CHEMICAL INVESTIGATION OF SOME INDIAN MEDICINAL PLANTS AND SYNTHESIS OF SOME BIOLOGICALLY ACTIVE COMPOUNDS

A THESIS

SUBMITTED TO THE

UNIVERSITY OF POONA

FOR THE DEGREE OF

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(IN CHEMISTRY)

BY

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TO

MY

PARENTS

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C O N T E N T S

Contd.

GENERAL REMARKS

Melting points are uncorrected and have been taken in capillaries. Ultraviolet spectra were taken in ethanol on a Perkin-Elmer model 350 spectrophotometer. Infrared spectra were recorded as nujol mulls, unless otherwise stated on a Perkin-Elmer model 221 spectrophotometer or Perkin-Elmer infracord. The maxima are recorded in cm^{-1}. Proton magnetic resonance spectra were recorded on a Varian A-60 and T-60 spectrometers, using tetramethylsilane as the internal standard. The shifts are reported in δ scale. Mass spectra were recorded on a CEC 21-110B double focussing mass spectrometer operating at 70 ev using direct inlet system. Optical rotations were determined on a Perkin-Elmer polarimeter in chloroform.

The reactions involving metallations were conducted under a dry argon or nitrogen atmosphere. Glassware was flashflamed and flushed with dry argon prior to use. Reagents were transferred by syringe using hypodermic needle and rubber septum. All reaction solvents were distilled and stored under nitrogen [tetrahydrofuran (THF) and ether from benzophenone ketyl, benzene from calcium hydride, triethylamine from barium-oxide, pyridine from potassium hydroxide and chloroform from calcium chloride].

n-Butyllithium in hexane was prepared [H.Gilman et al. J.Amer.Chem.Soc. 62, 2333-35 (1940)] from n-butyl chloride and lithium sand and its concentration determined by Gilman's double titration [H. Gilman et al. J.Amer.Chem.Soc. 66, 1515-16 (1944)].

C H A P T E R - I

EXPERIMENTS DIRECTED TOWARDS THE TOTAL SYNTHESIS

of

(±)-ZEARALENONE

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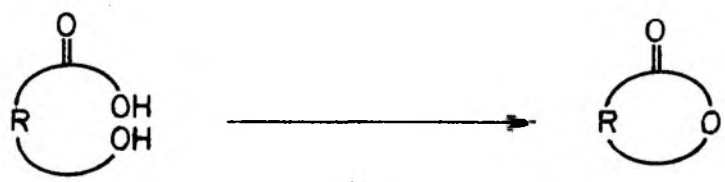
INTRODUCTION

The total synthesis of natural products with biological activity has always been a primary target of synthetic chemists. In the last two decades efforts were directed towards the synthesis of a vast number of important group of natural products such as steroids, terpenes, alkaloids. In the field of antibiotics total synthesis of penicillins, cephalosporins and tetracyclines has already been achieved, although, they continued to be of considerable interest because of their medicinal importance. The term 'macrolide', a class of natural products introduced by Woodward in 1957,[1a] has attracted worldwide attention owing to its immense physiological and pharmaceutical properties.

A macrolide is defined[1b] as a molecule having large ring lactone in its structure. Generally it is considered to be derived from the corresponding hydroxy acids by internal esterification as has been shown below.

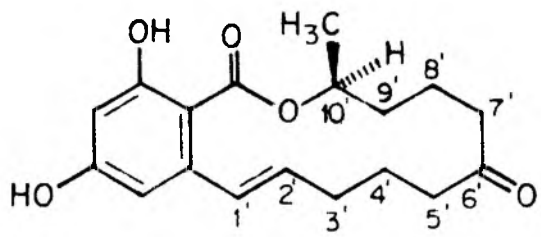


The biological and physiological activities of macrolides are currently under intense investigations and many of them have proved to be important therapeutic agents. Although their structures have been known for sometime, very little synthetic work has started till 1960 followed by intensive research in 1970.

Macrolides are classified into following groups:[2] (1) polyoxomacrolides, (2) polyenemacrolides, (3) ionophoric macrolides, (4) ansamycin, and (5) other macrolides. The last class includes several lactonic compounds of medium ring size. Most of these lactones are either mould or bacterial origin and exhibit varying biological activities, e.g. zearalenone, pyrenophorin, vermiculine and brefeldin A, etc.

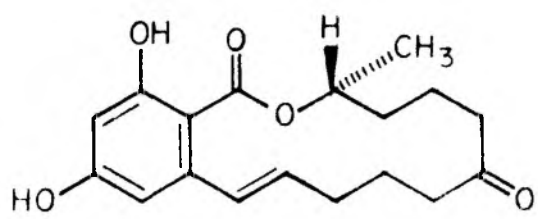
Zearalenone (I), the first naturally occurring β-resorcylic acid lactone (other examples are curvularin,[3a] radicicol[3b] and diaporthin[3c]),is a metabolite demonstrating pronounced anabolic and uterotrophic activity was isolated from the fungus Giberella zeae[4], which grows as a mould on the corn. Urry et al.[5] assigned structure (I) for it, and (S)-configuration at the C-1O' position was established by Kuo et al.[6]

Zearalenone and its derivatives find a number of uses in medicine. It is used as a growth stimulant[7] for meat animals (stears, sheep and pigs) and oral administration is more



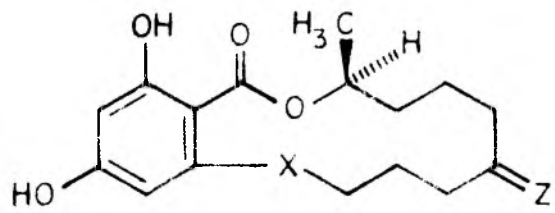
(S) – Zearalenone

(I)



(R) – Zearalenone

(II)



(III) $X = CH_2CH_2$, $Z = O$, Zearalanone

(IV) $X = CH_2CH_2$, $Z = H_2$, Zearalane

(V) $X = CH_2CH_2$, $Z = H, OH$, Zearalenol

effective than any other routes. Mirocha et al.[8] showed that zearalenone produced by the fungus under natural conditions caused marked uterotrophic response in rats, mice and guinea pigs. As mentioned earlier, the configuration[6] at C-10' in naturally occurring zearalenone has been determined as (S), Peter and Hurd[9] developed a synthetic sequence to invert this centre to (R)-configuration to study its biological activity. (R)-Zearalenone (II) showed no uterotrophic activity, but 1:1 mixture of (R)- and (S)-zearalenone gave approximately the same response as (S)-zearalenone. These results were confirmed by Hurd and Shah,[10] and they have shown that synthetic (R,S)-zearalenone has about the same uterotrophic activity as (S)-zearalenone. Inflammation in animals is treated[11] by administering effective doses of zearalenone (I), zearalanone(III), zearalane (IV) and zearalenol (V) (with or without protection of hydroxyl groups). Pregnancy in females is prevented by administering (I), (III) and (V), the last one gave good contraceptive results in human .[12] Zearalenone (I) and zearalane (IV) are clinically administered for human cholesterolemia.[13]

Zearalenone was perhaps the first naturally occurring macrolide to be synthesised. Potent steroid like anabolic and uterotrophic activity of zearalenone, prompted Merck[14,15] and Syntex[16-18] groups to develope synthetic routes(charts 1-5)

CHART - 1

Merck[14] 1968

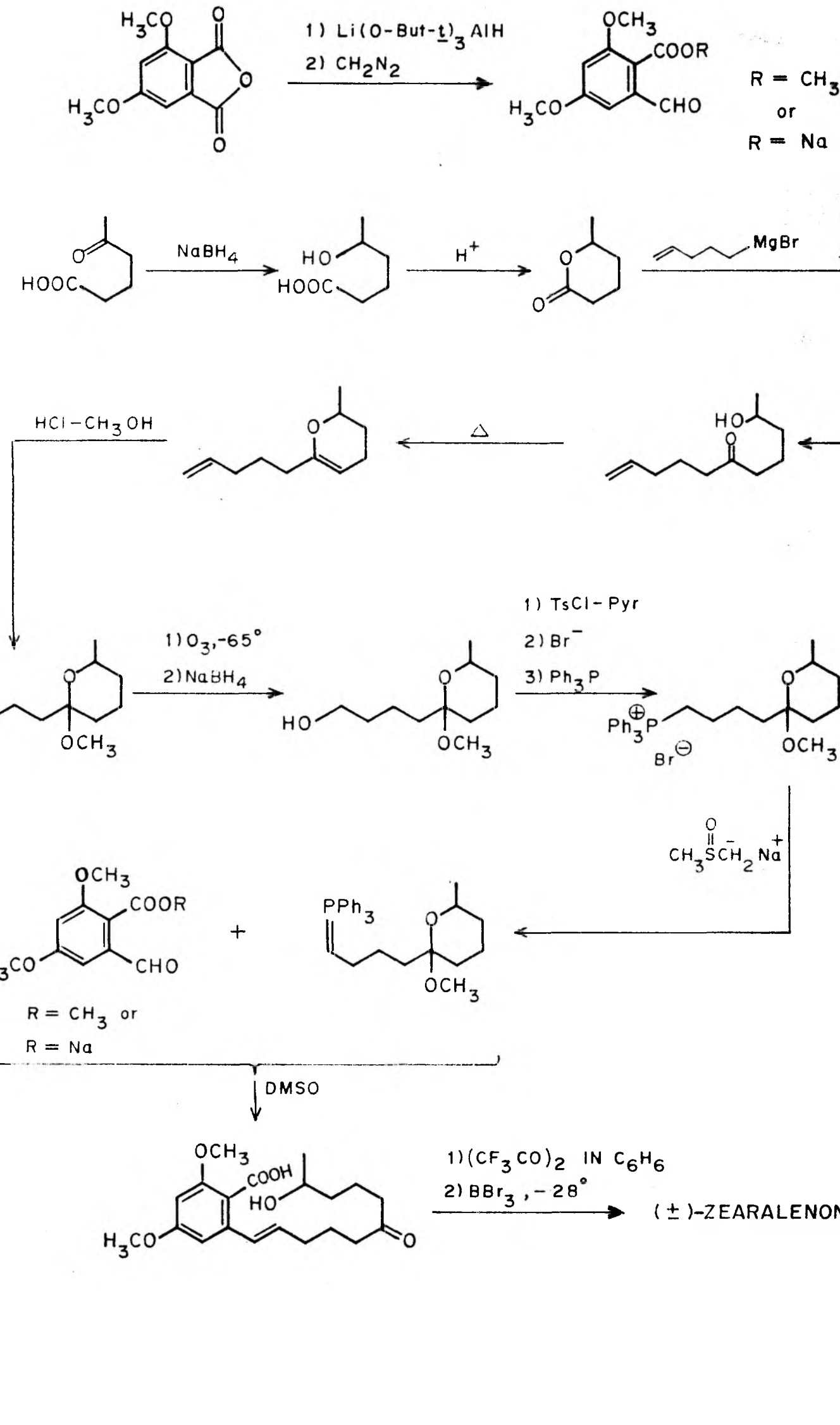
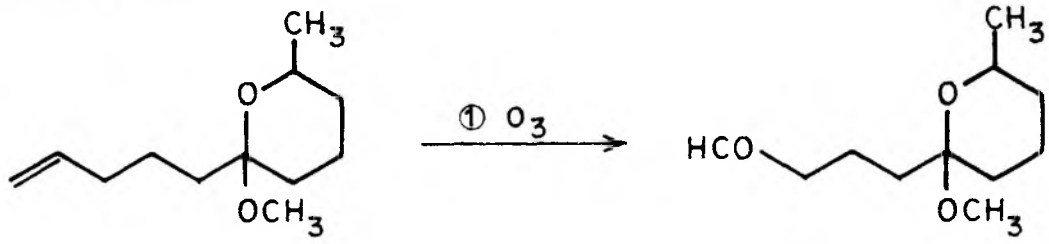
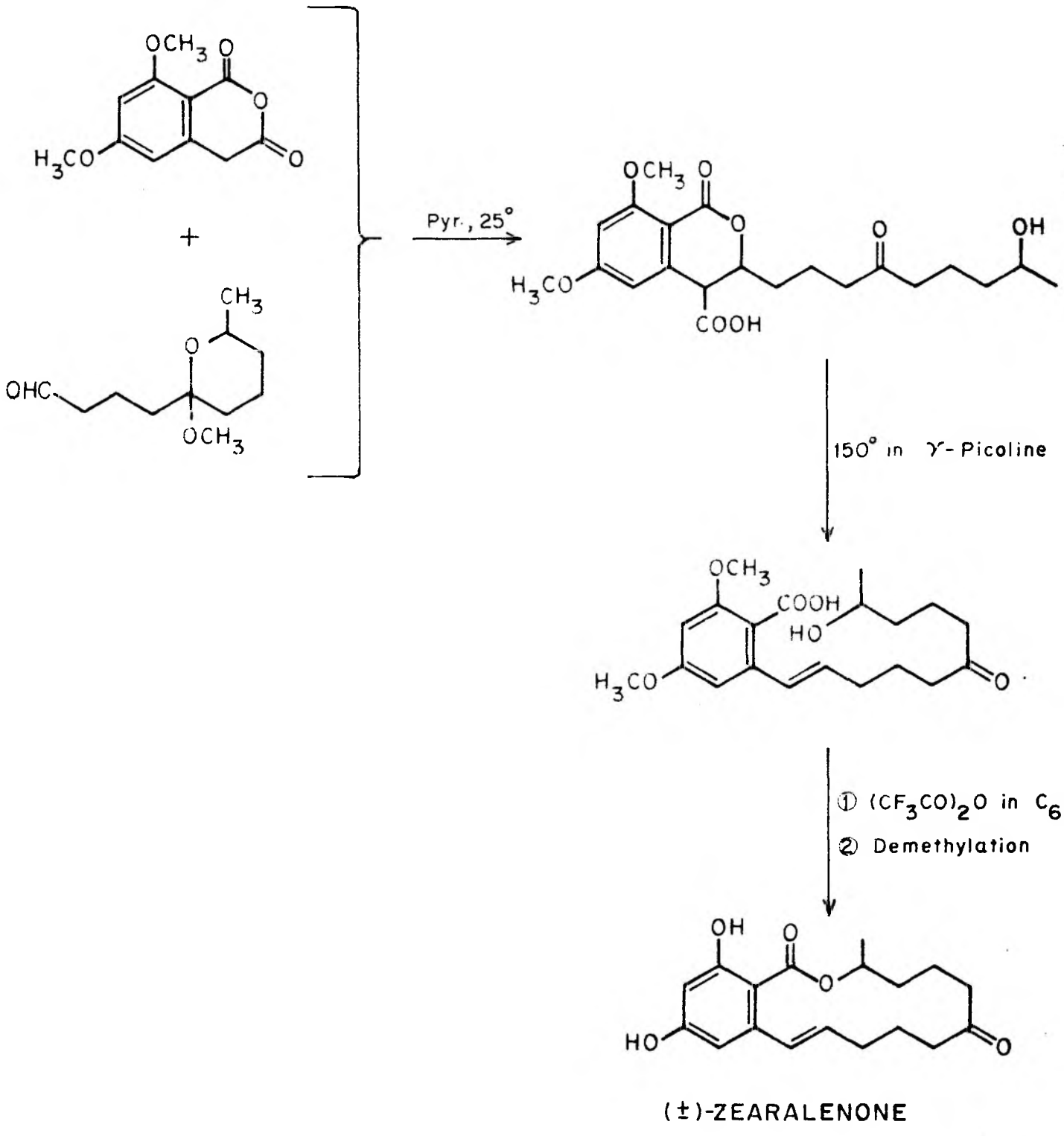


CHART - 2

Merck [15] 1967





(±)-ZEARALENONE

CHART-3

Syntex[16] 1968

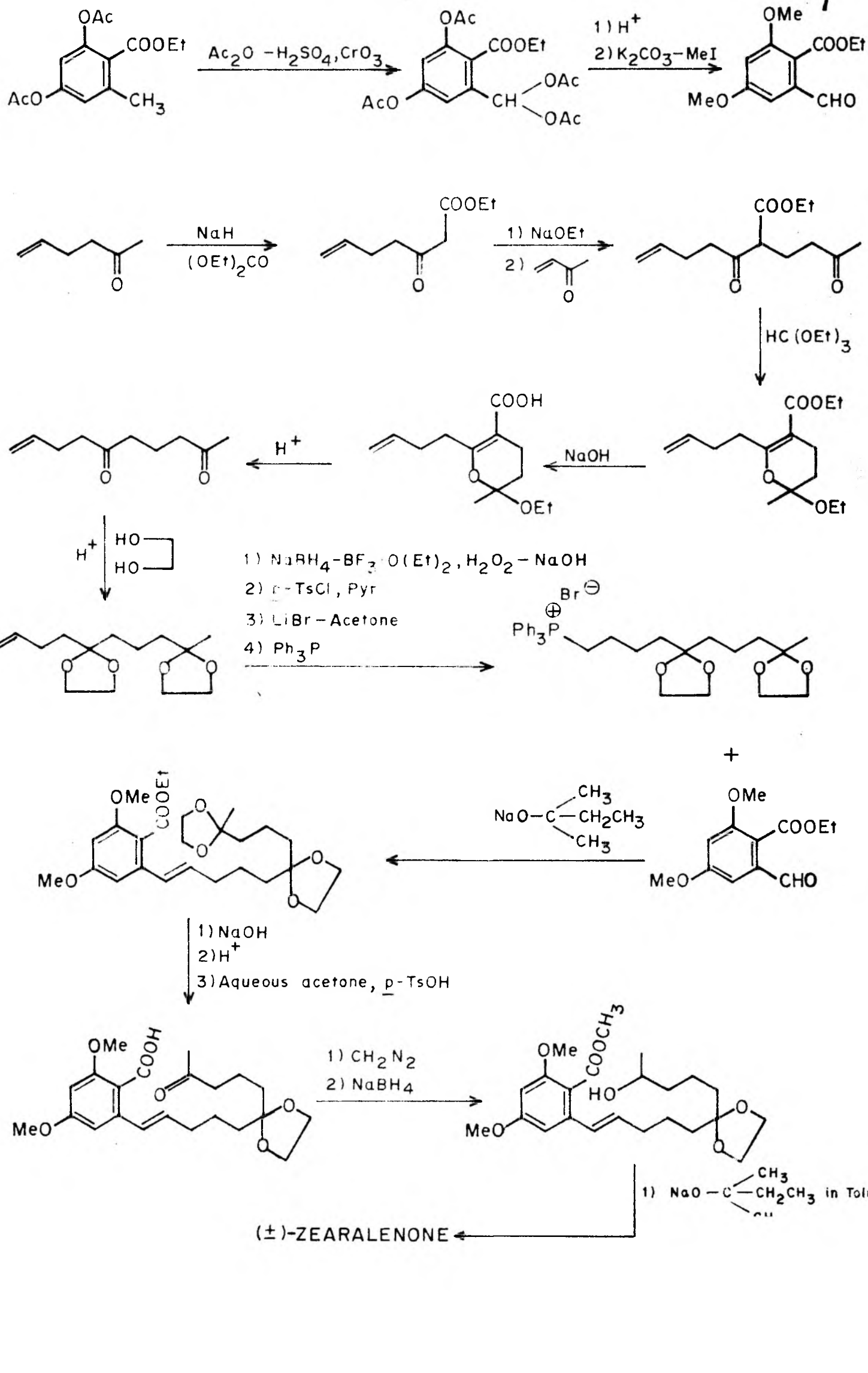
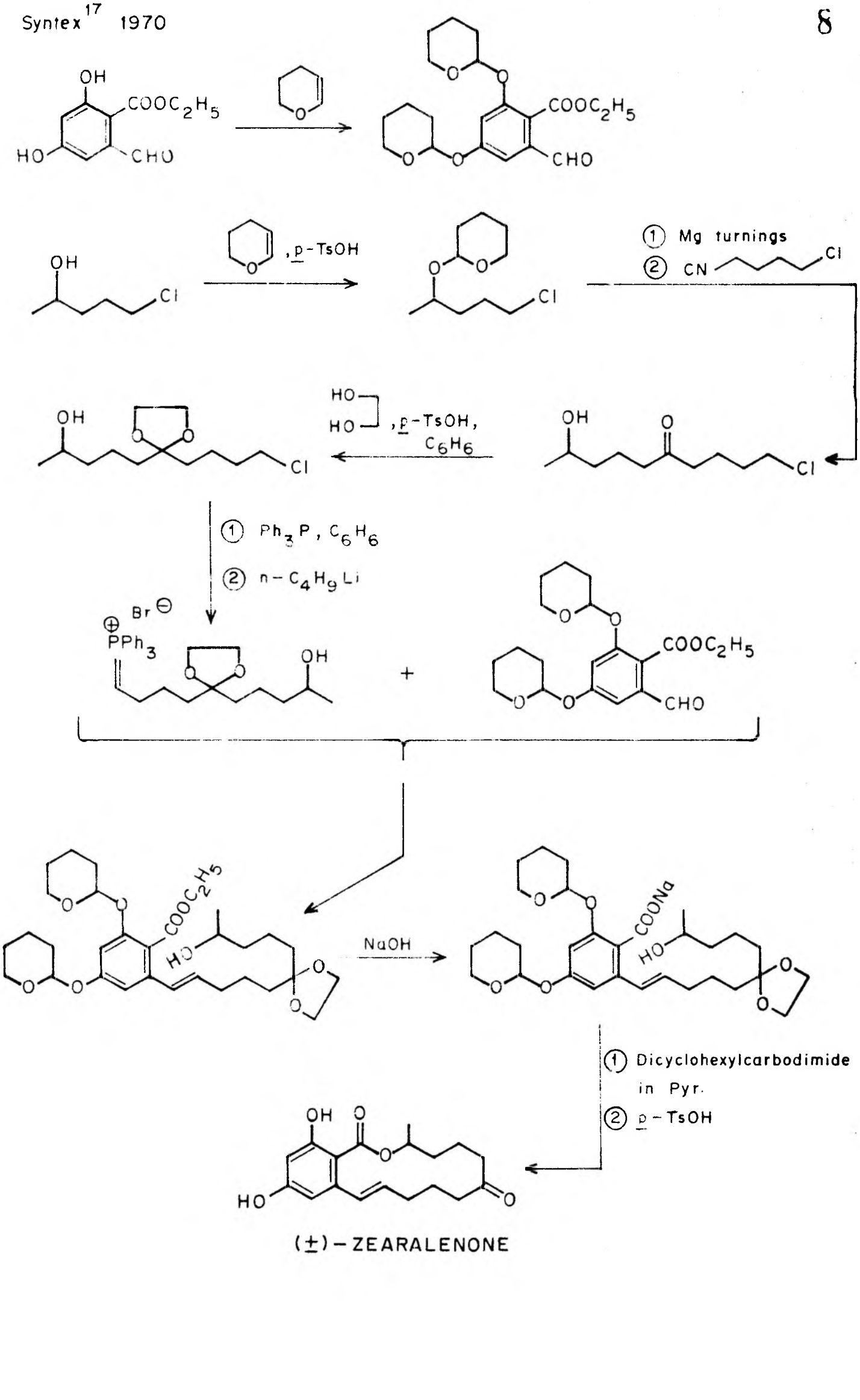


CHART - 4

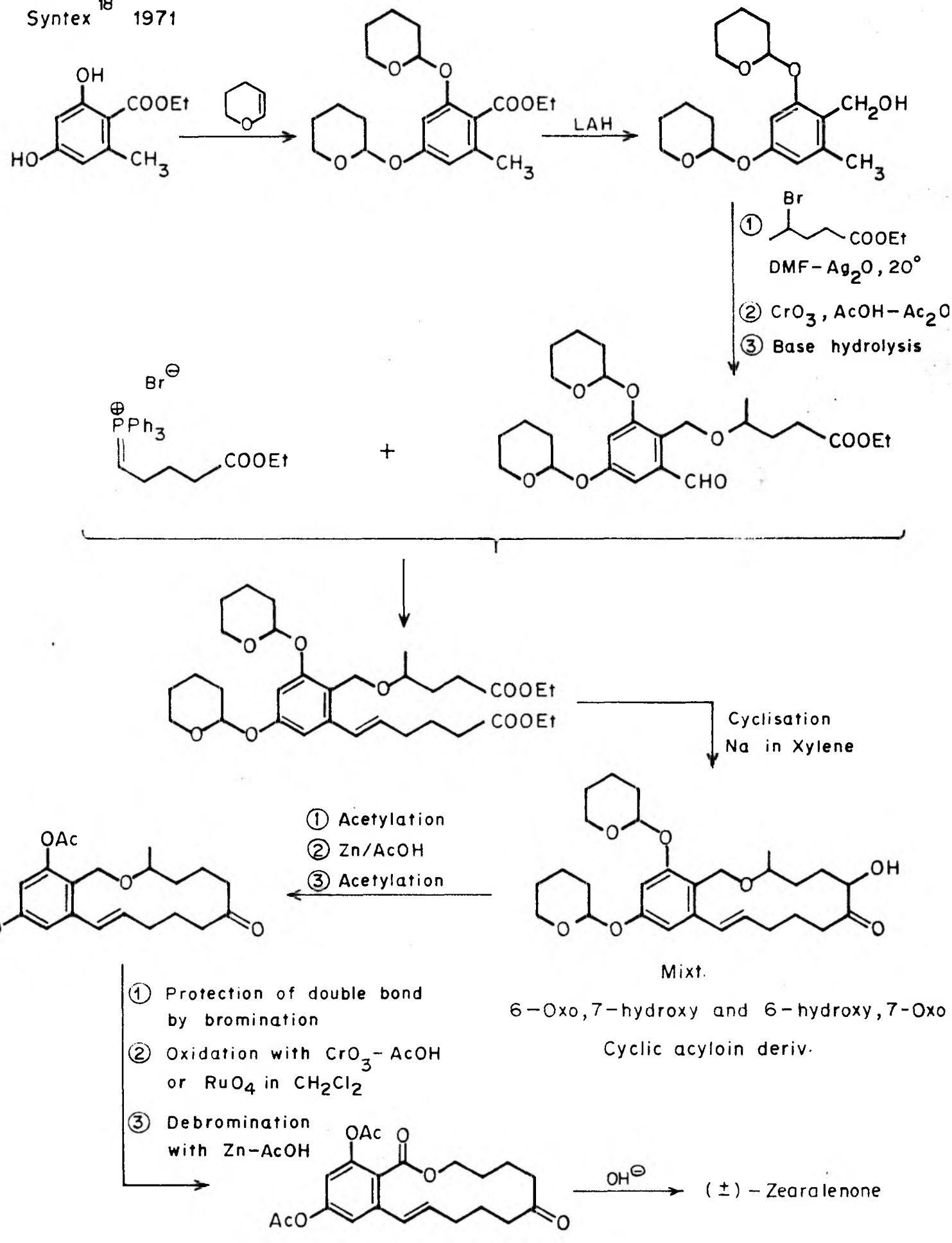
Syntex[17] 1970



(±)-ZEARALENONE

CHART-5

Syntex[18] 1971



for its synthesis. They have constructed aromatic and aliphatic portions separately and linked them by a Wittig reaction.

The Merck synthesis involved the use of trifluoroacetic-anhydride for the cyclisation of hydroxy acid and the same method had also been applied by Peters and Hurd,[9] for the construction of (R)-zearalenone (II). The yields obtained by this procedure are rather low and used strong acid conditions. The Syntex group effected the cyclisation[16] in low yield with sodium-t-amyloxide at elevated temperature. Further, the reaction conditions used by them for the cyclisation of α,ω-diesters namely sodium in xylene[18] do not appear promising as there is substantial loss in the yield of cyclisation product.[10]

PRESENT WORK

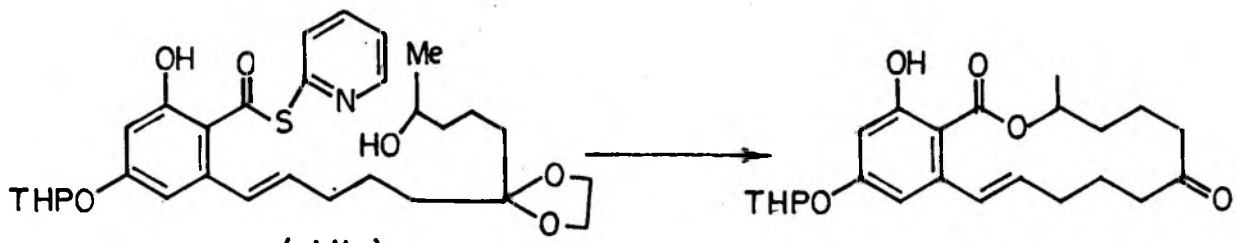
Zearalenone (I) continues to show the presence of a wide variety of pharmacological properties and is covered by numerous patents.[11-13,17,18] Further the synthesis of macrolides is gaining considerable interest and many well-known schools are engaged in their syntheses. It has been felt that it may be a good exercise to undertake the synthesis of zearalenone before venturing the synthesis of complex macrolides.

The Merck and Syntex[14-18] syntheses suffer from various disadvantages including poor yields at different stages. Both of them have adopted separate synthesis of aromatic and aliphatic portions and joined them by a Wittig reaction. The ultimate lactonisation (in low yield) has been carried out by using either trifluoroacetic anhydride[14,15] or sodium-*t*-amyloxide.[16]

For the last few years Corey's group at Harvard has been engaged in the synthesis of complex macrolides such as erythromycin.[19] In this connection they have worked out a new methodology to lactonise a long chain hydroxy acid. Corey and Nicolaou[21] have shown that 2-pyridinethiolester of a hydroxy acid when added in high dilution to a refluxing toluene or xylene can be subjected to intramolecular lactonisation as shown in scheme 1.

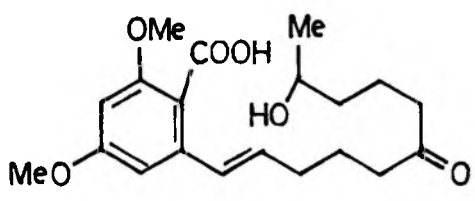
The use of 2-pyridinethiol ester for lactonisation has been successfully adopted in the synthesis of zearalenone from protected hydroxy acid derivative (VI), obtained from natural product by degradation.

Scheme – 1

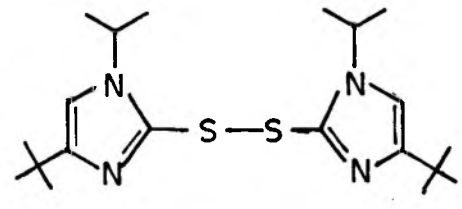


(VI)

The 2-pyridinethiolester (VI) was first prepared in benzene at 25° and subjected to cyclisation adopting high dilution technique in refluxing benzene and obtained zearalenone after removal of the protecting group in 75% yield. Subsequently Corey and Brunell[20] have introduced another reagent 2,2'-di-(4'-t-butyl-N-isopropyl)-imidazolyldisulfide (VIII) which is claimed to be superior to 2,2'-dipyridyldisulfide in lactonising long chain hydroxy acids at low temperature and in high yield. From this it is clear that lactonisation of hydroxy acid derivative to zearalenone can be achieved in excellent yield. So the main object lies in synthesising the hydroxy acid derivative (VII).



(VII)



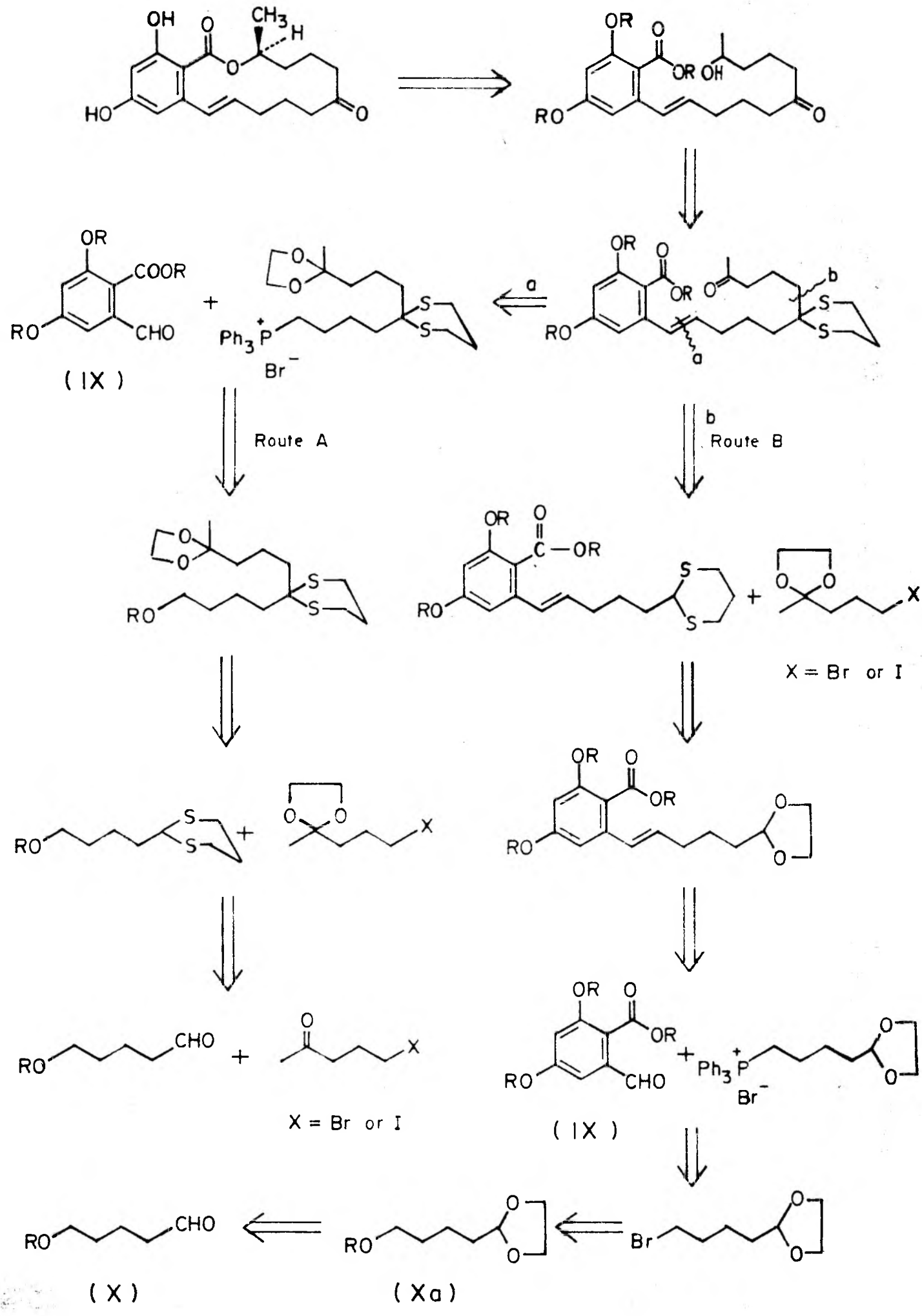
(VIII)

The best possible way of elaborating the synthesis of (±)-zearalenone is illustrated by the antithetic relationship indicated in scheme 2. Extensive antithetic analysis, as outlined in the scheme 2, revealed that the synthesis can be elaborated either by route A or route B. By this plan zearalenone can be achieved economically starting from easily accessible intermediates.

The method reported by Vlattas *et al.*[16] for the synthesis of aromatic part (IX) is relatively simple and can be duplicated. However, both Merck and Syntex[14-18] have synthesised the aliphatic part by a tedious route involving a large number of operations. In the present work efforts have been made to synthesise this aliphatic part in a simple straight forward way, confining the attention only to route A. The main synthetic strategy comprises the choice of protecting groups for the hydroxyl and two carbonyls, and should be such that each may be independently removed without affecting the other. The aliphatic part contains two ketonic functions at 5' and 9' positions, if protected by the same functional group, preferential removal of one over the other for the generation of required function is difficult. Hence one of these should be protected as ethyleneketal and another as thioketal which can be used for C-C bond formation. To achieve this goal, lower segment can be looked upon as hydroxyaldehyde, in which aldehydic function is masked by a thioketal group, and the

SCHEME -2

RETROSYNTHESIS OF ZEARALENONE

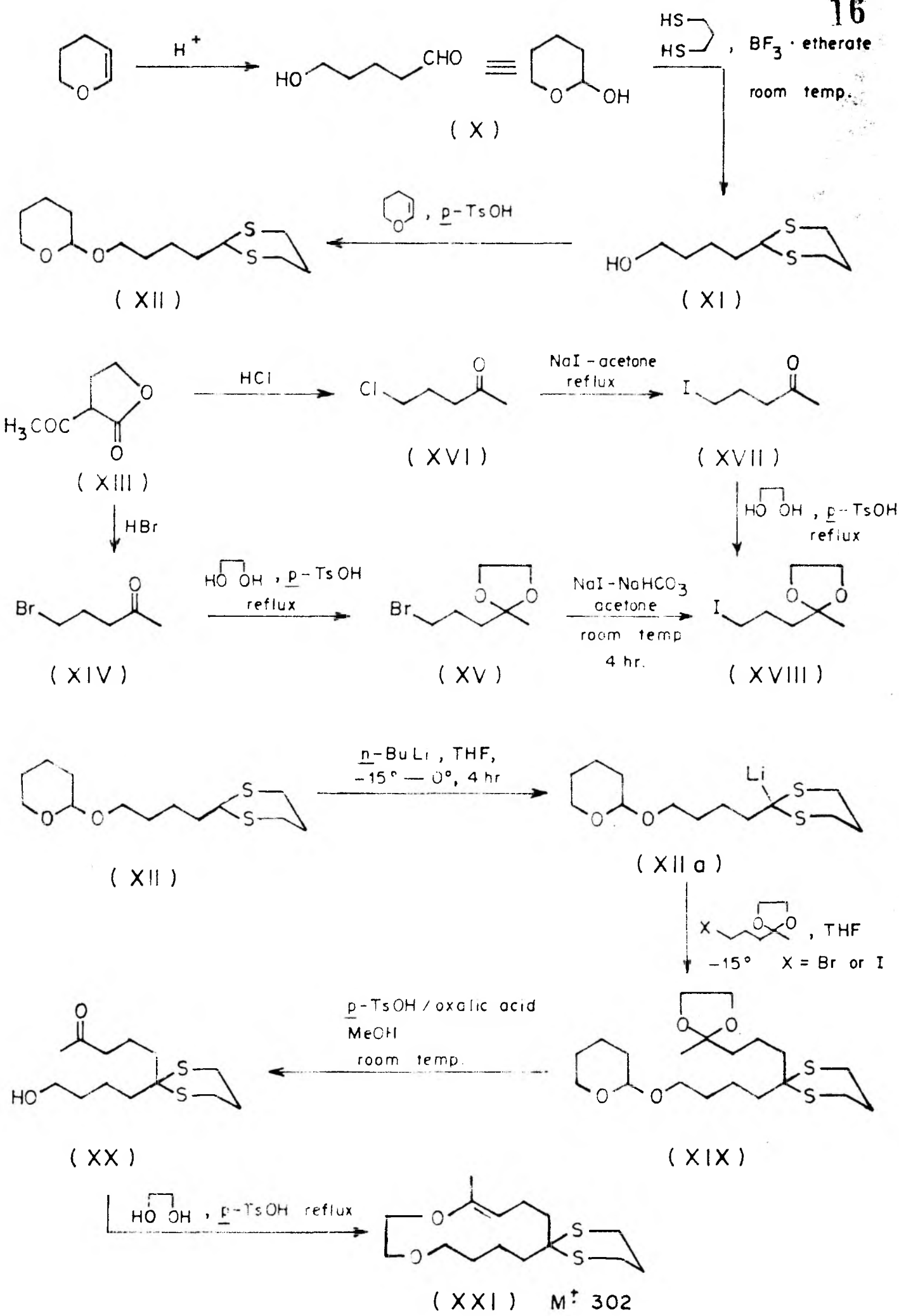


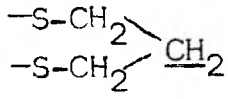
upper segment as bromoketone is protected by ethylene-ketal group.

The utility of 2-lithio-1,3-dithiane or 2-lithio-2-substituted-1,3-dithiane as the valuable intermediates for C-C bond formation is well known in synthetic methodology.[22,23] These sulfur stabilised anion reagents[24] are equivalent to acylion, which can be used effectively to reverse the electrophilicity of carbonyl carbon. Therefore C-C bond formation by dithiane route is definitely a method of preference, since dithianes are prepared in good yield, undergo reactions with electrophilic reagents (alkyl halides, carbonyl compounds, acids, etc.) and can be converted to ketone function under mild conditions.[24]

The major points of interest in the synthesis (schemes 3 and 4) are (1) construction of 2-substituted dithiane and protection of alcohol function, (2) C-C bond formation through metallation and alkylation of dithiane (3) the generation of functionality at desired position, i.e. preferential removal of protected hydroxyl group over the protected ketone group and difficulties encountered in this operation, and (4) the possible utilisation of specific ketone protecting group which survives deprotection in preference over the protected hydroxyl group.

SCHEME – 3 .


H+
HO
CHO
OH
HS
HS
, BF3 · etherate
room temp.
(X)
, p-TsOH
S
S
HO
(XII)
(XI)
HCl
Cl
NaI – acetone
reflux
I
H3COC
(XIII)
(XVI)
(XVII)
HBr
HO OH , p-TsOH
reflux
Br
HO OH , p-TsOH
reflux
NaI-NaHCO3
acetone
room temp
4 hr.
(XIV)
(XV)
(XVIII)
n-BuLi , THF,
–15° — 0°, 4 hr
Li
(XII)
(XII a)
X , THF
–15° X = Br or I
p-TsOH / oxalic acid
MeOH
room temp.
(XX)
(XIX)
HO OH , p-TsOH reflux
(XXI) M+ 302

2,3-Dihydropyran is an excellent starting material for the synthesis of an aldehyde containing five carbon atoms. Hydration[25] of dihydropyran with aqueous hydrochloric acid gave 5-hydroxypentanal (X). The IR spectrum of compound (X) shows very weak absorption at 1730, and intense band at 3300 cm^{-1}(-OH) indicating that the product exists[26] predominantly as a hemiacetal (2-hydroxypyran) and not as open chain (5-hydroxypentanal) form. Further it forms 2,4-DNP after a prolonged period, which shows that equilibrium shifts slowly to the open chain form. Attempted ethyleneacetal (Xa) formation of compound (X) (for the purpose of Wittig reaction with aromatic aldehyde (IX) as shown in scheme 2, route B) with ethyleneglycol in presence of acid catalyst, under reflux using a Dean-stark trap resulted in the formation of a polymeric black tarry material. However, on treatment with 1,3-propanedithiol (a strong nucleophile) in presence of boron-trifluoride-etherate, it converts into 2-(4 -hydroxybutyl)-1,3-dithiane (XI) in 86% yield. [IR(liquid film) 918 (characteristic dithiane band), 3200 cm^{-1} (alcoholic -OH). NMR(Fig.1) in CCl_4 shows expected signals: 4.00 (t, 1H, ), 3.57 (t, 2H, $-\underline{CH_2}OH$), 2.93-2.70 (m, 4H, $-S-\underline{CH_2}-$), 2.64 (s, 1H, -OH, exchanges with D_2O), 2.18-1.33 (m, 8H,  and 3 CH_2)].

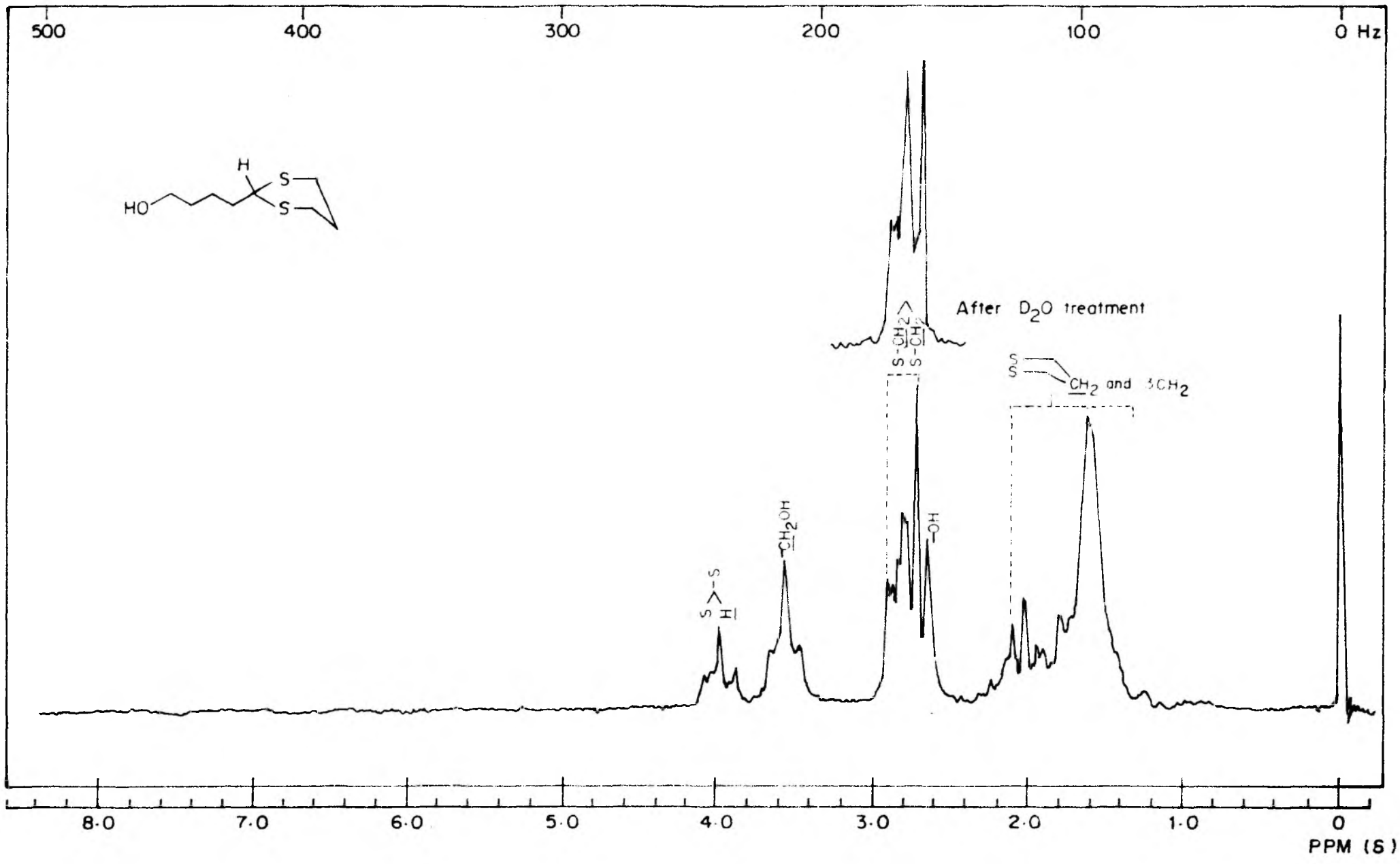


FIG. 1 . NMR SPECTRUM OF COMPOUND (XI) IN CCl_4

Protection of alcoholic function of the dithiane (XI) as its tetrahydropyranylether (THP) was effected by the reaction of the dithiane (XI) with 2,3-dihydropyran in presence of p-toluenesulfonic acid to give 2-(4-tetrahydropyranyloxybutyl)-1,3-dithiane (XII) in 74% yield. [IR(liq.film) shows absence of -OH group, 915 cm^{-1} (dithiane band); NMR (Fig.2) of compound(XII) in CCl_4 shows a single proton singlet at 4.54 (s,1H,THP-proton), 4.03-3.17 (m, 5H. 2-O$\underline{CH_2}$, $\underline{H}C(S-)_2$), 2.93-2.68 (m, 4H, $-S-\underline{CH_2}-S-\underline{CH_2}-$) 2.18-1.27 (m, 14H, 7 CH_2)]. The THP ether (XII) was further subjected to metallation and subsequent alkylation to generate carbon skeleton with the correctly positioned oxygen functions of the aliphatic portion of zearalenone.

2-Acetyl-Y-butyrolactone (XIII) on heating with 48% hydrobromic acid at reflux temperature gave 5-bromopentan—2-one[27] (XIV). Ketalisation[27] of carbonyl function of the compound (XIV), using ethyleneglycol and p-toluenesulfonic acid furnished 5-bromopentan—2-one ethyleneketal (XV) in 60% yield, which was used for the alkylation of 2-lithio-1,3-dithiane(XIIa).

Metallation of the THP ether (XII) with n-butyllithium at -15-0° followed by alkylation with 5-bromopentan—2-one-ethyleneketal (XV) at -15° provided 2-(4-tetrahydropyranyloxy-butyl)-2-(pentyl-4-one ethyleneketal)-1,3-dithiane (XIX) in 40% yield, after a prolonged period. The reaction might proceed

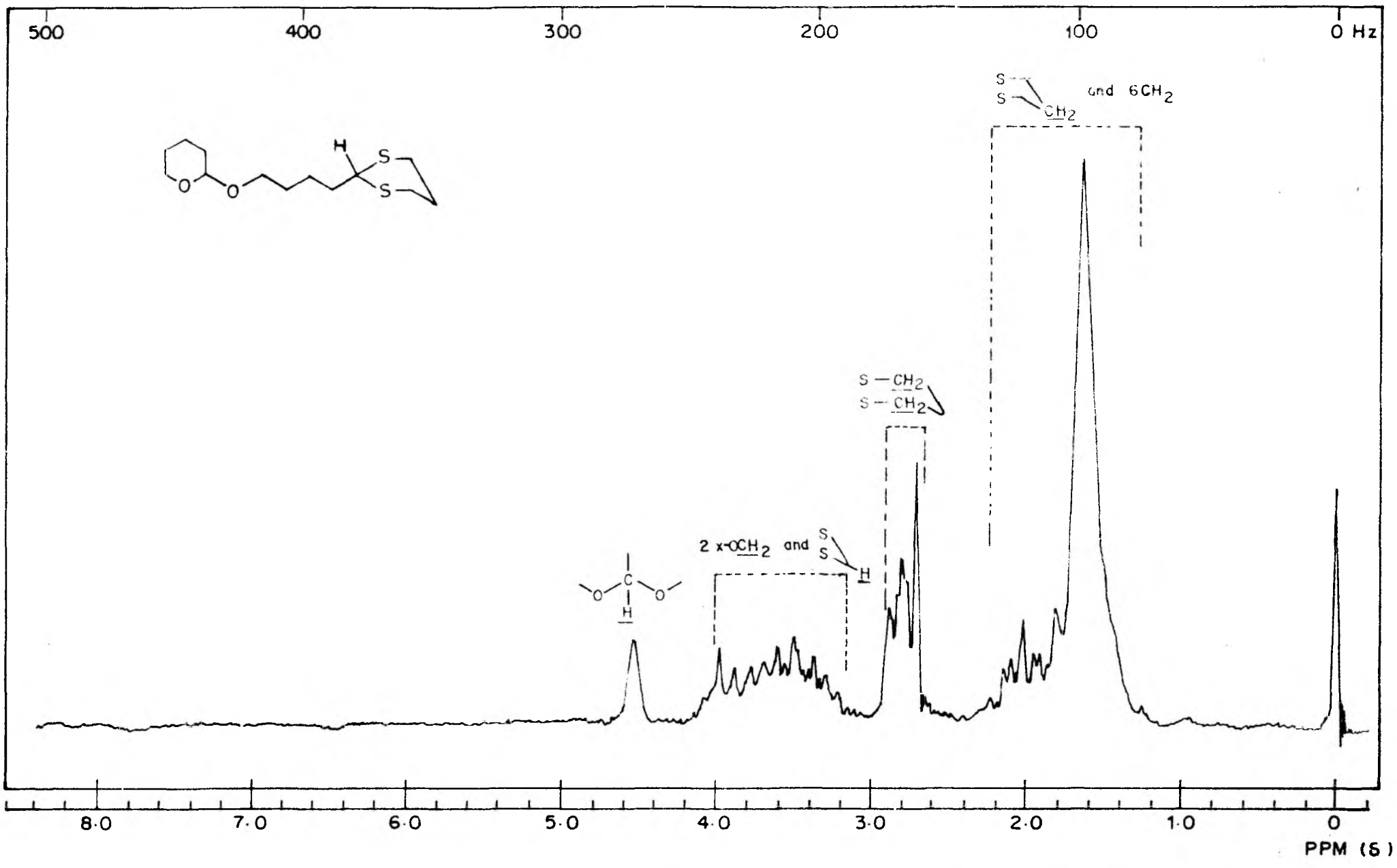


FIG. 2. NMR SPECTRUM OF COMPOUND (XII) IN CCl_4

better if iodide is used instead of alkyl bromide (XV). Accordingly 5-iodopentan—2-one ethyleneketal (XVIII) was prepared from 5-chloropentan—2-one[28] (XVI) (obtained from 2-acetyl-γ-butyrolactone and aqueous hydrochloric acid) as depicted in scheme 3 by a known procedure.[29] In this sequence of reactions the chloro compound (XVI) underwent exchange[29] at elevated temperature, which on subsequent ketalisation[29] with ethyleneglycol under standard conditions gave low yield of the compound (XVIII) due to decomposition. The sluggish reactivity of the chloro compound (XVI) at elevated temperature, gave poor yields of iodoethyleneketal (XVIII) and was obtained from 5-bromopentan—2-one-ethyleneketal (XV) by exchange with sodiumiodide in acetone, under neutral conditions (using sodium bicarbonate), which demonstrates that the bromoethyleneketal undergoes exchange reaction at room temperature and decomposition of the iodo compound (XVIII) is minimised, resulting in high yield of compound (XVIII).

Metallation of the THP ether (XII) with n-butyllithium (prepared as per the method described) and alkylation with freshly prepared 5-iodopentan—2-one ethyleneketal for 3.5 hr., under similar conditions as described above provided 2-(4-tetrahydropyranyloxybutyl)-2-(pentyl-4-oneethyleneketal)-1,3-dithiane (XIX) in 67% yield.

(XIX)

[The NMR of compound in CCl_4 (Fig.3): 4.54(s,1H,THP proton), 3.88(s, 4H, $\begin{matrix}-O-CH_2\\ \\ -O-CH_2\end{matrix}$), 3.80-3.17 (m, 4H, 2-OCH_2), 2.90-2.62 (m, 4H, $\begin{matrix}-S-CH_2-\\ \\ -S-CH_2-\end{matrix}$), 2.09-1.31 (bm, 20 H, 10 CH_2), 1.26(s, 3H, CH_3). Its mass spectrum shows molecular ion as base peak at m/e 404 and other prominent peaks at m/e 389 ($M-CH_3$), 319 (M-THP), 275 ($M-C_7H_{13}O_2$) and 247 ($M-C_9H_{17}O_2$)].

Treatment of the compound (XIX) with methanol containing p-toluenesulfonic acid at 0^o and room temperature to accomplish selective cleavage of THPether, resulted in the formation of a slower moving compound in quantitative yield, which was identified as 2-(4-hydroxybutyl)-2-(pentyl-4-one)-1,3-dithiane (XX) from its spectral properties [IR (liquid film) 920 cm^{-1} (characteristic dithiane band), 1710 (C=O) and 3280 cm^{-1} (-OH): NMR in CCl_4 (Fig.4): 3.60 (bt, 2H, $-CH_2OH$), 2.90-2.63 (m, 4H, $\begin{matrix}-S-CH_2-\\ \\ -S-CH_2-\end{matrix}$), 2.62-2.32 (bt, 3H, $-CH_2C=O$ and -OH: one of the signals exchanges with D_2O), 2.15 (s, 3H, $-COCH_3$), 2.00-1.30 (m, 12H, 6 CH_2). The mass spectrum shows molecular ion at m/e 276 and other peaks at m/e 203 ($M-C_4H_9O$), 191 ($M-C_5H_9O$)]. In order to avoid dioxalane cleavage in the compound (XIX) its hydrolysis with mild acid like oxalic acid was considered. Thus the compound (XIX) when treated with methanol containing catalytic amount of oxalic acid at 0^o and room temperature, resulted in the formation of (XX) in quantitative yield.

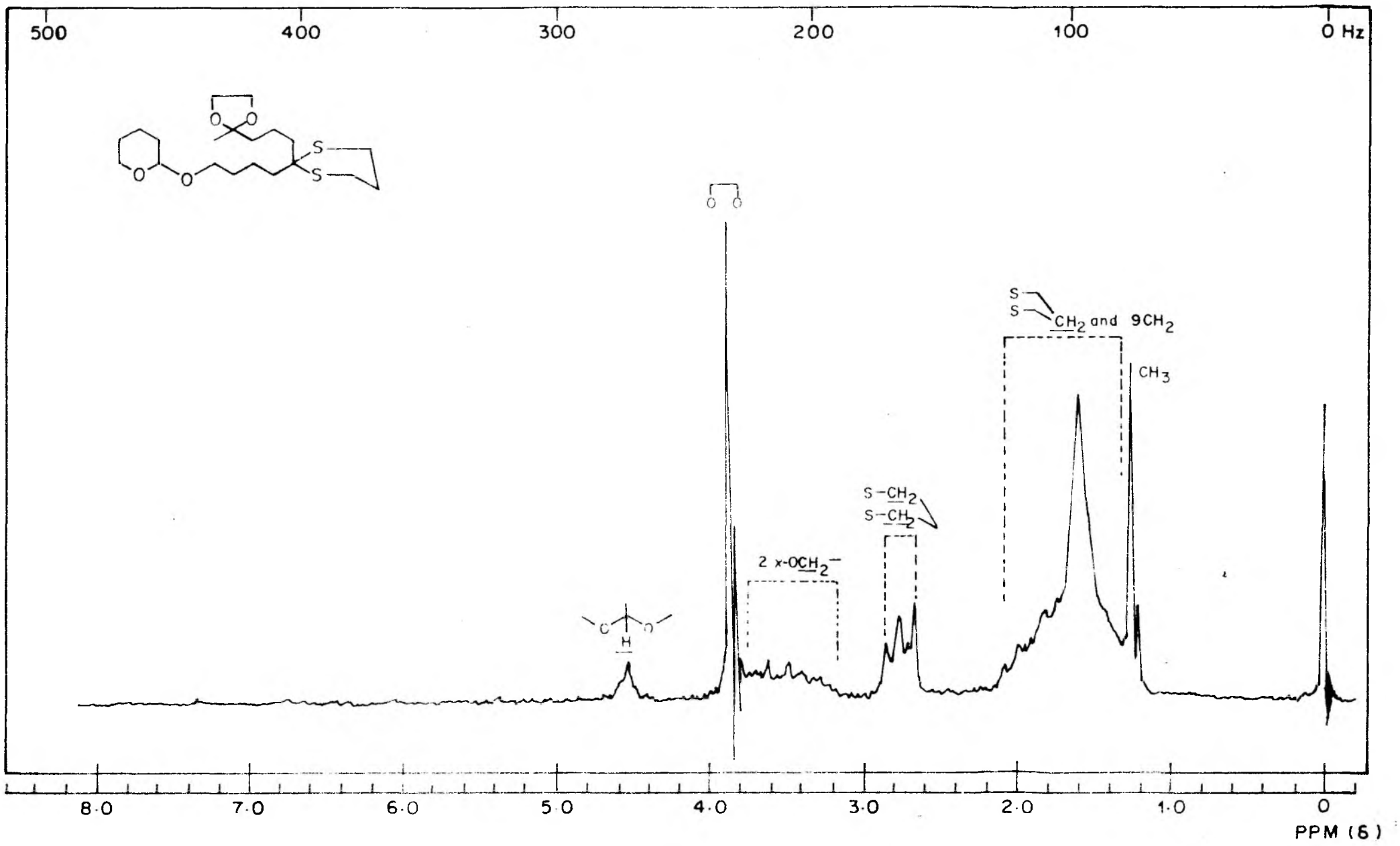


FIG. 3. NMR SPECTRUM OF COMPOUND (XIX) IN CCl_4

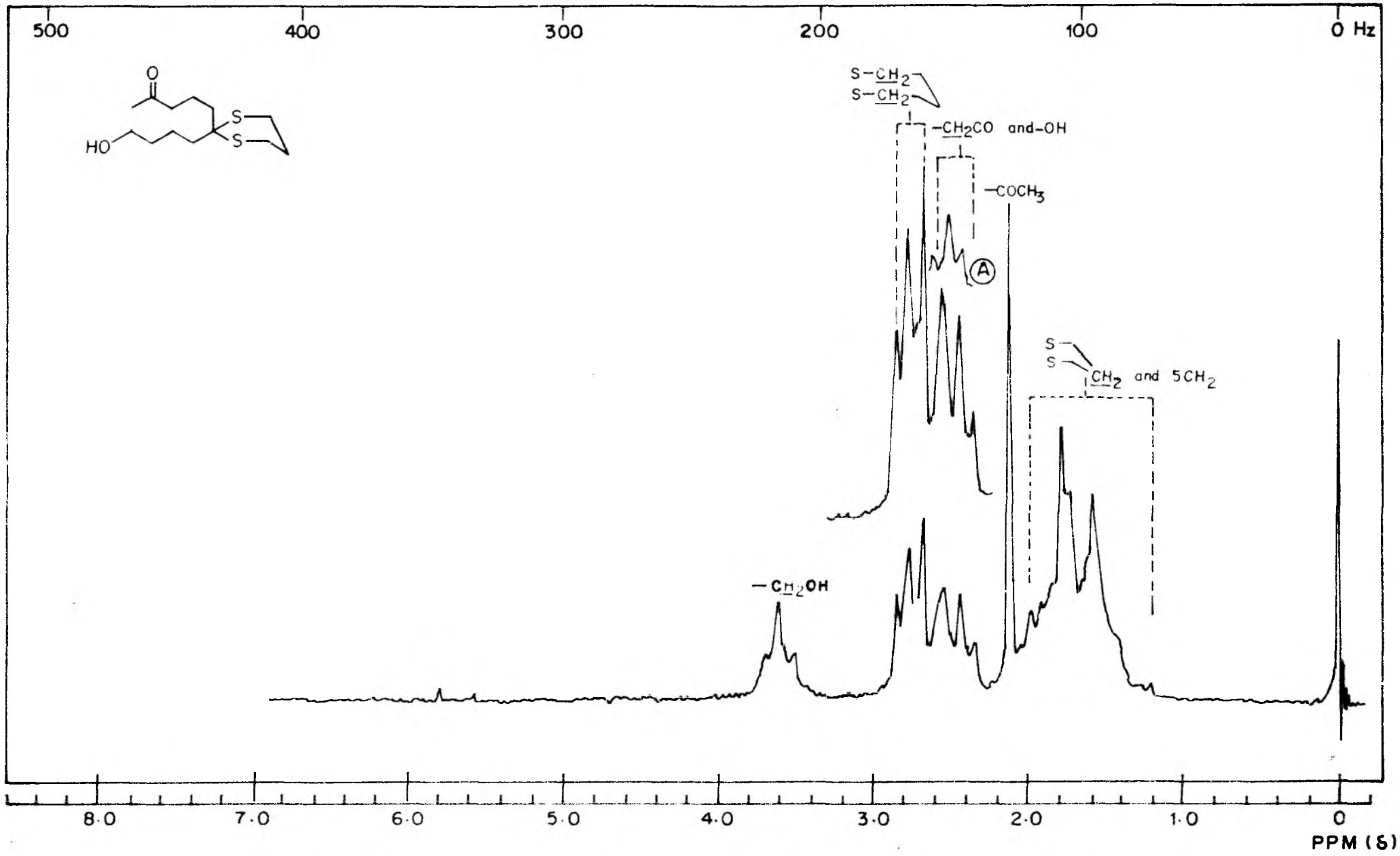


FIG. 4. NMR SPECTRUM OF COMPOUND (XX) IN CCl_4

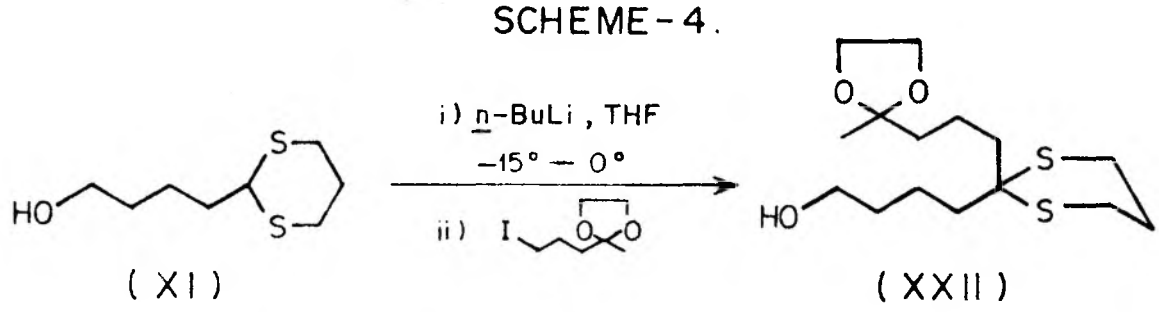
Ⓐ After D_2O treatment

It is known that THP ether can be removed under neutral conditions[30] without affecting the ketal group using ozone at low temperatures. However, it cannot be applied for the compound (XIX), as the sulfur atoms in the dithiane ring may be converted into sulfoxides (as ozone is an oxidising agent), thereby creating additional problems.

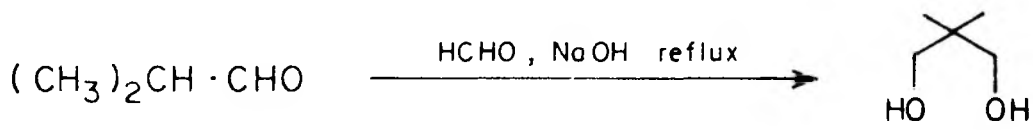
These observations indicate that preferential cleavage of THP ether over ethyleneketal is difficult, hence it was considered to introduce ketal group once again in the hydroxyketo compound (XX), under standard conditions. Thus the compound (XX) when subjected to ketalisation using ethylene glycol and p-toluenesulfonic acid for 76 hr. in the usual way, it was found that it resisted ketalisation with the formation of a faster moving product in minor quantity. Chromatography of the mixture gave unreacted hydroxyketo compound and a product in low yield, which is likely to be (XXI) (M^+ 302).

Although initial efforts to get the compound (XXII) from the THP ether (XII) met with several problems, attempts were made to metallate the alcohol (XI), thus circumventing the need for protection. Thus metallation of the alcohol (XI) with two moles of n-butyllithium at -15° for 4 hr. and its subsequent alkylation with freshly prepared 5-iodopentan—2-one ethyleneketal (XVIII) gave a mixture of compounds. The

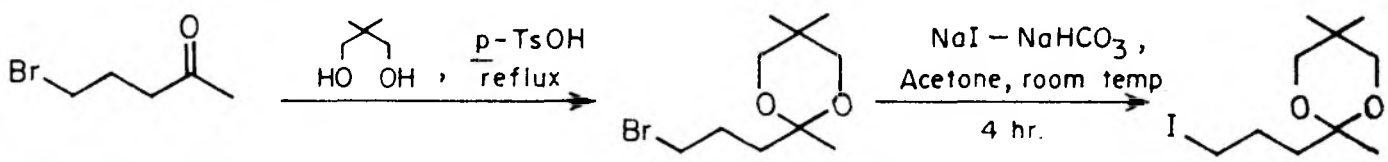
SCHEME-4.



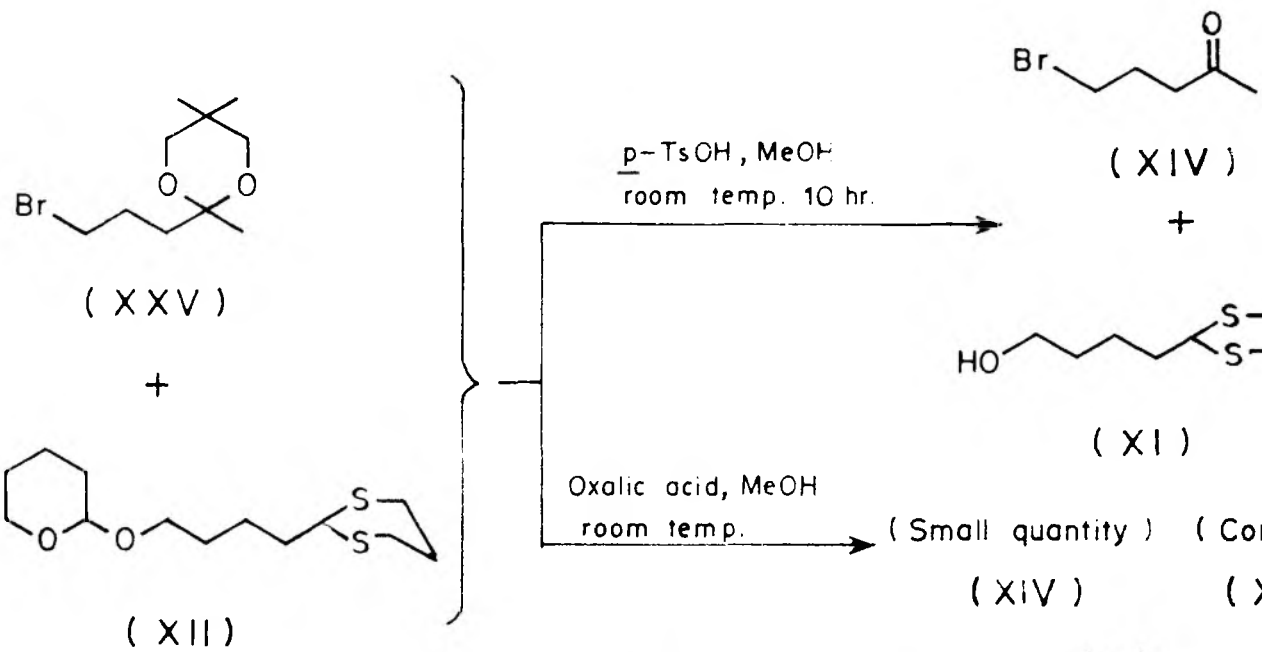
(XI) (XXII)



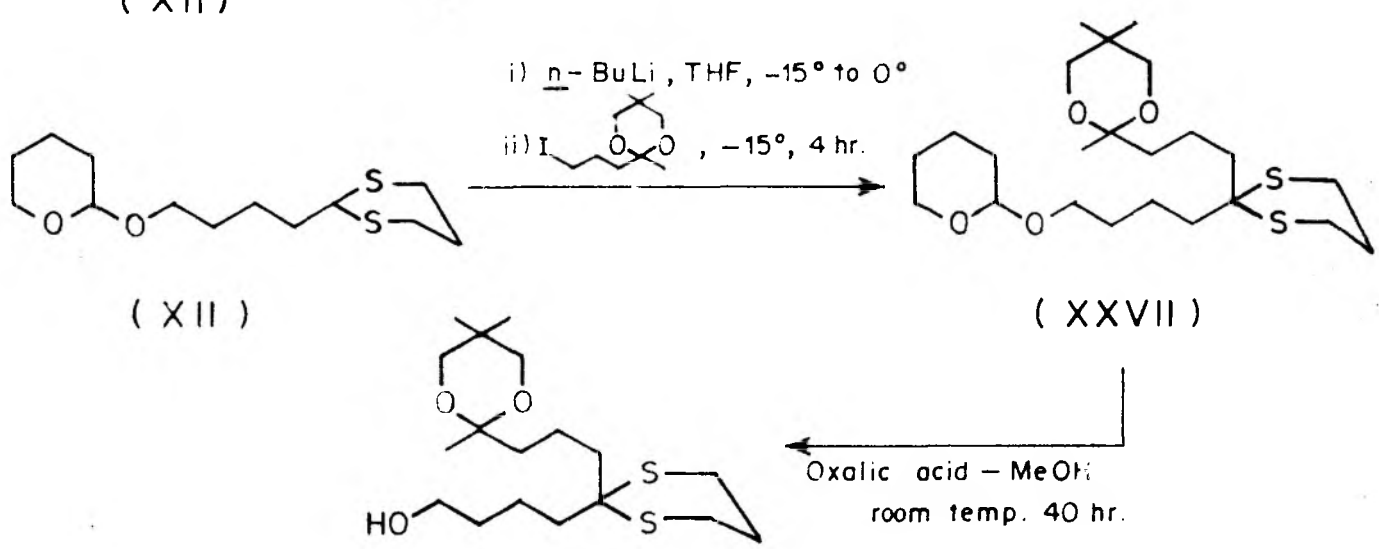
$(CH_3)_2CH \cdot CHO$ (XXIII) (XXIV)



(XIV) (XXV) (XXVI)



(XXV) + (XII) (XIV) + (XI)



(XII) (XXVII) (XXVIII)

column chromatography of the mixture furnished faster moving compound, which was anticipated to be o-alkylether and a slower moving 2-(4-hydroxybutyl)-2-(pentyl-4-oneethyleneketal)-1,3-dithiane (XXII) in poor yield.

The ketone function in compound (XXII) is protected and it bears a free hydroxyl group which can be made use of for further sequence of reactions. [IR (liquid film) 3300 (-OH), 925 cm^{-1} (dithiane band); NMR of compound (XXII) in CCl_4(Fig.5): 3.90 (s, 4H, $-O-\underset{|}{\overset{-O-CH_2}{CH_2}}$), 3.62 (t, 2H, $-CH_2OH$), 2.92-2.63 (m, 4H, $-S-CH_2-$), 2.15-1.36 (bm, 14H; 7 CH_2), 1.30 (s, 3H. CH_3). The $-S-CH_2-$

mass spectrum shows molecular ion at m/e 320 and other prominent peaks at m/e 276 (M-44), remaining fragmentation pattern is similar to that of 2-(4-hydroxybutyl)-2-(pentyl-4-one)-1,3-dithiane (XX)].

From the preceding discussion, it is clear that eventhough C-C bond formation is straightforward, specific cleavage of THP ether in compound (XIX) has been proved to be difficult. Moreover the ketohydroxy compound (XX) resists ketal formation and provided an unexpected product (XXI) in minor quantity. Direct metallation of the alcohol (XI) gave the desired product (XXII) in poor yield, alongwith other side products. Therefore, the protection of ketone in 5-iodopentan—2-one with a group (other than ethyleneketal or ethylenedithioketal)

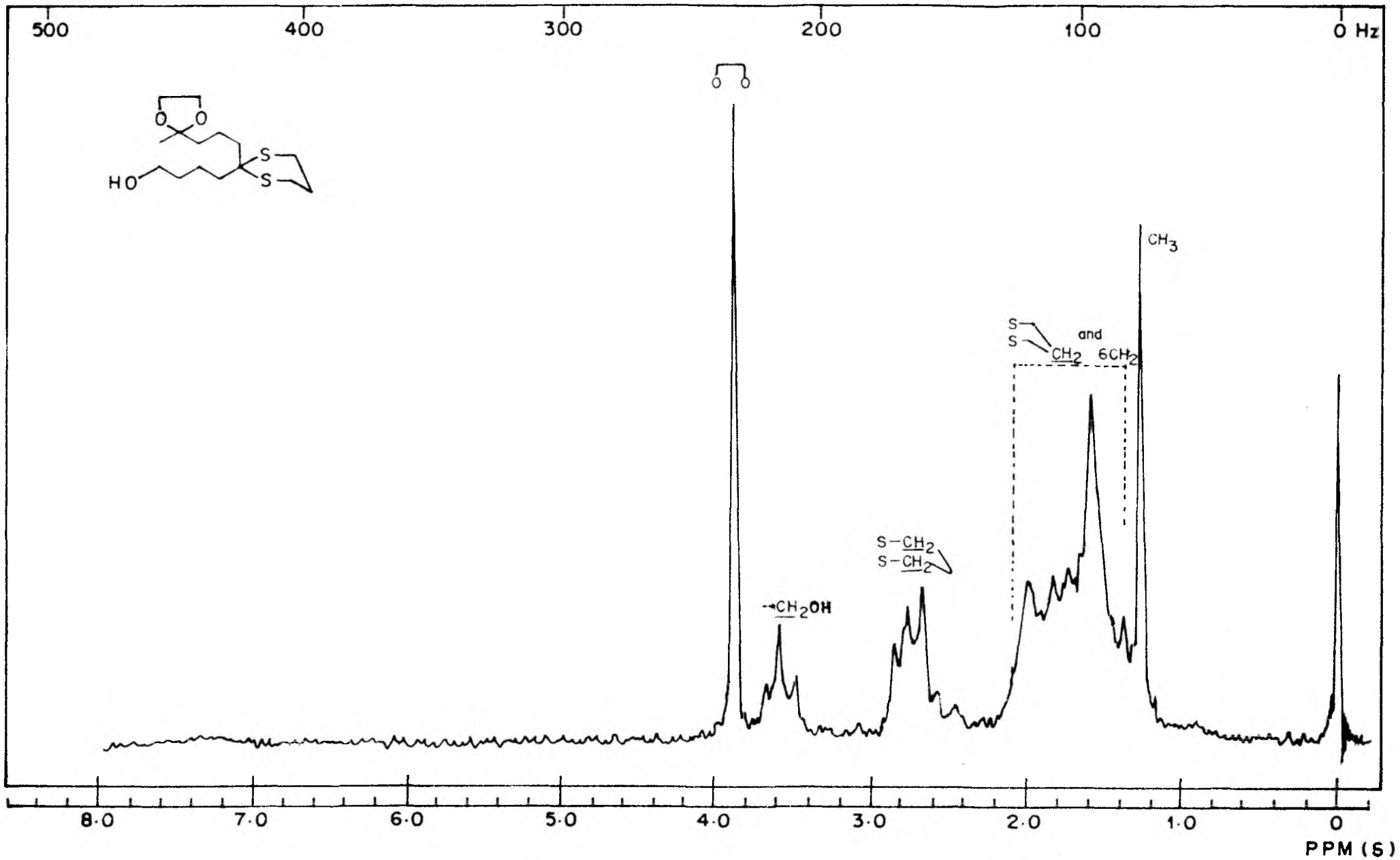


FIG. 5. NMR SPECTRUM OF COMPOUND (XXII) IN CCl_4

which can survive deprotection in preference over THP ether has been considered to be the desired approach.

A perusal of literature revealed several useful methods for the protection/deprotection of ketones including those involving hydrazones, N,N-dimethylhydrazones[31] (removed by periodates or methiodides) and 5-methylene-1,3-dioxane[32] (removed by $F^{\ominus}$). The first two methods involve oxidative hydrolysis using periodates and adoption of this method may lead to the formation of sulfoxides, while generating the ketone. Protection of ketone with 5-methylene-1,3-dioxane provided additional stabilisation due to the presence of double bond and hence requires fluoride ion for its hydrolysis to ketone. However, the preparation[32] of corresponding diol (2-methylene-propane-1,3-diol) from 5-norbornene-2-carboxaldehyde uses Cannizaro reaction followed by retro Diels-Alder reaction at 520°. Smith and Newman[33] first explored the possibility of the uses of 1,3-propanediol and 2,2-dialkyl-1,3-propanediol for the protection of carbonyl functions in steroids. They have shown that introduction of 2,2-dialkylsubstituents into 1,3-propanediol increases the stability and decreases the acid hydrolysis rates of the corresponding 1,3-dioxane. Therefore, the ketals derived from 2,2-dimethyl and 2,2-diethyl-1,3-propanediol are almost stable and possess hydrolytic stabilities superior to those of ethylene ketals. Further they have reported largest

ring stability for the ketals derived from 2,2-diisopropyl-1,3-propanediol. They attributed the hydrolytic stability to gem-dialkyl substituents.[33] The latter results are of interest and offer distinct advantage over ethyleneketals. Therefore, a brief investigation of 5,5-dialkyl-1,3-dioxane derived from 5-bromopentan-2-one (XIV) as an alternative method for protection of carbonyl function was undertaken as a model experiment in order to determine its stability compared to THP ether.

5-Bromopentan-2-one was converted to 5,5-dimethyl-1,3-dioxane derivative. Ketalisation of (XIV) with 2,2-dimethyl-1,3-propanediol (neopentyl glycol) (XXIV) [obtained from isobutyraldehyde[34] (XXIII) and formaldehyde under basic conditions] was achieved in the standard way by refluxing the ketone in benzene containing a catalytic amount of p-toluene-sulphonic acid for 4 hr. with azeotropic removal of water [The NMR of 2,5,5-trimethyl-2-(3-bromopropyl)-1,3-dioxane(XXV) in CCl_4 (Fig.6): 3.60-3.27 (bm, 4H, $\begin{matrix}-O-CH_2-\\-O-CH_2-\end{matrix}$), 3.16 (t, 2H, $-CH_2Br$), 2.10-1.53 (bm, 4H, 2 CH_2), 1.33, 1.06, 0.86 (3 s, 9H, 3 CH_3).

To ascertain the stability of 1,3-dioxane group over THP ether, a mixture of bromodioxane (XXV) and 2-(4-tetrahydro-pyranyloxybutyl)-1,3-dithiane (XII) in aqueous methanol was treated with p-toluenesulphonic acid at room temperature for 10 hr. which resulted in the cleavage of dioxane group as well as THP ether giving the respective ketone (XIV) and alcohol(XI).

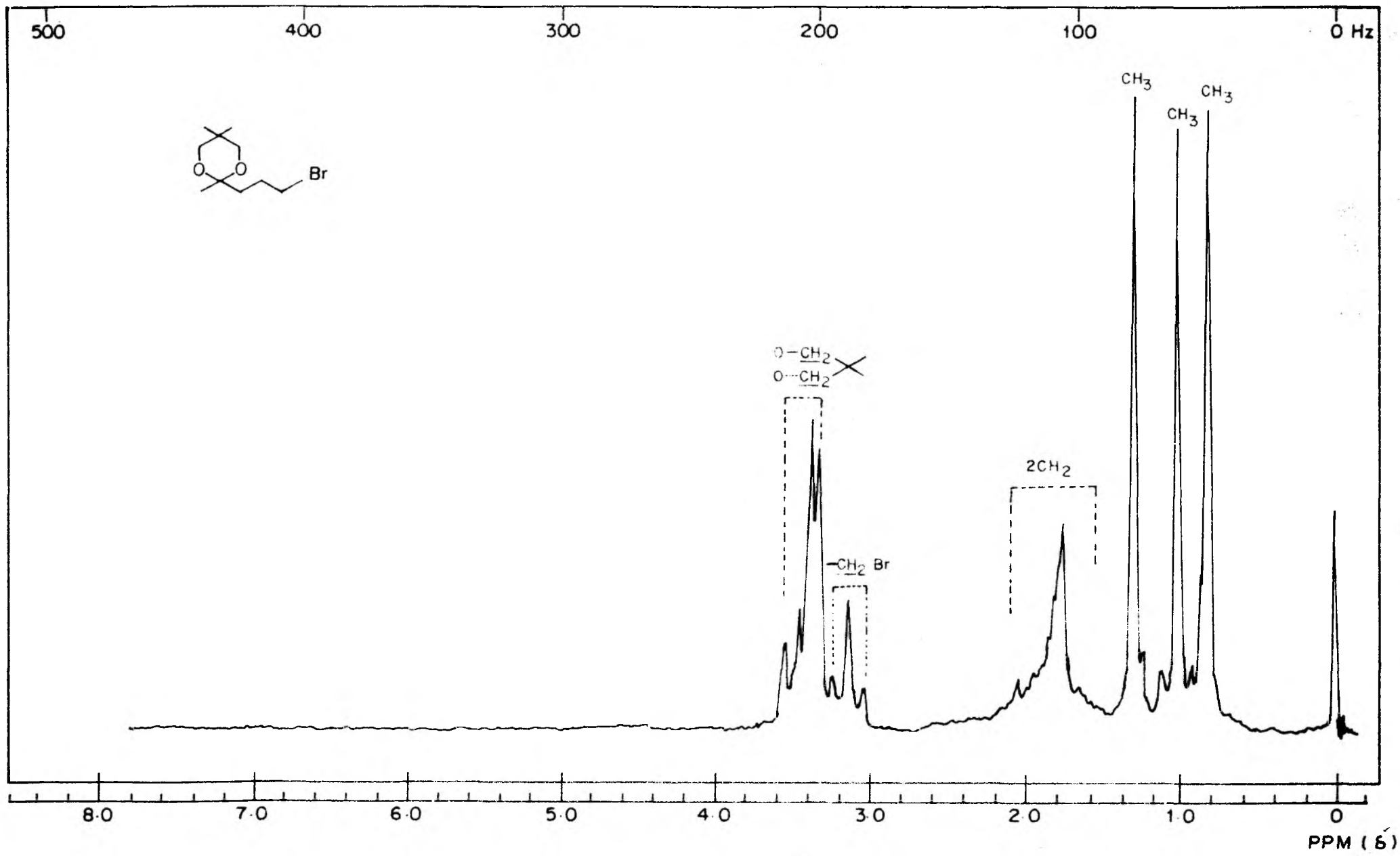


FIG. 6. NMR SPECTRUM OF COMPOUND (XXV) IN CCl_4

Hence the mixture in methanol was treated with oxalic acid at room temperature, proceeded in complete cleavage of THP ether during 7 hr., by contrast the corresponding keto compound(XIV) was formed only in small quantity after 16 hr. This indicates that preferential cleavage of THP ether in aqueous methanol catalysed by oxalic acid is superior to p-toluenesulphonic acid and can be achieved in a reasonable period of time. These results solved the previous problems and can be successfully applied to get the desired compound (XXVIII).

Metallation of 2-(4-tetrahydropyranyloxybutyl)-1,3-dithiane (XII) followed by alkylation with freshly prepared 2,5,5-trimethyl-2-(3-iodopropyl)-1,3-dioxane(XXVI) [prepared by the reaction of bromo compound(XXV) with sodium iodide in presence of sodium bicarbonate] under similar conditions, as described before, furnished 2-(4-tetrahydropyranyloxybutyl)-2-[4-(2,2-dimethyltrimethylenedioxy)-pentyl]-1,3-dithiane(XXVII) in 76% yield. [NMR (Fig.7) of compound(XXVII) in CCl_4 shows a single proton singlet at 4.42 assigned to tetrahydropyranyl proton, a broad multiplet in the region 4.0-2.33 integrating for 14 protons accounts for seven methylenes. The region 2.20-1.36 integrates for 24 protons comprising nine methylenesand two methyl groups and a singlet at 1.26 accounts for a methyl group]. All this data support

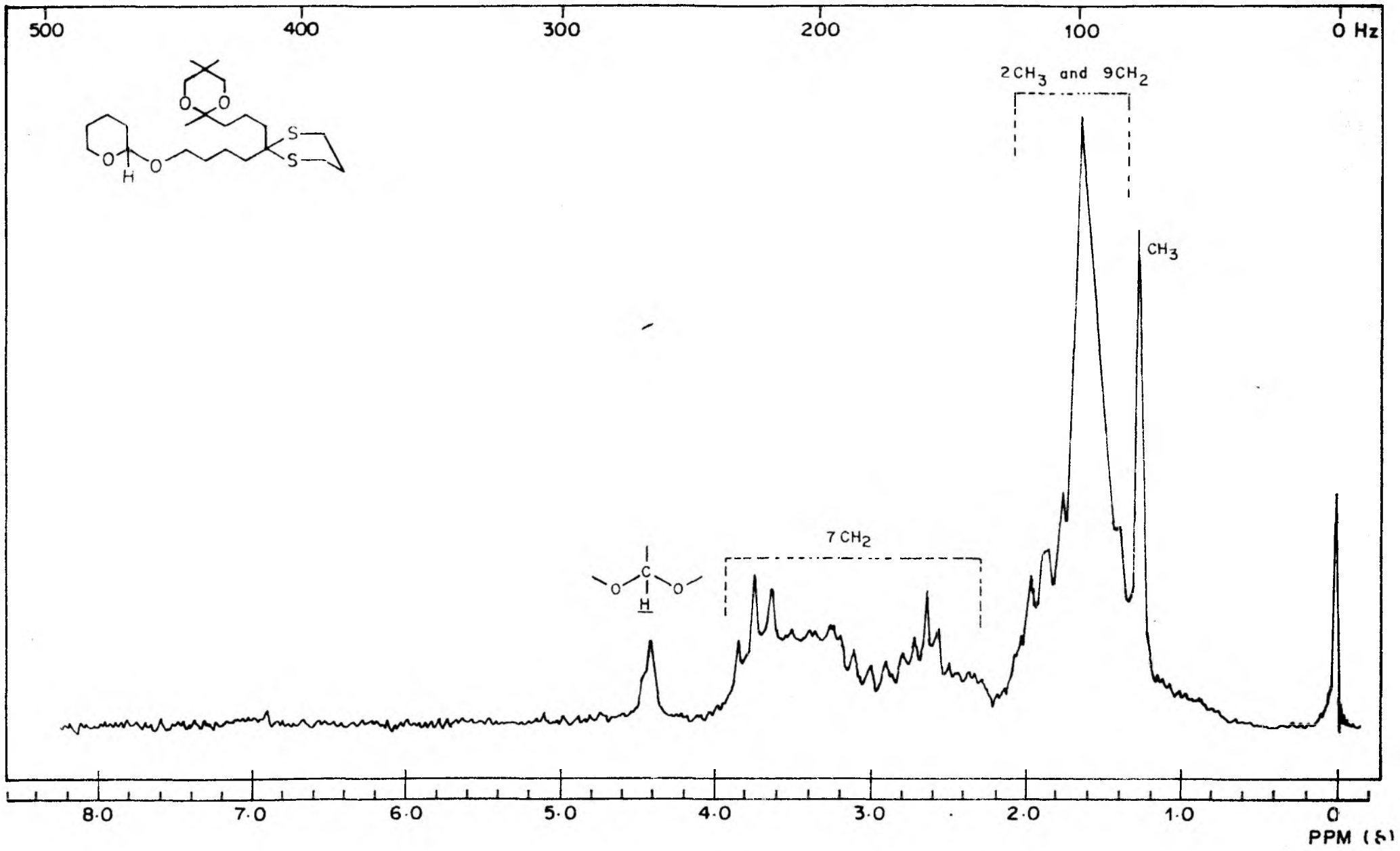


FIG. 7. NMR SPECTRUM OF COMPOUND (XXVII) IN CCl_4

structure (XXVII) for the compound. Analysis of the compound (XXVII) suggests its molecular formula to be $C_{23}H_{42}O_4S_2$ and molecular weight 446. In the mass spectrum molecular ion at m/e 446 is not detected and it shows prominent peaks at m/e 275 ($M-C_{10}H_{19}O_2$), 191 ($M - C_{10}H_{19}O_2$ - THP), corresponding to the fission α- to the dithiane, followed by loss of a tetrahydropyranyl group respectively].

Finally the compound (XXVII) on hydrolysis in aqueous methanol catalysed by oxalic acid, provided the desired 2-(4-hydroxybutyl)-2-[4-(2,2-dimethyltrimethylenedioxy)-pentyl]-1,3 dithiane (XXVIII) in 94% yield. However, the rate of selective cleavage of THP group is unexpectedly slow. [IR (liq.film) of the compound (XXVIII) shows strong bands at 915(characteristic dithiane band) and 3250 cm^{-1} (-OH). NMR(CCl_4, Fig.8): 3.93-3.32 (bm, 6H, $-CH_2OH$ and $-O-CH_2-$ $-O-CH_2-$), 3.23-2.28 (bm, 7H, $-S-CH_2-$ $-S-CH_2-$,-OH and a methylene), 2.20-1.40 (bm, 18H, 6 CH_2 and 2 CH_3 groups), 1.33 (s, 3H, CH_3). All this spectral data and elemental analysis ($C_{18}H_{34}O_3S_2$, molecular weight 362) strongly support structure (XXVIII) for the hydroxy compound. In the mass spectrum, molecular ion at m/e 362 is not seen and it shows prominent peaks at m/e 191 ($M-C_{10}H_{19}O_2$) and 119 ($M-C_{10}H_{19}O_2 - C_4H_8O$)].

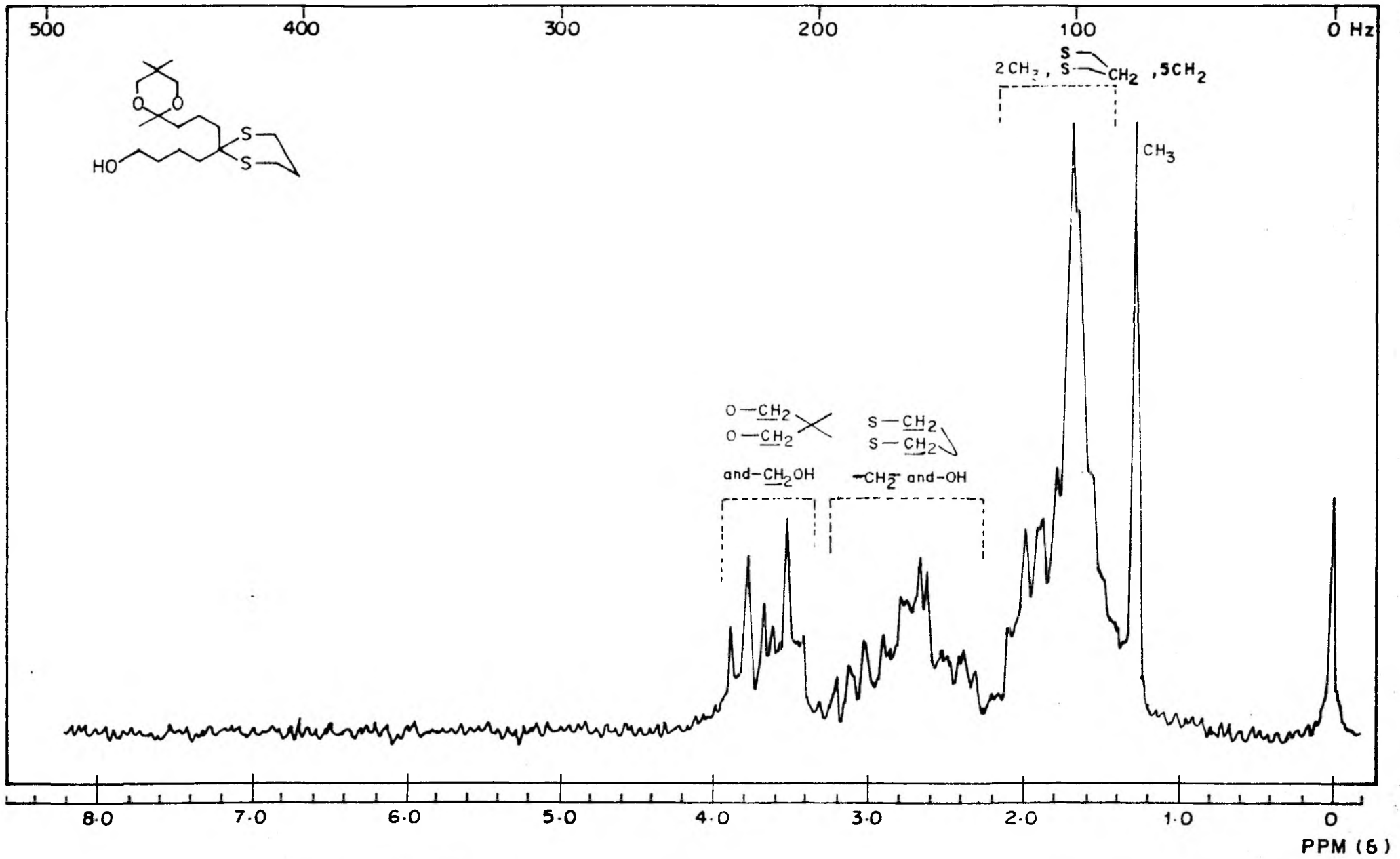
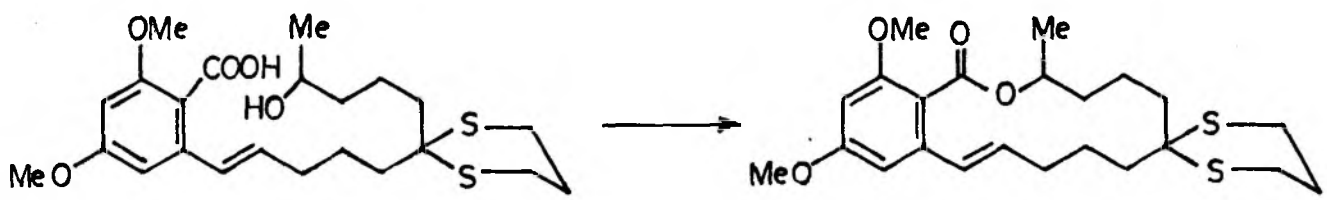


FIG. 8. NMR SPECTRUM OF COMPOUND (XXVIII) IN CCl_4

The aliphatic key intermediate (XXVIII) can be elaborated by established reactions as was outlined in the earlier synthesis by the Merck and Syntex groups to the hydroxy acid (XXIX), which on lactonisation should give the macrolide (XXX). Removal of the thioketal group followed by demethylation with borontribromide should result in (±)-zearalenone. Work in this direction is being carried out to complete its total synthesis.



(XXIX) (XXX)

EXPERIMENTAL

5-Hydroxypentanal (X)

A mixture containing water (60 ml), concentrated hydrochloric acid (5 ml) and 2,3-dihydropyran (20 g) was stirred at room temperature until the solution became homogeneous and again continued the stirring for 0.5 hr. After the addition of a few drops of phenolphthalein indicator to the mixture, it was neutralised with 20% sodium hydroxide till a faint pink colour persisted. The solution was then subjected to continuous extraction with ether for about 16 hr. The ether extract was dried (Na_2SO_4) and ether distilled off on a rotary evaporator. Distillation of the residue under reduced pressure gave 5-hydroxypentanal (16 g., 65%) as a clear, colourless, viscous oil at 65^{o}/10 mm. [lit.[25] b.p. $62\text{-}66^{o}$/9-10 mm).

2-(4-Hydroxybutyl)-1,3-dithiane (XI)

To a stirred mixture of 5-hydroxypentanal (5.10 g., 50 mmol) and 1,3-propanedithiol (5.4 g., 50 mmol) in 20 ml. dry chloroform, boron-trifluoride etherate (7.1 g., 50 mmol) was added at room temperature. The reaction was exothermic initially, then the contents stirred at room temp. for 7 hr. The reaction mixture was quenched (water 15 ml), diluted with chloroform, aqueous layer extracted with chloroform(2x20 ml) and the extracts mixed with chloroform layer. Chromatography

of the residue after evaporation of dried (sodium sulphate) chloroform extracts over a column of silica gel using benzene as an eluent furnished 8.160 g. (85%) of the dithiane (XI), as a colourless viscous oil. (Found: C, 49.81; H, 8.13; S, 33.0. $C_8H_{16}OS_2$ requires C, 50.00; H, 8.33; S, 33.34%).

2-(4-Tetrahydropyranyloxybutyl)-1,3-dithiane(XII)

To a stirred solution of the alcohol (XI) (6.27 g., 30 mmol) in 30 ml dry chloroform was added freshly distilled dihydropyran (9 g., excess), followed by a pinch of p-toluenesulphonic acid, and the mixture stirred at room temperature for 22 hr. The mixture was diluted with chloroform (20 ml) and the chloroform solution washed with sodium bicarbonate (5%), dried(potassium carbonate)and solvent distilled off on a rotary evaporator at 30-40°. Chromatography of the residue on neutral alumina(benzene) yielded the THP ether (XII) as a colourless oil (6.680 g., 74%) (Found: C, 56.46; H, 8.75; S, 23.01. $C_{13}H_{24}O_2S_2$ requires C, 56.52; H, 8.69; S, 23.19%).

5-Bromopentan-2-one (XIV)

2-Acetyl-γ-butyrolactone (10 g., 78.1 mmol) was added dropwise over a period of 1 hr. to a stirred and refluxing hydrobromic acid solution (25 ml of 48% and 12 ml water) in a flask surmounted by a Dean-Stark apparatus, when the product collected during the course of the reaction. The mixture was refluxed for additional 3 hr. The pale yellow oily layer of

the distillate was washed with water, dried (sodium sulphate) and distilled under reduced pressure to get 5-bromopentan -2-one (8 g., 62%), b.p.79°/21 mm (lit.[27] b.p.79-81°/21 mm.): IR(liq.film) 1700 cm^{-1} (C=O).

5-Bromopentan—2-one ethyleneketal (XV)

5-Bromopentan—2-one (5.5 g., 33.3 mmol), ethyleneglycol (7 ml, 100 mmol) and p-toluenesulphonic acid (0.055 g , 1 %) in 75 ml dry benzene was refluxed for 6 hr. under a Dean-Stark water separator. The mixture was cooled, washed with sodium bicarbonate solution (5%, 3 x 20 ml) and solvent distilled off under reduced pressure. Distillation of the residue under reduced pressure provided 5-bromopentan—2-one ethyleneketal (XV)(5 g., 72%),b.p.58°/1.5 mm. (lit.[27] b.p.103-105°/20 mm.). IR (liq.film) shows absence of carbonyll NMR(CCl_4) 3.90 (s, 4H, $-O-CH_2$ / $-O-CH_2$), 3.41 (t, 2H, $-CH_2Br$), 2.15-1.53 (m, 4H, 2 CH_2), 1.26 (s, 3H, CH_3).

5-Chloropentan—2-one (XVI)

The compound (XVI) was prepared from 2-acetyl-γ-butyrolactone (12.8 g), concentrated hydrochloric acid(15 ml) and water (17.5 ml) using the procedure as described for 5-bromopentan—2-one (XIV). The crude chloro compound (XVI) on distillation under reduced pressure yielded 9.57 g. (79%) pure 5-chloropentan—2-one (XVI), b.p.63°/10 mm. (lit.[28] b.p.70-72°/20 mm.). IR(liq.film) 1700 cm^{-1}(C=O): NMR(CCl_4): 3.54(t,2H, $-CH_2Cl$), 2.60(t,2H,$-CH_2CO$),2.10(s,3H, $-COCH_3$, 2.08-1.73(m,2H,CH_2).

5-Iodopentan—2-one (XVII)

A solution of 5-chloropentan—2-one (5 g., 50 mmol) in dry acetone (50 ml) was treated with sodium iodide (18 g., 60 mmol) and refluxed for 12 hr. Acetone was distilled off, residue treated with water and extracted with ether (3 x 30 ml). The extracts were washed with 0.1N sodium thiosulphate (2 x 10 ml), dried over anhydrous sodium sulphate and ether distilled off. The residue on distillation yielded (XVII) as a pale yellow liquid (5.2 g., 48%), b.p.94°/14 mm. (lit.[29] b.p. 94°/14 mm.). NMR(CCl_4) 3.27 (t, 2H, $-CH_2I$), 2.57 (t, 2H, $-CH_2CO$), 2.14 (s, 3H, $-COCH_3$), 2.10-1.78 (m, 2H. CH_2).

5-Iodopentan—2-one ethyleneketal (XVIII) from 5-iodopentan—2-one (XVII)

Ketalisation of the ketone (XVII) (5.3 g., 25 mmol) using ethyleneglycol (4.2 ml, 75 mmol) and p-toluenesulphonic acid (0.053g, 1%) in dry benzene (50 ml) at reflux temperature for 10 hr. as per the conditions described for 5-bromopentan—2-one ethyleneketal (XV) yielded 5-iodopentan—2-one ethyleneketal.[29] Purification of the product by chromatography (neutral alumina 25 g.) using pet.ether as an eluent yielded ketal (XVIII) as a pale yellow liquid (4.00 g., 62.50%).

5-Iodopentan—2-one ethyleneketal (XVIII) from 5-bromopentan—2-oneethyleneketal (XV)

A mixture of the bromoethyleneketal (XV) (0.836 g., 4 mmol), sodiumbicarbonate (1.08 g., 12 mmol) and sodium iodide (1.2 g., 8 mmol) in 10 ml. dry acetone was stirred at room temperature for 4 hr. The reaction was conducted in absence of light. Acetone was distilled off on a rotary evaporator at 30°, the residue treated with water and the separated yellow oil extracted with ether (3 x 10 ml). The extracts were washed with 0.1N sodium thiosulphate (2 x 10 ml), the solution passed through a pad of potassium carbonate and ether distilled off. The oily residue (0.750 g., 73%) of 5-iodopentan—2-one ethyleneketal (XVIII) was homogeneous on TLC (neutral alumina, benzene-hexane, 1:1). NMR(CCl_4): 3.90(s, 4H, $-O-CH_2-CH_2-O-$), 3.17 (t, 2H, $-CH_2I$), 2.09-1.54 (m, 4H, $2CH_2$), 1.27 (s, 3H, CH_3).

2-(4-Tetrahydropyranyloxybutyl)-2-(pentyl-4-one ethyleneketal)-1.3-dithiane (XIX)

To a stirred solution of the dithiane (XII) (0.552 g., 2 mmmol; dried by passing ethereal solution through a pad of potassium carbonate, solvent distilled off and residue dried at room temperature, 1.5 mm) in dry THF (5 ml), n-butyllithium in hexane (2.0 M, 1.6 ml, 3 mmol) was added dropwise at -15°, stirred at this temperature for 1 hr. and then at -15/ to 0° for 3 hr. (the dithiane was metallated quantitatively as determined by deuteration of aliquot of the reaction mixture). Then the

reaction mixture was cooled at -15° and freshly prepared (from 5-bromopentan—2-one ethyleneketal by NaI-$NAHCO_3$ method) 5-iodopentan—2-one ethyleneketal (XVIII) (0.768 g., 3 mmol, dried by passing through a short pad of K_2CO_3 and at room temperature, 1.5 mm) in dry THF (4 ml) added dropwise and stirred at this temperature until TLC analysis indicated maximum conversion of the dithiane (XII) to the product, ~4 hr.). Afterwards the mixture was poured into 1% aqueous sodiumbicarbonate (27 ml), aqueous solution extracted with ether (4 x 10 ml), extracts mixed, dried (K_2CO_3) and the solvent distilled off on a rotary evaporator at room temperature. Chromatography of the residue on a column of neutral alumina (10 g) with benzene as eluent and concentration of percolates at 30-40° on a rotary evaporator provided 0.541 g.(67%) of the pure alkylated product (XIX) as a colourless viscous oil. (Found: C, 59.72; H, 8.87; S, 15.63. $C_{20}H_{36}O_4S_2$ requires C, 59.40; H, 8.91; S, 15.84%). Metallation of the dithiane (XII) (0.138 g., 0.5 mml) in dry THF (2 ml) with n-butyllithium (2M, 0.38 ml, 0.75 mmol) as described above followed by alkylation with 5-bromopentan-2-one ethyleneketal (XV) (0.157 g., 0.75 mmol) in dry THF (1 ml), at -15° for 18 hr. gave the alkylated product (XIX) (0.080 g.,40%).

2-(4-Hydroxybutyl)-2-(pentyl-4-one)-1,3-dithiane(XX)

A solution of the compound (XIX) (0.541 g) in methanol (10 ml) containing a pinch of p-toluenesulphonic acid or oxalic acid was stirred at room temp. and 0° under argon until TLC analysis indicated the reaction to be complete (~7 hr. and 12 hr. respectively). The solvent was distilled off on a rotary evaporator at 30-40° and chromatography of the residue (silica gel, chloroform) yielded pure hydroxy-keto compound (XX) (0.369 g., 100%). (Found: C, 56.70; H, 8.59: S, 23.02. $C_{13}H_{24}O_2S_2$ requires C, 56.52; H, 8.70; S, 23.19%).

Reaction of 2-(4-hydroxybutyl)-2-(pentyl-4-one)-1,3-dithiane (XX) with ethyleneglycol

A mixture of the compound (XX) (0.050 g., 0.18 mmol), ethyleneglycol (0.050 g., 0.80 mmol) in dry benzene (10 ml) containing p-toluenesulphonic acid(0.006 g,10%) was stirred in a flask, equipped with a Dean-Stark water separator and refluxed for 76 hr. The reaction was followed by TLC (silica gel,benzene-acetone: 9:1) every 12 hr. The TLC showed very little product after 76 hr., with a lot of starting material. The reaction mixture was cooled to room temperature, benzene solution washed with 5% sodium bicarbonate (10 ml), dried (K_2CO_3) and solvent distilled off on a rotary evaporator. The residue was chromatographed on a column of silica gel (5 g), using benzene and

benzene-acetone mixture for elution. Elution of the column with benzene gave faster moving oily product (0.012 g., 20%) and it was assigned structure (XXI) on the basis of its spectral data (M^+. 302). Hydroxyketo compound (XX) (0.030 g) was obtained by eluting the column with benzene-acetone (9:1) mixture.

2-(4-Hydroxybutyl)-2-(pentyl-4-oneethylene ketal)-1,3-dithiane (XXII)

The dithiane (XI) (0.384 g., 2 mmol, dried by passing through a short K_2CO_3 column and then at room temperature, 1.5 mm) in dry THF (3 ml) was metallated exactly as described for the dithiane (XII), using n-butyllithium in hexane (1.57 M, 2.5 ml, 4 mmol) at -15° for 4 hr. Then freshly prepared 5-iodopentan-2-oneethyleneketal (0.512 g., 4 mmol, dried as mentioned before, 1.5 mm) in dry THF (5 ml) (precooled to -15°) was added dropwise with stirring and the reaction mixture maintained at -15° for 4 hr. The reaction mixture was quenched with 1% aqueous sodium bicarbonate (20 ml), diluted with ether and the aqueous phase extracted with ether (2 x 20 ml). The organic extracts were dried (K_2CO_3) and evaporation of ether under vacuum furnished crude mixture of products. Chromatography of the crude mixture on a column of silica gel (10 g) using benzene-acetone (9:1) for elution yielded pure desired 2-(4-hydroxybutyl)-2-(pentyl-4-oneethyleneketal)-1,3-dithiane (0.1 g., 15.63 %) as a colourless oil. (Found: C, 56.00; H, 8.92; S, 19.89. $C_{15}H_{28}O_3S_2$ requires C, 56.26; H, 8.75; S, 20.00%).

2,2-Dimethyl-1,3-propanediol (XXIV)

To a solution of potassium hydroxide (5.833 g., 104.2 mmol) in 95% ethyl alcohol (20 ml), was added dropwise with stirring a homogeneous solution containing 40% formaldehyde (10 ml) and isobutyraldehyde (6 g., 83.85 mmol) in ethyl alcohol (60 ml) over a period of 2 hr. The mixture was refluxed for 24 hr., The alcohol stripped off and the residue subjected to continuous ether extraction for 12 hr. After the removal of ether, the crude product was distilled at atmospheric pressure to obtain the diol (XXIV) at 175-205°, which on crystallisation from benzene-hexane gave colourless needles (5 g., 58%), m.p.130° (lit.[34a,34b] m.p. 130°, 126-27°). IR (liq.film) 3180 cm^{-1} (-OH); NMR($CHCl_3$) 4.20(s, 2H, 2 OH groups, exchanges with D_2O), 3.46 (s, 4H, 2 CH_2), 0.93 (s, 6H, 2 CH_3).

2,5,5-Trimethyl-2-(3-bromoprop-1,3-dioxane) (XXV)

A mixture of 5-bromopentan—2-one (0.825 g., 5 mmol), 2,2-dimethyl-1,3-propane diol (0.570 g., 5.5 mmol) and p-toluene-sulphonic acid (0.082 g, 10%) in dry benzene (20 ml) was refluxed in a flask surmounted by a Dean-Stark trap for 6-7 hr. The benzene solution was cooled, washed with 5% sodium bicarbonate (3 x 10 ml), dried over anhydrous potassium carbonate and the solvent distilled off on a retary evaporator. Purification of the residue on a column of neutral alumina (10 g) using

hexane for elution, furnished (XXV) (0.9 g., 71.77%) as a colourless oil (Found: C, 47.47; H, 7.69: Br, 32.12. $C_{10}H_{19}BrO_2$ requires C, 47.80; H, 7.57: Br, 31.87%).

Reaction of THP ether (XII) and 2,5,5-trimethyl-2-(3-bromopropyl)-1,3-dioxane (XXV) with p-toluenesulphonic acid.

A mixture of THP ether (XII, 0.040 g) and 1,3-dioxane (XXV, 0.040 g) in methanol (5 ml) was treated with a pinch of p-toluenesulphonic acid and stirred at room temperature. TLC analysis (silica gel, acetone-benzene, 1:9) of the mixture after 7 hr. and 10 hr. showed the formation of 2-(4-hydroxybutyl)-1,3-dithiane (XI) and 5-bromopentan-2-one (XIV) by comparisen with authentic samples respectively.

Reaction of THP ether (XII) and 2,5,5-trimethyl-2-(3-bromopropyl) 1,3-dioxane (XXV) with oxalic acid

A stirred mixture of THP ether (XII, 0.040 g) and 1,3-dioxane (XXV, 0.040 g) in methanol (5 ml) when treated with a pinch of oxalic acid at room temperature, indicated the formation of 2-(4-hydroxybutyl)-1,3-dithiane (XI) after 7 hr., and very little 5-bromopentan-2-one (XIV) after 16 hr. as shown by TLC comparison with authentic samples.

2,5,5-Trimethyl-2-(3-iodopropyl)-1,3-dioxane(XXVI)

A mixture of the compound (XXV) (0.251 g., 1 mmol), sodium bicarbonate (0.252 g., 3 mmol) and sodium iodide (0.300 g., 2 mmol) in 10 ml dry acetone was stirred at room temperature for 4 hr. (reaction was conducted in absence of light). Afterwards acetone was distilled off on a rotary evaporator at room temperature, residue treated with water and the iodo compound (XXVI) extracted with ether (3 x 10 ml). Ether extracts were passed through a short column of potassium carbonate, ether distilled off and the iodo compound (0.255 g., 85-87%) was dried under vacuum (1.5 mm). IR and NMR (CCl_4) is similar to that of bromodioxane (XXV).

2-(4-Tetrahydropyranyloxybutyl)-2-[4-(2,2-dimethyltrimethylenedioxy)-pentyl)]-1,3-dithiane (XXVII)

2-(4-Tetrahydropyranyloxybutyl)-1,3-dithiane(XII) (0.138 g., 0.5 mmol) was metallated exactly as described before, using n-butyllithium in hexane (2.5M, 0.3 ml, 0.75 mmol) in dry THF (2 ml) initially at -15° (1 hr.) and then at -15-0° (3 hr). The reaction mixture was then cooled to -15° and freshly prepared iododioxane (XXVI) (0.224 g., 0.75 mmol, dried at room temperature, 1.5 mm) in dry THF (2 ml) added dropwise with stirring, and maintained at this temperature for 4 hr. Then it was diluted with 12 ml water and ether (10 ml), aqueous phase extracted with ether (2 x 10 ml), extracts dried, ——————→

over potassium carbonate and filtered. Removal of ether and the residue on chromatography over a column of neutral alumina using benzene as an eluent provided compound (XXVII) as a colourless viscous oil (0.170 g., 76%). (Found: C, 62.00; H, 9.16; S, 14.28. $C_{23}H_{42}O_4S_2$ requires C, 61.89; H, 9.42; S, 14.35%).

2-(4-Hydroxybutyl)-2-[4-(2,2-dimethyltrimethylenedioxy)-pentyl]-1,3-dithiane (XXVIII)

A solution of compound (XXVII) (0.100 g) in methanol (5 ml) containing oxalic acid (0.01 g) was magnetically stirred in the atmosphere of argon until THP ether was converted into alcohol (~ 40 hr., at room temperature). Solvent was distilled off on a rotary evaporator at 30-40°, residue dissolved in chloroform (2 x 10 ml), solution washed with water, dried (K_2CO_3), filtered and evaporated on a rotary evaporator. Chromatography of the residue on a column of silica gel (acetone-benzene, 1:9), provided the desired alcohol (XXVIII) (0.077g, 94%) as a colourless oil. (Found: C, 59.48; H, 9.52; S, 17.60. $C_{18}H_{34}O_3S_2$ requires C, 59.68; H, 9.39; S, 17.68%).

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CHAPTER-II

CHEMICAL INVESTIGATION OF SOME INDIAN MEDICINAL PLANTS

Section 1

Toddalia aculeata.

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INTRODUCTION

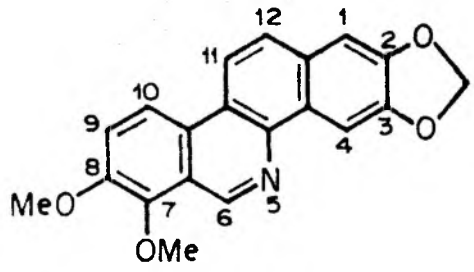
Toddalia aculeata[1-3] Pers. (Family: Rutaceae; sub-family: Toddalioideae) is a scandant shrub, known to the Sanskrit writers as "kanchan" (Golden) on account of its orange colour fruits, and also called "Dahana" because of pungency of its berries. The plant is distributed in sub-tropical Himalayas from Kumaon eastwards to Bhutan at an elevation of 5000 ft., also in Western and Southern India, China and Ceylon. Its branches are covered with prickles. The wood is porous yellowish white. Leaflets are crenulate, greatly varying in length in semi-evergreen shrub. Flowers are small, cream coloured. Fruits are orange coloured, globose.

The entire plant,[4] especially the root bark, finds use in medicine as a tonic, stimulant and antipyretic. The stem is also used in medicine to a limited extent. The fresh bark is administered by Tamil physicians for the cure of a sort of remmitent commonly known as "hill-fever". The plant acts as a good stomachic tonic improving the appetite and digestion. Because of these medicinal uses, the root and leaves were extensively studied and their constituents determined by a number of workers.

From the root bark Perkin and Hummel[5] isolated a yellow alkaloid considered to be the salt of berberine. Subsequently, Dey and Pillay[4] established the absence of berberine in the root bark and isolated two bases named toddaline and toddalinine. Govindachari and Thygarajan[6] isolated these two alkaloids (in pure crystalline form) from the root bark by a procedure less complicated than Dey and Pillay and showed identity of toddaline with chelerythrine, an alkaloid of papavaraceae. Desai *et al.*[7] examined the root bark and reported the isolation of toddaline (I), toddalinine (II) and acetone adduct of toddalinine (III). The latter compound was reported as an artefact produced by the action of acetone (used in isolation) on toddalinine. Govindachari and Viswanathan[8] isolated skimmianine (XIII), a furoquinoline, one of the widely distributed alkaloids from the root bark.

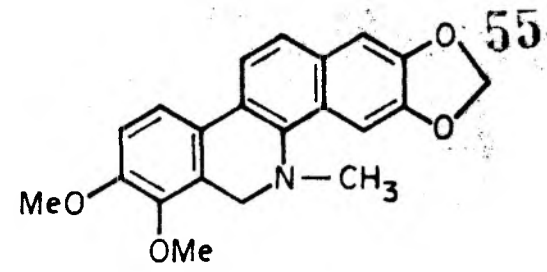
Dey and Pillay[9] found the coumarin, toddalolactone (VI) in the root bark and reviewed earlier data on this lactone. Dutta[10] examined the stem bark obtained from Darjeeling (Bengal) and reported the isolation of two coumarins, aculeatin (V) and aculeatin hydrate, isomeric with toddalolactone isolated earlier by Dey and Pillay.[9]

Combes *et al.*[11] described the isolation, structure and properties of a new coumarin, toddaculine[5,7-dimethoxy, 6(2-isopentyl)-coumarin] (IV) isolated either from the



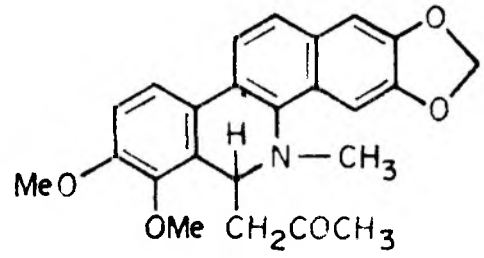
(I)

TODDALINE



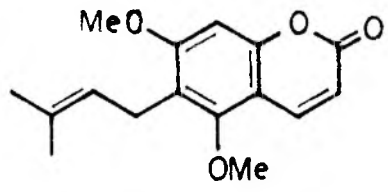
(II)

TODDALININE



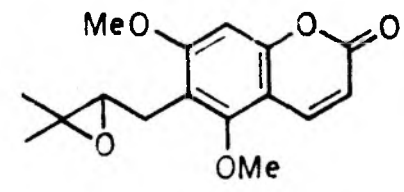
(III)

ACETONE ADDUCT OF TODDALININE



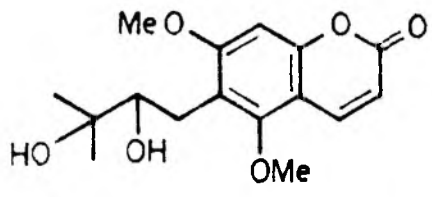
(IV)

TODDACULINE



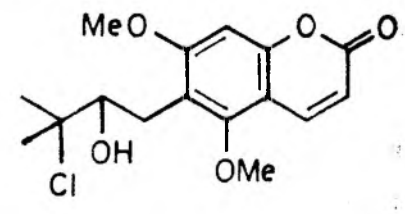
(V)

ACULEATIN



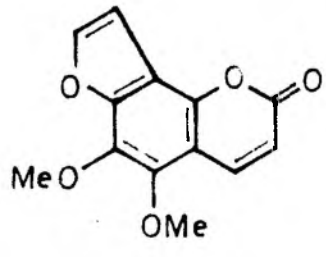
(VI)

TODDALOLACTONE



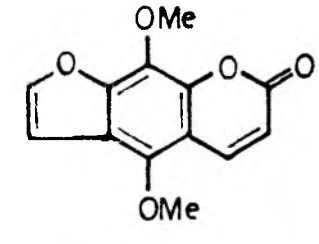
(VII)

6-(3-CHLORO-2-HYDROXY-3-METHYLBUTYL)-5,7-DIMETHOXYCOUMARIN



(VIII)

PIMPINELLIN



(IX)

ISOPIMPINELLIN

root or stem bark. Jacques et al.[12] used this coumarin in radiation protectants composition.

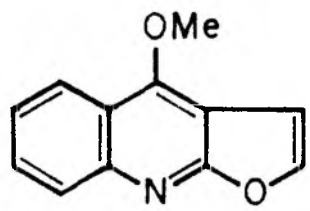
Desai et al.[7] have isolated pimpinellin (VIII), isopimpinellin (IX) and 6-(3-chloro-2-hydroxy-3-methylbutyl)-5,7-dimethoxycoumarin (VII) from the root bark. The last of these being an artifact arising from epoxycoumarin, aculeatin (V), by the action of hydrochloric acid used in alkaloid extraction. Wander et al.[13,14] isolated diosmin, the rhamnoglucoside of diosmetin. Lobstein and Hess[15] obtained oleoresin containing a mixture of acetone, benzoic acid, eugenol, citronellol, paraffin. b.p. 80°, along with saponifiable lipids, unsaponifiable lipids, glucose, sucrose, cirtic, malic and succinic acids from the roots. They have also isolated a dark-brown rosin which produces abortion in pregnant guinea pigs and causes contraction of uterus in non-pregnant ones.

PRESENT WORK

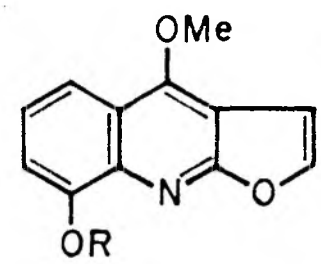
Literature survey of Toddalia aculeata revealed that various workers have reported mainly the isolation and characterisation of alkaloids and coumarins from roots and leaves. However, very little is known about the stem constituents of this plant. The present investigation deals with the stem of T. aculeata obtained from Algarah, Darjeeling (West Bengal).

From the stem two new coumarins, norbraylin [6-hydroxy-7,8-(2',2'-dimethylpyrano-5',6')-coumarin] (XVIII) and 5,7,8-trimethoxycoumarin (XVI) have been isolated, in addition to two alkaloids, toddalinine (II), skimmianine(XIII) and three known coumarins, toddalolactone (VI), pimpinellin (VIII), isopimpinellin (IX) reported earlier from the same plant. Three furoquinoline alkaloids, robustine (XI), dictamine (X) and γ-fagarine (XII) along with a furano-coumarin, bergapten (XIV) and two pyranocoumarins braylin (XVII) and luvangetin (XV) hitherto not reported from this source have been isolated.

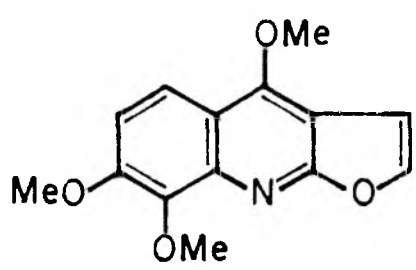
Cold extraction of the powdered stem with chloroform yielded a greenish syrupy extract (1.7%) which was successively extracted with hexane, benzene and chloroform in a soxhlet to give 0.5, 0.53 and 0.63 per cent extracts respectively.



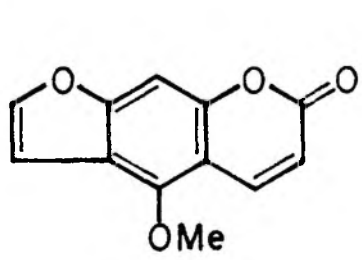
(X)
DICTAMINE



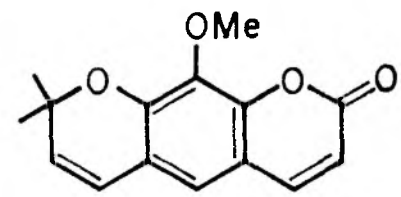
(XI) 8-HYDROXYDICTAMINE(ROBUSTINE)
R=H
(XII) γ-FAGARINE
R=Me



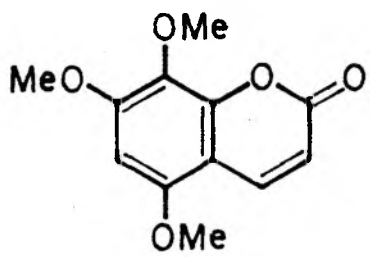
(XIII)
SKIMMIANINE



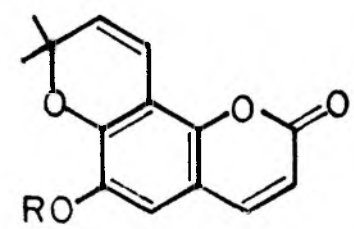
(XIV)
BERGAPTEN



(XV)
LUVANGETIN



(XVIA)
,7,8-TRIMETHOXYCOUMARIN



(XVII) BRAYLIN , R=Me
(XVIII) NORBRAYLIN , R=H

The hexane extract mainly contained waxes and oils and hence it was not worked up further. The benzene and chloroform extracts which showed identical TLC behaviour (silica gel, acetone-hexane, 2:8) were mixed and subjected to functional group separation. The mixed benzene-chloroform extract was successively extracted with hydrochloric acid (5%) and aqueous sodium hydroxide (10%) to give alkaloidal and phenolic parts respectively. The neutral part was chromatographed on a column of silica gel.

After the cold chloroform extraction, the stem powder was extracted with methanol. The methanol extract was adsorbed on extracted stem powder, which on successive extraction with chloroform, and methanol yielded 0.5 and 2.0% extractives respectively.

Examination of Alkaloidal part

The alkaloidal part was chromatographed on a column of silica gel. The column was eluted successively with benzene, benzene-chloroform and chloroform. A number of fractions were collected and examined by TLC (acetone-hexane). Like fractions were combined together and worked up, and the alkaloids (A-F) (belonging to benzophenanthridine and furoquinoline groups) in decreasing order of Rf values, have been isolated.

Alkaloid A (toddalinine), crystallised as yellow needles from acetone-hexane mixture, m.p. 165°, $C_{21}H_{19}O_4N$, M^+ 349. IR shows sharp band at 935 cm^{-1} (methylenedioxy group). The NMR spectrum in $CDCl_3$ shows a sharp singlet at 2.67 for $N\text{-}CH_3$, two singlets at 3.90 and 3.97 for two methoxyl groups, a singlet at 4.37 can be assigned to benzylic methylene, a sharp singlet at 6.10 for methylenedioxy group. Aromatic region integrates for six protons, comprising two singlets at 7.14 and 7.77; and four orthocoupled doublets (J=9 Hz) at 6.97, 7.50, 7.54 and 7.74. The mass spectrum shows base peak at m/e 349. The data is in agreement with toddalinine (II) first isolated by Dey and Pillay,[4] later by Govindachari and Thyagarajan[6] and Desai et al.[7] from the root bark.

Alkaloid B (8-hydroxydictamine), obtained as white plates from ethanol, m.p. 148°, $C_{12}H_9O_3N$, M^+ 215. Its IR spectrum shows band at 3430 cm^{-1} (-OH). The NMR spectrum in $CDCl_3$ shows a three proton singlet at 4.37 for methoxyl group. A pair of doublet (J = 2.5 Hz) at 6.97 and 7.54 account for C-2 and C-3 protons of a furan ring. The multiplets centered at 7.64, 7.30 and 7.07 integrate for three protons.

The alkaloid on methylation with diazomethane gave methyl ether, m.p. 142°, identical in all respects (TLC and mixed m.p.) with an authentic sample of γ-fagarine (lit.[25] m.p. 142°).

On the basis of above spectral and chemical data, alkaloid B is characterised as 8-hydroxydictamine (XI), which has been isolated for the first time from this plant.

Alkaloid C (skimmianine), yellow needles from methylene chloride-hexane. m.p. 176°, $C_{14}H_{13}O_4N$, M^+ 259. The NMR spectrum in $CDCl_3$ indicates the presence of two doublets (J=9 Hz) at 7.88 and 7.14 (2 Ar-H), two doublets (J=2.5 Hz) at 7.54 and 6.97 (C-3 and C-2 protons of a furan) and three singlets at 4.37, 4.12 and 4.01 (3OMe groups). All the above data correspond to the known alkaloid, skimmianine (XIII) earlier isolated by Govindachari and Viswanathan from the root bark.[8]

Alkaloid D (dictamine), crystallised from acetone-hexane mixture as colourless prisms, m.p. 132°, $C_{12}H_9O_2N$, M^+ 199. The NMR spectrum in $CDCl_3$ reveals the presence of a three proton singlet at 4.40(OMe group) and two doublets (J=2.5 Hz) at 6.97 and 7.54 (C-2 and C-3 furan protons). In the low field region of the spectrum four aromatic protons appear as multiplet between 7.20 and 8.27.

The mass spectrum shows a base peak at m/e 199, other prominent peaks are at m/e 184 (M-15), 156(M-43), 127(M-72), 130(M-69), 128 (M-71). The physical and spectral properties established its identity with that of dictamine(X) not reported earlier from this plant.

Alkaloid E (γ-fagarine) obtained as colourless prisms from ethanol, m.p. 142°, $C_{13}H_{11}O_3N$, M^+ 229. The NMR spectrum in $CDCl_3$ is similar to that of 8-hydroxydictamine (XI) except the appearance of an additional methoxyl at 4.04. Its IR spectrum shows absence of -OH absorption.

The above data show that alkaloid E is γ-fagarine (XII) isolated for the first time from this source. Although alkaloids, 8-hydroxydictamine, dictamine and γ-fagarine have been isolated from T. aculeata for the first time, they have been reported earlier from a number of Rutaceous plants.[8a]

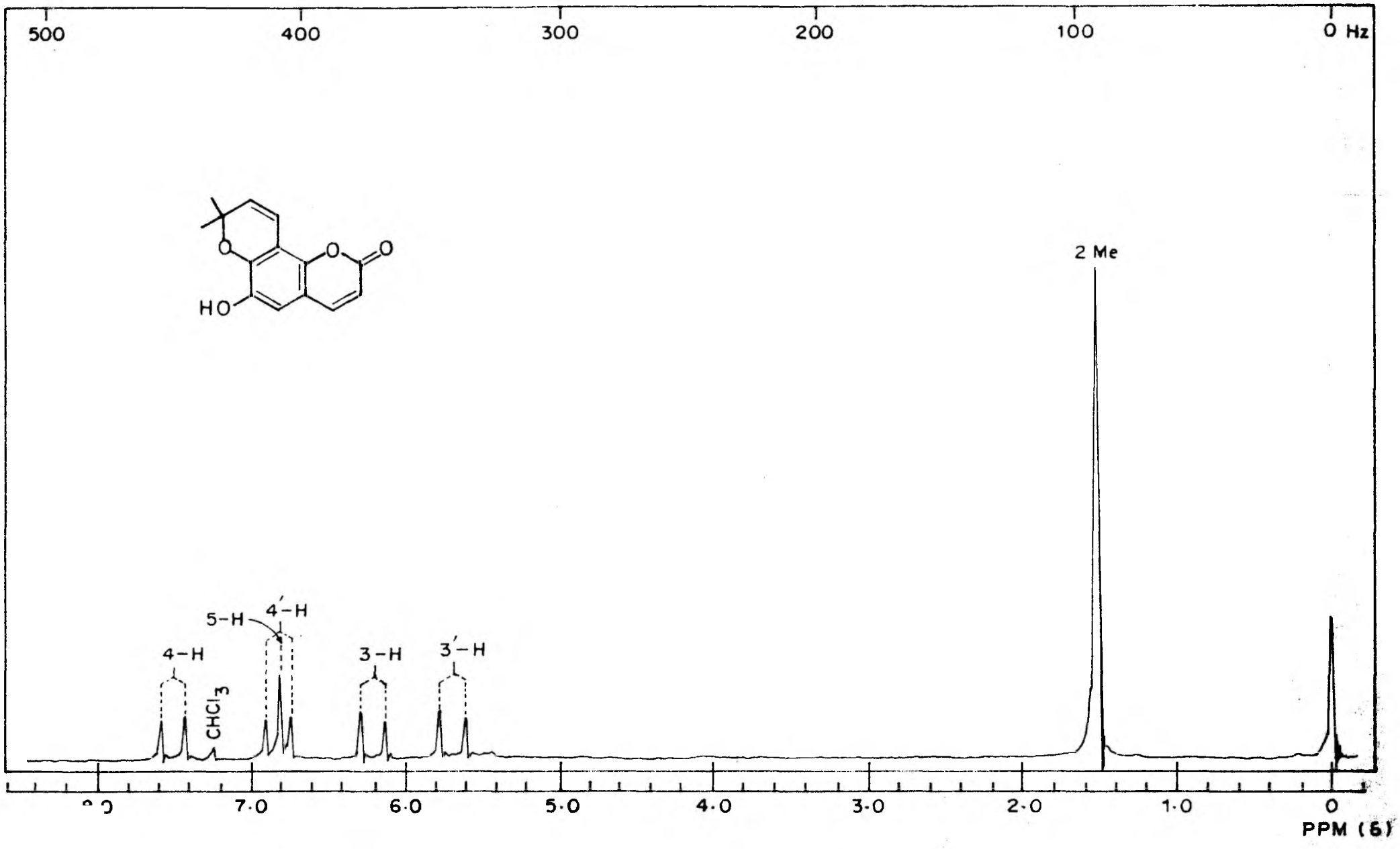
Alkaloid F, m.p. 196°. Its mass spectrum shows molecular ion at m/e 333 (base peak); other prominent peaks in the spectrum are at m/e 318 (M-15), 219 (M-15-28), 275 (M-15-28-15), 260 (M-73). Since the quantity of alkaloid was insufficient for detailed spectral and chemical analysis, it was not characterised further.

Phenolic part

The phenolic part of the mixed benzene-chloroform extract was chromatographed on a column of silica gel, monitoring the separation on TLC (silica gel, acetone-hexane 3:7). Initial fractions were eluted with hexane-acetone mixture and subsequent fractions eluted by increasing the percentage of acetone in hexane. Repeated chromatography of the initial

fractions gave a crystalline compound which on recrystallisation from acetone-hexane mixture yielded colourless needles, m.p. 144-45°, $M^{+}_{\cdot}$ 244. It was optically inactive, shown by analysis and its mass spectrum to have the formula $C_{14}H_{12}O_4$. It gave a negative ferric chloride test, but dissolved slowly in warm aqueous sodium hydroxide yielding a yellow solution from which it could be precipitated unchanged on acidification. Treatment with ethereal diazomethane yielded a methyl ether, $C_{15}H_{14}O_4$. This behaviour together with IR spectrum which exhibits bands at 1720 (C=O of α-pyrone) and 3535 cm^{-1} (phenolic -OH), suggests that the compound is a hydroxy coumarin. UV absorption $\lambda^{EtOH}_{max.}$ (log ε) 210 (2.87), 228(2.86). 277(2.61), 284(2.55).

The NMR spectrum (Fig.1) in $CDCl_3$ provides two vinylic protons as doublets (J=10 Hz) at 6.83 and 5.70 in conjugation with a singlet at 1.53for two methyl groups suggesting the presence of 2,2-dimethylchromene ring. This is substantiated in the mass spectrum (Fig.2) by a ready loss of a methyl radical from the molecular ion ($M^{+}_{\cdot}$ 244), giving a base peak at m/e 229, which is diagnostic for 2,2-dimethylchromene ring system in the molecule (chart 1). A pair of doublet (J=10 Hz) at 6.22and 7.50can be assigned to 3-H and 4-H of coumarin ring. A single proton singlet appearing at 6.80 remains unaccounted. The position of -OH group and an aromatic proton is to be fixed in the chromenocoumarin.



FIG·1: NMR SPECTRUM OF NORBRAYLIN (XVIII) IN $CDCl_3$

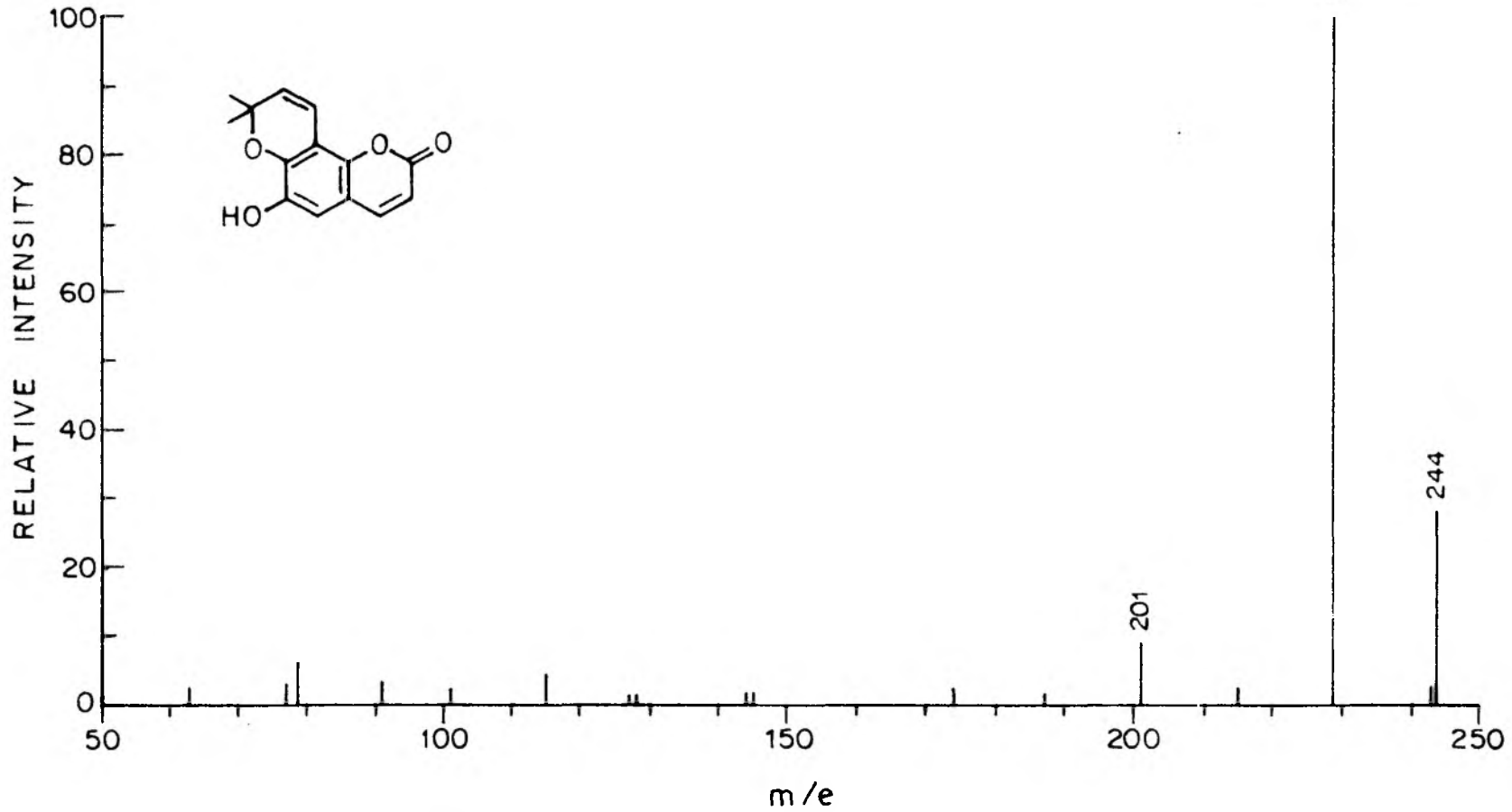
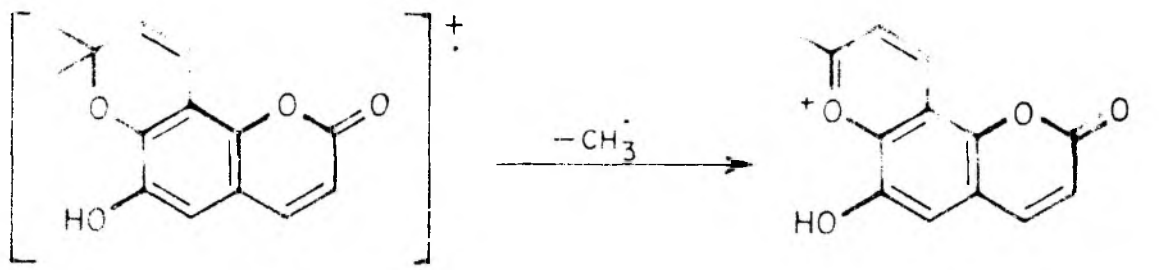

100
80
60
40
20
0
RELATIVE INTENSITY
50
100
150
200
250
m/e
201
229
244
O
O
O
HO

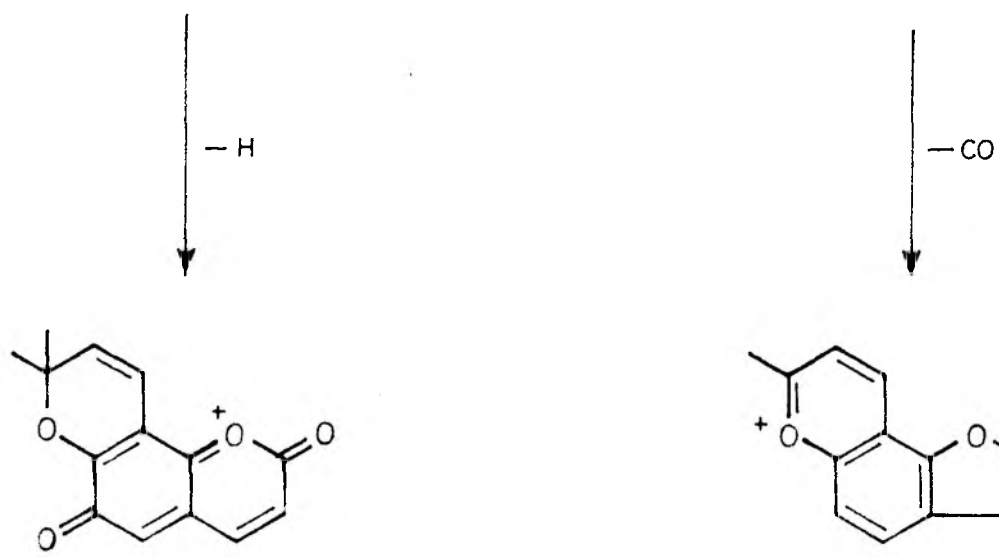
FIG. – 2 .

CHART – 1

MASS SPECTRAL FRAGMENTATION OF NORBRAYLIN



m/e 244 (28%) m/e 229 (100%)



m/e 243 (2%) m/e 201 (9%)

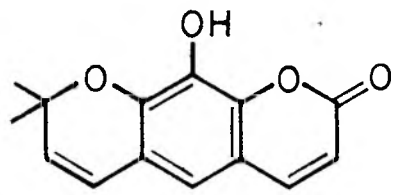
From the above data, six structures (XVIII-XXIII) have been considered for hydroxychromenocoumarin (chart 2).

The m.p. and TLC of the coumarin were not identical with demylluvangetin (XIX), hence this structure was ruled out. It has been well established that an oxygen substituent at the 5-position in coumarin ring shifts the resonance of adjacent C-4 proton to a much lower field (Ca. 0.5 ppm) (Table 1), because of the peri effect also marked in chromenes.[17] Since the hydroxychromenocoumarin does not show such a shift, structures XX-XXIII were rejected.

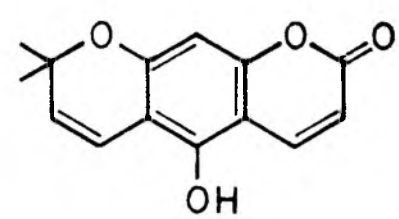
All the above data reveal structure (XVIII) for the hydroxychromenocoumarin, which shows that hydroxyl group is at 6-position of coumarin, consequently a singlet at 6.80 accounts for 5-H of chromenocoumarin. Further confirmation has been made by methylation with diazomethane to give its methyl ether, m.p. 150°, identical with braylin (XVII).

The coumarin shows molecular ion at m/e 244 in the mass spectrum. Other peaks in the spectrum are at m/e 243 (M-1), 229 (M-15, base peak), 201 (M-15-28). The mass spectral fragmentation outlined in the chart 1 is in complete agreement with structure (XVIII) for the hydroxychromenocoumarin, designated as norbraylin, a new pyranocoumarin.

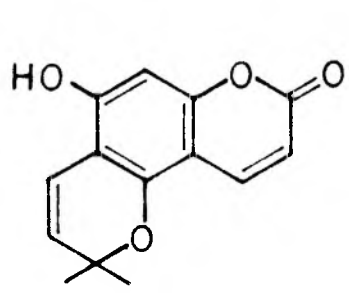
CHART - 2



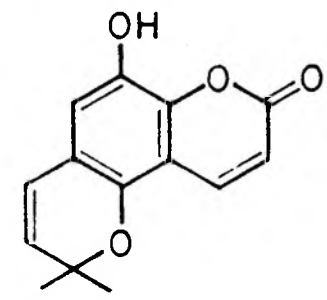
(XIX)



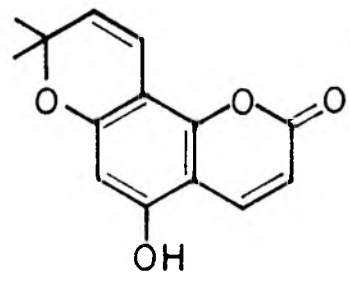
(XX)



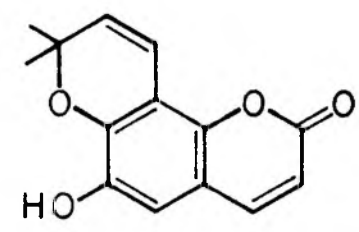
(XXI)



(XXII)

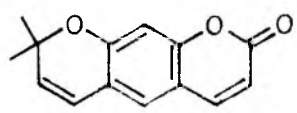
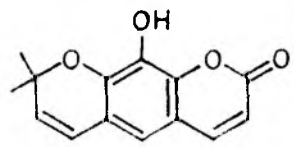
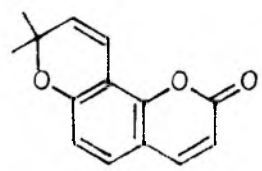
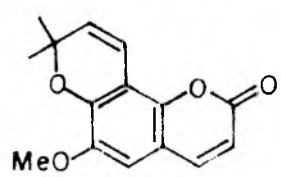
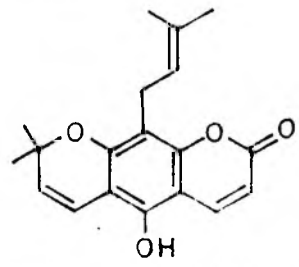
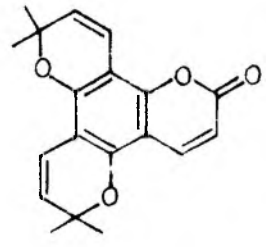
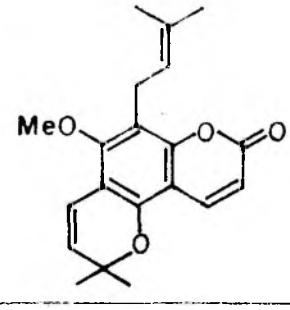


(XXIII)



(XVIII)

TABLE 1

NAME	STRUCTURE	CHEMICAL SHIFTS			
		3′-H	4′-H	3-H	4-H
XANTHYLETIN		5·67	6·33	6·19	7·56
DEMETHYLLUVANGETIN		5·70	6·40	6·20	7·60
SESELIN		5·68	6·82	6·10	7·54
BRAYLIN		5·73	6·80	6·20	7·56
TRACHYPHYLLIN		5·72	6·78	6·15	8·15
DIPETALOLACTONE		5·65 3″ 5·62	6·88 4″ 6·71	6·19	8·02
DIPETALINE		5·71	6·64	6·30	8·10

Neutral part

Neutral part of the mixed benzene-chloroform extracts was chromatographed on a column of silica gel, using a mixture of chloroform and benzene for elution with increasing polarity. A number of fractions were collected and monitored on TLC silica gel (acetone-hexane, 2:8). Like fractions were pooled together and final purification was achieved by rechromatography over a column of silica gel or neutral alumina. Seven compounds (1-7) were isolated in the decreasing order of their Rf values.

Compound 1 (bergapten), crystallised from acetone-hexane mixture in colourless needles, m.p. 188°, $C_{12}H_8O_4$, M^+ 216, IR spectrum in chloroform shows band at 1720 cm^{-1} indicating α,β-unsaturated lactone in the molecule. The NMR spectrum in acetone shows the characteristic pattern of furanocoumarin doublets (J=10 Hz) at 8.14 (4-H) and 6.20(3-H), and doublets (J = 2.5 Hz) at 7.80 (2'-H) and 7.24 (3'-H) together with two singlets at 7.14 (Ar-H) and 4.30 (OMe). The mass spectrum shows molecular ion as a base peak at m/e 216 and other peaks at m/e 201 (M-15), 188(M-28), 173(M-28-15) and 145 (M-71). Linear and angular structures were considered for this furanocoumarin, but the latter is rejected,as the melting point varies widely from compound 1. Thus from the above spectral and physical data, compound 1 has been characterised as bergapten (XIV), which was isolated earlier from Citrus bergamia.[23]

Compound 2 (braylin), obtained as colourless needles from benzene-hexane mixture, m.p. 148-49°, $C_{15}H_{14}O_4$, M^+ 258. Infrared spectrum in chloroform shows band at 1700 cm^{-1} for α,β-unsaturated lactone. The NMR spectrum in $CDCl_3$ is similar to that of new pyranocoumarin norbraylin(XVIII), except that it shows additional three proton singlet at 3.90 assignable to Ar-OMe. In its mass spectrum, it shows a molecular ion at 258 and other prominent peaks at m/e 243 ($M-CH_3$, 100%), 215 ($M-CH_3-CO$) and 228 which arises from 243, most probably by the loss of methyl radical from methoxyl group. Thus the above data are in conformity with a known pyranocoumarin braylin (XVII). Although isolation of the compound (XVII) from T. aculeata is for the first time, it was isolated ealier from Flindersia brayleyana.[26]

Compound 3 (luvangetin), crystallised from hexane in pale yellow needles, m.p. 109°, $C_{15}H_{14}O_4$, M^+ 258. IR shows band at 1700 cm^{-1} (α,β-unsaturated ketone). The NMR spectrum in $CDCl_3$ provides evidence for the 2,2-dimethylchromene ring (vinylic H at 6.33 and 5.79, d, J=10 Hz; Me_2C at 1.59, s), the coumarin ring (3-H and 4-H at 6.20 and 7.51, d, J=10 Hz); two singlets at 6.80(Ar-H), 3.90(OMe group). Thus compound 3 is characterised as luvangetin (XV). Although it has been reported from a number of Rutaceous plants, it is isolated for the first time from T. aculeata.

Compound 4 (isopimpinellin), pale yellow needles from benzene, m.p. 149°, $C_{13}H_{10}O_5$, M^+ 246. IR shows band at 1710 cm^{-1} (α,β-unsaturated lactone). The NMR spectrum in $CDCl_3$ shows characteristic pattern of furanocoumarin doublets(J=10Hz) at 8.15 (4-H), 6.27 (3-H) and doublets (J=2 Hz) at 7.64 (2'-H) and 7.0 (3'-H) together with a six proton singlet at 4.20 assignable to two methoxyl groups of a furanocoumarin. The physical and spectral data were found to be consistent with the reported data for isopimpinellin (IX) isolated by Desai et al.[7] from the root bark.

Compound 5 (pimpinellin) obtained as pale yellow needles from methanol, m.p. 117°, $C_{13}H_{10}O_5$, M^+ 246. Its IR spectrum shows band at 1710 cm^{-1} (α,β-unsaturated lactone). The NMR spectrum in acetone shows characteristic pattern for furanocoumarin, 6.20 and 8.15 (d, 1H each, J=10 Hz); 7.20 and 7.80 (d, 1H each, J = 2.5 Hz) along with two sharp three proton singlets at 4.24 and 4.17 representing two methoxyl groups. All these physical and spectral properties are found to be identical with a known furanocoumarin pimpinellin (VIII) reported earlier from the root bark by Desai et al.[7]

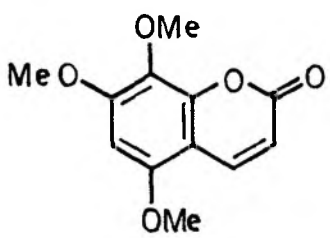
Compound 6 (5,7,8-trimethoxycoumarin) crystallised from benzene in colourless needles, m.p. 180°, M^+ 236. → $C_{12}H_{12}O_5$ IR shows band at 1715 cm⁻¹ (α,β-unsaturated lactone), characteristic of coumarin. UV spectrum shows absorption bands at $\lambda_{max.}^{EtOH}$ (log ε) 213 (4.35), 227 (4.0), 264 (3.94), 330(4.03). Its NMR

spectrum (Fig.3) in $CDCl_3$ reveals the presence of two doublets ($\underline{J}$ = 10 Hz) at 6.18 and 7.97 for 3-H and 4-H of a coumarin, a single proton singlet at 6.37 assignable to an aromatic proton and two singlets at 4.0 and 3.94, each integrating for three and six protons respectively represent three methoxyl groups.

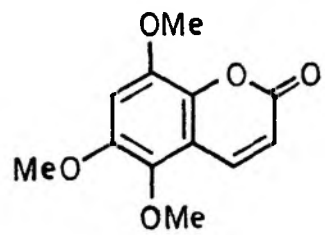
The occurrence of two doublets ($\underline{J}$= 10 Hz) appropriate to a cis-alkene, alongwith a single aromatic proton singlet and characteristic IR absorption for α-pyrone suggests that the compound is a trisubstituted coumarin.

Benzene-induced solvent shifts of methoxyl groups in the NMR spectrum (Fig.3) are seen at 3.80, 3.50 and 3.40 indicating that two methoxyls have suffered a singnificant upfield shift, suggesting that at least one adjacent position of the two methoxyl groups is unsubstituted.

From the above evidence trimethoxycoumarin can be represented by either (XVIA) or (XVIB). However, from the



(XVI A)



(XVI B)

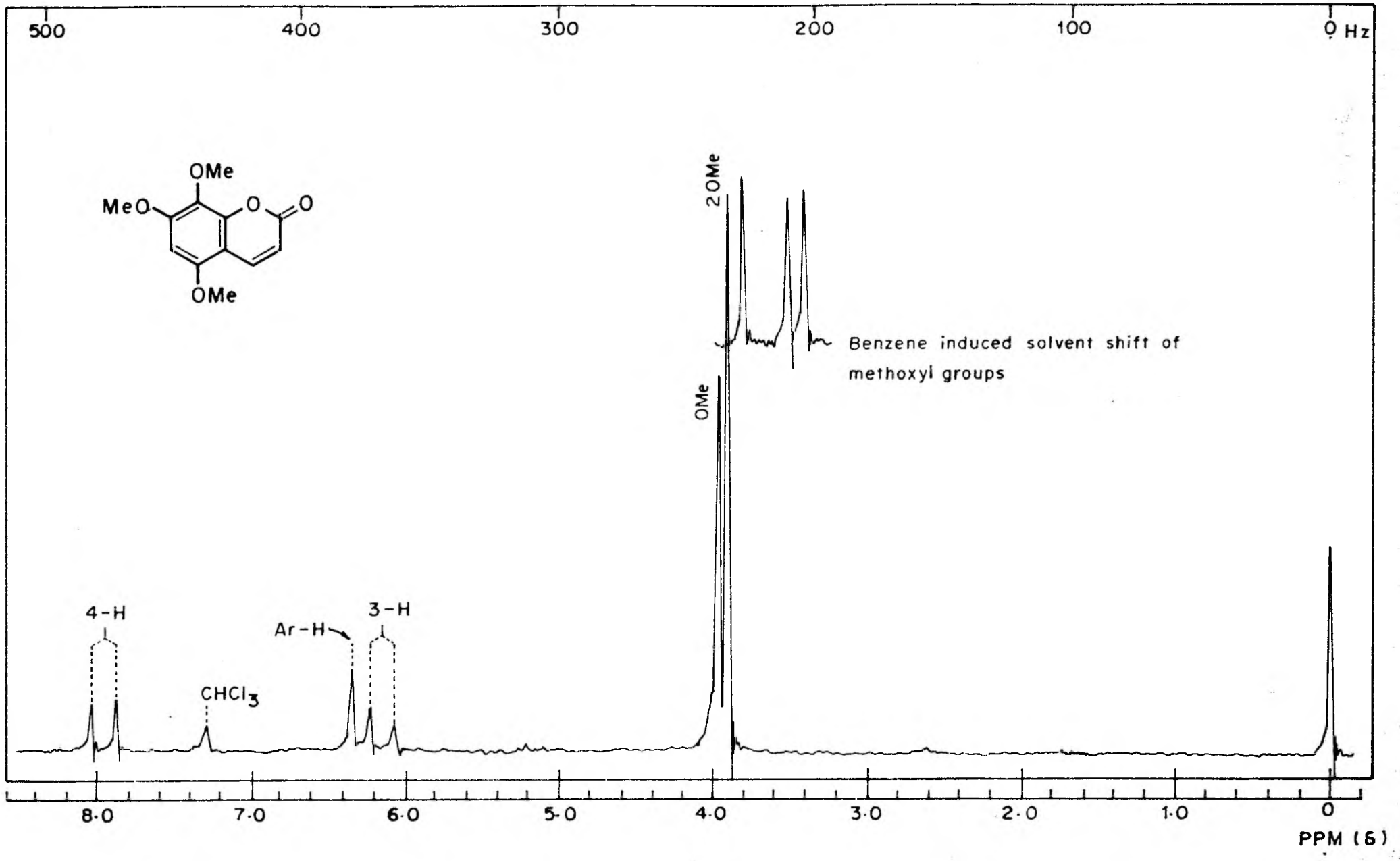
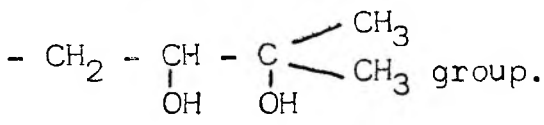


FIG. 3: NMR SPECTRUM OF 5,7,8-TRIMETHOXY COUMARIN (XVIA) IN $CDCl_3$

biogenetic considerations structure (XVIA) is more favourable. Further it was confirmed by the synthesis (see Section 4). The synthetic and natural products are identical in all respects.

The mass spectrum (Fig.4) shows molecular ion at m/e 236 which is a base peak and other intense peaks at m/e 221 (M-15), 195(M-41), 193(M-43), 178(M-58), 165(M-71), 150(M-86), 135(M-101) and 107 (M-129). The major fragments are shown in chart 3.

Compound 7 (toddalolactone), obtained as colourless plates from acetone-hexane mixture, m.p. 132°, $C_{16}H_{20}O_6$, M^+ 308, $\nu_{max.}$ 1720 (α,β-unsaturated lactone), 3310 cm^{-1} (-OH). Under standard conditions it forms a diacetate, m.p. 112°. The NMR spectrum in $CDCl_3$ indicates two doublets (J=10 Hz) at 7.90 and 6.20 for C-4 and C-3 coumarin protons, a singlet at 6.60(Ar-H), alongwith a sharp six proton singlet at 3.90(2 OMe groups). A single proton quartet at 3.64, two proton multiplet at 2.77, a broad singlet at 2.64 integrating for two protons (exchanges with D_2O) indicating two alcoholic hydroxyl groups, along with a singlet at 1.30 for two methyl groups, shows that the compound has



group.

From the above data compound 7 was characterised as toddalolactone (VI) reported earlier[4,6,10,26] from the root bark by Dey and Pillay.[9]

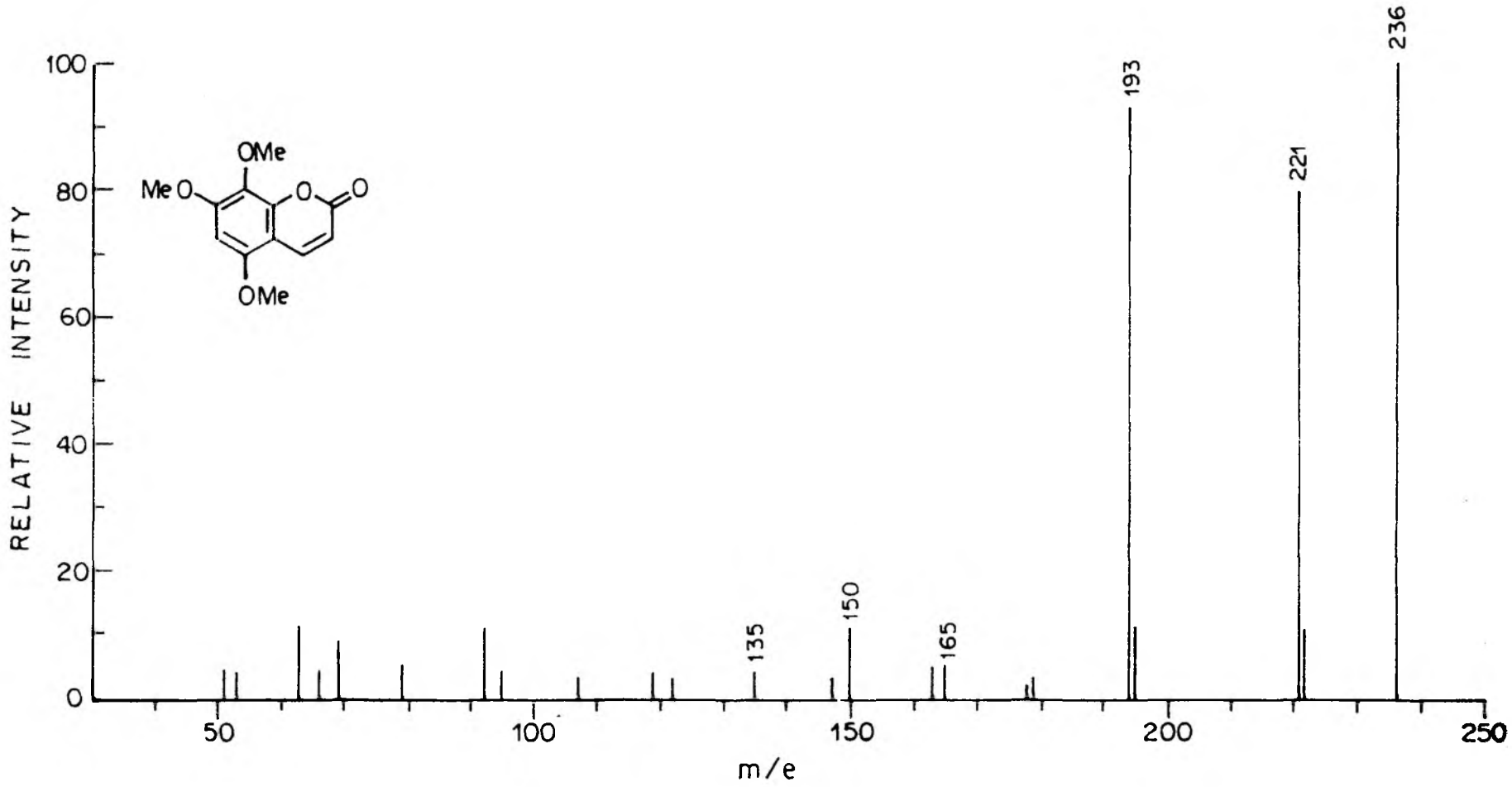
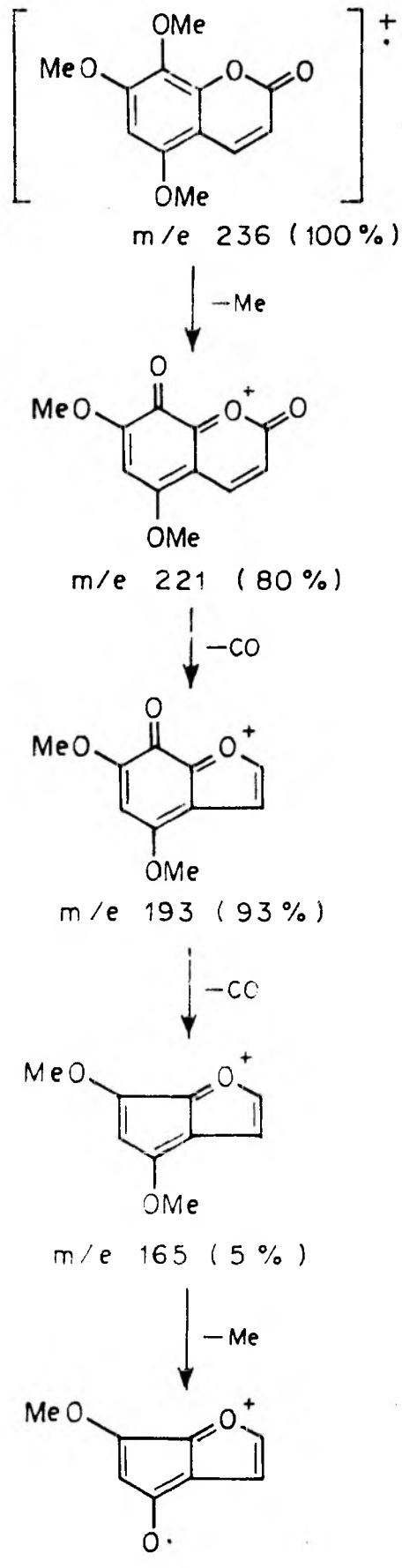

OMe
Me O
O
O
OMe
RELATIVE INTENSITY
100
80
60
40
20
0
193
221
236
135
150
165
50
100
150
200
250
m/e

FIG. — 4.

CHART-3.

MASS SPECTRAL FRAGMENTATION OF 5,7,8-TRIMETHOXYCOUMARIN



In this connection it may be interesting to note that eventhough Dragendorff's reagent is specifically used for the detection of alkaloids, it has been observed that coumarins develope orange colour with this reagent.

Methanol extract

The chloroform soluble part of the methanol extract was not examined further, as it contained same compounds as mixed benzene-chloroform extracts of the stem. Methanol extract gave a positive test for free sugars and glycosides. A part of the methanol extract was treated with cold water, water soluble part indicated the presence of glucose, rhamnose, arabinose and sucrose with authentic samples used for comparison (paper chromatography, n-butanol-ethanol-water). The water insoluble part did not show separation on different adsorbents (cellulose, polyamide, silica gel) using different solvent systems, hence it was not worked up further.

EXPERIMENTAL

Stem of Toddalia aculeata

Extraction:

The powdered stem (3 kg) was extracted with cold chloroform in an aspirator bottle. Removal of the solvent gave greenish syrupy extract (50 g). The extract was adsorbed on extracted stem powder (200 g) and successive soxhlet extraction with hexane, benzene and chloroform gave 15 g., 16 g. and 19 g. of extracts respectively.

After the cold chloroform extraction, the stem powder was extracted with methanol. Removal of methanol gave a brown residue, which was adsorbed on exhausted stem powder (300 g) and reextracted with chloroform and methanol to give 20 g. and 60 g. extracts respectively.

Hexane extract

The hexane extract (15 g) mainly contained waxes and oils, hence it was not worked up further.

Benzene and chloroform extracts

The benzene and chloroform extracts showed similar behaviour on TLC silica gel (hexane-acetone, 8:2) and were mixed together. The combined extract (35 g) was taken up in chloroform (700 ml) and extracted with 5% hydrochloric acid (500 ml). Aqueous layer was neutralised with sodium hydroxide,

maintaining the pH 7 of the solution, and reextracted with chloroform (1.5 lit.). The chloroform was distilled off to give the mixture of alkaloids (11 g). After the separation of alkaloids, chloroform solution was extracted with 5% sodium hydroxide (300 ml). Aqueous layer was separated, neutralised with hydrochloric acid (1:1) and reextracted with chloroform (500 ml). Removal of chloroform, furnished phenolic fraction (3 g). After the removal of alkaloid and phenolic fractions, the chloroform layer was washed with water, saturated brine and dried over anhydrous sodium sulphate. Removal of solvent gave a gummy neutral fraction (19 g).

Chromatography of alkaloidal fraction

Examination of the alkaloidal fraction showed a mixture of six compounds on TLC silica gel (benzene-chloroform, 1:1; acetone-hexane, 2:8). The alkaloidal fraction (11g) was chromatographed on a column of silica gel (250 g), using benzene, benzene-chloroform and chloroform for elution. Different fractions (500 ml each) were collected and monitored on TLC silica gel. Like fractions were mixed and worked up further.

Fractions 1-5 (1.56 g) were complex mixture of alkaloids with close Rf values and their separation on silica gel or alumina columns was unsuccessful.

Fractions 6-12 were found to be a mixture of three alkaloids A, B and C. Solvent was removed from these fractions

and the residue (1.2 g) was purified by passing through a column of silica gel (15 g). Elution of column initially with hexane and then increasing the percentage of acetone in hexane yielded three alkaloids in pure form. Alkaloid A crystallised from acetone-hexane mixture as yellow needles (0.035 g), m.p. 165°, and was found to be identical with a known alkaloid, toddalinine (II) (lit.[7] m.p.165°). (Found: C, 72.05; H, 5.41; N, 3.97. $C_{21}H_{19}O_4N$ requires: C, 72.21; H, 5.45; N, 4.01%). Alkaloid B was crystallised from ethanol in colourless plates (0.08 g), m.p. 148°. It was characterised as robustine (8-hydroxydictamine, XI). (lit.[20,21] m.p. 147-48°). (Found: C, 67.02; H, 3.71; N, 6.56. $C_{12}H_9O_3N$ requires C, 66.98; H, 4.18; N, 6.51%).

Methylation of rubustine

Robustine (0.040 g) in dry methanol (5 ml) was methylated by ethereal diazomethane at 0° overnight. Usual work up gave a gummy residue, which on crystallisation from ethyl alcohol gave colourless prisms (0.035 g), m.p. 141° (lit.[16] m.p.142°), identical with an authentic sample of γ-fagarine (XII).

Alkaloid C was crystallised from methylene-chloride-hexane in small yellow pyramids (0.1 g), m.p. 176°. It was identified as skimmianine (XIII) (lit.[16] m.p. 176°). (Found: C, 64.88; H, 5.17; N, 5.69. $C_{14}H_{13}O_4N$ requires C, 64.86; H, 5.05; N, 5.41%).

Fractions 13-21 (1.8 g) were a mixture of 8-hydroxy-dictamine and skimmianine which was not separated further.

Fractions 22-26 were a mixture of skimmianine and alkaloid D. Removal of the solvent from these fractions gave a residue (0.5 g), which was chromatographed on a column of neutral alumina (5 g) using hexane-acetone (8:2) mixture for elution. Initial fractions gave more of skimmianine. Alkaloid D was obtained from later fractions. It was crystallised from hexane-acetone mixture as colourless prisms (0.045 g), m.p.132^{o}, and was characterised as dictamine (X) on the basis of spectral and analytical data (lit.[16] m.p. 132-33^{o}) (Found: C, 72.56; H, 4.58; N, 7.11. $C_{12}H_9O_2N$ requires C, 72.44; H, 4.52· N, 7.07%).

Fractions 27-39 (2.2 g) were a complex mixture with very close Rf values and attempts to isolate pure compounds were failed.

Fractions 40-61 (Alkaloid E) were homogeneous on TLC silica gel (acetone-hexane 3:7). The alkaloid was crystallised from ethanol as colourless prisms (1.8 g), m.p. 141^{o}. It was identified as Y-fagarine (XII) (lit.[16] m.p.142^{o}). (Found: C, 68.37; H, 4.76; N, 6.32. $C_{13}H_{11}O_3N$ requires C, 68.13; H, 4.80; N, 6.11%).

Fractions 62-66 (0.4 g) contained Y-fagarine and alkaloid F in minor quantity.

Fractions 67 and 68 (0.1 g) were purified by passing through a column of silica gel (5 g) using acetone-hexane (3:7) as eluent. Alkaloid F (m.p.196°: 0.005 g) was obtained from initial fractions. It was not characterised further, as the quantity was insufficient.

Isolation of norbraylin (XVIII) from phenolic portion

The gummy phenolic portion (3 g) from mixed benzene-chloroform extracts showed the presence of six compounds with close Rf values on TLC (silica gel, hexane-acetone, 7:3). It was chromatographed on a column of silica gel (70 g) using hexane-acetone (8:2) mixture for elution. The fractions (150 ml) collected were examined on TLC silica gel (hexane-acetone, 7:3) and similar fractions pooled together.

Fractions 1-5 (0.4 g) contained a mixture of three compounds with close Rf values. Attempts to isolate these compounds by rechromatography and preparative layer chromatography were unsuccessful.

The residue (0.3 g) from fractions 6-9 was chromatographed over a column of silica gel (7 g), using hexane-acetone mixture (8:2) for elution. A colourless crystalline compound obtained was recrystallised from acetone-hexane mixture, as colourless needles (54 mg), m.p. 144-45°. It was assigned structure (XVIII) on the basis of spectral and chemical properties and characterised as norbraylin (Found: C, 68.98; H, 5.05. $C_{14}H_{12}O_4$ requires C, 68.85; H, 4.91%).

Methylation of norbraylin (XVIII)

Norbraylin (20 mg) was methylated with excess of ethereal diazomethane in dry methanol at 0° for 12 hr . The gummy methyl ether was purified by preparative layer chromatography (silica gel, acetone-hexane, 3:7) and subsequent crystallisation from hexane gave yellow needles (5 mg), m.p. 150°. It was identical with/known coumarin braylin (XVII) (lit.[22] m.p. 150°).

Chromatography of neutral portion

The neutral portion (19 g) of combined benzene-chloroform extract showed the presence of nine compounds on TLC silica gel (benzene-chloroform, 1:1; hexane-acetone, 7:3). It (15 g) was chromatographed on a column of silica gel and eluted with benzene-chloroform mixture with increasing percentage of chloroform. Like fractions (250 ml each) were mixed, monitoring the separation on TLC.

Fractions 1-8 (2 g) were not worked up further.

The residue (0.6 g) from fractions 9-14 contained compound 1 with traces of slower moving impurity. It was rechromatographed on a column of silica gel (6 g) and eluted with hexane-acetone (8:2) mixture, gave pure compound 1, crystallised from hexane-acetone as colourless silky needles (0.35 g), m.p. 188° and characterised as bergapten (XIV) (lit.[23] m.p. $188\text{-}89^{\circ}$) (Found: C, 66.71; H, 3.95. $C_{12}H_8O_4$ requires C, 66.7; H, 3.7%).

The next fractions 15-17 contained compounds 2 and 3 and were mixed, concentrated, and the residue (0.5 g) was rechromatographed over a column of silica gel (5 g) with hexane-acetone (8:2) as a solvent for elution. Compound 2 was crystallised from benzene-hexane mixture in colourless needles (0.040 g), m.p. 148-49° and was identified as braylin (XVII) (lit.[22] m.p.150°). (Found: C, 69.58; H, 5.73. $C_{15}H_{14}O_4$ requires C, 69.77; H, 5.43%). Compound 3 crystallised from hexane in pale yellow needles (0.055 g), m.p. 109°. This was identical with/a known coumarin, luvangetin, (XV) (lit.[24] m.p. 108-109°). (Found: C, 69.34; H, 5.98; $C_{15}H_{14}O_4$ requires C, 69.77; H, 5.43%).

Fractions 18-24 (0.8 g) contained exclusively luvangetin.

Fractions 25-28 (0.6 g) were a mixture of luvangetin and compound 4, which were not separated further.

The residue (0.380g) from fractions 29-32 was crystallised from benzene in pale yellow needles (0.260 g) (compound 4), m.p. 149°. It was found to be identical with a known furanocoumarin, isopimpinellin (IX) (lit.[7] m.p.150°). (Found: C, 63.16; H, 3.74. $C_{13}H_{10}O_5$ requires C, 63.4; H, 4.07%).

The residue (1.5 g) from fractions 33-41 contained a mixture of isopimpinellin and compound 5. The residue was purified by passing through a column of silica gel (15 g),

using benzene-acetone (9:1) for elution, yielded more of isopimpinellin and compound 5. The latter compound was crystallised from methanol as yellow needles (0.3 g), m.p.117°, and was characterised as pimpinellin (VIII) (lit.[7] m.p.118°). (Found: C, 63.16; H, 3.84. $C_{13}H_{10}O_5$ requires C, 63.4; H, 4.07%).

Fractions 42-49 were complex mixtures.

Fractions 50-52 were mixed (0.250 g) and purified by passing through a short column of neutral alumina (5 g) with hexane-acetone (8:2) as eluent. The compound 6 obtained on repeated crystallisation from benzene gave colourless needles (0.045 g), m.p. 180°, and was characterised as trimethoxy-coumarin for which two alternative structures have been proposed. (Found: C, 61.5; H, 5.3. $C_{12}H_{12}O_5$ requires C, 61.0; H, 5.1%).

Fractions 53-68 (2 g) (compound 7) were homogeneous on TLC (silica gel, hexane-acetone, 6:4). It was crystallised from hexane-acetone mixture as white plates (1.7 g), m.p.132° $(\alpha)_D$ + 55°, and was identified as toddalolactone (VI) (lit.[4,10] m.p. 132°) (Found: C, 62.53; H, 6.53. $C_{16}H_{20}O_6$ requires: C, 62.34; H, 6.49%).

Acetylation of toddalolactone

A mixture of toddalolactone (0.5 g), pyridine(2 ml) and acetic anhydride (3 ml) was heated on a water bath for 0.5 hr, and left at room temperature overnight. Usual work up gave a

solid which was crystallised from benzene-hexane as colourless needles (0.510 g), m.p. 112° (lit.[10] m.p. 111-112°).

Examination of methanol extract

The chloroform soluble part (15 g) of the methanol extract, showed the presence of same compounds isolated from benzene-chloroform extracts, hence was not examined further.

Methanol extract (60 g) was a complex mixture of glycosides and free sugars. The extract (10 g) was treated with cold water (150 ml), the solid (2 g) separated was filtered. The filtrate showed the presence of glucose, rhamnose, arabinose and sucrose with an authentic sample of sugars used for comparison on paper chromatogram (solvent system: butanol-ethanol-water, 4:1:5; silver nitrate in acetone and sodium hydroxide in ethanol as spray reagent). The solid did not show any separation on different adsorbents [silica gel, acetone-benzene (1:1); methanol-chloroform (4:6); polyamide, methanol; cellulose, acetic acid-water (3:7)] and hence was not examined further.

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Section 2

Skimmia laureola

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INTRODUCTION

Skimmia Thunb[1-5] (Family: Rutaceae, sub-family: Toddaliodeae) is a small genous of evergreen glabrous shrubs distributed in Himalayas from Kashmir to Kumaon and also found in China, Japan and Pakistan. The genus comprises of 5-6 species,[2,3] out of which four species, S. laureola, S. foremanii, S. fortunii, and S. wallichii, occur in India; the other two species, S.japonica and S. repens are found in Japan.[4]

Skimmia are grown for their handsome foliage and red berries.[1] They are suitable for borders of evergreen shrubberies and are popular for planting in cities. They grow preferably in a partly shaded situation. They are of rather slow growth and thrive best in a moist loamy soil, but also grow well in a stony clay soil.

Leaves of Skimmia species are widely used in perfumeries and ayurvedic medicines. They are used in small pox. The smoke produced by burning is said to purify the air. In Kashmir leaves are used as incense.[6] They are musk-scented and eaten in curries by hill tribes and also used for flavouring food.[1,6]

S. laureola Hook.

S. laureola commonly known as "Garhwal-Nair"[6] is an evergreen strongly aromatic shrub, found in temperate region of North Western Himalayas from Kashmir to Kumaon, Khasia hills of Assam, Swat State and Mathia Galli hills in Pakistan. It is often a small tree in Sikkim.[5] It is branched from the base. Wood is white, hard, heavy with concentric lines. Leaves alternate, simple, exceedingly variable in size. Flowers are yellowish white. The plant bears small oval shaped red fruits, which have not been put to any use so far.

In the indigenous system of medicine, burning of S. laureola leaves near small-pox patients is believed to have a curative effect.[7] The leaves are now used on large scale for the extraction of essential oil, which is an important commercial product.[8] The oil is potential source of linalyl acetate. It is used in perfumery as a substitute of petitgrain oil of French origin.[9]

Simonsen[10] isolated linalylacetate, in addition to an alcohol linalool and complex mixture of sesquiterpene alcohols and esters from the leaves. Chatterjee and Bhattacharya[11] while isolating skimmianine from the leaves found that the leaves contain a dimethoxyfurocoumarin, isopimpinellin(XVI), umbelliferone (V) and laureoline. They have examined the bark for the first time and found the same constituents as found in leaves.

From a sample of oil obtained by steam distillation of fresh leaves, Sharma et al.[8] isolated linalyl acetate as the chief constituent alongwith the alcohol linalool. Other constituents of the oil include geraniol, nerol, citral, citronellylformate, citronellylisobutyrate, ∝-pinene, myrecene, β-phellandrene and methylheptenone. Bhargav and Seshadri[13] reported the isolation of lupenone, lupeol, β-sitosterol, along with an alkaloid skimmianine (II) and coumarins, isolated by earlier investigators, and sucrose from the leaves. Bhattacharjee and Mullick[12] reported the isolation of skimmin (umbelliferone glucoside VI), umbelliferone and scopoletin(VII) from the trunk bark. Pasha et al.[4] have examined S.laureola seeds for their oil and protein constituents. They have shown that the oil contains palmitic, stearic, palmitoleic, oleic, linoleic, and linolenic acid and proteins are constituted of aspartic acid, argenine, alanine, glutamic acid, glycine, lucine, isolucine, lycine, prolein, methionine, serine and threonine. The presence of pelargonidin and an alkaloid skimmianine is also indicated.

Table 1 lists the alkaloids and coumarins of Skimmia species.

TABLE - 1

ALKALOIDS AND COUMARINS OF *SKIMMIA* SPECIES

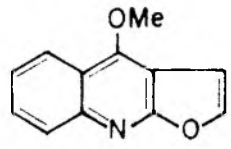
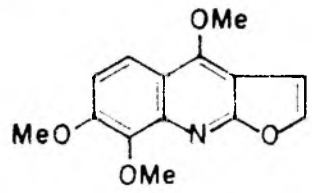
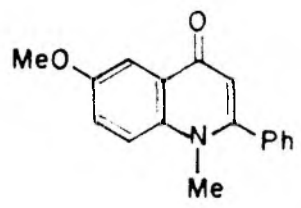
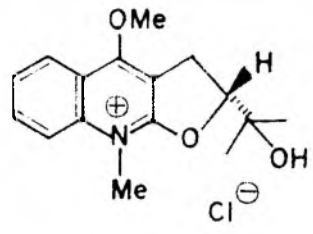
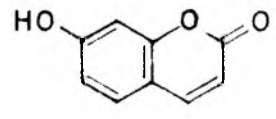
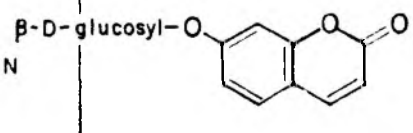
STRUCTURE NO.	NAME	STRUCTURE	SKIMMIA					REFERENCE
			foremanii	fortunii	japonica	laureola	repens	
I	DICTAMINE		+	–	+	–	+	14,15,16
II	SKIMMIANINE		–	–	+	+	–	15,4,11, 13,17
III	EDULINE		–	–	+	–	–	15
IV	PLATYDESMINIUM SALTS		–	–	+	–	–	15,16
V	UMBELLIFERONE		–	–	+	+	–	18,11,12
VI	SKIMMIN		–	+	+	+	–	21,20,12

TABLE-1 (CONTD.)

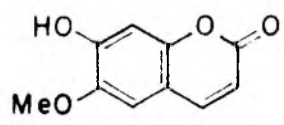
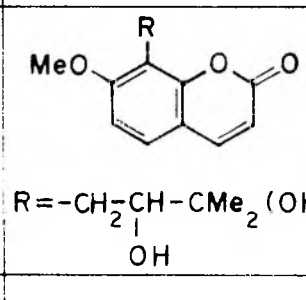
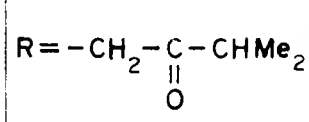
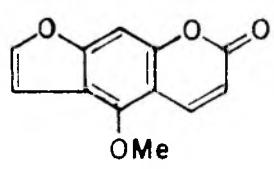
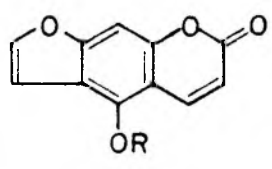
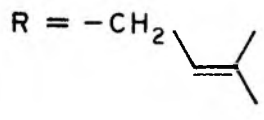
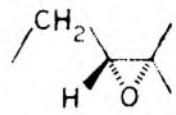
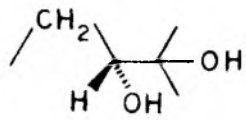
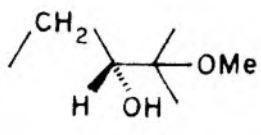
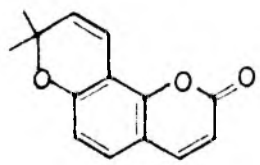
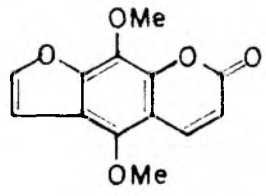
STRUCTURE NO.	NAME	STRUCTURE	SKIMMIA					REFERENCE
			foremanii	fortunii	japonica	laureola	repens	
VII	SCOPOLETIN		–	–	+	+	–	21,12
VIII	(+)-MERANZIN-HYDRATE		–	–	+	–	–	18
IX	ISOMERANZIN		–	–	+	–	–	18
X	BERGAPTEN		–	–	+	+	–	11,13
								
XI	ISOIMPERATORIN		+	–	+	–	–	14,20,1[illegible]

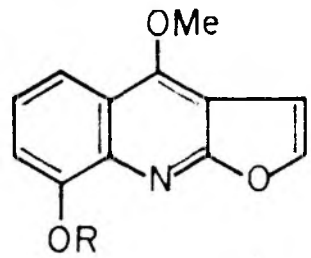
TABLE - 1 (CONTD.)

STRUCTURE NO	NAME	STRUCTURE	SKIMMIA					REFERENCE
			foremanii	fortunii	japonica	laureola	repens	
XII	(+)-OXY-PEUCEDANIN		+	–	+	–	–	14,20,18
XIII	(+)-OXYPEUCE-DANIN HYDRATE		–	–	+	–	–	18
XIV	(+)-OXYPEUCE-DANIN METHANO-LATE		–	–	+	–	–	18
XV	SESELIN		–	–	+	–	+	22,21
XVI	ISOPIMPINELLIN		–	–	–	+	–	11

PRESENT WORK

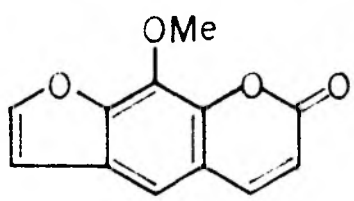
With our continued interest in the examination of medicinal plants belonging to family Rutaceae, chemical investigation of *Skimmia laureola* has been undertaken. Despite the extensive studies on the leaves and stems mainly for the isolation of essential oil, the alkaloids and coumarins have been studied relatively to a less extent. In the present work, the isolation of alkaloids and coumarins from the branches alongwith leaves of *S. laureola* is described.

The dried branches were obtained from M/s Mukherjee and Co., Darjeeling. The chemical investigation of branches led to the isolation of four known furoquinoline alkaloids: dictamine(I), 8-hydroxydictamine(XVII), Υ-fagarine(XVIII), not reported earlier from this plant, alongwith skimmianine(II), the principle alkaloid of *Skimmia* species. In addition to these, three coumarins, xanthotoxin (XIX), a furanocoumarin isomeric with bergapten (X), aesculetindimethyl ether(XX) and a dihydrofuranocoumarin, (+)-marmesin(XXI), have been isolated for the first time from this source. This is the first report of the occurrence of dihydrofuranocoumarin in *Skimmia* species. Besides these compounds, umbelliferone(V), isopimpinellin(XVI), bergapten (X), lupeol and β-sitosterol, have been isolated, which have been reported by earlier workers.[11-13]

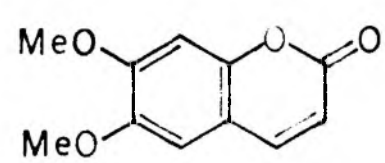


(XVII) 8-Hydroxy dictamine, R = H

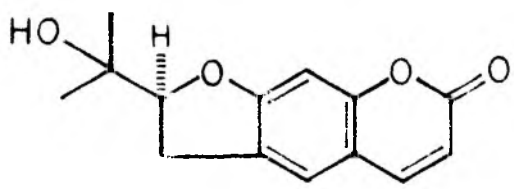
(XVIII) γ-Fagarine, R = Me



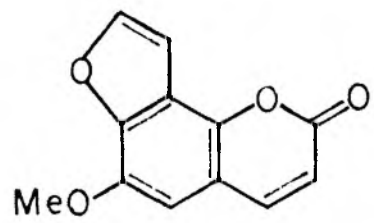
(XIX) Xanthotoxin



(XX) Aesculetin dimethyl ether



(XXI) (+)-(S)-Marmesin



(XIX A) Sphondin

The powdered branches were successively extracted in a soxhlet with hexane and chloroform. The solvents were distilled off to afford 0.5 and 1% extracts respectively.

Hexane extract: The chromatography of the hexane extract over a column of silica gel (hexane, hexane-benzene) afforded an oil and two homogeneous compounds which were identified as lupeol, m.p. 215° (M^+ 426), and β-sitosterol, m.p. 135°. Their identity was confirmed by direct comparison with the authentic samples. These two compounds were reported from leaves by Bhargav and Seshadri.[13]

Chloroform extract: Extraction of the chloroform extract with 10% sodium carbonate led to the isolation of a coumarin, unbelliferone, which crystallised from ethyl acetate as colourless needles, m.p. 232°, $C_9H_6O_3$, M^+ 162. $\nu_{max.}$ 1710 (α,β-unsaturated lactone), 3350(-OH) cm^{-1}. The NMR spectrum in acetone shows coumarin doublets (J = 10 Hz) at 6.20(3-H), 7.82(4-H), 6.77(s, 8-H), 6.87 (m, 6-H), 7.45 (d, J=9 Hz, 5-H). Umbelliferone was reported earlier from leaves by Chatterjee and Bhattacharyya[11] and later by Bhargav and Seshadri.[13]

The chloroform extract, after the separation of umbelliferone, was treated with 5% hydrochloric acid. Aqueous layer was neutralised (pH 7) with sodium hydroxide(10%) and reextracted with chloroform. Alkaloidal part showed a mixture of five alkaloids on TLC with silica gel (acetone-benzene, 1:9). Non-alkaloidal part showed a mixture of 6 compounds.

Alkaloidal part: Chromatography of the alkaloidal part over a column of silica gel using benzene and benzene-acetone mixture with increasing polarity for elution afforded five alkaloids (1-5).

Alkaloid 1, crystallised from benzene-acetone mixture as colourless plates, m.p. 148°, $C_{12}H_9O_3N$, M^+ 215, identified as 8-hydroxydictamine (XVII) by direct comparison with an authentic sample (mixed m.p. and TLC behaviour).

Alkaloid 2; obtained as yellow needles from methanol, m.p. 176°, $C_{14}H_{13}O_4N$, M^+ 259. Its identity with skimmianine (II) was confirmed by TLC comparison with an authentic sample and by mixed m.p.

Alkaloid 3, crystallised from benzene as colourless prisms, m.p.132°, $C_{12}H_9O_2N$, M^+ 199. It was identified as dictamine (I), (mixed m.p. and superposable IR).

Alkaloid 4, crystallised from benzene as colourless cubes, m.p. 142°, $C_{13}H_{11}O_3N$, M^+ 229. It was found to agree in all respects with the authentic sample of γ-fagarine(XVIII) (mixed m.p., TLC behaviour and superposable IR).

Alkaloid 5, crystallised from benzene as colourless needles, m.p. 138-40°, M^+ 235. The alkaloid was obtained in poor yield. Further work on this compound could not be done owing to the paucity of the material.

Examination of non-alkaloidal portion

Non-alkaloidal portion showed a mixture of six compounds with very close Rf values on silica gel TLC plates (benzene-acetone, 7:3). It was chromatographed on a column of silica gel, using benzene and benzene-acetone mixture for elution. Percentage of acetone was slowly increased to 20%. Fractions (250 ml each) were collected, monitored on TLC silica gel. Like fractions were pooled together and final purification achieved by rechromatography over a column of silica gel, neutral alumina or preparative TLC. Five coumarins (A-E) in the decreasing order of Rf values have been isolated.

Compound A (Bergapten), crystallised from benzene-hexane as pale yellow silky needles, m.p. 190°, $C_{12}H_8O_4$, M^+ 216. Its IR shows band at 1710 cm^{-1} (α,β-unsaturated lactone). Compound A was characterised as bergapten (X) by direct comparison with an authentic sample and mixed m.p.

Compound B (Xanthotoxin), was obtained in colourless needles from benzene-hexane mixture, m.p. 145-46°, $C_{12}H_8O_4$, M^+ 216, ν max. 1710 cm^{-1} (α,β-unsaturated lactone). The NMR in $CDCl_3$ reveals the presence of characteristic furanocoumarin doublets at 6.28 and 7.67 (d, 1H each, J=10 Hz, 3-H and 4-H of a coumarin); 6.75 and 7.64 (d, 1H each, J=2.5 Hz, 3'-H 2'-H of a furan), 7.25 (s, 1H, Ar-H) and 4.24 (s, 3H, Ar-OMe), 4-H of coumarin is not deshielded indicating the absence of oxygen function at C-5. Hence the methoxyl may be at C-6 or C-8.

Based on this data two structures (XIX) and (XIXA) were considered for the furanocoumarin. The angular structure is ruled out because of the general observation that the 3'-H of an angular furanocoumarin is seen around 7.10 to 7.20 compared with the chemical shift of the same proton in the linear furanocoumarin.[30] Moreover, structure (XIXA) corresponds to a known coumarin sphondin, m.p.[31] 189-90, which differs from compound B. Its mass spectrum shows molecular ion at m/e 216 (base peak) and other peaks at m/e 201 (M-15), 188 (M-28), 173 (M-15-28), 145(M-71). All these data correspond to a known furanocoumarin xanthotoxin (XIX), not reported previously from this source, although it has been isolated from a number of Rutaceous plants.[23,24]

Compound C (isopimpinellin), obtained as pale yellow needles from benzene, m.p. 149°, $C_{13}H_{10}O_5$, M^+ 246. IR indicates band at 1710 cm^{-1} (α,β-unsaturated lactone). It was identified as isopimpinellin (XVI) by comparison with the authentic sample and mixed m.p. It was isolated from leaves by Chatterjee and Bhattacharya[11], and later by Bhargav and Seshadri.[13]

Compound D (aesculetin dimethyl ether), colourless needles from benzene, m.p. 146°, $C_{11}H_{10}O_4$, M^+ 206. IR shows band at 1720 cm^{-1} (α,β-unsaturated lactone). The NMR spectrum in $CDCl_3$ shows characteristic coumarin doublets (J=10 Hz) at

6.27 (3-H) and 7.60(4-H) alongwith two three proton singlets at 3.90 and 3.97 (2 OMe groups). A two proton singlet appears at 6.84 (Ar-H). Its mass spectrum shows molecular ion as base peak at m/e 206 and other peaks at m/e 191 (M-15), 178(M-28), 163(M-15-28), 135(M-71), 120(M-86) and 107(M-71-28). On the basis of the above spectral data, compound D was characterised as aesculetindimethyl ether (XX). Although it is isolated for the first time from this source, it was reported earlier from Artemisia species and Zanthoxylum setosum.[24]

Compound E [(+)-(S)-marmesin], rhombic plates from ethyl alcohol, m.p. 186-87°, $C_{14}H_{14}O_4$, M^+ 246, $(\alpha)_D^{30}$ + 30° (in chloroform), ν_{max}. 1700 (conjugated lactone), 3450 cm^{-1} (-OH). On treatment with sodium acetate and acetic anhydride, it gave an acetate, m.p. 130°. Compound E was identified as (+)-(s)-marmesin (XXI) from its spectral and chemical properties. The NMR in $CDCl_3$ shows characteristic coumarin doublets (J=10 Hz) at 6.15 (3-H) and 7.50 (4-H): 7.15(s, 1H, 8-H), 6.70(s, 1H, 5-H), 4.70 (t, J=9 Hz, 1H, -CH), 3.20 (d, J=9 Hz, 2H, Ar-CH_2), 2.27 (s, exchanges with D_2O, -OH), 1.40 and 1.25 (two singlets, 3H each, -$C(CH_3)_2$). Its mass spectrum shows molecular ion at m/e 246 and peaks at m/e 188 (M-C_3H_6O), 187 (M-C_3H_7O or M-C_3H_6O-H, base peak), 228(M-H_2O), 213 (M-H_2O-CH_3). The S-configuration of (+)-marmesin has recently been established elegantly by chemical correlation experiments by Harda et al.[25]

EXPERIMENTAL

The dried branches alongwith leaves, obtained from M/s Mukherjee and Co., Darjeeling, were powdered (3 kg) and soxhletted successively with hexane and chloroform. Removal of the respective solvents gave 15 g. and 30 g. of the extracts.

Hexane extract: It yielded a brown oil (10 g), which was chromatographed on a column of silica gel (200 g) and eluted with benzene and benzene-acetone mixture. Percentage of acetone in benzene was slowly increased to 10%. Fractions (250 ml each) were collected and monitored by TLC silica gel (benzene-acetone, 8:2).

Fractions 1-20 (5.2 g) contained oil and waxes.

The residue (0.5 g) from fractions 21-23 was crystallised (0.350 g) from chloroform-methanol, m.p.214-15°. It gave a positive Libermann-Burchard test and was identified as lupeol, by direct comparison with an authentic sample and mixed m.p. (lit.[13] m.p. 212-13°).

Fractions 24-33 (1 g) were complex mixture.

The residue (1.3 g) from ffactions 34-40 was crystallised from methanol in colourless needles (1.05 g), m.p.135°, $(\alpha)_D$ - 30° (in $CHCl_3$) and identified as β-sitosterol by comparison with the authentic sample and mixed m.p. [lit.[13] m.p. 135-36°, $(\alpha)_D$ - 30.6° in $CHCl_3$].

Chloroform extract: It showed a complex mixture on TLC silica gel (acetone-benzene, 2:8) (15 g) and was taken up in chloroform (400 ml); and extracted with 10% sodium carbonate solution (5 x 30 ml). Aqueous layer was neutralised with hydrochloric acid (1:1) reextracted with chloroform (3 x 100 ml), and the chloroform extracts were mixed and dried over anhydrous sodium sulphate. Removal of chloroform gave 0.7 g. of the extract. It was chromatographed over a column of silica gel using benzene as a solvent and crystallised from ethyl acetate as colourless needles (0.3 g), m.p.232°, identified as umbelliferone (V) (lit.[13] m.p. 231-32°; 232°[12]). (Found: C, 66.4; H, 3.8. $C_9H_6O_3$ requires C, 66.67; H, 3.7%).

The chloroform extract after removal of umbelliferone was extracted with 5% hydrochloric acid (5 x 50 ml). Aqueous layer was neutralised with sodium hydroxide (10%), maintaining the pH of solution 7 and reextracting with chloroform (5 x 100 ml). Removal of chloroform afforded a mixture of alkaloids (3 g). Chloroform was distilled off from hydrochloric acid insoluble portion to give a residue (10 g), which showed a mixture of six compounds on TLC silica gel (acetone-benzene, 2:8).

Chromatography of alkaloidal fraction

The alkaloidal portion (3 g) of chloroform extract showed the presence of five alkaloids on TLC silica gel (acetone-benzene, 2:8). It was chromatographed on a column of

silica gel (70 g) and eluted with benzene and then with acetone-benzene mixture with increasing polarity. Fractions of 150 ml each were collected, examined by TLC and identical fractions pooled together.

Fractions 1-3 (0.2g) were not examined.

Fractions 4-9 (0.4 g) (alkaloid 1) were homogeneous on TLC, The residue after removal of solvent was crystallised from benzene-acetone as white plates, m.p. 148° and identified as 8-hydroxydictamine (XVII) by comparison with an authentic sample (lit.[26] m.p. 147-48°) (Found: C, 67.02; H, 4.3; N, 6.50. $C_{12}H_9O_3N$ requires C, 66.98; H, 4.18; N, 6.51%).

Fractions 10-14 (0.3 g) (Alkaloids 1 and 2) were a mixture of 8-hydroxydictamine and slower moving alkaloid. The residue from these fractions on chromatography over a column of neutral alumina (benzene) yielded the alkaloid. It was crystallised from methanol in yellow needles (0.050 g), m.p. 176°, characterised as skimmianine (II) by comparison with an authentic sample. Mixed m.p. with the authentic sample remained undepressed (lit.[11,13] m.p. 175-77°). (Found: C, 64.88; H, 5.17; N, 5.69. $C_{14}H_{13}O_4N$ requires C, 64.86; H, 5.05; N, 5.41%).

Fractions 15-18 (0.3 g) (Alkaloids 2 and 3) were a mixture of skimmianine and dictamine, which were not examined further.

Fractions 19-21 (0.1 g.) (alkaloid 3) crystallised from benzene as colourless prisms, m.p. 132°, characterised as dictamine (I) by direct comparison with the authentic sample and mixed m.p. (Lit.[27] m.p. 132-33°) (Found: C, 72.40; H, 4.53; N, 7.11. $C_{12}H_9O_2N$ requires C, 72.44; H, 4.52; N, 7.07%).

Fractions 22-38 (0.8 g.)(alkaloid 4) crystallised from benzene as colourless cubes, m.p. 142° and identified as γ-fagarine (XVIII) by comparison with the authentic sample and mixed m.p. (lit.[27] m.p. 142°). (Found: C, 68.4; H, 4.73; N, 6.30. $C_{13}H_{11}O_3N$ requires C, 68.13; H, 4.80; N, 6.11%).

Fractions 39-50 (0.6 g) were complex mixture.

The residue from fractions 51-52 (0.03 g) (alkaloid 5) was subjected to preparative layer chromatography (silica gel, acetone-benzene, 2:8) which gave an alkaloid, crystallised from benzene as colourless needles (0.003 g), m.p.138-40°, M^+ 235, and was not characterised further.

Chromatography of non-alkaloidal portion

The non-alkaloidal portion (10 g) was chromatographed on a column of silica gel (250 g) and was eluted with benzene and acetone-benzene mixture with increasing percentage of acetone. A number of fractions (250 ml each) were collected, monitored on TLC (silica gel, acetone-benzene, 2:8) and like fractions mixed together. The results are summarised in Table 1.

Table 1.

Fractions	Approximate weight (g)	Remarks
1-14	3.2	complex mixture(not examined)
15-24	0.8	Faster moving impurity + bergapten.
25-29	0.3	bergapten + xanthotoxin
30-32	0.1	xanthotoxin
33-40	0.6	xanthotoxin + isopimpinellin
41-52	1.5	complex mixture (not examined)
53-55	0.15	aesculetindimethyl ether
56-73	1.1	faster moving impurity + (+)-marmesin.
74-90	2	complex mixture(not examined).

The solvent was removed from fractions 15-24 and the residue (0.8 g) (compound A) chromatographed on a column of neutral alumina (5 g) using benzene as eluent. The slower moving homogeneous compound on crystallisation from benzene-hexane mixture gave pale yellow silky needles (0.2 g), m.p. 190°. It was identified as bergapten (X) by comparison with the authentic sample, and mixed m.p. (lit.[11,13] m.p. 188-90°, 187-89°) (Found: C, 66.63; H, 3.67. $C_{12}H_8O_4$ requires C, 66.7; H, 3.7%).

The residue (0.1 g) from fractions 30-32 (compound B), homogenous on TLC silica gel (acetone-benzene, 1:9) was crystallised from benzene-hexane mixture as colourless needles (0.080 g), m.p.145-46^{o} and characterised as xanthotoxin(XIX) on the basis of spectral data (lit.[19] m.p.145-46^{o}) (Found: C, 66.45; H, 3.63; $C_{12}H_8O_4$ requires C, 66.7; H, 3.7%).

Fractions 33-40 (0.6 g) (compounds B and C) were a mixture of two compounds with very close Rf values and were separated by repeated PLC (silica gel, acetone-hexane, 2:8). Two compounds were separated, the faster moving corresponds to xanthotoxin and the slower moving gave pale yellow needles (0.15 g) from benzene, m.p. 149^{o}; identified as isopimpinellin (XVI) by direct comparison with an authentic sample and mixed m.p. (lit.[11] m.p. 150^{o}).

Fractions 53-55 (0.15 g) (compound D) were crystallised from benzene as colourless needles (0.1 g), m.p. 146^{o}; identified as aesculetindimethyl ether (XX) on the basis of spectral data (lit.[24] m.p. 144-45^{o}) (Found: C, 63.84; H, 4.90. $C_{11}H_{10}O_4$ requires C, 64.07; H, 4.85%).

Fractions 56-73 (1.1 g) (compound E) were purified by passing through a column of silica gel (10 g) using acetone-benzene (1:9) for elution. Initial fractions contained a faster moving impurity. Removal of solvent from the later fractions gave a residue (0.7 g), which on crystallisation

from ethyl alcohol, afforded rhombic plates (0.6 g), m.p. 186-87°, $(\alpha)_D$ + 30° (in chloroform). It was identified as (+)-(S)-marmesin (XXI) on the basis of physical and spectral data[(lit.[25,28] m.p. 186-86.5°, $(\alpha)_D$ + 20.3° (in chloroform). 189.5°, $(\alpha)_D$ + 26.8° (in chloroform)] (Found: C, 68.23; H, 6.01. $C_{14}H_{14}O_4$ requires C, 68,29; H, 5.7%).

Acetylation of (+)-marmesin

A mixture of (+)-marmesin (0.1 g), aceticanhydride (2 ml) and freshly fused sodium acetate (0.25 g) was heated on a water bath for 2 hrs. and then the mixture kept at room temperature overnight. The mixture was poured on ice, separated solid filtered, washed with ice-cold water, and crystallised from benzene as colourless plates (0.08 g), m.p. 130° (lit.[28] m.p. 130°).

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Section 3

Boenninghausenia albiflora

INTRODUCTION

It is said that the genus Boenninghausenia[1] was named in honour of a German botanist, C.F. Von Boenninghausen (1785-1864).

The plant is a monotypic genus related to Ruta but differing by its smaller white flowers with a white stalked ovary and carpels connate only at the base. It is a native of Asia from Himalaya eastwards. The genus includes two species,[3,5] B. albiflora and B. japonica. The latter is found only in Japan.

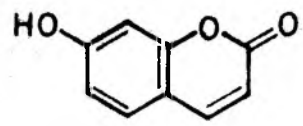
B. albiflora

B. albiflora[1-3] Reichb (synonym Ruta alba, Family: Rutaceae; subfamily: Rutoideae) is a perennial shrub or herb found in temperate Himalayas from Mari to Sikkim[4] and also in Japan. It is glaborous, branched with white shoots, leaves glaucous grey, leaflets numerous, with translucent oil glands. Flowers are small and white. The plant is beautiful and needs a well drained soil.

The benzene extract of B. albiflora showed a strong antifeeding activity against the insect larvae, Spodoptera litura.[6] Hosozawa et al.[6] identified these anti-feedants as furano- and pyranocoumarins, bergapten (VI) and xanthyletin (XIV) respectively. The entire plant has medicinal value. It is used as a tonic and a remedy for rheumatic pains and paralysis.

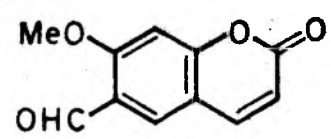
The occurrence of a number of coumarins[7-13] in B. albiflora has been reported, of which a dimeric coumarin, matsukaze lactone (XVII), (-)-nodakenetin acetate (XII) and 3-(1,1-dimethylallyl)-xanthyletin (XV), were reported to be novel.

Ohta et al.[7] have reported a furoquinoline alkaloid dictamine (XXI) and a coumarin bergapten (VI) in the plant; and Miyazaki et al.[8] found a new dimeric coumarin matsukaze lactone (XVII). Nayar et al.[9] examined the aerial parts and reported the isolation of 3-(1,1-dimethylallyl)-xanthyletin (XV), Talpatra et al.[10] isolated six coumarins, xanthyletin (XIV), bergapten (VI), isopimpinellin (VIII), (-)-nodakenetinacetate (XII), xanthotoxin (VII) and daphnetin-8-methyl ether (III) alongwith β-sitosterol from leaves and stem, collected from Darjeeling (W.Bengal). They have first isolated (-)-nodakenetin acetate from natural sources. B. albiflora is the first Rutaceous plant known to produce daphnetin-8-methyl ether (III). Further, they have reported[12] the isolation of three coumarins, angelical (II), 6-(trans-1-buten-3-onyl)-7-methoxycoumarin (IV) and daphnoretin(XVI) along with methyl-p--coumarate (XVIII). Bhan et al.[11] isolated two coumarins angenomalin (XIII) and micropubscin(V) from this plant. In the course of the studies on the fragrant



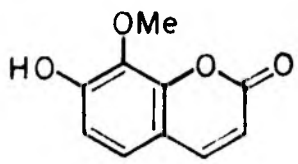
(I)

UMBELLIFERONE



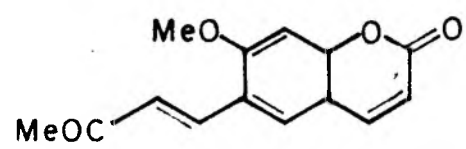
(II)

ANGELICAL



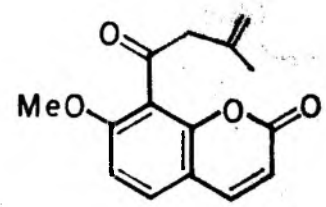
(III)

DAPHNETIN-8-METHYL ETHER



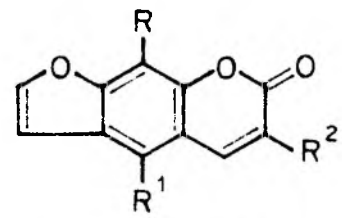
(IV)

6-(trans-1-BUTENE-3-ONYL)-7-METHOXYCOUMARIN



(V)

MICROPUBSIN

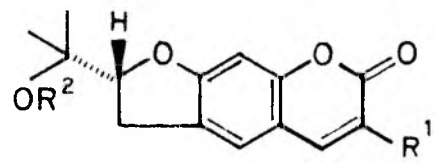


(VI) $R = R^2 = H$, $R^1 = OMe$ BERGAPTEN

(VII) $R = OMe$, $R^1 = R^2 = H$ XANTHOTOXIN

(VIII) $R = R^1 = OMe$, $R^2 = H$ ISOPIMPINELLIN

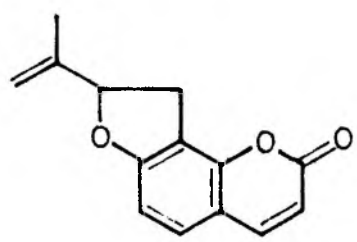
(IX) $R = R^1 = H$,
$R^2 = (CH_3)_2C-CH=CH_2$ CHALEPENSIN



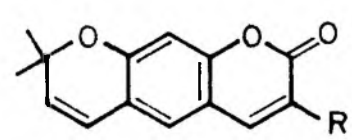
(X) $R^1 = (CH_3)_2C-CH=CH_2$,
$R^2 = COCH_3$ RUTAMARIN

(XI) $R^1 = H$, $R^2 = H$ (−)-NODAKENETIN

(XII) $R^1 = H$, $R^2 = COCH_3$ (−)-NODAKENETIN ACETATE

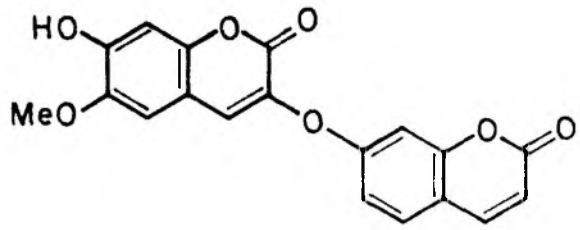


(XIII) ANGENOMALIN

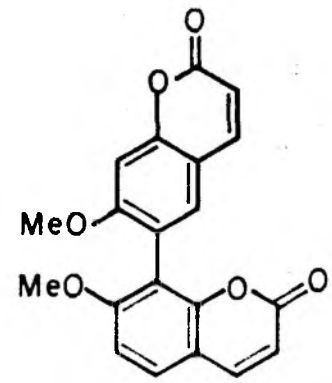


(XIV) R = H XANTHYLETIN

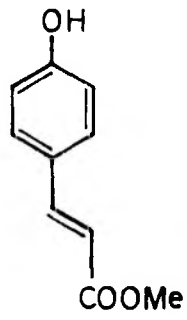
(XV) $R = (CH_3)_2C-CH=CH_2$
3-(1,1-DIMETHYLALLYL)-XANTHYLETIN



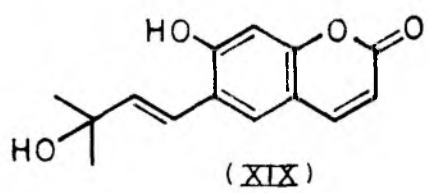
(XVI) DAPHNORETIN



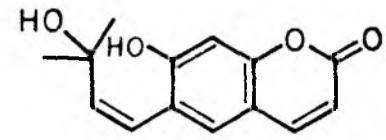
(XVII) MATSUKAZE LACTONE



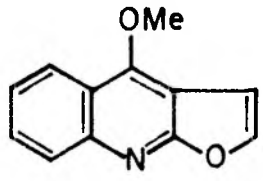
(XVIII) METHYL-p-COUMARATE



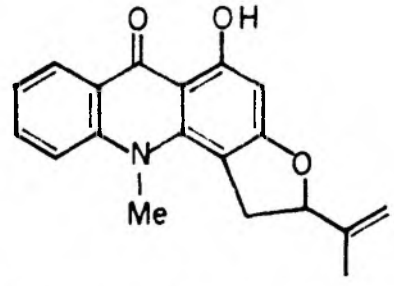
(XIX)
(E)-
7-HYDROXY-6-(3-HYDROXY-3-METHYL-
-1-BUTENYL)-COUMARIN



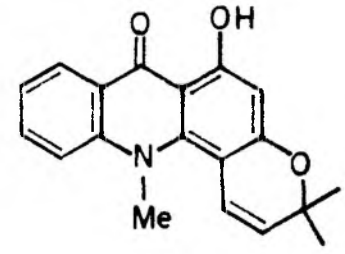
(XX)
(Z)-



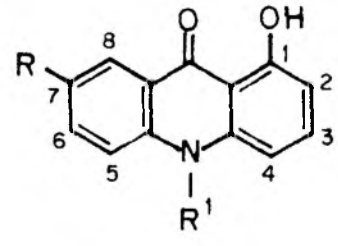
(XXI) DICTAMINE



(XXII) RUTACRIDONE



(XXIII) NORACRONYCINE



(XXIV) $R = R^1 = H$ 1-HYDROXYACRIDONE

(XXV) $R = H, R^1 = Me$ 1-HYDROXY-N-METHYLACRIDONE

(XXVI) $R = OH, R^1 = Me$ 1,7-DIHYDROXY-N-METHYLACRIDONE

components in the essential oil of leaves, Shibata et al.[13] have isolated two isomeric, coumarins (E)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-coumarin (XIX) and (Z)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-coumarin (XX).

In a preliminary communication, Rozsa et al.[14] reported the presence of rutamarin(X) (detected by TLC). They have isolated three acridone alkaloids, one of them was characterised as 1-hydroxy-N-methylacridone(XXV). Later they have also isolated[15] rutacridone(XXII), noracronycine(XXIII), 1-hydroxyacridone (XXIV) and 1,7-dihydroxy-N-methylacridone (XXVI) which were identified by physical and chemical properties, preparation of derivatives and synthesis.

Matsuno and Amano[16] isolated a flavone rhamnoside, rutin (3',4',5,7-tetrahydroxyflavonol-3-rhamnoside) from this plant. Takayuki et al.[17] found that the leaves and stem contained β-myrcene, α-phellandrene, β-caryophyllene, cadinene, cadalene, caryophylleneoxide, spathulenol, 7-methoxy-2,2-dimethylchromene, and 6,7-dimethoxy-2,2-dimethylchromene (angeratochromene).

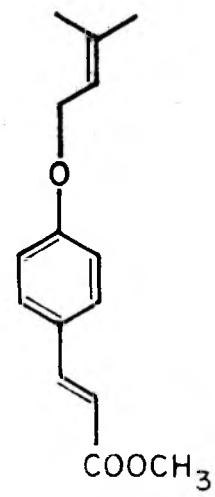
B. japonica

Kozwa et al.[18] isolated the following coumarins: umbelliferone(I), chalepensin(IX), (-)-nodakenetin(XI), bergapten (VI), xanthotoxin (VII), matsukaze lactone(XVII), rutamarin(X) and daphnoretin (XVI).

PRESENT WORK

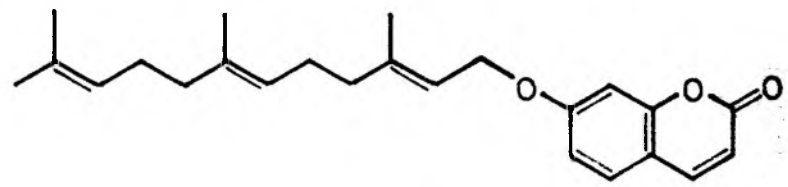
In the present work examination of the branches of B. albiflora is discussed. Three new compounds, 4-(3,3-dimethylallyloxy)-methylcinnamate (XXVII), byakangelecin acetonide (XXX) and 5,7,8-trimethoxycoumarin (XXIX) together with a known coumarin, umbelliprenin(XXVIII), have been isolated. In addition to these, the branches contained two known coumarins, rutamarin (X) and isopimpinellin(VIII) alongwith β-sitosterol, reported earlier from the same source. A lignan, justicidin-B (XXXII), has been isolated. This is the first report of the occurrence of justicidin B in a Rutaceae plant. It has been earlier isolated from Justicia species (Family: Acanthaceae), Cleistanthus collinus (Euphorbiaceae) and Heliopsis scabra (compositae).

The powdered branches and leaves of the plant were extracted with methanol, and the extract (3.4%) was concentrated to a small volume, adsorbed on exhausted powder and then successively reextracted with hexane, chloroform and methanol to give 1,2%, 0.75%, 1.5% of the extracts respectively.



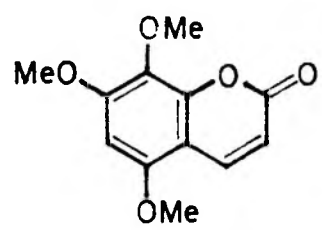
(XXVII)

4-(3,3-DIMETHYLALLYLOXY)
-METHYLCINNAMATE

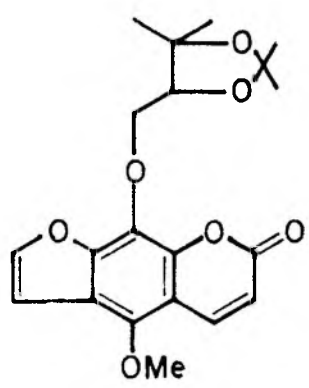


(XXVIII)

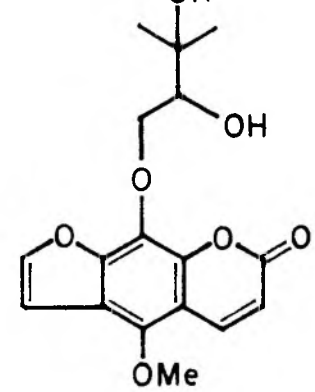
UMBELLIPRENIN or 7-O-FERNESYLCOUMARIN



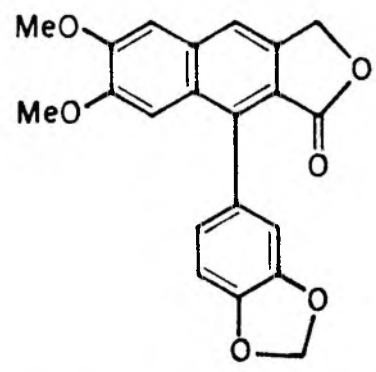
(XXIX) 5,7,8-TRIMETHOXYCOUMARIN



(XXX) BYAKANGELECIN-
ACETONIDE



(XXXI) BYAKANGELECIN



(XXXII) JUSTICIDIN — B

Hexane extract

The hexane extract was dissolved in a minimum amount of methanol and kept overnight at 0^{o}, when waxes and oil were separated out. It was then decanted to separate waxes and oil and again kept at 0^{o} for 24 hr. The solid which separated was filtered and recrystallised from methanol, m.p. 135^{o}, $(\alpha)_D$ - 30^{o}. It was identified as β-sitosterol by direct comparison with an authentic sample and mixed m.p.

The residue from hexane extract, after the removal of waxes, oil and β-sitosterol, was chromatographed on a column of silica gel using benzene and then increasing percentage of acetone for elution. Different fractions were collected and monitored by TLC (silica gel, acetone-benzene,2:8). Repeated column chromatography or PLC on silica gel of individual fractions resulted in the isolation of compounds A - E with decreasing Rf values.

Compound A [4-(3,3-dimethylallyloxy)-methylcinnamate], obtained as colourless cubes from hexane, m.p. 70^{o}, $C_{15}H_{18}O_3$, M^{+} 246, $\nu_{max.}^{cm^{-1}}$ 1700/(α,β-unsaturated ester, C=O). $\lambda_{max.}^{EtOH}$ (log ε) 205 (3.15), 230(3.09), 303(3.30), 312(3.33). The NMR spectrum in $CDCl_3$ (Fig.1) shows absorption typical of a prenyl group ($Me_2C=$ at 2.78; $-CH_2$ at 4.56, d, J=7 Hz; =CH at 5.47 bt). In addition to these signals, three proton singlet appears

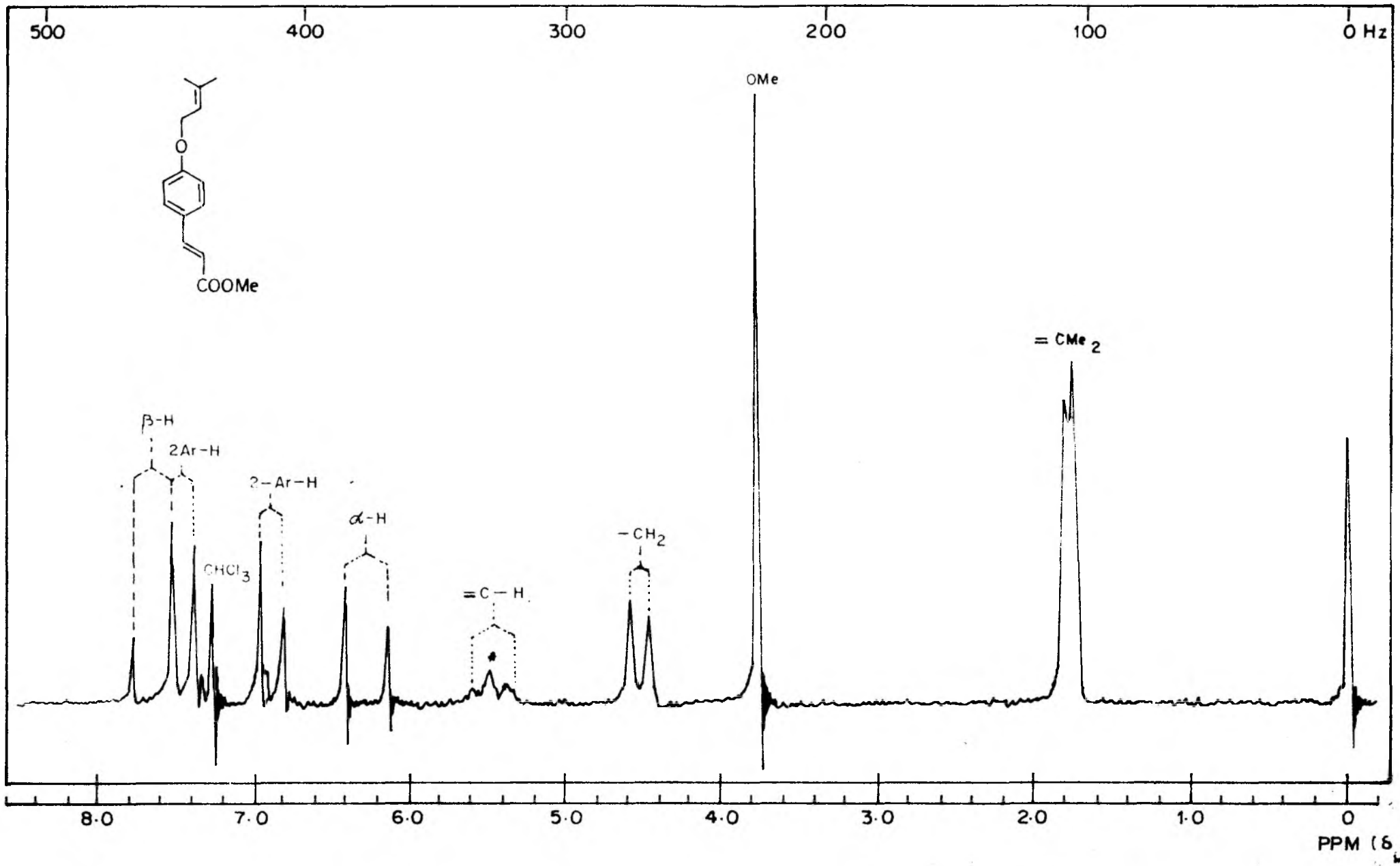


FIG. 1. NMR SPECTRUM OF 4-(3,3-DIMETHYLALLYLOXY)-METHYLCINNAMATE (XXVIII) IN $CDCl_3$

at 3.80 (OMe) together with two doublets ($\underline{J}$=16 Hz) at 6.3[illegible] and 7.64 corresponding to two <u>trans</u> olefinic protons (α- and β- to ester group). The aromatic region shows A_2B_2 pattern, comprising of four protons, appearing as two doublets($\underline{J}$=8.5 Hz) at 6.90 and 7.44. This suggests the presence of two substituents <u>para</u> to each other. All this data account for one isoprenyl group; α,β-unsaturated ester attached to benzene ring and these two are <u>para</u> to each other. The appearance of methylene doublet in the low field region at 4.56 can be ascribed to $-OCH_2$ and therefore the remaining oxygen is present in the form of ether linkage. The mass spectrum (Fig.2) shows molecular ion at m/e 246 and the other intense peaks appear at m/e 215 (M-31), 178(M-68) (base peak), 147(M-68-31), 119 (M-68-31-28), 91 (M-68-31-28-28). On the basis of the above spectral data, compound A was characterised as 4-(3,3-dimethylallyloxy)-methylcinnamate (XXVII).

<u>Compound B</u> (Umbelliprenin) obtained as colourless needles from hexane, m.p. 59-60°, $C_{24}H_{30}O_3$, M^+ 366, ν_{max}. 1720 cm^{-1} (α,β-unsaturated lactone). Compound B was identified as umbelliprenin (XXVIII) on the basis of its spectral properties. NMR in $CDCl_3$ reveals the presence of coumarin doublets ($\underline{J}$=10 Hz) at 6.23 and 7.57 (3-H and 4-H of coumarin); 7.33 (1H, <u>bd</u>, $\underline{J}$=9.5 Hz, 5-H), 6.80 (2H, <u>bm</u>, 6-H and 8-H of coumarin);

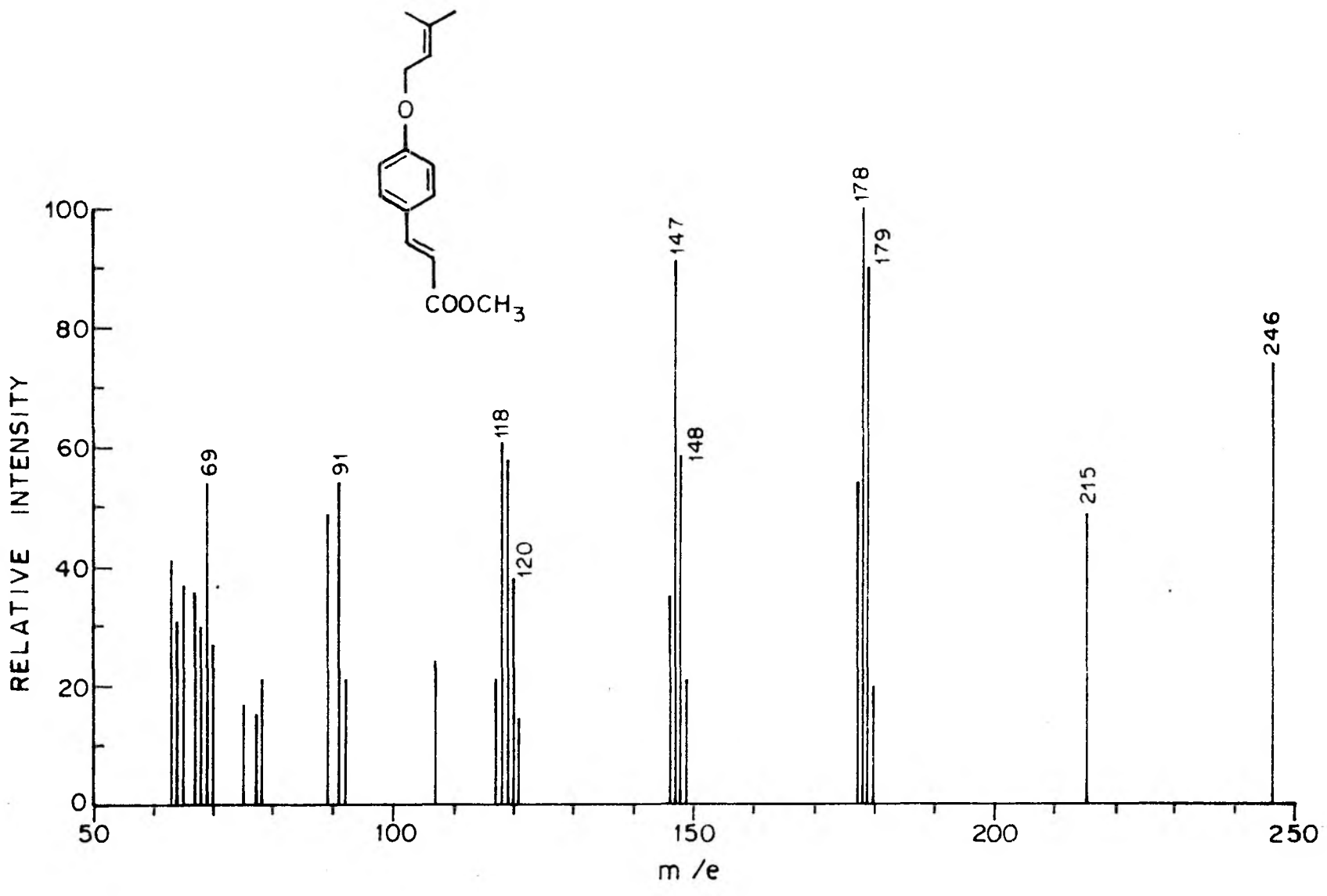

O
COOCH3
RELATIVE INTENSITY
100
80
60
40
20
0
50
100
150
200
250
m /e
69
91
118
120
147
148
178
179
215
246

FIG. – 2 .

5.47 (1H, bt, J=6 Hz, CH_2-CH=C); 5.12 (2H, bm, -CH_2-CH=C), 4.62 (2H, d, J=6 Hz, -OCH_2-CH=C), 2.2-1.87 (8H, four methylenes), 1.78 and 1.66 (6H, two s, two vinyl CH_3) and 1.60 (6H, s, two vinyl CH_3). The mass spectrum shows molecular ion at M^+ 366, and other peaks at m/e 204 (M-142), 162(M-204) (base peak), 134 (M-204-28), 106 (M-204-28-28) and 105(M-204-56-H).

Umbelliprenin was previously isolated from Thamnosma montana,[19] but isolated for the first time from B. albiflora.

Compound C was characterised as β-sitosterol.

Compound D(Rutamarin) crystallised from hexane as colourless needles, m.p. 107°, $C_{21}H_{24}O_5$, M^+ 356, ν_{max}. 1710 cm^{-1} (α,β-unsaturated lactone), 1740 cm^{-1} (C=O).

The NMR spectrum in $CDCl_3$ disclosed the presence of 1,1-dimethylallyl group: 4.06, q (J=10 Hz and 16 Hz) for vinylic H; 4.9-5.23, m, for $=CH_2$; 1.56 and 1.50(2s, for CMe_2); 4-,5- and 8-H of coumarin appearing as singlets at 7.40, 7.13 and 6.66; CH and CH_2 of dihydrofuran (one pron merged with signals of $=CH_2$ and multiplet at 3.16); and Ac and Me_2 of $-CMe_2Ac$ group at 2.0 and 1.46 respectively. On the basis of the above spectral data, compound D was characterised as rutamarin(X). It was detected earlier from the same source by Rozsa et al.[14]

Compound E, obtained as yellow needles from hexane benzene, m.p. 149°, $C_{13}H_{10}O_5$, M^+ 246, $\nu_{max.}$1710 cm^{-1} (α,β-unsaturated lactone). It was identified as isopimpinellin(VIII) by comparison with an authentic sample, mixed m.p. and superposable IR. Talapatra et al.[10] isolated isopimpinellin from the leaves and stem.

Chloroform extract

The chloroform extract which was a complex mixture of compounds with very close Rf values did not give a good separation on TLC silica gel or alumina, but showed a slightly better separation on polyamide. The extract was passed through a column of polyamide using benzene-chloroform (1:1) for elution. The residue from the percolate showed the presence of three major compounds with very close Rf values. It was chromatographed on a column of silica gel using benzene, benzene-acetone mixture for elution. A number of fractions were collected, monitored on TLC silica gel (acetone-benzene, 2:8) and worked up further.

Initial fractions gave more of isopimpinellin as confirmed by comparison with an authentic sample. Further fractions were complex mixtures and they were purified by chromatography over a column of neutral alumina using acetone-hexane for elution, and subsequent PLC (silica gel (acetone-

hexane, 2:8) gave three compounds (F-H) in the order of decreasing Rf values.

Compound F (5,7,8-trimethoxycoumarin), colourless crystals from benzene, m.p. 180°, M^+ 236 ($C_{12}H_{12}O_5$), ν_{max}. 1715 cm^{-1} (α,β-unsaturated lactone).

The NMR spectrum in $CDCl_3$ indicates characteristic coumarin doublets (J=10 Hz) at 6.18 and 7.92 for 3-H and 4-H of a coumarin, a single proton singlet at 6.37 (Ar-H), along with two singlets at 4.0 and 3.97 each integrating for three and six protons respectively (3 OMe groups). From this spectral data, it is evident that the compound is a trimethoxycoumarin. Benzene induced solvent shifts of the methoxyls show that the two groups suffer a significant upfield shift (0.5-0.6 ppm), suggestive of at least one free adjacent position at the two methoxyl groups. Therefore, two alternate structures, viz., 5,6,8- and 5,7,8- trimethoxycoumarin are proposed. TLC and m.p. were found to be identical with the new trimethoxycoumarin, isolated from Toddalia aculeata (Refer section 1). The trimethoxycoumarin was proved to be 5,7,8-trimethoxycoumarin by synthesis (Refer section 4).

Compound G (Byakangelecin-acetonide), obtained as colourless needles from benzene, m.p. 157°, $C_{20}H_{22}O_7$, M^+ 374. ν_{max}. 1720 cm^{-1} (α,β-unsaturated lactone), UV absorption

$\lambda_{max.}^{EtOH}$ (log ε) 226 (3.38), 247(3.12), 273(3.23) and 316(3.04). The NMR spectrum (Fig.3) in $CDCl_3$ exhibits a typical pattern of signals for furanocoumarin, a pair of doublets (J=10 Hz) at 7.97 and 6.20 of coumarin, and doublets (J=2.5 Hz) at 7.56 and 6.93 of a furan ring. A three proton signal at 4.16 indicates the presence of a methoxyl group and 1.56-1.22 region integrates for twelve protons (four methyl groups). The multiplet at 4.31 integrating for three protons, remains to be accounted for. The above data suggest that the compound is a linear furanocoumarin with oxygen function at 5-position, as chemical shift of 4-H of coumarin and 3'-H of furan are shifted downfield significantly.

Furanocoumarin nucleus with one methoxyl group accounts for twelve carbon atoms, which indicates that the remaining eight carbon atoms are present in the side chain. Out of seven oxygen-atoms, three are present in furanocoumarin skeleton and one in methoxyl group. The remaining three may be present on the side chain. IR shows the absence of hydroxyl absorption and hence they cannot be considered as alcoholic hydroxyl groups.

The above evidence is quite instructive of the presence of substituents both at 5- and 8-positions, because the protons of furanocoumarin are accounted for, considering that the three proton multiplet at 4.31 must be arising from the side chain

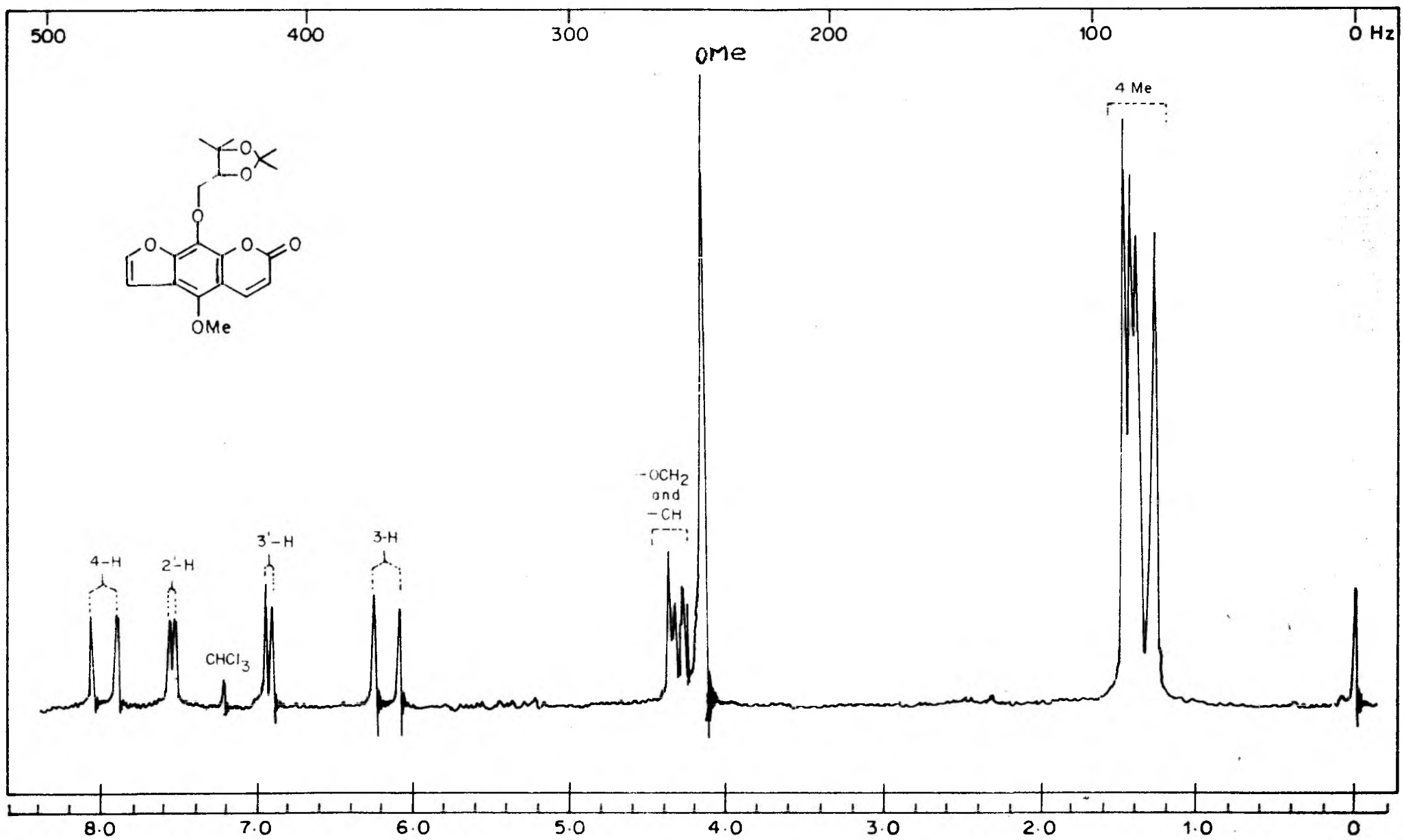


FIG. 3. NMR SPECTRUM OF BYAKANGELECINACETONIDE (XXX) IN $CDCl_3$

and not from the ring. The appearance of a three proton multiplet in the low field region can be ascribed to a methylene attached to oxygen (ether linkage) and methine proton. If C_5 side chain is considered, which accounts for two of the four methyls; from the NMR spectrum it seems probable that the remaining two methyls and two oxygens are to be involved in acetonide formation. This presumption is substantiated by the following reaction: Compound G on hydrolysis with methanolic hydrochloric acid gave the respective diol (XXXI). The spectral properties of the diol are in agreement with that of a known furanocoumarin byakangelecin.[20] Thus the spectral and chemical properties support structure (XXX) for the acetonide. The mass spectrum (Fig.4) of acetonide shows molecular ion as a base peak at m/e 374 and other prominent peaks at m/e 359(M-15), 317(M-15-42), 316(M-58), 232(M-142), 231(M-142-H), 217(M-142-15), 203(M-143-28) and 189 (M-142-15-28). The MS fragmentation is outlined in Chart 1.

It appears that the acetonide may be an artefact found during the separation of the products by column chromatography using benzene-acetone as a solvent for elution. However, the complete absence of the corresponding diol gave rise to some doubts about its formation with small quantities of acetone during the isolation procedure. The crude extract

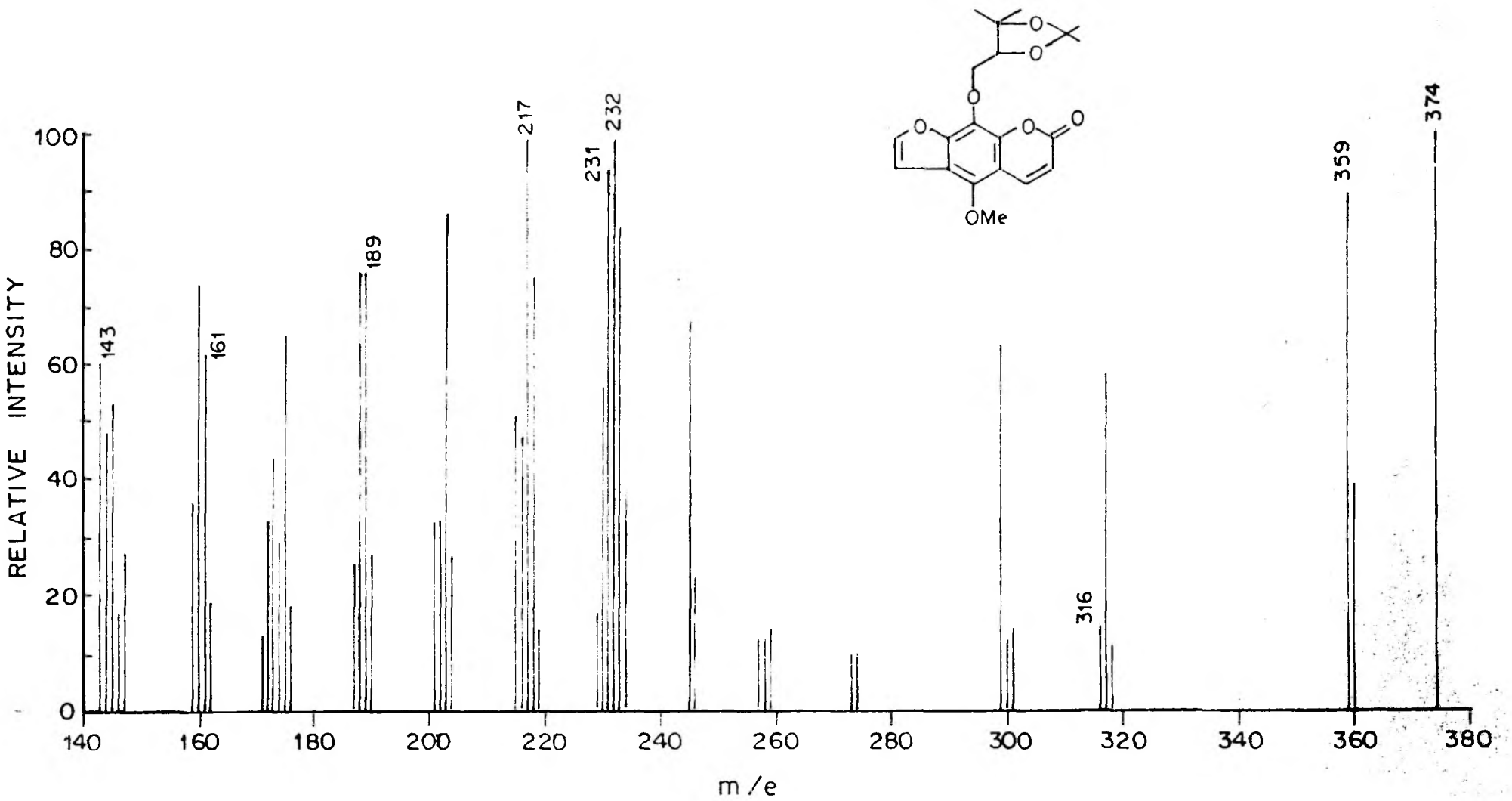
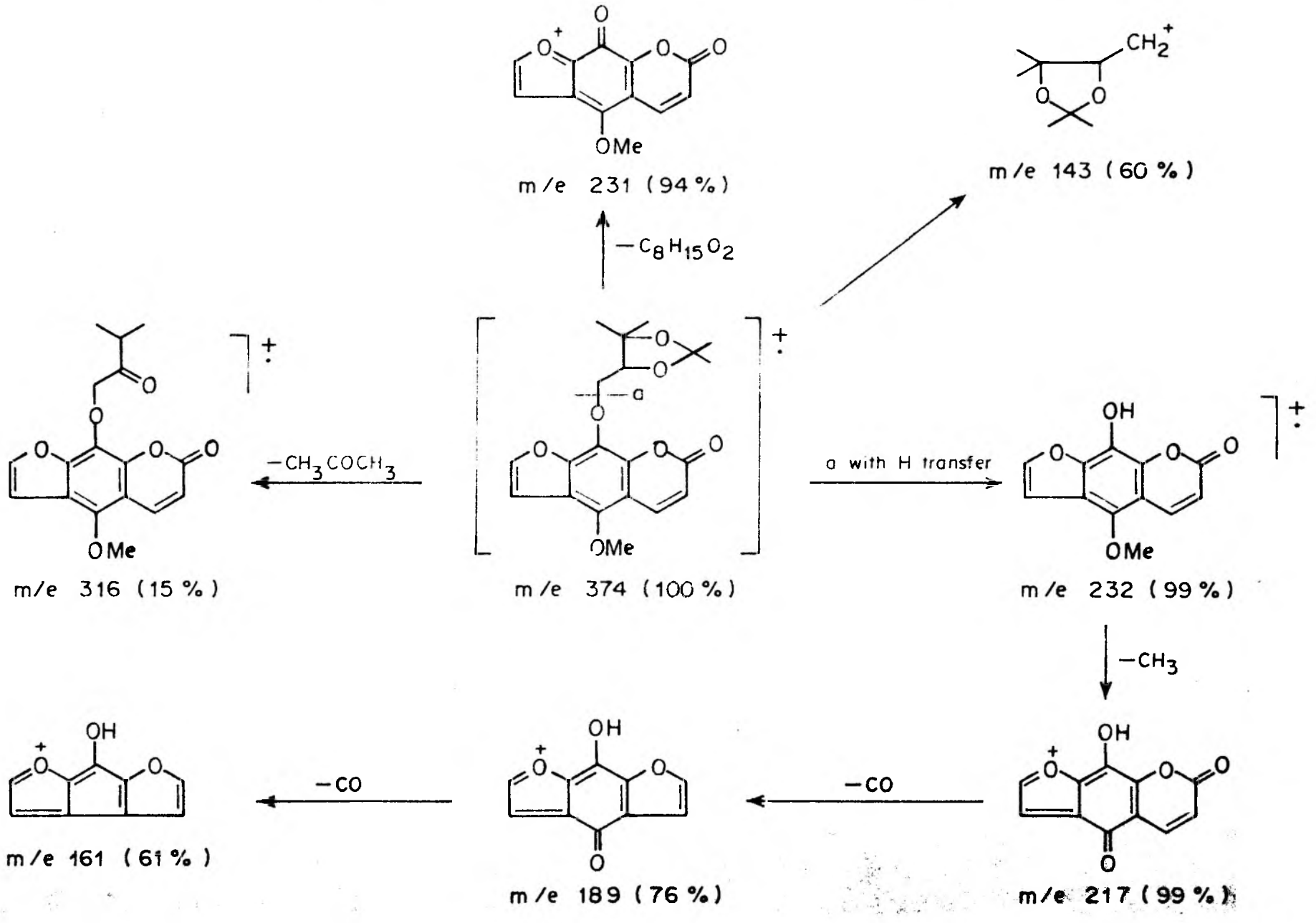

RELATIVE INTENSITY
100
80
60
40
20
0
140
160
180
200
220
240
260
280
300
320
340
360
380
m/e
143
161
189
217
231
232
316
359
374
OMe

FIG. 4 .

CHART –1.

MASS SPECTRAL FRAGMENTATION OF BYAKANGELECIN ACETONIDE

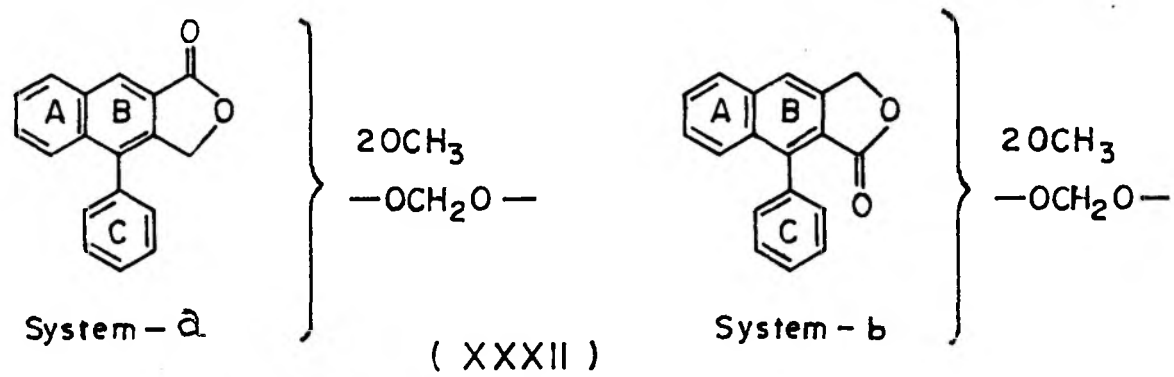


obtained by methanolic extraction indicated the presence of the acetonide (XXX) and no detectable spot on a TLC plate (acetone-benzene, 2:8; acetone-hexane, 3:7) corresponding to the diol (XXXI), was obtained, thus indicating the absence of the diol in the total extract. From this it appears that the acetonide is a natural product. A similar observation has also been made by Gonzalez et al.,[30] who isolated it simultaneously from Heracleum granatense (Family: Umbelliferae).

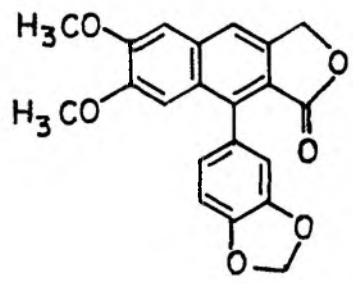
Compound H (Lignan - Justicidin B), colourless glistering plates from methylene chloride-hexane mixture, $C_{21}H_{16}O_6$, m.p. 240°, M^+ 364, $\nu_{max.}$ 1745 (C=O of five-membered lactone) and a sharp band at 935 cm^{-1} (methylenedioxy group), $\lambda_{max.}^{EtOH}$ 260, 295, 310, 350 nm.

The NMR spectrum in $CDCl_3$ exhibits two three-proton singlets at 3.81 and 4.10 ascribed to two methoxyl groups; a two proton quartet (J=1 Hz) at 6.06 for methylenedioxy group attached to an aromatic ring also suggested from its characteristic band at $\nu_{max.}$ 935 cm^{-1} in the IR spectrum. A two proton singlet at 5.37 can be assigned to a aromatic methylene attached to oxygen function. Aromatic region integrates for six protons, comprising of signals of ABX type at 6.83 and 7.05 (d, one proton each and a singlet merged with doublet at 7.05) and three single proton singlets at 6.80, 7.15 and 7.65.

The UV absorption indicates the presence of extended naphthelenic chromophore.[21] The NMR pattern and UV spectrum of compound H resembles to that of substituted 4-phenyl-naphthalide type.[23] This suggests the following two types of skeleton for compound H.



(XXXII)



(XXXII)

A survey of data for compounds of established structure initially made by Horii and his coworkers[22] and supplemented later by Holmes and Stevenson[23] indicate that methylene group in system a (XXXII) appears between 5.08 and 5.23, whereas in system b (XXXII) it appears at 5.32-5.54. Klemm et al.[24] reported that 1-H of lactone appears at 8.30(strongly deshielded by adjacent carbonyl) in system a, whereas the corresponding proton in isomer b appears at 7.76.

The lactone methylene of compound H is seen at 5.37 (not shielded) and 1-H appears at 7.75. These observations suggest system b for compound H. Now the methoxyl groups can be

fixed in ring A and methylenedioxy in ring C or vice-versa based on the NMR pattern. The appearance of an AB quartet (J=1 Hz) for methylenedioxy group indicates that they are non-equivalent[25] and prefers out of plane conformation of benzene ring bearing methylenedioxy group, hence it is in ring C at 3',4'-position. The upfield shift of the aromatic ABX pattern for ring C protons (6.83-7.05) is attributed to shielding caused by proximity of neighbouring aromatic ring (biphenyl system). The mass spectrum shows molecular ion at m/e 364. The spectral properties are in complete agreement with structure (XXXII) proposed for compound H. It was thus identified as justicidicin-B (XXXII), first isolated from Justicia hayatai[26] and its structure was confirmed by Munakata et al.[27] The m.p. and spectral data (UV, IR and NMR) of compound H (Justicidin B) is in agreement with the reported data, although a direct comparison has not been possible due to non-availability of authentic sample.

EXPERIMENTAL

Extraction of branches and leaves

Powdered branches (2 kg) were extracted with cold methanol and the removal of solvent gave a greenish syrupy extract (69 g). This was adsorbed on extracted powder, and on successive soxhlet extraction with hexane, chloroform and methanol, it gave 24 g., 15 g. and 30 g. of the extracts. respectively.

Hexane extract: The hexane extract (20 g) was dissolved in methanol (100 ml) and kept overnight at 0^{o}. The separated oil and waxes were removed by decantation. The decanted methanol solution was again kept at 0^{o} for 24 hr., when colourless needles separated out (5 g), m.p. 130^{o}. Recrystallisation from methanol gave a pure crystalline compound (3.5 g), m.p. 135^{o}, $(\alpha)_D$ - 30^{o}. It was characterised as β-sitosterol by comparison with an authentic sample and mixed m.p.

Isolation of 4-(3,3-dimethylallyloxy)-methylcinnamate(XXVII) umbelliprenin(XXVIII), rutamarin(X) and isopimpinellin(VIII) from hexane extract

The residue from hexane extract (13 g) after removal of waxes, oil and β-sitosterol was dissolved in benzene and chromatographed on a column of silica gel (250 g). The column

was initially eluted with benzene and then with benzene containing increasing percentage of acetone. A number of fractions (500 ml) were collected and examined over silica gel TLC plates (benzene-acetone, 9:1). Similar fractions were pooled together.

Fractions 1-5 (1.2 g) contained mostly waxes and have not been examined further.

Fractions 6-8 (1.0 g) (compound A) were purified by passing through a column of silica gel (10 g) with acetone-hexane (0.5:9.5) as eluent. The compound crystallised from hexane, m.p. 70° (0.220 g) and was characterised as 4-(3,3-dimethylallyloxy)-methylcinnamate (XXVII), not reported so far in literature (Found: C, 73.21; H, 7.40. $C_{15}H_{18}O_3$ requires C, 73.18; H, 7.32%).

Fractions 9-12 (1.2 g) were a complex mixture, which was not separated further.

Fractions 13-22 (2.1 g) (compound B) were chromatographed on a column of silica gel using acetone-hexane (0.5:9.5) mixture for elution. The compound was crystallised from hexane as colourless needles, m.p. 63° (0.256 g) and was found to be identical with a known coumarin, umbelliprenin (XXVIII) (lit.[19] m.p. 63°) (Found: C, 78.40; H, 8.0. $C_{24}H_{30}O_3$ requires C, 78.68; H, 8.2%).

Fractions 23-41 (2.0 g) contained exclusively

β-sitosterol.

Fractions 42-53 (2.7 g) (compounds C and D) on repeated PLC (silica gel, acetone-hexane, 0.5: 9·5) afforded compounds C and D. Compound C was identical with an authentic sample of β-sitosterol. Compound D yielded colourless needles from hexane, m.p. 107° (0.860 g) $(\alpha)_D$ 16.4° (in chloroform) and was identified as rutamarin (X) on the basis of spectral properties, (lit.[28] m.p.104-5°: 107-8°) $(\alpha)_D$ 14.3°, $(\alpha)_D$ 10.6°. (Found: C, 70.53; H, 6.68. $C_{21}H_{24}O_5$ requires C, 70.79; H, 6.74%).

Fractions 54-60 (0.7 g) were not worked up further, from which rutamarin was detected by TLC.

Fractions 61-69 (1.1 g) (compound E) were purified by column chromatography over a column of neutral alumina(10 g) using hexane-acetohe mixture (9.5:0.5) for elution and subsequent purification by PLC (silica gel, acetone-hexane 1:9) gave compound E. It was crystallised from hexane-benzene as yellow needles, m.p. 149° (0.5 g) and identified as isopimpinellin (VIII) by comparison with an authentic sample and mixed m.p. (lit.[10] m.p. 149°).

Chloroform extract. It was a complex mixture of compounds and did not give a separation on TLC silica gel (benzene-acetone,8:2) or alumina, however, it showed a slightly better separation on polyamide (benzene-chloroform,1:1). The chloroform extract (15 g) was chromatographed on a column

of polyamide (200 g) and the column was eluted with benzene-chloroform. All percolates were mixed and the solvent removed to give a residue (8.5 g). The residue showed the presence of isopimpinellin (VIII) along with three major compounds with very close Rf values in TLC on silica gel (benzene-acetone, 8:2). It (8.5 g) was chromatographed on a column of silica gel (150 g) using benzene and benzene-acetone mixture as eluent. Fractions (each 200 ml) were collected, monitored by TLC and identical fractions mixed together.

Fractions 1-10 (1.1 g) were a mixture of compounds having close Rf values and not examined further.

Fractions 11-18 (1.0 g) were homogeneous on TLC silica gel, readily crystallised from hexane-benzene as yellow needles (0.9 g), m.p. 149°, identified as isopimpinellin (VIII) by direct comparison with an authentic sample.

Fractions 19-33 (1.7 g) [isopimpinellin + compound F] were chromatographed on a column of neutral alumina (15 g) using acetone-hexane (2:8) as solvent. Faster moving compound was identified as isopimpinellin. PLC of the later fractions (silica gel, acetone-hexane, 2:8) gave compound F, crystallised from benzene, m.p. 180° (0.21 g). From its NMR, IR, mass spectra and synthesis, it was characterised as 5,7,8-trimethoxy-coumarin (XXIX) (Found: C, 61.32; H, 5.30. $C_{12}H_{12}O_5$ requires C, 61.0; H, 5.1%).

Fractions 34-39 (1 g) were a mixture of trimethoxy-coumarin and compound G. PLC (silica gel, acetone-hexane,3:7) of these fractions yielded only 100 mg of pure compound G, which on crystallisation from benzene gave colourless needles, m.p. 157°. It was characterised as byakangelein-acetonide(XXX) from its spectral and chemical properties (Found: C, 64.35; H, 5.75. $C_{20}H_{22}O_7$ requires C, 64.16; H, 5.75%).

Hydrolysis of byakangelecin acetonide, 50 mg. was dissolved in ethanolic solution of hydrochloric acid (5%) (5 ml) and refluxed for 4 hr. at water bath temperature. The solvent was distilled off, the residue was diluted with water and extracted with chloroform. The product was crystallised from benzene in colourless needles, m.p. 118° $(\alpha)_D$ + 20° in chloroform, (30 mg). It was identified as byakangelecin(XXXI) from its physical and spectral properties (lit.[29] m.p. 118°). (Found: C, 61.0; H, 5.71. $C_{17}H_{18}O_7$ requires C, 61.08; H, 5.39%).

Fractions 40-73 (3 g) (compound H) were homogeneous on TLC silica gel (benzene-acetone, 8:2), crystallised from methylenechloride-hexane mixture, as colourless glystering plates (1.3 g), m.p.240°. It was identified as justicidin-B (XXXII), a lignan of 4-phenylnaphthalide type on the basis of its spectral data (lit.[26,27] m.p.240, 247) (Found: C, 69.08; H, 4.78. $C_{21}H_{16}O_6$ requires C, 69.23; H, 4.39%).

The methanol extract was a complex mixture, hence it was not examined further.

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Section 4

Synthesis of 5,7,8-trimethoxycoumarin

INTRODUCTION

A recent review[1] on the naturally occurring plant coumarins includes about six hundred coumarins of diverse structural types. Oxygenation is known to occur at one or more of the six available positions on the coumarin nucleus, such oxygens being present in phenolic, ethereal and glycosidic groups.[1]

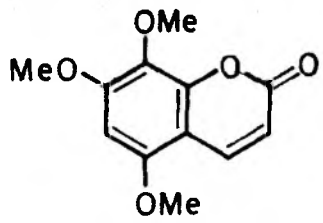
Some trends do appear to exist in the substitution pattern of coumarins. Oxygenation at more than two positions in the coumarin nucleus is relatively rare. A perusal of different reviews[1-3] on coumarins suggest that 5,6,7-, 5,7,8- and 6,7,8- oxygenation pattern is common in natural sources. However, 5,6,8 is not encountered so far. The 5,7,8- pattern being found in Ruta and the 6,7,8 mainly in Zanthoxylum (Family: Rutaceae). Until 1976, simple coumarins bearing hydroxyls in 5,7,8 positions either free or as methyl ether form has not been isolated from natural sources. In 1976 when the present work was in progress, Gonzalez et al.[4] isolated 5,7-dimethoxy-8-hydroxycoumarin from Ruta species (family: Rutaceae).

As discussed in Sections 1 and 3, the trimethoxy-coumarin isolated from Toddalia aculeata and Boenninghausenia albiflora is represented by two alternative structures (I) and (II) mainly on the basis of spectral data. From the

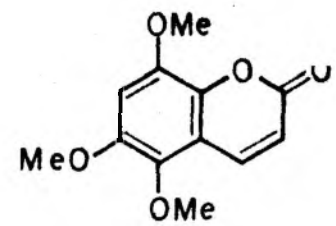
above discussion and biogenetic considerations, structure (I) seems to be more likely for trimethoxycoumarin. Hence 5,7,8-trimethoxycoumarin is synthesised (scheme 1) starting from pyrogallol to prove its identity with the natural product.

PRESENT WORK

Pyrogallol on benzylation[5] with benzylchloride and potassium carbonate in boiling acetone gave tribenzyl ether, (III) which on nitric acid oxidation[6] at 40° yielded 1:1 mixture of undesired 5-nitro-1,2,3-tribenzyloxybenzene (IV), m.p.139° and 2,6-dibenzyloxy-p-benzoquinone (V), m.p.200°. The latter was reduced[6,7] with saturated aqueous sodiumdithionite at 70-80° to 2,6-dibenzyloxy hydroquinone (VI), m.p. 113°. The hydroquinone on methylation[7] with dimethylsulphate in aqueous sodium hydroxide furnished 2,6-dibenzyloxy-1,4-dimethoxybenzene (VII), m.p. 80°. Gattermann reaction[8] of (VII), followed by debenzylation[8] *in situ* gave 2,4-dihydroxy 3,6-dimethoxy-benzaldehyde (VIII), m.p. 195°. Condensation[9] of aldehyde(VIII) with acetic anhydride and sodium acetate resulted in the formation of 7-acetoxy-5,8-dimethoxy coumarin (IX),m.p.180-81°, which on subsequent deacetylation[9] gave 7-hydroxy-5,8-dimethoxy-coumarin (X), m.p.190°. Methylation of hydroxycoumarin(X) with dimethylsulphate and potassium carbonate in boiling acetone furnished 5,7,8-trimethoxycoumarin(I), m.p.180° in quantitative yield, which is identical with natural coumarin in all respects (m.p., m.m.p., TLC, superposable IR and identical NMR).

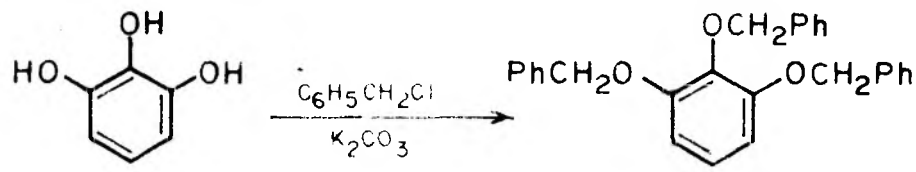


(I)



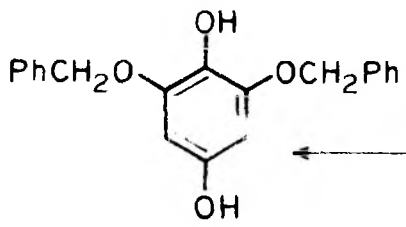
(II)

SCHEME - 1



(III)

OXIDATION WITH HNO_3



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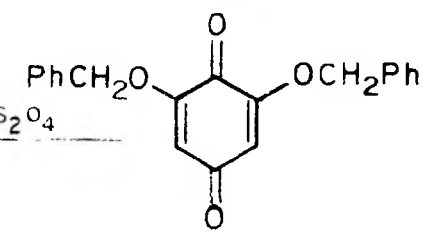

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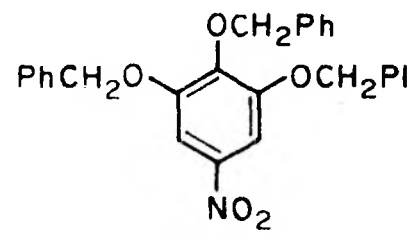
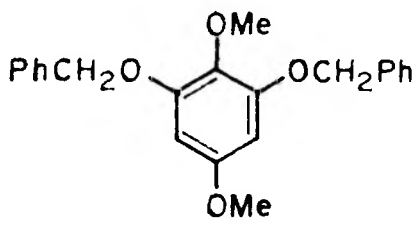


(IV)

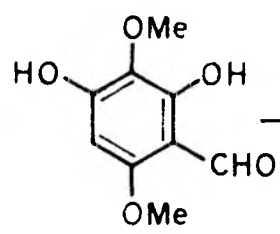
$Na_2S_2O_4$

DMS - NaOH



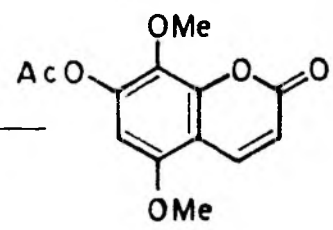
(VII)

1) GATTERMANN - REACTION
2) DEBENZYLATION



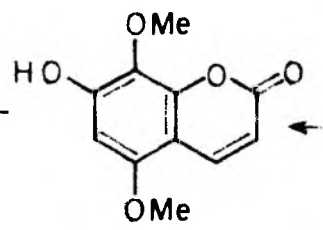
(VIII)

PERKIN CONDENSATIC



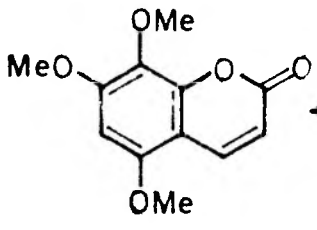
(IX)

NaOH - EtOH



(X)

DMS - K_2CO_3



(I)

5,7,8 - TRIMETHOXY -
COUMARIN

EXPERIMENTAL

Tribenzylether of pyrogallol (III)

A mixture of pyrogallol (50 g), benzyl chloride (18.2 ml) and anhydrous potassium carbonate (250 g) was refluxed in dry acetone (500 ml) for 40 hr. Acetone was distilled off, diluted with water and extracted in ether(500 ml). Ethereal solution was washed with sodium hydroxide (5%) to remove unreacted pyrogallol. Ether was distilled off and unreacted benzyl chloride steam distilled from the residue, when tribenzyl ether solidified on cooling. It was filtered and recrystallised from alcohol in colourless needles (82 g), m.p. 68-69° (lit.[5] 70°). (Found: C, 81.60; H, 6.06. $C_{27}H_{24}O_3$ requires C, 81.81; H, 6.0%).

2,6-Dibenzyloxy-benzoquinone (V)

Nitric acid (30%; 20 ml) was added dropwise to a stirred solution of pyrogallol tribenzyl ether (40 g) in 400 ml glacial acetic acid over a period of one hour at 40°. The reaction mixture was stirred at room temperature for 3 hr., the 5-mitro-1,2,3-tribenzyloxybenzene, m.p.139°,(lit.[6] 139°) separated was filtered. To the filtrate 30% nitric acid(20 ml) was added dropwise with stirring at 40° and allowed to stand at room temperature overnight. The crystalline 2,6-dibenzyloxy-

benzoquinone (35 g) contaminated with nitro compound was filtered and washed with water. Crude product on crystallisation from acetone furnished yellow needles (16.1 g) (50%), m.p.200° (lit.[6] 200-201°). $\nu_{max.}$ 1660, 1710 cm^{-1} (C=O). (Found: C, 75.2; H, 4.95. $C_{20}H_{16}O_4$ requires C, 75.01; H, 5.0%).

2,6-Dibenzyloxy hydroquinone (VI)

A saturated solution of sodiumdithionite (20 g) in 50 ml. water was added dropwise to a well stirred suspension of 2,6-dibenzyloxy-benzoquinone (10 g) in acetic acid(100 ml) and water (50 ml) at 70°. The reaction mixture was stirred for additional 2 hr. at this temperature, during which time the yellow suspension changes to light cream coloured solution. The solution on cooling gave pure 2,6-dibenzyloxyhydroquinone as colourless plates in quantitative yield, m.p. 115° (lit.[6,7] m.p. 115°). $\nu_{max.}$ 3250 cm^{-1} (-OH). (Found: C, 74.40; H, 5.60.$C_{20}H_{18}O_4$ requires C, 74.52; H, 5.59%).

2,6-Dibenzyloxy-1,4-dimethoxybenzene (VII)

A mixture of 2,6-dibenzyloxyhydroquinone(8 g), 10% sodium hydroxide (50 ml) and dimethylsulphate (5.9 ml) was refluxed for 5 hr., and allowed to stand at room temperature overnight. The deposited crystalline methyl ether was filtered and washed with water. It was recrystallised from

ethanol (6g, 69%), m.p.80° (lit.[7] m.p. 80°) (Found: C, 75.40; H, 6.20. $C_{22}H_{22}O_4$ requires C, 75.43; H, 6.28%).

2,4-Dihydroxy-3,6-dimethoxybenzaldehyde (VIII)

An ice-cold mixture of 2,6-dibenzyloxy-1,4-dimethoxy-benzene (5 g), zinc cyanide (9 g) and anhydrous ether (30 ml) was stirred and saturated with dry HCl, maintaining the ice cold condition for 15 hr. Then the ether layer was decanted, the residual oil decomposed with sodium hydroxide (about 75 ml, 2N) to keep the solution just acidic to congo red paper. The acidic solution was heated at 100° for 1.5 hr. to complete debenzylation, the cooled solid product filtered and washed with ether to remove benzyl chloride. The aldehyde was crystallised from methanol as golden yellow needles (2.240 g., 79.18%), m.p. 198° (lit.[8] m.p. 198°) (Found: C, 54.54; H, 5.05. $C_9H_{10}O_5$ requires C, 54.54; H, 5.05%).

7-Acetoxy-5,8-dimethoxycoumarin (IX)

A mixture of 2,4-dihydroxy-3,6-dimethoxybenzaldehyde (2.2 g), acetic anhydride (13.2 ml) and fused sodium acetate (3.7 g) was refluxed at 170-80° for 24 hr. The reaction mixture was cooled, poured on ice water, the precipitated compound was filtered and washed with water. Acetoxycoumarin (IX) was crystallised from methanol as colourless needles (2.4 g., 81.83%), m.p.180° (lit.[9] 180-81°) (Found: C, 59.10;H,4.60. $C_{13}H_{12}O_6$ requires C, 59.09; H, 4.54%).

7-Hydroxy-5,8-dimethoxycoumarin(X)

Sodium hydroxide (10%; 2 ml) was added to a suspension of acetoxycoumarin (IX) (2 g) in 10 ml ethyl alcohol and refluxed for 1.5 hr. The clear yellow solution was diluted with ice-cold water (10 ml), acidified with hydrochloric acid (1:1) and precipitated solid filtered. Purification of the product by column chromatography (silica gel, chloroform) yielded 7-hydroxy-5,8-dimethoxycoumarin (1.2 g: 71.36%) m.p. 190° (lit.[9] m.p. 191°) (Found: C, 59.30; H, 4.50. $C_{11}H_{10}O_5$ requires C, 59.46; H, 4.50%).

5,7,8-Trimethoxycoumarin (I)

A solution of 7-hydroxy-5,8-dimethoxycoumarin (1 g) in dry acetone (20 ml) was refluxed for 12 hr. with dimethyl-sulphate (0.9 ml) and anhydrous potassium carbonate (10 g). Acetone was distilled off from the reaction mixture, and the residue treated with water. The crude product was crystallised into colourless needles from benzene. Repeated crystallisation from benzene yielded 5,7,8-trimethoxycoumarin in quantitative yield, m.p. 180°, which is identical with natural coumarin in all respects (mmp, TLC silica gel, acetone-hexane 3:7, superposable IR). (Found: C, 61.5; H, 5.3. $C_{12}H_{12}O_5$ requires C, 61.0; H, 5.1%).

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C H A P T E R - III

SYNTHESIS OF n-TRIACONTANOL - A NEW PLANT GROWTH REGULATOR

INTRODUCTION

There have been numerous reports that many naturally occurring compounds possess growth inhibiting or promoting activities.[1] Extensive screening of natural products and synthetic organic compounds has shown that they possess plant growth regulatory properties.

Plant growth regulators are defined[2] as "organic compounds, other than nutrients, which in small concentrations, affect the physiological processes of plants." For practical purposes they are defined as either natural or synthetic compounds, that are applied directly to a plant, to enhance yields, improve quality or facilitate harvesting.

The wellknown plant hormones[1,2] (phytohormones) show plant growth regulating properties. They have high activity, specific action and function in the regulation of plant growth. They include auxins, cytokinins, gibberllins, abscisic acid and its derivatives. The term "plant hormone" is restricted to naturally occurring plant substances, however, the term "plant growth regulator" is not restricted to synthetic compounds, but also includes such hormones of natural origin.

The response[2] of plant or a plant part to a plant growth regulator may vary with variety of plants. Even a

single variety may respond differently, depending on its age, environment, physiological stage of development and its state of nutrition.

The regulation of plant growth can be useful in great many ways,[2] such as promote rooting and propogation of plant, promote or delay flowering, induce or prevent abscission, control fruit set and fruit development, control plant or organ size, prevent post harvest spoilage, regulate chemical composition of plants and colour of fruits, influence mineral uptake from soil, etc. Plant growth regulators are regarded as the most rapidly expanding field of agricultural chemical business.

A recent review[2] lists a number of organic compounds, which are used as plant growth regulators in a number of ways.

Despite the great practical importance of plant growth regulators as outlined above, chemical companies have considerable interest in the plant growth regulator field. For the plant growth regulation, the rate of application of chemical and the stage of plant growth, must be considered from the initial stages. Efforts are being made in commercial and academic research centres to discover such substances which will have many fold applications and develop cheap synthetic methods for their syntheses.

Recently Ries et al.[3] demonstrated that application of alfalfa (Medicago sativa L.) increased the yields of tomatoes, cucumber and lettuce. They have shown that several other crop species including rice, corn accumulate dry weight when alfalfa was applied in smaller concentration under various controlled environmental conditions. This observation led them to isolate the compounds in alfalfa that might be responsible for observed growth increases. Further they isolated the crystalline compound using gas liquid chromatography and identified by mass spectrum to be n-triacontanol which is the principle constituent of the wax derived from alfalfa leaves.[4] They have shown that it increased growth and water uptake of rice, corn and barley. Both the water uptake and dry weight increase with increasing amount of crystals applied in the nutrient solution or to the foliage. The response of both rice and tomatoes to synthetic sample was similar to that of natural n-triacontanol. The alcohol increases the dry weight of test plants in the dark, hence it cannot have an effect on photosynthesis.[5] Therefore, Ries speculated that it might function by increasing the uptake of nutrients. Estimates of the real value of n-triacontanol to agriculture will have to wait for verification of laboratory tests and the results of field trials currently underway.

n-Triacontanol was earlier prepared by lithium aluminium hydride or sodium-alcohol (ethanol or butanol) reduction of n-triacontanoic acid or its ester. Therefore, the methods reported (Chart 1-3) for the synthesis of n-triacontanoic acid (also called as mellissic acid) are summarised below.

The malonic ester synthesis constitutes a classical method for increasing the chain length of aliphatic acid by two carbon atoms. Bleyberg and Ulrich[6] synthesised n-triacontanoic acid from ethylbehenate (C_{22} acid ester) by repeating the malonic ester synthesis sequence four times. In this case yields are unsatisfactory in the first two steps. A complete decomposition of dicarboxylic acid became difficult to carry out with large quantities of material.

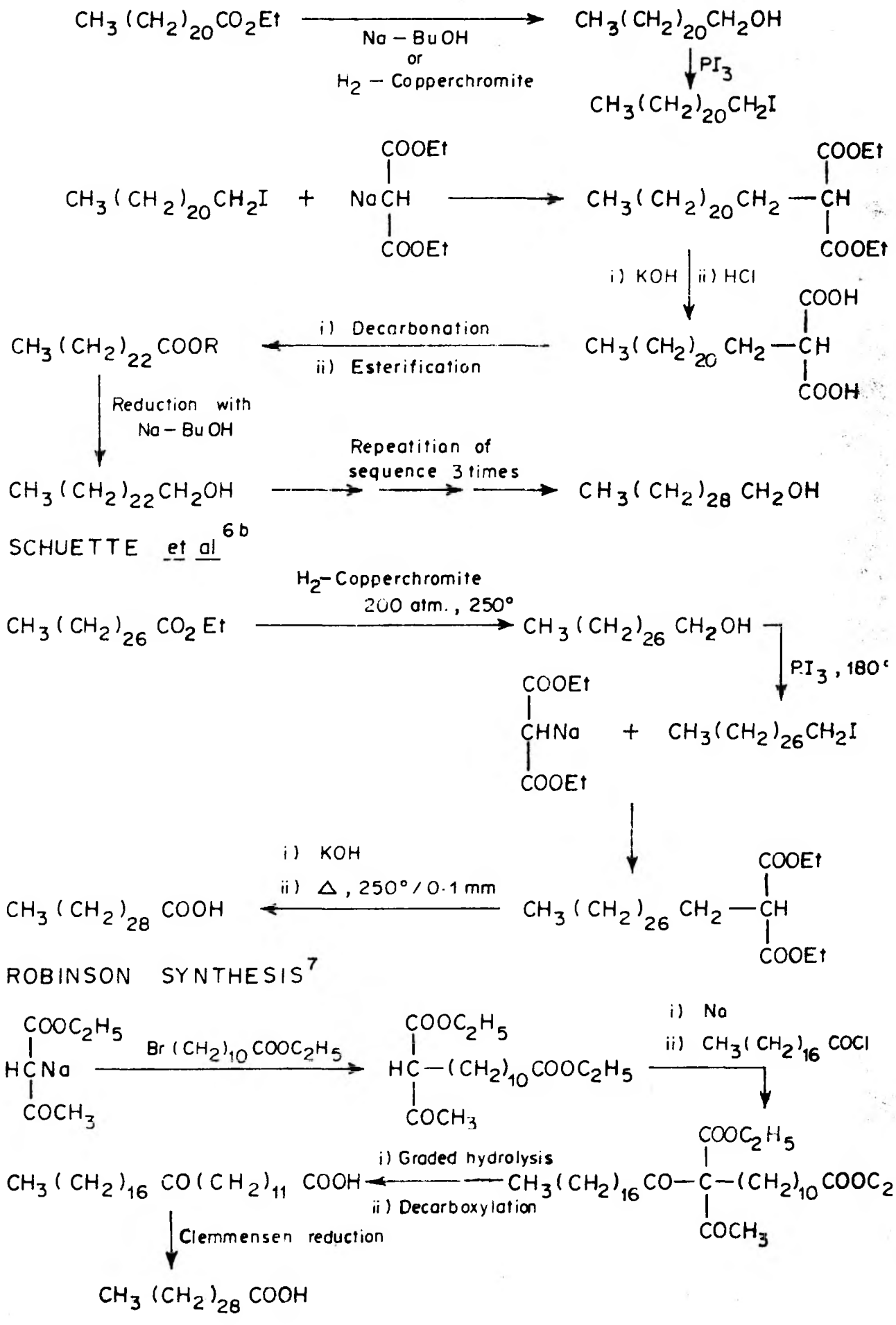
Robinson[7] prepared n-triacontanoic acid by alkylating ethylsodioacetoacetate with ethyl-ω-bromoundecanoate and reacting the condensation product with stearoyl chloride. Graded hydrolysis of the alkylated product gave 13-keto-triacontanoic acid, which was reduced to n-triacontanoic acid by Clemmensen reduction.

Jones[8a] found earlier methods for the synthesis of n-triacontanoic acid unsatisfactory. He prepared 12-oxotriacontanoic acid by the reaction of octadecylzincchloride (obtained by treating octadecylbromide with zinc chloride) with ω-carbethoxyundecanoylchloride, which on Clemmensen reduction

CHART – 1.

MALONIC ESTER SYNTHESIS

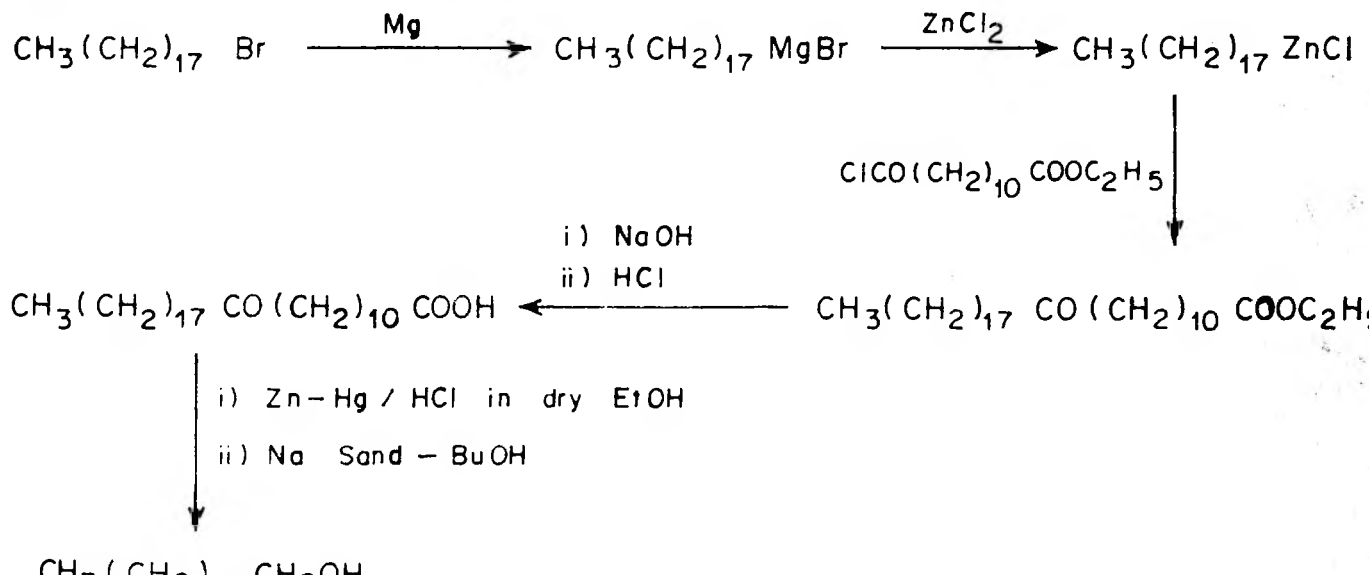
BLEYBERG AND ULRICH [6a]



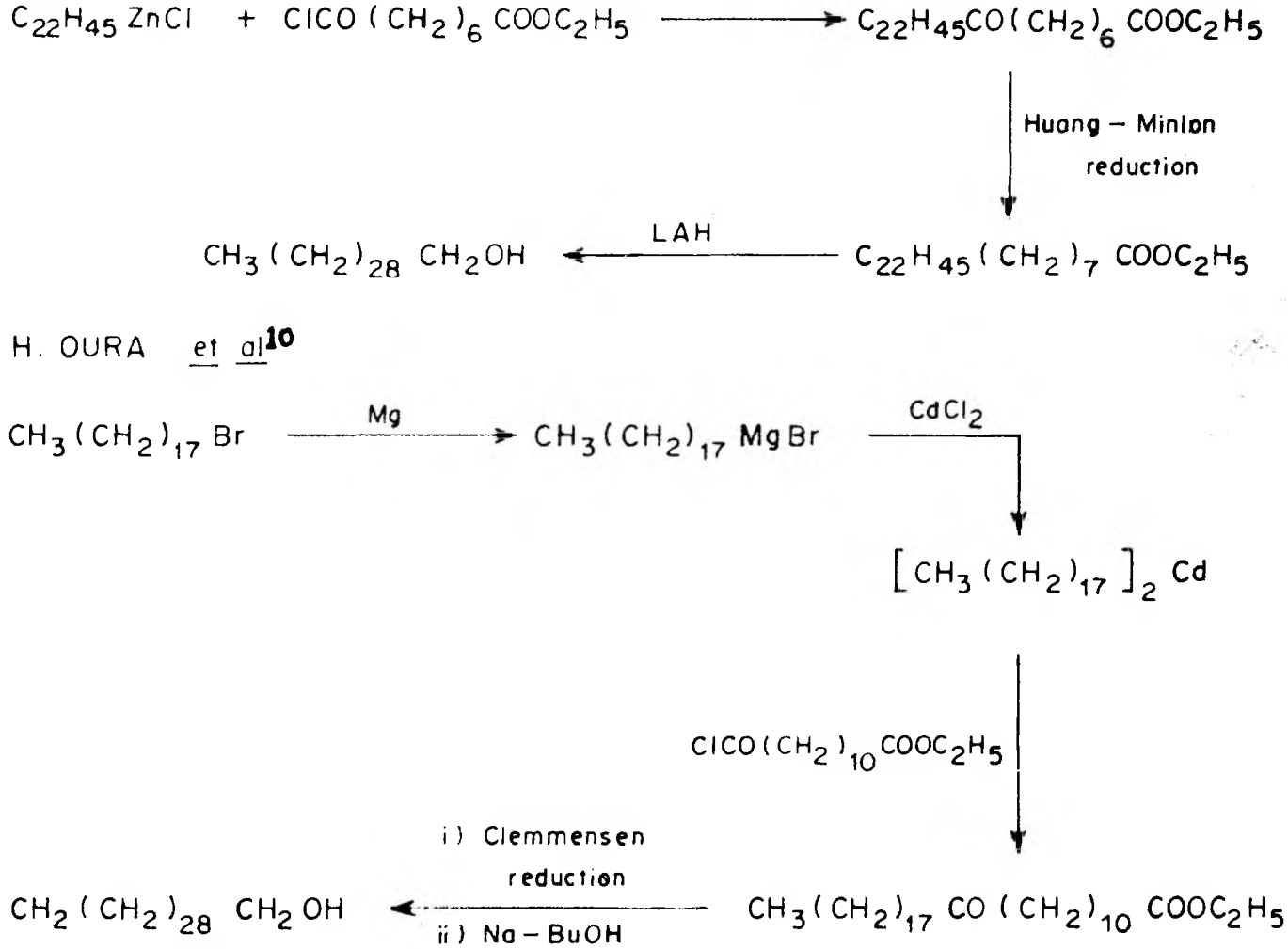
CHART—2.

METHODS INVOLVING ORGANOMETALLIC REAGENTS

JONES [8a]



SCHILDKNECHT AND RENNER [8b]



H. OURA et al[10]

in absolute ethanol as recommended by Schneider and Spielman[9] gave ethyl ester of triacontanoic acid which was reduced to n-triacontanol by sodium-butyl alcohol.

Oura et al.[10] synthesised n-triacontanoic acid, in two step sequence by the reaction of di-octadecylcadmimum with ω-carbethoxyundecanoylchloride gave 12-oxotriacontanoic acid, which on Clemmensen reduction gave n-triacontanoic acid.

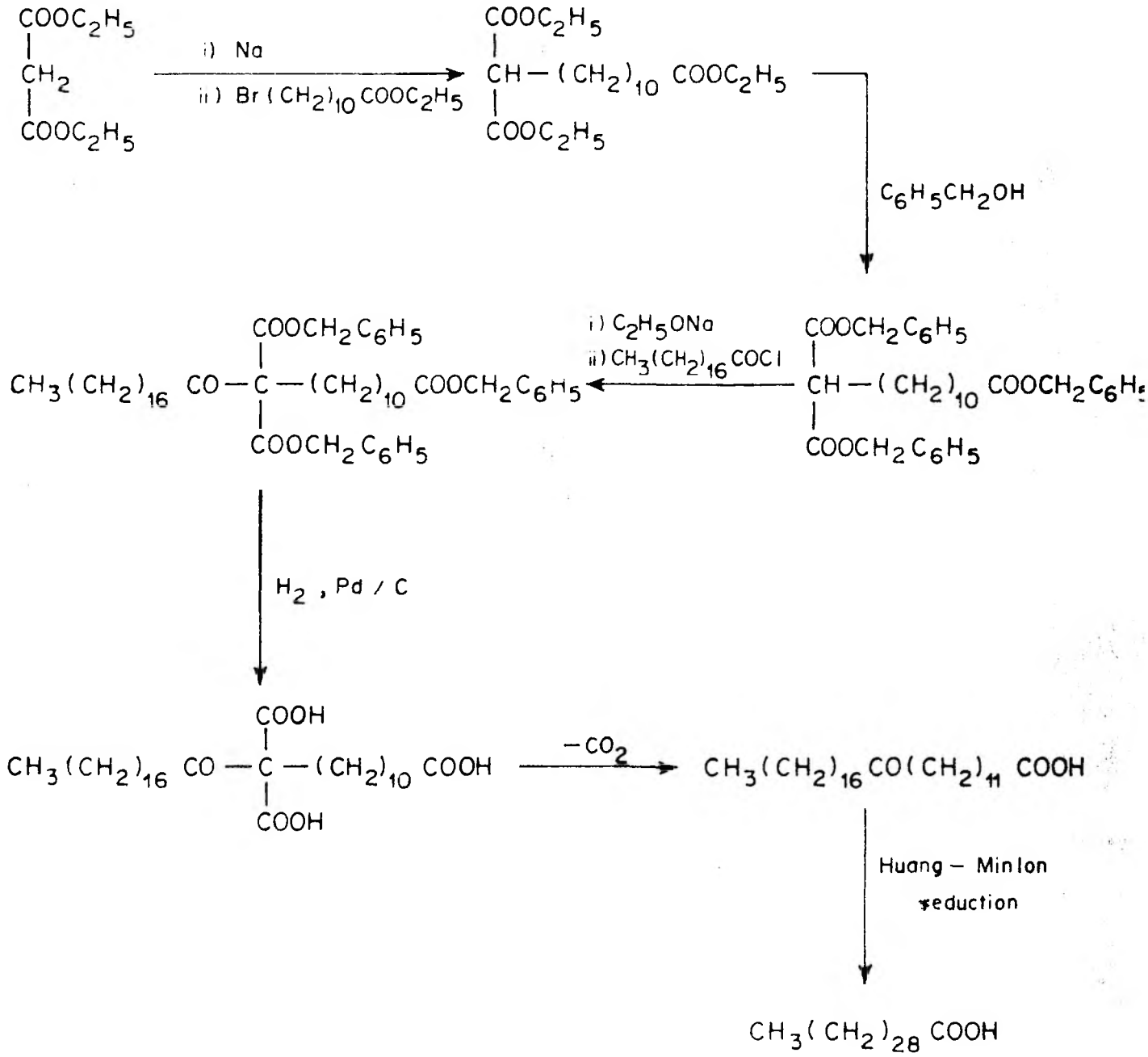
The utility of malonic ester synthesis was limited, because esterhydrolysis in the triester intermediate prior to decarboxylation, results in preferential hydrolytic cleavage at acyl to malonate bond and the formation of undesirable products. This limitation in the malonic ester synthesis was resolved by Bowman and coworkers[11] by substituting ethyl groups in diethylmalonate by benzyl groups and cleaving the benzyl to oxygen bond by hydrogenolysis. Watanabe[12] applied Bowmann's method with some improvement for the synthesis of n-triacontanoic acid using ethyl-ω-bromoundecanoate and stearoylchloride, as depicted in chart 3.

In an attempt to synthesise n-triacontanol through enamine reaction Schildknecht and Renner[8b] found that 2-tetracosanoylcyclohexanone underwent cleavage on treatment with sodium hydroxide giving n-tetracosanoic acid instead of the corresponding 7-oxotriacontanoic acid. Therefore they have abandoned this route and obtained n-triacontanol by the condensation of alkylzinc chloride and ω-carbethoxyacylchloride(chart 2).

CHART – 3.

BOWMAN'S SYNTHESIS

WATANABE [12]



PRESENT WORK

The foregoing discussion shows the importance of plant growth regulators and specifically n-triacontanol in agriculture. It is supplied by "Polyscience" in the U.S. at a price of 360 US dollars per gram. The methods reported for the synthesis of n-triacontanol are tedious, involving a number of steps; the yields are poor, and the work is of academic interest only. For field trials in this country, n-triacontanol is required, but the price is exhorbitant.

In the present investigation n-triacontanol is synthesised starting from stearic acid, which is available in plenty at a very low price, by two successive additions of six carbon atoms through enamine reaction. Its synthesis has been outlined in scheme 1. It involves the following four principle steps:

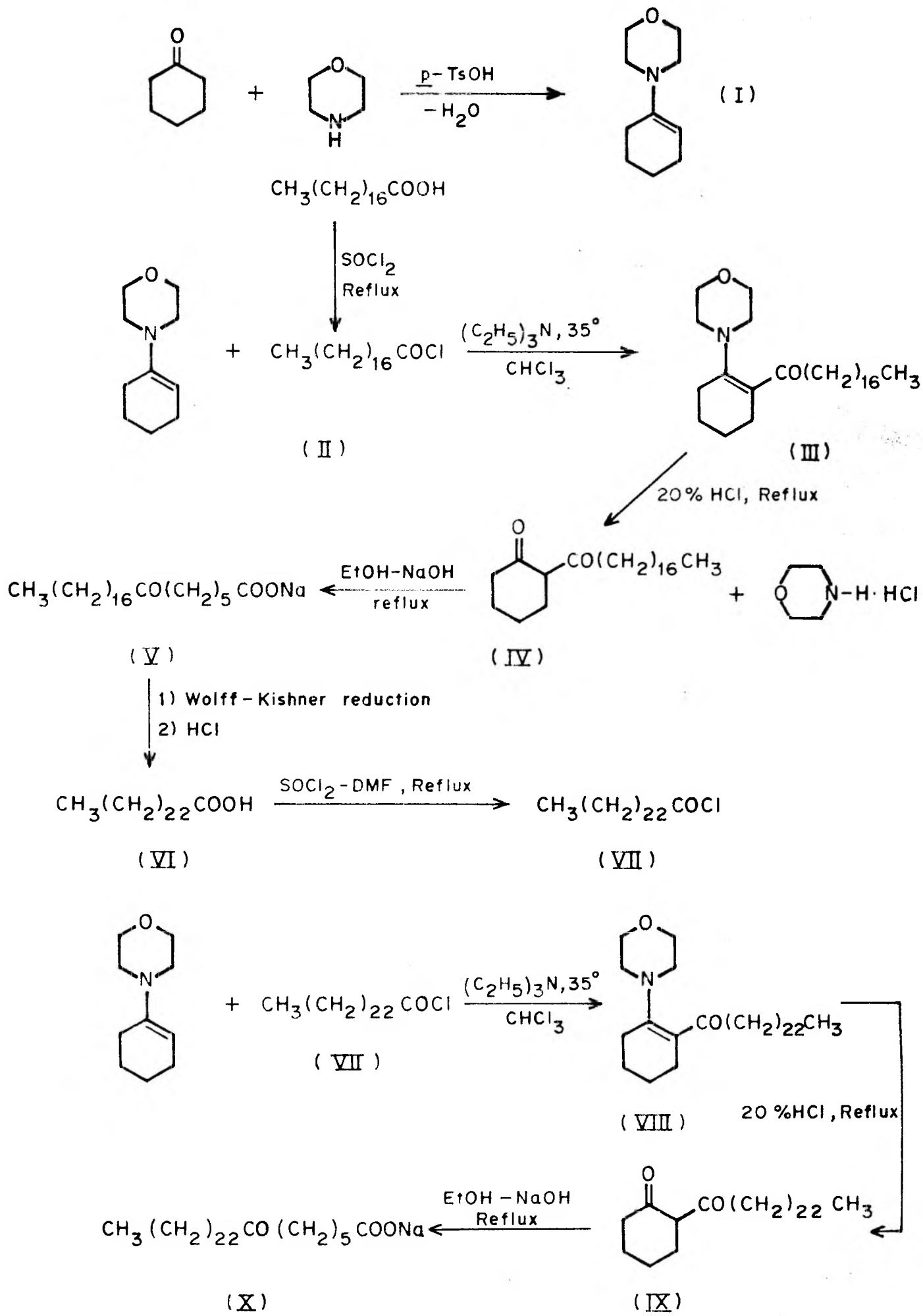
1. Acylation of cyclohexanone enamine,with carboxylic acid chloride in presence of triethylamine to 2-acylenamine, followed by its hydrolysis *in situ* to 2-acylcyclohexanone.
2. Alkali cleavage of 2-acylcyclohexanone to sodium salt of oxo acid and its subsequent conversion to free oxo-acid.
3. Reduction of sodium salt of oxoacid by Wolff-Kishner or through dithiane or ethylenedithioketal to the corresponding saturated acid.
4. Reduction of the ester function to alcohol.

1-Morpholino-1-cyclohexene (I) was prepared from cyclohexanone and morpholine condensation,[13] in presence of p-toluenesulphonic acid with azeotropic removal of water.

Stearoyl chloride[14] (II) obtained by the reaction of stearic acid and thionylchloride was condensed with (I) in chloroform solution in presence of triethylamine at 35°, and subsequent hydrolysis of 2-stearoyl -1-morpholino-1-cyclohexene (III) with hydrochloric acid in situ, furnished 2-stearoyl-cyclohexanone (IV), m.p. 46° in 95% yield. Compound (IV) on alcoholic alkali hydrolysis gave sodium salt of 7-oxotetracosanoic acid (V). The sodium salt (V) was then subjected to Wolff-Kishner reduction using 80% hydrazinehydrate and potassium hydroxide in ethyleneglycol yielded n-tetracosanoic acid, m.p.80-82°.

n-Tetracosanoic acid (VI) was converted to acid chloride (VII) by using thionylchloride in presence of dimethylformamide (the acid chloride was subjected to further reaction without distillation, as it decomposes even under vacuum) which on condensation with 1-morpholino-1-cyclohexene (I) under similar conditions, as described above, followed by subsequent hydrolysis with 20% hydrochloric acid in situ gave 2-tetracosanoylcyclo-hexanone (IX), m.p. 48-50°.

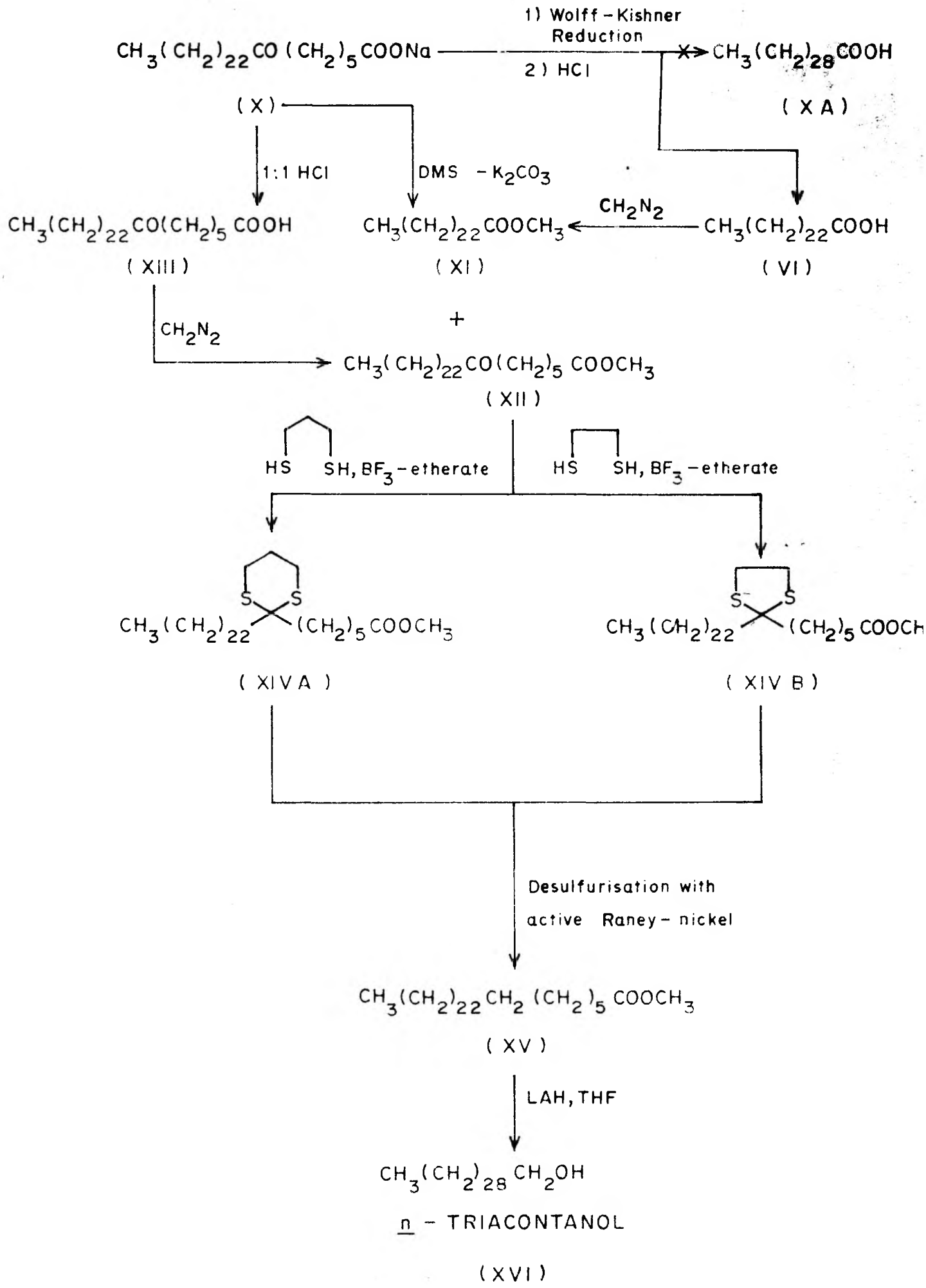
SCHEME-1


p-TsOH
-H2O
(I)
CH3(CH2)16COOH
SOCl2
Reflux
(C2H5)3N, 35°
CHCl3
CO(CH2)16CH3
(II)
(III)
20% HCl, Reflux
EtOH-NaOH
reflux
CO(CH2)16CH3
N-H·HCl
CH3(CH2)16CO(CH2)5COONa
(V)
(IV)
1) Wolff-Kishner reduction
2) HCl
SOCl2-DMF, Reflux
CH3(CH2)22COOH
CH3(CH2)22COCl
(VI)
(VII)
(C2H5)3N, 35°
CHCl3
CO(CH2)22CH3
(VII)
(VIII)
20 %HCl, Reflux
EtOH-NaOH
Reflux
CO(CH2)22 CH3
CH3(CH2)22CO(CH2)5COONa
(X)
(IX)

The diketone (IX) was hydrolysed with alcoholic alkali to sodium salt of 7-oxotriacontanoic acid (X). Attempts to reduce this sodium salt (X) by Wolff-Kishner reduction by usual method gave n-tetracosanoic acid (VI) instead of a desired n-triacontanoic acid (XA). Apparently in the Wolff-Kishner reduction, the hydrazine derivative might not have formed and the compound might have cleaved under the conditions with alkali. This is not surprising in view of the known behaviour of keto-acids[11a,15,16] of long chain compounds. They do not form easily either hydrazones or oximes.[8b] To overcome this difficulty, it has been resorted to other methods of reducing the carbonyl group. Earlier authors[8b] have used Clemmensen reduction to obtain n-triacontanoic acid from the 7-oxoacid in relatively low yield. As this method is not attractive and involves tedious operation, it was converted to a thioketal derivative and reduced by hydrogenation.

The sodium salt of 7-oxotriacontanoic acid (X) on methylation with dimethylsulphate and potassium carbonate in acetone at reflux temperature gave a mixture of methyl-n-tetracosanoate (XI) in major amount and methylester of 7-oxotriacontanoic acid (XII). Ester (XI) was found to be identical (mixed m.p. and TLC) with the ester prepared by esterification of n-tetracosanoic acid (VI) with diazomethane at 0°. From this it appears that 7-oxotriacontanoic acid

SCHEME-1 (Contd.)



is very sensitive with base and easily hydrolysed to n-tetracosanoic acid (VI). However, if the sodium salt (X) is converted to the corresponding acid (XIII) by treating with hydrochloric acid (1:1) and on subsequent esterification with diazomethane at 0^{o} gave the ester (XII) in quantitative yield. It was converted to the dithiane (XIVA) in quantitative yield by treatment with 1,3-propanedithiol in presence of borontrifluoride-etherate.

Although the dithiane (XIVA) was obtained in excellent yield, in practice, propanedithiol is used to only a limited extent as compared to ethanedithiol. Therefore ethylenedithioketal (XIVB) was also prepared. Condensation of the ester (XII) with ethanedithiol in presence of borontrifluoride-etherate gave ethylenedithioketal (XIVB) in 85% yield.

Desulfurisation of (XIVA) and (XIVB) with active Raney-nickel in ethanol at reflux temperature gave methyl-ester of n-triacontanoic acid (XV). The ester function in (XV) was reduced with lithium aluminium hydride in tetrahydforuan to give n-triacontanol (XVI) in quantitative yield.

EXPERIMENTAL

1-Morpholino-1-cyclohexene (I)

A solution of cyclohexanone (147 g., 1.5 moles), morpholine (170 g., 2 moles) and p-toluenesulfonic acid (1.5 g., 1%) in dry toluene (400 ml) was refluxed for 5 hr. in a flask equipped with a Dean-Stark water separator. Toluene was distilled off and the residue on distillation under vacuum furnished compound (I) (180 g., 72%) as colourless liquid at $118^{o}/10$ mm. (lit.[13] $118\text{-}120^{o}/10$ mm.).

Stearoyl chloride (II)

A solution of thionylchloride (47.6 g., 400 mmol) in dry benzene (20 ml) was added dropwise at room temperature to a stirred suspension of stearic acid (56.8 g., 200 mmol) in dry benzene (80 ml) during 0.5 hr. After the addition, the mixture was refluxed at 75^{o} for 2 hr. and then at 90^{o} for additional 2 hr. Excess thionyl chloride and benzene were distilled off under reduced pressure and the residue after drying under vacuum provided acid chloride (II) (60 g., 99%), b.p.$185^{o}/2$ mm. (lit.[14] b.p. $215^{o}/13\text{-}15$ mm). IR(liquid film) 1805 cm^{-1} (C=O of acid chloride) shows the absence of free acid.

2-Stearoylcyclohexanone (IV)

A solution of stearoylchloride (60.4 g., 200 mmol) in dry chloroform (60 ml) was added dropwise to a well stirred solution containing 1-morpholino-1-cyclohexene (36.74 g., 220 mmol) and dry triethylamine (20.2 g., 200 mmol) in dry chloroform (150 ml) over a period of 1 hr. at 35°. The mixture was stirred at this temperature for 3 hr., treated with hydrochloric acid (100 ml, 20%) and refluxed for 5 hr. The contents were cooled to room temperature, chloroform layer extracted with water (6 x 15 ml) and the extracts mixed with aqueous layer. The combined aqueous layer was adjusted to pH 5-6 with sodium hydroxide (25%), extracted with chloroform (5 x 20 ml) and extracts mixed with chloroform layer. The chloroform solution was dried (sodium sulphate) and passed through a dry column of silica gel (1 kg), using chloroform as an eluent; the product band moves rapidly and separates from impurities. Distillation of chloroform from the percolates provided a syrupy residue which on crystallisation from hexane gave colourless needles of (IV) (70 g., 95%), m.p. 46°. IR (CCl_4) 1740 (C=O of acyl-group), 1710 cm^{-1} (C=O of cyclohexanone), M^+ 364. (Found: C, 78.97; H, 12.0. $C_{24}H_{44}O_2$ requires C, 79.12; H, 12.09%).

Sodium salt of 7-oxotetracosanoic acid (V)

A mixture containing sodium hydroxide (8 g., 200 mmol) and commercial absolute ethanol (140 ml) was refluxed with stirring till sodium hydroxide dissolved. The solution was cooled to room temperature, a warm solution of 2-stearoyl cyclohexanone (36.4 g., 100 mmol) in absolute ethanol (50 ml) added and refluxed for 1 hr. The precipitated sodium salt was filtered and pressed as dry as possible. It was suspended in dry ethanol (100 ml) with stirring and filtered to get sodium salt (V) (35 g., 86%).

n-Tetracosanoic acid (VI)

Powdered potassium hydroxide (5.6 g, 100 mmol) in ethyleneglycol (60 ml) was refluxed till it dissolved and the solution cooled to 80-100°. Sodium salt (V) (20.2 g., 50 mmol) and hydrazinehydrate (8 ml, 80%) were added to this solution. The contents were warmed cautiously (reaction is strongly exothermic) and then refluxed for 1 hr. The water formed in the reaction and excess hydrazinehydrate were distilled off and the mixture refluxed for 1 hr. at 190-200°. It was cooled to 100-110°, poured into water (100 ml), acidified to congo-red with vigorous stirring, precipitated solid cooled and filtered. It was remelted in a beaker with water (200 ml) on a steam bath, cooled, filtered and crystallisation from acetic acid, afforded n-tetracosanoic acid (15 g., 81.53%), m.p. 87°,

(lit.[17] m.p. 87.5-88°). IR ($CHCl_3$) 1700 cm^{-1} (C=O of acid) M^+ 368. (Found: C, 78.26; H, 13.04. $C_{24}H_{48}O_2$ requires C, 78.26; H, 13.14%).

Preparation of n-tetracosanoylchloride (VII)

A solution of thionylchloride (5.95 g., 50 mmol) in dry benzene (15 ml) was added dropwise to a well stirred suepsnsion of n-tetracosanoic acid (3.68 g., 10 mmol) in dry benzene (10 ml) containing a few drops of DMF within 1 hr. at room temperature. The mixture was refluxed for 2 hr. at 75° and then at 90° for 6 hr. Benzene and excess thionyl-chloride were distilled off, the residue dried under vacuum and used without purification in the next step. IR (liquid film) 1700 (C=O of acid, weak band), 1805 cm^{-1} (C=O of acid chloride). In order to obtain high yields of acid chloride, variation in conditions did not ehnance the yield.

2-Tetracosanoylcyclohexanone (IX)

To a stirred mixture of 1-morpholino-1-cyclohexene (1.67 g., 10 mmol) and anhydrous triethylamine (1.39 ml, 10 mmol) in dry chloroform (15 ml) was added a solution of acid chloride (VII) (3.86 g., 10 mmol) in dry chloroform (15 ml), over a 20-minute period at 35°. The colour of the solution changed from orange to red. After an additional 3 hr. stirring at the same temperature, hydrochloric acid (10 ml, 20%) was added and

refluxed for 5 hr. with vigorous stirring. The contents were cooled to room temperature and worked up as described for 2-stearoylcyclohexanone (IV). Chloroform solution was dried (sodium sulphate), evaporated to dryness under reduced pressure and the brown oily residue subjected to chromatography on a column of silica gel (10 g). Elution of column with hexane yielded (IX), which on crystallisation from hexane afforded colourless plates (0.896 g.), m.p.48-50°. IR (nujol) shows weak band at 1700 and a strong band at 3200 cm^{-1}, thereby showing major proportion of keto-enol form, rather than the diketo form M^{+} 488. (Found: C, 79.64; H, 12.11. $C_{30}H_{56}O_2$ requires C, 80.05; H, 12.50%).

Sodium salt of 7-oxotriacontanoic acid (X)

Finely powdered sodium hydroxide (0.160 g., 4 mmol) was dissolved in absolute ethanol by boiling the mixture nearly for 15 min. To this solution was added a warm solution of 2-tetracosanoylcyclohexanone (IX) (0.896 g., 2 mmol) in absolute ethanol (2 ml) with stirring and refluxed for 1 hr. The reaction mixture was cooled, precipitated sodium salt filtered and pressed as dry as possible. The moist salt was suspended in absolute ethanol and filtered to get (X) (0.976 g., 100%).

Wolff-Kishner reduction of (X)

Powered potassium hydroxide (0.112 g., 2 mmol) was dissolved in ethylene glycol (5 ml) by heating. Sodium salt of 7-oxotriacontanoic acid (X,0.488 g., 1 mmol) and hydrazine hydrate (2 ml, 80%) were added to the solution, the reaction mixture warmed cautiously (reaction is exothermic) and then refluxed at 190° for 1 hr. The reaction was worked up exactly as described for (VI). The solid obtained was crystallised from benzene as colourless needles (0.215 g., 58%), m.p.87°, IR($CHCl_3$) 1700 cm^{-1} (C=O of **acid**), M^+ 368. It was chracterised as n-tetracosanoic acid from its mass spectral data, and mixed m.p.

Methylation of sodium salt of 7-oxotriacontanoic acid (X) using DMS-K_2CO_3

A mixture containing sodium salt (X) (0.682 g., 1.4 mmol), DMS (0.21 g., 1.7 mmol) and anhydrous potassium carbonate (5 g.) in dry acetone (25 ml) was refluxed for 4 hr. At the end of reaction, acetone was distilled off, the residue treated with water and pale yellow oil, extracted with chloroform. The chloroform solution was dried (sodium sulphate) and solvent removed under reduced pressure. The crude product was a mixture of two compounds, which were separated by chromatography, over a column of silica gel (6 g.) using benzene and benzene-acetone (9.8:0.2) for elution. The faster moving compound was

obtained by eluting the column with benzene, crystallised from hexane as colourless plates (0.5 g., 93%), m.p.60° (lit.[17] m.p. 59.5-60°) and characterised as methyl n-tetracosanoate (XI). IR($CHCl_3$) 1740 cm^{-1} (C=O of ester), M^+ 382.

Elution of the column with benzene-acetone furnished a slow moving compound, crystallised from hexane, m.p.62-64° and was identified as methyl-7-oxotriacontanoate (XII) on the basis of its spectral data.

7-Oxotriacontanoic acid (XIII)

Sodium salt (X) (0.976 g.) was suspended in dilute hydrochloric acid (10 ml, 1:1) with stirring and the keto acid extracted with chloroform (5 x 10 ml). The chloroform solution was washed with water, dried (sodium sulphate) and solvent removed under reduced pressure. The residue on crystallisation from benzene, provided acid (XIII) in colourless plates (0.93 g., 100%), m.p. 98° (lit.[8b] m.p. 98°), IR($CHCl_3$) 1710 cm^{-1} (C=O of ketone and acid). M^+ 466. (Found: C, 77.20: H, 12.60. $C_{30}H_{58}O_3$ requires C, 77.25; H, 12.45%).

Methyl 7-oxotriacontanoate (XII)

A solution of diazomethane was prepared from N-nitroso-N-methylurea (5.15 g., 50 mmol) in 30 ml ether and dried over potassium hydroxide. This ethereal solution was added to the ice cold suspension of 7-oxotriacontanoic acid (XIII) (0.466 g., 1 mmol) in dry methanol (5 ml) and kept in the refrigerator

overnight. After the removal of solvent and excess diazomethane by rotary evaporation, the residue was crystallised from hexane as colourless prisms in quantitative yield, m.p.64°. IR($CHCl_3$) 1705 (C=O of ketone), 1740 cm^{-1} (C=O of ester), M^+ 480. (Found: C, 77.38; H, 12.65. $C_{31}H_{60}O_3$ requires C, 77.50; H, 12.50%.

Dithiane (XIVA)

A solution of borontrifluoride-etherate (0.156 g., 1.1 mmol) in dry chloroform (1 ml) was added to a well stirred solution containing the methyl ester (XII) (0.480 g., 1 mmol) and 1,3-propanedithiol (0.118 g., 1.1 mmol) in dry chloroform(5 ml) and the mixture stirred at room temperature for 36 hr. The chloroform solution was washed with water, and dried (potassium carbonate). The residue obtained after evaporating the solvent on a rotary evaporator was chromatographed on a column of silica gel (10 g) using benzene-hexane (1:1) for elution provided the dithiane (XIVA) in quantitative yield as a colourless viscous oil. IR(CCl_4) 920 (characteristic dithiane band), 1740 cm^{-1} (C=O of ester). NMR(CCl_4): 3.40 (s,3H,OMe)2.60(m,4H, $\begin{matrix}S\text{-}CH_2\\S\text{-}CH_2\end{matrix}$) 1.97 (bt,2H,$CH_2CO$)1.20(bs,57H, 27 CH_2 and CH_3). M^+ 570. (Found: C, 71.58; H, 11.41; S, 11.08. $C_{34}H_{66}O_2S_2$ requires C, 71.58; H, 11.58; S, 11.23%).

Ethanedithioketal (XIVB)

A solution of borontrifluoride etherate (0.426 g., 3 mmol) in dry chloroform (2 ml) was added dropwise to a stirred mixture containing the ester (XII) (1.347 g., 2.8 mmol) and ethanedithiol (0.290 g., 2.9 mmol) in dry chloroform (20 ml). The contents were stirred at room temperature for 22 hr. Chloroform solution was washed with water, dried (sodium sulphate) and solvent distilled off on a rotary evaporator. The residue on chromatography over a column of silica gel (15 g) using benzene-hexane (1:1) as an eluent provided ethanedithioketal (XIVB) as colourlessoil (1.327 g., 85%). IR(CCl_4) shows a stfong band at 1740 cm^{-1} (C=O of ester) and absence of band at 1705 cm^{-1}: M^+ 556. (Found: C, 71.09; H, 11.47; S, 11.60. $C_{33}H_{64}O_2S_2$ requires C, 71.22; H, 11.51; S, 11.52%).

Methyl n-triacontanoate (XV)

(a) Desulfurisation of dithiane (XIVA)

A suspension containing the dithiane (XIVA, 0.320 g) and active Raney-nickel (3 g., i.e. 6 ml of settled suspension) in ethanol (20 ml, 95%) was refluxed for 9 hr. The suspension was filtered and nickel washed with hot ethanol. Evaporation of solvent under reduced pressure yielded the ester (XV) in quantitative yield, crystallised from eth yl alcohol as colourless plates, m.p.71° (lit.[18] m.p.71.5°). IR($CHCl_3$)

1740 cm^{-1} (C=O of ester), M^{+} 466. (Found: C, 79.68; H, 13.2. $C_{31}H_{62}O_2$ requires C, 79.82; H, 13.30%).

b) Desulfurisation of ethanedithioketal (XIVB)

A suspension of the compound ((XIVB) (1.277 g) and active Raney nickel (12 g., 24 ml of settled suspension in ethanol(75 ml) was refluxed for 7 hr. The hot suspension was filtered and Raney nickel washed with hot ethanol (2 x 50 ml). Evaporation of solvent on a rotary evaporator and the residue of ester (XV) on crystallisation from ethanol gave colourless plates (1.070 g., 100%), m.p.71°.

n-Triacontanol

A solution of ester (XV) (1.120 g., 2.4 mmol) in dry THF (15 ml) was added dropwise to a stirred suspension of lithium-aluminiumhydride (0.047g.,1.25 mmol) in dry THF (5 ml) at -10°. The temperature was allowed to raise to room temperature, over a period of 1 hr. and stirred at this temperature for 4 hr. After usual work up with aqueous sodium hydroxide, fine precipitate was filtered and washed with THF. The filtrate was dried (sodiumsulphate), solvent distilled off on a rotary evaporator and the crude alcohol (XVI) on crystallisation from hexane, furnished colourless plates (1.01 g., 95%), m.p.87° (lit.[3] m.p. 87-88°), mixed m.p. with an authentic sample remained undepressed. IR (Nujol) 3300 cm^{-1} (-OH); M^{+} 438; m/e 420 (M^{+} 18). (Found: C, 82.08; H, 13.10. $C_{30}H_{62}O$ requires C, 82.19; H, 13.30%).

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S U M M A R Y

Chapter I Experiments directed towards the total synthesis of (±)-zearalenone

Zearalenone (I), a metabolite of pathogenic fungi has been isolated by two different groups, one at Purdue and the other at University of Minnesota. It has shown potent steroid like anabolic and uterotrophic activity. Zearalenol (II), obtained by hydrogenation of (I), has shown to be an antifertility agent and is covered by numerous patents. In view of the interesting pharmacological properties, zearalenone (I) is perhaps the first naturally occurring macrolide to be synthesised by both Merck and Syntex groups.

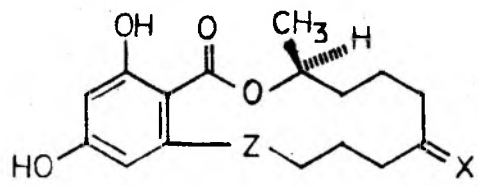
Recently Corey and Nicolaou have shown that the hydroxy acid (III) obtained by opening zearalenone can be lactonised in 75% yield through the 2-pyridinethiol ester. Subsequently Corey's group has developed better reagents, which can lactonise long chain hydroxy acids in high yield at low temperatures.

In the present work, an attempt has been made to synthesise hydroxy acid (IV), by a simple and straightforward method, starting from easily accessible intermediates. The main strategy in this work is to synthesise the aliphatic and aromatic portions separately and joining them by a Wittig reaction to give the hydroxy acid (IV), which can be lactonised to zearalenone.

As the Syntex approach for the synthesis of the aromatic part (V) is more straightforward, a convenient method for the synthesis of the aliphatic part(VI) is now explored.

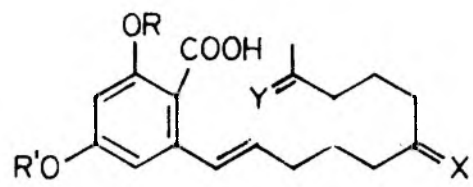
The synthesis involves the use of three different protective groups, which should be deprotected one after the other, without interference in the various synthetic operations. Antithetic analysis clearly shows the advantage of having 5-hydroxypentanal, protected by thioketalation (VIIa) (lower part), which itself can serve to generate the carbanion for C-C linkage. The upper part, which can be a derivative of 5-iodopentan-2-one (such as VIIIb), has been prepared starting from 2-acetyl-Y-butyrolactone.

5-Hydroxypentanal prepared from dihydropyran has been transformed into the dithiane derivative (VIIb) by sequential treatment with propanedithiol and dihydropyran. Metallation of the dithiane (VIIb) with n-butyllithium followed by alkylation with 5-iodopentan-2-one derivative (VIIIb) yielded the alkylated product (VIa). Although the C-C linkage has been achieved in good yield, exclusive cleavage of THP group in (VIa) has failed. Moreover, it resulted in the removal of both the THP and ethyleneketal, yielding (VIc). Direct metallation of the hydroxydithiane (VIIa) and subsequent alkylation with 5-iodopentan-2-one derivative (VIIIb) yielded the desired product (VIb) in very low yield.

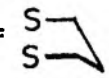


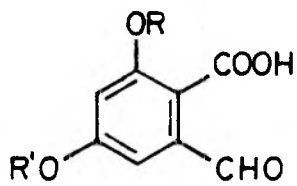
(I) Z = CH = CH , X = O

(II) Z = $CH_2 - CH_2$, X = H, OH

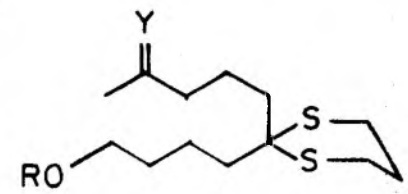


(III) R = H , R' = THP , X =  , Y = H, OH

(IV) R = R' = Me , X =  , Y = H , OH



(V)



(VI)

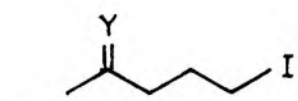
(VIa) R = THP , Y = 

(VIb) R = H , Y = 

(VIc) R = H , Y = O

(VId) R = THP , Y = 

(VIe) R = H , Y = 



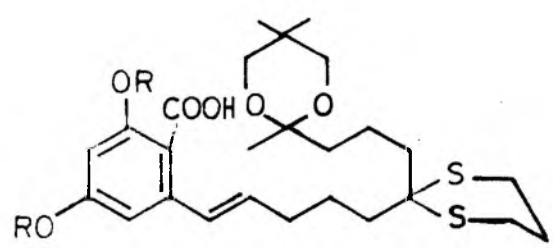
(VIIa) R = H

(VIIb) R = THP

(VIIIa) Y = O

(VIIIb) Y = 

(VIIIc) Y =



(IX) R = Me

Because of the incompatible nature of these two protecting groups (THP and ethyleneketal) with respect to hydrolysis, different protective groups for ketonic function in 5-iodopentan-2-one (VIIIa) have been examined. A perusal of literature indicated that the rate of hydrolysis of a substituted 1,3-dioxane derivative of a ketone is relatively much slow compared with a dioxalane group. Model studies with the THP ether (VIIb) and substituted dioxane derivative (VIIIc) using methanolic oxalic acid revealed that dioxane cleavage is moderately slow and THP group can be preferentially hydrolysed. Therefore the dioxane derivative (VIIIc) obtained from 5-iodopentan-2-one should serve the desired purpose.

Metallation of the dithiane (VIIb) with n-butyllithium and subsequent alkylation with the iodo compound(VIIIc) yielded the alkylated product (VId) in good yield. Selective cleavage of THP group has been achieved by mild hydrolysis using methanolic oxalic acid to give the aliphatic key intermediate (VIe) in 94% yield.

This method of synthesis of the aliphatic portion of zearalenone is simple and requires mild reaction conditions. Conversion of the hydroxy compound(VIe) to phosphorane followed by a Wittig reaction with aromatic portion (V) should result (IX). Hydrolysis of dioxane function and lactonisation followed by oxidative desulfurisation is expected to give (±)-zeralenone dimethylether. Work on these operations is now being perceived.

Chapter II: Chemical investigation of Indian medicinal plants

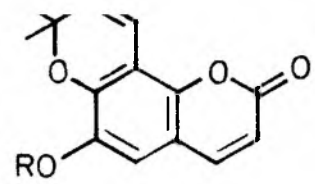
Family Rutaceae has been shown to be a rich source of compounds of diverse structural types like alkaloids, coumarins, lignans, etc. In recent years Rutaceous plants are being examined, because of their immense medicinal properties. A literature survey of three medicinal plants namely Toddalia aculeata, Skimmia laureola (subfamily: toddalioideae) and Boenninghausenia albiflora (subfamily: rutoideae) belonging to family Rutaceae and their medicinal uses have been presented. These plants obtained from M/s Mukherjee and Co., Darjeeling, have been examined for their chemical constituents.

Section 1: Toddalia aculeata

The total chloroform extract of the stem powder has been separated into alkaloidal, phenolic and neutral fractions.

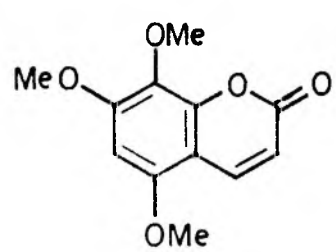
The chromatography of the alkaloidal part led to the isolation of five alkaloids, toddalinine, dictamine, robustine, γ-fagarine and skimmianine.

The phenolic part yielded a new coumarin, norbraylin (X), the NMR spectrum shows absorption typical of a chromeno-coumarin and a singlet for aromatic proton. The presence of 2,2-dimethylchromene ring has been supported by a ready loss of a methyl radical from the molecular ion. The structure (X) has been confirmed by converting it to a known pyranocoumarin, braylin (XI) by methylation with diazomethane.

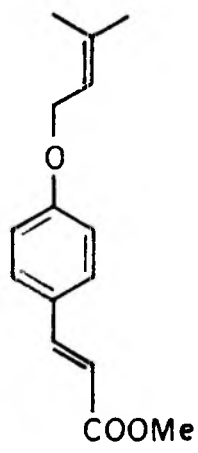


(X) R = H

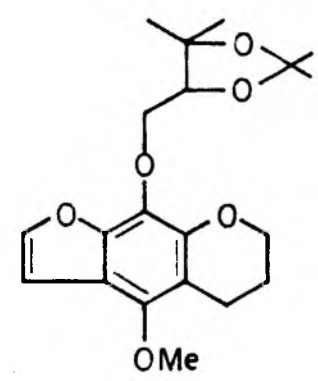
(XI) R = Me



(XII)



(XIII)



(XIV)

The neutral part contains six coumarins, pimpinellin, isopimpinellin, bergapten, braylin, luvangetin and toddalolactone. In addition, a new 5,7,8-trimethoxycoumarin (XII) has been isolated, its structure established by spectral data, benzene induced solvent shift of methoxyl groups including biogenetic considerations. Finally the structure has been proved by synthesis.

Water soluble part of the methanol extract of the stem contains glycosides and free sugars, glucose, rhamnose, arabinose and sucrose.

Section 2: Skimmia laureola

The powdered branches have been successively extracted with hexane and chloroform. Hexane extracted yielded lupeol and β-sitosterol. The chloroform extract is separated into sodium carbonate soluble, alkaloidal and non-alkaloidal part. The sodium carbonate soluble part yielded a coumarin, umbelliferone. The alkaloidal part contains four known alkaloids, dictamine, 8-hydroxydictamine, γ-fagarine and skimmianine. From the non-alkaloidal part five coumarins, xanthotoxin, aesculetin dimethyl ether, (+)-marmesin, bergapten and isopimpinellin have been isolated.

Section 3: Boenninghausenia albiflora

The methanol soluble part of branches and leaves has been extracted successively with hexane, chloroform and methanol.

The hexane extract yielded three coumarins, rutamarin, umbelliprenin and isopimpinellin alongwith β-sitosterol. In addition to these, a new 4-(3,3-dimethylallyloxy)-methyl-cinnamate (XIII) has been isolated. The structure (XIII) has been assigned to a new cinnamicacid ester, mainly on the basis of NMR and mass spectral data.

The chloroform extract contains byakangelecin acetonide (XIV), a lignan, justicidin B (4-phenylnaphthalide lignan) and a new trimethoxycoumarin (XII). The NMR and mass spectral data support structure (XIV) for the byakangelecin-acetonide. The presence of acetonide has been confirmed by acid hydrolysis to give the corresponding diol, the physical and spectral properties of which are in agreement with a known furanocoumarin, byakangelecin. The acetonide is a natural product.

The physical and spectral properties of trimethoxy-coumarin has been found to be identical with a new 5,7,8-trimethoxycoumarin (XII), isolated from Toddalia aculeata.

The structures of the isolated compounds have been determined on the basis of UV, IR, NMR, MS, microanalytical data and preparation of derivatives, wherever necessary.

It has been observed that Dragendorff's reagent, which is specific for the detection of alkaloids, also gives positive test for coumarins.

Section 4: Synthesis of 5,7,8-trimethoxycoumarin

5,7,8-Trimethoxycoumarin (XII) isolated from T.aculeata and B. albiflora is the first naturally occurring simple coumarin bearing methoxyl groups in 5,7,8-positions. The structure has been assigned on the basis of spectral data and biogenetic considerations. The structure has now been confirmed by synthesising it starting from pyrogallol. The synthetic sample is found to be identical with the natural product in all respects.

Chapter III: Synthesis of n-triacontanol - A new plant growth regulator.

A brief literature survey of the importance of plant growth regulators and their uses is presented. Recently Ries et al. have shown that, n-triacontanol (XV), a C_{30} straight chain alcohol, increases the yields of rice, tomatoes, corn, cucumber and barley. Since the recognition of the biological role of n-triacontanol in agriculture, this substance has occupied a major position in the field of plant growth regulators. A brief account of the various methods known for its synthesis has been narrated.

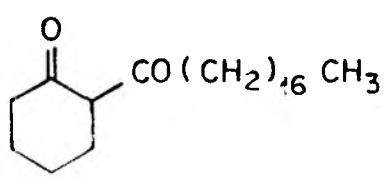
At present n-triacontanol is marketed by Polyscience (USA) at an exhorbitant price (US $ 360/g.). As it is interesting to study its effects on some of the Indian crops and is not easily accessible, a simple and convenient method has been developed for its synthesis starting from stearic acid.

2-Stearoylcyclohexanone (XVI), prepared from stearoyl-chloride through enamine reaction, on alkali hydrolysis followed by Wolff-Kishner reduction gave n-tetracosanoic acid (XVII). 2-Tetracosanoylcyclohexanone (XVIII) has been prepared similarly from tetracosanoylchloride through enamine reaction. The diketone (XVIII) is converted to sodium salt of oxo acid (XIXa) by alkaline hydrolysis. Attempted Wolff-Kishner reduction of (XIXa) yielded C_{24} acid (XVII) instead of a desired C_{30} acid (XXIIa). From this it appears that hydrazone intermediate might not have formed in the reduction and oxo acid salt (XIXa) is easily hydrolysed in the presence of a base. To overcome this difficulty, thioketals (XX and XXI) have been used as intermediates for the reduction of ketoester (XIXc) to saturated ester (XXIIb).

The sodium salt (XIXa) is converted to oxo acid (XIXb) which on esterification with diazomethane yielded the corresponding ester (XIXc). The keto ester (XIXc) on treatment with ethane or propanedithiol in presence of boron-trifluorideetherate yielded respective thioketals(XX and XXI),

$CH_3(CH_2)_{28}CH_2OH$

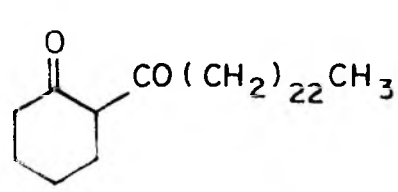
(XV)



(XVI)

$CH_3(CH_2)_{22}COOH$

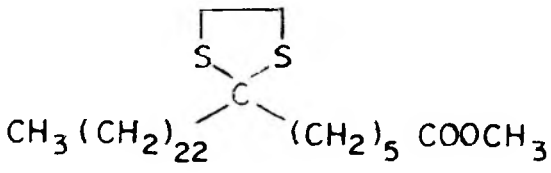
(XVII)



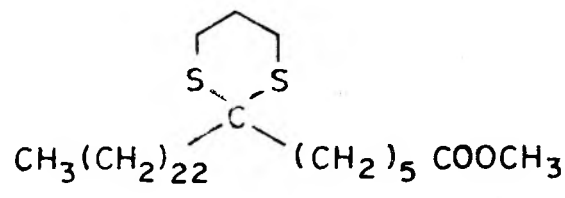
(XVIII)

$CH_3(CH_2)_{22}CO(CH_2)_5COOR$

(XIXa) R = Na
(XIXb) R = H
(XIXc) R = CH_3



(XX)



(XXI)

$CH_3(CH_2)_{28}COOR$

(XXIIa) R = H
(XXIIb) R = CH_3

which on desulfurisation (Raney nickel-ethanol) furnished the C_{30} acid ester (XXIIb). The ester has been reduced quantitatively to n-triacontanol, using lithium aluminium hydride.

The method is easy to scale up, and the yields are good. n-Triacontanol has been prepared on gram scale and is now submitted for field trials.

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

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