THE UNIVERSITY OF CALGARY

Synthesis of Lupane Geomarkers

by

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ABSTRACT

Many organic compounds which are found in crude oil extracts can be related to living organisms. These compounds are known as biomarkers. Some classes of biomarkers have been studied quite extensively but others, such as the lupanes, are only now being identified in crude oils. For positive identification, synthetic standards are often necessary, in this case, lupanes.

28-Norlupane was prepared. Starting with betulin, hydrogenation, oxidations to the keto-aldehyde, then to the keto-acid were carried out. Decarboxylation of the acid resulted in removal of the C-28 carbon atom and a Wolff Kishner reduction gave the required 28-norlupane.

The next compound prepared was bisnorlupane, in which a C-4 methyl group and the C-28 carbon atom had been removed. The C-28 carbon atom was removed by the same series of reactions used to prepare 28-norlupane. The C-4 methyl group was removed by a series of reactions involving formation of the oxime, the seconitrile, the epoxy-nitrile, ring closure and finally a Wolff Kishner reduction of the C-3 carbonyl to the methylene.

The last part of the work involved preparation of lupane dideuterated in the "A" ring. This was prepared by treatment of 3-oxolupane with sodium methoxide and deuterium oxide, followed by reduction, mesylation, elimination and finally hydrogenation to give the dideuterated compound.

ACKNOWLEDGEMENTS

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Special thanks are also extended to Lia Smit for the many hours she spent in typing this thesis.

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ABBREVIATIONS

CrO_3/Py	=	Chromium trioxide/pyridine
DMAP	=	p-Dimethylaminopyridine
DMF	=	N,N-Dimethylformamide
DMSO	=	Dimethyl sulfoxide
GC	=	Gas chromatography
IR	=	Infrared spectroscopy
$LiEt_3BH(D)$	=	Lithium triethylborohydride or (triethylborodeuteride) Super hydride (deuteride)
MS	=	Mass spectrum
MP	=	Melting point
NBS	=	N-bromosuccinimide
NMR	=	Nuclear Magnetic resonance
Py	=	Pyridine
PCC	=	Pyridinium chlorochromate

TLC	=	Thin layer chromatography
WK	=	Wolff Kishner reduction (Huang-Minlon modification)

CHAPTER 1

INTRODUCTION

I.1 Background

Petroleum exploration has been ongoing for the last hundred years or so. Since most of the easy-to-find and develop deposits have already been discovered, exploration today is being carried out in areas of difficult climatic conditions or in deep water, and so is very costly. Therefore ways of reducing these costs are continually being looked for, with the result that any proposed drilling site is very carefully studied. This means that the organic geochemistry of the area is important in terms of potential oil sources.

Crude oil originates from the organic waste of plants, algae, bacteria and other living matter that has been deposited in aquatic sediment, with less than 1% of this organic material being converted to fossil fuels[1]. "Initially in the sediment, it is subjected to microbial attack and various low-temperature chemical reactions. These microbial reactions occur in the water above and within the upper layers of the sediment and the organic material that survives is then incorporated into the sediment where it undergoes further alterations and reactions due to increasing temperature or thermal maturation"[1].

The general consensus of opinion is that the formation of petroleum takes place in three stages and these are catagorized by the temperature at which they occur and the products formed in each[2].

Diagenesis ($<50^{0}C$) - methanogenic bacteria cause the formation of methane from organic substances in the sediment. The oil found at this stage contains traces of C_2 - C_{14} hydrocarbons and about 15% of the C_{15} - C_{40} hydrocarbons are formed. It is at this time that many oxygen-containing functional groups are eliminated from organic precursors and heteroatomic bonds (primarily C-O bonds) broken to give carbon dioxide and water.

Catagenesis ($50-200^{0}C$) - with increasing temperatures and depth of burial, thermal alteration or maturation reactions occur. Approximately 85% of the oil and 75% of the gas are formed from the thermal cracking of organic molecules, mainly from kerogen in the sediment.

Metagenesis ($>200^{0}C$) - at this stage only methane is formed in any appreciable quantity.

Figure I[1] summarizes the changes and products from each of these three stages.

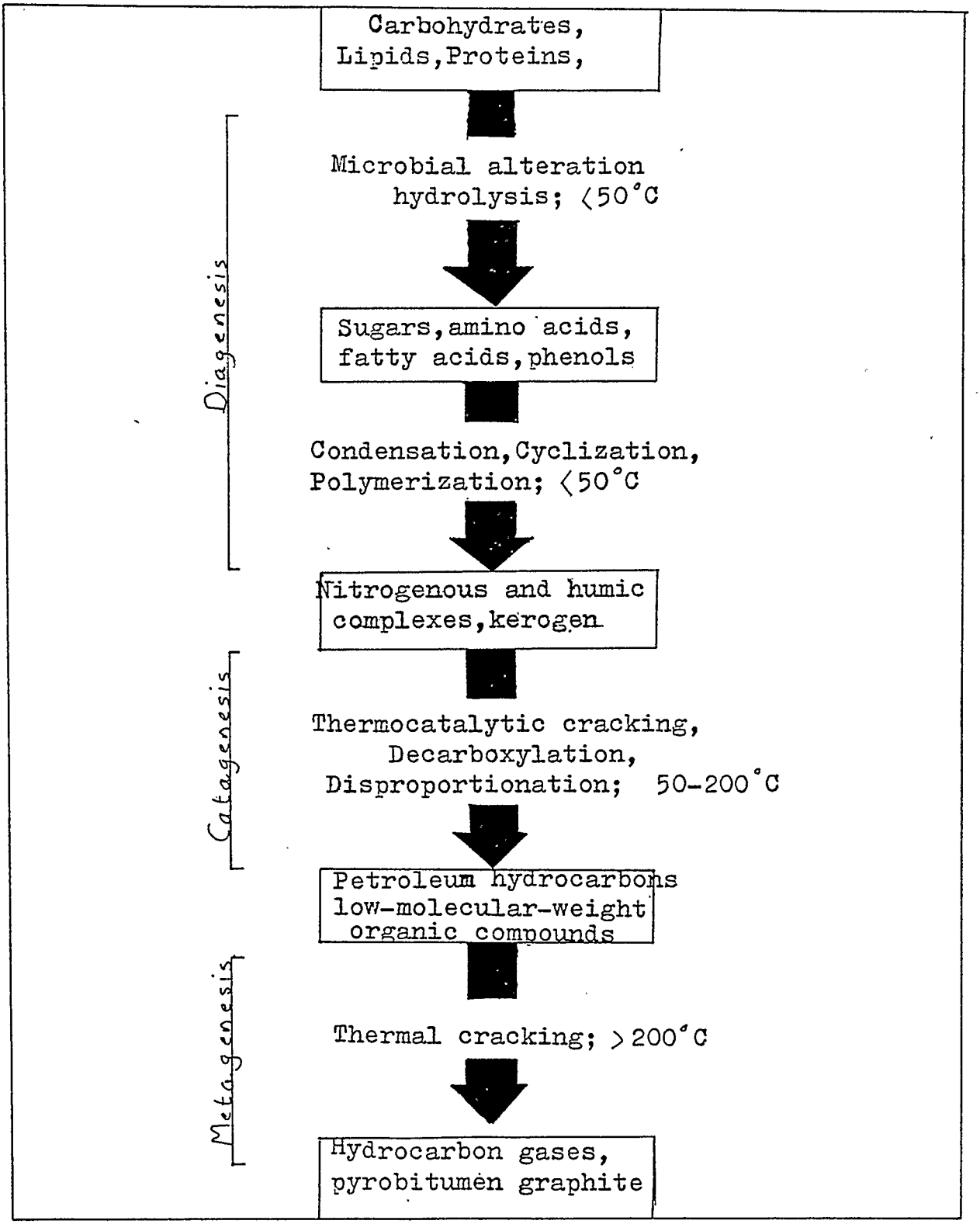


Figure 1[1] Evolution of organic matter

I.2. Biomarkers

I.2.1. Definition and examples

Crude oils are known to contain a wide variety of organic compounds such as hydrocarbons, aromatics and various nitrogen and sulfur containing compounds. In addition to these are other hydrocarbon and non-hydrocarbon compounds whose structures can be directly related to their living precursors. Such compounds are termed biomarkers. A biomarker then can be defined as: "an organic compound, present in the geological record, that has a carbon skeleton which can be related to a precursor molecule from a specific type of organism"[1].

They include such compounds as "n-alkanes, isoprenoids, sesquiterpenes, tricyclyclic terpenes, steroids and porphyrins"[1], and may be derived from terrestrial (mainly plant) or marine organisms. Biomarkers may stem from the living organism without any chemical alteration or with only minor changes which occur mainly during diagenesis e.g. the loss of functional groups, stabilization by hydrogenation or aromatization etc.

"If a precursor is to serve as a biomarker, the carbon skeleton of the said precursor must be maintained in the product"[1]. There can be changes in the stereochemistry but not in the actual carbon skeleton. "Biomarkers found in crude oils are generally derived from their oxygenated

precursor e.g. steranes from sterols"[1]. The biomarkers produced during diagenesis are usually saturated or aromatic hydrocarbons while those found in the catagenic zone are useful for correlating oils with other oils or with the source rock and to assess thermal maturity.

Figure 2[1] shows some of the more common compounds classed as biomarkers and examples of their precursors.

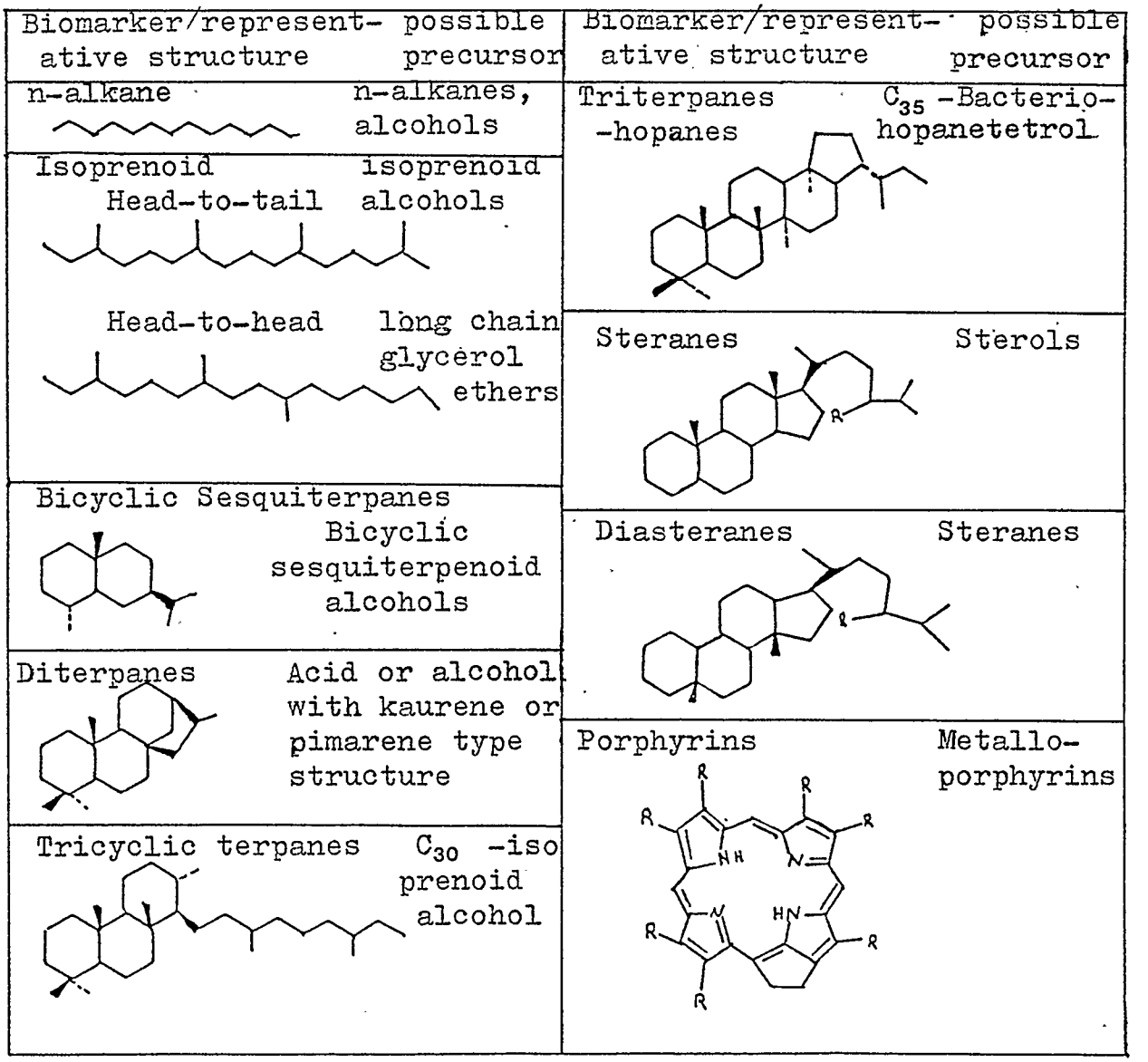


Figure 2. Compounds used as biomarkers

I.2.2. Their use in petroleum exploration

By virtue of their unique structures biomarkers can be used in a number of ways:

1. Since they are derived from compounds originating from living organisms, as a result of their biodegradation, biomarkers provide clues regarding the age of the oil and the degree to which it has been affected by heat and burial. The fact that the particular biomarker found is the saturated hydrocarbon instead of the unsaturated or oxygenated precursor indicates that it has undergone a greater degree of biodegradation than the precursor.

2. Their distribution can also provide information on the relative migration distances from source rock to the oil reservoir. In essence, they serve as a geological fingerprint for an oil containing specific organic compounds[1]. If the biomarker distribution in two oil wells in the same general area is similar, then it is quite likely that both oils have come from the same source. A correlation between a biomarker found in an oil sample and one found in a rock extract suggests that the rock is the source of the oil. This is illustrated in figure 3[1].

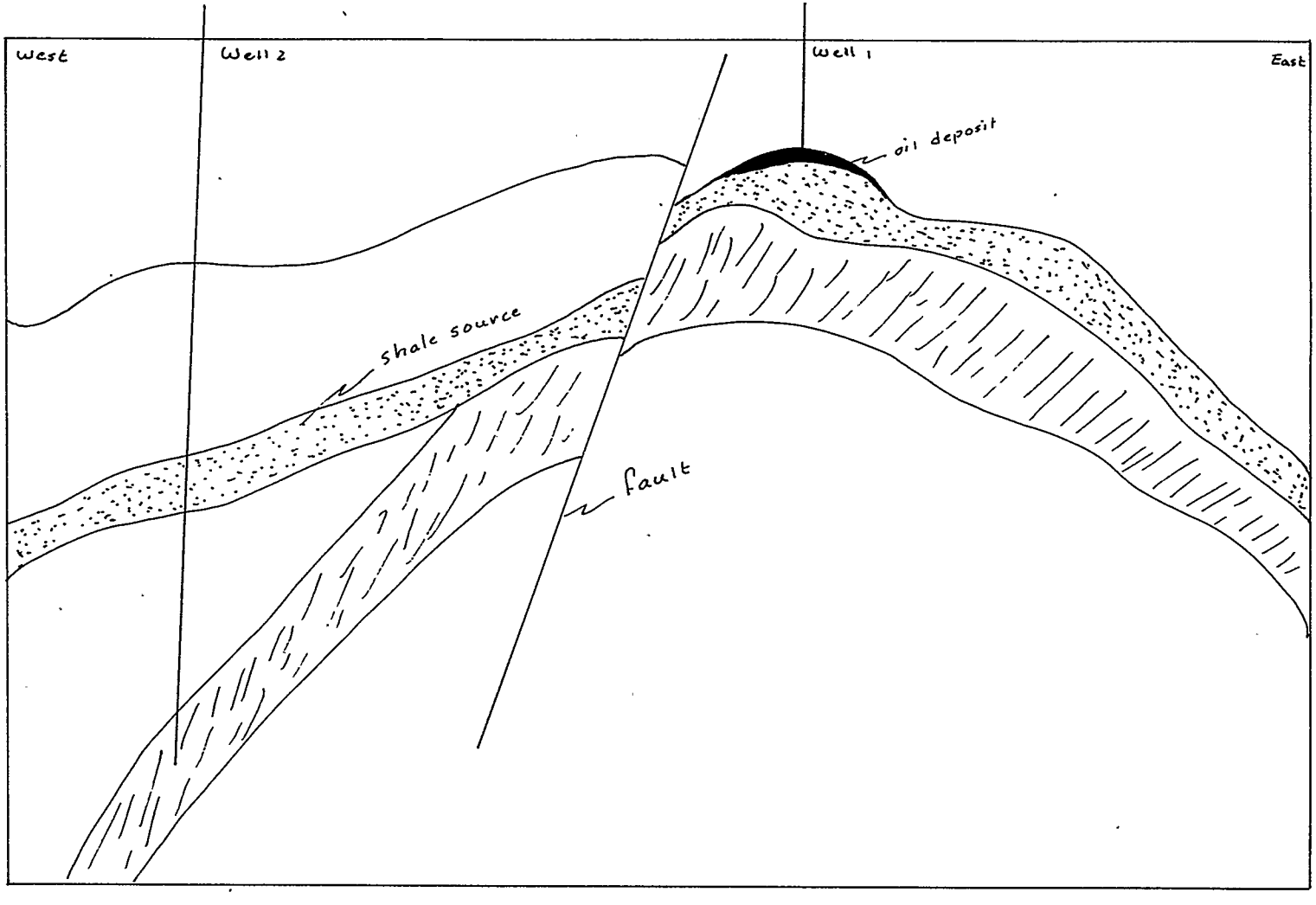


Figure 3. Relationship between the biomarkers found in different wells

Figure 3 shows that well #1 was drilled and struck oil whereas well #2 was dry. The second well has penetrated a shale sequence rich in organic material with the potential for forming oil. Even though there is a fault between the two wells, if there is a correlation betwen the oil in well #1 and the organic extract from the shale in the core from well #2 as a result of the distribution of certain classes of biomarkers then it is quite likely that at one time the two shales were continuous. It is also possible that the area to the west of well 2 might contain oil.

3. The finding of biomarkers can also help in the investigation of the biodegradation of the oil. In the early stages of crude oil break-down, n-alkanes are removed. "Extensive biodegradation of crude oils initially tends to remove more than 50% of the naturally occurring 20-R sterane epimer and ultimately removes all the steranes preferentially over the diasteranes"[1].

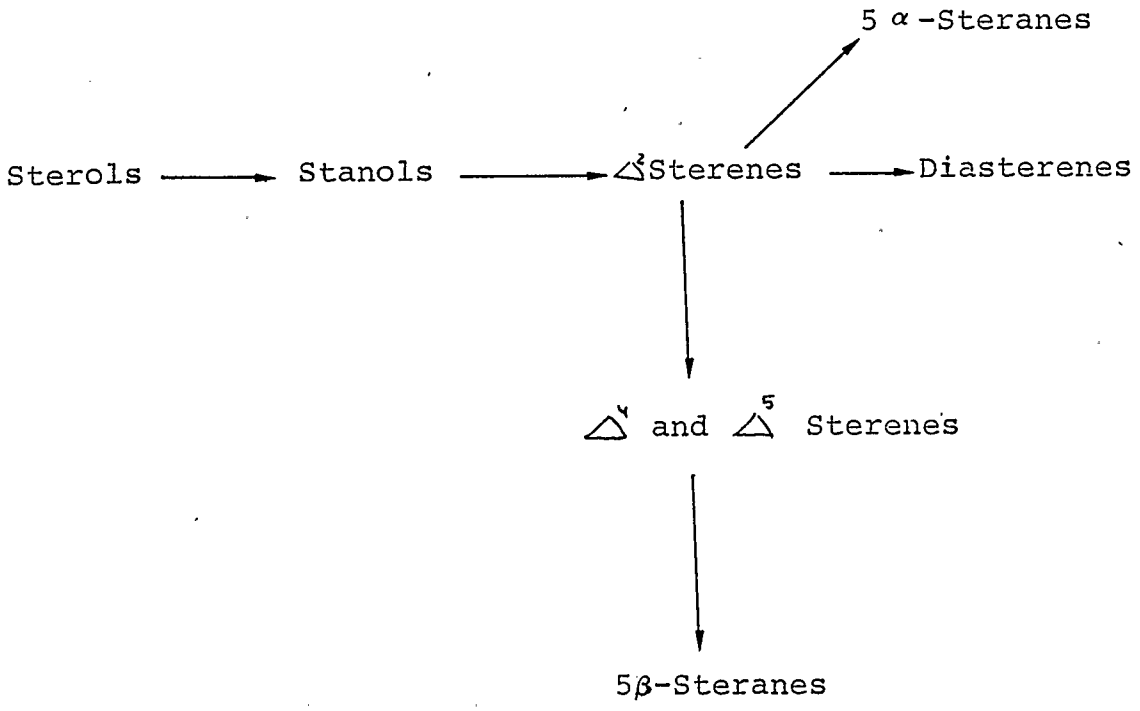
I.3. Classes of Biomarkers

Some of the more common types of biomarkers are:

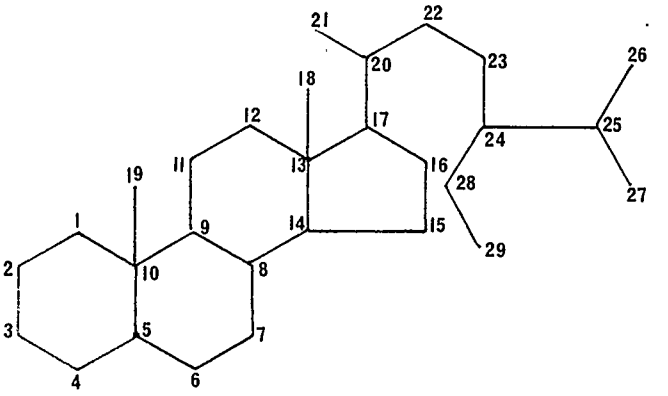
a) Acyclic isoprenoids
b) Steroids e.g. sterols
c) Porphyrins
d) Pentacyclic triterpenoids e.g. Hopanes, Lupanes.

I.3.1. Steroids e.g. Sterols

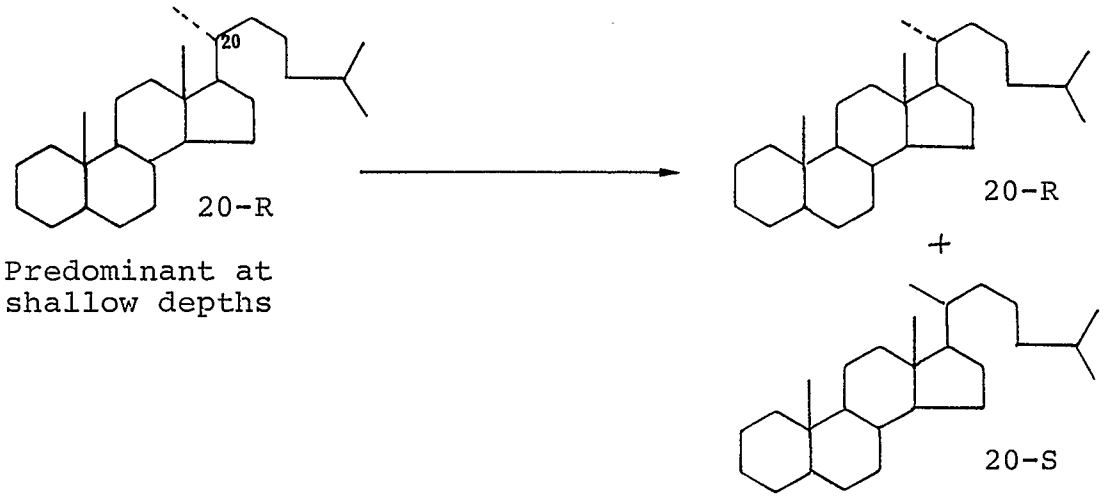
Perhaps the class of biomarkers that has been studied the most extensively to the present time is the sterols. They are usually found unaltered or only slightly altered in relatively immature older sediment. In sediments from marine sources, C_{27} sterols predominate while in those from land-derived sources C_{29} sterols predominate. The 20-R configuration is seen in immature, non-rearranged steranes. It is thought that the configuration at C-20 in the 5 α(H),14α(H),17 α(H) steranes relates to the extent of maturity[3]. The degradation of sterols is thought to follow a pathway similar to the one outlined below.



The numbering system used by all geochemists and chemists for steroids is shown below.



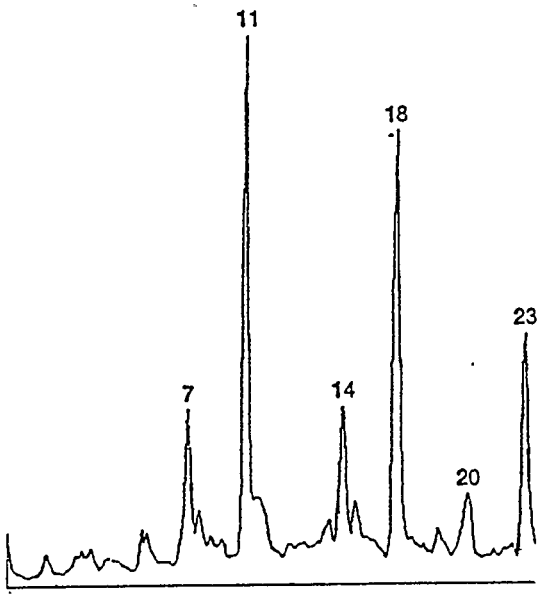
Since Δ^2 sterenes are frequently found in low maturity sediments it is generally thought that they originate from the sterol via the stanol by dehydration. The Δ^2 sterenes isomerize to the more stable Δ^4 or Δ^5 sterenes with increasing depth of burial and Δ^4 sterenes tend to predominate due to their greater stability[2]. Steranes initially formed during diagenesis retain the (5α(H),14α(H),17α(H),20-R) configuration of their sterol precursor but with increasing burial depth and temperature they tend to adopt the more stable 5α(H),14β(H),17β(H) configuration and to isomerize at C-20 to give such compounds as 5α(H),14α(H),17α(H),20-S cholestane. With increasing depth, the C_{20} steranes change from the 20-R, predominant at shallow depths, to equal amounts of 20-R and 20-S around the peak of oil generation.

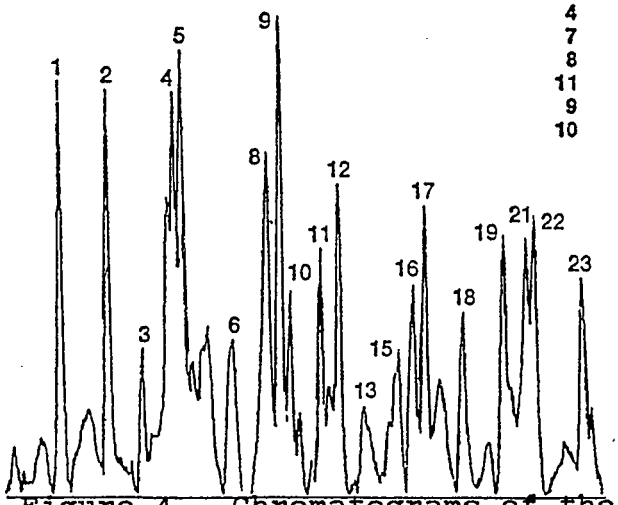


Since this change is slower than other stereochemical changes in isoprenoids and the C-17 and C-22 changes in hopanes, the 20-S/20-R + 20-S ratio can be used to assess the degree of maturity. The configuration at C-24 is a mixture of isomers, the predominance depending on the origin of the precursor. Sterols synthesized from algae give rise to 24-S steranes and those from higher plants give rise to 24-R steranes. Because steranes can exist with the C-5, C-14 and C-17 hydrogens in either the α or β configuration as well as the 20-R or 20-S configurations or as the 24-S or 24-R epimers, a particular crude oil sample can contain quite a complex mixture of steranes. However this can be used to advantage. The steranes in oils of low maturity (not exposed to high temperatures) have a fairly simple distribution with most of the isomers still showing biological stereochemistry. As maturity increases, the number of isomers and epimers increases, resulting in a complex distibution of steranes. With maturity, the distribution of particular isomers can change, for example, the amount of the 20-R isomer decreases with increasing age.

The fact that certain sterane isomers migrate more rapidly than others as a result of steric configuration means that they can provide information on relative migration distances from source rock to oil reservoir[1].

The chromatograms of figure 4[1] show how the sterane distribution changes from a relatively simple mixture in an immature oil to a complex mixture in a mature oil.





C_{27}	C_{28}	C_{29}	Stereochemistry
Peak number			
1	5	9	13β(H), 17α(H)-Diasterane (20*S*)
2	6	12	13β(H), 17α(H)-Diasterane (20*R*)
3	8	13	13α(H), 17β(H)-Diasterane (20*S*)
4	10	16	13α(H), 17β(H)-Diasterane (20*R*)
7	14	20	5β(H), 14α(H), 17α(H)-sterane (20*R*)
8	15	19	5α(H), 14α(H), 17α(H)-sterane (20*S*)
11	18	23	5α(H), 14α(H), 17α(H)-sterane (20*R*)
9	17	22	5α(H), 14β(H), 17β(H)-sterane (20*S*)
10	16	21	5α(H), 14β(H), 17β(H)-sterane (20*R*)

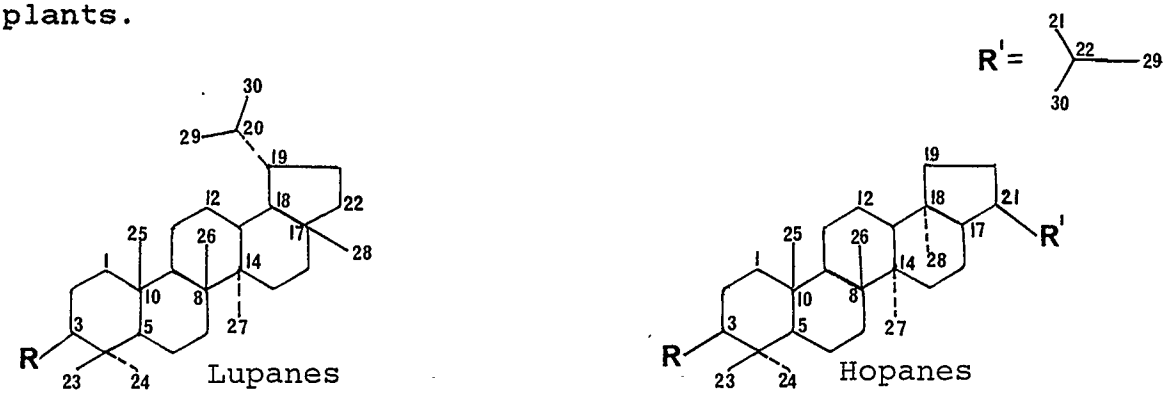
Figure 4. Chromatograms of the sterane distribution in an oil extract.

In geochemical samples different proportions of a homologous series of C_{27}, C_{28}, and C_{29} steranes predominate. In immature extracts each member is present as a mixture of 2 stereoisomers in a 1:4 ratio. The top chromatogram shows the distribution of steranes in an extract from an immature oil. Peaks 7 and 11 are from the C_{27} homolog, 14 and 18 from the C_{28} homolog and 20 and 23 from the C_{29} homolog.

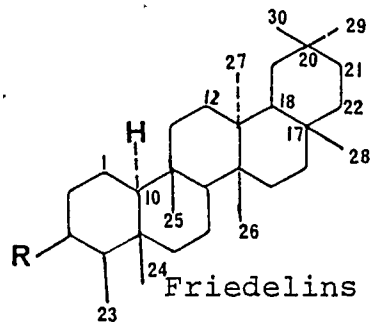
With diagenesis and increased maturity, the distribution of steranes is more complex as shown in the lower chromatogram. It is now possible to get 4 diasteranes and 4 steranes for each homolog. For the C_{27} homolog they are peaks 1--->4 and 8--->11. The accompanying table lists the stereochemistry and peak number of each component of the homolog.

I.3.2. Pentacyclic triterpenoids

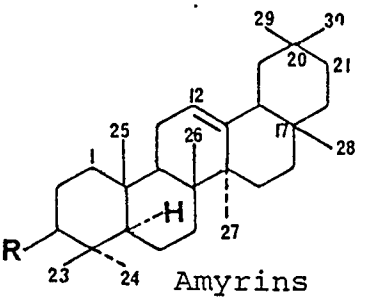
Since this project involves work on triterpenoids, in particular lupanes, it was thought appropriate to include some information on pentacyclic triterpenoids. Examples such as lupanes, hopanes, friedelins and amyrins containing an oxygen functionality at C-3 have all been extracted from higher plants.



R=an oxygen functionality



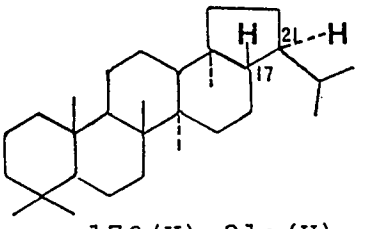
Friedelins



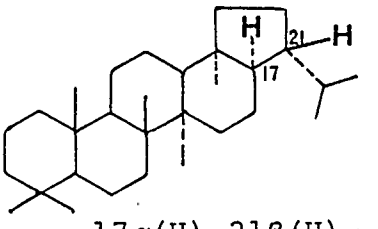
Amyrins

Hopanes

The saturated fraction of pentacyclic triterpenoids found in many crude oils consists primarily of hopanes and the naturally occurring ones normally contain 30 to 35 carbon atoms but to date C_{28} hopanes are not widely distributed[4]. These hydrocarbons are generally found in mature crude oils and are dominated by the thermally more stable 17 α(H),21β(H) isomer which does not occur in living systems. They also contain some of the 17β(H),21 α(H) isomer and a mixture of two diastereomers at the C-22 position for C-31 and higher homologs[5].

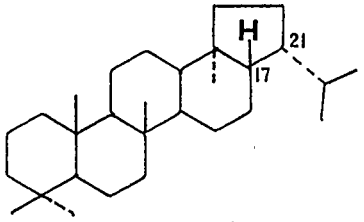


17β(H),21α(H)

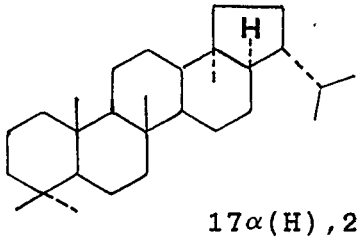


17α(H),21β(H)- the thermally more stable isomer

It is known that with increasing depth of burial (maturation) isomerization can occur at chiral centers.



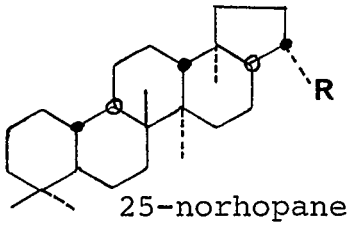
17β(H),21β(H)



17α(H),21β(H)

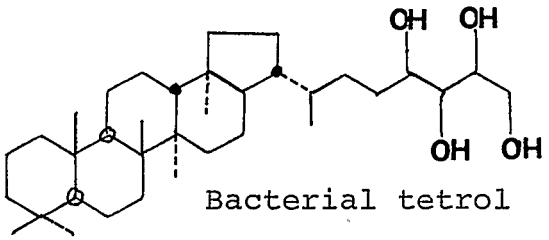
In hopanes, the naturally occurring 17β(H),21β(H) series is converted to the more stable 17α(H),21β(H) series and with increasing maturation this is followed by conversion (for members with greater than 30 carbons) of the preferred configuration at C-22 to a mixture of diastereomers[3]. At higher levels of maturity, the 17β(H),21α(H) isomer also converts to the 17α(H),21β(H) isomer. The fact that some geological samples have revealed the predominant hopane to be the 17β(H),21β(H) and not the more stable form would tend to suggest that the biomarkers in crude oils may not always be derived directly from the source rock, but may be acquired during migration or after accumulation in the reservoir[5].

The A/B ring demethylated hopanes found in biodegraded crude oils have been shown to be 17 α (H)-25-norhopanes. These are demethylated at the C-10 position and not at the C-4 position as previously thought[6,7]. It is suggested that they are produced during the last stages of crude oil biodegration and as such could be used as biomarkers for this stage[7].



25-norhopane

Hopanes with C-28 missing or a C_2 side chain have also been found. Hopanes are thought to be derived from a tetrol which has been isolated from several bacteria.



Bacterial tetrol

Hopanes have been divided into 3 series:

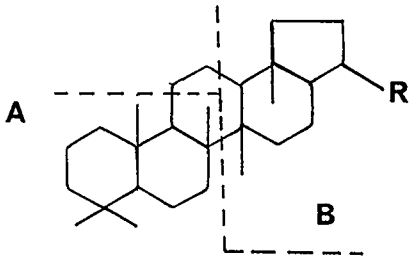
(17α,21β)H - $\alpha\beta$ hopanes

(17β,21α)H - $\beta\alpha$ hopanes

(17β,21β)H - $\beta\beta$ hopanes

The ββ-hopanes are always synthesized by living organisms and occasionally the βα type has been found in them, but to date, the αβ-hopanes have not been detected in living organisms[8]. Hence, the latter type must come from the isomerization of the ββ or βα-hopanes during diagenesis or early catagenesis. Thus the stereochemistry can be used as an indication of the maturation of the organic material.

Because biomarkers are present in such small quantities in any crude oil sample, the tool most widely employed in their detection and identification has been the gas chromatograph - mass spectrometer. Their identification has been largely based on the fragmentation pattern shown in their mass spectra and their elution times from the gas chromatograph. The following diagram shows the fragmentation pattern of the basic hopane skeleton.



A fragment: m/e 191

B fragment:

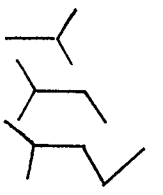
m/e 149 for C_{27} - R=H

m/e 177 for C_{29} - R=Et

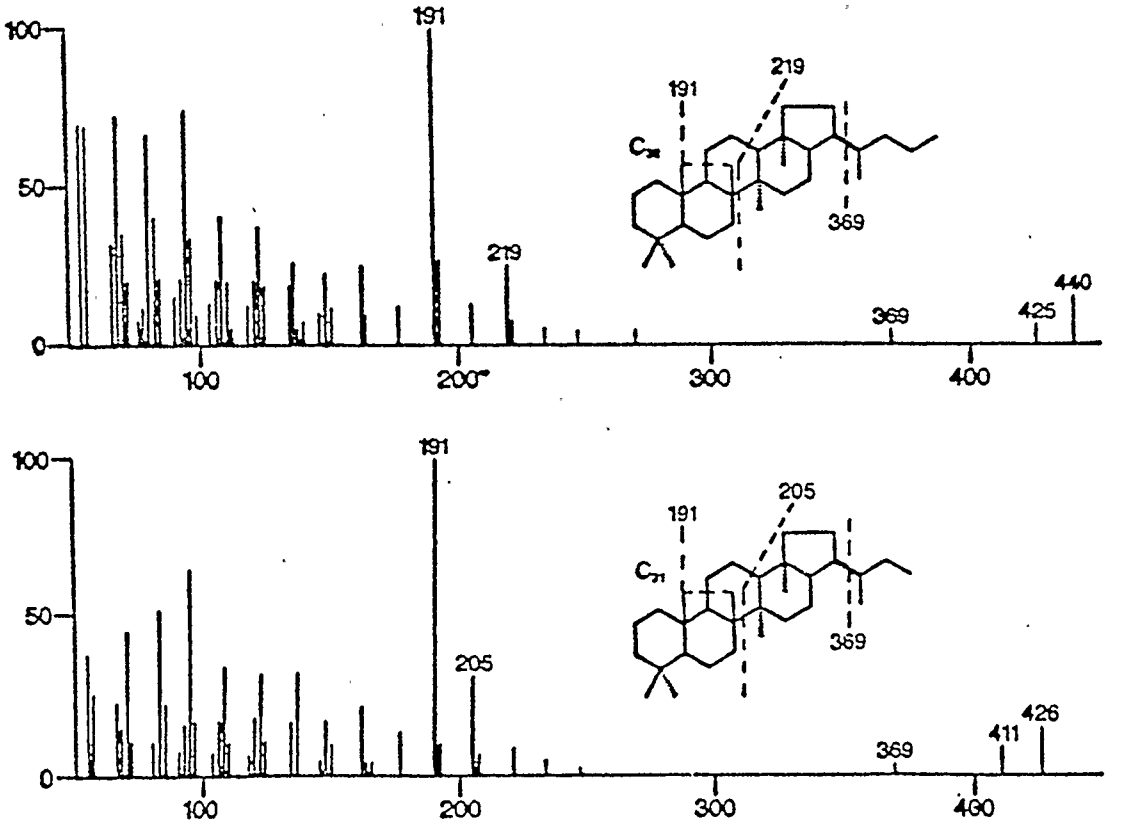
m/e 191 for C_{30} - R=

m/e 205 for C_{31} - R=

m/e 219 for C_{32} - R=



These fragmentation patterns are illustrated by the mass spectra of some representative examples[9] as shown in figure 5. The m/e 191 fragment intensity has been shown to be greater than that of fragment B for the 17 α(H),21β (H) series and is reversed for the 17β(H),21β(H) and 17β(H),21 α(H) (moretane) series[4]. For the $\beta\beta$ series, the B fragment intensity can be as much as twice that of the m/e 191 (A) fragment while for the $\beta\alpha$ series it is only slightly higher than fragment A[10]. The m/e 177 fragment also represents the A ring fragment of hopanes that have been demethylated in the A/B ring whether from C-4 or C-10.



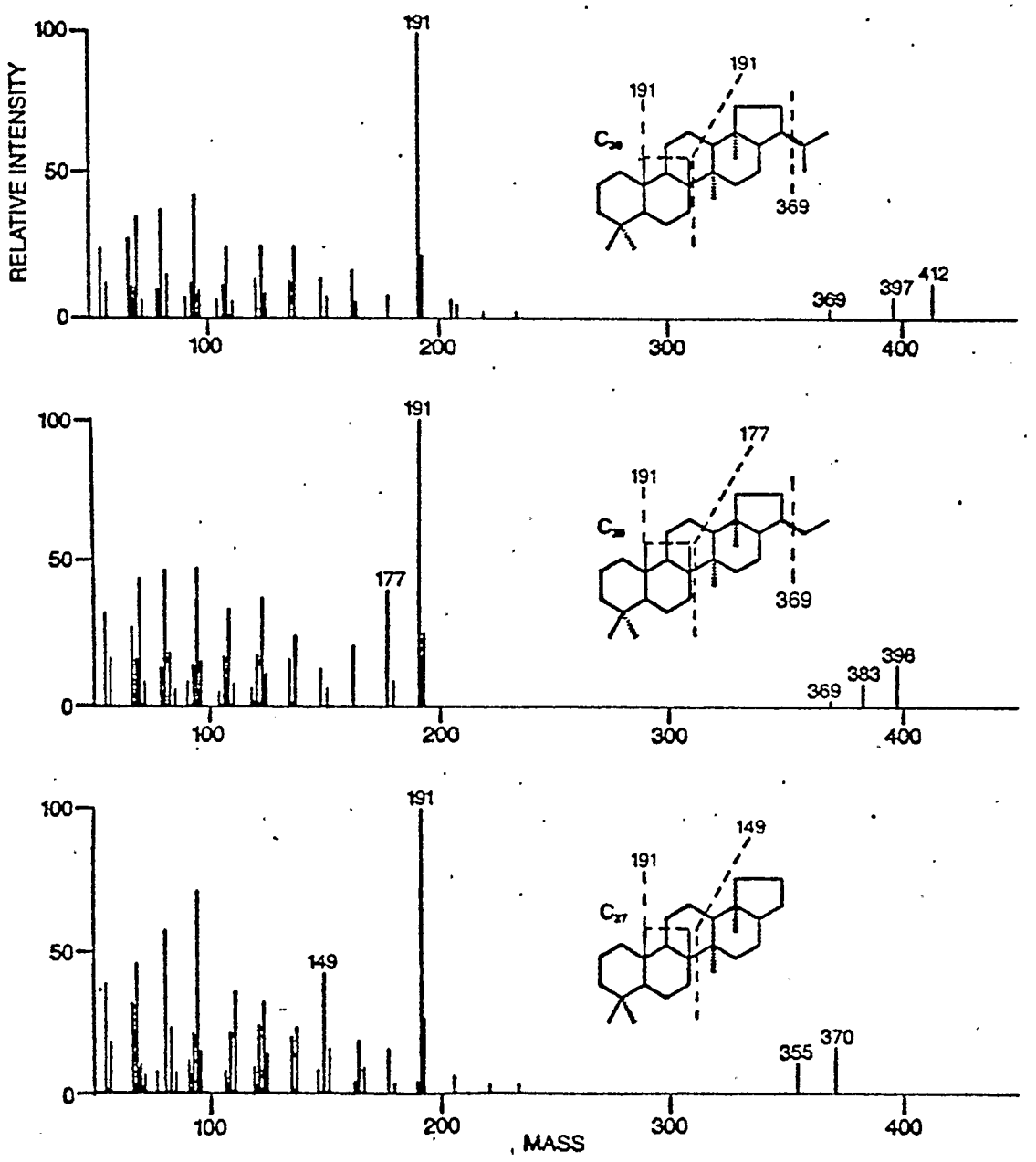
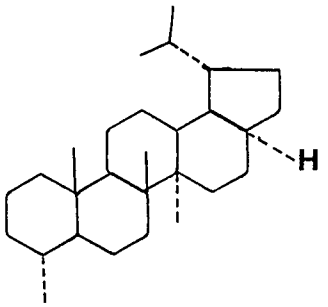


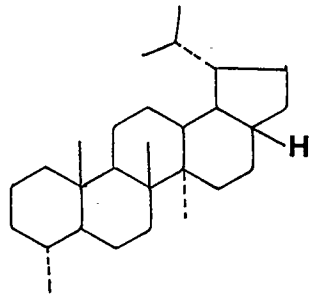
Figure 5. Mass spectra of some hopanes

Lupanes

Unlike many of the other biomarkers, the occurrence of lupanes in crude oils is rare. Two isomers of 23,28 - bisnorlupane have been found in high abundance in immature sediments in Greenland and the Suez[11,12] and only recently, isomeric C_{28} lupanes have been found in the offshore MacKenzie Delta oils[13].



17α(H), 4α(methyl)bisnorlupane



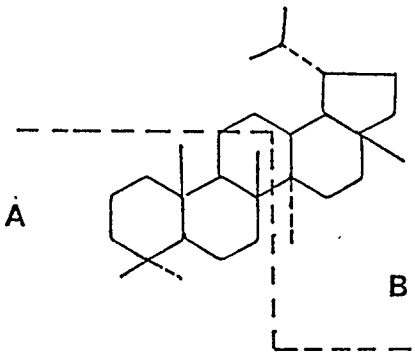
17β(H),4α(methyl)bisnorlupane

Most naturally occurring lupane derivatives are of the 17α(H),19β(H) configuration but the samples found in crude oils to date show a predominance of the 17β(H) isomer. It is too early to make any predictions regarding their occurrence, as to which compounds are going to be most prevalent in variously degraded oils or even their own degree of biodegradation. One might assume that they would follow a course similar to that of the hopanes.

I.4. Research Objectives

The structure of many of the biomarkers isolated from geological samples have been elucidated using GC-MS and NMR. Their occurrence in such minute quantities in complex mixtures makes it difficult for any other technique to be practical. Even so, in some cases the only way to use the NMR results was to compare the spectral data to known compounds with a structure similar to that proposed for the isolated material. Since very few lupanes have been identified in crude oils, little has been done in the way of synthesizing any standards. For this reason it was thought appropriate to synthesize the derivatives that so far have been identified in crude oils, since 17α(H) and 17β(H)-28-norlupane were used as the reference standard in their identification.

It was decided to make 23,28-bisnorlupane and lupane, di-deuterated in the A ring. In addition to these two compounds, 28-norlupane and lupane itself were prepared. Both 28-norlupane and lupane have been prepared previously[11,14]. The di-deuterated lupane was wanted as a standard for mass spectral studies. The MS of lupanes and derivatives, non-funtionalized in the A ring, give an "A" fragment of m/e 191. If the A ring could be dideuterated this fragment would occur at m/e 193 and this could be used for quantitative studies.


A
B

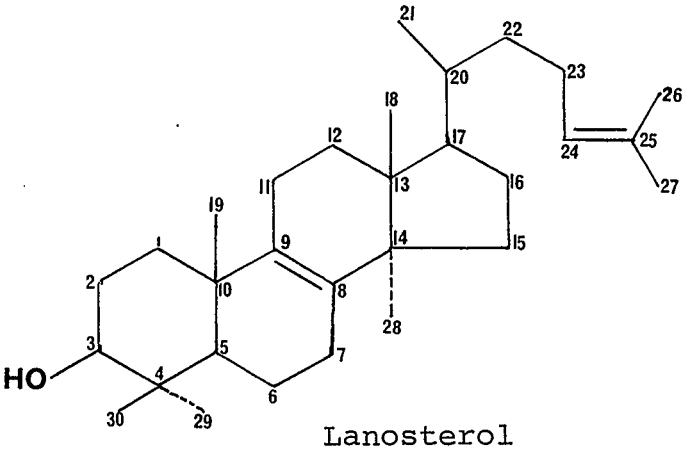
CHAPTER II

MODEL STUDIES

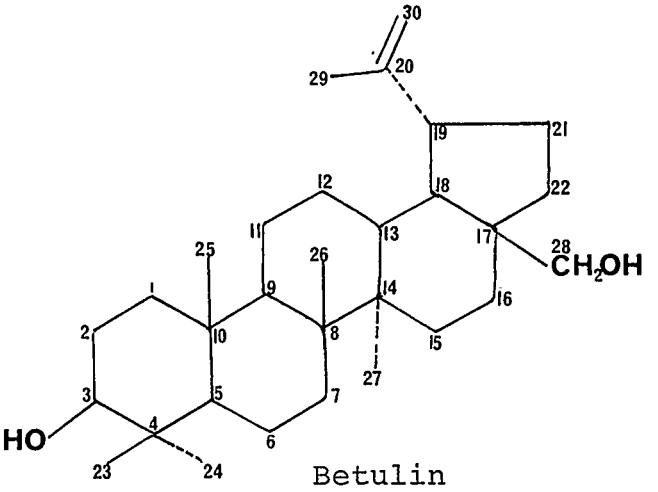
Betulin was chosen as the starting material in the synthesis of several lupane derivatives because it has the basic carbon skeleton needed and the proper orientation and position of methyl groups and hydrogens. The functional group changes necessary for the synthesis of these compounds were removal of a C-4 methyl group and the C-28 carbon atom. The preparation of bisnorlupane necessitated both of these modifications. Another goal was the incorporation of two deuteriums into the A ring of lupane.

Lanosterol was chosen as a model compound for some of the work because it has the same A, B ring system and the same orientation of hydrogens and methyls in these rings. It lacks the methyl at C-8 and has a double bond between C-8 and C-9 but it could still be used to test reactions for the elimination of the C-4 methyl and for attempting various deuteration methods. On examination this approach seemed quite reasonable and for the most part this was true.

The numbering system used for lupanes and lanosterols is depicted below.



Lanosterol



Betulin

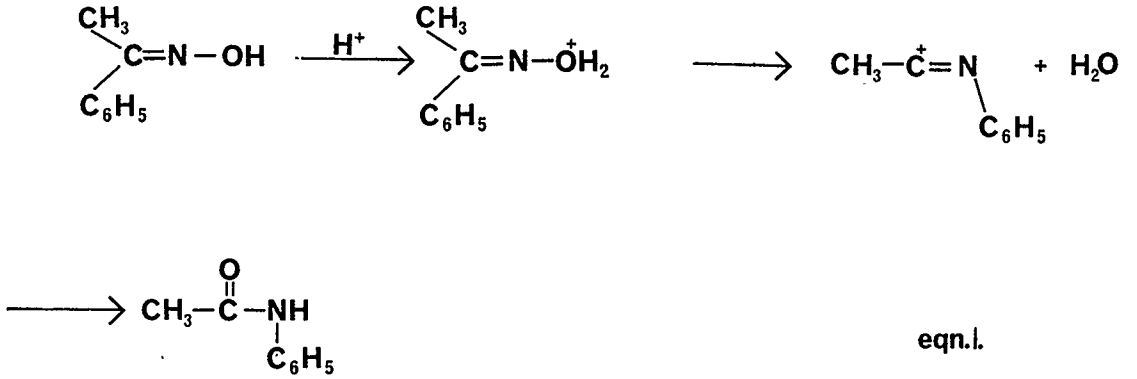
II.1 Removal of a methyl group from C-4 gem dimethyl terpenoids

Using 4,4-dimethyl-5α-cholestan-3-one as a model, Pinhey et al.[15] used the series of reactions shown in scheme 1 to remove a C-4 methyl group. They used the general idea of the scheme in the conversion of dihydrolanosterol to 4α,14α-dimethyl-5α-cholest-8-en-3-one and in the synthesis of 4β - demethylglycyrrhetinic acid. Because of its ready availability in quantity, lanosterol was chosen for model studies. Since commercial lanosterol is a mixture of lanosterol and dihydrolanosterol it was first necessary to hydrogenate the $\triangle^{24}$ double bond.

The disappearance of the signal at 130.87 and 125.26, attributed to the $\triangle^{24}$ double bond in the ^{13}C-NMR[16], as well as the appearance of a molecular ion at m/e 428 in the mass spectrum confirmed hydrogenation. The oxidation of dihydrolanosterol was initially carried out using CrO_3/pyridine[17] but later pyridinium chlorochromate (PCC)[18, 19] was used. Both methods gave yields of about 80% but the PCC route was cleaner in that no emulsion was formed as was the case with the CrO_3/pyridine method. The reaction mixture from the PCC oxidation was chromatographed on a basic alumina column to give a product that was sufficiently pure to carry out further reactions.

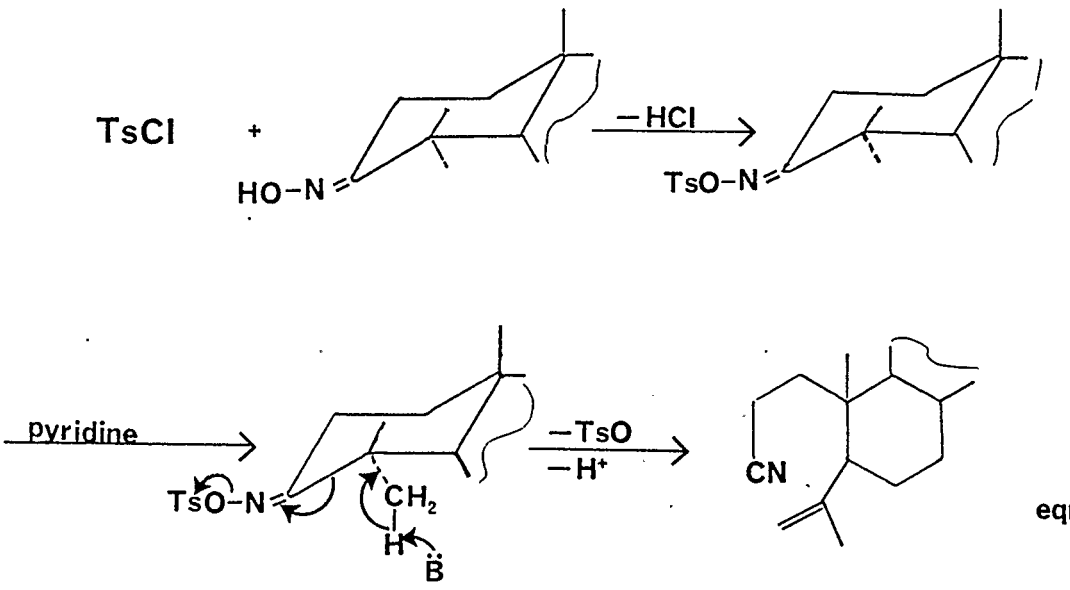
Oxidation of the C-3 hydroxyl group was followed by conversion to the oxime. The shift of the C-3 signal at 217.76 ppm in the ^{13}C-NMR for 3-oxo-5α-lanost-8-ene[20] to a signal at 167.17 ppm confirmed the formation of the oxime (C=N-OH). Oximes normally show signals in this region. The IR further confirmed the oxime by showing no carbonyl band but showing a band in the hydroxyl region and a C=N band at 1680 cm^{-1}.

The seco nitrile was prepared by an abnormal Beckmann rearrangement of the oxime by treating it with p-toluenesulfonyl chloride and pyridine. The normal Beckmann rearrangement is an acid catalysed transformation of a ketoxime to an amide (eqn.1).



eqn.1.

The abnormal Beckmann rearrangement could be envisioned to occur as in equation 2.



eqn.2.

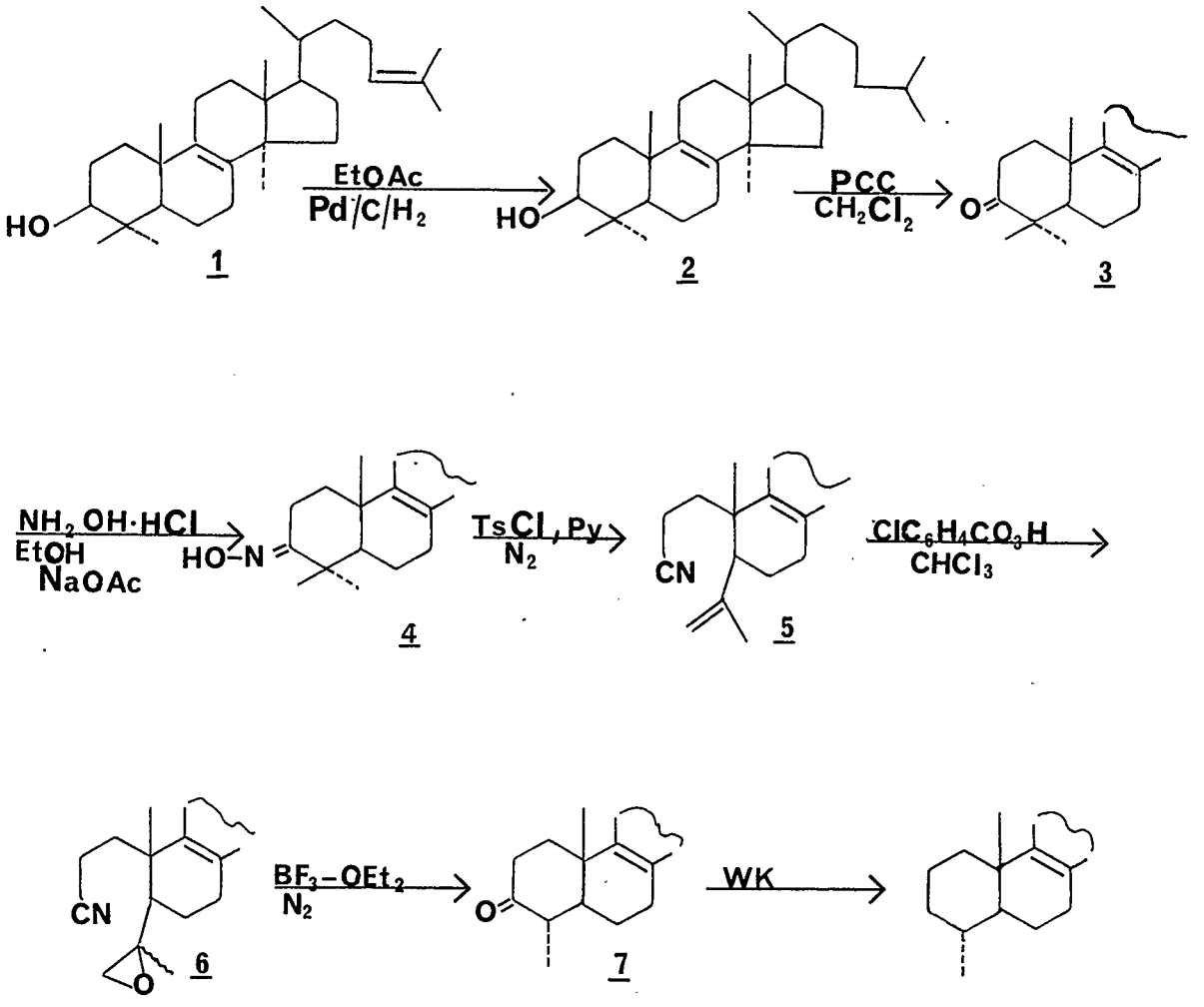
Using commercial lanosterol in the synthesis of 29-nor-lanostane derivatives, Takahashi et al.[21] published ^{1}H and ^{13}C-NMR data for the seco nitrile and the epoxy-nitrile of derivatives differing from the compound prepared here only in the side chain attached to the D ring. They attributed ^{1}H-NMR signals at δ1.77(3H,C=C-Me),δ4.69(1H) and 4.95(1H) to the isopropenyl group at C-5. The seconitrile of dihydrolanost-8-ene (5) showed a multiplet at 4.94 ppm, a multiplet at 4.67 ppm and a signal at 1.76 ppm in the ^{1}H-NMR, all due to the same isopropenyl group.

The ^{13}C-NMR spectrum for the seconitrile showed five signals in the 114-146 ppm region. The signals at 114.31

and 146.74 ppm belong to the double bond carbons in the isopropenyl group (C-4 and C-29) while carbons 8 and 9 show a single signal at 140.85 ppm. This leaves signals at 120.60 and 127.97 ppm either of which is due to the nitrile carbon atom, as nitriles show signals in this region.

Pinhey et al.[15] reported a ^{1}H-NMR signal at δ2.45 for the C-29-H_2 and at δ1.31 (3H,S,28-H_3) in 4,29-epoxy-3,4-seco-5α-lanost-8-en-3-nitrile while Takahashi et al.[21] reported ^{1}H-NMR signals at δ1.33(3H,S,C$\overset{O}{<}$C-Me) and δ2.69 (2H,S,C$\overset{O}{<}$C-H_2) for an epoxy-nitrile differing by having a hydroxyl group at C-24[21]. The epoxy-nitrile 6 synthesized here showed signals at 1.32 and 2.67 ppm due to the same epoxide protons. Normally the Δ^{8} double bond carbons show signals at 133 and 135 ppm in the ^{13}C-NMR spectrum but in the epoxy-nitrile they have been shifted to 140.56 ppm and the nitrile carbon appears at 127.69 or 120.02 ppm

3-Oxo-30-norlanost-8-ene (7) showed a signal for the C-3 carbonyl at 213.75 ppm in the ^{13}C-NMR spectrum while the corresponding signal for 3-oxolanost-8-ene occurs at 217.76 ppm. One would expect the loss of a C-4 methyl group to cause a shift in the position of the C-3 carbon signal. The C-29 signal has shifted from 21.38 for 3-oxolanost-8-ene[20] to 11.45 ppm for 7[22] and the C-4 carbon signal has also shifted from 47.46[20] to 45 ppm[22]. The appearance of a signal at 11.45 ppm and the disappearance of the signal at 26.36(C-30)[22] are evidence for the loss of a methyl group from C-4. The IR spectrum of 7 showed a strong carbonyl band not present in the epoxy-nitrile 6.



Scheme 1. Removal of a C-4 methyl group

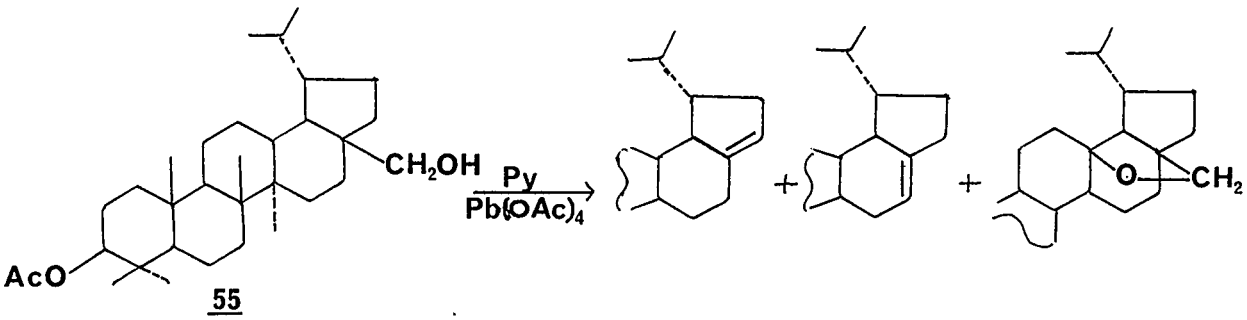
II.2 Removal of the C-28 carbon atom from betulin

Since lanosterol has no carboxylic acid functionality attached to a quaternary carbon atom, betulin itself had to be used. Different methods were tried in attempts to remove the C-28 carbon atom most efficiently.

II.2.1 Photo-oxidation

The method of Vystrčil and Protiva[23] was tried using 3-acetoxy-28-lupanol (55), described in Chapter 3, as starting material. Scheme 2 shows the products obtained by these authors. The ^{1}H-NMR of the reaction products indicated a similar mixture of compounds even after being chromatographed on silica gel impregnated with $AgNO_3$.

The ^{1}H-NMR showed a signal at 5.26 ppm due to the Δ^{16} double bond and another at 5.1 ppm due to the $\Delta^{(17-22)}$ double bond. These values correspond to those suggested by Protiva and Vystrčil[23]. The C-28 protons of the ether showed a pair of doublets at 3.62 and 3.47 ppm[23]. Hydroboration and protonolysis, with acetic acid, of the reaction mixture resulted in a loss of the signals due to the double bonds but those from the ether remained. No attempt was made to separate the ether from the saturated compounds since the overall % yield was low. Because of the poor yield this method was not pursued further.

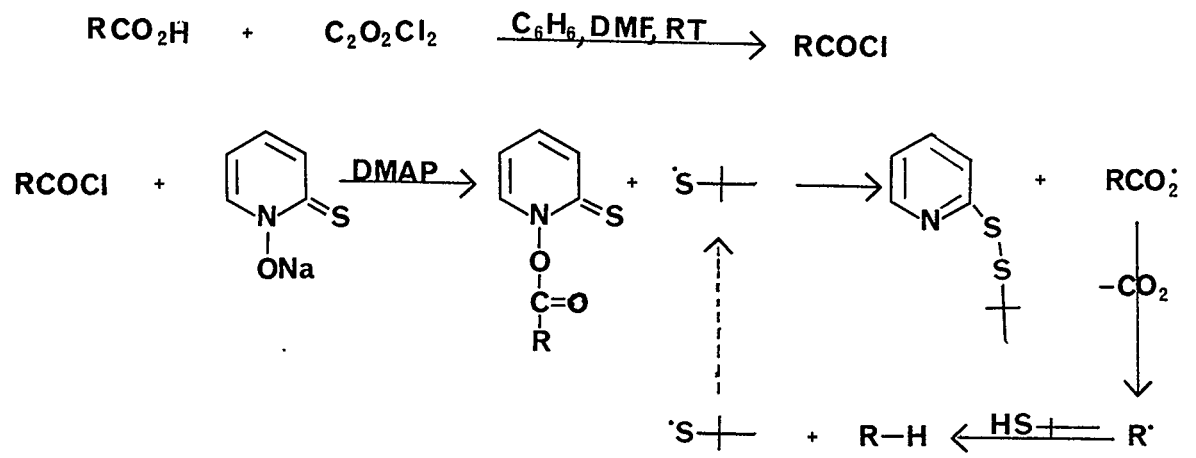


55

Scheme 2. Photo-oxidation reaction products.

II.2.2 Free radical method for removal of the C-28 carbon atom

The removal of the C-28 carboxyl group was carried out using the method of Crich and Barton[24]. The reaction follows a free radical mechanism as shown in Scheme 3. The acid chloride of 3-oxo-28-lupanoic acid was prepared and used immediately to form the thiocarbonyl ester from the reaction with the sodium salt of N-hydroxypyridine-2-thione in the presence of a catalytic amount of p-dimethylaminopyridine (DMAP). The ester was decomposed thermally in the presence of t-butylmercaptan via a free radical chain mechanism. The mercaptan acts as a radical trapping agent. The authors carried out decarboxylations on two similar compounds, both of which had an acetate at C-3 whereas the compound used here had a carbonyl group at that position.



Scheme 3. Free radical decarboxylation

A GC-MS of the product showed that a small amount (~10 %) of a 28-norlupene had also been formed. This should have been expected as a possible side product from a free radical reaction. The optimum reaction time was determined by following the reaction by TLC and 6 hours seemed to give the best results while longer reaction times gave a poor product yield. The saturated compound was separated from the norlupene by chromatographing the mixture on silica gel impregnated with $AgNO_3$.

The GC-MS of the first material eluted from the column showed a molecular ion at m/e 412 but no peak at m/e 410 which would be characteristic of any norlupene impurity present. The ^{1}H-NMR showed a complex multiplet at 2.48 ppm that is present in all 3-oxo-lupane derivatives. According to ^{1}H-NMR data on 3-keto-triterpenoids by Chari, Loganathan and Trivedi[25] this group of signals is due to the two protons on C-2 while a multiplet at 1.94 ppm could very well be from the C-1 protons[25].

The C-3 carbonyl signal appeared at 218.37 ppm in the ^{13}C-NMR and there was no evidence of any signals in the 120 - 140 ppm region where signals from unsaturated carbon atoms would be expected to appear. The signals at 18 ppm and 43.14 ppm for the C-28 methyl and C-17 carbon atoms, respectively, in 3-oxo-lupane[26] were both absent. Instead, there was a signal at 43.74 ppm which could be the C-17 carbon atom with only a hydrogen and no methyl group attached.

II.3 Di-deuteration of dihydrolanosterol derivatives

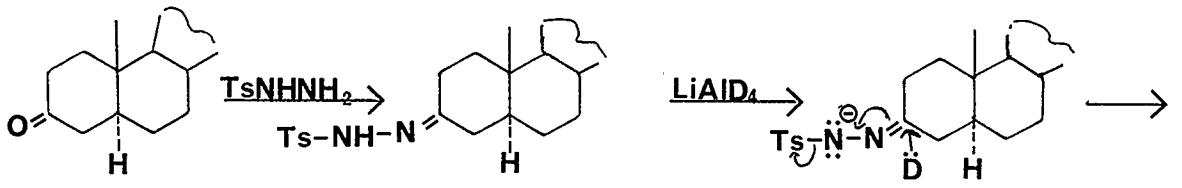
Several different approaches were attempted in trying to di-deuterate the A ring with a high degree of isotopic purity. A method was needed in which the reaction site would be at the C-3 alcohol or carbonyl, i.e. $\rangle C{=}O \rightarrow \rangle C{=}R \rightarrow \rangle C{-}D_2$ since it was necessary to have only protons or deuterons on the A ring carbon atoms. It was initially felt that if the deuterium atoms were introduced onto the α carbon reduction of the carbonyl to the methylene could result in the loss of these atoms. A 95 % or better degree of purity was desired. Isotopic purity was not a problem with various intermediates but conversion to the fully saturated, di-deuterated compound was not as easy to obtain. The different methods tried will be described.

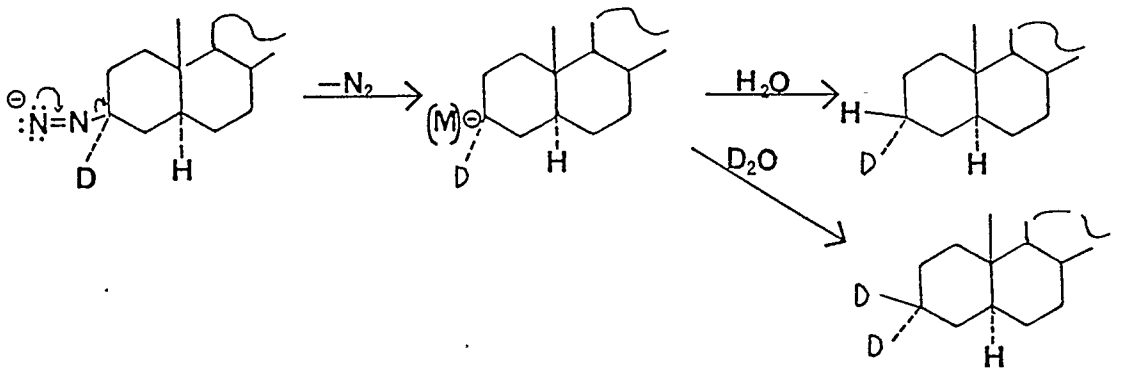
II.3.1 Tosylhydrazone reduction

According to the literature, a method that gives excellent isotopic purity is the reduction of the tosylhydrazone derivative of 3-keto-steroids[27]. Seemingly, the only problem

with reductions of this nature is the formation of olefinic side products. In fact, it is also a method for the preparation of alkenes[28]. Even if they were formed it was felt that they could be separated from the saturated product by chromatography on silica gel impregnated with $AgNO_3$. This proved to be the case. According to reference (27), the conversion of 5α -pregnane-3,20-dione-20-ethylene ketal into the 3,3-d_2 labelled analog was achieved with 94 % isotopic purity by the reaction of the tosylhydrazone with a large excess of lithium aluminium deuteride.

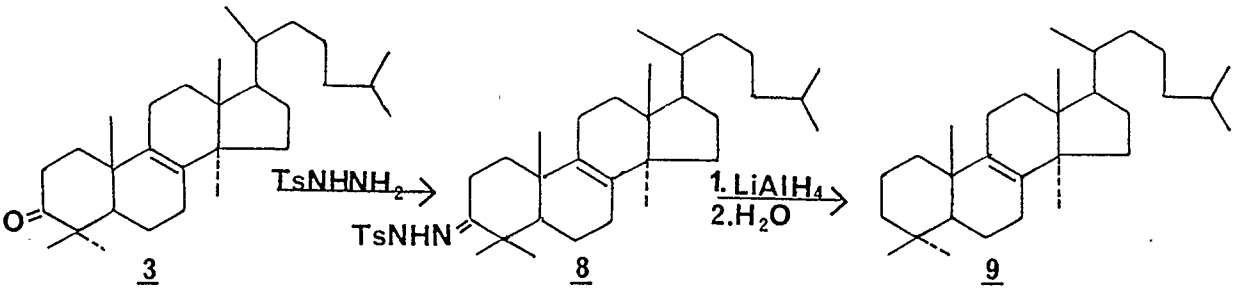
The initial step in the reaction is the abstraction of the acidic hydrazone proton, followed by hydride attack on the carbon of the C=N bond, mainly from the α-side, together with the simultaneous loss of the tosylate anion. Loss of the nitrogen then leads to formation of an insoluble complex which upon decomposition with water forms the methylene derivative. If $LiAlD_4$ is used and the complex decomposed with water the monodeuterated product is obtained. To prepare the di-deuterated compound both steps must be done with deuterated reagents, i.e. $LiAlD_4$ and D_2O for the decomposition step. Scheme 4 shows the reaction mechanism[27].





Scheme 4. Mechanism for the tosylhydrazone reduction.

The sequence of reactions attempted is shown in Scheme 5. The tosylhydrazone of 3-oxo-lanost-8-ene (3) was prepared and its structure confirmed by IR and ^{1}H-NMR. Tosylhydrazone 8 was treated with a 20 molar excess of $LiAlH_4$ and the excess reagent decomposed with water[29,30]. The product was chromatographed on silica gel impregnated with $AgNO_3$. The ^{1}N-NMR of the first compound eluted showed no unsaturation other than that at $\triangle^{8}$ and the ^{13}C-NMR showed only the double bond signals at 135,07 and 134.00 ppm for the C-8 and C-9 carbon atoms. The ^{1}H-NMR of the second compound eluted showed a multiplet at 5.46 ppm which was attributed to the proton on a double bond between carbon atoms 2 and 3. The ^{13}C-NMR of this compound showed signals at 135.03 and 133.01 ppm that showed off-resonance singlets, thus these are carbon atoms 8 and 9. Signals at 138.17 and 121.91 ppm both of which showed off-resonance doublets, indicative of methine carbons, and are therefore, due to carbon atoms 2 and 3. Since the first product was the desired lanost-8-ene it was assumed that the reaction would work equally well using $LiAlD_4$ and D_2O on 3-oxolupane as per reference (27).



Scheme 5. Tosylhydrazone reduction

Unfortunately when $LiAlD_4$ and D_2O were reacted with 3-oxolupane, a mixture of mono and di-deuterated products was obtained. All attempts to improve the yield of the di-deuterated product were unsuccessful.

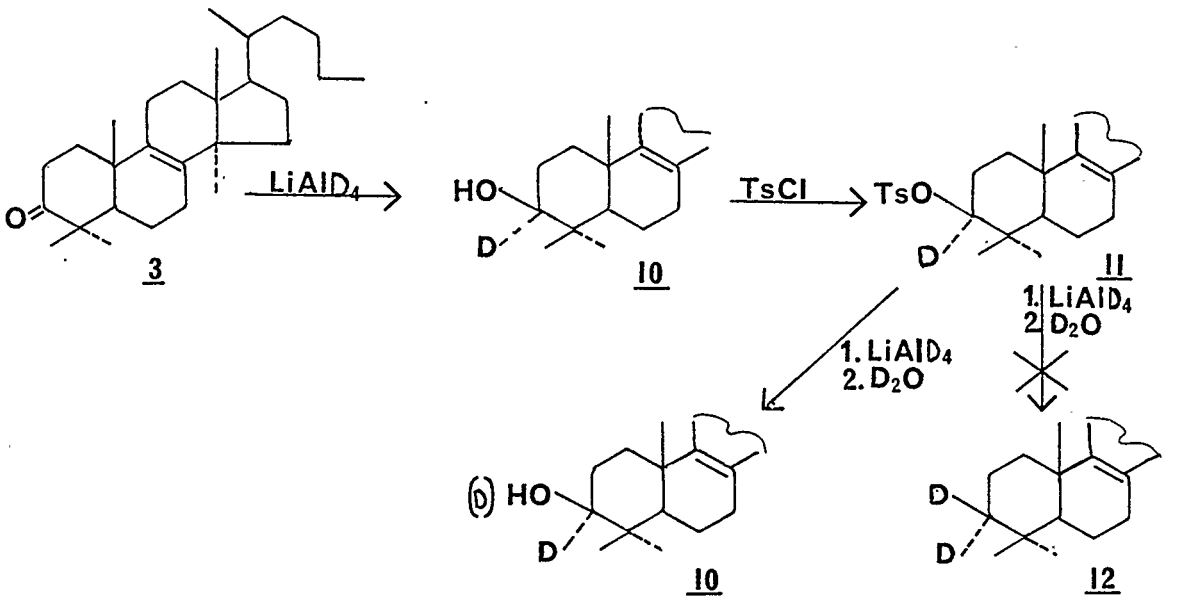
II.3.2 Elimination of the tosylate from (11)

The second approach was to react $LiAlD_4$ with 3-oxolanost--8-ene, introducing a deuterium onto C-3 and reducing the carbonyl to the hydroxyl group. The plan was to convert the alcohol to the tosylate and then treat this with $LiAlD_4$, replacing the tosyl group with deuterium. This approach is shown in Scheme 6.

The initial reduction with $LiAlD_4$ successfully reduced the carbonyl to the alcohol and introduced a deuterium onto the C-3 carbon atom. This was confirmed by IR which showed a hydroxyl but no carbonyl band and the ^{1}H-NMR which did not

show the doublet of doublets at 3.25 ppm shown by the C-3 proton when there is also an equatorial hydroxyl group attached to the C-3 carbon atom.

The tosylate was prepared but unlike cases in the literature[31], the final reduction with $LiAlD_4$ resulted in regeneration of the alcohol and not formation of the di-deuterated lanost-8-ene as was hoped. The MS showed peaks at m/e 429 consistent with the molecular ion of the alcohol.



Scheme 6. Elimination of the tosylate from C-3

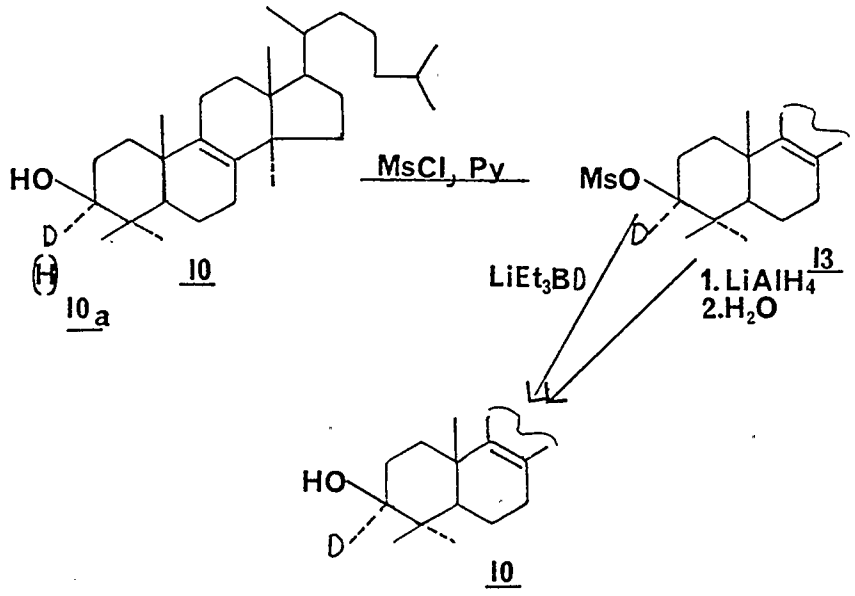
Considering the results from the last sequence of reactions, it was obvious that the two methyl groups on C-4 and the angular methyls at C-10, C-13 and C-14 provided too much steric hindrance for cleavage of the C-O bond in the tosylate, and rather, cleavage was between the sulfur and oxygen instead. The thought now was to use a smaller leaving group such as a mesylate or if that did not work with $LiAlD_4$, to use a more powerful reducing agent, e.g. lithium triethylborodeuteride ($LiEt_3BD$).

II.3.3 Elimination of the mesylate from (13)

The mesylate was prepared from the reaction of 10a with methanesulfonyl chloride[32] as outlined in Scheme 7. The ^{1}H-NMR spectrum showed a doublet of doublets at 4.37 ppm due to the C-3 proton coupling with the protons on C-2. These were absent in the ^{1}H-NMR spectrum of the deuterated mesylate 13. The methyl protons on the mesylate group showed a singlet at 3.03 ppm for both the deuterated and non-deuterated mesylates.

The ^{13}C-NMR signals for the C-8 and C-9 carbon atoms were at approximately 135 and 134 ppm for both mesylates. The non-deuterated mesylate showed a signal at 90.52 ppm for the C-3 carbon atom, which was absent in the deuterated form. Normally a deuterium on a carbon atom results in a decreased signal intensity in the ^{13}C-NMR, often to the point that the signal is absent. The remaining signals were in the same respective position for both mesylates.

Reaction of the mesylate with $LiAlH_4$ gave the same result as the tosylate. Since lithium triethylborohydride ($LiEt_3BH$) is a very powerful hydride donor (i.e. strong nucleophile for S_N2 reactions) and has been used successfully to replace the mesylate group with hydrogen[33], albeit none of the compounds used were hindered steroids, the reaction was repeated using it as the reducing agent. Again the same result was found - hydrogenolysis as shown by TLC and the IR spectrum of the product.

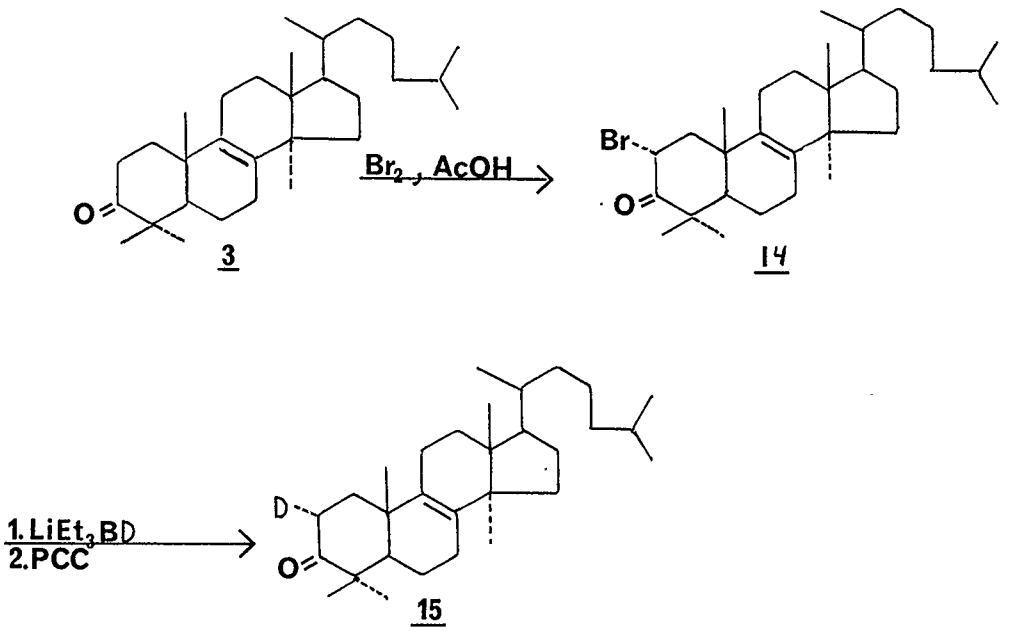


Scheme 7. Elimination of the mesylate from C-3.

II.3.4 Elimination of the mesylate from (19)

A different approach was attempted. Since elimination from C-3 was going to be difficult, it was decided to transpose the leaving group to C-2. In this case both adjacent carbons would carry protons and C-2 might be less hindered. A scheme such as 9 was envisioned but first it was necessary to see if a bromine on the A ring could be replaced with a deuterium as shown in Scheme 8. The standard method for introducing a bromine adjacent to a carbonyl was used[34]. The IR of the brominated product 14 showed a shift of the carbonyl band from 1707 cm^{-1} in 3-oxolanost-8-ene to 1729 cm^{-1}. The nucleophilic displacement of the bromine by deuterium was carried out using the method of H.C.Brown with $LiEt_3BD$[35]. The resultant alcohol was then oxidized back to the ketone, the IR of which showed the carbonyl band to again be at 1708 cm^{-1}.

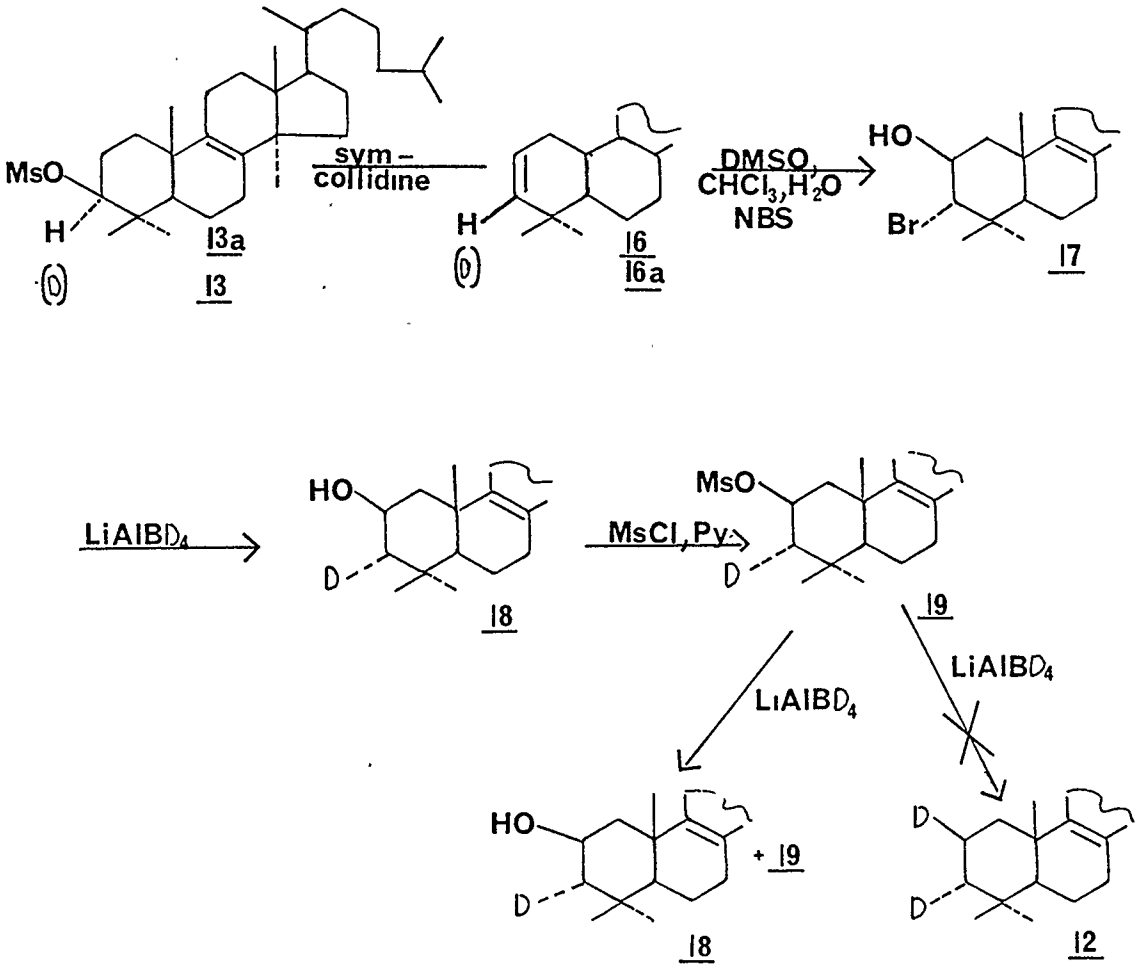
The ^1H-NMR of 2-bromo-3-oxolanost-8-ene (14) showed signals in the 4-5 ppm region which were absent in the spectrum for 2-deutero-3-oxolanost-8-ene (15). The ^{13}C-NMR data for the brominated ketone 14 was compared to that of brominated derivatives of 5α-cholestan-3-one and cholest-4-ene[36]. The C-3 signal had shifted to 203.31 ppm for 14 from the normal 217.8 ppm for 3-oxo-lanost-8-ene and the signals for the $\triangle^8$ carbon atoms had shifted to 144.96 and 137.64 ppm from 135.36 and 133.13 ppm respectively.



Scheme 8. Replacement of bromine with deuterium

It was thought that by starting with mesylate 13, already having a deuterium atom on C-3, a product containing 3 deuterium atoms could be prepared. Initially the ketone could be reduced with $LiAlD_4$ to form the deuterated alcohol from which the deuterated mesylate could be made. Elimination, followed by formation of the bromohydrin would result in C-3 having both a deuterium and a bromine atom. The IR of 17 showed the expected hydroxyl band while the ^{1}H-NMR showed a doublet at 4.55 ppm due to the proton on C-3. The spectrum also showed a multiplet at 2.03 ppm and a singlet at 1.56 ppm.

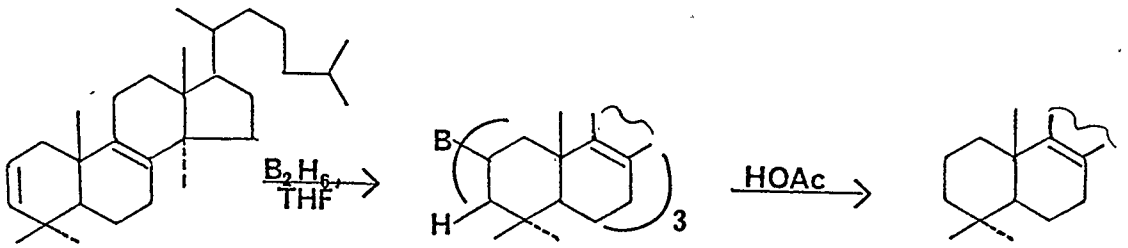
The bromohydrin was treated with $LiEt_3BD$, resulting in an exchange of bromine for deuterium. The mesylate 19 was prepared and subsequently treated with $LiEt_3BD$. This route proved to be even less successful than the reaction with the mesylate on C-3. Not only was the alcohol regenerated but some mesylate still remained. In the first case, all of the mesylate had reacted. This reaction sequence is shown in Scheme 9, starting with the non-deuterated mesylate 13a.



Scheme 9. **Elimination of the mesylate from C-2**

II.3.5 Hydroboration and protonolysis of lanosta-2,8-diene

Both deuterated and non-deuterated lanosta-2,8-diene were treated with diborane as in the literature (38-40), except that more reagent and longer reaction times were used. Deutero acetic acid was used to cleave the organoborane complex. From a comparison of the GC retention times of lanosta-2,8-diene and lanost-8-ene it appeared that a small amount of the latter was formed. This approach was also abandoned due to low yields.



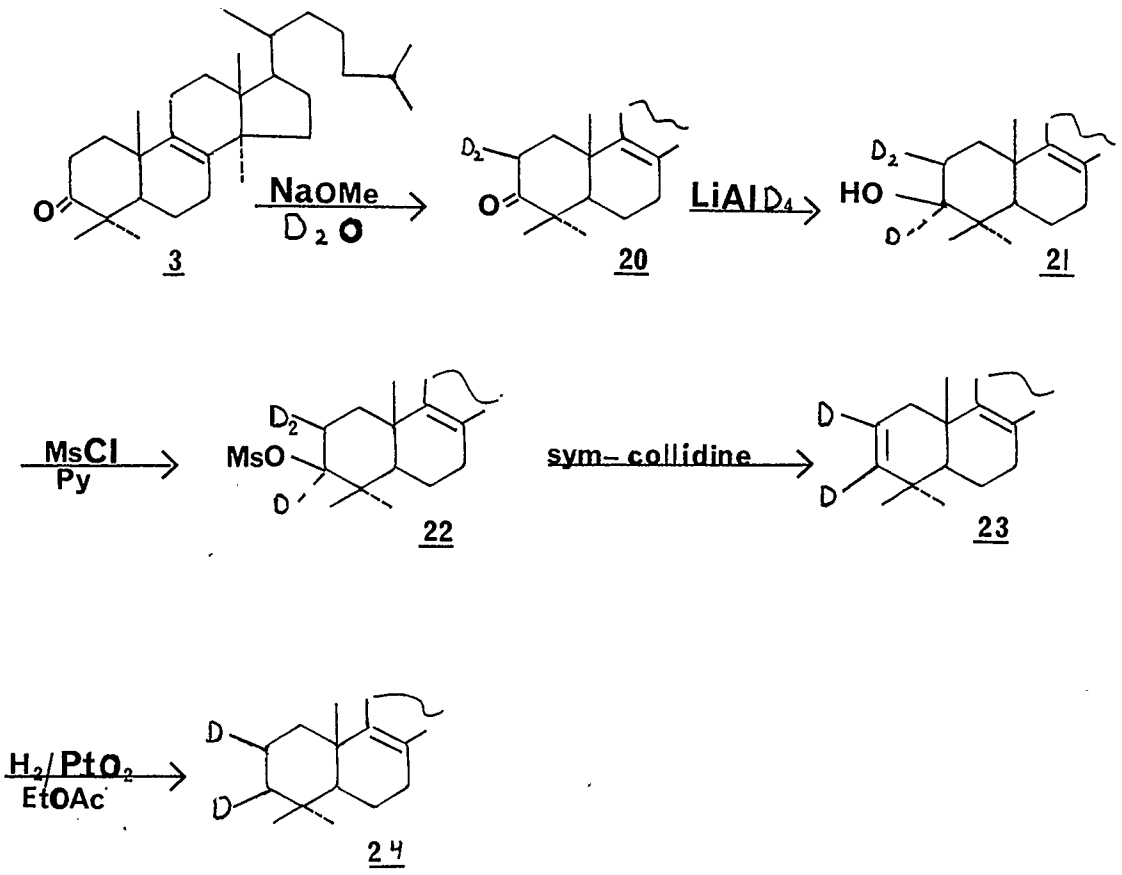
II.3.6 Deuterium exchange using NaoMe and D_2O

It was decided to prepare the di-deuterated lanost-8-ene from the reaction of 3-oxolanost-8-ene with sodium methoxide and deuterium oxide[41]. Reduction of the di-deuterated ketone by $LiAlD_4$ would give the tri-deuterated alcohol from which the mesylate could be prepared, followed by elimination and hydrogenation to give di-deuterated lanost-8-ene as shown in Scheme 10.

The ^{1}H-NMR of 3 showed a multiplet at 2.52 ppm due to the C-2 protons[25] which was absent in 20. The ^{13}C-NMR spectrum of 3 showed a signal at 34.69 ppm thought to be the C-2 signal[16]. The ^{1}H-NMR of 21 does not show the doublet of doublets at 3.25 ppm present in 3-hydroxylanost-8-ene (2). The ^{13}C-NMR spectrum of 2 showed a signal at 28.05 ppm due to the C-2 carbon atom and a C-3 signal at 79.12 ppm[16,42]. After reduction with $LiAlD_4$, the signal at 79 ppm for the tri-deuterated alcohol 21 was absent, but the C-2 signal at 28.07 ppm was still quite strong. From the literature[16,42], carbon atoms 25 and 29 show signals around 28 ppm.

The ^{1}H-NMR of the mesylate confirmed the fact that C-3 contained a deuterium atom. The non-deuterated mesylate 13a showed a doublet of doublets at 4.37 ppm due to the C-3 proton coupling with the C-2 protons which was absent in 22. The signal from the methyl protons on the mesylate occurred at 3.02 ppm for both mesylates. The signal at 25.7 ppm, in the ^{13}C-NMR, for the non-deuterated mesylate is probably from the C-2 carbon atom as it was absent in 22, as was the signal at 90.5 ppm for the C-3 carbon atom.

The GC-MS of 23 showed more than one component but it did indicate that the major component was di-deuterated. Since the lanosterol used was a technical grade, other products occur regularly in these reactions even though the TLC's only show one spot after they have been chromatographed on an alumina column.



Scheme 10. Dideuteration via NaOMe and D_2O of (3)

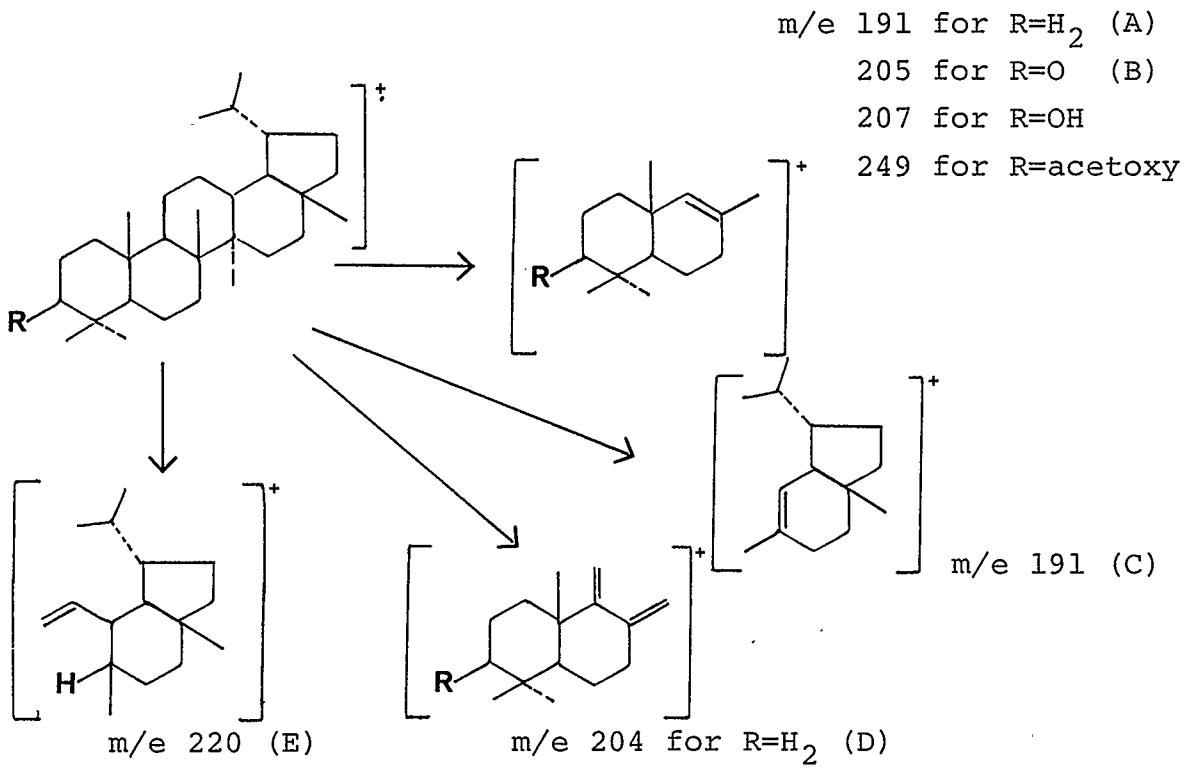
CHAPTER III

RESULTS and DISCUSSION

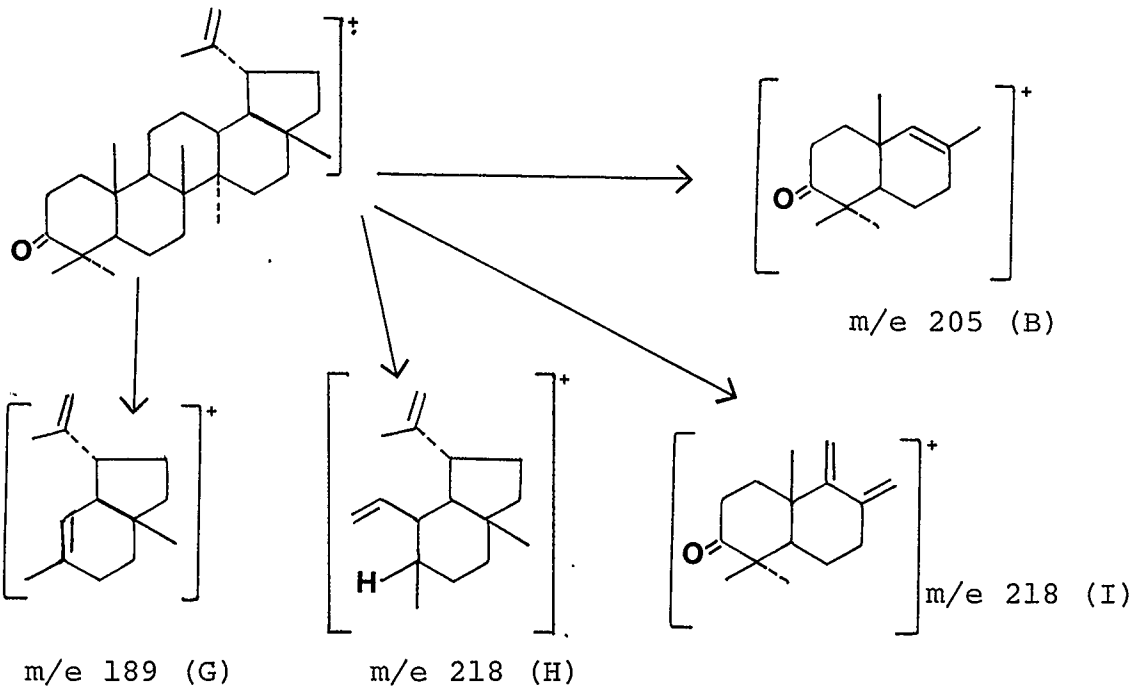
The purpose of this project was to prepare several lupane derivatives. There has been a lot of work done on modifying the basic lupane structure and much of the work has been done in the area of medicinal research.

III.1 The mass spectral fragmentation pattern for lupanes

Lupanes have a mass spectral fragmentation pattern that is well documented[43]. Figure 6 shows the mass spectral fragmentation of lupanes in general and as a specific example lup-20-en-3-one.



Lupanes



Lup-20-en-3-one

Figure 6. **The mass spectral fragmentation pattern for lupanes**

The (A) fragment, m/e 191, is the most characteristic fragment produced in saturated pentacyclic triterpenes. The loss of 43 mass units (C_3H_7) is pronounced in some members but minimal in highly substituted derivatives or in the presence of an isopropenyl group. Lupane has two fragments (A) and (C) with m/e 191, so this is the most abundant peak.

III.2 Synthesis of lupane (28)

Lupane has been prepared previously by Lehn and Ourisson[14], starting with lupeol benzoate from a natural extract. It was prepared here as shown in Scheme 11 using betulin as the starting material.

III.2.1 3β-Hydroxy-28-lupanol (dihydrobetulin) (26)

Betulin contains a double bond as part of an isopropenyl group attached to the E ring. The close proximity to the basic ring system made it more difficult to hydrogenate. It required more time and more catalyst compared to the hydrogenation of lanosterol. Dihydrobetulin melts at 275-276^{0}C[44] and betulin melts at 248-251^{0}C. The mass spectrum of betulin very clearly shows a molecular ion at m/e 442 while that of dihydrobetulin occurs at m/e 444. There was no peak at m/e 442 which would have been the case if there had been any betulin present.

The ^{1}H-NMR of both betulin and dihydrobetulin showed a doublet of doublets centered at 3.19 or 3.21 ppm, integrating for one proton, due to the axial proton on C-3 coupling with the C-2 protons[44]. Each also showed a pair of doublets; at 3.80 and 3.34 ppm for betulin, and at 3.79 and 3.31 ppm for dihydrobetulin. Each set of doublets integrated for one proton and these were due to the protons on the C-28 carbon atom which is attached to a hydroxyl group. The ^{13}C-NMR for dihydrobetulin showed the absence of any double bond carbon atoms. The signals for carbons 20 and 29 had shifted from 150.47 and 109.68 ppm to 29.3 and 15.38 ppm[26] respectively. They both showed a signal at 79 ppm for the C-3 carbon atom and at 60.6 ppm for the C-28 carbon atom[45].

III.2.2 3-Oxo-28-lupanal (27)

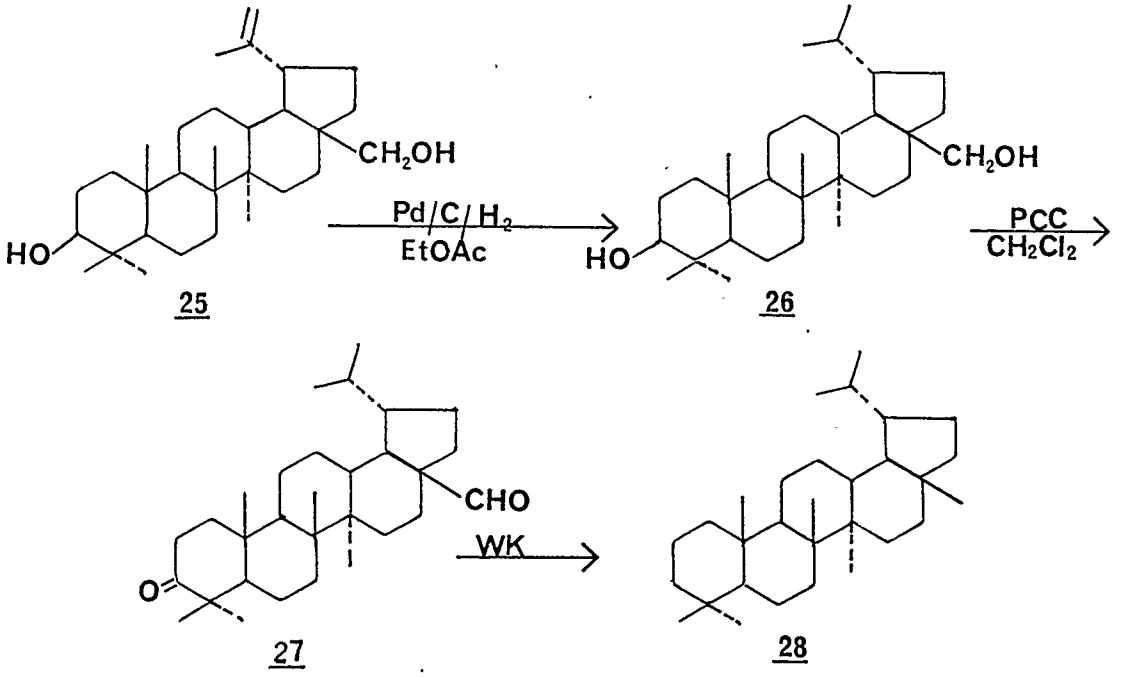
Oxidation of 26 with PCC resulted in the oxidation of

both alcohol groups, giving 3-oxo-28-lupanal (27). In the ^{1}H-NMR, the multiplet at 3.20 ppm due to the axial proton on C-3 was absent as was the pair of doublets at 3.31 and 3.79 ppm. Carbon atom 28 now contains an aldehydic proton which showed a singlet at 9.66 ppm instead. The multiplet due to C-2 protons adjacent to a carbonyl was at 2.48 ppm and the multiplet due to the C-1 protons was at 1.45 ppm[25].

Instead of signals at 78.9 and 60.6 ppm for carbon atoms 3 and 28 as in 26 these carbons now showed signals at 217.72 and 206.95 ppm[26]. A ketone carbonyl is generally shifted downfield relative to an aldehyde. The mass spectrum showed a molecular ion at m/e 440 and a dominant peak at m/e 205 which would be expected from fragment B.

III.2.3 Lupane (28)

A Wolff Kishner reduction (Huang-Minlon modification) of 27 was the final step in the synthesis of lupane. The IR showed no carbonyl bands and the ^{1}H-NMR showed no signal at 9.66 ppm for the aldehyde proton. The carbonyl signals at 217.22 and 206.95 ppm were absent in the ^{13}C-NMR but there were two new signals; at 42.20 and 18.11 ppm which correspond to literature values for C-3 and C-28 having only protons[45]. The signal at 43.2 ppm agreed closely to the literature values for the C-17 and C-14 carbon atoms[45]. The GC-MS showed that 28 was a single isomer having a molecular ion at m/e 412 and a base peak at m/e 191. This comes from fragments A and C as shown in Section III.1.



Scheme 11. Synthesis of lupane

III.3 Synthesis of 28-norlupane (31)

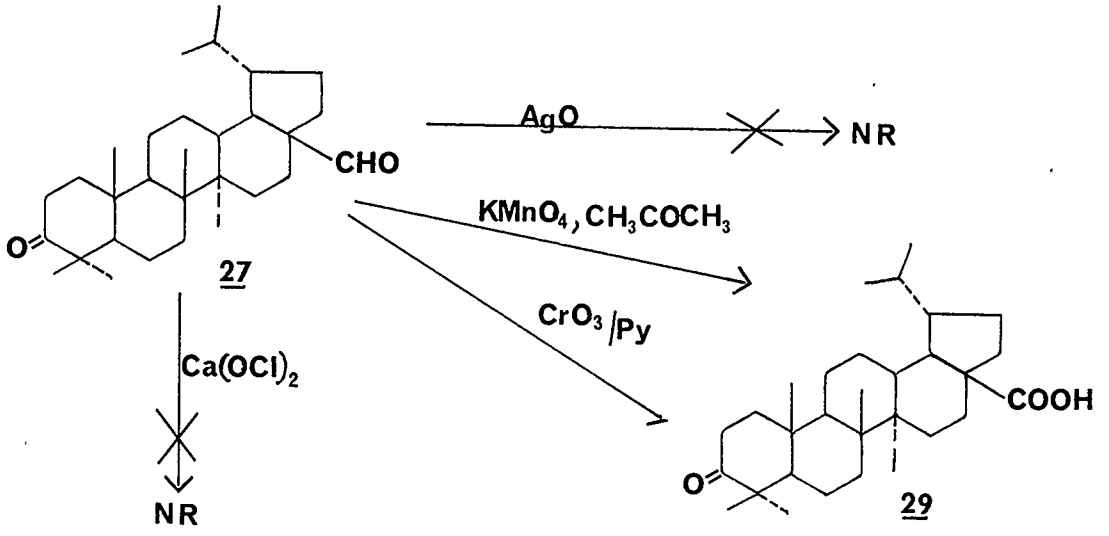
28-Norlupane has been prepared previously[11]. These workers started with betulin and made the diacetate, hydrogenated the product, then saponified it to yield the diol. Oxidation with n-bromosuccinimide followed by a Wolff Kishner reduction yielded lupan-28-ol which after photo-oxidation resulted in a mixture of 28-norlupenes. Column chromatographic separation and hydrogenation resulted in a mixture of the 17(α) and 17(β)-28-norlupenes.

A different approach was taken here. As outlined in scheme 12, hydrogenation of the double bond was the first

step. This was followed by two oxidation steps, then decarboxylation of C-28 as the acid function. A Wolff Kishner reduction completed the sequence. Photo-oxidation was tried but gave a very poor yield compared to the decarboxylation method.

III.3.1 3-Oxo-lupan-28-oic acid (29)

Starting with 27 oxidation to the acid was carried out. A number of different methods were tried in an attempt to oxidize the aldehyde to the acid. Oxidations with silver oxide and calcium hypochlorite yielded only the starting aldehyde. Prolonged reaction with CrO_3/pyridine did result in formation of the acid but only in 46 % yield. A more quantitative method was desired. The reaction with $KMnO_4$ gave yields of crude acid 29 of 90 % or better. The reaction was carried out by refluxing the aldehyde with $KMnO_4$ in acetone for one hour.



The IR of the acid showed a single carbonyl band at 1701 cm^{-1}. A broad band appeared in the hydroxyl region (3300 - 2500), characteristic of a carboxylic acid. The ^{1}H-NMR of the acid showed multiplets at 2.25 and 2.47 ppm. According to the literature[47], betulinic acid shows a signal for C-28 at 178.9 ppm and there was an upfield shift of the C-28 signal from 206.95 to 182.60 ppm. In betulinic acid the C-17 carbon signal occurs at 56.3 ppm[47] while in 29 it occurred at 57.0 ppm.

III.3.2 3-Oxo-28-norlupane (30)

The most critical step in the sequence of reactions with respect to yield was the decarboxylation of the acid. The two methods tried, have been discussed in Chapter II. The method of Barton and Crich[24] was chosen as it gave the best yield. The removal of the acid group was a two step process in which the acid chloride was formed and used immediately. The IR of the acid chloride showed no hydroxyl band and the carbonyl band had split into two bands; one at 1806 cm^{-1} for the C-28 carbonyl and the other at 1708 cm^{-1} for the C-3 carbonyl.

The ^{1}H-NMR of the crude decarboxylation product from the reaction showed that a small amount of 3-oxo-lupene had also been formed. Analysis by GC-MS also confirmed this; the first component eluted showed a molecular ion at m/e 410 which would correspond to 3-oxo-28-norlupene. 3-Oxo-28-norlupane showed a molecular ion at m/e 412. Comparing the ^{1}H-NMR to that of the products from the photo-oxidation reaction it seems that the norlupene formed from the method of Crich and Barton had the double bond between carbon atoms 17 and 22.

Barton et al. in their paper[24] make no mention of any other products being formed, in particular any unsaturated products, other than to say that aqueous extraction and flash chromatography over silica gel gave pure products. In this work about 10 % of unsaturated material was found.

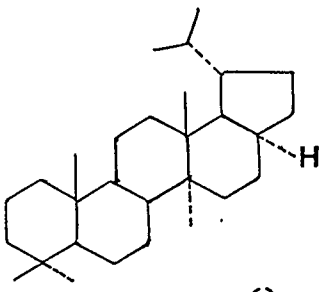
The crude product from this reaction was chromatographed on silica gel impregnated with $AgNO_3$. The first compound eluted showed a multiplet at 2.48 ppm due to the C-2 protons and another at 1.94 ppm likely due to the C-1 protons in the ^{1}H-NMR.

The ^{13}C-NMR of this compound showed the C-3 carbon signal to be at 218.37 ppm as expected but the signal at 182.60 ppm was absent. There was no evidence of unsaturation. The mass spectrum of 30 showed a molecular ion at m/e 412 and no peak at m/e 410. The base peak occurred at m/e 205 which would come from fragment B as shown in section III.1.

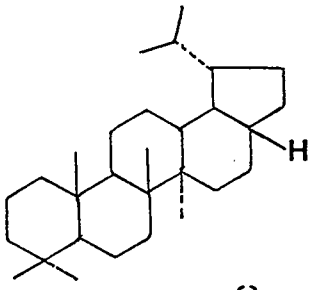
III.3.3 28-Norlupane (31)

The final step in the synthesis of 28-norlupane (31) was the Wolff Kishner reduction of 30 to convert the C-3 carbonyl to a methylene. The GC-MS of 31 showed it to be composed of two isomers, the second one to elute being the predominant one ($\sim$90 %). Generally the α-isomers tend to elute first. From the mass spectra of 28-norlupanes in the paper by Rullkötter et al.

the masses at 355 and 383 are of nearly equal intensity for the α-isomer. The molecular ion at m/e 398 was of a similar intensity. The mass spectrum of the β-isomer showed the mass at 355 to be of weaker intensity than that of 383 and the molecular ion mass was more intense than either.



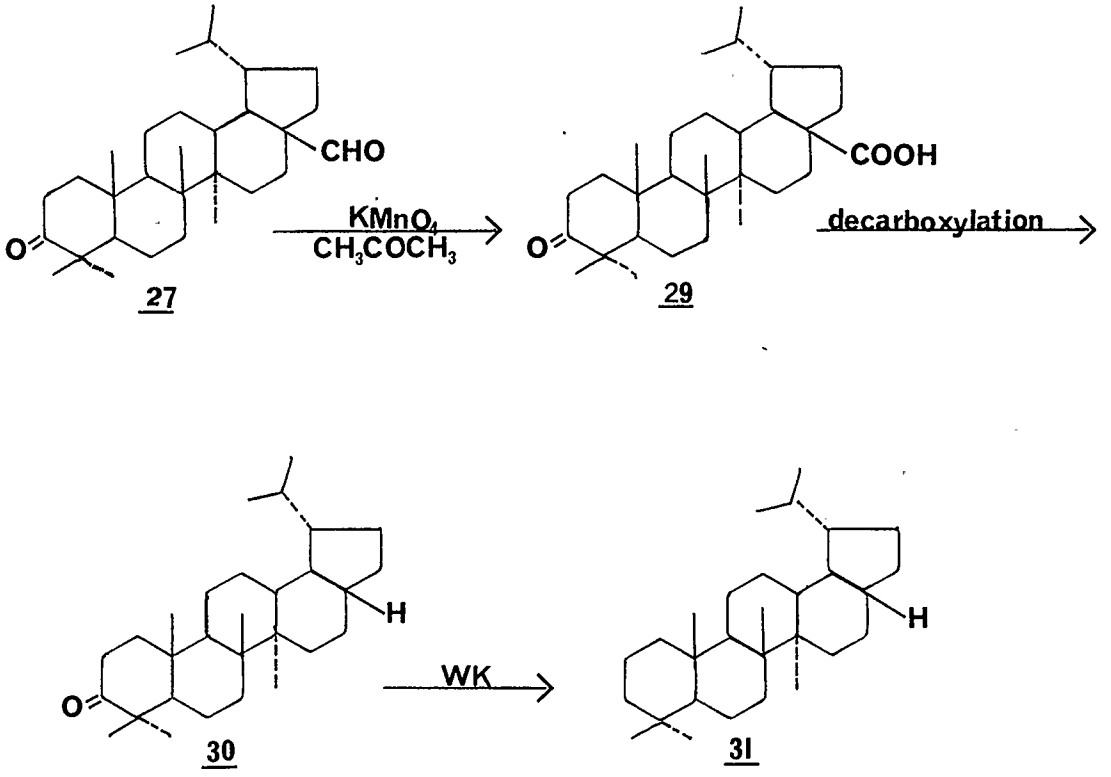
17α (H)



17β (H)

The mass spectra of the isomers of 31 showed the same general trend as those described by Rullkötter et al.. The mass spectrum of the first component to elute from the GC was similar to the mass spectrum of the α-isomer in the paper except that the intensity of the molecular ion was much stronger than the intensities of masses 355 and 383 which were similar. The mass spectrum of the second compound to elute from the GC was similar to that of the β-isomer from reference (11). From the comparison of the mass spectra of 31 with those shown in reference (11), the first compound was the α-isomer and the second one (the predominant isomer) was the β-isomer.

The ^{1}H-NMR pattern showed fewer signals in the 0.7-1 ppm region. There was no signal at 0.75 ppm for the 17β methyl. The ^{13}C-NMR of 31 showed no signal at 218.37 ppm but there was a new signal at 42.25 ppm which was probably due to the C-3 carbon atom[45].



Scheme 12. Synthesis of 17β(H)-28-norlupane

III.4 Synthesis of 23,28-bisnorlupane (36)

The removal of the C-28 carbon and one of the methyl groups on C-4 was necessary for the synthesis of bisnorlupane 36. It proved to be simpler than anticipated but the overall yield was low. The literature[24] quotes yields of 85 % or better for the decarboxylation step but all that could be achieved here was about 52 %.

Because two oxygen functions ultimately had to be removed, serious thought was given to whether it would be best to remove the C-28 carbon atom first or if it could be left until one of the C-4 methyl groups had been removed. Initially it was deemed best to remove C-28 first and this approach is outlined in Scheme 13. Later, other literature[44] indicated that it should not matter. The same series of reactions was tried, as shown in Scheme 14, but leaving the C-28 removal until after the ring closure step. It seemed to make little difference overall, but it was found that by using this alternate route, during formation of the seconitrile, an anhydride was also formed, which fortuitously was hydrolyzed during the work-up after the ring closure step. According to one paper[49] during the preparation of the seconitrile from betulinic acid, an anhydride was also formed.

III.4.1 3-Oximino-28-norlupane (32)

Starting with 3-oxo-28-norlupane (30), the oxime was prepared. Initially the method used to prepare the oxime of 3-oxolanost-8-ene was used[15] but in this case it required a

much longer reflux time (~60 hours) and more reagent (~3 times more). The yield was good but a more efficient procedure in terms of time was found[49,50] in which a better base, pyridine, was used. Using this method it was only necessary to reflux for one hour.

The IR now only showed a strong hydroxyl band at 3275 cm^{-1}. The mass spectrum showed a weak molecular ion at m/e 427 and a peak at m/e 177 indicating that one of the fragments is fragment (C) as shown in section III.1 but lacking the methyl group. NMR's were not run as a suitable deuterated solvent could not be found in which it was sufficiently soluble.

III.4.2 3-Seconitrile (33)

The seconitrile was prepared by the same method used to prepare the seconitrile of 3-oxolanost-8-ene, except that the reaction time was reduced to 6 hours. The crude product was chromatographed on silica gel or alumina with benzene. The IR showed bands at 3093 and 3036 cm^{-1} indicative of unsaturation. Bands at 1637 and 892 cm^{-1} show $-CR=CH_2$. The CN band appeared at 2249 cm^{-1}.

The ^{1}H-NMR showed two signals in the region for unsaturation (4-5 ppm); a complex multiplet at 4.89 ppm and another multiplet at 4.65 ppm, each integrating for one proton. They occurred at the same location as those for 5 for which data is known[15], and can be attributed to the isopropenyl group attached to C-5. The spectrum also showed multiplets at 2.30

ppm, integrating for two protons, at 1.73 ppm and a strong singlet at 1.56 ppm.

The ^{13}C-NMR showed double bond signals at 147.14 and 114.01 ppm The furthest upfield signal is due to the terminal methylene group. The fact that these signals did not appear in the spectrum of the epoxy-nitrile (next step) indicated that they were due to carbon atoms 4 and 24. There were two other signals in this region, one at 128.38 ppm and the other at 120.29 ppm. Again, the fact that only the latter one appeared in the epoxy-nitrile spectrum indicated that it was likely the one due to the nitrile carbon.

III.4.3 Epoxy-nitrile (34)

Epoxy-nitrile 34 was prepared by treating 33 with m-chloroperbenzoic acid. The IR showed a strong nitrile band at 2249 cm^{-1} but the bands at 3093 and 3036 cm^{-1} were absent. There was a strong band at 801 cm^{-1}, in the position anticipated for an epoxide. The ^{1}H-NMR showed a pair of doublets at 2.73 and 2.63 ppm due to the protons on the oxirane ring. These values compare to those of the epoxy-nitrile of 19β-28-epoxy-18α-oleanone reported by Klinot et al.[51]. The methyl group attached to the oxirane ring showed a strong singlet at 1.28 ppm and there was a complex multiplet at 2.20 ppm. The nitrile carbon showed a signal at 119.8 ppm in the ^{13}C-NMR.

III.4.4 3-Oxobisnorlupane (35)

The product from the reaction of the epoxy-nitrile with BF_3-etherate showed a very strong carbonyl band at 1708 cm^{-1} in the IR. The ^{1}H-NMR showed multiplets at 2.32, 2.04 and 1.41 ppm. The C-3 carbon atom showed a signal at 213.48 ppm in the ^{13}C-NMR.

III.4.5 23,28-bisnorlupane (36)

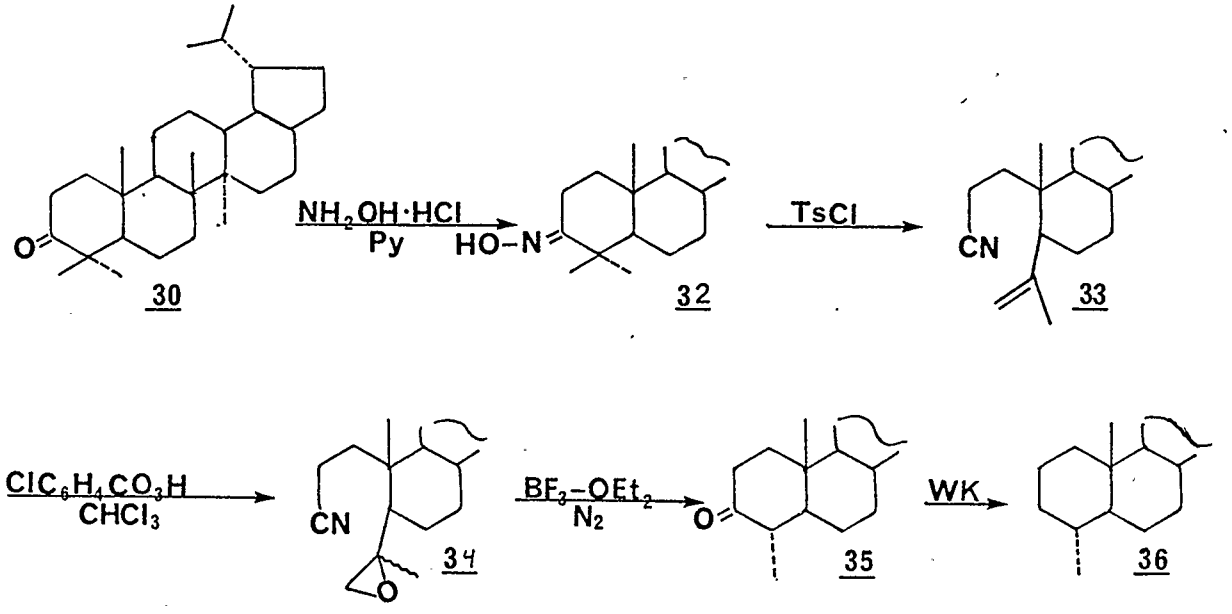
The final step was the Wolff Kishner reduction of 35. After chromatographing the product over alumina, the GC-MS showed it to be a mixture of three isomers; one predominating and the other two in minor quantities. There are theoretically four possible stereoisomers. Since C-28 was removed by a free radical mechanism, two isomers at C-17 could be expected, and there are two orientations that the C-4 methyl could take. In this case, the more stable position is the α-position.

In their paper reporting the isolation of 23,28-bisnorlupanes from crude oil[11], Rullkötter et al. assigned the mass spectra obtained to a specific structure based on comparison with the mass spectra of norlupanes and NMR comparisons with those of compounds having similar structures. The two isomers isolated were the 17β(H) and 17α(H), both having the C-4 methyl group in the α position. The mass spectrum of the 17β(H) isomer showed masses 341, 369 and 384 to have intensities of 7%, 11% and 35% respectively. The mass spectrum of the 17α(H) isomer showed the intensity of masses 341 and 384

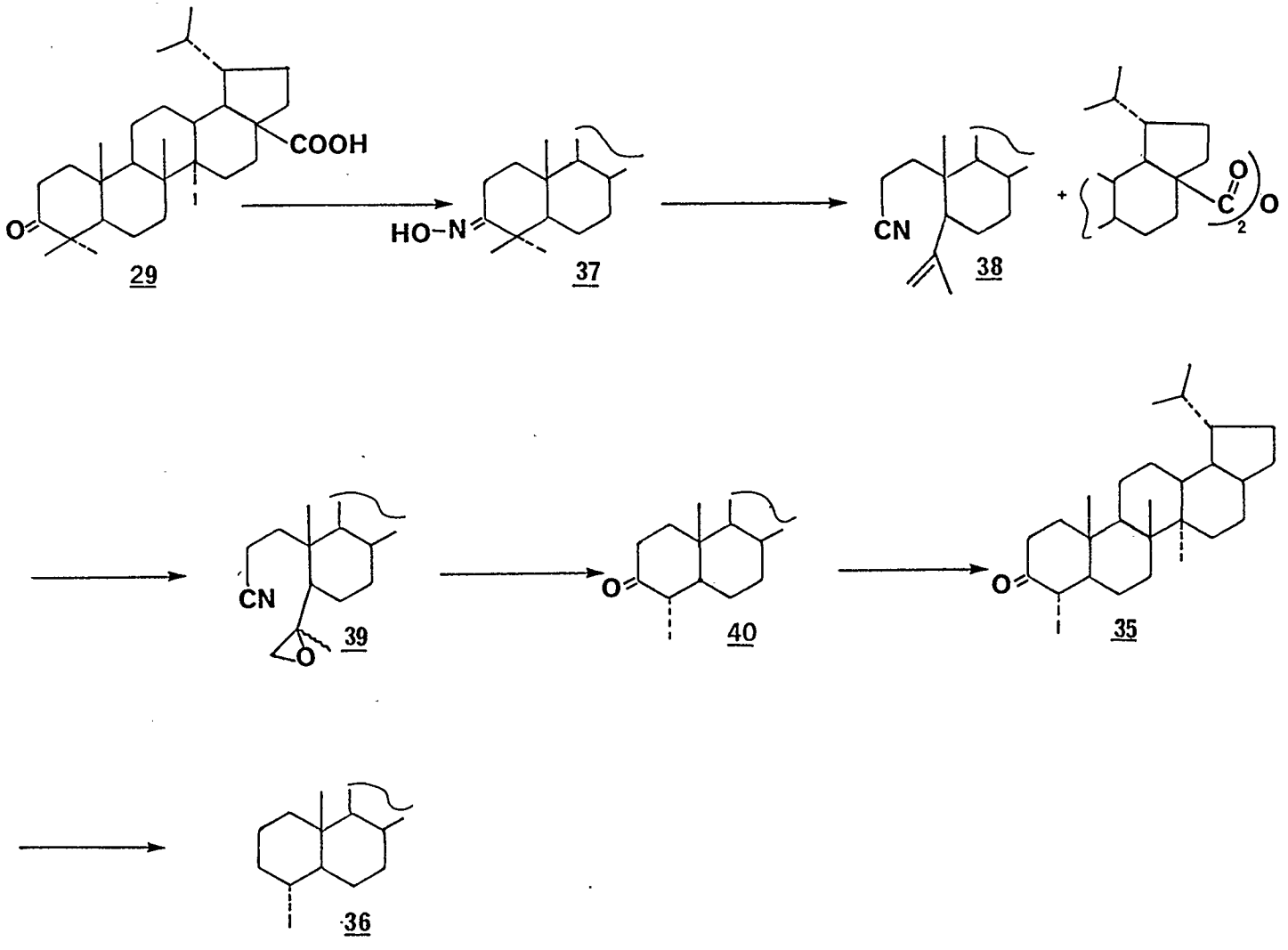
to be about the same and 369 to be weaker. The second of the three isomers to elute predominated (90%) and it showed the same relative intensities for masses 341, 369 and 384 as the 17β(H),4α(CH_3) isomer from Rullkötter's paper[11].

The mass spectrum of the first isomer to elute from the GC amounting to ~2%, showed masses 384, 369 and 341 to all be of a similar intensity. The intensities were 27%, 21% and 17% respectively. This is quite likely the α,α-isomer. The mass spectrum of the last isomer to elute, amounting to ~12%, was similar to that of the second isomer.

The ^{1}H-NMR showed only protons in the 0.5-2 ppm region. The ^{13}C-NMR showed the C-3 signal to now be at 42.25 ppm.



Scheme 13. Preparation of 23,28-bisnorlupane



Scheme 14. Preparation of 23,28-bisnorlupane, retaining the acid function until a later step.

III.5 Synthesis of 3-oxolupane (45)

3-Oxolupane (45) was required as starting material for the deuteration of the A ring of lupane. Compound 45 was prepared by first the diacetylation of dihydrobetulin and then hydrolysis such that C-28 was hydrolyzed predominantly. Oxidation of the monoacetate, followed by a Wolff Kishner reduction and a second oxidation yielded 45. This series of reactions is shown in Scheme 15.

III.5.1 Diacetylation of dihydrobetulin

The diacetate 41 was prepared by treating 26 with acetic anhydride and pyridine. The ^{1}H-NMR spectrum of dihydrobetulin showed a doublet of doublets at 3.22 ppm for the proton on C-3 which for the diacetate 41 had shifted to a multiplet at 4.48 ppm. The pair of doublets at 3.79 and 3.31 ppm for the C-28 protons in 26 had shifted to 4.25 and 3.82 ppm. The singlets at 2.07 and 2.05 ppm are due to the methyl protons on the two acetate groups. The IR showed no hydroxyl band but did show a carbonyl band at 1735 cm^{-1}. The signals for the acetate carbonyl carbons occurred at 171.58 and 170.96 ppm in the ^{13}C-NMR. The C-3 carbon atom showed a signal at 80.93 ppm and that for the C-28 carbon atom occurred at 62.86 ppm[45].

III.5.2 Monoacetate (42)

It was possible to selectively hydrolyze the diacetate 41 such that the predominant product only had the C-28 acetate hydrolyzed. It was not possible to obtain 100% monoacetate

but by following the progress of the reaction by TLC, one could find the reaction time producing the maximum amount of monoacetate and minimum amount of dihydrobetulin. A reaction time of 6-7 hours produced the optimum yield.

The IR of the monoacetate 42 showed a hydroxyl band at 3445 cm^{-1} and a carbonyl band at 1736 cm^{-1}. The multiplet at 4.48 ppm, for the C-3 proton, was still present in the ^{1}H-NMR but the signals for the C-28 protons had shifted back to the values shown by dihydrobetulin; 3.78 and 3.31 ppm. There was only one signal due to acetate protons and it was at 2.05 ppm. The ^{13}C-NMR showed only one carbonyl signal and it was at 171.07 ppm. The signals at 81.01 and 60.57 ppm were due to carbon atoms 3 and 28 respectively.

III.5.3 Oxidation of the monoacetate

The C-28 hydroxyl group was oxidized to the aldehyde 43. The IR of 43 showed two distinct carbonyl bands, one at 1736 cm^{-1} and the other at 1708 cm^{-1}. The ^{1}H-NMR showed the multiplet at 4.8 ppm and a signal at 9.65 ppm, due to the aldehyde proton on C-28. The methyl signal at 2.06 ppm was also present. The ^{13}C-NMR showed a signal at 206.32 ppm for the C-28 carbon atom and the acetate carbonyl still showed a signal at 170.12 ppm. The C-3 carbon signal occurred at 80.07 ppm.

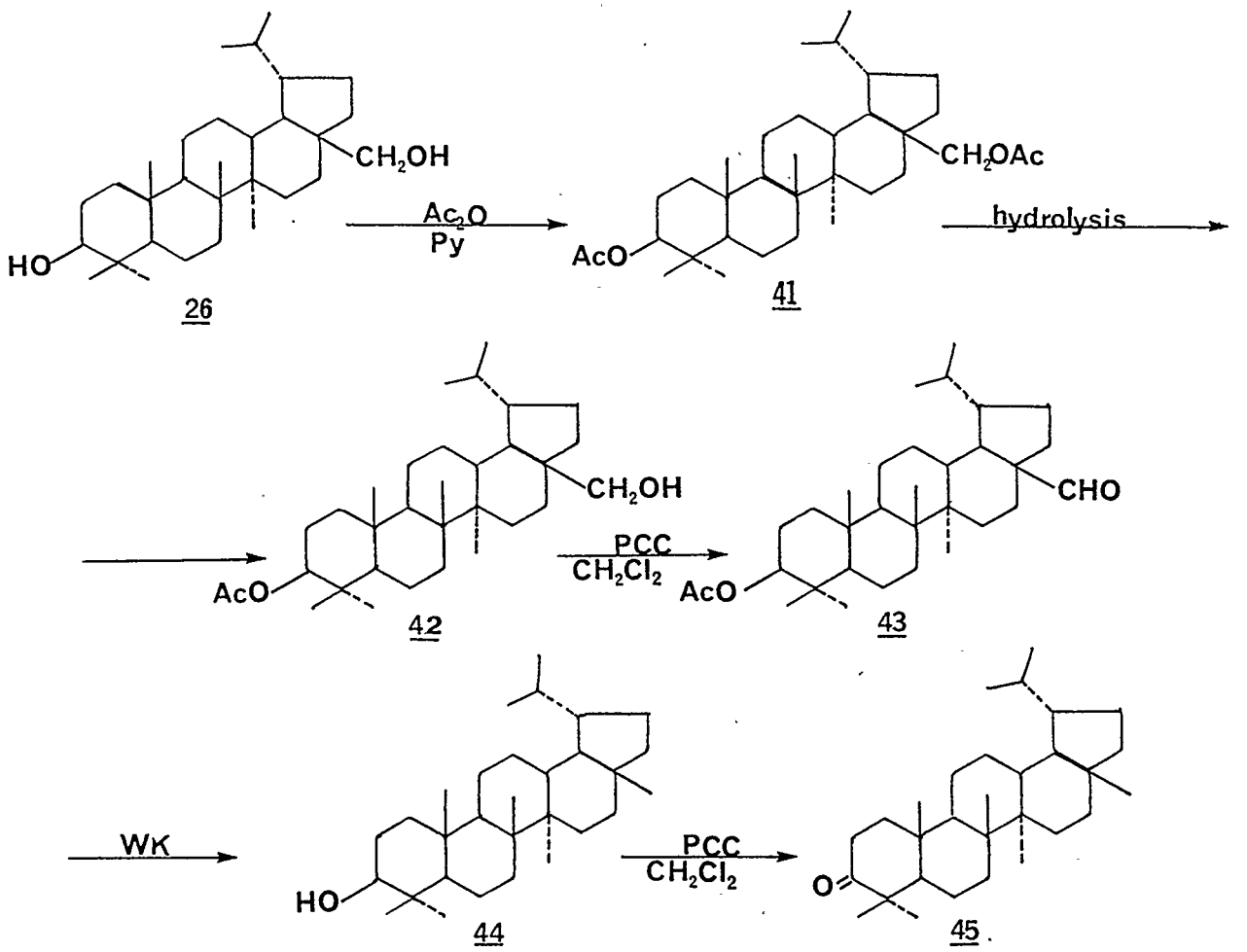
III.5.4 Wolff Kishner reduction of (43)

The Wolff Kishner reduction of 43 produced 3-hydroxylupane (44). The C-28 aldehyde was reduced to the methyl group and the C-3 acetate had hydrolyzed to the alcohol. The IR showed these changes with a hydroxyl band at 3374 cm^{-1} and no carbonyl band. The multiplet due to the C-3 proton had shifted upfield to a value of ~3.20 ppm in the ^{1}H-NMR. This agrees with the value for this proton in dihydrobetulin.

The ^{13}C-NMR showed a signal at 79.14 ppm for the C-3 carbon atom with a hydroxyl group attached. Again this agrees with the value for 26. According to the literature[45] the C-28 carbon should show a signal at 18.10 ppm[26] and the C-6 carbon appears at about 18.3 ppm and there were signals at 18.44 and 18.16 ppm. From the literature[26,45] it seems probable that the signal at 43.14 ppm is due to the C-17 carbon atom.

III.5.5 Oxidation of 3-hydroxylupane

Oxidation of the C-3 hydroxyl group to the carbonyl yielded 3-oxolupane (45). The IR showed a carbonyl band at 1701 cm^{-1}. The ^{1}H-NMR showed the multiplet at 2.47 ppm for the C-2 protons adjacent to a carbonyl[25]. The multiplet at 1.87 ppm is likely due to the C-1 protons[25]. The ^{13}C-NMR showed a signal at 218.06 ppm for the C-3 carbon atom.



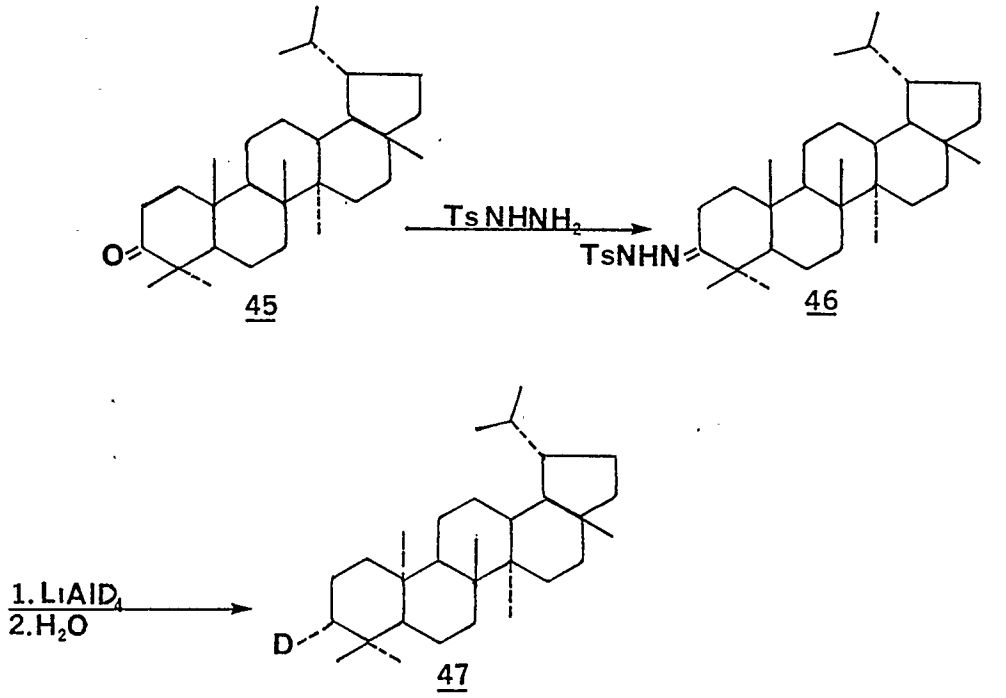
Scheme 15. **Preparation of 3-oxolupane**

III.6 Synthesis of deuterated lupanes

III.6.1 Monodeuterated lupane (47)

One of the methods tried in an attempt to dideuterate lupane gave a mixture of mono and dideuterated lupanes. This was the reaction of $LiAlD_4$ with the tosylhydrazone of 3-oxo-lupane. If instead of using D_2O, water was used in the second

step, the mono-deuterated product was obtained. This is shown in Scheme 16. Some olefinic side product was formed but it was easily removed by chromatography of the reaction products on $AgNO_3$ impregnated silica gel. The mass spectra of the first compound eluted showed a molecular ion at m/e 413 and a base peak at m/e 192. The GC-MS showed it to be only one compound. Both the ^{1}H-NMR and the ^{13}C-NMR showed no evidence of unsaturation.



Scheme 16. Preparation of mono-deuterated lupane 47

III.6.2 di-deuterated lupane (52)

3-Oxolupane was dideuterated by treating it with sodium methoxide and deuterium oxide [41]. Scheme 17 shows the steps followed in its synthesis, starting with 45. The ^{1}H-NMR of 48 showed no multiplet at 2.47 ppm for the C-2 protons indicating

that they had been replaced by deuterium atoms. The ^{13}C-NMR did not show the C-2 carbon signal at 34.23 ppm that was evident in 45[26,45] but analysis by GC-MS of the product showed that it was only about 80% di-deuterated. The product was treated with potassium t-butoxide and D_2O but in this case the mass spectrum showed even less of the di-deuterated material and more of the mono-deuterated 3-oxolupane. After further treatment with sodium methoxide and deuterium oxide, the GC-MS now showed the presence of about 5% of the mono-deuterated 3-oxolupane.

A review by H. Fuhrer[52] stated that cyclohexanone was di-deuterated on the two carbons α to the carbonyl to 94% purity, using potassium carbonate and deuterium oxide. This method was then tried on 45. The mass spectrum of the product indicated that there was still about 5% of the mono-deuterated material.

The IR of 49, obtained from the $LiAlD_4$ reduction of the di-deuterated ketone 48, showed a strong hydroxyl band but no carbonyl band. The ^{13}C-NMR of the tri-deuterated alcohol showed no signal at 79 ppm for the C-3 carbon atom since it now had a deuterium atom on it. There was no signal at 27.5 ppm, the signal thought to be due to the C-2 carbon atom when adjacent to a carbonyl. There was a signal at 27.44 ppm which was likely due to carbon atom 15[45].

The ^{1}H-NMR of the mesylate 50, formed from the reaction of 49 with methanesulfonyl chloride in pyridine, also showed no multiplet at 2.47 ppm nor did it show any multiplet at 4.4

ppm where the C-3 proton would be expected to show a signal. The ^{1}H-NMR of 51, formed by heating 50 with sym-collidine, showed weak signals about 4.79 ppm indicating the presence of a proton on a double bond carbon. The unsaturated lupene 51 was hydrogenated and the reaction product 52 chromatographed on silica gel. The ^{1}H-NMR of 52 showed no signals at 2.47 ppm and no indication of any unsaturated material. The ^{13}C-NMR showed no signal at 42.20 ppm which is the position for the C-3 carbon signal in lupane[45], but there was still a signal at 18.66 ppm. This is where one would expect to see the C-2 carbon signal but the C-6 carbon atom also shows a signal in this region. The GC-MS of 52 showed only ~4% of the mono-deuterated compound.

When the mass spectra of the deuterated lupanes were compared to the mass spectrum of lupane, some interesting facts came to light. The mass spectrum of lupane showed a molecular ion at m/e 412 and a base peak of mass 191. With lupane both fragment A (A/B ring) and fragment C (D/E ring have masses of 191.

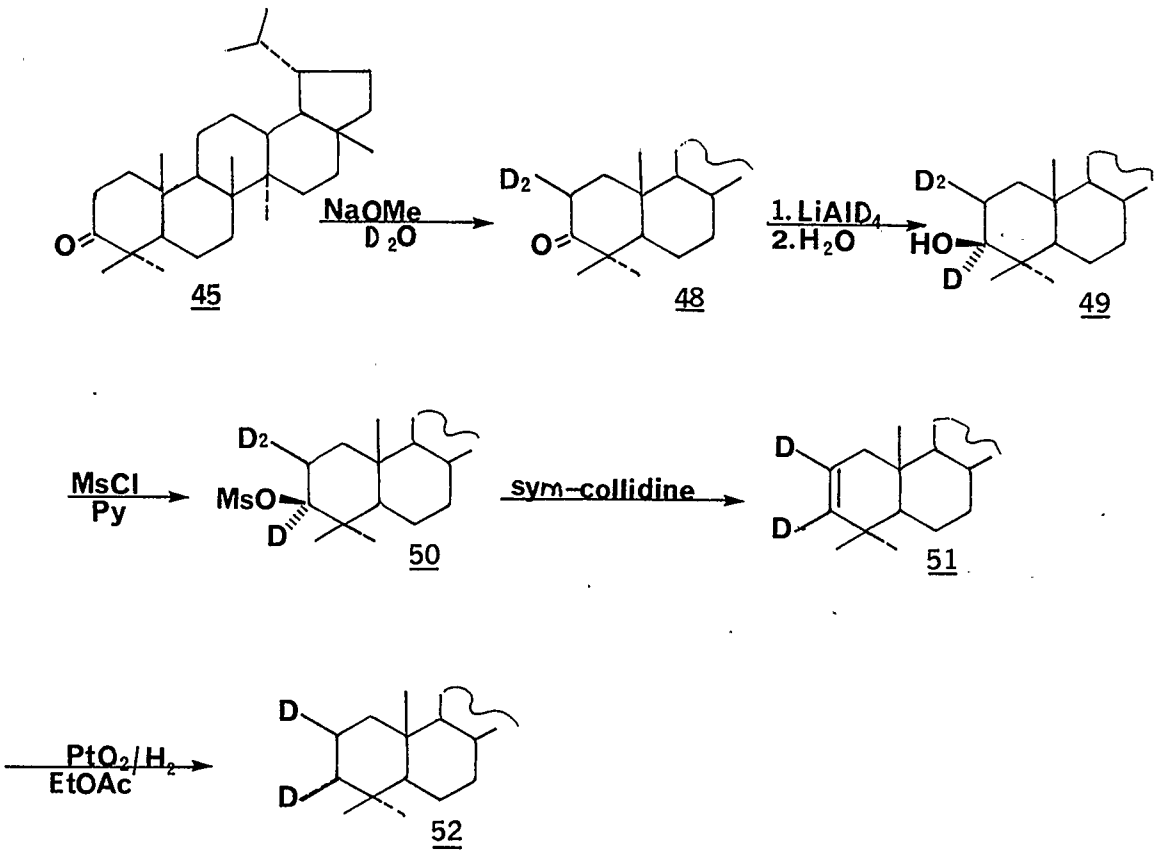
The mass spectrum of mono-deuterated lupane showed a molecular ion at m/e 413 and a base peak of mass 192. In this case the peak of mass 192 is due only to fragment A, since the deuterium is only in the A ring. Fragment C still has a mass of 191 with an intensity of 25%. The mass spectrum of di-deuterated lupane showed a molecular ion at m/e 414 and a base peak of mass 193. This is due to fragment A since the two

deuteriums are again in the A ring. Fragment C showed a mass of 191 with an intensity similar to that of the mono-deuterated lupane.

These results show that the intensity of fragment C is only 20-30% of the base peak and so if both fragments have the same mass then the largest proportion of it comes from fragment A. These results suggest that the base peak comes from the A/B ring.

Comparing further, with the mass spectra of other lupane derivatives, this trend is again apparent. The base peak of 28-norlupane occurred at mass 191, which is the mass of fragment A, and fragment C showed a mass of 177 with an intensity of 25%. 3-Oxo-28-norlupane showed a similar trend with the base peak at mass 205, again for fragment A and a mass of 177 with intensity of 20% for fragment C.

These observations can be used to compare the mass spectra of unknown samples with known samples. Only hopanes and lupanes have the isopropyl side chain and in hopanes the intensities of fragments A and C are the same while in lupane the intensity of fragment C is only of the order of 20-30%. It is much lower than the intensity of fragment A which in many cases is the base peak. This allows one to distinguish between hopanes and lupanes in which the A and C fragments are of different masses.



Scheme 17. Preparation of dideuterated lupane.

CHAPTER IV

EXPERIMENTAL

IV.1. Instrumental and reagents

IV.1.1. Instruments and techniques

Infrared spectra of solids were run as KBr discs of the samples on a Nicolet DX FT-IR connected to a 5 DX data processor and a 7470A Hewlett Packard plotter. The spectra of semi-solids or liquids were run as thin films on KBr plates.

Mass spectra of samples were obtained on a Kratos MS80(RFA) model spectrometer at 70ev. The mass spectral peaks (m/e) are given with relative intensities.

GC-MS of samples were run by Dr. P. Brooks at I.S.P.G. on a VG 70-SQ model controlled by a 112505 data system at 70ev. The GC used was a GC HP 5890 model with a J and W Scientific 30 meter DB5 column and a helium flow of 1 ml/minute.

Elemental analysis were carried out by Dr. W.S. Lin of the Department of Chemistry, University of Calgary, on a Perkin Elmer 240b Elemental analyser.

Melting points were taken on an Electrothermal melting point apparatus.

^{1}H and ^{13}C-NMR of many samples were run by Dr. R. Yamdagni of the Department of Chemistry, University of Calgary, using a Varian XL200 Spectrometer. Routine ^{1}H and ^{13}C-NMR spectra were run on a Bruker AC200 student model connected to a MP3200 graphtec XY plotter, using $CDCl_3$ as internal standard.

Chromatography:

Column chromatography was used as a method to purify and separate products from reactions, side products or mixtures. For routine column work, basic alumina (activity II-III E. Merck aluminum/oxide 90) was used with hexane or hexane/ether 9:3 or 9:5 as eluting solvent. Alumina columns were prepared by adding the dry alumina to the column filled with hexane. In the cases where silica gel was used (Davisil 62 Column chromatographic silica gel - a synthetic amorphous (non-crystalline) silica) it was added as a slurry in the appropriate solvent to the column. For the separation of unsaturated compounds, silica gel impregnated with 10-12.5% $AgNO_3$ was used. Normally 2-20 ml fractions were collected, spotted on TLC and visualized by developing the dampened plate in a tank containing SO_2Cl_2 and then drying the plate in an oven at $100^{o}C$.

TLC plates used were Merck DC Alufolien Kieselgel 60 F254.

GC was used to analyze products for purity. The instrument used was a Varian model 3700 GC connected to a Varian CDS 401 printer plotter. The column used was a DB5 capillary column.

IV.1.2. Reagents

Some solvents or reagents were purified before use by the methods listed below:

Benzene was dried over sodium prior to use as solvent in a reaction.

BF_3-etherate was distilled prior to use.

Diethylene glycol was distilled under vacuum prior to use.

Dioxane was chromatographed on alumina to remove peroxides and then distilled from $LiAlH_4$.

Pyridine was dried over KOH then distilled prior to use.

THF was distilled from $LiAlH_4$ prior to use.

IV.2. Synthetic procedures

IV.2.1. Hydrogenation of lanosterol

A mixture of commercial grade lanosterol (3.0 gm, 7.0 mmol), ethyl acetate (250 ml), and 10% Pd on charcoal (300 mg) was shaken at 25^{0}C for 0.5 hours under 2 atm. hydrogen. The catalyst was removed by filtration and the solvent removed under vacuum to yield 2.7 gm (90%) of dihydrolanosterol, melting at 141-144^{0}C, (lit.144.5-145.5^{0}C[53]).

IV.2.2. Oxidation of dihydrolanosterol to dihydrolanosterone (3)

Procedure a[17]

Chromium trioxide (6.0g, 60 mmol) was added to a stirred solution of 9.7 ml(120 mmol) of freshly distilled pyridine in 150 ml of methylene chloride. The flask was stoppered with a drying tube containing $CaCl_2$. Upon turning a deep burgundy color, the solution was stirred at 25 ^{0}C for 15 minutes, then 4.28 gm (10 mmol) of 2, dissolved in a minimum amount of methylene chloride, was quickly added and the solution stirred for a further 15 minutes at 25^{0}C. The solution was decanted into a separatory funnel, the residue remaining in the flask was washed with 200 ml ether and this was added to the separatory funnel. The combined organic solvents were washed with 3x100 ml portions of 5% NaOH, 100 ml of 5% HCL, 100 ml of 5% $NaHCO_3$ and 100 ml of saturated NaCl solution, then dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum.

The crude product was recrystallized from methylene chloride - methanol to yield 3.775 gm (88.5%) of 3 melting at 107.5 - 110^{0}C.

Procedure b[18]:

One g (2.3 mmol) of 2 was dissolved in 120 ml of methylene chloride, then 0.742 g (1.5 molar excess) PCC was added and the mixture stirred at 25^{0}C for 3 hours. The solution was then filtered through a short alumina column. The flask was rinsed with ether and this was added to the column. About 200 ml of ether was eluted through and the eluant washed with 3N HCl, 5% $NaHCO_3$ and water, then dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum. Yield: 0.800 gm (80%). mp = 107.5 - 110^{0}C (lit. 113 - 114^{0}C[53]).

IV.2.3. Preparation of oxime (4)[15]

Compound 3 (2.6 g, 6.6 mmol), anhydrous sodium acetate (1.011 g, 12.3mmol) and 0.635 gm (9.1 mmol) of hydroxylamine.HCl were added to 320 ml of 95% ethanol and refluxed for 24 hours. The solution was then cooled, diluted with water and extracted with chloroform. The chloroform extract was dried over anhydrous $MgSO_4$, filtered, and the solvent removed under vacuum. The crude product was recrystallized from a mixture of ethanol and chloroform to yield 2.30 gm (79%) of oxime 4, melting at 164.5 - 165^{0}C.

IR(KBr):3282, 1680(C=N), 948, 927 cm^{-1}.

MS:m/e(%), 441(M^+,12), 439(5), 427(23), 426(72), 145(16), 119(27), 95(43), 69(65), 43(100)

^{1}H-NMR:ppm, 1.25(s), 1.16(s), 1.10(s), 1.09(s), 0.90(s), 0.88(s), 0.85(s), 0.70(s)

^{13}C-NMR:ppm, 167.17(C=N-OH), 134.88 and 133.76 ($\triangle^8$).

IV.2.4. Preparation of seconitrile (5)[54]

The method of Shoppee et al.[54] was used to prepare seconitrile 5, using 2.2 gm(5.0 mmol) of 4, 6.16 gm(32.3 mmol) of p-toluenesulfonyl chloride and 123 ml of dry pyridine. The mixture was refluxed for 14 hours (compare ref. 15) and then chromatographed on silica gel with hexane/ether (7:3). The product recovered from chromatography was recrystallized from methanol to yield 1.24 gm(59%) of seconitrile 5 which melted at 92-93^{0}C (lit. 94-95^{0}C[54]).

IV.2.5. Preparation of epoxy-nitrile (6)[15]

Epoxy-nitrile 6 was prepared, using the method of Pinhey et al.[15], by treating seconitrile 5 with m-chloroperbenzoic acid at 0^o for 2 days. The crude material was recrystallized from methanol to give 1.04 gm(74%) of epoxy-nitrile 6, which melted at 93.5-95^{0}C (lit. 97-98^{o}C[15] for a mixture of epimers).

IV.2.6. Preparation of 3-oxo-4-desmethyl-5 -lanost-8-ene (7)[15]

Compound 7 was prepared according to the method of Pinhey et al. in which 1.2 ml of BF_3-etherate was added to a solution of the epoxy-nitrile 6 (750 mg) in toluene and refluxed for 6 hours. An eluting mixture of hexane/ether 9:5 was found to give better separation when the product was chromatographed. The product was then recrystallized from methylene chloride-methanol to yield 427 mg(60%) of 7, melting at 105.7-107.5°C (lit. 108-109°C[15]).

IV.2.7. Preparation of p-toluenesulfonyl hydrazine

Tosylhydrazine was prepared by the method outlined in reference (55), by reacting 12.5 g(0.07 m) of p-toluenesulfonyl chloride in 25 ml of methylene chloride with 8.5 ml of hydrazine hydrate. The crude material was recrystallized from water to yield 11.5 gm(94%) of tosylhydrazine which melted at 108-109°C (lit. 104-112°C[55]).

IV.2.8. Tosylhydrazone of 3-oxolanost-8-ene (8)[29,56]

One g(2.35 mmol) of 3 and 616 mg(3.3 mmol) of tosylhydrazine were placed in a flask and 25 ml of methanol and 0.5 ml of acetyl chloride were added. The mixture was refluxed for 20 minutes, water was added while the mixture was still hot, the mixture was cooled and the resulting precipitate filtered and air or vacuum dried. The crude material was recrystallized from 95% ethanol to give 774 mg(55%) of tosylhydrazone 8 which melted at 162-164°C.

IR(KBr): 3213, 2752, 1595,1476, 1384, 1166 cm^{-1}

^{1}H-NMR:ppm, 7.85(d,2H,J=8.3Hz), 7.32(d,2H,J=5.4Hz), 2.43(s,3H, CH_3-C_6H_4), 1.10(s), 0.97(s), 0.96(s), 0.89(s), 0.87(s), 0.86(s), 0.69(s)

IV.2.9. Reduction of tosylhydrazone (8) with $LiAlH_4$ [29,30]

Tosylhydrazone 8 (720 mg, 1.2 mmol) was dissolved in 37 ml of freshly distilled dioxane. $LiAlH_4$ (760 mg, 20 mmol) was added and the mixture was refluxed for 2 hours, cooled and the excess $LiAlH_4$ destroyed by the careful addition of water. Dilute HCl was added to dissolve any $Al(OH)_3$ formed. The aqueous layer was extracted with ether, the organic extract washed with 5% $NaHCO_3$ and water, dried over $MgSO_4$ filtered and the solvent removed under vacuum. The crude material was chromatographed on silica gel impregnated with 12.5% $AgNO_3$ with hexane and then hexane/ether (9:3). The compounds eluted from the column were recrystallized from methanol.

Lanost-8-ene was the first material eluted from the column with hexane/ether 9:3 to yield 82 mg; mp:72-73.5°C.
^{13}C- NMR:ppm; 135.07 and 134.00 ($\triangle^8$).

Lanosta-2,8-diene was the second material to elute from the column with hexane/ether (9:3) to give 96.5 mg; mp:80-81.5°C.

^{1}H-NMR:ppm; 5.46(m,2-H,3-H), weak signals in the 1-2 ppm region.

^{13}C-NMR:ppm; 138.17(C-3) shows an off-resonance doublet.
121.91(C-2) shows an off-resonance doublet.

135.03 and 133.01(Δ^8) show off resonance singlets

IR: 2966, 1468, 1370, 1258, 1087, 808, 731,716 cm^{-1}.

IV.2.10. Reduction of 3-oxolanost-8-ene with $LiAlD_4$[57]

3-Oxolanost-8-ene (250 mg, 0.59 mmol) was added to 100 ml of anhydrous ether, than $LiAlD_4$ (160 mg, 4 mmol) was added and the mixture refluxed under N_2 for 2 hours. The mixture was cooled, excess $LiAlD_4$ was destroyed by the careful addition of water, then dilute HCl was added to dissolve any $Al(OH)_3$ that had formed. Additional ether was added, the layers were separated and the organic layer was washed with 5% $NaHCO_3$ and water, then dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum. TLC showed no starting ketone. The alcohol (227 mg, 90%) was obtained.

IR: 3508, 2952, 2868, 1469, 1370, 1264, 1110, 1047, 941, 801 cm^{-1}

^{1}H-NMR:ppm; 2.02(m), 1.00(d,J=3.7 Hz), 0.91(s), 0.89(s), 0.86(s) 0.82(s), 0.70(s).

^{13}C-NMR:ppm; 134.51 (Δ^8).

IV.2.11. Preparation of the tosylate of dihydrolanosterol (11)[31]

Compound 10 (150 mg, 0.35 mmol) and p-toluenesulfonyl chloride (172 mg, 0.91 mmol) were added to dry, distilled pyridine (7.0 ml) and the mixture was stirred at 25°C for 48 hours, then diluted with water and extracted with ether.

The ether extract was washed with 3N HCl, 5% $NaHCO_3$ and water then dried over $MgSO_4$, filtered, and the solvent removed under vacuum. The crude product was chromatographed on silica gel (10 gm), eluting first with hexane, then hexane/ether (9:5). Tosylate 11 (142 mg, 70%) was recovered.

IR: 2900, 1595, 1469, 1363, 1265, 1173, 927, 876, 800, 667 cm^{-1}

IV.2.12. Reduction of tosylate (11) with $LiAlD_4$ to give (10)

Tosylate 11 was reduced to alcohol 10 with $LiAlD_4$ by the same procedure used to reduce tosylhydrazone 8. $LiAlD_4$ (100 mg, 2.6 mmol) was refluxed with 95 mg of 11 in 20 ml of dioxane for 48 hours. After removal of the solvent, the crude product was chromatographed on basic alumina, eluting with hexane/ether (9:5). Alcohol 10, (36 mg) was recovered. The TLC showed spots for two compounds, the most polar having the same R_f value as dihydrolanosterol and the less polar one being starting material.

The MS of the dihydrolanosterol showed a molecular ion at m/e 429.

IV.2.13 Preparation of mesylate (13a)

3-Hydroxylanost-8-ene(2) (2.0 gm, 0.5 mmol) was dissolved in 200 ml of freshly distilled pyridine. Methanesulfonyl chloride (4.0 ml, 0.05 m) was added and the mixture kept at $0^{\circ}C$ for 3 hours. The excess reagent was destroyed by adding water after which the mixture was extracted with ether.

The ether extract was washed with 3N HCl, 5% $NaHCO_3$ and water, then dried over $MgSO_4$ and removed under vacuum. The crude mesylate (2.13 gm, 90%) was obtained as a solid.

IR: 2952, 1469, 1370, 1335, 1173, 927, 906, 780, 512 cm^{-1}.
^{1}H-NMR:ppm; 4.37(dd,3-H), 3.03(s,3H,CH_3-mesylate),
1.26(s,2H), 1.03(d,6H,J=5.3Hz), 0.90(s),
0.89(s), 0.88(s), 0.87(s), 0.86(s), 0.85(s),
0.67(s,3H)
^{13}C-NMR:ppm; 134,78 and 133.84 (Δ^8), 90.52(C-3).

Data for mono-deutrolupane (13)

IR: 2952, 1469, 1335, 1173, 976, 906, 752, 534 cm^{-1}
^{1}H-NMR:ppm; 3.03(S,3H,CH_3-SO_3-), 1.6(s), ~1.1(d), 0.85(s,3H),
^{13}C-NMR:ppm; 134.90 and 133.96(Δ^8), 50.84, 50.57
mp: 131-135°C

IV.2.14. Reduction of mesylate (13) with $LiAlH_4$

Mesylate 13 (150 mg, 0.3 mmol) was added to 30 ml of freshly distilled THF. $LiAlH_4$ (200 mg, 5.2 mmol) was added and the mixture was refluxed under N_2 for 22 hours. Excess $LiAlH_4$ was decomposed by the careful addition of water, then dilute HCl was added to dissolve the $Al(OH)_3$ that had formed. The product was extracted with ether, the ether extract was washed with 5% $NaHCO_3$ and water, then dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum. Alcohol 10

(100 mg, 79%), was recovered. The TLC showed a spot with the same R_f as compound 2.

IV.2.15. Reduction of mesylate(13) with lithium triethylborohydride[33]

Mesylate 13 (144 mg, 0.28 mmol) in 5 ml of freshly distilled THF was introduced with a syringe to a dry N_2-flushed flask fitted with a reflux condenser and a rubber septum. Dry THF (20 ml) was added with a syringe, 1M $LiEt_3BD$ (0.56 ml) was added with a syringe and the mixture refluxed under N_2 for 60 hours. A further 2 ml of $LiEt_3BD$ were added after 18-20 hours. The reaction mixture was cooled, excess reagent was destroyed with water and the product was extracted with ether, the ether extract was dried over $MgSO_4$, filtered, and the solvent removed under vacuum to yield 110 mg of alcohol 10.

IR: 3360, 1468, 1370, 1227, 1068, 801, 758 cm^{-1}.

IV.2.16. Preparation of 2-bromo-3-oxolanost-8-ene (14)[34]

3-Oxo-lanost-8-ene (3) (400 mg, 0.94 mmol) was added to 13 ml of glacial acetic acid. One ml of 1 M Br_2 in acetic acid was added dropwise with stirring until a brown color remained. The mixture was stirred at 25°C for 3 hours then the solvent was removed under vacuum. The crude product was chromatographed on silica gel, eluting with hexane/ether (9:5). A light amber solid (308 mg, 64%) was recovered. It had a mp of ~100°C. TLC showed the product to have an R_f slightly higher than that of the starting material.

^{1}H-NMR:ppm; 5.0(m,2-H), 4.0(s), 2.67(s), 2.12(s), 1.56(s), 1.53(s).

^{13}C-NMR:ppm; 203.31(C-3), 144.96 and 137.64($\overset{8}{\triangle}$)

IV.2.17. Preparation of ketone(15) from bromo-ketone (14)

The bromo-ketone 14 was reduced to the alcohol and the bromine replaced by deuterium by treating with $LiEt_3BD$. The procedure used for the reduction of mesylate 13 was used; treating 14 (202 mg, 0.4 mmol) with a 4 molar excess (1.6 ml) of $LiEt_3BD$ in dry THF and refluxing it overnight. The crude product was chromatographed on alumina, eluting with hexane/ether (9:5). A light tan solid (~100 mg) was obtained. TLC showed a spot with the same R_f value as that of 3.

IR(KBr): 3416, 2959, 2875, 1455, 1377, 1061, 801 cm^{-1}

^{1}H-NMR:ppm; 1.26(s), 1.14(s), 1.13s), 1.11(s), 1.10(s), 1.07(s), 0.90(s), 0.86(s)

The alcohol was oxidized, using 75 mg PCC, by the method used to oxidize dihydrolanosterol, yielding 80 mg of 15.

IR(film): 2959, 2875, 1708, 1469, 1377, 1265, 1102, 759 cm^{-1}

The product had the same retention time when examined by GC as 3-oxolanost-8-ene.

IV.2.18 Elimination of mesylate (13a) with sym-collidine to form (16)[59]

Mesylate 13a (340 mg, 0.67 mmol) and 2 ml (15 mmol) of sym-collidine were refluxed under nitrogen for 5-6 hours. The mixture was cooled, diluted with 3N HCl and extracted with ether. The ether extract was washed with 3N HCl, 5% $NaHCO_3$ and water, then dried over $MgSO_4$, filtered, and the solvent removed under vacuum. The crude product was chromatographed on basic alumina with hexane. A white, waxy solid (160 mg, 58%) melting at 132-133°C was obtained.

IR(KBr or thin film): 2950, 1469, 1370, 1258, 1131, 1026, 808, 744 cm^{-1}

^{1}H-NMR:ppm; 5.44(m), 2.1(m, missing in 16a), 1.99(d,J=5.7Hz), 0.92(s), 0.90(s), 0.89(s), 0.86(s), 0.859(s), 0.73(s)

^{13}C-NMR:ppm; 138.17(C-3, missing in 16a), 135.00 and 133.00 ($\triangle^8$), 121.90(C-2)

IV.2.19. Preparation of the bromohydrin (17)[37]

Lanosta-2,8-diene(16)(400 mg, 0.73 mmol) was dissolved in 24 ml of chloroform and then 12ml of DMSO and 0.4 ml of water were added. NBS (350 mg, 2 mmol) was added in portions with swirling. The mixture was kept in the dark at 25°C for 24 hours, then diluted with water and washed 5-6 times with 5% $NaHCO_3$ and finally with water. The organic layer was dried over $MgSO_4$, filtered, and the solvent removed under vacuum. The resulting solid was chromatographed on silica gel with

benzene to yield about 120 mg (32%) of a yellow-green semi-solid.

IR(thin film): 3416, 2959, 1652, 1469, 1370, 1216, 759 cm^{-1}

^{1}H-NMR:ppm; 4.55(d,1H,J=10.7Hz,3-H), 4.0(m,1H), 2.04(m), 1.19(s), 1.10(s), 0.89(s), 0.86(s), 0.855(s), 0.70(s,3H)

IV.2.20. Reduction of bromohydrin (17) with $LiEt_3BD$ to form (18)

Bromohydrin 17 (120 mg, 0.2 mmol) in 15 ml of dry THF was treated with 0.4 ml(3.3 mmol) of $LiEt_3BD$ as with 13, refluxing for 18 hours. The product was chromatographed on basic alumina with benzene to give 65 mg of a pale yellow semi-solid.

IR(film): 3240, 2952, 2924, 2854, 1462, 1377, 1258, 1096(broad), 808 cm^{-1}

^{1}H-NMR:ppm; 3.3(d), 2.02(m), 1.44(s), 1.01(s), 0.99(s), 0.92(s), 0.89(s), 0.86(s), 0.70(s).

IV.2.21. Preparation of mesylate (19)

Mesylate 19 was prepared from alcohol 18 by the same method used to prepare mesylate 13. About 60 mg of a dark yellow solid were obtained.

^{1}H-NMR:ppm; 4.36(d,1H,J=11.8Hz), 3.02(s,3H,CH_3-SO_3), 1.26(s) 1.04(d,J=5.2Hz), 0.90(s), 0.89(s), 0.888(s), 0.878(s), 0.86(s), 0.855(s), 0.69(s)

^{13}C-NMR:ppm; 134,95 and 134.01($\triangle^{8}$), 90.67(C-2), 50.89, 5063

IV.2.22. Reduction of mesylate (19) with $LiEt_3BD$

Mesylate 19 was reduced with $LiEt_3BD$ by the method used to reduce 13. Sixty mg (0.12 mmol) of 19 was used and over 3 days, 2.4 ml of $LiEt_3BD$ were added periodically. The TLC showed spots for the mesylate and for the alcohol.
IR (KBr): 3381, 2924, 1462, 1384, 1279, 1215, 1054, 759 cm^{-1}.

IV.2.23. Hydroboration and protonolysis[38-40] of lanosta-2,8-diene

Lanosta-2,8-diene (100 mg, 0.24 mmol) and THF (10 ml) were added to a flask equipped with a N_2 inlet and a rubber septum. BH_3-THF (1 ml) was added with a syringe and the mixture was stirred at 25°C. Over 2 days a total of 7 ml of BH_3-THF was added in 1 ml portions every 6 hours. The reaction was followed by TLC. The THF was distilled off, then 5.0 ml of glacial acetic acid was added. The mixture was refluxed for 14 hours and then the excess acid was distilled off. The residue was chromatographed on alumina with hexane/ether (9:5). The eluted material was analysed by GC.

The crude product showed a carbonyl band in the IR. The GC showed lanost-8-ene and lanosta-2,8-diene and other products but the overall yield was low.

IV.2.24. Deuteration of 3-oxolanost-8-ene to give (20)

3-Oxolanost-8-ene (3) (500 mg, 1.2 mmol) was added to 10 ml of freshly distilled dioxane. Sodium methoxide (100 mg, 1.85 mmol) and 2 ml of deuterium oxide were added and the mixture refluxed under N_2 for 5 hours, then cooled and poured into dilute HCl. The aqueous solution was quickly extracted with ether; the ether extract was then washed with saturated NaCl, dried over $MgSO_4$, filtered, and the solvent was removed under vacuum. This procedure was repeated again. Yield, 436 mg of 20.

^{1}H-NMR:ppm; 2.01(m), 1.62(s), 1.26(s), 1.11(d,J=5.4Hz), 1.07(s), 0.89(s), 0.888(s), 0.72(s).

^{13}C-NMR:ppm; 217.73 (C-3), 135.54 and 133.33 ($\overset{8}{\triangle}$)

IV.2.25. Preparation of tri-deuterated 2-hydroxy-lanost-8-ene (21)

Ketone 20 was reduced with $LiAlD_4$ by the same procedure used with ketone 10. Compound 20 (436 mg) was treated with $LiAlD_4$ (400 mg) to give 255 mg of alcohol 21.

^{13}C-NMR:ppm; 134.62($\overset{8}{\triangle}$)

IV.2.26. Preparation of mesylate (22)

Mesylate 22 was prepared by the method used to make mesylate 13. Compound 21 (255 mg, 0.59 mmol) in 25 ml of pyridine was treated with methanesulfonyl chloride (0.5 ml, 6.5 mmol) to give 252 mg of mesylate 22.

^{1}H-NMR:ppm; 3.02 (s, 3H, CH_3-SO_3-), 2.0(m), 1.26(s), 1.04(d, 2H, J=5.2 Hz), 0.90(s), 0.89(s), 0.885(s), 0.88(s), 0.86(s), 0.857(s), 0.69(s,3H)

^{13}C-NMR:ppm; 134,98 and 134.07 ($\angle^{8}$)

IV.2.27. Preparation of di-deuterated lanosta-2,8-diene (23)

The reductive elimination of mesylate 22 was carried out using sym-collidine by the same method used to prepare 16. Compound 22 (200 mg, 0.39 mmol) was refluxed with sym-collidine (0.6 ml) to yield ~130 mg of 23, a white , waxy solid. After chromatography on alumina, GC-MS analysis showed the product to be a mixture but the major component to be predominantly dideuterated.

^{1}H-NMR:ppm; 0.99(d,J=5.4 Hz), 0.98(s), 0.92(s), 0.90(s), 0.86(s), 0.73(s)

^{13}C-NMR:ppm; 135.22 and 133.24 ($\angle^{8}$)

IV.2.28 Hydrogenation of betulin to give dihydrobetulin (26)

A mixture of betulin (310 mg), ethyl acetate (200 ml) and 10% palladium on charcoal (200 mg) was stirred at 25^{o}C for 2 hours under 2 atm. of hydrogen. The catalyst was removed by filtration and the solvent was removed under vacuum. Dihydrobetulin (300 mg) was obtained: mp: 275-277^{o}C (lit. 278-280^{o}C)[14].

^{1}H-NMR:ppm; 3.79(d,1H,J=10Hz, 28-H) 3.31(d,1H,J=11 Hz, C_{28}-H), 3.21(dd,1H,J=5.5 Hz and 10 Hz, 3-H), 1.04(s), 0.98(s), 0.99(d,s=3.2 Hz,20-$(Me)_2$), 0.98(s), 0.97(s), 0.87(s), 0.84(s), 0.83(s), 0.79(s), 0.77(s), 0.76(s)

^{13}C-NMR:ppm; 79.00(C-3), 60.62(C-28)

IV:2.29 3-Oxo-28-lupanal (27)[18,19]

Dihydrobetulin 26 (1.0 gm, 2.25 mmol) in 100 ml of methylene chloride was oxidized with a 3 molar excess PCC(1.45 gm)as described for 2. The oxidation yielded 870 mg of 26 melting at 190-195^{o}C (lit. 196-198^{o}C[60]).

^{13}C-NMR:ppm; 217.72(C-3), 206.95(C-28), 59.88(C-17)
MS:m/e(%): 440(M^{+}), 412(8), 411(18), 205(53), 177(36), 135(45), 109(57), 95(68), 81(85), 43(96).

IV.2.30. Wolff Kishner reduction of (27) to lupane (28)

Lupane 28 was prepared by the Wolff Kishner reduction[61] of 27. Crude 27 (400 mg, 0.9 mmol) and 40 ml of dry freshly distilled diethylene glycol were added to a flask, then 0.72 ml of 85% hydrazine hydrate and 0.98 g of KOH were added. The mixture was heated at 100^{o}C, under N_2 for one hour, then at 200-210^{o}C for 4-4 1/2 hours. The mixture was cooled, diluted with water and extracted with ether. The ether extract was washed with water, 5% $NaHCO_3$, and water, then dried over $MgSO_4$ filtered, and the solvent was removed under vacuum. The crude

product was chromatographed on basic alumina with hexane/ether 9:5 to yield 210 mg (56%) of lupane, melting at 186.5-188°C (lit. 184-185°C[14]).

IV.2.31. Oxidation of (27) to the Keto-acid (29)

Procedure 1[62]

Compound 27 (133 mg) was dissolved in 0.3 ml of 95% EtOH and 357 mg silver nitrate in 0.6 ml of water was added. NaOH (125 mg) was dissolved in 1.25 ml of water then added dropwise with stirring. Stirring was continued for 1 hour, the mixture filtered, the filtrate was acidified, filtered again, then extracted with ether. The ether extract was washed as usual, dried over $MgSO_4$ and the solvent was removed under vacuum. The ^{1}H-NMR still showed the presence of the aldehyde.

(1a) The procedure was repeated, allowing the mixture to stir overnight and acidifying it with 3N HNO_3, but the same results as before were found.

(1b) The procedure was modified by partially dissolving the aldehyde in ether and slowly adding this to the silver nitrate in 10% NaOH and stirring overnight. An ^{1}H-NMR of the product still showed the presence of the aldehyde proton.

Procedure 2[63]

$Ca(OCl)_2$(212) mg was added to 3 ml of water and 0.3 ml of glacial acetic acid added dropwise to get the $Ca(OCl)_2$ into

solution. Aldehyde 27 (350 mg) was dissolved in 15 ml of warm acetonitrile or CH_2Cl_2 and the $Ca(OCl)_2$ solution was added dropwise.

The resultant mixture was stirred at 25°C overnight. The solution was extracted with ether, the ether washed with 5% $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and then the solvent was removed under vacuum. An ^{1}H-NMR of the product still showed the aldehyde proton and the IR still showed bands at 1729 and 1701 cm^{-1}.

Procedure 3[64]

Procedure "a" for the oxidation of 2 was followed treating 150 mg of 27 with 189 mg of CrO_3 and 0.75 ml of pyridine to yield 45.4 mg (46%) of 28 having a mp:251-254°C (lit.258-260°C[14]).

Procedure 4[65]

Aldehyde 27 (500 mg, 1.1 mmol), $KMnO_4$ (1.33 gm, 8.4 mmol) and acetone (70 ml) were refluxed for 1 hour. The mixture was cooled, acidified with dilute H_2SO_4 and 5% $NaHSO_3$ was added until the brown color was gone. The aqueous mixture was extracted with chloroform , which was then washed with 5% $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and the solvent was removed under vacuum. The reaction yielded 474 mg (89.5%) of 28. Compound 28 (474 mg) was chromatographed on silica with $CHCl_3$:MeOH (95:5) to give 304 mg of fine, white needles melting at 251-254°C (lit.258-260°C[14]).

IR:(KBr): 2959, 2868, 1701, 1687, 1455, 1384, 1244, 1208, 759 cm^{-1}

^{1}H-NMR:ppm; 2.47(m), 2.25(m), 1.45(m), 1.08(s), 1.03(s), 0.97(s), 0.94(s), 0.87(d), 0.77(d)

^{13}C-NMR:ppm; 218.11 (C-3), 182.60 (C-28), 57.0 (C-17)

IV.2.32. Photo-oxidation of (42)

The method of Vystrčil and Protiva[23] was used, irradiating 42 (300 mg), benzene (75 ml), lead tetra-acetate (0.81 gm) and pyridine (0.31 ml) under 450W lamp for 5-6 hours. The product was chromatographed on silica gel with hexane/ether (9:5). GC-MS analysis and ^{1}H-NMR showed the product to be composed of an ether and two unsaturated compounds. The mixture was chromatographed on silica gel impregnated with $AgNO_3$ (10%). The mixture could not be separated into the individual components.

IV.2.33. Decarboxylation of Ketoacid (29)

a) Formation of the acid chloride

The acid chloride was prepared by treating ketoacid 29 (1.4 gm, 0.31 mmol), in benzene (25 ml) with oxalyl chloride (1.5 ml, 17.6 mmol) and DMF (3 drops). The mixture was stirred for 2 hours under N_2 at 25^{o}C, then the solvent was removed under vacuum and the acid chloride was used immediately.

b) Decarboxylation step

N-hydroxypyridine-2-thione Na salt (554 mg, 3.7 mmol), DMAP(36.6 mg, 0.3 mmol), dry benzene (30 ml) and t-butyl

mercaptan (1.5 ml, 13.3 mmol) were refluxed under N_2 for 5 minutes, then the acid chloride dissolved in 15 ml of benzene was added from an addition funnel over 20 minutes to the refluxing mixture. Refluxing was continued for 6 hours, the mixture was cooled, washed thoroughly with water and saturated NaCl solution, then dried over $MgSO_4$, filtered, and the viscous red-brown liquid was chromatographed on silica with hexane/ether (9:5) to give a semi-solid which could be made to precipitate by adding cold methanol. Compound 30 (650 mg, 52%), melting at 198-202°C, was obtained. This was chromatographed on silica gel impregnated with 10% $AgNO_3$ to remove the small amount of 3-oxonorlupene formed.

IR:(KBr); 2945, 2868, 1701, 1447, 1384, 1265, 801 cm^{-1}

^{1}H-NMR:ppm; 2.48(m,2-H_2), 1.94(m), 1.69(m), 1.48(m), 1.09(s), 1.04(s), 0.99(s), 0.94(s), 0.91(s), 0.85(s), 0.83(s), 0.826(s), 0.81(s)

^{13}C-NMR:ppm; 218.37(C-3)

MS:,m/e(%): 412(75), 369(53), 206(32), 205(100), 177(62), 135(75), 81(78)

IV.2.34. 28-norlupane (31)

28-Norlupane 31 was prepared by the Wolff Kishner reduction of 30, using the method employed to reduce 27. 3-Oxonorlupane 30 (170 mg, 0.4 mmol) KOH (237 mg), diethylene glycol (20 ml), and 85% hydrazine hydrate (0.18 ml) were allowed to react and the product was chromatographed on basic alumina with hexane/ether (9:5) to yield 58.7 mg (36.9%) of a

white, waxy solid melting at 157-159°C. GC-MS analysis showed the product to be composed of 2 isomers.

IR(film): 2952, 2868, 1455, 1384, 1082, 970 cm^{-1}.

^{1}H-NMR:ppm: 0.96(s), 0.91(s), 0.85(s), 0.83(s), 0.82(s), 0.80(s)

MS:m/e(%): 398(M^+,35), 383(16), 355(11), 260(10), 217(11), 192(36), 191(100), 177(27), 109(30), 95(40)
17β isomer (90%)

MS:m/e(%): 398(M^+,44), 383(10), 355(12), 260(25), 191(100), 177(43), 123(45), 81(60); 17α-H (10%)

Elemental analysis: Calc.: C,86.36%; H,12.64%
Found: C,87.88%; H,12.10%

IV.2.35. 3-Oximino-28-norlupane (32)

Procedure 1

The method used to prepare oxime 4 was used with some changes. The amount of reagents used had to be increased by 3 times; 30 (450 mg, 1.1 mmol), anhydrous Na acetate (484 mg, 5.9 mmol), hydroxylamine.HCl(300 mg, 4.3 mmol), and the reaction time was increased to 60 hours. The oxime (370 mg, 81%) was recrystallized from MeOH-$CHCl_3$ to yield 185 mg (40%) of fine, off-white,needles melting at 264-267°C.

IR(KBr): 3255, 2945, 2868, 1462, 1384, 1258, 1019, 927 cm^{-1}

Procedure 2.

The method of Valterová et al.[50] was used to react 30 (800 mg, 1.9 mmol) with hydroxylamine.HCl(800 mg, 11.5 mmol)

and pyridine (24 ml). After diluting with water, the mixture was extracted with chloroform and the organic layer was washed with 3N HCl, 5% $NaHCO_3$ and water then dried over $MgSO_4$, filtered, and the solvent was removed under vacuum. Oxime 32 (720 mg, 87%) was obtained and used without further purification.

IR: as for procedure 1

IV.2.36. Seconitrile of 28-norlupane (33)

The method of Klinot et al.[49] was used, refluxing oxime 32 (690 mg, 1.6 mmol) with p-toluenesulfonyl chloride (2.30 gm, 0.01 mmol) in pyridine (23 ml) for 6 hours under N_2. The red-brown, viscous liquid produced was chromatographed on basic alumina with benzene to yield 435 mg (67%) of a pale yellow solid.

IR: 2945, 2868, 2249, 1455, 1384, 1152, 1177, 808 cm^{-1}

^{1}H-NMR:ppm; 4.89(m,1H, J=1.74HZ), 4.65(m,1H), 2.30(m,2H), 1.56(s), 1.73(s), 1.00(s), 0.90(s), 0.86(s), 0.83(s), 0.826(s), 0.80(s)

^{13}C-NMR:ppm; 147.14(C-4), 128.38 or 120.29(CN), 114.01(C-24)

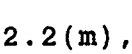
MS:m/e(%): 427(2), 409(14), 366(17), 177(31), 135(62), 95(71), 81(93)

IV.2.37. Epoxy-nitrile (34)

The method of Valterová et al,[48] was used in which seco-nitrile 33 (470 mg, 1.1mmol) was dissolved in 10 ml of $CHCl_3$ and cooled to 0°C. m-Chloroperbenzoic acid (376 mg, 2.2 mmol)

was dissolved in 10 ml of $CHCl_3$, cooled to 0°C, then added to the seco-nitrile solution. The mixture was kept at 0°C in the dark for 67 hours, then washed with 5% KI, 5% $NaHSO_3$, 5% $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under vacuum to yield 353 mg (73%) of epoxy-nitrile 34. A portion was chromatographed on basic alumina with hexane/ether 9:5 to give 54% of 34 melting at 170-172°C.

IR: 2952, 2868,2249(CN), 1722, 1455, 1384, 1265, 1152, 1089, 1019, 801, 519 cm^{-1}.

^{1}H-NMR:ppm; 2.73(d,1H,J=4.4HZ,C ), 2.63(d,1H,J=4.4 C ), 2.2(m), 1.28(s, CC-Me), 0.98(s), 0.96(s), 0.85(s), 0.83(s), 0.82(s), 0.80(s)

^{13}C-NMR:ppm; 119.8(3-CN)

Elemental analysis: Calc.: C,81.82%; H,11.13%; N,3.29%
Found: C,82.02%; H,11.28%; N,3.30%

IV.2.38 3-Oxo-23, 28-bisnorlupane (35)

According to the procedure of Valterová et al.[48], epoxy-nitrile 34 (184 mg, 0.4 mmol) was refluxed with BF_3-etherate (0.4 ml) in toluene (50 ml) for 6 hours under N_2. The reaction mixture was cooled, diluted with ether, the ether layer was then separated and washed with 3N HCl, 5% $NaHCO_3$ and water. After drying over $MgSO_4$ the solvent was removed under vacuum. The product was chromatographed on basic alumina with hexane/ ether 9:5 to give 108 mg (63%) of 35 melting at 160-164°C.

IR (film): 2938, 2868, 1708, 1455, 1384 cm^{-1}

^{1}H-NMR:ppm; 2.37(m), 2.05(m), 1.06(s), 1.04(s), 1.01(s), 0.98(s), 0.97(s), 0.94(s), 0.91(s), 0.89(s), 0.85(s), 0.83(s), 0.82(s)

^{13}C-NMR:ppm; 213.48(C-3)

MS:m/e(%); 398(M^{+},75), 355(63), 306(8), 263(7), 192(100), 191(95), 135(77), 109(56), 95(63), 81(81)

High resolution MS of molecular ion:

Calc.: 398.35508

Found: 398.35486

IV.2.39 Preparation of 23, 28-bisnorlupane (36)

Bisnorlupane 36 was prepared by the Wolff Kishner reduction of 35 using the procedure employed to reduce 27. Compound 35 (400 mg, 1.0 mmol) was reacted with KOH(490 mg), diethylene glycol (40 ml) and 85% hydrazine hydrate (0.36 ml) and the 224 mg (57%) of crude 36 obtained were chromatographed on silica impregnated with 10% $AgNO_3$ with hexane, hexane/ether (9:1) and hexane/ether (9:2) to yield 82 mg of 36, a white, waxy solid melting at 104-111^{o}C. Because the product was a mixture of isomers, as shown by GC-MS, chromatography on Kiesel gel silica - 5% $CaSO_4$ with cyclohexane and cyclohexane/ether (9:1) was tried by collecting with a fraction collector, but separation of the isomers could not be achieved.

IR(film): 2938, 2868, 1448, 1384, 1216, 759 cm^{-1}

^{1}H-NMR:ppm; 0.97(s), 0.91(s), 0.86(s), 0.83(d,J=1.6Hz), 0.80(s), 0.79(s), 0.78(s)

MS of isomers in order of elution from GC:

isomer 1:m/e(%); 384(M^+,27), 369(21), 341(17), 217(8), 192(22), 177(100), 135(32), 109(33), 95(42), 81(40) 17 α(H),4 α(CH_3)-2%

isomer 2:m/e(%); 384(M,28), 369(17), 341(7), 192(24), 177(100), 149(22), 135(36), 109(47), 95(38), 81(62) 17β(H),4 α(CH_3)-90%

isomer 3:m/e(%); 384(M^+,30), 369(13), 341(5), 217(7), 177(100), 149(13), 135(12), 109(23), 95(28), 81(26) either 17 α(H),4β(CH_3) or 17β(H),4β(CH_3) - 12%

High resolution MS of molecular ion: Calc.: 384.37584
Found: 384.37560

IV.2.40 3,28-diacetoxylupane (41)

Diacetate 41 was prepared by heating dihydrobetulin 26 (1.14 gm, 2.6 mmol), acetic anhydride (23 ml) and freshly distilled pyridine (57 ml) on a steam bath for 2 hours, then stirring at 25°C overnight. The mixture was diluted with water, extracted with ether and the organic layer was washed with 3N HCl, 5%$NaHCO_3$ and water. The ether extract was dried over $MgSO_4$, filtered, and the solvent was removed under vacuum. The product was either recrystallized from CH_2Cl_2-MeOH or chromatographed on basic alumina with hexane/ether (9:5) to yield 1.10 gm (82%) of diacetate 41 melting at 256-257°C (lit. 256- 259°C[14])

^{1}H-NMR:ppm; 4.48(dd.1H,J=5.8Hz,J=9.4Hz,3-H),
4.25(d,1H,J=11.2Hz,28-H_2)
3.82(d,1H,J=10.5Hz,28-H_2)
2.07(s,3H,CH_3CO), 2.05(s,3H,CH_3CO), 1.58(s),
1.04(s), 0.95(s), 0.86(s), 0.855(s), 0.847(s),
0.83(s), 0.77(d,J=6.6Hz,20-Me_2)

^{13}C-NMR:ppm; 171.58(acetate carbonyl), 170,96(acetate carbonyl), 80.93(C-3),62.82(C-28), 21.64(CH_3 on C-28 acetate), 21.37 (CH_3 on C-3 acetate)

Elemental analysis: Calc.: C,77.22%;H,10.6%
Found: C,77.37%;H,10.72%

IV.2.41. Preparation of monoacetate (42)

The monoacetate 42 was prepared by the partial hydrolysis of 41. Diacetate 41 (1.4 gm, 2.6 mmol), benzene (26 ml), and KOH(145 mg), dissolved in MeOH(10 ml) were stirred at 25°C for 5-7 hours. The reaction was followed by TLC. The mixture was diluted with ether, the ether was separated and washed with water, dried over $MgSO_4$, filtered, and the solvent was removed under vacuum. The product was chromatographed on basic alumina with hexane/ether (9:3) to give 580 mg (70%) of monoacetate 42 melting at 250.5-253.5°C. Also recovered were 37 mg of the diacetate 41 and a small amount of dihydrobetulin.

IR(KBr): 3445, 2952, 2868, 1736, 1469, 1370, 1244, 1026, 977 cm^{-1}

^{1}H-NMR:ppm; 4.48(m,1H,3-H), 3.78(d,1H,J=11.6Hz, 28-H),
3.31(d,1H,J=11.z,28-H), 2.05(s,1H,CH_3CO),
1.03(s), 0.96(s), 0.86(s), 0.85(s), 0.83(s),
0.79(s), 0.76(s)
^{13}C-NMR:ppm; 171.07 (acetate carbonyl), 81.01(C-3)

Elemental analysis: Calc.: C,78.96%, H,11.18%
Found: C,79.59%, H,11.34%

IV.2.42. Preparation of 3-acetoxy-28-lupanal (43)

The mono-acetate 42 was oxidized using the method for oxidizing 2 by treating 42 (590 mg, 1.2 mmol) with a 1.5 molar excess of PCC(376 mg) in CH_2Cl_2(40 ml). The product was chromatographed on basic alumina with hexane/ether (9:3) to give 586 mg (90%) of 43 melting at 209-212^{o}C.

IR(KBr): 2945, 2875, 1736, 1708, 1467, 1391, 1370, 1251, 1026,
977, 759 cm^{-1}.
^{1}H-NMR:ppm; 9.65(s,1H,CHO), 4.48(m,1H,3-H),
2.06(s,3H,CH_3CO-),
1.57(s), 0.95(s), 0.91(s), 0.90(s), 0.89(s),
0.85(s), 0.84(s), 0.81(s), 0.77(s)
^{13}C-NMR:ppm; 206.32(C-28), 170.12(acetate carbonyl),
80.07(C-3)

IV.2.43. Preparation of 3B-hydroxylupane (44)

Compound 44 was prepared by the Wolff Kishner reduction of 43, similar to the reduction of 27. Compound 44 (700 mg,

1.4 mmol) in diethylene glycol (60 ml) was treated with KOH(860 mg) and 85% hydrazine hydrate (0.64 ml) to yield 503 mg (82%) of 44 after chromatography on basic alumina with hexane/ether (9:5).
mp: 207-208°C (lit. 203-204°C[44]).

IV.2.44. Preparation of 3-oxolupane (45)

3β-Hydroxylupane 44 was oxidized with PCC as for 2 by treating 44 (400 mg, 0.93 mmol) with a 1.5 molar excess of PCC(300 mg). The product was chromatographed on basic alumina with hexane/ether (9:5) to yield 347 mg (88%) of 45 melting at 203.5-205.5°C (lit. 209.5-210°C[14]).

IV.2.45 Preparation of the tosylhydrazone of 3-oxolupane (46)

Tosylhydrazone 46 was prepared using the method to make tosylhydrazone 8 except that twice the amount of reagents had to be used. Compound 45 (100 mg, 0.28 mmol) was treated with tosylhydrazine (120 mg, 0.6 mmol), MeOH(5 ml) and acetyl chloride (1 ml). The product was chromatographed on basic alumina with hexane/ether (9:5) to yield 50 mg (45%) of 46 melting at 169-171°C.

IR(KBr): 3213, 2952, 2868, 1602, 1462, 1384, 1342, 1166, 1096, 1012, 815, 667, 547 cm^{-1}

^{1}H-NMR:ppm; 7.84(d,J=8.2Hz), 7.30(d,J=8.2Hz), 2.42(s,CH_3-C_6H_4), 1.08(s), 1.03(s), 0.94(s), 0.89(s), 0.86(s), 0.82(s), 0.78(s), 0.77(s)0.75(s)

IV.2.46 Preparation of mono-deuterated lupane (47)

Tosylhydrazone 46 was reduced with $LiAlD_4$, as for the reduction of 8 except refluxing was continued for 4 hours and water was used to decompose the excess $LiAlD_4$. Tosylhydrazone 46 (215 mg) was treated with $LiAlD_4$ (227 mg) to yield, after chromatography on silica followed by recrystallization from MeOH, giving 48.6 mg of product. This was chromatographed on silica impregnated with 10% $AgNO_3$ using hexane and hexane/ether (9:2) to give 47 (12 mg).

mp: 188-189°C
IR(KBr): 2931, 2868, 1455, 1384, 801, 731 cm^{-1}
MS:m/e(%): 413(m^+,29), 398(11), 370(11), 259(11), 231(12), 205(8), 193(27), 192(100), 191(26), 177(11), 123(37), 95(46), 81(42), 69(44), 55(32)
^{1}H-NMR:ppm; 1.62(m), 1.37(m), 1.04(s), 0.93(s), 0.85(s), 0.84(s), 0.80(s), 0.75(s).

IV.2.47 Deuteration of 3-oxolupane to give (48)

3-Oxolupane (45)was deuterated by the same method used to deuterate 3. Initially, 45 (611 mg, 0.4 mmol) was treated with NaOMe (100 mg, 1.8 mmol) and D_2O(2 ml). This reaction was repeated 3 more times. It was then treated with potassium t-butoxide (200 mg) and D_2O(2 ml), refluxing for 48 hours and then worked up as for 3. The 3-oxolupane was treated twice more with NaOMe and D_2O, then with K_2CO_3 (6.25 mg) and D_2O (0.94 ml)[52], refluxing for 52 hours. The mixture was

saturated with NaCl and then extracted with ether. The ether layer was dried over $MgSO_4$, filtered, and the solvent was removed under vacuum to yield 175 mg of 48.

MS:m/e(%): 428(M^+,17), 427(1.7), 385(17), 207(65), 163(52), 123(81), 95(88), 69(100)

^{1}H-NMR:pm; 1.66(s), 1.08(s), 1.04(s), 0.95(s), 0.947(s), 0.86(s), 0.83(s), 0.78(s), 0.77(s), 0.75(s),

^{13}C-NMR:ppm; 218 (C-3)

IV.2.48. Reduction of deuterated 3-oxolupane to give (49)

Alcohol 49 was prepared by treating 48 (175 mg, 0.4 mmol) with $LiAlD_4$ (170 mg, 4 mmol) as in the reduction of 10. The yield of 49, was 107 mg (60.8%).

^{1}H-NMR:ppm; 1.25(s), 1.03(s), 0.96(s), 0.91(s), 0.87(s), 0.84(s), 0.83(s), 0.81(s), 0.76(s), 0.755(s), 0.74(s), 0.73(s)

IV.2.49. Preparation of the mesylate of 3-hydroxylupane (50)

Mesylate 50 was prepared as for mesylate 13a, by treating 49 (107 mg, 0.25 mmol) with MsCl (0.4 ml) in pyridine (15 ml). The yield of mesylate 50 was 164 mg.

^{1}H-NMR"ppm; 3.02(s,CH_3-SO_3-), 1.03(d,J=4.4Hz), 0.92(s), 0.88(s), 0.86(s), 0.83(s), 0.78(s), 0.76(s)

IV.2.50. Elimination of the mesylate to give (51)

Elimination of the mesylate 50 was carried out as for the formation of 16. Mesylate 50 (164 mg, 0.3 mmol) was refluxed with sym-collidine (1 ml) to give 130 mg of 51. This was used without purification.

^{1}H-NMR:ppm; 4.79(weak), 1.08(s), 0.95(s), 0.89(s), 0.87(s), 0.83(s), 0.79(s), 0.77(s), 0.75(s)

IV.2.51. Hydrogenation of (51) to give (52)

A mixture of 51 (130 mg), ethyl acetate (100 ml) and 10% palladium on charcoal (150 mg) was shaken at 25°C for 2 days under 2 atm hydrogen. The catalyst was removed by filtration, the solvent removed under vacuum and the product chromatographed on alumina with hexane to yield 100 mg of the di-deuterated lupane, a white, waxy solid, melting at 129-148°C.

^{1}H-NMR:ppm; 1.05(s), 0.86(s), 0.855(s), 0.845(s), 0.83(s), 0.80(s), 0.78(s), 0.76(s), 0.75(s)

MS:m/e(%): 414(M^+,36), 413(1.5), 399(17), 397(1), 259(14), 231(13), 193(100), 191(20), 149(18), 123(37), 95(46), 81(37), 69(33).

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