RESEARCHES IN POLYACETYLENES

A Thesis submitted to the

Board of the Faculty of Physical Sciences

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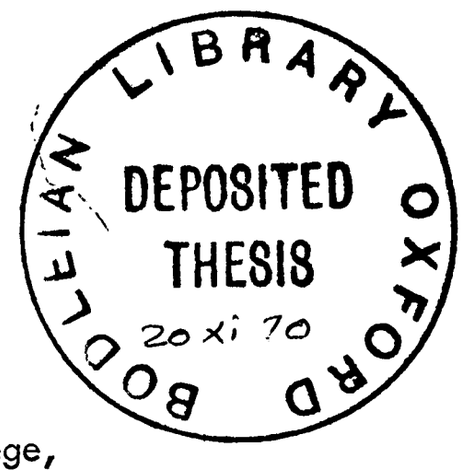
University of Oxford

in part fulfilment of the requirements for the

Degree of Doctor of Philosophy

by

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August, 1970

ABSTRACT

More than five hundred polyacetylenes have now been isolated from plants and fungi. Bu'Lock has proposed[1] that polyynes originate *in vivo* from oleate (1) via crepenynate (2). Those steps of polyacetylene biogenesis that have

$$Me.[CH_2]_7.CH{=}CH.[CH_2]_7.CO_2Me \qquad (1)$$

$$Me.[CH_2]_4.C{\equiv}C.CH_2.CH{=}CH.[CH_2]_7.CO_2Me \qquad (2)$$

been established through study of the incorporation of labelled compounds into natural systems are summarised in Part I of this thesis. This section also includes a review of natural acetylenes which do not obviously evolve from oleate (1).

Polyyne aldehydes (3) have often been utilised in syntheses of polyacetylenes,

$$R.[C{\equiv}C]_2.CHO \qquad (3)$$

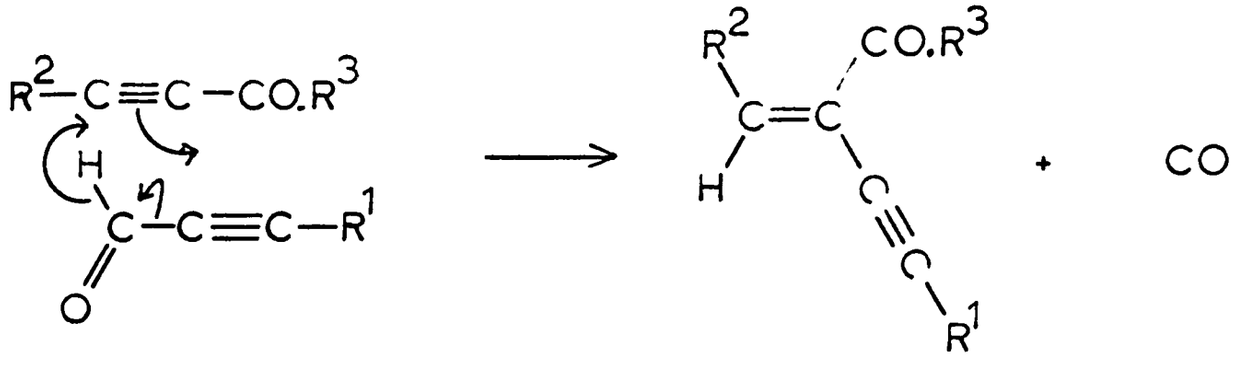
even though they are unstable in the condensed phase. Hexa-2,4-diynal (3; R = Me) was shown not only to polymerise but also to be converted into a C_{11} aldehyde of structure (4) (Part II). 3-Phenylprop-2-ynal 'dimerised' similarly on

$$Me.[C{\equiv}C]_2.\underset{\underset{HC.C{\equiv}C.Me}{\|}}{C}.CHO \qquad (4)$$

heating at 130° into both the stereoisomers of aldehyde (5). A scheme for this

Ph.C≡C.C(CHO)=CH.Ph (5)

reaction of α,β-acetylenic aldehydes is proposed (Scheme A; R^3 = H) which is



Scheme A

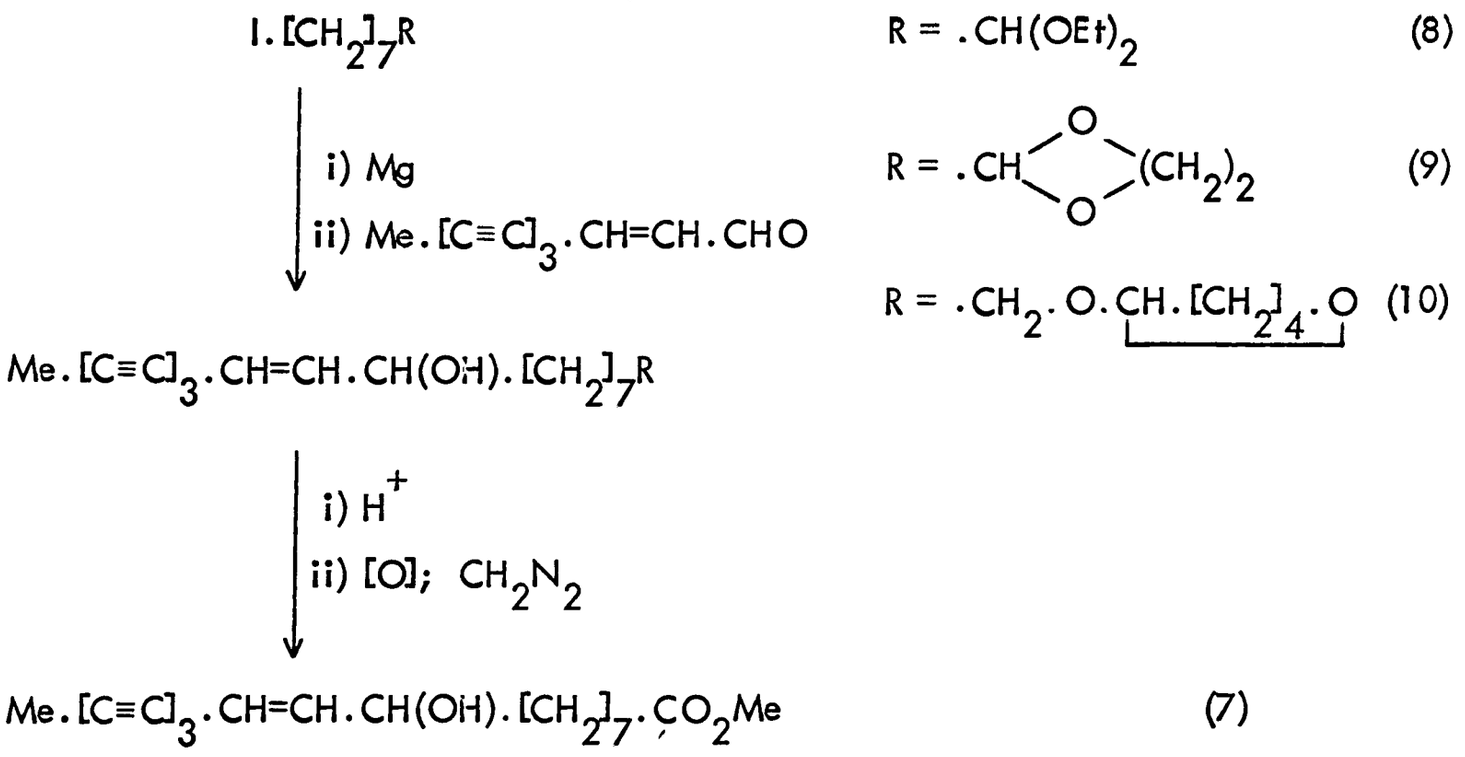
consistent with other evidence, e.g. use of labelled compounds and the small yield (3%) of cross-dimer (6; R^1 = Ph.C≡C; R^2 = Me.C≡C; R^3 = Me) obtained from a mixture of 5-phenylpenta-2,4-diynal and hepta-3,5-diyn-2-one.

The ester (7) is a likely precursor of some short-chain polyacetylenes in

$$Me.[C{\equiv}C]_3.CH{=}CH.CH(OH).[CH_2]_7.CO_2Me \qquad (7)$$

line with Bu'Lock's hypothesis,[1] and was to be prepared with radioactive label in the proximal half. Radioactive compounds which were to be isolated after administering this ester to a suitable fungus might throw light on the mode of chain shortening. Various routes suitable for the synthesis of a tritium-labelled proximal half are investigated in Part III of this thesis, for the C_{18} chain was to be built up by condensation of dehydromatricarianal and the Grignard reagent

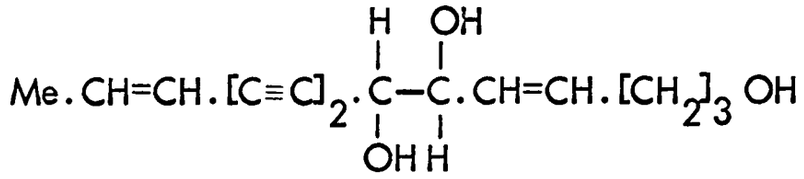
of an ω-iodoacetal (Scheme B). However, neither of the acetals (8) and (9) nor



Scheme B

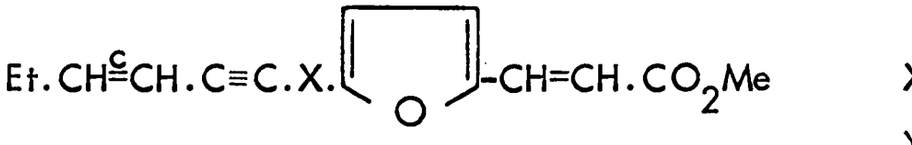
even the ether (10) would form a useful Grignard reagent. Possible reasons for this phenomenon, which prevented the synthesis of the ester (7), are discussed.

A systematic search for polyacetylenes in previously untested plant families is in progress in this department. As part of this, examinations of two species of the Lobeliaceae, Lobelia syphilitica L. and Lobelia cardinalis L., and their hybrid Lobelia x vedariensis were carried out (Part IV). The last two contained the new (6-R,7-R) triol (11), but apart from this, only traces of other polyacetylenes were detected.



(11)

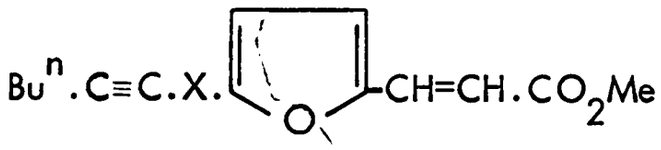
Wyerone (12) is a natural acetylene, with antifungal properties, that is



X = CO (12)

X = CH(OH) (13)

produced by the broad bean, Vicia faba L. It is of biosynthetic interest as present concepts of polyacetylene biogenesis (Bu'Lock's hypothesis) would not easily accommodate the presence of a cis-butenyl end group in such an acetylene. [1-^{14}C] Acetate and the doubly-labelled fatty acid esters, oleate (1) and crepenynate (2) [tritium at C(9) and C(10), carbon-14 at C(9), C(10) or C(1)] were incorporated into sprouting beans. The isolations of active wyerone (12) and the closely related metabolites (13-15) are described in part V. The incorporations



X = CO (14)

X = CH(OH) (15)

were positive (ca. 0.04%), and tritium isotope effects were observed. Possible pathways for wyerone biosynthesis are discussed.

1. J. D. Bu'Lock 'Comparative Phytochemistry', ed. T. Swain, Academic Press, New York, 1966, p.79; also J. D. Bu'Lock and G. N. Smith, J.Chem.Soc.(C), 1967. 332.

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I am also indebted to my colleagues in the Dyson Perrins Laboratory, many of whom are cited, somewhat humbly in the reference section. Those that are not include Dr. A. Fallis, Messrs. A. Cliffe and R. Bailey.

I am more than grateful to Misses M. Burt and P. Wilmot who typed this script.

This work was carried out under a tenure of a S.R.C. studentship.

R.A.M. Ross

Preface

In this thesis, schemes are used to their utmost in an attempt to present a clear picture. They are incorporated within the text, and in some cases appear on the page after that on which they are quoted.

Reactions envisaged but not carried out are portrayed in a scheme by a dotted line ------.

In certain cases, the terms cis and trans are included within a formula name. Because of a lack of artistic skill on the part of the author the figures are not strictly accurate. I apologise for these and any other discrepancies.

'Accuse not Nature, she hath done her part,

Do thou but thine.'

J. Milton, Paradise Lost, Bk.viii, l.561

PART I

GENERAL INTRODUCTION

The term "polyacetylenes" was originally coined for the group of natural products which contain one or more triple bonds in conjugation with an unsaturated system. The number reported has increased dramatically from 7 in 1950 to over 550 at the present time. Each carbon skeleton can be represented by a single unbranched chain which, though sometimes cyclised in various ways, evolved via oleate.

Good reviews on the synthesis and natural occurrence of polyacetylenes have appeared in the literature[1-5] and, in recent theses[6-11] from this department. In addition, two treatises[12,13] on acetylenic chemistry have been published within the last year. No attempt will therefore be made to cover well-documented grounds. Instead experimental data pertaining to polyacetylene biosynthesis will be surveyed. A second section reviews those natural acetylenes for which oleate is not the obvious precursor. Some of these non-polyacetylenic compounds may be valuable for the elucidation of the biosynthesis of the triple bond.

Polyacetylene biosynthesis

Polyacetylenes are distributed throughout the plant kingdom, mainly in the Compositae and Umbelliferae families. Lately the dihydromatricaria acid (I; R = H) was isolated[14] as the major component of the defensive secretion of

$$CH_3 \cdot CH^{c}{=}CH \cdot [C{\equiv}C]_2 \cdot CH_2 \cdot CH_2 \cdot CO_2R \qquad (1)$$

the beetle, Chauliognathus lecontei. The soldier beetle had, however, fed on Compositae species which contained the methyl ester (1; R = Me) and presumably metabolised this ester. The Basidiomycetes class of fungi are also prolific polyyne producers.

The more polar, fungal polyacetylenes tend to be shorter in chain length (centred on C_{10}) than the plant analogues (centred on C_{13}), though there are many structural similarities which inspired[15] hypotheses on their common biogeneses.

The schools of Bohlmann (Berlin) and Schulte (Münster) have tested these hypotheses using plants, whereas those of Bu'Lock (Manchester) and Jones (Oxford) have employed mainly fungi.

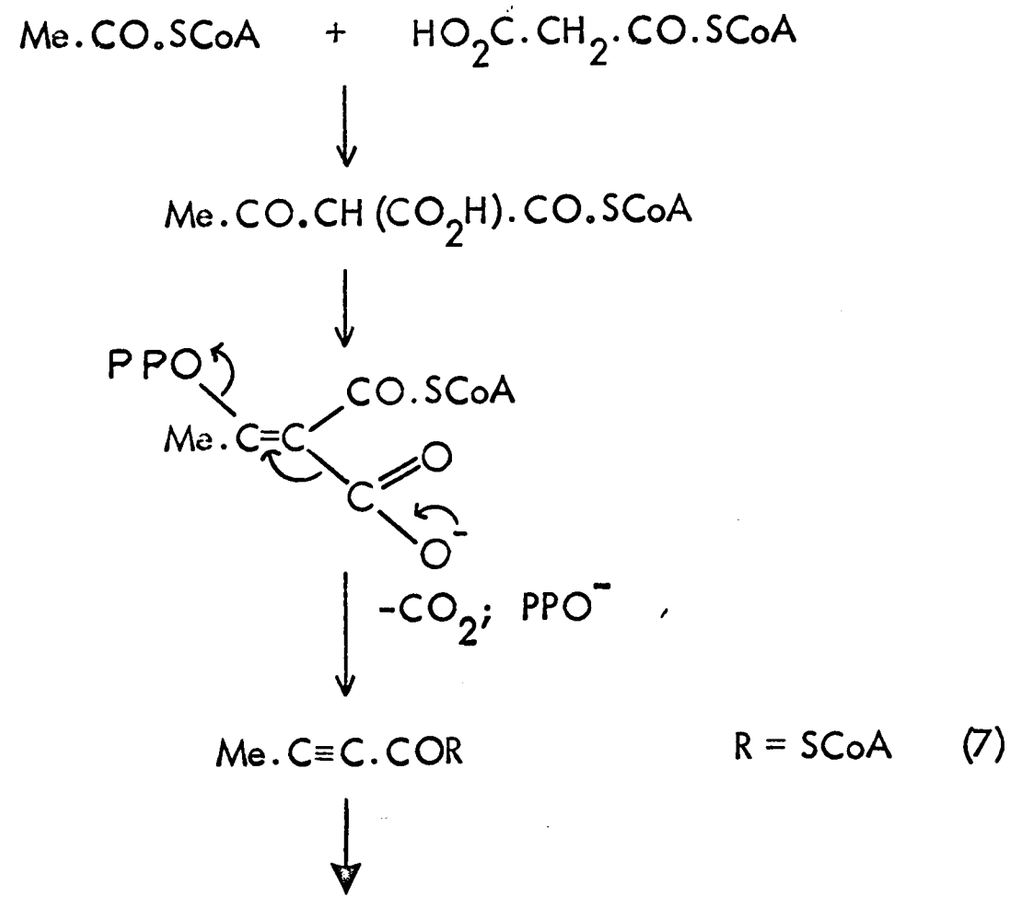
This review is broadly divided into three sections; the first deals with desaturation and chain shortening processes. The second is concerned with the secondary transformations required to convert one polyacetylene into another, and lastly the biosyntheses of phenyl and spiro-ketal enolether acetylenes are considered in the light of feeding experiments.

Acetate feedings and desaturation hypotheses

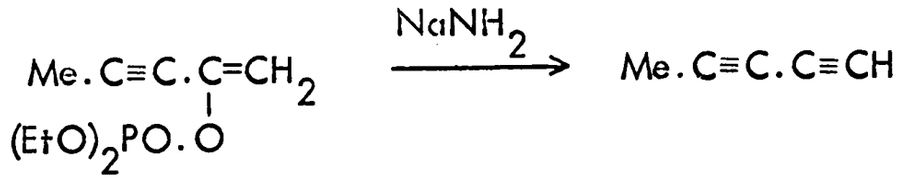
It was quickly established that sodium acetate was incorporated into both plant polyynes, e.g. (2)[16] (incorporation 0.002%), and fungal polyynes, e.g. (3)[17,18] (0.3%). These results typify the higher incorporation efficiencies

SCHEME A

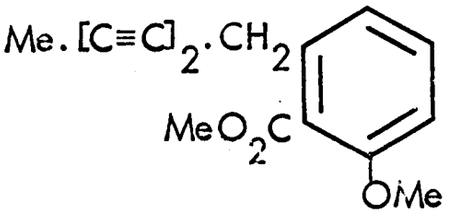
In vivo:[21]



In vitro:[22]



Triple bond formation via enol phosphates



(2)

$$Et.CH(OH).[C{\equiv}C]_2.CH(OH).CH_2OH \qquad (3)$$

obtained for fungal systems. Diethyl malonate has also been transformed into the polyynes (4)[17] (from Merulius lacrymans) and (5)[19] (from Coreopsis lanceolata L.).

$$HO_2C.[C{\equiv}C]_3.CH_2.CH_2.CO_2H \qquad (4)$$

$$Me.[C{\equiv}C]_3.Ph \qquad (5)$$

Degradation of the active metabolites revealed that each carbon skeleton was built up by the 'acetate + n-malonate' pathway as in fatty acid biosynthesis (the starter unit is drawn on the left of all formulae as in the fatty acid convention). [4-^{14}C] Acetoacetate acted[19] as the starter unit for phenyl triyne (5), but [1,4-^{14}C] succinate was degraded[20] by the Basidiomycete B 841 to acetate before it could be incorporated into nemotinic acid (6).

$$H[C{\equiv}C]_2.CH{=}C{=}CH.CH(OH).CH_2.CH_2.CO_2H \qquad (6)$$

In 1961, Jones[21] proposed that triple bond formation resulted from a variant in fatty acid biosynthesis, which involved concerted elimination and decarboxylation of enol phosphates (Scheme A). Craig and Moyle have demonstrated

that activated enol phosphates are smoothly transformed into acetylenes on treatment with alkali.[22] However only inactive polyacetylenes have been isolated[19,23] after feeding natural systems with labelled tetrolic acid (7; R = OH). This mechanism still appears attractive for the generation of single triple bonds present in some 'non-polyacetylenic' natural acetylenes.

Bu'Lock[4] suggested the enzymic dehydrogenation of a long chain fatty acid as an alternative process for triple bond formation, and pointed to the fatty acids found in the Olacaceae and Santalaceae[4,24] as examples of stepwise desaturation in the distal half of a fatty acid (Table 1).

Table 1

$Me.[CH_2]_5.CH{=}CH.C{\equiv}C.[CH_2]_7.CO_2H$

$Me.[CH_2]_3.[CH{=}CH]_2.C{\equiv}C.[CH_2]_7.CO_2H$

$Me.[CH_2]_3.CH{=}CH.[C{\equiv}C]_2.[CH_2]_7.CO_2H$

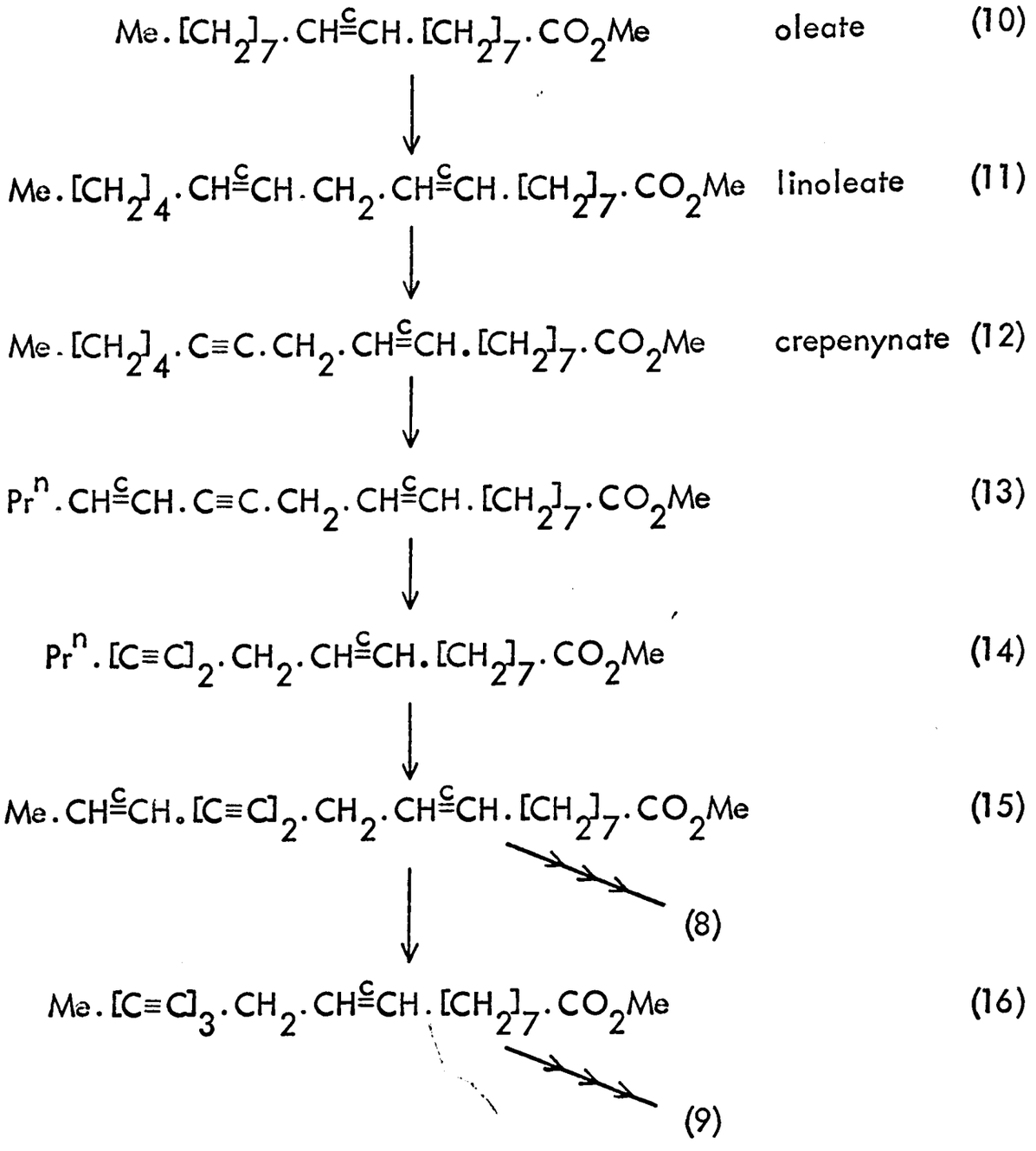
$Et.[CH{=}CH]_2.[C{\equiv}C]_2.[CH_2]_7.CO_2H$

$Et.CH{=}CH.[C{\equiv}C]_3.[CH_2]_7.CO_2H$

$H.[CH{=}CH]_2.[C{\equiv}C]_3.[CH_2]_7.CO_2H$

Sterolic acid derivatives found in Santalaceae and Olacaceae[4,24]

SCHEME B



Desaturation of oleate via crepenynate

The above process could be modified slightly (Scheme B) to account for the unsaturated system in polyacetylenes with the intriguing skipped enyne fragment (-C≡C.CH_2.CH=CH-), such as the plant polyyne (8)[25] and the fungal polyyne (9).[26]

$$Me.CH{=}CH.[C{\equiv}C]_2.CH_2.CH{=}CH.[CH_2]_6OH \qquad (8)$$

$$HO_2C.[C{\equiv}C]_3.CH_2.CH{=}CH.[CH_2]_3.CO_2H \qquad (9)$$

Methyl crepenynate (12), previously isolated[27] from the seed oil of Crepis foetida (Fam. Compositae) was envisaged as the key intermediate. Oleate (10) is desaturated progressively to either ester (15) or (16) after which chain shortening and secondary transformations engender the polyynes (8) or (9).

The isolated cis double bond might alternatively rearrange so as to conjugate with the polyyne system. Nearly every known polyacetylene can be then derived through this pathway, after invoking relatively few secondary transformations.

In vivo proof of Bu'Lock's hypothesis

Bu'Lock confirmed his hypothesis (Scheme B) by the feeding[28] of [10-^{14}C] oleate to Tricholoma grammopodium. The major metabolite, dehydromatricarianol (17), was labelled with all the activity at C(2).

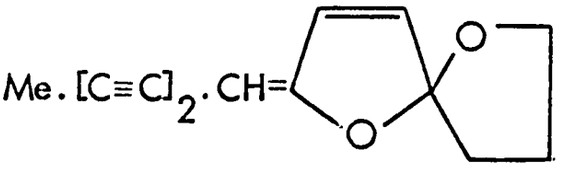
$$Me.[C{\equiv}C]_3.CH{=}CH.CH_2OH \qquad (17)$$

The oleate skeleton is easily obtained with labels of carbon-14 (in 1,9,10

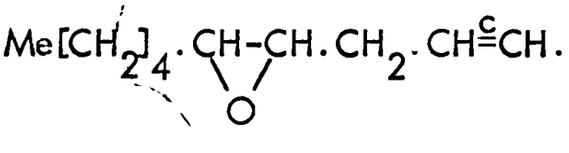
or 18 positions) or tritium (in 2,9,10 positions). The schools of Bohlmann and Jones have shown that oleate is transformed into many polyacetylenes which are labelled as predicted by scheme B, though feedings of doubly-labelled oleate[6,7,29] to fungi have resulted in unexpected $^{3}H:^{14}C$ ratios in the active polyynes.

The intermediacy of a double bond in the formation of a triple bond, e.g. of crepenynate, has still to be clarified. James[30] showed that oleate was converted into crepenynate by Crepis rubra L. seeds, and that linoleate was not an intermediate. Bohlmann,[32] on the other hand, has incorporated [9,10-^{3}H] linoleate into the polyynes (18) (Chrysanthemum flosculosum L.) and (5) with the same efficiency as [9,10-^{3}H] oleate. Both workers agreed that vernolate (19) was not involved. Linoleate (or any other intermediate) might be present in the

$$Me.[C\equiv C]_3.Ph \qquad (5)$$



(18)



(19)

seeds as a strongly bound enzyme-substrate complex, which would not exchange with the administered linoleate.

Methyl [10-^{3}H, 9-^{14}C] crepenynate has been converted[6,7,29,32,33] in vivo into fungal polyacetylenes. The incorporation efficiencies (ca. 1%) were

sometimes slightly less than comparison feedings with oleate, presumably as a result of transport differences between the esters to the enzyme active sites.

If the postulated biosynthetic pathway implies rearrangement of the crepenynate cis double bond in a secondary transformation, as for matricaria ester (20), then the feeding of acetate, oleate or crepenynate seems to be accompanied by tritium loss or an isotope effect (Table 2).

$$Me.CH{=}CH.[C{\equiv}C]_2.CH{=}CH.CO_2Me \qquad (20)$$

Table 2

Precursor	$^3H:^{14}C$	$^3H:^{14}C$ for (20)	
		Observed	Calculated
Sodium [2-^{3}H, 2-^{10}C] acetate	100	15.3	33.3
Methyl [9,10-^{3}H,10-^{14}C] oleate	520	231	260
Methyl [10-^{3}H,1,9-^{14}C] crepenynate	10.8	2.2	21.6

Tritium-carbon-14 ratios observed in feedings to *Polyporus anthracophilus*[6]

The European mugwort, Artemisia vulgaris L., transformed the diyne ester (14) into dehydromatricaria ester (22) in 0.13% yield.[34] Bohlmann[35] has

$$Pr^n.[C{\equiv}C]_2.CH_2.CH{\overset{c}{=}}CH.[CH_2]_7.CO_2Me \qquad (14)$$

$$Me.[C{\equiv}C]_3.CH{=}CH.CO_2Me \qquad (22)$$

incorporated the triyne ester (16) into the plant metabolites (23) (Chrysanthemum frutescens L.), (5) and (18), with efficiencies a hundred times greater than for oleate.

$$Me.[C\equiv C]_2.CO.Ph \qquad (23)$$

Feedings of esters (16) and (22) into Anthemis austriaca Jacq.[36] demonstrated that more than one pathway is open for polyacetylene biosynthesis. Dehydromatricaria ester (22) was converted into the mercaptans (24) and (25) more efficiently (0.6% and 2.2%) than the triyne ester (16) (0.1 and 0.5%). In the latter feeding the triyne ester was not incorporated into dehydromatricaria ester.

$$Me.[C\equiv C]_3.CH_2.CH\overset{c}{=}CH.[CH_2]_7.CO_2Me \qquad (16)$$

$$Me.[C\equiv C]_3.CH=CH.CO_2Me \qquad (22)$$

$$Me.C(SMe)=CH.C\equiv C.\underline{C=CH.CH\overset{c}{=}CH.CO_2}] \qquad (24)$$

$$Me.C(SMe)=C=C=CH.\underline{C=CH.CH\overset{c}{=}CH.CO_2}] \qquad (25)$$

Chain shortening

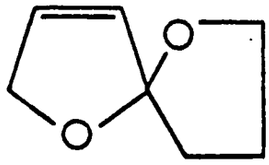
Bohlmann[35] has charted the effect of varying the proximal chain length (n) on the incorporation efficiencies of the skipped triynene (26) and monoene (27) ester homologues into the polyynes (5), (18), and (23). The results (Table 3)

$$Me.[C\equiv C]_3.CH_2.CH\overset{c}{=}CH.[CH_2]_n.CO_2Me \qquad (26,\ n = 2\text{-}7)$$

$$Me.[CH_2]_7.CH\overset{c}{=}CH.[CH_2]_n.CO_2Me \qquad (27,\ n = 2\text{-}7)$$

indicate that desaturation takes place before chain shortening or secondary transformations, and that the chain is reduced by alpha or beta oxidations in a set sequence determined by the total chain length (n + 11).

$$Me.[C\equiv C]_3.Ph \qquad (5)$$



(18)

$$Me.[C\equiv C]_2.CO.Ph \qquad (23)$$

Table 3

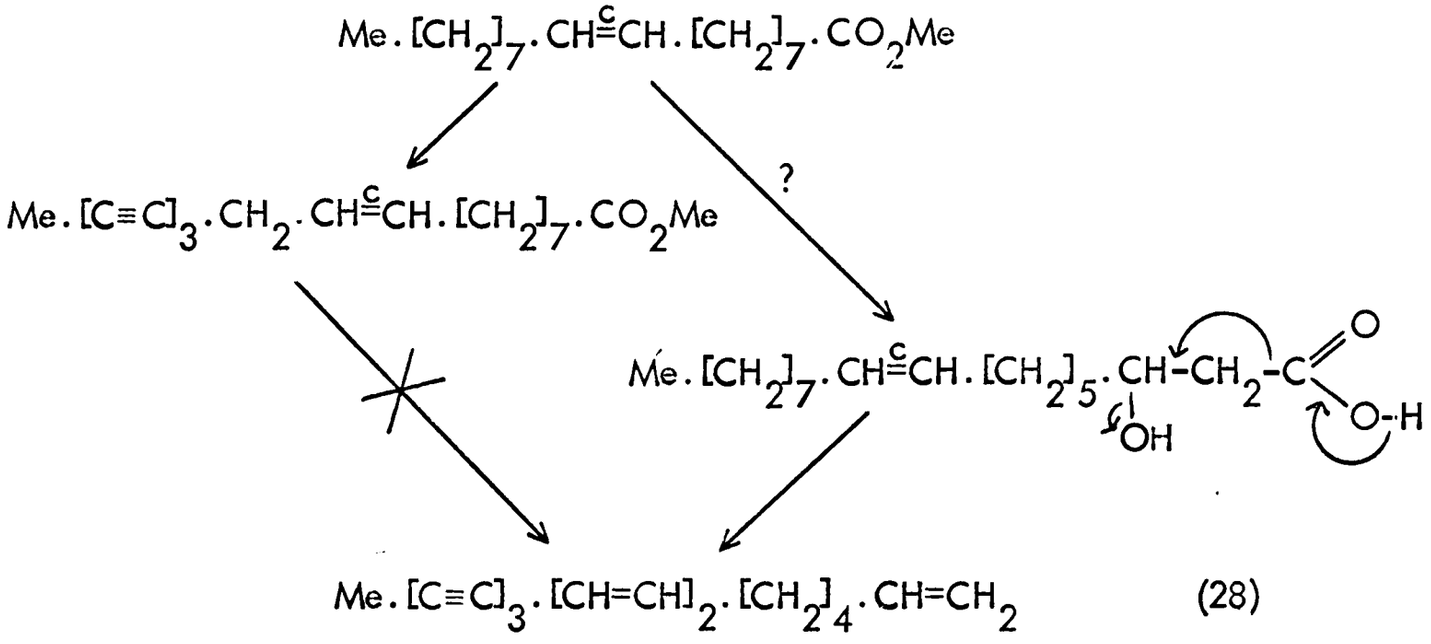
Polyyne	n values		
	(26)		(27)
(5)	3>5>7	>>	7>>3
(18)	4>6>7	>>	7>>3
(23)	2>4>6>7	>>	7>>3>2

n values not tabulated indicate negligible incorporations for that ester

Order of incorporation efficiencies of esters (26) and (27) into plant polyynes[35]

The triyne ester (16 ≡ 26; n = 7), unlike methyl 3-hydroxyoleate, was not converted by Artemisia vulgaris L.[37] into the C_{17} polyyne (28). Here the carboxyl group is removed by alpha oxidation before desaturation in the distal half occurs (Scheme C).

SCHEME C



Biosynthesis of Centaur X_3 (28)

Further experiments are required to establish the mechanism of beta oxidation, and whether an enzyme can sever more than two carbon atoms at any one time from the proximal half of the fatty acid ester (gamma and higher oxidations) (see page 56).

The triyne acids (29, n = 6, 8) were converted by Artemisia[38] and Chrysanthemum[34] species into dehydromatricaria ester (22) but not into metabolites

$$Me.[C\equiv C]_3.[CH_2]_n.CO_2H \qquad (29)$$

of longer chain length such as Centaur X_3 (28). This result, and the widespread abundance of the ester (22) throughout the Compositae, suggest that the C_{10} ester can be synthesised in vivo by routes other than Scheme B.

Secondary transformations

Secondary transformations alter the unsaturated esters, such as (26), into

$$Me.[C\equiv C]_3.CH_2.CH=CH.[CH_2]_n.CO_2Me \qquad (26)$$

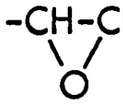
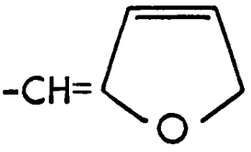
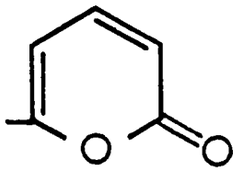
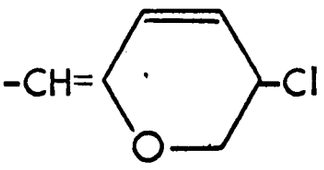
natural polyacetylenes. More often than not, these were well established laboratory reactions before they were repeated in vivo by feeding of radioactively labelled C_{10} to C_{14} polyynes to natural systems.

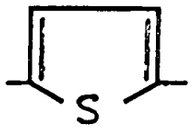
Early attempts to predict biogenetic polyacetylene interconversions from the weekly variations[9] in metabolite concentrations were ineffective since key intermediates were frequently present in undetectable amounts, as enzyme-bound substrate complexes.

Various secondary transformations, which seem unaffected by remote functional groups, are summarised below (Table 4). A maximum yield of incorporation is only noted if one transformation takes place. Otherwise a process is counted if all the other transformations occurring are already regarded as secondary processes and do not seem to interfere with the new modification.

Table 4

Secondary transformations in polyacetylene biosynthesis

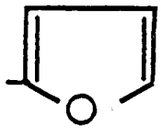
	Initial moiety	Transformed Product (with maximum incorporation)		Reference
Addition across a double bond				
1*	-CH=CH-		(0.2%)	34,42
2*	-CH=CH-	-CH(OH)-CH(OH)-	(0.8%)	43,44
3	$-CH=CH_2$	$-CHCl.CH_2OAc$		42,45,46,
4	$-CH=CH_2$	$-CH_2.CH_2OAc$	(0.14%)	46,47
5*	$-CH=CH_2$	$-CH(OH).CH_2OH$		46
Addition across a triple bond				
6*	$-C\equiv C.CH=CH.CH_2OH$			48,49
7*	$-C\equiv C.CH=CH.CO_2Me$			36
8	$-C\equiv C.CH=CH.CHCl.CH_2OAc$			48
9*a	-C≡C-	-CH=C(SMe)-	(0.9%)	36,46,50, 51,52,53

10	$-C\equiv CH$	$-CH=CHSMe$	(0.07%)	42
11[b]	$-C\equiv CMe$	$-CH=CH\ SMe$	(0.06%)	50
12*[c]	$-C\equiv C-C\equiv C-$		(0.05%)	38, 46, 54, 59

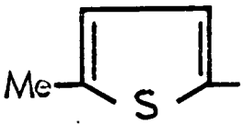
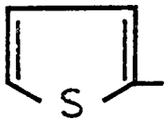
Hydrogenation

13*	$-C\equiv CH$	$-CH=CH_2$	(5.5%)	47
14*	$-C\equiv CMe$	$-CH=CH\ Me$		
		inoperable in plants		48
15*	$-CH=CH.CO-$	$-CH_2.CH_2.CO-$		55

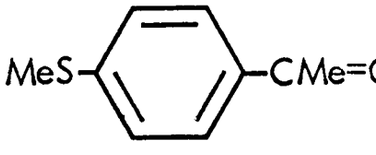
Dehydrogenation

16	$-CH=CH_2$	$-C\equiv CH$		47
17	$-CH=CH\ Me$	$-C\equiv CMe$	(inoperable)	43, 56
18	$-C\equiv C.CH_2.CH\overset{c}{=}CH.CH_2-$	$-C\equiv C.[CH=CH]_2-$	(0.3%)	48, 55, 56, 57
19	$R-C\equiv C.[CH=CH]_2-$	$-[C\equiv C]_2.CH=CH-$		
	$R = MeCH=CH.[C\equiv C]_2$	$-[C\equiv C]_3-$		48
20	$-CH(OH).CH_2.CH_2.CH_2OH$		$(1 \times 10^{-3}\%)$	34, 58

Terminal oxidation

21	$MeC{\equiv}C-$	$HOCH_2.C{\equiv}C-$		44,60,61
*	$HOCH_2.C{\equiv}C-$	$HO_2C.C{\equiv}C-$		
*	$HO_2C.C{\equiv}C-$	$HC{\equiv}C-$		
22				46,54
		(similar sequence to above)		
23	$MeCH{=}CH-$	$HOCH_2.CH{=}CH-$	(0.2%)	43,54,57
		(reverse inoperable)		

Rearrangements

24*	$-C{\equiv}C.CH_2-$	$-CH{=}C{=}CH-$		32
25*	$-C{\equiv}C.CH{=}CH-$	$-CH{=}C{=}C{=}CH-$		36
26	$-C{\equiv}C.CH_2.CH{=}CH-$	$-C{\equiv}C-CH{=}CH.CH(OH)-$	(0.4%)	57
27	$R.CH_2.CH\overset{c}{=}CH.[CH_2]_4OH$	$H[CH{=}CH]_2.[C{\equiv}C]_3.CH{=}CH.CH_2OAc$		
	$R = Me.[C{\equiv}C]_3$		(0.08%)	48
28	$R.CH{=}CH.CO_2Me$			51,53
	$R = Me.[C{\equiv}C]_3$		(0.03%)	

Notes
a) -SMe not derived from methionine[52]
b) c.f. transformation 21
c) $-C{\equiv}C-CH{=}CH_2$ dehydrogenates before H_2S addition[54]

Processes asterisked can be imitated in vitro

Several processes have been thought to be inoperable or, at least, to be very inefficient in vivo. These are tabulated if other active acetylenes were recovered in the same feeding, suggesting that the precursor was transported to an active site of the system.

Assumed transformations comprise alcohol oxidation,[39] ester reduction,

$$-CH_2.CH_2.CH_2.OH \rightleftharpoons -CH_2.CH_2.CHO \rightleftharpoons -CH_2.CH_2.CO_2Me$$

double-bond isomerisation,[40] hydrolysis and formation of esters. Significantly allylic oxidation of the skipped enyne system has not yet been confirmed in vivo,

$$-C\equiv C.CH_2.CH\overset{c}{=}CH- \longrightarrow -C\equiv C.CH(OH).CH\overset{c}{=}CH-$$

though much circumstantial evidence exists, especially in the falcarinone series. Thus Sylibum marianum Gaertn.[41] contains both the hydrocarbon (30) and alcohol (31).

$$Me.CH\overset{c}{=}CH.[C\equiv C]_2.CHX.CH\overset{c}{=}CH.[CH_2]_5.CH=CH_2$$

(30) X = H

(31) X = OH

Biogenesis of phenyl polyynes

Bohlmann has postulated two biosynthetic pathways for the phenyl plant polyacetylenes. One is suitable for fully conjugated analogues of phenyl triyne (5) and another for capillin (32) derivatives, in which the phenyl and acetylenic

$$Me.[C\equiv C]_3.Ph \qquad (5)$$

$$Me.[C\equiv C]_2.CO.Ph \qquad (23) \equiv (32)$$

linkages are separated by one methylene, or modified methylene, group. No plant has yet been discovered in which metabolites derived by both pathways have been found.[2]

Phenyltriyne type

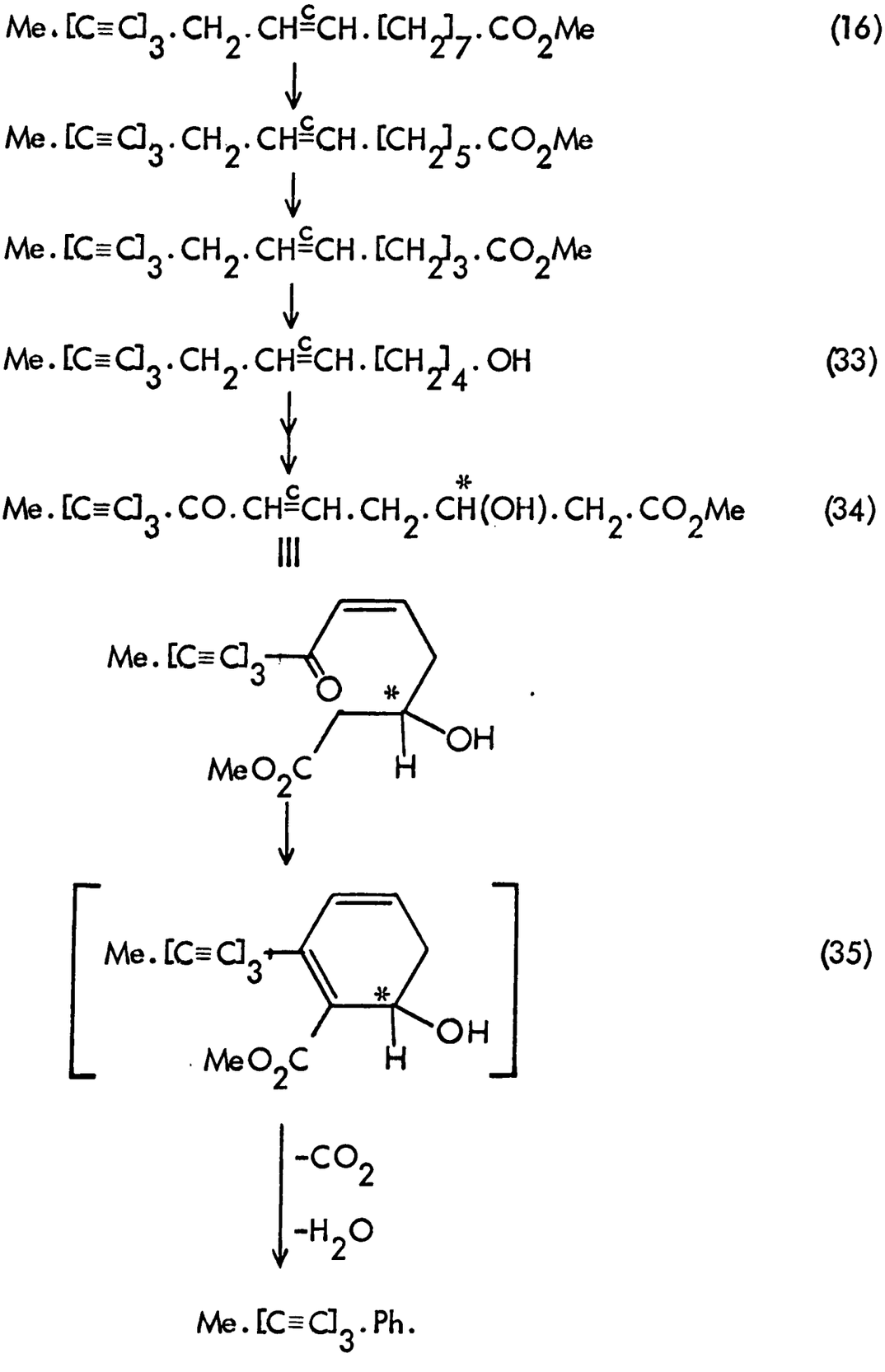
Scheme D outlines the proposed steps for the biogenesis of this type in tickseed (Coreopsis lanceolata L.).

The keto ester (34) which undergoes an intramolecular aldol condensation, has been isolated from the plant during one feeding,[61] but the cyclic product (35) has yet to be detected.

The presence of a secondary hydroxyl rather than a keto group at C(3) was inferred from tracer experiments with non-uniformly labelled [1,2,3,4-^{3}H, 14-^{14}C] alcohol (33) (^{3}H:^{14}C 2.27:1). Scheme D requires 28% tritium fraction in the ortho and para positions of the triyne (5) (observed 24%)[61] and a ^{3}H:^{14}C ratio for the metabolite 10.7:1 (observed 7.6:1). However Bohlmann did not consider the possibility of tritium isotope effects.

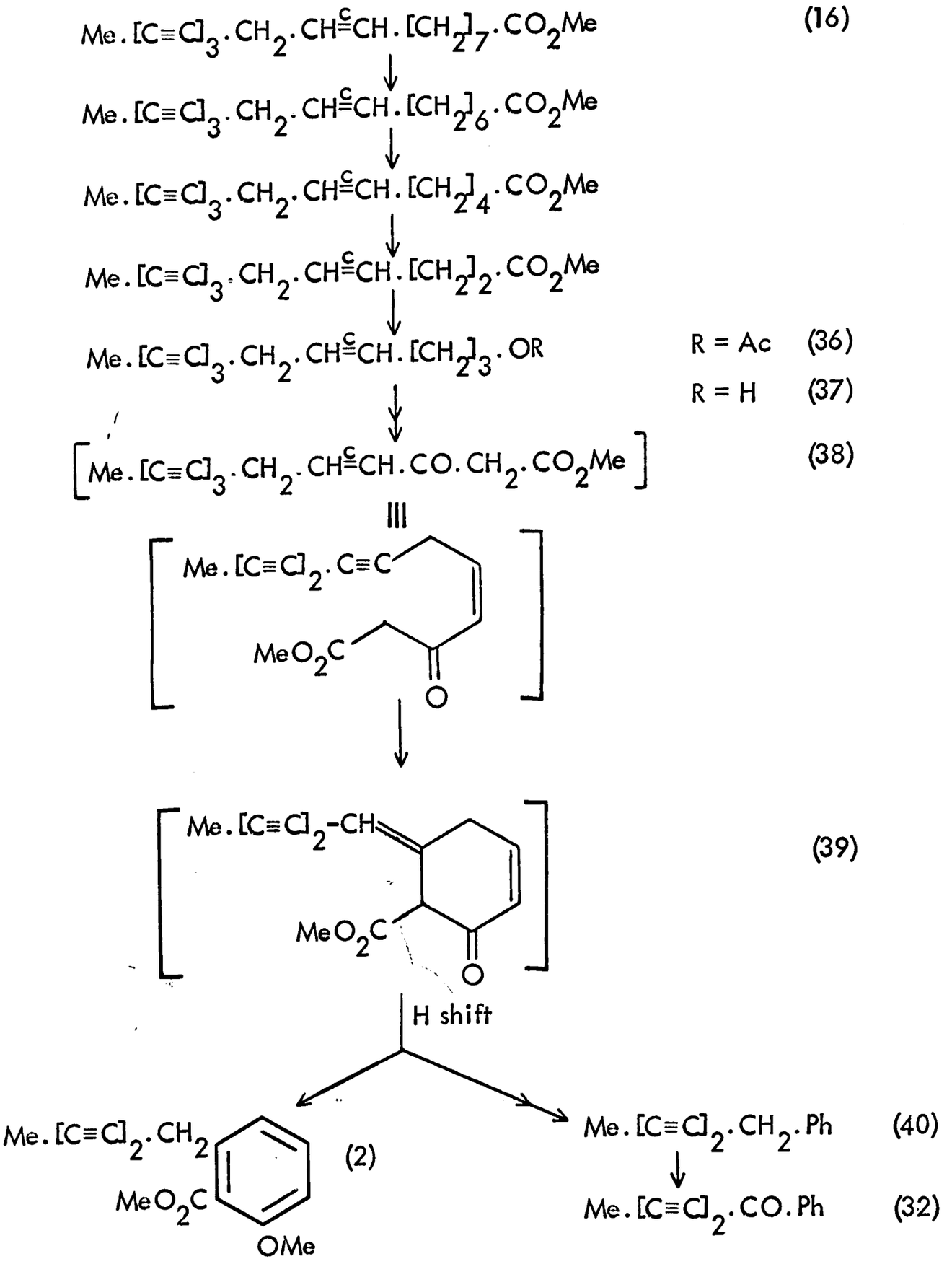
The projected intermediate (34) contains an asymmetric centre at C(3). A significantly larger incorporation into phenyl triyne of one optical isomer over the other would demonstrate enzyme specificity and confirm this pathway.

SCHEME D



Biogenesis of phenyl triyne[34,35,61]

SCHEME E



Biosynthesis of frutescin (2) and capillin (32)[34,35,58]

Capillin type

Many examples in this class, unlike those of the triyne type, display methoxy substitution meta to the polyyne chain, or methoxycarbonyl in the ortho position. One such derivative is frutescin (2) which coexists with capillen (40) and capillin (32) in some Chrysanthemum species.

In scheme E the C_{18} ester (16) is progressively shortened and oxidised to the keto C_{14} ester (38) (or keto alcohol) which then cyclises intramolecularly. A variant of scheme D is the retention of the keto function as precursor of the methoxy group.

Lonas annua L. converted[58] likewise the [4,5-^{3}H, 13-^{14}C] alcohol (37) (^{3}H:^{14}C 2.1:1) into capillin (32) which had a ^{3}H:^{14}C ratio 2.0:1.

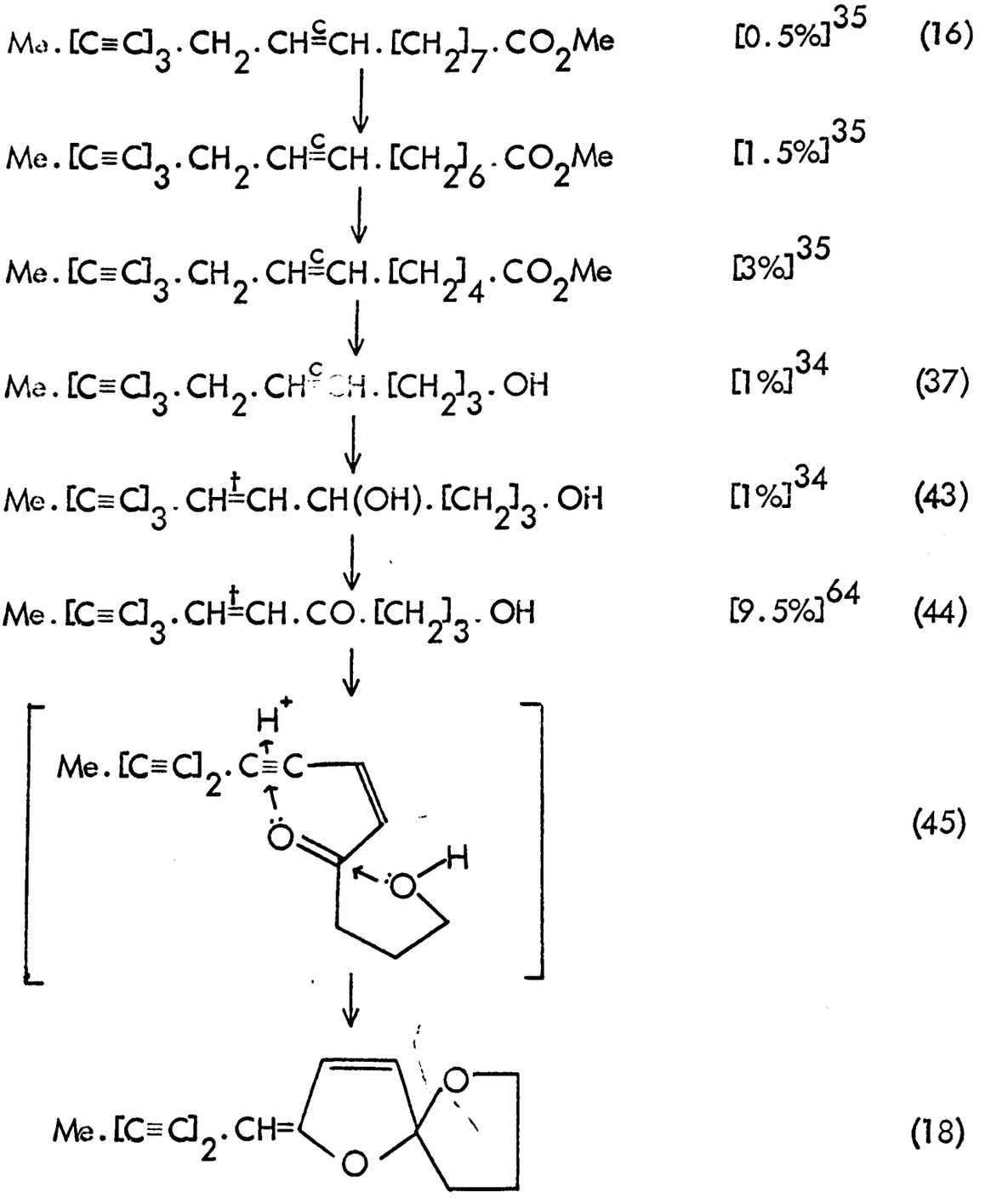
Capillen is easily oxidised by air to capillin,[62] and frutescin (2) itself has been synthesised[63] from the triyne keto-ester (41) by a pathway closely related to scheme E.

$$Me.[C{\equiv}C]_3.[CH_2]_3.CO.CH_2.CO_2Me \qquad (41)$$

Biosynthesis of spiroketal enolether acetylenes

Scheme F fits in with the feeding results with Chrysanthemum flosculosum L. for the polyyne (18). The corresponding (6, 5) spiroketals, e.g. (42) are also found in Chrysanthemum species, but their biosynthesis involves triyne esters (26) of different chain length than for the more common (5,5) spiroketals.[34,58]

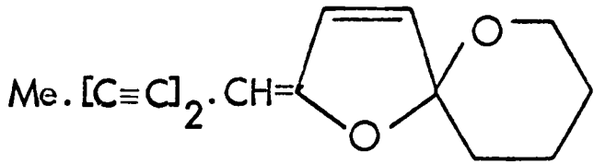
SCHEME F



Incorporations in parentheses

Biosynthesis of spiroketal enolether (18)

The combined isomerisation and cyclisation process (44→45→18) has been carried out in vitro.[64]

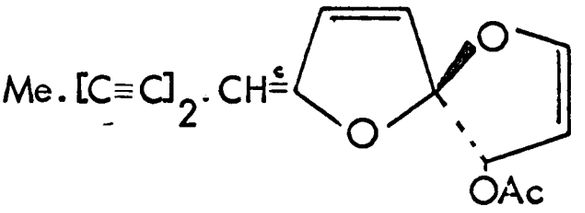


(42)

$$Me.[C\equiv C]_3.CH_2.CH^{c}{=}CH.[CH_2]_n.CO_2Me \qquad (26)$$

The aldehyde (46) has also been converted[58] into the polyyne (18) and the more unsaturated analogue (47), which is itself derived in vivo from the polyyne (18).[34]

$$Me.[C\equiv C]_3.CH_2.CH^{c'}{=}CH.CH_2.CH_2.CHO \qquad (46)$$



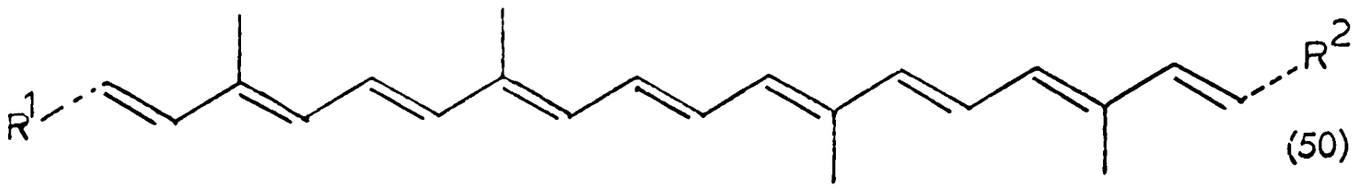
(47)[64a]

Future work on polyacetylene biosynthesis

The rationale behind the observed tritium isotope effects has still to be understood before the feeding results for doubly-labelled crepenynate are meaningful. However, the absence of tritium scrambling[7] in the fungal metabolites concerned is encouraging, and ultimately information about the rearrangement of the skipped enyne grouping $-C\equiv C.CH_2.CH^{c}{=}CH-$ might be forthcoming.

Bohlmann has shown[65] that at least two plant enzymes are needed to synthesise the spiroketal (18) but little progress has otherwise been made to determine the roles played by specific enzymes in polyacetylene biosynthesis. No feedings have been carried out with both optical isomers of a suspected precursor in order to test the stereospecificity of enzyme transformations.

Attempts to study the hydrogen abstraction mechanism in triple bond formation are being made in this department using the structurally simpler 'non-polyacetylenic' acetylenes as models.



(50)

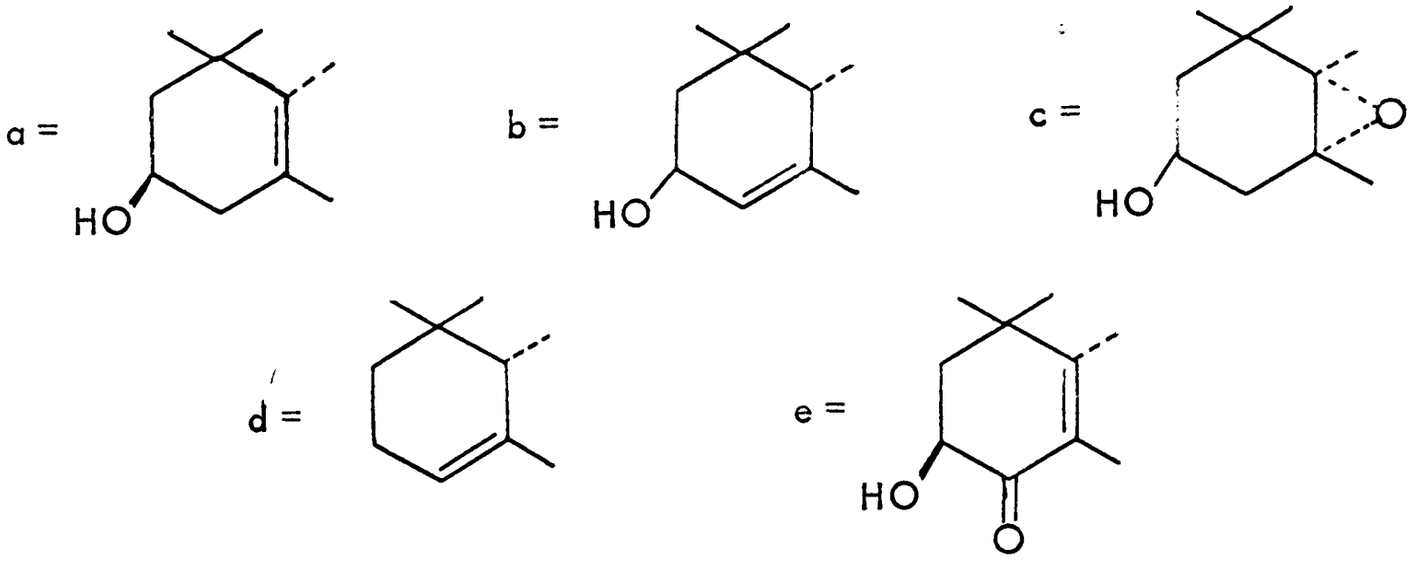


Table 5

Acetylenic Carotenoid	Structure			Ref	Polyene analogue (based on 50)
		R^1	R^2		
Alloxanthin	48	a	a	66,68,70	Zeaxanthin
Diatoxanthin	49	a	a	66,71	Zeaxanthin
Crocoxanthin	49	d	a	66	Zeinoxanthin
Monoadoxanthin	49	b	a	66	Lutein
Pectenolone	49	e	a	68	Adonixanthin
Diadinoxanthin	49	c	a	70,71	Antheraxanthin
Asterinic acid	48	e	e	69	Astaxanthin
	49	e	e		

Natural acetylenes of special biosynthetic interest

As the pattern of polyacetylene biosynthesis has been clarified over the last decade, it has become obvious that some natural acetylenes could not originate by the schemes outlined above. Infrared and n.m.r. spectra are often the only methods of detecting acetylenic moieties, so it is not surprising that the size of this group has expanded with the widespread use of these techniques.

These acetylenes are grouped conveniently into the following classes:

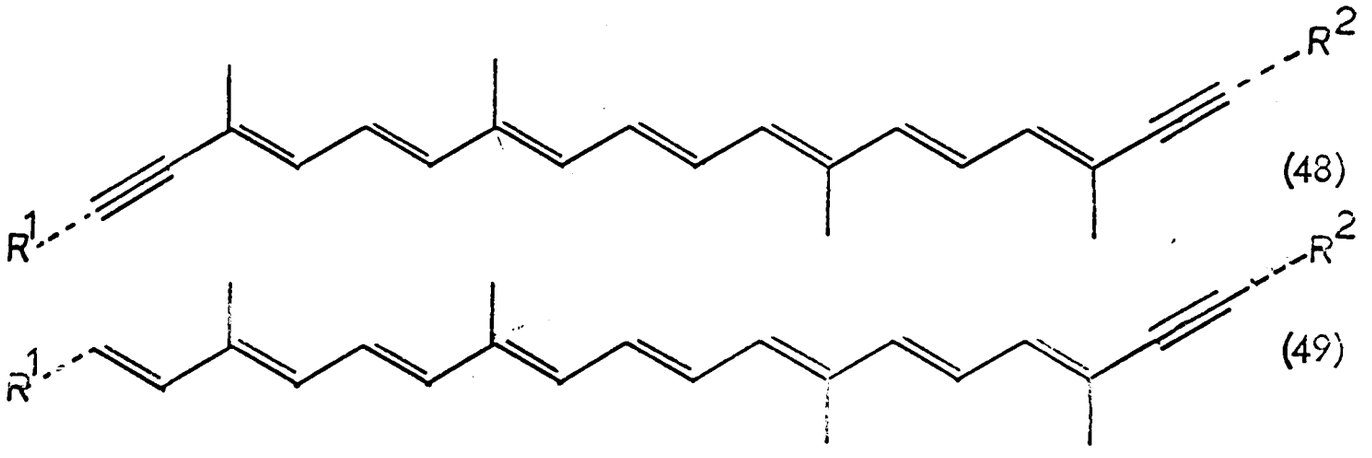
1) Carotenoids

2) Terpenoids

3) Miscellaneous acetylenes.

Acetylenic carotenoids

Alloxanthin (48, $R^1 = R^2 = a$) was the first acetylenic carotenoid to be fully characterised[66] in 1967. Since then algae and marine organisms have yielded seven carotenoids[67] containing acetylenic bonds, which always appear in the 7,8 position (Table 5).

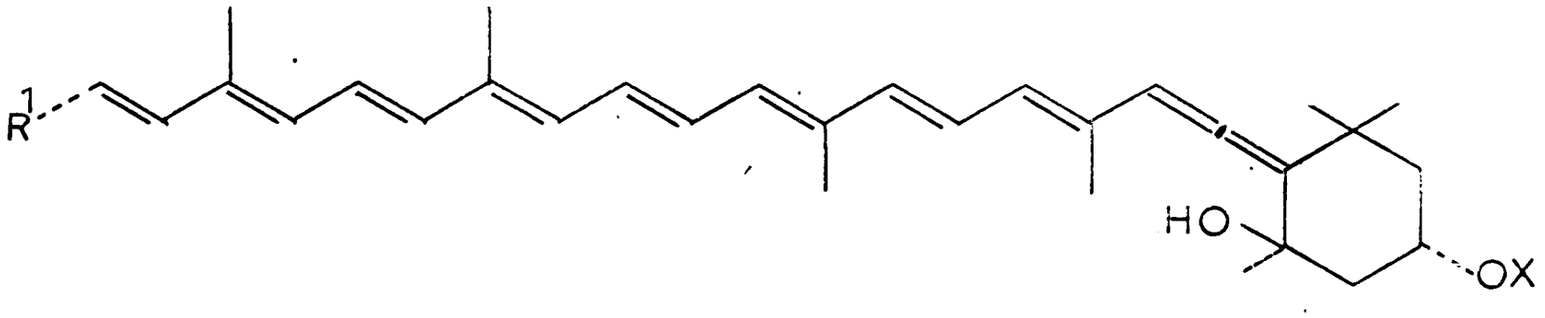


(48)

(49)

A literature search revealed that the all-trans polyene counterpart[67] for each acetylenic carotenoid has also been isolated from natural sources. This prompts the hypothesis that, say, alloxanthin (48; $R^1 = R^2 = a$) and diatoxanthin (49; $R^1 = R^2 = a$) are derived by dehydrogenation of zeaxanthin (50; $R^1 = R^2 = a$).

Modern spectroscopic techniques have shown that several well known xanthophylls,[67] like neoxanthin (51; R = c; X = H), contained an allene group in the 6,8 position.



(51) X = H or Ac

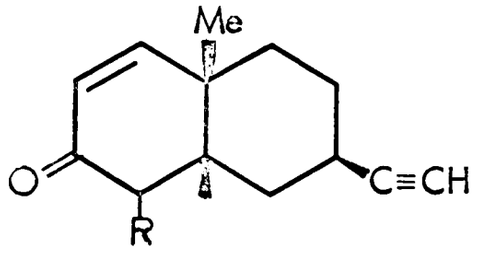
All these carotenoids have the dihydroxy or hydroxyacetoxy end group as in (51), and significantly no diallenic xanthophylls have yet been recorded. Allenic carotenoids are widely distributed in the higher plants and algae, and their discovery aroused interest in possible biological interconversion with acetylenic carotenoids.

Accordingly the allenic end group (51) has been transformed into an acetylenic end group[72] and vice versa.[73]

Other terpenoids

Several norsesquiterpenoids with an ethynyl substituent at C(7) were isolated (Table 6) by Nozoe,[74,75,76] from the Benihi tree Chamaecyparis

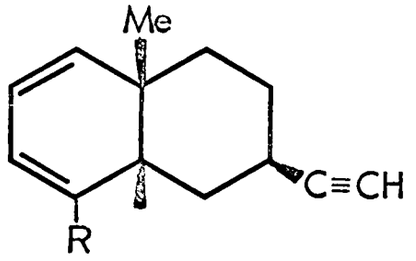
Table 6



R = α-Me Isochamaecynone[74]

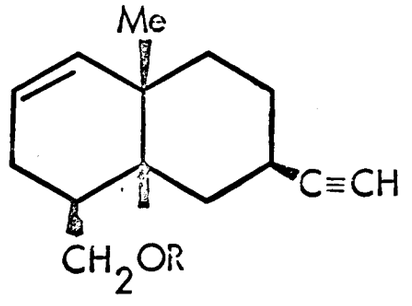
R = β-Me Chamaecynone[74]

R = α-OH Hydroxyisochamaecynone[74]



R = CH_2OH Dehydrochamaecynenol[75]

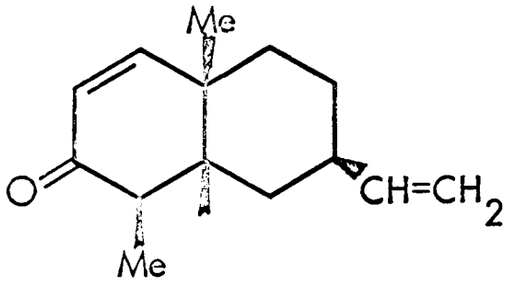
R = CHO Dehydrochamaecynenal[75]



R = H Chamaecynenol[76]

R = Ac Chamaecynenol acetate[76]

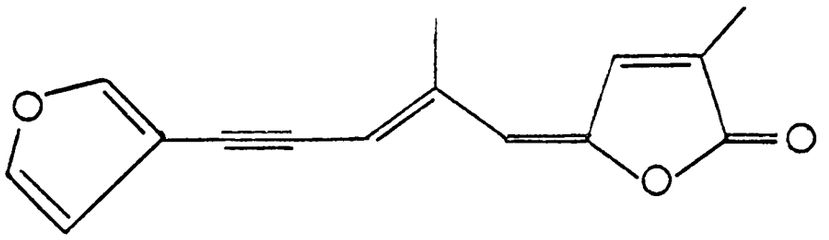
Ethynyl terpenes found in Benihi tree



(52)

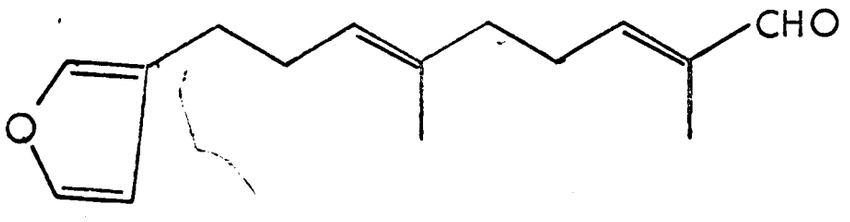
formosensis Matsum (Cupressaceae); the vinyl analogue (52) was also present. Hortmann[77] has speculated on the ring biosynthesis of these noreudesmanes.

Massy-Westropp[78] (1966) characterised freelingyne (53) amongst 'related' sesquiterpenes in the wood oil of the Angiosperm Eremophila freelingii (Fam.

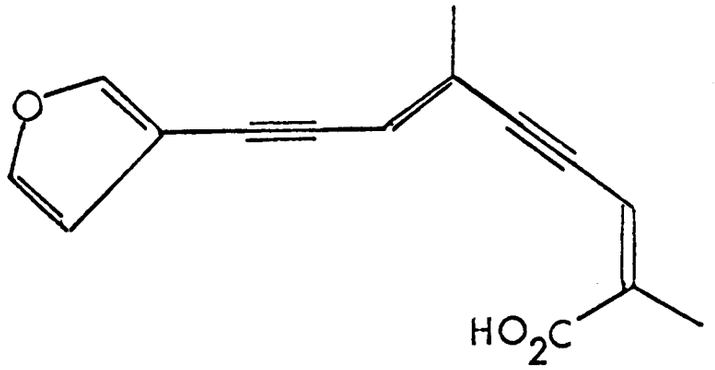


(53)

Myoporaceae). The skeleton was partially established on biosynthetic grounds, and clearly was thought to be derived from a modification of (say) torreyal[79] (54), by oxidation at C(1), C(4) and C(9) rather than derived from a diacetylenic intermediate (55); Torreyal has been isolated[79] from the wood oil of the Gymnosperm, Torreya nucifera (Fam. Taxaceae).



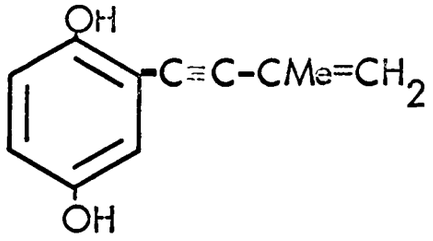
(54)



(55)

Miscellaneous acetylenes

Only two types in this miscellany have branched carbon skeletons. Siccayne (56) has been extracted[80] from the fungus imperfecti, Helminthosporium



(56)

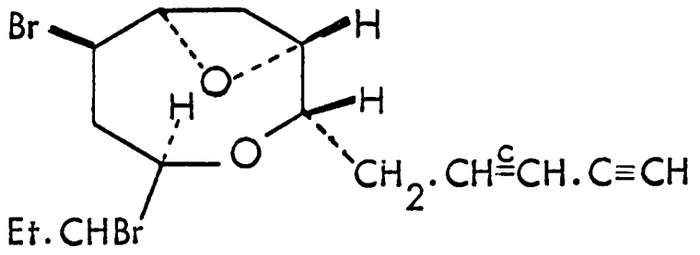
siccans g-207. The amino acids (57-59) have been discovered in the seed oil Euphoria longan[81] (Fam. Sapindaceae) and isoleucine was suggested as the precursor of the acetylene (57).

$HC{\equiv}C.CH(CH_2R).CH_2.CH(NH_2).CO_2H$ (57) R = H

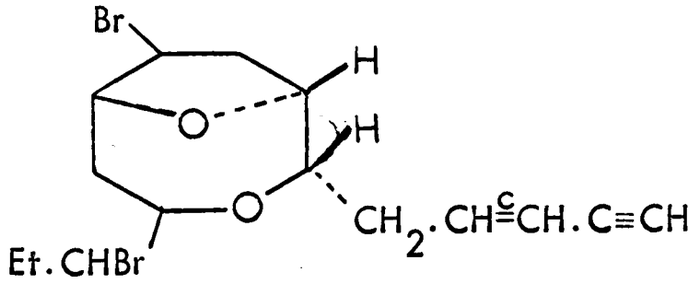
(58) R = OH

$HC{\equiv}C.CH(OH).CH_2.CH(NH_2).CO_2H$ (59)

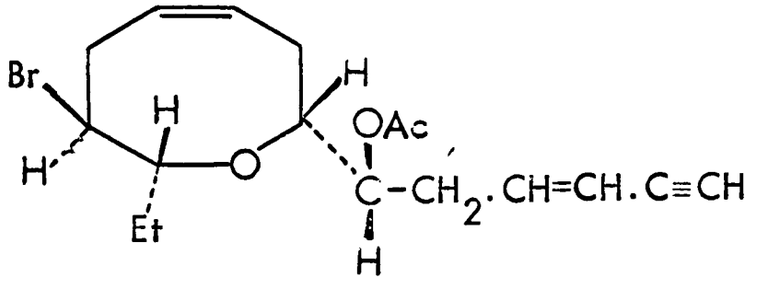
Acetylenic metabolites isolated from seaweed (Phylum Rhodophyta) include laureatin (60), isolaureatin (61) (Laurencia nipponica Yamada)[82,83] and laurencin (62) (Laurencia glandulifera Kützing).[84] Safe[85] has screened Laurencia pinnatitida for acetylenes in this department without success. These acetylenes all contain bromine and oxygen atoms attached to adjacent carbon atoms. An oxygen atom links different positions of a C_{15} chain for the trans enyne (62), and the cis enynes (60) and (61) to form the eight membered ring. Bu'Lock[86] has



(60)



(61)



(62)

suggested that hexadeca-4,7,10,13-tetraenoate, which has been detected in phylum Chlorophyta,[87] is epoxidised, then cyclises and undergoes transannular reactions to engender these structures.

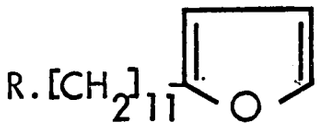
From the essential oil of the tree Litsea oderifera Valeton (Lauraceae), Matthews[88] obtained the methoxy-acetylene homologues (63; n = 7,9).

$$Me.CH(OMe).[CH_2]_n.C{\equiv}CH \qquad (63)$$

Recently Lifshitz[89] isolated eight related vinyl and ethynyl C_{17} compounds

(Table 7) from the seed of the avocado pear (variety Nabal or Effinger; Persea gratissima; Fam. Lauraceae).

Table 7



$R.[CH_2]_{11}.CO.CH_2.CH(OH).CH_2OAc$

$R.[CH_2]_{11}.CH(OH).CH_2.CH(OH).CH_2OAc$

$R.[CH_2]_{11}.CH(OH).CH_2.CH(OH).CH_2OH$

$R = -CH{=}CH_2$ or $-C{\equiv}CH$

Metabolites isolated from avocado pear[89]

Various micro organisms have been reported to produce monoacetylenes; however work in Oxford[90,91] has indicated that the yields depend very critically on culture conditions (Table 8).

Table 8

Microorganism (type)	Metabolite	Oxford trials	Ref
1. B. Coli (Bacteria)	$HC{\equiv}C.CO_2H$	-ve	92
2. Streptomyces griseus	$H_2N.CO.C{\equiv}C.CO.NH_2$	+ve	90, 93
(Actinomycetates)	$H_2N.CO.C(OEt){=}CH.CO.NH_2$		94
3. Rhodotorula glutinus	$H\ C{\equiv}C.[CH_2]_8.CO_2H$	-ve	95
var. lusitanica (Yeast)	$H_2C{=}CH.[CH_2]_8.CO_2H$		

Monoacetylenes and related compounds found in microorganisms

Several C_{18} acetylenic acids are known[96] which evidently originate from oleate via the stearolate (Table 1) or crepenynate (Scheme B) pathways. An exception is tairic acid (65), which has only been found in Genus Picramnia (Simaroubeaceae).

$$Me.[CH_2]_{10}.C{\equiv}C.[CH_2]_4.CO_2H \qquad (65)$$

Seed oils of the Sterculiaceae contain heptadec-8-ynoic acid (Sterculia foetida)[97] and sterculynic acid (66) (Sterculia alata Roxb.).[98]

$$HC{\equiv}C.[CH_2]_7.\underset{\diagdown\,\diagup}{C{=}C}\underset{CH_2}{}.[CH_2]_6.CO_2H \qquad (66)$$

PART II

Dimerisation of acetylenic aldehydes

Introduction

Acetylenic aldehydes have long been utilised in polyacetylene syntheses, e.g. 6,6-dimethylhepta-2,4-diynal was an intermediate in the preparation of long chain polyynes by Whiting[99] and Bohlmann.[100] In the neat state, these aldehydes were rapidly darkening liquids which decomposed during combustion analysis, though they appeared to be stable under conditions required for Wittig and Grignard reactions.

In this department Bentley[101] prepared 1-phenylocta—4,6-diyn-3-ol (Scheme G) through a Grignard reaction with hexa-2,4-diynal (67). A major

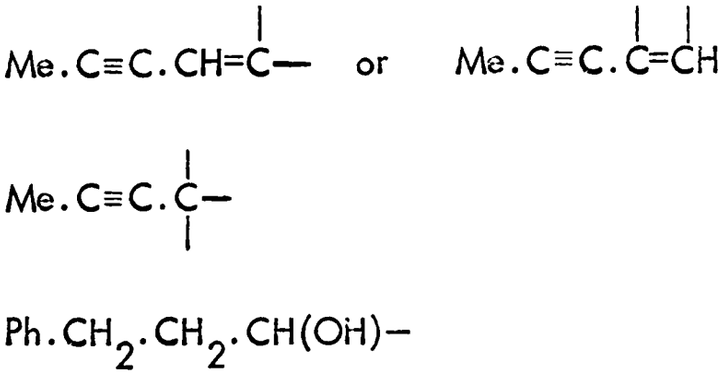
Scheme G

$$Me.C{\equiv}C.C{\equiv}C.CHO \quad (67)$$

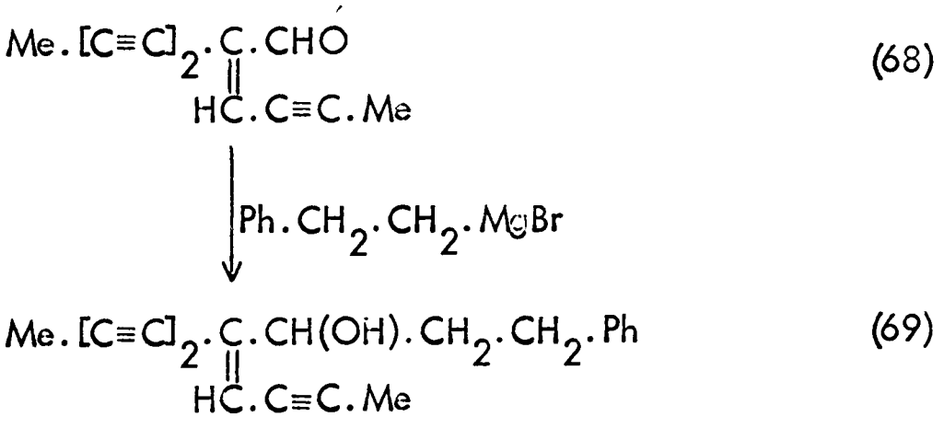
$$\downarrow \; Ph.CH_2.CH_2.MgBr$$

$$Me.C{\equiv}C.C{\equiv}C.CH(OH).CH_2.CH_2.Ph$$

by-product displayed a novel and unexpected chromophore, $\lambda_{max.}$ 303, 285, 269, 254, and 231 nm., which from the peak separation (ca. 2100 $cm.^{-1}$), was still polyacetylenic. Spectroscopic data suggested a $C_{19}H_{18}O$ alcohol which contained the following fragments:



The chromophore itself was unusual enough to provoke scrutiny into this by-product and its formation. Preliminary experiments, however, revealed that the aldehyde (67) changed into another acetylenic aldehyde (68) which participated in the Grignard condensation (Scheme G) and led to the by-product, the alcohol (69).



(68)

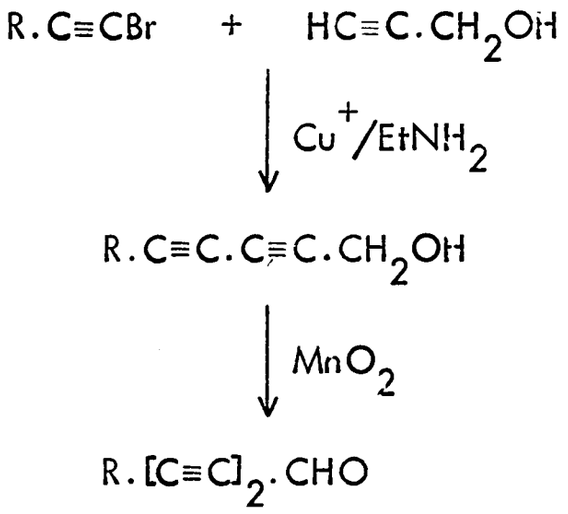
(69)

This section deals with the structure determination of the 'dimer' (68), and study of the 'dimerisation' reaction (67 → 68) which was shown to be general for activated αβ-acetylenic aldehydes.

The dimers from hexa-2,4-diynal

a) Isolation

Hexa-2,4-diynal (67), previously prepared[102] from the diethyl acetal, has been reported to be stable enough to distil at 25° (0.7 mm.), yet to detonate at 70°.[102] In the present investigation, this aldehyde was synthesised from the corresponding alcohol (Scheme Ha); the musty-smelling oil slowly solidified

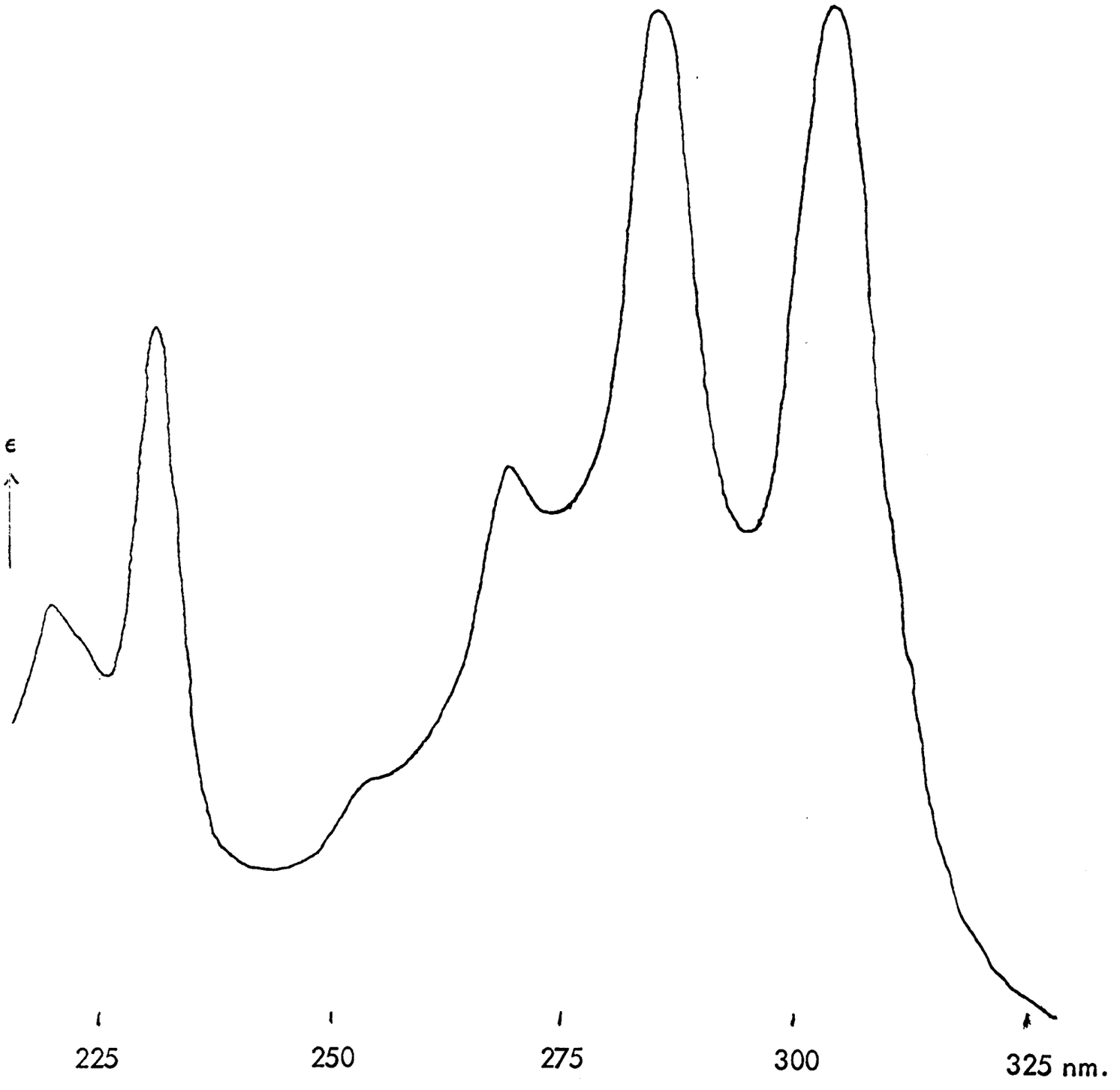


Scheme H

under nitrogen to a dark mass, a large portion of which still dissolved in hot light petroleum. Pale yellow crystals were deposited when this extract was cooled, and recrystallisation gave colourless needles, m.p 93-94°, which showed little decomposition over one year at 0°.

The analysis and high resolution mass spectrum indicated the formula $C_{11}H_8O$ for this product. Bands which could be attributed to disubstituted triple

Figure 1



Diynene chromophore

(obtained by treating hexadiynal dimer with acidic ethanol)

bonds (at $\nu_{max.}$ 2250 and 2220 cm.$^{-1}$) and a conjugated aldehyde (at $\nu_{max.}$ 2760 and 1695 cm.$^{-1}$) appeared in the infrared spectrum. The u.v. spectrum showed two broad maxima (319 and 254 nm.) (see figure 2, page 35),but changed dramatically after the addition of acidic ethanol into a sharp-peaked chromophore which was nearly equivalent to that exhibited by the Grignard reaction by-product (69) (figure 1). This phenomenon suggested that the formyl group was part of a cross-conjugated system with a linear polyacetylene which was, from consideration[103,7] of the longest wavelength maximum ($\lambda_{max.}$ 306 nm.), intermediate in conjugation between a triynene (338 nm.) and a diynene (286 nm.) (figure 1).

Structure (70) for the hexadiynal dimer was rejected since the n.m.r. spectrum showed an uncoupled formyl proton signal (τ-0.06). The small coupling

$$Me.[C{\equiv}C]_2.C(C{\equiv}C.Me){=}CH.CHO \qquad (70)$$

(2.5 Hz) observed between the vinyl proton (τ 3.1) and one set of methyl protons (τ 7.85) made the structure (71) the less likely of the remaining alternatives (71) and (72).

$$Me.[C{\equiv}C]_2.CH{=}C(CHO).C{\equiv}C.Me \qquad (71)$$

$$Me.[C{\equiv}C]_2.C(CHO){=}CH.C{\equiv}C.Me \qquad (72)$$

Two double bond isomers exist for each structure, and although isolation by crystallisation afforded a single isomer, isomerisation had been noted during

chromatography or storage in solution. This new isomer is, for convenience, discussed later (page 34 et seq.).

At this time, a preliminary communication was published by Gorgues and Levas,[104] which reported that 1,8-diphenyl-4-formyloct-3-ene-1,5,7-triyne (74) had been formed on concentration of 5-phenylpenta-2,4-diynal (73) in formic acid solution. The n.m.r. and i.r. data recorded, though incomplete, agreed

$$\mathrm{Ph.[C{\equiv}C]_2.CHO} \qquad (73)$$

$$\downarrow H^+$$

$$\mathrm{Ph.[C{\equiv}C]_2.C(CHO){=}CH.C{\equiv}C.Ph} \qquad (74)$$

well with the data for the hexadiynal dimer.

5-Phenylpenta-2,4-diynal (73) was now prepared (Scheme Hb), and this quickly solidified in the absence of solvent under neutral conditions. Work up as before gave brownish-yellow needles of the dimer (74), which melted at the temperature quoted by the French authors. The u.v. spectrum lacked fine structure just like that of the hexadiynal dimer, and so encouragingly supported the assumption of a similar formyl side chain.

In order to determine the position of the side chain, the perhydro methyl ester was prepared from the hexadiynal dimer and compared with a sample synthesised from diethyl malonate.

b) Conversion into the perhydro ester

Catalytic hydrogenation of the hexadiynal dimer resulted in hydrogenolysis and reduction of the formyl group, so the dimer was initially oxidised to the corresponding acid which was then converted to the perhydroester (77) (Scheme I)

SCHEME I

$$\underset{H,CHO}{Me.[C\equiv C]_2.\underbrace{C=C}.C\equiv C.Me} \quad (71) \text{ or } (72)$$

$$\downarrow CrO_3\text{-}Me_2CO$$

$$\underset{H,CO_2H}{Me.[C\equiv C]_2.\underbrace{C=C}.C\equiv C.Me} \quad (75)$$

$$\downarrow H_2\text{-}PtO_2$$

$$\underset{H,CO_2H}{Me.[CH_2]_4.\underbrace{CH.CH}.[CH_2]_2.Me} \quad (76)$$

$$\downarrow CH_2N_2$$

$$\underset{H,CO_2Me}{Me.[CH_2]_4.\underbrace{CH.CH}.[CH_2]_2.Me} \quad (77)$$

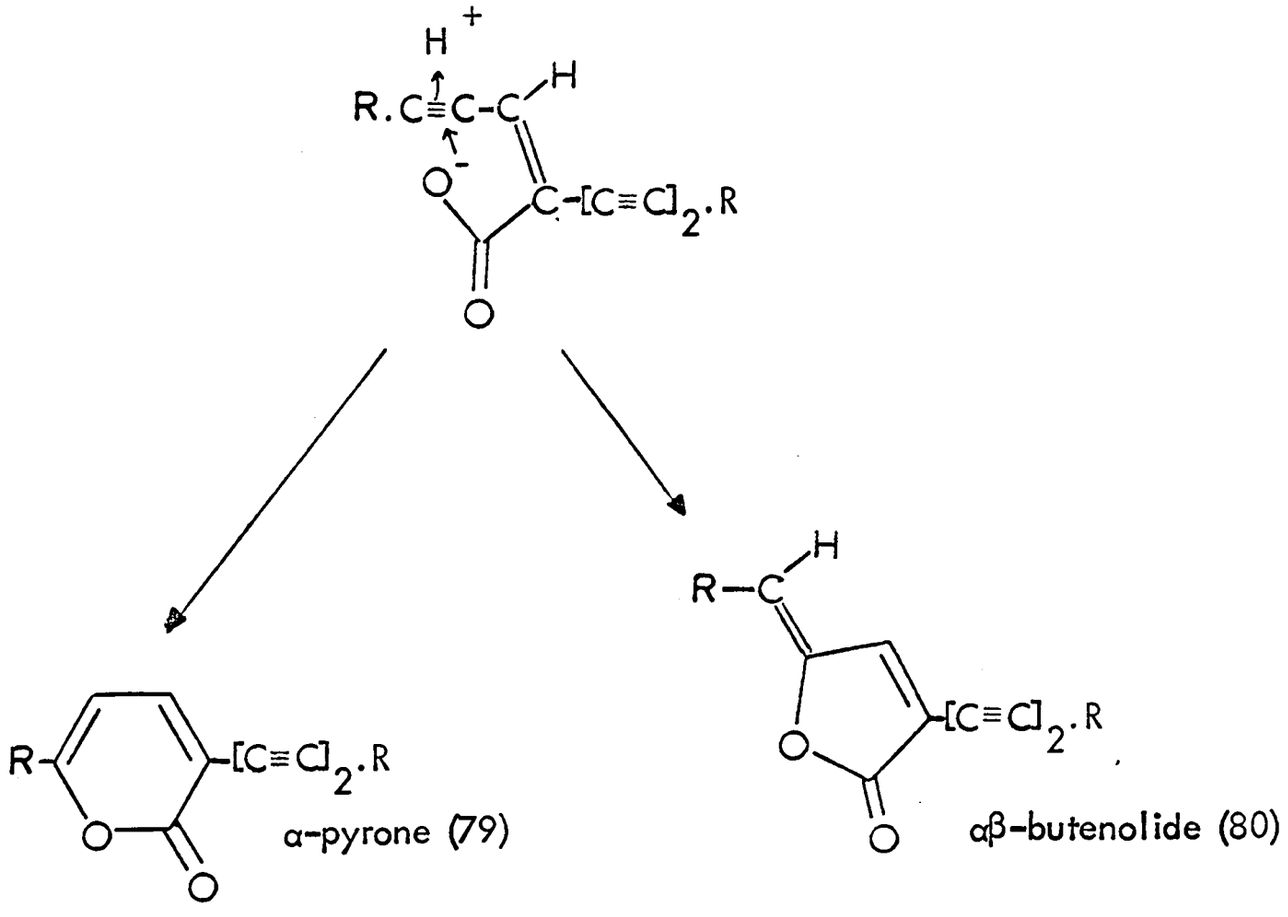
The recently published allylic oxidation[105] of aldehyde direct to the methyl ester via the cyanohydrin was the first process to be considered. However in the present work, the cyanide ion required to form the cyanohydrin in situ

also attacked the triple bonds of one typical polyacetylene aldehyde (78).

$$Me.[C\equiv C]_3.CH{=}CH.CHO \qquad (78)$$

The phenylpentadiynal dimer (74) was then used as model compound for oxidation by Jones reagent. As could be expected, lactone formation (Scheme J)

SCHEME J



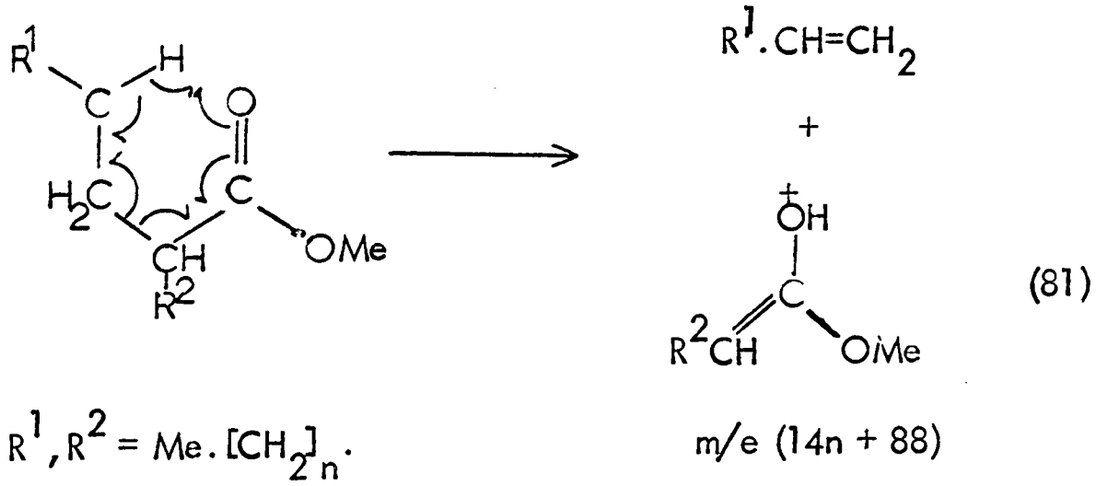
occurred; the carbonyl stretching band (1740 cm.$^{-1}$) indicated that the lactone[106] was the α-pyrone (79; R = Ph) rather than the αβ-butenolide (80; R = Ph) (predicted $\nu_{max.}$ ca. 1790 cm.$^{-1}$). This was minimised when separation of

of acidic and non-acidic products by alkaline extraction[107] was omitted and the crude ether extract was applied directly to thin layers.

Alkaline extraction succeeded in removing the last traces of lactone from the saturated acid (76), and methylation afforded an ester (77), homogeneous under t.l.c. and g.l.c. analysis.

In the mass spectrum, intense peaks appeared at m/e 130 and m/e 144 attributed to the ions (81; n = 3 and 4) arising from McLafferty rearrangement (Scheme K) with each α branch. Similar fragmentations had already been reported

SCHEME K



(81)

R^1, R^2 = Me.$[CH_2]_n$. m/e (14n + 88)

for methyl 2-hexyltridecanoate[108] and were confirmed in the present investigation using direct insertion probe conditions.

Only small peaks at m/e 158 and 116, expected for methyl 2-propyloctanoate [derived from structure (71)], were recorded and so for comparison methyl 2-butylheptanoate was synthesised in another way.

c) Synthesis of methyl 2-butylheptanoate

Methyl 2-butylheptanoate (84) was prepared by a typical malonate ester synthesis (Scheme L). The dicarboxylic acid (82) was purified by recrystallisation

SCHEME L

$CH_2.(CO_2Et)_2$

↓ NaOEt; Bu^nBr

$Bu^n.CH(CO_2Et)_2$

↓ NaH; $Me.[CH_2]_4.Br$

$Me.[CH_2]_4.C(CO_2Et)_2.Bu^n$

↓ KOH/EtOH

$Me.[CH_2]_4.C(CO_2H)_2.Bu^n$ (82)

↓ Δ

$Me.[CH_2]_4.CH.(CO_2H).Bu^n$ (83)

↓ $MeOH/H^+$

$Me.[CH_2]_4.CH(CO_2Me).Bu^n$ (84)

and the methyl ester (84) by preparative g.l.c.. This synthetic ester (84) and that (77) prepared from the hexadiynal dimer had superimposable infrared and mass spectra, and could not be separated on g.l.c.. Formula (72) hence represents the structure of the dimer which was analogous to that (74) from phenylpentadiynal.

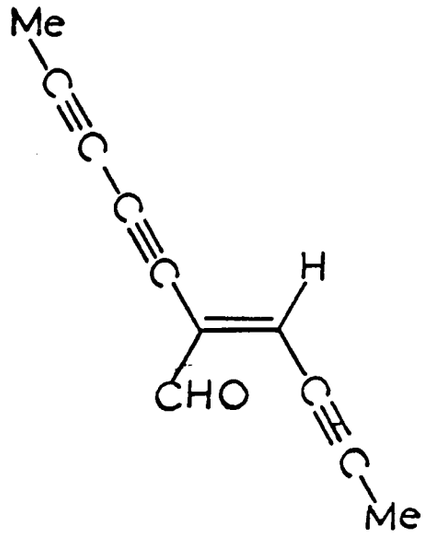
$$Me.[C{\equiv}C]_2.C(CHO){=}CH.C{\equiv}C.Me \qquad (72)$$

$$Ph.[C{\equiv}C]_2.C(CHO){=}CH.C{\equiv}C.Ph \qquad (74)$$

The acid (83) had already been prepared[109] by catalytic hydrogenation of 2,5-dipropyl-3-carboxythiophen, and characterised as the amide (m.p. 92°).[109] The amide of the acid prepared via scheme L had a higher melting point, 118°; this discrepancy has still to be resolved since a search for polymorphs with different melting points was unsuccessful.

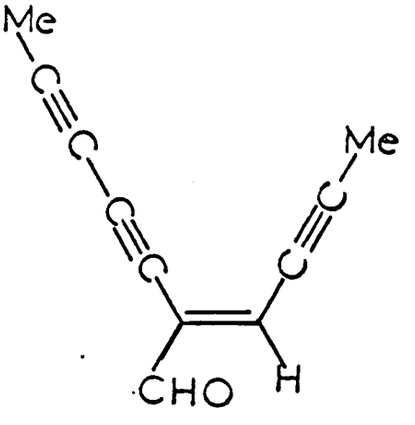
d) Stereochemistry of the double bond

The American Chemical Society's 'Entgegen-Zusammen' system[110] for double bond nomenclature was adopted for this work, as the relative priorities of the substituent groups were confusedly defined under other nomenclature rules. The hexadiynal dimer (72) (hereafter called isomer A) had one of the following configurations (72a and b):



(Z)-isomer

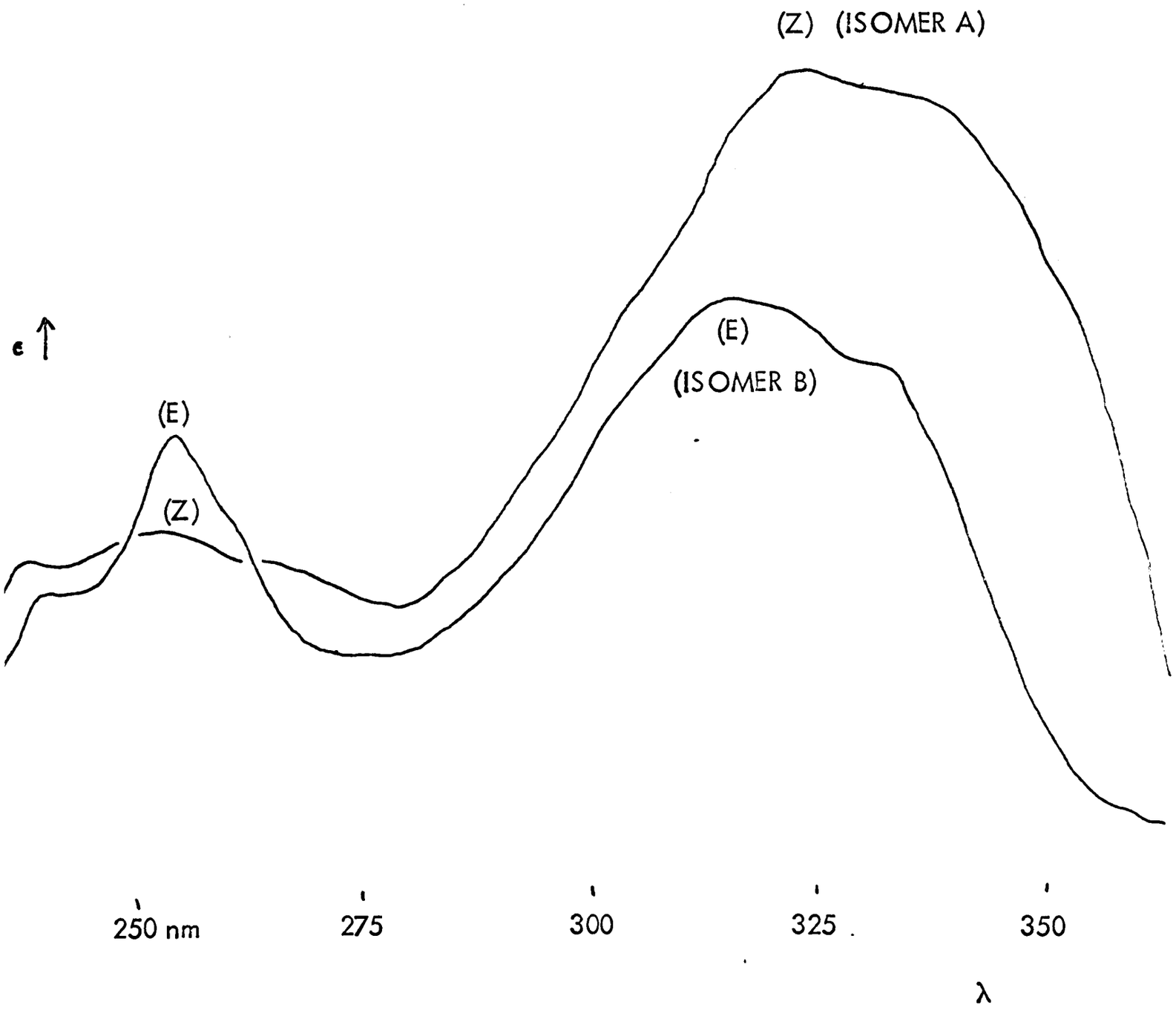
(72a)



(E)-isomer

(72b)

Figure 2



U.V. spectra of hexadiynal dimers (72a and b)

Fortunately the alternative geometrical isomer (isomer B) was isolated and direct comparison of spectral properties led to an unequivocal configuration assignment.

Ultraviolet irradiation of isomer A in carbon tetrachloride solution caused new signals to appear at τ 0.6, 3.3, 7.8 and 8.0, with splittings and areas consistent with 20% conversion into isomer B (no further isomerisation was noted after the solution had been exposed to sunlight for a further week). A second less mobile spot appeared when the mixture was chromatographed on silica gel layers; this was never completely resolved on multiple development as the spots appeared to diffuse into each other. It was shown with pure isomers that partial isomerisation (10% after one development) occurred during silica gel chromatography.

Isomer B was therefore prepared by recycling all but the tail end fractions of isomer A from a silica gel column. Concentration of the combined tail fractions yielded needles (m.p. 78-80°) of isomer B, which exhibited an identical mass fragmentation pattern to isomer A.

The low wavelength ultraviolet maximum was relatively more pronounced in isomer B, and thus the ratio of optical densities at 320 and 260 nm. afforded a quick method of differentiating isomer A (ratio 0.4) from isomer B (0.7) (figure 2).

Stereoisomeric mixtures of the dimers (72a and b) decomposed on g.l.c. (P.E.G.S. column), but could be reliably analysed from the formyl proton integrals.

In addition to equilibration on irradiation and silica gel chromatography, isomer A isomerised on storage in solution. When treated with acid isomer A yielded 40% of isomer B (with little loss of material), and on neutral alumina layers complete conversion of isomer A into B took place [here the monomer (67) travelled slower than the dimer].

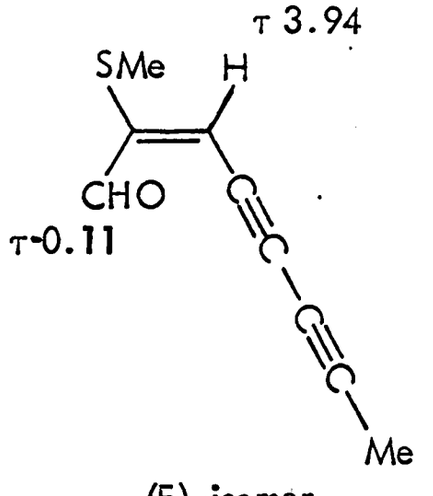
The chemical shifts of the formyl proton (Table 9) paralleled those for

Table 9

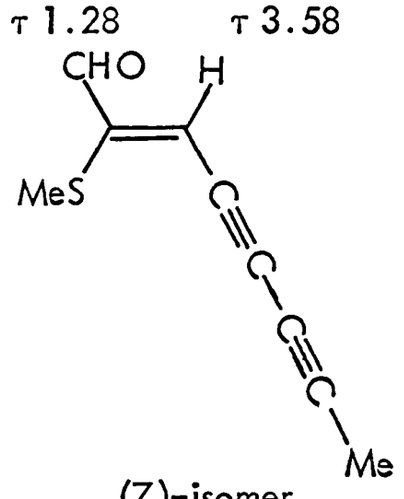
	τCHO	τ-CH=
Isomer A	-0.06	3.09
Isomer B	+0.58	3.28

Chemical shifts of hexadiynal dimers ($CDCl_3$)

other α,β-ethylenic aldehyde isomers. For example, Bohlmann[111] allocated the configurations of 2-(methylthio)oct-2-ene-4,6-diynal isomers (85) from the



(E)-isomer



(Z)-isomer

(85)

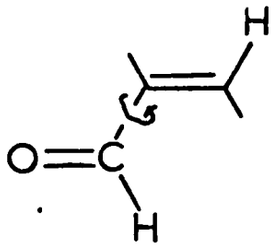
deshielding effect of the diyne system on the formyl proton. By analogy isomer B was assigned the (E)-configuration, and this assignment was confirmed through the observation of a nuclear Overhauser effect.

e) Nuclear Overhauser experiments on the hexadiynal dimers

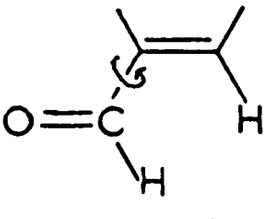
Since Anet[112] observed a significant intramolecular nuclear Overhauser effect (n.O.e.) between two protons, it has become a powerful technique[113] in structural elucidation, especially in the natural product field.

When a sample is dissolved in a magnetically inert solvent, which is freed from oxygen, two protons under 4Å[113] apart will predominantly relax one another through a dipole-dipole interaction. Irradiation of one causes a maximum signal area increase for the other of 50%,[114] though in practice 20-30%.

Isomers of the αβ-ethylenic aldehyde system (86) can be differentiated since this interaction only exists, despite rotation of the formyl group, for the cis-geometry (86b).



(86a)



(86b)

With the hexadiynal dimers, irradiation at a frequency equivalent to the centre of the vinyl proton resonance did not increase the half-width of the

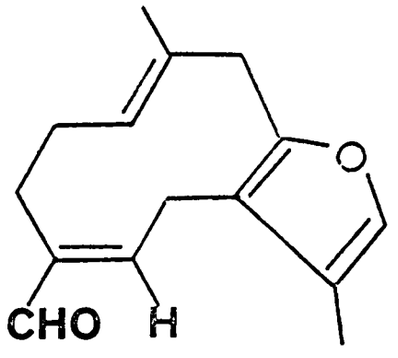
formyl proton signal, indicating that negligible spin-spin coupling existed between the two protons. Only for the new isomer (B) did the signal height increase [+18 (±2)%], and in the absence of spin-spin coupling this height increase corresponded to an area enhancement.

Irradiation of the aldehyde proton was easier to set up, but now it was difficult to check the signal height in the vinyl quartet due to the relative increase in noise.

In another experiment chloroform was added to the isomer B sample and the area ratio of the aldehyde proton and chloroform signals was measured using the electronic integrator (3.2:1). During irradiation the ratio increased to 3.7:1, corresponding to an enhancement of 16%.

It follows that only isomer B contained the cis configuration of vinyl and formyl groups (86b), and consequently has the (E)-configuration.

Recently an example of a n.O.e.[115] with the system (86) has been reported. Irradiation of the vinyl proton H(6) in the aldehyde (87) caused a 33% increase in the formyl signal area. Conversely, irradiation of the formyl proton effected a 22% increase in the vinyl proton signal.

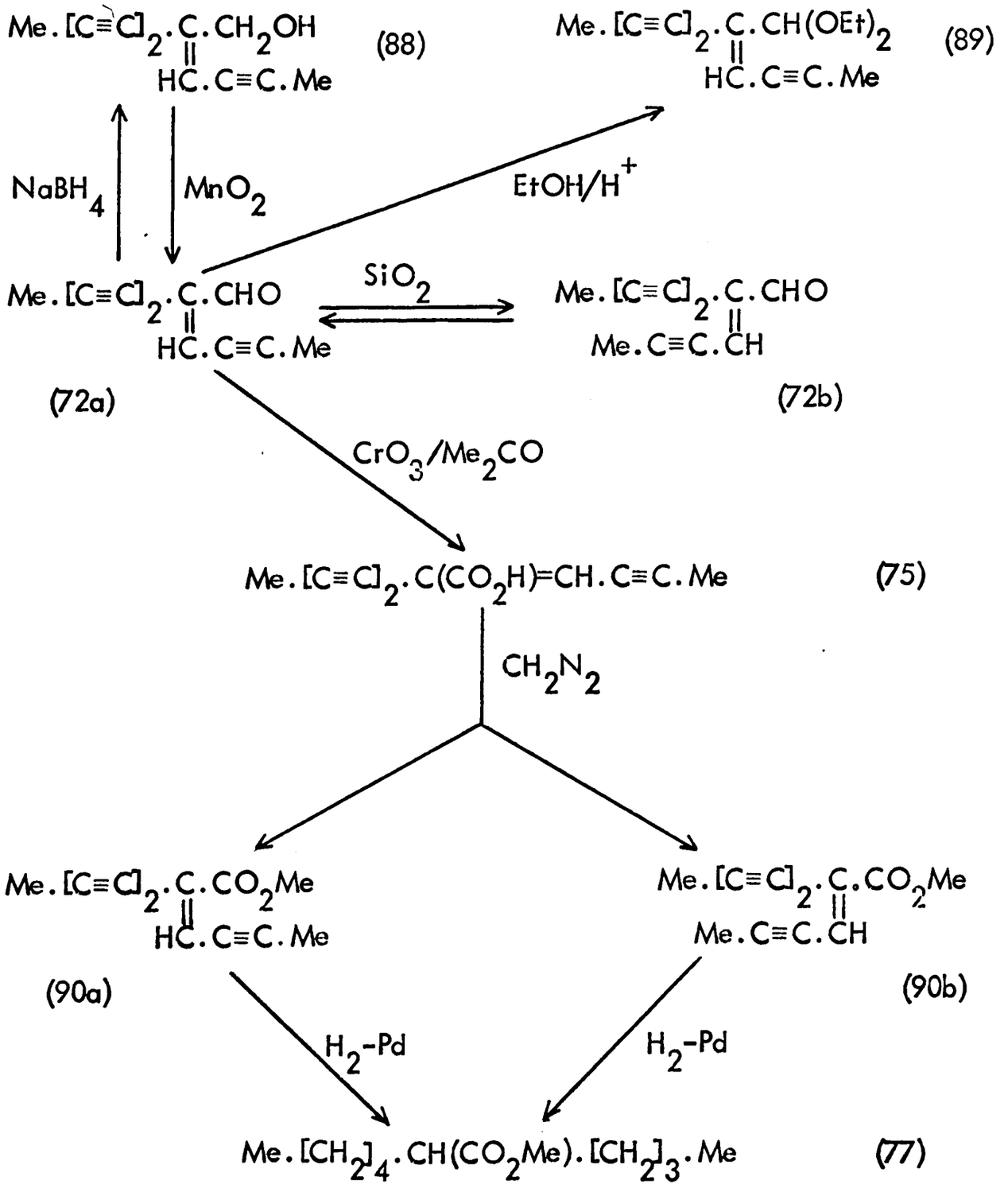


(87)

f) Other chemistry of the hexadiynal dimers

Concurrent with the structure elucidation of the hexadiynal dimer (72a), other derivatives were prepared (Scheme M).

SCHEME M



i) Alcohol and diethyl acetal

Treating the aldehyde (72a) with lithium aluminium hydride caused the ultraviolet absorption to disappear, presumably because hydrogenation of the αβ-double bond had occurred, which left only the less intense diyne chromophore intact.

Sodium borohydride reduction gave in good yield the (Z)-alcohol (88) [from which the (Z)-isomer (72a) could be regenerated by manganese dioxide oxidation]. Treatment of the aldehyde with acidic ethanol produced the diethyl acetal (89). Both derivatives exhibited very similar ultraviolet spectra, $\lambda_{max.}$ 305, 288, 271, 256, 233 and 221 nm., attributed to the diynenyne chromophore. The L series of bands were less intense than the A series (figure 1, page 28) in contrast to normal polyacetylenic chromophores, which generally correspond to triple bonds flanked by double bonds. The chemical shifts of the vinyl proton had moved upfield to τ 4.0 - τ 4.1, like the vinyl proton (τ 4.1) in the Grignard-reaction side product (69).

$$\begin{array}{l} Me.[C\equiv C]_2.C.CH(OH).CH_2.CH_2.Ph \\ \quad\quad\quad\

crystallised, yet low combustion analyses were obtained. The methyl ester isomers showed identical mass spectra with the predicted molecular ions (m/e 186).

In the ultraviolet the ester which travelled faster on silica gel layers, had the higher optical density ratio for the low and high wavelength maxima, i.e. the antithesis of the dimer aldehydes (72a and b). The less mobile isomer showed an appreciable upfield shift (0.58 p.p.m.) for the vinyl proton over the faster moving isomer (τ 3.07),which enabled the double bond configurations to be deduced.

Olefinic proton chemical shifts can be estimated using an additivity rule and deshielding constants for each substituent.[116] Predicted values for the aldehyde dimers were τ 2.73 (72a) and τ 3.21 (72b), whilst the observed shifts were τ 3.09 and 3.28 respectively. Tobey[117] has found that in highly conjugated systems interactions arise which upset these calculations, but better values are obtained if a suitable model is available to provide correction factors. The vinyl proton shifts for the methyl esters have been calculated from the additivity rule, and the correction factors derived from the model aldehydes (72a and b) have been applied to these values (Method A).

Ester shifts also were predicted by straight comparison with the known shifts[118] in the 2-methylpent-2-enals (91; R = H) and their corresponding methyl esters (91; R = OMe) (Method B).

The trends indicated by both methods (Table 10) designate the more mobile isomer as the (E)-isomer (τ 3.07); the less mobile had $\tau_{-CH=}$ 3.65. It follows

Et.CH=C(Me).CO.R (91)

Table 10

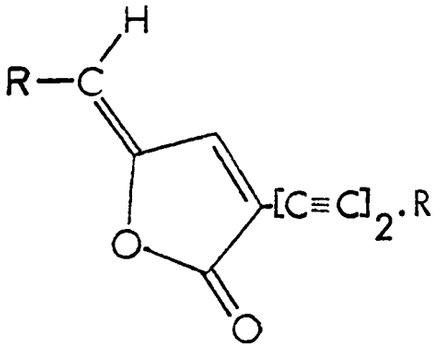
	(Z)-isomer	(E)-isomer
Method A	3.80	3.22
Method B	3.65	3.01

Vinyl proton shifts in the methyl esters (90)

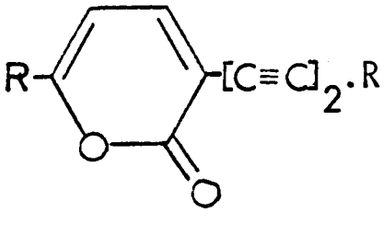
that their intensity changes in the ultraviolet are analogous to the aldehydes.

Hydrogenation of the esters gave the expected methyl ester (77) (g.l.c.).

A minor product from the methylation reaction was the αβ-butenolide (carbonyl $\nu_{max.}$ 1790 cm.$^{-1}$) (80; R = Me). The acetylenic band (2210 cm.$^{-1}$) was more intense than in the more symmetrically disposed pyrone (79; R = Me).



(80)



(79)

Table 11

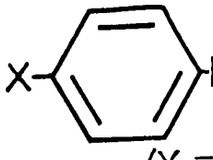
Monomer	Dimer				
$R.[C\equiv C]_2.CHO$	More mobile		Less mobile		Ref
	$^{\tau}.CHO$	$^{\tau}=CH-$	$^{\tau}.CHO$	$^{\tau}-CH=$	
Me *	-0.06	3.09	0.58	3.28	This work
Ph *	-0.11	2.93	0.52	3.04	104, This work
$Me.C\equiv C$ *	0.03	3.1	0.56	3.21	119
Pr^n *	0.01	3.17	0.6	-	119,121
Bu^n	0.0	3.14	†	†	120
$Ph.C\equiv C$	0.1	3.07	†	†	120

† Alternative isomer not known

* Prepared in this department

N.m.r. data for polyyne dimers

Table 12

Monomer	Ref
$Me.CH=CH.[C\equiv C]_2.CHO$	This work (p.196)
$HOCH_2.[C\equiv C]_2.CHO$	This work (p. 70)
 $[C\equiv C]_2.CHO$ (X = Me, Cl, Br and NO_2)	120
$Ph.C\equiv C.CHO$	This work (p. 45)

Further acetylenic aldehydes observed to dimerise

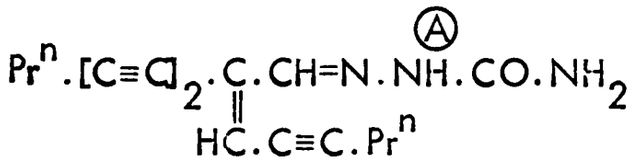
Dimerisation of other polyyne aldehydes

Not surprisingly, the (E)-phenylpenta-2,4-diynal dimer isomer was obtained after running the initially formed isomer (74) on silica gel layers. Dimers from other acetylenic aldehydes available in Oxford at this time (Table 11) were also prepared;[119] αβ-ethylenic aldehydes, e.g. dehydromatricarianal (78), did not undergo this reaction.

The dimer stereochemistries were deduced from the chemical shift of the formyl proton, which parallelled closely those of the hexadiynal dimers. On silica gel layers the (Z)-isomer always moved faster than the (E)-isomer. Their mass spectra all exhibited a strong molecular ion, and a peak at M -28; one of these was the base peak. Hydrogen abstraction for both ions was also evident.

The French group[120] reported later the dimerisation of further polyyne aldehydes under neutral conditions by heating benzene, toluene or carbon tetrachloride solutions under reflux (Table 11 and 12). From the n.m.r. data recorded, it can be seen[122] that these dimers are the (Z)-isomers (the work-up procedure of the French authors did not involve chromatography).

Octa-2,4-diynal[119] and nona-2,4-diynal[120] afforded oily dimers, which gave isomerically pure derivatives with hydrazines. The n.m.r. spectrum of the former aldehyde semicarbazone (92) was analysed in the present



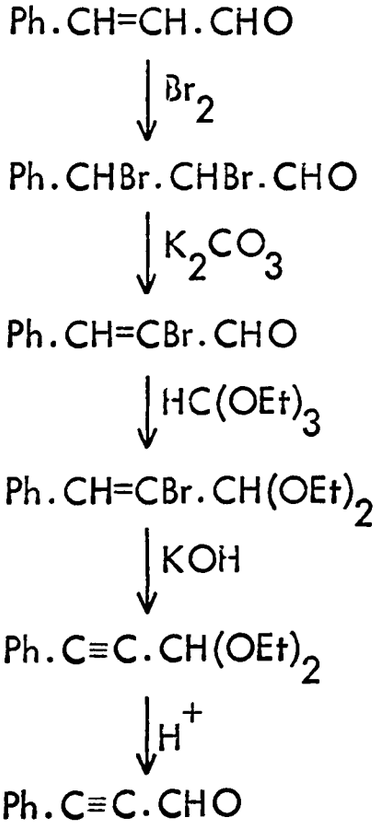
(92)

investigation. The electron withdrawing effect of the conjugated system promoted the rapid exchange of the amide proton (A) with deuterium oxide. In contrast equal exchange (90%) for the protons bound to nitrogen in cyclohexanone semicarbazone occurred only after 12 hours.

Dimers from 3-phenylprop-2-ynal

As each triyne and diyne aldehyde tested underwent dimersation, it seemed plausible that activated monoacetylenic aldehydes might also dimerise. Phenylpropargyl aldehyde (93)(prepared by scheme N) was initially tried since Claisen[123]

Scheme N



(93)

had reported that this aldehyde decomposed on distillation at 120° under water pump vacuum. At room temperature no dimer could be detected (n.m.r., u.v., t.l.c.) even after three months, but after prolonged heating at 130°, the phenyl aldehyde afforded a diffuse zone on thin layers which was less mobile than the monomer (93). The presence of two dimer isomers was suggested as two new aldehyde peaks at τ 0.45 and 0.17 with a non-integral ratio (1: 1.2) appeared in the n.m.r. spectrum.

Extraction of the front and rear of the diffuse zone gave the two oily dimer isomers (94a and b) which were more labile and unstable than the hexadiynal

$$Ph.C{\equiv}C.CHO \longrightarrow Ph.C{\equiv}C.C(CHO){=}CH.Ph$$

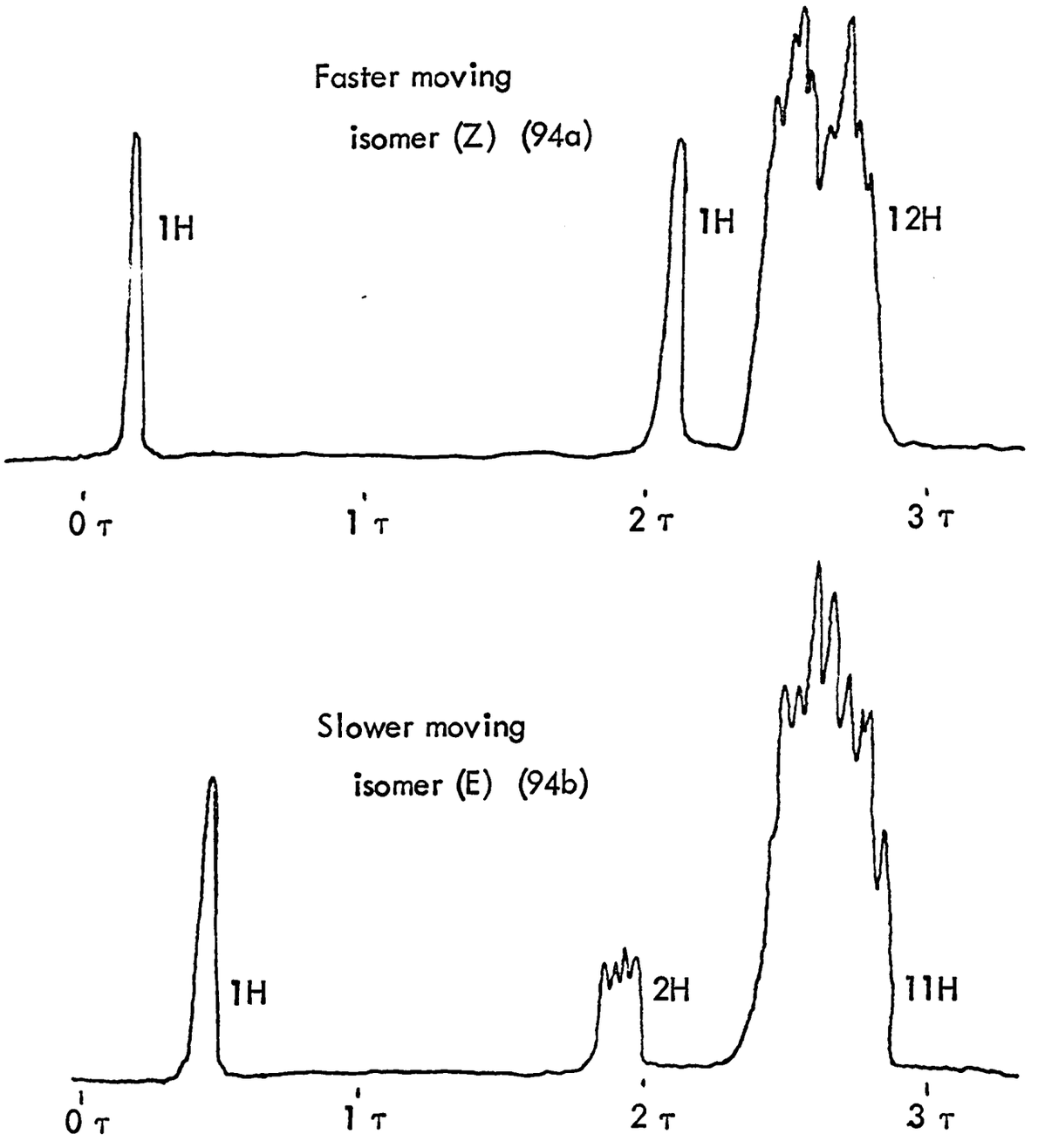
(94) a. front zone

b. rear zone

dimers (72a and b) (The observation of just one aldehydic proton in the n.m.r. spectrum was used as the criterion of purity). The dimer from the front zone (94a) gave a crystalline semicarbazone which analysed correctly for that of the aldehyde, $C_{17}H_{12}O$ (confirmed by mass spectra of the aldehydes).

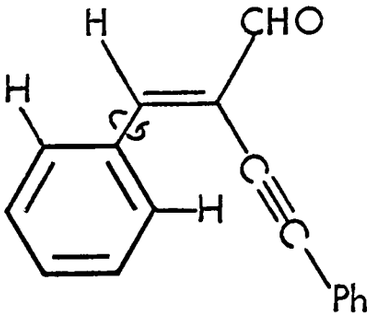
The ultraviolet spectra showed broad maxima at 322 and 259 nm.. The low wavelength maximum gradually became more intense, relative to that at $\lambda_{max.}$ 322 nm., on traversing from the front of the diffuse zone, i.e. (94a; ratio 0.75) to the rear (94b; ratio 0.9).

Figure 3



N.m.r. spectra of phenylpropynal dimers

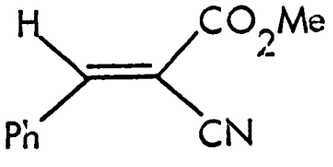
The intensity ratios, chromatography behaviour and formyl chemical shift (τ 0.17) suggested that the front of the zone contained the (Z)-isomer. Moreover the vinyl protons in the (Z)- and (E)-isomers were predicted[116] to resonate at τ 1.82 - (94a) had τ 2.1 - and 2.3. The shift observed for the (E)-isomer (94b) agreed with this trend as the vinyl signal was hidden under the aromatic proton multiplet at τ 2.7 (figure 3). For the latter isomer two aryl protons resonated 0.6 p.p.m. downfield to the remainder. Examination of Dreiding models (figure 4) revealed that the phenyl ring conjugated to the double bond can rotate and bring two of its protons close enough to the triple bond to be specifically deshielded.



(94b)

Figure 4

Similar phenomena have been encountered before in non-acetylenic systems, e.g. methyl α-cyanocinnamate[124] (95).



(95)

Bentley[119,121] has observed that mono-acetylenic aldehydes, e.g. but-2-ynal, do not dimerise.

Attempted dimerisation of ketones

Acetylenic ketones could not be induced to dimerise; thus the oily nona-3,5-diyn-2-one (96) simply decomposed on heating (u.v. and t.l.c.).

$$Pr^{n}.[C\equiv C]_{2}.CO.Me \qquad (96)$$

Ketones containing more unsaturated groups, like octa-3,5-diyn-2,7-dione and nona-3,5,7-triyn-2-one, polymerised in concentrated solution at room temperature. The diketone, on standing, inconsistently showed small additional bands ($\nu_{max.}$ 2180 and 1720 $cm.^{-1}$). After decomposing for 12 hours the triyne contained traces of at least three polar components which showed broad u.v. maxima at ca. 312 nm.. Treatment of the crude mixture with acidic ethanol did not change the spectrum into the sharp-peaked triynendiyne chromophore.[7] In these systems, dimers could be formed in the first stage of polymerisation but these might well be more unstable than their parent ketones.

When the ketone (96) and aldehyde (97) were mixed and kept for 80 hours

$$Pr^{n}.C\equiv C.C\equiv C.CHO \qquad (97)$$

under nitrogen, the major product was the expected octadiynal dimer (98). Chromatography of the reaction mixture revealed a minor zone (7%) less mobile

$$Pr^n.[C\equiv C]_2.C(CHO)=CH.C\equiv C.Pr^n \qquad (98)$$

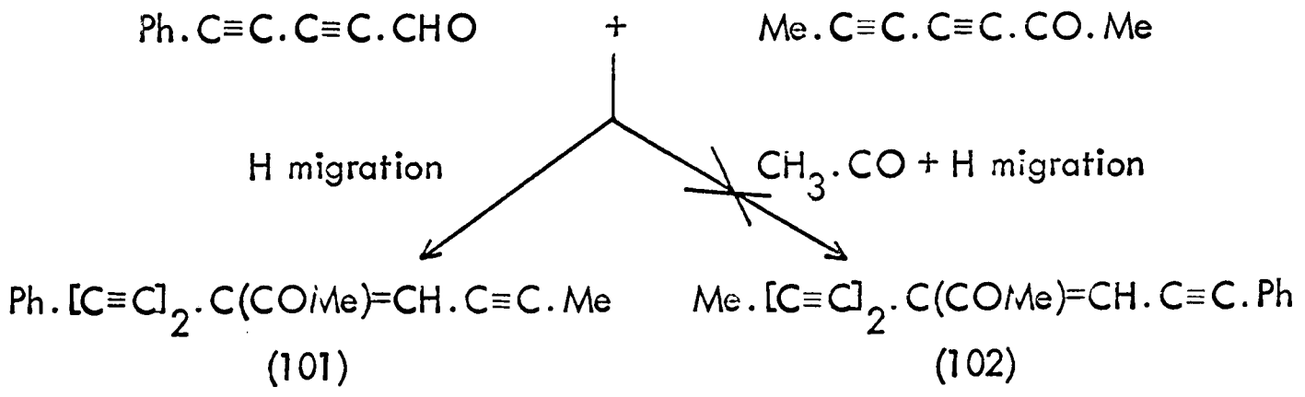
than the dimer (98), and which also exhibited the characteristic dimer u.v. spectrum. The molecular ion (m/e 226) of this oily product split off 28 a.m.u. (carbon monoxide), but the typical hydrogen abstraction was replaced by a loss of 15 a.m.u., corresponding to methyl abstraction. The n.m.r. spectrum indicated that the structure was the ketone (99) and not the aldehyde (100), but did not enable the stereochemistry of the double bond to be established (see page 35).

$$Pr^n.[C\equiv C]_2.C(COMe)=CH.C\equiv C.Pr^n \qquad (99)$$

$$Pr^n.[C\equiv C]_2.C(CHO)=CMe.C\equiv C.Pr^n \qquad (100)$$

To determine if acyl migration had taken place, the cross dimerisation was repeated with 5-phenylpenta-2,4-diynal (73) and hepta-3,5-diyn-2-one (Scheme O). The only cross dimer detected (3% yield) had structure (101) rather than (102), since n.m.r. spectroscopy showed that the vinyl and ethylidyne protons were coupled (2.5 Hz).

It follows that the ketone does not rearrange during the dimerisation and formally acts as a receptor of acetylenic and hydrogen fragments.

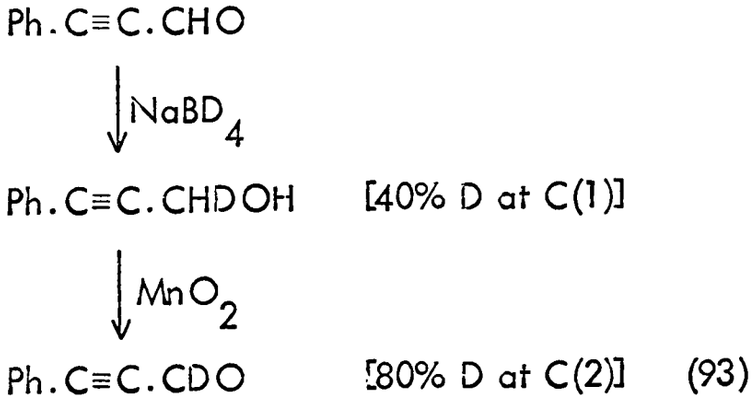


Scheme O

Mechanistic aspects of the dimerisation reaction

The origin of the vinyl proton was still not known; it could have come from the large amount of polymer that was always produced, or specifically from the aldehyde that also donated the acetylenic fragment. The latter hypothesis was confirmed through the dimerisation of [1-^{2}H] 3-phenylprop-2-ynal (93).

Deuterium was introduced in the formyl group on reduction with borodeuteride (Scheme P), and then the alcohol was reoxidised with manganese dioxide, during which the deuterium was preferentially retained. The dimer fraction produced



Scheme P

from this aldehyde (93) gave a fragmentation pattern in the mass spectrum totally consistent with the dideuterated structure (103).

$$Ph.C{\equiv}C.C(CDO){=}CD.Ph \qquad (103)$$

During dimerisations of hexadiynal and phenylpropynal, carbon monoxide was detected[125] in a nitrogen stream which had been passed slowly over the neat aldehydes. Carbon monoxide was not given off when no monomer remained, or from αβ-ethylenic aldehydes.

Hence the reaction appears to be a stoichiometric reaction between a receptor aldehyde (or ketone) and a decarbonylating aldehyde:-

$$R^1.C{\equiv}C.CHO \quad + \quad R^2.C{\equiv}C.CO\,R^3$$

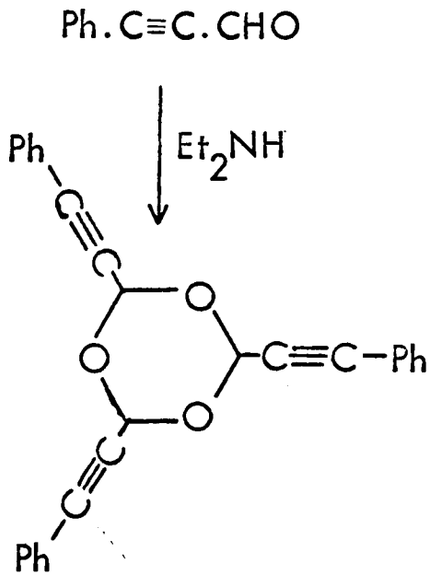
$$\downarrow$$

$$R^1.C{\equiv}C.C(COR^3){=}CH.R^2 \quad + \quad CO\uparrow$$

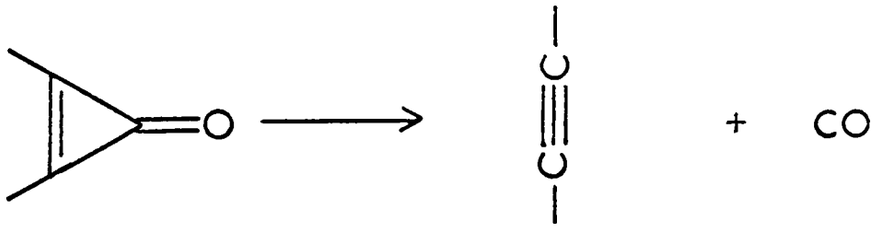
Attempts to increase the reaction rate and yield by the addition of decarbonylating catalysts, like palladium on charcoal, cuprous or silver salts, were all unsuccessful. Perhaps a catalyst like tris(triphenylphosphine)chlororhodium (I) which promotes[126] both decarbonylation and Strauss coupling would improve the yield of dimer at the expense of polymerisation.

Admixture of hexa-2,4-diynal with the free ethyne, penta-1,3-diyne, did not noticeably affect the reaction rate. Dimerisation was not acid or base catalysed;

in fact amines have long been known to induce trimerisation[127] of phenylpropynal:



Residues from phenylpropynal dimerisations were carefully screened for intermediates or side products, which could suggest the mode of dimerisation. One might reasonably contain a cyclopropenone ring which is known to pyrolyse[128] into carbon monoxide and an acetylene:-



The infrared spectrum of a crude dimerisation mixture did not, however, show a strained carbonyl stretching band ($\nu_{max.}$ 1840 cm.$^{-1}$), which was expected for such an intermediate.

Instead trace impurities present in the monomers were detected in the various dimerisation residues - phenylpropynal contained α-bromocinnamaldehyde

(Scheme N; page 44), and hexadiynal and phenylpentadiynal the analogous methyl esters. The methyl esters had been presumably derived from the methanol impurities in the dichloromethane utilised in manganese dioxide oxidations (Scheme H; page 27); the acetylenic hemiacetal formed therefrom still contains a propargylic hydroxyl function which was oxidised by the manganese dioxide (Scheme Q).

Scheme Q

$$R.C{\equiv}C.C{\equiv}C.CHO$$

$$\downarrow \; MeOH \text{ (from } CH_2Cl_2)$$

$$R.C{\equiv}C.C{\equiv}C.CH(OMe)OH$$

$$\downarrow \; MnO_2$$

$$R.C{\equiv}C.C{\equiv}C.CO_2Me$$

Formation of methyl esters from acetylenic aldehydes

R = Me, Ph

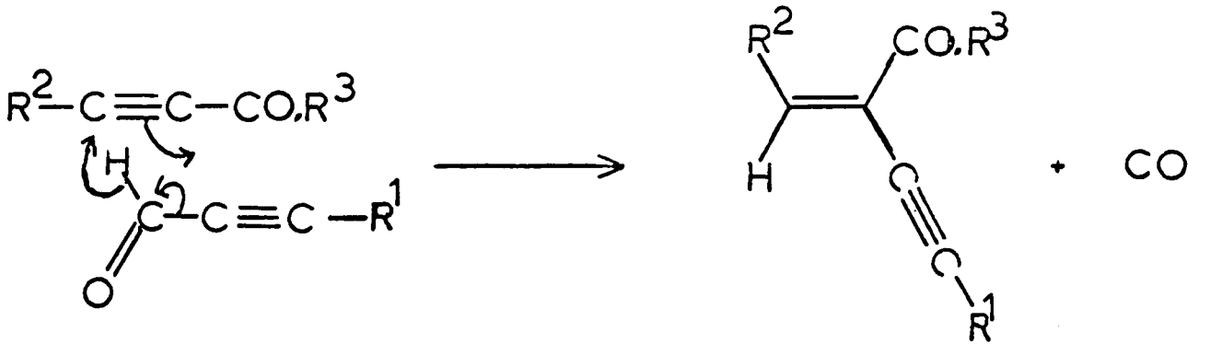
Free radicals were not detected by e.s.r. spectrometry in the dimerisation of hexadiynal or phenylpropynal.

In carbon tetrachloride solution hexadiynal dimerised solely into the (Z)-isomer. On the other hand both isomers of phenylpropynal dimer were present

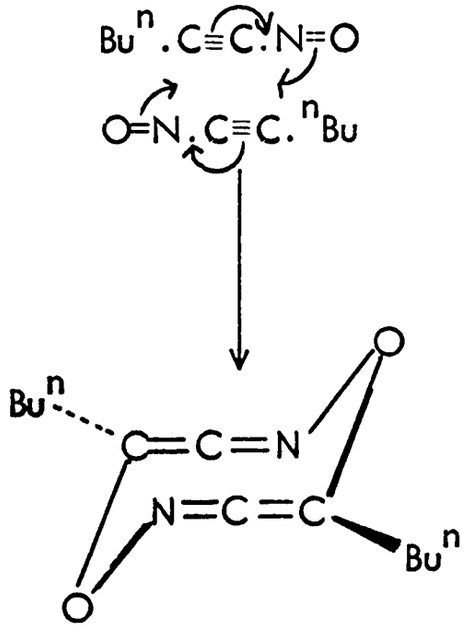
when the monomer had only partially dimerised; here the temperature (130°) required for a suitable reaction rate might cause isomerisation.

In harmony with the above results, a mechanism can be envisaged which simply involves the transference of a hydrogen atom from the decarbonylating aldehyde to the receptor acetylene (Scheme R).

Scheme R



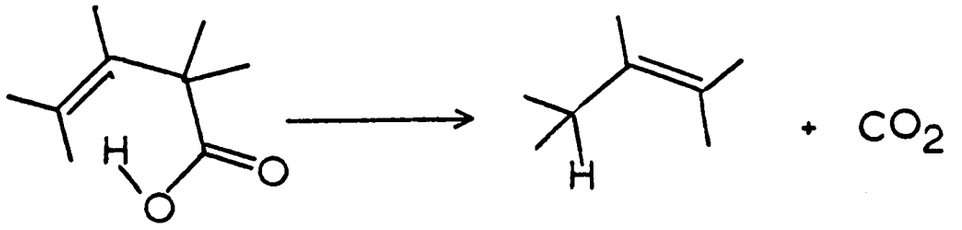
Formally scheme R implies the breaking of two σ bonds and one π bond in a concerted sigmatropic migration and cheletropic reaction.[129] No intermediate is involved and only the (Z)-dimer should be formed. Scheme R also necessitates the close approach and overlap of the two acetylenes; a similar orientation has been postulated[130] for the dimerisation of 1-nitrosohex-1-yne into the cyclic, unstable intermediate (104):



(104)

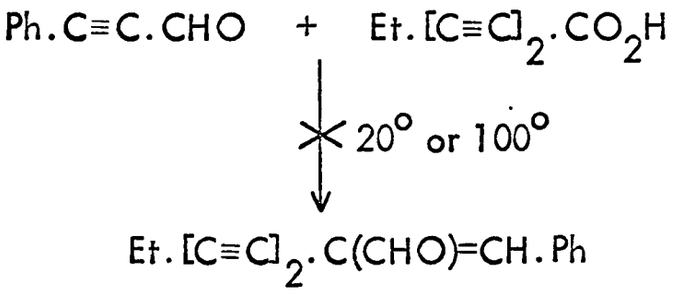
Analogous intermediates in the aldehyde dimerisations cannot be ruled out on the evidence available from this work.

A reaction allied to scheme R is the decarboxylation of skipped ene acids:[131]



Dimerisation in which carbon dioxide is lost instead of carbon monoxide has not yet been effected. 3-Phenylprop-2-ynal did not form a crossdimer with hepta-2,4-diynoic acid (Scheme S).

Scheme S



The dimerisation reaction, in preparative terms, is inefficient, though at this time it is the only synthesis available for these branched chain polyynes. The size of the end group, $R^3.CO$, (Scheme R) might be responsible for the low yield by sterically hindering the required orientation of the two acetylenes, though in Scheme R it is not actually involved in either bond fission or formation. Replacement with a smaller, electron withdrawing group, like cyano as in (105), should lead therefore to a higher yield of cross dimer.

$$R.C{\equiv}C.C{\equiv}C.CN \qquad (105)$$

However no cross dimer (106) was detected in a mixture of hexadiynal and the naturally occurring cyanoacetylene (105; R = $MeO_2C.CH{=}CH.$).

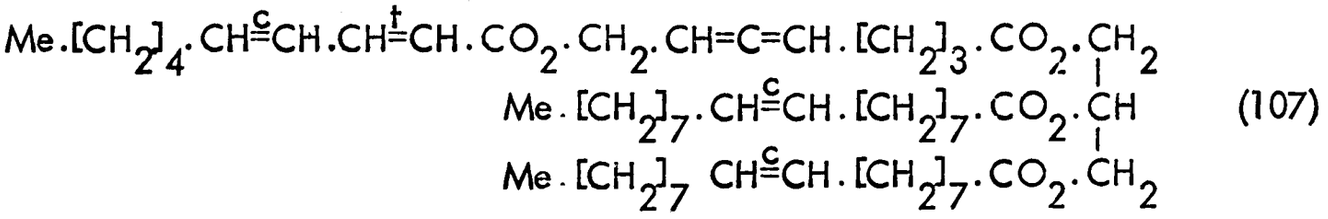
$$Me.[C{\equiv}C]_2.C(CN){=}CH.C{\equiv}C.CH{=}CH.CO_2Me \qquad (106)$$

PART III

Synthetic Studies with iodoacetals

Original aim

Alpha and beta oxidations have been confirmed[35] as chain shortening processes in polyacetylene biogenesis from oleate (see page 9), but it is still not known whether a chain containing more than two methylene groups can be cut off at any one time. Recently the lipid[132] (107) has been isolated from the Chinese tallow tree, and includes one C_{18} fatty acid sidechain which has undergone a cleavage of an eight-carbon chain resulting in an ester from C_{10}

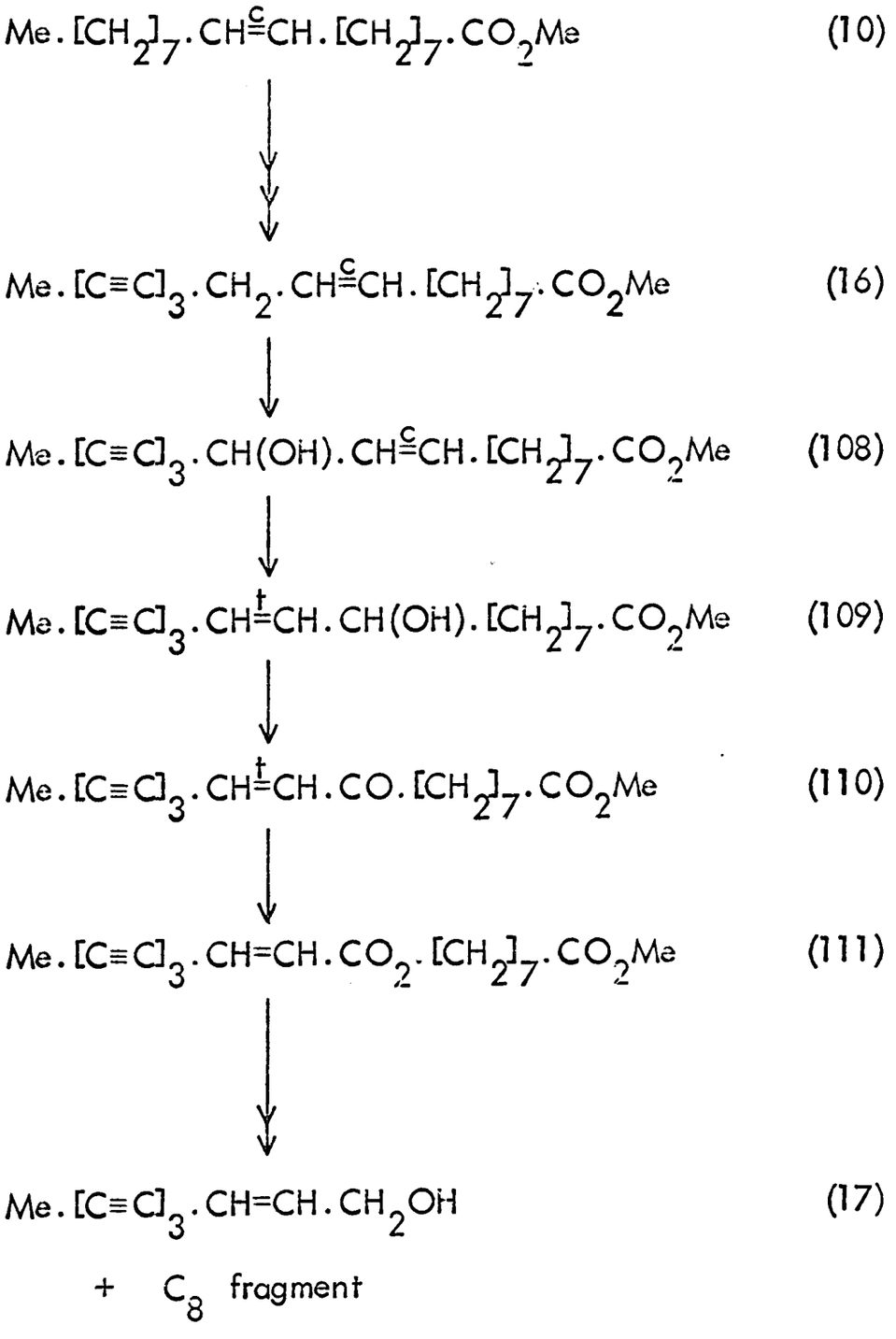


(107)

and C_8 fragments. Some 70% of fungal polyacetylenes have C_{10} and C_9 chain lengths and therefore Jones[133] suggested that a fungal C_{10} metabolite, say dehydromatricianol (17), might emanate from oleate (10) by severance of an eight carbon chain (Scheme T). As a corollary the oleate $\Delta^{9,10}$ bond rearranges before chain shortening takes place.

A similar set of transformations (16 → 111) performed on linoleate (11) instead of (16) would account for the distal half in the modified side chain of lipid (107).

Scheme I



The fungus Tricholoma grammopodium was considered a suitable system to test this hypothesis, since the main metabolite, dehydromatricarianol (17),

is produced in gram quantities. A C_8 fragment would then be generated in equivalent amounts. A C_{18} ester, say (109), labelled in the proximal half, would be transformed by this fungus into a C_8 chain or a mutation thereof, which could be located through its radioactivity.

Outline of synthetic schemes

The iodoacetal (112), radioactively labelled, was required to synthesise the ester (109) (Scheme U); the iodoester (113) was not expected to produce a normal Grignard reagent as it contained a functional group susceptible to attack by organomagnesium derivatives. This was checked with the homologue, methyl 9-iodononanoate which reacted sluggishly with magnesium under standard conditions.

$$I.[CH_2]_7R \qquad R = .CH(OAlk)_2 \quad (112)$$

$$= .CO_2Me \quad (113)$$

Scheme U

$I.[CH_2]_7R$ (112) or (113)

$\downarrow$ Mg

$IMg.[CH_2]_7R$

$\downarrow$ $Me.[C\equiv C]_3.CH{=}CH.CHO$

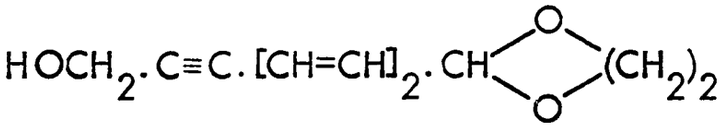
$Me[C\equiv C]_3.CH{=}CH.CH(O{:}H).[CH_2]_7R$

The ethylene acetal protecting group had already been employed under similar circumstances in the preparation of methyl crepenynate (12).[134] The acetal function was transformed into a methyl ester in 60% yield upon acid hydrolysis, silver oxide oxidation and methylation.

A straightforward method of introducing radioactivity into the alkyl chain of the acetal (112) was catalytic hydrogenation of an unsaturated linkage using tritium. Obviously, an extensive unsaturated system in the acetal would lead to a wide distribution of radioactive label in the proximal half of the C_{18} ester (109), and a higher chance of locating fragments engendered through Scheme T.

The iodoacetal synthesis can be subdivided into the preparation of a suitably unsaturated acetal and its conversion into the iodoacetal (112). Initially, readily accessible components were condensed to give three unsaturated acetals (114-116)

$$Br.[CH_2]_2.[C\equiv C]_2.CH_2.CH(OMe)_2 \qquad (114)$$



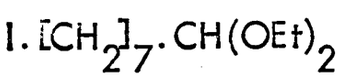
(115)

$$HO.[CH_2]_3.[C\equiv C]_2.CH(OEt)_2 \qquad (116)$$

in order to study the behaviour of different functionalities. The diyne (116) converted cleanly and efficiently into its saturated counterpart (117) and was

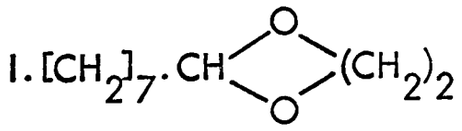
$$HO.[CH_2]_7.CH(OEt)_2 \qquad (117)$$

therefore prepared on a larger scale as precursor of the iodoacetal (118 ≡ 112; Alk = Et).

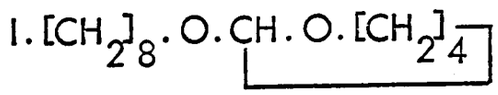


(118)

Unexpectedly this iodide did not form an organomagnesium derivative, so compounds of related geometry, (119) and (120), were prepared and likewise



(119)



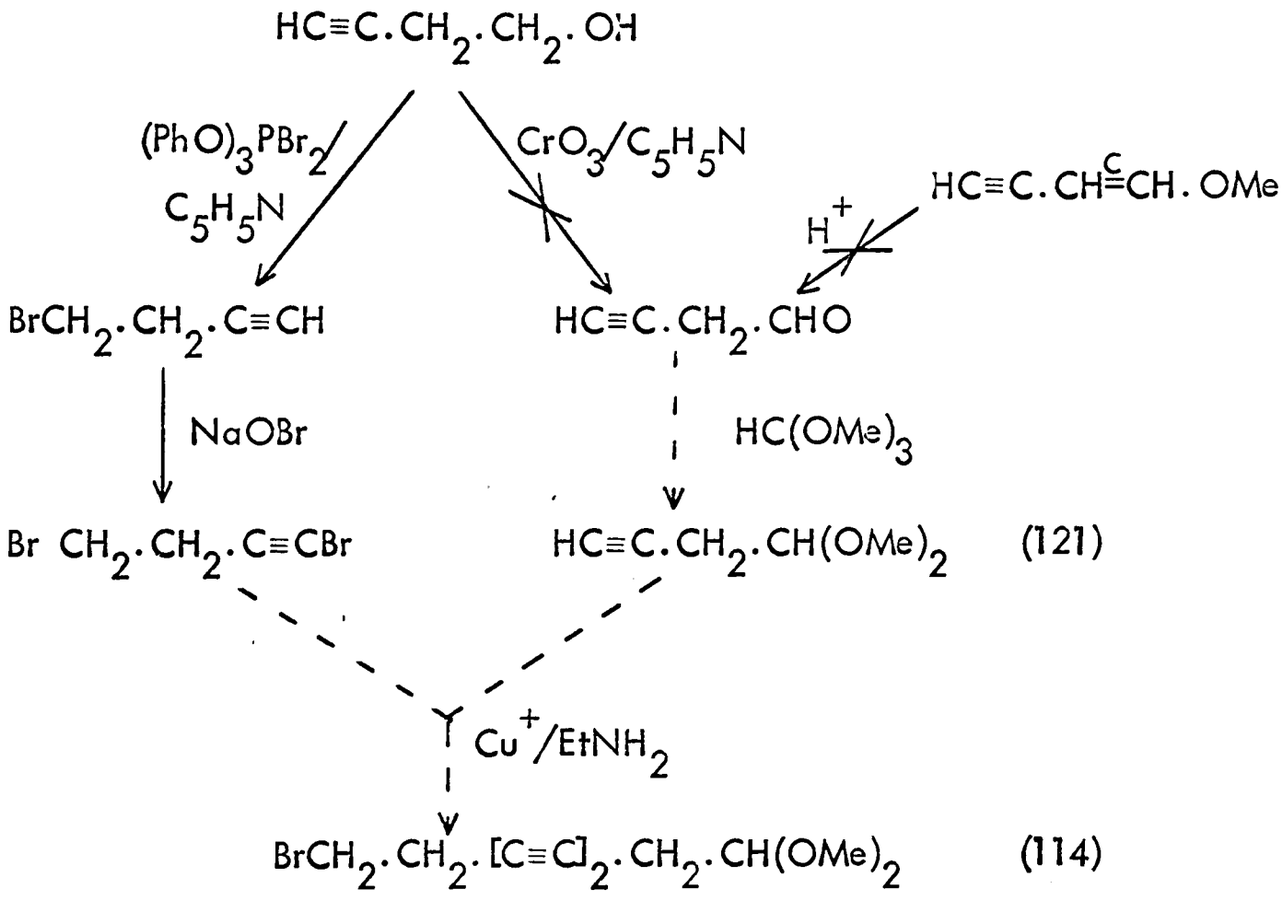
(120)

observed to be inert towards magnesium.

The synthesis of 8-bromoocta-3,5-diynal dimethyl acetal

The diyne (114) was to be prepared from but-3-yn-1-ol (Scheme V). But-3-ynal could not be obtained by Sarett oxidation of but-3-yn-1-ol, nor by acid hydrolysis of 1-methoxybut-cis-1-en-3-yne.[135,136] The hydrolysis product was, in fact, buta-2,3-dienal (n.m.r. and i.r.).

Scheme V



Winter[137] has demonstrated that hex-3-ynal exists in equilibrium with allenic and enol isomers (Scheme W; R = Et); but-3-ynal presumably isomerised

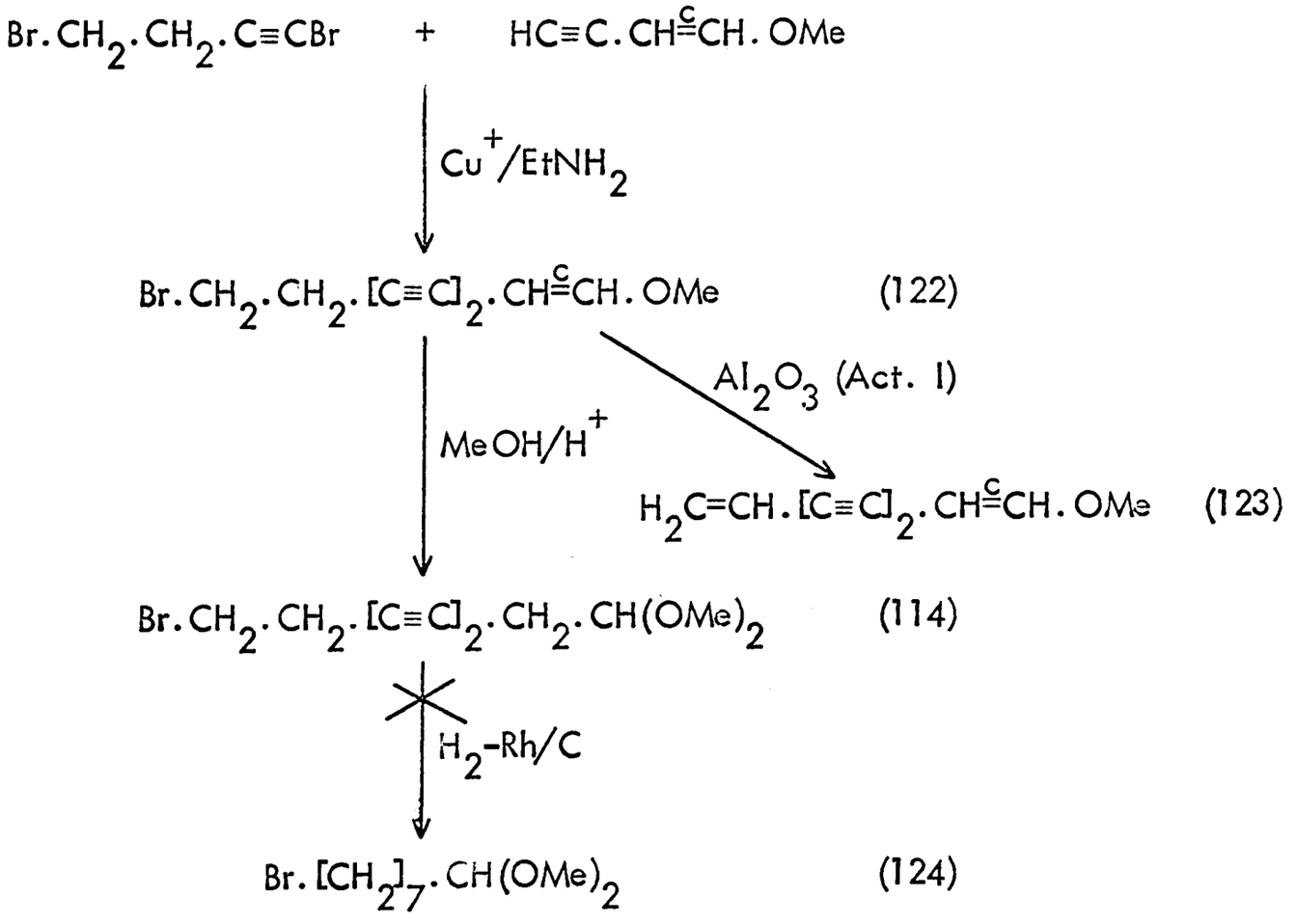
$$R.C{\equiv}C.CH_2.CHO \rightleftharpoons R.C{\equiv}C.CH{=}CH.OH \rightleftharpoons RHC{=}C{=}CH.CHO$$

Scheme W

into allenic aldehyde and a water soluble vinyl alcohol (Scheme W; R = H). Consequently the synthesis of the dimethyl acetal (121) from the aldehyde seemed unworkable.

Instead 1,4-dibromobut-1-yne was directly coupled with methoxybutenyne to afford the bromoether (122) (Scheme X). Methanol, on acid catalysis, added across the cis—double bond and gave the required bromoacetal (114). These C_8

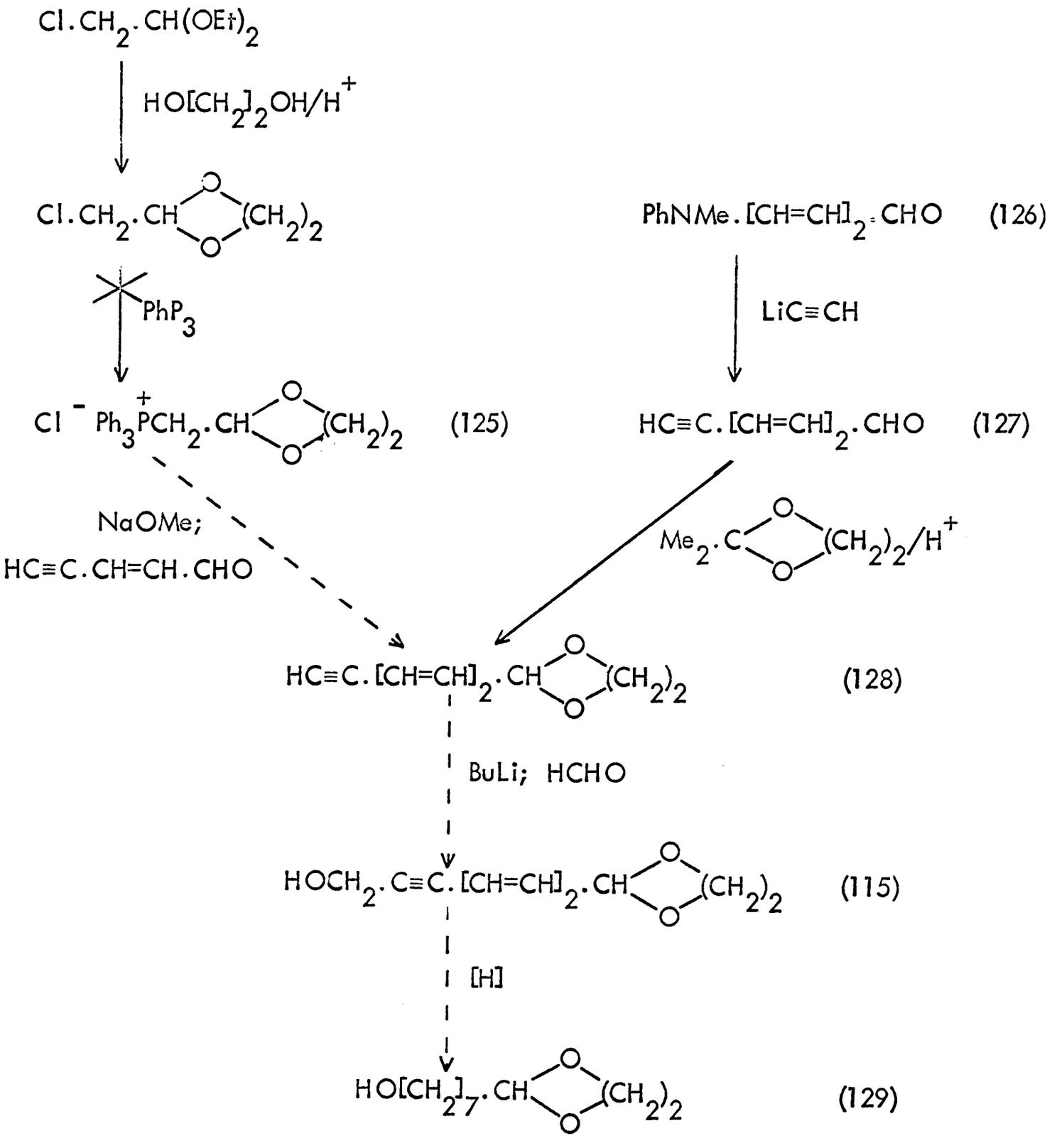
Scheme X



acetylenic bromides were purified on deactivated alumina (Act. III), since spontaneous dehydrohalogenation [of (122) to (123)] ensued on active alumina.

Extensive hydrolysis of the carbon-bromine bond was encountered in attempts to prepare the saturated bromoacetal (124); the synthesis of this acetal (124), in

Scheme Y


Cl.CH2.CH(OEt)2
HO[CH2]2OH/H+
Cl.CH2.CH(O)(O)(CH2)2
PhP3
Cl⁻ Ph3P⁺CH2.CH(O)(O)(CH2)2
(125)
PhNMe.[CH=CH]2.CHO
(126)
LiC≡CH
HC≡C.[CH=CH]2.CHO
(127)
NaOMe;
HC≡C.CH=CH.CHO
Me2.C(O)(O)(CH2)2/H+
HC≡C.[CH=CH]2.CH(O)(O)(CH2)2
(128)
BuLi; HCHO
HOCH2.C≡C.[CH=CH]2.CH(O)(O)(CH2)2
(115)
[H]
HO[CH2]7.CH(O)(O)(CH2)2
(129)

a manner parallel to that of the diethyl acetal (118) (Schemes Z and AD), was reported after this work was finished.[35]

Another functional group was required at C(8) which could be converted into halide after catalytic hydrogenation. Accordingly subsequent acetal preparations involved ω-hydroxyacetals.

Attempted synthesis of 8-hydroxyocta-2,4-dien-6-ynal ethylene acetal

The key intermediate in the synthesis of this yne-diene (115) was the ethynyl dioxolan (128) (Scheme Y). First attempts to prepare this through a Wittig reaction between the phosphonium salt (125) of chloromethyl-1,3-dioxolan and pent-trans-2-en-4-ynal broke down when the salt could not be crystallised. Moreover the crude salt did not produce a betaine colouration on treatment with base.

In an alternative, but inefficient synthesis (14%) of the dioxolan (128), ethynation of Zincke aldehyde, which was already available[9] in this department, yielded a stereoisomeric mixture of aldehydes (127). At -20° an ether-hexane solution deposited crystals of a previously known isomer (m.p. 92°), the configuration of which had not yet been rigorously determined.[9]

The 100 MHz n.m.r. spectrum for an acetone solution showed a first order splitting pattern for the vinyl protons. The observed coupling constants indicated that both double bonds had the trans configuration. N.m.r. spectra in carbon tetrachloride (at 60 and 100 MHz)[9] were too complex to interpret, and acetone

had induced specific downfield shifts (ca. 0.2 p.p.m.) for H(3) and H(5) (Table 13). Similar specific shifts were noted for the phenyl analogue (130; R = Ph.C≡C),

$$\overset{5}{R}.\overset{4}{CH}=\overset{3}{CH}.\overset{2}{CH}=\overset{1}{CH}.CHO \qquad (130)$$

Table 13

	Shift (p.p.m.)				
R	5	4	3	2	1
H.C≡C.	0.21	+0.05	+0.29	+0.04	+0.06
Ph.C≡C	+0.1	+0.03	+0.22	-0.02	0
Me	<0.02[a]	<0.02[a]	0	-0.1	0

[a] multiplet of very similar shape and shift for both solvents

Solvent shifts on changing from acetone to deuteriochlorform solution

but not for hexa-2,4-dienal (130; R = Me). Parallel behaviour had been recorded for ynenals like dehydromatricarianal (78), both in the present work and independently by Bohlmann.[138] Acetone seems to be a useful solvent for the spectral analysis of such aldehydes.

$$Me[C\equiv C]_3.CH=CH.CHO \qquad (78)$$

The aldehyde (127) was scarcely transformed into the acetal (128) by

treatment with acidic ethanediol, in contrast to the immediate conversion with 2,2-dimethyl-1,3-dioxolan. The transacetalisation reaction afforded a side product (131), arising from an aldol condensation between hepta-2,4-dien-6-ynal and acetone.

$$H\ C{\equiv}C.[CH{=}CH]_2.CH(OH).CH_2.CO.Me \qquad (131)$$

The acetal (128) turned out to be quite unstable and further preparation by Scheme Y in the quantities necessary for conversion to the hydroxyacetal (115) was not practicable. This synthesis was carried out concurrently with that of the diyne (116) and was abandoned in favour of the latter.

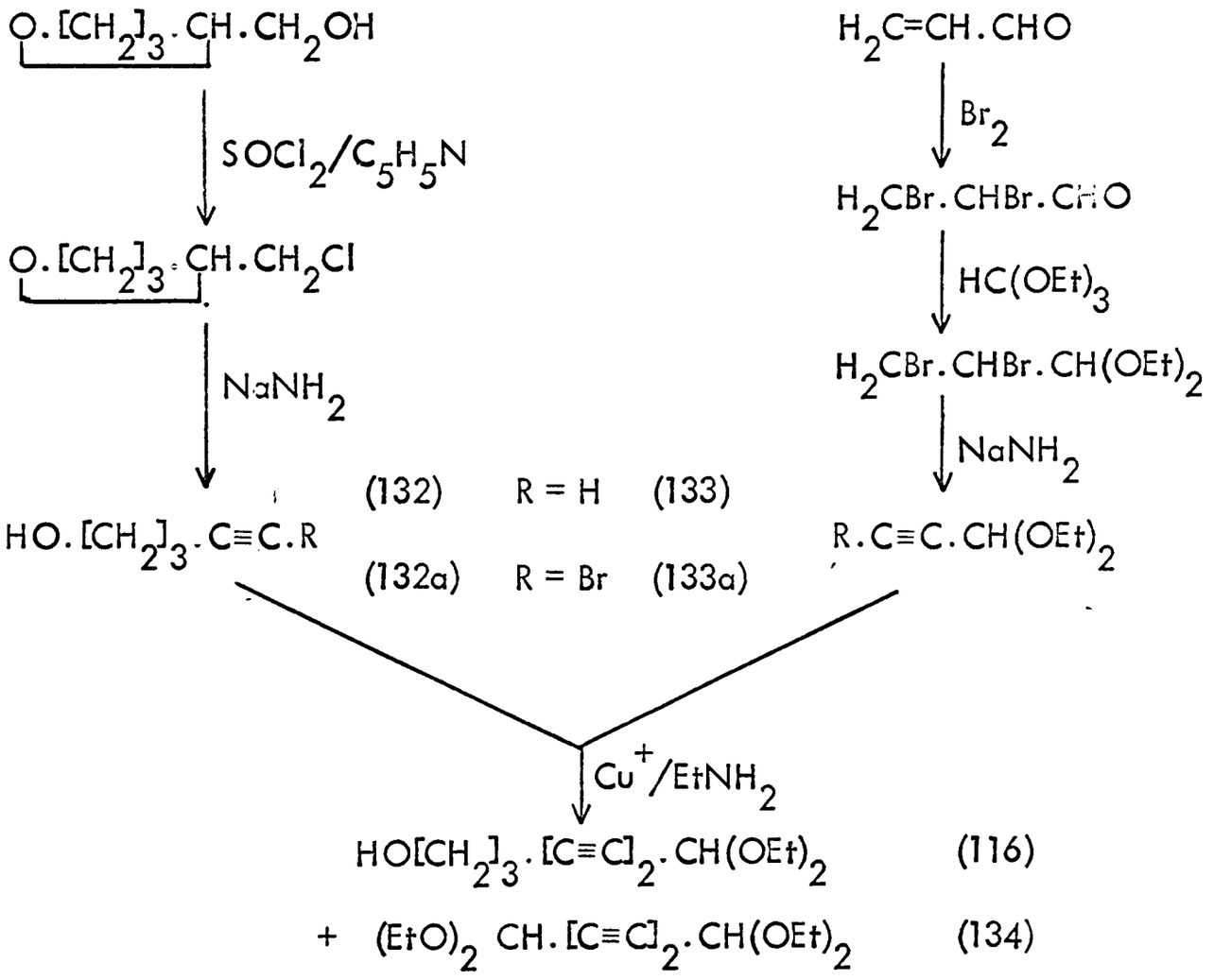
The synthesis of 8-hydroxyocta-2,4-diynal diethyl acetal

The diyne (116) was synthesised by straightforward reactions (Scheme Z) from pent-4-yn-1-ol (132) and prop-2-ynal diethyl acetal (133), both of which were stable enough to be handled in gram quantities.

Cadiot-Chodkiewicz coupling of the bromoalcohol (132a) with the acetal (133) gave a slightly better yield (33%) than the alcohol (132) with the bromoacetal (133a) (27%). The more efficient route also avoided manipulation of the bromo-acetal (133a) which inflicted severe skin dermatitis on the author.

On silica gel, pentynol (132) moved only slightly faster than the diyne (116). Its presence in the product was initially suspected from odd integrals in the n.m.r.

Scheme Z



spectrum, though the ethynyl proton was clearly seen for benzene solution. The band attributed to free ethynyl ($\nu_{max.}$ 3300 cm.$^{-1}$) was only observed in dilute samples since it was effectively concealed under the broad bonded-hydroxyl band (3400 cm.$^{-1}$). Column chromatography on alumina (Act. III) was sufficient to remove impurities which previously had caused acetal hydrolysis on silica gel thin layers.

The hydroxyacetal (116) decomposed on g.l.c. columns, so its purity was checked by the determination of the diyne chromophore extinction coefficients over successive fractions.

Another diyne acetal (i.r. and u.v.) was eluted before the hydroxyacetal in the work-up of the Cadiot-Chodkiewicz coupling. The n.m.r. spectrum, practically identical to that of the acetal (133) without the ethynyl proton signal, suggested the dimer structure (134).

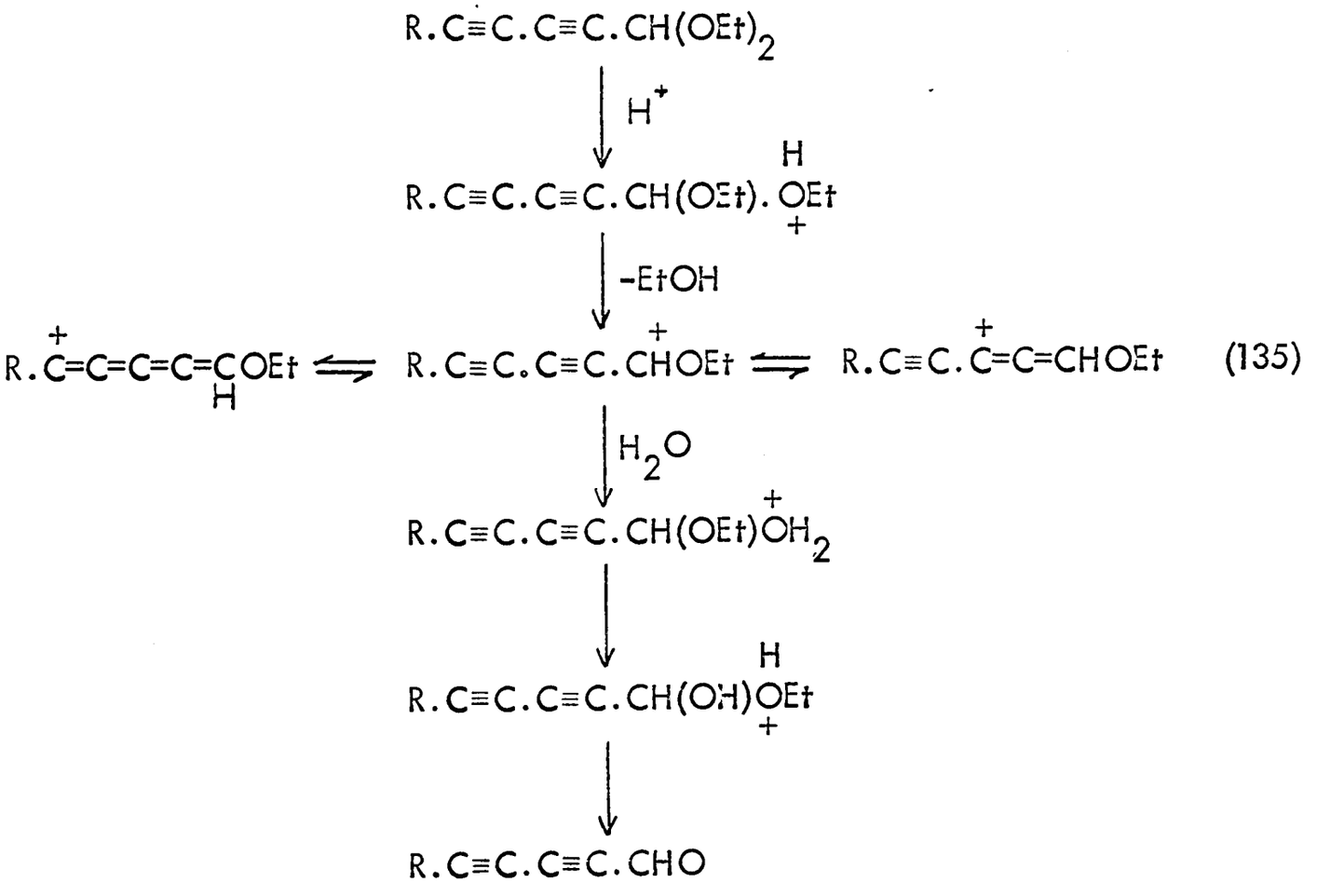
$$(EtO)_2CH.C{\equiv}C.C{\equiv}C.CH(OEt)_2 \qquad (134)$$

In succeeding preparations, advantage was taken of the stronger acid and longer time needed to hydrolyse the dimer (134) to differentiate between it and the wanted hydroxyacetal (116). No ene-diyn-ene chromophore was ever observed, presumably indicating that hexa-2,4-diynedial spontaneously decomposed in the acidic conditions.

The marked difference in hydrolysis rates can be attributed to the relative stabilities of the intermediate carbonium ion (135) in the hydrolysis mechanism[139] (Scheme AA).

The positive charge is delocalised over the diyne linkage in (135) and partial positive charges are induced on C(3) and C(5). The electron withdrawing acetal group in the dimer (134) destabilises the positive charge [135; R = $CH(OEt)_2$] in contrast to the positive inductive effect imparted by the hydroxypropyl group of (116) to [135; R = $[CH_2]_3OH$].

Scheme AA

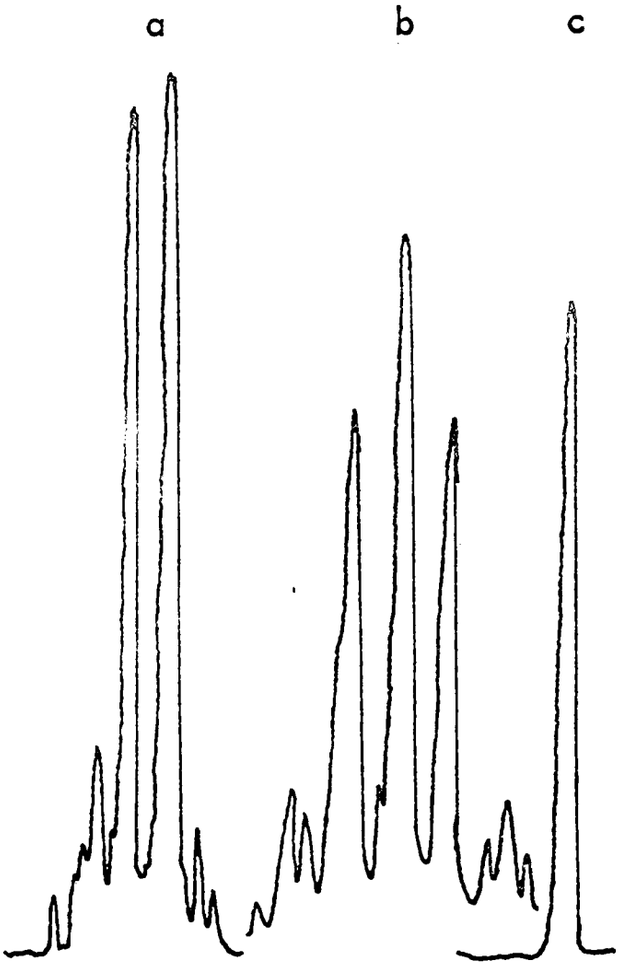


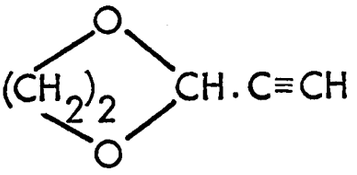
(135)

Acetal hydrolysis mechanism[139]

Diethyl acetals are well known for their intrinsic internal asymmetry.[140] Despite rotation of the ethoxy sidechains the protons on the methylene groups are magnetically non-equivalent [e.g. (133) in figure (5)]. Thus two ^{13}C-H coupling constants have been observed for each methylene group[141] and the spectrum does not change on cooling the sample.[140]

Figure 6



a $-O.C\underline{H}_2C\underline{H}_2.O-$ in 

b $-OC\underline{H}_2.CH_3$ in $(EtO)_2CH.C{\equiv}C.H$

c $-OC\underline{H}_3$ in $(MeO)_2.CH.CH_2.[C{\equiv}C]_2.CH_2.CH_2Br$

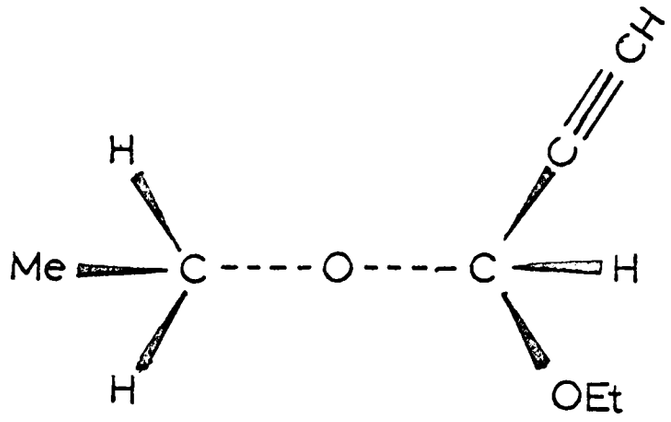


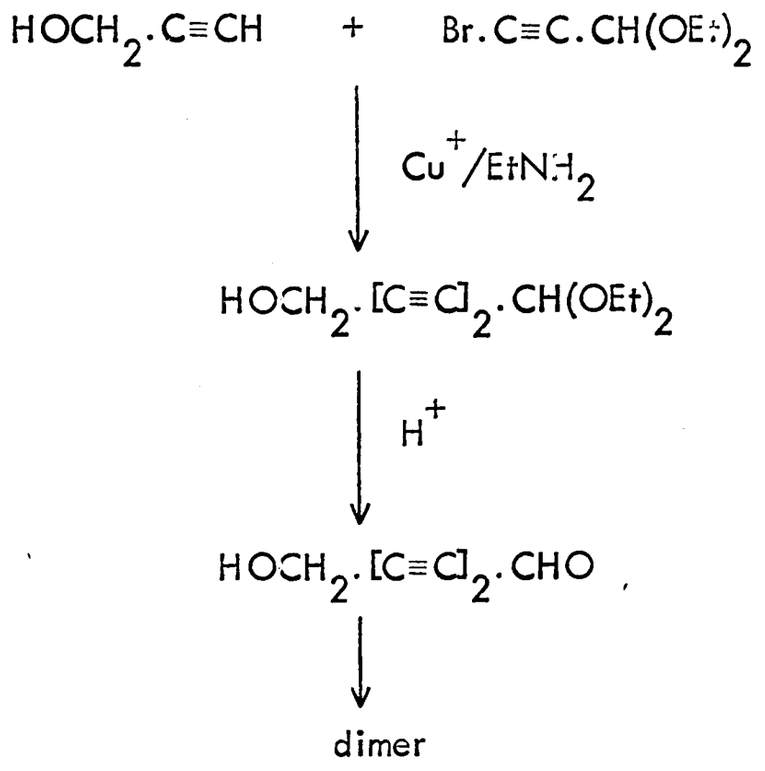
Figure 5

Accordingly the n.m.r. signal for the methylene protons appeared very complex (figure 6) and for benzene solutions even the methyl protons (τ 8.9) resonated as double triplets. Protons adjacent to electron withdrawing groups, e.g. hydroxyl and halide, resonate in this region (τ 6.2-6.8) and so their signals were obscured by the methylene protons' multiplet. This phenomenon led to confused analysis of spectra and was only sidestepped through the use of ethylene and dimethyl acetal analogues (figure 6).

Synthesis of further diyne acetals

A preliminary Cadiot-Chodkiewicz coupling reaction between the bromo-acetal (133a) and the readily available propargyl alcohol had been carried out to obtain a model hydroxyacetal (Scheme AB).

Scheme AB

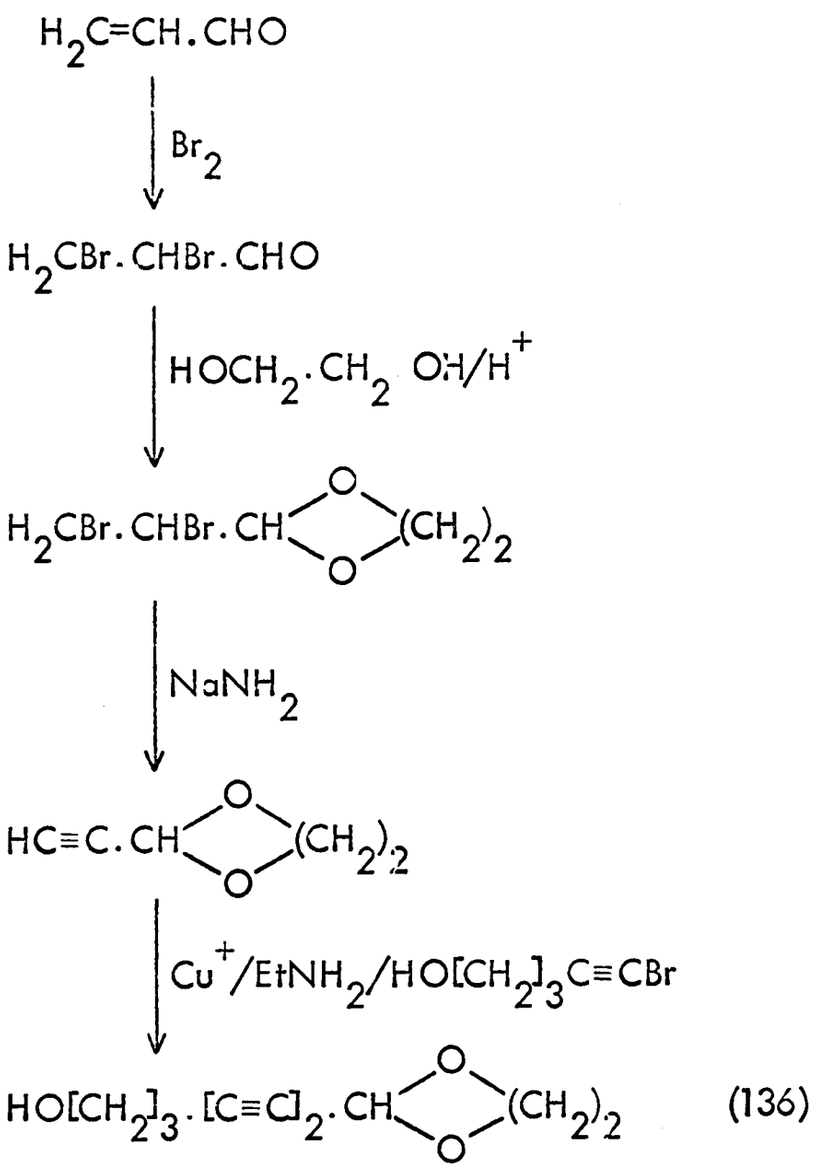


Acid hydrolysis of the acetal gave the corresponding aldehyde, which quickly dimerised (t.l.c. and u.v.) (see Table 12, page 43). This decomposition suggested that transacetalisation reactions, e.g. with ethanediol, which involved free diynals as intermediates would be contaminated with side products.

Transacetalisation of the diethyl acetal (116) with ethanediol was negligibly slow at 20°, and at higher temperatures polymerisation predominated; therefore the ethylene acetal (136) was prepared, as in Scheme Z, from C_3 and C_5 components (Scheme AC).

Models show that ring formation restricts the flexibility of the acetal end

Scheme AC



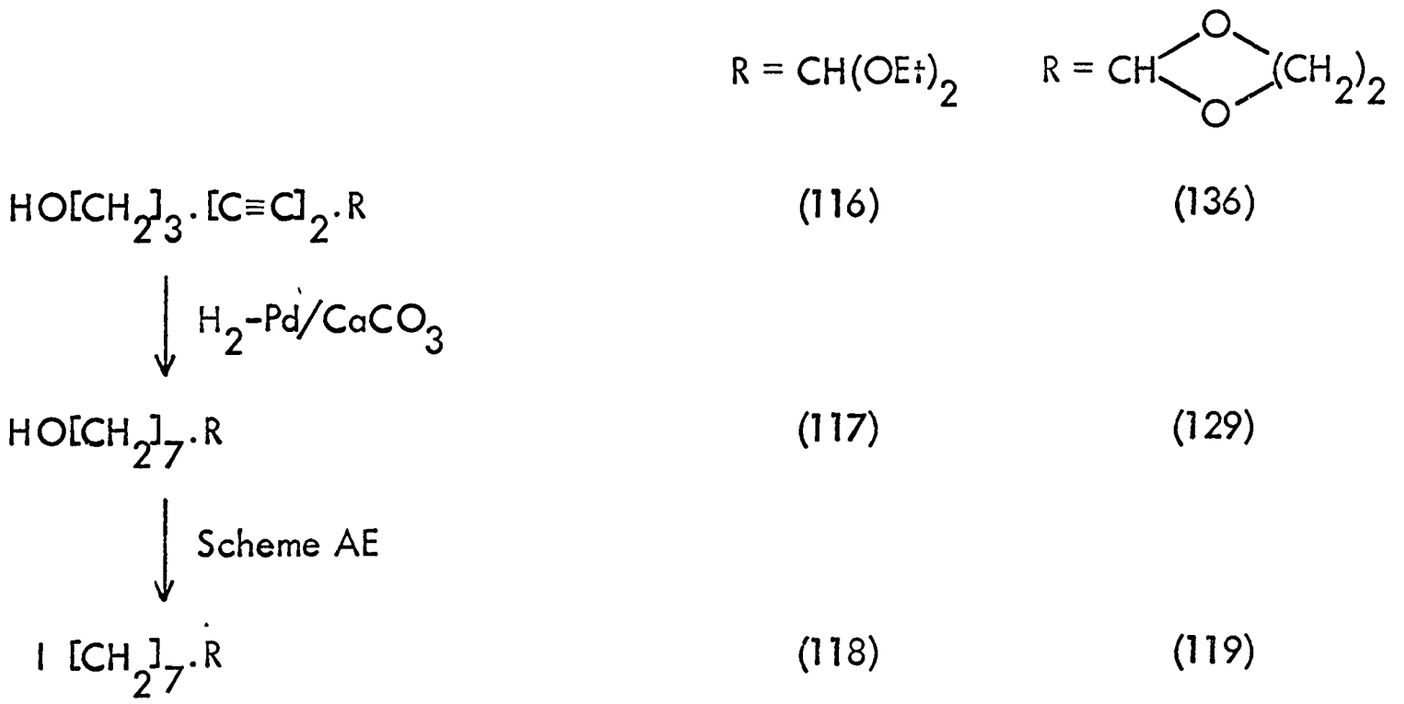
(136)

group and the oxygen lone pairs are more exposed than in the diethyl analogue. This was reflected in the greater instability of the ethylene acetals, less mobility on silica gel and a downfield shift (0.5 p.p.m.) for the methine proton signal. However acid hydrolysis of the ethylene acetal (136) was comparable in rate to the diethyl analogue.

Transformations of hydroxyoctadiynal acetals

Eventually the hydroxy-diyne (116) or (136) was to be saturated with tritium and converted into iodide (118) or (119) (Scheme AD). These reactions had

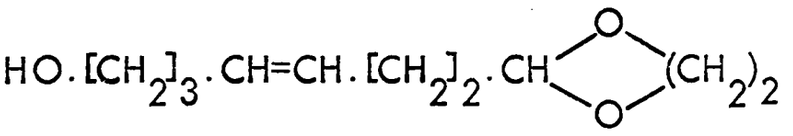
Scheme AD



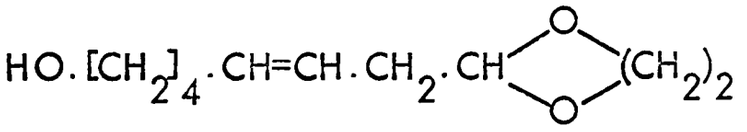
necessarily to work efficiently on the small scale, and be carried out under neutral or basic conditions in order to preserve the acetal functions.

a) Hydrogenation

Saturation of the ethylene acetal over a mild catalyst, e.g. palladium on calcium carbonate, stopped after the uptake of three equivalents of hydrogen, and gave one of or both the olefins (137) and (138) (n.m.r.). Bentley[121] has demonstrated that the absorption of three equivalents of hydrogen by hexa-2,4-

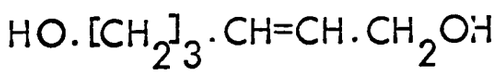


(137)



(138)

diyn-1,6-diol over the same catalyst afforded mainly hex—2-ene-1,6-diol (139).



(139)

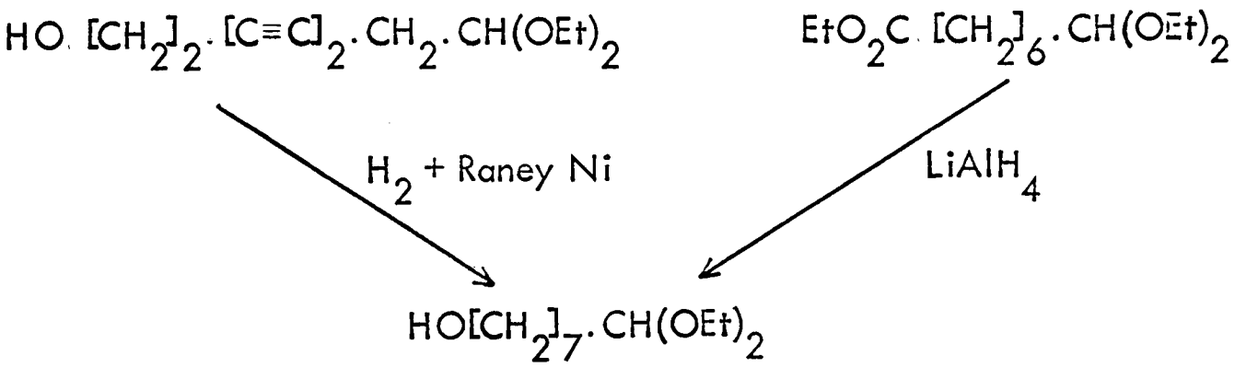
If a hydroxyl function three methylene groups distant is considered to hinder hydrogenation of a double bond, then structure (137) appears more plausible. This phenomenon, less marked with the diethyl acetal (116), could be related to the incomplete hydrogen uptake by the Lobelia triol acetonide (page 97).

The remaining double bond was saturated after either the catalyst concentration had been increased (2% to 10% w/w) or a more active catalyst, palladium on charcoal, had been added.

The purity of the hydroxyacetals could not be checked by g.l.c. analysis as the acetals decomposed on columns of apiezon, carbowax, silicon oil, and poly(ethylene glycol)succinate. Both 8-hydroxyoctanal and the diethyl acetal (117) exhibited the same chromatograms, comprising two peaks of variable intensity and retention time.

The diethyl acetal (117) had been previously prepared from the analogous

acetal-ester[142a] and the corresponding 3,5-diyne acetal:[142b]



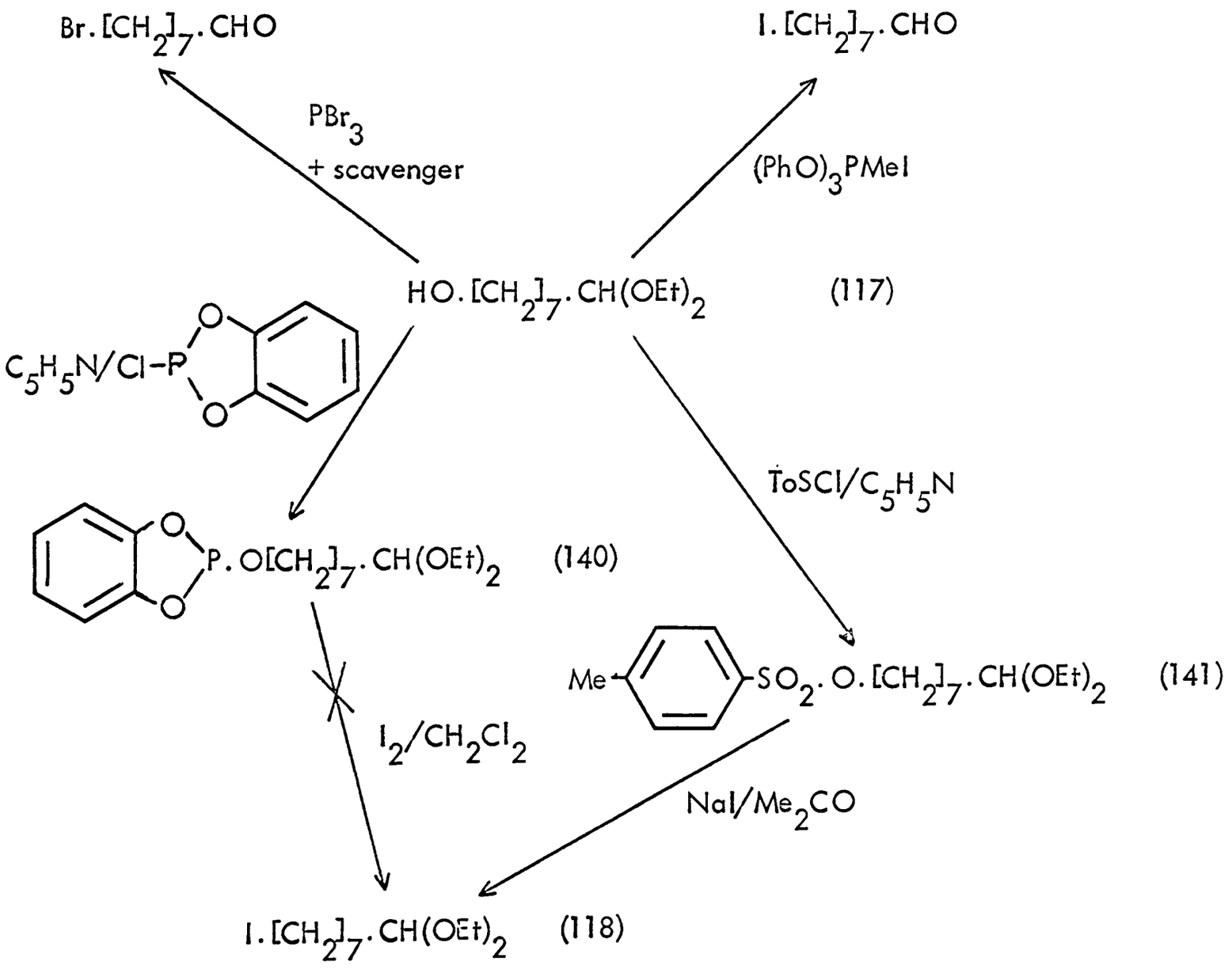
b) Halogenation

The necessity for non-acidic reaction conditions excluded many halogenation methods, because acidic side-products were not adequately neutralised by scavengers. Initial attempts to optimise conditions (Scheme AE) were conducted with the first available acetal (117).

Considerable hydrolysis to aldehyde was observed on treatment of the acetal with phosphorous tribromide in ether, even in the presence of potassium carbonate, pyridine or acetamide,-and also with triphenyl phosphite methiodide. Action of iodine on the o-phenylene phosphite ester (140) yielded an unexpected and unstable iodide.

Just as the o-phenylene ester was formed without simultaneous hydrolysis from o-phenylene phosphorochloridite in pyridine, the analogous tosylate (141) was successfully prepared from tosyl chloride in pyridine. The required iodide (118) was then prepared by nucleophilic displacement.

Scheme AE

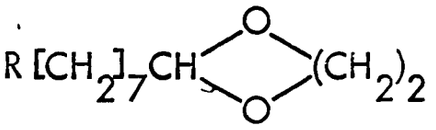


Halogenation methods with the hydroxyacetal (117)

Analysis, low frequency infrared and mass spectra suggested that the product was a mixture of iodide (118) and chloride (142). Repetition of the tosylation and

$$Cl\,[CH_2]_7\,CH(OEt)_2 \qquad (142)$$

iodination stages with the ethylene acetal (129) gave an oil whose n.m.r. spectrum for carbon tetrachloride solution showed two triplets at τ 6.5 and 6.8. That these signals were not due to two conformations of the iodoacetal (119) was demonstrated by rerunning the n.m.r. spectrum for dimethyl sulphoxide solution whereupon the relative areas of the two peaks did not alter. Accurate comparison spectra confirmed that an almost equimolar mixture of chloride (143) and iodide (119) had been obtained.



R = Cl (143)

R = I (119)

Control experiments ruled out chloride formation during the iodoacetal work-up, and implied that the chloro-impurity must be derived from the crude tosylate mixture (141), even though no free chloroacetal was detected (t.l.c.). T.l.c. pure tosylate afforded iodide free from chloride, but the overall yield was reduced by half. No attempt was made to remove the chloride precursor in the tosylation mixture since chlorides were presumed to be just as efficient as iodides in Grignard reactions.

The structure of the iodoacetal (118) was confirmed by comparison (n.m.r., i.r., m.s. and t.l.c.) with a sample unambiguously synthesised from ω-bromo-chloroheptane (Scheme AF).

Scheme AF

$$Cl.[CH_2]_7.Br$$

$$\downarrow Mg$$

$$Cl.[CH_2]_7.MgBr$$

$$\downarrow HC(OEt)_3$$

$$Cl.[CH_2]_7.CH(OEt)_2 \qquad (142)$$

$$\downarrow NaI/Me_2CO$$

$$I.[CH_2]_7.CH(OEt)_2 \qquad (118)$$

Nucleophilic displacement by iodide on the tosylate (141) or chloride (142) at elevated temperatures inconsistently produced the aldol condensation product (144).

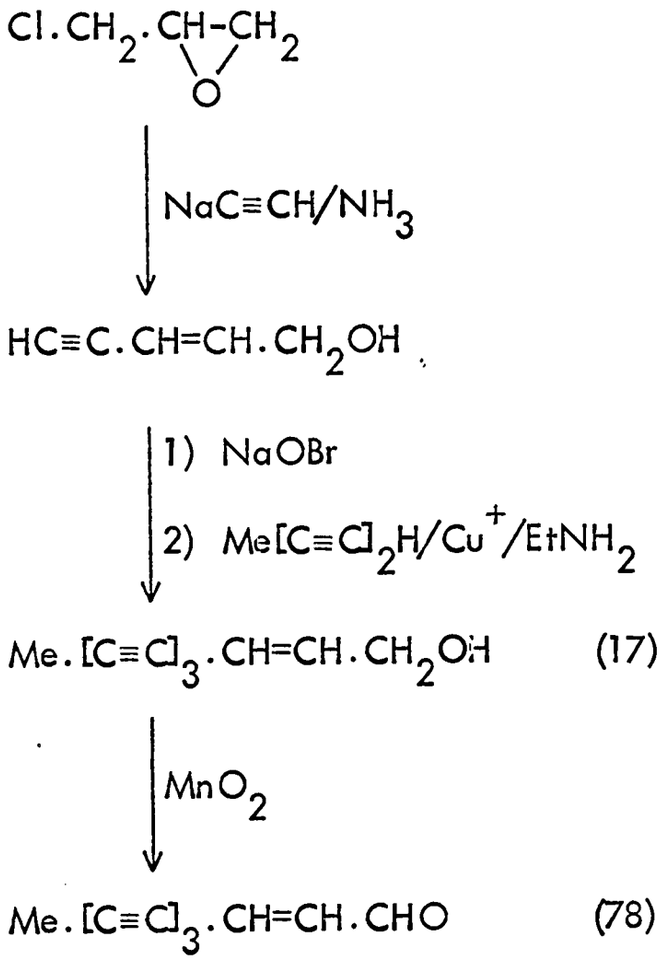
$$I.[CH_2]_7.CH(OH).CH_2.CO.Me \qquad (144)$$

Lately Stork[143] has described a new method of chloride formation via the tosylate, which is mild enough for dimethyl acetals.

Coupling reactions of dehydromatricarianal with halides

Dehydromatricarianal (78) was prepared from epichlorohydrin (Scheme AG).

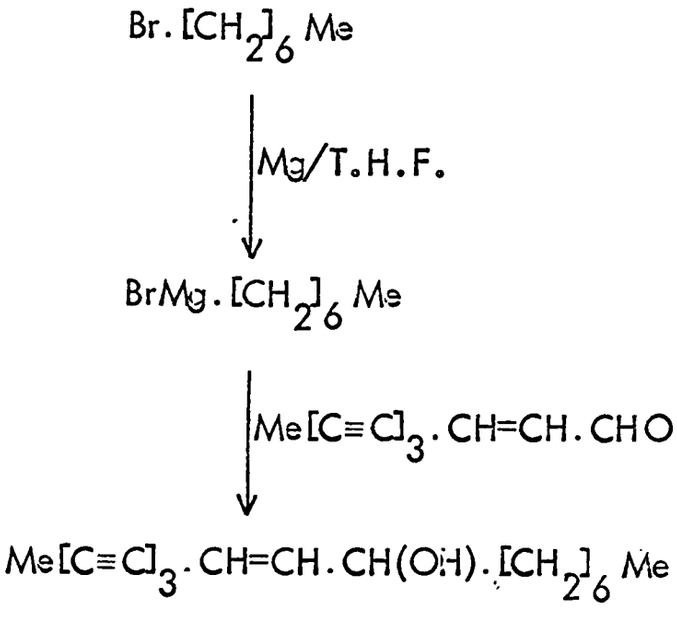
Scheme AG



Good yields for the condensation of sodium (or lithium) acetylide with epichlorohydrin were only obtained when the ammonia solvent had been distilled into the reaction vessel off sodium. This distillation presumably removed traces of water.

The aldehyde was initially condensed with the organomagnesium derivative from heptyl bromide to give the C_{17} alcohol (145) in 40% yield (1 mmole scale).

Scheme AH



(145)

The unstable product ran together with the starting aldehyde (78) on silica gel. After borohydride reduction of the mixture, the alcohols separated easily on column chromatography.

The iodoacetals (118) and (119), either pure or mixed with the analogous chloroacetal, refused to react with magnesium (0.5 mmole scale).

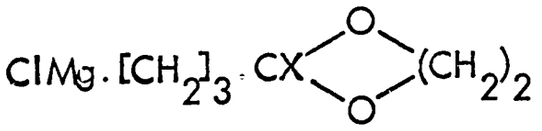
Strenuous attempts had been taken to avoid contamination from moisture - the solvent, either diethyl ether or tetrahydrofuran, had been distilled prior to use from lithium aluminium hydride, and a benzene solution of the iodoacetal had been evaporated to dryness. Trace quantities of ethylene dibromide and methyl iodide rapidly reacted with the magnesium in the presence of the iodoacetal. Activation by local heating, scratching, addition of either mercuric chloride or

iodine was without effect, and only slow and inconsistent bubbling was observed after the addition of entrainment reagent (ethylene dibromide).

The unreacted iodoacetal was recovered in good yield (90-95%), but recoveries were lower (70-80%) after entrainment reagent and aldehyde (78) had been added. No coupled product was detected.

The inability of these iodoacetals to form a Grignard reagent can be attributed to poisoning of the metal surface by either an impurity or a Grignard reagent by-product, or to an idiosyncrasy of the iodide's molecular geometry.

Short chain haloethers have been reported[144] to produce Grignard derivatives which preferentially undergo beta elimination or cyclisation. For example the organomagnesium derivative of the chlorodioxolan (146; X = Me) rearranged[145] in ether to the cyclobutane (147; R = $HOCH_2 \cdot CH_2$). The Grignard



(146)

$RO.\underline{C(Me).[CH_2]_3}$ (147)

reagent in tetrahydrofuran, and that of the nor-homologue (146; X = H) reacted[146,147] normally. The presence of a functional group susceptible to attack by Grignard reagents, as in 5-chloropentan-2-one (148; R = Me), did not prevent solvation of the magnesium,[148] though the product in this case was again a cyclobutane

$$Cl.[CH_2]_3.CO.R \qquad (148)$$

(147; R = H). In contrast Bucaille[149] has indicated that the chloroester (148; R = OMe), like methyl 9-iodononanoate (see page 58), did not attack magnesium; perhaps this is a result of surface contamination by a by-product similar to those mentioned above.

In the present investigation the magnesium did not dissolve when treated with the iodoacetals (118) or (119), so interaction by the terminal ether group was not ruled out. Hence 1-(2'-tetrahydropyranoxy)-8-iodooctane (120) was

$$I.[CH_2]_7.R \qquad R = CH(OEt)_2 \qquad (118)$$

$$= CH\begin{matrix}O\\ \diagup\ \diagdown\\ O\end{matrix}(CH_2)_2 \qquad (119)$$

prepared (Scheme AI), which contains iodine and oxygen atoms arranged as in the iodoacetals. This should, like the homologue (150),[150] form a Grignard reagent.

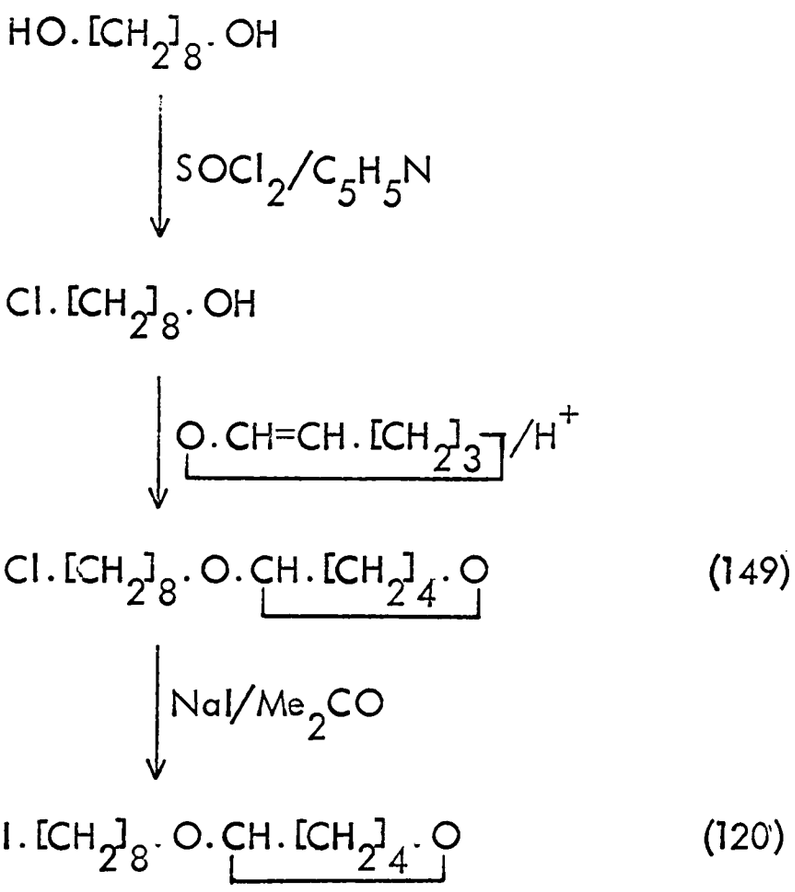
$$Cl.[CH_2]_4.O.\underline{CH.[CH_2]_4.O} \qquad (150)$$

Moreover, the coupled product (151) with dehydromatricarianal, after acid hydrolysis, oxidation and methylation, would be the wanted C_{18} ester (109).

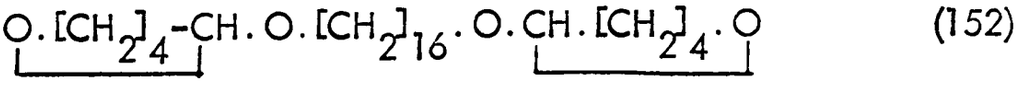
$$Me.[C\equiv C]_3.CH{=}CH.CH(OH).[CH_2]_8.O.\underline{CH.[CH_2]_4.O} \qquad (151)$$

$$Me.[C\equiv C]_3.CH{=}CH.CH(OH).[CH_2]_7.CO_2Me \qquad (109)$$

Scheme AI



No sign of Grignard reagent formation was noted (6 mmole scale),and after the addition of entrainment reagent and aldehyde (78) a mixture of triyn-ene alcohols was obtained. The components were separated after manganese dioxide oxidation of the mixture, and spectral evidence demonstrated that the coupled product (151) had been produced, albeit in 1% yield. Even the tetrahydropyranyl dimer (152), generated by Würtz-type coupling, had been formed in greater amount (3% yield).

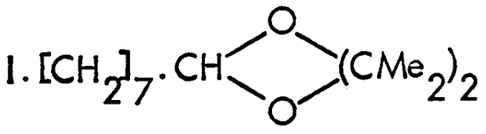


On the other hand, steric hindrance by a terminal tetrahydropyranyl group has been invoked[134] to explain the immunity of the alcohol (153) to tosylate formation.

$$HOCH_2.CH{=}CH.[CH_2]_8.O.\underline{CH.[CH_2]_4.O} \qquad (153)$$

Thus the terminal group in the acetals or tetrahydropyranyl ether may bend around and interfere sterically (or through the oxygen lone pairs) with the approach of the iodine atom onto the metal surface.

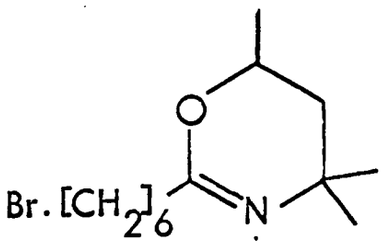
The tetramethyl ethylene acetal (154) contains a more bulky terminal group than (119), but here the oxygen atoms are flanked by methyl groups. Grignard



(154)

reagent formation may well be possible with this iodide, but the vigorous conditions[151] needed to hydrolyse these acetals ensure that coupling with a polyyne aldehyde would be of academic interest only.

Recently the dihydro-1,3-oxazine group[152] has been used for protecting long chain ω-bromocarboxylic acids. These derivatives, e.g. (155), have been



(155)

reported to form Grignard reagents, which suggest their use in future syntheses of the C_{18} ester (109).

PART IV

Polyacetylenes from the Lobeliaceae

Previous work

Over 1,000 species in the Compositae and Umbelliferae families have been screened for polyacetylenes, but few other plant families have been examined.[1] Recently in this department, systematic screening of further plant families has been started in the hope of detecting new polyacetylenes, the structures of which could be of biosynthetic interest.

Bentley and Jenkins have recently examined several species of the Campanulaceae. Roots of Campanula glomerata L. contained[153] small amounts (8 mg./kg.) of the previously unknown tetrahydropyranyl alcohols (156a and b). In Campanula medium L. and Campanula pyramidalis L. complex mixtures of similar tetrahydropyranyl ethers (156b, c, d) and their open chain analogues (157b, c, d) were discovered.[90,121] Independently Lam[154] detected polyynes

$$R.[C\equiv C]_2.CH=CH.\underbrace{CH.CH_2.CH_2.CH_2.CH_2.O} \qquad (156)$$

$$R.[C\equiv C]_2.CH=CH.CH(OH).CH_2.CH_2.CH_2.CH_2OH \qquad (157)$$

a $R = HOCH_2.CH_2.CH_2$

b $HOCH_2.CH=CH$

c $H_2C=CH.CH(OH)$

d $Me.CH_2.CH_2(OH)$

in other Campanula species, which were found to be identical with some of our samples (156a and b). In Trachelium caeruleum L. the tetrahydropyranyl polyyne (156b) was detected,[90,121] together with corresponding ene-diynes in which the 8,9-double bond was oxygenated like (158).

$$HOCH_2.CH{=}CH.[C{\equiv}C]_2.CH(OH).CH(OH).\underbrace{CH.[CH_2]_4.O}$$ (158)

The Lobeliaceae are closely related[155] to the Campanulaceae, but had not previously been examined for polyacetylenes.

Present work

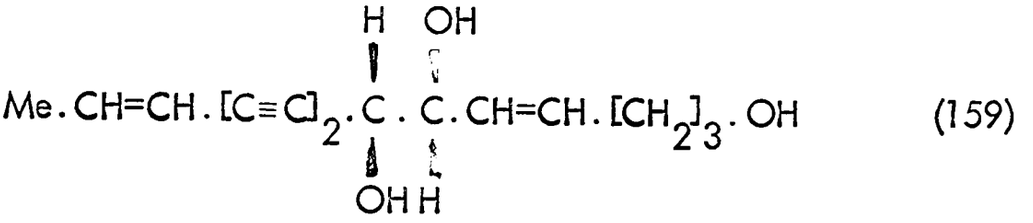
Lobelia cardinalis L., Lobelia syphilitica L. and their hybrid Lobelia x vedariensis were grown in Oxford. The undried above-ground and below-ground parts were separately investigated for polyacetylenes.

Lobelia cardinalis L.

The aerial parts of this species contained larger amounts of polyacetylenes (45 mg./kg.) than the roots (3 mg./kg.). As for the latter, the polyyne fraction (2 mg.) was embedded in so much plant pigment (1 g.) after column chromatography on silica gel that purification was not continued; its mobility on silica gel and u.v. spectrum suggested it to be a diyn-enediol.

The major component (80%) from the above-ground parts was fully

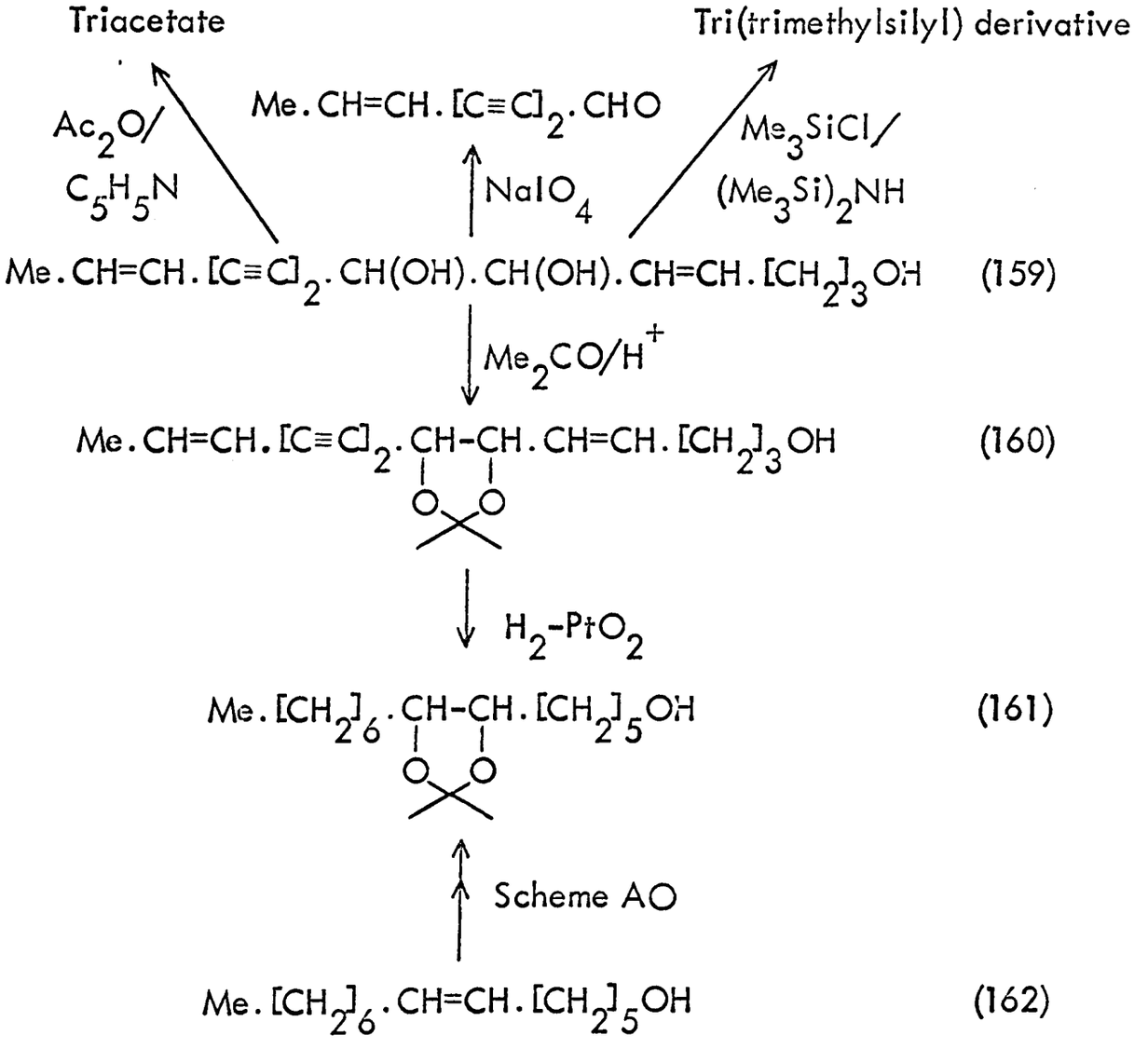
characterised as the triol (159). Its structure was deduced from data obtained on



(159)

the triol and its derivatives (Scheme AJ). The relative stereochemistry of the

Scheme AJ



Outline of reactions used for structure elucidation of the triol (159)

vic diol grouping was determined by the preparation of the saturated threo dioxolan from both the triol (159) and tetradec-6-en-1-ol (Scheme AJ). The absolute configuration followed by analogy from the optical rotations of the saturated dioxolan (161) and those of related compounds of known configuration. The structural elucidation is discussed in detail later.

Traces of a diyne-diene and a triyn-ene were also detected. T.l.c. behaviour suggested that the former was a tetraol; however attempted acetylation caused decomposition. The triyn-ene had the polarity of a triol on silica gel layers, but the amount remaining after purification was too small for further investigation.

Lobelia syphilitica L.

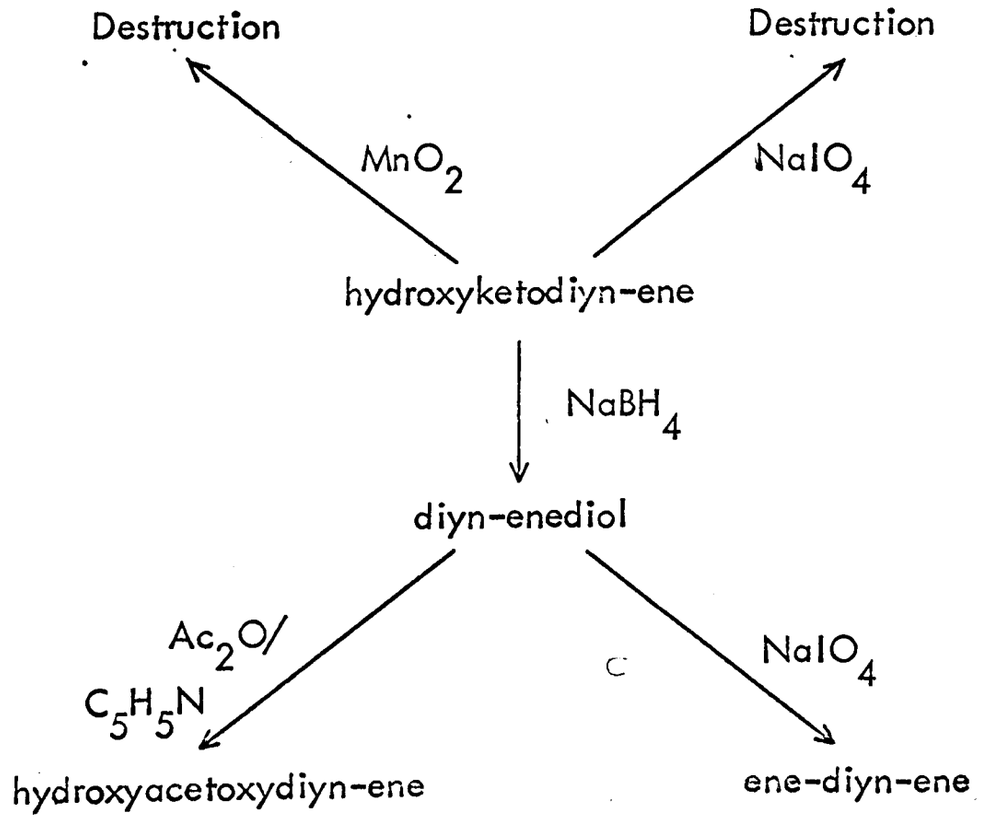
The roots of this species contained small quantities of diyn-enetriols (3 mg./kg.), none of which had similar t.l.c. behaviour to the triol (159). No polyyne chromophores were detected in the above-ground parts (1 kg.). Future work on this plant obviously requires more plant material.

Lobelia x vedariensis

The triol (159) was isolated from the aerial parts (20 mg./kg.) and the roots (65 mg./kg.) of this hybrid.

A less polar fraction (6 mg./kg.), which appeared homogeneous on thin layers when developed with ether-light petroleum and chloroform, was obtained from the root extract. Spectral data indicated the presence of a hept-trans-5-ene-1,3-diynyl fragment (n.m.r. and u.v.), a carbonyl group ($\nu_{max.}$ 1725 cm.$^{-1}$) and a hydroxyl function associated with a strong hydrogen bond. No further functionalities were revealed by chemical reactions (Scheme AK), and no definite

Scheme AK



Transformations on hydroxyketodiyn-ene fraction from

Lobelia x vedariensis

molecular ions were observed in the mass spectra of the metabolite and the acetylated reduction product. In fact, these transformations suggested that the original fraction was a mixture of diyn-enes,as minor products were noted in both the reduction and acetylation reactions. Further work was prevented by lack of material.

Structural elucidation of the triol (159)

a) Spectroscopic and degradative data

The ene-diyne fraction from Lobelia cardinalis L. contained a large amount of chlorophyll. Passage down a column, dry-packed with t.l.c. grade silica gel, effectively removed most of the non-acetylenic impurities, and then subsequent thin layer chromatography was not marred by the streaking associated with plant pigments.

The oily diyn-enetriol, $[\alpha]_D$ +33°, had extinction coefficients significantly lower (ca. 30%) than typical values.[153] Characteristic C-O stretching bands for both primary (1020 $cm.^{-1}$) and secondary (1040 $cm.^{-1}$) alcohols were evident in the infrared spectrum, though no persistent bonded-hydroxyl band (3400 $cm.^{-1}$) was observed in diluted samples. Bands, at $\nu_{max.}$ 1660, 1620, 970, 945 and 910 $cm.^{-1}$, indicated the presence of conjugated and non-conjugated trans substituted double bonds.

The n.m.r. spectrum (Table 14) was most informative, although signals for only two vinyl protons could be unequivocally assigned, and H(6) and H(7)

Table 14

N.m.r. data for Lobelia triol (159)

Me.CH=CH.[C≡C]$_2$.CH(OH).CH(OH).CH=CH.CH$_2$.CH$_2$.CH$_2$OH
14 13 12 7 6 5 4 3 2 1

H	τ		Integral	Description
	$CDCl_3$-D_2O	$(CD_3)_2SO$		
1	6.33	6.54	2	triplet, J 7 Hz
2	8.34	8.5	2	multiplet
3	7.84	7.94	2	double triplet, J 7 Hz
4	4.1	4.3*	1	double triplet, J 16 and 7 Hz
5	4.4*	4.3*	1	
6, 7	5.8	6.0	2	multiplet (AB system)
12	4.4*	4.3*	1	
13	3.62	3.60	1	double quartet, J 16 and 6.5 Hz
14	8.16	8.19	3	double doublet, J 6.5 and 1.5 Hz

* Unresolved signals

appeared as an AB system for both deuteriochloroform and dimethyl sulphoxide solutions. The mass spectrum of the metabolite did not show prominent peaks above m/e 200, whereas the triacetate exhibited a molecular ion (8%) equivalent to $C_{14}H_{18}O_3$ as the chemical formula for the triol.

When the triol was treated with aqueous periodate, the ultraviolet spectrum shifted bathochromically by 30 nm.. Octa-trans-6-ene-2,4-diynal (163) (prepared as in Scheme AL) was shown to be the only polyyne product. The other, more

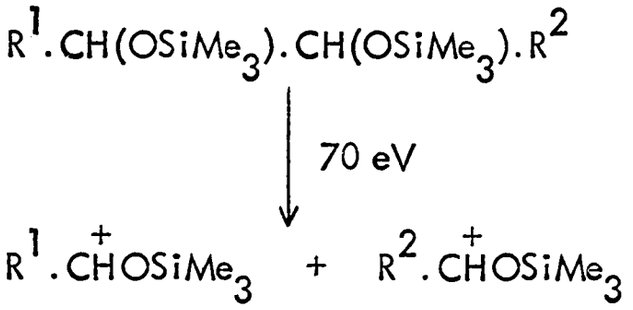
Scheme AL

$$\begin{array}{l} Me.CH{=}CH.C{\equiv}CH \quad + \quad Br.C{\equiv}C.CH_2OH \\ \qquad\qquad \Big\downarrow \; Cu^{+}/EtNH_2 \\ Me.CH{=}CH.[C{\equiv}C]_2.CH_2OH \\ \qquad\qquad \Big\downarrow \; MnO_2 \\ Me.CH{=}CH.[C{\equiv}C]_2.CHO \end{array} \qquad (163)$$

polar aldehyde, was extracted with chloroform, but was too unstable to permit comparison of g.l.c. and t.l.c. behaviour with synthetic hydroxy-aldehydes.

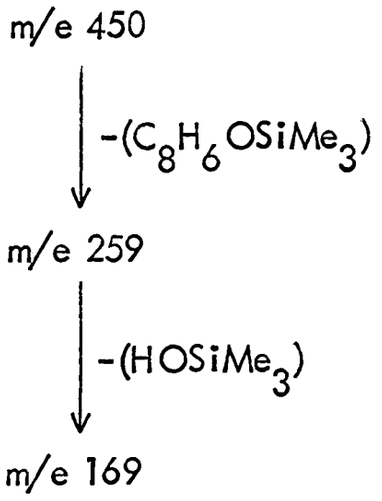
Fragmentation analogous to periodate cleavage[156] occurs for the di(trimethyl-silyl)ether of a vic-diol in the mass spectrometer (Scheme AM).

Scheme AM



The mass spectrum of the trimethylsilyl derivative showed dominant peaks at m/e 259 and 169. The former ion (164) had the predicted mass for the non-acetylenic fragment derived by Scheme AM, and lost trimethylsilyl alcohol to afford the latter ion (the expected metastable peak was observed). The relative intensities[157] of the isotopic peaks confirmed these molecular formulae (Scheme AN),

Scheme AN



and hence the ion (164) had structure (164a or b).

$C_{12}H_{27}O_2Si_2$ (164)

$Me_3SiO\overset{+}{C}H.CH=CH.[CH_2]_3.OSiMe_3$ (164a)

$Me_3SiO\overset{+}{C}H.CH_2.CH=CH.[CH_2]_2.OSiMe_3$ (164b)

In this cleavage, the polyacetylenic fragment was the one which was not detected.

From the above data, the partial structure (165) was inferred for the triol.

$$Me.CH=CH.[C\equiv C]_2.CH(OH).CH(OH)\begin{cases}.CH_2.\\.CH=CH.CH_2.\\.CH_2OH\end{cases} \quad (165)$$

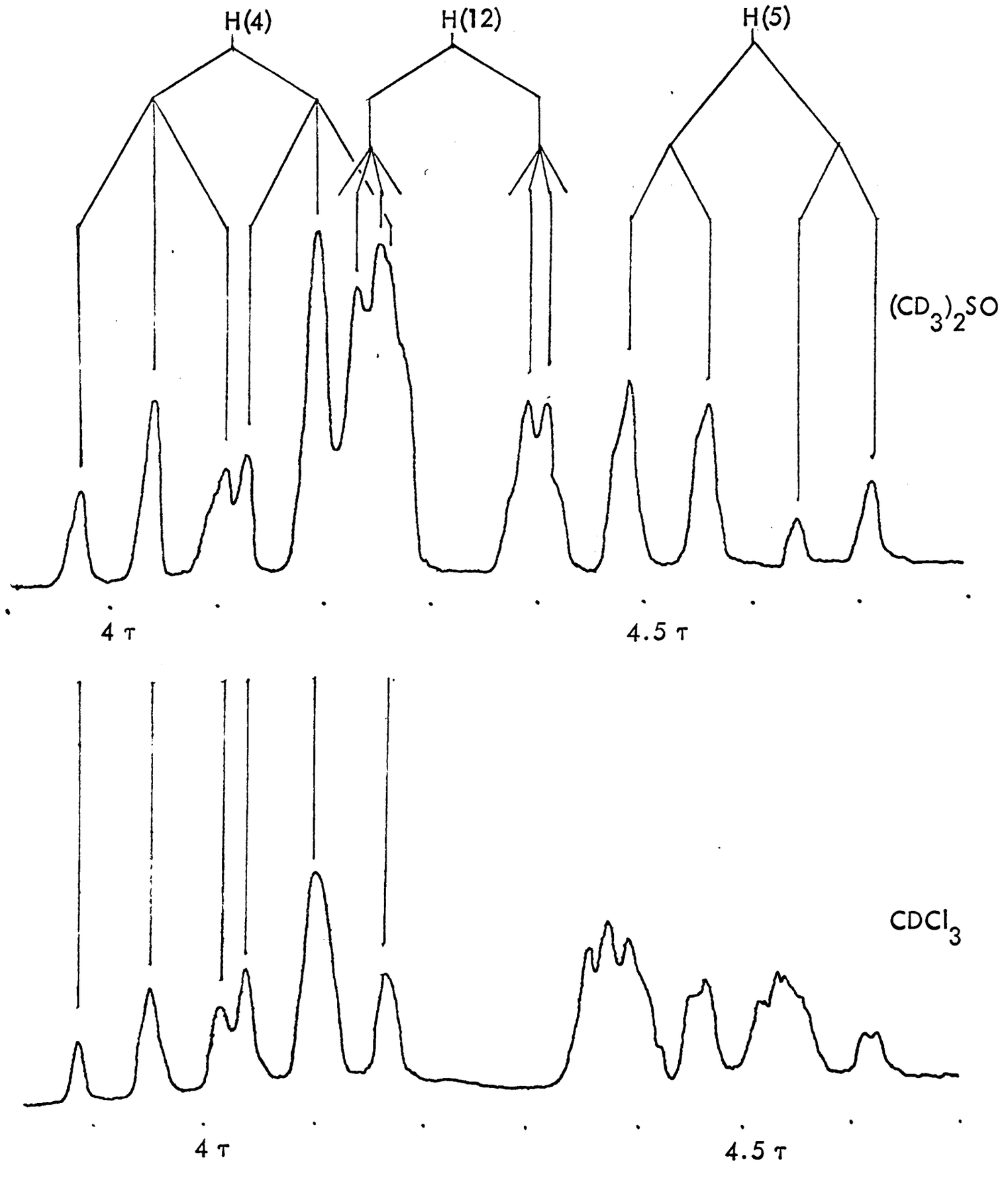
The exact location of the isolated double bond would have followed from the identification of both fragments from the periodate cleavage, or complete analysis of the vinyl region in the nuclear magnetic resonance spectrum (spin decoupling was not possible).

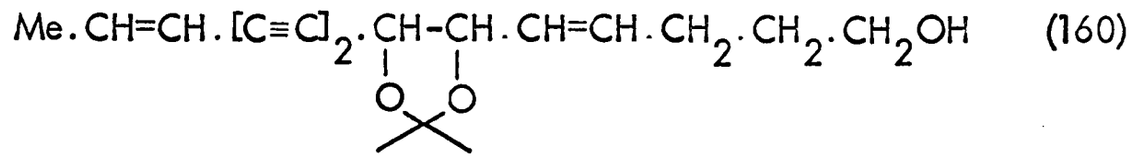
b) Data obtained from the triol acetonide (160) and perhydroderivative (161)

Acetone in the presence of anhydrous copper sulphate and p-toluene sulphonic acid reacted preferentially with the vic-diol group and yielded a 1,3-dioxolan (160 or 166). The dioxolan, which was more stable than the triol, did not crystallise and gave a carbon value from combustion analysis 3% too low for either structure.

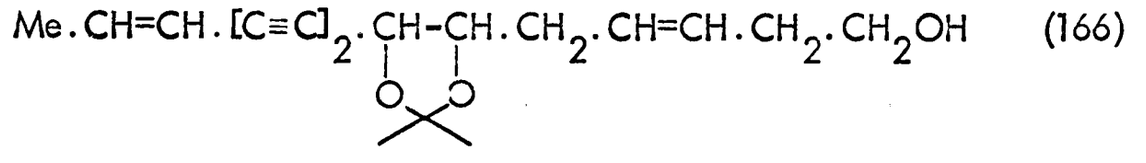
Figure 7

Partial n.m.r. spectra of Lobelia triol acetonide (160)





(160)

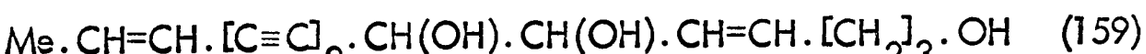


(166)

In an attempt to obtain a crystalline derivative the p-phenylazobenzoyl ester was prepared, but this also failed to crystallise. Hydrogenation of the ester resulted in a complex mixture of perhydroderivatives.

The n.m.r. spectrum (Table 15) of the isopropylidene derivative in dimethyl sulphoxide, unlike deuteriochloroform, clearly showed resonances for the protons of the isolated double bond as a double triplet and a double doublet (figure 7). Structure (160) must therefore represent the isopropylidene derivative of the natural triol. Would structure (166) be correct, the vinyl proton signals would appear as two double triplets. Whilst the shifts for H(12) and H(13) were unaltered from those for the triol, the double triplet H(4) was slightly deshielded by the 1,3-dioxolan ring, as for the H(6) and H(7) signals. The last two remained as an AB system for acetonitrile, deuteriochloroform and dimethyl sulphoxide solutions.

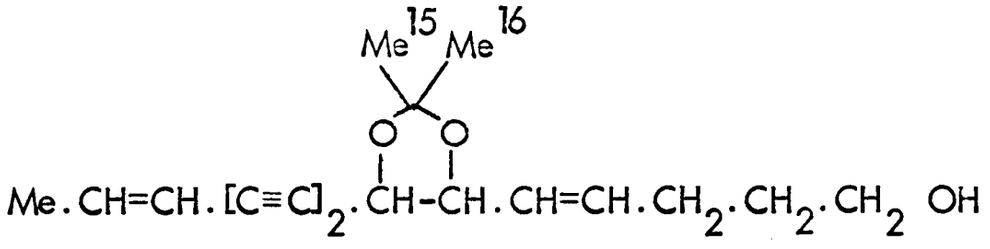
Thus the structure of the Lobelia triol must be represented by formula (159).



(159)

Table 15

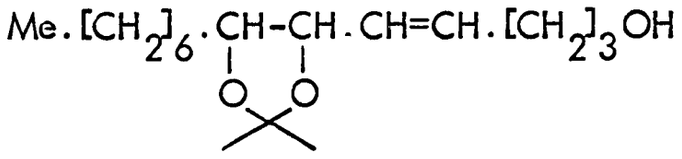
N.m.r. data for Lobelia triol acetonide (160)



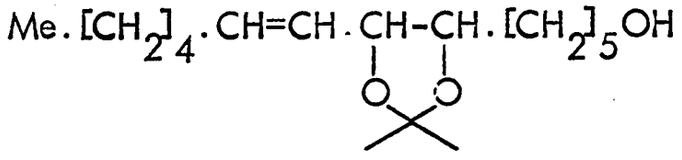
H	τ $CDCl_3$-D_2O	τ $(CD_3)_2SO$	Integral	Description
1	6.35	6.61	2	triplet, J 6.5 Hz
2	8.29	8.47	2	multiplet, J 7 Hz
3	7.8	7.94	2	double triplet, J 7 Hz
4	4.03	4.12	1	double triplet, J 16 and 7 Hz
5	4.5*	4.6	1	double doublet, J 16 and 7 Hz
6, 7	5.6	5.55	2	AB system
12	4.5*	4.34	1	double quartet, J 16 and 1.5 Hz
13	3.61	3.54	1	double quartet, J 16 and 6.5 Hz
14	8.17	8.21	3	double doublet, J = 6.5 and 1.5 Hz
15	8.60	8.66	3	singlet
16	8.53	8.63	3	singlet

* not resolved (see figure 7)

Hydrogenation of the acetonide (160) over palladium on calcium carbonate stopped at the monoene stage, though n.m.r. spectroscopy on the product could not differentiate between the structures (167) or (168) (see discussion in the iodoacetal section, p. 72).



(167)



(168)

One ring methine proton clearly showed up as a quartet (τ 6.1, J 8 Hz); its position was a little upfield from the shift of a typical erythro dioxolan (τ 6.05).[158] The coupling constant also alluded to the threo configuration.[159] Moreover, the ring methyl protons resonated as a singlet, whereas in the erythro isomer these were anticipated to appear as a doublet.[158]

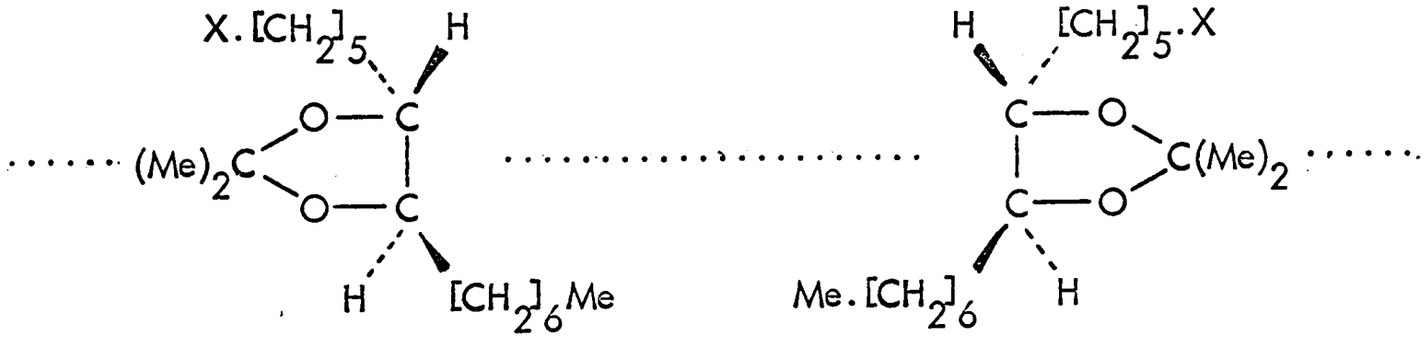
Further saturation over platinum oxide gave a small amount (3.5 mg.) of the required isopropylidene derivative (161). This perhydro derivative, t.l.c. and g.l.c. pure, had specific rotation of the same order and sign (+) as the triol (159) but smaller than the rotation of the more rigid unsaturated analogue (160).

Consideration of the symmetry elements in the symmetrical acetonide, 2,2-dimethyl-4,5-diheptyl-1,3-dioxolan (169), reveals that only the erythro

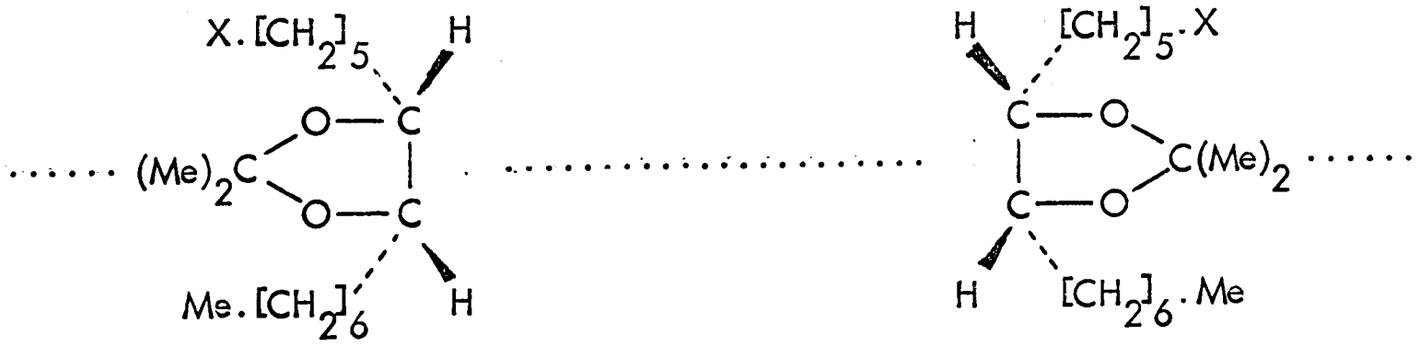
isomer is internally compensated, since a mirror plane can be drawn perpendicular to the mean ring plane conformation through the ring methyls (figure 8). As a result

Figure 8

Threo isomers (a)



Erythro isomers (b)

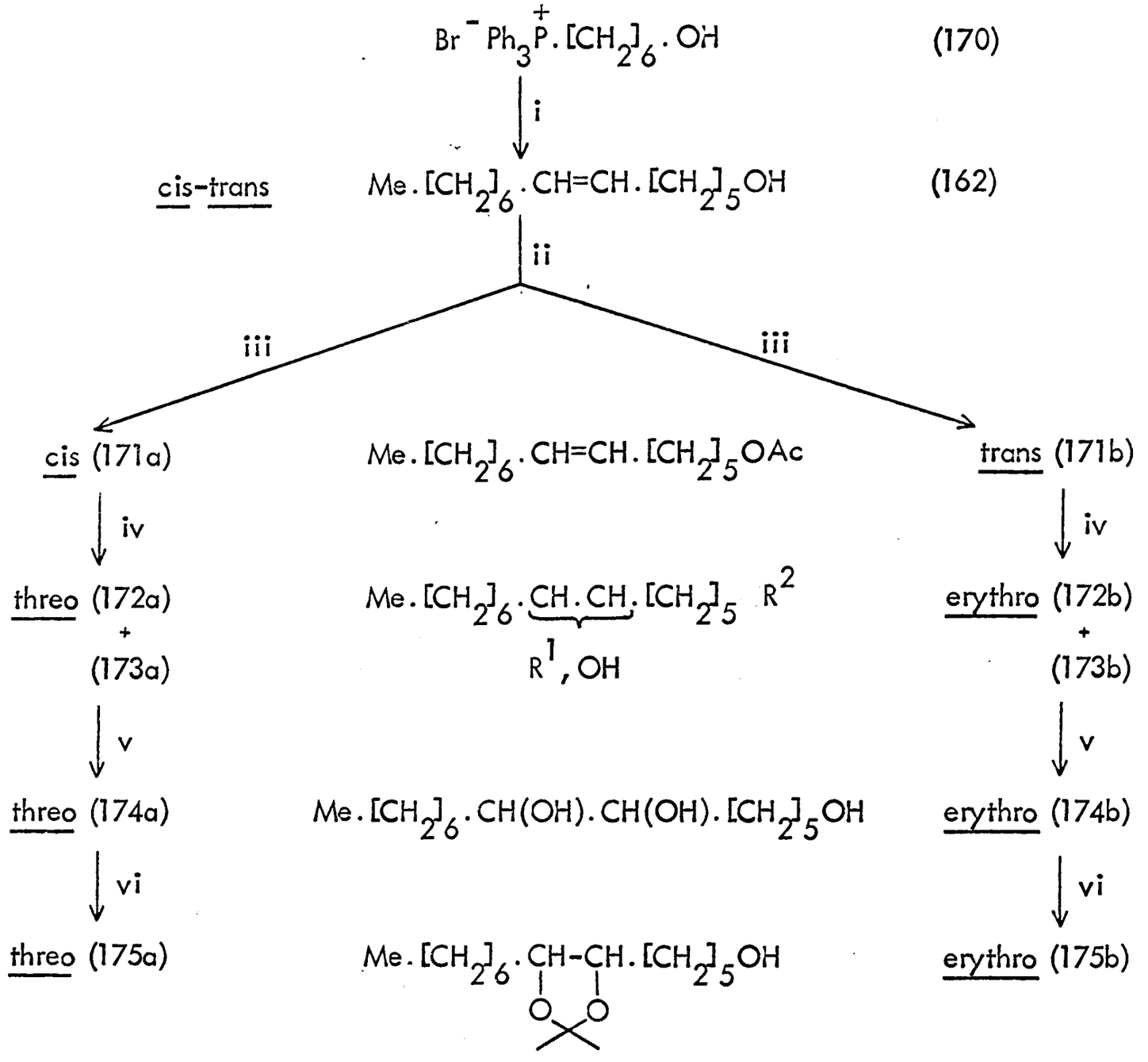


(The dotted line represents the mirror plane, perpendicular to the page and passing through the ring methyls which are located above and below the page.)

Stereoisomers of acetonides (161; X = OH) and (169; X = Et)

of Tschugaeff's distance rule,[160] the erythro hydroxy dioxolan (161) is anticipated to possess small optical rotation; the vic diol had therefore the threo configuration.

Scheme AO



R^1 =OAc (172), OH (173); R^2 =OAc

or R^1 =OCHO; R^2 =OCHO (172), OH (173)

Reagents: i) BuLi; Me.$[CH_2]_6$.CHO; ii) Ac_2O/C_5H_5N; iii) SiO_2-$AgNO_3$; iv) $MeCO_3H$ or H_2O_2-HCO_2H; v) KOH-MeOH; vi) $Me_2CO/CuSO_4$

Synthesis of perhydro derivative (161)

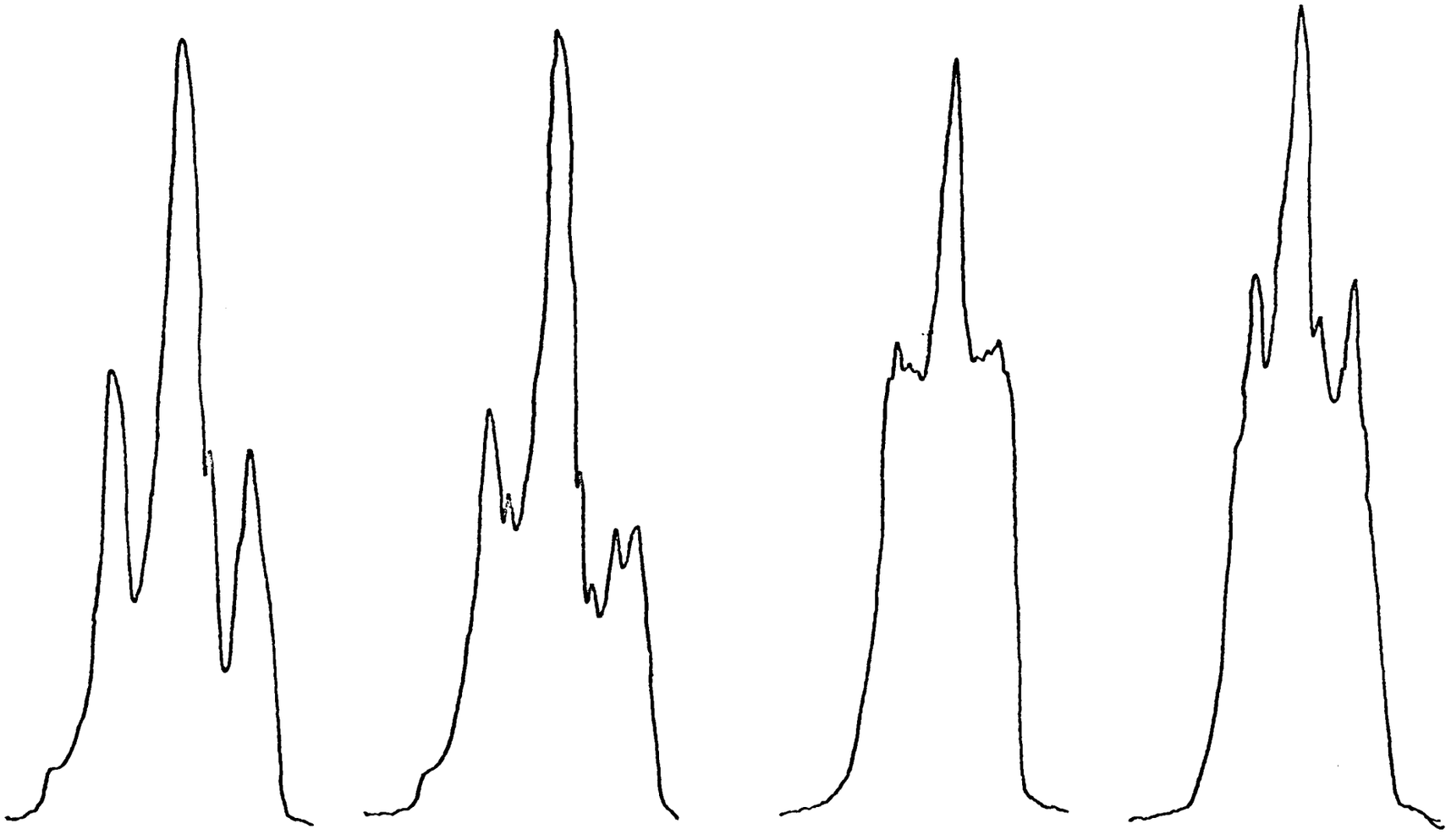
The stereoisomers of the acetonide (175a and b) were prepared as in Scheme AO. Wittig reaction[8] between 6-hydroxyhexyl triphenylphosphonium salt (170) and octanal gave a cis/trans tetradec-6-en-1-ol (162) mixture (3:1). The isomers were separated as acetates (171) on layers of silica gel impregnated with silver nitrate. The acetates (171a and b) had identical retention times, but could be differentiated by their infrared spectra. The line shapes of the vinyl proton resonances of the acetates (171a and b) and their fatty acid counterparts, oleate and elaidate were also distinctive for each double bond isomer (figure 9).

Originally the double bond was to be epoxidized, the ring opened in a trans fashion by acid hydrolysis, and the dioxolan (175) formed from the vic-diol. In practice the remaining steps in Scheme AO were carried out with the cis:trans acetate mixture (3:1) and the acetonide product (175) so obtained was a 3:1 threo-erythro mixture. The pure trans olefin (171b) led, by the same sequence, to the erythro isomer (175b) identical with the minor component in the previous preparation.

Treatment of the acetate (171) with peracetic or performic acid afforded mainly hydroxydiesters (172) and dihydroxyesters (173). Small amounts of epoxides (176) were also isolated from the peracetylation reaction mixture, and could be

$$Me.[CH_2]_6.\underset{\diagdown\,O\,\diagup}{CH-CH}.[CH_2]_5\,OAc \qquad (176)$$

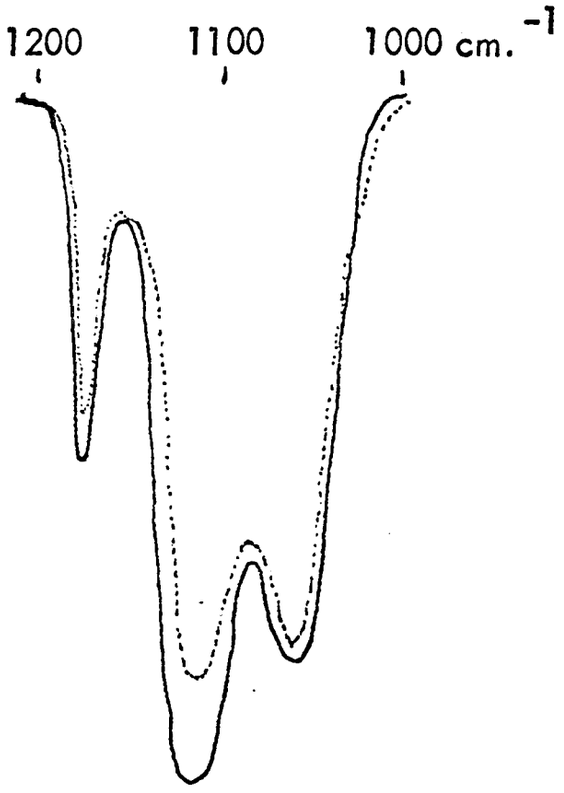
Figure 9



1-acetoxytetradec-6-ene

Vinyl proton resonances at 100 MHz

Figure 10



Partial infrared spectra of erythro (····) and threo (——)

2,2-dimethyl-4-heptyl-5(5'-hydroxypentyl)-1,3-dioxolan (175)

obtained isomerically pure by preparative layer chromatography. The cis-epoxide (176a) showed a downfield shift for the methine protons (τ 7.3) over the trans (threo) isomer (τ 7.53), as for the acetonide counterparts (175a and b) (see page 97); the configurations were upheld by the generation of the erythro acetonide (175b) after carrying out Scheme AO on the trans epoxide.

The retention time of the threo acetonide (175a) was significantly shorter than the erythro isomer (175b). Although a predominantly threo mixture was being compared with the pure erythro acetonide, the subtle intensity changes of the C-O bands in the 1200-1000 $cm.^{-1}$ region noted by Ewing[158] were confirmed (figure 10). In addition the intensity of the (M-15) peak was noted to be greater for the erythro isomer.[161]

Comparison of the retention times of the isomers (175a and b) with that of the dioxolan (161) confirmed that the natural product had the threo stereochemistry.

Absolute configuration

Relevant analogies can be drawn from examples in the fatty acid field to show that specific and molar rotations of long chain threo 1,2-glycols do not change drastically on:

i) chemical transformations operating on groups far distant from the diol groups, e.g.:

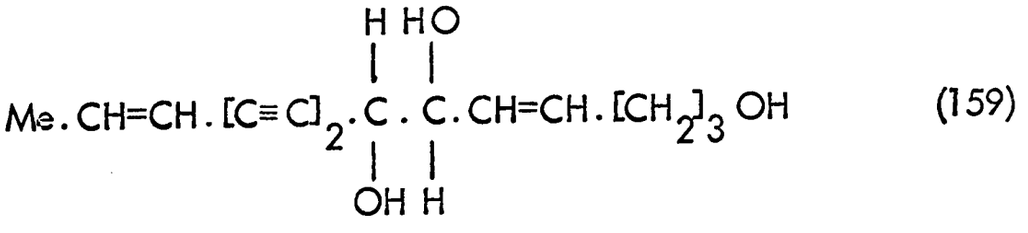
threo 9,10-Dihydroxystearic acid	*threo* Octadeca-1,9,10-triol[162]
$[\alpha]_D$ +23.5°	$[\alpha]_D$ +25.5°
$[M]_D$ +74°	$[M]_D$ +77°

ii) acetonide formation

threo 9,10-Dihydroxypalmitic acid	O-Isopropylidene derivative[158]
$[\alpha]_D$ -22.2°	$[\alpha]_D$ -26.8°
$[M]_D$ -64°	$[M]_D$ -88°

Recently Morris predicted[163,164] the configuration of (+) *threo*-9,10-dihydroxystearic acid as (9-R,10-R) and proved[163] the configuration of (+) *threo*-12,13-dihydroxyoleic acid ($[\alpha]_D$ +21.1°, $[M]_D$ +66°) as (12-R,13-R). Independently Irie has shown that (+) *threo*-pentadeca-6,7-diol[83] ($[\alpha]_D$ +25.5°, $[M]_D$ +62°) has the (6R, 7R) configuration.

The perhydro derivative (161) had $[\alpha]_D$ +25°, $[M]_D$ +71°, and the full structure of the (6R, 7R) triol is therefore that given by formula (159).



(159)

Table 16

vic-Diols (from plants)	Relative stereochemistry	Source	Ref
Ph.[C≡C]$_2$.[CH(OH)]$_2$.CH$_2$OH	threo	Dahlia hybrid - 'Dolce Vita'	43
Ph.[C≡C]$_2$.[CH(OH)]$_2$.Me	threo	or 'Preference'	
Me.CH=CH.[C≡C]$_2$.CH=CH.[CH(OH)]$_2$.CH=CH$_2$	*	Dahlia hybrid - 'Deerplay'	11
Me.[C≡C]$_3$.CH=CH.[CH(OH)]$_2$.CH=CH$_2$	*	Pentzia grandiflora L	165
Me.CH=CH.[C≡C]$_2$.CH=CH.[CH(OH)]$_2$.Et	*	Äthusa cynapium L.	166
Me.[CH(OH)]$_2$.CH=CH.C≡C.[CH=CH]$_2$.Et	*	Äthusa cynapium L.	57
HOCH$_2$.CH=CH.[C≡C]$_2$.[CH(OH)]$_2$.CH.[CH$_2$]$_4$.O	*	Trachelium caerulium L.	90
vic-Polyols (from fungi)			
H[C≡C]$_3$.[CH(OH)]$_2$.CH$_2$OH	erythro	Coprinus quadrifidus	167
H[C≡C]$_2$.CH=CH.[CH(OH)]$_2$.CH$_2$OH	threo	Aleurodiscus roseus	168
Me[C≡C]$_4$.[CH(OH)]$_3$.CH$_2$OH	2,3 threo 3,4 erythro	Fistulina hepatica	169

* Not known

Polyacetylenic vic-diols isolated from nature

Biogenetic considerations

Several non-terminal vic-diols have been isolated previously from plants (Table 16), but for only 7-phenylhepta-4,6-diyne-1,2,3-triol and 7-phenylhepta-4,6-diyne-2,3-diol have the relative stereochemistries (both threo) been determined.[43]

Of these diols only the tetrahydropyranyl polyyne (158) (Trachelium caeruleum L.) contains the vic-diol group in the middle of the carbon skeleton. It would be of interest to determine if this has threo stereochemistry.

vic-Triols and tetraols have also been detected in fungi (Table 16).

Scheme AP is an attempt to rationalise the biosynthesis of the triol (159) along present concepts of polyacetylene biogenesis. The exact order of transformations cannot at present be forecast, especially for the later stages, and in scheme AP chain shortening is assumed to take place before hydroxylation.

The diene-diyn-ene (177) has already been isolated from the Dahlia[9] (Fam. Compositae).

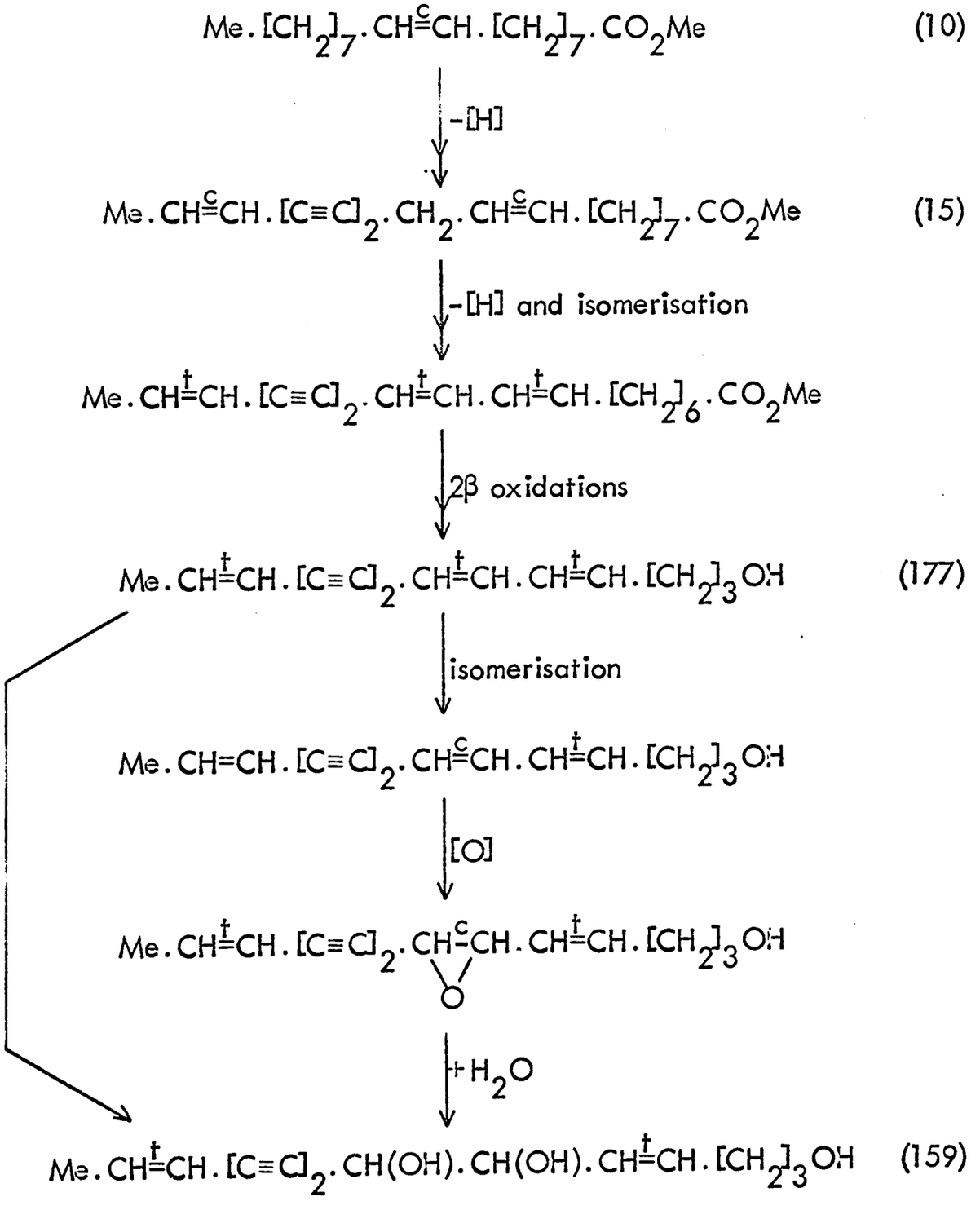
The generation of a diol via an epoxide has already been proposed,[40] but threo stereochemistry necessitates prior isomerisation of the C(6)-(7) double bond. Only recently has a precedent for this stage appeared; Jenkins[90] isolated the triyne-dienes (178a and b) from Urospermum dalchampii L. (Fam. Liguliflorae).

$$Me[C\equiv C]_3.CH{=}CH.CH{=}CH.CH(OH).[CH_2]_5OH$$

c t (178a)

t t (178b)

Scheme AP



Possible biogenesis of Lobelia triol (159)

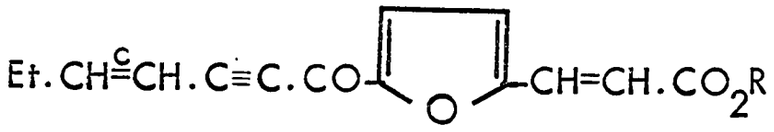
An alternative mechanism, by no means improbable, would be the direct cis-hydroxylation of the C(6)-(7) trans double bond in the alcohol (177).

PART V

Studies in the biosynthesis of wyerone

Introduction

In 1961, Wain and coworkers[170] isolated a crystalline metabolite with high antifungal activity against Alternaria brassicicola from the broad bean Vicia faba L. (Fam. Papilionaceae), and called it wyerone. The furanoid structure (179) was established and confirmed by synthesis in this department.[171]

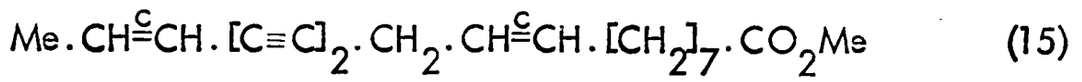


R = Me (179)

R = H (180)

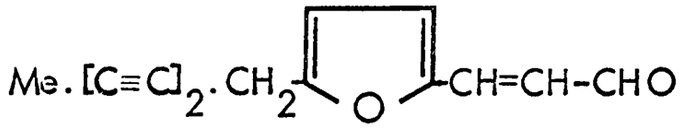
The corresponding acid (180) has recently been detected[172] in tissues of Vicia faba L. infected by the parasites Botrytis cinerea and fabae.

The presence of a cis butenyl end group in a natural acetylene is rather unusual; the expected biosynthetic pathway from the ester (15) would lead to a

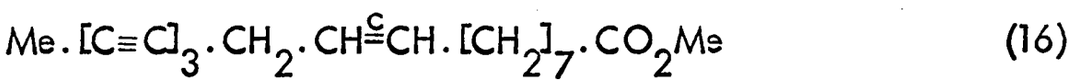


(15)

terminal cis-propenyl group. Thus the disubstituted furan (181) (Chrysanthemum silvaticium Hoffmgg. L.K.)[173] is postulated to evolve from the triyne ester (16).[2]



(181)



(16)

Acetylene biosynthesis in the family Papilionaceae might well involve linoleate (11) and linolenate (182), which have already been found in the fatty acid fraction of the broad bean.[174]

$$Me.[CH_2]_4.CH{\overset{c}{=}}CH.CH_2.CH{\overset{c}{=}}CH.[CH_2]_7.CO_2Me \qquad (11)$$

$$Et.CH{\overset{c}{=}}CH.CH_2.CH{\overset{c}{=}}CH.CH_2.CH{\overset{c}{=}}CH.[CH_2]_7.CO_2Me \qquad (182)$$

This section deals with feeding experiments on germinating broad bean using labelled acetate, oleate and crepenynate.

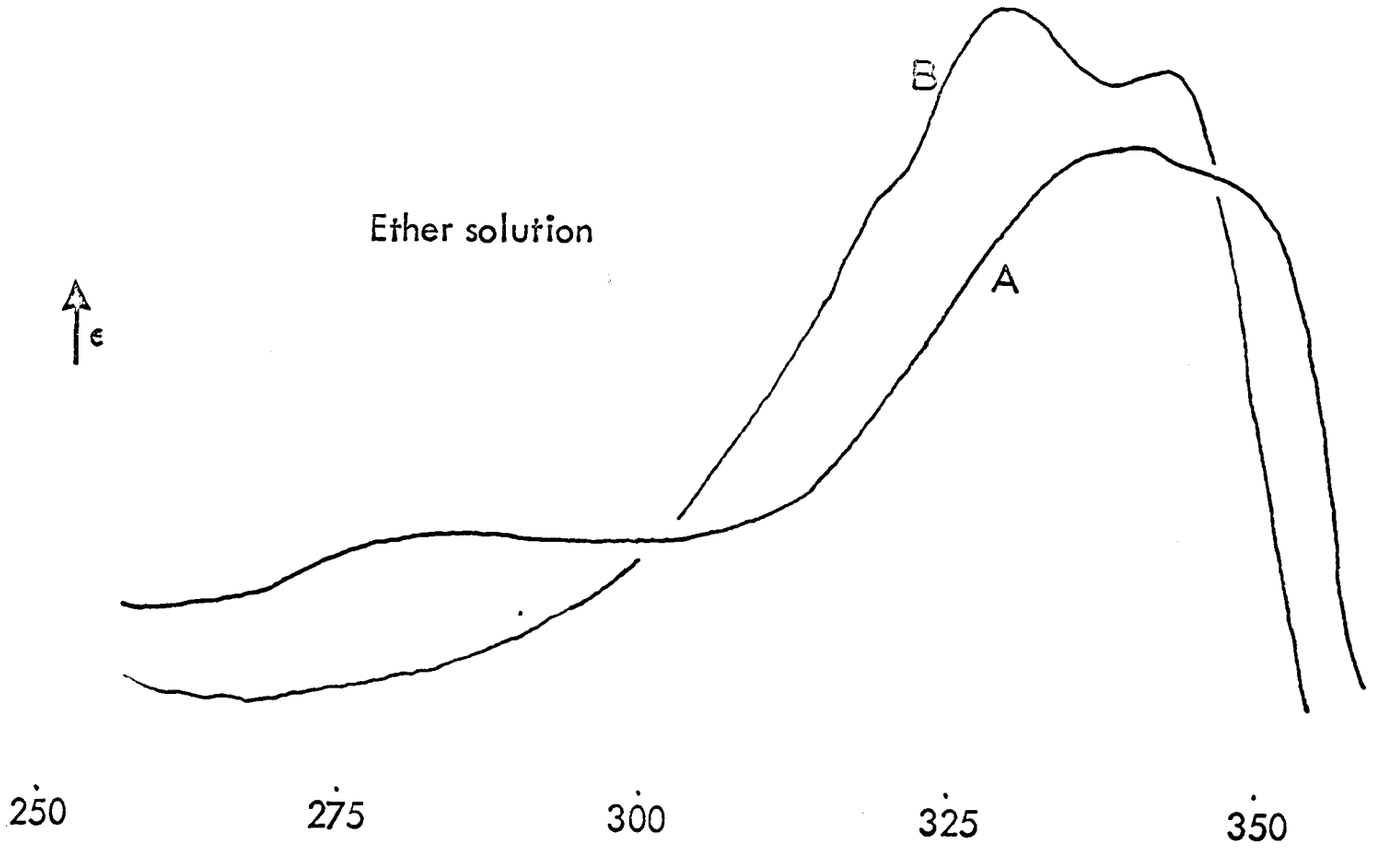
[10-^{3}H,9-^{14}C]-Linoleate (11) is now being prepared[33] in this department and when the results of this feeding are known, definite conclusions can be formulated regarding the biosyntheses of wyerone and closely related metabolites.

Bean culture and metabolite isolation

In initial experiments[171] the beans were grown between sterilised sacks, but this arrangement did not permit efficient administration of radioactive compounds to the beans. When germinating on sterilised tissues in covered trays, the beans were easily spotted but now became encased with a Mucorales-type mould. Surface sterilisation with mercuric chloride solution (5%) retarded bean growth, and the beans were still attacked by a Penicillium-type mould after six days.

When the cotyledons were stripped of their skins, bean growth was accelerated and fungal attack was slower. Spotting the cotyledon surface with

Figure 11



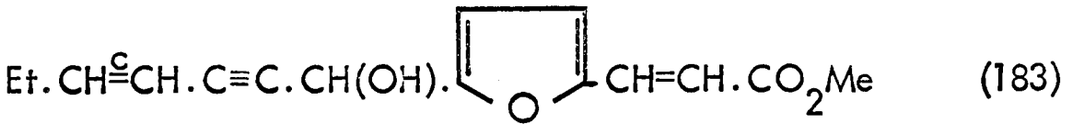
U.v. spectra of:

A Wyerone

B Dihydrowyerone

water soluble dye demonstrated that penetration into the tissue was highest around the base of the root.

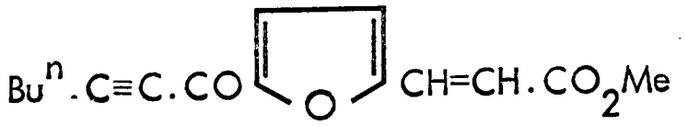
Wyerone (179) and wyerol (183) were isolated from macerated tissues by



(183)

solvent extraction and preparative layer chromatography. The optically active alcohol had not previously been isolated in the pure state from the broad bean, though its presence had been established.[171] The alcohol concentration, approximately 1 mg. per 100 beans, hardly varied with germination time yet the wyerone concentration increased from ca. 2 mg. (per 100 beans) after 2 days to 20 mg. after 10 days.

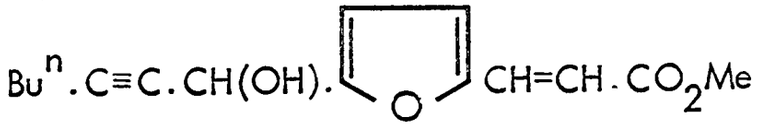
Mass spectral evidence had suggested[171] that dihydrowyerone (184) was



(184)

present in the wyerone fraction. The ultraviolet spectra for ether solutions of dihydrowyerone and wyerone were sufficiently different (figure 11) that the ratio of wyerone to dihydrowyerone in solution could be estimated, at least to 10%, by comparison of accurately determined peak shapes with those of standard mixtures. When an active crude wyerone fraction was diluted with synthetic dihydrowyerone, the

dihydrowyerone that crystallised out was active. Conversely, the addition of synthetic wyerone to the wyerone fraction (containing 20% dihydrowyerone) reduced contamination from the dihydro analogue in the recrystallised material to better than 5%. The mass spectrum of uncrystallised natural wyerol (M^+ 260) suggested that the dihydro-alcohol (185) (M^+ 262) was also produced by the broad bean.



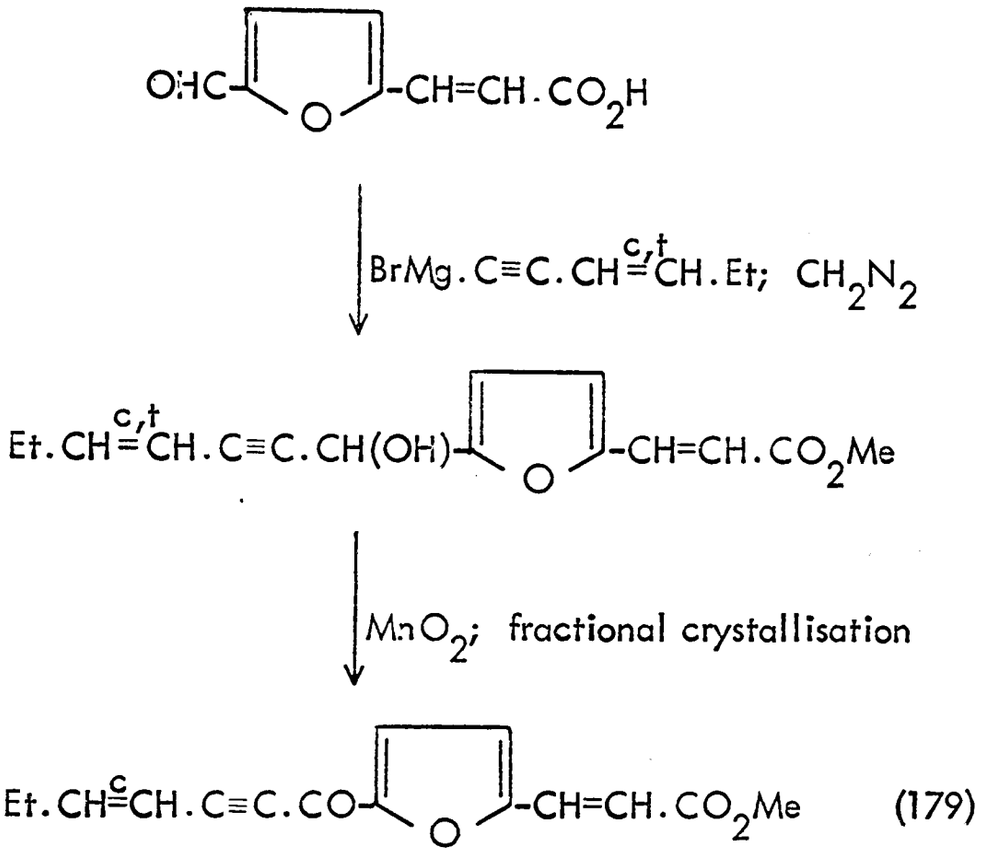
(185)

Wyerone, wyerol or wyerol trimethylsilyl derivatives were not resolved from their respective dihydro-analogues on silica gel layers even when impregnated with caffeine or silver nitrate. Wyerol acetate moved slightly slower than dihydrowyerol acetate on silver nitrate impregnated silica gel. Although the acetates were prepared in high yield on the milligram scale, they decomposed overnight in solution. Hence purification of wyerone from dihydrowyerone by thin layer chromatography showed little promise.

Synthetic work

Wyerone (179) was required in gram amounts to co-crystallise with the crude wyerone isolated from the broad bean. It was prepared essentially as in previous preparations[171] (Scheme AQ) save that hexenyne was used as a

Scheme AQ



stereoisomeric mixture (cis:trans 3:2). (cis)-Wyerone was then obtained by fractional crystallisation of the product mixture, thus avoiding the tedious and inefficient separation of cis and trans hexenyne isomers by p.g.l.c.

trans-Wyerone crystallised out first and moved slightly slower than the cis isomer on thin layers. Isomerisation did not take place on either silica gel or irradiation as for the hexadiynal dimer (72a; page 35).

Radioactivity measurement in the metabolites

The ketones (179) and (184) quenched the scintillation process in t-butyl-P.B.D./toluene solution, so the calibration values for the Beckman L.S.100 counter, the external standard ratios (e.s.r.), were consequently too low (<10.5) for accurate measurement of tritium and carbon-14 activities. The ketones were either reduced by borohydride and counted as the corresponding non-quenching alcohols, or else the samples were manipulated in the following way.

Naphthalene was dissolved in the scintillator solution containing the active sample, and the dissolved air displaced with nitrogen. The increased efficiency imparted to the light transmission process generally resulted in an e.s.r. enhancement of ca. 10%. If the solution was coloured by yellow impurities, addition of P.O.P.O.P. to the solution shifted the wavelength of the transmitted light to 415 nm. (from 365 nm.), which was accompanied by a sufficient increase in the e.s.r. value (to >10.5). Since the instrument's photomultiplier response was invariant over this wavelength range,[175] no error should be introduced in the measurement of tritium and carbon-14 labelled samples. The activities calculated by each method did compare favourably.

Feeding results

The results of incorporating acetate, oleate and crepenynate into wyerone are summarised in Table 17. Acetate, fed as the sodium salt, was incorporated

into wyerone (0.03%), wyerol (0.008%) and dihydrowyerone (0.007%).

Table 17

	Precursor	Label Positions		$^3H : ^{14}C$	Wyerone Incorp. %	$^3H : ^{14}C$	Notes
		^3H	^{14}C				
1	Acetate	-	1	0	0.027	0	a,b
2	Oleate	9,10	10	5.5 : 1	0.06	2.67 : 1	b
3		10	9	6.8 : 1	0.06	10.7 : 1	a
4		10	9	2.7 : 1	0.04	2.1 : 1	
5		10	1	14.7 : 1	0.002	> 150 : 1	
6	Crepenynate	10	9	25 : 1	0.02	9.1 : 1	b
7		10	9	6.7 : 1	negligible	-	a

a. dihydrowyerone isolated

b. wyerol isolated

Incorporations of radioactive compounds into wyerone

The incorporation efficiencies of oleate and crepenynate, despite their administration as emulsions, were also in these three ranges (apart from feedings 5 and 7).

In feeding 5 the low incorporation followed more from the small amount of wyerone isolated (1.2 mg. instead of ca. 10 mg.) rather than low specific activity in the metabolite. In feeding 7, in which inactive wyerone and dihydrowyerone were isolated, the total activities of the extracts were unusually high (approximately 30% instead of 10%). This suggested that the crepenynate ester might not have been properly transported into the cotyledons.

The wyerol incorporations are tabulated in table 18.

Table 18

	Precursor	Wyerone		Wyerol	
		sp.Act.	incorp.%	sp.Act.	incorp.%
	($^3H:^{14}C$)	μC/mmole	($^3H:^{14}C$)	μC/mmole	($^3H:^{14}C$)
1	Acetate	75	0.027	7	0.008
2	Oleate	0.2	0.06	0.22	0.009
	(5.5)		(2.67)		(2.8)
6	Crepenynate	0.17	0.02	0.47	0.013
	(25)		(9.1)		(10.2)

Incorporations into both wyerone and wyerol

Except for the acetate feeding, the wyerol specific activities were higher than those of wyerone and decreases in the expected $^3H:^{14}C$ ratio lower.

These results, taken together with the increase in wyerone, but not wyerol, concentration on bean growth, suggest that the alcohol is an intermediate for the ketone. This does not rule out a simultaneous equilibrium between ketone and alcohol.

The dihydrowyerone that co-crystallised out from the wyerone fraction in the oleate feeding had a similar $^{3}H:^{14}C$ ratio to the wyerone metabolite, and, apart from one case, the specific activities and (minimum) incorporations were an order of magnitude lower than for wyerone (Table 19). Wyerone may be reduced in vivo to form dihydrowyerone (see secondary transformation 15; page 11).

Table 19

	Precursor	Wyerone		Dihydrowyerone	
		sp.Act.	Incorp.%	sp.Act.	Incorp.%
	($^{3}H:^{14}C$)	μC/mmole	($^{3}H:^{14}C$)	μC/mmole	($^{3}H:^{14}C$)
1	Acetate	75	0.027	1.2	0.007
3	Oleate	0.016	0.06	0.04	0.004
	(6.8)		(10.7)		(11.0)
7	Crepenynate	inactive		inactive	

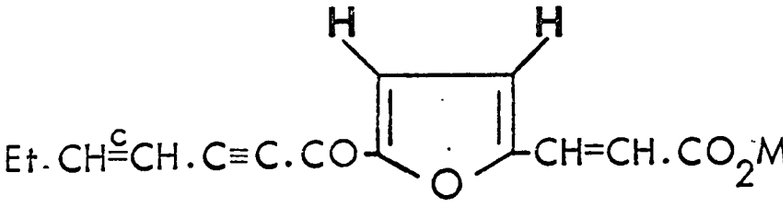
Incorporations into both wyerone and dihydrowyerone

Although the results were consistent for each feeding, a peculiarity was the varying $^{3}H:^{14}C$ ratios which, in all but feeding (3), were lower than expected. If the fatty acid esters had been broken down within the cells to, say, acetate, which had then been resynthesised into wyerone then the odd $^{3}H:^{14}C$ ratios could be accounted for. However, the feeding of [10-^{3}H,1-^{14}C] oleate (feeding 5) and isolation of wyerone containing only tritium label appears to disprove this possibility.

The C_{18} esters and metabolites were then degraded to demonstrate that the radioactive label had not been scrambled in some other way.

Degradation of wyerone

On the assumption that the terminal methyl group in wyerone (179) corresponds to the terminal methyl in the fatty acids, tritium on C(9) or C(10) should appear as the furan ring hydrogens (see page 118). These positions were not predicted to

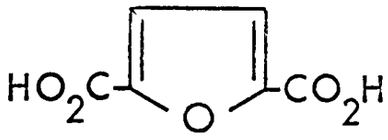


(179)

be labile; deuterium (as deuterated acidic acid) did not exchange with the ring hydrogens in acid conditions. However, it was observed that the tritium content

c. wyerone samples kept in ether solution decreased gradually, e.g. from $^{3}H:^{14}C$ 9.1:1 to 5:1 over 3 months.

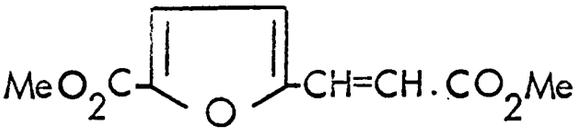
Wyerone has been transformed into 2,5-dicarboxyfuran (187) by the action of alkaline permanganate.[171] Although this diacid would be an ideal degradation



(187)

product to confirm the specificity of tritium labelling, in small scale reactions the diacid (187) could not be isolated. In the present work it was discovered that the diacid (187) was itself oxidised by the permanganate.

Alkaline hydrogen peroxide only severed the acetylene-carbonyl bond, and after methylation of the product, the reaction yielded the diester (188).



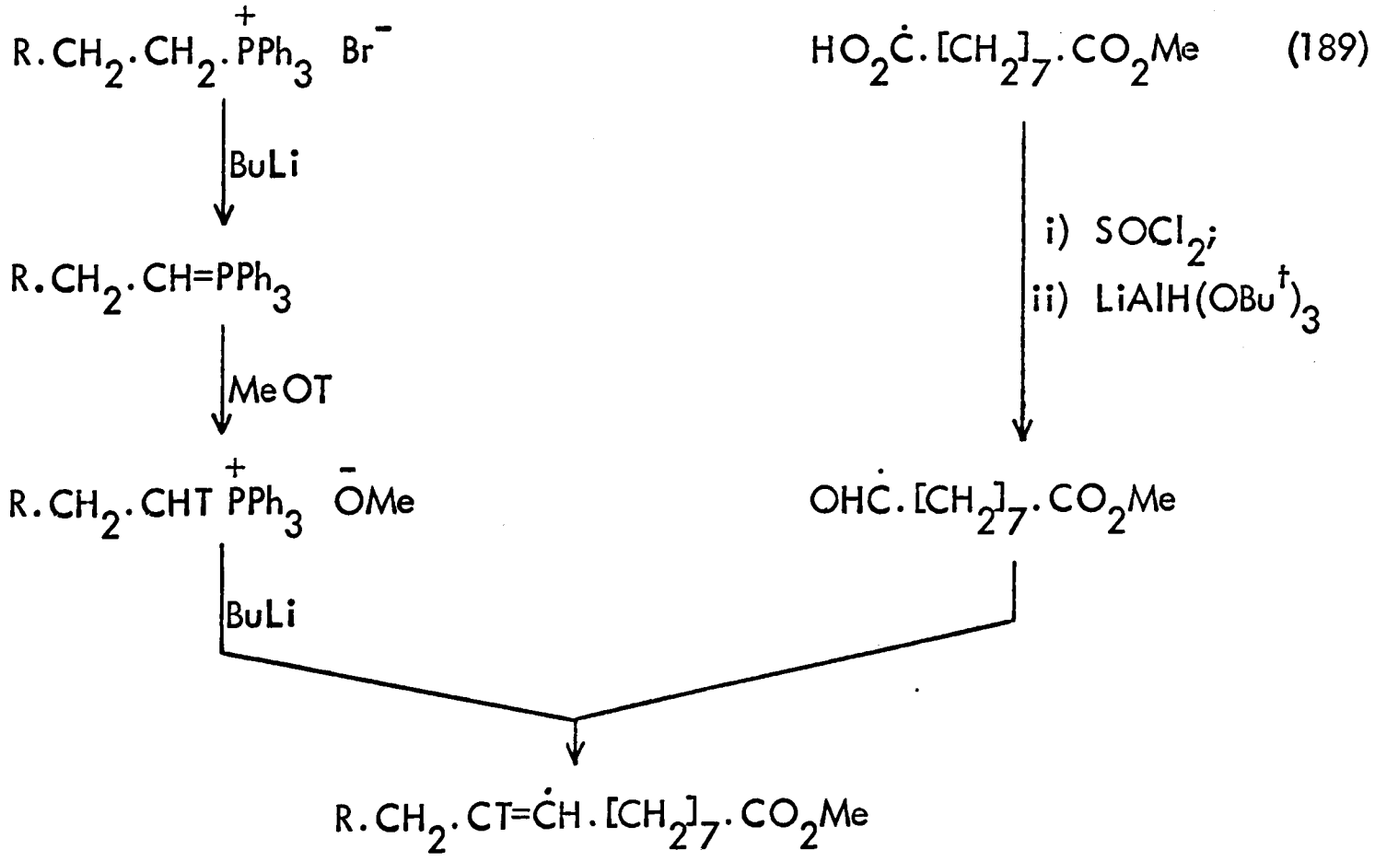
(188)

Moreover this procedure avoided heterogeneous conditions, so fair yields (30%) were obtained on the milligram scale. Degradation of wyerone samples from feedings 2 and 6 afforded the dimethyl ester (188) with the same tritium and carbon-14 specific activities. The small activities counted implied that these results would only be accurate to approximately 10%.

Labelling specificity in the fatty acids

The inconsistent ratios could have been caused by non-specific labelling in the oleate and crepenynate precursors. O'Neill has demonstrated[29] that not more than 95% of the total activity is associated with the double bond in [9,10-^{3}H] oleate. Degradations of polyacetylenes[7] which had incorporated [10-^{3}H] crepenynate, and mass spectral studies on [10-^{2}H] crepenynate[7] have not suggested that introduction of hydrogen isotopes at C(10) by scheme AR is unspecific. [10-^{3}H] Oleate was prepared by essentially identical reactions.[33]

Scheme AR



Flowchart for preparing [10-^{3}H, 9-^{14}C] oleate (R = Me.[CH_2]$_6$.) and crepenynate (R = Me.[CH_2]$_4$.C≡C.)[7]

Carbon-14 was introduced at C(9) via the acid ester (189) (Scheme AR). During storage in solution trans esterification might have taken place:

$$HO_2\dot{C}.[CH_2]_7.CO_2Me \rightleftharpoons MeO_2\dot{C}.[CH_2]_7.CO_2H$$

which would lead to activity at C(1) in the C_{18} ester. Consequently a $^3H:^{14}C$ ratio in wyerone [H(9), H(10), C(9) retained; C(1) lost] would be higher than expected, e.g. feeding 3. Schmidt degradation on doubly labelled oleate (Scheme AS) afforded inactive barium carbonate [from C(1)], and so no exchange had in fact occurred.

Scheme AS

$$Me.[CH_2]_7.CT=\overset{9}{C}H.[CH_2]_7.\overset{1}{C}O_2Me \qquad ^3H:^{14}C\ 6.8:1$$

$$\downarrow H_2\text{-}Pd/C$$

$$Me.[CH_2]_7.CHT.\overset{9}{C}H_2.[CH_2]_7.\overset{1}{C}O_2Me$$

$$\downarrow KOH/MeOH$$

$$Me.[CH_2]_7.CHT.\overset{9}{C}H_2.[CH_2]_7.\overset{1}{C}O_2H$$

$$\downarrow NaN_3/H_2SO_4$$

$$Me.[CH_2]_7.CHT.\overset{9}{C}H_2.[CH_2]_7.NH_2 \qquad ^3H:^{14}C\ 6.8:1$$

$$+$$

$$CO_2 \qquad \text{Inactive (as } BaCO_3\text{)}$$

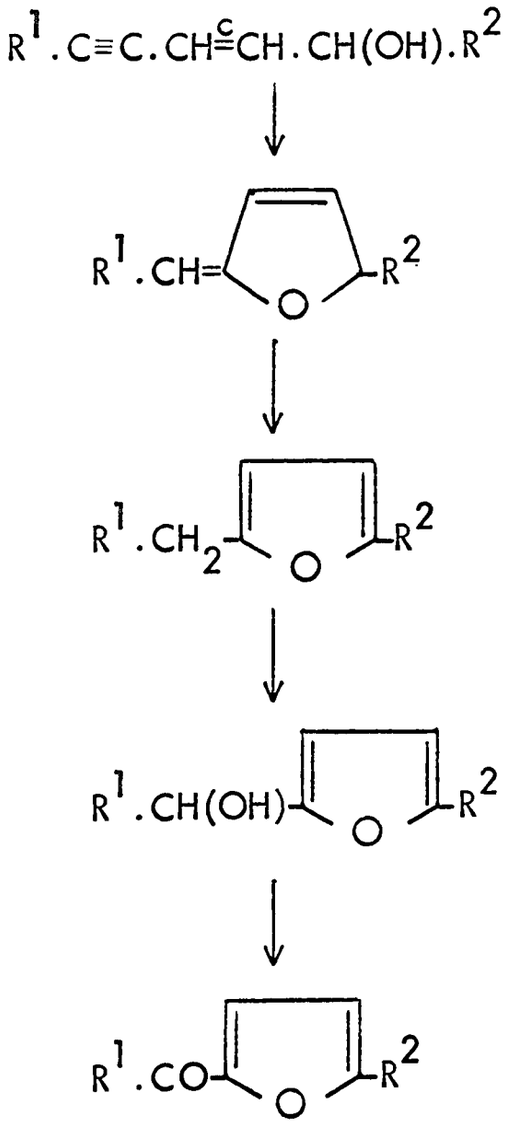
Degradation of [10-^{3}H, 9-^{14}C] oleate

[1-^{14}C] and [10-^{14}C] Oleate were commercially available samples, which were synthesised by routes unlikely to promote scrambling of the radioactive label.

Biosynthetic considerations

The furan ring can be envisaged to originate from a 4-yn-cis-2-en-1-ol system (Scheme AT).

Scheme AT



(190)

The activated methylene group in (190) is oxidised to an alcohol (wyerol) and a ketone (wyerone). R^1 thus corresponds to the hexenyne fragment in wyerone, which presumably arises from the distal half of the fatty acid ester. A rational biosynthesis, including scheme AT, can be formulated:-

$$Me.[CH_2]_7.\overset{10}{CH}\overset{c}{=}\overset{9}{CH}.[CH_2]_7.CO_2Me \qquad (10)$$

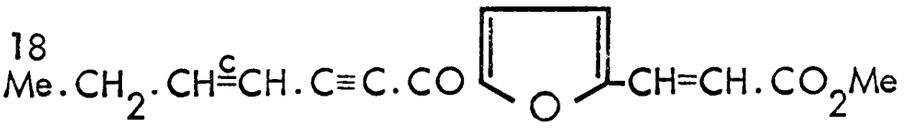
$$\downarrow$$

$$Me.[CH_2]_4.C\equiv C.CH_2.\overset{10}{CH}\overset{c}{=}\overset{9}{CH}.[CH_2]_7.CO_2Me \qquad (12)$$

$$\downarrow$$

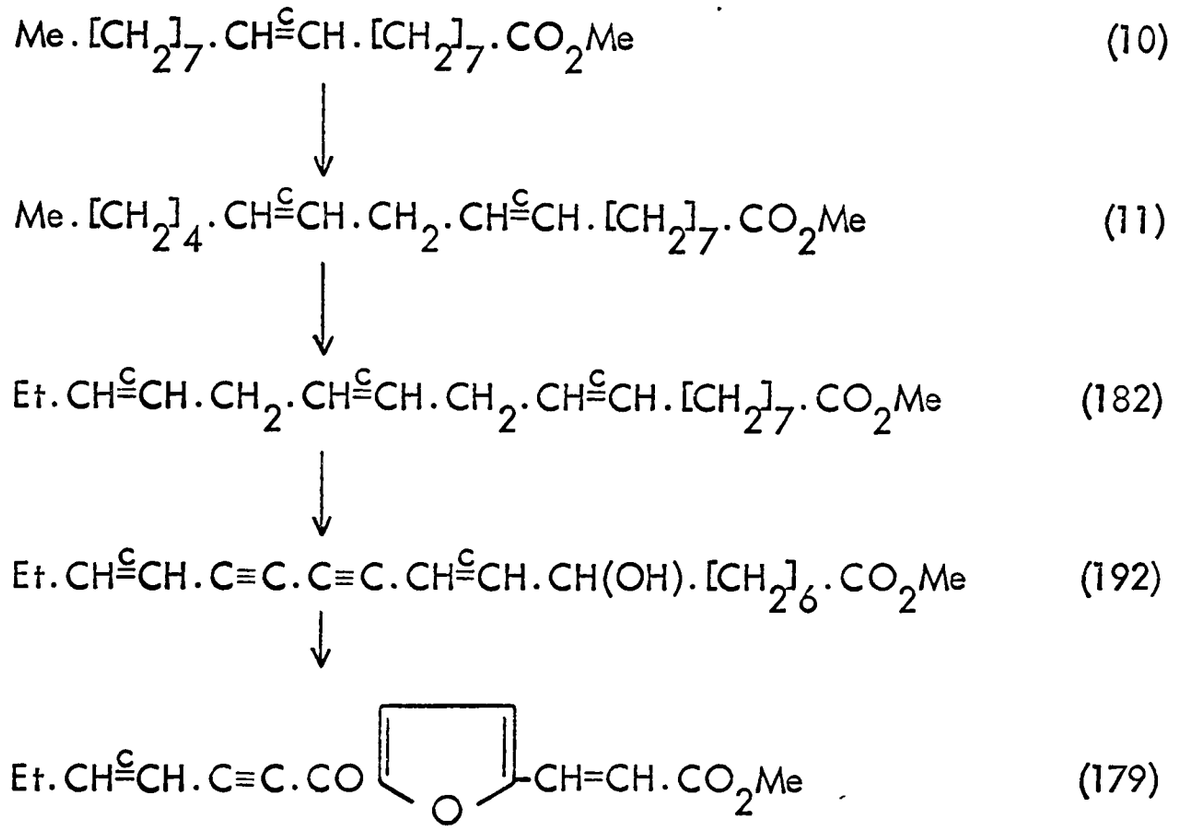
$$Me.[CH_2]_5.C\equiv C.\overset{10}{CH}\overset{c}{=}\overset{9}{CH}.CH(OH).[CH_2]_6.CO_2Me \qquad (191)$$

$$\downarrow$$

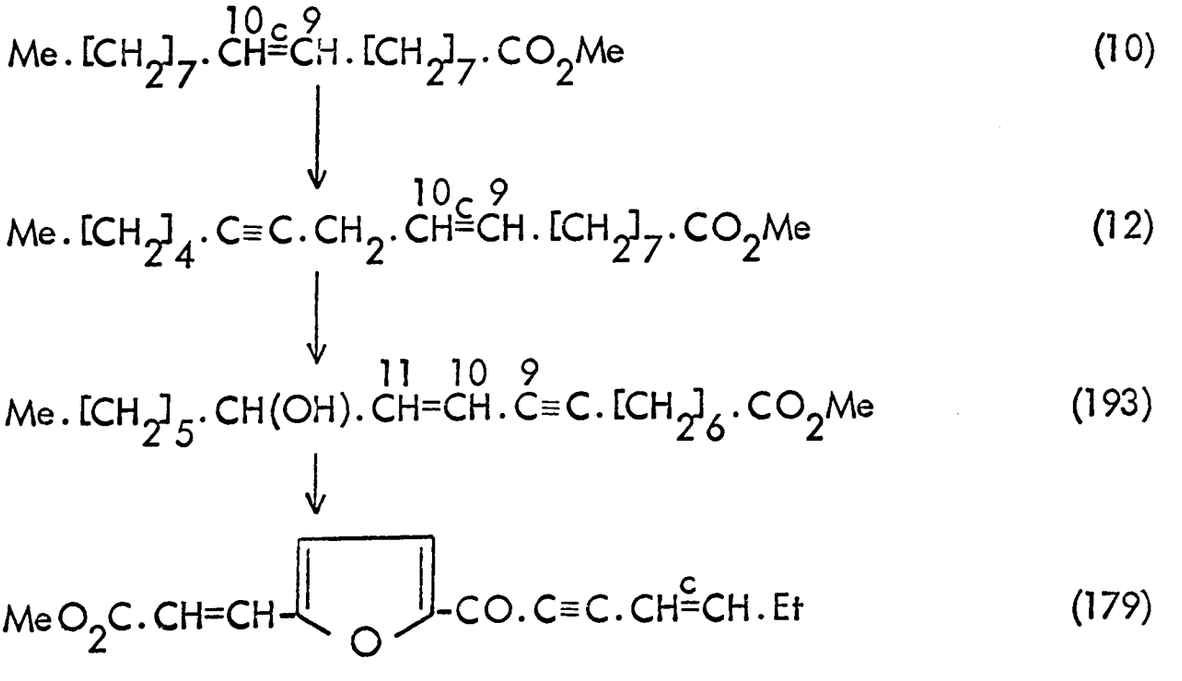


This implicates a triple bond shift in crepenynate to generate (191), which could account for the low incorporations obtained with this ester. The oleate cis double bond has rearranged during the furan ring formation (Scheme AT) and hence tritium isotope effects are expected (see page 7).

Linolenate (182) contains the same cis butenyl end group found in wyerone and may be converted into the ene-diyn-ene ester (192), which seems an obvious precursor of wyerone:



Alternatively the fatty acid ester could decarboxylate and the proximal half engender R^1 (Scheme AT):-



Now an even more complex rearrangement of crepenynate to generate the en-yne (193) is required.

No new acetylenes were detected in Vicia faba in this work and further light on wyerone biosynthesis must await the results of feedings with other radioactive precursors.

EXPERIMENTAL SECTION

GENERAL EXPERIMENTAL TECHNIQUES

Ultraviolet (u.v.) absorption spectra of pure new stable crystalline compounds were determined in hexane solution on a Cary Model 14M double beam recording spectrometer. For unstable compounds, u.v. spectra were recorded in diethyl ether solutions on a Unicam SP800 spectrophotometer, the solution evaporated to dryness and the residue taken as the weight of compound for extinction coefficient (ϵ, 1 mole^{-1} cm.$^{-1}$) calculations. Polyyne concentrations were reckoned from the optical densities of the longest wavelength band.

Infrared (i.r.) spectra of pure compounds were measured on a Perkin Elmer 257 grating spectrometer. Routine i.r. spectra were recorded on Perkin Elmer 157 or Unicam SP200 spectrometers. Low frequency spectra (below 700 cm.$^{-1}$) were determined on a Perkin Elmer 521 instrument for 50% solutions in KRS5 cells of path length 0.1 mm. Carbon tetrachloride, unless otherwise stated, was used as solvent and all spectra were calibrated with a polystyrene standard.

Proton magnetic resonance (n.m.r.) spectra at 60 or 100 MHz (Perkin Elmer R10 or R14 spectrometers) were recorded by Mrs. R. E. Richards at 34° using tetramethylsilane as internal standard. Carbon tetrachloride, unless otherwise stated, was employed as solvent.

Specific rotations were measured in ethanol on a Perkin Elmer 141 polarimeter. Mass spectra (m.s.) were run by Dr. R. T. Aplin on A.E.I. MS9 and

Atlas CH7 mass spectrometers operating at 70 eV in the temperature range 50-200° using direct insertion probes.

Melting points (m.p.) were determined on a Kofler Hot stage apparatus and are corrected.

Refractive indices of stable liquids were measured on an Abbé refractometer.

Analyses were performed by Drs. G. Weiler and F. Strauss of this department.

Chromatography: Analytical gas liquid chromatography (g.l.c.) was carried out on a Pye '104' series model 24 instrument equipped with 5 ft. columns and using an argon flow of 40 ml./min.. Columns were designated:
A - 10% poly(ethylene glycol succinate); B - 10% dinonyl phthalate;
C - 10% apiezon; D - 30% silicon oil. All stationary phases were supported on Embacel (60-100 mesh; acid washed). Integrations of peak areas were computed by a Kent Chromalog 2 digital integrator.

Preparative gas chromatography (p.g.l.c.) was performed on a Pye '104' series gas chromatograph adapted for preparative use in this department. An injection volume of 50 μl. was passed down a 20 ft. 20% P.E.G.S. column (A-Prep) or 15% silicon oil column (D-Prep) at an inlet gas flow of 150 ml./min..

Column chromatography was carried out on silica gel Whatman SG31, or, if so stated, on alumina Camag 'Neutral' (Activity I) equilibrated with 8% w/w

water (for Activity III). Unless otherwise stated, ether-light petroleum mixtures were used as the eluting solvent. The polarity increase was either stepwise or continuous (gradient elution).

Silica gel $HF_{254 \text{ and } 366}$ (Merck) was used as sorbent for thin layer chromatography (t.l.c.) in 0.3 mm. layers. Preparative layer chromatography (p.l.c.) was carried out on 1 mm. layers of silica gel [$PF_{254 + 366}$] or if stated, alumina [$PF_{254 \text{ and } 366}$ (Merck)] on 20 x 20 or 100 x 20 cm. plates. Repeated development was used as necessary. Polyacetylenic and most other bands could be detected by their quenching of fluorescence under 254 or 366 nm. light. Positions of further bands could be determined using iodine vapour absorption or molybodic acid spray.

Techniques: Solutions containing polyacetylenes were concentrated under reduced pressure on a rotary film evaporator away from direct sunlight below 40°. Such solutions were stored at 0° and pure compounds in the neat state at -40°. Acetylenes were distilled in a nitrogen atmosphere at high vacuum in the presence of a little hydroquinone.

Hydrogenations were carried out at atmospheric pressure and room temperature. 'Isolation, extraction or work up with ether', accompanied by no further extraction details refers to dilution of the reaction mixture with a suitable volume of water, extraction with ether, washing the ether extract with saturated

brine and drying. Small amounts of material were recrystallised in Craig[176] tubes at -40° and the mother liquor was removed by centrifugation at 0°.

Manganese dioxide was prepared by the method of Attenburrow et al.[177] and its activity was tested on cinnamyl alcohol. Jones reagent[178] refers to a solution of chromium trioxide (267 g.) in concentrated sulphuric acid (230 ml.) made up to 1 l. with water.

Nitrogen (white spot) was further purified by passing through, in succession, Fieser's[179] solution, concentrated sulphuric acid and potassium hydroxide pellets. Acetylene was purified by passing through a sequence of two cold traps (acetone-dry ice), two concentrated sulphuric acid bubblers, a column (30 cm.) of activated alumina (4-8 mesh) and a tower containing potassium hydroxide pellets.

Light petroleum refers to fraction b.p. 30-40°; it was redistilled from phosphorous pentoxide before use and had absorption of less than 0.1 between 225 and 275 nm. in a 1 cm. cell.

Ether was diethyl ether, peroxide free and sodium dried. Pyridine was dried by standing over potassium hydroxide pellets. Acetone was distilled before use, after standing over Linde 4A molecular sieve.

Tetrahydrofuran (THF) was passed down an alumina column and dried with sodium wire in the dark. Prior to use it was distilled from lithium aluminium hydride.

All other solvents were purified according to standard procedures.[179,180] Solutions were dried with magnesium sulphate, unless otherwise stated.

Preparation of p.l.c. plates of silver nitrate impregnated silica gel:- A slurry of silica gel (85 g.; Merck $PF_{254 + 366}$) in aqueous silver nitrate (10%; 200 ml.) was spread over three 50 x 20 cm. plates. The layers were dried overnight at 20° and activated prior to use at 110° for not longer than 5 min.. The maximum load applied to each plate was ca. 100 mg..

GENERAL PROCEDURES

The procedures described below are applicable in more than one section of this thesis. Other general procedures, specific to one topic, are sited at the beginning of the relevant experimental section and are also listed in the index.

Manganese dioxide oxidation of allylic or propargylic alcohols:-

The alcohol (1 g.) was shaken with manganese dioxide (10 g.) in dichloromethane (100 ml.). After 6 hr. shaking, the solution showed a u.v. spectrum with no alcohol chromophore. The manganese dioxide was removed by filtration through a bed of magnesium sulphate with suction, washed with hot dichloromethane (100 ml.) and the concentrated filtrate was applied to a silica gel column (100 g.). The aldehyde was eluted with dichloromethane; solvent enrichment with ether was required for elution of any remaining alcohol.

Bromination of ethynyl compounds:-

Sodium hydroxide (19 g., 0.475 mole) in water (100 ml.) and ice (50 g.) was stirred whilst bromine (10 ml., 0.2 mole) was slowly added to give a solution of sodium hypobromite (0.2 mole). The terminal alkyne (1 mol.) in ether at 0° under nitrogen was treated dropwise with sodium hypobromite solution (2.5 mol.) and the heterogeneous mixture was stirred vigorously for 2 hr. at 0° in the dark.

The ether layer was separated and the yellow aqueous layer extracted with ether. The combined ether extracts were washed with saturated ammonium chloride solution, and concentrated to yield a solution of crude 1-bromoalk-1-yne. An aliquot evaporated to an oil showed no $\nu_{max.}$ (thin film) 3300 cm.$^{-1}$.

Cadiot-Chodkiewicz coupling:-

The ethynyl compound (7.5 mmole) was dissolved in methanol (30 ml.) with cuprous chloride (10 mg.), hydroxylamine hydrochloride (180 mg.) and ethylamine (10 ml., 30% w/w aqueous solution) under a nitrogen atmosphere in the dark. The crude bromoacetylene (from above; 7.5 mmole) was added dropwise at 15°, unless otherwise stated, whilst the reaction mixture was magnetically stirred. The stirring was continued for 2 hr. during which time the copper ions were kept in the cuprous state by the addition of further amounts of hydroxylamine hydrochloride. Potassium cyanide (20 mg.) was then added to complex the copper salts. Isolation with ether, followed by column chromatography, afforded the coupled acetylene.

Nucleophilic displacement with iodide:-

Sodium iodide (May and Baker Ltd.) was dried at 100°. The chloride or tosylate (1 mmole) was stirred in acetone (20 ml.) with sodium iodide (5 mmole) under nitrogen at a bath temperature of 60°. Isolation with ether, including a

wash with sodium thiosulphate (0.1N; 10 ml.) gave an oil after concentration. Thin layer chromatography yielded the oily iodide as the least polar zone after development with ether-light petroleum (1:4).

PART II

SCHEME Ha

1. 1-Bromoprop-1-yne:-

Sodium hydroxide (40 g., 1 mole) in ice (100 g.) and water (100 ml.) was treated with bromine (22 ml., 0.44 mole) to give sodium hypobromite solution. Propyne was passed slowly through the major part of this solution (150 ml.), which was vigorously stirred in a flask (250 ml.) fitted with an acetone-dry ice condenser. When the solution had become colourless (2 hr.) the rest of the hypobromite solution (50 ml.) was added and the propyne was replaced by nitrogen for 30 min.. The heavy oil was separated from the yellow aqueous layer and washed twice with saturated ammonium chloride solution (20 ml.) to give the crude bromopropyne (39 g., 75%). The bromoalkyne was stored away from direct sunlight, otherwise on contact with air the material spontaneously ignited, $\nu_{max.}$ (thin film) 2240, 2220 and 2060 cm.$^{-1}$ (C≡C). τ 8.15 (s, CH_3.).

2. Hexa-2,4-diyn-1-ol:-

1-Bromoprop-1-yne (25 g., 0.21 mole) was coupled with redistilled prop-2-yn-1-ol (10.75 g., 0.19 mole; b.p. 110-111°) at 35°. Ether-light petroleum (1:2) eluted hexa-2,4-diyn-1-ol (15.2 g., 84%) as colourless plates m.p. 43-44° (from hexane; lit.,[181] 47°) which turned bright red on exposure to light, $\lambda_{max.}$ 254 (ϵ 245), 241 (390), and 230 nm. (395), $\nu_{max.}$ 3600 and 1020 (OH), 2260 (C≡C) and 1385 cm.$^{-1}$ (CH_3), τ 8.03 (t, J 1 Hz, -CH_3), 6.22br (s, disappears on D_2O addition, -OH), and 5.74 (q, 1 Hz, -CH_2-).

3. Hexa-2,4-diynal (67):-

Hexa-2,4-diyn-1-ol (940 mg., 10 mmole) was oxidised with manganese dioxide. Hexa-2,4-diynal[102] (0.6 g., 65%) was isolated as a colourless oil, rapidly darkening in light, $\lambda_{max.}$ (CH_2Cl_2) 287 (ε 8500), 272 (9500), 257 (6000) and 245 nm. (2500), $\nu_{max.}$ (CH_2Cl_2) 2210, 2140 (C≡C) and 1660 cm.$^{-1}$ (CHO), $\nu_{max.}$ (hexane) 1678 cm.$^{-1}$ (C=O), τ 7.89 (s, -C$\underline{H}_3$) and 0.81 (s, -C$\underline{H}$O).

4. Dimerisation of hexa-2,4-diynal:-

a) Isolation of (Z)-5-formyldec-4-ene-2,6,8-triyne (72a): A solution of hexa-2,4-diynal (200 mg.) in methylene chloride (5 ml.) was kept under nitrogen in the dark for 7 days. The solvent was removed under reduced pressure, and the residue repeatedly extracted with hot light petroleum (100 ml.; 40-60°). Pale yellow crystals of (Z)-5-formyldec-4-ene-2,6,8-triyne, m.p. 90-92°, separated out on cooling. Concentration of the mother liquors afforded a second crop whilst unreacted hexa-2,4-diynal (24 mg.) remained in the supernatant solution. Recrystallisation from hexane gave the dimer (90 mg., 60% based on hexa-2,4-diynal reacted), m.p. 93-94° (Found: C, 83.8; H, 5.3%; M^+, 156.0578 ± 0.0004. $C_{11}H_8O$ requires C, 84.6; H, 5.2%, M, 156.0575), $\lambda_{max.}$ 319 (ε 15,600), 268 (4700), 260sh, 254 (6200), 239 (6100), and 227 nm. (4500), $\nu_{max.}$ (CS_2) 2250, 2220, 2210sh (C≡C), 2760, 1695 and 1125 cm.$^{-1}$ (unsat. C=O), τ ($CDCl_3$) 7.99 (s, C$\underline{H}_3$.C≡C.C≡C.), 7.85 (d, J 2.5 Hz, C$\underline{H}_3$.C≡C.CH=), 3.09 (q, J 2.5 Hz, -C$\underline{H}$=) and -0.06 (s, .C$\underline{H}$O), τ (C_6H_6) 8.66, 8.61 and 0.55 (assignment order as before).

b) Detection of carbon monoxide: A nitrogen gas stream was passed slowly over the neat hexadiynal; carbon monoxide was detected in the exit stream through:

i) blackening of cotton wool soaked in palladium chloride solution within 2 min., and silver nitrate paper within ten minutes.[125c]

ii) the oxyhaemoglobin reaction.[125a,b] The exit gas was bubbled through an aqueous solution of 'old' human blood for 12 hr.. An aliquot (0.5 ml.) of this solution with freshly prepared aqueous solutions of pyrogallol (2%, 1 ml.) and tannic acid (2%, 1 ml.) was diluted with water to 25 ml.. After ten minutes a blank produced a grey suspension, whereas the test solution developed a distinct carmine colour. An aliquot of the blood solution diluted with 0.4% ammonium hydroxide solution had $\lambda_{max.}$ 561 (rel. E 1.0), 543 (1.0) and 406 nm. (11.5). The blank had $\lambda_{max.}$ 570 (1.1), 544 (1.0) and 407 nm. (11.5).

SCHEME Hb

5. 5-Phenylpenta-2,4-diyn-1-ol:-

Bromoethynyl benzene [prepared from phenyl acetylene (5.1 g., 50 mmole)] was coupled with propargyl alcohol (2.8 g., 50 mmole). Column chromatography of the ether extract and recrystallisation from ether-hexane gave needles of the alcohol (4.7 g., 60%), m.p. 40-41° (lit.,[182] 41°).

6. 5-Phenylpenta-2,4-diynal (73):-

5-Phenylpenta-2,4-diyn-1-ol (156 mg., 1 mmole) was oxidised with

manganese dioxide to yield 5-phenylpenta-2,4-diynal[104] (70 mg., 45%) as an unstable oil, $\lambda_{max.}$ 325 (rel. E 1.0), 306 (1.2), 298 (1.3), 271 (1.2), and 243 nm. (2.6), $\nu_{max.}$ 1655 cm.$^{-1}$ (C=O), τ 2.5 (m, 5 Ar-H) and 0.7 (s, -CHO).

7. (Z)-1,8-Diphenyl-4-formyloct-3-ene-1,5,7-triyne (74):-

The aldehyde (73) (70 mg., 0.45 mmole) was transformed after 12 hr. under nitrogen into a yellow-brown residue which was worked up as in experiment 4 to give yellow brown crystals of (Z)-1,8-diphenyl-4-formyloct-3-ene-1,5,7-triyne,[104] (30 mg., 47%), m.p. 130-131° (lit.,[104] 129.5-131.5°), $\lambda_{max.}$ 371 (ϵ 28,000), 290 (13,600), 274 (14,800), 243 (25,200) and 227 nm. (26,400), $\nu_{max.}$ 2200, 2180 (C≡C) and 1700 cm.$^{-1}$ (C=O), τ 2.93 (s, -CH=), 2.6 (m, 10 Ar-H) and -0.11 (s, -CHO).

The phenyl dimer (74) after standing in dilute solution for one week, or after chromatography on silica gel [multiple development, ether-light petroleum (1:4)], showed new signals at τ 3.04 (s, -CH=) and 0.52.(s, -CHO). Integration of the aldehyde proton region indicated a 60% conversion to the (E)-isomer after repeated development (4 times) on silica gel plates.

8. Hydrogenation of (Z)-5-formyldec-4-ene-2,6,8-triyne:-

The aldehyde (72a) (16 mg., 0.1 mmole) was hydrogenated in ethyl acetate (20 ml.) over 5% palladium on charcoal (10 mg.). The t.l. chromatogram (ether-light petroleum 1:2) of the filtered solution showed no spots under u.v. light, but

on development with iodine spots at R_F 0.9, 0.4 and 0.2. Hexadecane had R_F 0.9, n-decanol R_F 0.23 on the same plate.

9. Attempted oxidation[105] of dehydromatricarianal (78):-

The aldehyde (78) (72 mg., 0.5 mmole) was shaken with potassium cyanide (32 mg., 0.5 mmole), acetic acid (10 mg.) and manganese dioxide (0.5 g.) for 12 hr. in methanol (20 ml.). The mixture was filtered through a bed of magnesium sulphate and extracted with ether. The ether extract had $\lambda_{max.}$ 325 (rel. E 0.8), 305 (1.0), 287 (0.75), 270sh, 253 (0.7), 238 (1.5) and 226 nm. (1.2) (broad peaks) and was subjected to p.l.c. (ether). One zone (R_F 0.9) had a polyacetylenic chromophore (as above) and had τ 6.2 (s, $.CO_2\ \underline{CH_3}$), 4.5-3.0 (m, 3H, vinyl H), and 7.94 (s, $CH_3.C{\equiv}C$). No formyl proton observed (τ -0.1 to +0.1).

10. Oxidation of 1,8-diphenyl-4-formyloct-3-ene-1,5,7-triyne (74):-

The aldehyde (20 mg., 0.072 mmole) in 'Analar' acetone (15 ml.) was treated dropwise at 0° under nitrogen in the dark with Jones reagent until the supernatant solution remained orange. After adding water (100 ml.), the mixture was extracted with ether (2 x 20 ml.), and the extracts washed with saturated sodium hydrogen carbonate solution (2 x 20 ml.) at 0° to remove the acids. The bicarbonate phase was acidified at 0° and extracted with ether (2 x 20 ml.). The dried and concentrated ethereal acid fraction was cooled in hexane to -40° and yielded a gum. This gum afforded after p.l.c. (ether-light petroleum, 1:3, three

elutions), a yellow zone (R_F 0.4) and a polar fraction ($R_F < 0.2$).

P.l.c. of the neutral ethereal extract afforded a yellow zone (identical in R_F value to the yellow zone present in the acid fraction) and a trace of acid.

The yellow zone was extracted, and deposited at -40° from ether-hexane crystals (10 mg.) of 3-(4'-phenylbuta-1',3'-diynyl)-6-phenylpyran-2-one (79; R = Ph), M^+ 296. ($C_{21}H_{12}O_2$ requires M 296), $\lambda_{max.}$ 357 (rel. E 1.0) and 272 (1.4), $\nu_{max.}$ 2210w, 2190w (C≡C), 1740 (C=O) and 1050 $cm.^{-1}$ (C-O). The polar fraction, which could not be freed from the pyrone had $\lambda_{max.}$ 355 nm., $\nu_{max.}$ 3540br, 1700s, 1100 (CO_2H) and 2200s $cm.^{-1}$ (C≡C).

On treatment with sulphuric acid (2N; 5 ml.) the polar fraction (ca. 2 mg.) in ether (5 ml.) developed immediately a deep yellow colouration. After stirring for 4 hr. under nitrogen in the dark, the mixture was extracted with ether (10 ml.). The ether extract was chromatographed on thin layers and gave a trace zone on the base line and a major band, which corresponded to the pyrone (79; R = Ph) (t.l.c., u.v. and i.r.).

SCHEME I

11. Methyl 2-butylheptanoate from the dimer (72a):-

Jones reagent (0.165 ml.) was slowly added to (Z)-5-formyldec-4-ene-2,6,8-triyne (104 mg., 0.67 mmole) in 'Analar' acetone (5 ml.) under nitrogen in the dark. A white precipitate appeared immediately, and slowly green chromium salts separated from the orange reaction mixture. After 15 min., the mixture was

centrifuged, the supernatant phase was separated off and the precipitate was washed with acetone. The combined washings were worked up with ether and solution gave on evaporation an oil. P.l.c. [ether-light petroleum (1:4); three developments] produced three zones.

A band (R_F 0.6) afforded unreacted aldehyde [42 mg., (Z):(E) ratio 2:1] and a trace zone, R_F 0.55, $\lambda_{max.}$ 319 and 257 nm., $\nu_{max.}$ 2220w (C≡C), 1740 (C=O), 1380 (Me) and 1050 cm.$^{-1}$ (C-O) represented 3-(penta-1',3'-diynyl)-6-methylpyran-2-one (79; R = Me).

The major band (R_F < 0.2) was extracted with ether and crystallised from ether-hexane at -20°. 5-Carboxy-dec-4-ene-2,6,8-triyne (75) was obtained as white crystals (38 mg., 56%), m.p. 143-145.5° (decomp.) which rapidly turned yellow in light (Found: C, 74; H, 4.3. $C_{11}H_8O_2$ requires C, 77; H, 4.7%), $\lambda_{max.}$ 309 (ε 20,000), 269sh, 254sh, and 244 nm. (8150), $\nu_{max.}$ 3630, 3540br, 1700 (CO_2H) and 2240 cm.$^{-1}$ (C≡C).

The crude acid fraction (10 mg., 0.058 mmole) in ether (25 ml.) was hydrogenated over Adams catalyst (5 mg.) for 14 hr.. The catalyst was filtered off and the acid (76) extracted with sodium carbonate solution (2N; 2 x 10 ml.). After acidification the aqueous phase was saturated with sodium chloride and continuously extracted with ether (50 ml.). The ethereal solution was treated with excess diazomethane at 0°, concentrated and chromatographed on 0.3 mm. silica gel layers [ether-light petroleum (1:4)]. A zone (R_F 0.7) was obtained which corresponded to methyl 2-butylheptanoate (77) (3 mg., 26%); it had similar t.l.c.

and g.l.c. behaviour as a sample prepared in experiment 16.

SCHEME L

12. Diethyl butylmalonate:-

Diethylmalonate (105 g., 0.66 mole; b.p. 92-92.5°/14 mm.) was alkylated[183] with n-butyl bromide (87 g.; 0.64 mole, 99.9% pure by g.l.c.) and sodium ethoxide [from sodium (14.5 g., 0.63 mole)] in ethanol. Distillation of the crude product gave a fraction, b.p. 124.5°/17 mm., which was shaken with potassium hydroxide solution (25%) for 1 min. and redistilled to yield diethyl butylmalonate (115 g., 84%), b.p. 122.5-123°/16 mm. (lit.,[183] 130-135°/20 mm.), n_D^{21} 1.4230 (lit.,[184] n_D^{20} 1.425) (Found: C, 61.15; H, 9.6. Calc. for $C_{11}H_{20}O_4$: C, 61.1; H, 9.3%), $\nu_{max.}$ 1760, 1740 cm.$^{-1}$ (C=O), τ 9.1 (t, J 7 Hz, $C\underline{H}_3.CH_2.CH_2$), 8.75br (s, 10H, with triplet, J 7 Hz at 8.75, 2 x $O.CH_2.C\underline{H}_3$), 8.25 [p, J 8 Hz, $.C\underline{H}_2.CH(CO-)_2$], 6.85 [t, J 8 Hz, $CH_2.C\underline{H}(CO-)_2$] and 5.86 (q, J 7 Hz, 2 x $.OC\underline{H}_2.CH_3$), m/e 216 (M^+, 0.5%) and 160 [$(M-C_4H_8)^+$, 100%].

13. Diethyl butylpentylmalonate:-

Diethyl butylmalonate (10.8 g., 0.05 mole) was slowly added under nitrogen to a stirred suspension of sodium hydride (2.6 g., 0.054 mole; 50% dispersion in oil) in dry dimethyl formamide (15 ml.) with ice bath cooling. After 30 min. at 20°, the sodium salt at 60° was treated dropwise with pentyl bromide

(8.9 g., 0.059 mole; b.p. 125.5-126°/760 mm.; n^{15} 1.4465; 94% pure by g.l.c.), and sodium bromide slowly came out of solution. The mixture was heated at 100° for 6 hr. and concentrated at 70°/20 mm. to remove excess bromide and solvent. The dialkyl ester was isolated with ether, distilled and the fraction b.p. 108°/0.05 mm. was collected. Further distillation of this fraction gave diethyl butylpentylmalonate (10.1 g., 71%) b.p. 128-129°/2 mm., $n_D^{18.5}$ 1.4339 (lit.,[185] 185-193°/55 mm.; n_D 1.4312), (Found: C, 67.3; H, 10.35. Calc. for $C_{16}H_{30}O_4$: C, 67.1; H, 10.55%), $\nu_{max.}$ 1740 $cm.^{-1}$ (C=O), τ 9.1 (t, J 6 Hz, 2 x $C\underline{H}_3.CH_2.CH_2.$), 8.7br (20H, with triplet τ 8.78, J 7 Hz, 2 x $.OCH_2C\underline{H}_3$) and 5.87 (q, J 6.5 Hz, 2 x $.OC\underline{H}_2CH_3$), m/e 286 (M^+, 0.2%), 241 (20), 230 (35), 216 (50) and 173 (100).

14. Butylpentylmalonic acid (82):-

Diethyl butylpentylmalonate (3.2 g., 11.2 mmole) in an ethanolic sodium hydroxide solution (20 ml.; 25% w/w) was heated under reflux until t.l.c. indicated absence of ester (10 hr.). The ethanol was then removed under reduced pressure and the clear aqueous solution was washed with ether.

The aqueous layer, after acidification with sulphuric acid (4N), was saturated with ammonium chloride and the free acid isolated with ether. Butylpentylmalonic acid (82) (2.4 g., 93%) recrystallised repeatedly from ether-hexane and ethanol-water, melted at 143-144° (decomp.) [The dimethyl ester, obtained by action of excess diazomethane onto the diacid, had R_T (A, 120°) 19.0 min.

(96% pure)] (Found: C, 62.4; H, 9.35. $C_{12}H_{22}O_4$ requires C, 62.6; H, 9.65%), $\nu_{max.}$ ($CHCl_3$) 1715 cm.$^{-1}$ (C=O), τ ($CDCl_3$) 9.12 (t, J 7 Hz, 2 x $CH_3.CH_2$.), 8.7br (10H), 8.07 [m, $(-CH_2)_2.C(CO-)_2$], and 0.6br (s, disappears on addition of D_2O, 2 x OH), m/e 228 [$(M-2)^+$, 0.5%], 186 (37), 143 (50), 129 (80), 117 (75), 87 (99) and 73 (100%).

15. 2-Butylheptanoic acid (83):-

Butylpentylmalonic acid (3.25 g., 14.1 mmole) was heated at 150°, first at 300 mm. Hg, then increasing the vacuum gradually until effervescence had terminated (3 hr.), and finally it was heated under reflux at 0.5 mm. for 1 hr.. Distillation gave 2-butylheptanoic acid (2.1 g., 80%), b.p. 128-129°/1 mm. [lit.,[109] 170-173°/28 mm.; attempted distillation at 180° (bath)/15 mm., resulted in extensive decomposition], $n_D^{19.5}$ 1.4340 (Found: C, 71.0; H, 12.15. Calc. for $C_{11}H_{22}O_2$: C, 70.9; H, 11.9%), $\nu_{max.}$ (thin film) 1710 cm.$^{-1}$ (C=O), m/e 186 (M^+, 0.4%) 130 (57), 116 (87), 87 (55), 73 (100%).

16. Methyl 2-butylheptanoate (84):-

2-Butylheptanoic acid (1.43 g., 7.7 mmole) was heated under reflux with 4% methanolic sulphuric acid (25 ml.) for 14 hr.. Excess methanol was removed under reduced pressure and the ester isolated with ether. Distillation yielded methyl 2-butylheptanoate, (1.3 g., 85%) b.p. 118-119°/23 mm., n_D^{20} 1.4242 (Found: C, 72.05; H, 12.25. $C_{12}H_{24}O_2$ requires C, 71.95;

H, 12.1%), R_T (A: 76°) 13.8 min., $\nu_{max.}$ 1740 cm.$^{-1}$ (C=O), m/e 200 (M^+, 2%), 144 (45%), 130 (59), 101 (40), 87 (100), 74 (2), 69 (5) and 59 (5).

Preparative g.l.c. (A-Prep) gave a purer sample (99% by g.l.c.) which showed an identical mass spectrum to above. Treatment of the monoacid with diazomethane afforded the ester (84) identical in all respects to above sample.

The mass and infrared spectra and g.l.c. retention times were identical to the sample of methyl 2-butylheptanoate prepared from the dimer (72a) (Experiment 11).

17. 2-Butylheptanamide:-

2-Butylheptanoic acid (245 mg., 1.3 mmole) was heated with thionyl chloride under reflux. Excess chloride was removed under reduced pressure and the residual oil [$\nu_{max.}$ 1795 cm.$^{-1}$ (COCl)] in ether (10 ml.) was treated with gaseous ammonia. The solution was filtered and the filtrate concentrated yielding 2-butylheptanamide (230 mg., 94%). Recrystallisation from ether-hexane or methanol-water gave the pure amide in long needles, m.p. 117-118° (lit., [109] 92°; from methanol-water) (Found: C, 71.25; H, 12.7; N, 7.75. Calc. for $C_{11}H_{23}NO$: C, 71.3; H, 12.5; N, 7.55%), $\nu_{max.}$ 3540, 3520, 3430 (N-H) and 1690 cm.$^{-1}$ (C=O).

18. Methods of isomerising (Z)-hexadiynal dimer (72a) into (E)-isomer (72b):-

a) Irradiation. The (Z)-isomer (20 mg., 0.13 mmole) in 'Analar' carbon tetra-

chloride (0.4 ml.) was irradiated (10 in. away from a medium pressure 450 W mercury discharge lamp) under nitrogen at 42-45° for 3 hr.. The n.m.r. spectrum of the solution showed a (Z)-:(E)-isomer ratio 4:1, which did not change after one week in the sunlight. The recovered yield of dimer was 18 mg..

b) Chromatography [Isolation of (E)-isomer]. Column chromatography (silica gel; 100 g.) of the (Z)-isomer (47 mg., 0.3 mmole) with ether-light petroleum (1:4) afforded more polar fractions in which the smaller maximum ($\lambda_{max.}$ 255 nm.) was more pronounced. These fractions were retained, and the earlier fractions combined, concentrated and rechromatographed. This procedure was repeated three times, and the combined tail fractions yielded (E)-5-formylidec-4-ene-2,6,8-triyne (30 mg., 64%) on fractional crystallisation, m.p. 78-80° (hexane), $\lambda_{max.}$ 317 (ε 12,200), 255 (8200) and 242 nm. (5600), $\nu_{max.}$ (CS_2) 2260, 2230, 2220 (C≡C), 1705 and 1140 $cm.^{-1}$ (C=O), τ ($CDCl_3$) 7.96 (s, CH_3.C≡C.C≡C.), 7.80 (d, J 2.5 Hz, CH_3.C≡C.CH=), 3.28 (q, J 2.5 Hz, -CH=C) and 0.58 (s, CHO), τ (C_6H_6) 8.66 (s, CH_3.C≡C.C≡C), 8.46 (d, J 2.5 Hz, CH_3C≡C.CH=) and 0.58 (s, .CHO), m/e 156 (M^+, 100%) and 128 [$(M-CO)^+$, 43].

c) Acid. The (Z)-isomer (20 mg., 0.13 mmole) was stirred in dioxan (10 ml.) with sulphuric acid (2N, 1 ml.) for 30 min. under nitrogen. Isolation with ether afforded a mixture (18 mg.) of the dimers (72a) and (72b) in ratio 5:3 (n.m.r.).

19. Nuclear Overhauser Effect for isomers of 5-formyldec-4-ene-2,6,8-triyne (72a and b):-

1) At 60 MHz on Perkin Elmer R14 spectrometer irradiation at vinyl proton resonance frequency (τ 3)affected the aldehydic proton signal as follows:

a) for the more mobile isomer on silica gel (Z-isomer): < 2% increase in height; band width constant at 0.7 (± 0.2) Hz..

b) for the less mobile isomer (E-isomer): 18% (± 2%) increase in height; band width constant at 1.0 (± 0.1) Hz.

2) At 100 MHz on a Varian HA 100 spectrometer, integrated area ratios of aldehydic proton to chloroform proton resonances were:-

Before irradiation of vinylic proton 3.2 (± 0.15)

After irradiation of vinylic proton 3.7 (± 0.2).

corresponding to an increase of 16%.

SCHEME M

20. (Z)-5-Hydroxymethyldec-4-ene-2,6,8-triyne (88):-

The (Z)-dimer (72a) (51 mg., 0.3 mmole) in ethano. (10 ml.) was stirred with excess sodium borohydride (25 mg.) under nitrogen for 6 hr.. Isolation with ether and subsequent p.l.c. (ether-light petroleum 1:1) afforded a zone at R_F 0.4. Extraction gave needles of (Z)-5-hydroxymethyldec-4-ene-2,6,8-triyne (30 mg., 58%), m.p. 80-81° (from hexane) (M^+ 158. $C_{11}H_{10}O$ requires M 158),

$\lambda_{max.}$ 305 (ε 19,000), 288 (19,000), 271 (11,000), 256sh, 233 (13,800) and 222sh nm., $\nu_{max.}$ ($CHCl_3$) 3605, 1035 (OH), 2210 and 2180 $cm.^{-1}$ (C≡C), τ ($CDCl_3$) 7.99 (s, $\underline{CH}_3.C{\equiv}C.C{\equiv}C$), 7.96 (d, J 2.5 Hz, $\underline{CH}_3.C{\equiv}C.CH{=}$), 6.00br (s, disappears with D_2O, -O<u>H</u>), 5.31 (m, $\underline{CH}_2OH$) and 4.00 (m, -C<u>H</u>=C).

A sample of the alcohol (<u>ca.</u> 2 mg.) oxidised by manganese dioxide (20 mg.) gave a product with identical u.v. spectrum and t.l.c. behaviour to (<u>Z</u>)-isomer (72a).

21. (Z)-<u>5-Diethoxymethyldec-4-ene-2,6,8-triyne</u> (89):-

The (<u>Z</u>)-dimer (72a) (16 mg., 0.1 mmole) and <u>p</u>-toluenesulphonic acid (1 mg.) were dissolved in ethanol (5 ml.) and kept at 20° for 2 days. Potassium carbonate (10 mg.) was added and the acetal isolated with ether. Purification on silica gel layers gave after two elutions of ether-light petroleum (1:9) a zone (R_F 0.4) which represented the oily <u>5-diethoxymethyldec-4-ene-2,6,8-triyne</u> (10 mg., 42%) (M^+ 230. $C_{15}H_{18}O_2$ requires M 230), $\lambda_{max.}$ 306 (rel. E 2.0), 289 (2.0), 271 (1.0), 256sh, and 233 nm. (1.1), τ 8.82 (t, J 7 Hz, 2 x $\underline{CH}_3.CH_2$.), 7.98 (s, $\underline{CH}_3.C{\equiv}C.C{\equiv}C$), 7.96 (d, J 2.5 Hz, $\underline{CH}_3.C{\equiv}C.CH{=}$), 6.4 (m, 2 x -$\underline{CH}_2$.O), 4.8 [m, -C<u>H</u>(O-)$_2$] and 4.1 (m, -C<u>H</u>=).

22. (Z)- <u>and</u> (E)-<u>5-Methoxycarbonyldec-4-ene-2,6,8-triyne</u> (90a and b):-

a) <u>Isolation</u>. The acid (75) fraction (experiment 11) (25 mg., 0.145 mmole) was treated at 0° with diazomethane solution (0.14 mmole). P.l.c. with ether-light

petroleum (1:2) gave a zone (R_F 0.4) which represented a mixture of the esters (90a and b) [1.1:1 (Z)- to (E)-isomer ratio from areas of signals at τ 3.07 and 3.65]. A trace of free acid (75) was still present on the base line.

Multiple elution resolved the two esters, giving a more mobile band assigned to (E)-5-methoxycarbonyldec-4-ene-2,6,8-triyne (90b) (9 mg.) m.p. 96-97° (from hexane) (Found: C, 70.3; H, 4.7. $C_{12}H_{10}O_2$ requires: C, 77.5; H, 5.4%), $\lambda_{max.}$ 315 (ε 12,500) and 248 nm. (8500), $\nu_{max.}$ 2230, 2200 (C≡C), 1720 (C=O), 1160 and 1070 cm.$^{-1}$ (C-O), τ 7.96 (s, CH_3.C≡C.C≡C), 7.83 (d, J 2.5 Hz, CH_3.C≡C.CH=), 6.21 (s, CH_3.OOC) and 3.07 (q, J 2.5 Hz, -CH=), m/e 186 (M^+, 100%) and 187 [$(M+1)^+$, 15%]. The less mobile zone yielded (Z)-5-methoxycarbonyldec-4-ene-2,6,8-triyne (90a) (9 mg.), m.p. 107-108° (from hexane), $\lambda_{max.}$ 315 (ε 14,500) and 248 nm. (7000), $\nu_{max.}$ 2230, 2200 (C≡C), 1730, 1160 and 1070 cm.$^{-1}$ (CO_2Me), τ 7.97 (s, CH_3.C≡C.C≡C), 7.85 (d, J 2.5 Hz, CH_3.C≡C.CH=), 6.22 (s, CH_3O_2C.) and 3.65 (q, J 2.5 Hz, -CH=), m/e 186 (M^+, 100%) and an identical fragmentation pattern to the above isomer.

A thin zone, more polar than the esters, gave a solid (ca. 2 mg.), $\lambda_{max.}$ 317 nm., $\nu_{max.}$ 2210m (C≡C), 1790s (C=O) and 1025 cm.$^{-1}$ (C-O) consistent with the structure 2-(penta-1',3'-diynyl)hexa-2,4-dien-4-olide (80, R = Me).

Hydrogenation of the methyl esters (5 mg.) over Adams catalyst in ether afforded a saturated ester with identical retention time as methyl 2-butylheptanoate (77) (experiment 16).

b) Predicted values for vinyl resonances in esters (90a and b):-

Method A[116,117]

Additivity rule calculations:-

$$\delta = 5.25 + Z_{gem} + Z_{cis} + Z_{trans}$$

Aldehyde (72):

(Z)-isomer: $\delta = 5.25 + 0.47 + 0.38 + 1.17 = 7.27$ ppm.

$\tau_{calc.}$ 2.73 $\tau_{obs.}$ 3.09

Correction factor = + 0.36 ppm.

(E)-isomer: $\delta = 5.25 + 0.47 + 0.95 + 0.12 = 6.79$ ppm.

$\tau_{calc.}$ 3.21 $\tau_{obs.}$ 3.28

Correction factor = + 0.07 ppm.

Ester (90):

(Z)-isomer: $\delta = 5.25 + 0.47 + 0.38 + 0.46 = 6.56$ ppm.

$\tau_{calc.}$ 3.44 + 0.36 = 3.80

(E)-isomer: $\delta = 5.25 + 0.47 + 1.01 + 0.12 = 6.85$ ppm.

$\tau_{calc.}$ 3.15 + 0.07 = 3.22

Method B[118]

	τ-CH= (Z)	τ-CH= (E)
Aldehyde (91; R = H)	3.54	3.55
Ester (91; R = OMe)	4.10	3.28
Correction factors	+0.56	-0.27
Aldehyde dimers (72)	3.09	3.28
Ester (90)	3.65	3.01

23. (Z)-7-Formyltetradec-6-ene-4,8,10-triyne semicarbazone (92):-

(Z)-7-Formyltetradec-6-ene-4,8,10-triyne[119] (15 mg., 0.07 mmole) on treatment with semicarbazide hydrochloride[186] gave a semicarbazone (11 mg., 59%), m.p. 155-157° (methanol-water), (Found: C, 71.7; H, 7.0%, M^+ 269. $C_{16}H_{19}N_3O$ requires: C, 71.35; H, 7.1%, M 269), $\lambda_{max.}$ 329 (ε 35,200), 317 (35,500), 276 (17,300), 259sh, and 231 nm. (15,200), τ ($CDCl_3$) 9.0 (t, J 6.5 Hz, 2 x CH_3), 8.45 (m, 2 x $CH_2.CH_2.CH_3$), 7.65 (m, 2 x $CH_2.C\equiv$), 4.08br (s, remains on addition of D_2O, $.NH_2$), 3.79 (t, J 2.5 Hz, $C\equiv C.CH=$), 2.10 (s, $-CH=N$) and -0.2br (s, removed on addition of D_2O, $N.NH.CO$).

24. Exchange of amide protons in cyclohexanone semicarbazone:-

Cyclohexanone similarly yielded a semicarbazone,[186] m.p. 165-166° (lit.,[186] 167°), τ ($CDCl_3$) 8.37 [m, $CH_2.(CH_2)_3.CH_2.$], 7.7 [m, $(-CH_2)_2C=N$],

4.04br (s, CO.N$\underline{H}_2$, not removed with D_2O) and 0.9br (s, N$\underline{H}$.CO.NH_2, not removed with D_2O).

Deuterium oxide caused approximately 90% decrease in absorbance in the τ 4.04 and 0.9 signals after 12 hr..

SCHEME N

25. 3-Phenylprop-2-ynal (93):-[187]

Cinnamic aldehyde (88 g., 0.67 mole; b.p. 99.5-100.5°/1 mm.) was treated with bromine[187] and dehydrobrominated with potassium carbonate to yield α-bromocinnamic aldehyde (124 g., 88%), m.p. 70-71° (lit.,[187] 72-73°; ethanol-water), τ 2.5 (m, 2 $\underline{m}$ + 1 $\underline{p}$ Ar-H), 2.12 (s, -C$\underline{H}$=), 2.05 (2 $\underline{o}$ Ar-H) and 0.68 (s, C$\underline{H}$O).

The bromoaldehyde (41 g., 0.19 mole) and triethoxymethane (50 ml., 0.3 mole) afforded[187] the diethyl acetal (34.4 g.; 62%), b.p. 146-150°/5 mm., n_D^{18} 1.5579 (lit.,[187] 146-149°/5 mm.; n_D^{22} 1.5500).

The diethyl acetal (25.0 g., 0.088 mole) was treated[187] with potassium hydroxide to yield phenylpropynal diethylacetal (13.0 g., 73%), b.p. 153-156°/ 19 mm. (lit.,[187] 153-156°/19 mm.), $\nu_{max.}$ (thin film) 2200 (C≡C), 1110 and 1060 $cm.^{-1}$ (diethyl acetal).

The diethyl acetal (10.5 g., 0.051 mole) was treated[187] with aqueous sulphuric acid to yield phenylpropynal (6.1 g., 91%), b.p. 47-48°/ 0.05 mm., $n_D^{17.5}$ 1.5999 (lit.,[187] 114-117°/17 mm., n_D^{25} 1.6032), R_T (A; 159°) 4.1 min.

(one peak), $\lambda_{max.}$ 285sh, 275 (ε 16,500), 264sh and 234 nm. (8500), $\nu_{max.}$ 2230, 2190 (C≡C) and 1655 cm.$^{-1}$ (CHO), τ 2.54 (m, 5 Ar-H) and 0.62 (s, .CHO), m/e 131 (11%), 130 (M^+, 100%), 129 (76%) and 102 [M^+-CO, 85%].

26. a) Dimerisation of 3-phenylprop-2-ynal (93):-

The aldehyde (93) (700 mg., 5.4 mmole) was heated in a nitrogen atmosphere at 130° for 7 hr.. Initially slight effervescence was observed and carbon monoxide was detected as in experiment 4b in the exit gas. The dark residual oil was taken up in ether (5 ml.) and chromatographed on silica gel layers (100 x 20 cm.) with ether-light petroleum (1:9) (developed 6 times). The fastest moving band (R_F 0.7) afforded the starting material (93) (240 mg.). A broad zone (R_F 0.6-0.5), more concentrated at the front and tail, contained both dimers (94a and b) (1.2:1).

The front of the band gave the unstable oily (Z)-2-formyl-1,4-diphenyl-but-1-en-3-yne (94a) (180 mg., 44% based on reacted aldehyde), $\lambda_{max.}$ 322 (ε 12,000) and 259 nm. (9000), $\nu_{max.}$ 2750, 1690 (CHO) and 2240 cm.$^{-1}$ (C≡C), τ 2.6 (m, 10 Ar-H), 2.1 (s, -CH=) and 0.17 (s, .CHO), m/e 232 (M^+, 100%), 231 (79), 230 (43), 215 (25), 203 [(M^+-HCO), 80%] and 202 [(M^+-H_2CO), 98%]. The aldehyde (94a) gave a crystalline semicarbazone, m.p. 182-184° (decomp.) (from methanol-water), (Found: C, 74.9; H, 5.4%, M^+ 289. $C_{18}H_{15}N_3O$ requires C, 74.7; H, 5.25%, M, 289). The tail gave the E-isomer (100 mg., 25%), $\nu_{max.}$ 2720, 1705 (CHO) and 2225 cm.$^{-1}$ (C≡C), τ 2.6 (m, 8 Ar-H + -CH=), 1.92 (m, o Ar-H in C_6H_5.CH=) and 0.45 (s, .CHO), m/e 232 (M^+, 100%)

with an identical fragmentation to above isomer, $\lambda_{max.}$ 329 (ϵ 12,000) and 258 nm.(11,000). A band (R_F 0.65) yielded α-bromocinnamic aldehyde (5 mg.), m.p. 69-71° (lit.,[187] 72-73°).

b) Stereochemical assignment of phenylpropynal dimers (94):-

From additivity rules,[116] the vinyl proton shifts are predicted:-

$$\delta = 5.25 + Z_{gem} + Z_{cis} + Z_{trans}$$

(Z)-isomer: $\delta = 5.25 + 1.38 + 0.38 + 1.17 = 8.18$ ppm.

$\tau_{calc.} = 1.82$ $\tau_{obs.} = 2.1$

(E)-isomer: $\delta = 5.25 + 1.38 + 0.95 + 0.12 = 7.70$ ppm.

$\tau_{calc.} = 2.30$

27. Nona-3,5-diyn-2-one (96):-

1-Bromobut-1-yn-3-ol [prepared from but-1-yn-3-ol (3.5 g., 50 mmole)] was coupled with pent-1-yne (3.9 g., 57 mmole). Nona-3,5-diyn-2-ol (2.4 g., 35%) was eluted with ether-light petroleum (1:2) as a colourless oil, (Found: C, 78.55; H, 8.55. $C_9H_{12}O$ requires C, 79.35; H, 8.9%), $\nu_{max.}$ 3400 (OH) and 2230 cm.$^{-1}$ (C≡C).

Manganese dioxide oxidation of the alcohol gave the ketone (90%), $\lambda_{max.}$ 283 (rel. E 0.8), 267 (1.0), 253 (0.7), 241 (0.4) and 230sh nm., $\nu_{max.}$ 2230, 2130 (C≡C) and 1670 cm.$^{-1}$ (C=O), τ 8.9 (t, J 7 Hz, $C\underline{H}_3.CH_2$), 8.3 (m, J 7 Hz, $CH_3.C\underline{H}_2$), 7.6 (s, $C\underline{H}_3.CO$) and 7.6 (t, J 7 Hz, $.C\underline{H}_2.C{\equiv}C$).

Dimerisation:-

The ketone (100 mg.) was kept under nitrogen without solvent for 24 hr. at 60°. The u.v. spectrum of the mixture showed end absorption with superimposed diyn-one chromophore. T.l.c. did not show any extra spots apart from polymer and remaining ketone.

28. Octa-3,5-diyn-2,7-dione:-

Octa-3,5-diyne-2,7-diol[188] (500 mg., 3.6 mmole) was oxidised by manganese dioxide in dichloromethane-ether (1:1; 200 ml.). The corresponding diketone (20 mg.) was the least polar product; it was a very unstable oil, $\lambda_{max.}$ 293, 277, 262 and 247 nm., $\nu_{max.}$ 2110 (C≡C) and 1690 cm.$^{-1}$ (C=O), τ 7.6 (s, .CH_3).

The infrared spectrum was run 30 min. later and showed new bands at $\nu_{max.}$ 2180 and 1720 cm.$^{-1}$. In the neat state, the diketone decomposed (> 90%) in 1.5 hr..

29. Nona-3,5,7-triyn-2-one:-

Nona-3,5,7-triyn-2-ol[189] (50 mg., 0.38 mmole; m.p. 38°) was oxidised by manganese dioxide to the ketone (45 mg.), a rapidly polymerising oil, $\lambda_{max.}$ 333, 312, 293, 276, 260, 247 and 230 nm., $\nu_{max.}$ 2220, 2190, 2110 (C≡C) and 1670 cm.$^{-1}$ (C=O), τ 7.93 (s, CH_3.C≡C) and 7.65 (s, CH_3.CO.).

The triyne (20 mg.) was kept in dichloromethane (0.5 ml.) under nitrogen

for 12 hr., T.l.c. [multiple development ether-light petroleum (1:4)] indicated three spots (R_F 0.4, 0.35 and 0.27) behind the triyne (R_F 0.6; 0.5 mg.). Treatment of the crude mixture did not change the u.v. spectrum, $\lambda_{max.}$ 312 nm..

30. Crossdimerisation of octa-2,4-diynal (97) and nona-3,5-diyn-2-one (96):-

The aldehyde[119] (144 mg., 1.2 mmole) and ketone (161 mg., 1.2 mmole) were kept under nitrogen for 80 hr. at 20°. The residue was taken up in ether, filtered and chromatographed on a 100 x 20 cm. plate with ether-light petroleum (1:30) using multiple development. The most mobile zone (R_F 0.7) yielded the aldehyde dimer (98) (27 mg.); the starting ketone (45 mg., 28% recovery) was slightly more polar. A minor zone (R_F 0.5) afforded the unstable cross dimer 7-acetyltetradec-6-ene-4,8,10-triyne (99) (14 mg., 7% based on recovered ketone), $\lambda_{max.}$ 322 (rel. E 1.3) and 253 nm. (1.0), $\nu_{max.}$ 2240, 2210, 2160 (C≡C) and 1710 $cm.^{-1}$ (C=O), τ ($CDCl_3$) 8.9 (t, J 7 Hz, 2 x $\underline{CH}_3.CH_2$), 8.4 (sextet, J 7 Hz, 2 x $CH_3.\underline{CH}_2.CH_2$.), 7.6 (m, with singlet $\underline{CH}_3.CO$, 2 x $\underline{CH}_2.C{\equiv}C$) and 3.75 (t, J 2.5 Hz, $=\underline{CH}.C{\equiv}C.CH_2$.), m/e 226 ($M^+$, 22%), 211 [$M^+-CH_3$, 17%], 198 [($M^+-CO$), 100%] and 43 (70%).

31. Hepta-3,5-diyn-2-ol:-

1-Bromopropyne (4.8 g., 40 mmole) was coupled with but-3-yn-2-ol (2.8 g., 40 mmole) at 25°. Ether-light petroleum (1:4) eluted oily hepta-3,5-diyn-2-ol (2.2 g., 51%), b.p. 50-56° (block)/10 mm., n_D^{20} 1.5212 (lit.,[190]

b.p. 84-6°/14 mm.). (Found: C, 77.4; H, 7.65. Calc. for C_7H_8O: C, 77.75; H, 7.45%), $\lambda_{max.}$ 255 (ε 200), 242 (400) and 230 nm. (420), $\nu_{max.}$ 3600, 3500 (OH), 2230 (C≡C), 1380, 1360 (Me) and 1035 cm.$^{-1}$ (C-O), τ 8.57 [d, J 6.5 Hz, CH_3.CH(OH),], 8.03 (s, CH_3.C≡C), 6.45br (s, removed on D_2O addition, OH) and 5.48 [q, J 6.5 Hz, -CH(OH).C≡].

32. Hepta-3,5-diyn-2-one:-

Manganese dioxide oxidation of hepta-3,5-diyn-2-ol (1.08 g., 10 mmole) afforded hepta-3,5-diyn -2-one (490 mg., 48%), $\lambda_{max.}$ 280 (ε 7000), 265 (8900), 252 (6400) and 238 (2750), $\nu_{max.}$ 2230, 2150 (C≡C), 1675, 1120 (C=O) and 1360 cm.$^{-1}$ (Me), τ 7.93 (s, CH_3.C≡) and 7.70 (s, CH_3.CO).

SCHEME O

33. Crossdimerisation of 5-phenylpenta-2,4-diynal (73) and hepta-3,5-diyn-2-one:-

The aldehyde (100 mg., 0.65 mmole) was kept with the ketone (1.06 g., 10 mmole) for 30 hr. at 20° under nitrogen. The residue was taken up in ether (10 ml.), applied to two 100 x 20 cm. silica gel plates, and developed three times with ether-light petroleum (1:8). A diffuse zone (R_F 0.6 to 0.8) contained the starting ketone and 1,8-diphenyl-4-formyloct-3-ene-1,5,7-triyne (74).

A narrow band (R_F 0.35) afforded an unstable oil, $\lambda_{max.}$ 337 and 273 nm., which was purified by repeated chromatography on silica gel layers using chloroform-light petroleum as solvent and yielded 5-acetyl-9-phenylnon -4-ene-

2,6,8-triyne (7 mg., 3% based on starting ketone), $\lambda_{max.}$ 340 (rel. E 1.0) and 275 nm. (0.9), $\nu_{max.}$ 3020 (Ph), 2210 (C≡C), 1700 and 1120 cm.$^{-1}$ (C=O), τ 7.85 (d, J 2.5 Hz, CH_3.C≡C.CH.), 7.6 (s, CH_3.CO), 3.65 (q, J 2.5 Hz, =CH.C≡C.Me), 2.6 and 2.4 (m, 5, Ar-H), m/e 232 (M^+, 52%), 217 [(M^+-CH_3), 15%] and 204 [(M^+-CO), 100%].

SCHEME P

34. [1-^{2}H]-3-Phenylprop-2-yn-1-ol:-

The 3-phenylprop-2-ynal (93) (1 g., 7.7 mmole) in ethanol (20 ml.) was reduced with sodium borodeuteride (100 mg.) for 2 hr.. Isolation with ether afforded the oily deuterated alcohol (985 mg., 97%), τ 6.4br (s, .OH), 5.6 (s, -CH_2-) and 2.73 (m, 5 Ar-H) with no aldehydic proton signal. Integration of the n.m.r. signals showed 38% deuterium substituted at C(1).

35. [1-^{2}H]-3-Phenylprop-2-ynal:-

The deuterated alcohol (950 mg., 7.2 mmole) was oxidised with manganese dioxide, and the deuterated aldehyde was separated from the starting material by preparative layer chromatography with ether-light petroleum (1:3). The less polar zone gave the aldehyde which was bulb-distilled, 130-140° (block temp.)/10 mm., to yield the g.l.c. pure [1-^{2}H]-3-phenylprop-2-ynal (310 mg., 2.4 mmole), $\nu_{max.}$ 2220, 2120 (C-D and C≡C), 1670, 1650 cm.$^{-1}$ (C=O), m/e 131 (M^+, 100%), 130 [(M^+-1), 59%] and 129 [(M^+-2), 100%]. Integration of the n.m.r. signals

(see experiment 25) suggested 81% deuteration at C-1.

36. Dimerisation of [1-^{2}H]-3-phenylprop-2-ynal:-

[1-^{2}H]-3-Phenylprop-2-ynal (300 mg., 2.3 mmole) was heated as in the dimerisation procedure in experiment 26. The deuterated dimers (103) had m/e 235 (16%), 234 (100), 233 (100), 232 (42), 231 (8), 217 (15), 216 (11), 206 (11), 205 (38), 204 (65), 203 (72), 202 (32) and 201 (13).

37. Effect of possible catalysts on dimerisation rate:-

3-Phenylpropynal controls did not contain any dimer [t.l.c.; ether-light petroleum (1:4)].

a) Cuprous and silver ions: 3-Phenylpropynal (10 mg.) and either silver nitrate or cuprous chloride (5 mg.) were stirred for 24 hr. in methanol (5 ml.). T.l.c. indicated complete absence of dimer.

b) Palladium on charcoal:[191] 3-Phenylpropynal (50 mg.) was heated at 80° under nitrogen with 5% palladium on charcoal (50 mg.) for 2 hr.. The residue was taken up in ether and t.l.c. indicated no increased dimer formation over a blank sample.

c) p-Toluene sulphonic acid: 3-Phenylpropynal (50 mg.) and the acid (2 mg.) were kept under nitrogen for 7 days. T.l.c. indicated negligible dimer formation.

d) Pyridine: 3-Phenylpropynal (50 mg.) and pyridine (10 mg.) were kept under nitrogen for 7 days. T.l.c. indicated negligible dimer formation.

e) Penta-1,3-diyne: Hexa-2,4-diynal (10 mg.) and pentadiyne (10 mg.) in

dichloromethane (1 ml.) were kept in the dark for 24 hr.. The u.v. spectrum showed an intensity ratio for maxima at 288 and 320 nm. of 1:1.2, similar to that of a control experiment.

38. Isolation of methyl 5-phenylpenta-2,4-diynoate from monomer aldehyde (73) sample:-

5-Phenylpent-2,4-diynal (200 mg., 1.3 mmole) (from experiment 6) was reduced with excess sodium borohydride (200 mg.) in ethanol (30 ml.). The mixture was worked up with ether and applied to silica gel layers. Development with ether-light petroleum (1:8) gave a band of similar polarity to that of the starting aldehyde. Extraction yielded the oily 5-phenylpenta-2,4-diynoate, (M^+ 184, $C_{12}H_8O_2$ requires M 184), $\lambda_{max.}$ 306, 298, 271 and 243, $\nu_{max.}$ 1670 $cm.^{-1}$ (C=O), τ 6.1 (s, $CO_2\underline{CH}_3$) and 2.7 (s, 5 x Ar-$\underline{H}$).

SCHEME S

39. Attempted cross-dimerisation between 3-phenylpropynal (93) and hepta-2,4-diynoic acid:-[192]

a) The aldehyde (500 mg., 3.8 mmole) and acid (700 mg., 5.7 mmole) were mixed and kept under nitrogen for 3 days at 20°. The residue was taken up in ether (5 ml.) and applied to thin layers. No component of similar t.l.c. behaviour to that of the hexadiynal dimer (72) was observed.

b) A similar mixture was heated at 99° for 24 hr. Work-up afforded traces of the phenylpropynal dimers (94), but no cross dimer.

40. Attempted cross dimerisation between hexa-2,4-diynal (67) and methyl 9-cyanohept-trans-2-ene-4,6-diynoate (diatetryne II):-

The aldehyde (92 mg., 1 mmole) and diatetryne II[91] (60 mg., 0.38 mmole) in dichloromethane (0.3 ml.) were kept under nitrogen for 3 days. The solution was applied to a plate which was run using multiple development [ether-light petroleum (1:15)] and yielded four bands.

The two most mobile bands (R_F 0.75 and 0.6) corresponded to the aldehyde (67) (5 mg.) and diatetryne II (52 mg.) respectively. A diffuse zone at R_F 0.4 comprised the hexadiynal dimers (72) (20 mg.); its tail appeared to include another band. Further chromatography on thin layers afforded the hexadiynal dimers (72) together with a minor zone, $\lambda_{max.}$ 337, 316 and 296 nm., which was present as an impurity in the diatetryne II sample.[91]

The hexadiynal dimer fraction was reduced with sodium borohydride. T.l.c. indicated no spot moving faster than the reduced dimer (88). A trial experiment demonstrated that diatetryne II decomposed under these conditions.

PART III

General procedure for attempted reactions of iodides with magnesium:-

a) Initiation: A 50 ml. flask containing magnesium turnings (1 mol.) was flamed whilst a dry nitrogen stream passed through via two needles in serum caps. The exit needle was removed and the flask was allowed to cool down in this atmosphere. The solvent, either tetrahydrofuran or ether, was freshly distilled from lithium aluminium hydride, and added so that it just covered the magnesium.

A solution of the iodide (1 mol.) in benzene (sodium dried; 5 ml.) was evaporated to dryness under reduced pressure, and the residue was introduced into the flask dissolved in the Grignard-solvent (1 ml.).

Initiation of the Grignard reagent formation was assumed to be successful if bubbles emanated continuously from the magnesium surface, concurrent with solvation of the turnings. Promotion by scratching, addition of iodine and warming was also attempted.

b) Work up after aldehyde addition: Saturated ammonium chloride (10 ml.) was added to the mixture, which was stirred until all magnesium had dissolved. Isolation with ether in the usual way yielded an oil, which was chromatographed on a column (silica gel; 30 g.), using gradient elution. Fractions (30 ml.) were concentrated to 2 ml. and monitored by t.l.c..

1. Methyl 9-iodononanoate:-

Methyl 9-bromononanoate[193] (2.5 g., 10 mmole) was heated under reflux according to the procedure for displacement with iodide. Work up afforded an oil, which was not chromatographed, but distilled to give oily methyl 9-iodononanoate (2.5 g., 83%) b.p. 100-101°/0.1 mm., n_D^{25} 1.4878 (lit.,[194] 124-127°/2 mm., n_D^{20} 1.4911) (Found: C, 40.3; H, 6.3. Calc. for $C_{10}H_{19}IO_2$: C, 40.3; H, 6.4%), τ 8.6br [s, .[CH_2]$_6$.CH_2I], 7.9 (t, J 7 Hz, .CH_2.CO_2Me), 6.83 (t, J 6.5 Hz, .CH_2I) and 6.4 (s, .CO_2CH_3).

Methyl 9-iodononanoate (1.5 g., 5 mmole) was subjected to the general procedure for Grignard reagent formation in tetrahydrofuran. No significant dissolution of magnesium or effervescence was observed.

SCHEME V

2. 1-Bromobut-3-yne:-

But-3-yn-1-ol (7 g., 0.1 mole; b.p. 65-66°/69 mm.) in pyridine (7.9 g., 0.1 mole) was added dropwise at 0° under nitrogen to triphenylphosphite dibromide[195] (52 g., 0.11 mole) which was vigorously stirred with a Hershberg stirrer. After 2 hr. the mixture became homogeneous and ether (70 ml.) was introduced. The ether fraction was decanted. The residue was dissolved in water (100 ml.); the aqueous layer was saturated with ammonium chloride and extracted with ether (2 x 25 ml.). The ether extracts were washed with ice-cold sulphuric acid (10 ml.; 2N), aqueous sodium hydrogen carbonate (10 ml.; 2N), water (10 ml.)

and brine (10 ml.), dried and concentrated to an oil which was distilled through a short column. The fraction, (7 g.) b.p. 46°/300 mm., was redistilled to give 1-bromobut-3-yne (4.7 g., 35%), b.p. 107-108°/760 mm. (lit.,[196] 107°/740 mm.), R_T (A; 99°) 1.1 min., $\nu_{max.}$ 3300 and 2110 cm.$^{-1}$ (C≡CH), τ 8.03 (t, J 3 Hz, HC≡C.), 7.35 (dt, J 7 and 3 Hz, .CH_2.C≡C), and 6.63 (t, J 7 Hz, CH_2Br).

3. Attempted oxidation of but-3-yn-1-ol:-

But-3-yn-1-ol (500 mg., 7.1 mmole) in pyridine (5 ml.) was treated with Sarett reagent[197] [chromium trioxide (1.1 g.) in pyridine (10 ml.)] and the mixture kept at 20° under nitrogen in the dark for 8 hr. The mixture was poured into water (200 ml.) and extracted with ether (2 x 50 ml.). The combined ether extracts were washed, in turn, with aqueous sulphuric acid (2N; 50 ml.), saturated aqueous sodium hydrogen carbonate (2 x 10 ml.), water (20 ml.), brine (20 ml.) and dried. The solvent was slowly evaporated off through a Durfton column (7"), affording an oil (ca. 1 gm.) which still contained pyridine. I.r. spectroscopy indicated free ethynyl ($\nu_{max.}$ 3300 cm.$^{-1}$) but n.m.r. spectroscopy revealed absence of aldehyde (no signal τ-0.5-1.0).

4. 1-Methoxybut-cis-1-en-3-yne:-

A commercial sample was distilled twice, b.p. 52-54°/20 mm. and 62-63°/100 mm., n_D^{22} 1.4615 (lit.,[135] 63°/80 mm.; n_D^{20} 1.462) to give a clear liquid which rapidly reddened on standing in air, $\nu_{max.}$ (thin film) 3300, 2080,

(C≡CH), 1630, 740 (cis CH=CH), 1110 and 1020 cm.$^{-1}$ (OMe), τ 7.18 (d, J 2 Hz, HC≡C), 6.22 (s, .CH_3), 5.6 (dd, J 6.5 and 2 Hz, =CH.C≡) and 3.75 (d, J 6.5 Hz, .O.CH=).

5. Acid hydrolysis of methoxybutenyne:-[135]

Distillation of an ethereal solution (5 ml.) of 1-methoxybut-cis-1-en-3-yne (100 mg.) from sulphuric acid (2N; 10 ml.) at 50° gave a fraction, b.p. 42°/400 mm. which darkened rapidly, $\nu_{max.}$ (thin film) 1960, 1940 (CH=C=CH) and 1690 cm.$^{-1}$ (CHO), τ (neat liquid) 4.67 (d, J 6 Hz, .CH_2=), 4.23 (m, =CH.CHO) and 0.55 (d, J 7 Hz, .CHO).

SCHEME X

6. 1-Bromo-8-methoxyoct-cis-7-ene-3,5-diyne (122):-

1-Methoxybut-cis-1-en-3-yne (660 mg., 8 mmole) and 1,4-dibromobut-1-yne [from 1-bromobut-3-yne (1.35 g., 0.01 mole)] were coupled at 30°. In the work up quick elution [ether-light petroleum (1:9)] of the reaction products from alumina (Act. III) yielded a compound exhibiting a diynene chromophore. This fraction was purified on thin layers to give the oily and unstable 1-bromo-8-methoxy-oct-cis-7-ene-3,5-diyne (270 mg., 15%), $\lambda_{max.}$ 292 (rel. E 1.23), 275 (1.45), 261 (1.0) and 224 nm. (3.25), $\nu_{max.}$ 2250, 2230 (C≡C), 1630 (CH=CH), 1110 cm.$^{-1}$ (OMe), τ 7.15 (t, J 7 Hz, .CH_2.C≡C), 6.6 (t, J 7 Hz, .CH_2Br), 6.19 (s, .OCH_3), 5.55 (d, J 8 Hz, =CH.C≡C) and 3.69 (d, J 8 Hz, .OCH=).

7. Dehydrohalogenation of 1-bromo-8-methoxyoct-cis-7-ene-3,5-diyne (122):-

The bromide (122) (30 mg.) was chromatographed on a column of alumina (Act. I; 10 g.) and eluted [ether-light petroleum (1:15)] as unstable 8-methoxy-octa-cis-1,7-diene-3,5-diyne (123), $\lambda_{max.}$ 318 (rel. E 1.4), 299 (1.7), 282 (1.4), and 273 nm. (1.0), $\nu_{max.}$ 2220, 2140 (C≡C), 1110 (OMe), 1665, 1625, 1030, 970 and 930 $cm.^{-1}$ (cis-CH=CH and $CH=CH_2$), τ 6.19 (s, CH_3·), 5.48 (d, J 7 Hz, .CH=CHOMe), 4.5 (dd, J 10 and 5 Hz, $-CH=CH_2$), 4.2 (m, $CH_2=$) and 3.67 (d, J 7 Hz, O-CH=).

8. 8-Bromoocta-3,5-diynal dimethyl acetal (114):-

1-Bromo-8-methoxy-oct-cis-7-ene-3,5-diyne (150 mg., 0.7 mmole) was heated under reflux for 3 hr. with 4% sulphuric acid in methanol (50 ml.) under nitrogen. The cooled solution was poured into water (120 ml.) and the mixture extracted with ether (3 x 50 ml.). The combined extracts were washed with aqueous sodium hydrogen carbonate (20 ml.; 2N), water (20 ml.), brine (20 ml.) and dried. The concentrated solution was chromatographed on an alumina column; ether-light petroleum (1:48) eluted a trace of diynene, $\lambda_{max.}$ 281, 265, 251 and 238 nm.. Oily 8-bromoocta-3,5-diynal dimethyl acetal (120 mg., 70%) was eluted with ether-light petroleum (1:9) and had $\lambda_{max.}$ 256 (ε 230), 243 (420) and 230 nm. (400), $\nu_{max.}$ 2260 (C≡C), 1120 and 1080 $cm.^{-1}$ (methyl acetal) τ 7.48 [d, J 5.5 Hz, $.CH_2.CH(OMe)_2$], 7.18 (t, J 7 Hz, $.CH_2.CH_2Br$), 6.67 (s, 2 x CH_3O.), 6.57 (t, J 7 Hz, $.CH_2.Br$) and 5.5 [t, J 5.5 Hz, $.CH(OMe)_2$].

9. Hydrogenation of 8-bromoocta-3,5-diynal dimethyl acetal:-

8-Bromoocta-3,5-diynal dimethyl acetal (54 mg., 0.22 mmole) in methanol (20 ml.) was hydrogenated for 2 hr. over 5% rhodium on charcoal (50 mg.). The mixture was filtered and the filtrate concentrated to yield an oil (35 mg.), which was examined by t.l.c. [ether-light petroleum (1:4); multiple development]. Five spots resulted, none of which had the expected t.l.c. behaviour of 8-bromooctanal dimethyl acetal (R_F 0.8).

SCHEME Y

10. 2-Chloromethyl-1,3-dioxolan:-

Chloroacetaldehyde diethyl acetal (153 g., 1 mole) and ethanediol (62 g., 1 mole) were heated under reflux for 5 hr. with concentrated hydrochloric acid (1 ml.). Solid potassium carbonate (2 g.) was added to neutralise the acid, and the mixture was distilled through a column (10 inch Durfton). The initial fractions, b.p. $< 80^{\circ}$, contained ethanol (75 ml.) and were discarded. The chlorodioxolan (82 g., 67%) was then collected, b.p. 158°, n_D^{21} 1.4430 (lit.,[198] 155-159°/740 mm., n_D^{25} 1.4465), $\nu_{max.}$ 1190, 1040 and 950 $cm.^{-1}$ (dioxolan), τ 6.52 (d, J 5 Hz, $C\underline{H}_2.Cl$), 6.04 (m, $.C\underline{H}_2.C\underline{H}_2.$) and 4.89 [t, J 5 Hz, $-C\underline{H}(O-)_2$].

11. Attempted Wittig salt formation (125):-

The chlorodioxolan (2.5 g., 20 mmole) and excess triphenyl phosphine (10 g.) were heated at 100° or under reflux in acetone, or benzene. After 3 days,

the solvent was removed and the residue washed repeatedly with ether. The remaining brown gum was triturated with ether, and at best a small yield of amorphous solid (20 mg.) was obtained on recrystallisation from ethyl acetate-ether. The crude salt (10 mg.) was treated with sodium methoxide [from sodium (10 mg.) and methanol (10 ml.)]. No colouration was observed.

12. Hepta-trans, trans-2,4-dien-6-ynal (127):-

The ethynyl aldehyde was prepared[199] through the action of lithium acetylide ethylene diamine (1 g., 11.6 mmole) on Zincke aldehyde (126) (1 g., 5.3 mmole) in benzene (20 ml.) under acetylene. The work-up gave a crude ethereal solution which after chromatography (silica gel; 30 g.), yielded hepta-2,4-dien-6-ynal (155 mg., 27%) as a stereoisomeric mixture, τ ($CDCl_3$) 6.62 and 6.41 (each d, J 2 Hz, H$\underline{C}\equiv$C: ratio 2:1), 4.3-2.1 (m, 4 x -C$\underline{H}$=), 0.38 and 0.26 (each d, J 8 Hz, .C$\underline{H}$O; ratio 2:1). The trans, trans aldehyde crystallised out at -20°, m.p. 95-97° (lit.,[9] 92°; [200] 51°) from ether-light petroleum, $\lambda_{max.}$ 296 (ϵ 38,000) and 284 nm. (40,000), $\nu_{max.}$ ($CHCl_3$) 3300, 2130 (HC≡C), and 1680 $cm.^{-1}$ (CHO), τ ($CDCl_3$) 6.64 (d, J 2 Hz, H$\underline{C}\equiv$C.), 3.92 (dd, J 15.5 and 2 Hz, =C$\underline{H}$.C≡C), 3.74 (dd, J 15.5 and 8 Hz, =C$\underline{H}$.CHO), 3.05 and 2.91 (2H, AB system, =C$\underline{H}$.C$\underline{H}$=) and 0.36 (d, J 8 Hz, C$\underline{H}$O), τ (Me_2CO) (assignment order as above) 6.06, 3.71, 3.70, 3.00 (dd, J 15.5 and 11 Hz, C$\underline{H}$=CH.C≡), 2.62 (dd, J 15.5 and 11 Hz, .C$\underline{H}$=CH.CHO) and 0.30.

13. N.m.r. data for model all-trans dienals:-

a) 7-Phenylhepta-2,4-dien-6-ynal (130; R = Ph.C≡C) had m.p. 70-71° (lit.,[200] 71°). τ ($CDCl_3$) 3.73 (dd, J 15.5 and 7 Hz, C<u>H</u>.CHO), 3.65 (d, J 15.5 Hz, Ph.C<u>H</u>=), 3.1 (dd, J 15.5 and 11 Hz, .C<u>H</u>=CH.Ph), 2.82 (dd, J 15.5 and 11 Hz, .C<u>H</u>=CH.CHO), 2.7 (m, 5 Ar-<u>H</u>) and 0.35 (d, J 7 Hz, .C<u>H</u>O), τ (Me_2CO) 3.75, 3.55, 3.07, 2.6, 2.55, 0.35 (assignment order as before).

b) Hexa-2,4-dienal[201] (130; R = Me) had τ 8.05 (d, J 5.5 Hz, C<u>H</u>$_3$.), 4.0 (dd, J 15 and 8 Hz, =C<u>H</u>.CHO), 3.75 (m, C<u>H</u>=C<u>H</u>.Me), 3.0 (dd, J 15.5 and 10 Hz, C<u>H</u>=CH.CHO) and 0.48 (d, J 8.0 Hz, .C<u>H</u>O); τ (Me_2CO) 4.1, 3.75 and 3.0 (assignment order of vinyl protons as before).

14. 2,2-Dimethyl-1,3-dioxolan:-

Ethanediol (124 g., 2 mole) 'Analar' acetone (58 g., 1 mole) and light petroleum (200 ml.; b.p. 30-40°) were vigorously stirred and heated under reflux for 60 hr. After removal of the upper layer, the diol fraction was further extracted with light petroleum (2 x 100 ml.). The extracts were dried and distilled from potassium carbonate through a column (5 inch; glass bead) to yield the dioxolan (47 g., 46%), b.p. 93-94°, n_D^{20} 1.3991 (lit.,[202] b.p. 89°; n_D^{20} 1.3992), $\nu_{max.}$ (thin film) 1150, 1060 and 945 $cm.^{-1}$ (1,3-dioxolan), τ 8.72 (s, 2 x C<u>H</u>$_3$), and 6.17 (s, .C<u>H</u>$_2$.C<u>H</u>$_2$.).

15. Hepta-2,4-dien-6-ynal ethylene acetal (128):-

i) Hepta-2,4-dien-6-ynal (127) (150 mg., 1.4 mmole) in 2,2-dimethyl-1,3-dioxolan (20 ml.) was kept at 20° under nitrogen with p-toluene sulphonic acid (2 mg.). After 48 hr., the acid was neutralised with solid potassium carbonate (10 mg.), insoluble salts were filtered off, and the filtrate concentrated to an oil. Chromatography on thin layers [multiple development: ether-light petroleum (1:3)] resulted in two bands.

The more mobile zone (R_F 0.6) arose from the ethylene acetal (110 mg., 52%), $\lambda_{max.}$ 290sh, 270sh and 258 nm. (ε 25,000), $\nu_{max.}$ ($CHCl_3$) 3300, 2100 (C≡CH), 1140, 1060 and 980 $cm.^{-1}$ (1,3-dioxolan), τ ($CDCl_3$) 7.03 and 6.8 (d, J 2 Hz, HC≡C isomers: ratio 7:3), 6.12 (m, $.CH_2.CH_2$), 4.75 [m, $-CH(O-)_2$] and 5.0-3.1 (m, 4 x -CH=).

The less mobile band (R_F 0.2) afforded 4-hydroxydeca-5,7-dien-9-yn-2-one (131) (12 mg., 5%) (M^+ 164. $C_{10}H_{12}O_2$ requires M 164), $\nu_{max.}$ 3500 (bonded OH), 3310, 2100 (HC≡C), 1720 (saturated ketone) and 1630 $cm.^{-1}$ (CH=CH).

ii) Hepta-2,4-dien-6-ynal (50 mg., 0.5 mmole), ethanediol (1 ml.), p-toluene sulphonic acid (2 mg.) were dissolved in dry methyl cyanide (50 ml.). Linde 4A molecular sieve was added, and the solution kept in the dark for 60 hr.. No change in the u.v. spectrum, $\lambda_{max.}$ 284 nm., was noted.

SCHEME Z

16. Tetrahydrofurfuryl chloride:-

Thionyl chloride (75 g., 0.64 mole; G.P.R.) was added[203] dropwise to a vigorously stirred mixture of tetrahydrofurfuryl alcohol (60 g., 0.59 mole) and pyridine (53 g., 0.67 mole) at 20° and yielded tetrahydrofurfuryl chloride (55 g., 77%), b.p. 45-46°/24 mm. (lit.,[203] 42°/12 mm.) n_D^{20} 1.4560 (lit.,[204] n_D^{20} 1.4560).

17. Pent-4-yn-1-ol (132):-

Tetrahydrofurfuryl chloride (54.5 g., 0.45 mole) on treatment with[205] sodamide [prepared from sodium (36 g., 1.55 mole)] in liquid ammonia (500 ml.) gave pent-4-yn-1-ol (32 g., 85%), b.p. 75-77°/35 mm., n_D^{21} 1.4450 (lit.,[205] b.p. 71°/29 mm.; n_D^{23} 1.4443), R_T (column A; 92°) 5.9 min. (one peak), $\nu_{max.}$ 3670, 3450 (OH) and 2110 cm.$^{-1}$ (C≡C), τ ($CDCl_3$-D_2O) 8.3 (m, $CH_2.\underline{CH}_2.CH_2$), 8.0 (t, J 2 Hz, $\underline{H}C\equiv C$), 7.7 (m, $.\underline{CH}_2.C\equiv C$) and 6.3 (t, J 6 Hz, $.\underline{CH}_2O.$), τ (C_6D_6) 8.1 ($\underline{H}C\equiv C$), τ (C_5H_5N) 7.4 ($\underline{H}C\equiv C.$).

18. 2,3-Dibromopropanal:-

Acrolein (56 g., 1 mole) was treated[206] dropwise with bromine (160 g., 1 mole) in ether at 20°. The mixture was evaporated under reduced pressure with benzene and the residue distilled through a short column to give 2,3-dibromopropanal (201 g., 93%), b.p. 82-83°/22 mm. (lit.,[206] 80°/20 mm.), τ 6.24 (m, $.\underline{CH}_2Br$),

5.5 (m, -CHBr) and 0.67 (d, J 3 Hz, .CHO).

19. 2,3-Dibromopropanal diethyl acetal:-

Dibromopropanal (162 g., 0.75 mole) was stirred in redistilled triethoxymethane (235 g., 1.6 mole) and absolute ethanol (100 ml.) for 4 hour. Removal[207] of solvent afforded an oil which was distilled to give the diethyl acetal (142 g., 65%), b.p. 99-100°/3 mm., n_D^{21} 1.4850 (lit.,[207] b.p. 100-113°/10 mm.; n_D^{20} 1.4894), τ 8.8 (t, J 6 Hz, 2 x .CH_3), 6.3 (m, 2 x OCH_2. + CH_2Br), 5.9 (dt, J 6 and 5 Hz, .CHBr.) and 5.48 [d, J 5 Hz, .$CH(OEt)_2$].

20. Prop-2-ynal diethyl acetal (133):-

The dibromo acetal (118 g., 0.4 mole) was treated[207] with sodium (23 g., 1 mole) in liquid ammonia (500 ml.) and afforded the ethynyl acetal (25 g., 48%), b.p. 124-127°/330 mm., n_D^{25} 1.4097 (lit.,[207] b.p. 40-42°/11 mm., n_D^{20} 1.4118), $\nu_{max.}$ (thin film) 3300, 2150 (HC≡C), 1110 and 1060 $cm.^{-1}$ (unsaturated diethyl acetal), τ 8.8 (t, J 7 Hz, 2 x .CH_3), 7.68 (d, J 2 Hz, HC≡C.), 6.4 (m, 2 x .OCH_2.) and 4.86 [d, J 2 Hz, .$CH(OEt)_2$].

The yield was 53% when sodium was replaced by lithium (7 g., 1 mole).

21. Bromoprop-2-ynal diethyl acetal (133a):-

The acetal (133) (0.92 g., 7.2 mmole) was converted by the standard bromination method into the bromoalkyne[208] (1.4 g., 94%), $\nu_{max.}$ 2200 (C≡C),

1100 and 1060 cm.$^{-1}$ (unsaturated acetal), τ 8.8 (t, J 8 Hz, 2 x .CH_3), 6.4 (m, 2 x .OCH_2.) and 4.86 [s, .$CH(OEt)_2$].

22. 8-Hydroxyocta-2,4-diynal diethyl acetal (116):-

i) 3-Bromoprop-2-ynal diethyl acetal (133a) (1.5 g., 7.2 mmole) was coupled with pent-4-yn-1-ol (132) (630 mg., 7.5 mmole) according to the Cadiot-Chodkiewicz coupling procedure. The crude reaction product was chromatographed on alumina; fractions 4-5 [ether-light petroleum (1:9)] and 8-13 (1:3) both exhibited diyne absorption.

Fractions 8-13 were combined, concentrated and rechromatographed on alumina (50 g.) to give the hydroxyacetal (116) (405 mg., 27%) as a clear liquid, τ 8.8 (3H, t, J 8 Hz, 2 x .CH_3), 8.2 (2.6H, m, $HC{\equiv}C$. + $CH_2.CH_2.CH_2$.), 7.6 (2H, m, .$CH_2.C{\equiv}C$), 6.7br (1H, s, removed by D_2O, OH), 6.4 (3.9H, m, 2 x .OCH_2. + .CH_2OH) and 4.82 [0.5H, s, .$CH(OEt)_2$], τ (C_6D_6) 8.9 (m), 8.6 (.$CH_2.CH_2.CH_2$.), 8.1 ($HC{\equiv}C$), 7.9, 6.5 and 4.8. This spectrum indicated that the product was contaminated with pent-4-yn-1-ol. P.l.c. [ether-light petroleum (1:2)] gave a band, R_F 0.3, corresponding to 8-hydroxyocta-2,4-diynal, $\lambda_{max.}$ 287, 272 and 257 nm..

Fractions 4 and 5 were chromatographed on thin layers to yield 1,1,6,6-tetraethoxyhexa-2,4-diyne (134) (200 mg., 22%), $\nu_{max.}$ 2180 (C≡C) and 1020 cm.$^{-1}$ (unsaturated acetal), τ 8.8 (t, J 7 Hz, 4 x .CH_3), 6.4 (m, 4 x .OCH_2) and 4.8 [s, -$CH(OEt)_2$].

ii) 5-Bromopent-4-yn-1-ol (132a) [prepared from pent-4-yn-1-ol (840 mg., 10 mmole)] was coupled with prop-2-ynal diethyl acetal (133) (1.3 g., 10.2 mmole). The product after work up was chromatographed twice on alumina (100 g.) using gradient elution [light petroleum enriched with ether-light petroleum (1:1)] to afford two groups of fractions with the diyne chromophore. The more polar group was purified on thin layers [4 x 100 cm. plates; 3 elutions, ether-light petroleum (1:1)] and gave a zone (R_F 0.4) corresponding to 8-hydroxyocta-2,4-diynal diethyl acetal (690 mg., 33%), b.p. 135-140° (block)/0.02 mm., n_D^{21} 1.4973, (Found: C, 68.95; H, 8.7. $C_{12}H_{18}O_3$ requires C, 68.55; H, 8.65%), $\lambda_{max.}$ 256 (ϵ 210), 242 (360) and 227 nm. (375), $\nu_{max.}$ 3670, 3400br (OH), 2240 (C≡C), 1100 and 1060 $cm.^{-1}$ (unsaturated diethyl acetal), τ 8.8 (t, J 7 Hz, 2 x CH_3.), 8.2 (m, J 7 Hz, .CH_2.CH_2.CH_2-), 7.8br (s, removed by D_2O, OH), 7.6 (t, J 7 Hz, CH_2.C≡C), 6.4 (m, CH_2OH + 2 x .OCH_2.) and 4.82 [s, .$CH(OEt)_2$].

23. i) Hydrolysis of 8-hydroxyocta-2,4-diynal diethyl acetal (116):-

The hydroxyacetal (116) (0.5 mg.; $\lambda_{max.}$ 257, 244 and 232 nm.) in ether (2 ml.) was shaken with sulphuric acid (2N; 2 ml.) for 20 min. The u.v. spectrum of the ethereal layer had $\lambda_{max.}$ 287, 272 and 257 nm.

ii) Hydrolysis of 1,1,6,6-tetraethoxyhexa-2,4-diyne (134):-

Shaking the diacetal (134) (0.5 mg., $\lambda_{max.}$ 257, 244 and 232 nm.) under conditions described above produced no visible change in the u.v. spectrum of the upper layer. After shaking 24 hr. with sulphuric acid (10N; 2 ml.)

the ethereal layer exhibited $\lambda_{max.}$ 286, 270 and 256 nm. No further shift was observed after more shaking.

SCHEME AB

24. 6-Hydroxyhexa-2,4-diynal diethyl acetal:-

3-Bromoprop-2-ynal diethyl acetal (1.5 g., 7.2 mmole) was coupled with prop-2-yn-1-ol (0.63 g., 11.2 mmole) and after work up the reaction product was chromatographed on alumina. A diyne alcohol was eluted with ether-light petroleum (1:9) which was purified further by preparative t.l.c. to yield oily 6-hydroxyhexa-2,4-diynal diethyl acetal (770 mg., 59%), $\lambda_{max.}$ 257 (ϵ 220), 242 (410) and 230 nm. (440), $\nu_{max.}$ 3630, 3440br (OH), 2180, 2150 (C≡C), 1100, 1050 and 1020 $cm.^{-1}$ (diethyl acetal), τ 8.8 (t, J 7 Hz, 2 x CH_3.), 7.7br (s, removed by D_2O, OH), 6.4 (m, 2 x OCH_2), 5.73 (s, CH_2.C≡C.) and 4.8 [s, .CH(OEt)$_2$].

Hydrolysis:- The hydroxyacetal (700 mg., 3.8 mmole) was stirred with sulphuric acid (2N; 2 ml.) and dioxan (50 ml.) for 24 hr. at 20°. Isolation with ether and p.l.c. purification afforded 6-hydroxyhexa-2,4-diynal (350 mg., 84%), $\lambda_{max.}$ 286, 270, 255 and 243 nm.. After 8 hr. the ultraviolet spectrum of the neat aldehyde showed $\lambda_{max.}$ 320br nm., and t.l.c. indicated the presence of a more polar compound, presumably 5-formyldec-4-ene-2,6,8-triyne-1,10-diol.

25. Attempted transacetalisation of 8-hydroxyocta-3,5-diynal diethyl acetal with ethane-diol:-

i) The hydroxyacetal (35 mg., 0.19 mmole) and p-toluene sulphonic acid (1 mg.) were kept under nitrogen in ethanediol (1 ml.) for 24 hr. T.l.c. (ether) did not show a spot with t.l.c. behaviour corresponding to the hydroxy-ethylene acetal (136).

ii) A duplicate reaction mixture was heated at 60° for 2 hr. in the dark. T.l.c. (ether) indicated just starting material and polymer.

SCHEME AC

26. 2,3-Dibromopropanal ethylene acetal:-

2,3-Dibromopropanal (190 g., 0.88 mole) in benzene (300 ml.) was vigorously stirred with ethanediol (90 ml.) and p-toluene sulphonic acid (500 mg.) under reflux for 36 hr.. The hetereogenous mixture was cooled, the diol layer separated off and extracted with benzene (2 x 100 ml.). The benzene layer and extracts (900 ml.) were combined, washed with sodium hydroxide (2N; 200 ml.), water (200 ml.), dried and concentrated to an oil. Distillation afforded the acetal (185 g., 81%), b.p. 88-90°/3 mm., n_D^{20} 1.5310, $\nu_{max.}$ (thin film) 1160 $cm.^{-1}$ (1,3-dioxolan), τ ($CDCl_3$) 6.2 (m, $C\underline{H}_2Br.C\underline{H}Br.$ + $O.C\underline{H}_2.C\underline{H}_2O.$) and 4.84 [m, $.C\underline{H}(O-)_2$].

27. Prop-2-ynal ethylene acetal:-

The above dibromo acetal (85 g., 0.33 mole) was treated with sodium (16 g., 0.7 mole) in ammonia (500 ml.) as described in experiment 20 and yielded prop-2-ynal ethylene acetal (20 g., 62%), b.p. 111-112°/260 mm., n_D^{18} 1.5556, (Found: C, 60.5; H, 6.15%; M^+ 98. $C_5H_6O_2$ requires C, 61.2; H, 6.15%; M 98), R_T (column A: 60°), 13.7 min. (one peak), $\nu_{max.}$ (thin film) 3300, 2120 (C≡CH), 1100, 1020 and 920 $cm.^{-1}$ (unsaturated dioxolan), τ 7.47 (d, J 2 Hz, HC≡C.), 6.08 (m, .CH_2.CH_2-) and 4.43 [d, J 2 Hz, .CH(O-)$_2$].

28. 8-Hydroxyocta-2,4-diynal ethylene acetal (136):-

5-Bromopent-4-yn-1-ol [prepared from pent-4-yn-1-ol (840 mg., 10 mmole)] was coupled with the ethylene acetal (980 mg., 10 mmole) as in experiment 22. The reaction product gave a zone (R_F 0.3) on thin layers which yielded 8-hydroxy-octa-2,4-diynal ethylene acetal (0.79 g., 44%), b.p. 115-117° (block)/ 0.02 mm., n_D^{22} 1.5321 (Found: C, 66.15; H, 6.9. $C_{10}H_{12}O_3$ requires C, 66.65; H, 6.7%), $\lambda_{max.}$ 256 (ε 230), 243 (380), 230 (350) and 216 nm. (290), $\nu_{max.}$ ($CHCl_3$) 3600, 3400 (OH), 2230, 2130 (C≡C) and 1100 $cm.^{-1}$ (unsaturated dioxolan), τ 8.15 (p, J 6 Hz, CH_2.CH_2.CH_2), 7.62 (t, J 7 Hz, .CH_2.C≡C), 7.2br (s, removed by D_2O, -OH), 6.4 (t, J 6 Hz, .CH_2OH), 6.0 (m, J 3 Hz, O.CH_2.CH_2.O) and 4.3 [s, .CH(O-)$_2$]. The p-phenylazobenzoyl ester separated as needles from hexane, m.p. 104.5-105.5°, (Found: C, 70.9; H, 5.0. $C_{23}H_{20}N_2O_4$ requires C, 71.1; H, 5.2%), τ ($CDCl_3$) 7.9 (m, .CH_2.CH_2.CH_2-), 7.5 (t, J 7 Hz,

.$\underline{CH}_2$.C≡C), 6.08 (m, .O.$\underline{CH}_2$.$\underline{CH}_2$.O.), 5.6 (t, J 7 Hz, .$\underline{CH}_2$.O.CO), 4.4 [s, -$\underline{CH}$(O-)$_2$], 2.6 (m, 5 terminal Ar-H), 2.08 and 1.82 [each d, J 8 Hz, each 2H in $\underline{p}$-N-($C_6\underline{H}_4$).CO.].

An aliquot of the alcohol (136) shaken for 20 min. with sulphuric acid (2N; 2 ml.) and ether (2 ml.) had $\lambda_{max.}$ (ether) 287, 273, 256 and 244 nm., $\nu_{max.}$ ($CHCl_3$) 1680 cm.$^{-1}$ (C=O).

SCHEME AD

29. 8-Hydroxyoctanal diethyl acetal (117):-

8-Hydroxyocta-2,4-diynal diethyl acetal (116) (1.05 g., 5 mmole) was hydrogenated (105% theoretical uptake) in absolute ethanol (200 ml.) over 5% palladium on calcium carbonate (400 mg.; Kochlight) for 2 hr.. The mixture was filtered (Whatman No.1), and the catalyst washed well with boiling ethanol (270 ml.). After concentration, the solution was chromatographed on thin layers [four elutions with ether-light petroleum (1:4)] to give a band at R_F 0.75 [(116) had R_F 0.7 on the same plate], corresponding to the saturated acetal (1 g., 92%), $\nu_{max.}$ 3650, 3500 (OH), 1120 and 1060 cm.$^{-1}$ (diethyl acetal), τ 8.65br [.[$\underline{CH}_2$]$_6$.CH_2O. with superimposed triplet τ 8.85, J 7 Hz, 2 x .$\underline{CH}_3$], 7.7br (s, removed by D_2O, O$\underline{H}$), 6.5 (m, .$\underline{CH}_2$OH + 2 x O$\underline{CH}_2$.) and 5.62 [t, J 5 Hz, -$\underline{CH}$(O-)$_2$]. G.l.c. (column A; 100-160°) indicated two peaks of variable intensity and retention time (ca. 5 and 8 min.) whereas apiezon, carbowax and silicon oil columns caused decomposition. 2,4-Dinitrophenyl hydrazone had

m.p. 90-92° (lit.,[209] 91-93°).

30. 8-Hydroxyoctanal ethylene acetal (129):-

8-Hydroxyoctadiynal ethylene acetal (1 g., 5.6 mmole) in absolute ethanol (150 ml.) was hydrogenated over 5% palladium on calcium carbonate (400 mg.) for 1 hr. until hydrogen uptake (76% theoretical) stopped. The mixture was worked up as in experiment 29 and gave an oil, $\nu_{max.}$ (thin film) 1660 cm.$^{-1}$ (CH=CH), τ ($CDCl_3$) 8.7br [.[$\underline{CH}_2$]$_4$], 7.1br (s, removed by D_2O, O$\underline{H}$), 6.4 (t, J 6 Hz, .$\underline{CH}_2$OH), 6.1 (m, -O$\underline{CH}_2$.$\underline{CH}_2$.O), 5.15 [t, J 5.5 Hz, -$\underline{CH}$(O-)$_2$] and 4.5 (m, -$\underline{CH}$=$\underline{CH}$-), attributed to 8-hydroxyoct-4(or 3)-enal ethylene acetal (137 or 138).

The oil was rehydrogenated to completion over 5% palladium on calcium carbonate (2 g.) for 8 hr., or 5% palladium on charcoal (400 mg.) for 1 hr.. Isolation (as before) yielded oily 8-hydroxyoctanal ethylene acetal (500 mg., 48%) which partially polymerised on distillation, b.p. 110-111°/0.2 mm., n_D^{21} 1.4560 (Found: C, 62.8; H, 10.7. $C_{10}H_{20}O_3$ requires C, 63.8; H, 10.7%), $\nu_{max.}$ 3640, 3520 (OH), 1120, 1030 and 940 cm.$^{-1}$ (1,3-dioxolan), τ ($CDCl_3$) 8.6br [($\underline{CH}_2$)$_6$.CH_2OH], 6.4 (t, J 6 Hz, .$\underline{CH}_2$OH), 6.3br (s, removed by D_2O equilibration, O$\underline{H}$), 6.2 (m, .O$\underline{CH}_2$.$\underline{CH}_2$.O.) and 5.15 [t, J 5.5 Hz, -$\underline{CH}$(O-)$_2$].

31. 8-Hydroxyoctanal:-

The diethyl acetal (117) (105 mg., 0.48 mmole) in ether (5 ml.) was shaken

with sulphuric acid (2N; 10 ml.) for 10 hr. Isolation with ether, and purification of the reaction product by preparative t.l.c. [ether-light petroleum (7:3)] gave two bands, R_F 0.4 and 0.3. The more mobile zone corresponded to the starting material (65 mg., 62%) and the slower moving zone afforded oily 8-hydroxyoctanal (16 mg., 23%) which solidified overnight, m.p. 63-66° (lit.,[209] 66-68°) $\nu_{max.}$ 3650, 3500 (OH), 1720 (.CHO) and 1040 cm.$^{-1}$ (OH), τ ($CDCl_3$) 8.8br [.$(C\underline{H}_2)_5$.$CH_2O\underline{H}$], 7.7 (t, J 6 Hz, -$C\underline{H}_2$.CHO), 6.4 (t, J 6 Hz, .$C\underline{H}_2$OH), 6.0br (s, O$\underline{H}$ removed by D_2O) and 0.3 (s, .C$\underline{H}$O). G.l.c. (column A; 137°) behaviour was identical to acetal (117) and on admixture the chromatogram showed two peaks (typically 7.6 and 4.2 min.).

SCHEME AE

32. Treatment of 8-hydroxyoctanal diethyl acetal with phosphorous tribromide:-

The diethyl acetal (117) (35 mg., 0.16 mmole) in ether (5 ml.) was treated[134] with freshly distilled phosphorous tribromide (16 mg., 0.06 mmole) in ether (2 ml.) in the presence of scavengers like potassium carbonate (50 mg.; 100 mesh), pyridine (1 drop) or acetamide (20 mg.). Work up with ether afforded an oil which was purified on thin layers [ether-light petroleum (1:4)]. The least polar band (R_F 0.85) gave 8-bromooctanal, (typically 8 mg.), $\nu_{max.}$ 1720 cm.$^{-1}$ (CHO). More polar zones yielded starting material (13 mg.) and 8-hydroxyoctanal (8 mg.).

33. Treatment of triphenyl phosphite methiodide with 8-hydroxyoctanal diethyl acetal:-

The diethyl acetal (117) (110 mg., 0.5 mmole) in dry dimethyl formamide (10 ml.) was treated dropwise with a solution of triphenyl phosphite methiodide[210] (226 mg., 0.5 mmole) in dimethyl formamide (5 ml.) under nitrogen. The mixture was heated to 50°, cooled and partitioned between ether (20 ml.) and water (30 ml.). The ethereal portion was washed with sodium hydroxide (3 x 20 ml.; 2N), water (10 ml.), sodium thiosulphate (10 ml., 0.1M), brine (10 ml.) and dried. Thin layer chromatography of the iodides [ether-light petroleum (1:1)] afforded a zone (R_F 0.8) corresponding to 8-iodooctanal (20 mg., 16%), $\nu_{max.}$ 2710 and 1720 $cm.^{-1}$ (CHO), τ 8.6br [.$(CH_2)_5$.CH_2I], 7.62 (t, J 6 Hz, .CH_2.CHO), 6.82 (t, J 6 Hz, CH_2I) and 0.27 (s, CHO). The aldehyde gave a 2,4-dinitrophenylhydrazone, m.p. 78-79° (from hexane), (Found: C, 39.05; H, 4.4; N, 12.8. $C_{14}H_{19}IN_4O_4$ requires C, 38.75; H, 4.4; N, 12.9%).

The zones below R_F 0.4 were extracted and treated with Brady's reagent. The extract did not contain a component of similar t.l.c. behaviour to the iodooctanal or hydroxyoctanal dinitrophenyl hydrazones.

Similar treatment with triphenyl phosphite methiodide on the ethylene acetal (129) yielded a 3:1 mixture of iododioxolan (119) and iodooctanal (11% yield) which could not be resolved on preparative p.l.c. (ether-light petroleum).

34. 8',8'-Diethoxyoctyl o-phenylene phosphite (140):-

The diethyl acetal (117) (220 mg., 1 mmole) in ether (2 ml.) was slowly added to o-phenylene phosphorochloridite[211] (175 mg., 1 mmole) in ether (1 ml.) and pyridine (0.07 ml.) at 0°. The suspension was stirred[212] under nitrogen at 0° for 1 hr., then at 20° for 14 hr. Pyridine hydrochloride was filtered off through a bed of magnesium sulphate, and washed quickly with ether (10 ml.). The filtrate was evaporated with benzene under reduced pressure to give the oily phosphite ester (318 mg., 89%), $\nu_{max.}$ ($CHCl_3$) 1100 and 1050 (diethyl acetal), 1490, 1010, 980 and 810 cm.$^{-1}$ (P-O), τ ($CDCl_3$) 8.7br [s, $(CH_2)_6 CH_2$.O with superimposed triplet, τ 8.8, J 7 Hz, 2 x CH_3], 6.5 (m, .CH_2-OP + 2 x .OCH_2.CH_3), 5.5 [t, J 5 Hz, .$CH(OEt)_2$] and 2.96 (m, 4 x Ar-H).

Iodination of the ester: The method of Corey[212] was carried out. Iodine (216 mg., 0.85 mmole) was slowly added to a stirred solution of the crude phosphite ester (0.89 mmole) in dichloromethane (5 ml.). The colouration disappeared quickly for the initial additions, although 48 hr. after the final addition the mixture was still brown. The mixture was shaken with sodium hydroxide (2N; 25 ml.) for 30 min., the lower organic layer separated off, and the aqueous layer re-extracted with dichloromethane (2 x 10 ml.). The combined organic extracts were washed with sodium thiosulphate (0.1N; 10 ml.), brine (10 ml.) and dried. Thin layer chromatography [ether-light petroleum (1:2)] gave a zone (R_F 0.7) from which an unstable brown oil was extracted, $\nu_{max.}$ 500, 475 cm.$^{-1}$ (primary and tertiary C-I),

τ ($CDCl_3$) 8.7br (3H) and 6.6 (m, J 6.5 Hz, IH).

35. 8',8'-Diethoxyoctyl p-toluenesulphonate (141):-

Recrystallised[213] p-toluene sulphonyl chloride (1.1 g., 5.8 mmole), 8-hydroxyoctanal diethyl acetal (1.23 g., 5.63 mmole) in pyridine (5 ml.) were maintained at 20° for 6 hr.. The mixture was diluted with aqueous sodium hydrogen carbonate (2N; 5 ml.) and stirred for 10 min., The reaction products were extracted with ethyl acetate in the usual way to afford on evaporation with benzene the crude oily tosylate (1.3 g., 62%), $\nu_{max.}$ ($CHCl_3$) 1190 and 1180 (tosylate), 1120, 1060 (diethyl acetal); (CS_2) 820 (5 adjacent Ar-H) and 720 cm.$^{-1}$ [$(CH_2)_4$], τ ($CDCl_3$) 8.7br [.$(CH_2)_6$.CH_2O with superimposed triplet, τ 8.85, J 7 Hz, 2 x CH_3], 7.56 (s, CH_3.Ar), 6.5 (m, $OCH_2.CH_3$), 6.0 (t, J 7 Hz, .CH_2.OS), 5.67 [t, J 5 Hz, .$CH(OEt)_2$], 2.67 (d, J 8 Hz, 2 Ar-H ortho to Me) and 2.25 (d, J 8 Hz, 2 Ar-H meta to Me).

36. 8',8'-(Ethylene dioxoxy)octyl p-toluene sulphonate:-

8-Hydroxyoctanal ethylene acetal (755 mg., 4 mmole) was converted, as described in experiment 35, into the corresponding p-toluenesulphonate (1.16 g., 85%), $\nu_{max.}$ (thin film) 1190, 1180 (tosylate), 1140, 1040 and 940 cm.$^{-1}$ (1,3-dioxolan), τ ($CDCl_3$) 6.48 (t, J 7 Hz, -CH_2Cl). T.l.c. indicated absence of tosyl chloride and 8-chlorooctanal ethylene acetal.

T.l.c. purification [ether-light petroleum (1:1), multiple elution R_F 0.4]

gave a colourless oil, τ ($CDCl_3$) 8.6br [$(C\underline{H}_2)_6.CH_2O$], 7.54 (s, $C\underline{H}_3$.), 6.19 (m, $-O.C\underline{H}_2.C\underline{H}_2.O$), 6.03 (t, J 6 Hz, $C\underline{H}_2$-OToS), 5.28 [t, J 5.5 Hz, $-C\underline{H}(O-)_2$], 2,68 (d, J 7 Hz, Ar-H ortho to Me) and 2.23 (d, J 7 Hz, 2 Ar-H meta to Me).

37. 8-Iodooctanal diethyl acetal (118):-

The crude diethyl tosylate (1 g., 2.7 mmole) was converted by iodide substitution into 8-iodooctanal diethyl acetal (732 mg., 83%) which had R_F 0.8 (Sudan yellow had R_F 0.7 on same plate), b.p. 74-76° (block)/0.05 mm. (Found: C, 48.05, 46.55; H, 8.45, 7.7; I, 30.7, 34.9. $C_{12}H_{25}IO_2$ requires C, 43.9; H, 7.65; I, 38.65%. $C_{12}H_{25}ClO_2$ requires C, 60.8; H, 10.65%), $\nu_{max.}$ (thin film) 1120, 1060 (diethyl acetal); (CCl_4) 655 (C-Cl), 595, 500 $cm.^{-1}$ (C-I), τ 8.7br [$(C\underline{H}_2)_6.CH_2I$ with superimposed triplet, J 7 Hz, 2 x $C\underline{H}_3$], 6.82 (t, J 7 Hz, $C\underline{H}_2I$), 6.45 (m, 2 x $.C\underline{H}_2.O$) and 5.55 [t, J 5 Hz, $C\underline{H}(OEt)_2$], m/e 327 [(M^+-1), 0.5%], 283 [(M^+-45), 45%], 191 [30%] and 103 (100%). G.l.c. (column A) did not give reproducible results. 2,4-Dinitrophenyl hydrazone had m.p. 78-79° (from ethanol-water). A band (R_F 0.5) afforded a gum (10 mg.) with identical i.r. spectrum to the tosylate. The remaining zone (R_F 0.2) corresponded to 8-hydroxyoctanal diethyl acetal (20 mg.).

38. 8-Iodooctanal ethylene acetal (119):-

The crude ethylene acetal tosylate (900 mg., 2.64 mmole) was stirred in acetone (5 ml.) at 25° for 24 hr. following the procedure for iodide substitution.

After the usual work up the band at R_F 0.4 [ether-light petroleum (1:8)] gave the crude iodide (615 mg., 78%), τ ($CDCl_3$) 8.7br [$(C\underline{H}_2)_6.CH_2.I$], 6.82 (t, J 6.5 Hz, 0.5 x $.C\underline{H}_2I$), 6.5 (t, J 6.5 Hz, 0.5 x $.C\underline{H}_2Cl$), 6.17 [m, $O[C\underline{H}_2]_2.O$], 5.25 [t, J 5.5 Hz, $-C\underline{H}(O-)_2$], τ [$(CD_3)_2SO$] (assignment order as before), 8.7, 6.74, 6.4, 6.2 and 5.25.

When the tosylate (140 mg., 0.41 mmole) was maintained under the above conditions at 45° for 18 hr. 8-iodooctanal ethylene acetal (105 mg., 86%) was obtained, $\nu_{max.}$ 1140, 1045 and 950 $cm.^{-1}$ (dioxolan), which had an n.m.r. spectrum identical to above but with no signal at τ 6.5 and the triplet, τ 6.82, now integrated for two protons.

The t.l.c. pure tosylate afforded quantitatively the iodoacetal (119) (with no triplet at τ 6.5).

39. Control experiments

a) Effect of brine: The iodoacetal (119) was stirred with brine for 24 hr. Isolation with ether as for the diethyl acetal afforded the iodoacetal (97%) uncontaminated with chloride (n.m.r.).

b) But-3-ynyl p-toluene sulphonate[196] (100 mg., 0.45 mmole) was subjected to the iodide displacement conditions for 4 hr., and yielded but-3-ynyl iodide (28 mg., 22%) uncontaminated with chloride (n.m.r.).

SCHEME AF

40. 8-Chlorooctanal diethylacetal (142):-

ω-Chloro-bromoheptane[8] had n_D^{18} 1.4828 (lit.,[214] n_D^{20} 1.4880) (Found: C, 39.35; H, 6.7; Br, 37.45; Cl, 15.9. Calc. for $C_7H_{14}BrCl$: C, 39.4; H, 6.6; Br, 37.45; Cl, 16.6%); R_T (column A; 155°) 2.7 min.

Triethoxymethane (5.1 g., 34.5 mmole; distilled off sodium) was added dropwise over 0.5 hr. to the mono-Grignard reagent prepared from 1-chloro-7-bromoheptane (7.55 g., 35 mmole) and magnesium (850 mg., 35 mmole) in ether (100 ml.). The solution was stirred at 50° for 16 hr., cooled to 0°, and diluted with a saturated aqueous solution of potassium tartrate (20 ml.). Isolation with ether in the usual way afforded on concentration an oil, which was distilled collecting 8-chlorooctanal diethyl acetal (1.6 g., 20%), b.p. 120-130°/1 mm., n_D^{22} 1.4399 (lit.,[215] b.p. 80°/0.2mm., n_D^{20} 1.4360), $\nu_{max.}$ (thin film) 1120 and 1060 cm.$^{-1}$ (diethyl acetal), (CCl_4) 655 cm.$^{-1}$, τ 8.8br [$(C\underline{H}_2)_6.CH_2.Cl$ with superimposed triplet, J 7 Hz, 2 x $C\underline{H}_3$], 6.5 (m, $.C\underline{H}_2Cl$ + 2 x $.OC\underline{H}_2$-) and 5.6 [t, J 5.5 Hz, $.C\underline{H}(OEt)_2$], m/e 191, 189 (20%) and 103 (100%).

2,4-Dinitrophenyl hydrazone, m.p. 83-84° (from either ethanol-water or aqueous acetic acid) (lit.,[215] 88°) (Found: C, 49.15; H, 6.0; N, 16.4. Calc. for $C_{14}H_{19}ClN_4O_4$: C, 49.05; H, 5.6; N, 16.35%).

8-Iodooctanal diethyl acetal (118):-

The above chloride (1.0 g., 4.2 mmole) was displaced with iodide, and

work up in the usual way afforded the iodoacetal (970 mg., 70%), b.p. 73-74° (block)/0.005 mm. (Found: C, 56.55; H, 9.8; I, 11.15%; see experiment 37).

41. Condensation of acetone with 8-iodooctanal diethyl acetal:-

Prolonged continuation (ca. 40 hr.) of an iodination of chloro or tosyl octanal diethyl acetal at 50° afforded solely a polar oil, R_F 0.4 (ether). 4-Hydroxy-11-iodoundecan-2-one (144) had m.p. 18-20°, b.p. 125-130° (block)/0.02 mm. (Found: C, 43.1; H, 6.75; I, 40.6%; M^+ 312. $C_{11}H_{21}IO_2$ requires C, 42.3; H, 6.8; I, 40.65. M, 312), $\nu_{max.}$ 3500br (bonded OH, no unbonded observed) and 1705 $cm.^{-1}$ (C=O), τ 8.7br [$(C\underline{H}_2)_6.CH_2I$], 7.87 (s, $C\underline{H}_3.CO$), 7.63 [d, J 7 Hz, $.CO.C\underline{H}_2.CH(OH).$], 6.83 (t, J 6.5 Hz, $.C\underline{H}_2I$) and 6.07 [m, $.C\underline{H}(OH).$].

SCHEME AG

42. 1,4-Dichlorobut-2-yne:-

Treatment[216] of but-2-yne-1,4-diol (86 g., 1 mole) with thionyl chloride (240 g., 2 mole) at 40-50° gave 1,4-dichlorobut-2-yne (typical yield 115 g., 94%), b.p. 87-89°/53 mm., n_D^{19} 1.5080 (lit.,[216] b.p. 68-69°/17 mm., n_D^{20} 1.5072).

43. Penta-1,3-diyne:-

1,4-Dichlorobut-2-yne (63 g., 0.5 mole) was treated[217] with sodamide

[prepared in situ from sodium (37 g., 1.6 mole)] in liquid ammonia (600 ml.) and methyl iodide (70 g., 0.5 mole) was added. The worked-up product was distilled from decalin, as a concentrated ether solution, b.p. 23-25°/250 mm. (9 g., 30%). The concentration was estimated from n.m.r. spectroscopy, τ 8.11 (s, ≡CH) and 8.07 (s, CH_3) or g.l.c., R_T (column C; 35°) 2.5 min.

44. trans-Pent-2-en-4-yn-1-ol:-[218]

Epichlorohydrin (62.5 g., 0.67 mole) was treated with sodium acetylide [prepared in situ from sodium (32 g., 1.4 mole) and excess acetylene] in liquid ammonia (500 ml.) and afforded trans-pent-2-en-4-yn-1-ol (26 g., 47%) b.p. 58-59°/10 mm. (lit.,[218] 95-96°/54 mm.).

45. trans-Dec-2-ene-4,6,8-triyn-1-ol; (dehydromatricarianol) (17):-

Penta-1,3-diyne (1.6 g., 25 mmole, 10% ether solution) was coupled with trans-5-bromopent-2-en-4-yn-1-ol [from trans-pent-2-en-4-yn-1-ol (2.05 g., 25 mmole)]. The oil, after work up, was chromatographed on silica gel [ether-light petroleum (1:3)] to afford the alcohol (1.2 g., 33%) which crystallised as unstable yellow crystals, m.p. 127-129° (lit.,[8,219] 129°), $\lambda_{max.}$ 328 (ε 11,500), 307 (16,500), 287 (14,000), 272 (8500), 257 (4000), 241 (110,000) and 230 nm. (72,000), $\nu_{max.}$ ($CHCl_3$) 3600, 3440 (OH), 2130 (C≡C) and 950 cm.$^{-1}$ (trans CH=CH), τ ($CDCl_3$) 8.00 (s, CH_3), 6.8br (s, removed on D_2O addition, OH), 5.8 (dd, J 6 and 2 Hz, CH_2OH), 4.35 (dt, J 16 and 2 Hz, =CH.C≡C) and

3.55 (dt, J 16 and 4.5 Hz, =C<u>H</u>.CH_2OH).

46. trans-<u>Dec-2-ene-4,6,8-triynal (dehydromatricarianal)</u> (78):-

Dehydromatricarianol (125 mg., 0.87 mmole) was oxidised by manganese dioxide (1.1 g.) in dichloromethane. The reaction product was chromatographed on silica gel with dichloromethane yielding dehydromatricarianal (120 mg., 98%) m.p. 103-105° (lit.,[219] 109°), $\lambda_{max.}$ 348 (ϵ 34,000), 325 (43,000), 305 (26,000), 288 (11,500), 258 (73,000) and 245 nm. (49,000), $\nu_{max.}$ 1680 and 1110 (CHO), 2220, 2160, 2100 (C≡C) and 955 $cm.^{-1}$ (<u>trans</u> CH=CH), τ 7.94 (s, $C\underline{H}_3$), 3.45 (m, .C<u>H</u>=C<u>H</u>.), 0.40 (dd, J 4 and 3 Hz, .C<u>H</u>O), τ (Me_2CO) 3.65 (dd, J 16 and 6 Hz, =C<u>H</u>.CHO), 3.32 (d, J 16 Hz, C≡C.C<u>H</u>) and 0.62 (d, J 6 Hz, .C<u>H</u>O).

A trace of unchanged alcohol (2 mg.) was eluted with ether-dichloromethane (1:2).

<u>SCHEME AH</u>

47. trans-<u>Heptadec-9-ene-11,13,15-triyn-8-ol</u> (145):-

The Grignard reagent from 1-bromoheptane (180 mg., 1 mmole), G.P.R. reagent) and magnesium (25 mg.) in tetrahydrofuran (2 ml.) was treated dropwise with the aldehyde (78) (142 mg., 1 mmole) in tetrahydrofuran (10 ml.). The mixture was stirred under nitrogen for 6 hr. in the dark, aqueous ammonium chloride (2N; 10 ml.) was added and the mixture was worked up with ether in the usual way. Chromatography of the concentrated ether extract on silica gel afforded

a mixture of the alcohol (145) and starting aldehyde (ether-light petroleum 1:5). Elution with a more polar mixture gave dehydromatricarianol (0.2 mmole).

The alcohol mixture was reduced with excess sodium borohydride (50 mg.) in ethanol (10 ml.) over 0.5 hr.. Isolation with ether and working up as before resulted in the elution of the unstable alcohol (77 mg., 40%), $\lambda_{max.}$ 329 (ϵ 13,000), 309 (19,000), 290 (15,000), 273 (8200), 243 (120,000) and 232 nm. (74,000), $\nu_{max.}$ 3640 (OH), 2230 (C≡C) and 955 cm.$^{-1}$ (trans-CH=CH), τ 9.12 (t, J 6 Hz, $CH_3.CH_2$), 8.7br [s, $(CH_2)_6.Me$], 8.01 (s, $CH_3.C{\equiv}C$), 5.85 [m, .CH(OH).], 5.7 (s, broad, OH removed on D_2O addition), 4.29 (d, J 16 Hz, =CH.C≡C) and 3.65 [dd, J 16 and 6 Hz, CH(OH).CH=].

48. Attempted Grignard reaction with 8-iodooctanal diethyl acetal (118):-

The iodoacetal (164 mg., 0.5 mmole) and magnesium (16 mg., 0.67 mmole) were brought together under the Grignard forming conditions in either tetrahydrofuran or ether (0.3 ml.) for 1 hr., No reaction was observed, and isolation with ether recovered the iodoacetal (160 mg.).

In another experiment, ethylene dibromide (20 mg.) in tetrahydrofuran (0.2 ml.) was added to the iodide-solvent-magnesium mixture. After the effervescence had subsided, the mixture was cooled to 0° and the aldehyde (78) (70 mg., 0.5 mmole) in tetrahydrofuran (5 ml.) was slowly added. The mixture was stirred for 1 hr.. The worked-up product was then applied to a silica gel solumn (30 g.) and the iodoacetal was recovered in 70% yield (typical of four

experiments). Ether-light petroleum (1:5) eluted the aldehyde (78), whilst a trace of triynene (1:4) was detected which had identical t.l.c. behaviour and i.r. spectrum to trans-dec-2-ene-4,6,8-triyn-1-ol (17).

49. Attempted Grignard reaction with 8-iodooctanal ethylene acetal (119):-

8-Iodooctanal ethylene acetal (100 mg., 0.34 mmole), magnesium (8 mg., 0.33 mmole) in tetrahydrofuran (5 ml.) were subjected to the Grignard reagent initiation procedure, but without success. When aldehyde (78) (140 mg., 1 mmole) had been introduced, chromatography of the crude mixture did not separate the starting materials without borohydride reduction, whereupon the iodoacetal (119) was recovered in 80% yield.

SCHEME AI

50. 8-Chlorooctan-1-ol:-

Treatment[8] of octane-1,8-diol (58 g., 0.4 mmole) with thionyl chloride (29 ml., 0.40 mole) in pyridine (32 ml.) afforded the chloro-alcohol (28.5 g., 43%), b.p. 139-140°/14 mm., n_D^{22} 1.4567 (lit.,[220] 129°/11 mm; n_D^{25} 1.4563) (Found: C, 58.3; H, 10.4. Calc. for $C_8H_{17}ClO$: C, 58.35; H, 10.4%), R_T (column A: 148°) 8.4 min. (one peak), $\nu_{max.}$ (thin film) 3400 and 1055 cm.$^{-1}$ (OH); (CCl_4) 655 cm.$^{-1}$ (C-Cl), τ (CCl_4-D_2O) 8.6br [-(CH_2)$_6$.CH_2Cl] and 6.5 (m, CH_2Cl + CH_2OH).

51. 1-(2'-Tetrahydropyranyloxy)-8-chlorooctane (149):-

8-Chlorooctan-1-ol (6.6 g., 40 mmole), dihydropyran (3.4 g., 40 mmole) were stirred at 0° with phosphoryl chloride (10 mg.) for 12 hr.. After neutralisation with aqueous sodium hydrogen carbonate (2N; 10 ml.) the mixture was worked up with ether in the usual way, and the colloidal extract filtered through a bed of magnesium sulphate. The filtrate was concentrated and distilled off potassium carbonate to yield the tetrahydropyranyl ether (8 g., 80%), b.p. 117-118°/1 mm., n_D^{20} 1.4627 (Found: C, 63.0; H, 10.4. $C_{13}H_{25}ClO_2$ requires C, 62.75; H, 10.15%), $\nu_{max.}$ (thin film) 1130, 1115, 1070 and 1030 cm.$^{-1}$ (tetrahydropyran), τ 8.6br [$(CH_2)_6.CH_2$ + $CH_2.CH_2.CH_2.CH(O-)_2$], 6.5 (m, CH_2-O-CH.O.CH_2· with superimposed triplet τ 6.5, J 6 Hz, .CH_2Cl) and 5.46br [$CH(O-)_2$].

52. 1-(2'-Tetrahydropyranyloxy)-8-iodooctane (120):-

The chloroether (3 g., 12 mmole) was stirred at 50° for 50 hr. in acetone (50 ml.) under conditions for iodide substitution. The crude oil was purified on thin layers, and distilled to yield the iodo-ether (2.6 g., 71%), b.p. 130-131°/0.01 mm., n_D^{24} 1.4990 (Found: C, 46.35; H, 7.25; I, 37.2. $C_{13}H_{25}IO_2$ requires C, 45.9; H, 7.4; I, 37.3%), $\nu_{max.}$ (thin film) 1120, 1080, 1030, 990 and 910 cm.$^{-1}$ (tetrahydropyran), $\nu_{max.}$ (CCl_4) 500 cm.$^{-1}$ (-CH_2-I), τ 8.6br [.$(CH_2)_6.CH_2I$ + $CH_2.CH_2.CH_2.CH(O-)_2$], 6.8 (t, J 7 Hz, .CH_2I), 6.4 (m, $CH_2.O.CH.O.CH_2$) and 5.45br [-$CH(O-)_2$].

53. Grignard condensation of the iodo-ether (120) with dehydromatricarianal (78):-

The iodo ether (2.04 g., 6 mmole) was brought into contact with magnesium (144 mg., 6 mmole) and dry tetrahydrofuran under Grignard initiation conditions. No continuous bubbling was observed so the aldehyde (140 mg., 1 mmole) was introduced and the mixture stirred for 12 hr.,

Work up afforded the unchanged iodo-ether (85%) though more polar fractions [ether-light petroleum (1:1)] exhibited a trace of triynene chromophore. These fractions were combined and purified on thin layers to give the unstable trans-10-hydroxy-18-(2'-tetrahydropyranyloxy)-octadec-8-ene-2,4,6-triyne (151) (14 mg., 0.2%), $\lambda_{max.}$ 328 (rel. E 1.0), 308 (1.5) and 290 nm. (1.2), $\nu_{max.}$ 3600 (OH), 2220, 2180 (C≡C), 1130, 1115, 1070 and 1030 $cm.^{-1}$ (tetrahydropyran), τ 8.6br [s, $(C\underline{H}_2)_7.CH_2.O.CH(O-).(C\underline{H}_2)_3$], 8.00 (s, $C\underline{H}_3.C{\equiv}C$), 6.5 (m, $C\underline{H}_2.O.CH.O.C\underline{H}_2$), 5.85 [m, $C\underline{H}(OH)$], 5.5 [s, $C\underline{H}(O-)_2$], 4.2 (dd, J 16 and 5 Hz, $=C\underline{H}.C{\equiv}C$) and 3.6 [m, $=C\underline{H}.CH(OH)$].

Oxidation with manganese dioxide (20 mg.) in dichloromethane, and purification on thin layers gave the corresponding diyn-en-one ketone (M^+ 354, $C_{23}H_{30}O_3$ requires M 354), m/e 156 (12%), 155 (45) and 85 (100%). A less mobile band found on a plate used for purifying fractions of the iodoether (120) corresponded to 1,16-di(2'-tetrahydropyranyloxy) hexadecane (152) (36 mg., 3%) (M^+ 426. Required for $C_{26}H_{50}O_4$, M 426), $\nu_{max.}$ 1120, 1030 and 990 $cm.^{-1}$ (tetrahydropyran), τ 8.6br (40H), 6.5 (m, $-C\underline{H}_2.O.CH(-CH_2-).O.C\underline{H}_2-$ x 2), and 5.56 [s, $-C\underline{H}(O-)_2$ x 2].

PART IV

General conditions for plant culture

All plants were grown from commercially available seed in an Oxford garden in 1968.

The species assignments were confirmed by comparison with specimens available in The Field Druce Herbarium OXF.

General procedure for isolation of polyacetylenes

Plants (harvested in at the termination of flowering) were divided into above-ground and below-ground parts, and each group was independently examined.

The aerial parts (or roots) were cleaned, cut up and soaked in ether at 20°. The extract was decanted after 7 days and the procedure was repeated with fresh ether. The combined extracts were dried and concentrated at reduced pressure to a residue which was applied to a silica gel column (200 g.). The column was developed by gradient elution [first with dichloromethane (500 ml.) enriched with ether (750 ml.) and then with methanol-ether (1:9; 500 ml.)]. Fractions (50 ml.) were collected and assayed spectrophotometrically (estimating amounts for a polyacetylene M, 200); those of similar polarity and u.v. absorption were combined and rechromatographed on dry packed silica G columns.

1. Examination of Lobelia Cardinalis L. (variety Queen Victoria):-

a) The extract from chopped roots (750 g.) was chromatographed. Only fractions 7,8 showed typical diynene absorption, $\lambda_{max.}$ 280, 266 and 253 nm., (2 mg.) in a pigment residue (ca. 1 g.).

b) The residue (1.3 g.) from the extract of aerial parts (400 g.) gave two groups of polyacetylene fractions (fractions 23-27; and 40).

Fractions 23-27 were combined and rechromatographed on a dry silica G column. The major polyacetylene component was purified on thin layers [ether-methanol (9:1) and chloroform-methanol (9:1)] to yield the oily pale green (6-R,7-R)-trans,trans-tetradeca-4,12-diene-8,10-diyne-1,6,7-triol (159) (18 mg., 0.005%), $[\alpha]^{20}$ +33° (589 nm.), +35° (578 nm.), +40° (546 nm.), +71° (436 nm.) and +194° (365 nm.) (c 0.745 in ethanol), $\lambda_{max.}$ (MeOH) 284 (ϵ 8400), 268 (11,400), 254 (8200), 241 (4300) and 215 nm. (36,000), i.r. data ($CHCl_3$) see p. 90, n.m.r. spectrum see Table 14, p. 91.

Later fractions from the t.l.c. silica column gave a trace of triynene ($\lambda_{max.}$ 320, 303, 283, 268 and 251 nm.).

Fraction 40 was subjected to thin layer chromatography [methanol-ether (1:20)] and gave a zone (R_F 0.3) for a diynediene (4 mg., 0.001%), $\lambda_{max.}$ 301, 283 and 268 nm. which decomposed during acetylation with acetic anhydride (0.5 ml.) and pyridine (1 drop) as in experiment 4a.

2. Screening of Lobelia syphilitica L.:-

a) The extract from chopped roots (0.56 kg.) was chromatographed. Only fractions 10,11,12 showed weak polyyne absorption, $\lambda_{max.}$ 282, 267 and 252 nm.. Thin layer chromatography on these fractions yielded three zones (R_F 0.3-0.45) after 2 elutions with ether-light petroleum (4:1) (total amount 2 mg., 0.0003%).

Treatment with manganese dioxide or sodium periodate had no effect on the polyynes' u.v. spectrum.

b) The extract from leaves (1.0 kg.) was chromatographed but no fractions contained polyacetylenes (u.v.).

3. Isolation of polyacetylenes from Lobelia x vedariensis:-

a) The extract from aerial parts (1 kg.) was chromatographed; fractions eluted with ether-methanol (20:1) exhibited polyyne absorption, $\lambda_{max.}$ 284, 268 and 253 nm.. Further purification as for Lobelia triol (experiment 1b) gave an oil (20 mg., 0.002%), identical in all respects to the Lobelia triol (159).

b) The extract from chopped roots (1 kg.) was chromatographed; two groups of fractions (1-3, and 13-22) exhibited polyyne absorption. Fractions 13-22 were combined and purified on silica gel layers [ether-methanol (25:1)] to give a zone (R_F 0.6), containing a polyyne (65 mg., 0.007%) identical in all respects to the Lobelia triol (159).

Fractions 1-3 were combined and purified further on silica gel layers [ether-light petroleum (1:1) and chloroform] to give a zone containing diynene

fraction (6 mg.; 0.0006%), $\lambda_{max.}$ 283 (rel. E 0.8), 268 (1.0), 253 (0.7) and 240 nm. (0.35), $\nu_{max.}$ 3400 (bonded OH), 2230, 2150 (C≡C), 1725 (C=O), 1360 (Me) and 1040 cm.$^{-1}$ (OH), τ 8.16 (d, J 6.5 Hz, $\underline{CH}_3$.CH=), 6.9 (m, 4H), 6.2 (p, J 7 Hz, 2H), 5.7 (t, J 7 Hz, 1H), 4.4 (dq, J 16 and 1.5 Hz, $\underline{CH}$=CH.Me) and 3.62 (dq, J 16 and 6.5 Hz, Me.$\underline{CH}$=CH) and other unresolved signals >τ 7.8 (6H).

SCHEME AK: A portion (3 mg.) was reduced with sodium borohydride (3 mg.) in ethanol (2 ml.). Work up with ether, saturating the aqueous layer with ammonium chloride, gave a diynene fraction which showed four bands on t.l.c.. P.l.c. purification [ether-methanol (9:1)] gave a zone (2 mg.), R_F 0.3, $\nu_{max.}$ ($CHCl_3$) 3400 cm.$^{-1}$.

This reduced alcohol was acetylated in ether (2 ml.) with acetic anhydride (1 ml.) and pyridine (1 drop) for 24 hr.. Work up with ether gave a diynene acetate (1 mg.), $\lambda_{max.}$ 284, 268 and 253 nm., $\nu_{max.}$ 1740 (AcO) and 3450 cm.$^{-1}$ (OH).

The reduced alcohol (2 mg.) in ether (5 ml.) was shaken with aqueous sodium periodate (2N; 5 ml.) for 6 hr.. The ether layer had $\lambda_{max.}$ 318, 300, 284 and 269 nm., and contained a polyyne of similar t.l.c. behaviour to trans-oct-6-ene-2,4-diynal.

Duplication of the periodate reaction with the initial alcohol led to an ether layer, $\lambda_{max.}$ 325 nm..

4. SCHEME AJ: Structural determination of the triol (159)

a) Triacetate:-

The triol (5 mg.) in ether (1 ml.) was treated with acetic anhydride (1 ml.) and pyridine (1 drop) at 0°. The mixture was left at 20° for 12 hr., then poured into sulphuric acid (1N; 5 ml.). The ether layer was separated off, washed with aqueous sodium hydrogen carbonate (2N; 5 ml.), dried and the oily triacetate (3 mg.) was obtained after p.l.c. purification [ether-light petroleum (1:1)], $\lambda_{max.}$ 283 (ε 8200), 267 (11,100), 253 (8200), 240 (4400) and 215 nm. (30,000), $\nu_{max.}$ ($CHCl_3$) 3020, 1630, 960, 950 (conjugated and non-conjugated trans-CH=CH-), 2240 (C≡C) and 1735 $cm.^{-1}$ (MeCO.O), m/e 360 (M^+; 8%), 301 (10), 276 (8), 258 (17), 199 (12), 162 (35), 157 (11), 139 (35), 119 (35), 102 (10) and 97 (100).

b) Tri(trimethylsilyl)ether (Scheme AN):-

The triol (5 mg.) in pyridine (5 drops) was treated with hexamethyl disilylazane (2 drops) and trimethyl chlorosilane (1 drop). After 0.5 hr., the pyridine was evaporated with carbon tetrachloride under reduced pressure to leave a residue of crude tri-(trimethylsilyl)ether, $\lambda_{max.}$ 283 (rel. E 1.0), 267 (1.4) and 253 nm. (1.0), $\nu_{max.}$ ($CHCl_3$) 3030, 1670, 1630, 965, 930 (conjugated and non-conjugated trans CH=CH), 2230 (C≡C), 1250 and 1100 $cm.^{-1}$ ($SiOCH_3$), m/e 450 (M^+; 0.2%), 259 (80), 260 (21%, $C_{12}H_{27}O_2Si_2$ requires 80 x 0.23 = 18%), 169 (100), 170 (18%, $C_9H_{17}OSi$ requires 15%); metastable observed m/e 110.3 (m/e 259 to 169 requires 110.27).

c) Periodate cleavage:-

The triol (20 mg., 0.085 mmole) and sodium metaperiodate (200 mg.) in water (25 ml.) was shaken at 20° for 2 hr.. The aqueous solution was extracted with ether in the usual way. Concentration of the ether extract gave an oily residue (10 mg.) which was identified as trans-oct-6-ene-2,4-diynal (163) by comparison of t.l.c. behaviour, i.r. and n.m.r. spectra with synthetic material (experiment 4d). The remaining aqueous layer was subsequently extracted with chloroform and afforded on concentration an oil (2 mg.), R_T (A; 100°) 7.7 min. (one peak).

d) trans Oct-6-ene-2,4-diynal (163) (Scheme AL):-

3-Bromoprop-2-yn-1-ol (270 mg., 2 mmole) was coupled with trans pent-3-en-1-yne[91] (100 mg., 1.5 mmole). The reaction mixture was worked up and afforded on silica gel chromatography [ether-light petroleum (1:1)] crude oct-trans-6-ene-2,4-diyn-1-ol, $\lambda_{max.}$ 283, 267 and 253 nm., which was immediately oxidised with manganese dioxide (800 mg.). Work up and chromatography (dichloromethane) afforded the pure aldehyde (163) (40 mg., 26%), $\lambda_{max.}$ 318 (ϵ 8000), 300 (8500), 284 (7000), 269 (5500), 253 (4000), 235 (23,000) and 228 nm. (12,000), $\nu_{max.}$ ($CHCl_3$) 2700, 1660 (CHO), 2200, 2115 (C≡C), and 950 $cm.^{-1}$ (trans CH=CH), τ 8.07 (dd, J 6.5 and 1.5 Hz, CH_3.CH=CH.), 4.38 (dq, J 16 and 1.5 Hz, .CH=CH.Me), 3.50 (dq, J 16 and 7 Hz, =CH.Me) and 0.75 (s, .CHO). The spectroscopic data are in agreement with those previously reported.[221]

Dimerisation:-

The aldehyde (10 mg.) in the condensed state solidified overnight. T.l.c. analysis [ether-light petroleum (1:1)] of the ether extract of this aldehyde residue indicated the presence of a less mobile spot, ascribed to 7-formyltetradeca-2,6,12-triene-4,8,10-triyne.

e) Isopropylidene derivative (160):-

The triol (20 mg., 0.085 mmole) was shaken with acetone (20 ml.), anhydrous copper sulphate (500 mg.) and p-toluene sulphonic acid (2 mg.) for 12 hr. under nitrogen in the dark. Potassium carbonate (10 mg.) was added, the inorganic salts were filtered off and the concentrated filtrate was applied to a thin layer plate. Development with ether-light petroleum (1:1) (five times) yielded one band (R_F 0.7) corresponding to the oily trans-(4R, 5R)-2,2-dimethyl-4-(hept-5'-ene-1',3'-diynyl)-5-(5''-hydroxypent-1''-enyl)-1,3-dioxolan (20 mg., 85%). A sample was distilled for analysis at 130-140° (block)/0.005 mm. (Found: C, 71.75, 71.55, 71.85; H, 7.65, 8.25, 8.15%; M^+ 274. $C_{17}H_{22}O_3$ requires C, 74.5; H, 8.1%; M, 274) and the distillate solidified to a gum, m.p. 25-27°, $[\alpha]^{20}$ +139° (589 nm.), +145° (578 nm.), +166° (546 nm.), +302° (436 nm.) and 534° (365 nm.) (c 0.79 in ethanol), $\lambda_{max.}$ 286 (ε 8600), 270 (14,200), 255 (10,000), 243 (5100), 216 (47,000) and 209 nm. (39,000), $\nu_{max.}$ 3620, 1040 (OH), 2240 (C≡C), 960, 935, 910 (conjugated and unconjugated trans CH=CH), 1385 and 1375 $cm.^{-1}$ (CMe_2), and for n.m.r. spectrum [$CDCl_3$ and $(CD_3)_2CO$] see Table 15, page 96; τ (CH_3CN) 5.9 [d, J 5 Hz, -CH(O-).CH(O-)-], m/e 274

(0.4%), 160 (36) and 102 (100).

The p-phenylazobenzoyl ester was obtained as a gum (t.l.c. pure) which did not crystallise at -20° (hexane or carbon tetrachloride), $\lambda_{max.}$ 322 (ε 15,300), 283 (12,000), 253 (8600) and 239 nm. (sh.), $\nu_{max.}$ 2220w (C≡C) and 1725 $cm.^{-1}$ (CO), τ 8.62 and 8.52 [s, $(CH_3)_2.\dot{C}$], 8.44 (dd, J 6.5 and 1.5 Hz, $CH_3.CH=CH.$), 8.1 (m, J 7 Hz, $CH_2.CH_2.CH_2.$), 7.8 (m, $.CH_2.CH=CH.$), 5.6 [m, $.CH(O-).CH(O-).$ + $.CH_2OCO.$], 4.41 (m, $.CH=CH.CH_2.$ + $Me.CH=CH.$), 4.1 (dt, J 16 and 7 Hz, $.CH=CH.CH_2.$), 3.6 (dq, J 16 and 6.5 Hz, $Me.CH=CH.$), 2.54 (m, 5H on terminal phenyl group), 2.0 and 1.8 [d, J 8 Hz, 2 Ar-H + 2 Ar-H in p-$.N-(C_6H_4)CO.$].

f) Hydrogenation of isopropylidene derivative (160):-

The acetonide (17 mg., 0.073 mmole) in ethyl acetate (15 ml.) was hydrogenated over 5% palladium on calcium carbonate for 8 hr.. The solvent was removed under reduced pressure and p.l.c. [ether-light petroleum (1:1)] of the residual oil (13 mg.) gave a band, slightly less polar than the starting material, $\nu_{max.}$ 935 $cm.^{-1}$ (trans CH=CH) and τ 9.1 (t, J 7 Hz, $CH_3.CH_2.$), 8.7 [singlet, half width 2 Hz, $(CH_3)_2C.$) superimposed on a broad singlet (7 x $.CH_2.$)], 7.9 (m, $.CH_2.CH=CH.$), 6.4 [t, J 6.5 Hz, $CH_2.CH(O-)-$ + CH_2OH)], 6.3br (s, disappears with D_2O, OH), 6.1 [q, J 8 Hz, $=CH.CH(O-)-$], 4.8-4.1 (m, $.CH=CH.$) attributed to a crude mixture of olefins (167) or (168).

The hydrogenation was continued over platinum oxide (5 mg.) in ether (70 ml.) for 48 hr.. The reaction mixture was filtered, concentrated and purified by p.l.c. [ether-light petroleum (1:1)]. The main band (R_F 0.4) was extracted

with ether and yielded oily (4R,5R)-2,2-dimethyl-4(5'-hydroxypentyl)-5-(heptyl)-1,3-dioxolan (161) (3.5 mg., 17%), $[\alpha]^{20}$ +25, 24° (589 nm.), +28.5, 25.2° (578 nm.), +31, 28.6° (546 nm.), +48, 43° (436 nm.) and +67, 63° (365 nm.) (c 0.17 and 0.16 in ethanol), no $\lambda_{max.}$ > 210 nm., $\nu_{max.}$ 3600 (OH), 1385 and 1375 cm.$^{-1}$ (CMe_2), m/e 271 (M^+-CH_3; 100%), 211 (10), 59 (90) and 43 (95).

g) Hydrogenation of the p-phenylazobenzoyl ester of acetonide (160):-

The ester (5 mg.) in methanol (10 ml.) was hydrogenated over 5% palladium on charcoal (5 mg.) for 10 hr., T.l.c. [ether-light petroleum (1:4)] on the product indicated five spots (visible only after treatment with iodine) at R_F 0.27, 0.3, 0.4 and 0.45, all of which were less mobile than the starting material.

5. SCHEME AO

a) cis,trans-Tetradec-6-en-1-ol (162):-

n-Butyl lithium (15% hexane solution; 19 mmole) was added slowly to a stirred suspension of 6-hydroxyhexyltriphenylphosphonium iodide[8] (170) (4.9 g., 10 mmole; m.p. 131-132°) in dry tetrahydrofuran (150 ml.) under nitrogen until a lcear red solution finally remained. Octanal (1.1 g., 8.6 mmole) was introduced during 0.5 hr. until the colouration was just destroyed. The mixture was stirred for 2 hr., and then diluted with saturated aqueous ammonium chloride (75 ml.). The product was isolated with ether, and purified on 1 mm. layers [ether-light petroleum (1:1)] to afford the oily tetradec-6-en-1-ol (1.4 g., 77%), R_T (A; 140°) 11.0 min. (one peak) which appeared homogeneous on silica gel but resolved into

two spots on silver nitrate impregnated silica gel layers.

b) 1-Acetoxytetradec-6-ene (171):-

The above alcohol (1.2 g., 5.7 mmole) was added dropwise to a stirred solution of pyridine (10 ml.) and acetic anhydride (10 ml.) at 0°. After standing for 48 hr. at 20°, the mixture was poured into water (50 ml.), and extracted with ether (2 x 30 ml.). The combined ether extracts were washed with sodium hydrogen carbonate solution (2N; 20 ml.), sulphuric acid (2N; 20 ml.), water (20 ml.), brine (20 ml.) and dried.

The oily residue obtained on concentration was chromatographed on silica gel layers [four elutions; ether-light petroleum (1:19)]. The main zone (R_F 0.8) was extracted and yielded an oily mixture of cis and trans 1-acetoxytetradec-6-ene (1.2 g., 83%), R_T (A; 160°) 5.3 min. (one peak), $\nu_{max.}$ (thin film) 3000 (CH=CH), 1735, 1220, 1020 (acetate), 965 (trans-CH=CH) and 720 $cm.^{-1}$ (cis CH=CH), τ 9.1 (t, J 7 Hz, $C\underline{H}_3.CH_2.$), 8.7br ($CH_3.[C\underline{H}_2]_5.$ + $.[C\underline{H}_2]_3.CH_2O.$), 8.02 (m, $.C\underline{H}_2.CH{=}CH.C\underline{H}_2.$ with superimposed singlet $C\underline{H}_3.CO.$), 6.01 (t, J 6.5 Hz, $.C\underline{H}_2.O.CO.$) and 4.7 (m, $.C\underline{H}{=}C\underline{H}.$).

The mixture of cis and trans acetates (600 mg.) was resolved by multiple development [ether-light petroleum (1:25)] on layers of silver nitrate impregnated silica gel. The less polar, trans-1-acetoxytetradec-6-ene (171b) (150 mg.; R_F 0.7) was distilled at 160-165° (block)/3 mm., n_D^{20} 1.4462, $\nu_{max.}$ 965 $cm.^{-1}$ (trans CH=CH), τ 4.62 ($.C\underline{H}{=}C\underline{H}.$; half width 8 Hz).

The more polar cis-1-acetoxytetradec-6-ene (171a) (420 mg.,; R_F 0.3)

did not crystallise at -40°, but distilled at 170-175° (block)/3 mm., n_D^{20} 1.4478, (Found: C, 75.8; H, 12.0. $C_{16}H_{30}O_2$ requires C, 75.8; H, 11.9%), $\nu_{max.}$ (thin film) 720 cm.$^{-1}$ (cis CH=CH), τ 4.62 (.CH=CH.; half width 12 Hz).

c) Performylation of 1-acetoxytetradec-6-ene (171):-

The cis, trans (3:1) mixture (20 mg., 0.095 mmole) of acetate isomers was stirred with hydrogen peroxide (0.1 ml.; 30 vol.) and formic acid (40 mg.) at 40° for 1 day. The mixture was diluted with saturated aqueous sodium hydrogen carbonate (30 ml.) and the products extracted with ether. T.l.c. of the residual oil revealed the presence of two bands at i) R_F 0.25 (12 mg.) for (mainly) threo 1,7-dihydroxytetradec-6-yl formate or 1,6-dihydroxytetradec-7-yl formate (173), $\nu_{max.}$ (thin film) 3400br (OH) and 1720 cm.$^{-1}$ (OCHO), τ 9.1 (t, J 7 Hz, $CH_3.CH_2$.), 8.6br [$CH_3[CH_2]_6$. + .$[CH_2]_4.CH_2OH$], 6.42 [m, .CH_2OH + .CH(OH).], 5.1 [m, CH(O.CHO)] and 1.90 [s, .CH(OCHO).]; ii) R_F 0.6 (8 mg.) for (mainly) threo-7-hydroxytetradec-1,6-yl diformate or 6-hydroxytetradec-1,7-yl diformate (172) which had identical infrared spectrum to above, save that band ($\nu_{max.}$ 1720 cm.$^{-1}$) was more intense, τ 9.1 (t, J 7 Hz, $CH_3.CH_2$.), 8.7br [Me $[CH_2]_6$. + .$[CH_2]_4.CH_2O$], 6.42 [m, .CH(OH).], 5.85 (t, J 6.5 Hz, .CH_2.OCHO), 5.1 [m, .CH(OCHO).], 1.98 (s, .CH_2.OCHO) and 1.90 [s, .CH(OCHO)].

d) Peracetylation of 1-acetoxytetradec-6-ene (171):-

The mixture of cis and trans (3:1) acetates (180 mg., 0.75 mmole) was shaken with peracetic acid (1 ml.; 40% w/v) and chloroform (10 ml.) for 48 hr..

The solution was poured into saturated aqueous sodium hydrogen carbonate (5 ml.) and the products isolated with ether. The concentrate was separated by multiple development on thin layers [ether-light petroleum (1:1)] into four zones,

i) trans-6,7-epoxytetradecyl acetate (176b) (34 mg.; R_F 0.95 after one elution) $\nu_{max.}$ (thin film) 1735, 1240, 1040 (acetate) and 900 cm.$^{-1}$ (trans epoxide), τ 9.1 (t, J 7 Hz, $CH_3.CH_2.$), 8.6br [.$[CH_2]_6$.CH$\overset{O}{\frown}$CH.$[CH_2]_4$.], 8.03 (s, CH_3.CO.), 7.53 (m, trans.CH$\overset{O}{\frown}$CH.) and 6.0 (t, J 6.5 Hz, .CH_2.OAc).

Acid hydrolysis and selective acetonisation (experiment 6) gave erythro dioxolan (175b).

ii) cis-6,7-epoxytetradecyl acetate (176a) (17 mg.; R_F 0.9 after one elution), infrared spectrum as for (176b) except for band at 840 cm.$^{-1}$ (cis.CH$\overset{O}{\frown}$CH) replacing $\nu_{max.}$ 900 cm.$^{-1}$, n.m.r. spectrum also identical with τ 7.3 (m, cis.CH$\overset{O}{\frown}$CH.) instead of τ 7.53.

iii) (mainly) threo 6-hydroxytetradec-1,7-yl diacetate or 7-hydroxytetradec-1,7-yl diacetate (172) (80 mg.; R_F 0.65 after three elutions), $\nu_{max.}$ 3600w (OH) and 1735v.s cm.$^{-1}$ (acetate), τ 9.1 (t, J 7 Hz, $CH_3.CH_2.$), 8.6br [Me$[CH_2]_6$. + .$[CH_2]_4$.CH=, 8.03 (s, CH_2.OCO.CH_3), 7.98 [s, .CH(OCO.CH_3).], 6.5 [m, .CH(OH).], 5.98 (t, J 6.5 Hz, .CH_2.OAc) and 5.25 [m, .CH(OAc).].

iv) (mainly) threo 6,7-dihydroxytetradecyl acetate (173) (100 mg.; R_F 0.3 after three elutions) as a clear oil which crystallised at 20°, $\nu_{max.}$ 3600, 3580m (OH), 1735s, 1235 and 1040 cm.$^{-1}$ (acetate), τ 9.1 (t, J 7 Hz, $CH_3.CH_2.$), 8.6br [.$[CH_2]_6$.CH(OH).CH(OH).$[CH_2]_4$.], 8.03 (s, CH_3.CO.), 6.5 [m, erythro and

threo.CH(OH).CH(OH).] and 5.98 (t, J 6.5 Hz, .CH_2.OAc).

6. Threo-2,2-Dimethyl-4(5'-hydroxypentyl)-5-heptyl-1,3-dioxolan (175a):-

The (mainly) threo hydroxy esters (172a and 173a) (200 mg.) and potassium hydroxide (2 g.) in methanol (25 ml.) were kept for 24 hr. at 40°. The mixture was extracted with ether (including an acid wash) and afforded, after removal of solvent, the (mainly) threo triol (174a) (120 mg.) as an amorphous solid, $\nu_{max.}$ ($CHCl_3$) 3600 $cm.^{-1}$ (OH), τ ($CDCl_3$) 9.1 (t, J 7 Hz, $CH_3.CH_2$.), 8.6br [$CH_3[CH_2]_6$. + .$[CH_2]_4.CH_2OH$], 8.0br (s, disappears with D_2O, 3 x OH), 6.56 [m, .CH(OH).CH(OH).] and 6.34 (t, J 6.5 Hz, .CH_2OH).

The above triol (174a) (100 mg., 0.4 mmole) was shaken with anhydrous copper sulphate (1 g.) in dry acetone (10 ml.) for 10 hr. The mixture was filtered through magnesium sulphate and evaporated to dryness. The residual oil was extracted with ether; purification by preparative t.l.c. yielded a single band [R_F 0.3, ether-light petroleum (1:1)] for (mainly) threo-2,2-dimethyl-4(5'-hydroxypentyl)-5(heptyl)-1,3-dioxolan (105 mg., 90%), which was distilled at 125-145° (block)/0.05 mm., n_D^{20} 1.4539 (Found: C, 71.1; H, 12.2. $C_{17}H_{34}O_3$ requires C, 71.3; H, 11.95%), $\nu_{max.}$ 3600 (OH), 1375, 1365 (CMe_2), 1170, 1100 and 1050 $cm.^{-1}$ (C-O), τ 9.1 (t, J 7 Hz, $CH_3.CH_2$.), 8.7br [Me$[CH_2]_6$. + .$[CH_2]_4CH_2OH$ with superimposed singlet, τ 8.7 $CH_3.\dot{C}.CH_3$] and 6.4 [t, J 6.5 Hz, .CH_2OH with m, .CH(O-).CH(O-).] (CMe_2 calculated[222] to resonate at τ 8.6), R_T (A; 160°) 24 min. (70%; threo), identical, on admixture,

to natural acetonide (161) and,32 min (30%; erythro) identical to isomer prepared in experiment 7, m/e 271 (95%), 211 (9), 59 (100) and 43 (95).

7. Erythro-2,2-Dimethyl-4-(5' hydroxypentyl)-5(heptyl)-1,3-dioxolan (175b):-

trans-1-Acetoxytetradec-6-ene (171b) (25 mg., 0.1 mmole) was peracetylated as described for the cis-trans isomer mixture and afforded oily erythro-tetradeca-1,6,7-triol (174b) after alkaline hydrolysis. Selective acetonisation yielded the isopropylidene derivative, erythro-2,2-dimethyl-4(5' hydroxypentyl)-5(heptyl)-1,3-dioxolan (16 mg., 57%), which was distilled at 150-170° (block)/0.05 mm., n_D^{23} 1.4550 (Found: C, 71.55; H, 11.7. $C_{17}H_{34}O_3$ requires C, 71.3; H, 11.95%), i.r. spectrum identical to threo isomer save in relative intensities of 1050 $cm.^{-1}$ peak (figure 9 , p.100), τ 9.1 (t, J 7 Hz, $\underline{CH}_3.CH_2.$), 8.6br $[CH_3[\underline{CH}_2]_6. + .[\underline{CH}_2]_4.CH_2OH$, with superimposed singlets at τ 8.66 and 8.76, $C(\underline{CH}_3)_2]$, 6.43 (t, J 6.5 Hz, $.\underline{CH}_2OH$) and 6.05 [m, $.\underline{CH}(O-).\underline{CH}(O-).$] ($CMe_2$ calculated[222] to resonate at 8.68 and 8.78), R_T (A; 160°) 32 min. (one peak), m/e 271 (100%), 211 (20), 59 (94) and 53 (95%).

PART V

General experimental

Counting techniques

Samples (of known weight) of radioactive material were dissolved in scintillator solution [10 ml.; 0.6% 2-(4'-t-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole (Butyl P.B.D.) solution in toluene] and counted in all three channels (^{3}H, ^{14}C without ^{3}H, ^{14}C and ^{3}H) in a Beckmann LS-100 scintillation counter. The sample was automotically calibrated through the external standard ratio (e.s.r.); addition of naphthalene (ca. 1 g.), P.O.P.O.P. (2 mg.) and degassing with nitrogen were used as necessary to give an e.s.r. > 10.5.

At least a thousand counts were recorded in each channel (standard deviation 2%) and the counts recorded were adjusted for the background rate prior to conversion[7] into activities of tritium and carbon 14.

Constant specific activity implies specific activities between two successive recrystallisations differed by less than 3%.

Incorporations are quoted, if possible, on carbon-14-values:

$$\text{Incorporation (\%)} = \frac{\text{Dilution factor} \times \text{Sp. Act.} \times \text{Amount recovered} \times 100}{\text{Total activity fed}}$$

Wyerone was sometimes reduced, prior to counting, to wyerol by borohydride reduction, and subsequent p.l.c. purification as in experiment 1a.

1. CHEMICAL SYNTHESES

a) Dihydrowyerol (185):-

Dihydrowyerone (184) (26 mg., 0.1 mmole) in ethanol (10 ml.) was stirred with excess sodium borohydride (25 mg.) for 0.5 hr. under nitrogen whilst the yellow colour gradually disappeared. Isolation with ether gave on concentration an oil which was purified on thin layers, [R_F 0.4, ether-light petroleum (3:1)], to give dihydrowyerol (25 mg.; 95%), m.p. 61-62° (ether-hexane), $\lambda_{max.}$ (EtOH), 310 nm. (ε 23,200), $\nu_{max.}$ 3580 (OH), 1715 (CO), 1605 (CH=CH), 1200, 1180 and 1170 (furan ring) and 1050 $cm.^{-1}$ (OH), τ 9.1 (irr. t., J 7 Hz, $C\underline{H}_3.CH_2$), 8.7 (m, $CH_3.C\underline{H}_2$), 8.5 (m, $Et.C\underline{H}_2$), 7.72 (irr. t., J 7 Hz, $C\underline{H}_2.C{\equiv}C$), 7.3br (s, O<u>H</u>, disappears on D_2O addition), 6.21 (s, $CO_2.C\underline{H}_3$), 4.56br [m, -C<u>H</u>(OH)-], 3.6 (d, J 16 Hz, CH=C<u>H</u>.CO_2Me), 3.49 [d, J 3 Hz; -C<u>H</u>=C(O-).CH(OH)], 3.43 [d, J 3 Hz, -C<u>H</u>=C(O-).CH=] and 2.58 (d, J 16 Hz, -C<u>H</u>=CH.CO_2Me).

b) T.l.c. comparisons of wyerone and dihydrowyerone and analogues

Wyerone and dihydrowyerone moved at the same rate on silica gel or alumina thin layers when developed many times with ether-light petroleum (1:18), acetone-light petroleum (1:19) benzene, or ethyl acetate-light petroleum (1:10). A mixture appeared homogeneous in silica gel layers impregnated with caffeine[223] or silver nitrate. Wyerol and dihydrowyerol also moved with identical rates under the above conditions.

c) Trimethyl silyl derivatives of wyerol (183) and dihydrowyerol (185):-

The acetylenic alcohol (26 mg., 0.1 mmole) in pyridine (5 drops) was treated dropwise with hexamethylsilylazane (2 drops) and trichloromethylsilane (1 drop). After standing in nitrogen for 2 hr., the mixture was evaporated under reduced pressure with carbon tetrachloride, $\lambda_{max.}$ 310 nm., $\nu_{max.}$ 1265 cm.$^{-1}$ (Si-O).

The trimethylsilyl esters of both alcohols were unstable on t.l.c. and moved at the same rate with ether-light petroleum(1:3).

d) Acetates of wyerol (183) and dihydrowyerol (185):-

The acetylenic alcohol (26 mg., 0.1 mmole) was kept with pyridine (5 drops) and acetic anhydride (10 drops) for 10 hr., The mixture was diluted with methanol-water (10 ml.; 1:1) and extracted with ether (2 x 10 ml.). The combined ether extracts were washed with sulphuric acid (5 ml.; 2N), aqueous sodium hydrogen carbonate (5 ml.; 2N), water (5 ml.) and dried. P.l.c. [ether-light petroleum (1:1)] gave the unstable acetate (R_F 0.5), $\lambda_{max.}$ 304 nm., $\nu_{max.}$ 1750 (OAc) and 1725 cm.$^{-1}$ (CO_2Me).

Multiple development with ether-light petroleum on caffeine and untreated silica gel layers indicated that wyerol acetate moved slower than dihydrowyerol acetate.

e) Scheme AQ

Wyerone (179):-

Hex-3-en-1-yne (3.2 g., 40 mmole; cis-trans ratio 3:2) in dry tetrahydro-

furan (10 ml.) was added dropwise to a solution of ethyl magnesium bromide [from magnesium (922 mg., 38 mmole) and ethyl bromide (2.4 g., 22 mmole)] stirred at 30° under nitrogen. The mixture was stirred for 0.5 hr., and trans 3-(5'-formyl-2'-furyl)prop-2-enoic acid (1.6 g., 9.65 mmole) was added in a single portion. The slurry was stirred at 20° for 2 hr., and the magnesium complexes were destroyed on the addition of sulphuric acid (100 ml.; 2N). Extraction with ether afforded on concentration the hydroxy acid which was treated with diazomethane solution (2N) until gas evolution ceased. The crude hydroxy ester was oxidised with manganese dioxide (2 g.) for 6 hr. in dichloromethane (100 ml.). The solution was filtered, concentrated and chromatographed on silica gel (50 g.), cis/trans wyerone (280 mg., 11%) was eluted with ether-light petroleum (1:1), and t.l.c. analysis of these fractions (nine elutions; ether-light petroleum 1:19) gave cis wyerone at R_F 0.27 and trans wyerone R_F 0.21.

Fractional crystallisation (ether-hexane) gave initially trans wyerone m.p. 95-101°, recrystallised from hexane as crystals m.p. 105-109°, $\lambda_{max.}$ 332 nm. (ε 23,000), $\nu_{max.}$ 2200 (C≡C), 1725, 1645 (C=O), 1625, 970, 960 (trans CH=CH) and 1015 $cm.^{-1}$ (furan), τ 8.88 (t, J 7.5 Hz, $C\underline{H}_3.CH_2$), 7.5 (m, $.C\underline{H}_2.CH=$), 6.22 (s, $C\underline{H}_3O_2C.$), 4.27 (d, J 16 Hz, $=C\underline{H}.C\equiv$), 3.44 (d, J 16 Hz, $=C\underline{H}.CO_2Me$), 3.35 (m, $=C\underline{H}.CH_2$), 3.29 [d, J 3 Hz, $.C\underline{H}=C(O-).CH=$], 2.78 [d, J 3 Hz, $C\underline{H}=C(O-).CO.$] and 2.55 (d, J 16 Hz, $C\underline{H}=CH.CO_2Me$). A second crop yielded cis wyerone m.p. 62-63° (from hexane) (lit.,[171] 65 °), $\lambda_{max.}$ 336 nm. (ε 22,600).

f) Dihydrowyerone (184):-

Dihydrowyerone was prepared according to the above procedure, replacing hexenyne with redistilled hex-1-yne (3.2 g., 40 mmole; b.p. 71.5°). The dihydrowyerone (1.75 g., 70%) was recrystallised from ether hexane, m.p. 80-81° (lit.,[171] 80-80.5°), $\lambda_{max.}$ 342 (ϵ 24,300) and 327 nm. (26,800).

2. Isolation of Wyerone, wyerol and detection of dehydroanalogues

Culture of beans on Kleenex tissues

Dried beans (typically 100, ca. 140 g.) of Vicia faba L. (variety Green Windsor) (from Wye College, Kent) were soaked in sterilised water (300 ml.) for 24 hr.. The swollen beans were washed repeatedly with sterilised water, and their skins removed. The bare cotyledons were washed in sterilised water and laid out on 'Kleenex' tissues in a tray, 60 x 60 x 3 cm.. The beans germinated in this tray encased in a polythene bag away from light at 16-23°.

Extraction of acetylenic furanoid metabolites

After approx. 120 hours, the seedlings (ca. 95% germination) were macerated ("Automix" electric blender) in benzene (1.5 l.) and extracted with benzene (2 l.) at 20° for 12 hr.. The mixture was filtered through a bed of magnesium sulphate under vacuum and the tissue washed with benzene (1 l.). The combined benzene filtrates were concentrated to a waxy oil (ca. 1 g.) which was taken up in ether (10 ml.) and filtered. Preparative thin layer chromatography [three 100 cm. plates; three elutions with ether-petroleum (1:1)] separated out zones corresponding to

wyerone (R_F 0.6; 10 mg.), dihydrowyerone (R_F 0.4; 1 mg.) and wyerol.

The keto-and hydroxy-esters were purified further on silica gel layers using chloroform-light petroleum (1:1) and ether-light petroleum (1:3) as developing solvents. Dilution with synthetic wyerol and wyerone on repeated crystallisation (ether-hexane) afforded samples of constant melting point, and in the case of radioactive feedings, constant specific activity.

Wyerone had m.p. 62-62.5° (lit.,[171] 63.5-64.5°) and wyerol m.p. 36-37° (lit.,[171] 55-56°).

The undiluted crude wyerol, $[\alpha]_D^{22}$ -5°, showed m/e 262 (M^++2), 261 (M^++1), 260 (M^+, 100%). Intensity ratio of peaks at m/e 262:261 = 1:6 ($C_{15}H_{16}O_4$ requires 1:22).

3. Incorporation experiments

Administration of radioactive precursors to beans

The fatty acid ester (ca. 30 mg.) was emulsified in an aqueous Tween 80 solution (1 ml.; 1%); sodium acetate was dissolved in water (1 ml.). The aqueous mixture was administered in drops from a capillary pipette onto the cotyledons.

The beans were extracted as before, save that the extract was estimated for radioactivity after concentration to ca. 250 ml..

3.1 Acetate feeding:-

Sodium [1-^{14}C] acetate (0.5 mC.; 49 mg.) (Radiochemical Centre, Amersham) was administered to 49 beans which had been soaked for 48 hr. and

germinated for 36 hr., After a further 110 hr. germination the beans were worked up to give a benzene extract (130 ml.; 220 μC 44% incorporation), which was concentrated to an oil (230 mg.). After chromatographic purification the crude wyerone (4.6 mg.) and wyerol (1.5 mg.) fractions were purified on thin layers and crystallised as follows:-

a) The wyerone fraction (2.68 mg.) was diluted with inactive natural material (24.8 mg.) and recrystallised to constant specific activity (7.34 μC/mmole). The incorporation of acetate $= \frac{27.5}{2.68} \times \frac{7.34}{500} \times \frac{4.6}{258} \times 10^2 = 0.027\%$.

b) The mother liquors of the first wyerone crystallisation were estimated by u.v. to contain wyerone (2.5 mg.) and dihydrowyerone (0.7 mg.). Dihydrowyerone (17 mg., by weight) was added and crystallised out of solution to constant melting point, m.p. 80-81°, and specific activity (0.0487 μC/mmole). The minimum incorporation was $\frac{17.7}{0.7} \times \frac{0.0487}{500} \times \frac{0.7}{260} \times 100 = 0.007\%$.

c) Wyerol (0.87 mg.) was diluted with synthetic material (2.92 mg.; m.p. 56-57°) and crystallised to constant melting point 36-36.5° (and constant specific activity (1.6 μC/mmole). The incorporation was $\frac{3.8}{0.87} \times \frac{1.6}{500} \times \frac{1.5}{260} \times 100 = 0.008\%$.

d) The Kleenex tissues on which the beans were grown, were dried in air, soaked in water (400 ml.) and the decanted solution concentrated to 260 ml. The activity of an aliquot (1 ml.) was 0.07 μC corresponding to $\frac{260 \times 0.07}{500} \times 100 = 3.6\%$ of the fed activity.

Methyl [9,10-^{3}H];[10-^{14}C];[1-^{14}C] oleate:-

The acid (1 mol.) (Radiochemical Centre, Amersham) in ether (15 ml.) was treated with excess ethereal diazomethane at 0°. The solution was washed with aqueous sodium hydroxide (5 ml.; 2N), brine (5 ml.) and dried. Chromatography on silica gel layers [ether-light petroleum (1:9)] afforded a single radioactive zone (R_F 0.8), corresponding to methyl oleate (99%), R_T (A: 200°) 16.2 min. (one peak).

Methyl [10-^{3}H];[9-^{14}C] oleate:-

The samples[33] were purified on thin layers, as above, as the sole radioactive zone, R_T (A: 200°), 16.2 min. (85% of total area).

3.2 Methyl [9-10-^{3}H,10-^{14}C] oleate feeding:-

Methyl [10-^{14}C] oleate (20 μC) was mixed with methyl [9,10-^{3}H] oleate (110 μC) to give an ester mixture (110 mg.; ^{3}H:^{14}C 5.5:1) which was administered over 6 hr. to 150 beans which had soaked for 24 hr., and germinated for 24 hr.. After a further germination for 8 days, the beans were worked up to give a concentrated benzene extract (250 ml.; total ^{3}H activity 13.2 μC, 12%; total ^{14}C activity 2.15 μC 11%; ^{3}H:^{14}C 6.13:1).

A second benzene extract (210 ml.) of the tissues had total ^{3}H activity 1.45 μC (1.3%), total ^{14}C activity 0.27 μC (1.3%), ^{3}H:^{14}C 5.4:1.

The combined benzene extracts were concentrated to an oil (1.2 g.) which was separated on thin layers into wyerone (14.8 mg.) and wyerol (2.16 mg.).

a) Further thin layer chromatography and recrystallisation gave wyerone (2.55 mg.)

which was diluted with natural material (6.88 mg.) and crystallised to constant activity (^{3}H 0.147 μC/mmole; ^{14}C 0.055 μC/mmole; ^{3}H:^{14}C 2.67:1). Incorporation was $\frac{9.43}{2.55} \times \frac{0.055}{20} \times \frac{14.8}{258} \times 100 = 0.06\%$.

b) Further thin layer chromatography gave wyerol (0.8 mg.) which was diluted with synthetic material (6.19 mg.) and recrystallised to constant activity (^{3}H 0.071 μC/mmole; ^{14}C 0.0254 μC/mmole; ^{3}H:^{14}C 2.8:1). Incorporation of methyl oleate was $\frac{7.0}{0.8} \times \frac{0.0254}{20} \times \frac{2.16}{260} \times 100 = 0.009\%$.

c) The Kleenex tissues, dried at 20°, were extracted with ether (200 ml.) and had total ^{3}H activity 3.34 μC (3%), total ^{14}C activity 0.5 μC (2.5%), ^{3}H:^{14}C 6.7:1. The tissues were re-extracted with methanol (300 ml.) and had total ^{3}H activity 3.75 μC (3.4%), total ^{14}C activity 0.5 μC (2.5%), ^{3}H:^{14}C 7.5:1.

Methyl [10-^{3}H,9-^{14}C]oleate feedings:-

Methyl [10-^{3}H] oleate was mixed with methyl oleate [9-^{14}C] oleate. 3.3 Methyl [10-^{3}H,9-^{14}C] oleate (36 mg.) (^{3}H-136 μC, ^{14}C-20 μC; ^{3}H:^{14}C 6.8:1) was administered to 140 beans, which had been soaked for 18 hr., and germinated for 12 hr.. After a further 150 hr. germination, the beans were worked up to give a concentrated benzene extract (180 ml.) total [^{3}H activity 15 μC (11%); total ^{14}C activity 2.5 μC (13%); ^{3}H:^{14}C 6:1] which afforded a waxy solid (810 mg.) on removal of solvent. The solid was taken up in ether (50 ml.) and separated into wyerone (20.6 mg.) and wyerol (1 mg.) fractions.

a) The wyerone fraction was further chromatographed on thin layers and crystallised to constant activity (^{3}H 0.17 μC/mmole; ^{14}C 0.016 μC/mmole; $^{3}H:^{14}C$ 10·7). A portion (1 mg.) was reduced with borohydride to wyerol which had the same activity. The incorporation of methyl oleate was

$$1 \times \frac{0.016}{20} \times \frac{20.6}{258} \times 100 = 0.06\%.$$

b) The mother liquor sample from the wyerone recrystallisation was estimated (u.v.) to contain wyerone (2.7 mg.) and dihydrowyerone (0.52 mg.). Dihydrowyerone (8.5 mg.) was added and crystallised to constant melting point 80-81°, and constant activity (^{3}H 0.025 μC/mmole; ^{14}C 0.0022 μC/mmole; $^{3}H:^{14}C$ 11·0). The (minimum) incorporation of methyl oleate was $\frac{9.0}{0.52} \times \frac{0.0022}{20} \times \frac{0.52}{260} \times 100 = 0.004\%$.

3.4 Methyl [10-^{3}H,9-^{14}C] oleate (15 mg.) (^{3}H-190 μC; ^{14}C-70 μC; $^{3}H:^{14}C$ 2.7) was administered to 200 beans which had been soaked for 24 hr. and germinated for 20 hr. After germinating for a further 5 days, the seedlings were worked up to give a concentrated benzene extract (140 ml.; total ^{3}H activity 17.5 μC (9.0%); total ^{14}C activity 6.3 μC (9%); $^{3}H:^{14}C$ 2.8:1), which on removal of solvent afforded an oil (670 mg.).

Preparative layer chromatography gave wyerone (14.4 mg.) and wyerol (1 mg.) fractions.

The wyerone fraction was further purified on thin layers, and crystallised to constant melting point and activity (^{3}H 1.05 μC/mmole; ^{14}C 0.51 μC/mmole; $^{3}H:^{14}C$ 2.1). Incorporation of methyl oleate was therefore

$\frac{0.51}{70} \times \frac{14.4}{258} \times 100 = 0.04\%.$

3.5 Methyl [10-^{3}H,1-^{14}C] oleate

Methyl [10-^{3}H] oleate (880 μC) and methyl [1-^{14}C] oleate (60 μC) were mixed (^{3}H:^{14}C 14.7:1) and administered to 195 beans which had soaked for 24 hr., and germinated for 24 hr.. After a further 125 hr. germination the beans were worked up to give a benzene extract (100 ml.) (total ^{3}H-activity 12.5 μC incorporation 1.4%; total ^{14}C-activity 0.87 μC incorporation 1.4%; ^{3}H:^{14}C 14.4), which was concentrated to an oil (760 mg.). Preparative layer chromatography separated out the wyerone fraction (1.2 mg.), which was diluted with synthetic material (8 mg.), and crystallised to constant melting point. A fraction (4.4 mg.) was reduced with sodium borohydride, to give wyerol (3.2 mg.) (^{3}H-0.58 μC/mmole, ^{14}C inactive). The incorporation was $\frac{9.2}{1.2} \times \frac{0.58}{880} \times \frac{1.2}{258} \times 100 = 0.002\%.$

Methyl [10-^{3}H,9-^{14}C] crepenynate feedings

Methyl [10-^{3}H] crepenynate and methyl [9-^{14}C] crepenynate were mixed and run on thin layers [ether-light petroleum (1:9)]. The only active zone had identical R_F value (0.8) to authentic methyl crepenynate.

3.6 Methyl [10-^{3}H,9-^{14}C] crepenynate (^{3}H 300 μC; ^{14}C 12 μC; ^{3}H:^{14}C 25:1) was administered to 100 beans which had germinated for 29 hr.. After further germination (104 hr.), the beans were worked up to give a concentrated benzene extract (200 ml.) [total ^{3}H activity 30 μC (10%), total ^{14}C activity 1.5 μC (13%);

^{3}H:^{14}C 20.2] which afforded an oil (500 mg.) on evaporation of solvent. The oil was separated on thin layers into wyerone (3.6 mg.) and wyerol (0.83 mg.) fractions.

a) Further chromatography on silica gel layers yielded active wyerone (1.6 mg.) which was diluted with synthetic material (11.0 mg.) and crystallised (five times) to constant activity (^{3}H 0.19 μC/mmole; ^{14}C 0.021 μC/mmole; ^{3}H:^{14}C 9.1:1). The incorporation of methyl crepenynate was $\frac{12.6}{1.6} \times \frac{0.021}{12} \times \frac{3.6}{258} \times 100 = 0.02\%$.

b) Further chromatography on silica gel layers afforded active wyerol (0.67 mg.) which was diluted with synthetic material (6.5 mg.) and crystallised to constant melting point and activity (^{3}H 0.45 μC/mmole; ^{14}C 0.044 μC/mmole; ^{3}H:^{14}C 10.2). Incorporation of methyl crepenynate was $\frac{7.2}{0.67} \times \frac{0.044}{12} \times \frac{0.83}{260} \times 100 = 0.013\%$.

3.7 Methyl [10-^{3}H, 9-^{14}C] crepenynate (30 mg.) (^{3}H 160 μC; ^{14}C 24 μC; ^{3}H:^{14}C 6.7:1) was administered to 200 beans that had been growing for 36 hr.. After a further 50, and 110 hr., germination the beans were worked up in equal lots (A and B respectively) to give concentrated benzene extracts.

Batch A

The benzene extract (200 ml.) had total ^{3}H activity 10.1 μC (13%); total ^{14}C activity 6.32 μC (53%); ^{3}H:^{14}C 1.6:1, and afforded on removal of solvent an oil (440 mg.). Rough chromatographic purification gave wyerone (2.6 mg.) and wyerol (1.75 mg.). The wyerone fraction was diluted with inactive natural

material (5 mg.) and crystallised to give inactive material, m.p. 62–62.5°.

Batch B

The benzene extract (200 ml.) had total ^{3}H activity 26.5 μC (33%); total ^{14}C activity 3.7 μC (31%); ^{3}H:^{14}C 7.2:1), and afforded an oil (480 mg.) on removal of solvent. Wyerone (4.7 mg.) and wyerol (1.65 mg.) fractions were isolated by p.l.c..

Wyerone was further purified on thin layers (3.62 mg.) and diluted with synthetic material (9.05 mg.). Recrystallisation yielded material, m.p. 62–62.5°. The liquors from the wyerone recrystallisations (ca. 5 mg. containing ca. 5% dihydrowyerone by u.v.) were mixed with dihydrowyerone (12 mg., by weight). Crystallisation yielded inactive dihydrowyerone, m.p. 80–81°.

4. DEGRADATION STUDIES

a) trans Methyl 3-(5'-methoxycarbonyl-2'-furyl)prop-2-enoate (188):-

trans 3-(5'-Methoxycarbonyl-2'-furyl)prop-2-enoic acid[224] (100 mg., 0.51 mmole) was esterified by 4% sulphuric acid in methanol for 48 hr.. Work up with ether gave an oil which was recrystallised from ethanol-water to give the methyl ester (100 mg., 93%) m.p. 143–144° (Found: C, 56.8; H, 4.8. $C_{10}H_{10}O_5$ requires C, 57.1; H, 4.8%), $\lambda_{max.}$ 321 (ε 20,000) and 303 nm. (25,000), $\nu_{max.}$ 1730 cm.$^{-1}$ (ester CO), τ 6.19 (s, $CH_3.O_2C.CH=$), 6.09 [s, $CH_3.O_2C(O-)=$], 3.43 (d, J 16 Hz, $CH.CO_2Me$), 3.31 [d, J 3.5 Hz, $-CH=C(O-)-CH=$], 2.78 [d, J 3.5 Hz, $CH=C(O-).CO_2Me$], and 2.52 [d, J 16 Hz, $=CH.C(O-)=CH$].

b) Wyerone degradations

Wyerone (7 mg., 0.027 mmole), dissolved in 5% potassium hydroxide in methanol (3 ml.), was treated dropwise with hydrogen peroxide (30%; 1 ml.). The mixture was kept under nitrogen for 12 hr., and then evaporated to dryness at 40°/20 mm.. The residue was taken up in water (5 ml.) and washed with ether (2 x 2 ml.). The aqueous layer was acidified with sulphuric acid (10N) and extracted with ethyl acetate (2 x 10 ml.). The combined ethyl acetate washings were washed with brine (5 ml.), dried and reduced to dryness. The solid residue was treated with excess ethereal diazomethane and the solution transferred to 0.3 mm. layers. Development with ether-light petroleum (1:1) gave a zone (R_F 0.6) corresponding to methyl 3-(5'-methoxycarbonyl-2'-furyl)prop-2-enoate (2 mg., 26%). I.r. and u.v. spectra were identical with an authentic sample (experiment 4a); mixed melting point 140-142°.

Degradation of wyerone from methyl crepenynate feeding (3.6):-

Active wyerone (1.7 mg.) was mixed with synthetic material (8.8 mg.) and recrystallised to needles m.p. 62-3° (^3H 0.017 μC/mmole; ^{14}C 0.0034 μC/mmole; ^3H:^{14}C 5:1). Degradation by hydrogen peroxide, and methylation of the acid yielded on work up the dimethyl ester (3 · mg.) (^3H activity 0.017 μC/mmole, ^{14}C 0.0033 μC/mmole; ^3H:^{14}C 5.1:1).

Degradation of wyerone from oleate feeding (3.2):-

Active wyerone (3.25 mg.) was mixed with synthetic material (6.65 mg.) and recrystallised to constant melting point 62-62.5° (^3H 0.044 μC/mole;

^{14}C 0.018 μC/mole; ^{3}H:^{14}C 2.4:1). The acid fraction from the hydrogen peroxide degradation was methylated to give the dimethyl ester (2 · mg.) (^{3}H activity 0.043 μC/mmole; ^{14}C 0.018 μC/mmole; ^{3}H:^{14}C 2.4:1).

c) SCHEME AS

Hydrogenation and hydrolysis of methyl [9-^{14}C], [10-^{3}H] oleate:-

Methyl oleate [ex-feeding (3.3)] (34 mg., 0.115 mmole), was hydrogenated over Adams catalyst (10 mg.) in ethyl acetate (10 ml.) for 12 hr.. Filtration through Whatmann No.1., removal of solvent and p.l.c. purification [R_F 0.8; ether-light petroleum (1:12)] yielded a solid (^{3}H:^{14}C 6.8:1). The crude methyl stearate was warmed under reflux of methanol (10 ml.) with potassium hydroxide (0.1 g.) for 4 hr.. The alkaline solution was washed with ether, acidified with sulphuric acid (2N) and the acid isolated with ether in the usual way. The stearic acid was dissolved in ether and made up to 50 ml..

Schmidt degradation of stearic acid:-

An aliquot (20 ml.) was evaporated and dissolved in concentrated sulphuric acid (1 ml.). The solution was cooled to 0° and sodium azide (65 mg., 1 mmole) added. A nitrogen stream was led over the solution through potassium permanganate solution (25% in 2N sulphuric acid) and bubbled through filtered barium hydroxide solution. The mixture was heated slowly to 100° when vigorous effervescence occurred. After keeping the temperature constant for 1 hr., the mixture was cooled, and made alkaline with aqueous sodium hydroxide (10N). 1-Heptadecamine (9 mg., 20%) was isolated with ether and had ^{14}C:^{3}H 6.8:1.

The barium carbonate isolated by filtration was inactive (counted^{225} with Cab-O-Sil).

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