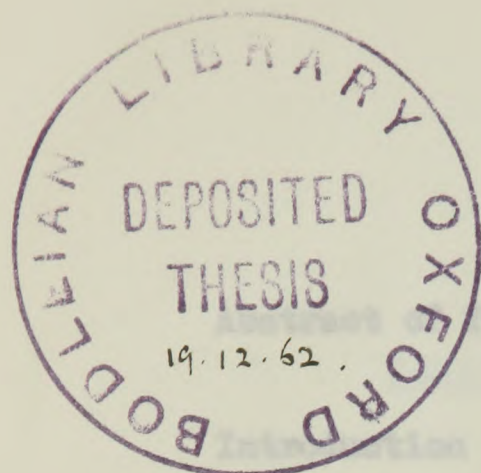




POLYACETYLENES from MICRO-ORGANISMS

Page

(1)

A Thesis submitted to the

Board of the Faculty of Physical Sciences

The Correlation of Optical Rotations in the
Absolute Configuration - A Summary

21

University of Oxford

The Polyacetylenes from Glomus bicolor - Discussion

27

for the

The Polyacetylenes from Glomus bicolor - Experimental

31

Degree of Doctor of Philosophy

The Isomerisation of the Allenic Metabolites from

Glomus bicolor

by

The Polyacetylenes from Glomus bicolor - Discussion

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B. E. Lowe, B.Sc. (Lond.)

The Polyacetylenes from Glomus bicolor - Experimental

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Appendix - The Synthesis of

Deca-2,4,6-triyno-1,10-dioic Acid

101

The Queen's College

The Dyson Perrins Laboratory,

Oxford

June, 1962

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I am also very much indebted to Dr. G. Lowe for all his thoughtful guidance and encouragement.

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Finally, I wish to acknowledge receipt of a grant from the Department of Scientific and Industrial Research.

Abstract of Thesis

More than fifty polyacetylenic compounds produced by micro-organisms have been isolated and characterised since the discovery of the allenic polyacetylene, mycomycin, in 1952. A review of the sixteen natural allenic polyacetylenes which have been isolated and their structures elucidated, is included. This review is followed by a summary of the methods published by Brewster in 1959 for the correlation of observed optical rotations with the configurations of compounds having simple asymmetric centres.

Four allenic polyacetylenes (I-IV) have been isolated from the culture medium of the fungus Odontia bicolor. In addition,

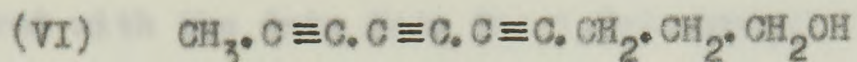
- (I) $\text{HC}\equiv\text{C}.\text{C}\equiv\text{C}.\text{CH}=\text{C}=\text{CH}.\text{CH}_2.\text{CH}_2.\text{CH}_2\text{OH}$
 (VII) (II) $\text{HC}\equiv\text{C}.\text{C}\equiv\text{C}.\text{CH}=\text{C}=\text{CH}.\text{CH}_2.\text{CH}_2.\text{CH}_2.\text{CH}_2\text{OH}$
 (III) $\text{HC}\equiv\text{C}.\text{C}\equiv\text{C}.\text{CH}=\text{C}=\text{CH}.\text{CH}(\text{OH}).\text{CH}_2.\text{CH}_2\text{OH}$
 (IV) $\text{HC}\equiv\text{C}.\text{C}\equiv\text{C}.\text{CH}=\text{C}=\text{CH}.\text{CH}(\text{OH}).\text{CH}_2.\text{CH}_2.\text{CH}_2\text{OH}$
 (VIII)

substantial evidence has been presented for assigning the structure isolated from micro-organisms to being the allenic polyacetylene (V) to a non-allenic polyacetylene which is also produced by the fungus.

- (V) $\text{HC}\equiv\text{C}.\text{C}\equiv\text{C}.\text{CH}=\text{CH}.\text{CH}=\text{CH}.\text{CH}_2.\text{CH}_2.\text{CH}_2\text{OH}$

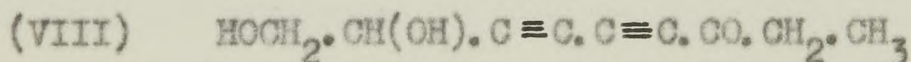
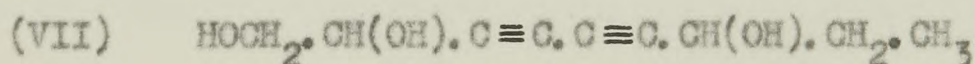
The allenic metabolites isomerised readily in dilute alkali to form triynes since the allene group in each case is conjugated to a diacetylene unit. The triynes formed from the diols had a terminal ethynyl group, whereas those formed from the alcohols had no terminal

ethynyl group. The isomerised C_{10} alcohol (I) was shown to be identical to synthetical specimen with the structure (VI). The mechanisms of these isomerisations are discussed.



An isopropylidene derivative of the C_{10} 1,3-diol (III) was prepared and fully characterised. The absolute configuration of the C_3 secondary alcohol centre in this diol was deduced to be (S) from a consideration of observed optical rotations and the use of Brewster's methods. Certain characteristics of the infrared spectra of the diols and the 1,3-isopropylidene derivatives have been noted.

Two polyacetylenes have been isolated from the culture medium of the fungus Clitocybe rhizophora and shown to have the structures (VII-VIII). They are unique among the polyacetylenes so far

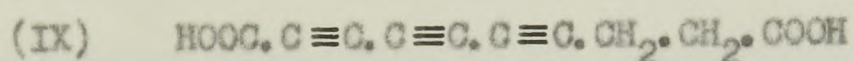


isolated from micro-organisms in being the first diacetylene and the first ketone to be isolated. The structural determination for the diyne-triol (VII) was confirmed by investigating the nuclear magnetic resonance spectrum of its isopropylidene derivative and of the products of hydrogenation and of sodium borohydride reduction of this derivative.

The absolute configurations of the secondary alcohol centres at C_2 in both the natural compounds and at C_7 in the triol were

deduced to be (S) from observed optical rotations and the use of the methods of Brewster. Certain characteristics in the infrared spectra of the 1,2-isopropylidene derivatives were noted and compared with the data from the 1,3-isopropylidene derivatives of the allenic C₁₀ 1,3-diol (III) from Odontia bicolor.

The synthesis of a polyacetylenic diacid (IX) from Merulius lachrymans has been described in an appendix.



Introduction

The term "polymerization" is used to describe a chemical reaction in which small molecules (monomers) combine to form a long chain molecule (polymer). The process of polymerization is a fundamental reaction in chemistry and is of great importance in the study of organic chemistry. The first polymerization to be described was the polymerization of ethylene to form polyethylene. This reaction was discovered by Ziegler and Natta in 1954. Since then, many other polymerizations have been discovered, and the study of polymerization has become a major branch of chemistry. The first polymerization to be described was the polymerization of ethylene to form polyethylene. This reaction was discovered by Ziegler and Natta in 1954. Since then, many other polymerizations have been discovered, and the study of polymerization has become a major branch of chemistry.

INTRODUCTION

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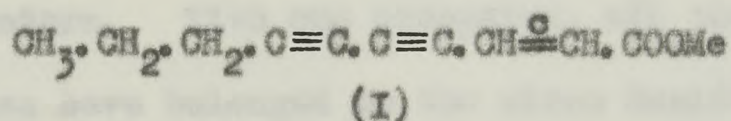
Goljov in 1935,⁽²⁾ as a constituent of the essential oil of *Isobutyrola racemosa*, a plant in the Compositae family. The first polymerizations⁽³⁾ have since been isolated from the essential oil of *Isobutyrola racemosa* and also a few from the *Isobutyrola* family and from various other sources. Recently, certain micro-organisms have been found to produce polymerizable substances, and it is in this sphere that the most important work has been carried out.

The synthesis of polymers in natural polyacetylenes are not only important in their own right, but frequently with other structural features as well. As a result of conjugation, the compounds exhibit optical properties which are of considerable interest.

Introduction.

The term "polyacetylene" is used to describe a molecule containing more than one acetylenic carbon-carbon linkage. The question of the properties of a molecule which has a succession of these linkages, and therefore chains consisting only of carbon atoms, led Baeyer in 1885 to conduct several syntheses of conjugated acetylenes. ⁽¹⁾ From his observations of their extremely unstable and often explosive nature, he made a brief enunciation of the strain theory, one of the fundamental concepts of organic chemistry.

The first polyacetylene to be isolated from natural sources was lachnophyllum ester (I), characterised as such by Willjams, Smirnov and



Goljmov in 1935, ⁽²⁾ as a constituent of the essential oil from Lachnophyllum gossypinum, a plant in the Compositae family. Many more polyacetylenes ⁽³⁾ have since been isolated from the Compositae family and also a few from the Umbelliferae family and from certain seed oils. More recently, certain micro-organisms have been found to produce polyacetylenic metabolites, and it is in this sphere that the work described in this thesis has been carried out.

The acetylenic linkages in natural polyacetylenes are not only conjugated with each other, but frequently with other unsaturated linkages as well. As a result of conjugation, the compounds exhibit light absorption properties in the ultraviolet region which are

characteristic of the number and identity of the individual members of the conjugated group or chromophore. The intensity of absorption often varies markedly with change in wavelength to give a series of sharp maxima whose separation is ca. $2,000\text{ cm}^{-1}$. The extinction coefficients of these maxima frequently fall within the range 5,000-100,000, making it possible to detect minute quantities of these compounds at low concentrations (ca. 0.2 mg./l.)

The structures of more than fifty different polyacetylenic compounds produced by micro-organisms have been elucidated during the last decade. Originally isolated as antibiotics,^(4,5) a systematic screening programme for the detection of polyacetylenic metabolites through their characteristic light absorption in fungal culture fluids was undertaken by Jones⁽⁶⁾ and his collaborators. With one exception, all the fungi found to produce polyacetylenes have belonged to the class Basidiomycetes. The structures elucidated for these highly unstable natural products share some common characteristics which are shown in Table 1. The compounds as a whole are seen to contain oxygen functions which would enable the organism to rid itself of these by-products by rendering them fat-insoluble, but soluble in water. They may then be easily discharged into the aqueous culture medium.

Until 1960, it was observed that natural polyacetylenes having an odd numbered carbon chain invariably possessed a terminal ethynyl group; conversely, no polyacetylene of even-numbered carbon chain had been isolated which contained a terminal ethynyl group. In 1960, Jones and Leeming⁽⁷⁾ reported the isolation of a C_{11} polyacetylene from

TABLE 1.

Summary of Structures of Polyacetylenes from
micro-organisms

Straight chains	C_8-C_{14}
Unsaturation	$-C \equiv C-, -CH=CH-$ $-CH=C=CH-(16)$
Hydrogen uptake	8-16H
Terminal C-functions	$HC \equiv C-(19); CH_3-(15)$ $H_2C=C=CH-(1)$ but never $H_2C=CH-$
Terminal O-functions	$-COOH(21) \quad -CHO(3)$ $-CH_2OH(25) \quad -COOMe(6)$ rarely $-CONH_2, -CN.$
Other O-functions (at 2,3,4 and 7)	$-OH(8)$ ketone (1) lactone (2) epoxide (1)

Figures in parentheses indicate the number
of examples so far isolated.

Drosophila subatrata which did not have a terminal ethynyl group.

Other exceptions to this generalisation were recently described by Bew,⁽⁸⁾ and more are described in this thesis. All the known exceptions to this "odd-even rule" are listed in Table 2.

The biogenesis and metabolic changes of these compounds are now receiving attention. Bu'Lock⁽⁹⁾ has shown by incorporating radioactivity (C^{14}) into nemotinic acid from carboxyl-labelled acetate that 6 molecules are involved and that the labelling pattern is identical with that of the fatty acids. The C_{11} nemotinic acid contained 6 labelled atoms, suggesting that odd-numbered carbon chains are produced from even chains by loss of one carbon atom. It has been shown⁽¹⁰⁾ that Coprinus quadrifidus contains an enzyme system which can decarboxylate an $\alpha\beta$ -acetylenic acid (see Fig. 1.)

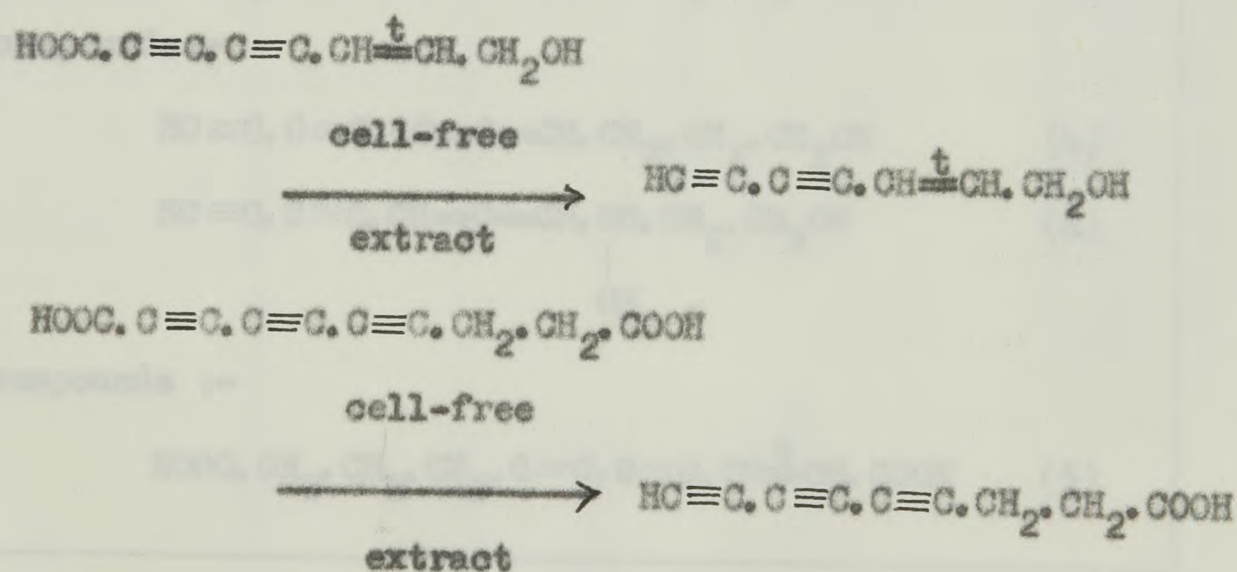


Fig. 1

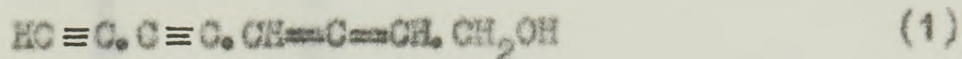
The biogenesis of polyacetylenes which do not conform to the "odd-even rule" mentioned above is discussed later in this thesis.

The structural relationship between some metabolites is shown

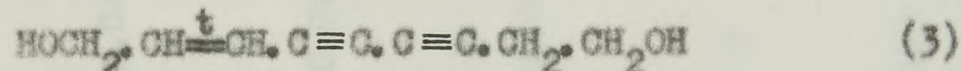
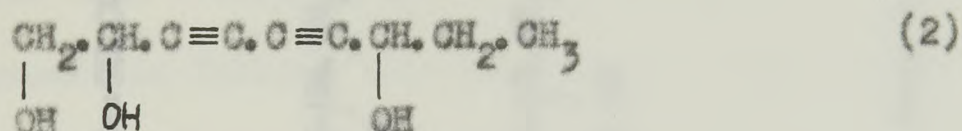
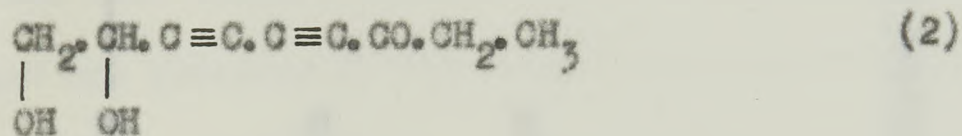
TABLE 2.

Acetylenic Compounds from Micro-Organisms which
do not conform to the "odd-even" rule.

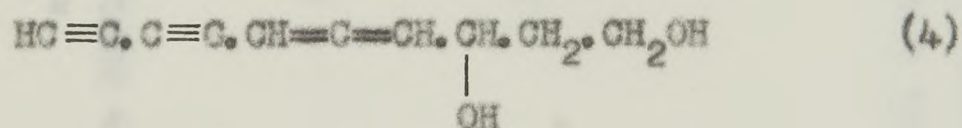
C_8 compounds:-



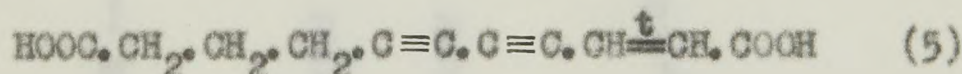
C_9 compounds:-



C_{10} compounds:-



C_{11} compounds :-



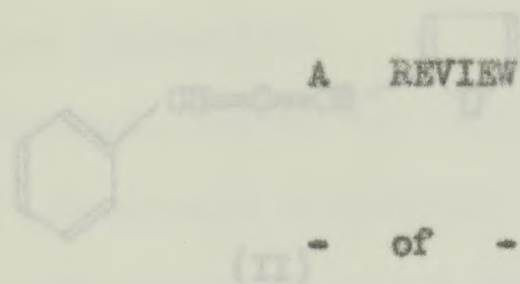
(1) *Cortinellus berkeleyanus* (2) *Clitocybe rhizophora*
 (3) *Poria sinuosa* (4) *Odontia bicolor*, "B. 285",
Flammula sapinea ? (5) *Drosophila subatrata*.

in Table 3, representing the hypothesis that the less saturated C_{10} compounds are formed through the progressive reduction of a tetra-acetylenic acid and that the C_9 compounds arise from terminal oxidation and decarboxylation. Further reduction beyond the stages indicated would produce compounds which would normally escape notice through their lack of characteristic light absorption. The only diyne so far isolated from fungal cultures was produced in exceptional quantities and not detected by the usual ultraviolet absorption screening procedure (see this thesis, p.84). It is noteworthy that the three central compounds in Table 3 were found in the culture medium of Coprinus quadrifidus, and that their stereochemistry is that which would be expected on the basis of in vitro conversions. Moreover, the origin of the allene bond in the C_9 alcohol is paralleled by the in vitro reduction of vinylacetylenes to allenes. (11)

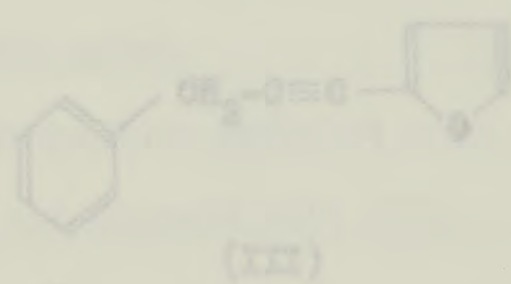
A Review of Natural Allenes.

Allenes form a distinct group within the general classification of natural polyacetylenes. This group has attracted considerable attention, mainly through the high optical activity and elegant isomerization reactions of its members.

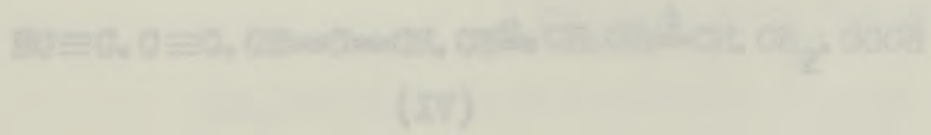
Sessler, in 1859, isolated carline oxide from Carlina acuta,⁽¹²⁾ and assigned to it the structure (II) on the basis of its refractivity.



The proposed allenic structure was, however, shown to be incorrect, and the structure (III) was established in 1935 by Kuhn, Plattet, Plattner and Sauer.⁽¹³⁾



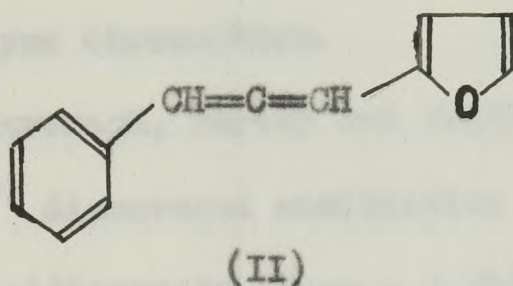
It was not until 1952 that a natural allenic compound was isolated and fully characterized, when Colner and Solomon obtained the antibiotic pyrolysin (IV) from the culture fluid of the Actinomyces pyrolysin.⁽¹⁴⁾ This compound is remarkable in that it exhibits



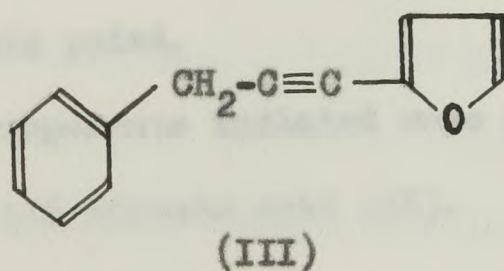
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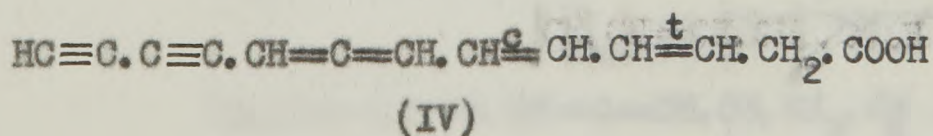
Semmler, in 1889, isolated carlina oxide from Carlina aculis,⁽¹²⁾ and assigned to it the structure (II) on the basis of its refractivity.



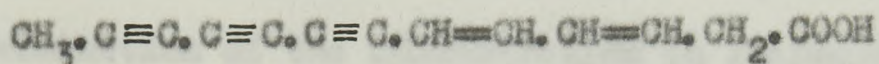
The proposed allenic structure was, however, shown to be incorrect, and the structure (III) was established in 1935 by Pfau, Pictet, Plattner and Susz.⁽¹³⁾



It was not until 1952 that a natural allenic compound was isolated and fully characterised, when Celmer and Solomons obtained the antibiotic mycomycin (IV) from the culture fluid of the Actinomycete Norcardia acidophilus.⁽⁴⁾ This compound is remarkable in that it exhibits



every known type of carbon-carbon unsaturation, the allene group accounting for its high optical activity ($[\alpha]_D -130^\circ$). Mycomycin formed colourless, highly unstable needles, and was isomerised in the presence of potassium hydroxide solution to another crystalline acid, isomycomycin (V). This was accompanied by a complete change in the

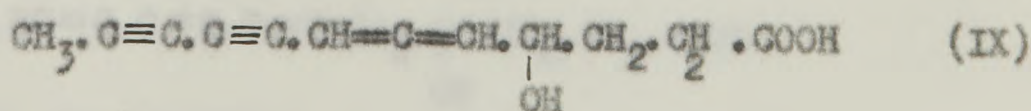
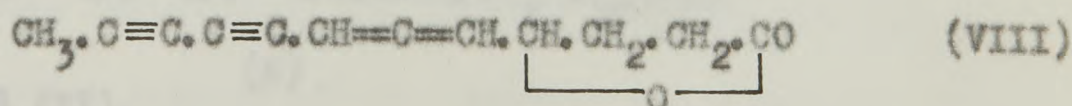
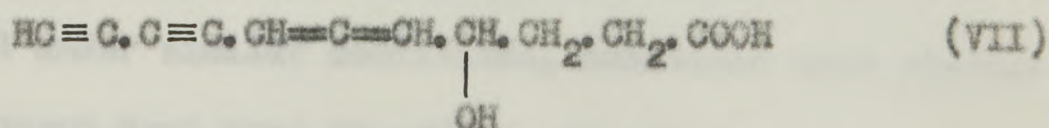
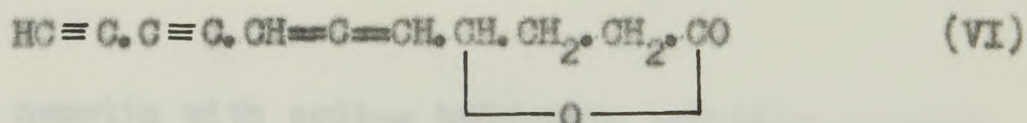


(V)

ultraviolet absorption spectrum from that of enediyne + triene to that of the dienetriyne chromophore.

In 1950, Kavanagh, Hervey and Robbins^(5a) and Anchel, Polatnik and Kavanagh^(5b) discovered antibiotics in the culture fluid of the unidentified Basidiomycete "fungus B. 841". The isolation and characterisation of the four allenic polyacetylenes by Bu'Lock, Jones, Leeming and Thompson⁽¹⁴⁻¹⁶⁾ is typical of the general method employed for the majority of natural allenes, and can be summarised with advantage at this point.

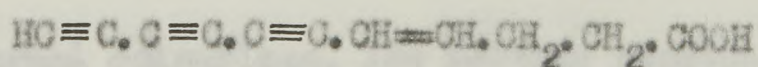
The four components isolated were nemotin (VI), nemotinic acid (VII), odyssin (VIII) and odyssic acid (IX).



Nemotinic and odyssic acids were separated by counter-current distribution and nemotin and odyssin separated similarly using a different solvent system. None of the compounds (VI)-(IX) could be obtained crystalline and were extremely unstable in concentrated solutions at room temperature. Quantitative data had to be obtained through determination of the concentration in standard solutions of the polyacetylenes by the mean of three weighings of the residues from the evaporation of aliquot samples of the solutions in tared flasks. The ultraviolet absorption extinction coefficients given were the highest obtained after exhaustive purification, and analyses were performed on the resinous evaporation residues. The concentrations of solutions in general were deduced from the difference in optical density between the longest wavelength ultraviolet absorption maximum and minimum.

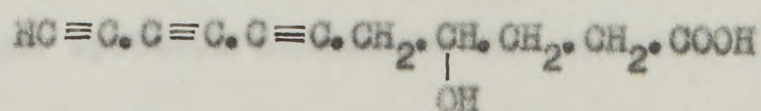
The ultraviolet absorption data indicated enediyne chromophores, and infrared data indicated the presence or absence of a terminal ethynyl group, γ -lactone, free hydroxyl and carboxyl groups, and the consistent presence of an allene group. The chain lengths were confirmed by hydrogenation and comparison of the products with authentic specimens.

Treatment of nemotin with sodium hydroxide solution yielded nemotin A (X), and under similar conditions, nemotinic acid yielded



(X)

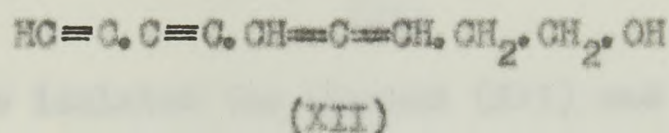
isonemotinic acid (XI).



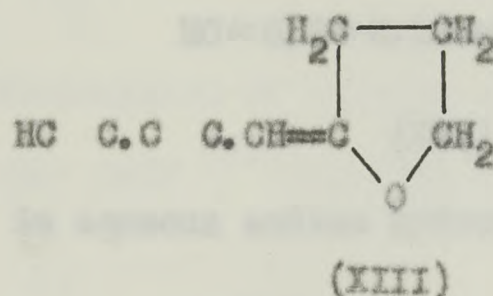
(XI)

With acid, isonemotinic acid yielded the corresponding lactone which, in alkali, isomerised to nemotin A. The initial isomerisations (VI)-(X) and (VII)-(XI) were accompanied by spectacular changes in the ultraviolet absorption spectra, and a large drop in magnitude of the optical rotation (from $[\alpha]_D$ ca. -300° to ca. -3°).

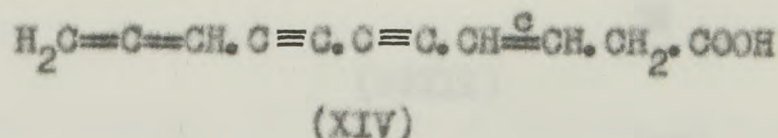
In 1959, Bendz described the isolation and characterisation of the allenic polyacetylene marasin (XII) from Marasmius ramealis.⁽¹⁷⁾ In



sodium hydroxide solution, this compound isomerised to isomarasin (XIII).

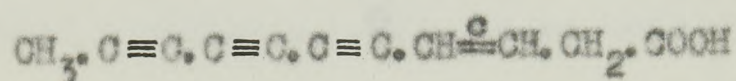


In 1960, Jones and Leeming reported the characterisation of a terminal allenic polyacetylene, drosophilin D, from Drosophila subatrata.⁽⁷⁾ This fungus had been investigated in 1950 by Anchel, Polatnik and Kavanagh.^(5b) The compound was isolated, purified and completely characterised as (XIV)



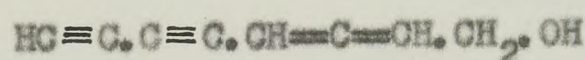
Drosophilin D formed colourless, low melting needles, and had an ultraviolet absorption typical of the enediyne chromophore. The infrared absorption spectrum indicated the presence of an unconjugated acid (confirmed by the formation of a methyl ester with carbonyl

absorption at 1745 cm^{-1}). There was no ethynyl hydrogen band, but bands at $1960, 1930$ and 838 cm^{-1} indicated the presence of a terminal allenic group. ⁽¹⁸⁾ cis-Ethylenic hydrogen absorption was also present. Hydrogenation followed by reduction with lithium aluminium hydride gave n-undecanol. Isomerisation of dropsophilin D in aqueous sodium hydroxide gave (XV).



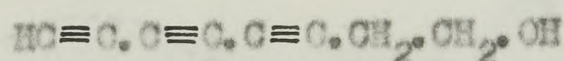
(XV)

In 1961, Bew isolated the alcohol (XVI) and also marasin (XII) from Cortinellus berkeleyanus. ⁽⁸⁾ The alcohol (XVI) isomerised to the



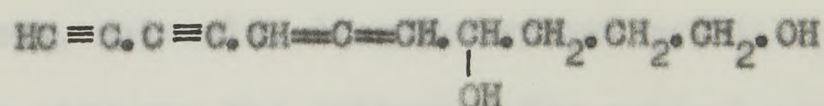
(XVI)

triyne (XVII) in aqueous sodium hydroxide.



(XVII)

The C_{10} and C_{11} homologues of (XVI) and the diol (XVIII), reported by Bew as metabolites of "fungus B. 285" are discussed later in this thesis.



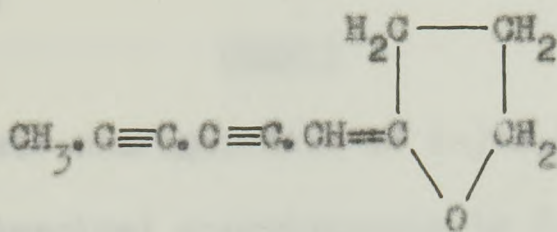
(XVIII)

Recently isolated, by Mr. J. R. Chapman, ⁽¹⁹⁾ from growths of Daedalea juniperina on a sulphur-free medium is the allenic alcohol (XIX) which conforms to the "odd-even rule" (see Introduction), having an even numbered carbon chain and no terminal ethynyl group. As might

be expected, this isomerises in an analogous fashion to marasin



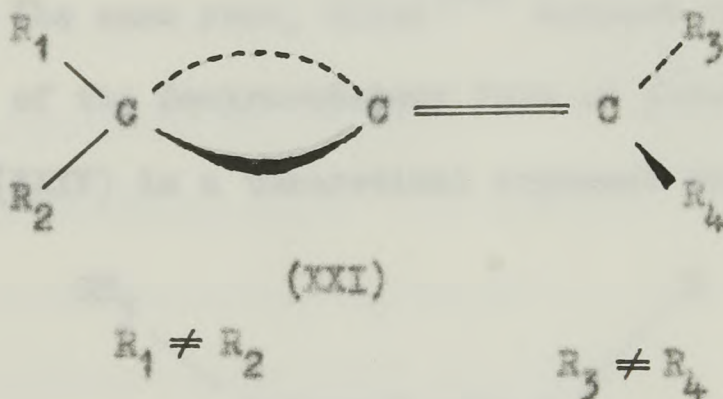
(see p. 11), to form a tetrahydrofuran derivative (XX).



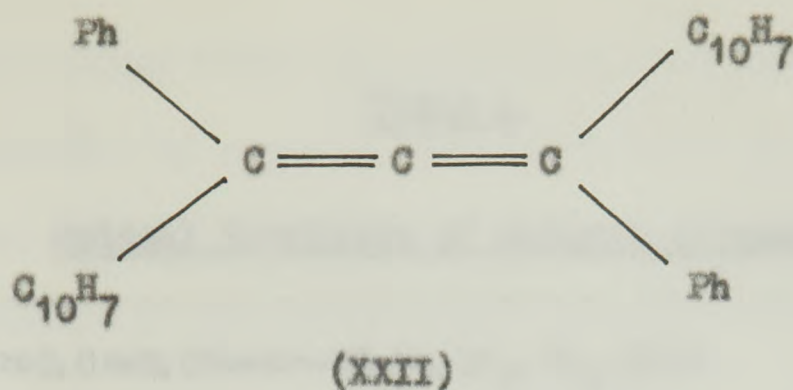
(XX)

The two main features of interest in the naturally occurring allenes are their high optical activity and ready isomerisation in dilute base.

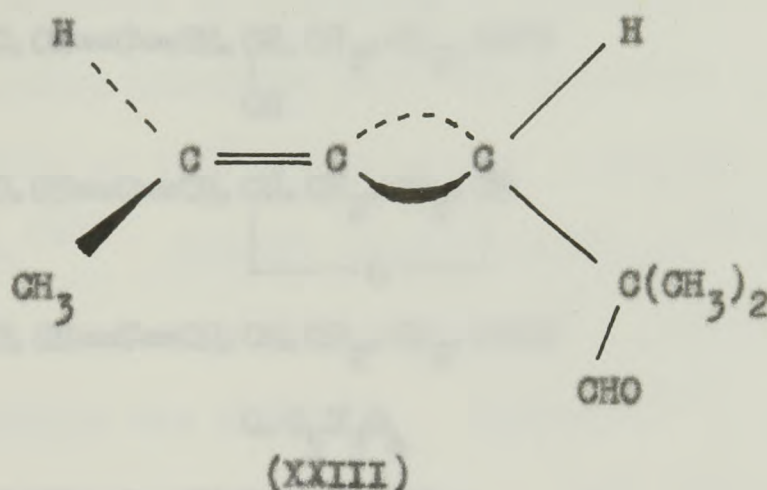
The enantiomorphism of allenes of the general formula (XXI) was predicted by van' Hoff ⁽²⁰⁾ in 1875 with the proposal that, as a result



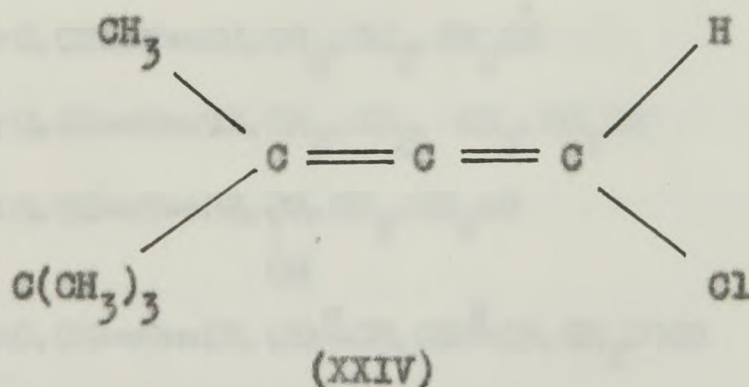
of his tetrahedral theory, molecular dissymmetry and not the presence of an asymmetric carbon atom was the criterion for optical activity. This was proposed without the known existence of any allenic structure, and was not verified until 1936 when Waitland and Mills ⁽²¹⁾ (simultaneous with similar work by Kohler and Fishler ⁽²²⁾) resolved 1,3-dinaphthylallene (XXII).



The absolute configuration of (-)-2,2-dimethylhexa-3,4-dienal (XXIII) was determined unambiguously by Jones, Loder and Whiting⁽²³⁾



in 1960. In the same year, Eliel⁽²⁴⁾ deduced the absolute configuration of the dextrorotatory form of 3-methyl-3-t-butyl-1-chloroallene (XXIV) in a theoretical argument based on the preparation



by Landor and Taylor-Smith⁽²⁵⁾ and the calculations of Brewster.⁽²⁶⁾

We now know the optical rotations of a number of naturally occurring allenes, and these are listed in Table 4. In each of these examples there are two common factors, the presence of a diyne-allene

TABLE 4.

Optical Rotations of Natural Allenes.

$\text{CH}_3 \cdot \text{C} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \underset{\text{OH}}{\text{CH}} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$	+ 360°
$\text{CH}_3 \cdot \text{C} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \underset{\text{O}}{\text{CH}} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CO}$	+ 300°
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \underset{\text{OH}}{\text{CH}} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$	+ 380°
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \underset{\text{O}}{\text{CH}} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CO}$	+ 320°
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$	+ 237°
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$	+ 385° (a)
$\text{HO} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$	- 385° (b)
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \text{CH}_2 \cdot \text{OH}$	- 380° (1)
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$	- 290°
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$	- 300 (1)
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \underset{\text{OH}}{\text{CH}} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}$	- 210°
$\text{HC} \equiv \text{C} \cdot \text{C} \equiv \text{C} \cdot \text{CH} = \text{C} = \text{CH} \cdot \text{CH} \overset{\text{O}}{=} \text{CH} \cdot \text{CH} \overset{\text{t}}{=} \text{CH} \cdot \text{CH}_2 \cdot \text{COOH}$	- 130°

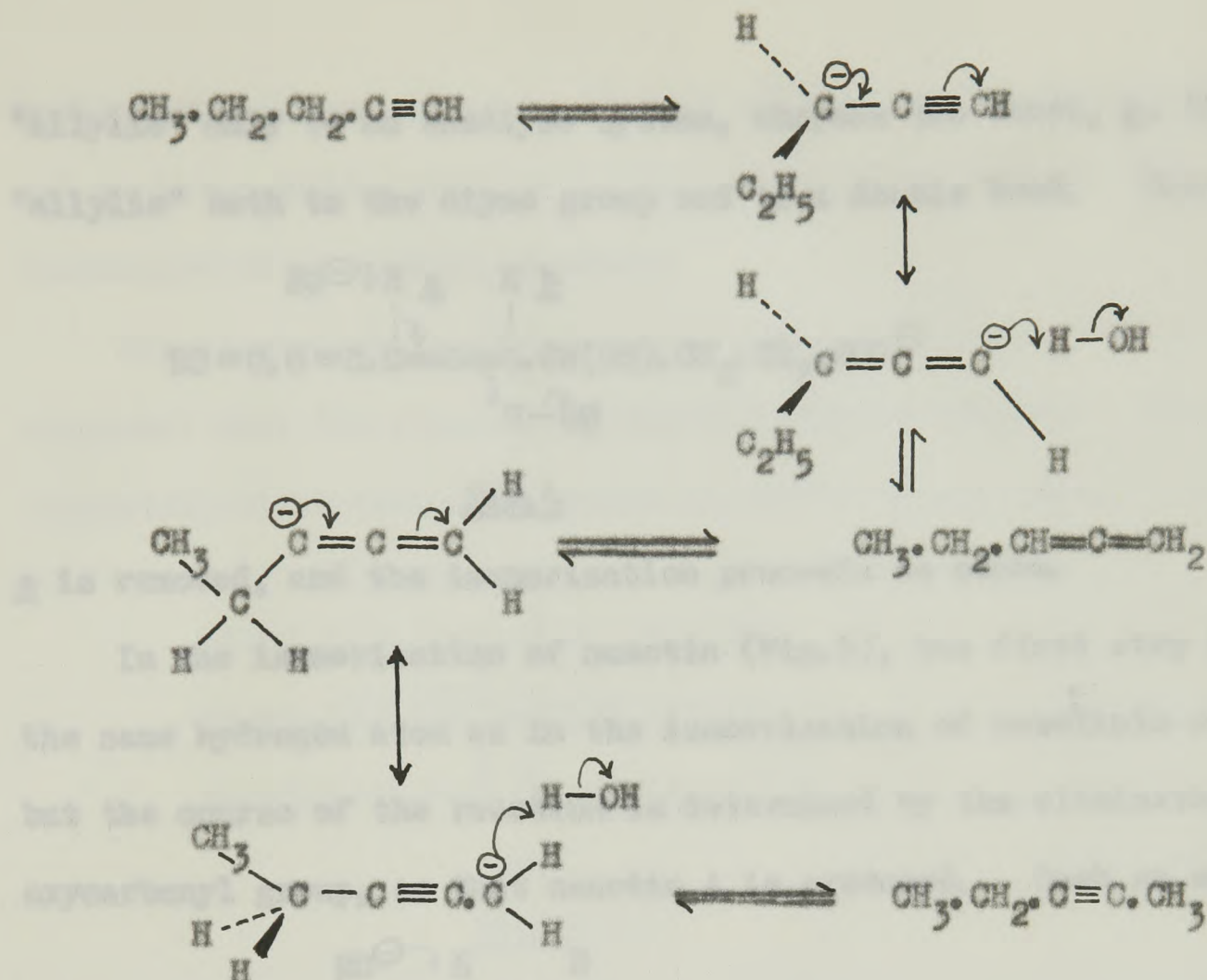
(1) in CH_2Cl_2

All others in EtOH.

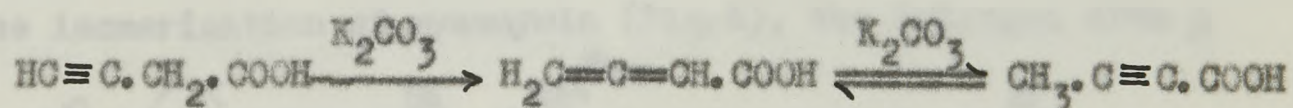
(a) from Aleurodiscus roseus(b) from Marasmius ramealis

group, and a large value (ca. $\pm 300^\circ$) for the optical rotation. It seems reasonable to suppose, therefore, that the optical rotation in this series is predominantly due to the diyne-allene group, regardless of the presence of any other centre of asymmetry, the contribution to the rotation made by such a centre being small compared to the effect of the diyne-allene group.

The rearrangement of 1-alkynes to 2-alkynes in the presence of alcoholic potassium hydroxide has been known for some time. (27-33) It occurs readily at 170° and is faster at higher alkali concentrations. (28) Jacobs, Akawie and Cooper (34) showed that an allene was present among the products of the reaction, and that an equilibrium mixture was produced. Favorskii (27) suggested that the rearrangement involved the addition and elimination of alcohol, but this theory was shown to be unlikely by Jacobs, Akawie and Cooper, (34) who agitated a mixture of 1-pentyne and powdered potassium hydroxide for 20 hours at 175° , and obtained the equilibrium mixture. Conclusive evidence against this hypothesis was provided by subjecting 2-ethoxy-1-pentene to equilibration conditions with alcoholic potassium hydroxide when no pentynes or pentadiene were found. (30) The latter workers suggested that the mechanism involved the removal of a proton by the base as postulated for the interconversion of 1,3-diarylpropenes. (35-37) From this postulate, the equilibrium could be represented as in Fig. 2.

Fig. 2

The presence of unsaturated substituents greatly facilitates the removal of a proton, as exemplified by the ready isomerisation of but-3-ynoic acid⁽³⁸⁾ (Fig. 3).

Fig. 3

In the case of natural allenes, which often have two such substituents, the isomerisation takes place readily in dilute alkali at room temperature. The isomerisation reactions of mycomycin, nemotin and nemotinic acid are three variants of the allene-acetylene rearrangement, $-\text{CH} = \text{C} = \text{CH}- \rightleftharpoons -\text{CH}_2 - \text{C} \equiv \text{C}-$, which is described above. In the isomerisation of nemotinic acid (Fig. 4), the hydrogen atom b is

"allylic" only to an enediyne system, whereas the other, a, is "allylic" both to the diyne group and to a double bond. Consequently,

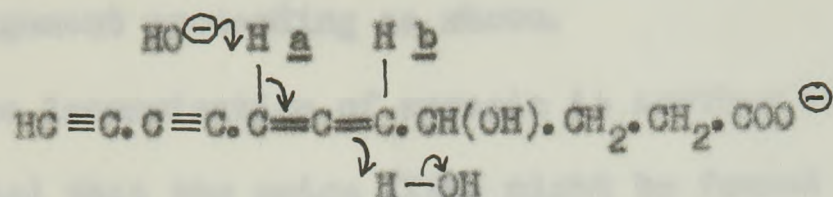


Fig. 4

a is removed, and the isomerisation proceeds as shown.

In the isomerisation of nemotin (Fig. 5), the first step involves the same hydrogen atom as in the isomerisation of nemotinic acid, but the course of the reaction is determined by the elimination of the oxycarbonyl group, so that nemotin A is produced. Such an effect

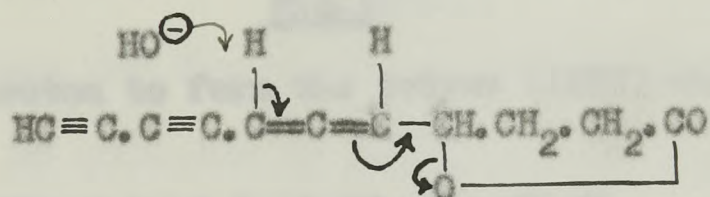


Fig. 5

would be much less likely in the case of nemotinic acid because the carboxylate ion is a better leaving group than the hydroxyl ion.

In the isomerisation of mycomycin (Fig. 6), the hydrogen atom a

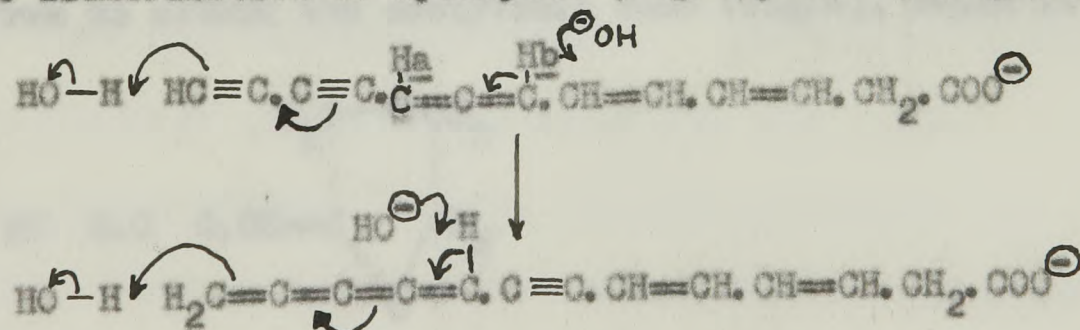
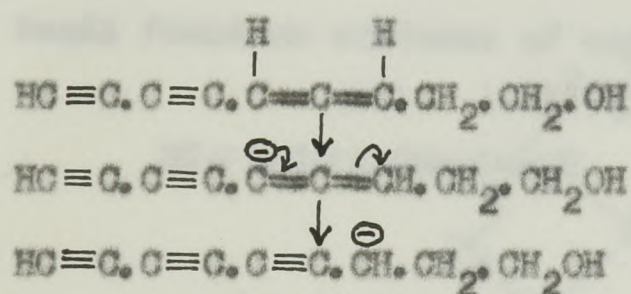


Fig. 6

is "allylic" to diyne and triene groups, and atom b is "allylic" to

enediyne and diene groups. Removal of b gives the more stable anion and so in mycomycin this is the initial point of attack, the rearrangement proceeding as shown.

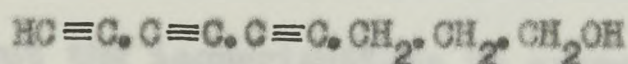
The isomerisation of marasin is another variant, where Bendz⁽¹⁷⁾ suggested that the anion (XXV) might be formed (Fig.7). This, Bendz suggested, might take up a proton in either of two ways. Water could



(XXV)

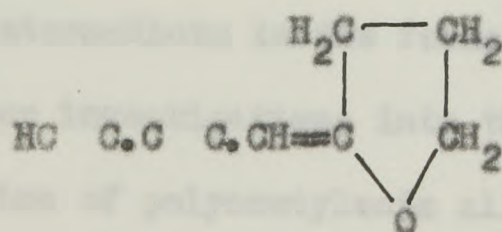
Fig. 7

donate a proton to form the triyne (XXVI) which could then ring close



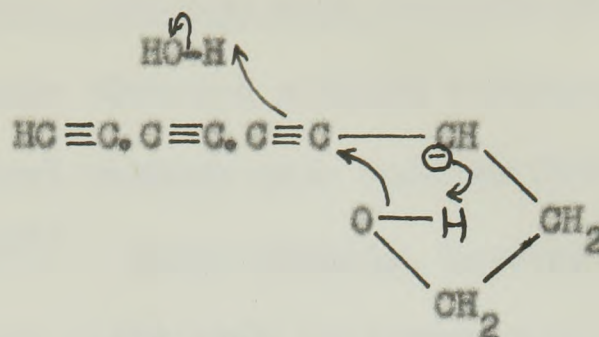
(XXVI)

to the tetrahydrofuran derivative, isomarasin (XIII). Alternatively, a proton might come from the neighbouring hydroxyl group, leaving the $\text{-O}^\ominus$ group free to attack the acetylenic bond (Fig.8), which is known

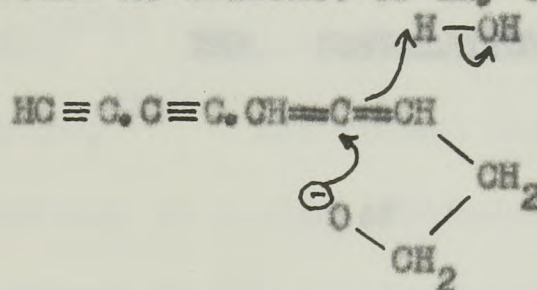


(XIII)

to be reactive towards nucleophilic reagents.⁽³⁹⁾ Bew⁽⁸⁾ considered a

Fig. 8

third mechanism of direct attack by an alkoxide anion, as shown in Fig. 9. Bendz found no evidence of any triyne formation during the

Fig. 9

rearrangement, but Bew found some 25% in the normal isomerisation product. He attempted to decide between the possible mechanisms by carrying out the isomerisations in aqueous and in D_2O solutions, but the isomarasins infrared absorptions were the same in each case. However, he found that the triyne (XXVI) required vigorous conditions to form the enol-ether. He concluded that the formation of triyne and of enol ether are competing reactions. The triyne is unlikely to be an intermediate in the formation of the enol ether.

Further investigations into the possible mechanisms of isomerisation of polyacetylenic allenic alcohols are described in this thesis.

The Correlation of Optical Rotation with Absolute Configuration

Attempts to correlate observed optical rotation with absolute configuration by empirical methods were made in 1936 by Harter⁽⁴⁰⁾ and in 1956 by Whiffen.⁽⁴¹⁾ These methods, however, were somewhat restricted and it was not until 1959 that Brewster outlined an empirical approach⁽⁴²⁾ which was clearly successful in a much wider application. Brewster has so far applied his technique to (a) open chain compounds,^(42a) (b) cyclic compounds,^(42b) and (c) endocyclic olefins.^(42c) The method has been used in the present work tentatively to assign of configurations to three asymmetric secondary alcohol groups in the polyacetylenes isolated from Clusia bigler and Clusia optima. This exercise, a detailed examination of the cyclic ketol derivatives was found to present considerable difficulty with the arguments presented for the configurational assignments do not require estimation of these absolute values. ABSOLUTE : CONFIGURATION with Brewster's method, as it applies to open chain compounds. This section therefore serves the form of a summary of Part I of Brewster's series of publications.

- A Summary -

Absolute Configuration

Brewster suggests that a centre of optical activity can be described as an asymmetrical array pattern of polarisability distributed according to the placing of each substituent around that centre. In Fig. 10, the centre C has four substituents whose order of polarisability is $A > B > C > D$. Brewster describes this centre as having a right-handed array pattern of polarisability in the projection

The Correlation of Optical Rotation with Absolute Configuration

Attempts to correlate observed optical rotation with absolute configuration by empirical methods were made in 1936 by Marker⁽⁴⁰⁾ and in 1956 by Whiffen.⁽⁴¹⁾ These methods, however, were somewhat restricted and it was not until 1959 that Brewster outlined an empirical approach⁽⁴²⁾ which was clearly successful in a much wider application. Brewster has so far applied his techniques to (a) open chain compounds,^(42a) (b) saturated cyclic compounds^(42b) and (c) endocyclic olefins.^(42c) The method has been used in the present work tentatively to assign configurations to three asymmetric secondary alcohol groups in the polyacetylenes isolated from Odontia bicolor and Glitocybe rhizophora. In this exercise, a detailed examination of the cyclic ketal derivatives was found to present considerable difficulty, and since the arguments presented for the configurational assignments do not require calculation of these absolute values, we were concerned only with Brewster's method as it applies to open chain compounds. This section therefore takes the form of a summary of Part I of Brewster's series of publications.

Atomic Asymmetry

Brewster suggests that a centre of optical activity can be described as an asymmetric screw pattern of polarisability distributed according to the placing of each substituent around that centre. In Fig. 10, the centre x has four substituents whose order of polarisability is $A > B > C > D$. Brewster describes this centre as having a right-handed screw pattern of polarisability in the projection

shown in Fig. 10. The overall pattern for the molecule is obtained

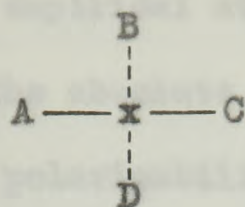


Fig. 10

by inspection along the two axes at 90° to the axis of projection of Fig. 10, and taking an average. Since the latter two patterns are "left-handed screws", then the centre has an overall "left-handed screw pattern of polarisability" in the configuration shown.

An empirical sequence of polarisabilities was obtained by noting the sign of $[M]_D$ of configurationally related compounds which can reasonably be expected to show essentially pure atomic asymmetry (i.e. compounds in which the asymmetric atom is not part of a ring or flexible chain, and the substituents do not interact by hydrogen bonding). The empirical sequence observed is shown in Table 5., and is the same as that obtained by considering the atomic

Table 5

Substituent	R_D of attachment atom
Br	8.741
Cl	5.844
CN, (C C)	3.580
C_6H_5 , (CH=)	3.379
CO_2H	3.379
CH_3	2.591
NH_2	2.382
OH	1.518
H	1.028

refraction values of the substituent attachment atoms (Table 5). Brewster thus obtained the empirical atomic asymmetry rule that an asymmetric carbon atom in the absolute configuration shown in Fig. 10 is dextrorotatory when the polarisabilities of the attachment atoms decrease in the order $A > B > C > D$. According to this rule, a formally asymmetric carbon atom carrying two or more alkyl groups will be considered to be per se optically inactive although it may provide a sterically asymmetric environment conducive to the development of conformational asymmetry.

Conformational Asymmetry.

A twisted chain of four atoms A-C-C-A' displays its asymmetry most clearly when it is viewed along the C-C bond, as in the Newman projections in Fig. 11. The conformational asymmetry

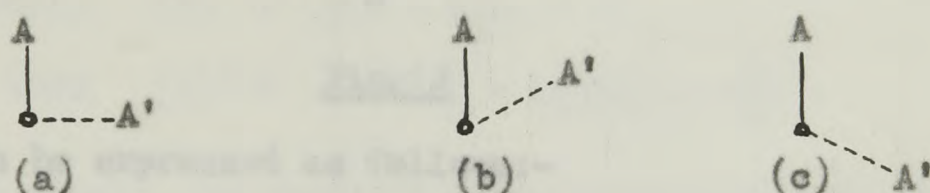


Fig. 11

of a complex structure is most conveniently analysed by systematic inspection of all the bonds for units of this type. It was found empirically by Brewster that the conformational units 11(b) and 11(c) can be considered to be dextrorotatory in the absolute configurations shown and to make contributions to $[M]_D$ which can be expressed mathematically as follows:-

$$\Delta [M] = + k.A.A'$$

where A and A' are functions of the polarisabilities of the terminal

atoms and k is a constant which appears to be the same for (b) and (c). Brewster showed that the dextrorotatory unit A-C-C-A' could be described as a "left-handed screw pattern of polarisability" in a like manner to the asymmetric atom. He noted also that the magnitude of the rotatory effect of one of these conformational units would be increased if one or both of the terminal bonds were multiple. The assumption is made that rotatory effects of conformational units are additive in full conformations as in Fig. 12. Thus the rotatory power of the full conformation in

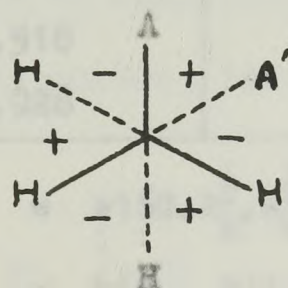


Fig. 12

Fig. 12 can be expressed as follows:-

$$\begin{aligned}
 [M] &= +k(A.A' - A.H - A'H + H.H) \\
 &= +k(A - H)(A' - H)
 \end{aligned}$$

It was found empirically that rotatory powers could be assigned to these full conformations. They are given in Table 6.

When used in conjunction with a simple method of conformational analysis, they allow predictions to be made of the sign and magnitude of rotation of simple open chain compounds. The rotatory powers were also roughly calculated by Brewster from atomic refractions (see Table 6), using the empirical equation:-

Table 6

Substituent (A)	R_D (see Table 5)	Conformational Rotatory Powers $k(C - H)(A - H)$	
		Observed	Calculated
Br	8.741	180	192
Cl	5.844	170	139
CN	3.580	160	87
C C	3.580	-	87
C_6H_5	3.379	140	82
$CH=CH$	3.379	-	82
CO_2H	3.379	90	82
CH_3	2.591	60	60
NH_2	2.382	55	53
OH	1.518	50	23
H	1.028	0	0

$$\Delta M = +160 \cdot R_A^{\frac{1}{2}} \cdot R_C^{\frac{1}{2}}$$

$$\text{Since } [M] = k(C - H)(A - H)$$

$$\text{then } [M] = +160(R_C^{\frac{1}{2}} - R_H^{\frac{1}{2}})(R_A^{\frac{1}{2}} - R_H^{\frac{1}{2}})$$

Unfortunately, no observed value is recorded by Brewster for the case when A is the attachment atom of an acetylenic linkage. The calculated value is the same as for the CN attachment carbon, where the observed value is 160° . The rules outlined by Brewster for conformational analysis are as follows:

1. Only conformations corresponding to energy minima will be prevalent enough to produce appreciable rotatory effects.

2. The five atom conformation:-



is prohibited when the terminal atoms are both larger than hydrogen.

3. The conformation:-
is prohibited when the atoms
A, B and C are all larger than hydrogen.



4. In the first approximation, all allowed conformations are considered equally probable.

In addition it was assumed that the rotatory contributions of individual conformations are additive. The method was illustrated for the case of 3-methylhexane (see Fig. 13), where the significant bonds are designated: $C_2-C_3 = I$, $C_3-C_4 = A$ and $C_4-C_5 = B$. The three staggered conformations of bond A are shown in Fig. 13(a), (b) and (c) with C_3 , the asymmetric centre, forward.

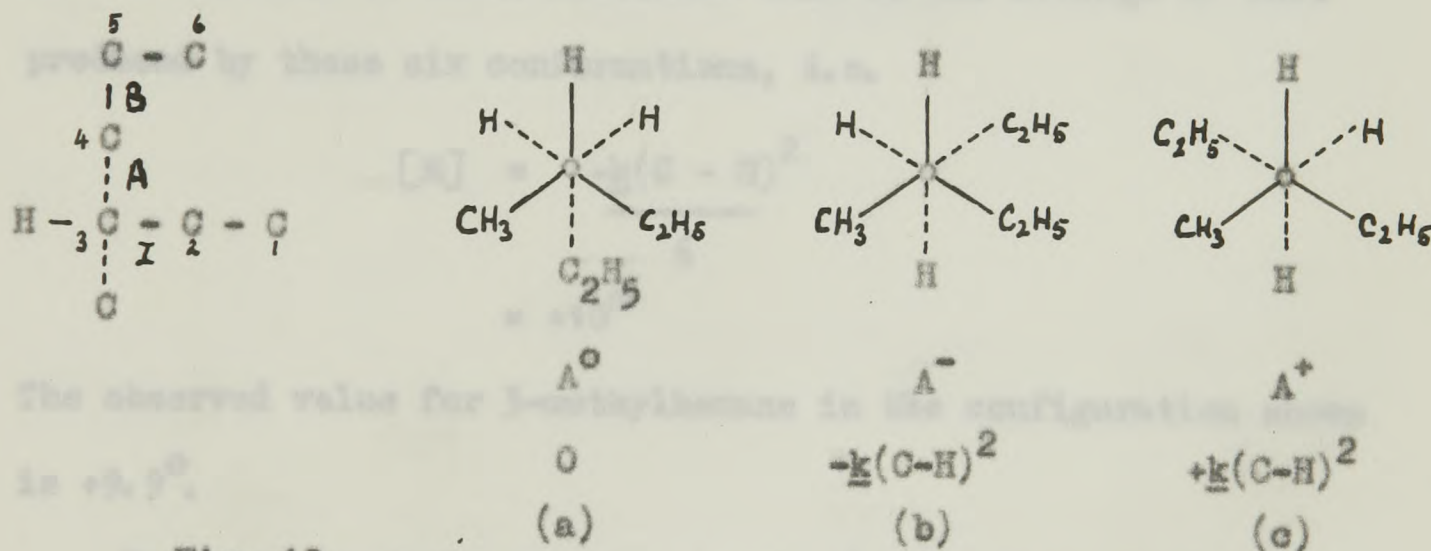


Fig. 13

(13a) is prohibited by rule 3 (above), the other two conformations have equal and opposite rotations (from Table 6, $\pm 60^\circ$). Inspection of models reveals that the combinations A^+B^- , A^-B^+ and A^-I^+ are prohibited by rule 2 (I^0 is prohibited by rule 3). There are then only the six molecular conformations shown in Table 7; these conformations are equal in probability (rule 4) so that the predicted rotation

Table 7

Bond Conformation			[M]
I	A	B	
+	+	+	$+3k(C-H)^2$
+	+	o	$+2k(C-H)^2$
-	+	+	$+k(C-H)^2$
-	+	o	0
-	-	o	$-2k(C-H)^2$
-	-	-	$-3k(C-H)^2$

Total, 6 conformations = $+k(C-H)^2$

of a collection of these molecules will be the average of that produced by these six conformations, i.e.

$$[M] = \frac{+k(C-H)^2}{6}$$

$$= +10^\circ$$

The observed value for 3-methylhexane in the configuration shown is $+9.9^\circ$.

Brewster concludes his treatment of open chain compounds by considering the groups of compounds shown in Fig. 14.

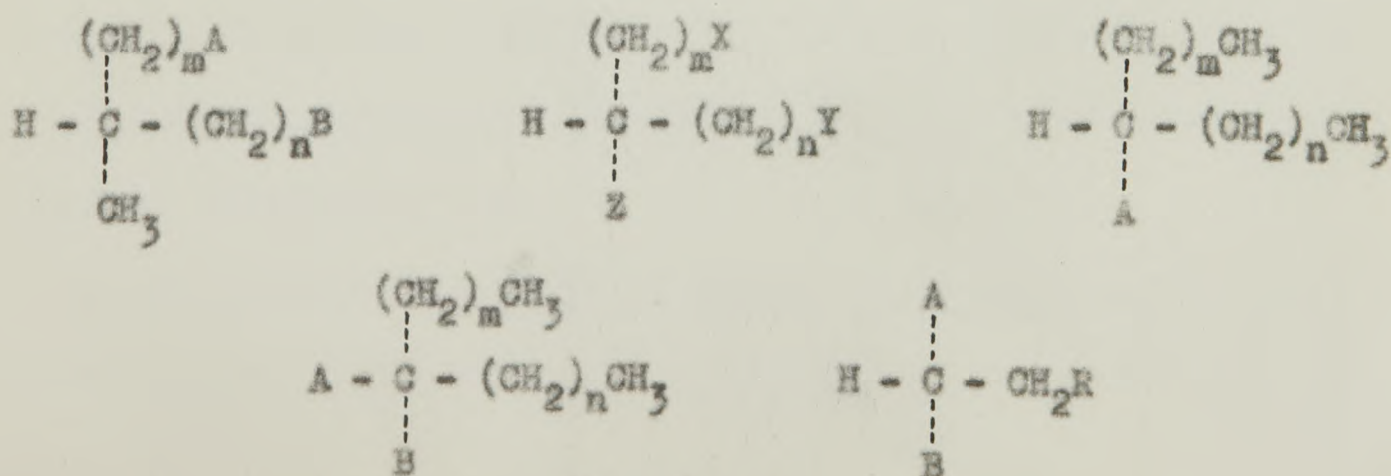


Fig. 14

Expressions have been derived dependent only on the magnitudes of $\underline{m}$ and $\underline{n}$ and the nature of the substituents A,B,X,Y,Z or R, thus avoiding the laborious conformational analysis and inspection of models for compounds in these particular groups.

The Polyacetylenes from *Odontia bicolor*

The structures have been elucidated for four allylic polyacetylenes (XIVII)-(XXI) produced during the growth of the fungus *Odontia bicolor*. In addition, one fungus has



THE POLYACETYLENES



been found to produce small, from cultures of *Odontia bicolor* and at least one other polyacetylenic microorganism.

Growth of *Odontia bicolor* ODONTIA BICOLOR

Polyacetylene (acetylene) production by the fungus varied considerably with each growth, the maximum from several cultures being 9.5 mg./litre. This could arise as a result of reworking the mycelium.

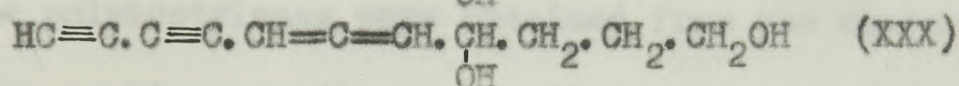
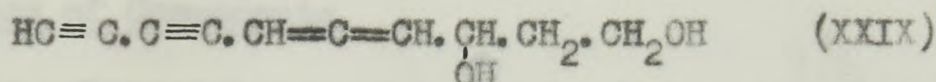
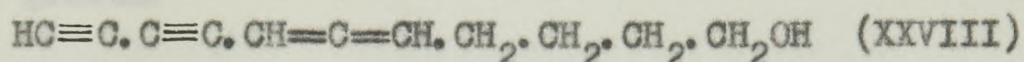
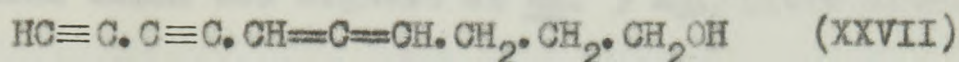
- Discussion -

Yields of up to 10 mg./litre from the first and 5.5 mg./litre from the second broth. A third reworked broth produced the same amount of acetylene as the first. It was established that this acetylene was of biological origin and that on treatment with dilute alkali, a characteristic reaction with that of *Agropyron* was produced. The results are in accordance with this conclusion.

By contrast, the growth of *Agropyron* on these cultures gave a periodic production of polyacetylene. The acetylene

The Polyacetylenes from *Odontia bicolor*

The structures have been elucidated for four allenic polyacetylenes (XXVII)-(XXX) produced during the growth of the fungus *Odontia bicolor*. In addition, the fungus has



been found to produce small, irregular amounts of mycomycin, and at least one other polyacetylenic metabolite.

Growth of *Odontia bicolor*

Polyacetylene (enediyne) production by the fungus varied considerably with each growth, the maximum from normal static cultures being 9.5 mg./litre. This could often be improved by reflooding the mycelial mat with an acetate medium to give yields of up to 10 mg./litre from the first and 8.5 mg./litre from the second reflood. A third reflood was found to produce the chromophore characteristic of mycomycin (IV). It was established that this chromophore belonged to an acidic molecule, and that on treatment with dilute alkali, a chromophore identical with that of isomycomycin was produced. No further work was done on this metabolite.

By contrast, the growth of *Odontia bicolor* as shake cultures gave a periodic production of polyacetylenes. The mycomycin

chromophore (see above) was first produced, reaching a maximum at 7 days, after which it disappeared, to be replaced by enediyne absorption, greatest after 17 days at ca. 13 mg./litre. This also rapidly disappeared, and was followed by further enediyne production which was highest at ca. 5 mg./litre after a total of 34 days growth.

Isolation of Components

The polyacetylenes were obtained from the aqueous culture fluids by continuous ether extraction and were separated into acidic and neutral components. Apart from traces of mycomycin, the acidic fraction showed no ultraviolet light absorption and was rejected. The neutral components were then subdivided into arbitrary "polar" and "non-polar" fractions by extraction of a benzene solution of the neutral components with water. The water-soluble "polar" fraction was distributed between benzene and water on a counter-current distribution apparatus to yield the diols (XXIX) and (XXX). The benzene-soluble "non-polar" fraction was distributed between light petroleum (b.p. 40-60°) and water/30% methanol on a similar apparatus to yield the alcohols (XXVII) and (XXVIII).

Further purification of the diol (XXIX) was carried out by forming an isopropylidene compound and chromatographing this on alumina. The alcohols (XXVII) and (XXVIII) were both further purified by chromatography on short columns of alumina.

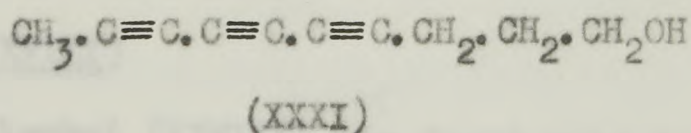
All the components were extremely unstable and decomposed even

on storing at -10° in dilute solutions. Attempts to get the alcohols (XXVII), (XXVIII) and the diol (XXIX) to crystallise only resulted in the separation of highly labile oils.

The Alcohol (XXVII)

This component was found in concentrations of up to 1.2 mg./litre in the culture fluid. The ultraviolet absorption data indicated an enediyne chromophore having a terminal ethynyl group. The alcohol group was identified from its infrared spectrum (3610 and 1055 cm^{-1}), which also contained allene bands at 1947 and 858 cm^{-1} . Ethynyl hydrogen was confirmed by a sharp absorption at 3295 cm^{-1} . Hydrogenation of the alcohol (XXVII) gave n-decanol, identified from a single peak on a vapour phase chromatogram. In this and subsequent cases, the identity of the hydrogenated product was confirmed by obtaining a single peak on a vapour phase chromatogram of a mixture of the product with the standard or synthetic specimen.

The alcohol (XXVII) isomerised readily in dilute alkali to a crystalline triyne-alcohol (XXXI), m.p. $69.5-71^{\circ}$, which showed no



ethynyl hydrogen in its infrared spectrum and whose structure was confirmed by synthesis. The synthetical route followed was that given in Fig. 15, chosen because of the availability of

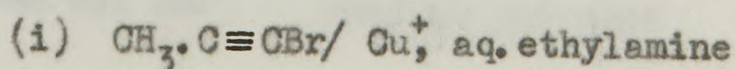
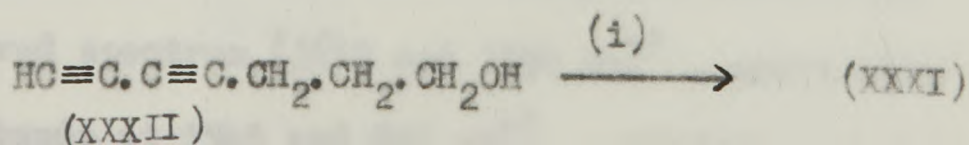


Fig. 15

the alcohol (XXXII), an intermediate in the synthesis of deca-4,6,8-triyne-1,10-dioic acid (see Appendix). The product had infrared absorption identical with that of the natural isomer, and an undepressed mixed melting point was obtained. The mechanism of this isomerisation is discussed in a later section (p. 78). Some further isomerisation occurs to give traces of a diyne-enol-ether chromophore.⁽⁴³⁾ This most probably results from alkoxide ion attack by the terminal hydroxyl group to give the tetrahydrofuran derivative (XX) as in Fig. 16.

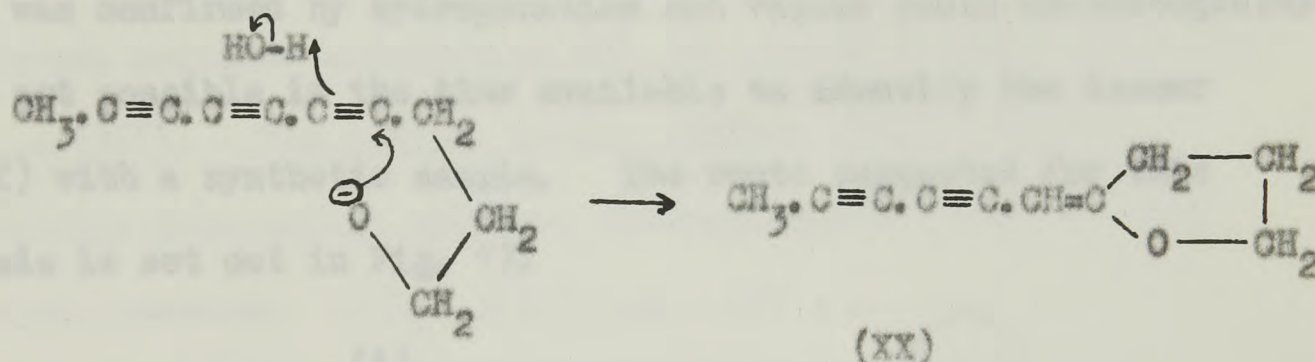


Fig. 16

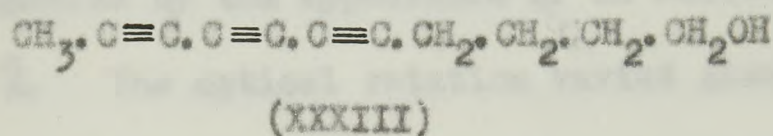
This alcohol is one of the micro-organism polyacetylenes whose structure does not conform to the "odd-even rule" (see Introduction), having a C_{10} chain and a terminal ethynyl group. The possible biogenesis of this compound is discussed on p. 52 of this thesis.

The Alcohol (XXVIII)

The C_{11} alcohol (XXVIII) was found only in concentrations of up to 0.4 mg./l. in the culture fluid. The ultraviolet absorption data indicated an enediyne chromophore having a terminal ethynyl group. The alcohol group was identified from the infrared spectrum (3610 and 1050 cm^{-1}), which also showed allene bands at 1945 and 857 cm^{-1} . Ethynyl hydrogen

was confirmed by a sharp absorption at 3295 cm^{-1} . Hydrogenation of the alcohol (XXVIII) gave n-undecanol, identified from a single peak on a vapour phase chromatogram.

The alcohol (XXVIII) isomerised readily in dilute alkali to a crystalline tri-yne-alcohol (XXXIII), m.p. $38-40^{\circ}$, which showed no ethynyl hydrogen in its infrared spectrum. The chain



length was confirmed by hydrogenation and vapour phase chromatography. It was not possible in the time available to identify the isomer (XXXIII) with a synthetic sample. The route suggested for this synthesis is set out in Fig. 17.

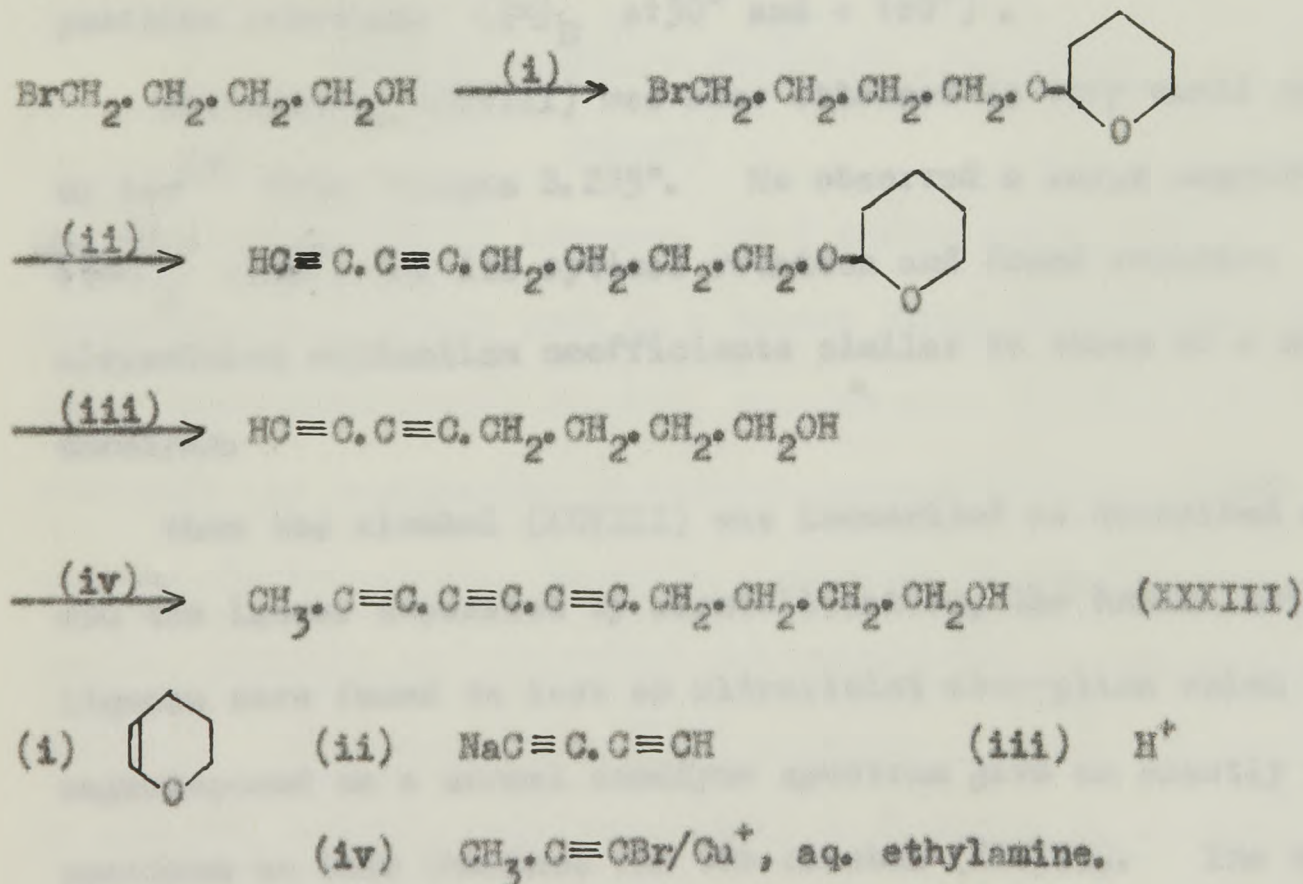


Fig. 17

The mode of formation of the isomer (XXXIII) is discussed in a

later section (p. 80).

The ultraviolet absorption data for the alcohol (XXVIII) were somewhat distorted in several of the maximum intensities compared with those for the normal enediyne chromophore. The difference varied according to the particular growth from which the metabolite had been obtained, and a large distortion was invariably accompanied by the appearance of an additional broad maximum at 3030 Å. The optical rotation varied similarly. The sample having an ultra-violet absorption spectrum nearest to the normal enediyne intensities had the largest negative rotation ($[\alpha]_D - 170^\circ$). Two samples having spectra which deviated appreciably from normal enediyne absorption were found to have positive rotations ($[\alpha]_D +130^\circ$ and $+160^\circ$).

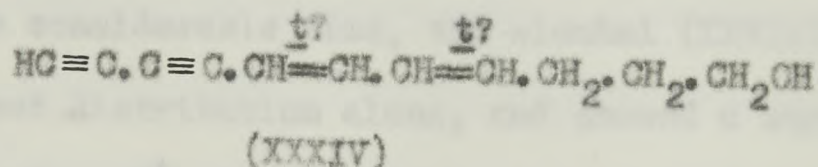
The alcohol (XXVIII) was also obtained in very small quantities by Bew⁽⁸⁾ from "fungus B.285". He observed a large negative value ($[\alpha]_D -300^\circ$) for its optical rotation and found relative ultraviolet extinction coefficients similar to those of a normal enediyne.

When the alcohol (XXVIII) was isomerised as described above and the isomer separated by crystallisation, the hexane mother liquors were found to have an ultraviolet absorption which when superimposed on a normal enediyne spectrum gave an exactly similar spectrum to that obtained for the alcohol (XXVIII). The new absorption compared very well in maxima and their relative extinction coefficients with that reported by Bohlmann⁽⁴⁴⁾ for a

dienediyne chromophore. The residue after evaporation of the mother liquors had no measurable rotation, but the infrared spectrum showed ethynyl hydrogen (3310 cm^{-1}) and weak absorptions at 1628 and 981 cm^{-1} . From this evidence, it would seem that there are at least three components in the extract which had been purified by counter-current distribution and alumina chromatography.

- (i) The alcohol (XXVIII)
- (ii) A compound with a dienediyne chromophore.
- (iii) A strongly destrorotatory species which loses its optical activity on treatment with dilute alkali.

There is considerable evidence for assigning the structure (XXXIV) to the molecule containing a dienediyne chromophore.



The chromophore itself accounts for 8 carbon atoms; the appearance of a single peak on a vapour phase chromatogram for the hydrogenated impure alcohol, coupled with the fact that the compounds are inseparable by counter-current distribution or alumina chromatography, suggests a molecule of similar chain length, polarity and absorption characteristics to the alcohol (XXVIII). The separation of the alcohols (XXVIII) and (XXXIV) by physical methods would reasonably be expected to be difficult.

Treatment of the residues from the mother liquors with cupric acetate in aqueous pyridine gave a product with a series of 8 detectable bands in the ultraviolet and visible absorption spectrum.

It is suggested that this is the result of self coupling of the alcohol (XXXIV) to form a diene-tetrayne-diene chromophore (see Fig. 18)

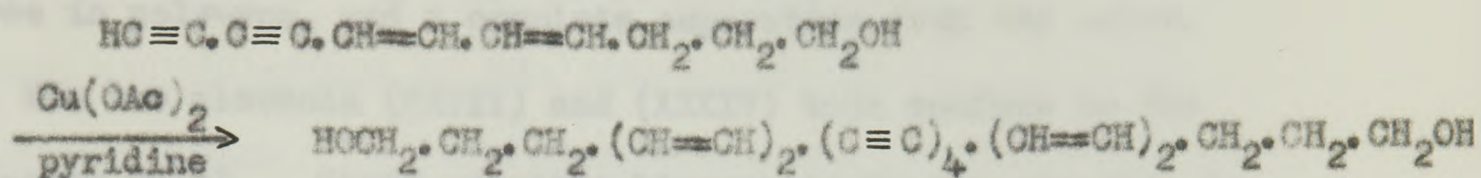


Fig. 18

No record of this chromophore has been found in the literature.

The dextrorotatory impurity may well be allenic, since its optical activity is lost on treatment with dilute alkali. It seems unlikely that it is associated with the dienediyne molecule; it is probably yet another metabolite which is inseparable from the alcohol (XXVIII) by the purification procedures used.

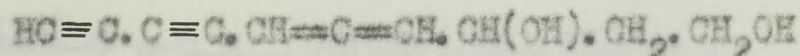
For some considerable time, the alcohol (XXVIII) was purified by counter-current distribution alone, and showed a strong saturated ester band (1733 cm^{-1}) in the infrared spectrum. Several attempts were made to compare the hydrogenated material with synthetic hydroxy-esters, until it was noticed that the retention time on vapour phase chromatography corresponded exactly to that of n-undecanol. The natural material was therefore redistributed using a different (ether-glycol) solvent system, resulting in considerable reduction of the ester band compared to the allene and ethynyl hydrogen bands. Initial attempts to separate the polyacetylenic metabolites from Odontia bicolor on alumina had resulted in over 50% loss of polyyne and no separation of the components. Having separated the alcohol (XXVIII) from the C_{10} homologue (XXVII) but not from the ester

impurity by counter-current distribution, the mixture was rapidly chromatographed on a short alumina column to give only slight losses in poly-yne, and a complete separation from the ester.

The two alcohols (XXVII) and (XXXIV) both conform to the "odd-even rule". Their possible biogenesis by the organism is discussed on p. 52 of this thesis.

The Diol (XXIX)

The diol (XXIX) was found in concentrations of up to



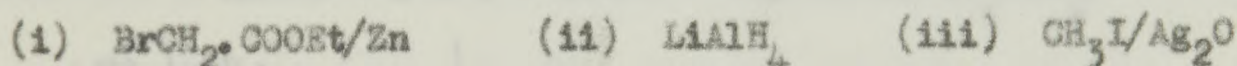
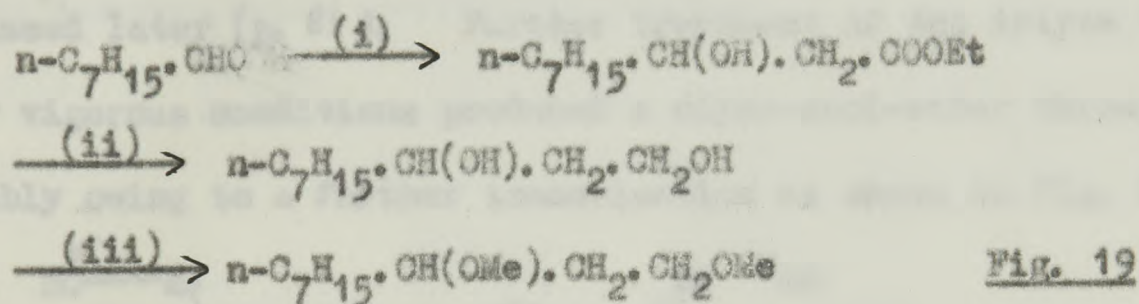
(XXIX)

4.5 mg./l. in the culture fluid. The ultraviolet absorption data indicated an enediyne chromophore having a terminal ethynyl group. The diol grouping was identified from the infrared spectrum (3584 and 3497 cm^{-1} , primary and secondary C-hydroxyl) which also had allene bands at 1944 and 851 cm^{-1} . Ethynyl hydrogen was confirmed by a sharp absorption at 3289 cm^{-1} .

The formation of a typical ene-one chromophore on treatment of the diol (XXIX) with manganese dioxide established the position of one of the hydroxyl groups next to the allene group (or an isolated double bond). Failure to react with sodium periodate solution indicated that the hydroxyl groups were not adjacent. The diol (XXIX) was then hydrogenated in ethanol/hydrochloric acid over platinum and the product found to have the same retention time as n-decanol on vapour phase chromatography. The allene-adjacent hydroxyl group had been hydrogenolysed to leave n-decanol,

establishing a C_{10} skeleton for (XXIX).

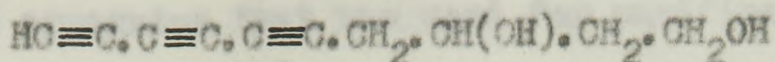
The structure was confirmed by methylation of the product from hydrogenation of the diol (XXIX) in ethanol over palladium/charcoal, and comparison of this dimethyl ether with synthetic 1,3-dimethoxydecane. 1,3-Dimethoxydecane was synthesised as shown in Fig. 19. The C_{10} chain and 1,3-bifunctionality were obtained



by a Reformatsky extension of n-octanal. The diol was methylated by the Purdie method to give the required dimethyl ether. The dimethyl ether of perhydro-(XXIX) and the synthetic sample gave similar single peaks on vapour phase chromatography, and a mixture of the two gave one single peak.

The hydrogenated diol from the allene (XXIX) was obtained crystalline, but the crystals melted below 15° , in contrast to synthetic decane-1,3-diol which melted at $29-30^\circ$. The compounds differ in that the diol from (XXIX) is optically active ($[\alpha]_D -5^\circ$), whereas the synthetic product is a racemic mixture.

The diol (XXIX) isomerised readily in dilute alkali to form a highly unstable triyne (XXXV). At 15° , this compound became an intractable black resin within 20 seconds of being isolated in the condensed state.



(XXXV)

The triyne-diol (XXXV) was characterised by its ultraviolet ($\lambda_{\text{max.}}$ 2070 Å) and infrared ($\nu_{\text{max.}}$ 3584, 3500, 1080 and 1060 cm^{-1}) absorption spectra. The infrared spectrum also showed ethynyl hydrogen at 3279 cm^{-1} . The mechanism for this isomerisation is discussed later (p. 81). Further treatment of the triyne (XXXV) under vigorous conditions produced a diyne-enol-ether chromophore,⁽⁴³⁾ probably owing to a further isomerisation as shown in Fig. 20

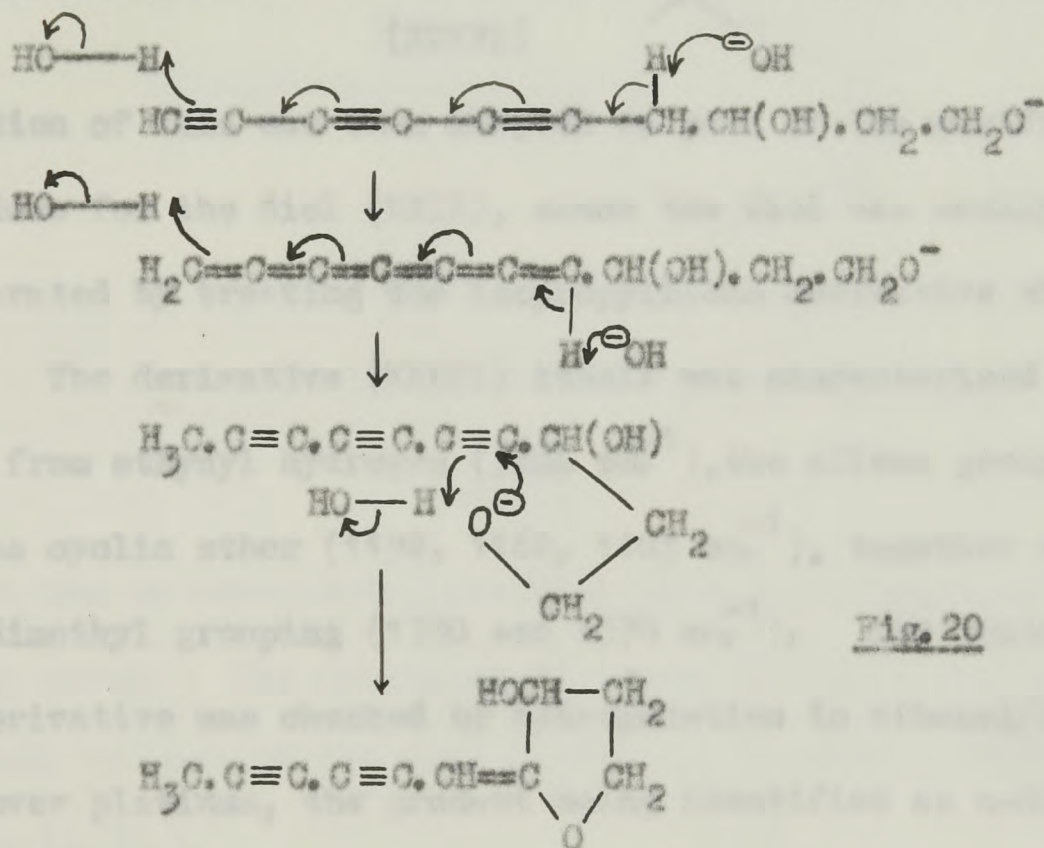
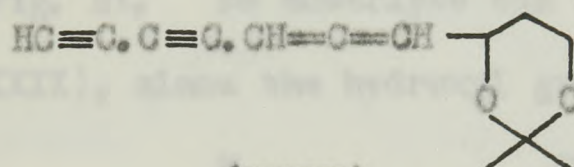


Fig. 20

Work on the diol (XXIX) was carried out initially on samples purified only by counter-current distribution. When the presence of a 1,3-dihydroxy grouping seemed likely, the diol (XXIX) was refluxed for 12 hours with excess acetone in the presence of a little *p*-toluenesulphonic acid. The products were distributed using the same counter-current distribution solvent

system and apparatus that was used for purification of the diol. Most of the enediyne was found in the leading top phase solutions, and only a very small amount was detected in the middle tubes usually occupied by the diol (XXIX). The compound had obviously become much less polar, and it was found that it could be chromatographed on neutral "Woelm" alumina. The purified product was identified as the isopropylidene derivative (XXXVI).



Formation of this was then adopted as part of the purification procedure for the diol (XXIX), since the diol was easily regenerated by treating the isopropylidene derivative with dilute acid. The derivative (XXXVI) itself was characterised by infrared bands from ethynyl hydrogen (3284 cm^{-1}), the allene group (1951 cm^{-1}) and the cyclic ether ($1192, 1162, 1103\text{ cm}^{-1}$), together with a gem-dimethyl grouping (1380 and 1370 cm^{-1}). The chain length of the derivative was checked by hydrogenation in ethanol/hydrochloric acid over platinum, the product being identified as n-decanol (from regeneration of the diol in acid solution, followed by hydrogenolysis) by vapour phase chromatography. The derivative (XXXVI) was also hydrogenated over palladium in ethanol, and the product identified as 1,3-O-isopropylidenedecane-1,3-diol by comparison with a synthetic sample (from decane-1,3-diol, see above) by vapour phase chromatography.

The derivative (XXXVI) readily isomerised in dilute alkali to form a triyne (XXXVII). The infrared spectrum showed ethynyl

if n-decanol was shaken with acetic/hydrochloric acid over platinum catalyst, the same two peaks were produced.

The optical rotations of the diol (XXIX) and its derivatives were measured, and are set out in Fig. 22.

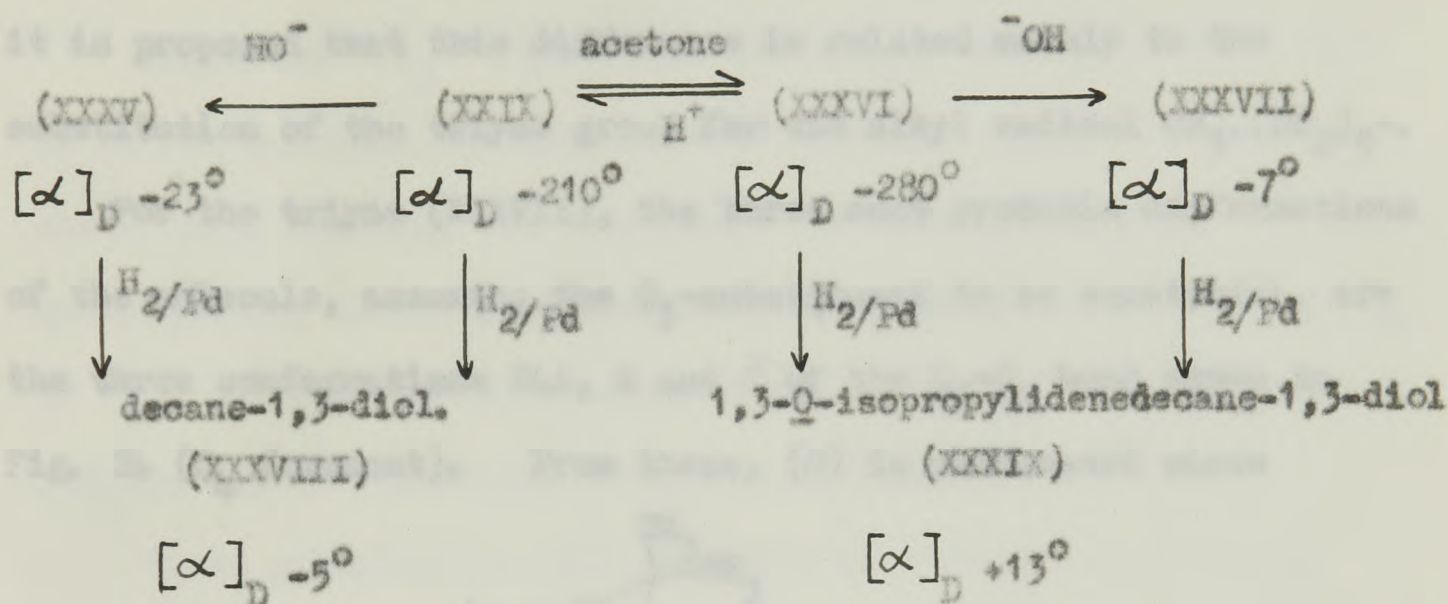


Fig. 22

It was of interest to see if Brewster's methods ⁽⁴²⁾ could be applied to determine the absolute configuration of the C₃ atom in these compounds. Those of interest in this respect are the isomers and hydrogenated compounds shown in Fig. 23. The

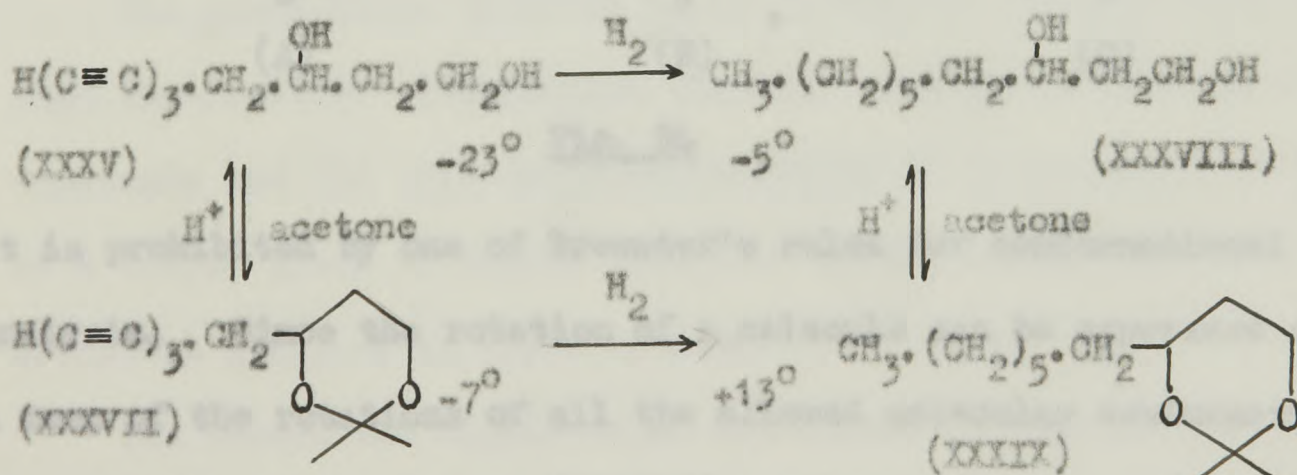


Fig. 23

difference (18°) between the triyne-diol (XXXV) and the hydrogenated

diol (XXXVIII) is paralleled by the difference (20°) between the triyne-isopropylidene derivative (XXXVII) and the hydrogenated isopropylidene derivative (XXXIX), and is of the same sign (+) when moving from triyne to hydrogenated material. From this observation it is proposed that this difference is related mainly to the substitution of the triyne group for the alkyl radical $\text{CH}_3 \cdot (\text{CH}_2)_5 \cdot$.

For the triyne (XXXVII), the three most probable conformations of the molecule, assuming the C_3 -substituent to be equatorial, are the three conformations 24A, B and C of the C_3 - C_4 bond shown in Fig. 24 (C_4 foremost). From these, (C) is eliminated since

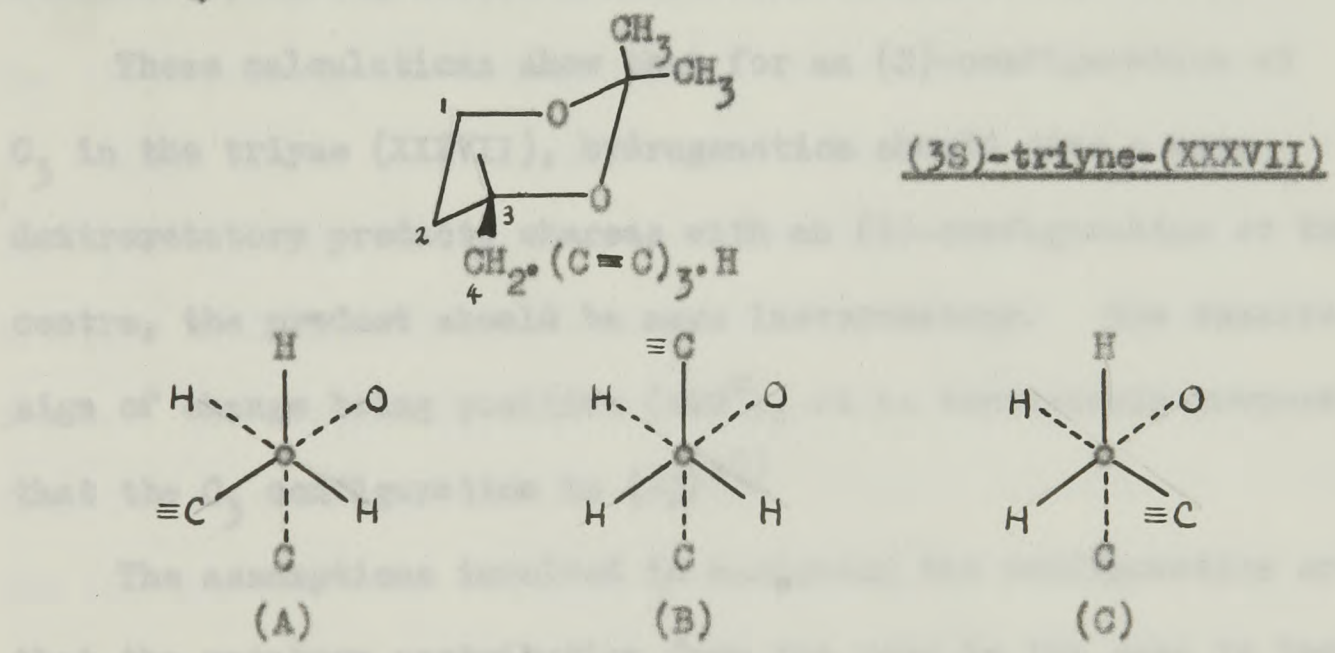


Fig. 24

it is prohibited by one of Brewster's rules for conformational analysis. Since the rotation of a molecule can be expressed as a mean of the rotations of all the allowed molecular conformations, the rotation of the triyne (XXXVII) can be expressed as follows:-

$$\frac{(x + a) + (x + b)}{2}$$

where $\underline{x}$ = contribution of the ring itself, and $\underline{a}$ and $\underline{b}$ the contributions from the allowed bond conformations 24A and 24B. From Brewster's data, $\underline{a} = +\frac{5}{6} \cdot 160^\circ$; $\underline{b} = -160^\circ$ for the above (S)-configuration.

Therefore $[M]_D$ calc. for the triyne (XXXVII) = $(x - 14)^\circ$. If we now consider the hydrogenated material (XXXIX), it can be shown that, assuming the ring makes the same size ($\underline{x}$) of contribution, then the allowed conformations give a value for $[M]_D$ of $(x - 5)^\circ$ for the (S)-configuration. The corresponding values for the (R)-configuration are $(14 - x)^\circ$ and $(5 - x)^\circ$.

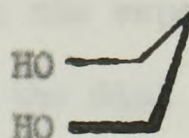
These calculations show that for an (S)-configuration at C_3 in the triyne (XXXVII), hydrogenation should give a more dextrorotatory product, whereas with an (R)-configuration at the same centre, the product should be more laevorotatory. The observed sign of change being positive $(+20^\circ)$, it is tentatively proposed that the C_3 configuration is (S).⁽⁴⁵⁾

The assumptions involved in assigning the configuration are (a) that the rotatory contribution from the ring is the same in both compounds and (b) that the polarisability of the terminal carbon atom of a triyne group is higher than that of the carbon atom of a methylene group of an alkyl radical.

The calculated magnitude of the change in rotation on hydrogenation is increased if a higher value for the rotatory contribution of the acetylene attachment atom is taken. The value used is that given by Brewster for the terminal carbon atom of a

CN group. For a triyne this would certainly be higher, and would bring the calculated difference closer to the observed value.

An exactly similar treatment was extended to the diols (XXXV) and (XXXIX), assuming that the conformation



represents an energy minimum and is entirely responsible for the rotatory contribution from the C_1-C_3 unit. Using Brewster's values, the calculated rotation for the hydrogenated diol (3S)-(XXXIX) was -5° , and that of the triyne diol (3S)-(XXXV) -14° (a minimum value, calculated from the data for CN group; see above). Similarly, for the (3R)-isomers, the values are $+5^\circ$ and $+14^\circ$.

These latter calculations support those for the isopropylidene derivatives, but are based on the assumption that, through intramolecular hydrogen bonding, all molecules have been one C_1-C_3 conformation shown above. However, it is felt that there is reasonable overall evidence from the calculations and experimental results tentatively to propose the (S)-configuration for the C_3 centre in these compounds. A projection of the configuration is shown in Fig. 25.

(S)-configuration at the C_3 centre

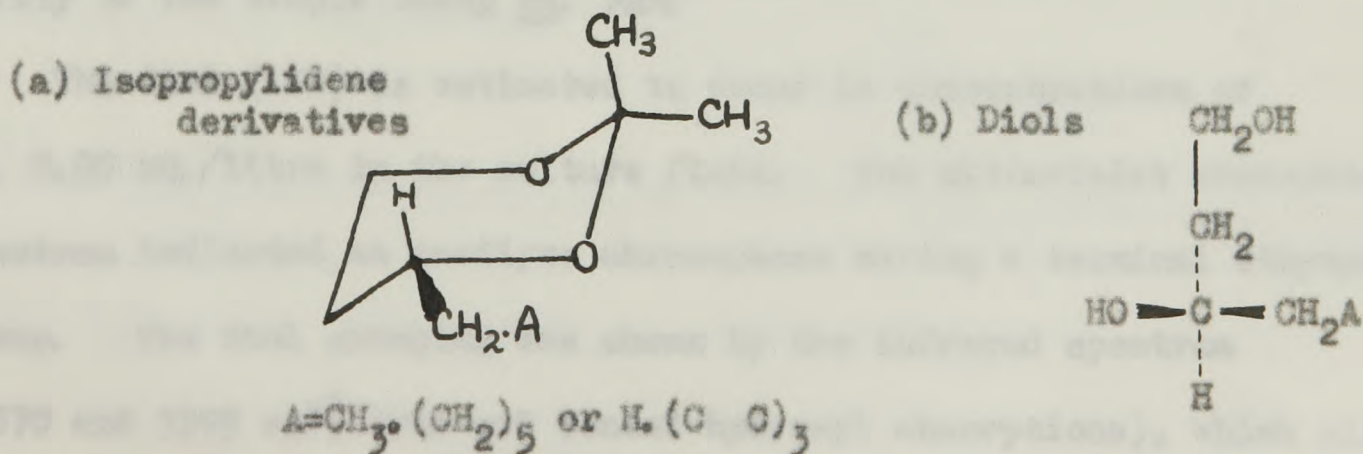
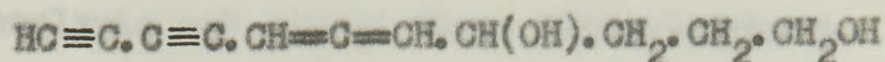


Fig. 25

The Diol (XXX)

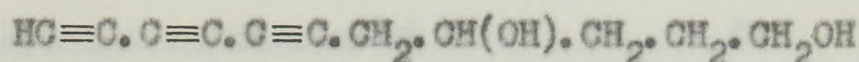


This minor component was first detected from the appearance of a small peak corresponding to n-undecanol in the vapour phase chromatography of hydrogenolysed samples of the diol (XXIX) which had not been through an isopropylidene derivative. It was detected again on counter-current distribution of a large batch of the crude diol (XXIX), when the distribution curve showed a shoulder on the less polar side (see Experimental Section). The contents of the tubes giving rise to this shoulder were worked up and part of the extract was hydrogenated in ethanol/hydrochloric acid over platinum and examined by vapour phase chromatography. The two peaks corresponding to n-decanol and n-undecanol were in the ratio 5 : 1 (resp.). The remainder of the residue was refluxed with acetone in the presence of p-toluenesulphonic acid, and a sample of the polar products hydrogenated (EtOH-HCl/Pt). The ratio was then 2 : 5 (in favour of n-undecanol), and the total poly-yne remaining was estimated at ca. 2 mg. This was the best sample of the diol (XXX) which could be obtained in the time available, and so it was investigated as described below, the purity of the sample being ca. 70%.

The diol (XXX) is estimated to occur in concentrations of ca. 0.05 mg./litre in the culture fluid. The ultraviolet absorption spectrum indicated an enediyne chromophore having a terminal ethynyl group. The diol grouping was shown by the infrared spectrum (3570 and 3395 cm^{-1} , free and bonded hydroxyl absorptions), which also had allene bands at 1941 and 857 cm^{-1} . Ethynyl hydrogen was confirmed

with a sharp absorption at 3279 cm^{-1} . The chain length was already known from hydrogenolysis and vapour phase chromatography.

The diol (XXX) isomerised readily in dilute alkali to form the triyne-diol (XL). Only just enough material was available to obtain



(XL)

an infrared spectrum (in 1 cm. cells) of the crude isomer, but the relative intensities of free and bonded hydroxyl (3600 and 3420 cm^{-1}), and ethynyl hydrogen (3279 cm^{-1}) gave reasonable support for the structure (XXXIX). The isomer was then hydrogenated in ethanol over palladium on charcoal and the product methylated by the Purdie method and examined by vapour phase chromatography. Two peaks were obtained in the ratio 1 : 9 respectively, one unresolvable from 1,3-dimethoxydecane and the other unresolvable from 1,4-dimethoxy-undecane.

1,4-Dimethoxy-undecane was synthesised by the basic route used by Bu'Lock, Jones and Leeming ⁽¹⁴⁾ to synthesise γ -undecanolactone. The lactone was reduced with lithium aluminium hydride, and the resulting 1,4-diol methylated by the Purdie method to give 1,4-dimethoxy-undecane. The scheme is set out in Fig. 26

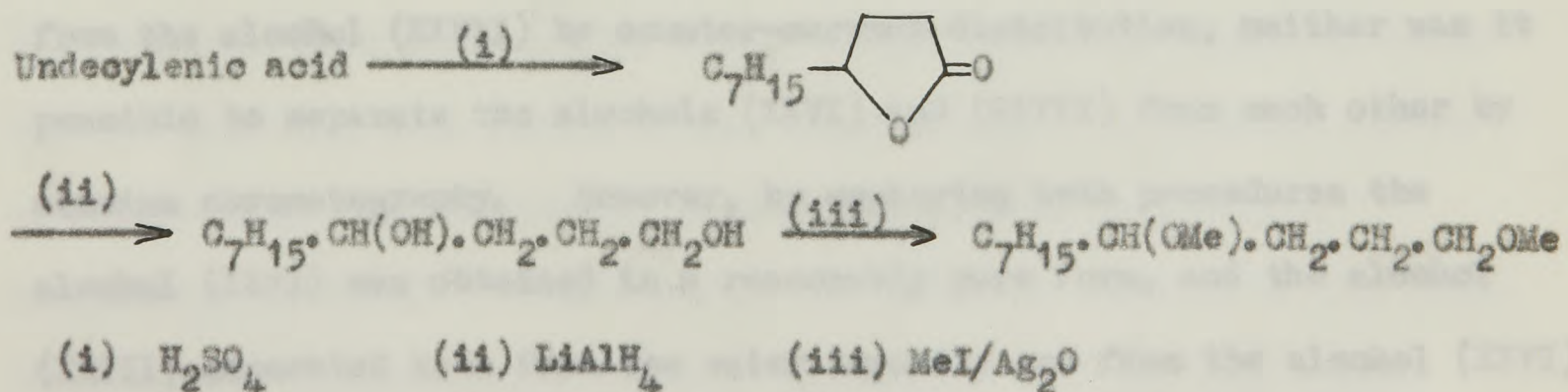
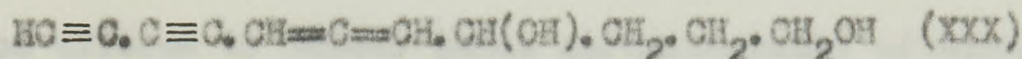
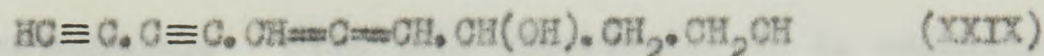
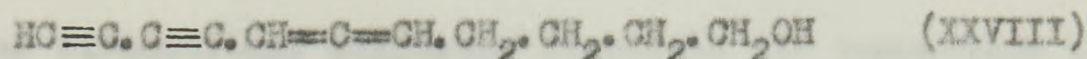
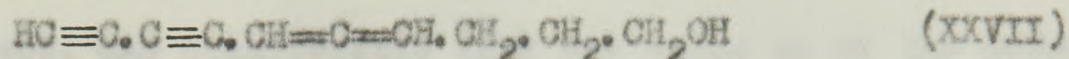


Fig. 26

General Comments



The difficulties experienced in the examination and characterisation of these Odontia bicolor metabolites has necessitated careful consideration of the certainty which can be attached to the results of experiments carried out on this scale. In almost every case, the validity of the result is very dependent on the purity of the starting material. The purification procedure for non-crystalline compounds, especially the allenes, should be as comprehensive as possible although instability and the quantities involved limit the techniques which can be employed.

It is desirable that at least two methods of purification should be used, a counter-current distribution alone is not always sufficiently selective to enable the polyacetylenes to be isolated as pure components. An example of this is the failure to separate an ester (?) impurity from the alcohol (XXVII) by counter-current distribution, neither was it possible to separate the alcohols (XXVI) and (XXVII) from each other by alumina chromatography. However, by employing both procedures the alcohol (XXVI) was obtained in a reasonably pure form, and the alcohol (XXVII) separated both from the ester impurity and from the alcohol (XXVI). Similarly, the diol (XXIX) was purified both by counter-current distribution and, via its isopropylidene derivative, by alumina

chromatography.

The chain lengths of the alcohol (XXVII) and diol (XXIX) remained long in doubt, and considerable time was taken up by the preparation of saturated bifunctional aliphatic standards for comparison by vapour phase chromatography. From this experience, it is emphasised that the evidence offered by vapour phase chromatography of minute amounts of material can be accepted only when the following conditions are satisfied: (i) That there is only one peak (other than the solvent peak) recorded from the solution under examination.

(ii) If there are two or more peaks, then all the additional peaks should be identified both from mixed runs with known standards, and from at least one other source, such as light absorption data. To eliminate the possibility of multiplicity caused by solvent or reagent impurities, "blanks" should be run.

(iii) The similarity of retention time of a compound under investigation with that of a known standard should be checked by examining a mixture of the unknown with the standard in roughly equal concentrations to give a single unresolved peak.

The dimethyl ethers prepared as V.P.C. standards were prepared from the synthetic diols by the Purdie method which was simple to carry out and generally gave yields of the order of 80%. A fourfold excess of silver oxide was the minimum necessary to assist in complete methylation of diols. Occasionally, the infrared spectrum of the crude products had several strong bands in the region $1400-900\text{ cm}^{-1}$, which may have been caused by the formation of a cyclic ethers of the type

shown in Fig 27, possibly from the presence of traces of formaldehyde

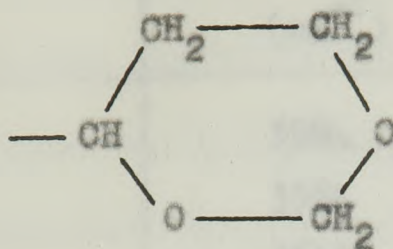


Fig. 27

in the methyl iodide. The bands disappeared on treating the mixture with dilute acid, and were replaced by weak hydroxyl absorption.

This "by-product" was never found from methylation of undecane-1,4-diol which, if it were to cyclise in this fashion, would involve the formation of a 7-membered ring.

Bew reported the isolation of the alcohols (XXVI), (XXVII) and (XXX) from "fungus B.285",⁽⁸⁾ but was unable to characterise them fully, since they were produced in very low yields by the fungus. The chain length of eleven carbon atoms assigned to the diol he isolated was based only on hydrogenation of the natural diol in acetic/hydrochloric acid over platinum catalyst, and the peak obtained on vapour phase chromatography may well have been that of n-decyl acetate (see p. 41).

The infrared data obtained during work on the diol polyacetylenes from Odontia bicolor are of some interest, and are set out in Table 8. The correlation of the difference between the maxima of free and intramolecular hydroxyl absorptions with the spacing of the hydroxyl groups along the flexible carbon chain is discussed by L. P. Kuhn,⁽⁴⁶⁾ who gives values for the three polymethylene ω -glycols as below:-

n	$\Delta\nu$ cm. ⁻¹
2	32
3	78
4	150

for $\text{HO}(\text{CH}_2)_n\text{OH}$:-

Compound	Free Hydroxyl (cm.^{-1})	Bonded Hydroxyl (cm.^{-1})	$\Delta \nu$ (cm.^{-1})
allene (XXIX)	3584	3497	87
allene (XXXV)	3584	3500	84
per-H-(XXIX)	3610	3510	100 *
Decane-1,3-diol	3630	3540	90
triyne (XXX)	3570	3395	175
triyne (XXXVIII)	3600	3420	180
Undecane-1,4-diol	3620	3455	165

* = measured in 1 mm. cells; maxima masked by some intermolecular H-bonds.

Table 8

Differences in the range 80-90 cm.^{-1} are reported by Sohleyer⁽⁴⁷⁾ for various 2-substituted propane-1,3-diols. The two maxima from the diols (XXIX) and (XXXV) were both well defined in spectra of solutions in carbon tetrachloride in 1 cm. cells, and their difference values fall in the expected range for 1,3-diols. Similarly, the values for the 1,4-diols are reasonably consistent although spread over a wider range.

The isopropylidene derivatives were also found to show some common bands, set out in Table 9.

Table 9.

Compound	Observed ν_{max} values. (cm.^{-1})		
	Gem.-dimethyl	Ketal Ring	
allene (XXXVI)	1380, 1370	1192, 1162	1103
triyne (XXXVII)	1378, 1367	1196, 1159	1106
perhydro-(XXXVI)	1378, 1366	1195, 1162	1105
1,3-O-isopropylidene-decane-1,3-diol	1383, 1371	1200, 1168	1111

The absorptions $1200-1100\text{ cm}^{-1}$ were very strong and are most probably skeletal vibrations of the ketal ring. In addition, the separations of the gem.-dimethyl maxima was consistently ca. 11 cm^{-1} . These characteristics for 1,3-O-isopropylidene derivatives are compared with those observed for 1,2-O-isopropylidene derivatives on p. III of this thesis.

Biogenetic Considerations.

The fungus Odontia bicolor has been found to produce five polyacetylenic metabolites in the following order of quantity:-

Components.		Occurrence mg./litre.	% Total Poly-yne
Allenes	(XXIX) C_{10} -diol	4.5	73
	(XXVII) C_{10} -alcohol	1.2	19
	(XXVIII) C_{11} -alcohol	0.4	6.5
	(XXX) C_{11} -diol	0.05	1.0
	(XXXIV) C_{11} -alcohol	0.02	0.5

It is interesting to note that the metabolites which do not conform to the "odd-even rule" are the major products, the C_{11} metabolites together forming only 8% of the total poly-yne production.

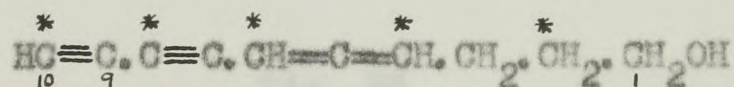
It is already reasonably well established that polyacetylenes are built up in a fashion analogous to that of the fatty acids,⁽⁹⁾ and that odd-numbered chains are produced by removal of a carbon atom from an even-numbered precursor.⁽¹⁰⁾ It seems most likely, therefore, that the C_{11} metabolites from Odontia bicolor are derived from a C_{12} unit through loss of one carbon atom, and that some 90% of the product loses

yet another carbon atom to form the C_{10} derivatives, since both of these have a terminal ethynyl function. The question arises as to whether the two carbon atoms are removed from the same end of the chain, or from opposite ends. The alternative explanation that the C_{10} compounds are produced from a C_{10} precursor such as dehydromatricarianol

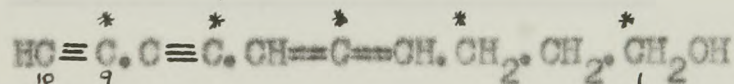
$$CH_3.C\equiv C.C\equiv C.C\equiv C.CH=CH.CH_2OH \rightarrow HC\equiv C.C\equiv C.CH=C=CH.CH(OH).CH_2.CH_2OH$$

is so far without analogy and seems much less probable.

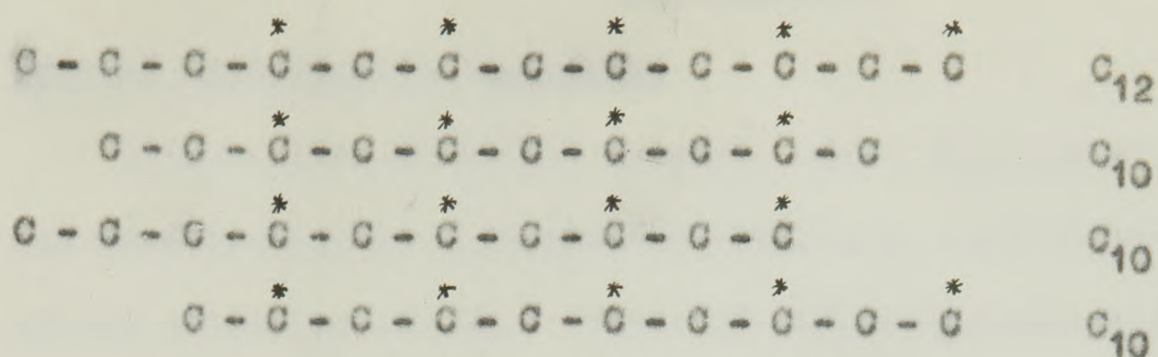
Yuan, in this laboratory, ⁽⁴⁸⁾ found that when carboxyl-labelled (C^{14}) acetate was fed into the culture medium Odontia bicolor, one fifth of the total activity of the C_{10} alcohol (XXVII) was found in the C_9-C_{10} unit, and none in the C_1 (carbinol) unit. If, as is likely, there is alternate atom labelling, then the pattern should be:



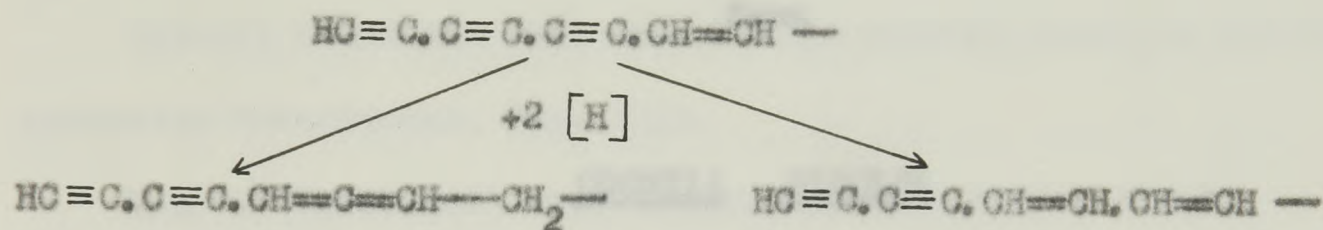
Derivation from a C_{10} molecule such as dehydromatricarianol should have given a labelled C_1 -atom, since the expected labelling pattern would then be:-



Experiments are also in progress to determine the labelling pattern after feeding the organism with carboxyl-labelled (C^{14}) malonate. The hypothetical C_{12} molecule should then have three inactive carbon atoms at one end, since malonate builds on a acetate primer. The possible labelling patterns of the C_{10} product according to where the carbon atoms are removed, are shown in Fig. 28.

Fig. 28

It is of further interest that the two C_{11} alcohols may be derived from the same less saturated precursor, by different reductive processes (see Fig. 29).

Fig. 29

Experimental

General Experimental Procedures

Ultraviolet absorption spectra, where these of chromatographic and counter-current distribution fractions, were measured in ethanol, unless otherwise stated. Those of chromatographic and distribution fractions were measured in ether or the relevant solvent. Ultraviolet absorption spectra were obtained on a Cary Recording Spectrophotometer Model 14M.

THE POLYACETYLENES

Infrared absorption spectra were obtained in carbon tetrachloride, unless otherwise stated, on a Perkin-Elmer Model 51 spectrophotometer.

from

Optical rotations were measured in ethanol using an EEL-400 Automatic Polarimeter, Type 1434.

E.p.'s (corrected) = ODONTIA BICOLOR Keller Block.

Alumina used for chromatography was either Fisher Reagent Grade "N", deactivated by treatment with 5% of 10% acetic acid, referred to in this thesis as "alumina (S)"; or Fisher "neutral" alumina, activity grade IV (10% water added), referred to as "alumina (N)".

- Experimental -

Vapour phase chromatography was carried out on a Pye Argon 614, with a 4 ft. column of 10% apiezon "L" supported on "Rohmco" (AD-400 acid-washed).

The chemical drying agent employed was anhydrous magnesium sulphate unless otherwise stated.

All evaporations were carried out under reduced pressure.

Calculations of approximate polyacetylene concentrations in solutions were based on the value of the difference in extinction coefficient between the longest wavelength ultraviolet absorption maxima and minima.

Experimental

General Experimental Procedures

Ultraviolet absorption spectra, other than those of chromatographic and counter-current distribution fractions, were measured in ethanol, unless otherwise stated. Those of chromatographic and distribution fractions were measured in ether or the relevant solvents. Ultraviolet absorption spectra were obtained on a Cary Recording Spectrophotometer Model 14M.

Infrared absorption spectra were measured in carbon tetrachloride, unless otherwise stated, on a Perkin-Elmer Model 21 spectrophotometer.

Optical rotations were measured in ethanol using an ETL-NPL Automatic Polarimeter, Type 143A.

M.p.'s (corrected) were determined on a Kofler block.

Alumina used for chromatography was either Peter Spence Grade "H", deactivated by treatment with 5% of 10% acetic acid, referred to in this thesis as "alumina (S)"; or Woelm "neutral" alumina, activity grade IV (10% water added), referred to as "alumina (W)".

Vapour phase chromatography was carried out on a Fye Argon G.C.A. with a 4 ft. column of 10% apiezon "L" supported on "Embacel" (60-100 mesh, acid-washed).

The chemical drying agent employed was anhydrous magnesium sulphate unless otherwise stated.

All evaporations were carried out under reduced pressure.

Calculations of approximate polyacetylene concentrations in solution were based on the value of the difference in extinction coefficient between the longest wavelength ultraviolet absorption maximum and minimum.

Growth of *Odontia bicolor*. -

The culture of "*Odontia bicolor* (A. & S.) Bres" was obtained from the Forest Products Research Laboratory and was numbered 165 in their type culture collection. The fungus was grown as surface cultures on a medium of 3% malt extract, 0.8% sodium acetate. The poly-yne content was highest at periods varying from 21 to 42 days. The maximum concentration of enediynes poly-yne varied, for different growths from 2.8 mg./litre to 9.5 mg./litre. The yield of poly-yne was increased by reflooding with 4% glucose, 0.8% sodium acetate solution when the poly-yne concentration was highest after a further 17-21 days at ca. 10 mg./litre. A second reflood with 4% glucose, 1.6% sodium acetate yielded 5.8-8.5 mg./litre after 18-19 days. Reflooding a third time with 4% glucose, 1.6% acetate, produced the mycomycin chromophore, maximum at ca 0.5 mg./litre 19 days after reflooding.

Odontia bicolor was also grown as shake cultures on a medium of 3% malt, 0.8% sodium acetate. The poly-yne content first reached a maximum after 7 days with the mycomycin chromophore. It then went to zero, and after 17 days reached a second maximum of ca 13 mg./litre having now the enediyne chromophore. The poly-yne content again fell to zero before rising to a third maximum (enediyne) of ca 5 mg./litre after a total of 34 days growth.

Isolation of Polyacetylenic Components. -

The procedure for a particular growth is described here, representing the general method for all growths.

The culture fluid (23 l.) was continuously extracted with ether

(2.5 l.) for 24 hr. The extract was evaporated to 200 c.c. in a darkened flask, and separated into acidic and neutral fractions by extraction with sodium hydrogen carbonate solution. The ultraviolet absorption spectrum of the acidic fraction indicated mycomycin (ca. 0.1 mg.). The neutral fraction was dried, evaporated to dryness, and the residue taken up in benzene (100 c.c.). The benzene solution was washed with water (3 x 100 c.c.) and the aqueous extracts saturated with salt and extracted with ether (3 x 300 c.c.) These extracts were combined and dried, and found to contain ca. 100 mg. neutral "polar" enediyne. The benzene solution was dried and evaporated and the residue taken up in ether. This solution contained ca. 36 mg. neutral "less polar" enediyne.

Routine Purification of the Neutral "Polar" Fraction.-

An ethereal solution of the neutral "polar" fraction (100 mg.) was evaporated into benzene (80 c.c.). This solution was placed in the first two tubes of a 50 tube Craig-type counter-current distribution apparatus (volumes of upper and lower phases 40 c.c.) and distributed between benzene and water. After 50 transfers, the distribution of polyacetylenic material was as follows:-

Tubes 10-30 enediyne (XXIX) 78 mg.

The organic layers were evaporated to dryness and taken up in ether. The aqueous layers were extracted with ether (3 x equal volume), the dried extracts evaporated and the residues taken up in ether. The combined ethereal solutions were examined for ultraviolet light absorption from which the quantity of polyacetylenic material was estimated.

When the structure of the major "polar" component was first proposed as a 1,3-diol, (XXIX), reaction with acetone to form an isopropylidene compound was attempted and, being successful, was then adopted as a normal purification procedure for this component. The isopropylidene compound was chromatographed on alumina (see p. 67), and then treated with 2N hydrochloric acid (50 c.c.) and kept in the dark at 15° for 15 min. The mixture was then extracted with ether and the extracts dried to give a solution of the diol (XXIX).

Large-scale Purification of the Neutral "Polar" Fraction.-

The neutral "polar" material (190 mg.) was distributed between benzene and water on a 120 tube Craig-type counter-current distribution apparatus (volumes of upper and lower phases, 40 c.c.). After 150 transfers, the distribution of polyacetylenic material was as follows:-

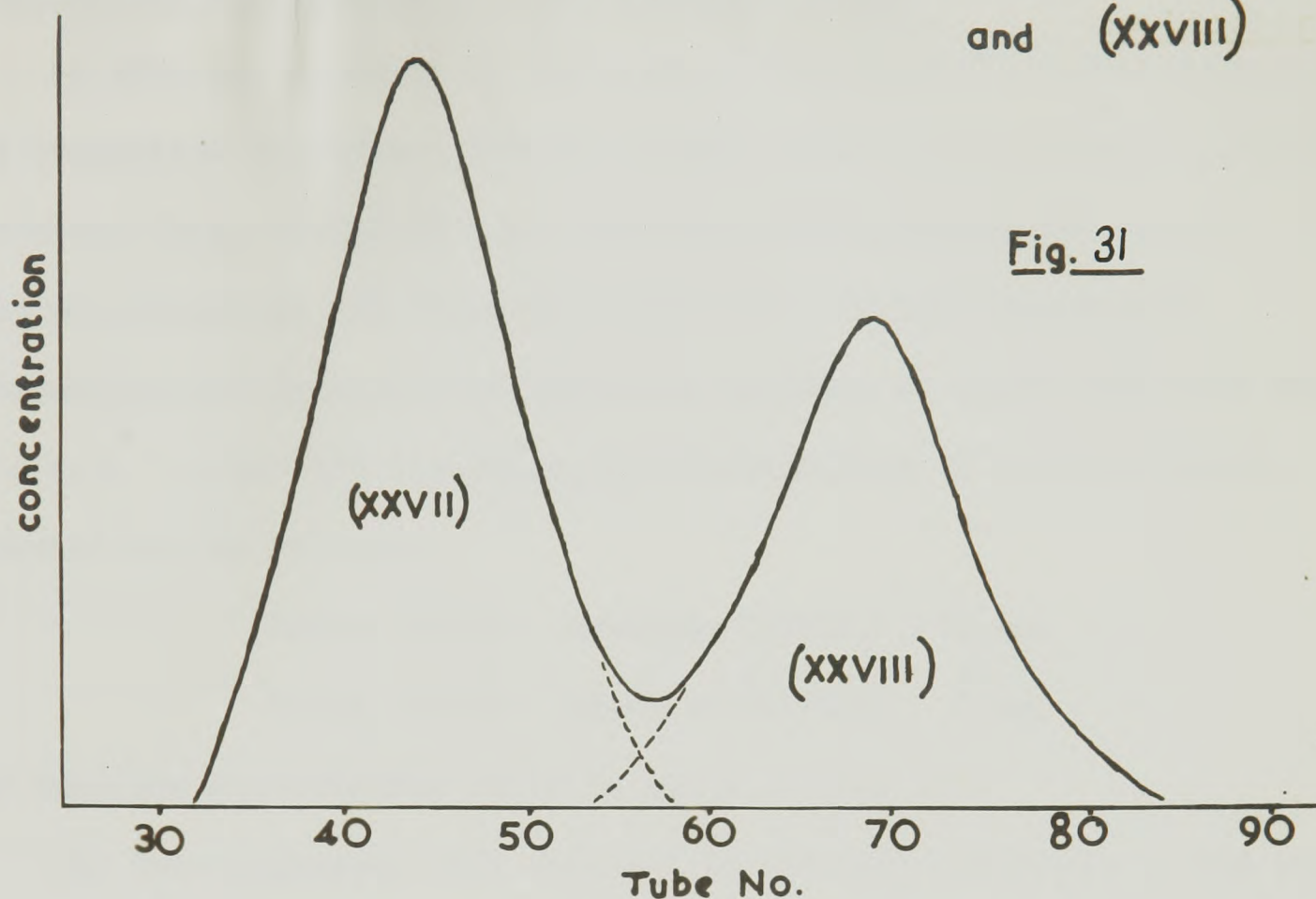
Tubes 33-65 enediyne (XXIX) 178 mg.

Tubes 66-80 enediyne (XXIX) and (XXX) 8 mg.

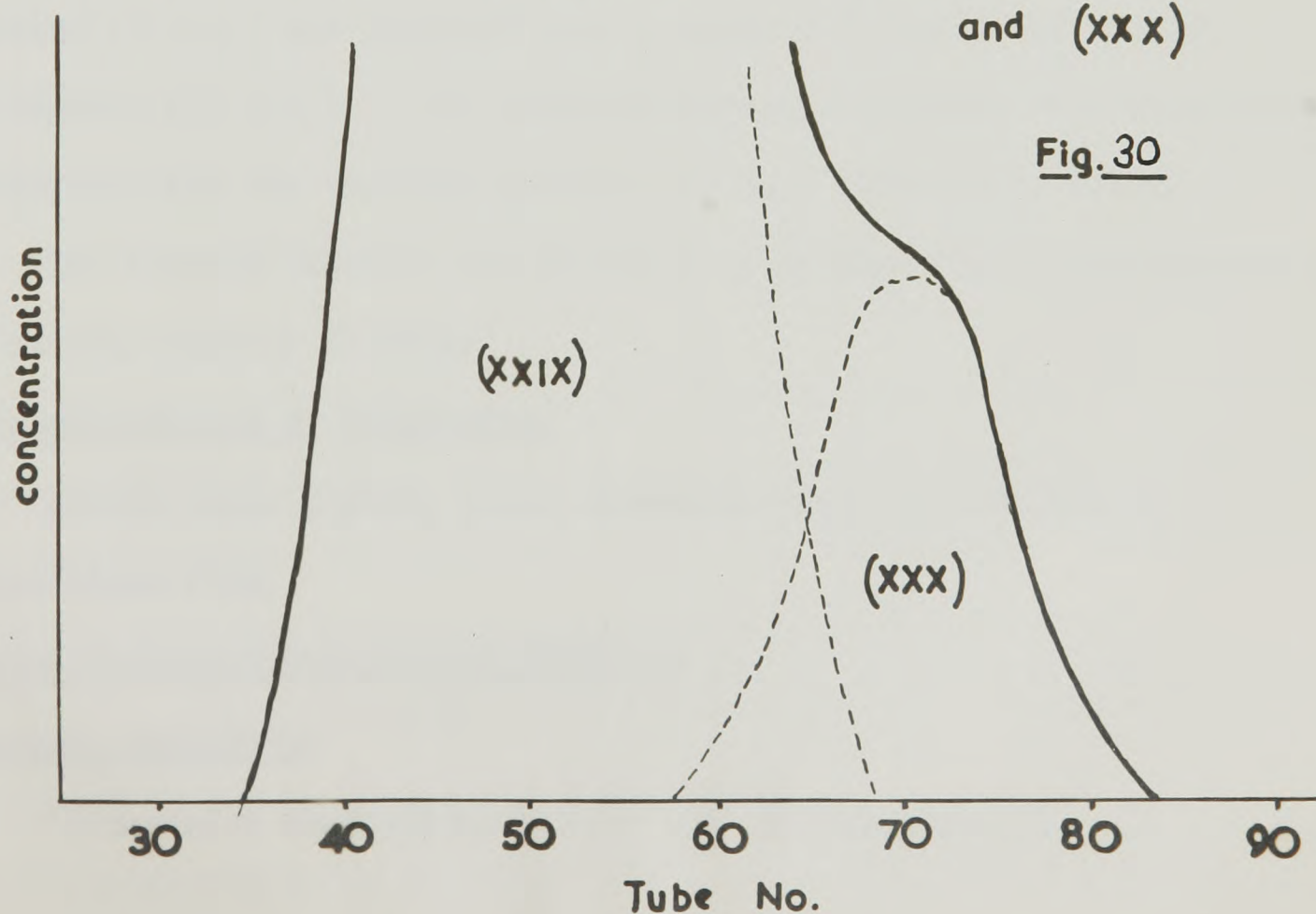
The complete distribution curve is shown in Fig. 30.

The final ethereal solution of the components (8 mg.) in tubes 66-80 was evaporated to dryness and the residue taken up in acetone (25 c.c.) with *p*-toluenesulphonic acid (1.5 mg.) and kept at 15° in the dark for 18 hr. Excess solid sodium carbonate was added, and the mixture kept at 15° for 1 hr., when it was filtered free from sodium carbonate and evaporated to dryness. The residue was extracted with light petroleum (b.p. 30-40°, 2 x 25 c.c.) and then with ether (2 x 25 c.c.). The ethereal extracts contained ca. 2.6 mg. enediyne, shown by V.P.C. (see p. to contain ca. 1.8 mg. of the diol (XXX).

Counter-Current Distribution of the Alcohols (XXVII)
and (XXVIII)



Counter-Current Distribution of the Diols (XXIX)
and (XXX)



Purification of the Neutral "Less Polar" Fraction. -

An ethereal solution of the neutral "less polar" fraction (30 mg.) was evaporated to dryness and the residue taken up in a mixture of light petroleum (b.p. 40-60°, 80 c.c.) and water/30% methanol (80 c.c.). This was placed in the first two tubes of a 100 tube Craig-type counter-current distribution apparatus (volumes of upper and lower phases 40 c.c.). After 109 transfers, the distribution of polyacetylenic material was as follows:-

Tubes 30-50 enediyne (XXVII) 18 mg.

Tubes 60-89 enediyne (XXVIII) 5 mg.

The complete distribution curve is shown in Fig. 31.

The two components were obtained in ethereal solutions by the same procedure as for the neutral "polar" fraction (p. 57).

The alcohol (XXVII) (18 mg.) in light petroleum (b.p. 40-60°)/30% benzene (2 c.c.) was adsorbed onto alumina (S)(12g.), and eluted in benzene (60 c.c.). The combined benzene fractions were evaporated to dryness and the residual alcohol (17 mg.) taken up in ether.

The alcohol (XXVIII) was purified by alumina (S) chromatography in an exactly similar fashion.

Characterisation of Components.

Except where stated, these compounds were not obtained in crystalline form.

Deca-4,5-diene-7,9-diyn-1-ol (XXVII). -

Physical Constants.

Ultraviolet Absorption: ($\epsilon \times 10^{-3}$ in parentheses)

$\lambda_{\text{max.}}$: 2775 (11.0), 2625 (14.1), 2490 (9.2), 2365 (4.9) 2240[#](3.1),
2070 Å (44.5). ([#] denotes inflexion)

Infrared Absorption:

$\nu_{\text{max.}}$: 3610 (O-H), 3295 ($\equiv\text{C-H}$), 2208 ($\text{C}\equiv\text{C}$), 1947 ($-\text{CH}=\text{C}=\text{CH}-$),
1055 (C-O-H), 858 cm^{-1} ($-\text{CH}=\text{C}=\text{CH}-$).

Optical Rotation:

$[\alpha]_{\text{D}}^{21} -290^{\circ}$ (c 0.012)

Chain Length Determination.-

The alcohol (XXVII; 10 mg.), was taken into ethanol (15 c.c.) and hydrogenated over 5% palladium on charcoal (5 mg.) for 1 hr. The product was filtered free from catalyst, evaporated to dryness, and the residue taken up in ethanol (0.2 c.c.). The perhydro-alcohol was identified as n-decanol by vapour phase chromatography. [Retention time 5.04 min. on a column of 25% Apiezon "L" supported on "Embacel" at 192°. Column length 4 ft. Retention time of authentic n-decanol 5.08 min.]

Isomerisation of the Alcohol (XXVII) in 0.1N Sodium Hydroxide Solution.-

The alcohol (XXVII ; 15 mg.) was taken into sodium hydroxide solution (0.1N, 100 c.c.) and kept at 15° in the dark for 75 min. The solution was acidified to pH 4 and extracted with ether (2 x 100 c.c.). The combined extracts were washed with ammoniacal silver nitrate (10% in dilute ammonia solution) and water, and then dried. This solution had the following ultraviolet absorption characteristics.

$\lambda_{\text{max.}}$: 2920 (shoulder, 1.0), 2780 (1.7), 2630 (1.8), 2500 (1.45),
233 (1.55), 223 (1.50), 2090 Å (110).

After evaporation of the ether at 15° the crude product was extracted

with hexane, and on cooling to -10° , this extract yielded colourless plates (7.5 mg.), m.p. $69.5-71^{\circ}$, which slowly turned pink. Mixed m.p. with synthetic deca-4,6,8-triyn-1-ol, $69.5-71^{\circ}$ (Found: C, 81.5; H, 7.65. $C_{10}H_{10}O$ requires C, 82.15; H, 6.90%) $\lambda_{\max}$: (ether) $2090 \text{ \AA}$ (ϵ 121,000). $\nu_{\max}$: 3600 (O-H), 2212 ($C\equiv C$), 1053 cm^{-1} (C-O-H). Mixed m.p. with synthetic deca-4,6,8-triyn-1-ol, $69.5-71^{\circ}$.

Undeca-5,6-diene-8,10-diyn-1-ol (XXVIII).-

Physical Constants.

Ultraviolet Absorption: ($\epsilon \times 10^{-3}$ in parentheses) $\lambda_{\max}$: 2780 (11.0), 2630 (12.4), 2490 (8.4), 2370 (6.9), 2244 (4.9), $2080 \text{ \AA}$ (46.6)

Infrared Absorption: $\nu_{\max}$: 3610 (O-H), 3295 ($\equiv C-H$), 2203 ($C\equiv C$), 1945 ($-CH=C=CH-$), 1050 (C-O-H), 857 cm^{-1} ($-CH=C=CH-$).

Optical Rotation $[\alpha]_D^{25}$ -160° (c 0.017)

Chain Length Determination.-

The alcohol (XXVII) (5 mg.) was taken into ethanol (17 c.c.) and hydrogenated over 5% palladium on charcoal (20 mg.) for 6 hr. The product was filtered free from catalyst, evaporated to dryness, and the residue taken up in ethanol (0.2 c.c.). The perhydro-alcohol was identified as undecanol by vapour phase chromatography. [Retention time 10.5 min. at 142° . Retention time of authentic n-undecanol 9.8 min. A mixture of the product with n-undecanol gave a single peak, retention time 10.7 min.]

Isomerisation of the Alcohol (XXVIII) in 0.1N Sodium Hydroxide Solution.-

The alcohol (XXVIII ; 10 mg.), was taken into sodium hydroxide solution (0.1N, 25 c.c.) and kept at 30° for 40 min. The solution was then acidified to pH 4, extracted with ether (2 x 30 c.c.), and

the extracts washed with water and dried. After evaporation of the ether at 15° , the crude product was extracted with hexane and this on cooling to -10° yielded pale yellow plates (3 mg.), m.p. $38-40^{\circ}$.

$\lambda_{\text{max.}}$: $2090 \text{ \AA}$ (ϵ 102,000). $\nu_{\text{max.}}$: 3630 (O-H), 2220 ($\text{C}\equiv\text{C}$), 1053 cm^{-1} (C-O-H).

Chain Length Determination of the Isomerisation Product (XXXIII).-

The alcohol (XXXIII), (2 mg.) obtained above was taken into ethanol (15 c.c.) and hydrogenated over 5% palladium on charcoal (5 mg.) for 2 hr. The product was filtered free from catalyst, evaporated to dryness, and the residue taken up in ethanol (0.05 c.c.). The perhydro-alcohol was identified as n-undecanol by vapour phase chromatography. [Retention time 22.9 min. at 162° Retention time of an authentic specimen 22.5 min.]

Examination of the Mother Liquors after Crystallisation of the Isomer (XXXIII).-

The residues after evaporation of the hexane mother liquors had the following light absorption:

Ultraviolet absorption: (relative ϵ in parentheses)

$\lambda_{\text{max.}}$: (ether) 3040 (1.00), 2885 (1.15), 2800 (1.20), 2650 (0.98), 2510 (0.63), 2375 (1.31), $2265 \text{ \AA}$ (1.05).

Infrared absorption:

$\nu_{\text{max.}}$: 3623 (O-H), 3310 ($\equiv\text{C-H}$), 2220 ($\text{C}\equiv\text{C}$), 1628 ($-\text{CH}=\text{CH}-$), 1054 (C-O-H), 981 cm^{-1} ($-\text{CH}=\text{CH}-$).

Optical rotation: none observed.

Eglinton Coupling Reaction.-

The residues examined above were treated with a solution of

cupric acetate (0.2 g.) in pyridine (1.1 c.c.) and water (1.2 c.c.), and the mixture kept for 42 hr. at 15°, when it was poured into water (25 c.c.) and extracted with ether (3 x 25 c.c.). The extracts were washed with 2N. hydrochloric acid, then with water, and then dried.

$\lambda_{\text{max.}}$: (relative ϵ in parentheses) 4260 (1.00), 3890 (1.51), 3600 (1.50), 3370 (2.88), 3155 (2.89), 3010 (3.00), 2800 (2.75), 2640 Å (2.91).

Deca-4,5-diene-7,9-diyne-1,3-diol (XXIX)

Physical Constants.

Ultraviolet Absorption: ($\epsilon \times 10^{-3}$ in parentheses)

$\lambda_{\text{max.}}$: 2785 (11.8), 2630 (15.3), 2495 (9.6), 2370 (4.8), 2250^{*} (2.9), 2080 Å (51.7). (^{*} denotes inflexion)

Infrared Absorption:

$\nu_{\text{max.}}$: 3584 (O-H), 3497 (sharp; intramolecular H-bond), 3289 ($\equiv$ C-H), 2203 (C $\equiv$ C), 1944 (-CH=C=CH-), 1099 (sec. C-O-H), 1050 (pri. C-O-H)

Optical Rotation: $[\alpha]_D^{22}$ -210° (c 0.058)

Manganese Dioxide Oxidation.-

An ethereal solution of the diol (XXIX ; 0.2 mg.) was evaporated to dryness the residue was taken up in methylene dichloride (10 c.c.) and activated manganese dioxide (10 mg.) added. The mixture was shaken for 20 hr. at -10°, and then filtered. A curve was plotted of the difference between the extinction at a given wavelength before and after treatment with manganese dioxide against the wavelength.

This curve had a single maximum ($\lambda_{\text{max.}}$: 2205 Å; ϵ 11,000) characteristic of the ene-carbonyl chromophore.

Attempted Periodate Oxidation.-

To a solution of the diol (XXIX ; 10 mg.) in ethanol (5 c.c.) was added a solution of sodium periodate (5 c.c. of a solution containing sodium periodate (1075 mg.) in water (250 c.c.)). The solution was kept at 15° and sampled (2 c.c. aliquots) after 1 hr. and after 21 hr. To each sample was added a solution of sodium arsenite (0.101N, 1 c.c.) together with an excess of sodium hydrogen carbonate and of 20% potassium iodide solution. The mixture was then titrated with a standard solution of iodine (0.096N) using starch indicator. After 1 hr., 0.502 c.c. of the iodine solution was required; after 21 hr., 0.505 c.c. was required. An identical experiment was carried out in the absence of the diol, and 0.495 c.c. of the iodine solution was required before an end point was reached. These results correspond to 3% oxidation of the diol after 1 hr., and 4% after 21 hr.

Chain Length Determination.-

The diol (XXIX ; 11 mg.), purified by counter-current distribution only, was taken up in ethanol/20% hydrochloric acid (15 c.c.) and hydrogenated over platinum (from 10 mg. of platinic oxide) for 4 hr. The solution was filtered, and then evaporated to ca. 5 c.c., when it was poured into water (50 c.c.) and extracted with ether (3 x 50 c.c.). The ethereal extracts were then washed with water and dried. The ethereal solution was evaporated to dryness and the residue taken up in ethanol (0.15 c.c.). The product was identified as a mixture of decanol (ca. 95%) and undecanol (ca. 5%) by vapour phase chromatography. [Retention times 10.4 and 19.2 min. at 144.5°. Retention times of authentic n-decanol and n-undecanol 10.0 and

19.2 min. respectively. A mixture of the product with authentic n-decanol gave a single peak at 10.0 min. and a much relatively diminished peak at 19.2 min.]

Perhydro-diol (XXXVIII).-

This hydrogenation product of the diol (XXIX) was obtained from the perhydro-isopropylidene compound (see p. 67; 40 mg.), by treating the latter for 15 min. with 2N hydrochloric acid (20 c.c.). The mixture was extracted with ether (2 x 25 c.c.), the ethereal extracts washed with water, and dried. The ethereal solution was evaporated to dryness to give a colourless oil (31 mg.). This, from ether/hexane at -40° , yielded crystals of m.p. $< 0^{\circ}$. $\gamma_{\text{max.}}$: 3571 (O-H), 3491 (sharp; intramolecular H-bond), 1119 (sec. C-O-H), 1064 cm.^{-1} (pri. C-O-H). $[\alpha]_{\text{D}}^{19} -5^{\circ}$ (c 0.61)

Formation of the Dimethyl Ether.-

The above diol (5 mg.) was dissolved in methyl iodide (10 c.c.) and heated under reflux with dry silver oxide (55 mg.) for 12 hr. The product was filtered and the solution evaporated to dryness. The residue was taken up in ethanol (0.2 c.c.). The product was identified as 1,3-dimethoxydecane by vapour phase chromatography. [Retention time 9.4 min. at 168° . Retention time of authentic 1,3-dimethoxydecane, 9.35 min. A mixture of the product with 1,3-dimethoxydecane gave a single peak at 9.5 min.]

Isomerisation of the Diol (XXIX) in Sodium Hydroxide Solution.-

An ethereal solution of the diol (XXIX; 30 mg.), (purified by counter-current distribution and through the isopropylidene compound)

was evaporated into sodium hydroxide solution (0.1N, 50 c.c.) and kept at 15° in the dark for 45 min. The solution was acidified to pH 4 and extracted with ether (2 x equal vol.). The combined ethereal extracts were washed with water and dried. A portion was evaporated to dryness at 15° and the residue became black and intractible within 20 sec.

$\lambda_{\text{max.}}$: 2070 Å (ϵ 126,000). $\nu_{\text{max.}}$: 3584 (O-H), 3500 (sharp intramolecular H-bond), 3279 ($\equiv\text{C-H}$), 2215 cm^{-1} ($\text{C}\equiv\text{C}$). In carbon disulphide; 1080 (sec. C-O-H), 1060 cm^{-1} (pri. C-O-H). $[\alpha]_{\text{D}}^{23}$ -23° (c 0.089).

Isomerisation of the Diol (XXIX) in Sodium Hydroxide/Aqueous Ethanol.-

An ethereal solution of the diol (XXIX ; 25 mg.), was taken into sodium hydroxide in aqueous ethanol (50 c.c., 2% sodium hydroxide, 10% water) and heated under reflux under nitrogen for 1 hr.

Considerable darkening occurred to give a dark brown solution, which was evaporated to small bulk at 15° and then poured into water (100 c.c.) and extracted with ether (2 x 100 c.c.). The combined ethereal extracts were washed and dried, and showed the following light absorption. (Relative ϵ in parentheses) $\lambda_{\text{max.}}$: 2910 (1.00), 2765 (1.24), 2635 (0.95), 2510 (0.63), 2235 (1.73), 2135 (1.49).

The ethereal solution was evaporated under reduced pressure to give a highly viscous brown oil which could not be crystallised. The ultraviolet absorption showed ca 5 mg. enol-ether to be present.

Conversion of the Diol (XXIX) to the Isopropylidene compound (XXXVI).-

The diol (XXIX ; 65 mg.) was kept in acetone (50 c.c.) with p-toluenesulphonic acid (5 mg.) at 15° for 12 hr. Excess sodium carbonate was added, and the mixture kept for a further hour at 15°. The solution was then filtered and evaporated to dryness under reduced

pressure. The residue was extracted with light petroleum (b.p. 30-40°) and chromatographed on alumina (10g.). The required product (46 mg.) was eluted in the same solvent (150 c.c.). $\lambda_{\text{max.}}$: ($\epsilon \times 10^{-3}$ in parentheses) 2775 (11.5), 2630 (14.8), 2490 (9.3), 2365 (4.8), 2240^{*} μ (3.0). (* denotes inflexion). $\nu_{\text{max.}}$: 3284 ($\equiv \text{C-H}$), 2210 ($\text{C}\equiv\text{C}$), 1951 ($-\text{CH}=\text{C}=\text{CH}-$), 1380 and 1370 ($\text{C}(\text{CH}_3)_2$), 1162 and 1103 cm^{-1} (C-O-C) $[\alpha]_{\text{D}}^{23}$ -280°. (d 0.032)

Chain Length Determination. -

The isopropylidene compound (XXXVI ; 15 mg.) was taken into ethanol /20% hydrochloric acid (15 c.c.) and hydrogenated over platinum (from 12 mg. platinum oxide) for 4 hr. The solution was filtered, and then evaporated to ca 5 c.c. when it was poured into water (50 c.c.) and extracted with ether (3 x 50 c.c.). The ethereal extracts were washed with water and dried. The ethereal solution was evaporated to dryness and the residue was taken up in ethanol (0.2 c.c.). The product was identified as decanol by vapour phase chromatography.

[Retention time 14.0 min. at 162°. Retention time of authentic n-decanol 14.3 min. A mixture of the product with authentic material gave a single peak, retention time 14.1 min.]

Hydrogenation over Palladium. -

The isopropylidene compound (XXXVI ; 15 mg.) was taken into ethanol (15 c.c.) and hydrogenated over 5% palladium on charcoal (15 mg.). The solution was filtered, evaporated to dryness, taken up in light petroleum (b.p. 30-40°) and chromatographed on alumina (5g.). The required product was eluted in the same solvent (50 c.c.) $\nu_{\text{max.}}$: 1378 and 1366 ($\text{C}(\text{CH}_3)_2$), 1195, 1162 and 1105 cm^{-1} (C-O-C) $[\alpha]_{\text{D}}^{19}$ +13° (d 0.75).

Identified as 1,3-O-isopropylidenedecane-1,3-diol by vapour phase chromatography. [Retention time 24.7 min. at 144° . Retention time of authentic material 24.7 min. A mixture of the hydrogenation product with authentic material gave a single peak, retention time 13.1 min. at 168°]

Isomerisation of the Isopropylidene Compound (XXXVI) in 0.1N Sodium Hydroxide Solution.-

An ethereal solution of the isopropylidene compound (15 mg.) was evaporated into sodium hydroxide solution (0.1N, 10 c.c.) and kept at 15° for 40 min. The solution was then acidified to pH 4, extracted with ether, and the extracts washed with water and dried. This solution had the following ultraviolet light absorption, $\lambda > 2200 \text{ \AA}$: (relative ϵ in parentheses). $\lambda_{\text{max.}}$: 3290 (1.00), 3080 (1.41), 2890 (1.24), 2730 (0.88), 2405 (6.36), 2290 $\text{\AA}$ (5.11). Spectroscopic estimation, 0.3 mg. triynene. The ethereal solution was evaporated to dryness, the residue taken up in light petroleum (b.p. $30-40^{\circ}$) and chromatographed on alumina (5 g.). The isomer (13 mg.) was eluted in the same solvent (120 c.c.), the triynene chromophore appeared on elution with ether. Constants for the isomer: $\lambda_{\text{max.}}$: 2070 $\text{\AA}$ (ϵ 120,000). $\nu_{\text{max.}}$: 3279 ($\equiv \text{C-H}$), 2215 ($\text{C}\equiv\text{C}$), 1378 and 1367 ($\text{C}(\text{CH}_3)_2$) 1196, 1159 and 1106 cm^{-1} (C-O-C) $[\alpha]_{\text{D}}^{22} -7.0$ (c 0.25)

Undeca-5,6-diene-8,10-diyne-1,4-diol (XXX)

Physical Constants

Ultraviolet Absorption: ($\epsilon \times 10^{-3}$ in parentheses)

$\lambda_{\text{max.}}$: 2790 (10.8), 2635 (13.2), 2500 (8.7), 2370 (5.3), 2250 (4.2), 2080 $\text{\AA}$ (36.5).

Infrared Absorption

$\nu_{\text{max.}}$: 3570 (O-H), 3395 (sharp; intramolecular H-bond), 3279 ($\equiv\text{C-H}$), 2201 ($\text{C}\equiv\text{C}$), 1941 ($-\text{CH}=\text{C}=\text{CH}-$), 1048 (broad; C-O-H), 857 cm^{-1} ($-\text{CH}=\text{C}=\text{CH}-$).

Optical Rotation:

$[\alpha]_D^{21} -50^{\circ}$ (c 0.047)

Chain Length Determination.-

The diol (XXX ; 0.3 mg.) was taken into ethanol/20% hydrochloric acid (15 c.c.) and hydrogenated over platinum (from 2 mg. platinum oxide) for 4 hr. The solution was filtered, and then evaporated to ca. 5 c.c. when it was poured into water (50 c.c.) and extracted with ether (3 x 50 c.c.). The ethereal extracts were washed with water and dried. The ethereal solution was evaporated to dryness, and the residue taken up in ethanol (1 small drop). The product was identified as a mixture of decanol (ca. 30%) and undecanol (ca. 70%) by vapour phase chromatography. [Retention times 6.70 min. and 9.55 min. at 163.5° . Retention times of authentic n-decanol and n-undecanol 6.75 and 9.45 min. respectively.]

Isomerisation of the Diol (XXX) in Sodium Hydroxide Solution.-

An ethereal solution of the diol (XXX ; 1.5 mg.) was evaporated into sodium hydroxide solution (0.1N, 10 c.c.), and kept at 15° in the dark for 90 min. The solution was acidified to pH 4 and extracted with ether (2 x equal vol.). The combined ethereal extracts were washed with water and dried. Evaporation of the ethereal solution afforded insufficient material for a quantitative ultraviolet light absorption estimation or optical rotation.

$\lambda_{\text{max.}}$: 2080 μ . $\nu_{\text{max.}}$: 3600 (O-H), 3240 (sharp; intramolecular H-bond), 3289 ($\equiv$ C-H), 2217 cm^{-1} ($\text{C}\equiv\text{C}$).

Hydrogenation and Methylation.-

The isomer obtained above (XL ; 0.7 mg.) was hydrogenated in ethanol (15 c.c.) over 5% palladium on charcoal (5 mg.) for 4 hr. The solution was filtered, and evaporated to dryness. The residue was taken up in methyl iodide (6 c.c.) and heated under reflux with dry silver oxide (40 mg.) for 12 hr. The solution was filtered, evaporated to dryness, and the residue taken up in ethanol (0.02 c.c.). The product was identified as a mixture of 1,4-dimethoxyundecane (90%) and 1,3-dimethoxydecane (10%) by vapour phase chromatography.

[Retention times 17.0 and 27.4 min. at 164° . Retention times of synthetic 1,3-dimethoxydecane and 1,4-dimethoxyundecane 16.9 min. and 27.5 min. respectively.]

Related Syntheses

Deca-4,6,8-triyn-1-ol.-

To a stirred solution of cuprous chloride (2 mg.) in aqueous ethylamine (33%, 0.6 c.c.) was added, under nitrogen, hepta-4,6-diyn-1-ol (86 mg., see Appendix) in ethylamine (0.6 c.c.) together with hydroxylamine hydrochloride to keep the copper in the reduced form. 1-Bromoprop-1-yne (130 mg.) in methanol (0.5 c.c.) was added dropwise over 10 min. Hydroxylamine hydrochloride was added periodically with the bromopropyne, and when addition of the latter was complete, stirring was continued for 15 min. Solid potassium cyanide was added to dissolve the copper salts, and the mixture acidified with dilute hydrochloric acid, poured into water, and extracted with ether.

The washed and dried ethereal extracts were evaporated at 15° to give crude orange crystals (41 mg.). These crystals were extracted with light petroleum (b.p. $40-60^{\circ}$) and on cooling, colourless plates of the required alcohol were precipitated (25 mg.), m.p. $68-70.5^{\circ}$.

$\lambda_{\text{max.}}$: $2095 \text{ \AA}$ (ϵ 147,000). $\nu_{\text{max.}}$: 3600 (O-H), 2212 ($\text{C}\equiv\text{C}$), 1056 cm.^{-1} (C-O-H). (Found: C, 82.15; H, 6.5. $\text{C}_{10}\text{H}_{10}\text{O}$ requires C, 82.15; H, 6.9%).

1,3-Dimethoxydecane

Ethyl 3-hydroxydecan-1-oate.-

A solution of n-octanal (6.0 g.) and ethyl bromoacetate (9.0 g.) in benzene/30% ether was added slowly, with stirring under anhydrous conditions, to zinc powder (3.3 g., washed with 2% hydrochloric acid, water, ethanol, acetone and ether, and finally dried for 90 min. at 100°). The mixture was heated under reflux for 1 hr., when it was cooled and poured into cold sulphuric acid (10%, 100 c.c.). The organic layer was washed with sulphuric acid (5%, 2 x equal volume), sodium carbonate solution (10%), sulphuric acid (5%) and water. The acidic extracts were combined and washed with ether (2 x 50 c.c.) and the organic extracts combined and dried. After evaporation of the solvents from these extracts, the residue was maintained at 100° under reduced pressure for 1 hr., when it was fractionated to give the hydroxy-ester as a colourless oil (3.2 g. 31%), b.p. $106-108^{\circ}/1\text{mm.}$, n_{D}^{20} 1.4400. (Lit. ⁽⁴⁹⁾ b.p. $130-135^{\circ}/10 \text{ mm.}$, n_{D}^{20} 1.4365)

Decane-1,3-diol.-

A solution of ethyl 3-hydroxydecan-1-oate (2.96 g.) in ether (20 c.c.) was added slowly to a stirred slurry of lithium aluminium hydride (0.70 g.) in ether (50 c.c.), and the mixture boiled under reflux for 30 min. The mixture was cooled, excess lithium aluminium hydride destroyed, and then poured into 2N sulphuric acid (50 c.c.). The two layers were separated and the aqueous layer extracted with ether (2 x equal volume). The ethereal extracts were combined, washed with water, and dried. Evaporation of the extracts afforded a pale yellow oil (2.28 g.) which could not be crystallised.

This oil (0.90 g.) was then heated under reflux in acetone (100 c.c.) with p-toluenesulphonic acid (0.05 g.) for 24 hr. Solid anhydrous sodium carbonate was added, and the mixture kept at 20° for 1 hr. The filtered solution was evaporated, and the residue extracted with light petroleum (b.p. 30-40°). The petroleum extracts were washed with water, dried, and evaporated to small bulk. The product was chromatographed on alumina (25 g.) and eluted in the same solvent (120 c.c.). The colourless oil (0.72 g. 61%) was identified as an isopropylidene compound by its infrared spectrum. $\nu_{\text{max.}}$: 1383 and 1371 ($\text{C}(\text{CH}_3)_2$), 1200, 1168 and 1111 cm^{-1} . This oil was dissolved in ethanol (20 c.c.) and concentrated hydrochloric acid (2 c.c.) added. The mixture was kept at 20° for 30 min., when it was poured into water (200 c.c.), extracted with ether (3 x 250 c.c.), and the extracts washed with water and dried. Evaporation of the ethereal solution afforded a pale yellow semi-solid residue (0.56 g.) which, when

crystallised from light petroleum (b.p. 30-40°), produced colourless needles (0.51 g, 54%) of decane-1,3-diol, m.p. 29-30°. (Found: C, 69.3; H, 12.75. $C_{10}H_{22}O_2$ requires C, 68.9; H, 12.7%).

$\nu_{\max.}$: 3390 (broad, intermolecular H-bonds), 1130 (secondary C-O-H), 1063 cm^{-1} (primary C-O-H). Diluted: $\nu_{\max.}$: 3630 (O-H), 3540 cm^{-1} (sharp intramolecular H-bonds).

1,3-Dimethoxydecane. -

Decane-1,3-diol (16 mg.) was heated under reflux in methyl iodide (15 c.c.) with dry silver oxide (300 mg. dried at 100° for 12 hr.) for 24 hr. The solution was filtered and evaporated to dryness, and the residue taken up in light petroleum (b.p. 30-40°) and chromatographed on alumina (5g.). The required dimethyl ether (12 mg.) was eluted in the same solvent, and identified by its infrared spectrum $\nu_{\max.}$: 1117, 1090 cm^{-1} (C-O-C).

Decane -1,2-diol. -

Methyl 2-hydroxydecanoate (39 mg.) in ether (10 c.c.) was added slowly to a stirred slurry of lithium aluminium hydride (50 mg.) in ether (15 c.c.) and the mixture refluxed for 30 mins. After cooling, the excess lithium aluminium hydride was destroyed and the mixture poured into 2N sulphuric acid (25 c.c.). The two layers were separated, and the aqueous layer extracted with ether (2 x equal volume). The ethereal extracts were combined, washed with water and dried. Evaporation of the extracts afforded colourless plates (32 mg.), m.p. 44-45°, identified by their infrared spectrum. $\nu_{\max.}$: 3380 (bonded O-H), 1130 (sec. C-O-H) and 1065 cm^{-1} (pri. C-O-H). (Lit.⁽⁵⁰⁾ m.p. 45-48°)

1,4-Dimethoxy-undecane.- γ -Undecanolactone.-

Undecylenic acid (20 g.) was stirred with concentrated sulphuric acid (20 g.) at 90° for 8 hr. The mixture was cooled, crushed ice (100 g.) added, and then heated to 90° for 10 min., when it was poured into water (1 l.) and extracted with ether. The ethereal extracts were washed with saturated sodium hydrogen carbonate solution and water and then dried. The residue, after evaporation of ether, was fractionated to give a colourless oil (3 g., 15%), b.p. $160-163^{\circ}/13\text{mm.}$, n_D^{20} 1.4500. (Lit. ⁽¹⁴⁾ b.p. $164-166/13\text{mm.}$, n_D^{20} 1.4514).

Undecane-1,4-diol.-

γ -Undecanolactone (1.00 g.) in ether (50 c.c.) was added slowly to a stirred slurry of lithium aluminium hydride (0.60 g.) in ether (100 c.c.), and the mixture boiled under reflux for 30 min. After cooling, the excess lithium aluminium hydride was destroyed and the mixture poured into 2N sulphuric acid (100 c.c.). The two layers were separated, and the aqueous layer extracted with ether (2 x equal volume). The ethereal extracts were combined, washed with water, and dried. Evaporation of the extracts afforded a semi-solid residue (1.00 g.)

This residue (0.5 g.) was crystallised from hexane to give colourless needles (0.34 g. 68%) of undecane-1,4-diol m.p. $30-31^{\circ}$. (Found: C, 70.4; H, 12.65. $C_{11}H_{24}O_2$ requires C, 70.15; H, 12.85%)

$\nu_{\text{max.}}$: 3620 (O-H), 3455 (sharp; intramolecular H-bonds), 1130 (secondary C-O-H), 1053 cm.^{-1} (primary C-O-H).

1,4-Dimethoxy-undecane.-

Undecane-1,4-diol (0.22 g.) was heated under reflux with silver

oxide (dried at 100° for 12 hr.) in methyl iodide (10 c.c.) for 18 hr. The filtered solution was evaporated, and the residual oil (0.25 g.) was distilled from sodium to give a colourless ether (0.14 g., 73%), b.p. $120^{\circ}/15\text{mm.}$ (Found, C, 72.1; H, 13.05. $\text{C}_{13}\text{H}_{28}\text{O}_2$ requires C, 72.15; H, 13.05%) $\nu_{\text{max.}}$: 1117, 1094 cm.^{-1} (O-CH₃).

1,5-Dimethoxytridecane.-

n-Octyl Bromide.-

n-Octanol (26 g.) was heated under reflux with hydrobromic acid (47%, 120 g.) and concentrated sulphuric acid (31g.) for $2\frac{1}{2}$ hr. The mixture was then cooled and water (150 c.c.) added. The two layers were then separated and the upper organic layer washed with cold concentrated sulphuric acid (25 c.c.), water (25 c.c.) and sodium carbonate solution (10%, 25 c.c.). It was then dried (CaCl_2), and after filtration, the crude product (35 g.) was fractionated to give the bromide as a colourless oil (30 g., 78%), b.p. $184-186^{\circ}$, n_D^{20} 1.4504. (Lit. (51) b.p. 201.5° n_D^{25} 1.4503).

5-Ketotridecan-1-oic acid.-

A few drops of a solution of n-octyl bromide (10.0 g.) in tetrahydrofuran (20 c.c.) was added to a stirred mixture of magnesium turnings (1.4 g, washed with ether, dried at 100° for 12 hr). Gentle warming initiated a smooth reaction, maintained by slow addition of the rest of the bromide solution. When addition was complete, the mixture was heated under reflux for 35 min., then cooled to 20° . It was decanted from residual turnings and added, under nitrogen, over 2 hr. to a stirred solution of glutaric anhydride

(11.4 g.) in tetrahydrofuran (50 c.c.) at 0° . Stirring was continued for a further 2 hr., when saturated ammonium chloride solution was added, and the mixture kept for 30 min. at 15° . The two layers were separated, and the aqueous phase washed with ether (2 x equal volume). The ether washings and tetrahydrofuran solution were combined and extracted with saturated sodium hydrogen carbonate solution (3 x equal volume). These extracts were acidified with hydrochloric acid, precipitating a solid which was extracted with ether (4 x equal volume) and the extracts washed with water and dried. Evaporation of the solution yielded a solid, m.p. $55-61^{\circ}$, which gave 5-ketotridecan-1-oic acid as colourless prisms (2.2 g., 19%), m.p. $69-70^{\circ}$, from light petroleum (b.p. $40-60^{\circ}$). (Found, C, 67.95; H, 10.25. $C_{13}H_{24}O_3$ requires C, 68.4; H, 10.6%). $\nu_{\max.}$: 3510 (O-H), 2653 (O-H), 1757 (C=O, monomer), 1715 cm^{-1} (broad, strong; C=O, ketone and dimer acid). The Semicarbazone formed needles from aqueous ethanol, m.p. $119-119.5^{\circ}$ (Found, C, 58.9; H, 9.2. $C_{14}H_{27}O_3N_3$ requires C, 58.9; H, 9.55%).

Methyl 5-ketotridecan-1-oate. -

5-Ketotridecan-1-oic acid (0.50 g.) in ether (50 c.c.) was treated with excess diazomethane at 0° . The solution was then shaken with dilute hydrochloric acid, washed with water and dried. Evaporation of the ethereal solution afforded the methyl ester as prisms (0.49 g., 92%), m.p. $28-29^{\circ}$ (Found, C, 68.2; H, 10.65. $C_{14}H_{26}O_3$ requires C, 69.4; H, 10.8%). $\nu_{\max.}$: 1740 (C=O ester) and 1719 cm^{-1} (C=O, ketone).

Tridecane-1,5-diol. -

Methyl 5-ketotridecan-1-oate (0.36 g.) was added slowly in ether

(12 c.c.) to a stirred slurry of lithium aluminium hydride (0.2 g.) in ether (10 c.c.). The mixture was heated under reflux for 45 min. After cooling, excess lithium aluminium hydride was decomposed and the complex broken up by shaking the mixture with dilute sulphuric acid. The layers were separated, the aqueous layer washed with ether (3 x 50 c.c.) and the ethereal solutions combined, washed with water, and dried. Evaporation of this solution afforded a white solid (0.23 g.), m.p. $42-44^{\circ}$, crystallised from ether-hexane to give prisms (0.20 g., 62%) of, m.p. $45-46.5^{\circ}$. (Found, C, 71.65; H, 12.7. $C_{13}H_{28}O_2$ requires C, 72.15; H, 13.05%). $\nu_{\max.}$: 3600 (free O-H), 3290 (broad, intermolecular H-bonds), 1052 cm^{-1} (C-O-H).

1,5-Dimethoxytridecane.-

Tridecane-1,5-diol (92 mg.) was heated under reflux in methyl iodide (9 c.c.) with dry silver oxide (280 mg.) for 12 hr. The mixture was cooled, filtered, and the methyl iodide evaporated to give a pale yellow oil (95 mg.). This was distilled (bath temp. ca 140° ; 15 mm.) to give the ether (44 mg.) (Found, C, 73.0; H, 13.2, $C_{15}H_{32}O_2$ requires C, 73.7; H, 13.2%). $\nu_{\max.}$: 1120, 1097 cm^{-1} (C-O-CH₃).

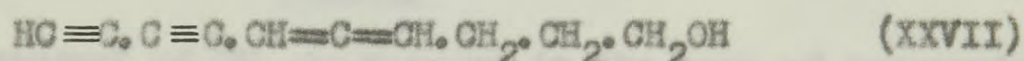
n-Decyl acetate.-

n-Decanol (0.79 g.) was kept in dry pyridine (10 c.c.) with acetic anhydride (5 c.c.) at 15° for 24 hr. The products were then poured into water (100 c.c.), and extracted with ether (2 x 150 c.c.). The extracts were washed with 2N hydrochloric acid, saturated sodium hydrogen carbonate solution and water, and dried. The ethereal solution was evaporated and the colourless residual acetate identified from its infrared absorption. $\nu_{\max.}$: 1730 cm^{-1} (C=O), 1232 cm^{-1} (C-O-C, acetate.)

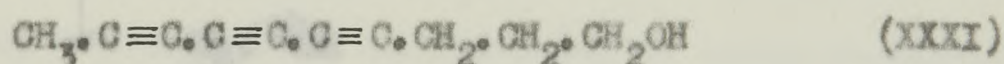
The Isomerisation of the Allenic Metabolites from *Odontia bicolor*.

The isomerisation of allenes in general has been discussed on p. 16 of this thesis. This section comprises a brief discussion of the isomerisation of the allenic metabolites from *Odontia bicolor* since two of these form products which at first sight are unexpected for the molecules concerned. A mechanism for the formation of the observed products is proposed, and a means of verification of this proposal via kinetic studies is outlined.

The Isomerisation of the Alcohol (XXVII).



The isomerisation was observed to take place readily in 0.1N aqueous sodium hydroxide at room temperatures, the disappearance of enediyne absorption in the ultraviolet spectrum being complete in ca. 75 min. The crystalline product exhibited typical triyne ultraviolet absorption, but no ethynyl hydrogen in the infrared



spectrum. The identity of the product was established as the triyne (XXXI) by comparison with a synthetic specimen (see p. 31).

The initial step in allene-acetylene isomerisation is the removal of an allenic proton and in the general discussion of these reactions, it was noted that in the absence of further conjugation, H_a in Fig. 32 was more easily removed than H_b, due to activation of

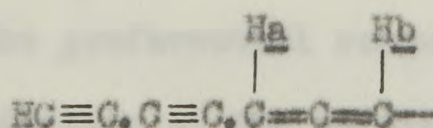


Fig. 32

Ha by the diyne group and a double bond. Such an initial step leads to the formation of a terminal ethynyl triyne as shown in Fig. 33.

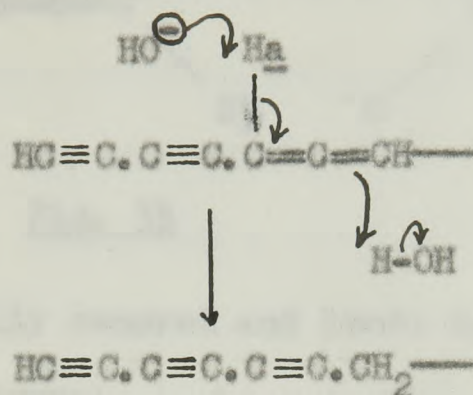


Fig. 33

The preferential removal of Hb, as happens in the case of mycomycin, would give rise to a triyne group displaced in-chain by one carbon atom so that there would be no terminal ethynyl group (see Fig. 34.).

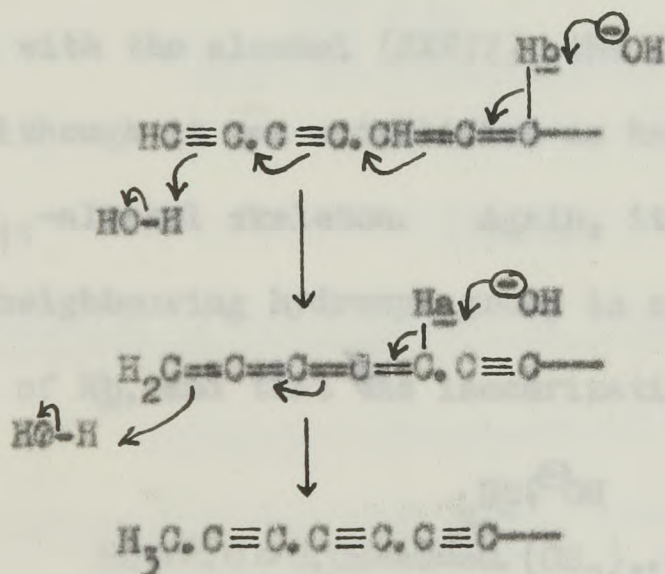


Fig. 34

The production of the non-terminal triyne (XXXI) on isomerisation of the allene-diyne (XXVII) indicated that a factor was operating which facilitated the preferential removal of Hb. This factor is probably the participation of the neighbouring hydroxyl group since the flexibility of this end of the molecule allows the hydroxyl

group to approach quite close to Hb , as shown in Fig. 35

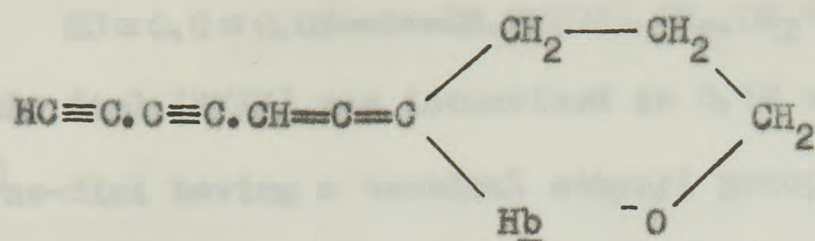
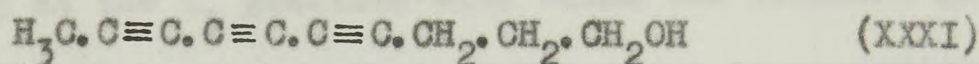
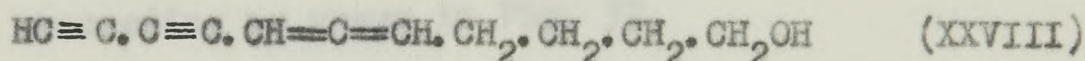


Fig. 35

Hb is then preferentially removed and leads to the non-terminal ethynyl triyne isomer (XXXI) as shown in Fig. 34.



The Isomerisation of the Alcohol (XXVIII)



As with the alcohol (XXVII), the isomer has no terminal ethynyl group although it was established as having a triyne chromophore and a C_{11} -alcohol skeleton. Again, it is proposed that participation of the neighbouring hydroxyl group is operating to cause preferential removal of Hb , and that the isomerisation proceeds as shown in Fig. 36.

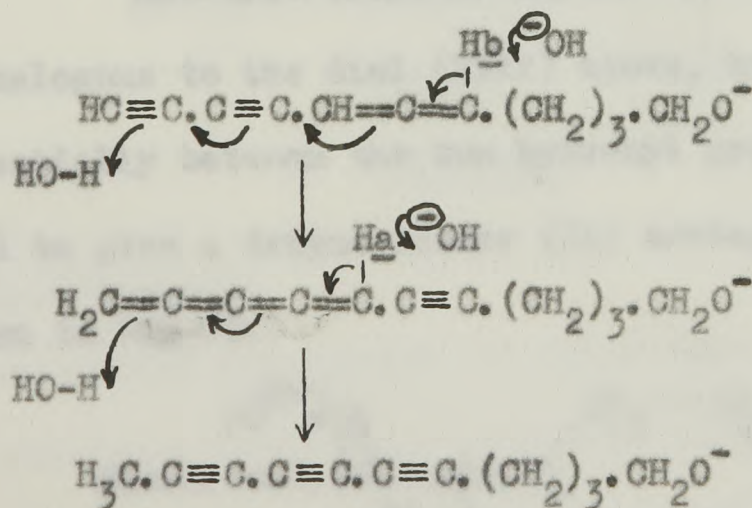
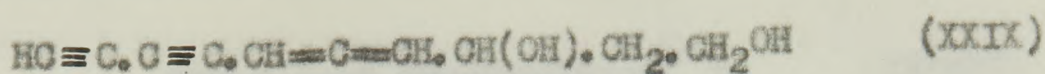


Fig. 36

The Isomerisation of the Allenic Diol (XXIX)



The allenic diol (XXIX) was isomerised in 0.1N sodium hydroxide to a triyne-diol having a terminal ethynyl group. This is the expected product involving the preferential removal of H_a (see Figs. 32 and 33). The diol (XXIX) has a terminal hydroxyl group in the same position relative to the allene as in the alcohol (XXVII), and some assistance of H_b removal might be expected, but there is an additional hydroxyl group at the C_3 position with which the terminal group can hydrogen-bond. Thus for the diol (XXIX) there is no activation of H_b by hydrogen bonding, and H_a is preferentially removed by base, the isomerisation proceeding as shown in Fig. 37.

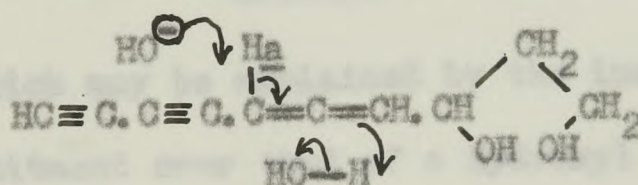
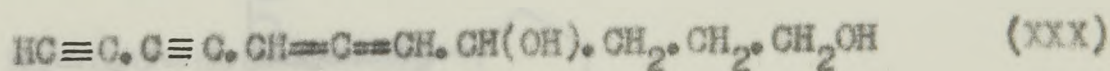


Fig. 37

Isomerisation of the Allenic Diol (XXX)



Analogous to the diol (XXIX) above, hydrogen bonding occurs preferentially between the two hydroxyl groups so that H_a is first removed to give a triyne isomer (XL) having a terminal ethynyl group as shown in Fig 38.

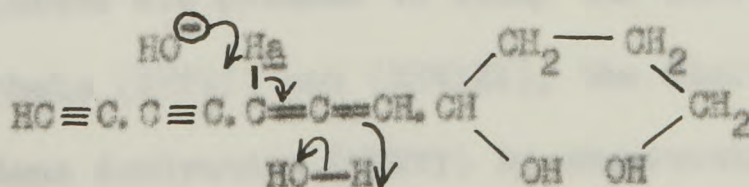
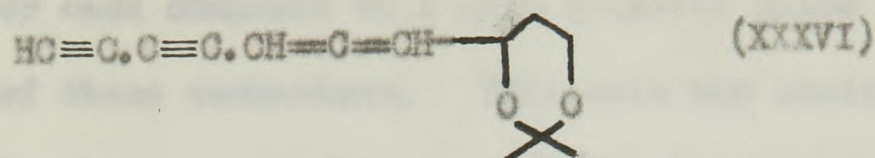


Fig. 38

Isomerisation of the Allenic Isopropylidene Derivative (XXXVI)



The isopropylidene derivative (XXXVI) of the diol (XXIX) has no hydroxyl groups. Isomerisation therefore proceeds via removal of H_a first, to give a terminal ethynyl triyne isomer (XXXVII), as shown in Fig. 39. In addition, a trace of enetriyne is formed,

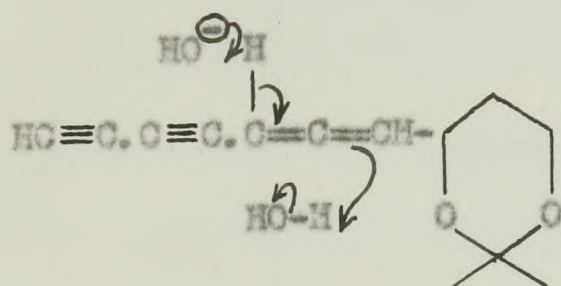


Fig. 39

(see p. 41), which may be explained by the increased electronegativity of the C_3 -substituent over that of a hydroxyl group resulting in a very small amount of "nemotin-nemotin A" type of isomerisation (Fig. 40).

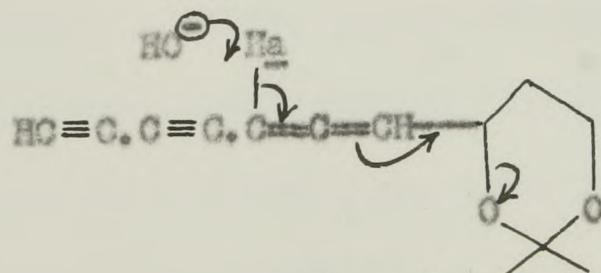


Fig. 40

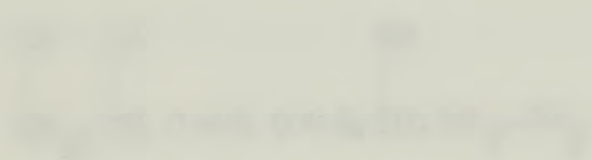
Planned Experiments

Experiments are planned to study the kinetics of isomerisation of the alcohols (XXVII) and (XXVIII), the diol (XXIX) and the isopropylidene derivative (XXXVI) by observation of the decrease in intensity of ultraviolet light absorption at $2780 \text{ \AA}$ at known

and thermostatically controlled temperatures. Evaluation of the change in entropy for each compound will give a useful guide as to the correctness of these mechanisms. This work now awaits the arrival of a suitable thermostatically-controlled ultraviolet absorption cell holder.

The Polyacetylenes from Clitocybe

The structure of the polyacetylene was determined by the use of infrared spectroscopy during the course of the investigation. The infrared spectra of the polyacetylenes were determined by using the



THE POLYACETYLENES



First polyacetylenic ketone to be isolated from macro-organisms, and from the fact that its structure was determined solely by the use of nuclear magnetic resonance. CLITOCYBE RHIZOPHORA (III) is the first diacetylenic derivative to be isolated from macro-organisms.

Growth of the Fungus

The original static growth of the fungus Clitocybe Rhizophora produced up to 6 mg./litre - Discussion - and 22 mg./litre diene-triol (III). First, second and third extracts of the mycelium wet with 4% glucose gave yields of (i) 22 and 17 mg./litre (ii) 26 and 5 mg./litre and (iii) 3 and 4 mg./litre of the diene-triol (III) and diene-triol (III) respectively.

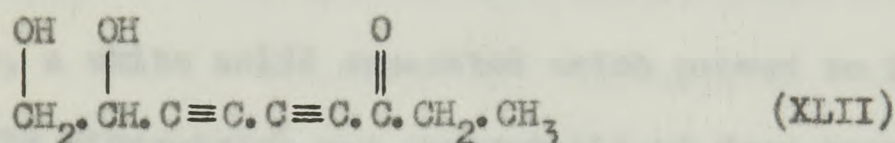
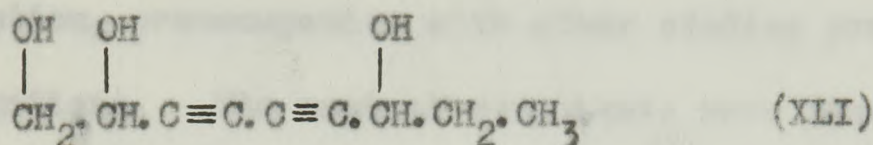
The fungus was also grown on whole malted wheat, and the ethanolic light extracts of the several extracts indicated the presence of only very small quantities of diene-triol. These extracts were further investigated.

Isolation of Diene-triol

The polyacetylenes were isolated from the mycelium in the culture

The Polyacetylenes from Clitocybe Rhizophora

The structures (XLI) and (XLII) have been elucidated for two polyacetylenes produced during the growth of the fungus Clitocybe rhizophora. The component (XLII) is somewhat unique in being the



first polyacetylenic ketone to be isolated from micro-organisms, and from the fact that its structure was determined mainly by the use of nuclear magnetic resonance spectroscopy. The triol (XLI) is the first diacetylenic derivative to be isolated from micro-organisms.

Growth of the Fungus

The original static growths of the fungus Clitocybe rhizophora produced up to 8 mg./litre diyne-ketone (XLII) and ca 45 mg./litre diyne-triol (XLI). First, second and third refloods of the mycelial mat with 4% glucose gave yields of (i) 28 and 11 mg./litre (ii) 24 and 5 mg./litre and (iii) 8 and 4 mg./litre of the diyne-ketone (XLII) and diyne-triol (XLI) respectively.

The fungus was also grown as shake cultures, but the ultraviolet light absorption of the ethereal extracts indicated the presence of only very small quantities of diyne-ketone. These extracts await further investigation.

Isolation of Components

The polyacetylenes were obtained from the aqueous culture medium

by continuous ether extraction and were separated into neutral and acidic components. The acidic fraction showed no characteristic ultraviolet light absorption and although it was realised that the extract might contain polyacetylenes (e.g. diacetylenes) with low intensity absorption, preoccupation with other studies prevented further investigations. The neutral components were then transferred to a benzene solution, which, by chance, was kept cool for 24 hours. During this time, a white solid separated which proved to be the diyne-triol (XLI). The diyne-triol was recrystallised from benzene or from ether-hexane.

The diyne-ketone was separated from less polar components by extraction with water and re-extraction with ether. A trial formation of an isopropylidene derivative of the diyne-keto-diol (XLII) proved successful and was adopted as a general purification procedure, the derivative being chromatographed on alumina. The diyne-ketone was examined principally in this form, but the parent keto-diol (XLII) could be regenerated by treatment with dilute acid. It formed low-melting needles from ether-hexane.

Extracts from refluxes were washed with sodium hydrogen carbonate solution, and the neutral components were treated with acetone, and the two isopropylidene derivatives were separated by alumina chromatography.

The Diyne-Triol (XLI)

This component separated from benzene as needles and was almost completely stable at 15° in a specimen tube. Recrystallised samples tended to become pink and to form yellowish solutions after exposure

to the atmosphere at 15° for several months. It is thus one of the most stable of polyacetylenes to be isolated from fungal culture media. As such it was of interest to test its biological activity against Staphylococcus aureus (Oxford strain). This was kindly carried out by Dr. E.P. Abraham of the Sir. William Dunn School of Pathology, and the diyne-triol showed no biological activity.

Carbon-hydrogen analysis gave an empirical formula of C_3H_4O , and with a "Rast" molecular weight of 168, the molecular formula $C_9H_{12}O_3$ was tentatively nominated. Ultraviolet absorption data indicated the presence of a diyne chromophore; infrared data suggested hydroxyl as the only oxygen functions and the absence of a terminal ethynyl group. On hydrogenation, the natural compound (assuming M.W. 168.2) required 3.8 mols of hydrogen.

The hydrogenated molecule consumed 0.95 mol. of sodium periodate over 24 hr., and formaldehyde was established as one of the fission products of both the natural and hydrogenated material. Periodate cleavage of the natural material and removal of formaldehyde left a highly unstable residue which had ultraviolet absorption characteristic of the diyne-aldehyde chromophore. The infrared spectrum supported this with unsaturated carbonyl (1668 cm^{-1}) and aldehydic C-H (2732 cm^{-1}) absorptions, and in addition showed a free hydroxyl absorption at 3597 cm^{-1} . Periodate oxidation of the saturated molecule and chromatography of the products as 2,4-dinitrophenylhydrazones gave two main fractions, one of formaldehyde 2,4-dinitrophenylhydrazone, and one which analysed for the 2,4-dinitrophenylhydrazone of the hydroxy-aldehyde $C_8H_{16}O_2$.

From the above evidence, the grouping $\text{HOCH}_2\cdot\text{CH}(\text{OH})\cdot\text{C}\equiv\text{C}\cdot\text{C}\equiv\text{C}-$ was established, leaving three possible structures for the complete molecule as shown in Fig. 41.

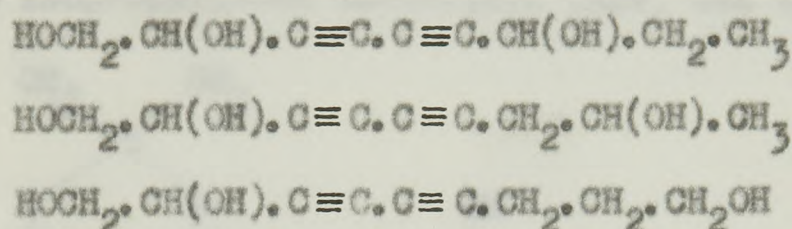


Fig. 41

The hydrogenated material was treated with periodate/permanganate reagent⁽⁵²⁾ and then oxidised with chromic acid in acetone to give a product which after crystallisation from hexane had m.p. $49-51^\circ$, and showed acidic and extremely strong carbonyl absorption in its infrared spectrum. It formed a semicarbazone, m.p. $189-190^\circ$, which established it as the keto-acid (XLIII) from the three possibilities shown in Fig. 42.

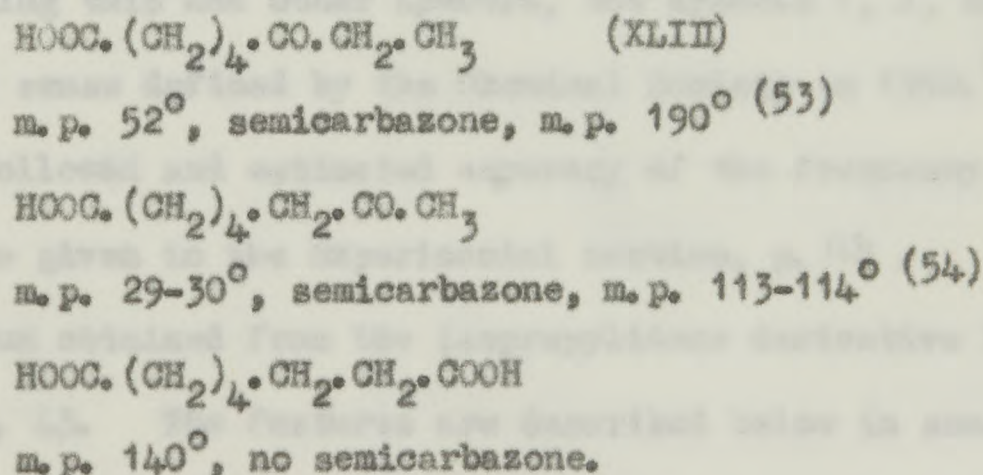
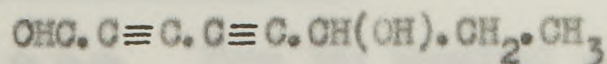


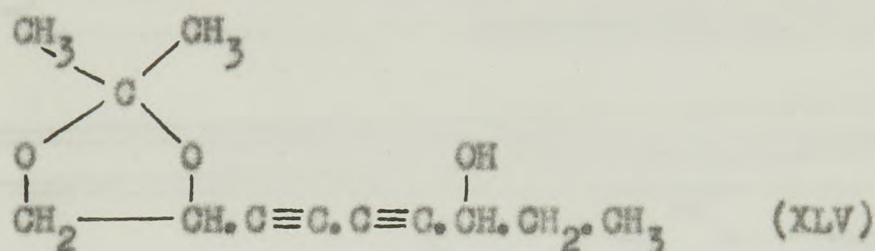
Fig. 42

This evidence established the structure of the natural product as (XLI), and therefore the structure of the unsaturated periodate oxidation product as the diyne-aldehyde (XLIV)



(XLIV)

Partly to provide a check on the structure determination outlined above, and partly to investigate the possibilities of its application to acetylenic studies, the nuclear magnetic resonance spectrum of the isopropylidene derivative (XLV) was investigated.



It has been appreciated for some years that the nuclear magnetic resonance spectra of polyacetylenes should be highly characteristic because of the small numbers and individuality of the protons present. However, the investigation of these spectra requires solutions of high concentration (ca. 30 mg/0.2 c.c.) and this is the first occasion when it has been possible to obtain enough natural material of suitable stability for examination under these conditions.

In discussing this and other spectra, the symbols τ , J , and δ are used in the sense defined by the Chemical Society in 1960.⁽⁵⁵⁾ The procedure followed and estimated accuracy of the frequency measurements are given in the Experimental section, p. 113.

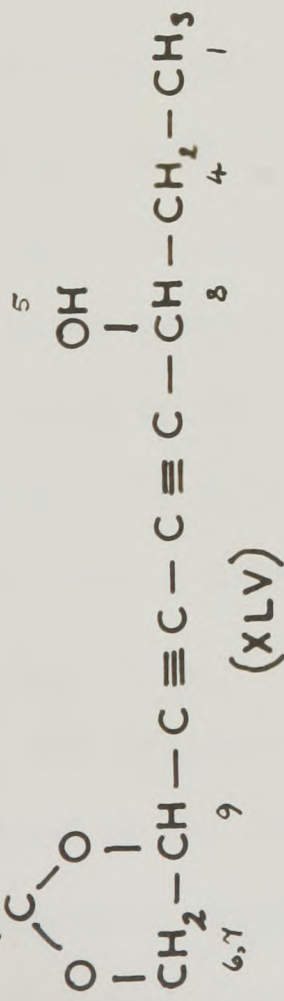
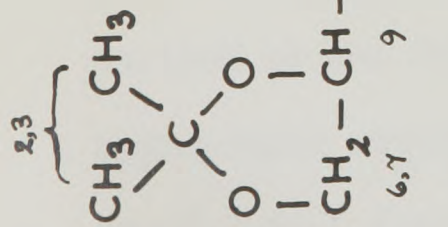
The spectrum obtained from the isopropylidene derivative (XLV) is shown in Fig. 43. The features are described below in some detail, followed by the data obtained from model compounds for correlation purposes. The absorptions are considered in bands, numbered in sequence from high to low fields as they occur in the spectrum.

The Isopropylidene Derivative (XLV)

Band 1 $\tau = 8.99$ p.p.m. From the terminal methyl protons; split

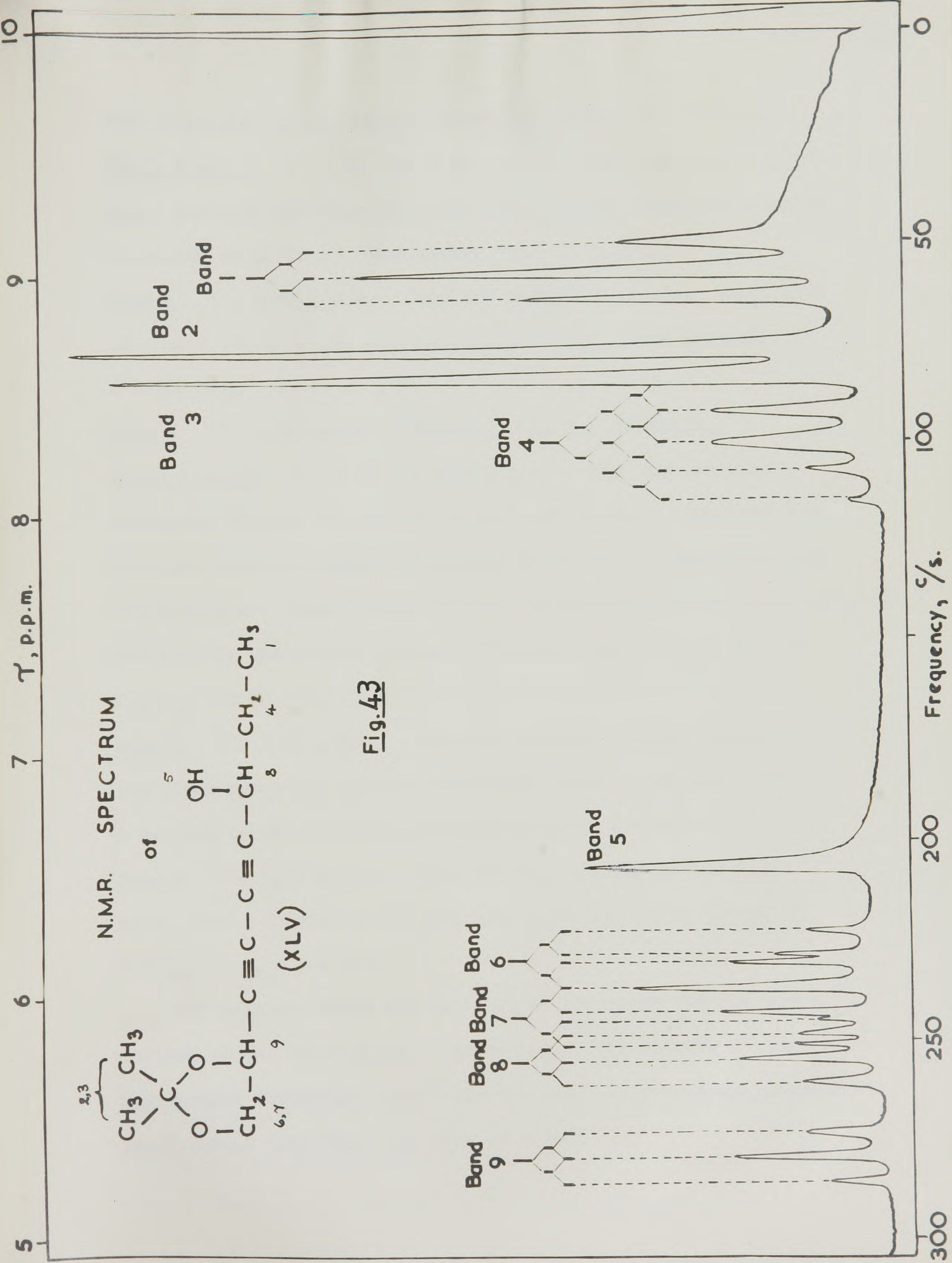
N.M.R. SPECTRUM

of



(XLV)

Fig. 43



into a triplet by an adjacent methylene group. $J = 7.2$ c/s.

Bands 2 and 3 $\tau = 8.66$ and 8.55 p.p.m. From six protons of the gem.-dimethyl grouping; resolved into discrete bands for reasons discussed on p. 92. Band separation 0.11 p.p.m.

Band 4 $\tau = 8.30$ p.p.m. From two methylene protons; split into five main lines by the adjacent methyl and carbinol protons.

$J = 7.2$ c/s.

Band 5 $\tau = 6.50$ p.p.m. Probably from the -OH proton.

Bands 6 and 7 $\tau = 6.10$ and 5.90 p.p.m. From the two ring methylene protons "A" and "B" of an "ABX" system. Resolved into discrete bands for reasons discussed on p. 92. Band separation = 0.20 p.p.m. Each proton (A or B) is split (i) by the other (B or A) and (ii) by the methine proton (X) in the ring. $J_{AB} = 8.1$ c/s.

$$J_{AB}/\delta_{AB} = 0.68$$

Band 8 $\tau = 5.68$ p.p.m. From the carbinol proton, adjacent to the diyne group and with an β -hydroxyl substituent; split into a triplet by the adjacent methylene group. $J = 6.2$ c/s.

Band 9 $\tau = 5.27$ p.p.m. From the ring methine proton (X); split into a triplet by the adjacent methylene group (A and B).

$$J = J_{AX} = J_{BX} = 5.9 \text{ c/s.}$$

The molecule (XLV) can usefully be considered in two parts, divided by the diyne group. Accordingly, the spectra of the two appropriate acetylenes pent-1-yn-3-ol and 1,2-O-isopropylidenebut-3-yne-1,2-diol (see Fig. 44.) were examined.

Table 11

Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	8.99	3	$\text{CH}_2\text{---CH}(\text{C}(\text{CH}_3)_2)\text{C}\equiv\text{C}\text{---C}\equiv\text{C}\text{---CH}(\text{OH})\text{CH}_2\text{CH}_3$	7.3
2	8.28	5		6.9
3	7.60	2		2.2
4	6.02	1		-
5	5.73	6		6.2
				2.0

1,2-O-isopropylidene-3-ethynyl-5-hydroxypent-1-yn-3-ol

(see Fig. 45 and Table 12) Fig. 44

The data for these compounds are set out in Tables 10, 11 and 12. It was found that replacement of the ethynyl hydrogen with bromine in 1-bromopent-1-yn-3-ol did not affect the positions of the various bands, and had the advantage of eliminating the absorption and couplings of the ethynyl proton.

Table 10 N.M.R. Bands for Pent-1-yn-3-ol

Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	8.99	3	$-\text{CH}_2\text{---CH}_3$	7.3
2	8.28	5	$-\text{CH}(\text{OH})\text{---CH}_2\text{---CH}_3$	6.9
3	7.60	2	$\text{HC}\equiv\text{C}\text{---CH}(\text{OH})\text{---}$	2.2
4	6.02	1	$-\text{OH}$?	-
5	5.73	6	$\text{HC}\equiv\text{C}\text{---CH}(\text{OH})\text{---CH}_2\text{---}$	6.2
				2.0

Subtraction of the spectrum of 1-bromopent-1-yn-3-ol from that of the isopropylidene derivative (XLV) leaves bands 2, 3, 6, 7 and 9 which should therefore be found in the spectrum of

Table 11

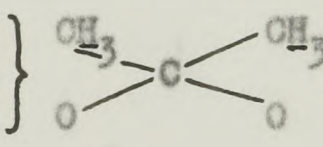
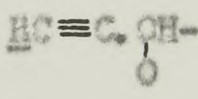
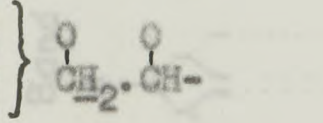
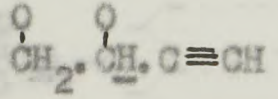
N.M.R. Bands for 1-Bromopent-1-yn-3-ol

Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	9.00	3	$-\text{CH}_2-\text{CH}_3$	6.8
2	8.30	5	$-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}_3$	7.2
3	5.93	1	$-\text{OH}$?	-
4	5.73	3	$\equiv\text{C}-\text{CH}(\text{OH})-\text{CH}_2-$	6.2

1,2-O-isopropylidenebut-3-yne-1,2-diol. The spectrum obtained (see Fig. 45 and Table 12) confirmed this prediction, showing clearly the ABX system of ketal ring protons and the split gem-dimethyl absorption. These characteristics are a result of the rigidity of the cyclic structure and the proximity of the acetylene linkage; they are worthy of some attention at this point.

Table 12

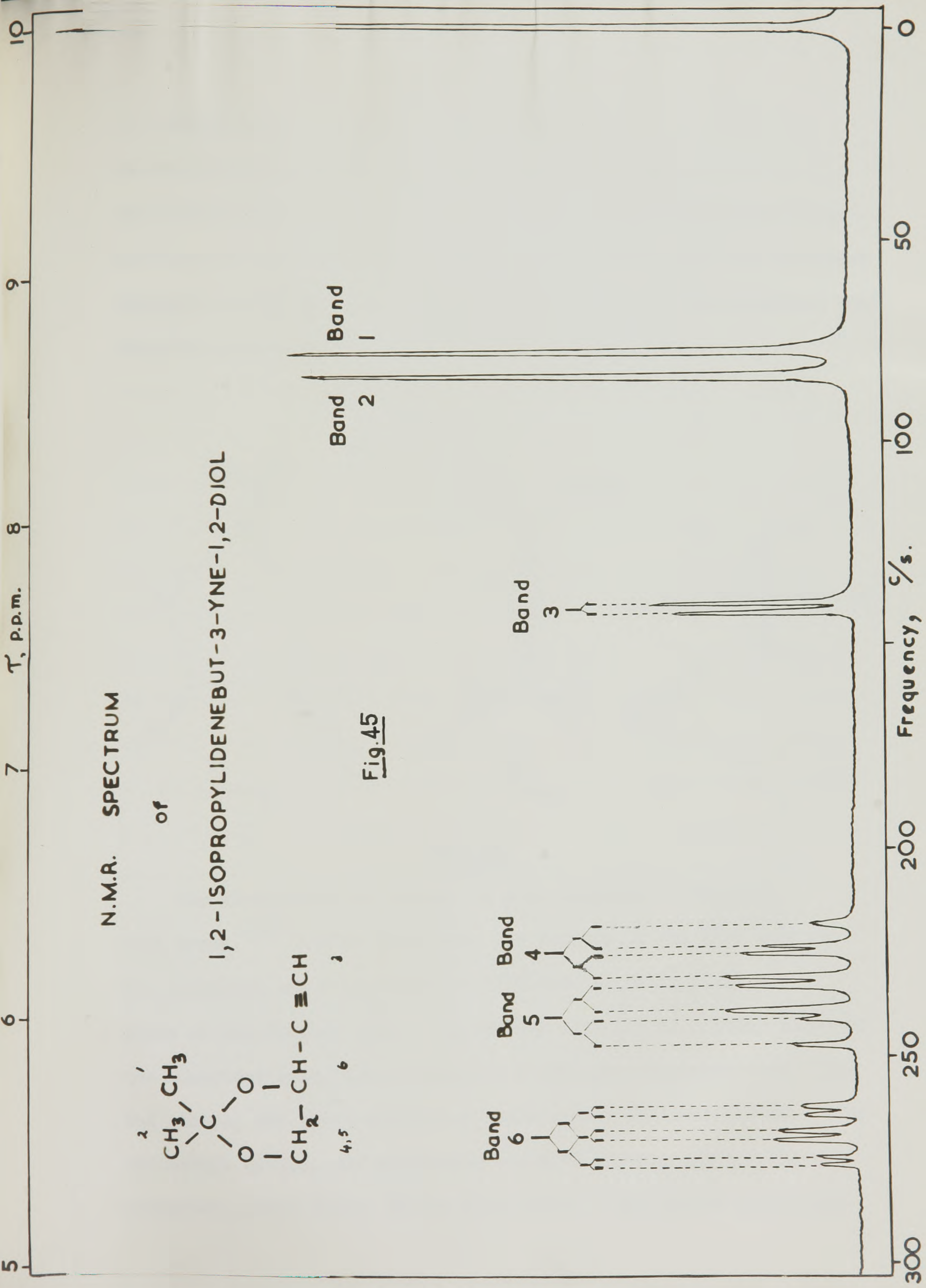
N.M.R. Bands for 1,2-O-Isopropylidenebut-3-yne-1,2-diol

Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	8.69	1		-
2	8.59	1		-
3	7.64	2	$\text{HC}\equiv\text{C}-\text{CH}-$ 	2.2
4	6.22	4 *		7.4
5	5.98	4 *		7.7
6	5.48	6 *		6.2 2.0

* = Bands of an ABX system. $J_{AB} = 7.6$ c/s. $J_{AX} = J_{BX} = 6.2$ c/s.

$$J_{AB}/\delta_{AB} = 0.56$$

$$\delta_{AB} = 0.23 \text{ p.p.m. (r.m.s. value)}$$



Inspection of a model (see Fig. 46) showed that if the ring is considered as planar, then the acetylene linkage is held to one side of this plane. One methyl group and one methylene proton are also on this side and therefore (a) are much nearer the acetylene linkage and (b) lie at a wider angle to the axis of the acetylene bond than the protons on the other side of the ring plane.

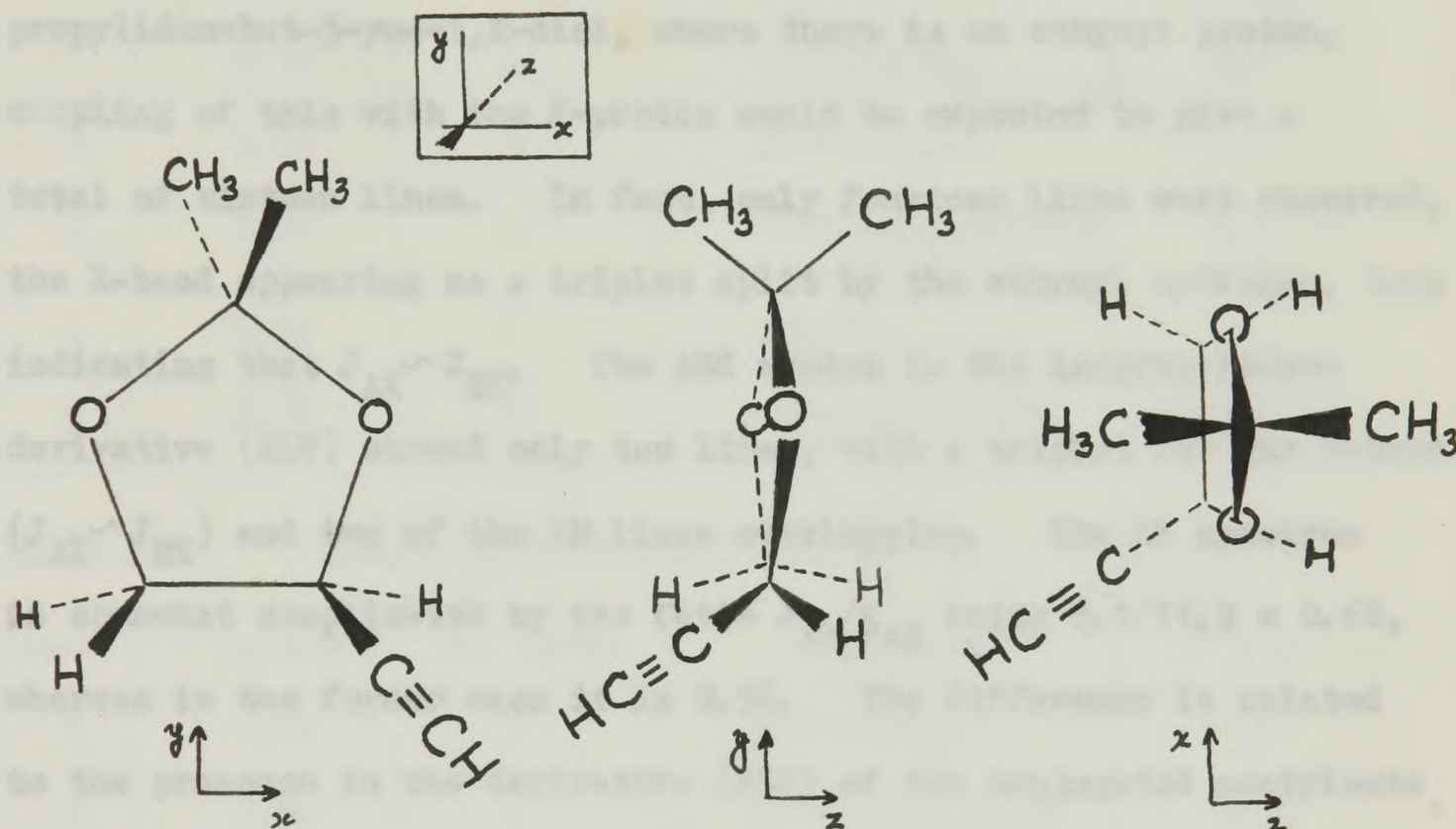
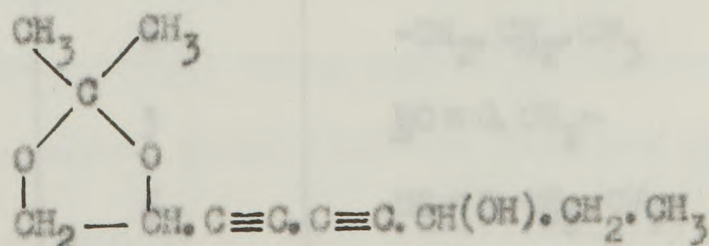


Fig. 46

The diamagnetic anisotropy of the acetylenic linkage is well known,⁽⁵⁶⁾ and in this case, the protons on the same side as the acetylene would experience a different magnetic field to those on the further side. Since they also lie at a wider angle to the acetylene axis, they experience a stronger resultant field than the others, and their absorption bands appear at lower fields. For the methyl groups, the separation was 0.10 p.p.m., and for the two methylene proton bands, it was 0.23 p.p.m. The latter separation

is larger since the methylene protons are together nearer the acetylene linkage than the methyl protons. In the isopropylidene derivative (XLV), the gem.-dimethyl separation was 0.11 p.p.m., and the methylene proton split 0.20 p.p.m.

The three ring protons form an ABX resonance system which normally has twelve visible lines. In the case of 1,2-O-isopropylidenebut-3-yne-1,2-diol, where there is an ethynyl proton, coupling of this with the X-proton would be expected to give a total of sixteen lines. In fact, only fourteen lines were observed, the X-band appearing as a triplet split by the ethynyl hydrogen, thus indicating that $J_{AX} \sim J_{BX}$. The ABX system in the isopropylidene derivative (XLV) showed only ten lines, with a triplet for the X-band ($J_{AX} \sim J_{BX}$) and two of the AB lines overlapping. The AB spectrum is somewhat complicated by the ratio J_{AB}/δ_{AB} being $8.1/11.9 = 0.68$, whereas in the former case it is 0.56. The difference is related to the presence in the derivative (XLV) of two conjugated acetylenic linkages with a β -hydroxy-substituent as against a single acetylenic bond in the model compound. This difference also explains the change in position of the X-band from 5.48 p.p.m. in the model compound to 5.27 p.p.m. in the derivative (XLV). All the bands in the spectrum of the derivative (XLV) are thus accounted for, and the structure (XLV) upheld well by the analysis.



(XLV)

Further to this structural confirmation, three readily available acetylenic compounds were examined and shown to have relatively simple spectra of highly individual character. The bands are listed in Tables 13-15.

Table 13 N.M.R. Data for Pent-1-yn-4-ol

Band	τ (p. p. m.)	No. lines	Assignment	J c/s.
1	8.74	2	$-\text{CH}(\text{CH})\cdot\text{CH}_3$	5.9
2	8.02	3	$\text{HC}\equiv\text{C}\cdot\text{CH}_2-$	2.8
3	7.70	4	$\text{HC}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}(\text{OH})-$	6.1 2.5
4	6.09	1	$-\text{OH} ?$	-
5	6.08	6	$-\text{CH}_2\cdot\text{CH}(\text{OH})\cdot\text{CH}_3$	5.9

Table 14 N.M.R. Data for But-1-yn-3-ol

Band	τ (p. p. m.)	No. lines	Assignment	J c/s.
1	8.00	3	$\text{HC}\equiv\text{C}\cdot\text{CH}_2-$	2.5
2	7.60	6	$\text{HC}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}_2\text{OH}$	6.7 2.6
3	6.32	3	$-\text{CH}_2\cdot\text{CH}_2\text{OH}$	6.7
4	5.71	1	$-\text{OH} ?$	-

Table 15 N.M.R. Data for Pent-1-yne

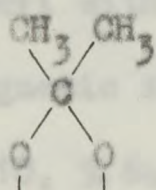
Band	τ (p. p. m.)	No. lines	Assignment	J c/s.
1	8.99	3	$-\text{CH}_2\cdot\text{CH}_3$	6.6
2	8.46	6	$-\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3$	6.5
3	8.21	3	$\text{HC}\equiv\text{C}\cdot\text{CH}_2-$	2.6
4	7.87	6	$\text{HC}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}_2-$	6.2 2.4

The data suggest that there may be some doubt attached to the accepted position of 7.65 p.p.m. for acetylenic protons in unconjugated hydrocarbons. The evidence here suggests that it may be nearer 8.00 p.p.m. There is a marked move to lower fields with the approach of oxygen functions (see Table 16).

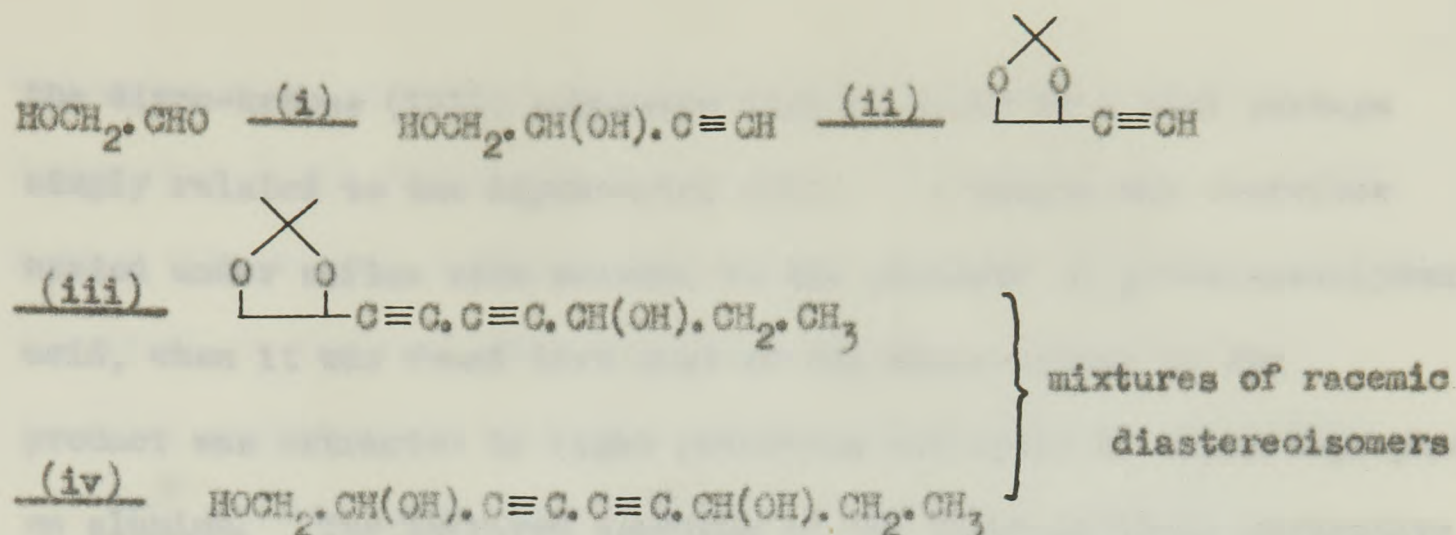
Table 16

The Position of Ethynyl Proton Absorption in Various Terminal

Ethynyl Compounds

Compound	Ethynyl Proton Band τ , (p.p.m.)
$\text{HC}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3$	8.21
$\text{HC}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}(\text{OH})\cdot\text{CH}_3$	8.02
$\text{HC}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}_2\text{OH}$	8.00
	7.64
$\text{HC}\equiv\text{C}\cdot\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{CH}_3$	7.60
$\text{HC}\equiv\text{C}\cdot\text{CO}\cdot\text{CH}_2\cdot\text{CH}_3$	6.64

The structure assigned to the natural diyne-triol (XLI) was finally ratified by synthesis, carried out by the route shown in Fig. 47. The but-3-yne-1,2-diol was isolated in bulk from stage (i), the crude product being converted directly to an isopropylidene derivative which could be purified by alumina chromatography. A portion of this compound was used for the



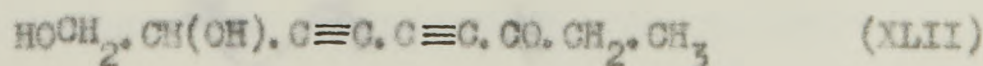
- (i) $\text{HC}\equiv\text{CMgBr}$ (ii) Acetone/p-toluenesulphonic acid
 (iii) $\text{BrC}\equiv\text{C}\cdot\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{CH}_3/\text{Cu}^+, \text{EtNH}_2$ (iv) H^+

Fig. 47

examination of its nuclear magnetic resonance spectrum (see above). The synthetic racemic mixture of isopropylidene derivatives was found to have ultraviolet, infrared and nuclear magnetic resonance spectra which agreed well with those of the optically active natural product. The nuclear magnetic resonance bands appeared at 8.98, 8.68, 8.57, 8.32, 6.52, 6.10, 5.84, 5.68 and 5.28 p.p.m. (cf. data on p. 89).

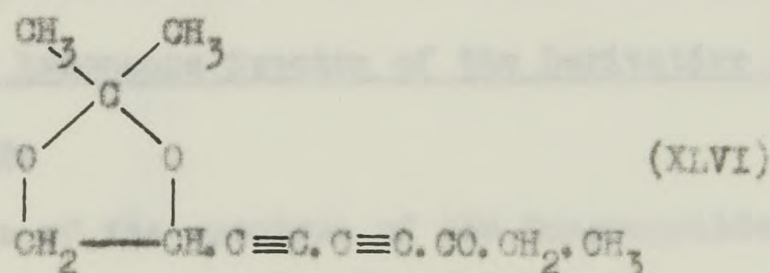
The synthetic mixture of triols formed low-melting needles, giving only broad absorption envelopes in its infrared spectrum which corresponded with the more intense bands of the natural product. The ultraviolet absorption spectra of natural and synthetic material were closely similar.

The Diyne-Ketone (XLII)

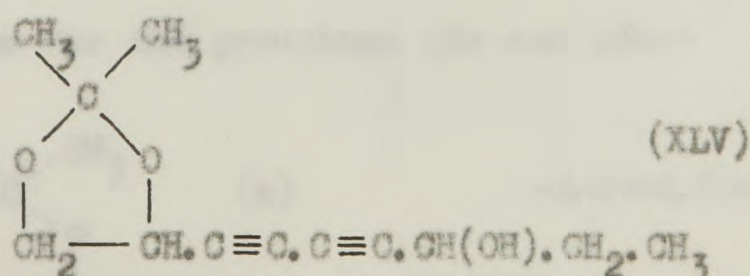


The investigation of this component was started when the structure of the diyne-triol (XLI) was reasonably certain. The polarity of

the diyne-ketone (XLII) suggested that it could be a diol perhaps simply related to the diyne-triol (XLI). A sample was therefore heated under reflux with acetone in the presence of *p*-toluenesulphonic acid, when it was found that most of the diyne-ketone in the product was extracted by light petroleum and could be chromatographed on alumina. The infrared spectrum of the isopropylidene derivative thus formed had two extremely strong acetylene bands at 2230 and 2141 cm^{-1} , unsaturated carbonyl at 1675 cm^{-1} , *gem*-dimethyl bands at 1379 and 1371 cm^{-1} , and characteristics of a ketal ring at 1206, 1147 and 1065 cm^{-1} . The diyne-ketone chromophore was already established from the ultraviolet spectrum, and sufficient material was available to investigate the nuclear magnetic resonance spectrum. Two possible structures were suggested by the spectrum, and one of these (XLVI)



was established after obtaining spectra of the hydrogenated material and the product from sodium borohydride reduction. The latter had ultraviolet, infrared and nuclear magnetic resonance spectra closely similar to those of the isopropylidene derivative (XLV) from the diyne-triol (XLI). The relationship between the



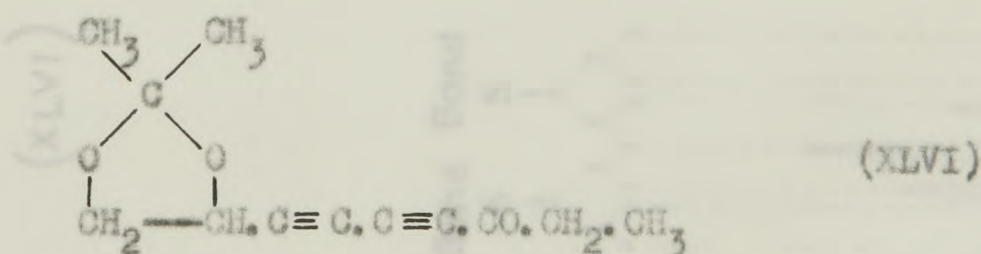
two metabolites was fully established when the isopropylidene derivative (XLV) of the triol (XLI) was oxidised to give a product having ultraviolet, infrared and nuclear magnetic resonance spectra identical to those of the derivative (XLVI). In addition, the sign and magnitude of optical rotation were found to be the same.

The natural keto-diol (XLII) was regenerated from the isopropylidene derivative (XLVI) and could be crystallised, with difficulty and in very small amount, from ether/hexane as needles, m. p. 34.5-35.0°

Chemical evidence for the structure (XLII) was difficult to obtain. The diol group was not easily cleaved by periodate in aqueous solution, and the compound was found to be extremely unstable towards base. A very small amount of formaldehyde (Identified as its dimedone derivative) was formed in aqueous periodate.

Nuclear Magnetic Resonance Spectra of the Derivative (XLVI) and Related Compounds.

The features of the spectrum of the isopropylidene derivative (XLVI) are shown in Fig. 48 and Table 17. From light absorption



data, and from its difference in polarity from the natural product, there was evidence for the groupings 49a and 49b:-

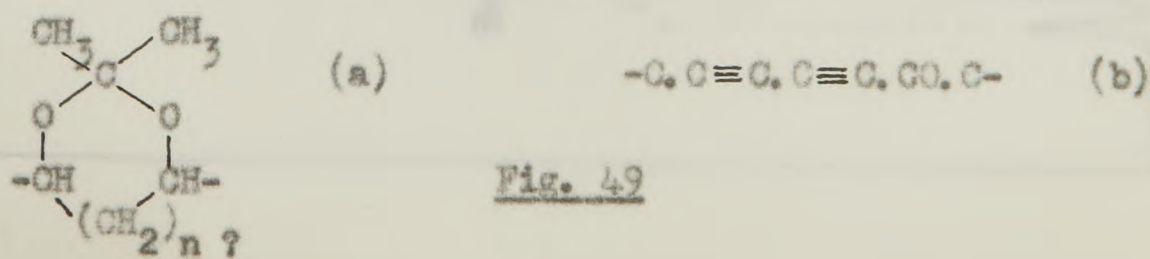


Fig. 49

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of

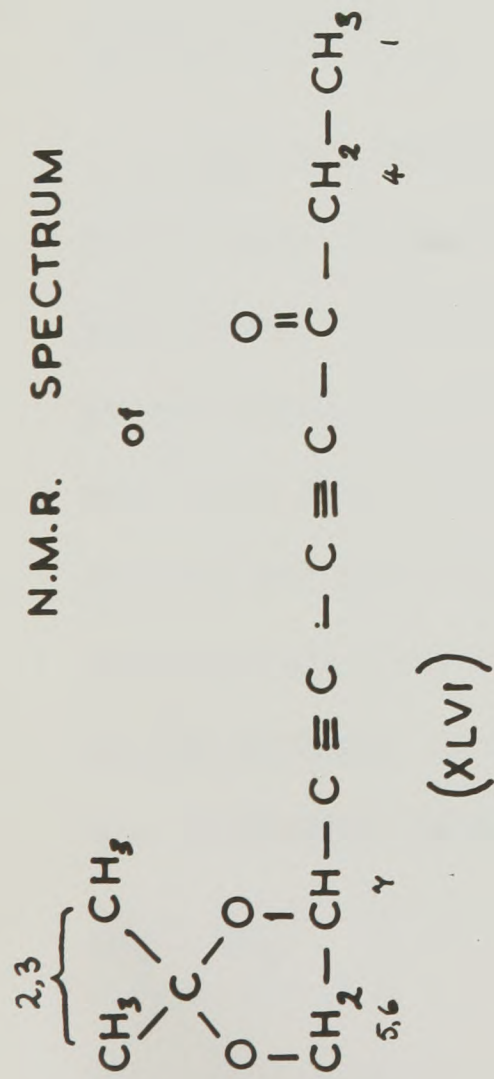


Fig. 48

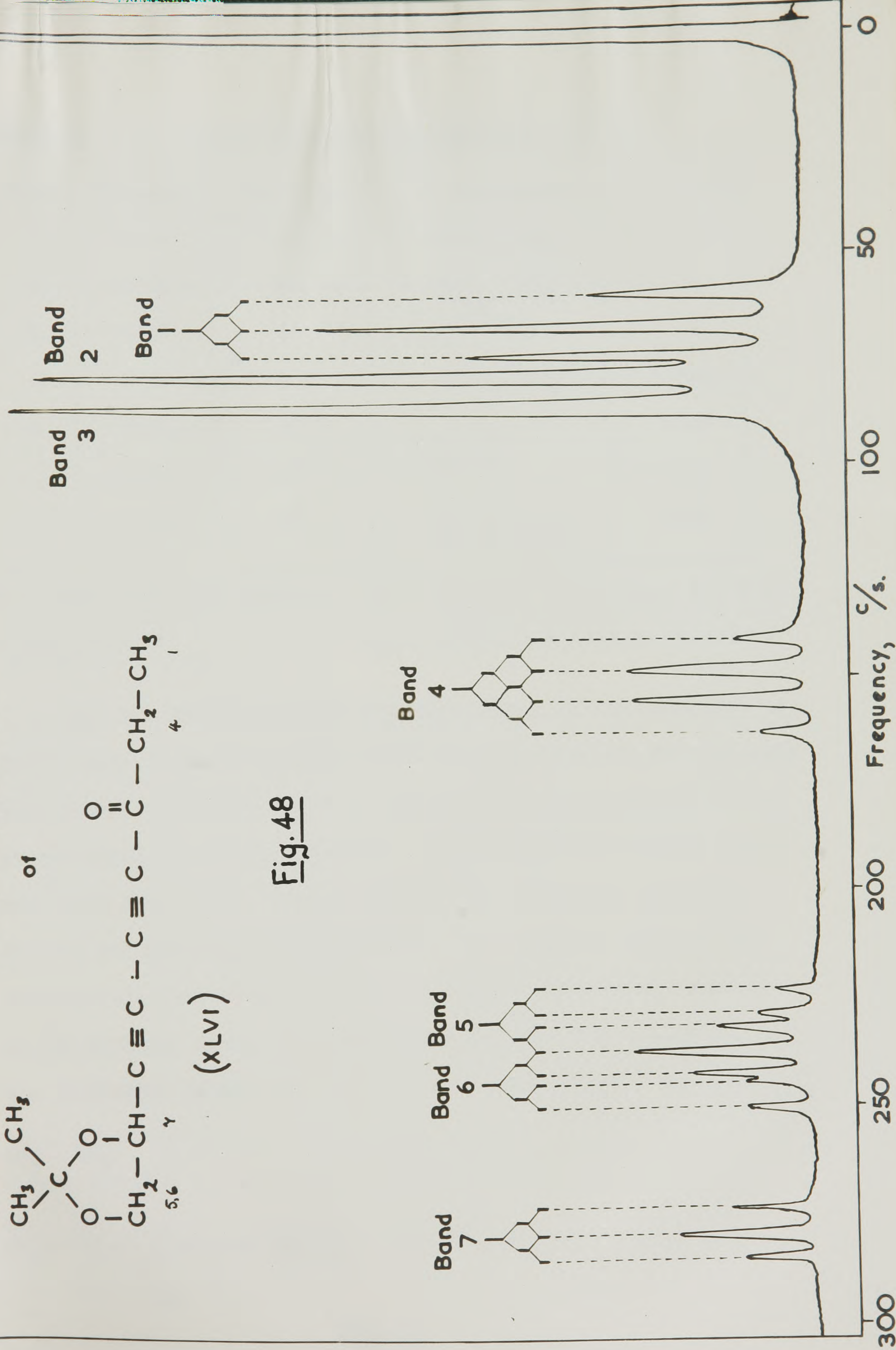


Table 17

N.M.R. Data for the Derivative (XLVI)

Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	8.88	3	$-\text{CO} \cdot \text{CH}_2 \cdot \text{CH}_3$	6.7
2	8.68	1	$\left\{ \begin{array}{c} \text{CH}_3 \quad \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{C} \\ \diagup \quad \diagdown \\ \text{O} \quad \text{O} \end{array} \right.$	-
3	8.56	1		-
4	7.47	4	$-\text{CO} \cdot \text{CH}_2 \cdot \text{CH}_3$	7.4
5	6.14	4*	$\left\{ \begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{CH}_2 \cdot \text{CH} \end{array} \right.$	8.6
6	5.96	4*		7.3
7	5.32	3*	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{CH}_2 \cdot \text{CH} \cdot \text{C} \equiv \text{C}- \end{array}$	5.7

* = Bands of an ABX system. $J_{AB} = 8.0$ c/s. $J_{AX} = J_{BX} = 5.7$ c/s.
 $J_{AB}/\delta_{AB} = 0.72$ $\delta_{AB} = 0.18$ p.p.m. (r.m.s. value)

From the nuclear magnetic resonance data, it was found that as in the case of the derivative (XLV), the gem-dimethyl grouping gave two absorptions (Bands 2 and 3), and the ketal ring protons formed an ABX system (Bands 6, 7 and 9). The presence of a methyl group was established with a methyl triplet and a methylene quartet sharing the same coupling constants. The chemical shift of the quartet suggested close proximity to either an acetylenic bond or to the carbonyl group, thus giving two possible full structures for the derivative (XLVI) (see Fig. 50 - (a) and (b)). Hydrogenation

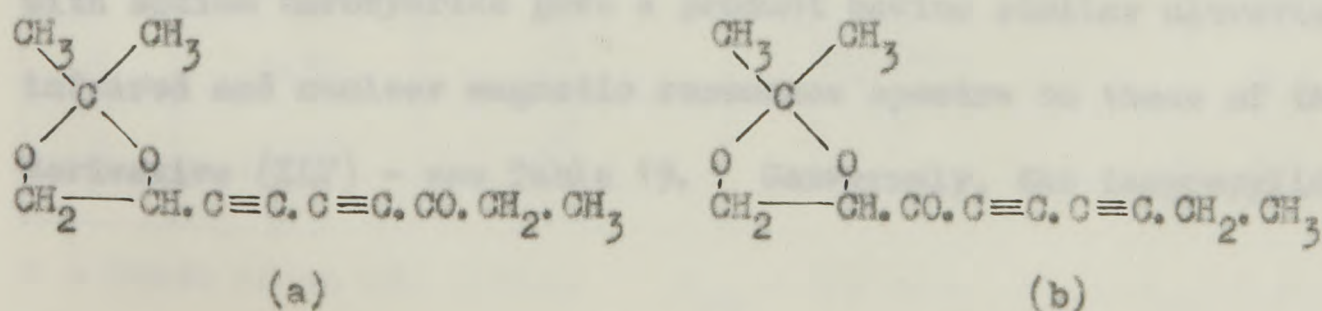


Fig. 50

was expected to give a product which, if derived from structure (a) would retain the methylene quartet of the ethyl group in the same position in its nuclear magnetic resonance spectrum, but if derived from structure (b) would not have this characteristic in its spectrum.

It can be seen from the data in Table 18 that the methylene quartet was retained at 7.60 p.p.m., superimposed on a triplet at 7.60 p.p.m. The latter band is due to the methylene group on the other side of the carbonyl group being split by a further methylene pair. A small separation of the gem.-dimethyl bands was still present (0.04 p.p.m.) due to the asymmetric substitution of the ketal ring.

Table 18 N.M.R. Data for Perhydro-(XLVI). Derivative (XLVII)

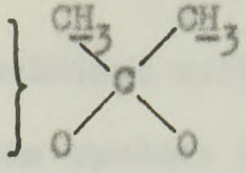
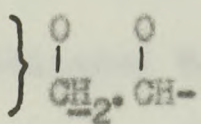
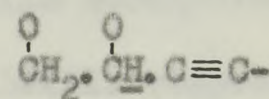
Band	τ (p. p. m.)	No. lines	Assignment	J c/s.
1	8.96	3	$-\text{CO} \cdot \text{CH}_2 \cdot \text{CH}_3$	7.4
2	8.69	1		-
3	8.65	1		-
4	7.60	3	$-\text{CH}_2 \cdot \text{CH}_2 \cdot \text{CO}-$	6.2
5	7.60	4	$-\text{CO} \cdot \text{CH}_2 \cdot \text{CH}_3$	7.3

Reduction of the unsaturated isopropylidene derivative (XLVI) with sodium borohydride gave a product having similar ultraviolet, infrared and nuclear magnetic resonance spectra to those of the derivative (XLV) - see Table 19. Conversely, the isopropylidene

Table 19

N.M.R. Data for Derivative (XLVIII)

(Product from NaBH₄ reduction of Derivative (XLVI))

Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	8.98	3	$-\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{CH}_3$	7.0
2	8.65	1		-
3	8.53	1		-
4	8.27	5	$-\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{CH}_3$	6.4
5	6.95	1	$-\text{OH}$?	-
6	6.03	4*		8.4
7	5.83	4*		8.1
8	5.65	3	$\equiv\text{C}\cdot\text{CH}(\text{OH})\cdot\text{CH}_2-$	6.4
9	5.25	3*		5.7

* = Bands of an ABX system.

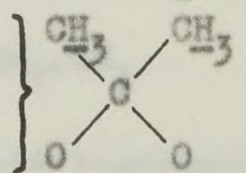
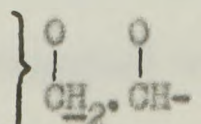
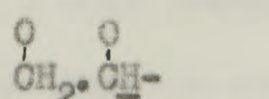
$$J_{AB} = 8.2 \text{ c/s.} \quad J_{AX} = J_{BX} = 5.7 \text{ c/s.}$$

$$J_{AB}/\delta_{AB} = 0.68$$

$$\delta_{AB} = 0.20 \text{ p.p.m. (r.m.s. value)}$$

Table 20

N.M.R. Data for the derivative (XLVI) from the
Oxidation of Derivative (XLV)

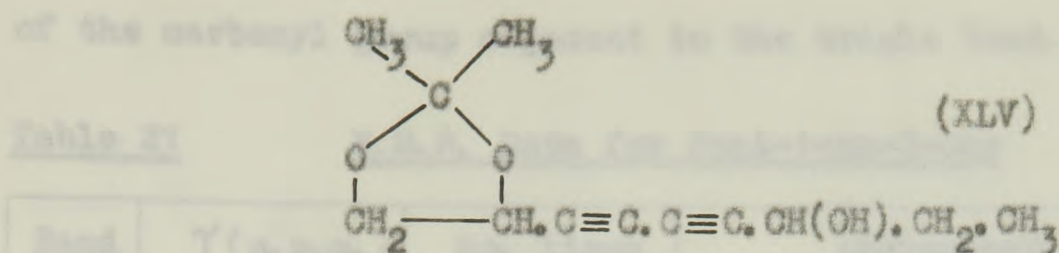
Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	8.90	3	$-\text{CO}\cdot\text{CH}_2\cdot\text{CH}_3$	7.1
2	8.71	1		-
3	8.60	1		-
4	7.52	4	$-\text{CO}\cdot\text{CH}_2\cdot\text{CH}_3$	6.7
5	6.12	4*		8.0
6	5.94	4*		7.1
7	5.40	3*		5.4

* = Bands of an ABX system.

$$J_{AB} = 7.6 \text{ c/s.} \quad J_{AX} = J_{BX} = 5.4 \text{ c/s.}$$

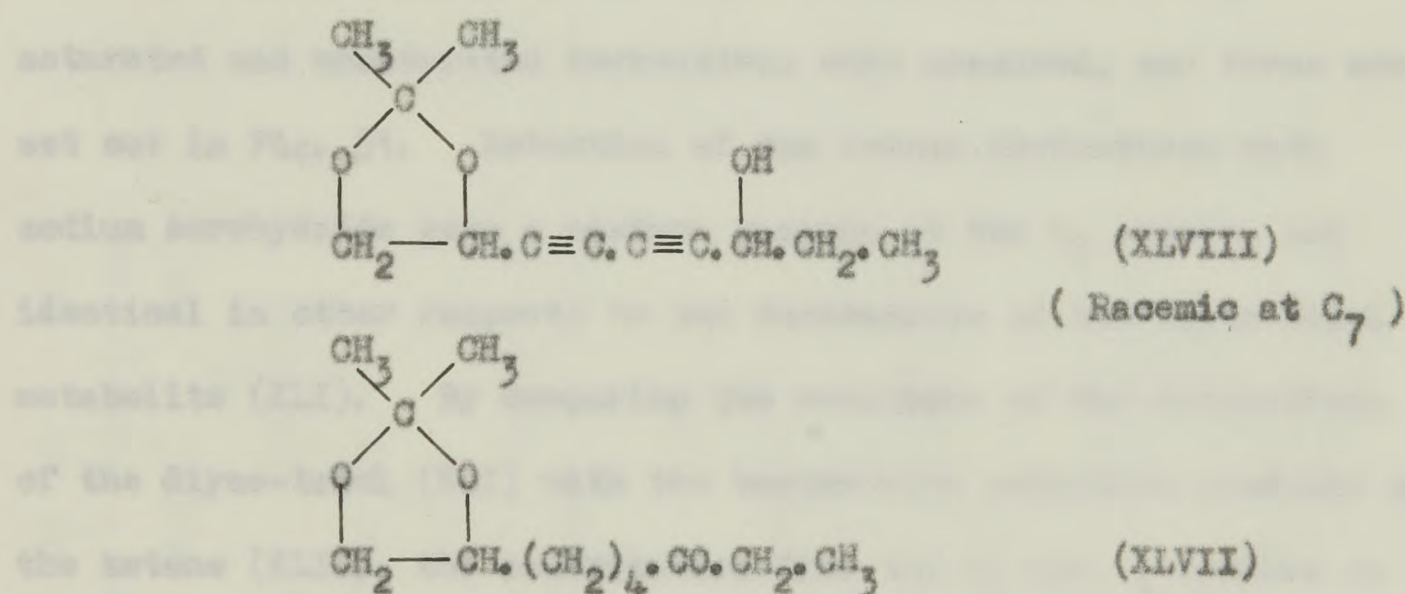
$$J_{AB}/\delta_{AB} = 0.74$$

$$\delta_{AB} = 0.17 \text{ p.p.m. (r.m.s. value)}$$



derivative (XLV) of the triol was oxidised with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and the product found to have identical ultraviolet, infrared and nuclear magnetic resonance spectra to those of the derivative (XLVI) of the ketone (see Table 20).

Hence the structure (XLII) was established for the natural product from that of its isopropylidene derivative (XLVI). The structures of the hydrogenated material and sodium borohydride reduction product are therefore (XLVII) and (XLVIII) respectively.



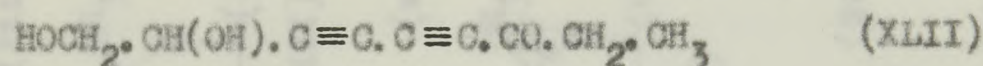
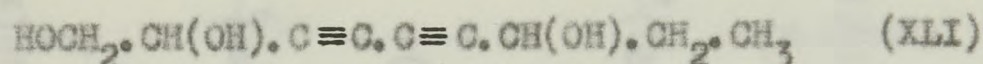
To complete the nuclear magnetic resonance correlation data, pent-1-yn-3-ol was oxidised with chromic acid in ether⁽⁵⁷⁾ to pent-1-yn-3-one, which showed the expected $-\text{CO} \cdot \text{CH}_2 \cdot \text{CH}_3$ multiplets (see Table 21), and also evidence of a much deshielded ethynyl proton. This is probably due to the electron-withdrawing properties

of the carbonyl group adjacent to the triple bond.

Table 21 N.M.R. Data for Pent-1-yn-3-one

Band	τ (p.p.m.)	No. lines	Assignment	J c/s.
1	8.85	3	$-\text{CO} \cdot \text{CH}_2 \cdot \text{CH}_3$	7.9
2	7.39	4	$-\text{CO} \cdot \text{CH}_2 \cdot \text{CH}_3$	7.5
3	6.64	1	$\text{HC} \equiv \text{C} \cdot \text{CO}-$	-

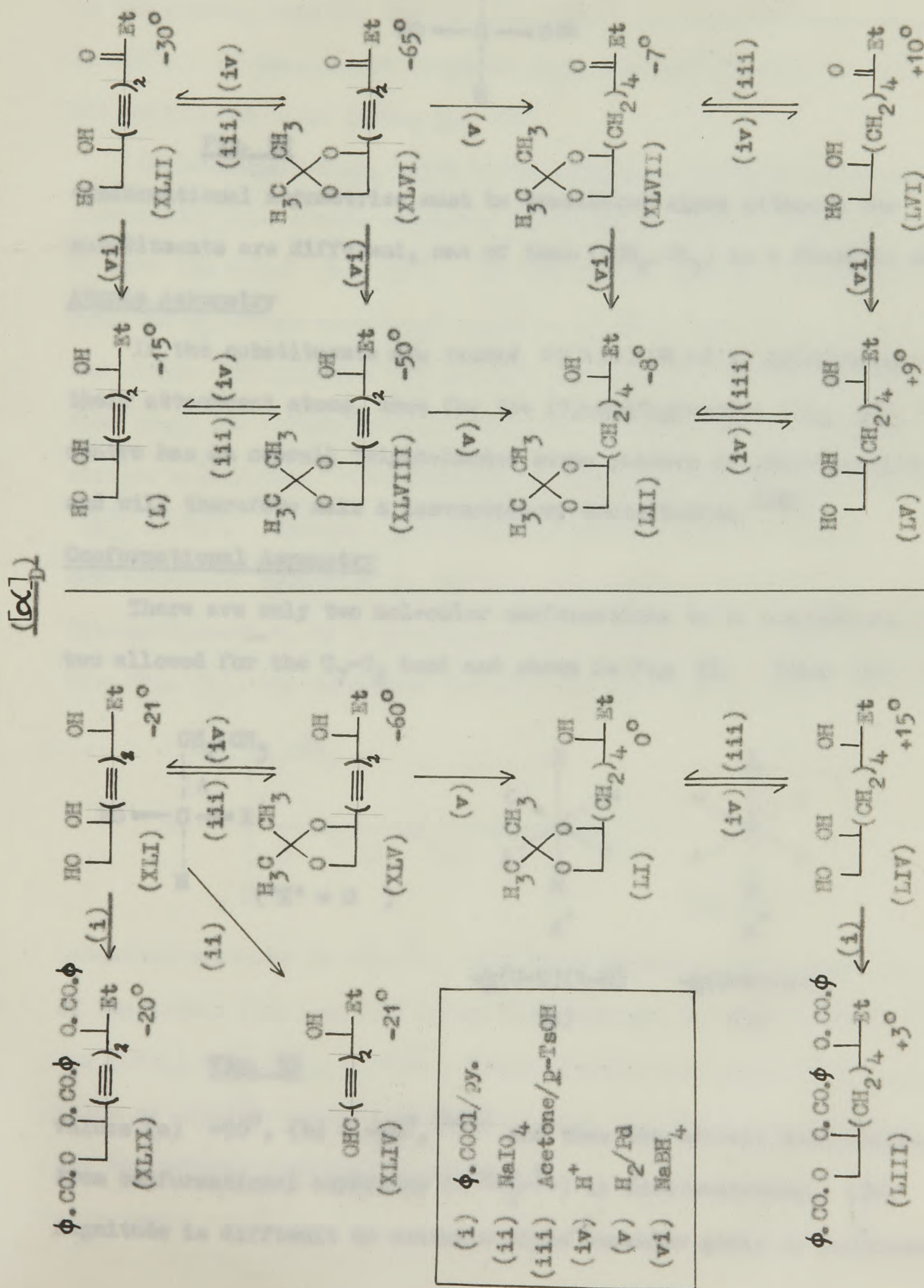
General Comments

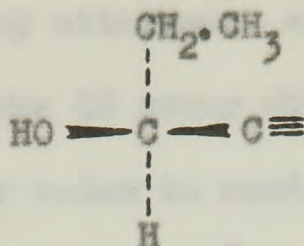


The optical rotations of the two metabolites and their saturated and unsaturated derivatives were measured, and these are set out in Fig. 51. Reduction of the ketone derivatives with sodium borohydride gave a mixture racemic at the C_7 centre, but identical in other respects to the derivatives of the diyne-triol metabolite (XLI). By comparing the rotations of the derivatives of the diyne-triol (XLI) with the borohydride reduction products of the ketone (XLII), the contribution from the C_2 and C_7 centres in the diyne-triol (XLI) and its derivatives could be deduced. It then became of interest to discover whether Brewster's methods⁽⁴²⁾ could be used to determine the absolute configuration at these centres.

The C_7 Centre

The (S)-configuration⁽⁴⁵⁾ is shown in Fig. 52. Both atomic and



Fig. 52

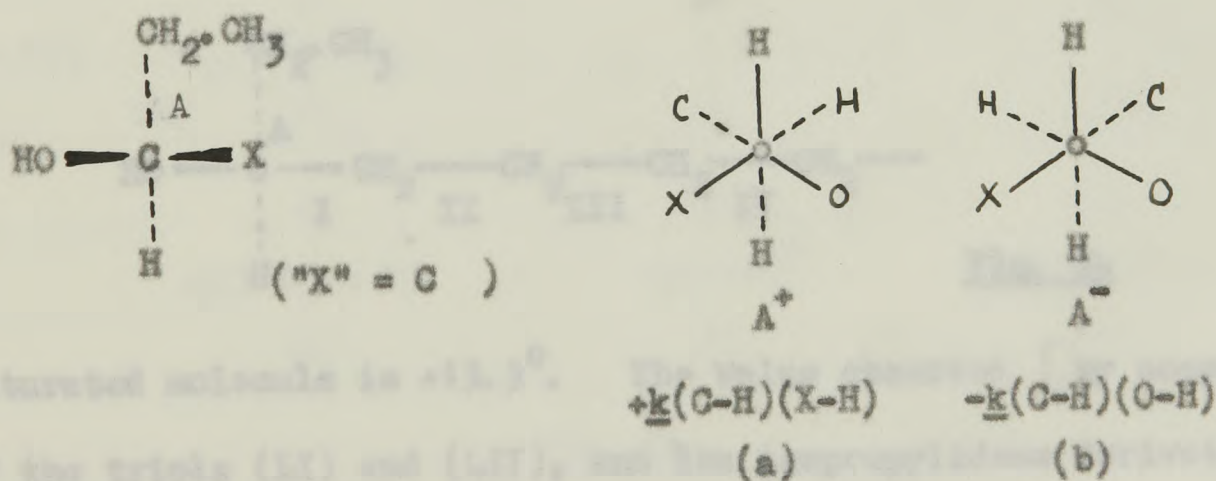
conformational asymmetries must be considered since although the substituents are different, one of them ($-\text{CH}_2\cdot\text{CH}_3$) is a flexible chain.

Atomic Asymmetry

If the substituents are ranked $\equiv\text{C} > \text{Et} > \text{OH} > \text{H}$ in polarisability of their attachment atoms, then for the (S)-configuration (Fig. 52), the centre has an overall "right-handed screw pattern of polarisability", and will therefore make a laevorotatory contribution. (42a)

Conformational Asymmetry

There are only two molecular conformations to be considered, the two allowed for the $\text{C}_7\text{-C}_8$ bond and shown in Fig. 53. These have the

Fig. 53

values (a) $+50^\circ$, (b) $= -50^\circ$, (42a) and thus the overall contribution from conformational asymmetry ($= \frac{a+b}{2}$) is dextrorotatory. The magnitude is difficult to estimate since Brewster gives no experimental

values for acetylene group attachment atoms. The calculated value (87°) is the same as that for the CN group where the observed value is given as 160° . If this latter value is used, the contribution is $+55^\circ$, the calculated value giving ca. $+20^\circ$.

(2R)-But-3-yn-2-ol, which could reasonably be expected to show essentially pure atomic asymmetry was found by Jones, Loder and Whiting⁽²³⁾ to have the value $[M]_D +31^\circ$. If the atomic asymmetry contribution from the C_7 centre considered above is of this order of magnitude, then for either configuration the overall rotation will be of unpredictable sign since the atomic and conformational asymmetries are opposed and of similar magnitudes. The observed contribution from this centre is $[M]_D = -15^\circ$. However, hydrogenation gives a compound where conformational asymmetry is the only contributing factor. For the (S)-configuration, by inspection of the bonds A, I, II, III and IV (See Fig. 54), the estimated contribution from the C_7 centre in the

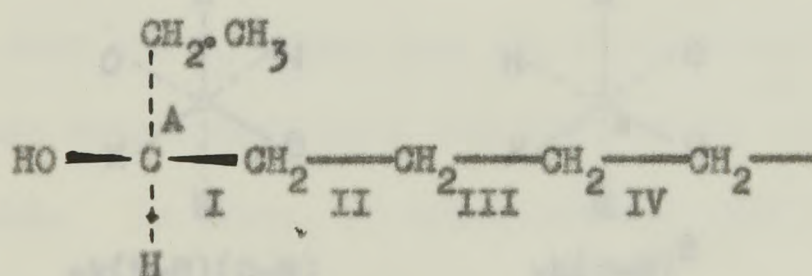


Fig. 54

saturated molecule is $+13.3^\circ$. The value observed [by comparison of the triols (LI) and (LII), and the isopropylidene derivatives (LIV) and (LV)] was $[M]_D = +15^\circ$. Since the (R)-configuration gives a value of -13.3° , the (7S)-configuration is tentatively put forward for the natural diyne-triol (XLI).

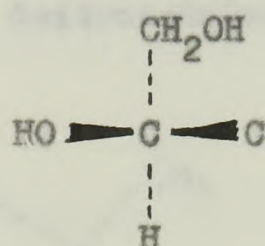
The C₂ Centre

Here again, for the unsaturated molecule, there is both atomic and conformational asymmetry to be considered.

Atomic Asymmetry

For the (S)-configuration, (see Fig 55), there is a "right-handed

Fig. 55



screw pattern", and therefore a laevorotatory contribution, when the substituents are ranked $\equiv\text{C} > \text{CH}_2\text{OH} > \text{OH} > \text{H}$ in polarisability.

Conformational Asymmetry

Conformational asymmetry is exhibited by the C₁-C₂ bond which has the possible conformations shown in Fig. 56 (C₂ forward).

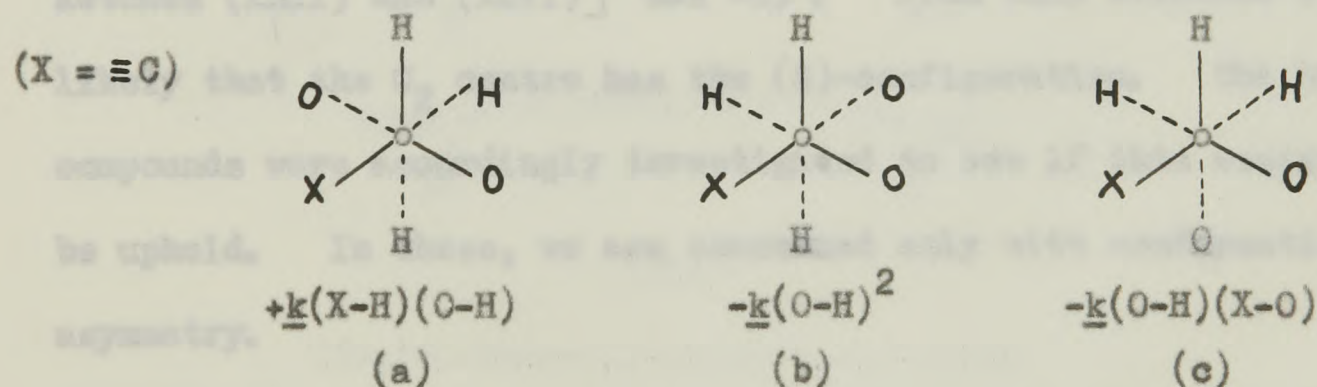


Fig. 56

Configuration (c), normally prohibited by Brewster's rules (see rule 3, p.26, this thesis) could be allowed here on the grounds of the likelihood of hydrogen bonding and the relatively small size of the oxygen atom. Allowing this conformation as equally probable, the

estimated contribution is very small and laevorotatory. Considering only 56 (a) and (b) gives a dextrorotatory contribution. However, on formation of an isopropylidene derivative, there is only one possible conformation, shown in Fig. 57, which gives a large laevorotatory value. Similarly, the formation of an isopropylidene derivative of the (R)-form gives a large dextrorotatory value.

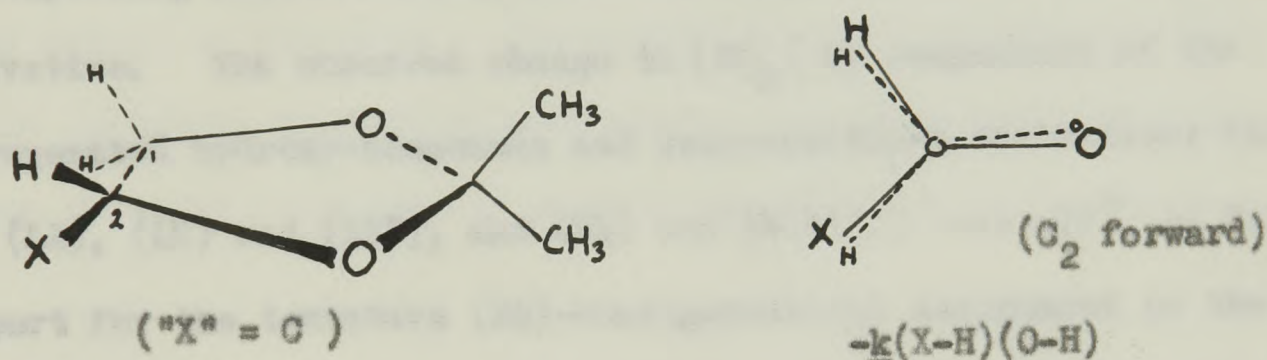


Fig. 57

The observed change in $[M]_D$ from open-chain to a cyclic ketal [comparing the diynes (XLI) and (XLV), (L) and (XLVIII), and the ketones (XLII) and (XLVI)] was -85° . From this evidence it seems likely that the C₂ centre has the (S)-configuration. The hydrogenated compounds were accordingly investigated to see if this assignment could be upheld. In these, we are concerned only with conformational asymmetry.

For the (S)-configuration, by inspection of the bonds A, I, II, III, and IV (see Fig. 58), the estimated contribution of the C₂ centre

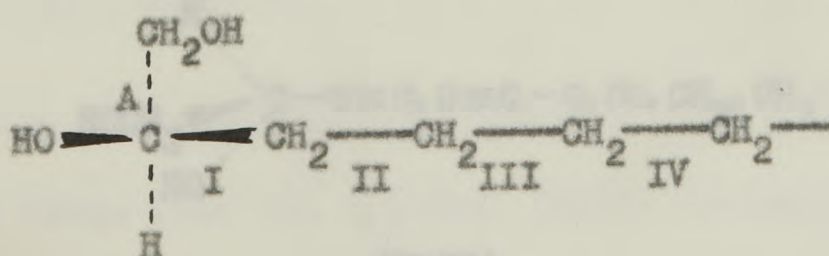
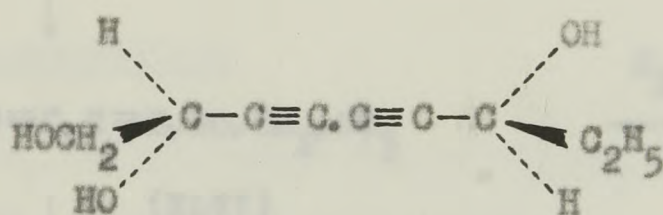


Fig. 58

is again dependent on whether the "disallowed" conformation of bond A

(C₁-C₂, see Fig. 56) is "allowed" through the likelihood of hydrogen-bonding. Allowing all three conformations of A, $[M] = +3^\circ$, and allowing only two conformations, $[M] = +15^\circ$. However, with formation of an isopropylidene derivative, the conformation of bond A becomes fixed (cf. unsaturated compounds above), and the estimated contribution of the C₂ centre becomes -48° . For the (R)-form, there is a corresponding dextrorotatory shift on formation of an isopropylidene derivative. The observed change in $[M]_D$ [by comparison of the hydrogenated hydroxy-compounds and isopropylidene derivatives (LIV) and (LI), (LV) and (LII), and LVI) and (XLVII)] was -35° , in further support for the tentative (2S)-configurational assignment to the diyne-triol (XLI) and the diyne-ketone (XLII) and their derivatives.

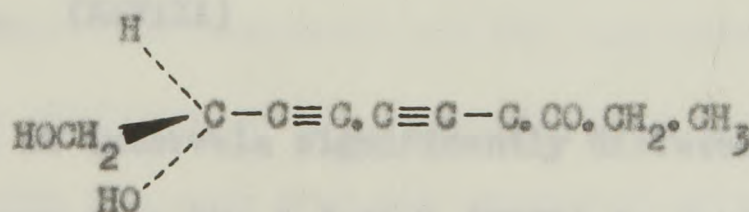
The proposed full structures of the two metabolites are shown in Fig. 59.



(XLI)

(2S,7S)-Nona-3,5-diyne-1,2,7-triol

Fig. 59

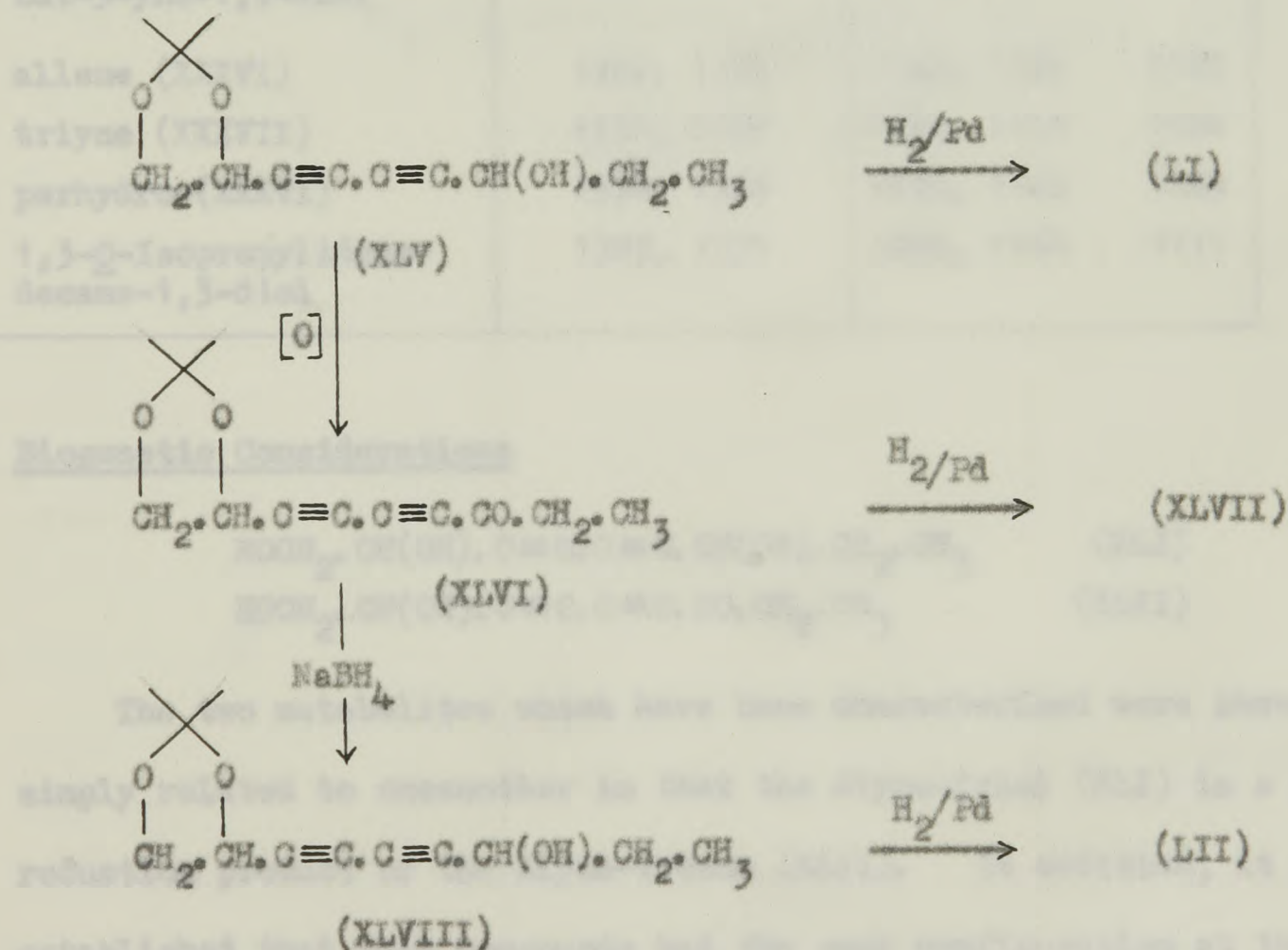


(XLII)

(2S)-Nona-3,5-diyn-7-one-1,2-diol

Infrared Data from the Isopropylidene Derivatives

In the discussion of the polyacetylenic metabolites from Odontia bicolor (p. 51), attention was drawn to certain consistent bands in the infrared spectra of the isopropylidene derivatives of the 1,3-diol (XXIX). These bands, in the region $1100-1200\text{ cm}^{-1}$, were attributed to skeletal vibrations of the ketal ring, and should therefore be dependent to some extent on its size. The isopropylidene derivatives formed from the Clitocybe rhizophora metabolites have five-membered rings and were observed to produce a similar set of bands appearing in the

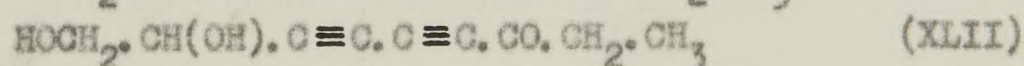
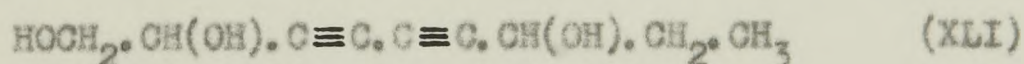


same region but at intervals significantly different from those of the six-membered rings from the 1,3-diol (XXIX) derivatives. The data are recorded in Table 22.

Table 22

Compound	Observed $\gamma_{\max.}$ values. (cm^{-1})		
	Gem.-dimethyl	Ketal Ring	
diyne-alcohol (XLV)	1381, 1373	1210, 1151	1065
Synthetic (XLV)	1377, 1369	1206, 1148	1063
diyne-alcohol (XLVIII)	1378, 1370	1205, 1148	1063
diyne-ketone (XLVI)	1379, 1371	1206, 1147	1065
alcohol (LI)	1380, 1372	1211, 1157	1061
alcohol (LII)	1380, 1372	1211, 1157	1060
ketone (XLVII)	1379, 1371	1210, 1156	1062
1,2-Isopropylidene-but-3-yne-1,2-diol	1378, 1370	1206, 1151	1063
allene (XXXVI)	1380, 1370	1192, 1162	1103
triyne (XXXVII)	1378, 1367	1196, 1159	1106
perhydro-(XXXVI)	1378, 1366	1195, 1162	1105
1,3-O-Isopropylidene-decane-1,3-diol	1383, 1371	1200, 1168	1111

Biogenetic Considerations



The two metabolites which have been characterised were shown to be simply related to one another in that the diyne-triol (XLI) is a simple reduction product of the diyne-ketone (XLII). In addition, it was established that both compounds had the same configuration at the C_2 centre, since oxidation of the isopropylidene derivative (XLV) gave a product having the same sign and magnitude of optical rotation as the isopropylidene derivative (XLVI) from the ketone (XLII).

Experiments are planned to study the labelling pattern produced by

feeding labelled (C^{14}) acetate to the fungus. The metabolites can be degraded by periodate fission.

It is possible that these C_9 polyacetylenes are derived from a C_{10} tetra-acetylenic acid, and a hypothetical sequence is shown in Fig. 60.

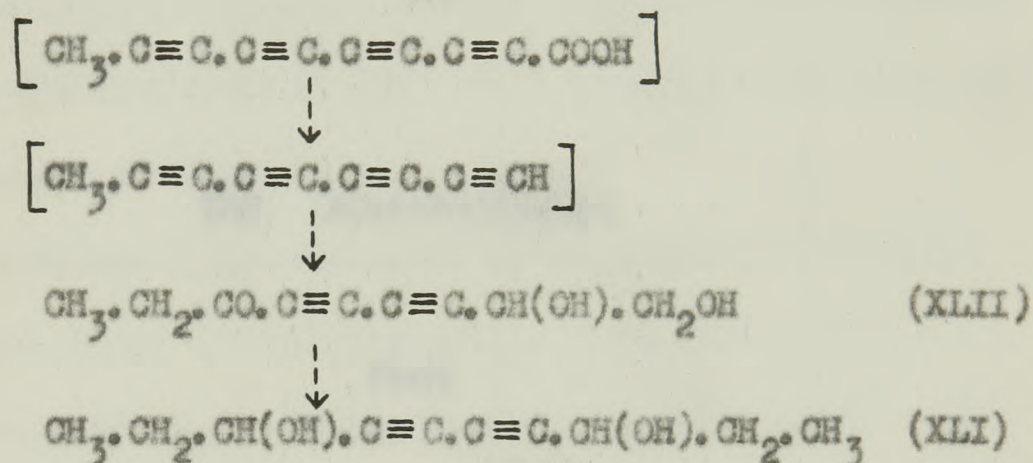


Fig. 60

Experimental

Procedures adopted for the examination of Nuclear Magnetic Resonance Spectra

All samples were examined in carbon tetrachloride solution (minimum 15% in 0.2 c.c.) on an AEI RS 2 Spectrometer with an operating frequency of 60 Mc/s.

Tetramethylsilane (TMS) was used as an internal reference standard, and the shift of the absorption lines from this standard was measured by the "superimposition technique". In this method, the TMS absorption line is moved to coincide with a strong line in the spectrum. The movement of the reference peak can be controlled to 1 c/s., and the last figure is estimated by the operator from the height of the superimposed lines on an oscilloscope. Assuming a linear sweep, all other lines in the spectrum are calibrated from TMS by appropriate interpolation or extrapolation of the value obtained for the one strong line. Distances on the chart paper could be measured visually with an estimated accuracy of ± 0.5 mm., which corresponds to ca. 0.6 c/s. for most of the spectra.

the polyacetylene extracted from the polymer was found to be

Isolation of polyacetylene from polymer

(a) Original Sample

The sample (10g) was extracted with carbon tetrachloride (2 L.) for 24 hr. The extract was evaporated to dryness and the

Experimental

The general experimental procedures are given on p. 55

Growth of *Clitocybe rhizophora*. -

The culture of "*Clitocybe rhizophora* Velen" was obtained from the Centraalbureau voor Schimmelcultures, Baarn (Netherlands). The fungus was grown as surface cultures on a medium of 3% malt extract. The poly-yne content was highest at periods varying from 35 to 44 days. The maximum concentration of diyne-ketone varied for different growths from 3 mg./litre to 8 mg./litre, and the maximum concentration of diyne-triol was ca. 45 mg./litre. The following yields of diyne-ketone and diyne-triol (resp.) were obtained from three refloods of the mycelial mat with 4% glucose: (i) 16-28 mg./litre and 11 mg./litre after 13-23 days, (ii) 17-24 mg./litre and 5 mg./litre after 18-19 days, (iii) 8 mg./litre and 4 mg./litre after 26 days.

Clitocybe rhizophora was also grown as shake cultures on a medium of 3% malt. The diyne-ketone ultraviolet light absorption reached a maximum after 34 days, and again after a further 14 days following reflooding with 4% glucose solution. The intensity of ultraviolet light absorption is very low compared to that from static cultures; the polyacetylenic metabolites have not yet been investigated.

Isolation of Polyacetylenic Components. -

(a) Original Growths

The culture fluid (20.1) was continuously extracted with ether (2 l.) for 24 hr. The extract was evaporated to 200 c.c. in a

darkened flask and separated into acidic and neutral fractions by extraction with sodium hydrogen carbonate solution. The ultraviolet absorption spectrum of the acidic fraction indicated that there was no acidic absorbing species present. The neutral fraction was dried, evaporated to dryness, and the residue extracted with benzene (200 c.c.). The benzene was cooled and filtered free from precipitated diyne-triol. The diyne-triol (XLI) was recrystallised either from benzene or from ether/hexane. The benzene solution was then extracted with water (2 x equal volume) and the aqueous extracts saturated with sodium chloride and extracted with ether (3 x equal volume). The ethereal extracts were dried, evaporated, and the residue taken up in ether again to give a solution of the neutral diyne-ketone (50 mg., U.V. estn.)

Purification of the Diyne-Ketone (XLII)

An ethereal solution of the diyne-ketone (XLII), (40 mg.), was evaporated to dryness and the residue taken up in acetone (40 c.c.). This solution was heated under reflux with *p*-toluenesulphonic acid (5 mg.) for 18 hr. when excess solid sodium carbonate was added and the mixture kept at 15° for 1 hr. The solution was filtered and evaporated to dryness, and the residue extracted with light petroleum (b.p. 30-40°, 3 x 20 c.c.) and chromatographed on alumina (W) (10 g.). The purified diyne-ketone isopropylidene compound (XLVI) (31 mg.) was eluted in light petroleum (b.p. 30-40°, 150 c.c.)

(b) Glucose Refloods

The culture medium was extracted with ether and the extracts washed with sodium hydrogen carbonate solution as for the original growths. The extracts were then dried and evaporated to dryness

and the residues treated with acetone and *p*-toluenesulphonic acid as above. After filtration from added sodium carbonate and evaporation of the acetone solution, the product was taken up in benzene (2 c.c.) and adsorbed onto alumina (W) (18 g.). The diyne-ketone isopropylidene compound (XLVI) was eluted in light petroleum (b.p. 30-40°, 250 c.c.), and the diyne-triol isopropylidene compound (XLV) in light petroleum (b.p. 40-60°)/50% benzene (120 c.c.)

The isopropylidene compounds were treated separately with 2N hydrochloric acid (10 c.c.) for 30 min. and the mixture extracted with ether (3 x 20 c.c.). The extracts were dried to give solutions of the original natural products (XLII) and (XLI).

Characterisation of Components.-

Nona-3,5-diyne-1,2,7-triol (XLI).-

Physical Constants

Needles, m.p. 71.5-72.5°

Found: C, 64.5; H, 7.45. $C_9H_{12}O_3$ requires C, 64.25; H, 7.2%.

"Rast" molecular weight, 168. $C_9H_{12}O_3$ requires M.W. 168.2

Ultraviolet Absorption: (ϵ in parentheses)

λ_{max} : 2560 (230), 2420 (370), 2280 (450), 2200 Å (410)

Infrared Absorption: (in nujol)

ν_{max} : 3130 (broad; intermolecular H-bonds), 1082, 1028 cm^{-1}
(C-O-H).

Optical Rotation:

$[\alpha]_D^{26} = 21^\circ$ (c 0.53)

Quantitative Hydrogenation.-

The diyne-triol (XLI; 6.40 mg.) was hydrogenated in ethanol over

platinum (from 9 mg. platonic oxide) in a Hosli microhydrogenator. The uptake of hydrogen at N.T.P. was 3.22 c.c., 95% of that calculated for a diacetylene M.W. 168.2. In a second run the diyne-triol (XLI) (6.57 mg.) absorbed 3.28 c.c. at N.T.P., 94% of the calculated value.

Reaction with Sodium Periodate.-

(a) The diyne-triol (XLI; 57.0 mg., 0.34 mm.) in excess sodium periodate solution (ca. 4 g./l., 40 c.c.) was kept at 15° for 12 hr. The solution was then distilled, leaving behind about one third of the original volume, and the distillate collected under saturated aqueous dimedone solution (ca. 0.8 mm.). The distillate mixture was kept at ca. 95° for 10 min. and then set aside at 0°. The pale pink precipitate (80.4 mg.), m.p. 150-165° and 190-192°, was recrystallised from aqueous ethanol. Colourless needles (44.9 mg., 45.3%), m.p. 191-192°, (undepressed by admixture with authentic formaldehyde dimedone derivative) were deposited.

Separately, an aqueous solution of formaldehyde (42.1 mg.) was two-thirds distilled and the distillate collected under saturated aqueous dimedone solution. The derivative was collected as colourless needles (69.3 mg., 42.5%), m.p. 191-192°.

(b) The diyne-triol (XLI; 32 mg.) was treated with excess sodium periodate solution (20 c.c., 4 g./l.) at 15° for 16 hr., when the solution was extracted with ether (3 x 25 c.c.) and the ethereal extracts washed with water (3 x 100 c.c.) and dried. The ethereal solution was evaporated to yield an unstable pale yellow oil (27 mg.). (An ethanolic solution of the oil (0.02% w./v.) lost 95% of its ultraviolet light absorption intensity at 15° over 8 hr.)

$\lambda_{\text{max.}}$: ($\epsilon \times 10^{-3}$ in parentheses) 2865 (4.5), 2705 (5.6), 2565 (4.1), 2435 (2.6), 2325 Å (1.7). $\nu_{\text{max.}}$: 3597 (O-H), 2732 (C-H; aldehyde), 2232, 2174 and 2210 ($\text{C}\equiv\text{C}$), 1668 cm^{-1} ($\text{C}=\text{O}$). $[\alpha]_{\text{D}}^{19} -21^{\circ}$ (d 0.70).

Formation of the Tribenzoate of the Diyne-Triol (XLI).-

The diyne-triol (XLI; 100 mg.) together with benzoyl chloride (400 mg.) and dry pyridine (0.5 c.c.) was dissolved in benzene (5 c.c.) and heated under reflux for 15 min. The mixture was cooled, and ether (30 c.c.) added. The solution was then washed with 2N hydrochloric acid, 2N sodium hydroxide solution, and water (2 x 40 c.c. of each). It was then dried, evaporated and the residual oil chromatographed on alumina (S), (25 g.) in benzene (5 c.c.). The tribenzoate was eluted in the same solvent (100 c.c.). Attempts to crystallise produced needles which melted below 0° . $\lambda_{\text{max.}}$: ($\epsilon \times 10^{-3}$ in parentheses) 2810 (1.6), 2735 (2.0), 2300 Å (32.6). $\nu_{\text{max.}}$: 1730 ($\text{C}=\text{O}$), 1266 and 1090 cm^{-1} (C-O-, benzoate). $[\alpha]_{\text{D}}^{21} -20^{\circ}$ (d 0.63).

Large scale Hydrogenation of the Diyne-Triol (XLI).-

The diyne-triol (XLI; 393 mg.) was hydrogenated in ethanol (100 c.c.) over 5% palladium on charcoal (42 mg.) for 3 hr. The solution was filtered free from catalyst and evaporated to yield an oil which could not be crystallised (409 mg.). $\nu_{\text{max.}}$: (liquid film) 3330 (broad; intermolecular H-bonds), 1119 and 1071 cm^{-1} (C.O.H). $[\alpha]_{\text{D}}^{22} + 15^{\circ}$ (d 0.78). The tribenzoate, prepared as for the diyne-triol (XLI) above, was also an oil. $\nu_{\text{max.}}$: (liquid film) 1720 ($\text{C}=\text{O}$), 1276 and 1109 cm^{-1} (C-O-; benzoate). $[\alpha]_{\text{D}}^{20} + 3^{\circ}$ (d 5.25, visual polarimeter).

Reaction of the Hydrogenated Triol (LIV) with Aqueous Sodium Periodate.-

(a) To a solution of the perhydro-triol (LIV; 43 mg.) in aqueous

ethanol (25 c.c.) was added sodium periodate solution [25 c.c. of a solution of sodium periodate (1075 mg.) in water (250 c.c.)]. This solution was kept at 15° and sampled after 1 hr. and after 24 hr.

The procedure for titration of samples and evaluation of results is given on p. 64 of this thesis. The titrations indicated consumption of 81% of 1 mole of periodate for each mole of perhydro-triol

(b) The perhydro-triol (LIV; 100 mg.) was treated with excess sodium periodate solution (50 c.c., 4 g./l.) at 15° for 20 hr. The mixture was then distilled to give a cloudy distillate (15 c.c.). To this distillate (3 c.c.) was added saturated aqueous dimedone solution. The dimedone derivative which separated out had m.p. 189-191° (m.p. of formaldehyde dimedone 190°). The remaining distillate (12 c.c.) was treated with excess 2,4-dinitrophenylhydrazine in methanolic perchloric acid. The precipitated dinitrophenylhydrazones (60 mg.) were chromatographed on alumina (S), (25 g.), from benzene (5 c.c.). The formaldehyde derivative was eluted in benzene (20 c.c.) and identified from its infrared absorption spectrum $\nu_{\text{max.}}$: (carbon disulphide) 1188, 949 cm^{-1} . The other major component of the mixture, the 2,4-dinitrophenylhydrazone of 6-hydroxyoctanal was eluted in benzene/10% ether (75 c.c.) and was crystallised to give yellow needles, m.p. 114-115°. (Found: C, 51.5; H, 6.55. $\text{C}_{14}\text{H}_{20}\text{O}_5\text{N}_4$ requires C, 51.85; H, 6.2%)

(c) The perhydro-triol (LIV; 176 mg., 1.00 mM.) was dissolved in sodium periodate solution (188 c.c., 4 g./l., 3.50 mM.) containing sodium carbonate (415 mg. 3.00 mM) and potassium permanganate (21 mg., 0.13 mM.) and kept at 15° for 16 hr. The solution was acidified,

extracted with ether, and the extracts dried and evaporated. The residue (166 mg.) was dissolved in acetone (15 c.c.) and 8N/12N chromic sulphuric acid (0.5 c.c.) added with stirring over 35 min. The mixture was stirred at 15° for a further 2 hr. when the acetone solution was decanted and evaporated to 5 c.c. The solids were washed with ether, and the ethereal and acetone solutions combined and extracted with saturated sodium hydrogen carbonate solution (2 x equal vol.). These extracts were acidified and extracted with ether, the combined ethereal extracts washed with water and dried. Evaporation of the extracts yielded a semi-solid mass which was extracted with hot hexane (2 x 10 c.c.). On cooling the hexane solution, colourless prisms (5 mg.) of 6-keto-octanoic acid were deposited, m.p. 49-51°. $\nu_{\text{max.}}$: 3500 (free O-H), 2642 (bonded O-H), 1751, 1712 (shoulder) and 1703 cm^{-1} (C=O; carbonyl and carboxyl). (Lit. ⁽⁵³⁾ m.p. 52°). Semicarbazone: colourless needles from aqueous ethanol, m.p. 189-190°. (Lit. ⁽⁵³⁾ m.p. 190°)

The Isopropylidene Derivative (XLV) of the Diyne-Triol

The crude diyne-triol (XLI; 98 mg.) was dissolved in acetone (50 c.c.) and heated under reflux with p-toluenesulphonic acid (20 mg.) for 18 hr. Excess solid sodium carbonate was then added and the mixture kept at 15° for 2 hr., when it was filtered, and the acetone solution evaporated to dryness. The residue was adsorbed from light petroleum (b.p. 40-60°)/50% benzene onto alumina (W), (15 g.) and the isopropylidene compound (59 mg.) eluted in the same solvent (100 c.c.)

$\lambda_{\text{max.}}$: (ϵ in parentheses) 2575 (350), 2440 (560), 2315 (580), 2200 Å (470). $\nu_{\text{max.}}$: 3584 (O-H), 2150 (weak, C≡C), 1381 and

1373 ($\text{C}(\text{CH}_3)_2$), 1210, 1151 and 1065 cm^{-1} (C-O-C). $[\alpha]_D^{24} = 61^\circ$ (c 0.23).

Hydrogenation of the Isopropylidene Compound (XLV).-

The isopropylidene compound (XLV; 27 mg.) was taken up in ethanol (15 c.c.) and hydrogenated over 5% palladium on charcoal (20 mg.) for 4 hr. The solution was filtered free from catalyst and evaporated to dryness. The product was chromatographed on alumina (W), (9 g.) and eluted in benzene as a colourless oil (15 mg.). $\nu_{\text{max.}}$: 3610 (O-H), 1380 and 1372 ($\text{C}(\text{CH}_3)_2$), 1211, 1157, and 1061 cm^{-1} (C-O-C). $[\alpha]_D^{21} 0^\circ$ ($\pm 0.3^\circ$; c 0.38)

Oxidation of the Isopropylidene Compound (XLV).-

The isopropylidene compound (XLV; ca. 200 mg.) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (387 mg.) were kept at 15° in benzene (30 c.c.) for 40 hr. The mixture was then adsorbed directly onto alumina (W) (10 g.), and eluted with carbon tetrachloride (50 c.c.). The eluate was found to contain the diyne-ketone (28 mg., 14%) $\lambda_{\text{max.}}$: ($\epsilon \times 10^{-3}$ in parentheses) 2825 (4.03), 2665 (5.64), 2525 (4.86), 2395 (3.65) 2260 Å (2.96) $\nu_{\text{max.}}$: 2230, 2137 ($\text{C}\equiv\text{C}$), 1677 ($\text{C}=\text{O}$), 1381, 1373 ($\text{C}(\text{CH}_3)_2$), 1210, 1148 and 1066 cm^{-1} (C-O-C). $[\alpha]_D^{23} -57^\circ$. (c 1.1; visual polarimeter)

Nona-3,5-diyne-7-one-1,2-diol. (XLII).-

Physical Constants.

Needles (ether/hexane), m.p. $34.5-35^\circ$.

Ultraviolet Absorption: ($\epsilon \times 10^{-3}$ in parentheses).

$\lambda_{\text{max.}}$: 2825 (4.3), 2670 (5.7), 2535 (4.2), 2410 (2.3), 2275 Å (1.4).

Infrared Absorption:

$\nu_{\text{max.}}$: 3553 (broad, O-H), 2240 and 2150 ($\text{C}\equiv\text{C}$), 1683 ($\text{C}=\text{O}$), 1103 and 1068 cm^{-1} (C-O-H).

Optical Rotation:

$[\alpha]_{\text{D}}^{24} -30^{\circ}$ (c 2.52)

Reaction with Aqueous Sodium Periodate to yield Formaldehyde.-

The diyne-ketone (XLII; 41 mg.) in excess aqueous sodium periodate (ca. 4 g.l., 40 c.c.) was shaken for 12 hr. at 15° . The mixture remained cloudy and had become yellow. It was distilled to leave one third of the original volume, and the distillate collected under saturated aqueous dimedone solution. The distillate mixture was heated at 95° for 10 min., and then set aside at 0° . The yellow precipitate (21 mg.), m.p. $165-170^{\circ}$ and $186-189^{\circ}$, was recrystallised from aqueous ethanol. Colourless needles (13 mg., 18%), m.p. $190-192^{\circ}$ (undepressed by admixture with authentic formaldehyde dimedone derivative) were deposited.

A second experiment, conducted under these conditions, revealed no change in the diynone chromophore. The ketone (XLII) was seen to be immiscible with aqueous periodate, and decomposing slightly with shaking at 15° .

Hydrogenation Product (LVI) of the Diyne-Ketone (XLII).-

The diyne-ketone isopropylidene compound (XLVI; 150 mg.) was hydrogenated over 5% palladium on charcoal (43 mg.) in ethanol (15 c.c.) at 15° for 6 hr. The solution was filtered free from catalyst, and evaporated to yield an oil (158 mg.) which was chromatographed on alumina (W), (10 g.) The hydrogenated isopropylidene compound was

eluted, in light petroleum (b.p. 40-60°) 15% benzene, as a colourless oil (120 mg.). This was treated with 2N hydrochloric acid (20 c.c.) for 15 min. at 15°, when the mixture was extracted with ether (3 x 25 c.c.) and the extracts washed with water and dried. $\nu_{\max.}$: 3497 (broad, O-H), 1716 (C=O), 1098, 1078 and 1048 cm^{-1} (C-O-H bonds). $[\alpha]_D^{21} +10^\circ$ (c 0.51).

Reduction of the Diyne-Ketone (XLII) with Sodium Borohydride.-

The diyne-ketone (XLII; 45 mg.) was treated with excess sodium borohydride in methanol (10 c.c.) at 0° for 30 min. The mixture was then evaporated to 2-3 c.c. and poured into water (40 c.c.) and extracted with ether. The dried extracts on evaporation yielded a colourless uncrystallisable oil (32 mg.). $\lambda_{\max.}$: (ϵ in parentheses) 2555 (350), 2420 (540), 2310 (530), 2200 Å (420). $\nu_{\max.}$: 3400 (broad, O-H), 1096, 1042 and 1016 cm^{-1} (C-O-H bonds.) $[\alpha]_D^{23} -15^\circ$ (c 1.58).

The Isopropylidene Derivative (XLVI) of the Diyne-Keto-Diol (XLII).-

The procedure followed was that described for the formation of the derivative as part of the general isolation and purification programme on p. . A sample of the derivative was examined.

$\lambda_{\max.}$: ($\epsilon \times 10^{-3}$ in parentheses). 2825 (4.7), 2670 (6.5), 2525 (4.7), 2400 (2.5), 2270 Å (1.6). $\nu_{\max.}$: 2230, 2141 (C≡C), 1675 (C=O), 1379 and 1371 (C(CH₃)₂), 1206, 1147 and 1065 cm^{-1} (C-O-C). $[\alpha]_D^{21} -65^\circ$ (c 0.35).

Hydrogenation of the Isopropylidene Derivative (XLVI).-

The procedure followed was that described for obtaining the perhydro-ketone, first part (See p. 122). $\nu_{\max.}$: 1723 (C=O),

1379 and 1371 ($\text{C}(\text{CH}_3)_2$), 1210, 1156 and 1061 cm^{-1} (C-O-C).

$[\alpha]_D^{23} -7^\circ$ (c 0.24).

Reduction of the Isopropylidene Derivative (XLVI) with Sodium Borohydride.-

The isopropylidene derivative (XLVI; 84 mg.) was treated with excess sodium borohydride as described for the ketone (XLII) above, and the product isolated via ether. $\lambda_{\text{max.}}$: (ϵ in parentheses) 2575 (350), 2435 (540), 2315 (560), 2190 Å (490). $\nu_{\text{max.}}$: 3571 (O-H), 1378 and 1370 ($\text{C}(\text{CH}_3)_2$), 1205, 1148 and 1063 cm^{-1} (C-O-C).

$[\alpha]_D^{22} -50^\circ$ (c 0.29).

Reduction of the Hydrogenated Isopropylidene Derivative (XLVII) with Sodium Borohydride.-

Procedure as described above for the diyne-isopropylidene derivative (XLVI). The hydrogenated isopropylidene derivative (80 mg.) was reduced to give a colourless oil (67 mg.), after elution from alumina (W) (10 g.) in light petroleum (b.p. 40-60°)/30% benzene.

$\nu_{\text{max.}}$: 3610 (O-H), 1380 and 1372 ($\text{C}(\text{CH}_3)_2$) 1211, 1157 and 1059 cm^{-1} (C-O-C), $[\alpha]_D^{22} -8^\circ$ (c 0.63).

Acidification.-

The above product was treated with 2N hydrochloric acid (10 c.c.) at 15° for 30 min, and the product isolated via ether. $[\alpha]_D^{23} +9^\circ$ (c.0.66)

Synthesis.Nona-3,5-diyne-1,2,7-triol.1,2-O-Isopropylidenebut-3-yne-1,2-diol.-

Ethyl bromide (2.28 g., redistilled and dried over calcium chloride) in tetrahydrofuran (11 c.c., redistilled from lithium aluminium hydride) was added in portions, under nitrogen, to a stirred mixture of magnesium turnings (0.53 g., washed with ether and dried at 100°) in dry tetrahydrofuran (20 c.c.) at 15° over 30 min. No reaction was visible but the mixture became grey in colour and the magnesium was slowly consumed. The mixture was heated under reflux for 1 hr. then cooled and filtered through glass wool into a tap funnel, from which it was added dropwise under acetylene to a stirred saturated solution of acetylene (previously passed for 20 min. at 0°) in dry tetrahydrofuran (60 c.c.) The addition was accompanied by slight gas evolution and the appearance of a transitory pink colour, and was complete in 30 min. after which the solution was stirred for a further 30 min. under acetylene. The acetylene was then replaced by nitrogen and a solution of glycollic aldehyde (0.45 g.) in dry tetrahydrofuran (35 c.c.) added dropwise over 1 hr. Stirring was continued for 16 hr. when the solution had become clear and orange in colour. Saturated aqueous ammonium chloride solution (50 c.c.) was added, and the mixture stirred for a further 30 min. when the two layers were separated. The top layer was evaporated to small bulk, and the aqueous phase extracted continuously with ether for 12 hr. The ethereal and tetrahydrofuran solutions were combined, dried and evaporated. The residue was dissolved in acetone (60 c.c.) containing p-toluenesulphonic

acid (0.02 g.) and heated under reflux for 15 hr. Excess sodium carbonate was then added, and the mixture kept at 15° for 1 hr. The filtered solution was evaporated, and the residue chromatographed in light petroleum (b. p. 30-40°) on alumina (W) (8 g.). The required isopropylidene derivative (0.13 g., 14% from glycollic aldehyde) was eluted in the same solvent as a colourless mobile fluid.

$\nu_{\max.}$: 3274 ($\equiv\text{C-H}$), 1378 and 1370 ($\text{C}(\text{CH}_3)_2$) 1206, 1151 and 1063 cm^{-1} (C-O-C). (Found: C, 66.75; H, 8.35 $\text{C}_7\text{H}_{10}\text{O}_2$ requires: C, 66.65; H, 8.00%).

A sample of the crude but-3-yne-1,2-diol, recrystallised from carbon tetrachloride formed plates, m. p. 38-39°. [Lit. (58) m. p. 39.5-40.5°]

1-Bromopent-1-yn-3-ol.-

Pent-1-yn-3-ol (2.00 g.) was dissolved in a few drops of 2N sodium hydroxide and sodium hypobromite solution (14 c. c. of a solution from ice (20 g.), 10N sodium hydroxide (10 c. c.), bromine (2.2 c. c.) and water (4 c. c.) was added over 5 min. with stirring. Stirring was continued for a further 30 min., when the two layers were separated, and the upper aqueous layer extracted with ether. The ethereal extracts were combined with the lower layer and washed once with water and dried. Evaporation of the solution yielded the bromo-alcohol, (2.64 g., 68%) as a pale yellow oil $\nu_{\max.}$: 3610 (O-H), 2208 cm^{-1} ($\text{C}\equiv\text{C}$).

1,2-O-Isopropylidenenona-3,5-diyne-1,2,7-triol.-

To a stirred solution of cuprous chloride (2 mg.) in aqueous ethylamine (33%, 0.5 c. c.) was added 1,2-O-isopropylidene-but-3-yne-1,2-diol

(104 mg.) in aqueous ethylamine (1.0 c.c.) under nitrogen at 15°. Hydroxylamine hydrochloride was added in sufficient quantity to maintain the copper in the reduced form. 1-Bromopent-1-yn-3-ol (170 mg.) in methanol (0.7 c.c.) was added dropwise over 10 min. hydroxylamine hydrochloride being added periodically as required. The solution was stirred for 15 min., when excess potassium cyanide was added, and the mixture poured into water (25 c.c.). The product was extracted with ether (3 x 25 c.c.) and dried. After evaporation of the ethereal solution, the residue was extracted with light petroleum (b.p. 40-60°)/5% benzene and adsorbed onto alumina (W) (5 g.). The required isopropylidene derivative was eluted in light petroleum (b.p. 49-60°)/50% benzene as a colourless oil, (55 mg.). $\lambda_{\text{max.}}$: (ϵ in parentheses) 2575 (340), 2440 (580), 2315 (600), 2195 Å (520). $\nu_{\text{max.}}$: 3565 (O-H), 1377 and 1369 ($\text{C}(\text{CH}_3)_2$), 1206, 1148, and 1063 cm^{-1} (C-O-C).

Nona-3,5-diyne-1,2,7-triol. -

The isopropylidene derivative (prepared as above; 20 mg.) was treated with 2N hydrochloric acid (8.c.c.) at 15° for 1 hr. The required triol (12 mg.) was isolated via ether, and crystallised from ether/hexane (through a seed from the natural triol (XLI)) to form colourless needles, m.p. 18-26°. $\lambda_{\text{max.}}$: (ϵ in parentheses) 2560 (240), 2420 (390), 2285 (460), 2190 Å (460). $\nu_{\text{max.}}$: 3210 (O-H), 1070-1030 (C-O), 960 cm^{-1} (all broad envelopes of absorption). (Found: C, 63.4; H, 7.3 $\text{C}_9\text{H}_{12}\text{O}_3$ requires: C, 64.25; H, 7.2%)

- APPENDIX -

The Synthesis

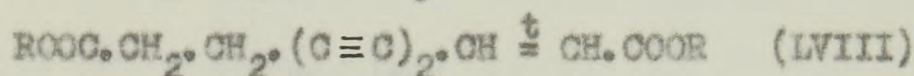
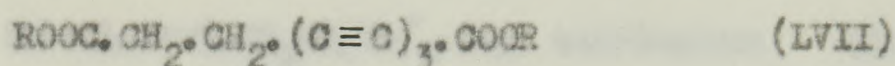
of

Deca-2,4,6-triyne-1,10-dioic Acid

Appendix.

Synthesis of Deca-2,4,6-triyn-1,10-dioic acid.

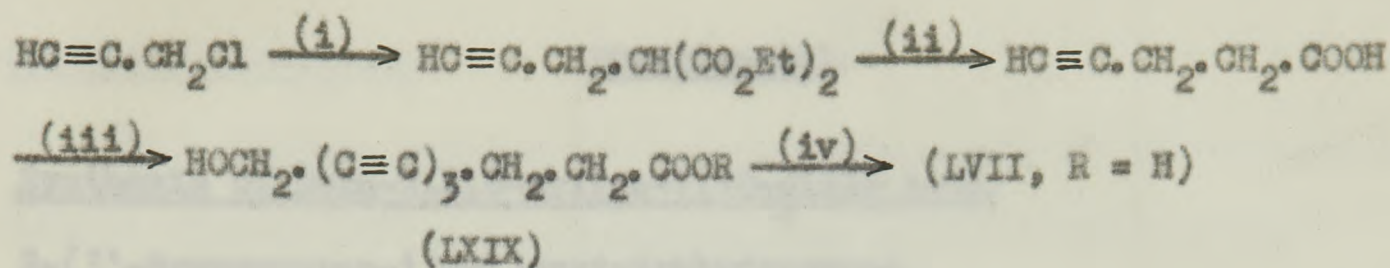
Certain strains of the "dry rot" fungus Merulius Lachrymans were found by Gardner⁽⁵⁹⁾ to produce the acids (LVII and LVIII, R = H).



The acid (LVII, R = H) was esterified and identified as a dimethyl ester, and a sample of the parent acid was obtained from hydrolysis of the ester. Gardner proposed three routes for the synthesis of this metabolite (Figs. 61-63), but abandoned the first one after repeated unsuccessful attempts to carry out the final oxidation.

Reactions of chromic acid under various conditions with either the hydroxy-acid (LXIX, R = H) or the hydroxy-ester (LXIX, R = Me) resulted in total destruction of starting material. Work on the route shown in Fig. 62 was then started and reported up to the formation and characterisation of the tetrahydropyranyl ether (LX). Later unpublished work revealed that the route was practicable and a small quantity of the diester (LVII, R = Me) was obtained by chromatography after treatment of the crude products of the coupling reaction (vi) with diazomethane.

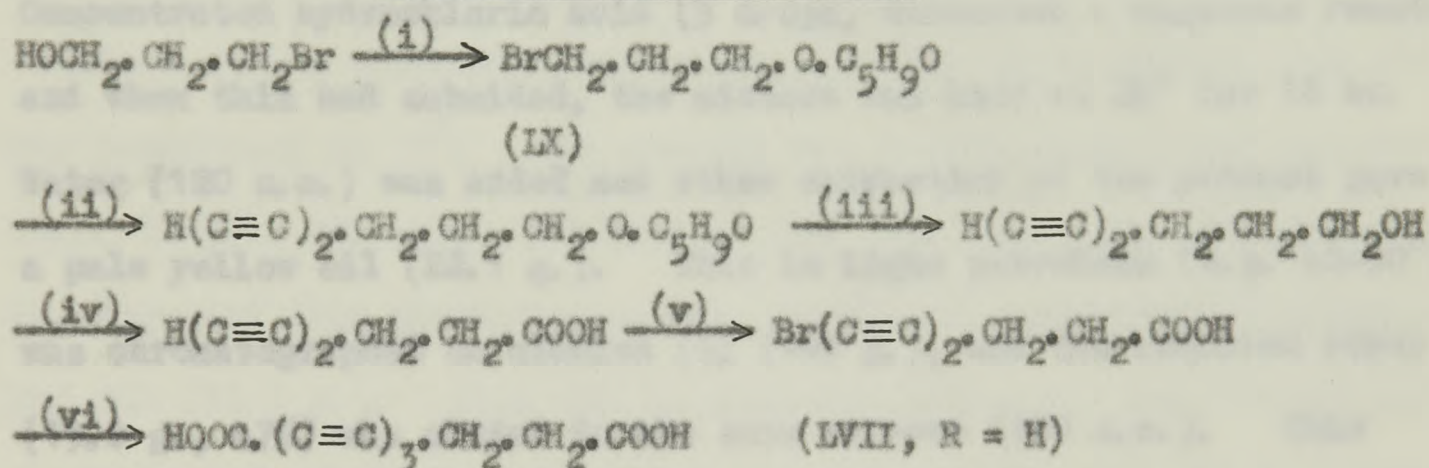
In the present work, this route was repeated to give a specimen of the diacid (LVII, R = H) having characteristics comparable with those of the natural product.



(i) $\text{NaCH}(\text{CO}_2\text{Et})_2$ (ii) Alkaline hydrolysis; heat, $-\text{CO}_2$

(iii) NaOBr ; $\text{HC}\equiv\text{C}\cdot\text{C}\equiv\text{C}\cdot\text{CH}_2\text{OH}/\text{Cu}^+$, aq. ethylamine (iv) CrO_3

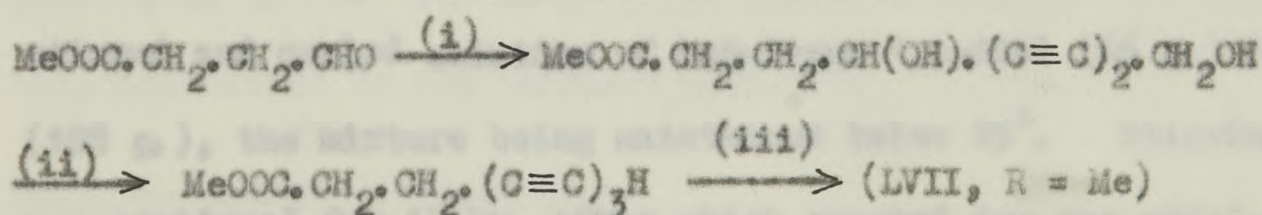
Fig. 61



(i) H^+ , 2,3-dihydropyran (ii) $\text{NaC}\equiv\text{C}\cdot\text{C}\equiv\text{CH}$ (iii) H^+

(iv) CrO_3 (v) NaOBr (vi) $\text{HC}\equiv\text{C}\cdot\text{COOH}/\text{Cu}^+$, aq. ethylamine

Fig. 62



(i) $\text{BrMg}\cdot(\text{C}\equiv\text{C})_2\cdot\text{CH}_2\text{OMgBr}$ (ii) $\text{SOCl}_2/\text{pyridine}$; base

(iii) $\text{EtMgBr}/\text{CO}_2$; $\text{H}_2\text{SO}_4/\text{MeOH}$

Fig. 63

Experimental.

Synthesis of Deca-2,4,6-triyn-1,10-dioic Acid.

2-(3'-Bromopropan-1'-yl)oxytetrahydropyran.

3-Bromopropan-1-ol (23.5 g.) (freed from acidic impurities by washing an ethereal solution with sodium hydrogen carbonate solution) was added to 2,3-dihydropyran (17.0 g.) and the mixture cooled to 0°. Concentrated hydrochloric acid (5 drops) initiated a vigorous reaction and when this had subsided, the mixture was left at 20° for 16 hr. Water (120 c.c.) was added and ether extraction of the product gave a pale yellow oil (22.1 g.). This in light petroleum (b.p. 40-60°) was chromatographed on alumina (S) (100 g.), and the required ether (19.0 g., 47%) was eluted in the same solvent (200 c.c.). This product was sufficiently pure for reaction with monosodium diacetylide.

1,4-Dichlorobut-2-yne.

Thionyl chloride (300 g.) was added dropwise over 4 hr. to a stirred and cooled solution of but-2-yne-1,4-diol (66 g.) in pyridine (108 g.), the mixture being maintained below 25°. Stirring at 20° was continued for 17 hr. after which crushed ice was added, and the mixture extracted with ether. The ethereal extracts were washed with water, 2N sodium hydroxide and sodium hydrogen carbonate solutions and again with water, each of these solutions extracted with ether, and the combined ethereal extracts dried. The ether was then removed up a Fenske column, and the residual red oil (72 g.) fractionated to give a colourless oil (56 g., 56%), b.p. 56.8-57.0°/13mm. n_D^{17} 1.5066. (Lit. (60) b.p. 78°/24mm., n_D^{20} 1.5072).

Hepta-4,6-diyn-1-ol.

To a stirred solution of the monosodium salt of diacetylene in liquid ammonia (250 c.c.), prepared from sodium (13.2 g.) and 1,4-dichlorobut-2-yne (22.4 g.), was added 2-(3'-bromopropan-1'-yl)-oxytetrahydropyran (18 g.) over 5 min. under nitrogen. Stirring was continued for 14 hr. when ammonium chloride and ether were added, and the ammonia allowed to evaporate. The ether layer was decanted, the residual solids extracted with ether, and the total extract (700 c.c.) was evaporated at 15°. The residue was treated with light petroleum (b.p. 30-40°, 200 c.c.) and kept at -10° for 2 hr. when the petroleum was decanted from precipitated oil, dried, and evaporated to yield a red oil (12.2 g.). This oil in methanol (120 c.c.) was treated with concentrated sulphuric acid (15 c.c.) and kept at -10° for 20 hr. when the mixture was poured into water. The product was isolated by extraction with ether, the extracts dried, and the volatile material removed at 20°/0.1 mm. to give the required alcohol as a residual red oil, identified by its infrared spectrum.

Hepta-4,6-diyne-1-oic acid.

To a stirred solution of the above alcohol (3.30 g.) in acetone (210 c.c.) was added 8N chromic acid (17 c.c.) over 30 min., the temperature being maintained at <10°. The mixture was then stirred for a further 3 hr. at 15°, when the acetone layer was decanted off and concentrated to 100 c.c. The solids were extracted with ether, and these extracts combined with the acetone solution and extracted with sodium hydrogen carbonate solution. Acidification of the

alkaline solution, ether extraction and evaporation of the extract at 20° gave pale yellow needles (2.75 g.). This product was sufficiently pure for bromination. A sample was recrystallised from hexane to give colourless needles which rapidly turned orange-pink, m.p. $85-89^{\circ}$. $\nu_{\text{max.}}$: 3290 ($\equiv\text{C-H}$), 1755 (weak) and 1715 (strong) cm^{-1} (C=O).

7-Bromohepta-4,-6-diyne-1-oic acid.

A stirred solution of the above acid (0.84 g.) was cooled to 0° , and sodium hypobromite [7 c.c. of a solution from ice (100 g.), sodium hydroxide (50 c.c., 10N), bromine (11 c.c.) and water (20 c.c.)] added over 5 min. Stirring was continued for a further 25 min. when the mixture was cautiously acidified with concentrated hydrochloric acid. Isolation of the crude product by ether extraction and solvent removal at 20° afforded yellow needles (1.30 g.) which rapidly turned pink. A portion was recrystallised from hexane to give colourless plates (0.19 g.), m.p. 108° (decomp.), which rapidly turned pink, identified by its infrared spectrum. $\nu_{\text{max.}}$: (carbon disulphide) 1760 and 1718 cm^{-1} (C=O).

Deca-2,4,6-triyne-1,10-dioic acid.

To a stirred solution of cuprous chloride (3 mg.) in aqueous ethylamine (33%, 1.0 c.c.) was added, under nitrogen, propiolic acid (70 mg.) in ethylamine solution (0.9 c.c.) together with sufficient hydroxylamine hydrochloride to keep the copper in the reduced form. The mixture was maintained at 10° and 7-bromohepta-4,6-diyne-1-oic acid (140 mg.) in methanol (0.5 c.c.) added dropwise over 10 min.

Hydroxylamine hydrochloride was added periodically with the

bromo-acid, and when addition of the latter was complete, stirring was continued for 15 min. Solid potassium cyanide was added to dissolve the copper salts and while still at 10° , the mixture was acidified with concentrated hydrochloric acid, poured into water, and extracted with ether. After evaporation of the ether at 20° the crude product was extracted with hexane containing 30% ether, and on cooling, this extract afforded the white amorphous diacid, decomp. 169° , which rapidly turned purple. (Found: C, 62.7; H, 3.85. Calc. for $C_6H_{10}O_4$: C, 63.15; H, 3.2%) $\lambda_{\max.}$: 3265, 3055, 2870, 2705, 2570 μ . $\nu_{\max.}$: (in nujol) 1690 cm^{-1} (broad, C=O). Lit. (59) $\lambda_{\max.}$: 3270, 3060, 2870, 2715, 2570 μ $\nu_{\max.}$: (in nujol) 1690 cm^{-1} (unconjugated and conjugated carboxyl)

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