THE NATURAL PYRETHRINS AND CAROTENOID PIGMENTS

by

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A thesis submitted to the University of Nottingham in candidature for the degree of Doctor of Philosophy.

November, 1983.

Abstract

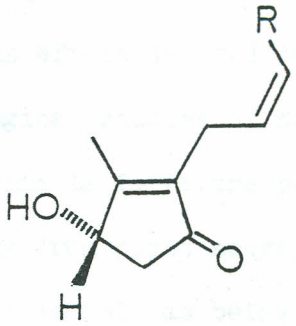
The thesis describes the chemistry of some commercial natural products obtained from plants indigenous to Kenya. The natural products are the insecticidal 'pyrethrins' obtained from flower heads of Chrysanthemum cinerariaefolium, and the food colouring pigment 'bixin', the main constituent of 'annatto' extracted from seeds of Bixa orellana.

Chapter I first summarises the more important previous synthetic approaches to the 'rethrolones' the alcohol portions of natural pyrethrin esters. This is followed by a discussion of a new synthetic approach we have developed to the rethrolones and their analogues. The new approach has as its central theme, a novel and facile 4-ylidenebutenolide-tocyclopentene-1,4-dione rearrangement. Thus, the 4-ylidenebutenolides (152a - c, 153a - c) in the presence of sodium methoxide in methanol were found to smoothly rearrange to the cyclopent-2-ene-1,4-diones (154a - c). The latter, when heated in the presence of sodium chloride in aqueous dimethyl sulphoxide gave rise to the 1,4-diones (155a - c), which were then reduced using zinc powder in acetic acid to the required dihydrorethrolones (15a - c). In a similar manner, the rearrangement of 2-methyl-3-methoxy-4-methoxycarbonylmethylidene-butenolides (163a - b) produced the 1,4-dione (164) which was demethoxycarbonylated to give the 1,4-dione (165). The latter dione (165) was protected selectively by methoxylation using methyl orthoformate leading to the ketal (167) which was then alkenylated by Grignard addition leading to allethrone (174). The allethrone was reduced selectively to allethrolone (175).

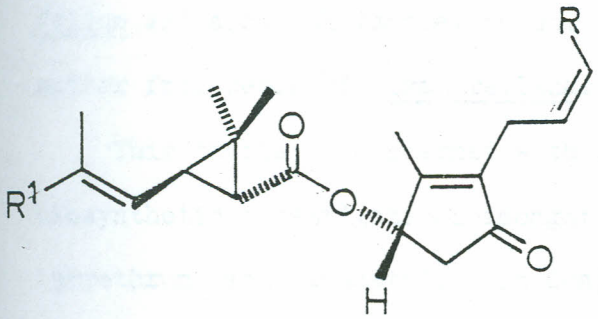
Chapter II briefly presents the uses, chemistry and a possible biogenetic source of bixin, the main pigment of 'annatto'. Although a number of chemical investigations of bixin have been carried out,

very little is known about the origin of this interesting 9'-cis-apocarotenoid in Nature. As a contribution to this problem an extensive chromatographic examination of the components of the extracts of seeds of Bixa orellana has been carried out, resulting in the isolation and structural elucidation of the following interesting compounds: methyl 9'-cis-apo-1-bixinal ester (218), all-trans geranylgeraniol (219), farnesylacetone (220), all-trans geranyl-geranyl formate (221), all-trans geranylgeranyl octadecanoate (222), and δ-tocotrienol (223).

Quantitative gas liquid chromatographic analysis of the hexane extract of Bixa seeds has incidentally revealed that the seeds are the richest known natural source of all-trans geranylgeraniol.



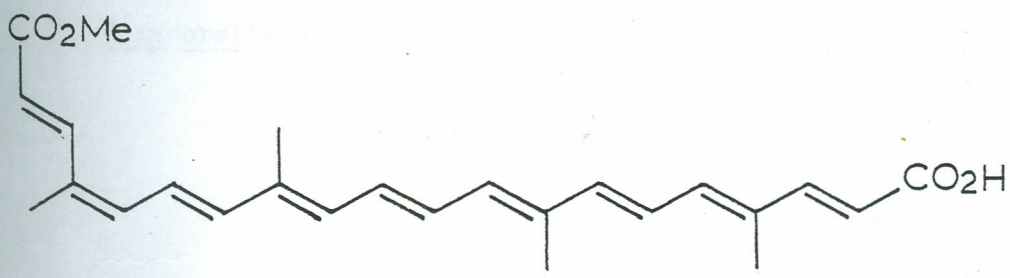
I



II

R = Me, Et or CH:CH$_2$

R^1 = Me or CO$_2$Me



III

Introduction

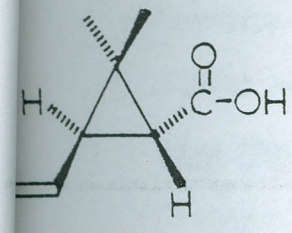
During the last one hundred years there has been a tremendous effort devoted to structural, synthetic, biosynthetic and biological studies of natural products. In an agricultural country such as Kenya, the production of natural products, especially from plant sources, has been an important source of employment as well as being a useful earner of foreign currency.

Kenya now produces fifty percent of the world market requirement of 'pyrethrum' the insecticide found in Chrysanthemum cinerariae-folium, and also substantial amount of 'annatto' the food colouring matter from seeds of Bixa orellana.

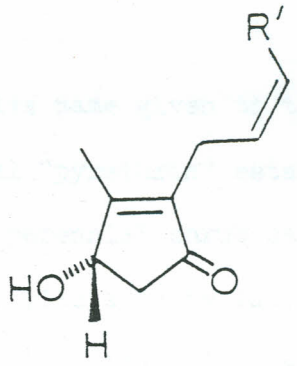
This thesis is concerned with structural, synthetic and biosynthetic investigations amongst natural products found in 'pyrethrum' and 'annatto'. In Chapter I, we describe our studies of a new approach to the synthesis of the rethrolone portions (I) of the pyrethrin insecticide (II) present in Chrysanthemum cinerariaefolium. In Chapter II we summarise our structural investigations of biosynthetically significant secondary metabolites that co-occur with the apo-carotenoid bixin (III) in seeds of Bixa orellana.

Chapter I

A New Approach to the Synthesis of Rethrolones



1



2

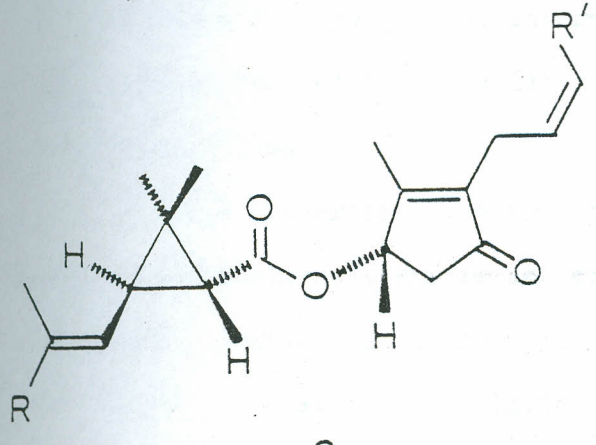
R = Me, (+) trans(1R, 3R)-chrysanthemic acid

R = CO_2Me, (+)-trans-(1R,3R)-pyrethric acid

a, R′ = Me, 4S-(+)-Z-cinerolone

b, R′ = Et, 4S-(+)-Z-jasmololone

c, R′ = $CH=CH_2$, 4S-(+)-Z-pyrethrolone



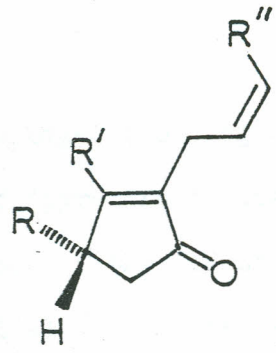
3

Pyrethrum ester	R	R′
a, Cinerin I	Me	Me
b, Cinerin II	CO_2Me	Me
c, Jasmolin I	Me	Et
d, Jasmolin II	CO_2Me	Et
e, Pyrethrin I	Me	$CH=CH_2$
f, Pyrethrin II	CO_2Me	$CH=CH_2$

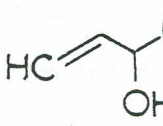
1.1 Introduction:

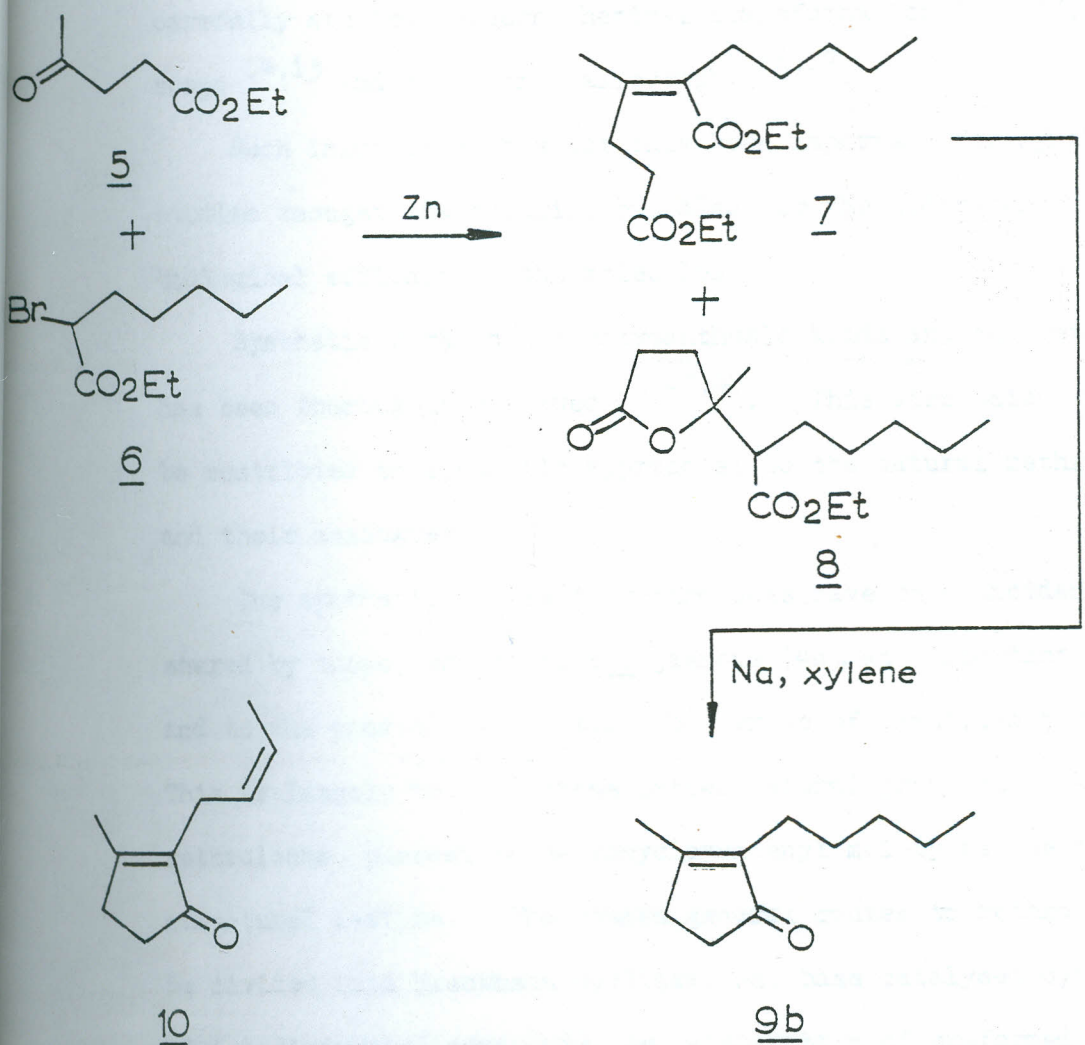
Rethrolone is the collective name given to the alcohol portions of the six insecticidal "pyrethrin" esters found in the flower heads of the herbacious perennial shrub called Chrysanthemum cinerariaefolium [1a]. The carboxylic acid residues of the "pyrethrins" are chrysanthemic acid (1a) and pyrethric acid (1b), which on esterification with the rethrolones form chrysanthemates ("pyrethrin I's") and pyrethrates ("pyrethrin II's") respectively. The esters thus formed between the acids and natural rethrolones [cinerolone (2a) jasmololone (2b) and pyrethrolone (2c)] are called cinerins I and II [(3a) and (3b) respectively], jasmolins I and II, [(3c) and (3d) respectively] and pyrethrins I and II [(3e) and (3f) respectively]. Flower heads of the pyrethrum plant are extracted with light petroleum to obtain an oleoresin which contains 20 - 30% of the esters (3a - f); the oleoresin is further refined and formulated for use as insect repellents and insecticides.

The commercial pyrethrum plant is a native shrub of Dalmatia (now Yugoslavia), and is now grown in Kenya, Tanzania, Ecuador, Japan, Rwanda, New Guinea, Brazil, Zaire, Indonesia, India, U.S.S.R., Taiwan, Zimbabwe, Yugoslavia and parts of South Africa. Most of these countries export ninety percent of their output to the United States of America and to Western Europe [1a]. Kenya produces over 50% of the world output under the sound supervision of the Pyrethrum Board of Kenya. This agricultural enterprise benefits over eightyfive thousand families, each of which owns less than 10 acres of land and yet uses $\frac{1}{4}$ to 5 acres for pyrethrum farming. Plenty of hand labour is still a very important condition for pyrethrum farming.



4

	R	R'	R''
a cis-jasmone	H	Me	Et
b Prostaglandins B_2	H		$(CH_2)_3CO_2H$



Scheme 1

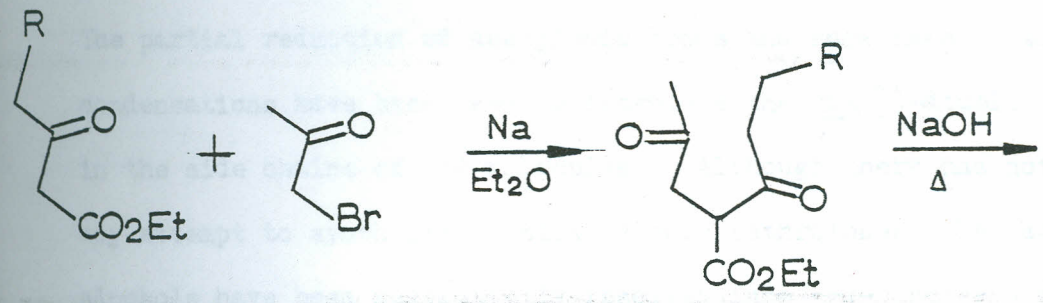
Knowledge of biological activities of the chemical constituents of 'pyrethrum' dates back to antiquity [4], making the rethrin esters some of the oldest organic insecticides. The broad insecticidal [5] and repellent [6] properties of the rethrins, combined with their low toxicity to warm blooded animals and facile biodegradability to non-toxic compounds [7] have made the use of these compounds against insect vectors and pests environmentally acceptable. These properties and their novel structures have made rethrins exciting natural products to chemists.

The methods of isolation [8,9,10] and analysis [18 - 22] of the pyrethrins and their constituents are well documented and structural, stereochemical and chiral properties of the six esters have been carefully studied through chemical transformation [11 - 13], spectroscopy [14,15] and X-ray crystallography [16,17].

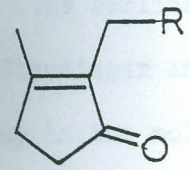
Such information has not only been important for synthetic studies amongst the rethrins but also for the understanding of the biological efficacy of the molecules.

Synthetic work on the chrysanthemic acids and the rethrolones has been thoroughly reviewed [23,47,48]. This discussion will however be restricted to synthetic approaches to the natural rethrolones and their analogues.

The synthetic routes to rethrolones have been incidentally shared by those leading to cis-jasmone (4a), an important perfume, and to the prostaglandin (e.g. 4b), group of mammalian hormones. This is largely because these latter natural products, like the rethrolones, possess an 3-oxocyclopentenyl moiety as their main structural feature. The common general routes to rethrolones can be divided into Dieckmann cyclisations, base catalysed cyclisations of 1,4-dicarbonyl compounds, and elaboration of preformed cyclopentane-1,2,4-triones and 3-alkoxy-3-methyl-2-vinylcyclopentanones.



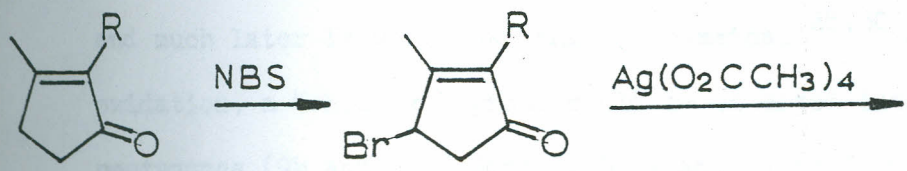
11 12 13



9 a R = 

b R = 

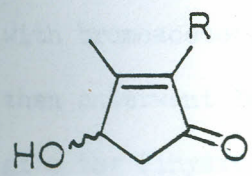
Scheme 2



9 14

a R =  a R = 

b R =  b R = 



15

b R = 

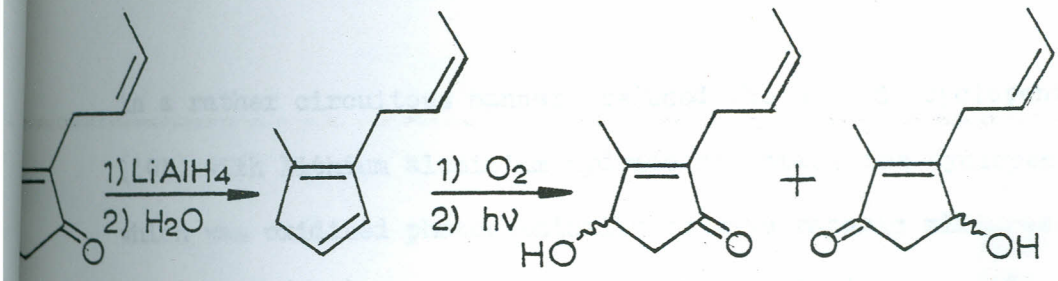
c R = 

Scheme 3

The partial reduction of acetylenic bonds and sometimes Wittig condensations have been used to introduce the cis(Z)-double bonds in the side chains of the molecules. Although there has not been any attempt to synthesise optically pure rethrolones, the racemic alcohols have been successfully resolved into (+)- and (-)- forms by conventional methods [48].

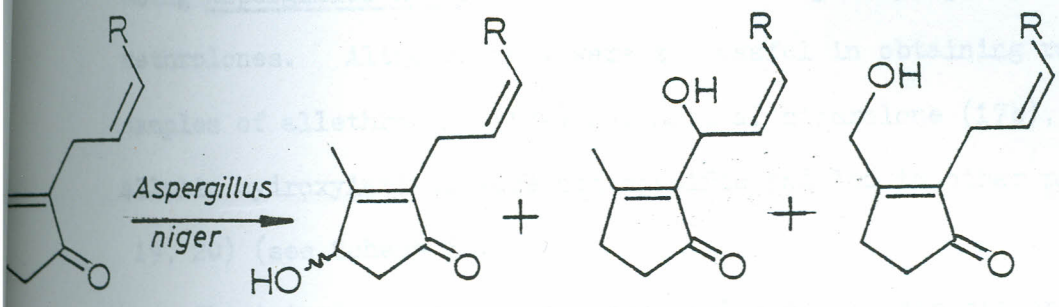
The earliest synthetic route to the rethrolones was developed by Staudinger and Ruzicka [8] even before the position of the hydroxyl group in the molecule was correctly established. A Reformatsky reaction between ethyl 4-oxopentanoate (5) and ethyl 2-bromohept-anoate (6) furnished diethyl 2-n-pentyl-3-methylhex-2-ene-1,6-dioate (7), which then underwent Dieckmann cyclisation to give tetrahydropyrethrone (9b) in poor yield. Treff and Werner [24] later improved this general approach to obtain cis- cinerone (10), and much later it was shown that by chemical [28,30] and biological [31] oxidation, a 4-hydroxyl group could be inserted into these cyclo-pentenones (9b and 10) (Schemes 3, 4 and 5) to form rethrolones.

The most successful and general approach to rethrolones is the base catalysed cyclisation of a suitably substituted 1,4-dicarbonyl compound. The pioneer of this approach was Hunsdiecker [25, 26] who reacted the carbanion derived from the substituted acetoacetate (11) with bromoacetone (12) to obtain α-acyllaevulic esters (13), which then underwent base catalysed cyclisation and decarboxylation to give the dihydrorethrones (9a, 9b) in good yields (Scheme 2). Groups working with Harper, Wenkert [27,28] and LaForge [29] independently improved upon Hunsdiecker's route by allylic hydroxylation of the dihydrorethrones (9a, 9b), via bromination with N-bromosuccinimide and hydrolysis, to obtain dihydrocinerolone (15b) and tetrahydropy-rethrolone (15c) (Scheme 3). This method however, failed to give natural cinerolone and pyrethrolone. Later on, Le Mahieu et al.[30]



10b 16 17b 18b

Scheme 4

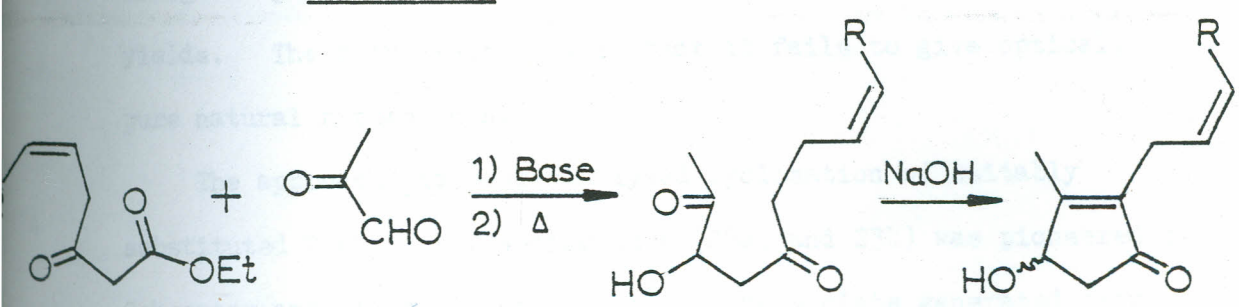


10
a R = H
b R = Me
c R = Et

17
a R = H
b R = Me
c R = Et

19 20

Scheme 5



21 22

23 a R = H
b R = Me

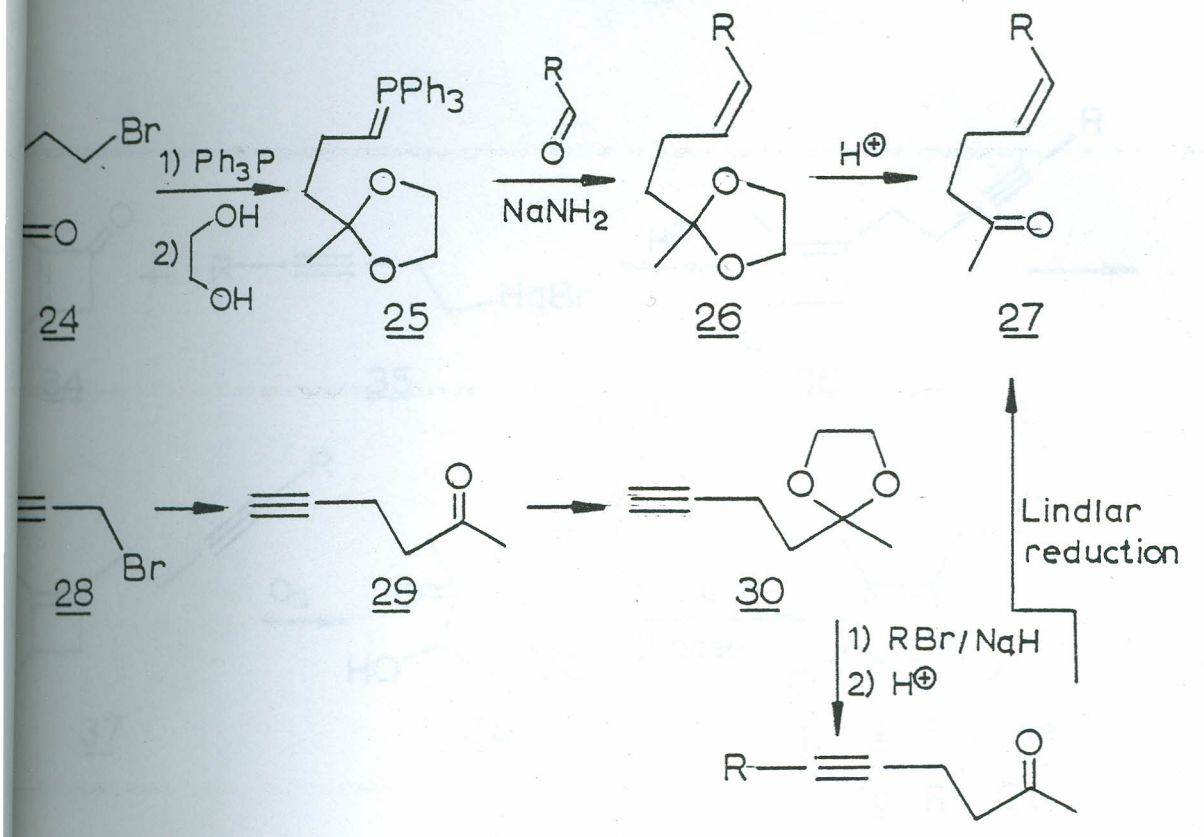
17 a R = H
b R = Me

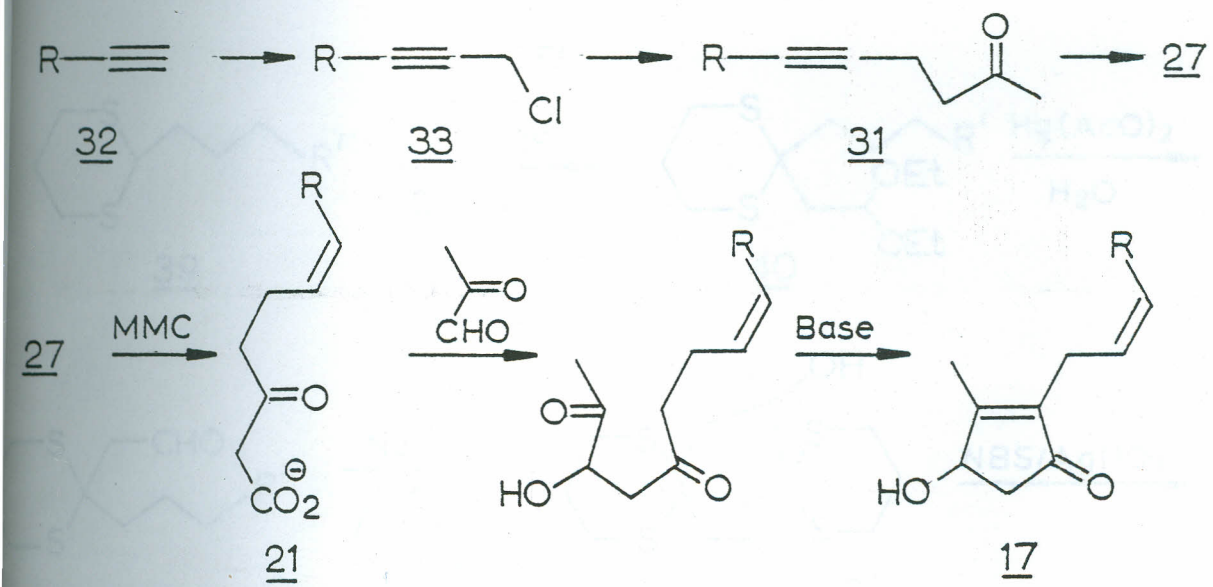
Scheme 6

in a rather circuitous manner, reduced the α, β -cyclopentenone (10b) with lithium aluminium hydride to obtain the cyclopentadiene (16) which was oxidised photochemically to give racemic mixtures of cinerolone (17b) as well as its hydroxy regio-isomer (18b) (Scheme 4); this route was not extended to jasmololone and pyrethrolone. To overcome this difficulty, Le Mahieu's group [31] employed micro-biological hydroxylation of allethrone (10a) and cinerone (10b), using Aspergillus niger to introduce the 4-hydroxyl group in the rethrolones. Although they were successful in obtaining racemic samples of allethrolone (17a) and natural cinerolone (17b), the allylic hydroxylations were not specific and led to other products (e.g. 19, 20) (see Scheme 5).

The introduction of the 4-hydroxyl group on the cyclopentenones carrying unsaturated side chains had serious limitations, and thus other routes were developed. In one of these, the hydroxyl group was inserted before cyclisation of the suitably substituted 1,4-diketones or 1,4-ketoaldehydes. In fact this approach is flexible enough to give the three natural rethrolones in reasonable overall yields. The only limitation is that it fails to give optically pure natural rethrolones.

The approach via base catalysed cyclisation of suitably substituted 2-hydroxy-1,4-diketones (23a, and 23b) was pioneered by Schechter and his collaborators [32]. The enolate generated from the 4-alkenyl-3-oxobutyrate (21) was found to react smoothly in a salt free medium with pyruvaldehyde (22) to give an intermediate that simultaneously underwent decarboxylation to give the 4-hydroxy-1,4-diketones (23a and 23b); these diketones then cyclised in the presence of sodium hydroxide to afford allethrolone (17a) and cinerolone (17b) (Scheme 6). This scheme is now used for the industrial [41] production of allethrolone, an important analogue of natural rethrolones.





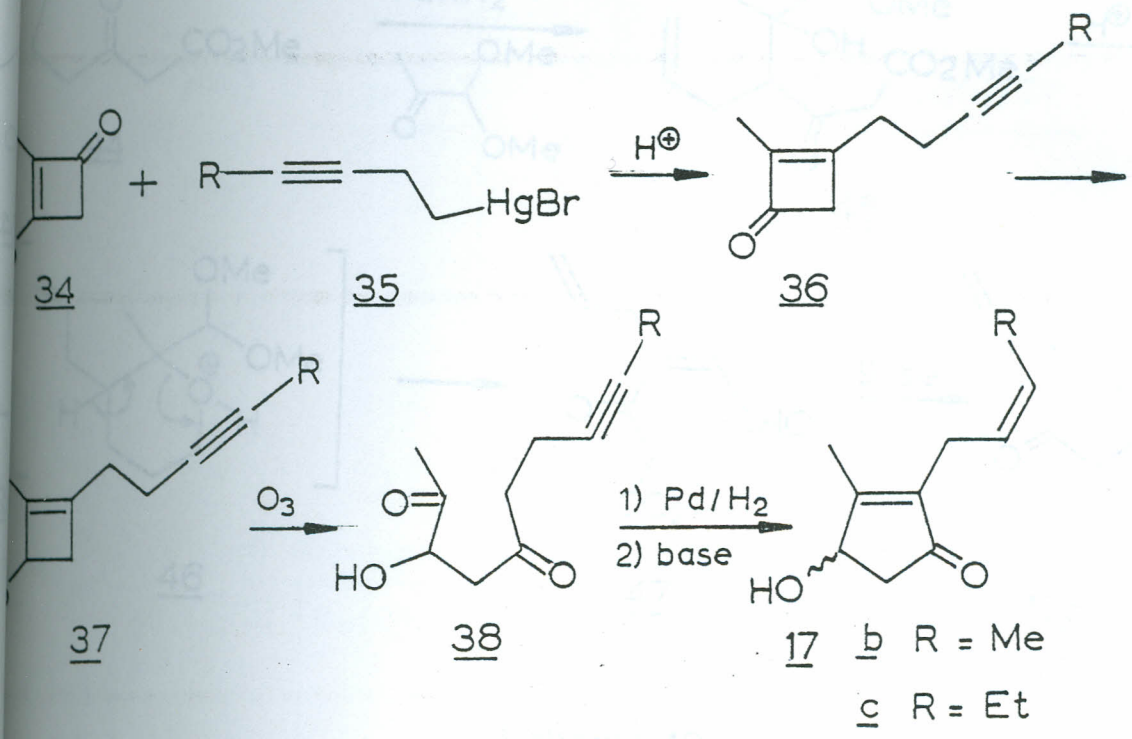
a R = H

b R = Me

c R = Et

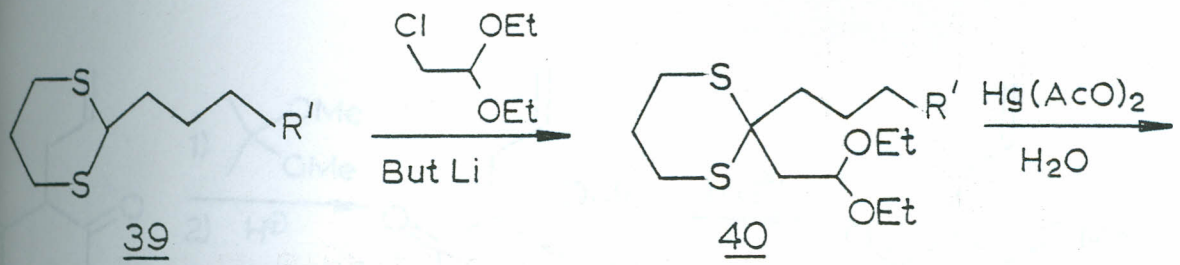
d R = CH=CH$_2$

Scheme 7

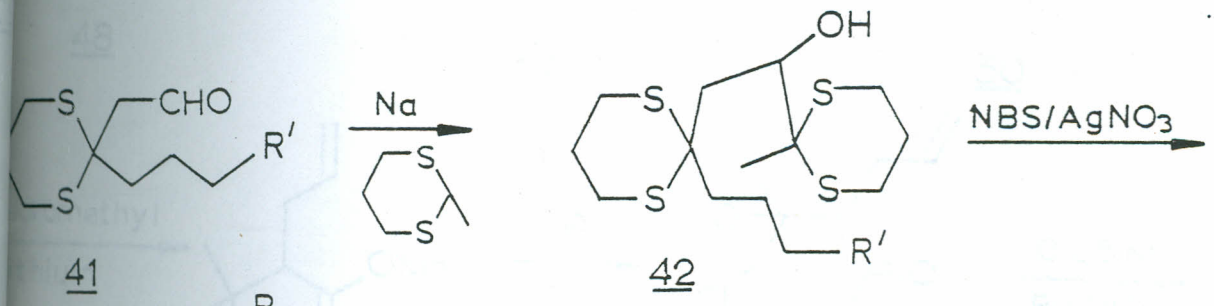


34 35 36 37 38 17 b R = Me c R = Et

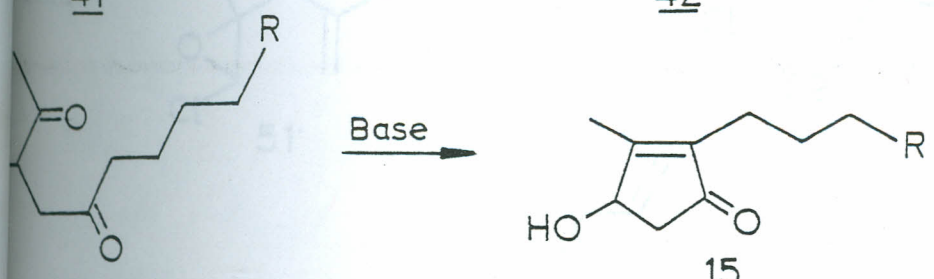
Scheme 8



39 40

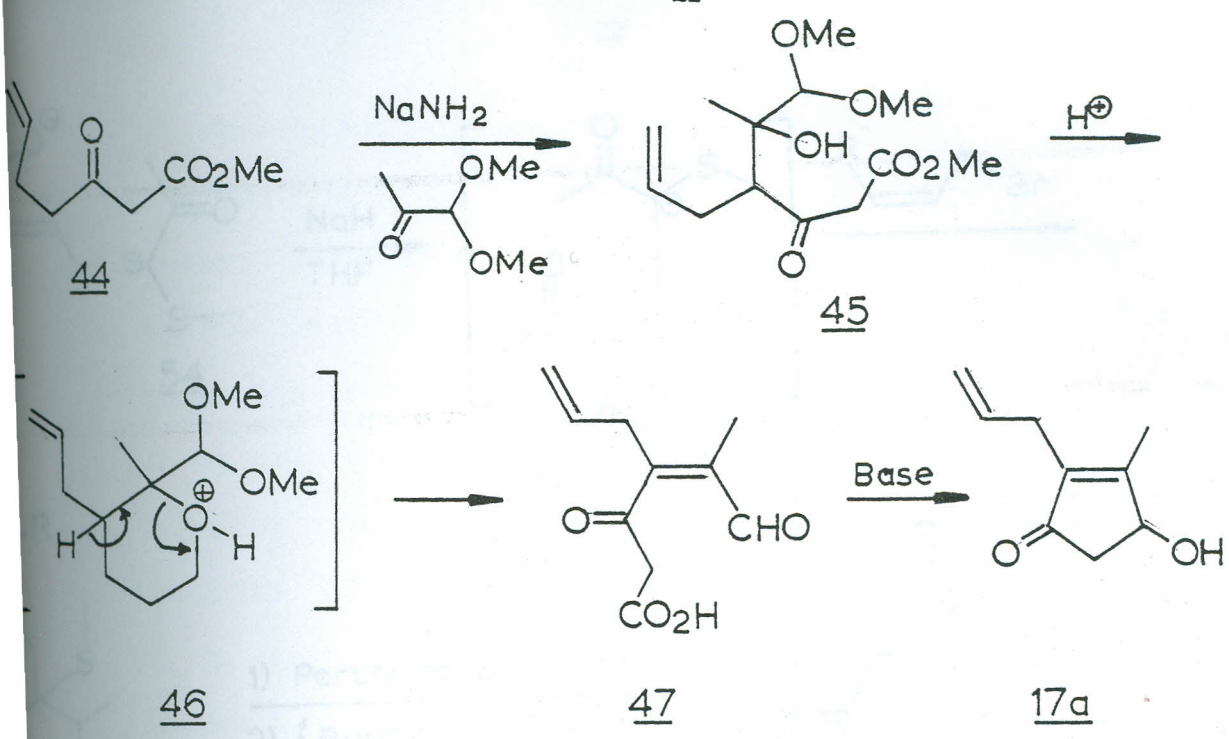


41 42

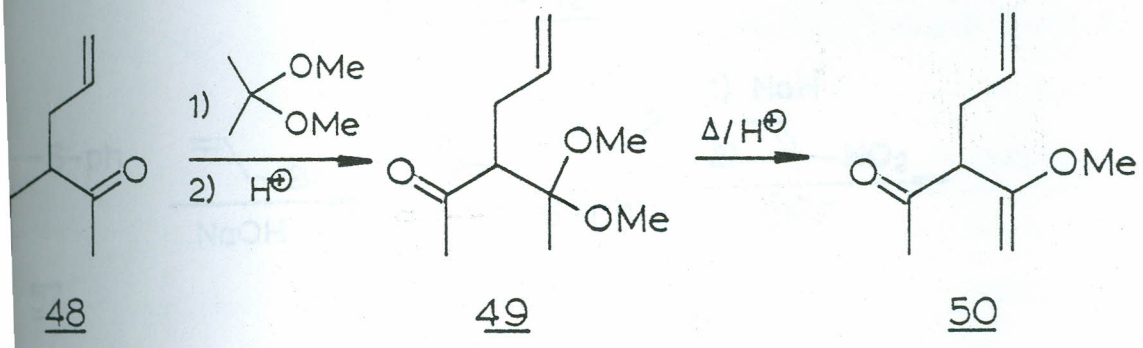


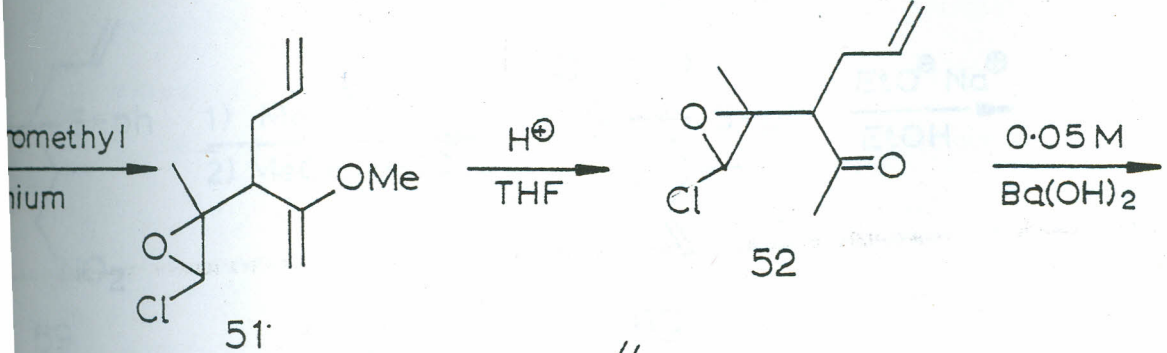
43 15 a R = H b R = Me

Scheme 9

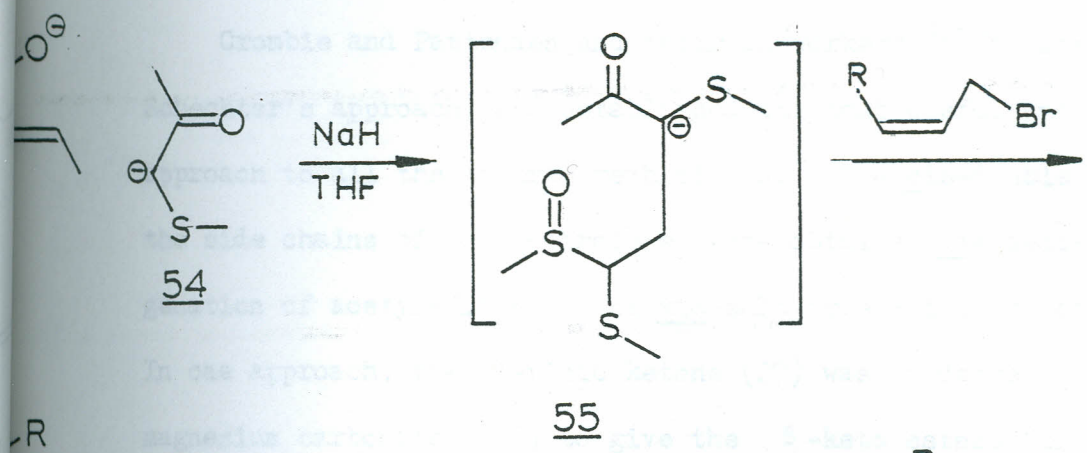


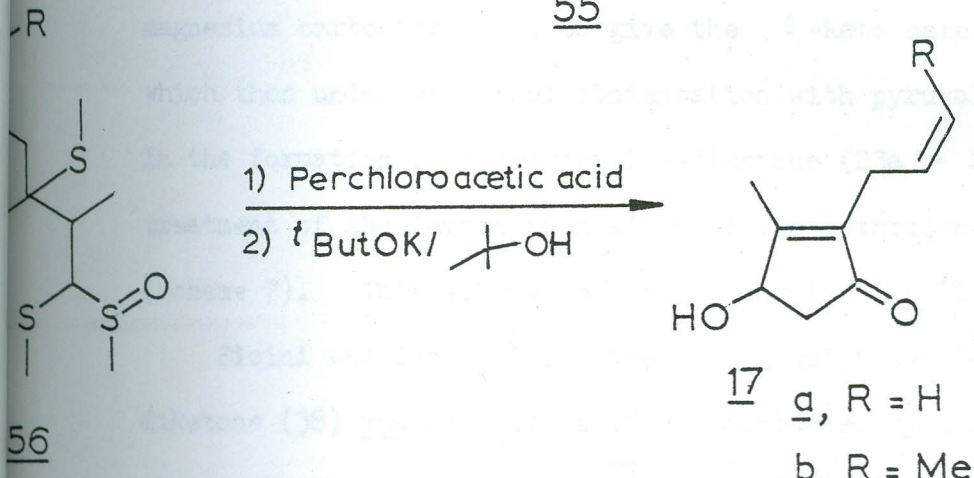
Scheme 10



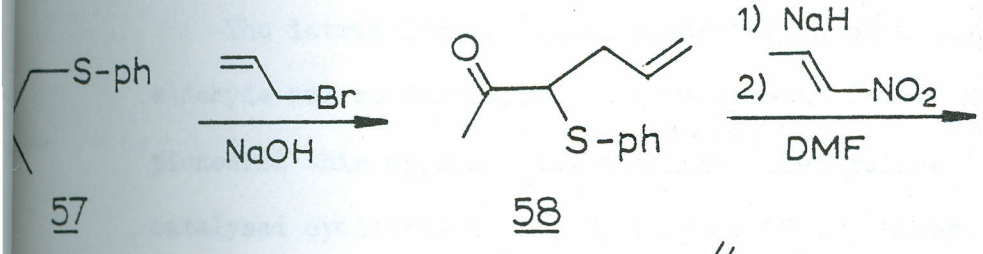


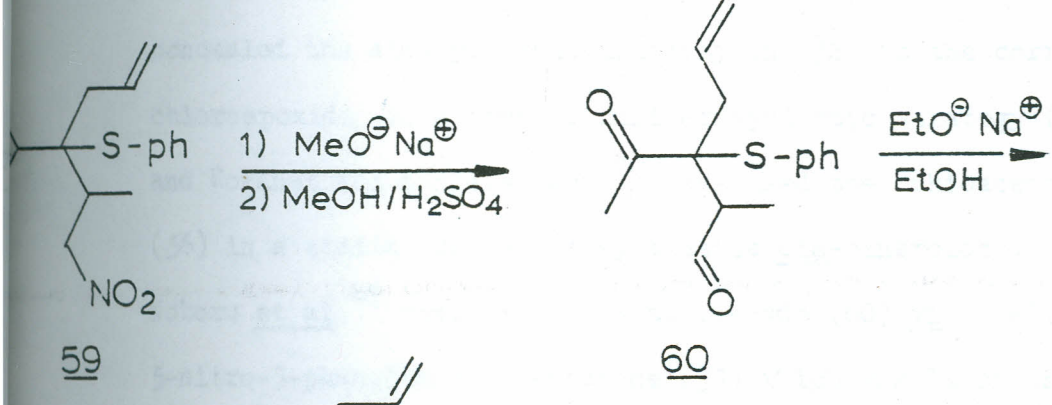
Scheme 11

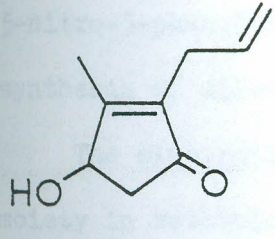




Scheme 12







17a

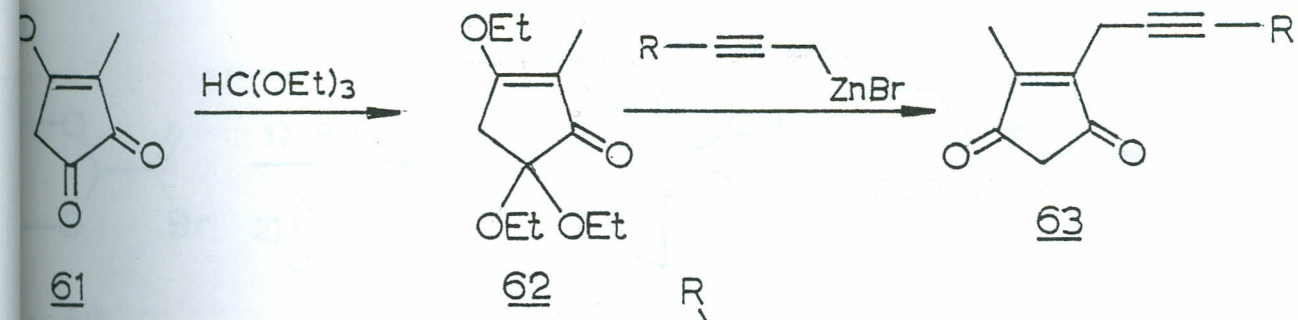
Scheme 13

Crombie and Pattenden and their co-workers [33-35] later improved Schechter's approach, and established the most useful and versatile approach to all the natural rethrolones. The cis-double bonds in the side chains of the rethrolones were obtained via semi-hydrogenation of acetylenic bonds or via salt free Wittig reactions. In one approach, the olefinic ketone (27) was condensed with methylmagnesium carbonate (MMC) to give the β-keto esters (21a - 21d) which then underwent aldol condensation with pyruvaldehyde resulting in the formation of 3-hydroxy-1,4-diketone (23a - 23d); base treatment of the latter then afforded the rethrolones (17a - 17d) (Scheme 7). This scheme led to pyrethrolone in 21% overall yield.

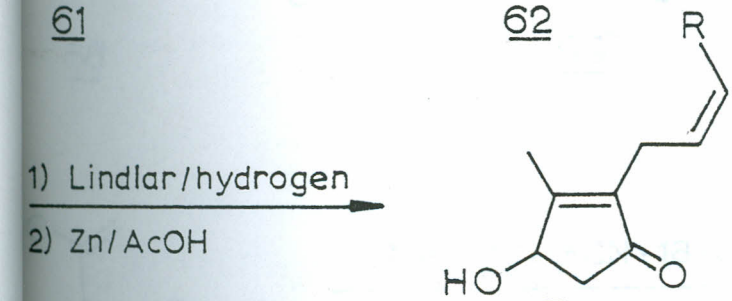
Ficini and Genet [36] obtained the substituted 3-hydroxyl-1,4-diketone (38) via ozonolysis of a substituted cyclobutene (37), whereas Woessner and Ellison [37] prepared the 3-hydroxy-1,4-diketone (43) via dithiane intermediates. The diketones were then cyclised in base to the rethrolones (15a-15b, 17b-17c)(Schemes 8 and 9).

The intramolecular aldol condensation of a substituted 1,4-ketoaldehyde has so far failed to give pyrethrolone. Martel and Nomine [38] pioneered this approach and obtained allethrolone (17a) on base catalysed cyclisation of (47) (Scheme 10). Later, Büchi et al.[39] concealed the aldehyde functionality in (52) as the corresponding chloroepoxide to achieve a similar synthetic strategy (Scheme 11) and Romanet and Schlessinger [40] have used the thioacetal monoxide (56) in a similar manner to synthesise cis-cinerolone. Recently, Noboru et al.[41] obtained the ketoaldehyde (60) via 3-allyl-4-methyl-5-nitro-3-phenylthio-2-pentanone (59) which was later used in their synthesis of allethrolone (17a) (Scheme 13).

The elaboration of the side chain on the preformed cyclopentane moiety in rethrolones was initially and independently explored by Vandewalle [45] and Fujisawa [46] who inserted an anion from a substituted



61 62 63



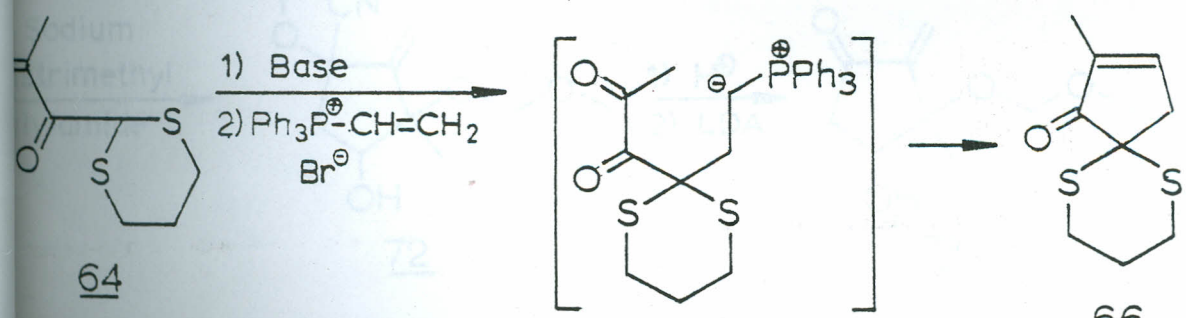
17

a R = H

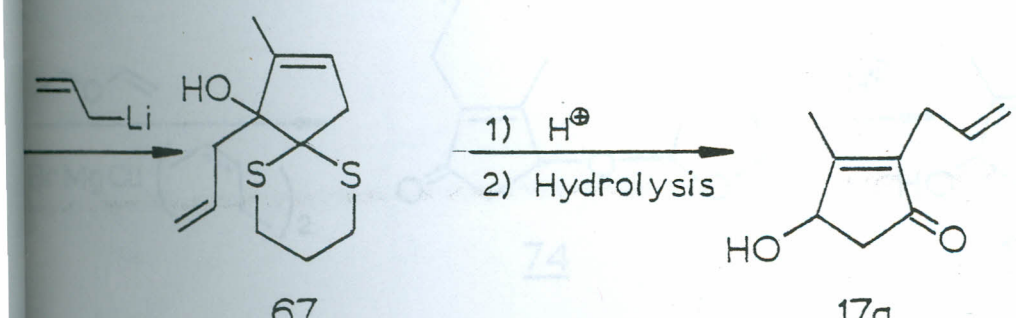
b R = Me

c R = Et

Scheme 14

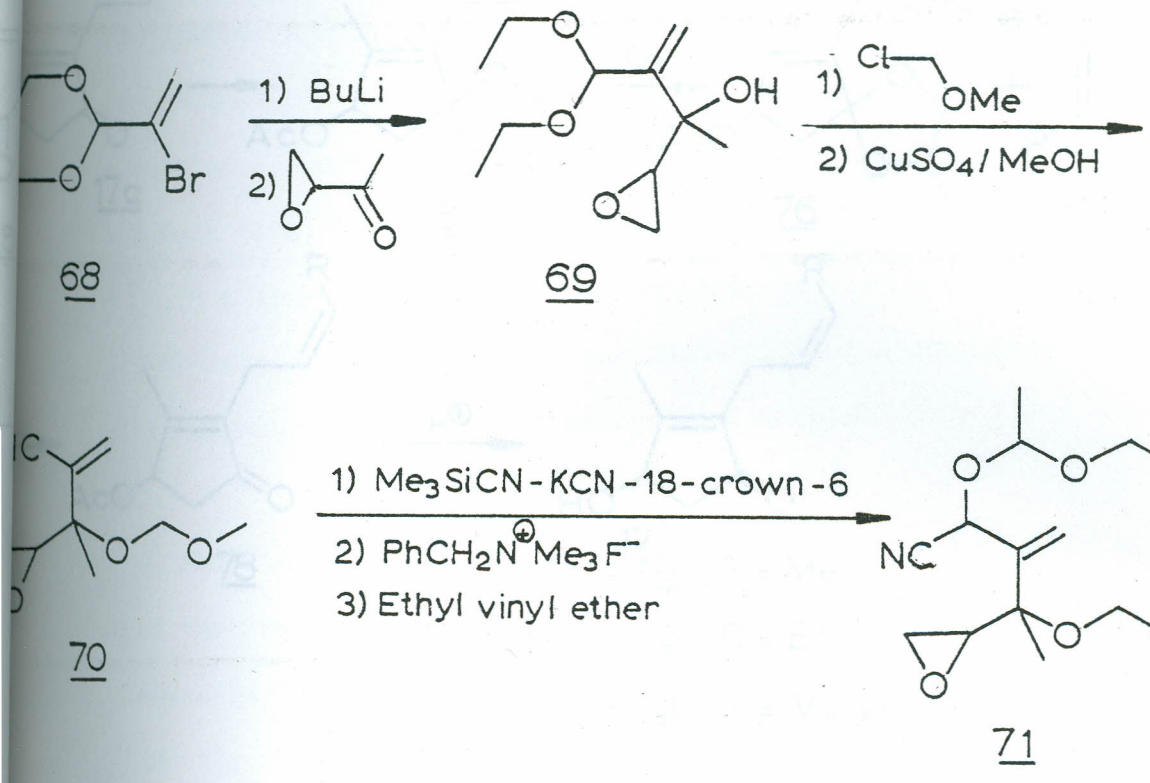


64 65 66

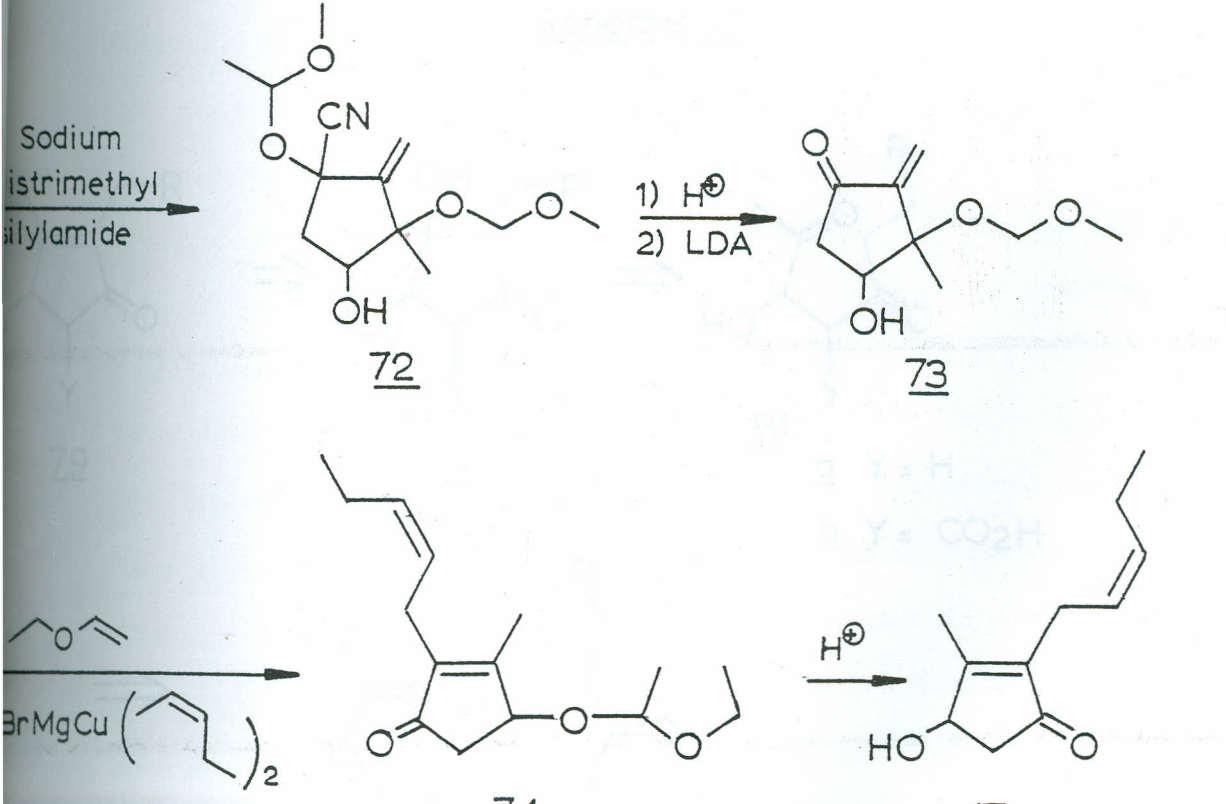


67 17a

Scheme 15

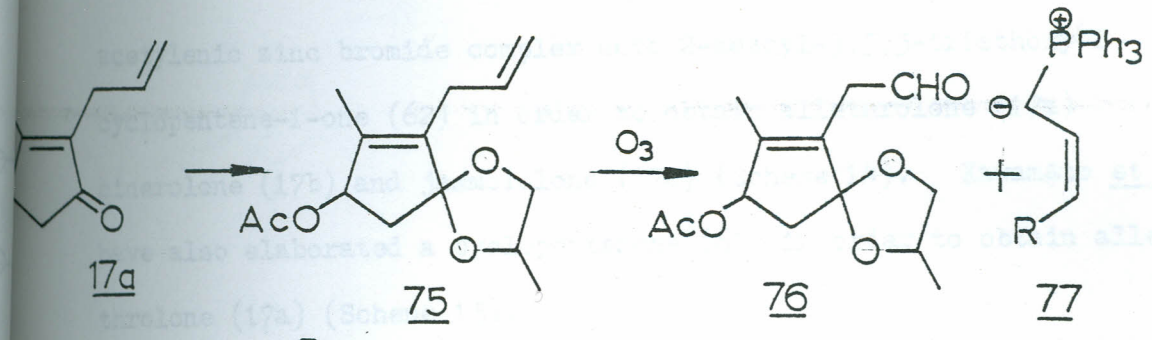


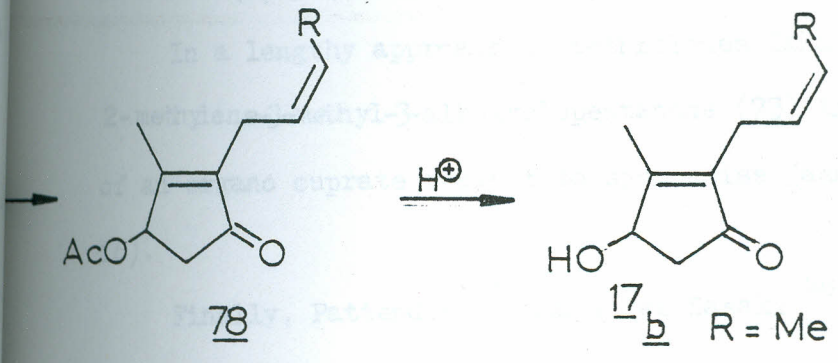
68 69 70 71



72 73 74 17c

Scheme 16



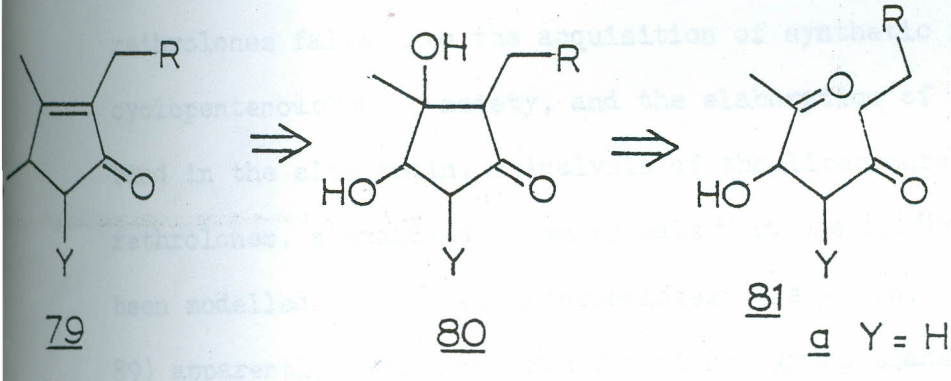


17 b R = Me

c R = Et

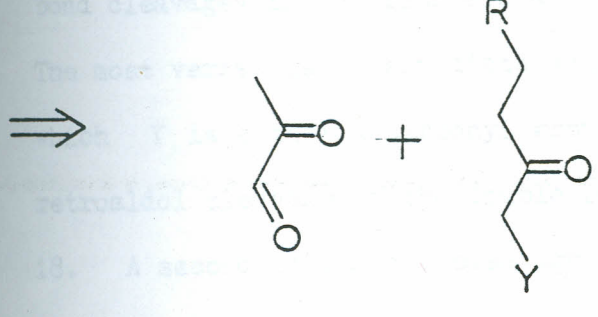
d R = Vinyl

Scheme 17



a Y = H

b Y = CO_2H



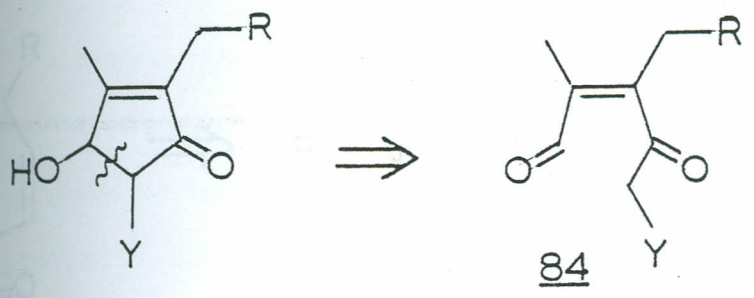
Scheme 18

acetylenic zinc bromide complex onto 2-methyl-3,5,5-triethoxy-2-cyclopentene-1-one (62) in order to obtain allethrolone (17a) cinerolone (17b) and jasmololone (17c) (Scheme 14). Kawamato et al.[42] have also elaborated a cyclopentenone (66) in order to obtain allethrolone (17a) (Scheme 15).

In a lengthy approach to rethrolones Takahashi et al.[50] employed 2-methylene-3-methyl-3-alkoxyclopentanone (73) in a conjugate addition of an organo cuprate reagent to synthesise jasmololone (17c) (Scheme 16).

Finally, Pattenden [43] and later Sasaki [44] have demonstrated that the readily available protected allethrolone (75) can be elaborated into cinerolone (17b), jasmololone (17c) and pyrethrolone (17c) via oxidative cleavage of side chain in the presence of osmium tetroxide or ozone followed by Wittig reaction (Scheme 17).

Generally, the problem of retrosynthetic analysis of the rethrolones falls into the acquisition of synthetic routes to a cyclopentenolone moiety, and the elaboration of the cis-double bond in the side chain. Analysis of the literature approaches to rethrolones, summarised above, reveals that the synthetic strategies have been modelled on the key intermediates (81a - 81b, 84a - 84c, 86 and 89) apparently conceived from functional group transformations and bond cleavages in the rethrolone as illustrated in Schemes 18 - 21. The most versatile intermediate would seem to be (81a - 81b), in which Y is either a carbonyl group or hydrogen atom produced from retroaldol cleavage of the double bond in (79) as shown in Scheme 18. A second retroaldol cleavage of the C - C bond between the hydroxyl group and the carbonyl centre in the intermediates (81a - 81b) then leads to synthons of the type (82 and 83) which have been used in the synthesis of all the natural rethrolones as represented in Schemes 6, 7 and 8.



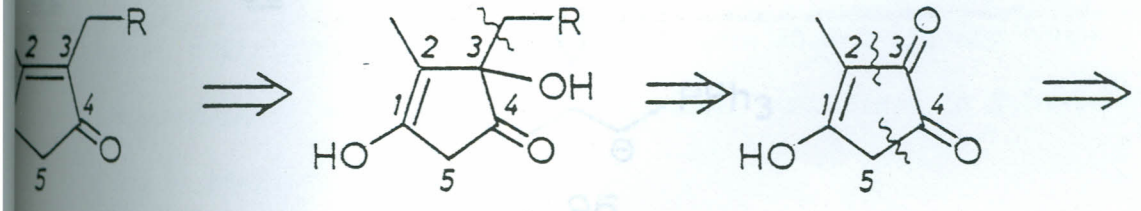
79 84

a Y = H

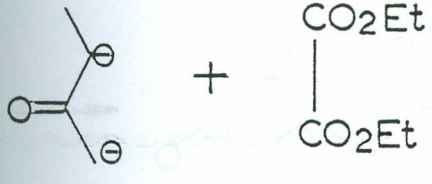
b Y = CO_2H

c Y = CN

Scheme 19

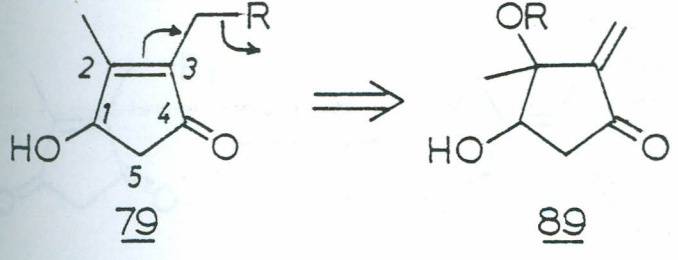


79 85 86



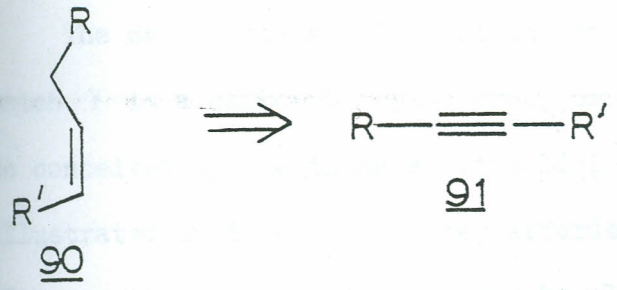
87 88

Scheme 20



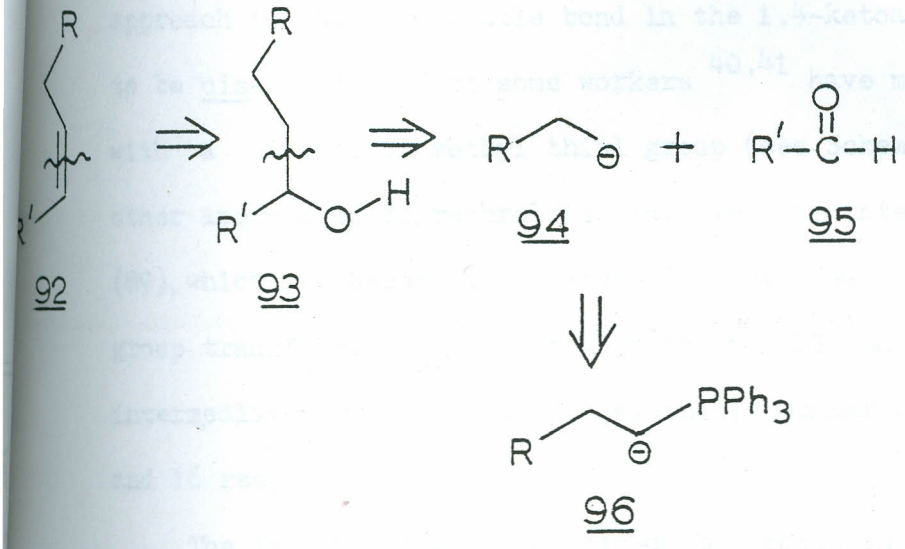
79 89

Scheme 21



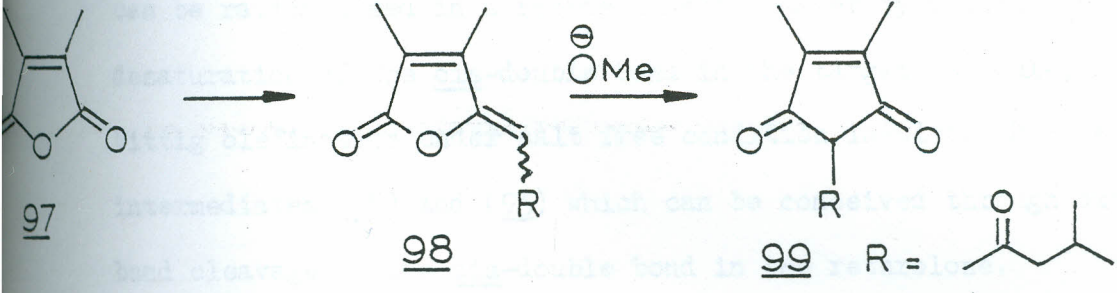
90 91

Scheme 22



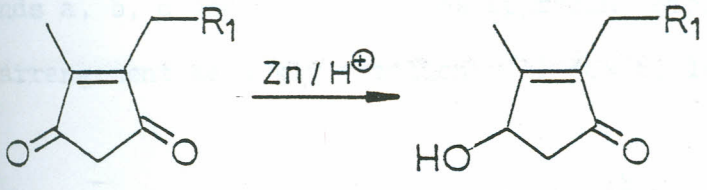
92 93 94 95 96

Scheme 23



97 98 99

Scheme 24



100 101

Scheme 25

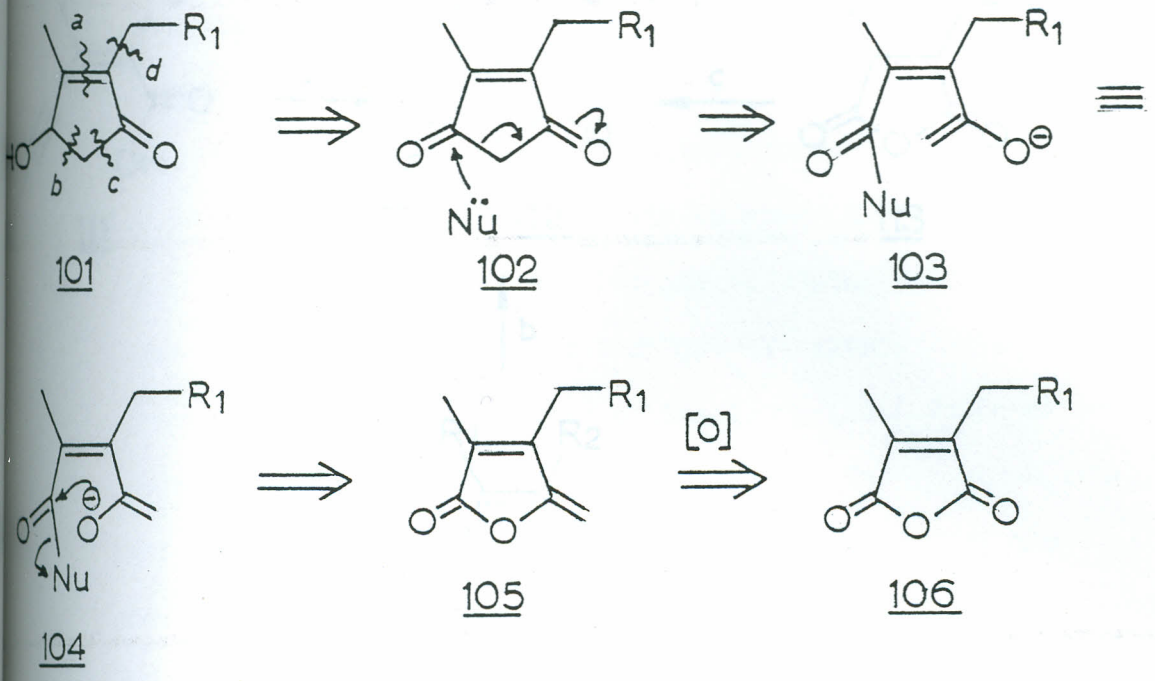
The second class of useful intermediates are (84a - 84c), in which Y is a carboxyl, cyano group or a hydrogen atom. These can be conceived by imagining a retroaldol C - C bond cleavage as illustrated in Scheme 19; they afforded high yields of allethrolone (Schemes 10, 13 and 16) which can be elaborated to the natural rethrolones (Scheme 17). The main disadvantage of this latter approach is that the double bond in the 1,4-ketoaldehydes (84a-c) has to be cis-, and in fact some workers [40,41] have masked this double bond with a phenyl or methyl thiol group (see Schemes 12 and 13). The other approaches to rethrolones involve the intermediates (86) and (89), which are assumably obtained by bond cleavages and functional group transformations depicted in Schemes 20 and 21, and these intermediates were used in the syntheses summarised in Schemes 14 and 16 respectively.

The introduction of the cis-double bonds in the side chains of rethrolones involves the use of acetylenic intermediates (91) can be rationalised in a retrosynthetic manner by assuming further desaturation of the cis-double bond in the target molecule, while Wittig olefination under salt free condition involves the use of intermediates (94) and (95) which can be conceived through oxidative bond cleavage of the cis-double bond in the rethrolone.

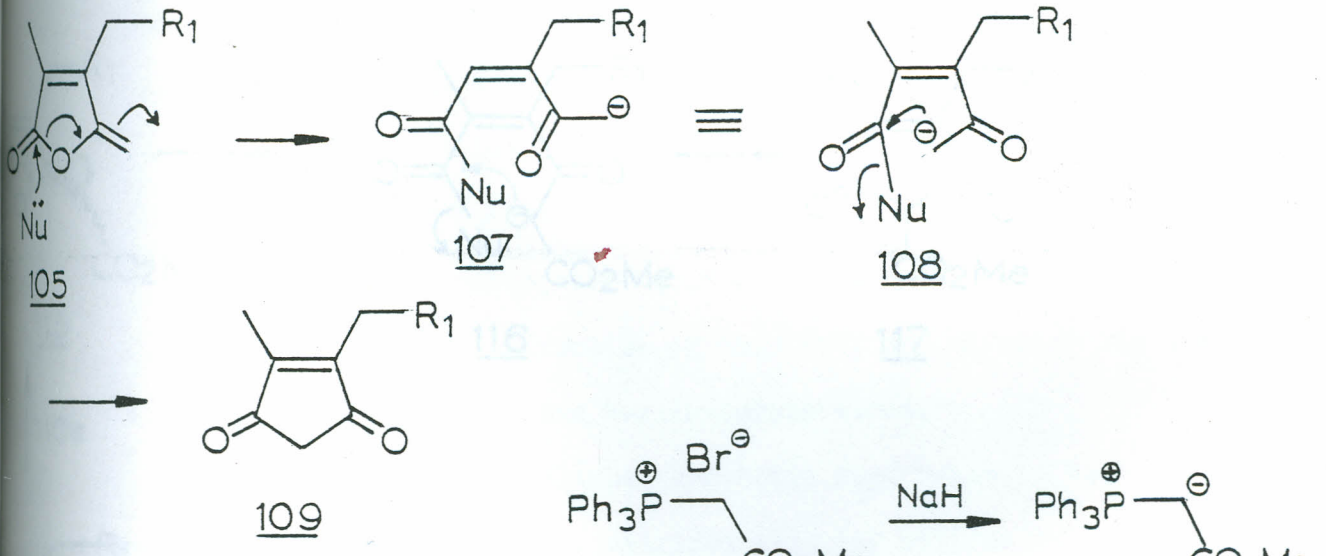
In our new approach to the rethrolones, we have conceived a disconnection from the target molecule (101) involving cleavage of bonds a, b, c and d. This new approach, employing a novel butenolide rearrangement as a key reaction will now be described.

1.2 A New Approach to Rethrolone Synthesis [1b].

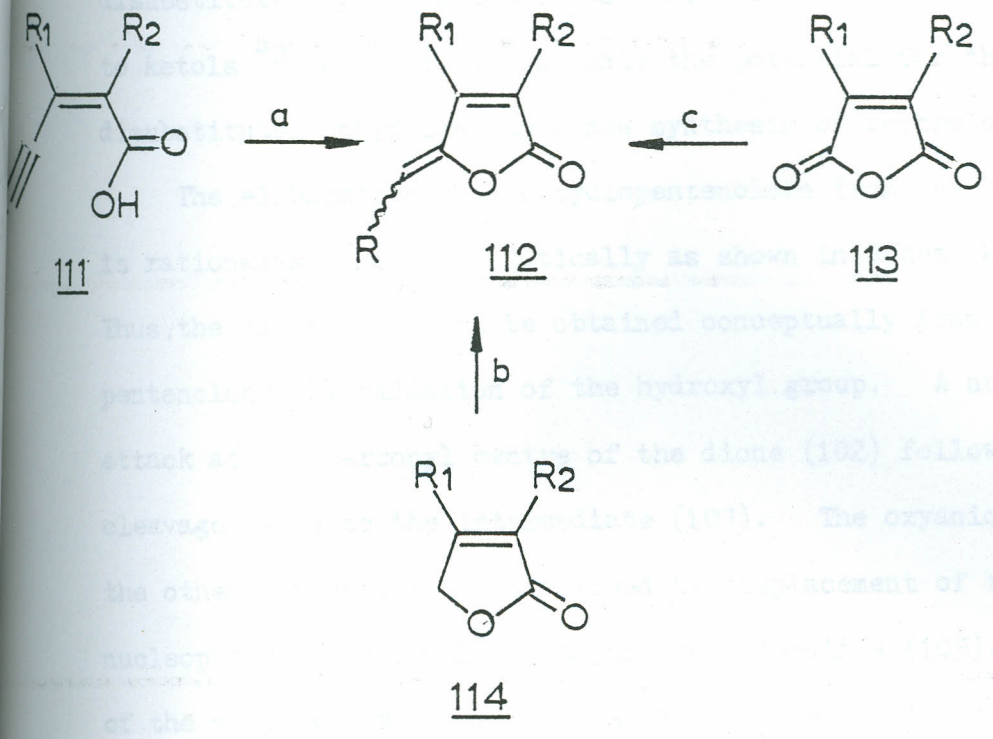
The observation [51] that 4-ylidenebutenolides (98), obtained from the maleic anhydride (97), can be rearranged in a facile manner to the cyclopent-2-ene-1,4-dione (99) combined with the fact that the



Scheme 26

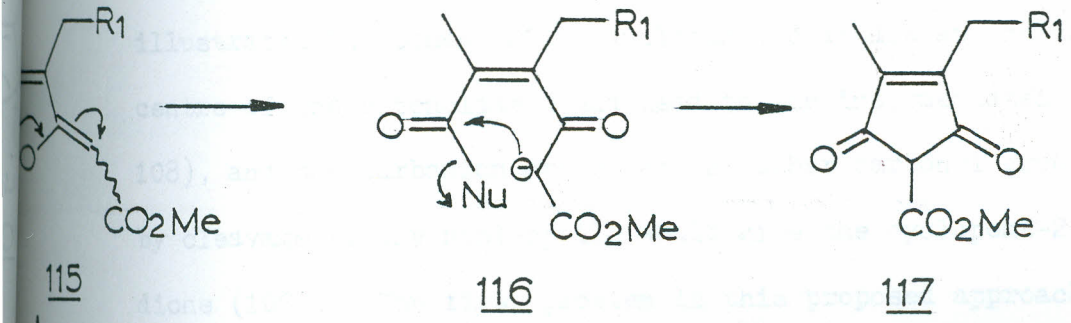


Scheme 27



111 112 113 114

Scheme 28



115 116 117

106

a, R_1 = n-alkyl

b, R_1 = Alkenyl

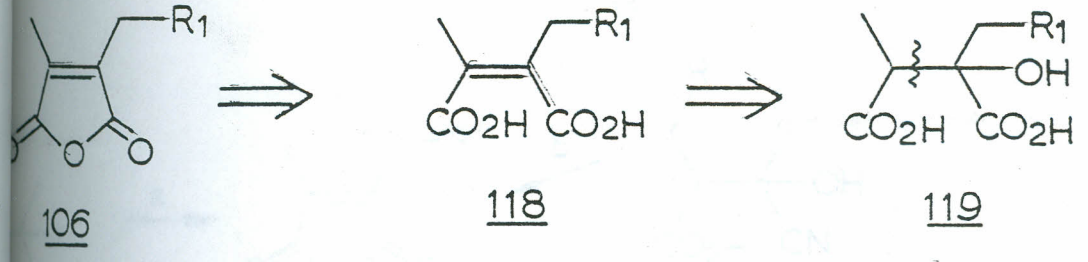
Scheme 29

disubstituted cyclopentenediones (100) can be selectively reduced to ketols [45] led us to investigate the potential for the use of disubstituted anhydrides in a new synthesis of rethrolones.

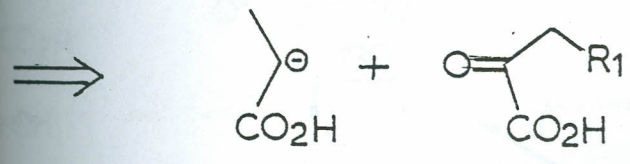
The elaboration of the cyclopentenolone from the butenolide is rationalised retrosynthetically as shown in Scheme 26. Thus, the dione (102) can be obtained conceptually from the cyclopentenolone via oxidation of the hydroxyl group. A nucleophilic attack at the carbonyl centre of the dione (102) followed by bond cleavage leads to the intermediate (103). The oxyanion attack at the other carbonyl centre, followed by displacement of the nucleophile would finally lead to the butenolide (105). "Oxidation" of the vinyl group finally leads back to the maleic anhydride (106) (Scheme 26).

In a forward sense, the rearrangement of the butenolide (105) to the cyclopent-2-ene-1,4-dione (109) can also be rationalised as illustrated in Scheme 27. Nucleophilic attack at the carbonyl centre of the butenolide would lead to the intermediates (107 and 108), and the carbanion attack at the other carbonyl group followed by cleavage of the nucleophile would give the cyclopent-2-ene-1,4-dione (109). The first problem in this proposed approach was then to design a synthesis of the 4-ylidenebutenolide.

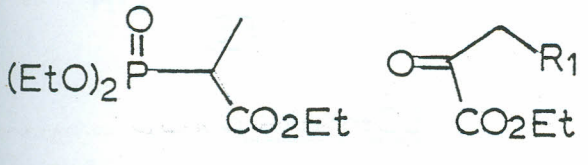
There are several published synthetic approaches to the butenolides; these can be summarised as: (a) cyclisations of Z-but-2-en-4-ynoic acids (111), [57] (b) aldol type condensations using carbanions formed from suitable lactones (114) [58], and (c) condensations between maleic anhydrides (113) and Grignard or Wittig reagents, [55,59] as illustrated in Scheme 28. The first two suffer from the disadvantage of low yields, whereas the use of Grignard reagents was unsuitable for our strategy. The newly developed synthetic approach [55] to the butenolides that accommodated our



106 118 119

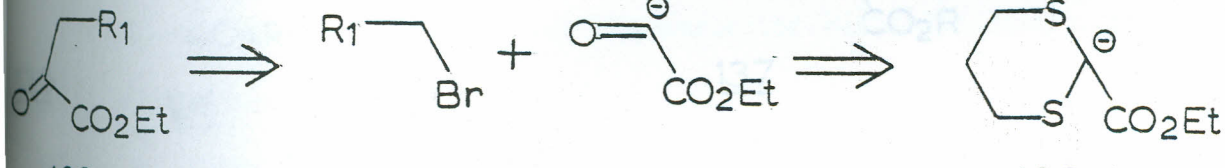


120 121



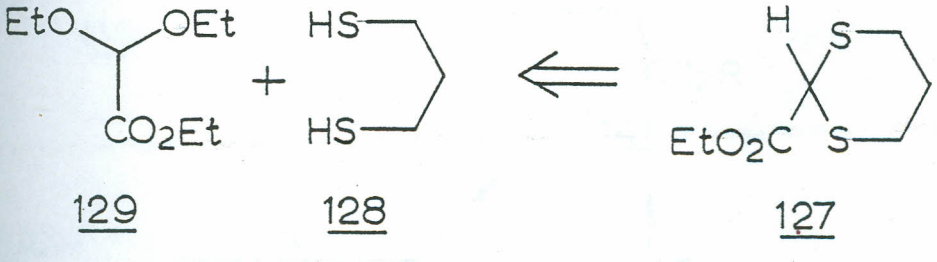
122 123

Scheme 30



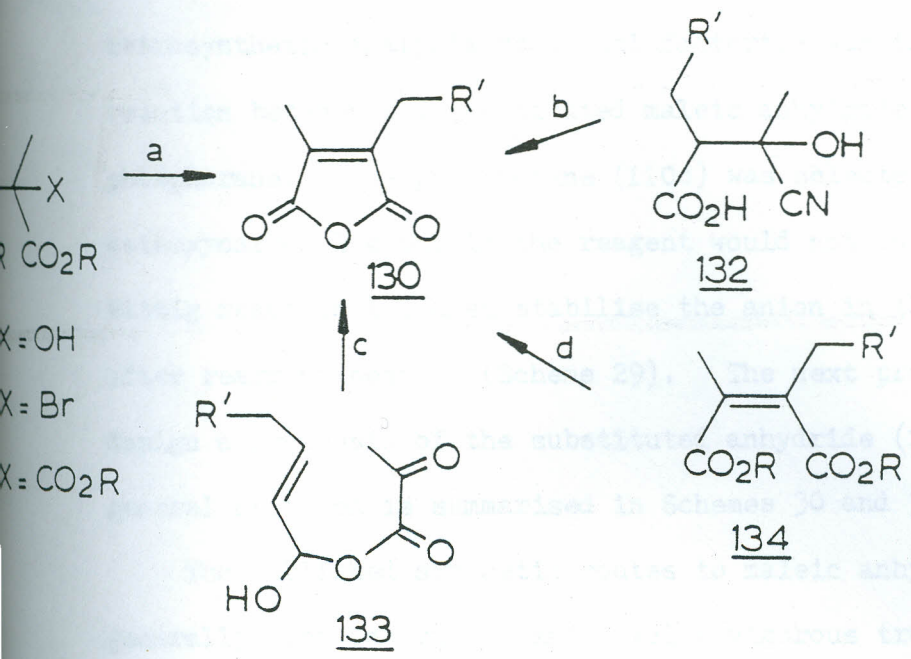
123 124 125 126

a) $R^1 = n$-Pr b) $R^1 = n$-Bu
c) $R^1 = n$-Pent. d) R^1 = allyl

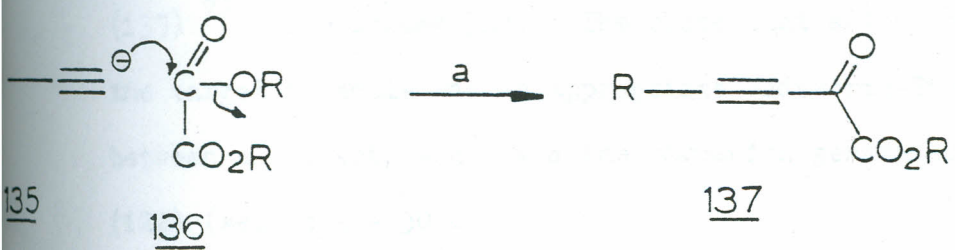


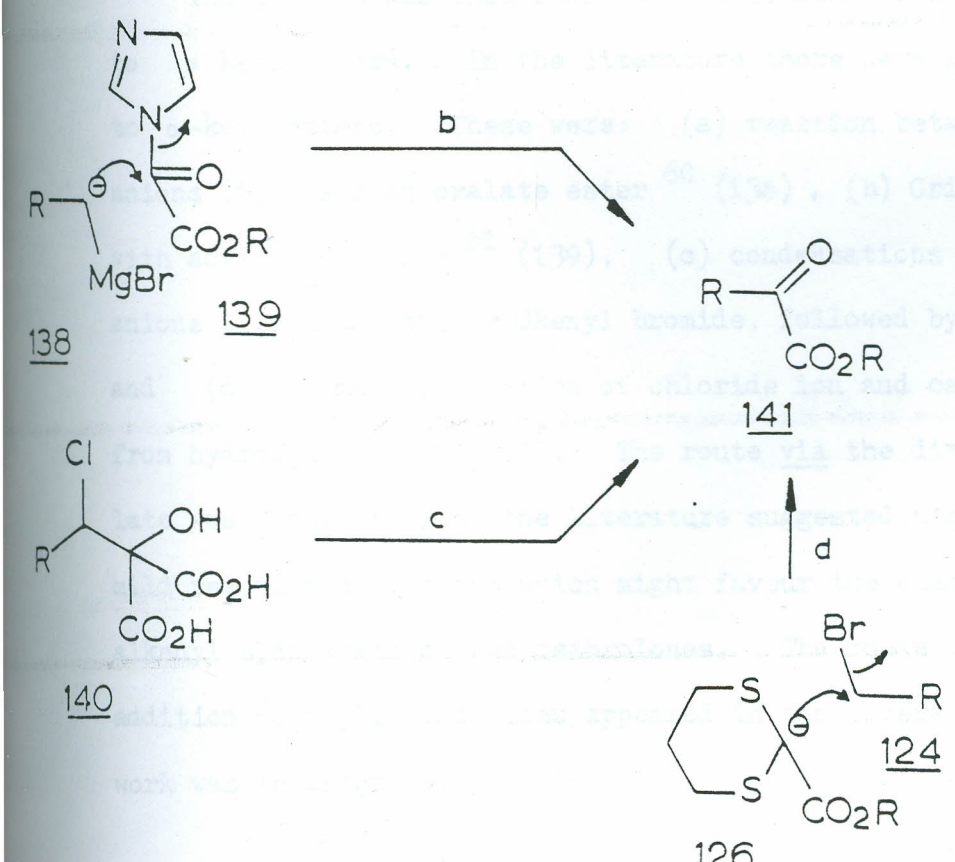
129 128 127

Scheme 31



Scheme 32



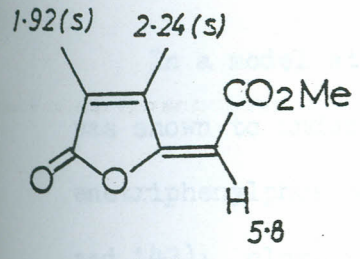


Scheme 33

retrosynthetic analysis most satisfactorily was the Wittig reaction between a disubstituted maleic anhydride and a suitable phosphorane. The phosphorane (110c) was selected since the methoxycarbonyl group in the reagent would not only permit a facile Wittig reaction, but also stabilise the anion in the product (116) after rearrangement (Scheme 29). The next problem was to design a synthesis of the substituted anhydride (106), and our general approach is summarised in Schemes 30 and 31.

The published synthetic routes to maleic anhydrides suffer generally from low yields and involve vigorous treatments; they can be summarised as: (a) cyclodehydration and elimination effected on substituted succinates (131a - 131c) [67,69,75] or cyanohydrins (132) [76] and (b) thermal rearrangement of acyclic anhydrides (133) [77], (see Scheme 32). The route that suited our needs for the target molecule was an appropriate Wadsworth-Emmons reaction [68] between an α-keto ester and the carbanion generated from a phosphonate (122) (see Scheme 30).

The problem was then reduced to a search for synthetic routes to α-keto esters. In the literature there were four useful routes to α-keto esters. These were: (a) reaction between acetylide anions (135) and an oxalate ester [60] (136), (b) Grignard reaction with acylimidazolides [61] (139), (c) condensations between dithiane anions (126) and alkyl or alkenyl bromide, followed by desulphurisation [52], and (d) thermal elimination of chloride ion and carboxyl group from hydroxydiacid (140) [62]. The route <u>via</u> the dithiane-carboxylate was chosen because the literature suggested high yields under mild reaction conditions which might favour the elaboration of the alkenyl side chain of the rethrolones. The route involving Grignard addition to acylimidazolides appeared in the literature when our work was in progress.



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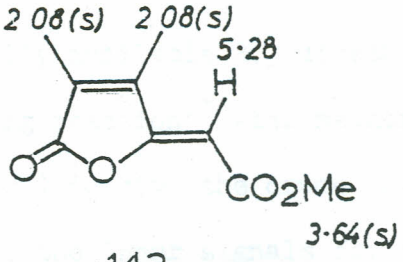

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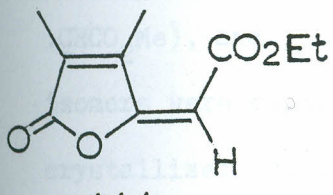

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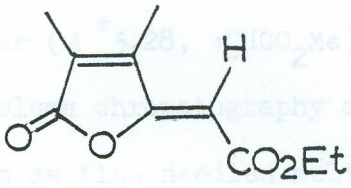

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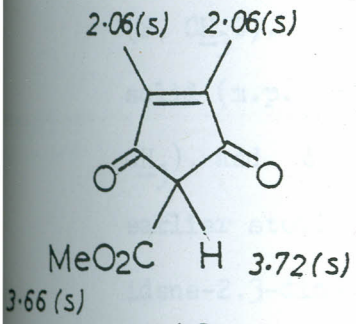

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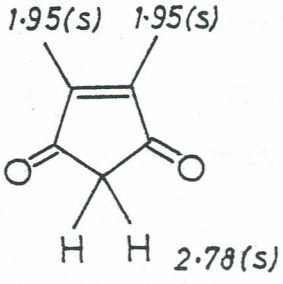

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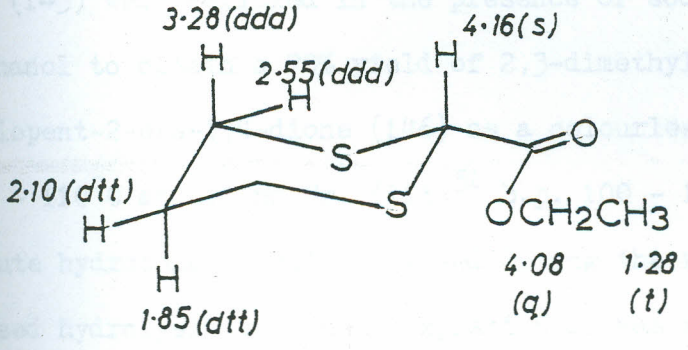
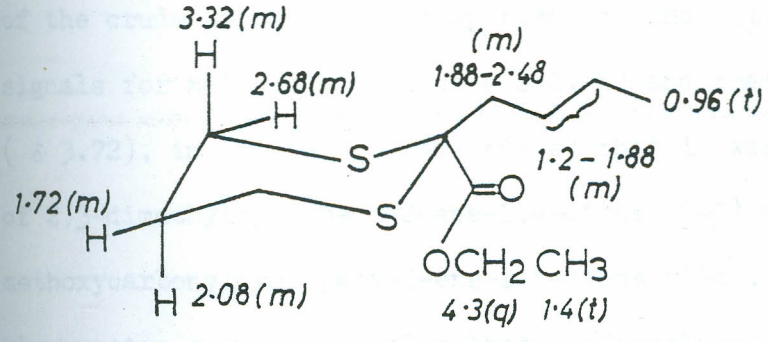


127



148 b

In a model study, readily available 2,3-dimethylmaleic anhydride was shown to undergo a Wittig reaction[55] with methoxycarbonylmethylenetriphenylphosphorane (110c) to give the crude butenolides (142 and 143); close analysis of the ^{1}Hnmr signals for olefinic protons showed the composition of the butenolides to be 50% (E) isomer (δ *5.8, :CHCO$_2$Me), and 50% (Z)-isomer (δ *5.28, :CHCO$_2$Me). The geometrical isomers were separated by column chromatography and the E-isomer (142) crystallised from chloroform as fine needles melting at (34 - 35°C); it showed ^{1}Hnmr signals at δ 1.92 (S, CH$_3$), δ 2.24 (S, CH$_3$) and δ3.64 (S, CH$_3$O). The Z-isomer (143) was also obtained as a low melting solid (m.p. 35°C) and showed the ^{1}Hnmr signals at δ 2.08 (S, 2 x CH$_3$), and δ 3.64 (S, CH$_3$O). These observations corroborated earlier studies with (E)- and (Z)-isomers of 4-ethoxycarbonylmethylidene-2,3-dimethylbut-2-enolide (144 and 145)[78].

For the rearrangement reaction both the (E) and (Z)-isomers (142) and (143) were refluxed in the presence of sodium methoxide in methanol to obtain a 70% yield of 2,3-dimethyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (146) as a colourless liquid boiling at 105 - 110°C at 0.1 mm Hg. (lit.[51] b.p. 100 - 105°C/ 0.05 mmHg). The dilute hydrochloric acid employed during the work-up of the reaction caused hydrolysis and decarboxylation as was evident from the signal at δ2.76 due to the methylene protons in (147) in the ^{1}Hnmr spectrum of the crude product. A comparison of the integral values of the signals for methylene protons (δ 2.76) and that of methine protons (δ 3.72), in the ester (146) showed that it was a 1 : 1 mixture of 2,3-dimethylcyclopent-2-ene-1,4-dione (147) and 2,3-dimethyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (146). In fact this observation gave us the clue that a "decarboxylation cocktail" involving water and chloride ion might be useful. Hence 2,3-dimethyl-

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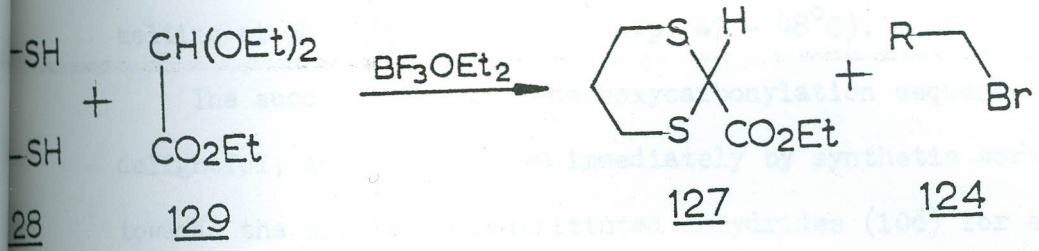
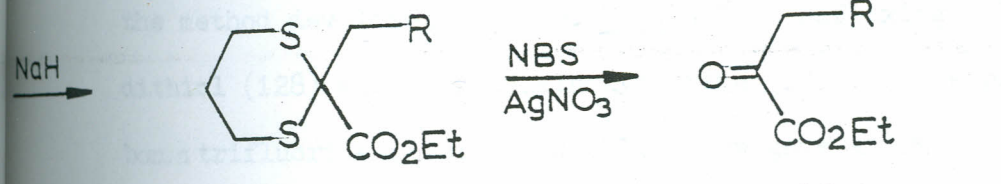
* All n.m.r. shifts are quoted in δ values measured from TMS as internal standard in dilute solutions of samples in deuterochloroform.





148

a, R = Et

b, R = nPr

c, R = nBu

d, R = vinyl

123

a, R = Et

b, R = nPr

c, R = nBu

d, R = vinyl

Scheme 34

5-methoxycarbonylcyclopent-2-ene-1,4-dione in aqueous dimethyl sulphoxide containing sodium chloride gave an 80% yield of 2,3-dimethylcyclopent-2-ene-1,4-dione, obtained as a colourless oil which solidified and recrystallised from pentane as cubes, melting at 34 - 35°C (lit.[63], m.p. 47 - 48°C).

The success of this demethoxycarbonylation sequence was quite delightful, and was followed immediately by synthetic work directed towards the suitably disubstituted anhydrides (106) for elaboration to the natural rethrolones.

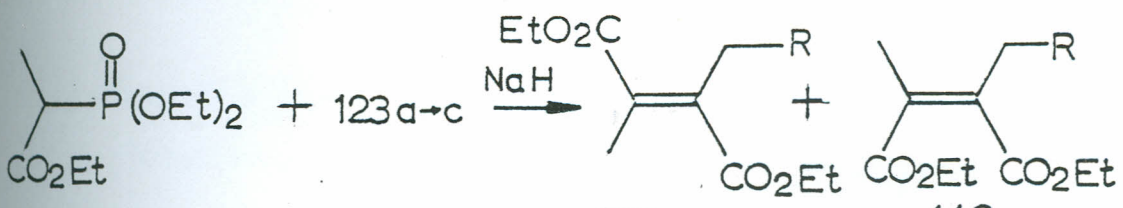
The α-keto esters (123a - 123c) were synthesised by following the method developed by Eliel et al.[52] Refluxing 1,3-propane dithiol (128) and ethyl diethoxyacetate (129) in the presence of boron trifluoride etherate in chloroform gave a 70% yield of an oil, ethyl 1,3-dithiane-2-carboxylate (127) which distilled at 106 - 107 / 1.5 mmHg (lit.[52], 75 -77°C / 0.2 mmHg). The dithiane had ^{1}Hnmr data[64] assigned in (127) given on page 37 while the ir spectrum had strong signals at 920 cm^{-1} for the dithiane moiety, besides the C = O signal at 1730 cm^{-1}.

The carbanion (126), generated with sodium hydride from the dithiane carboxylate in benzene and N,N-dimethylformamide, was successfully alkylated with a series of alkyl bromides (124a - 124c) and with allyl bromide (124d) at room temperature to afford the substituted dithianes (148a - 148c) and ethyl 2-allyl-1,3-dithiane-2-carboxylate (148d) as oils in 75 - 83% yield (see Scheme 34).

The oily alkyl dithianes prepared were: ethyl 1,3-dithiane-2-n-propyl-2-carboxylate (148a), ethyl 1,3-dithiane-2-n-butyl-2-carboxylate (148b), ethyl 1,3-dithiane-2-n-pentyl-2-carboxylate (148c) and ethyl 1,3-dithiane 2-allyl-2-carboxylate (148d), and they were purified by vacuum distillation at 90 - 100°C/3 mmHg, 100 - 110°C / 2 mmHg (lit.[52], b.p. 90 - 95°C / 0.1 mmHg), 110 - 115°C /

2 mmHg and 85 - 90°C / mm Hg respectively. The accurate mass measured confirmed their elemental composition, whereas their ir spectra had strong dithiane signals at 920 cm^{-1} besides bands at 1730 and 1030 cm^{-1} attributed to the ester group. Ethyl 1,3-dithiane-2-allyl-2-carboxylate had ^{1}Hnmr signals at δ 2.80, 5.96 and 5.24 confirming the presence of the allyl substituent, whereas its ir spectrum had an absorption at 1675 cm^{-1} attributed to the double bond. The alkyl substituted dithianes had characteristic dithiane signals in their ^{1}Hnmr spectra as well as the signals that were attributed to the alkyl groups, as exemplified for ethyl 1,3-dithiane-2-n-butyl-2-carboxylate (see 148b) on page 37.

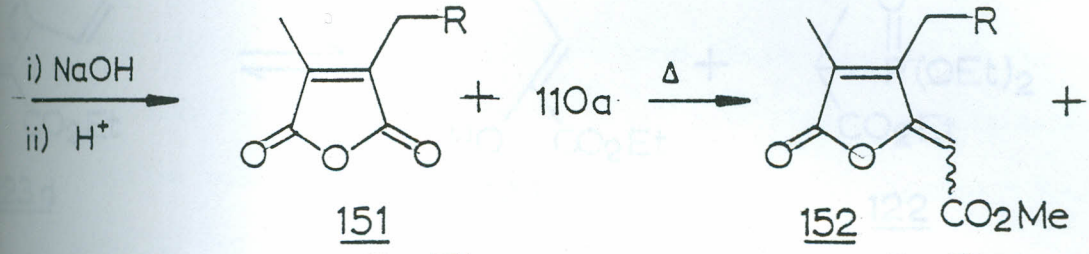
The dithianes were next treated with silver nitrate and N-bromosuccinimide in the absence of light to obtain the alkylated α-keto esters (123a - 123d) in 80 - 90% yields; the ethyl 2-allyl-1,3-dithiane-2-carboxylate (148d) was desulphurised to ethyl 2-oxo-pent-4-enoate (123d) in only 60% yield. The α-keto esters ethyl 2-oxopentanoate [66] (123a), ethyl 2-oxohexanoate (123b), ethyl 2-oxoheptanoate (123c) and ethyl 2-oxopent-4-enoate (123d) were purified by vacuum distillation at 50°C / 0.5 mmHg, 90 - 95°C / 15 mmHg, (Lit. [52] 80 - 92°C / 12 mmHg), 95 - 100°C / 15 mmHg (lit.[65], 54°C / 0.07 mmHg), and 40°C / 0.5 mmHg respectively. Once again the accurate mass measurements confirmed the molecular formulae of the α-keto esters, whereas the ir spectra had strong bands at 1730 cm^{-1} and 1040 cm^{-1} associated with their C = O and C - O groups respectively. Further evidence for these functionalities came from ^{1}Hnmr spectra, for example the underlined methylene protons in $CH_3\underline{CH_2}O$ and $CH_3CH_2\underline{CH_2}C{=}O$ found in ethyl 2-oxopentanoate resonated at δ 2.8(t) and at δ4.28(q) respectively. The ir spectrum of ethyl 2-oxopent-4-enoate had strong bands at 1675 and 1642 cm^{-1} associated with the double bonds which was also shown by the presence of signals for olefinic



122

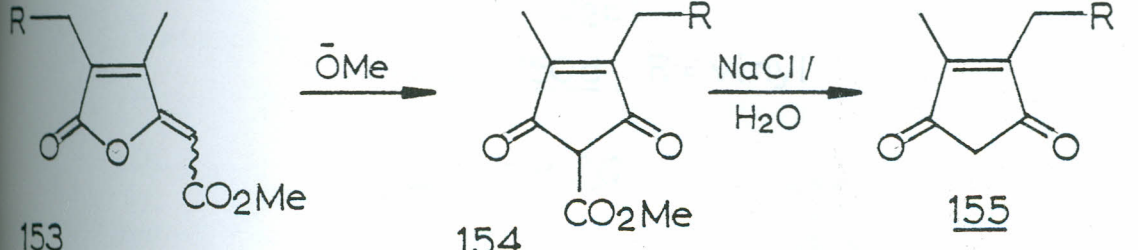
150
a, R = Et
b, R = nPr
c, R = nBu

149
a, R = Et
b, R = nPr
c, R = nBu



151
a, R = Et
b, R = nPr
c, R = nBu

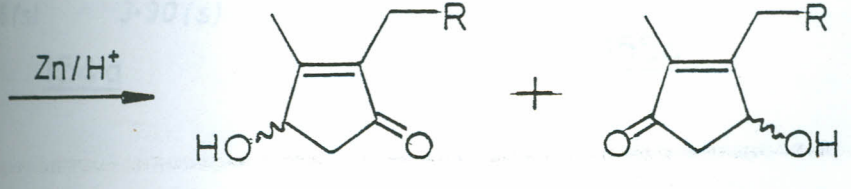
152
a, R = Et
b, R = nPr
c, R = nBu



153
a, R = Et
b, R = nPr
c, R = nBu

154
a, R = Et
b, R = nPr
c, R = nBu

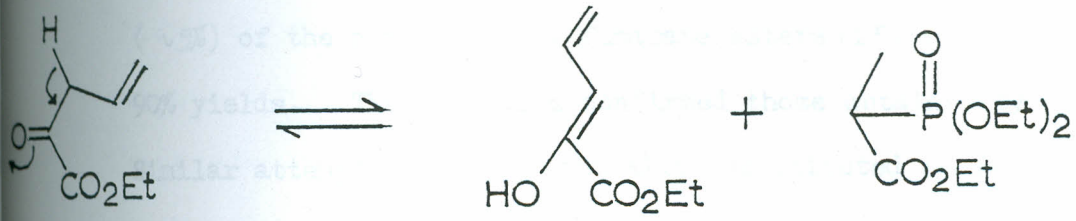
155
a, R = Et
b, R = nPr
c, R = nBu



15 a, R = Et
b, R = nPr
c, R = nBu

156 a, R = Et
b, R = nPr
c, R = nBu

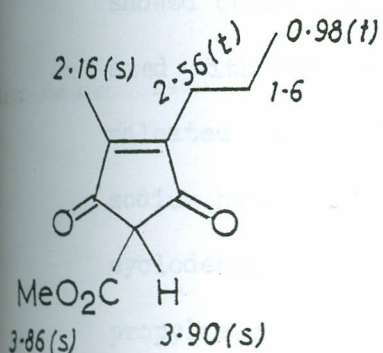
Scheme 35



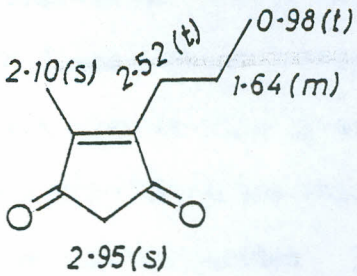
123d 123e 122

149d + 150d, R = vinyl

Scheme 36



154a



155a

protons at δ 5.88 (m, : CH-) and δ5.2 (m, : CH_2) in the ^{1}Hnmr spectrum.

Olefination of the alkyl substituted α-keto esters (123a - 123c) with diethyl 1-ethoxycarbonylethylphosphate (122) [53] in the presence of sodium hydride in 1,2 dimethoxyethane gave mainly the substituted maleate esters (149a - c) with negligible amounts (∿5%) of the corresponding fumarate esters (150 a - c), in 36 - 90% yields. These results confirmed those obtained by Sutherland[54]. Similar attempts to obtain the allyl substituted maleate ester (149d) failed because enolate formation from the ethyl 2-oxopent-4-enoate (123d) reduced its electrophilicity under base conditions (Scheme 36). The maleates, diethyl 3-methyl-2-n-propylmaleate (149a), diethyl 2-n-butyl-3-methylmaleate (149b) and diethyl 3-methyl-2-n-pentylmaleate (149c) were purified by distillation at 0.01 mmHg and collecting fractions boiling at 80 - 90°C, 98 - 100°C and 110 - 120°C respectively. The ir spectra had strong bands at 1730 cm^{-1} and 1650 cm^{-1} associated with α, β-unsaturated esters, and the ^{1}Hnmr spectra showed characteristic proton signals at δ 1.98 (S) and 2.32 (t) associated with the groups (CH_3-C=) and (-CH_2C=) respectively. The maleates (149a - c) were next treated with 2N-aqueous ethanolic sodium hydroxide, followed by acetic anhydride which effected cyclodehydration to produce the oily anhydrides 3-methyl-2-n-propylmaleic anhydride (151a) [69], 2-n-butyl-3-methylmaleic anhydrides (151b) [68] and 3-methyl-2-n-pentylmaleic anhydride (151c) in 47%, 75% and 86% yields respectively after column chromatographic purification. The ir spectra of the anhydrides showed strong bands at 1860, 1820, 1760 and 1690 cm^{-1} characteristic of α, β-unsaturated five membered ring anhydrides, and their ^{1}Hnmr spectra showed resonances at δ 2.08 (t) and at δ 2.52(t) associated with (-CH_3C:) and (-CH_2C:) respectively.

The Wittig reactions between the maleic anhydrides (151 a - c)

and methoxycarbonylmethylenetriphenylphosphorane (110) in refluxing chloroform resulted in the formation of 68 - 89% yields of mixtures of (Z)(major) and (E)-(minor) isomers of the ylidene-butenolides [55], as oils, which were purified by column chromatography; the (Z)- and (E)-isomers were easily separated. Inspection of the ^{1}Hnmr spectra of the crude products showed two signals for the olefinic protons; the higher field one was associated with the (Z)-isomer, and the lower field one was due to the (E)-isomer, and their integral ratios were used to determine the proportions of (Z) and (E) isomers. It was not possible to work out the regioisomers from the data available for the butenolides. The crude product for 3-methyl-2-n-propyl-4-methoxycarbonylmethylidene-but-2-enolide (152a or 153b) had olefinic proton signals at δ 5.52 (S) and 6.12 (S) indicating that both (Z) and (E) isomers had been produced in a 6 : 1 ratio. Spectra of products of 2-methyl-3-n-butyl-4-methoxycarbonylmethylidenebut-2-enolide (152b,153c) and 3-methyl-2-n-pentyl-4-methoxycarbonylmethylidenebut-2-enolides (152c, 153c) had olefinic proton signals at δ 5.44 (S), δ 5.6 (S) for the (Z)-isomers, and δ 5.94 (S) and δ 6.12 (S) for the (E)-isomers respectively. The integration ratio of the signals at δ 5.44 to δ 5.94, and that of the signals at δ 5.6 (S) to δ 6.12 (S) showed that the (Z)- to (E)-ratio of the butenolides (152b, 153b)(152c, 153c) was 3:1. These findings were comparable to those observed earlier[55,78]. The stability of the (Z)-isomer over the (E)-isomer is probably due to steric factors, since the methoxycarbonyl group in the (Z)-isomer experiences less interaction with the side chain substituents. The olefinic proton of the (E)-isomer also experiences more deshielding from the butenolide oxygen than the corresponding proton in the (Z)-isomer; hence the difference in their shift values. The ir spectra of the butenolides showed strong bands at 1780, 1720 and 1650 cm^{-1},

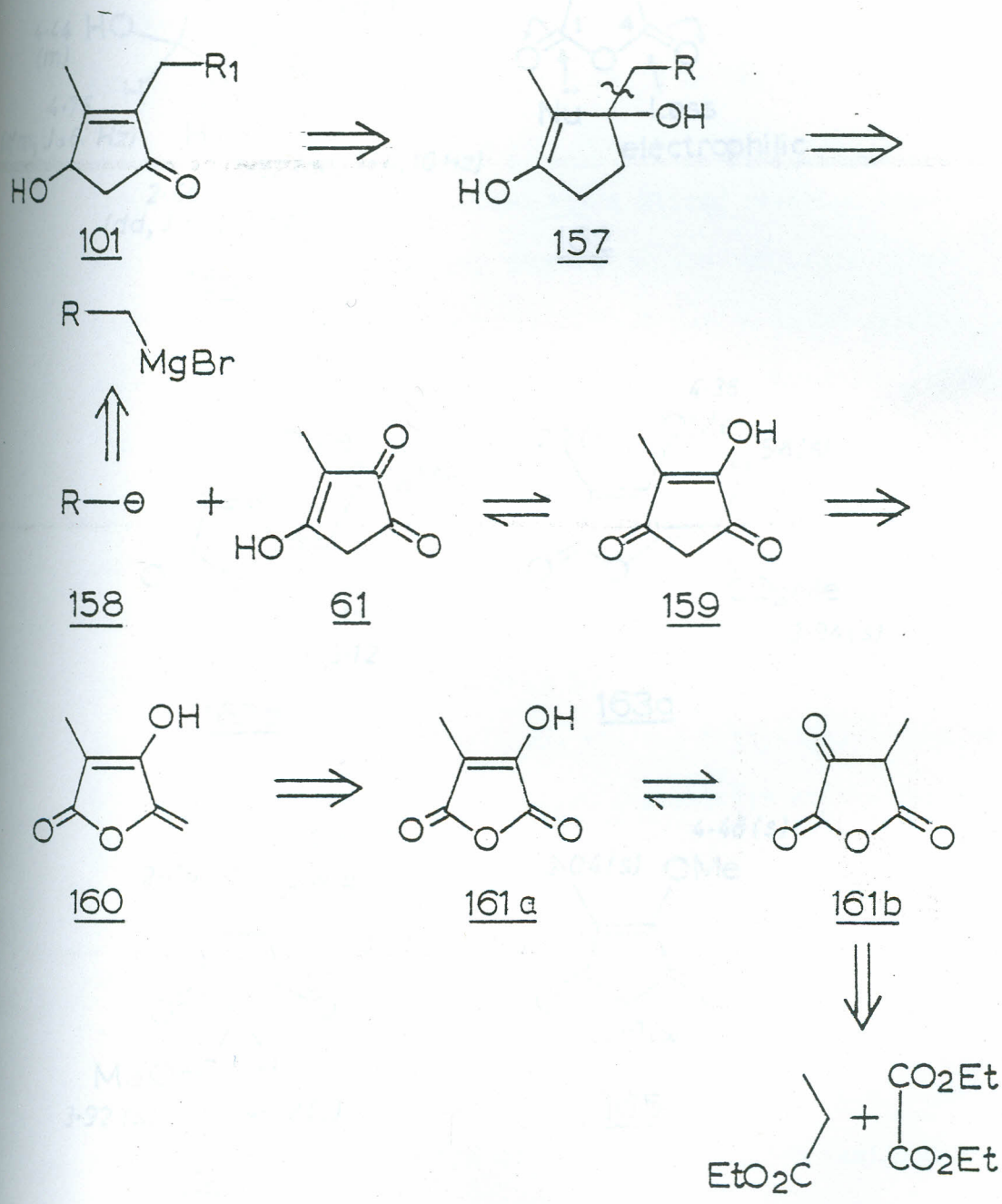
associated with enol- γ-lactones [55,78]. Both (E)- and (Z)-isomers of each of the butenolides were collectively treated with sodium methoxide in methanol at 0°C whereupon, warming to reflux, they rearranged to 3-methyl-2-alkyl-5-methoxycarbonylcyclopent-2-ene-1,4-diones (154a - c) in 70 - 80% yield. The diones showed absorption at 1760, 1730, 1700 and 1630 cm^{-1} in their infrared spectra, which were associated with the ester group and the α, β - unsaturated cyclopentenedione. Their ^{1}Hnmr spectra were homogeneous and the assignment of the proton signals is exemplified by 3-methyl-2-n-propyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione as shown in (154a) on page 43.

The esters (154a - c) were then demethoxycarbonylated, in the presence of sodium chloride on warming in aqueous dimethylsulphoxide, to obtain almost in quantitative yields 3-methyl-2-alkylcyclopent-2-ene-1,4-diones (155a - c) which were purified by column chromatography. The ir spectra of the samples had absorptions at 1745, 1700 and 1630 cm^{-1} associated with the presence of α, β - unsaturated five membered ring diones, and the accurate mass measurements confirmed the molecular formulae of the diones. The assignment of ^{1}Hmnr data can be exemplified for dihydroallethrone (155a) see page 43. The diones were analysed isothermally at 200°C by g.l.c. on both 10% SE 30 and 5% OV - 17 and were found to be homogeneous. Dihydroallethrone (155a) had the same retention time as an authentic sample on co-injection during the g.l.c. analysis. 2-Methyl-3-n-propylcyclopent-2-ene-1,4-dione(155a) and 2-n-butyl-3-methylcyclopent-2-ene-1,4-dione (155b) had ir, ms and ^{1}Hnmr data closely comparable to those published.[43, 70, 71]

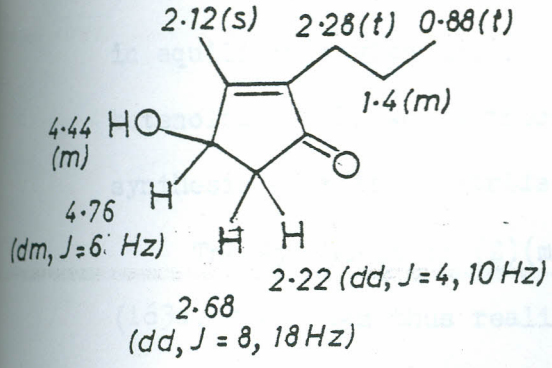
Reduction of the diones (155a - c), using zinc and acetic acid in dichloromethane, and following the work of Vandewalle et al.[45] was found to be regioselective, producing largely the racemic

mixture of dihydrorethrolones (15a - c) accompanied by small amounts (~10%) of the positional isomers (156a - c). The products were purified by column chromatography and found to be homogeneous on g.l.c. analysis (10% SE 30 and 5% OV - 17, column temperature 200°C), except for the peak that was shown to be the corresponding regioisomers of the dihydrorethrolones. Dihydroallethrolone (15a) had the same elution time as an authentic sample when co-injected under these g.l.c. conditions. The comparison of the ratio of integration values for signals for olefinic methyl protons of the two isomers also confirmed the g.l.c. composition percentage. For example, the ratio of integral value of singlet signal at δ 2.09 (major isomer 15c) to that at δ 1.76 (minor isomer 156c) was 20:1 as was observed for the reduction products from 2-methyl-3-n-pentylcyclopent-2-ene-1,4-dione (155c). The ratio of the integral value of methyl proton singlet signal at δ 2.12 (major isomer 15a) to that at δ 1.72 (minor isomer, (156a) was 9 : 1 for the reduction products of 3-methyl 2-n-propylcyclopent-2-ene-1,4-dione. It was a general observation that the selectivity during reduction of the diones improved with increasing length of the alkyl side chain. A typical ABX proton signal observed for the cyclopentenolones is illustrated for dihydroallethrolone (15a)(page 49). Finally the ir, ms and ^{1}Hnmr data observed for all the dihydrorethrolones were closely comparable to those already published [43,72].

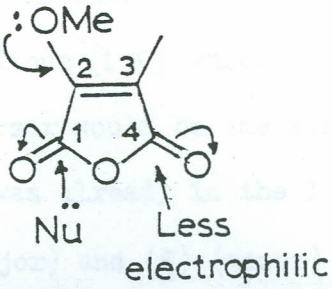
The failure of the original strategy to afford allyl substituted maleate (149, R = vinyl) prompted a further parallel investigation into the potential of maleic anhydrides in the synthesis of rethrolones. This parallel investigation involved the disconnection of the target molecule as illustrated in Scheme 37. In this strategy it was imagined that water attacked the double bond of the rethrolone (101) resulting into the alcohol (157) which is set for


R1
HO
O
101
R
OH
HO
157
R
MgBr
R—⊖
158
+
HO
O
O
61
OH
O
O
159
OH
O
O
160
OH
O
O
161a
O
O
O
161b
CO2Et
CO2Et
EtO2C
+

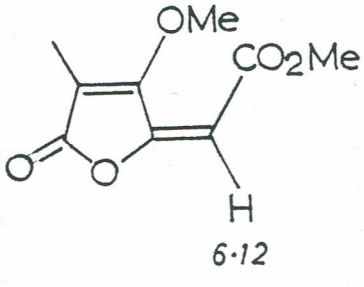
Scheme 37



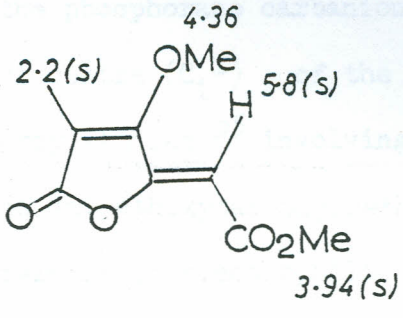
15a



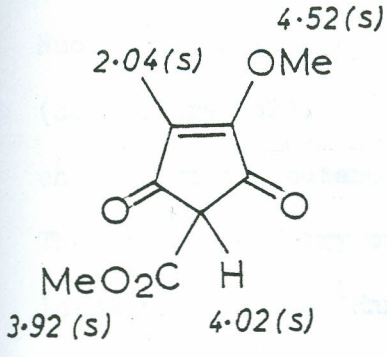
162



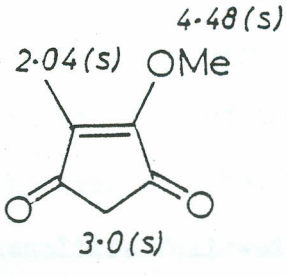
163b



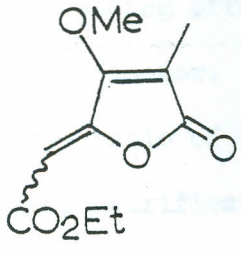
163a



164



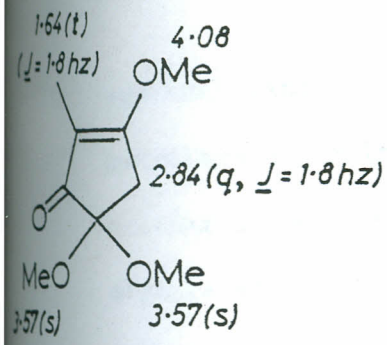
165



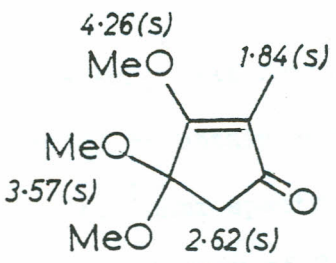
166

retroaldol process to obtain the dione (61). This 1,2-dione occurs in equilibrium with the 1,3 dione (159) which is isomeric to the butenolide (160) whose precursor would be the anhydride (161a - b). The synthesis of this anhydride was already in the literature [51].

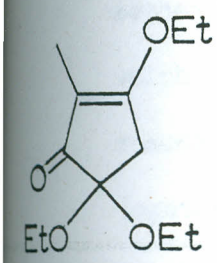
The synthesis of (Z)(major) and (E) (minor) 4-ylidenebutenolides (163a, 163b) was thus realised via a Wittig reaction between the readily available 2-methoxy-3-methylmaleic anhydride (162) [51] and methoxycarbonylmethylenetriphenylphosphorane in 88% yield. The regioselectivity of the attack of the phosphorane carbanion (110b) at the most electrophilic carbonyl centre (C_1-) of the anhydride (162) was inferred from the X-ray studies [55] involving (166); the reaction products between this anhydride and ethoxycarbonylmethylenetriphenylphosphorane (110c). The difference in electrophilicity of the two carbonyl centres is most likely due to facile electron donation from the oxygen atom of methoxy group via the 'vinylogous' bond towards C_4 other than C_1, hence reducing electrophilicity at the former carbon. Nucleophilic attack is therefore more favoured at C_1 than at C_4 (see figure 162). The infrared spectrum of the product showed characteristic butenolide absorptions at 1785, 1730, 1710 and 1650 cm^{-1}. The stereochemistry of the butenolides followed from a close inspection of the ^{1}Hnmr spectrum of the crude product which had olefinic proton resonances at δ 6.12 and δ 5.8 assigned to protons of (E) and (Z) isomers respectively. The butenolide oxygen seems to exert a more deshielding effect on the olefinic proton in the (E)-isomer than in the (Z)-isomer. Integration values of these resonances showed that the ratio of (Z) isomer to (E) isomer was 5 : 3. Column chromatographic purification of the crude product on silica gel using a chloroform-acetone mixture (4 : 1) as eluant separated the two isomers, but during this work-up the (E)-isomer isomerised into the more stable (Z) isomer, and this prevented acquisition of its



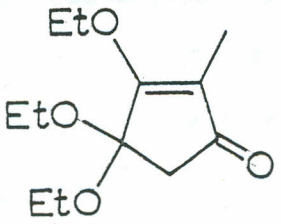
167



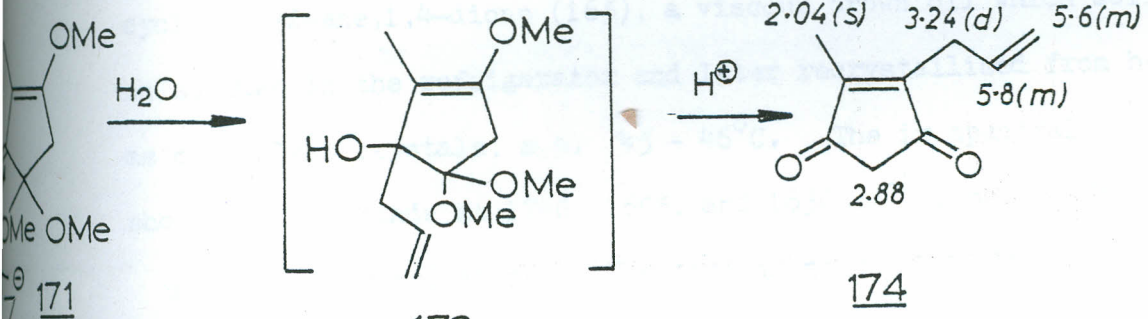
168



169



170



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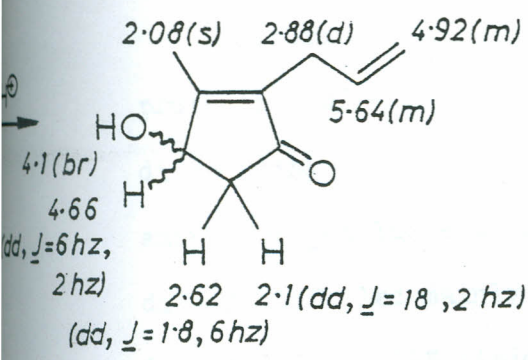
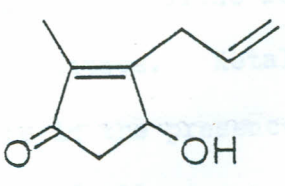


175



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Scheme 38

spectral data. The (Z)-isomer was recrystallised from benzene-petroleum ether (60 - 80°C) to obtain rod-like colourless crystals, melting at 40 - 42°C. The ^{1}Hnmr spectrum of the butenolide showed signals assigned for the (Z) isomer of 2-methyl-3-methoxy-4- methoxycarbonylmethylidenebut-2-enolide as illustrated in figure (163a).

The butenolide (163a) was then treated with sodium methoxide in methanol at 0°C, and on boiling to reflux it rearranged to 2-methoxy-3-methyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (164) which was obtained as an oil in 92% yield. The ^{1}Hnmr spectral resonances of the dione were as given in the figure (164), which together with ir and mass spectral data, were closely comparable to those in the literature [51]. Refluxing the methoxycarbonylated dione (164) with sodium chloride in aqueous dimethylsulphoxide [56] then resulted in the formation (86% yield) of 2-methoxy-3-methyl-cyclopent-2-ene,1,4-dione (165), a viscous brown oil which solidified on storage in the refrigerator and later recrystallised from hexane as colourless crystals, m.p. 45 - 46°C. The ir spectral data showed strong bands at 1740, 1695, and 1630 cm^{-1} expected for an α, β-unsaturated dione. The assignment of the ^{1}Hnmr signals is illustrated in the figure (165), and the accurate mass measured at 140.0477 (expected 140.0473) confirmed its molecular mass whereas the fragmentation pattern was similar to that published [73].

Elaboration of the side chain on the dione required selective protection of one of the carbonyl centres. Ketalisation of the dione using trimethyl orthoformate in the presence of a catalytic amount of p-toluenesulphonic acid and allowing methyl formate to distill off, led mainly to 2-methyl-3,5,5-trimethoxycyclopent-2-ene-1-one (167) in 74% yield. The sample was purified by distillation; gas liquid chromatographic analysis on 3% SE 30, running isothermally at 180°C gave two peaks whose ratio of their areas was

9: 1. The major isomer (167) was thus separated from the minor isomer (168). This ratio was also observed by comparing the integration values of methyl proton signal at δ 1.64 (t) (for major isomer, 167) and δ 1.84 (S) (for minor isomer 168). The ^{1}Hnmr data for the major isomer represented in figures (167) and (168) assigned with reference to parallel studies with the ethoxylated analogues (169, 170) [74]. The signal at δ 1.64, attributed to the methyl protons of (167), appeared as a triplet due to allylic splitting, whereas the signal at δ 1.84 due to methyl protons of (168), appeared as a broad singlet because the homoallylic splitting was not strong enough to be observed. The ir spectrum of the cyclopentenone had strong bands at 1690 and 1620 cm^{-1} usually expected for α,β-unsaturated five membered ring ketone. The distilled ketal was then allylated with allylmagnesium bromide (172) and dehydrated and hydrolysed in the presence of dilute sulphuric acid to obtain 2-methyl-3-allylcyclopent-2-ene-1,4-dione (174) in 54% yield. The distillate was homogeneous and had similar chromatographic properties to those of an authentic sample. The ir, ms and ^{1}Hnmr data were closely similar to those described in the literature [71]. The assignment of the ^{1}Hnmr spectral data is illustrated in figure(174). Reduction of 2-methyl-3-allylcyclopent-2-ene-1,4-dione (174), in the presence of zinc dust and acetic acid in dichloromethane at -10°C then gave an 80% yield of a racemic mixture of allethrolone(175), contaminated by about 5% of the corresponding regioisomer (176) as revealed by g.l.c. analysis. The major peak had the same elution time as the authentic sample. The ir, ms and ^{1}Hnmr data closely resembled those published [71]. The figure (175) shows the proton n.m.r. signals of the synthetic cyclopentenolone.

Chapter I

Experimental

Methoxycarbonylmethylenetriphenylphosphorane (110a). -

A mixture of triphenylphosphine (25 g) and methyl bromoacetate (15.98 g) in toluene (300 mls) was warmed at 70°C for 0.25 hr and then kept at 17°C for 2 hr. The crystalline salt (30.6 g, 71%) m.p.159°C (Lit.[80] m.p. 161°C) was filtered off and the crystals were then suspended in toluene (800 mls). Aqueous 0.38 M sodium hydroxide solution (800 mls) was added to this suspension and the mixture was then stirred until the two phases became clear. The aqueous phase was discarded while the toluene layer was dried over anhydrous sodium sulphate. Evaporation of the filtered toluene solution in vacuo left an amorphous powder which was recrystallised from a 1 : 1 mixture of ethyl acetate and petroleum ether (40 - 60°C) to give the phosphorane as colourless crystals (21.9 g, 67%), m.p. 161°C (Lit.[80] m.p. 162 - 163°C); ν max ($CHCl_3$), 1610, 1430, 890 cm^{-1}; δ 3.56 (S, OCH_3), 7.44 - 7.96 (m, phenyl and :CH); (m/e 334.1121, $C_{21}H_{19}O_2P$ M requires 334.1123).

2,3-Dimethyl-4-methoxycarbonylmethylidenebut-2-enolide (142 and 143). -

A solution of 2,3-dimethylmaleic anhydride (2.72 g) in dry chloroform (100 mls) was added dropwise to a solution of methoxycarbonylmethylenetriphenylphosphorane (6.77 g) in dry chloroform (100 mls), and the mixture was then refluxed in a stream of nitrogen for 16 hr. The solvent was evaporated at reduced pressure and the residue was then extracted successively with petroleum ether (40 - 60°C) (3 x 300 mls) and diethyl ether (2 x 100 mls) at boiling point. The organic extracts were combined and then cooled in ice whereupon a solid (2.6 g) deposited. The crude

mixture of solid butenolides was purified by chromatography on silica gel, using chloroform as eluant to give : a) the E-butenolide (142), (2.26 g, 46%), which crystallised from chloroform as colourless crystals m.p. 44.5°C; ν max ($CHCl_3$), 1785, 1730 and 1650 cm^{-1}; δ 3.64 (S, OMe), 5.8 (S, :CH), 2.24 (S, :CCH_3), 1.92 (S, :CCH_3); (m/e 182.0560, $C_9H_{10}O_4$ requires M 182.0580), and b) the Z-butenolide (143) 2.25 g, 46%), m.p. 35°C; ν max 1780, 1730, and 1650 cm^{-1}; δ 3.64 (S, OCH_3), 5.28(S, :CH), 2.08 (S, 6H, :CCH_3); m/e 182.0568, $C_9H_{10}O_4$ requires M 182.0580).

2,3-Dimethyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (146). -

A solution of 2,3-dimethyl-4-methoxycarbonylmethylidenebut-2-enolide (3.18 g) in dry methanol (100 mls) was added dropwise to a solution of sodium methoxide [from sodium (2.95)] in dry methanol (250 ml) at 4°C. The resulting orange mixture was stirred and refluxed for 0.5 hr before transferring it into ice-water (250 mls) where it was acidified with 2 M hydrochloric acid until the colour became pale yellow. The methanol was evaporated *in vacuo* and the aqueous residue was extracted with ether (3 x 300 mls). The ether layer was washed with brine (100 ml), and then dried over anhydrous sodium sulphate. Evaporation of the ether extract *in vacuo* left a yellow oil (2.25 g, 70%) which was purified by chromatography on silica gel, using 4% acetic acid in chloroform as eluant to give the dione as a colourless oil, b.p. 105 - 110°C/ 0.1 mm Hg. (Lit.[51] b.p. 100 - 105°C/0.05 mm Hg); ν max ($CHCl_3$) 1730, 1650, 1375, 1055 cm^{-1}; δ 2.06 (S, 2 x :CMe), 3.66 (S, OMe), 3.72 (S, -CH-); (m/e 182.0572, $C_9H_{10}O_4$ requires M 182.0580).

2,3-Dimethylcyclopent-2-ene-1,4-dione (147). -

2,3-Dimethyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (0.7 g) was added to dimethylsulphoxide (10 mls) containing sodium chloride (0.29 g) and water (0.2 g). The mixture was refluxed for 1 hr, after which time no more escaping carbon dioxide could be detected by precipitation in barium hydroxide solution. The mixture was diluted with water (20 mls), and then extracted with chloroform (3 x 50 mls). The solvent was evaporated, and the residue was extracted with boiling n-pentane (3 x 50 mls). The pentane extracts were dried over anhydrous sodium sulphate and then evaporated to leave pale yellow oil (0.48 g, 80%) which solidified on storage in the refrigerator. Recrystallisation from pentane gave the dione as cubic crystals, m.p. 34 - 35°C (Lit.[63], m.p. 47 - 48°C); ν max ($CHCl_3$) 1760, 1735, 1690, 1635, 1380 cm^{-1}; δ 1.95 (S, 2 x MeC:), 2.78 (S, $-CH_2-$); (m/e 124.0520, $C_7H_8O_2$ requires M 124.0524).

Ethyl 1,3-dithiane-2-carboxylate (127). -

A solution of 1,3-propanedithiol (11.45 g) and ethyl diethoxyacetate (18.23 g) in dry chloroform (20 mls) was added gently over a period of 18 minutes to a refluxing solution of boron trifluoride etherate (28.66 g) in dry chloroform (60 mls) under an atmosphere of nitrogen. The mixture was refluxed for 35 minutes, before cooling and washing successively with water (80 mls), 20% aqueous potassium carbonate (80 mls), and water (80 mls). The chloroform layer was dried over anhydrous magnesium sulphate and then evaporated under reduced pressure to leave a residue which distilled to give the dithiane as a

colourless oil (11.35 g, 70%), 106 - 107°C/ 1.5 mm Hg, (Lit.[52], b.p. 75-77°C at 0.2 mm Hg); ν max ($CHCl_3$) 1730, 1040, 920 cm^{-1}; δ 1.28 (t, J7*, $-CH_3$), 4.08 (q, J8, $-CH_2O$), 4.16 (S, -CH-) 3.16 - 3.48 (m, $-CH_2-S$), 2.40 - 2.68 (m, $-CH_2-S$), 1.84 - 2.20 (m, $-CH_2-$); (m/e 192.0278, $C_7H_{12}O_2S_2$ requires M 192.0279).

Ethyl 1,3-dithiane-2-alkyl-2-carboxylates (148a - d):

Typical procedure. - Ethyl 1,3-dithiane-2-(prop-2-enyl)-2-carboxylate (148). -

A solution of ethyl 1,3-dithiane 2-carboxylate (11.02 g) and 1-bromoprop-2-ene (8.33 g) in dry N,N-dimethylformamide (22 mls) was added dropwise to a stirred suspension of sodium hydride (4 g) in dry benzene (62 mls) under an atmosphere of nitrogen. The resulting yellow mixture was initially stirred for 1 hr at 0°C before allowing it to warm to room temperature where it was stirred for a further 17 hr. The mixture was then washed with water (2 x 100 mls), and the aqueous layer was further extracted with benzene (2 x 50 mls). The organic extracts were washed with saturated sodium chloride solution then dried over anhydrous magnesium sulphate, and evaporated to leave a yellow oil. Distillation gave the substituted dithiane (10.5 g, 79%) as an oil b.p. 85-90°C/ 0.2 mm Hg, ν max 1725, 1675, 1030, 920 cm^{-1}; δ 1.28 (t, J 7, $-CH_3$), 4.28 (q, J 7.5, $-CH_2-$), 5.24 (m, $:CH_2$), 5.96 (m, :CH-), 2.80 (d, J 8, $:CH-CH_2-$), 3.2 - 3.46 (m, $-CH_2-S$), 2.56 - 2.88 (m, $-CH_2-S$), 1.60 - 2.32 (m, 2 x $-CH_2-$); (m/e 232.0591, $C_{10}H_{16}O_2S_2$ requires M 232.0592).

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* Coupling constants are quoted in Hertz (Hz) throughout this thesis.

Ethyl 1,3-dithiane-2-(n-propyl)-2-carboxylate (148a). -

Following the typical procedure, ethyl 1,3-dithiane-2-carboxylate (4.12 g) reacted with 1-bromopropane (3.0 g) in the presence of sodium hydride (1.0 g) to yield a colourless oil (6.0 g, 82%), b.p. 90 - 100°C/ 3 mm Hg; ν max ($CHCl_3$) 1720, 920, 1030 cm^{-1}; δ 1.32 (t, J 7, $-CH_3$), 0.98 (t, J 7, $-CH_3$), 1.2 - 1.80 (m, $-CH_2-$), 1.8 - 2.40 (m, $-CH_2-$), 3.28 (m, $-CH_2-S$), 2.64 (m, $-CH_2-S$), 1.88 - 2.40 (m, $-CH_2-$), 4.20 (q, J 8, CH_2O); (m/e 234.0745, $C_{10}H_{18}O_2S_2$ requires M 234.0748).

Ethyl 1,3-dithiane-2-(n-butyl)-2-carboxylate (148b)[52]. -

Following the typical procedure a mixture of ethyl 1,3-dithiane-2-carboxylate (5.76 g) and sodium hydride (1.38 g) reacted with 1-bromobutane (4.11 g) to yield a liquid, which was distilled at 100 - 110°C/ 2 mmHg to obtain colourless oil (5.6 g, 75%), ν max ($CHCl_3$) 1730, 1030, 920 cm^{-1}; δ 1.40 (t, J 7.5, $-CH_3$), 0.96 (t, J 7, $-CH_3$), 4.30 (q, J 8, $-CH_2O$), 1.2 - 1.88 (m, 2 x $-CH_2-$), 1.88 - 2.48 (m, 2 x $-CH_2-$), 2.88 - 3.00 (m, $-CH_2-S$), 3.16 - 3.64 (m, $-CH_2-S$); (m/e 248.0903, $C_{11}H_{20}O_2S_2$ requires M 248.0905).

Ethyl 1,3-dithiane-2-(n-pentyl)-2-carboxylate (148c). -

Following the typical procedure, a mixture of ethyl 1,3-dithiane-2-carboxylate (4.35 g), sodium hydride (1.38 g) and 1-bromopentane (4.16 g) reacted to give a liquid, which was distilled at 110 - 115°C/ 2 mm Hg to obtain a colourless oil (4.9 g, 83%) ν max ($CHCl_3$) 1730, 1035, 920 cm^{-1}; δ 1.34 (t, J 7, $-CH_3$),

0.96 (t, J 7 $-CH_3$), 4.32 (q, J 8 $-CH_2O$), 3.12 - 3.6 (m, $-CH_2-S$), 2.52 - 2.84 (m, $-CH_2-S$), 2.02 - 2.52 (m, 2 x $-CH_2-$), 1.32 - 1.72 (m, 3 x $-CH_2-$); (m/e 262.1051, $C_{12}H_{22}O_2S_2$ requires M 262.1061).

Ethyl 2-oxopent-4-enoate (123d). -

A solution of ethyl 1,3-dithiane-2-(prop-2-enyl)-2-carboxylate (3.24 g) in acetonitrile (50 mls) was added dropwise to a stirred suspension of freshly recrystallised N-bromosuccinimide (8.21 g) and silver nitrate (8.77 g) in 80% aqueous acetonitrile (180mls)under an atmosphere of nitrogen in the dark. The mixture was stirred for two minutes at 0°C, then allowed to warm to room temperature where it was stirred for a further half hour. An equal mixture (200 mls) of chloromethane and hexane was then added to the reaction mixture, followed by successive addition of saturated solutions of sodium sulphate (10 mls), sodium carbonate (20 mls) and sodium chloride (10 mls). This mixture was stirred for 10 minutes, then filtered and the organic layer was separated and washed twice with water (100 mls) before drying over anhydrous magnesium sulphate. The solvent was evaporated *in vacuo* to leave a yellow oil which on distillation gave the α-keto ester as a colourless oil, b.p. 40°C at 0.5 mm Hg, (1.63, (60%), ν max ($CHCl_3$) 1725, 1675, 1640 and 1030 cm^{-1}; δ 1.28 (t, J 7, $-CH_2\underline{CH_3}$), 4.3 (q, J 8, $CH_3\underline{CH_2}O$) 3.60 (q, J 7, CH_2CO), 5.6 - 6.2 (m, $\underline{CH}$: CH_2), 5.1 - 5.4 (m, $:CH_2$), (m/e 142.0628, $C_7H_{10}O_3$ requires M 142.0630).

Ethyl 2-oxoalkanoates: Typical procedure. - Ethyl 2-oxopentanoate (123a). -

A solution of ethyl 1,3-dithiane-2-(n-propyl)-2-carboxylate (2.34 g) in acetonitrile (80 mls) was added dropwise over 5 min to a stirred mixture of silver nitrate (7.15 g) and N-bromosuccinimide (6.64 g) in 80% aqueous acetonitrile (150 mls) under nitrogen in the dark at 4°C. The mixture was stirred for 0.5 hr, then the temperature was allowed to rise to room temperature where the mixture was stirred for a further 2 hr. Water (100 mls) was added to the mixture, which was then extracted with chloroform (4 x 50 mls). The chloroform layer was washed successively with water (2 x 100 mls), 1% ammonium acetate solution (2 x 50 mls), water (2 x 50 mls) and finally saturated sodium chloride (50 mls). The organic layer was dried over anhydrous sodium sulphate and then evaporated in vacuo to leave an oil (1.3 g, 90%) which distilled to give the α-keto ester b.p. 50°C at 0.5 mm Hg; ν max (film) 1725, 1040 cm^{-1}; δ 0.92 (t, J 7.5, $CH_2\underline{CH_3}$), 1.36 (t, J 7 $OCH_2\underline{CH_3}$), 1.68 (sextet, J 7.5, $CH_2\underline{CH_2}CH_3$), 2.8 (t, J 8 $CH_2\underline{CH_2}CO$), 4.28 (q, J 7, $O\underline{CH_2}CH_3$); (m/e 144.0779, $C_7H_{12}O_3$ requires M 144.0787).

Ethyl 2-oxohexanoate (123b)[52]. -

Following the typical procedure desulphurisation of ethyl 1,3-dithiane-2-(n-butyl)-2-carboxylate (5 g) was performed in the presence of N-bromosuccinimide (13.5 g) and silver nitrate (13.0 g) suspended in 80% aqueous acetonitrile (240 mls) to yield the α-keto ester as an oil (2.72 g, 85%), b.p. 90 - 95°C at 15 mm Hg; ν max (film) 1730, 1040 cm^{-1}; δ 4.32 (q, J 7, $O\underline{CH_2}CH_3$), 2.84 (t, J 8, $CH_2\underline{CH_2}CO$), 1.16 - 1.8 (m, 7 H), 0.96 (t, J 7, $CH_2\underline{CH_3}$);

(m/e 158.0933, $C_8H_{14}O_3$ requires M 158.0943).

Ethyl 2-oxoheptanoate (123c)[65]. -

Following the typical procedure, ethyl 1,3-dithiane-2-(n-pentyl)-2-carboxylate (3.5 g) was desulphurised in the suspension of N-bromosuccinimide (6.75 g) and silver nitrate (6.8 g) in 80% aqueous acetonitrile (180 mls) to obtain a colourless oil (2.08 g, 90%), b.p. 95 - 100°C at 15 mm Hg; ν max (film) 1730, 1030 cm^{-1}; δ 4.28 (q, J 7, OCH_2CH_3), 2.8 (t, J 8, $-CH_2CH_2CO$), 1.12 - 1.8 (m, 9 H), 0.92 (t, J 7, CH_2CH_3); (m/e 172.1094, $C_9H_{16}O_3$ requires M 172.1095).

Triethyl α-phosphonopropanoate (122)[53]. -

A quarter of total amount of triethylphosphite (17.82 g) was added dropwise to ethyl 2-bromopropanoate(18.24 g) at room temperature. The mixture was heated to boiling before the addition of the rest of the triethylphosphite at a rate ensuring gentle reflux under nitrogen. The temperature of the reaction mixture was maintained at 150°C for 1.5 hr, and to abtain an oil (18.98 g, 80%) distilled at 90 - 100°C at 1 mm Hg, (Lit.[53] b.p. 93 - 95° at 0.85 mm Hg); ν max 2990, 1730, 1315 cm^{-1}; δ 2.92 (dq , J 24, 7 P-$CHCH_3$), 1.44 (d, J 7, P-CH-CH_3), 1.32 (t, J 7.5, 9 H), 4.04 (q, 6 H, CH_3CH_2O); (m/e 238, $C_9H_{19}O_5P$).

Diethyl 2-methyl-3-n-alkylmaleates: Typical procedure:-

Diethyl 3-methyl-2-n-propylmaleate (149a).-

A solution of triethyl α -phosphonopropanoate (1.46 g) in dry 1,2-dimethoxyethane (13 mls) was added gently to a stirred slurry of sodium hydride (0.37 g) in dry 1,2-dimethoxyethane (17 mls) under an atmosphere of nitrogen. When the evolution of hydrogen had ceased, ethyl 2-oxopentanoate (0.88 g) in dry 1,2-dimethoxyethane (5 mls) was added to the mixture, which was then stirred for 24 hr. Water (40 mls) was added to the reaction mixture, before it was extracted with diethyl ether(3 x 50 mls). The organic layer was washed with saturated solution of sodium chloride (20 mls), and then dried over anhydrous magnesium sulphate. Evaporation of the ether in vacuo left an oil, which distilled to give the maleate as a colourless oil(0.64 g, 46%), b.p. 80 - 90°C at 0.01 mm Hg; ν max 1770, 1730, 1650, 1040 cm^{-1}; δ 4.2 (q, J 8, 4 H, $CH_3\underline{CH_2}O$), 1.32 (t, J 7, 6 H, $\underline{CH_3}CH_2O$), 0.96 (t, J 7, $CH_2CH_2\underline{CH_3}$), 1.96 (S, CH_3C:), 2.32 (t, J 8, $CH_2\underline{CH_2}C$:), 1.12 - 1.4 (m, $\underline{CH_2}CH_3$); (m/e 228, $C_{12}H_{20}O_4$).

Diethyl 2-n-butyl-3-methylmaleate (149b).[54] -

Following the typical procedure, triethyl α -phosphonopropanoate (3.8 g) reacted in a Wadsworth-Emmons manner with ethyl 2-oxohexanoate (2.5 g) to give the maleate as an oil (1.40 g, 36%), b.p. 98 - 100°C at 0.01 mm Hg; ν max 1730, 1640, 1375, 1030 cm^{-1}; δ 4.20 (q, J 8, 4 H, $CH_2\underline{CH_2}O$), 2.32 (t, J 7, $CH_2\underline{CH_2}C$:), 1.94 (S, CH_3C:), 0.90 (t, J 7, $CH_2\underline{CH_3}$), 1.12 - 1.6 (m, 4 H, $\underline{CH_2CH_2}CH_3$), 1.32 (t, J 7, 6 H, $OCH_2\underline{CH_3}$); (m/e 242, $C_{13}H_{22}O_4$).

Diethyl 3-methyl-2-n-pentylmaleate (149c). -

Following the typical method, Wadsworth-Emmons reaction was effected between ethyl 2-oxoheptanoate (1.72 g) and triethyl α -phosphonopropanoate (2.38 g) in the presence of sodium hydride (0.48 g) in 1,2-dimethoxyethane (35 mls) to obtain an oil (1.5 g, 90%), b.p. 110 - 120°C at 0.01 mm Hg; ν max (film) 1730, 1640, 1030 cm^{-1}; δ 4.22 (q, J 8, 4 H, $CH_3\underline{CH_2}O$), 2.32 (t, J 7, $CH_2\underline{CH_2}C$:), 1.96 (S, CH_3C:), 1.2 - 1.6 (m, $\underline{CH_2CH_2CH_2}$), 1.36 (t, J 7, $\underline{CH_3}CH_2O$), 0.96 (t, J 7, $CH_2\underline{CH_3}$); (m/e 256, $C_{14}H_{24}O_4$).

2-Alkyl-3-methylmaleic anhydrides: Typical procedure:

3-Methyl-2-n-propylmaleic anhydride (151).-

A solution of diethyl 2-methyl-3-n-propylmaleate (0.5 g) in ethanol (4 mls) was added to 2N aqueous sodium hydroxide solution (2 mls) and the mixture was stirred for 7 hr. The mixture was diluted with water (8 mls), and then extracted with diethyl ether (4 x 10 mls). The ether layer was discarded, while the aqueous layer was acidified with dilute hydrochloric acid and then extracted with diethyl ether (5 x 15 mls). The ether layer was washed with saturated sodium chloride solution, and then dried over anhydrous magnesium sulphate. Evaporation of the ether in vacuo left the crude maleic acid (0.25 g, 74%) which was immediately added to anhydrous acetic anhydride (8.64 g) and heated under reflux for 15 hr. before evaporating to dryness. Chromatography of the residue on silica gel impregnated with formic acid and using benzene as eluant, gave the anhydride (0.17 g, 89%) as a colourless oil, b.p. 132 - 135°C at 15 mm Hg (Lit.[69] b.p. 131 - 133°C at 12 mm Hg), ν max (film) 1860, 1820, 1760, 1690 and 1030 cm^{-1}; δ 2.52 (t, J 7, $CH_2\underline{CH_2}C$:), 2.06 (S, CH_3C:) 1.28 (m, $-CH_2-$), 1.0 (t, J 7, $\underline{CH_3}CH_2-$); (m/e 154.0615, $C_8H_{10}O_3$ requires M 154.0617).

2-n-Butyl-3-methylmaleic anhydride (151b). -

Following the typical procedure, diethyl 2-n-butyl-3-methylmaleate (1.2 g) was hydrolysed with 2N sodium hydroxide solution (5 mls) in ethanol (10 mls), and cyclodehydrated in the presence of anhydrous acetic anhydride (8 g) and purified by chromatography to obtain an oil (0.44 g, 75%), b.p. 100 - 105°C at 0.1 mm (Lit.[68] b.p. 105 - 110°C at 0.2 mm Hg); ν max (film) 1860, 1820, 1760, 1730, 1620 and 1030 cm^{-1}; δ 2.08 (S, $:CCH_3$), 2.48 (t, J 7.5, $CH_2\underline{CH_2}C:$), 1.12 - 1.5 (m, $-\underline{CH_2CH_2}-$), 0.98 (t, J 7, $-CH_2\underline{CH_3}$); m/e 168.0787, $C_9H_{12}O_3$, requires M 168.0786).

3-Methyl-2-n-pentylmaleic anhydride (151c). -

Following the typical procedure, diethyl 3-methyl-2-n-pentylmaleate (1.28 g) was hydrolysed with 2N sodium hydroxide solution (5 mls) and cyclodehydrated in the presence of anhydrous acetic anhydride (9.0 g) and was chromatographically purified as described, to obtain a colourless oil (0.77 g, 88%), b.p. 110 - 115°C at 0.1 mm Hg; ν max 1860, 1820, 1760, 1730, 1690, 1030, and 930 cm^{-1}; δ 2.08 (S, $CH_3C:$), 2.52 (t, J 8, $CH_2\underline{CH_2}C:$), 1.12 - 1.5 (m, $\underline{CH_2CH_2CH_2}CH_3$) 0.96 (t, J 7, $CH_2\underline{CH_3}$); (m/e 182.0932, $C_{10}H_{14}O_3$ requires M 182.0943).

2-Alkyl-3-methyl-4-methoxycarbonylmethylidenebut-2-enolides: Typical procedure: - 3-Methyl-2-n-propyl-4-methoxycarbonylmethylidenebut-2-enolide (153a). -

A solution of 3-methyl-2-n-propylmaleic anhydride (0.16 g) in dry chloroform (10 mls) was added dropwise to a solution of methoxycarbonylmethylenetriphenylphosphorane (0.37 g) in dry chloroform (10 mls). The solution was heated under reflux and nitrogen for 20 hr, and the solvent was then evaporated in vacuo. The residue was extracted with boiling diethyl ether (3 x 20 mls). The solvent was evaporated in vacuo and the resulting residue was then chromatographed on silica gel and using 4 : 1 dichloromethane-hexane as eluant giving: a) Z-butenolide as an oil (0.13 g, 59%), ν max (film) 1780, 1720, 1650, 1615, 1060 and 1120 cm^{-1}; δ 3.9 (S, OCH_3), 5.52 (br, S, :CH), 2.12 (S, CH_3C:), 2.44 (t, J 8, $\underline{CH_2}CH_2C$:), 1.28 (m, $\underline{CH_2}CH_2C$:), 0.98 (t, J 7, $CH_2\underline{CH_3}$); m/e 210.0904, $C_{11}H_{14}O_4$ requires M, 210.0892), and , b) E-butenolide as an oil (0.02 g, 10%), ν max (film), 1780, 1720, 1650, 1610, 1060 and 1120 cm^{-1}; δ 3.92 (S, CH_3O), 6.12 (S, :CH), 2.04 (S, :CCH_3), 2.44 (t, J 8, $CH_2\underline{CH_2}C$:), 1.68 (m, $\underline{CH_2}CH_2C$:), 0.98 (t, J 7, CH_3); (m/e 210.0901, $C_{11}H_{14}O_4$ requires M 210.0892).

2-n-Butyl-3-methyl-4-methoxycarbonylmethylidenebut-2-enolide (153b). -

Following the typical procedure, a Wittig reaction between 2-n-butyl-3-methylmaleic anhydride (0.44 g) and methoxycarbonylmethylenetriphenylphosphorane (0.88 g) in refluxing chloroform (30 mls) under nitrogen gas for 20 hr and after usual work up and chromatography, gave the Z isomer of the butenolide as an oil (0.35 g, 59%), ν max (film) 1780, 1720, 1650, and 1380 cm^{-1}; δ 5.44 (br, S, :CH), 3.80 (S, OCH_3), 2.36 (t, J 8, $CH_2\underline{CH_2}C$:), 2.12 (S, CH_3C:), 1.12 - 1.6 (m, $\underline{CH_2CH_2}CH_3$), 0.88 (t, J 7, $CH_2\underline{CH_3}$); (m/e 224.1046, $C_{12}H_{16}O_4$ requires 224.1048), and the E-isomer of the butenolide as an oil (0.1 g, 17%), ν max (film) 1775, 1725, 1650 and 1385 cm^{-1}; δ 5.94 (S, :CH), 3.85 (S, OCH_3) 2.36(t, J7, $CH_2\underline{CH_2}C$:), 2.04 (S, CH_3C:), 1.12 - 1.6 (m, $\underline{CH_2CH_2}$), 0.88 (t, J 7.5, $CH_2\underline{CH_3}$); (m/e 224.1047, $C_{12}H_{16}O_4$ requires M 224.1048).

3-Methyl-2-n-pentyl-4-methoxycarbonylmethylidenebut-2-enolide (153c). -

Following the typical procedure, a Wittig reaction was effected between 3-methyl-2-n-pentylmaleic anhydride (0.66 g) and methoxycarbonylmethylenetriphenylphosphorane (1.24 g) in dry chloroform (35 mls) to give Z-and E-isomers of the butenolides (0.76, 87%) which were separated by chromatography as usual, to obtain: a) Z-isomer, an oil (0.6 g) ν max (film) 1780, 1720, 1650, 1380 cm^{-1}; δ 3.94 (S, OCH_3), 5.6 (S, :CH), 2.12 (CH_3C:), 2.44 (t, J 8, $CH_2\underline{CH_2}C$:), 1.12 - 1.7 (m, $\underline{CH_2CH_2CH_2}CH_3$) 0.96 (t, J 7, $CH_2\underline{CH_3}$); (m/e 238.1206, $C_{13}H_{18}O_4$ requires M

238.1205); b) E-isomer, an oil (0.16 g) ν max (film) 1780, 1720, 1630, 1385 cm^{-1}; δ 3.90 (S, OCH_3), 6.12 (S, :CH), 2.04 (S, CH_3C:), 2.44 (t, J 8, $CH_2\underline{CH_2}C$:), 1.12 - 1.7 (m, $\underline{CH_2CH_2CH_2}$), 0.96 (t, J 7, $CH_2\underline{CH_3}$), (m/e 238.1204, $C_{13}H_{18}O_4$ requires M 238.1205).

2-Alkyl-3-methyl-5-methoxycarbonylcyclopent-2-ene-1,4-diones: Typical procedure: - 3-Methyl-2-n-propyl-5-methoxycarbonyl-cyclopent-2-ene-1,4-dione (154a).-

A solution of 3-methyl-2-n-propyl-4-methoxycarbonyl-methylidenebut-2-enolide (0.13 g) in dry methanol (10 mls) was added to an ice-cooled solution of sodium methoxide [from sodium (0.05 g)] in dry methanol (15 mls) under an atmosphere of nitrogen. The resulting orange solution was refluxed for 1 hr then poured into iced-water (50 mls) where it was acidified to pH 1 with dilute hydrochloric acid. The turbid grey mixture was concentrated in vacuo to remove methanol, and the aqueous portion was then extracted with diethyl ether (3 x 20 mls). The ether layer was dried over anhydrous magnesium sulphate, then evaporated in vacuo to afford a yellow oil which was purified by chromatography on silica gel, using ethyl acetate as the eluant to give the dione as a colourless oil (0.11 g, 85%), νmax (film) 1745, 1700, 1630, 1600, 1380 and 1130 cm^{-1}; δ 3.86 (S, OCH_3), 3.90 (S, CH), 2.16 (S, CH_3C:), 2.56 (t, J 8, $CH_2\underline{CH_2}C$:), 1.60 (m, $\underline{CH_2}CH_2C$:), 0.98 (t, J 7, $CH_2\underline{CH_3}$); (m/e 210.0890, $C_{11}H_{14}O_4$ requires M 210.0892).

2-n-Butyl-3-methyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (154b).-

Following the typical procedure a solution of 2-n-butyl-3-methyl-4-methoxycarbonylmethylidenebut-2-enolide (0.16 g) underwent a smooth rearrangement in the presence of sodium methoxide [from sodium (0.05 g)] in dry methanol (20 mls), then worked up and chromatographed as usual, to obtain a colourless oil (0.12 g, 75%, ν max (film) 1700, 1630 and 1380 cm^{-1}; δ 2.04 (S, $:CCH_3$), 3.90 (S, -CH-), 2.48 (t, J 7.5, $CH_2\underline{CH_2}C:$), 1.4 (m, $\underline{CH_2CH_2}$), 0.90 (t, J 7 $CH_2\underline{CH_2}$), 3.88 (S, OCH_3); (m/e 224.1046, $C_{12}H_{16}O_4$ requires M 224.1048).

3-Methyl-2-n-pentyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (154c).-

Following the typical procedure, 3-methyl-2-n-pentyl-4-methoxycarbonylmethylidenebut-2-enolide (0.70 g) was rearranged in the presence of sodium methoxide from sodium metal (0.15 g) in dry methanol (30 mls), then worked up and chromatographed as usual to obtain a colourless oil (0.42 g, 70%), ν max (film) 1760, 1725, 1700, 1630, 1600, 1125 and 1385 cm^{-1}; δ 3.88 (S, OCH_3), 3.92 (S, -CH-), 2.16 ($:CCH_3$), 2.46 (t, J 8, $CH_2\underline{CH_2}C:$), 1.40 (m, $\underline{CH_2CH_2CH_2}CH_3$), 0.96 (t, J 7, $CH_2\underline{CH_3}$), (m/e 238.1221, $C_{13}H_{18}O_4$ requires M 238.1205).

3-Methyl-2-alkylcyclopent-2-ene-1,4-diones: Typical procedure: 3-methyl-2-n-propylcyclopent-2-ene-1,4-dione (155a). -

A mixture of 3-methyl-2-n-propyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (0.1 g), sodium chloride (0.08 g), and water (0.8 g) in dimethylsulphoxide (5 ml) was refluxed for 0.5 hr, until no more carbon dioxide was evolved. The mixture was diluted with water (2.4 mls) and extracted with chloroform (3 x 10 mls). The chloroform layer was separated and rinsed successively with water (10 mls), and a saturated solution of sodium chloride (10 mls) then dried over anhydrous magnesium sulphate. The extract was then evaporated *in vacuo* and the residue was purified on a silica gel column using ethyl acetate as solvent to obtain the dione as a colourless oil (0.06 g, 85%), ν max (film) 1740, 1700, 1635, and 1385 cm^{-1}; δ 2.95 (S, $OCCH_2CO$), 2.10 (S, CH_3C:), 2.52 (t, J 7.5, $CH_2\underline{CH_2}C$:), 1.64, (m, $CH_2\underline{CH_2}CH_3$), 0.98 (t, J 7, $CH_2\underline{CH_3}$); (m/e 152.0841, $C_9H_{12}O_2$ requires M 152.0837). The sample was found to be homogeneous and had the same elution time as the authentic dione on g.l.c. analysis under the following conditions: 10% SE 30 and 3% OV - 17, column temperature 200°C, flame ionisation detector and injector temperature 210°C and 200°C respectively, and nitrogen gas flow rate, 30 ml/min.

2-n-Butyl-3-methylcyclopent-2-ene-1,4-dione (155b).-

Following the typical procedure, a mixture of 2-n-butyl-3-methyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (0.11 g), sodium chloride (0.08 g), and water (0.8 g) in dimethylsulphoxide (5 ml) was refluxed to obtain the product, worked up and purified as usual to obtain the dione as a colourless liquid (0.07 g, 77%), ν max (film) 1745, 1705, 1635, 1605 and 1390 cm^{-1}; δ 2.08 (S, CH_3C:), 2.48 (t, J 8, $CH_2\underline{CH_2}C$:), 2.88 (S, $OCCH_2CO$), 1.40 (m, $\underline{CH_2}\underline{CH_2}CH_3$), 0.98 (t, J 7.5, $CH_2\underline{CH_3}$); (m/e 166.0993, $C_{10}H_{14}O_2$ requires M 166.0994). The sample was homogeneous on the g.l.c. conditions given under the typical procedure.

3-Methyl-2-n-pentylcyclopent-2-ene-1,4-dione (155c).-

Following the typical procedure, a mixture of 3-methyl-2-n-pentyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (o.4 g), sodium chloride (0.1 g) and water (1 g) in dimethylsulphoxide (5 mls) was refluxed, worked up and purified as usual to obtain the dione as a colourless oil (0.28 g, 93%), ν max (film) 1745, 1735, 1700, 1630, 1385 and 1020 cm^{-1}; δ 2.10 (S, CH_3C:), 2.54 (t, J 8, $CH_2\underline{CH_2}C$:), 2.95 (S, $OCCH_2CO$), 1.40 (m, $(CH_2\underline{CH_2}\underline{CH_2})$, 0.94 (t, J 7, $CH_2\underline{CH_3}$); (m/e 180.1149, $C_{11}H_{16}O_2$ requires M 180.1150).

2-Alkyl-3-methyl-4-hydroxycyclopent-2-en-1-ones: Typical procedure:- 4-Hydroxy-3-methyl-2-n-propylcyclopent-2-en-1-one (15a). -

Zinc dust (1.5 g) was gently added over a period of 5 minutes to a stirred solution of 3-methyl-2-n-propylcyclopent-2-ene-1,4-dione (0.89 g) and acetic acid (10 mls) in dichloromethane (20 mls) kept at -10°C, and the resulting mixture was stirred for 1.5 hr. The solvent was evaporated in vacuo and the residue was extracted with diethyl ether (2 x 50 mls) and filtered. The filtrate was washed successively with 20% sodium carbonate (30 mls), water (30 mls) and a saturated solution of sodium chloride (30 mls), before drying over anhydrous magnesium sulphate and filtered. The filtrate was evaporated in vacuo to give a residue which was chromatographed on silica gel, using ethyl acetate as eluant to obtain dihydroallethrolone as a colourless oil (0.6 g, 67%), ν max (film) 3450, 1710, 1650, 1620, 1600 and 1385 cm^{-1}; δ 2.12 (S, CH_3C:), 2.28 (t, J 8, $CH_2\underline{CH_2}C$:), 1.40 (m, $CH_2\underline{CH_2}CH_3$), 0.88 (t, J 7.5, $CH_2\underline{CH_3}$); 4.44 (br, s, O - H), 4.76 (dd, J 5, 2, CHOH), 2.68 (dd, J 20, 5, CHCO), 2.22 (dd, J 20, 2, CHCO); (m/e 154.0992, $C_9H_{14}O_2$ requires M 154.0994). Gas liquid chromatographic (g.l.c.) analysis of the sample gave two peaks in the ratio of 9 : 1. A co-injection of the sample with authentic dihydroalletholone gave the same result under the following g.l.c. conditions: 10% SE 30 on diatomite, column temperature 200°C, flame ionisation detector and injector temperature 210°C and 200°C respectively and nitrogen gas flow rate, 30 ml/min.

2-n-Butyl-4-hydroxy-3-methylcyclopent-2-en-1-one (15b). -

Following the typical procedure, 2-n-butyl-3-methyl-cyclopent-2-ene-1,4-dione (0.06 g) was reduced in the presence of zinc dust (0.2 g) and acetic acid (10 mls) in dry dichloro-methane (20) kept at -10°C to obtain the product which was worked up and purified as usual to obtain dihydrocinerolone as an oil (0.05 g, 82%), ν max (film) 3600, 1700, 1650 and 1380 cm^{-1}; δ2.09 (S, CH_3C:), 2.18 (t, J 8, $CH_2\underline{CH_2}C$:), 1.33 (m, $\underline{CH_2}\underline{CH_2}CH_3$) 0.9 (t, J 7, $CH_2\underline{CH_3}$), 4.85 (br. s, OH), 4.73 (dd, J 5, 2, CHOH), 2.79 (dd, J 20, 5, CHCO), 2.27 (dd, J 20, 2, CHCO); (m/e 168.1140, $C_{10}H_{16}O_2$ requires M 168.1150). G.l.c. analysis gave two peaks in the ratio of 10 : 1.

4-Hydroxy-3-methyl-2-n-pentylcyclopent-2-en-1-one (15c). -

Following the typical procedure, 2-n-pentyl-3-methylcyclo-pent-ene-1,4-dione (0.1 g) was reduced in the presence of zinc powder (0.35 g) and acetic acid (10 mls) in dry dichloromethane (20 ml) kept at -10°C to obtain the product which was worked up and purified as usual to obtain dihydrojasmololone as an oil (0.07 g, 75%), ν max (film) 3600, 1710, 1650, 1390 cm^{-1}; δ2.09 (S, CH_3C:), 2.17 (t, J 8, $CH_2\underline{CH_2}C$:), 1.34 (m, $CH_3\underline{CH_2}\underline{CH_2}\underline{CH_2}$), 0.88 (t, J 7, $CH_2\underline{CH_3}$), 4.85 (br.s, OH), 4.75 (dd, J 5, 2, CHOH), 2.78 (dd, J 20, 5, CHCO), 2.28 (dd, J 20, 2, CHCO); m/e 182.1283, $C_{11}H_{18}O_2$ requires M 182.1306); g.l.c. analysis gave two peaks in the ratio of 20 : 1.

2-Methoxy-3-n-methylmaleic anhydride (162). -

Following Schreiber's work [55], a condensation reaction between ethyl propanoate (10 g) and diethyl oxalate (14.6 g) in the presence of sodium ethoxide [from sodium metal (2.3 g) in ethanol (50 ml)] in refluxing xylene (150 mls) over a period of 3 hr. gave an adduct which was acidified with dilute sulphuric acid and extracted into xylene.

The xylene extract was rinsed with 20% potassium bicarbonate solution and dried over magnesium sulphate. The solvent was removed in vacuo to leave a residue which was cyclodehydrated with concentrated sulphuric acid (9 g) at room temperature over a period of 48 hr to obtain 2-methyl-3-oxosuccinic anhydride (146), crystallised from benzene as plates (8 g, 64%) m.p. 112 - 113 °C (Lit.[55] m.p.113°C), ν max (KBr), 1850, 1775 and 1715 cm^{-1}, The plates (7 g) were methylated with dimethyl sulphate (10.3 g) in the presence of potassium carbonate (10.3 g) in acetone (150 mls) at room temperature for 3 hr to obtain the anhydride, which crystallised from methanol as colourless plates (7 g, 78%), m.p. 45°C (Lit.[55] m.p. 44.5 - 45°C); νmax (KBr) 1850, 1775, 1675 cm^{-1}; δ 4.26 (S, OCH_3), 1.07 (S, $:CCH_3$); (m/e 142.0264, $C_6H_6O_4$ M, requires 142.0266).

2-Methyl-3-methoxy-4-methoxycarbonylmethylidenebut-2-enolide (163a). -

A solution 2-methoxy-3-methylmaleic anhydride (1.78 g) in dry chloroform (65 mls) was added dropwise to a solution of methoxycarbonylmethylenetriphenylphosphorane (4.2 g) in dry chloroform (65 mls) under nitrogen gas. The resulting solution was refluxed for 16 hr and the solvent was evaporated in vacuo to leave a residue which was successively extracted with petroleum ether (40 - 60°C) (3 x 200 mls) and diethyl ether (2 x 100 mls) at boiling point. The organic extracts were combined and then cooled in ice where upon a solid (2.6 g) deposited. The crude mixture of solid butenolides was purified by chromatography on silica gel, using chloroform/acetone (4 : 1) as eluant to give the Z-butenolide (2.17, 88%); m.p. 40 - 42°C; ν max (KBr) 1785, 1730, 1710, 1650, 1390 and 1020 cm^{-1}; δ 2.20 (S, CH_3C:), 4.36 (S, OCH_3), 3.94 (S, CO_2CH_3), 5.8 (s, :CH); (m/e 198.0537, $C_9H_{10}O_5$ requires M 198.0528); the E isomer (δ 6.12 S, :CH) isomerised to the more stable Z-isomer during the chromatographic work up.

2-Methoxy-3-methyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (164). -

A solution of 2-methoxy-3-methyl-4-methoxycarbonylmethylidene-but-2-enolide (1.2 g) in dry methanol (50 ml) was added dropwise into a solution of sodium methoxide (from sodium metal 2.5 g) in dry methanol (120 mls) at 0°C. The resulting orange solution was refluxed for 1.5 hr before transferring it

into ice-water (250 ml) where it was acidified with 2M hydrochloric acid until the colour became pale yellow. The methanol was evaporated *in vacuo* and the aqueous residue was extracted with diethyl ether (3 x 300 mls). The ether layer was washed with brine (100 mls) and then dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated *in vacuo* to obtain an oily residue which was chromatographed on silica gel using 4% formic acid in chloroform as the eluant to obtain a pale yellow oil (1.11 g, 92%), ν max (film) 1760, 1730, 1695, 1625, 1385, 1140 cm^{-1}; δ 2.04 (S, CH_3C:), 4.25 (S, OCH_3), 3.92 (S, CO_2CH_3), 4.02 (S, CH); (m/e 198.0528 $C_9H_{10}O_5$ requires M 198.0528).

2-*Methoxy-3-methylcyclopent-2-ene-1,4-dione* (165). -

A solution of 2-methoxy-3-methyl-5-methoxycarbonylcyclopent-2-ene-1,4-dione (1.1 g) in dimethylsulphoxide (5 mls) was added to a mixture of sodium chloride (0.3 g) and water (0.25 g) in dimethylsulphoxide (10 mls). The mixture was refluxed for 1.5 hr after which time no more escaping carbon dioxide was detected by precipitation in barium hydroxide solution. The mixture was diluted with water (30 mls) and then extracted with chloroform (3 x 50 mls). The organic layer was then evaporated *in vacuo* to dryness and the residue was extracted with boiling *n*-pentane (3 x 50 mls). The pentane extracts were dried over anhydrous sodium sulphate and then evaporated *in vacuo* to give an oil which solidified and recrystallised from hexane as colourless rods (0.62 g, 86%) m.p. 45 - 46°C, νmax ($CHCl_3$),

1740, 1695, 1630, 1600, 1385, 1340 and 1160 cm^{-1}; δ 2.04 (S, CH_3C:), 4.48 (S, OCH_3), 3.0 (S, CH_2), (m/e 140.0477 $C_7H_8O_3$ requires M 140.0473).

2-Methyl-3,5,5-trimethoxycyclopent-2-en-1-one (167). -

A solution of 2-methoxy-3-methylcyclopent-2-ene-1,4-dione (0.6 g) and trimethyl orthoformate (0.84 g) with a catalytic amount of p -toluenesulphonic acid monohydrate (0.005 g) in dry methanol (20 mls) was refluxed at bath temperature, 100°C for 1.5 hr and allowing methyl formate (2 mls) to distill off. The methanol was evaporated in vacuo to leave a thick oily residue which was extracted with chloroform (2 x 30 mls) and washed with 10% sodium carbonate solution (10 mls). The organic layer was dried over anhydrous magnesium sulphate and the filtrate was evaporated in vacuo to yield a yellow oily residue. The oil distilled at 80 - 90°C and 1 mm Hg to obtain a colourless oil (0.64 g, 74%) which was then purified by g.l.c. (3% SE 30, column temperature 180°C, nitrogen gas flow 30 ml/min) to obtain the major and required product as an oil (0.50 g, 63%) ν max (film) 1690, 1620, 1380, 1150 and 1050 cm^{-1}, δ 4.08 (S, $:COCH_3$), 3.57 (S, 6H, OCH_3), 2.84 (q, J 1.80, CH_2), 1.64 (t, J 1.80, CH_3C:); (m/e 1860.0890, $C_9H_{14}O_4$ requires M 186.0892); the minor fraction (0.1 g, 10%) was thought to be the wrong isomer (168) ν max (film) 1685, 1630, 1385, 1150 and 1030 cm^{-1}; 4.26 (S, $:COCH_3$), 1.84 (S, CH_3C:), 2.62 (S, CH_2), 3.57 (S, 6 H, OCH_3); (m/e 186.0889, $C_9H_{14}O_4$ requires M 1896.0892).

3-Methyl-2-(prop-2-enyl)cyclopent-2-ene-1,4-dione (174). -

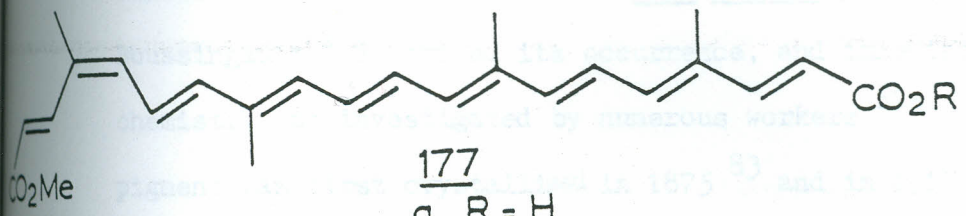
A solution of 2-methyl-3,5,5-trimethoxycyclopent-2-en-1-one (0.32 g) and allyl bromide(2.42 g) in dry diethyl ether (20 mls) was added portionwise to a suspension of magnesium turnings (0.49 g) in dry ether (15 mls), stirred under nitrogen gas. The mixture was heated under reflux for 6 hr and then stirred at room temperature for a further 12 hr. The resulting solution was acidified with 1 N H_2SO_4 (20 mls), and diluted with water (40 mls), then the ether layer was separated. The aqueous layer was further extracted with diethyl ether (3 x 50 mls). The combined ether extracts were washed with saturated sodium chloride solution (2 x 20 mls) and dried over anhydrous magnesium sulphate and filtered. The filtrate was then evaporated *in vacuo* to leave a yellow oily residue which was purified by distillation at 100 - 110°C and 0.1 mm Hg (Lit.[71] b.p. 94°C/ 0.05 mm Hg) to give a pale yellow oil (0.14 g, 54%) ν max (film) 1705, 1640, and 1385 cm^{-1}; δ 2.04 (S, CH_3C:), 2.88 (S, $OCCH_2CO$), 3.24 (d, *J* 7, :$CHCH_2$), 5.80 (m, :CH), 5.6 (m, :CH_2); (m/e 150.0682, $C_9H_{10}O_2$ requires *M* 150.0681); the sample was homogeneous and had the same elution time as the authentic sample on the following g.l.c. conditions: 5% OV - 17 on Chromosorb W, and 3% SE 30 on diatomite, column temperature 202°C, and carrier gas (N_2) flow rate; 30 ml/min, flame ionisation detector and injector temperature 210°C and 202°C respectively.

4-Hydroxy-3-methyl-2-(prop-2-enyl)cyclopent-2-en-1-one (175). -

Allethrone (0.05 g) was dissolved in dry dichloromethane (10 mls) and added to a suspension of zinc dust (0.1 g) and acetic acid (2 ml) in dry dichloromethane (15 mls) under nitrogen gas. The mixture was stirred at -10°C for 2 hr and the solvent was evaporated *in vacuo* to dryness. The residue was extracted with ether (2 x 50 mls). The ether filtrate was washed with 10% sodium carbonate solution (20 mls) and dried over anhydrous magnesium sulphate and filtered. The filtrate was then evaporated *in vacuo* to leave a residue which was purified by column chromatography on silica gel, using ethyl acetate as the solvent to obtain a pale yellow oil (0.045 g, 80%), ν max (film) 3600, 2900, 1720, 1650, and 1375 cm^{-1}; δ2.08(S, CH_3C:), 2.88 (d, *J* 7, :CHCH_2), 4.92 (m, :CH_2), 5.64 (m, :CH), 4.10 (br, S, OH), 4.66 (dd, *J* 6, 2, CHOH), 2.62 (dd, *J* 18, 6, CHCO), 2.10 (dd, *J* 18, 2, CHCO); (m/e 152.0835, $C_9H_{12}O_2$ *M* requires 152.0837). The sample was homogeneous and had the same elution time as the authentic sample on the following g.l.c. conditions: 3% SE 30 on diatomite, column and injector temperature, 202°C, the flame ionisation detector temperature, 210°C and carrier gas (N_2) flow rate, 30 ml/min.

Chapter II

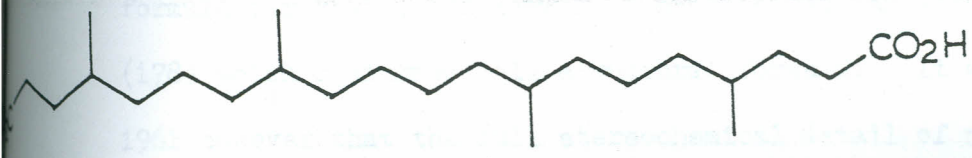
Terpenoids and Carotenoids of *Bixa orellana*



177

a R = H

b R = Me



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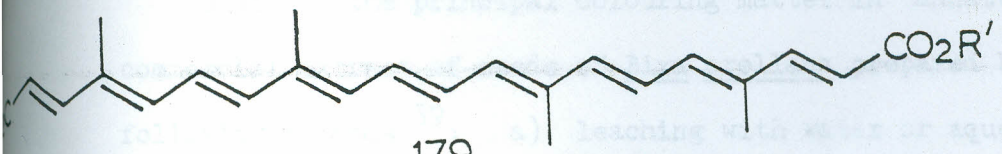
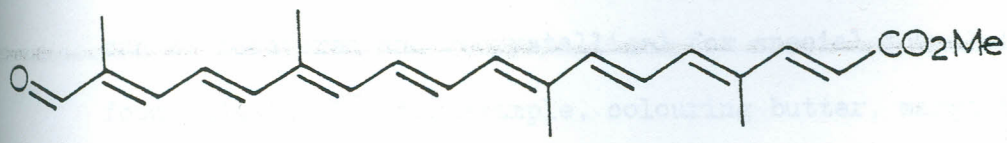


179

a R = H, R' = Me

b R = Me, R' = Me



180

2.1 Introduction:

Bixin is a C_{25}-apocarotenoid, methyl hydrogen 9'-cis-6,6'-diapocarotene-6,6'-dioate (177a) which is found as the major carotenoid pigment in fruits of Bixa orellana. In 1825 Boussingault[81] described its occurrence, and immediately its chemistry was investigated by numerous workers [82a - g]. The pigment was first crystallised in 1875 [83] and in 1917 Heiduschka and Panzer [84] carried out a careful elemental analysis, which provided the empirical formula, $C_{25}H_{30}O_4$. During the period 1928 - 1933 Kuhn and his co-workers [85] arrived at a structural formula for bixin, and Karrer [86] synthesised perhydronorbixin (178) which confirmed this structural formula. It was not until 1961 however that the full stereochemical detail of natural bixin was completely established by studies of its proton n.m.r. spectrum[87] and the 9'-cis stereochemistry was vindicated by a total synthesis reported in 1970 [88].

Bixin is the principal colouring matter in "annatto", the commercial extract of seeds of Bixa orellana prepared by one of the following methods [89]: a) leaching with water or aqueous alkaline solution; b) extraction with edible oils or alkaline propylene glycol or volatile solvents such as acetone, methanol or chlorinated alkanes; c) esterification, followed by extraction with solvents or edible oils. From any of these crude extracts, bixin can be recovered and recrystallised for special formulation in the food industry [89], for example, colouring butter, margarine, cheese, ice-cream, bakery products and edible oils. Other minor uses [89] include colouring rice, gravies and stews by people in Latin America, decoration of pots and repulsion of insects by indigenous Brazilians, and colouring of floor-wax, furniture and shoe polishes, nail gloss, brass lacquer, hair oil and wood stains by some

ethnic groups in the Philipines.

It has been demonstrated that "annatto" elicits cytological staining properties [90], pro-vitamin A activity [91] and inhibition [92] of growth of Clostridium botulinum besides having medicinal and cosmetic properties [93].

Bixa orellana is the only species of the family Bixeae and is cultivated [94] in Bolivia, Brazil, Sri Lanka, Dominica Republic, Ecuador, Guyana, India, Jamaica, Mexico, Angola, Kenya and Tanzania. These countries export most of their products as "annatto" to Britain, United States of America, Denmark, Netherlands and New Zealand.

The economic importance and the wide ranging biological activity of "annatto" have contributed to an immense amount of interest in the chemistry of bixin. The isolation of a more stable carotenoid having a higher wavelength maximum than that of bixin from seeds of Bixa orellana, by Herzig and Faltis [95] in 1913 triggered the detailed stereochemical studies on bixin. Karrer and his collaborators [96] coined 'labile bixin' for the previously known bixin and 'stable bixin' for the latter one, and suggested that the two bixins had a cis-trans relationship. This work was followed by stereomutation studies [97] on 'labile bixin' (177a) in the presence of iodine which produced 'stable bixin' (179a). It was then accepted that 'stable bixin' had the all-trans configuration while 'labile bixin' had a mono-cis configuration. The intriguing question which took many years to solve was, where was the cis double bond positioned in the polyene chain of bixin?

Carefully controlled potassium permanganate oxidation [98] of 'labile bixin' and 'stable bixin' produced a different apo-1-norbixinal methyl ester (methyl 8'-oxo-6,8'-diapocaroten-6-oate)*

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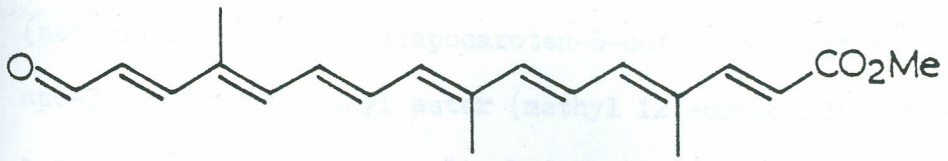
*Nomenclature used is that recommended by Commission on Biochemical Nomenclature, Bichemistry 14, 1975, 1803.



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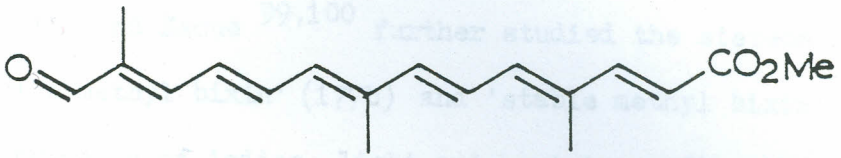

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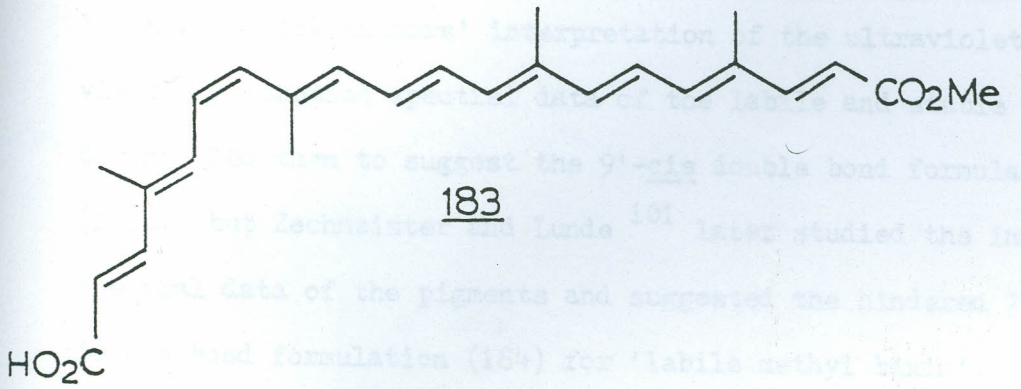

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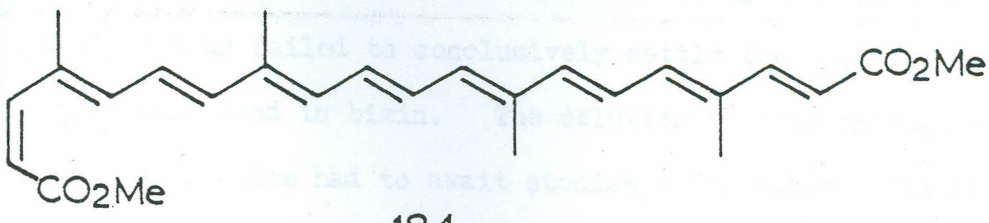


184

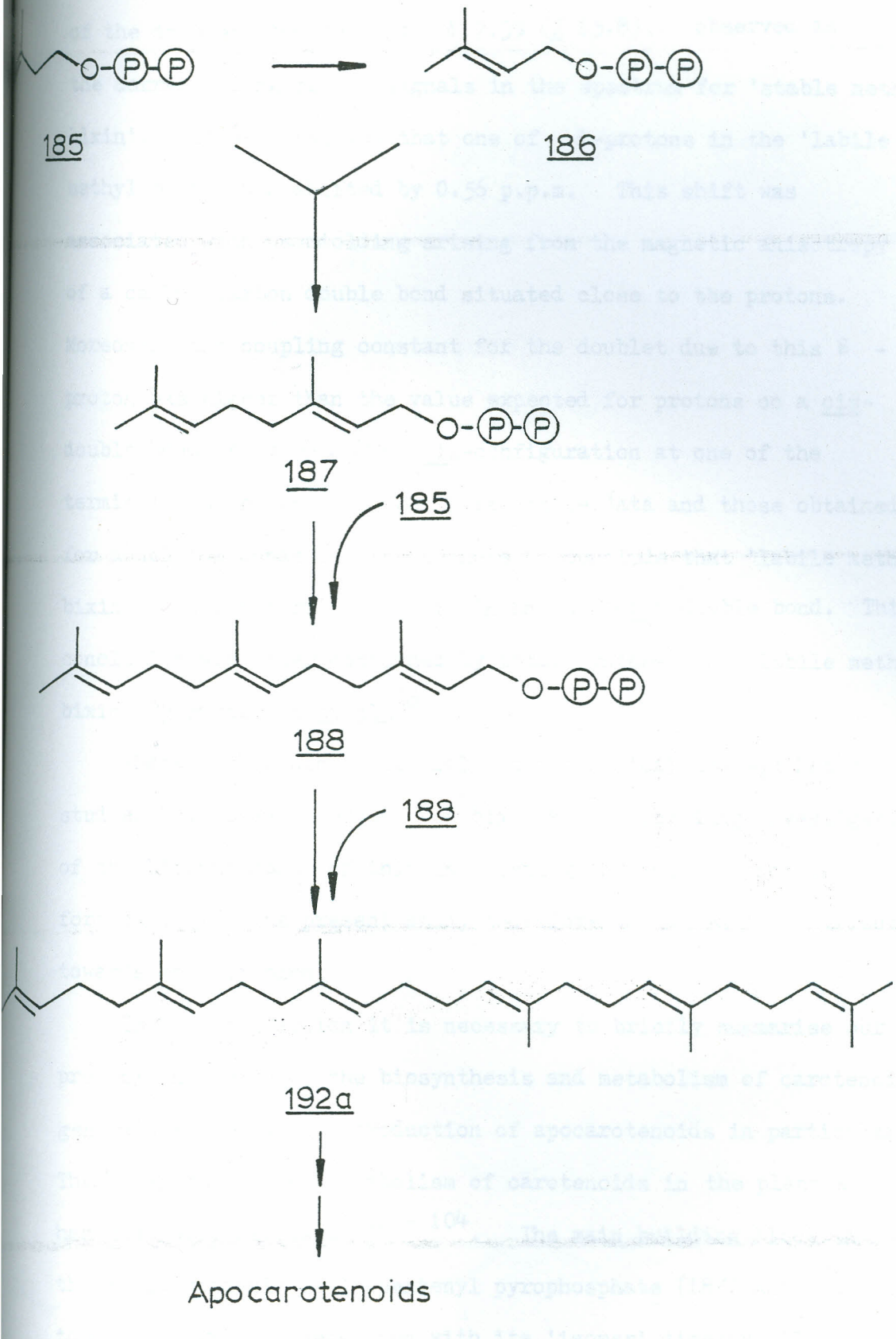
ural methyl bixin ⇌ Neomethyl bixin C

⇅

trans methyl bixin ⇌ Neomethyl bixin A

Scheme 39

(180), and a very probably different apo-2-norbixinal methyl ester (methyl 10'-oxo-6,10'-diapocaroten-6-oate (181) and an identical apo-3-norbixinal methyl ester (methyl 12'-oxo-6,12'-diapocaroten-6-oate (182). These results led the authors to the conclusion that labile bixin had a hindered 11'-cis double bond (183). Zechmeister and Escue [99,100] further studied the stereomutation of 'labile methyl bixin' (177b) and 'stable methyl bixin' (179b) in the presence of iodine, light and heat to confirm the reversibility of the cis-trans-relationship given in Scheme 39 and during these studies they isolated new crystalline isomers, which they termed 'neomethyl bixin A', 'neomethyl bixin B' and 'neomethyl bixin C'. The authors' interpretation of the ultraviolet and visible absorption spectral data of the labile and stable methyl bixins, led them to suggest the 9'-cis double bond formulation (177b), but Zechmeister and Lunde [101] later studied the infrared spectral data of the pigments and suggested the hindered 7'-cis-double bond formulation (184) for 'labile methyl bixin'. Results from ultraviolet-visible and infrared absorption spectroscopic studies thus failed to conclusively settle the position of the cis-double bond in bixin. The solution to this intriguing stereochemical problem had to await studies of ^{1}H n.m.r. data of the pigment [87]. The shifts at δ 3.76 (S), 1.97 (S), 6.30 - 7.00, and 5.88d (J 15.8) in the spectra of both 'labile methyl bixin' and 'stable methyl bixin', assigned to methoxyl, vinyl methyl, in chain olefinic and the two α-olefinic protons respectively were common. The spectra had some important differences however. The spectrum for 'labile methyl bixin' had doublet resonances at δ 7.37 (J 15.8) and δ 7.93(J 15.8), assigned to the two β-olefinic protons split by α-protons. The intensity of each of these signals was equivalent to half of the intensity

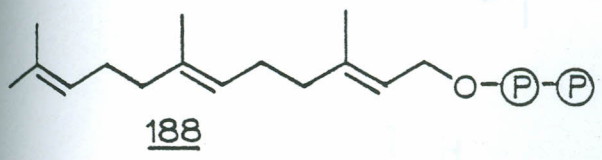


Scheme 40

of the doublet centred at δ 7.39 (J 15.8), observed as the corresponding proton signals in the spectrum for 'stable methyl bixin'. It was observed that one of β-protons in the 'labile methyl bixin' was shifted by 0.56 p.p.m. This shift was associated with deshielding arising from the magnetic anisotropy of a carbon-carbon double bond situated close to the protons. Moreover, the coupling constant for the doublet due to this β-proton was higher than the value expected for protons on a cis-double bond; this excluded a cis-configuration at one of the terminal double bonds. Therefore, these data and those obtained for model compounds led the authors to conclude that 'labile methyl bixin', hence natural labile bixin had a 9'-cis-double bond. This conclusion was later confirmed by total synthesis of 'labile methyl bixin' by Pattenden et al.[88]

Whereas thorough structural, stereochemical and synthetic studies have been undertaken on bixin, a corresponding investigation of the biosynthesis of this interesting molecule has not been forthcoming. The present study therefore was aimed at contributing towards this problem.

In this connection it is necessary to briefly summarise our present knowledge of the biosynthesis and metabolism of carotenoids in general, and the natural production of apocarotenoids in particular. The biosynthesis and metabolism of carotenoids in the plant kingdom has been well reviewed [102 - 104]. The main building block in their biosynthesis is isopentenyl pyrophosphate (185) which by head-to-tail enzymic condensation with its 'isomer' dimethylallyl pyrophosphate (186) first leads to geranyl pyrophosphate (187). The sequential condensations between isopentenyl pyrophosphate and geranyl pyrophosphate then forms farnesyl pyrophosphate (188) which is then converted to geranylgeranyl pyrophosphate (189).



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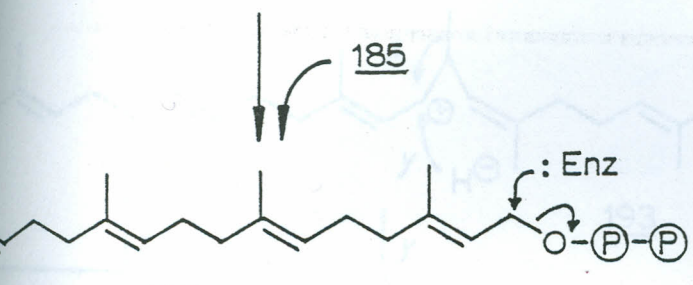

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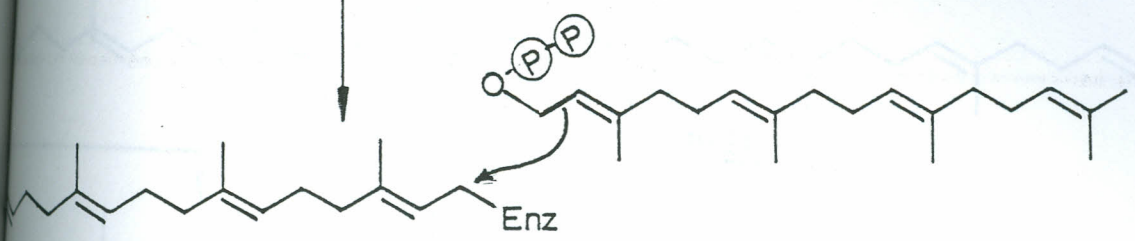

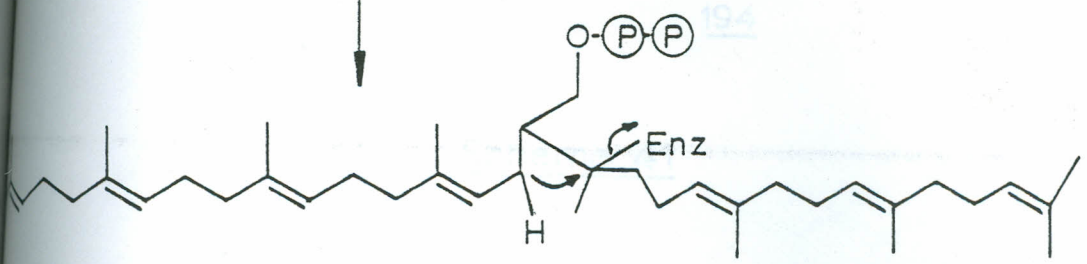

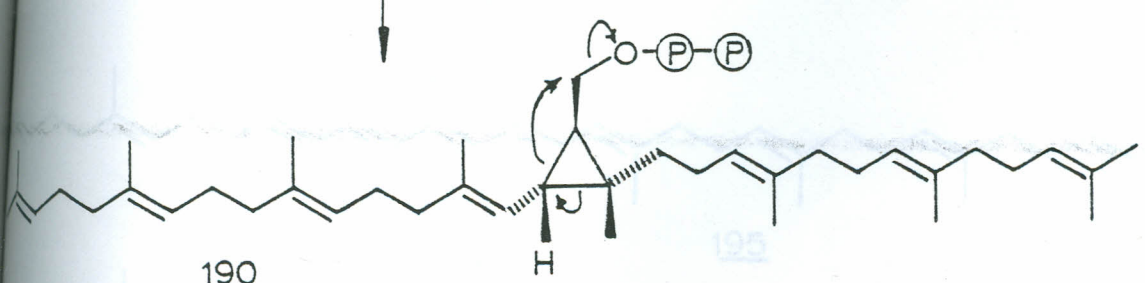

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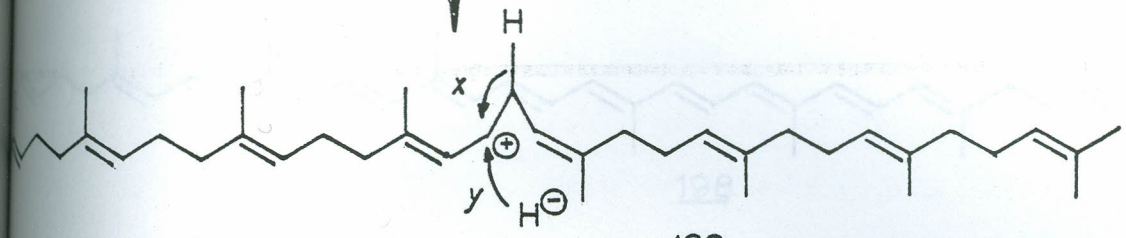

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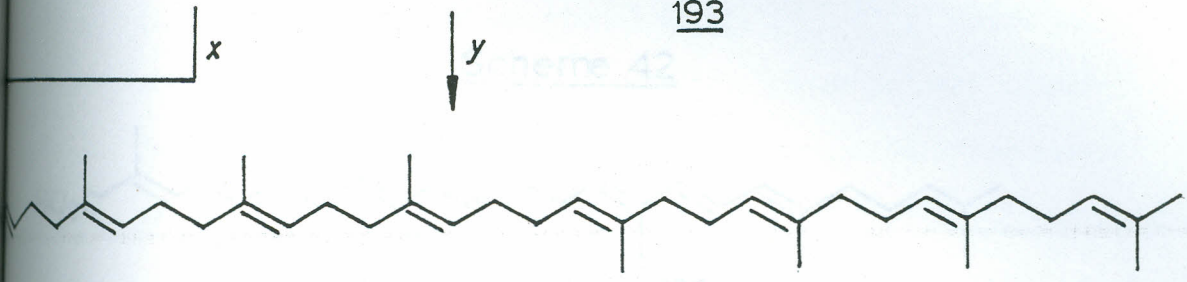

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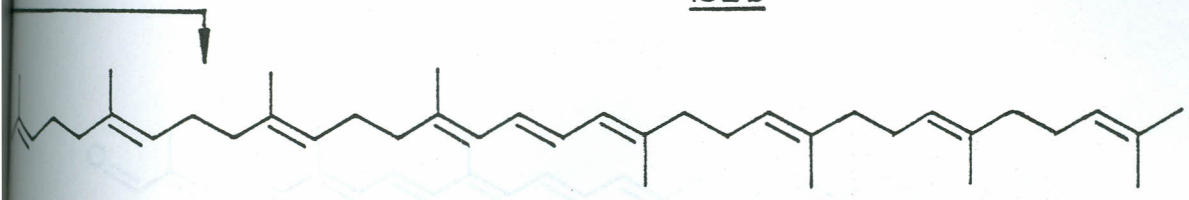

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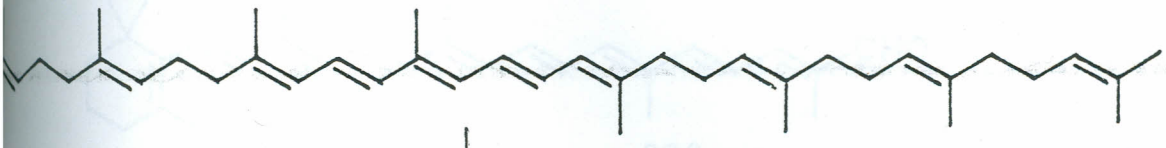
Scheme 41

194



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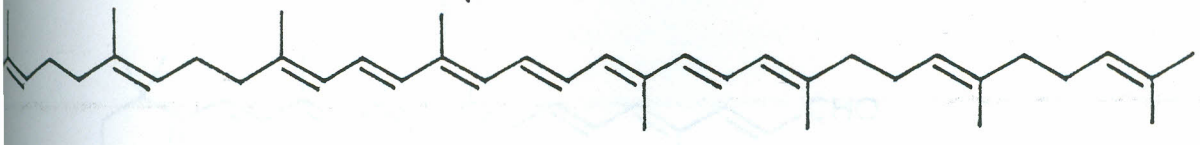

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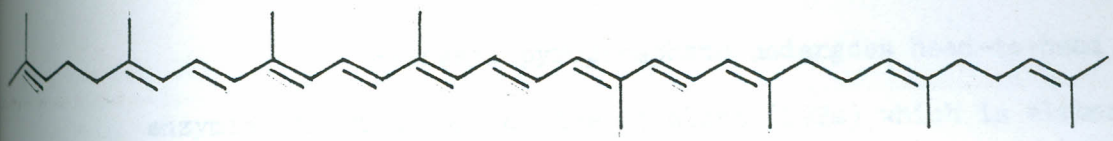

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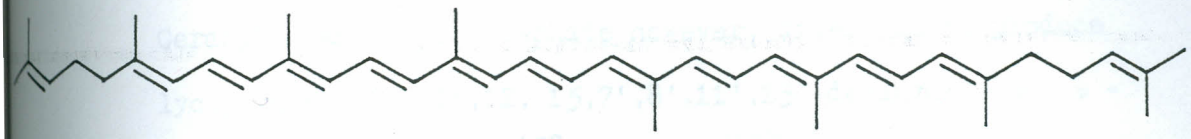

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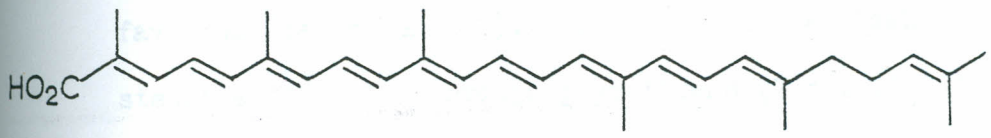
Scheme 42



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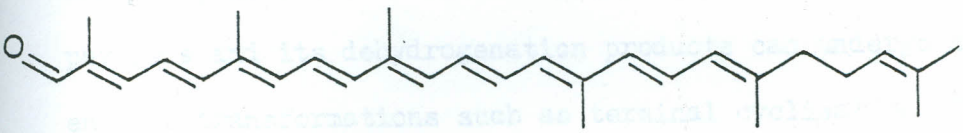

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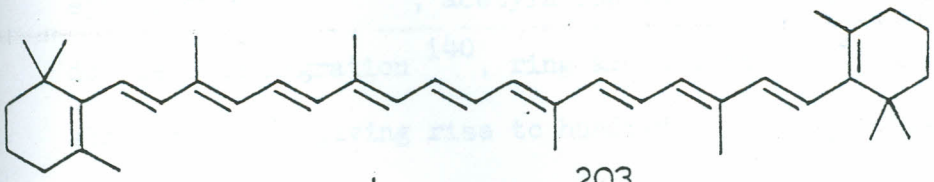

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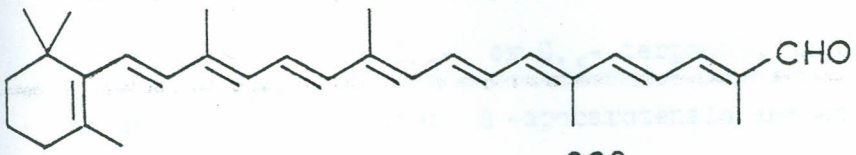

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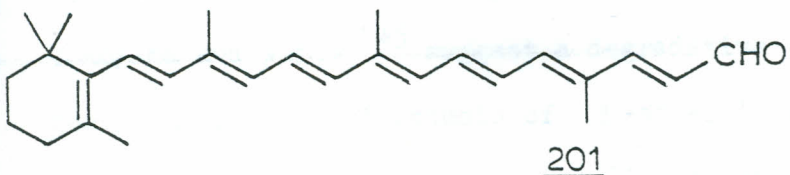
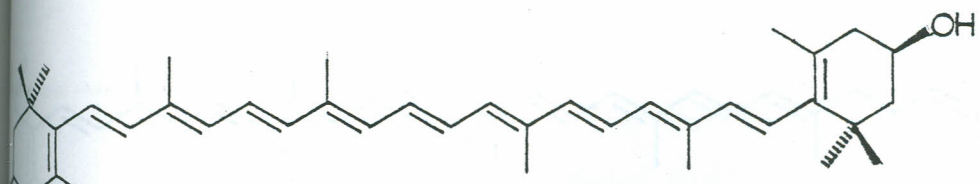


201

Scheme 43

Farnesyl pyrophosphate undergoes head-to-head enzymic dimerisation to give squalene (192a) which is elaborated to steroids or sometimes to carotenoids [105] such as 4,4'-diapo-neurosporen-4-oic acid (199) and 4,4'-diapolycopen-4-al (200). Geranylgeranyl pyrophosphate however, dimerises to produce lycopersene (7,8,11,12, 15,7',8',11',15'-decahydro- ψ , ψ -carotene)(192b) [106 - 108] and/ or phytoene (7,8,11,12,7',8',11', 12'-octahydro- ψ , ψ -carotene) (194) [109 - 117]; there is substantial evidence to show that the latter pathway is more favoured (see scheme 41). Phytoene is then 'dehydrogenated' stepwise [118 - 120] through phytofluene (7,8,11,12,7',8'-hexahydro- ψ , ψ -carotene (195), ζ -carotene (7,8,7',8'-tetrahydro- ψ , ψ -carotene (196), neurosporene (7,8-dihydro- ψ, ψ -carotene) (197) and lycopene (ψ , ψ -carotene) (198) (see Scheme 42). Both phytoene and its dehydrogenation products can undergo several enzymic transformations such as terminal cyclisation [120 - 126], ring contraction [102], hydration [127 - 128], hydroxylation [129 - 131], epoxidation [131 - 136], acetylation and allene formation [137 - 139], double bond migration [140], ring aromatisation [141] and chain elongation [142] giving rise to hundreds of carotenoids.

Theoretically, apocarotenoids are thought to be formed by oxidative degradation of any of the above carotene hydrocarbon and modified carotenoids, or by oxidation of a modified product of dimerisation of the C_{10}- or C_{15}- terpenoid unit. The isolation [143] of structurally related β -apocarotenals and apolycopenals from natural sources has led Isler and his co-workers [144] to suggest that β -oxidation mechanisms may be involved in their formation. Yokoyama and White [145] suggest a degradative transformation as a mechanism for the biogenesis of β -apo-10'-carotenal (10'-apo- β - β caroten-10'-al) (201) and β -apo-8'-carotenal (8'-apo- β -caroten-



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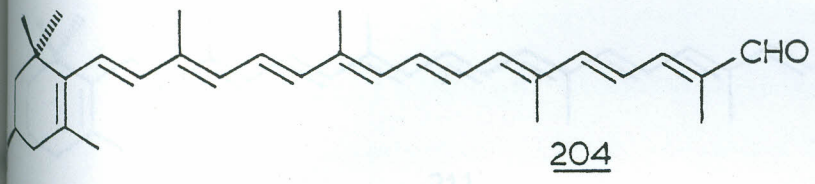

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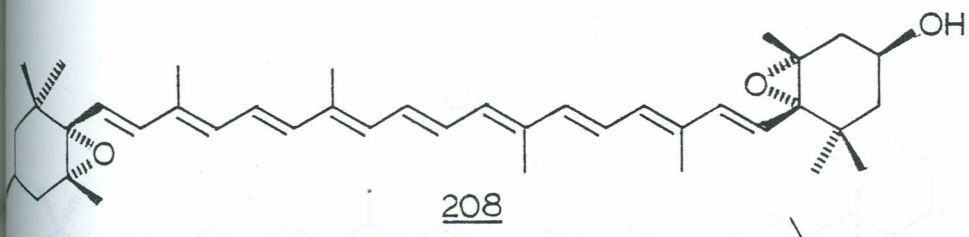
Scheme 44



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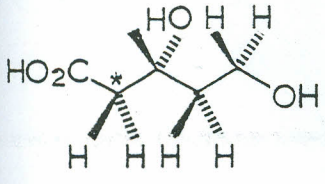

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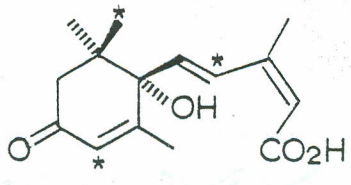

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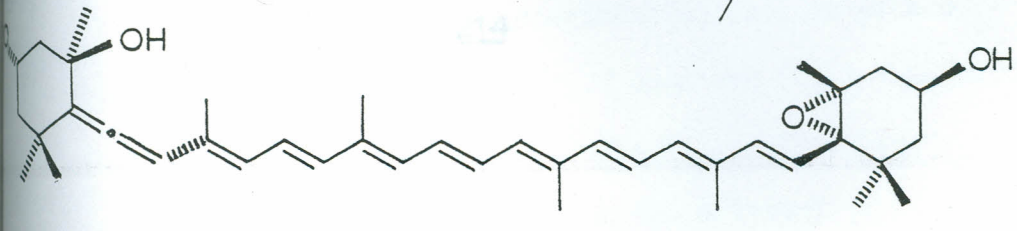

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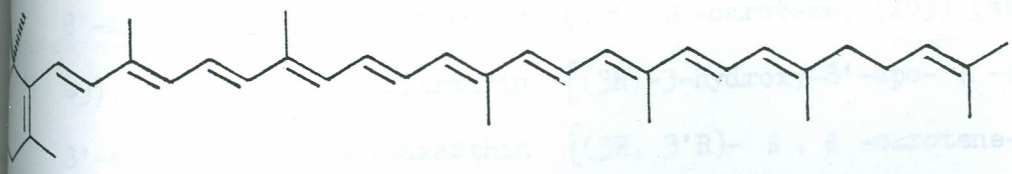
Scheme 45



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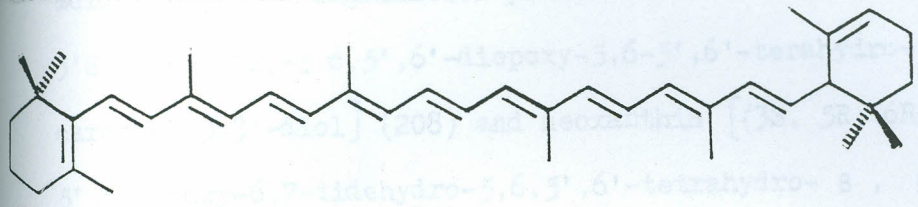

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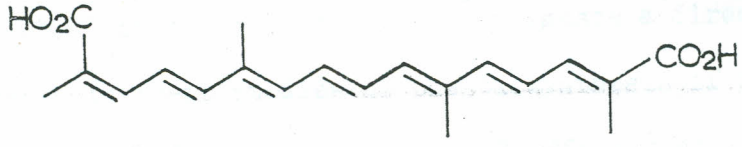

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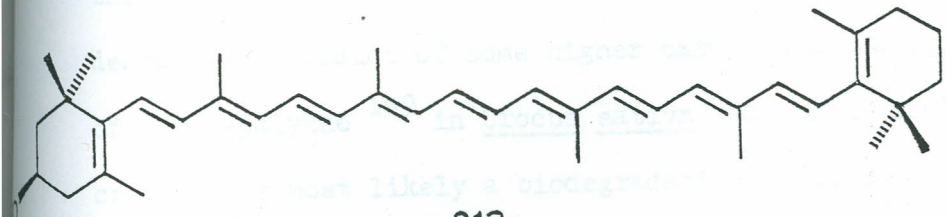

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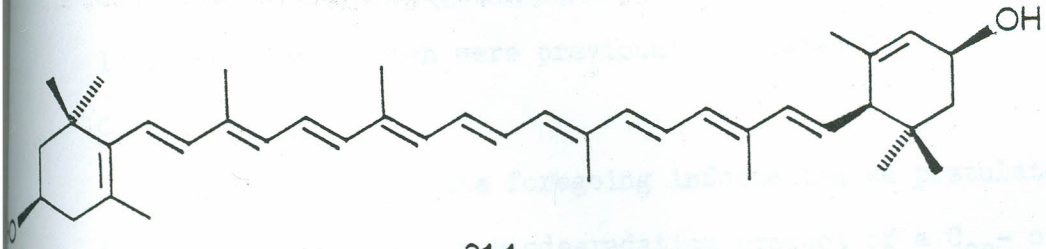

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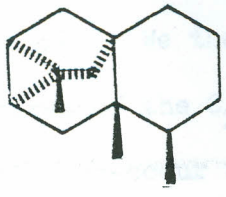
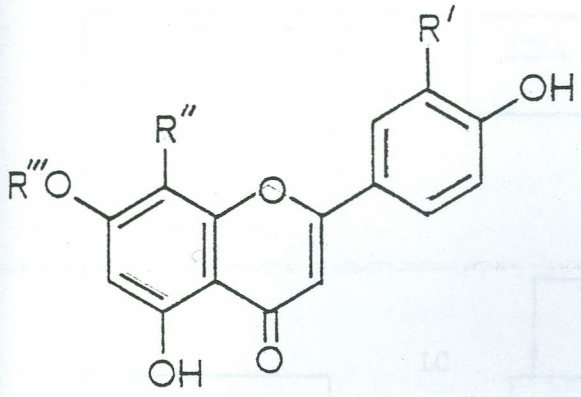


215

8'-al) (202) from β-carotene (β , β -carotene) (203) (see Scheme 43),and that of β-citraurin [(3R)-3-hydroxy-8'-apo- β -caroten-8'-al)] (204) from zeaxanthin [(3R, 3'R)- β , β -carotene-3,3-diol] (205) (see Scheme 44). There is good evidence[146-147] that abscisic acid (206) is a degradation product of violaxanthin [(3S, 5R, 6S, 3'S, 5'R, 6'S)-5,6,5',6'-diepoxy-5,6-5',6'-terahydro- β , β -carotene-3,3'-diol] (208) and neoxanthin [(3S, 5R, 6R, 3'S, 5'R, 6'S)-5',6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro- β , β-carotene-3,5,3'-triol] (209), while the incorporation of 2 - ^{14}C mevalonic acid (207) into abscisic acid [148] suggests a direct biosynthesis. Recently in their studies of the biosynthesis of crocetin (8,8'-diapocarotene-8,8'-dioic acid) (212) Pfander et al.postulated that this dioic acid could be either a biosynthetic product or a biodegradation product of some higher carotenoid analogues. The absence of a C_{20}-polyene [149] in Crocus sativa made them conclude that crocetin is most likely a biodegradation product of the carotenoids; β -carotene (203), γ-carotene (ψ , β -carotene) (210), α -carotene (6'R)- β , ε-carotene (211), zeaxanthin (205) or lycopene (198), which were previously isolated [150 - 151] from Crocus sativa.

In the light of the foregoing information we postulated that bixin was most likely a biodegradation product of a C_{30}- or a C_{40}-carotenoid. The elaboration of squalene (C_{30}) into carotenoids has been observed only in bacteria [152]. This observation made us believe that bixin is most unlikely, a byproduct of carotenoids elaborated from squalene. We therefore set to isolate and elucidate the structures of the C_{20}-acyclic terpenoids and C_{20}- to C_{40}-carotenoids that co-occur with bixin and its isomers in the seeds of Bixa orellana.

At the onset of this project there were no reports on chemical



216

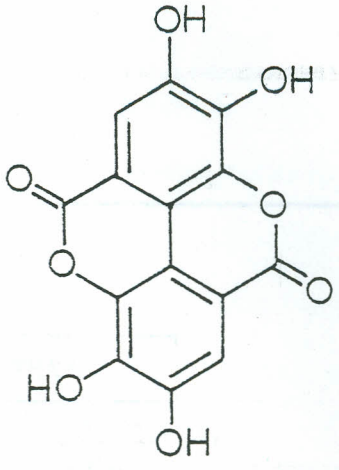
a $R' = R'' = H$, $R''' =$ bisulphate

b $R' = R'' = H$, $R''' =$ glucosyl

c $R' = OH$, $R'' = H$, $R''' =$ bisulphate

d $R' = OH$, $R'' = H$, $R''' =$ glucosyl

e $R' = OH$, $R'' =$ o-bisulphate, $R''' = H$



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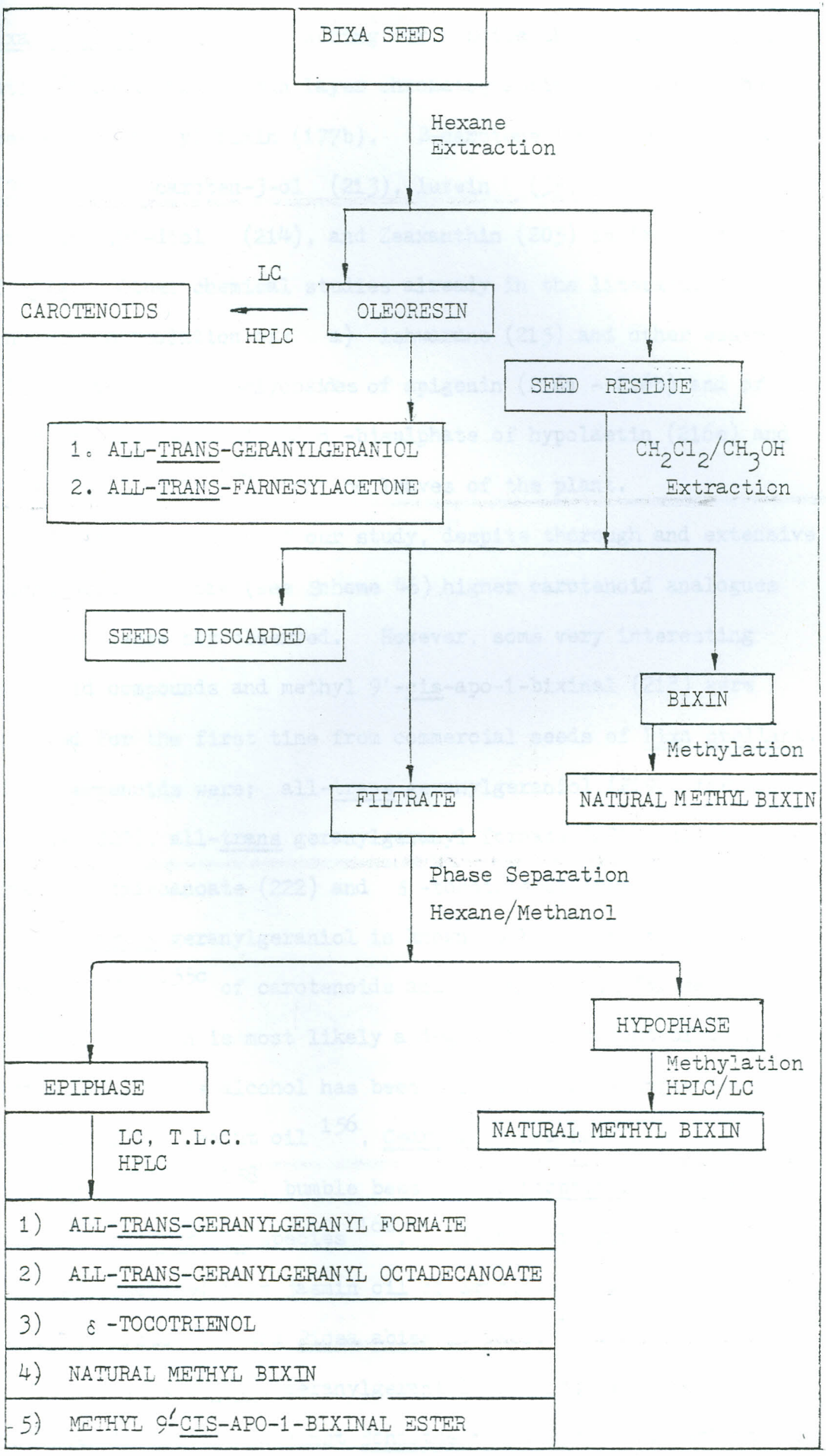


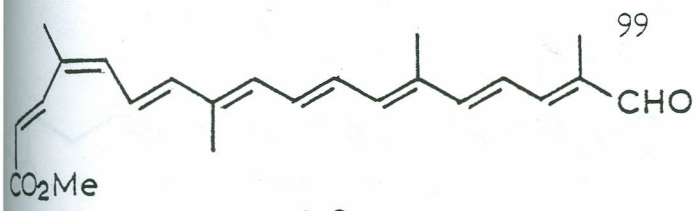
Scheme 46 : Isolation of Apocarotenoids and their precursors in seeds of Bixa orellana

investigations of carotenoid co-metabolites of bixin in seeds of Bixa orellana. However, during our studies there was a communication[93] relating to thin layer chromatographic evidence of the presence of methyl bixin (177b), β-carotene (203), cryptoxanthin (3R)- β, β -caroten-3-ol (213), lutein (3R, 3'S, 6'R)- β, ε -carotene-3,3'-diol (214), and Zeaxanthin (205) in fresh seeds of Bixa. Other chemical studies already in the literature concern the isolation of: a) ishwarane (215) and other essential oils [153] and b) 7-glycosides of apigenin (216a - 216b) and of luteolin (216c - 216b) and 8 -bisulphate of hypolaetin (216e) and ellagic acid (217) [154] from the leaves of the plant.

During the course of our study, despite thorough and extensive chromatographic work (see Scheme 46) higher carotenoid analogues of bixin were not observed. However, some very interesting terpenoid compounds and methyl 9'-cis-apo-1-bixinal (218) were isolated for the first time from commercial seeds of Bixa orellana. These terpenoids were; all-trans geranylgeraniol (219), farnesyl-acetone (220), all-trans geranylgeranyl formate (221), all-trans geranyl-geranyl octadecanoate (222) and δ -tocotrienol (223).

All-trans geranylgeraniol is known to be an important precursor [155a - 155c] of carotenoids and therefore reinforces our belief that bixin is most likely a degradation product of a C_{40}-carotenoid. This alcohol has been isolated, for example from linseed oil and peanut oil [156], Cedrela toona Roxb [157], Dicranum elongatum [158], bumble bees [159], Phytophthora cactorum [160], ants [161] and Pterodon species [162]. Its isomer, geranyllinalool (224) was isolated from jasmin oil of Egyptian origin, and from Norwegian spruce [163] and Picea abies L. Karst [164].

Although all-trans geranylgeraniol does not have chemoprophylactic activity against Schistosoma mansoni, its derivatives



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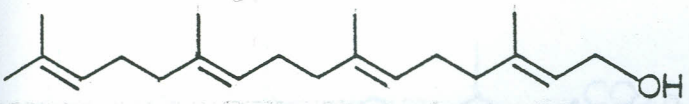

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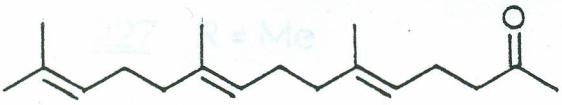

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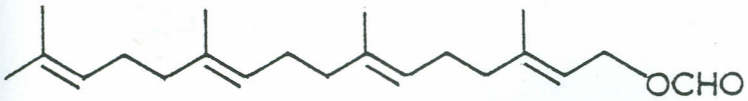

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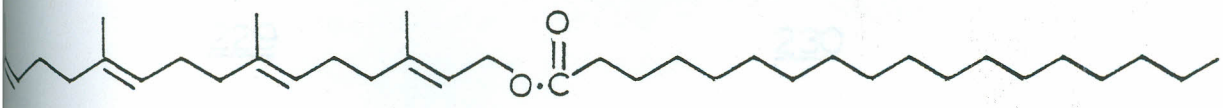

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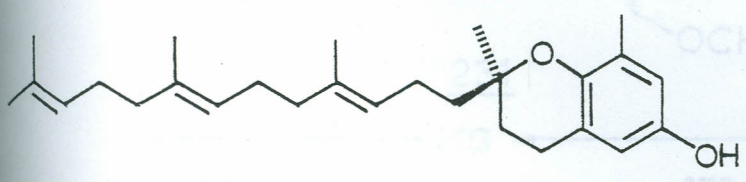

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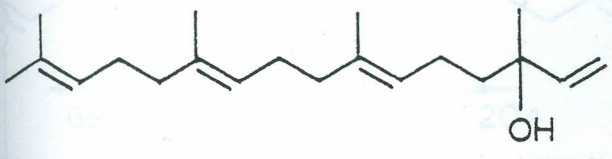

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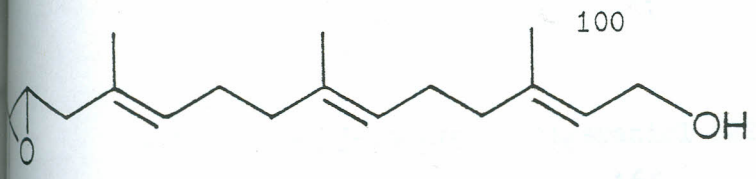

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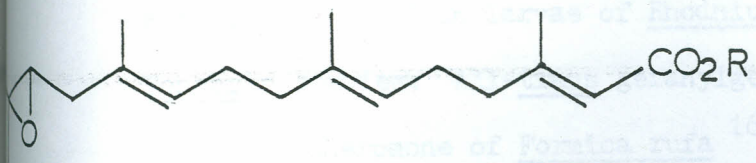
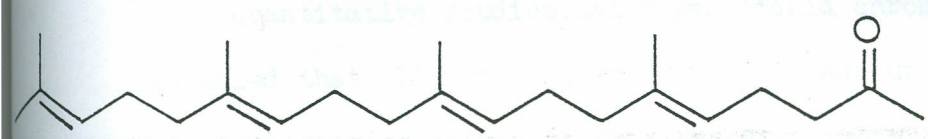


226 R = H

227 R = Me



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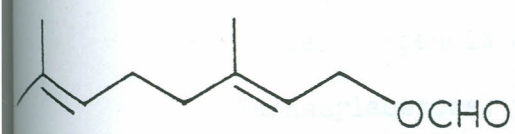

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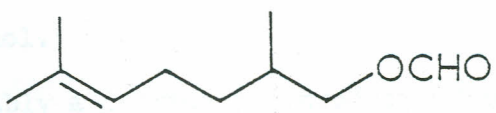

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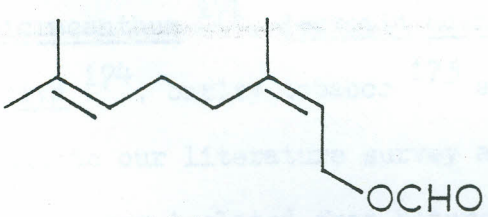

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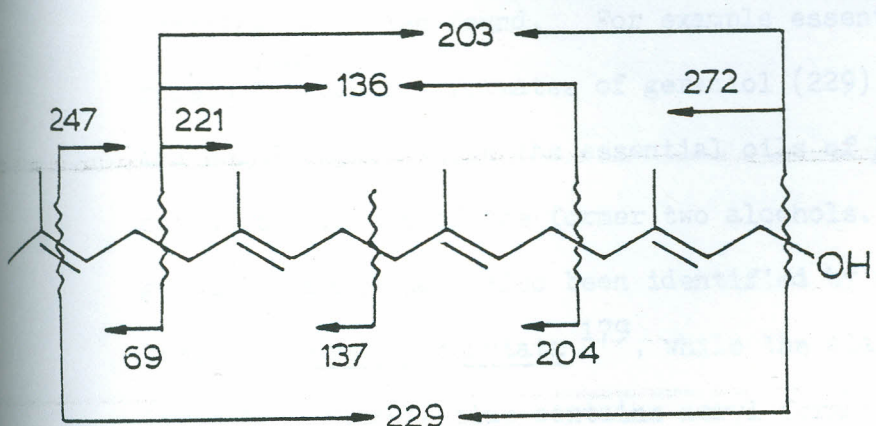


232

such as 14,15-epoxygeranylgeraniol (225), 14,15-epoxygeranylgeranoic acid (226) are highly active [165]. It has been demonstrated that methyl 14,-15-epoxygeranylgeranoate (227) elicits Juvenile hormone activity [162, 166] on larvae of Rhodnius, Culex, Pyrrhocoris and Tenebrio species. All-trans geranylgeraniol is a constituent of the alarm pheromone of Formica rufa [167] while geranylgeranylacetone (228) has been shown to possess antiulcer activity in rats [168]. Finally, geranylgeraniol has been found to improve tobacco aroma [169].

Quantitative studies using gas liquid chromatography (g.l.c.) Fig.1 revealed that all-trans geranylgeraniol made up more than 50% of the oleoresin extracted from the Bixa seeds. It was also shown that about 0.8% of dry seeds was made up of free all-trans geranylgeraniol. This makes Bixa orellana, the richest known source of the natural terpenoid alcohol.

Farnesylacetone, probably a degradation product of geranylgeraniol, has been demonstrated to elicit Juvenile hormone activity on larvae of Tenebrio species [170] and has been isolated from Sargassum micracanthum [171], Cecropia adenopus [172], tomato [173], Cannabis sativa [174], burley tobacco [175] and Carphephorus species [176].

According to our literature survey all-trans geranylgeranyl formate has not been isolated from Nature; however, other terpenoid formates have been found. For example essential oils of Pelargonium roseum [177] contain formates of geraniol (229), citronellol (230) and nerol (231), while the essential oils of Pelargonium asperum [178] contains formates of the former two alcohols. Neryl formate and geranyl formate have also been identified by g.l.c. in oils of leaves of Monarda punctata [179], while the alarm pheromone of Tyrophagus putrecentiae contains neryl formate [180]. Other esters of geranylgeraniol have, however, been found in Nature. For example, all-trans geranylgeranyl octadecanoate (222) was found to occur in

essential oils of Picea abies [181]. It has also been demonstrated that all-trans geranylgeraniol is one of the alcohols esterifying bacteriophyll (a) of Rhodospirillum rubrum [182], and chlorophyll of newly formed leaves of Aesculus hippocastanium [183a] and barley [184].

In Nature, all-trans geranylgeraniol not only esterifies acids, but can also be involved in prenylation of quinones, as was demonstrated by the isolation of tocotrienols from palm oil [183b] Havea braziliensis [184], Sargassum species [185] and some leguminous seeds[186]. Tocotrienols have been shown to influence settling of swimming larvae of Coryne uchidae [185] and to possess antioxidation properties[187]. The presence of δ-tocotrienol in "annatto" may influence the stability of bixin against oxidation.

The isolation of methyl 8-oxo-9'-cis-8,6'-diapocaroten-6'-oate (218) from the seeds of Bixa orellana indicates that the cis-end of natural bixin is more resistant to further degradation than the free acid-all-trans end. Probably it is the cis-double bond that stops further β-oxidative cleavage. This observation causes a temptation to suggest that the cis configuration in bixin is probably present in its precursor. This aldehyde was probably methyl apo-1-norbixinal ester (180) obtained through permanganate oxidation of 'labile bixin' by Karrer [98]. Their ultraviolet absorption data are closely comparable to those observed by us. Weedon [87] et al, repeating Karrer's work, isolated this aldehyde and their ultraviolet absorption and proton nuclear magnetic resonance data are similar to those observed for our sample.

FIGURE 1 : Gas Liquid Chromatogram of Oleoresin.

Conditions:

Column : 3% OV-1 on Chromosorb B 60-80 mesh, 2M x 5mm.

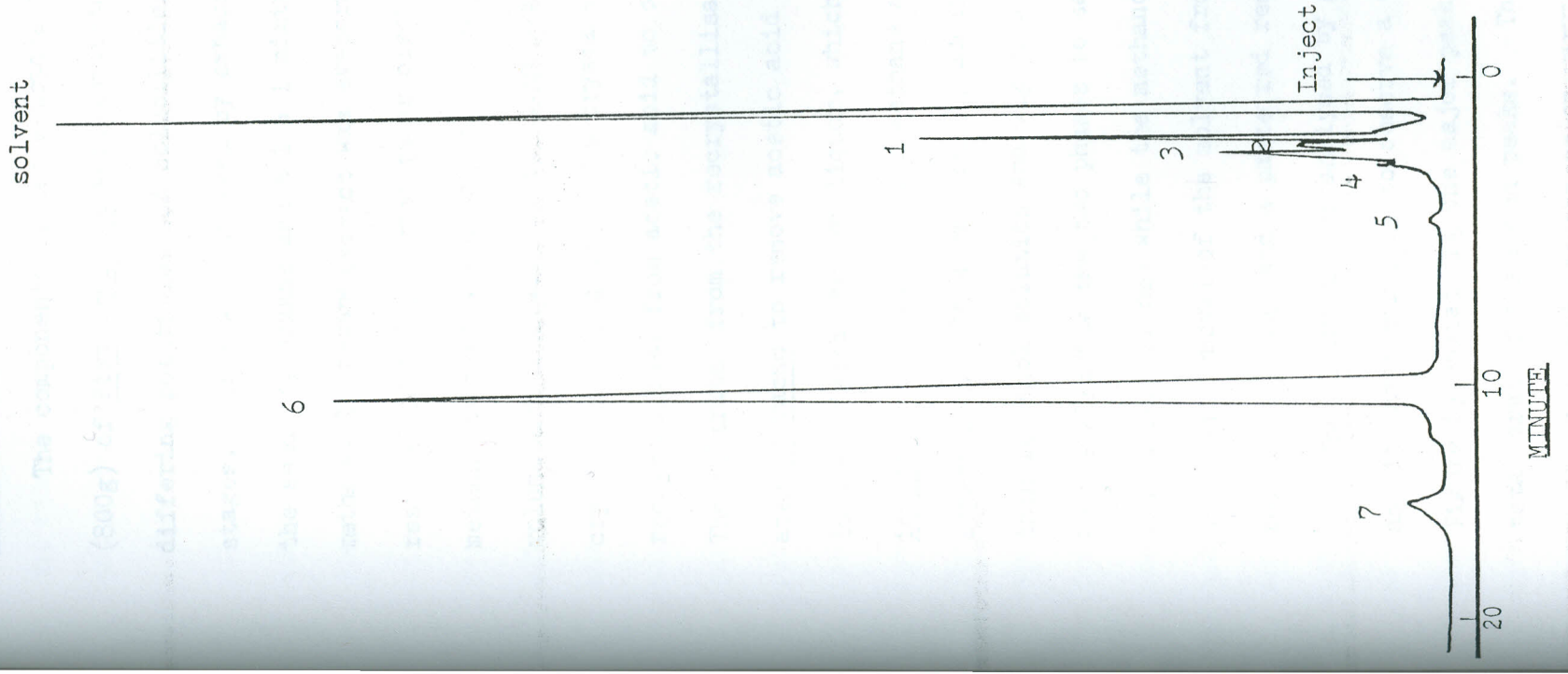
Oven Temperature : Isothermal at 210°C.

Flame Ionisation Detector and Injector temperature : 250°C

Carrier Gas (Nitrogen) flow rate : 30 ml/min.

Chart speed : 0.5 cm/min.

Sample size : 0.3 microlitre of dilute solution of oleoresin in ethyl acetate.

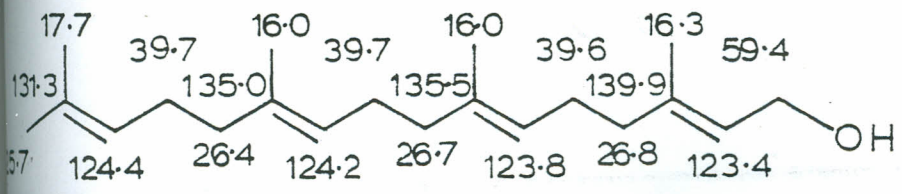


2.2 Isolation and Structural Elucidation of Terpenoids and Apocarotenoids of Bixa orellana (see Scheme 46):

The components of the extracts of dry commercial seeds (800g) of Bixa orellana were separated according to their differing polarities and solubilities during a series of extraction stages. This was achieved by exhaustive successive extractions of the seeds by hexane and a 1 : 1 mixture of methanol and dichloromethane. The hexane extract was evaporated *in vacuo* to leave an oily residue (12.2g) referred to as oleoresin. The methanol-dichloromethane extract was also evaporated *in vacuo* to half its original volume and then allowed to stand overnight at -10°C whereupon crystals slowly grew. The crystals (11g) were filtered off, and recrystallised from acetic acid to obtain natural bixin (177a). The filtrate from the recrystallisation process was first evaporated *in vacuo* to remove acetic acid and the residue was transferred to the original mother liquor, which was then further evaporated *in vacuo* to remove dichloromethane and then subjected to phase separation. This was done by adding an equal volume of hexane to this methanol solution and vigorously shaking the resulting mixture before allowing the two phases to separate. The hexane phase was called epiphse while the methanol phase was called the hypophase, and removal of the solvent from each of the phases gave a red solid (6.2g) and a paste red residue (30g) respectively.

The oleoresin was analysed by g.l.c. for its terpenoid content, and it was surprising to observe a very simple g.l.c. trace (see Figure 1) containing one major peak, comprising about 50% of the total area of the seven peaks. The number of peaks and their proportions did not change on varying the column temperature between 160 - 210°C. At temperatures between 100°C and 160°C, all peaks appeared except the major one. These observations showed that the

oleoresion contained mainly the components resolved when running the chromatograph at 210°C. It was decided to isolate and characterise the main component. The oleoresin was distilled at 0.6 mm Hg and a fraction boiling between 130 - 170°C was collected, leaving a dark-red coloured resin. The distillate was loaded onto a silica gel column, which was eluted successively with petroleum ether (40 - 60 °C), 30% diethyl ether in petroleum ether (40-60°C), 40% diethyl ether in petroleum ether (40 - 60°C) and finally with diethylether to obtain the pale yellow oil. This oil was rechromatographed on a silicagel column and thin layer plates to obtain a pale yellow oil which was homogeneous on t.l.c. and g.l.c. analysis. The spectral data (m.s. ^{1}H n.m.r., ^{13}C n.m.r. and i.r.) were analysed and together they fitted the structure of all-trans geranylgeraniol (219). Moreover, these data corroborated those published by Nagasampagi [157] for natural all-trans geranylgeraniol, and by Coates [190] for the synthetic sample. The accurate mass observed at m/e 290.1612 fitted the molecular formula, $C_{20}H_{34}O$ for which the calculated accurate mass was 290.2609. Four double bond equivalents were calculated for the molecular formula. The mass fragmentation pattern revealed the isoprenoid nature of the sample (see 232). Doubly allylic cleavages, and those associated with hydroxyl group competed, and sometimes occurred together as was evidenced by the appearance of ions at m/e 69, 204, 272, 203 and 136. To a minor extent double bond cleavages gave rise to peaks at m/e 247 and 229. The ions which had lost oxygen were more abundant than those with oxygen. The base peak, observed at m/e 69 confirmed the isoprenoid nature of the sample while the 'dehydration ion' at m/e 272 showed that the sample had an hydroxyl group. The interpretation of the fragmentation pattern was done according to published work in related terpenoid mass spectroscopy [191,192].



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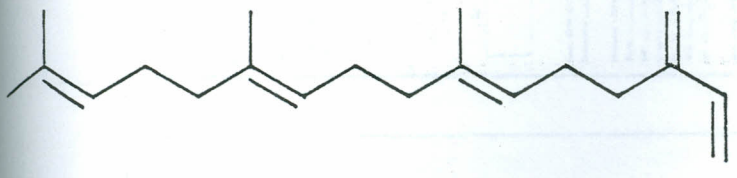

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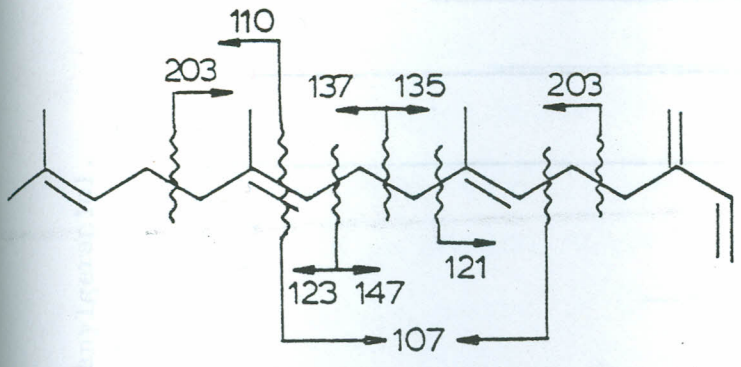

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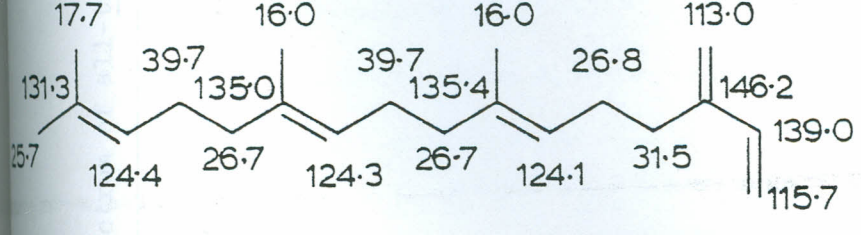

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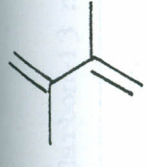

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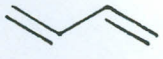

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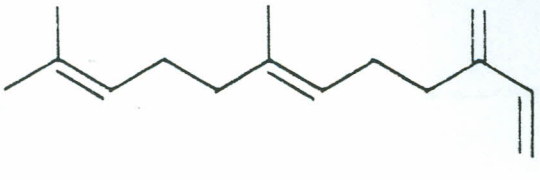

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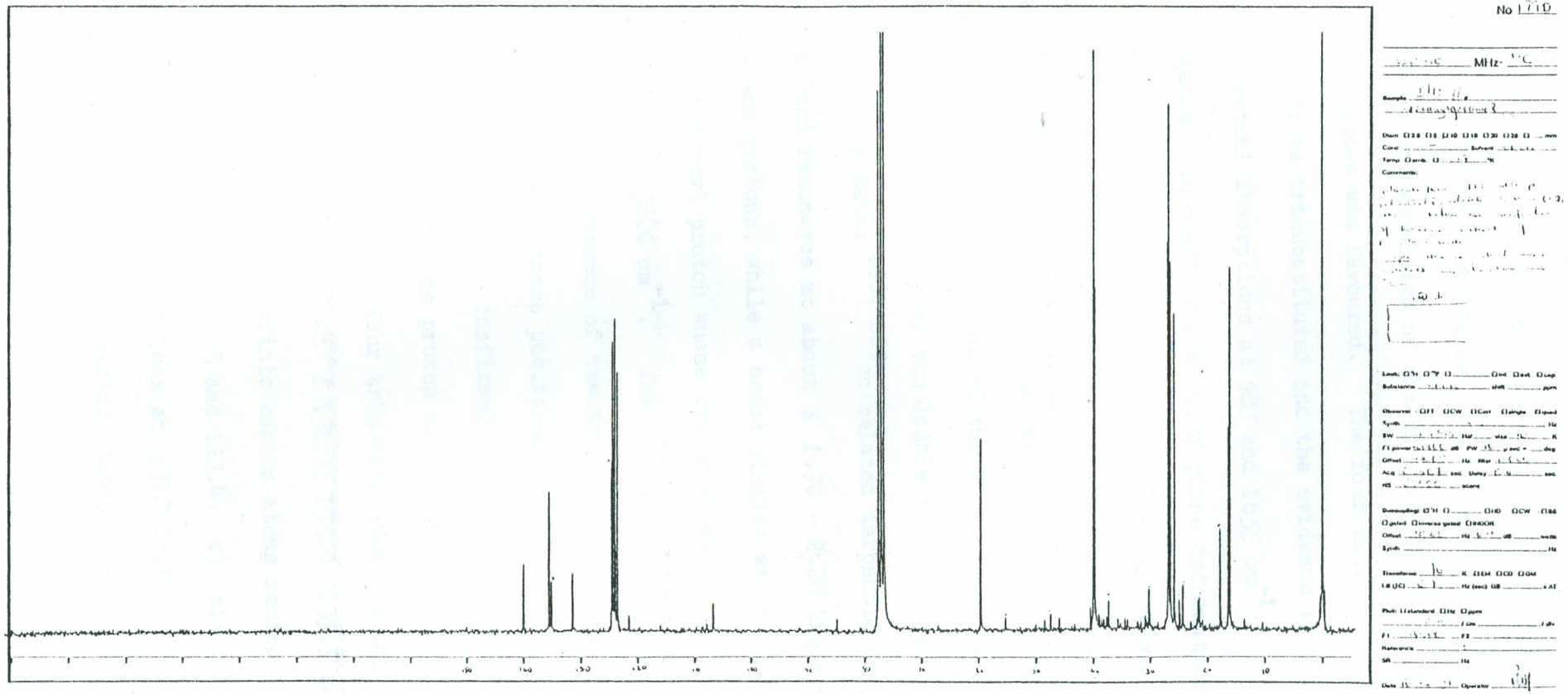
Figure 2 : Carbon-13 n.m.r. Spectrum of all-trans Geranylgeraniol.

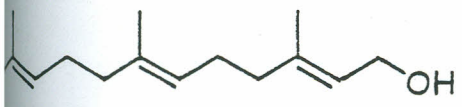


Inspection of the ^{1}H n.m.r. spectrum of the alcohol indicated that the total integration value for the olefinic proton signals at δ 5.45 and 5.13 was equivalent to four protons, and taken together with the four double bond equivalents calculated for the sample and knowledge of the biosynthesis of terpenoids, an acyclic system was favoured. The four double bonds were therefore assumed to be trisubstituted and the evidence was observed in the i.r. spectral absorptions at 985 and 1655 cm^{-1}; bands usually associated with olefinic C-H out of plane deformation and C = C stretching of the trans double bonds respectively. Whereas the doublet resonance at δ 4.15 (J 7 Hz) was assigned to two C_1-protons, the 3:2 ratio of the integral values of the signals at δ 1.62 and 1.70 assigned to fifteen protons of the vinyl methyl groups, confirmed the all-trans geometry of the double bonds in agreement with studies published by Bates [188, 189] on related terpenoid compounds. The broad band resonance at about δ 1.90 - 2.20 integrated for twelve methylene protons, while a broad singlet at δ 1.51 was attributed to the hydroxyl proton whose evidence was further given by the i.r. absorption at 3600 cm^{-1}. The ^{13}C n.m.r. spectrum (see Figure 2) confirmed the presence of twenty carbon atoms and their shift values (233) agreed with those published by Coates [190]. The absorption at δ 59.4 assigned to C_1, confirmed the presence of an hydroxyl group. Inspection of both the proton coupled and decoupled spectra showed the presence of: a) four trisubstituted olefinic carbon atoms resonating as singlets at δ 131.3, 135.0, 135.5 and 139.9, b) four monosubstituted olefinic carbon atoms resonating as doublets at δ 124.4, 124.2, 123.8 and 123.4, c) six methylene carbon atoms resonating as triplets at δ 39.7, 26.4, 39.7, 26.7, 39.6, and 26.8 and d) the five olefinic methyl carbon atoms resonating as quartets at δ 25.7, 17.7, 16.0, 16.0 and 16.3.

The percent composition of all-*trans* geranylgeraniol in the oleoresin was studied by g.l.c. analysis. The oleoresin was distilled to eliminate involatile components. The distillate (b.p. 0.6 mm Hg/ 130 - 170°C) was 52% of the oleoresin which was 1.5% of the dry seeds. A dilute solution of the distillate was injected five times into the g.l.c. column and the average areas of the resulting peaks were computed. It was then calculated that the major peak due to all-*trans* geranylgeraniol was 88% of the sum of the areas of the peaks observed. Thus further calculation showed that 0.7% of dry commercial seeds was made up of free all-*trans* geranylgeraniol. In a parallel experiment 100g of seeds were extracted by hexane to yield oleoresin (1.75g, 1.75%). A solution (1.89 mg/ml) of this oleoresin was prepared in ethyl acetate and a known amount of geraniol was added as an internal standard to the solution. The solution was then injected four times into the g.l.c. column and the average area of each peak was calculated. From these results the percent composition of free all-*trans* geranylgeraniol was found to be 57% using the formula given by Szepesy [200] (see experimental). Further calculation thus revealed that about 1% of the dry seeds was made up of free all-*trans* geranylgeraniol. The chromatogram is given in Figure 1.

Although the amount of esters of geranylgeraniol in the dry seeds was not computed, *Bixa* seeds are the richest natural source of free all-*trans* geranylgeraniol known to us. Since *Bixa* seeds are already on commercial production we suggest that this discovery has revealed an attractive and commercially viable source of all-*trans* geranylgeraniol; a very important compound. The only other reasonable natural source of the alcohol is from *Cedrela toona* Roxb [157] in which it is said to form 4% of extractible oleoresin.

Attempts to establish g.l.c. conditions for the preparative isolation



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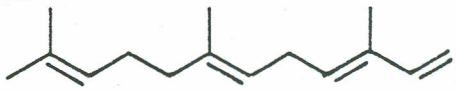

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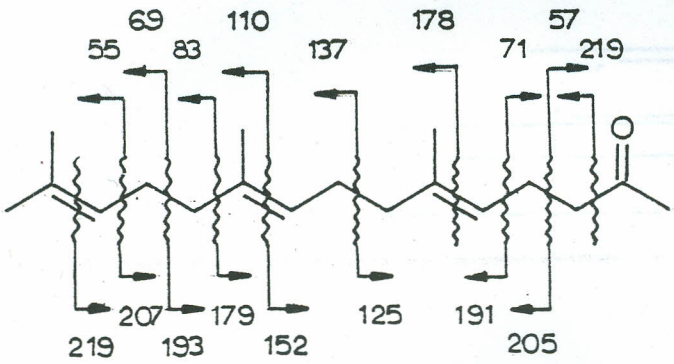

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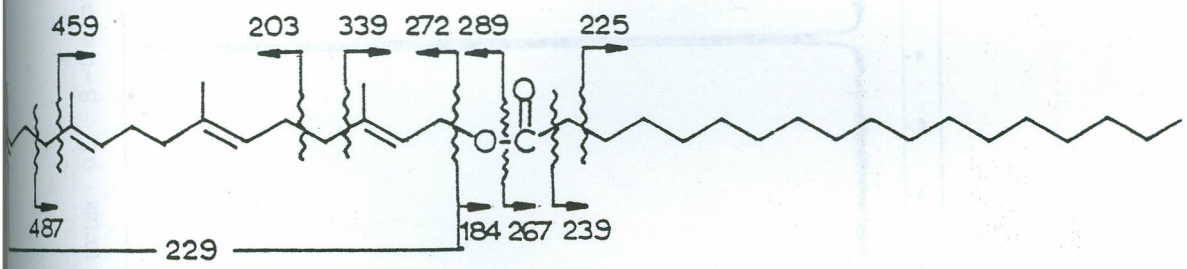

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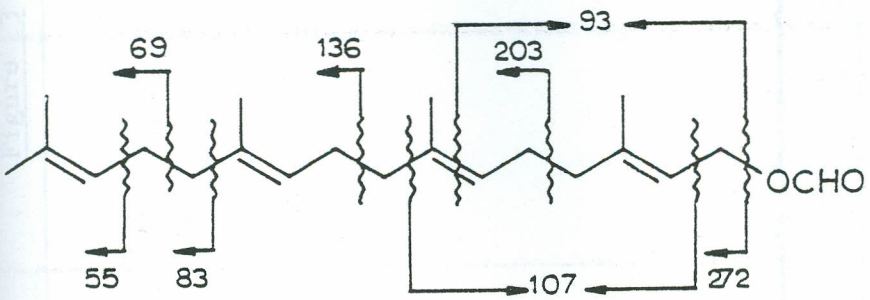

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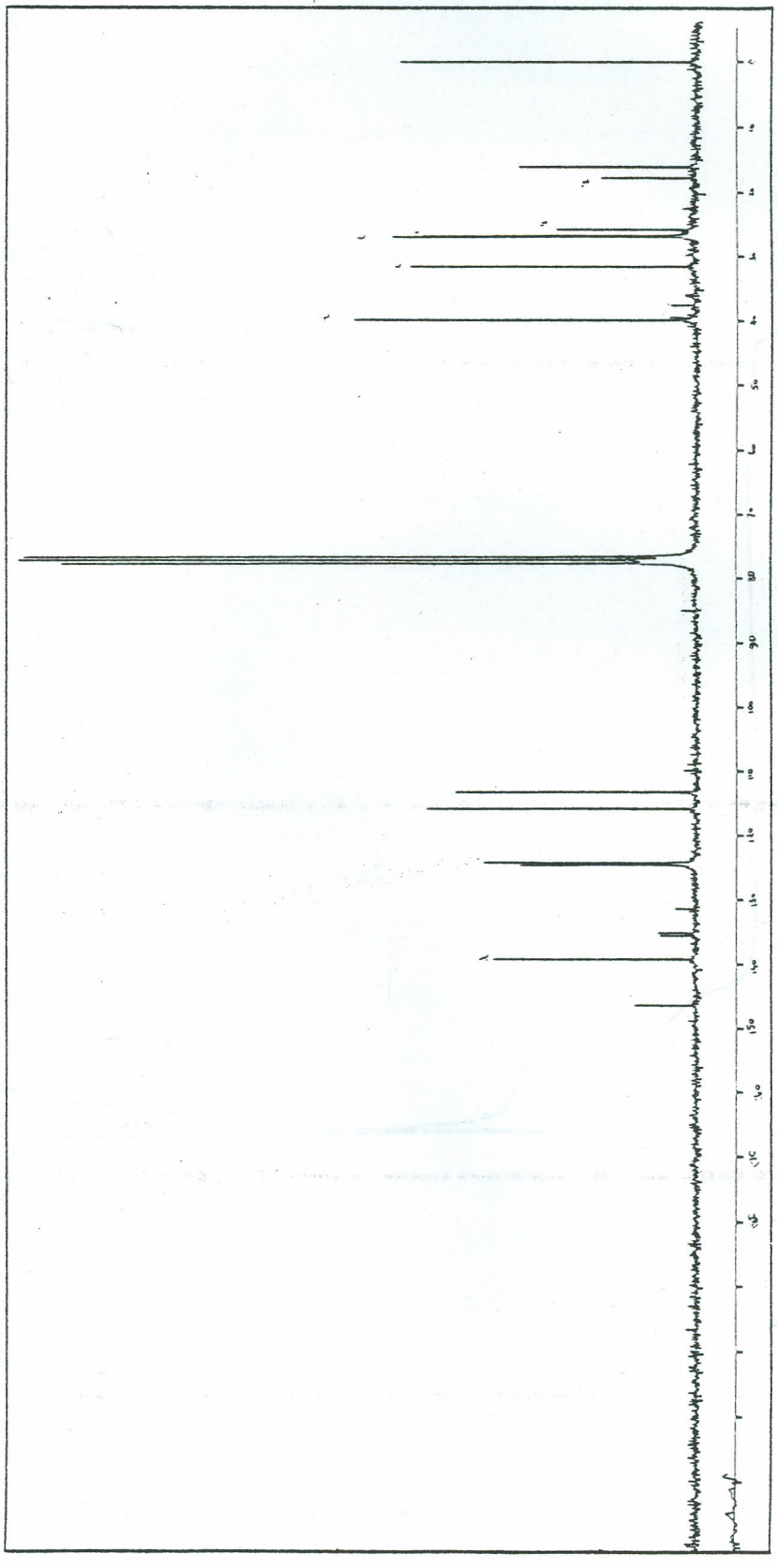
Figure 3 : Carbon-13 n.m.r. Spectrum of β-Geranylgeranene.



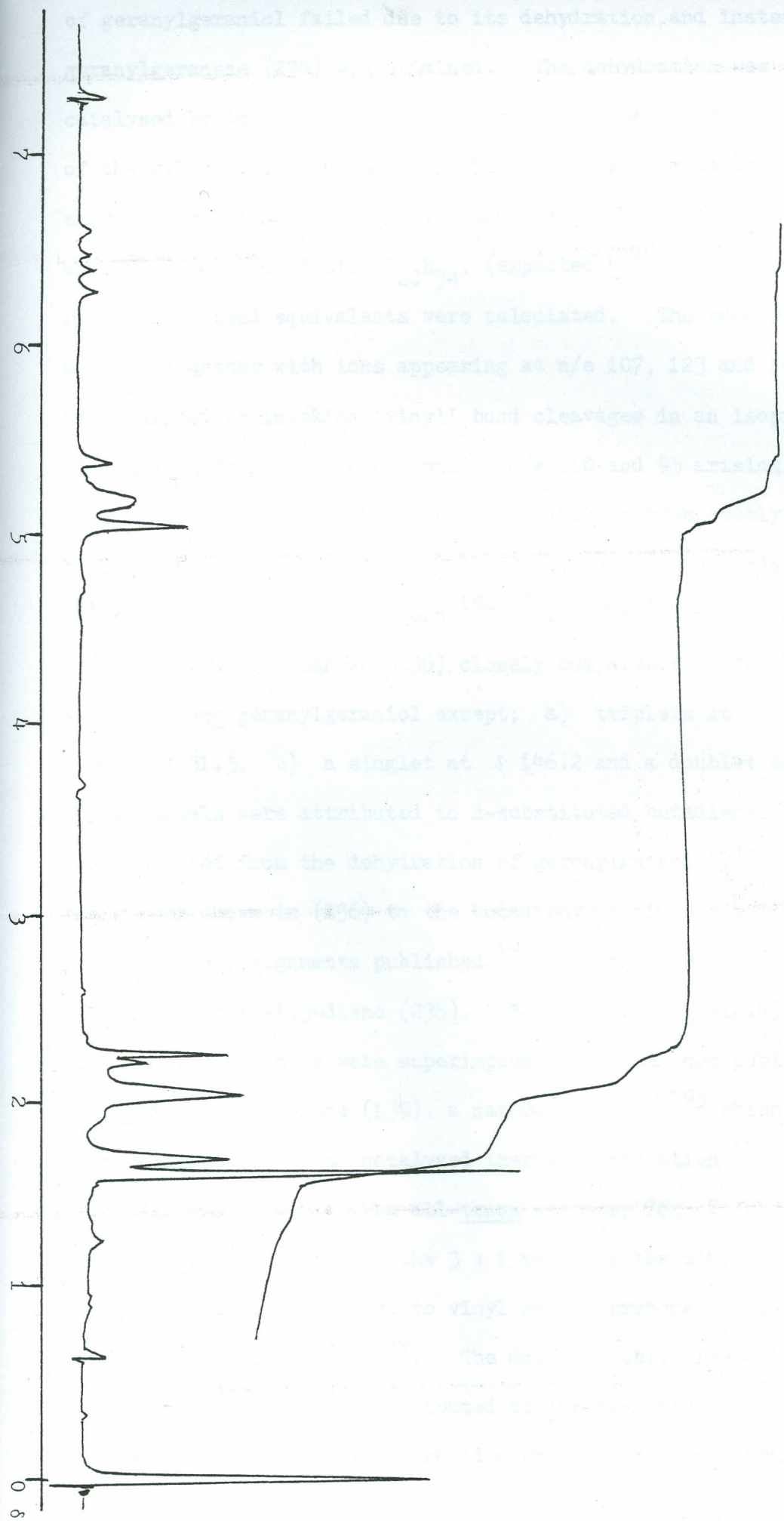


Figure 4 : Proton n.m.r. Spectrum of β-Geranylgeranene.

of geranylgeraniol failed due to its dehydration, and instead β-geranylgeranene (234) was obtained. The dehydration was possibly catalysed by potassium hydroxide, usually a component of Carbowax W of the g.l.c. column we used. The resulting hydrocarbon had a shorter retention time on g.l.c. and had a highest accurate mass at m/e 272.2500 which fitted $C_{20}H_{34}$, (expected M 272.2504) - from which four double bond equivalents were calculated. The base peak at m/e 121 together with ions appearing at m/e 107, 123 and 147 were rationalised by invoking 'vinyl' bond cleavages in an isoprenoid compound. There were also ions at m/e 110 and 95 arising from cleavages of the double bonds while cleavage across doubly allylic bonds led to peaks at m/e 135 and 203 (see 235) in accordance with terpenoid fragmentation pattern [191,192]. The ^{13}C n.m.r. spectrum (Figure 3) had resonances (236) closely comparable to those observed for all-trans geranylgeraniol except; a) triplets at δ 113.0, 115.7 and 31.5, b) a singlet at δ 146.2 and a doublet at δ 139.0. These signals were attributed to 2-substituted butadienyl residue that resulted from the dehydration of geranylgeraniol. The assignment shown in (236) to the butadienyl residue was done by reference to assignments published [194a] for buta-1,3-diene (237) and 2,3-dimethylbuta-1,3-diene (238). Both ^{1}H n.m.r. (Fig.4) and i.r. spectra of the sample were superimposable on the ones published for all-trans- β-farnesene (139), a natural product [195] which has also been obtained from base catalysed thermal dehydration [196] of all-trans farnesol (240). The all-trans geometry for β-geranylgeranene was inferred from the 3 : 1 ratio of the singlet signals at δ1.60 and 1.70 assigned to vinyl methyl protons in agreement with Bates' studies [188, 189]. The double doublet resonance observed at δ 6.44 (J 10 Hz) was attributed to the olefinic C_2-proton of the 2-substituted butadienyl residue whose other vinyl protons were

assigned to a singlet signal at δ 5.0 and a multiplet band at δ 5.10 - 5.35. The integration value showed that the multiplet resonance at δ 5.10 - 5.35 included the other three olefinic protons. The absence of a triplet at δ 2.80 observed for doubly allylic protons of α -farnesene (241) [196] together with the observation that the integral value for the multiplet at δ 1.8 - 2.23, assignable to methylene protons of our sample ruled out the presence of α -geranylgeranene (242). The U.V. band at 228 nm, and the sharp singlet band at 1600 cm^{-1} in the i.r. spectrum were characteristic of the conjugated double bond in the buta-1,3-dienyl residue, whereas absorptions at 910 and 1000 cm^{-1} were attributed to a terminal 'vinyl' group. The bands at 1670 and 1385 cm^{-1} provided evidence for the isolated double bonds and 'vinyl' methyl groups respectively.

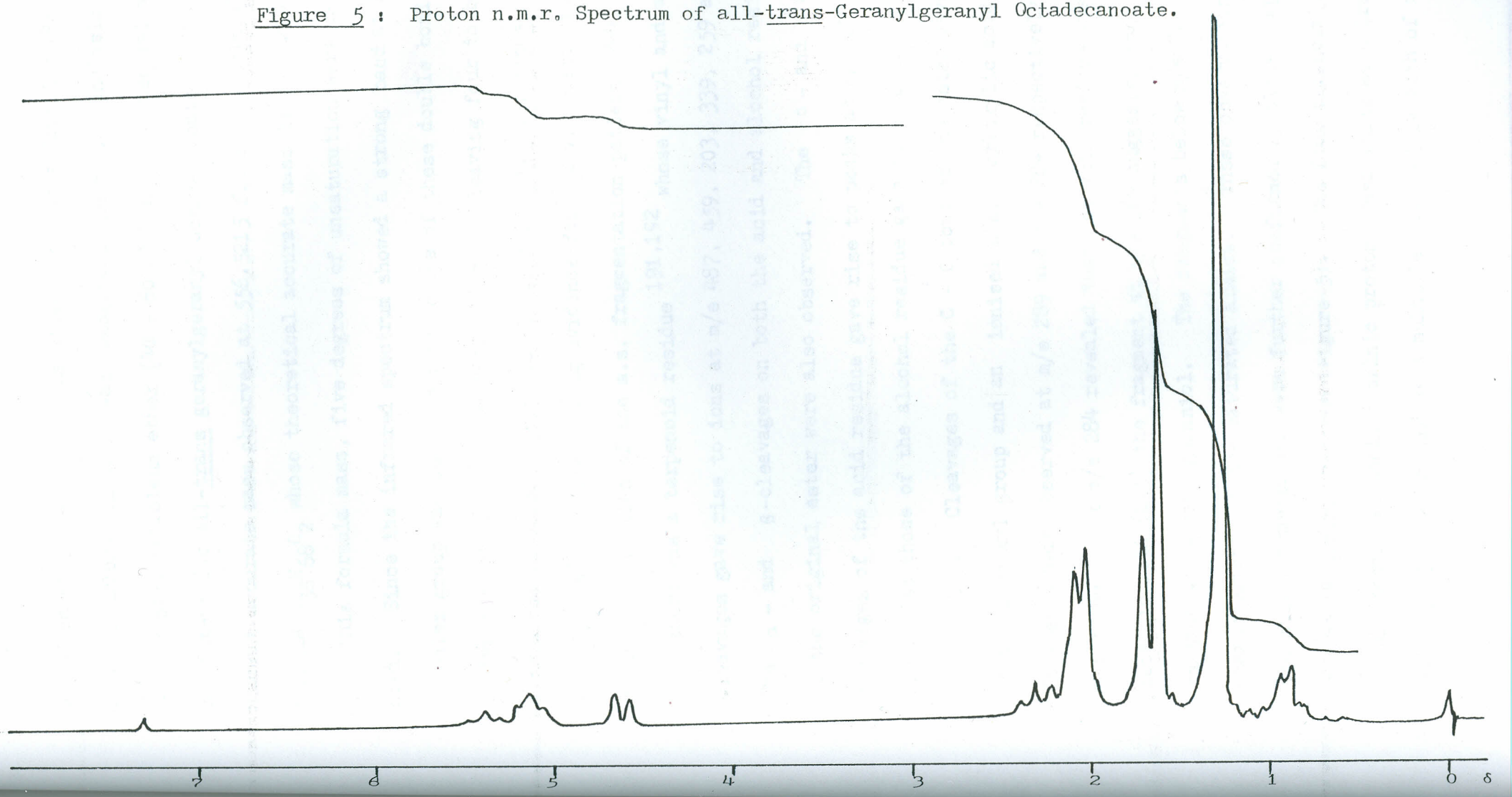
In the course of column purification of all-trans geranyl-geraniol from the distillate, a fraction eluted with a 3 : 1 mixture of petroleum ether (40 - 60°C) and diethyl ether was further re-chromatographed on another silica gel column, using a 3 : 1 mixture of petroleum ether (40 - 60°C) and diethyl ether to obtain a t.l.c. (silica gel,25% diethyl ether in petroleum ether)homogeneous pale yellow oil whose spectral data were closely comparable to those published for all-trans farnesylacetone (220)[171]. Moreover its ^1H n.m.r. and i.r. spectra were superimposable on those reported for geranylacetone. The accurate mass observed at m/e 262.2298 was attributed to the molecular formula $C_{18}H_{30}O$ for which the theoretical molecular formula is 262.2297. The mass fragmentation pattern of the sample was dominated by isoprenoid cleavages (243) [191,192]. The doubly allylic cleavages gave rise to ions at m/e 69, 137, and 205 attributed to the hydrocarbons and those at m/e 57, 125 and 193 were assigned to ions containing oxygen. The α -

cleavage influenced by the carbonyl group was evidenced by the ion at m/e 219. The double bond cleavages also gave rise to ions containing oxygen at m/e 152 and 84 as well as the hydrocarbon ions at m/e 110 and 178. Four double bond equivalents were calculated from the molecular mass and the i.r. band at 1710 cm^{-1} showed that one of the double bond equivalents was due to the carbonyl group of the ketone; the rest were attributed to isolated trans trisubstituted double bonds, as was evidenced by the i.r. bands at 1665 cm^{-1} and the 3 : 1 ratio of the integral values for singlet signals in the ^1H n.m.r. spectrum at δ 1.62 and 1.70 associated with 'vinyl' methyl protons. This n.m.r. evidence was in agreement with studies done by Bates [188,189]. The evidence for the methyl alkenyl ketone was provided by the resonance at δ 2.12 attributed to protons of a ketonic methyl group and a triplet at δ 2.40 (J 7 Hz) was assigned to methylene protons alpha- to the carbonyl group. Further evidence for the presence of three C = C double bonds came from the multiplet band at δ 5.0 - 5.25 integrating for three olefinic protons and the multiplet resonance at δ 1.96 - 2.10 integrating for ten allylic protons.

We next turned our attention to an examination of the constituents of the epiphase from the Bixa extraction. The epiphase (5g) was chromatographed on a silica gel column using chloroform as eluant under high pressure to obtain twenty fractions. Further purifications, by both column and thin layer chromatography (t.l.c.) of fractions 1, 2, 6 and 9 using appropriate solvents led to the isolation of all-trans geranylgeranyl octadecanoate (222), all-trans geranylgeranyl formate (221), methyl 9'-cis-1-apobixinal ester (218) and δ -tocotrienol (223).

Fraction one (0.35g) from the initial separation was loaded onto a silica gel column and eluted under medium pressure using

Figure 5 : Proton n.m.r. Spectrum of all-trans-Geranylgeranyl Octadecanoate.

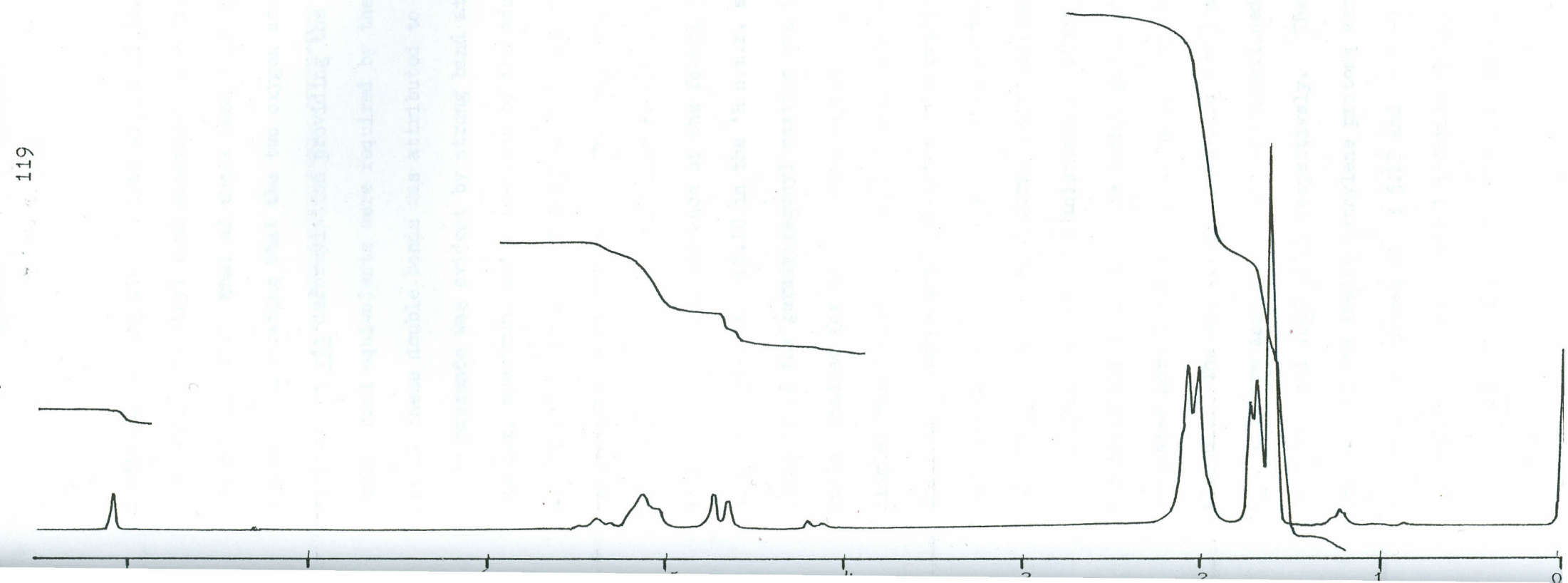


petroleum ether (40 - 60°C) as eluant to give a fraction (42 mg), as a colourless oil which was homogeneous in t.l.c. analysis (silica gel, petroleum ether (40 - 60°C)) and had spectral data consistent for all-trans geranylgeranyl octadecanoate. The highest accurate mass observed at 556.5215 fitted the molecular formula $C_{38}H_{68}O_2$ whose theoretical accurate mass is 556.5219. From this formula mass, five degrees of unsaturation were calculated. Since the infrared spectrum showed a strong band at 1740 cm^{-1} an ester group was suggested; hence one of these double bond equivalents was due to the carbonyl group, leaving four to be attributed to carbon-carbon double bonds. The i.r. bands at 1670 and 1395 cm^{-1} provided strong evidence for methyl substituted double bonds. Inspection of the m.s. fragmentation pattern (244) revealed that there was a terpenoid residue [191,192] whose vinyl and allylic cleavages gave rise to ions at m/e 487, 459, 203, 339, 259 and 297. The α - and β-cleavages on both the acid and alcohol residues of the original ester were also observed. The α - and β-cleavages of the acid residue gave rise to peaks at m/e 239 and 225 whilst those of the alcohol residue gave rise to ions at m/e 272 and 259. Cleavages of the C - O bond of the ester gave rise to an alkenoxyl group and an ionised alkyl carboxylic acid whose fragments were observed at m/e 289 and m/e 284 respectively. While the ion at m/e 284 revealed that the acid residue was octadecanoic acid (245) the fragment at m/e 289 suggested that the alcohol residue was geranylgeraniol. The fragments below m/e 200 were characteristic of the saturated alkane. These observations from the mass fragmentation were further confirmed by close inspection of the ^1H n.m.r. spectrum (Figure 5). The four trisubstituted double bonds had their olefinic protons resonating as a broad triplet at δ 5.35 (J 7 Hz) and as a multiplet at δ 5.14 both of which had

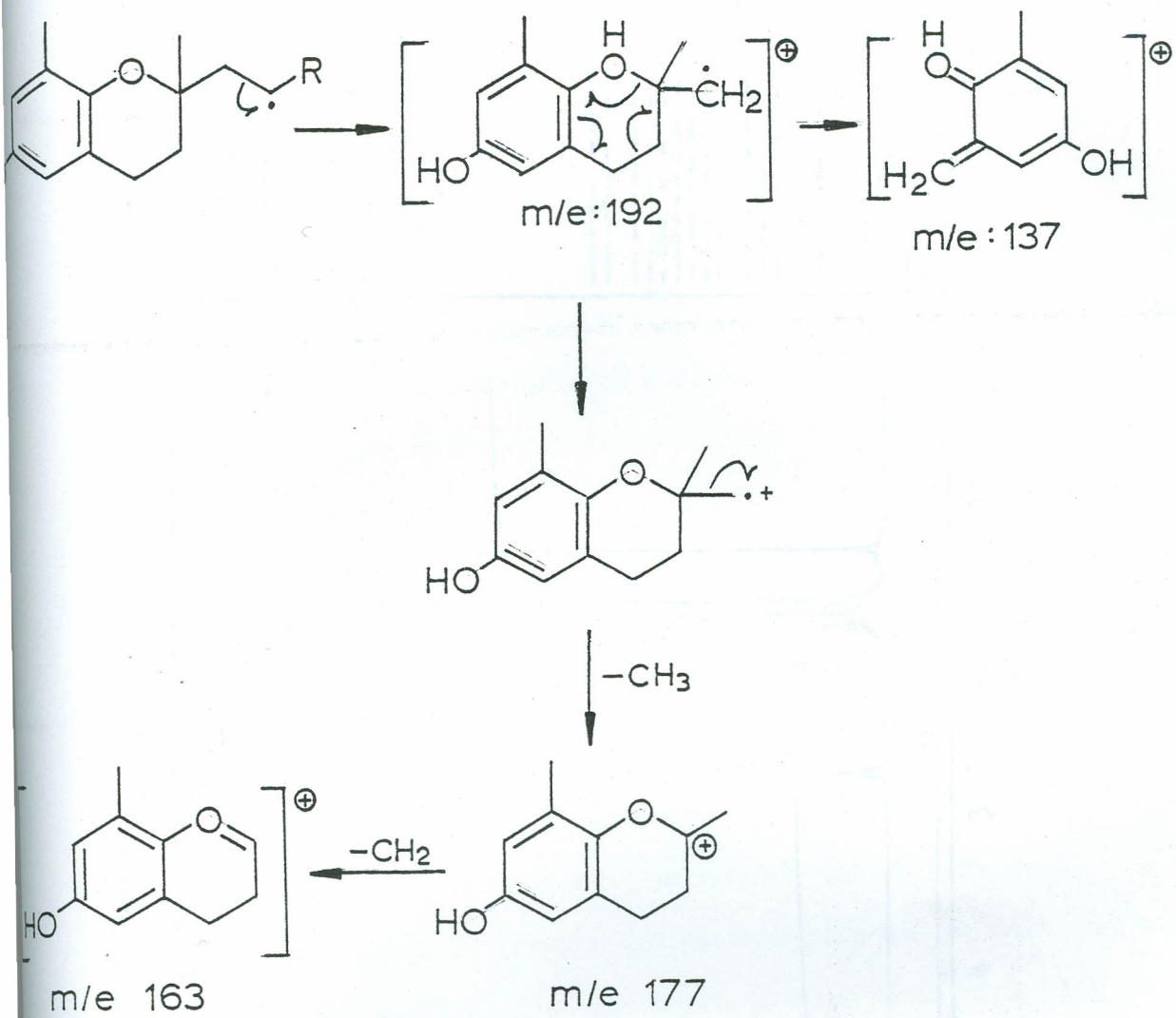
their integral values equivalent to four protons. The all-trans geometry of the alcohol residue was inferred from the 3 : 2 ratio of the resonances at δ 1.61 and δ 1.70 assigned to the vinyl methyl protons respectively, in agreement with Bates' observation [188,189]. A doublet centred at δ 4.62 (J 7 Hz) was assigned to the methylene protons of C_1- of the alcohol residue; the downfield shift for this methylene group is characteristic of the deshielding effect of the ester group. The triplet centred at δ 2.33 (J 7 Hz) was assigned to the group ($CH_2\underline{CH_2}CO_2R$), which reinforced the same observation. The resonance of the rest of the allylic methylene protons was observed as a broad band between δ 2.0 - 2.2 while a broad band centred at about δ 1.26 showed an integration value equivalent to thirtytwo methylene protons of the acid residue. The terminal methyl protons of this residue resonated as a triplet at δ 0.9 (J 6 Hz). The strong i.r. band at 2950 cm^{-1} was further evidence for these alkyl protons.

Fraction two (60 mg) from the initial separation under high pressure liquid chromatographic purification of the epiphase was further purified on a column of silica gel, eluting under medium pressure, successively with petroleum ether (40 - 60°C) and 5% diethyl ether in petroleum ether (40 -60°C). This led to a fraction (26 mg) which was finally purified on a silica gel plate using 10% diethyl ether in petroleum ether (40 - 60°C) as solvent. This then provided a homogeneous band which was scraped off and eluted into acetone to obtain a light yellow oil (16 mg) whose spectral data were consistent with all-trans geranylgeranyl formate (221), a natural product isolated for the first time. The accurate mass observed at m/e 318.2554 fitted the molecular formula $C_{21}H_{34}O_2$ (calculated M, 318.2559) and the m.s. fragmentation pattern (246) was characteristic of a terpenoid compound [191,192]. The bis-allylic bond cleavages

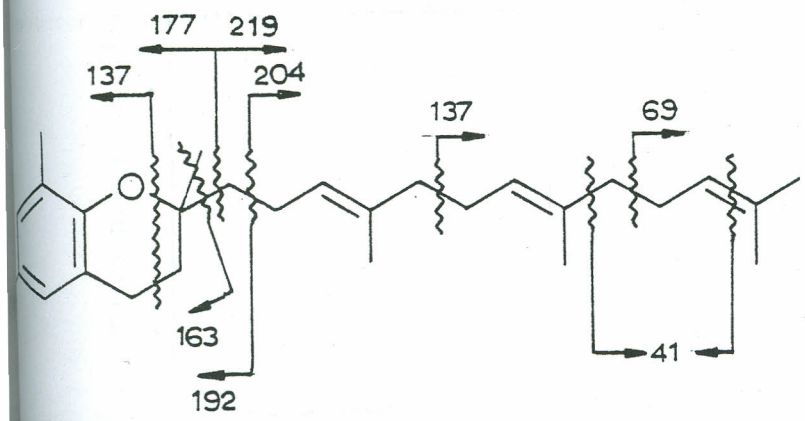
Figure 6 : Proton n.m.r. Spectrum of all-trans Geranylgeranyl Formate.



dominated, giving rise to ions at m/e 69 (the base ion), 136 and 203, while the vinyl bond cleavages gave rise to ions at m/e 55, 83 and 107. Most of these ions corresponded to hydrocarbon fragments indicating that the two oxygen atoms in the formate were easily lost *via* deformylation providing the ion at m/e 272. Five double bond equivalents were required by the molecular formula. One of these double bonds was attributed to the ester group, whose presence was evident by strong band at 1720 and 1170 cm^{-1} in the i.r. spectrum, while the rest of the double bonds were attributed to trisubstituted double bonds evidenced by the weak bands at 1665 and 915 cm^{-1}. The occurrence of methyl substituents on the double bonds was inferred from the strong infrared maximum at 1380 cm^{-1}. The presence of the formyl group was confirmed by a singlet signal at δ8.10 in the ^{1}H n.m.r. spectrum (Fig.6). The all-*trans* geometry of the geranylgeranyl residue was inferred from the 3 : 1 : 1 ratio obtained for the integral values of the protons of vinyl methyl singlet resonances at δ1.60, 1.70 and 1.72 in the ^{1}H n.m.r. spectrum. This was in agreement with Bates' observation [188,189] and our own analysis of the ^{1}H n.m.r. spectrum of authentic geranyl formate and all-*trans* geranylgeranyl formate synthesised in our laboratory. It was observed that the anisotropic effect of the formyl group deshields the protons of the methyl group on C_3. These protons resonated more downfield than the corresponding ones on the free alcohol. The doublet at δ 4.70 (*J* 7 Hz) and the triplet at δ 5.4 (*J* 7 Hz) were associated with the underlined protons in the groups; $CHC\underline{H}_2OCHO$ and $:C\underline{H}CH_2OCHO$ respectively. The other three olefinic protons and the twelve methylene protons were associated with multiplet resonances at δ 5.12 and δ 1.90 - 2.20 respectively. To confirm all the spectral information and the structure of the new natural product, both geranyl formate (229) and all-*trans*



Scheme 47



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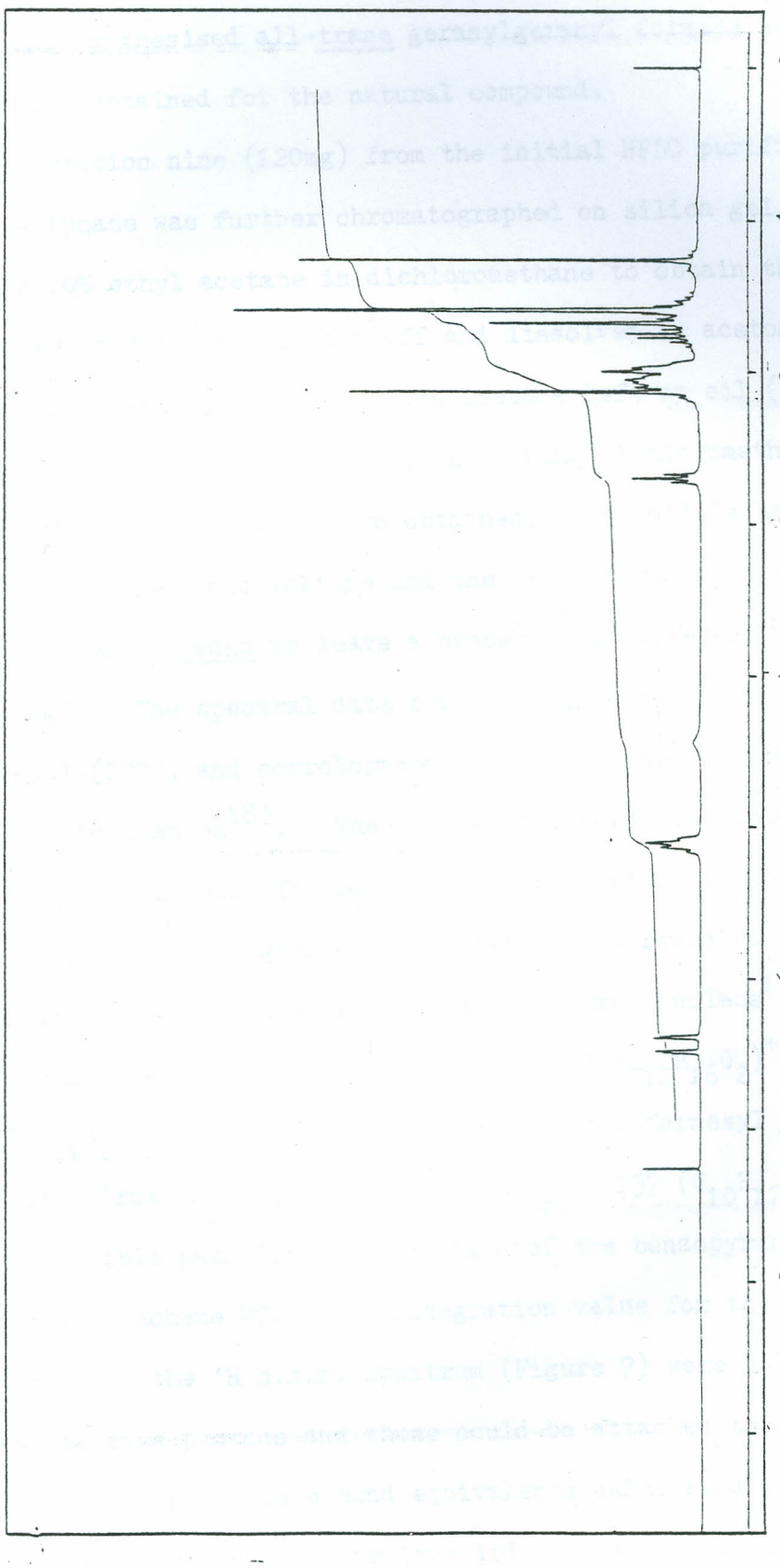
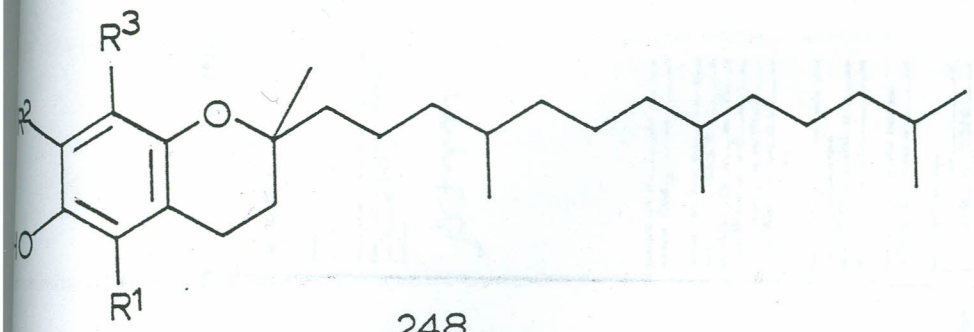


Figure 7 : Proton n.m.r. Spectrum of -Tocotrienol.

geranylgeranyl formate were synthesised by reacting the corresponding alcohols with acetic-formic anhydride[194b]. Inspection of their 'H n.m.r. spectra confirmed that the natural sample has the all-trans geometry and the 'H n.m.r., i.r. and m.s. spectral data for the synthesised all-trans geranylgeranyl formate were identical to those obtained for the natural compound.

Fraction nine (120mg) from the initial HPLC purification of the epiphase was further chromatographed on silica gel plates using 10% ethyl acetate in dichloromethane to obtain three bands. The second band was scraped off and dissolved in acetone. Filtration, followed by removal of the acetone left an oil (90 mg) which was finally purified by t.l.c., now using dichloromethane as solvent and three bands were obtained. The middle band was scraped off and eluted into acetone and the extract was filtered and evaporated in vacuo to leave a homogeneous pale-yellow coloured oil (25 mg). The spectral data for this oil were consistent with α-tocotrienol (223), and corroborated those published for the natural and synthetic samples[185]. The highest accurate mass observed was at m/e 396.3042 which fitted $C_{27}H_{40}O_2$ (requiring M, 396.3028). Close analysis of the fragmentation pattern (247) revealed the presence of a terpenoid [191,192] side chain on a benzopyran nucleus[193]. The chroman nucleus showed prominent peaks at m/e 192 $(C_{12}H_{16}O_2)^+$, 163 $(C_{10}H_{11}O_2)^+$, 177$(C_{11}H_{13}O_2)^+$, and 137 $(C_8H_9O_2)^+$, while the farnesyl side chain was evident from the peaks at m/e 69 $(C_5H_9)^+$, 137 $(C_{10}H_{17})^+$ and 204 $(C_{15}H_{24})^+$. The possible path for fragmentation of the benzopyran nucleus is illustrated in Scheme 47. The integration value for the olefinic proton signals in the 'H n.m.r. spectrum (Figure 7) were collectively equivalent to five protons and these could be attached to six double bonds of the total eight double bond equivalents calculated from the molecular formula. Since the i.r.spectrum did not reveal the presence of a carbonyl

group, the other two double bond equivalents were attributed to the two cyclic systems for which both the mass fragmentation pattern and the ultraviolet absorption maximum at 197 nm ε (ethanol) 2100 suggested a hydroxylated benzopyran system. The evidence for the presence of a hydroxyl group substituent on the chroman also came from the sharp band at 3625 cm^{-1} in the infrared spectrum, while the phenyl group and the isolated trisubstituted double bonds were attributed to the bands at 920, 1390, 1485, and 1620 cm^{-1}; a C - O stretching frequency was also observed at 1155 cm^{-1}. The ^1H n.m.r. spectrum displayed doublet (J 2 Hz) signals at δ 6.30 and δ 6.47, which were easily assigned to the two meta-protons on the phenyl ring which was also established as having a methyl group substituent by the presence of a singlet at δ 2.12. Since the two phenyl protons were meta- to each other the methyl and hydroxyl groups must also be meta- to one another. The study of the biogenesis [184] of chromans suggests that methylation initially occurs at C_8 and therefore the hydroxyl group must be at C_6. The broad triplet resonance at 2.69 (J 6 Hz) and the double doublet signal at δ1.7 (J 6 Hz) were assigned to the C_3 and C_4 pyran methylene protons respectively. The assignment of these protons was aided by an irradiation experiment. Irradiation of the protons at δ 2.69 made the resonance at δ 1.70 become a doublet confirming the interaction between the methylene protons at C_3 and C_4. The singlet resonance at δ1.26 was characteristic of C_2 methyl protons. The α-configuration at C_2 was deduced from comparison with the published [185] optical rotation, $[\alpha]_D^{20^\circ C}$ + 22.15° of a natural sample with our observed figure of $[\alpha]_D^{20^\circ C}$ + 20.5°. The ^1H n.m.r. study confirmed the presence of the C_2 substituent as the farnesyl group, which then accounted for the remaining three double bond equivalents. The broad band between δ 5.0 - 5.20, integrating for three protons was characteristic of a terpenoid group.



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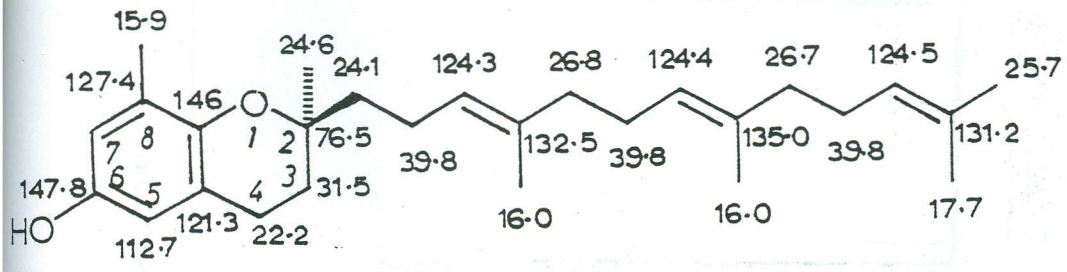
Type	α	β	γ	δ
R^1	Me	Me	H	H
R^2	Me	H	Me	H
R^3	Me	Me	Me	Me



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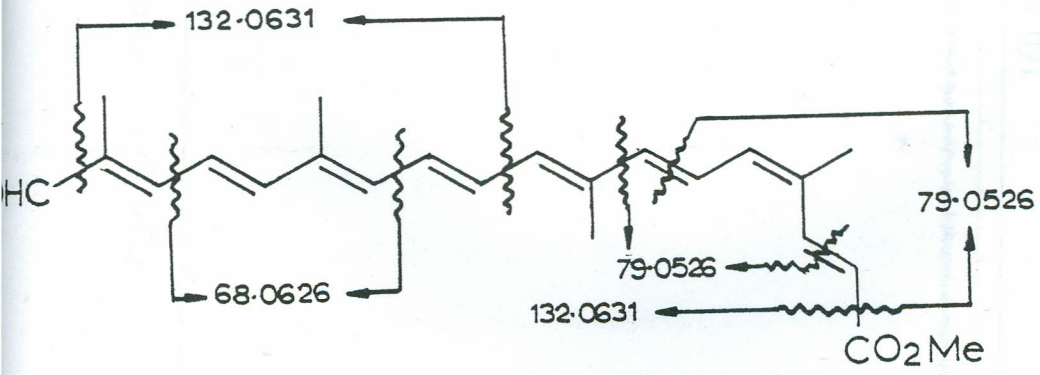

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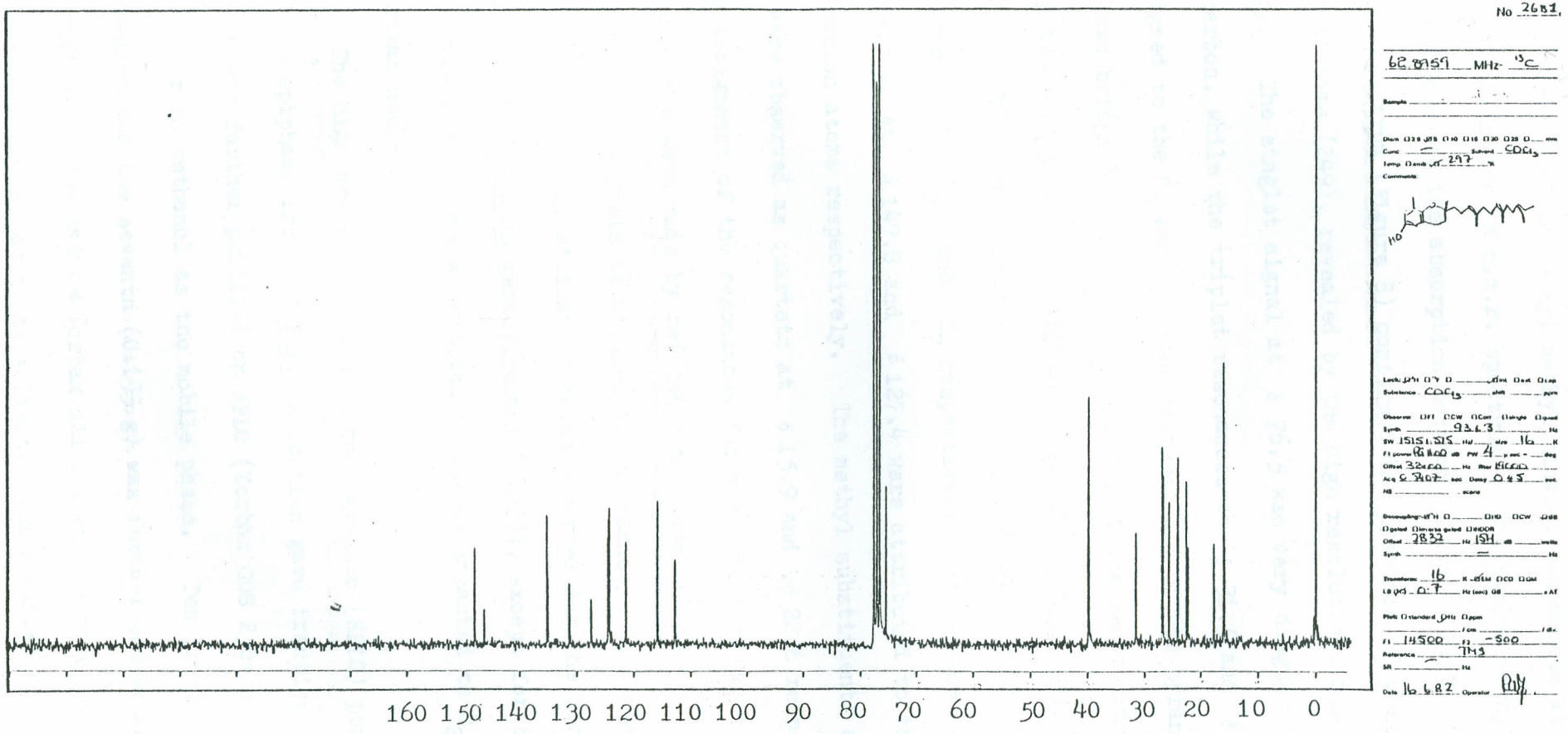
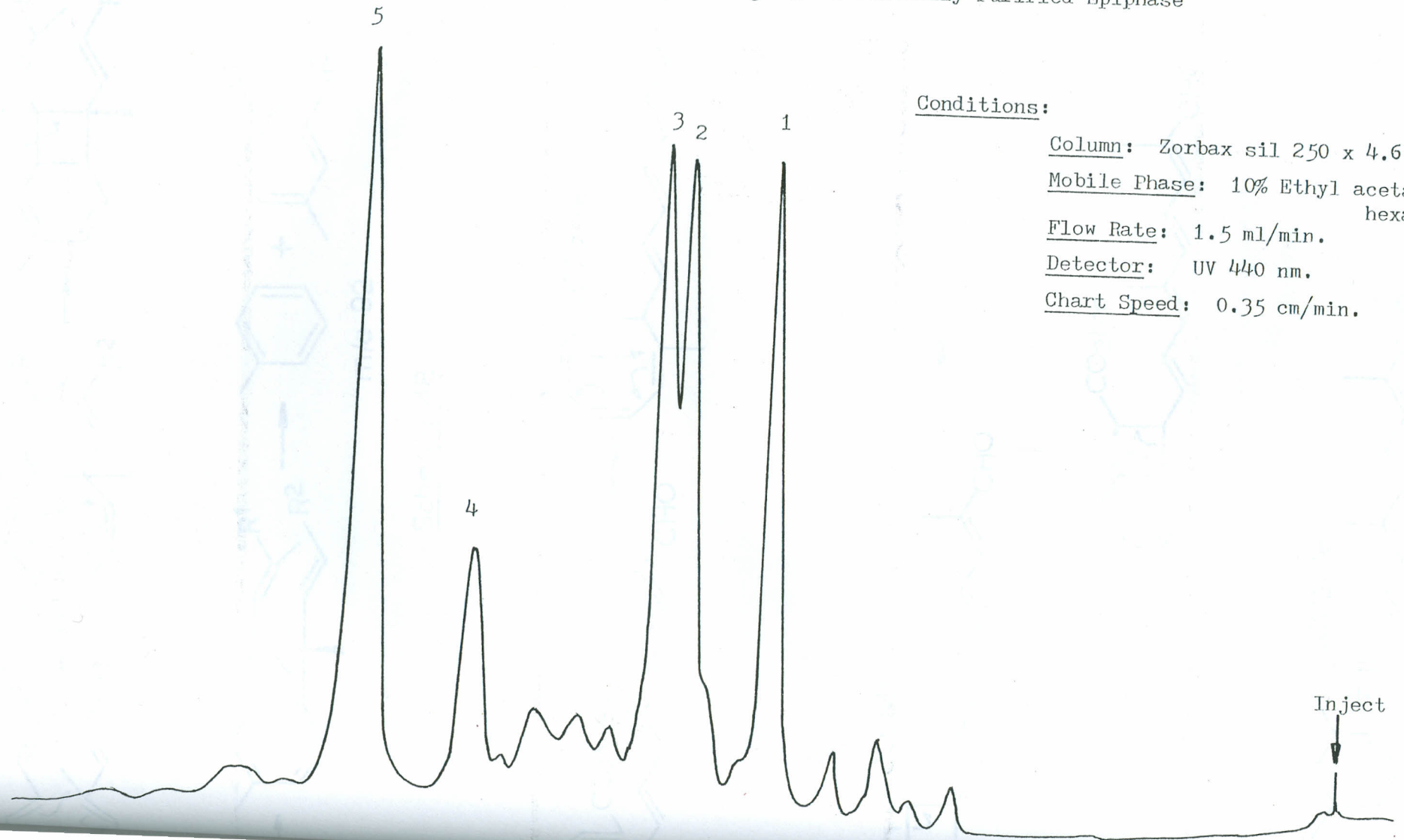
Figure 8 : Carbon-13 n.m.r. Spectrum of -Tocotrienol



The all-trans geometry of the farnesyl side chain followed from the 3 : 1 ratio of the vinyl methyl singlet resonances at δ 1.59 and δ 1.68 in the ^{1}H n.m.r. spectrum. The twelve methylene protons were assigned to the absorption between δ 1.96 - 2.10. The ^{13}C n.m.r. spectrum (Figure 8) confirmed the presence of twenty-seven carbon atoms (249), revealed by the high resolution mass spectrometry. The singlet signal at δ 76.5 was very diagnostic for the C_2-carbon, while the triplet resonances at δ 31.5 and δ 22.5 were assigned to the C_3 and C_4 atoms respectively of the pyran residue. The two bridge carbon atoms C_1, and C_4, of the benzopyran system were assigned to singlet signals at δ 146.0 and δ 121.3 respectively. The doublet resonances at δ 112.7 and δ115.7 could be assigned to C_5 and C_7 respectively, whereas the singlet resonances at δ 147.8 and δ 127.4 were attributed to the C_6 and C_8 carbon atoms respectively. The methyl substituents at C_8 and C_2 were observed as quartets at δ 15.9 and δ 22.6 respectively. The assignment of the resonances for the benzopyran systems and its substiuents were made by reference to those published for vitamin E[197] (248). The signals attributed to the farnesyl side chain shown in (249) were closely similar to those observed for the corresponding portion of all-trans geranylgeraniol (233), except for the signal at δ 24.1 which was attributed to carbon attached to C_2 of the chroman nucleus.

The high pressure liquid chromatographic (HPLC) purification of the epiphase from the Bixa extraction gave fraction six (0.26g) which was further purified on HPLC (Zorbax ODS 25cm x 9.4 mm) using 5% water in methanol as the mobile phase. Ten fractions were obtained and the seventh (0.133 g) was further subjected to HPLC purification, now using a Zorbax sil column (25 cm x 4.6 mm) and 10% ethyl acetate in hexane as solvent. This resulted in the separation of

Figure 9 : High Pressure Liquid Chromatogram of Partially Purified Epiphase



Conditions:

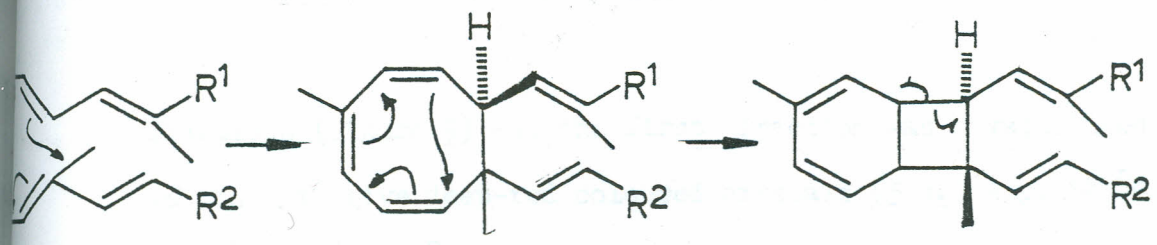
Column: Zorbax sil 250 x 4.6 mm

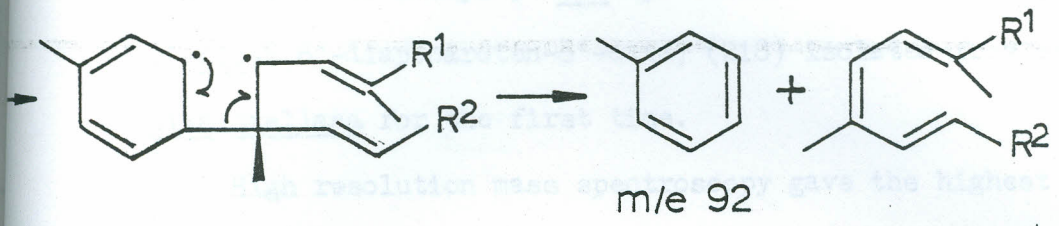
Mobile Phase: 10% Ethyl acetate in hexane.

Flow Rate: 1.5 ml/min.

Detector: UV 440 nm.

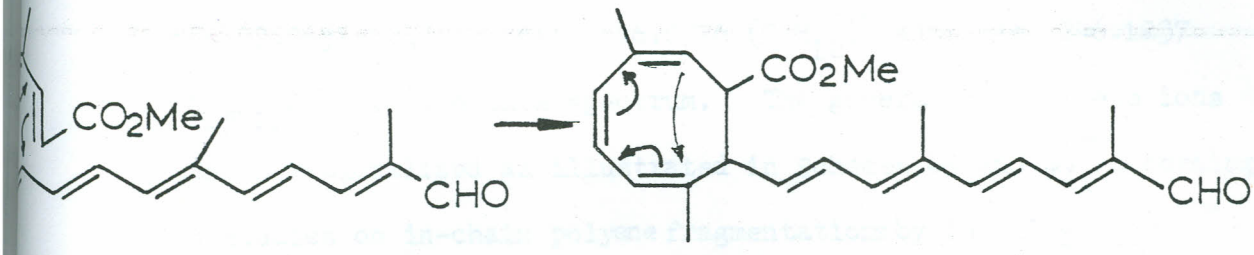
Chart Speed: 0.35 cm/min.

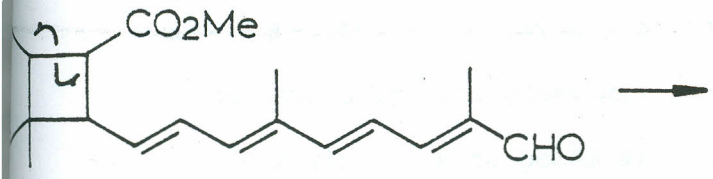


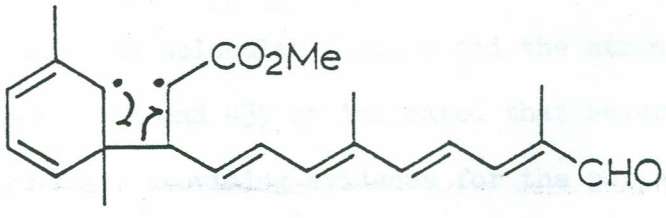


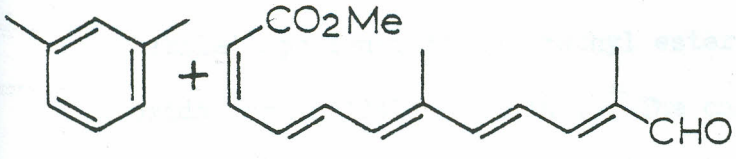
m/e 92

Scheme 48







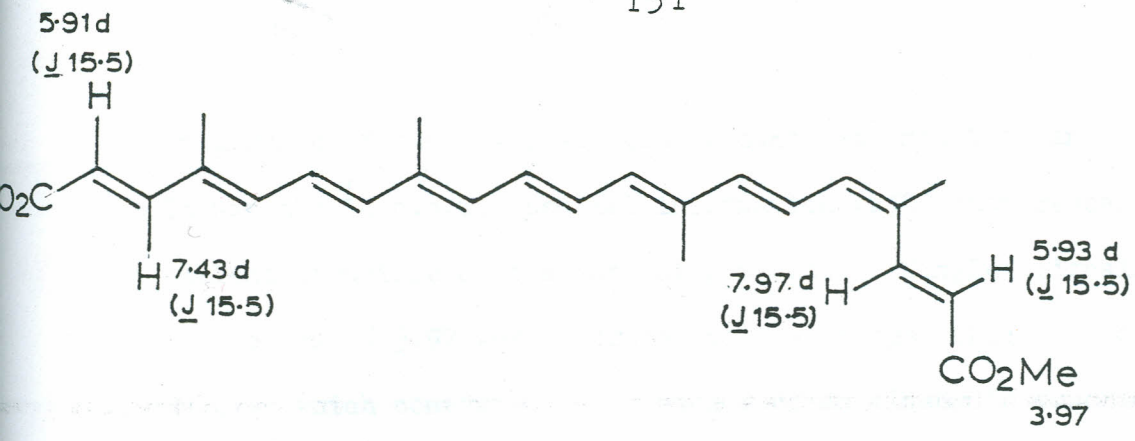


m/e 106 m/e 246

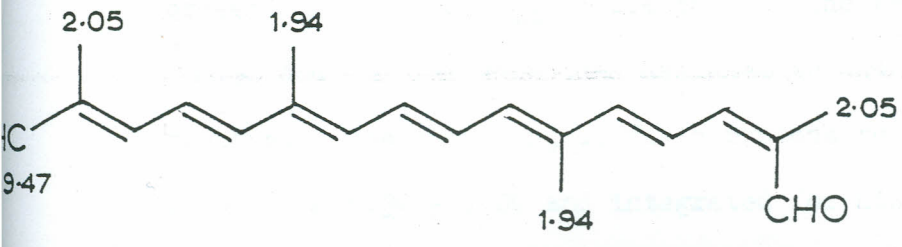
Scheme 49

fractions (Figure 9) and the first fraction was crystallised from methanol to give deep-red coloured crystals (5 mg) m.p. 145°C (lit.[87] 148 - 149°C). The spectroscopic data obtained were conclusive for methyl 9'-cis-apo-1-bixinal ester (methyl 8-oxo-9'-cis-8,6'-diapocaroten-6'-oate) (218) isolated from seeds of Bixa orellana for the first time.

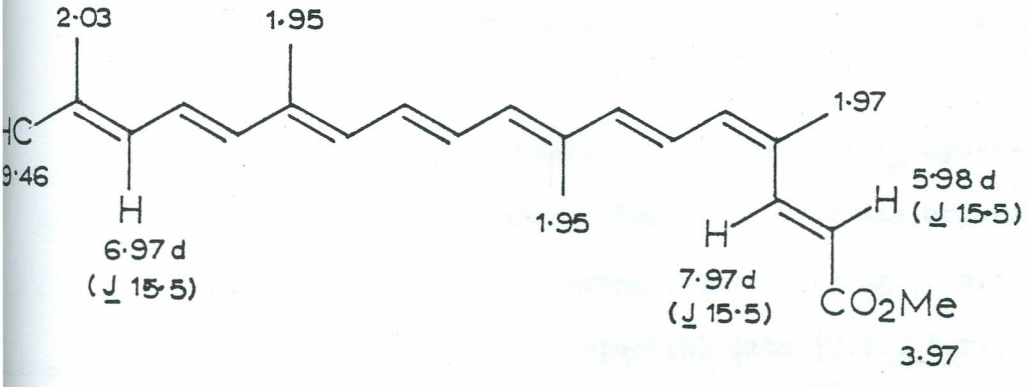
High resolution mass spectroscopy gave the highest accurate mass as the base peak at m/e 352.2042 fitting the molecular formula, $C_{23}H_{28}O_3$, requiring M 352.2038. The polyene nature of the sample was evident from the presence of fragment peaks at m/e 92.0631 $(C_7H_8)^+$, m/e 106.0776 $(C_8H_{10})^+$ and m/e 246.1237 $(C_{15}H_{18}O_3)^+$ in the mass spectrum. The generation of these ions can be rationalised as illustrated in Schemes 48 and 49 according to studies on in-chain polyene fragmentations by Enzell et al.[198] Thermal extrusion of toluene and meta-xylene seem to have been triggered by the 9'-cis double bond. The fragmentation influenced by the two carbonyl end groups was also observed (see 250). The α -, β -, and δ - cleavages of the carbonyl end groups occurred simultaneously with cleavages of the in-chain, single and double bonds giving rise to peaks at m/e 68.0626 $(C_5H_8)^+$, m/e 79.0526 $(C_6H_7)^+$ and m/e 132.0631 $(C_{10}H_{12})^+$. Ten double bond equivalents were calculated from the molecular formula and the strong ultra-violet bands at 497, 462 and 439 nm indicated that several bonds were conjugated, hence, providing evidence for the polyene character of the natural product. These bands compared favourably with those published by Barber et al.[87] and Karrer et al.[98] for their 'labile' apo-1-norbixinal methyl ester prepared by permanganate oxidation of 'labile bixin'. The carbonyl functionality in the sample gave rise to an infrared maximum at 1710 cm^{-1}, whereas the conjugated carbon-carbon double bonds were associated with the


5·91 d
(J 15·5)
H
O2C
H 7·43 d
(J 15·5)
7·97 d
(J 15·5)
H
H
5·93 d
(J 15·5)
CO2Me
3·97

251


2·05
1·94
C
9·47
2·05
1·94
CHO

252


2·03
1·95
C
9·46
H
6·97 d
(J 15·5)
1·95
1·97
H
5·98 d
(J 15·5)
H
7·97 d
(J 15·5)
CO2Me
3·97

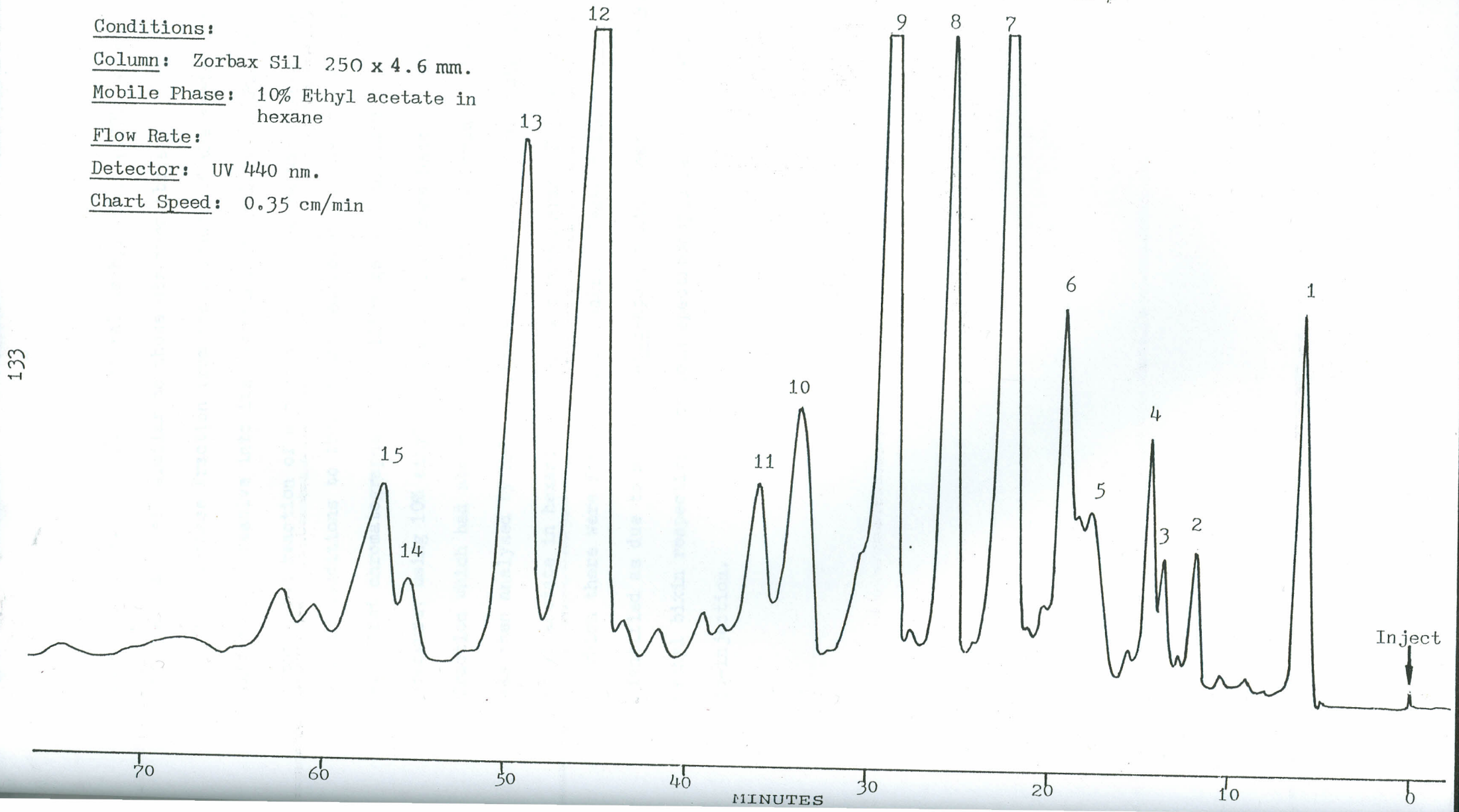
253

occurrence of the C = C stretching bands at νmax 1665 and 1610 cm^{-1}. It was the ^1H n.m.r. spectral information (253) that revealed the salient structure of the natural product. Singlet signals at δ 9.46 and δ 3.97 were ascribed to the groups OHC:C- and CO_2Me which confirmed the presence of an aldehydic functionality and an ester group respectively. The occurrence of the doublet signal at δ 7.97 (J 15.5 Hz), we observed for the $C_{8'}$-proton of 9'-cis methyl bixin (251) led us to the conclusion that the sample was a degradation product of 9'-cis-methyl bixin with the preservation of the cis-double bond. The remaining doublets at δ5.98 and δ 6.97 were then assigned to the $C_{7'}$- and $C_{10'}$-olefinic protons. The remaining olefinic protons resonated as a multiplet between δ 6.30 - 7.00 and integrated for nine protons. The singlet resonance at δ1.95 integrated for six protons and was associated with the vinyl methyl groups at C_{13} and $C_{13'}$, while the singlets at δ 2.03 and 1.97 were associated with the vinyl methyl groups at C_9 and $C_{9'}$ respectively. These assignments were made with reference to those published for 8,8'-diapocarotene-8, 8'-dial (252) [199] and those observed by us for the natural methyl bixin (251).

During the purification of methyl 9'-cis-apo-1-bixinal ester by HPLC Zorbax sil column, fraction three was crystallised from methanol to obtain glittering crystals (3 mg); m.p. 162°C (Lit. [88], m.p. 163°C) and afforded spectral data (U.V., i.r., ^1H n.m.r. and ms) which corroborated those published [88] and observed by us for natural methyl bixin (177b).

The bixin fraction obtained from the extraction of the seeds was homogeneous on t.l.c. (20% ethyl acetate in dichloromethane) and HPLC (Zorbax ODS 25 cm x 9.4 mm, dichloromethane-ethyl acetate-methanol 3 : 1 : 1). A portion of the sample was methylated [194b] with

Figure 10 : High Pressure Liquid Chromatogram of Partially Purified Hypophase.



dimethyl sulphate to obtain natural methyl bixin (177b) whose spectral data were similar to those observed above.

The hypophase fraction from the extracts of the seeds proved difficult to resolve into its components by t.l.c. or HPLC, and thus a methylation reaction of a portion of the sample was carried out under similar conditions to those described above. The methylated sample was first chromatographed on a silica gel column under medium pressure, using 10% ethyl acetate in dichloromethane to obtain a fraction which had absorption in the visible spectrum. The fraction was then analysed by HPLC (Zorbax sil, 25 cm x 4.6 mm) using 10% ethyl acetate in hexane to obtain a chromatogram (Figure 10) in which there were fifteen major peaks. Peaks 8 and 9 were identified as due to methyl 9'-cis-apo-1-bixinal ester and natural methyl bixin respectively by both spectroscopic data and HPLC co-injection.

Chapter II.

Experimental

Materials and General Information:- Dry seeds of Bixa orellana were obtained from the Kenya Cereal Board Authority through the generous assistance of Dr. Dismas A. Otieno.

All the solvents used for extraction and chromatography were purified by standard methods. Silica gel from Merke was used for column and thin layer chromatography. Special precautions against the effects of light, heat, acid, base, peroxides and atmospheric oxygen were taken during handling of the extracts from the Bixa seeds. Samples were kept under nitrogen gas and containers were wrapped with aluminium foil. Solvents were evaporated under vacuum at temperatures below 30°C, and kept in the refrigerator at -10°C. Glass columns and thin layer chromatographic tanks were wrapped with aluminium foil to eliminate light. The high pressure liquid chromatographic separations were carried out using Dupont chromatographic columns; Zorbax ODS 250 x 4.6 mm, 250 x 9.4 mm, Zorbax Sil 250 x 4.6 mm and Water's Pre Pak 500 sil, on a Waters HPLC instrument.

Gas liquid chromatographic analysis were carried out on glass columns (2M x 5 mm) packed with 3% OV-1, on Chromosorb B, 60 -80 mesh fitted to a Pye Unicam 104 GCD instrument. The oven temperatures were varied between 100°C and 210°C while the injector and flame ionisation detectors were kept at 250°C. The carrier gas, nitrogen, was kept at 30 ml/min , the recorder chart speed was tuned to 5 mm/min, and the detector was attenuated at 16 x 10^2. Dilute solutions (0.3 µl) in an appropriate solvent were injected onto the column. Preparative gas liquid chromatographic separations were run on an aluminium column (10 M x 5 mm) packed with 7.5% KOH in Carbowax 20M on Chromosorb W, 60 - 80 mesh. The column temperature was kept at 200°C while those of the detector (flame ionisation) and injector were kept at 250°C. The flow rate of the carrier gas

(nitrogen) was 30 ml/min while the recorder chart speed was kept at 4.2 mm/min with the detector attenuation at X2. The sample size used was 5 µl of a dilute solution, and the instrument used was an Autoprep Model B-700.

The structures of the purified compounds were determined by spectroscopic data. High resolution mass spectral data were obtained through direct insertion of samples into the ion chamber kept at 200°C and the electron acceleration energy of 70 eV. The instrument used was a VG Micromass 7070E or A.E.I. MS902. The infrared absorption, presented in cm^{-1} were from a Perkin-Elmer infrared spectrophotometre 710B, using dilute solutions in chloroform or film, while the visible and ultraviolet spectroscopic data, presented in nm, were recorded from benzene, ethanol or hexane dilute solutions using a Unicam SP 800 spectrophotometer. The ^{1}H n.m.r. and ^{13}C n.m.r. spectral data were obtained for dilute solutions in deuteriochloroform in the presence of trimethylchlorosilane (TMS) as internal standard. The spectrometers used were either a 90 MHz or a 250 MHz. Data are presented in delta (δ) values from TMS and the resonances are described as singlet, doublet, triplet, multiplet or broad abbreviated as S, d, t, m, or br respectively. The coupling constants are quoted in Hertz (Hz). Finally optical activity was obtained from Optical Activity AA-10 Equipment, Serial number 79-08-30/A100, using 1dm tube.

Preparation of Oleoresin, Bixin fraction, Epiphase and Hypophase from Seeds of Bixa orellana:-

The dry commercial seeds (800 g) were shaken under distilled hexane (1.6 litres) for four hours at room temperature under nitrogen gas with exclusion of light. The solution was decanted, and the seeds were further shaken under hexane (0.8 litre) for two hours, then decanted. The combined hexane extracts were filtered and

the solvent was then removed in vacuo to leave a deep red coloured oleoresin (12.2 g).

The post-hexane extracted seeds were shaken under 1.6 litres of a 1 : 1 mixture of methanol and dichloromethane for four hours at room temperature with the exclusion of light. The extract was decanted and the seeds were again similarly treated, but using 0.6 litre of the same solvent system for two hours. The remaining seeds had no pigments on them and were therefore discarded. The combined extracts were filtered and the solvents were then evaporated in vacuo to half their original volume. The concentrated extract was allowed to stand overnight at -10°C whereupon a deposit of deep red-coloured crystals (11.0 g) formed. The crystals were filtered off, and washed with cold methanol before recrystallisation from acetic acid to obtain bixin fraction (5 g) m.p. 195°C (Lit.[83] m.p. 196°C); λ max (benzene) 503 (ε = 11500), 470 (ε = 12500), and 444 (ε = 83,400) nm.; ν max 3400 (broad), 1720, 1660, 1600, 1385, 1300 and 900 cm^{-1}; δ 7.80 (d, J 15.8, :CH), 7.22 (d, J 15.8, :CH), 6.30 - 7.00 (m, 10H, :CH), 5.8 (d, J 15.8, :CH), 5.68 (d, J 15.8, :CH), 3.66 (S, OMe), 1.80 - 2.00 (m, 12H, $:CCH_3$); m/e 394 ($C_{25}H_{30}O_4$).

The filtrate from the bixin separation above, was further concentrated to 400 mls and then extracted with hexane (4 x 400 mls) to obtain two layers called 'epiphase' and 'hypophase'. The phases were evaporated under vacuum to leave a red-coloured solid (6.15 g) as epiphase fraction, and a red-coloured thick pasty material (30g) as hypophase fraction.

Preparation of Methyl Bixin from Natural Bixin:-

Bixin (1.0 g) was dissolved in a 1 : 1 mixture (30 mls) of methanol and benzene. The resulting solution was then added to a suspension of potassium hydroxide pellets (0.5 g) in anhydrous methyl acetate (30 mls). Dimethyl sulphate (0.5 g) was added to the suspension, and the mixture was then allowed to stand overnight at room temperature, whereupon bright red coloured crystals grew. The crystals were filtered off, washed with cold methanol and recrystallised from methanol to give the methyl bixin as crystals (0.8 g, 80%), m.p. 161°C (Lit.[88] m.p. 163°C); λ max (benzene) 501 (ε = 109,900), 469 (ε = 124,000), 442 (ε = 84,000) nm; ν max ($CHCl_3$), 1700, 1660, 1600, 1280, 1125, 1000, 985, 900 and 860cm^{-1}; δ 7.97 (d, J 15.5, :CH), 7.4 (d, J, 15.5, :CH), 6.30 - 7.00 (m, 10H, :CH), 5.93 (d, J 15.5, :CH), 5.89 (d, J 15.5, :CH), 3.80 (S, OMe), 3.79 (S, OMe), 1.95 and 2.0 (S, 12H, :CCH_3); m/e 408.2313, $C_{26}H_{32}O_4$, requires M, 408.2301.

Methylation of Hypophase Fraction:-

Using a similar procedure the hypophase (1.0g) was methylated by dimethyl sulphate (1.0 g) in the presence of potassium hydroxide (1.0 g) to obtain a sample (0.5 g) which was then chromatographed on a silica gel column using 10% ethyl acetate in dichloromethane to obtain fraction 3 (0.3 g), which showed visible spectral absorption above 400 nm. The sample was then analysed and preparatively fractionated by HPLC using a Zorbax sil column (25 cm x 4.6 mm) and 10% ethyl acetate in hexane as the solvent system. Fifteen main peaks (see Figure 10) resulted. Peaks 8 and 9 were collected and shown by spectroscopic data and coinjection to be due to methyl 9'-cis-apo-1-bixinal ester (218) and natural methyl bixin (177b) respectively.

Gas Liquid Chromatographic Analysis of the Oleoresin:-

Oleoresin (10 mg) was dissolved in hexane (10 mls) and 0.3 µl of the solution was injected into a glass column (2 M x 5 mm) containing 3% OV-1 on Chromosorb B, 60 - 80 mesh. The column, detector and injector temperatures were kept at 210°C, 250°C and 270°C respectively, while the carrier gas (nitrogen) swept through the column at 30 ml/min. The hydrogen gas and the air pressures were kept at 1.4 Kg/cm^2 and 1.18 Kg/cm^2 respectively. An isothermal run produced a chromatogram containing seven peaks (Figure 1), with the sixth peak showing a retention time of 10 minutes. This peak comprised over 50% of the total area of the peaks and was shown to be due to all-trans geranylgeraniol.

Isolation and Characterisation of Farnesylacetone, all-trans Geranylgeraniol, and β-Geranylgeranene:-

Oleoresin (3 g) was distilled at 0.6 mm Hg and a fraction (1.56 g) boiling between 130 - 170°C was collected, leaving a dark residue (1.07 g). The distillate (1.1 g) was chromatographed on a silica gel column, eluting with petroleum ether (40 - 60°C) (50 ml), then 30% diethyl ether in petroleum ether (40 - 60°C) (150 ml) to give a fifth fraction as a pale yellow oil (23 mg) which was homogeneous on thin layer chromatography (t.l.c.), (silica gel, 30% diethyl ether in petroleum ether (40 - 60°C)). The fraction showed spectral data consistent with farnesylacetone (220); λ max (ethanol) 211 nm, ν max ($CHCl_3$), 2930, 1715, 1665, 1440, 1390, and 1140 cm^{-1}; δ 1.62 (S, 9H, $:CCH_3$), 1.70 (S, $:CCH_3$), 1.96 - 2.10 (br.m., 10H, $-CH_2-$), 2.40 (t, J 7, $-CH_2CO$), 2.12 (S, CH_3CO), 5.0 - 5.25 (m, 3H, :CH); m/e 262.2298, $C_{18}H_{30}O$, requires M 262.2297; other fragmentation ions observed are:

m/e	55	57	69	71	83	84	110	111	123	125
% abundance	33	18	100	9	11	10	3	3	16	19

m/e	137	139	151	152	179	191	193	205	219	262
% abundance	17	3	4	1	2	2	4	2	3	5

Further elution of the above column, first with 40% diethyl ether in petroleum ether (40 - 60°C) (50 mls) and finally with diethyl ether (200 mls) gave a yellow oil (780 mg) as the fifteenth fraction. A portion of this fraction (80 mg) was finally purified by streaking on silica gel plates(20 x 20 x 0.025 cm) and developing with 30% diethyl ether in petroleum ether (40 - 60°C) to obtain a homogeneous middle band which was eluted into acetone. The extract was filtered and the solvent was evaporated under vacuum to give a pale yellow oil (50 mg), found to be homogeneous on g.l.c. and its spectral data were consistent for 3,7,11,15-tetramethylhexadeca (E, E, E)-tetra-2,6,10,14-en-1-ol (219); ν max ($CHCl_3$) 3600, 2930, 1665, 1440, 1390, 1140, 1095, 985 cm^{-1}; δ 5.45 (brt, J 7, H, C - 2), 5.13 (br.m, 3H, :CH), 4.15 (d, J 7, 2 H, C - 1), 1.90 - 2.20 (m, 12 H, $-CH_2-$), 1.62 (S, 9H, $:CCH_3$), 1.70 (S, 6 H, $:CCH_3$), 1.51 (S, OH), δ carbon : 17.7 (q), 25.7 (q), 131.3 (S), 124.4 (d), 39.7 (t), 26.4 (d), 135.0 (S), 16.0 (q), 124.2 (d), 39.7 (t), 26.7 (d), 135.5 (S), 16.0 (q), 123.8 (d), 39.6 (t), 26.8 (t), 139.9 (S), 16.3 (q), 123.4 (d), 59.4 (t); m/e 290.2612, $C_{20}H_{34}O$ requires M 290.2609; other fragmentation ions observed are:

m/e	69	85	117	136	161	173	203	204	221	229
% abundance	100	15	3	60	60	5	50	70	30	10

m/e	247	272	290
% abundance	5	15	10

β-Geranylgeranene (16,11,15-Trimethyl-3-vinylhexadeca (E,E,E)-tetra-1,5,10,14-ene)(234):-

A portion of fraction fifteen (50 mg) obtained as above from the distillate of oleoresin was dissolved in hexane (5 ml) and 5 µl portions were injected into a preparative g.l.c. column (for details see material and general information) and four fractions were collected. The main fraction (25 mg) with a retention time of 24 minutes was shown to be homogeneous in analytical g.l.c. (see material and general information) and showed a retention time of 3 minutes. Its spectral data follow:

λ max (ethanol) 228 (ε = 8,000) nm; ν max ($CHCl_3$) 2930, 1670, 1600, 1445, 1385, 1000, 910 cm^{-1}; δ 6.4 (dd J, 17, 10,1H, C - 2), 5.10 - 5.35 (m, 5 H, :CH and :CH_2), 5.0 (br.S, :CH_2, C - 3), 2.20 - 2.23 (m, -CH_2-), 1.80 - 2.18 (m, 10 H, -CH_2-), 1.70 (S, 3 H, :CCH_3), 1.60 (S, 9 H, :CCH_3); δ carbon: 25.7 (q), 17.7 (q), 131.3 (S), 124.4 (d), 39.7 (t), 26.7 (t), 135.0 (S), 16.0 (q), 124.3 (d), 39.7 (t), 26.7 (t), 135.4 (S), 16.0 (q), 124.1 (d), 26.8 (t), 31.5 (t), 146.2 (S), 113.0 (t), 139.0 (d), 115.7 (t); m/e 272.2500, requires M, 272.2504 and other fragmentations observed are:

m/e	95	107	110	121	123	133	135	137	147	203	272
% abundance	19	21	4	100	5	25	13	5	11	5	10

Quantitative Analysis of Free all-trans Geranylgeraniol in Oleoresin:-

The oleoresin (12.2 g) was calculated to be 1.5% of the dry seeds of Bixa while the distillate was found to be 52% of the oleoresin. This distillate (10 mg) was dissolved in ethyl acetate (10 ml) and 0.2 µl of the solution was injected five times into a g.l.c. column (3% OV - 1 on Chromosorb B, 60 - 80 mesh). The chromatograms were run isothermally at 210°C (for details see under material and general information) and the areas of the seven peaks (Figure 1) were calculated by triangulation and the percentage composition for each peak was calculated from their total areas (Table 1). For example the percentage composition of free geranylgeraniol in the distillate was therefore

$$\frac{623}{780} \times 100 = 88\%.$$

Since the percentage composition of the distillate from the oleoresin was 52%, percentage composition of free geranylgeraniol in the oleoresin was then,

$$\frac{52 \times 88}{100} = 46\%,$$ and the proportion of free geranylgeraniol in dry Bixa seeds was calculated as

$$\frac{1.5 \times 46}{100} = 0.7\%.$$

In a parallel quantitative study, 100 g of Bixa seeds were extracted exhaustively with hexane (200 ml) over a period of 12 hr. The extraction was repeated four times and the extracts were then filtered and the solvent was removed in vacuo to leave oleoresin (1.75 g, 1.75%). A stock solution (5 ml) containing the oleoresin (1.89 mg/ml) and geraniol (3.5mg/ml)as internal standard was prepared in ethyl acetate. The solution (0.2 µl) was injected into a g.l.c. glass column (2 M x 5 mm) packed with 3% OV - 1 on

Chromosorb B, 60 - 80 mesh. The column was swept with nitrogen gas at 30 ml/min, keeping the injector and the flame ionisation detector temperature at 250°C while the oven temperature was programmed from 160°C with 2 minutes initial delay, then allowed to rise at 20°C per minute to finally reach 210°C at which it was kept constant until all the peaks were eluted. The response attenuation was set at 16×10^2 while the chart speed was 0.5 cm/min. The area of the peaks due to geranylgeraniol and geraniol, displaying retention times of 4.5 minutes and 1 minute respectively, were then calculated. The formula [200]

$$i\,\% = \frac{Ai \times Wis}{Ais}$$ where

i% = percentage composition of all-<u>trans</u> geranylgeraniol in stock solution.

Ai = area of peak due to all-<u>trans</u> geranylgeraniol

Wis = percent weight of geraniol in stock solution

Ai = area of peak for geraniol, was used to obtain the percentage composition of all-<u>trans</u> geranylgeraniol in the oleoresin.

The experiment was repeated four times to obtain the data in Table 2. The percentage composition of geraniol in the stock solution was;

$$Wis = \frac{3.5}{3.5 + 1.89} \times 100 = 64.9\%$$

and using the above formula, the percentage composition of all-<u>trans</u> geranylgeraniol in oleoresin was calculated as;

$$i\,\% = \frac{64.9 \times 156 \times 100}{100 \times 174} = 58\%\ .$$

Finally the average proportion of all-<u>trans</u> geranylgeraniol in the oleoresin was found as:

$$\frac{58 + 53 + 63 + 55}{4} = 57\%$$, and this figure was

used to calculate the percentage composition of free all-trans geranylgeraniol in commercial Bixa seeds as follows:

$$\frac{1.75 \times 57}{100} = 0.997\% = 1\% .$$

Peak	1	2	3	4	5	6	7	Total
Area in sq. mm.	42	6	9	11	2	623	15	708
% Composition	6	0.8	1.3	1.6	0.3	88	2	100

Table 1: Areas and percentage composition of peaks of g.l.c. chromatograph of distillate from oleoresin.

Experiment	1	2	3	4
Peak area for geraniol (sq. mm)	174	164	169	178
Peak area for all-trans geranylgeraniol (sq. mm)	156	136	163	169
Percentage Composition of all-trans geranylgeraniol in oleoresin	58	53	63	55

Table 2: Percentage composition of free all-trans geranyl-geraniol in the oleoresin.

Isolation and Structural Elucidation of all-trans Geranylgeranyl Octadecanoate (222), all-trans Geranylgeranyl Formate (221) and δ -Tocotrienol (223): -

The epiphase fraction (5 g) from Bixa seed extraction was dissolved in chloroform (25 mls) and injected into a high pressure liquid chromatographic preparative column (Waters Pre Pak 500 Sil No. 12137767) mounted on a Waters Prep LC System 500. The components of the extract were eluted with freshly distilled chloroform, at 100 ml/min and 20 fractions were detected by the refractive index detector and subsequently collected. The chart speed was tuned at

5 min/cm.

all-trans Geranylgeranyl Octadecanoate (222):- Fraction 1 (0.35g) obtained above, was loaded onto a silica gel column and eluted with petroleum ether (40 - 60°C) under medium pressure (aquarium pump), collecting 20 ml portions. The fifth fraction (42 mg), a colourless oil, was homogeneous on t.l.c. (silica gel, petroleum ether (40 - 60°C) and had the following spectral data; λ max (ethanol) 210 nm, νmax ($CHCl_3$), 1740, 1670, 1120, 2950 and 1395 cm^{-1}; δ 5.35 (t, J 7, :CH), 5.14 (m, 3 H, :CH), 4.62 (d, J 7, $-CH_2O$, 2.0 - 2.2 (m, 12 H, $-CH_2-$), 1.7 (S, 6 H, $:CCH_3$), 1.61 (S, 9 H, $:CCH_3$), 2.33 (t, J 7, $-CH_2CO$), 1.26 (m, 32 H, $-CH_2-$), 0.9 (t, J 6, $-CH_3$); m/e 556.5215, $C_{38}H_{68}O_2$ requires M, 556.5219; other fragmentation ions are:

m/e	202	203	229	239	259	267	272	283
% abundance	65	100	40	10	7	5	60	14

m/e	284	289	297	459	487	556
% abundance	36	3	4	5	3	4

all-trans Geranylgeranyl Formate (221):- Fraction two (60 mg) obtained from initial HPLC purification of the epiphase was loaded onto a silica gel column and eluted under medium pressure (aquarium pump), first with petroleum ether (40 - 60°C) (60 mls), and finally with 5% diethyl ether in petroleum ether (40 - 60°C) (100 mls) to obtain a fraction two (26 mg). This fraction was further purified on a silica gel plate using 10% diethyl ether in petroleum (40 - 60°C) to obtain three bands; the middle band was scraped off and extracted into acetone. The extract was filtered and the solvent

was evaporated *in vacuo* to leave a colourless oil (16 mg) which was homogeneous on t.l.c. [silica gel, 10% diethyl ether in petroleum ether (40 - 60°C)]. The spectral data obtained for the sample were; λ max (ethanol) 210 nm; ν max ($CHCl_3$) 1720, 1665, 1450, 1380, 1170 and 915 cm^{-1}; δ 8.1 (S, OCHO), 5.4 (t, J 7, :CH), 5.12 (m, 3 H, :CH), 4.70 (d, J 7, CH_2O), 1.90 - 2.20 (m, 12, $-CH_2-$), 1.60 (S, 9 H, $:CCH_3$), 1.70 (S, 3 H, $:CCH_3$), 1.72 (S, 3 H, $:CCH_3$); m/e 318.2554, $C_{21}H_{34}O_2$ requires M, 318.2559; other fragmentation ions are:

m/e	52	55	69	83	93	107	121	135
% abundance	21	64	100	25	78	36	36	41

m/e	136	161	203	272	318
% abundance	57	14	11	6	7

Geranyl Formate (229):- A 3-neck flask fitted with a condenser, a stirrer and a dropping funnel was charged with sodium bicarbonate (18.8 g), geraniol (15.4 g) and dry ether (30 mls). To this suspension was added acetic-formic anhydride [194b] (17.6 g) and the mixture was then stirred for 1.5 hr. and filtered. The solvent was evaporated *in vacuo* to leave a pale yellow oil which was distilled to give the ester (14.1 g, 77%) as a colourless oil; b.p. 63 - 4°C at 0.8 mm Hg; λ max (ethanol) 214 nm; ν max (film), 2950, 1725, 1665, 1450, 1380, 1170, 1050, and 915 cm^{-1}; δ 8.10 (S, OCHO), 5.32 (t, J 6, :CH), 5.14 (m, :CH), 4.62 (d, J 7, CH_2O), 1.90 - 2.20 (m, 4 H, $-CH_2-$), 1.72 (S, $:CCH_3$), 1.64 (S, $:CCH_3$), 1.60 (S, $:CCH_3$), m/e 182.1304, $C_{11}H_{18}O_2$ requires M, 182.1307.

all-trans Geranylgeranyl Formate (221):- Following the procedure outlined above, the naturally derived all-trans geranylgeraniol (0.05 g) reacted with acetic formic anhydride (0.035 g) in the presence of sodium bicarbonate (0.03 g) in anhydrous ether (10 mls) to give the crude formate. The ester was purified on a silica gel plate using 10% diethyl ether in petroleum ether (40 - 60°C) to obtain a pale yellow oil (0.04 g, 80%); λ max (ethanol) 211 nm; ν max (film) 2950, 1720, 1660, 1450, 1380, 1170, 1050, and 915 cm^{-1}; δ 8.10 (S, OCHO), 5.40 (t, J 7, :CH), 5.14 (m, 3 H, :CH), 4.70 (d, J 7, $-CH_2O$), 1.90 - 2.20 (m, 12 H, $-CH_2-$), 1.72 (S, 3 H, $:CCH_3$), 1.70 (S, $:CCH_3$), 1.60 (S, 9 H, $:CCH_3$); m/e 318.2558, $C_{21}H_{34}O_2$ requires M, 318.2559.

δ-Tocotrienol[3,4-dihydro-2,8-dimethyl-2(4,8,12-trimethyl-3,7,11-(E,E,)tridecatrienyl)-2H-1-benzopyran-6-ol] (223):-

Fraction nine (120 mg) obtained from the initial HPLC purification of the epiphase fraction, was dissolved in a 9 : 1 mixture (0.5 mls) of dichloromethane and ethyl acetate, and the solution was streaked on silica gel plates. The streaks were developed by the same solvent system to give three bands identified by the use of a U.V. lamp at 366 nm. The middle band was scraped off and eluted into acetone. After filtration the solvent was removed in vacuo to leave a pale yellow oil (90 mg). The oil was finally purified on silica gel plates using dichloromethane as solvent to give three bands; the middle one was eluted into acetone. The extract was filtered and evaporated in vacuo to leave a pale yellow oil (25 mg) which was homogeneous in t.l.c. analysis (silica gel, dichloromethane) and had the following spectral data; λ max (ethanol) 297 (ε = 2100); ν max ($CHCl_3$) 3625, 2950, 1620, 1485, 1390, and 1155 cm^{-1}; δ 1.26 (S, $-CH_3$), 1.59 (S, 9 H, $:CCH_3$), 1.68 (S, $:CCH_3$), 1.70 (q, J 7, $-CH_2-$), 1.96 - 2.10 (m, 12 H, $-CH_2-$),

2.12 (S, :CCH_3), 2.69 (t, J 7, -CH_2-), 5.00 - 5.20 (m, 3 H, :CH), 6.38 (d, J 2.0, ph-H), 6.47 (d, J 2.0, ph-H), 4.45 (brs, OH); δcarbon: 25.7 (q), 17.7 (q), 131.2 (S), 124.5 (d), 39.8 (t), 26.7 (t), 135.0 (S), 16.0 (q), 124 (d), 39.8 (t), 26.8 (t), 135.2 (S), 16.0 (q), 124.3 (d), 39.8 (t), 24.6 (t), 76.5 (S), 22.6 (q), 31.5 (t), 22.2 (t), 127.4 (S), 146 (S), 112.7 (d), 115.7 (d), 147.8 (S), 121.3 (S), 15.9 (q), optical rotation $[\alpha]_D^{20°C}$ (ethanol) + 20.5°(C, 0.02 g/ml); m/e 396.3042, $C_{27}H_{40}O_2$, requires M 396.3028; other m/e and their percent abundance are:

m/e	41	69	45	109	121	137	163	175	177	192
% abundance	37	85	18	22	20	95	14	21	50	20

m/e	204	219	396
% abundance	8	4	100

Methyl 8-oxo-9'-cis-8,6'-diapocaroten-6'-oate (218):- Fraction six (260 mg) from the HPLC purification of the epiphase was dissolved in 5% water in methanol (1.5 ml) and injected into a Zorbax ODS column (25 cm x 9.4 mm) mounted on a Waters HPLC equipment. Using high pressure (1000 psi) and the same solvent system as the eluant ten fractions were detected by U.V. spectrophotometry at 280 nm. The flow rate was 1.5 ml/min while the chart recorder speed was 0.5 cm/min. The seventh fraction (133 mg) was then purified by HPLC, using a Zorbax sil (25 cm x 4.6 mm) column and 10% ethyl acetate in hexane and detecting the eluates at 440 nm. Five fractions (Figure 9) were obtained and the first one was recrystallised from methanol to give deep red-coloured crystals (5 mg);

m.p.145°C (Lit.[87] 148 - 149°C); λ max (benzene) 497 (ε = 84,000), 462 (ε = 100,000), 439 (ε = 70,000); ν max ($CHCl_3$), 1710, 1665, 1610, 1380, 1080 and 915 cm^{-1}; δ 9.46 (S, CHO), 6.97 (d J 15.5, :CH), 5.98 (d, J 15.5, :CH), 7.97 (d, J 15.5, :CH), 2.03 (S, $:CCH_3$), 1.95 (S, 6 H, $:CCH_3$), 1.97 (S, $:CCH_3$) 6.30 - 7.00 (m, 9 H, :CH), 3.97 (s, OMe); m/e 352.2042, $C_{23}H_{28}O_3$ requires M, 352.2038; other m/e and percent abundance are:

m/e	68	69	79	91	119	132	143	159	196	352
% abundance	10	80	44	60	44	22	45	18	10	100

Methyl 9'cis-Bixin(Dimethyl 9'cis 6,6'-diapocarotene-6-6'-dioate)(177b);-

The third fraction from the latter column above was crystallised from methanol to obtain glittering red crystals (3 mg) m.p. 162°C (Lit.[88] 163°C), λ max (benzene) 501 (ε = 109,900), 469 (ε = 124,000), 442 (ε = 84,000) nm; ν max ($CHCl_3$) 1700, 1280, 1125, 1000, 985, and 860 cm^{-1}; δ 7.97 (d, J 15.5, :CH), 7.40 (d, J 15.15, :CH), 6.30 - 7.00 (m, 10 H, :CH), 5.93 (d, J 15.5, :CH), 5.89 (d, J 15.5, :CH), 3.80 (S, OMe), 3.97 (S, OMe), 1.95 and 2.0 (S, 12 H, $:CCH_3$); m/e 408.2313, $C_{26}H_{32}O_4$, requires M 408.2301. For the chromatogram see Figure 9.

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