STUDIES IN THE TRANSFORMATION OF STEROIDS

A THESIS SUBMITTED

To The Ahmadu Bello University

For The Degree of Mster of Science

in Chemistry

BY

SAMUEL ADENIYI IBIYEMI, B.Sc.

Department of Chemistry,
AHMADU BELLO UNIVERSITY,
Zaria, NIGERIA.

December, 1968

ABSTRACT

A series of experiments were planned to convert a steroid into a tetracyclic triterpenoid.

As preliminary to these experiments, a number of systematic reactions of related types and conpounds were carried out. N-Mono- and Di-alkylation of a primary aromatic amine (1-naphthy1amine) was first carried out. Next alkylation with different alkyl groups on different types of hydroxyl groups were conducted, Alkylation was done with methyl and ethyl groups on phenolic as well as carboxylic hydroxyl groups, AS podocarpic acid contains both these types of hydroxyl groups, that was the model compound taken for these experiments, Six out of the eight possible forms of the mone and dialkylated products with the two different alkyl groups and the two different hydroxyl groups of podocarpic acid were prepared and characterized.

Three experiments were conducted to convert cholesterol into a nor compound by removing the methylene group at C_4 and converting ring A into a six membered ether. An A/B trans rused and A/B cis fused ether were planned. Out of this, the A/B-trans fused ether was prepared by oxidizing cholesterol to cholesteaone, further oxidizing cholestenone to a ring A a-lactone, then reducing this lactone to a diol and then cyclising this diol to a o-membered ether.

To prepare the cis fused ether, the starting material required was free hydroxy acid to be obtained by hydrolysing the δ-lactone. This free acid could not however, be isolated. Therefore the cis. fused ether could not be prepared.

Cholesterol was oxidized to cholestenone and this was alkylated to 4,4-dimethyl-cholest-5-en-3-one, with methyl iodide and potassium tertiary butoxide. Model experiments were then conducted to methylate C_8. Attempts to prepare it from cholest-5-en-7-one-3β-yl acetate and 6,6-dimetyl-cholest-4-en-7-one were unsuccessful. The former gave elimation, hydrolysis and O-methylation products; while with the later no methylation took place. Therefore, the product initially prepared viz., 4,4-dimethyl-cholest-5-ene-3-one, itself was taken for further experiments. Its C_3-ketone was protected as an ethylene ketal, it was then oxidized at the allylic position to a ketone and was then methylated using a modified procedure. The product isolated was characterized as having an additional methyl group at C_8. From its spectral and other properties it is concluded that in the bulk of the product this methyl group has entered from the α-side.

A C K N O W L E D G E M E N T

I wish to express my deep and very sincere gratitude to Dr. C. R. Narayanan, Reader-in-Chemistry, for suggesting the problem, and for his untiring inspiration and his guidance throughout this investigation.

I am deeply indebted to Professor D. A. H. Taylor, Department of Chemistry, University of Ibadan, for his kind help in undertaking the N.M.R., Mass and Infra-red spectra and the elemental analysis of the compounds described in this thesis. I am also indebted to Dr. D. E. U. Ikong of the Chemistry Department of the University of Ibadan for the same.

I wish to express my thanks to Messrs Joshua K. Olaku and Isaac A. Odeshola for neatly typing this thesis in a very short time.

The constant encouragement and help from my wife and Stephen Afolayan are also acknowledged.

I am indeed deeply indebted to Professor A.R. Mathieson, Head of the Department of Chemistry, for making it possible for me to do my research in this department; and to the University Council and Northern States Government Scholarship Board for the busary and all other forms of financial assistance.

AHMADU BELLO UNIVERSITY, ZARIA.

A Ibiyomi
Candidate.

CONTENTS

I. ABSTRACT. i

II. ACKNOWLEDGEMENT. iii

III. INTRODUCTION. 1

IV. PRESENT WORK AND DISCUSSION. 10

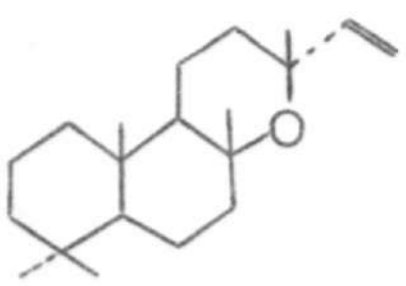
1. N-Alkylation Studies with an Aromatic Primary Amine. 10
2. O-Alkylation Studies with Hydroxyl Groups Having an Acidic Hydrogen. 14
3. Oxidation, Reduction and Cyclization Studies with a Steroid. 20
4. Methylation of Cholesterol to get 4,4-Dimethyl Derivative. 26
5. Experimental. 50

V. REFERENCES. 76

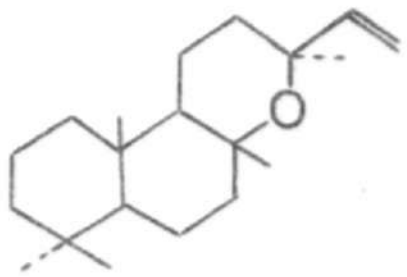
I N T R O D U C T I O N

Some tricyclic diterpenes like manoyl oxide I, epimanoyl oxide II, and diterpene derivatives like isoagathane dicarboxylic acid III, or the scalerol derivative IV, contain an angular methyl group at C-8, besides the one at C-10. In pentacyclic triterpenes belonging to the major groups α-amyrin V, β-amyrin VI, Iupeol VII[1] and zeorin VIII[2] there is invariably an angular methyl group at C-8, unless the triterpenoid is having a rearranged skeleton. However, the principal groups of tetracyclic triterpenes formed by the normal biogenetic cyclisation of squalene as lanosterol IX, euphol X, tirucallol XI, and butyrospermor XII have their angular methyl groups at C-13 and C14, besides the one at C-10[1]. But the nuclear double bond in these comporids is so located that acid catalyzed isomerisation can readily shift the methyl group from C-13 to C-14 to C-8, moving the double bond to the 14, 15 position. From euphol this change would lead to apoeuphol XIII. Similar rearrangement with oxidation at C-7 has actually been achieved in the laboratory with dihydrobytyrospermol acetate leading to a 7-oxo derivative of apoeuphol[3]. After extensive studies conducted by an international collaboration of some of the top organic chemists of the world, the structure of a complex triterpene bitter principle limonin has been recently elucidated[4] as XIV, and was thus found to be derivable from apoeuphol or butyrospermol.

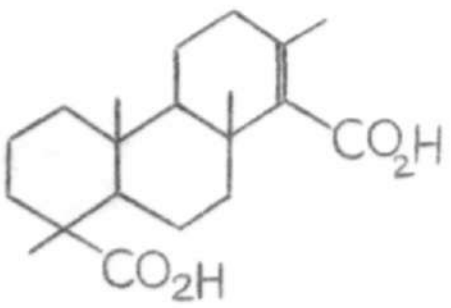
CHART 1



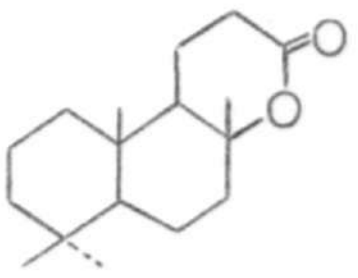
I



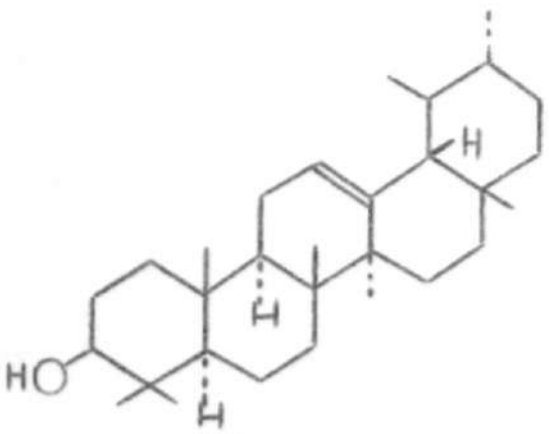
II



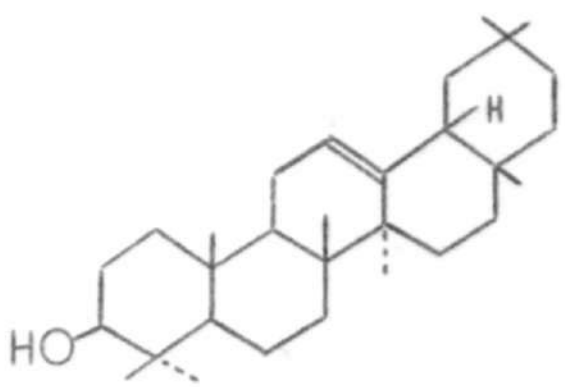
III



IV

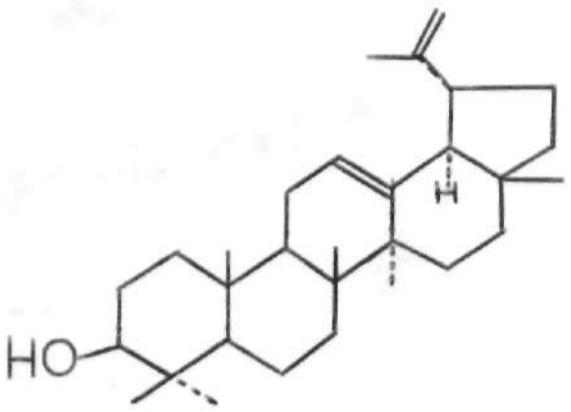


V

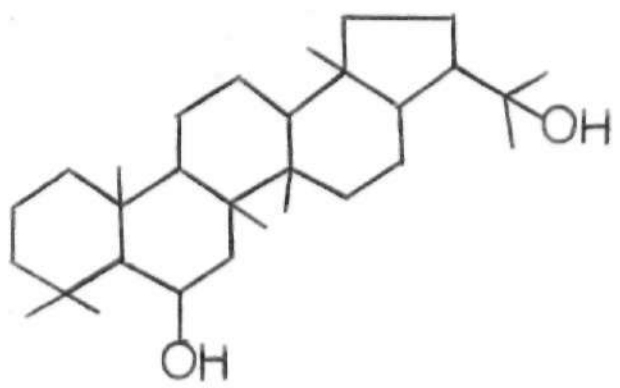


VI

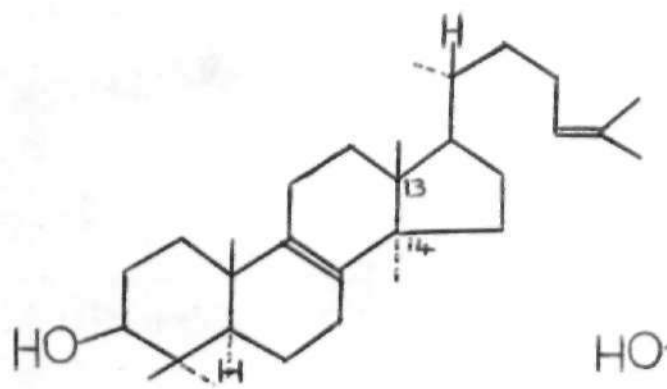
CHART 2



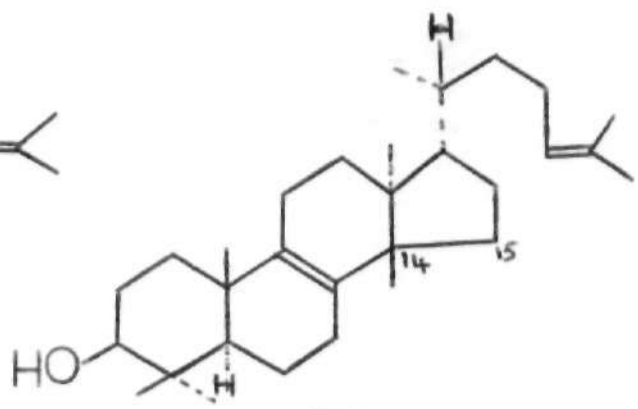
VII



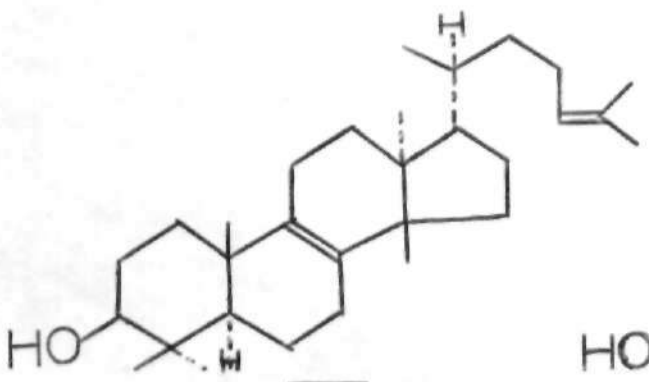
VIII



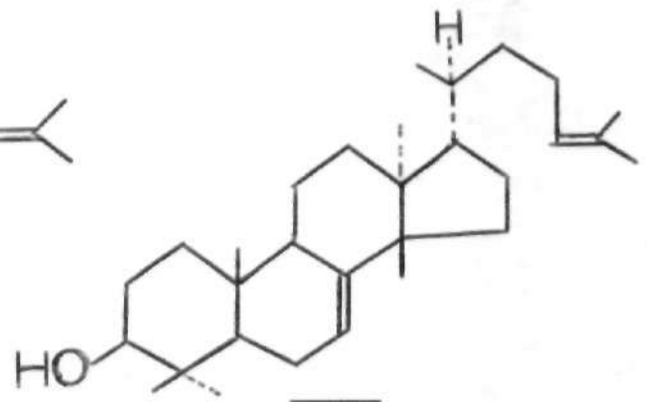
IX



X



XI



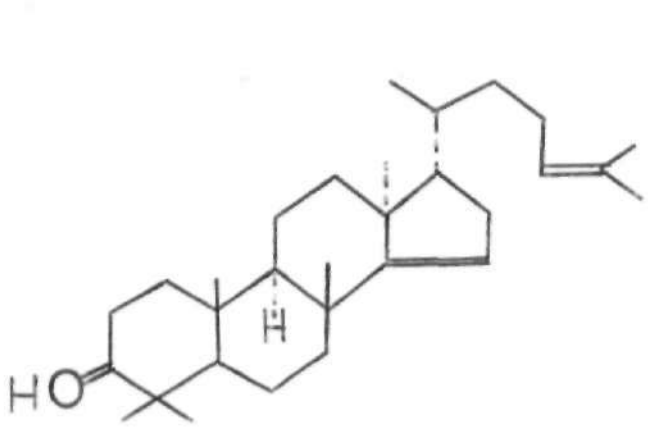
XII

This stimulated work on a number of other complex triterpene bitter principles, the structures of which have very recently been elucidated. They were all found to be related to limonin, but were at different oxidation levels and have one or more of the four ring systems in them oxidized and cleaved.

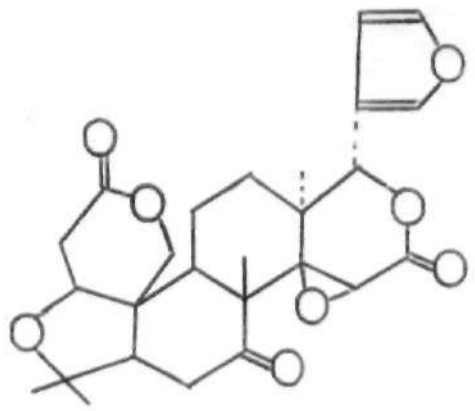
Thus cedarlone XV, has all the four rings intact and ring D five membered[5,6]. In nimbin XVI, and related compounds[7], ring C is oxidized and cleaved. In andirobin[8] XVII, and in methyl angolensate[9,10] XVIII, ring B is oxidized and cleaved. There are a variety of these complex compounds, wherein the A,B,C rings have several oxygen functions, but the rings not cleaved, but the D ring is oxidized to a δ-lactone. Gedunin XIX[11], and Khivorin XX[12], are some relatively simple examples of these.

The synthesis of even some of the simpler members of this group would be a challenging problem to a synthetic organic chemist. One of the problems in such a synthetic approach would be to put in an angular methyl group at C-8, and an oxygen function at C-7, which are invariably found in all these compounds. The abundantly available steroids (which have already been synthesized[13,14]) have got the required tetracyclic system, although the stereochemistry at C-13 and C-17 is different. These could be altered at a subsequent stage.

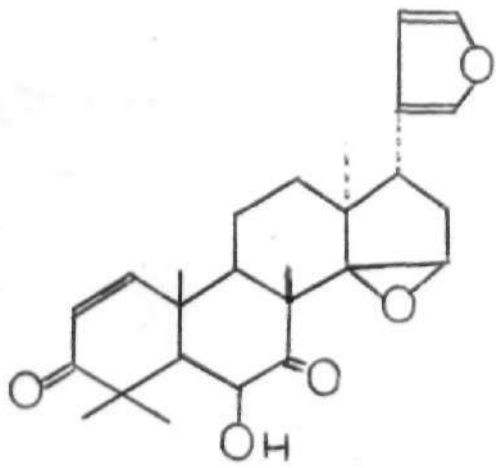
CHART 3



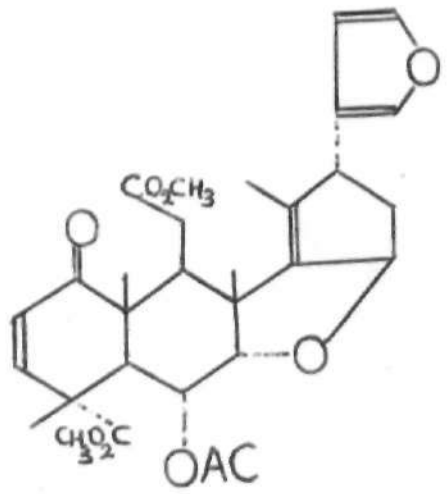
XIII



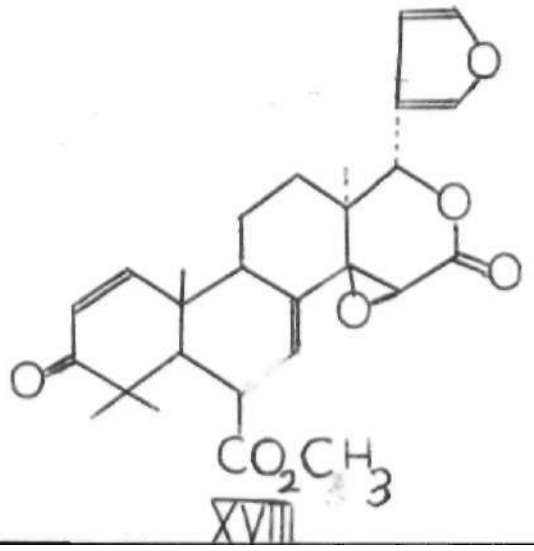
XIV



XV

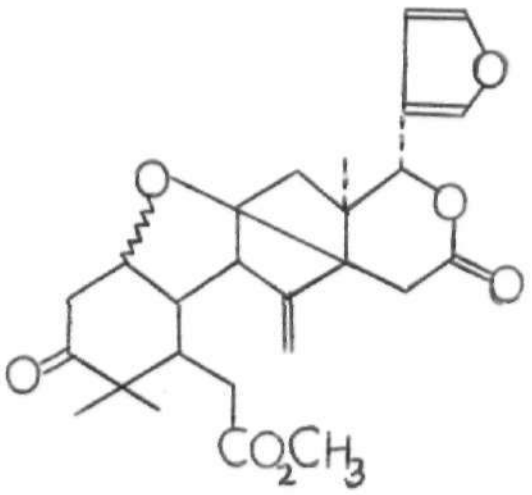


XVI

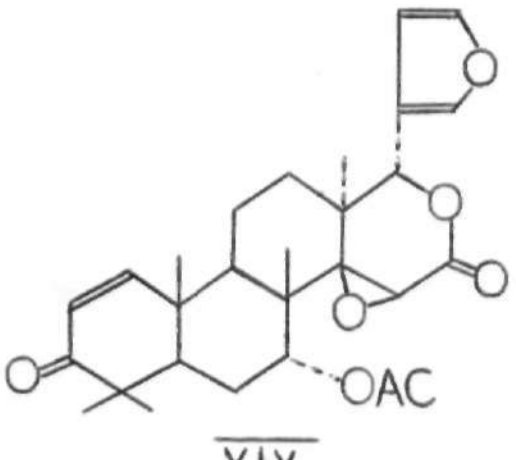


XVII

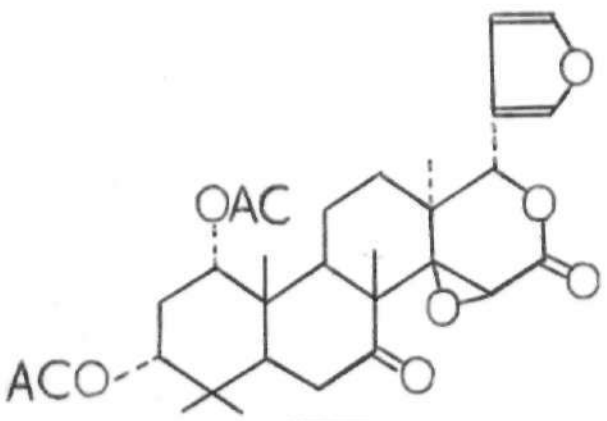
CHART 4



XVIII



XIX



XX

Therefore as a first approach to this problem, it was considered worthwhile to make attempts to introduce a gem dimethyl group at C-4; an angular methyl group at C-8 and an oxygen function at C-7, in the tetracyclic steroid skeleton so as to convert it into an analogous triterpenoid. Approaches made to achieve this objective are described in the next section

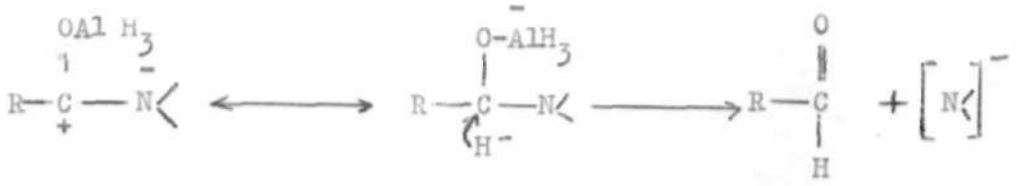
REFERENCES

1. P.de Mayo, The Higher Terpenoids, Interscience Publishers Inc. New York, 1959.
2. D.H.R. Barton, P.de Mayo and J.C. Orr, Journ. Chem. Soc. 2239 (1958).
3. F.S. Spring, W. Lawrie, W. Hamilton and H.S. Watson; Journ. Chem. Soc. 3272 (1956).
4. D.D. Arigoni, D.H.R. Barton, E.J. Corey, O. Jeger, L.Caglioti, S.Dev, P.G. Ferrini, E.R. Glazier, A. Melera, S.K. Pradhan, K. Schaffner, S. Sternhell, J.F. Templeton and S. Tobinaga; Experientia, 16, 41, (1960).
5. H. Hodges, S.G. Me Geochin and R.A. Raphael, Journ. Chem. Soc. 2515, (1960).
6. K.W. Gopinath, T.R. Govindachari, P.C. Parthasarathy, N. Viswanathan, D. Arigoni, and W.C. Wildman; Proc. Chem. Soc. 446, (1961).
7. C.R. Narayanan, and R.V. Pachapurker, Tet. Letters 2607 (1961).
8. W.D. Ollis, A.D. Ward, and A. Zelnick, Tet. Letters 2607 (1964)
9. E.C.W.L. Bevan, J.W. Powell, D.A.H. Taylor, T.G. Halsall, P. Toft, and M. Welford; Journ. Chem. Soc. (C) 1963, (1967).
10. W.R. Chan, K.E. Magnus, and B.S. Mootoo; Journ. Chem. Soc. (C) 171, (1967).
11. A. Akinsanya, C.W.L. Bevan, J. Hirst, T.G. Halsall, and D.A.H. Taylor, Journ. Chem. Soc. 3705, (1961).
12. C.W.L. Bevan, T.G. Halsall, N.N. Nwaji, and D.A.H. Taylor, Journ. Chem. Soc. 768 (1962).

13. R.B. Woodward, F, Sondheimer, D. Taub, K. Heusler and W.N. Maclamore; Journ. Chem. Soc. 74, 4223 (1952) and connected papers.

14. W.S. Johnson, R. Pappo, and W.F. Johns; Jour. Am. Chem. Soc. 78, 6339, (1956) and connected papers.

It has been shown[4,5] that when the electron donating tendency of the nitrogen atom is decreased by resonance stabilization, the adjacent amido carbon atom assumes a more positive character. Then the attack of the hydride ion alone[6], or in conjunction with a shift of electrons from the oxygen atom would lead to a cleavage [7-11] of the carbon-nitrogen bond as shown below:-



In the present case resonance of the pair of electrons on the nitrogen atom with the adjacent naphthalene ring can lead to the above result. The carbonyl compound produced would be readily attacked and reduced by the excess of lithium aluminium hydride present. The N-methyl-α-naphthylamine produced was separated by acid, base treatment from other products, purified by chromatography and characterized by its infra-red and N.M.R. spectra.

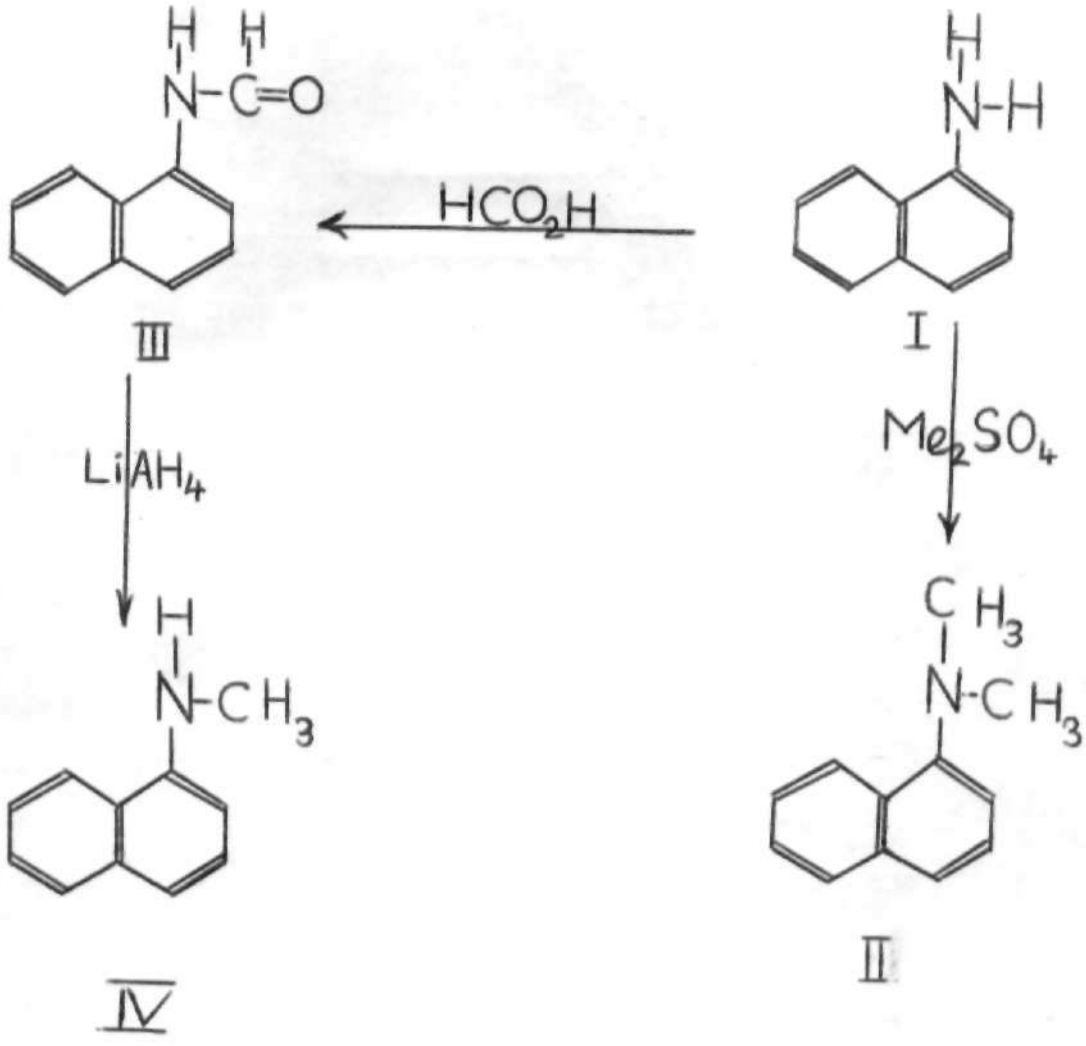
PRESENT WORK AND DISCUSSION

N-Alkylation Studies with an Aromatic Primary Amine

To gain some experience in methylation processes, α-naphtylamine, which is a simple aromatic amine, was taken and was methylated to obtain the dimethyl and monomethyl derivatives, under different conditions. By methylating with dimethyl sulphate in the presence of a base, the dimethyl derivative was readily obtained in about 68 per cent yield. The liquid product so obtained was purified by distillation under very low pressure and was characterized[1] by its boiling point and spectral properties.

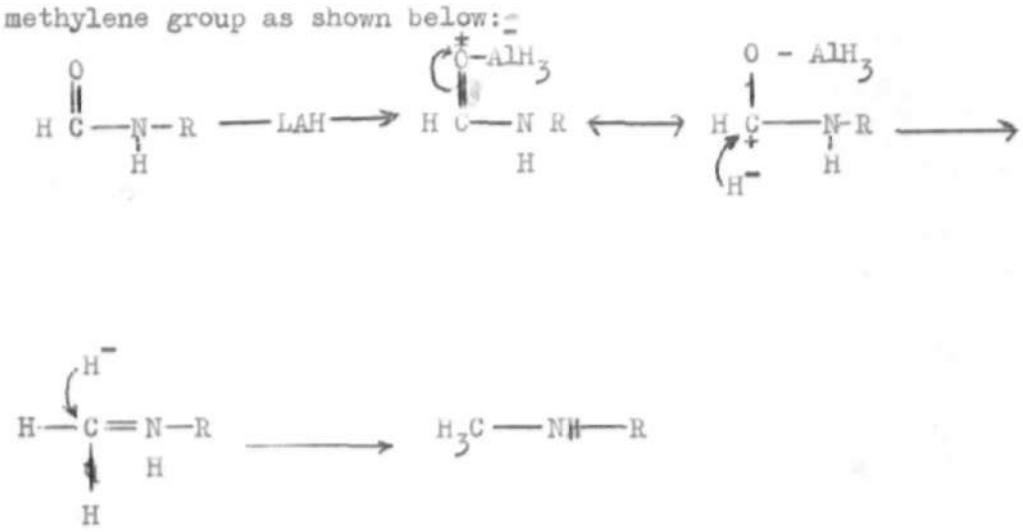
The monomethyl derivative however was not easy to prepare. The procedures given in literatures for its preparation are not very straightforward, and do not appear to lead to easy isolation of the pure product. One procedure is to methylate α-naphthylamine with the methyl ester of p-toluene sulphonic acid into a mixture of the mono- and dimethyl derivatives; this is followed by converting it to a mixture of sulphate salts with sulphuric acid, and then separating the two sulphate salts by fractional crystallization[2]. The other procedure consists in methylating α-naphthylamine to the N,N-dimethylnaphthylamine, and then carrying out a Von Braun reaction on it with cyanogen bromide to give the N-methyl-α-naphthylamine in two or three steps.

CHART I



The yield of the final product is not given[3]. Besides, the preparation of cyanogen bromide, and conducting the reaction with cyanogen bromide has not been very convenient.

A novel method was therefore adapted to prepare the compound (vide chart I). By heating with formic acid at water-bath temperature, α-naphthylamine was converted to the N-formamido derivative. This compound was isolated, purified by crystallization, and characterized by its melting point and infra-red spectrum. The formamide was then subjected to reduction with lithium aluminium hydride to give the monomethyl derivative by the reduction of the carbonyl to a methylene group as shown below:-



However, the yield of the monomethyl derivative in this case was not very high. This should probably arise from a side reaction in which the carbon nitrogen bond would get cleaved during the reduction.

O-Alkylation Studies With Hydroxyl Groups Having An Acidic Hydrogen

After the successful mono-and di-N-alkylation with an aromatic amine, O-alkylation, with two different alkyl groups was conducted on a phenolic and a carboxylic hydroxyl group. Since both the type of hydroxyl groups are present in the diterpene podocarpic acid, each of them was in turn alkylated with a methyl and ethyl group. With the two different types of hydroxyl groups in the molecule and the two different alkylating groups, it is possible to obtain four different monoalkylated products viz. 1, methyl podocarpate, 2, ethyl podocarpate, 3, O-methyl podocarpic acid, and 4, O-ethyl podocarpic acid, and also four different dialkylated products viz: 5, methyl, O-methyl-podocarpate, 6, methyl, O-ethyl podocarpate, 7, ethyl, O-methyl-podocarpate, and 8, ethyl, O-ethyl podocarpate. Out of these the last six were prepared and fully characterized. Since the preparation of the first two would have been more involved and tedious, and also since it would not have given any additional new type of experience, attempts were not made tp prepare them.

Their preparation is schematically shown in Chart II. Methylation of podocarpic acid V with excess dimethyl sulphate in the presence of a base[12] readily gave methyl, O-methyl podocarpate VI[13]. Hydrolysis of this ester under strongly alkaline conditions[14-15] hydrolysed the ester group and gave O-methyl podocarpic acid VII[16]. As the yield of this product was poor, several attempts were made to hydrolyse the ester under varying conditions.

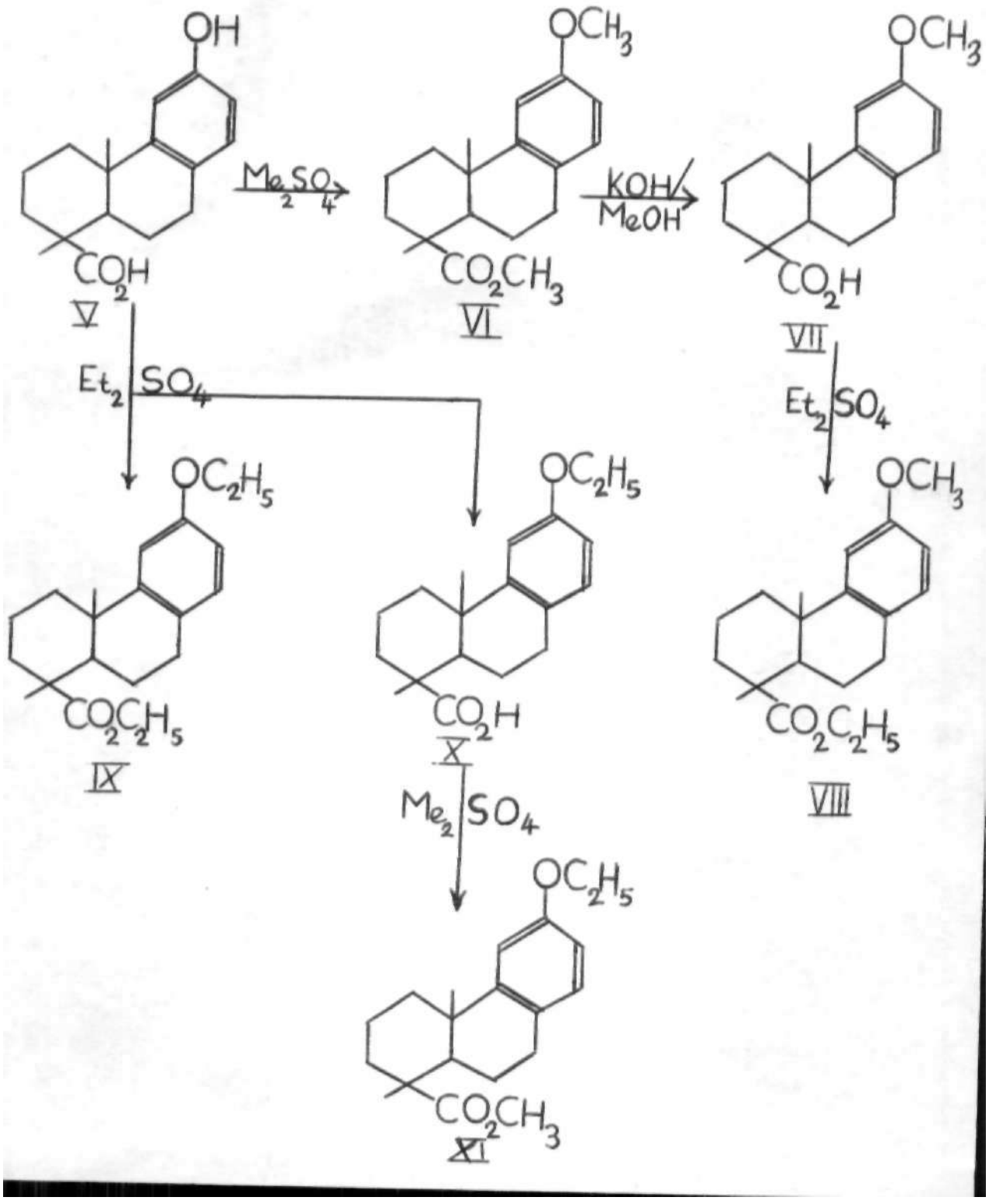
Increasing the percentage of the alkali, changing the composition of the methanol dioxane mixture to increase the proportion of the higher boiling dioxane and thus raising the reflux temperature, and increasing the hours of reflux, were the variations tried. The results are summarized in Table 1.

Table 1

Exp. No.	Podocarpic Acid. (mg).	KOH (Gm)	Solvents MeoH (ml)	Dioxane (ml)	Time (Hours)	Unreacted Methyl, O-Methyl podocarpate (mg)	O-Methyl Podocarpic Acid (mg)	(%)	Remarks
1	400	20	100	-	16	300.8	80	20	
2	259	20	100	-	24	150	100	39.3	
3	517	25	100	-	36	282	200	38.8	
4	395	25	40	60	36	243.1	87.4	22.4	The crystals were coloured brown.
5.	727.4	25	30	70	36	400	28.8	35.4	The solution and crystals were coloured brown.
6.	1 gm.	25	25	75	36	700	110	11	Two phased solution, shaken to mix them.

(Hydrolysis of methyl, O-methyl Podocarpate)

CHART 2


OH
Me_2SO_4
OCH_3
KOH/MeOH
OCH_3
CO_2H
V
CO_2CH_3
VI
CO_2H
VII
Et_2SO_4
Et_2SO_4
OC_2H_5
OC_2H_5
OCH_3
$CO_2C_2H_5$
IX
CO_2H
X
$CO_2C_2H_5$
VIII
Me_2SO_4
OC_2H_5
CO_2CH_3
XI

Increasing the proportion of dioxane and thus raising the reflux temperature appear to raise the yield, from 22.4 to 35.4 as would be seen from experiment Nos 4 and 5. Much higher proportion of dioxane did not give a homogenous solution and hence the yield dropped down on further increasing the percentage of dioxane as is seen in experiment 6. However, addition of dioxane, may though, raise the reflux temperature, would probably reduce the base strength of the medium, since potassium hydroxide in methanol would be a powerful base than potassium hydroxide in dioxane, and hence the overall effect of addition of dioxane would be to dampen the rate of hydrolysis. That must be the reason why the hydrolysis is poor in experiment No.4 (22%) compared to 3 (38.8%), whereas the only difference in 4, from 3, is the addition of dioxane. A total reflux time of 24 hours seems to be the optimum; and increasing the strength of the potassium hydroxide solution from 20 to 25 per cent does not seem to have any significant effect, as could be judged from experiment Nos. 2 and 3. Less than 24 hours, as 16 hours, is insufficient for maximum hydrolysis (experiment No.1).

The O-methyl podocarpic acid was ethylated with diethyl sulphate[17] and alkali to give ethyl, O-methyl podocarpate VIII. Ethylation of podocarpic acid with a small excess of diethyl sulphate and sodium hydroxide under the conditions of complete methylation with dimethyl sulphate did not completely ethylate the phenolic acid.

It gave 51 per cent of the ethyl, O-ethyl podocarpate IX. as an oil, and 47 per cent of O-ethyl podocarpic acid X as a nicely crystalline solid. The compounds were characterized by their infra-red and N.M.R. spectra. As ethylation was found to be sluggish, compared to methylation, the reaction was conducted for a much longer time, adding small amounts of diethyl sulphate at intervals[18]. Thus it was possible to achieve complete ethylation to IX in one step. Experiments conducted under varying conditions to achieve complete ethylation are given in Table 2. The results show that addition of small amounts of diethyl sulphate at several intervals thereby increasing the quantity added, and heating for a longer time, brought ethylation to completion.

Table 2

Exp. No.	Podo-carpic acid (mg).	Diethyl-sulphate (ml)	Reaction Time	Percentage ethyl, O-ethyl Podo-carpate	Yield of O-ethyl Podocarpic Acid.
1	1.003g	1.2	1 hour	38	61
2	502	1.2	2 nours	51	48
3	490	1.2	2 hours	50	46
4	1.0gm	2.5	2 hours	75	20
5	500	2.0	6 hours	100	0.0

Since O-ethyl podocarpic acid X was obtained initially as one of the products of ethylation of podocarpic acid the tedious process of hydrolysis of the axial ethyl ester had not to be undertaken to obtain the O-ethyl derivative.

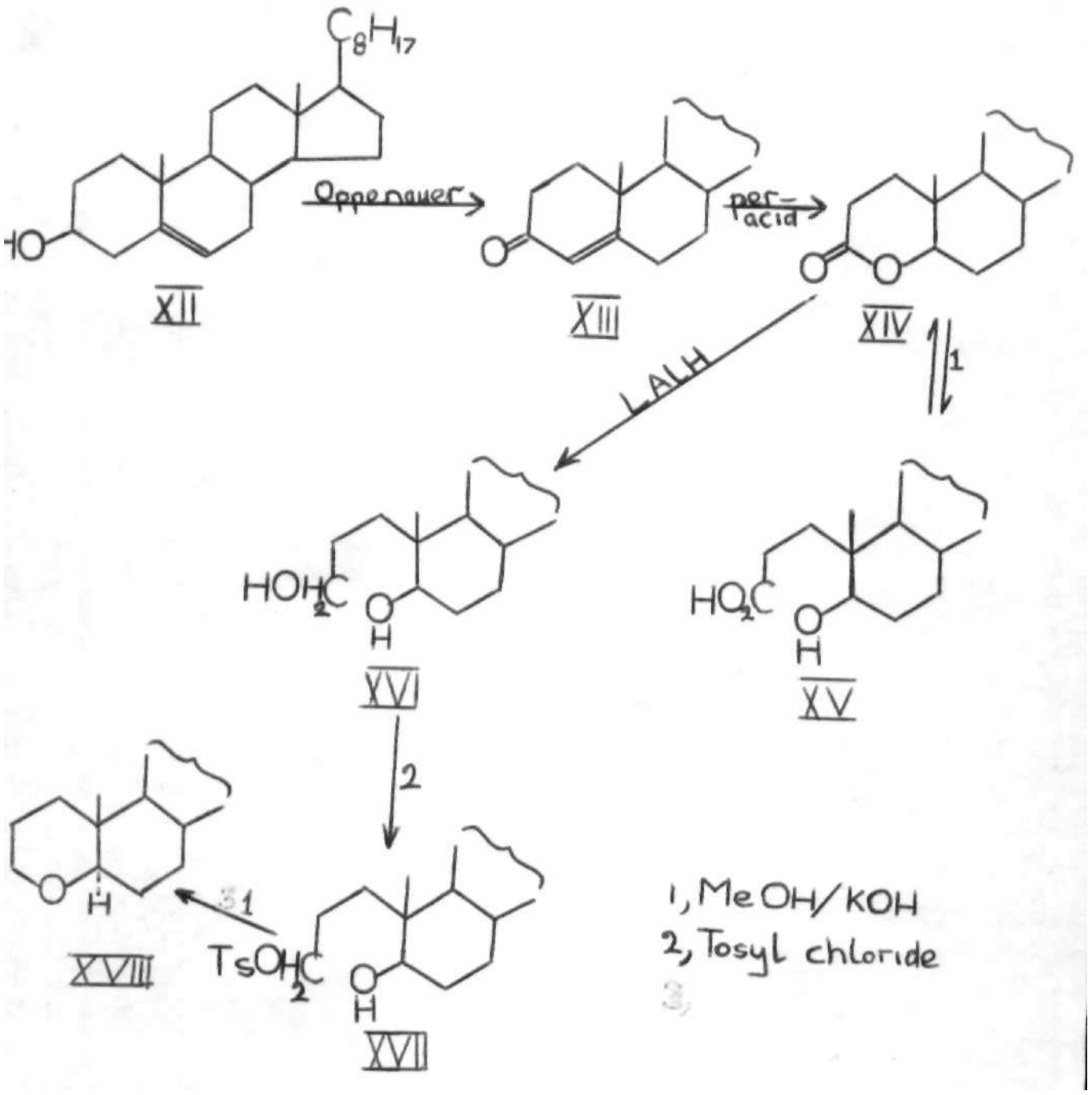
The ether X was methylated under the usual conditions[5] of methylation of podocarpic acid to obtain methyl, O-ethyl podocarpate XI. All the compounds were characterized by their infra-red and NMR spectra and analysis.

Oxidation, Reduction and Cyclization Studies with a Steroid.

To familiarize with the handling of steroids and their reactions which have to be attempted later, a series of oxidation, reduction and cyclization reactions with cholesterol were planned. Two products were aimed to be produced by breaking the carboxylic ring A of cholestanol viz. (1) a trans-fused heterocyclic Ring A-ether and (2) a cis-fused heterocyclic ring A-ether. For this, cholesterol XII, was to be converted to cholest-4-ene-3one by Oppenauer oxidation (vide chart 3). This α,β-unsaturated ketone, XIII, was to be converted to a ring A δ-lactone (a non-compound having carbon atom 4 removed) by oxidation of cholestenone XIII, with per sulphuric acid, which is a known procedure[19,21]. As the yield of this product in the first attempt (17%) was much less than the reported (33%), and as the product was required in some bulk for the series of reactions envisaged (charts 3 and 4), this oxidation reaction was repeated a few times to improve the yield. In the last instance, it gave a new crystalline product m.p. 166°, I.R. spectral bands at 1724 (non conjugated ketone), no U.V. absorption, but as the yield of this product was very low it was not fully characterized. In this last experiment done of this oxidation, the product was chromatographed.

In literature, this lactone is assumed to be trans-fused to the B-ring, but there is no evidence given for it[19-21].

CHART 3

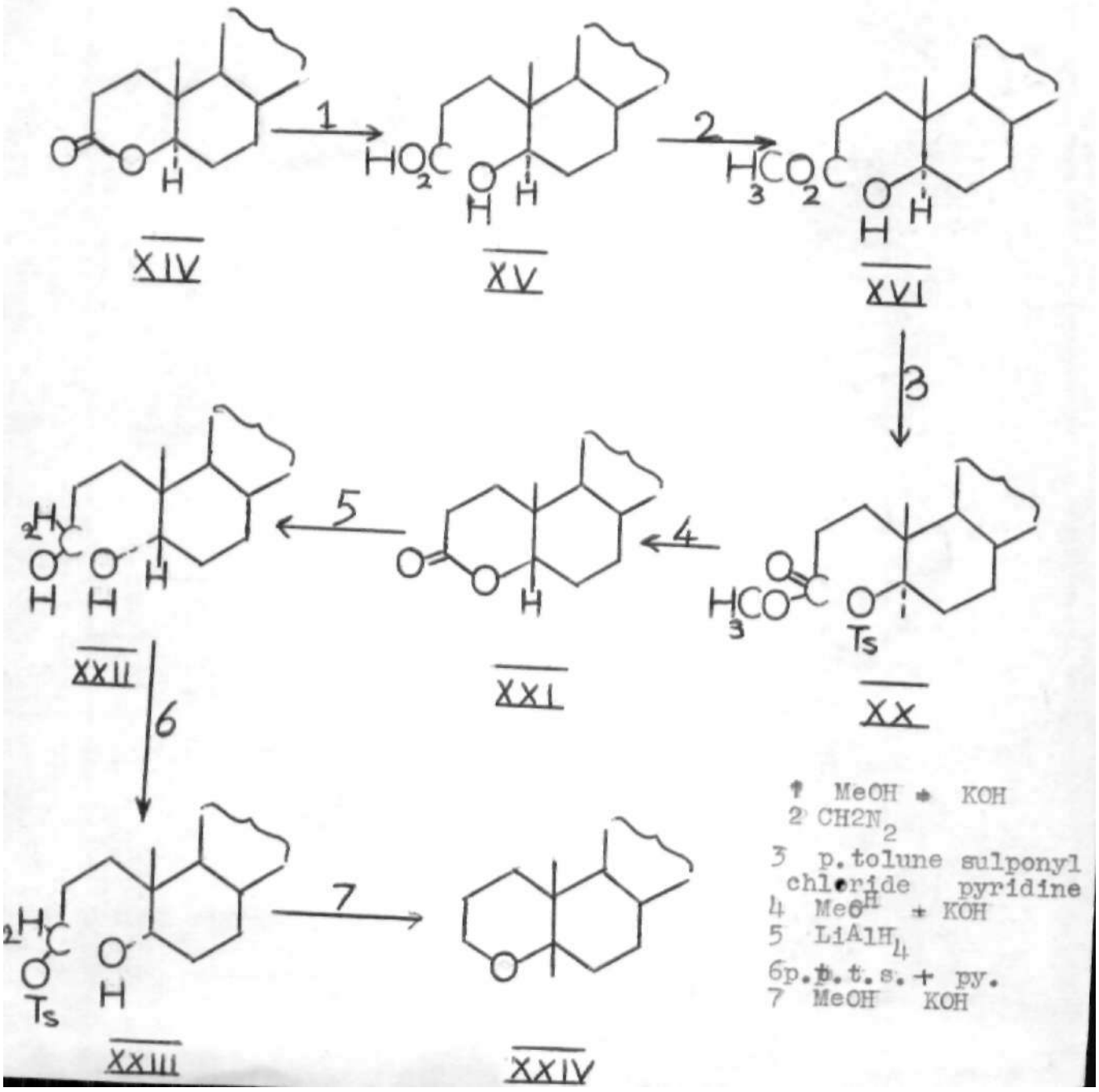

C_8H_{17}
HO
Oppenauer
per-acid
XII
XIII
XIV
LAlH
1
HOH$_2$C
O
H
XVI
HO$_2$C
XV
2
TsOH$_2$C
XVII
31
XVIII
1, MeOH/KOH
2, Tosyl chloride
3,

The mechanism of the reaction converting the 4-en-3-one to a ring A δ-lactone XIII to XIV, in one oxidation step with persulphuric acid is rather obscure; it was considered necessary to find the stereochemistry of this lactone fusion. This was all the more important since the reactions planned to make the two ether, viz. the trans-fused ether XVIII, and the cis-fused ether XXV, were on the basis that the original lactone XIV is trans-fused. Therefore the N.M.R. spectra of the lactone was determined in $CDCl_3$ solution and the region around δ_4 was enlarged. The spectra showed the signal of the C_5-H as a quartet centred at δ 3.88, J = 11.5 cps, showing thereby that the lactone is really trans-fused. It was then reduced with lithium aluminium hydride to give a nicely crystalline C_3,C_5-diol XVI which was characterized by its infra-red spectra band at 3245 and 2857 cm^{-1} and its analysis. The diol was then treated with one mole of para toluene sulphonyl chloride in dry pyridine when the more reactive primary hydroxy group at C_3 was expected to form a monotosylate. The tosylate was not isolated and characterized since it was likely to be an unstable compound. Hence the purified extract from the tosylation reaction was mixed with 10% methanolic potassium hydroxide and shaken at room temperature for an hour to obtain the trans ether XVIII, by the displacement of the C_3-tosyl ester by the C_5 β-hydroxyl group. The product was obtained as expected, but the yield was very poor, and it had to be chromatographed from the reaction mixture.

The infra-red spectra of the crystalline material did not show any absorption characteristic of hydroxyl bands or of aromatic double bands XVII, but showed only an ether C-O-band absorption at 1088 and 1103 cm^{-1}.

Hence to proceed for the preparation of the ether XXIV, it was necessary to obtain a reasonably good amount of the acid XV. For this purpose the lactone XIV was dissolved in 5% methanolic potassium hydroxide and heated on steam bath for half an hour and then the mixture was cooled to 0^{o} and acidified with dilute hydrochloric acid. Under these conditions the lactone in santonin has been hydrolysed to the corresponding hydroxyl acid[22]. However, the product isolated in the present case was the starting lactone. Later attempts to obtain the free hydroxyl acid after hydrolysing with 10% methanolic potassium hydroxide and cooling the hydrolysis product to -10^{o} and then acidifying with just the theoretical amount required to neutralize the alkali with dilute sulphuric acid or acetic acid also were not successful. In one attempt a small amount (57mg) of the acid was obtained. This was characterized as the acid by its infra-red absorption peaks at 3344.6 and 1732.3 cm^{-1} (carboxylic acid), its effervescence with saturated sodium bicarbonate solution, and by its relactonization on acidification to the starting lactone. However, this experiment could not be repeated, the lactone should certainly be hydrolysed by heating with 10% methanolic potassium hydroxide on the water bath, since the solution was homogenous from the beginning and such unhindered lactones are readily hydrolysed under such condition(22).

CHART 4


XIV
XV
XVI
XXII
XXI
XX
XXIII
XXIV
1 MeOH + KOH
2 CH2N2
3 p.tolune sulponyl chloride pyridine
4 MeOH + KOH
5 LiAlH4
6 p.t.s. + py.
7 MeOH KOH

It must however be relactonising on neutralization of the alkali, since the carboxyl group is very favourably situated to form the trans-6-membered lactone ring. Some lactones have similarly been found to be very difficult to obtain as the free hydroxy-acid, i.e.g. the lactone from santonin has never been obtained as the free hydroxy acid, but as the sodium salt of the acid, although those from dihydro and tetrahydro santonin have been hydrolysed to the corresponding hydroxy acids[23,24]. Hence attempts to prepare the ring A cis ether as envisaged in chart 4 had to be abandoned.

Methylation of Cholesterol to get 4,4-Dimethyl Derivative

The main difference between steroids and the tetracyclic triterpenes, is as has been stated before, the presence of a gem-dimethyl group at C_4 and another additonal group at C_{14} or C_8 in the triterpene. Of these in apo-euphol and in the triterpene bitter principles, derivable from it, the third additonal methyl group at C_8. Hence in an attempt to convert the steroid to a triterpenoid, two methyl groups have to be introduced at C_4 and one at C_8. The limonoid-type bitter principles have either an acetate or other ester, or a free hydroxyl, or an ether or a ketone present at C_7[25]. No such bitter principle has so far been isolated which does not have an oxygen function at C_7. Hence as a first attempt in the approach for the synthesis of such a compound, it was decided to introduce the gem dimethyl group at C_4.

The known procedure for this step is to oxidise cholesterol to cholest-4-en-3-one XIII, and then treat it with methyl iodide in the presence of a strong base[27-30]. When methylation takes place at C_4 to give the 4,4-dimethyl derivative XXV (Chart 5). It should be assumed from the location of the ketone at C_3 and of the double bond at C_5 and C_6 in the methylated product XXV that the first step in the methylation should be the removal of the double bond from C_4, C_5 to C_5, C_6 (as shown through XXVI to XXVIII in Chart 5) so that C_4 is more activated both by the C_3-ketone and the C_5 double bond, and is also free for methylation.

It has also been demonstrated[31] recently that with the same base used for methylation, viz. potassium tert. butoxide, the double bond in cholest-4-en-3-one is removed from the α,β-position to the β,γ position and the product as such, viz. cholest-5-en-3-one, XXVIII, could be isolated in high yield by acidification of the conjugate ion with acetic acid. It therefore appeared that if cholest-5-en-3-one XXVIIIis used as the starting material for the methylation at C_4, the yield of the methylated product would be better than that obtained in the reported procedure.

Therefore to obtain the product the 5,6-double bond cholesterol was protected by bromination to give the 5α, 6β-dibromocholesterol (XXIX), which was then oxidized with sodium dichromate to give 5α, 6β-dibromocholestane-3-one XXX. The bromine was removed by stirring with zinc dust, thus regenerating the double bond at 5,6, to give XXVI. This product was then subjected to methylation with potassium tertiary butoxide and methyl iodide under the usual conditions. After work up, the crystalline product isolated in 90% yield was surprisingly found to be the starting material XXVI. Since under the conditions of the reaction, one should expect that the α,β, and the β,γ -unsaturated ketone be in equilibrium either starting with the conjugated or the non-conjugated ketone; it is not clear why methylation did not take place when the reaction was started with the non-conjugated ketone.

CHART 5A

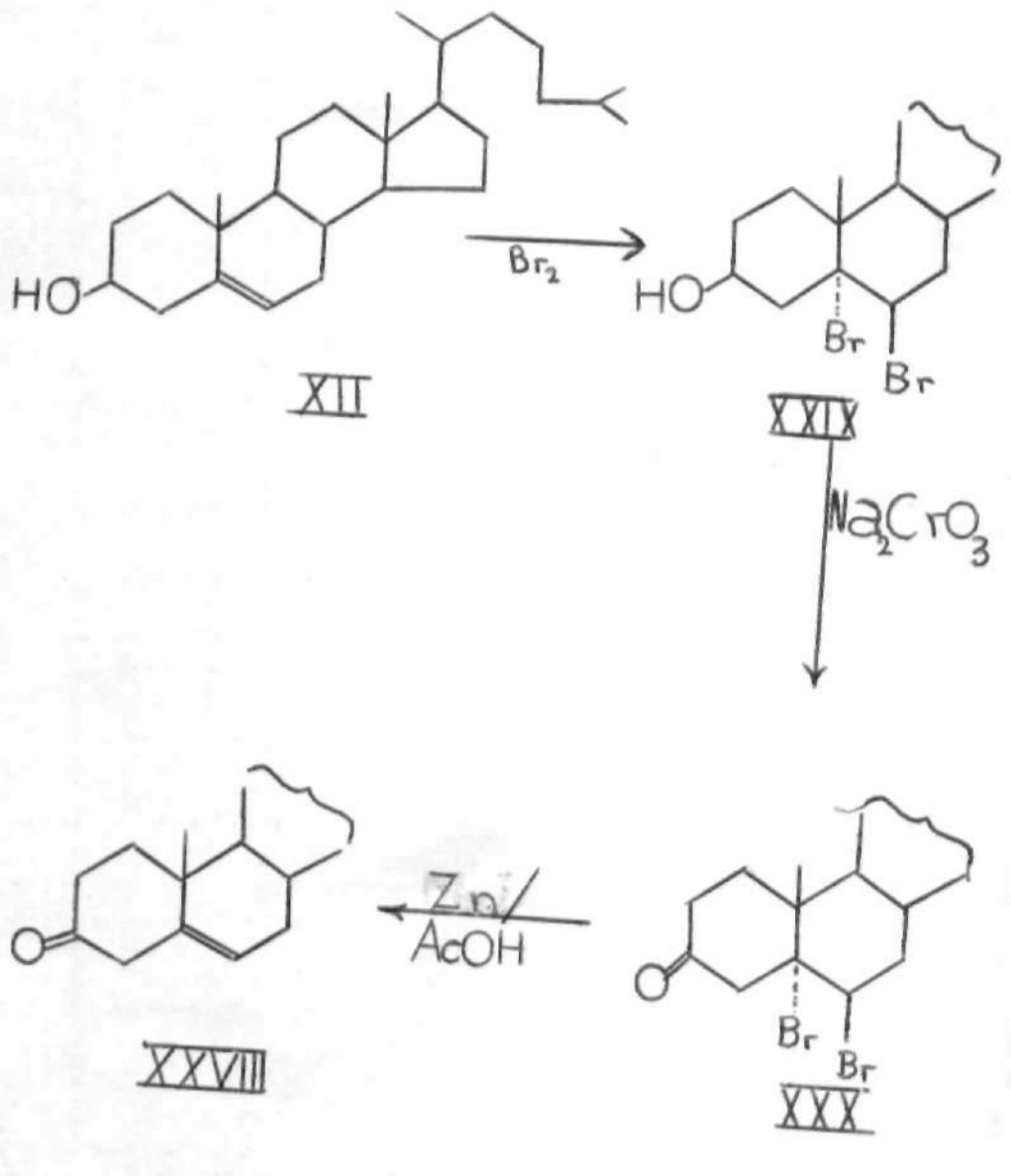
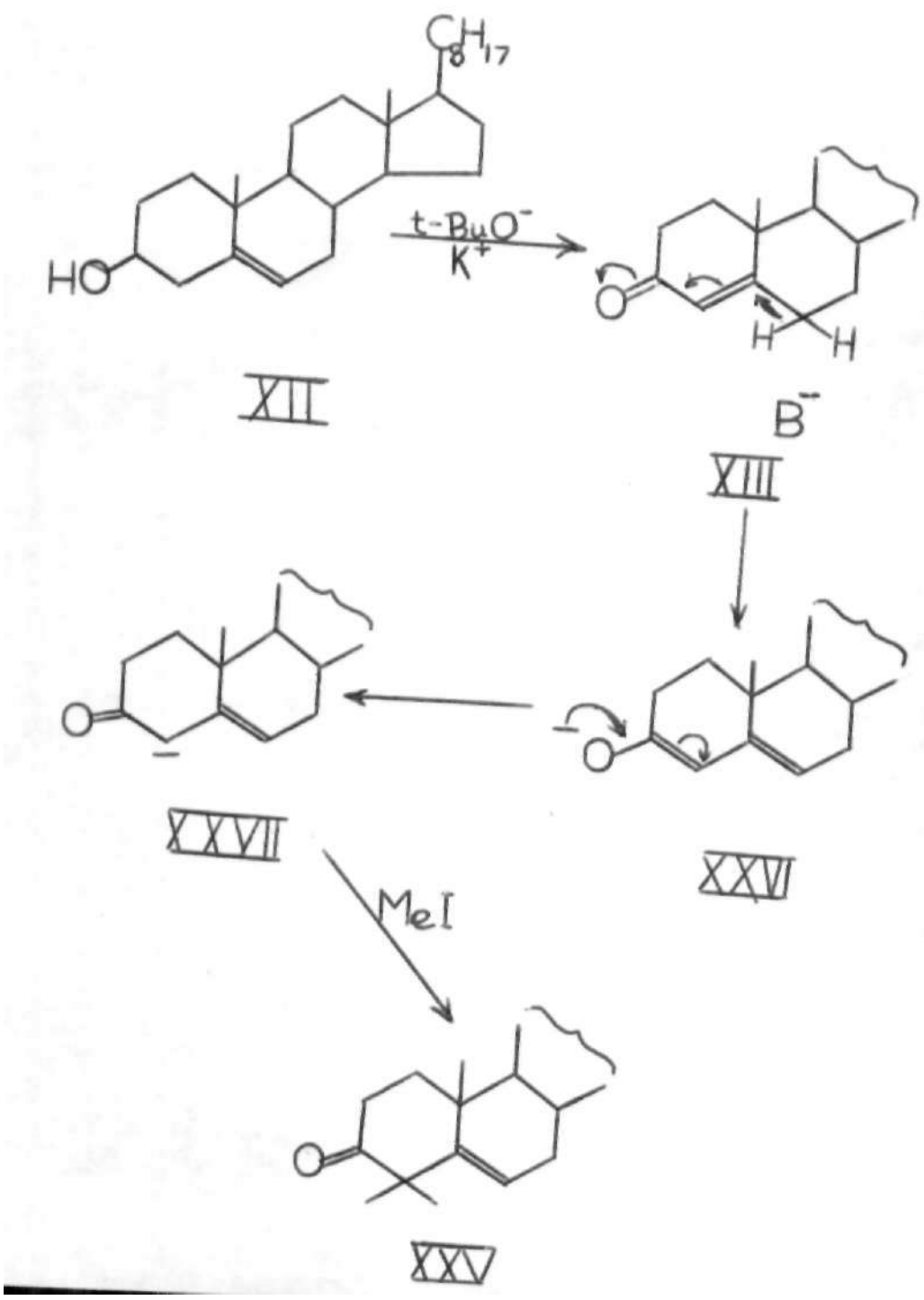


CHART 5B



It also appears that under basic conditions, the β,γ-position or the 5,6, position is more stable for the double bond as is the case with cholesterol or even with cholest-4-en-3-one when the C_3 ketone gets tied up as a ketal or as any other ketone derivative[32-34]. Still this does not explain the unreactivity of C_4 for methylation in the above experiment. One possible explanation could be that, perhaps, since the C_3-ketone in cholestanol is known to enolise towards C_2[35-38], in the case of cholest-5-en-3-one, it might enolise both towards C_2 and C_4, with the result that there may not be enough concentration of the anion at any one place at a time to attack the methyl iodide for methylation.

As the methylation experiment with XXVI failed under the conditions in which cholest-4-en-3-one gives the methylated product in high yield, it was considered that even if methylation would take place under some modified conditions, it may not be a superior method to that with cholest-4-en-3-one XIII, and therefore no modifications of the above experiment was tried. The conjugated ketone XIII was then methylated with potassium tertiary butoxide and methyl iodide. Of the two procedures, one refluxing for only five minutes[28] after the addition of methyl iodide and the other for one hour; the latter, which is the original procedure of Barton, et.al[30], gave better results.

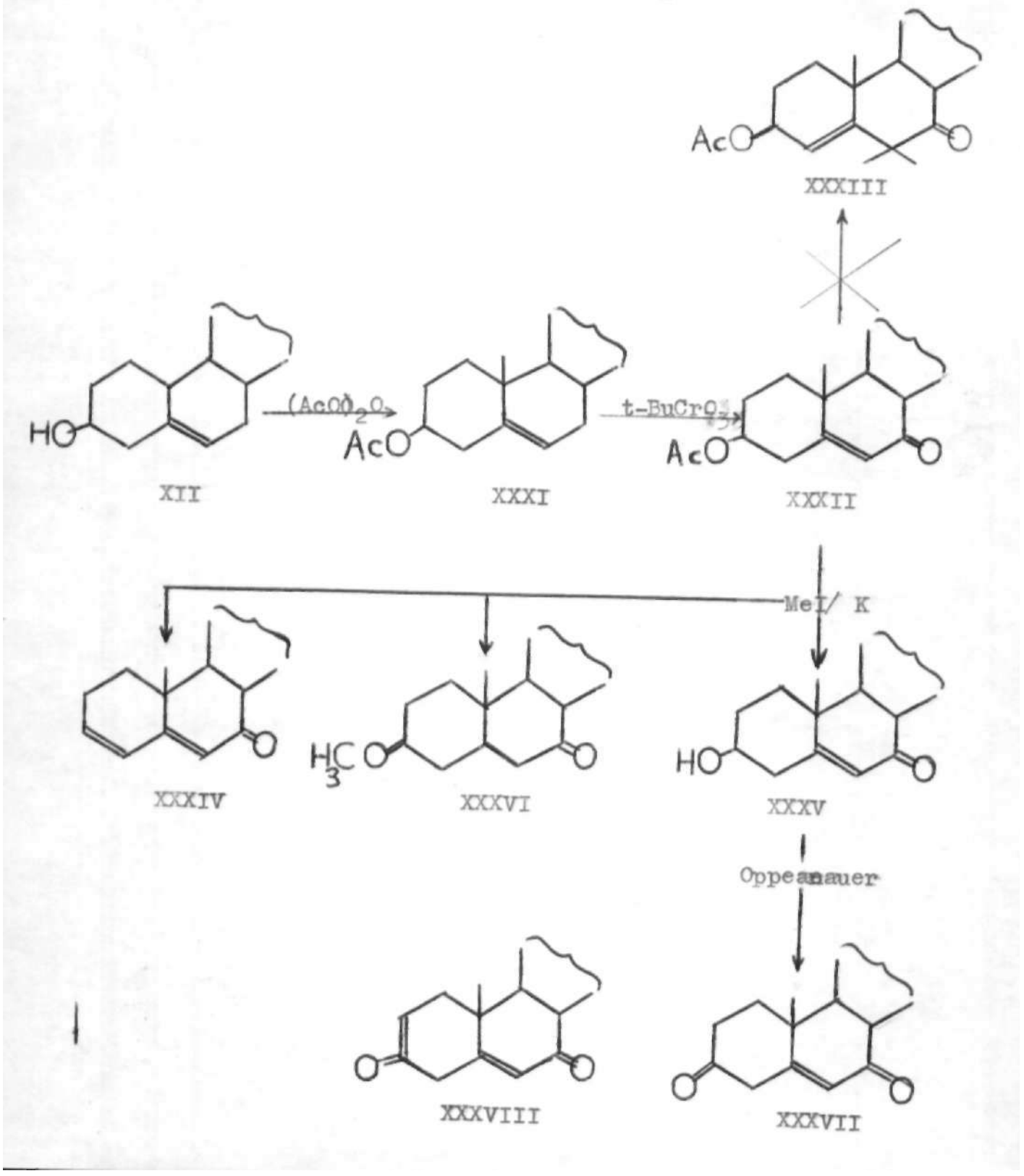
Introducing an Oxygen Function at C_7 and Methylation of C_8 Carbon-Atom

The next steps involved introducing an oxygen function at C_7 and methylation at C_8. Before proceeding with the C_4 methylated product, it was considered worthwhile to attempt experiments for methylation at C_8, by introducing a ketone at C_7 and blocking the alternate position C_6. Some support for this argument can be adduced from the fact that even cholest-4-en-3-one is found to enolise either towards C_6 or C_2 according to the experimental conditions. In these experiments, deuterium has been found incorporated either at C_2 or at C_6 depending upon the conditions used(39).

The successful method in this could then be later applied to the required 4,4-dimethyl steroid. Introducing a gem dimethyl group at C_6 would be an easy way or blocking that position. For this purpose the experiments planned are shown in chart 6.

Cholesterol would be acetylated to the C_3-acetate XXXI, which is then oxidized with tertiary butyl chromate(40-42) to cholest-4-en-7-one-3β-yl-acetate. Cholesteryl acetate XXXI, on oxidation as envisaged above, gave the 7-keto derivative XXXII, in about 56% yield. This product on methylation in the usual way as suggested above, however, did not give the required methylated product XXXIII. The crystalline product obtained from this reaction melted at 112°, showed infra red absorption at 1613 and 1642 cm^{-1}, doubly unsaturated ketone, and U.V. absorption at 280mμ, ε = 20310. This was therefore

CHART 6


AcO
O
XXXIII
HO
(AcO)2O
AcO
t-BuCrO3H
AcO
O
XII
XXXI
XXXII
MeI/ K
XXXIV
H3C
XXXVI
HO
XXXV
Oppenauer
XXXVIII
XXXVII

concluded to be the cholest-3-5-dien-7-one[41,42] XXXIV. The expected specific rotation of the product is-306, the product obtained has $[\alpha]_D$ -293°. An authentic sample of this product was prepared by acid catalysed β-elimination of the acetate group C_3, in the acetate XXXII by the known procedure in the literatere[42,43] to give XXXIV. Both compounds had identical melting points and mixed melting points. The N.M.R. spectra of the product showed one vinyl proton signal at δ5.6 and two vinyl protons at δ6.13, thus fully confirming the structure of the product obtained. There was no significant change in the product composition by carrying out the methylation in benzene solution, instead of tertiary butyl alcohol, thus reducing the polarity of the solvent medium.

In another attempt the temperature of the reaction was lowered and the reaction time with the base was also reduced. The ketone XXXII was first dissolved in benzene at 40°; the solution was cooled to room temperature; then potassium metal in tertiary butyl alcohol was added to the solution and the mixture was heated to 60° and maintained at that temperature for an hour. After the usual work up, the amorphous mixture obtained was chromatographed over silica gel. Three different crystalline products were obtained. The product that was eluted first was characterised as 3,5,dien-7-one XXXIV. The product that came last had m.p. 170°, showed absorption for a hydroxyl group and conjugated ketone in the infra red (3044 and 1640 cm^{-1}) and showed the ultra violet absorption for an α,β-unsaturated ketone

(λmax 238mμ, εmax = 9400). These suggested that the compound obtained was cholest-5-en-7-one-3β-ol XXXV presumably formed by the hydrolysis of the C_3-acetate group under alkaline conditions. This was fully confirmed by acetylating this product with acetic anhydride and pyridine, when the starting cholest-5-en-7-one-3β-yl-acetate XXXII was obtained.

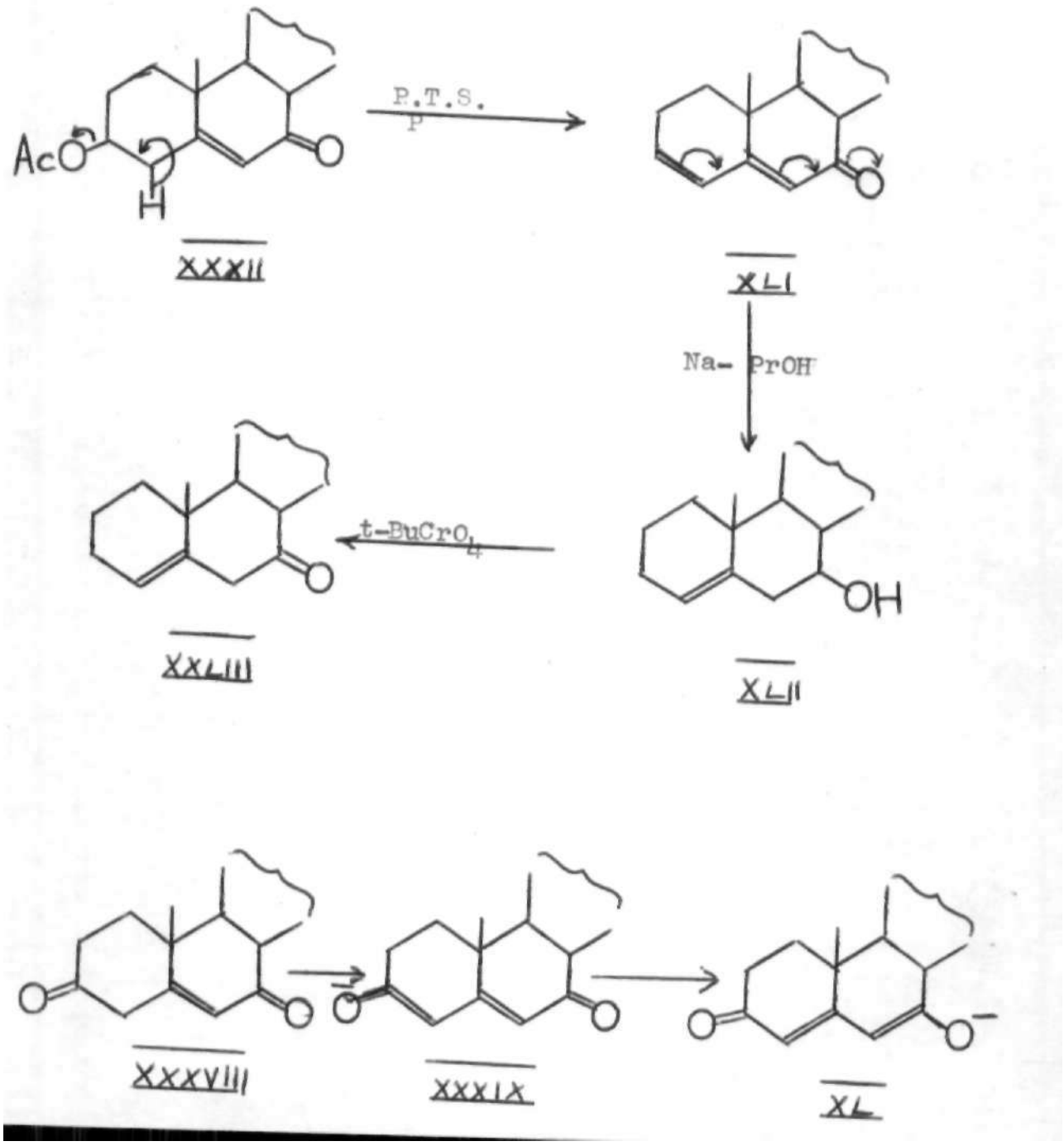
The crystalline product obtained in between these two had m.p. 124°, and showed U.V. absorption for an α,β-unsaturated ketone, λmax 238mμ, εmax = 11100. It did not show any absorption band characteristic of the hydroxyl or acetate group in the infra red. At this stage a compound that would be envisaged to arise from the starting material XXXII, and would fit the data obtained is only the non-conjugated dienone XXXVII. This could arise either by elimination of the acetate to form a C_2, C_3 - double bond, or by the C_3, C_4 double bond in XXXIV, going out of conjugation to C_2, C_3. Both these reaction paths to lead to the product XXXVII seemed unlikely and therefore an N.M.R. spectrum of the compound was determined to obtained further information about it. The spectra showed a methoxy methyl proton resonance at δ3.93 and a broad vinyl proton resonance at δ5.55 suggesting thereby that the compound is cholest-5-en-7-one-3-methyl ether, as the signal of the C_3-H appeared as a multiplet centred at δ3 which is characteristic of the axial proton of cholesteryl methyl ether,[44] the methoxy group is equatorial and therefore β-oriented at C_3. The compound is therefore cholest-5-en-7-one-3β-yl-methyl ether XXXVI.

This was confirmed by its mass spectra which gave the mass peak of the molecule as 414, corresponding to XXXVI. The compound must have arisen by O-alkylation of the C_3-hydroxyl group at XXXV (produced in the reaction) with methyl iodide under the strong basic conditions used.

It is known that in cholesterol[45], the double bond is more stable at 5,6, than at 4,5. However, when a ketone is introduced at C_3, the double bond shifts its positions from 5,6 to 4,5. It was therefore interesting to know which would be the position of the double bond when there are ketones at C_3, as well as C_7 staying in either of the two positions, would give conjugation with one of the ketones. Hence the question might be conjugation with which of the two ketone makes the double bond more stable.

Having compound XXXV viz, cholest-5-en-7-one-3β-ol in hand, it was easy to ascertain this. This was converted by Oppenauer oxidation to en-dione m.p. 184° whose infra red spectrum[46] showed the absence of any hydroxyl group but the presence of two ketones, one absorbing at 1722 cm^{-1}, a non-conjugated 6-membered ketone, and another at 1668 cm^{-1}, a conjugated 6-membered ketone. This compound could be either cholest-5-en-3, 7-dione or cholest-4-en-3, 7-dione. Ultra violet spectra of the compound showed the high intensity absorption at 239mμ, ε = 3650 which can fit it to either of the above two compounds. The U.V. spectra of the compound shifted its λmax from 239mμ to 289mμ on addition of two drops of 10% of potassium hydroxide to the solution

CHART 7


AcO
H
O
P.T.S.
P
Na- PrOH
t-BuCrO4
OH
XXXII
XLI
XXXIII
XLII
XXXVIII
XXXIX
XL

then absorbed at 295mμ on acidification. This is characteristic of the binalogues of β-diketones and should nearly be the same for XXXIX or XL, which are tautomeric forms and could arise from either of the enediones - the one having double bond at 5,6 or at 4,5.

This enedione was refluxed with paratoluene sulphonic acid for one hour to equilibrate the double bond and the product was then worked up as usual to give 90 per cent of the starting material. This shows that after the Oppenauer oxidation, the enedione had already had its double bond at the more stable position. The rotation of the compound was then determined to deduce its structure[48]. The specific rotation of cholest-4-en-3-one is $+89^{\circ}$ and hence its M_D is $\frac{89 \times 393}{100} = +354^{\circ}$, in the eno-dione. Since the Molecular Rotation contribution of cholest-7-one is $+223^{\circ}$, the M_D of the ene-dione with the double bond at C_4, C_5, would be the sum of these two (354 + 223) i.e. $+577^{\circ}$. Similarly, since the specific rotation of cholest-5-ene-7-one is -127°, its M_D is -508° in the ene-dione. As the M_D for cholesten-3-one is $+71^{\circ}$, the M_D of the ene-dione with the double bond at C_5, C_6 would be the sum of these two, viz. -437°. Since the specific rotation of the en-dione in the stable position of the double bond as shown by the equilibrium reaction was found to be -252°, its M_D is -501. Hence the double bond is at Δ^{5-6} in the dione with conjugation with the 7-ketone.

Since this attempt of methylating XXXI at C_6 failed because of

the easily removable acetate group at C_3, it was decided to remove the oxygen function at C_3. Hence the dienone XXXIV, was prepared on a larger scale, by acid catalyzed elimination of the C_3- acetate in XXXII. The dienone was reduced with sodium and alcohol to give cholest-4-ene-7β-ol, by α,ω addition of hydrogen to the oxygen and at C_3 in XLI. The alcohol XLII was readily oxidized to the non-conjugated ketone XLIII with chromic acid. This compound was methylated with potassium tertiary butoxide and methyl iodide in the usual way, but no methylation took place and only the starting material was recovered from the reaction. It has previously been observed that the β,δ-unsaturated ketone, cholest-5-en-3-one also gave back starting material on attempted methylation. It therefore appears that for the successful alkylation of enones, deconjugation and alkylation has to take place, more or less simultaneously.

The next step was to prepare the conjugated ketone cholest-5-en-7-one, with no group at C_3 to get eliminated and bring in complications, and then to attempt to methylate it under the conditions of the successful methylation of cholest-4-en-3-one. This was achieved by the series of reactions envisaged in chart 8.

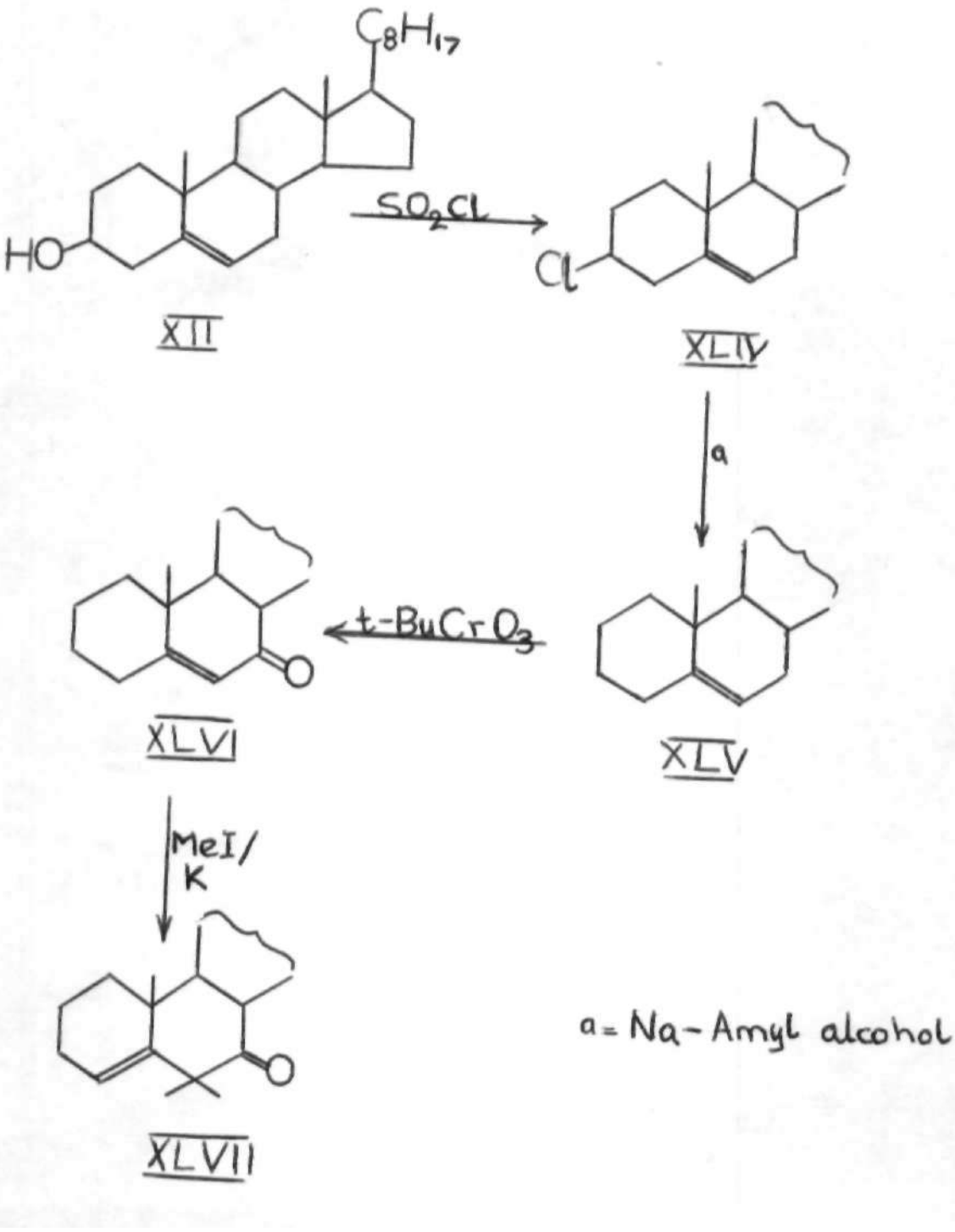
Cholesterol by treatment with thionyl chloride was converted to 3β-chloro-cholest-5-ene XLIV in about 90% yield. By treating XLIV with sodium and propyl alcohol, the chloride was reduced to cholest-5-ene XLV. This compound on oxidation with tertiary butyl chromate[40] as before was converted to cholest-5-ene-7-one XLVI[50] which was charac-

terised by its m.p. 131°, infra red absorption at 1667 cm^{-1} (conjugated ketone) and ultra violet absorption at λmax 240mμ, ϵ = 7932. It is interesting to note that whether there is an ester or ether[49] function at C_3 or not, on oxidation with tertiary butyl chromate, the oxidation takes place at the C_7 position only and not at the alternate allylic position C_4. This might be explained as due to the ease of approach to one of the axial positions amongst C_4, and C_7. Since C_4 is hindered due to the angular methyl group at C_{10}, C_7 would become the preferred position. However, this explanation would not hold good when we consider selenium dioxide oxidation. Oxidation of cholesterol or cholesteryl acetate with selenium dioxide introduces a new axial hydroxyl group at C_4[51-53] and no product with oxidation at C_7. Hence the reasons for the oxidation allylic sites by these and other oxidising agents are yet to be found.

The conjugated ketone XLVI was subjected to methylation with potassium tertirary butoxide and methyl iodide for a much longer time than with cholest-5-en-3-one since from the work of Ringold, et.al., it is known that the time taken for the enolate ion to form may vary from case to case. The product obtained in about 35% yield was characterized as 6,6-dimethyl-cholest-4-en-7-one[54] XLVII from its infra red spectra band at 1710 cm^{-1} (non conjugated ketone), absence of strong ultra violet absorption, unlike the starting conjugated ketone, and by its elemental analysis.

This compound was subjected to methylation at C_8 with the same methylating agents and under stronger alkaline conditions as used for

CHART 8


C_8H_{17}
HO
SO_2Cl
Cl
XII
XLIV
a
t-$BuCrO_3$
XLVI
XLV
MeI/
K
XLVII
a = Na - Amyl alcohol

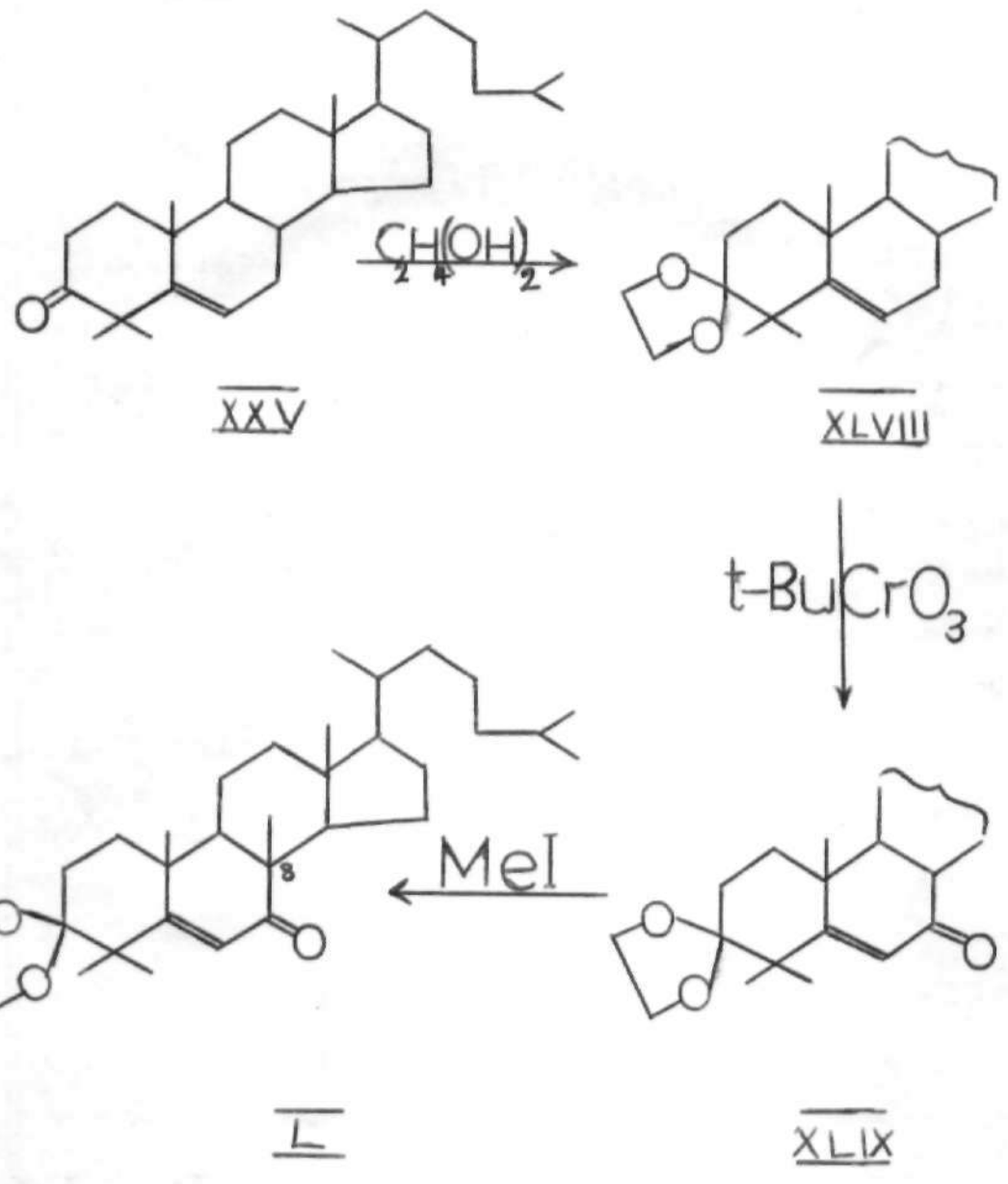
methylation at C_6. However, the product isolated in about 80% yield was characterized as the starting material XLVII. Thin layer chromatography of the mother liquor showed that there was a faint spot that was different from the starting material, but since no crystalline material was obtained and since the amount of material present was so small, this was not precessed further.

In this case it is quite possible that the enolate is not formed easily from C_8, and / or methylation at C_8 in this particular case would be more difficult, since the approach of the reagent would be hindered by the bulky methyl groups at C_6 and C_{10}. It was therefore considered that instead of trying further model experiments, it might be better to proceed directly with the 4,4-dimethyl-compound XXV (chart 5) already prepared to introduce an oxygen function at C_7 and methylation at C_8.

The experiments described for this purpose are schematically represented in chart 9. The C_3-ketone in 4,4-dimethyl-cholest-5-en-3-one was to be protected by ketalation with ethylene glycol as shown in XLVIII, so that on further oxidation, to produce a ketone at C_7, the C_2-methylene group which is activated by the C_3 ketone, would not get attacked. This compound was to be subjected to tertiary butyl chromate oxidation to give the corresponding 7-ketone XLIX, which on methylation should yield L.

The ketalation proceeded in about 80% yield and the product was characterised as XLVIII by the absence of absorption for a ketone in

CHART 9



XXV

XLVIII

L

XLIX

its infra-red spectrum. This compound gave no difficulty in tertiary butyl chromate oxidation at the allylic position C_7, which incidentally is the only one available for attack in this molecule, since the alternate allylic position C_4 is blocked. The ketone XLIX; was isolated in about 45% yield. It was characterized by its infra-red bands at 1667 and 1608 cm^{-1} (conjugated ketone and double bond) and its ultra violet spectrum λ_{max}^{EtOH} 243mμ, ε_{max} 10100. This compound was subjected to prolonged methylation as in the previous case. The reaction product was worked up and subjected to thin-layer chromatography with the starting material. The reaction product did not show a spot with a different rf from the starting material. However, the product was crystallized from methanol. The crystalline product obtained had a m.p. 180°, which is markedly different from the starting ketone m.p. 115°. Its infra red spectrum had absorption band at 1667 and 1608 cm^{-1} corresponding to conjugated ketone-5-en-7-one- showing that the chromaphore is intact in the molecule, but its U.V. spectrum showed a reduced intensity of 5800 at λ_{max}^{EtOH} 244mμ. Its N.M.R. spectrum showed the resonances at δ 3.95 for the ethylene dioxy group at C_3, and at δ 5.9 for the vinyl proton at C_6 as in the starting material showing thereby that these functional groups are intact in it. Besides, it showed an additional methyl signal at δ 1.2 showing thereby that there is an additional methyl group with a chemical shift appropriate for a methyl group adjacent to a ketone[55-57]. This compound must therefore have a methyl group at C_8 and hence must be 3,3-ethylene dioxy-

4,4-dimethyl-8-methyl-cholest-5-en-7-one L, the required product. This was confirmed by its mass spectrum which showed the highest peak corresponding to a mass of 484, different from the starting material having M^+ 470, in agreement with their respective molecular formulae. However, the thin layer chromatography of this compound and the starting material did not show any significant difference in their rf values as is shown in plate I despite the fact that different solvents were tried. This would only mean that the addition of a methyl group at C_8 did not alter the polarity of the molecule to any significant extent.

There is one more aspect in the formation of this compound which deserves serious consideration. In the alkylation studies by Johnson, et.al.,(58-61) in the synthesis of steroids it was found that alkylation at the position α to the ketone often does not give an axial alkyl group, and if it is at a ring junction, the major product would be to make the ring cis fused rather than trans fused, (see chart 10). Steric hindrance due to the two axial hydrogen atoms have been considered to be responsible for this, since removal of one of them improved the yield of the axial alkyl group with a trans ring junction.(62) Hence in this case also since there are axial methyl groups at C_{10} and C_{13}, it is very likely that the major product is the one in which the methyl group enter at the α-side at C_8. This is supported by the N.M.R. spectrum of the compound. Although the signals of the methyl groups and the methylene dioxy group do not show any serious shift,

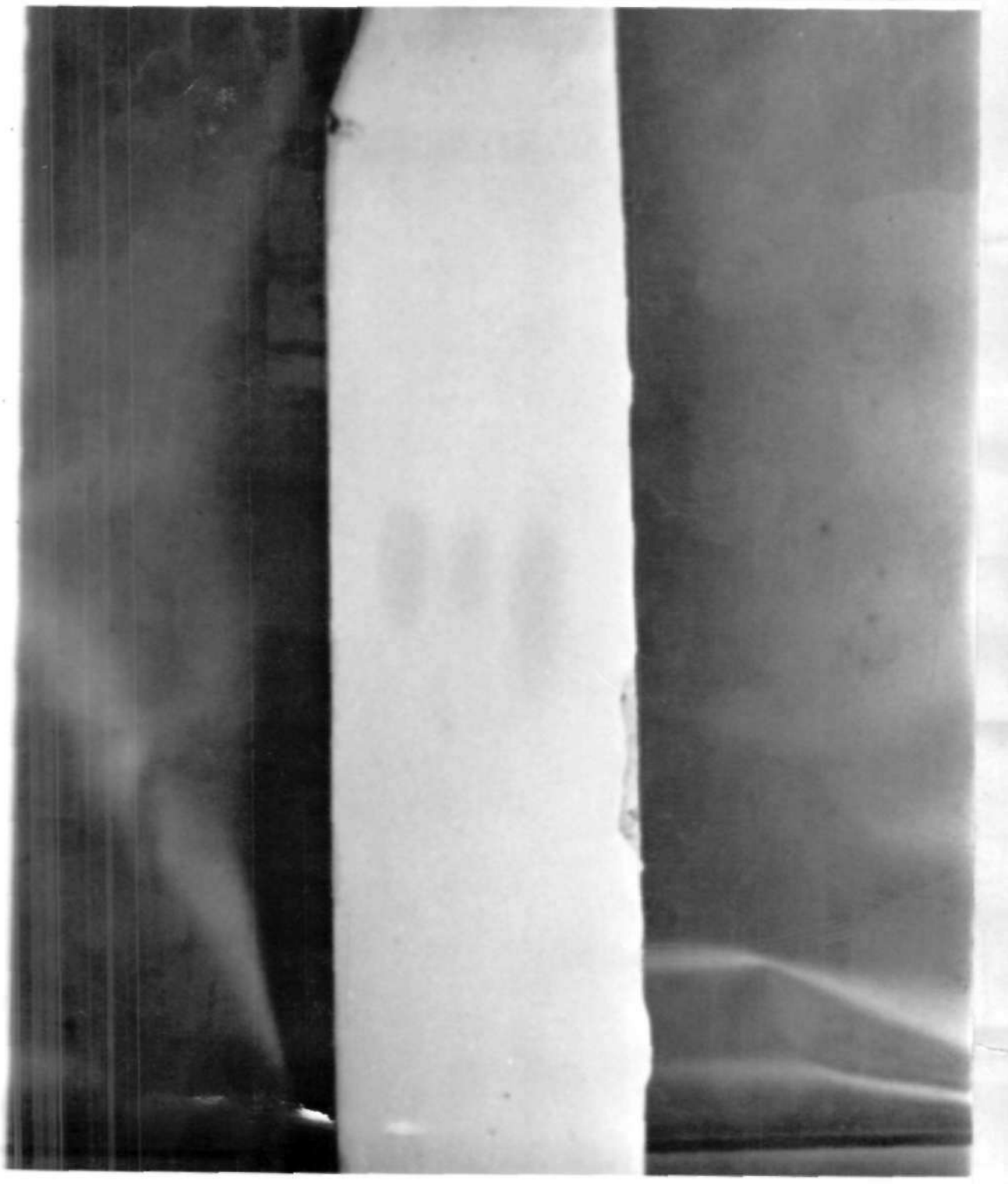
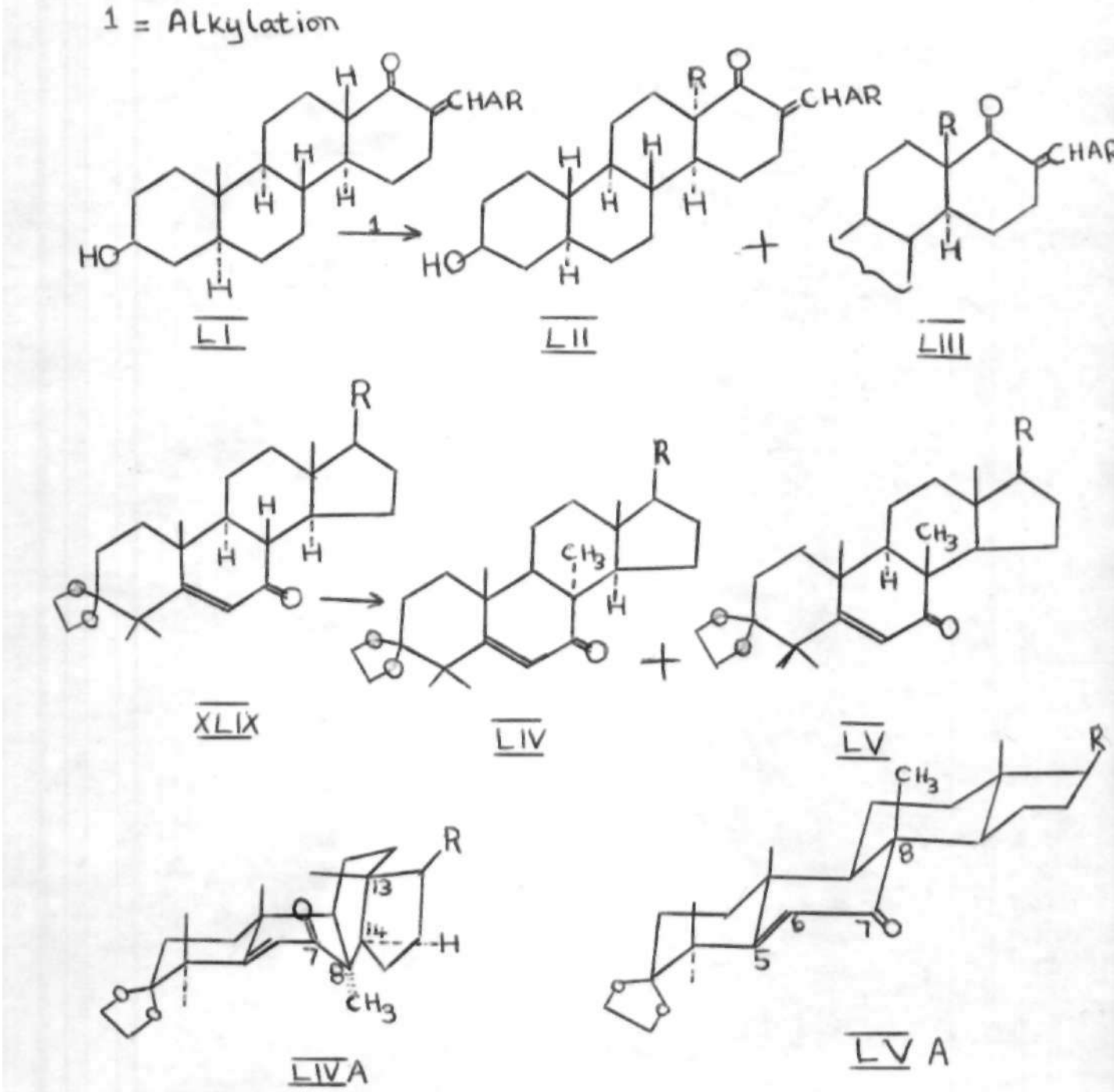


PLATE 1

The Unmethylated C_8 Product Spots on each side of the Spot of the Methylated C_8 Product.

CHART 10



the signal of the vinyl proton at C_5 in the methylated product is found as two signals, one at δ 5.9 i.e. the same place as that of the unmethylated product, and the other at a higher field at δ 5.5. The intensities of the two signals are in the ratio of about 1:2, and the areas of both together would only make that for one proton. Hence, this would mean that the compound isolated with m.p. 180°, is a mixture of two compounds LIV and LV, i.e. with the methyl groups α at C_8 and β at C_8 respectively. Since a β-methyl group would give the same conformation to the ring systems as the starting ketone XLIX, it is not expected to affect the chemical shift of C_5-H. An α C_8-CH_3 however would change the conformation of the whole molecule, since the B ring has to go into a half conformation as shown in LIV A. (A conformation with the C-ring in the boat form would not be possible to exist, since in that case, the methyl groups at C_{10} and C_{13} have no space to stay). The conformational change in the molecule as a whole as shown in the perspective formula LIV A, is quite likely to affect the chemical shift of C_5-H, giving it an up field shift as observed. Since the signal of the C_5-H in LIV A is more than double that of the one in LV A, C_8 α-methylated product should be more than two thirds of the isolated product. The large change in the specific rotation from -95 to -152 in going from XLIX, to the methylated product reflect the stereochemical and conformational change involved in the bulk of the product, as envisaged. The reduction of the intensity of the absorption

in the ultra violet spectra of the 5-en-7-one from 10130 in the C_8 unmethylated product XLIX to 5795 in the C_8 methylated product LIV A, also supports the above conclusion. Reduction[63,65] of the intensity of absorption of chromophores on the introduction of strain into the system is well known. For example, in the steroids, homo annular dienes have a much reduced intensity of absorption than hetero annular dienes. Similarly, when a 7-en-6-one has an intensity of absorption of the order 14000, a 5-en-6-one has an intensity of the order of 6000 only.

It is unlikely that the signal of a vinyl proton at δ 5.4 is due to an extrananeous impurity and that the one at δ 5.9 only represents the truely methylated product, since the molecular ion peak for the starting material at 470 and that for the methylated product melting at 180° (whose N.M.R. spectra was discussed above) at 484, are of comparative intensities. If there was an extraneous impurity present, whose signal is seen at δ 5.4, and not that due to a C_8 α-methylated product, then that impurity should form the bulk of the product as per the intensities of the two signals as discussed above and hence the mass ion peak at 484 should be of very low intensity, since there is no other position to get methylated in the molecule. Also the elemental analysis of the product would not have fitted with the molecular formula of the methylated product.

There are many examples in steroids, wherein two related but different compounds forming a molecular complex and showing a sharp

melting point, have also been found difficult to separate. Attempts were made to separate the isomers present in the product under discussion, both thin layer and column chromatography were unsuccessful. Elaborate chromatography of the product on silica gel which extended for a period of six days got the ethylene ketal at C_3 - opened up. The eluted product gave a carbonyl peak for a saturated ketone in the infra red, thus making this chromatography useless in this case.

EXPERIMENTAL

Melting points are uncorrected and have been taken on a Gallenkamp melting point apparatus. Optical rotations were determined in 1% chloroform solution on Bellingham and Stanley Model A-P 3 polarimeter. Ultra violet spectra were taken on a Unicam Spectrophotometer, model SP.500. Infra-red spectra were recorded as nujol mull on a Perkin Elmer spectrophotometer model 221 or in chloroform solution on Unicam spectrophotometer model SP.1200. Nuclear magnetic resonance spectra were recorded on a varian A-60 spectrometer in deutero-chloroform solutions, using tetramethyl silane as the internal standard. Thin layer chromatography was carried out on silica gel mixed with plaster of Paris as binder. The plates were 0.9mm thick and were fixed in iodine.

I. NN-Dimethyl-1-Naphthylamine.

To a mixture of 24.85g. of pure[66] α-naphthylamine and 19g. of sodium bicarbonate was added 45.1g. of dimethyl sulphate. This was refluxed at 180° (oil bath) for seven hours. When cooled, it was extracted with benzene, and the solvent was distilled off under a reduced pressure.

The dimethyl derivative II was distilled at 140°/8mm of Hg. (lit 155°/10mm)[67] I.R. ν_{max} 3100, 2965, 2850 and 2820 cm^{-1} ($-N(CH_3)_2$) N.M.R. δ 2.83 (6H of $-N-(CH_3)_2$).

N.-Formyl-1-Naphthylamine III.

Pure[66] 1-naphthylamine was refluxed in 5ml. of formic acid (90%) for two hours. This, when poured into some ice-cold water gave white precipitates. These were filtered, washed with cold water, drained thoroughly and let to dry; then recrystallized from pet-ether-benzene (1:1) mixture. From this were obtained long thread-like crystals of a formyl derivative III.

Melting point 138° (lit 138.5°)[67]
I.R. ν_{max} 1675 cm^{-1} (carbonyl group) 3450 cm^{-1} (>NH)

Lithium Aluminium Hydride Reduction of N-Formyl-1-Naphthylamine[68]

4g. of LAlH in 200ml. of tetrahydrofuran was heated for 30 minutes. To this hot solution was poured a solution of the formate, III (4.g), in tetrahydrofuran, (250ml), in bits over a period of two hours, then refluxed for additional six hours. This was cooled in ice-salt mixtures and chips of ice were added until there was no more reaction.

Addition of a saturated solution of sodium-potassium-tartarate (10ml) caused coagulation of the inorganic compounds. This after settling was filtered with slight suction, washed with benzene, dried (anhydrous Na_2SO_4) and then evaporated to dryness. The crude product obtained was dissolved in chloroform and then extracted with 7 x 10ml. of 6N hydrochloric acid, the combined acid extracts was washed with chloroform, then the acid solution was basified with 20% sodium hydroxide solution, then chloroform extracted. The chloroform extract was washed with water (1 x 10ml), dried (Na_2SO_4) and evaporated to dryness. The crude product obtained was chromatographed over 150g. of silica gel. Pure benzene eluted a red liquid found to contain the monomethyl derivative IV and some other compounds uncharacterized. Pet-ether-benzene (70:30) mixture eluted largely pure monomethyl-naphthylamine IV.

I.R. νmax 3471 cm^{-1} (amine).

N.M.R. δ 2.9 (NH - $\underline{CH_3}$), 4.14 (>NH) (1.22 ?)

II. Preparation of Methyl, O-Methyl Podocarpic Acid.

A mixture of 500mg. of podocarpic acid V, 700mg. of freshly distilled dimethyl sulphate[69] and 250mg. of sodium hydroxide in 2ml. of 50% aqueous alcohol was refluxed for 15 minutes. On cooling, the reaction mixture solidified. This was dissolved in aqueous methanol and then extracted with chloroform, dried, and brought to dryness.

The crude product obtained in 460mg. yield (m.p. 125°) was crystallized from methanol-acetone. 450mg. of the methyl, O-methyl podocarpate VI, melting at 128° (lit. 128°) was produced.

U.V. λ_{max}^{ETOH} = 249 mμ, ϵmax 215

I.R. νmax 1729 cm^{-1} (carbonyl)

$[\alpha]_D$ = + 124.9° (C = 0.01 $CHCl_3$)

Hydrolysis of Methyl, O-Methyl Podocarpate.

The ester, O-ether VI (516mg.) was dissolved in a solution of 25g. of potassium hydroxide in methanol (100ml). This was then refluxed for 36 hours. The solution, when cooled, was diluted with 300ml. of water, extracted with chloroform (4 x 25ml); the combined chloroform solution was washed with water, dried (Na_2SO_4) and brought to dryness by distilling off the solvent. By this method 282mg. of the unreacted starting material was recovered. The aqueous solution remains (after chloroform extraction) was acidified blue to Congo red, extracted with chloroform, and worked up as usual. O-methyl podocarpic acid VII was obtained in 200mg. yield, melting at 154° (lit 154°).

U.V. λ_{max}^{ETOH} 248 mμ εmax 184

I.R. νmax 1575, 1600, 1674 cm^{-1} (carboxyl carbonyl)

$[\alpha]_D$ = + 136.3° (C = 0.01 $CHCl_3$).

Ethyl, O-Methyl Podocarpate VIII.

To 472mg. of O-methyl podocarpic acid VII in 5ml. of 50% aqueous alcohol containing 500mg. of sodium hydroxide was added 0.3ml x 4 of pure[69] diethyl sulphate at intervals of 15 minutes. After the last addition, the solution was refluxed for an additional hour. This was diluted with some water, extracted with chloroform; this chloroform solution was washed with water, and worked up to get 162mg. of crystalline ethyl, O-methyl podocarpate VIII, melting at 86°.

I.R. ν max 1674 (carboxylic carbonyl)

U.V. λ^{EtOH} max 248 mμ ε max 183.6

$[\alpha]_D$ = 128.4° (C = 0.01 $CHCl_3$)

N.M.R. τ 6.53 - 7.2 (benzenoid protons) 3.93 - 4.29 (ethyl group of the ester), 3.7 (methyl protons of the O-ether).

Analysis, found : C = 76.4 H = 9.16

required C = 75.9 H = 8.92 $C_{20}H_{28}O_3$

The aqueous solution remaining was acidified, extracted with chloroform, and after the usual work-up, 175mg. of crystalline unreacted starting material was obtained. (m.p. 154°).

Ethylation of Podocarpic Acid.

To 1.0g of podocarpic acid in 8ml. of 50% aqueous alcohol containing 500mg. of sodium hydroxide, was added 0.6ml. of freshly distilled diethyl sulphate at intervals of 15 minutes (total 2.4ml).

After the last 0.6ml. of the alkylating agent was added, refluxing was maintained for additional one hour. The reaction mixture was cooled, extracted with chloroform, and after the work-up described earlier, ethyl, O-ethyl podocarpate IX, an oil, was obtained in 395mg. yield.

I.R. ν_{max} 1722 (carbonyl)

U.V. λ^{EtOH}_{max} 249 mμ ε_{max} 322

$[\alpha]_D$ = + 122° (C = 0.011 $CHCl_3$).

N.M.R. δ 6.53 - 7.2 (benzenoid protons), 3.8 - 4.3 (ethyl protons of ester group).

Analysis, found C = 76.9 H = 9.6

required C = 76.3 H 9.2 C_{21} H_{30} O_3

The aqueous solution remains, when acidified and extracted in the usual manner, afforded 615mg. of O-ethyl podocarpic acid X in beautiful crystalline form. (m.p. 136°).

I.R. ν_{max} 1600 and 1675 cm^{-1} (carboxyl carbonyl)

U.V. λ^{EtOH}_{max} 248 mμ ε_{max} 181

$[\alpha]_D$ = + 138° (C = 0.012 $CHCl_3$)

N.M.R. δ = 6.53 - 7.2 (benzenoid protons), 3.8 - 4.2 (ethyl protons of ester group)

Analysis, found C = 75.8 H = 9.1

required C = 75.5 H = 8.7 C_{19} H_{26} O_3.

Methylation of O-Ethyl Podocarpic Acid

500mg. of O-ethyl podocarpic acid was methylated by the method described earlier for the methylation of podocarpic acid. The crude product obtained was crystallized from acetone yielding 140mg. of XI, melting at 143-144°.

I.R. ν_{max} 1715 cm^{-1} (carboxyl carbonyl)

U.V. λ^{EtOH}_{max} 247.5 mμ ϵ_{max} 220

$[\alpha]_D$ = + 129.6° (C = 0.009 $CHCl_3$).

N.M.R. δ 6.53 - 7.23 (benzenoid protons), 3.8 - 4.2 (ethyl protons of ethyl ether), 3.65 (methyl proton of ester group).

Analysis, found C = 76.4 H = 9.1

required C = 75.9 H = 8.9 $C_{20}H_{28}O_3$.

III. Peracid Oxidation of cholest-4-en-3-one to 3-Oxo-4-Oxa-5α-Cholestane[20].

A mixture of potassium persulphate (9g) and concentrated sulphuric acid (10g) was prepared in a mortal, this was then diluted with glacial acetic acid (150ml). This mixture was then added to a solution of the cholest-4-en-3-one XIII in 150ml. of acetic acid, in a flask painted black, and kept at room temperature, with occasional shaking, for seven days. Then the flask was immersed in ice-bath and 50% potassium hydroxide (40ml) was added cautiously.

The precipitates formed were filtered, and the filtrate brought to dryness at 60° under suction. The residue was dissolved in ether, filtered to remove inorganic solids; and the filtrate washed successively with water, carbonate (5%) and water, dried (Na_2SO_4) and brought to dryness. Crystallization of the residue from ethanol yielded colourless, prismic crystals of lactone* XIV, 540mg. melting at 109°. Recrystallization improved the melting point to 114° (lit 116°).

I.R. ν_{max} = 1728.6 cm^{-1} (saturated carbonyl group).

$[\alpha]_D$ = + 79.27° (C = 0.011 $CHCl_3$)

N.M.R. δ 3.81 - 4.08 (quartet signals of the C_5-H) 2.48 - 2.74 (methylene protons).

* To Mr. Paul Thomas of this Department; I am grateful for the supply of one gramme of pure lactone.

Lithium Aluminium Hydride Reduction of the Lactone XIV[70-72]

400 mg. of Lithium aluminium hydride (LAH) was refluxed in 40ml. of dry ether[73] for 30 minutes, then 500mg. of the lactone in 10ml. of dry ether was added over a period of 30 minutes. Refluxing was continued for additional six hours. To this, in the cold, was added ethyl acetate cautiously in small lots. The solution was finally tested with chips of ice to ensure that all unreacted LAlH was destroyed. The suspension was separated by a separatory funnel, the organic layer was washed with water, (3x10ml) dried and brought to dryness. A white pasty product obtained was crystallized from methanol yielding 315mg. of the diol, XVI; melting at 134°.

I.R. ν_{max} 3245 cm^{-1} (- OH groups)

$[\alpha]_D$ = + 9.05 (C = 0.010 $CHCl_3$).

N.M.R. δ 3.62 (methylene protons at C_3)

Analysis, found C = 79.8 H = 12.5

required C = 79.5 H = 12.3 ($C_{26}H_{50}O_2$).

Tosylation of the Diol XVI.

400mg. of the diol XVI was dissolved in 7ml. of dry pyridine, and 170mg. of toluene-para-sulphonyl chloride in 7ml. of dry pyridine, both were cooled at 0° for 30 minutes.

The tosyl chloride solution was then poured into the steroid solution and kept at 15° for 24 hours. The solution was then poured into 80ml. of water, and ether extracted (5 x 20ml). The etherate was washed with water (5 x 20ml), then shaken continuously with 20ml of 10% methanolic potassium hydroxide for one hour. The KOH solution was separated, then the ether solution was washed with water (5 x 20ml), with 2N hydrochloric acid (3 x10ml), and finally with water until free of acid. The solution was dried over anhydrous sodium sulphate and evaporated to dryness. The product obtained was chromatographed (30g. silica gel). Hexane eluted a crystalline tosylate XVII (36mg.), melting at 86°. Methanol eluted unreacted starting material XVI (358mg.).

The tosylate showed infra-red absorption bands at 1088 and 1103 (ether C - O band).

$[\alpha]_D$ = + 96.03 (C = 0.01 $CHCl_3$)

N.M.R., δ 7.42 - 7.8 (benzenoid proton of the tosyl group)

δ 4.05 - 4.42 (C_3 methylene protons),

δ 2.61 (vinyl proton of the C_5),

δ 2.15 (methyl group of the tosyl group).

Analysis found C = 82.5 H = 12.7

required C = 83.4 H = 12.3 (C_{33} H_{55} O_4 S).

Hydrolysis of Lactone XIV to a Carboxylic Compound.

100mg. of the lactone XIV plus 2ml. of 5% methanolic potassium hydroxide were refluxed for 30 minutes. This was cooled in ice-salt mixture for about 30 minutes, then glacial acetic acid was added dropwise with stirring until the solution turned clear, with a little more acetic acid fresh white precipitates were formed again. This neutralization by acetic acid was also controlled by testing the solution, at close intervals, with Congo Red paper; the acid was added until the paper turned faint blue. The precipitates formed were filtered, washed with water, then crystallized from ethanol, yielding 57.4 mg. of the hydroxyl carboxylic compound melting at 150°. Recrystallization improved the melting point of the acid XV to 172°.

I.R. ν_{max} 3344.6 and 1732.3 (carboxyl carbonyl).

During a second attempt, 498mg. of the lactone was hydrolysed by an identical method employed above, but unfortunately the product obtained was found to be the starting material.

In another attempt, the reaction solution was neutralized with dilute hydrochloric acid, yet the starting material was obtained.

In a final attempt, using the same conditions as previously employed, but neutralized, the reaction solution with a calculated equivalent amount of glacial acetic acid. The starting material was equally obtained in about 90% yield.

VI. Methylation on a Methylene Group Activated by Ketone.

A. Bromination of Cholesterol to 5α, 6β-Dibromide XXIX[74a].

1.5g of cholesterol XII was dissolved in 10ml of dry ether by brief warming in a beaker, this was cooled to 25°, then a solution of anhydrous sodium acetate (50mg) and bromine (70mg) in acetic acid (6ml) was added. The solution was stirred briskly. The solution turned stiff yellow paste of the dibromide, XXIX. The mixture was allowed to cool to 20°, filtered in Hirsch funnel under suction. The precipitates were washed thoroughly with acetic acid until the filtrate was colourless. These precipitates of the dibromide[XXIX] were used moist in the following experiment, of course a small amount of this was kept to dry so as to justify the purity of the compound by its melting point determination; melting at 108°.

Oxidation of the 5α, 6β-Dibromide XXIX to 5α, 6β-Dibromo-Cholestan-3-en-XXX[74,b].

A major portion of the dibromide XXIX, while moist was suspended in 20ml of acetic acid in a 250ml flask equipped with a stirrer at room temperature, and a solution, preheated to 90°, of 0.8g of sodium dichromate dihydrate in acetic acid (20ml) was added in a fume cupboard. The warm mixture became a fairly clear solution after sometimes.

Then the flask was cooled in an ice-bath, stirring was stopped, and the mixture allowed to stand in the ice-bath without further stirring for 15 minutes, to allow the dibromoketone XXX to separate in an easily separatable crystals. With stirring resumed, the temperature was raised to 25° then 4ml of water was added and the temperature lowered to 15°. The product was collected in a Hirsch funnel and the filter-cake drained; suction was stopped, then the walls of the funnel was washed with methanol, then 2ml of methanol was added to the filter-cake. After about 5 minutes standing, suction was applied and the crystals were drained thoroughly, then washed with more portions of methanol until the filtrate was completely colourless. A small portion of this dibromo-ketone XXX was kept to dry and used for melting point determination; melting 74-76° (lit. = 73-75°).

Debromination of the Dibromo ketone XXX to cholest-5-en-3-one XXVII.

The moist filter cake of the dibromo ketone XXX was transferred to a 250ml flask, and covered with 20ml of dry ether, and acetic acid (0.3ml). The suspension was stirred mechanically, and then lowered into an ice-bath to control the temperature to 15°. The ice-bath was then removed and 0.05g of zinc-dust was then added in one lot. As soon as the reaction of debromination started (ebullution), the temperature was controlled to 15° by cooling, 400mg of more zinc-dust were added in about five minutes.

The bath was removed and stirring continued for 15 minutes longer. With continued stirring 0.4ml. of pyridine was added to precipitate a white zinc salt. The mixture was filtered, and the filter-cake was washed thoroughly with ether. The colourless filtrate was washed with water (6ml), then shaken thoroughly with 6ml. of 5% of aqueous sodium bicarbonate solution until free of acetic acid. The solution was dried and evaporated to about 10ml, then 5ml. of methanol was added; this was allowed to crystallize at room temperature and finally at 0°. Large prismic crystals of the ketone were formed. 400mg. of the cholest-5-en-3one XXVIII, melting at 124 - 127.5°. (lit. 124-129°). were obtained.

Methylation of cholest-5-en-3-One.

The ketone, XXVIII (250mg.), in dry tertiary butyl alcohol (20ml) at 40° was treated with a solution of potassium (1g) in the same solvent (10ml.) At once methyl iodide (5ml) in the same solvent was added and the solution was refluxed on water bath for one hour. The solvents were distilled off and the product was ether extracted, then the ether was distilled off. The crude product obtained was crystallized from acetone-methanol mixture yielding 200mg. of a crystalline product characterized as the unreacted starting material.

Oppenauer Oxidation of Cholesterol to Cholest-4-en-3-one[75,76]

To a 250 ml three-necked flask, equiped with a sealed mechanical stirrer, a dropping funnel, and a take-up reflux condenser, were added 20 ml of sulphur-free toluene and two boiling chips. A portion of the toluene (2 ml) was distilled off in order to dry the system azeotropically, then 1.0g of cholesterol in 5 ml of purified cyclohexanone[77] were added to the refluxing flask. After an additional 0.5ml of toluene had been distilled, a solution of aluminium isopropoxide[78] (0.28g) in 4ml of dry toluene was added dropwise over a period of about 30 minutes. During this time, toluene was distilled at a rate slightly greater than the rate of addition of the catalyst solution, so that when the addition was complete about 6ml of toluene had distilled. An additional 3 ml of toluene was distilled, and the murky orange coloured mixture was then allowed to cool, 4 ml of a saturated aqueous solution of sodium-potassium tartarate was added to the mixture, and the organic layer became clear and orange. The stirrer assembly was removed, and the mixture was collected. The residual mixture was cooled and extracted with chloroform (3 x 20 ml). The combined extracts was washed (water), dried (Na_2SO_4) and distilled at reduced pressure to remove the solvent. The residual viscous amber oil was dissolved in 1.5 ml of methanol (a small portion of which was kept on a watch glass and scratched to give crystals). The crystals formed in the watch glass were used to induce crystallization

by seedling. The solution was kept at 0° for 24 hours for total crystallization. Recrystallization from acetone-methanol gave 776 mg of 4-cholest-4-en-3-one, melting at 78.5-79° (lit. 80°). U.V. λ_{max}^{EtOH} 242mμ (lit 241mμ) ϵ_{max} 17,640 (lit 18,000) $[\alpha]_D$ = +86.08° (lit = +89°) (C = 0.01, $CHCl_3$).

4,4-Dimethyl-Cholest-5-en-3-one[28]

A solution of 150 mg of potassium (3 molar equivalent) in 10 ml of dry tert. butanol was added to a boiling solution of the ketone XIII (0.5g) in 30 ml of benzene. Methyl iodide (1.5 ml) in benzene (5 ml) was then added and refluxing continued for 5 minutes. The solution was then cooled, 0.25 ml of water added, and the solvents were distilled off. Ether was added and insoluble potassium iodide filtered. The filtrate was evaporated to dryness. The crude product was crystallized from methanol yielding 252.4 mg of the dimethyl derivative XXV; melting at 169° (lit 172°).

A second method[30] which produced a higher yield of the dimethyl derivatice involved dissolving 500 mg of the ketone XIII in dry tert. butanol, to this was added a solution of 1g of potassium and methyl iodide (5ml) in the same solvent. This was then refluxed for one hour, then worked-up as in above described method. A yield of 65% was obtained.

V. 3β-CHLORO-CHOLEST-5-ENE

Cholesterol (2.5g) was placed in a 50 ml flask, fitted with a calcium chloride guard-tube. Purified thionyl[79] (2ml) was added rapidly with shaking. The dark green solution was allowed to stand at room temperature for 18 hours. Excess thionyl chloride was distilled off with suction at 45°. The residue on crystallization from methanol gave 1.50g of 3β-chloro-cholest-5-ene, XLIV, melting at 94° (lit 95°).

Conversion of XLIV to Cholest-5-ene[80] XLV

The chloride XLIV (1.7g) in boiling amyl alcohol (20ml) was treated with 1.5g of sodium, added in small pieces over a period of two hours. The solution was refluxed for further 1.5 hours and then cooled overnight. The next morning the content was warmed and thoroughly washed, until neutral to litmus paper, with warm water (warm condition was employed to minimize emulsion formation). This solution was dried, filtered and brought to dryness. The crude product was recrystallized from ethanol yielding 753mg of cholest-5-ene, XLV; melting at 88° (lit 90°).

Oxidation of Cholest-5-ene to Cholest-5-en-7-one XLVI

930mg of cholest-5-ene was dissolved in 25 ml of dry carbon tetrachloride[(42)] and warmed to 80°. Under vigorous stirring (sealed

stirrer) a mixture of 10ml of tert-butyl chromate solution[82] and 4 ml of glacial acetic, and acetic anhydride (1.5ml) was added in drops. Refluxing and stirring was mainted for ten hours. The cold reaction mixture was treated with 2.5g of oxallic acid in 25 ml of water, while cooled in an ice-bath, within 45 minutes. After 15 minutes, 1.8g of solid oxallic acid was then added in four portions and stirring continued for another two hours while the solution warmed to room temperature. The pale yellow carbon tetrachloride layer was separated and the aqueous layer extracted with carbon tetrachloride (2 x 15 ml); the combined tetrachloride solution was washed with water (2 x 15ml), sodium bicarbonate (3 x 15 ml) and water (3 x 15ml). This was dried, and then brought to dryness. The crude product was crystallized from acetone-methanol yielding 800mg of the ketone melting at 124°. This was chromatographed over silica gel; elution with benzene gave crystalline cholest-5-en-7-one, melting at 131° (lit. 132°)

I.R. ν_{max} . 1657 (unsaturated ketone)

U.V. λ_{max}^{EtOH} 240mμ (lit 238mμ) ε_{max} 8,000 lit 10,000) $[\alpha]_D$ = -138°

(C = 0.01 $CHCl_3$) (lit. -128°).

6,6-Dimethyl-Cholest-4-en-7-one[54]

Cholestenone, XLVI (480mg) was dissolved in 2.5M solution of potassium tert. butoxide in benzene, this was refluxed for 24 hours. Then 10 ml of methyl iodide was added to the solution at once and

refluxing was continued for additional two hours. After this, the solvents were distilled off and the crude product was worked up as usual. This was chromatographed, hexane elluted the crystalline 6,6-dimethyl derivative, XLVII, 250mg, melting at 72-73° (lit = 69°).

I.R. ν_{max} 1710 (saturated ketone)

U.V. λ_{max}^{EtOH} 298.5mμ (lit 296) ε_{max} 86 (lit 93).

Then 150mg of the 6,6-dimethyl ketone XLVII was dissolved in 5ml of dry tert. butanol, to this solution was added 150mg of potassium in 3 ml of the same alcohol, then 3 ml of methyl iodide in 2 ml of the same alcohol was added. This reaction solution was refluxed for six hours, then worked up as usual. A sticky product which sluggishly crystallized was obtained. Thin layer chromatography showed a major spot corresponding to the starting material and a very faint spot uncharacterized.

Acetylation of Cholesterol

500mg of cholesterol was dried azeotropically with dry benzene, this was then dissolved in dry[83] pyridine (4ml). To this was added 4ml of dry[84] acetic anhydride, and the solution kept overnight. Next morning, the solution was poured into 70ml of ice-cold water, and left at room temperature for two hours. The precipitates formed were filtered, washed, drained thoroughly, and crystallized from acetone. Crystalline cholesteryl acetate (m.p. 114°) was obtained in 80% yield.

Tert-Butyl-Chromate Oxidation of XXXI to 3-Acetoxyl-Cholest-5-en-7-one XXXI

The acetate, XXXI, (508mg) was oxidized, following the Marshall, et.al.,[42]. This involved dissolving the acetate in a dry carbon tetrachloride with a mixture of acetic acid and acetic anhydride, then refluxed at 80° for ten hours and then worked up as previously described. From the crude product was obtained 201mg of colourless prismic crystals on crystallization from acetone; melting at 155° (lit 159°).

U.V. λ_{max}^{EtOH} 235.5mμ (lit. 235) ϵmax 11,040 (lit 10700)

I.R. νmax 1240, 1727 (acetate) 1628 (conjugated ketone) cm^{-1}

$[\alpha]_D$ = +73° (C = 0.0116, $CHCl_3$).

Methylation of the 3-Acetoxy-Cholest-5-en-7-one

500mg of the ketone XXXII was dissolved in dry tert-butanol, to this was added 500mg of potassium in dry tert. butanol (3ml). To the hot solution was added 1.5ml of methyl iodide in 1ml of the same solvent. A crystalline product obtained was characterized as cholest-3,5-dien-7-one. In another attempt 1g of the ketone XXXII dissolved in 10ml of benzene was refluxed with 1g of potassium dissolved in 10ml of tert. butanol, almost immediately 10ml of methyl iodide was added and refluxing continued for an hour on a steam-bath. The crystalline product obtained after the usual work up was also characterized to be cholest-3,5-dien-7-one.

In a final attempt 3g of the ketone XXXII was dissolved in

10ml of dry benzene at 60°, methyl iodide (10ml) was added and then cooled to room temperature. 1g of potassium in 10ml of dry tert. butanol was added, then refluxed for one hour, at 60°. The solution was worked up as usual. The crude product was chromatographed over silica gel (150g). Six eluates (100ml each) of pet-ether-benzene (95:5) gave 750mg, of a crystalline product melting at 112°. This was characterized to be cholest-3,5-dien-7-one by:

I.R. νmax 1612.5, 1639 (unsaturated conjugated ketone)
U.V. λ_{max}^{EtOH} 276mμ (lit 280) εmax 20,310 lit (25,400)
$[\alpha]_D$ = -293° (lit -306) (C = .010, $CHCl_3$)
NMR δ 6.13 (vinyl protons)

Four eluates of pet-ether-benzene (75:25), 100ml each, gave an oily product which crystallized from methanol in 80mg yield. Melting at 124°.

I.R. νmax 1654 cm^{-1} (conjugated ketone) 1122 cm^{-1} (?)
U.V. λ_{max}^{EtOH} 237.8mμ, εmax 11,140
$[\alpha]_D$ = -108.5 (C = 0.01, $CHCl_3$)
N.M.R. δ 3.37 (methoxyl protons), Mass 444

Analysis C = 79.5 H = 10.5 found,
required C = 81.1 H = 11.19 $C_{28}H_{46}O_2$

This compound was therefore characterized as cholest-5-en-7-one-3-methyl ether, XXXVI. Washing the column with about one litre of benzene-acetone (95:5), a third component was obtained. This crystallized readily as long needles in acetone, in 1.3g yield.

M.pt. 170° (lit 170-172°)[85].
I.R. νmax 1637.5 cm^{-1} (conjugated ketone), 3044.3 cm^{-1} (-OH).
U.V. λ_{max}^{EtOH} 238mμ εmax 9417
$[\alpha]_D$ = -78.2° (C = 0.011 $CHCl_3$) (lit -74°)

This compound was therefore characterized as cholest-5-en-3-ol-7-one XXXV.

149mg of the ketone XXXII was acetylated in a mixture of acetic

anhydride and pyridine, then worked up as usual. An acetate melting at 157° (lit. 159°), showing no depression in the melting point when mixed with an authentic sample, was obtained in 68% yield.

400 mg of the same ketone, XXXII, was oxidized by Oppenauer method[75-76]. A pale yellow crystalline product was obtained in 100mg yield, melting at 184°.

I.R. ν_{max} 1664, 1668 (conjugated unsaturated ketone)

1725 (non-conjugated unsaturated ketone)

U.V. λ_{max}^{EtOH} 230mμ ϵ_{max} 2410.

$[\alpha]_D = -86.08°$ (C = 0.014 $CHCl_3$)

This compound was characterised as cholest-5-en-3,7-dione. 30 mg of the endione was dissolved in 5 ml of dry benzene, warmed to dissolve. To this solution was added 10mg of toluene-para-sulphonic acid. This was refluxed for an hour on water-bath. This solution was washed with sodium bicarbonate (3 x 7ml), then by water (4 x 10ml), dried and brought to dryness. The crude product was crystallized from methanol yielding 22mg of a powdery product.

I.R. ν_{max} 1725 cm^{-1} (non conjugated unsaturated ketone)

1664 and 1668 (conjugated ketone)

<u>Acid-Catalyzed β-Elimination of 3β-Acetoxyl-Cholest-5-en-7-one to cholest-3,5-dien-7-one.</u>

100mg of the ketone XXXII was dissolved in acetic acid (0.6ml) and 3mg of tolune-para-sulphonic acid was added. The mixture was

refluxed for 50 minutes, chilled and then 5 ml of water was added slowly with shaking. This was ether extracted (8 x 10ml), and the etherate was washed with water (5 x 10ml), bicarbonate (4 x 10ml), and water (3 x 10ml), successively. It was dried, brought to dryness and the residue was norited in ether. The crude product obtained crystallized from methanol with a yield of 62.2mg of the dienone ; melting at 114° (lit 116°).

I.R. ν_{max} 1612, 1640 (conjugated ketone)

U.V. λ_{max}^{EtOH} 280mμ (lit 276), ε_{max} 20,310, (lit 25400)

$[\alpha]_D = -297.3°$ (lit -306) (C = 0.010 $CHCl_3$).

Reduction of the Dienone XLI to α-Cholesterol XLII[86]

1.21g of the dienone XLI and 22 ml of dry n-propanol were heated to boiling and 1.6g of metallic sodium was added over a period of about 4 hours. After the addition of sodium, the mixture was refluxed for an additional hour, cooled and diluted with 70ml of water. The mixture was then ether extracted, washed with water, dried (Na_2SO_4) and evaporated to dryness with suction. The crude product was crystallized from acetone-methanol affording white crystals of α-cholesterol, XLII, in 430mg yield; melting at 118° (lit 120°).

Oxidation of Cholest-4-en-7-ol to Cholest-4-en-7-one XLIII[86]

A solution of 400mg of cholest-4-en-7-ol and 550 ml of dry acetone was cooled to 10-15°, and with stirring under an atmosphere of nitrogen, 5.6ml of tert. butyl chromate was added rapidly. After 5 minutes, the reaction mixture was poured into a saturated aqueous sulphur dioxide solution, followed by the addition of saturated aqueous solution of potassium carbonate solution and ether. The ether layer was separated and the aqueous phase was extracted thrice with ether, the combined ethereal solution was washed with water, dried and brought to dryness. The residue was crystallized from acetone-water to give a crude product (400mg) melting at 82°. Recrystallization from acetone yielded cholest-4-en-7-one, 350mg, melting at 97° (lit 98°).

150mg of this ketone XLIII was dissolved in ter-butanol-potassium tert. butoxide system, methyl iodide in the same alcohol was added. This solution was refluxed for one hour, worked up as usual; affording a crystalline product in about 90% yield. This product was characterized as the unreacted starting material.

Ketalization of 4,4-Dimethyl-Cholest-5-en-3-one to 3-Methylene dioxy-4,4-Dimethyl-Cholest-5-ene

The ketone XXV was dried azeotropically with benzene, then dissolved in 5ml of benzene, toluene-para-sulphonic acid (5mg) and ethane diol (0.2ml) were added. The benzene solution was refluxed for 18 hours with a Diene-Stark-Head. The cold solution was washed

with saturated sodium bicarbonate solution, water, dried (Na_2SO_4) and then brought to dryness. The residue on crystallization from methanol readily gave 97.6mg of the ketal XLVIII, melting at 124°. Three time recrystallization improved the melting point to 126°.

I.R. no absorption between 1500 and 2000 cm^{-1} region.

$[\alpha]_D = -74.32°$ (C = 0.01, $CHCl_3$)

NMR. δ 5.54 (methylene dioxy protons)

Analysis found C = 81.8 H = 11.8

required C = 81.52 H = 11.5 $C_{31}H_{52}O_2$.

Tert. Butyl Chromate Oxidation of the 3-Methylene dioxy-4,4-Dimethyl Cholest-5-ene

1.55g or the ketal XLVIII was oxidized following Marshall, et.al.,[42] by tert. butyl chromate solution in 25ml of carbon tetrachloride, containing acetic anhydride and acetic acid in a catalytic quantity. This solution was refluxed for 10 hours at 80°, worked up in the usual manner described earlier. From this reaction was obtained a crystalline ketone XLIX, in 385mg yield; melting at 115°.

I.R. νmax = 1667 (conjugated ketone)

U.V. λ_{max}^{EtOH} 243mμ εmax 10130

$[\alpha]_D = -94.7°$ (C = 0.01; $CHCl_3$)

Mass = 470

NMR δ 5.9 (protons of the methylene dioxy group)

Analysis found C = 78.5 H = 10.8

required C = 79.1 H = 10.7 $C_{31}H_{50}O_3$.

C_8-Methylation of the Ketone XLIX

The ketone, XLIX (226mg) in dry tert. butanol (20ml) at 40° was treated with a solution of 1g of potassium in the same solvent. At once 5ml of methyl iodide in the same solvent was added and this was refluxed for an hour. The solvents were distilled off, and the dry product etracted with ether. The ether solution was distilled and the oily crude product was crystallized from methanol. 149mg of the 3-ethane dioxy-4,4-dimethyl-8-methyl-cholest-5-en-7-one L, melting at 180° was obtained.

I.R. ν_{max} = 1662 (conjugated [illegible] ketone)

U.V. λ_{max}^{EtOH} 244mμ, ϵ_{max} 5795.

$[\alpha]_D$ −151.6 (C = 0.010 $CHCl_3$)

Mass = 484.

NMR δ 5.90 (protons of the methylene dioxy group) δ 1.20 (C_8 methyl)

Analysis found C = 79.1 H = 11.1

required C = 79.28 H = 10.8 $C_{32}H_{52}O_3$

REFERENCES

1. Gokhle, Mason, Journ. Chem. Soc. (1930) 1957.
2. W.N. Rodianow, and W.E. Vvedenskij; 45 109, 121, 1929.
3. Eugen Muller, Meth. der Organischin Chem., (Houben Weyl). Nitrogen Compounds Vol. II, George Thieme Verlag; Stuttgard. 4th Edition (1957) p. 983.
4. Gaylord N.G. Experientia 10 166, 1954.
5. Gaylord N.G. Reduction with complex metal hydrides. Interscience Publishers New York (1956) p.544.
6. Paddock N.L. Nature 167, 1070 (1951).
7. Micovic V.M. and Mihailovic M.L. Journ. Org. Chem. 18 1190 (1953).
8. Moessenron, M.R. Jacquier, M. Mousseron-Canet, and R. Zagdoun; Bull. Soc. Chem. France 19, 1042 (1952).
9. Wittig G., Hornberger P., Ann. 577, 11, (1952).
10. Matlow, Unpublished Work, through G.W. Brown, in Org. Reactions Vol. VI, p.535.
11. Blucke, F.F. and Chi-Jung Lu. Journ. Amer. Chem. Soc. 74 3933 1952.
12. N.K. Venkatasubramanian Ph.D. thesis, Poona University (1966).
13. I.R. Sheerwood, and W.F. Short; Journ. Chem. Soc. 1006 (1938).
14. N.K. Iyer Ph.D. thesis, University of Bombay 1965.
15. N.R. Bhadane Ph.D. thesis, Poona University 1968.
16. W.P. Campbell and D. Todd, Journ. of Amer. Chem. Soc. 64 928 1942 and F.E. King, T.S. King, J.G. Topliss. Chem. and Ind. 113, 1956.
17. I.A. Vogel, A Textbook of Practical Organic Chemistry, 3rd edition, Longmans p.665.
18. I.A. Vogel, ibid, p.670.

19. A. Saleman, Physiol. Chem. 272, 61, 1941.

20. G.R. Pettit and T.R. Kasturi, Journ. Org. Chem. 26 4557 (1961).

21. ibid., 26, 4553 1961.

22. D.H.R. Barton, P. de Mayo and M. Shafiq, Journ.Chem.Soc. 142 (1958).

23. W. Cocker and T.B.H. McMurry, Tet. 8 181, (1960).

24. ibid., Proc. Chem.Soc. 147 (1958).

25. I.R. Sherwood and W.F. Short., Journ.Chem.Soc. 1006, (1938).

26. M.K. Iyer, Ph.D. thesis, Bombay University, (1965).

27. Y.M.Y. Haddad, W.T. Pike, G.H.R. Summer, and W. Klyne, Journ.Chem. Soc. 6117 (1965).

28. Sondheimer, I., Mazur, Y., Journ.Chem.Soc. 79, 2906, (1957).

29. W.W. Wells, and D.H. Neiderhiser, Journ.Amer.Chem.Soc. 99, 6569 (1957).

30. R.B. Woodward, and A.A. Patchett, D.H.R. Barton, D.A. Ives and R.B. Kelly, Journ.Amer.Chem.Soc., part 1, p.1138 (1953).

31. H.J. Ringold and S.K. Malhortra, ibid, 84, 3402 (1962).

32. H. Hauptmann, ibid, 69, 562 (1947).

33. L.F. Fieser, ibid, 76, 3704, (1954).

34. H.J. Dauben, (Jr)., B. Loken, and H.J. Ringold. ibid 76 1359 (1954)

35. R.N. Jones, P.Humphries, E.Packard and K. Dobriner, ibid, 72, 86, (1950).

36. C. Djerassi and C.R. Scholf, ibid, 70, 417, (1948).

37. E.T. Stiller and O. Rosenheim, Journ.Chem.Soc. 353, (1938).

38a. A. Butenandt, G.Schramm, A. Wolff and N. Kudszus, Ber. 69, 2779 1936).

b. C. Doree, Journ.Chem.Soc. 95, 649 (1909).

39. Malhortra S.K., and Ringold, Journ.Am.Chem.Soc. 86, 1997 (1964).

40. L.F. Fieser, et.al., Journ.Amer.Chem.Soc. 71, 2228, (1949).

41. K. Heusler and Wettstein, Hel.Chem.Acta. 36, 1613, 1953.

42. C.W. Marshall, R.E. Ray, I. Laos, and Riegel; Jour.Amer.Chem.Soc. 79, 6308, (1957).

43. J.W. Rallsp R.M. Dodson, and B. Riegel, ibid, 71, 332o, (1949); and J. Romo, G. Rosenkranz, and C. Djerassi, Journ.Org.Chem. 17, 1413, (1952).

44. C.R. Narayanan and K.N. Iyer, Tet. lett. 3741, (1965).

45. L.F. Fieser and M.Fieser, Steroid, Reinhold; New Yonk.

46. R.N. Jones and F. Herling, Journ.Org.Chem. 19, 1252 (1954);
R.N. Jones, D.A. Ramasey, D.S. Keir, K. Dobriner, Journ.Amer. Chem.Soc. 74,80, (1952).
R.N. Jones, A.R.H. Cole ibid 74, 5648, (1952).
R.N. Jones, A.R.H. Cole, and B.Nolin ibid, 74, 5662 (1952).

47. The Interpretations of the Ultra Violet Spectra of Natural Products by A.I. Scott, Pergamon Press, 1964.

48. W. Klyne in E.A. Brande and F.C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press 1955; and H.P. Sig, and T. Reichstein, Hel. 39, 1507, (1956).

49. N.K. Iyer Ph.D. thesis, University of Bombay, 1965; D.H. Gould, K.H. Shaaf and W.L. Ruigh, Journ.Amer.Chem.Soc. 73, 1263 (1951).

50. D.H.R. Barton and C.H. Robinson, Journ. of Chem.Soc. 3046, (1954).

51. O. Rosenhein, and W.W. Starling, Journ.Chem.Soc. 377, (1937).

52a. V. Petrow, O. Rosenheim and W.W. Starling. ibid, 135 (1943) and

b. B. Pete, Coll. Czech, 22, 1457 (1957).

53. A. Butenandt, and E. Hansman, Ber. 70 1154 (1937).

54. G. Ourisson et. F. Lederer, Bull Chem.Soc., France, 1295 (1965).

55. R.F. Zurcher, Hel.Chem. Acta 44, 1380, (1961), 46, 2054 (1963).

56. N.S. Bhacca, and D.H. Williams, Applications of N.M.R. spectroscopy in Organic Chemistry.

57. L.N. Jackman, Applications of NMR Spectroscopy in org.chem., Pergamon Press. (1959).

58. W.S. Johnson, D.K. Banerjae, W.P. Schneider, C.D. Gutsche, W.E. Shelberg, L.F. Chinn, Journ.Amer.Chem.Soc. 74, 2832, (1952).

59. W.S. Johnson, B. Bannister and R. Pappo, ibid, 78, 6331, (1956).

60. W.S. Johnson, R. Pappo, and W. Johns, ibid, 78 6339 1956.

61. W.S. Johnson, B. Bannister, R. Pappo and J.E. Pike, ibid. 78, 6354, 1956.

62. W.S. Johnson and D.S. Allen, ibid, 79, 1261, (1957).

63. H. Dannesberg Abhandl, preciss. Akad. Wiss 21,3,(1939).

64. L.F. Fieser and M. Fieser, 'Natural Products Related to Phenanthrene,' 3rd edition. Reinhold - (1949).

65. I.A. Scott. Interpretation of the Ultra Violet Spectra of Natural Products. Pergamon Press (1964).

66. 1-naphthylamine was purified by crystallising it twice from alcohol.

67. J.R.A. Pollock, Dictionary of Organic Compounds. 4th edition Vol. 2., Eyre and Spottiswoode, Publishers, London.

68. Nystrom R.F., Brown W.G. Journ.Amer.Chem.Soc. 70, 3739 (1948).

69. A.I. Vogel; A Textbook of Practical Organic Chemistry, 3rd edition, Longmans (1964) p.804.

70. Collin-Asselinean, C.E. Lederer, D. Mercier, and J. Polonsky, Bull. Soc. Chem., France 17, 720, (1950).

71. Hund. M., and Stoll, M., Helv. Chem. Acta, 33, 1345 (1950).

72. Deitrich, P., and E. Lederer, ibid, 35, 1148, (1952).

73. Dried by pressing sodium wire.

74a. Norman Rabjohn, Org. Syn. Coll. IV (1963) p.193.

b. ibid, p.196.

c. ibid, p.197.

75. I.A. Vogel, A Textbook of Practical Organic Chemistry, 3rd edition, Longman (1964), p.888.

76. N. Rabjohn, Org. Syn. Coll. IV, p.192-193.

77. I.A. Vogel, A Textbook of Practical Organic Chemistry, 3rd edition, Longman, (1964) p.342. An alternative method employed was simple distillation of the solvent at a reduced pressure.

78. ibid, p. 883.

79. ibid, p.189.

80. N.K. Venkasubramanian, thesis, Poona,University, 1966, Chapter 3.

81. G.J. Kent and E.S. Wallis, Journ.Org.Chem. 24, 1237 (1959).

82. K. Heusler and Wettstein, Helv. Chem. Acta. 36, 1613 (1953).

83. I.A. Vogel, A Textbook of Practical Organic Chemistry, 3rd edition, Longman (1964) p.175.

84. ibid, p.371.

85. Sune Bargstrons and O. Wintersteiner, Journ. Biol. Chem. 141, 597 (1941).

86. N.K. Venkatasubramian, personal notebook, Vol.III, p.110, unpublished work.