A THESIS

presented by

ALAN KEITH PRINCE

for the

DEGREE OF DOCTOR OF PHILOSOPHY

in the

VICTORIA UNIVERSITY OF MANCHESTER

Manchester College of Science
and Technology,
MANCHESTER.

September 1962.

ProQuest Number: 10997148

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

ProQuest 10997148

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

L 7525. Trans.

The University of
Manchester Institute of
Science and Technology
12 NOV 1970
LIBRARY

The work contained in this thesis, unless otherwise stated, is that of the author and has not been submitted in support of an application for another degree in this, or any other, university.

The author graduated at the Victoria University of Manchester in July 1959, with an honours degree (B.Sc.(Tech), Class I) in Chemistry, and has since been engaged in research under the guidance of Dr. J. E. Davies, leading to the presentation of this thesis.

ACKNOWLEDGEMENTS

The author takes this opportunity to thank Dr. J. E. Davies most sincerely for the continued guidance and encouragement freely given throughout the course of this work. He also wishes to thank Dr. A. R. Thompson for valuable discussions of ultraviolet spectra, Dr. A. E. Williams of I.C.I. Ltd. (Blackley) for a mass spectrum, Professor R. N. Haszeldine for the facilities necessary for this research to be undertaken, and the Department of Scientific and Industrial Research for a research studentship.

TO IRENE

THE CHEMISTRY OF SOME FUNGAL METABOLITES

CONTENTS

	Page
SUMMARY	1
Part I, INTRODUCTION	
Foreword	1
Theories of Biosynthesis of Microbial Metabolites	5
The Oxygen Heterocycles	19
References	48
Part II, THE STRUCTURE OF RADICININ	
Introduction	54
Discussion	60
Preliminary Chemical Investigations	61
Acetylation and Methylation Studies on Radicinin	63
The Chemistry of the Side Chain in Radicinin	65
The Hemiacetal System of Radicinin	74
The Chromophoric System of Radicinin	79
Reduction of Radicinin with Complex Metal Hydrides	82
Evidence for the Existence of a 2-pyrone System in Radicinin	90
Attempted Degradation of Radicinin	101

Consideration of Spectroscopic Data

1. The Ultraviolet Spectral Data 103

2. The Infrared Spectra of Radicinin and its Derivatives 120

Conclusion 126

The Mass Spectrum of Radicinin 128

Part II, EXPERIMENTAL 130

References 171

Part III, THE CHEMISTRY OF SOME SUBSTITUTED THIOPHENES ....

Introduction 173

Discussion 177

Formylation of Thiophene 179

Condensation Reactions with Metal Acetylides 187

Part III, EXPERIMENTAL 199

References 217

SUMMARY

Since the commencement of the first systematic examination of the chemistry of microorganisms some forty years ago, an astonishingly large number of compounds of widely variegated structures have been isolated and their chemistry elucidated.

Due to the relatively simple techniques involved, the study of fungi and moulds has attracted considerable attention. Initially the work carried out was mainly concentrated on the elucidation of the structures of new metabolites, but with increasing knowledge, certain structural correlations could be made which lead to more fundamental conclusions on the mode of biosynthesis of metabolites. Such information is likely to provide valuable information, leading to a deeper understanding of the chemistry of cells.

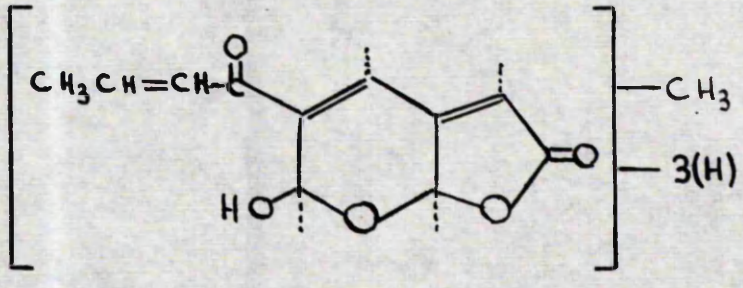
In the introduction to this thesis the present state of knowledge with regard to the biosynthesis of fungal metabolites has been briefly reviewed, showing that important advances are to be made in the biochemical aspects of natural products.

In addition, the fungal metabolites containing heterocyclic oxygen systems have been classified into groups according to their chemical structures and their chemistry briefly reviewed. It has been shown that although

many such metabolites at first sight appear relatively simple in structure, the chemistry involved in elucidation of their structures has, on occasion, been extremely complicated.

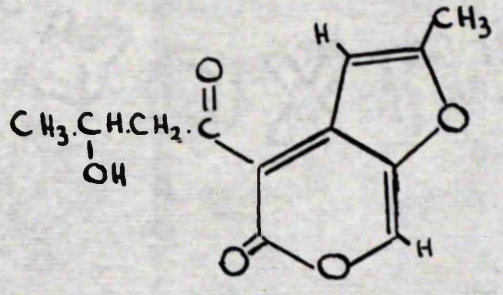
It is further shown that a study of such heterocyclic oxygen systems has given an insight into several general biosynthetic mechanisms, and has contributed to the concept of "biogenetically patterned" syntheses of natural compounds.

The main work comprising this thesis (Part II) is concerned with a reconsideration of the structure of one such metabolite, radicinin, which was reported (1) to possess an unusual arrangement of oxygen atoms in a cyclic structure (I).



The original objective of this work was to confirm and complete the postulated structure, but in the light of new evidence, this structure was found to be untenable. A consideration of the chemical and physical

properties of radicinin has led to the proposal of a structure which is in better agreement with the known facts, namely:



The experiments upon which this conclusion was based were as follows.

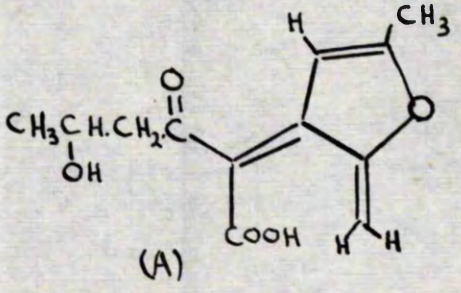
1. On treatment of the dihydro, dibromo-and the hydrogen chloride adducts of radicinin with alkali, acetaldehyde was evolved.

2. Treatment of radicinin with ozonised oxygen did not give acetaldehyde, and on oxidation of dibromoradicinin, no α,β -dibromobutyric acid could be isolated.

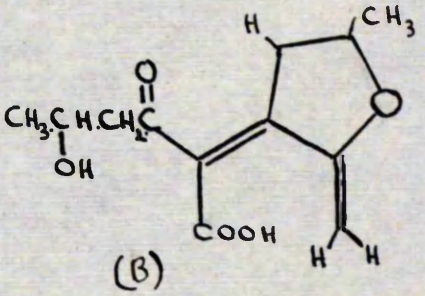
3. Spectroscopic evidence was contrary to the presence of the proposed crotonyl side chain in radicinin.

4. Radicinin gave none of the reactions expected of a hemiacetal system as postulated in (I).

5. On treatment of radicinin and dihydroradicinin with lithium aluminium hydride, carboxylic acids were obtained which analysed for the addition of two atoms of hydrogen to the parent compounds, a reaction characteristic of 2-pyrones. The structures proposed for these two products are respectively (A) and (B).



(A)

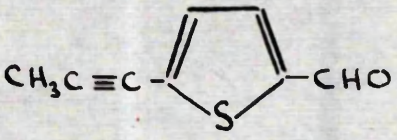


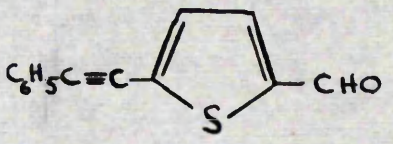
(B)

6. The stability of dihydroradicinin and compound B to sulphuric acid over prolonged periods, in contrast to the instability of radicinin and compound A, is considered to be in accord with the postulated cyclic ether structures and vinyl ether structures of the two pairs of compounds respectively.

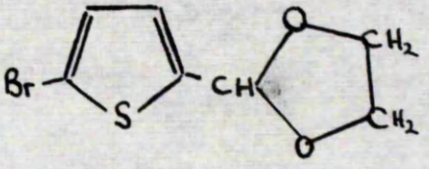
7. The ultraviolet and infrared spectroscopic data for radicinin and its derivatives are discussed, and shown to be compatible with the structures proposed.

Part III of this thesis is concerned with a study of coupling reactions between metal acetylides and bromothiophene compounds as an approach to the synthesis of the fungal thiophene compound, Junipal (2) and its phenyl analogue:





2-bromo-5-formylthiophene was prepared by a number of routes and converted into its ethylene ketal:



In attempts to couple this compound with phenylacetylene under a variety of conditions, starting materials only were recovered. Attempts to synthesise model systems by similar reactions were also unsuccessful.

References

(1) Clarke and Nord, Arch.Biochem.Biophys., 1955, 59, 269.

(2) Birkinshaw & Chaplen, Biochem.J., 1955, 60, 255.

PART I

INTRODUCTION

FOREWORD

Microorganisms form a continuum from plants to animals, and include bacteria, fungi, yeasts, rickettsiae, viruses and protozoa. Taxonomically they are widely different, but a study of their chemistry and biochemistry quickly reveals remarkably close interrelationships which warrant microorganisms being studied as a whole, rather than in separate groups. The ubiquitous character of many biochemical processes serves to emphasise the essential unity of nature. Thus the biochemical cycles which operate in simpler forms of life are known to operate in the plant and animal kingdom, a situation perhaps not surprising in view of modern concepts of primeval evolution.

During the last forty years the study of microbial metabolites, especially those arising from fungi, has been mainly concerned with the chemical problems of structure determination. The biochemistry of the cells involved has been treated as a separate issue. It is now becoming more clearly realised that this separation is not justified, and indeed studies of these metabolites and their precursors has revealed important biochemical pathways in the cell. The successful isolation of several antibiotics from microbial

cultures no doubt stimulated interest in this region of natural product chemistry, and many structural investigations have been carried out. However, present trends of research centre round the biochemical aspects of the subject.

The metabolites produced may be classified into two types. Firstly the primary metabolites, which are essential for cell growth, and include complex enzyme systems, lipids, phosphorylated entities, etc., common to all cell systems. In addition, there are the so-called secondary metabolites, which are mainly excreted from the cell prior to detection, and for which no full biochemical role has yet been discovered.

The role that the latter play in the growth processes of microorganisms is obscure. Raistrick (1), in considering mould metabolites, pointed out that the large yields of many of these compounds would indicate that they played an important biological role. Birch (2) has suggested that the metabolites, which have no apparent role in the metabolism of the microorganism, and which are excreted from the cell, might be detoxicants whose function is to remove poisonous entities from the cell. This idea must be considered doubtful in view of the fact that certain mutants with blocked pathways still grow with unreduced

efficiency (3). Nord (4), Bu'Lock (5), and Dalgliesh (6) have pointed out that the metabolites are often produced with yields in excess of normal functional requirements, and may therefore accumulate because some enzyme systems have become saturated with respect to the substrate. This theory is supported by the observation that the secondary metabolites only begin to appear after an induction period, during which essential factors for cell growth and maintenance are produced (5).

Microorganisms are capable of producing a wide variety of chemical compounds. Attempts have been made to classify these products in the hope of finding some correlation between chemical type and taxomomic properties. No useful generalisations have been obtained from studies of the lower molecular weight metabolites, but the larger molecules produced by microorganisms have proved more useful. Strains and species can sometimes be distinguished by the presence or otherwise, of metabolites from various cell tissues, exotoxins, or other high molecular weight exudates (7). It is also possible to classify microbial metabolites according to their mode of biosynthesis, although such a method is too general because many different types may arise from the same biogenetic route. Classification according to chemical structure is more

convenient, although of less fundamental value from a biochemical point of view.

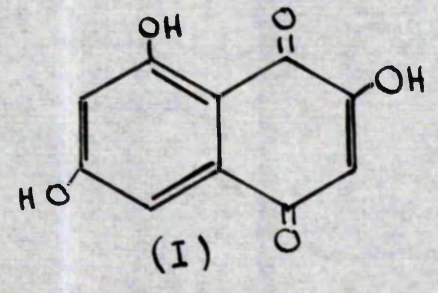
In general, although microorganisms can produce interrelated groups of compounds, different types of microorganisms often produce typical compounds. Thus bacteria produce a larger number of nitrogen and sulphur heterocycles than moulds. On the other hand the oxidative metabolism of moulds leads to many oxygenated compounds, including quinones, and many oxygen heterocycles.

Undoubtedly many types of microbial metabolites still remain to be isolated. Antibiotics and easily isolated metabolites may have received too much attention, while others more difficult to isolate still remain. Among the latter group are the low molecular weight (probably phosphorylated) compounds directly involved in the metabolism of the microorganism. Thus generalisations on the chemistry of microorganisms and classification of their metabolites may be premature at this time.

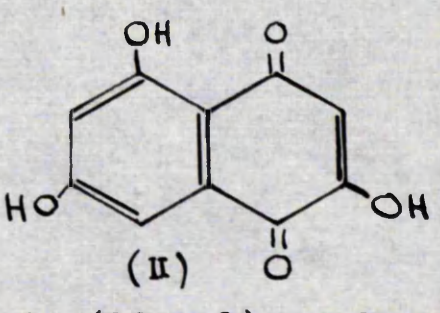
THEORIES OF BIOSYNTHESIS OF MICROBIAL METABOLITES

1. The acetate theory

The biochemical origin of microbial metabolites has been the focus of much attention for the past ten years, and a general pattern is being built up to show the relationship between the final metabolite and its precursors. The revival by Robinson and Birch (8,9) of the acetate theory of biogenesis, first postulated by Collie in 1893 (10,11), was the first really successful attempt to study the origin of natural products, and has been especially successful in the field of mould chemistry. This theory provides a useful aid to structural determination; thus a decision between two alternative structures can often be made by considering the head-to-tail condensation of acetate units. The mould metabolite flaviolin provides an example of the application of the acetate theory to the determination of structure. Of the two possibilities (I) and (II), only structure (II)



(I)

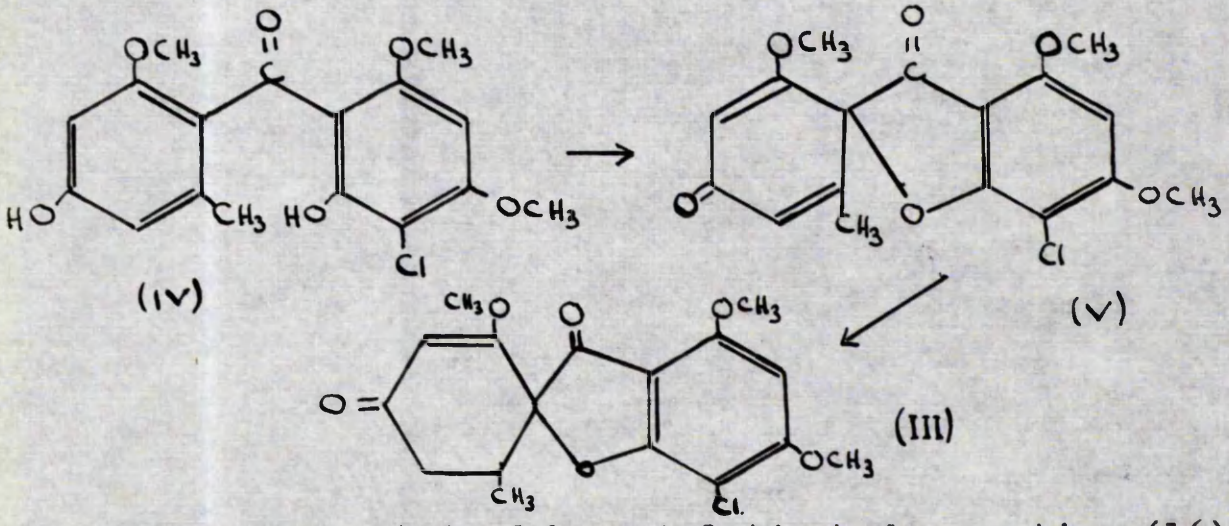


(II)

satisfied the acetate hypothesis (12, 13), and was considered to be the correct structure. This was

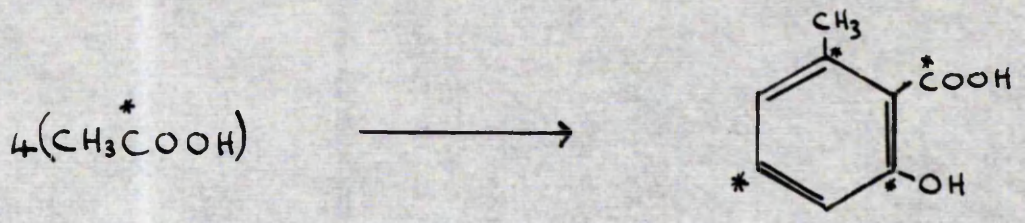
confirmed by synthesis of both isomers (14).

A further useful application of biosynthetic theories is found in the synthesis of natural products. Considerable attention has been paid to "biogenetically-patterned" syntheses (15), and some very interesting syntheses have been devised. For example, the total synthesis of griseofulvin (III) involved phenol coupling (see page 21) of the benzophenone (IV), which gave the cyclohexadienone (V), from which



griseofulvin was obtained by catalytic hydrogenation (16).

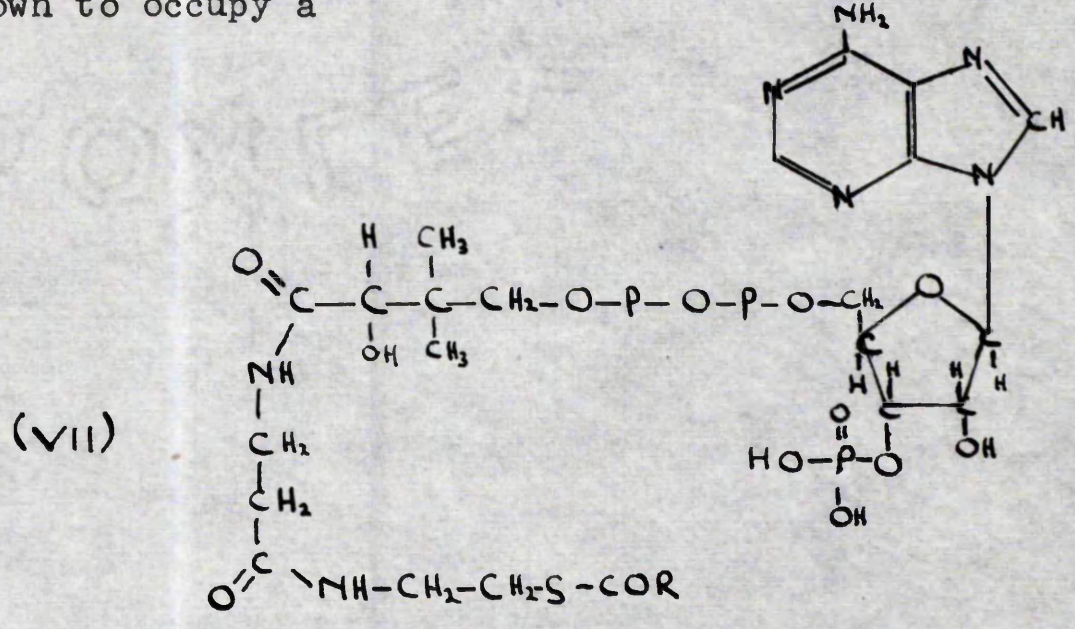
There is now a considerable body of evidence from radio-tracer work, etc., that the acetate theory is based on sound biochemical principles. For example, it was shown that isotopically labelled acetate was incorporated from the culture medium of P. griseofulvum into 2-hydroxy-6-methylbenzoic acid (VI).



(VI)

The pattern of labelling was found to be as predicted by the acetate theory (17).

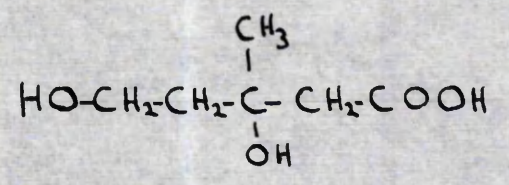
In microorganisms it is likely that the carbohydrate and fatty acid pathways act as a source of acetate. During the controlled oxidation of glucose by either the Embden-Meyerhof or the pentose-phosphate pathways, combined with the citric acid cycle, acetyl-coenzyme-A (VII, R = CH_3-) is produced (18, 19). This compound is known to occupy a



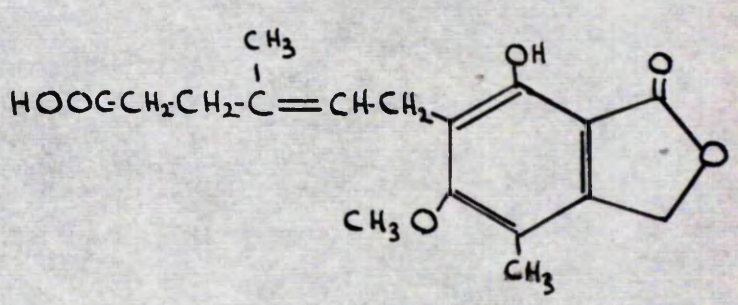
(VII)

central role in metabolism, and is converted into malonyl-coenzyme-A (VII, R = -CH_2 COOH), which is the fundamental

unit of fatty acid and glyceride biosynthesis (20). Acetyl-coenzyme-A has been shown to be involved in the biosynthesis of steroids and terpenes (21, 22) via mevalonic acid (VIII), which is also a precursor of isoprenoid mould metabolites. For example, P.brevi-compactum (Dierck) was shown to incorporate labelled mevalonate in the production of mycophenolic acid (IX)(23).



(VIII)



(IX)

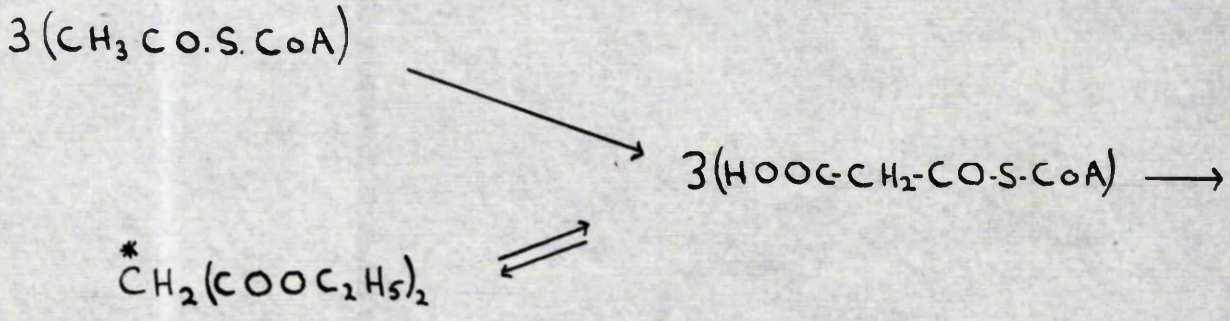
Thus, it appears that "acetate" is utilised in the cell as either acetyl-coenzyme-A, or malonyl-coenzyme-A. Recent work has clarified the role of malonyl-coenzyme-A as the active precursor of metabolites other than fatty acids. Bentley and Keil have reported its function as a precursor of orsellinic acid (56) (see page 28). Bu'Lock and Smalley have shown that 6-methylsalicylic acid, produced from [2 - ^{14}C] malonate, is labelled only in the aromatic ring (24), whereas from [1 - ^{14}C] acetate the labelling occurs equally in the four

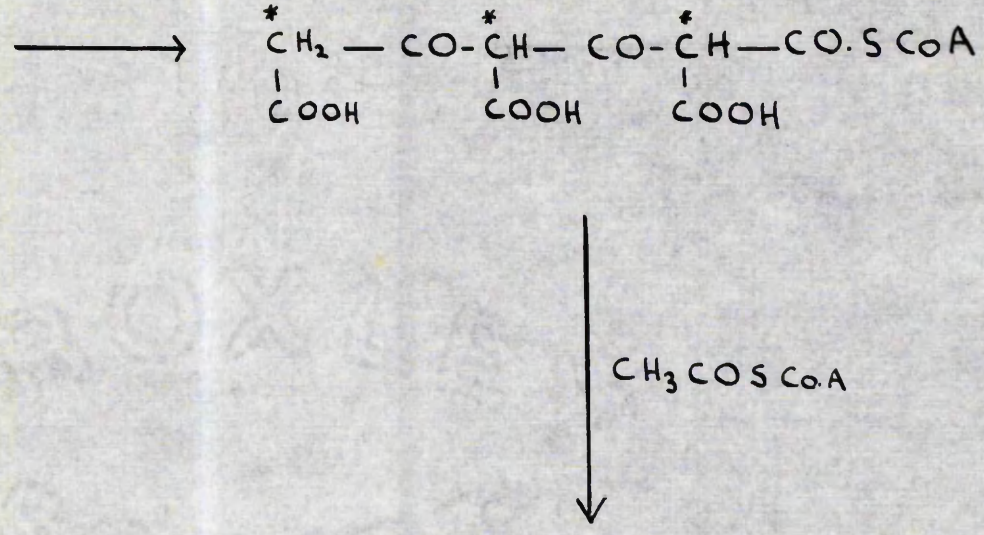
constituent 'C_2 units' (17). From this evidence it was concluded that the condensation of three malonate units followed by condensation with an acetate unit, gave a poly-β-keto-compound, which could cyclise and aromatise to give either 6-methylsalicylic acid (VI), or orsellinic acid (X) (25, 56) (see Figure 1).

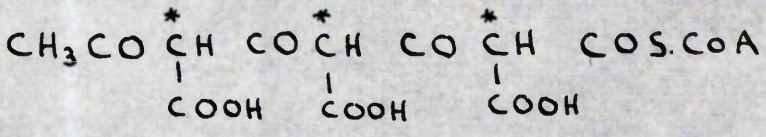
The high activity of the penultimate carbon atom of the β-polyketo acid chain observed in some metabolites (2), was considered by Birch and colleagues (25) to be due to the incorporation of this acetate unit by a different route.

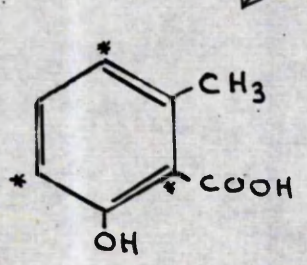
Experiments with P. urticae, on the production of 6-methylsalicylic acid from [1 - ^{14}C] acetate and unlabelled malonate, confirmed that unlabelled malonate was utilised to extend the carbon chain with the methyl-terminal group being derived from acetate.

Figure 1

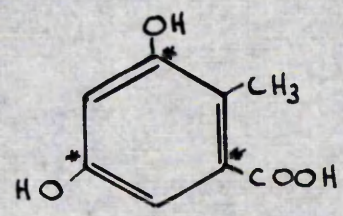



CH2 — CO- CH— CO- CH—CO.S CoA
COOH COOH COOH
CH3 COS CoA


CH3CO CH CO CH CO CH COS.CoA
COOH COOH COOH

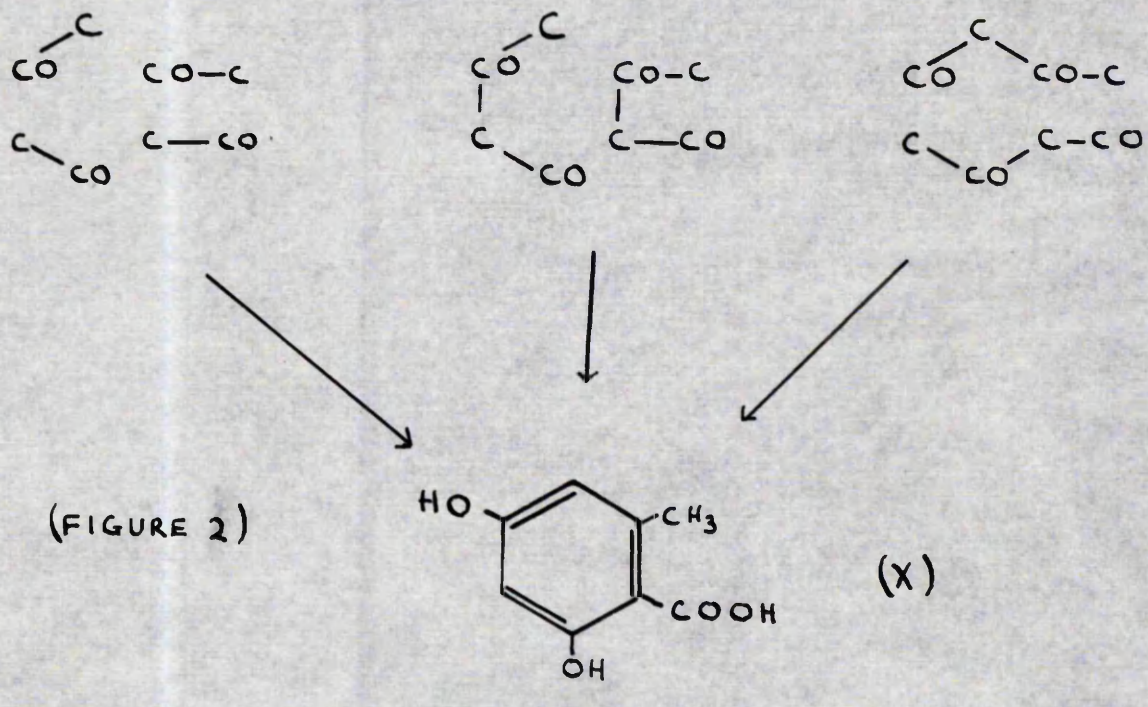

CH3
COOH
OH

(VI)


OH
CH3
HO
COOH

(X)

At the present time, little is known of the intermediate steps between acetate or malonate and the final metabolite. Gatenbeck and Mosbach (26) showed that orsellinic acid (X), in the fungus Chaetomium cochliodes, is produced by the condensation of acetate or, malonate units. The intermediate was considered to be a β-polyketo-acid, which would not be obtained by the oxidation of a fatty acid. Thus, condensation of the acetate units may proceed by either successive condensation of four "C_2 units", or by coupling of two C_4 compounds, such as acetoacetyl-coenzyme-A (Figure 2). Later work has indicated that orsellinic acid is produced by condensation of both malonate and acetate units (56) (see above).

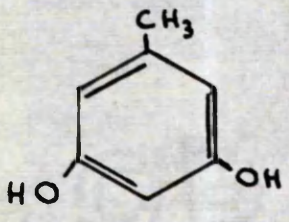


(FIGURE 2)

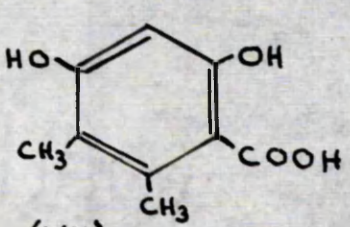
(X)

It appears that the biosyntheses of metabolites similar to orsellinic acid and fatty acids are related. In the former case condensation of acetate or malonate units gives the β -polyketo-acid, but in the latter case stepwise condensation and reduction of the units occurs to give a fully saturated chain.

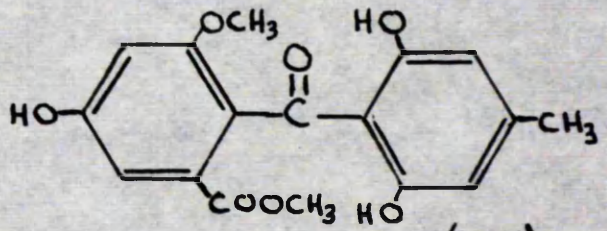
A study of mould mutants would seem to offer the most promising method available for the isolation of intermediates in biosynthesis. The microorganism is subjected to radiation, which may destroy one or more of the enzymes involved in the metabolism of the organism, thus blocking the biosynthetic route to the metabolite. By culture of the mutant organism, the intermediates accumulated prior to the blockage may be isolated, and in some cases identified. This approach is exemplified by the classic work of Davis, Sprinson and Tatum on the biosynthesis of aromatic amino acids (see page 13). Hassall, working with mutamt strains of A. terreus (5), has shown that orsellinic acid (X), orcinol (XI), and a new metabolite (XII), are precursors of sulochrin (XIII).



(X I)



(XII)



(XIII)

In addition to the small units so far mentioned, orsellinic acid has been proposed as a precursor of lichen depsides, depsidones and mould metabolites (27, 28). In view of the work of Gatenbeck and Mosbach (page 8), and the above mentioned work with mutants, it seems likely that in at least some cases "orsellinic acid-like" molecules may indeed be intermediates in acetate biosynthesis.

It is noticeable that the evidence, so far, obtained from studies of mutants gives information about the later stages of biosynthesis. Intermediates (in the early stages) related to the polyketo-acids have not been isolated. In view of the instability of this type of compound, this is not surprising.

2. The shikimate route

An alternative and perhaps concurrent biosynthetic pathway involves shikimic acid intermediates, which give rise to aromatic compounds. There is evidence that the acetate-shikimate pathways occur together in some micro-organisms, for example P.patulum utilises glucose as the sole carbon source to produce two families of aromatic compounds by these two routes (29). The shikimate sequence was elucidated by Davis, Sprinsen and Tatum in work with mutant strains of the mould Neurospora crassa, and with the bacteria Aerobacter aerogenes and Escherichia coli (30, 31, 32). The sequence is linked, as is the acetate

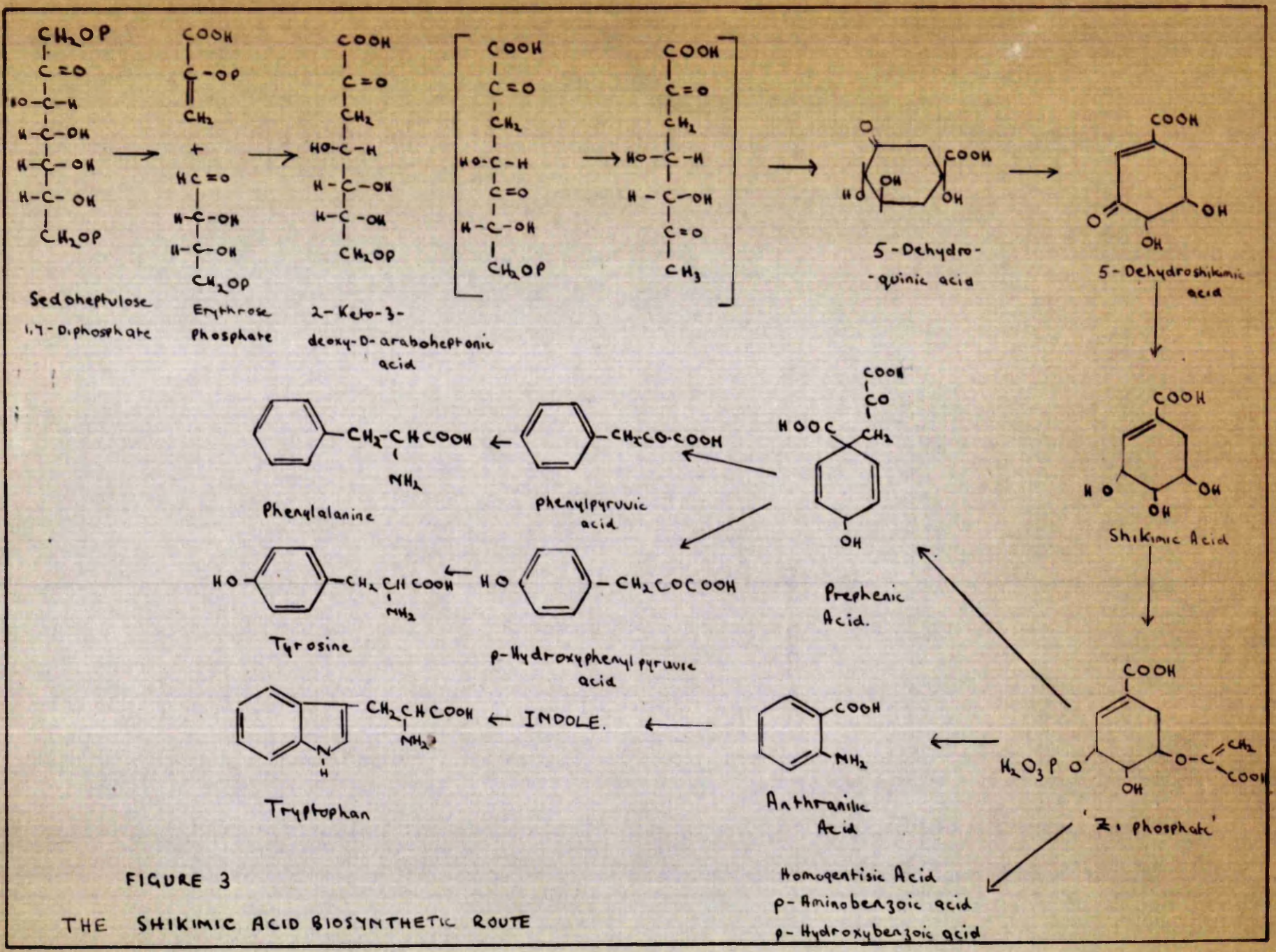


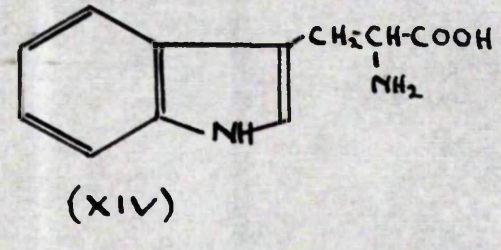
FIGURE 3

THE SHIKIMIC ACID BIOSYNTHETIC ROUTE

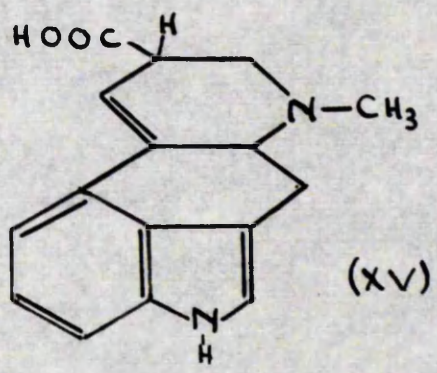
route, with the pentose-phosphate cycle, but no breakdown to acetyl-coenzyme A is involved. The pathways involved in the biosynthesis of some aromatic compounds are shown in Figure 3. These compounds could then undergo modification to orsellinic acid type intermediates, and yield a large variety of metabolites.

3. <u>The biosynthesis of nitrogen and sulphur-containing metabolites</u>

The shikimate route is one method by which nitrogen is incorporated into microbial metabolites. This is achieved by the formation of p-aminobenzoic acid, phenylalamine, tyrosine and tryptophan. The utilisation of these compounds in further biochemical reactions leads to a variety of nitrogen-containing metabolites. Thus tryptophan (XIV) is utilised by the fungus <u>Claviceps purpurea</u> to produce the ergot alkaloids, which contain the lysergic acid moiety (XV) (33).



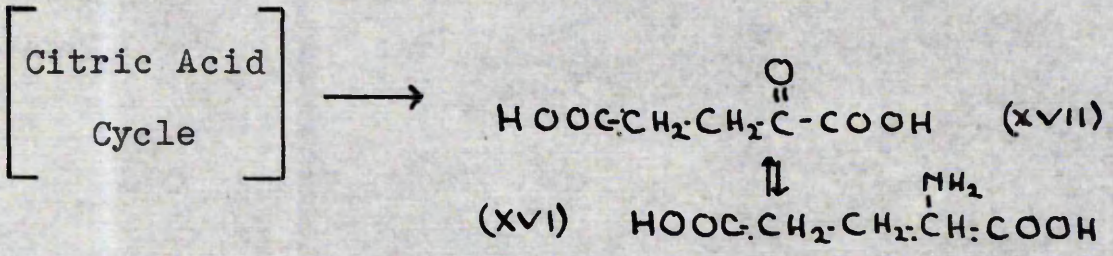
(XIV)



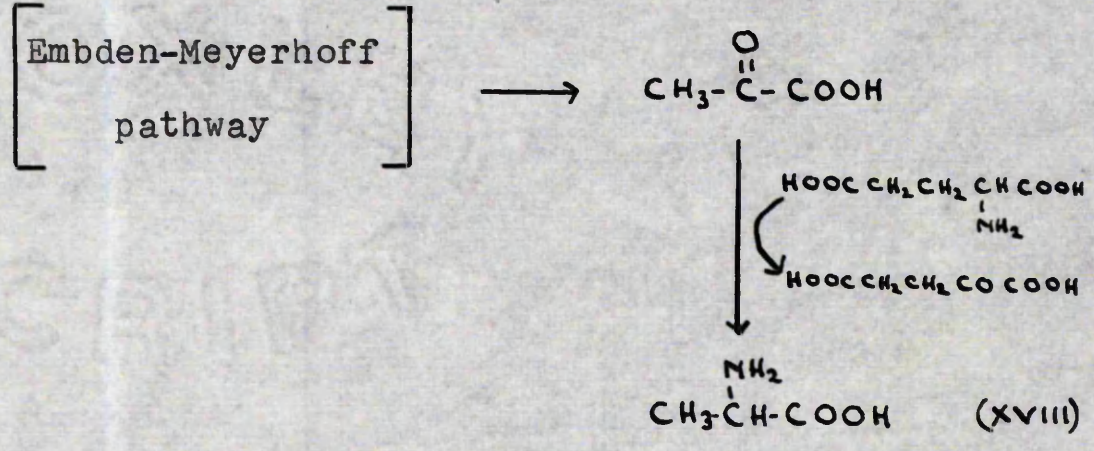
(XV)

It is known that glutamic acid (XVI) occupies a key role in nitrogen metabolism (34, 35), being

connected with the citric acid cycle via α-ketoglutaric acid (XVII).



By means of transamination reactions, glutamic acid provides amino-nitrogen for the production of a large number of compounds, for example α-alanine (XVIII) is synthesised from pyruvate by transamination.



Only a small number of sulphur-containing metabolites have been isolated, although they are very important in biochemistry. For example coenzyme-A operates as a "carrier" of acetate units, and methionine as S-adenosylmethionine (XIX) (36), takes an important part in one-carbon metabolism.

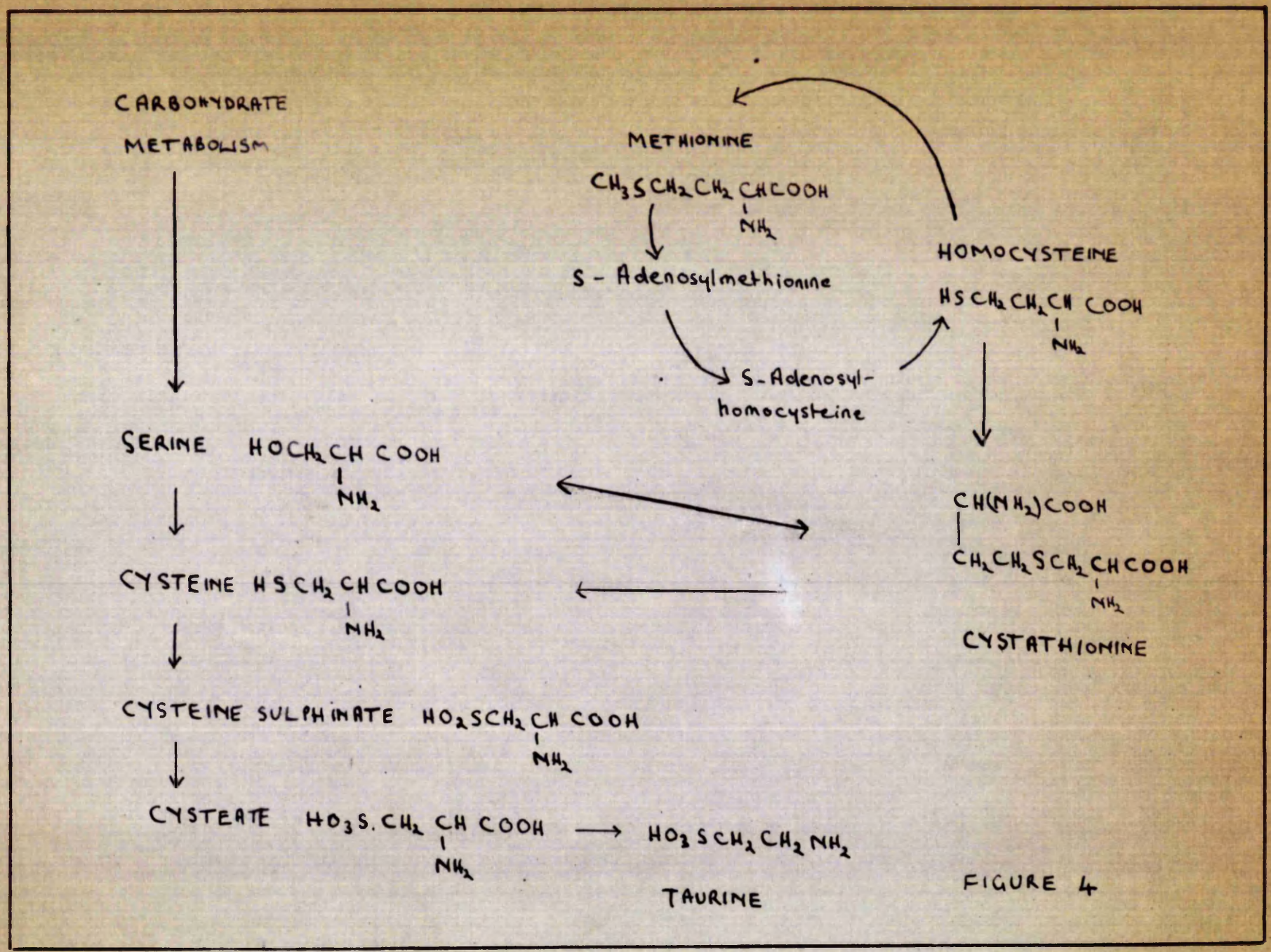
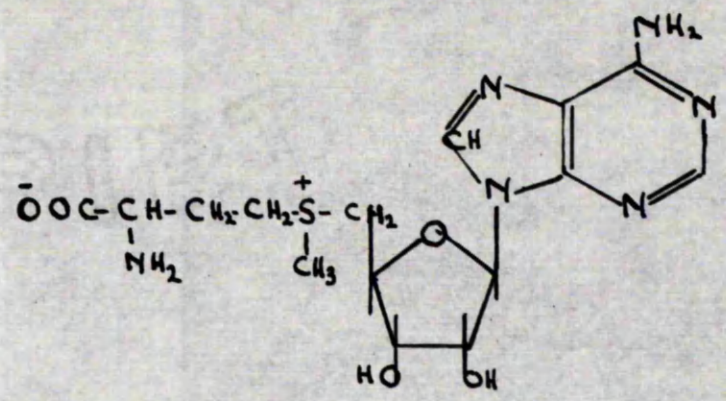
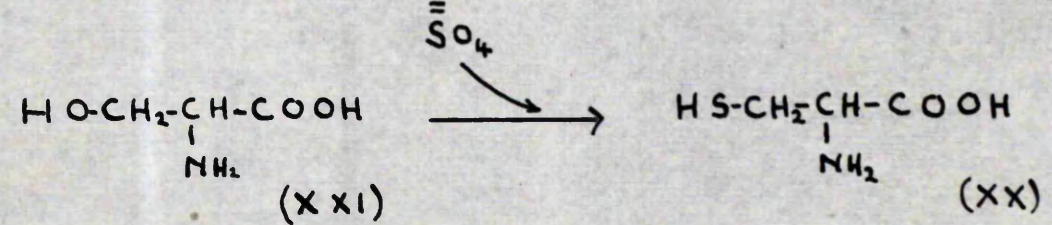

CARBOHYDRATE
METABOLISM
METHIONINE
$CH_3SCH_2CH_2CH(NH_2)COOH$
S - Adenosylmethionine
S-Adenosyl-
homocysteine
HOMOCYSTEINE
$HSCH_2CH_2CH(NH_2)COOH$
SERINE $HOCH_2CH(NH_2)COOH$
CYSTEINE $HSCH_2CH(NH_2)COOH$
$CH(NH_2)COOH$
$CH_2CH_2SCH_2CH(NH_2)COOH$
CYSTATHIONINE
CYSTEINE SULPHINATE $HO_2SCH_2CH(NH_2)COOH$
CYSTEATE $HO_3S.CH_2CH(NH_2)COOH$
$HO_3SCH_2CH_2NH_2$
TAURINE

FIGURE 4



(XIX)

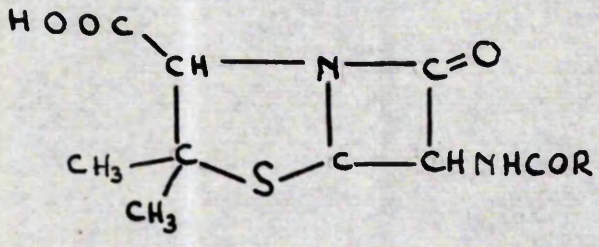
The relationship between the various sulphur-containing amino acids is shown in Figure 4. Most micro-organisms can utilise sulphate as the sole sulphur source, by which means cysteine (XX), a key compound in sulphur metabolism, is formed from serine (XXI). Thus there is a close relationship between sulphur and nitrogen metabolism.



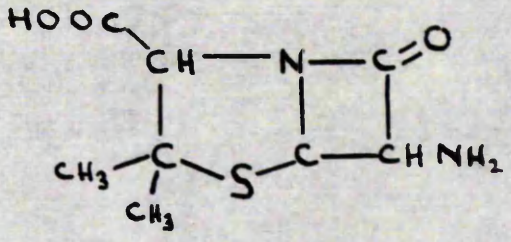
(XXI) (XX)

Very little information is available on the intermediates involved in the formation of complex heterocyclic compounds from amino acids in nature, although there has been much conjecture on the subject. A few compounds have, however, been studied in detail. Thus 6-aminopenicillanic acid (XXII) the precursor of the penicillins (XXIII) (37, 38), is itself derived from

L-cysteine and L-valine (39, 40). Much work has been published on the biosynthesis



(XXIII)

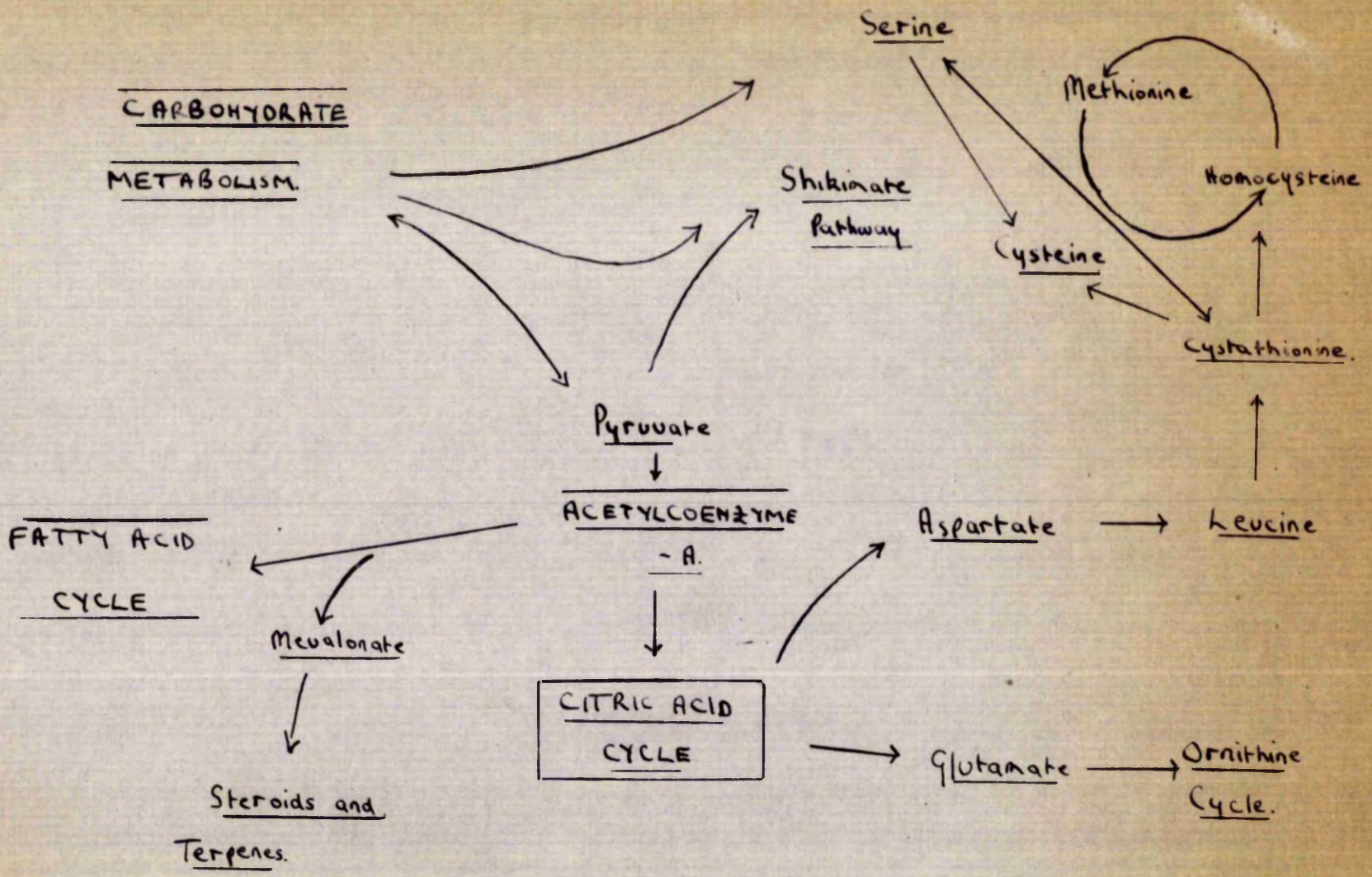


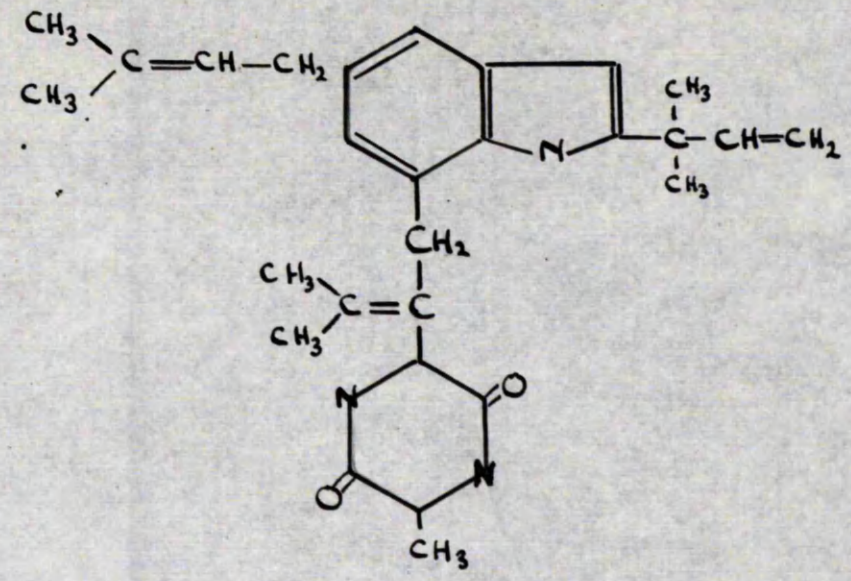
(XXII)

of complex substances like the porphyrins etc. because of their vital role in biological systems, but information is still lacking on the origin of simpler heterocycles.

In some complex metabolites specific portions of the molecule are elaborated by different biosynthetic pathways. Thus the mould A. amstelodami, in the production of echinulin(XXIV), was shown (41) to incorporate [2 - ^{14}C] mevalonic lactone and [1 - ^{14}C] acetate. It also incorporated [1 - ^{14}C] alanine and [1 - ^{14}C] glycine through its amino acid metabolism. In addition, the indole nucleus is probably formed from tryptophan in an analogous manner to the biosynthesis of the ergot alkaloids.

FIGURE 5


CARBOHYDRATE
METABOLISM.
Shikimate
Pathway
Serine
Methionine
Homocysteine
Cysteine
Cystathionine.
Pyruvate
ACETYLCOENZYME
- A.
FATTY ACID
CYCLE
Mevalonate
Steroids and
Terpenes.
CITRIC ACID
CYCLE
Aspartate
Leucine
Glutamate
Ornithine
Cycle.

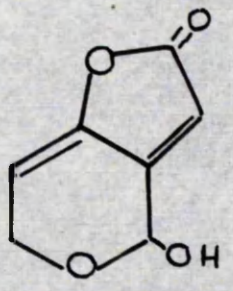


(XXIV)

Figure 5 shows the biochemical pathways encountered in a study of microorganisms as outlined in the foregoing introduction, and emphasises the wide range of biochemistry covered by such a study.

THE OXYGEN HETEROCYCLES

Many oxygen heterocycles are produced by microorganisms. Some unexpectedly complex structures occur providing the chemist with difficult problems in structure determination and synthesis. Thus the structural investigation of patulin (I), at first sight a relatively simple molecule, provided problems of great complexity.



(I)

For convenience, the oxygen heterocycles have been divided into eight groups according to their main structural features, Figure 6.

1. Sugars and related compounds.
2. Furans.
3. Lactones including the macrolides.
4. Tetronic acids.
5. Pyrans : { γ -Pyrones; α -Pyrones and derivatives; Quinonoid pyrones; Chromenopyrones; Azaphilones.
6. Depsidones.
7. Xanthones.
8. The Spirans.

Figure 6.

In some cases particular metabolites may belong to more than one group because of their complexity of structure.

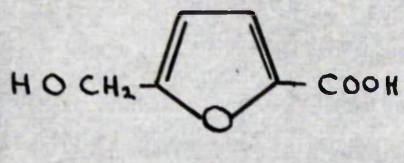
1. Sugars and related compounds

The chemistry of the sugars is normally considered to be a special topic in itself, and would be out of place in this review. It is not surprising that such compounds are important, since carbohydrate metabolism is general throughout nature. Thus a fungus grown on a glucose substrate could produce a large number of different sugars, as well as other metabolites *via* the various biochemical pathways.

The sugars found in antibiotics are included in this group. Many rare sugars have been found as moieties of *streptomycete* antibiotics (42, 7). Other unusual sugars have been obtained by hydrolysis of the polysaccharides and mucopolysaccharides which occur in cell tissue.

2. Furans

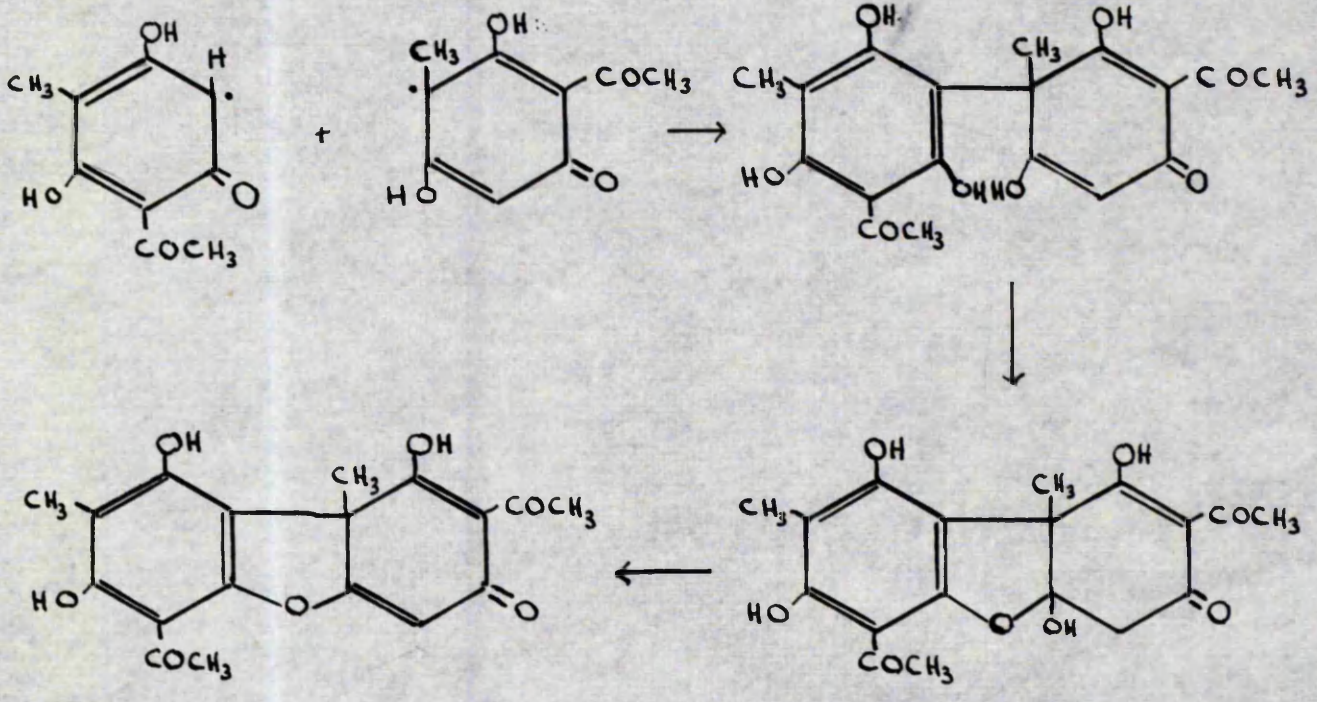
Only five simple furans have been so far isolated from microorganisms, the best known being Sumiki's acid (II), produced by moulds of the *Aspergillus* species.



(II)

No work has been carried out on the biosynthesis of these compounds, although they are presumably related to the sugars in their furanose forms. This is emphasised by the fact that erythrose has been shown (43) to be a possible furan precursor.

Dibenzofurans constitute a class of natural products found only in lichens, usnic acid (III) being the most widely distributed. These compounds are of particular interest because their biosynthesis involves phenol coupling, which has also been postulated to occur in the formation of a number of other natural products. Usnic acid is considered (44) to arise from phenolic precursors according to the following scheme:

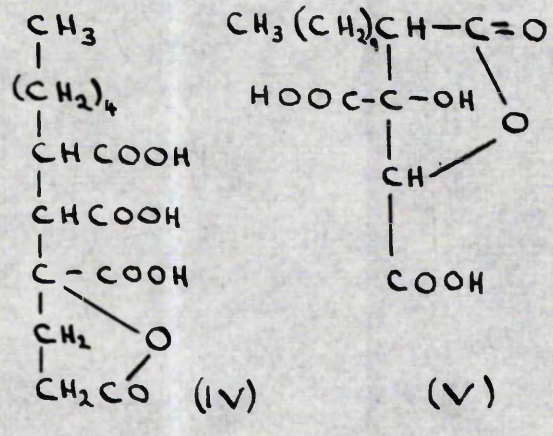


(III)

Phenol coupling reactions have been admirably reviewed by Barton and Cohen, and Lewis (44a).

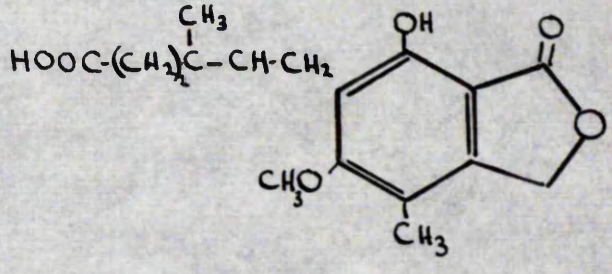
3. Lactones

Lactones are quite common in nature, the most usual being γ-lactones. Examples include spiculisporic acid (IV), mineoluteic acid (V), and mycophenolic acid (VI). In many cases the presence of a lactone grouping has been indicated by the production of one equivalent of acid on alkaline hydrolysis, and by consideration of infrared spectra.



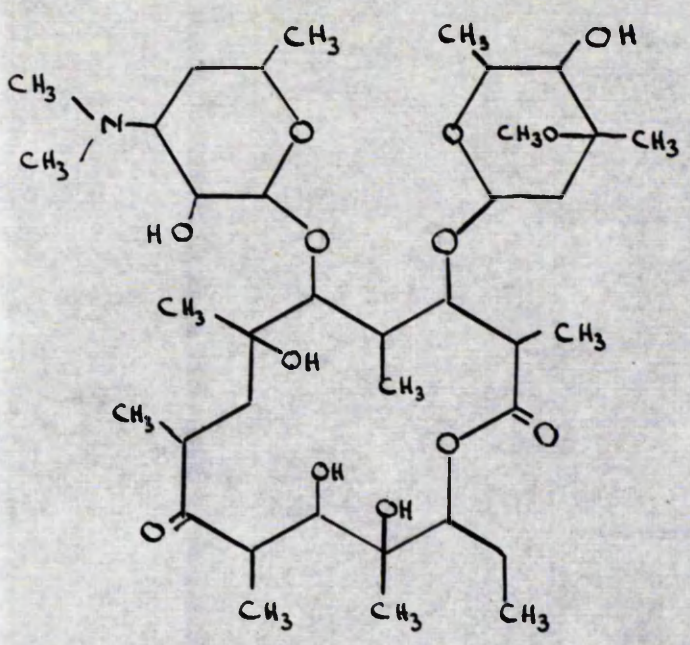
(IV)

(V)



(VI)

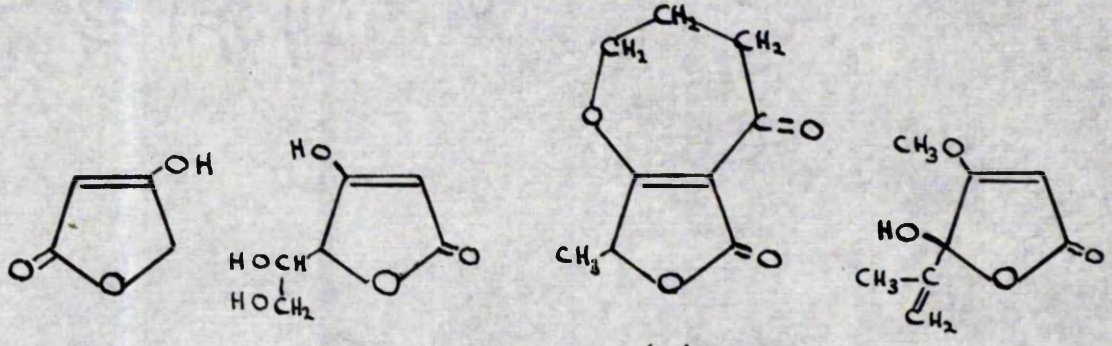
The macrolide lactones are produced by the genus streptomyces. These are lactones of long chain fatty acids, probably acetate-derived, in combination with one or more sugar moiety. An example is erythromycin (VII), the biosynthesis of which has been investigated thoroughly. A labelling and degradation study has shown that propionate is the true precursor (45).



(VII)

4. Tetronic Acids

The tetronic acid nucleus (VIII) occurs naturally in vitamin C (IX), in a group of lichen colouring matters, and in a series of mould metabolites related to carolic acid (X), and penicillic acid (XI).



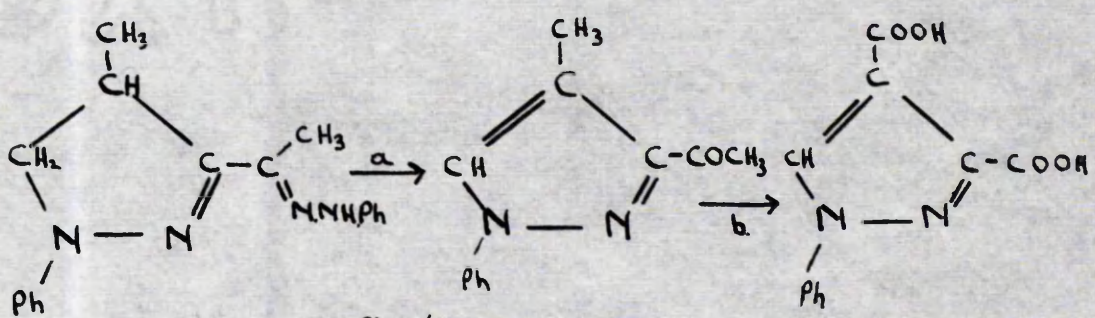
(VIII) (IX) (X) (XI)

The chemistry of tetronic acids has recently been reviewed by Haynes and Plimmer (46).

The difficulties involved in the determination

of the structures of these compounds may be illustrated by a consideration of the structure of penicillic acid. Physical methods, particularly ultraviolet spectroscopy, proved to be extremely valuable, and the final proof of the structure, by synthesis, provides an interesting application of the use of acetylenic compounds in organic synthesis.

Considerable light was thrown on the structure of penicillic acid by the isolation (47) of a pyrazoline compound from the reaction of penicillic acid with two moles of phenylhydrazine. This pyrazoline was shown to have structure (XII), as on oxidation it gave 1-phenylpyrazole-3:4-dicarboxylic acid (XIII).



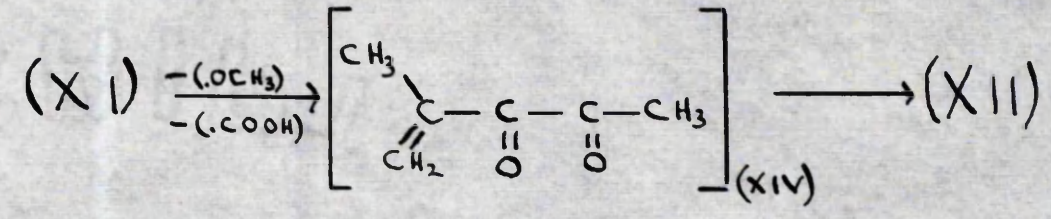
(a) PbO_2/HOAc ; H_2SO_4

(b) I_2/Alkali ; $KMnO_4$.

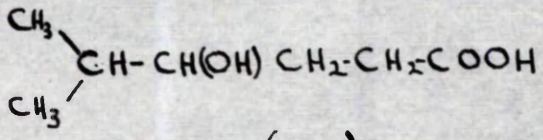
XII XIII

It was deduced that the pyrazoline (XII) was formed from penicillic acid by elimination of one methoxyl and one carboxyl group, and reaction of the

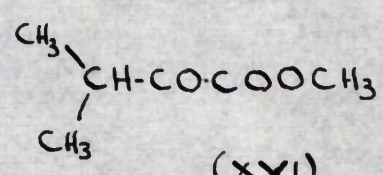
resulting diketone (XIV) with two moles of phenylhydrazine.



Further information was obtained by treatment of penicillic acid with hydriodic acid, which gave rise to the lactone of γ-hydroxy-δ-methylhexanoic acid (XV), Oxidation with potassium permanganate gave methyl dimethylpyruvate (XVI) and oxalic acid.



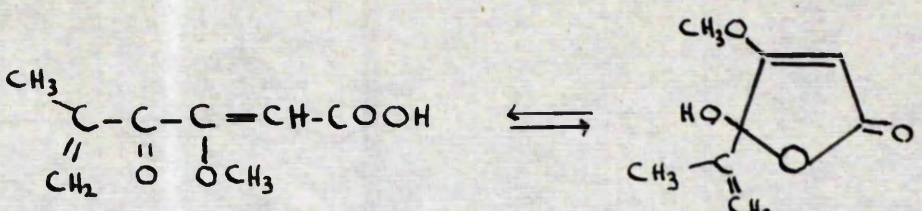
(XV)



(XVI)

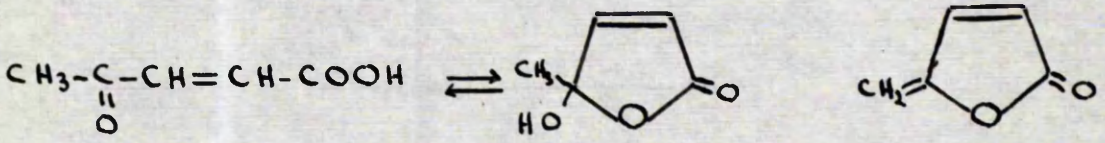
From this information, plus the fact that penicillic acid formed a neutral acetyl derivative, it was concluded that penicillic acid could be represented as an equilibrium between structures (XVII) and (XVIII). However, comparison of the ultraviolet spectrum of penicillic acid with the spectra of acetylacrylic acid (XIX), and protoanemonin (XX) (48, 49), showed that the cyclic acetal structure

explained all the properties of penicillic acid.



(XVII) (XVIII)

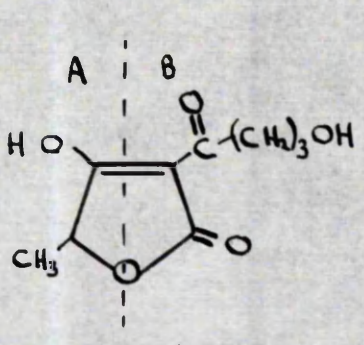
This was confirmed by the synthesis of penicillic acid and its dihydro-derivative by Raphael (50).



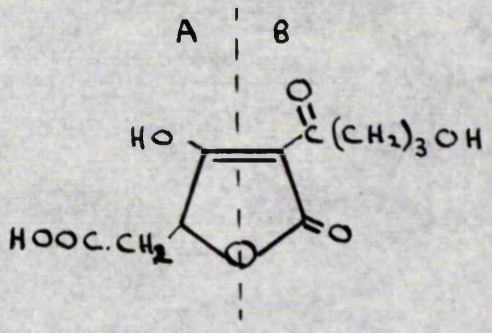
(XIX) (XX)

Despite the considerable number of tetronic acids known to occur naturally, little work has yet been done on their biosynthesis. The available evidence is contradictory, suggesting that tetronic acids can be formed by a number of routes. Thus, ascorbic acid has been shown (51) to be formed from glucuronic acid (available from normal carbohydrate metabolism) in A niger, without breaking the sugar skeleton. With several tetronic acids, however, one part of the molecule seems to be acetate-derived, whilst the other part is derived from another source, probably carbohydrate. Thus Ehrensvärd, and Lybing & Reio (52), considered carolic acid (XXII) and carlosic

acid (XXIII) to be formed from three acetate units (part B) and a further C_4 unit (part A).

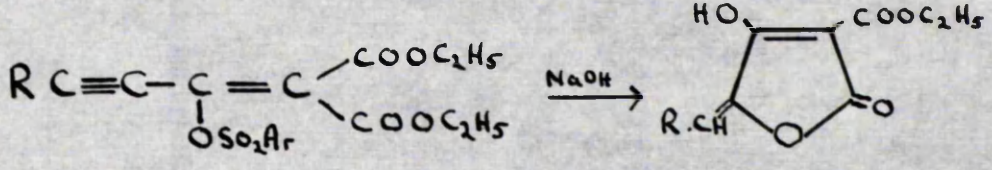


(XXII)



(XXIII)

A biogenetically patterned synthesis of tetronic acids, which may prove to occur <u>in vivo</u>, was recently carried out by Fleming and Harley-Mason (53). Starting from a suitable acetylenic acid, the product obtained was reported to be a substituted tetronic acid, as below:



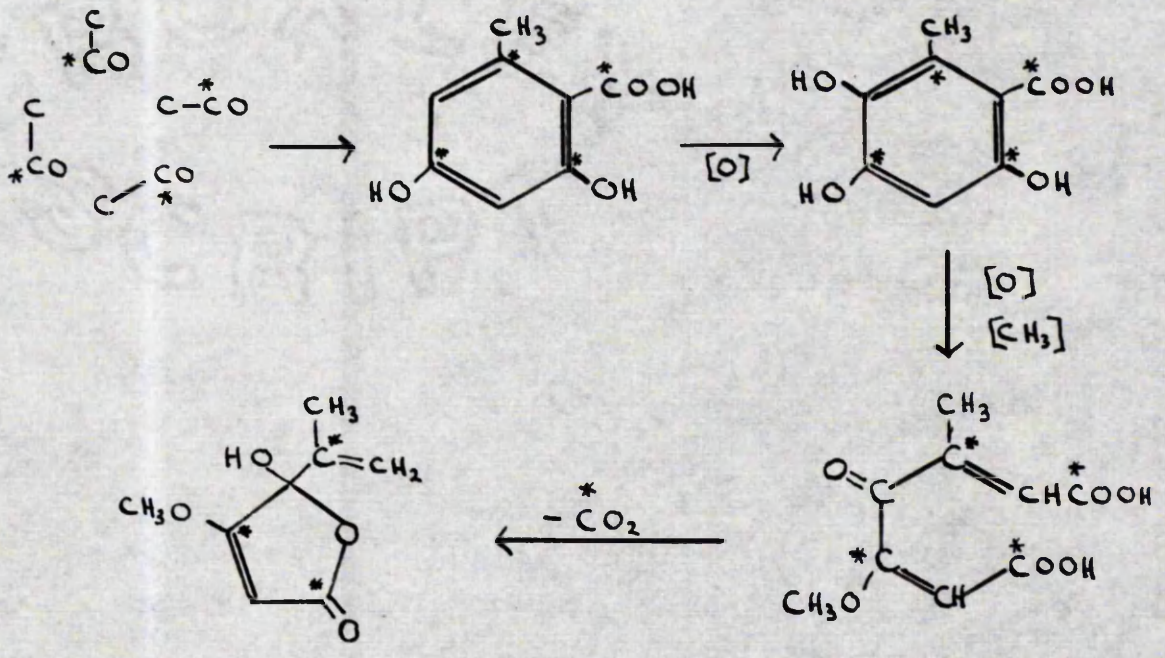
(R = C_6H_5–)

Unfortunately acetylenic compounds have not been isolated from the tetronic acid—moulds, so no evidence for this scheme is available.

Penicillic acid would appear to be derived from acetate and dimethyl pyruvate, or similar precursors. However the labelling pattern produced in penicillic acid

when P. cyclopium was grown on [1 - ^{14}C] acetate was not in agreement with any of the routes proposed above. It was suggested (54) that an orsellinic acid precursor of the type postulated by Seshadri and Birkinshaw (27, 28) (see page 13) was involved, and the scheme shown in Figure 7 accounts for the observed labelling pattern in penicillic acid (XXIV).

Figure 7.



(XXIV.)

Mosbach confirmed this pathway to penicillic acid in P barnense (55), and Bentley and Keil have identified orsellinic acid in P. cyclopium cultures by paper chromatography (56).

The oxidative ring opening of an orsellinic acid-

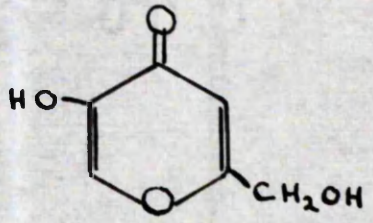
like precursor (compare page 13) may be a fairly common biosynthetic pathway, thus patulin has been shown to originate from 6-methylsalicylic acid (page 35).

5. The Pyrans

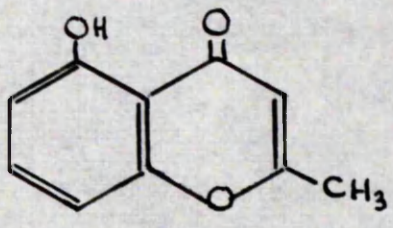
This group contains a large number of extremely variegated structures, which are difficult to classify. For convenience, the pyranose metabolites have been divided into five subgroups (Figure 6), with considerable overlapping between the groups. As might be expected for a group of such diverse structures, no general scheme of biosynthesis has been elaborated; there is of course a formal relationship to glucopyranose.

5.i. The α - and γ - pyrones

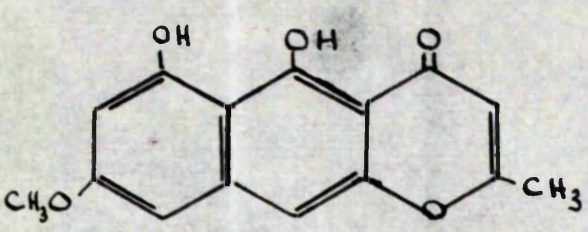
γ-pyrones are well represented among microbial metabolites, examples from fungi are kojic acid (XXV), and 5-hydroxy-2-methylchromone (XXVI). Rubrofusarin (XXVII), and asperxanthone (XXVIII), recently the subjects of some controversy (57, 58), are now thought to be naphtha-4-pyrones, rather than xanthones as previously postulated (59, 60).



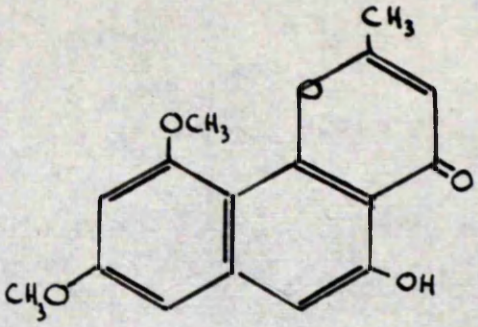
(XXV)



(XXVI)

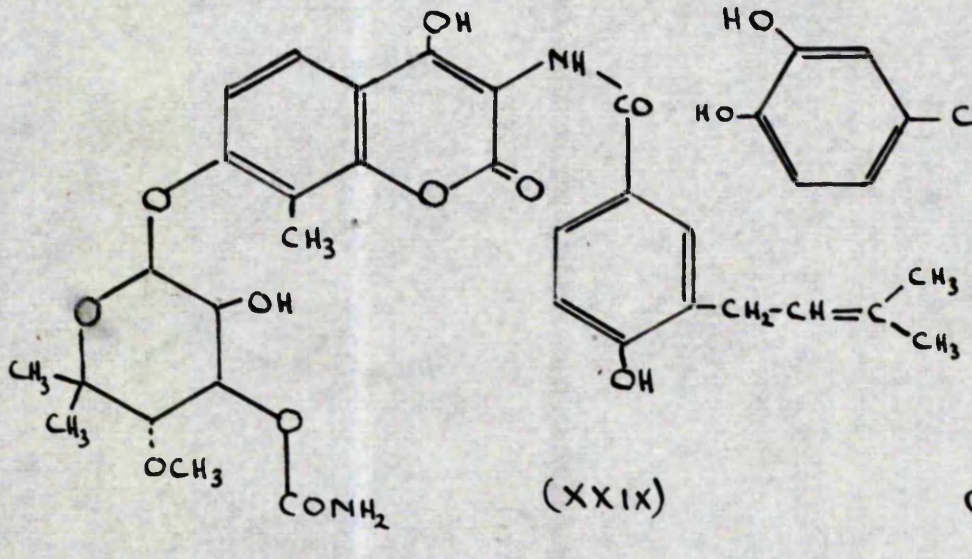


(XXVII)

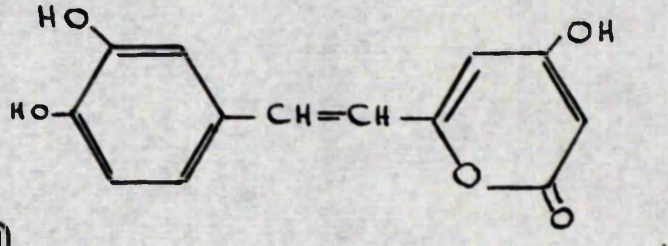


(XXVIII)

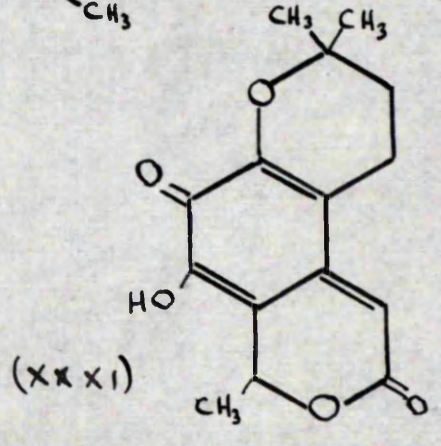
In contrast, only two compounds containing α-pyrone nuclei are known among microbial metabolites. Novobiocin (XXIX) is an antibiotic from Streptomyces niveus, and recently hispidin, a metabolite of the higher fungus Polyphorus hispidus (Bull.) Fr., has been shown (61, 62, 63) to possess structure (XXX). Fuscin (XXXI), although it has been considered to be a chromenopyrone (64), may be classified as a dihydro-α-pyrone.



(XXIX)



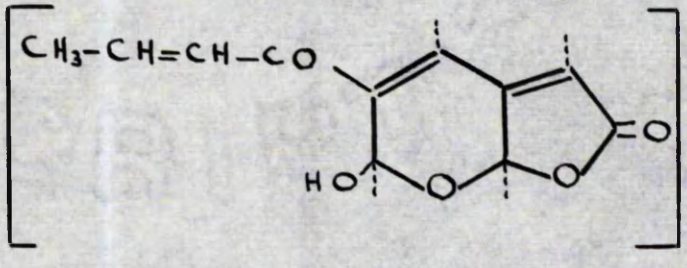
(XXX)



(XXXI)

Among the γ -pyrones, kojic acid has long attracted interest because it is obtained in high yield from several aspergillus and bacterium species. The work of Arnstein and Bentley (65) has shown that in fungi, glucose is converted to kojic acid without breakage of the carbon chain, as in the biosynthesis of ascorbic acid (page 26). The isolation of these γ -pyrones from both bacteria and fungi emphasises the close biochemical relationship between these organisms.

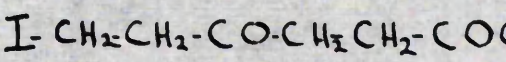
Patulin (I) is considered as a pyranose derivative, although it could be included in the lactone or, tetronic acid groups. Studies on this compound are of particular interest in relation to my own work, since patulin contains structural features in common with those postulated (66) for radicinin (XXXII.) (see discussion,page 54).



(XXXII)

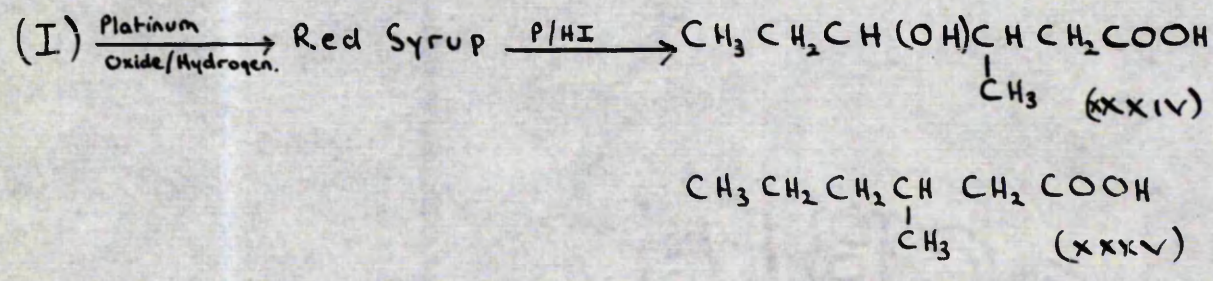
The determination of the structure of patulin may be outlined as follows. The presence of a lactone group was recognised from its reaction with alkali. Reaction of patulin with hydriodic acid gave γ-keto- ε -iodohexanoic acid (XXXIII), showing the presence of a carbonyl group γ

to a potential carboxyl group (there being no carboxyl group in patulin). This iodoacid had one carbon atom less than patulin;



$$I\text{-}CH_2CH_2\text{-}CO\text{-}CH_2CH_2\text{-}COOH \quad (XXXIII)$$

further information was obtained by reduction of fully hydrogenated patulin with red phosphorus and hydriodic acid as in the scheme shown:

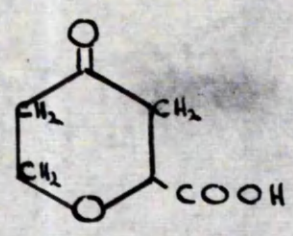


$$(I) \xrightarrow[\text{Oxide/Hydrogen.}]{\text{Platinum}} \text{Red Syrup} \xrightarrow{P/HI} CH_3CH_2CH(OH)\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2COOH \quad (XXXIV)$$

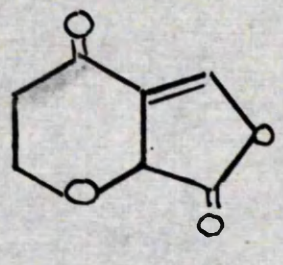
$$CH_3CH_2CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2COOH \quad (XXXV)$$

The product was the lactone of β-methyl-γ-hydroxy-n-hexanoic acid (XXXIV), and its reduction product, methylcaproic acid (XXXV). This indicated the presence of a substituent on the carbon atom γ to the potential carboxyl group.

The isolation of formic acid and tetrahydro-γ-pyrone-2-carboxylic acid (XXXVI), by hydrolysis of patulin with dilute sulphuric acid, led to structure (XXXVII) for patulin (67). This structure was apparently confirmed by the work of Bergel (68), who found that a ketochloro-acid was obtainable from patulin on reaction with ethanolic hydrochloric acid.

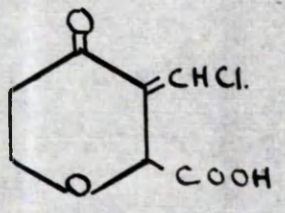


(XXXVI)

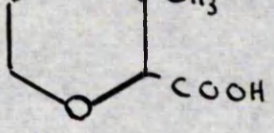


(XXXVII)

The ketochloro-acid was given structure (XXXVIII), and on reduction it was shown to give a chlorine-free ketoacid which was assigned structure (XXXIX).

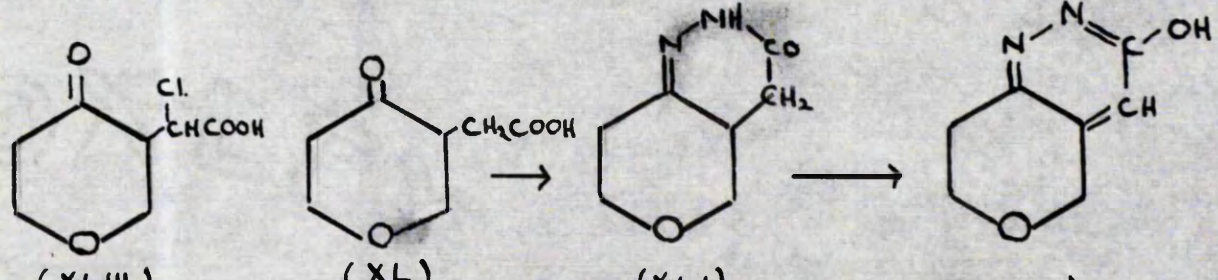


(XXXVIII)



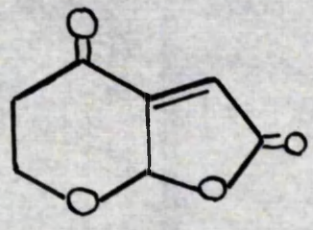
(XXXIX)

Subsequently (XXXVII) was synthesised (69, 70, 71), and found to be different from patulin. In 1949, Plattner and his colleagues (72) showed that the chlorine-free ketoacid gave a pyridazone derivative with hydrazine hydrate, which could be converted to an oxypyridazine. This result was incompatible with the proposed structure (XXXIX) for the ketoacid, and a new structure (XL) was proposed. This acid would give rise to the pyridazone (XLI), and oxypyridazine (XLII) as in the scheme:

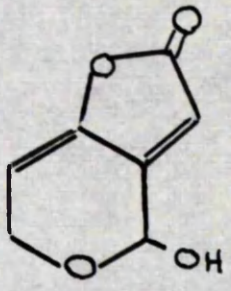


(XLIII) (XL) (XLI) (XLII)

The ketochloro-acid would thus be represented by (XLIII), and consideration of the formation of compounds (XLIII) and (XL) led Plattner to postulate structure (XLIV) or (XLV) for patulin.

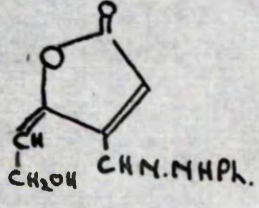


(XLIV)



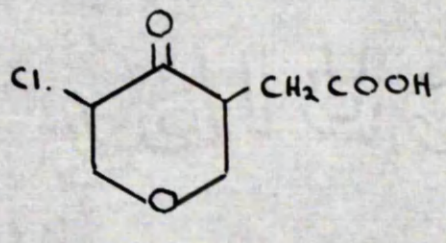
(XLV)

Additional evidence was adduced from the infrared and ultraviolet spectra of patulin, which supported structure (XLV) (73). The fact that patulin and patulin acetate formed the same phenylhydrazone provided further support for the hemiacetal structure.



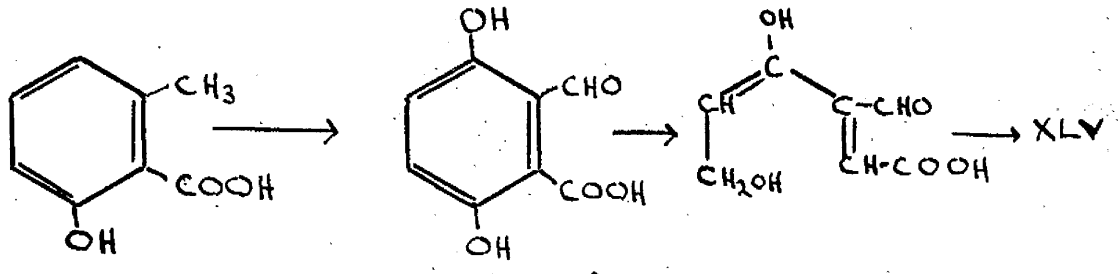
(XLVI)

Woodward and Singh (74) reinterpreted the previous work and concluded that the ketochloro-acid was in fact an α-chloroketo-acid (XLVII), and confirmed this by synthesis. Final proof of structure (XLV) for patulin was provided by an unambiguous synthesis from the acid. (XLVII).



(XLVII)

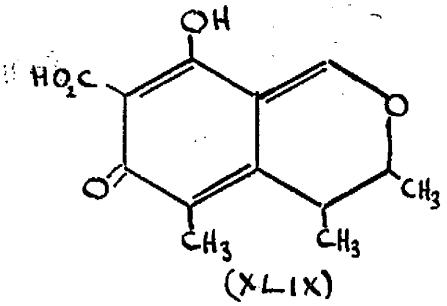
The biosynthesis of patulin, as previously mentioned (page 29), is similar to that of penicillic acid, involving oxidative ring fission and recyclisation of an orsellinic acid-like precursor (XLVIII) (28, 75).



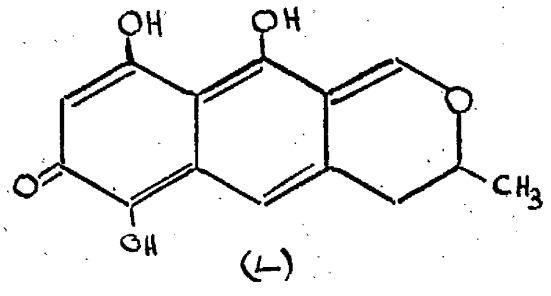
(XLVIII)

5.ii. The Quinonoid-pyrones

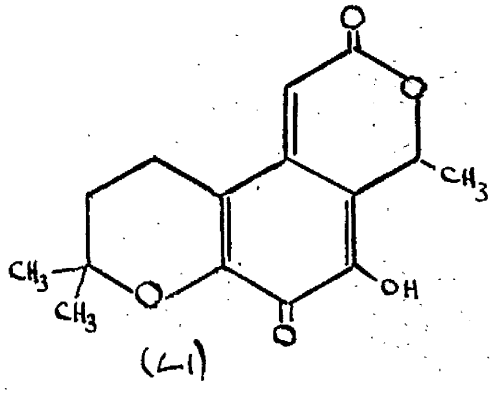
A few metabolites have pyrone moieties in combination with quinonoid nuclei, such that the conjugation of the quinone chromophore is extended. Examples of these are citrinin (XLIX), purpurogenone (L) and fuscin (LI), which has already been mentioned as an example of an α-pyrone derivative; and the azaphilones, which constitute a separate group (see page 39).



(XLIX)



(L)

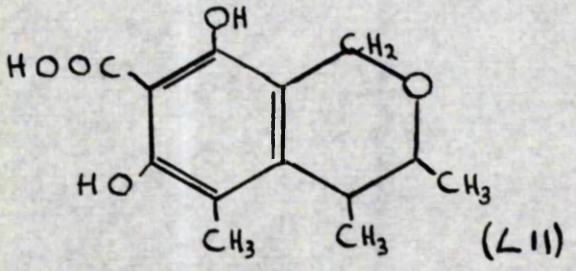


(LI)

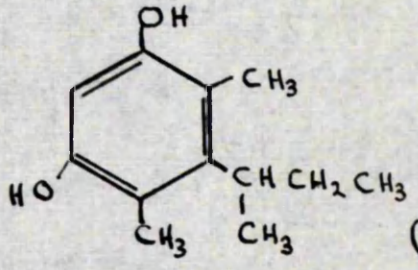
Citrinin was first isolated in 1931 (76), but it was not until 1949 that its structure was confirmed by synthesis (77, 78, 79, 80). The determination of the

structure of citrinin has been reviewed by Bracken (81) and Whalley (64). Of the many degradation products obtained, three phenolic compounds provided vital evidence for the structure of citrinin.

When dihydrocitrinin (LII) was treated with red phosphorous and hydriodic acid, it yielded a dihydric phenol, which was shown (78), by direct comparison with a synthetic speciman, to be 2-(3, 5-dihydroxy-2, 6-dimethylphenyl) butane (LIII).

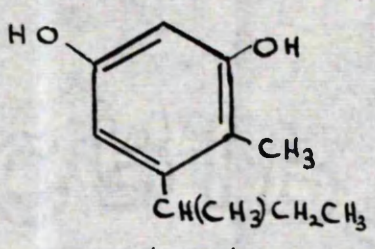


(LII)

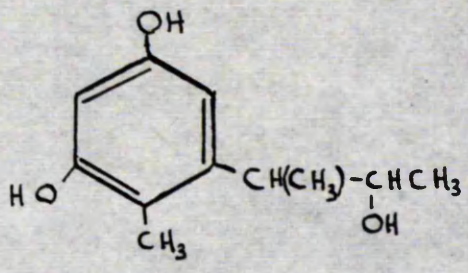


(LIII)

Hydrolysis of citrinin with hot dilute acid or alkali gave a laevo-rotatory phenol (phenol-A) and its optically inactive isomeride (76). The carbon skeleton of phenol-A was established by phosphorous-hydriodic acid reduction (78), when a dihydric phenol was obtained, which was shown by comparison with a synthetic speciman to have structure (LIV). From this evidence it was concluded that phenol-A was l-3-(4, 6-dihydroxy-o-tolyl)-butan-2-ol (LV), this being confirmed by synthesis of its dimethylether p-nitrobenzoate.



(LIV)

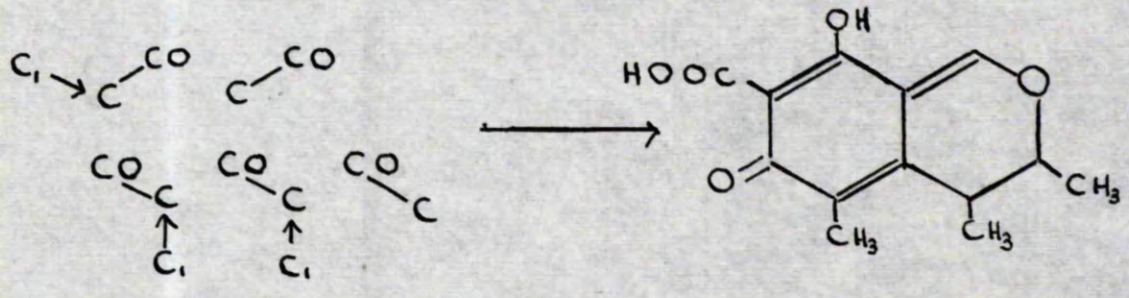


(LV)

As the only other products obtained by hydrolysis of citrinin were formic acid and carbon dioxide, a -C=CH-O- group, (eliminated as HCOOH) and a carboxyl group must be present in the molecule. The hemiquinonoid structure (XLIX) was thus considered to best explain all the properties of citrinin.

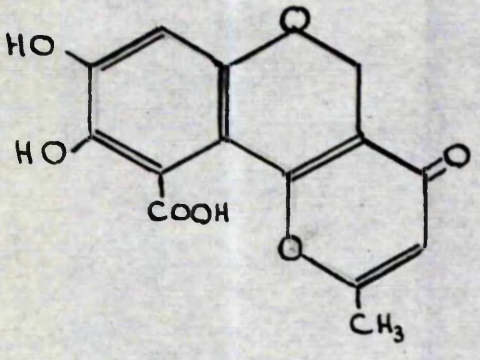
Final confirmation of the structure came from the total synthesis of citrinin from o-toluic acid via phenol-A (77, 78, 79); and also from the synthesis of dihydrocitrinin (80).

The biosynthesis of citrinin is interesting because it provides an example of the introduction of C_1 units during biosynthesis, probably from methionine (see page 15). In the case of citrinin, three C-methyl groups are introduced, one being oxidised to a carboxyl group (82):

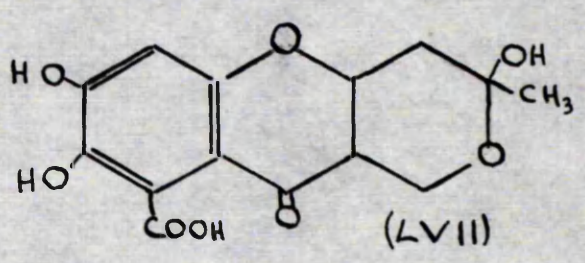


5.iii. The Chromenopyrones

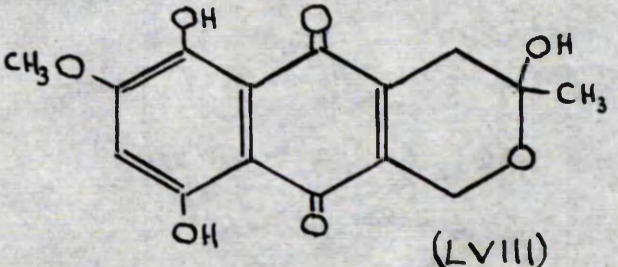
Citromycetin (LVI), fulvic acid (LVII), and fusarubin (LVIII) were considered by Whalley (64) as a separate group of oxygen heterocyclic compounds, presumably because they contain a pseudo-chroman or chromone ring system.



(LVI)

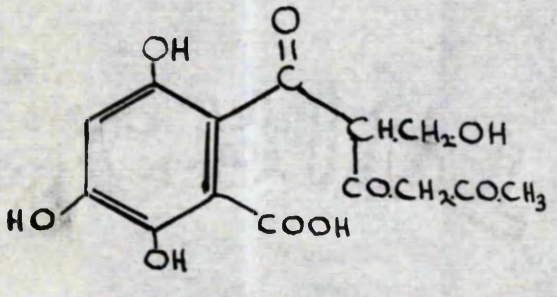


(LVII)

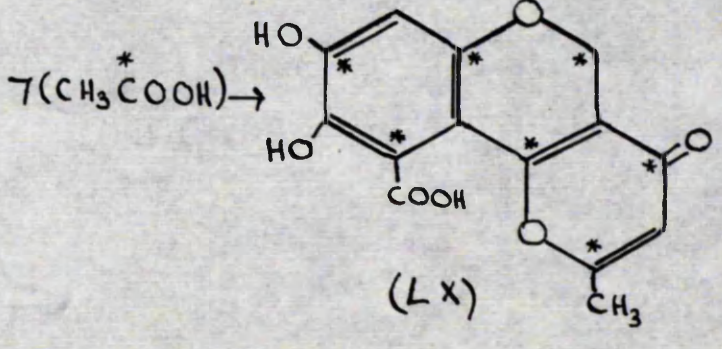


(LVIII)

The biochemistry of the chromenopyrones has attracted attention because a common precursor such as (LIX) can be envisaged (83). Biogenetic work to date has been confined to citromycetin, which was shown (82) to incorporate seven acetate units to give the labelling pattern shown (LX);



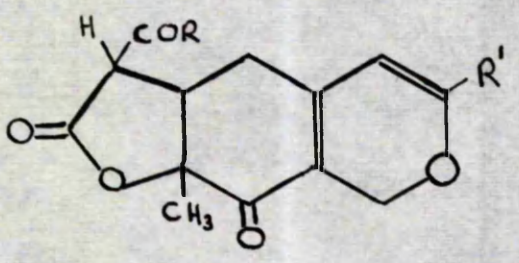
(LIX)



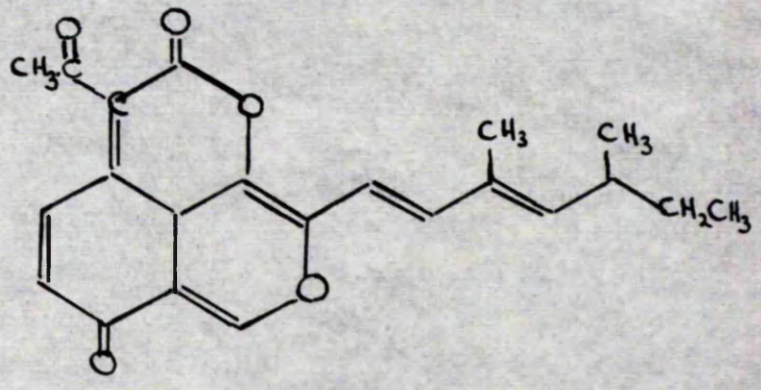
(LX)

5. iv. The Azaphilones

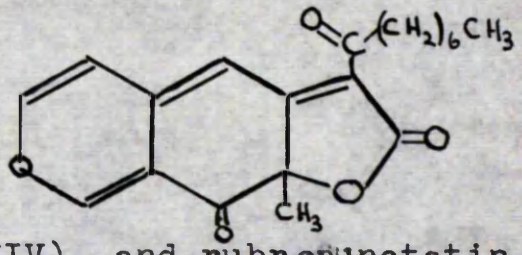
This group of mould pigments, so named because most of them react readily with ammonia, has been elucidated mainly by the work of Robertson, Whalley and their colleagues. The work was first reviewed in 1956 (84), at which time very little information was available. Tentative structures have now been postulated for monascin (LXI), rotiorin (LXII), and monascorubrin (LXIII).



(LXI), $\begin{cases} R = C_5H_{11} \\ R' = -CH=CHCH_3 \end{cases}$

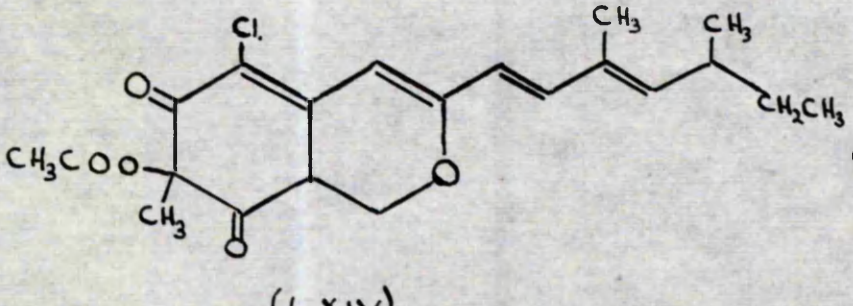


(LXII)

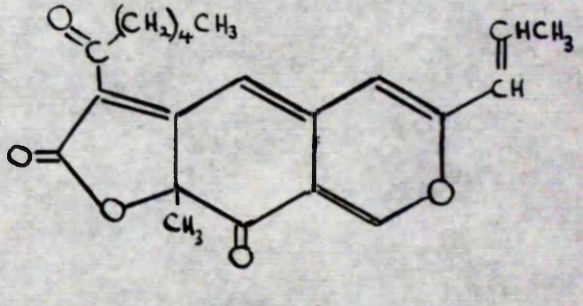


(LXIII)

The structures of sclerotiorin (LXIV), and rubropunctatin (LXV) are known with more certainty, but synthetic confirmation of these structures is awaited.

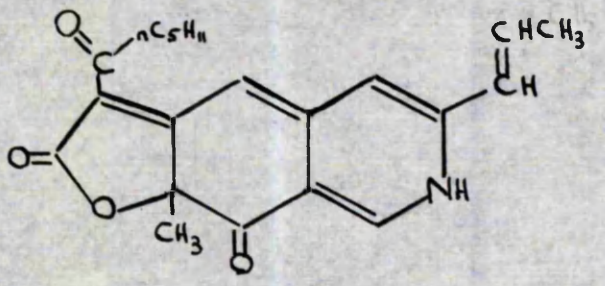


(LXIV)

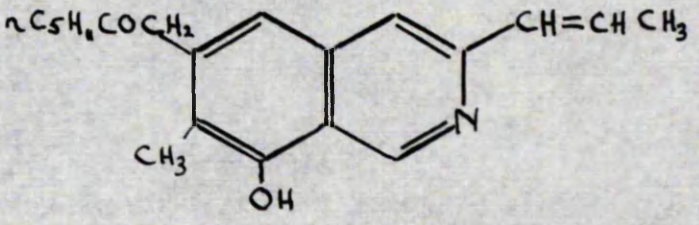


(LXV)

Some recently reported work on azaphilones has been concerned with rubropunctatin (85, 86). In common with the azaphilones (except monascin) rubropunctatin gave an "ammonia compound", rubropunctatamine (LXVI), in which the functional groups of the parent compound were retained.

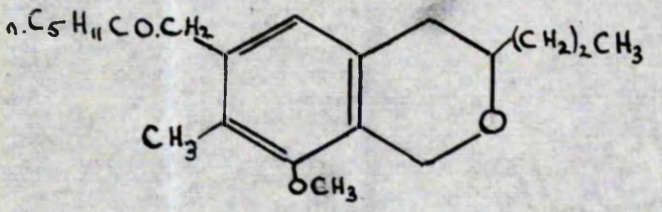


(LXVI)

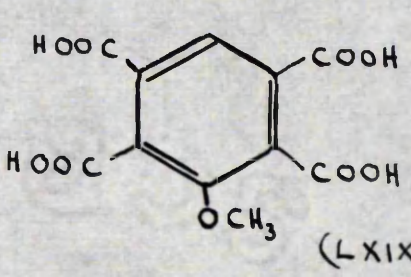


(LXVII)

On reduction of (LXVI) with zinc and acetic acid, a colourless compound, aporubropunctatamine was obtained. This was shown to be a substituted isoquinoline of structure (LXVII). Oxidation of hexahydro-O-methylaporubropunctatin, which was thought to have structure (LXVIII), gave anisole-2,3,5,6-tetracarboxylic acid (LXIX), which evidence supported the structures proposed for rubropunctatin and its derivatives.

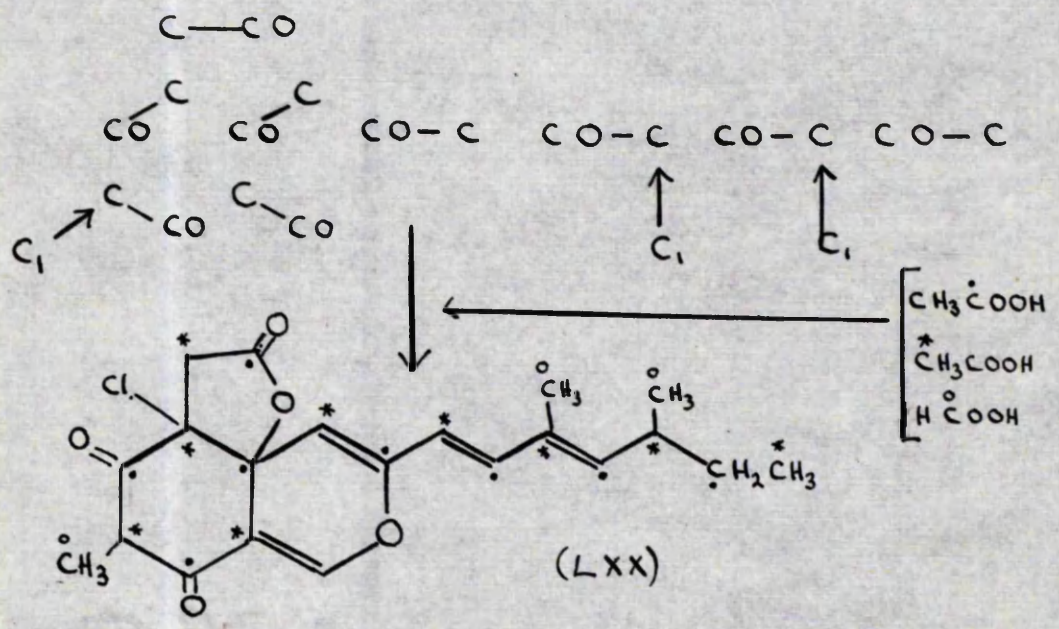


(LXVIII)



(LXIX)

The biosynthesis of sclerotiorin has been shown (82) to involve the condensation of nine C_2 units, with three nuclear methylations occuring. This was originally interpreted in accordance with structure (LXX), which is now known to be incorrect.

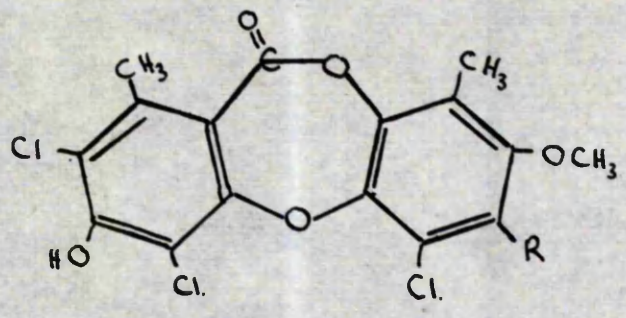


(LXX)

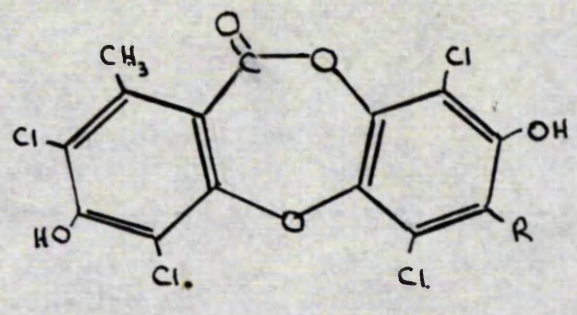
The data, however, is in agreement with structure (LXIV) now accepted for sclerotiorin.

6. The Depsidones

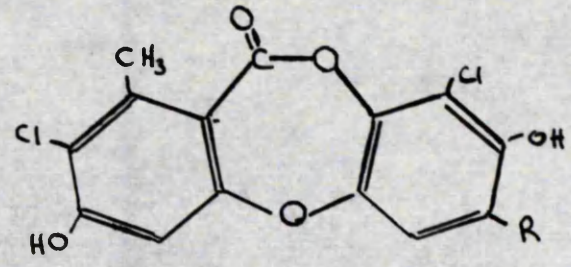
The main source of depsidones is lichens, and as these organisms are symbiotic combinations of algae and fungi, it is appropriate to consider depsidones as fungal metabolites. However, nidulin (LXXI), nornidulin (LXXII), and dechloronornidulin (LXXIII), are metabolites produced by a strain of A. nidulans.



(LXXI)



(LXXII)

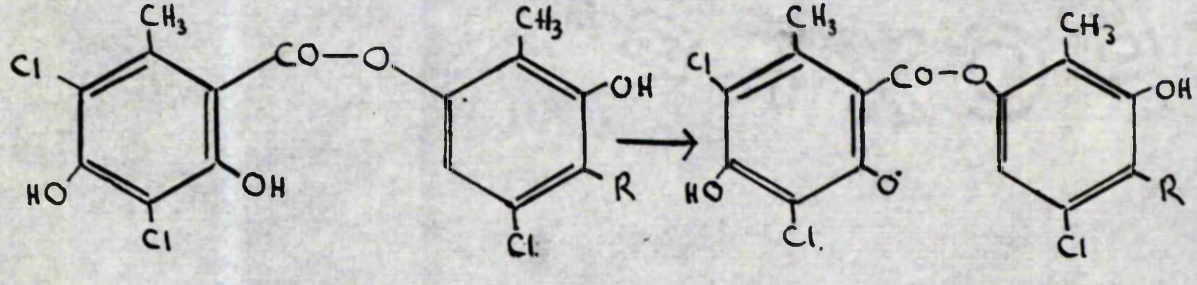


$\left[R = -\overset{CH_3}{\underset{|}{C}}=CHCH_3\right]$

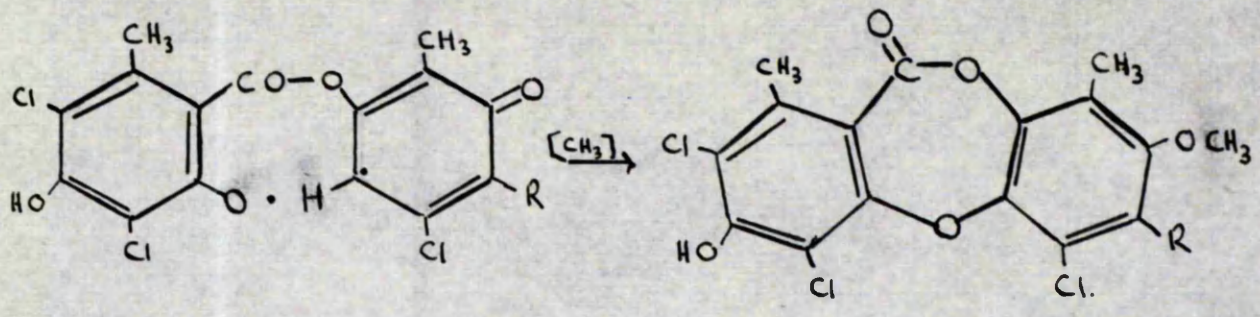
(LXXIII)

The determination of the structure of a depsidone may be carried out by cleavage of the lactone ring with alkali, and oxidation to phenolic acids (64).

Depsidones are most probably formed in nature by a phenol coupling reaction (reference 44, page 21). Thus the biosynthesis of nidulin may be represented as follows:



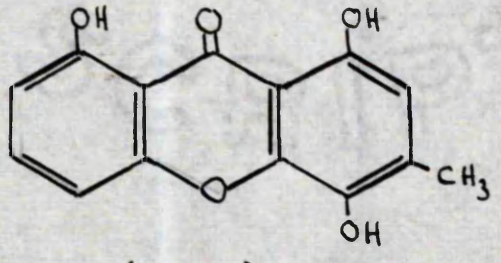
$\left[R = -\overset{CH_3}{\underset{|}{C}}=CHCH_3\right]$



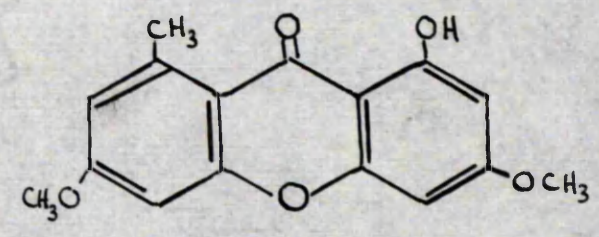
(LXXI)

7. Xanthones

Only three genuine xanthone derivatives have been isolated from micro-organisms. These include ravenelin (LXXIV), and lichexanthone (LXXV). The curious

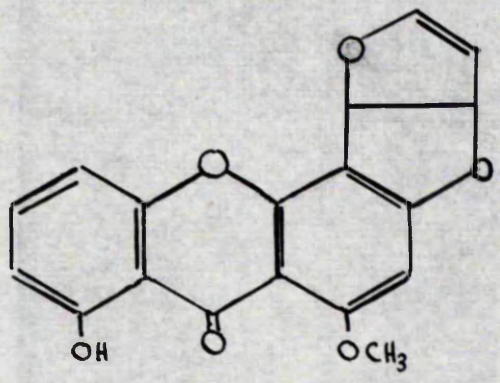


(LXXIV)



(LXXV)

structure postulated (87) for sterigmatocystin (LXXVI) has recently received further support by the synthesis of one of its degradation products (88).

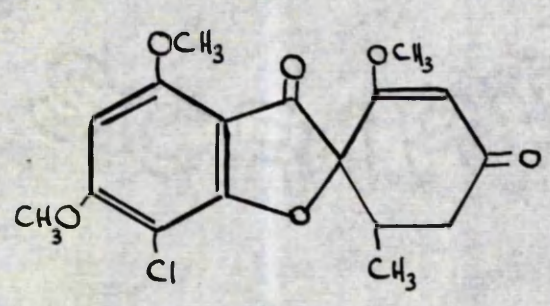


(LXXVI)

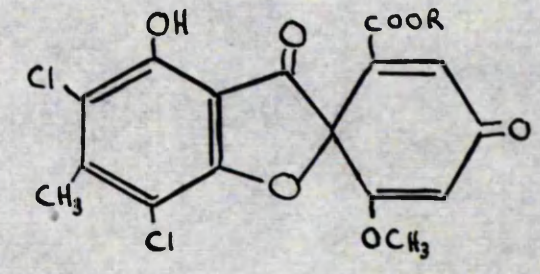
8. The Spirans

Moulds are known to produce four closely related spirocoumaran-3-ones, they are griseofulvin (LXXVII),

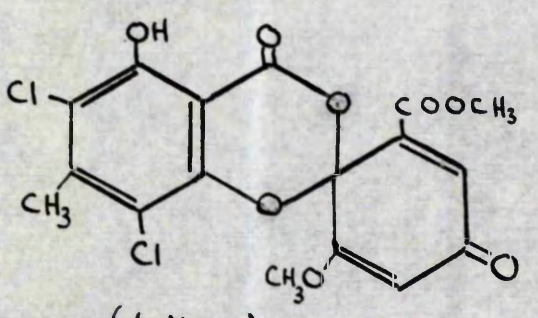
geodin, erdin (LXXVIII R = CH_3 or H), and geodoxin (LXXIX). In addition, picrolichenic acid, a lichen acid, is thought to possess structure (LXXXI) (89).



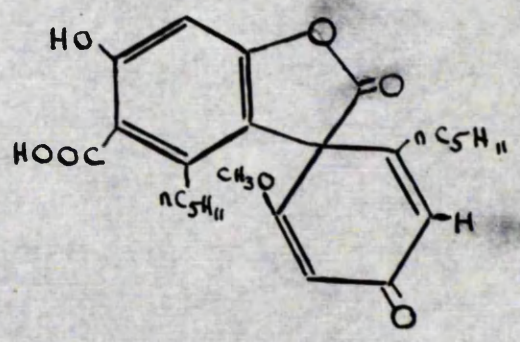
(LXXVII)



(LXXVIII)



(LXXIX)

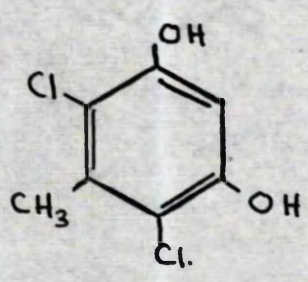


(LXXX)

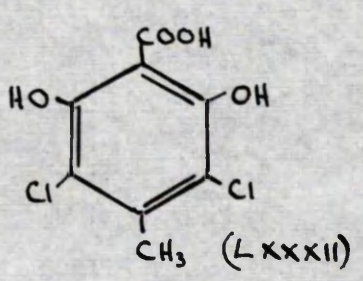
The most recently reported structural work on spiran heterocycles was concerned with geodoxin (90). Since this metabolite is produced by a mutant of A terreus, which elaborates geodin and erdin, a close biochemical relationship between these compounds was predicted.

Pyrolysis of geodoxin gave 4, 6-dichloro-5-methylresorcinol (LXXXI), and oxidation with alkaline hydrogen peroxide gave the dichloro-p-orsellinic acid (LXXXII). The production of methyl-2, 5-dihydroxy-3-

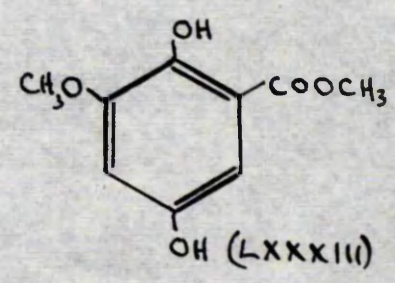
methoxybenzoate (LXXXIII), on treatment of dihydrogeodoxin with 80% sulphuric acid, suggested structure (LXXXIV) for the dihydro compound. Comparison of the spectral data of geodoxin with other spirans led to the proposal of structure (LXXIX) for geodoxin.



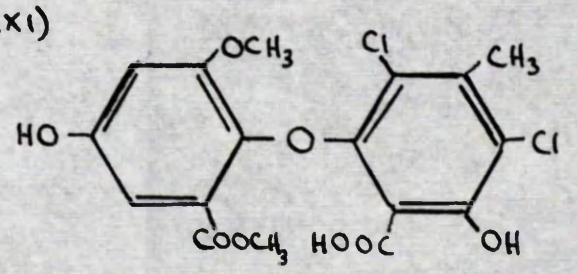
(LXXXI)



(LXXXII)

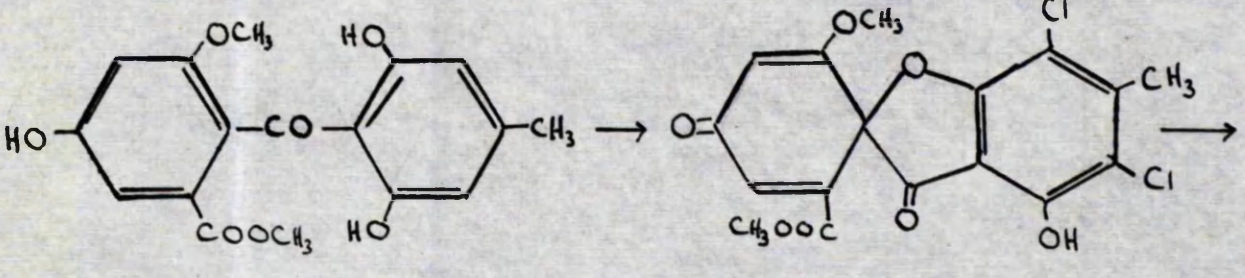


(LXXXIII)



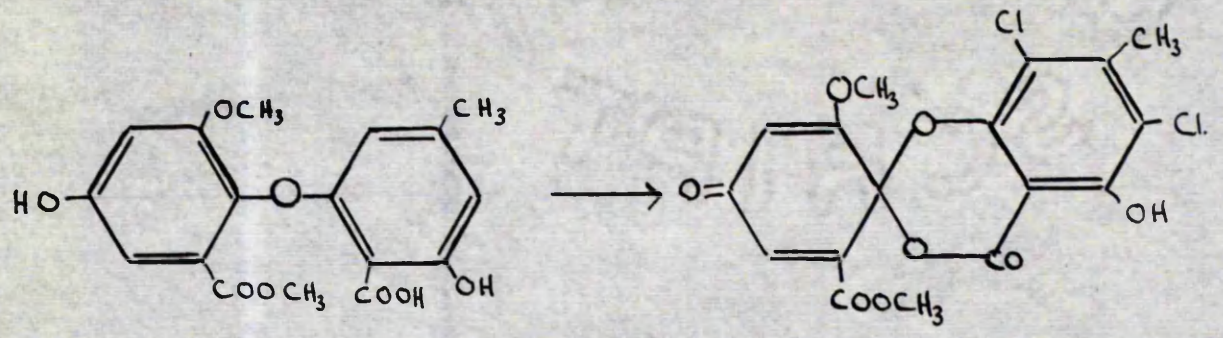
(LXXXIV)

The spirans might be expected to show close biogenetic relationships, and recently, evidence has been advanced in support of this. Thus Curtis and Hassall (91), working with A. terreus, have postulated that geodoxin (LXXIX) is produced in a biosynthetic sequence involving sulochrin (LXXXV), geodin (LXXVIII), and asterric acid (LXXXVI) as illustrated.



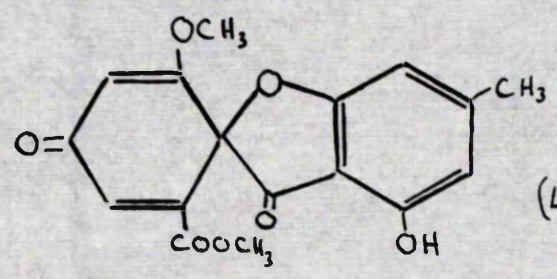
(LXXXV)

(LXXVIII)



(LXXXVI) (LXXIX)

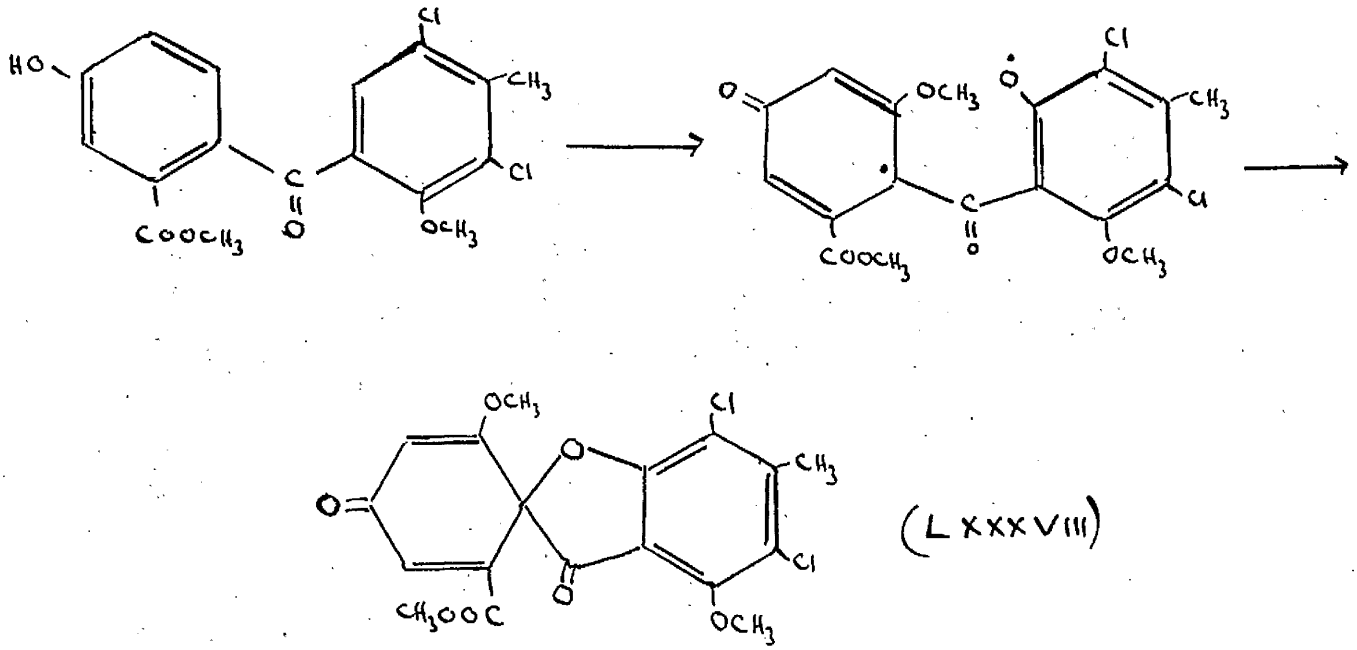
Somerfield and his colleagues concluded that dechlorogeodin (LXXXVII) is a common intermediate in the biosynthesis of geodin and asterric acid, and that the nature of the end products is determined by the extent to which this compound is further chlorinated.



(LXXXVII)

Birch et. al. (93) have shown that griseofulvin may be biosynthesised from acetate. Present observations seem to favour the biogenetic pathway proposed by Barton and Cohen (44a), in which a β -polyketone synthesis is followed as far as the benzophenone stage, with subsequent radical coupling. The latter stages have been admirably illustrated by the total synthesis of griseofulvin (see page 6) and partial synthesis of (±)-geodin methylether (LXXXVIII) (94), by Scott and his colleagues, from the corresponding

benzophenones, as illustrated below:



(LXXXVIII)

It is hoped that this survey, although mainly restricted to oxygen heterocyclic metabolites, has served to illustrate the enormous sythetic ability of the 'humble fungi' and the important contributions towards closer understanding of the chemical and biochemical processes of all metabolism made by studies of such micro-organisms.

REFERENCES

1. Raistrick, Proc.Roy.Soc., 1931, 220B, 9.
2. Birch and Smith, Chem.Soc.Special Publ., 1958,12,15.
3. Ehrensvärd, Chem.Soc.Special Publ., 1958,12,29.
4. Nord and Clarke, Arch.Biochem.Biophys., 1955,59,285.
5. Bu'Lock, Chemical Society Symposium, Sheffield, 1962.
6. Dalgliesh, Chem.Soc.Special Publ., 1958,12,14.
7. Miller, "The Pfizer Handbook of Microbial Metabolites", McGraw Hill, New York, 1961.
8. Birch and Donovan, Austral.J.Chem., 1953,6,370.
9. Robinson, "Structural Relations of Natural Products", Clarendon Press, 1955.
10. Collie, J., 1893, 63, 329.
11. Collie and Chrystall, J., 1907, 91, 1803.
12. Astill and Roberts, J., 1953, 3302.
13. Birch and Donovan, Chem. and Ind., 1954, 1047.
14. Davies, King and Roberts, J., 1955, 2782.
15. van Tamelen, Fortschr.Chem.Org. Naturstoffe, "Biogenetic-type synthesis of Natural Products", 1961,XIX,242.
16. Day, Nabney and Scott, Proc.Chem.Soc.,1960, 284.
17. Birch, Massey-Westropp and Roye, Austral.J.Chem., 1955,8,539.
18. Gunsalles, Barton and Gruber, J.Amer.Chem.Soc.,1956,78,1763.
19. Breslow, Chem. and Ind., 1957, 893.

20. Stumpf, Ann.Rev.Biochem., 1960,29,261

Wakil and Ganguly, J.Amer.Chem.Soc., 1959,2597.

Wakil, McLain and Warshaw, J.Biol.Chem., 1960,235,PC 26,31-32.

21. Lynen, Reichert and Rueff, Annalen, 1951, 574, 1.

22. Lynen, et. al., J.Amer.Chem.Soc., 1959,81,1254
Angew.Chem., 1958, 70, 738.

23. Birch, English, Massey-Westropp and Smith, J., 1958, 369.

24. Bu'Lock and Smalley, Proc.Chem.Soc., 1961, 209.

25. Birch, Cassera and Rickards, Chem. and Ind., 1961, 792.

26. Gatenbeck and Mosbach, Acta Chem. Sc. and., 1959,13,1561.

27. Aghoramurthy and Seshadri, Proc. Indian Acad.Sci., 1949,20A, 1.
J.Sci. Ind. Res., India, 1954,13A,114.

28. Birkinshaw, Ann. Rev. Biochem., 1953,122,379.

29. Bassett and Tanenbaum, Experimentia, 1958, 14, 38.

30. Davis, Adv. Enzymol., 1955, 16, 247.

31. Meister, "Biochemistry of the Amino Acids", Academic Press, Inc., New York, 1957, 346.

32. Sprinson et al, J. Biol. Chem., 1959, 234, 713, 716.

33. Stoll, Fortschr. Chem. Org. Naturstoffe, 1952, 9, 14.

34. Meister, "Biochemistry of the Amino Acids", Academic Press, Inc., New York, 1957, 266.

35. Fruton and Simmonds, "General Biochemistry", John Wiley and Sons, Inc., New York, 1958, 771.

36. Challenger, "Aspects of the Organic Chemistry of Sulphur", Buterworths Scientific Publications, 1959.

37. Kato, J.Antibiotics (Japan), 1953, 6A, 130, 184.

38. Batchelor, Doyle, Nayler and Robinson, Nature, 1959, 183, 257.

39. Arnstein and Grant, Biochem. J., 1954, 57, 353.

Arnstein and Crawhill, ibid, 1957, 67, 180.

Stevens, Inamine and Delong. J.Biol Chem., 1956, 219, 405.

Arnstein and Margreiter, Biochem.J., 1958, 68, 339.

Grau and Halliday, ibid, 1957, 69, 205.

40. Arnstein and Clubb, ibid, 1957, 65, 618.

Stevens and Delong, J. Biol Chem., 1958, 230, 991.

41. Birch et. al., J., 1961, 3128.

42. Foster and Horton, "Advances in Carbohydrate Chemistry", Academic Press, New York, 1959.

43. Wenkert, Experimentia, 1959, 15, 165.

44. Barton, Deflorin and Edwards, J., 1956, 532.

44a. Barton and Cohen, Festschrift Arthur Stoll, 1957, 117.

Lewis Chem. and Ind., 1962, 159.

45. Corcoran, Kaneda and Butte, J. Biol. Chem., 1960, p.c.29.

46. Haynes and Plimmer, Quart.Rev., 1960, XIV, 292.

47. Birkinshaw, Oxford and Raistrick, Biochem.J., 1936, 30, 394.

48. Shaw, J., 1946, 68, 2510.

49. Paist, Blout, Uhle and Elderfield, J.Org.Chem., 1941,6,280.

50. Raphael, J., 1947, 805.

51. Sanstry and Sarma, Nature, 1957, 179, 44.

52. Ehrensvärd, Chem.Soc.Special Publ., 1958,12,14.

Lybing and Reio, Acta Chem. Scand., 1958,12,1575.

53. Fleming and Harley-Mason, Chem. and Ind., 1962,561.

54. Birch, Blance and Herchel-Smith, J., 1958,4582.

55. Mosbach, Acta Chem. Scand., 1960,14,457.

56. Bentley and Keil, Proc.Chem. Soc., 1961,111.

57. Stout, Dreyer and Jensen, Chem and Ind., 1961, 289.

58. Bycroft, Dobson and Roberts, J., 1962, 40.

59. Mull and Nord, Arch. Biochem., 1944,4,419.

60. Lund, Robertson and Whalley, J., 1953,2434.

61. Bu'Lock and Smith, Experimentia, 1961,17,553.

62. Bu'Lock, Leeming and Smith, J., 1962,2085.

63. Edwards, Lewis and Wilson, J., 1961,4995.

64. Whalley, "Oxygen Heterocyclic Fungal Metabolites" Progr.Org.Chem., Ed. Cook, 1958,4,96.

65. Arnstein and Bentley, Biochem.J., 1956,62,403.

66. Clarke and Nord, Arch.Biochem.Biophys.,1955,59,269.

67. Birkinshaw, Bracken, Michael and Raistrick, Lancet, 1943,2,625.

68. Bergel et.al., J., 1944,415.

69. Attenburrow et.al., J., 1945,571.

70. Foldi, Fodor and Denyen, J., 1948,1295.

71. Puetzer, Nield and Barry, J.Amer.Chem.Soc., 1945,67,832.

72. Engel, Brzeski and Plattner, Helv.Chim.Acta, 1949,32,1166.

73. Dauben and Weisenborn, J.Amer.Chem.Soc., 1949,71,3853.

74. Woodward and Singh, Nature, 1950,928.

75. Bu'Lock and Ryan, Proc.Chem.Soc., 1958,222.

76. Hetherington and Raistrick, Phil.Trans.B., 1931,220,269.

77. Whalley et.al., J., 1949,563.

78. Whalley et.al., J., 1949,859.

79. Johnson, Roberts and Whalley, J., 1950, 2971.

80. Warren, Dougherty and Wallis, J.Amer.Chem.Soc., 1949,71,3422.

81. Bracken, "The Chemistry of Microorganisms" Pitmans Press, 1955.

82. Birch, Fitton, Pride, Ryan, Smith and Whalley, J., 1958,4576.

83. Dean, Eade, Moubasher and Robertson, Nature, 1957,179,366.

84. Powell, Robertson and Whalley, Chem.Soc.Special Publ.,1956,5,27.

85. Haws, Holker, Kelly, Powell and Robertson, J., 1959,3598.

86. Haws and Holker, J., 1961,3820.

87. Davies, Kirkaldy and Roberts, J., 1960,2169.

88. Roberts and Underwood, J., 1962,2060.

89. Wachtmeister, Acta Chem.Scand., 1958,12,147.

90. Hassell and McMorris, J., 1959,2831.

91. Curtis and Hassall, Chem. and Ind., 1961,1360.

92. Rhodes, McGonagle and Somerfield, Chem. and Ind., 1962,611.

93. Birch, Massey-Westropp, Rickards and Smith, J., 1958,360.

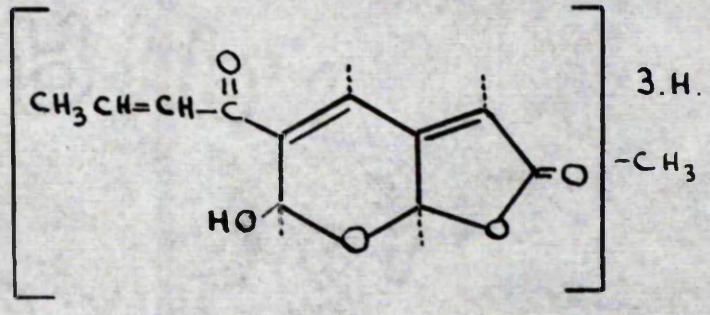
94. Scott, Proc.Chem.Soc., 1958,195.

PART II

THE STRUCTURE OF RADICININ

INTRODUCTION

The isolation of the metabolite radicinin, from the mould Stemphylium radicinum (Sterad), was reported by Clarke and Nord (1,2), and a tentative partial structure, (I), was suggested on the basis of its chemical and physical properties.



(I)

The original objective of this work was to confirm these proposals and to determine the position of the methyl group. However, in the light of new evidence, which has rendered structure (I) untenable, alternative structures have been considered, which might better explain the properties of the metabolite.

The evidence on which structure (I) was based was as follows. Molecular weight and analytical data indicated the molecular formula $C_{12}H_{12}O_5$ for radicinin, in which there were two terminal methyl groups, but no methoxyl groups. The compound was optically active, having a specific rotation ($[\alpha]_D^{27}$) of -175.7° in ethanol.

Radininin was shown to possess no free carboxyl

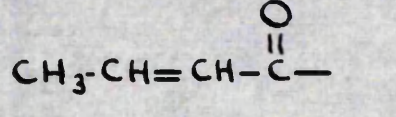
group or phenolic hydroxyl group by the fact that it did not liberate carbon dioxide from sodium carbonate, or sodium bicarbonate solutions. The presence of a hydroxyl group was indicated by formation of a monoacetate and a monomethyl ether. A carbonyl group was also present, as shown by the formation of a mono-2,4-dinitrophenylhydrazone, and this was considered not to be an aldehyde or a methyl ketone.

The presence of a reactive double bond in radicinin was revealed by its ready reaction with one mole of bromine to form a crystalline dibromo derivative, and the formation of a hydrogen chloride adduct. In both these compounds the halogen was covalently bound. On reduction of radicinin, with 5% palladium-on-charcoal as catalyst, a dihydro compound was obtained, but no analytical figures were quoted.

Radicinin dissolved in cold, dilute aqueous sodium hydroxide, giving a pink solution, and on heating this solution under reflux in a stream of nitrogen, acetaldehyde was produced. Acidification of the resulting solution yielded 0.4 mole of carbon dioxide and 0.3 equivalents of volatile acid. No larger fragments of the molecule could be identified as the only other product was a resin. It was considered that the formation of acetaldehyde was the result of an oxidation, since passage of the nitrogen over

a heated copper gauze reduced the yield of acetaldehyde obtained.

The metabolite proved very sensitive to oxidising agents such as permanganate, ozone, chromic acid etc., large quantities of these reagents being consumed, but no characterisable products could be obtained. However, it was reported that oxidation of dibromoradicinin with alkaline potassium permanganate gave α,β - dibromobutyric acid, although no yields were quoted. The acid was identical with the stereoisomer obtained by bromination of trans-crotonic acid (3). On the basis of this evidence the presence of a crotonyl side chain in radicinin (II) was postulated.



(II)

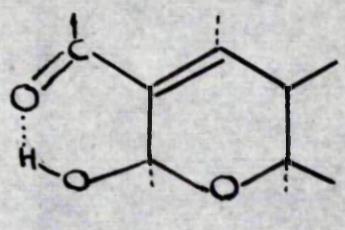
Further unsaturation in the molecule was revealed by the absorption of 3.4 moles of hydrogen in the presence of platinum oxide as catalyst, on prolonged hydrogenation in glacial acetic acid plus methanol (1:1) as solvent. Under these same conditions, the dibromo and the hydrogen chloride adducts absorbed 2.3 moles of hydrogen.

One mole of carbon dioxide per mole of radicinin was liberated on heating under reflux in diphenyl ether,

and therefore a potential carboxyl group was thought to be present in radicinin.

On the basis of chemical evidence, therefore, a free hydroxyl group, three carbon-to-carbon double bonds, a carbonyl group, a potential carboxyl group, and two terminal methyl groups could be detected in the molecule. The ring system in radicinin remained to be established.

The infrared absorption spectrum of radicinin and its derivatives confirmed some of the above conclusions. Radininin had an absorption band at 2.9 μ., which was attributed to a hydrogen-bonded hydroxyl group. An absorption band at 6.02 μ. indicated a conjugated carbonyl group, and a further absorption at 5.66 μ. was attributed to the presence of a five-membered lactone ring. Bands at 6.21 μ. and 6.55 μ. showed the presence of conjugated carbon-to-carbon double bonds (4). On the basis that the hydroxyl absorption band showed no shift when measured in chloroform solution (as compared to nujol mull), it was concluded that the hydrogen bonding was intramolecular (III).



(III)

Acetylation of radicinin resulted in the loss of the absorption band at 2.9μ and the appearance of a new one at 5.75μ , as would be expected for the formation of an acetate (4). The bromine and hydrogen chloride adducts showed the same carbonyl absorptions as radicinin.

The ultraviolet absorption spectrum of radicinin was found to possess an intense maximum at 343m.μ(ε ,18.6 x 10^3), and less intense maxima at 270m.μ(ε ,6.2 x 10^3), 280m.μ.(ε ,4.2 x 10^3) and 220.5m.μ.(ε ,16.6 x 10^3). This was considered to indicate an extensive system of conjugated carbon-to-carbon double bonds.

The ultraviolet absorption spectrum of radicinin acetate was found to be almost identical with that of radicinin, thus indicating that the hydroxyl group was not conjugated with the chromophore. This phenomenon has also been observed in patulin (5).

Clarke and Nord considered that the possibility of radicinin possessing a pyrone or hydroxyfuran ring system was unlikely as the compound gave no colour with alcoholic ferric chloride solution, and by a process of elimination concluded that the structure which best accomodated the known facts was the hemiacetal structure (I).

It was considered that the shift to shorter

wavelength of the principal ultraviolet absorption bands on formation of the dihydro, dibromo and hydrogen chloride adducts of radicinin was consistent with saturation of the α,β -unsaturated ketone chromophore. By a study of ultraviolet absorption spectra, radicinin and its adducts were shown to undergo irreversible reaction with alkali. Thus, the spectrum of radicinin showed at first a bathochromic shift ($\Delta\lambda$ = 21 mμ.), when radicinin was dissolved in methanolic sodium hydroxide solution, but on allowing to stand, the new peak decreased in intensity and two new peaks appeared at 315 mμ. and 255 mμ. . After a further period of time, the spectrum possessed a single absorption maximum at 255 m.μ., and showed no further changes. This behaviour was considered to indicate that a cyclic β - diketone was being formed (6), but as the end products were intractable gums, no chemical studies could be carried out.

Radicinin mono-2,4-dinitrophenylhydrazone possessed a maximum at 416 m.μ (ε ,24.0 x 10^3), which indicated that the carbonyl group was part of an extended conjugated system. It was considered that this derivative might be formed from the potential carbonyl group of the hemiacetal system, rather than the carbonyl group of the side chain and this was thought to be consistent with the proposed structure (I) for radicinin.

DISCUSSION

The isolation of radicinin

Radicinin was obtained essentially as described by Clarke and Nord. Stemphylium radicinum (Sterad) (I.M.I. 63223) was grown, in large scale surface culture, on a potato extract-dextrose solution. Optimum yields of the crude yellow pigment were found to be obtained after three weeks incubation of the cultures at 30° C, after which time the metabolite began to crystallise out of the medium in small amounts. Further incubation decreased the yield of radicinin obtained.

The culture liquor yielded radicinin (0.1-0.2 gms/litre) by acidification (2N.HCl) and extraction with chloroform or ether. The latter solvent gave purer samples of radicinin, but had the disadvantage that the metabolite was not very soluble in ether.

Extraction of the dried and finely ground mycelium (5 gms./litre) with light petroleum, ether, chloroform, acetone, and alcohol respectively, yielded a fat fraction, small amounts of radicinin from the ether extract, and small amounts of brown oil, from the other extractions, which were not investigated further.

Repeated recrystallisation of the crude metabolite

from methanol, or ethanol, followed by sublimation (140° / 1mm) gave very pale yellow needles, m.pt., 214-215° (Clarke and Nord reported 220°), with constant analyses. Attempts were made to detect the presence of any compound, other than radicinin, in the recrystallised materials by fractional sublimation, and chromatography on silica gel films, silicic acid columns and paper. The material, however, was shown to be homogeneous.

The analytical data, chemical properties, infrared and ultraviolet absorption spectra of the metabolite thus obtained, were identical with those previously reported for radicinin; there was, therefore, no doubt about the authenticity of the materials used.

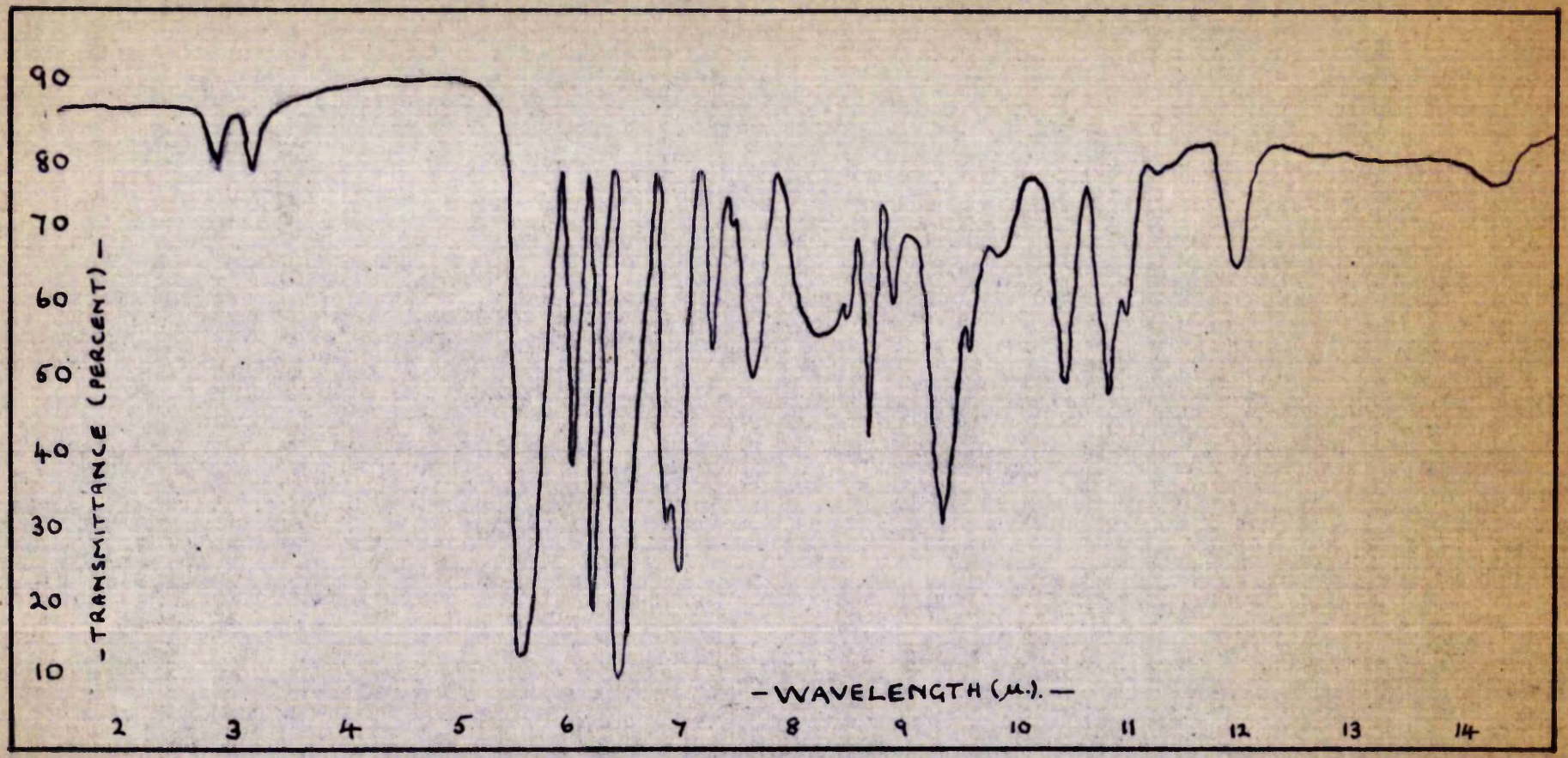
Analysis and molecular weight determinations confirmed the molecular formula of radicinin to be $C_{12}H_{12}O_5$, containing two terminal methyl groups and no methoxyl groups.

Preliminary chemical investigations

Radicinin was sparingly soluble in ether, alcohol, methanol, ethyl acetate, and glacial acetic acid, but more soluble in chloroform and dimethyl sulphoxide. It was confirmed that radicinin did not liberate carbon dioxide from saturated solutions of sodium carbonate or sodium bicarbonate, showing it to contain neither a free carboxyl

FIGURE 1.

THE INFRARED SPECTRUM OF RADICININ
(IN CHLOROFORM SOLUTION).



group, nor a phenolic hydroxyl group. In dilute aqueous sodium hydroxide, it rapidly dissolved in the cold, yielding a blood red solution, from which radicinin could not be recovered on acidification. No colour was obtained with ferric chloride in alcoholic solution.

The infrared spectra of radicinin in chloroform solution, nujol, and potassium bromide, were essentially identical, and showed maxima as follows: 2.88μ (W., possibly a hydrogen bonded hydroxyl group), 3.35μ (W., carbon-hydrogen streching), 5.7μ (S., possibly a lactone or 5-membered ring ketone), 6.05μ(M., α,β- unsaturated ketone), 6.25μ, 6.55μ(S., possibly carbon-to-carbon double bonds), 6.9μ(S.), 6.98μ(S.), 7.25μ (M.), 7.45μ (W.), 7.58μ(M.) (carbon-hydrogen, carbon-methyl and methylene groups), see also Figure 1.

Thus, there are at least two carbonyl functions present in radicinin. The absorption band at 5.7μ is broad and of exceptionally high intensity, and could be a multiple peak.

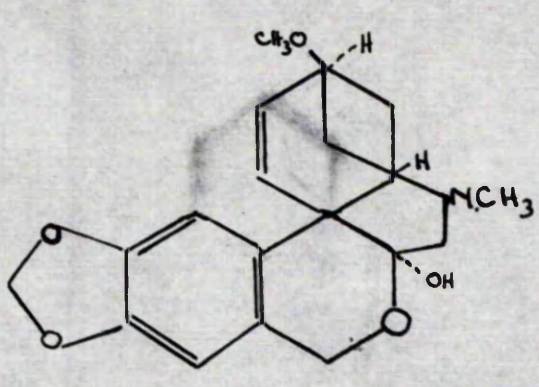
The presence of an aldehydic or ketonic carbonyl group was confirmed by the formation of a mono-2,4-dinitrophenylhydrazone, which gave correct analyses. No other carbonyl derivatives could be obtained, however, the products being gums (probably due to further attack of the

reagents at the carbonyl-activated double bonds). Tests for an aldehyde group were negative, but oxidation of radicinin with an alkaline solution of iodine in potassium iodide gave a precipitate of iodoform, thus indicating the possible presence of a CH_3CO- or a $CH_3CH(OH)-$ group, contrary to evidence previously reported.

Acetylation and methylation studies on radicinin

The formation of radicinin monoacetate (m.pt. 197°) confirmed the presence of a single hydroxyl group, as reported by Clarke and Nord. The infrared absorption spectrum of this compound showed no hydroxyl absorption, but contrary to previous reports, no acetyl-carbonyl absorption band at 5.75μ was detected, this probably being masked by the high intensity carbonyl absorption band at 5.7μ . Infrared absorption maxima obtained for radicinin monoacetate were as follows: 3.35μ (W., carbon-hydrogen streching), 5.7μ (S., very broad band), 6.07μ (M., α,β -unsaturated lactone), 6.25μ, 6.55μ (S., carbon-to-carbon-double bonds), 6.92μ, 7.0μ (S.), 7.23μ (W.) 7.3μ (W.), 7.66μ (M) (carbon-hydrogen, carbon-methyl and methylene groups). Changes in the spectrum between 6.9μ and 7.5μ indicated the presence of additional carbon-hydrogen and carbon-methyl bonds in radicin acetate as expected.

Attention was drawn to the possibility that further acetylation products might be obtainable from radicinin as, according to Clarke and Nord, high acetyl values had been obtained on analysis of the acetylation product of radicinin. Tsuda and Uyeo (7) recently studied the acetylation of the alkaloid tazettine (IV) under vigorous conditions, and obtained a number of products.



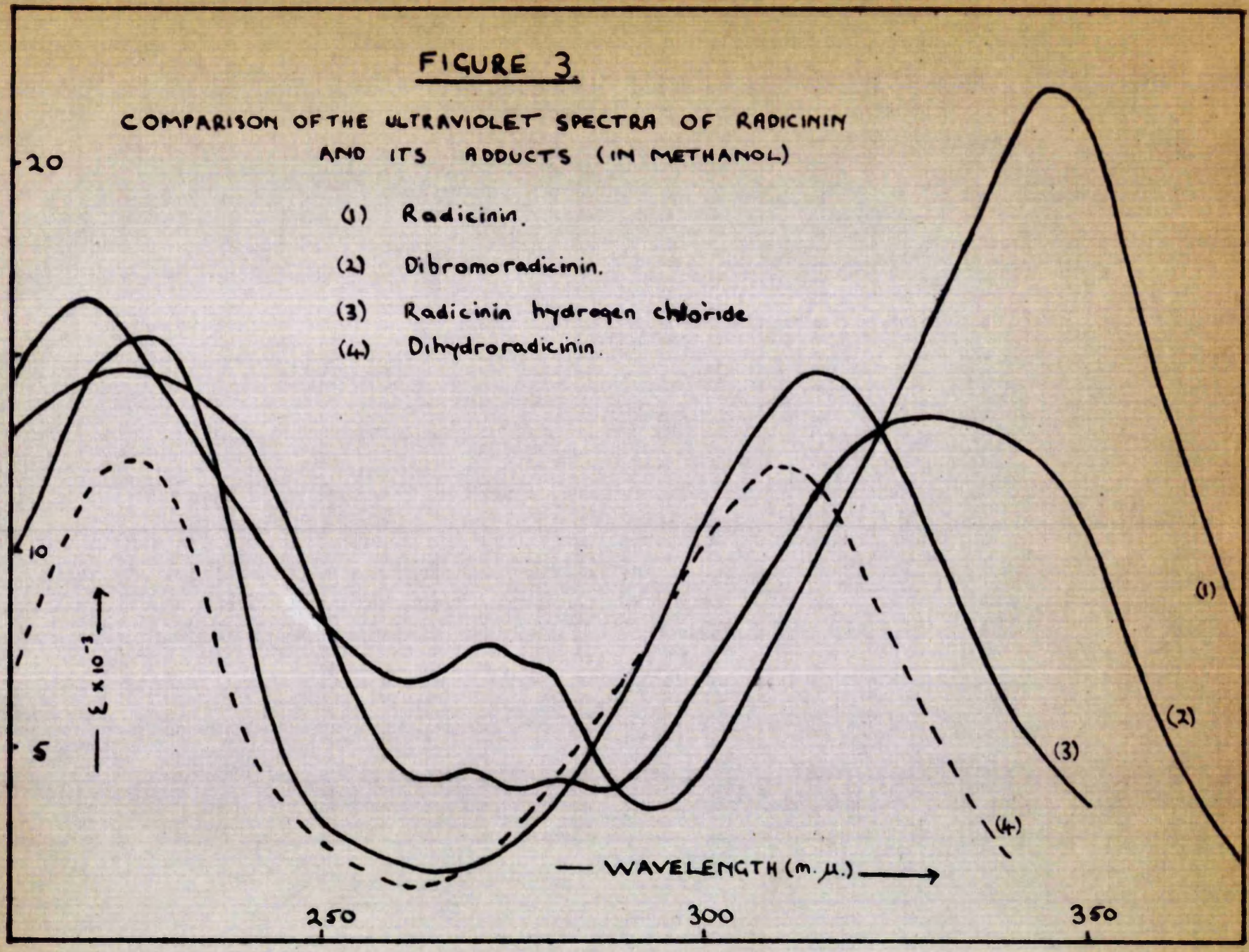
(IV)

When these vigorous acetylation methods were applied to radicinin, only radicinin monoacetate was obtained, together with decomposition products as the conditions were intensified. The high acetyl values obtained by the previous workers were probably due to formation of a volatile acid on hydrolysis; this will be more fully discussed later (page 68).

It was previously reported that radicinin could be methylated with dimethyl sulphate in acetone, in the presence of anhydrous potassium carbonate, however, on no occasion could this result be reproduced and in

FIGURE 3.

COMPARISON OF THE ULTRAVIOLET SPECTRA OF RADICININ AND ITS ADDUCTS (IN METHANOL)



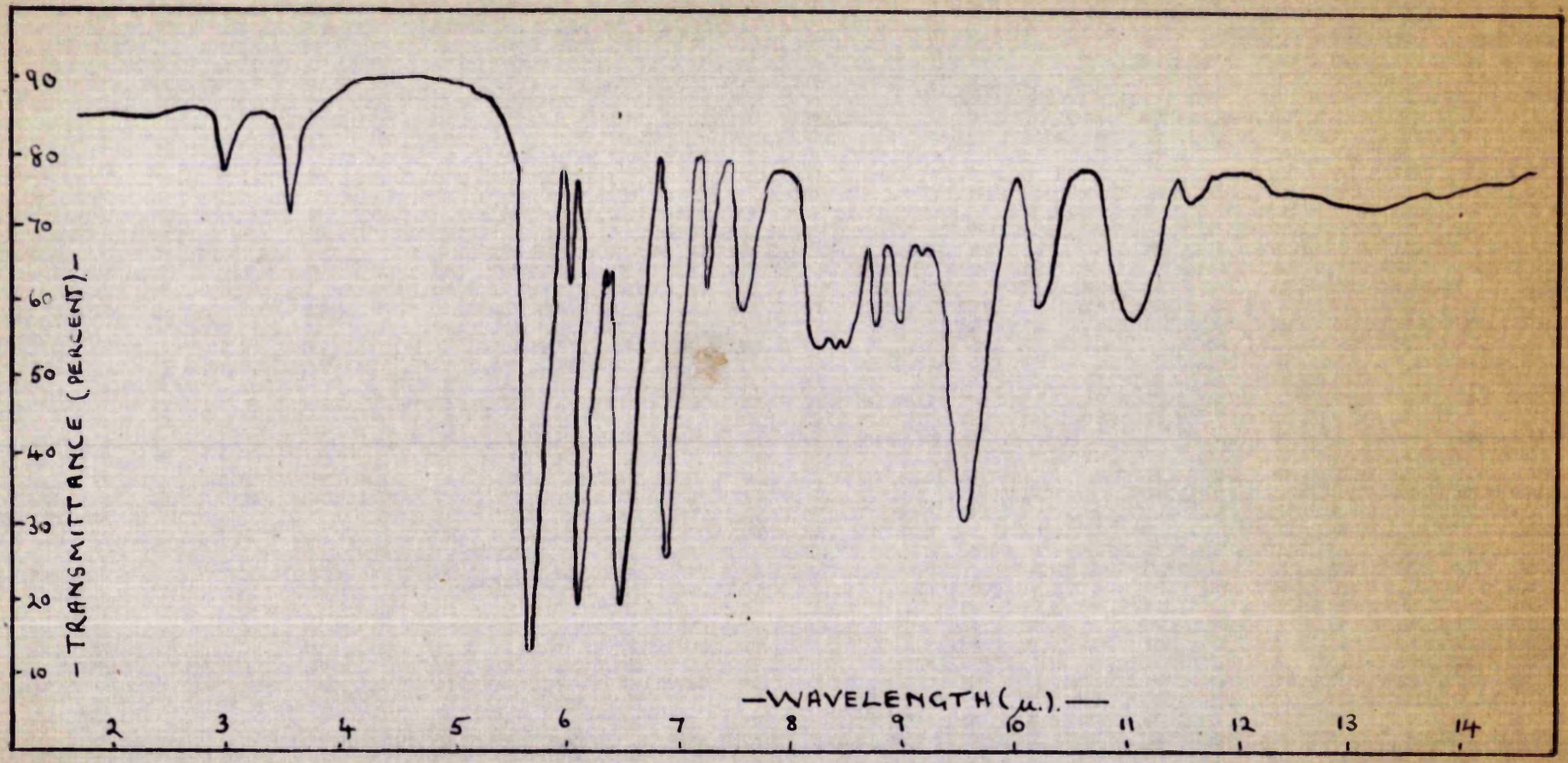
every case radicinin was recovered unchanged. In addition, a number of attempts were made to obtain the methyl ether by the use of methyl iodide, but again only starting material was recovered. With diazomethane in ether, radicinin yielded a bright red gum, which could not be purified, and it was concluded that this was probably formed by the attack of diazomethane at the double bonds or ether oxygen atoms. By treating a solution of radicinin in aqueous alkali with dimethyl sulphate, however, a crude tan solid was obtained. All attempts to purify this material have been unsuccessful and therefore analytical data is not available. But, by chromatographic methods it was shown that the compound was different from radicinin.

The chemistry of the side chain in radicinin

Radicinin reacted with bromine, hydrogen chloride and hydrogen to give the adducts previously reported. Dihydroradicinin was successfully characterised by its infrared and ultraviolet spectra, and satisfactory analyses were obtained. As reported by Clarke and Nord, the ultraviolet absorption spectrum of dihydroradicinin, when compared to that of radicinin shows a hypsochromic shift, of 34 m.μ., of the main absorption band (Figure 3). The infrared spectrum of dihydroradicinin, when compared to that of radicinin, only shows significant changes in the region ascribed to the unsaturated parts of the molecule.

FIGURE 2.

THE INFRARED SPECTRUM OF DIHYDRORADICININ
(IN CHLOROFORM SOLUTION).



Thus, the hydroxyl and carbonyl bands still appear at 2.88μ and 5.7μ respectively, but the region 6μ to 6.5μ shows shifts to shorter wavelength. Thus, there are bands at 5.99μ (m.), 6.14μ (S.), 6.33μ (V.W.) and 6.5μ (S.) See Figure 2. It appeared from these data, that addition of two atoms of hydrogen not only affected the α,β -unsaturated carbonyl system, but also had a strong effect on the other conjugated system in the molecule. These results were somewhat surprising in view of the fact that structure (I) suggests that one double bond is in effect isolated from the main chromophore.

The hydrogen chloride adduct showed the same characteristic shifts in the conjugated carbon-to-carbon double bond region of the infrared as in dihydroradicinin. Thus bonds were recorded at 2.9μ (W.), 3.33μ (W.), 5.73μ (S.), 6.0μ (M.), 6.1μ (S.), 6.5μ (S.). The dibromo adduct showed similar shifts in its intrared spectrum, but closer similarity to radicinin than the other adducts. Thus, the main absorption bands were as follows: 2.86μ (W.), 3.31μ (W.), 5.68μ (S.), 5.99μ (W.), 6.1μ (S.), 6.25μ (M.), 6.49μ (shoulder) and 6.52μ (S.).

The ultraviolet spectra (Figure 3) of these adducts lend weight to the conclusions drawn from the infrared data. Thus, the ultraviolet spectrum of dihydro-

radicinin, when compared to that of radicinin, shows the greatest hypsochromic shift ($\Delta\lambda$ = -34m.μ.), and is therefore least like radicinin; the hydrogen chloride adduct gives the next greatest shift ($\Delta\lambda$ = -28m.μ), and is therefore essentially like radicinin; while dibromoradicinin, giving the smallest hypsochromic shift ($\Delta\lambda$ = -13m.μ.), is very similar to radicinin. These observations may be explained by consideration of the increasing electronegativity of the substituents. The large shifts observed in the ultraviolet bands of the adducts of radicinin were surprising, since the site at which addition takes place was reported to be an isolated double bond, rather than part of the main chromophore.

Clarke and Nord offered proof that bromination occurred in the side chain, but a comparison of the above data for dihydroradicinin and dibromoradicinin led to the conclusion that hydrogenation might occur elsewhere in the molecule, thus accounting for the large spectral shifts observed for dihydroradicinin. However, a sample of dihydroradicinin absorbed no bromine under the conditions used for the bromination of radicinin, and dibromoradicinin absorbed no hydrogen in the presence of 5% palladium-on-charcoal. Thus, it was concluded that the additions must all occur across the same reactive double bond.

A series of hydrolyses of radicinin and its

adducts revealed some important information on the parent compound. On hydrolysis of radicinin with hot 1% aqueous potassium hydroxide under nitrogen, 0.4 mole of acetaldehyde was obtained by trapping it as its 2,4-dinitrophenylhydrazone, as reported by Clarke and Nord. On acidification of the resulting alkaline solution with 2N. sulphuric acid, 1 mole of carbon dioxide was released and 0.8 equivalent of volatile acid was obtained on exhaustive steam distillation. Recent results obtained in this laboratory (8) have indicated that this acid is acetic acid. It was shown, in separate experiments, by potentiometric titration, that 1 mole of radicinin reacted with only 1 mole of sodium hydroxide in the cold, even after prolonged standing. On heating, however, a further mole of alkali was consumed. The products of the hydrolyses in hot or cold alkali were yellow oils which resisted all attempts at purification.

If, as Clarke and Nord postulated, the reactive double bond of radicinin is in the side chain, and is cleaved under alkaline conditions to produce acetaldehyde, then dibromoradicinin, dihydroradicinin and the hydrogen chloride adduct, in which this bond is saturated, would be expected to consume one mole of sodium hydroxide, and no acetaldehyde would be produced. However, these adducts

were shown potentiometrically to consume two moles of alkali on heating, and liberated acetaldehyde under these conditions. Unfortunately the main products of these hydrolyses were again intractable oils and were not further studied.

In view of the fact that the acetaldehyde was previously considered to be derived by oxidation of the crotonyl side chain of radicinin, its formation from compounds containing saturated side chains was surprising. It was therefore concluded that, either the side chain existed in the form proposed by Clarke and Nord, in which case addition to radicinin occurs elsewhere in the molecule, or else the molecule does not contain a crotonyl side chain, and some other system present accounts for the formation of acetaldehyde on hydrolysis.

The above conclusions led to a careful re-examination of the main evidence advanced for the existence of a crotonyl side chain, namely the reported oxidation of dibromoradicinin with alkaline potassium permanganate to yield α,β -dibromobutyric acid. This oxidation was repeated a number of times by the method reported (9), but on no occasion was any bromo-acid isolated. In common with all oxidations of radicinin and derivatives, much carbon dioxide was evolved, and, in this case, 5-6 moles

per mole of radicinin oxidised. In addition 0.6 mole of acetaldehyde was obtained, although it was not possible to determine whether this arose by oxidation, or by hydrolysis under the alkaline conditions. The product of the oxidation in every case was a brown oil which could not be purified by the usual methods.

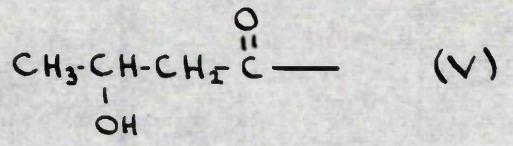
In attempts to identify the oxidation products by chromatography, α,β -dibromobutyric acid was prepared from crotonic acid (3), and compared with the oxidation product by thin layer chromatography on silica gel. The observed R_F of α,β -dibromobutyric acid was 0.46, whereas the oxidation product was shown to be a mixture of components, none having R_F values close to 0.46.

Milder oxidation gave similar results, potassium permanganate in acetone giving recovered starting material, together with an oil which was again shown to contain no α,β -dibromobutyric acid. It was thought that the removal of some of the oxidisable sites in radicinin prior to oxidation might help in the isolation of a recognisable material. Thus dibromoradicinin was reduced with hydrogen over platinum oxide to give an oil (two moles of hydrogen were absorbed) and oxidation of this with alkaline potassium permanganate gave a brown oil which showed no recognisable fractions by chromatography.

If a crotonyl group was present in radicinin, then acetaldehyde would be produced on ozonolysis. However, in several experiments, ozonolysis of radicinin produced only intractable materials with no acetaldehyde. Crotonic acid, on ozonolysis under the same conditions, produced a good yield of acetaldehyde, characterised as its 2,4-dinitrophenylhydrazone derivative.

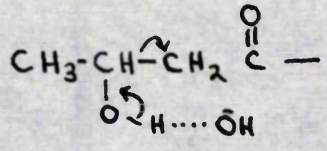
Lemieux and his colleagues have reported that sodium periodate in the presence of catalytic amounts of potassium permanganate is a useful mild oxidising agent, by which carbon-to-carbon double bonds are oxidised first by the permanganate to hydroxyketones, which are then cleaved by the periodate (10, 11). The permanganate is apparently not reduced beyond the manganate state under the pH. conditions of the reaction, and is regenerated by the periodate. This reaction was considered to present possibilities of more specific and milder oxidation than the normal reagents, but it was found that no reaction occurred with radicinin, starting material being recovered quantitatively.

In the light of the above results, it was concluded that radicinin did not contain a crotonyl side chain, as proposed by Clarke and Nord. It was considered that the side chain was more likely to be the hydrated crotonyl group (V).

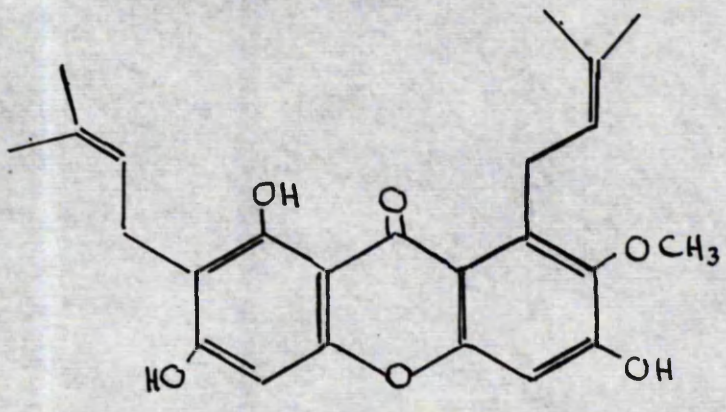


(V)

The presence of this side chain explains the formation of acetaldehyde on treatment with base as being the result of a reverse aldol reaction:



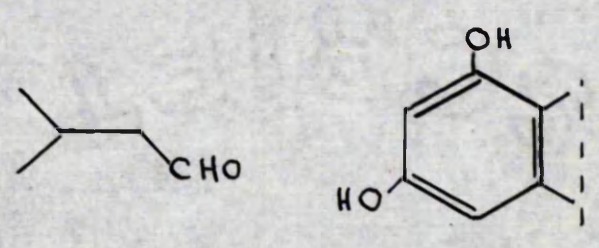
Thus acetaldehyde would be obtained also by alkaline treatment of the adducts of radicinin, but not on ozonolysis. The fact that radicinin gives a positive iodoform test is also in accord with this formulation.



(VI)

It is interesting to note that mangostin (VI) undergoes reverse aldol reactions on fusion with base, the more vigorous conditions being required to hydroxylate the molecule prior to the elimination of isovaleraldehyde (VII) as shown below (12).

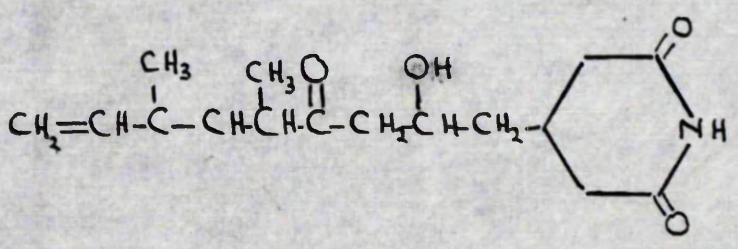
(VI)



(VII)

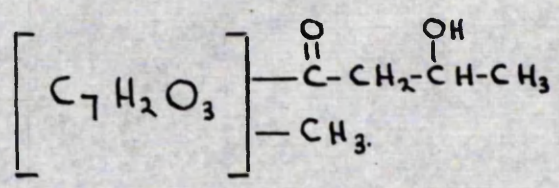
It is significant too, that streptimidone (VIII), itself a β -hydroxyketone, undergoes reverse aldol cleavage readily, to give aldehyde derivatives of the β -ethylglutarimide moiety (13).

(VIII)



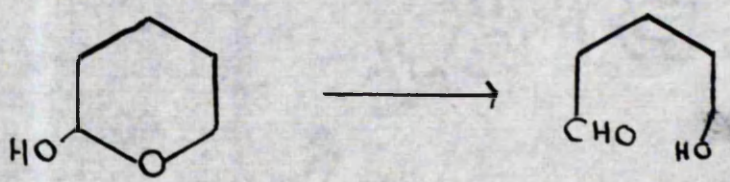
At this stage it was not possible to decide whether other carbon atoms were interposed between the C_4 moiety and the remainder of the molecule. It was suggested that the remainder of the molecule was a ring system to account for the large proportion of oxygen in the molecule. Two of the oxygen atoms have now been located in the side chain,

and thus radicinin may be represented by partial structure (IX). This implies that the ring system of structure (I) is not correct since the single hydroxyl group is now known to be situated in the side chain, rather than forming part of a hemiacetal system. Thus experiments to clarify this situation were carried out.

(IX) 

The "hemiacetal" system of radicinin

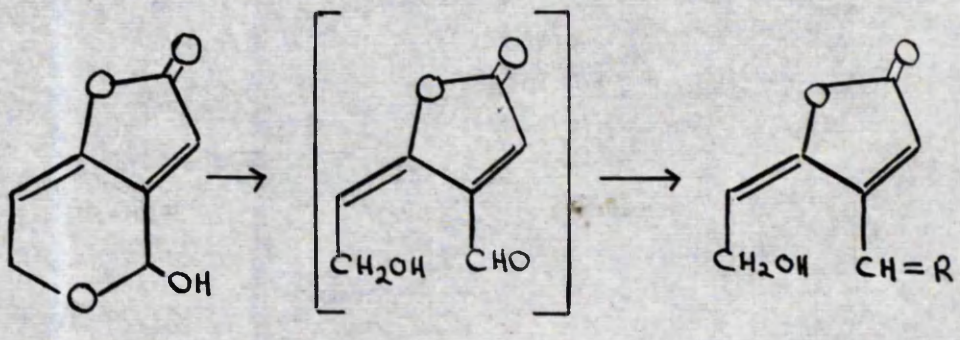
It is known that hemiacetals are relatively stable to boiling alkali, yet easily hydrolysed by hot dilute acids (14) thus:



The reaction of radicinin with acid was incompatible with this, as it was recovered unchanged after treatment with hot dilute acid for one hour. The metabolite was, however, broken down on heating under reflux with acid for several hours.

One of the interesting features of the chemistry

of patulin (X), was the formation of both an oxime and a phenylhydrazone (15), although it was shown that no aldehyde or ketone group was present in the molecule. It was considered that these derivatives (XI) were formed from the potential carbonyl group of the hemiacetal system (5) as shown:

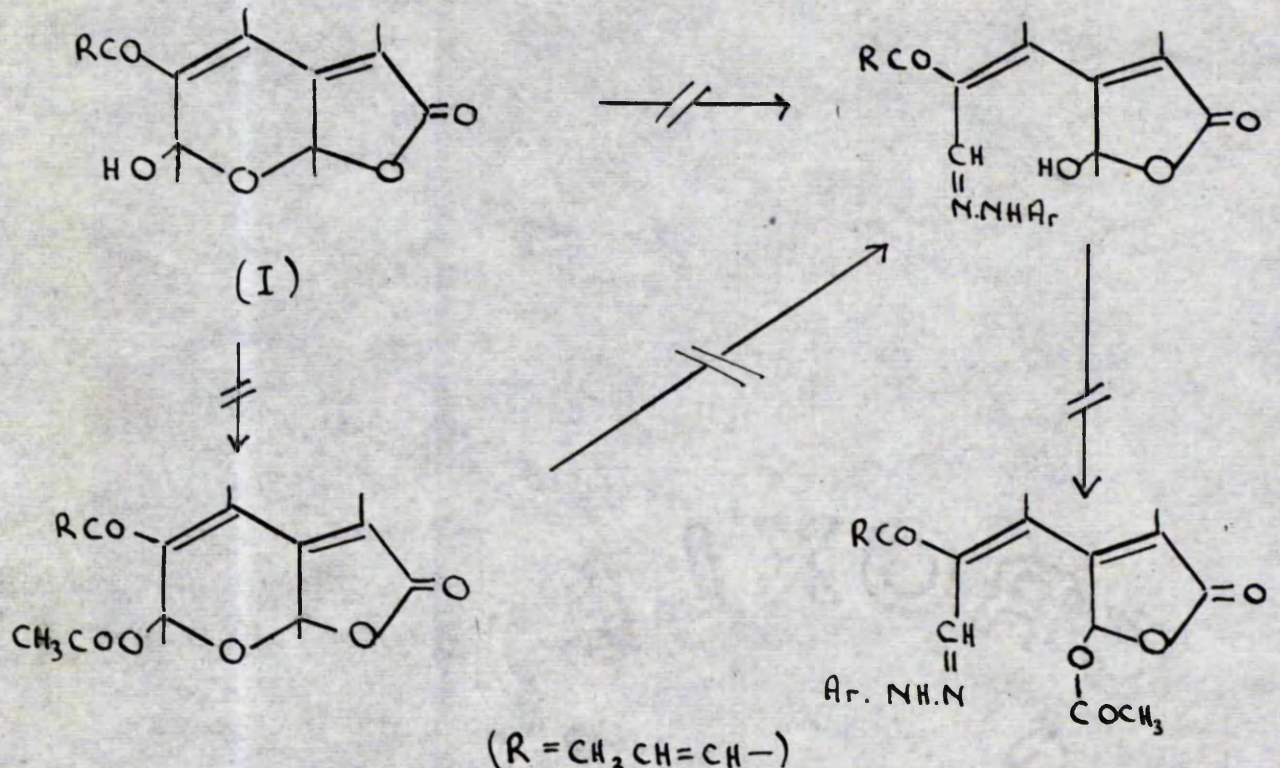


(X) R=NOH; N.NH C_6H_5 (XI)

The properties of these derivatives showed that the lactone ring of patulin was unopened. The formation of the same phenylhydrazone (XI, R = N.NH Ph) from patulin acetate was considered to be due to elimination of the acetyl group prior to ring cleavage of the hemiacetal.

It was considered that if radicinin does contain a hemiacetal moiety it also should undergo a ring cleavage of this type, prior to the formation of the 2,4-dinitrophenylhydrazone, and therefore the product should be a di-2,4-dinitrophenylhydrazone. However, analysis showed the product of the reaction of radicinin with 2,4-dinitrophenylhydrazine hydrochloride to be a mono-derivative. In order to determine whether this derivative was formed from

the free carbonyl group of the side chain, or from the potential carbonyl group of the "hemiacetal" system, the 2,4-dinitrophenylhydrazone of radicinin acetate was prepared. This proved to be different from radicinin 2,4-dinitrophenylhydrazone, but identical with the product obtained by acetylation of radicinin 2,4-dinitrophenylhydrazone. Both these derivatives analysed for one acetyl and one 2,4-dinitrophenylhydrazone group. These results were thus contrary to those expected for a compound of structure (I), which would react as shown below, in a similar manner to patulin. On these grounds alone, the presence of a hemiacetal ring in radicinin was thought to be unlikely.



(R = $CH_3CH=CH-$)

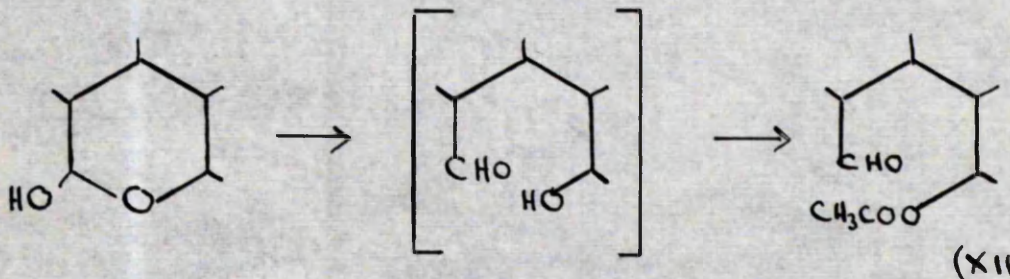
In addition, if the conclusions of Nord and Clarke were correct, dihydroradicinin would be an α,β-unsaturated ketone, as distinct from radicinin which is an α,β - α',β' - unsaturated ketone. Thus dihydroradicinin might be expected to form a di-2,4 dinitrophenylhydrazone, assuming that the mono derivative of radicinin arises from the potential carbonyl group of the hemiacetal ring. However, reaction of dihydroradicinin with 2,4-dinitrophenylhydrazine hydrochloride, by the method of Wilds and Nelson (16), gave only a monoderivative.

It has been shown by Ross, that the 2,4-dinitrophenylhydrazones of simple carbonyl compounds possess no infrared absorption bands in the region 5-6μ., but invariably maxima appear between 6.05 and 6.2μ. (1%). The infrared spectra of radicinin and dihydroradicinin-2,4-dinitrophenylhydrazones both have high intensity bands (5.8μ and 5.84μ respectively) in the same region as radicinin, and thus it was concluded that these bands represent a non-ketonic carbonyl function or functions. It was not possible, from the infrared spectra, to determine whether the α,β -unsaturated carbonyl absorption band at 6.05μ in the spectrum of radicinin is absent in the 2,4-dinitrophenylhydrazone, since this region is masked in such derivatives. However it was assumed that the derivative

was formed from this function.

Cyclic acetals may be conveniently ring-opened by treatment with a mixture of acetic anhydride, acetic acid and sulphuric acid (18, 19).

If radicinin did indeed possess the hemiacetal structure postulated by Clarke and Nord, acetolysis should result in opening of the ring and the product should be a monoacetate (XII) different from the monoacetate obtained by mild acetylation of radicinin. Several reactions were carried out, but in every case the product obtained was identical with radicinin monoacetate. It was observed that the infrared spectrum of radicinin acetate possessed none of the bands characteristic of an aldehyde group, and gave only a mono-2,4-dinitrophenylhydrazone.



(XII)

The hemiacetal ring system postulated for radicinin resembles the cyclic form of sugars and as such, it should be possible to methylate radicinin by the Fischer method (20). However the reaction of radicinin with methanolic

hydrogen chloride led to the formation of radicinin hydrochloride only.

A further characteristic reaction of sugar acetals is the formation of crystalline osozones with phenylhydrazine hydrochloride, the failure to isolate such a product again suggests that radicinin does not possess a cyclic acetal structure.

The chromophoric system in radicinin

With the establishment of partial structure (IX) for radicinin, it was clear that considerable unsaturation was present in the molecule, as evidenced by the rapid uptake of three moles of hydrogen in the presence of platinum oxide in methanol. Clarke and Nord observed the uptake of 3.4 moles of hydrogen over platinum oxide in methanol and glacial acetic acid. The extra fraction of a mole of hydrogen absorbed was thought to be due to hydrogenolysis of carbon-to-oxygen bonds, and this was borne out in the present work, in which the absorption of approximately 4 moles of hydrogen occurred on prolonged catalytic hydrogenation.

Dihydroradicinin absorbed two moles of hydrogen over platinum oxide, thus showing that radicinin contains three carbon-to-carbon double bonds, one more reactive than

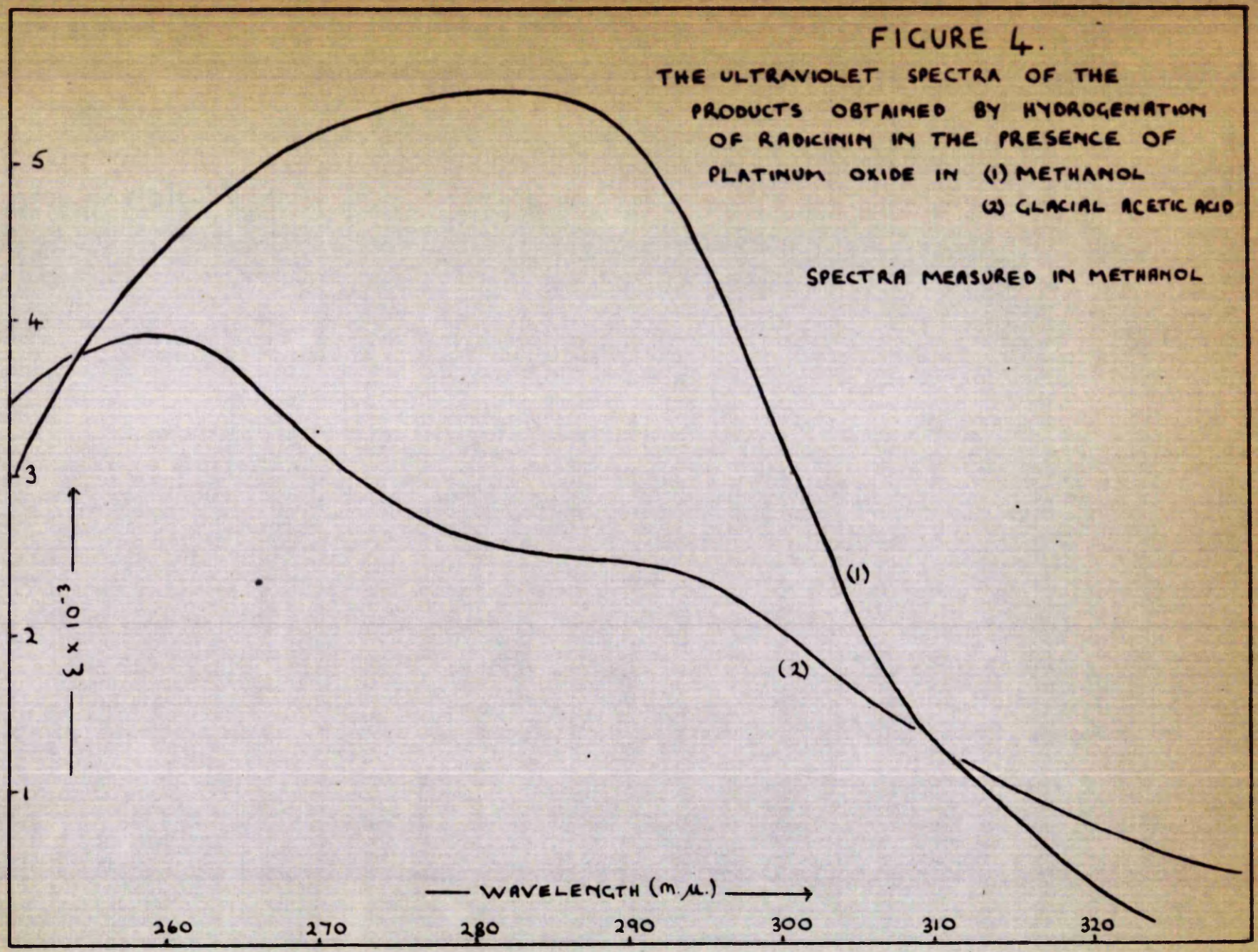


FIGURE 4.
THE ULTRAVIOLET SPECTRA OF THE PRODUCTS OBTAINED BY HYDROGENATION OF RADICININ IN THE PRESENCE OF PLATINUM OXIDE IN (1) METHANOL (2) GLACIAL ACETIC ACID
SPECTRA MEASURED IN METHANOL
5
4
3
2
1
ε x 10⁻³
(1)
(2)
WAVELENGTH (mμ.)
260
270
280
290
300
310
320

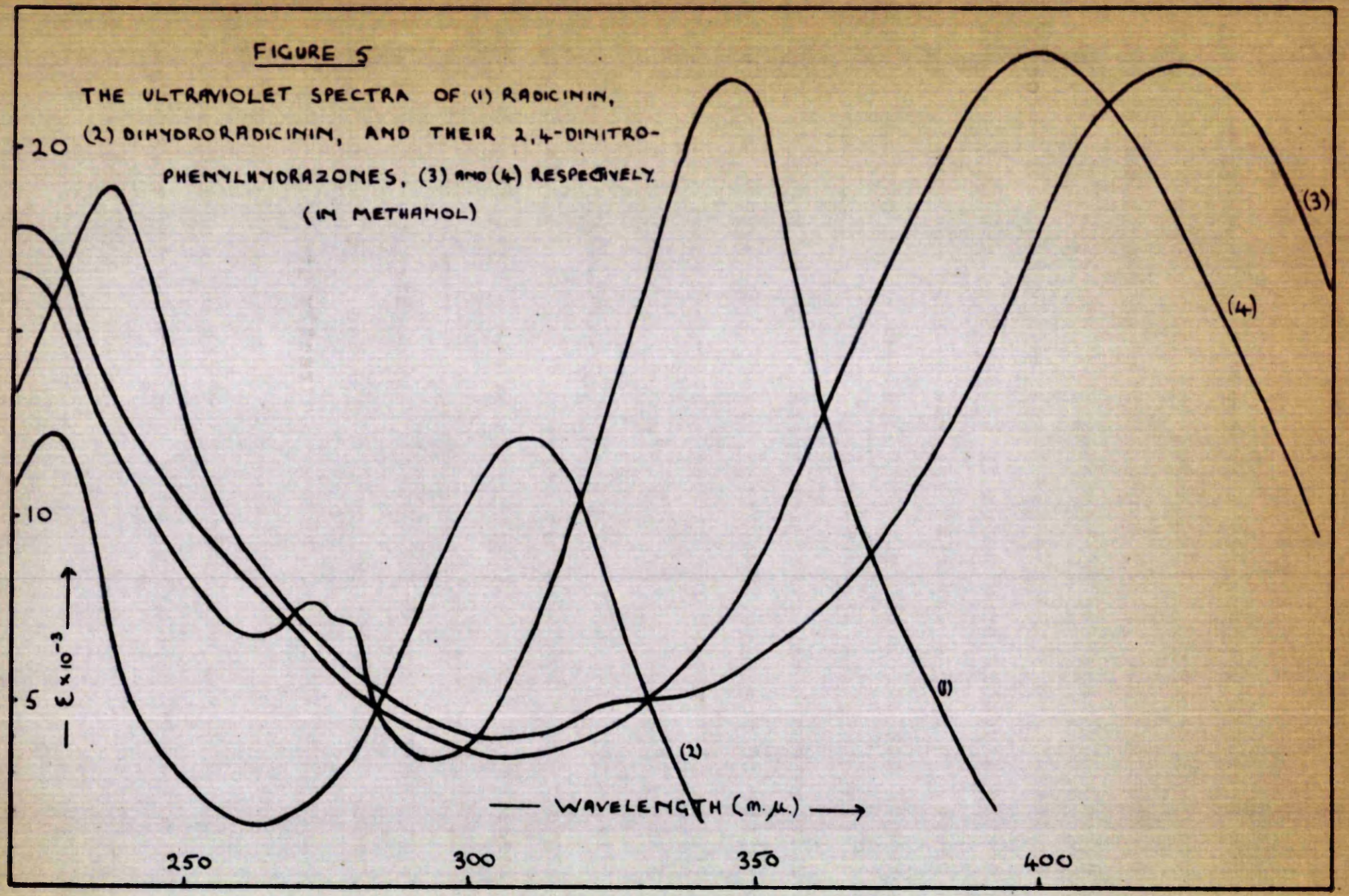
the other two. The completely hydrogenated compounds were clear yellow oils, which resisted all attempts at purification.

The ultraviolet spectrum of radicinin in methanol, as reported by Clarke and Nord was confirmed (Figure 3), and indicated the presence of a long conjugated or highly polar system. The presence of more than one absorbing system was indicated by the appearance of four peaks in the observable spectrum, although the diagnostic value of the absorption band in the 220 m.μ region in radicinin and its derivatives is doubtful, as it appears practically unchanged in all of these compounds. That the three carbon-to-carbon double bonds present in radicinin were responsible for most of its ultraviolet absorption was confirmed by the fact that the ultraviolet spectra of the oil obtained by full reduction of radicinin, showed only low intensity absorption bands at shorter wavelength than radicinin (Figure 4). From the ultraviolet spectrum of this oil it was concluded that the product obtained by complete hydrogenation in methanol, and in glacial acetic acid were not the same. It is likely that hydrogenolysis occurs in the latter but not the former case.

At this stage, a further consideration of the

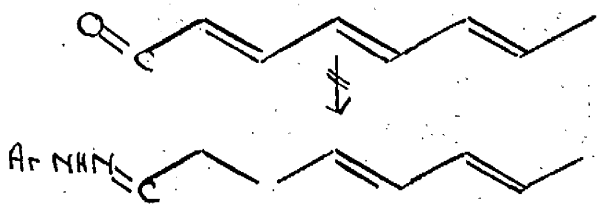
FIGURE 5

THE ULTRAVIOLET SPECTRA OF (1) RADICININ, (2) DIHYDRORADICININ, AND THEIR 2,4-DINITROPHENYLHYDRAZONES, (3) AND (4) RESPECTIVELY (IN METHANOL)



chromophoric system present in radicinin is enlightening. Now that the presence of a crotonyl side chain in radicinin has been shown to be unlikely, the large hypsochromic shift of the main absorption band observed on comparison of the ultraviolet spectra of radicinin and dihydroradicinin, may be satisfactorily explained by assuming reduction to occur across a double bond which is attached to the main chromophore, in such a way that saturation does not completely remove the chromophore. That the side chain carbonyl groups of radicinin and dihydroradicinin were conjugated to the main chromophore, was demonstrated by large bathochromic shifts of the main absorption bands, observed on comparison of the ultraviolet spectra of radicinin and dihydroradicinin 2,4-dinitrophenylhydrazones with the parent compounds (Figure 5) ($\Delta\lambda$ = -78 mμ and -90 mμ respectively). The hypsochromic shift, noted on comparison of the ultraviolet spectrum of the dinitrophenylhydrazone of dihydroradicinin with that of radicinin (Figure 5) ($\Delta\lambda$ = -23 mμ), implies that the reactive double bond is conjugated to the carbonyl group. In addition it may be concluded that the reactive double bond in radicinin is not directly attached to the carbonyl group as shown below (XIII), since this would result in no bathochromic shift of the main chromophore, on comparison of the ultraviolet spectra of dihydroradicinin and its 2,4-dinitrophenylhydrazone. From this evidence,

the most readily reducible double bond in systems of the type (XIII) would probably be the terminal double bond. This reduction would leave a conjugated system of sufficient length and polarity to account for the high wavelength of the absorption band in dihydroradicinin (310 mμ).



(XIII)

It therefore seemed likely that the unsaturated system in radicinin constitutes one or more chromophore, the carbonyl group being attached to at least one of these. Attempts were then made to reduce this carbonyl group with the object of determining its position relative to the carbon-to-carbon double bonds.

Reduction of radicinin with complex metal hydrides

Reduction was first attempted with sodium borohydride in aqueous methanol or ethanol, but from a series of reactions under a variety of conditions (table 5, page 151), no products could be characterised. However, the infrared spectra of the gums obtained showed the absence of absorption at 5.7 μ found in the infrared spectrum of radicinin, and ascribed to the presence of a lactone ring by Clarke and Nord. This observation was surprising in

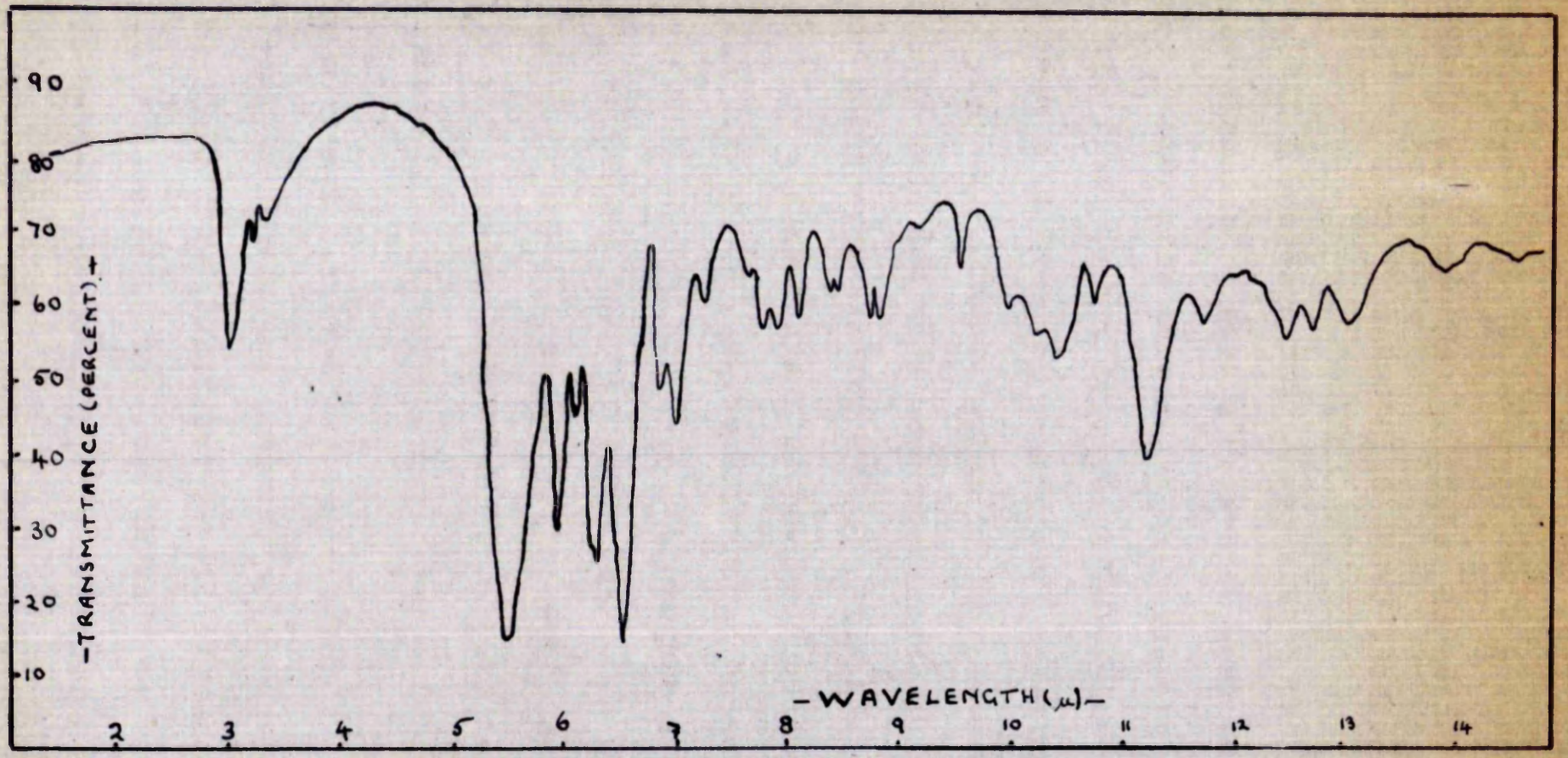
view of the fact that lactones are usually inert to sodium borohydride (21).

Reduction of radicinin with varying proportions of lithium aluminium hydride, by heating under reflux in dry ether, gave no clearly defined product. The failure of these reactions was thought to be due to the lack of specificity of the reagent, and it was considered that a reaction carried out by the reverse addition technique might lead to a one stage reduction. Since radicinin is only sparingly soluble in ether, this reaction was carried out by the Soxhlet extraction method. The reaction product was worked up in the usual manner, to give a white, crystalline solid, $C_{12}H_{14}O_5$, m.pt 167° in about 20% yield. Considerable difficulty was encountered in repeating this reaction as it was found that satisfactory yields could only be obtained by working on a 100 mg. scale. Attempts to "scale up" the reaction led to increasing amounts of oily impurities. A summary of the various conditions used for the reduction of radicinin with lithium aluminium hydride will be found in table 4, page 156.

The product of this reaction (compound A) was initially thought to arise by reduction of the side chain carbonyl group, or a lactone group, or both. However, as the analyses indicated addition of only two atoms of hydrogen

FIGURE 6.

THE INFRARED SPECTRUM OF COMPOUND A
(IN POTASSIUM BROMIDE DISC).



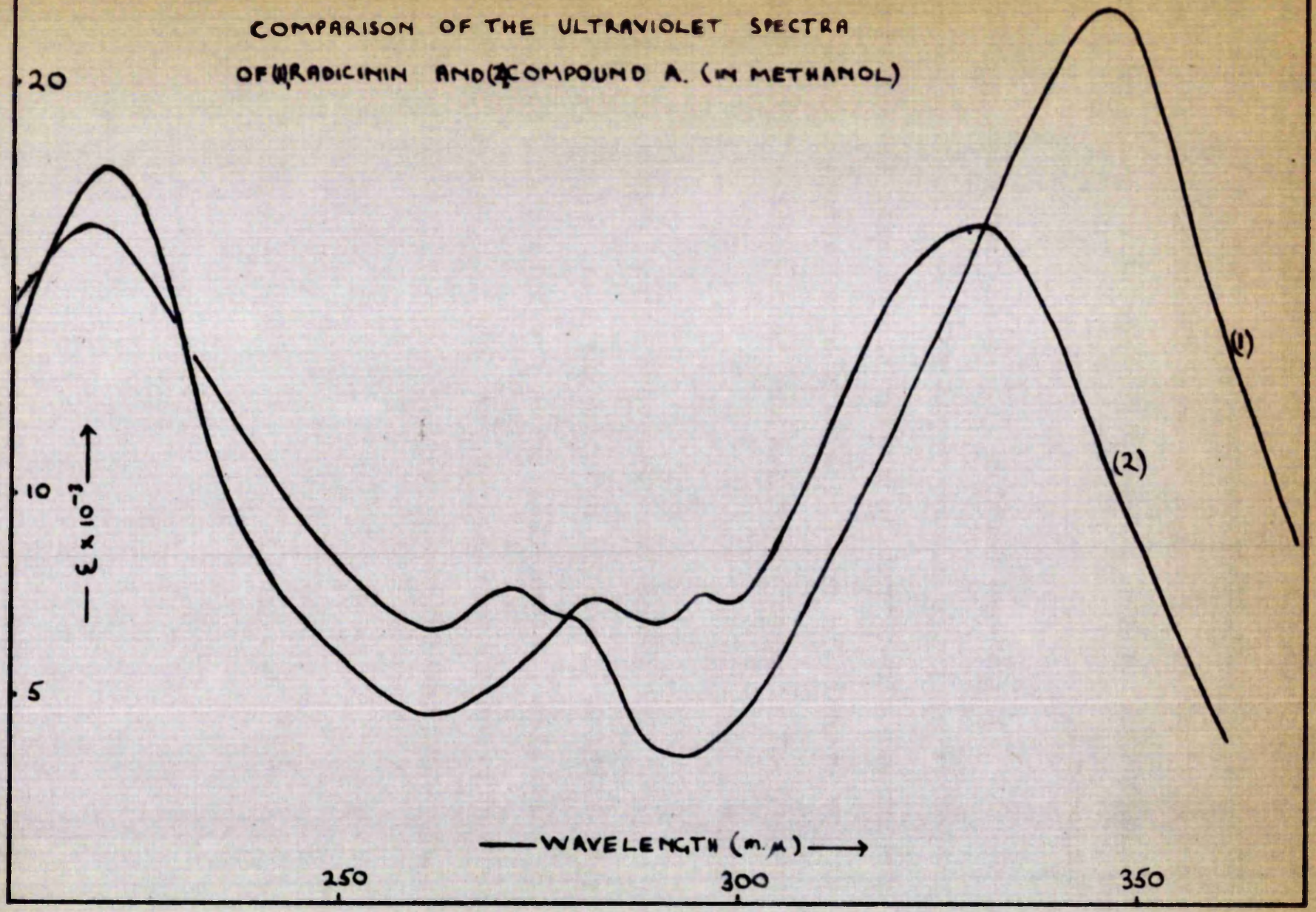
to radicinin, the former seemed most likely. The acetate of compound A was prepared by the method used for the acetylation of radicinin, and analysis showed it to be a monoacetate, $C_{12}H_{13}O_4.OCOCH_3$.

It was found that compound A liberated carbon-dioxide from saturated sodium bicarbonate solution and gave an acidic solution on warming with water, thus indicating the presence of a carboxyl group. This was confirmed by the infrared spectrum of compound A in potassium bromide, which gave the following bands: 3.0μ (m., typical broad hydroxyl absorption band obtained from carboxylic acids), 3.1μ and 3.25μ (w., satellite absorption bands as frequently observed in the hydroxyl region of the infrared spectra of carboxylic acids), 5.67μ (s., similar to the band in radicinin), 5.91μ (s., α,β -unsaturated carboxylic acid), 6.06μ (m., possibly $\alpha-\beta$, unsaturated ketone), 6.26 6.3μ (s., doublet), 6.46μ (s.). The appearance of a strong band at 11.35μ , the region expected for hydroxyl deformation, also indicated the presence of a carboxylic acid (22), Figure 6

Otherwise, the infrared spectrum of compound A showed no major changes, and the ultraviolet spectrum showed the same profile as radicinin, with the main chromophore shifted to shorter wavelength, 330mμ (ε = 16.5 x 10^3). The lower intensity absorption bands appeared at slightly longer

FIGURE 7.

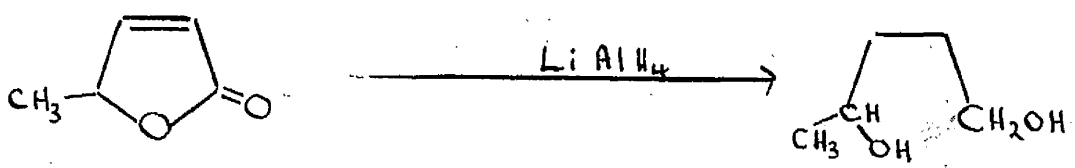
COMPARISON OF THE ULTRAVIOLET SPECTRA OF (1) RADICIMIN AND (2) COMPOUND A. (IN METHANOL)



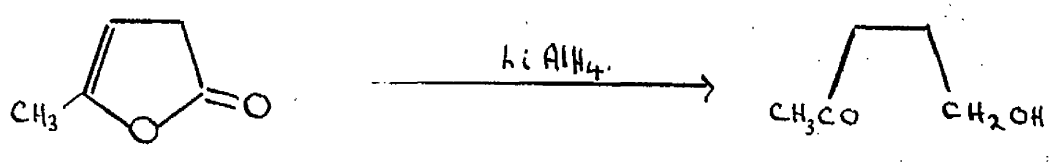
wavelengths than those of radicinin, 295 mμ ($\epsilon = 7.8 \times 10^3$) and 2.82 mμ ($\epsilon = 7.6 \times 10^3$). These data were in agreement with the finding that compound A was formed by the addition of two atoms of hydrogen to some function. Figure 7 shows a comparison of the ultraviolet spectra of radicinin and compound A.

The above results suggested that some type of reductive cleavage had occurred to give a carboxyl group, and it was clear that no hydrolytic process was involved, as for example in the hydrolysis of a lactone, since the analytical figures precluded the introduction of a further oxygen atom.

The reduction of several unsaturated lactones with lithium aluminium hydride has been reported. Thus β -angelica lactone (XIV) gave the saturated diol (XV) on reduction in tetrahydrofuran, while α -angelica lactone (XVI) gave the intermediary ketoalcohol (XVII) (23).

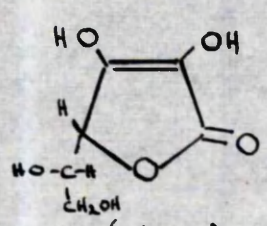


(XIV) (XV)

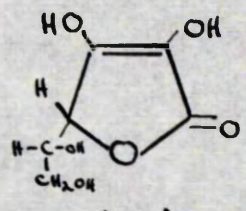


(XVI) (XVII)

However, it has been reported that l-ascorbic acid (XVIII) and isoascorbic acid (XIX) were recovered unchanged on reaction with lithium aluminium hydride in ether (24).



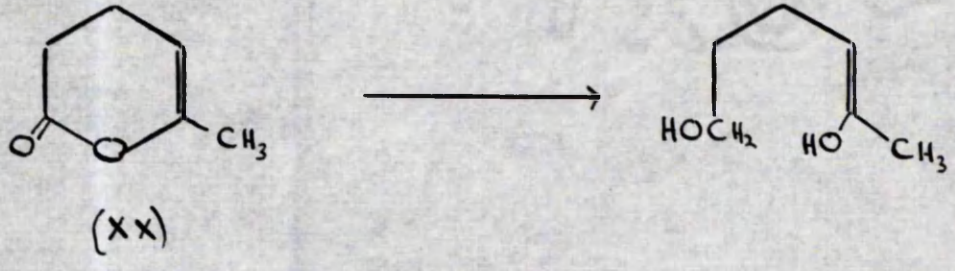
(XVIII)



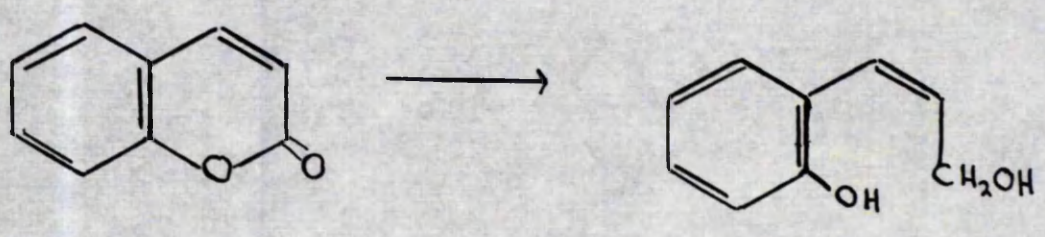
(XIX)

It can thus be seen that the reduction of an unsaturated lactone gives unpredictable results, but it is very unlikely that a carboxylic acid would be produced.

Furthermore, reduction of the unsaturated 6-membered lactones 6-methyl-3,4-dihydro-2-pyrone (XX) and coumarin (XXI) takes place at the carbonyl group to cleave the ring with retention of the enol oxygen (25,25).

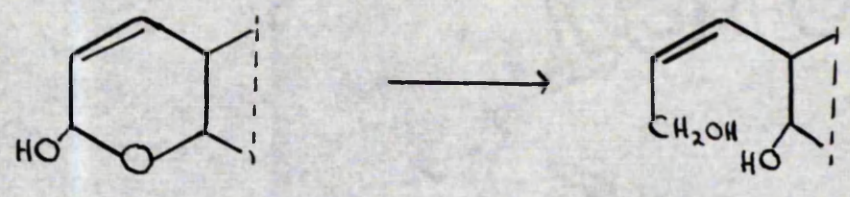


(XX)



XXI

Hemiacetal systems give rise to diols on reduction with lithium aluminium hydride:

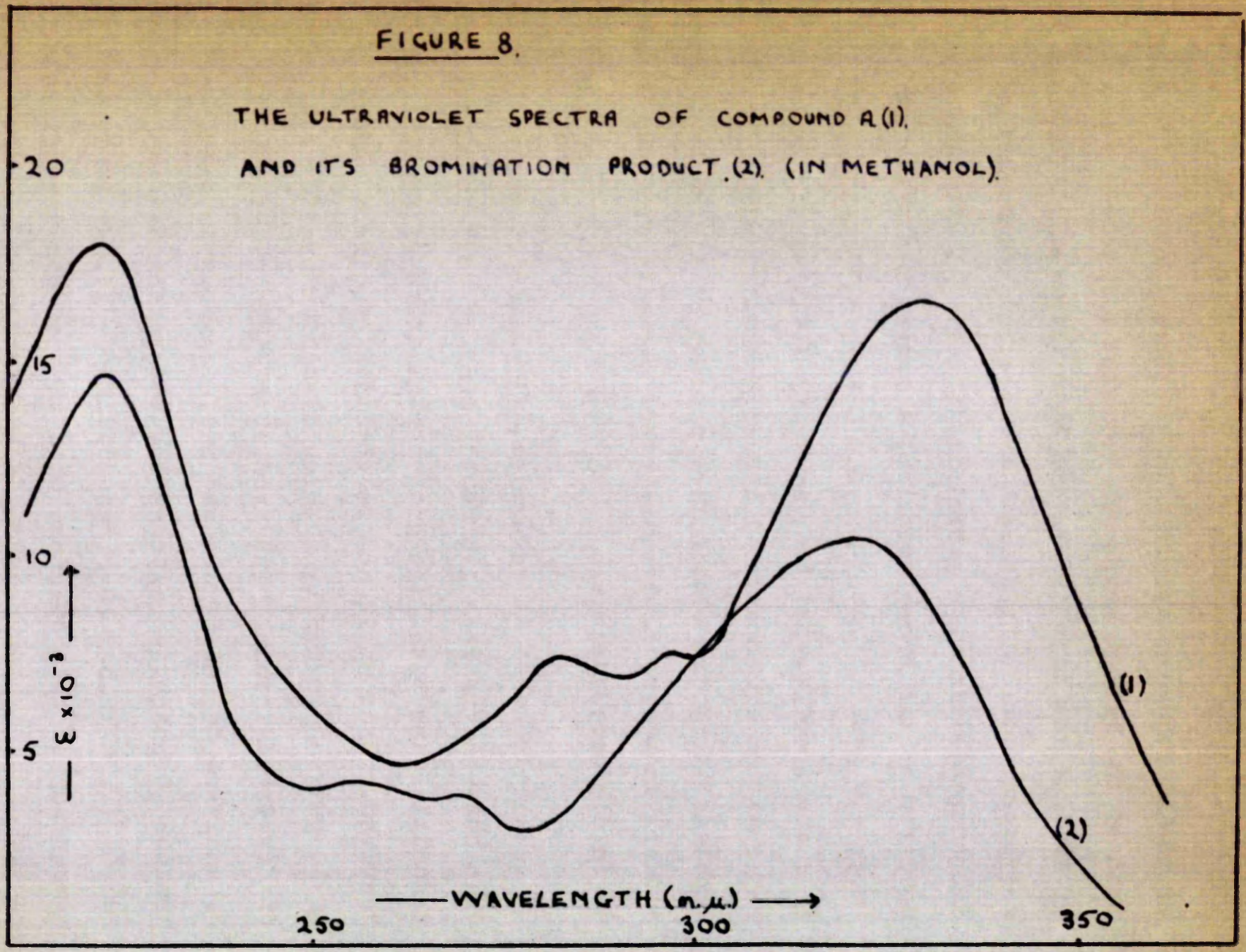


The main chemical features of radicinin were not affected by conversion to the acid A, as alkaline hydrolysis gave acetaldehyde, a 2,4-dinitrophenylhydrazone could be obtained, and as previously mentioned, a mono-acetyl derivative was prepared. However, on reduction of A in the presence of 5% palladium-on-charcoal, three moles of hydrogen were absorbed to produce a clear glass-like solid. Thus, the three carbon-to-carbon double bonds in A were of similar order of reactivity, in sharp contrast to those in radicinin, and this was thought to be due to ring opening which would alter the environment of the double bonds and thus affect their reactivity.

In contrast, bromination of A, with 1 molecular proportion of bromine in chloroform, gave a crystalline compound, mpt. 180° , which bore the same spectral relationship to compound A as dibromoradicinin did to radicinin. Thus its ultraviolet absorption spectrum revealed maxima

FIGURE 8.

THE ULTRAVIOLET SPECTRA OF COMPOUND A (1).
AND ITS BROMINATION PRODUCT (2). (IN METHANOL).



at 320 mμ.(ε = 10.4 x 10^3), 268 mμ.(ε = 3.9 x 10^3), 256 mμ (ε = 4.1 x 10^3) and 221 mμ.(ε = 17.9 x 10^3). Comparison of the ultraviolet spectrum of the bromo-adduct of A with that of the parent compound, revealed a hypsochromic shift of 10 mμ of the main absorption band (Figure 8), compared to a hypsochromic shift of 13 mμ on spectral comparison of dibromoradicinin with radicinin (Figure 3). The infrared spectrum of the bromo adduct of A showed bands at 2.96 μ. (m.), 5.7 μ.(s.), 5.99 μ.(m), 6.1 μ(s.), 6.5 μ.(s.), 6.92 μ. (s.) and incorporated the important features of the infrared spectrum of compound A and dibromoradicinin. It was concluded that the bromination product of A was the dibromo-adduct, in which the bromine atoms occupied the same sites as in dibromoradicinin.

In order to determine, if possible, the relationship between the reactive double bond of radicinin, and the reducible ring site, the dihydroderivative of A would be required. This could not be obtained by direct reduction of A, but was formed by lithium aluminium hydride reduction of dihydroradicinin. The product of this reaction (compound B) analysed for $C_{12}H_{16}O_5$, and was a carboxylic acid. Compound B possessed ultraviolet absorption maxima at 296 mμ (ε = 12.0 x 10^3) and 207 mμ(ε = 15.6 x 10^3), and comparison of this spectrum with that of dihydroradicinin

FIGURE 9

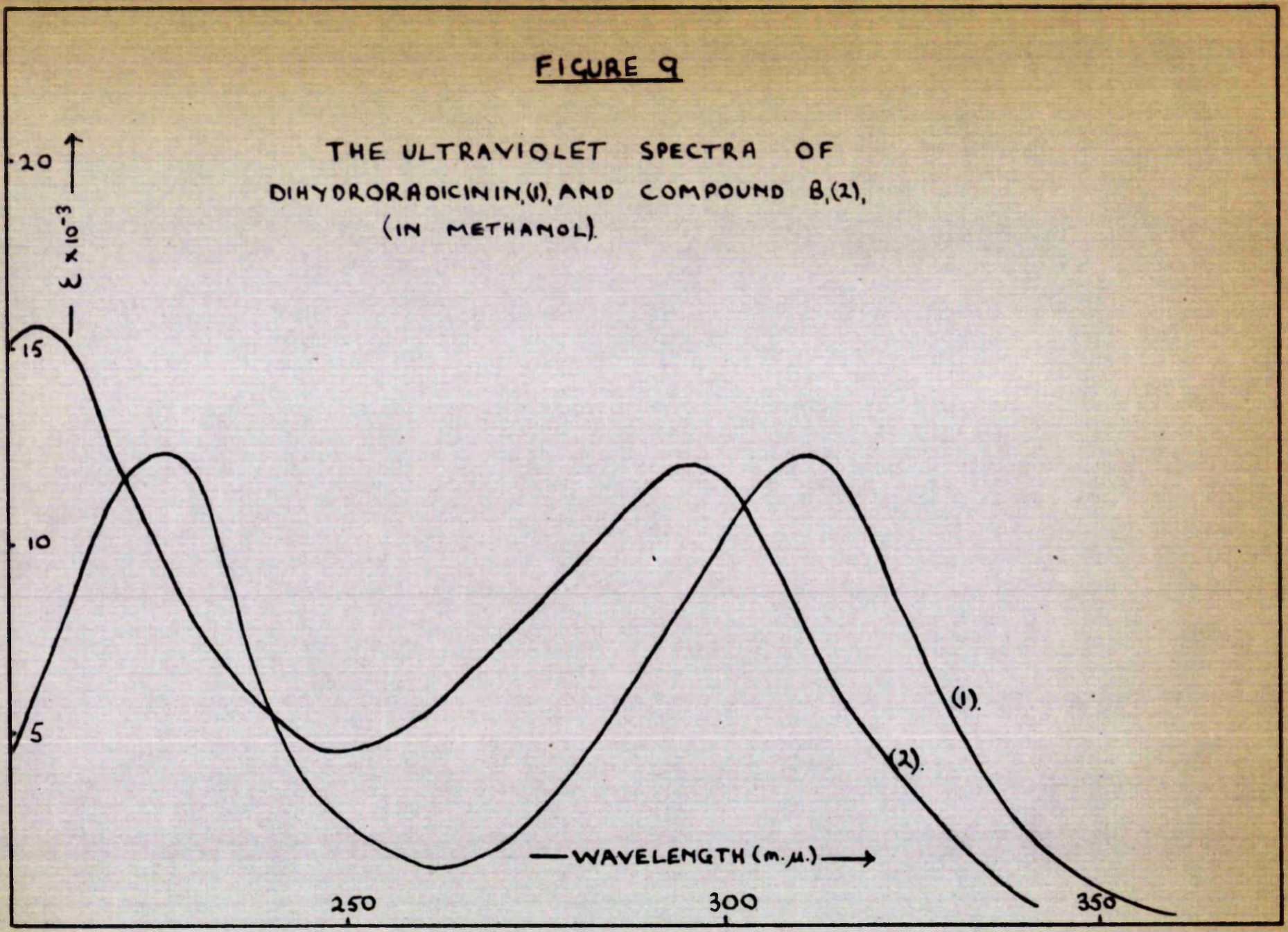
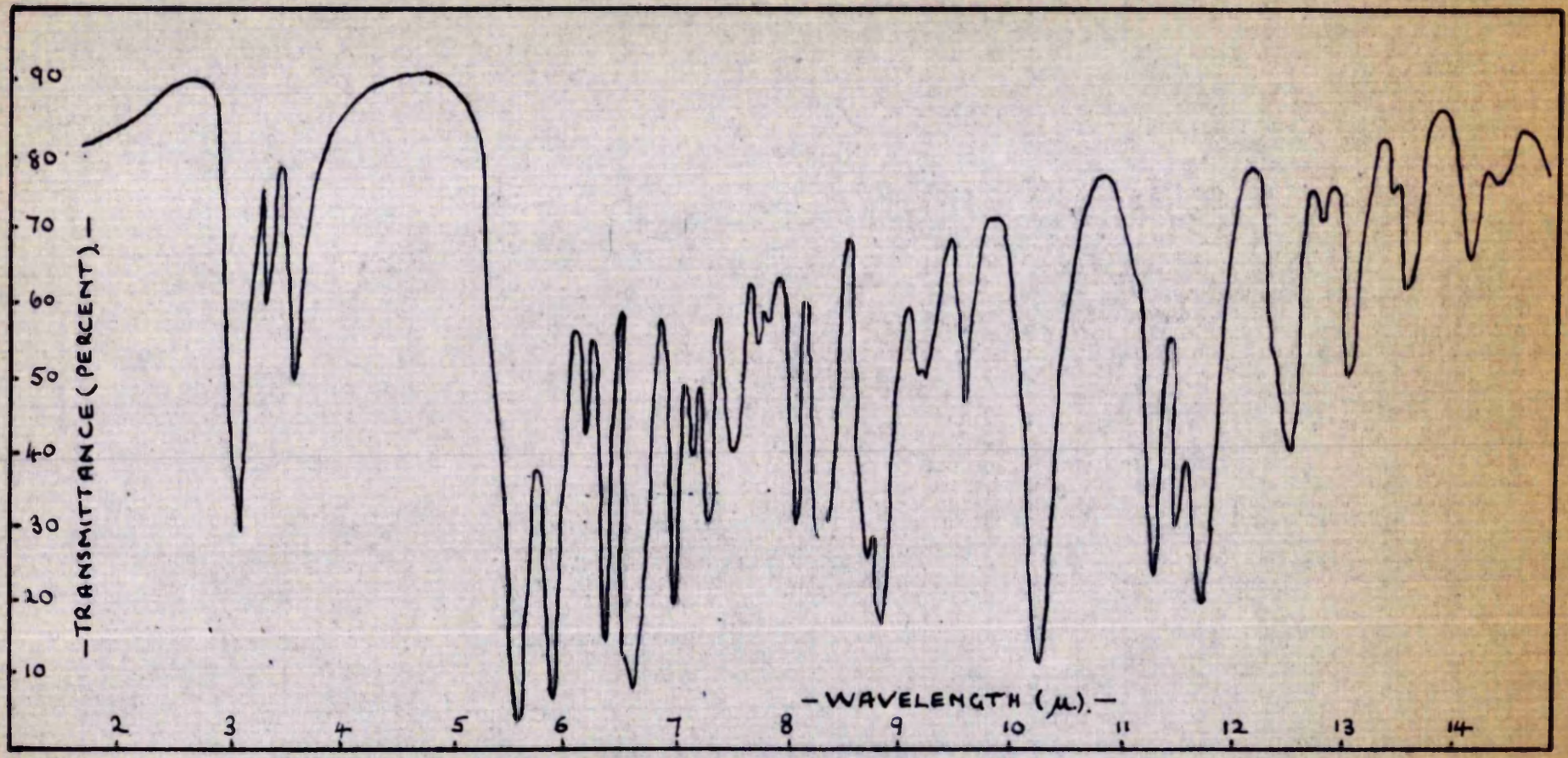

THE ULTRAVIOLET SPECTRA OF
DIHYDRORADICININ, (1), AND COMPOUND B, (2),
(IN METHANOL).
20
15
10
5
ε x10⁻³
(1).
(2).
WAVELENGTH (m.μ.)
250
300
350

FIGURE 10.

THE INFRARED SPECTRUM OF COMPOUND B
(IN POTASSIUM BROMIDE DISC).

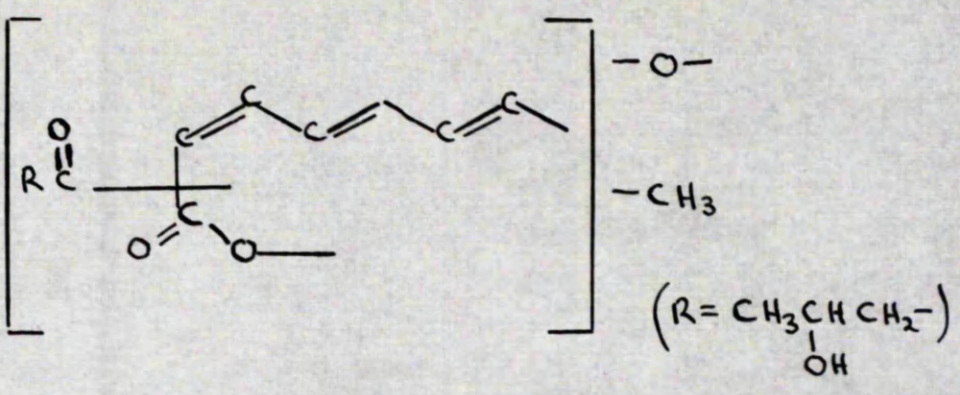


showed a hypsochromic shift of 15 mμ (Figure 9). This was remarkably close to the shift (-14 mμ) observed on comparison of the ultraviolet spectra of compound A and radicinin. The infrared spectrum of B (see Figure 10), with bands at 3.0μ (m.), 5.75μ (s.), 5.93μ (s.), 6.23μ (s.), 6.43μ and 6.48μ (doublet), incorporates the salient spectral features of dihydroradicinin and compound A, as anticipated.

From this spectroscopic data, it was concluded that the reactive double bond in radicinin was not directly attached to the potential carboxyl group, since the hypsochromic shift observed on ring cleavage was the same for radicinin as for dihydroradicinin. It has already been established (page 81) that the reactive double bond was not adjacent to the free carbonyl group.

The above evidence indicated that the unsaturated system (probably two carbon-to-carbon double bonds) immediately conjugated to the potential carboxyl group in radicinin was stabilised in some way. A partial structure (XXII) could be written for radicinin at this stage.

(XXII).

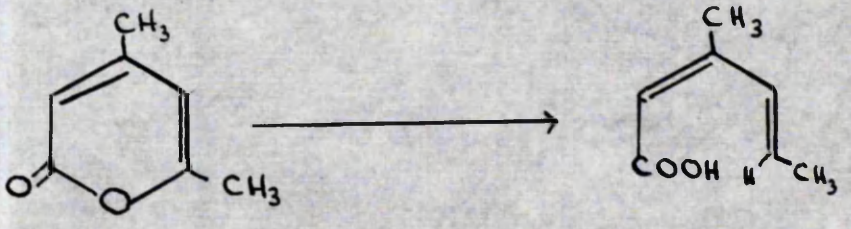


The nature of the cyclic structure which is responsible for the low reactivity of the double bonds may now be considered. The possibility of a benzene nucleus had been eliminated by Clarke and Nord on the basis of the hydrogenation experiments, and the infrared spectrum of radicinin precludes an aromatic system. No phenolic or other aromatic degradation products have been isolated from the molecule.

Evidence for the existence of a 2-pyrone ring in radicinin

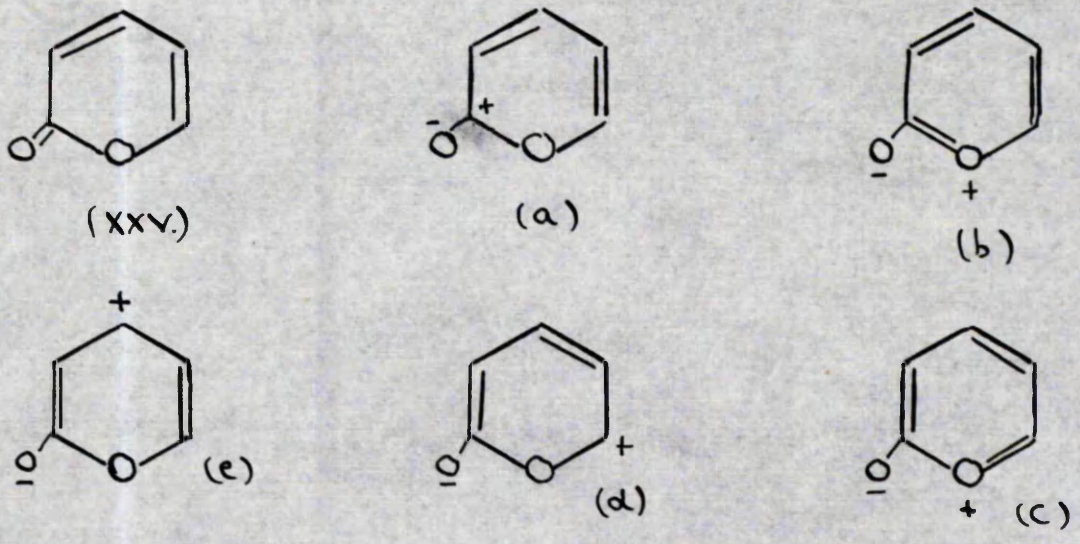
A system which may be considered, which possesses a potential carboxyl group and two stabilised double bonds is the 2-pyrone system. 2-Pyrones are essentially unsaturated lactones, but unfortunately little is known regarding the behaviour of such systems in the presence of complex metal hydrides, except as previously mentioned (page 86), it had been reported that coumarin and 3,4-dihydro-2-pyrone behaved as normal lactones, giving diols on reduction. However, it has been recently reported that 2-pyrones are susceptible to hydride ion attack at the 6-

position, resulting in ring cleavage, with the formation of α,β -unsaturated acids (27). For example, 4,6-dimethyl-2-pyrone (XXIII) gave a 47% yield of β-methylsorbic acid (XXIV) on treatment with 0.4 mole of lithium aluminium hydride.



(XXIII) (XXIV)

The mechanism of this reaction is clear if one considers the various possible canonical forms of 2-pyrone (XXV).



(XXV) (a) (b) (e) (d) (c)

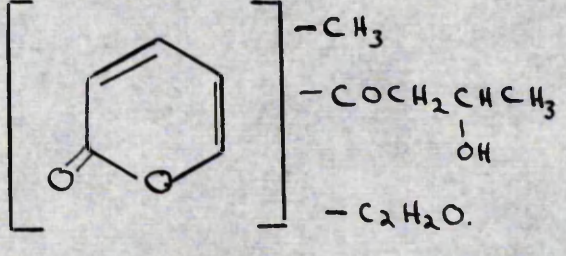
Thus if structure (XXVd) makes any contribution to the resonance hybrid, the 2-pyrone nucleus will bear a

fractional positive charge at the 6-position, thus allowing the attack of nucleophilic reagents at this position. This type of reaction is further exemplified by methylation of methyl-2-pyrone-5-carboxylate with diazomethane to yield methyl-6-methyl-2-pyrone-5-carboxylate (28).

These results allow a rationalisation of the reaction of radicinin with lithium aluminium hydride. On reduction of radicinin with an excess of lithium aluminium hydride in ether, an ether insoluble complex was seen to form immediately the reaction started. This is believed to be a complex salt of the carboxylic acid with lithium aluminium hydride.

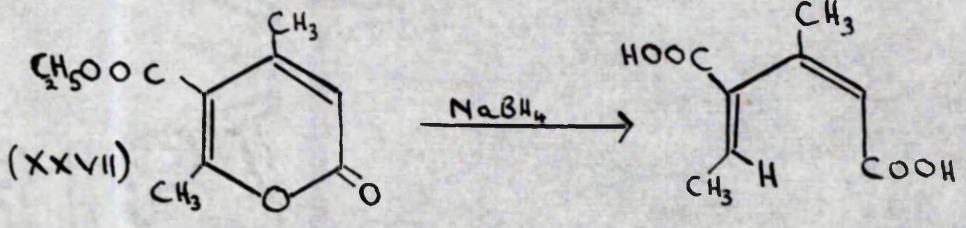
The presence of a 2-pyrone system in radicinin therefore offers a satisfactory explanation for the formation of the carboxylic acid, and partial structure (XXVI) may now be written for radicinin.

(XXVI.)



Further evidence for this formulation was obtained by a re-examination of the products obtained by reduction of radicinin with sodium borohydride. It was found that reaction of radicinin with 0.75 moles of sodium borohydride,

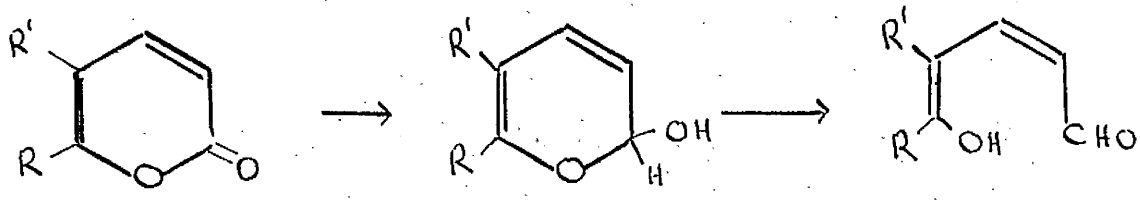
in aqueous ethanol, gave a yellow glass-like solid from which compound A could be obtained in very small yield (table 5 , page 157). Since it has been shown that ethyl-4,6-dimethyl-2-pyrone-5 carboxylate (XXVII) undergoes reductive ring cleavage by sodium borohydride, whereas 4,6-dimethyl-2-pyrone only undergoes cleavage with lithium aluminium hydride, it seems reasonable to suggest that 2-pyrones with electron-withdrawing substituents are labilised to attack by sodium borohydride(27). Thus the observation that radicinin undergoes reductive ring cleavage with sodium borohydride would support the proposal that radicinin possessed a 2-pyrone nucleus with a ketonic electron-withdrawing side chain.



It was considered that this electron-withdrawing group could occupy any site in the ring, in order to increase the reactivity of the 2-pyrone to nucleophilic reagents, although positions 3 or 5 were possibly more likely than positions 4 or 6.

In addition to the above work, the reduction of radicinin with lithium aluminium hydride in tetrahydrofuran at low temperature was carried out according to the method

recommended by Dagley (29), who reported that in the reduction of 2-pyrones, the reaction could be controlled by the use of low temperatures and calculated quantities of lithium aluminium hydride, to give the corresponding hydroxy-aldehydes (XXVIII).



(XXVIII)

a. $R = R^1 = H$

b. $R = COOH$, $R^1 = H$

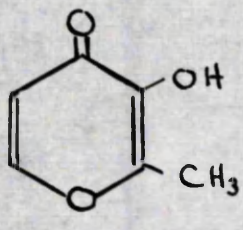
c. $R = H$, $R^1 = COOH$

d. $R = R^1 = COOH$.

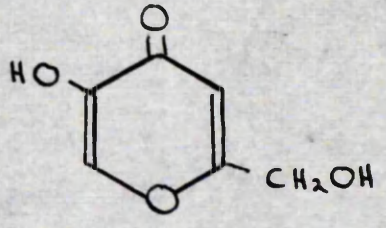
When this method was applied to radicinin the product obtained was a solid mixture containing five components, one of which was compound A, as shown by thin film chromatography. That the other products probably possessed free carbonyl groups was shown by the infrared spectrum of the mixture, but purification of these compounds could not be achieved. The reaction was therefore not pursued further. For a summary of the conditions used see table 4, page 156 .

All attempts to further reduce compounds A and B with lithium aluminium hydride or sodium borohydride failed, starting materials being recovered, due probably to complexing of the carboxyl group with the reagents.

As a means of comparison, the behaviour of 4-pyrones in the presence complex metal hydrides was also studied. Thus, maltol (XXIX), and kojic acid (XXX) were treated with lithium aluminium hydride and sodium borohydride.

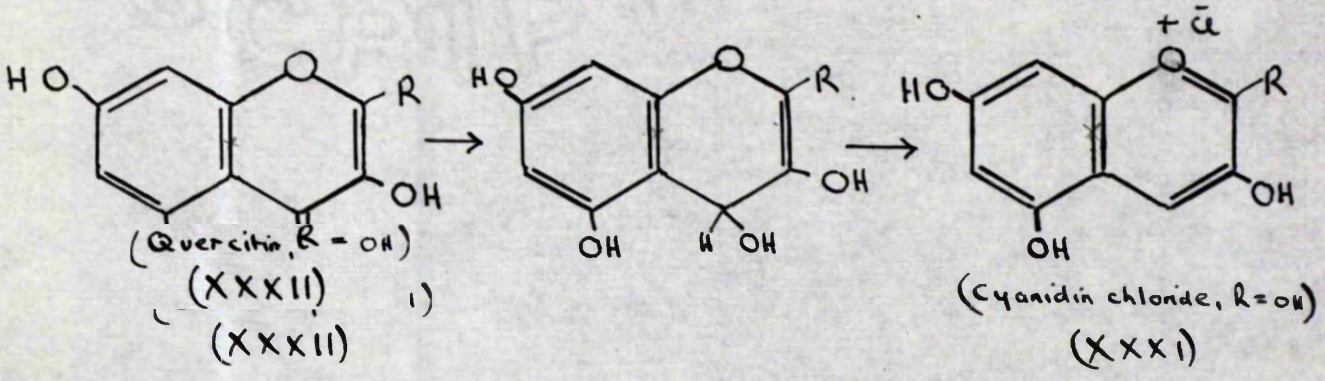


(XXIX)



(XXX)

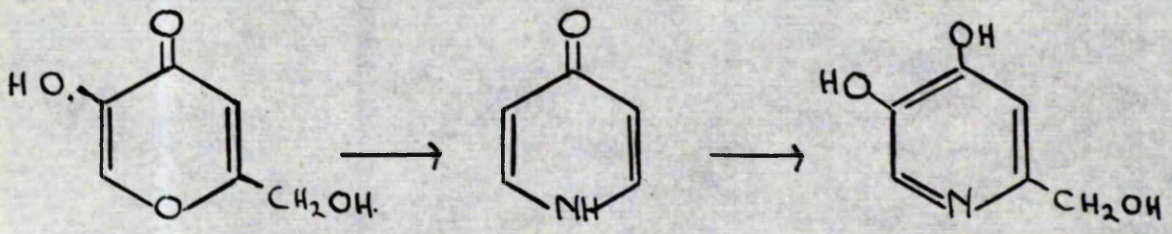
The conditions used and the results obtained are summarised in table 6, page 164 . Sodium borohydride was found not to react with these compounds, but reaction with lithium aluminium hydride, by direct or reverse addition, gave clear yellow oils which were not acidic and which gave positive carbonyl tests. These results were contrary to the reported production of pyrilium salts on reduction of 2-pyrones with lithium aluminium hydride (30), thus anthocyanidins (XXXI) could apparently be obtained from flavones (XXXII).



In addition, it was found that patulin, on reduction with lithium aluminium hydride, by reverse and direct methods, also gave non-acidic carbonyl containing products, but again the products were oils which could not be purified.

These experiments show that radicinin does not react with complex metal hydrides in a manner comparable with 4-pyrones or hemiacetals. No further work has been carried out along these lines, although it is considered that, in view of the lack of information on this topic, a considerable amount of work needs to be done.

It is well known that simple 2-and 4-pyrones react with ammonia to give the corresponding pyridones (31). Characterisation of the pyridine compound so formed provides valuable information on the constitution of the parent pyrone. Thus kojic acid was converted into the pyridine (XXXIII), the structure of which confirmed the constitution of kojic acid.



(XXXIII)

With this object in mind radicinin was heated under reflux with 5N. ammonium hydroxide. The product obtained was a brown, nitrogen-containing gum. Attempts to purify this material were abortive, and it was assumed that radicinin was attacked at more than one site. When radicinin was dissolved in .880 ammonia, a bright red solution was obtained (as with sodium hydroxide solution), which on evaporation yielded an intractable brown gum.

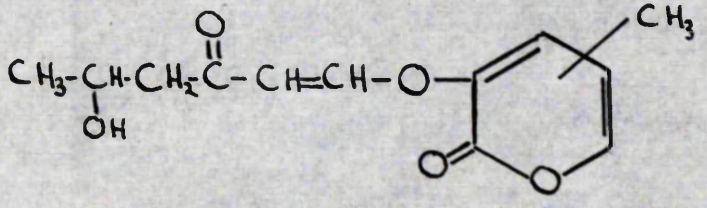
The presence of a furan or dihydrofuran ring in radicinin

Having proposed that radicinin is a derivative of 2-pyrone, there remains only the C_2H_2O residue, and the position of the methyl group to be considered.

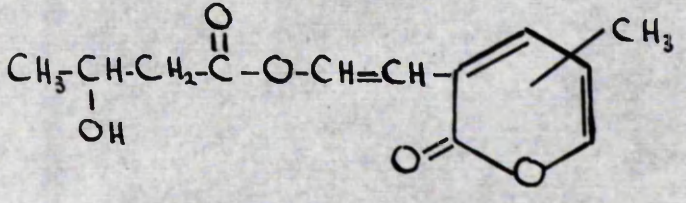
It seems most likely that the reactive double bond in radicinin is part of the C_2H_2O fragment, this being arrived at by the process of elimination. The only other possible position for the double bond would be if it

were interposed between the hydrated crotonyl side chain and the 2-pyrone nucleus. To incorporate the remaining oxygen atom one has to consider structures of the type (XXXIV), (XXXV), (XXXVI). However, these structures are all eliminated by consideration of spectroscopic and chemical evidence.

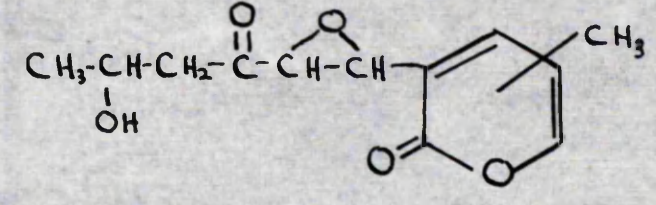
(XXXIV)



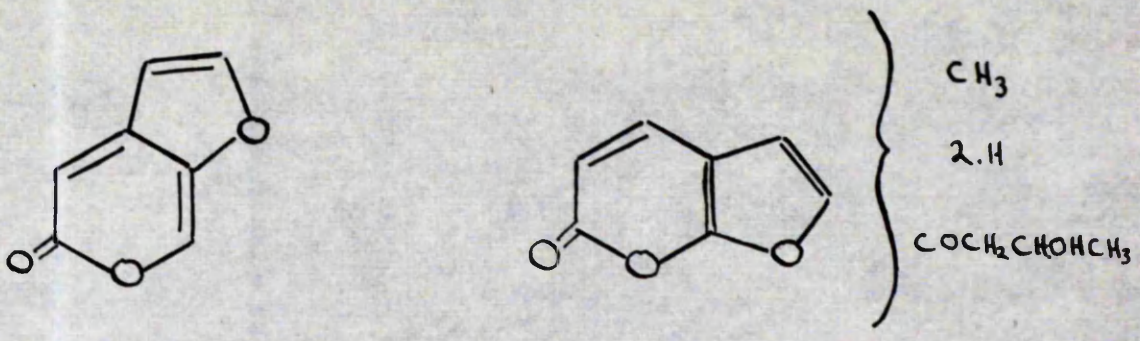
(XXXV)



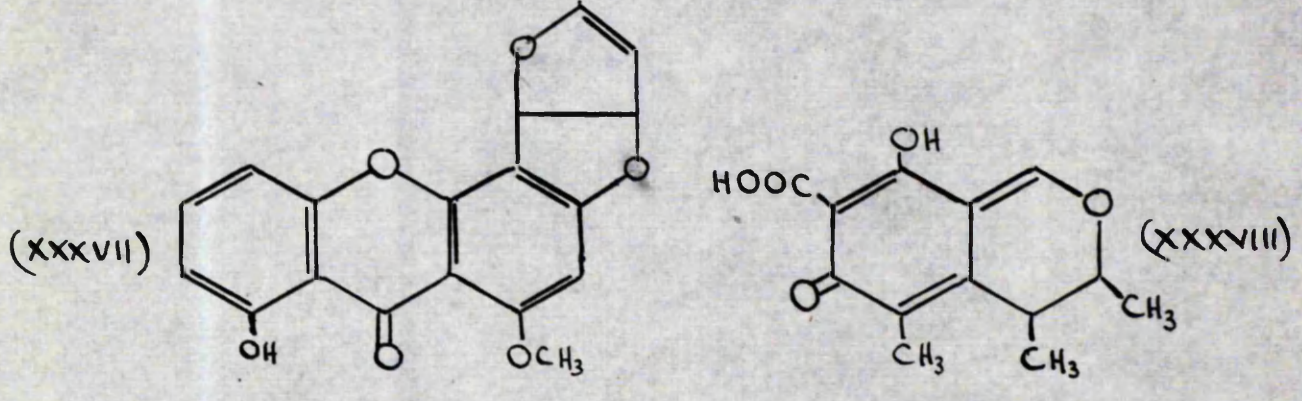
(XXXVI)



A structure which would better explain the properties of radicinin would be one in which the reactive double bond and the remaining oxygen atom form a fused furan or dihydrofuran ring system, examples of which are shown below.



The observed stability of radicinin to acids, except on prolonged treatment with hot dilute acids and prolonged treatment with concentrated sulphuric acid in the cold, may not be incompatible with the proposed furanose structure which contains a vinyl ether linkage. Thus sterigmatocystin (XXXVII) and citrinin (XXXVIII), which contain vinyl ether groups are stable to acid, except under extreme conditions.



It has been shown by several workers (32, 33) that those compounds containing a vinyl ether grouping show characteristic absorption in the infrared at wavelengths of ca. 6.25μ, 7.85 - 8.35μ, and 9.3 - 9.8μ. The infrared spectrum of radicinin shows a band at 6.25μ

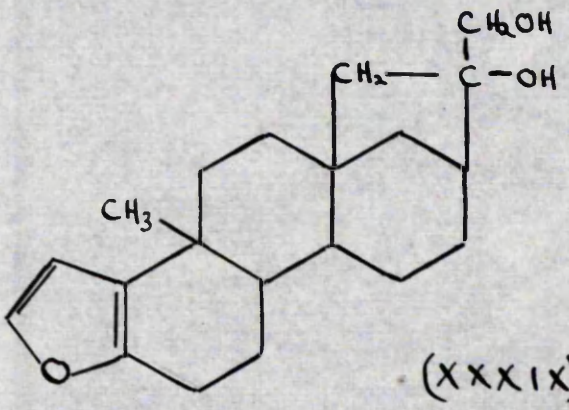
which is missing in dihydroradicinin. This result would be compatible with the presence of a vinyl ether group in radicinin undergoing reduction. Similar changes observed in this region on comparison of the infrared spectra of the dibromo and hydrogen chloride adducts can now be ascribed to the loss of a vinyl ether linkage and the production of the respective halogenated furan system.

In addition the three adducts of radicinin possess a single strong absorption maximum in the region 9.3 to 9.6 μ, whereas radicinin possesses a doublet at 9.48 and 9.71 μ. It has been reported (22) that tetrahydrofuran possesses a band at 9.3 μ in the infrared and therefore the changes observed in this region on comparison of the infrared spectra of radicinin and its adducts suggest that a furan or dihydrofuran ring is present.

The infrared spectra of the adducts of radicinin show differences from the spectrum of radicinin in the 8.05 - 8.4 μ region. These data would also be in agreement with the presence of a furan or dihydrofuran system, although consideration of this region of the spectra is complicated by the fact that the lactonic C-O-C system of a pyrone also absorbs in this region. This would account for the complexity of this region in the infrared spectra

of radicinin and its derivatives.

Similar infrared spectroscopic data has been interpreted in favour of furanose systems in sterigmatocystin (34) and cafestol (XXXIX) (35).



(XXXIX)

It must be emphasised that due to the complex structural features of radicinin, its infrared spectrum possesses a large number of absorption bands such that overlapping is to be expected, rendering interpretation difficult.

Attempted degradation of radicinin

The inability to obtain recognisable degradation products of radicinin has made structural work on this compound difficult. As alkaline hydrolysis failed to give any recognisable product, hydrolysis with dilute acids was attempted. When radicinin was heated under reflux for 4 hours with 2N. hydrochloric acid, a gum was obtained from which the hydrogen chloride adduct of radicinin was separated in 30% yield after chromatography on silicic

acid. In spite of many attempts the residual gum remained intractable. On treatment of radicinin with 2N. sulphuric acid for 5 hours, 1 mole of carbon dioxide and 0.3 mole of volatile acid per mole of radicinin were produced. The main product of this hydrolysis was also an intractable gum which could not be purified chromatographically.

Since compounds A and B were considered to be α,β unsaturated acids, attempts were made to decarboxylate small quantities of A by the method of Sondheimer et.al. (36) using copper chromite in quinoline. The evolution of 1 mole of carbon dioxide was detected, but the product was a brown gum which could not be purified.

Reduction of highly oxygenated compounds with sodium or lithium in liquid ammonia has often provided useful structural information (37). Accordingly, radicinin was treated with lithium in liquid ammonia. In no case, however, was it possible to obtain a solid product from the resultant yellow oils.

One of the classical methods by which useful degradation products have been obtained from natural products is reduction with red phosphorus and hydriodic acid. For example, fully reduced patulin was found to yield β-methyl-γ-hydroxy-n-hexanoic acid and β-methyl caproic acid by this method (page 32). Reduction of

fully reduced radicinin with phosphorous and hydriodic acid gave a pale yellow oil which partly crystallised to give minute quantities of oily crystals (yield 0.2%), m.pt. 118-120°. The yield of this product obtained was so small that no further work could be carried out. The residual oil could not be characterised by techniques available.

Also by analogy with patulin, the action of hydriodic acid on radicinin was studied, but no characterisable iodo-acid was obtained, although the dark red oil produced was shown to contain iodine.

Consideration of the spectroscopic data for radicinin and its derivatives in light of the chemical evidence

1. The ultraviolet absorption spectral data

The conclusions reached on the structure of radicinin, on chemical grounds, are as follows. The 2-pyrone nucleus contains a hydrated crotonyl side chain, attached at some undetermined point, and is fused to a dihydrofuran ring.

Consideration of the various combinations of these three moieties leads to twelve possible structures for radicinin. Of these, all but four may be eliminated by consideration of three previously mentioned pieces of evidence.

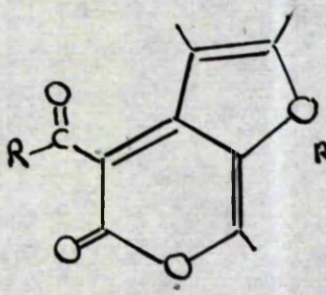
1. On reduction of radicinin in the presence of 5% palladium-on-charcoal, 1 double bond only is saturated. It is unlikely that the 2-pyrone system would be reduced in preference to an exocyclic carbon-to-carbon double bond. (Thus fulvoplumierine, see page 107, undergoes preferential reduction in the side chain and cyclopentene ring.). This is substantiated by the fact that dihydroradicinin undergoes reductive ring cleavage with lithium aluminium hydride as expected for a 2-pyrone. Thus the reactive double bond must be situated in the furan ring.

2. The double bond is conjugated with the main chromophore, as indicated by the large hypsochromic shift observed on comparison of the ultraviolet spectra of dihydroradicinin and radicinin.

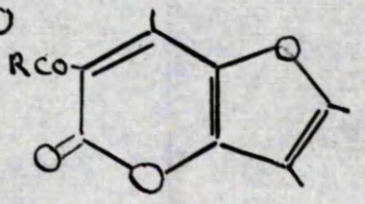
3. The carbonyl group of the side chain is conjugated to the main chromophore and to the reactive double bond, as shown by comparison of the ultraviolet spectra of the 2,4-dinitrophenylhydrazones of radicinin and dihydroradicinin.

The only structures which comply with the above requirements are (XL) to (XLIII). It has been shown by

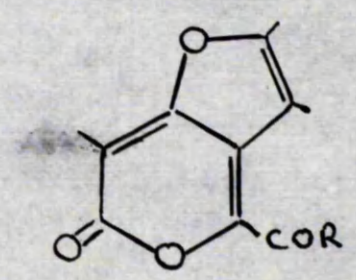
POSITION OF 2(H) AND 1(CH_3) UNDETERMINED.*



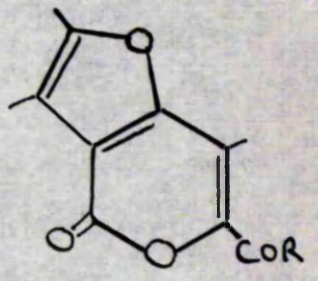
(XL)



(XLI)



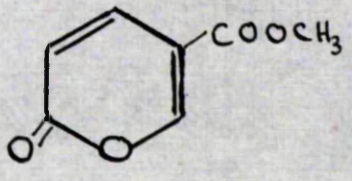
(XLII)



(XLIII)

R = CH_3 CH CH_2–
OH

Schmidt and Benez (38) that methyl-2-pyrone-5-carboxylate (XLIV) shows an ultraviolet absorption maximum at 285 mμ. ($\varepsilon = 4.6 \times 10^3$) and 240 mμ ($\varepsilon = 8.9 \times 10^3$).

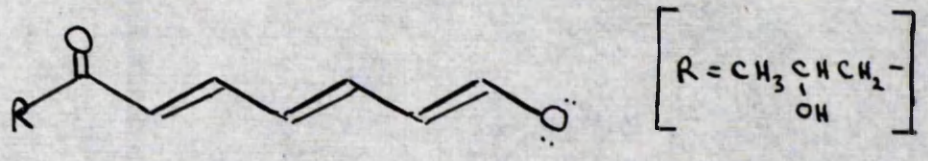


(XLIV)

Thus it is clear that further unsaturation in radicinin must be conjugated with the 2-pyrone system to account for the high intensity wavelength of its main absorption band. Of the four possible structures, (XLI) and (XLIII) contain the longest conjugated systems being of the type (XLV), which might give rise toalong wavelength ultraviolet

* Applies throughout the following discussion (see page 126).

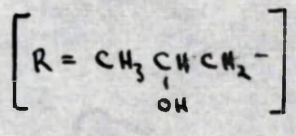
absorption band. As a parallel it is worth noting that 2,4,6-octatrienal gives an absorption band at 315 m.μ., ($\varepsilon = 37.0 \times 10^3$).

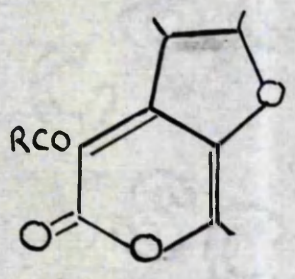


XLV

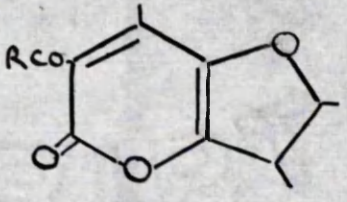
Structures (XLI) and (XLIII) contain this system with an additional -OR group placed in such a position as to give the longest conjugated system possible, i.e. at the end of the chain. Thus the system could well give rise to an absorption band at 344 μ.

The possible structures for dihydroradicinin, which correspond to the structures for radicinin, are (XLVI) to (XLIX). Of these (XLVII) and (XLIX) would best account for the large hypsochromic shift observed on comparison of the ultraviolet spectra of dihydroradicinin and radicinin, because a long conjugated system is broken.

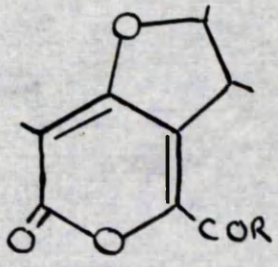




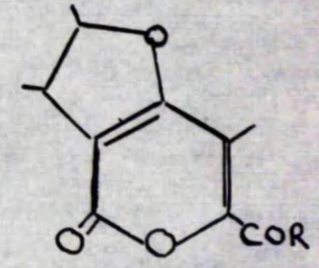
(XLVI)



(XLVII)



(XLVIII)

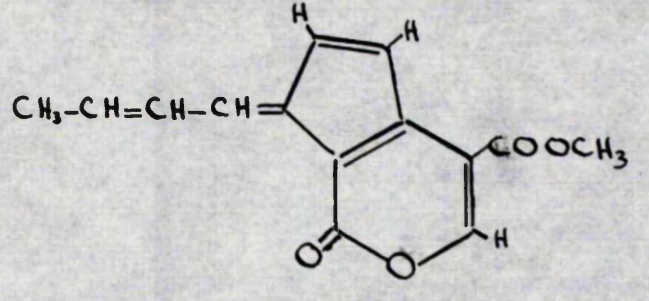


XLIX

However, as no rigorous treatment of the ultraviolet spectra of such complex molecules is possible, structures (XLVI) and (XLVIII) cannot be eliminated.

The ultraviolet spectrum of dihydroradicinin lacks the low intensity absorption bands of radicinin at 270 mμ and 280 mμ. This may indicate the loss of shorter, cross conjugated systems originally present in radicinin.

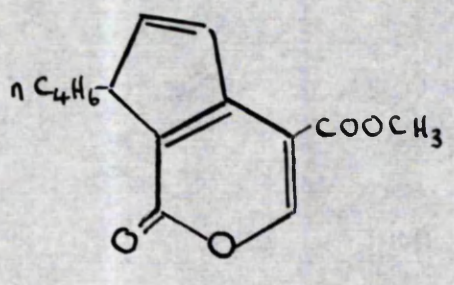
Each of the postulated structures for dihydroradicinin possesses a 2-pyrone nucleus substituted by an electron withdrawing group. The ultraviolet absorption spectrum of dihydroradicinin shows close similarities to that of methyl-2-pyrone-5-carboxylate (XLIV), which was used as a model compound in the study of the naturally occurring 2-pyrone, fulvoplumierine (L) (38,39).



(L)

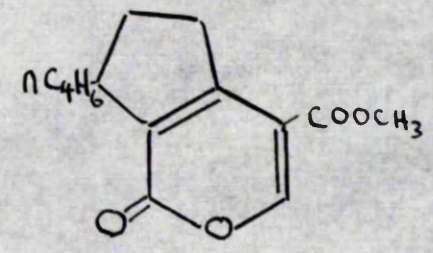
This compound has ultraviolet absorption bands at 360 m.μ. ($\varepsilon = 44.6 \times 10^3$), 275 m.μ ($\varepsilon = 8.0 \times 10^3$) and 220 m.μ ($\varepsilon = 20 \times 10^3$). The long wavelength of the main absorption band is due to the conjugated side chain, but the spectrum of this compound is remarkably similar to that of radicinin if due

allowance is made for further conjugation. The tetrahydro and hexahydro derivatives of fulvoplumierine (LI) and (LII) have ultraviolet absorption maxima as shown:



(LI)

330 mμ (ε = 5 x 10^3)
258 mμ (ε = 5 x 10^3)

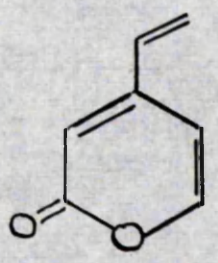


(LII)

295 mμ (ε = 6.3 x 10^3)
250.5 mμ (ε = 8.9 x 10^3)

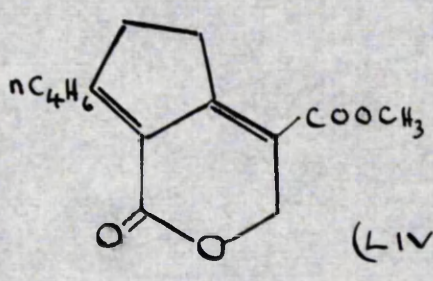
It will be seen that tetrahydrofulvoplumierine has its main ultraviolet absorption maximum at a shorter wavelength than radicinin, but lacks the —OR group conjugated to the main chromophore, which probably accounts for the higher wavelength absorption in radicinin. It will also be seen that in tetrahydrofulvoplumierine, the double bond of the cyclopentene ring is conjugated with only one double bond of the 2-pyrone ring (discounting the small contribution of positively charged canonical forms to the ultraviolet spectrum of the whole system). Despite this, reduction of this double bond involves a hypsochromic shift of 35 mμ, which is almost exactly the shift found on comparison of the ultraviolet spectra of dihydroradicinin

and radicinin. Thus it is possible that radicinin, like tetrahydrofulvoplumierine, contains a system of the type (LIII), and therefore (XL) and (XLII) must still be considered as possible structures for radicinin. The fact that dihydroradicinin has an ultraviolet absorption spectrum similar to those of both hexahydrofulvoplumierine and methyl-2-pyrone-5-carboxylate supports the finding that dihydroradicinin is a substituted 2-pyrone.



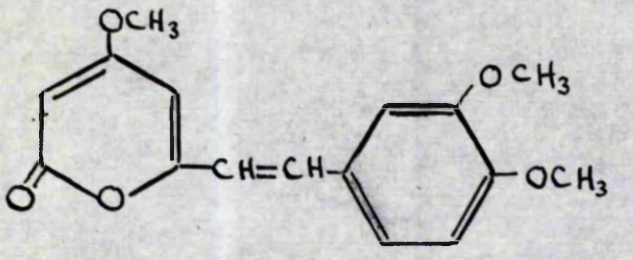
(LIII)

Schmidt and Bencz also reported the formation of isohexahydrofulvoplumierine (LIV), which has an absorption band at 285mμ($\varepsilon = 16 \times 10^3$). This value is quite close to that recorded for dihydroradicinin, but a structure such as this for radicinin would require 1:4 addition and destruction of the 2-pyrone system, which has been shown to be unlikely.



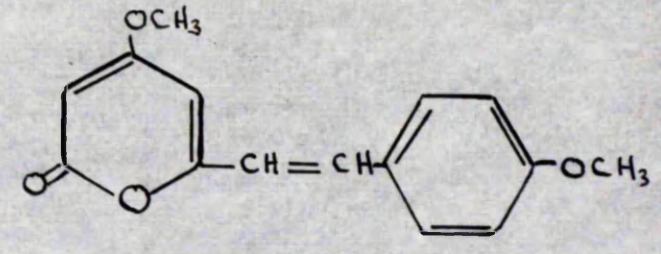
(LIV)

Hispidin (LV), a recently reported fungal 2-pyrone (page 30), and the related plant product yangonin (LVI), both have ultraviolet absorption spectra similar to radicinin, but it is difficult to compare the effect of the styryl side chains with the side chain unsaturation in the proposed structures for radicinin.



(LV)

366 m.μ. ($\varepsilon = 25.1 \times 10^3$)



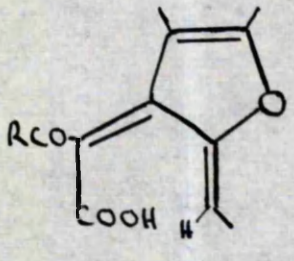
(LVI)

360 ($\varepsilon = 25.1 \times 10^3$)

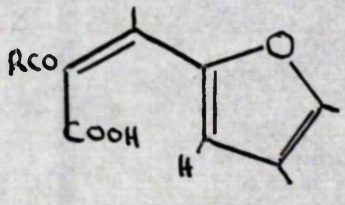
The ultraviolet absorption spectra of the bromine and hydrogen chloride adducts of radicinin, as previously discussed (page 67), confirm in general the above conclusions about the structure of radicinin, due allowance being made for the effect of the halogen substituents on the ultraviolet spectrum of the saturated furan produced.

Comparison of the ultraviolet spectra of radicinin with that of the carboxylic acid A, produced by reductive ring cleavage of radicinin, shows a hypsochromic shift of 14.4 m.μ., accompanied by a reduction of intensity of the main absorption band (Figure 7). This

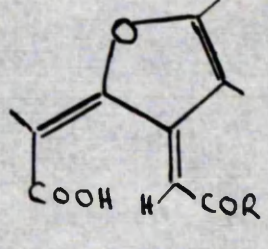
is understandable, as acids in general are expected to have ultraviolet absorption maxima at lower wavelengths than those of the corresponding ester, in alcoholic solution. Apart from the shift of the main band, the ultraviolet spectrum of compound A shows a similar profile to that of radicinin, and thus it would appear that no major structural change has occurred,as no chromophoric group has apparently been lost. Consideration of the four possible structures for radicinin would indicate structures (LVII) to (LX) for compound A. It will be noted that two of these are furan derivatives while the other two are dihydrofurans.

XL → 

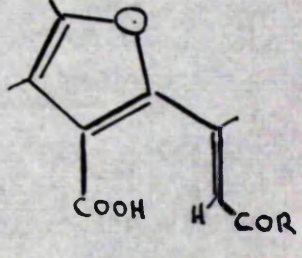
(LVII)

XLI → 

LVIII

XLII → 

(LIX)

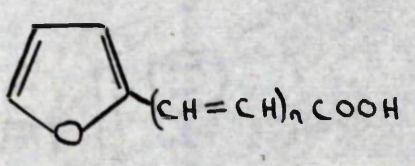
XLIII → 

(LX)

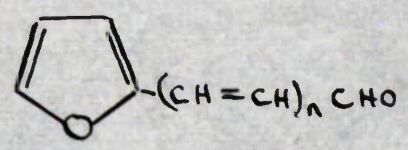
($R = CH_3CH(OH)CH_2-$)

At this juncture it is interesting to compare the absorption maxima of some 2-furylpolyene acids (LXI), 2-furylpolyene aldehydes (LXII), and polyene carboxylic

acids, aldehydes and ketones (LXIII, LXIV, LXV) (40) with the data obtained for compounds A and B, see table I



(LXI)



(LXII)

$CH_3(CH=CH)_nCOOH$

LXIII

$CH_3(CH=CH)_nCHO$

XIV

$CH_3(CH=CH)_2COCH_3$

LXV

$CH_3CH=CH\ COCH_3$

LXVI

$CH_2=CH-CH=CH\ COOH$

LXVII

The structures (LVIII) and (LX), postulated for compound A, bear similarities to compounds (LXII, n=1) and (LXI, n=1), and although the furan aldehyde possesses an ultraviolet absorption band closer to the value for radicinin than does the corresponding acid, differentiation between the two furan structures for compound A is not possible, because both structures also contain a ketonic carbonyl group. In the case of (LX) the ketonic and carboxylic functions are at opposite ends of a diene system, but in the case of (LVIII), both functions

Ultraviolet absorption bands observed in some 2-furylpolyene acids and related compounds

Compound	λ max(m.μ).	$\varkappa \times 10^{-3}$	Solvent
A	330	38.0	Methanol
B	296	27.6	Methanol
LXI, n=1	300	50.0	Hexane
LXI, n=2	315	80.0	Hexane
LXI, n=3	350 372	90.0	Hexane
LXII, n=0	272	30.0*	Alcohol
LXII, n=1	314	52.0*	Alcohol
LXII, n=2	350	59.0*	Alcohol
LXIII, n=1	204	26.9*	Alcohol
LXIII, n=2	261	57.0	Alcohol
LXIII, n=3	294	84.0	Alcohol
LXIV, n=1	220	34.5	Alcohol
LXIV, n=2	271	57.5	Alcohol
LXV	260	48.5	Hexane
LXVI	215	24.0	Hexane
LXVII	245	22.0	Alcohol

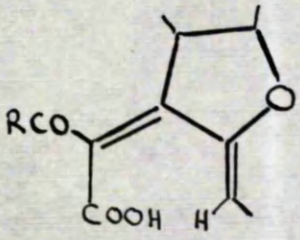
*. Approximate values only.

Table I

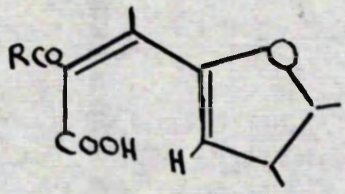
are attached to the terminal carbon atom of a furylethylene system. Thus of the two systems,(LVIII) should be the more polar.

The dihydrofuran structures (LVII) and (LIX) postulated for compound A contain similar diene systems to (LXIV, n=2) and (LXV), but these latter compounds possess absorption bands of considerably lower wavelength than those observed for compound A. It appears therefore, that the furan structures would better account for the spectral properties of A, although the influence of a carbonyl group and further conjugation cannot be predicted.

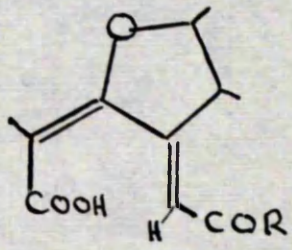
Considering the structures (XLVI) to (XLIX) postulated for dihydroradicinin, compound B, formed by reductive ring cleavage of dihydroradicinin, must have one of the following structures:



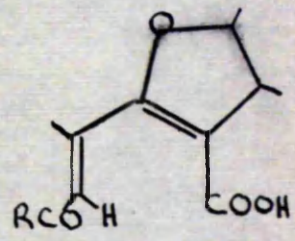
(LXVIII)



(LXIX)



(LXX)



(LXXI)

$\left(R = CH_3\,\underset{OH}{CH}\,CH_2-\right)$

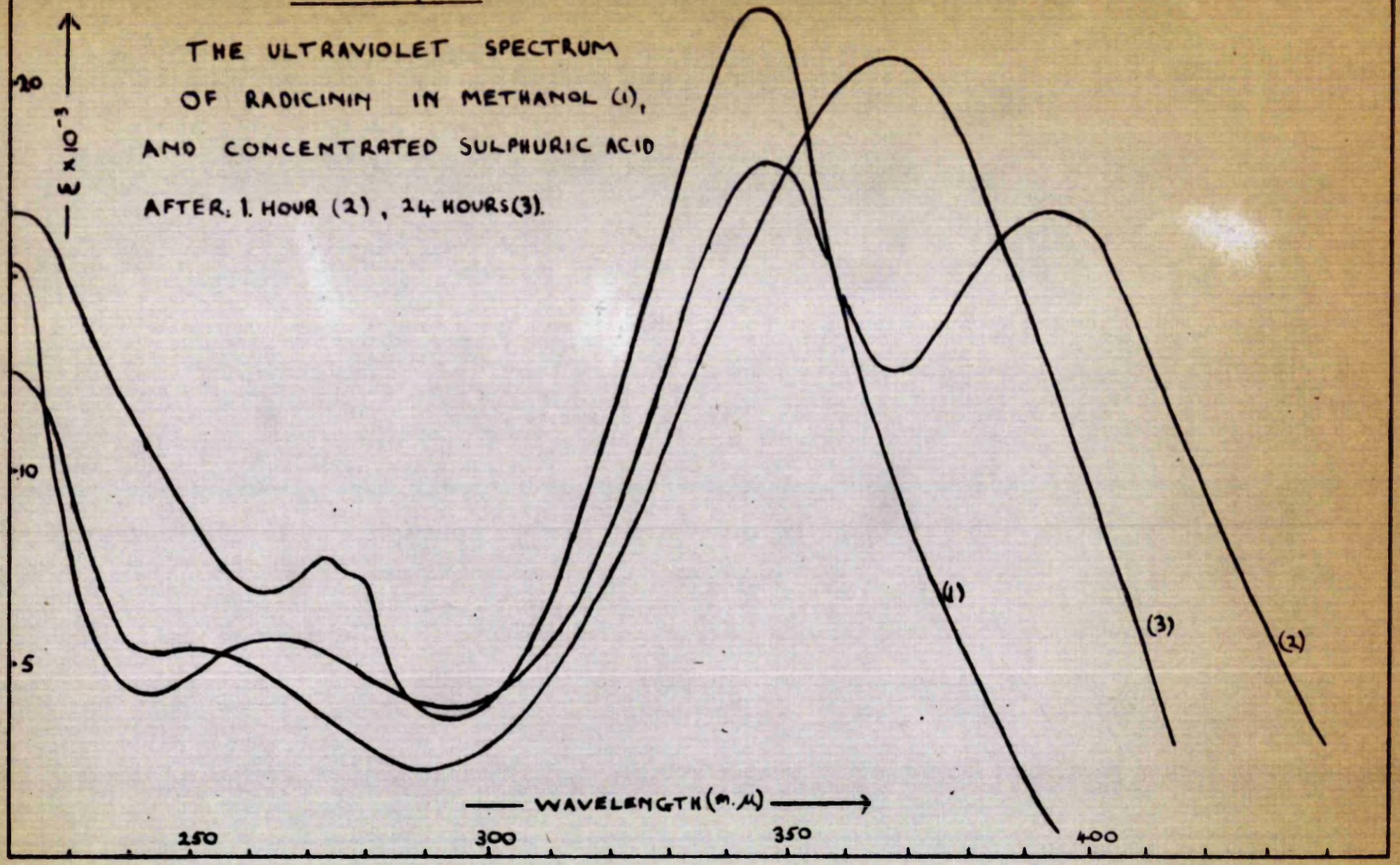
All four possess a conjugated diene chromophore, but whereas (LXVIII) and (LXIX) are β-ketocarboxylic acids, and in effect two absorbing systems, structures (LXX) and (LXXI) possess structures in which the ketonic group and the carboxylic acid group are linked through a conjugated diene system. The latter two structures would therefore possess a single ultraviolet band of high absorption and wavelength (e.g. muconic acid shows a band at 266 m.μ, $K = 60 \times 10^3$), whereas the former structures would show either two very closely situated absorption bands, or a doublet, too closely situated to be resolved into its bands.

In an attempt to resolve any multiple absorption bands in the spectra of radicinin and its derivatives, a series of ultraviolet absorption spectra were measured in concentrated sulphuric acid solution. Prior to this it had been shown that radicinin could be dissolved in concentrated sulphuric acid and recovered after short periods. The spectra obtained could be divided into two distinct types.

On the one hand the main absorption band, in the ultraviolet absorption spectrum of radicinin and the acid A, was split into two absorptions of higher wave-

FIGURE, 11.

THE ULTRAVIOLET SPECTRUM OF RADICININ IN METHANOL (1), AND CONCENTRATED SULPHURIC ACID AFTER: 1. HOUR (2), 24 HOURS (3).



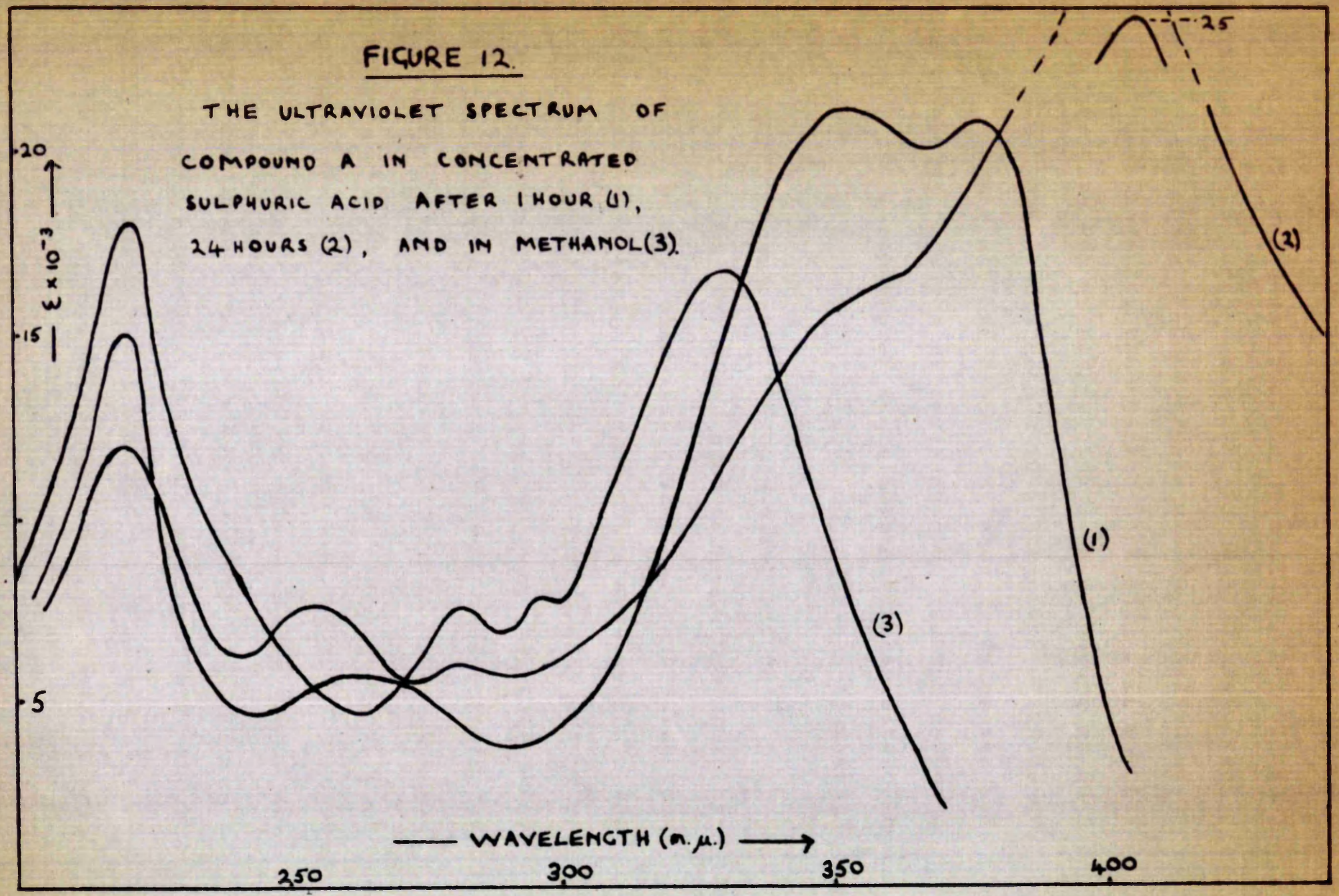


FIGURE 12.
THE ULTRAVIOLET SPECTRUM OF COMPOUND A IN CONCENTRATED SULPHURIC ACID AFTER 1 HOUR (1), 24 HOURS (2), AND IN METHANOL (3).
20
15
5
ε x 10⁻³
·25
(1)
(2)
(3)
WAVELENGTH (m.μ.)
250
300
350
400

length. Thus, for radicinin two bands appeared in its ultraviolet spectrum at 395 mμ. and 374 mμ., corresponding to bathochromic shifts of 51 mμ. and 3 mμ. respectively (Figure 11). The same pattern was observed for compound A which showed bathochromic shifts in its ultraviolet spectrum to 350 mμ. and 380 mμ. ($\Delta\lambda$ = 6 mμ. and 36 mμ. respectively) (Figure 12). Both radicinin and compound A showed further changes after 24 hours in concentrated sulphuric acid, presumably due to decomposition (See Figures 11 and 12).

On the other hand, the spectra of dihydroradicinin and compound B in concentrated sulphuric acid, were split into two absorption bands, one of higher and one of lower wavelength than that observed in methanol. As in the case of the spectra of radicinin and compound A, the shifts were more pronounced in the spectra of the parent compound. Thus dihydroradicinin in concentrated sulphuric acid had two ultraviolet absorption maxima, one at 283 mμ(ε = 17.2 x 10^3), and another at 332 mμ(ε = 4.3 x 10^3), see Figure 13., whereas the ultraviolet spectrum of compound B showed shifts to 282 mμ.(ε = 12.0 x 10^3) and 325 mμ (ε = 4.7 x 10^3), see Figure 14. In marked contrast to the spectra of radicinin and compound A, those of dihydroradicinin and compound B in concentrated sulphuric acid remained unchanged after 24 hours.

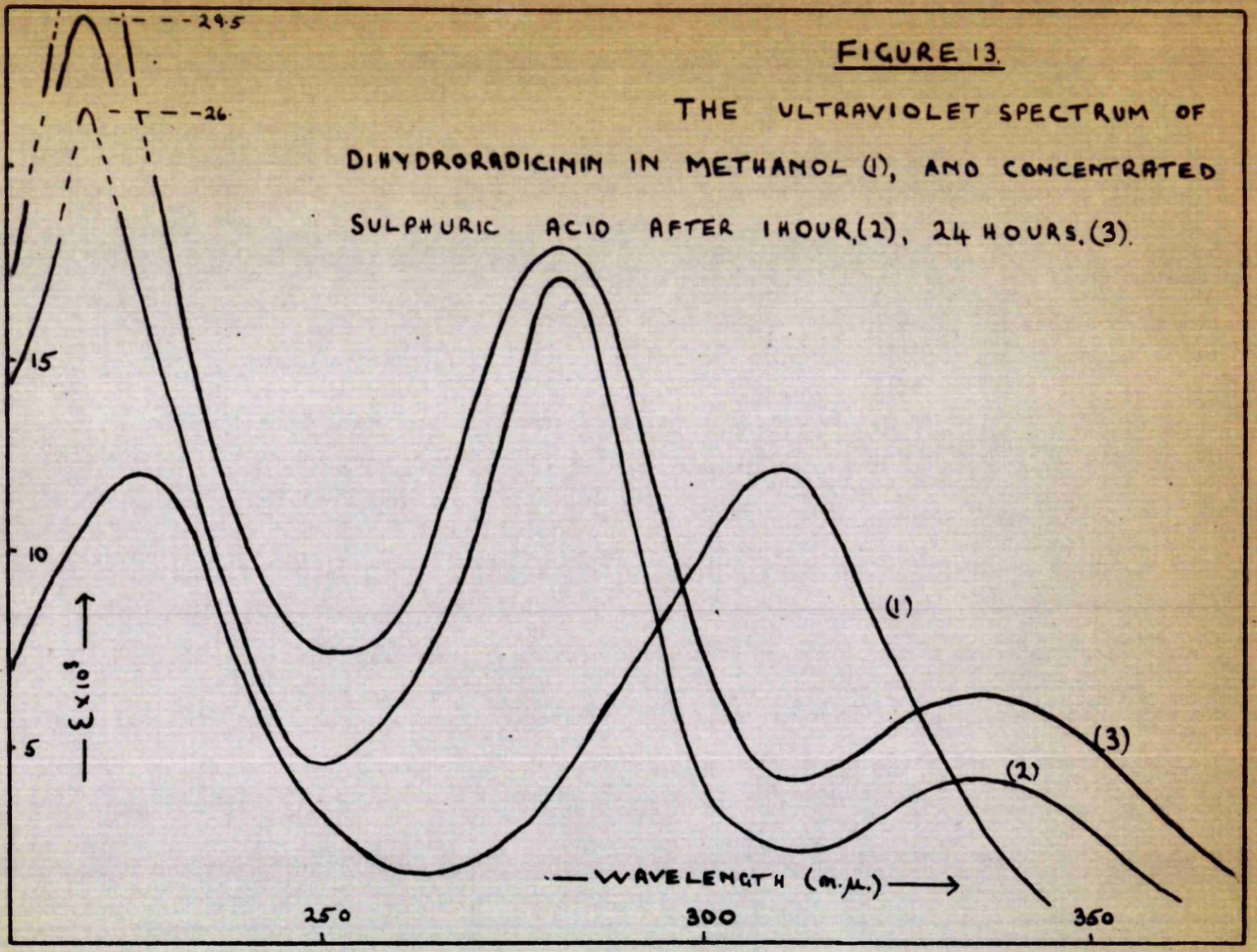


FIGURE 13.

THE ULTRAVIOLET SPECTRUM OF DIHYDRORADICININ IN METHANOL (1), AND CONCENTRATED SULPHURIC ACID AFTER 1 HOUR (2), 24 HOURS (3).

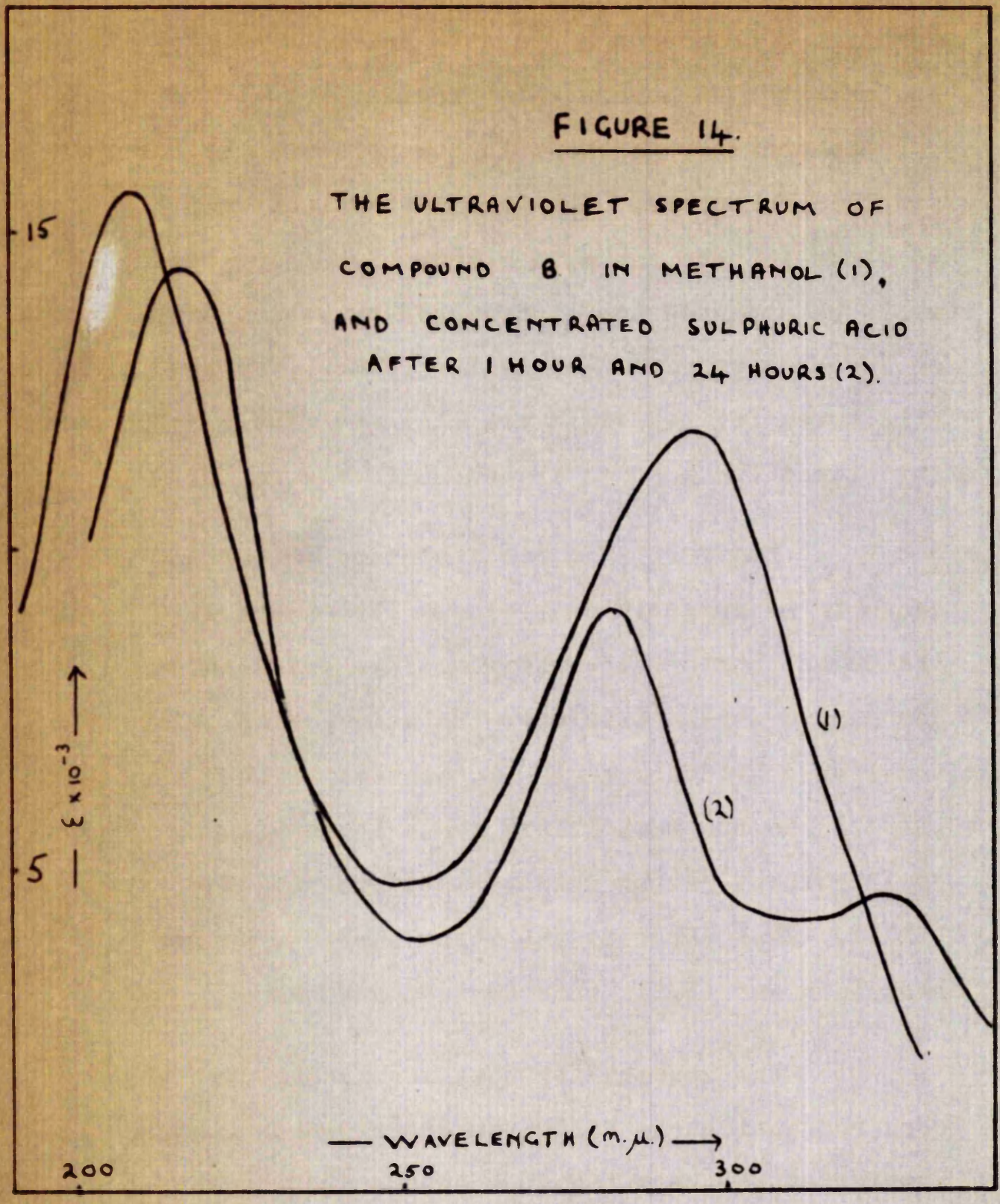


FIGURE 14.
THE ULTRAVIOLET SPECTRUM OF COMPOUND B IN METHANOL (1), AND CONCENTRATED SULPHURIC ACID AFTER 1 HOUR AND 24 HOURS (2).
15
5
$\varepsilon \times 10^{-3}$
(1)
(2)
WAVELENGTH (m.μ.)
200
250
300

The fact that the ultraviolet absorption spectrum of compound B could be split by protonation in concentrated sulphuric acid, indicated that two similar chromophores were present in the molecule, rather than a single chromophore. This eliminated (LXX) and (LXXI) for B, since in both, the main ultraviolet absorption band would be expected to be unchanged by protonation. Hence on spectroscopic and chemical evidence, the most likely structures for radicinin are (XL) and (XLVI). The possible structures for A and B are shown in Figure 15.

The fact that radicinin and compound A were shown to be unstable on prolonged treatment with concentrated sulphuric acid, whereas dihydroradicinin and compound B were stable over the same periods, may be considered evidence that, of the possibilities for B, (LXVIII) is the most likely since it contains a saturated cyclic ether, rather than a vinyl ether system, as in (LXIX). Thus (XL) is the most likely structure for radicinin and (LVII) that for compound A.

The possibility that a rearrangement had occurred during the formation of compounds A and B was considered unlikely, in view of the close spectral similarities between radicinin, dihydroradicinin and compounds A and B, and also because the properties of

The alternative structures for radicinin and derivatives

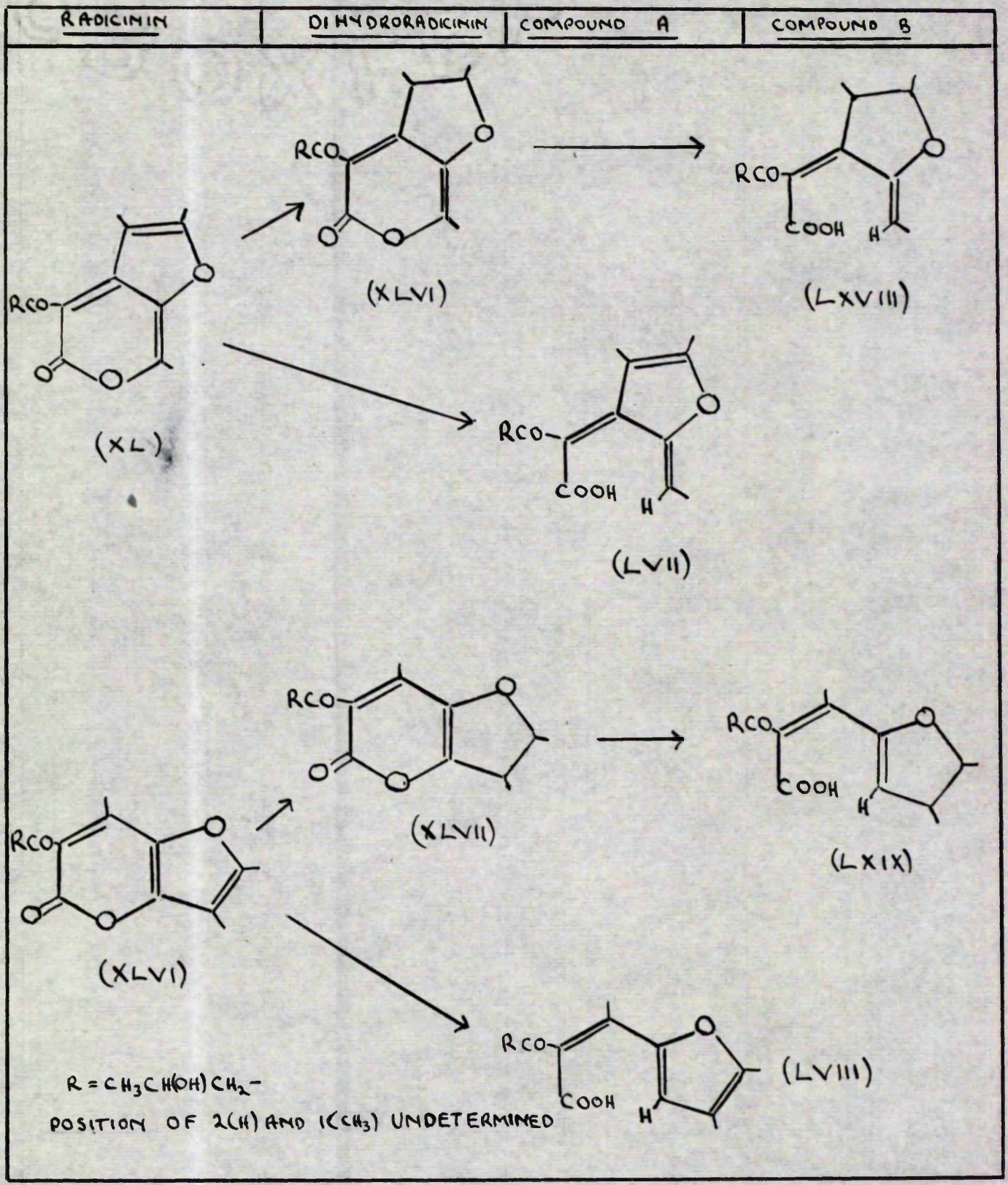
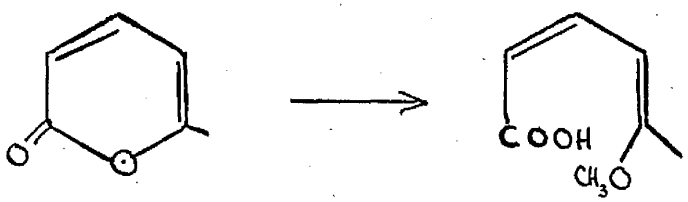


Figure 15

compounds A and B could be explained in terms of structures directly derived from the parent compounds. It was also clear that in the conversion of radicinin to A, no carbon-to-carbon double bond was saturated by a process similar to that observed in the reduction of α, and β-angelica lactones (page 85), since the spectroscopic evidence precluded any change in the chromophoric systems.

At this juncture, it is of interest to re-examine the spectroscopic data reported for the methyl ether of radicinin prepared by Clarke and Nord, even though the preparation of this compound could not be repeated. This compound showed a band in the ultraviolet spectrum at 334 mμ (ϵ = 14.1 x 10^3). In view of the fact that the hydroxyl group has been shown to be isolated from the chromophoric systems of radicinin, the methyl ether would be expected to have an ultraviolet absorption spectrum almost identical with that of radicinin, as was found for the acetate. The hypsochromic shift observed in comparing the ultraviolet spectra of radicinin and the methyl ether therefore, is not compatible with structure I. This shift is however almost identical with that observed on comparing the ultraviolet spectra of radicinin and compound A, and therefore it is considered that the 2-pyrone formulation for radicinin

better explains the properties of the methyl ether, since it is possible that on methylation, the 2 pyrone ring undergoes hydrolytic cleavage and methylation as shown below:



The change in the ultraviolet spectrum would then be due only to ring cleavage. This argument is substantiated by the observation that the only method by which a methylation product could be obtained was by prior treatment with alkali. It must be emphasised that these conclusions are only tentative, in view of the inability to reproduce Clarke and Nord's experiment.

2. <u>The infrared spectra of radicinin and its derivatives</u>

Infrared data have already been discussed, where relevant, in the above work, however the following points are worthy of further consideration.

Jones and his colleagues have recently studied the infrared absorption spectra of a number of unsaturated

lactones, including some 2-pyrone derivatives (41). It was found that all the lactones studied, in which the carbonyl group was conjugated to a carbon-to-carbon double bond, exhibited a doublet absorption band in the region 5.6 to 6.1 μ . The values obtained by Jones and his co-workers for certain compounds of interest in the present work are quoted in table 2 . Thus, the very broad, high intensity band, observed in the infrared spectrum of radicinin at 5.7 μ , might well be a multiple peak, which could not be resolved on the instruments available.

One piece of evidence was, in fact, obtained which indicated that the infrared absorption band at 5.7 μ of radicinin was a doublet. In attempts to obtain infrared spectra of radicinin and dihydroradicinin 2,4-dinitrophenylhydrazones, which are not very transparent to infrared radiation, the ordinate scales of the spectra were expanded so that accurate wavelength readings could be obtained, in so doing, the high intensity absorption band in the 5.7 μ to 5.8 μ region was split into a closely situated doublet (5.82 μ and 5.87 μ).

Further, the infrared spectrum of fulvoplumierine (L) exhibits a doublet at 5.8 and 5.9 μ , and another absorption at *ca.* 6.1 μ (38), suggesting

Carbonyl absorptions of some unsaturated lactones

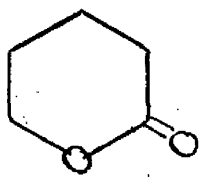
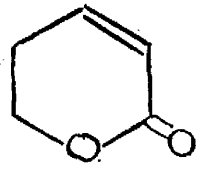
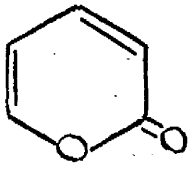
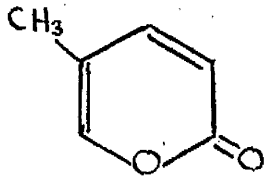
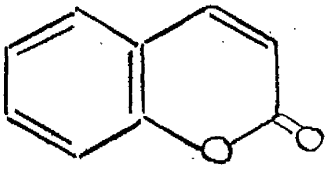
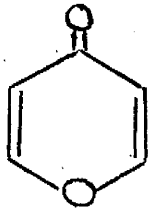
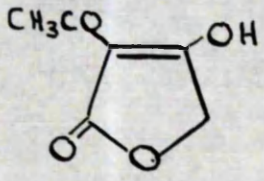
Compound	Doublet absorption	Others
	-	5.78(w)
	-	5.78(m)
	5.75(s) 5.83(s)	5.6(w) 6.1(w) 6.15(w)
	5.73(w) 5.83(w)	6.07(w) 6.1(w) 6.48(m)
	5.68(w) 5.78(s)	-
	5.94 6.03	-
All spectra in chloroform solution		

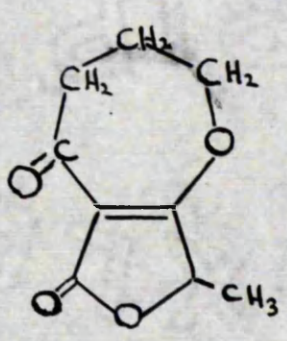
Table 2

that an acyl substituted 2-pyrone would show typical infrared absorption at 5.8$\mu$and 6.1 μ., which lends further support to the structure assigned to radicinin.

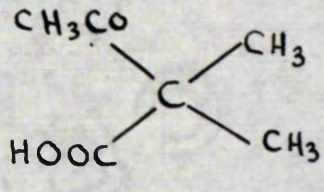
The structures suggested for compounds A and B both contain a non-enolisable β-ketoacid system. Similar systems are encountered in α,α-dimethyl-acetoacetate (LXXII) (22), α-acetyltetronic acid (LXXIII), terrestricacid (LXXIV) and carolinic acid (LXXV) (42), and a comparison of the infrared absorption of these compounds in the region 3-6.5μ is useful, see table 3. It will be seen that the values bear close similarities to those for compounds A and B and thus support the structures proposed for these compounds.



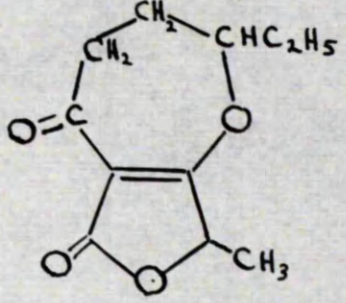
(LXXIII)



(LXXIV)



(LXXII)



(LXXV)

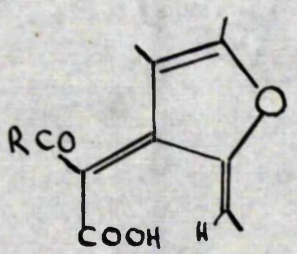
The infrared spectra of some non-enolisable β - ketoacid systems in the region 3-6.5 μ.

Compound	Infrared bands (μ.)	
	Solid	Chloroform solution
LXXII	5.83 5.76	
LXXIII	3.25 5.68 5.96 6.25	 5.64 5.86 5.96 6.21
LXXIV LXXV	5.73 5.84 6.2	5.68 5.84 6.2
Compound A	5.67 5.91	5.7 5.8
Compound B	5.73 5.93	

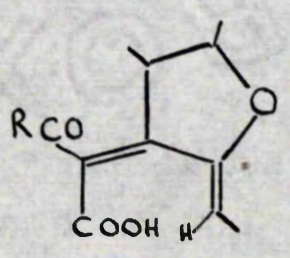
Table 3

Further evidence for the formulation of A as a β -ketoacid may be derived by a consideration of the infrared spectrum of its 2,4-dinitrophenylhydrazone. This possesses absorption bands at 3.1 μ. (probably hydroxyl hydrogen-bonded with the carboxylic acid group), and 6.0 μ , the remainder of the infrared region being masked by the bands from the 2,4-dinitrophenylhydrazine moiety. The shift of the carbonyl absorption to 6.0 μ is as would be expected for an α,β -unsaturated acid.

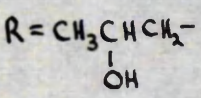
The infrared spectra of radicinin and dihydroradicinin have already been compared with similar data for furanose systems (page 99), and it is useful now to compare compounds A and B with these systems.



A



B



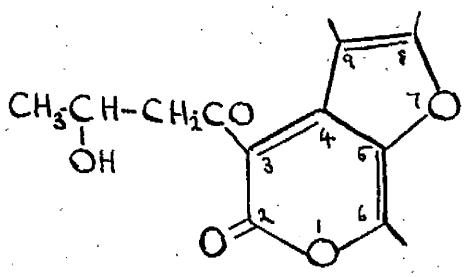
B may be considered to be a substituted tetrahydrofuran, the exocyclic double bond constituting part of a vinyl ether system, as shown by the infrared absorption band at 6.23 μ . A, on the other hand is the corresponding

dihydrofuran derivative, containing a divinyl ether system, and shows a doublet at 6.26 and 6.3 μ in the infrared, which may be assigned to two differently substituted double bonds. The infrared spectra of A and B also show differences in the 7.8 μ to 8.4 μ region, on comparison with the spectrum of radicinin, due to changes in the vinyl ether systems.

It was considered that the above spectroscopic data for A and B were consistent with the structures proposed and further, the similarity of these spectra to those of radicinin and dihydroradicinin was to be expected, since all four compounds possess essentially the same functions.

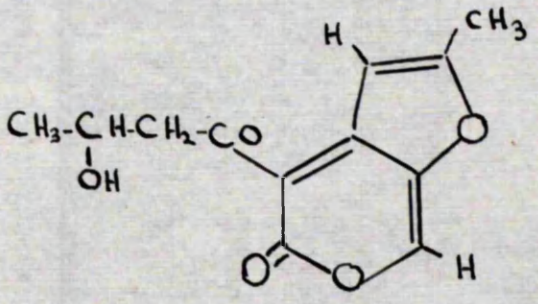
Conclusion

It has been established, from the information available, that the most probable structure for radicinin is (XL), where only the position of the methyl group remains to be determined.



(XL)

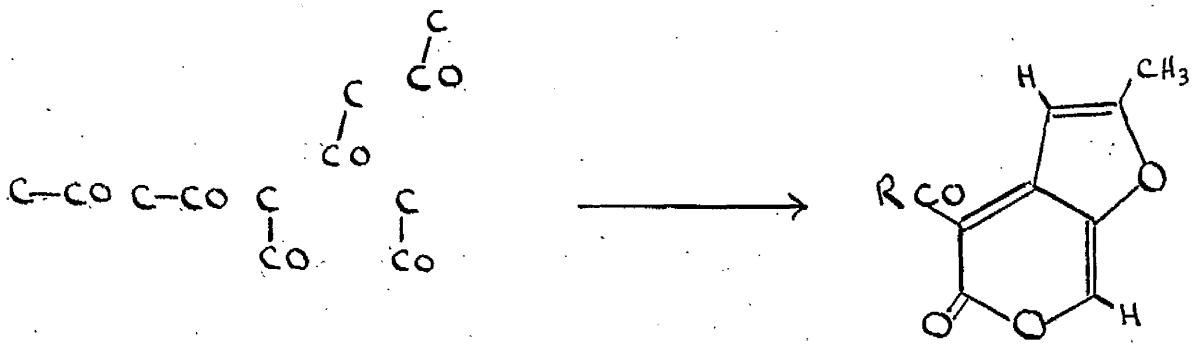
The three possible positions for this group are on C_6 C_8 or C_9. It was reported however (page 68), that alkaline hydrolysis of radicinin gave a volatile acid, which has recently been shown to be acetic acid. Of these possibilities, it is considered that the formation of acetic acid is best accounted for, if the methyl group is on C_8, and thus radicinin is considered to have structure (LXXVI).



(LXXVI).

Finally, having arrived at (LXXVI) by chemical and physical methods, additional evidence may be adduced by a consideration of the possible biosynthetic routes to such a structure.

If radicinin is considered to be acetate derived, then (LXXVI) may be built up by head-to-tail condensation of six acetate units as shown:



(LXXVI).

It is notable that a similar scheme can be drawn for the possible structure in which the methyl group occupies position 6, but not if the methyl group is attached to position 9.

These conclusions on the structure of radicinin are in agreement with the finding of Clarke and Nord, that radicinin was synthesised, in Stemphylium radicinin, from intact carbohydrate molecules, since, as was previously discussed (page 31), it is known that pyrones are synthesised in nature by utilisation of carbohydrates without breakage of the carbon skeleton.

The mass spectrum of radicinin

Since the completion of this discussion, the following results have been obtained during attempts to determine the fragmentation pattern of radicinin in a mass spectrometer.

At 300°, the only significant ions detected were due

to water and carbon dioxide. It would therefore appear that radicinin is thermally unstable under the conditions required to obtain a mass spectrum.

Other peaks appeared at mass 41, mass 43, mass 69, mass 84. Of these the peak at mass 43 was a doublet which was thought to be due to the species C_3H_7 and C_2H_3O, and that at mass 69 was thought to be a mass deficient ion probably C_4N_5O, while the other peaks could not be evaluated.[1]

These ions are all present in the spectra of methyl propenylketones however, and it is considered that these data support the proposed structure for radicinin, since under the conditions required to obtain a mass spectrum, the side chain of radicinin would undergo loss of water to produce a propenylketone.

PART II

EXPERIMENTAL

Isolation of radicinin

From an authentic culture of *Stemphylium radicinum* (Sterad) (I.M.I.63223), potato dextrose agar flats were inoculated, and incubated at 30°. After three days the mycelium was a black felt and the organism was considered mature after six days further incubation. To each flat was added, as required, sterile distilled water (30 mls.), and the mycelial spores were displaced with a sterile platinum wire. The spore suspension thus obtained was used immediately.

Penicillin flasks containing 500 mls. of a sterile solution of potato extract (4 g./litre) and dextrose (20 g./litre) were inoculated with 1-2 mls. of the spore suspension, by use of a sterile graduated pipette fitted with a sterile rubber bulb. The neck of each culture flask was flamed before and after inoculation.

After being incubated for three weeks at 30°, the mature mycelial felts were harvested, and the pale yellow substrate filtered through layers of cotton wool. It was found in several large scale cultures that longer periods of incubation led to diminished yields of radicinin.

The culture filtrate (70 litres) was concentrated to 7 litres by evaporation in a climbing film evaporator,

acidified to litmus (dilute hydrochloric acid), and exhaustively extracted with chloroform by shaking in a large separating funnel. The aqueous solution was then saturated with salt, and extracted with chloroform over an extended period in a large liquid-liquid extractor. Extraction with ether proved to be a more lengthy process, as radicinin was not very soluble in this solvent, the material obtained however was purer than that obtained by chloroform extraction.

Evaporation of the combined chloroform (10 litres) or ether extracts gave a crude, bright yellow product (c.a. 19 g.), which after four recrystallisations from methanol gave a pale yellow solid of melting point 214-215° (Clarke and Nord reported 220° (dec.)).

The mycelial mats were dried in a current of air at 30° and ground to a fine powder (220 g). Extraction with light petroleum (b.p. 40-60°), in a Soxhlet extractor yielded a fat fraction (10 g.), extraction with ether yielded small quantities of radicinin (0.5 g.), and subsequent extraction with chloroform, acetone and alcohol respectively, gave small amounts of a brown oil which were not further investigated.

Sublimation of the recrystallised sample of

radicinin at 140° (1 mm) yielded pale yellow needles, m. pt. 215°. Radicinin could also be purified by chromatography on a column of silicic acid by elution with chloroform. Recrystallisation of the product thus obtained gave radicinin of m.pt. 214-215°.

Purity of the material isolated

Fractional sublimation at 1 mm pressure over a temperature range of 100 to 200° gave a series of fractions of melting point 214-215°. No evidence was found for the presence of other compounds.

Chromatography on silica gel films, using chloroform 0.5% methanol as eluent, gave a single spot, R_F = 0.38 on spraying with concentrated sulphuric acid and subsequent baking in an oven at 100°.

Descending chromatography on Whatman No.1 paper, using a butanol:acetic acid:water mixture (4:1:5) and development with 2.N sodium hydroxide gave a single spot, R_F = 0.85.

General properties of radicinin

Pure radicinin obtained as described above, m.pt. 214-215°. Found on sample dried at 80° in a vacuum: C, 60.53; H, 5.13; C-methyl 11.43; N,0; O-methyl, 0%. mol. wt., 261 (Rast). $C_{12}H_{12}O_5$ requires C, 61.01; H, 5.12;

N, O; C-methyl, 12.7; O-methyl, 0%. mol.wt., 236.

A solution of radicinin in methanol showed the following ultraviolet absorption maxima, max 344 mμ., 279 mμ, 271 mμ, 220 mμ, ($\varepsilon \times 10^{-3}$ = 22.0, 6.8, 7.7 and 16.6 respectively), see Figure 3 (page 65). The infrared absorption curve obtained for radicinin is shown in Figure 1 (page 62).

Radicinin was insoluble in water, sparingly soluble in ether, alcohol, methanol, dioxan, ethyl acetate and glacial acetic acid; slightly more soluble in chloroform, and readily soluble in dimethyl sulphoxide. The pigment was insoluble in sodium bicarbonate and sodium carbonate solutions, but rapidly dissolved in cold dilute (2N) sodium hydroxide solution, to give a blood red solution. On acidification, this solution became colourless, but no material precipitated out. Extraction of the neutral solution with chloroform gave a yellow oil which resisted all attempts at purification.

Preliminary chemical tests confirmed that radicinin gave no colour with alcoholic ferric chloride solution. A solution of radicinin in alcohol, when treated with Brady's reagent, slowly gave an orange precipitate. Radicinin did not reduce Fehling's solution or ammoniacal silver nitrate solution, but on treatment of a solution of

radicinin in dioxan/water (1:1) with alkali and potassium iodide-iodine reagent, a yellow precipitate of iodoform was obtained, m.p. 120°, confirmed by mixed melting point with an authentic sample of iodoform.

A solution of radicinin in ethanol showed a specific rotation, $[\alpha]_D^{20}$, of -166° (Clarke and Nord reported $[\alpha]_D^{27} = -175.7°$).

Acetylation of radicinin

1. Radicinin acetate was obtained by the method of Clarke and Nord (2), m.p., 197° (reported m.p. 197°).

Found: C, 60.1; H,5.1; $C_{14}H_{14}O_6$ requires C, 60.43; H, 5.14%.

Radicinin monoacetate in methanol solution, showed ultraviolet absorption bands at 344mμ ($\varepsilon = 21.3 \times 10^3$) 278mμ ($\varepsilon = 5.7 \times 10^3$), 269mμ ($\varepsilon = 6.85 \times 10^3$) and 220mμ ($\varepsilon = 16.1 \times 10^3$).

The infrared spectrum in chloroform solution possessed the following bands: 3.35μ (m.), 5.7μ (s.), 6.07μ (m.), 6.25μ (s.), 6.55μ (s.), 6.92μ (shoulder), 7.0μ (s.), 7.23μ and 7.3μ doublet, 7.66μ (w.), 7.96μ to 8.4μ (broad absorption band), 8.61μ (m.), 9.1μ (w.), 9.37μ (s.), 9.7μ (w.).

On chromatography on Whatman No. 1 paper, using butanol:acetic acid:water (4:1:5) as solvent, radicinin acetate had an R_F = 0.95, detected by spraying with 2% sodium hydroxide solution.

2. More vigorous conditions for acetylation, as recommended by Tsuda and Uyeo (7), were also used:

To a solution of radicinin (0.1 gm) in acetic anhydride (3 mls.) was added, in three separate experiments, concentrated sulphuric acid ((i) 3 drops, (ii) 0.5 ml., (iii) 1.0 ml). The solutions were allowed to stand for 48 hours and then poured into iced water. The tan precipitates were filtered off, washed with cold water, dried <u>in vacuo</u>, and crystallised from ethyl alcohol. Each of the products melted between 195 and 197°, possessed identical infrared spectra to that of radicinin monoacetate, and by paper chromatography (Whatman No. 1, butanol:acetic acid: water, 4:1:5), were shown to be identical with radicinin monoacetate (R_F = 0.95). The filtrates were neutralised with sodium bicarbonate solution (5N) and extracted with chloroform. The chloroform solutions were dried (Na_2SO_4) and evaporated to give brown gums which could not be purified. The yield of gum increased from (i) to (iii).

<u>Attempted methylation of radicinin</u>

1. The method reported by Clarke and Nord (2) was repeated

a number of times, but on each occasion starting material was recovered, identified by mixed melting point, infrared spectra and chromatography.

2. Radicinin (0.1 g.) was dissolved in dry acetone (10 mls.) and heated under reflux for 24 hours in the presence of anhydrous potassium carbonate (0.2 g.) and dimethyl sulphate ((i) 0.4 ml., (ii) 1.0 ml.), the apparatus being protected from moisture by a drying tube. The reaction mixture was cooled and filtered, and the pale pink solid obtained by evaporation of the solvent was crystallised from ethyl alcohol. It was shown by melting point, mixed melting point and chromatography to be radicinin.

3. Radicinin (0.1 g.), methyl iodide (1 ml.) and anhydrous potassium carbonate (0.2 g.) were heated under reflux in dry acetone (18 ml.) for 6 hours. The solution was cooled, filtered and the solvent evaporated. The product was washed with water and crystallised from ethanol to give a product, m.p. 213-215°, which was shown by mixed melting point and chromatography to be radicinin. In another experiment, the time of reflux was increased to 14 hours, with the same result.

4. To a solution of radicinin (0.2 g.) in a chloroform-ether mixture (1:1, 20 mls.) was added a solution of

diazomethane in ether (5%, 5 ml). The mixture was allowed to stand in an ice bath for 4 hours, and then at room temperature for 12 hours. Evaporation of the solvent gave a bright red gum. Attempts to purify this compound by chromatography on a column of silicic acid were unsuccessful.

5. To a solution of radicinin (0.1 g.) in sodium hydroxide solution (4%, 1 ml.) was added dimethyl sulphate (0.5 ml.), and the mixture was shaken for 5 minutes. Water (1 ml.) was added and the resulting solution warmed at 60°, until the red colour was discharged, and then allowed to cool. The solution was extracted with chloroform, and the chloroform extract was evaporated to give a brown oil, which was treated with dilute ammonia (5 ml.) at 60°. The alkaline solution was acidified with 2N. sulphuric acid and extracted with chloroform. Removal of the chloroform gave a brown oil, which on trituration with ether gave an orange-brown solid, m.p. 130-134°. This product was chromatographed on a thin film of silica gel (eluent, chloroform/0.5% methanol), which on spraying with concentrated sulphuric acid and baking at 120° gave two spots, R_F 0.28 and 0.38. The latter was shown to be radicinin by comparison with an authentic sample. Attempts to separate this mixture by sublimation or chromatography on silicic acid columns were not successful.

Dibromoradicinin

Was prepared and purified by the method of Clarke and Nord (2), m.p., 149-150° (Clarke and Nord reported m.p., 151-152°).

Found, C. 36.3; H, 2.7; Br, 41.6 %; $C_{12}H_{12}O_5Br_2$ requires C, 36.39; H, 3.05; Br, 40.36%.

Dibromoradicinin in methanol solution showed ultraviolet absorption bands at 331mμ(ε = 13.5 x 10^3), 281mμ (ε = 4.2 x 10^3), 268mμ(ε = 4.4 x 10^3), 225mμ(ε = 14.6 x 10^3), figure 3, page 65.

The infrared spectrum of dibromoradicinin in chloroform solution showed maxima at 2.86μ (w.), 3.31μ (w.), 5.68μ (s.), 6.1μ (m.), 6.25μ (m.), 6.49μ and 6.52μ (doublet), 6.9μ(s.), 7.25μ(m.), 7.57μ(m.), 7.92μ to 8.35μ(broad band), 8.6μ and 8.67μ (w., doublet), 8.95μ (w.), 9.5μ (s.).

Radicinin hydrogen chloride

Was prepared as previously reported (2), m.pt 154-155° (Clarke and Nord reported 156°, dec.).

The hydrogen chloride adduct of radicinin in methanol solution showed ultraviolet absorption bands at 316mμ(ε = 14.6 x 10^3), 255mμ(inflection, ε = 2.5 x 10^3), and 288mμ(ε = 15.5 x 10^3), figure 3, page 65.

The infrared spectrum of radicinin hydrogen chloride

as a nujol mull showed absorption bands at 2.9μ (m.), 5.74μ (s.), 6.1μ (m.), 6.5μ (s.) 6.88μ (s.), 7.0μ (w.), 7.12μ (w.), 7.3μ (w.), 7.53μ (w.), 7.8 and 7.9μ (m., doublet), 8.25μ (m.), 8.48μ (m.), 8.62μ (w.), 8.95 and 9.03μ (m., doublet), 9.35μ (m.).

Attempted bromination of dihydroradicinin

To a solution of dihydroradicinin (0.1 g.) in chloroform (5 ml), was added a solution of bromine in chloroform (1.25%, 2 ml.). The mixture was allowed to stand for twelve hours, then washedwith sodium bicarbonate solution (4%, 5 ml.) and dried over anhydrous magnesium sulphate. Evaporation of the solvent and crystallisation of the residue from ethanol gave unchanged dihydroradicinin, confirmed by mixed melting point with an authentic sample of dihydroradicinin.

Hydrolysis of radicinin

1. Radicinin (0.2 g.) was dissolved in 0.1N. sodium hydroxide solution (20 mls.) and allowed to stand at room temperature. Aliquots (5 ml.) were taken after 2, 3, 4 and 24 hours and titrated potentiometrically against standard hydrochloric acid. It was found that under these conditions, even after 24 hours, radicinin consumed only 1 mole of alkali.

2. Radicinin (0.1 g.) was heated under reflux with

potassium hydroxide solution (0.158N, 15 mls.) for 5 hours in a stream of nitrogen. The mixture was cooled and titrated against standard hydrochloric acid as described above, when it was shown that two moles of alkali had been consumed.

3. In a separate experiment, radicinin (0.1 g.) was heated under reflux with potassium hydroxide solution (1%, 30 ml.) in a stream of nitrogen. The effluent gases were passed through a solution of 2,4-dinitrophenylhydrazine hydrochloride. After 30 minutes, an orange precipitate started to form, and after 5 hours no further precipitate was formed. After filtering, the crude product (30 mg., 10%) was crystallised from methanol to give orange needles, m.p. 159-160°, unaltered on admixture with acetaldehyde-2,4-dinitrophenylhydrazone.

The alkaline hydrolysis solution was cooled, and acidified (2N. sulphuric acid). The carbon dioxide evolved was collected in barium hydroxide solution. When the evolution of carbon dioxide had ceased, the precipitated barium carbonate was filtered off, and dried. The weight of barium carbonate produced was equivalent to 0.4 moles of carbon dioxide.

Steam distillation of the remaining acidified solution produced a volatile acid (0.8 mole per mole of

radicinin as estimated by titration).

4. Radicinin (0.1 g.) was heated with potassium hydroxide solution (1%, 30 mls.) for 5 hours. The solution was cooled, acidified with dilute sulphuric acid and extracted with chloroform. Evaporation of the chloroform solution gave a yellow oil which could not be crystallised or purified by chromatography. This oil gave a positive iodoform test, but as this may have been given by aldol products, it is not a significant result.

Hydrolysis of the dihydro, dibromo and hydrogen chloride adducts of radicinin

1. Under the conditions described in experiments 1 and 2 above, these adducts were found to consume one mole of alkali in the cold and two on heating.

2. Acetaldehyde (0.4 mole) and carbon dioxide (1 mole) were produced from the adducts when treated according to the conditions in 3 (above). No other identifiable product could be isolated.

Oxidation of dibromoradicinin

1. To a suspension of dibromoradicinin (0.32 g.) in potassium permanganate solution (1.2 g. in 24 mls. water) was added a solution of sodium hydroxide (1%, 3 mls.), and the mixture heated under reflux in a stream of nitrogen

until the purple colour had been discharged (2 hours). The effluent gases were passed through barium hydroxide and 2,4-dinitrophenylhydrazine hydrochloride solutions. The solution was then cooled, carefully acidified, and the evolved carbon dioxide collected in barium hydroxide solution.

All precipitates were collected, washed and weighed, from which it was found that 4 moles of carbon dioxide were evolved during heating and a further 2 moles on acidification. The 2,4-dinitrophenylhydrazone (0.6 mole) was shown by melting point and mixed melting point, to be acetaldehyde-2,4-dinitrophenylhydrazone (quantities with respect to 1 mole of starting material).

The residual solution was clarified by addition of 10% aqueous sodium thiosulphate solution, and extracted with ether, benzene and chloroform respectively. Evaporation of the organic solutions to dryness gave small amounts of brown oils which could not be purified. The aqueous layer was evaporated to dryness in vacuo at 30° , and the residue extracted with ethyl acetate, evaporation of the dried (K_2CO_3) solution gave a small yield of brown oil.

Attempts to prepare acid derivatives of these oils were unsuccessful. Thin layer chromatography on silica gel showed the presence of at least four components with R_F

values 0.97, 0.08, 0.05, 0.2 respectively. A sample of α,β-dibromobutyric acid prepared by bromination of trans-crotonic acid (3), when chromatographed under the same conditions, gave an R_F value of 0.46.

2. Dibromoradicinin (0.3 g., 1 mole) was allowed to react with potassium permanganate (0.067 g., 1 mole) in the presence of 0.1% sodium hydroxide (1.5 ml.) as described above. The product obtained was a brown oil (0.2 g.) which could not be purified. Silica gel chromatography showed this product to contain no α,β-dibromobutyric acid, components being detected with R_F values of 0.8, 0.2 and 0.04 respectively.

3. Dibromoradicinin (0.1 g.) was dissolved in acetone (20 ml.) and potassium permanganate (0.04 g., .0003 mole) was added over 1 hour, with stirring. The mixture was allowed to stand for two hours, and the solvent was removed in vacuo to give a yellow solid. The product isolated by extraction with chloroform and crystallised from ethyl alcohol was shown to be unchanged dibromoradicinin.

4. Oxidation of fully reduced dibromoradicinin

a. Preparation of fully reduced dibromoradicinin

Dibromoradicinin (0.1 g.) was shaken in methanol solution (50 ml), in the presence of platinum oxide (0.013 g.) in an atmosphere of hydrogen. Absorption was complete after

two hours, when 12.0 mls. (2 moles) of hydrogen had been absorbed. After removal of the catalyst by filtration, and evaporation of the solvent, a reddish oil was obtained (0·09 g.). This product reacted slowly with aqueous silver nitrate solution at room temperature.

b. Oxidation of the reduction product

Oxidation of tetrahydro-dibromoradicinin (90 mg.) with potassium permanganate was carried out as described above (1). The products obtained by extraction of the reaction mixture with ether, benzene, chloroform, and ethyl acetate respectively were oils which showed no recognisable fractions after chromatography on silicic acid. Comparison with α,β-dibromobutyric acid by thin layer chromatography on silica gel showed that α,β-dibromobutyric acid was absent from the oils.

Ozonolysis of radicinin

1. A stream of ozonised oxygen (1-2%) was passed through a solution of radicinin (0.2 g.) in ethyl acetate (150 ml.) at 0 . After 5 hours, pure oxygen was passed through the solution to sweep out unreacted ozone, 5% palladium-on-charcoal (0.05 g.) was added, and the mixture shaken in an atmosphere of hydrogen. In several experiments the volume of hydrogen absorbed was variable, but on completion of reduction, the solution was filtered and the ethyl acetate

distilled off carefully. Each successive portion (5 mls.) of solvent removed was tested with a solution of 2,4-dinitrophenylhydrazine hydrochloride in phosphoric acid-ethanol mixture. No precipitate was obtained on standing or on concentration of the solutions.

The residues, after removal of the solvent from the reduced ozonides, were clear yellow oils (ca. 40 mg.), which could not be purified by chromatography. Attempts to isolate derivatives of acidic components were unsuccessful.

2. The ozonolysis was also carried out in chloroform solution. Catalytic reduction of the ozonide produced no recognisable products.

3. Ozonolysis of crotonic acid

Under identical conditions to (1) and (2) above, crotonic acid was ozonised and gave acetaldehyde which was identified by formation of its 2,4-dinitrophenylhydrazone (confirmed by m.pt. and mixed m.pt. with an authentic sample)

Permanganate-periodate oxidation of radicinin (10, 11)

1. To a solution of radicinin (0.1 g.) in aqueous ethanol (1:1, 50 mls.), were added potassium carbonate (0.05 g.), sodium periodate (0.09g.), and potassium permanganate (0.007 g.). The mixture was thoroughly shaken and allowed to stand for 24 hours at room temperature. The solution was then acidified

(2 ml. of 2N. sulphuric acid) and reduced to one quarter volume. The distillate gave a negative test for aldehyde. The residual solution yielded a yellow solid (0.09 g.), on extraction with chloroform, which on recrystallisation proved to be unreacted radicinin.

2. The above reaction was repeated with the following modifications; (a) aqueous dioxan (1:1) was used as solvent (b) a large excess of permanganate-periodate in aqueous dioxan (1:1) was used, and the mixture allowed to stand for 3 days. On both occasions radicinin was recovered in high yield.

Radicinin —2,4-dinitrophenylhydrazone

2,4-Dinitrophenylhydrazine hydrochloride (0.25 g.) was dissolved in hot alcohol (5 ml.) containing concentrated hydrochloric acid (0.5 ml.). To this warm solution, a solution of radicinin (0.125 g.) in ethyl alcohol (10 ml.) was added. The mixture was warmed for 5 minutes and allowed to cool. The resulting orange precipitate was filtered off, washed with (1) alcohol (2) water, and recrystallised from pyridine. Three recrystallisations gave orange needles, m.pt. 237-239° (dec.) (reported m.pt., 235-238°).

Found, C.51.6, H, 3.8%, calculated for $C_{18}H_{16}N_4O_8$, C, 51.93; H, 3.87%.

The infrared spectrum of radicinin-2,4-dinitrophenylhydrazone in nujol was obtained by ordinate scale expansion. Bands were obtained as follows: 5.82μ. and 5.87μ.(s., doublet), 6.07μ.(m.), 6.22μ.(m.), 6.3μ. and 6.35μ.(w., doublet).

The ultraviolet absorption spectrum of radicinin, 2-4-dinitrophenylhydrazone in ethanol solution showed absorption bands at 423m.μ.(ε = 22.3 x 10^3), 328m.μ.(inflexion) (ε = 5.1 x 10^3), and 237m.μ.(ε = 19.1 x 10^3), see Figure 5,pg.81.

2,4-Dinitrophenylhydrazone of radicinin acetate

This derivative was prepared as described above for radicinin-2,4-dinitrophenylhydrazone. Recrystallisation from pyridine gave orange needles, m.pt. 223-224°, which on admixture with radicinin-2,4-dinitrophenylhydrazone gave m.p 210-215°.

Found, C, 52.8; H, 4.4%; $C_{20}H_{18}N_4B_9$ requires C, 52.7; H, 4.18%.

Acetylation of radicinin-2,4-dinitrophenylhydrazine

Radicinin-2,4-dinitrophenylhydrazone (0.11 g.) was dissolved in a mixture of acetic anhydride (3.0 mls.) and concentrated sulphuric acid (2 drops). After allowing to stand for 1 hour, the solution was poured into iced water (10 mls.), and the resulting orange precipitate filtered off

and recrystallised from pyridine to give orange needles, m.pt. 223°; mixed melting point with radicinin-2,4-dinitrophenylhydrazone, 216-220°, admixture with the 2,4-dinitrophenylhydrazone of radicinin acetate gave no depression.

Dihydroradicinin-2,4-dinitrophenylhydrazone

Prepared by the method of Wilds and Nelson (16).

A solution of dihydroradicinin (0.21 g.) in ethanol (7 mls.), was added to a solution of 2,4-dinitrophenylhydrazine hydrochloride (0.5 g.) in ethanol (15 mls.) containing concentrated hydrochloric acid (1.4 mls.), and heated under reflux for 10 minutes. On allowing to cool, an orange precipitate formed, which, on recrystallisation from pyridine gave orange needles m.pt. 159-160°. Sample dried in vacuo at 80°C.

Found: C, 53.0; H, 4.7%; $C_{18}H_{18}O_8N_4$ requires C, 51.7%; H, 4.3%.

The ultraviolet spectrum of dihydroradicinin-2,4-dinitrophenylhydrazone in ethanol, showed absorption maxima at 399mμ (ε = 22.7 x 10^3) and 220mμ (ε = 17.8 x 10^3). The infrared spectrum in nujol showed bands at 5.85μ (s.), 6.11μ (w.), 6.24μ (s.), 6.35μ (s.).

Attempted acetolysis of radicinin

1. Radicinin (0.1 g.) was dissolved in a mixture of an acetic anhydride, glacial acetic acid and concentrated sulphuric acid (35:15:1.5 mls.), and allowed to stand at room temperature for 12 hours. The mixture was then poured into iced water, and the precipitate filtered off. A tan solid was obtained, which was shown by paper chromatography, using butanol:acetic acid:water mixture (4:1:5), to be identical with radicinin monoacetate. Chromatography on a column of silicic acid on elution with chloroform, gave a fraction, m.p. 194-196°, which was shown by a mixed melting point determination to be radicinin monoacetate. Elution with mixtures of chloroform and ethanol gave small quantities of brown gum which could not be purified.

2. Using the same quantities as above, the mixture was heated under reflux for one hour to give a dark brown coloured solution. This was cooled and poured into water to give a small quantity of a solid product, which was shown by chromatographic methods to be, essentially, radicinin monoacetate. The filtrate was neutralised with sodium carbonate solution (5N.) and extracted with chloroform. The brown oil obtained by evaporation of the dried ($MgSO_4$) solution resisted all attempts at purification.

Reaction of radicinin with methanolic hydrogen chloride

Radicinin (0.1 g.) was dissolved in methanolic

hydrogen chloride (3%, 15 ml.) and heated under reflux for one hour. The solvent was removed *in vacuo*, and the yellow solid crystallised from methanol to give white needles, m.p.,156°. This material was shown by mixed melting point and infrared spectra to be radicinin hydrochloride.

Attempted osazone formation

A solution of radicinin (0.1 g.), phenylhydrazine hydrochloride (0.2 g.) and sodium acetate (0.3 g.) in aqueous ethanol (1:1, 20 mls.) was heated under reflux for one hour. The mixture was allowed to stand at room temperature for 24 hours, and the solvent was then removed to give a red gum, which could not be induced to crystallise. Attempts to purify this material by chromatography were unsuccessful.

Catalytic reduction of radicinin

1. Radicinin (0.5 g.) was dissolved in methanol (170 mls.) and shaken in an atmosphere of hydrogen in the presence of 5% palladium-on-charcoal (0.01 g.). Absorption was complete after one hour, when 54.5 mls. (1 mole) of hydrogen had been absorbed. The solution was filtered and the solvent evaporated to give a pale pink solid, which after three recrystallisations from ethanol gave dihydroradicinin as white needles, m.p., 156°.

Found: C, 60.1 ; H, 5.1%. $C_{12}H_{14}O_5$ requires

C, 60.5; H, 5.88%.

The ultraviolet absorption spectrum of dihydroradicinin in methanol showed bands at 310m.μ ($\varepsilon = 12.4 \times 10^3$) and 226m.$\mu$ ($\varepsilon = 12.2 \times 10^3$), see Figure 3, page 65 . The infrared absorption spectrum of dihydroradicinin is shown in Figure 2, page 66 .

2. Radicinin, when reduced with hydrogen in the presence of platinum oxide, in methanol/acetic acid solution, according to the method of Clarke and Nord (2), absorbed 3.4 moles of hydrogen. The product obtained was an oil, which could not be crystallised and no derivatives could be prepared.

3. On shaking a solution of radicinin (0.1 g.) in methanol (50 ml.), with platinum oxide (0.014 g.), in an atmosphere of hydrogen at atmospheric pressure for one hour, 28.5 ml. of hydrogen were absorbed, corresponding to an uptake of 3 moles of hydrogen. The catalyst was filtered off and on removal of the solvent, a clear, yellow oil remained which could not be purified.

The ultraviolet spectrum of this oil in methanol showed a single broad absorption band at 284m.μ ($\varepsilon = 5.5 \times 10^3$) (Figure 4, page 80).

4. On shaking a solution of radicinin (0.1 g.) in glacial

acetic acid (50 mls.) with 5% palladium-on-charcoal (0.01 g.) for 24 hours, 33 mls. of hydrogen (approx 3.4 moles) were absorbed. Filtration and removal of the solvent <u>in vacuo</u> gave a clear oil which could not be purified by the techniques available. It was shown however to be different from the product obtained in (3) above since its ultraviolet spectrum possessed two bands, one at 260mμ ($\varepsilon = 3.9 \times 10^3$) and the other at 292m$\mu$ ($\varepsilon = 2.5 \times 10^3$) (Figure 4, page 80).

5. A solution of radicinin (0.1 g.) in methanol (150 mls.) on shaking in an atmosphere of hydrogen with platinum oxide (0.01 g.) for 2 days, absorbed 38 mls. of hydrogen (approx. 4 moles). The product of this reaction, a clear oil, was subjected to reduction with red phosphorous and hydriodic acid. See page 169.

<u>Catalytic reduction of dihydroradicinin</u>

A solution of dihydroradicinin (0.1 g.) in methanol (150 mls.), on shaking in an atmosphere of hydrogen in the presence of platinum oxide (0.01 g.), absorbed 19.0 mls of hydrogen (approximately 2 moles). The product obtained in the usual manner was a clear, yellow oil, which was shown to be identical with the product from (3) above, by comparison of ultraviolet spectra.

Reduction of radicinin with lithium aluminium hydride

1. Preparation of carboxylic acid A

Radicinin (0.1 g.) in a Soxhlet extraction apparatus was extracted into a solution of lithium aluminium hydride (0.06 g.) in ether (180 ml.). After 20 hours all the radicinin had been extracted into solution and the mixture was allowed to cool and carefully acidified with 2N. sulphuric acid. The ether layer was separated, dried ($MgSO_4$) and evaporated to give a yellow oily solid, which on extraction with carbon tetrachloride, followed by recrystallisation from benzene or carbon tetrachloride gave white needles, m.pt. 158-159°.

Found: C, 60.8; H, 5.8%, $C_{12}H_{14}O_5$ requires C, 60.5; H, 5.88%.

The ultraviolet spectrum of compound A in methanol showed absorption bands at 330m.μ.(ε = 16.5 x 10^3), 295m.μ. (ε = 7.8 x 10^3), 282m.μ.(ε = 7.6 x 10^3) and 221m.μ.(ε = 17.9 x 10^3), See Figure 7, page 85.

The infrared spectrum of compound A as a potassium bromide disc showed absorption at : 2.96μ.(m., broad), 5.67μ.(s.), 5.91μ.(m.) 6.06μ.(w.), 6.26 and 6.3μ(m., doublet), 6.46μ(s.), 6.82μ(s.), 6.98μ.(s.), 7.27μ.(w.), 7.5μ.(w.) 7.63μ.(w.), 7.72μ.(w.), 7.8μ(w.) 7.95μ(w.), 8.17μ. and 8.29μ.

(w., doublet), 8.62μ (m.), 8.8μ (m.), 9.2μ (w.), 9.5μ (m.), See Figure 6, page 84.

2. Reductions carried out by direct reaction of radicinin with lithium aluminium hydride in ether gave a variety of products depending upon the conditions used. For results of these experiments see table 4 .

3. Reduction with lithium aluminium hydride at low temperatures.

Several reactions were carried out according to the method recommended by Dagley (29). A solution of radicinin (0.1 g.) in dry tetrahydrofuran (20 mls.) was cooled in an ice-salt mixture, and a solution of lithium aluminium hydride in tetrahydrofuran was added slowly over a 30 minute period. When addition was complete, the mixture was stirred for one hour, then acidified (2N. sulphuric acid) and extracted with chloroform. Evaporation of the solvent gave solid products which were examined by thin layer chromatography on silica gel. Chromatography on silicic acid columns gave as recognisable fractions, only radicinin and compound A.

In addition, this reaction was carried out at room temperature and at reflux (ca. 60°). In each case the product obtained was a solid mixture, which was submitted to thin layer chromatography on silica gel. Several components were detected, but only radicinin and compound A could be isolated by silicic acid chromatography.

The product of reaction 11 was a solid mixture, which was shown to contain radicinin R_F, 0.46), compound A R_F, 0.25 and three further products of R_F, 0.33, 0.74 and 0.82 by chromatography on silica gel films using chloroform/1% methanol as eluent. The infrared spectrum of this mixture showed enhanced absorption at 5.9μ and reduced absorption at 5.7μ compared to that of radicinin, but as no separation could be achieved by column chromatography the experiment was abandoned.

4. The reduction of radicinin with sodium borohydride.

Several reactions were carried out using the following general procedure:

A solution of radicinin (0.1 g.), in alcohol (see table 5) (25 mls.), was heated under reflux, and sodium borohydride (4-15 mg.) was added in small portions during 30 minutes. The solution was then boiled for a further 1-3 hours, cooled, acidified (2N sulphuric acid), and extracted with chloroform. The dried chloroform extract (magnesium sulphate) was evaporated to dryness, and the product worked up in the usual way. Table 5 summarises the conditions used and products obtained.

Reaction 2 gave a yellow gum as product, which showed the following infrared absorption bands: 2.85μ (w.), 3.5μ (m.), 5.93μ (s.), 6.05μ (w.), 6.2μ (w.), 6.35μ (s.), the spectrum being measured in chloroform solution.

Reductions of Radicinin with Lithium Aluminium Hydride

Table 4.

	Method of addition	Solvent	Proportion of $LiAlH_4$ (mg.)	Temperature of reaction	Reaction Time (hours)	Products
1	Reverse	Ether	60	Reflux	22	Compound A
2	"	"	180	"	22	Compound A
3	Direct	"	33	"	9	oil
4	"	"	9.3	"	4	Radicinin + Compound A
5	"	"	4	"	12	Radicinin
6	"	"	6	"	12	Radicinin + traces of A
7	"	T.H.F.	1	ice-salt	1.5	Radicinin
8	"	"	4	ice-salt	2.0	Radicinin
9	"	"	4	reflux	1	Radicinin
10	"	"	8.8	"	3	Radicinin + compound A
11	"	"	12	ice-salt	24	Radicinin + compound A + other compounds

Key: a. "Reverse" refers to the method of reverse addition by extraction of radicinin into a solution of lithium aluminium hydride.

continued.

b. "Direct" refers to reaction by heating radicinin lithium aluminium hydride under reflux.

c. All reactions carried out on 100 mg. of radicinin.

Table 4.

The products of reactions 4 and 5 were examined by thin layer chromatography on silica gel, using chloroform/1% methanol as eluent. The two new components detected in the product from reaction 5 had R_F values of 0.16 and 0.66. However chromatography on silicic acid yielded only fractions of radicinin and compound A.

Sodium borohydride reductions of radicinin

	Solvent	Quantity of sodium borohydride (mg.)	Reaction Time	Products
1	Aqueous ethanol	4.3	2 hours	Radicinin
2	Aqueous methanol	6.0	2.5 hours	Yellow gum
3	Methanol	7.2	1 hour	Radicinin + oil
4	Aqueous ethanol	8.9	2 hours	Radicinin + compound A (traces)
5	Aqueous ethanol	12.3	2 hours	Traces of radicinin, compound A + 2 other compounds

Table 5.

Acetylation of compound A

A solution of compound A (0.05 g.), and 1 drop of concentrated sulphuric acid in acetic anhydride (2 mls.) was allowed to stand at room temperature for 1 hour. The brown solution was then poured into iced water, and the yellow precipitate filtered off. Evaporation of the aqueous filtrate to half volume gave a further small quantity of yellow solid. Recrystallisation of the combined solids from ethanol twice and ethyl acetate gave a white microcrystalline solid, m.pt. 147-148°. The sample was dried *in vacuo* at 68°.

Found: C, 60.6; H, 5.12; $C_{12}H_{13}O_4.OCOCH_3$ requires C, 60.0, H, 5.7. The infrared spectrum of the acetate, as potassium bromide disc showed the following bands 3.26μ., 3.36μ., 3.45μ.(broad triplet), 5.7μ.(s.) 5.9μ.(m.), 6.38μ., 6.47μ.(s., doublet) 6.9μ.(shoulder), 7.0μ.(s.), 7.34μ.(s.), 7.65μ.(m.), 7.8μ.(m.), 7.94μ.(m.), 8.2μ.(s.), 8.5μ.(m.), 8.6μ.(w., shoulder), 8.8μ.(s.), 9.1μ.(s.), 9.5μ.(s.).

Reaction of compound A with alkali

A solution of compound A (40 mg.) in aqueous potassium hydroxide solution (1% 10 mls.) was heated under reflux under nitrogen for 5 hours. The effluent gases were passed through a solution of 2,4-dinitrophenylhydrazine

hydrochloride. The resulting precipitate was filtered off and crystallised from methanol to give orange needles, m.p. 168° unaltered on admixture with acetaldehyde-2,4-dinitrophenylhydrazone.

The alkaline hydrolysis solution was carefully acidified with dilute sulphuric acid and extracted with chloroform. The dried extracts ($MgSO_4$) were evaporated to dryness to give a yellow oil which could not be purified.

2,4-Dinitrophenylhydrazone of compound A

A solution of 2,4-dinitrophenylhydrazine hydrochloride (0.06 g.), concentrated hydrochloric acid (3 drops), and compound A (0.03 g.) ethanol (6 mls.) was heated for 30 minutes at 60° . The solution was allowed to cool, and the orange precipitate filtered off. One recrystallisation gave orange plates (10 mg.) m.p. 174-176°.

The infrared spectrum of this derivative, as a nujol mull, showed absorption bands at 3.1μ (w.), 6.0μ (m.), 6.25μ (s.), 6.35μ(m.), 6.4μ (w.).

Catalytic reduction of compound A

Compound A (0.08 g.), was dissolved in methanol (100 ml.) and shaken with 5% palladium-on-charcoal (0.005 g.) in an atmosphere of hydrogen. Absorption was complete after

24 hours, when 22 mls. (ca. 3 moles) of hydrogen had been absorbed. The solution was filtered and the solvent evaporated, to give a clear glass-like solid which could not be purified.

Bromination of compound A

To a solution of compound A (0.05 g.) in chloroform (3 ml.) was added a solution of bromine in chloroform (1.25%, 1 ml.). The mixture was allowed to stand for twelve hours, then washed with dilute sodium bicarbonate solution (3 ml), and dried over anhydrous magnesium sulphate. Evaporation of the solvent gave a yellow solid (36 mg.), which was crystallised from acetone, followed by ethanol, to give a white micro-crystalline solid, m.p. 140-142°.

The ultraviolet absorption spectrum of this compound, in methanol, possessed bands at 320m.μ (ε = 10.4 x 10^3), 268m.μ (ε = 3.9 x 10^3), 256m.μ (ε = 4.1 x 10^3) and 221m.μ (ε = 17.9 x 10^3). (Figure 8, page 88).

The infrared spectrum as a potassium bromide showed bands at 2.96μ (m., broad band), 5.7μ (s.), 6.0μ (w.) 6.1μ (m.), 6.5μ (s.), 6.92μ (s.), 7.16μ (w.) 7.26μ (w.), 7.6μ (v.w.), 7.9 and 7.97μ (m., doublet), 8.27μ (s.), 8.47μ (w.), 8.65μ and 8.74μ (m., doublet), 8.95μ and 9.0μ (m., doublet), 9.4μ (s.), 9.74μ (m.).

Lithium aluminium hydride reduction of dihydroradicinin-compound B

Dihydroradicinin (0.1 g.) in a Soxhlet extraction apparatus was extracted into a solution of lithium aluminium hydride (0.07 g.) in ether (180 mls.). After twenty hours, when all the dihydroradicinin had been extracted into solution, the yellow suspension was cooled and carefully acidified (2N. sulphuric acid). The ether layer was separated, dried ($MgSO_4$), and evaporated to dryness. The yellow oily residue was extracted twice with boiling light petroleum (b.p., 100-120°, 15 mls.) and the combined extracts allowed to cool, when a solid precipitated. The latter, after three recrystallisations from benzene gave a white microcrystalline solid, m.p. 105-106°.

Found, C, 60.26; H, 6.9%; $C_{12}H_{16}O_5$ requires C, 60.0%; H, 6.66%.

The ultraviolet spectrum of compound B in methanol was found to possess bands at 296m.μ.(ε = 12.0 x 10^3) and 207m.μ.(ε = 15.6 x 10^3) (Figure 9 page 89).

The infrared spectrum of compound B, in potassium bromide, showed bands as follows, 3.0μ.(m. broad band), 3.26μ. (w.), 3.4μ.(m.), 5.75μ.(s.), 5.93μ.(s.), 6.23μ.(m.), 6.43 and 6.48μ.(s., doublet), 6.84μ.(s.), 7.1μ.(m.), 7.25μ.(m.),

7.5μ (m.), 7.7μ (m.), 7.8μ (w.), 8.0μ (m.), 8.22μ, 8.3μ (s. doublet), 8.64μ (m.), 8.84μ (s.), 9.15 and 9.2μ (m., doublet), 9.48μ (s.), 10.4μ (s.). See Figure 10, page 89.

Attempted reduction of compounds A and B

1. With lithium aluminium hydride, compounds A and B were treated as follows.

The compound (0.05 g., (i) compound A, (ii) compound B) was extracted from a Soxhlet apparatus into a solution of lithium aluminium hydride (0.035 g.) in ether (80 mls.) After six hours the compound had been completely extracted into solution, to form a yellow suspension, which was cooled and carefully acidified (2N. sulphuric acid). The ether layer was separated, dried ($MgSO_4$) and evaporated to dryness. The oily solid obtained was shown by thin layer chromatography on silica gel (eluent: chloroform/1% methanol) to be mainly compound A, R_F 0.21, in case (i) and compound B, R_F 0.12, in case (ii).

2. Attempted sodium borohydride reduction of compound A

Compound A (0.04 g.) was heated under reflux in aqueous ethanol (1:1, 10 mls.), and sodium borohydride (0.003 g.) was added in small quantities during one half hour. After two hours the pink solution was allowed to cool, acidified (2N. sulphuric acid) and extracted with chloroform.

The dried extract ($MgSO_4$) was evaporated to dryness, and the crude solid product (0.035 g.) shown, by thin layer chromatography on silica gel (eluent, chloroform/1% methanol) to be unchanged compound A.

Complex metal hydride reductions of maltol, kojic acid, and patulin

A series of reactions were carried out at the reflux temperature of the solvent employed. Methods used were identical with those described above for the reduction of radicinin. Table 6 summarises the conditions used and the products obtained.

The product of reactions (6) and (9) (table 6) gave positive tests with Brady's reagent and showed the following infrared absorption bands in chloroform solution, (6): 2.85μ (m.), 3.3μ (s.) 5.8μ (s.), 6.12μ (s.), 6.4μ (w.) 6.85μ (m.), 7.25μ (m.), 7.4μ (m.), 8.2μ (w.), 8.36μ (w.), (9): 2.95μ (s.), 3.4 and 3.47μ (s., doublet), 4.15μ (w.), 5.86μ (s.), 6.12μ (w., shoulder), 6.9μ (m.), 7.2μ (w., shoulder), 7.3μ (m.), 8.2 to 8.4μ (s., broad band).

The product of reaction (7) also gave a positive test with Brady's reagent, and showed the following infrared absorption bands: 2.82μ (w.), 3.1μ (m.), 3.5 and 3.55μ (s., doublet), 5.72μ (s.), 6.1μ (m.), 6.2 and 6.3μ (m.,

Complex metal hydride reductions of maltol, kojic acid and patulin

Table 6.

	Compound	Reagent	Solvent	Method	Product
1	Maltol	S.B.H.(40) $NaBH_4$	Aqueous Ethanol	Reflux (2 hrs.)	Unreacted maltol
2	Maltol	(120) $NaBH_4$	Aqueous Ethanol	Reflux (2 hrs)	" "
3	Kojic acid	$NaBH_4$ (120)	Aqueous Ethanol	Reflux (2 hrs)	" "
4	Patulin	$NaBH_4$	Aqueous Ethanol	Reflux (2 hrs)	Unreacted patulin
5	Maltol	(50) $LiAlH_4$	Ether	(12 hrs) Reverse	Maltol recovered
6	"	"(70)	Ether	(2 days) Reverse	Oil
7	Patulin	$LiAlH_4$ (60)	Ether	Reverse (12 hrs)	Oil
8	Kojic acid	$LiAlH_4$ (70)	Ether	Reverse (12 hrs)	Oil
9	Maltol	"	Ether	Reflux (2 days)	Oil

Key Aqueous ethanol used was a 1:1 mixture.

"Reflux" refers to reactions carried out by direct heating of the reagent and compound in a solvent.

"Reverse" refers to reactions carried out by reverse addition, by the extraction method.

Second column: figures in parentheses refer to weight (mg.) of complex metal hydride used.

Fourth column, figures in parentheses refer to period of heating.

doublet), 6.8μ. (s.), 7.25μ. (m.)

Reaction of radicinin with ammonia

1. A mixture of radicinin (0.1 g.) and ammonium hydroxide (10 mls.) was heated under reflux for forty minutes. After ten minutes heating, all the radicinin had dissolved to produce a bright red solution. This solution was evaporated to dryness *in vacuo* to give a brown gum, which resisted all attempts at purification. The material gave no clearly defined infrared absorption bands, but gave positive tests for nitrogen (Lassaigne).

2. Radicinin (0.1 g.) was dissolved in concentrated ammonium hydroxide solution (0.880, 5 mls.) to give a bright red solution. After allowing to stand for fifteen minutes the solvent was evaporated (*in vacuo*), to produce a dark brown gum which contained nitrogen (Lassaigne). Trituration with small quantities of ether or petroleum ether gave a semi-solid which could not be purified further.

Hydrolysis of radicinin with dilute hydrochloric acid

A mixture of radicinin (0.5 g.), and hydrochloric acid (2N., 30 mls.) was heated under reflux in an atmosphere of nitrogen. After two hours, the radicinin had all dissolved and traces of a brown gum began to separate out. After four hours, the mixture was cooled, evaporated to approximately

one third its volume, and extracted with chloroform. Evaporation of the dried chloroform solution ($MgSO_4$), gave a tan-coloured gum (0.4 g.) which could not be crystallised. The aqueous solution was evaporated to dryness and the residue (a dark brown tar) extracted successively with chloroform, ether, and ethyl acetate. The dried solutions ($MgSO_4$), on evaporation yielded further small quantities of the tan-coloured gum.

Chromatography of the bulk of the product (0.4 g.) on a column of silicic acid (10.0 g.) gave five distinct fractions.

	Eluent	Product	Weight
(1)	Chloroform	brown oil	0.03 g.
(2)	Chloroform	brown oil	0.02 g.
(3)	Chloroform	yellow solid	0.09 g.
(4)	Chloroform/1% methanol	yellow oil	0.01 g.
(5)	Chloroform/2% methanol	yellow oil	0.2 g.

On evaporation of the solvent from fraction 3, a yellow solid was obtained, which on washing with small quantities of cold ether, gave a white solid, m.p. 153-154°. Crystallisation from ethanol gave white needles, m.p. 154-155°, unchanged on admixture with authentic radicinin hydrochloride.

The remaining fractions could not be purified further.

Hydrolysis of radicinin with dilute sulphuric acid

A mixture of radicinin (0.1 g.), and sulphuric acid (2N., 15 mls.) was heated under reflux in a stream of nitrogen, and the effluent gases passed through solutions of 2,4-dinitrophenylhydrazine, and barium hydroxide. After two hours, all the radicinin had dissolved, and heating was continued for a further three hours. The precipitated barium carbonate was collected, washed and weighed, from which it was found that one mole of carbon dioxide was evolved during the reaction. Only slight traces of a dinitrophenylhydrazone precipitate were formed.

The acidic solution was steam distilled, producing a volatile acid (0.3 mole, as estimated by titration).

In a similar experiment, the acidic solution was extracted with chloroform, ether, and ethylacetate. Evaporation of the dried ($MgSO_4$) solutions gave yellow oils (combined weight 0.08 g.) which could not be purified by chromatography.

Attempted decarboxylation of compound A

A mixture of compound A (0.08 g.), copper chromite (0.01 g.) and redistilled quinoline (10 mls.) was heated under reflux in a stream of nitrogen for one hour, the effluent gases being passed through barium hydroxide solution. The barium

carbonate formed was filtered, dried and weighed, indicating the liberation of one mole of carbon dioxide.

Addition of water to the cooled quinoline solution produced no solid precipitate, and the mixture was made homogeneous by the addition of concentrated hydrochloric acid. The acidic solution was extracted with ether, the ether was dried ($MgSO_4$) and evaporated to give a dark brown oil (0.05 g.) which could not be purified.

Reduction of radicinin with lithium in liquid ammonia

In a round bottomed flask, equipped with a magnetic stirrer, dropping funnel and dry ice condenser, was placed radicinin (0.2 g.), methyl alcohol (10 mls.) and liquid ammonia (50 mls.). Lithium was added to the rapidly stirred solution until a permanent blue colouration was obtained. The solution was allowed to stand for one hour, when excess lithium was destroyed by the addition of methyl alcohol (2-3 mls.). The ammonia was then allowed to evaporate at room temperature, and water was (20 mls.) added to the residue. The resulting solution was extracted successively with chloroform, ether and benzene. The aqueous layer was then acidified and extracted with ethyl acetate. Evaporation of the dried ($MgSO_4$) solutions yielded yellow oils (total weight 0.15 g.), which proved intractable.

Attempted phosphorous-hydriodic acid reduction of radicinin

A mixture of radicinin (0.5 g.), red phosphorous

(0.47 g.) and freshly distilled hydriodic acid (S.G. 1.7; 5 mls) was heated under reflux in an atmosphere of nitrogen for five hours. The mixture was cooled and poured into iced water (20 mls.), the iodine colour destroyed with a little sodium thiosulphate solution (4%, 5 mls.), and the suspended solid filtered off and extracted with chloroform and ether. The dried ($MgSO_4$) solutions were evaporated to dryness to give minute traces of brown oils.

The aqueous solution was extracted with chloroform and ether, then evaporated to one-quarter volume and re-extracted with ethyl acetate. The dried organic solutions were evaporated to dryness, yielding fractions of yellow oil (combined weight, 0.3 g.), which could not be purified further.

Attempted phosphorous-hydriodic acid reduction of hexa hydroradicinin

Hexahydroradicinin (0.5 g.) (prepared as previously described, page 152) was treated with red phosphorous and hydriodic acid as described above for radicinin. The product was a yellow oil (0.33 g.), which on standing in a refrigerator for 24 hours, partially crystallised to yield colourless plates, which after filtration and washing with petroleum ether (0-40°) gave m.p. of 118-120° (yield, 0.0006 g. 0.2%). The residual oil could not be purified.

Reaction of radicinin with hydriodic acid

A mixture of radicinin (0.15 g.) and hydriodic acid (S.G., 1.7, 10 mls.) was heated under reflux for two hours, poured into iced water (20 mls.) and the iodine destroyed by the addition of sodium thiosulphate solution (4%). The aqueous solution was extracted with chloroform and the dried ($MgSO_4$) solution evaporated to dryness. A dark red tar (0.15 g.) was obtained, which gave a positive test for iodine, but which could not be purified.

The action of concentrated sulphuric acid on radicinin

A solution of radicinin (0.07 g.) in concentrated sulphuric acid (3.0 mls.) was allowed to stand for one hour, poured into iced water (10 mls.), and the aqueous solution extracted with chloroform. The dried ($MgSO_4$) solution was evaporated to dryness and the crude product (0.06 g.) was crystallised from ethyl acetate, to give pale yellow crystals m.p. 210-212°, undepressed on admixture with radicinin.

Melting points were taken on a Kofler block and are uncorrected.
Ultraviolet absorption spectra were measured on a Hilger Uvispek Spectrophotometer (model 700.305.)
Infrared spectra were measured on a Perkin Elmer model 21 double beam infrared spectrophotometer.

REFERENCES

1. Clarke and Nord, Arch.Biochem.Biophys.,1953,45,469.
2. Clarke and Nord, Arch.Biochem.Biophys.,1955,59,269.
3. James, J., 1910,97,1565.
4. Colthup, J.Opt.Soc.Amer.,1950,40,397.
5. Woodward and Singh, Experimentia,1950,6,238.
6. Woodward and Blout, J.Amer.Chem.Soc.,1943,65,562.
7. Tsuda and Uyeo, J.,1961,1060.
8. Davies and Wilcox, Private communication.
9. Schriner and Fuson, "The Systematic Identification of Organic Compounds", John Wiley and Sons, Inc., New York, 4th edition, pg.250.
10. Lemieux and Bauer, Analyt.Chem.,1954,26,920.
11. Lemieux and Rudloff, Canad.J.Chem.,1955,33,1701.
12. Yates and Stout, J.Amer.Chem.Soc.,1958,1691.
13. Frohardt et.al. J.Amer.Chem.Soc.,1960,83,5500.
14. Wild, "The Characterisation of Organic Compounds", Cambridge University Press, 1958, pg.142.
15. Raistrick et.al., Lancet, 1943,245,625.
16. Wilds and Nelson, J.Amer.Chem.Soc., 1953,75,5360.
17. Ross, Analyt.Chem., 1953,25,1288.
18. Haskins, Hann & Hudson, J.Amer.Chem.Soc., 1942,64,134.
19. Ness, Hann and Hudson, J.Amer.Chem.Soc., 1943,65,2215.
20. Dyke, "The Carbohydrates", Interscience Publications, 1960,32.

21. Gaylord, "Reduction with complex metal hydrides", Interscience Publications, New York, 1956.

22. Bellamy, "The infrared spectra of complex molecules" Methuen and Co., London, 1958.

23. Hochstein, J.Amer.Chem.Soc.,1949,71,305.

24. Putuely & Bauer, Monatsh., 1952,83,758.

25. Susherina et.al. Zhur.Obshelei Khim, 1961,31,1076.

26. Karrer and Banerjea, Helv.Chim.Acta., 1949,32,1692.

27. Vogel, Chem.and Ind., 1962, 268.

28. Fried and Elderfield, J.Org.Chem., 1941,6,677.

29. Dagley, Private communication.

30. Mirza & Robinson, Nature, 1950,166,929,997.

31. Elderfield, "Heterocyclic Compounds" John Wiley & Sons, inc., New York, 1950, vol.1,385.

32. Meakins, J., 1953,4170.

33. Barton, J., 1956,142.

34. Davies, Kirkaldy and Roberts, J., 1960,2169.

35. Haworth, Jubb and McKenna, J., 1955, 1953.

36. Sondheimer, J.Amer.Chem.Soc., 1955,77,4145.

37. Birch and Herchel-Smith, Quart.Revs.1958,XII, pg17.

38. Schmidt & Bencz, Helv.Chim.Acta.1953,36,205,1468.

39. " " " " " 1962,

40. Smakula, Angew.Chem.,1934,38,657.

41. Jones et al. Canad.J.Chem., 1959,37,2007.

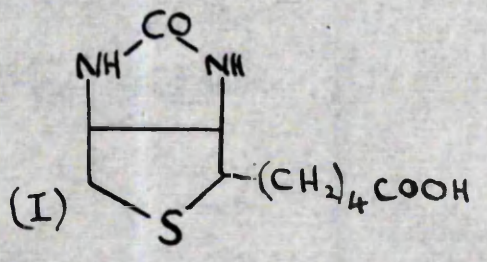
42. Duncanson, J.,1953,1207.

PART III

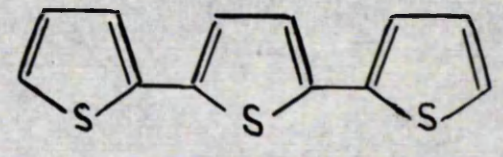
THE CHEMISTRY OF SOME SUBSTITUTED THIOPHENES

INTRODUCTION

Apart from biotin (I) and thiophene itself, which occurs in coal tar, no other naturally occurring thiophene compound was known until 1947, when α-terthienyl (II) was isolated from Indian marigold (1). However, in 1955, the isolation of another thiophene compound, junipal (III), was reported from the wood-destroying fungus Daedalea juniperina, Murr (2). It was obtained, together with anisaldehyde and a second, as yet unidentified thiophene compound by steam distillation of the culture liquor.

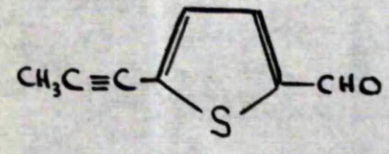


(I)

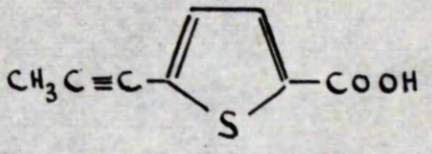


(II)

The infrared and ultraviolet spectra of junipal indicated the presence of an acetylenic linkage, and an aldehyde group.



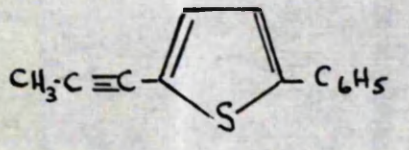
(III)



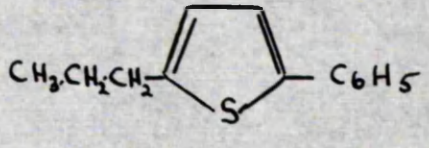
(IV)

Oxidation of junipal with Doeuvre reagent yielded juniperic acid (IV), which gave thiophene-2,5-dicarboxylic acid on oxidation with potassium permanganate. Thus, it was evident that junipal had the constitution (III).

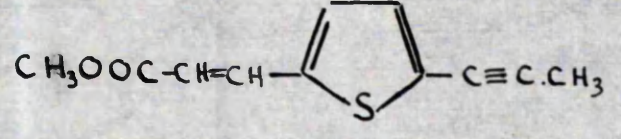
Although junipal remains the only known fungal thiophene (apart from biotin), a number of other thiophene derivatives have been isolated from natural sources (3). For example, a compound isolated from Coreopsis grandiflora, has been shown (3a) to be 5-(α-propynyl)-2-phenylthiophene (V), by comparison of its infrared spectrum with that of junipal, and synthesis of its tetrahydro derivative (VI). A further example is the compound isolated from the roots of Chrysanthemum vulgare, Bernh., which has been identified as cis-methyl-5-(1-propynyl)-2-thienylacrylate (VII) by spectral data and by hydrogenation experiments, (4).



(V)



(VI)

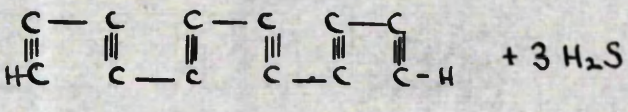


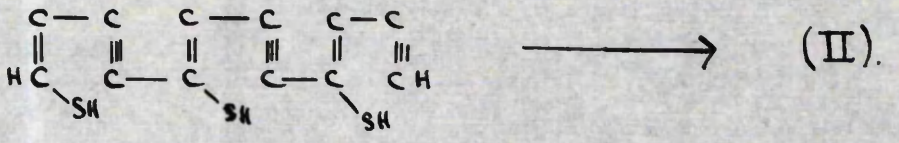
(VII)

The biological significance of thiophene compounds

Challenger (5) pointed out that the simultaneous occurrence of α-terthienyl and polyacetylenic compounds in members of the compositae, may not be coincidental. It was suggested that α-terthienyl may arise by the interaction of hydrogen sulphide with a polyacetylene or

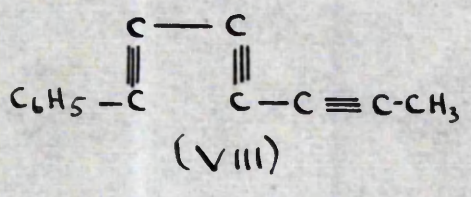
acetylenic-olefinic system as follows:

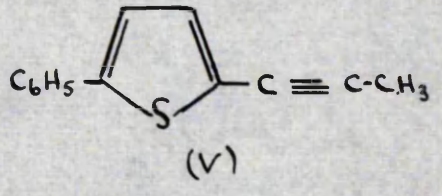




The isolation of junipal, a compound containing both a thiophene residue and a triple bond, was considered by Birkinshaw and Chaplen (2) to lend support to this postulate. In addition it was suggested that anisaldehyde, which is isolated in conjunction with junipal, may be derived from a common acetylenic precursor.

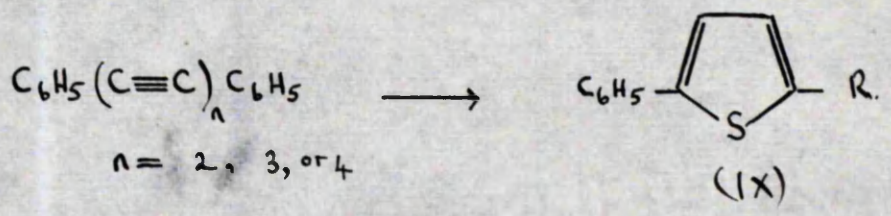
Compound (VII) was also isolated from a member of the <u>compositae</u> family, whilst (V) was accompanied in the flowers and leaves of <u>coreopsis grandiflora</u>, by a poly-acetylene (VIII), which could be considered the precursor of (V).


(VIII)


(V)

However, there is no evidence that thiophene compounds do arise from acetylenes in nature by this method. That the route is chemically feasible has been shown by the synthesis of a number of thiophene derivatives in the

laboratory by the action of alkaline hydrogen sulphide on polyacetylenes (6). Thus 2,5-diphenylthiophene (IX,R=Ph), 2-phenyl-5-phenylethynylthiophene (IX,R=-C≡C-Ph), and 2-phenyl-5-[4'-phenylbutadiynyl (1')]-thiophene (IX,R=-$(C{\equiv}C)_2$-Ph) were obtained from the corresponding polyacetylenes (n= 2,3,4 respectively).



(IX)

Recent work, on the biosynthesis of acetylenic acids (7,8,9), has led Fleming and Harley-Mason (10) to postulate a route for the formation of tetronic acids, which was supported by biogenetically patterned synthesis of tetronic acid derivatives from suitable acetylenic acids (see introduction page 27.). It is possible that thiophene compounds arise naturally by a similar route; and thus the biosyntheses of tetronic acids, polyacetylenes and thiophene compounds may be closely related, although no evidence to support this hypothesis is available.

DISCUSSION

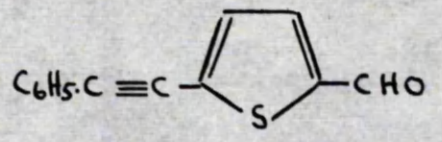
The object of this work was the unambiguous synthesis of junipal. This object was not attained as whilst work was in progress, several independent syntheses of junipal were published along similar lines, and the project was abandoned. The work is of interest however, as the applicability of various general synthetic methods to thiophene compounds was studied.

The synthesis of heterocyclic compounds containing acetylenic substituents has not received much attention, thus the main problem in the synthesis of junipal lies in the introduction of the propynyl side chain. Of the two main methods for preparing substituted acetylenes, namely dehydrohalogenation and alkylation methods, the former has been most studied (11).

Despite the statement by Raphael (12), "aryl and vinyl halides are extremely resistant to nucleophilic attack and therefore unaffected by metal acetylides", several instances of the condensation of such compounds with metal acetylides, especially Grignard reagents, have been cited in the literature.

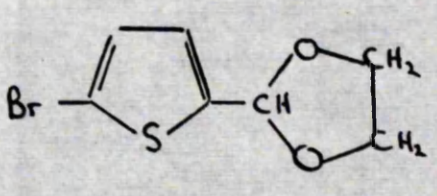
It was considered that the carbon-bromine bond of α-substituted bromothiophenes might be sufficiently

reactive to undergo such a condensation. Experiments along these lines were carried out, but because of the difficulties involved in the preparation and use of methylacetylene (13), it was considered advisable to test the proposed reaction with model systems. Thus initially, attempts were made to prepare the homologue of junipal (X).



(X).

The formyl group was introduced into the thiophene nucleus first, because it was considered that an acetylenic residue might possibly be attacked during formylation. And indeed, in the work of Janda (page 198), it was found that the acetylenic bond was hydrated during chloromethylation. It was considered necessary to protect the formyl group prior to condensation of the bromoaldehyde with acetylene derivatives. Thus the bromoketal (XI) was prepared and used for subsequent condensation reactions.



(XI)

1. Formylation of thiophene

The following are the methods available for the synthesis of thiophene aldehydes, Unfortunately these are not particularly efficient, even in favourable cases.

a. The Gatterman-Grignard reaction with formic esters.

b. The Sommelet reaction.

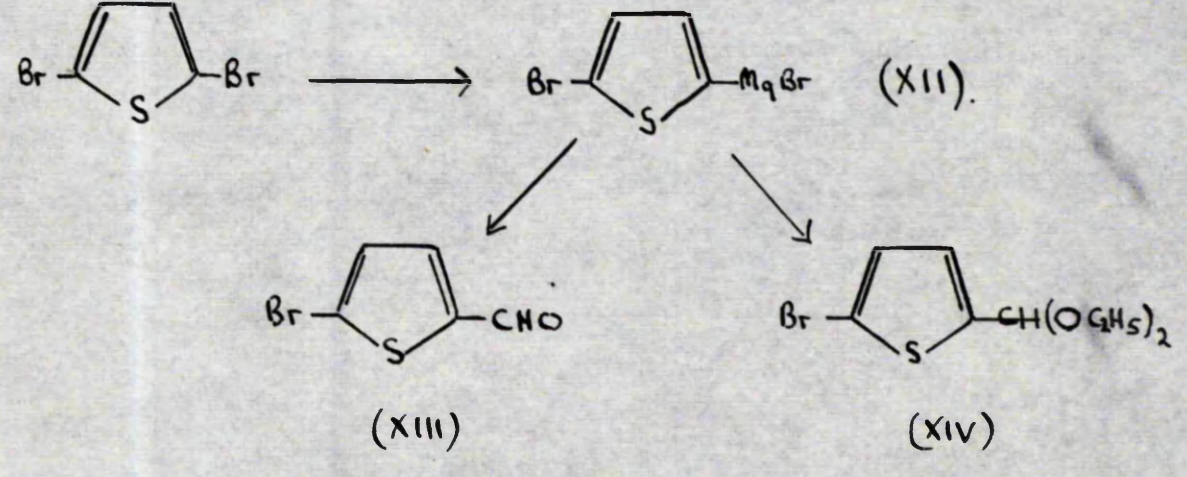
c. Formylation with N-methylformanilide.

d. Rosenmund reduction of the acid chloride (very low yield).

e. Gatterman synthesis using HCN/HCl. (very low yield).

a. The Gatterman-Grignard reaction.

This requires the reaction of a Grignard reagent with either ethylformate, or ethylorthoformate to give the aldehyde, or the acetal respectively. Thus, the proposed synthesis involved the preparation of 2-bromothiophene-5-magnesium bromide (XII) and conversion to either 2-bromo-5-formylthiophene (XIII) or its diethylacetal (XIV).



(XIII) (XIV)

2,5-Dibromothiophene was prepared in 60% yield by the method of Mozingo (14) and Lawesson (15). The bromothiophene compounds were characterised for reference by vapour-phase chromatography, as well as by infrared and ultraviolet spectroscopy.

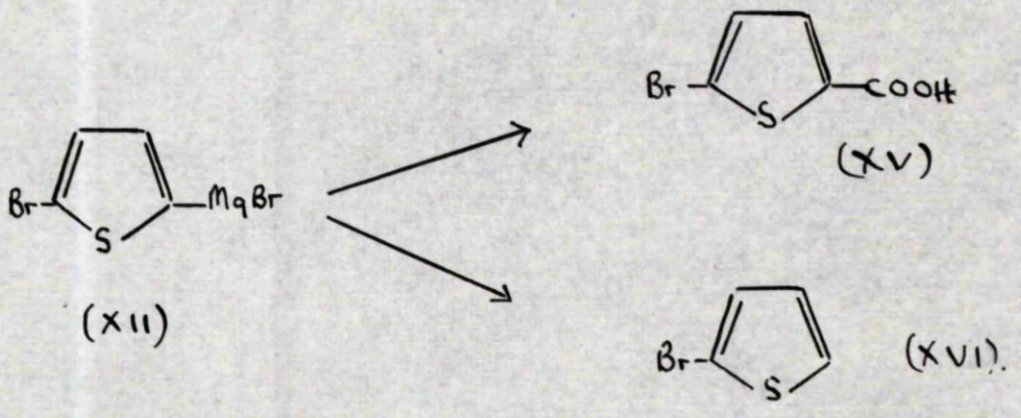
According to Lawesson (16), the preparation of Grignard reagents of substituted thiophene halides is difficult under normal conditions, due partly to the insolubility of polyhalothiophenes. It was shown, however, that the method of entrainment increased the yield of the required Grignard reagent. The entrainment technique was introduced by Steinkopf (17) and Grignard (18), who proposed that a halogen-magnesium exchange occurred according to the equilibrium shown (19), where R'X is the unreactive halide.

$$RMgX + R'X \rightleftarrows R'MgX + RX$$

The position of the equilibrium will thus depend on the proportion of entraining reagent used; this was confirmed by Lawesson's work on thiophene Grignard reagents, in which optimum proportion of 5 moles of entraining reagent per mole of thiophene halide were used.

Before attempting the Gatterman-Grignard reaction, the formation of the Grignard reagent (XII) by direct and

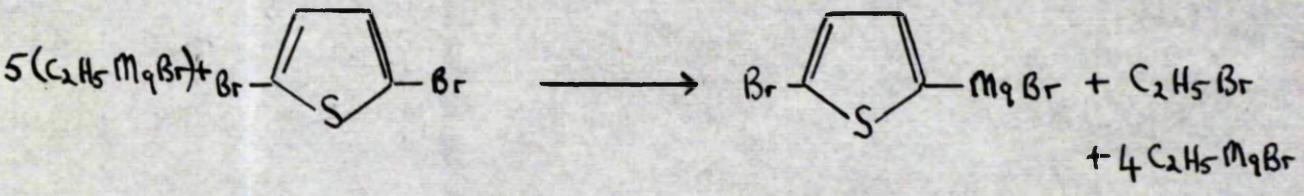
entrainment methods was checked. Carboxylation of the Grignard intermediate obtained by both methods gave 2-bromothiophene-5-carboxylic acid (XV), while hydrolysis gave 2-bromothiophene (XVI) (estimated by vapour-phase chromatography).



The yields of these compounds indicated 65% and 80% formation of the Grignard reagent, respectively.

The reaction between ethylorthoformate and Grignard reagents was first discovered by Bodroux (20), whilst attempting to prepare triarylmethanes. It was shown that removal of the solvent from a solution of Grignard reagent and ethylorthoformate in ether caused a vigorous reaction, with the production of the acetal (21). It was later discovered that the reaction led to further substitution (22, 23). These observations were confirmed in the benzene series, and conditions for optimum yields were established (24, 25, 26).

From a practical point of view the entrainment method had the disadvantage of giving 2-bromothiophene-5-magnesium bromide contaminated with four moles of ethylmagnesium bromide, and one mole of ethylbromide.

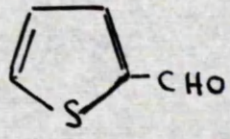


Thus the reaction with formic esters would give a mixture of aldehydes, and in several cases where entrainment was used, reaction with ethylorthoformate gave propionaldehyde-diethylacetal, although no thiophene-acetal was formed. In attempts to obtain a mixture of both acetals, 5 moles of ethylorthoformate were used, but again the required acetal was not formed.

In view of the possibility of the reaction proceeding beyond the required stage, the method of reverse addition was used, although this had not previously been reported in the literature in connection with the preparation of ethylacetals. However, this led only to the recovery of unchanged starting materials. Table 1 (page 200) sumarises the variety of conditions used for the reaction.

In view of the lack of success with ethylortho-

formate it was decided to attempt formylation with ethylformate. The preparation of aldehydes by the reaction of ethylformate with Grignard reagents was first reported by Gatterman (27, 28). Amongst other aldehydes, 2-bromo-5-formylthiophene was reported in 10% yield. Later workers (29, 30) repeated the reaction in the preparation of 2-formylthiophene (XVII), and reported correspondingly low yields.

(XVII) 

Trial reactions showed that with phenylmagnesium bromide and ethylformate, benzaldehyde was obtained, but only by reverse addition, using three moles of ester to one mole of Grignard reagent. Gatterman's experiments with 2-bromothiophene-5-magnesium bromide were carried out using the entrainment method as previously mentioned, but yields greater than 10% of the required aldehyde could not be obtained. For a summary of results, see table 1 (page 200).

b. The Sommelet reaction

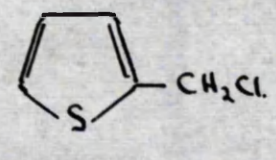
Aldehydes may also be obtained by the action of hexamethylene tetramine (hexamine) on halomethyl-compounds. The preparation of quaternary salts from benzyl halides and hexamine was first reported by Delépine (31), who noted

that on strong acid hydrolysis, a primary amine, ammonia, and formaldehyde were obtained. However, on hydrolysis with water, an aromatic aldehyde was obtained (32). The reaction has been shown to be general for aromatic aldehydes. It is considered that the reaction involves the formation of a primary amine, which on dehydrogenation and hydrolysis gives the aldehyde, as shown (33, 34, 35).

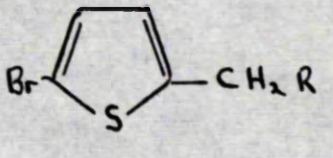
$$RCH_2Cl \longrightarrow RCH_2NH_2 \rightarrow RCH{=}NH \rightarrow RCHO + NH_3$$

Further evidence in support of this sequence has been obtained by Graymore and Davies (36).

In the thiophene series the Sommelet reaction has been used for the preparation of 2-formylthiophene (XVII) (37, 38) from 2-chloromethylthiophene (XVIII). Thus in order to prepare 2-bromo-5-formylthiophene, it was necessary to obtain 2-bromo-5-halomethylthiophene (XIX, R=Halogen). The synthesis of the latter (R=Cl) from 2-bromothiophene and formaldehyde in hydrochloric acid has been reported in the patent literature, but no physical properties were recorded (39, 40, 41).



(XVIII)



(XIX)

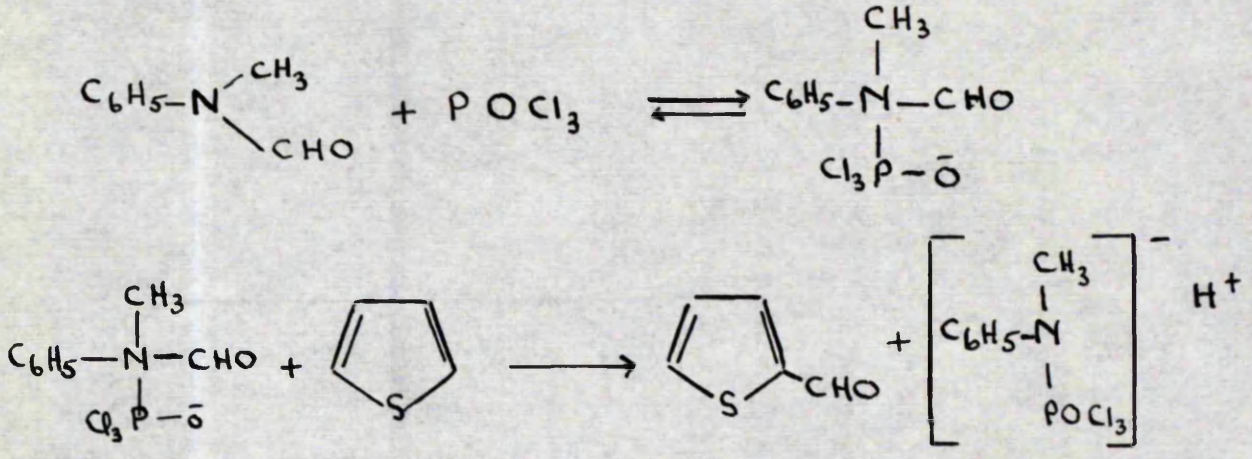
These experiments were repeated under a variety of conditions and the results are summarised in table 3 (page 205). The best results were obtained by addition of formaldehyde to a rapidly stirred mixture of concentrated hydrochloric acid and 2-bromothiophene, through which hydrogen chloride gas was passed. The use of zinc chloride as catalyst was also found to be advantageous. The chloromethyl compound thus obtained was somewhat unstable, liberating chlorine on standing, and undergoing ready hydrolysis with dilute aqueous sodium hydroxide solution. However, it could be purified by distillation under reduced pressure, and satisfactory analyses were obtained.

The preparation of (XIII) from the chloromethyl compound was carried out according to the method described for the preparation of 2-formylthiophene (37), in which the intermediate quaternary salt was filtered off, air dried and then steam distilled to achieve hydrolysis. The yields obtained by this method were of the order of 30%.

c. Formylation using N-methylformanilide

Direct formylation of the thiophene nucleus by the action of N-methylformanilide and phosphorylchloride has been reported by King and Nord (42), and others (43). Formylation is usually promoted by phosphoryl chloride, although other Lewis acids may be used. The mechanism

involves formyl ion transfer to moderately reactive aromatic systems (44), thus the 2-position of thiophene is susceptible to attack.



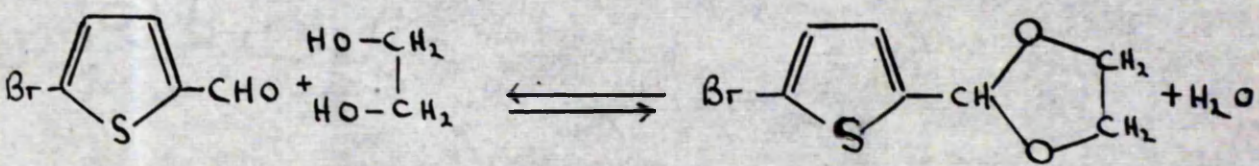
N,N-Dimethylformamide has been reported as a substitute for N-methylformanilide. Tyson and Shaw (45) obtained a 72% yield of 3-indolecarboxaldehyde by this method, and the application to thiophene compounds has been patented (46). This reagent has the advantage of being cheaper than N-methylformanilide, but the yields of the aldehydes obtained by its use as a formylating agent are generally lower than in the case of N-methylformanilide.

N-methylformanilide was prepared from formic acid and N-methylaniline (47), and 2-formylthiophene and 2-bromo-5-formylthiophene were prepared by the method of Nord and King (48), in yields of 61% and 50% respectively. Gronowitz (49) showed that bromination of 2-formylthiophene

occurs in the unsubstituted α-position, thus providing an alternative route to the required bromoaldehyde.

2. Protection of the formyl group

Aldehydes condense with ethylene glycol in the presence of an acid catalyst to form ethylene ketals (50). The most convenient method for the preparation of these ketals is to heat the reagents under reflux in dry benzene, collecting the water formed in a separator as its benzene azeotrope (51). p-Toluenesulphonic acid has proved to be an efficient catalyst (48). The reaction between an aldehyde and a glycol is reversible, involving an equilibrium as shown, thus to obtain good yields of the ketal, water formed during the reaction is removed. The ethyleneketal (XI) of 2-bromo-5-formylthiophene (XIII) was accordingly prepared, and proved to be a stable liquid, easily purified by distillation under reduced pressure, and characterised by its infrared spectrum. Satisfactory analyses were obtained.

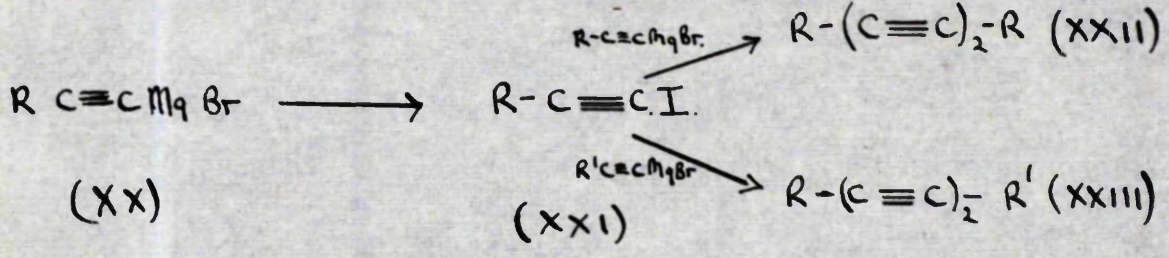


(XIII) XI.

3. Condensation reactions with metal-acetylides

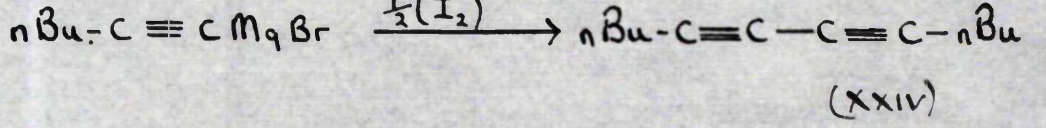
It was first suggested by Grignard (53,54), that the use of alkynyl Grignard reagents (XX) provided a

convenient synthesis of diacetylenes. Thus the reagent (XX), on treatment with a half-molecular proportion of iodine, formed some of the alkynyl iodide (XXI), which reacts with (XX) to yield a symmetrical diyne (XXII).



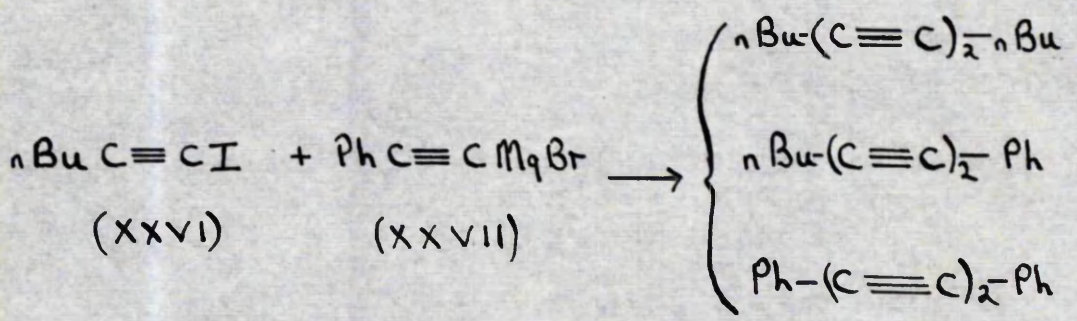
Claims to have prepared an unsymmetrical diyne (XXIII) by introduction of a second Grignard reagent (R^1-C≡CMgBr) were later discredited.

Black and Weedon (55) re-examined Grignard's experiments, and showed that dodecadiyne (XXIV) was produced in very small yield from hexynylmagnesium bromide (XXV).



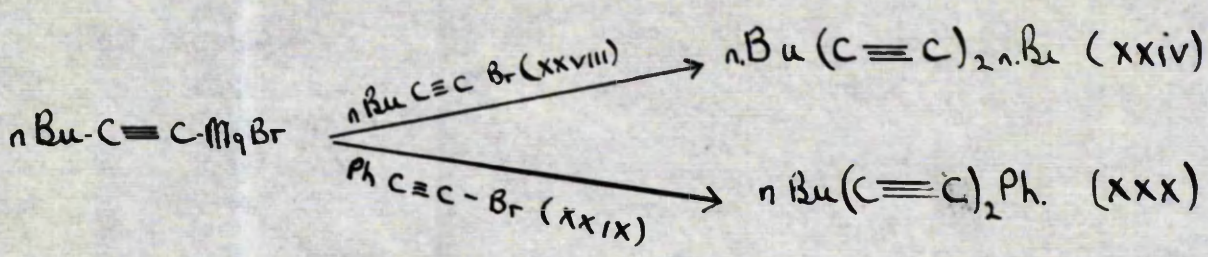
The coupling of alkynylmagnesium halides with allyl bromide under the influence of cuprous chloride catalyst had earlier been shown to proceed in good yield (56,57). Black, Horn and Weedon (58) tried this catalyst in the formation of dodecadiyne from hexynylmagnesium

bromide (XXV), and found the reaction to proceed in 70% yield. However, in the preparation of unsymmetrical diynes, side reactions became more prominent. Thus, the reaction between iodohexyne (XXVI) and phenylethynylmagnesium bromide (XXVII) in the presence of cuprous chloride produced a mixture:

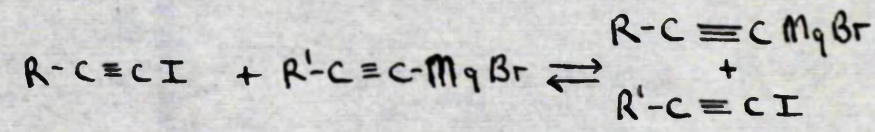


The unsymmetrical product was formed in only 35% yield.

Kharasch and his colleagues showed that small amounts of cobaltous chloride exert a strong influence on the course of many reactions utilising alkyl and aryl Grignard reagents (59). Weedon and his colleagues (58) went on to show that the condensation of hexynylmagnesium bromide (XXV) with 1-bromo-hex-1-yne (XXVIII) or, 1-bromo-2-phenylacetylene (XXIX) in the presence of cobaltous chloride, gave 30% yields of dodecadiyne (XXIV) and phenyloctadiyne (XXX) respectively.

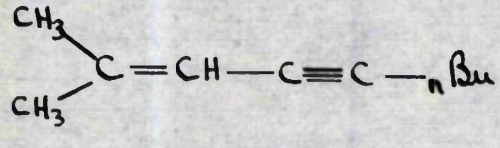


The simultaneous formation of symmetrical diynes as by-products during the preparation of unsymmetrical diynes is probably due to the establishment of an equilibrium of the type:

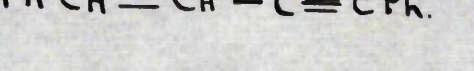


Similar interchanges have been observed in the reaction of alkyl and aryl Grignard reagents with organic halides both in the presence and absence of catalysts (60).

This reaction has been extended (58) to the condensation of alk-1-enyl halides with acetylenic Grignard reagents, under the influence of cobaltous chloride. Thus hexynylmagnesium bromide (XXV) was condensed with isobutenyl bromide, and phenylethynylmagnesium bromide (XXVII) with styryl bromide to give the vinylacetylenes (XXXI), and (XXXII) respectively.



(XXXI)



(XXXII)

Condensation reactions reported in the literature

Grignard reagent	Halide	Catalyst	Product
$PhC{\equiv}CMgBr$	β -bromostyrene	Cobaltous chloride	1,4-diphenylbutenyne
$BuC{\equiv}CMgBr$	butyl bromide	Cobaltous chloride	dec-5-yne
MeMgBr	$PhC{\equiv}C\text{-}Br$	Cobaltous chloride	phenylpropyne phenylacetylene
BuMgBr	$PhC{\equiv}C\text{-}Br$	Cobaltous chloride	1-phenyl-hex-1-yne
BuMgBr	$PhC{\equiv}C\text{-}I$	Cobaltous chloride	1-phenyl-hex-1-yne
BuMgBr	$PhC{\equiv}CBr$	Cuprous chloride	1-phenyl-hex-1-yne
BuMgBr	$PhC{\equiv}CBr$	No catalyst	phenyl acetylene butyl bromide
PhMgBr	$PhC{\equiv}CBr$	Cobaltous chloride	$PhC{\equiv}C\text{-}Ph$ $Ph(C{\equiv}C)_2Ph$
PhMgBr	$PhC{\equiv}CBr$	No catalyst	phenyl acetylene diphenylbutadiyne

Table (I)

Formation of alkyl and aryl-acetylenes by condensation reactions

Aryl and alkylmagnesium halides have been condensed with dichloroacetylene to give chloroacetylenes (XXXIII, R=p.CH_3-C_6H_4-, cyclohexyl, dodecyl) (61).

$$RMgX + Cl\text{-}C\equiv C\text{-}Cl \longrightarrow R\text{-}C\equiv C\text{-}Cl \quad (XXXIII)$$

However, it was reported that various haloacetylenes undergo metathesis with Grignard reagents.

$$R\text{-}C\equiv C\text{-}Br + R'MgBr \longrightarrow R\text{-}C\equiv C\,MgBr + R'Br$$

It has been reported in a few cases, that the addition of cobaltous chloride catalyses the condensation reaction. Thus, phenylhexyne (XXXIV) was obtained by the reaction of n-butylmagnesium bromide with 1-bromo-2-phenylacetylene, and phenylpropyne (XXXV) was prepared by the reaction of methylmagnesium bromide with 1-bromo-2-phenylacetylene (62)

$$PhC\equiv C\,Br + n\,Bu\,MgBr \longrightarrow PhC\equiv C\text{-}n\,Bu$$

$$PhC\equiv C\,Br + CH_3MgBr \longrightarrow PhC\equiv C\text{-}CH_3$$

However, in the absence of cobaltous chloride, the former reaction gave only phenylacetylene and n-butyl bromide by an exchange reaction. Table (1) shows some of the results obtained by Black, Horn and Weedon (58).

The use of sodium acetylides

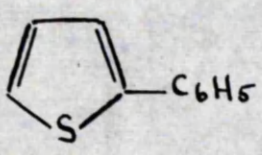
Except in liquid ammonia the sodium derivatives

of acetylenes are inert towards alkyl halides, and vigorous decomposition occurs at the high temperatures required to bring about attack of the halide by the alkynyl nucleophile (63). However, ready metathesis of sodium acetylides with alkyl halides occurs in liquid ammonia to produce substituted acetylenes. The reaction is restricted to primary halogeno-compounds containing the group $-CH_2CH_2X$, secondary, tertiary and primary derivatives branched at the second carbon atom undergo dehydrohalogenation to produce ethylenes (12).

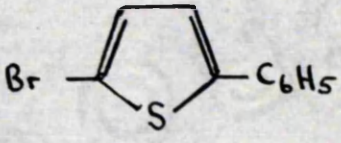
The reaction between allylic halides and sodium acetylide is complex, the initially formed acetylene readily undergoes metallation at the active methylene group, with further substitution leading to branched chain hydrocarbons (12).

Thus, it seemed likely that a Grignard reagent would provide the most efficient way of attaching a phenylethynyl group to the thiophene ring, and experiments were carried out accordingly. Several condensations were tried with the sodium salt of phenyl acetylene, both in organic solvents and in liquid ammonia. Kharasch and Fuchs (62) showed that when phenylmagnesium bromide and n-butyl bromide were allowed to react together, only diphenyl was obtained. The conclusion they drew from this fact was that the radicals formed from the Grignard reagent, if stabilised by resonance,

as in the case of the phenyl radical, would undergo self-coupling, rather than coupling with another radical. It was initially considered that thiophene radicals might be sufficiently stabilised to undergo coupling with phenyl radicals, in order to obtain 2-phenylthiophene (XXXVI), and 2-bromo-5-phenylthiophene (XXXVII). However the work Kharasch and Fuchs was substantiated by the results obtained. Thus from an attempted reaction between phenylmagnesium bromide and 2-bromothiophene, only diphenyl and unchanged bromothiophene were obtained, presumably because the phenyl radical is stabilised by resonance to a greater extent than the thienyl radical.



(XXXVI)



(XXXVII)

When bromobenzene was caused to react with 2-bromothiophene-5-magnesium bromide, a vigorous reaction occurred on addition of the catalyst, and the product obtained was a tar, containing bromine and sulphur, which was thought to arise from the self-condensation of 2-bromothiophene-5-magnesium bromide. Experimental conditions and results are summarised in table 4 , page 214-216.

In spite of the lack of success in these

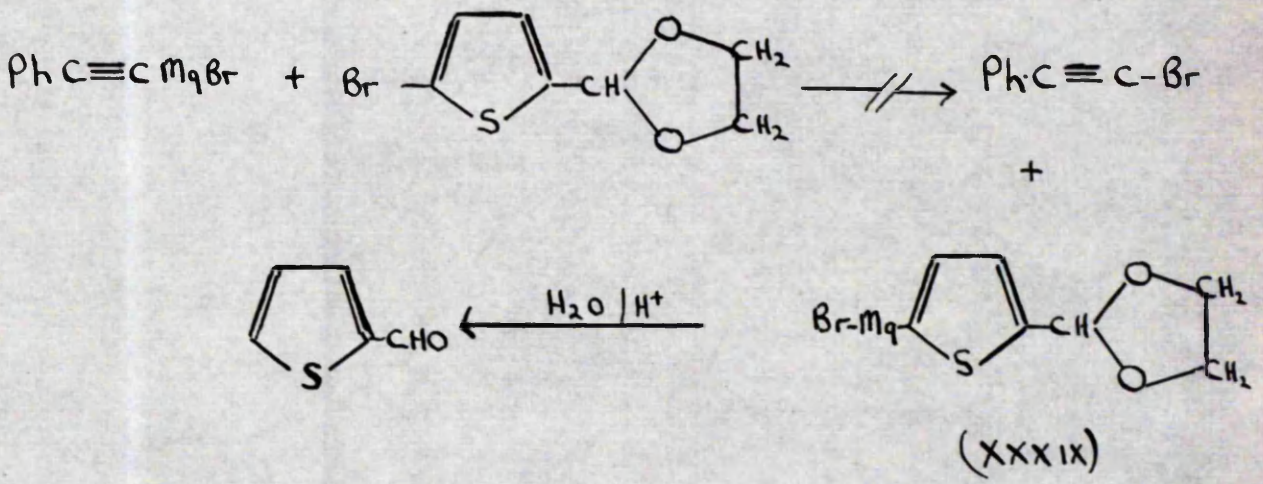
experiments, it was decided to attempt coupling reactions between phenylacetylene derivatives and thiophene compounds. The formation of the required phenylacetylene Grignard reagent, by the reaction of ethylmagnesium bromide with phenylacetylene, was confirmed by the isolation of phenylpropiolic acid (XXXVIII) in 80% yield on carboxylation:

$$PhC{\equiv}CH + C_2H_5MgBr \longrightarrow PhC{\equiv}CMgBr + C_2H_6$$

$$PhC{\equiv}CMgBr + CO_2 \longrightarrow Ph\text{-}C{\equiv}C\text{-}COOH \quad (XXXVIII)$$

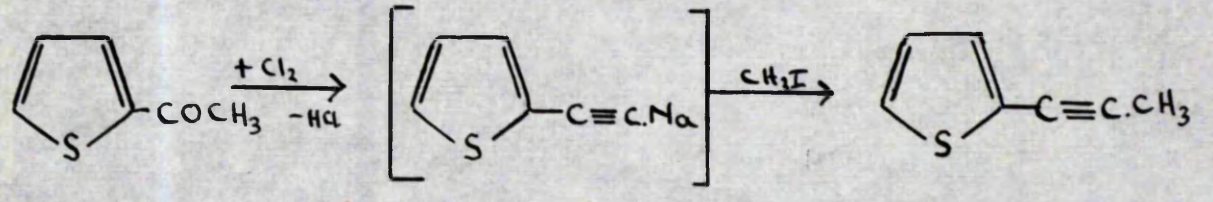
In a series of reactions, the bromoketal (XI) was added to a cooled solution of the Grignard reagent in ether, in the presence of 10 mole percent cobaltous chloride or, cuprous chloride (All reactions were carried out at moderate temperatures to avoid decomposition of phenylethynylmagnesium bromide). Reverse addition was employed in some cases, i.e. the Grignard reagent was added to a solution of the bromoketal in ether containing suspended catalyst. On occasion, traces of a solid were obtained which did not contain either sulphur or a carbonyl group, which was assumed to be diphenylbutadiyne, In no case did the expected condensation appear to take place, and there was no evidence for the formation of the required acetylenic thiophene compound. The reagents appeared to be unreactive, starting material being recovered in most cases. The conditions used,

together with methods of working up, are summarised in table 4, page 214. The possibility that a metathesis reaction, as discussed previously (page 190), could have occurred in these reactions was ruled out, because this would have led to the formation of the thiophene Grignard reagent (XXXIX). This latter on hydrolytic work-up would give 2-formylthiophene and none of this compound could be detected in the reaction products.



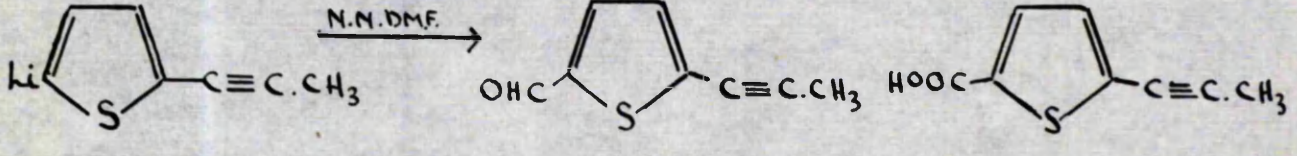
Similar, negative results were obtained in attempts to react sodium phenylacetylide with the bromoketal (XXXIX) in ether or in liquid ammonia. In both cases only 2-bromo-5-formylthiophene was obtained by hydrolysis of the reaction mixture. A similar attempt to couple the sodium salt of phenylacetylene with 2-bromothiophene in liquid ammonia also gave unchanged starting material. These results are included in table 4 (page 214-215).

Shortly after the commencement of this work, a synthesis of junipal was reported by Skattebøl (64), being obtained as an intermediate in the synthesis of (VII) (see page 174). The acetylenic function was introduced via 2-acetylthiophene (XL) by chlorination and dehydrochlorination (65). The ethynyl compound thus produced was not isolated, but reacted further, as the sodium salt, with methyl iodide to give 2-propynylthiophene (XLI).



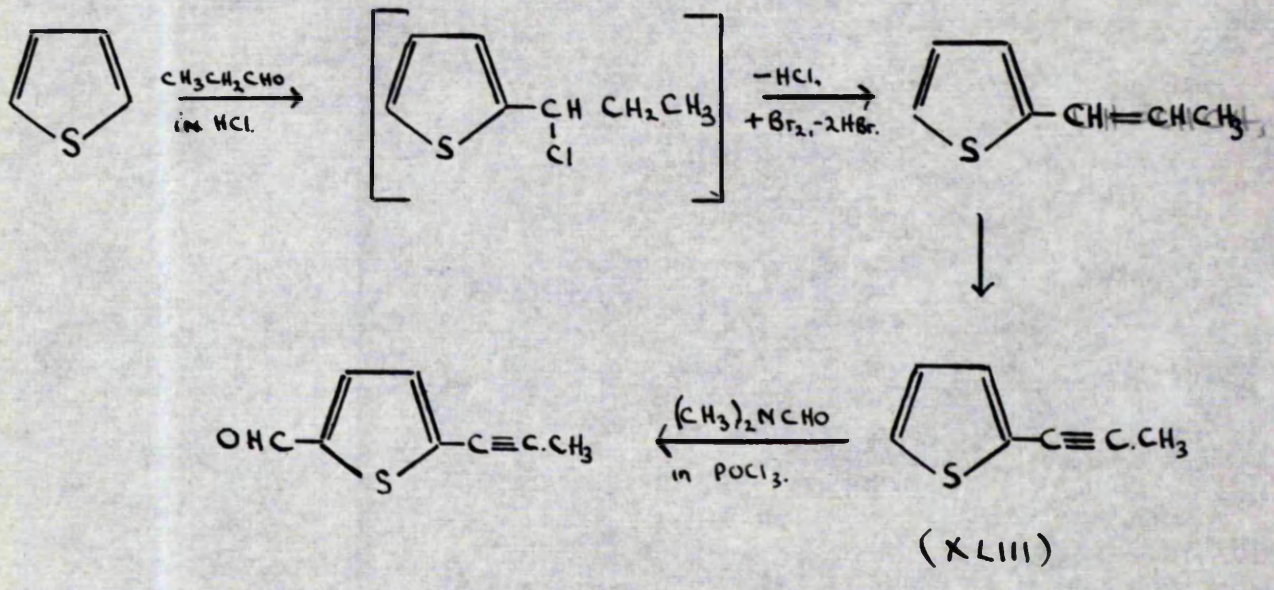
(XL) (XLI)

This was converted into the lithium compound (XLII), by treatment with butyllithium (66), and allowed to react with N,N-dimethylformamide (67) to give a product, which was identical with junipal. Carboxylation of the lithium compound (XLII) gave juniperic acid (IV).



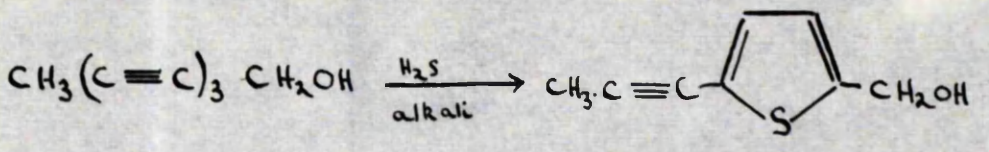
(XLII) (IV)

An alternative synthesis of junipal was reported by Schulte and Jantos (68), who first prepared 2-[α -propynyl]thiophene (XLIII) by reaction of thiophene with propionaldehyde, followed by dehydrohalogenation (69), bromination and dehydrobromination. This compound was successfully converted into junipal by formylation with N,N-dimethylformamide in phosphoryl chloride, and characterised as its 2,4-dinitrophenylhydrazone.



(XLIII)

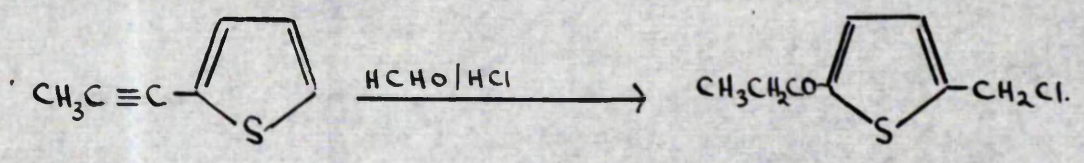
Among other 2,5-disubstituted thiophenes, Höpner (70) prepared α-terthienyl, β-[5-propynylthienyl-(2)]-acrylic acid, and the thienyl alcohol (XLIV) in good yield, by the action of alkaline hydrogen sulphide on the appropriate polyacetylenic compound. Junipal was prepared by oxidation of (XLIV), obtained by the reaction of octatriyne-(2,4,6)-ol-(1) (XLV) with alkaline hydrogen sulphide.



(XLV) (XLIV)

The laboratory synthesis of natural thiophene compounds by this route gives no insight into the actual biochemistry of the incorporation of sulphur into such compounds. It appears unlikely that the organism involved is able to use hydrogen sulphide directly in the reaction outlined above.

Recently, Janda has published an attempt to synthesise junipal (71) in a study of the chloromethylation of thiophene compounds (72, 73). However, it was shown that acetylenic side chains are susceptible to attack under the conditions of chloromethylation. The product obtained, therefore, on reaction of 2-propynylthiophene with formaldehyde in hydrochloric acid, was not junipal but 5-chloromethyl-2-propionylthiophene (XLVI).



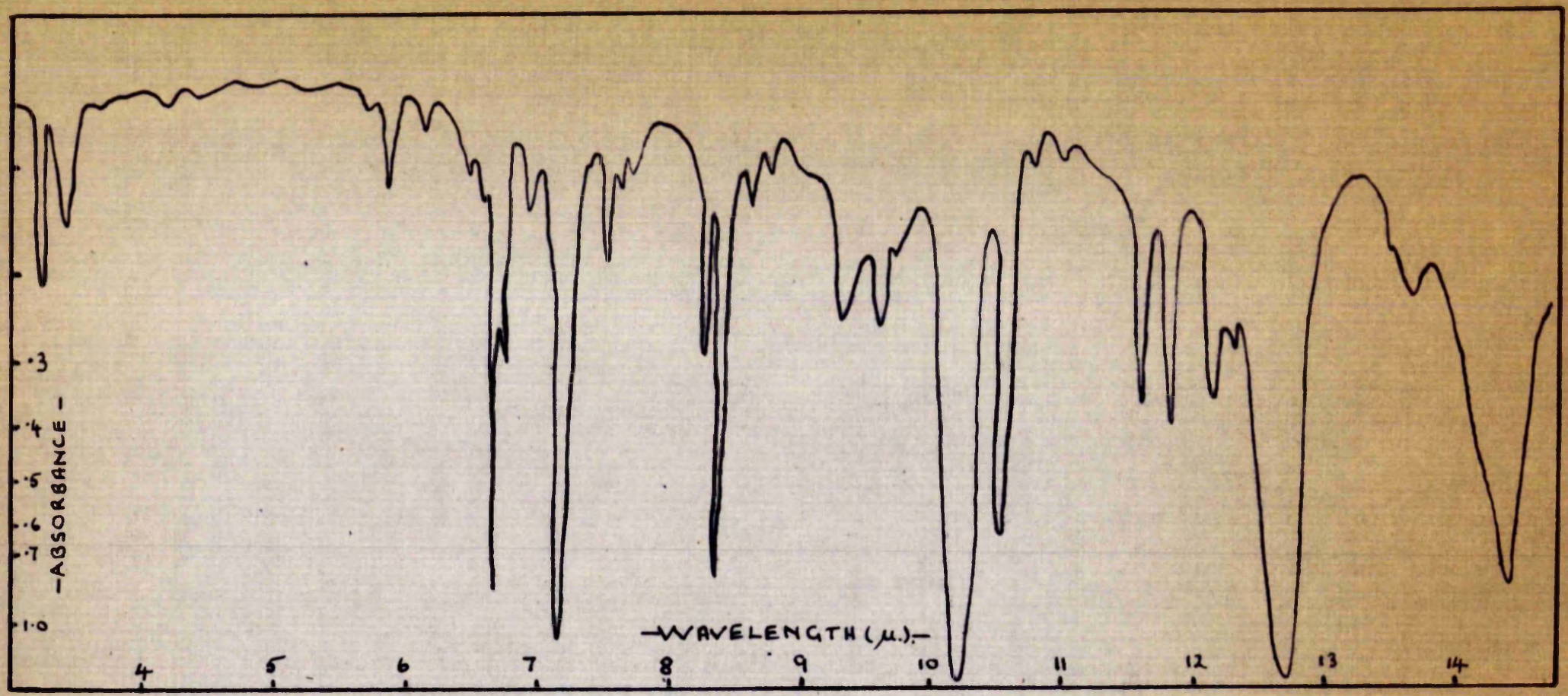
(XLVI)

PART III

EXPERIMENTAL

FIGURE 1.

THE INFRARED SPECTRUM OF 2,5-DIBROMOTHIOPHENE,
(LIQUID FILM).



2,5-Dibromothiophene

Was prepared by the method of Mozingo (14) and Lawesson (15). Fractional distillation of the crude product gave 2-bromothiophene (b.p. 74-76°/30 mm.) in 10% yield, and 2,5-dibromothiophene (b.p. 145°/50 mm) in 60% yield. Vapour phase chromatography of 2,5-dibromothiophene (2m. silicone "MS550" column, temp. 220°) showed a single peak with an elution time of 288 seconds. The infrared spectrum of 2,5-dibromothiophene is shown in Figure 1.

2-Bromothiophene-5-magnesium bromide (XII)

Anhydrous conditions under an atmosphere of nitrogen were used throughout. 2-Bromothiophene-5-magnesium bromide, used in subsequent experiments, was prepared as required, by one of the general methods recorded below and used immediately.

a. Direct preparation. Magnesium (1 mole) was activated by warming with a crystal of iodine, and a solution of 2,5-dibromothiophene (1 mole) in an equal volume of dry ether was added, and the mixture carefully warmed until the magnesium started to dissolve. Dry ether was then added (400 mls.) and the mixture heated under reflux until all the magnesium had dissolved (ca. five hours).

b. Entrainment method. Magnesium (5 moles) was activated by warming with a crystal of iodine and then heated gently

with ethyl bromide (b.p. 37-39°, 5 moles) in an equal volume of dry ether until a vigorous reaction commenced; the mixture was cooled and dry ether (2 litres) was added. The mixture was then heated under reflux and when all the magnesium had dissolved (ca. 5 hours), a solution of 2,5-dibromothiophene (1 mole), in an equal volume of dry ether, was added during 45 minutes with stirring, and the mixture heated under reflux for a further five hours.

2-Bromothiophene-5-carboxylic acid (XV)

Prepared according to the method of Lawesson (15) from 2-bromothiophene-5-magnesium bromide, m.p.138° (Lawesson reported m.p., 139°). Yield 65-80%.

Hydrolysis of 2-bromothiophene-5-magnesium bromide

A solution of the Grignard reagent (XII) in ether was prepared by methods (a) and (b) above (0.025 mole), and water (10 mls.) was added slowly with stirring. The ether layer was separated, dried ($MgSO_4$), and the solvent distilled in vacuo. The products were estimated by gas chromatography (2 m. silicone "MS550" column, temperature 220°). 2,5-Dibromothiophene (elution time, 288 secs), yield: (a) 30%, (b) 20% and 2-bromothiophene (elution time, 126 secs), yield: (a) 60% (b) 75%, were found to be present.

Attempted preparation of the diethylacetal of 2-bromo-5-formylthiophene

Table 2 summarises the widely differing conditions

Reaction of formic esters with Grignard reagents

Reactants		Method of prep. Grignard	Reaction Temp.	Reaction Time (hrs)	Method		Products
Ester	Grignard				Reaction	Work up	
1. EOF.	XII	Direct	Reflux	3	Direct	Hydrol	MBT DBT
2. EOF.	XII	Entrain.	Reflux	6	Direct	Hydrol	MBT DBT + PA
3. EOF.	XII	Entrain.	Reflux	24	Direct	Hydrol	MBT DBT + PA
4. EOF.	XII	Entrain.	Reflux	6	Direct	Hydrol	MBT + PA
5. EOF.	XII	Entrain.	Reflux	6	Direct	Distil of solvent	MBT + EOF
6. EOF (5)	XII	Direct	Reflux	1.5	Reverse	"	DBT MBT EOF
7. EF.	XII	Entrain.	Initial cooling Reflux	2	Direct	Hydrol	MBT
8. EF.	PhMgBr	Direct	Reflux	2	Direct	Hydrol	Secondary Alcohol
9. EF. (3)	PhMgBr	Direct	-50°	0.5	Reverse	Hydrol	PhCHO (40%)
10. EF. (3)	XII	Direct	-50°	0.5	Reverse	Hydrol	XIII (10%)
11. EF. (3)	XII	Direct	Reflux	0.5	Reverse	Hydrol	No XIII

Table 1

Key.to Table 1

EOF. = Ethylorthoformate, EF. = ethylformate, Roman numerals refer to compounds in the discussion of this work. Figures in parentheses in the first column refer to mole ratio of ester/Grignard reagent where not equal to 1. In the third column the method of preparation of the Grignard reagent (Direct or entrainment methods) is referred to. The sixth column refers to the method of addition of reagents, i.e. direct (by addition of ester to Grignard reagent), or reverse (by addition of Grignard reagent to ester). The seventh column refers to the method of working up the reaction mixture (either by normal hydrolysis of Grignard reaction mixtures, or distillation of the solvent).

MBT. = 2-bromothiophene, DBT. = 2,5-dibromothiophene, PA. = propionaldehyde diethylacetal (b.p. 113°)

---

used for reactions of (XII) with ethylorthoformate and ethylformate.

(1) to (4) To a boiling solution of the Grignard reagent (0.025 mole) in ether (page 199), a solution of ethylorthoformate (0.025 mole) in an equal volume of dry ether was added dropwise over thirty minutes. The mixture was then heated for a further period (table 1), cooled and treated with ammonium chloride solution (5%, 20 mls.). The ether layer was separated, dried ($MgSO_4$), and the solvent removed <u>in vacuo</u>. The residual liquid was then distilled.

(5) and (6). The reaction was carried out as above, but was worked up by concentrating the reaction mixture to low volume (5 ml). After treatment with ammonium chloride solution, the product was isolated in the usual manner.

Reaction of phenylmagnesium bromide with ethylformate

1. This was carried out by the method of Gatterman (27, 28), to give benzaldehyde (b.p., 58°/8 mm.), yield: 40% [(9) table 2].

2. In addition, the following experiment was carried out. Magnesium (3.06 g.) was activated with iodine, and a solution of bromobenzene (20 g.), in an equal volume of dry ether, was added slowly with stirring. The mixture was warmed until a vigorous reaction commenced, then cooled, and dry ether (30 mls.) was added. The mixture was heated under reflux for two hours, when all the magnesium had dissolved. This solution was cooled in an ether-solid carbon dioxide bath, and ethylformate (b.p. 54-56°, 9.5 g.) in an equal volume of dry ether was added slowly with stirring, during thirty minutes. The solution was then allowed to warm to room temperature, hydrolysed with dilute hydrochloric acid, and the ether layer separated. The solvent was distilled from the dried ($MgSO_4$) ether solution, yielding a viscous oil (10 g.), which gave no positive test for an aldehyde group.

Reaction of (XII) with ethylformate

A solution of the Grignard reagent (XII, 11.0 g.) in dry ether (prepared by the direct method as described on page 199) was added dropwise with stirring, during thirty minutes to a cooled (ether-solid carbon dioxide) solution

of ethylformate (9.2 g.) in an equal volume of dry ether. The viscous solution was allowed to warm to room temperature, hydrolysed with dilute hydrochloric acid, and the ether layer separated. The aqueous layer was extracted with ether, the combined ethereal solutions dried ($MgSO_4$), and the solvent distilled <u>in vacuo</u>. The product (8.0 g.), which gave a positive test with Brady's reagent, was shown by vapour phase chromatography (2 m. silicone "MS.550" column, temperature 220°) to contain 2,5-dibromothiophene (39%), 2-bromothiophene (15%), and a new product, elution time 486 seconds, estimated yield 10%.

The crude product (8 g.) in ethyl alcohol (10 mls.) was treated with a solution of phenylhydrazine hydrochloride (0.5 g.) and sodium acetate (0.8 g.) in water (5 ml.), and warmed at 80° for half an hour. The solution was cooled and the solid filtered off; one crystallisation from ethyl alcohol gave yellow plates, m.p. 112° (lit. m.p. 111-112° (49) of the phenylhydrazone of 2-bromo-5-formylthiophene) overall yield 8%.

Attempts to increase the yield of aldehyde, by using longer reaction periods and higher temperatures led only to decreased yields of aldehyde (table 2 page 200-201).

Preparation of 2-bromo-5-chloromethylthiophene (XIX, R = Cl)

This was carried out by methods reported in the patent literature (39,40,41). The best yield of (XIX, R = Cl) was obtained as follows.

A solution of formalin (37% w/v.) in concentrated hydrochloric acid (1:1, 5 mls.), previously saturated with hydrogen chloride gas at 0-10°, was added dropwise during two hours to a stirred mixture of 2-bromothiophene (8.8 g.) and anhydrous zinc chloride (0.1 g.), through which hydrogen chloride gas was passed, at 35-40°. The mixture was diluted with water (50 mls.), extracted with ether, the ethereal solution washed with sodium bicarbonate solution (3%) and dried ($CaCl_2$). The solvent was removed in vacuo and the brown oil distilled (through a 9" Vigreux column) to give 2-bromo-5-chloromethylthiophene, b.p. 122-125°/14 mm., in 53% yield.

Found, C, 28.9; H, 1.7%., C_5H_4BrClS requires C, 28.4; H, 1.9%.

A quantity of 2-bromothiophene was recovered as fore-run (1.5 g., 17%).

The conditions used and yields obtained in other similar chloromethylations are recorded in table 3.

Chloromethylation of 2-bromothiophene.

Ref	Proportion of Reagents MBT(g)	HCHO(mls)	Solvent and Volume (mls)	Catalyst	Reaction Time and Temp.	Yield
40	10	6.15	HCl.(5.8)	-	2½ hrs. 0-5°.	20%
41	8.8	8	HCl.(8)	$ZnCl_2$	2 hrs., 35-40°	53%
39	43	19	HCl.(23) + Light petroleum (0-40°)	$ZnCl_2$	3 hrs., 0-10°	22%

Table 3.

Key MBT = 2-bromothiophene

HCHO = formaldehyde solution (37% w/v.)

Ref. 39 third column, light petroleum was used to remove the product from formaldehyde solution in attempts to increase yield.

In all experiments the solvents were saturated with hydrogen chloride gas, see references.

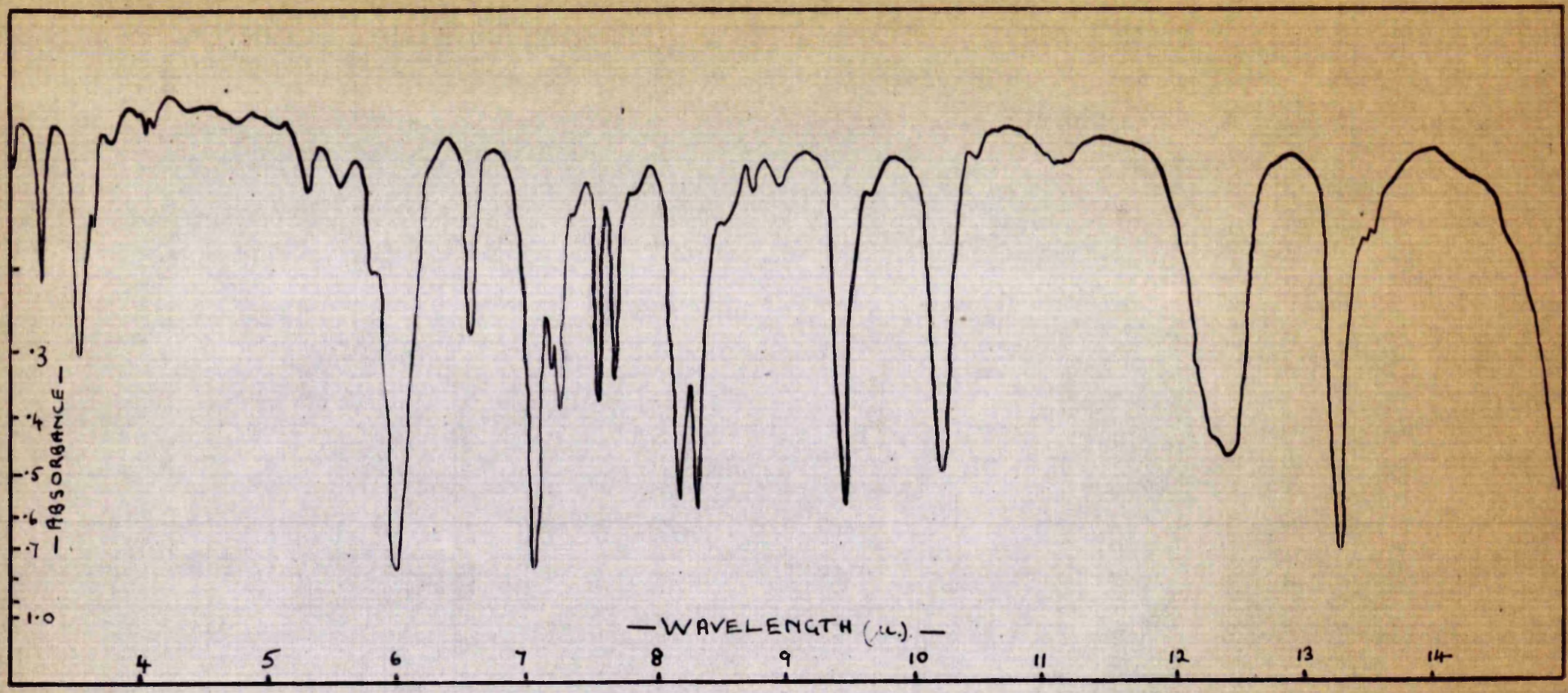
The conversion of 2-bromo-5-chloromethylthiophene into 2-bromo-5-formylthiophene (XIII)

2-Bromo-5-chloromethylthiophene (XIX, R = Cl) (33.3 g.) was dissolved in chloroform (120 mls.), and heated under reflux with hexamethylene tetramine (23 g.) for two hours. The mixture was cooled and the white solid filtered off. The

FIGURE 2.

THE INFRARED SPECTRUM OF 2-BROMO-5-FORMYLTHIOPHENE.

(LIQUID FILM)



air dried product (54 g; 98%), was dissolved in hot water (250 mls.), steam distilled rapidly, and the distillate (2 litres) acidified (dilute hydrochloric acid), saturated with salt and extracted with ether. The ethereal solution was dried ($MgSO_4$) and evaporated to give 2-bromo-5-formylthiophene (6.9 g., 25%), b.p. 89-92°/5 mm, which was characterised as its phenylhydrazone, m.p. 112°. Gronowitz (49) gives m.p. 111-112°. The infrared spectrum of the aldehyde is shown in Figure 2.

N-methylformanilide

- was prepared as reported (47), b.p. 118-125°/ 9 mm.yield 90%.

2-formylthiophene

-was prepared according to the method of Nord and King (48). The crude product (yield ca. 61%) was not purified but was brominated directly, as below.

Bromination of 2-formylthiophene

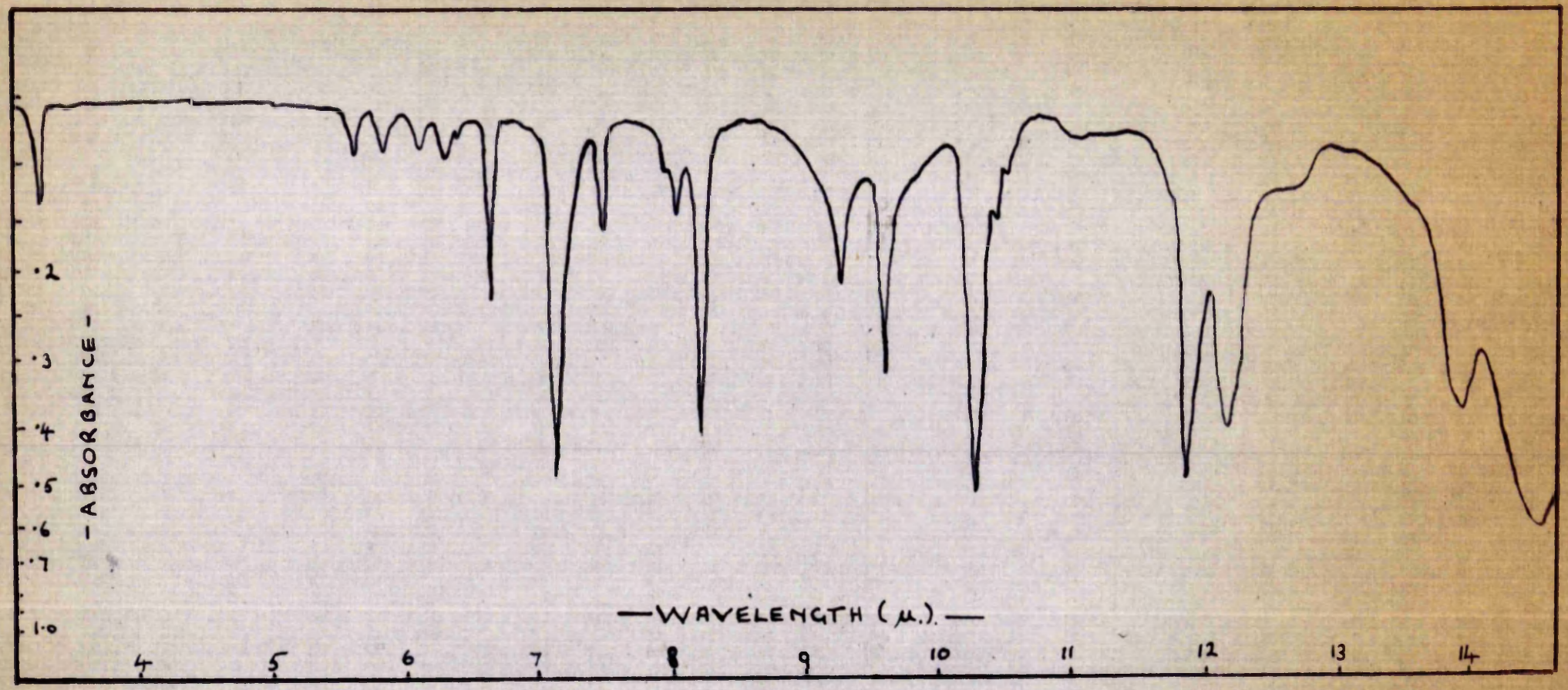
-was carried out by the method of Gronowitz (49), fractional distillation of the crude product gave 2-bromo-5-formylthiophene b.p. 82-85°/3 mm. in 55.5% yield.

Preparation of 2-bromothiophene

This was obtained as a by-product in the preparation of 2,5-dibromothiophene.

FIGURE 3.

THE INFRARED SPECTRUM OF 2-BROMOTHIOPHENE,
(LIQUID FILM).



Further samples were obtained by the methods of Buu-Hoi (74) and Krauze and Renwanz (75), b.p. 50°/15 mm., in yields of 20% and 50% respectively.

The infrared spectrum of 2-bromothiophene is shown in Figure 3.

Formylation of 2-bromothiophene

Was carried out by the method of Nord and King (48), fractional distillation of the crude product gave 2-bromo-5-formylthiophene, b.p. 80-83°/1 mm., in 50% yield.

The ethyleneketal (XI) of 2-bromo-5-formylthiophene

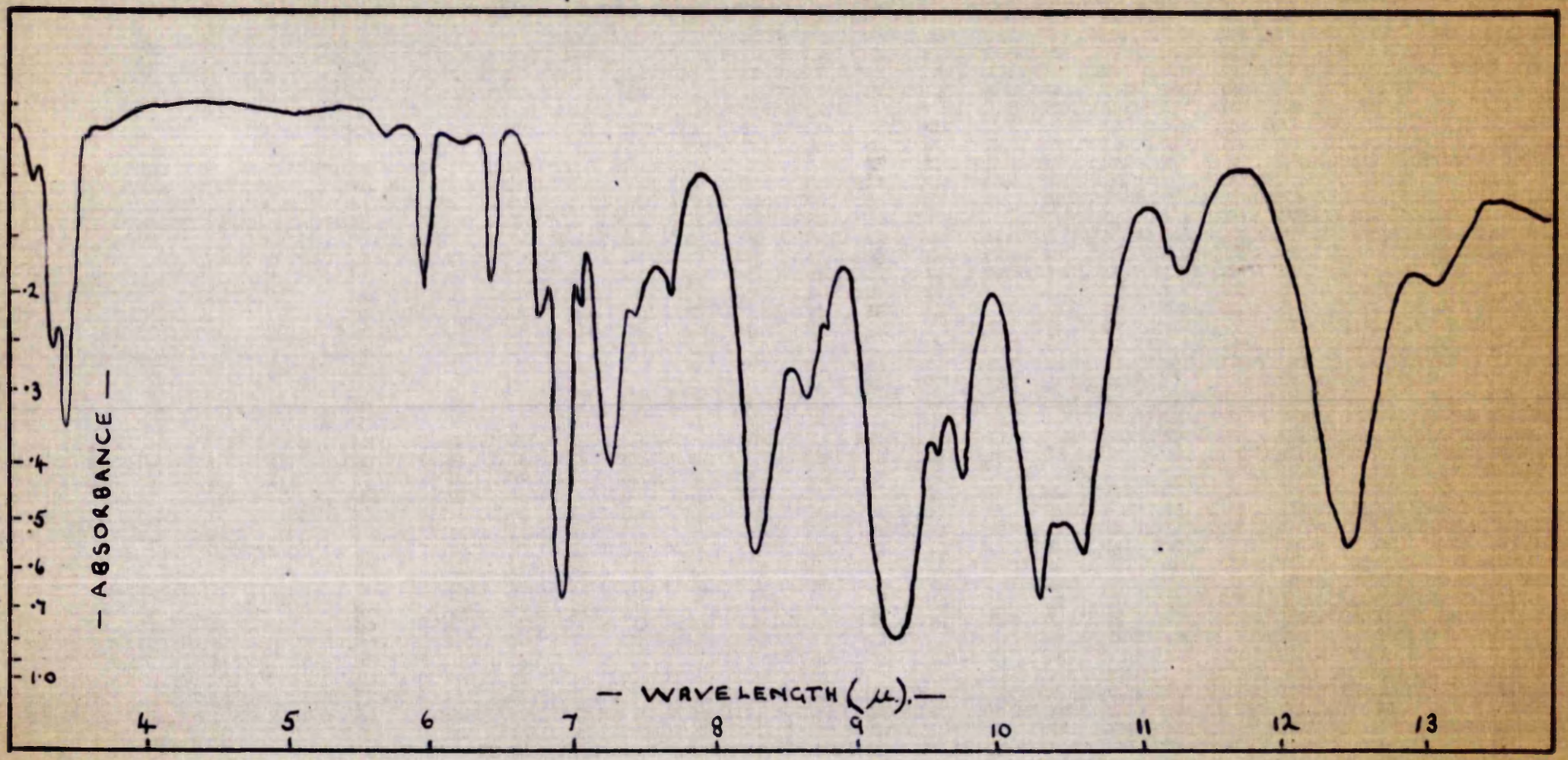
A mixture of 2-bromo-5-formylthiophene (7.0 g.), ethylene glycol (b.p. 194°, 2.5 g.) and toluene-p-sulphonic acid (0.2 g.), in dry benzene (20 mls.), was heated under reflux for five hours, the water formed being separated, in a Dean and Starke apparatus, as the benzene azeotrope. The reaction mixture was then cooled, anhydrous potassium carbonate (0.5 g.) added, and allowed to stand for twelve hours. After filtration, the benzene was distilled in vacuo. Distillation of the residue gave 2-bromo-5-formylthiophene ethylene ketal, (7.7 g., 90%) b.p. 109-116°/3-4 mm.

Found: C, 35.6; H, 2.8%, $C_7H_6O_2BrS$ requires C, 35.8; H, 3.0%.

The infrared spectrum of this compound is shown in Figure 4.

FIGURE 4

THE INFRARED SPECTRUM OF THE ETHYLENE KETAL OF 2-BROMO-5-FORMYL THIOPHENE (LIQUID FILM).



Attempted coupling reactions

All reactions were carried out in an atmosphere of nitrogen.

1. The coupling of phenylmagnesium bromide and 2-bromothiophene

To a solution of phenylmagnesium bromide in ether, prepared from bromobenzene (9.6 g.) and magnesium (1.5 g.) as described on page 201, was added cobaltous chloride (0.4 g., previously dried at 150°/4 mm.). A solution of 2-bromothiophene (10 g.) in an equal volume of dry ether was then added dropwise, with stirring at room temperature, during half an hour. The dark brown mixture was heated under reflux for five hours, cooled and hydrolysed with sulphuric acid (2N). The ether layer was separated, and the aqueous layer extracted with ether. The combined ethereal solutions were dried ($MgSO_4$), and the solvent removed in vacuo. Distillation of the residue gave recovered 2-bromothiophene (6.0 g., 60%) and diphenyl (3.0 g., 64%) identified by its infrared spectrum and mixed melting point with an authentic sample.

2. The coupling of bromobenzene with 2-bromothiophene-5-magnesium bromide

A solution of 2-bromothiophene-5-magnesium bromide in ether was prepared from 2,5-dibromothiophene (15.g.) and magnesium (1.6 g.) as described in (a), page 199 . Anhydrous cobaltous chloride (0.4 g.) was added to the cooled solution,

when a vigorous reaction ensued, and then bromobenzene (15.6 g.) was added rapidly with stirring and the mixture heated under reflux for two hours. The mixture was cooled, hydrolysed with 2N. sulphuric acid (a black tar precipitated at this point), the ether layer separated and the aqueous layer extracted with further ether, the combined ethereal solutions were dried ($CaCl_2$), and the solvent removed *in vacuo*. Distillation of the dark red residue gave bromobenzene b.p. 34°/5 mm (11 g.) in 70% yield and 2,5-dibromothiophene, b.p. 70-74°/15 mm., (4.9 g., recovered 39%) confirmed by comparison of infrared spectra with spectra of genuine samples.

The black tar gave positive tests for bromine and sulphur, but no purification was achieved.

Preparation of phenylethynylmagnesium bromide (XXVII) and its conversion to phenylpropiolic acid (XXXVIII)

Magnesium (0.82 g.) was activated by warming with a crystal of iodine, and a solution of ethyl bromide (b.p. 37-39°, 3.7 g.), in an equal volume of dry ether, was added and the mixture warmed until a vigorous reaction commenced. The mixture was cooled and dry ether (30 mls.) was added. The mixture was then heated under reflux until all the magnesium had dissolved (*ca* 5 hours), and then a solution of phenylacetylene (b.p. 21.5/4mm., 3.9 g.) in an equal volume of dry ether was added to the cooled solution during fifteen minutes with stirring. The resulting solution was allowed to stand

until evolution of gas had ceased (1 hour), and then heated under reflux for three hours.

The solution of the acetylenic Grignard reagent was then poured on to an excess of solid carbon dioxide, the mixture allowed to reach room temperature, hydrolysed with hydrochloric acid (2N.), and the yellow ether layer separated. The aqueous layer was extracted with a further quantity of ether, the combined solutions dried ($MgSO_4$) and the solvent distilled in vacuo. The resultant yellow oil crystallised on standing and the solid was recrystallised from water to give colourless needles, m.p. 133-134° (lit, m.p. 135-136°), in yields of 80%.

3. Coupling of phenylethynylmagnesium bromide with ketal (XI)

a. A solution of phenylethynylmagnesium bromide in ether was prepared from ethyl bromide (3.7 g.), magnesium (0.82 g.), and phenylacetylene (3.89 g.) as described on page 209.

This solution was cooled (ice-salt), and anhydrous cobaltous chloride (0.3 g.) added with stirring, followed by a solution of the ketal (XI) (8.0 g.) in an equal volume of dry ether during one hour. The mixture was stirred at room temperature for twelve hours, and hydrolysed by addition of ammonium chloride solution (10%, 20 mls.). The ether layer was separated and the aqueous layer extracted with further quantities of ether. The combined ethereal solutions were

dried ($MgSO_4$) and the solvent removed *in vacuo*, the red oil obtained being distilled to give phenylacetylene, b.p., 26°/4 mm., (2 g., 60%) and bromoketal (XI), b.p. 126-130°/3-4 mm., (5 g., 62.5%). The identity of these fractions was confirmed by a comparison of their infrared spectra with those of the genuine materials.

Prior to distillation, the crude reaction mixture on standing, gave traces of a brown solid, m.p. 73-76°, which gave no positive test for sulphur and did not contain an aldehyde group, (diphenylbutadiyne, lit. m.p. 88°).

There remained in the flask, after distillation, a high boiling residue, which decomposed on attempted distillation.

b. The above reaction was modified as follows:

A solution of the Grignard reagent in ether was cooled in a solid carbon dioxide-methylated spirits bath, and after addition of cobaltous chloride a solution of ketal (XI) in ether was added slowly with stirring, over two hours. The mixture was then allowed to warm to room temperature, and heated under reflux for two hours. After cooling, the mixture was hydrolysed with hydrochloric acid (5N., 10 mls.), the ether layer separated, and the aqueous layer saturated with salt and extracted with ether. The solvent was removed from

the dried ($MgSO_4$) ethereal solutions to yield a brown oil.

Sodium bisulphite solution (a saturated aqueous solution containing 70% ethanol; 15 mls.) was added to the oil, and the solid obtained by refrigeration of the mixture was filtered, washed with a little alcohol and ether, air dried and shaken with sodium carbonate solution (2N., 30 mls.). The solution was extracted with ether, the ethereal solution dried ($MgSO_4$) and the solvent removed <u>in vacuo</u> to yield a yellow oil (6.0 g.) which when distilled gave 2-bromo-5-formylthiophene b.p. 86-87°/3-4 mm., (5.6 g., 78% yield). The absence of aldehydic and acetylenic components in the ether extracted steam distillate was shown by physical methods.

c. A further modification of the reaction required the use of the reverse addition technique:

An ethereal solution of phenylethynylmagnesium bromide was prepared from magnesium (1.0 g.), ethyl bromide (3.7 g.) and phenylacetylene (3.0 g.) as described, page 209

This solution was added dropwise at 0°C to a stirred suspension of anhydrous cobaltous chloride (0.3 g.) in the bromoketal (XI) (6.3 g.). On completion of addition, the mixture was heated under reflux for three hours, cooled, hydrolysed with hydrochloric acid (2N., 10 mls.) and the

ether layer separated. The aqueous layer was extracted with ether, the combined ethereal solutions dried ($MgSO_4$) and the solvent removed in vacuo to yield a yellow oil (10 g.).

Treatment of the oil with sodium bisulphite, as described above, gave 2-bromo-5-formyl thiophene (3.4 g., 59%), as indicated by infrared spectroscopy.

d. The reaction was repeated exactly as above, using cuprous chloride (0.3 g.) in place of cobaltous chloride as catalyst. Again only 2-bromo-5-formylthiophene was obtained.

Table 4, page 214-215, summarises the conditions used and results of the coupling reactions.

Attempted coupling with metal acetylides

a. Phenylacetylene (3.5 g.) was added dropwise to small pieces of sodium (0.8 g.) covered with dry ether (40 mls.); hydrogen was evolved and a white suspension formed. The mixture was gently heated under reflux for two hours, then the ketal (XI) (8 g.) was added dropwise in an equal volume of dry ether, and the suspension heated under reflux for two hours. Water (10 mls.) was then added dropwise with vigorous stirring, the ether layer was separated and the aqueous layer extracted with ether. The combined ethereal solutions were dried ($MgSO_4$) and the solvent distilled in. vacuo.

The residual oil (9.0 g.), which gave no positive test for an aldehyde group, was hydrolysed by shaking at room temperature with hydrochloric acid (2N., 15 mls.), extracted with ether, and the ether layer separated. The solvent was removed from the dried ($MgSO_4$) ethereal solution, giving a yellow oil (8.0 g.) which gave a positive test with Brady's reagent.

The aldehyde fraction of this oil (5.5 g., ca. 60%), obtained by treatment with sodium bisulphite as described above, was shown by infrared spectroscopy to be 2-bromo-5-formylthiophene.

b. A suspension of sodium phenylacetylide in ether was prepared from phenylacetylene (7.7 g.) and sodium (1.7 g.) as described above. Most of the ether was removed in vacuo and liquid ammonia (140 mls.) was added. The ethyleneketal (XI) (14.1 g.) was added dropwise during one hour with stirring. After five hours, the ammonia was allowed to evaporate, and the residual yellow solid hydrolysed carefully by addition of hot water, to produce a red solution which was acidified to litmus (2N.HCl), saturated with salt and extracted with ether. The solvent was removed from the dried ($MgSO_4$) ethereal solution in vacuo, to give a yellow oil (15.g.).

The aldehyde fraction of this oil (9 g., ca, 55%),

Attempted coupling reactions.

Reactants	Solvent and Catalyst	Reaction temp.	Reaction time (hrs.)	Isolated Products
PhMgBr MBT	Ether $CoCl_2$	Reflux	5	diphenyl + MBT
PhBr Br.Th.MgBr	Ether, $CoCl_2$	Reflux	2	Tar + PhBr
Phc=cMgBr (XI)	Ether, $CoCl_2$	a)-5 to 0° b)Room temp.	a) 1 b) 12	Phc=CH and the ketal (XI)
Phc=cMgBr (XI)	Ether, $CoCl_2$	a)ca.-60° b)reflux	a) 2 b) 2	2-bromo-5-formylthiophene
Phc=cMgBr (XI) reverse addition	Ether, $CoCl_2$	a)-5 to 0° b)reflux	a)45 mins b)3.5	2-bromo-5-formylthiophene
Phc=cMgBr (XI)	Ether, Cu_2Cl_2	a)-5 to 0° b)reflux	a)45 mins b)3.5	2-bromo-5-formylthiophene
Phc=cNa (XI)	Liquid ammonia	reflux	5	2-bromo-5-formylthiophene
Phc=cNa (XI)	Ether	reflux	2	2-bromo-5-formylthiophene

Key

PhMgBr = phenylmagnesium bromide.

MBT = 2-bromothiophene.

PhBr = bromobenzene.

Br.Th.MgBr = 2-bromothiophene-5-magnesium bromide.

Roman numerals refer to compounds in text.

Table 4

separated by treatment with sodium bisulphite as described above, was shown by infrared spectroscopy to be 2-bromo-5-formylthiophene.

All melting points were taken on a Kofler block and are uncorrected.

All infrared absorption spectra were measured on a Perkin Elmer 'infracord' spectrophotometer.

Gas chromatography was carried out using a Perkin Elmer fractometer, no. 116.

REFERENCES

1. Sease and Zechmeister, J.Amer.Chem.Soc.,1947,69,270,273.
2. Birkinshaw and Chaplen, Biochem,J., 1955,60,255.
3. Sörensen, M.A. Proc.Chem.Soc.,1961,98.
3a. Sörensen and Sörensen, Acta Chem.Scand.,1958,12,771.
4. Guddal and Sörensen, Acta Chem.Scand.,1959,13,1185.
5. Challenger and Holmes, J., 1953,1837.
6. Schulte Reische and Hörner, Angew, Chem.,1960,72,920.
7. Jones, Chem.Eng.News,1961,39,46.
8. Flemming and Harley-Mason, Proc.Chem.Soc., 1961,245.
9. Craig and Royle, Proc.Chem.Soc., 1962,149.
10. Fleming and Harley-Mason, Chem.and Ind., 1962,560.
11. Keskin, Miller and Nord, J.Org.Chem., 1951,199.
12. Raphael, "Acetylenic compounds in organic synthesis", Butterworths, 1955.
13. Heath, Technical memorandum no.R.P.13155. Royal Aircraft Establishment, Westcott, and private communication.
14. Mozingo et.al., J.Amer.Chem.Soc.,1945,67,2092.
15. Lawesson, Arkiv Kemi, 1957,11,373.
16. Lawesson, Arkiv Kemi, 1957,11,317.
17. Steinkopf, Annalen, 1934,512,136.
18. Grignard, Compt.rend., 1934,198,625.
19. Urion, Compt,rend., 1934,198,665.

20. Bodroux, Compt.rend., 1904,138,92.

21. Tschitschibabin, Ber., 1904,37,856.

22. Tschitschibabin and Jelgasin, Ber., 1914,48,1843.

23. Bodroux, Compt.rend., 1904,138,700.

24. Wood and Comely, Chem.and Ind., 1923,42,429T.

25. Smith and Baylis, J.Org.Chem., 1941,6,437.

26. Smith and Baylis, J.Org.Chem., 1941,6,489.

27. Gatterman, Annalen, 1912,215,230.

28. Gatterman, Ber., 1903,36,4153.

29. Barger and Easson, J., 1938,2100.

30. du Vigneaud et.al., J. Biol.Chem., 1945,159,387.

31. Délépine, Bull.Soc.Chim.France, 1895,13 iii 358.

32. Sommelet, Compt.rend., 1913,157,852.

33. Sommelet, Bull.Soc.chim.France, 1915,17,82.

34. Sommelet, Bul.Soc.chim.France, 1917,23,96.

35. Angyal and Rassack, J, 1949,2700.

36. Graymore and Davies, J, 1945,293.

37. Dunn, Waugh and Dittmer, J.Amer.Chem.Soc., ,68,2118.

38. Wiberg and McShane, Org.Synth., 1949 ,29,31.

39. U.S.Patents, 2,425,721.

40. U.S.Patents, 2,639,285.

41. U.S.Patents, 2,776,980.

42. King and Nord, J.Org.Chem., 1948,13,635.

43. Weston and Michaels, J.Amer.Chem.Soc., 1950,72,1422.

44. Cram and Hammond, "Organic Chemistry", McGraw Hill 1959, 381.

45. Tyson and Shaw, J. Amer. Chem. Soc., 1952, 74, 2273.

46. Emerson and Patrick, U.S. Patents, 1952, 2, 581, 009.

47. Fieser and Jones, Org. Synth. 1940, 20, 66.

48. Nord and King, J. Org. Chem., 1948, 13, 635.

Nord and King, J. Org. Chem., 1949, 14, 405.

49. Gronowitz, ArkivKemi, 1955, 8, 87.

50. Délépine, Bull. Soc. chim. France, 1900, 23, 915.

51. Haworth and Lapworth, J., 1922, 121, 81.

52. Salmi, Ber., 1938, 71, 1803.

53. Grignard and Perrichon, Ann. Chim. (France), 1926, 5, 13.

54. Grignard and Tchéoufait, Compt. rend., 1929, 188, 357.

55. Black and Weedon, J, 1953, 1785.

56. Daneby, Killiam and Nieuwland, J. Amer. Chem. Soc., 1936, 58, 611.

57. Grignard and Lapayre, Bull. Soc. chim. France, 1928, 43, 141.

58. Black, Horn & Weedon, J., 1954, 1704.

59. (Reviews by) Hey, Ann. Reports, 1944, 41, 195. 1948, 45, 160.

60. Kharasch and Fuchs, J. Org. Chem., 1945, 10, 292.

61. Ott and Bassaller, Ber., 1943, 76, 88.

62. Kharasch and Fuchs, J. Org. Chem., 1945, 10, 298.

63. Jacobs, Org. Reactions, 1949, 33, 1.

64. Skattebøl, Acta. Chem. Scand. 1959, 13, 1460.

65. Nord and Vaitiekunas, J. Org. Chem., 1954, 19, 902.

66. Gilman and Morton, Org. Reactions, 1954, 8, 258.

67. Evans, J., 1956,4691, and Chem.and Ind., 1957,1596.

68. Schulte and Jantos, Arch.Pharm., 1959,292,536.

69. Emerson and Patrick, J.Org.Chem., 1948,13,729.

70. Hörner, Angew.Chem., 1962,1,58.

71. Janda and Dvorak, Coll.Czech.Chem.Comm.,(1962) 27, 372.

72. Janda, Coll.Czech.Chem.Comm.,1960,25,1058.

73. Janda, Coll.Czech.Chem.Comm.,1961,26,1889.

74. Buu-Hoi, Annalen,1944,5561.

75. Krause and Renwanz, Ber., 1929,62,1710.