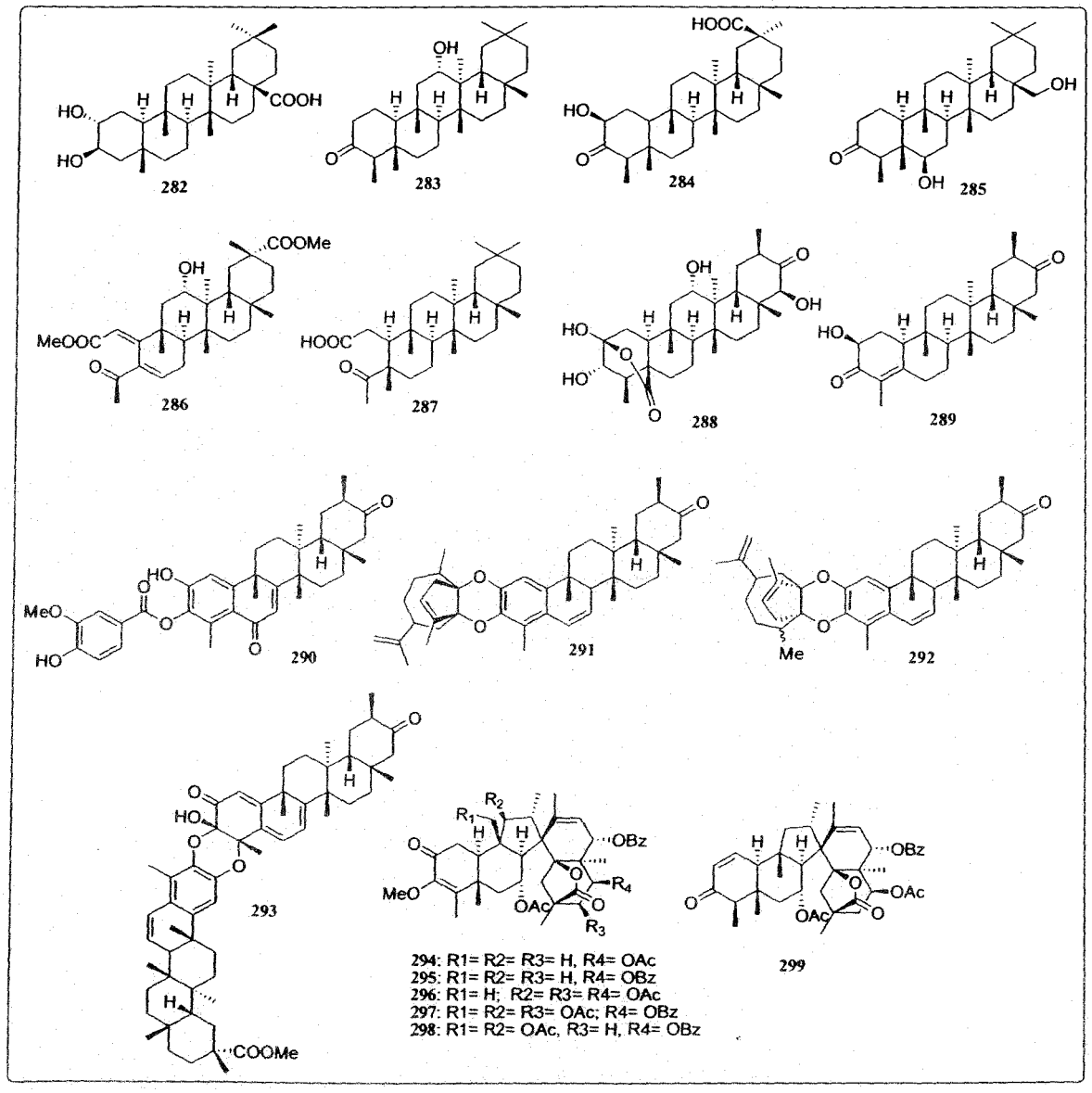


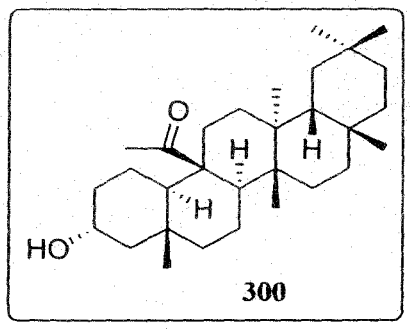
Figure 15. 2α,3β-Dihydroxyfriedelan-28-oic acid (**282**), 12α-hydroxyfriedelan-3-one (**283**), 2β-hydroxy-3-oxofriedelan-30-oic acid (**284**), 28-hydroxyzeylanol (**285**), dzununcanone (**286**), trifloralactone **288**, triptocalline B **289,** milicifolines A–D (**290-293**) and spirocarcolitones G–L (**294-299**).

Moreover, friedelane triterpenoids 2α,3β-dihydroxyfriedelan-28-oic acid (**282**) from the leaves of *Marila pluricostata*,[48] 12α-hydroxyfriedelan-3-one (**283**) from *Maytenus gonoclada*[49]

and 2β-hydroxy-3-oxofriedelan-30-oic acid (**284**) from *Dichapetalum barteri*,[50] 28-hydroxyzeylanol (**285**) from *Dichapetalum gelonoides*,[51] Dzununcanone (**286**), a 3,24-dinor-2,3-seco-friedelane derivative from *Hippocrate excels*,[52] **287** from *Passiflora wilsonii*[53] were isolated. Other norfriedelane derivatives include trifloralactone **288** and triptocalline B **289** isolated from *Microtropis triflora*,[54] and milicifolines A–D (**290-293**) obtained from *Maytenus ilicifolia*.[55] Milicifolines B (**291**) and -C (**292**) were related to the cheiloclines reported by the same group in 2005. Spirocarcolitones G–L (**294-299**), isolated from the bark of *Ruptiliocarpon caracolito*, was actually rearranged friedelane derivatives (**Figure 15**).[56]

Apart from the plant origin, the first report of natural occurrence of friedelane triterpenoid in fungi was the presence of euphorcinol (**300**) in the fermented broth of the fungus *Leptosphaeria maculanns* (**Figure 16**).[14] Compound **100** was also found in marine endophytic fungus.[15]

Many more of the natural friedelane triterpenoids as mentioned above, along with their occurrence, showed potent biocidal activities which also include α-glucosidase inhibitory,[57] anti-inflammatory,[58] anti-HIV,[59] anti-tumor-promoting[60] activities etc.



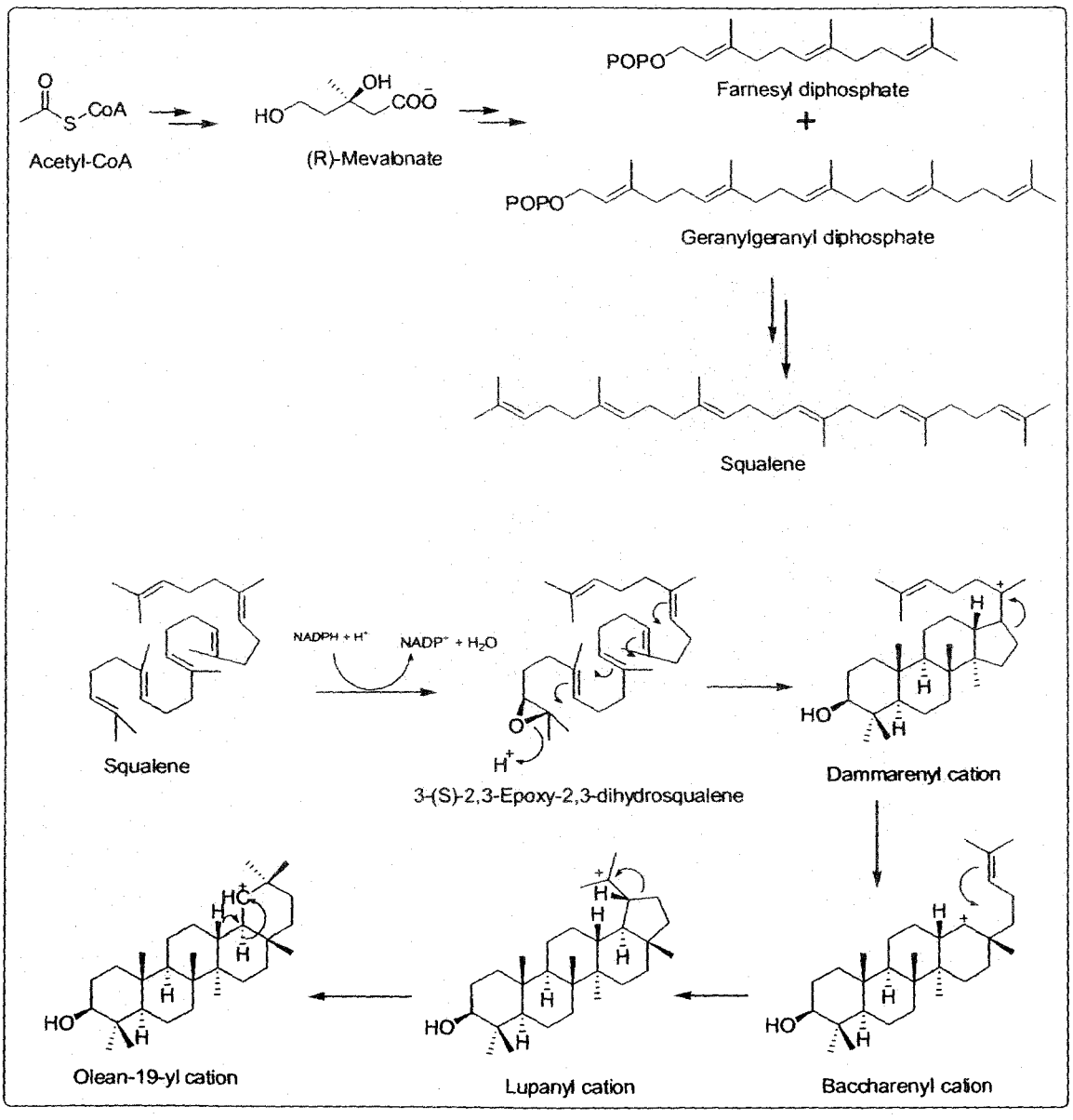
300

Figure 16. Euphorcinol (**300**).

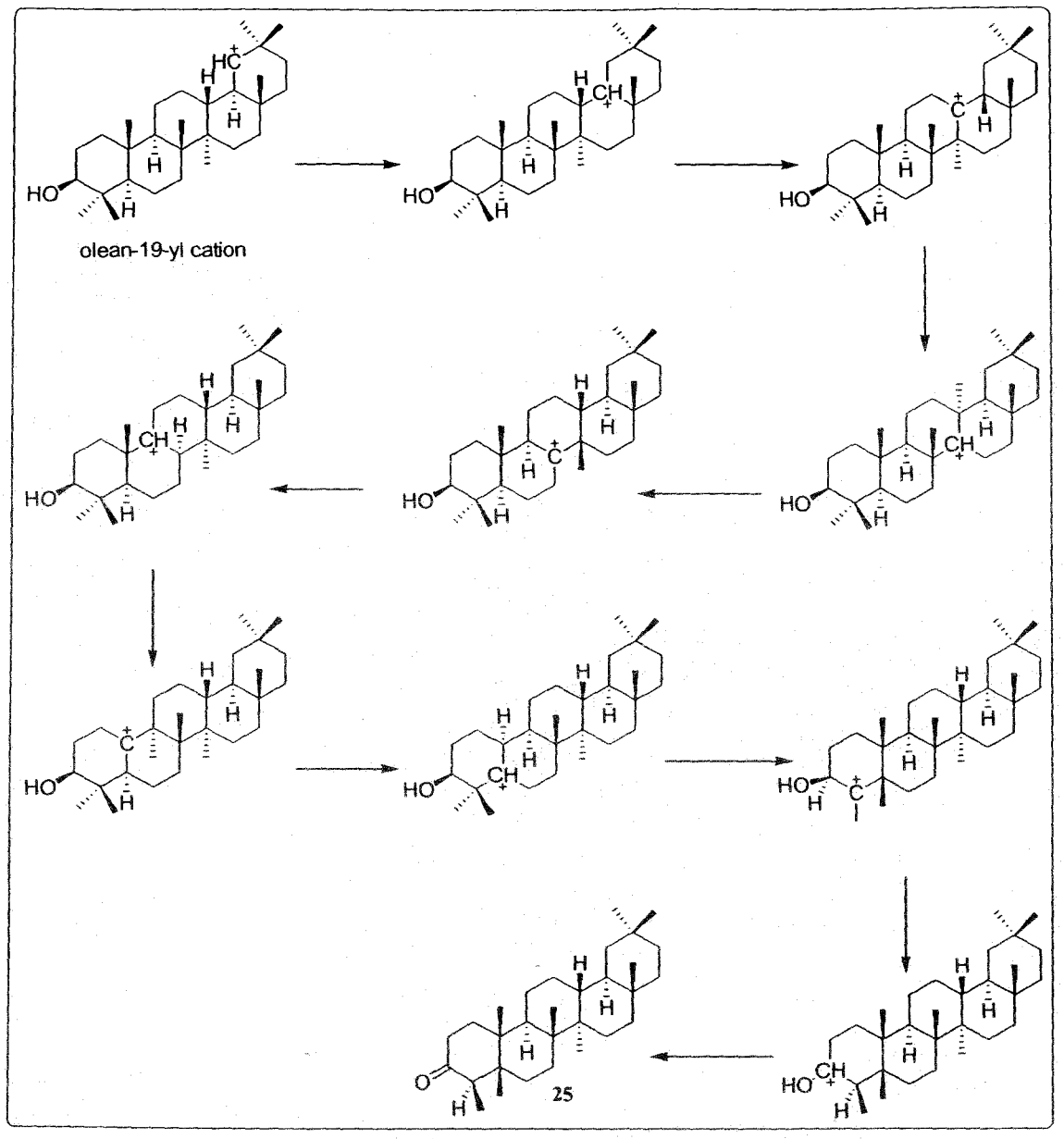
IV.A.1.2 Biosynthesis of friedelin

Cyclization of epoxysqualene, leading to a single enzyme-catalysed reaction is the source of triterpenoid biosynthesis in eukaryotes and it forms an initial compound $C_{30}H_{50}O$ which can contain 1-5 rings.[61] Many organisms have single product-specific oxidosqualene cyclase (OSC) enzyme,[62] but plants have arrays of cyclase enzymes and these enzymes compete for the same substrate resulting diverse steroids and pentacyclic triterpenoids.[62-64] This cyclization is considered to be a branch point between primary and secondary metabolism. Biosynthesis of triterpenoid cyclization is consisted of four steps including protonation, a polyene addition, 1,2-

hydride or -methyl shifts and finally deprotonation.[3] Detailed information regarding this biosynthesis is beautifully described in the literature.[65] Here, formation of olean-19-yl cation from Acetyl-CoA is shown in **Scheme 2** and formation of friedelin (**25**) from olean-19-yl cation is shown in **Scheme 3**.



Scheme 2. Biosynthetic route to olean-19-yl cation from Acetyl-CoA.



Scheme 3. Biosynthesis of friedelin from olean-19-yl cation.

IV.A.1.3 Laboratory syntheses, transformations and biological activities of friedelane triterpenoids

IV.A.1.3.1 Syntheses and transformations based on rearrangements

After a series of consecutive 1,2-rearrangements of methyl groups and hydrogens, α-amyrine or β-amyrine (**301**) (**Figure 17**), produced friedelin (**25**). Reduction of friedelin (**25**) with lithium

aluminum hydride produces 3β-hydroxy friedelane (**261a**). Treatment of **261a** with hydrogen chloride in phenol at 110°C caused a remarkable multi-group rearrangement which afforded olean-13(18)-ene (**302**). Compound **302** was again prepared from α- or β-amyrine by consecutive oxidation, Wolff-Kishner reduction and acid catalysed isomerization. This olean-13(18)-ene (**302**), when was treated with *m*CPBA, corresponding peroxide **302a** was formed which upon BF_3-etherate treatment afforded olean-11,13(18)-diene (**303**) (**Scheme 4**).[66]

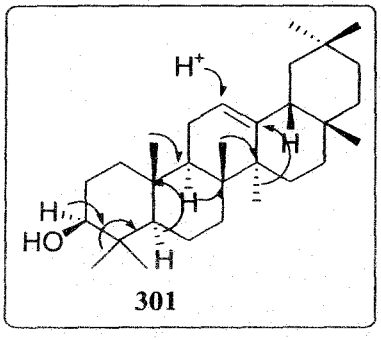
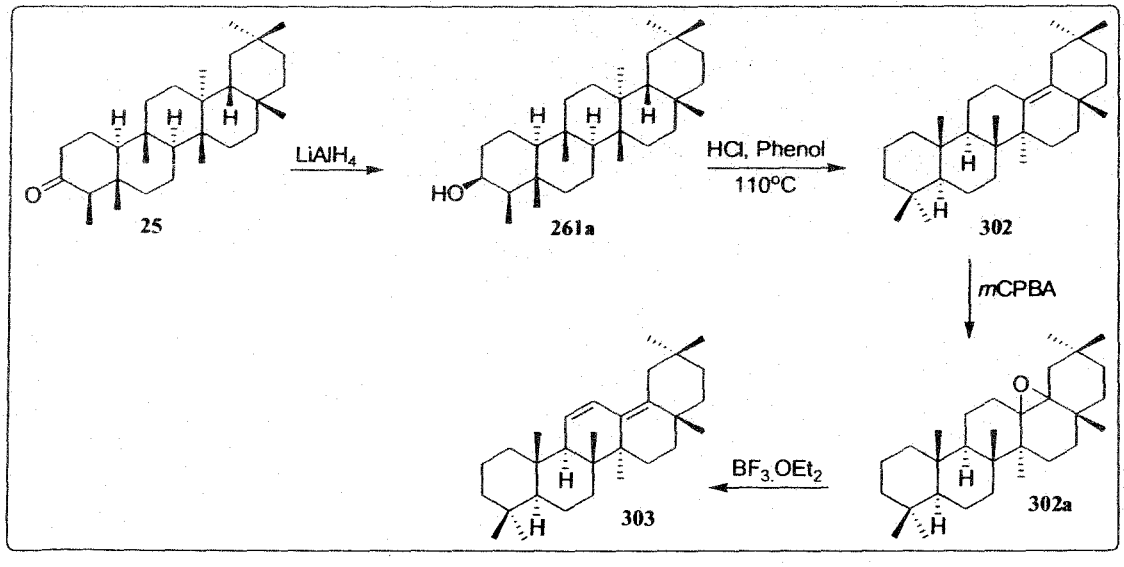


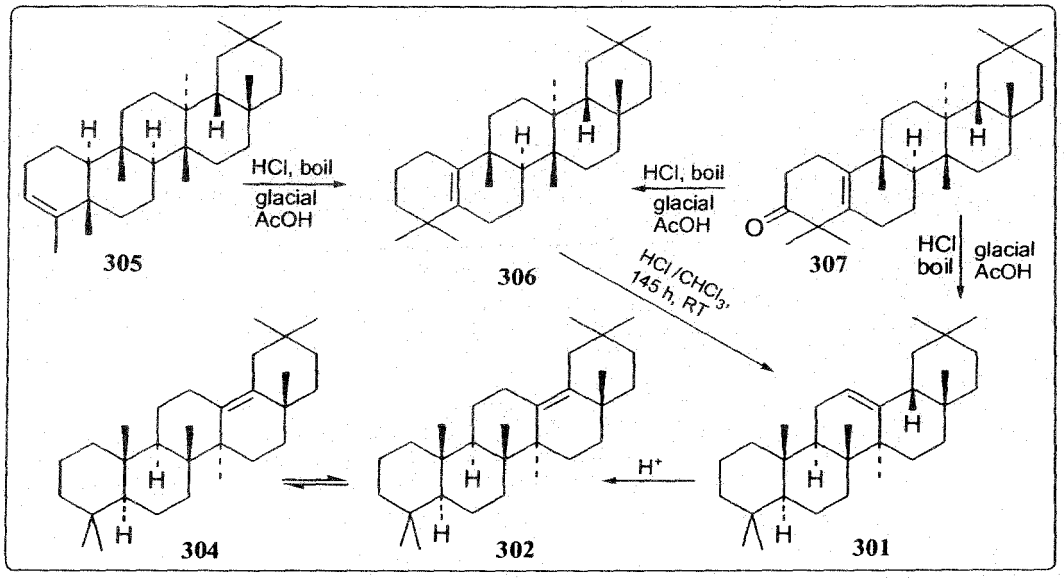
Figure 17. β-Amyrine (**301**, arrows show the consecutive 1,2-rearrangements toward friedelin).



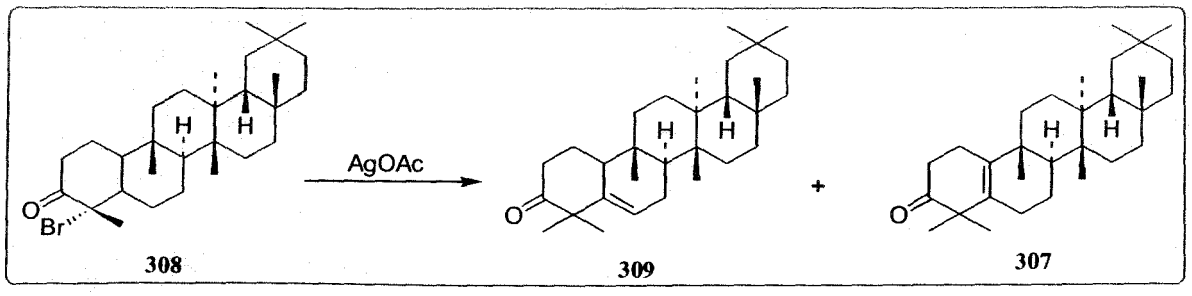
Scheme 4. Friedelin (**25**) to olean-11,13(18)-diene (**303**).

In the friedelene-oleanene rearrarrangement, a number of significant steps such as proton addition, 1,2-shifts of methyl and hydrogens, proton elimination, double bond shifts etc.

occurred and then ultimately olean-13(18)-ene (**302**) and 18α-olean-12-ene (**304**) were resulted.[67] When friedel-3-ene (**305**) in boiling acetic acid was treated with hydrogen chloride, glutin-5(10)-ene (**306**) was obtained. This on vigorous acid treatment yielded a mixture of olean-13(18)-ene (**302**) and 18α-olean-12-ene (**304**). Glutin-5(10)-en-3-one (**307**) on several hours of treatment with HCl in boiling glacial acetic acid gave a mixture of glutin-5(10)-ene (**306**) and olean-12-ene, i.e.; β-amyrine (**301**). Glutin-5(10)-ene (**306**) after 145 hrs of treatment with HCl in chloroform at room temperature yielded olean-12-ene (**301**), though **301** itself remained unaffected by the same condition. Then, compound **301**, on acid treatment, was found to result compounds **302** and **304** in equilibrium mixture (**Scheme 5**).[12,68,69]



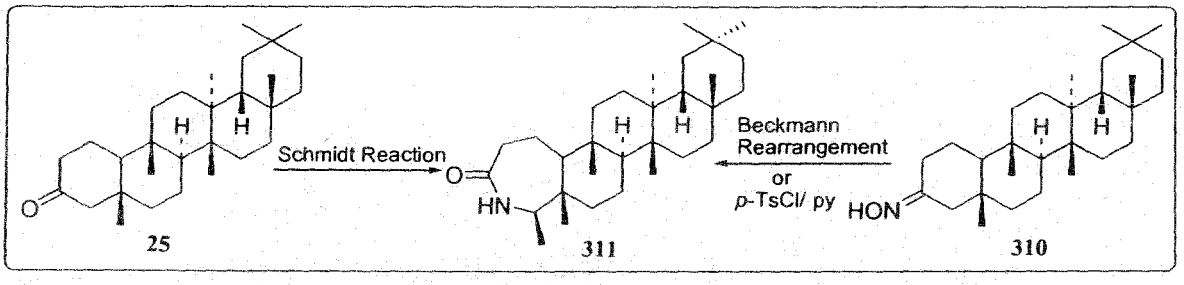
Scheme 5. Friedelene-oleanene rearrangement.



Scheme 6. Molecular rearrangement of 4α-bromofriedelin (**308**) by silver acetate.

Another rearrangement was reported by the action of silver acetate on 4α-bromofriedelin (**308**) to yield a a mixture of compounds **309** and **307** (**Scheme 6**).[70]

Drake and Shrader reported the Beckmann rearrangement of friedelin oxime (**310**)[6] but the structure was established actually as 4-aza-A-homofriedelan-3-one (**311**) by Stevenson in the year 1963.[71] He also isolated the same product by the treatment of *p*-tolunesulfonyl chloride on the oxime in pyridine solution. The same product was also obtained by the Schmidt reaction of friedelin (**25**) (**Scheme 7**).[71]



Scheme 7. 4-Aza-A-homofriedelan-3-one (**311**) from both friedelin oxime (**310**) and friedelin (**25**).

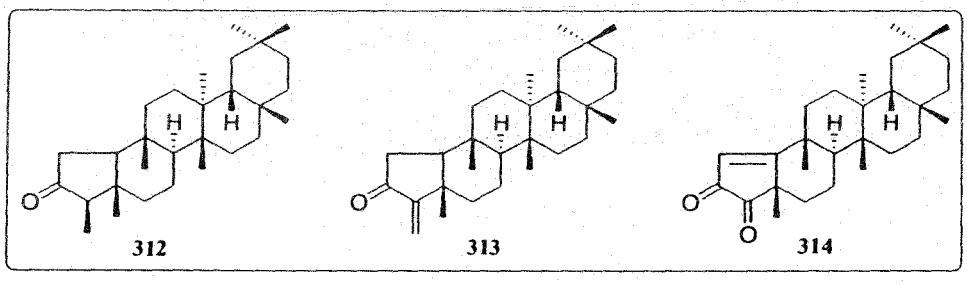


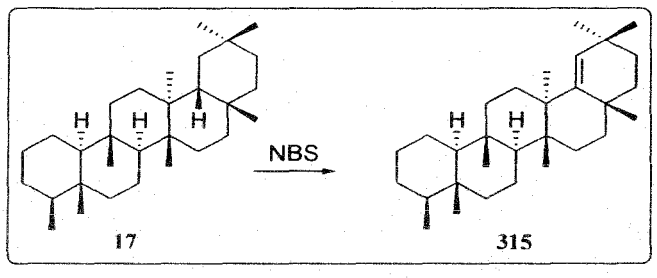
Figure 18. Norfriedelanone (**312**), A(1)-norfriedel-4(23)-en-3-one (**313**) and A(4)-nor-23-norfriededel-1(10)-ene-2:3-dione (**314**).

IV.A.1.3.2 Syntheses and transformations based on oxidation reactions

Chromic acid oxidation of friedelin to produce friedelonic acid[8,9] and dehydrogenation of one of the epimeric secondary alcohols (derived from reduction of friedelin) by selenium at 315°C to produce 1,2,7-trimethylnaphthalene, 1,2,8-trimethylphenanthrene and 1,8-dimethylpicene were studied earlier.[7] Oxidation of friedelin (**25**) gave a dicarboxylic acid and anhydride of the acid on pyrolysis gave norfriedelanone (**312**). This on reflux in glacial acetic acid with selenium dioxide gave norfriedelenone, established as A(1)-norfriedel-4(23)-en-3-one (**313**) and more drastic

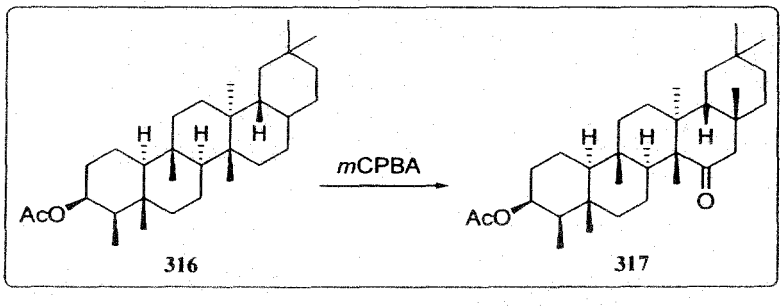
oxidation with selenium dioxide in dioxane at 200°C gave norfriedelendione[72-74] which was later identified as A(4)-nor-23-norfriededel-1(10)-ene-2,3-dione (**314**) (**Figure 18**)[75].

Kohen and Stevenson reported that the saturated hydrocarbon friedelane (**17**) was oxidized by NBS to the olefin friedel-18-ene (**315**) along with an unstable bromofriedelane, probably, 18-bromofridelane which yielded readily **315** (**Scheme 8**).[76]



Scheme 8. NBS oxidation of friedelane.

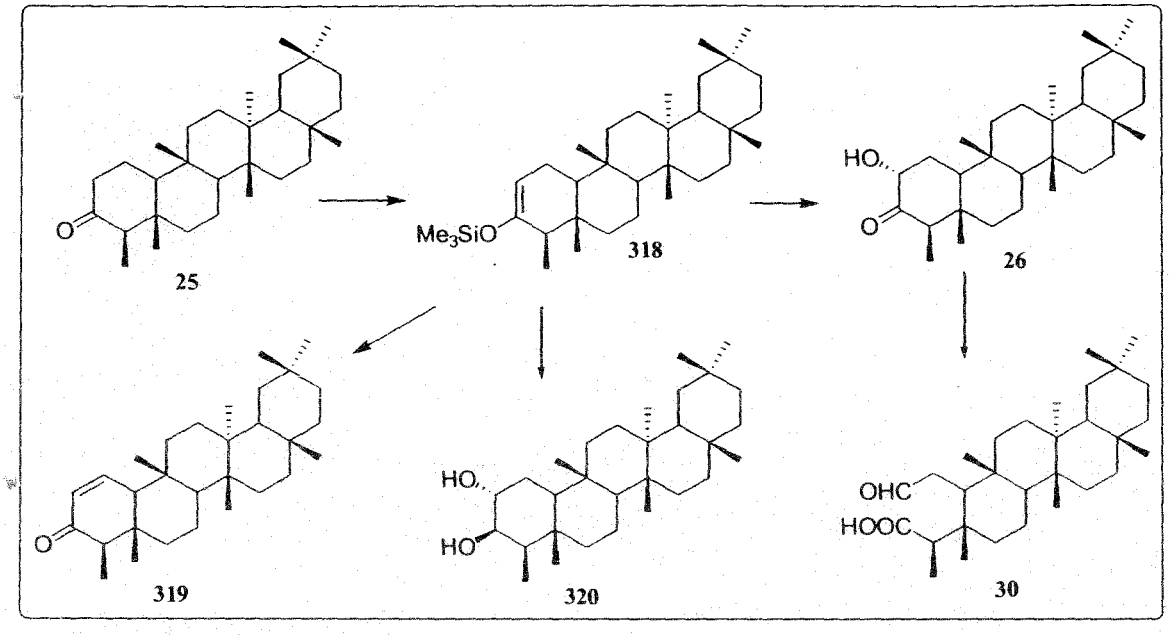
3β-Acetoxy friedelane (**316**) was found to form 3β-Acetoxy-15-oxofriedelane (**317**) when treated with *m*-chloroperbenzoic acid as is reported by Asakawa et al. [77] (**Scheme 9**)



Scheme 9: *m*-Chloroperbenzoic acid oxidation of 3β-acetoxy friedelane (**316**).

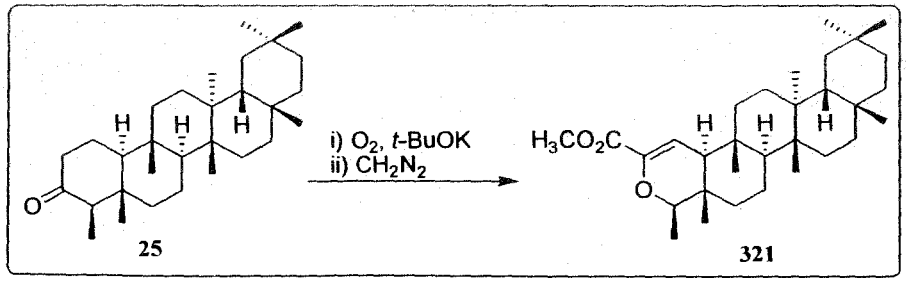
In another set of reactions, first friedelin (**25**) on controlled silylation was transformed into 3-trimethylsiloxyfriedel-2-ene (**318**) in high yields. Compound **318** was then oxidized with OsO_4/NMMO to produce 2α-hydroxyfriedelan-3-one (known as cerin, **26**) which by periodic acid oxidation resulted 2,3-secofriedelan-2-al-3-oic acid (**30**). Oxidation of **318** with DDQ afforded friedel-1-en-3-one (**319**). Compound **318** on reductive ozonolysis furnished 2α,3β-dihydroxyfriedelane, also known as pachysandiol A (**320**). Compound **30** was proved to be a

potent inhibitor of human lymphocyte proliferation (IC_{50} 10.7 μM) and of the growth of a human cancer cell line (GI_{50} 5.4-17.2 μM) (**Scheme 10**).[78]



Scheme 10. Silylation of friedelin leading to different friedelane derivatives (Ref. 78).

Brownlie, Spring, Stevenson and Strachan in their thorugh study with friedelin revealed that dehydration of friedelanol gives an unsaturated hydrocarbon, friedel-3-ene (**305**).[12] Again, friedelin (**25**) on oxidation with potassium *tert*-butoxide followed by treatment with diazomethane was found to result an unsaturated ester **321** (**Scheme 11**).[79]



Scheme 11. Treatment of friedelin (**25**) with *t*-BuOK and diazomethane.

3β-Hydroxy friedelane (**261a**) was oxidized with lead tetraacetate and iodine in dry benzene to furnish a tetrahydropyridine **322a**, an iodo-ether **322b** and an α-acetoxytetrahydrofuran **322c** derivatives (**Figure 19**).[80]

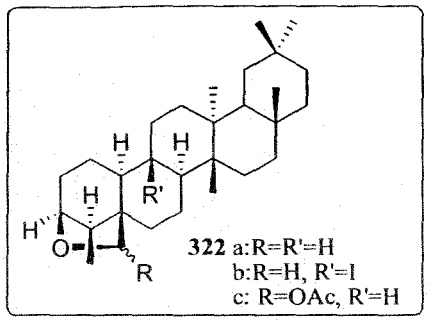
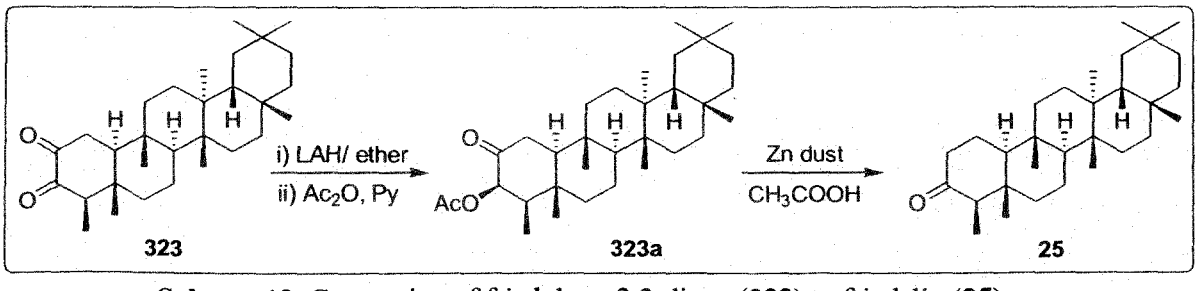


Figure 19. Tetrahydropyridine **322a**, iodo-ether **322b** and α-acetoxytetrahydrofuran **322c**.

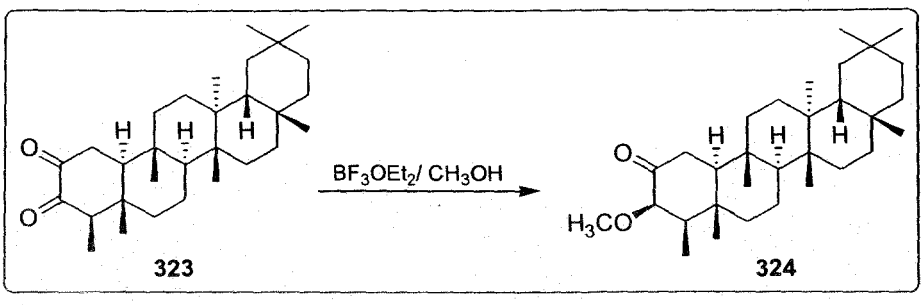
IV.A.1.3.3 Syntheses and transformations based on reduction reactions

In the year 1960, Kane and Stevenson, isolated friedelane-2,3-dione (**323**) from Cork Smoker Wash Solids and they characterized it as monoacetate, monobenzoate, monomethyl and quinoxaline derivatives. Huang-Minlon reduction of the dione **323** yielded friedelane whereas selective reduction produced friedelin (**Scheme 12**). Friedelane-2,3-dione (**323**) was also converted to the monomethyl ether **324** by refluxing with boron trifluride etherate in methanol solution (**Scheme 13**).[81]



Scheme 12. Conversion of friedelane-2,3-dione (**323**) to friedelin (**25**).

Stevenson, in the year 1961, described a convenient isolation method of friedelin from cork smoker wash solids. He also found that friedelin oxime on treatment with nitrous acid readily yields compound **325** (**Figure 20**), and can then be converted again to friedelin by heating in aqueous dioxane.[82]



Scheme 13. Conversion of friedelane-2,3-dione (**323**) to 3-methoxy-2-oxofriedelane (**324**).

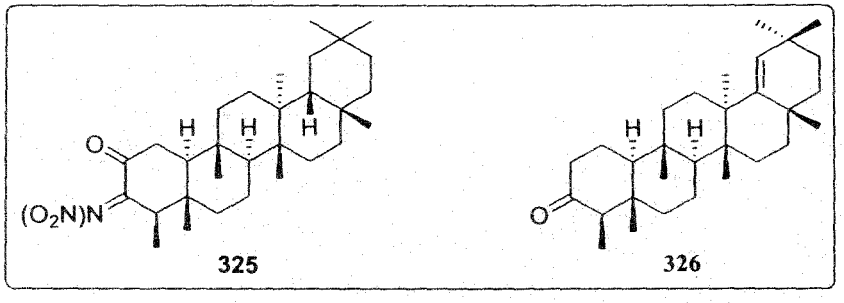


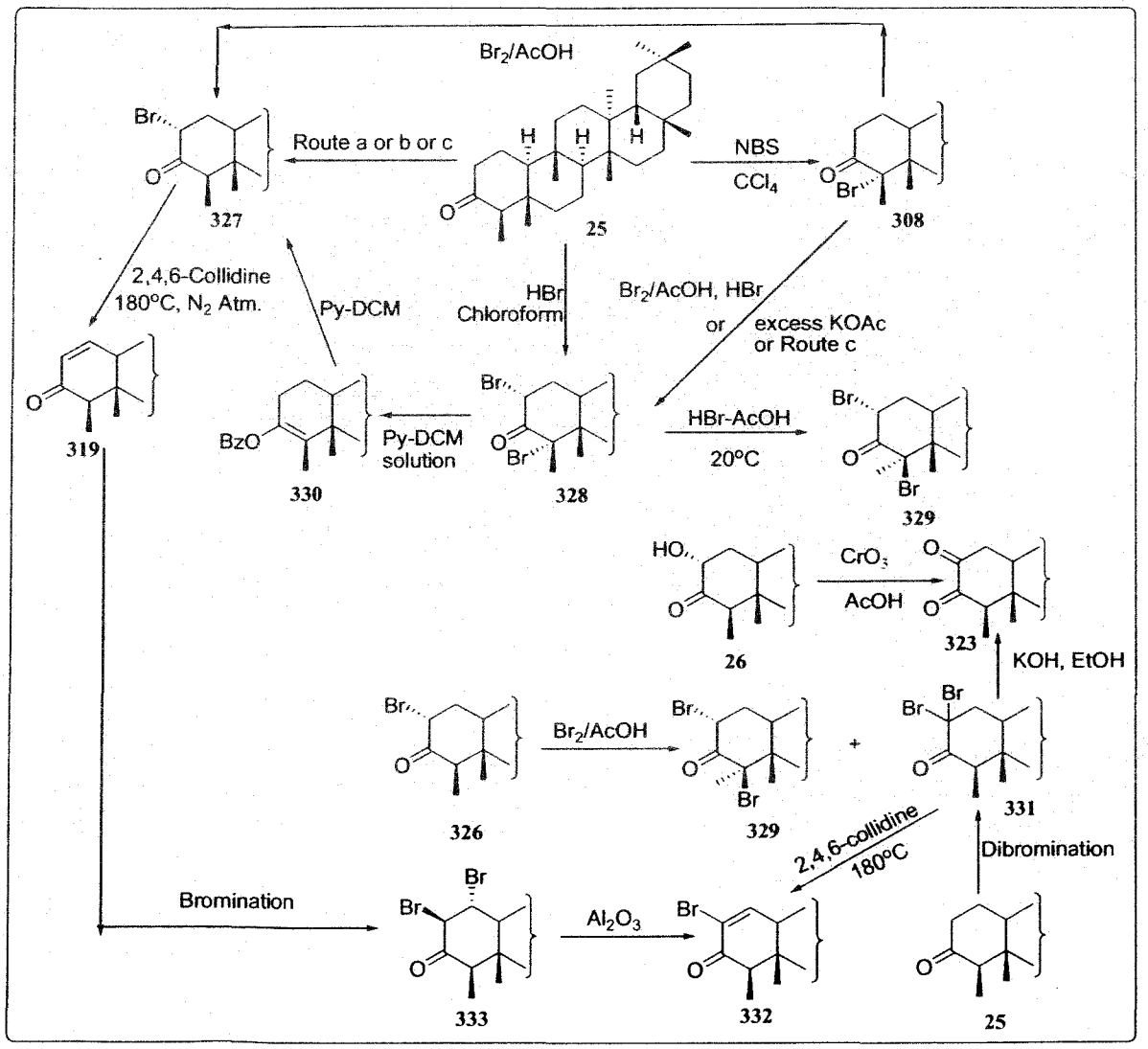
Figure 20. Compound **325** and friedel-18-en-3-one (**326**).

A number of ransformative reactions such as lithium aluminium hydride reduction, acetylation of the product formed, Huang-Minlon reduction on compound Friedel-18-ene-3-one (**326**, **Figure 20**) were also carried out by Kane and Stevenson. Detailed regarding this can be found in their descriptive paper.[83]

IV.A.1.3.4 Syntheses, transformations and rearrangements of bromo-friedelane triterpenoids

Friedelin (**25**) yielded 4α-bromofriedelin (**308**) on treatment with *N*-bromosuccinimide in CCl_4,[83] but produced 2α-bromofriedelin (**327**) by either monobromination with bromine in $CHCl_3$ in presence of HBr[10] or, base catalysed monobromination in acetic acid or by treatment with pyridinium bromide-dibromide in acetic acid.[84] Again, friedelin (**25**) when treated with HBr in $CHCl_3$, 2α,4α-dibromo friedelin (**328**) was produced[10] which was transformed readily into the more stable 2α,4β-dibromo isomer (**329**) in HBr-AcOH at 20°C.[70,85] The 2α,4α-dibromo derivative **328** was also obtained from the 4α-bromo compound **308** by either the treatment of

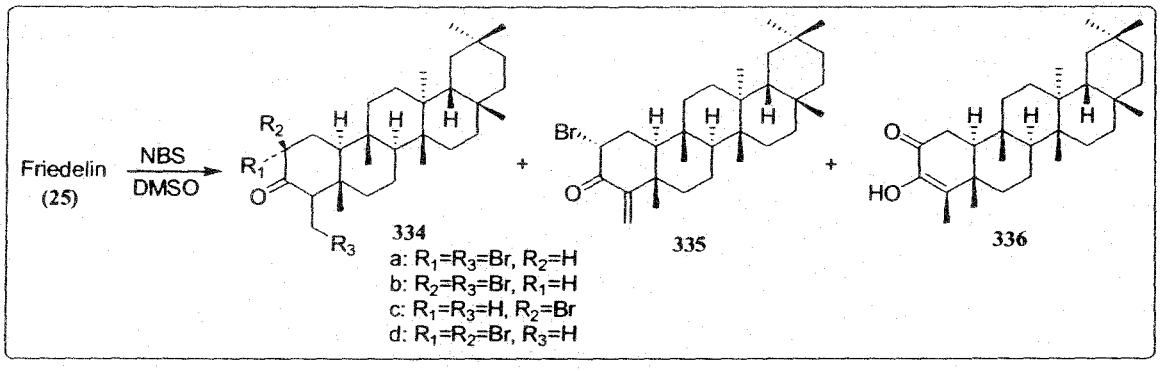
Br_2 in acetic acid in presence of HBr or with excess potassium acetate, and again from the enol benzoate derivative of friedelin (**330**) in pyridine-dichloromethane solution.[84] 2α-Bromo derivative **327** was also the other product obtained in the last process.[84] 4α-Bromofriedelin (**308**) when treated with Br_2 in acetic acid yielded 2α-bromofriedelin (**319**)[84,85] which when treated again with the same reagent formed either 2α,4β-dibromo friedelin (**329**) or 2,2-dibromo



Scheme 14. Synthetic routes of different bromo-derivatives of friedelin. [**Route a**: monobromination with Br_2 in chloroform in presence of HBr, **Route b**: base catalysed monobromination in AcOH and **Route c**: pyridinium bromide-dibromide in acetic acid.]

friedelin (**331**), as was assumed by Djerassi et al.,[86] though Shoppee and Johnston obtained the latter compound by the same process and also by dibromination of friedelin (**25**).[84] This 2,2-dibromo compound when refluxed with KOH in ethanol yielded the 2,3-diketo derivative (**323**),[83] identical with that obtained from cerin (**26**) by chromium trioxide oxidation in acetic acid.[72] Again, this 2,2-dibromo compound upon treatment with 2,4,6-collidine at 180°C resulted 2α-bromo friedel-1-ene-3-one (**332**). The same reagent under nitrogen atmosphere converted 2α-bromo derivative into friedel-1-ene-3-one (**319**) and bromination of it followed by Al_2O_3 treatment resulted 2-bromofriedel-1-ene-3-one (**332**) *via* 1α,2β-dibromo friedelin (**333**).[84] All these transformations are illustrated in **Scheme 14**.

Pradhan et al. also reported a group of bromo- derivatives of friedelin, using NBS, and their associated rearrangements as shown below[87-89] (**Scheme 15**).



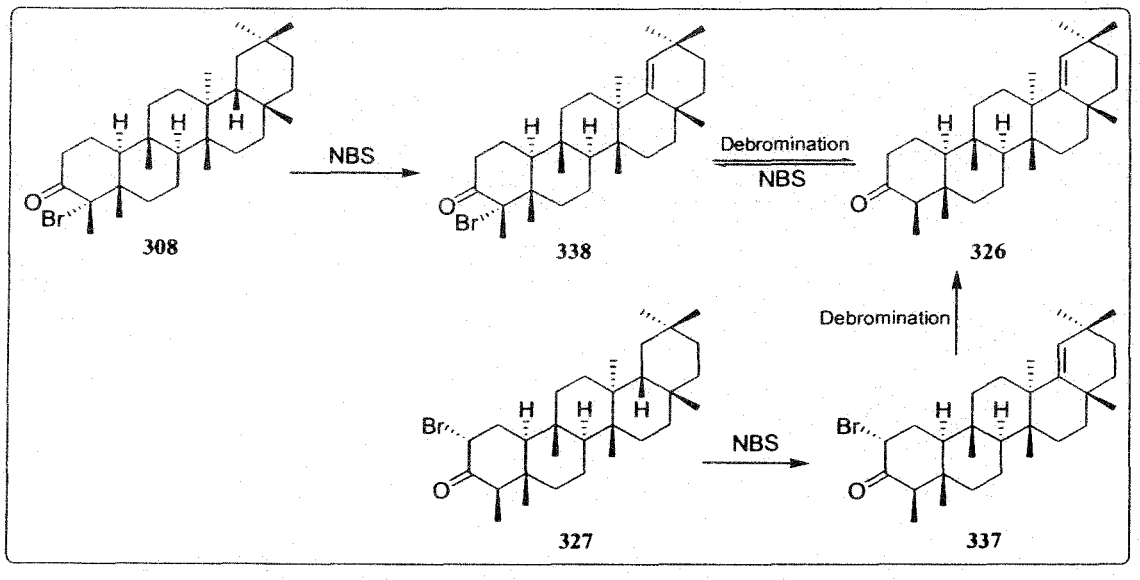
Scheme 15. NBS on friedelin.

Kane and Stevenson also reported that friedel-18-ene-3-one (**326**) was obtained by debromination of **337** and **338**, produced respectively from 2α-bromofriedelin (**327**) and 4α-bromofriedelin (**308**) by the action of NBS. And compound **326** regenerated **338** on treatment with NBS (**Scheme 16**).[83]

IV.A.1.3.5 Syntheses and transformations based on both oxidation/ reduction reactions

Cerin (**26**) was primarily assumed to be 2β-hydroxyfriedelin, which was later on proved actually to be the 2α-hydroxy isomer by Stevenson and his group.[90] They prepared pachysandiol-A (**320**), i.e., 2α,3β-dihydroxy friedelane by reducing cerin (**26**) with sodium borohydride. Cerin after

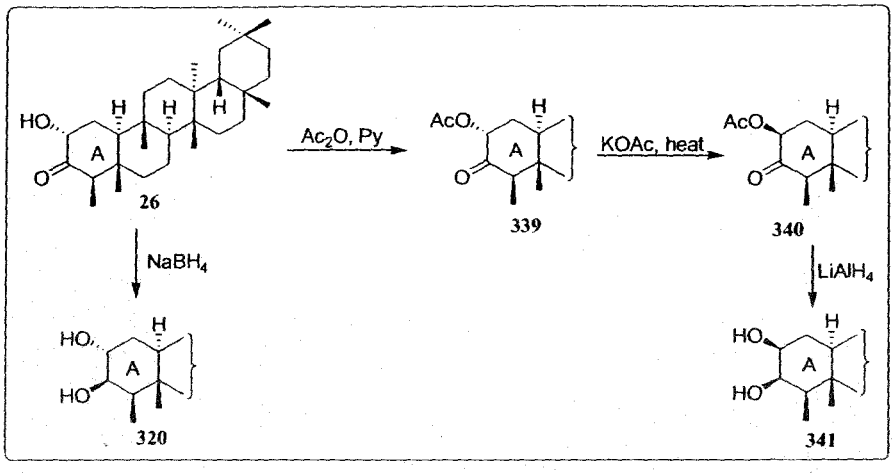
acetylation was heated for prolonged time with potassium acetate to result 2β-acetoxyfriedelin (**340**) which on reduction with lithium aluminium hydride yielded 2β,3β-dihydroxy friedelane (**341**). Friedelin (**25**) was also converted to friedelane-3β-yl benzoate (**342**) by reduction followed by benzoylation. This benzoate on pyrolysis produced friedel-2-ene (**343**). This upon treatment with *m*CPBA, followed by perchloric acid treatment and acetylation resulted pachysandiol-A diacetate (**344**). Hydrolysis of it formed the corresponding diol (**320**). Again, friedel-2-ene (**343**) was converted to 2α,3α-dihydrxyfriedelane (**345**) by the action of osmium tetraoxide, followed by cleavage of the ester by lithium aluminium hydride (**Scheme 17** and **Scheme 18**).[90]



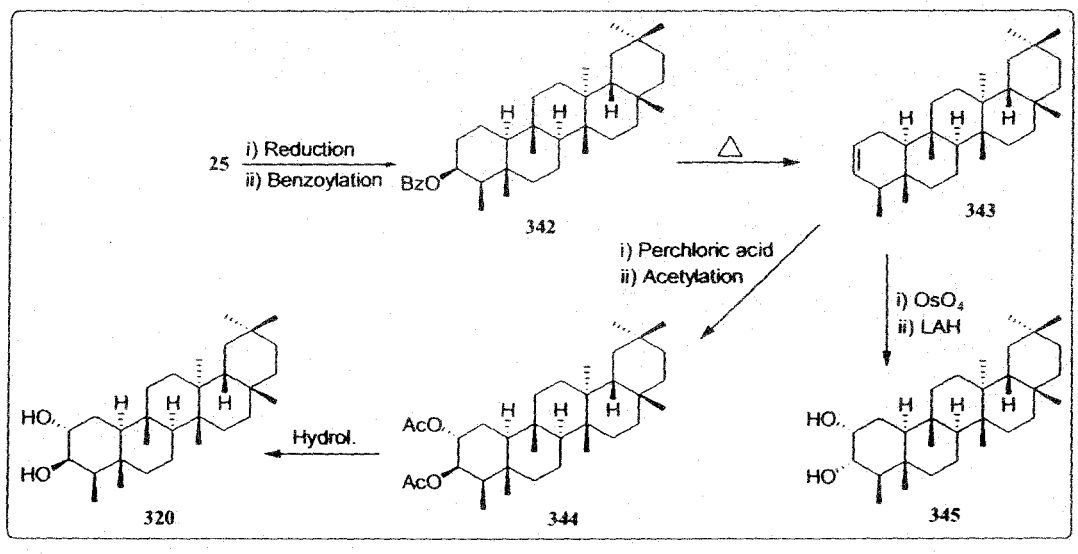
Scheme 16. Molecular rearrangements of bromo-derivatives of friedelin.

IV.A.1.3.6 Photochemical syntheses and transformations

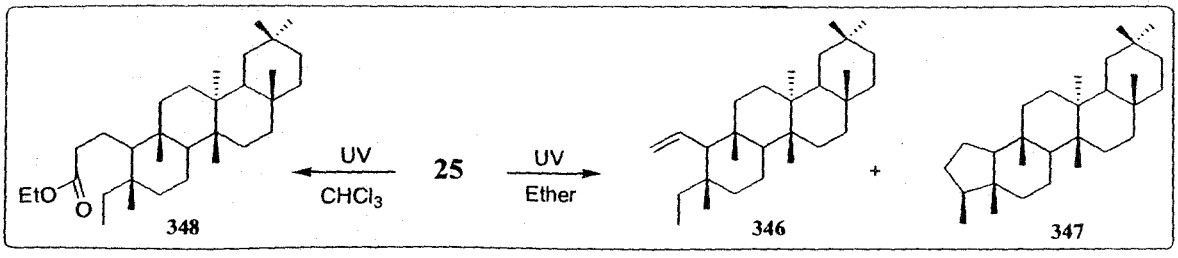
Stevention and his group reported the ultraviolet irradiation of friedelin (**25**) in ether and in chloroform solution. In the former case, 5-ethyl-10β-vinyl-des-A-friedelane (**346**) and norfriedelane (**347**) were obtained whereas in the latter test, ethyl 3,4-secofriedelane-3-oate (**348**) was produced which was characterized by hydrolysis to the corresponding acid and reduction to the corresponding alcohol (**Scheme 19**).[91]



Scheme 17. Transformation of cerin into different dihydroxy derivatives.

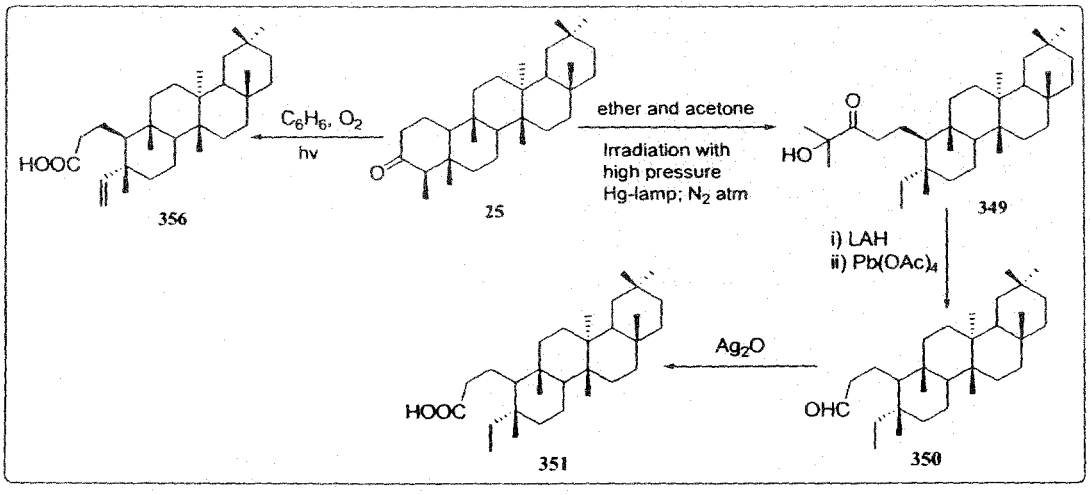


Scheme 18. Transformation of friedelin into different dihydroxy derivatives.



Scheme19. Ultraviolet irradiation products of friedelin in ether and in chloroform.

Photoreaction on friedelin was carried out by many other research groups.[92] Shirasaki, Tsuyuki, Takahashi and Stevenson irradiated friedelin (**25**) in ether containing acetone with a high pressure mercury lamp under nitrogen atmosphere in a quertz vessel. The product **349** was successively treated with LAH and $Pb(OAC)_4$ to result aldehyde **350** which was further treated with Ag_2O to yield **351** and the transformations, in fact, confirmed the structure of compound **349** as 5α-ethyl-10β-(4-hydroxy-4-methyl-3-oxopentyl)-des-A-friedelane.[93] The other major friedelane triterpenoids which were produced photochemically are 2-oxo-3-oxa-friedelane (**352**),[79] epoxyfriedelane (**353**),[94,95] friedelan-3-on-24-al (**354**),[96] 3β-hydroxy friedelane (**161a**) and 3α- hydroxy friedelane (**161b**),[97,98] and 4-*epi*friedelin (**160**) and 4-*epi*shionone (**355**),[99,100] and some nitrogen containing friedelanes.[101] A one step synthesis of putranjivic acid (**356**) from friedelin by photochemical oxidation in benzene under oxygen was also accomplished (**Scheme 20** and **Figure 21**).[102]



Scheme 20. Photochemical reactions on friedelin (**25**).

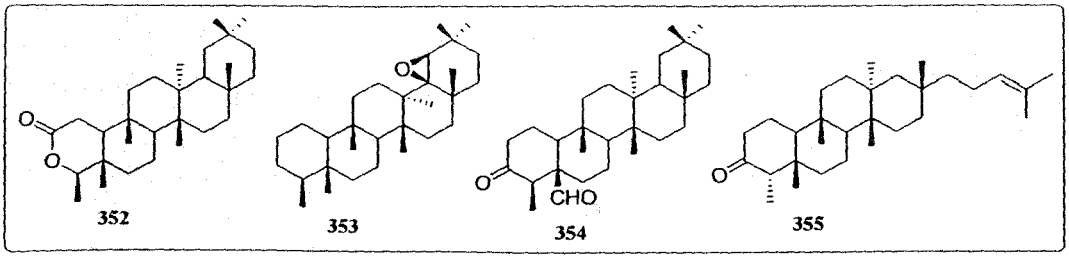
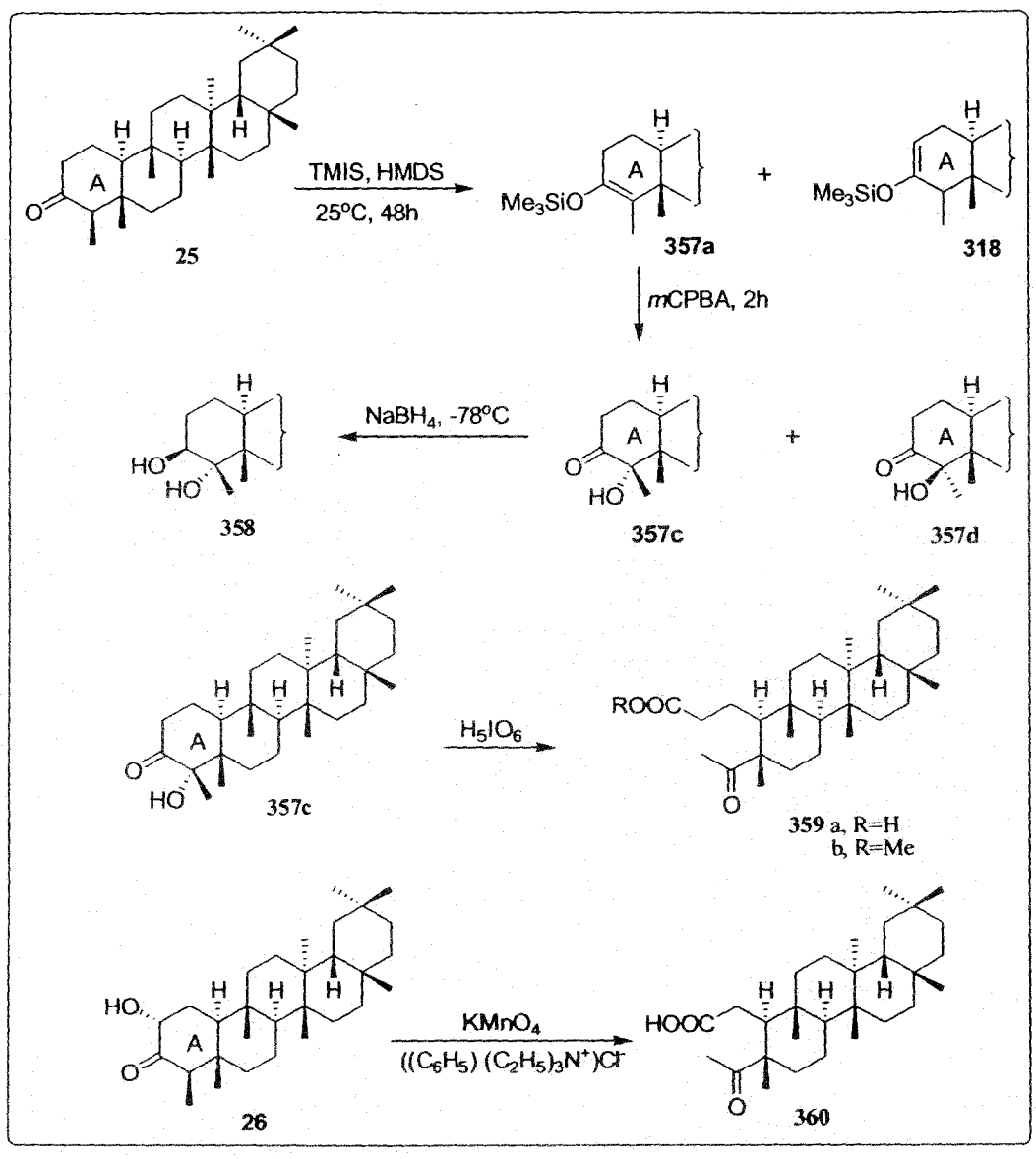


Figure 21. 2-Oxo-3-oxa-friedelane (**352**), epoxyfriedelane (**353**), friedelan-3-on-24-al (**354**), and 4-*epi*shionone (**355**).



Scheme 21. A-ring modifications along with seco-friedelanes.

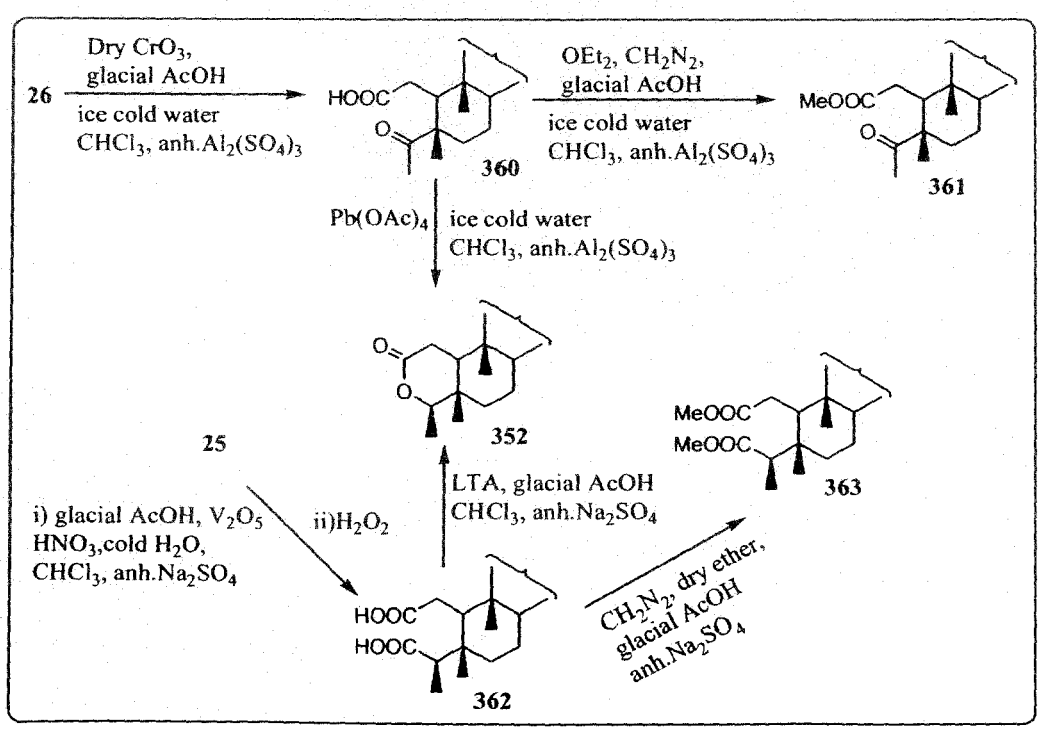
IV.A.1.3.7 Miscelleneous syntheses and transformations

Amyrin **(301)**,[103] or a readily available tetracyclic triterpene[104] was the starting material for chemical synthesis of friedelin. A total synthesis of friedelin was accomplished successfully in 31 steps and at 0.3% yield by Ireland and Walba in 1976.[105]

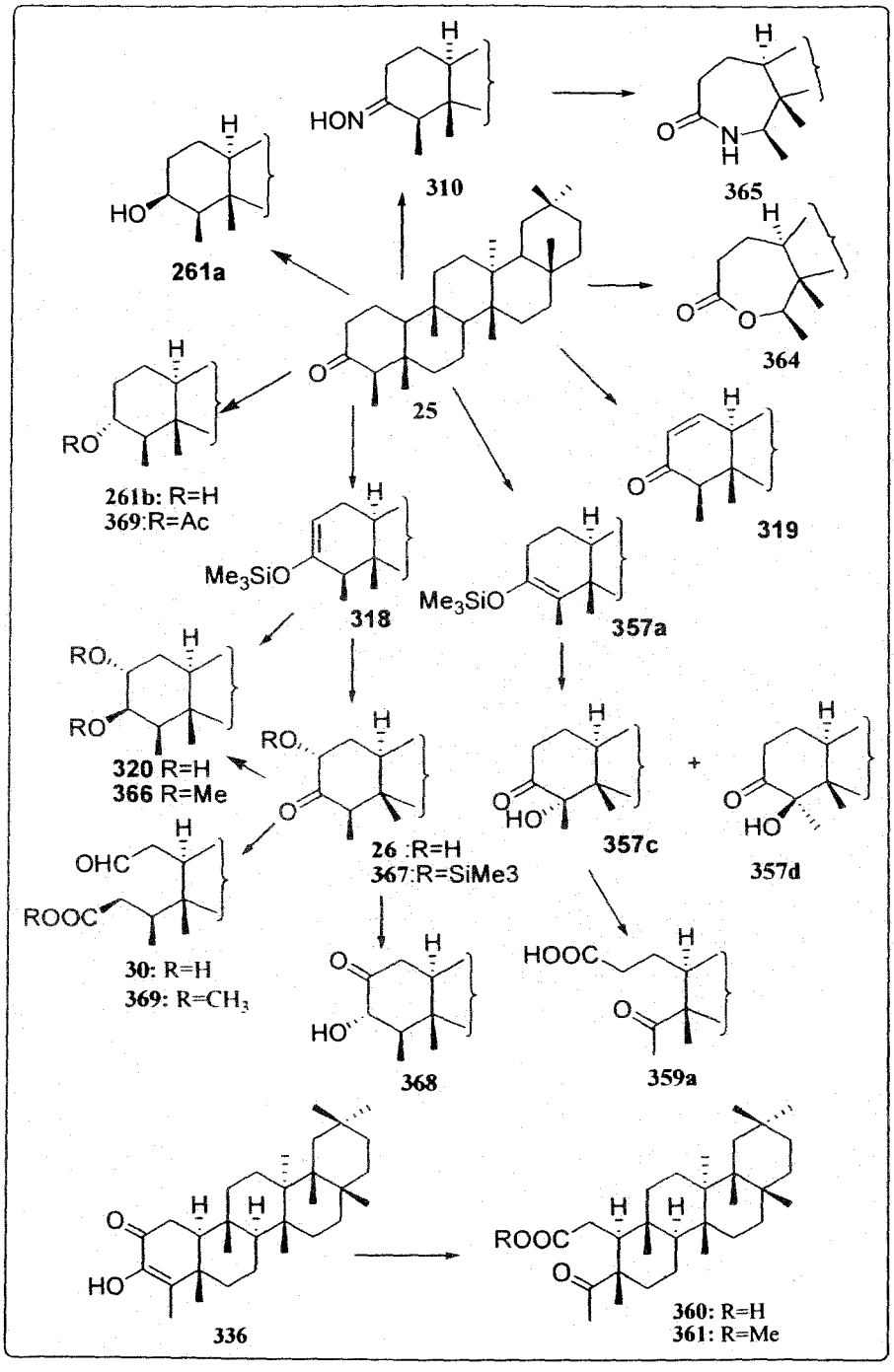
Moiteiro et al. synthesized secofriedelane triterpenoids stereoselectively in high yields. The ability of the compounds to inhibit *in vitro* the growth of three human tumor cell lines, MCF-7

(breast adenocarcinoma), NCI-H460 (nonsmallcell lung cancer), and SF-268 (CNS cancer) were evaluated and only compounds **359** and **360** were found to possess significant growth inhibitory effects, exhibiting GI_{50} values that range from 24.6 to 32.8 μM and 10.9 to17.6 μM, respectively (**Scheme 21**).[106]

Our laboratory also has reported the synthesis of five oxygenated friedelane triterpenoids four of them being seco derivatives. Lactone **352** was synthesized both from cerin (**26**) and friedelin (**25**); seco dioic acid **362** and its dimethyl ester (**363**) were synthesised using friedelin (**25**); and seco keto acid **360** and its methyl ester were prepared using cerin (**26**). The 3D molecular docking of the derivatives in the 'central catalytic domain of topoisomerase IIα (1bgw PDB for topoisomerase IIα)' was also performed which described the binding nature and type of interactions between the enzyme and the synthesized friedelane triterpenoids and the topoisomerase IIα inhibitory activity was confirmed by *in vitro* experiments (**Scheme 22**).[107]



Scheme 22. Topoisomarase IIα inhibitory seco-friedelanes.

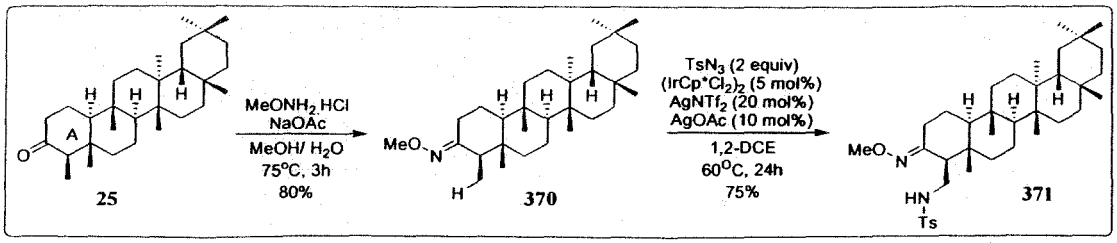


Scheme 23. Biologically active derivatives of friedelin[b]

[b] Details of the scheme, ref. 108.

It was observed that in most of the studies, A-ring of friedelin was modified, though there are very few reports of changes in ring-B or even in ring C, D or E. A number of A-ring modified friedelin derivatives were found to possess potent biocidal activities as were studied by a large number of research groups. Synthesis, bioactivity screening and structure-activity relationships of various natural and synthetic triterpenoids such as friedelin (**25**), 2α-trimethylsiloxyfriedelan-3-one (**367**), 3-hydroxyfriedel-3-en-2-one (**336**), friedelin-2,3-lactone (**364**), friedelin-3-oxime (**310**) and friedelin-3,4-lactam (**365**) were studied. Insecticidal and phytotoxic potential of these compounds, their selective cytotoxic effects on insect and mammalian cells, and their antiparasitic effects were also described. Structurally modified A-ring derivatives such as **26**, 2,3-secofriedelan-2-al-3-oic acid (**30**), its acetylated derivative **369**, 3β- and 3α-hydroxyfriedelane (**261a and 261b**), 3α-hydroxyfriedel-2-one (**368**), 4β-hydroxyfriedel-3-one (**357d**), 3,4-secofriedelan-4-oxo-3-oic-acid (**359a**), lactone **364**, and the oxime **310** were found to be stronger insecticides than the parent compound. Methyl-3-nor-2,4-secofriedelan-4-oxo-2-oic acid (**360**) and its acetylated derivative **370** also showed insecticidal activity in contrast to their inactive parent compound **336**. The post ingestive effects and cytotoxicity of these compounds suggested a multifaceted insecticidal mode of action. These structural modifications did not result in better phytotoxic agents than the parent compounds except for lactam **365** and yielded several moderately active antiparasite derivatives (seco acids **30**, **359a**, **360a** and 4β-hydroxyfriedel-3-one **357d**) with cytotoxic effects on mammalian cells (**Scheme 23**).[108]

Very recently, in 2014, Kang et al. revealed a novel process of direct amidation of sp^3 C-H bonds and they applied the technique also on friedelin (**25**) (**Scheme 24**).[109]



Scheme 24. Direct amidation of unactivated 24-methyl on friedelin.

IV.A.1.3.8 Some more important bioactive derivatives

Apart from these triterpenoids many more biologically potent derivatives of friedelane triterpenoids are also reported. Some of them are reproduced here in short.

Compound **372** (**Figure 22**) was found to be tenfold more active than paracetamol or aspirin, when tested on mice in a dose dependent manner[110] and also showed a strong anti-ulcerogenic activity against the ulcer of mice induced by HCl/EtOH.[111]

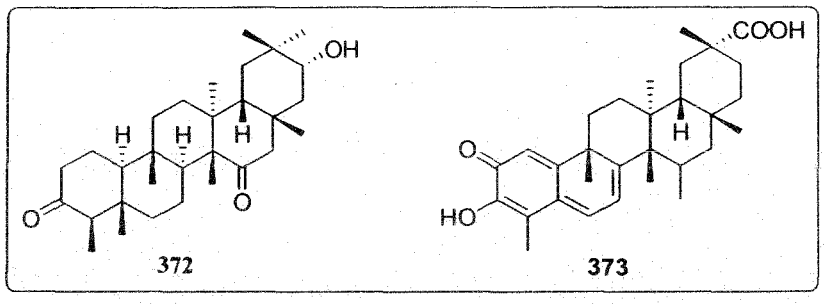


Figure 22. Potent friedelane-based drugs: compound **372** and Celastrol (**373**).

A series of synthesized seco-friedelin triterpenoids were found to have moderate cytotoxicity and insecticidal activities.[112-114] Celastrol **373** (**Figure 22**) was found to have potent anti-inflammatory and neuroprotective effect and investigation was carried out for the treatment of *Parkinson's* and *Alzheimer's* diseases. Anticancer activity of this compound was also tested for different tumor cell lines, as well as in preclinical animal model.[115-123]

Another two biologically important friedelane triterpenoids are maytenfolone A (**374**) and celasdin B (**375**) (**Figure 23**). From the biological evaluation, compound **374** was found to be cytotoxic against hepatoma and nasopharynx carcinoma and compound **375** was found to exhibit anti-HIV replication activity in H9 lymphocyte cells with an EC_{50} value of 0.8 mg/ml.[124]

Friedelin (**25**) was found to be capable of binding the human receptors for endothelin A (ETA) and angiotensin 1 (AT1)[125] and it also showed significant protective activities under different conditions, against AA, CCl_4, $CdCl_2$ induced hepatotoxicity or in the case of cyclophosphamide, cardiotoxicity, in mouse or rat models.[126-131]

Against *Staphylococcus aureus*, Netzahualcoyone (**376**, **Figure 24**)) exhibited stronger inhibitory activity (with a MIC value of 1.5– 1.6 mg/ml) in comparison to some of the antibiotics used in clinical practice.[132]

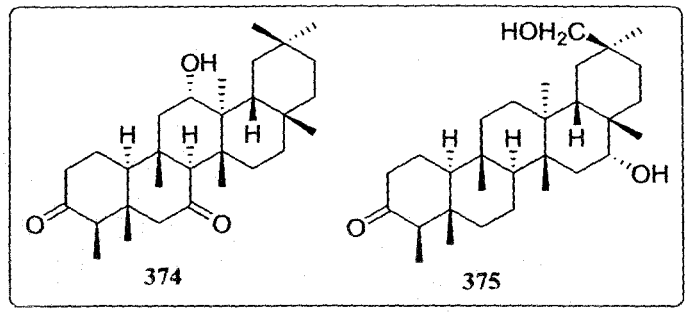


Figure 23. Bioactive maytenfolone A (**374**) and celasdin B (**375**).

Compound **377** was found to possess potential analgesic and potassium channel-blocking activity.[133] Correolide (**378**) was found to block KV1.3 voltagegated potassium channel with an IC_{50} value of 86 nM,[134] and the compound also inhibited human T-cell proliferation with an EC_{50} value of 307 nM (**Figure 24**).[134,135]

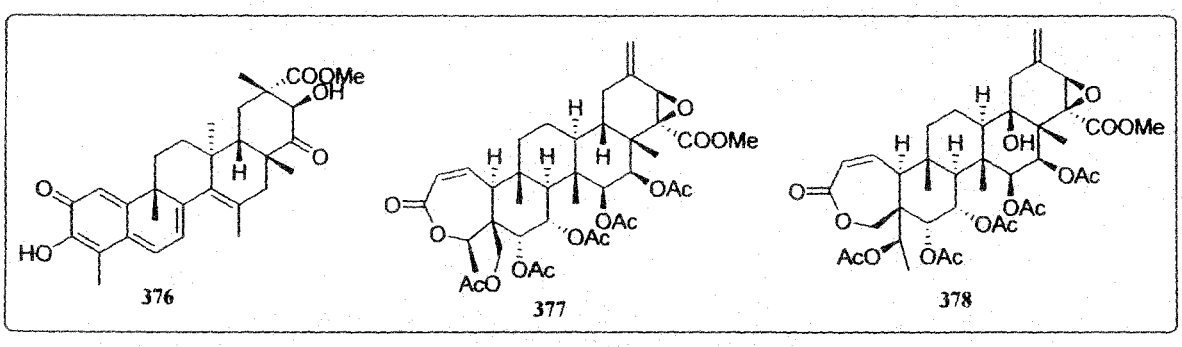


Figure 24. Bioactive netzahualcoyone (**376**), compounds **377** and correolide (**378**).

In summary, recent studies showed that the natural compound friedelin (**25**) and particularly, its derivatives have anti-cancer activity, analgesic and anti-inflammatory capability, anti-bacterial activity and can act as vascularizing agent. Some derivatives can potentially be used in phermaceuticals and functional foods for the treatment or prevention of cardiovascular and cerebrovascular diseases and tumors. Their use in cosmetics and as agro chemicals are also well pronounced.[136]

Hence on the valuable perspective towards the chemical synthetic scope of structural modifications as well as promising useful biological potentiality of friedelin and its derivatives, the author became interested in this field of the natural products chemistry and thus undertook the present work as discussed below.

IV.B Present work

IV.B.1 Background and abstract of the work

Friedelin (**25**) and its derivatives are well known among the PTs to exhibit potential biological effects as is evident from the above review of the friedelane triterpenoids. Herein, a simple but unique protocol for a library of A-ring modified friedelane PTs are described. The key route of the derivatizations is the one-pot $BF_3.OEt_2$-mediated oxidative transformation of friedelin (**25**) into friedel-2-ene (**343**), friedel-3-enol acetate (**379,** the major product) and 2-keto-friedel-3-enol acetate (**380**). A number of common reaction strategies, e.g., oxidation, reduction, acetylation, oximination were employed to the enol acetate to produce more C3- and C4-functionalised friedelane derivatives. Besides, the same reaction protocol was also applied on friedelin oxime (**310**) and 3β-hydroxy friedelane (**261a**). Moreover, some simple transformative reactions of cerin (**26**) have been carried out to prepare sets of compounds which are actually isomeric to some of the prepared friedelane triterpenoids. These sets of isomeric compounds would be helpful to carry out structure activity relationship studies (SAR). The friedelane triterpenoids were then evaluated for their anticipated phytotoxicity.

IV.B.2 Results and Discussion-*Chemical*

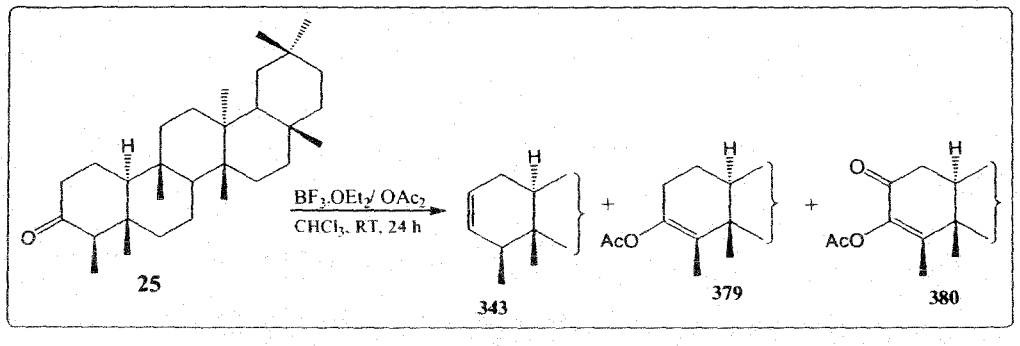
IV.B.2.1 Extraction and isolation of friedelin from *Quercus suber* bark

3 Kg of finely powdered cork (*Quercus suber*) was extracted with petroleum ether in a soxhlet apparatus for 72h. The crude yellowish solid obtained, after removal of the solvent by distillation, was dissolved in minimum volume of chloroform and chromatographed over silica gel column (200 g). Eluent of the column with 2% ethyl acetate in petroleum ether yielded pure white solid characterized as friedelin.

IV.B.2.2 The key-transformative reaction towards diverse A-ring modified friedelane triterpenoids: action of $BF_3.OEt_2$/ Ac_2O on friedelin: Synthesis of friedel-2-ene (**343**), friedel-3-enol acetate (**379**) and 2-ketofriedel-3-enol acetate (**380**).

$BF_3.OEt_2$ is a well known reagent for carrying out versatile transformational changes of different substrate molecules. It was found to be an effective reagent along with acetic anhydride and hydrogen peroxide for α-acetoxylation of ketones.[137] This reagent was also effective in carbon-carbon bond formation[138] in Friedel-Crafts arylation reaction or direct alkylation or arylation of

functionalized pyridine using Grignard reagent[139] or hindered *tert*-alkylamine preparation with organolithium reagent.[140] Considering the accelerating and versatile effect of this reagent we tried a transformation of friedelin with $BF_3.OEt_2$ and Ac_2O, in chloroform, at room temperature and as a result friedel-2-ene (**343**, 7%), friedel-3-enol acetate (**379**, 42%) and 2-keto-friedel-3-enol acetate (**380**, 22%) were isolated (**Scheme 25**).

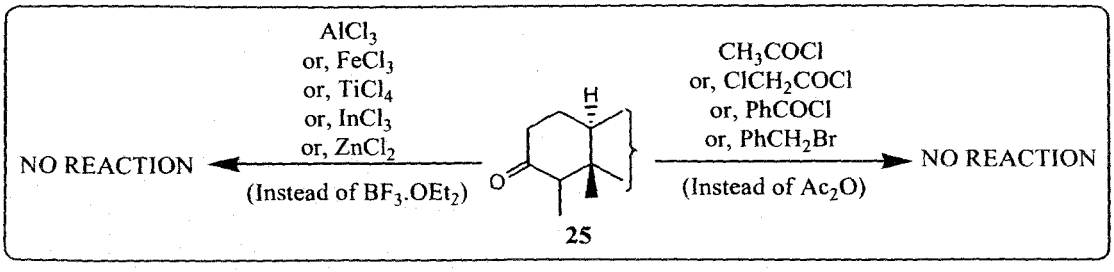


Scheme 25. Treatment of $BF_3.OEt_2$/ OAc_2 on friedelin (**25**).

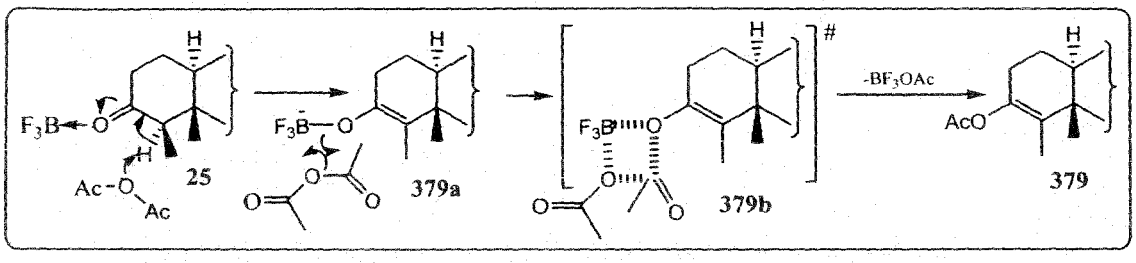
The reaction was conducted also with other potential Lewis acids *viz.*, anhydrous aluminium chloride, ferric chloride, titanium trichloride, magnesium chloride, zinc chloride and nickel chloride along with acetic anhydride; but surprisingly, little transformation too did not take place (**Scheme 26**). We used these Lewis acids at 100 mol% as well as in excess (5-10 eqv.). The reaction was then also tested by varying time and temperature. Allowing the reaction for longer period of time it yielded the enol acetate **379** better (RT, 48h, yield: 60%) though change in the condition from RT to reflux reduced the percentage yield of product **379** (Reflux, 8h, yield: 8%) and increase that of **380** (Reflux, 8h, yield: 32%).

We have also carried out the reaction with different other oxidants instead of acetic anhydride. But none of them, in the same reaction condition, were able to furnish the expected products (**Scheme 26**).

A probable mechanism was thus postulated for the formation of the enol acetate **379**. Using 4α-H of friedelin and considering the Lewis acidity of $BF_3.OEt_2$, these can form 3-enol trifluoroborate moiety **379a**, which can then follow a transition state **379b** involving a new acetic anhydride molecule to result ultimately the 3-enol acetate derivative **379** (**Scheme 27**).



Scheme 26. Reaction of friedelin with different Lewis acids and different oxidants.



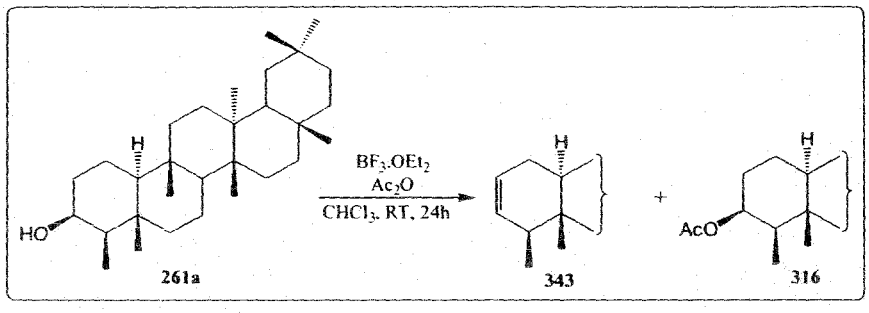
Scheme 27. Probable mechanism for the formation of enol acetate **379**.

The enol acetates are excellent reactive synthons for the functionalization of the corresponding precursor carbonyl compounds and it is, indeed, remarkably evident from a number of transformative reactions. These include aldol reactions,[141,142] the catalytic α-alkylation,[143] catalytic asymmetric hydrogenation,[144,145] halogenation, synthesis of chiral acetates,[146] regioselective hydroxymethylation,[147] acyl transfer reactions,[148] methylation of enol acetates,[149] formation of chiral acetates[150] etc. Besides, enol acetate based syntheses of some natural products are also reported whereas in some cases it has been found that the enol acetates are biologically more potent than the corresponding carbonyl compounds.[151]

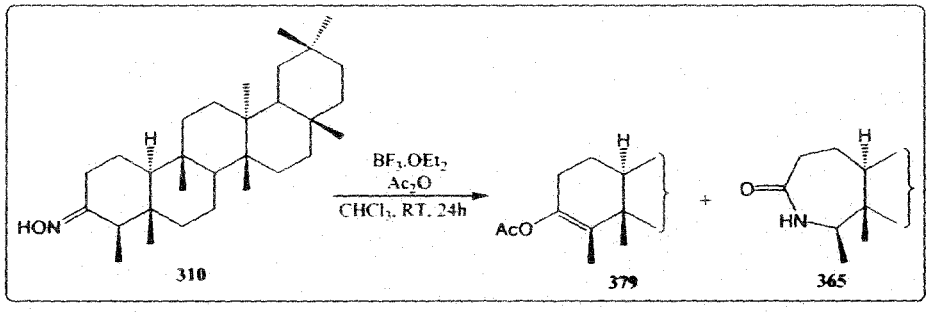
To have a library of A-ring modified friedelane triterpenoids, different easy transformative reactions on the enol acetate **379** were also carried out.

IV.B.2.3 Action of $BF_3.OEt_2$/ Ac_2O on friedelin oxime (310) and 3β-hydroxy friedelane (261a): First, friedelin oxime (**310**, 88%, m.p. 289-291°C) and 3β-hydroxy friedelane (**261a**, 72%, m.p. 290°C) were prepared separately from friedelin following the usual method (please follow the experimental section for general detailed process). The $BF_3.OEt_2$/ Ac_2O reaction protocol was then applied on 3β-hydroxy friedelane (**261a**) and friedelin oxime (**310**) separately.

Compound **261a** furnished a hydrocarbon friedel-2-ene (**343**, 16%) and the acetate derivative of the starting material, i.e., 3β-acetoxy friedelane (**316**, 30%) (**Scheme 28**) whereas the oxime resulted friedel-3-enol acetate (**379**, 18%) and the lactam (**365**, 67%) (**Scheme 29**). The formation of the enol actate from the oxime, can be visualized as the deoxymination (to generate friedelin, **25**) followed by fresh reaction with friedelin and the lactam **365** was formed following the Beckmann rearrangement of the oxime.



Scheme 28. Treatment of $BF_3.OEt_2$/ OAc_2 on 3β-hydroxy friedelane (**261a**).

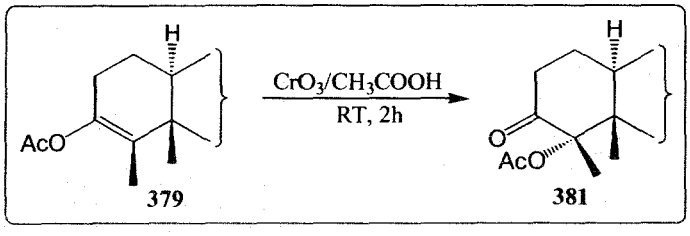


Scheme 29. Treatment of $BF_3.OEt_2$/ Ac_2O on friedelin oxime (**310**).

IV.B.2.4 Transformative reactions toward diverse A-ring modifications based on the compounds prepared as above

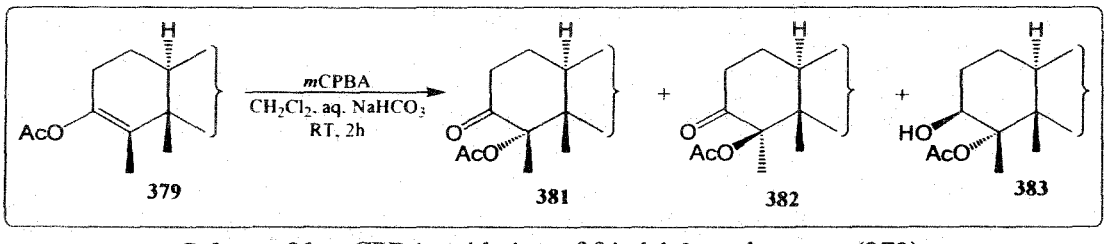
IV.B.2.4.1 Oxidation of friedel-3-enol acetate (379) with CrO_3/ CH_3OOH: Chromic acid is an easily available and strong oxidizing agent. It is very well known that it can easily oxidize the side chain of benzene ring to benzoic acid derivatives whereas in basic condition, such as $CrO_3.py_2$ generally oxidizes the allylic position of alkenes. In the present case, when friedel-3-enol acetate (**379**) was treated with CrO_3/CH_3OOH, it was interesting to note that migration of

the acetate group from C3 to C4 occurred to result 4α-acetoxy friedel-3-one (**381**, 41%) (**Scheme 30**).



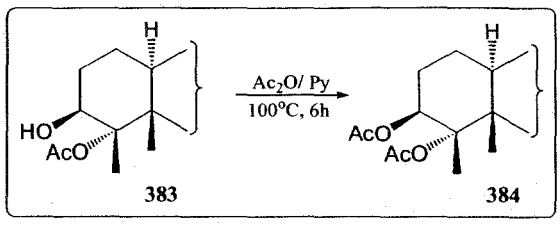
Scheme 30. CrO_3/CH_3COOH oxidation of friedel-3-enol-acetate (**379**).

IV.B.2.4.2 Oxidation of friedel-3-enol acetate (379) with *m*CPBA: Reaction of an alkene with a peroxy acid leads generally to the formation of an epoxide ring and *m*CPBA is a common, quite stable and widely used reagent for the alkene epoxidation. When the enol acetate **379** was treated with *m*CPBA, in milder reaction condition, three products were isolated all having the acetyl/ acetoxy group migrated from C3 to C4. The products isolated were 4α-acetoxy friedelan-3-one (**381**, 12%), 4β-acetoxy friedelan-3-one (**382**, 35%) and 4α-acetoxy-3β-hydroxy friedelane (**383**, 27%) (**Scheme 31**). The first one (**381**) is actually identical with the product obtained by chromic acid oxidation and moreover, compound **382** is a C4-epimer of compound **381** (**Scheme 31**).



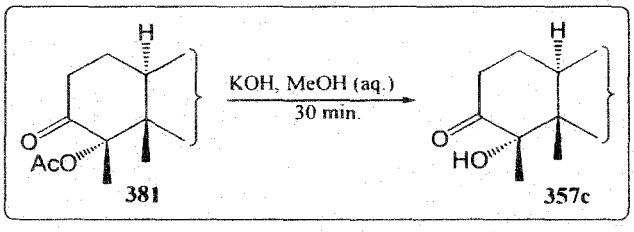
Scheme 31. *m*CPBA oxidation of friedel-3-enol acetate (**379**).

IV.B.2.4.3 Acetylation of the 4α-acetoxy-3β-hydroxy friedelane (383): Compound **383** was acetylated with acetic anhydride in pyridine to produce 3β,4α-diacetoxy friedelane (**384**, 95%) (**Scheme 32**).



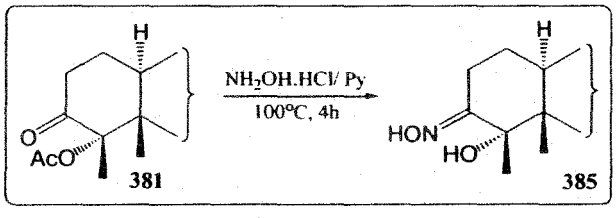
Scheme 32. Acetylation of **383**.

IV.B.2.4.4 Alkali hydrolysis of 4α-acetoxy friedelan-3-one (381): Compound **381** was treated with KOH in aqueous MeOH to produce 4α-hydroxy friedelan-3-one (**357c**, 84%) (**Scheme 33**).



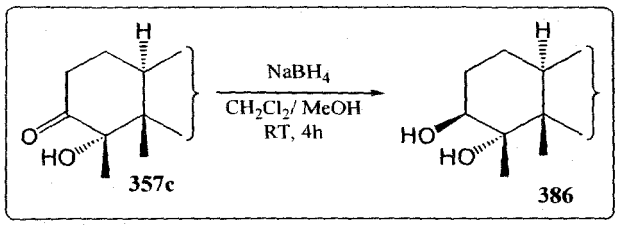
Scheme 33. Formation of 4α-hydroxy friedelan-3-one (**357c**) from **381**.

IV.B.2.4.5 Oximination of keto acetate 381: To prepare the corresponding oxime derivative the keto acetate **381** was treated with hydroxylamine hydrochloride in pyridine. In the reaction condition the acetate group was found to be hydrolyzed and finally 4α-hydroxy friedelane-3-oxime (**385,** 88%) was isolated (**Scheme 34**).



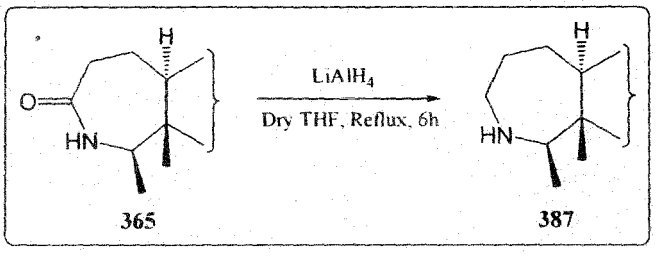
Scheme 34. Simultaneous oximination and deacetylation of keto acetate **381**.

IV.B.2.4.6 Reduction of 4α-hydroxy friedelan-3-one (357c) with $NaBH_4$: Compound **357c** was reduced with $NaBH_4$ to produce 3β,4α-dihydroxy friedelane (**386**, 62%, m.p. 240-241°C lit[88], 240-241°C) (**Scheme 35**).



Scheme 35. $NaBH_4$ reduction of **357c**.

IV.B.2.4.7 Reduction of friedelin lactam 365 with $LiAlH_4$: Lactam **365** was reduced with lithium aluminium hydride to yield a seven-membered cyclic amine **387** (63%) **(Scheme 36)**.



Scheme 36. $LiAlH_4$ reduction of lactam **365**.

IV.B.2.5 Synthesis of some 2-substituted friedelane derivatives which are isomeric to the above compounds

Considering the scope of the structure-activity relationships (SAR) of the different isomeric friedelane triterpenoids (modifications based on A-ring only), cerin, **26**, isomeric to 4α-hydroxy friedelan-3-one (**357c**, **Scheme 33**) was isolated from bark cork and some of its common derivatives which were indeed isomeric to at least one of the prepared friedelane triterpenoids based on the $BF_3.OEt_2$/ Ac_2O-mediated key transformative reaction (**Scheme 25**), were also then prepared.

IV.B.2.5.1 Extraction and isolation of cerin (26) from *Quercus suber* bark

Cerin, a friedelane triterpenoid, structurally 2α-hydroxy friedelin (**26**, **Figure 25**)[1-2] is available from the same source where from friedelin was isolated. Ethyl acetate as eluent in the column chromatography of the cork extracts (please follow section **IV.B.2.1** for detailed extraction and isolation method) produced pure white crystalline solid characterized as cerin (m.p. 252-256°C, after repeated recrystallization from $CHCl_3$). Again, structurally cerin is isomeric to compound

357c (i. e., 4α-hydroxy friedelan-3-one) which was synthesized, in three steps, from friedelin (**Schemes 25**, **30** and **33**).

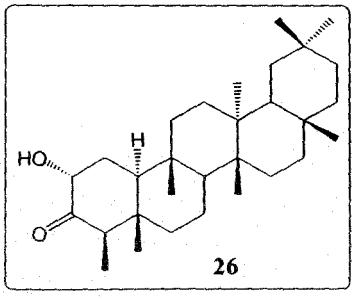
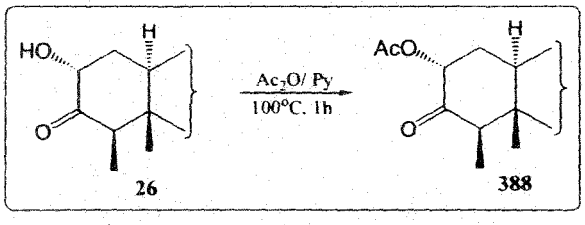


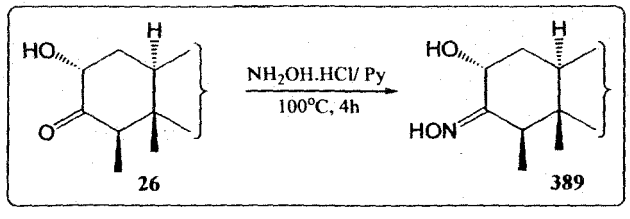
Figure 25. Cerin (**26**).

IV.B.2.5.2 Acetylation of cerin (26): Treatment of cerin (**26**) with acetic anhydride in pyridine yielded 2α-acetoxy friedelan-3-one (**388**, 78%, **Scheme 37**) which is actually isomeric to the compounds 4α-acetoxy friedelan-3-one (**381**) and 4β-acetoxy friedelan-3-one (**382**) synthesized, in two steps, from friedelin (**Schemes 25** and **31**).



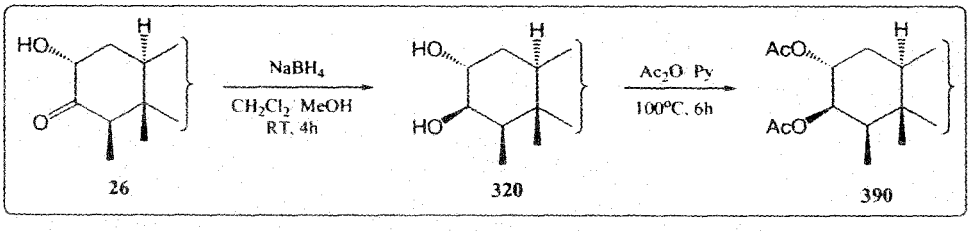
Scheme 37. Acetylation of cerin (**26**).

IV.B.2.5.3 Oximination of cerin (26): Hydroxylamine hydrochloride converted cerin (**26**) into 2α-hydroxy friedelan-3-oxime (**389**, 95%) (**Scheme 38**) which is indeed isomeric to 4α-hydroxy friedelane-3-oxime (**385**) which was synthesized, in four steps, from friedelin (**Schemes 25, 30, 33** and **34**).



Scheme 38. Oximination of cerin (**26**).

IV.B.2.5.4 $NaBH_4$ reduction of cerin (26): 2α,3β-Dihydroxy friedelane (**320**), another natural product also named as pachysandiol A was obtained (68%) when cerin (**26**) was reduced with $NaBH_4$. Acetylation of the diol **320** resulted 2α,3β-diacetoxy friedelane (**390**, 97%) (**Scheme 39**). The diol **320** and its diacetate **390** are isomeric to the compounds 3β,4α-dihydroxy friedelane (**386,** synthesized from friedelin in three steps, **Schemes 25, 30** and **35**) and 3β,4α-diacetoxy friedelane (**384,** synthesized, in three steps, from friedelin, **Schemes 25, 31** and **32**) respectively.



Scheme 39. Synthesis of pachysandiol A (**320**) and its diacetate (**390**).

IV.B.3 Results and discussion-*Biological.*

The synthesized friedelane triterpenoids were evaluated for their phytotoxicity effect and the plant species *Oriza sativa* was selected as the experimental target. The effects of the compounds on germination and radicle and shoot length of *O. sativa* were evaluated after 10 days. The tests were carried out in three different sets. In the first set of experiments compounds **25, 379, 380, 310, 365, 261a, 316, 343**; in the second set compounds **320, 26, 388, 389** and in the third set of experiments compounds **381, 385, 357c, 383, 387** were selected. In each set of the experiments two different concentrations of each compound was employed (except **261a, 383, 357c** and **385**). Germination, radicle and shoot length of *O. sativa* seeds of three different sets of experiments are given in **Tables 1-3**, **Tables 4-6** and **Tables 7-9** respectively.

Table 1: Germination experiment for the first set of compounds

Compounds	Concentration (μg)	No. of seeds germinated	Germination (%)
Control	-	38	100
25	50	31	82
	25	31	82

Table 1: (contd.)

Compounds	Concentration (μg)	No. of seeds germinated	Germination (%)
379	50	16	42
	25	25	66
380	50	30	79
	25	27	71
310	50	28	74
	25	26	68
365	50	35	92
	25	34	90
261a	50	32	84
316	50	39	103
	25	35	92
343	50	34	90
	25	32	84

Table 2. Root length measurement for the first set of compounds

Compounds (μg)	Root length (cm.)	Mean	Inhibition (%)
Control	2.4, 3.1, 3.0, 3.1, 3.1, 2.0, 0.4, 1.0, 0.6, 1.0	1.97	-
25 (50)	3.6, 1.4, 1.2, 0.1, 1.6, 0.0, 0.4, 0.3, 0.5, 0.0	0.91	54
25 (25)	1.1, 1.0, 0.8, 1.0, 0.3, 0.5, 0.5, 0.0, 0.0, 0.0	0.54	73
379 (50)	1.3, 0.5, 0.4, 0.6, 0.4, 0.9, 0.1, 0.2, 0.0, 0.0	0.44	78
379 (25)	0.5, 0.5, 0.7, 0.0, 1.0, 1.2, 0.1, 0.0, 0.1, 0.2	0.43	78
380 (50)	0.9, 0.1, 2.1, 1.1, 0.6, 1.7, 2.0, 0.5, 0.7, 0.1	0.98	50
380 (25)	0.2, 0.3, 0.7, 1.5, 0.4, 0.9, 0.0, 0.7, 0.4, 0.1	0.52	74
310 (50)	0.5, 0.7, 0.7, 0.4, 1.4, 0.5, 2.1, 1.2, 0.3, 1.2	0.90	54
310 (25)	0.3, 1.0, 2.2, 0.1, 2.6, 0.3, 1.0, 0.9, 0.5, 0.6	0.95	52
365 (50)	1.0, 0.4, 1.7, 0.0, 1.0, 2.7, 0.0, 0.4, 0.8, 0.0	0.80	59
365 (25)	0.9, 0.9, 1.3, 0.4, 1.7, 0.9, 1.2, 0.6, 0.4, 0.4	0.87	56

Table 2. (contd.)

Compounds (μg)	Root length (cm.)	Mean	Inhibition (%)
261a (50)	0.6, 2.0, 1.4, 0.7, 0.7, 2.1, 0.7, 2.1, 0.2, 0.2	1.07	46
316 (50)	0.5, 0.6, 0.9, 1.4, 2.4, 1.4, 1.9, 0.9, 0.6, 0.9	1.15	42
316 (25)	0.8, 1.5, 1.3, 0.7, 0.2, 1.0, 0.7, 0.7, 0.4, 0.8	0.81	59
343 (50)	1.0, 1.0, 1.1, 1.4, 1.6, 1.4, 1.1, 1.7, 0.3, 0.8	1.14	42
343 (25)	0.8, 2.0, 1.0, 1.8, 0.5, 1.5, 1.4, 0.8, 0.7, 0.4	1.09	45

Table 3. Shoot length measurement for the first set of compounds.

Compounds (μg)	Shoot length (cm.)	Mean	Inhibition (%)
Control	1.0, 1.1, 0.8, 1.7, 1.4, 0.9, 1.3, 0.7, 0.7, 0.9	1.05	-
25 (50)	1.8, 0.7, 0.6, 0.6, 0.3, 0.8, 0.4, 0.3, 0.5, 0.8	0.68	35
25 (25)	0.7, 0.5, 0.7, 0.5, 0.7, 0.4, 0.3, 0.4, 0.7, 0.7	0.56	47
379 (50)	0.6, 0.6, 0.7, 0.8, 0.4, 0.7, 0.7, 0.5, 0.4, 0.2	0.56	47
379 (25)	0.8, 0.8, 0.7, 1.0, 1.1, 0.7, 0.8, 0.8, 0.6, 0.5	0.78	26
380 (50)	0.5, 1.2, 1.0, 0.7, 0.8, 0.9, 1.0, 0.6, 0.6, 0.6	0.79	25
380 (25)	0.5, 0.8, 0.7, 1.0, 0.7, 0.8, 0.5, 0.7, 0.7, 0.7	0.71	32
310 (50)	0.5, 0.6, 0.5, 0.6, 0.4, 0.7, 0.5, 0.6, 0.4, 0.7	0.55	48
310 (25)	0.2, 1.2, 0.7, 0.6, 1.4, 0.4, 0.6, 0.4, 0.4, 0.4	0.63	40
365 (50)	1.2, 0.4, 0.7, 0.5, 0.6, 0.5, 0.6, 0.7, 0.8, 0.6	0.67	36
365 (25)	0.7, 0.6, 1.0, 0.7, 1.2, 0.7, 0.5, 0.5, 0.4, 0.9	0.72	31
261a (50)	0.6, 0.4, 1.4, 0.7, 0.7, 1.1, 0.9, 0.4, 0.6, 0.6	0.74	30
316 (50)	0.5, 0.2, 0.7, 0.7, 0.5, 1.7, 0.7, 0.7, 0.4, 0.5	0.66	37
316 (25)	0.6, 0.8, 0.8, 0.7, 0.7, 1.3, 0.7, 0.7, 0.6, 0.8	0.77	27
343 (50)	0.6, 0.7, 0.9, 0.7, 0.9, 0.7, 0.8, 0.7, 0.6, 0.8	0.74	30
343 (25)	0.2, 0.7, 0.7, 0.8, 0.7, 0.5, 0.5, 0.7, 0.7, 0.6	0.61	42

Table 4. Germination experiment for the second set of compounds

Compounds	Concentration (μg)	No. of seeds germinated	Germination (%)
Control	-	45	100
320	50	45	100
	25	45	100
26	50	48	107
	25	48	107
388	50	49	109
	25	47	104
389	50	45	100
	25	49	109

Table 5. Root length measurement for the second set of compounds

Compounds (μg)	Root length (cm.)	Mean	Inhibition (%)
Control	13.6, 17.5, 15.6, 12.7, 11.9, 14.0, 14.0, 13.7, 13.7, 13.2	13.99	-
320 (50)	14.9, 14.4, 9.6, 11.9, 6.7, 12.0, 14.2, 13.2, 7.6, 12.8	11.73	16
320 (25)	11.3, 13.8, 13.5, 9.9, 9.6, 15.3, 8.2, 10.2, 11.2, 10.2	11.32	19
26 (50)	0.6, 8.9, 13.3, 11.4, 10.4, 9.7, 11.7, 8.7, 11.5, 12.1	10.83	23
26 (25)	14.6, 17.7, 5.0, 11.5, 10.2, 14.6, 6.7, 11.9, 5.0, 8.6	10.58	24
388 (50)	10.3, 13.5, 9.2, 5.9, 14.2, 12.0, 8.1, 7.6, 11.0, 16.0	10.51	25
388 (25)	9.0, 12.4, 10.2, 13.0, 10.4, 10.7, 13.2, 10.3, 8.5, 7.4	10.78	23
389 (50)	8.2, 14.1, 10.1, 11.4, 10.5, 11.3, 11.1, 14.0, 6.9, 15.8	11.34	19
389 (25)	12.2, 10.7, 9.4, 14.6, 14.0, 10.1, 12.2, 11.3, 11.5, 12.5	11.85	15

Table 6. Shoot length experiment for the second set of compounds

Compounds (μg)	Shoot length (cm.)	Mean	Inhibition (%)
Control	9.1, 9.7, 8.9, 8.7, 8.5, 8.7, 8.5, 9.7, 5.2, 5.6	8.25	-
320 (50)	7.8, 7.5, 8.0, 8.2, 5.2, 6.2, 5.8, 8.4, 6.4, 6.2	7.09	14
320 (25)	6.7, 4.3, 6.7, 6.8, 8.0, 7.8, 7.7, 6.6, 6.0, 8.0	6.99	15
26 (25)	7.3, 8.2, 8.9, 4.0, 8.0, 10.1, 7.0, 7.7, 5.9, 7.9	6.62	20

Table 6. (contd.)

Compounds (μg)	Shoot length (cm.)	Mean	Inhibition (%)
26 (50)	6.7, 8.2, 9.1, 6.6, 8.0, 7.5, 7.0, 7.3, 5.9, 7.5	7.06	15
388 (50)	7.2, 9.1, 9.0, 7.4, 8.3, 6.6, 7.5, 7.5, 9.6, 5.6	7.08	14
388 (25)	6.1, 6.7, 7.3, 8.5, 7.4, 6.7, 7.9, 8.5, 5.7, 4.5	6.95	16
389 (50)	3.5, 7.4, 7.0, 8.1, 6.0, 7.8, 7.0, 8.2, 4.1, 8.5	6.76	18
389 (25)	8.6, 5.8, 7.5, 7.2, 7.1, 6.5, 5.9, 8.6, 4.6, 6.8	6.86	17

Table 7. Germination experiment for the third set of compounds

Compounds	Concentration (μg)	No. of seeds germinated	Germination (%)
Control	-	48	100
381	50	43	90
	25	46	96
385	25	50	104
357c	25	48	100
383	25	43	90
387	50	49	102
	25	45	94

Table 8. Root length experiment for the third set of compounds

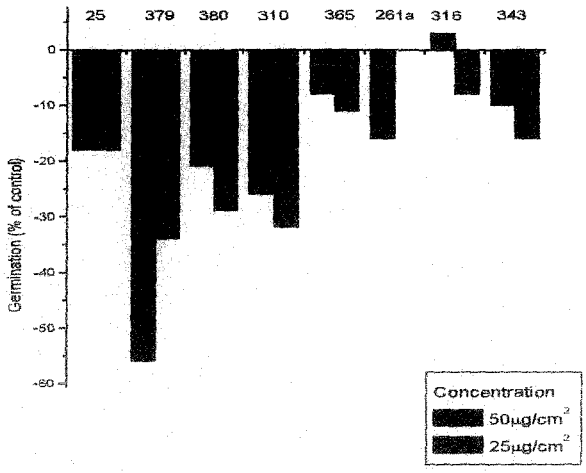
Compounds (μg)	Root length (cm.)	Mean	Inhibition (%)
Control	12.5, 11.9, 11.2, 14.1, 11.6, 15.4, 10.7, 11.1, 12.3, 12.4	12.32	-
381 (50)	12.1, 10.7, 7.4, 12.2, 8.5, 12.3, 11.4, 10.2, 11.2, 6.8	10.28	17
381 (25)	11.2, 11.3, 12.1, 9.5, 10.5, 13.4, 11.3, 7.6, 9.2, 8.0	10.25	17
385 (25)	12.4, 8.4, 9.2, 12.8, 12.0, 11.8, 13.2, 12.95.2, 10.9	10.16	18
357c (25)	12.8, 6.1, 12.7, 10.0, 11.2, 12.6, 13.6, 6.6, 9.3, 9.0	10.39	16
383 (25)	11.1, 7.7, 12.6, 8.6, 13.4, 11.8, 11.5, 12.0, 9.7, 9.6	10.80	18
387 (50)	9.0, 6.3, 9.9, 8.7, 9.1, 8.7, 8.2, 13.1, 11.5, 8.8	9.87	20
387 (25)	8.7, 10.7, 8.8, 8.5, 11.2, 9.2, 9.3, 11.7, 11.2, 11.2	10.05	18

Table 9. Shoot length experiment for the third set of compounds

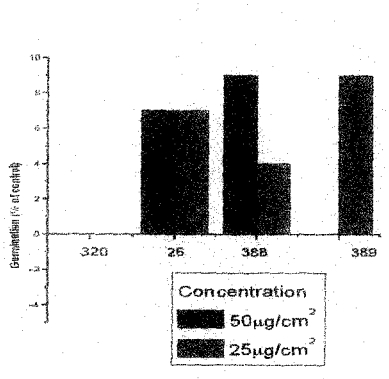
Compounds (μg)	Shoot length (cm.)	Mean	Inhibition (%)
Control	7.2, 9.2, 8.0, 6.6, 9.5, 7.2, 8.9, 7.0, 7.6, 6.7	7.79	-
381(50)	7.6, 6.0, 7.0, 6.9, 6.9, 7.3, 6.3, 6.9, 7.4, 7.7	7.70	10
381(25)	9.3, 7.3, 5.0, 5.5, 5.7, 7.3, 3.6, 7.2, 4.2, 4.2	5.93	24
385 (25)	7.1, 5.8, 8.4, 7.6, 4.8, 7.8, 7.1, 5.5, 3.3, 7.8	6.52	16
357c (25)	8.1, 6.7, 6.4, 5.7, 6.7, 8.2, 7.5, 5.0, 3.6, 6.1	6.40	18
383 (25)	6.9, 6.7, 5.9, 8.0, 8.3, 6.2, 5.2, 8.1, 6.3, 5.5	6.71	14
387 (50)	5.9, 5.0, 5.3, 4.9, 4.6, 4.7, 7.2, 7.9, 5.9, 4.9	5.79	26
387 (25)	6.0, 5.0, 4.7, 5.3, 8.3, 4.8, 4.3, 5.2, 5.6, 5.4	5.46	30

From the graphs below (**Figure 26**), it was clear that in comparison to the mother compound friedelin (**25**, inhibition: 18%), the synthesized derivatives friedel-3-enol acetate (**379**) and 2-ketofriedel-3-enol acetate (**380**) inhibited the germination of *O. sativa* seeds more (inhibition: 34% and 29% respectively) and in fact, compound **379** at higher concentration (50 μg) was most effective (inhibition: 58%). But compared to friedelin oxime (**310**, inhibition: 26%) its lactam derivative (**365**) was found to be less effective (inhibition: 8%) although both **310** and **365** were better effective at lower concentration (25 μg, inhibition: 32% and 10% respectively). On the other hand, friedel-2-ene (**343**) at lower concentration (25 μg) showed equal effect like its starting material 3β-hydroxy friedelane (**261a**) at higher concentration (50 μg) (inhibition: 12% and 10% respectively), but 3β-acetoxy friedelane (**316**) at higher concentration (50 μg) showed stimulative effect (stimulation: 3%). For second set of experiments cerin (**26**), 2α-acetoxy friedelan-3-one (**388**) (at both concentrations, 50 μg and 25 μg) and 4α-hydroxy friedelan-2-one (**389**) (at lower concentration, 25 μg) showed stimulating effect (stimulation: 7%, 7%; 9%, 4%; and 9% respectively) on germination except **389** at higher concentration (50 μg) and 2α,3β-dihydroxy friedelane (**320**) (in both concentrations) which had identical effect as control. In the third case, 4α-aceatoxy friedelan-3-one (**381**), inhibition: 10% (50 μg) and 4% (25 μg); 4α-acetoxy friedelan-3β-ol **383** (inhibition: 10% at 25 μg concentration) and the cyclic amine **387** (inhibition 6% at 25 μg concentration) were found to be effective but 4α-hydroxy friedelan-3-

oxime (**385**) and **387** had stimulation effect at 50 μg concentration (stimulation: 4% and 2% respectively).



(A)



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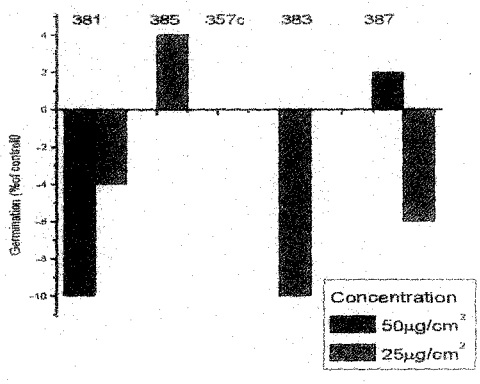
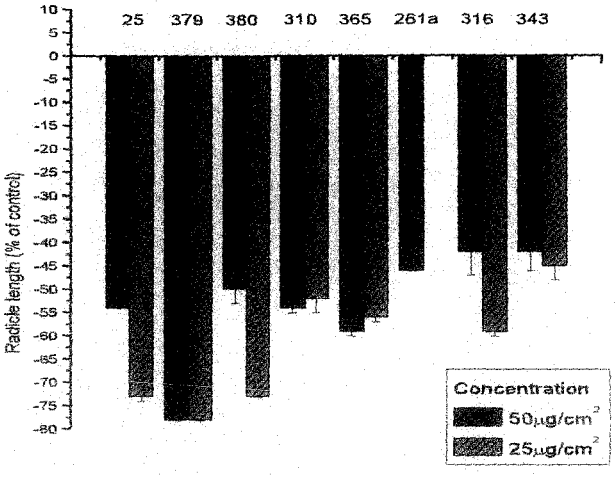


(C)

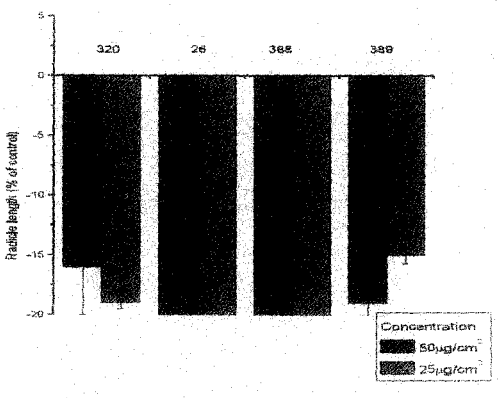
Figure 26. Effect of first set (A), second set (B) and third set (C) of compounds on germination of *O. sativa* seeds. Values are presented as percentage differences from the control: zero representing an observed value identical to the control, a positive value representing stimulation and a negative value representing inhibition.

From the experiments, it was also found that acetoxy group at C-4 position of friedelin skeleton, as in 4α-aceatoxy friedelan-3-one (**381**) increased the activity of the compound

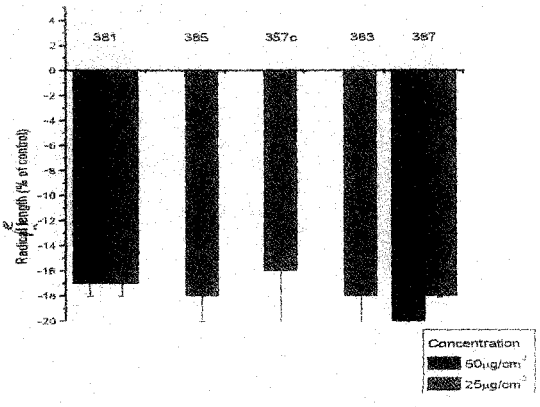
compared to the hydroxyl group at the same position, as in 4α-hydroxy friedelan-3-one (**357c**) which actually showed identical effect as control on the germination of *O. sativa.*



(D)



(E)



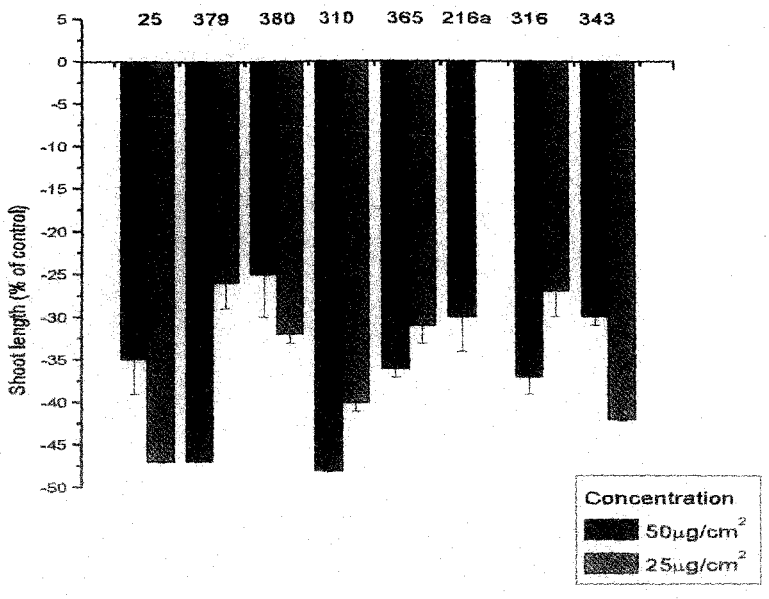
(F)

Figure 27. Effect of first set (D), second set (E) and third set (F) of compounds on the radicle length of *O. sativa.* Values are presented as percentage differences from the control: zero representing an observed value identical to the control, a positive value representing stimulation and a negative value representing inhibition.

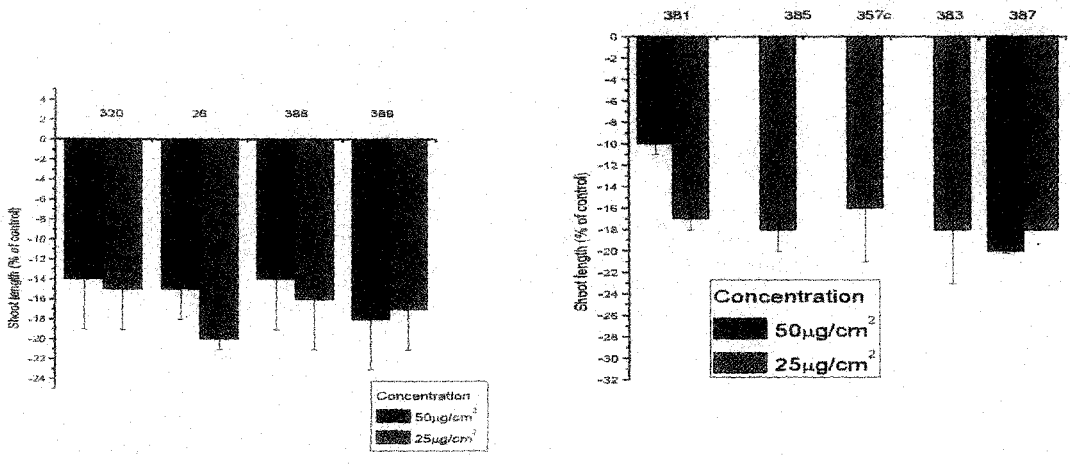
The graphs (**Figure 27**) for the radicle length measurements indicated that in comparison to the mother compound friedelin (**25**, inhibition: 54% and 73% at 50 µg and 25 µg concentration

respectively), its derivatives friedel-3-enol acetate (**379**) inhibited the root length of *O. sativa* most (inhibition: 78% in both the concentrations) although friedel-2-keto-3-enol acetate (**380**) was found to be more effective at lower concentration only (inhibition: 74%). Among friedelin oxime (**310**, inhibition: 54% at 50 μg and 52% at 25 μg) and its lactam derivative (**365**) (inhibition: 59% at 50 μg and 56% at 25 μg) the latter one was little bit more effective at both the concentrations. On the other hand, friedel-2-ene (**343**) at both concentrations (inhibition: 42% and 45%) and 3β-acetoxy friedelane (**316**) at higher concentration (inhibition: 42%) showed less effectivity than 3β-hydroxy friedelane (**261a**) at higher concentration (inhibition: 46%). Only 3β-acetoxy friedelane (**316**) at lower concentration showed better inhibitory effect (inhibition: 59%) on the radicle length. For second and third set of compounds all showed inhibitory effect but these were found to be less effective compared to the first set of compounds and friedel-3-enol acetate (**379**) was found most potent among the three sets of compounds towards the inhibition of the radicle length of *O. sativa*.

While analysing the effect of the compounds on the shoot length of *O. sativa* seeds after germination (**Figure 28**), friedel-3-enol acetate (**379**) at higher concentration (50 μg) was found to be effective (inhibition: 47%) although the mother compound friedelin (**25**) at lower concentration (25μg) showed the equal activity whereas friedel-2-keto-3-enol acetate (**380**) was less effective (inhibition: 25%, 50 μg; and 32%, 25 μg). In comparision to friedelin oxime (**310**) its lactam derivative (**365**) was found to be less effective (inhibition: 48%, 40% and 36%, 31% in both the concentrations respectively). On the other hand, friedel-2-ene (**343**) at lower concentration (25μg) and 3β-acetoxy friedelane (**316**) at higher concentration (50μg) were more effective (inhibition: 42% and 37% respectively) than 3β-hydroxy friedelane (**261a**) at higher concentration (inhibition: 30%). For second and third sets of compounds all showed inhibitory effect, the cyclic amine **387** being the most effective among them (inhibition: 30%), but not as much as the first set of compounds.



(G)



(H) (I)

Figure 28. Effect of first set (G), second set (H) and third set (I) of compounds on the shoot length of *O. sativa*. Values are presented as percentage differences from the control: zero representing an observed value identical to the control, a positive value representing stimulation and a negative value representing inhibition.

IV.C Experimental

IV.C.1 General:

Melting points were measured in open capillary methods and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on BruckerAvance 300MHz FT-NMR spectrometer using 5 mm BBO probe. $CDCl_3$ or DMSO-d_6 were used as solvent and TMS as reference material. Data are presented as follows: Chemical shift -in ppm on the scale relative to $\delta_{TMS} = 0$; coupling constant-*J*/Hz. Infrared spectra were recorded either in Shimudzu FT-IR 8300 Spectrometer or in Perkin Elmer FT-IR Spectrum *RX I* Spectrometer as neat or thin films (KBr or Nujol) as indicated in the experimental procedures, and at room temperature. Frequencies are given in wave numbers (cm^{-1}). Mass spectra were recorded on a Qtof Micro YA263 high-resolution mass spectrometer. For column chromatography, silica gel G, 60-120 mesh was used with petroleum ether- ethyl acetate mixture as the eluent. For thin layer chromatography (TLC), freshly made silica gel plates (using silica gel for TLC and petroleum ether) were used and visualization was achieved by staining with iodine.

IV.C.2 General procedure for the $BF_3.OEt_2$ mediated reactions (towards the synthesis of the compounds **316**, **343**, **365**, **379** and **380**):

Compound (either **25** or **310** or **261a**; 1 mmol) was dissolved in $CHCl_3$ (10 mL). Acetic anhydride (5 mmol) followed by $BF_3.OEt_2$ (5 mmol) was added to the solution and was allowed to stir for 24h at room temperature. 10 mL chloroform and 10 mL aqueous sodium acetate solution were added to the reaction mixture and was allowed to reflux for 30 minutes. The reaction mixture was cooled, extracted with chloroform (3x 25 mL), washed with saturated brine solution (2x 25 mL) followed by water (1x 25 mL), and dried (Na_2SO_4). The solvent was removed by distillation to obtain the reddish solid which after coloum chromatography furnished the products. In each case the separated compounds were further purified by crystallization.

IV.C.3 Oxidation of friedel-3-enol acetate with CrO_3/ CH_3OOH:

Friedel-3-enol acetate (**380,** 100 mg, 0.21 mmol) was dissolved in glacial acetic acid (1 mL). CrO_3 (1.5 eqv.) was added to it and stirred for 2 h at room temperature. The reaction mixture was poured into ice cold water (50 mL), filtered and washed with water repeatedly and dried

(vaccuum). The residue obtained, after column chromatography produced 4α-acetoxy friedelan-3-one (**381,** 42 mg, 41%) which was further recrystallized from chloroform-methanol.

IV.C.4 Oxidation of friedel-3-enol acetate (380) with *m*CPBA:

Friedel-3-enol acetate (100.0 mg, 0.21 mmol) was dissolved in CH_2Cl_2 (10 mL), and then *m*-CPBA (84 mg, 0.37 mmol) and $NaHCO_3$ (aqueous, 0.5 M, 5 mL) were added. The mixture was stirred for 2h at room temperature. $CHCl_3$ (10 mL) was poured and the organic layer was separated, washed with saturated solution of $NaHCO_3$ (2x 15 mL), and with water (2x 15 mL), and then concentrated (vaccuum) and dried (Na_2SO_4). Column chromatography followed by recrystallization yielded compounds 4α-acetoxy friedelan-3-one (**381,** 12%), 4β-acetoxy friedelan-3-one (**382,** 35%) and 4α-acetoxy friedelan-3β-ol (**383,** 27%).

IV.C.5 General procedure for the acetylation reactions (to synthesize compounds **384**, **388 and 390**):

10 mg of the compound was dissolved in pyridine (1 mL) and acetic anhydride (0.5 mL) was added to it and allowed to heat at 100°C for specific time (please follow the respective Schemes for the reaction times). The reaction mixture was cooled, poured into ice cold water (50 mL), filtered and washed with cold water repeatedly and the solid was vacuum dried. The residue was column chromatographed and further recrystalised from chloroform-methanol to obtain the corresponding pure acetyl derivative.

IV.C.6 General procedure for the oximination reactions:

10 mg of the compound was dissolved in pyridine (1 mL) and hydroxylamine hydrochloride (1.5 eqv.) was added to it and allowed to heat at 100°C for 4h. The reaction mixture was cooled, poured into ice cold water (50 mL), filtered and washed with water repeatedly and the solid was vacuum dried. The residue was recrystallised from chloroform-ethanol or ethanol to obtain the corresponding pure oxime derivative.

IV.C.7 Hydrolysis with KOH/ MeOH:

4α-Acetoxy friedelan-3-one (**381,** 17 mg, 0.035 mmol) was dissolved in aqueous methanol (MeOH: H_2O= 9:1, 10 mL) and solid KOH (3 eqv.) was added to it. The reaction mixture was

refluxed for 30 minutes and then methanol was evaporated out and was cooled. Chloroform (25 mL) was added to the residue, washed with water (3x 20 mL), brine (1x 20 mL) and again with water (1x 20 mL) and was dried (Na_2SO_4). The solvent was removed by distillation to obtain pale yellowish solid which after coloum chromatography furnished the pure product 4α-hydroxy-friedel-3-one (**357c,** 13 mg, 84%).

IV.C.8 General procedure for the reduction with $NaBH_4$:

Compound (10 mg) was dissolved in CH_2Cl_2-MeOH (1:1, 10 mL) and $NaBH_4$ (1.2 equivalent) was added. The solution was stirred for 4 hours at room temperature. Sodium hydroxide (1 M, 10 mL) was added and the reaction mixture was extracted with $CHCl_3$ and after usual work-up (washed, dried, solvent evaporated), silica gel column chromatography furnished the expected pure products.

IV.C.9 $LiAlH_4$ reduction of lactam:

Friedelin lactam **365** (40 mg, 0.09 mmol) was dissolved in dry THF (10 mL) and $LiAlH_4$ (10 mg, 0.28 mmol) was added to it. The reaction mixture was refluxed for 6 h, cooled and water (10 mL) was added dropwise to destroy excess $LiAlH_4$. THF was then evaporated out (rotavap) and the residue was extracted out in chloroform (30 mL), washed (with water: 1x 15 mL; with brine: 2x 15 mL; again water: 1x 15 mL) and dried (Na_2SO_4). Coloum chromatography of the solid residue produced the seven-membered cyclic amine **387** (22 mg, 63%) which was further purified by recrystallization with aqueous ethanol.

IV.C.10 Bioassay:

O. sativa seeds were purchased from commercial shops. All undersized and damaged seeds were discarded. Germination and growth were conducted in 90 mm petri dishes containing a 4.4 cm sheet of Whatman no. 1 filter paper as support. Compounds were dissolved in chloroform and spread over the Whatman no. 1 filter paper uniformly. Then these were allowed to dry completely. Then 50 seeds were placed and 10 mL of distilled water was poured in each petri dishes and each was then covered with a thin and uniform layer of cotton. These were kept at 20-25°C for 10 days in a controlled growth chamber, where light/dark ratio was maintained as 10:14

in hours. In each set of experiments one petri dish without compound was used as control. After 10 days germinated seeds were counted and root and shoot lengths were measured.

IV.C.11 Data analysis:

The effects on germination and growth are given as percent differences from control, and they consists of the differences (in cm.) between mean values of seeds with tested compounds and mean values for control (seeds grown without addition of tested compounds)/ mean values for control × 100. Thus, zero represents the control, positive values represent stimulation of the studied parameter, and negative values represent inhibition. The data were evaluated applying Student's *t*-tests, using T-Test statistics calculator available on www.http://studentstest.com and the differences between the experiment and control were significant at a value of $P < 0.05$. Taking the values of germination, inhibition (or stimulation), in percentages, different graphs were plotted using origin software.

IV.C.12 Charecterization of the products

IV.C.12.1 Friedelin (25): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. White crystals, m. p. 262-263°C (Pet. ether- ethyl acetate), (lit.[152] 262-263°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.73 (s, 3H, Me-24), 0.87 (s, 3H, Me-25), 0.89 (s, 3H, Me-23), 0.95(s, 3H, Me-30), 1.00 (s, 6H, Me-26 and Me-29), 1.05 (s, 3H, Me-27), 1.18 (s, 3H, Me-28), 1.91- 2.02 (m, 1H, H-1), 2.20-2.47 (m, 3H, H-2 and H-4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 6.80 (C-23), 14.61 (C-24), 17.91 (C-25), 18.18 (C-7), 18.64 (C-27), 20.22 (C-26), 22.24 (C-1), 28.12 (C-20), 29.93 (C-17), 30.45 (C-12), 31.74 (C-29), 32.04 (C-28), 32.35 (C-21), 32.69 (C-15), 34.99 (C-30), 35.28 (C-19), 35.55 (C-11), 35.94 (C-16), 37.37 (C-9), 38.23 (C-14), 39.20 (C-22), 39.63 (C-13), 41.21 (C-6), 41.49 (C-2), 42.11 (C-5), 42.70 (C-18), 53.03 (C-8), 58.15 (C-4), 59.38 (C-10), 213.37 (C-3). FTIR (nujol, cm^{-1}): ν 1714, 1380, 1302, 1257, 1103, 1071, 1046, 1004, 795, 719.

IV.C.12.2 3β-Hydroxy friedelane (261a): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: 72%. White crystals, m. p. 290°C (Pet ether- ethyl acetate), (lit.[153] 290-291°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (s, 3H, Me-25), 0.93 (s, 3H, Me-24), 0.95 (d, *J*=1.2 Hz, 3H, Me-23), 0.96 (s, 3H, Me-30), 0.99 (s, 6H, Me-26 and Me-29), 1.00 (s, 3H, Me-27), 1.17 (s, 3H, Me-28), 1.75 (dt, *J*=12.8 and 3.2 Hz, 1H, H-6,), 3.75 (m, 1H, H-3). ^{13}C NMR (75 MHz,

$CDCl_3$): δ 11.63 (C-23), 15.76 (C-1), 16.39 (C-24), 17.52 (C-7), 18.24 (C-25), 18.66 (C-26), 20.12 (C-27), 28.17 (C-20), 30.00 (C-17), 30.62 (C-15), 31.78 (C-29), 32.07 (C-28), 32.29 (C-21), 32.76 (C-12), 35.03 (C-30), 35.13 (C-2), 35.31 (C-19), 35.52 (C-11), 36.04 (C-16), 37.06, 37.79 (C-9), 38.33 (C-13), 39.26 (C-22), 39.64 (C-14), 41.67 (C-5), 42.76 (C-6), 49.12 (C-4), 53.16 (C-8), 61.29 (C-10), 72.75 (C-3). FTIR (nujol, cm^{-1}): ν 3474, 1381, 1174, 1044, 999, 943, 916, 719.

IV.C.12.3 Friedel-2-ene (343): Eluent in column chromatography: petroleum ether. Yield: 7-16%. m.f. $C_{30}H_{50}$, white prism like crystals, m.p. 246°C (chloroform-methanol, lit.[90] 246-248°C). Though m. p. of the crystals was same to that of the literature, little impurities were found in the ^1H- as well as in ^{13}C-NMR spectra. ^1H NMR (300 MHz, $CDCl_3$): δ 0.75 (s, 3H, Me-24), 0.80 (s, 3H, Me-25), 0.86 (s, 3H, Me-23), 0.94 (s, 3H, Me-30), 1.03 (s, 3H, Me-26 and Me-29), 1.09 (s, 3H, Me-27), 1.15 (s, 3H, Me-28), 2.27 (d, *J*=13.8 Hz, 2H, H-1), 2.65 (d, *J*=10.5 Hz, 1H, H-4), 4.86 (s, 1H, H-2), 5.13-5.21 (m, 1H, H-3).^{13}C NMR (75 MHz, $CDCl_3$): δ 16.36, 17.80, 18.72, 18.79, 21.43, 21.69 (2), 23.83, 24.14, 25.17, 26.55, 32.38, 33.27, 33.34, 33.47, 34.61, 34.87, 35.53, 36.74, 37.66, 38.71, 39.47, 40.51, 41.29, 42.14, 44.77, 50.87, 56.50, 133.02 (C-2), 134.67 (C-3). FTIR (nujol, cm^{-1}): ν 1376, 1363, 1343, 1257, 1166, 1142, 1085, 1036, 967, 820, 803, 720.

IV.C.12.4 Friedel-3-enol-acetate (379): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 8-60%. m.f. $C_{32}H_{52}O_2$ white crystals, m.p. 262°C ($CHCl_3$-MeOH). ^1H NMR (300 MHz, $CDCl_3$): δ 0.85 (s, 3H, Me-25), 0.95 (s, 3H, Me-30), 1.00 (s, 6H, Me-26 and Me-29), 1.01 (s, 3H, Me-27), 1.02 (s, 3H, Me-24), 1.18 (s, 3H, 28-Me), 1.59 (s, 3H, Me-23), 2.12 (s, 3H, -CH_3 of -OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.55, 17.46, 18.16, 18.26, 18.67, 20.10, 20.69, 20.88, 28.20, 28.39, 30.07, 30.61, 31.83, 32.16, 32.39, 32.90, 35.04, 35.25, 35.42, 36.11, 37.08, 38.22, 38.39, 38.66, 39.31, 39.84, 42.95, 52.75, 56.10, 130.57 (C-4), 141.31 (C-3), 168.98 (>C=O of -OAc). FTIR (nujol, cm^{-1}): ν 667, 759, 1070, 1222, 1382, 1456, 1749 (>C=O of -OAc), 2337. Mass: 491.32 (M^++ Na^+), (33%), 489.31 (1008%), 413.22 (13%), 301.13 (14%), 149.01 (14%), 100.10 (12%). Analysis calcd: C, 81.99; H, 11. 18. Found: C, 81.56; H, 10.89.

IV.C.12.5 2-Ketofriedel-3-enol-acetate (380): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: 22-32%. m.f. $C_{32}H_{50}O_3$, yellowish white solid, m.p. 280-282°C

($CHCl_3$-MeOH) (lit.[154] 283-285°C). ^1H NMR (300 MHz, $CDCl_3$): δ 0.93 (s, 3H, Me-25) , 0.95 (s, 3H, Me-30), 0.99 (s, 6H, Me-26 and Me-29), 1.01 (s, 3H, Me-27), 1.16 (s, 3H, Me-24), 1.18 (s, 3H, Me-28), 1.76 (s, 3H, Me-23), 2.24 (s, 3H, CH_3 of -OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.46, 17.64, 17.95, 18.59, 19.11, 20.13, 20.37, 28.14, 29.99, 30.21, 31.76, 32.10, 32.19, 32.73, 34.04, 34.52, 35.01, 35.31, 35.88, 36.89, 37.83, 38.23, 39.22, 39.72, 40.54, 42.74, 52.41, 54.55, 140.87 (C-4), 157.02 (C-3), 168.58 (-CH_3 of -OAc), 191.89 (C-2). FTIR (neat, cm^{-1}): ν 752, 916, 1006, 1089, 1219, 1325, 1383, 1462, 1523, 1682, 1761, 2862, 2929. Mass: 483.25 (M+1), (32%), 465.26 (65%), 413.16 (47%), 315.09 (51%), 301.08 (100%), 245.03 (27%), 152.09 (12%), 148.99 (68%). Analysis calcd: C, 79.62; H, 10.44. Found: C, 79.78; H, 10. 52.

IV.C.12.6 3β-Acetoxy friedelane (316): Eluent in column chromatography: 1% ethyl acetate in petroleum ether. Yield: 30%. m.f. $C_{32}H_{54}O_2$ m.p. white crystals, m. p. 298°C (chloroform-methanol) (lit.[153] 299°C) ^1H NMR (300 MHz, $CDCl_3$): δ 0.75 (s, 3H, Me-24), 0.77 (s, 3H, Me-25), 0.82 (s, 3H, Me-23), 0.95 (s, 3H, Me-30), 0.99 (s, 6H, Me-26, Me-29), 1.01 (s, 3H, Me-27), 1.17 (s, 3H, Me-28), 1.78 (dt, *J*=12.8 and 3.2 Hz, 1H, H-6), 2.01 (s, 3H, -$OCOCH_3$), 2.03- 2.12 (m, 3H, H-2 and H-4), 4.62 (m, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.93 (C-23), 14.50 (C-24), 17.85 (C-25), 18.12 (C-7), 18.65 (C-27), 19.33 (-CH_3 of -OAc), 20.16 (C-26), 21.36 (C-1), 28.18 (C-20), 30.01 (C-17), 30.57 (C-12), 31.80 (C-29), 32.10 (C-28), 32.35 (C-15), 32.71 (C-2), 32.80 (C-21), 35.02 (C-30), 35.32 (C-19), 35.53 (C-11), 36.05 (C-16), 37.00 (C-9), 38.30 (C-14), 38.39 (C-5), 39.27 (C-22), 39.68 (C-13), 41.33 (C-6), 42.81 (C-18), 50.03 (C-4), 52.98 (C-8), 59.89 (C-10), 75.19 (C-3), 170.99 (>C=O of -OAc). FTIR (nujol, cm^{-1}): ν 1736, 1379, 1049, 1019, 972, 754, 721. Analysis calcd: C, 81.64; H, 11.56. Found: C, 81.25; H, 11.13.

IV.C.12.7 Lactam (365): Eluent in column chromatography: 35% ethyl acetate in petroleum ether. Yield: 67%. m.f. $C_{30}H_{52}NO$, yellow amorphous solid, m.p. 266-267°C (lit[108] 266-268°C), ^1H NMR (300 MHz, $CDCl_3$): δ 0.81 (s, 3H, Me-24), 0.81 (s, 3H, Me-25), 0.95 (s, 3H, Me-30), 0.98 (s, 3H, Me-23), 0.99 (s, 3H, Me-29), 1.00 (s, 3H, Me-26), 1.04 (d, *J*=6.6Hz, 3H, Me-27), 1.17 (s, 3H, Me-28), 1.73 (brs, 1H, H-10), 2.38 (m, 2H, H-2), 3.26 (q, *J*=6.6 Hz, 1H, H-4), 5.28 (brs, 1H, N-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.88 (C- 24), 16.03 (C-23), 17.95 (C-25), 18.35 (C-1), 18.44 (C-7), 18.58 (C-26), 20.21 (C-27), 28.17 (C-20), 29.99 (C-17), 30.66 (C-12), 31.75 (C-29), 32.04 (C-15), 32.40 (C-28), 32.76 (C-21), 35.03 (C-30), 35.29 (C-11), 35.50 (C-19),

36.01 (C-16), 36.52 (C-2), 38.43 (C-9), 38.51 (C-14), 39.24 (C-22), 39.27 (C-5 and C-6), 39.86 (C-13), 42.72 (C-18), 52.77 (C-8), 59.02 (C-4), 65.24 (C-10), 177.07 (C-3). FTIR (nujol, cm^{-1}): ν 2928, 2867, 1672 (C=O), 1458 (N-H), 1362 (C-N), 734 (N-H).

IV.C.12.8 4α-Acetoxyfriedel-3-one (381): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 12-41%. m.f. $C_{32}H_{52}O_3$ white crystals, m.p. 174-176°C ($CHCl_3$-MeOH). ^1H NMR (300 MHz, $CDCl_3$): δ 0.80 (s, 3H, Me-25), 0.86 (s, 3H, Me-24), 0.96 (s, 3H, Me-30), 1.00 (s, 6H, Me-26 and Me-29), 1.07 (s, 3H, Me-27), 1.18 (s, 3H, Me-28), 1.30 (s, 3H, Me-23), 1.93- 2.02 (m, 1H, H-1), 2.26-2.52 (m, 2H, H-2), 2.14 (-CH_3 of -OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.64, 15.68, 17.95, 18.13, 18.73, 20.19, 21.50, 22.98, 28.20, 30.03, 30.55, 31.84, 32.12, 32.38, 32.85, 34.29, 35.00, 35.34, 35.95, 36.05, 37.29, 38.03, 38.32, 39.28, 39.70, 42.88, 46.05, 50.34, 52.47, 89.09 (C-4), 170.11 (-CH_3 of -OAc), 208.30 (C-3). FTIR (nujol, cm^{-1}): ν 722, 1119, 1245, 1377, 1508, 1736, 2345. Analysis calcd: C, 79.29; H, 10.81. Found: C, 79.52; H, 10.63.

IV.C.12.9 4β-Acetoxyfriedel-3-one (382): Eluent in column chromatography: 5% ethyl acetate in petroleum ether. Yield: 35%. m.f. $C_{32}H_{52}O_3$, white crystals, m.p. 228-230°C ($CHCl_3$-MeOH). ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (s, 3H, Me-25), 0.91 (s, 3H, Me-24), 0.96 (s, 3H, Me-30), 1.00 (s, 3H, Me-26), 1.01 (s, 3H, Me-29), 1.05 (s, 3H, Me-27), 1.18 (s, 3H, Me-28), 1.84 (s, 3H, Me-23), 2.08 (-CH_3 of -OAc), 2.29-2.40 (m, 1H, H_α-2), 2.51-2.59 (m, 1H, H_β-2) ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.02, 17.64, 17.99, 18.73, 19.18, 20.33, 21.71, 22.84, 28.18, 30.02, 30.49, 31.76, 32.12, 32.49, 32.80, 33.22, 35.05, 35.39, 36.02, 36.08, 37.40, 37.45, 38.34, 39.29, 39.73, 42.85, 47.00, 51.98, 53.23, 89.83 (C-4), 169.69 (-CH_3 of -OAc), 205.79 (C-3). FTIR (nujol, cm^{-1}): ν 1755, 1723, 1462, 1377, 1235, 1192, 1118, 1090, 1017, 605, 500. Analysis calcd: C, 79.29; H, 10.81. Found: C, 79.71; H, 10.25.

IV.C.12.10 4α-Acetoxy-3β-hydroxy friedelane (383): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 27%. m.f. $C_{32}H_{54}O_3$, pale yellow solid, m.p. 230°C. ^1H NMR (300 MHz, $CDCl_3$): δ 0.83 (s, 3H, Me-25), 0.94 (s, 3H, Me-30), 0.99 (s, 6H, Me-26 and Me-29), 1.14 (d, *J*= 1.2 Hz, 3H, Me-27), 1.15 (s, 3H, Me-24), 1.16 (s, 3H, Me-28), 1.20 (d, *J*=1.5 Hz, 3H, Me-23), 1.75-1.78 (m, 1H, H-10), 2.07 (q, *J*=1.5 Hz, -CH_3 of -OAc), 2.15 (t, *J*=3.9 Hz,

H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.18 (C-23), 15.25 (C-24), 17.70 (C-25), 17.82 (C-1), 18.04 (C-7), 18.71 (C-27), 20.17 (C-26), 21.02 (CH_3 of -OAc), 27.86 (C-6), 28.15 (C-20), 30.00 (C-17), 30.49 (C-12), 31.76 (C-29), 32.10 (C-28), 32.33 (C-15), 32.77 (C-21), 35.05 (C-30), 35.34 (C-11), 35.52 (C-16), 36.00 (C-22), 36.46 (C-19), 36.66 (C-9), 38.27 (C-14), 38.36 (C-5), 39.25 (C-2), 39.70 (C-13), 42.78 (C-18), 48.10 (C-8), 52.36 (C-10), 71.65 (C-3), 88.05 (C-4), 169.51 (>C=O of -OAc). FTIR (nujol, cm^{-1}): ν 615,722, 838, 1041, 1148, 1213, 1241, 1377, 1560, 1654, 1742. Analysis calcd: C, 78.96; H, 11.18. Found: C, 78.13; H, 10.93.

IV.C.12.11 3β,4α-Diacetoxy friedelane (384): Eluent in column chromatography: petroleum ether. Yield: 95%. m.f. $C_{34}H_{56}O_4$, white solid, m.p. 209-210°C. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (s, 3H, Me-25), 0.91 (s, 3H, Me-25), 0.95 (s, 3H, Me-30), 1.00 (s, 3H, Me-30), 1.06 (d, *J*=1.8 Hz, 3H, Me-27), 1.18 (s, 3H, Me-28), 1.26 (s, 3H, Me-23), 2.07 (s, 3H, -CH_3 of -$OCOCH_3$), 2.08 (s, 3H, -CH_3 of -$OCOCH_3$), 2.90-3.06 (m, 1H, H-3). FTIR (nujol, cm^{-1}): ν 1749, 1734, 1717, 1459, 1375, 1233, 1112, 1014, 955, 800, 719, 599.

IV.C.12.12 4α-Hydroxy friedelan-3-one (357c): Eluent in column chromatography: 10% ethyl acetate in petroleum ether. Yield: 84%. m.f. $C_{30}H_{50}O_2$, needle shaped crystals, m.p. 268-270°C (lit.[106] 269-271°C). ^1H NMR (300 MHz, $CDCl_3$): δ 0.81 (s, 3H, Me-25), 086 (s, 3H, Me-24), 0.95 (s, 3H, Me-30), 0.99 (s, 6H, Me-26 and Me-29), 1.06 (d, *J*=3.9 Hz, 3H, Me-27), 1.16 (s, 3H, Me-23), 1.17 (s, 3H, Me-28), 2.11 (d, *J*=12.6 Hz, 1H, H-10), 2.22 (d, *J*= 14.1 Hz, 1H, H-2), 2.95 (m, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.74 (Me-23), 16.91 (Me-24),17.86 (C-1), 18.02 (Me-25), 18.77 (Me-27), 20.29 (Me-26), 21.72 (C-7), 28.21 (C-20), 30.06 (C-17), 30.57 (C-12), 31.79 (Me-29), 32.13 (Me-28), 32.51(C-15), 32.86 (C-21), 33.50 (C-19), 35.05 (Me-30), 35.42 (C-11), 35.83 (C-16), 36.09 (C-22), 37.08 (C-6), 37.25 (C-9), 38.33 (C-14), 39.31 (C-2), 39.77 (C-13), 42.89 (C-18), 44.63 (C-5), 49.38 (C-8), 52.45 (C-10), 81.08 (C-4), 212.92 (C-3). FTIR (nujol, cm^{-1}): ν 3447, 1702, 1377, 1108, 1068, 746, 722. Analysis calcd: C, 81.39; H, 11.38. Found: C, 88.95; H, 11.63.

IV.C.12.13 4α-Hydroxy friedelane-3-oxime (385): Yield: 88%. m.f. $C_{30}H_{51}NO_2$, pale yellow solid. ^1H NMR (300 MHz, DMSO-d6): δ 0.73 (s, 3H, Me-24), 0.81 (s, 3H, Me-25), 0.96 (s, 3H, Me-30), 0.99 (s, 6H, Me-26 and Me-29), 1.12 (s, 3H, Me-27), 1.17 (s, 3H, Me-28), 1.26 (s, 3H,

Me-23), 1.70-2.01 (m, 3H, H-2 and H-10), 4.39 (br s, 1H, -OH), 10.38 (br s, 1H, -NOH). ^{13}C NMR (75 MHz, DMSO-d6): δ 17.16, 18.12, 18.67, 18.93, 19.85, 20.36, 28.32, 30.06, 30.59, 32.16, 32.35, 32.99, 33.75, 35.24, 35.80, 36.11, 36.97, 38.25, 39.50, 39.77, 40.05, 40.33, 40.61, 40.89, 42.90, 43.47, 49.44, 52.37, 76.62 (C-4), 161.14 (C-3). FTIR (nujol, cm^{-1}): ν 3442, 3301, 2723, 1378, 1302, 1178, 1045, 992, 944, 919, 769, 725, 572.

IV.C.12.14 Cyclic amine 387: Eluent in column chromatography: methanol. Yield: 63%. m.f. $C_{30}H_{53}N$, white crystalline solid, m.p. 228°C (aqueous ethanol). ^1H NMR (300 MHz, $CDCl_3$): δ 0.84 (s, 3H, Me-24), 0.87 (s, 3H, Me-25), 0.92 (d, *J*= 3 Hz, 3H, Me-23), 0.94 (s, 3H, Me-30), 0.97 (s, 3H, Me-27), 0.99 (s, 3H, Me-26), 1.00 (s, 3H, Me-29), 1.17 (s, 3H, Me-28), 1.79-1.86 (m, 1H, H_β-3), 2.13 (dd, J=12 Hz, 6 Hz; 1H, H-4), 2.71-2.81 (m, 1H, H_α-3), 3.07-3.14 (m, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.94 (C-24), 17.55 (C-23), 17.76 (C-25), 18.30 (C-7), 18.69 (C-27), 20.22 (C-26), 21.08 (C-1), 28.18 (C-20), 28.99 (C-2), 30.04 (C-17), 30.57 (C-12), 31.81 (C-29), 32.09 (C-28), 32.43 (C-15), 32.87 (C-21), 34.88 (C-11), 35.01 (C-30), 35.33 (C-19), 36.16 (C-16), 38.38 (C-14), 38.73 (C-9), 39.28 (C-22), 39.46 (C-13), 40.24 (C-6), 41.67 (C-5), 42.85 (C-18), 47.94 (C-3), 52.78 (C-8), 60.28 (C-10), 70.52 (C-4). FTIR (nujol, cm^{-1}): ν 669, 786, 1145 (br., C-N str.), 1384, 1510, 1558, 1651 (N-H, def.), 2337, 2362, 3340, 3423 (N-H, str.). Analysis calcd: C, 84.24; H, 12.49. Found: C, 83.93; H, 12.25.

IV.C.12.15 2α-Acetoxy friedelan-3-one (388): Eluent in column chromatography: 2% ethyl acetate in petroleum ether. Yield: 78%. m.f. $C_{32}H_{52}O_3$, white solid, m.p. 268-269°C (lit.[90] 269.5-271°C). ^1H NMR (300 MHz, $CDCl_3$): δ 0.71 (s, 3H, Me-24), 0.84 (s, 3H, Me-25), 0.89 (d, *J*= 6.6 Hz, 3H, Me-23), 0.96 (s, 3H, Me-30), 1.00 (s, 6H, Me-26 and Me-29), 1.07 (s, 3H, Me-27), 1.18 (s, 3H, Me-28), 2.13 (s, 3H, $-CH_3$ of $-COCH_3$), 2.67 (q, *J*= 6.6 Hz, 1H, H-4), 4.94 (d, *J*= 3.3 Hz, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 6.50, 14.13, 17.91, 18.28, 18.63, 20.14, 21.12, 28.19, 28.26, 30.04, 30.38, 31.77, 31.89, 32.16, 32.41, 32.90, 34.97, 35.35, 35.58, 36.09, 36.99, 38.41, 39.28, 39.77, 41.14, 42.93, 43.24, 53.10, 53.40, 54.47, 169.69 ($-CH_3$ of -OAc), 207.98 (C-3). FTIR (nujol, cm^{-1}): ν 1744, 1720, 1377, 1365, 1257, 1229, 1184, 1164, 1041, 1023, 997, 933, 795, 758, 722. Analysis calcd: C, 79.29; H, 10.81. Found: C, 78.92; H, 11.23.

IV.C.12.16 2α-Hydroxy friedelan-3-oxime (389): Yield: 95%. m.f. $C_{30}H_{51}NO_2$, pale yellow plate like crystals, m.p. 265-271°C (EtOH) (lit.[5] 266-272°C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.71 (s, 3H, Me-24), 0.74 (s, 3H, Me-25), 0.84 (d, *J*=2.7 Hz, 3H, Me-23), 0.95 (s, 3H, Me-30), 1.00 (s, 3H, Me-26 and Me-29), 1.03 (s, 3H, Me-27), 1.18 (s, 3H, Me-28), 1.92-2.10 (m, 2H, H-2), 2.50-2.58 (m, 1H, H-4), 3.62- 3.80 (m, 1H, H-2). FTIR (nujol, cm^{-1}): ν 3286, 3168, 2724, 1380, 1261, 1147, 1093, 1023, 943, 801, 724.

IV.C.12.17 2α,3β-Dihydroxy friedelane (320): Eluent in column chromatography: 50% ethyl acetate in petroleum ether. Yield: 68%. m.f. $C_{30}H_{52}O_2$, white crystals, m.p. 288-290°C (ethyl acetate, lit[90] 287-290°C), ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (s, 3H, Me-24), 0.88 (s, 3H, Me-25), 0.93 (s, 3H, Me-23), 0.95 (s, 3H, Me-30), 0.99 (s, 3H, Me-26), 1.01 (s, 3H, Me-29), 1.07 (s, 3H, Me-27), 1.17 (s, 3H, Me-28), 3.55 (s, 1H, H-2), 3.99 (d, *J*=2.7 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.90, 15.93, 17.54, 18.17, 18.74, 20.15, 23.96, 28.19, 30.05, 30.63, 31.80, 32.14, 32.36, 32.84, 35.05, 35.37, 35.52, 36.09, 36.55, 37.82, 38.42, 39.30, 39.73, 41.41, 42.87, 43.71, 52.31, 53.27, 71.43 (C-2), 77.22 (C-3). FTIR (nujol, cm^{-1}): ν 3443, 3300, 2724, 1377, 1301, 1179, 1045, 992, 943, 918, 768, 724, 572. Analysis calcd: C, 81.02; H, 11.79. Found: C, 81.32; H, 11.35.

IV.C.12.18 2α,3β-Diacetoxy friedelane (390): Eluent in column chromatography: 1% ethyl acetate in petroleum ether. Yield: 97%. m.f. $C_{34}H_{56}O_4$, white crystals, m.p. 226-228°C (lit[90] 226-227°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (s, 3H, Me-24), 0.88 (s, 3H, Me-25), 0.91 (s, 3H, Me-23), 0.94 (s, 3H, Me-30), 0.99 (s, 3H, Me-26 and Me-29), 1.01 (s, 3H, Me-27), 1.17 (s, 3H, Me-28), 2.06 (s, 3H, -CH_3 of -OCOCH_3), 2.08 (s, 3H, -CH_3 of -OCOCH_3), 4.02-4.4.33 (m, 1H, H-2), 4.84 (dd, *J*=21.3 Hz and 2.4 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.49, 15.32, 17.67, 18.04, 18.52, 20.06, 21.00, 21.73, 21.29, 28.18, 30.07, 30.41, 31.47, 31.87, 32.14, 32.34, 32.91, 34.97, 35.43, 36.13, 36.50, 37.55, 38.45, 39.29, 39.70, 41.36, 42.93, 43.86, 53.04, 53.17, 70.90, 73.97, 169.63 (-CH_3 of C_2-OAc), 169.95 (-CH_3 of C_3-OAc). FTIR (nujol, cm^{-1}): ν 1734, 1717, 1464, 1376, 1243, 1227, 1201, 1032, 965, 891, 719.

IV.D Conclusion: A new reaction protocol has been accomplished, to use as the key transformative reaction, to prepare a library of friedelane triterpenoids. The key route of the

derivatizations is the one-pot $BF_3.OEt_2$/ Ac_2O-mediated oxidative transformation of friedelin (**25**) into friedel-2-ene (**343**), friedel-3-enol acetate (**379,** the major product) and 2-keto-friedel-3-enol acetate (**380**). Then, a number of common reaction strategies, e.g., oxidation, reduction, acetylation, oximination, etc., were employed to achieve a number of C3- and C4-functionalised friedelane derivatives. Besides, the same reaction protocol was also applied on friedelin oxime (**310**) and 3β-hydroxy friedelane (**261a**). Moreover, cerin (**26,** structurally 2α-hydroxy friedelan-3-one) was isolated and then some of its simple transformative reactions were carried out to prepare sets of compounds which are actually isomeric to some of the prepared friedelane triterpenoids. The friedelane derivatives were then biologically evaluated as their anticipated phytotoxicity taking *Oriza sativa* as the targeted entity. The sets of the isomeric compounds were very much helpful too to analyze the structure activity relationship studies (SAR) during the evaluation of the phytotoxic effect of the compounds. The phytotoxicity studies revealed the potential activity of the synthesized derivatives in comparison to the starting natural products which, indeed, imply presumably, the scope of the compounds to possess enhanced useful cytotoxicity and to use as agrochemicals.

IV.E Supporting spectra (NMR, Mass):

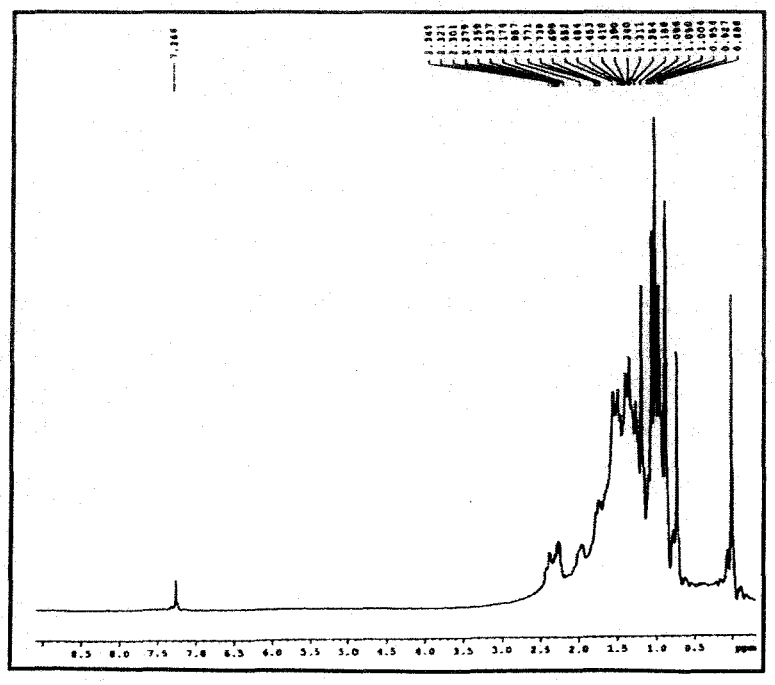


Figure 29. ^1H NMR spectrum of friedelin (**25**).

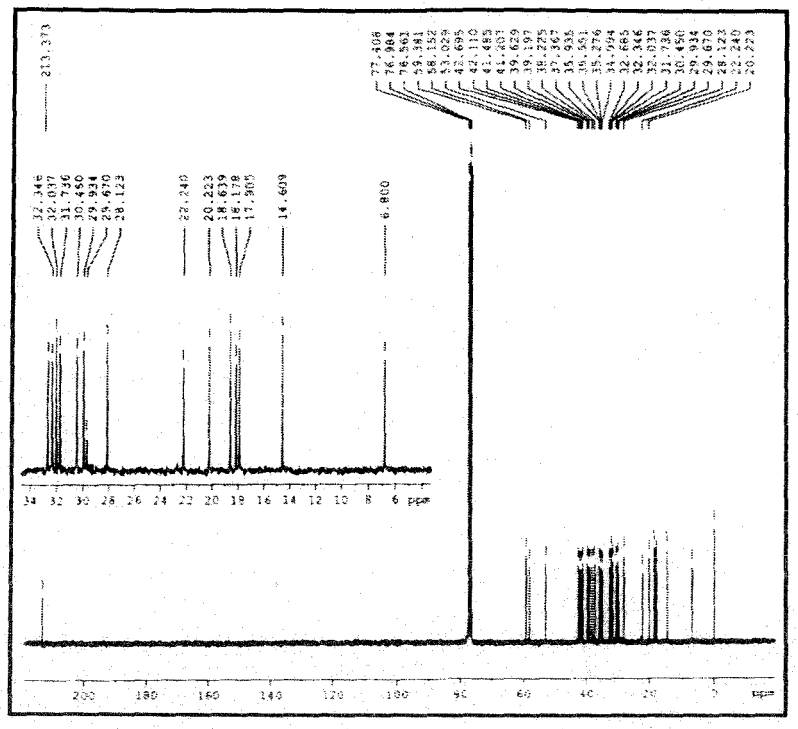


Figure 30. ^{13}C NMR spectrum of friedelin (**25**).

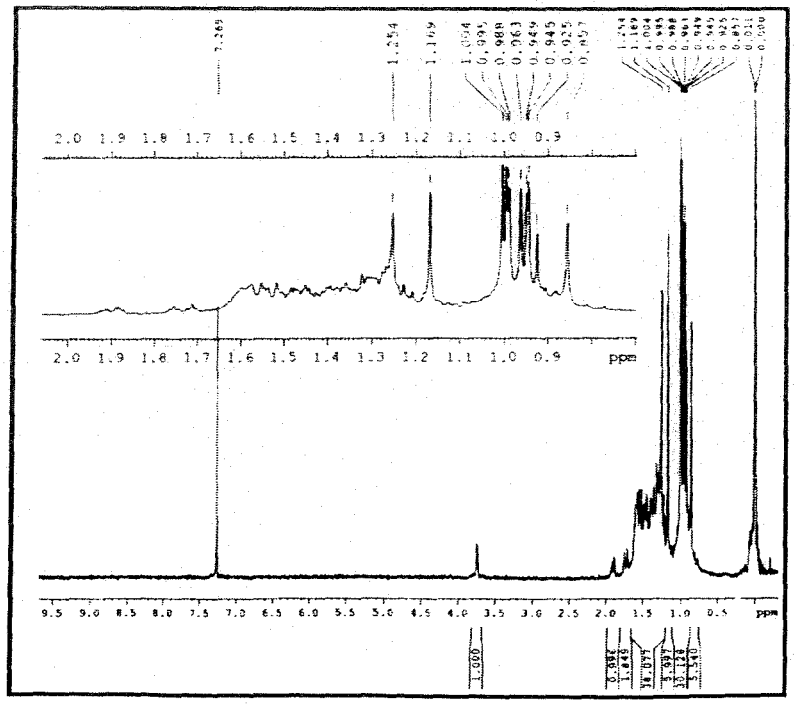


Figure 31. ^{1}H NMR spectrum of 3β-hydroxy friedelane (**261a**).

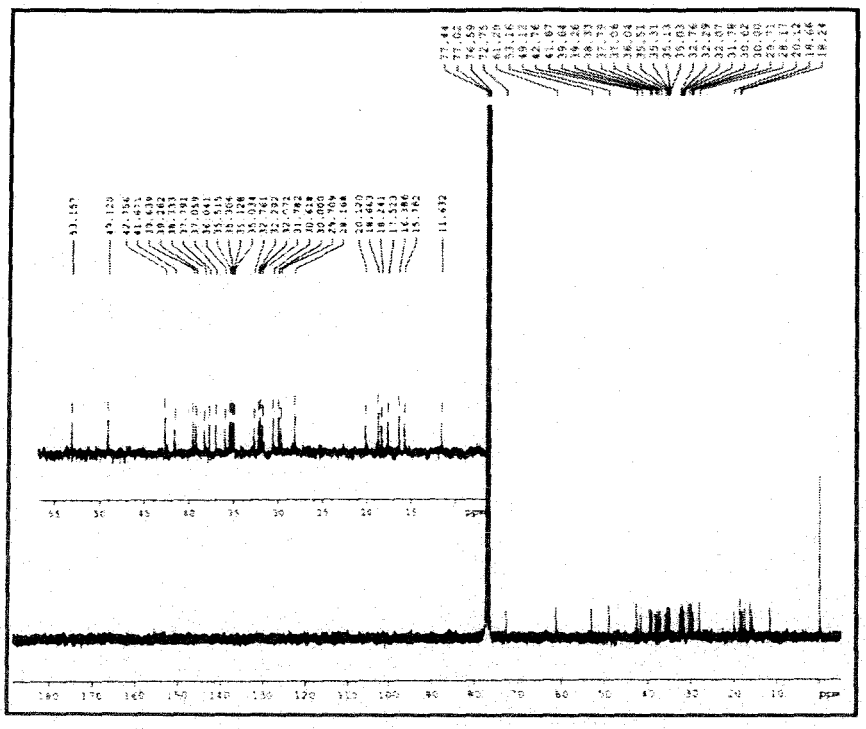


Figure 32. ^{13}C NMR spectrum of 3β-hydroxy friedelane (**261a**).

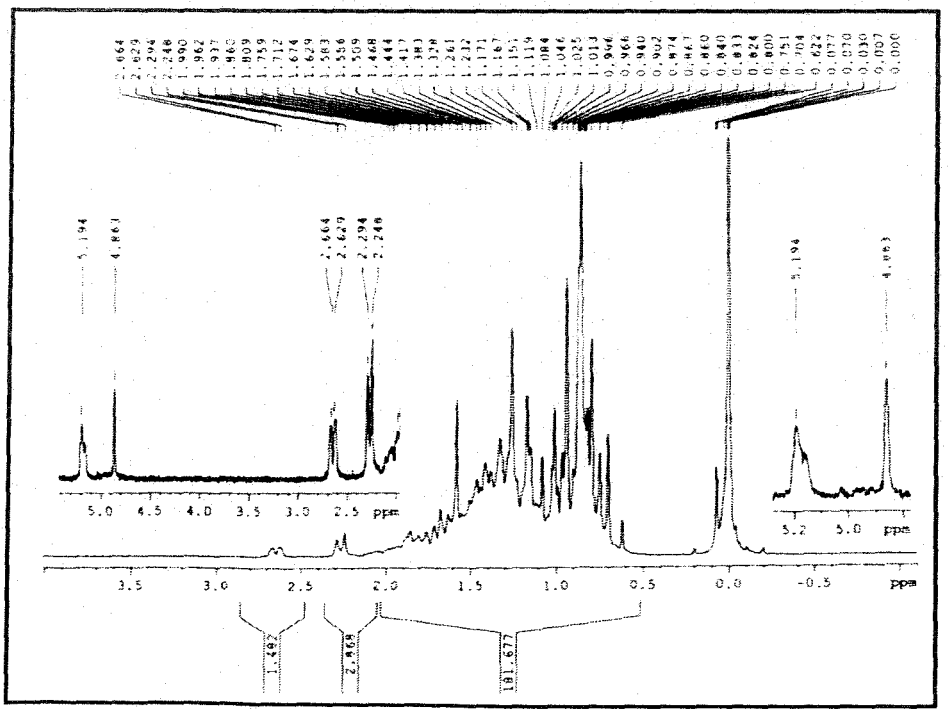


Figure 33. ^{1}H NMR spectrum (with little mixture) of friedel-2-ene (**343**).

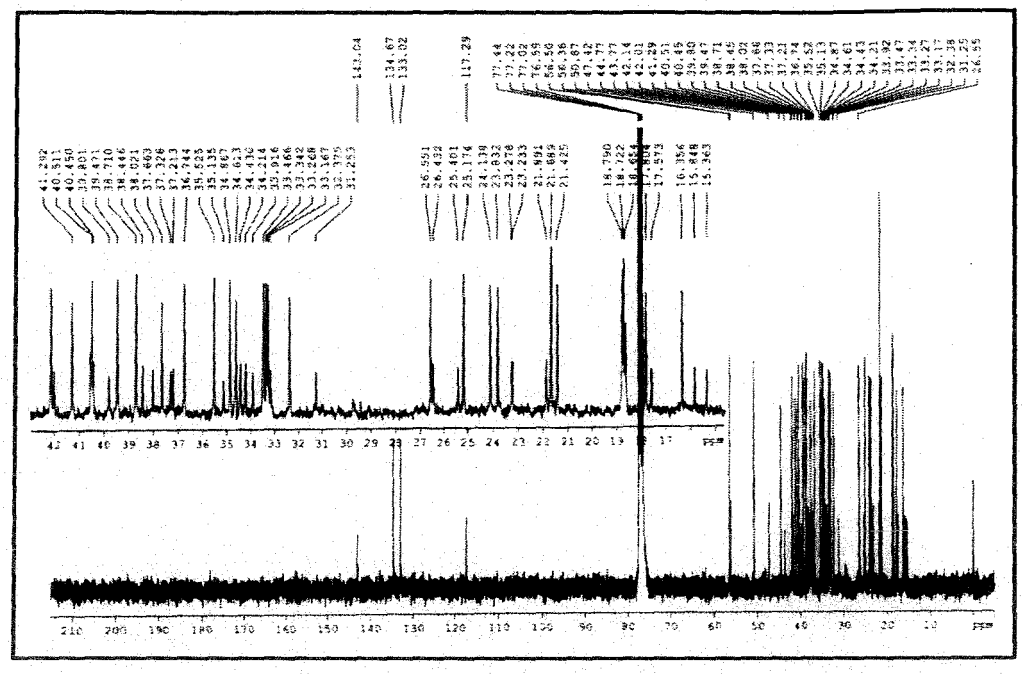


Figure 34. ^{13}C NMR spectrum (with little mixture) of friedel-2-ene (**343**).

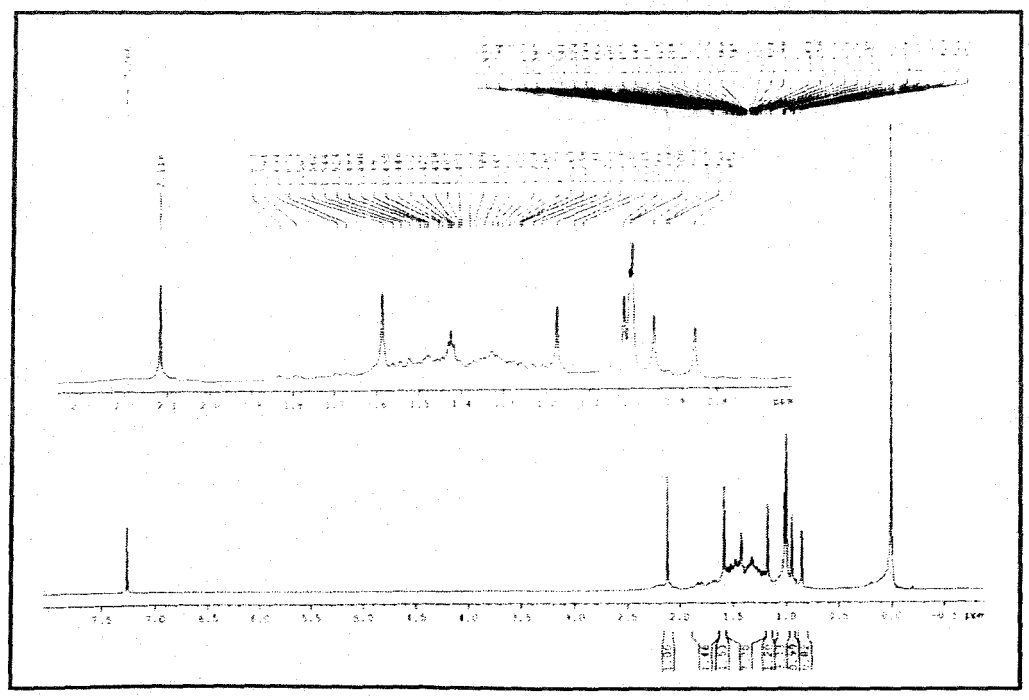


Figure 35. ^{1}H NMR spectrum of friedel-3-enol acetate (**379**).

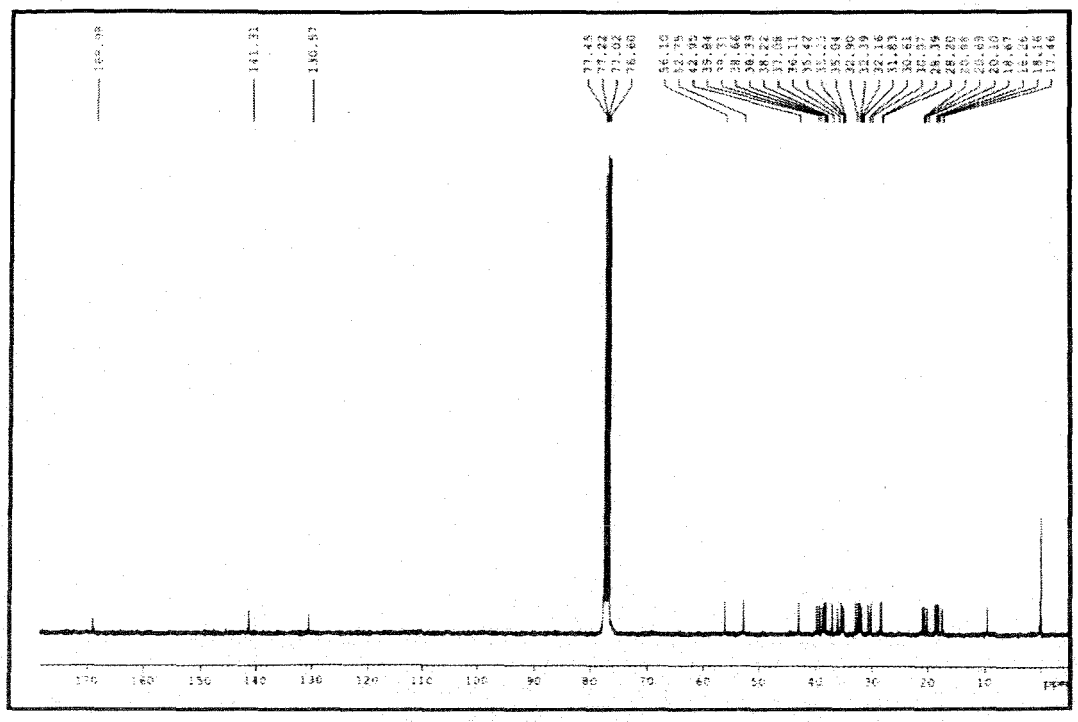


Figure 36. ^{13}C NMR spectrum of friedel-3-enol acetate (**379**).

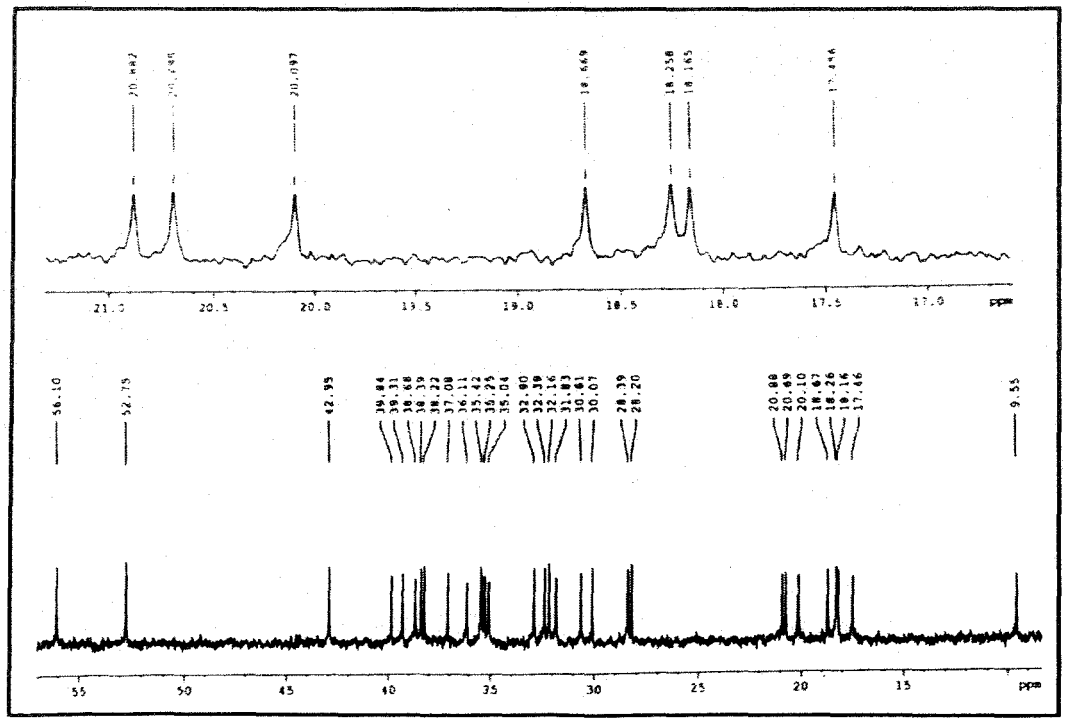


Figure 37. ^{13}C NMR spectrum (partially expanded) of friedel-3-enol acetate (**379**).

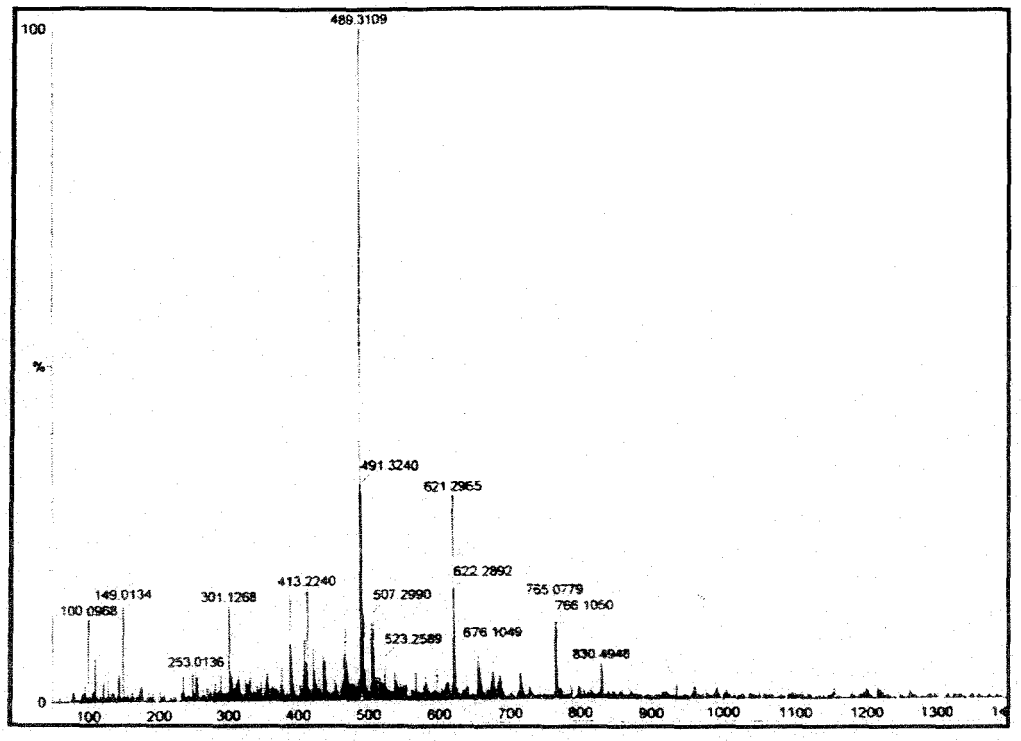


Figure 38. Mass spectrum of friedel-3-enol acetate (**379**).

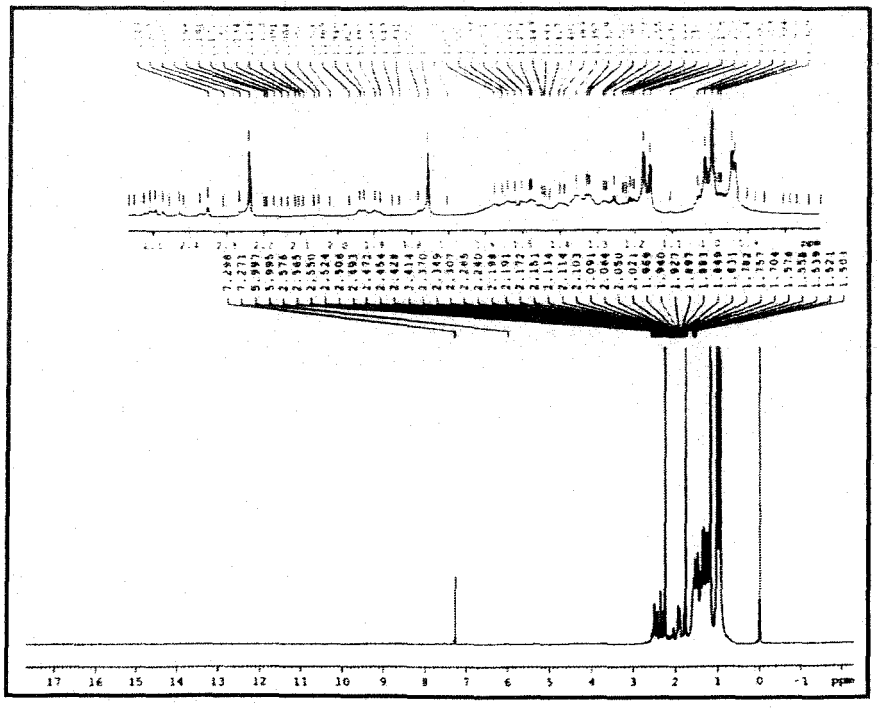


Figure 39. ^{1}H NMR spectrum of friedel-2-keto-3-enol acetate (**380**).

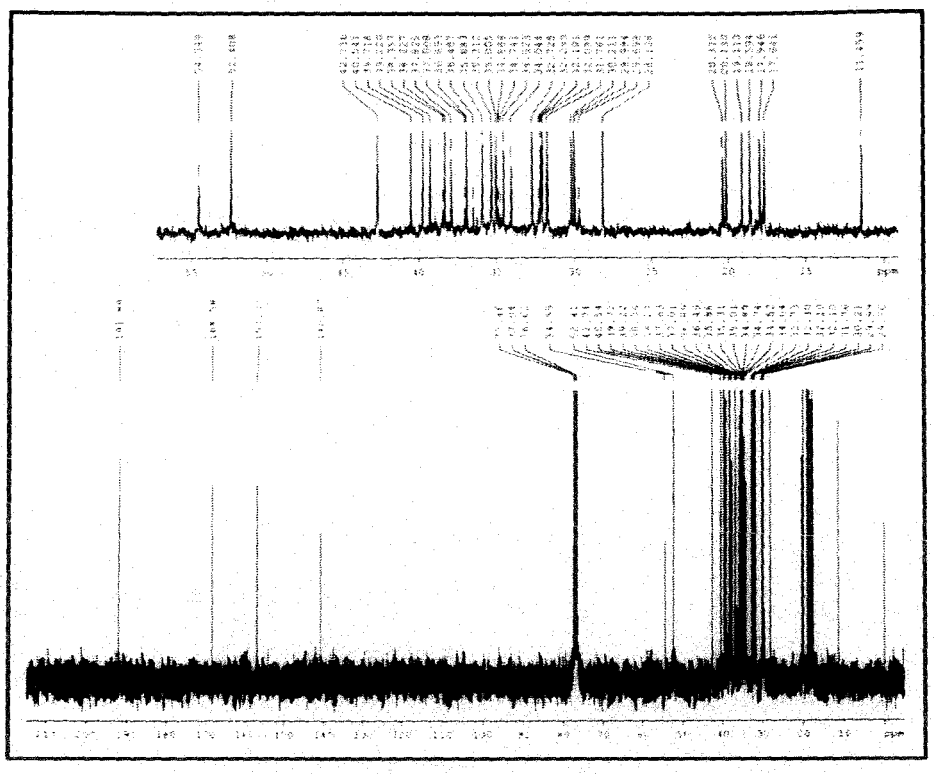


Figure 40. ^{13}C NMR spectrum of friedel-2-keto-3-enol-acetate (**380**).

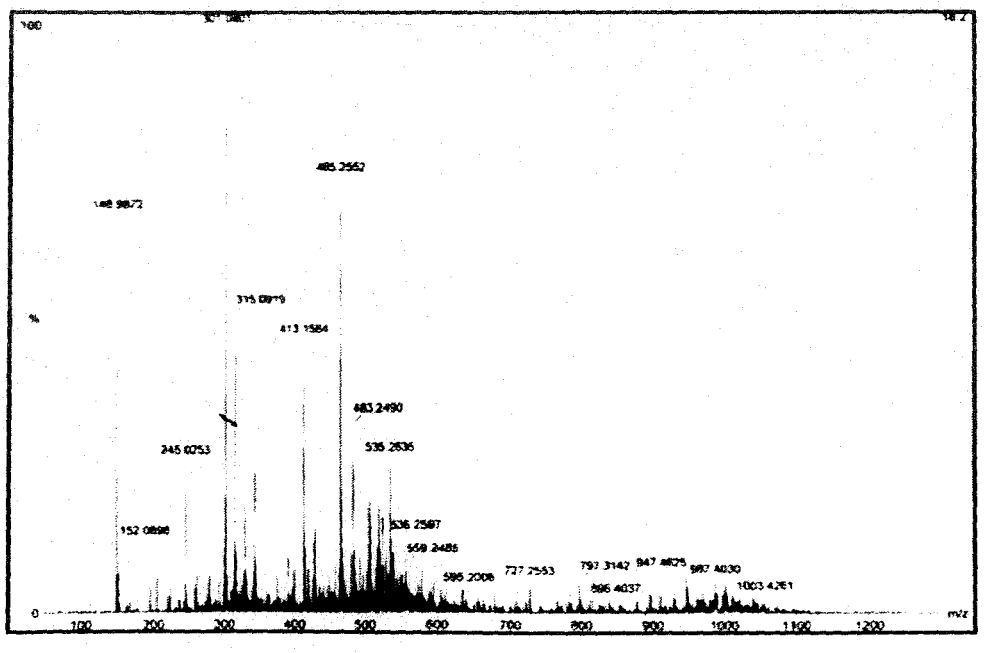


Figure 41. Mass spectrum of friedel-2-keto-3-enol acetate (**380**).

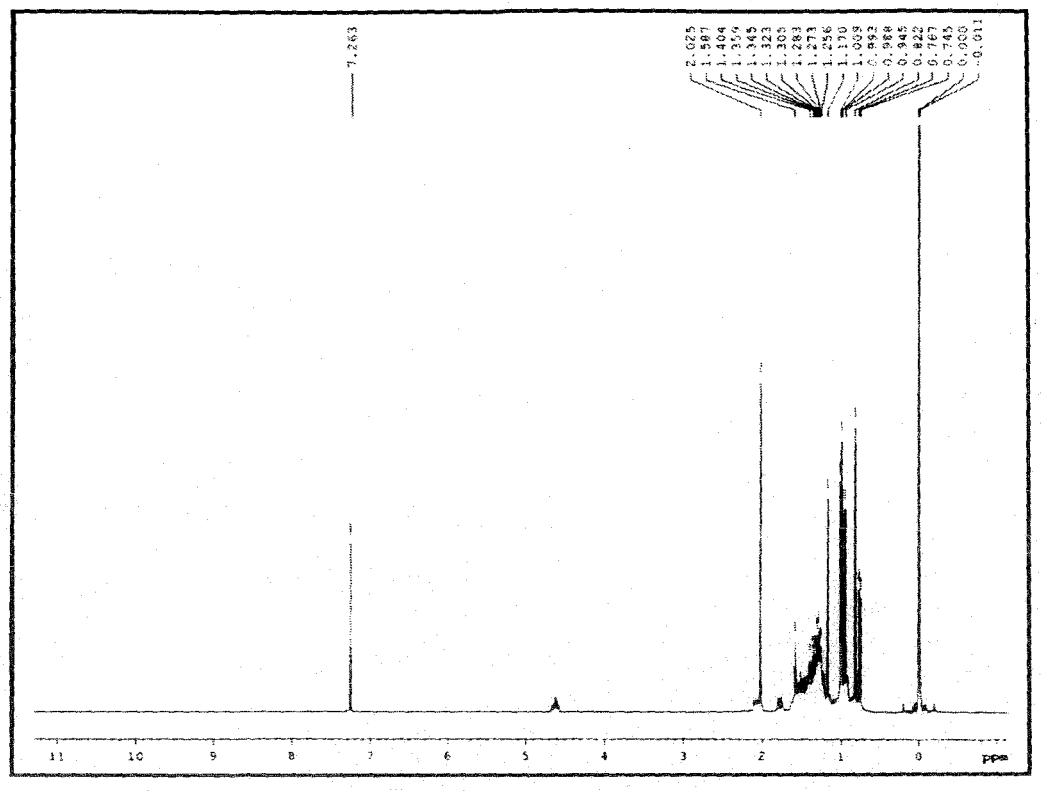


Figure 42. ^{1}H NMR spectrum of 3β-acetoxy friedelane (**316**).

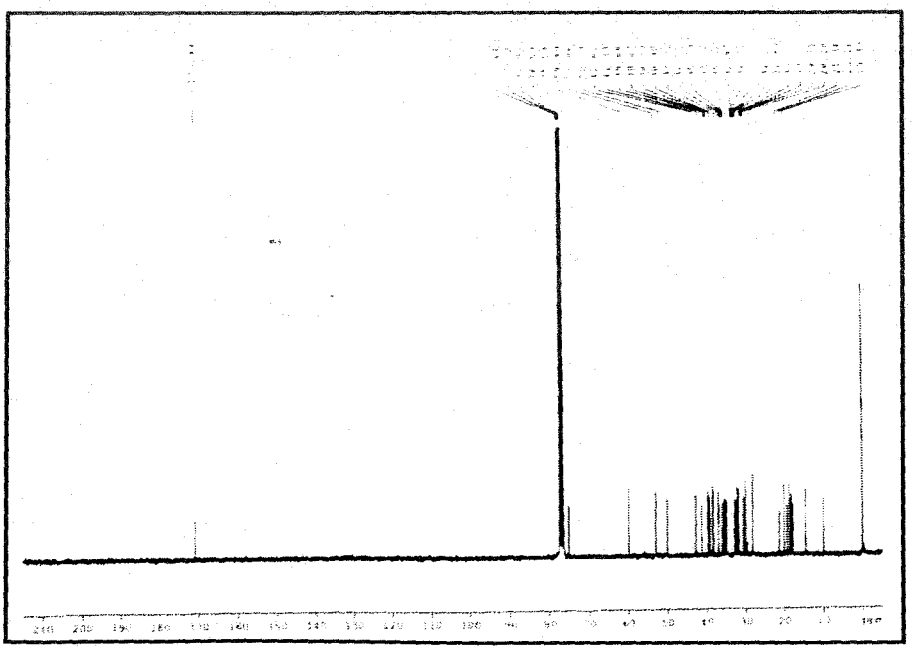


Figure 43. ^{13}C NMR spectrum of 3β-acetoxy friedelane (**316**).

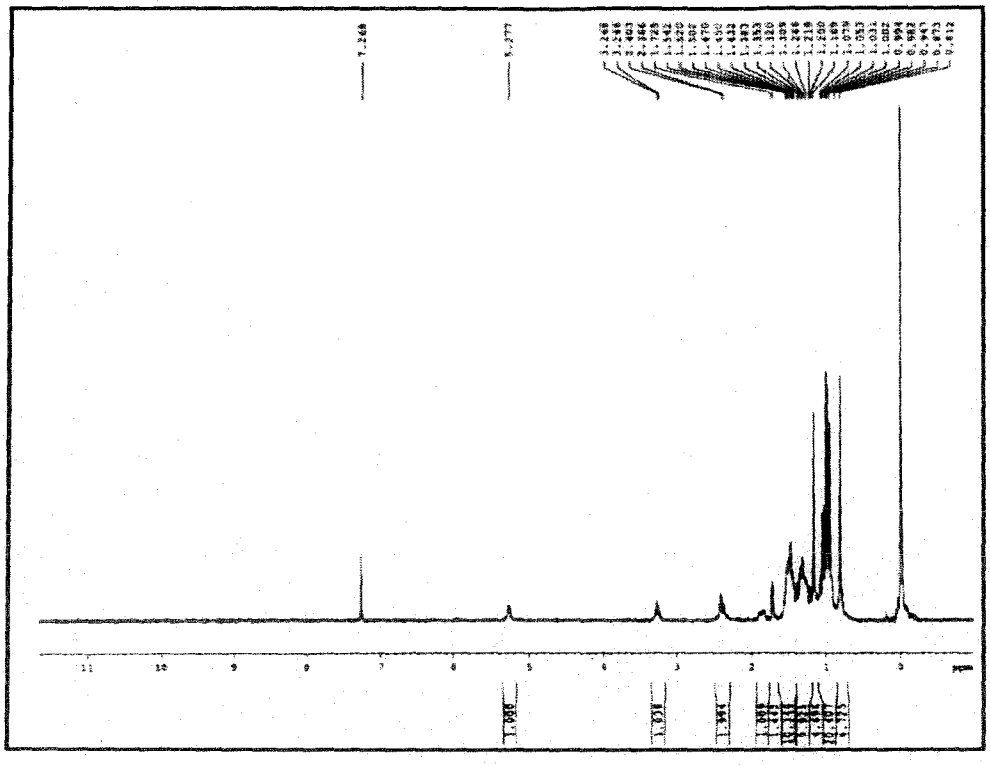


Figure 44. ^{1}H NMR spectrum of lactam **365**.

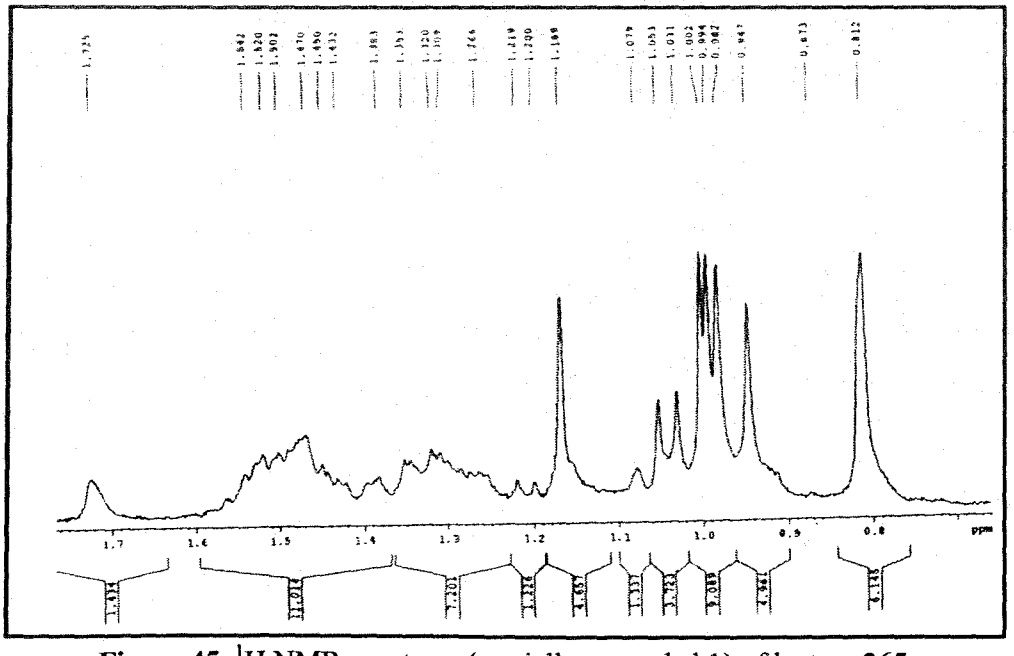


Figure 45. ^{1}H NMR spectrum (partially expanded 1) of lactam **365**.

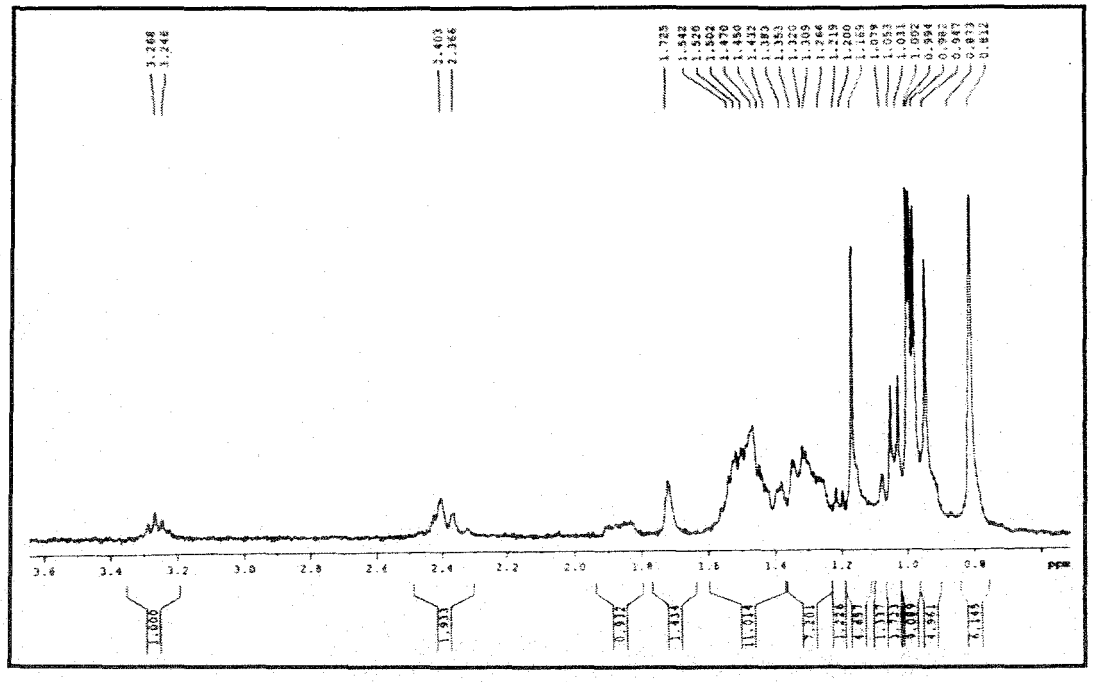


Figure 46. ^{1}H NMR spectrum (partially expanded 2) of lactam **365**.

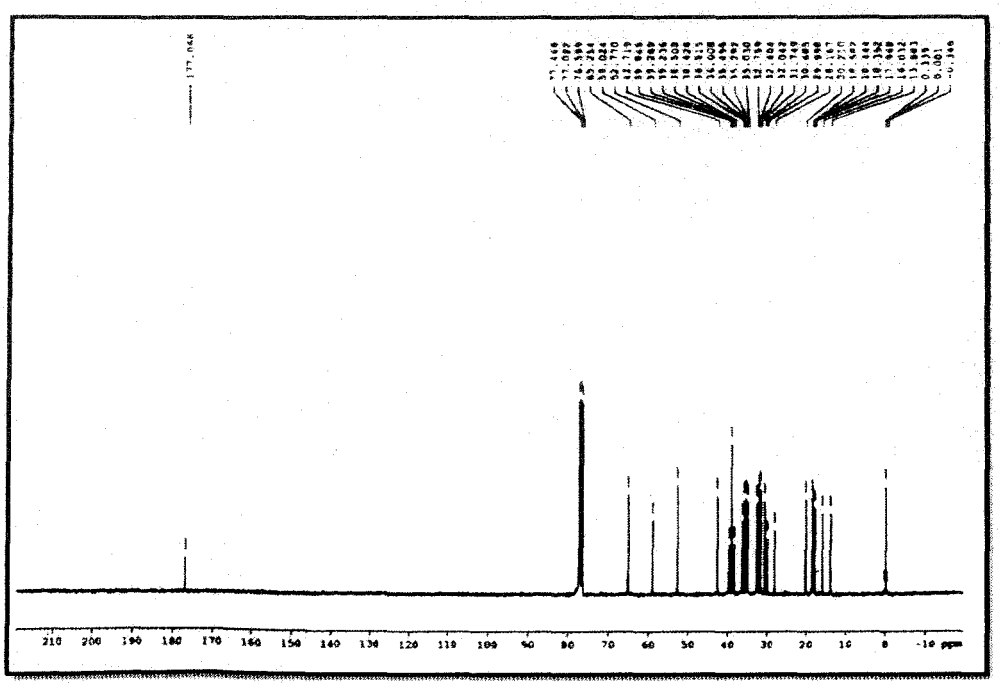


Figure 47. ^{13}C NMR spectrum of lactam **365**.

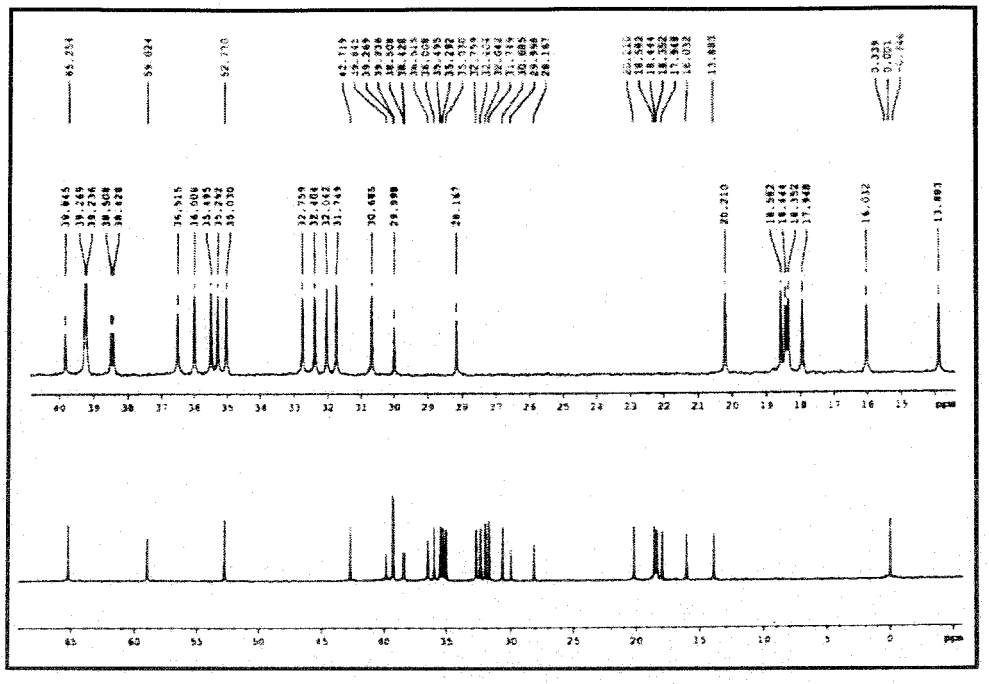


Figure 48. ^{13}C NMR spectrum (partially expanded) of lactam **365**.

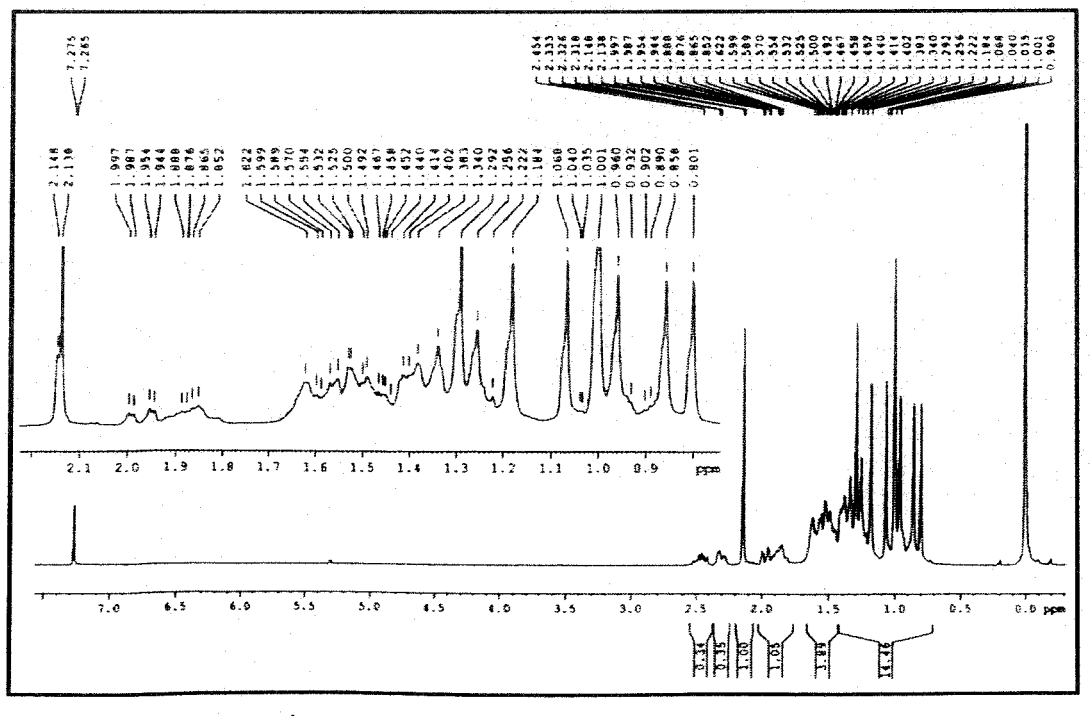


Figure 49. ^{1}H NMR spectrum of 4α-acetoxy friedel-3-one (**381**).

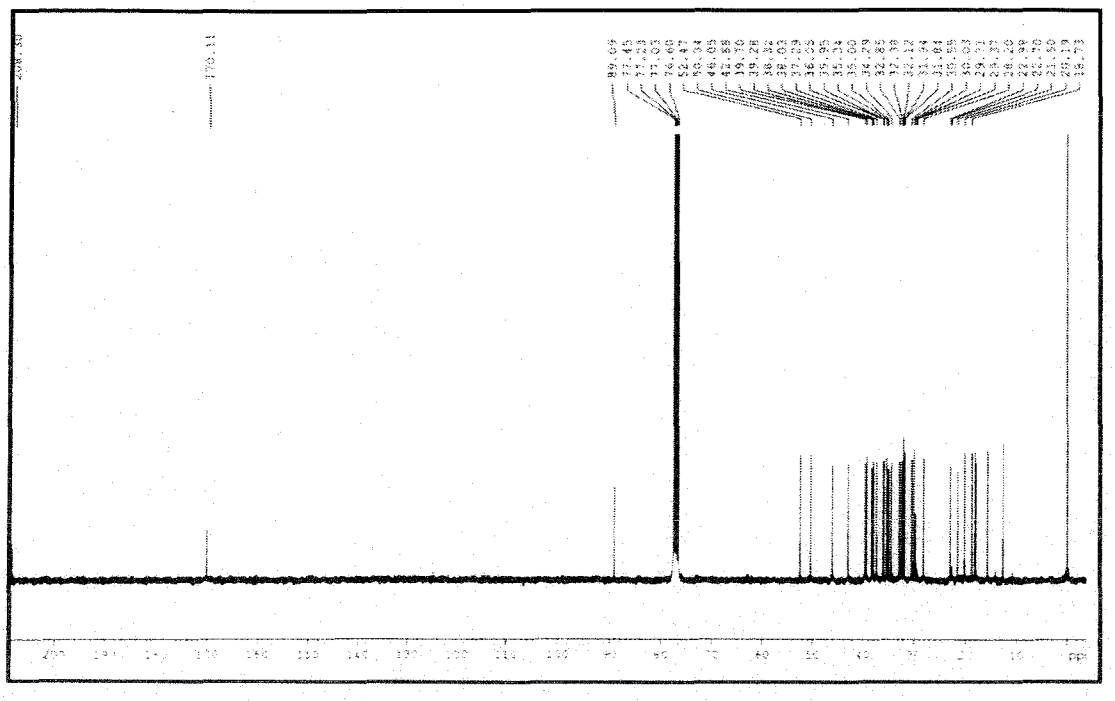


Figure 50. ^{13}C NMR spectrum of 4α-acetoxy friedel-3-one (**381**).

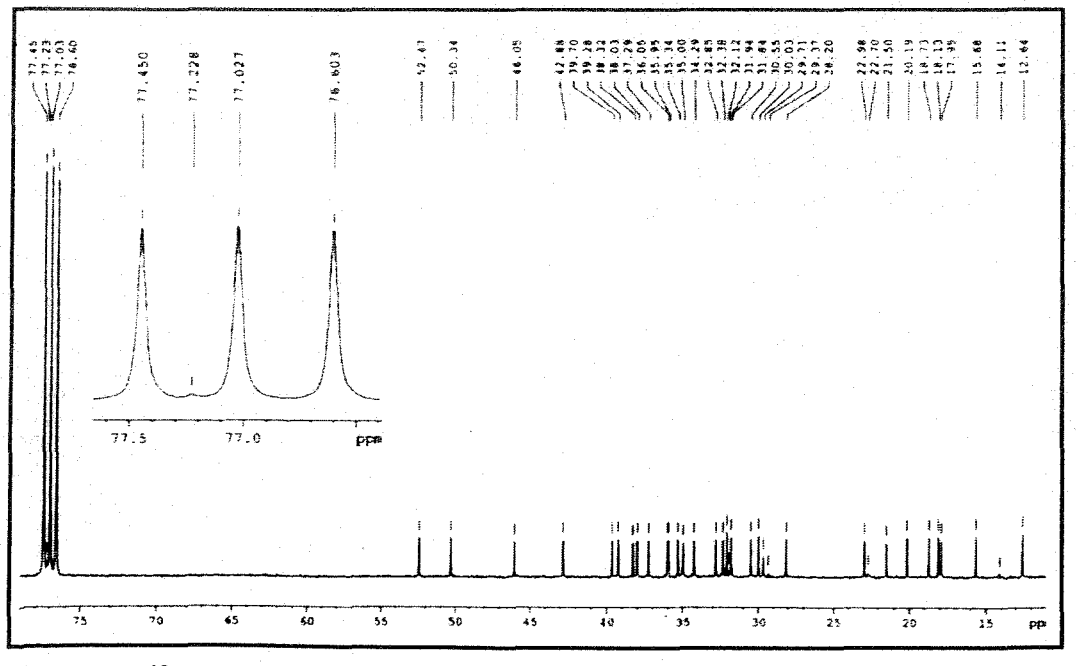


Figure 51. ^{13}C NMR spectrum (partially expanded) of 4α-acetoxy friedel-3-one (**381**).

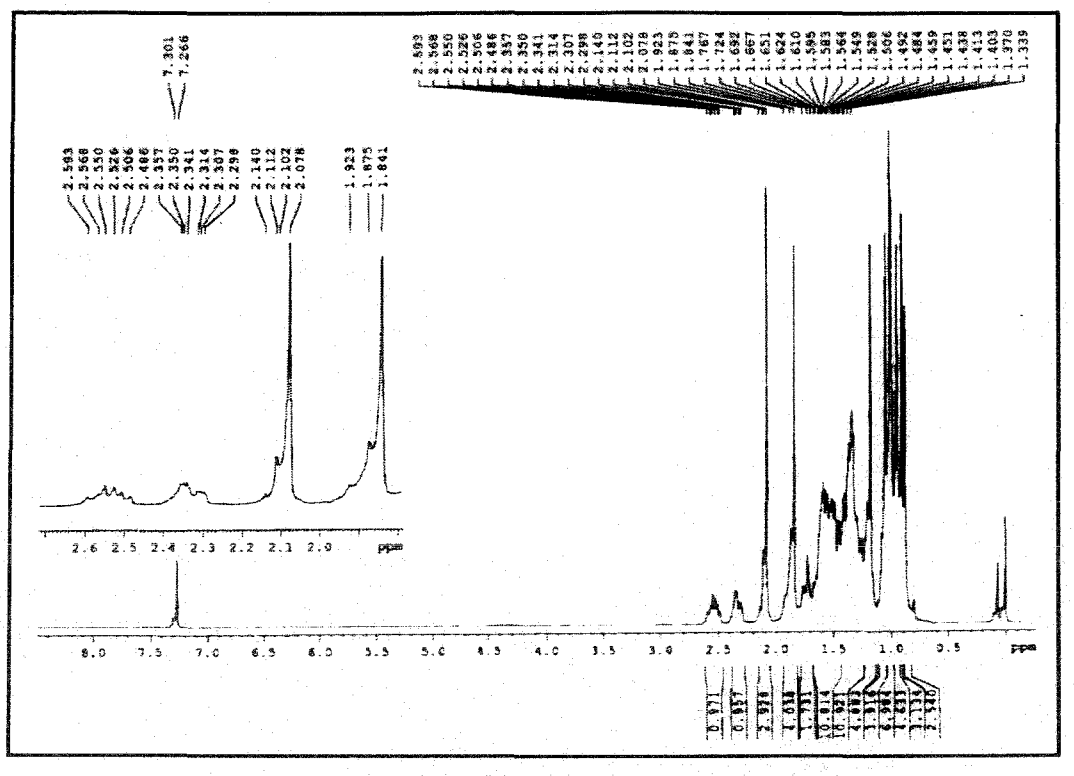


Figure 52. ^{1}H NMR spectrum of 4β-acetoxy friedel-3-one (**382**).

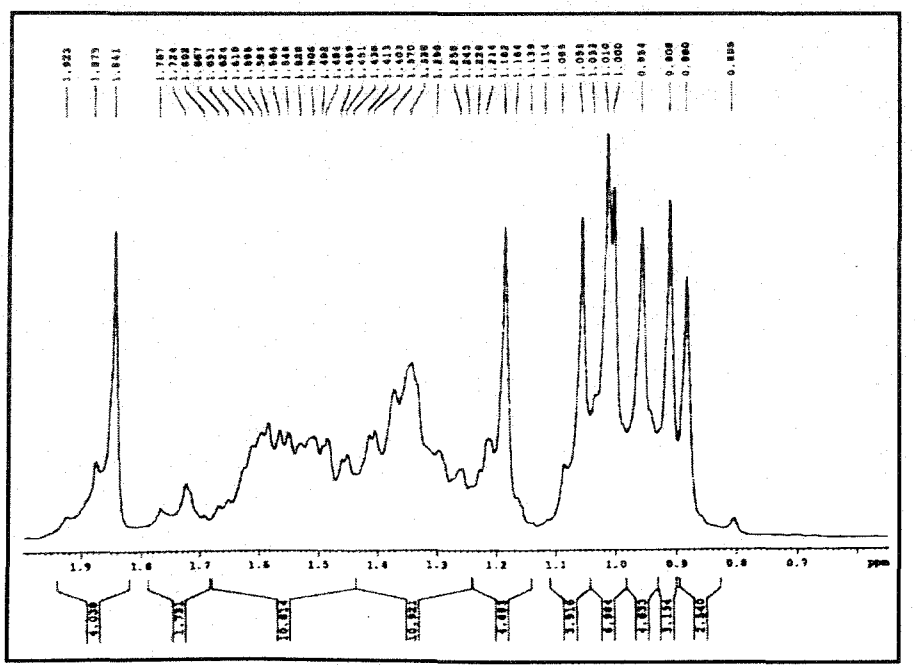


Figure 53. ^{1}H NMR spectrum (partially expanded) of 4β-acetoxy friedel-3-one (**382**).

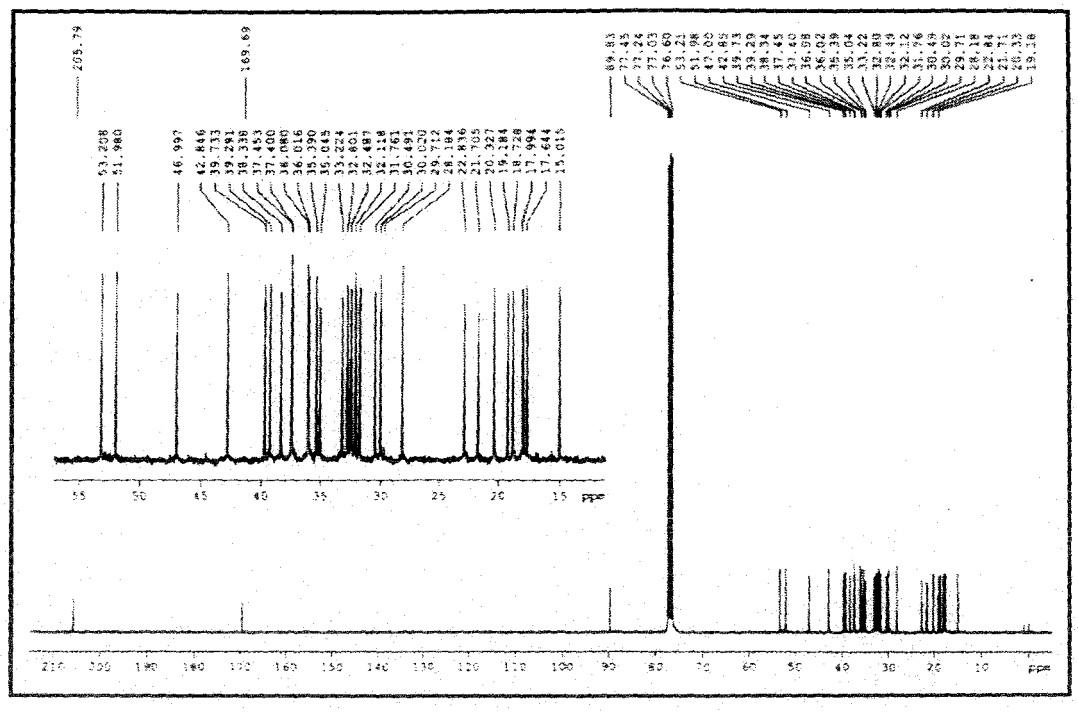


Figure 54. ^{13}C NMR spectrum of 4β-acetoxy friedel-3-one (**382**).

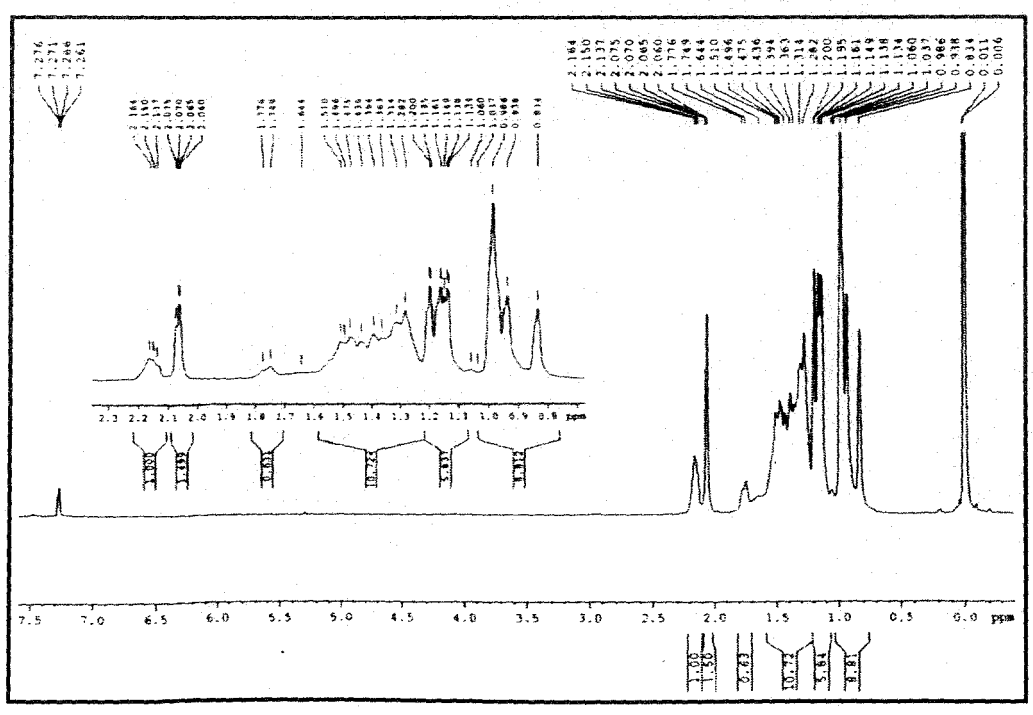


Figure 55. ^{1}H NMR spectrum of 4α-acetoxy-3β-hydroxy friedelane (**383**).

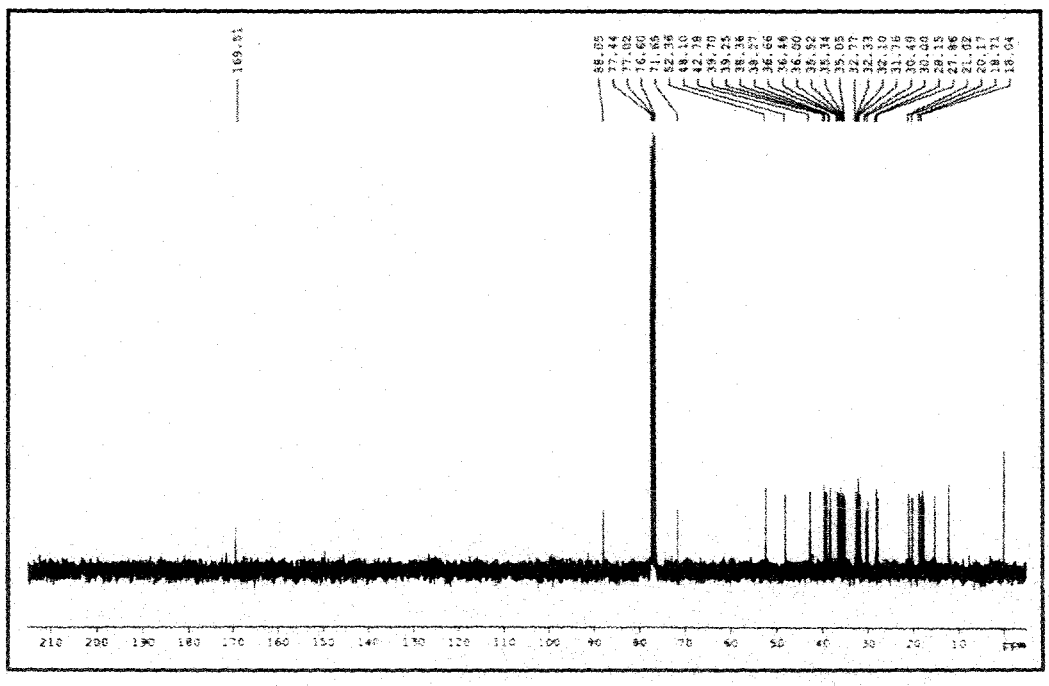


Figure 56. ^{13}C NMR spectrum of 4α-acetoxy-3β-hydroxy friedelane (**383**).

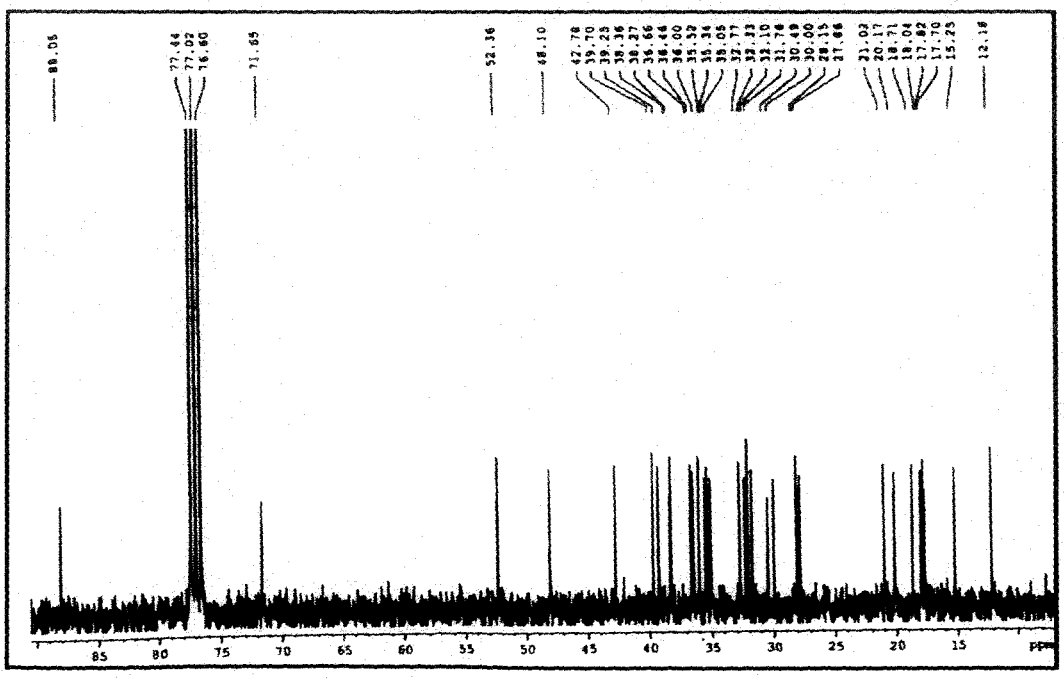


Figure 57. ^{13}C NMR spectrum (partially expanded) of 4α-acetoxy-3β-hydroxy friedelane (**383**).

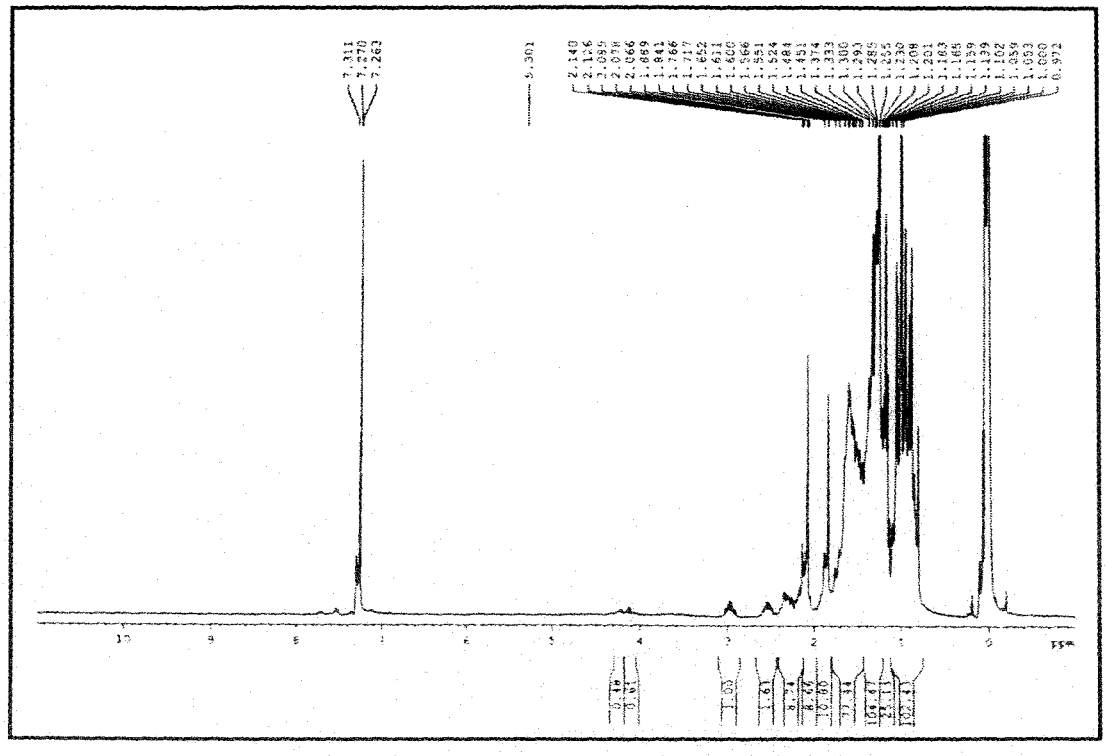


Figure 58. ^{1}H NMR spectrum of 3β, 4α-diacetoxy friedelane (**384**).

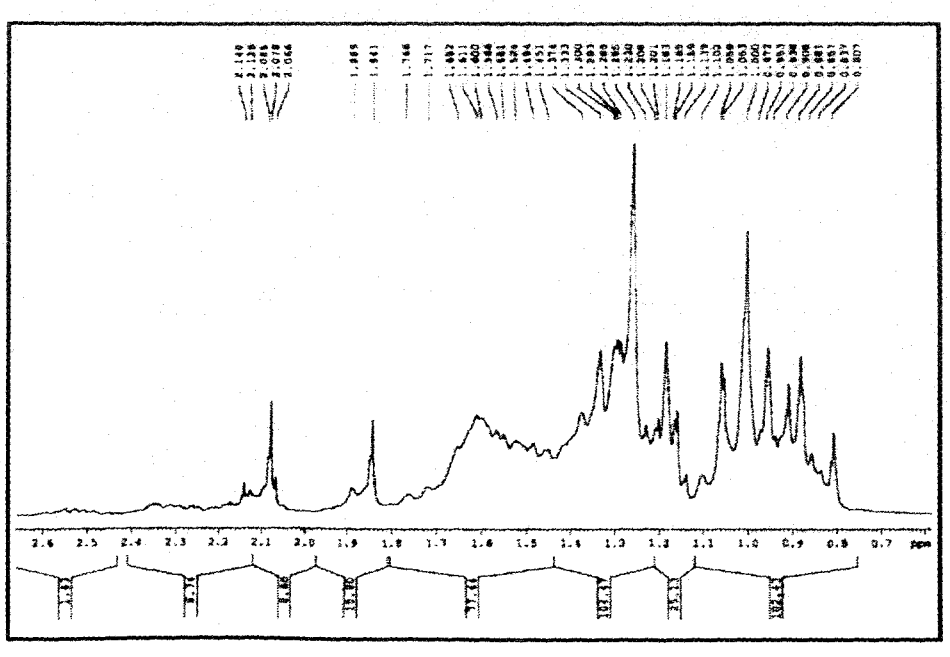


Figure 59. ^{1}HNMR spectrum (partially expanded) of 3β, 4α-diacetoxy friedelane (**384**).

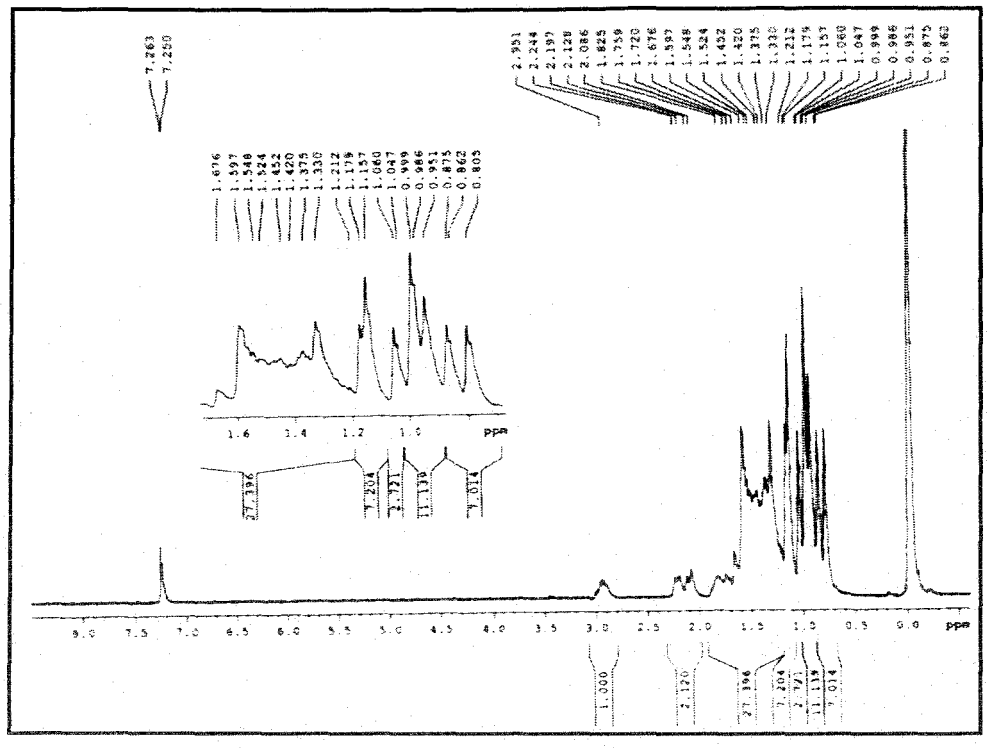


Figure 60. ^{1}H NMR spectrum of 4α-hydroxy friedel-3-one (**357c**).

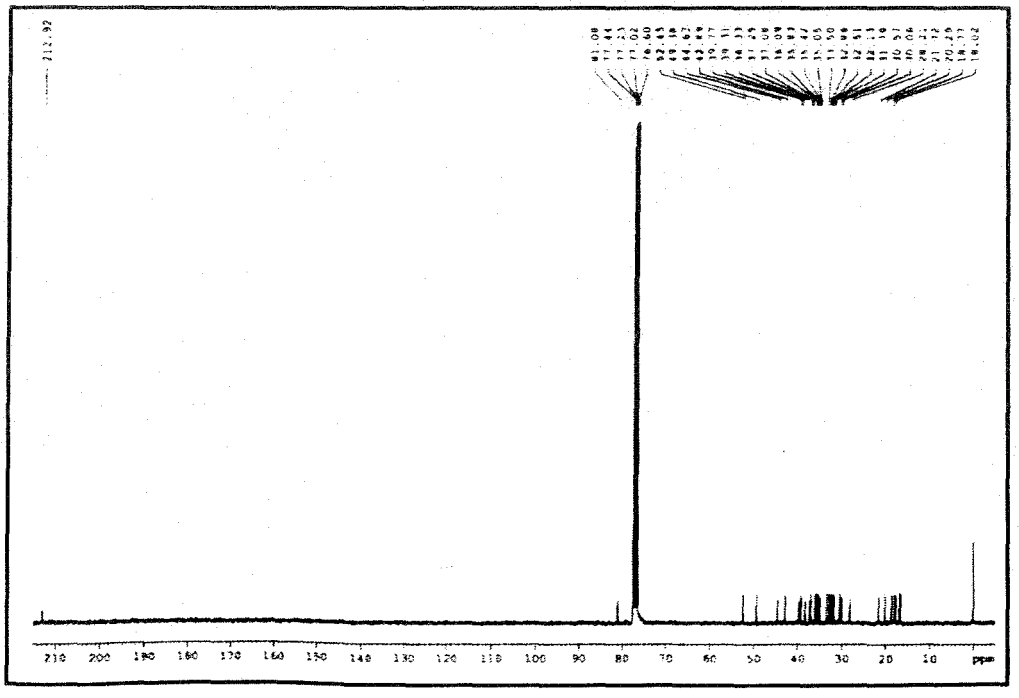


Figure 61. ^{13}C NMR spectrum of 4α-hydroxy friedel-3-one (**357c**).

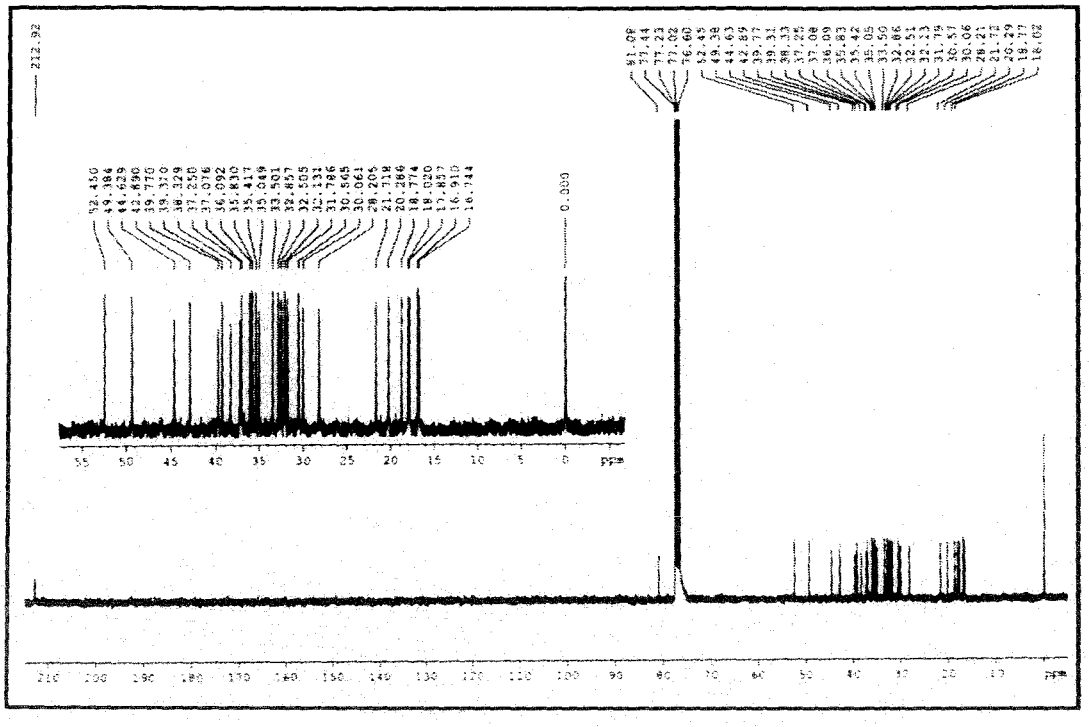


Figure 62. ^{13}C NMR spectrum (partially expanded) of 4α-hydroxy friedel-3-one (**357c**).

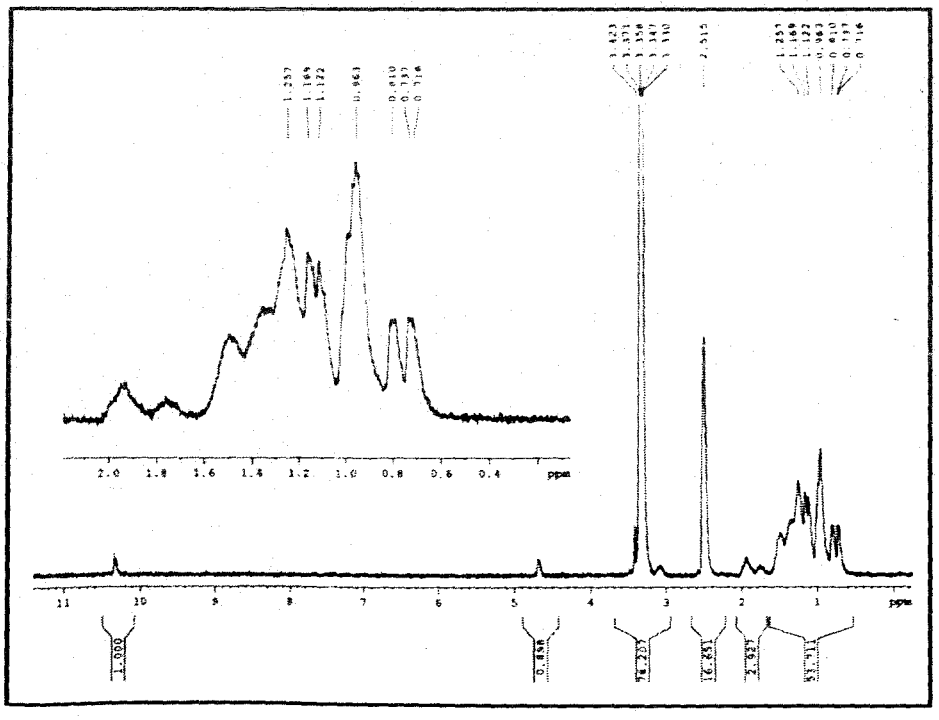


Figure 63. ^{1}H NMR spectrum of 4α-hydroxy friedelane-3-oxime (**385**).

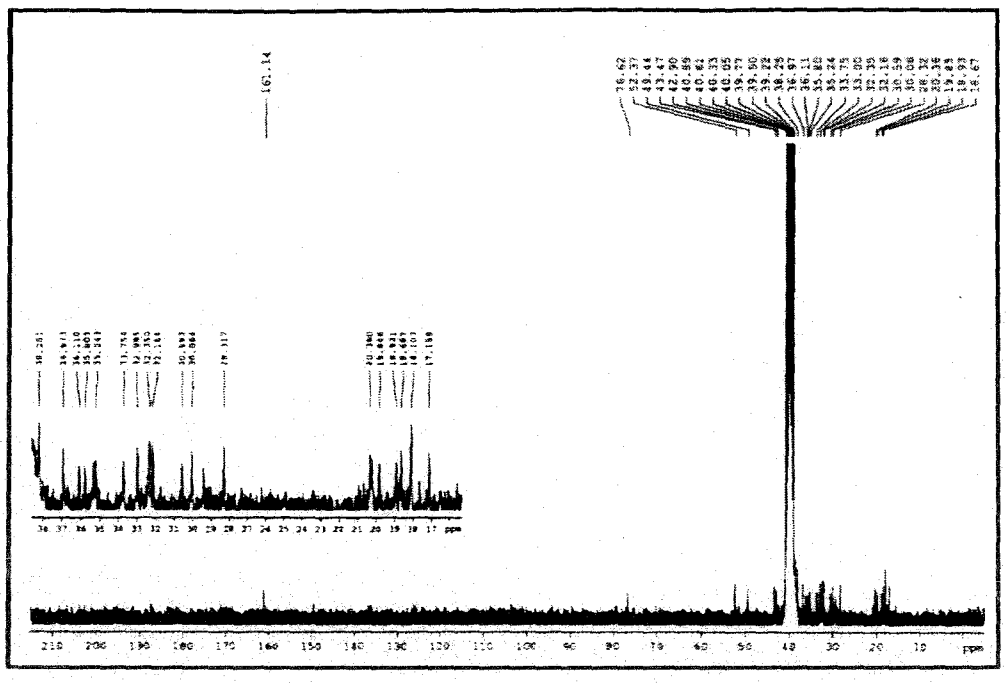


Figure 64. ^{13}C NMR spectrum of 4α-hydroxy friedelane-3-oxime (**385**).

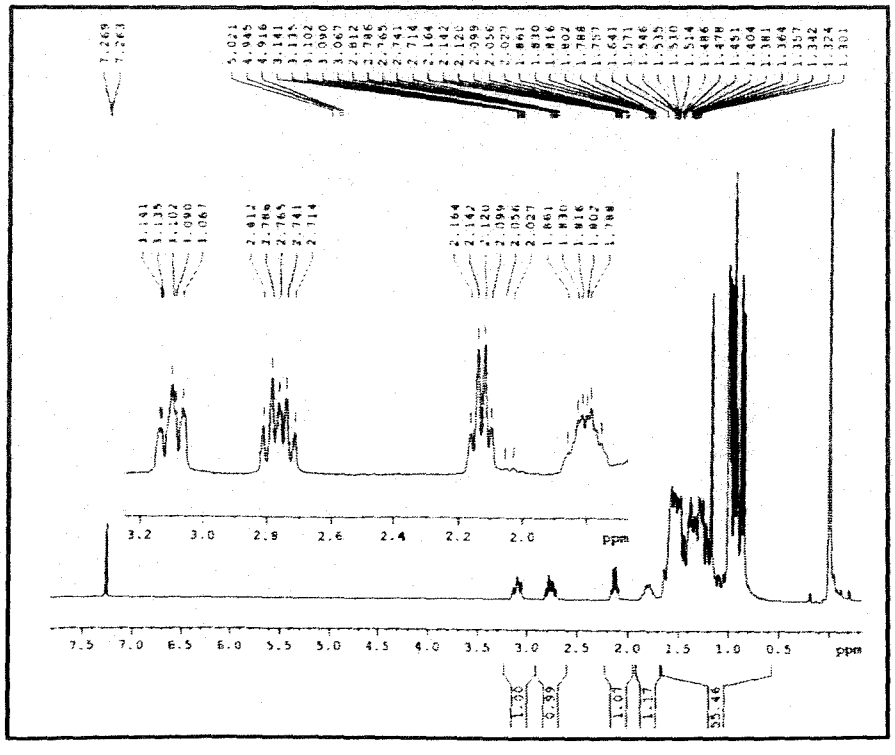


Figure 65. ^{1}H NMR spectrum of cyclic amine **387**.

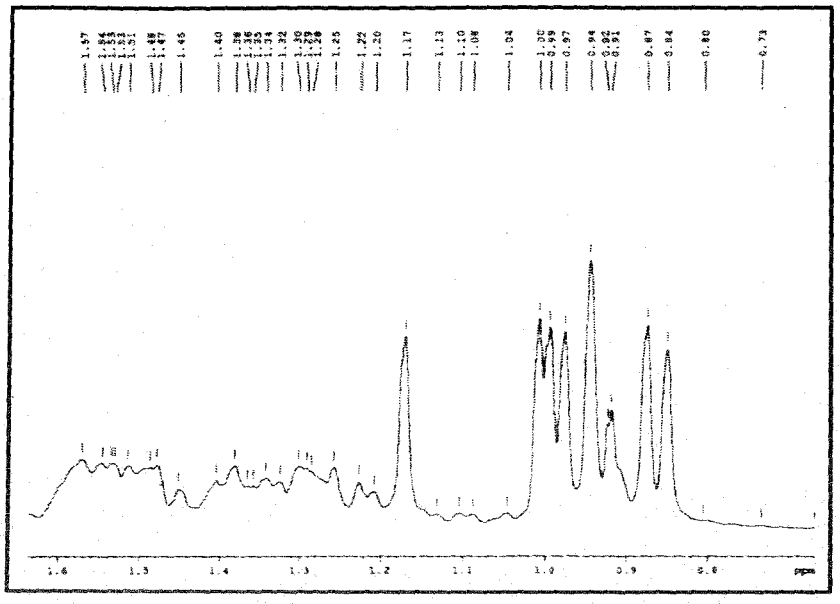


Figure 66. ^{1}H NMR spectrum (partially expanded) of cyclic amine **387**.

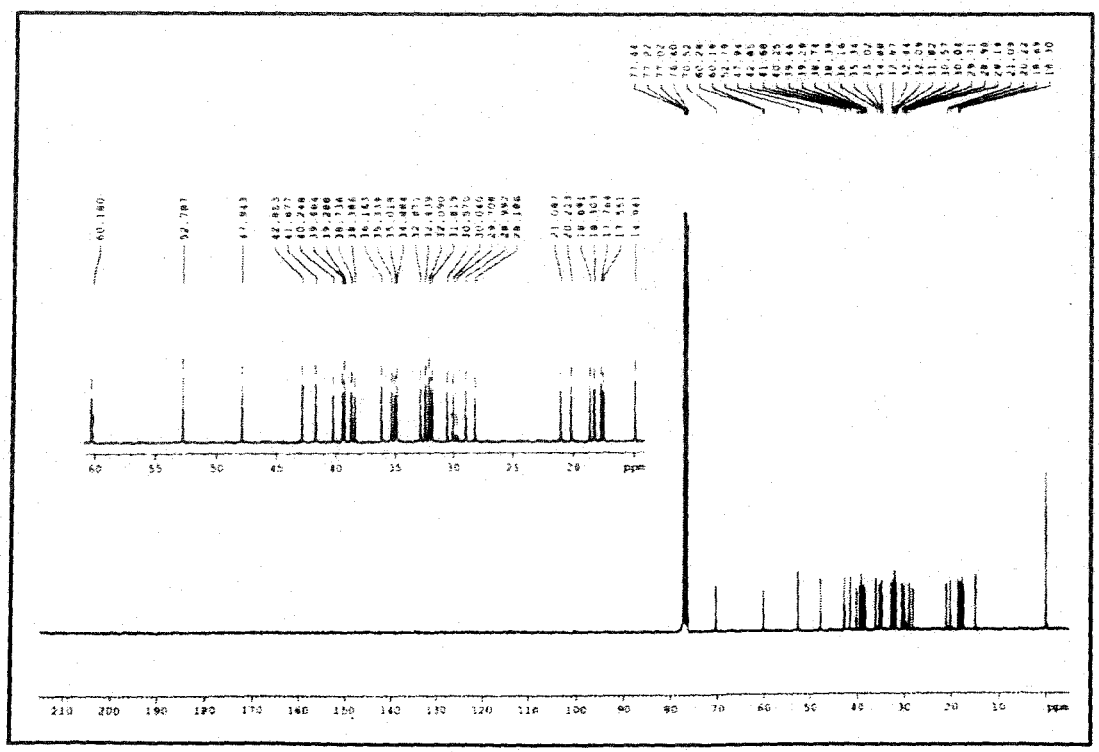


Figure 67. ^{13}C NMR spectrum of cyclic amine **387**.

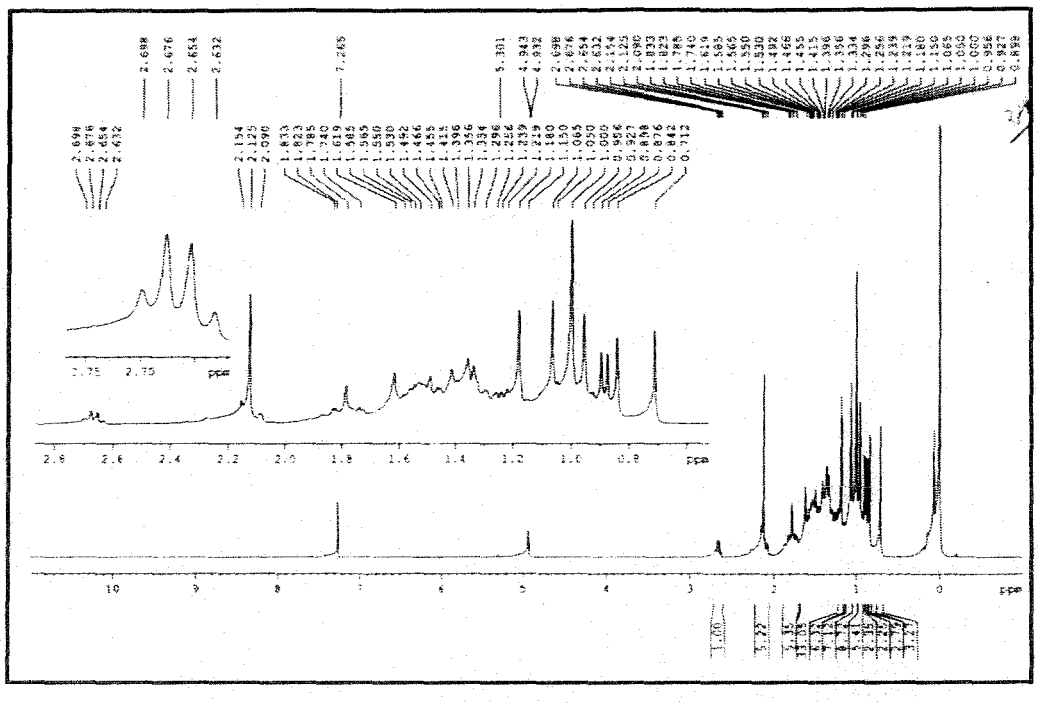


Figure 68. ^{1}H NMR spectrum of 2α-acetoxy friedelan-3-one **(388)**.

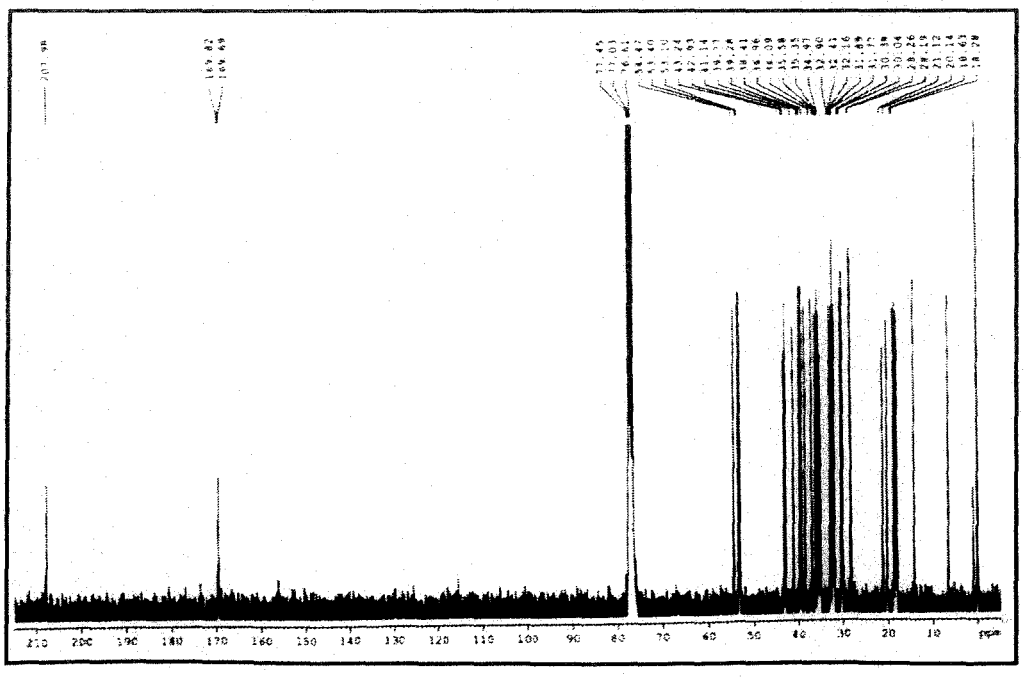


Figure 69. ^{13}C NMR spectrum of 2α-acetoxy friedelan-3-one **(388)**.

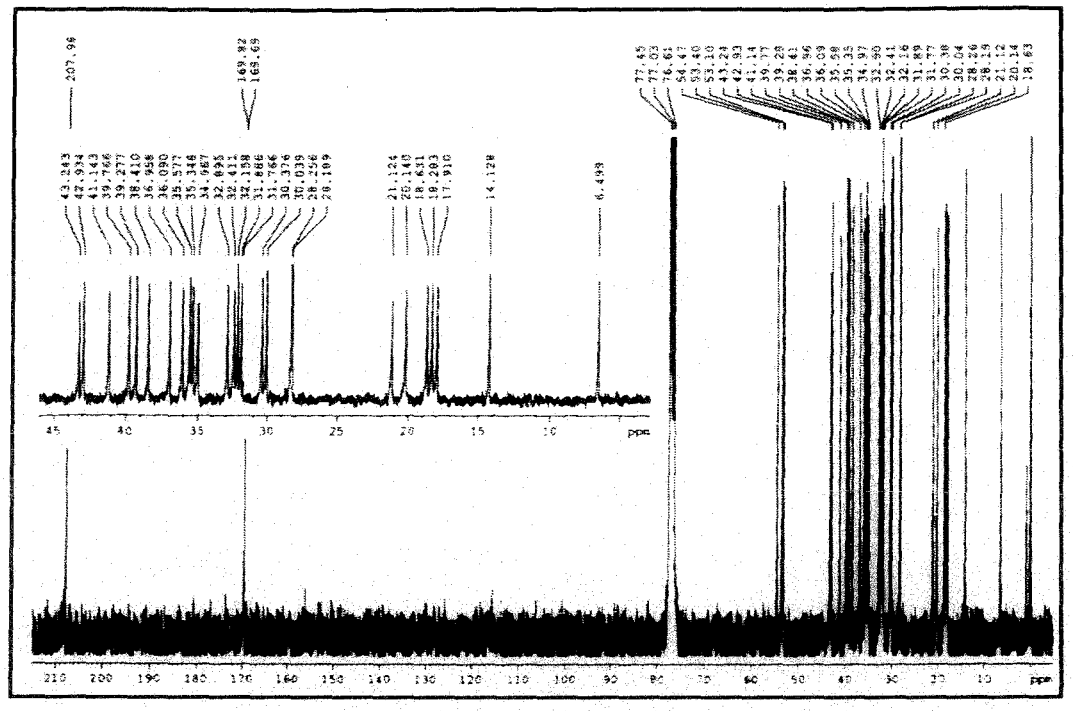


Figure 70. ^{13}C NMR spectrum (partially expanded) of 2α-acetoxy friedelan-3-one (**388**).

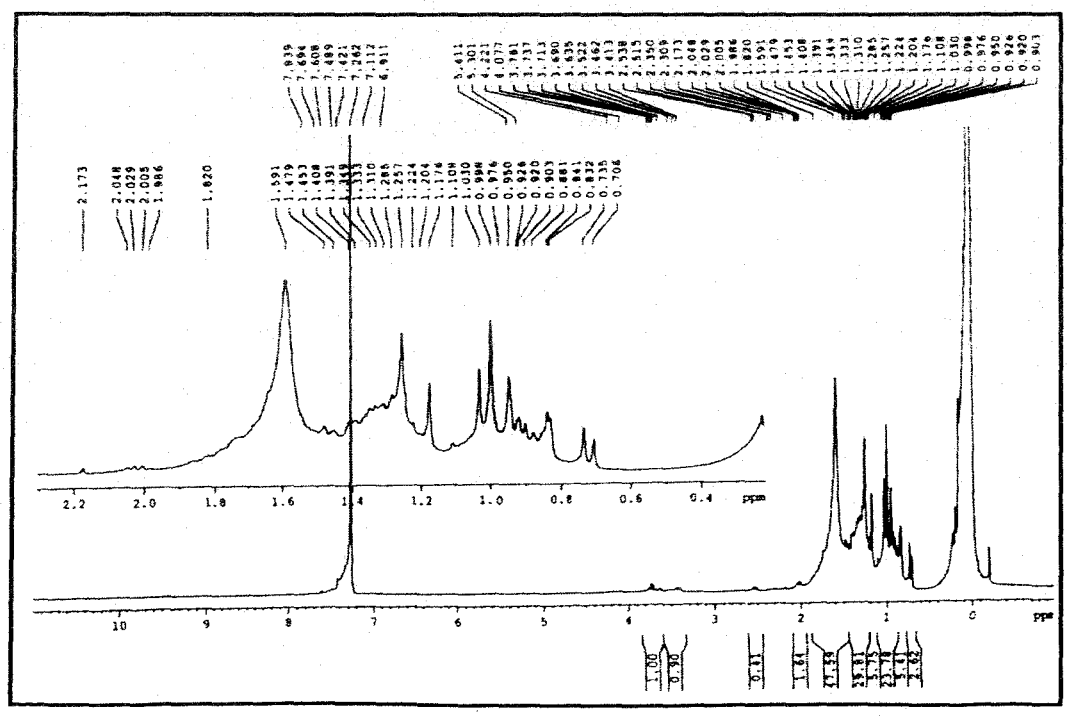


Figure 71. ^{1}H NMR spectrum of 4α-hydroxy friedelan-3-oxime (**389**).

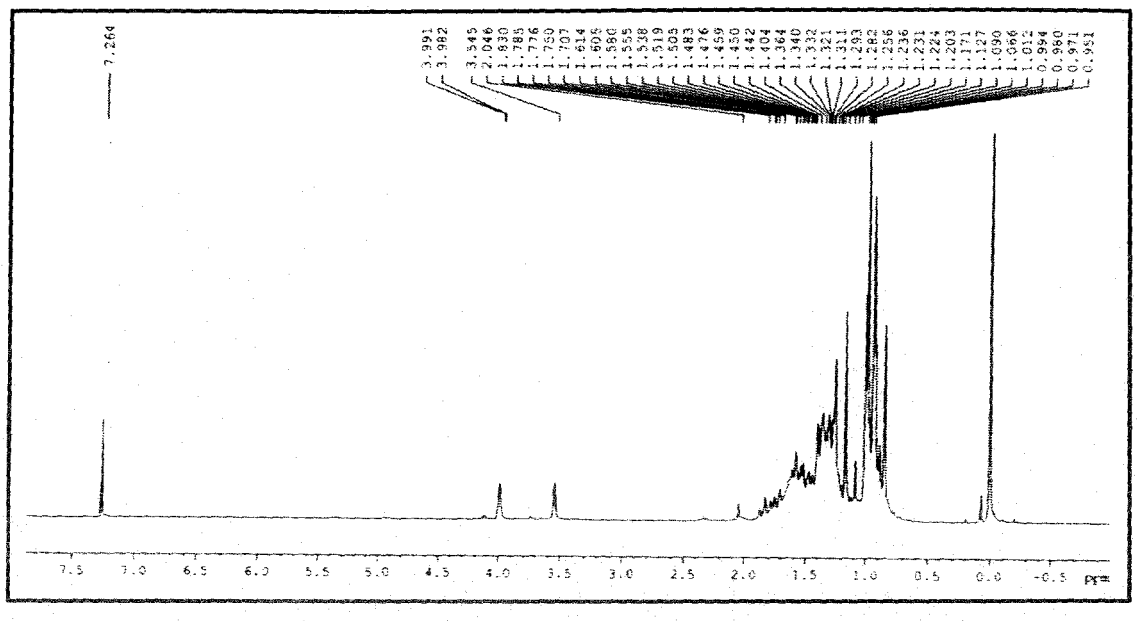


Figure 72. ^{1}H NMR spectrum of 2α,3β-dihydroxy friedelane (**320**).

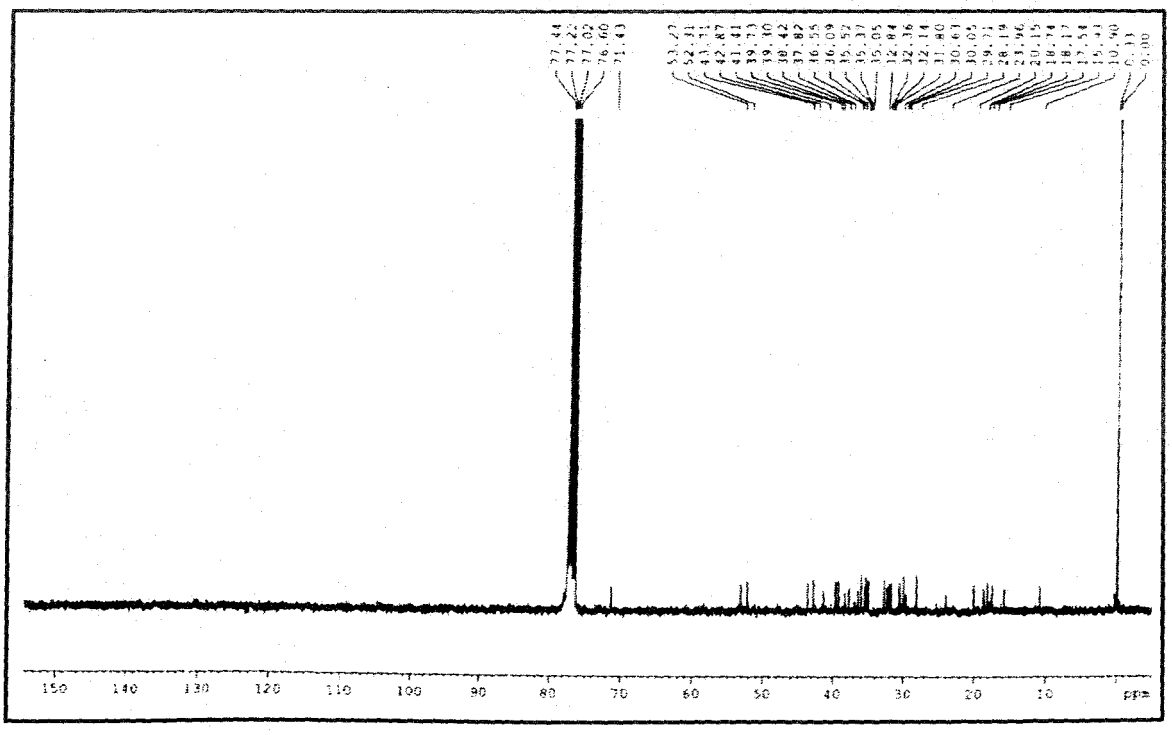


Figure 73. ^{13}C NMR spectrum of 2α,3β-dihydroxy friedelane (**320**).

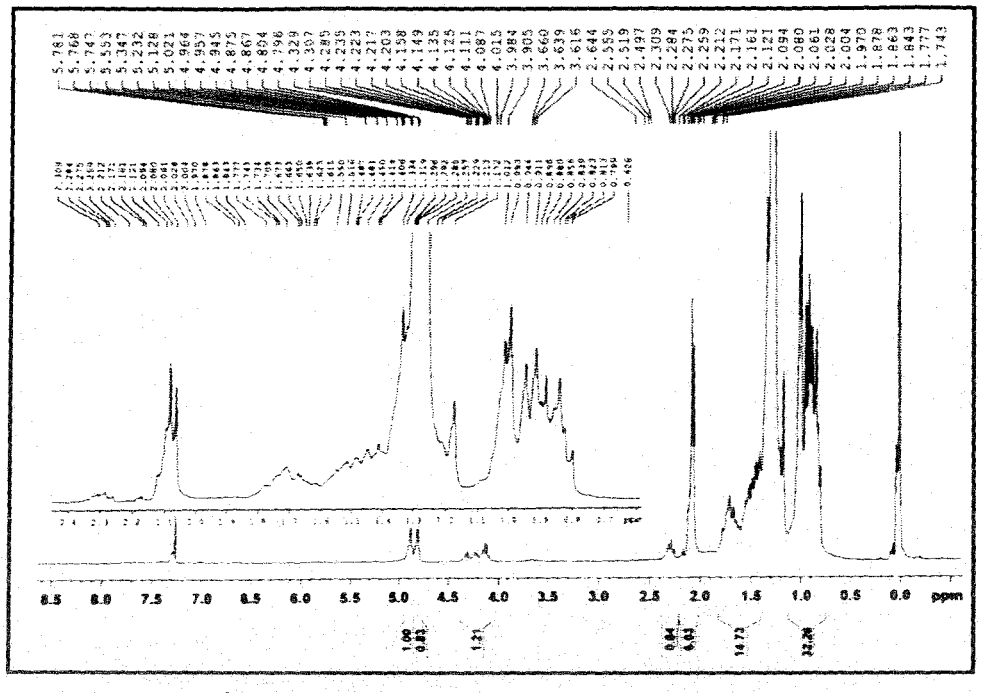


Figure 74. ^{1}H NMR spectrum of 2α,3β-diacetoxy friedelane (**390**).

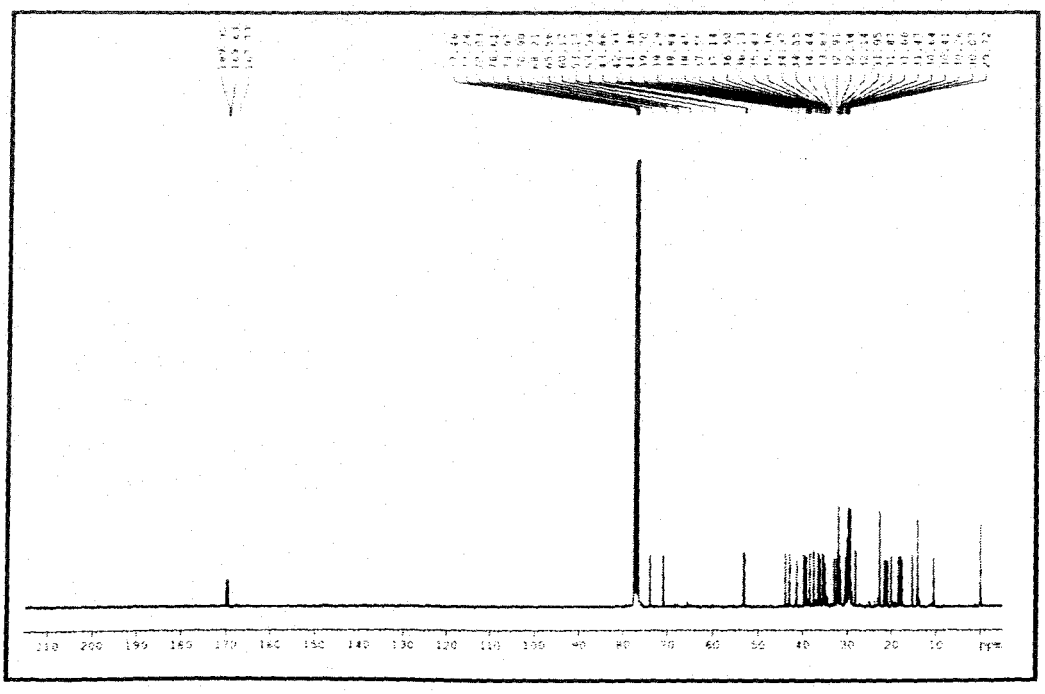


Figure 75. ^{13}C NMR spectrum of 2α,3β-diacetoxy friedelane (**390**).

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