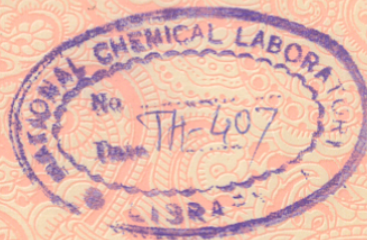


SYNTHESIS OF SOME BIOLOGICALLY ACTIVE COMPOUNDS

COMPUTERISED

A THESIS
SUBMITTED TO THE
UNIVERSITY OF BOMBAY

FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY



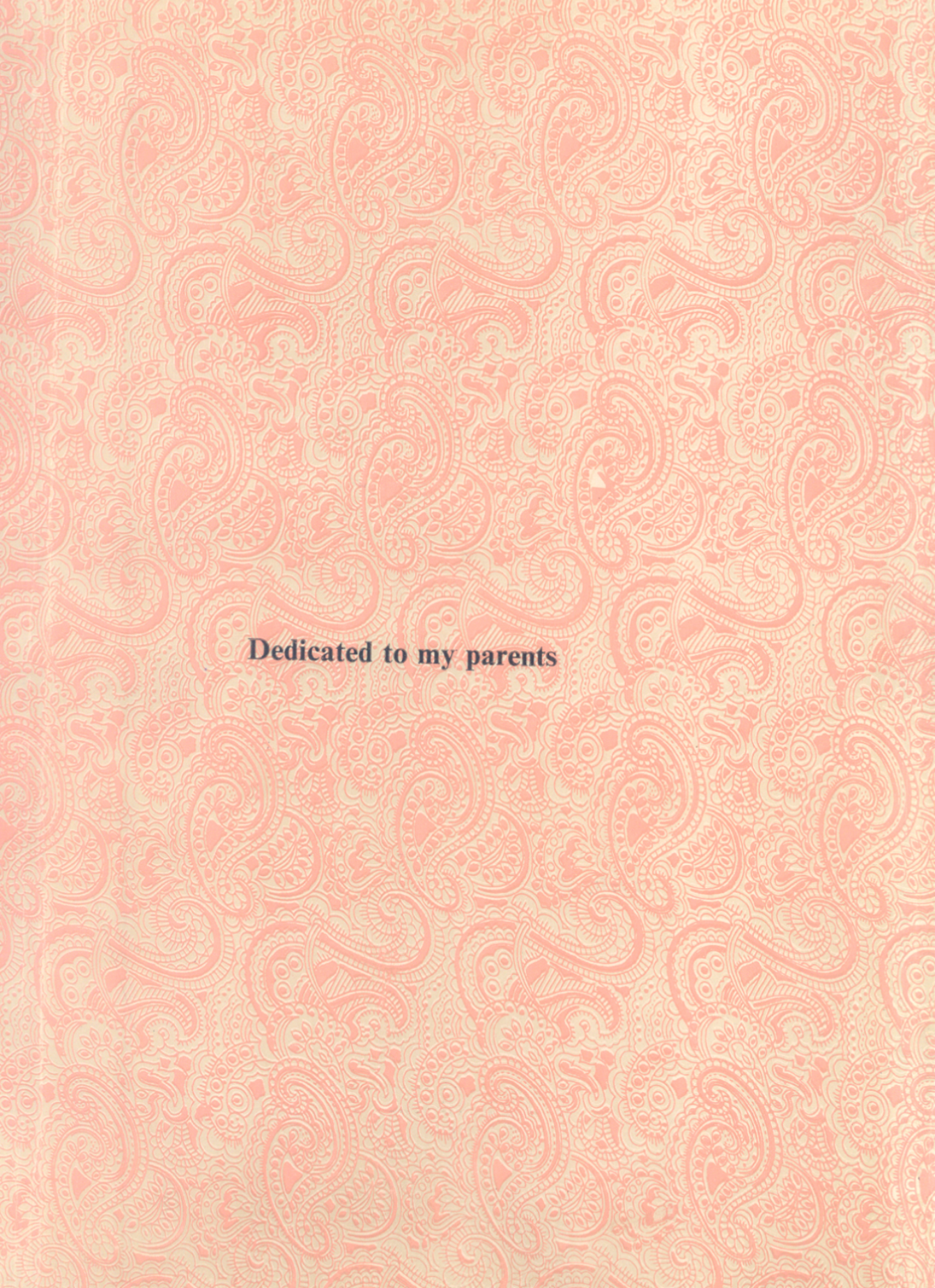
BY
K. RAVICHANDRAN
B. Sc. (Hons.), B. Sc. (Tech.)

547.673 (043)

RAV

NATIONAL CHEMICAL LABORATORY
PUNE - 411008 (INDIA)

1984



Dedicated to my parents

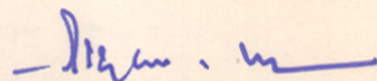
Statement Required to be Submitted Under Rule 0.413 of the University of Bombay

No part of this work has been submitted for a degree or diploma or other academic award. The literature concerning the problem investigated has been surveyed and all the necessary references are given. The experimental work has been carried out entirely by me. In accordance with the usual practice, due acknowledgement has been made wherever the work presented is based on the results of other workers.

National Chemical Laboratory,
Pune 8, India



K. RAVICHANDRAN
Candidate



A. V. Rama Rao
Research Guide

P R E F A C E

The chapters of this thesis cover important fields of research (where) 'DRUGS' and 'PLANT GROWTH REGULATOR' have proved to be of key importance.

Man is heir to many ills which have plagued, tormented and killed him throughout all of written history and beyond. Among the older ones, few of them, however, have been more pernicious and more (b)ethal than tuberculosis; and recently 'cancer', the scourge of mankind, has been on a rampage. As the investigation, development and manufacture of medicinal chemicals, giving way to the realities of the bottom line, have become productive and intellectually rewarding, newer approaches towards the synthesis of established drugs have been presented in this dissertation.

At rare intervals into the systems of chemical research come concepts which are intriguing to the biological mind. In general 'excursions' of investigators working on drug synthesis into the 'gardens' of investigations involved in the development of other types of bioactive compounds such as 'plant growth regulators' may lead to a fruitful cross fertilization of ideas. And nothing can be more thrilling than recognising a ~~substance~~ substance whose properties serve a dual purpose - medicinal as well as regulation of plant growth. This has been achieved by bridging the wide gulf which is thought to separate chemical and biological sciences. For the ~~physiology~~ physiology of plants cannot be studied in isolation; it must be dealt in conjunction with chemical science, into which, of course, it merges imperceptibly.

Each chapter reflects the individual approach and also serves to introduce a particular field of study. Nevertheless an introductory narrative has been included with the hope that it will aid in circumventing the associated problem.

Though the work has been carried out at the **National Chemical Laboratory**, facilities of the Botany ^Ddept., Pune University have also been utilized for 'plant physiology experiments'. Infrared Spectra (IR) were determined on a Perkin-Elmer 683 spectrophotometer. The maxima are recorded in cm^{-1} . Proton magnetic resonance (PMR) spectra were recorded on a Varian T-60 or Varian FT-80A or Bruker WH-90 spectrometer. All chemical shifts are reported in parts per million (δ) downfield from internal standard tetramethylsilane. Mass spectra were recorded on a CEC-21-110B double focussing mass spectrometer operating at 70 eV using direct inlet system. HPLC was done on a Waters Associates ALC/GPE - 202/R 401/M 440 system, with two M - 6000 A pumps controlled by M 660 solvent programmer. Melting points were determined in open capillaries and are uncorrected. Progress of the reactions was monitored by thin layer chromatography (tlc) on 0.2 mm layers of silica gel, prepared with Swambe chemicals silica gel 'H' and the chromatograms were exposed to iodine vapours for visualisation. Column chromatography was carried out by using silica gel (60-120 mesh, Acme make).

This thesis would not have been possible were it not for the efforts of my teacher and revered guide, **Dr. A. V. Rama Rao** who, in unmasking the problems by schematic reasoning and logical deductions provided ultimate sunshine and joy. It is with all sincerity and a deep sense of gratitude that I express my indebtedness and thankfulness to him.

I also want to thank **Dr. V. H. Deshpande** who, besides being a source of encouragement and support, guided me over the rough spots and helped shape this thesis.

To **Drs. Sujata Ranade** for helping in the plant physiology experiments, **B. R. Rao** for helping in some of the chemical experiments, **J. S. Yadav**

for fruitful criticism and perceptive comments and to **S. S. Khuspe** for conducting the field trials; I owe a deep debt of gratitude.

While I gratefully acknowledge the many persons, I alone am responsible for any errors or inadvertent improprieties that may have escaped recognition.

The financial assistance by a grant from the Science & Technology Cell, Education & Youth Services Department, Maharashtra State is gratefully acknowledged.

Finally, I wish to thank the **Director**, National Chemical Laboratory, Pune, for allowing me to submit this work in the form of a thesis.

National Chemical Laboratory
Pune 411008, India

K. RAVICHANDRAN

April 1984

CONTENTS

| | Page |
|--|------|
| | 1 |
| Summary | |
| I ANTHRACYCLBMONES | 10 |
| General Introduction | |
| A) Synthesis of (±) 4-demethoxydaunomycinone using | |
| (i) 1,4 - Anthraquinone [DCB+ A] | |
| Introduction | 17 |
| Present Work | 25 |
| Experimental | 32 |
| (ii) (±) 2-acetyl - 5, 8 - dimethoxy - 1,2,3,4 - tetrahydro-2-riaphthol [AB + CD] | |
| Introduction | 36 |
| Present Work | 46 |
| Experimental | 68 |
| B) Synthesis of (±) 4-demethoxy-11 -deoxy daunomycinone | |
| Introduction | 84 |
| Present Work | 92 |
| Experimental | 108 |
| R e f e r e n c e s | 120 |
| II PLANT GROWTH REGULATORS | 126 |
| General Introduction | |
| A) Synthesis of 1-triacontanol | 133 |
| Introduction | |
| Present Work | 144 |
| Experimental | 151 |

| | Page |
|--|------|
| B) The evaluation of 1-triacontanol | 155 |
| Introduction | 157 |
| Experimental Results | 165 |
| Conclusion | |
| C) Menadione Bisulfite : A Promising Plant Growth Regulator | |
| (i) 'Preliminary Scan | |
| Introduction | 168 |
| Experimental | 173 |
| Discussion | 184 |
| (ii) 'Mode of Action' | |
| Introduction | 186 |
| Experimental | 189 |
| Discussion | 193 |
| D) Synthesis of Menadione | 211 |
| Introduction | |
| Present Work | 218 |
| Experimental | 232 |
| R e f e r e n c e s | 241 |
| III SYNTHESIS OF PYRAZINAMIDE | 250 |
| Introduction | |
| Present Work | 253 |
| Experimental | 257 |
| R e f e r e n c e s | 260 |
| List of Publications | 262 |

S U M M A R Y

This thesis consists of three chapters, divided into seven sections.

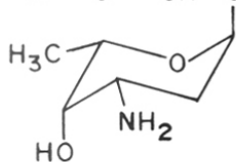
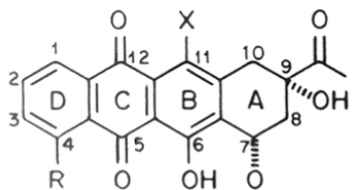
CHAPTER 1 - ANTHRACYCLINONES

Part A: Synthesis of (+)4-demethoxydaunomycinone

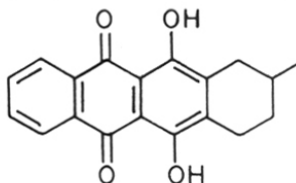
Current interest in the total synthesis of the clinically important anthracycline antibiotic daunomycin (1a) has led to the development of a variety of strategies for the construction of this complex molecule. Recently it has been shown that 4-demethoxydaunomycin (1b), a synthetic analogue of 1a, besides exhibiting greater activity than 1a is also effective for oral therapy. As there is no possibility of obtaining 1b by fermentation and as several routes to L-daunosamine, the amino sugar moiety have been reported and its coupling to the aglycone of 1b [4-demethoxydaunomycinone (2a)] has been well established, much attention has been focussed towards the synthesis of 2a.

Synthesis of the aglycone, 4-demethoxydaunomycinone (2a), an intermediate in the synthesis of 1b, has been discussed in this part.

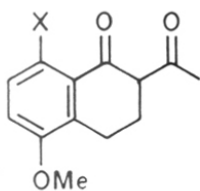
In the first attempt, the Diels-Alder approach utilizing 1,4-anthraquinone (having DCB rings already incorporated) had been exploited, rather unsuccessfully to build



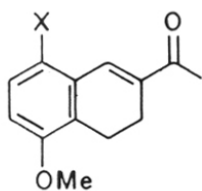
- 1 a) R = OCH₃, X = OH 2 a) R = H, X = Y = OH
 b) R = H, X = OH b) R = X = H, Y = OH
 c) R = OCH₃, X = H c) R = Y = H, X = OH
 d) R = X = H d) R = X = Y = H



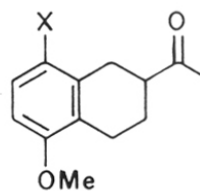
3



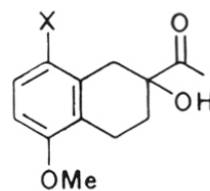
- 4 a) X = OMe
 b) X = H



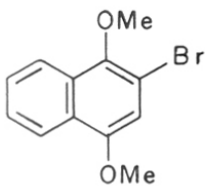
- 5 a) X = OMe
 b) X = H



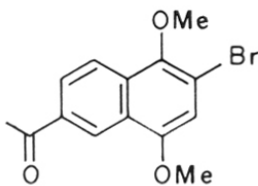
- 6 a) X = OMe
 b) X = H



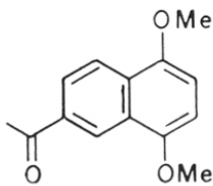
- 7 a) X = OMe
 b) X = H



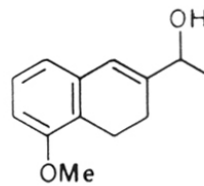
8



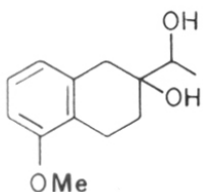
9



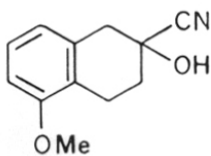
10



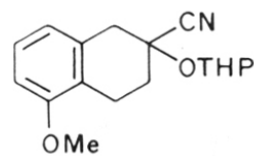
11



12



13



14

the 'A'-ring. A number of unexpected problems were faced during the elaboration of the side chain. The feasibility of building the tetracyclic system by AB + CD method was studied by synthesizing a new model compound 3, and as it smoothly gave the desired tetracyclic system, efforts were directed towards the construction of (+)2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydro-2-naphthol (7a), representing AB rings, as it could be easily condensed by acylation with phthalic anhydride, corresponding to CD rings to directly provide (+)4-demethoxy-7-deoxydaunomycinone (2c). 2c was hydroxylated at C-7 position to provide (+)4-demethoxydaunomycinone (2a).

Compound 7a was obtained by two different approaches.

In the first approach, 2-acetyl-5,8-dimethoxy-1-tetralone (4a) was made from 5,8-dimethoxy-1-tetralone by condensing with BF_3 -etherate- Ac_2O . The acetyl ketone group after selective protection and then reduction, followed by acid work-up and dethioketalization gave 2-acetyl-5,8-dimethoxy-3,4-dihydronaphthalene (5a). Hydrogenation of 5a using Pd-C (10%) as catalyst gave 6a. Oxidation of 6a in t-butanol with potassium t-butoxide and oxygen, followed by reduction (Zn-AcOH) gave the desired 7a in excellent overall yield.

In another approach, 2-bromo-1,4-dimethoxy naphthalene (8) (obtained from 1,4-dimethoxy naphthalene) afforded

4

exclusively 2-bromo-6-acetyl-1,4-dimethoxy naphthalene (9) on acetylation (Ac_2O , AlCl_3 , EDC). Compound 10, obtained by debrominating 9 was subjected to metal ammonia reduction to afford 6a which was converted to 7a as described earlier.

Part B - Synthesis of (+)4-demethoxy-11-deoxydaunomycinone

Recently it has been shown that 4-demethoxy-11-deoxydaunomycin (1d) has better antitumor activity than the natural 11-deoxydaunomycin (1c). Thus the synthesis of the aglycone 4-demethoxy-11-deoxydaunomycinone (2b) was carried in accordance with the versatile AB + CD method, by making use of the key synthon, 2-acetyl-2-hydroxy-5-methoxy-1,2,3,4-tetrahydronaphthalene (7b).

This synthon 7b was obtained by two different approaches.

In the first approach, 5-methoxy-1-tetralone was easily prepared from α -naphthol and converted to 5b, by appropriate chemical conversions as described earlier. NaBH_4 reduction of 5b in methanol gave 11, which after epoxidation and reduction (LAH) gave 12. The diol 12 was oxidised selectively using Feizson's reagent (Ag_2CO_3 over celite powder) to give 7b.

In the second approach, 5-methoxy-2-tetralone was converted to cyanohydrin, 13. Its tetrahydropyranyl ether

14 was reacted with methyl magnesium iodide to give the desired 7b.

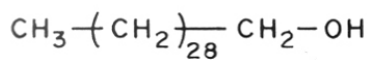
Fusion of 7b with phthalic anhydride under identical conditions as described earlier provided 2d. As the conversion of 2d to 4-demethoxy-11-deoxydaunomycinone (2b) is well established, the synthesis of 2d can in effect constitute a total synthesis of 2b.

CHAPTER II - PLANT GROWTH REGULATORS

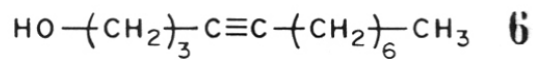
Part A - Synthesis of 1-Triacontanol

The recent efficacy of 1-triacontanol (15) as a plant growth stimulant, coupled with the fact that the responses of both rice and tomatoes to a synthetic sample are similar to that of natural triacontanol has directed attention towards its synthesis. This part describes an elegant method for the preparation of 15, conceptually different from previous approaches.

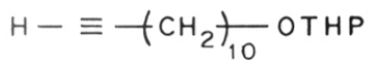
Alkylation of the dianion of 4-pentyl-1-ol with n-heptyl bromide afforded 4-dodecyn-1-ol (16), which after subjecting to acetylene-zipper reaction, followed by treatment with dihydropyran in dichloromethane provided 1-[(tetrahydro-2H-pyran-2yl)-oxy]-11-dodecyne (17). Alkylation of the anion of 17 with 1-bromooctadecane provided the desired C-30 unit, which after hydrogenation and acid work up gave 15.



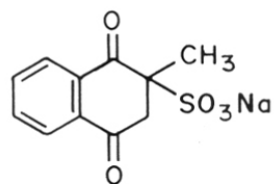
15



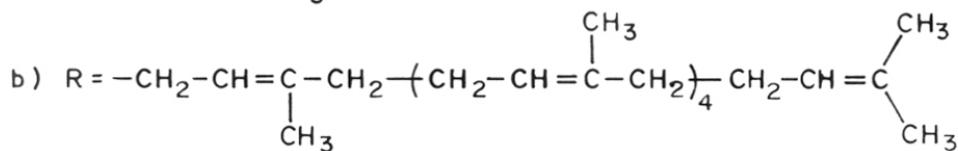
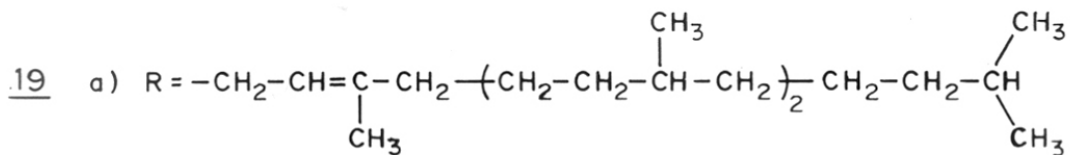
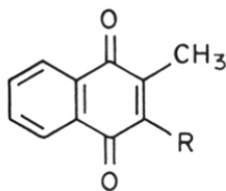
16



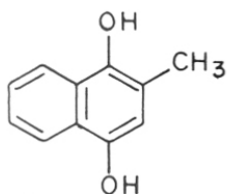
17



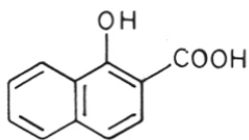
18



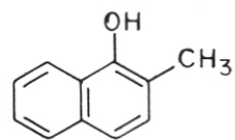
c) $\text{R} = \text{H}$



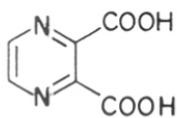
20



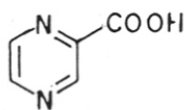
21



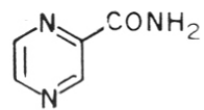
22



23



24



25

Part B - The Evaluation of 1-Triacontanol

The growth regulatory effect of 1-triacontanol was evaluated in a system comprising three components namely (1) Callus tissues (2) Isolated plant parts and organs such as those used in bioassays (3) Whole plants. The results of the various experiments are presented in this part with the conclusion that 1-triacontanol failed to bring about growth promoting activity in all the systems tried.

Part C - Menadione Bisulfite : A promising Plant Growth Regulator

Recent controversies regarding 1-triacontanol (first isolated from alfalfa) as a plant growth regulator, coupled with its failure to evoke growth promoting activity in many of the systems we tried, led us to probe into the other constituents of alfalfa. As alfalfa is rich in Vitamin K, and Vitamin K₃ (19c) being more active than Vitamin K₁ (19a) and/or Vitamin K₂ (19b) in its pharmacological properties its water soluble form viz. Menadione bisulfite (18) was assessed for its growth regulatory activity in a number of plants and tissues, where it was seen to bring about significant increase in growth. Studies were carried out to investigate its role in auxin metabolism. Besides lowering the activities of the various oxidases, which are known to participate directly or indirectly in regulating IAA levels, menadione bisulfite (18)

also brought about a 3-4 fold increase in the IAA levels in plants as determined by HPLC studies. The possible commercial utility of 18 was assessed by carrying out field trials using tomato plants, where the increase in yield was approx. 60% as compared to the untreated plants. This part deals with all these aspects in detail.

Part D - Synthesis of Menadione

Ever since Fieser reported that 2-methyl-1,4-naphthoquinone (Menadione/Vitamin K₃) (19c) has been found to be more active than Vitamin K₁ (19a) and/or Vitamin K₂ (19b), much attention has been focussed towards its preparation. This 19c being used in the preparation of 18 was required in appreciable large quantities. This part exemplifies numerous approaches for the preparation of 19c, conceptually different from previously described, and some of which are potentially flexible for large scale operation.

Diels-Alder reaction between 1-acetoxybutadiene and toloquinone provided the desired adduct, which on acid work up gave 2-methyl-1,4-naphthalenediol (20). Compound 20 on air-oxidation with salcomine gave 19c.

In another approach, α -naphthol was carboxylated by a new method to afford 21 which on treatment with triethylamine and ethyl chloroformate, followed by NaBH₄ reduction gave 22. Air oxidation of 22 provided 19c.

Vapour phase alkylation of α -naphthol under specific conditions provided 22 as the major product, which was converted smoothly to 19c.

19c was directly obtained in one step by alkylation of 1,4-naphthoquinone by decarboxylation of acetic acid with the silver ion peroxydisulfate system.

CHAPTER III - SYNTHESIS OF PYRAZINAMIDE

Considerable interest is attached to the synthesis of pyrazinoic acid (24), since this compound is the key intermediate in the preparation of the tuberculostat pyrazinamide (25). This part describes a simple method for the preparation of 24. It involves a condensation reaction between 2,3-diaminosuccinic acid and glyoxal, and an oxidation carried out in the same medium, employing air as an oxidising agent. The resulting compound 23 was easily decarboxylated to 24. As the conversion of 24 to 25 is well established, this approach constitutes a total synthesis of 25.

CHAPTER I
ANTHRACYCLINONES

A GENERAL INTRODUCTION

About 20 per cent of the deaths in major countries are currently ascribed to neoplastic diseases, i.e. those associated with growth of new abnormal body tissues commonly referred to as "cancer". This disease being the scourge of mankind, has engaged the world-wide attention of a variety of research workers.

Radiation and surgery have certainly a curative effect as long as it is detected at an early stage and localized. But unfortunately by the time it is detected, the disease often spreads to other organs of the body and then the answer lies in chemotherapy either exclusively or in combination with surgery and radiation.

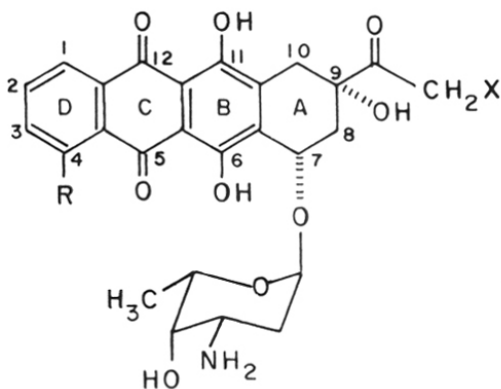
A large number of anti-cancer drugs are now being used in medical practice which have been approved by the National Cancer Institute (USA). Further many are undergoing clinical trials. All these drugs can be broadly classified into (1) alkylating agents (2) antimetabolites (3) antibiotics and (4) miscellaneous compounds.

Among the various compounds which are promising as antitumor agents, natural products, either of plant or microbial origin, are showing much more specificity in their anti-cancer properties. Many of them possess structures and

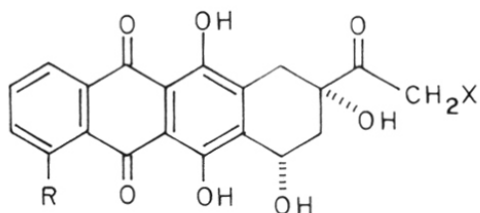
clinical properties which suggest that they may act by selective alkylation of growth regulatory macromolecules.

The usefulness of certain anthracycline antibiotics as anti-neoplastic agents are now widely accepted. Both daunomycin¹ (1a) and adriamycin² (1b) produced by Streptomyces peucetius (Family: Streptomycetacea) and a mutant strain respectively, have shown pronounced anticancer activity in some types of human cancer³. Daunomycin has achieved a significant place in the treatment of acute lymphocytic and myelogenous leukemias. Recently, carminomycin^{3,4} (1c), isolated from Actinomadura carminata sp. nov. and Streptosporangium sp. has shown to be more effective than other two antibiotics (1a and 1b) in inhibiting DNA synthesis⁵. Further, carminomycin is found to suppress the growth of a murine bronchogenic lung carcinoma to indicate that it has less severe cardiotoxicity and is better absorbed from gastrointestinal tract than daunomycin³.

The structures of these compounds were established by a combination of spectral analyses^{5a} and chemical degradations⁶ and further confirmed by X-ray analysis⁷ which revealed these molecules to consist of a tetracyclic aglycone attached to the amino sugar L-daunosamine via an α -glycosidic bond. The stereochemistry of both asymmetric centres of the aglycone is of the S-configuration and the amino sugar is



- 1a) Daunomycin : $R = \text{OCH}_3$; $X = \text{H}$
- b) Adriamycin : $R = \text{OCH}_3$; $X = \text{OH}$
- c) Carminomycin : $R = \text{OH}$; $X = \text{H}$
- d) 4-Demethoxydaunomycin: $R = \text{H}$; $X = \text{H}$



- 2 a) Daunomycinone: $R = \text{OCH}_3$; $X = \text{H}$
- b) Adriamycinone : $R = \text{OCH}_3$; $X = \text{OH}$
- c) Carminomycinone: $R = \text{OH}$; $X = \text{H}$
- d) 4-Demethoxydaunomycinone: $R = \text{H}$; $X = \text{H}$

of the L-configuration.

The biological activity of the antitumor anthracyclines is related to their ability to bind with DNA. Adriamycin displayed a more favourable therapeutic index than daunomycin in different experimental tumors in laboratory animals and particularly an impressive broad spectrum of activity. Adriamycin however is not devoid of serious side effects such as dose limiting myelosuppression and cardiomyopathy etc. All toxic effects, with the exception of cardiotoxicity which in its fatal form, congestive cardiac failure, effects approximately 1% of patients, are dose related and reversible. Much effort has been directed to obtain either new derivatives or develop new dose regimes that show decrease in undesirable side effects and/or increased anticancer activity and selectivity. Such goals are common to many areas of cancer chemotherapy; however, in the case of daunomycin and adriamycin, it is now possible to take account of recent knowledge concerning their mode of action.

A detailed examination of the daunomycin DNA model reveals that intercalation of the chromophore is only partial and one might speculate the removal of bulky methoxy group which would result in a molecule that could intercalate more effectively. In fact 4-demethoxydaunomycin (1d) binds

to DNA somewhat better than its parent compound. Significantly, in vivo testing of these demethoxy derivatives in mouse cancer shows that it is as effective as daunomycin itself, but at dose levels four to eight times lower⁸.

Total synthesis of these compounds by itself is a major line of research directed towards the exploration of structural modification on different parameters of anthracycline antitumor activity. The problems which arise here are: (a) development of a synthetic procedure which can be adopted at least upto a large bench scale resulting in gram amounts of the aglycones, (b) synthesis of the amino sugar L-daunosamine from a relatively accessible natural sugar, (c) development of suitable methods for the synthesis of the glycosidic bond and (d) the necessity of obtaining these products in substantial quantities in optically pure form.

As there are several practicable methods for the synthesis of L-daunosamine from sugar and non-sugar precursors and the coupling of aglycone with L-daunosamine is well-established, much efforts were directed to develop a preparative method for the synthesis of anthracyclines, the aglycones of anthracyclines. In consequence there appeared a good number of methods for the synthesis of aglycones. In the year 1979, Kelly⁹ has published a comprehensive review,

"On synthetic approaches to anthracyclines", in which it was discussed under three parts (i) synthesis of anthracyclonones [daunomycinone (2a), adriamycinone (2b), carminomycinone (2c) and analogues such as 4-demethoxydaunomycinone (2d) etc.] (ii) synthesis of L-daunosamine and (iii) glycosidation of aglycone with the sugar moiety. Synthetic methods on anthracyclonones, appearing after 1979, are dealt as reviews in different theses¹⁰, from this laboratory.

The principal synthetic challenge posed by the aglycones include generation of the tetracyclic skeleton, introduction of the A-ring functionality and achievement of "correct" (i.e. natural) regiochemical juxtaposition of the substituents in the A- and D-rings. The demonstration¹¹ that the natural, cis orientation of the 7- and 9-hydroxyl groups is thermodynamically preferred (6:1) ratio and that epimerization of the C-7 position is effected by CF_3COOH H has decreased the need to address aglycones stereochemistry directly. The recent finding¹² that the 4-demethoxy analogues of (1a) and (1b) are approximately eight times as potent as (1a) and (1b) (they are also more toxic) may well have diminished the practical need for finding an efficient solution to the problem of regiochemistry.

Nonetheless, efforts to achieve regiochemically controlled

routes towards the synthesis of the anthracyclines have provided a chemical harvest which is already bounteous and continues to be reaped. Overall yields for the total synthesis of the intact, chiral natural products (1a) and (1b) as well as their demethoxy analogues are now on the order of 5% in the best cases. Whether total (or partial) synthesis will emerge as the practical solution for the future is, however, a question whose ultimate answer depends on many as yet incompletely resolved factors which include the relative economics of fermentative and total synthesis, the potential therapeutic superiority of non-natural anthracyclines, and future advances in total synthesis.

A) SYNTHESIS OF
(±)-4-DEMETHOXYDAUNOMYCINONE
USING -----

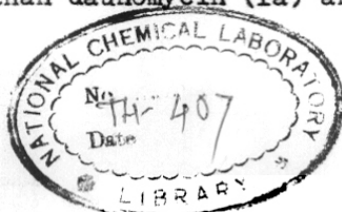
(i) 1,4-ANTHRAQUINONE

[DCB + A]

INTRODUCTION

The anthracycline antibiotics, daunomycin (1a) and adriamycin (1b) are of topical interest in view of their activity against various experimental tumors as well as their clinical effectiveness in the treatment of many types of human cancers¹². Their primary site of action is considered to be at the tumor cell level through an interference with DNA synthesis and functionality. These compounds, however, suffer from a very serious drawback of having cumulative dose dependent cardiotoxicity¹³.

These broadspectrum anticancer agents are presently made by microbiological fermentation methods, but the yields of these reactions are very low, thus making these drugs rather inaccessible and expensive¹⁴. The lack of an efficient biosynthetic process¹⁵ coupled with their therapeutic importance have prompted the investigations of chemical synthesis to alleviate the scarcity of these drugs. These aspects have led to continual chemical interest aimed at the development of an efficient total synthesis that would be advantageous than the fermentation process and offer a way to provide configurationally and/or functionally modified analogues, having improved therapeutic indices. For example it has been shown that 4-demethoxydaunomycin (1d) is eight times more active than daunomycin (1a) and the results of its



547.673(043)
RAY

clinical trials are reported to be promising⁸. As several suitable syntheses of L-daunosamine and its coupling¹⁶ to daunomycinone (2a) have been accomplished, much efforts have been directed to the synthesis of the aglycone moieties (anthracyclines) of these antitumor antibiotics.

The best possible way of elaborating the synthesis of anthracyclines is illustrated by Smith et al.^{11c}, in which the degradation of daunomycin (1a) to a nonasymmetric tetracyclic ketone (6) and its refunctionalization of the A ring to daunomycin (1a) and adriamycin (1b) were carried as depicted in Scheme 1.

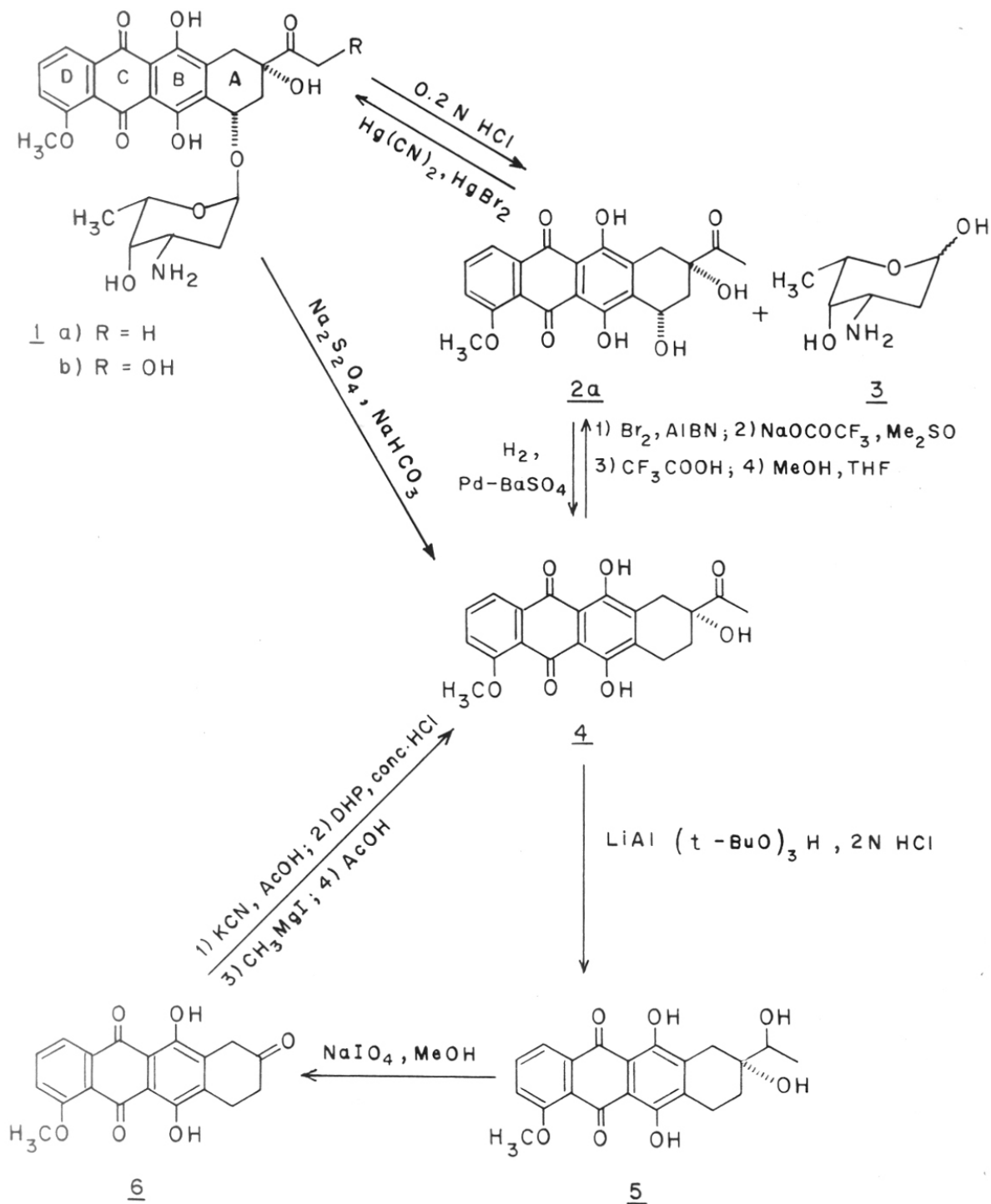
Mild acid hydrolysis¹⁷ of (1a) gave daunomycinone (2a) and daunosamine (3), whereas treatment with sodium dithionite resulted in reductive cleavage of the glycosidic bond to afford 7-deoxydaunomycinone (4).

Since there is no possibility of obtaining 4-demethoxydaunomycin (1d) by fermentation and its total synthesis from 4-demethoxydaunomycinone (2d) is well established¹⁸, the main strategy is centred around the synthesis of 4-demethoxydaunomycinone. The methods so far reported for its synthesis are not practicable. This part mainly concerns with attempts towards new synthesis of 4-demethoxydaunomycinone, (either as DCB + A or AB + CD) starting from cheap and easily

SCHEME - 1

19

19



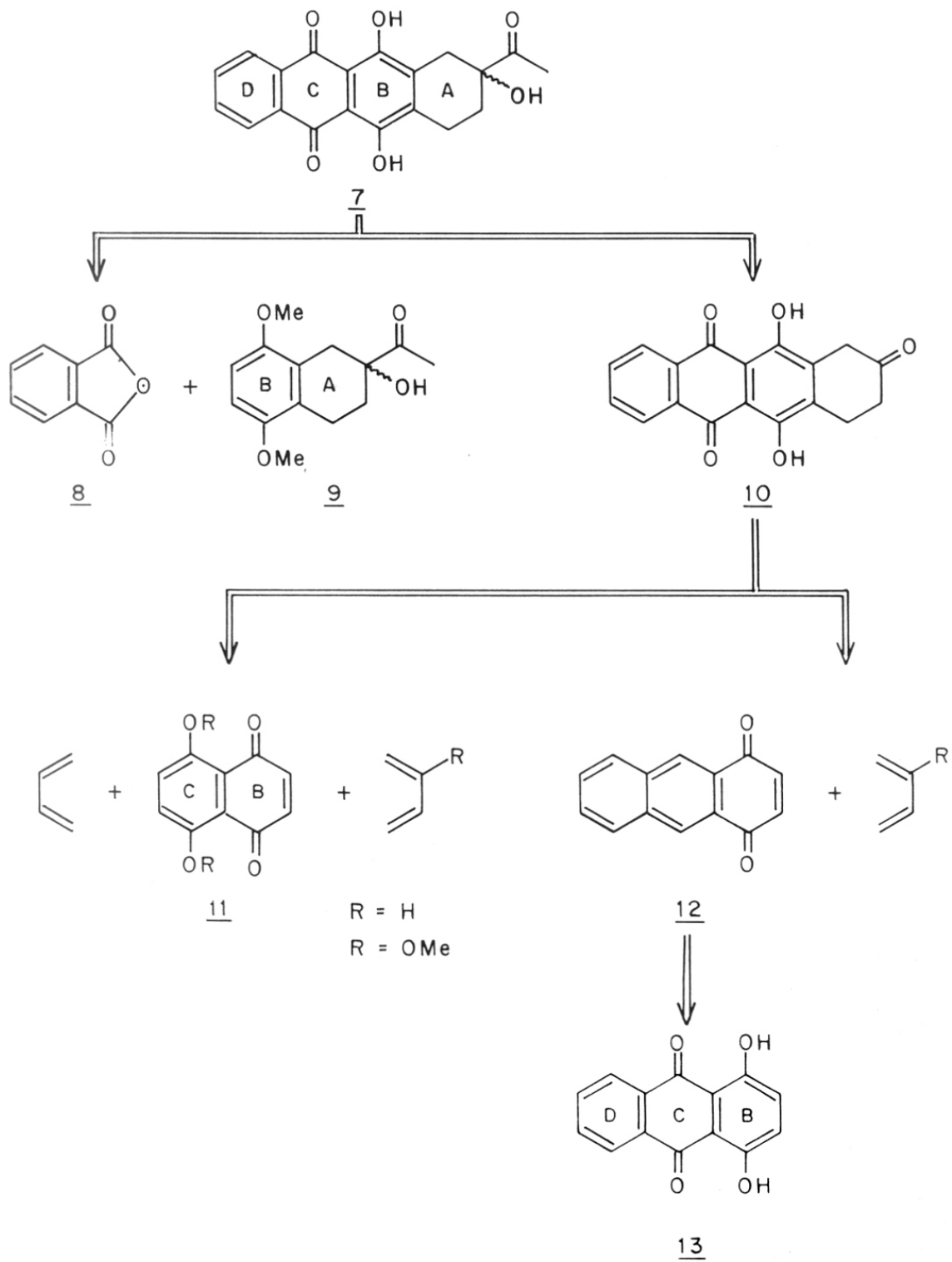
accessible synthons such as 1,4 anthraquinone precursor [DCB + A].

The retrosynthesis of the key intermediate 7 (as shown in Scheme-2) led to the belief that anthraquinone precursors could act as a plausible starting material for anthracycline synthesis.

Anthraquinone derivatives could be easily obtained from microbial¹⁹ or plant sources²⁰ or as a dye intermediate²¹ and more so could be converted into suitably substituted derivatives. Moreover, in anthraquinone the rings 'B', 'C' and 'D' of anthracyclines are already present and the ring 'A' could be generated by Diels Alder reaction with suitably substituted butadiene.

The obvious application of the Diels Alder reaction to build up the tetracyclic ring system of the anthracyclines has been widely explored, and it represents one of the most, if not the most, attractive routes leading to economically feasible preparations of such compounds.

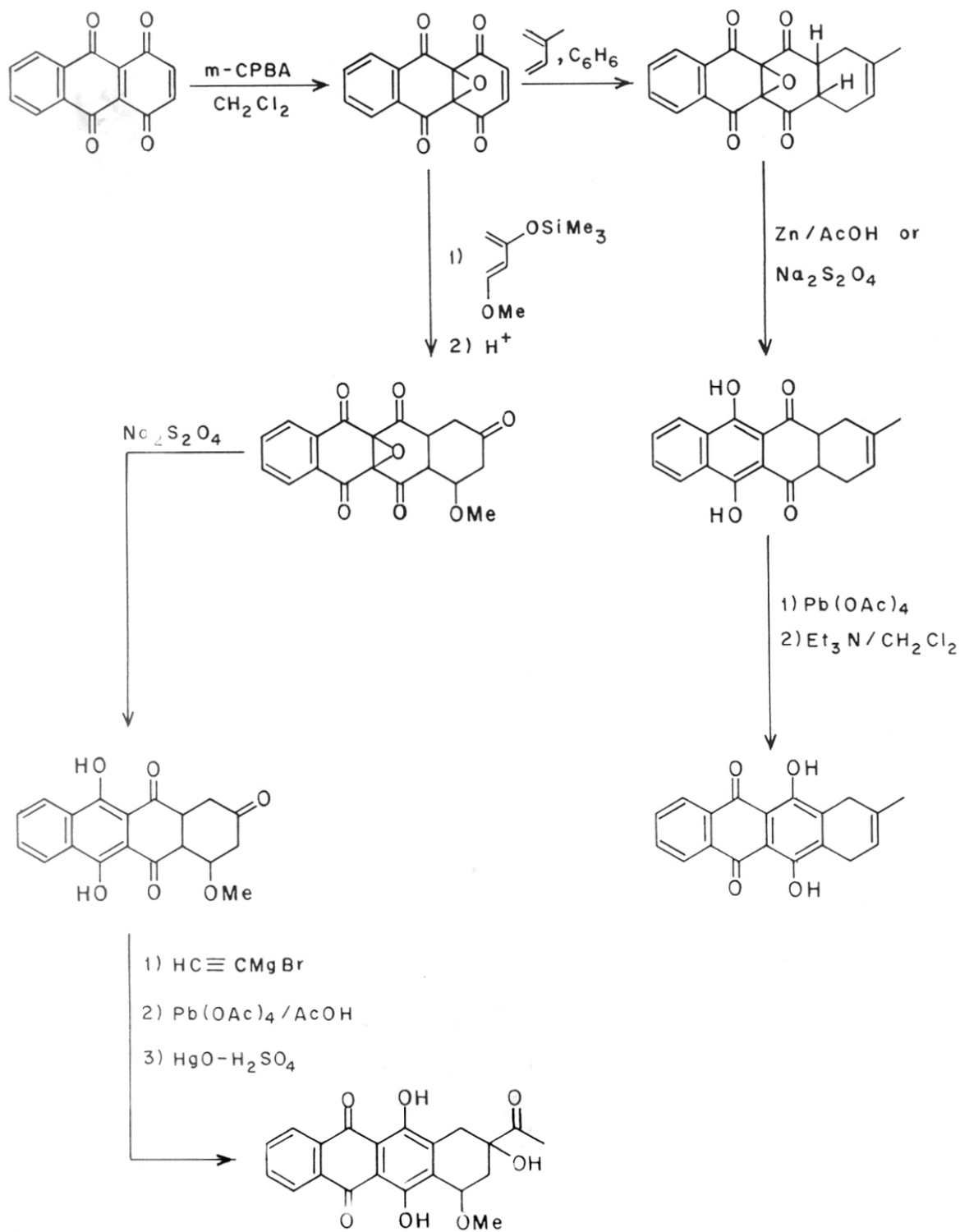
Stoodley et al.²² developed a method where quinizarin quinone (15) was oxidised with m-chloroperbenzoic acid to give an internal 4a, 9a epoxide, which upon treatment with isoprene in a Diels-Alder reaction afforded the tetracyclic adduct. They further extended²³ this



strategy to other substituted anthracyclines as depicted in Scheme-3. The 9-hydroxy-1,4 anthraquinone and 9-chloro-10-hydroxy anthraquinone upon Diels-Alder reaction with isoprene in presence of borontriacetate gave the regio-selective adduct (Scheme-4)²⁴.

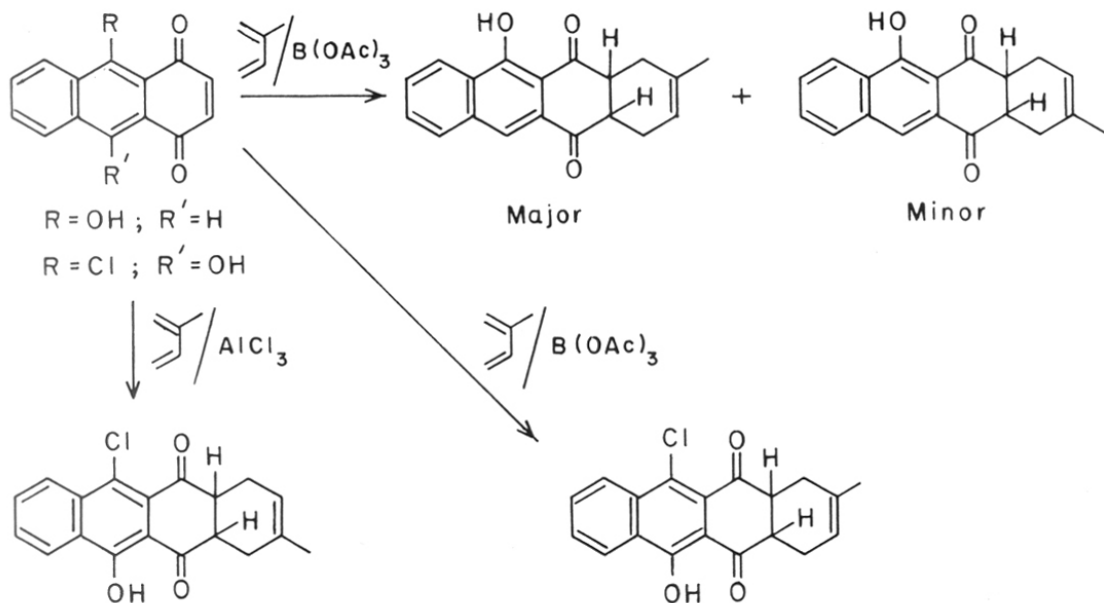
Inhoffen et al.²⁵ studied the Diels-Alder reaction of quinizarin quinone (15) (dienophile) with substituted butadienes (Scheme 5). They observed that since quinizarin-quinone was bifunctional, the Diels-Alder reaction occurred at both internal and terminal double bonds. Later Sauer²⁶ suggested a hypothesis based on Inhoffen's as well as on his own work that the internal addition was favoured with electron-rich dienes, whereas terminal addition occurred with unsubstituted or slightly electron-poor dienes. Thus, with 1-acetoxybutadiene and 2-methoxy or 2-ethoxybutadiene addition to quinozarinquinone predominantly took place at the internal double bond giving rise to 16a and 16b, whilst with 1,3-butadiene or 2-acetoxybutadiene addition at the terminal double bond predominated to afford 17a and 17b respectively. Although in the latter case addition at the terminal bond did occur, the yields were not significant and moreover, the reaction was reported to be sluggish.

Thus it was concluded that quinizarin quinone (15) was not particularly suited as a dienophile for the present requirements.

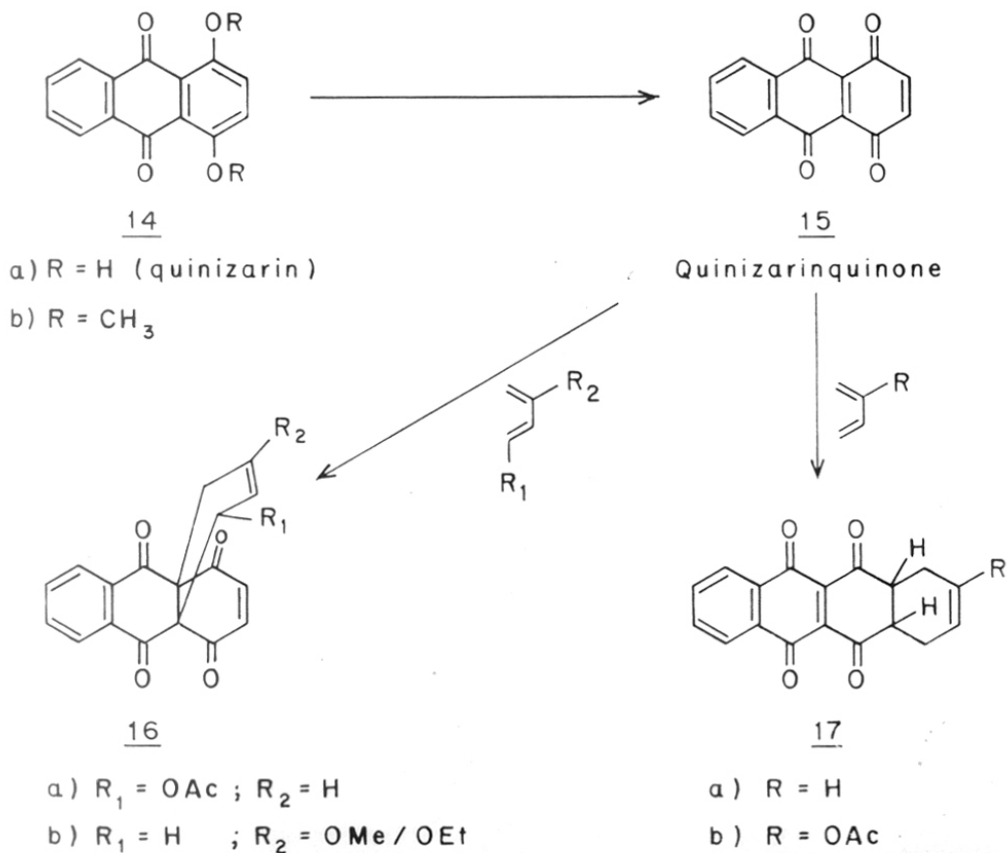


SCHEME - 4

24



SCHEME - 5



PRESENT WORK

1,4-Anthraquinone was considered as an ideal dienophile, because in 1,4-anthraquinone (18) the possibility of addition of a diene to internal double bond did not exist.

Although 1,4-anthraquinone was known for a long time it had not been exploited as a dienophile in Diels-Alder reactions* and it was felt worthwhile exploring its dienophilicity.

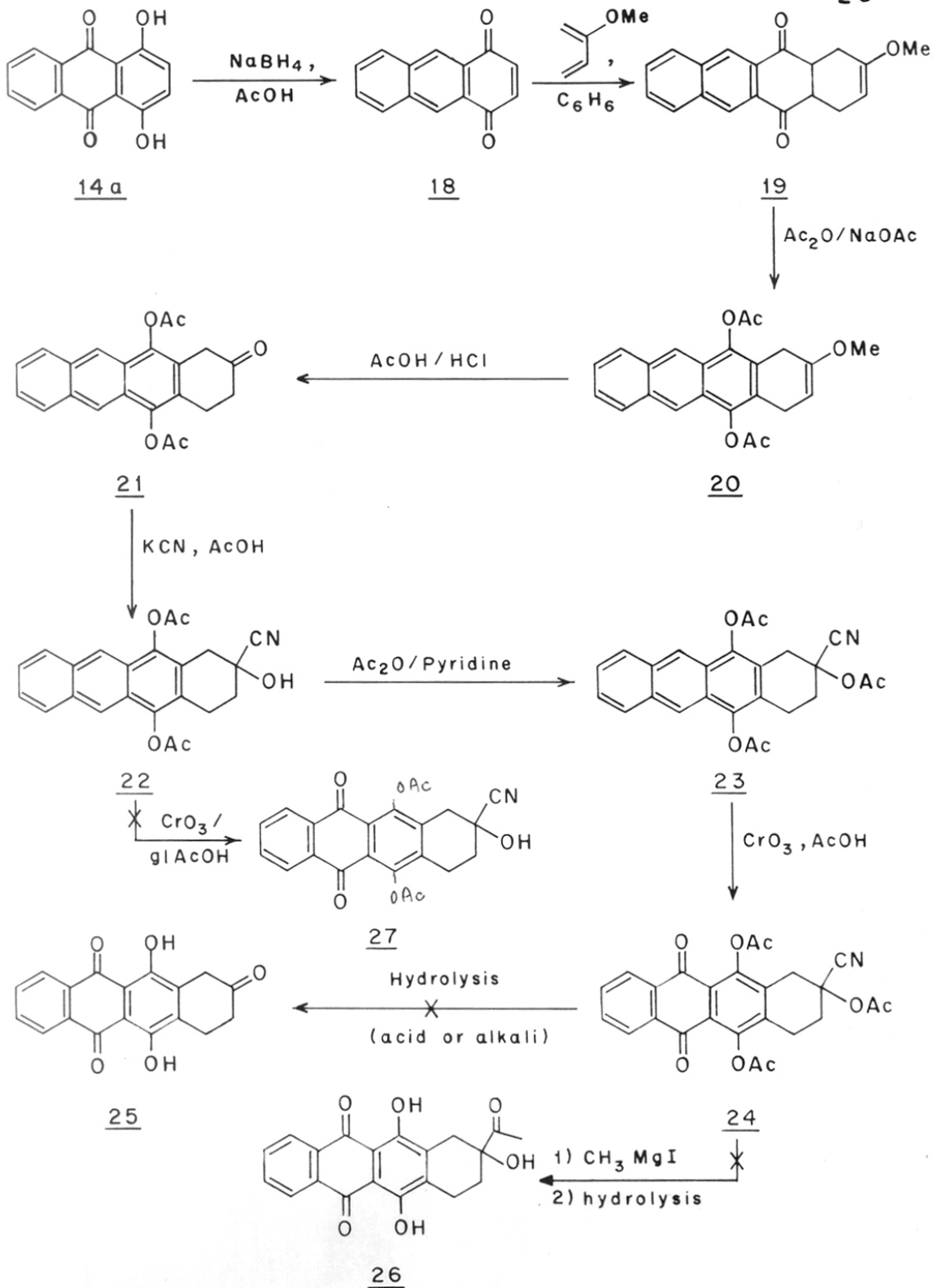
The strategy adopted for the synthesis of (+)4-demethoxy-daunomycinone from 1,4-anthraquinone (18) consisted of the following steps: (Scheme-6).

- 1) Explore the D.A. reaction of 1,4-anthraquinone with 2-methoxybutadiene.
- 2) O-alkylation or O-acylation of the adduct.
- 3) Conversion of the ketone to cyanoacetate via cyanohydrin.
- 4) Oxidation to tetracyclinone.
- 5) Hydrolysis to trione and then conversion of the ketone to acetyl group via ethynylation or direct conversion of cyanoacetate to acetyl group using CH_3MgI .

*After 2 yr of initiation of this work, a paper by D.N. Gupta, P. Hodge and N. Khan, J. Chem. Soc. Perkin Tran.I 689 (1981) appeared in which D.A. reactions with 1,4-anthraquinone have been reported.

SCHEME - 6

26



1,4-Anthraquinone (18) could be easily obtained from quinizarin (14a) by sodium borohydride reduction in acetic acid²⁷.

2-Methoxybutadiene prepared²⁸ starting from methyl vinyl ketone adds to 1,4-anthraquinone in benzene to give 2-methoxy-1,4,13,14-tetrahydronaphthacene-5,12-dione (19) in 96% yield. The PMR spectrum (Fig.1) of the compound (19) in CDCl_3 shows a complex multiplet of two methylene groups at δ 2.5 and a multiplet at δ 3.3 merging with a methoxyl signal at δ 3.53, representing two protons (13,14-H). The vinyl proton (3-H) triplet is seen at δ 4.63. A multiplet of A_2B_2 pattern is centred at δ 7.9 representing four protons at δ 8.63 can be assigned for C-ring protons.

The adduct (19) on acetylation with sodium acetate and acetic anhydride at 98° for 2.5 hr yielded 5,12-diacetoxy-1,4-dihydro-2-methoxy naphthacene (20). The PMR spectrum (Fig.2) of this compound (20) in CDCl_3 shows a singlet at δ 2.5 representing two acetoxy groups. The enolic methoxyl singlet is seen at δ 3.6 and the vinylic proton triplet at δ 4.8. Demethylation of 20 with traces of hydrochloric acid in acetic acid gave the diacetoxy ketone (21), whose structure was confirmed from PMR by the absence of methoxyl singlet and vinylic proton. Treatment of 21 with $\text{KCN}/\text{CHCl}_3/\text{EtOH}$ provided the required cyanohydrin (22). The IR spectrum of 22

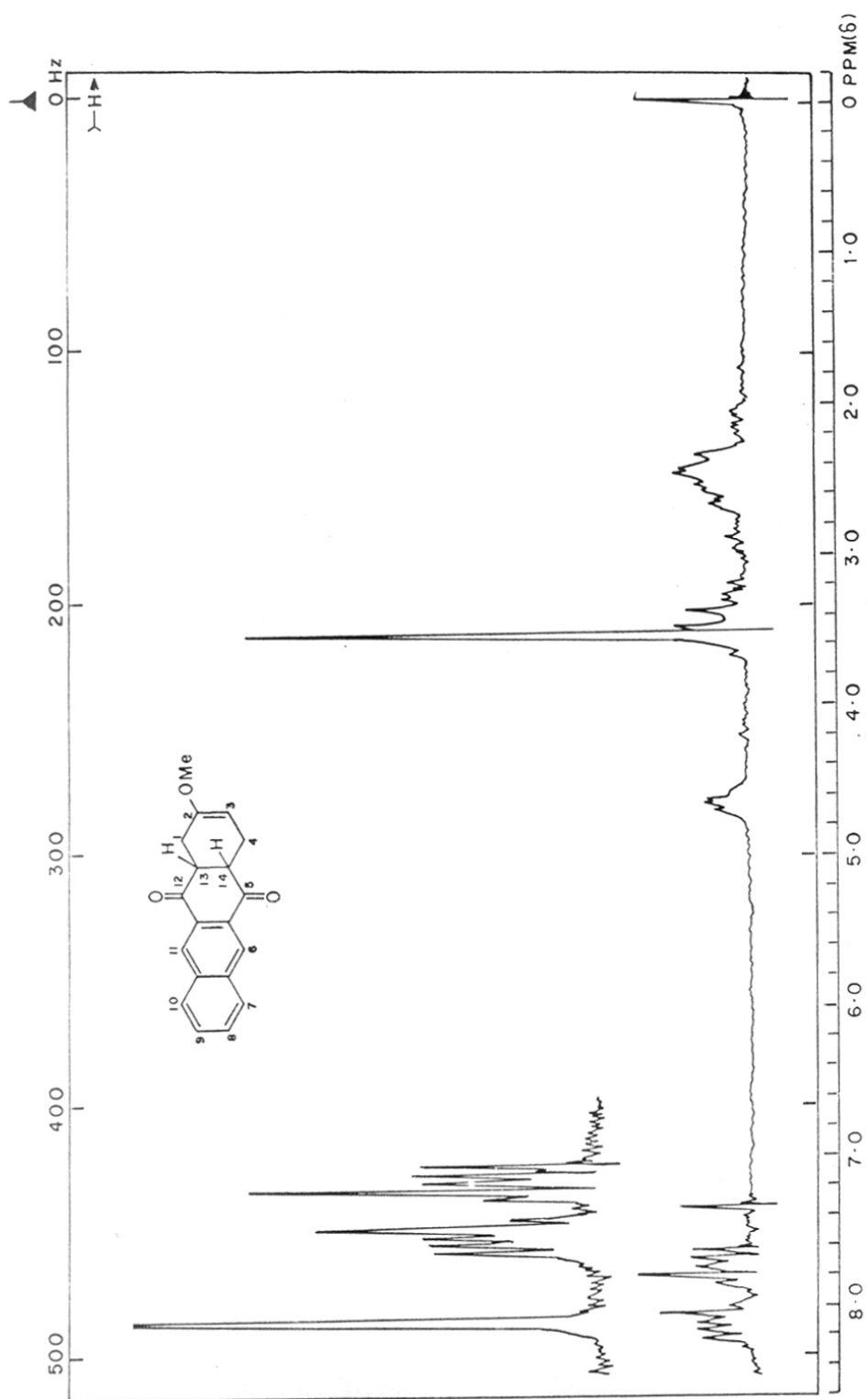
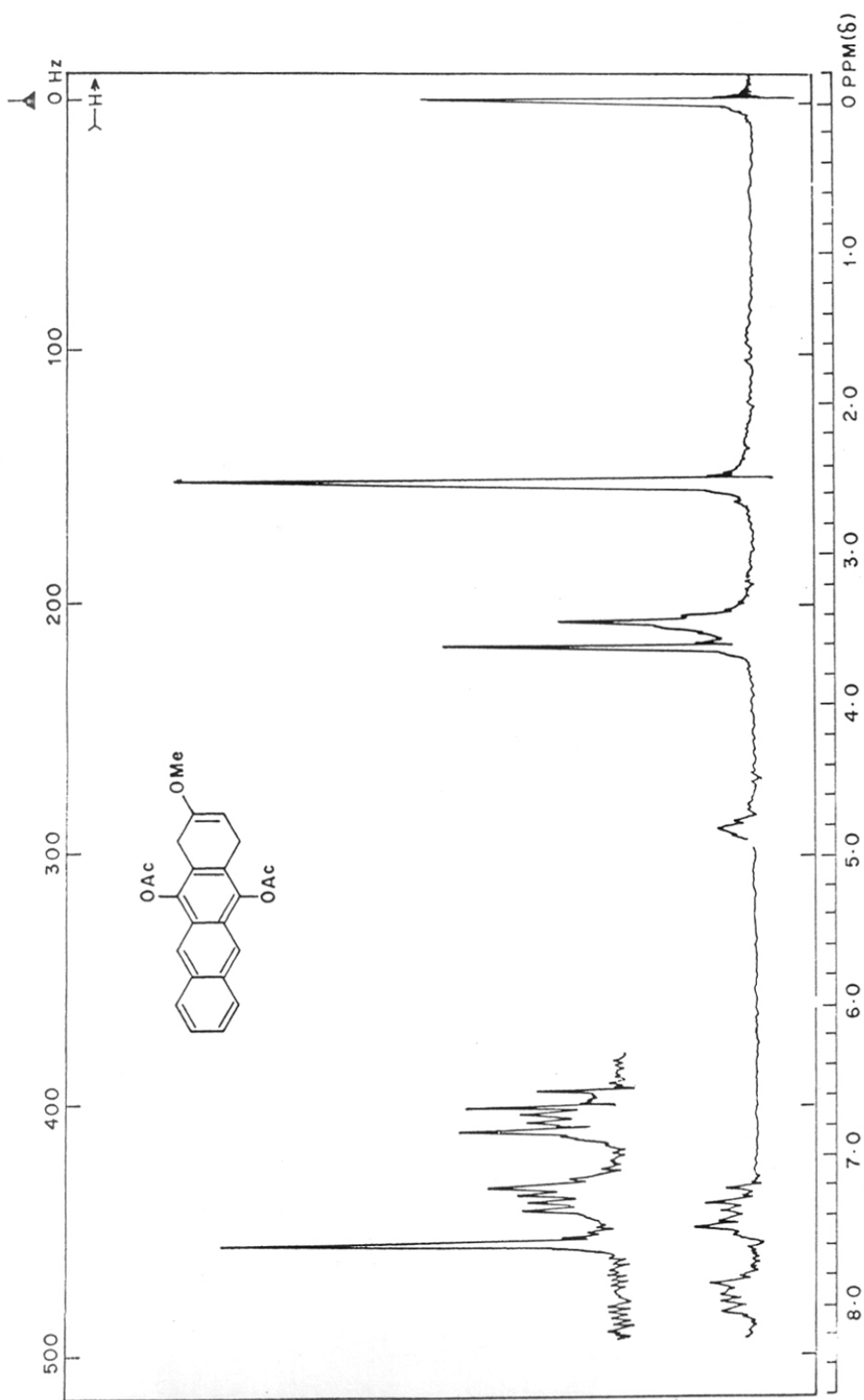


FIG.1 PMR SPECTRUM OF COMPOUND 19 IN CDCl₃

FIG. 2 PMR SPECTRUM OF COMPOUND 20 IN CDCl_3

in nujol showed the presence of $\text{-C}\equiv\text{N}$ and -OH groups at 2240 cm^{-1} and 3400 cm^{-1} respectively.

Compound (22) was treated with Ac_2O /pyridine to yield 2-cyano-2,5,12-triacetoxy-1,2,3,4 tetrahydronaphthacene (23). The PMR of 23 in CDCl_3 shows a singlet at δ 2.0 representing the tertiary acetoxy group. The other two acetoxy's group (occurring in 5,12 positions) appear as two singlets at δ 2.50 and 2.60 respectively. A multiplet of A_2B_2 pattern extends from δ 7.2 - δ 8.0 representing four protons of D-ring. A singlet of two protons at δ 8.63 can be assigned for the C-ring protons. Oxidation of 23 with $\text{CrO}_3/\text{gl. AcOH}$ provided the triacetoxy dione (24). The disappearance of the singlet at δ 8.63 corresponding to the two protons of C-ring confirmed the structure of 24 by PMR.

Attempts to convert 22 to 27 by oxidation with $\text{CrO}_3/\text{gl. AcOH}$ resulted in the aromatisation of A-ring (as seen from PMR) and it became obvious that in order to oxidise the 6,11 positions without aromatising the A-ring, the tertiary hydroxy had to be protected as 23.

Mild acid or alkali hydrolysis of 24 failed to provide the required trione (25) as indicated by PMR in which the A-ring had aromatised. Treatment of 24 with methyl magnesium iodide, followed by hydrolysis did not provide

25, but instead afforded again the non-desired A-ring aromatised product. This problem encountered could be attributed to the extended conjugation of the tetracyclic system.

[The diene, 2-methoxybutadiene was replaced by thioketal protected acetyl butadiene* and then subjected to D.A. reaction with 1,4-anthraquinone. Though the D.A. reaction proceeded smoothly, all attempts to dethioketalize the D.A. adduct resulted again in the aromatisation of A-ring.

The problem could be avoided* if the A-ring is built first on a naphthalene derivative dienophile and then bridged ultimately to form the B-ring of the anthracyclinone moiety].

*For details see Rama Rao et al., JOC 48, 1552 (1983). It forms a part of the Ph.D dissertation of S.M. Jaweed (Poona University), 1983.

E X P E R I M E N T A L

2-Methoxybutadiene

This was prepared from methyl vinyl ketone, methyl orthoformate and anhydrous methanol according to the known procedure.

1,4-Anthraquinone (11)

1,4-Anthraquinone was prepared from quinizarin by following the reported procedure²⁷.

2-Methoxy-1,4,13,14-tetrahydronaphthacen-5,12-dione (19)

The mixture of 1,4-anthraquinone (18) (2.0 g, 0.01 mole), 2-methoxy-1,3-butadiene (2.50 g, 0.03 mole), dry benzene (40 ml) was sealed in a thick corning tube and heated at 90° for 24 hrs. The solvent was distilled off under reduced pressure and the residue crystallised from the mixture of benzene-pet. ether (2:1) as pale yellow needles (2.80 g, 96%). m.p. 192-93°, M^+ 292. PMR (CDCl₃) δ 2.50 (m, 4H, 2 X CH₂), 3.30 (m, 2H, 13,14-H), 3.53 (s, 3H, OMe), 4.63 (t, 1H, 3-H), 7.90 (m, 4H, 7,8,9 and 10-H), 8.63 (s, 2H, 6,11-H).

Analysis: Calculated for C₁₉H₁₆O₃: C, 78.0; H, 5.48; Found: C, 77.77; H, 5.93%.

5,12-diacetoxy-2-methoxy-1,4-dihydronaphthacene (20)

The mixture of the dione 19 (1.8 g, 0.006 m), anhydrous sodium acetate (3.0 g, .036 mole) acetic anhydride (20 ml) was heated on water bath for 2.5 hr and poured over crushed ice.

The yellow precipitate was filtered, and crystallized from methanol to yield the diacetate 20 [2.20 g, 95%] as light yellow needles. m.p. 182°, M⁺ 376. PMR (CDCl₃): δ 2.50 (s, 6H, 2X COCH₃), 3.50 (m, 4H, 2X CH₂), 3.60 (s, 3H, OMe) 4.80 (t, 1H, 3-H), 7.80 (m, 4H, 7,8,9 and 10-H).

Analysis: Calculated for C₂₃H₂₀O₅: C, 73.4; H, 5.32; Found: C, 73.80; H, 5.20%.

Diacetoxy ketone 21

A mixture of 20 (1.8 g, .005 mole), acetic acid (2.5 ml) and three drops of conc. hydrochloric acid was stirred for 5 minutes at room temperature and then poured over crushed ice. The precipitate was filtered, washed with water, dried and crystallized from methanol to yield the ketone 21 as pale yellow needles (1.56 g, 90%).

m.p. 220° (decomp.), M⁺ 362. PMR (CDCl₃): δ 2.30 (m, 4H, 2 X CH₂), 2.50 (s, 6H, 2X COCH₃), 3.21 (bs, 2H, 1 X CH₂), 7.80 (m, 4H, 7,8,9 and 10-H), 8.63 (s, 2H, 6 and 11-H).

Analysis: Calculated for C₂₂H₁₈O₅: C, 72.9; H, 4.97; Found: C, 72.5; H, 5.20%.

2-Cyano-2-hydroxy-5,12-diacetoxy-1,2,3,4-tetrahydronaphthacene
(22).

The diacetoxy ketone 21 [1.0 g, .003 mole] and potassium cyanide (5.0 g) were placed in chloroform (125 ml), Ethanol (37.5 ml) and cooled to 0°C. Acetic acid (7.5 ml) was added

over 10 minutes. The mixture was diluted with EtOH (25 ml) and stirred at room temp. for 5 hr. The mixture was diluted with H₂O (150 ml) and extracted with chloroform (3 x 40 ml). The combined extracts were dried and solvent distilled off under reduced pressure. Crystallization from methanol provided the required cyanohydroxy compound 22 [0.85 g, 80%]. m.p. 142°. M⁺ 389. IR (Nujol): 2240 cm⁻¹ (-C≡N), 3400 cm⁻¹ (-OH).

Analysis: Calculated for C₂₃H₁₉NO₅: C, 70.95; H, 4.88; N, 3.59; Found: C, 71.0; H, 4.92; N, 3.48%.

2-Cyano-2,5,12-triacetoxy-1,2,3,4-tetrahydronaphthacene (23)

A mixture of 22 (0.5 g, .0013 mole), acetic anhydride (5.0 ml) and pyridine (3.0 ml) was stirred for 10 hr at room temp. and then poured over crushed ice. The precipitate was filtered, washed with water, dried and recrystallized from benzene to yield 23 as crystalline material (0.5 g, 90%). m.p. 165°. PMR (CDCl₃): δ 2.0 (s, 3H, COCH₃), 2.10 - 3.0 (m, 4H, 2X CH₂), 2.50 (s, 3H, COCH₃), 2.60 (s, 3H, COCH₃), 3.50 (s, 2H, CH₂), 7.2 - 8.0 (m, 4H, 7,8,9,10-H), 8.63 (s, 2H, 6,11-H).

Analysis: Calculated for C₂₅H₂₁NO₆: C, 69.60; H, 4.87; N, 3.24; Found: C, 65.52; H, 4.80; N, 3.18%.

Triacetoxy dione 24

To a solution of 23 (0.5 g, .0012 mole) in 10 ml of

glacial acetic acid under stirring, in a nitrogen atmosphere was added CrO_3 (0.6 g) in 20 ml of gl. acetic acid at room temp. After stirring for 10 hr, the reaction mixture was neutralized with aqueous sodium bicarbonate solution and extracted with chloroform (3 x 25 ml). The combined extracts were washed with H_2O (3 x 50 ml), dried over anhydrous sodium sulphate evaporated under reduced pressure and chromatographed over silica gel column (5% acetone-benzene) to give the tri-acetoxy dione 24 which was crystallized from benzene-pet.ether (2:1) as orange needles (0.32 g, 60%). PMR(CDCl_3): δ 2.0 (s, 3H, COCH_3), 2.50 (s, 6H, 2X COCH_3), 2.82 (m, 4H, 2X CH_2), 3.40 (s, 2H, 1X CH_2), 7.92 (m, 4H, 7,8,9,10-H). m.p. 130° . M^+ 419 [M-42 (CH_2CO)].

Analysis: Calculated for $\text{C}_{25}\text{H}_{19}\text{O}_8\text{N}$: C, 65.07; H, 4.12; N, 3.03; Found: C, 65.22; H, 4.24; N, 2.75%.

(ii) (±)-2-ACETYL-5,8-DIMETHOXY-1,2,3,4-
TETRAHYDRO-2-NAPHTHOL

[AB + CD]

INTRODUCTION

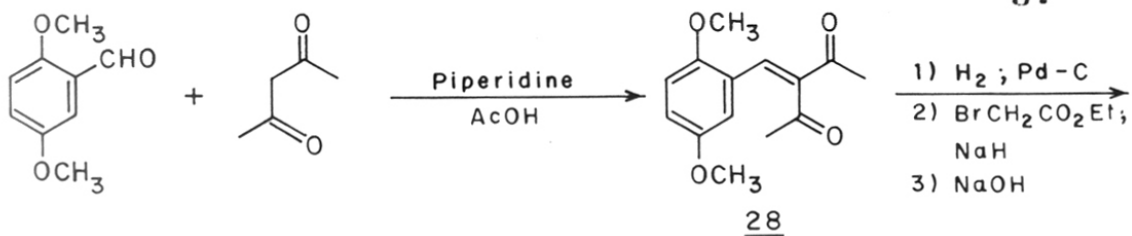
In the previous part, the Diels-Alder approach utilising 1,4-anthraquinone (having DCB rings already incorporated) had been exploited, rather unsuccessfully to build the A-ring. A number of unexpected problems were faced during the elaboration of the side chain.

It is clear from Scheme-2 that transformation of naphthaquinone derivative (11) or anthraquinone derivative (12) to 4-demethoxy-7-deoxydaunomycinone (7) requires many steps, whereas the substituted tetralin 9 can be converted to 7 in a single step by fusion with a phthalic acid derivative.

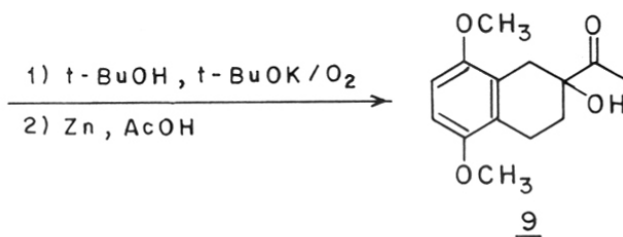
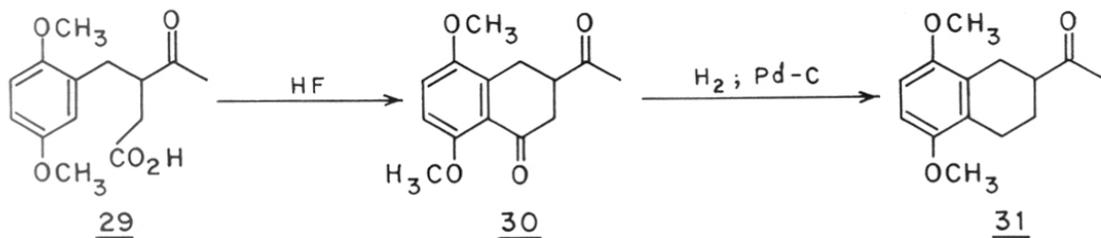
Thus 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydro-2-naphthol (9), an important intermediate representing AB rings, has not escaped⁹ the notice of practitioners of anthracycline synthesis, as it can be condensed by acylation with a phthalic acid derivative, corresponding to CD rings in tetracyclic system. In fact in many cases 9 was further converted to anthracyclines⁹. Among these Wong et al.²⁹ reported the first synthesis of 9 in the year 1971 (Scheme-7) 2,5-dimethoxybenzaldehyde on condensation with 2,4-pentanedione gave the diketone 8, which was transformed to keto-acid 29 on four steps. Cyclisation of 29 with HF provided 30. Hydrogenolysis of 30 with 5% Pd-C in acidic ethanolic solution gave the

SCHEME - 7

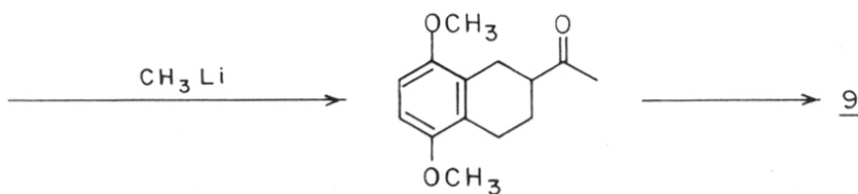
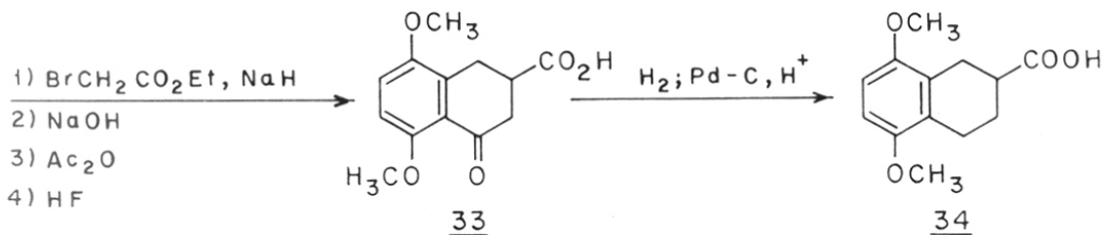
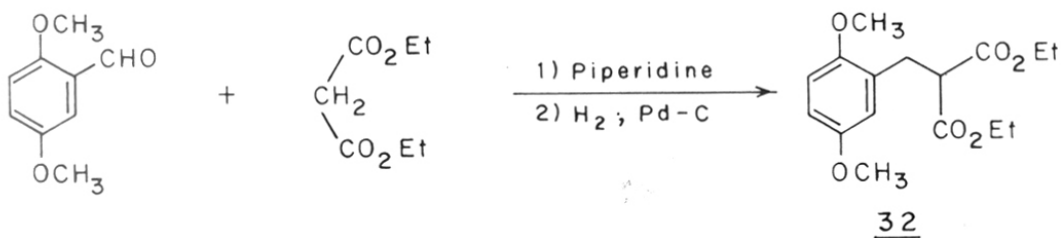
37



1) H_2 ; Pd-C
 2) $\text{BrCH}_2\text{CO}_2\text{Et}$;
 NaH
 3) NaOH



SCHEME - 8



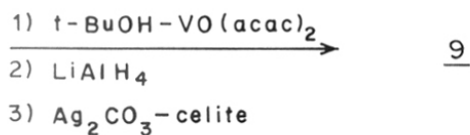
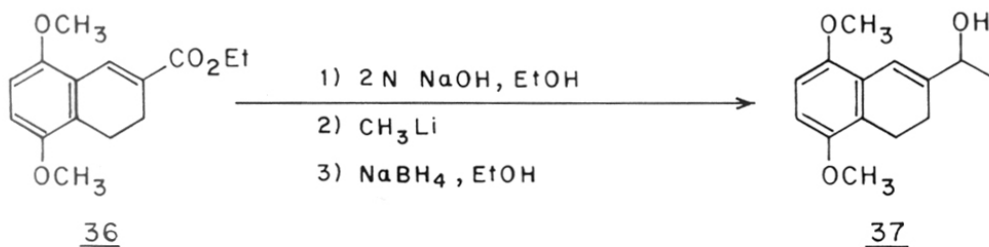
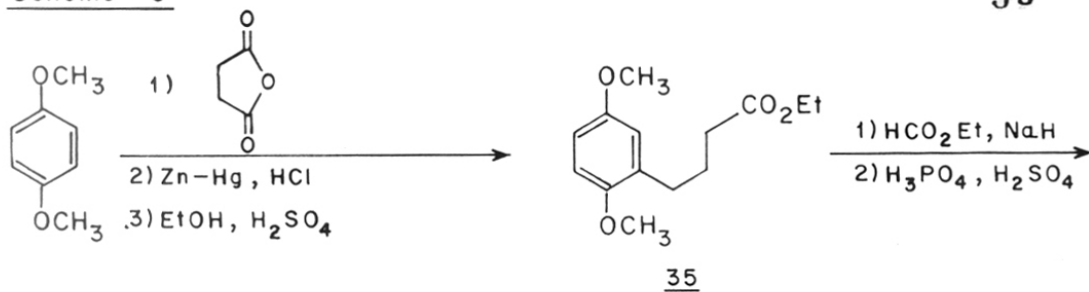
tetralin 31, which upon oxidation in *t*-butyl alcohol with potassium *t*-butoxide and oxygen followed by reduction with Zn-AcOH afforded 9.

Wong et al.^{11a} further reported a modified method (Scheme-8) in which the 2,5-dimethoxybenzaldehyde was condensed with diethylmalonate followed by reduction with H₂, Pd-C to give the diester 32 in 80% yield. Ketoacid 33 was obtained in three steps from 32. Hydrogenolysis of 33 with 5% Pd-C gave the tetralin 34, which upon treatment with methyl lithium produced the acetyl tetralin 31. Oxidation of 31 by the above method gave 9.

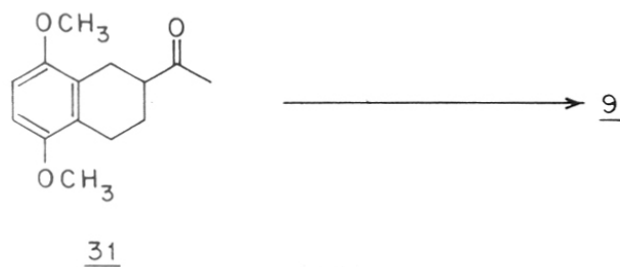
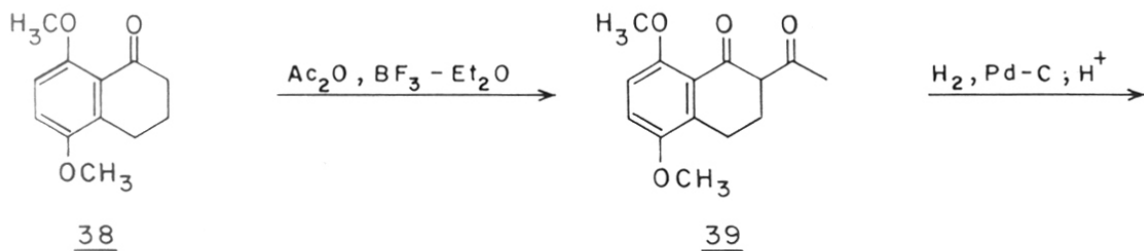
Terashima et al.³⁰ reported a synthesis of 9 starting from 1,4-dimethoxybenzene (Scheme-9). According to their study 35 was obtained in 63% over-all yield from 1,4-dimethoxybenzene. Conversion of 35 to 36 was accomplished by condensation with ethylformate followed by acid-promoted cyclization. 37 obtained in three steps from 36 was converted to 9.

A new approach was developed by Blade and Hodge³¹, starting from 5,8-dimethoxy-1-tetralone (38) [Scheme-10]. Friedel-Crafts acetylation of 38 with acetic anhydride and BF₃-etherate gave the diketone 39, which on selective hydrogenolysis afforded the Wong's ketone (31). Conversion of 31 to 9 was carried according to Wong's procedure.

Scheme - 9



Scheme - 10

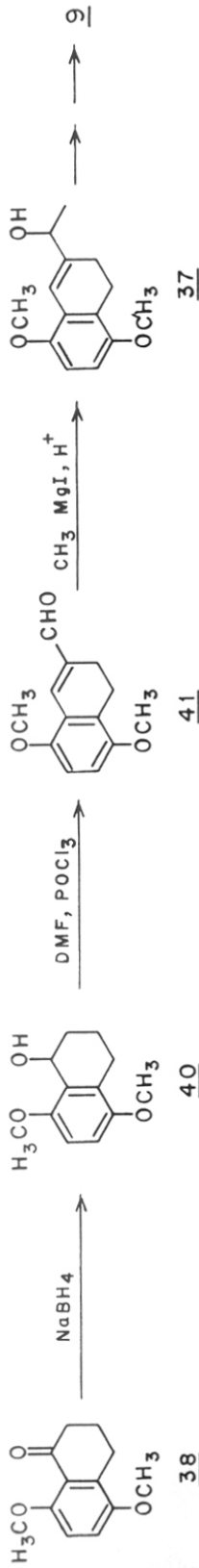


Krishna Rao and Reddy³² reported the synthesis of 2- α -hydroxyethyl-5,8-dimethoxy-3,4-dihydronaphthalene (37), a late stage precursor to 9 starting from 5,8-dimethoxy-1-tetralone (38) (Scheme-11). The dihydronaphthalene (40) obtained in two steps from the corresponding tetralone, was subjected to Vilsmeier reaction with DMF on POCl₃ to give the dihydro aldehyde (41). Grignard reaction of 41 with CH₃MgI, followed by aqueous work-up afforded 37.

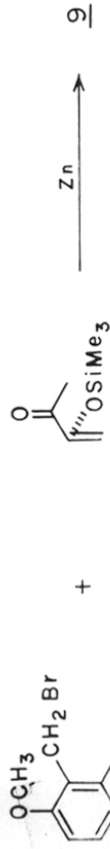
Cava et al.³³ reported a two step synthesis of 9 (Scheme-12) by a Diels-Alder reaction. The O-quinodimethane, derived from a substituted benzylbromide (42) by treatment of zinc, was treated with substituted methyl vinyl ketone to give 9 after hydrolysis in 18% yield.

Rama Rao et al.^{34,35} reported two synthesis for 9. In one method³⁴ (Scheme-13) 2,5-dimethoxybenzyl bromide was condensed with acetyl acetone in presence of potassium carbonate to afford the diketone 43. Conventional operations transformed 43 to 29, which was smoothly elaborated to 9 by Wong's procedure. In another³⁵ method (Scheme-14), 2-acetyl-5,8-dimethoxynaphthalene (44) (a minor product obtained in the acylation of 1,4-dimethoxynaphthalene) was subjected to metal-ammonia reduction to afford 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (31), which was transformed to 9 according to Wong's procedure.

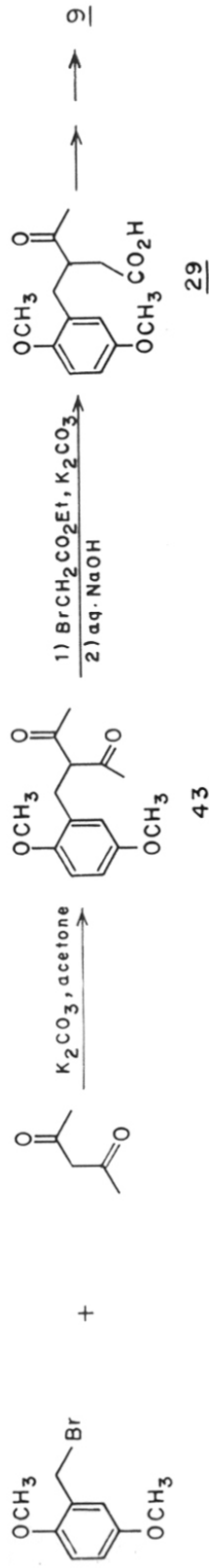
Scheme - 11



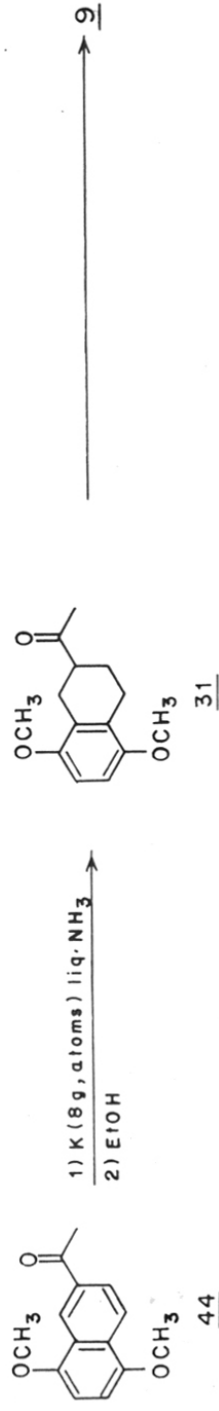
Scheme - 12



Scheme - 13

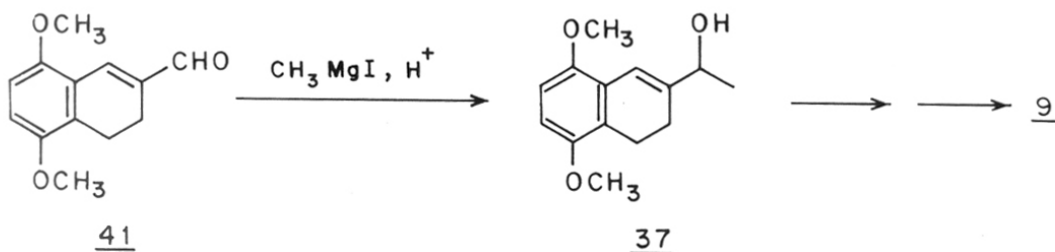
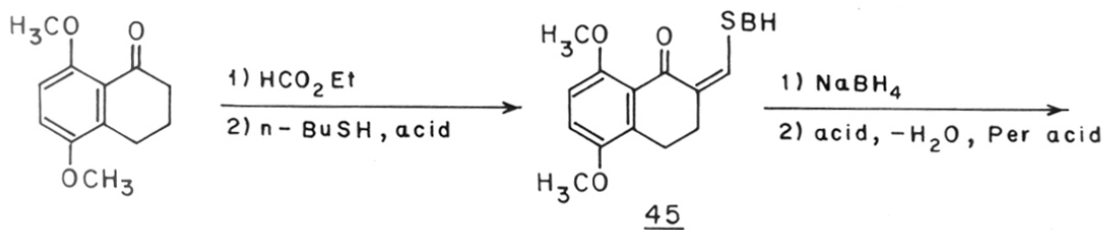


Scheme - 14

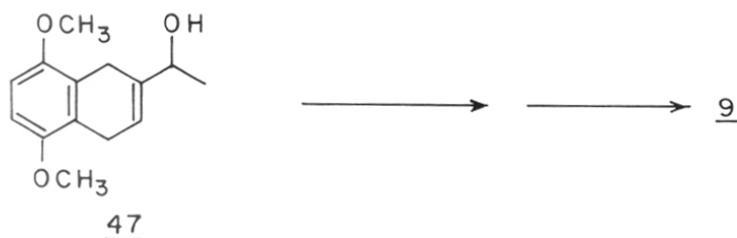
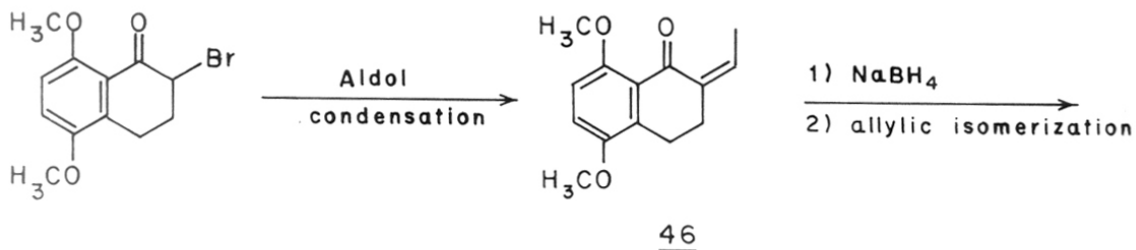


Arcamone³⁶ reported the synthesis of 9 by three different routes. According to the first approach (Scheme-15), the 5,8-dimethoxy-1-tetralone was treated with ethylformate in presence of a base to give, after acid catalysed addition of n-butyl thiol, compound 45. The dihydroaldehyde 41, obtained in two steps from 45 was treated with CH_3MgI followed by hydrolysis to give 37. Epoxidation of 37 followed by reductive opening of epoxide and finally oxidation afforded 9 in 55% overall yield. The second synthesis to 9 (Scheme-16) involved the aldol condensation of 5,8-dimethoxy-2-bromo-1-tetralone, which on acid treatment afforded 46. Reduction of 46 followed by allylic isomerization gave 47 which on epoxidation, followed by reductive opening of epoxide and finally oxidation yielded 9 in 56% overall yield. The third route (Scheme-17) involved a Diels-Alder reaction between p-benzoquinone and 2-(α -hydroxyethyl)-1,3-butadiene to give the same adduct 47 after methylation. 47 was further elaborated to 9 in a similar manner in 51% overall yield.

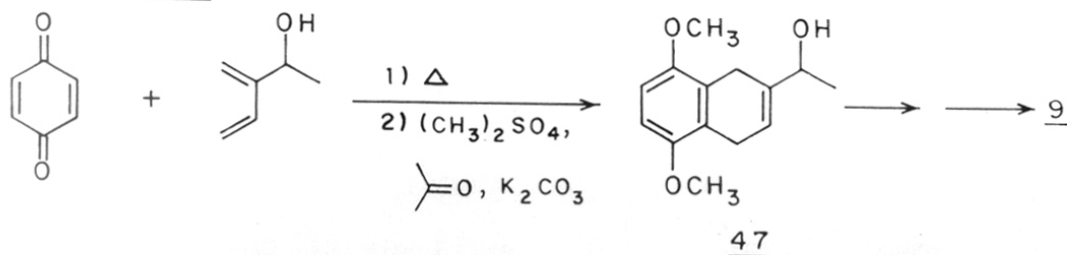
The ketone functionality in 5,8-dimethoxy-2-tetralone (48) was exploited by many chemists for sidechain elaboration to give 9. In general these methods could be classified either as two carbon homologations or two sequential one carbon



Scheme - 16



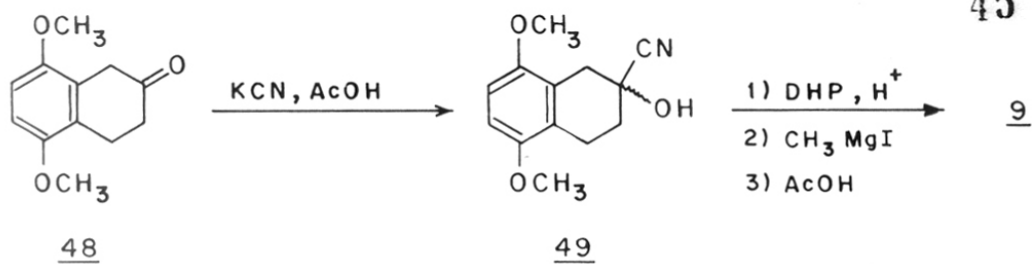
Scheme - 17



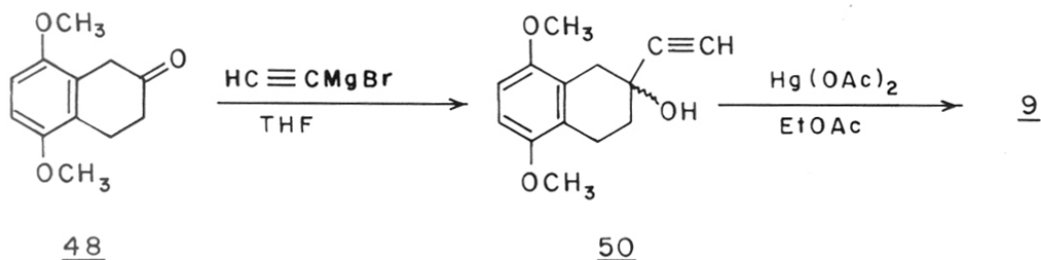
homologation. Smith et al.^{11c} converted the 5,8-dimethoxy-2-tetralone (48) into its cyanohydrin derivative (49), which after THP protection was treated with excess methylmagnesium iodide and subsequently hydrolysed to produce 9 (Scheme-18). Ethynylation of 48 with ethynylmagnesium bromide was reported by Kende et al.³⁷ to give 50 which was elaborated smoothly to 9 (Scheme-19). Wiseman et al.³⁸ treated 48 with methoxy vinyl lithium, followed by aqueous work-up to give 51, which on hydrolysis with perchloric acid gave 9 (Scheme-20).

Rama Rao et al.³⁹ treated 48 with an acyl anion equivalent such as 2-lithio-2-methyl-1,3-dithiane and transformed the resultant intermediate by conventional operations to obtain 9, as depicted in Scheme-21.

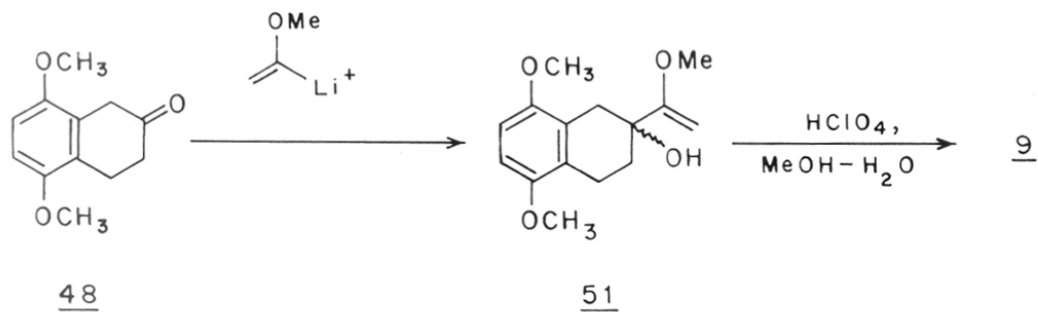
Scheme - 18



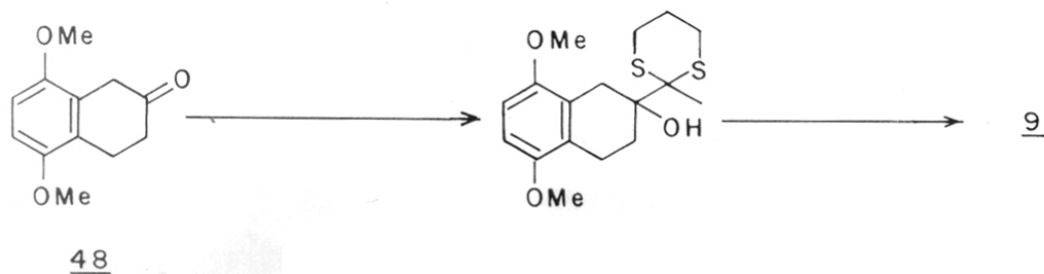
Scheme - 19



Scheme - 20



Scheme - 21



PRESENT WORK

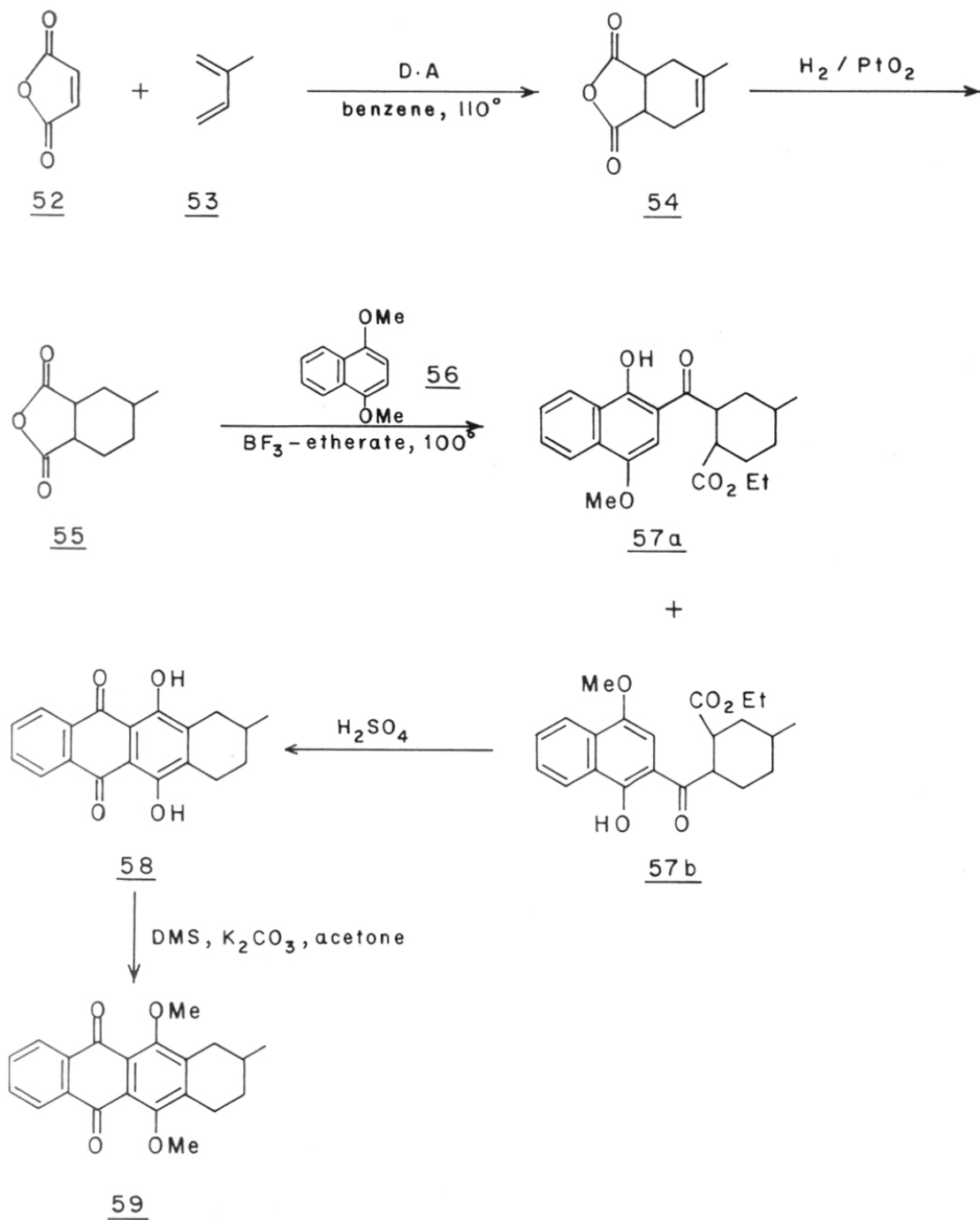
The numerous methods described earlier exemplify the importance of 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydro-2-naphthol (9), as it can be condensed with a phthalic acid derivative to afford the required tetracyclic system.

The feasibility of building the tetracyclic system by AB + CD method was studied by synthesizing a new model compound 58* (Scheme-22) from easily available starting materials such as maleic anhydride (52) and isoprene (53).

Isoprene (53) adds to maleic anhydride (52) in benzene to give the adduct, 4-methyl-4-cyclohexene-1,2-dicarboxylic acid anhydride (54) in quantitative yield. m.p. 65° (lit.⁴⁰ m.p. 63-64°). The PMR spectrum (Fig.3) of 54 shows the methyl signal as a singlet at δ 1.8. A multiplet of two methylene groups appear at δ 2.4. Another multiplet is centred at δ 3.4, representing two protons (1,2-H). The vinyl proton (5-H) multiplet is seen at δ 5.6. Hydrogenation of 54 in ethyl acetate/Ac₂O at atmospheric pressure for 20 hr using PtO₂ as catalyst⁴¹ afforded 55 as a viscous mass in quantitative yield. Acetic anhydride

*The same compound was prepared in this laboratory by another route. For details see S.M. Jaweed, Ph.D thesis (University of Poona), 1983.

SCHEME - 22



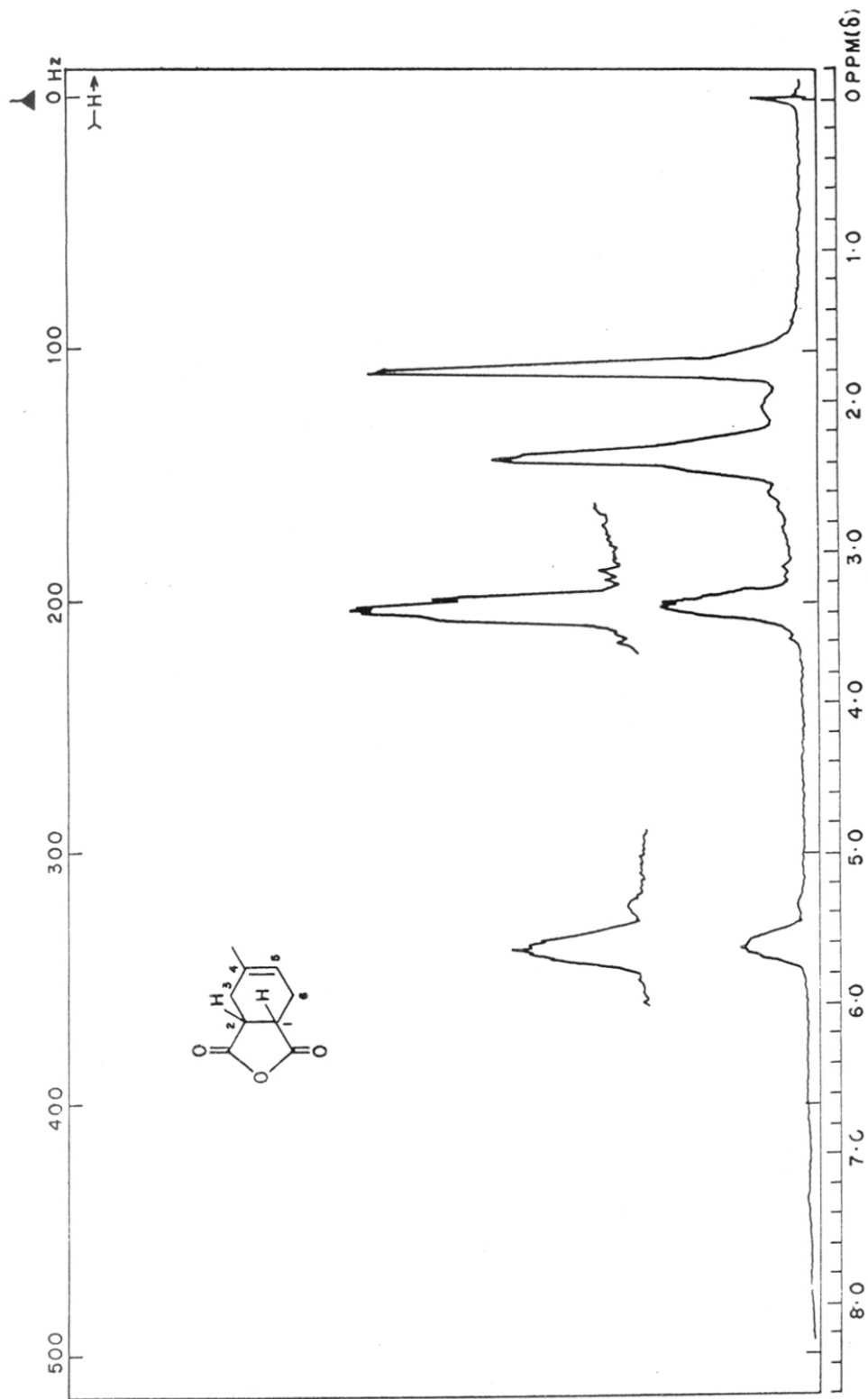


FIG. 3. PMR SPECTRUM OF COMPOUND 54 IN CCl₄

was used to prevent the opening of anhydride to acid. The absence of the vinylic proton (5-H) at δ 5.6 confirmed the structure of 55 by PMR spectrum. The remaining protons resonated at expected chemical shifts. Condensing 55 with 1,4-dimethoxynaphthalene (56) (prepared from 1,4-naphthoquinone by reduction with $\text{Na}_2\text{S}_2\text{O}_4$ followed by conventional methylation using dimethyl sulphate, K_2CO_3 in boiling acetone) in presence of BF_3 -etherate at 100° for 16 hr provided 57. TLC (10% acetone in benzene) indicated 2 spots with close Rf values, showing +ve FeCl_3 test. The PMR spectrum of 57 in CDCl_3 showed the presence of two isomers in equal ratio. For example the methyl group was expected to resonate as a doublet but it appeared as two broad doublets. The shoulder (δ 1.63) to the doublet (δ 1.70) could be attributed to the methyl group of the other isomer. Similarly the broad multiplet for A_2B_2 pattern for aromatic protons at δ 7.5 and 8.1 also confirmed the presence of two isomers. The remaining signals were not amenable to first order analysis. No attempts were made to separate these isomers, because subsequent cyclization would lead to a single symmetrical product. 57 in conc. H_2SO_4 afforded 58, whose PMR spectrum in CDCl_3 indicated that cyclization with concomitant demethylation and isomerization had taken place in one step. Further support for the structure of 58 was gleaned by conventional methylation (dimethyl sulphate, potassium carbonate and

acetone) which afforded the dimethyl ether (59) and confirmed by mass and PMR spectrum (Fig.4). For example a doublet at δ 1.13 was assigned to methyl group, a singlet at δ 3.87 assigned to two methoxyls, multiplets in the region of δ 1.0 to δ 3.3 corresponded to three methylenes and one methine proton, and A_2B_2 pattern between δ 7.5 - δ 8.5 assigned to aromatic protons.

Thus as the AB + CD method gave the desired tetra-cyclic system rather smoothly, efforts were directed towards the construction of 9. In spite of numerous approaches for 9 there seems to be ample scope for constructing this key intermediate, constituting AB rings, by new methodology. This part deals with the synthesis of 9 by two different approaches and its subsequent elaboration to (+)4-demethoxy-daunomycinone (2d).

Method 1 (Scheme-23)

5,8-Dimethoxy-1-tetralone (38) was prepared from 1,4-dimethoxy benzene (60) according to the procedure reported by Moore et al.⁴².

1,4-Dimethoxy benzene (60) (prepared from hydroquinone by conventional methylation using dimethyl sulphate, acetone and K_2CO_3) underwent Friedel Craft's acylation with succinic anhydride to yield 2-[2',5'-dimethoxybenzoyl]propionic acid (61) in 94% yield. Clemmensen reduction of

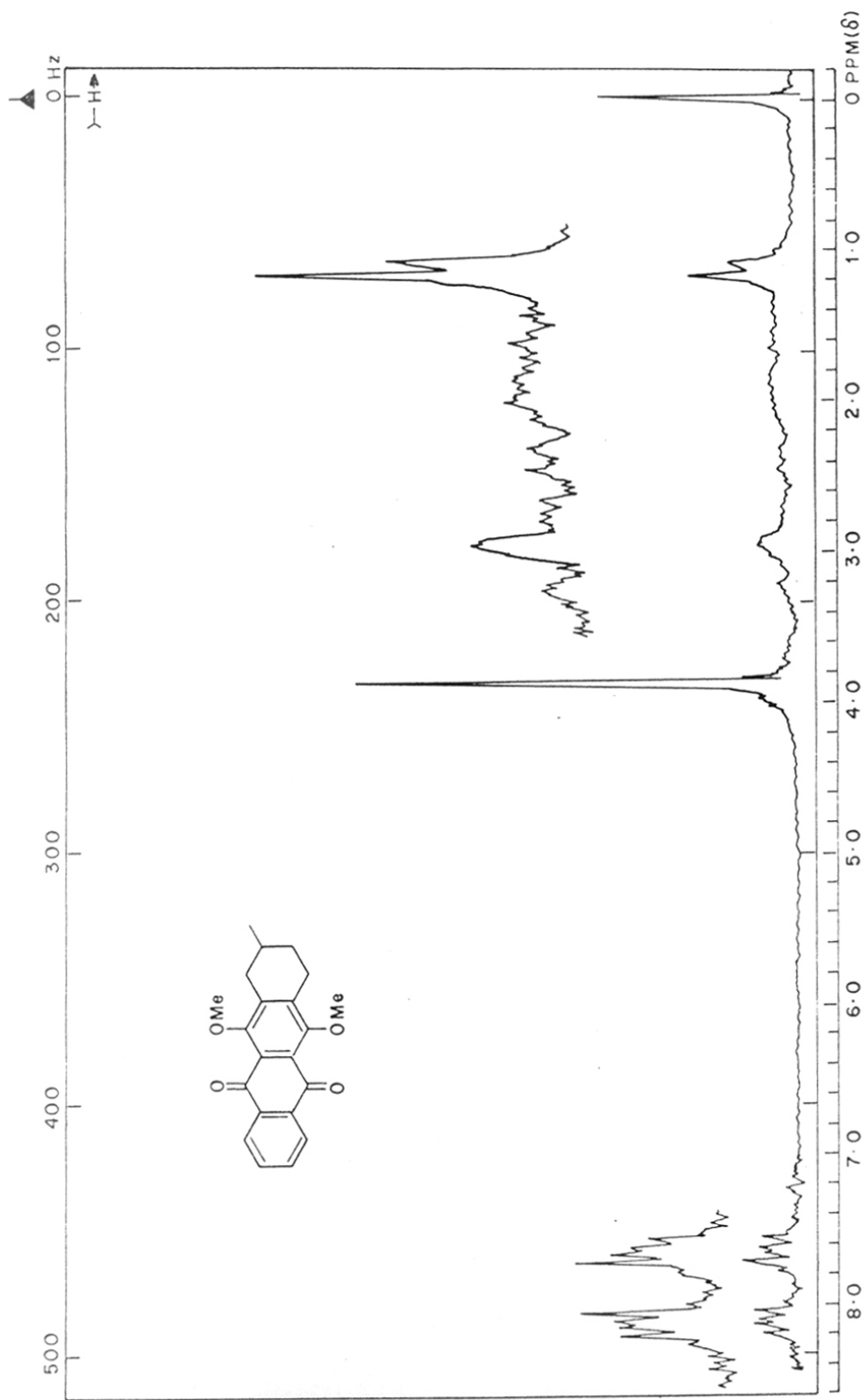
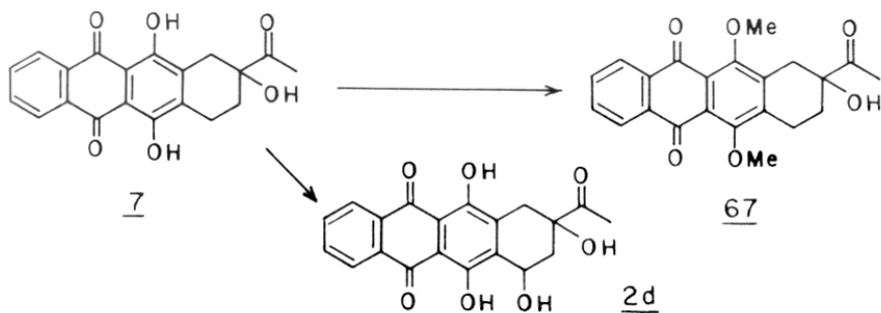
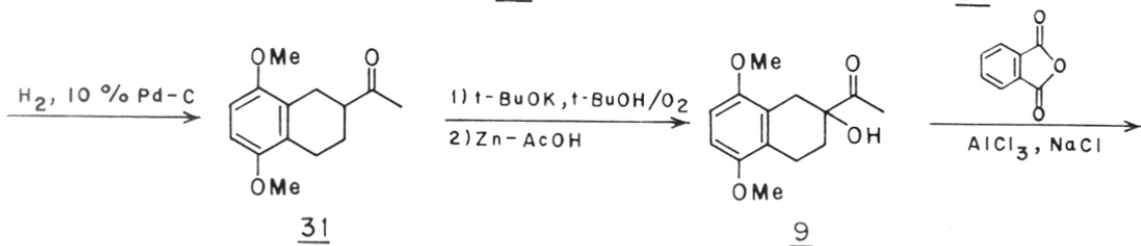
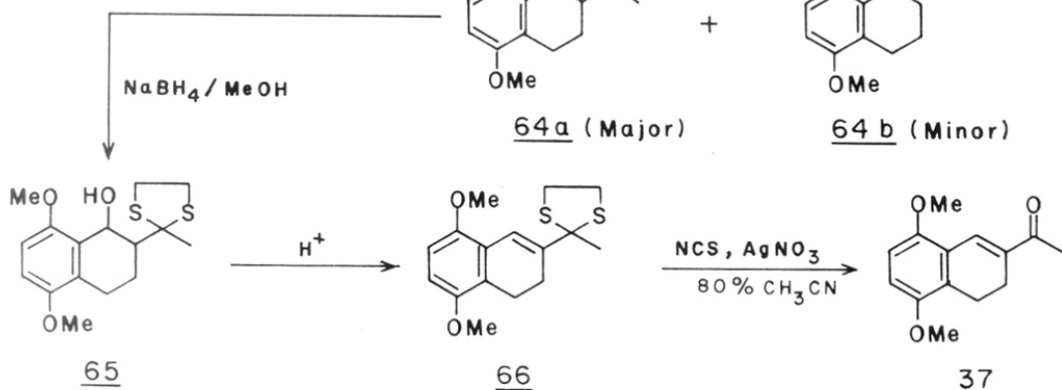
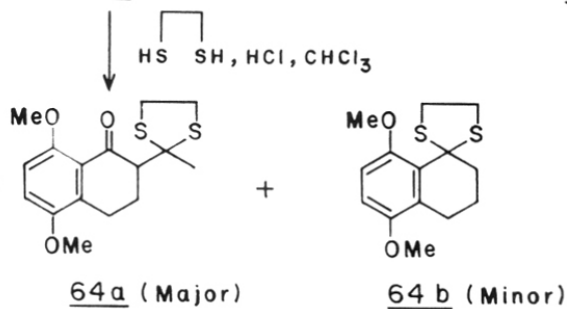
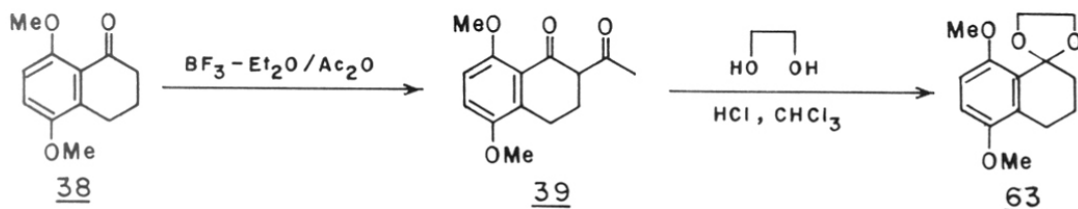
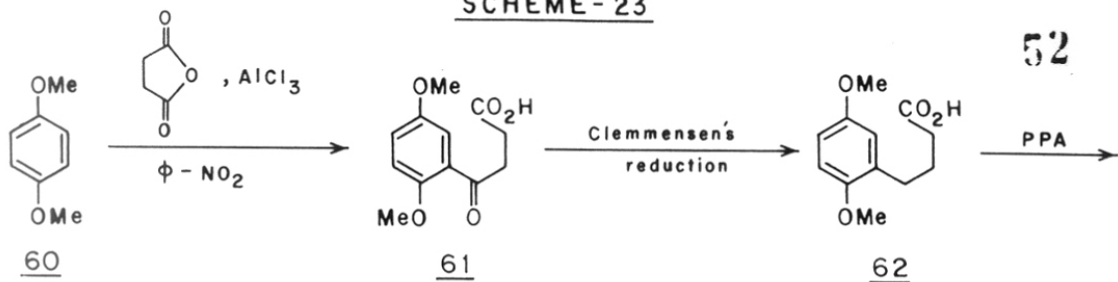


FIG.4 PMR SPECTRUM OF COMPOUND 59 IN CDCl₃

SCHEME - 23

52



61 afforded 4-[2',5'-dimethoxyphenyl]butyric acid (62) which when subjected to PPA cyclization gave 5,8-dimethoxy-1-tetralone (38) in 50% overall yield.

2-Acetyl-5,8-dimethoxy-1-tetralone (39) was made from 38 in 85% yield by condensing with BF_3 -etherate - Ac_2O according to Hodge method³¹. The acetyl ketone group was then selectively protected (1,2-ethane dithiol, HCl gas, CHCl_3 , room temp. 10 hr) to provide the ketone 64a as the major product, along with small amount of 64b (identified from PMR spectrum) which could be separated by silica gel chromatography. This was in contrast to protection of 39 with ethylene glycol under identical conditions, wherein the acetyl group was cleaved to give 63 exclusively. The PMR spectrum of 64a in CCl_4 (Fig.5) shows the presence of methyl group as a sharp singlet at δ 1.70. The two methoxyl groups appear as a singlet at δ 3.46. The methylene groups are extended as a multiplet from 1.90 - 2.80. A singlet at 2.90 represents the methylenes present in $[-\text{S}-(\text{CH}_2)_2-\text{S}]$. The aromatic protons appear at δ 5.96, 6.16 as doublets ($J = 9$ Hz). Reduction of 64a with $\text{NaBH}_4/\text{MeOH}$ at room temp. for 24 hr provided 65. m.p. 98-100°C. The PMR spectrum of 65 in CCl_4 (Fig.6) shows the presence of methyl group as a sharp singlet at δ 1.93. The methoxyl groups appear as two singlets at δ 3.73 and δ 3.80. The methylene groups are

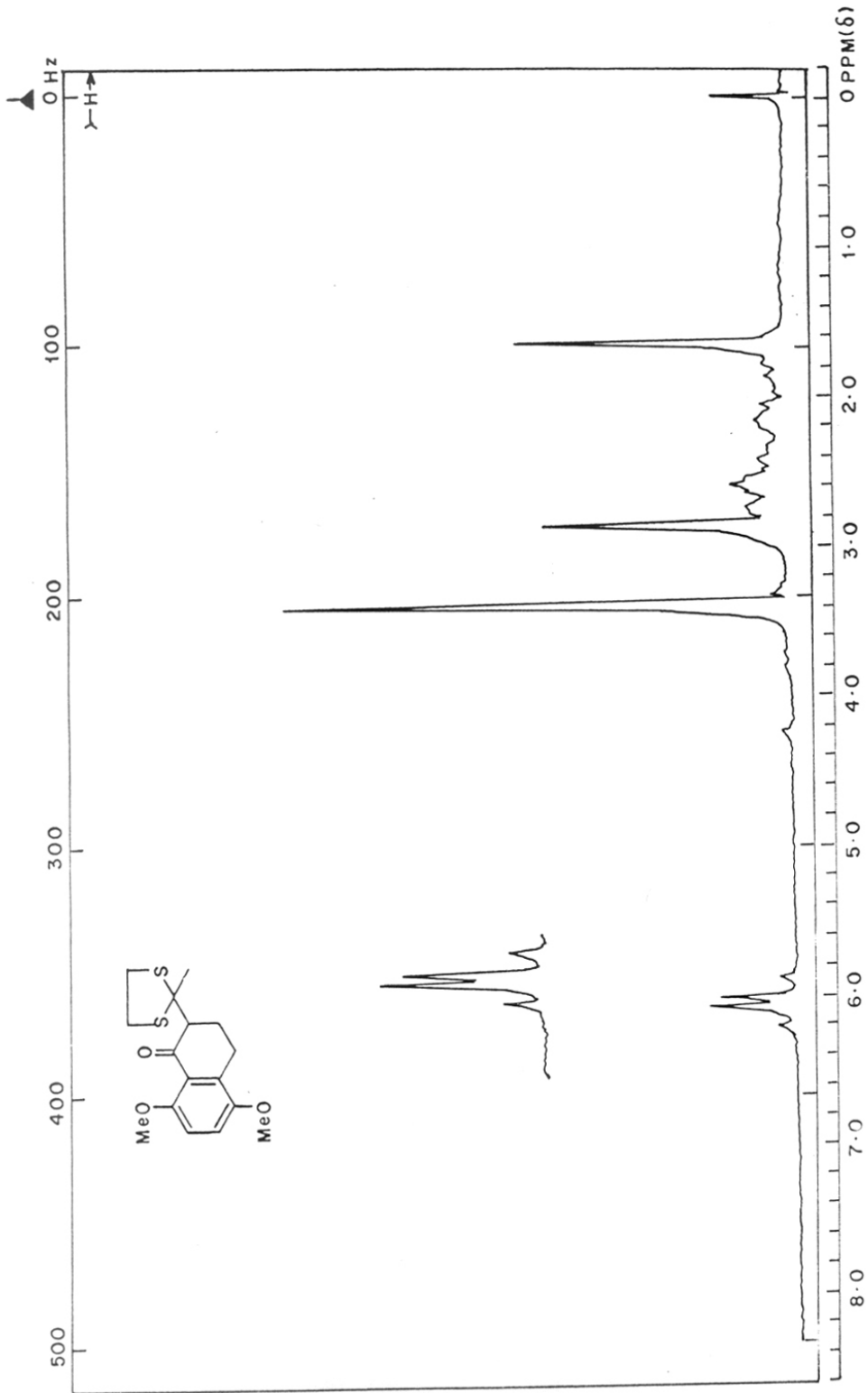


FIG.5 PMR SPECTRUM OF COMPOUND 64 a in CCl₄

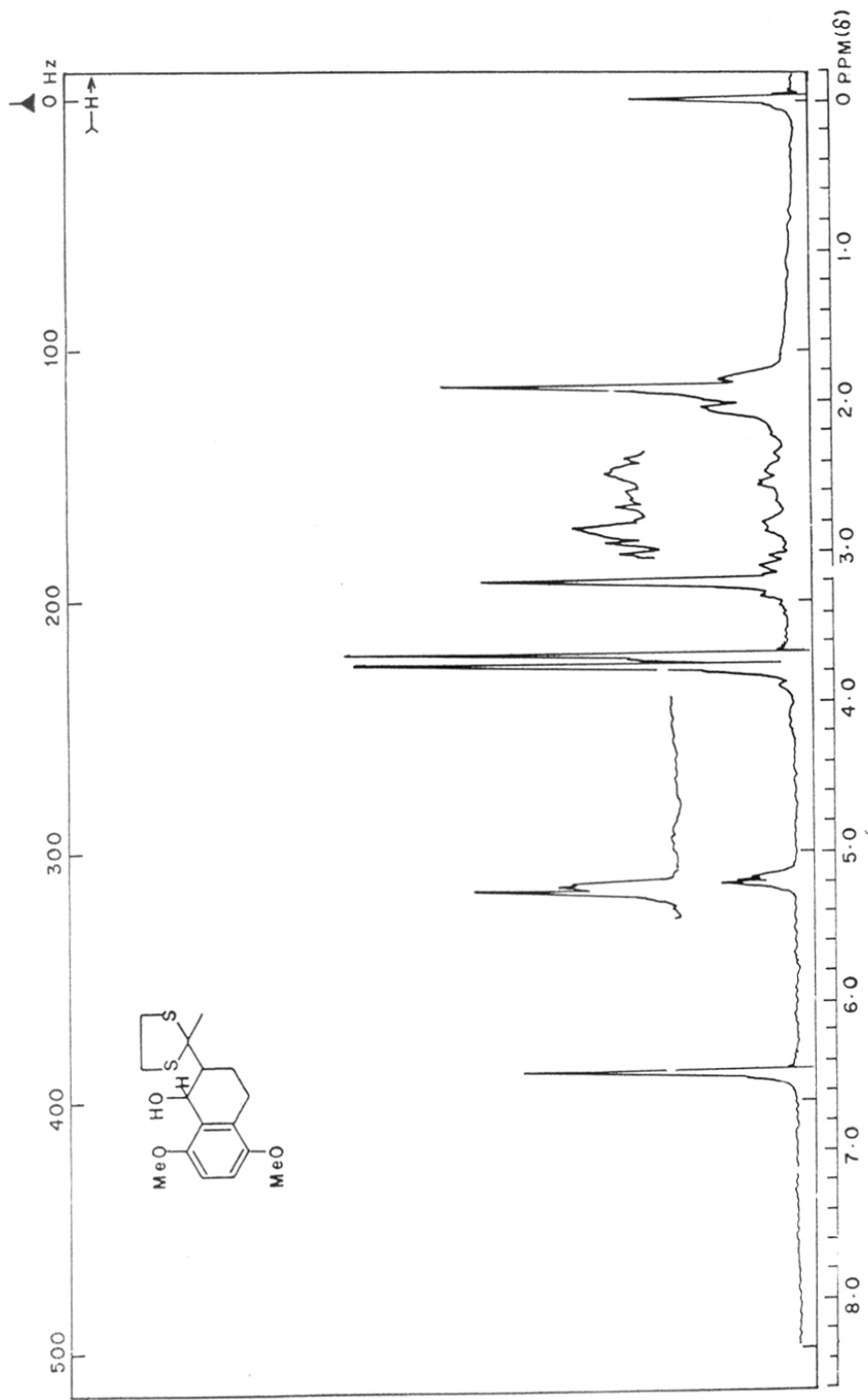


FIG. 6 PMR SPECTRUM OF COMPOUND 65 IN CCl_4

extended as a multiplet from δ 2.00 - δ 2.90. A singlet at 3.23 represents the methylenes present in $[-S-(CH_2)_2-S-]$. The multiplet at δ 5.23 represents a single proton (1-H). The two aromatic protons appear as a singlet at δ 6.50. IR spectrum showed stretching at 3300 cm^{-1} (OH). Acid treatment of 65 provided 66 in 76% yield. m.p. $95-97^\circ$. The PMR spectrum of 66 [Fig.7] in CCl_4 shows the methyl group as a singlet at δ 2.00. The methylenes are centred as a multiplet at δ 2.53. The remaining protons resonated at expected chemical shifts. Dethioketalization of 66 (NCS, $AgNO_3$, 80% CH_3CN , room temp. 20 minutes) gave the desired 2-acetyl-5,8-dimethoxy-3,4-dihydronaphthalene (37) in 73% yield. m.p. $102-103^\circ$ [lit.^{43,32} m.p. $104-105^\circ$, m.p. $102-103^\circ$]. The PMR spectrum of 37 in CCl_4 shows the methyl group as a singlet at δ 2.36. The methylenes are centred as a multiplet at δ 2.60. The methoxyls appear as two singlets at δ 3.76 and δ 3.83. The aromatic protons appear as doublets at δ 7.53, δ 7.76 ($J = 9\text{ Hz}$) and a broad singlet at δ 7.70 accounts for the vinylic proton. IR spectrum (Nujol) shows stretching at 1660 cm^{-1} ($>C=O$). Hydrogenation of 37 with 10% Pd-C as catalyst at 20 p.s.i. for 5 hr provided 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (31) in 90% yield. m.p. $82-83^\circ$ (lit.²⁹ m.p. $81-82^\circ$). The absence of vinylic proton at δ 7.70 confirmed the structure of 31 by PMR spectrum (Fig.8). The IR spectrum($CHCl_3$) showed

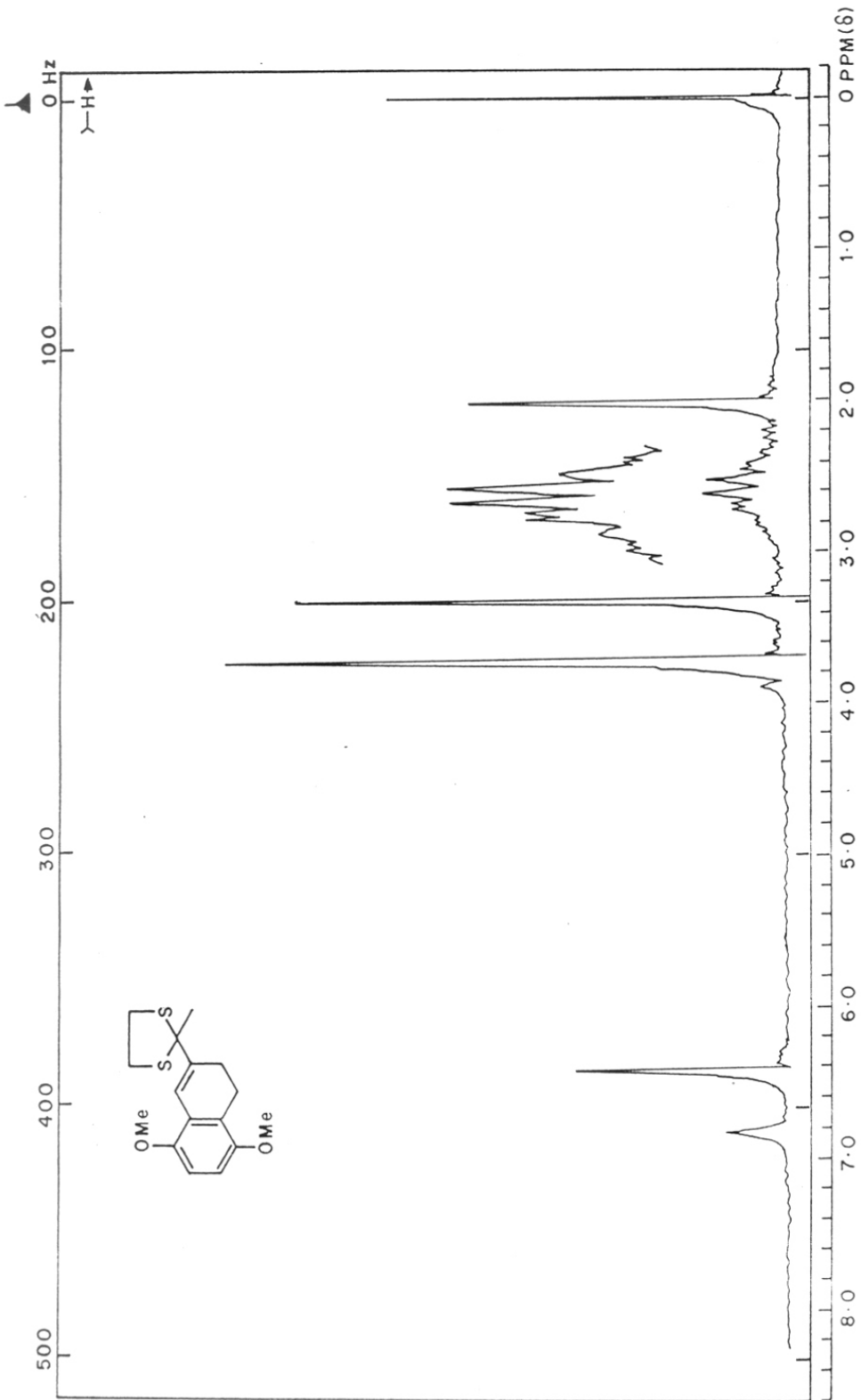


FIG.7 PMR SPECTRUM OF COMPOUND 66 IN CCl_4

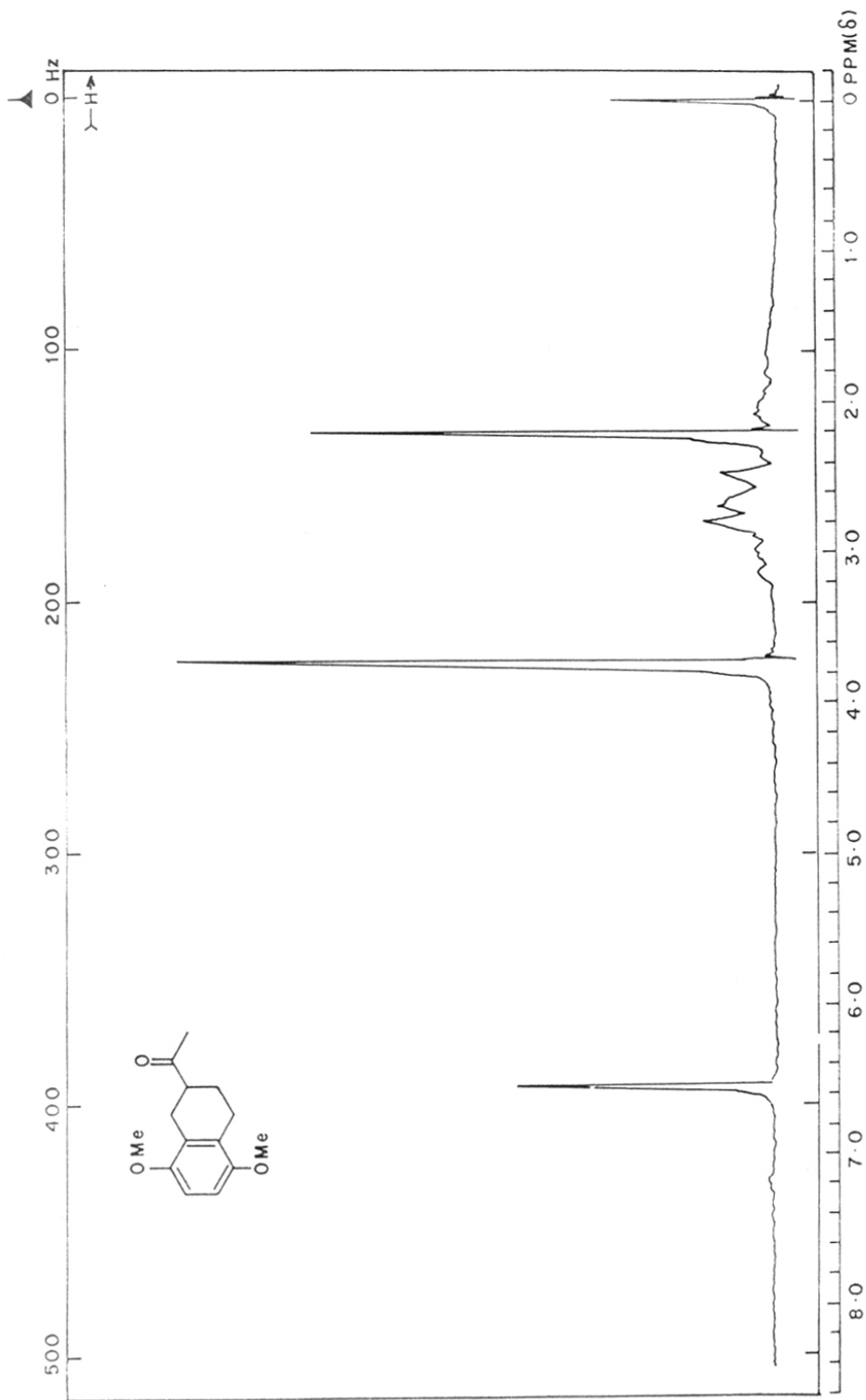


FIG. 8 PMR SPECTRUM OF COMPOUND 31 IN CCl₄

stretching at 1710 cm^{-1} ($>\text{C}=\text{O}$). Conversion of 31 to 9 in 60% yield, m.p. 99° (lit.²⁹ m.p. 97°) was carried according to Wong's procedure by oxidation of 31 in t-butanol with potassium t-butoxide and oxygen followed by reduction with zinc-acetic acid. The PMR spectrum of 9 in CDCl_3 showed a singlet at δ 2.30 and broad singlet at δ 3.40 (D_2O exchangeable) for $-\text{COCH}_3$ and $-\text{OH}$ protons respectively. The methoxyl groups appeared as two sharp singlets at 3.66 and 3.73. The remaining protons resonated at expected chemical shifts. IR spectrum (Nujol) showed absorptions at 1685 cm^{-1} ($>\text{C}=\text{O}$) and 3200 cm^{-1} ($-\text{OH}$).

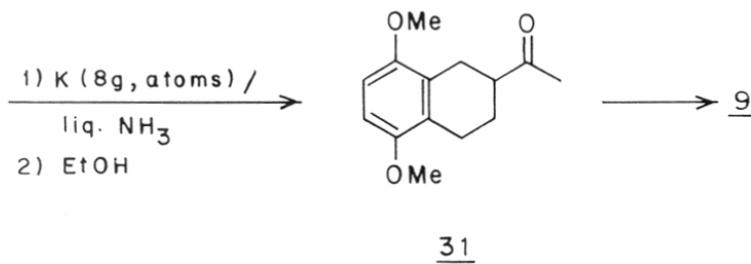
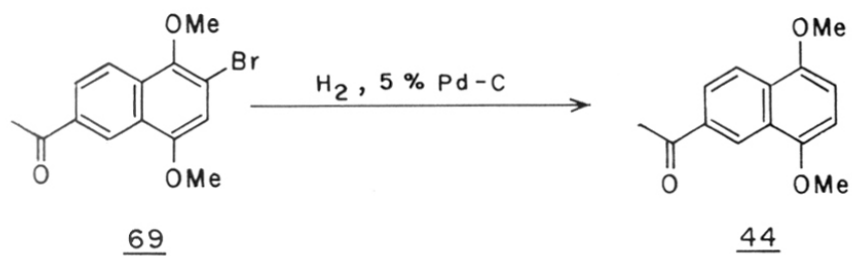
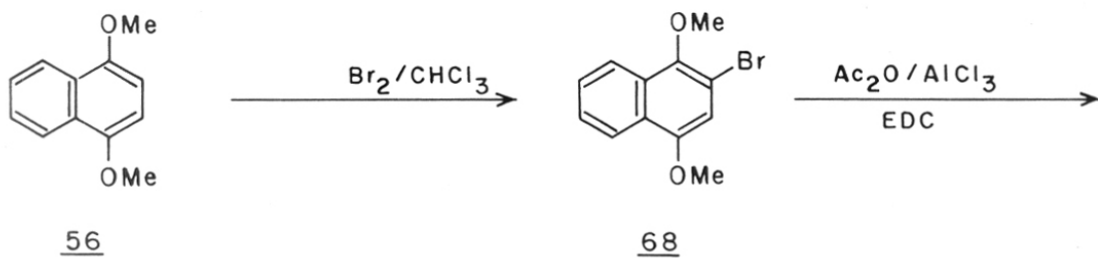
Method-2

Earlier in this laboratory 9 was synthesized³⁵ starting from 2-acetyl-5,8-dimethoxynaphthalene (44) (Scheme-14). However, this compound 44 was obtained in poor yields (30%), during the acylation of 1,4-dimethoxy naphthalene (56).

This part mainly deals with a modified method for obtaining 44 in good yields and thereby its elaboration to (+)4-demethoxydaunomycinone (2d). The synthetic approach is shown in Scheme-24.

1,4-Dimethoxy naphthalene (56) (prepared from 1,4-naphthaquinone by reduction with $\text{Na}_2\text{S}_2\text{O}_4$, followed

SCHEME - 24

**9****31**

by conventional methylation using dimethyl sulphate and K_2CO_3 in boiling acetone) was brominated using $Br_2/CHCl_3$ under N_2 bubbling to afford 2-bromo-1,4-dimethoxy naphthalene (68) in 90 yield. m.p. 57° (lit.⁴⁴ m.p. $58-59^\circ$). Acylation of 68 with acetic anhydride (1.2 eq) using anhydrous $AlCl_3$ (2.2 eq) in ethylene dichloride at 60° for 6 hr. afforded 2-bromo-6-acetyl-1,4-dimethoxy naphthalene (69) in 70% yield; m.p. 97° . [That acylation has occurred at 6 rather than 7 position was based on others work* and chemical reasoning that the presence of bromine constitutes a para orienting effect whereby the 6 position is more electron rich than 7].

The PMR spectrum of 69 in $CDCl_3$ shows the acetyl group as a singlet at δ 2.63. The methoxyl groups appear as two singlets at δ 3.83 and δ 3.86. A singlet at δ 6.66 corresponds to (3-H) proton. A doublet at δ 8.38 ($J = 2$ Hz) represents the (5-H) proton, and the multiplet centred at δ 7.78 represents (7,8-H) protons. Debromination of 69 by hydrogenation using 5% Pd-C afforded 6-acetyl-1,4-dimethoxy naphthalene, herewith named for convenience as 2-acetyl-5,8-dimethoxy naphthalene (44) in 80% yield m.p.

*Acylation of 2-bromonaphthalene goes to 6 position. For details see: Lauk U, Skrabal, P and Zollinger H, Helv. Chim Acta 66(5), 1574 (1983).

110° (lit.³⁵ m.p. 111-112°). The PMR spectrum [Fig.9] of 44 in CDCl₃ showed a singlet at δ 2.63 for acetyl protons. The two singlets at δ 3.83 and δ 3.86 were assigned for two methoxyl groups. The aromatic C-6 and C-7 protons appeared as a singlet at δ 6.46. The remaining three aromatic protons appeared as follows: C-3 proton as a doublet of doublet at δ 7.77 (J = 9 and 2 Hz), C-4 proton as a doublet at δ 8.0 (J = 9 Hz) and C-1 proton as a doublet at δ 8.60 (J = 2 Hz).

Metal ammonia reduction of naphthalenes and its derivatives was studied extensively⁴⁵. It was shown earlier that reduction of naphthalenes depends mainly on the nature of substituents in the ring. In general electron donating groups such as -OCH₃, -CH₃ and -N(CH₃)₂ at 1-position of the naphthalene directs the reduction in the adjacent ring, while 2-substituted naphthalenes were reduced having the substituents in the ring. However the presence of electron withdrawing groups such as -COOH, -COCH₃ are known to result in the reduction of the rings having such substituents. Thus one can anticipate that the reduction of 2-acetyl naphthalene can give either 2-acetyl-1,4-dihydronaphthalene or 2-acetyl-1,2,3,4-tetrahydronaphthalene.

By this analogy it was felt that the metal ammonia

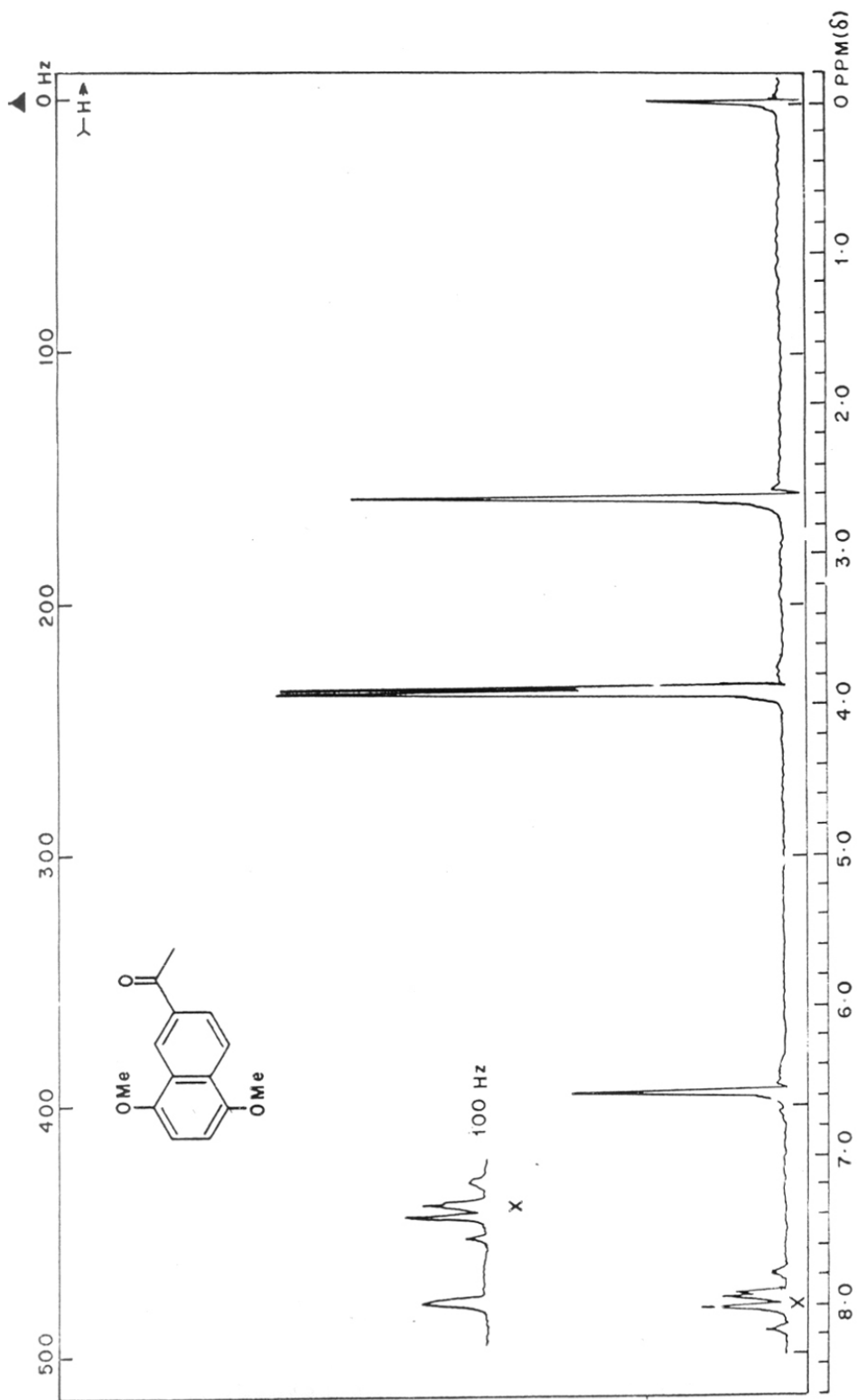


FIG. 9 PMR SPECTRUM OF COMPOUND 44 IN CCl₄

reduction of 2-acetyl-5,8-dimethoxynaphthalene (44) can give either 2-acetyl-5,8-dimethoxy-1,4-dihydronaphthalene or 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (31).

Subba Rao et al.⁴⁶ have shown that the metal-ammonia reduction of 2-acetyl naphthalene in presence of anhydrous FeCl_3 gives only the dihydro product. Recently, however, Rama Rao et al.³⁵ have optimised the conditions for the conversion of 44 to 31.

The 2-acetyl-5,8-dimethoxynaphthalene (44) was best converted directly in a single step to 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (31) by treating with 8 g. atom equivalents of potassium in 50 fold excess of liquid ammonia at -33° for 30 min. and quenching the reaction mixture with ethanol. Extraction with chloroform and chromatography over a short column of silica gel (2% acetone in pet. ether) afforded the desired product, 31 in 75% yield. The compound 31 was identical in all respects with the authentic sample obtained by the first method. 31 was converted to 9 by Wong's method²⁹ as described in Method I.

Having obtained 9 which constitutes the AB synthon the next step was to elaborate it to (+)4-demethoxy-7-deoxydaunomycinone (7).

9 was converted directly to 4-demethoxy-7-deoxydauno-

mycinone (7) by fusion¹⁸ with an intimate mixture of phthalic anhydride, aluminium chloride-sodium chloride melt for 5 minutes followed by treatment of the resultant reddish mass with a saturated solution of oxalic acid. The solid separated was chromatographed on silica gel to give the desired product 7 as reddish plates in 70% yield. m.p. 212-215° (lit.³⁸ m.p. 210-12°). In all respects, this product 7 was in agreement with the reported data.

For further confirmation of 7, its methylation was carried out with dimethyl sulphate in presence of potassium carbonate in boiling acetone to afford the dimethyl ether (67) in 95% yield m.p. 184-86° (lit.⁴⁷ m.p. 184-86°). In the PMR spectrum (Fig.10) of 67 the methoxyl protons appeared as two sharp singlets at δ 3.80 and δ 3.83. The acetyl group appears as a singlet at δ 2.33. The singlet at δ 3.77 (D_2O exchangeable) was assigned for the hydroxyl group. The IR spectrum (nujol) showed stretching at 3350 cm^{-1} (-OH) and 1700 cm^{-1} (>C=O), 1670 cm^{-1} (quinone).

Further (+)-4-demethoxy-7-deoxydaunomycinone (7) was hydroxylated at C-7 position according to the known procedure^{37,11b}. Benzylic bromination of 7 was achieved by using five equivalents of bromine in refluxing CCl_4 with 2,2'-azobisisobutyronitrile (AIBN) as radical initiator. Under these conditions enol bromination at C-14 was suppressed

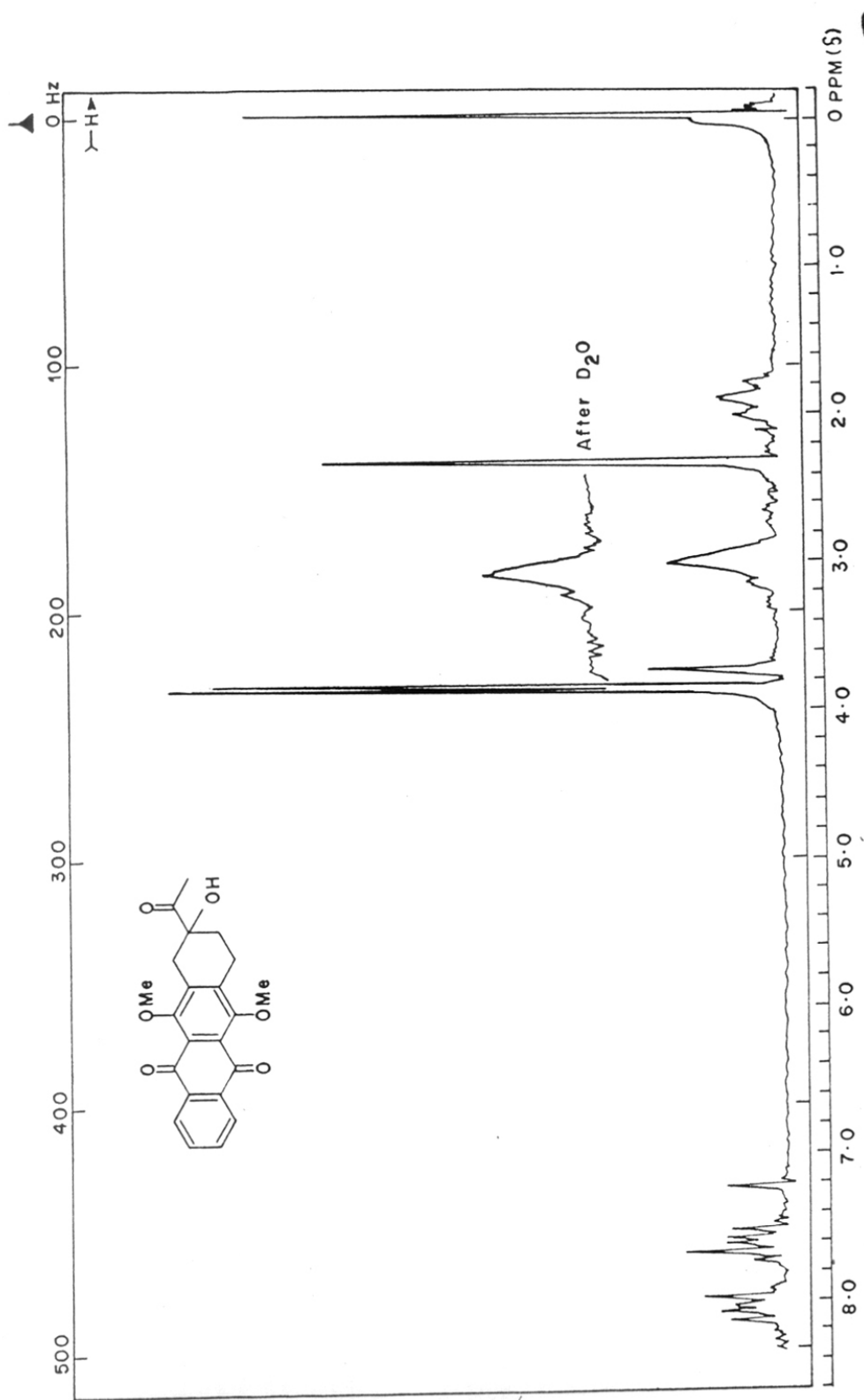


FIG. 10 PMR SPECTRUM OF COMPOUND 67 IN CDCl₃

and the product was that of free radical bromination at C-7. Hydrolysis of this very labile bromine was carried out by treating it with moist silica gel in acetone to give a mixture of three compounds. The epimerization of 4-demethoxy-7-epidaunomycinone to (+)4-demethoxydaunomycinone (2d) was achieved by treating the mixture with TFA. The reaction mixture after aqueous work up and chromatographic purification afforded (+)4-demethoxydaunomycinone (2d) in 23% yield. m.p. 180-183° (lit.⁴⁸ m.p. 183-185°). The compound was identical in all respects with the reported data⁴⁸.

E X P E R I M E N T A L

4-Methyl-4-cyclohexene-1,2-dicarboxylic acid anhydride (54)

The mixture of maleic anhydride (52) [2.0 g, .02 mole] isoprene (53) [1.6 g, .024 mole], dry benzene (20 ml) was sealed in a thick corning tube and heated at 90° for 22 hrs. The solvent was distilled off under reduced pressure and the residue crystallised from the mixture of benzene-pet. ether (2:1) as white needles (3.4 g, quantitative). m.p. 65° [lit.⁴⁰ m.p. 63-64°]. PMR:(CCl₄): 1.8 (s, 3H, CH₃), 2.4 (m, 4H, 2X CH₂), 3.4 (m, 2H, 1,2-H); 5.6 (m, 1H, 5-H).

Analysis: Calculated for C₉H₁₂O₄: C, 58.6; H, 6.5;
Found: C, 59.03; H, 6.5%.

4-Methyl-4-cyclohexane-1,2-dicarboxylic acid anhydride (55)

A solution of the adduct (54) [2.0 g, .012 mole], dry ethyl acetate (15 ml), acetic anhydride (5 ml) (Ac₂O was used to prevent the ring opening of the adduct 54 to free acid) and PtO₂ [50 mg] was hydrogenated at room temp. and at atmospheric pressure with mechanical shaking for 20 hr. The catalyst was filtered, washed with dry ethyl acetate (10 ml) and the combined ethyl acetate solution evaporated under reduced pressure to afford 55 as a viscous substance (not free from acetic anhydride) in quantitative yield. PMR (CDCl₃): 1.00 (d, 3H, -CH₃), 1.30 - 2.32 (m, 7H,

3X CH₂ + CH) 3.4 [m, 2H, 1,2-H].

Preparation of 58

A mixture of the hydrogenated product 55 [1.50 g, .008 mole], 1,4-dimethoxynaphthalene (56) [1.75 g, .0096 mole] and BF₃-etherate [5 ml] was heated at 100° for 16 hr. Saturated sodium acetate solution (50 ml) was added to the above mixture and the resultant solution heated on water bath for 45 minutes. It was extracted with methylene chloride [3 x 25 ml], washed with water [4 x 25 ml], dried over anhydrous Na₂SO₄ and solvent distilled off under reduced pressure. To the reddish viscous substance so obtained, conc. H₂SO₄ (2.5 ml) was added and then left at room temp. for 20 hr. It was poured over crushed ice, the precipitate filtered, washed with water, dried and crystallized from chloroform to yield the cyclized product 58 (1.90 g, 70%) as red coloured solid, m.p. 250°, M⁺ 308.

Preparation of dimethyl ether 59

The dihydroxy compound 58 (1.50 g, .0048 mole), dimethyl sulphate (1.2 ml, .012 mole) and anhydrous K₂CO₃ (5 g) in dry acetone (75 ml) were refluxed on a steambath for 6 hr, followed by usual work-up to give the dimethyl ether 59 (1.20 g, 73% yield). PMR (CDCl₃) 1.0 - 3.3 (bm, 10H, -CH₂- X3 + =CH + -CH₃), 3.87 (s, 6H, OMe X2), 7.50 - 8.50

(m, 4H, aromatic), M^+ 336.

Analysis: Calculated for $C_{21}H_{20}O_4$: C, 75.0; H, 5.95;
Found: C, 75.04; H, 5.92%.

2-[2',5'-Dimethoxybenzoyl]propionic acid (61)

To an ice-cold solution of p-dimethoxybenzene (60) [10.5 g, .076 mole], succinic anhydride (8.8 g, .088 mole) in nitrobenzene (65.0 ml), aluminium chloride (22.5 g, .168 mole) was added and the contents were stirred for 3.5 hr at room temp. The reaction mixture was poured over crushed ice, containing conc. HCl and the organic layer was separated. The solvent was removed by steam distillation. The crude ketoacid was purified by dissolving it in 10% sodium bicarbonate solution and extracted with ethyl acetate. The aqueous layer was neutralized by conc. HCl and the solid separated was filtered to afford the keto acid (61) (17.5 g, 94%), m.p. 101-103° [lit.⁴² m.p. 101-102°].

Analysis: Calculated for $C_{12}H_{14}O_5$: C, 60.50; H, 5.88; Found: C, 60.71; H, 5.93%.

4-[2',5'-Dimethoxyphenyl]-butyric acid (62)

Zinc wool (7.5 g), mercuric chloride (0.5 g), conc.HCl (0.5 ml) and water (8 ml) were shaken efficiently. Contents were decanted and to the zinc amalgam so obtained, the keto acid (61) [2.4 g, .01 mole), toluene (30 ml), conc. HCl (7.0 ml) and water (5.0 ml) was added. The mixture was heated on a steam-bath for 30 hr. During this period conc. HCl (7.0 ml) was added after every 6 hr. The organic layer was separated, washed with water (4 x 25 ml), dried over anhydrous Na_2SO_4 and solvent

distilled off under reduced pressure to afford 4-[2',5'-dimethoxyphenyl]-butyric acid (62) as a crystalline solid (2.0 g, 88%), m.p. 67-68° (lit.⁴² m.p. 68-69°). M⁺ 224.

Analysis: Calculated for C₁₂H₁₆O₄: C, 64.28; H, 7.14; Found: C, 64.45; H, 7.28%.

5,8-Dimethoxy-1-tetralone (38)

Polyphosphoric acid (PPA) was prepared from P₂O₅ (90 g) and phosphoric acid (40 ml) by heating at 80° for 1 hr and to this the dimethoxy acid 62 (2.50 g, .011 mole) was added in one lot. This was thoroughly stirred at this temperature for 5 minutes and the homogeneous reaction mixture was left aside at 80° for another 45 min. without stirring. The reddish brown product, while still hot, was at once poured on crushed ice. The material was left aside for 12 hr (overnight) to decompose the complex. The product was taken up in chloroform (50 ml), washed with water (2 x 50 ml), 10% NaOH solution in water (2 x 25 ml), brine (2 x 25 ml), dried over anhydrous Na₂SO₄ and finally concentrated to give 38 (1.37 g, 60%) as a crystalline material, m.p. 60-62° (lit.⁴² m.p. 58-62°). PMR (CCl₄): 1.8 - 2.9 (m, 6H, 3X CH₂), 3.77 (s, 3H, OMe), 6.53, 6.76 (dd, J=9, 2H, aromatic).

Analysis: Calculated for C₁₂H₁₄O₃: C, 55.34; H, 6.79; Found: C, 55.62; H, 6.84%.

2-acetyl-5,8-dimethoxytetralone (39)

A solution of 5,8-dimethoxy-1-tetralone (38) (3.2 g, .015 mole), acetic anhydride (50 ml) and BF_3 -etherate (12.0 ml) was stirred at room temp. for 1 hr. The reaction mixture was diluted with water (100 ml) and the bright yellow coloured complex was filtered and dissolved in methanol (50 ml). Saturated aqueous solution of sodium acetate (40 ml) was added and the contents refluxed on a steam bath for 2 hr. The solvent was distilled off under reduced pressure and the residue diluted with H_2O (200 ml). The solid separated was filtered, washed with cold H_2O , dried and crystallized from benzene: pet.ether (2:1) as yellow crystalline compound (3.26 g, 85%), m.p. $130-132^\circ$ (lit.³¹ m.p. $131-132^\circ$). PMR (CDCl_3): 2.20 (s, 3H, COCH_3), 2.3 - 2.9 (m, 4H, 2X CH_2), 3.73 (s, 3H, 1X OMe), 3.80 (s, 3H, 1X OMe). 6.4, 6.9 (dd, $J = 9$ Hz, 2H, ArH), 17.55 (s, 1H, enolic OH). M^+ 248.

Analysis: Calculated for $\text{C}_{14}\text{H}_{16}\text{O}_4$: C, 67.74; H, 6.45.
Found: C, 67.89; H, 6.59%.

Preparation of ketone 64a

To a solution of 2-acetyl-5,8-dimethoxy-1-tetralone (39) (3.5 g, .014 mole) and 1,2-ethanedithiol (1.316 g, .014 mole) in dry chloroform (50 ml), under stirring at room temp. dry HCl was passed for 10 hr. The reaction mixture was successively washed with water (4 x 50 ml); 2% aqueous NaOH solution

(3 x 50 ml) and brine (2 x 100 ml). The clear organic layer was dried over anhydrous Na_2SO_4 , distilled off under reduced pressure and chromatographed over silica gel column (8% acetone in pet. ether) to give 64a (3.20 g, 70% yield). M^+ 324. PMR (CCl_4): 1.70 (s, 3H, CH_3), 1.90 - 2.80 (m, 5H, CH and 2X CH_2), 2.90 (s, 4H $-\text{S}(\text{CH}_2)_2-\text{S}-$), 3.46 (s, 6H, 2X OMe), 5.96, 6.16 (dd, $J = 9$ Hz, 2H, ArH).

Analysis: Calculated for $\text{C}_{16}\text{H}_{20}\text{O}_3\text{S}_2$: C, 59.25; H, 6.17; S, 19.75. Found: C, 59.12; H, 6.30; S, 19.62%.

PMR(CCl_4) of 64b: 1.50 - 2.50 (m, 6H, 3X CH_2), 3.10 (s, 4H, $-\text{S}(\text{CH}_2)_2-\text{S}-$), 3.33 (s, 3H, OMe), 3.40 (s, 3H, OMe), 6.00 (s, 2H, aromatic).

Reduction of ketone (64a) to 65

To a stirred solution of 64a (2.8 g, .009 mole) in 50 ml of dry methanol, at room temp. NaBH_4 (1.4 g, .036 mole) was gradually added portionwise in small quantities. The resultant mixture was further stirred for 24 hr at the same temp. The solvent was removed under reduced pressure and to the semi-solid so obtained, water (60 ml) was added. It was extracted with methylene chloride (4 x 25 ml), dried over anhydrous Na_2SO_4 , and evaporated under reduced pressure to afford the reduced product (65) (2.8 g, quantitative yield) which being sufficiently pure (as indicated by TLC

and PMR spectrum) was immediately used for the next step.

m.p. 98-100°. PMR (CCl₄): 1.93 (s, 3H, CH₃), 2.00 - 2.90 (m, 5H, CH and 2X CH₂), 3.23 (s, 4H, -S(CH₂)₂S-), 3.73 (s, 3H, OMe), 3.80 (s, 3H, OMe), 5.23 (m, 1H, 1-H), 6.50 (s, 2H, ArH). IR (Nujol): 3300 cm⁻¹ (OH).

Preparation of 66

An acetone solution (50 ml) containing 65 (2.8 g, .0085 mole) and conc. HCl (0.5 ml) was stirred at room temp. for 24 hr. The acetone was then distilled off under reduced pressure and water (50 ml) was added to it. It was then extracted with methylene chloride (4 x 25 ml) and dried over anhydrous Na₂SO₄. Removal of solvent under reduced pressure and chromatography over silica gel column (5% acetone in pet. ether) gave 66 (2.0 g, 76%) as a crystalline solid. M⁺ 308; m.p. 95-97°. PMR (CCl₄): 2.0 (s, 3H, CH₃), 2.53 (m, 4H, 2X CH₂), 3.36 (s, 4H, -S-(CH₂)₂-S-), 3.76 (s, 6H, 2X OMe), 6.43 (s, 2H, ArH), 6.83 (bs, 1H, vinylic C-H).

Analysis: Calculated for C₁₆H₂₀O₂S₂: C, 62.33; H, 6.49; S, 20.77. Found: C, 62.18; H, 6.78; S, 20.64%.

2-Acetyl-5,8-dimethoxy-3,4-dihydronaphthalene (37)

Silver nitrate (4.6 g, .027 mole) was dissolved in 80% aqueous acetonitrile (40 ml) and to that N-chlorosuccinimide (3.20 g, .024 mole) was added with stirring at room temp. To the turbid solution thus obtained, the compound 66 (1.9 g,

.006 mole) in 80% aqueous acetonitrile (40 ml) was added dropwise and stirring continued for 20 minutes.

To the above reaction mixture, the following solutions were added: saturated aqueous solutions of Na_2SO_3 (3.0 ml), Na_2CO_3 (3.0 ml), NaCl (3.0 ml) and pet. ether (40 ml) and methylene chloride (40 ml). The contents were stirred for an additional 10 minutes; solid separated by filtration, washed thoroughly with methylene chloride (4 x 15 ml). The organic layer was separated from the combined washings and dried over anhydrous Na_2SO_4 . Removal of solvent under reduced pressure and chromatography of the residue over silica gel (5% acetone in pet. ether) afforded the title compound 37 (1.0 g, 73%) m.p. 102-103° (Lit.^{43,32} m.p. 104-105°, m.p. 102-103°). M^+ 232. PMR (CCl_4): 2.36 (s, 3H, CH_3), 2.60 (m, 4H, 2X CH_2), 3.76, 3.83 (2s, 6H, 2X OMe), 7.53, 7.76 (dd, $J = 9$ Hz, 2H, ArH), 7.70 (bs, 1H, vinylic C-H). IR (Nujol): 1660 cm^{-1} ($>\text{C}=\text{O}$).

Analysis: Calculated for $\text{C}_{14}\text{H}_{16}\text{O}_3$: C, 72.41; H, 6.89. Found: C, 72.36; H, 6.32%.

2-Acetyl-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (31)

A solution of the dihydronaphthalene 37 (1.0 g, .004 mole) in dry ethanol (25 ml) and 10% Pd/C (.100 g) was hydrogenated at room temp. and 20 psi with mechanical shaking for 5 hr. The catalyst was filtered, washed with a ethyl alcohol (10 ml)

and the combined alcohol solution evaporated to dryness under reduced pressure. The colourless residue was crystallised from ethyl alcohol and petroleum ether (2:1) to give the monoketone (31) [0.9 g, 90%], m.p. 82-83° (lit.²⁹ m.p. 81-82°). IR (CHCl₃): 1710 cm⁻¹ (C=O). M⁺ 234. PMR (CDCl₃): 2.20 (s, 3H, COCH₃), 2.20 - 3.20 (m, 7H, 3X CH₂ and C-H), 3.73 (s, 6H, 2X OMe), 6.46 (s, 2H, ArH).

Analysis: Calculated for C₁₄H₁₈O₃: C, 71.79; H, 7.69. Found: C, 71.80; H, 7.72%.

2-Acetyl-5,8-dimethoxy-1,2,3,4-tetrahydro-2-naphthol (9)

The acetyl tetralin (31) [0.57 g, 2.43 m.mole] in dry t-butyl alcohol, (5 ml) was added to an oxygen saturated solution of potassium t-butoxide in t-butanol [prepared from potassium (0.8 g) and t-butyl alcohol (40 ml)]. Dry oxygen was bubbled into the solution through a gas dispersion tube, for 5 min. while keeping the reaction temp. at 35°. The solution was then cooled to 0° and made acidic with gl. acetic acid. Zinc dust (2.0 g) was added immediately and solution stirred at room temp. for 6 hr. Zinc dust was removed by filtration and the filtrate evaporated under reduced pressure. The residue was diluted with water (50 ml) and extracted with chloroform (4 x 25 ml). The combined extracts were dried over anhydrous Na₂SO₄, solvent distilled off under reduced pressure and the residue crystallized from

methanol-ether (2:1) to give the hydroxy ketone 9
(0.364 g, 60%) as colourless crystalline needles, m.p. 99°
(lit.²⁹ m.p. 97°), M^+ 250. PMR (CDCl_3): 1.85 (t, 2H,
3- CH_2 -), 2.30 (s, 3H, COCH_3), 2.76 (t, 2H, ArCH_2), 2.80 (bs,
2H, ArCH_2), 3.40 (s, 1H, OH; D_2O exchangeable), 3.66 (s,
3H, OCH_3), 3.73 (s, 3H, $-\text{OCH}_3$), 6.60 (s, 2H, ArH). IR (CHCl_3):
 1685 cm^{-1} ($>\text{C}=\text{O}$) and 3200 cm^{-1} (OH).

Analysis: Calculated for $\text{C}_{14}\text{H}_{18}\text{O}_4$: C, 67.18; H, 7.25;
Found: C, 66.99; H, 7.16%.

2-Bromo-1,4-dimethoxynaphthalene (68)

To a stirred solution of 1,4-dimethoxynaphthalene (56) (1.8 g, .0096 mole, prepared from 1,4-naphthaquinone by reduction with $\text{Na}_2\text{S}_2\text{O}_4$ followed by conventional methylation using dimethyl sulphate and K_2CO_3 in boiling acetone) in CHCl_3 (10 ml) containing iron filings (25 mg) was added dropwise a solution of bromine (1.53 g, .0096 mole) in CHCl_3 (15 ml) at room temp. while a slow stream of N_2 was passed through the solution.

Stirring was continued for 30 minutes after addition was complete and then N_2 was bubbled rapidly through the solution to expel HBr. The solution was filtered and poured into H_2O (50 ml). The CHCl_3 layer was separated, washed with 10% KOH solution (2 x 50 ml), dried over anhydrous Na_2SO_4 and solvent removed in vacuum.

Crystallization of the crude residue from MeOH afforded 2-bromo-1,4-dimethoxynaphthalene (68) (2.28 g, 90%), m.p. 57° (lit.⁴⁴ m.p. $58-59^\circ$). PMR (CCl_4): 3.83, 3.92 (s, 3H, OMe) 6.73 (s, 1H, 3-H), 7.36 (m, 2H, 6,7-H), 7.93 (m, 2H, 5,8-H).

2-Bromo-6-acetyl-1,4-dimethoxynaphthalene (69)

To a stirred solution of 2-bromo-1,4-dimethoxynaphthalene (68) [0.5 g, .0018 mole] and freshly powdered AlCl_3 (0.55 g, .0039 mole) in ethylene dichloride (25 ml) was added acetic anhydride (0.23 g, .0021 mole). The reaction

mixture was warmed at 60° for 6 hr and then poured over crushed ice (50 g) containing conc. HCl (10 ml). The organic layer was separated, washed with water (2 x 50 ml), dried over anhydrous Na₂SO₄ and solvent removed under reduced pressure. Purification of the crude product over silica gel column using 2% acetone in benzene as the eluent afforded [0.406 g, 70%] of the title compound (69). m.p. 97°; M⁺ 308, 310. PMR (CDCl₃): 2.63 (s, 3H, COCH₃), 3.83 (s, 3H, OMe), 3.86 (s, 3H, OMe), 6.66 (s, 1H, 3-H), 7.78 (m, 2H, 7,8-H), 8.38 (d, J = 2 Hz, 1H, 5-H).

Analysis: Calculated for C₁₄H₁₃O₃Br: C, 54.37; H, 4.21; Br, 25.89; Found: C, 54.20; H, 4.18; Br, 25.71%.

2-Acetyl-5,8-dimethoxynaphthalene (44)

2-Bromo-6-acetyl-1,4-dimethoxynaphthalene (69) (0.2 g, .0006 mole) in dry ethyl acetate (15 ml) containing 5% Pd/C (50 mg) was hydrogenated at room temp. and atmospheric pressure with mechanical shaking for 20 hr. The catalyst was filtered, washed with ethyl acetate (10 ml) and the combined solution evaporated to dryness under reduced pressure. Crystallisation of the residue from benzene:pet.ether (2:1) gave 2-acetyl-5,8-dimethoxynaphthalene (44) [.118 g, 80%] as yellow needles, m.p. 110° [lit.³⁵ m.p. 111-112°]. M⁺ 230. PMR (CDCl₃): 2.63 (s, 3H, COCH₃), 3.83 (s, 3H, OMe), 3.86 (s, 3H, OMe), 6.46 (s, 2H, 6,7-H), 7.77 (dd, J = 9 and 2 Hz, 1H, 3-H), 8.00

(d, $J = 9$ Hz, 1H, 4-H), 8.60 (d, $J = 2$ Hz, 1H, 1-H).

Analysis: Calculated for $C_{14}H_{14}O_3$: C, 73.02; H, 6.13;
Found: C, 73.07; H, 6.13%.

2-Acetyl-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (31)

To a stirred and cooled (-33°) solution of 2-acetyl-5,8-dimethoxynaphthalene (44) [1.0 g, .004 mole] in liquid ammonia (50 ml) was added potassium metal (1.35 g, 8 g. atom) in portions over a period of 10 minutes. After stirring for 30 minutes at -33° , the reaction was quenched by adding absolute ethanol. The ammonia was evaporated, water added and the precipitated solid was filtered, washed thoroughly with water, dried and crystallized from ethanol: pet. ether (2:1) mixture to give 2-acetyl-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (31) [0.76 g, 75%] as colourless needles, m.p. $81-82^\circ$ [lit.²⁹ m.p. $81-82^\circ$]. M^+ 234. IR:($CHCl_3$): 1710 cm^{-1} ($>C=O$). PMR ($CDCl_3$): 2.20 (s, 3H, $COCH_3$), 2.20 - 3.20 (m, 7H, 3X CH_2 and C-H), 3.73 (s, 6H, 2X OMe), 6.46 (s, 2H, ArH).

(+)-4-demethoxy-7-deoxydaunomycinone (7)

An intimate mixture of hydroxyketone 9 [0.25 g, .001 mole], phthalic anhydride (0.25 g, .0016 mole), $AlCl_3$ [2.5 g] and NaCl (0.5 g) was heated at 180° for 5 min. and the resultant reddish mass was treated with a saturated solution of oxalic acid (25 ml) to give a red coloured solid. It was filtered, washed with water, dried and chromatographed on a

silica gel column using 2% acetone in benzene as eluent to give (+)4-demethoxy-7-deoxydaunomycinone (7) (0.245 g, 70%) as reddish plates m.p. 212-215° (lit.³⁸ m.p. 210-212°).

(+)4-Demethoxy-7-deoxydaunomycinone-dimethyl ether (67)

4-Demethoxy-7-deoxydaunomycinone (7) [0.180 g, 0.5 m.mole], dimethyl sulphate (1 ml) and potassium carbonate (2.0 g) in acetone (25 ml) was refluxed for 12 hr. Usual work up, followed by crystallization of the crude residue from methanol afforded the dimethyl ether 67 [0.185 g, 95%] as yellow crystalline material. m.p. 184-186° [lit.⁴⁷ m.p. 184-186°]. M^+ 380. PMR(CDC1₃) 1.90 (t, 2H, -CH₂), 2.33 (s, 3H, -COCH₃), 3.09 (broad s, 4H, 2X CH₂), 3.77 (s, 1H, 6H, D₂O exchangeable), 3.80 (s, 3H, 1X OMe), 3.83 (s, 3H, 1X OMe), 7.53 (m, 2H, 2,3-ArH), 8.00 (m, 2H, 1,4-ArH). IR (Nujol): 3350 cm⁻¹ (-OH) and 1700 cm⁻¹ (>C=O), 1670 (quinone).

Analysis: Calculated for C₂₂H₂₀O₆: C, 69.46; H, 5.30.
Found: C, 69.64; H, 5.43%.

(+)4-Demethoxydaunomycinone (2d)

4-Demethoxy-7-deoxydaunomycinone (7) [.050 g, 0.145 m.mole] was dissolved in carbon tetrachloride (100 ml) containing catalytic amount of 2,2'-azobisisobutyronitrile (AIBN) and nitrogen was bubbled through the solution. Bromine (0.150 g, 0.85 m.mole) in CCl₄ (25 ml) was added dropwise over a period of 3 hr, while irradiating the reaction mixture with a sun lamp.

The mixture was cooled, washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was treated with moist silica gel (1 g) in acetone (25 ml) for 2 hr, filtered and washed with acetone (25 ml). The filtrate was evaporated to dryness and extracted with chloroform (2 x 25 ml). The organic solvent was washed with water (3 x 25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was treated with trifluoroacetic ^{acid} anhydride (3 ml) at room temp. for 2 hr. The solvent was removed under reduced pressure, diluted with 10% aqueous sodium bicarbonate solution (20 ml) and extracted with chloroform (2 x 25 ml). It was washed with water (2 x 25 ml), brine (1 x 25 ml), dried over anhydrous Na_2SO_4 and finally distilled off under reduced pressure. Chromatography of the residue over a column of silica gel using 5% acetone in pet. ether afforded (+)4-demethoxydaunomycinone (2) [0.0132 g, 23%], m.p. 180-183° [lit.⁴⁸ m.p. 183-185°]. PMR (CDCl_3): 2.28 (m, 2H, 1X CH_2), 2.40 (s, 3H, COCH_3), 3.00 (m, 2H, 1X CH_2), 4.56 (s, 1H, 1X OH (D_2O exchangeable)), 5.39 (m, 1H, $-\text{C}-\underline{\text{H}}$), 7.84 (m, 2H, 2,3-ArH), 8.33 (m, 2H, 1,4-ArH), 13.29 (s, 1H, 1X OH), 13.90 (s, 1H, 1X OH).

Analysis: Calculated for $\text{C}_{20}\text{H}_{16}\text{O}_7$: C, 65.21; H, 4.38; Found: C, 65.17; H, 4.43%.

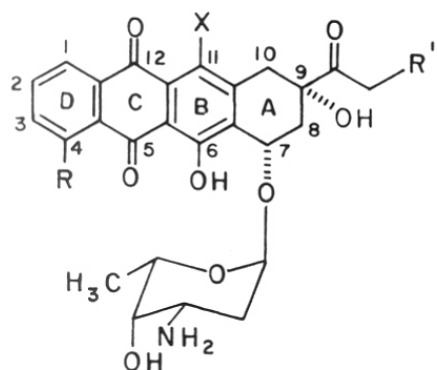
B) SYNTHESIS OF

(±)-4-DEMETHOXY-11-DEOXYDAUNOMYCINONE

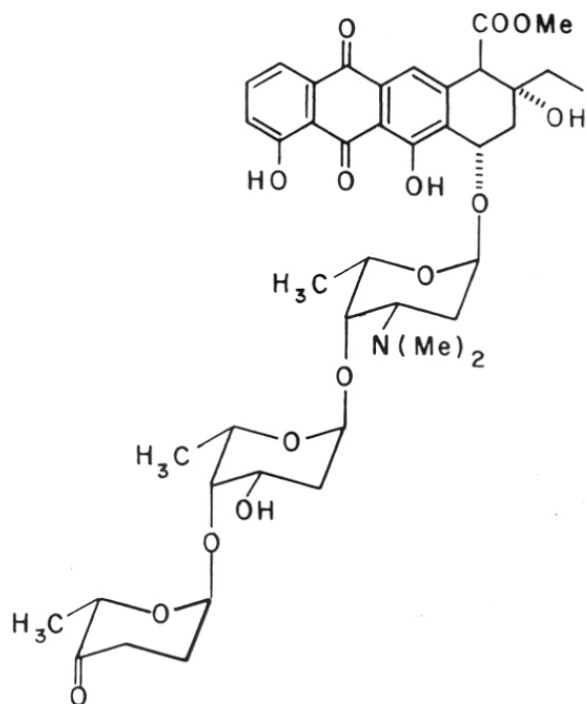
INTRODUCTION

Continuous search for new compounds that show decreased side effects and/or increased antitumour activity than daunomycin (1a), adriamycin (1b) and carminomycin (1c) has resulted in the isolation of a new group of anthracycline antibiotics lacking a hydroxyl group at the C-11 position (11-deoxydaunomycin (1e) etc.). These compounds were isolated from Micromonospora peucetica⁴⁹ related to aclacinomycin A. Clinical studies of aclacinomycin A⁵⁰ showed that it has a low incidence of cumulative dose dependent cardiomyopathy compared to the clinically important agent adriamycin (1b). In contrast to adriamycin, which interferes with DNA synthesis and function⁵¹, aclacinomycin A appears to preferentially inhibit RNA synthesis. The lack of an efficient biosynthetic process coupled with their therapeutic importance have attracted the attention of synthetic organic chemists to alleviate the scarcity of these drugs. Although many syntheses of daunomycinone (2a) have appeared in the literature, very few are reported for the 11-deoxydaunomycinone (2e) [aglycone of 11-deoxydaunomycin (1e)].

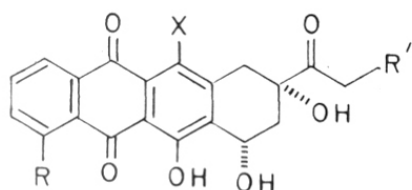
It will be appropriate to mention that synthetic anthracycline antibiotics are likely to be an exception to the general concept, that it is difficult to find a



- 1 a) $R = \text{OCH}_3$, $R' = \text{H}$, $X = \text{OH}$
 b) $R = \text{OCH}_3$, $R' = \text{OH}$, $X = \text{OH}$
 c) $R = \text{OH}$, $R' = \text{H}$, $X = \text{OH}$
 d) $R = \text{H}$, $R' = \text{H}$, $X = \text{OH}$
 e) $R = \text{OCH}_3$, $R' = \text{H}$, $X = \text{H}$
 f) $R = R' = X = \text{H}$



III



- 2 a) $R = \text{OCH}_3$; $R' = \text{H}$, $X = \text{OH}$
 b) $R = \text{OCH}_3$; $R' = \text{OH}$, $X = \text{OH}$
 c) $R = \text{OH}$, $R' = \text{H}$, $X = \text{OH}$
 d) $R = \text{H}$, $R' = \text{H}$, $X = \text{OH}$
 e) $R = \text{OCH}_3$, $R' = X = \text{H}$
 f) $R = R' = X = \text{H}$

totally synthetic analogue of an antibiotic produced through the natural process of fermentation⁸⁶, to be more effective in its pharmacological properties compared to the natural antibiotics. Recent findings indicate that 4-demethoxy-11-deoxydaunomycin (1f) is more effective compared to the natural 11-deoxydaunomycin (1e)⁵² and the results of its clinical trials are reported to be promising.

The first synthesis of 4-demethoxy-11-deoxydaunomycin (1f) was developed by Umezawa et al.⁵² starting from 5-methoxy-2-tetralone (70) (Scheme-25).

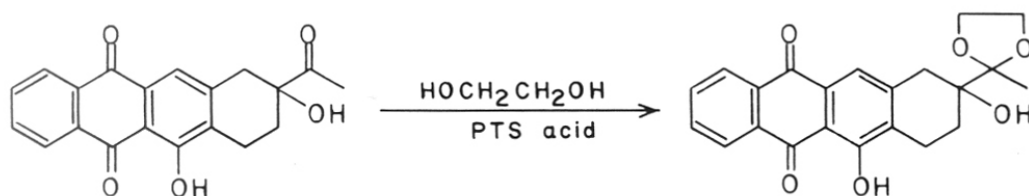
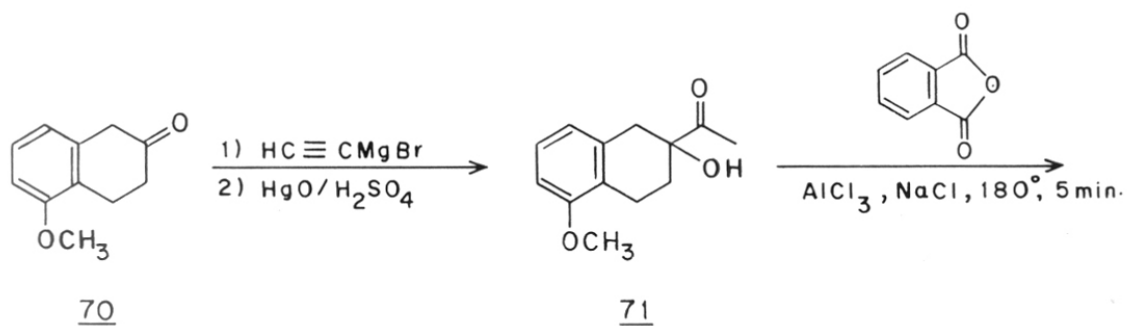
Earlier in this laboratory (+)4-demethoxy-11-deoxydaunomycinone (2f) has been synthesized by three different, yet efficient and flexible approaches.

In the first approach⁵³ (Scheme-26) the requisite AB synthon 73 (made from m-cresol in eight steps) was converted to 2-acetyl-5-hydroxy-8-bromo-1,2,3,4-tetrahydronaphthalene (74) by first protecting the acetyl ketone (ethylene glycol), followed by Wolf-Kishner reduction. 74 was then converted directly to 2-acetyl-1,2,3,4-tetrahydro-12-bromonaphthacene-6,11-dione (75) by fusing with an intimate mixture of phthalic anhydride, $AlCl_3:NaCl$ at 180° for 10 minutes and treating the resultant mass with cold dil. HCl (5%).

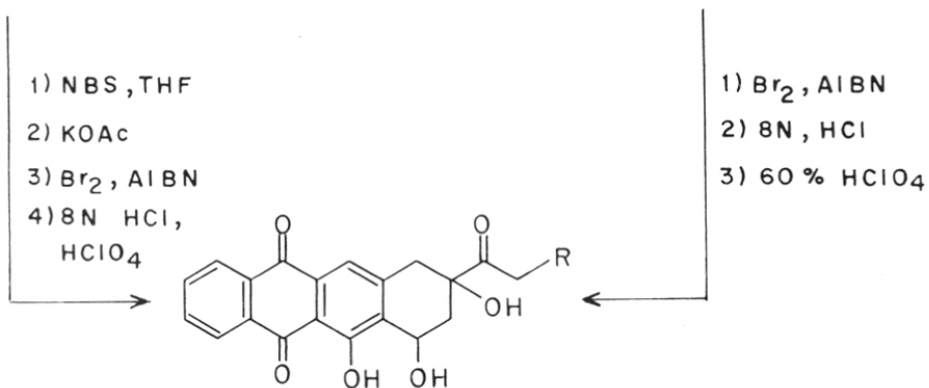
The bromo compound, 75 was dehalogenated by hydro-

SCHEME - 25

87



72



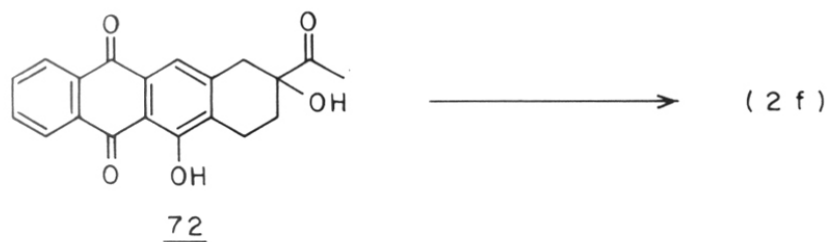
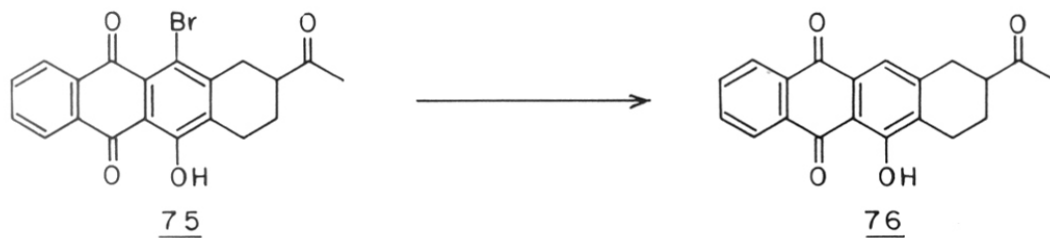
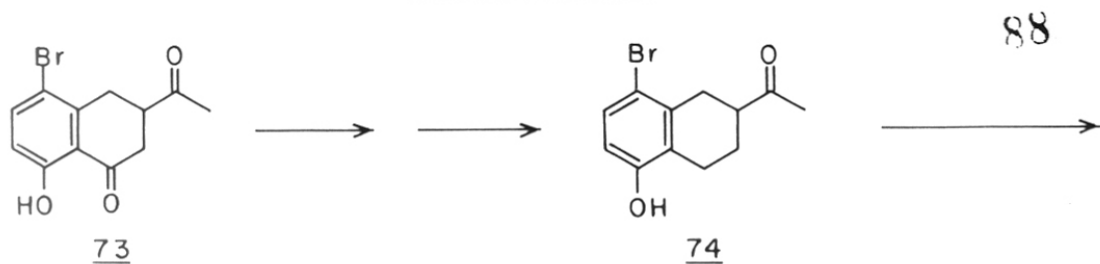
R = H (2f)

R = OH

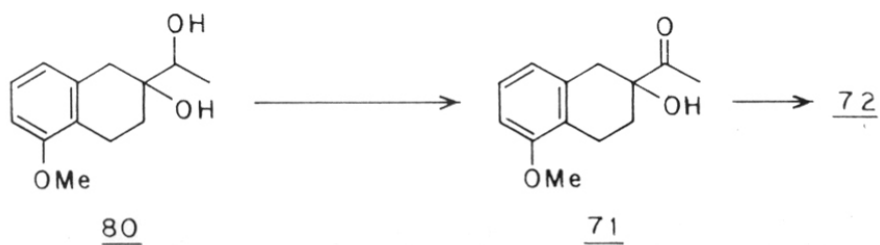
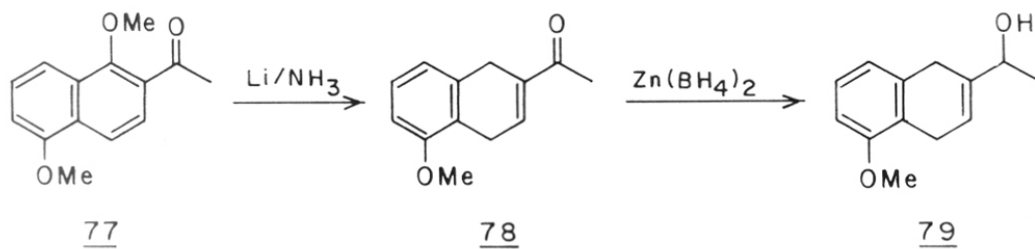


(1f)

SCHEME - 26



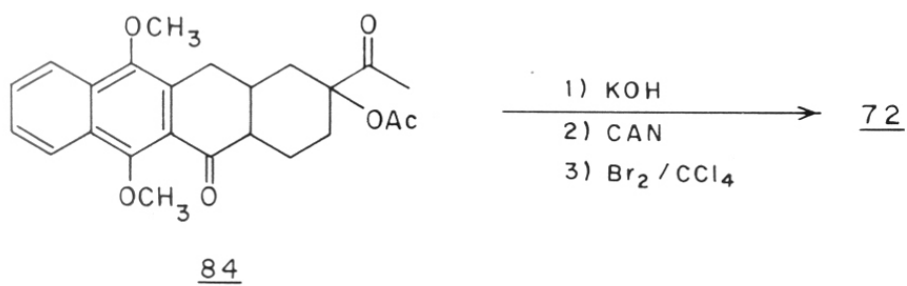
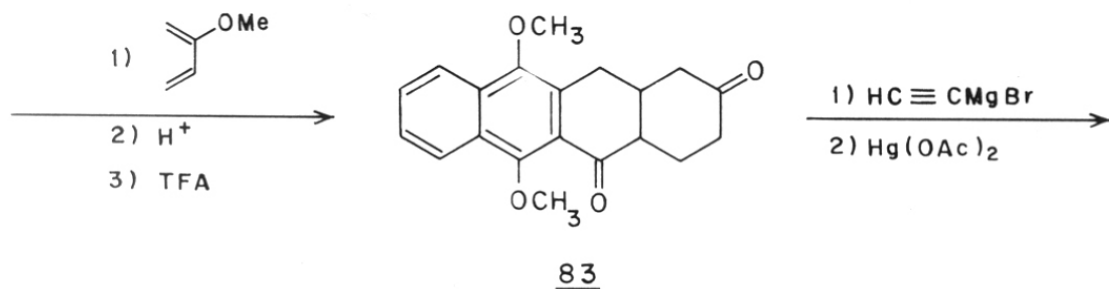
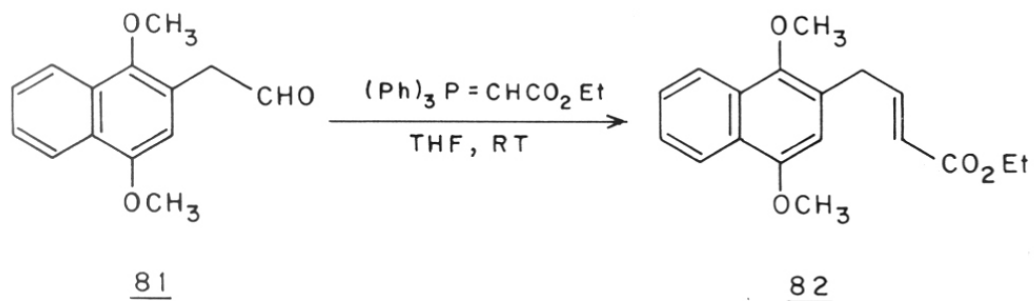
SCHEME - 27



genation to give 76. Hydroxylation at tertiary carbon (C-9) was accomplished by preparing the enol acetate (PTS acid and Ac_2O), followed by epoxidation and the resultant epoxy acetate was then treated successfully by base and acid to give (+)4-demethoxy-7,11-dideoxydaunomycinone (72) which was elaborated to (2f) according to the known procedure⁵⁴.

In the second approach⁵⁵ (Scheme-27) 2-acetyl-1,5-dimethoxynaphthalene (77) [made from 1,5-dihydroxynaphthalene] was reduced with Li/NH_3 to afford 2-acetyl-5-methoxy-1,4-dihydronaphthalene (78) as the major product. Reduction of 78 with $\text{Zn}(\text{BH}_4)_2$ gave 2-(hydroxyethyl)-5-methoxy-1,4-dihydronaphthalene (79). Epoxidation of 79 with m-chloroperbenzoic acid, followed by LAH reduction gave the diol (80). Selective oxidation of 80 with Fetizon's reagent gave 71, which on fusion with phthalic anhydride gave (+)4-demethoxy-7,11-dideoxydaunomycinone (72).

Another approach^{10a} (Scheme-28) constituted a Diels-Alder reaction. The aldehyde 81 [obtained in 4 steps from 1,4-dimethoxynaphthalene] on condensation with triphenylcarboethoxymethylene phosphorane afforded the α,β -unsaturated ester 82. Diels-Alder reaction of 82 with 2-methoxybutadiene, followed by acid work up and cyclization gave 83. Ethynylation of 83 followed by treatment with



mercuric acetate gave 84. Deacetylation of 84, followed by oxidation, bromination and aqueous work up gave 72.

PRESENT WORK

In spite of the fact that (+)4-demethoxy-11-deoxy-daunomycin (1f) is more effective in its pharmacological properties compared to the natural 11-deoxydaunomycin (1e)⁵² [constituting the so-called "Second generation Anthracycline Antibiotics"] much efforts have not been directed towards its total synthesis. This is clearly exemplified by the fact that only few approaches are known, (as discussed in the introductory part) most of which originated from this laboratory^{53,55}.

Since there is no possibility of obtaining 4-demethoxy-11-deoxydaunomycin (1f) by fermentation and its total synthesis from 4-demethoxy-11-deoxydaunomycinone (2f) is well established⁵², the main strategy is centred around the synthesis of 2f.

In the present study, the versatile method of Wong et al.²⁹ of AB + CD coupling was made use for assembling the tetracyclic system. This is also clear from Scheme-2 in which the transformation of naphthoquinone or anthraquinone derivative to the desired tetracyclic system requires many steps, whereas the substituted tetralin can be converted in a single step by fusion with a phthalic acid derivative.

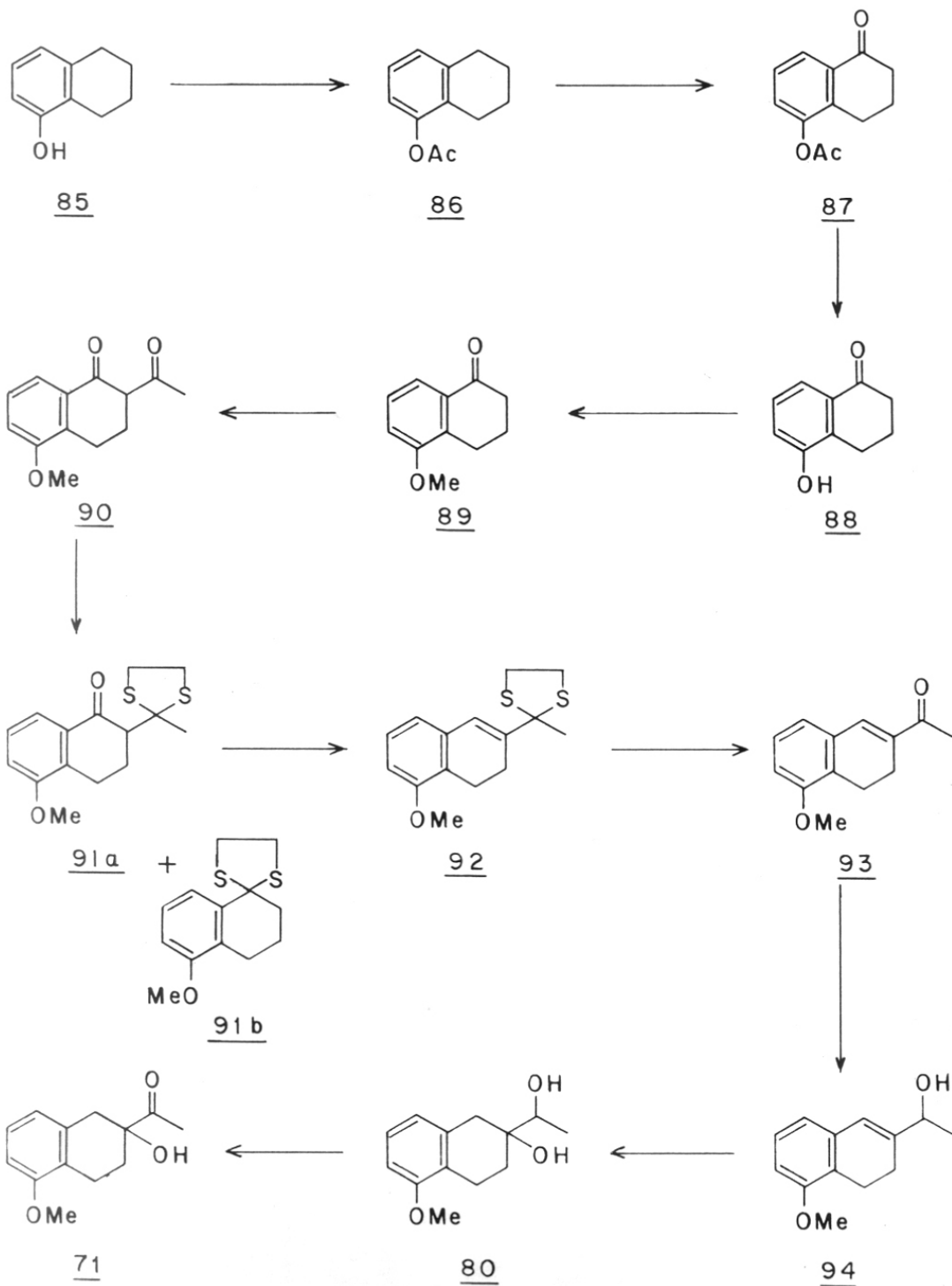
Thus much attention was focussed towards the

preparation of 2-acetyl-5-methoxy-1,2,3,4-tetrahydro-2-naphthol (71), an important intermediate representing AB rings, as it can be condensed by acylation with a phthalic acid derivative, corresponding to CD rings in tetracyclic system.

This part mainly deals with the synthesis of 71 by two new approaches, conceptually different from previous described ones, and its further elaboration to 2f.

In the first approach (Scheme-29) (analogous to Scheme-23) 5-hydroxy-1-tetralone (88) was made from α -naphthol by adopting the known procedure⁵⁶. Reduction of α -naphthol using Na/amy alcohol provided α -hydroxy tetralin (85) which was smoothly converted to α -acetoxy tetralin (86). Chromic acid oxidation of 86 gave 5-acetoxy-1-tetralone (87), which was hydrolysed under alkali conditions to provide 5-hydroxy-1-tetralone (88). Methylation of 88 by the conventional method using dimethyl sulphate and potassium carbonate in boiling acetone provided 5-methoxy-1-tetralone (89) in 90% yield m.p. 87° (lit.⁵⁷ m.p. 87-89°).

2-Acetyl-5-methoxy-1-tetralone (90) was made from 89 by condensing with BF_3 -etherate- Ac_2O (room temp. 2 hr) in 80% yield. m.p. 104-106°, M^+ 218. The PMR spectrum of 90 in CCl_4 (Fig.11) shows the presence of acetyl group as a



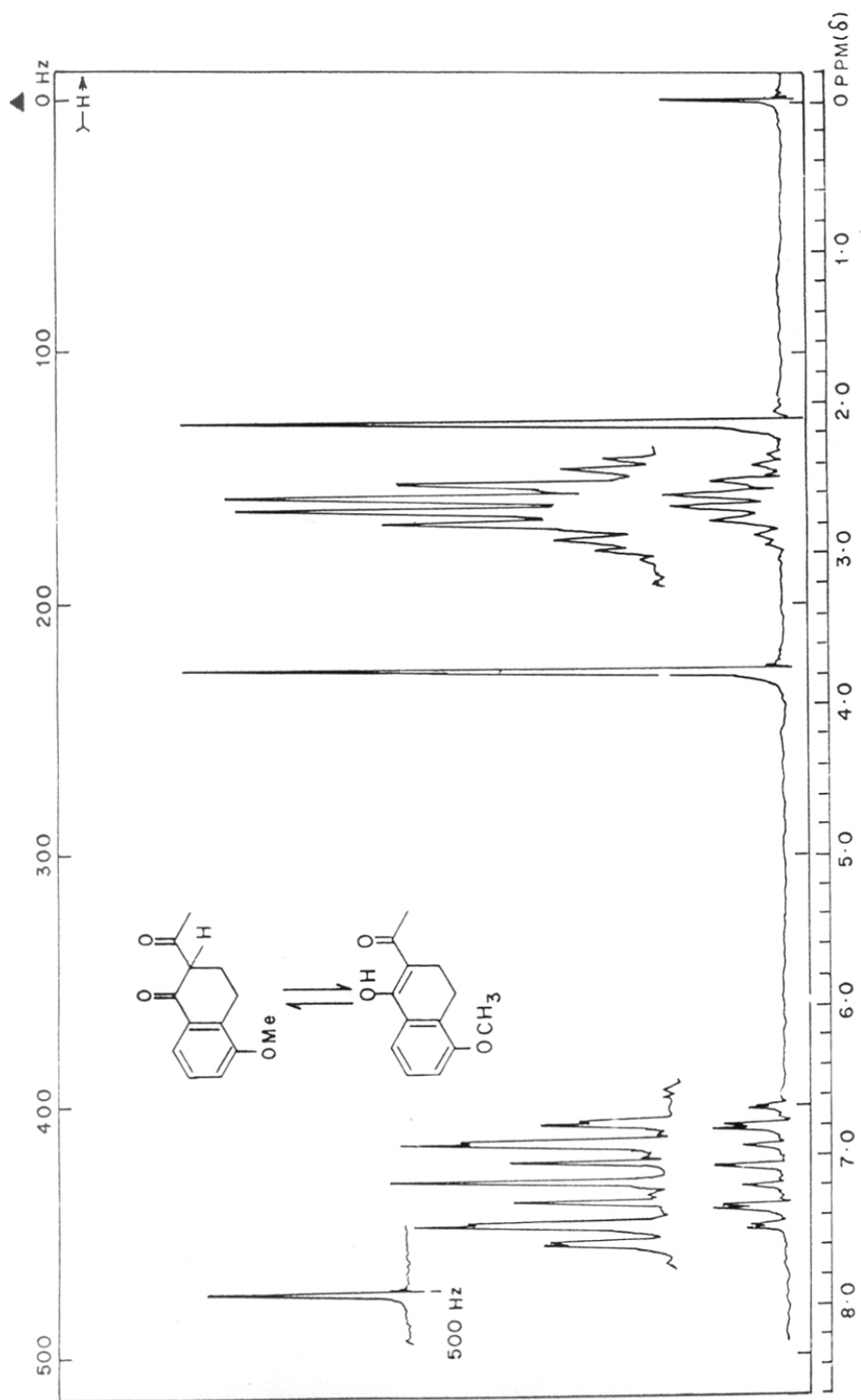


FIG. 11 PMR SPECTRUM OF COMPOUND 90 IN CCl_4

sharp singlet at 2.13. The methylene groups are extended as a multiplet from 2.3 - 2.9. The methoxyl appears as a singlet at 3.80. The aromatic protons are distributed as follows. H-6 as a doublet of doublet at 6.73 ($J = 8 \text{ Hz}$ and 2 H_3), H-7 as a triplet at 7.1 and H-8 as a doublet of doublet at 7.43 ($J = 8 \text{ Hz}$ and 2 H_3). The enolic proton appears as a singlet at 16.13. The acetyl ketone group was then selectively protected (HS-CH₂-CH₂-SH, HCl gas, CHCl₃, room temp. 10 hr) to afford the tetralone (91a) as the major product in 85% yield, m.p. 130-132°; along with a small amount of 91b (identified from PMR spectrum) which could be separated by column chromatography. The PMR spectrum of 91a in CCl₄ showed the methyl group as a sharp singlet at 1.86. The two methylenes and methine were extended as a multiplet from 2.0 - 3.1. A singlet at 3.23 represented the methylenes present in [-S-(CH₂)₂-S]. The methoxyl appeared as a singlet at 3.83. The aromatic protons resonated at expected chemical shifts.

Compound 91a was then subjected to reduction (NaBH₄, MeOH, reflux temp. 10 hr) followed by boiling in benzene with PTS acid to give 92 in 90% yield m.p. 90°, M^+ 278. The PMR spectrum of 92 in CDCl₃ showed the methoxyl and methyl groups as singlets at 3.81 and 2.00 respectively. The methylenes extended as a multiplet from 2.37 - 2.93. A

singlet at 3.37 represented the methylenes present in $-S-(CH_2)_2-S-$. The vinylic together with the aromatic protons appeared as a multiplet from 6.62 - 7.14. Dethio-ketalization of 92 (NCS, $AgNO_3$, 80% CH_3CN , room temp. 1 hr) gave the desired 2-acetyl-5-methoxy-3,4-dihydronaphthene (93) in 70% yield, m.p. 91-93°, M^+ 202. The PMR (CCl_4) of 93 [Fig.12] shows the presence of methyl and methoxyl groups as singlets at 2.36 and 3.83 respectively. The methylenes extend as a multiplet from 2.4 - 2.9. The vinylic proton appears as a singlet at 7.16 and the aromatic protons resonate at expected chemical shifts.

Compound 93 was then converted to 2-acetyl-2-hydroxy-5-methoxy-1,2,3,4-tetrahydronaphthalene (71) by the following sequence of reactions.

$NaBH_4$ reduction of 93 in MeOH (reflux 4 hr) gave 94 M^+ 204, as a semi-solid mass which was immediately put for the next step. The PMR of 94 in CCl_4 (Fig.13) shows a doublet at 1.26 corresponding to the methyl group. A broad singlet at 1.76 (D_2O exchangeable) represents the hydroxyl proton. The methylenes are extended as a multiplet from 2.1 - 2.8. The methoxyl appears as a singlet at 3.76. The quartet at 4.23 represents \underline{CH} proton. The aromatic protons together with the vinylic proton are extended as a multiplet from 6.1 - 7.1.

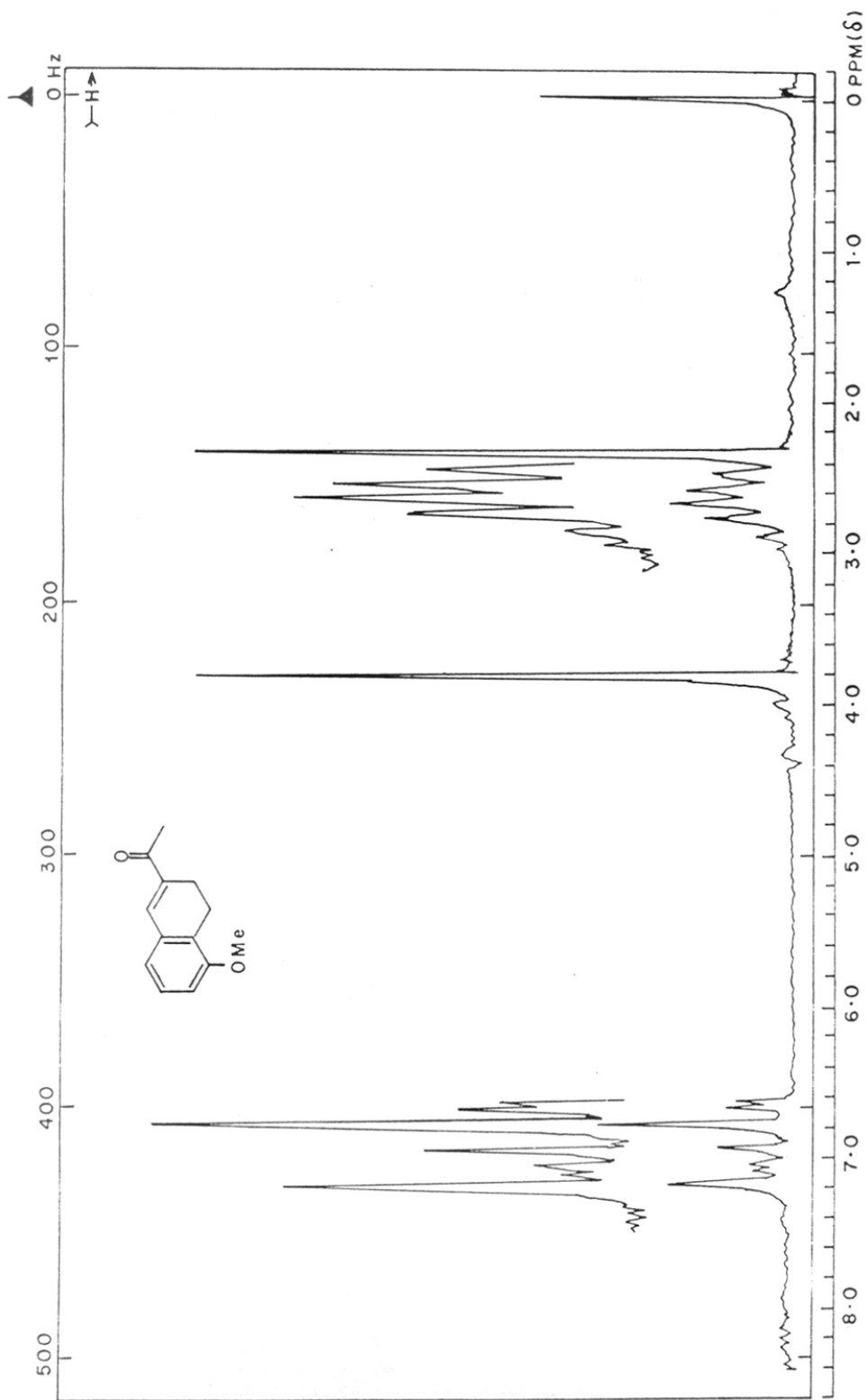


FIG. 12 PMR SPECTRUM OF COMPOUND 93 IN CCl₄

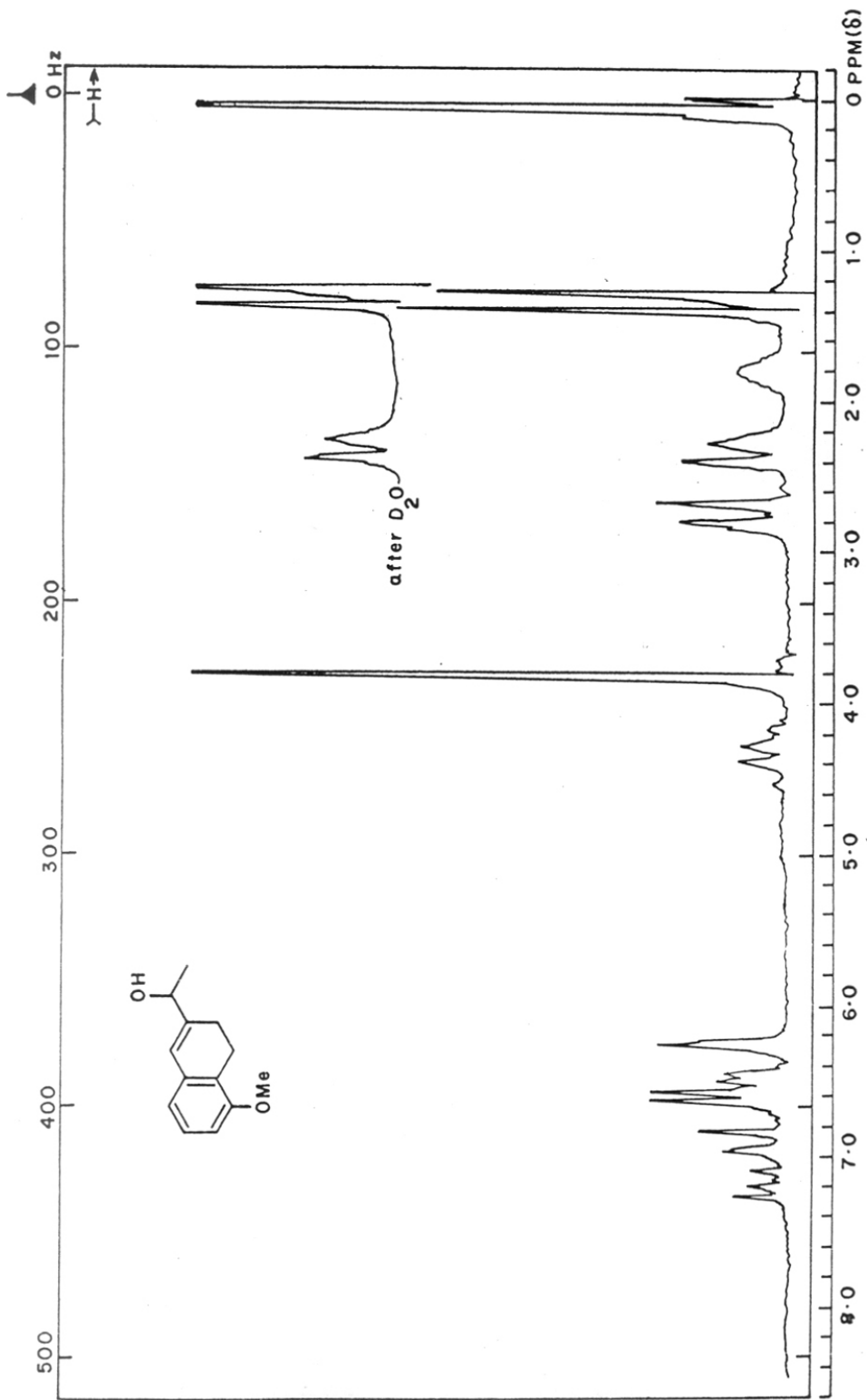


FIG. 13 PMR SPECTRUM OF COMPOUND 94 IN CCl_4

Compound 94 was subjected to epoxidation by the vanadium oxyacetylacetonate method using tert butyl hydroperoxide in benzene (2 hr) to give the epoxide, which was directly reduced (LAH, THF, room temp. 4 hr) to yield the diol (80) in 83%. m.p. 88-90°. M^+ 222. [Attempts to isolate the epoxide proved futile, and it became clear that the epoxide was highly unstable and hence should be used immediately for the next step viz. LAH reduction]. The PMR spectrum of 80 in $CDCl_3$ showed a doublet at 1.26 representing the methyl group. Both the hydroxyl protons appeared as a broad singlet at 2.10 (D_2O exchangeable). The multiplet at 1.83 represented a single methylene and that at 2.76 two methylenes. The $-CH-OH$ proton appeared as a quartet at 3.73. The methoxyl appeared as a singlet at 3.80. The multiplet from 6.46 - 7.13 accounted for the aromatic protons. IR (Nujol) showed stretching at 3420 cm^{-1} (OH).

The diol 80 was oxidised selectively using Fetizon's reagent⁵⁸ (benzene reflux 2 hr) to give 71 in 80% yield. m.p. 62-63° (lit.⁵² m.p. 63-65°). M^+ 220. The PMR spectrum of 71 in $CDCl_3$ [Fig.14] shows a multiplet centred at 1.76 representing a single methylene group. The other two methylenes are centred as a multiplet at 2.72. The remaining protons resonate at expected chemical shifts.

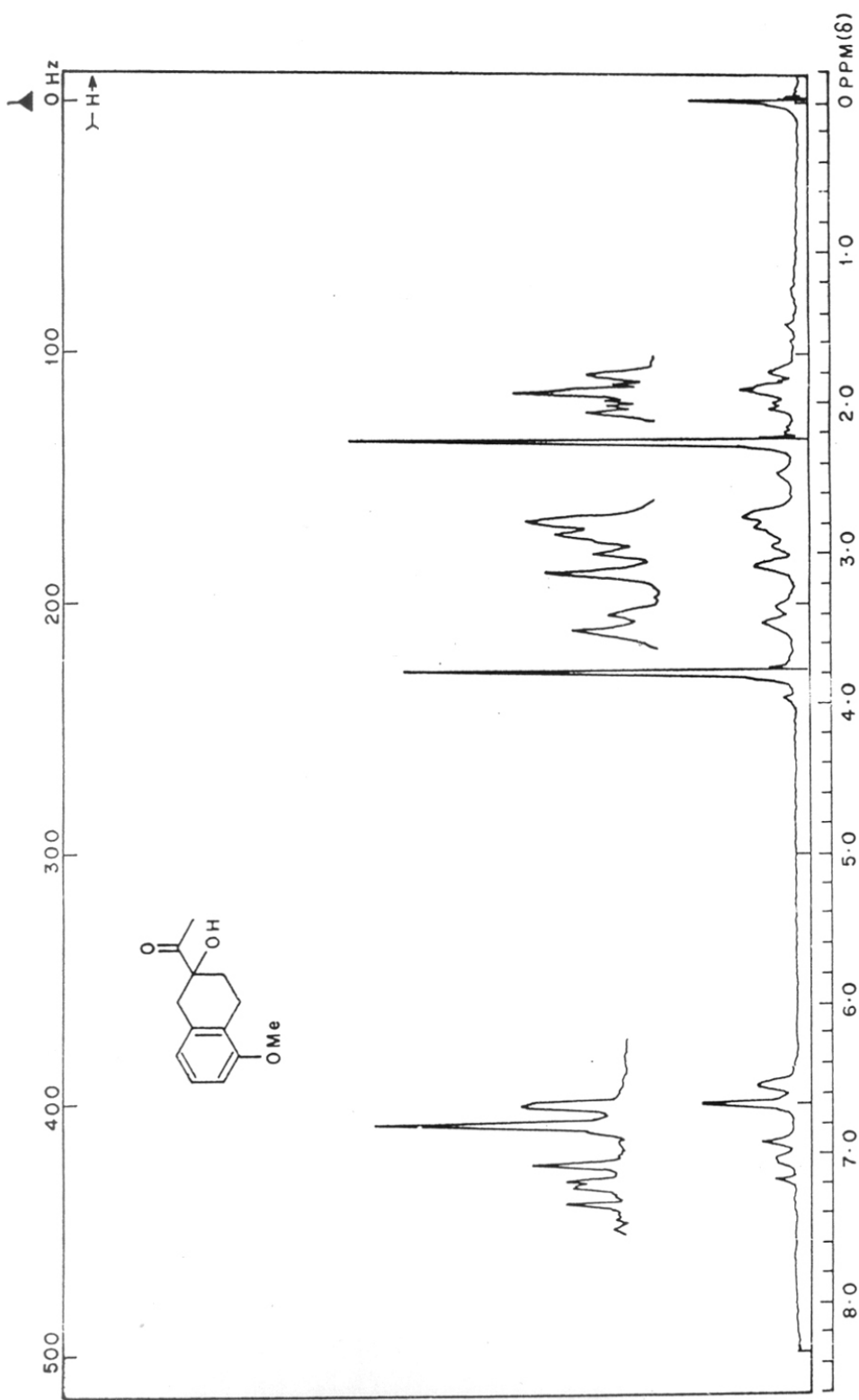


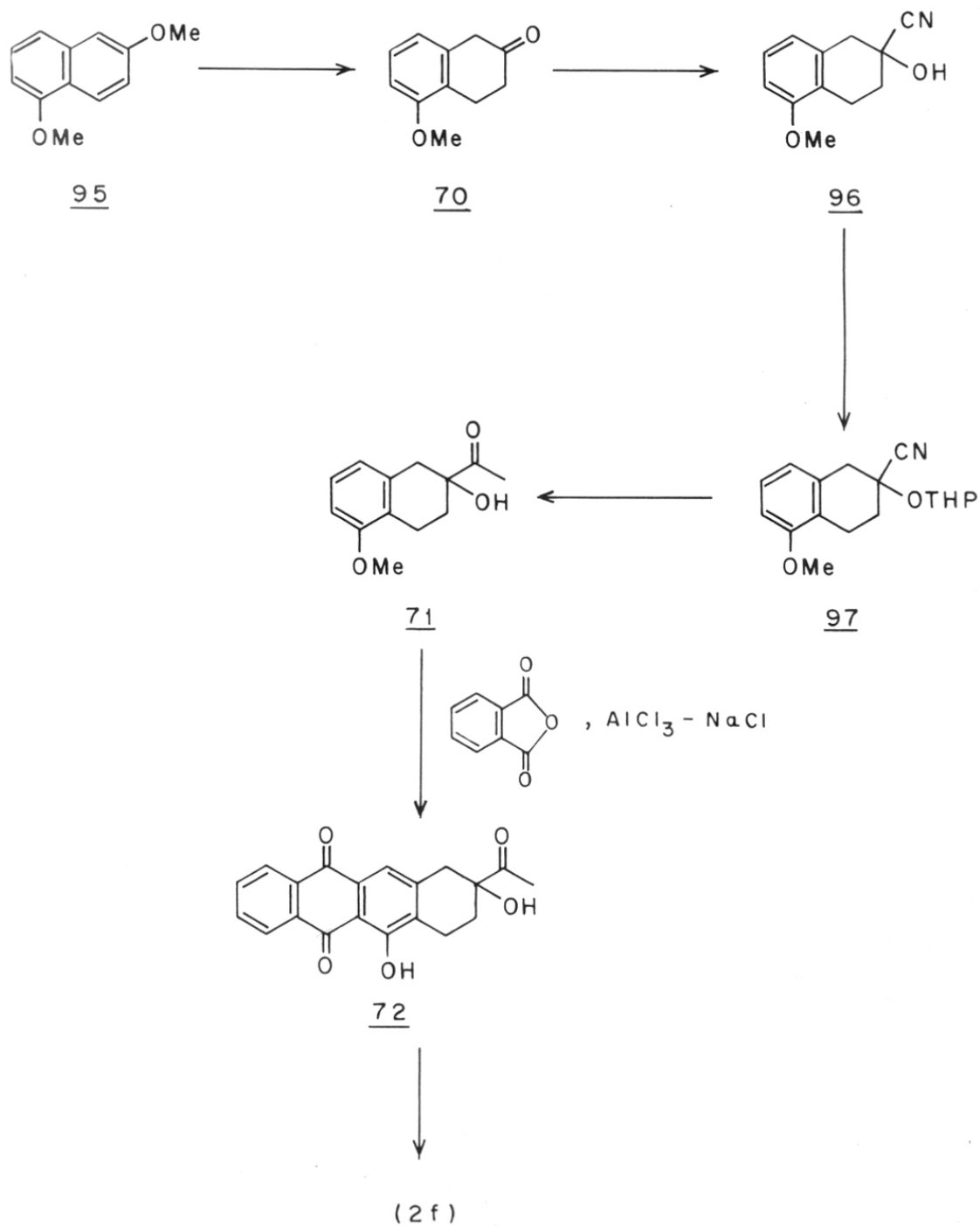
FIG. 14 PMR SPECTRUM OF COMPOUND **71** IN CDCl_3

In the second approach (Scheme-30) 71 was synthesized in a very simple manner by exploiting the ketone functionality in 5-methoxy-2-tetralone (70) for sidechain elaboration by carbon homologation.

The commercially available 1,6-dihydroxynaphthalene on methylation (by conventional method using dimethyl sulphate and potassium carbonate in boiling acetone) provided 1,6-dimethoxy naphthalene (95) in 90% yield. m.p. 60° (lit.⁵⁹ m.p. $60-61^{\circ}$).

Compound 95 was easily converted to 5-methoxy-2-tetralone (70) according to the known procedure⁶⁰ by heating with Na/alcohol. Treatment of 70 with KCN in $\text{CHCl}_3/\text{EtOH}$ (room temp. 8 hr) provided 2-cyano-2-hydroxy-5-methoxy-1,2,3,4-tetrahydronaphthalene (96) in 70% yield. m.p. 96° . M^+ 203. The PMR (CDCl_3) of 96 showed a singlet at 3.75 representing methoxyl group. A triplet at 2.13 represented one methylene (at 3-position) and the other two methylenes along with the hydroxyl proton extended as a multiplet from 2.73 - 3.30. The aromatic protons extended as a multiplet from 6.42 - 7.16. IR (Nujol) [Fig.15] shows stretching at 3400 cm^{-1} (OH) and 2250 cm^{-1} (CN).

Compound 96 was converted to its tetrahydropyranyl ether (97) in 86% yield. M^+ 287, whose structure was confirmed by PMR spectrum [Fig.16] in which all the protons



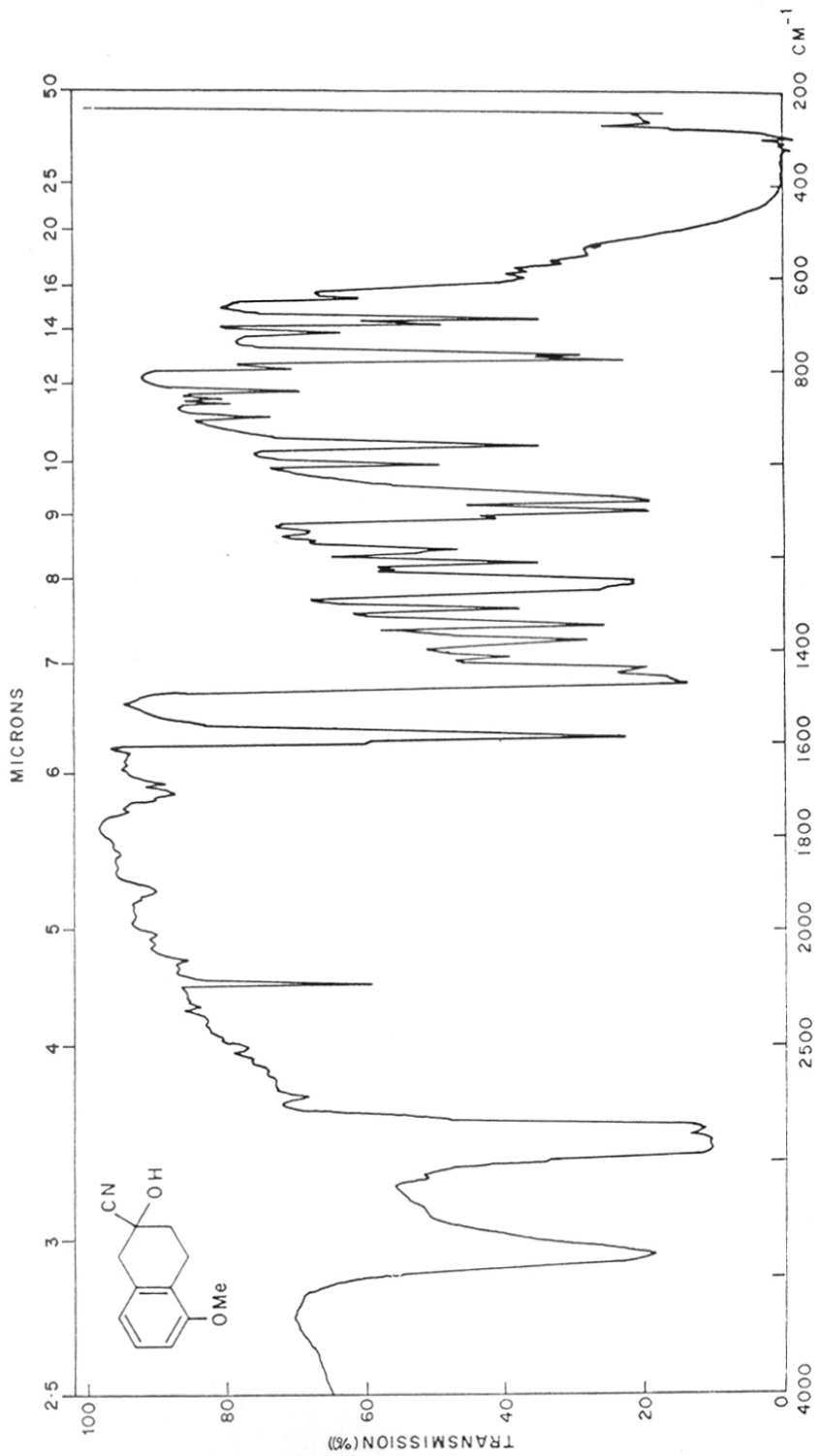
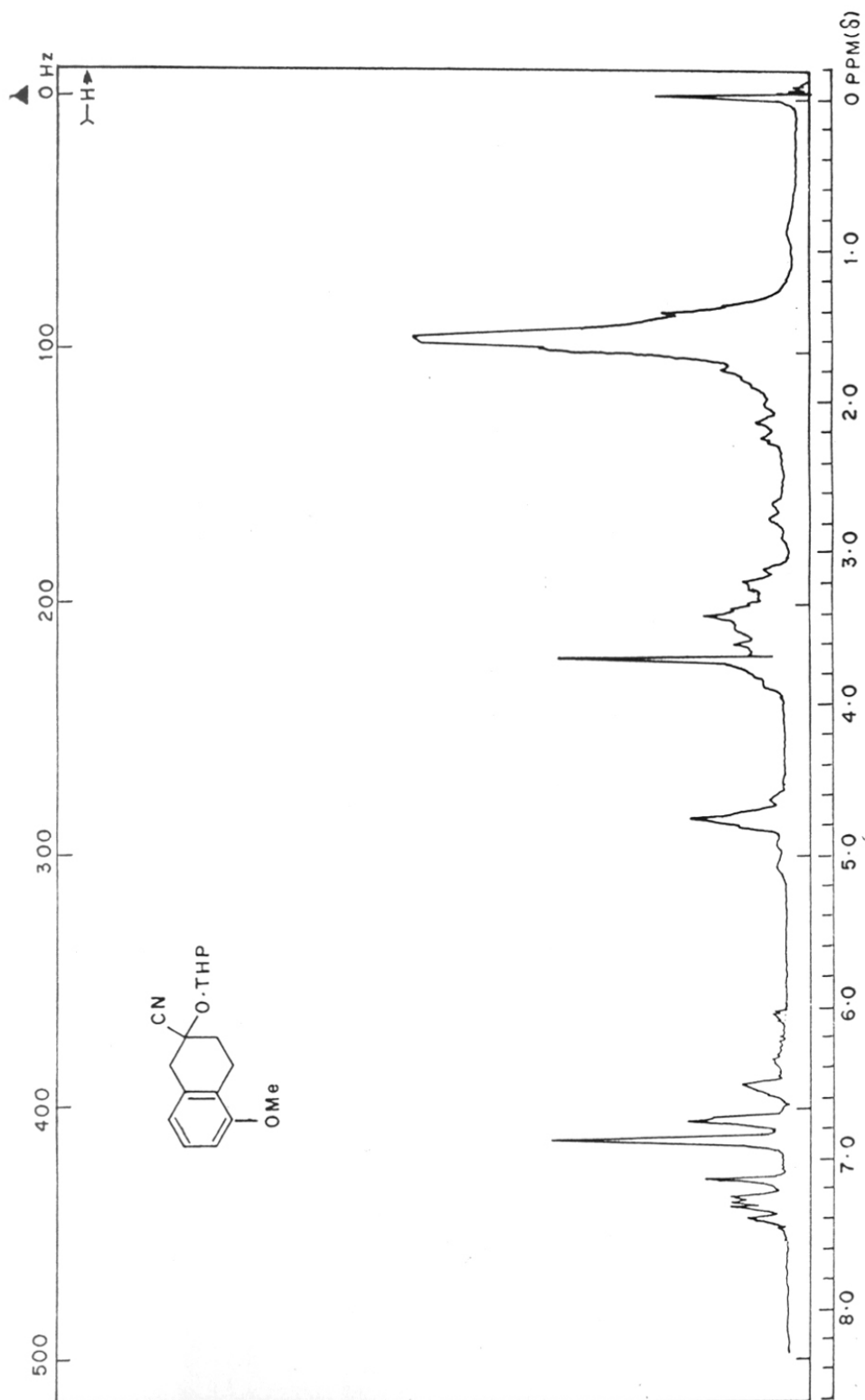


FIG. 15 IR SPECTRUM OF COMPOUND 96 IN NUJOL

FIG. 16 PMR SPECTRUM OF COMPOUND 97 IN CCl_4

resonated according to their expected chemical shifts. The IR (Nujol) showed stretching at 2250 cm^{-1} (CN). [There was absence of hydroxyl stretching].

Treatment of 97 with 5 moles excess of methyl magnesium iodide, followed by acid treatment provided 71 in 70% yield, which was identical in all respects with the authentic sample obtained from the first method.

71 was converted directly to (+)4-demethoxy-7,11-dideoxydaunomycinone (72) by fusion⁵² with an intimate mixture of phthalic anhydride, aluminium chloride-sodium chloride melt for 5 minutes, followed by treatment of the resultant reddish mass with a saturated solution of oxalic acid. Purification of the crude residue on silica gel column afforded 72 in 15% yield. m.p. 214° (decomp.) (Lit.⁵² m.p. 208-214 (decomp.)). M^+ 336. The PMR spectrum of 72 in CDCl_3 [Fig.17] shows a singlet at 2.37 for acetyl protons and a singlet at 3.67 (D_2O exchangeable) for C-9 hydroxy. The phenolic hydroxy was located at 13.00 as a singlet and the rest of the peaks were in full agreement with the assigned structure.

As the conversion of 72 to (+)4-demethoxy-11-deoxy-daunomycinone (2f) is well established⁵², the present synthesis in effect constitutes the total synthesis of 2f.

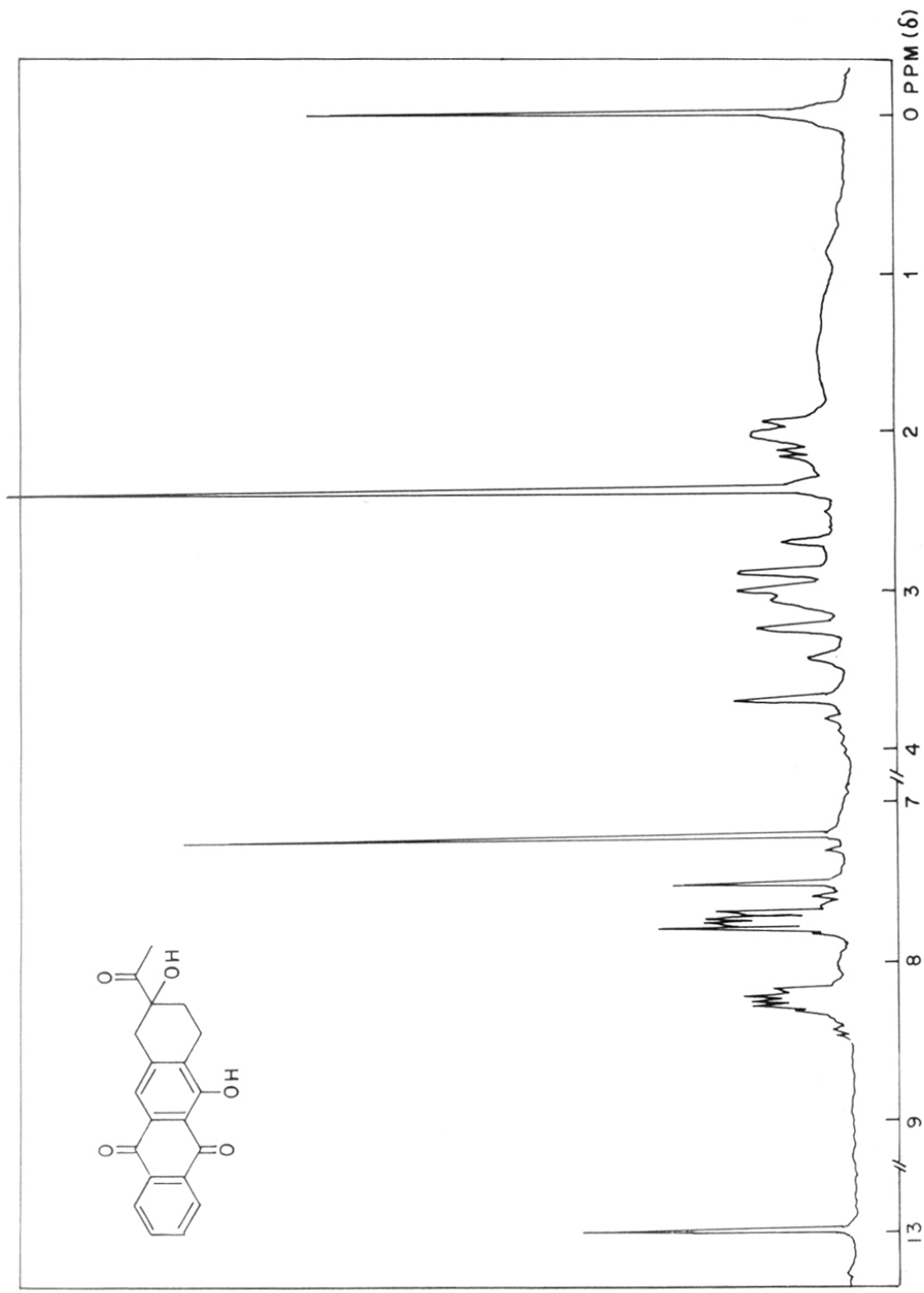


FIG. 17 PMR SPECTRUM OF COMPOUND 72 IN CDCl₃

E X P E R I M E N T A L

5,6,7,8-tetrahydro-1-naphthol (85)

Sodium (18.0 g) was added to a preheated solution of α -naphthol (10.0 g) in n-amyl alcohol (250 ml) (maintained at 150°C) with stirring. Addition was completed within 1 hr. After the complete addition, temp. of the reaction was raised to 160° and maintained there for another 2 hr. Later it was cooled, diluted with cold water (100 ml) and steam-distilled to remove the amyl alcohol. Aqueous alkaline solution was filtered, neutralised with conc. HCl and extracted with chloroform (3 x 50 ml). The organic layer was dried over anhydrous Na₂SO₄, and finally distilled under reduced pressure to yield 10 g (quantitative) of the title compound, 85 m.p. 68° (lit.⁶¹ m.p. 68.5 - 69°).

 α -Acetoxy tetralin (86)

To a solution of 30 g of α -hydroxy tetralin (85) in 100 cc of Ac₂O, 1 cc of conc. H₂SO₄ was added and allowed to stand overnight. After decomposing the excess anhydride with ice water (100 ml), the separated oily substance was extracted with ether (4 x 50 ml). The combined ethereal solution was washed successively with aqueous NaHCO₃ (3 x 50 ml) and H₂O (4 x 50 ml), dried over anhydrous Na₂SO₄ and concentrated. The residue was distilled in vacuo b.p. 119-21°

and recrystallised from MeOH to 2.5 plates of m.p. 40° (36 g, 93%) (lit.⁵⁶ m.p. $40-40.5^{\circ}$).

Analysis: Calculated for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42.
Found: C, 75.81; H, 7.48%.

5-Acetoxy-1-tetralone (87)

To a solution of 30 g of α -acetoxytetralin (86) dissolved in a mixture of 120 cc of AcOH and 90 cc of Ac_2O , solution of 43 g of CrO_3 dissolved in 150 cc of 90% AcOH was added with stirring during 1 hr at $35-40^{\circ}$. The reaction mixture was stirred for additional 5 hr and allowed to stand overnight. The mixture was diluted with H_2O (100 ml) and extracted with ether (4 x 50 ml). The combined ethereal solution was washed successively with Na_2CO_3 (3 x 50 ml) and H_2O (4 x 50 ml) and dried over anhydrous Na_2SO_4 . After removal of the solvent, the separated crystals were recrystallized from MeOH to afford 87 (18.0 g, 56%) as yellow needles. m.p. 127° (lit.⁵⁶ m.p. 127°).

Analysis: Calculated for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92; Found: C, 70.25; H, 5.80%.

5-Hydroxy-1-tetralone (88)

A solution of 18.0 g of 5-acetoxy-1-tetralone (87) dissolved in a mixture of 70 cc EtOH, 15 cc of H_2O and 12 g of NaOH, was refluxed for 15 minutes in N_2 atmosphere. After distillation of EtOH, the mixture was acidified with

10% HCl and the separated crystals were recrystallized from MeOH to afford 88 (11.0 g, 77%) as colourless plates, m.p. 204° [lit.⁵⁶ m.p. 204-205°].

5-Methoxy-1-tetralone (89)

5-Hydroxy-1-tetralone (88) [3.5 g, .022 mole] dimethyl sulphate (2.5 ml, .024 mole) and anhydrous K_2CO_3 (10 g) in dry acetone (100 ml) were refluxed on a steam bath for 6 hr. Usual work up, followed by crystallization (benzene) of the crude product gave 5-methoxy-1-tetralone (89) [3.4 g, 90%] as yellow needles. m.p. 88° [lit.⁵⁷ m.p. 85-87°].

Analysis: Calculated for $C_{11}H_{12}O_2$: C, 75.00; H, 6.8; Found: C, 75.12; H, 6.77%.

2-Acetyl-5-methoxy-1-tetralone (90)

A solution of 5-methoxy-1-tetralone (89) [2.5 g, .014 mole], acetic anhydride (25 ml) and BF_3 -etherate (7.5 ml) was stirred at room temp. for 2 hr. The reaction mixture was diluted with water (100 ml) and the yellow coloured complex was filtered and dissolved in methanol (50 ml). Saturated aqueous solution of sodium acetate (40 ml) was added and the contents refluxed on a steam bath for 2 hr. The solvent was distilled off under reduced pressure and the residue diluted with H_2O (100 ml). The oily material that separated was extracted with methylene chloride (2 x 50 ml) dried over anhydrous Na_2SO_4 and

finally concentrated. Purification over a silica gel column using 2.5% acetone in pet. ether as eluent gave 90 [2.5 g, 80%] as a pale yellow crystalline material. m.p. 104-106°, M^+ 218. PMR (CCl_4): 2.13 (s, 3H, COCH_3), 2.3 - 2.9 (m, 4H, 2X CH_2), 3.80 (s, 3H, OMe), 6.73 (dd, $J = 8$ Hz and 2 Hz), 1H, H-6), 7.1 (t, 1H, H-7), 7.43 (dd, $J = 8$ Hz and 2 Hz, 1H, H-8), 16.13 (s, 1H, enolic OH).

Analysis: Calculated for $\text{C}_{13}\text{H}_{14}\text{O}_3$: C, 71.55; H, 6.42; Found: C, 71.72; H, 6.38%.

Preparation of tetralone (91)

To a solution of 2-acetyl-5-methoxy-1-tetralone (90) [4.30 g, .019 mole] and 1,2-ethanedithiol [1.85 g, .019 mole] in dry chloroform (75 ml), under stirring at room temp. dry HCl was passed for 10 hr. The reaction mixture was successively washed with water (4 x 50 ml); 2% aqueous NaOH solution (3 x 50 ml) and brine (2 x 100 ml). The clear organic layer was dried over anhydrous Na_2SO_4 , distilled off under reduced pressure and chromatographed over silica gel column (2% acetone in pet. ether) to give 91b and 91a (4.90 g, 85%). m.p. 130-132°, M^+ 294. PMR (CCl_4) of 91b: 1.60 - 2.40 (m, 6H, 3X CH_2), 3.10 (s, 4H, $-\text{S}-(\text{CH}_2)_2-\text{S}$), 3.80 (s, 3H, OMe), 7.14 (m, 3H, ArH). PMR (CCl_4): 1.86 (s, 3H, CH_3), 2.0 - 3.1 (m, 5H, CH and 2X CH_2), 3.23 (s, 4H, $-\text{S}-(\text{CH}_2)_2-\text{S}-$), 3.83 (s, 3H, OMe), 6.83 (dd, $J = 8$ Hz and 2 Hz, 1H, H-6), 7.16

(t, 1H, H-7), 7.53 (dd, J = 8 Hz and 2 Hz, 1H, H-8).

Analysis: Calculated for $C_{15}H_{18}O_2S_2$: C, 61.22; H, 6.12; S, 21.76. Found: C, 61.60; H, 6.44; S, 21.52%.

Preparation of 92:

To a stirred solution of 91a (2.4 g, .008 mole) in 50 ml of dry methanol, at room temp. $NaBH_4$ (1.24 g, .032 mole) was gradually added portionwise in small quantities. The resultant mixture was further refluxed for 10 hr. The solvent was removed under reduced pressure and to the semi-solid so obtained, water (100 ml) was added. It was extracted with methylene chloride (3 x 25 ml), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. To the resultant residue in dry benzene (30 ml). p-toluenesulfonic acid (25 mg) was added and refluxed on a steam bath using Dean-Stark apparatus for 1 hr. The organic layer was washed with saturated $NaHCO_3$ solution (2 x 50 ml), H_2O (2 x 50 ml) and dried over anhydrous Na_2SO_4 . Solvent removal and purification of the crude residue over a silica gel column using 2% acetone in pet. ether provided 92 (1.9 g, 90% m.p. 90° , M^+ 278. PMR ($CDCl_3$): 2.00 (s, 3H, CH_3), 2.37 - 2.93 (m, 4H, 2X CH_2), 3.37 (s, 4H, $-S-(CH_2)_2-S-$), 3.81 (s, 3H, OMe), 6.62 - 7.14 (m, 4H, ArH and Ar-CH=).

Analysis: Calculated for $C_{15}H_{18}OS_2$: C, 64.74; H, 6.47; S, 23.02. Found: C, 64.67; H, 6.67; S, 23.24%.

2-Acetyl-5-methoxy-3,4-dihydronaphthalene (93)

AgNO_3 (4.6 g, .027 mole) was dissolved in 80% aqueous CH_3CN (40 ml) and to that NCS (3.20 g, .024 mole) was added with stirring at room temp. To the turbid solution thus obtained, the compound 92 (1.9 g, .007 mole) in 80% aqueous CH_3CN (40 ml) was added dropwise and stirring continued for 1 hr.

To the above reaction mixture, the following solutions were added: saturated aqueous solutions of Na_2SO_3 (3.0 ml), Na_2CO_3 (3.0 ml) and NaCl (3.0 ml); pet. ether (40 ml) and methylene chloride (40 ml). The contents were stirred for an additional 10 minutes, solid separated by filtration, washed thoroughly with methylene chloride (4 x 15 ml). The organic layer was separated from the combined washings and dried over anhydrous Na_2SO_4 . Removal of solvent under reduced pressure and chromatography of the residue over silica gel [5% acetone in pet. ether] afforded the title compound 93 [0.970 g, 70%] m.p. 91-93°, M^+ 202. PMR (CCl_4):

2.36 (s, 3H, Me); 2.4 - 2.9 (m, 4H, 2X CH_2); 3.83 (s, 3H, OMe), 6.66 (dd, $J = 8$ Hz and 2 Hz, 1H, H-6); 6.76 (t, 1H, H-7); 7.10 (dd, $J = 8$ Hz and 2 Hz, 1H, H-8), 7.16 (s, 1H, ArCH=).

Analysis: Calculated for $\text{C}_{13}\text{H}_{14}\text{O}_2$: C, 77.22; H, 6.9; Found: C, 77.04; H, 7.10%.

Preparation of 94

A mixture of 93 [0.74 g, .004 mole], NaBH_4 (.3 g) and dry MeOH (25 ml) was refluxed on a steam bath for 4 hr. The solvent was removed under reduced pressure and diluted with H_2O (50 ml). It was extracted with chloroform (2 x 25 ml) and dried over anhydrous Na_2SO_4 . Removal of solvent under reduced pressure provided 94 [0.8 g, quantitative] M^+ 204^o as a semi-solid which was immediately put for the next step.

PMR (CCl_4): 1.26 (d, $J = 7$ Hz), 3H, CH_3); 1.76 (bs, 1H, OH (D_2O exchangeable)); 2.1 - 2.8 (m, 4H, 2X CH_2); 3.76 (s, 3H, OCH_3); 4.23 (q, 1H, $>\text{CH}$), 6.1 - 7.1 (m, 4H, ArH and vinylic).

IR (Nujol): 3380 cm^{-1} (OH).

Preparation of diol (80)

To a solution of 94 [0.2 g, .0009 mole) in dry benzene (10 ml), vanadium oxyacetylacetonate (5.0 mg) was added and contents stirred under N_2 atmosphere at room temp. for 5 minutes. tert. butyl hydroperoxide was added gradually whereby the colour of the solution turned from bright yellow to red and the stirring continued (2 hr) till the colour became light yellow. Water (10 ml) was added and the contents further stirred for 5 minutes. The organic layer was separated, dried over anhydrous Na_2SO_4 and finally concentrated. The resultant residue in THF (5 ml) was added to a mixture of LAH (150 mg) suspended in THF (5 ml) under N_2 atmosphere,

at room temp. After stirring for 4 hr, the excess of LAH was decomposed by initial addition of ethyl acetate (10 ml), followed by successive addition of H₂O (1 ml), 15% aqueous NaOH (2 ml) and again H₂O (1 ml). The inorganic solid that separated was filtered, washed with ethylacetate (3 x 10 ml). The combined organic extracts were dried over anhydrous Na₂SO₄ and solvent removed under reduced pressure to yield the diol (80) [0.180 g, 83%] as colourless crystalline product, m.p. 88-90°. M⁺ 222. PMR (CDCl₃): 1.26 (d, J = 7 Hz, 3H, Me), 1.83 (m, 2H, CH₂); 2.10 (bs, 2H, 2X OH), 2.76 (m, 4H, 2X CH₂); 3.73 (q, 1H, -CHOH); 3.80 (s, 3H, OMe); 6.46 - 7.13 (m, 3H, ArH). IR (Nujol): 3420 cm⁻¹ (OH).

Analysis: Calculated for C₁₃H₁₈O₃: C, 70.27; H, 8.10; Found: C, 70.54; H, 8.22%.

Fetizon's reagent

This was prepared from activated celite powder, silver nitrate and sodium carbonate according to the known procedure⁵⁸.

(+)-2-acetyl-5-methoxy-1,2,3,4-tetrahydro-2-naphthol (71)

A mixture of 80 (.110 g, .0005 mole) Fetizon's reagent (Ag₂CO₃ over celite powder) (1.0 g) and dry benzene (20 ml) was refluxed on a steam bath for 2 hr. The contents filtered, residue washed with dry benzene (20 ml) and finally evaporated

to dryness. Purification of the crude residue over silica gel column using 15% ethyl acetate in pet. ether gave 71 [.080 g, 80%] as a crystalline material. m.p. 62-63° (lit.⁵² m.p. 63-65°). M^+ 220. PMR ($CDCl_3$): 1.76 (m, 2H, CH_2); 2.21 (s, 3H, $COCH_3$), 2.72 (m, 4H, 2X CH_2); 3.48 (bs, 1H, OH (D_2O exchangeable)); 3.80 (s, 3H, OMe); 6.3 - 7.1 (m, 3H, ArH). IR (Nujol): 3480 cm^{-1} (OH); 1715 cm^{-1} ($>C=O$).

Analysis: Calculated for $C_{13}H_{16}O_3$: C, 70.91; H, 7.27. Found: C, 70.60; H, 7.32%.

5-Methoxy-2-tetralone (70)

1,6-Dimethoxynaphthalene (95) [6.0 g, .032 mole] (prepared from 1,6-dihydroxynaphthalene by conventional methylation using dimethyl sulphate and K_2CO_3 in boiling acetone) was dissolved in 75 ml of dry absolute alcohol and refluxed for 30 minutes. 6.5 g of Na metal was added gradually within 15 minutes. After refluxing for further 30 minutes, the mixture was cooled, solvent removed under reduced pressure and diluted with 100 ml of H_2O . Resultant mixture was heated on a water bath for 15 minutes, cooled, acidified with conc. HCl, and extracted with chloroform. It was dried over anhydrous Na_2SO_4 and solvent removed under reduced pressure. Purification over silica gel column using 2% acetone in pet. ether as the eluent provided 70 [4.0 g, 72%] as a colourless oily substance. M^+ 176.

PMR (CCl₄): 2.40 (t, 2H, 3-H); 3.10 (t, 2H, 4H); 3.50 (s, 2H, 1H), 3.93 (s, 3H, OMe); 6.70 - 7.56 (m, 3H, ArH).

Analysis: Calculated for C₁₁H₁₂O₂: C, 75.01; H, 6.81. Found: C, 74.96; H, 6.79%.

2-Cyano-2-hydroxy-5-methoxy-1,2,3,4-tetrahydronaphthalene (96)

5-Methoxy-2-tetralone (70) [2.8 g, 16 m.mole] and potassium cyanide [15.0 g, 230 m.mole] were placed in chloroform (375 ml)/Ethanol (113 ml) and cooled to 0°. Acetic acid (23 ml) was added over 10 minutes. The reaction mixture was diluted with ethanol (75 ml) and stirred at 25° for 8 hr. The mixture was diluted with water (150 ml), organic layer separated, dried over anhydrous Na₂SO₄ and solvent removed under reduced pressure. Purification on silica gel column using 5% acetone in pet. ether as eluent afforded 96 (1.9 g, 70%) as colourless crystalline product m.p. 96°. M⁺ 203. IR (Nujol): 3400 cm⁻¹ (OH), 2250 cm⁻¹ (CN). PMR (CDCl₃) 2.13 (t, 2H, 3-CH₂), 2.73 - 3.30 (m, 5H, OH and 2X CH₂), 3.75 (s, 3H, OMe), 6.42 - 7.16 (m, 3H, ArH).

Analysis: Calculated for C₁₂H₁₃NO₂: C, 70.96; H, 6.40; N, 6.98. Found: C, 71.42; H, 6.57; N, 7.4%.

2-Cyano-5-methoxy-2-(2'-tetrahydropyranloxy)-1,2,3,4-tetrahydronaphthalene (97)

The above cyanohydrin 96 [1.5 g, 7.38 m.mole], freshly

distilled dihydropyran (15 ml), chloroform (25 ml) and PTS-acid (2.0 mg) were stirred at room temp. for 6 hr. The reaction mixture was diluted with water (25 ml), extracted with chloroform (2 x 20 ml) washed with saturated NaHCO_3 solution (3 x 25 ml) and H_2O (2 x 50 ml). The organic layer was dried over anhydrous Na_2SO_4 and finally concentrated under reduced pressure. Purification of the crude product over a short alumina column using 2% acetone in pet. ether as the eluent gave 97 [1.8 g, 86%]. M^+ 287. PMR (CCl_4): 1.27 - 1.69 (m, 6H, 3X CH_2), 2.21 (m, 2H, CH_2), 2.69 (m, 2H, CH_2), 3.21 (s, 2H, CH_2), 3.42 (m, 2H, CH_2), 3.66 (s, 3H, OMe), 4.75 (m, 1H, 2'-H), 6.15 - 7.12 (m, 3H, Ar-H). IR (Nujol): 2250 cm^{-1} (CN).

(+)-2-Acetyl-2-hydroxy-5-methoxy-1,2,3,4-tetrahydronaphthalene
(71).

To the above compound 97 [1.0 g, 35 m.mole] in dry THF (15 ml) was added methyl magnesium iodide (7 ml, 5 moles excess) under N_2 atmosphere and stirred at 25° for 16 hr. The reaction mixture was added to 60% aqueous acetic acid (100 ml) and heated on water bath for 45 minutes. The mixture was allowed to cool, extracted with chloroform (3 x 25 ml) washed with H_2O (3 x 50 ml), dried over anhydrous Na_2SO_4 and finally concentrated under reduced pressure. Purification of the crude residue over silica gel column

using 2% acetone in benzene as the eluent afforded 71 (0.52 g, 70%) as a crystalline material. m.p. 62-63° (lit.⁵² m.p. 63-65°). M^+ 220. (Identical in all respects with an authentic sample).

(+)-4-Demethoxy-7,11-dideoxydaunomycinone (72)

An intimate mixture of 71 (0.22 g), phthalic anhydride (0.23 g), NaCl (0.46 g) and $AlCl_3$ (2.3 g) was heated at 180° for 5 min. The mixture was cooled, saturated oxalic acid solution (25 ml) added and extracted with chloroform (3 x 25 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the crude residue over silica gel column afforded 72 [0.05 g, 15%] as crystalline material, m.p. 214 (decomp.) [lit.⁵² m.p. 208-214° (decomp.), M^+ 336. PMR ($CDCl_3$): 2.03 (t, 2H, CH_2), 2.37 (s, 3H, $COCH_3$), 2.77 - 3.330 (AB q, 2H, CH_2). 3.00 (t, 2H, CH_2), 3.67 (s, 1H, -OH D_2O exchangeable), 7.50 (s, 1H, H-11), 7.78 (m, 2H, H-2 and H-3), 8.23 (m, 2H, H-1 and H-4), 13.00 (s, 1H, 6-OH, d_2O exchangeable). IR (Nujol): 1465, 1585, 1630, 1670, 1710 cm^{-1} .

Analysis: Calculated for $C_{20}H_{16}O_5$: C, 71.43; H, 4.76.
Found: C, 71.34; H, 4.93%.

REFERENCES

- 1 a) F. Arcamone, G. Franceschi, P. Orezzi, G. Cassinelli, W. Barbiere and R. Mondelli
J. Am. Chem. Soc., 86, 5334 (1964);
b) J. Bernard, R. Paul, M. Boiron, C. Jacquillat and R. Miral,
Ed. "Rubidomycin", Springer Verlag, New York (1969).
c) R.B. Livingston and S.K. Carter
"Daunomycin" Chemotherapy Fact Sheet,
National Cancer Institute, Bethesda, Md. (1970).
- 2 a) F. Arcamone, G. Franchesch and S. Penco
Tetrahedron Lett., 1007 (1969).
b) R.H. Blum and S.K. Carter
Ann. Intern. Med, 86, 249 (1974).
- 3 a) M.G. Brazhnikova, V.B. Zbarsky, V.I. Ponomarenko and N.P. Potapova
J. Antibiotics 27, 254 (1974);
b) G.F. Gause, M.G. Brazhnikova and V.A. Shorin
Cancer Chemother. Rep. Part I 58, 255 (1974).
- 4 a) M. Wani, H.L. Taylor, M.E. Wall, A.T. McPhail and K.P. Onan
J. Am. Chem. Soc., 97, 5955 (1975).
b) G.R. Pettit, J.J. Einck, C.L. Herald, R.H. Ode, R. Von Dreele, P. Brown, M.G. Brazhnikova and G.F. Gause, *ibid.* 97, 7387 (1975).
- 5 a) D.W. Henry
Cancer Chemotherapy Reports, Parts 2,3,4 (1974);
b) R.H. Iwamoto, P. Lim and N.S. Bhacca
Tetrahedron Lett., 3891 (1968).
- 6 F. Arcamone, G. Franceschi, S. Penco, P. Orezzi and R. Mondelli
Tetrahedron Lett., 3349 (1968).
- 7 R. Angiuli, E. Foresti, L. Riva di Sanseverino, N.W. Isaacs, O. Kennard, W.D.S. Motherwell, D.L. Wampler and F. Arcamone
Nature (London), 234, 78 (1971).

- 8 a) F. Arcamone, L. Bernardi, P. Giardino,
B. Patelli, A. Dimarco, A.M. Casazza, G. Pratesi
and P. Reggiani
Cancer Treatment Rep., 60, 829 (1976).
b) S. Neidle
Nature, 268, 195 (1977).
- 9 T.R. Kelly
Ann. Rep. Med. Chem., 14, 288 (1979).
- 10 a) N. Laxma Reddy
Ph.D Thesis, University of Poona (1983);
b) K. Bal Reddy
Ph.D Thesis, University of Poona (1983);
c) S.M. Jaweed
Ph.D Thesis, University of Poona (1983).
- 11 a) C.M. Wong, R. Schwenk, D. Papier and T.L.Ho
Can. J. Chem., 51, 466 (1973);
b) A.S. Kende, Y. Tsay and J.E. Mill
J. Am. Chem. Soc., 98, 1967 (1976);
c) T.H. Smith, A.N. Fujiwara, W.W. Lee, H.Y. Wu
and D.W. Henry
J. Org. Chem., 42, 3653 (1977).
- 12 F. Arcamone
Llyodia, 40, 45 (1977) and references cited therein.
- 13 a) L. Lenaz and J.A. Page
Cancer Treat. Rep., 3, 111 (1976);
b) D.D. Von Hoff, M. Layard, M. Boseneweig and
F.M. Muggia
Cancer Treat. Rep., 61, 1411 (1977).
- 14 M. Ghione
Cancer Chemother. Rep., 3, 83 (1975).
- 15 F. Arcamone, G. Cassinelli, G. Fantini, A. Grein,
P. Orezzi, C. Pol and C. Spallo
Biotechnol. Bioeng., 11, 1109 (1969).
- 16 a) E.M. Acton, A.N. Fujiwara and D.W. Henry
J. Med. Chem., 17, 659 (1974);
b) F. Arcamone, S. Penco and A. Vigevani
Cancer Chemother. Rep., 6, 123 (1975).
c) T.H. Smith, A.N. Fujiwara, W.W. Lee and D.W. Henry
J. Am. Chem. Soc., 98, 1969 (1976).

- 17 a) J.P. Marsch, C.W. Mosher, E.M. Acton and
L. Goodman
Chem. Commun., 973 (1967);
b) T. Yamaguchi and M. Kojima
"Symposium on the Chemistry of Natural Products",
Abstracts, Tokyo, 205 (1973);
c) D. Horton and W. Weekerle
Carbohydr. Res., 44, 227 (1975);
d) F. Arcamone
Carbohydr. Res., 46, 3 (1976).
- 18 F. Arcamone, L. Bernardi, B. Patelli, P. Giardino,
A. Dimarco, A.M. Casazza, C. Soranzo and G. Pratesi
Experientia, 34, 1255 (1978).
- 19 W.B. Turner
"Fungal Metabolites", Academic Press, New York, N.Y.(1971).
- 20 R.H. Thomson
"Naturally occurring Quinones", 2nd Ed. Academic Press,
New York, N.Y. (1971).
- 21 J. Houben
"Das Anthracen und die Anthrachinone"
George Thieme, Leipzig (1929).
- 22 M. Chandler and R.J. Stoodley
J. Chem. Soc. Perk Tran I, 1007 (1980).
- 23 D.A. Jackson and R.J. Stoodley
J. Chem. Soc. Chem. Commun., 478 (1981).
- 24 a) R.A. Russell, G.J. Collin, M. Stern, R.N. Warrenner
Tetrahedron Lett., 4229 (1979);
b) R.C. Gupta, D.A. Jackson and R.J. Stoodley
J. Chem. Soc. Chem. Commun., 928 (1982).
- 25 H.H. Inhoffen, H. Muxfeldt, V. Koppe and
J. Heiman Trosien
Chem. Ber., 90, 1448 (1957).
- 26 J. Sauer
Angew Chem. Int. Ed. Eng. 6, 16 (1967).
- 27 A.N. Griner, I.S. Protopov and A.A. Cherkasova
J. Org. Chem. USSR, 8, 220 (1972).

- 28 L.J. Dolby and K.S. Marshall
Org. Prep. Proceed. 1(4), 229 (1969).
- 29 C.M. Wong, D. Popien, R. Schwenk, and J.T. Raa
Can. J. Chem., 49, 2712 (1971).
- 30 S. Terashima, N. Tanno and K. Koga
Tetrahedron Lett., 4937 (1978).
- 31 R.J. Blade and P. Hodge
J. Chem. Soc. Chem. Commun., 85 (1979).
- 32 G.S. Krishna Rao and M.P. Reddy
Tetrahedron Lett., 22, 3549 (1981).
- 33 M.P. Cava, R.J. Ardecky and F.A.J. Kerdesky
J. Org. Chem., 46, 1483 (1981).
- 34 A.V. Rama Rao, B.M. Chanda and H.B. Borate
Tetrahedron, 38, 3555 (1982).
- 35 A.V. Rama Rao, V.H. Deshpande and N. Laxma Reddy
Tetrahedron Lett. 23, 4373 (1982).
- 36 F. Arcamone
Bull. Soc. Chim. Belg., 91, 1003 (1982).
- 37 A.S. Kende, D.P. Curran, Y.S. Tsay and J.E. Mills
Tetrahedron Lett., 3537 (1977).
- 38 J.R. Wiseman, N.I. French, R.K. Hallmark and K.G. Chiong
Tetrahedron Lett., 3765 (1978).
- 39 A.V. Rama Rao, V.H. Deshpande and N. Laxma Reddy
Tetrahedron Lett., 21, 2661 (1980).
- 40 Boeseken and Vander Grach
Rec. trav. Chim., 56, 1203 (1937).
- 41 PtO₂ was prepared from chloroplatinic acid. For
details see: Vogel's textbook of "Practical Organic
Chemistry", 4th ed. p.311 (1978).
- 42 J.A. Moore and M. Rahm
J. Org. Chem., 26, 1109 (1961).
- 43 S. Terashima, N. Tanno and K. Koga
Tetrahedron Lett., 21, 2753 (1980).

- 44 John B. Data and Raymond D. Bennett
J. Med. and Pharm. Chem., 4, 327 (1961).
- 45 a) R.G. Harvey
Synthesis 161 (1970);
b) A.J. Birch and G.S.R. Subba Rao
Adv. in Org. Chem. 8, 1-65 (1972).
- 46 G.S.R. Subba Rao and N. Shyam Sunder
J. Chem. Soc. Perk. Tran I, 875 (1982).
- 47 F.A.J. Kerdesky and M.P. Cava
J. Am. Chem. Soc., 100, 3635 (1978).
- 48 J.S. Swenton, D.K. Anderson, D.K. Jackson and
L. Narasimhan
J. Org. Chem., 46, 4825 (1981).
- 49 F. Arcamone, G. Cassinelli, F. Dimatteo,
S. Forenza, M.C. Ripamonti, G. Rivola, A. Vigevani,
J. Clardy and T. Mecabe
J. Am. Chem. Soc., 102, 1462 (1980).
- 50 a) T. Oki
Biotech. and Bioeng., 22, Suppl.1,83 (1980);
b) T. Oki
J. Antibiotics (Japan), 30, 70 (1977).
c) H. Tanaka, T. Yoshioka, Y. Shimauchi,
Y. Matsuzawa, T. Oki and T. Inui
J. Antibiotics, 33, 1323 (1980).
- 51 S. Neidle
"Topics in antibiotic chemistry"
P.G. Sammes, Ed. Halsted Press, New York, Vol.2,
Chapter 4 (1978).
- 52 H. Umezawa, Y. Takahashi, M. Kinoshita, H. Naganawa,
K.Tatsuta and T. Takeuchi
J. Antibiotics, 33 (12), 1581 (1980).
- 53 A.V. Rama Rao, A.R. Mehendale and K. Bal Reddy
Tetrahedron Lett., 23, 2415 (1982).
- 54 S.D. Kimball, D.R. Walt and F. Johnson
J. Am. Chem. Soc., 103, 1561 (1981).
- 55 A.V. Rama Rao, Bhanu M. Chanda and H.B. Borate
Synth. Commun., 1983 (in press).

- 56 Tsutomu Momose and Yosuke Ohkura
Pharm. Bull.(Japan), 4, 209 (1956).
- 57 J.W. Huffman
J. Org. Chem., 24, 1759 (1959).
- 58 A.McKillop and D.W. Young
Synthesis, 401 (1979).
- 59 Claus
J. Prakt. Chem. 39, 316 (1889).
- 60 J.W. Cornforth, R.H. Cornforth and R. Robinson
J. Chem. Soc., 689 (1942).
- 61 E. Bamberger and M. Althausse
Ber., 21, 1892 (1888).

CHAPTER II

PLANT GROWTH REGULATORS

A GENERAL INTRODUCTION

The complex phenomenon of plant development could be said to comprise the following processes:

- 1) Growth
- 2) Differentiation
- 3) Pattern formation
- 4) Regressive changes (embryonisation)
- 5) Regeneration

Growth occupies an unique position amongst all these because it is involved either directly or indirectly with each of these developmental process. It could be said that growth is a summation of all the physiological attributes of organisms¹. In the study of growth it becomes necessary to formulate some sort of a restricted definition of growth as a means to evaluate the results obtained.

In reviewing the various concepts of growth, one comes across different parameters in evaluation² such as (i) increase in protoplasm and the subsequent increase in size and/or substance e.g. protein (ii) increase in the complexity of organisation (iii) increase in cell number or the ability of the cells to multiply.

Any of these aspects could involve both a qualitative and quantitative parameter and a worker could select those

parameters which most concern his studies. As growth is intimately connected with various metabolic stages, it becomes quite difficult to assess the factors which contribute to the control of growth at every stage of change. Growth basically depends on an appropriate supply of various inorganic and organic compounds, and the deficiency of one or more of these, individually or relatively, becomes the chemical factor limiting that aspect of growth. These compounds together form a heterogeneous group of chemical growth factors. Thus some major groups can be recognised easily: (1) Inorganic compounds supplying essential elements (2) organic compounds supplying the radicals necessary as building blocks of major constituents of cells, called bulk building materials (3) organic compounds required in small amounts which are incorporated in active systems within the cell, in more or less unchanged forms and which are indispensable for growth.

In higher plants³ a self supporting system operates with respect to these factors, because of mutual exchange of substances between the various parts which have different synthesizing powers and hence different requirement of these factors. Therefore one has to speak of requirements not in terms of whole plants but its parts, tissue and cells. Tissue and organ culture have

proved to be invaluable tools permitting the study of plants under artificially produced heterotrophic conditions. Thus it has been seen that isolated plant parts require not organic and mineral nutrients but also growth factors (viz. the three groups mentioned earlier). They include vitamins and amino acids, nucleic acid constituents, fatty acids etc.

There are other types of growth factors which act as co-ordinators of growth and development of plants and are active in minute amounts. These include 'phytohormones' which move from the site of production to the site of action to evoke characteristic responses. These also include compounds from intact plants which regulate growth when supplied exogenously to the same or different plants, but their movement to the site of action intact plants has not been established. They are called the "naturally occurring growth regulators". There is a third group of chemicals which are synthetic and show growth regulatory properties when supplied exogenously. They are called "synthetic growth regulators".

The beginning of the concept of growth regulators dates back to the 19th century when Sachs⁴ spoke of certain 'chemical messengers' responsible for co-ordinating growth and development in plants, later⁵ the name 'hormone' was

assigned. The well known plant hormones⁶ (phytohormones) show plant growth regulating properties. They have high activity, specific action and function in the regulation of plant growth. Today five classes of such hormones have been characterized chemically. They are (i) Auxins (ii) Gibberellins (iii) Cytokinins (iv) Abscissic acid and (v) Ethylene. 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.

Plant growth regulators are defined⁷ 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. Growth regulators, both natural and synthetic are seen to be identified by certain operational terms, like auxins⁸ which cause an increase in size, cytokinins⁹ which cause an increase in the cell number, florigens¹⁰ which mediate flowering and morphactions¹¹ which are morphologically active etc.

The response¹² 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 ways¹², such as promote rooting and propagation 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 number of organic compounds are used as plant growth regulators¹² in a number of ways.

The properties of the growth regulators in relation to their mode of action can be studied at two levels (i) The role of growth regulators acting singly or in combination and their effects on growth at all developmental stages of the plant; (ii) The role of growth regulators in regulating the more fundamental biochemical processes. It is likely that such a clear-cut division of functions may not be possible. In many cases it is not. Letham¹³ distinguishes between mechanisms and mode of hormone action. He speaks in terms of an initial trigger reaction(s)

which he calls 'mechanism' and the series of steps leading to the physiological response which he calls 'mode' of action.

Other than the problem of growth regulation by phytohormones (and substances like Fusicoccin), there are a host of naturally occurring substances which seem to control both metabolism and growth, but their actual mode of action is not known. Perhaps they have escaped notice because their effect is not as dramatic as observed with growth regulators.

Whatever approach is made to the problem of growth, it depends largely on the particular stage of development and on the particular phenomenon under consideration. The lowest level of control and the largest and most complex units are seen in field experiments. These experiments yield results which can have direct application in an empirical way. The treatment effect here is the effect of all the components - it tells us nothing of how the treatment works. To know that, one needs to reduce the size and complexity of the experimental units that can be used. This improves precision and in some sense simplifies interpretation. However, it also introduces a different set of components and makes the results less applicable to what happens in nature or agricultural practices.

It seems that we have a choice between obtaining results that we can apply, but not understand or those which we think we understand but cannot apply. The only way out of this dilemma seems to have experiments all up and down the range between field and test tube and to argue back and forth from one to the other.

A) SYNTHESIS OF

1-TRIACONTANOL

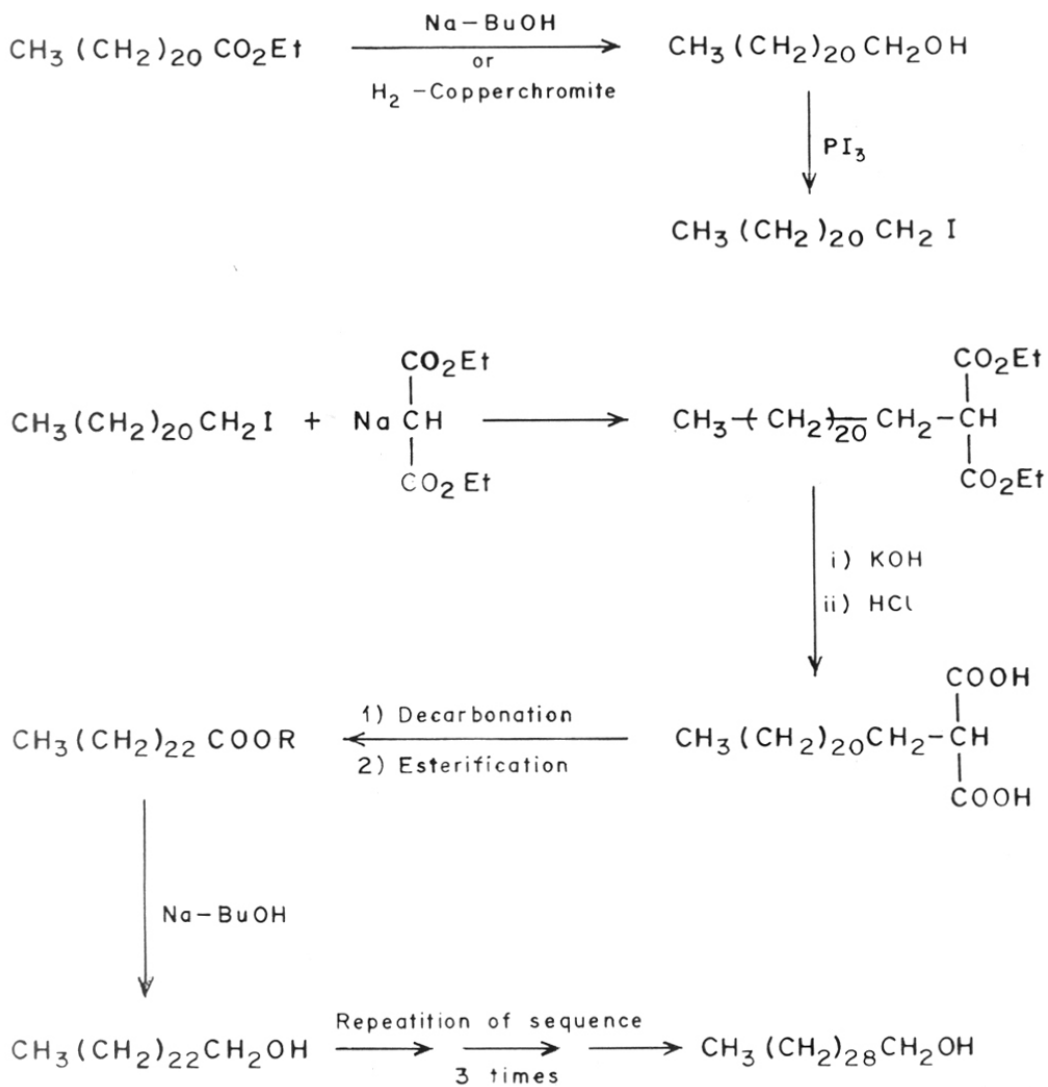
INTRODUCTION

Though 1-triacontanol (1) has been known for a long time¹⁴, much attention has been drawn towards it, only recently; due to its potential use as a plant growth stimulant. It was Ries et al.¹⁵ who first demonstrated that application of alfalfa (Medicago sativa L.) increased the yields of tomatoes, cucumber, lettuce, rice, corn and several other crop species. The active principle responsible for the enhanced growth was identified as 1-triacontanol (1), the main constituent of the wax derived from alfalfa leaves¹⁶. As this compound increased the dry weight of test plants in the dark, thereby having no effect on photosynthesis¹⁷, it was speculated that it might function*by increasing the uptake of nutrients.

1-Triacontanol was earlier prepared by lithium aluminium hydride (LAH) or sodium-alcohol (ethanol or butanol) reduction of n-triacontanoic acid or its ester. n-triacontanoic acid (also called as mellissic acid) (2) has been synthesised by a variety of tedious methods.

Bleyberg and Ulrich¹⁴ (Scheme 1) first synthesised it from ethyl behenate (C₂₂ acid ester) by repeating the malonic ester synthesis sequence four times. Besides poor yield, it became difficult to carry out the complete decomposition of dicarboxylic acid with large quantities

SCHEME - 1



①

of the material.

Robinson¹⁸ prepared n-triacontanoic acid (2) (Scheme-2) by alkylating ethyl sodio aceto acetate (3) with ethyl- ω -bromoundecanoate (4) and reacting the condensation product with stearoyl chloride. Graded hydrolysis and decarboxylation of the alkylated product gave 13-keto-triacontanoic acid (5), which was reduced to 2 by Clemmensen reduction.

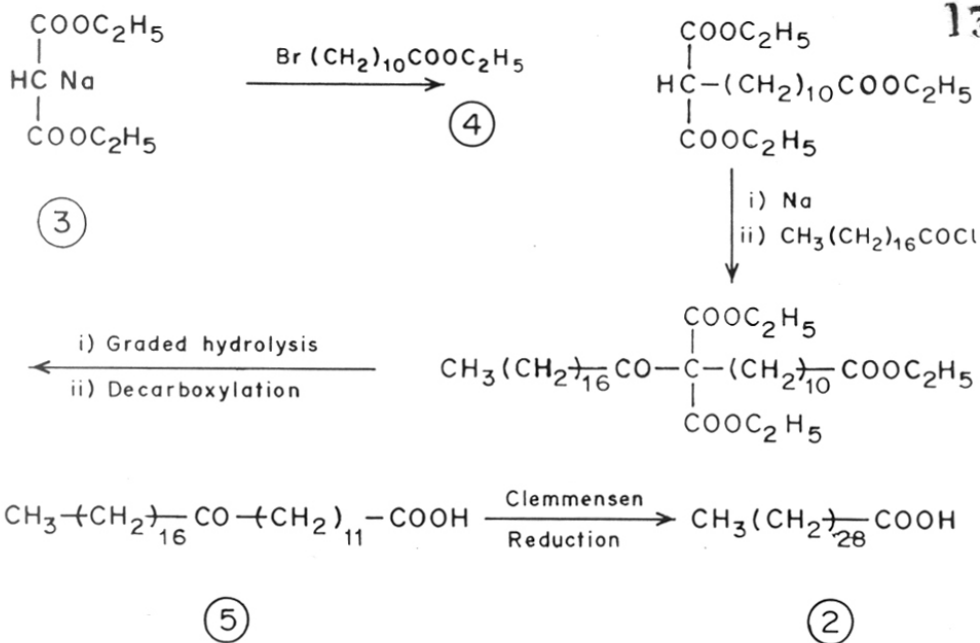
Jones¹⁹ prepared 12-oxotriacontanoic acid (8) (Scheme-3) by reaction of octadecylzincchloride (6) with ω -carbethoxyundecanoyl chloride (7), followed by graded hydrolysis. 8 on Clemmensen reduction in absolute ethanol gave ethyl ester of triacontanoic acid which was reduced to 1 by sodium-butyl alcohol.

Oura et al.²⁰ synthesized 1 by two step sequence (Scheme-4). Reaction of di-octadecyl cadmium with ω -carbethoxyundecanoyl chloride (7) gave 12-oxotriacontanoic acid (8) which on Clemmensen reduction and sodium-butyl alcohol treatment gave 1.

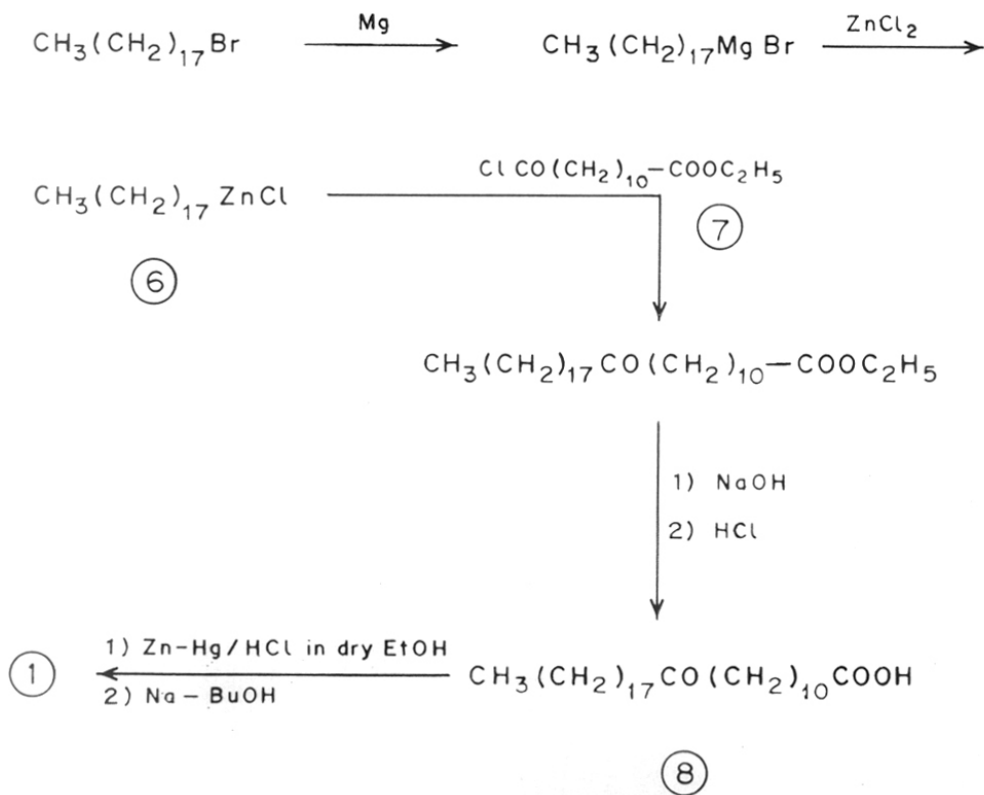
Hunter et al.²¹ (Scheme 5) modified Robinson's procedure¹⁸, by condensing 1-morpholino cyclododecene (9) with stearoyl chloride. Alkali hydrolysis of 2-hexadecyl-1,3-cyclotetradecane dione (10) gave sodium-13-ketotria-

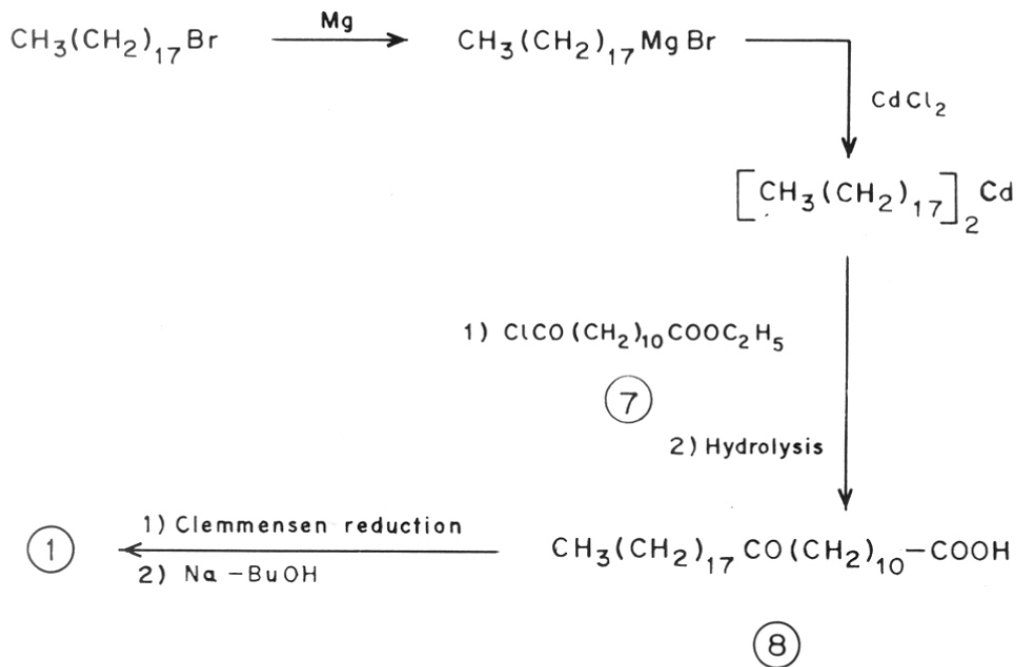
SCHEME - 2

136

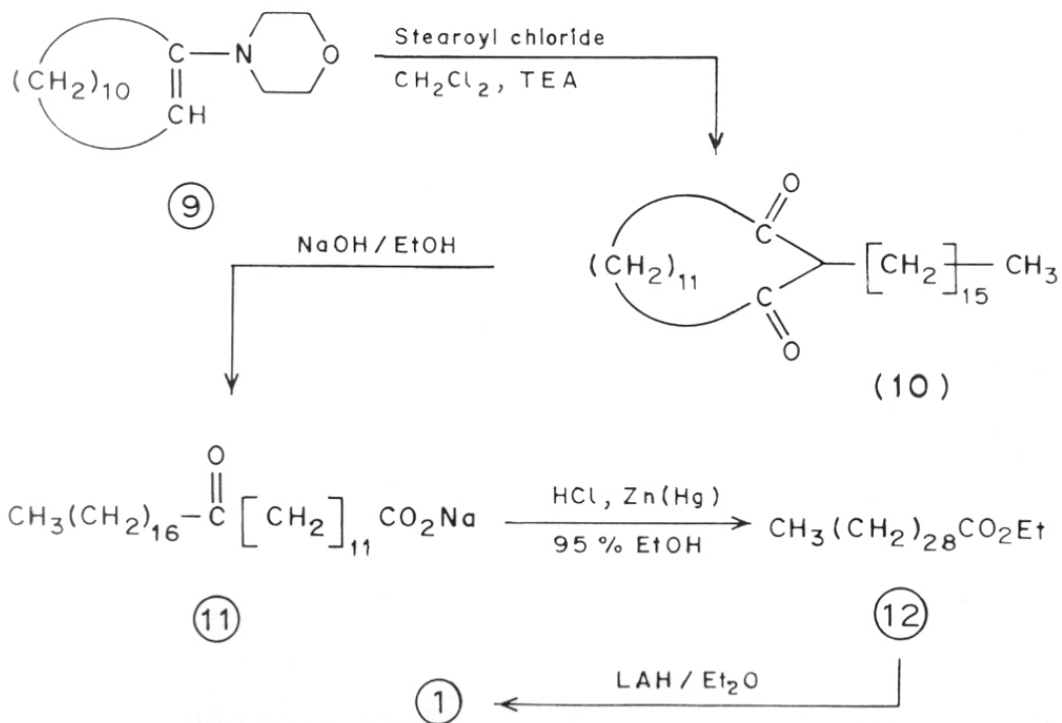


SCHEME - 3





SCHEME - 5

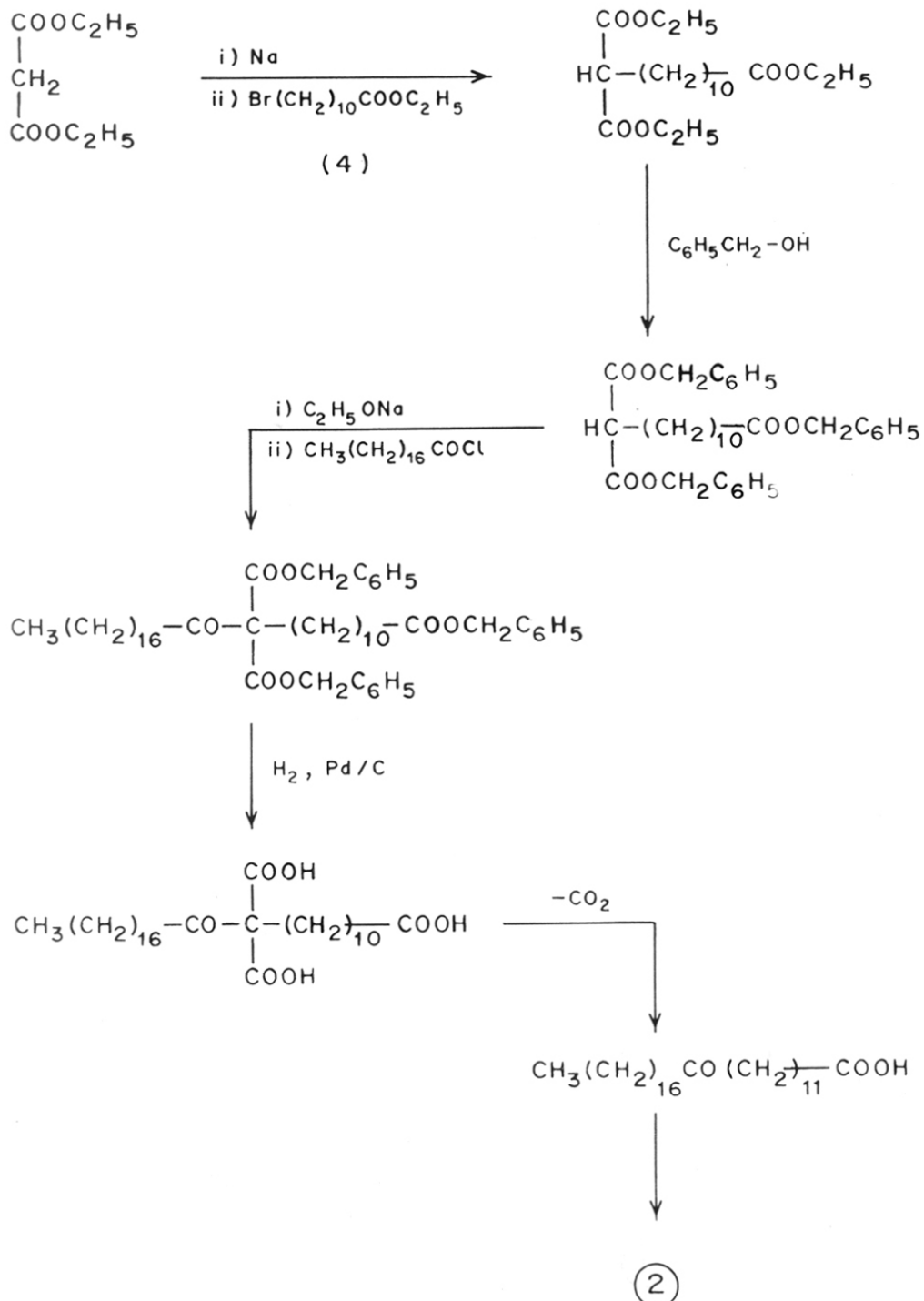


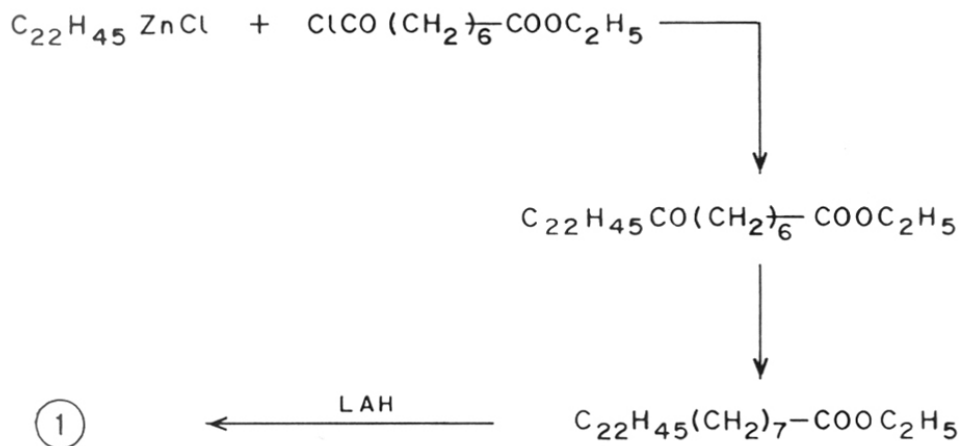
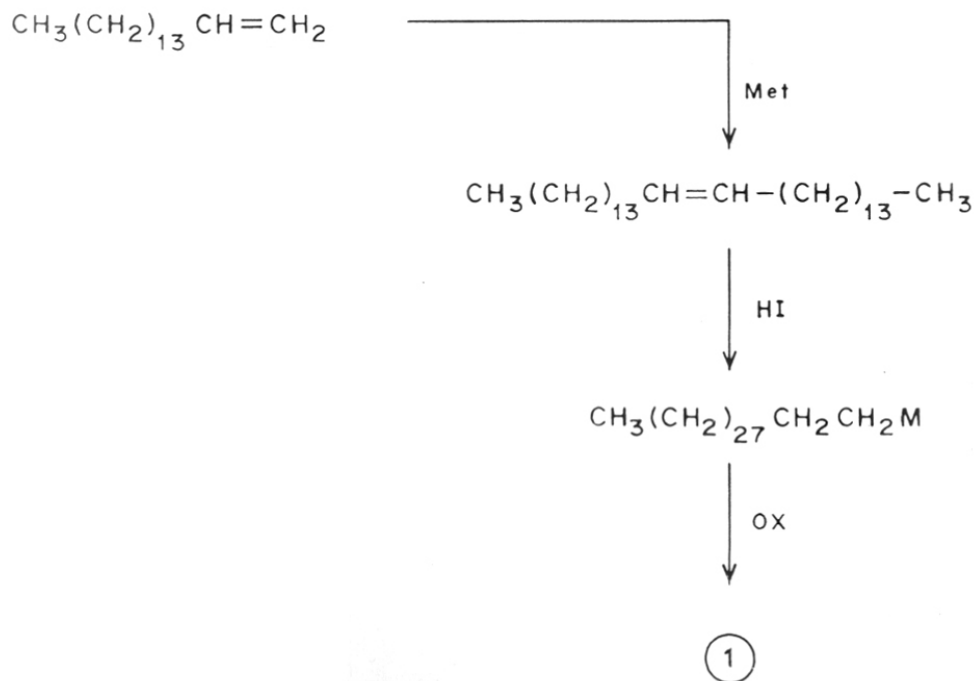
contanoate (11) which was reduced to the ester 12, with amalgamated Zn and HCl bubbling.

The utility of malonic ester synthesis was limited, because ester hydrolysis in the tri-ester 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 Bouman and coworkers²² by substituting ethyl groups in diethyl malonate by benzyl groups and cleaving the benzyl to oxygen bond by hydrogenolysis. Watanabe²³ applied Bowman's method with some modifications by using ethyl- ω -bromoundecanoate (4) and stearoyl chloride as depicted in Scheme-6.

In an attempt to synthesize 1 through enamine reaction, Schildknecht and Renner²⁴ found that 2-tetracosanoylcyclohexanone underwent cleavage on treatment with NaOH giving n-tetracosanoic acid instead of the corresponding 7-oxotriacontanoic acid. Therefore they have abandoned this route and obtained 1 by the condensation of alkyl zinc chloride and ω -carbethoxyacyl chloride (Scheme-7).

Maruyama et al.²⁵ have investigated a practical procedure for 1 via metathesis (Met) of 1-hexadecene followed by hydrometalation-isomerization (HI) and oxidation (OX) as shown in Scheme-8. Earlier in this laboratory 1



SCHEME-7SCHEME-8

has been synthesised by two different approaches^{26,27}.

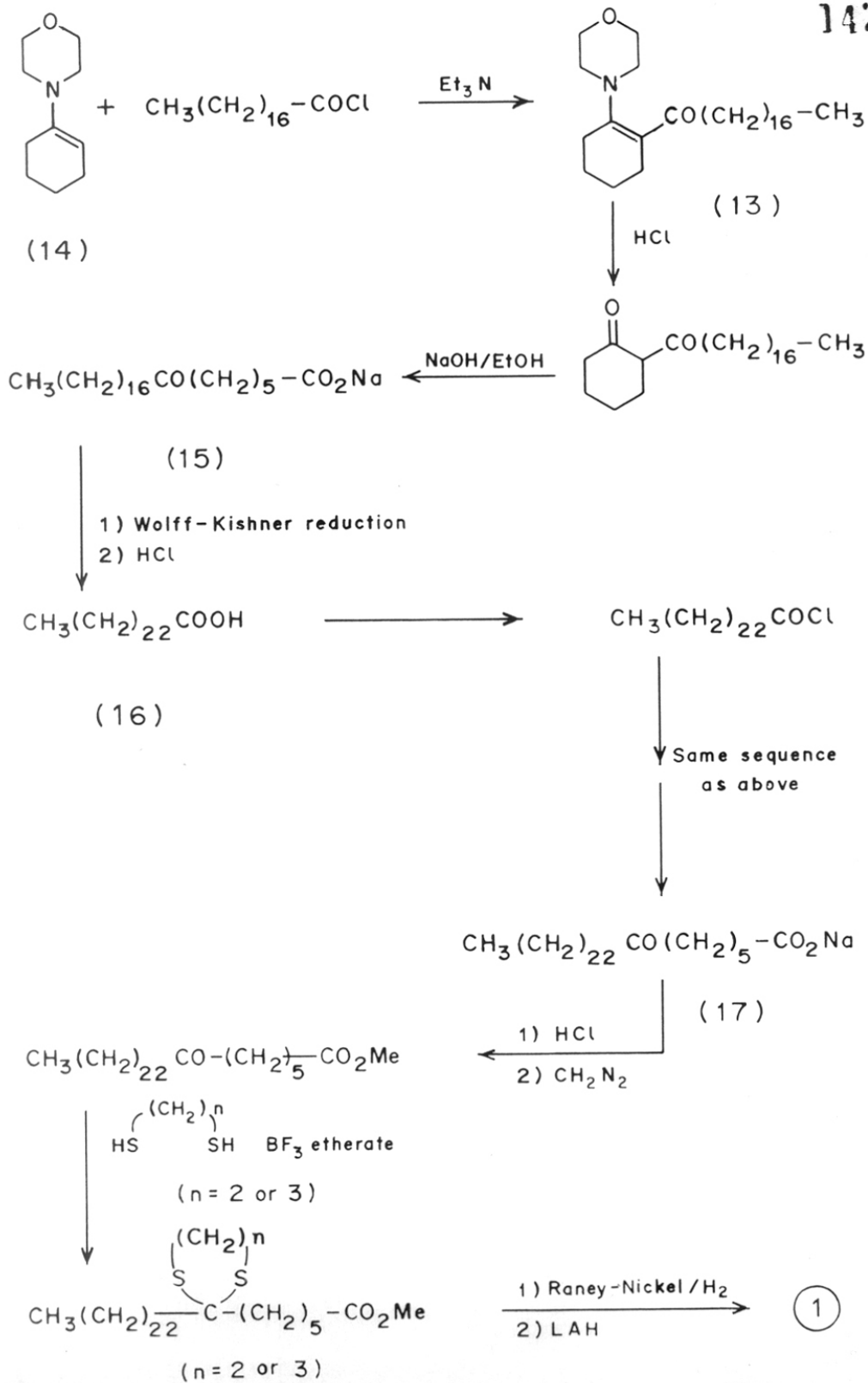
In the first approach²⁶ (Scheme 9) 1 was synthesised from stearic acid by two successive additions of six carbon units through enamine intermediates. 2-Stearoyl-1-morpholino-1-cyclohexene (13) was obtained by condensing 1-morpholino-1-cyclohexene (14) with stearoyl chloride. Graded hydrolysis of 13 gave the sodium salt of 7-oxotetracosanoic acid (15), which when subjected to Wolff-Kishner reduction gave n-tetracosanoic acid (16). The acid chloride of 16 was subjected to the same sequence of reactions with 14 to finally give sodium salt of 7-oxotriacontanoic acid (17). 17 was smoothly elaborated to 1, through various steps.

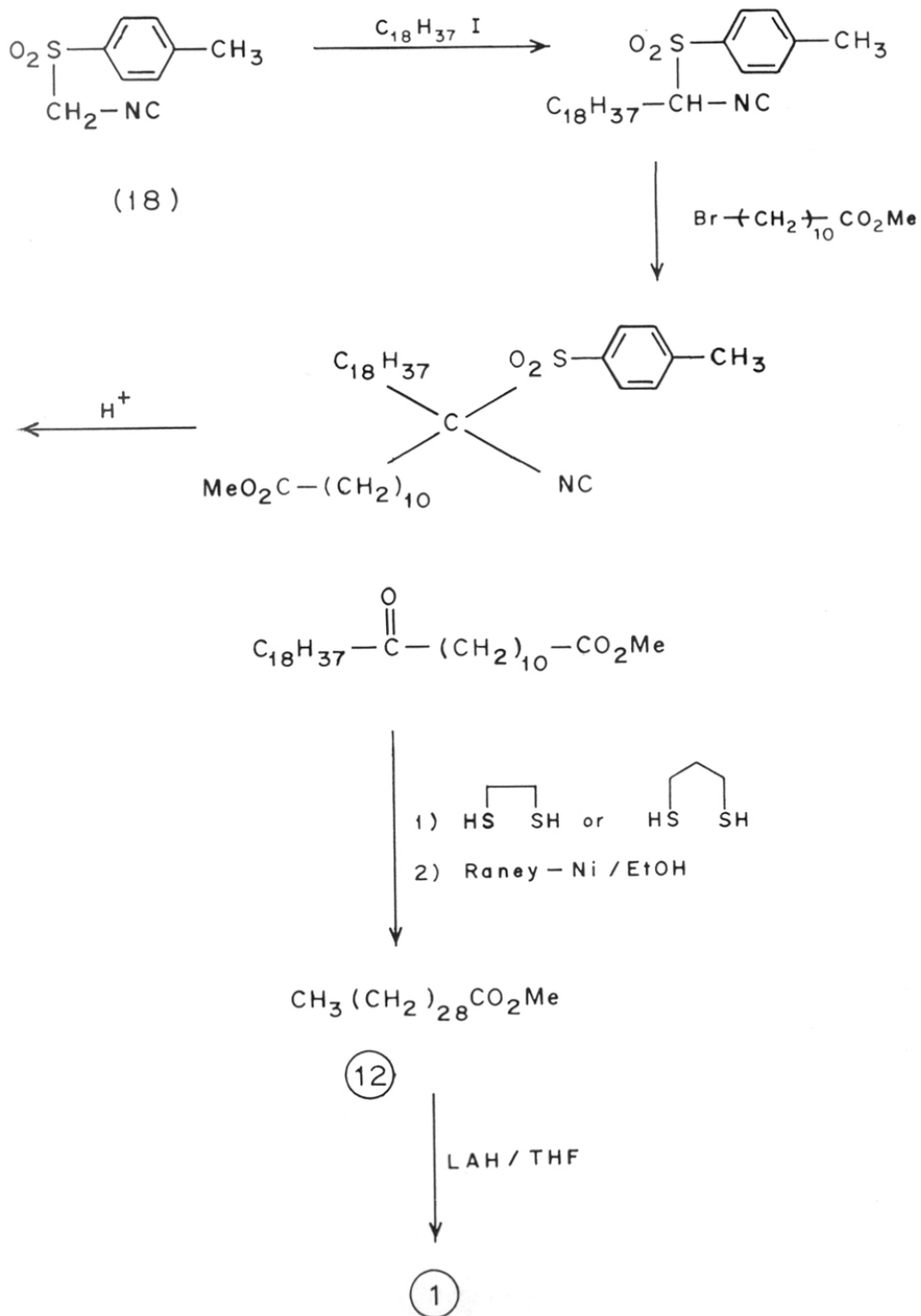
In the second approach²⁷ (Scheme 10) the strategy was based on two successive alkylations of tosylmethyl isocyanide (18)[TosMIC]. TosMIC was monoalkylated with stearyl iodide and the second alkylation was performed using methyl 11-bromoundecanoate subsequent hydrolysis of the resulting product and reduction of the keto ester 12, gave 1.

Recent synthesis by Chadha et al.²⁸ involves coupling between stearyl magnesium bromide and 12-bromo-1-tetrahydropyranyloxydodecane in the presence of Li_2CuCl_4 followed by acid hydrolysis of the coupled product.

SCHEME-9

142





PRESENT WORK

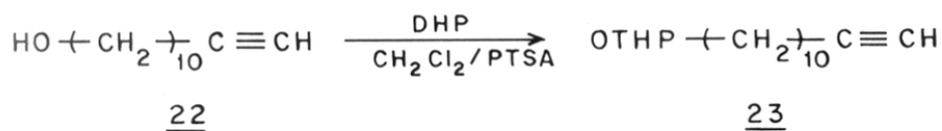
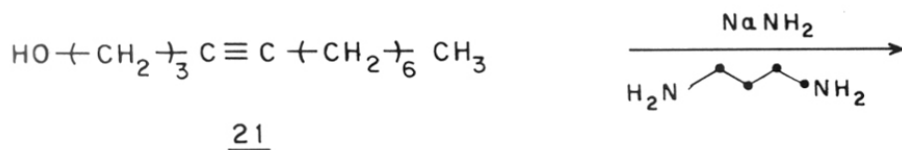
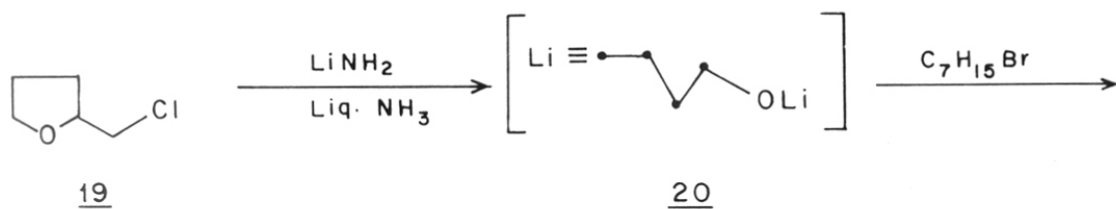
The recent efficacy of n-triacontanol (1) as a plant growth stimulant¹⁵ coupled with the fact that the responses of both rice and tomatoes to a synthetic sample¹⁷ were similar to that of natural triacontanol has directed attention towards its synthesis. In order to carry out a comparative study of the effect of triacontanol, synthetic routes were developed in this laboratory^{26,27}. However, as both the earlier routes^{26,27} from this laboratory make use of IAH and since 1 has the potential for commercial use, new approaches were looked upon with emphasis being placed on simple procedures.

This part deals with a simple synthesis of 1 as exemplified in Scheme 11, conceptually different from previous approaches and totally free from expensive reagents.

Tetrahydrofurfuryl chloride (19) was prepared from the inexpensive and commercially available tetrahydrofurfuryl alcohol, by adopting the known procedure²⁹.

Tetrahydrofurfuryl chloride (19) on treatment with lithium amide in liquid ammonia gave rise to the dianion of 4-pentyn-1-ol (20)³⁰ which was directly reacted with n-heptyl bromide (prepared from n-heptanol³¹) to afford 4-dodecyn-1-ol (21) in 75% yield, b.p. 108°/1 mm. M⁺ 182. The PMR (CCl₄) of 21 [Fig.1] shows a distorted

SCHEME - 11



(1)

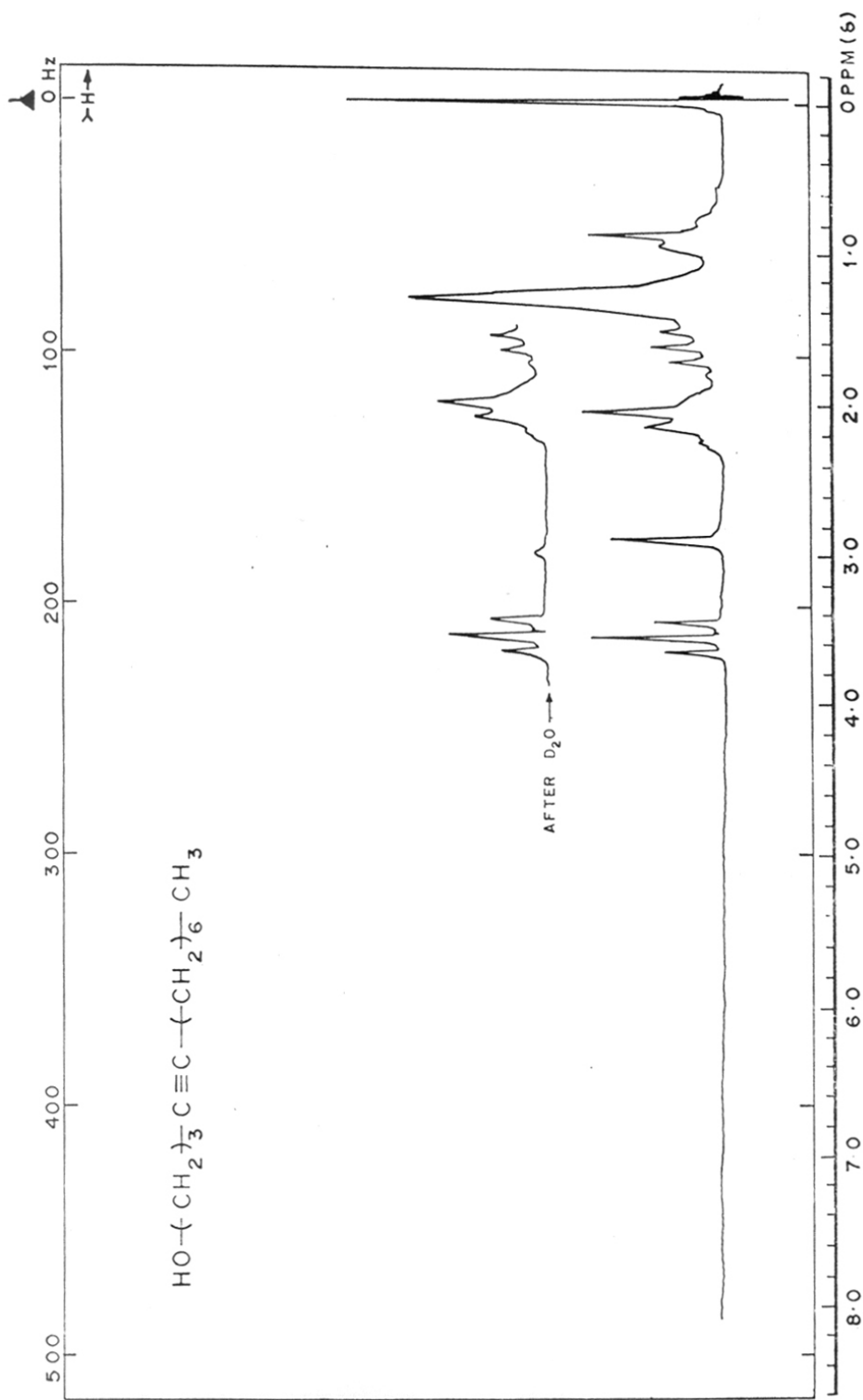


FIG. 1. PMR SPECTRUM OF COMPOUND 21 IN CCl_4

triplet at 1.00 representing the methyl group. The broad singlet at 1.33 and a multiplet centred at 2.16 represents 6 methylenes and 2 methylenes respectively. A singlet at 2.90 (D_2O exchangeable) represents the hydroxyl proton and the triplet at 3.60 represents a single methylene group. IR (Neat) showed stretching at 3330 cm^{-1} (OH).

Alcohol 21 was subjected to acetylene-zipper reaction³² employing an excess (3 moles) of sodium amide in 1,3-diamino propane to give 11-dodecyn-1-ol (22) in 90% yield. The structure of 22 was confirmed by PMR (CCl_4) [Fig.2] in which the broad singlet at 1.26 represents 8 methylenes. The terminal acetylenic proton ($=C-\underline{H}$) appears as a triplet at 1.73. A multiplet at 2.00 accounting for 3 protons represents the hydroxyl proton and a methylene group. Another methylene appears as a multiplet at 3.40. IR (Neat) showed stretching at 3320 cm^{-1} (OH) and 2120 cm^{-1} ($-C=\underline{C}-H$).

Compound 22 on reaction with dihydropyran in dichloromethane containing a catalytic amount of p-toluene sulfonic acid (PTSA) gave 1-[(tetrahydro-2H-pyran-2-yl)-oxy]-11-dodecyne (23) in 90% yield. M^+ 266. IR (neat) showed stretching at 2120 cm^{-1} ($-C=\underline{C}-H$). [There was no hydroxyl stretching band]. The structure of 23 was further confirmed by PMR (CCl_4) (Fig.3) in which all the protons resonate in

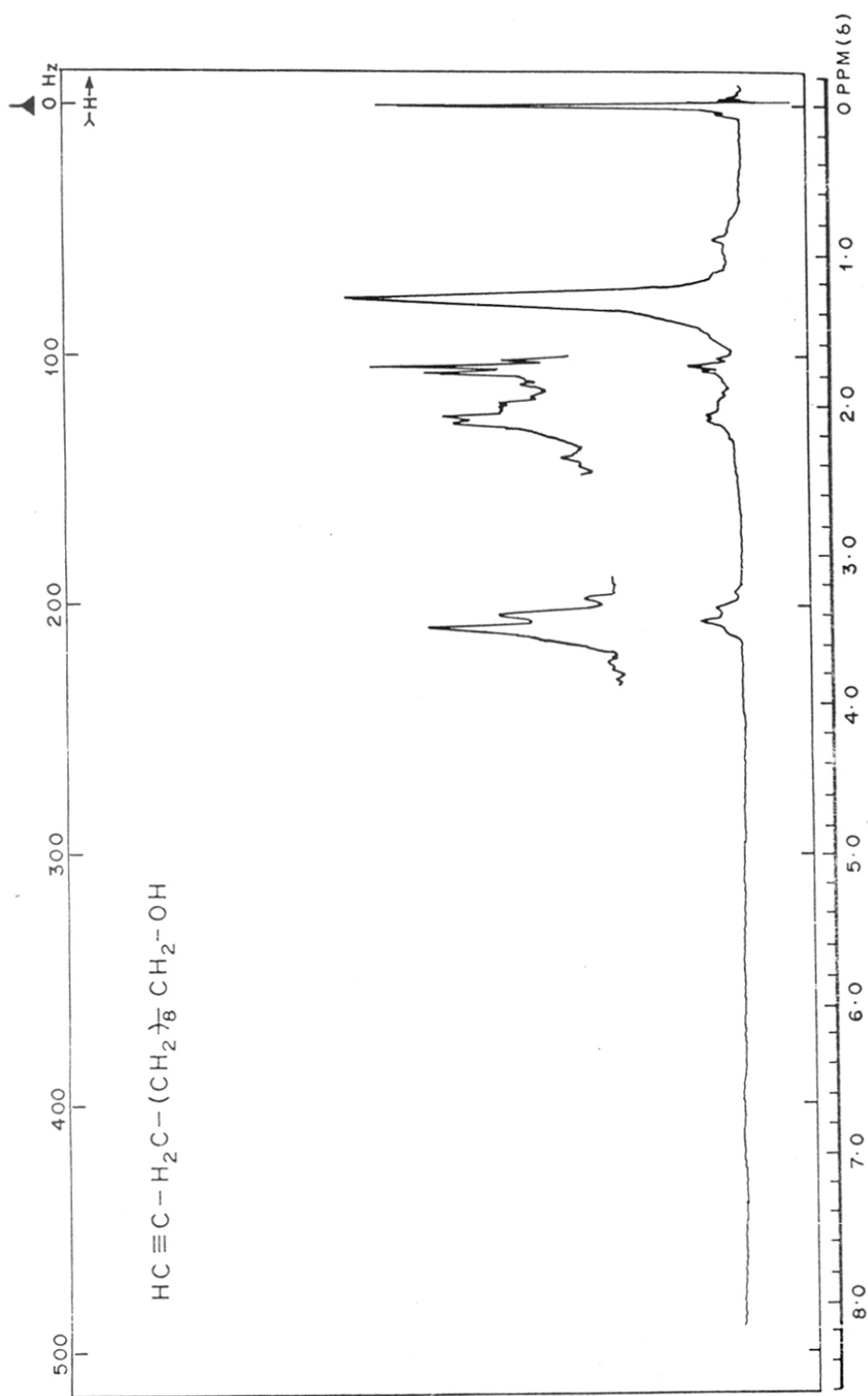


FIG. 2. PMR SPECTRUM OF COMPOUND 22 IN CCl_4

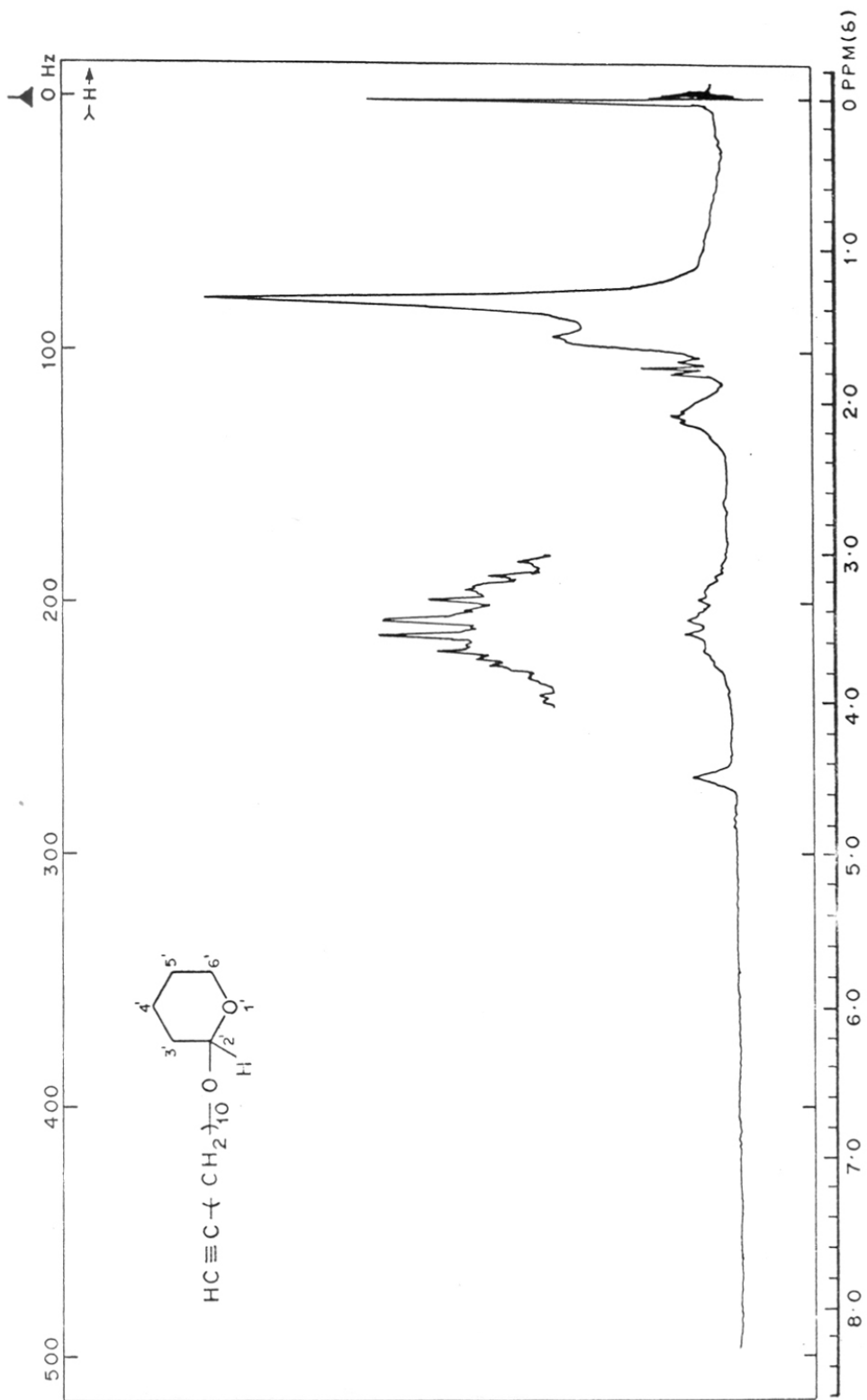


FIG. 3. PMR SPECTRUM OF COMPOUND 23 IN CCl_4

accordance with the expected chemical shifts.

Compound 23 was then converted to 1-triacontanol (1) by the following sequence of reactions. Alkylation of the anion of 23, generated in situ from lithium amide in liquid ammonia with 1-bromooctadecane (1-2 moles excess, prepared from 1-octadecanol³¹) provided the desired C-30 chain) [that alkylation had taken place was confirmed from IR by the absence of stretching at 2120 cm^{-1} ($-\text{C}=\text{C}-\text{H}$)], which was directly subjected to hydrogenation in ethyl acetate using 10% Pd/C at room temp. and atmospheric pressure with mechanical shaking for 12 hr. Acid work up of the hydrogenated product and purification of the crude product by column chromatography using 5% acetone in benzene as the eluent afforded 1 (0.5 g, 60%) as a crystalline white material. m.p. 87° (lit.²⁶ m.p. $87-88^{\circ}$), mixed m.p. with an authentic sample remained undepressed. M^+ 438. IR (Nujol) 3300 cm^{-1} (OH); superimposable with an authentic sample.

E X P E R I M E N T A L

Sodium amide was prepared freshly from liquid ammonia and sodium³³.

Tetrahydrofurfuryl chloride (19) was prepared from tetrahydrofurfuryl alcohol²⁹.

1-Bromoheptane was prepared from 1-heptanol, and 1-Bromooctadecane from 1-octadecanol according to the known procedure³¹.

4-Dodecyn-1-ol (21)

Lithium (0.15 g atom) in the presence of ferric nitrate (50 mg) was dissolved in freshly distilled ammonia (250 ml) which is indicated by the disappearance of blue colour. To this freshly prepared lithium amide solution was added tetrahydrofurfuryl chloride (6.0 g, 0.05 m) during 10 minutes and was stirred for 2 hr at -33°C . After all the tetrahydrofurfuryl chloride had reacted, 1-bromoheptane (8.95 g, .05 mole) dissolved in tetrahydrofuran (10 ml) was added dropwise to the stirred and cooled (-33°C) reaction mixture. It was stirred for an additional 0.5 hr and the ammonia was allowed to evaporate by bringing it to room temp. The residue was treated with saturated ammonium chloride solution (100 ml), extracted with ether (6 x 30 ml) and dried over anhydrous Na_2SO_4 . Removal of ether and

distillation (108°/1 mm) gave 21 (6.82 g, 75%). M^+ 182.
 PMR (CCl₄): 1.00 (distorted t, 3H, CH₃), 1.33 (bs, 12H, 6X CH₂); 2.16 (m, 4H, 2X CH₂), 2.90 (s, 1H, OH (D₂O exchangeable), 3.60 (t, 2H, CH₂). IR (neat): 3330 cm⁻¹ (OH).

Analysis: Calculated for C₁₂H₂₂O: C, 79.12; H, 12.08;
 Found: C, 79.36; H, 12.12%.

11-Dodecyn-1-ol (22)

A suspension of sodium amide (1.17 g, 30 m.mol) and 4-dodecyn-1-ol (21) (1.82 g, 10 m.mol) in 1,3-diaminopropane (16 ml) was heated at 80° for 2.5 hr. The reaction mixture was cooled to room temp. ice-cold water (150 ml) added and extracted with ether (4 x 25 ml). The organic layer was washed successively with H₂O, dil. HCl and brine; and dried over anhydrous Na₂SO₄. Removal of solvent under reduced pressure provided (1.63 g, 90%) of 22, which being sufficiently pure (as indicated by TLC and PMR) was used as such for the next step. PMR (CCl₄): 1.26 (bs, 16H, 8 x CH₂); 1.73 (t, 1H, ≡C-H); 2.00 (m, 3H, 1X CH₂ + OH), 3.40 (m, 2H, CH₂). IR (neat): 3320 cm⁻¹ (OH), 2120 cm⁻¹ (-C≡CH).

1-[(tetrahydro-2H-pyran-2-yl)-oxy]-Dodecyne (23)

The mixture of 22 (1.82 g, 10 m.mol) freshly distilled dihydropyran (20 ml), chloroform (30 ml) and PTS acid (2 mg) were stirred at room temp. for 6 hr. It was diluted with water (50 ml), extracted with chloroform (3 x 25 ml),

washed with saturated NaHCO_3 solution (3 x 25 ml) and H_2O (2 x 50 ml). The organic layer was dried over anhydrous Na_2SO_4 and finally concentrated under reduced pressure. Purification of the crude product over a short alumina column using 2% acetone in pet. ether as the eluent gave 23 (2.34 g, 96%). M^+ 266. PMR: 1.50 (m, 22H, 11X CH_2), 1.80 (t, 1H, =C-H); 2.10 (m, 2H, 1X CH_2), 3.50 (m, 4H, 2X CH_2), 4.50 (m, 1H, $\text{-}\overset{\text{I}}{\underset{\text{I}}{\text{C}}}\text{-H}$). IR (neat): 2120 cm^{-1} ($\text{-C}\equiv\text{C-H}$).

1-Triacontanol (1)

Lithium (0.006 g atom) in presence of ferric nitrate (25 mg) was dissolved in freshly distilled ammonia (75 ml) which is indicated by the disappearance of blue colour. To this freshly prepared lithium amide solution was added 23 (0.5 g, 1.88 m.mole) during 5 minutes and was stirred for 2 hrs at -33°C . 1-Bromooctadecane (0.75 g, 2.25 m.mole) dissolved in tetrahydrofuran (10 ml) was added dropwise to the stirred and cooled (-33°) reaction mixture. It was stirred for an additional 0.5 hr and ammonia was allowed to evaporate by bringing it to room temp. The residue was treated with saturated NH_4Cl solution (50 ml), extracted with ether (3 x 15 ml), dried over Na_2SO_4 and solvent removed under reduced pressure. To the residue so obtained, dry ethyl acetate (20 ml) and 10% Pd/C (50 mg) were added and hydrogenated at room temp. and atmospheric pressure with

mechanical shaking for 12 hr. The catalyst was filtered and washed with ethyl acetate (10 ml). To the combined ethyl acetate solution, conc. HCl (0.5 ml) was added and stirred at room temp. for 4 hr. The organic layer was washed with H₂O (2 x 25 ml), dried over anhydrous Na₂SO₄ and finally evaporated to dryness. Purification of the crude residue over silica gel column using 5% acetone in benzene as the eluent gave (0.5 g, 60%) of n-triacontanol (1) M⁺ 438. m.p. 87° (lit.²⁶ m.p. 87-88°, mixed m.p. with an authentic sample remained undepressed. IR (Nujol): 3300 cm⁻¹ (OH), superimposable with an authentic sample.

Analysis: Calculated for C₃₀H₆₂O: C, 82.19; H, 13.30;
Found: C, 82.08; H, 13.10%.

B) THE EVALUATION OF 1-TRIACONTANOL

INTRODUCTION

In recent years, no other plant growth regulator has evoked so much attention, as much as n-triacontanol. In green house studies, both foliar and root applications of n-triacontanol (1) promoted the growth of many plant species by 20-50%¹⁵. Synthetic triacontanol applied as a foliar spray enhanced the growth and yield of several important food crops. The average dry weight increases in corn and rice were 15.1% and 12.4% respectively¹⁷. Triacontanol increased the growth of rice within 6 hr.¹⁷ and its effect was regulated by the concentrations of CO₂ and O₂ in the atmosphere.

Besides whole plants the effect of n-triacontanol on callus cultures of tobacco, tomato and a number of other plants was also reported and the increase in growth was attributed to an increase in cell number¹⁵.

At this stage of knowledge there were a number of gaps which could not be explained particularly because there were variations in responses of plants due to applications of triacontanol. Variations were also reported in callus cultures. Keeping this in mind, a system was so evolved for testing the effects of the growth regulator taken up for study, that the application of the regulator in the field could be somewhat assessed along with some

information regarding the mode of action of the regulator.

The system consisted of three components:

- 1) Callus tissues
- 2) Isolated plant parts and organs such as those used in bioassays.
- 3) Whole plants.

The three components exhibit an increasing order of tissue organisation.

The effect of triacontanol was tested out in this system initially, to get a gross idea of the nature and magnitude of response. This was called a preliminary scan. Depending on the response obtained in this scan, further experiments were designed to probe into the mode of action.

EXPERIMENTAL RESULTS*

Samples of naturally occurring n-triacontanol were avoided because of the possibility of contamination with analogous long chain compounds which may be inhibitory to the activity of triacontanol at equimolar or lower concentrations. Moreover the purity of the sample manifests the extent of activity. Hence the synthetic sample was used.

Triacontanol applications have been posing difficulties because of its insolubility in water. Solutions of the required concentrations were made by first dissolving triacontanol in chloroform and then adding aliquots of this to measured amounts of distilled water containing Tween 20, so as to form a uniform dispersion of triacontanol.

Lucerne callus tissue was used as the first system, because it was fast growing and uniform. For bioassay

*In accordance with Rule 0.413 of the Univ. of Bombay, the results incorporated in this part are of the experiments carried by the author in collaboration with the Botany Dept. University of Poona, to get acquainted with the various 'Plant Physiology' experimental techniques. It forms a part of the Ph.D dissertation of Sujata Ranade, University of Poona (1983). The purpose of this section is to exemplify the logical reasoning which inspired to develop a new plant growth regulator. Menadione Bisulphite, which will be discussed in detail in the next section.

systems, considering the growth-promotory role of triacontanol, the systems used for assaying growth promotory phytohormones were chosen for comparing the actions. For this purpose wheat coleoptile segment growth and rooting of mung bean cuttings, were chosen for assaying auxin like activity of triacontanol. Hypocotyl elongation in cucumber and lettuce seedlings were chosen for assaying gibberellin like activity. Radish cotyledon expansion and betacyanin formation in *Amaranthus* seedlings were chosen for assaying cytokinin like activity.

Whole plants of tomato and rice seedlings were chosen for the third system. Growth parameters corresponding to test chosen, were measured in each of the systems to get a gross idea of the nature of response and its magnitude.

Callus system: Triacontanol failed to show any 'dramatic' effect on this system (Table 1). There was a maximum of 19% increase in fresh weight over controls which was observed at a concentration of 10^{-6} M. At 10^{-4} M and 10^{-5} M, only slight increases were shown. Dry weight increases were even lower, the maximum being about 14% over controls at 10^{-6} M. None of these results were, however, statistically significant, indicating a high variance which discredited the percentage difference between means. There was no change in the morphology

TABLE 1 : EFFECT OF TRIACONTANOL ON LUCERNE CALLUS

| Treatment | Fresh weight (g) | Dry Weight (g) | Proteins mg/g dry weight |
|-------------------------|---------------------|-------------------|-----------------------------|
| Control | 0.992 ± 0.103 | 0.059 ± 0.004 | 6.084 |
| 10 ⁻⁶ M Tria | 1.178 ± 0.054 | 0.067 ± 0.003 | 6.388 |
| 10 ⁻⁵ M Tria | 1.145 ± 0.092 | 0.066 ± 0.004 | 6.075 |
| 10 ⁻⁴ M Tria | 1.114 ± 0.065 | 0.065 ± 0.003 | 5.446 |
| LSD at 0.05 level | 0.335 | 0.0103 | - |

of the callus, due to triacontanol treatment. Cell size did not vary from controls and there was no differentiation either.

Bioassay systems: In the six bioassay systems experimented with, triacontanol failed to show the characteristic response obtained with indole acetic acid, gibberellin or cytokinin. As seen from the graphs [Fig.4] it can be inferred that in the assay system for IAA and cytokinin, triacontanol showed a very low magnitude promotory effect in comparison with the hormones. In the gibberellin assay systems, triacontanol was more or less inhibitory or neutral in its action.

Whole plant systems: In the whole plants of rice and tomato, triacontanol again proved ineffective [Tables 2 and 3]. Rice seedlings were treated with 10^{-7} M and tomato with 10^{-8} M. Concentration of triacontanol. These concentrations were chosen on the basis of observations made by Ries (personal communication). Dry weight increases in tomato were observed but they were statistically insignificant. In rice triacontanol showed a slight reduction in dry weight, though the decrease again was statistically insignificant. Protein increases were rather meagre of the order of 1.3% in tomato and 1.9% in rice. Chlorophyll content was decreased by 2.4% in tomato and 10% in rice. A Tween-20 control was maintained to ensure that the response produced was due to

Fig.4 : Effect of TRIA in several bioassay systems.

—— hormone ----- TRIA

Auxin bioassay systems

- (a) Wheat coleoptile segment elongation system
- (b) Mung bean rooting system

Gibberellin bioassay systems

- (c) Cucumber hypocotyl elongation system
- (d) Lettuce hypocotyl elongation system

Cytokinin bioassay systems

- (e) Radish cotyledon expansion system
- (f) *Amaranthus* betacyanin formation system.

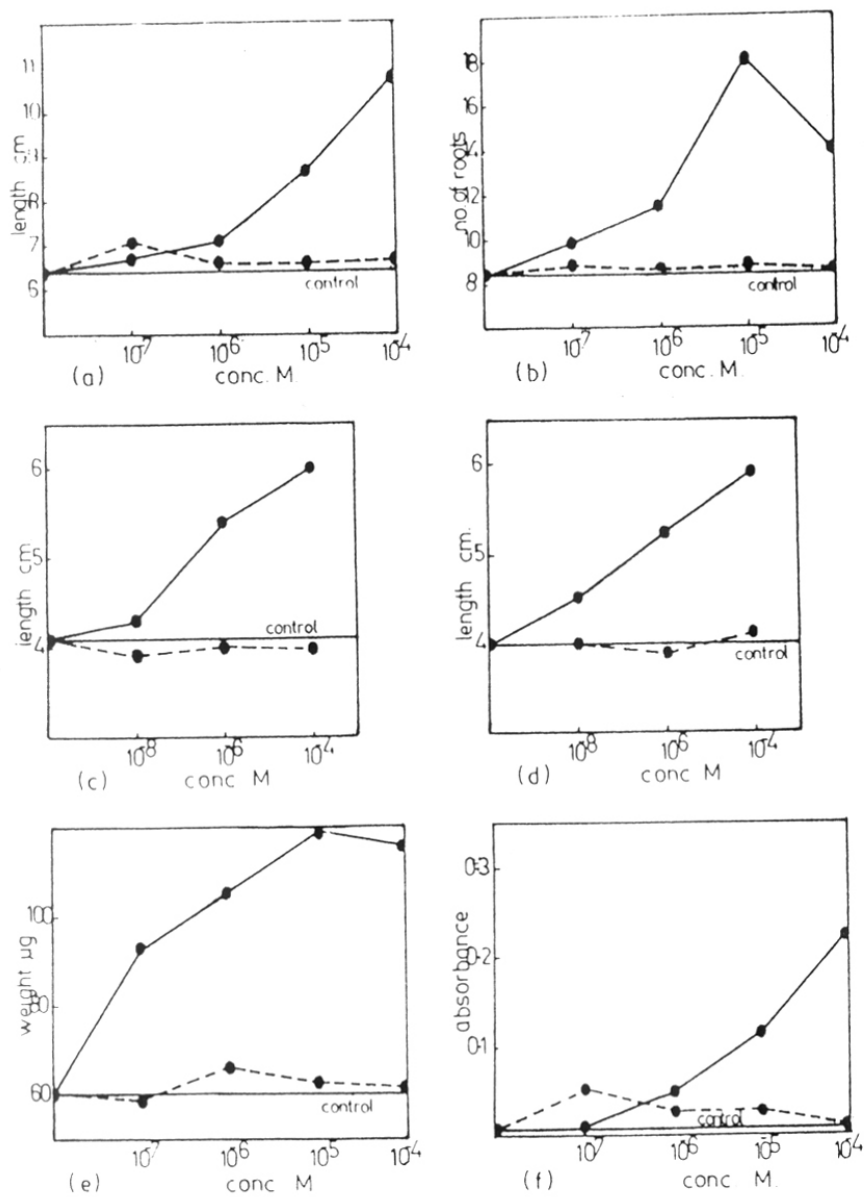


FIG. 4

TABLE 2 - EFFECT OF 10^{-7} M TRIACONTANOL ON THE GROWTH OF RICE SEEDLINGS

| Treatment | Dry weight/g Fresh weight | Chlorophyll mg/g fresh weight | Proteins mg/g dry weight |
|-----------------------------|------------------------------|-------------------------------------|-----------------------------|
| Control | 0.295 \pm 0.008 | 0.73 | 7.75 |
| Tween 20 | 0.284 \pm 0.011 | 0.72 | 7.05 |
| Triacontanol 10^{-7} M | 0.287 \pm 0.009 | 0.66 | 7.90 |

TABLE 3 - EFFECT OF 10^{-8} M TRIACONTANOL ON GROWTH OF TOMATO PLANTS

| Treatment | Dry weight/g fresh weight | Chlorophyll mg/g fresh weight | Proteins mg/g dry weight |
|------------------|------------------------------|-------------------------------------|-----------------------------|
| Control | 0.113 \pm 0.002 | 0.79 | 7.63 |
| Tween-20 | 0.114 \pm 0.002 | 0.83 | 7.15 |
| Tria 10^{-8} M | 0.115 \pm 0.001 | 0.77 | 7.73 |

triacontanol alone. However, the tween-20 control differed from the normal control and this made the results confusing, nevertheless, this control was necessary for evaluation.

CONCLUSION

The response observed in all the three systems designed to assess the regulatory role of triacontanol was poor. This could be attributed to:

- a) Unsuitability of such a system for assessing growth regulation.
- b) The lack of any growth regulatory activity shown by triacontanol. In fact, the later reports and publications on triacontanol resolved this, for even the authors claiming triacontanol to be a promising plant growth stimulant have made equivocal statements.

According to Ries and Wert³⁴, the application of triacontanol rapidly increased leaf area, total nitrogen, water soluble proteins and reducing sugars, but over a period of time these differences between controls and treated plants were not noticeable. Evidently the responses, if any, were apparently only at the initial stages of observations.

Erickson et al.³⁵ reported that triacontanol regulates processes related to photosynthesis, and that the effect was different, in different plants. There was no evidence, however, of dark fixation of CO₂.

Triacontanol was shown to be ineffective on germination and early stages of plant growth³⁶ but was seen to

inhibit responses to gibberellin in lettuce seed germination³⁷.

A number of speculations arise regarding the mode of action of triacontanol. The 'speed' of the responses suggest that triacontanol may be rapidly absorbed into the plant and probably is active in an unaltered form. Triacontanol may be activating an enzyme or altering a membrane site resulting in increased metabolism and accumulation of various intermediary substances. The most likely theory³⁴ suggests that water is metabolically incorporated via carbohydrate and/or fat hydrolysis, or there may be an increase in hydroscopicity (physically bound water) leading to large increases in free amino acids and reducing sugars which constitute about 30% of the dry weight.

With controversies^{36,37,38} regarding the activity of triacontanol, there are chances that a compound claimed by some workers as a promising plant growth regulator may not evoke the same results with others. Then the only solution to justify this ambiguous dilemma is to seek the luck of the farm or farmer.

It seems more likely that the inactivity of triacontanol in our experiments arises due to its controversial nature as a growth regulator, as against the unsuitability

of the proposed system for assessing growth regulatory activity. This view is validated when in further work using this system, interesting results were obtained (see next section).

C) MENADIONE BISULPHITE
A PROMISING PLANT GROWTH REGULATOR

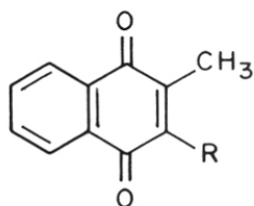
(i) PRELIMINARY SCAN

INTRODUCTION

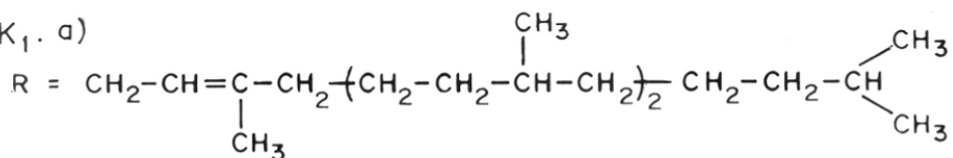
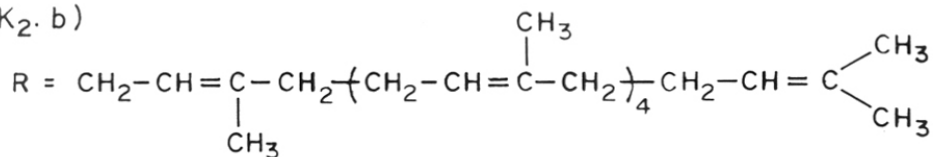
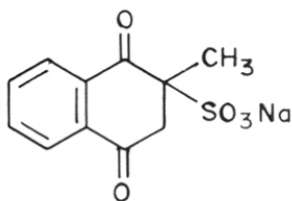
Global demand for higher crop production coupled with constant increase in the cost of nitrogen fertilizers has led in recent times to an increased interest in plant growth regulators. For the plant growth regulation, the rate of application of chemical and the stage of plant growth, must be considered 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 synthesis.

The role of 1-triacontanol (1) (first isolated from alfalfa¹⁶ has not been fully ascertained. While some groups report its growth promotory role³⁴ there are others who contradict it^{36,37,38}. In order to carry out a comparative study of the effect of 1-triacontanol, it was synthesized, (Discussed in IIA), but failed to bring about growth promoting activity in many of the systems evaluated (Discussed in IIB). This ambiguity provoked to probe into the known chemical constituents of alfalfa which may be responsible as plant growth regulators. One of the interesting observations was that vitamin K₁ (24a) was first isolated from the same source.

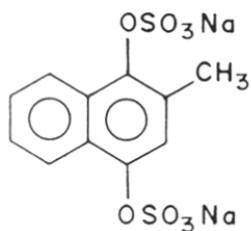
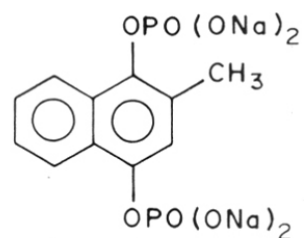
To determine whether vitamin K₁ (24a) may be responsible



24 (VITAMIN K)

Vit K₁. a)Vit K₂. b)Vit K₃. c) R = H (MENADIONE)

MENADIONE BISULFITE (25)

2627

for the observed growth enhancement on application of alfalfa to some of the crops, a systematic study has been undertaken. Menadione* (2-methyl-1,4-naphthoquinone/Vitamin K₃) (24c) being more active than Vitamin K₁ (24a) and/or Vitamin K₂ (24b) in its general biological properties⁴⁰ was preferred to the other forms of Vitamin K.

As 1-triacontanol applications have been posing difficulties because of its insolubility in water, it was felt worthwhile to use the water soluble form of menadione (24c). Menadione bisulfite (25) was preferred to the other water soluble forms, namely sodium-2-methyl-1,4-naphtho-hydroquinone disulfate (26)⁴¹ and/or sodium-2-methyl-1,4-naphthoquinone diphosphate(27)⁴¹ because in menadione bisulfite the quinone nature is retained and quinones have a more or less universal distribution as components of electron transport systems in plants, where they play an active role in metabolism⁴², besides occurring as secondary metabolites. Besides participating in photosynthetic electron transport reactions, quinones are components of the respiratory chain in plants.

*Synthesis of menadione will be dealt separately in Section IID.

In addition to this role, the quinones also occur in the form of Vitamin K in all plants but its role is not clear, although suggestive of a more generalized function.

McNew and Burchfield⁴³ made attempts to find out the biological activity of quinones and reported on their mode of action as constituting a vital redox system, that may be important in stabilizing the cell protoplasts. Addition of synthetic quinones would drift the redox balance in these systems sufficiently to interfere with normal cell functions. They were also known to block vital enzymes by forming additional products with the free sulphhydryl groups⁴⁴. Quinones have been surveyed as one of the groups of phenolics⁴⁵, which affect several physiological processes in plants. Mandava⁴⁶ includes quinones under polyphenols on the basis of their similar activities in inhibition of IAA oxidase activity.

However, quinones have remained somewhat neglected in the assessment of their growth regulatory activity, when actually they are perhaps one of the few groups of phenolics which play an active role in metabolism, besides occurring as secondary metabolites.

Menadione bisulfite (25) was prepared from menadione (24c)

and sodium bisulfite according to the known procedure⁴⁷. To test the growth regulatory role of 25, a scanning system was designed which consisted of -

- 1) isolated plant parts and organs such as those used in bioassays.
- 2) callus tissues.
- 3) whole plants.

The three components exhibit an increasing order of tissue organisation. The effect of menadione bisulfite was tested in this system as a 'PRELIMINARY SCAN' to get a gross idea of the nature and magnitude of response. Depending on the response obtained in this scan, further experiments were designed to probe into 'MODE OF ACTION' of the regulator.

EXPERIMENTAL *

I. BIOASSAY SYSTEMS: The effect of menadione bisulfite was tested in bioassay systems commonly used for assaying the activity of growth promotory hormones namely auxins, gibberellins and cytokinins. The range of concentrations scanned was 10^{-6} M - 10^{-4} M.

1) For Indole Acetic Acid (IAA)

a) Wheat coleoptile segment elongation method of Nanda and Kaur⁴⁸ was employed. Wheat grains (MACS-9: variety) were soaked and allowed to germinate on sieves for 72 hrs in the dark. The coleoptiles of uniformly grown seedlings were placed on a glass slide and after the removal of 2 mm tips, the first 5 mm segments were excised. They were placed in solutions containing 1% sucrose and known concentrations of menadione bisulfite. Results were recorded after 24 and 48 hrs of incubation in the dark. As there was no significant difference in growth at 48 hr from growth

* Though the author has collaborated with the Botany Department, University of Poona for getting acquainted with the "Plant Physiology" experimental techniques, the results incorporated here have not been submitted by anybody else for any degree or diploma or other academic award. It is not included in the Ph.D dissertation of Sujata Ranade, University of Poona (1983).

at 24 hr, further readings were taken only after 24 hr.

b) Mung bean (var. Vaishakhi) seedlings were grown in vermiculite and after 8 days, uniformly growing seedlings were cut 4 cm below the cotyledon. These were immersed in tubes (4" x 1") wrapped with black paper, having known concentrations of menadione bisulfite and incubated at $25 \pm 2^{\circ}$ and normal diffused daylight. The number of adventitious roots initiated were counted after 8 days, in 10 randomly selected cuttings for each treatment⁴⁹.

2) For Gibberellin

a) The method of Halvey and Cathey⁵⁰ using cucumber hypocotyls was modified and used seeds of 'Poona Khira' variety of cucumber were placed in petriplates on filter papers wetted with 5 ml solutions of known concentrations of menadione bisulfite. They were incubated at $25 \pm 2^{\circ}$ in diffused white light. Hypocotyl length was noted 84 hr after germination.

b) Lettuce seeds (Variety Pariscos) were sown in the dark and after 2 days, seedlings having radicles of a similar length were selected. They were placed in petriplates, on filter papers wetted with 5 ml solutions of known concentrations of menadione bisulfite. They were incubated at $25 \pm 2^{\circ}$ in diffused white light. Hypocotyl length was noted,

120 hr after germination⁵¹.

3) For Cytokinin

- a) Radish cotyledon expansion method of Letham⁵² was used with suitable adaptations. Seeds of 'Purefabl kalmi' variety were germinated in the dark for 36 hr at $25 \pm 2^{\circ}$. The smaller of the cotyledons were then excised from uniformly grown seedlings and placed in petriplates on filter paper wetted with 5 ml solution of known concentrations of menadione bisulfite. Incubation was for 48 hr in diffused white light at $25 \pm 2^{\circ}$. Weights of the cotyledons were recorded to the nearest mg.
- b) Amaranthus caudatus L. Seeds were sown on moistened filter paper for 72 hr at $25 \pm 2^{\circ}$ in the dark. The seed coats were then removed and explants consisting of the upper portion of hypocotyls along with the cotyledons were cut from the seedlings. These were placed in petriplates containing two layers of filter paper moistened by $M/75 PO_4$ buffer at pH 6.3 containing 1 mg/ml of tyrosine, and various concentrations of menadione bisulfite. The plates were incubated at $25 \pm 2^{\circ}$ for 18 hr in the dark after which the explants were removed and placed in 3 ml of distilled water. The intensity of colour formation was measured by reading the absorbance⁵³ at 542 nm.

RESULT:

Out of all these assays, menadione bisulfite showed a positive response in the mung bean rooting system, where there was an increase over 100% in the number of adventitious roots formed at a concentration of 10^{-5} M [Table 4 and Fig.5]. Since 10^{-5} M concentration was seen to be the most favourable the same concentration was retained for further evaluation.

II. Callus system:

The effect of menadione bisulfite was tested on lucerne and tomato callus. They were obtained from hypocotyls of 4-5 day old seedlings and raised on Murashige and Skoog's basal medium⁵⁴. The callus were incubated for 25 days in the dark on the callusing medium containing 10^{-5} M menadione bisulfite and growth was noted at the end of this period. The callus was then kept in an oven at 60° and since the weight of the tissue remained constant after 48 hr, dry weight estimations were made after this time.

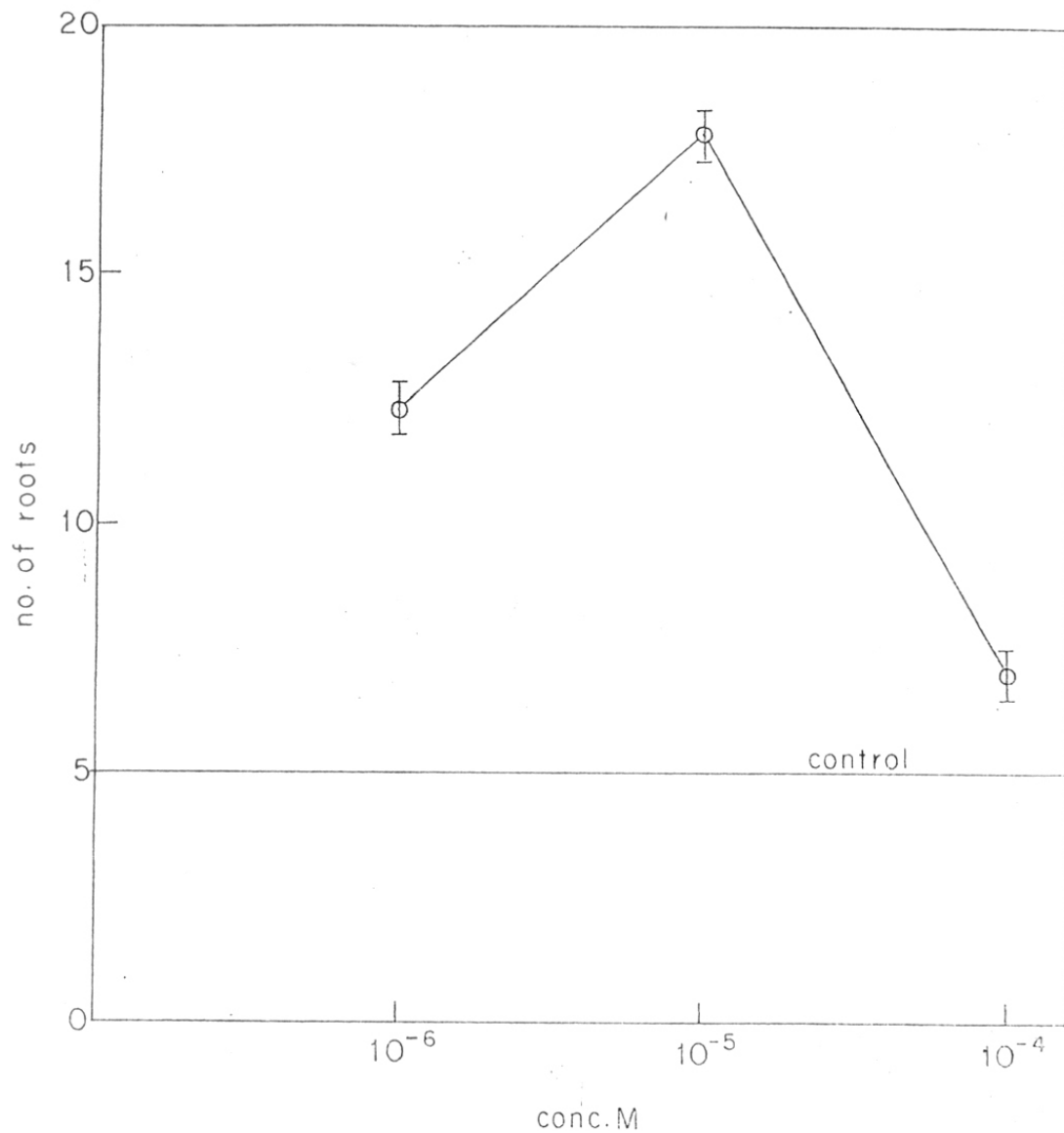
Proteins were estimated from this callus using Lowry's method⁵⁵. The dried callus masses were immersed in 80% ethanol overnight and then homogenized. The supernatant liquid was discarded and to the residue 0.5N perchloric acid was added and boiled at 80° for 20 minute. The supernatant containing the nucleic acids and other phosphates was discarded, and after washing the residue

TABLE 4 - EFFECT OF MENADIONE BISULFITE ON MUNG BEAN ROOTING. RESULTS ARE A MEAN OF 10 REPLICATES

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
|------------------|----|----|----|----|----|----|----|----|----|----|-------------|
| Control | 1 | 4 | 4 | 5 | 4 | 6 | 4 | 5 | 4 | 5 | 5 ± 0.47 |
| 10 ⁻⁴ | 9 | 8 | 7 | 8 | 8 | 7 | 6 | 5 | 6 | 6 | 7 ± 0.37 |
| 10 ⁻⁵ | 16 | 14 | 20 | 19 | 18 | 20 | 19 | 18 | 16 | 18 | 17.8 ± 0.56 |
| 10 ⁻⁶ | 14 | 11 | 12 | 14 | 13 | 10 | 12 | 11 | 14 | 12 | 12.3 ± 0.42 |

+ values significant at 99% confidence limits.

Fig.5



The effect of Menadione bisulfite on the number of adventitious roots formed in the mung bean rooting system.

thoroughly with distilled water, it was suspended in 1N NaOH containing 2% Na_2CO_3 . To ensure complete extraction of the proteins, the suspension was left standing overnight, after which the supernatant was used for protein estimation. The readings were taken on Spectronic 20 (Bausch and Lomb) at 750 nm and the protein concentration calculated from a standard BSA curve.

RESULT: As seen from Table 5 and Table 6 it is evident that 10^{-5} M menadione bisulfite brought about significant increases in the growth of callus cultures of Lucerne and tomato.

At this stage it became essential to eradicate the doubt, as to whether the effect of menadione bisulfite was not due to either only the inorganic sodium bisulfite and/or only menadione. Hence experiments were carried on the same callus cultures viz. lucerne and tomato using separately 10^{-5} M menadione and 10^{-5} M sodium bisulfite. In both the cases, no increases were observed, whereby it became clear that the moiety menadione bisulfite as a whole is essential for plant growth stimulation.

III. Whole Plants:

For the third system plants covering a broad range of families were used viz. tomato and capsicum (solanaoae), Mung bean (Leguminosae), cucumber (cucurbitaceae) and corn

TABLE 5 - EFFECT OF 10^{-5} M MENADIONE BISULFITE ON LUCERNE CALLUS. READINGS ARE A MEAN OF 5 REPLICATES

| | Dry Weight (g) | | | | | Proteins | |
|-------------|----------------|------|------|------|------|----------------|--------------|
| | 1 | 2 | 3 | 4 | 5 | Mean | mg/g dry wt. |
| Control | .058 | .057 | .055 | .080 | .065 | .063 ± .004 | 11.0 |
| 10^{-5} M | .090 | .078 | .085 | .088 | .087 | .086 ± .002 | 12.75 |

+ve values significant at 99% confidence limits

TABLE 6 - EFFECT OF 10^{-5} M MENADIONE BISULFITE ON TOMATO CALLUS. READINGS ARE A MEAN OF 5 REPLICATES

| | Dry Weight | | | | | Proteins | |
|-------------|------------|-------|-------|-------|-------|-----------------|--------------|
| | 1 | 2 | 3 | 4 | 5 | Mean | mg/g dry wt. |
| Control | 0.71 | 0.62 | 0.67 | 0.60 | 0.65 | 0.65 ±0.017 | 12.4 |
| 10^{-5} M | 0.921 | 0.952 | 0.999 | 0.907 | 1.084 | 0.973 ± .029 | 19.4 |

+ve values significant at 99% confidence limit.

(Graminae).

Uniformly grown, 15 day old seedlings of the various plants were transplanted into pots having soil and compost mixture (1:1). A nutrient solution comprising various major and minor elements as suggested by Bidwell⁵⁶ was added to the soil (to prevent any growth changes brought about by mineral deficiency) once immediately after transplanting and then after interval of 15 days. The plants were sprayed with 10^{-5} M conc. menadione bisulfite in water after 45 days i.e. just before flowering. The spraying was repeated after a week. Each plant received about 50 ml solution i.e. it was sprayed till drip point. Tween-20, at a concentration of 0.5 mg/L. was added as a surfactant. The plants were harvested a week after the second spray. Parameters chosen for studying the effect of menadione bisulfite were fresh weight and dry weight. The harvested plants were kept in an oven at 60° , and since the weight remained constant after 48 hr, dry weight estimations were made after this period.

RESULT: As seen from Table 7, menadione bisulfite at a concentration of 10^{-5} M, brought about a significant increase in growth as estimated by an increase in fresh and dry weights, the most promising being in 'solanaceae'. This could probably be due to their role as 'auxin protectors',

TABLE 7 - EFFECT OF 10^{-5} M MENADIONE BISULFITE ON THE GROWTH OF VARIOUS PLANTS. READINGS ARE A MEAN OF 3 REPLICATES

| Plant | Fresh Wt.(g) | | Dry Wt. (g) | |
|----------|--------------|---------|-------------|---------|
| | Control | Treated | Control | Treated |
| Tomato | 7.6 | 10.8 | 0.65 | 0.97 |
| Capsicum | 6.75 | 10.5 | 2.0 | 3.0 |
| Mungbean | 5.2 | 5.3 | 1.6 | 1.9 |
| Cucumber | 31.6 | 36.6 | 3.3 | 4.5 |
| Corn | 32.8 | 36.1 | 4.6 | 7.6 |

Though this arguement cannot be put forward emphatically as yet, in view of the limited evidence.

DISCUSSION

Generally in bioassay systems, the phenolics (quinones) are known to affect the responses in the auxin-assay systems. In some instances they are seen to inhibit the growth of coleoptile segments, whereas in others they promote it⁵⁷. Some quinones inhibit the development of lateral roots in cuttings, while others promote it⁵⁸. In the bioassay systems assessed, not only specific for auxins, but also for gibberellins and cytokinins, menadione bisulfite (10^{-5} M) showed a response only in the mung bean rooting system, which could imply their auxin like activity. They were, however, without effect in the coleoptile segment bioassay which is also characteristic for the auxins.

It could be possible that the activity of menadione bisulfite involves an interference with the Indole acetic acid (IAA) content of the tissues, and hence in decapitated coleoptiles which have low IAA levels, their responses were not significant. In mung bean cuttings, however, endogenous IAA levels were already high enough and menadione bisulfite 'reacted' on these levels of IAA.

The role of quinones in callus cultures has also been studied⁵⁹ especially in view of the fact that endogenously produced quinones inhibit callus growth by leaching into the medium and accumulating there. The quinones are considered to be similar in their activity to the polyphenols, in view

of the ease in which they can be reduced. Quinones, as oxidised forms of polyphenols, have been characterized as 'auxin-protectors'⁶⁰ where they bring about an enhancement in growth, by preventing the destruction of auxins. The effect of polyphenols supplied exogenously through the medium has indicated a marked increase in fresh weights of callus and negligible increases in dry weights. Quinones are also known to enhance shoot formation in tobacco callus culture⁶¹.

Menadione bisulfite (10^{-5} M) brought about a significant increase in dry weights, probably by 'protecting' the auxins.

From the results obtained in the 'PRELIMINARY SCAN' the growth promotory activity of menadione bisulfite seems quite doubtless, though very empirical. Their role in growth promotion can be indirectly inferred from the nature of responses obtained in these experiments, and the literature available on this subject. However, the basis for drawing such inferences is not sufficiently substantiated by experimental evidence.

An important indication that is worthwhile pursuing is the one suggesting their possible involvement in auxin metabolism, and this has been taken up for further studies in the section entitled 'MODE OF ACTION'.

(ii) MODE OF ACTION

INTRODUCTION

The role that quinones play in growth regulation has been well documented⁶² and their mode of action is based on:

- i) Their role in auxin metabolism
- ii) Their role in general metabolic processes involved in the expression of growth.

It is suggested by many workers⁶³ that quinones are implicated as components of phenol-quinone systems which control, directly or indirectly, auxin metabolism. The enzymatic degradation of IAA has been reviewed by Galston and Hillman⁶⁴. The role of peroxidase in degrading auxin has been demonstrated by Galston et al.⁶⁵. Three theories emerge regarding IAA oxidation.

- a) That there are two separate and distinct enzymes (IAA oxidase and peroxidase) responsible for degrading IAA and other substrates.
- b) That the IAA oxidase activity site and peroxidase activity site resides on the same enzyme molecule having two active centres.
- c) That IAA oxidase activity resides on one member of the family of peroxidases.

There are evidence in favour of all the theories⁶⁶,

though the last one seems to be more accepted.

Phenolics are considered as cofactors for the activity of IAA oxidase. Monophenols are generally seen to enhance IAA oxidase activity, and polyphenols are seen to inhibit it⁴⁶. Various models have been given to account for the phenolic inhibition of the peroxidase catalysed degradation of IAA⁶⁷. The most accepted theory for the role of quinones in such a system is the trapping of free radical intermediates which would otherwise contribute to the oxidation of IAA⁶⁷.

There are reports suggesting the role of ascorbic acid in the peroxidase catalysed degradation of IAA⁶⁸. Both polyphenols and ascorbic acid are said to act as auxin protectors.

The effect of menadione bisulfite (10^{-5} M) on the growth of different tissue organisations indicated its possible role in auxin metabolism, as stated earlier. To understand better such a role of quinones, their effect on the enzymatic oxidation of IAA was taken up for study. Since there is adequate evidence for the involvement of peroxidase (PO), Indole acetic acid oxidase (IAAO), polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO), in the regulation of IAA levels in plants, all these oxidases were studied, using the same crude enzyme extract and specific substrates for each

oxidase.

As mentioned earlier, tomato plants treated with 10^{-5} M menadione bisulfite were used for the enzyme studies one week after the second spraying. The activities of IAAO, PPQ and AAO were estimated by measuring the oxygen uptake in a Warburg respirometer. PO activity was observed spectrophotometrically.

EXPERIMENTAL

Tomato plants were grown and treated with 10^{-5} M menadione bisulfite as discussed earlier.

Studies on IAAO, PPO and AAOa) Preparation of the crude enzyme extract

Fresh tomato tissue (5-10 g) was taken for extraction. The tissue was homogenised with 10 ml chilled distilled water in a cold room (4°). The homogenate was centrifuged for 10 minute. To the supernatant, an equal volume of cold acetone was added till a precipitate appeared. This was then again centrifuged for 10 minute. The supernatant was discarded and the precipitate suspended in 10 ml of citrate-phosphate buffer (0.1 M citric acid, 0.2 M Na_2HPO_4) pH 5.6. This was used as the crude enzyme extract.

b) IAAO, PPO AND AAO activity: For estimating the activity of these enzymes, the method of Sanwal⁶⁹ was used with some modifications. The crude enzyme extract was placed in the main compartment of the Warburg vessel along with 1 ml of 0.05M phosphate buffer, pH 7.0, for AAO and PPO, and citrate phosphate buffer, pH 5.6, for IAAO. The side arms of the vessels received 0.5 ml of 200 $\mu\text{g}/\text{ml}$ solutions of ascorbic acid, catechol and IAA respectively. The central well contained 0.4 ml of 20% KOH and a wick of filter paper was placed in it. For control 0.5 ml distilled water was used instead

of the substrate. The temperature of the bath was maintained at 25° and the flasks were shaken. After equilibration (10 min.) the contents of the side arms were tipped into the main vessel. The amount of O₂ uptake was measured at 10 minute intervals. Activity was expressed as μ l of O₂ taken up/g. fresh wt./hr.

RESULT: As seen from Table 8 menadione bisulfite decreased the activities of indole acetic acid oxidase (IAAO), ascorbic acid oxidase (AAO) and polyphenol oxidase (PPO), the most significant decrease being in IAAO levels (40%).

Studies on Peroxidase (PO)

a) Preparation of the enzyme extract:

Fresh tissue (5-10 g) was homogenized under cold conditions in 0.05 M phosphate buffer, pH 7.5, containing 0.8M KCl and 0.05 M ascorbic acid. This was centrifuged for 10 minute at 2°. The supernatant was then dialysed against cold distilled water for 24 hr. The dialysed extract was used for estimating the PO activity as suggested by Lee⁶¹.

b) PO activity:

The method of Hackett⁷⁰ was adopted using guaiacol as a substrate for the enzyme. To 7.9 ml of phosphate buffer (0.1 M, pH 7.0), 1 ml of enzyme extract was added and also 1 ml of guaiacol (200 mg/L) as substrate. Before taking the

TABLE 8 - EFFECT OF 10^{-5} M MENADIONE BISULFITE ON THE ACTIVITIES OF VARIOUS OXIDASES. THE RESULTS ARE A MEAN OF TWO EXPERIMENTS ON TOMATO PLANTS

| Treatment | μl of O_2/g fresh wt./hr | | |
|-------------|---|-----|-----|
| | IAAO | PPO | AAO |
| Control | 810 | 550 | 225 |
| 10^{-5} M | 500 | 520 | 200 |

(Fresh wt. taken = 10 g

Time = 1 hr)

readings, 0.1 ml of 10 mM H_2O_2 was added and the colour development followed by measuring the absorbance at 470 nm on a spectronic-20 instrument. Blank readings were taken without adding H_2O_2 . One unit of enzyme was considered as the amount of enzyme required, to bring a change in OD by 0.010. The peroxidase specific activity was calculated as units/min/mg protein.

Calculation [C = control, M = treated with menadione bisulfite]

| | |
|--|-----------------|
| Difference in OD over 10 min. | C = 1.046 |
| | M = 0.553 |
| Activity in units | C = 0.1046 |
| | M = 0.0553 |
| Activity in units/min | C = 0.01046 |
| | M = 0.00553 |
| Proteins/ml | C = 460 μ g |
| | M = 440 μ g |
| Specific activity in units/ min/mg. protein | C = 0.023 |
| | M = 0.013 |

RESULT: The decrease in the peroxidase activity was also significant, indicating a regulation of IAA levels by affecting IAA oxidase/peroxidase system.

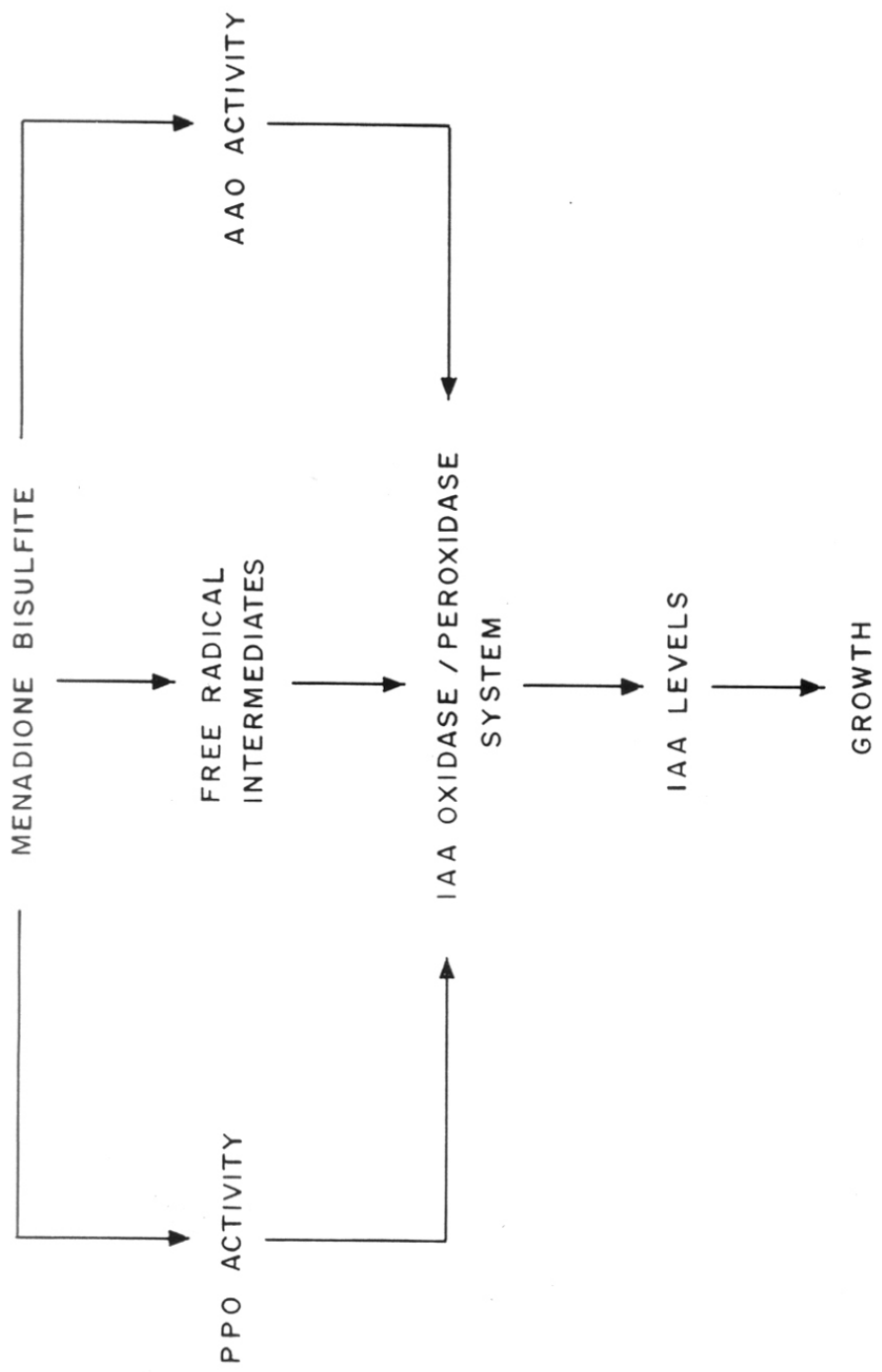
DISCUSSION

To account for the suppression of these enzymes by Menadione bisulfite, several pathways may be possible [Fig.6].

a) One could deal as charge transfer reactions between IAA and quinones. The IAA is oxidised by peroxidases, and the peroxidases are in turn regulated by quinones⁶⁰. The phenolic compounds regulate peroxidases by acting as either electron acceptrs or electron donors. It has been observed by Fieldes and Tyson⁷¹ that an inhibition of peroxidase activity by phenolics could lead to regulation of IAA levels. Quinones could possibly inhibit peroxidase activity by regulating the redox levels in the cell by their strong oxidising potential. They may thus be responsible for the decrease in the formation of free-radical intermediates (produced by peroxidase activity) which may otherwise bring about the oxidation of IAA.

b) The regulation of the levels of the phenolic compounds is carried out by polyphenol oxidase. The polyphenol oxidase system has been known to play a role in regulation of IAA levels⁷². The quinones may possibly be responsible for a decrease in PPO activity by a feedback mechanism. A decrease in PPO activity may bring about an accumulation of phenols which could act as auxin protectors.

FIG. 6.



SCHEMATIC PRESENTATION OF THE PROBABLE MODE OF ACTION OF MENADIONE BISULFITE

c) Ascorbic acid has been recently implicated as an auxin protector by forming an Ascorbic acid-IAA complex⁶⁸. It has also been shown that the efficiency of PO-IAAO is modulated by a slight change in the ascorbic acid concentration or IAA:AA ratio.⁶⁸ The inhibition of AAO by quinones could mean an increase in IAA levels which may be responsible for auxin protection.

Contrary to the mechanism which have been cited in support of increased IAA levels, through the suppression of these oxidases by quinones, there could be other mechanisms, involving some activity other than the regulation of IAA levels which could result in the expression of increased growth. Hence, besides inhibition of these enzymes, quinones could possibly condense with amino acids of other proteins in a system which could lead to an increase in growth⁴³.

To know whether the increase in growth observed is in fact due to IAA levels regulated by the four oxidases, it would be relevant to study the IAA levels, rather, the change in IAA levels brought about by menadione bisulfite treatment. Attempts have been made to correlate the endogenous IAA levels and the growth of plants⁷³, and in some plants it has clearly been shown that IAA levels are highest when the rate of dry-weight increases is maximum⁷⁴. A correlation of IAA levels and growth has also been studied

in mature plants, where a decrease in IAA levels is observed in the ageing leaves⁷⁵.

In the growth of plants, more than the control of IAA levels through its synthesis, the control of IAA levels through its destruction has been clearly observed⁷⁶. This destruction or inactivation is brought about by oxidation involving the IAA oxidase/peroxidase system. Quinones bring about an increase in IAA levels by protecting the IAA from being oxidised by IAA oxidase and as mentioned earlier, ascorbic acid can also act as an agent in auxin protection.

Another way by which quinones can affect the levels of IAA is irrespective of whether they enhance or inhibit IAA oxidase activity. They are seen to enhance the levels of free IAA in plants by affecting the binding properties of IAA⁶³. Quinones have been shown to condense with IAA and hence destroy its activity in vitro⁷⁷ but their action in vivo is equivocal.

In the light of the above argument, it would be logical to examine the changes in the free IAA levels within plants treated with menadione bisulfite, for increased IAA levels could then be correlated to the increase in growth obtained in earlier experiments with menadione bisulfite (10^{-5} M). This would then enable to assign a growth promotory role

to menadione bisulfite, in view of their involvement in the endogenous growth regulation brought about by auxins.

IAA levels were estimated in tomato plants treated with menadione bisulfite and compared with those in untreated plants similar correlations between growth and IAA levels were extended to Capsicum (Solanaceae), Cucumber (Cucurbitaceae) and Corn (Graminae).

The plants were treated with 10^{-5} M menadione bisulfite at regular intervals as mentioned earlier. After harvesting they were subjected to a preliminary purification⁷⁸ as discussed below.

Preparation of the sample: The tissue was homogenized in methanol, the ratio of solvent used being 5 ml to every gram of tissue. The choice of solvent was restricted to methanol since the best yields of IAA have been obtained with it⁷⁸. Extraction was allowed to proceed for 18-20 hr at room temp. the period being kept constant for each sample. This was necessary because more than the absolute values of IAA, the change in IAA levels was to be noted. This also avoided the various problems associated with the quantitation of IAA¹³.

The extract was then filtered and the filtrate concentrated at 40° under reduced pressure. To the aqueous

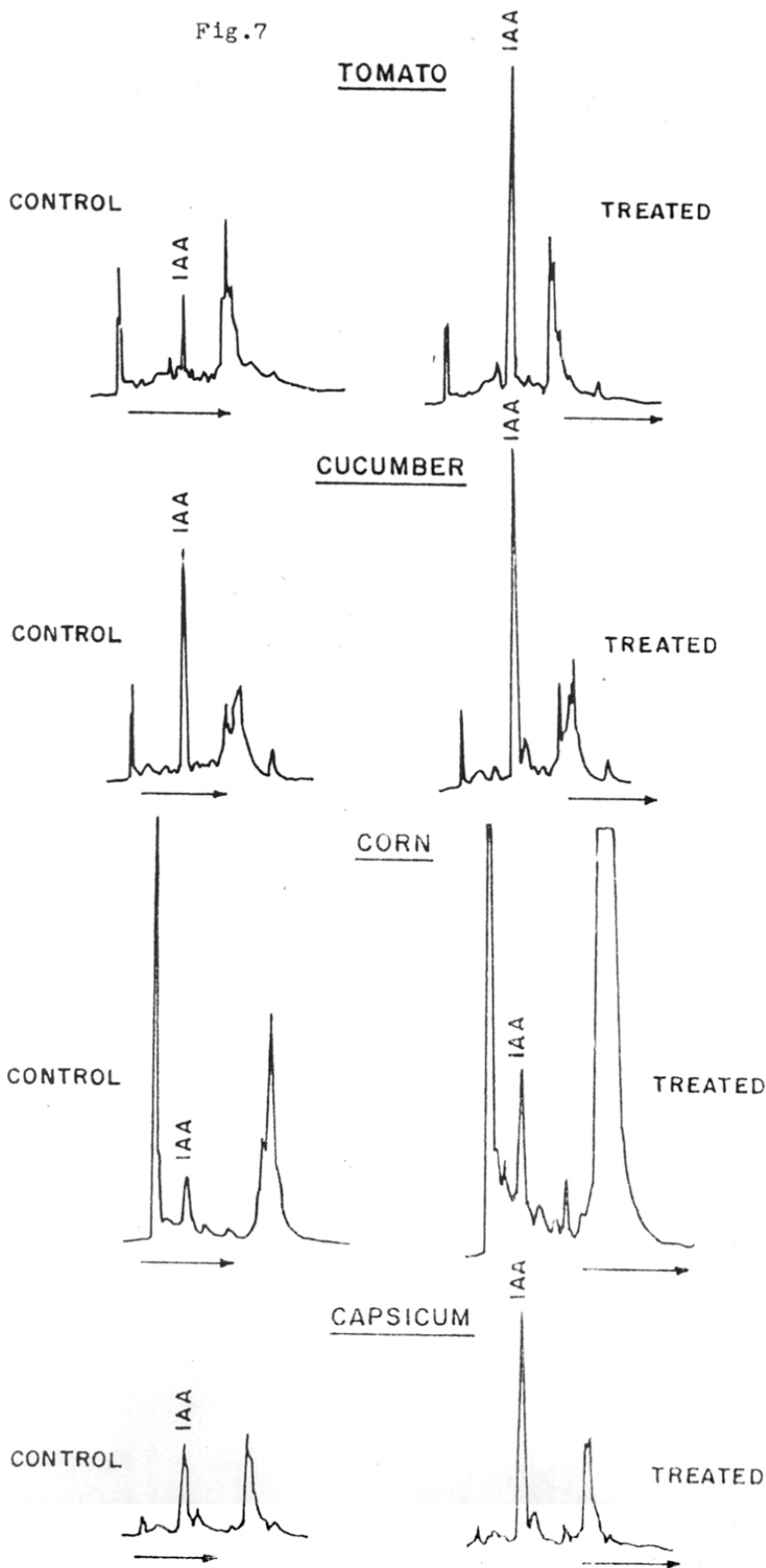
extract, a few drops of NaOH solution were added to make the pH 8.0. This aqueous extract was then partitioned against diethyl-ether aliquots, and the ether fractions discarded. This was done to remove any basic indoles from the extract. The pH was then lowered to 3 with HCl, and the aqueous extract partitioned against diethyl-ether once again, This time the ether fractions contained acidic indoles which included IAA. Ether was evaporated under reduced pressure. The residue remaining in the flask was taken up in a few ml of spectro-photometrically pure methanol and the final volume noted. This served as the sample for IAA estimation.

Since interest was vested in the relative values, rather than absolute values of IAA content, no internal standards were used. However, care was taken to extract the IAA from both controls and treated plants under exactly similar conditions, so as to minimize error fluctuations.

The samples were then chromatographed on Waters HPLC system using a Radical Pak C₁₈ cartridge (8 mm i.d. x 10 cm). A gradient programme was run from 10% methanol in water to 55% methanol in water for 15 minute and then elution was continued in the isocratic mode for about 20 min. at a flow rate of 1.5 ml/min. An UV detector operating at 254 nm was used and the chromatogram plotted on a data module (Fig.7).

Different concentrations of indole acetic acid (IAA)

Fig.7



Comparative study of IAA levels in various plants, 'untreated' (control) and 'treated' with menadione bisulfite solution (10^{-5} M)

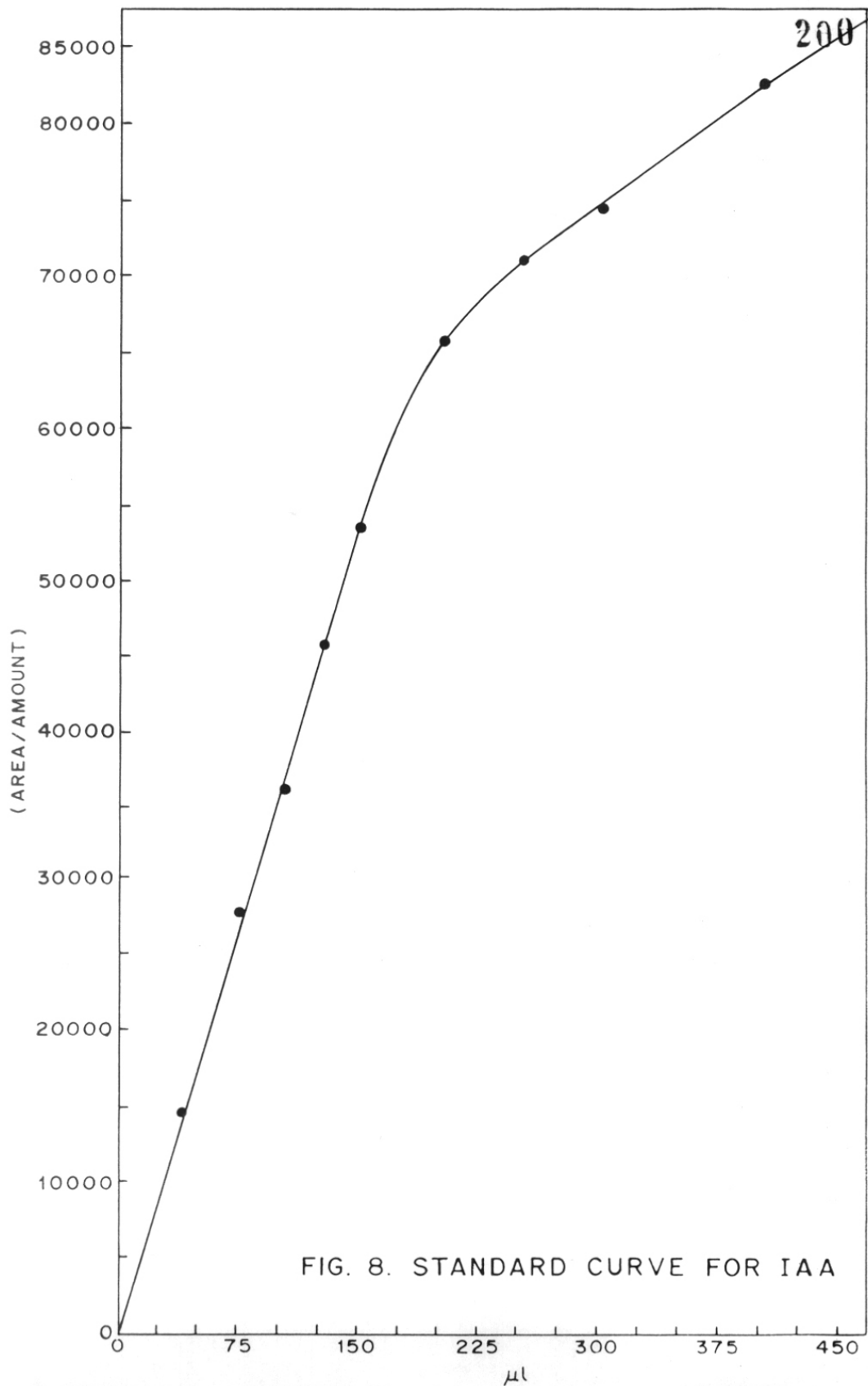


FIG. 8. STANDARD CURVE FOR IAA

TABLE 9 - PEAK AREA/AMOUNTS

| Plant | Control = C | Treated = M | Corresponding value from std. curve | |
|-------------|----------------|----------------|--|----------------|
| | | | Control = C | Treated = M |
| 1) Cucumber | 62740 | 118277 | 175 | 440 |
| 2) Capsicum | 32951 | 87332 | 90 | 410 |
| 3) Tomato | 43920 | 87301 | 120 | 430 |
| 4) Corn | 70747 | 87352 | 220 | 450 |

were injected and the corresponding peak area/amounts noted. A standard curve was plotted (Fig.8) and the relative values of indole acetic acid in control and menadione bisulfite treated plants was deduced from it as depicted in Table 9.

Calculations based on Table 9

C = Control; M = Treated with menadione bisulfite 10^{-5}

[For standard curve 1 mg (IAA) \rightarrow 1000 ml]

i) Cucumber

Amount injected = 25 μ l

Total extract = 2 ml

Therefore, values for 2 ml extract : C = 14.00 μ g

M = 35.20 μ g

Fresh weight of tissue take for extraction = 250 g

∴ Values corresponding to 1000 g

C = 56 μ g/Kg

M = 140 μ g/Kg

ii) Capsicum

Amount injected = 25 μ l

Total extract = 2 ml

∴ Values for 2 ml extract: C = 7.2 μ g

M = 32.8 μ g

Fresh weight of tissue taken for extraction = 200 g

∴ Values corresponding to 1000 g

C = 36.0 μ g/Kg

M = 164.0 μ g/Kg

iii) Tomato

Amount injected = 25 μ l

Total extract = 1 ml

Values corresponding to 1 ml extract: C = 4.8 μ g

M = 180 μ g

Fresh weight of tissue taken for extraction = 140 g

.'. Values corresponding to 1000 g fresh wt.

C = 34 μ g/Kg

M = 130 μ g/Kg

Corn

Amount injected = 25 μ l

Total extract = 2 ml

Values corresponding to 2 ml extract: C = 17.6 μ g

M = 36 μ g

Fresh weight of tissue taken for extraction = 500 g

.'. Values corresponding to 1000 g fresh wt.

C = 35.2 μ g/Kg

M = 72 μ g/Kg

Results: All plants treated with 10^{-5} M menadione bisulfite showed increases in free levels of IAA in the order of 3-4 times the integral values in controls. This increase was observed to be the same on repeating the experiment. On comparing the peak areas obtained in the controls with those

obtained with standard IAA, the values were seen to fall within the ranges reported for IAA levels in the respective plants⁷⁹.

Such correlations between growth increases and IAA levels, have been reported earlier^{73,74} and hence the increased IAA levels observed in the above experiments could be responsible for the growth increases observed.

Finally to assess the possible commercial utility of menadione bisulfite, field trials were carried out using tomato plants and growth in terms of fruit yield increases of about 40-80% were observed in experiments repeated in different seasons (Table 10).

CONCLUSION

The results obtained from the various experiments enabled to draw several inferences about the nature of menadione bisulfite effect on plants. Previous experiments showed an involvement of menadione bisulfite in the enzymatic oxidation of IAA. To confirm whether this oxidation of IAA leads to an increase in IAA levels (ultimately growth) the IAA levels were estimated in treated and unreacted plants. There was a 3-4 fold increase in IAA levels, together with a corresponding increase in fresh and dry weights.

Reports⁸⁰ of the effect of phenolics on the IAA content of tomato plants indicate that phenolics bring about

an increase in IAA content.

These results strengthened the postulated mode of action as depicted in Fig.6, and it seems therefore, that the most probable way in which menadione bisulfite affects IAAO activity and hence IAA levels, is by decreasing the formation of free intermediates which otherwise bring about an oxidation of IAA. This could be possible by the inhibition of peroxidase activity through regulation of redox levels of cells and hence prevent the formation of free radical intermediates which are products of peroxidase activity.

In general the findings have indicated a positive growth regulatory property of menadione bisulfite and probably vitamin K could also be one of the important constituents present in alfalfa responsible for the observed increases in growth on its application.

TABLE 10

Effect of 10^{-5} M menadione bisulfite on the yields of tomato plants. The plants were grown in randomized blockdesign and sprayed as mentioned earlier. The yields were calculated over 8 pickings at weekly intervals. The experiments were performed in two different seasons.

TABLE 10A

| | |
|----------------------------------|---|
| Place: | N. C. L., Pune |
| Plot size: | 3 x 2 m |
| Statistical design: | R.B.D. (Randomize Block Design) |
| No.of replicates: | Four |
| Date of sowing: | 10th Feb. 1983 |
| Date of germination: | 20th Feb. 1983 |
| Date of transplanting: | 4th March 1983 |
| Soaking treatment: | 24 hr (9-2-83) |
| Menadione treatment: | 10^{-5} M in water |
| Spraying of Menadione bisulfite: | 1) 45 days after sowing 2) 52 days after sowing. |

| Treatment | Mean Yield/Plot Kg | % Increase over control |
|--|-----------------------|----------------------------|
| Dry seed (control) | 4.349 | - |
| Seed soaked in menadione bisulfite | 4.525 | - |
| Seed soaked in water + spraying of menadione bisulfite | 4.264 | - |
| Seed soaked in menadione bisulfite + spraying of menadione bisulfite | 4.979 | 14.48 |
| Dry seeds + spraying of menadione bisulfite | 8.004 | 84% |

Standard error/plot = ± 0.78

Critical Difference/plot = ± 2.358

TABLE 10B

| | |
|-------------------------------------|---|
| Place: | N.C.L., Pune |
| Plot size: | 4 x 2.7 m |
| Statistical design: | R.B.D. (Randomize Block Design) |
| No.of replicates: | Four |
| Date of sowing: | 17-7-83 |
| Date of germination: | 27-7-83 |
| Date of transplanting: | 8-8-83 |
| Soaking treatment: | 24 hr (16-7-83) |
| Menadione treatment: | 10^{-5} M |
| Spraying of menadione Bisulfite: | 1) 45 days after sowing 2) 52 days after sowing. |

TABLE 10B (Contd.)

| Treatment | Mean Yield/Plot Kg | % Increase over control |
|--|-----------------------|----------------------------|
| Dry seed (control) | 21.321 | |
| Seed soaked in menadione bisulfite | 31.436 | 46.37 |
| Seed soaked in water + spraying of menadione bisulfite | 29.813 | 38.83 |
| Seed soaked in menadione bisulfite + spraying of menadione bisulfite | 28.971 | 34.90 |
| Dry seeds + spraying of menadione bisulfite | 30.980 | 45.28 |

Standard Error (SE)/Plot = ± 1.465

Critical difference/plot = ± 5.1563

CONTROL

TREATED

D) SYNTHESIS OF MENADIONE

INTRODUCTION

In 1929, during a study of cholesterol metabolism in chicks, Dam⁸¹ observed that chicks fed exclusively on ether-extracted diets developed a deficiency syndrome of the blood, characterized by subcutaneous and intramuscular hemorrhages, anemia and prolonged blood clotting time. Mcfarlane et al.⁸² confirmed these observations; chicks fed on diets containing fish meal as the sole source of fat soluble vitamins showed good growth and low mortality rates, whereas those maintained on diets supplemented with ether extracted fish meal showed a high mortality from hemorrhage, particularly during the 3rd and 4th weeks of life.

Dam demonstrated⁸³ that the syndrome did not result from either a cholesterol deficiency or a deficiency of vitamins A, C, D or E and postulated the existence of a new fat soluble factor necessary for blood clotting, which he designated vitamin K (Koagulations faktor)⁸⁴. Almquist et al.⁸⁵ confirmed this conclusion by producing the same syndrome in chicks using diet consisting of ether-extracted fish meal, ether-extracted brewers yeast, polished rice, cod liver oil and essential salts.

In 1935, Schonheder⁸⁶ postulated that vitamin K activity was associated with the maintenance of sufficient concentrations of the blood clotting accelerator, prothrombin.

Vitamin K was concentrated from natural sources such as fats, alfalfa and fish meal⁸⁷. Vitamin K₁ (24a) was isolated from dehydrated alfalfa⁸⁸ and Vitamin K₂ (24b) from desiccated fish meal⁸⁹.

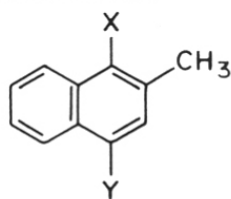
Many modified forms of Vitamin K, have been tested for antihemorrhagic activity in chicks. Of all the fat soluble forms tested, however only menadione (2-methyl-1,4-naphthoquinone/Vitamin K₃) (24c) has been found to be more active than 24a and/or 24b in its pharmacological properties⁴⁰.

Menadione (24c) is effective in preventing hemorrhage, especially in cases designated as 'hypoprothrombinemic'. This term is descriptive of a state in which the blood clotting time is prolonged either by a deficiency of prothrombin, the precursor of the blood clotting enzyme, or by a deficiency of either of two plasma factors. Besides being active in granuloma and edema⁹⁰, menadione shows in vitro antibiotic properties against tubercle bacillus⁹¹.

Many efforts have been made to devise a simple route to menadione (24c). One method employs the oxidation of either 2-methyl naphthalene⁹² or corresponding 1, or 1,4-substituted⁹³ amino, hydroxy or methoxy derivative using CrO₃, Mn₂(SO₄)₃, H₂O₂, FeCl₃ and HNO₃ as the oxidizing agents (Table 11).

The Diels-Alder approach employs condensation between

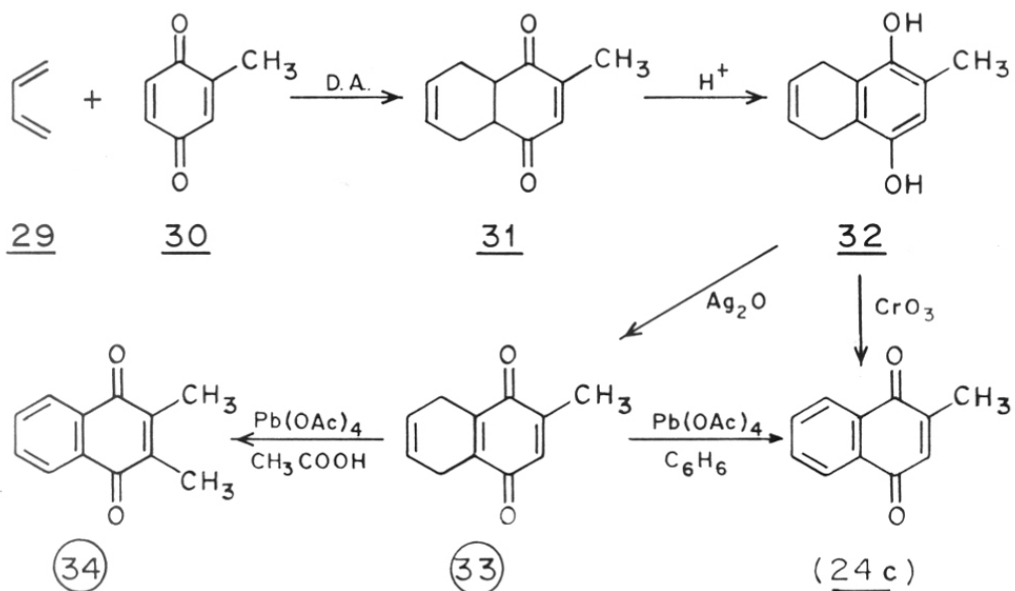
TABLE-11



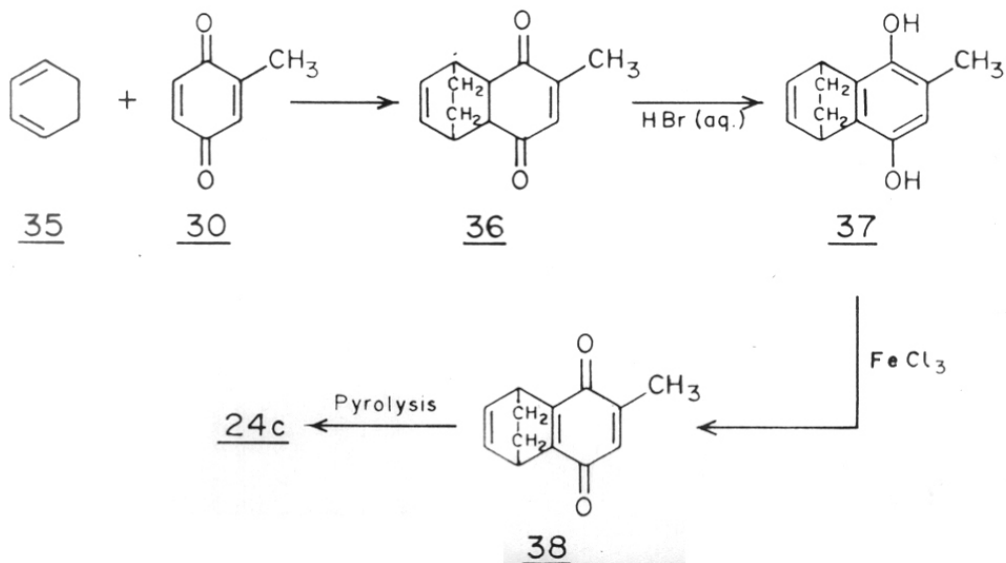
213

X and Y = H, NH₂, OH, OCH₃

SCHEME-12



SCHEME-13



a suitable diene and 2-methyl-1,4-benzoquinone (p-tol-quinone) (30). The intermediate tetrahydro-2-methyl-1,4-naphthoquinone (31) is then isomerized and oxidized to 24c.

Butadiene (29) has been condensed⁹⁴ with 30 giving 5,8,9,10-tetrahydro-2-methyl 1,4-naphthoquinone (31). Fieser et al.⁴¹ isomerized 31 to 5,8-dihydro-2-methyl-1,4-naphthohydroquinone (32) which on chromic acid oxidation gave menadione (24c) in about 50% yield. The hydroquinone 32 was also oxidised with silver oxide, giving 5,8-dihydro-2-methyl-1,4-naphthoquinone (33), which when oxidized with lead tetraacetate in benzene solution gave 24c. On the other hand, treatment of 33 with excess lead tetraacetate in acetic acid solution gave 2,3-dimethyl-1,4-naphthoquinone (34). These reactions are outlined in Scheme 12.

Menadioné (24c) has also been synthesized by the condensation of 1,3-cyclohexadiene (35) with 30 (Scheme 13)⁹⁵. The product of this condensation, 5,8-ethylene-5,8,9,10-tetrahydro-2-methyl-1,4-naphthoquinone (36) was enolized catalytically with aqueous HBr to 5,8-ethylene-5,8-dihydro-2-methyl-1,4-naphthohydroquinone (37), and then oxidized with ferric chloride to 5,8-ethylene 5,8-dihydro-2-methyl-1,4-naphthoquinone (38). Deethanation of 38 by pyrolysis provided 24c.

A total synthesis has been accomplished⁹⁶ by

condensing benzene by means of AlCl_3 with anhydride of methyl succinic acid, which is obtained from citric acid or d tartaric acid. Reaction product α -methyl-benzoyl propionic acid (39) was reduced by Clemmensen method to yield α -methyl-phenylbutyric acid (40). Ring closure of its chloride with AlCl_3 gave 2-methyl- α -tetralone (42) which after subsequent reactions (as shown in Scheme 14) was converted to 24c.

In another classical example of total synthesis (Scheme 15)⁹⁷ naphthalene was sulfonated on the β position to yield β -naphthalene sulfonic acid (44), which was converted to β -naphthoic acid (46) via β -naphthoic acid nitrile (45). Ba salt of 46 upon distillation with Ba formate gave the aldehyde 47 which was elaborated to 24c.

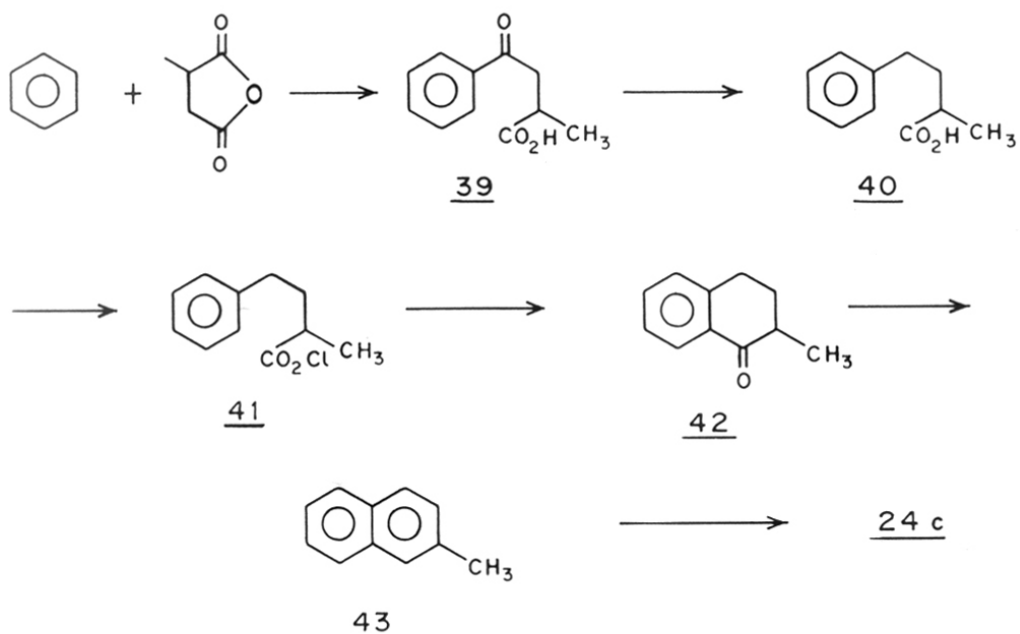
By a new annelation reaction involving crotonaldehyde and p-toloquinone (30) in presence of ethoxymagnesiumbromide, Casnati et al.⁹⁸ obtained 24c in 15% yield.

Vapour phase oxidation of 2-methyl naphthalene (43) with various catalysts has been investigated⁹⁹. However, the yields of the desired product, 24c are unsatisfactory.

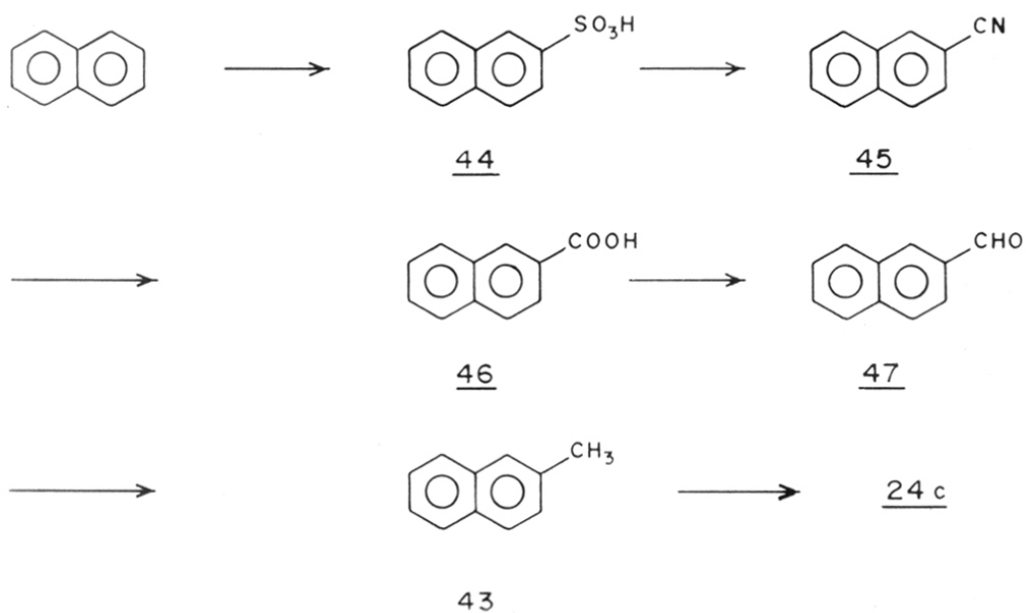
Recently, Torii et al.¹⁰⁰ have synthesised 24c by electrolysis of 2-methyl-5,8-dihydro-1,4-naphthalenediol (32)

SCHEME - 14

216



SCHEME - 15



in a MeCN-t BuOH (9/1) - LiClO₄-(Pt or C electrodes) system.

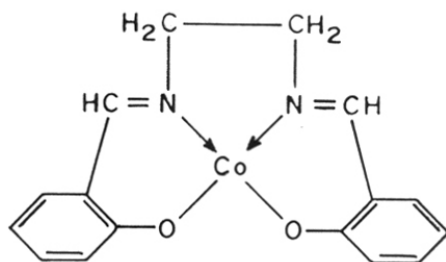
Miller et al.¹⁰¹ have exploited 1,4-dimethoxy-1,3-butadiene as a donor diene in diels-alder cycloadditions for obtaining 24c.

PRESENT WORK

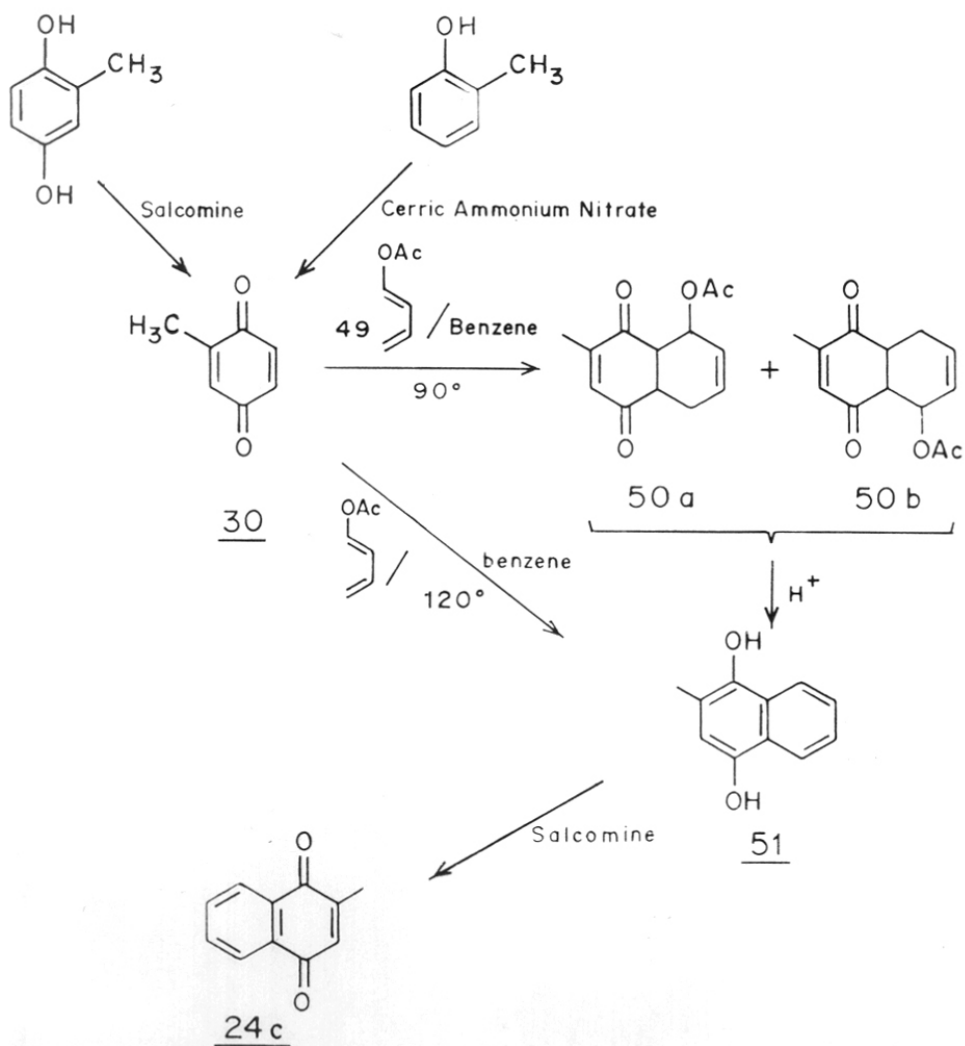
Ever since Fieser⁴⁰ reported that 2-methyl-1,4-naphthoquinone (24c) (Menadione/Vitamin K₃) has been found to be more active than vitamin K₁ (24a) and/or vitamin K₂ (24b) in its pharmacological properties, much attention has been focussed towards its preparation. This 24c being used in the preparation of menadione bisulfite (25) was required in appreciable large quantities [Menadione bisulfite shown to be a promising plant growth regulator (see II-C) has the potential for commercial use].

In spite of numerous approaches, (as discussed in the introductory part) a synthesis that provides a practicable and proven basis for preparing large quantities of 24c seems to be elusive. Infact the oldest method of obtaining 24c by chromic acid oxidation of 2-methyl naphthalene (43), in about 40% yield continues to be the commercial method of choice. In conjunction with the aforesaid problem, numerous approaches were developed, conceptually different from previously described and some of which are potentially flexible for large scale operation.

In general it was found that the oxygen carrier bis-(salicylidene)-ethylenediiminocobalt (II) (salcomine) (48) is a very good catalyst for air oxidation^{102,103} in organic media. Salcomine was prepared according to the known procedure¹⁰⁴.



SALCOMINE (48)

SCHEME-16

2-Methylbenzoquinone (30) is usually prepared from o-cresol using either Fremy salt¹⁰⁵ or iodobenzoic acid¹⁰⁶ or anodic oxidation¹⁰⁷. Preparation of 30 from 2-methylhydroquinone involves either potassium dichromate or trifluoroacetic acid as the oxidising agents.

However in the first approach towards menadione (24c) as depicted in Scheme 16, the requisite starting material 2-methyl benzoquinone (30) was obtained by two different and unknown methods.

Oxidation of o-cresol with ceric ammonium nitrate provided 30 in 60% yield, whereas oxidation of 2-methyl hydroquinone with catalytic amount of salcomine in presence of oxygen using DMF as solvent provided 30 in 93% yield. In both the cases the compound 30 was identical with the published data¹⁰⁸ m.p. 67° (lit.¹⁰⁸ m.p. 68°). The PMR of 30 in CCl₄ showed the methyl group as a doublet at 2.06 (J = 1 Hz). A multiplet at 6.50 represented the proton at 3 position, whereas the protons at 5 and 6 positions appeared as a singlet at 6.60.

1-Acetoxybutadiene (49) was prepared from crotonaldehyde acetic anhydride and freshly fused potassium acetate according to the known procedure¹⁰⁹. Diels-Alder condensation of 30 with 49 at 90° for 16 hr (sealed tube reaction) using dry benzene as solvent provided the adduct,

as isomeric mixture of 50a and 50b (as indicated by PMR). No efforts were made to separate these isomers as subsequent acid isomerization would lead to a single product. Adduct 50 (a and b) after warming with stannous chloride/dil.HCl and usual work up gave 2-methyl-1,4-naphthalenediol (51) in 75% yield. m.p. 170° (lit.¹¹⁰ m.p. 175°, lit.¹¹¹ m.p. 160-178°). M⁺ 174. The PMR of 51 [Fig.9] in acetone-d₆ shows the methyl group as a singlet at 2.16. Another singlet at 6.39 corresponds to 3-H proton. Singlets at 6.79 and 7.79 (both D₂O exchangeable) accounts for the two hydroxyl protons. The remaining aromatic protons are centred as multiplets at 7.00 and 7.76.

In the PMR spectrum of the adduct 50 (a and b), obtained when the Diels-Alder reaction was carried at 90°, besides the required signals, unexpected signals were visible weakly in the aromatic region, indicating the tendency of the compound towards aromatization. This provoked to perform the condensation at an elevated temp.

Interestingly and as expected, the Diels-Alder condensation of 30 with 49 at 120° (sealed-tube) for 30 hr using benzene as the solvent directly provided 51, which was identical in every respect with an authentic sample obtained by the aforesaid method.

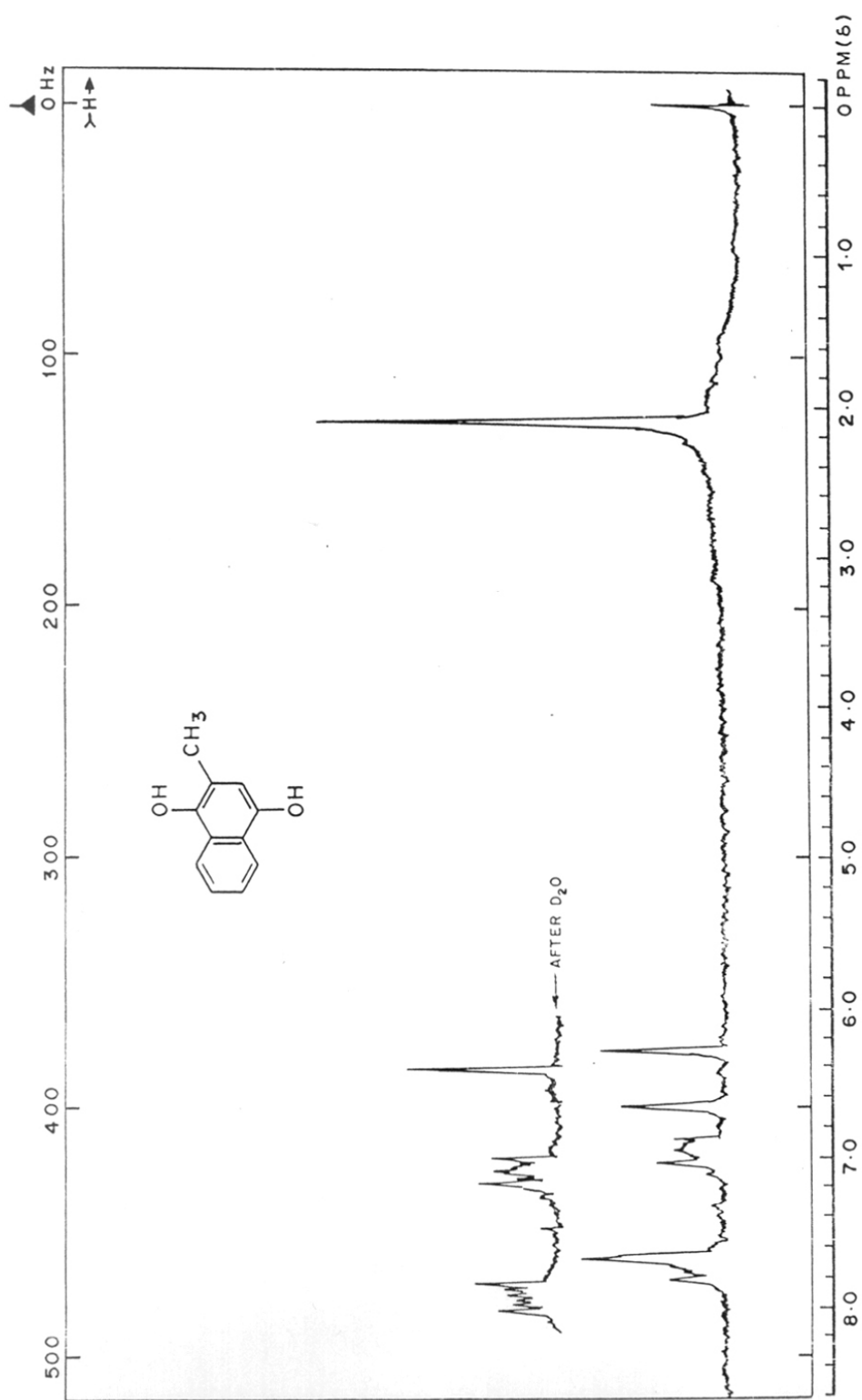


FIG. 9. PMR SPECTRUM OF COMPOUND 51 IN ACETONE- d_6

Oxidation of 51 with catalytic amount of salcomine in presence of oxygen using DMF as solvent and crystallization of the crude product from benzene provided the desired menadione (24c) in 75% yield. m.p. 105° (lit.¹¹² m.p. $105-107^{\circ}$). The PMR of 24c in CCl_4 [Fig.10] shows the methyl group as a doublet ($J = 1 \text{ Hz}$) at 2.16. The vinylic proton appears as a triplet at 6.72 and the aromatic protons extend as a multiplet from 7.5 - 8.10. The mass spectrum showed M^+ at 172.

In the second approach [Scheme-17] 1-hydroxy-2-carboxynaphthalene (52) was required as a starting material. Compound 52 can be synthesized via the carboxylation of α -naphthol. Carboxylation of α -naphthol is classically done by the Kolbe-Schmitt reaction using known literature procedures.

This method is normally quite satisfactory, but is adversely affected by many factors. A practical difficulty in carboxylating the naphthoxide is the maintenance of a strictly anhydrous, uniformly mixed and finely divided solid state. If these conditions are not met, low yields, superheating and the subsequent byproducts often result. Another drawback of the method is the hard caking and charring that usually accompanies the reaction, causing difficult product isolation.

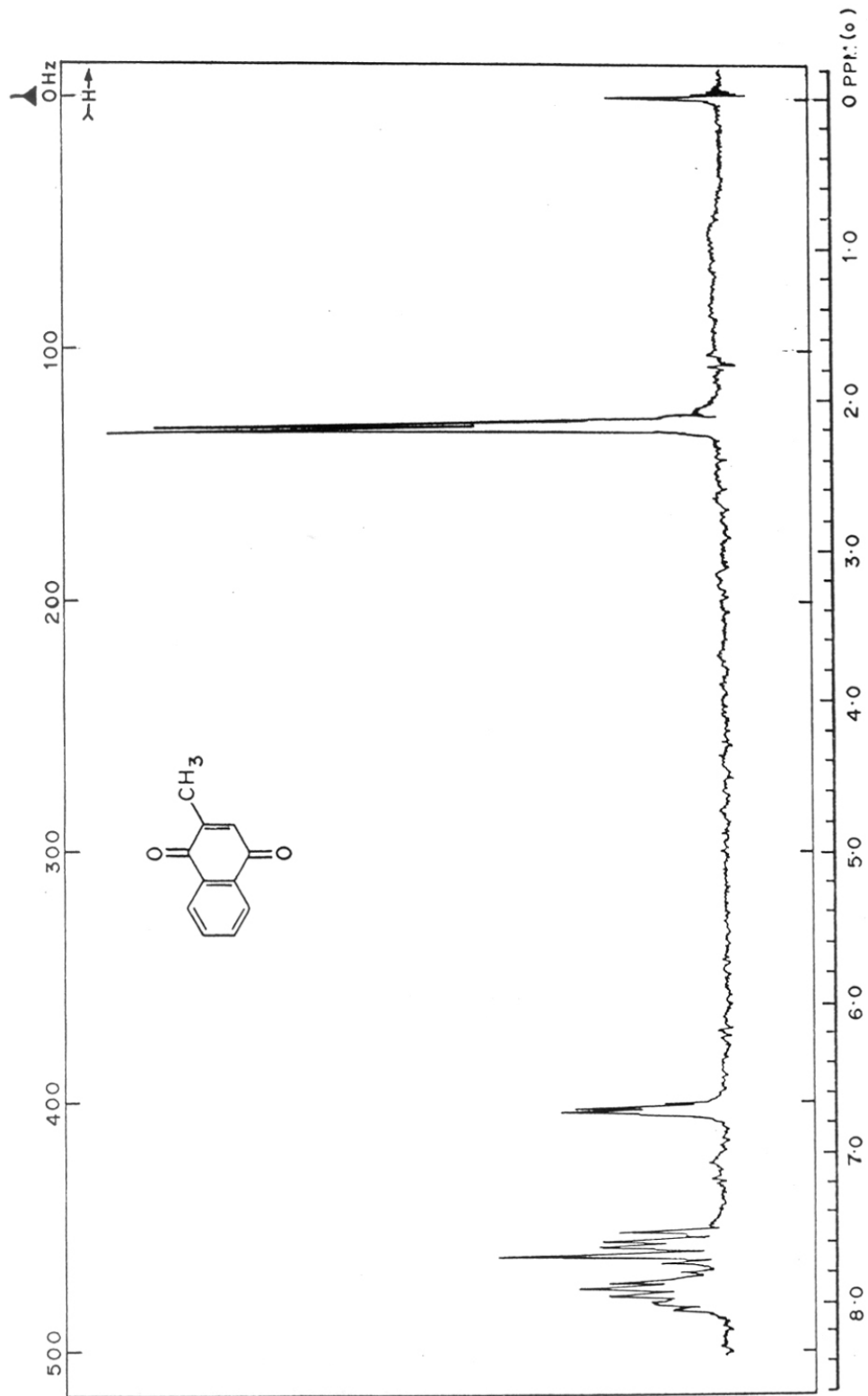
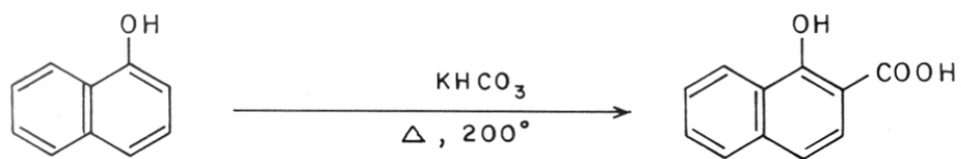


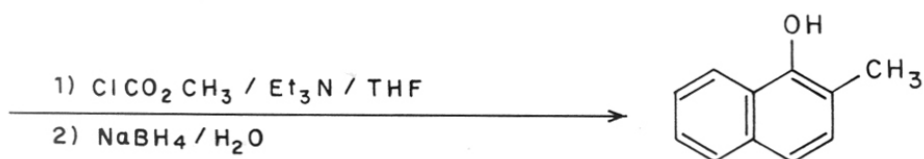
FIG. 10. PMR SPECTRUM OF COMPOUND 24c IN CCl₄

SCHEME - 17

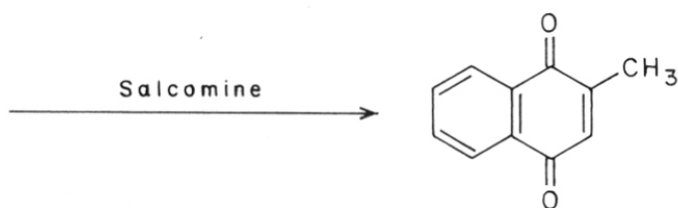
225



52

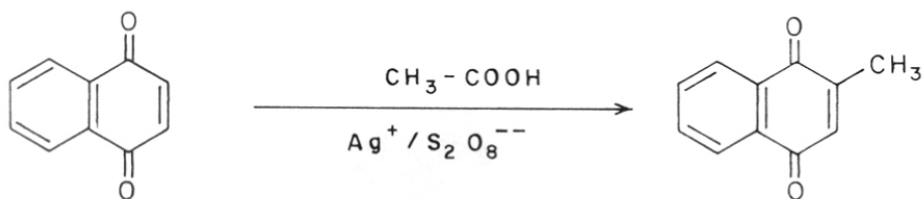


53



24c

SCHEME - 18



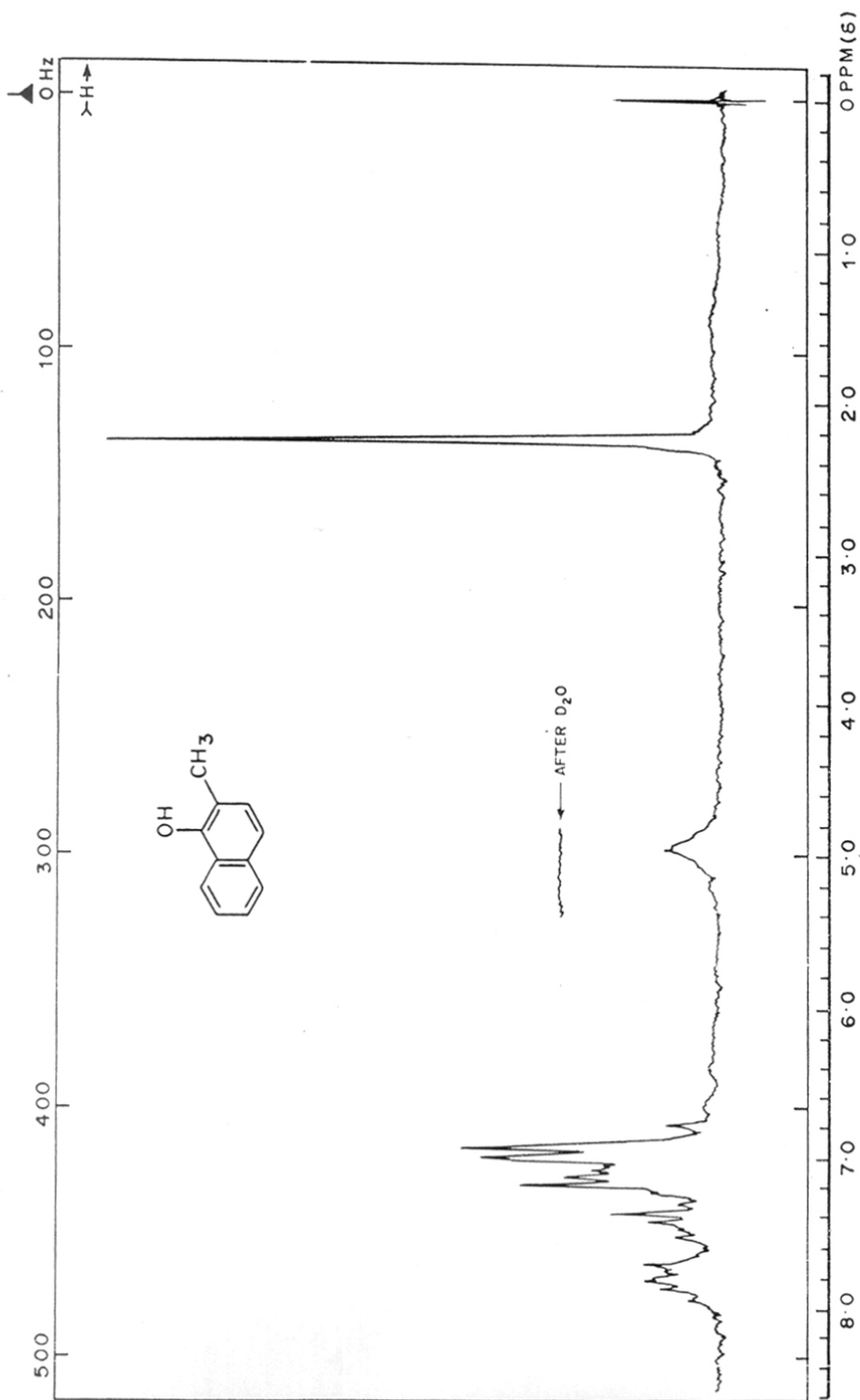
24c

The recently reported¹¹⁴ carboxylation using Stiles reagent, besides being tedious is not economical, mainly because of the difficulty in preparing the reagent. This provoked to seek an alternate route for the preparation of 52.

α -Naphthol was heated with twice its weight of KHCO_3 at 200°C for 5 hr (stainless steel bomb being used) to afford 52 in 60% yield. m.p. 193° [lit.^{115,116} m.p. $190-191^\circ$, m.p. $191-2^\circ$]. The mass spectrum showed M^+ at 188. IR (Nujol) showed stretching at 2920 cm^{-1} (OH) and 1670 cm^{-1} (COOH).

The unreacted α -naphthol was easily recovered and the process recycled. Apparently it seems that increasing the amount of KHCO_3 would enhance the yield. However using excess of KHCO_3 (four times the weight of α -naphthol) did not significantly increase the yield.

Compound 52 on treatment with methyl chloroformate and triethylamine in THF as solvent, followed by sodium borohydride reduction directly provided 2-methyl-1-naphthol (53) in 80% yield. m.p. 60° (lit.¹¹⁷ m.p. 61°). The PMR spectrum of 53 in CCl_4 [Fig.11] shows the methyl group as a singlet at 2.23. A broad singlet at 5.0 (D_2O exchangeable) accounts for the hydroxyl proton. The remaining aromatic protons extend as a multiplet from 6.89 - 7.90. The IR (Nujol) showed stretching at 3650 cm^{-1} (OH).

FIG. 11. PMR SPECTRUM OF COMPOUND 53 IN CCl₄

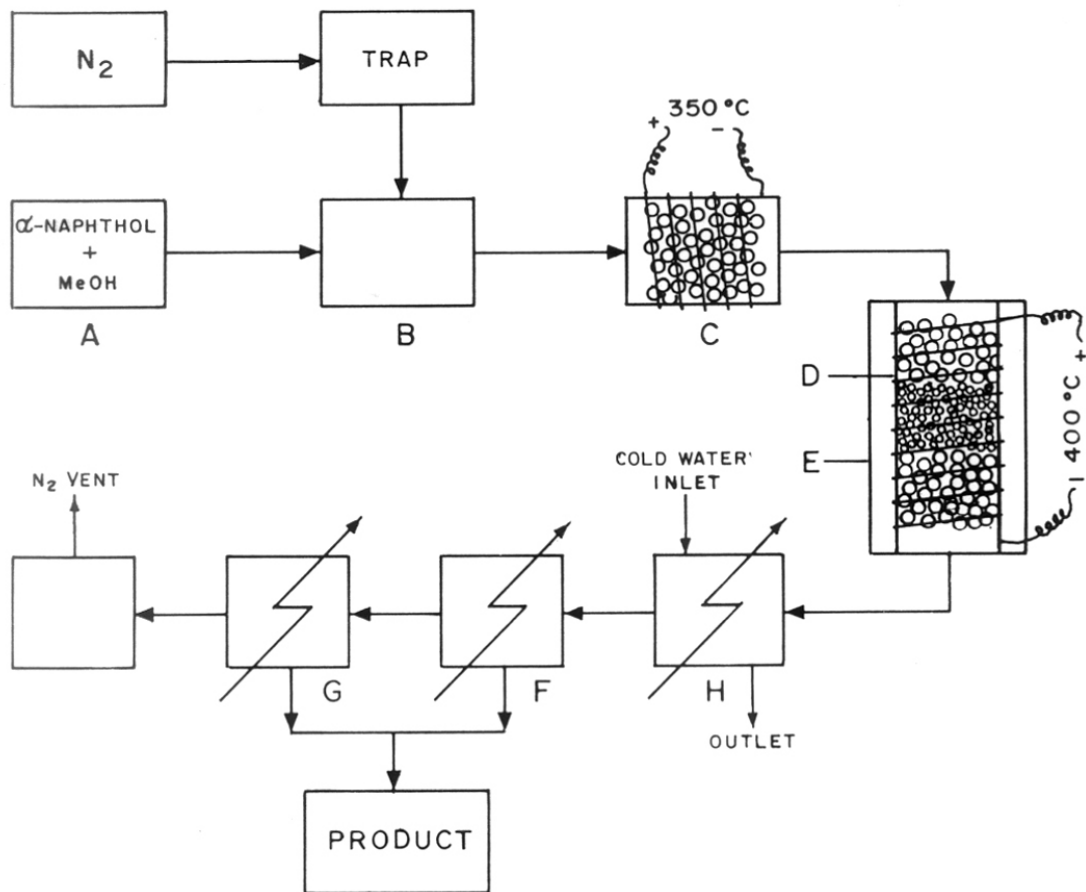
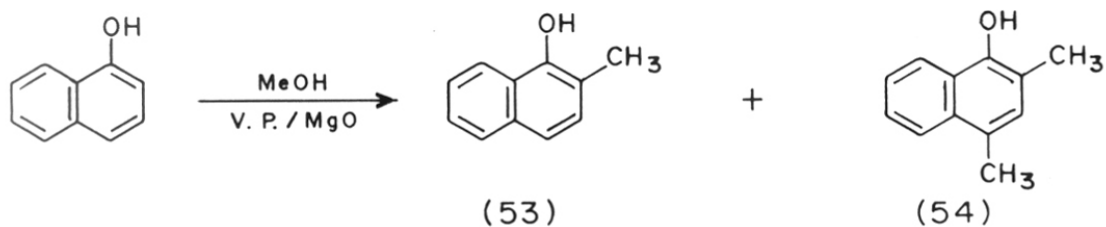
Horii et al.¹¹⁷ oxidised 53 with CrO_3 /glacial acetic acid at 20° for 48 hr to yield menadione (24c) in 37% yield.

However, in order to further exploit the utility of salcomine, compound 53 was oxidised with catalytic amount of salcomine, in presence of oxygen using DMF as solvent to provide in the first instance 25% menadione (24c) [Remaining being starting material]. Thus salcomine had the predominant advantage over other oxidizing agents in providing an overall 80% of the required menadione (24c) (on the basis of the unreacted starting material, 2-methyl-1-naphthol (53) which could be recovered by alkali extraction, acidifying and recycling the entire process).

The menadione (24c) thus obtained was identical in every respect with an authentic sample obtained by the first method.

Vapour phase alkylation of α -naphthol in absolute methanol (Fig.12) using MgO as catalyst provided a product consisting a mixture of 2-methyl-1-naphthol (53) and a dimethyl-1-naphthol, both having the same R_f value on the TLC and hence unseparable by column chromatography. The PMR spectrum of the product showed two singlets at 2.10 and 2.12 corresponding to the two methyls of the dimethyl product and a singlet at 2.50 corresponding to the methyl

FIG. 12. BLOCK DIAGRAM FOR VAPOUR-PHASE ALKYLATION 229



- A REACTANTS (α -NAPHTHOL AND METHANOL)
- B RECEIVER
- C PREHEATER CONTAINING PORCELAIN BEADS
- D SILICA TUBE CONTAINING MgO (CATALYST)
- E REACTOR ENCLOSING THE TUBE
- H CONDENSER FOR CIRCULATING COLD WATER
- F & G-COOLING SYSTEM FOR COLLECTING THE PRODUCT

group of the monomethyl product. The remaining signals corresponding to the aromatic protons were not amenable to a single product.

In order to ascertain whether the dialkyl product was 2,4-dimethyl-1-naphthol (54) or the second alkylation had occurred in the other ring, the product mixture was subjected to CrO_3/AcOH oxidation with the logical reasoning that if the second alkylation had occurred in the 4-position giving 54, then it cannot undergo any oxidation.

After oxidation, the Rf values of the products, as seen from TLC were significantly different to be separated by column chromatography.

The major product was identified (PMR, m.p.) as menadione (24c) which was identical in every respect with an authentic sample, thereby indicating that 2-methyl-1-naphthol (53) was the major product during alkylation.

The other product, having the same Rf value on TLC corresponding to the starting product mixture was identified as 2,4-dimethyl-1-naphthol (54) m.p. 82° (lit.¹¹⁸ m.p. 84°). The PMR of 54 in CCl_4 showed two singlets at 2.10 and 2.28 corresponding to the 2-methyls. The hydroxyl group appeared as a broad singlet at 5.10. The 3-H proton appeared as a singlet at 6.90 and the remaining aromatic

protons extended as a multiplet from 7 - 7.80.

In yet another approach [Scheme 18] menadione (24c) was obtained in one step, in 60% yield, by alkylation of 1,4-naphthoquinone with radical obtained from the decarboxylation of glacial acetic acid with the silver ion peroxodisulfate system.

When acetic acid was taken in 1.3 moles excess, the unreacted naphthoquinone was separated from the required menadione (24c) by column chromatography. Too much excess (greater than 1.5 moles) of gl. acetic acid was avoided to prevent formation of dialkyl products.

The menadione thus obtained was identical in every respect with an authentic sample (m.p., PMR).

E X P E R I M E N T A L

Preparation of Salcomine (48)

Salcomine was prepared according to the known procedure¹⁰⁴.

Preparation of 2-methyl benzoquinone (30)A) Using Cerric ammonium nitrate (CAN)

A solution of C.A.N. (2.48 g, .0046 mole) in 12 ml H₂O was added portionwise and with stirring to a solution of o-cresol (0.2 g, .0018 mole) in 10 ml of acetonitrile. The mixture was further stirred at room temp. for 12 hr and poured into ice water (50 ml). It was extracted with methylene chloride (2 x 25 ml), dried over anhydrous Na₂SO₄ and solvent removed under reduced pressure. Crystallization of the crude product from benzene gave ³30 (0.135 g, 60%) m.p. 67° (lit.⁶ 108 m.p. 68°). [PMR (CCl₄): δ 2.06 (d, J = 1 Hz, 3H, CH₃), 6.50 (m, 1H, 3H), 6.60 (s, 2H, 5,6-H).] δ

Analysis: Calculated for C₇H₆O₂: C, 68.85; H, 4.91; Found: C, 68.62; H, 4.89%.

B) Using salcomine

In a 50 ml R.B. flask equipped with a balloon containing oxygen were placed 1.24 g (.01 mole) of methyl hydroquinone in 7 ml of DMF and 0.125 g (0.4 m.mole) of salcomine. After stirring for 2 hr at room temp. the reaction

mixture was poured into 50 g of crushed ice containing 5 ml of 4N HCl. The brown precipitate that separated was collected by suction filtration, washed with 1N HCl (3 x 10 ml) and H₂O (3 x 25 ml). Crystallization from benzene gave (1.14 g, 93%) of 30 m.p. 67° (lit. ⁶108 m.p. 68°).

The compound 30 was identical in every respect with an authentic sample obtained by the previous method.

② Preparation of 1-acetoxybutadiene (49)

1-Acetoxybutadiene was prepared from crotonaldehyde, acetic anhydride and potassium acetate according to the known procedure ¹⁰⁹.

③ Preparation of Diels-Alder adduct 50 at 90°

Method A The mixture of 2-methyl-1,4-benzoquinone (30) [0.6 g, 4.9 m.mole], 1-acetoxybutadiene (49) (0.66 g, 5.9 m.mole), dry benzene (15 ml) were sealed in a thick corning tube and heated at 90° for 16 hr. The solvent was distilled off under reduced pressure, to afford the adduct, 4, which was immediately put for the next step.

2-methyl-1,4-naphthalene diol (51)

The adduct 50, (1.0 g, .0042 mole) in 15 cc alcohol was warmed on a steam bath with a mixture of 0.1 g of stannous chloride in 2 cc of conc. HCl and 5 cc of H₂O. After 1 hr, the solution was diluted with H₂O (50 ml),

extracted with methylene chloride (4 x 25 ml) and dried over anhydrous Na_2SO_4 . Removal of the solvent and crystallization of the crude product from MeOH afforded 51 (0.55 g, 75%) m.p. ¹⁷⁴170° (lit. ¹¹⁰m.p. 175°, lit. ¹¹¹m.p. 160-178°). PMR (acetone- d_6) δ 2.16 (s, 3H, CH_3), 6.39 (s, 1H, H-3), 6.79 (s, 1H, OH (D_2O exchangeable)), 7.00 (m, 2H, H-6 and 7), 7.76 (m, 2H, H-5 and 8), 7.79 (s, 1H, OH (D_2O exchangeable)) M^+ 174.

Analysis: Calculated for $\text{C}_{11}\text{H}_{10}\text{O}_2$: C, 75.86; H, 5.74; Found: C, 75.80; H, 5.69%.

Method B

2-methyl-1,4-naphthalenediol (51) via Diels-Alder

The mixture of 2-methyl-1,4-benzoquinone (30) [0.6 g, 4.9 m.mole], 1-acetoxybutadiene (49) [0.66 g, 5.9 m.mole], dry benzene (15 ml) were sealed in a thick corning tube and heated at 120° for 30 hr. The solvent was distilled off under reduced pressure. The crude product so obtained was crystallized from methanol to afford 51 (0.510 g, 60%).

The compound 51 obtained by this method was identical with the authentic sample obtained by the aforesaid method.

2-methyl-1,4-naphthoquinone (24c)

In a 50 ml R.B. flask equipped with balloon containing oxygen were placed 51 (0.9 g, 5.2 m.mole) and salcomine (.065 g, 0.18 m.mole) in 7 ml of DMF. After stirring for

6 hr at room temp. the reaction mixture was poured into crushed ice (100 g) containing 5 ml of 4N HCl. The brown precipitate which separated was collected by suction filtration, washed with 1N HCl (3 x 10 ml) and H₂O (3 x 25 ml). Usual

work up and
Crystallization of the crude product from benzene afforded 224c (0.660 g, 75%), m.p. 105° (lit. ¹¹² m.p. 105-107°).

PMR (CCl₄): δ 2.16 (d, J = 1 Hz, 3H, CH₃), 6.72 (t, 1H, H-3), 7.50 - 8.10 (m, 4H, ArH), M⁺ 172.

Analysis: Calculated for C₁₁H₈O₂: C, 76.76; H, 4.65;
Found: C, 77.06; H, 4.75%.

1-Hydroxy-2-carboxynaphthalene ⁶ (52)

α-Naphthol (5.0 g) and KHCO₃ (10.0 g) were finely powdered and heated to 200° for 5 hr in a metal bomb. Saturated solution of sodium bicarbonate (100 ml) was added to the contents and stirred for 0.5 hr. The unreacted α-naphthol was filtered off.

The resultant filtrate was acidified with HCl, to yield (3.8 g, 60%) of ⁶ 52 as a white crystalline material m.p. 193° (lit. ^{115, 116} ¹² m.p. 190-1°, m.p. 191-2°), M⁺ 188. IR (Nujol) 2920 cm⁻¹ (OH), 1670 cm⁻¹ (COOH).

Analysis: Calculated for C₁₁H₈O₃: C, 70.21; H, 4.25;
Found: C, 70.11; H, 4.20%.

2-methyl-1-naphthol ⁷ (53)

Methyl chloroformate (1.95 g, .018 mole) was added *omit*

[to a solution of ⁶52 (1.83 g, 9.7 m.mole) and triethylamine (1.82 g, .018 mole) in tetrahydrofuran (20 ml) at 0° over a period of 30 minute. After standing for 45 minute at the same temperature, the white precipitate (triethyl ammonium chloride) was filtered off and washed with tetrahydrofuran (10 ml). The combined filtrate was added to a solution of sodium borohydride (1.21 g, .032 mole) on water (25 ml) with stirring at 0-5° over a period of 1 hr. When the addition was complete, the reaction mixture was stirred at room temp. for 3 hr, then diluted with H₂O (100 ml); made acidic with dil. HCl and extracted with benzene (2 x 25 ml). The benzene layer was extracted with 5% aqueous NaOH (2 x 25 ml). The aqueous layer was neutralized with dil. HCl and extracted with benzene (3 x 25 ml).]

The benzene layer was washed with H₂O (3 x 25 ml), 5% aqueous NaHCO₃ (2 x 25 ml) and dried over anhydrous Na₂SO₄.

Removal of the benzene in vacuo provided (1.20 g, 80%) of 53. m.p. 60° (lit. ¹¹⁷m.p. 61°). [PMR (CCl₄): δ 2.23 (s, 3H, CH₃), 5.0 (bs, 1H, OH (D₂O exchangeable), 6.89 - 7.90 (m, 6H, ArH). M⁺ 158. IR (Nujol): 3650 cm⁻¹ (OH).]

2-methyl-1,4-naphthoquinone (²24c)

A mixture of compound 53 (⁷0.4 g, .002 mole), salcomine (.015 g, .048 m.mole) and dry DMF (25 ml) were taken in a 50 ml R.B. flask fitted with an oxygen balloon and stirred at

room temp. for 14 hr. ⁸ The TLC checked in 5% acetone in benzene indicated the formation of menadione (24c) (corresponding to an authentic spotting) along with the unreacted starting material, 53. The reaction mixture was poured into 100 g of crushed ice containing 5 ml of 4N HCl. This was ~~and~~ extracted with methylene chloride (3 x 25 ml).

The organic layer was washed with 10% aqueous NaOH (4 x 25 ml),

The ~~organic layer was~~ concentrated and the crude product so obtained was crystallized from benzene to afford menadione (24c) (.108 g, 25%) which was identical in every respect with an authentic sample obtained by the previous method.)

The alkali layer was acidified with dil. HCl, extracted with methylene chloride (3 x 25 ml) and dried over anhydrous Na_2SO_4 . It was ~~distilled off under reduced pressure and~~ the product 53 so obtained (0.3 g) was again put for oxidation (process recycled).

Finally 24c (.348 g) was obtained in overall 80% yield.

cont. on page 239

Preparation of MgO catalyst¹¹⁹

90 g of MgCO_3 was suspended in 450 ml of H_2O and heated to 60° with mechanical stirring for 1 hr. Stirring was continued for 1 hr more without heating. It was cooled,

filtered, dried initially at room temp. and then at 110° for 1 hr.

50 g was taken for calcination and the calcined MgO was pelletised, crushed and sieved in 22 mesh size.

Vapour phase alkylation

A silica tube, 19 mm internal diameter was filled to a height of 20 cm with MgO catalyst, which was supported with chunks of porcelain beads. This tube was enclosed by a reactor. To the tube was fitted a condenser (with cold water circulated) at the bottom and a preheater made of glass, at the top. The preheater was packed with chunks of porcelain beads. The silica tube and preheater, contacted with thermocouples, were insulated with asbestos, wound with nichrome electric heaters and further insulated with asbestos.

While the preheater was maintained at 350°C and reactor at 400°C , 1 g of α -naphthol dissolved in 50 cc of absolute methanol was added dropwise (2 drops per minute) from an adjustable syringe. Vapour emerging from the tube was well condensed (an additional trap was set to prevent escape) and collected.

To the product obtained, in gl. AcOH (10 ml) was added a solution of CrO_3 (1.2 g) in 80% AcOH (10 cc) at such a rate that temp. did not exceed 20° . After the addition

the contents were stirred at room temp. for 30 hr and poured into ice water. It was extracted with methylene chloride (3 x 25 ml), dried over anhydrous Na_2SO_4 and finally distilled off under reduced pressure. Purification of the crude product over silica gel column using 5% acetone in benzene as the eluent afforded 24c (0.47 g) as the major product, which was identical in every respect with an authentic sample. *and*

The other compound (0.28 g) was identified as 2,4-dimethyl naphthol (54) m.p. 82° (lit.¹¹⁸ m.p. 84°). PMR (CCl_4) 2.10 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 5.10 (s, 1H, OH (D_2O exchangeable)), 6.90 (s, 1H, H-3), 7.0 - 7.80 (m, 4H, ArH). M^+ 172. *38*

2-methyl-1,4-naphthoquinone (24c) *21* ✓

To the vigorously stirred solution of 1,4-naphthoquinone (0.79 g, .005 mole), gl.AcOH (0.39 g, .0065 mole), silver nitrate (0.5 g) in acetonitrile (15 ml) and H_2O (15 ml) at 80°C was added ammonium peroxydisulfate (1.48 g, 0.065 m) in H_2O (20 ml) during 15 minute. After stirring at 80° for further 4 hr, mixture was cooled to room temp. and H_2O (100 ml) was added. It was extracted with methylene chloride (3 x 25 ml) dried over anhydrous Na_2SO_4 and finally distilled off under reduced pressure. Purification of the crude mixture over silica gel column using 5% acetone in

benzene as the eluent afforded ²24c (0.510 g, 60%) and the remaining (40%) being the starting material. //

Ⓢ [The menadione (²24c) obtained was identical in every respect with an authentic sample obtained by the previous approaches.]

REFERENCES

- 1 R. Bloch
"Encyclopaedia of Plant Physiology"
Vol. XIV, pages 1-14, Ed. W. Ruhland, Springer-Verlag,
Berlin (1961).
- 2 F.C. Steward
"Growth and Organisation in Plants"
pages 1-37, Addison Wesley, Reading, Mass. (1968).
- 3 H. Burstrom
"Encyclopaedia of Plant Physiology"
Vol. XIV, pages 325-329, Ed. W. Ruhland,
Springer-Verlag, Berlin (1961).
- 4 J. Sachs,
Arb. bot. inst., 2, 689 (1882).
- 5 H. Fitting
S. Bot., 1, 1 (1909).
- 6 B.G. Pickard
Bot. Rev., 39, 172 (1973).
- 7 H.B. Tukey, F.W. Went, R.M. Muir and J. Van Overbeck
Plant Physiol., 29, 307 (1954).
- 8 K.V. Thimann
"The Physiology of Plant Growth and Development"
Pages 3-45, Ed. M.B. Wilkins, McGraw-Hill, New York (1969).
- 9 F. Skoog and D.J. Armstrong
Ann. Rev. Plant Physiol., 21, 359 (1970).
- 10 M.K. Chailakhyan
Ann. Rev. Plant Physiol., 19, 1 (1968).
- 11 E.A. Schneider and F. Wightman
Ann. Rev. Plant Physiol., 25, 487 (1975).
- 12 L.G. Nickell
Chemical and Engg. News, 56(41), 18 (1978).
- 13 D.S. Letham, T.J.V. Higgins, P.B. Goodwin and J.V.
Jacobsen
"Phytohormones and related compounds" - A comprehensive
treatise, Vol. I, pages 1-27, Ed. Letham D.S./North
Holland Biomedical Press, Amsterdam (1978).

- ✓14 W. Bleyberg and H. Ulrich
Chem. Ber., 64B, 2504 (1931).
- 15 S.K. Ries, V. Wert, C.C. Sweely and R.A. Leavitt,
Science, 195, 1339 (1977).
- 16 A.C. Chibnall, E.F. Williams, A.L. Latner and
S.H. Piper
Biochem. J., 27, 1885 (1933).
- 17 S.K. Ries and W. Violet
Planta, 135, 77 (1977).
- ✓18 G.M. Robinson
J. Chem. Soc., 1543 (1934).
- ✓19 R.G. Jones
J. Am. Chem. Soc., 69, 2350 (1947).
- ✓20 H. Oura, J. Hase, K. Honda and S. Fukai
J. Pharm. Soc. Japan, 76, 1433 (1956).
- ✓21 N.R. Hunter and J.L. Charlton
Org. Prep. and Procedures Int., 13(1), 19 (1981).
- ✓22 R.E. Bowman, D.E. Ames and R.G. Mason
J. Chem. Soc., 174 (1950).
- ✓23 A. Watanabe
Bull. Chem. Soc. Japan, 34, 398 (1961).
- ✓24 H. Schildknecht and G. Renner
Felte Seifen Anstrichmittel, 66, 176 (1964).
See also Chem. Abstr., 61, 15966h (1964).
- ✓25 K. Maruyama, K. Terada and Y. Yamamoto
J. Org. Chem., 45, 737 (1980).
- ✓26 A.V. Rama Rao, M.N. Deshmukh and M. Kamalam
Tetrahedron, 37, 227 (1980).
- ✓27 A.V. Rama Rao, J.S. Yadav and G.S. Annapurna
Synth. Commun., 13(4), 331 (1983).
- ✓28 M.S. Chadha, V.R. Mamdapur and S.M. Kulkarni
Indian J. Chem., 22B, 683 (1983).
- 29 L. Brandsma
"Preparative Acetylenic Chemistry", New Publishing
Company, p.188 (1971).

- 30 P. S. Reddy and J.S. Yadav
Synth. Commun., 1983 (in press).
- 31 E.M. Reid, J.R. Ruhoff and R.E. Burnett
Organic Synthesis, Coll. Vol.2, p.246 (1946).
- 32 C.A. Brown and A. Yamashita
J. Chem. Soc. Chem. Commun., 959 (1976).
- 33 L. Brandsma
"Preparative Acetylenic Chemistry", New Publishing Co.
page 20 (1971).
- 34 S.K. Ries and K. Wert
J. Plant Growth Regulation 1, 117 (1982).
- 35 A.B. Erickson, G. Sellden, D. Skogen and S. Nilsen
Planta, 152, 44 (1981).
- 36 R.E. Hoagland
Bot. Gaz., 141, 53 (1980).
- 37 S. Lewak and E. Skowronska
Physiol. Plant., 55, 1983 (1982).
- 38 T.H. Maugh
Science, 212, 33 (1981).
- 39 H.J. Almquist
J. Biol. Chem., 114, 241 (1936).
- 40 L.F. Fieser, M. Tushler and W.L. Sampson
J. Biol. Chem., 137, 659 (1941).
- 41 L.F. Fieser and E.M. Fry
J. Am. Chem. Soc., 62, 228 (1940).
- 42 D.B. Knaff, R. Malkin, J.C. Myron and M. Slotter
Biochim. Biophys. Acta, 459, 402 (1977).
- 43 G.L. McNew and H.P. Burchfield
Contrib. Boyer. Thompson Inst., 16, 357 (1950) [Reports].
- 44 C.F. Van Sumere, J. Albrecht and A. Dedonder
"The Chemistry and Biochemistry of Plant Proteins"
pages 211-264. Ed. J.B. Harborne and Van Sumera,
cf. Academic Press New York (1975).

- 45 F.C. Steward and A.D. Krikorian
"Plants, Chemicals and Growth", pages 37-39,
Academic Press, New York (1971).
- 46 N.B. Mandava
"Plant Growth Substances", pages 135-213, ed.
N.B. Mandava, Symposium series, III, American Chemical
Society, Washington DC (1979).
- 47 R.R. Baker, T.H. Davies, L. McElroy and G.H. Carlson
J. Am. Chem. Soc., 64, 1096 (1942).
- 48 K.K. Nanda and P. Kaur
Indian J. Plant Physiol., 10, 1 (1967).
- 49 A.K. Dhawan, D.M. Paton and R.R. Willing
Planta, 146, 419 (1979).
- 50 A.H. Halvey and H.M. Cathey
Bot. Gaz., 122, 63 (1960).
- 51 P.W. Brian, H.C. Hemming and D. Lowe
Ann. Bot., 28, 369 (1964).
- 52 D.S. Letham
Physiol. Plant, 25, 391 (1971).
- 53 N.L. Biddington and T.H. Thomas
Planta, 111, 183 (1973).
- 54 T. Murashige and F. Skoog,
Physiol. Plant, 15, 473 (1962).
- 55 O.H. Lowry, N.J. Rosenbrough, A.L. Farr and
R.J. Randell
J. Biol. Chem., 193, 265 (1951).
- 56 R.G.S. Bidwell
"Plant Physiology", p.257, IInd Ed., Macmillan
Publishing Co. Inc., New York (1979).
- 57 H. Klusak
Biologia (Batisl.), 31, 283 (1977).
- 58 F. Wightman, E.A. Schneider and K.V. Thimann
Physiol. Plant, 49, 304 (1980).
- 59 W. Feucht and P.P.S. Schmid
Physiol. Plant, 50, 309 (1980).

- 60 T. Stonier and H. Yang
Plant Physiol., 51, 391 (1973).
- 61 T.T. Lee and F. Skoog
Plant Physiol., 47, 181 (1971).
- 62 E.K. Demos, M. Woolwine and R.H. McMillan
Am. J. Bot., 62, 97 (1975).
- 63 T.T. Lee
Physiol. Plant, 50, 107 (1980) and references cited therein.
- 64 A.W. Galston and W.S. Hillmann
"Handbook of Plant Physiology", Vol.XIV, p.647-670,
ed. W. Ruhland, Springer, Berlin (1961).
- 65 A.W. Galston, J. Bonner and R.S. Baker
Arch. Biochem. Biophys., 42, 456 (1953).
- 66 M.C. Hoyle
Plant Physiol., 50, 15 (1972).
- 67 D.A. Gelinas
Plant Physiol., 51, 967 (1973).
- 68 S. Palmieri and F. Giovinazzi
Physiol. Plant, 56, 1 (1982).
- 69 B.D. Sanwal
Proc. Ind. Acad. Science, 44B, 257 (1956).
- 70 D.P. Hackett
"Modern Methods of Plant Analysis", Vol.VII,
pages 384-386, Ed. K. Paech and M.V. Tracy,
Springer-Verlag, Berlin (1964).
- 71 M.A. Fieldes and H. Tyson
Z. Pflanzenphysiol., 66, 385 (1972).
- 72 M. Tomaszewski and K.V. Thimann
Plant Physiol., 41, 1443 (1966).
- 73 P.B. Goodwin
"Phytohormones and related compounds" - A comprehensive
treatise, Vol.II, p.31-173. Ed. D.S. Letham, P.B.
Goodwin and T.J.V. Higgins, Elsevier/North Holland
Biomedical Press Amsterdam (1978).

- 74 A. Dunberg
Physiol. Plant, 38, 186 (1976).
- 75 R.H. Wetmore and W.P. Jacobs
Am. J. Bot., 40, 272 (1953).
- 76 L.V. Runkova, E.K. Lis, M. Tomaszewski and
R. Antoszewski
Biol. Plant, 14, 71 (1972).
- 77 W.R. Briggs and P.M. Ray
Plant Physiol., 31, 165 (1956).
- 78 J. McDoughall and J.R. Hillman
"Isolation of Plant Growth Substances p.1-25,
Ed. J.R. Hillman, Cambridge University Press,
Cambridge (1978).
- 79 E.A. Schneider, R.A. Gibson and F. Wightman
J. Expt. Bot., 23, 152 (1972).
- 80 G. Marigo and A.M. Boudet
Z. Pflanzenphysiol., 92, 33 (1979).
- 81 a) H. Dam
Biochem. Z., 220, 158 (1930);
b) H. Dam
Biochem. Z., 215, 475 (1929).
- 82 a) W.D. McFarlane, W.R. Graham and G.E. Hall,
J. Nutrition, 4, 331 (1931) and
b) W.D. McFarlane, W.R. Graham and F. Richardson
Biochem. J., 25, 358 (1931).
- 83 H. Dam
Nature, 133, 909 (1934).
- 84 H. Dam
Nature, 135, 652 (1935).
- 85 H.J. Almquist and E.L.R. Stokstad
J. Nutrition, 12, 329 (1936).
- 86 F. Schonheyder
Nature, 135, 653 (1935).
- 87 H.J. Almquist
J. Biol. Chem., 117, 517 (1937).

- 88 P.A. Karrer, R. Geiger, A. Legler and H. Salomon
Helv. Chim. Acta, 22, 1464 (1939).
- 89 D.W. MacCorquodale, S.B. Binkley, R.W. McKee,
S.A. Thayer and E.A. Doisey
Proc. Soc. Exptl. Biol. Med., 40, 482 (1939).
- 90 P. Gorog, I.B. Kovacs, L. Szporny and G. Fekete
Arzneim-Forsch, 18, 227 (1968).
- 91 A. Kimler
J. Bacteriol., 60, 469 (1950).
- 92 a) M. Periaswamy and M.V. Bhatt
Tetrahedron Lett., 4561 (1978);
b) L.F. Fieser
J. Biol. Chem., 133, 391 (1940);
c) R.T. Arnold and R. Larson
J. Org. Chem., 5, 250 (1940).
- 93 a) M. Wakae and K. Konishi
Repts. Ind. Research Inst. Osaka Project
7/2, 59 (1955) through Chem. Abstr. 50, 12005c (1956).
b) V.A. Zagorevskii and D.A. Zykov
Zh. Prikl. Khim., 32, 2815 (1959) through
Chem. Abstr., 54, 9818e (1960).
c) L. Syper, K. Klóc, J. Mlochowski and Z. Sulc
Synthesis, 521 (1979).
d) M. Shimizu and G. Ouchi
J. Pharm. Soc. Japan, 71, 963 (1951).
- 94 C.K. Chuang and C.T. Han
Ber., 68, 876 (1935).
- 95 M.R. Grdinic and V.K. Jugovic
Archiv Khem., 23, 73 (1951) through Chem. Abstr.,
46, 11165g (1952).
- 96 P.P.T. Sah and Wilhelm Brull
Ber., 73, 1430 (1940).
- 97 P.P.T. Sah, Wilhelm Brull and Herbert Holzen
Ber., 73, 762 (1940).
- 98 C.G. Casnati and G. Salerno
J. Chem. Soc. Chem. Comm., 955 (1972).
- 99 S.K. Ray, G.S. Murty and H.S. Rao
Proc. Symp. Chem. Oil Coal, 320 (1969); Chem. Abstr.,
80, 135736s (1974).
- 100 S. Torii, H. Tanaka and S. Nakane
Bull. Chem. Soc. Japan, 55, 1673 (1982).

- 101 S.I. Miller and H. Hiranuma
J. Org. Chem., 47, 5083 (1982).
- 102 H.M. Van Dort and H.J. Geursen
Recueil, 86, 520 (1967).
- 103 V.M. Kothari and T.J. Tazuma
J. Catalysis, 41, 180 (1976).
- 104 H. Diehl and C.C. Hach
Inorganic synthesis, Vol.III, 196 (1950).
- 105 H.J. Teuber and W. Rau
Ber., 86, 1036 (1953).
- 106 A.Siegel and F. Antony
Monatshefte Fur Chemie, 86, 292 (1955).
- 107 Alvin Ronlan
J. Chem. Soc. Chem. Comm., 1643 (1971).
- 108 H.W. Underwood and W.L. Walsh
J. Am.Chem. Soc., 58, 646 (1936).
- 109 K.K. Georgeieffe and A. Dupre
Can. J. Chem., 38, 1070 (1960).
- 110 Buu-Hoi and Denise Lavit
J. Chem. Soc., 1743 (1956).
- 111 D.W. MacCorquodale, L.C. Cheney, S.B. Binkley,
W.F. Halcomb, R.W. McKee, S.A. Thayer and E. Doisy
J. Biol. Chem., 131, 357 (1939).
- 112 a) L.F. Fieser
J. Biol. Chem., 133, 391 (1940);
b) L.F. Fieser, W.P. Campbell, E.M. Fry and M.D.Gates
J. Am. Chem. Soc., 61, 3216 (1939).
- 113 A.S. Lindsey and H. Jeskey
Chem. Rev., 57, 583 (1957).
- 114 Laurence A. Cate
Syn., 385 (1983).
- 115 R.T. Arnold and Joseph Sprung
J. Am. Chem. Soc., 60, 1163 (1938).

- 116 H. Deisebach, O. Wanger and A. Stockalper
Helv. Chim. Acta, 14, 355 (1931).
- 117 Z. Horii, T. Tanaka and Y. Murakami
Pharm. Bull., 5, 82 (1957).
- 118 R.H. Cornforth
J. Chem. Soc., 168 (1943).
- 119 Kirk and Othmer
"Encyclopaedia of Chemical Technology" Vol.VIII,
p.596, Interscience Publishers Inc., New Yoark (1952).

CHAPTER III

SYNTHESIS OF PYRAZINAMIDE

INTRODUCTION

The tuberculostatic activity of pyrazinecarboxamide (pyrazinamide) (1) was first announced by Kushner and his coworkers¹, who prepared it by oxidation of quinoxaline (2) to pyrazine-2,3-dicarboxylic acid (3), decarboxylation of the latter to pyrazinoic acid (4), esterification of the acid and conversion of the ester to the amide. Besides being several times more active than PAS and niacinamide², pyrazinamide (1) in combination with isoniazid is superior to any other drug or combination of drugs³.

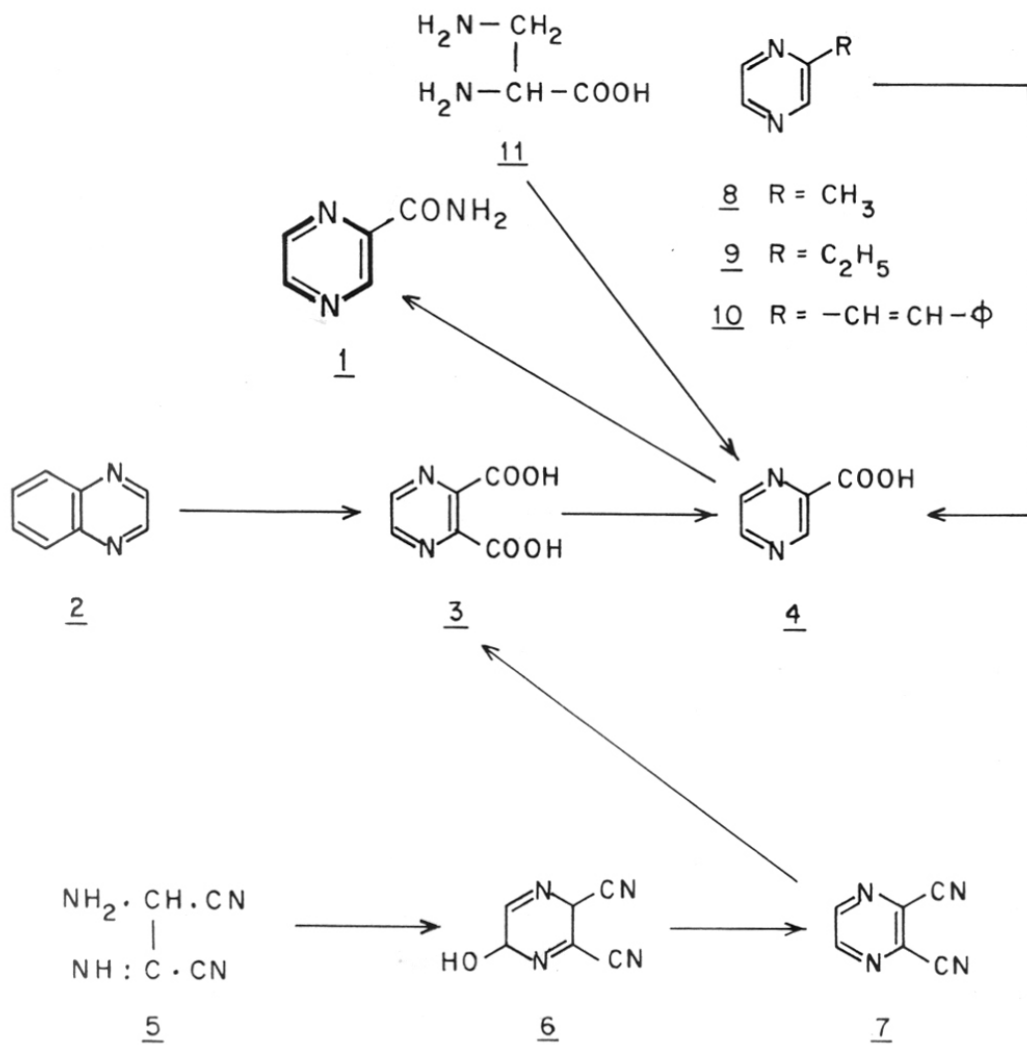
Considerable interest is attached to the synthesis of pyrazinoic acid (4), since this compound is the key intermediate in the preparation of the tuberculostat pyrazinamide (1). The acid 4 is obtained generally by

I. Decarboxylation of pyrazine-2,3-dicarboxylic acid (3)

Acid 3 was obtained by oxidation of quinoxaline (2) using either alkaline KMnO_4 ⁴, or sodium hypochlorite and KMnO_4 ⁵ or electrolytic oxidation⁶ in a solution containing KMnO_4 and NaOH with a copper anode.

3 has also been obtained by the oxidation of "pyrazinotropone oxime"⁷ with alkaline KMnO_4 .

Hinkel et al.⁸ obtained 3 by making use of the polymer, aminoiminosuccinonitrile (5). Condensation of glyoxal in aqueous or alcoholic solution with 5 provided



6-hydroxy-2,3-dicyanodihydropyrazine (6) which after dehydration to 2,3-dicyanopyrazine (7) was hydrolysed to acid 3.

Decarboxylation of 3 to 4 has been achieved by heating⁹ 3 in a vacuum sublimation chamber at 210°.

Heating 3 with solvents like AcOH¹⁰, or toluene/xylene/chlorobenzene¹¹ or nitrobenzene¹² gives 4.

II. Oxidation of alkyl pyrazine

Methyl pyrazine (8) has been oxidised with KMnO_4 ¹³ or selenious acid¹⁴ or $\text{Na}_2\text{Cr}_2\text{O}_7$ ¹⁵ to provide pyrazinoic acid (4).

Ethyl pyrazine (9) and styryl pyrazine (10) have also been oxidised with KMnO_4 ¹⁶ to provide 4.

III. Condensation reaction

Acrylic acid and α,β -diaminopropionic acid (11) have also been utilized in condensation reactions¹⁷ with glyoxal for obtaining 4.

Homolytic amidation of pyrazine to pyrazinamide can be effected¹⁸ by using conc. H_2SO_4 with carbamoyl radicals ($\cdot\text{CONH}_2$) generated from the action of H_2O_2 and ferrous sulphate on formamide.

Dehydrogenation¹⁹ of 2-carbamoyl piperazine in a current of N_2 over Pd-C at 290-305° gives 1.

PRESENT WORK

In spite of extensive investigations, a synthesis that provides a practicable and proven basis for preparing large quantities of pyrazinamide (1), with ease and suitable for commercialization seems to be elusive.

The present commercially adopted quinoxaline method⁴, besides being costly (utilizing 6 moles excess of KMnO_4 as the oxidizing agent) is also tedious, since the manganese dioxide produced in the reaction makes it difficult to recover the pyrazine-2,3-dicarboxylic acid (3) formed.

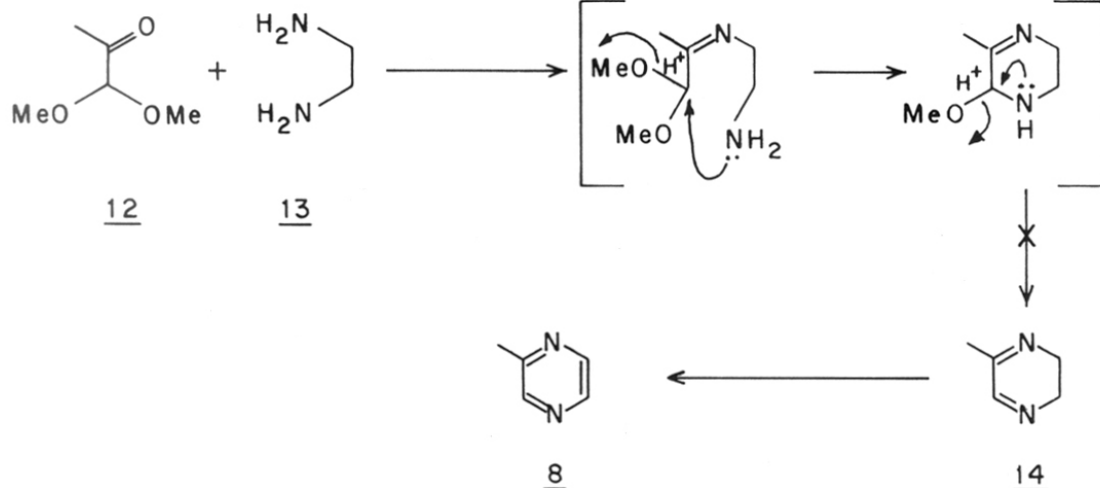
In conjunction with the aforesaid problem, attempts were made in exploring newer methods for obtaining 1.

In the first approach, attempts were made, however unsuccessful to condense pyruvaldehyde dimethyl acetal (12) and ethylenediamine (13) under acidic conditions to afford 2-methyl pyrazine (8), after aromatisation of the resulting product 14 as anticipated and depicted in Scheme-1.

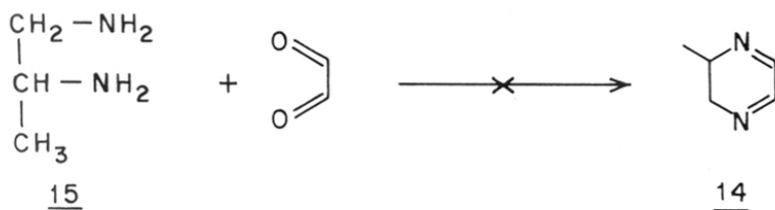
When the condensation of 12 and 13 in equimolar ratio were carried using PTS acid (catalytic amount) in solvents like methanol or ether or benzene at room temp. for 24 hr only starting materials were recovered.

Elevation in temp. during condensation reactions, using all the above mentioned solvents resulted in polymeric products (as indicated by nature of the product, and further

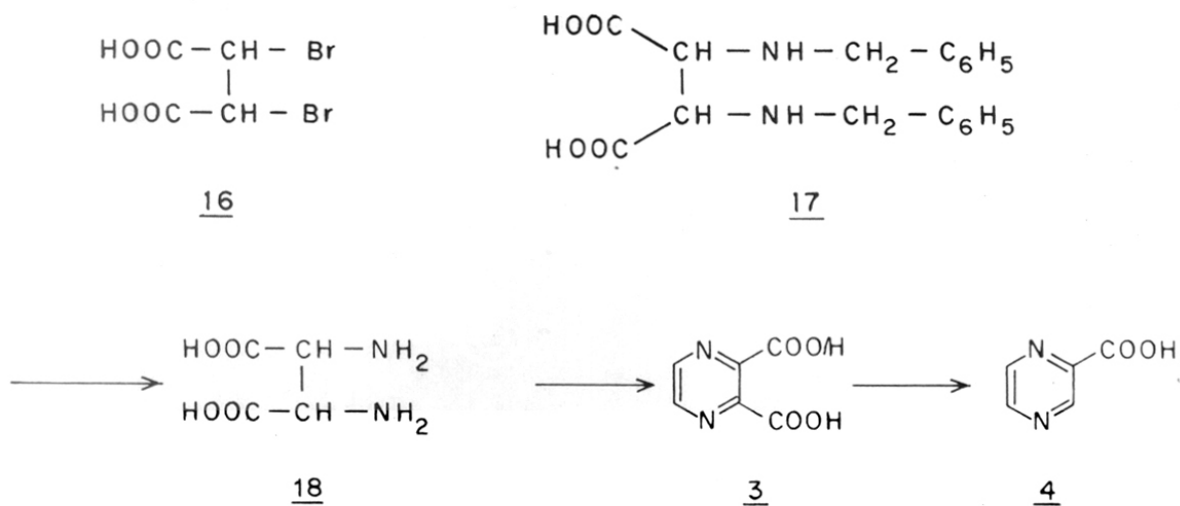
SCHEME - 1



SCHEME - 2



SCHEME - 3



confirmed by PMR).

Refluxing 12 and 13 in benzene with catalytic amount of PTS, using Dean-Stark apparatus again resulted in the formation of polymeric material.

As the condensation between 2,3-diaminopropionic acid (11) and glyoxal was established¹⁷, attempts were made to condense 2,3-diaminopropane (15) and glyoxal as shown in Scheme-2. However all efforts to get the condensed product 14 failed, much to the chagrin.

Interestingly, however, the condensation of 2,3-diaminosuccinic acid (18) with glyoxal proceeded smoothly (Scheme-3). This further involved an oxidation carried out in the same medium employing air as the oxidizing agent, which besides being available free of cost did not produce any side products.

The requisite starting material 18 was obtained by slight modifications.

Bromination of fumaric acid by adopting the conventional organic synthesis procedure²⁰ failed to yield the desired product 16, but instead resulted in the recovery (100%) of the starting material. This failure was in well agreement with previous observations²¹ that α,β -dibromosuccinic acid (16) cannot be prepared in the presence of excess of water. This difficulty was overcome by taking fumaric acid in 20%

HBr and treating with Br_2 at $70-80^\circ$ for 1 hr to yield 16 in 95% yield. m.p. $260-64^\circ\text{C}$ (decomp.) [lit.²² m.p. $265-68^\circ$ (decomp.)].

16 was then converted to 18 according to the known procedure^{23,24}. Compound 16 on treatment with benzylamine in alcohol provided α,β -bis(benzylamino)succinic acid (17), which was smoothly converted to 2,3-diamino succinic acid (18) by hydrogenolysis under acidic conditions.

Condensation of 18 with 40% glyoxal in methanol under alkaline conditions and an oxidation carried out in the same medium employing air as an oxidizing agent provided pyrazine-2,3-dicarboxylic acid (3) in 70% yield. m.p. $180-82^\circ$ (decomp.) [lit.⁴ m.p. $180-85^\circ$ (decomp.)].

Mono decarboxylation of the dicarboxylic acid 3 in boiling $\text{AcOH}/\text{H}_2\text{SO}_4$ (catalytic amount of H_2SO_4 being used) provided pyrazinoic acid (4) in 85% yield. m.p. $223-27^\circ$ (decomp.) [lit.²⁵ m.p. 225° (decomp.)].

As the conversion of 4 into pyrazinamide (1) is well established¹, this new approach in effect constitutes a total synthesis of 1.

E X P E R I M E N T A L

 α , β -Dibromosuccinic acid (16)

2.8 g (0.035 mole) of Br_2 was added dropwise to the suspension of 2.0 g (0.173 mole) of fumaric acid in 5.3 g (0.065 mole) of 20% HBr with stirring at 80° . After the addition, the mixture was further stirred at the same temp. for 1 hr. The flask was then surrounded with ice water and cooled to 10° with stirring. Product was then collected on large Buchner funnel, washed thoroughly with cold water to remove Br_2 liquor and dried at room temp. to afford 4.6 g (95%) of 16 m.p. $260-64^\circ$ [lit.²² m.p. $265-68^\circ$].

Analysis: Calculated for $\text{C}_4\text{H}_2\text{Br}_2\text{O}_4$: C, 17.5; H, 0.72; Br, 58.39. Found: C, 17.1; H, 0.54; Br, 58.10%.

 α , β -bis(benzylamino)succinic acid (17)

To a stirred solution of 12.5 g (.045 mole) of 16 in 100 ml of alcohol at room temp. 42.75 g (0.4 mole) of benzylamine were added slowly. After complete addition, the mixture was heated on a steam bath for 6 hr. After cooling to about 40° , 50 ml of H_2O was added, followed by small portions of conc. HCl until pH 1-2 was obtained. The pH was then adjusted with sodium acetate solution to pH 4-5. The mixture was filtered by suction and crystals washed with alcohol and H_2O until colourless. Recrystallisation from $\text{AcOH}/\text{H}_2\text{O}$ gave (12.0 g, 80%) of 17 m.p. 225°

[lit.²³ m.p. 218-230°].

Analysis: Calculated for $C_{18}H_{20}N_2O_4$: C, 65.85; H, 6.10; N, 8.54. Found: C, 65.53; H, 5.94; N, 8.10%.

2,3-diaminosuccinic acid (18)

Compound 17 (5.0 g, .015 m) was dissolved in a mixture of 25 ml of gl. AcOH and 20 ml of conc. HCl.

Hydrogenolysis was effected at room temp. and atmospheric pressure in presence of 0.5 g of 10% Pd-C. An equal volume of H_2O was added and catalyst removed by filtration. The filtrate was concentrated to a syrupy mass at the water pump. The residue was dissolved in dil. NaOH. Upon treatment with gl. AcOH to make the pH 5-6, solution deposited (2.0 g, 90%) of 18 as colourless crystals. m.p. 297-300° (decomp.) [lit.²⁴ m.p. 304 (decomp.)].

Analysis: Calculated for $C_4H_8N_2O_4$: C, 32.43; H, 5.44. Found: C, 31.74; H, 5.58%.

2,3-Pyrazine dicarboxylic acid (3)

0.740 g (.005 mole) of 18 was dispersed in 15.0 ml of methanol. The dispersion was heated to 50° and 2-18 ml of a 2.96N solution of NaOH in 10 ml of MeOH (8 mole equivalents of 18) were gradually added with stirring. While stirring was continued, and the temperature was maintained at 50°, 0.5 ml of an aqueous 40% glyoxal solution was added and stirring continued for further 0.5 hr. A

vigorous stream of air was passed through the solution for about 3 hr while it was being refluxed. It was then evaporated to dryness in a vacuum. After acidifying and removal of solvent the residue was extracted with boiling dry acetone, decolourised with charcoal, filtered and concentrated to yield (0.58 g, 70%) of 3 as a pale brown material. m.p. 180-82° (decomp.) [lit.⁴ m.p. 180-85° (decomp.)]. M⁺ 168.

Analysis: Calculated for C₆H₄N₂O₄: C, 42.86; H, 2.38; N, 16.67. Found: C, 42.76; H, 2.57; N, 16.8%.

Pyrazinoic acid (4)

A mixture of 3 (0.5 g, .003 mole), 2.5 ml gl.AcOH and 0.5 ml conc. H₂SO₄ was refluxed for 2 hr. The mixture was poured into 25 ml of ice cold water whereby dark brown solid separated. It was filtered and recrystallised from water to yield (0.310 g, 85%) of 4. m.p. 223-25° (decomp.). Lit.²⁵ m.p. 225° (decomp.). M⁺ 124.

Analysis: Calculated for C₅H₄N₂O₂: C, 48.38; H, 3.23; N, 22.50. Found: C, 48.26; H, 3.10; N, 22.30%.

REFERENCES

- 1 S. Kushner, H. Dalalian, J.L. Sanjurjo, F.L. Bach, S.R. Safir (Jr.), V.K. Smith (Jr.) and J.H. Williams
J. Am. Chem. Soc., 74, 3617 (1952).
- 2 E.F. Rogers, W.J. Leanza, H.J. Becker, A.R. Matzuk, R.C. O'Neill, K.J. Basso, G.A. Stein, M. Solotorovsky, F.J. Gregory and K. Pfister
Science, 116, 253 (1952).
- 3 C. Muschenham, W. McDermott, R.M. McCune, K. Deuschle, L. Ormond and R. Tompsett
Trans. Assoc. Am. Physicians, 67, 224 (1954).
- 4 R.G. Jones and K.C. McLaughlin
Org. Synth., 30, 86 (1950).
- 5 B. Hoffman and A. Zmojdzin
Polish Pat. 81,899 through Chem. Abs., 86, 55491 (1977).
- 6 T. Kimura, S. Yamada, K. Yoshizue and T. Nagoya
J. Pharm. Soc. Japan, 77, 891 (1957).
- 7 S. Ito
Sci. Rep. Tohoku Univ. First Ser., 42, 247 (1958)
through Chem. Abst., 54, 5666 (1960).
- 8 L.E. Hinkel, G.O. Richards and O. Thomas
J. Chem. Soc., 1432 (1937).
- 9 R.P. Linstead, E.G. Noble and J.M. Wright
J. Chem. Soc., 911 (1937).
- 10 M. Asai and R. Takasaki
Japan Pat. 8187 through Chem. Abs., 54, 4634 (1960).
- 11 W.L. McEwen
U.S. Pat. 2,675,384 through Chem. Abs., 49, 4730 (1955).
- 12 L. Bernardi and G. Lavini
Ann. Chim. (Italy) 48, 239 (1958).
- 13 C. Stoehr
J. Prakt. Chem. [2], 51, 449 (1895).
- 14 H. Gainer
J. Org. Chem., 24, 691 (1959).

- 15 L.H. Beck
U.S. Pat. 3154, 549 through Chem. Abs. 62, 1673 (1965).
- 16 S. Yamada and M. Negishi
Japan Pat. 3368 (1961) through Chem. Abs., 57, 3458 (1962).
- 17 a) M. Litmanowitsch, E. Felder and D. Pitre
Swiss Pat. 458361 (1968) through Chem. Abs., 70,
20083c (1969);
b) Eprova Ltd.
British Pat., 1016468 (1965) through Chem. Abs., 64,
9745h (1966);
c) S. Baloniak, B. Hoffmann, A. Lukowski, A. Mroczkiewics,
B. Zyczynska and A. Marszewski
Pol. Pat. 100262 (1976) through Chem. Abs., 91,
175397r (1979).
- 18 F. Minisci, G.P. Gardini, R. Galli and F. Bertini
Tetrahedron Lett. 15 (1970).
- 19 F.L. Bach, S. Kushner and J.H. Williams
J. Am. Chem. Soc., 77, 6049 (1955).
- 20 H.S. Rhinesmith
Org. Syn. Coll. Vol.II, 177 (1950).
- 21 I.M. Mathai
J. sci. industr. Res., 15B, 582 (1956).
- 22 Chemische Fabrik Kalk. G.m.b.H.,
French Pat. 1468014 (1967) through Chem. Abs.,
67, 90401s (1967).
- 23 W. Wenner
J. Org. Chem., 13, 26 (1948).
- 24 H. McKennis (Jr. and S. Yard
J. Org. Chem., 23, 980 (1958).
- 25 a) S.A. Hall and P.E. Spoerri
J. Am. Chem. Soc., 62, 664 (1940);
b) S. Gabriel and A. Sonn,
Ber., 40, 4850 (1907).

LIST OF PUBLICATIONS

1. **Simple synthesis of 2-acetyl-5,8-dimethoxy-3,4-dihydro-naphthalene, a key intermediate for the synthesis of optically active anthracylinones.**
A. V. Rama Rao, V. H. Deshpande, B. Ramamohan Rao and K. Ravichandran
Tetrahedron Lett., 23,1115 (1982).
2. **Synthesis of 4-demethoxy-7, 11-dideoxydaunomycinone**
A. V. Rama Rao, V. H. Deshpande, K. Ravichandran and B. Ramamohan Rao
Synth. Commun., 13 (14), 1219 (1983).
3. **Synthesis of (±) 4-demethoxy-7, 11-dideoxydaunomycinone**
V. H. Deshpande, K. Ravichandran and B. Ramamohan Rao
Synth. Commun. 1984 (in press).
4. **An efficient synthesis of 1-Triacontanol.**
A. V. Rama Rao, K. Ravichandran and N. Laxma Reddy
Synth. Commun., 1984 (in press).
5. **Menadione Bisulfite : A promising plant growth regulator.**
A. V. Rama Rao, K. Ravichandran, S. B. David and Sujata Ranade
Plant Growth Regulation, 1984 (in press).
6. **Synthesis of Pyrazinamide.**
A. V. Rama Rao, J. S. Yadav, K. Ravichandran, A. B. Sahasrabudhe and S. S. Chaurasia
Ind. J. Chem., 1984 (in press).

Note : Publications 1-3 are discussed in **Chapter I**; 4 and 5 in **Chapter II**, and 6 in **Chapter III**.