β–Damascenone Precursors in Grapes and Wines

A Thesis

Submitted in Partial Fulfilment

of the Requirements for the Degree

of

Doctor Of Philosophy

by

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(B. SC. Hons.)

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The University of Adelaide

1991

As no better man advances to take
this matter in hand, I promise nothing
complete; because any human thing supposed
to be complete must for that very reason infallibly
be faulty.

(from *Moby Dick*, HERMAN MELVILLE)

Contents

Acknowledgements

I would like to acknowledge the two most important people who I dedicate this thesis to, my father Kyriakos Kostis Skouroumounis and my mother Evangelia Skouroumounis. This thesis would not have been written if it were not for the financial support in the last eight months of my studies and I thank them for their support. Although they were deprived of an education they have worked hard to enable all their children the opportunity to be educated. I would like to thank my brother Kostas and sisters Anastasia, Maria, Despina and Michelle for putting up with me and their support and encouragement throughout.

I wish to thank my supervisor and friend Dr. Ralph A. Massy-Westropp for his guidance and valuable discussion of my work. I also gratefully acknowledge friend and colleague Dr. Mark A Sefton of the Australian Wine Research Institute (A W R I) for his help and valuable advice, especially to the hydrolytic work. My thanks also go to the other members of the academic and technical staff and my fellow students at the University of Adelaide for their valuable assistance.

A special thanks must go to my honours supervisor Dr. Ernest W. Della for creating my enthusiasm and interest in organic chemistry.

I acknowledge Drs. C. R. Strauss and P. J. Williams of the A W R I for initiating the project and Dr. Terry H. Lee (Director of the A W R I) and the A W R I for giving me the opportunity to do my PhD. The Grape and Wine Research is also acknowledged for their financial support in funding my scholarship.

Statement

To the best of my knowledge, this thesis contains no material previously submitted for a degree or diploma and contains no material previously published except where due reference is made.

George Skouroumounis. (B. Sc. Hons.)

Publications

Some of the work described in this thesis has been reported in the following publication:

M. A. Sefton, G. K. Skouroumounis, R. A. Massy-Westropp, P. J. Williams. *Aust. J. Chem.* , 1989, **42**, 2071.

ABSTRACT

The syntheses of four dinorisoprenoids and the β-D-glucosides of three of these compounds were undertaken to investigate the role of these compounds and their glycosidic derivatives as precursors of 3,5,(8E)-Megastigmatrien-7-one [β-damascenone] (38).

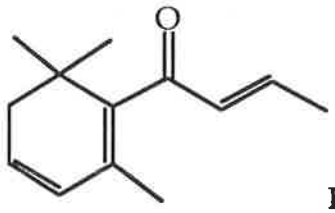
These syntheses included four dinorisoprenoids of known relative configuration: (3SR,5SR,6RS,9RS)-7-Megastigmyne-3,6,9-triol (55a), (3SR,9RS)-5-Megastigmen-7-yne-3,9-diol (58a), 3-hydroxy-5,(8E)-Megastigmadien-7-one [3-hydroxy-β–damascone] (39) and (3SR,5SR,6RS,9RS)-6,7-Megastigmadiene-3,5,9-triol (43a).
The relative configuration of compound (55a) was determined from an X-ray crystal structure analysis. In addition, the compounds 3,5,5-trimethyl-3-cyclohexen-1-ol (107) and 4-(1'-cyclohexenyl)-3-butyn-2-ol (99) were prepared as model compounds.

Several modern glycosidation techniques were investigated for the formation of β-D-glucopyranosides (58b), (39a), (107a), (99a), (153a), (43c) and (1a).

During this research the compounds (58a), (39a), (43a) and (tentatively) (153a) have been identified as natural products in grape juice/wine for the first time.

From the hydrolytic studies of the compounds (55a), (58a), (39), (43a), (107), (99),(1) and also the glucosides (58b), (39a), (107a), (99a), (1a) at different temperatures and various pH values it was generally found that ,at lower temperatures, the β-glucosides hydrolyzed at a slower rate than their respective aglycones. Glucosides are considered to be acid labile compounds, however,the results from this study clearly indicate that they show some resistance to acid, especially at grape juice/wine pH and ambient temperature.

Compounds (55a), (58a), and (43a), have been shown to undergo rearrangement to yield β-damascenone (38) under acidic conditions. However, the major product obtained in these transformations was 3-hydroxy-β-damascone (39). Importantly, the glucoside (58b) gave an increased amount of β-damascenone (38), in relation to 3-hydroxy-β-damascone (39), however the latter compound was still the predominant product. The rate of hydrolysis of the glucoside (58b) was slower than (58a). The hydrolysis of glucoside (39a) gave mainly 3-hydroxy-β–damascone (39) and no β-damascenone (38) was observed. See following page for diagrams of compounds.



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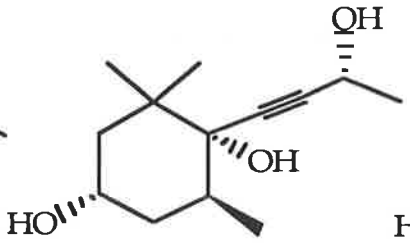
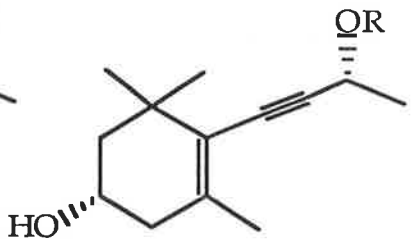
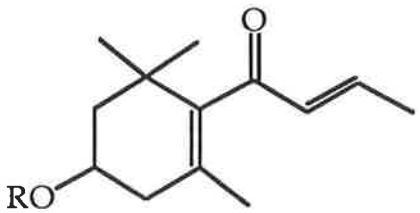


(55a)



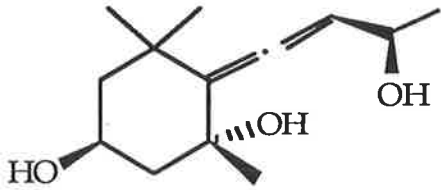
(58a) R = H

(58b) R= β–D-glucopyranosyl



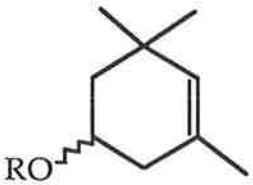
(39) R = H

(39a) R= β–D-glucopyranosyl



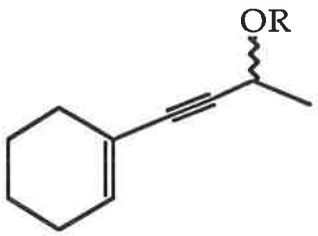
(43a) R = H

(43c) R= β–D-glucopyranosyl



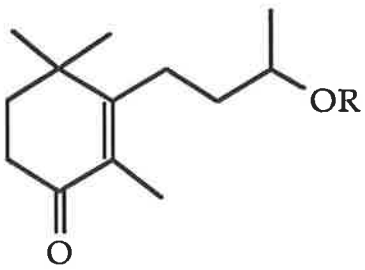
(107) R = H

(107a) R= β–D-glucopyranosyl



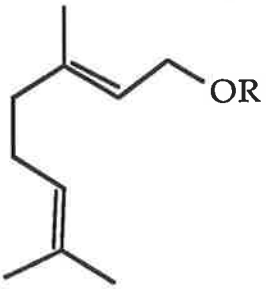
(99) R = H

(99a) R= β–D-glucopyranosyl



(153) R = H

(153a) R= tetra-O-acetyl-β–D-glucopyranosyl



(1) R = H

(1a) R= β–D-glucopyranosyl

Abbreviations

CI	chemical ionization
ppb	parts per billion
ppm	parts per million
GC	gas chromatography
DCCC	droplet counter current chromatography
n m r	nuclear magnetic resonance
GC / MS	gas chromatography / mass spectroscopy
MHz	mega hertz
FAB	fast atom bombardment
J	coupling constant
h p l c	high pressure liquid chromatography
t.l.c.	thin layer chromatography
Rf	retention front
Å	amstrongs
UV	ultra violet
COSY	correlation spectroscopy
C18RP	octadecyl silyl reverse phase
FID	flame ionization detector
IR	infra red
^{13}C	carbon isotope of atomic mass 13
eV	electron volts

INTRODUCTION

Flavour is probably the single most important factor that contributes to the enjoyment of wine. The flavour of wines is made up of a combination of taste, aroma and mouth-feel sensations brought about by a large number of wine components. Wine aroma is not attributed to a single aroma compound but rather a multitude of odoriferous components frequently present in trace concentrations. Aroma is exclusively associated with volatile molecules with the highest molecular weight found so far for an odorant being 294[1]. The total content of known aroma compounds in wine amounts to approximately 0.8 to 1.2 grams per litre, which is equivalent to 1% of the ethanol concentration[2]. Wine aroma components may originate from any of the following four sources[3].

1 Originating from the grapes,
2 Produced during the crushing of grapes by the action of certain enzymes,
3 Produced by yeast fermentation and
4 Produced during maturation of wine.

In recent years much research has been directed to such aroma / flavour compounds especially those derived from the Vitis vinifera grape cultivars as these are the principal wine grape varieties used in wine making in European and Australasian countries. In 1979 Schreier[4] reported approximately 550 volatile components that have been identified in grapes and wine and many of them contribute to its aroma. Ten years later Rapp[2] reported that the number of volatile components of wine is as high as 800.

The majority of volatiles in grape and wine are also present in many other fruits. The concentration and types of components can be influenced by environmental factors (climate, soil), cultivar, the condition of the fruit, the

conditions during the fermentation stage (pH, temperature, juice nutrients, microflora) and finally, the various post-fermentation treatments (clarification, blending, oak barrel maturation, etc.)[5].

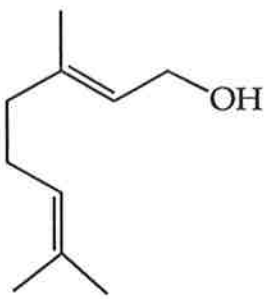
Using suitable extraction methods[6] grape / wine aroma compounds can be concentrated without formation of artefacts and analysed using capillary column gas chromatography coupled with mass spectrometry. With the aid of Fourier transform ^{1}H n m r and mass spectrometric techniques, identification of many of these components is possible.

Most known flavour components which are derived from grapes can be categorized into three biogenetic classes. These are the monoterpenes, shikimate derived metabolites and a group of norisoprenoids which are thought to be degraded carotenoid products. Until recently, research into grape flavour has concentrated on the muscat varieties which are mainly dependent on monoterpenes for their flavour[7].

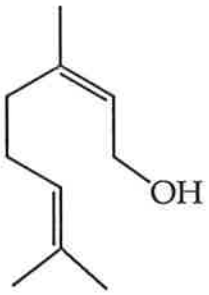
These muscat varieties can have monoterpene concentrations as high as 6 milligrams per litre of juice. Grape varieties such as muscat of Alexandria and Italia fall into this category. Non-muscat but floral varieties, which contain monoterpene concentration of 1-4 milligrams per litre, include Riesling, Traminer and Wurzer. Among the other grape varieties, the non-floral group such as Chardonnay, Sauvignon Blanc and Semillion are independent of monoterpenes for flavour. These cultivars rely on other secondary metabolites for their flavour.

At present more than 50 monoterpene alcohols and derivatives are known in muscat cultivars (Muscat of Alexandria)[5]. The most prominent terpene alcohols are geraniol (1), nerol (2), linalool (3), hotrienol (4), α-terpineol (5), and the pyran and furan forms of linalool oxides (6) and (7)[7]. These are also

the most abundant naturally occurring oxygen-containing monoterpenes in many essential oils[5].



(1)



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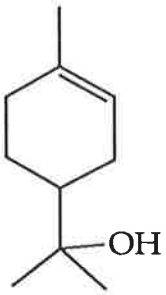

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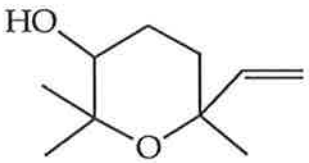

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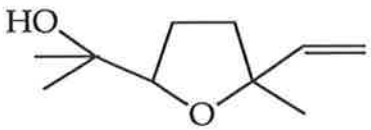
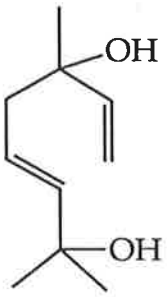


(7)

In addition to these volatile monoterpenes, muscat grapes also contain many polar odourless polyhydroxylated monoterpenes[8], e.g. compounds (8)-(11) which when heated under mild acid conditions readily rearranged and / or dehydrated to give additional volatile aroma compounds.



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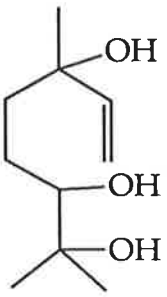

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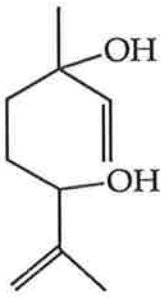

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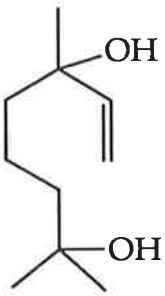
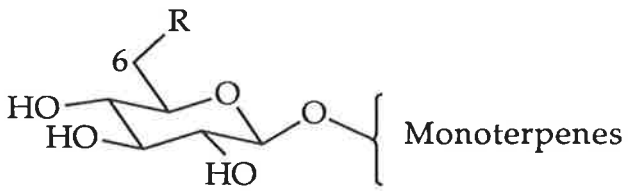


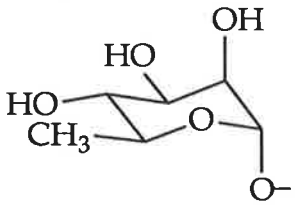
(11)

Cordonnier and Bayonove[9] had suggested that certain grape varieties contained non-volatile, acid labile, "bound" forms of flavour compounds and

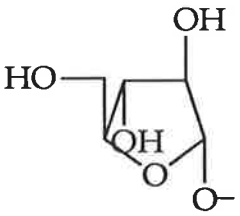
these could be glycosides. This proposal was substantiated when Williams[10] isolated glycosidic derivatives of monoterpenes by (C18 RP) liquid chromatography from grape juice. The glycones of the glycosidic monoterpenes were shown to comprise glucopyranosides and dissacharides in which position 6 of a glucose was linked to either α-L-arabinofuranoside, or α-L-rhamnopyranoside[11].



R= –OH ,



α-L-rhamnopyranosyl ,



α-L-arabinofuranosyl

Williams later showed that model monoterpene β-glucosides, when hydrolyzed at grape juice pH, gave product distributions similar to those from natural monoterpene disaccharides of Vitis vinifera [12].

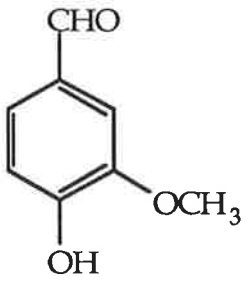
Additionally, it has been observed that grape monoterpene polyols also occur as glycosidic conjugates in the grape[13,7]. More recent evidence indicates that glycosylation takes place after the extra hydroxyl groups are introduced into the monoterpene skeleton which is consistent with the terminal step of any biosynthetic pathway[14]. In muscat varieties, dependent on monoterpenes for their flavour, glycosides accumulate in the berries to a greater extent than the non glycosylated compounds.[15,16]

Interestingly, Wilson[17] observed an increase in monoterpene glycosides during grape maturation and noticeably some decrease in certain free monoterpenes. Therefore flavour compounds are not the end products of monoterpene biosynthesis in grapes. Oxidative pathways which lead to these polyhydroxylated flavourless forms in monoterpenes are active in Vitis vinifera . These, in turn, together with the mono alcohols, are converted to glycoconjugates. However, these processes do not necessarily denude the finished wine of flavour as these polyols and glycoconjugates are in turn acid labile precursors to other flavour components. Thus the study of flavour precursors in grapes has become more important in light of the observed glycoside accumulation in berries.

In non-floral grape varieties, i.e. those with low monoterpene content , the flavour is often delicate and subtle, and knowledge of the chemical composition of the flavour is almost non-existent. Although these grape varieties make up the bulk of the world's premium wines not much information on their flavour is available. One reason is that only tiny quantities of volatiles are present in juices of these non-floral grapes.

Recent research has shown that the low molecular weight secondary metabolites of these varieties also accumulate as glycosides and that mild acid hydrolysis of these glycoconjugates is a source of important flavour compounds[18,19]. The majority of these glycoconjugates were monoterpenes, thirteen-carbon and other norisoprenoids together with shikimate-derived aromatic compounds[16]. It was also apparent that the acid hydrolysates of these glycoconjugates were more complex than the respective enzymatic hydrolysates. The norisoprenoid, and shikimate derived compounds were predominantly observed in the cultivars Chardonnay and Semillion. The shikimate derivatives were aromatic

compounds with one to four carbon side chains and substituted with hydroxy and / or methoxy substituents directed para, meta / para and dimeta / para. Compounds such as vanillin (12) and raspberry ketone (13), which are known as important flavour compounds, were amongst the derivatives.



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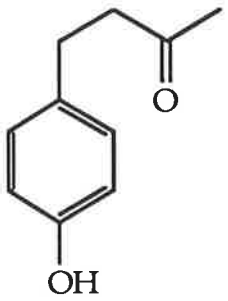
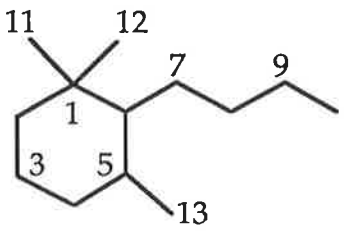


(13)

1.1 Norisoprenoids as Grape Components.

Increasing numbers of norisoprenoids, probably carotenoid degradation products, are being reported as grape components[18,20]. Carotenoid degradation products are particularly important to the flavour of tobacco, tea and many fruits[21]. They are based on the megastigmane carbon skeleton (M) [22] or are rearranged megastigmanes. Many of these compounds have been patented as flavour addition or perfume components in the food, tobacco and perfume industries[23].



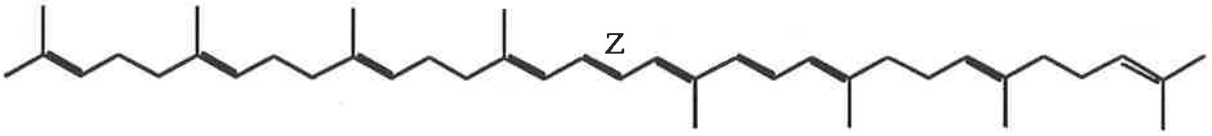
(M)

This numbering will be used throughout the text of this thesis.

C13 norisoprenoid research in grapes has increased in the last decade since the isolation of glycosidic precursors from grape juice and in particular from non-muscat or non-floral varieties. Many of the C13 norisoprenoids

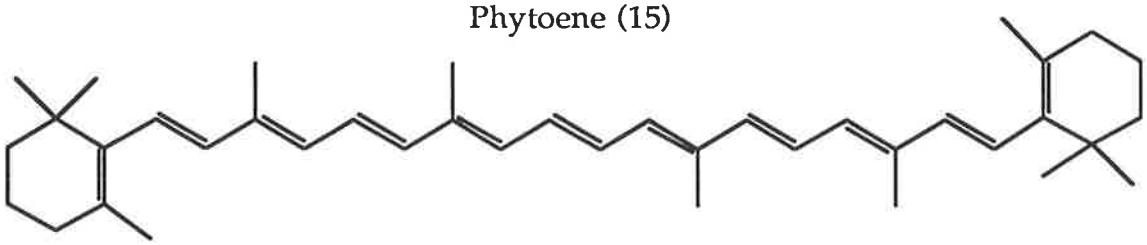
isolated are thought to have initially been formed via breakdown of corresponding carotenoids in their biosynthesis.

The large majority of naturally occurring norisoprenoids were first identified as tobacco constituents. In tobacco for instance, there are eleven known carotenoids (14-24) that have been isolated from plants[24].

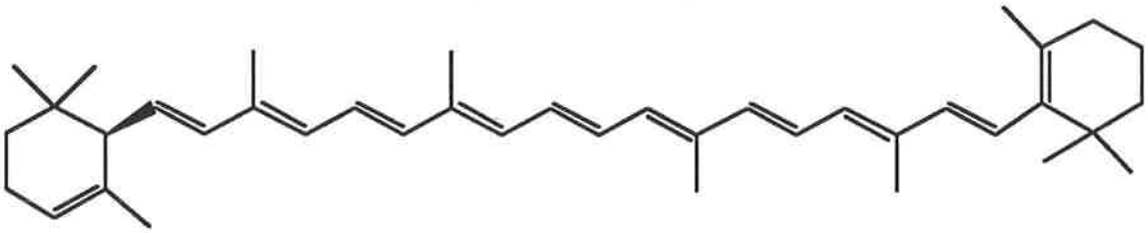


Phytofleuene (14)

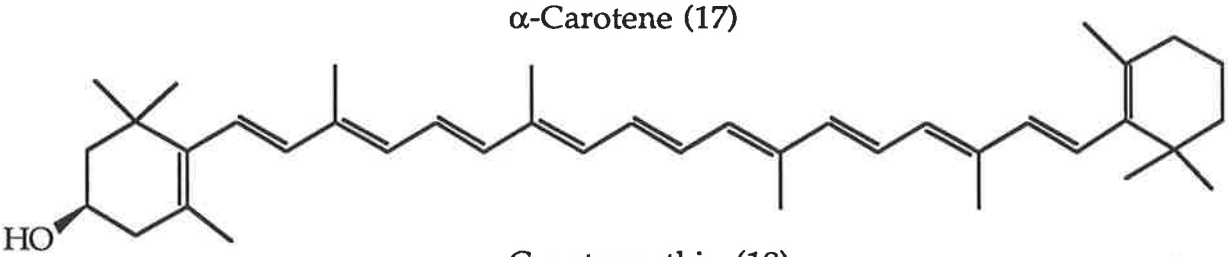
Phytoene (15)



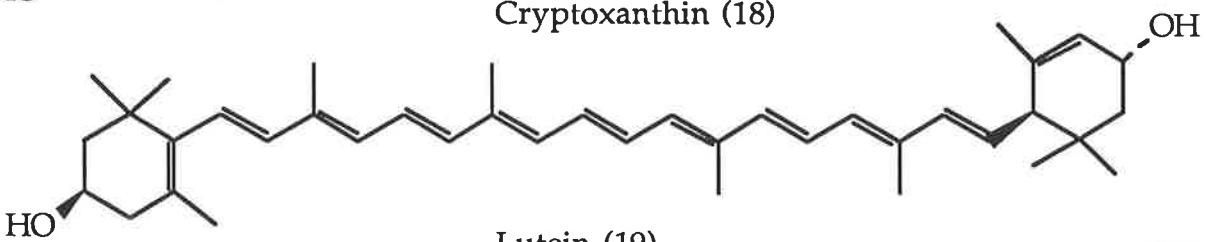
β-Carotene (16)



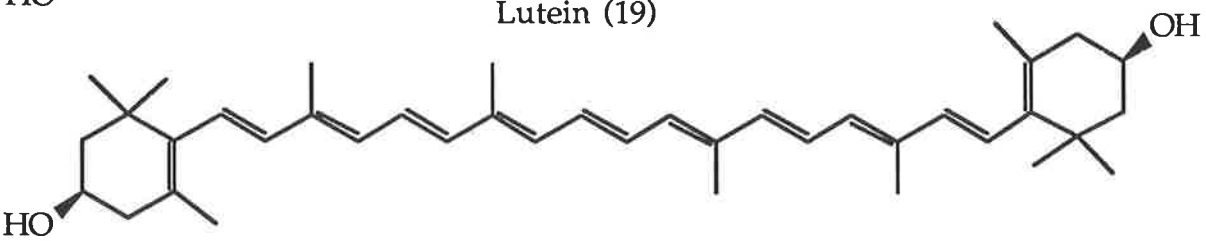
α-Carotene (17)



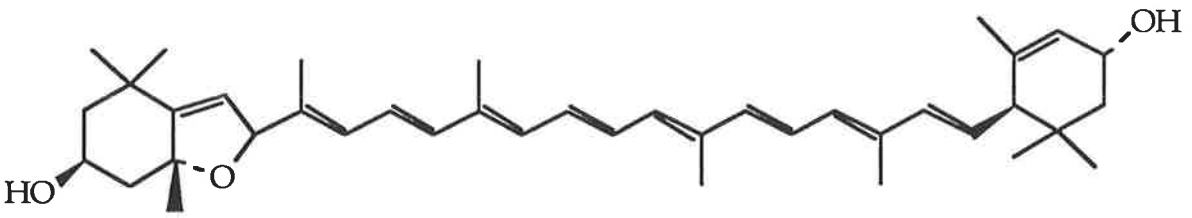
Cryptoxanthin (18)



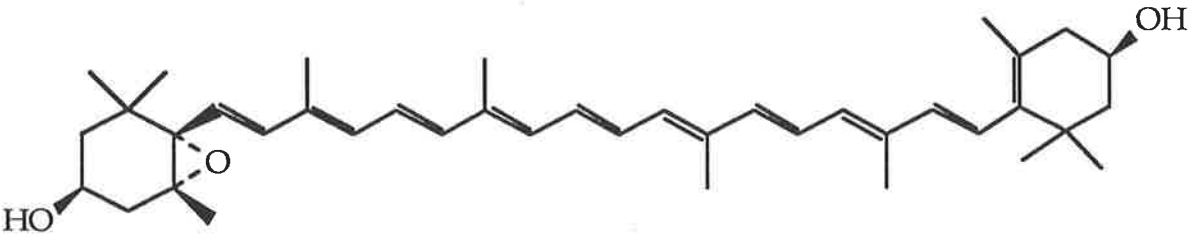
Lutein (19)



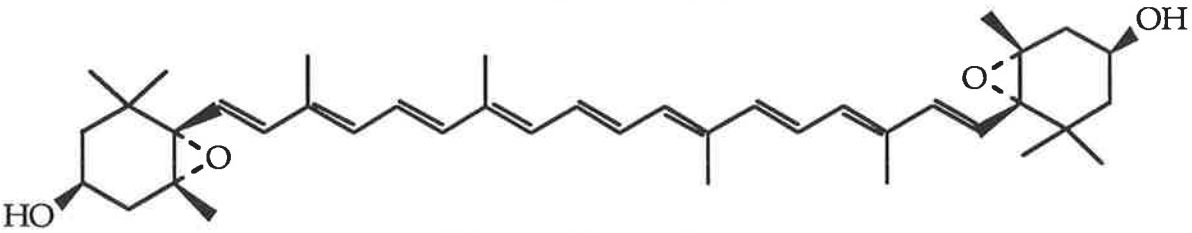
Zeaxanthin (20)



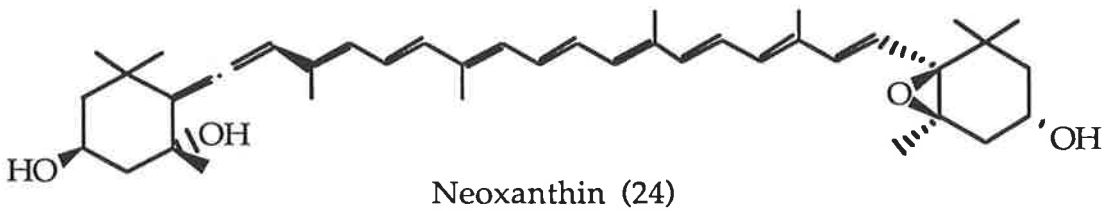
Flavoxanthin (21)



Antheraxanthin (22)

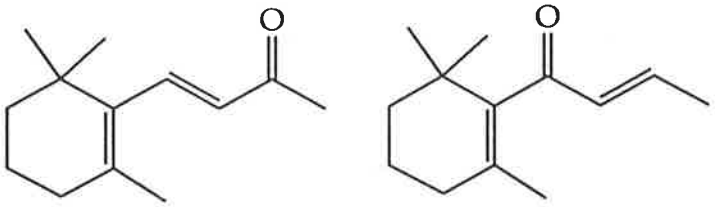


Violaxanthin (23)



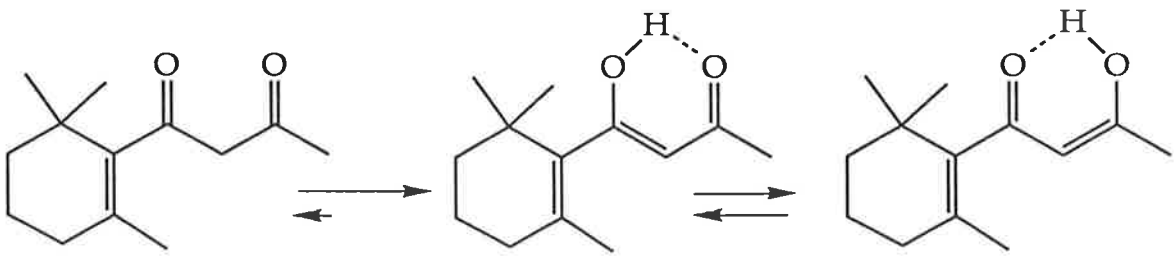
Neoxanthin (24)

The four major carotenoids always found in green leaves are β-carotene (16), lutein (19), violaxanthin (23) and neoxanthin (24). There are 200 or more[25] C13 norisoprenoids found in leaf condensates and 83 are assumed to be carotenoid metabolites. Many of the C13 norisoprenoids found in tobacco and various fruits are also found in grape juice and have the oxygen at C9 in the megastigmane skeleton.



(25) (26)

For example β-ionone (25) which has an oxygen at C9 is frequently found in many sources and has a low odour threshold (0.007ppb) in water[26]. It has been described as having the characteristic fragrance of violets. Studies have shown by enzymic in-chain cleavages, that an enzyme preparation from tea, in the presence of tea flavonols, can convert ^{14}C-labelled β-carotene into β-ionone (25) and several other volatile compounds[27], including some that are derived exclusively from the central part of the polyene chain[24]. A few C13 norisoprenoids, e.g. β-damascone (26)[28], have an oxygen at C7. This C13 norisoprenoid (26) also has a low odour threshold (0.09ppb) [29] and has been described as having a completely different and complicated odour profile in which fruity-flowery, exotic-spicy and chrysanthemum-like elements predominate. Numerous syntheses have been directed at these and similar compounds due to their importance in the flavour and perfume industries. One such example is the diketone (27) oxygenated at C7 and C9[30].

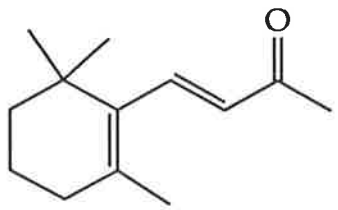


(27)

This diketone is present as a tautomeric mixture at equilibrium between the two enol forms and thus combines the functional elements of β-ionone (25)

and β-damascone (26) as well as the odour qualities of both ketones which are observed.

The C13 norisoprenoids can be postulated as breakdown metabolites of carotenoids, arising from enzyme-assisted, singlet oxygen or by autoxidation, followed by further enzymatic or chemical transformations[24]. Of the seven C13 norisoprenoid ketones that are expected to arise from C9-10 double bond cleavage of the nine alicyclic carotenoids in tobacco (28-34), only four have been encountered in tobacco[24]. The three that have not been reported are the so-called grasshopper ketone (31) (from the end group of neoxanthin), 3-hydroxy-α-ionone (32) (from the end group of flavoxanthin and lutein) and *(3S,5R,8S)* -5,8-epoxy-3-hydroxy-6-megastigmen-9-one (33) (from flavoxanthin).



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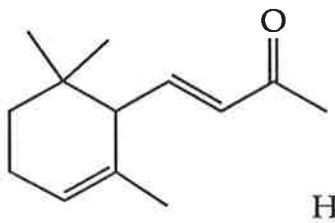

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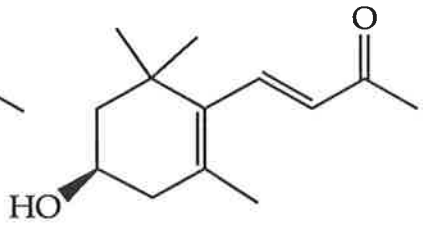

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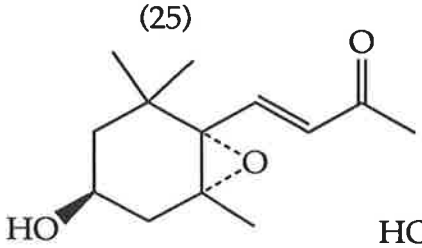

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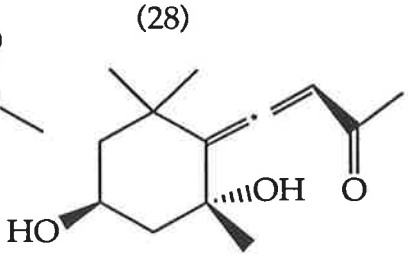

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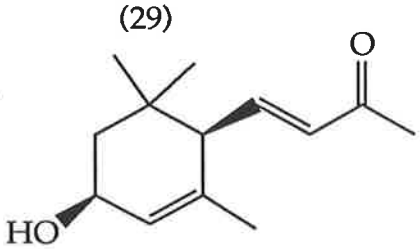

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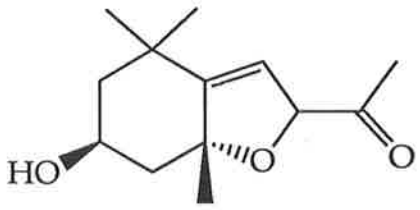
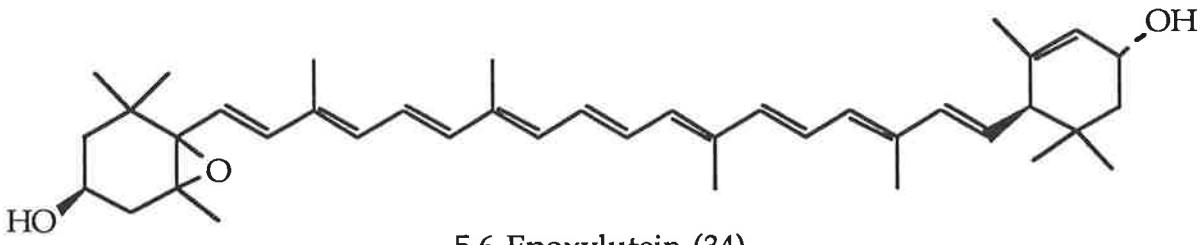


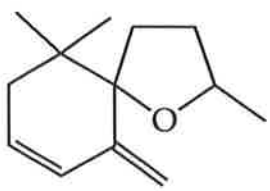
(33)

The relative abundance of C13 norisoprenoids in the grape cultivars Chardonnay, Semillion and Sauvignon Blanc suggest that these compounds are also important to the flavour of these varieties. Although much work has been carried out on carotenoids in various tobacco leaves and fruits[31] few studies have concerned grapes. Grape carotenoids are mainly congregated at the skin of the pulp. Detected so far are β-carotene (16), lutein (19), 5,6-epoxylutein (34) and neoxanthin (24)[32].



5,6-Epoxylutein (34)

Among the grape and wine norisoprenoids so far identified many are found mainly in wines only, or are formed from hydrolysis of grape glycosidic fractions under mild acidic conditions simulating bottle aging. Examples of this group include vitispirane (35) and 1,1,6-trimethyl-1,2-dihydronaphthalene (36) (TDN). The latter compound, which has a detection threshold in wine of 20-30 ppb is responsible for the kerosene-like odour which develops in many aged Riesling wines. Another product of the bottle aging of Riesling wines is the "Riesling acetal" (37) which has a fruity and ionone-like aroma.



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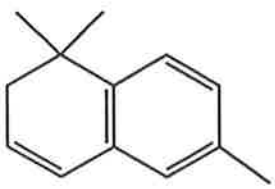

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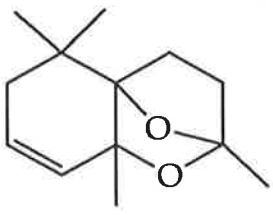
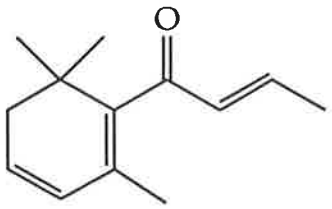


(37)

An important compound of many wines, also thought to be formed by acid hydrolysis reactions from hitherto unidentified precursors, is the 7-oxygenated megastigmane, β-damascenone (38). This substrate has an odour threshold of 0.002 ppb in water, making it among the most potent of all known wine flavour compounds.

This thesis reports the synthesis, identification as grape components, and mild acid hydrolysis of several β-damascenone (38) precursors. The thesis also reports the synthesis and hydrolytic studies of glycoconjugates of these precursors.

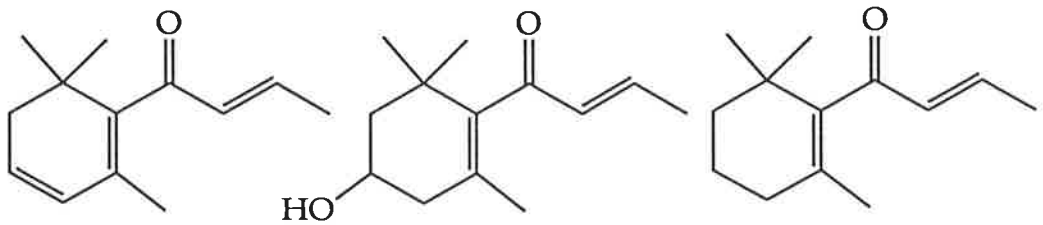


(38)

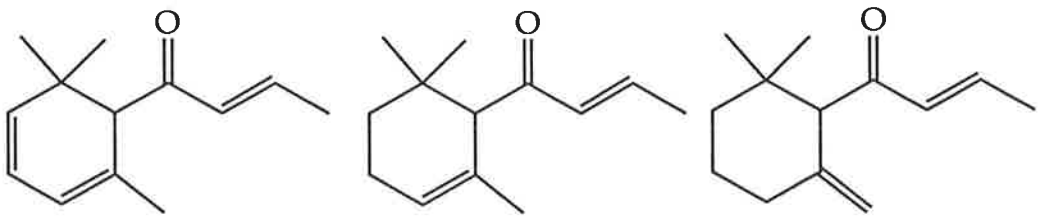
1.2 β-Damascenone (38).

β-Damascenone (38) is one of the most frequently observed C13 norisoprenoid compounds in non-floral grapes. It has an odour threshold of 0.002[33] ppb and was first isolated from Bulgarian rose oil by Demole[34]. β-Damascenone (38) has a complex aroma which has been described as flowery and ionone-like[26]. There are at least 190 cited publications on its detection in various fruits. For example β-damascenone (38) has been detected in apricot, cherry, tomato paste, honey, starfruit, green coffee, oils, blackberry juice, apples, fig leaf, Brie and Camembert cheese, mango, soy protein, passionfruits, tobacco, kiwi fruit, wine and probably also occurs in many fruits yet to be studied. Because of its essence and flavour numerous syntheses have been reported in the literature[35]. Schreier[36] was the first to detect β-damascenone (38) in grapes and wine extracts using gas

chromatography (GC), and since then, it has also been detected in beer and other alcoholic beverages[37], and in the aroma composition of Chardonnay wine[38]. β-Damascenone (38) belongs to a family known as the rose ketones. The name originates from the analysis of Bulgarian rose oil by Kovats where up to 275 constituents were identified. The production of rose ketones, six in all, of which β-damascenone (38) is one, had reached approximately ten tonnes per year, reflecting their industrial importance[29].

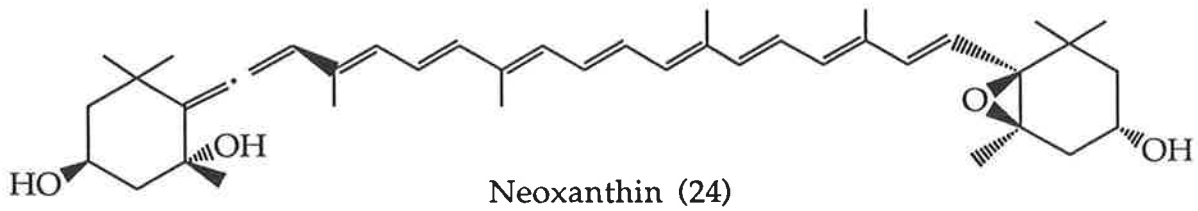


(38) (39) (26)



(40) (41) (42)

It has been suggested[39,40,18] that β-damascenone (38) arises biogenetically from degradation of the carotenoid neoxanthin (24) which is a common plant constituent.



Neoxanthin (24)

Neoxanthin has recently been isolated by Razungles[32] from the skin and pulp of three *vin* vinifera grapes and found to decrease in abundance during grape maturation. These workers also noted the absence of neoxanthin in the juice. They postulated that the carotenoid is extremely

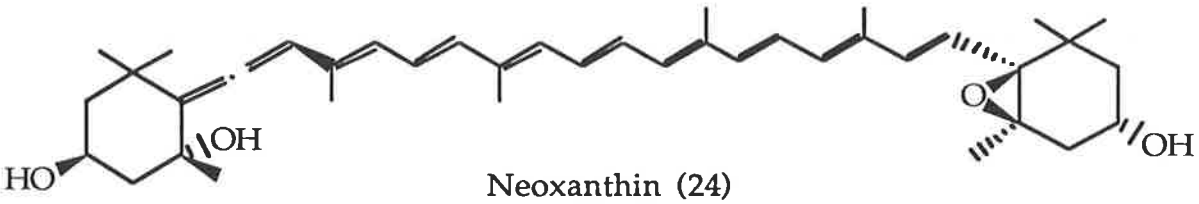
lipophilic and would diffuse only with difficulty in aqueous juice. However this carotenoid could undergo rapid photochemical oxidation under the effect of catechol oxidase or lipoxygenase as soon as whole berries are broken up by crushing or during maceration[32].

1.3 Precursors of β-Damascenone (38)

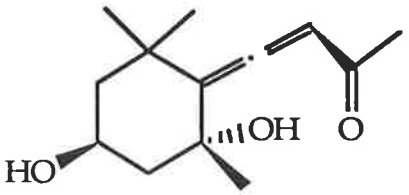
Masuda[37] detected a two to three fold increase in β-damascenone (38) upon heat treatment of wort at low pH and postulated the existence of an acid labile precursor. Strauss[41] observed that β-damascenone (38) does not exist free to any great extent in grape juices or young wines. The compound arises in juices and wines during ageing, presumably also by hydrolysis of one or more as yet unidentified precursors which are natural constituents of the fruit. Precursors of β-damascenone (38) have also been recognized in other grape varieties such as Vitis labruscana and Vitis rotundifolia, by Acree[42] but are not yet identified. Importantly, in many of the products in which β-damascenone (38) occurs its origin has been attributed to acid-catalysed hydrolysis of a precursor[43]. In work on C13 norisoprenoids in grapes, Strauss[41] has shown that a number of these compounds arise from glycosidically conjugated precursors.
In Vitis vinifera grape juices, it has been shown that β-damascenone (38) arises only by acid catalyzed hydrolysis of the juice or isolates at pH 1 - 3.8. Enzymatic hydrolysis of glycosidic fractions gave no β-damascenone (38).

The majority of the C13 norisoprenoids isolated to date have the oxygen in the megastigmane skeleton at C9. Simple carotenoid degradation could not alone generate 7-oxygenated compounds such as β-damascenone (38). The important group of rose ketones all have the oxygen at C7 and this has stimulated research in determining how oxygen at C9 might be transposed to C7.

Over the past two decades, several biogenetic studies have been carried out in an attempt to determine the origin of β-damascenone (38). The results from two groups, one led by Ohloff[39] and the other by Isoe[40], are particularly significant. These authors postulated the formation of β-damascenone (38) via the allenic triol (43) which could in turn be derived from the grass-hopper ketone (31) Scheme 1.



Neoxanthin (24)



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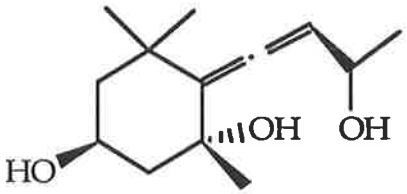

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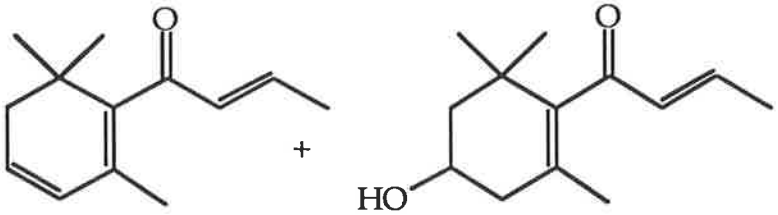
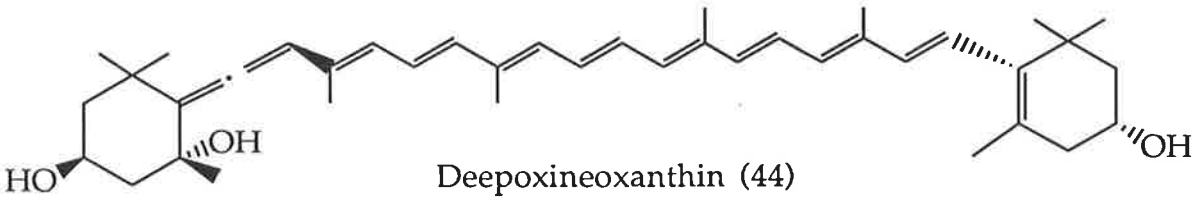


(38) (39)

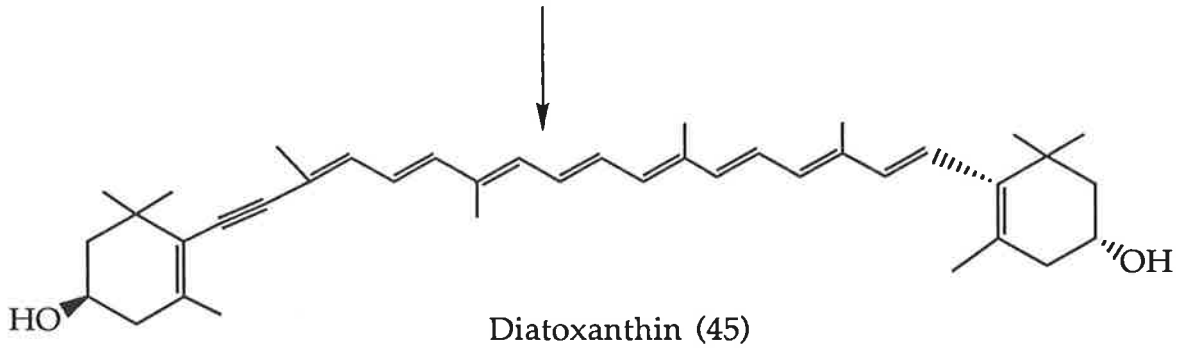
Scheme 1.

Glycoconjugates[44,45,46,47], of grasshopper ketone (31), have subsequently been isolated from several plant sources, including grapes[18].

Ohloff[39] used acetylenic triols in a biomimetic study as these compounds are known to equilibrate with the allenic derivative. For example, deepoxineoxanthin (44) is converted to diatoxanthin (45) (an acetylenic substrate) under acidic conditions[48].

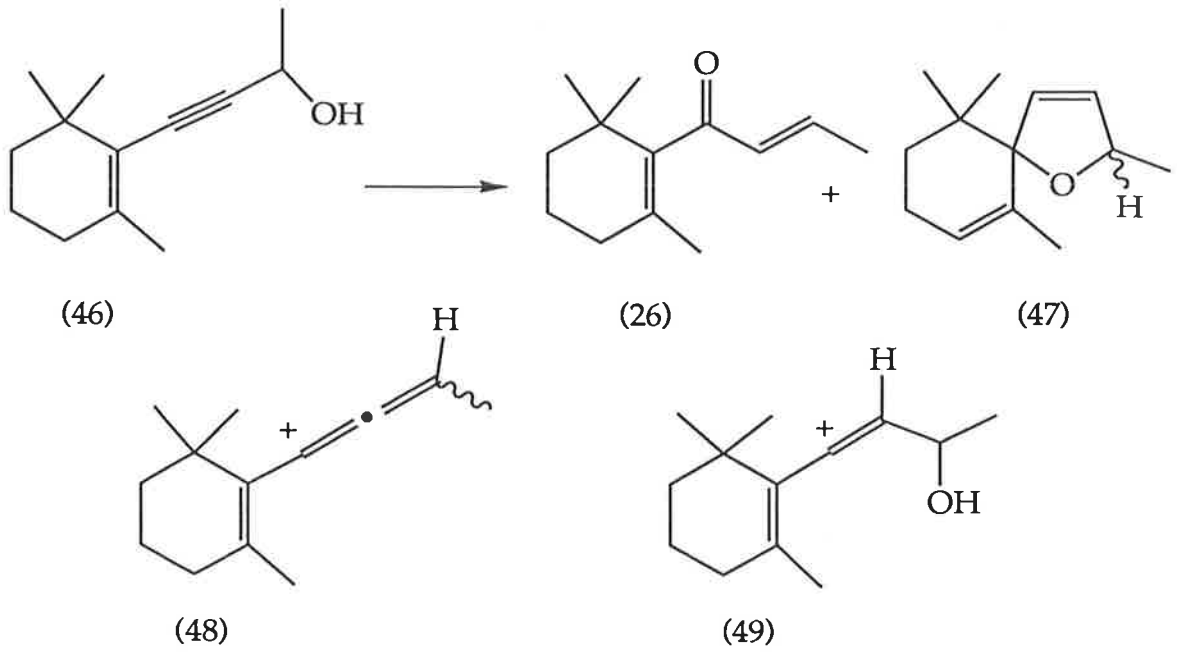


Deepoxineoxanthin (44)



Diatoxanthin (45)

They examined the acid-catalysed rearrangement of the alkyne alcohol (46) which was found to be transformed to damascone (26) and diastereomeric 7,8-dehydrotheaspiranes (47). The transposition of oxygen from C9 to C7 was achieved either via a Meyer Schuster[49] or a Rupe[49] rearrangement. Scheme 2.

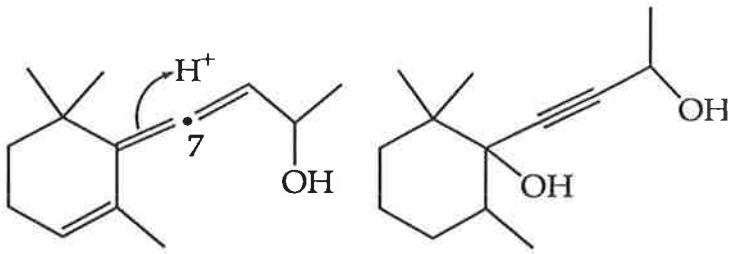


Scheme 2.

The Meyer-Schuster rearrangement is the isomerization, by a formal 1,3 hydroxyl shift, of secondary or tertiary α-acetylenic alcohols to give α,β unsaturated carbonyl compounds, via an allenyl cation, in this case, via intermediate (48).

The Rupe rearrangement is the acid catalyzed rearrangement of tertiary α-acetylenic alcohols leading to the formation of α,β unsaturated ketones, with enynes as intermediates. For example intermediate (49) would be expected from protonation of (46).

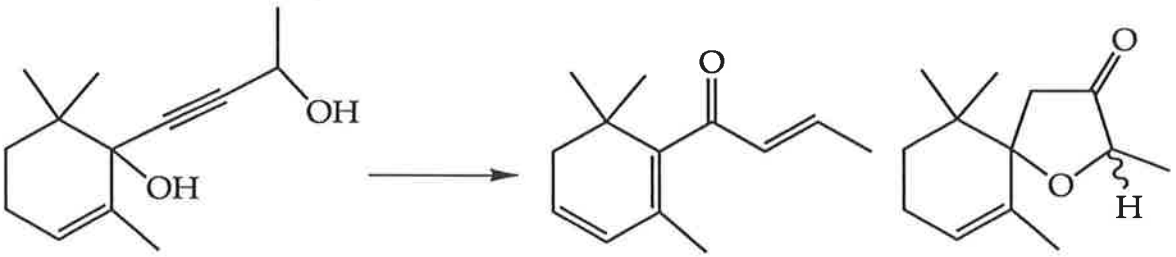
The formation of the minor component, the spirodihydrofurane (47), was thought to be derived from allene intermediate (50) which can be converted to the spirodihydrofurane (47) upon protonation at C7 which is then trapped to give the end product.



(50) (51)

The same results were obtained with the acetylenic diol (51).

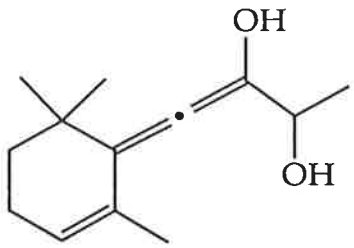
Ohloff also studied the hydrolysis of acetylenic diol (52) under acidic conditions and observed the formation of β-damascenone (38) and 8-oxotheaspiranes (53) in a ratio of 1:9. Scheme 3. Again the Meyer Schuster type rearrangement occurs in the formation of β-damascenone (38) which involved the transposition of oxygen at C9 to C7. Scheme 3. Here, β-damascenone (38) was obtained as the minor component and 8-oxotheaspiranes (53) as the major.



(52) (38) (53)

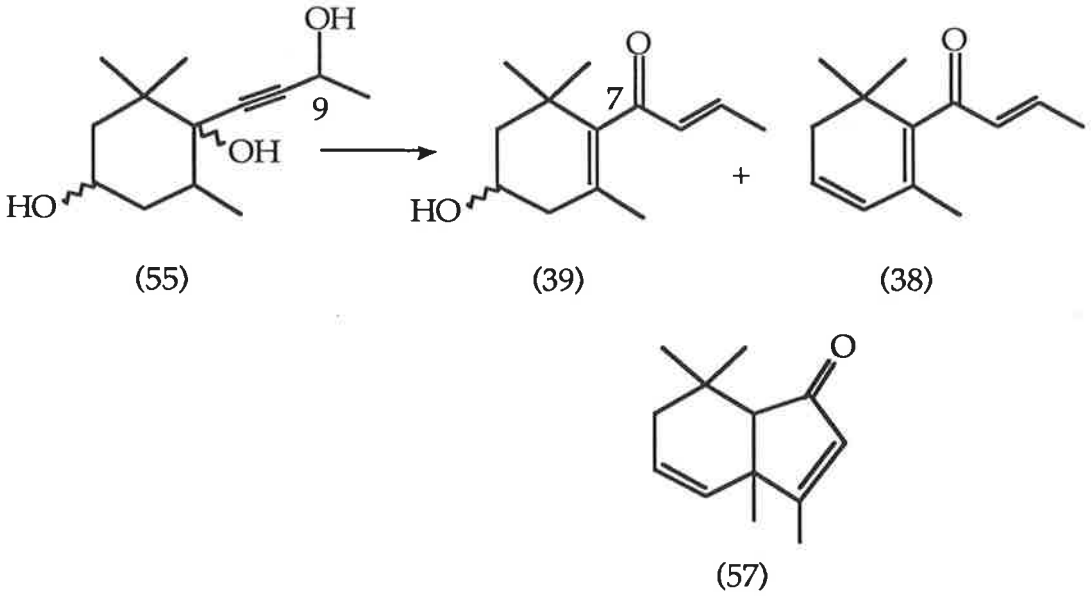
Scheme 3

Formation of the spiroketone (53) was attributed to initial loss of the tertiary hydroxyl group followed by alkyne-allene isomerization to an allenic intermediate (54), which underwent hydration to give (53) after tautormerization. The spiroketone (53) was a mixture of two diastereoisomers.



(54)

Finally Ohloff examined the hydrolysis of acetylenic triol (55). The acid-catalysed rearrangement of the acetylenic triol (55) gave β-damascenone (38), although the predominant product obtained was 3-hydroxy-β-damascone (39). Scheme 4.

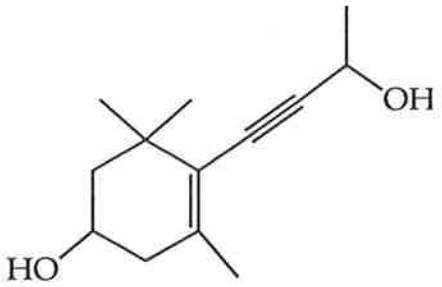


(55) (39) (38)

(57)

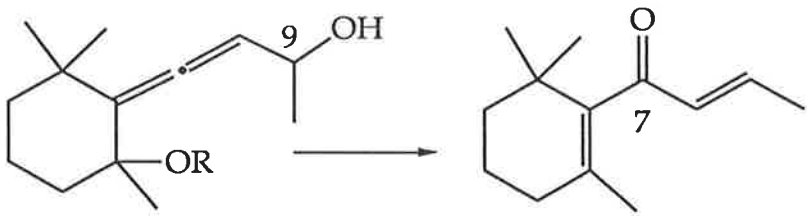
Scheme 4

The indenone (57) was formed by a Nazarov cyclization[50] process. Ohloff later postulated that the enynediol (58) could be an intermediate in the hydrolysis of the acetylenic triol (55). The enynediol (58) has been observed as a tobacco constituent[25]. However the acetylenic triol (55) has not yet been seen as a natural product.



(58)

Isoe[40] studied the allenic diol (59) (R = H) as a model compound to see if oxygen could be transposed from C9 to C7 under acidic conditions to form the damascenone side chain. Acid hydrolysis of the allenic diol (59) (R = H) gave a 35%yield of β-damascone (26). Scheme 5. No details were given as to whether other products were obtained. Interestingly when the tertiary tetrahydropyranyl ether (59a) (R = OTHP) was hydrolyzed under the same conditions, an increase in the yield of β-damascone (26) was seen.



(59) R=H

(59a) R=THP

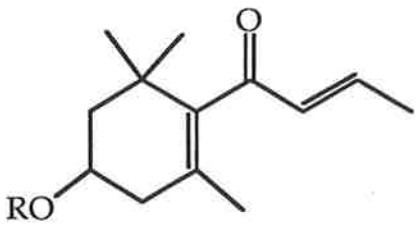
(26)

Scheme 5.

Recently Winterhalter[19] used two-dimensional droplet counter current chromatography (DCCC) to analyse the glycoconjugates of a Riesling wine and demonstrated that several glycoconjugates were involved in β-damascenone (38) formation. Multiple precursors of β-damascenone (38) have also been suggested earlier for non-vinifera grapes[51].

The aim of the work reported in this thesis was to synthesize possible precursors of β-damascenone (38), namely (39), (43), (55), (56) and (58)

and their glycoconjugates, to study their hydrolytic behaviour at wine pH and to furnish reference compounds to ascertain whether these compounds are also grape constituents.



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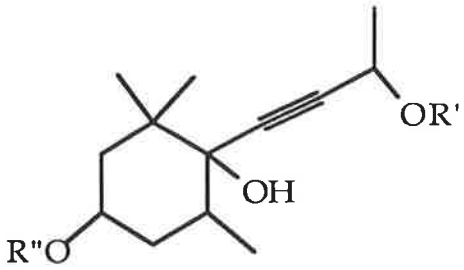
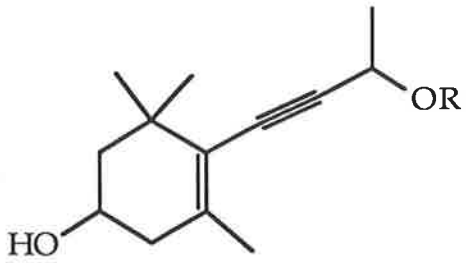


(55) R" = H
(56) R" = THP



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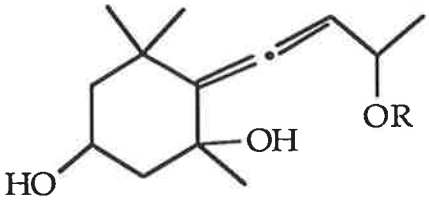


(43)

R = H
R = β-D-glucopyranoside

Should the precursor(s) of β-damascenone (38) be identified and an assay developed, then it may become possible to optimise the formation of such a precursor by viticultural techniques and thereby improve the flavour of certain wines.

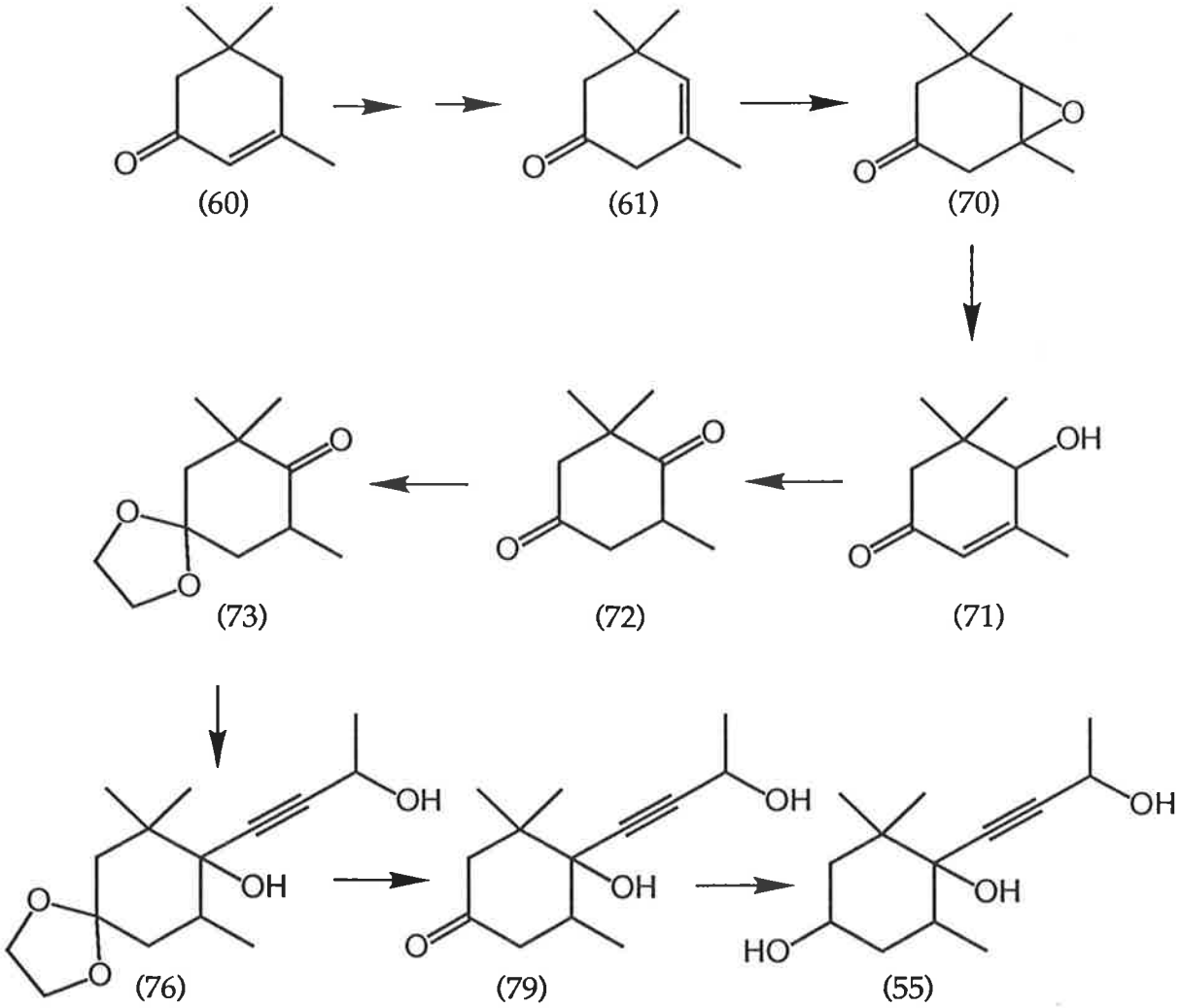
Preparation of Aglycones For Hydrolytic Studies.

The syntheses of the aglycones in this chapter are based on work by Weedon's group on the synthesis of the so-called grasshopper ketone (31).[52,53,87,89] Although the same synthetic sequence has been followed, many alternative reactions have been investigated, leading to increased yields of target aglycones. Many of the stereochemical features of these reactions, which were not determined previously, have been elucidated.

2.1 Synthesis of (*3S',5S',6R',9R'*)'-7-megastigmyne-3,6,9-triol (55a)

2.1.1 Introduction and Scheme.

The synthesis of (55) involved a nine step sequence beginning from isophorone (60), as shown in Scheme 6. Note that all products are racemates.



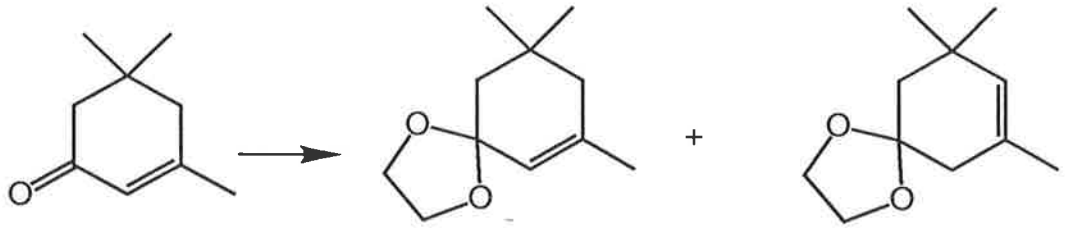
Scheme 6.

2.1.2 Synthesis of β-Phorone (61)

Several methods were investigated for the preparation of this key starting material.

2.1.2.1 Synthesis of Acetals (62) and (63) from Isophorone (60)

In our hands, conversion of isophorone (60) to a mixture of acetals (62) and (63) by the method of Babler[53] (with ethylene glycol and p-toluenesulphonic acid in refluxing toluene) gave variable yields of (62) and (63) (30-60%) in a ratio of 3:7 respectively as determined by ^1H n.m.r. spectroscopy.

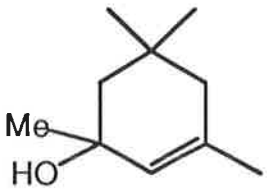


(60) (62) (63)

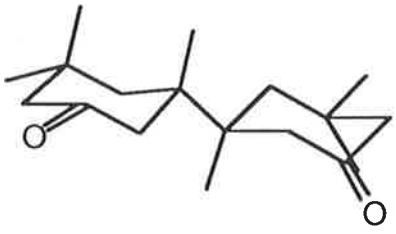
Isolation of the required acetal (63) was achieved by chromatography after selective hydrolysis of the unwanted isomer (62) to starting material (60). However, this procedure was slow and difficult to reproduce. Many variations of the procedure were attempted including use of benzene and 1,2 dichloroethane as azeotropic solvents, different acid catalysts such as pyridinium p-toluenesulphonate and a Dowex 50W acid resin. However, all these attempts were to no avail. The method of Constatino[54], involving direct distillation of water / toluene in the presence of an acid catalyst and the use of larger amounts of ethyl orthoformate, proved to be moderately successful, although lower yielding than reported. Acetal (63) was smoothly converted to β-phorone (61) upon treatment with acetic acid / water. The methods above were abandoned for other alternatives.

2.1.2.2 Deconjugation of Isophorone (60)

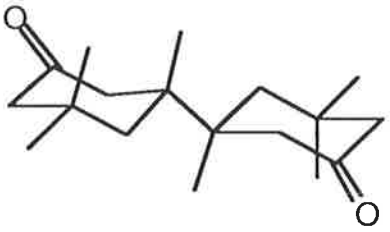
An alternative, one-pot, method of Meinwald[55] for preparing ketone (61) from isophorone (60) involved deconjugation of isophorone with methyl magnesium bromide in the presence of a catalytic amount of ferric chloride. This was more successful and gave a 65% yield of β-phorone (61). However, reproducibility of the reaction was poor and the 1, 2-addition product (64) was obtained together with reductive dimers (65) and (66)[55].



(64)



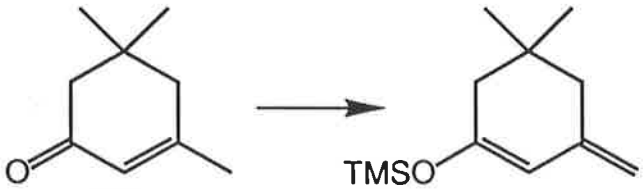
(65)



(66)

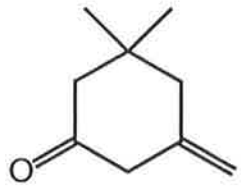
The use of ethylmagnesium bromide as a Grignard reagent proved unsatisfactory for the conversion of (60) to (61). The predominant products obtained were the reduced dimers observed by Meinwald[55], 1,2, and 1,4 reduction products, starting material and minor amounts of 1,2 and 1,4 ethyl addition products. These products were tentatively identified by GC / MS. Similarly, the use of methyl iodide for the preparation of the Grignard reagent gave a complex mixture of products and this alternative was not pursued further.

Application of the method of Krafft[56] for the generation of the endocyclic dienol silyl ether (trimethyl silyl ether, TMS) from enones, involving generation of zero-valent iron and Grignard reagent (Kharasch reagent)[57] in tandem, was also unsuccessful as the major product was the silyl ether (67).



(60) (67)

Deconjugation was also attempted by the method of Ringold[58] which involved the treatment of isophorone (60) in refluxing tertiary butanol in the presence of potassium tert-butoxide for two hours followed by kinetic protonation with acetic acid. This gave the exocyclic double bond isomer (68)[59] together with isophorone (60) and polymeric material.

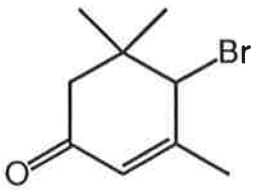


(68)

Finally the method of Haubenstock[60] which involved the deconjugation of isophorone (60) with a catalytic amount of base (a pellet of sodium hydroxide), at reflux using a fractionating column, produced mainly polymeric material, isophorone (60) and trace amounts of β-phorone(61). This method was also abandoned.

2.1.2.3 An Improved Synthesis of β-Phorone (61)

The method which was most successful and reproducible was the method of Marx[61] involving the reductive elimination of 4-bromoisophorone (69) to give β-phorone (61) and isophorone (60) in a ratio of 5:1 respectively. Separation was easily achieved by fractional distillation. Crystalline 4-bromoisophorone (69)[62] was synthesized by the treatment of isophorone (60) with N-bromosuccinimide in carbon tetrachloride. Recrystallization from ether yielded compound (69) in 50%.

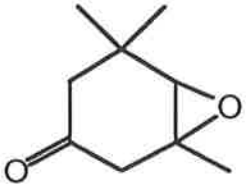


(69)

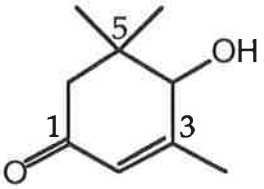
Reduction of compound (69) with buffered chromous acetate gave β-phorone (61) as the major product. Distillation of the mixture afforded β-phorone (61) in approximately 70% yield.

2.1.3 Synthesis of 3,3-Ethylenedioxy-7-megastigmyne-6,9-diol (76)

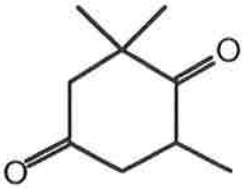
3,4-Epoxy-3,5,5-trimethylcyclohexanone (70) was obtained by reaction of β-phorone (61) with m-chloroperoxybenzoic acid in ether (or dichloromethane) at 5°C for 12 h. Attempts to obtain the product (70) in pure form were unsuccessful because of difficulties in removing a trace amount of m-chloroperoxybenzoic acid. Treatment of the crude epoxyketone (70) with base[63] gave 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (71).



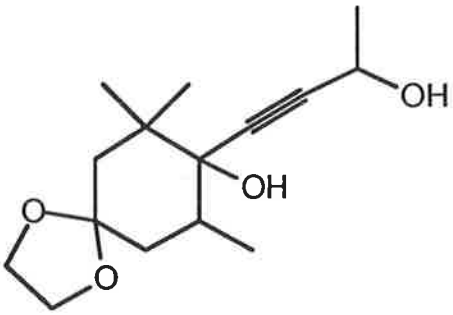
(70)



(71)

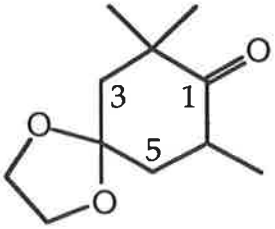


(72)

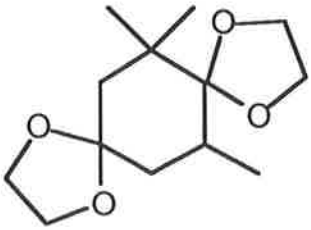


(76)

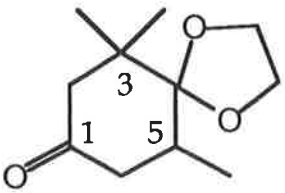
Equilibration of the conjugated hydroxyketone (71) with p-toluenesulphonic acid in benzene led to formation of diketone (72). The reaction of diketone (72) with ethylene glycol and p-toluenesulphonic acid[64] then gave a mixture of the three derivatives (73-75).



(73)



(74)



(75)

The mixture of acetals (73-75) was analysed by GC / MS and each acetal showed its molecular ion. After 2.5 hours at reflux with 1.4 equivalents. ethylene glycol, the ratio of (73):(74):(75) was found to be approx. 96:3:1. Early experiments following the method of Isler and co-workers[64],in which longer reaction times and a larger quantity of ethylene glycol were utilized, gave increased yields of (74).

Starting material (72) was separated from the crude mixture by chromatography. Separation of acetal (73) from acetals (74) and (75) was achieved by bisulphite addition[65] to unwanted acetal (75) followed by separation of acetal (73) from the bis acetal (74) by fractional distillation.

Treatment of diketone (72) in boiling 2-ethyl-2-methyl-1,3-dioxolane (as a form of acetal exchange in the absence of water), catalyzed by p-toluenesulphonic acid, resulted in a slight enhancement in the yield of acetal (73). However, a mixture of the three acetals was still obtained. For large scale syntheses the mixture of products was used directly in the next step. The monoacetal (73) was distinguished from its isomer (75) by its ^{1}H n.m.r. and mass spectra. Isolation and identification of pure (75) was accomplished by complete conversion of (72) to the bis acetal (74), followed by a mild hydrolysis to yield (75) as the sole product. In the ^{1}H n.m.r. spectrum, the C3 and C5 protons of (73) (δ1.85-1.95) are considerably upfield from the corresponding signals (C6 and C2) for (75) (δ1.8-2.8). Important mass spectral differences between (73) and (75) were the m/z 127 and 86 ions observed only with (73). See Figure 1 and 2.

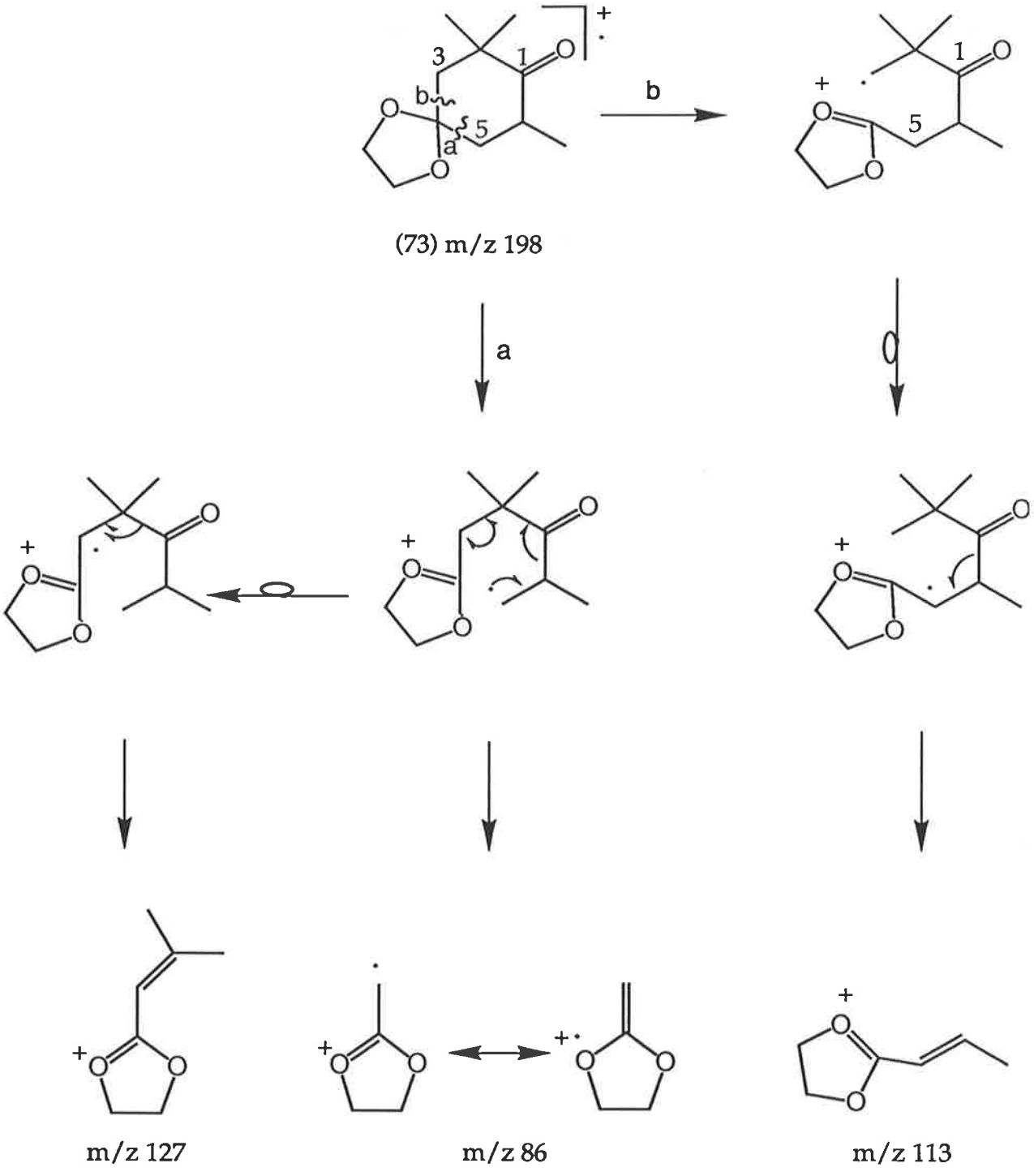


Figure 1.

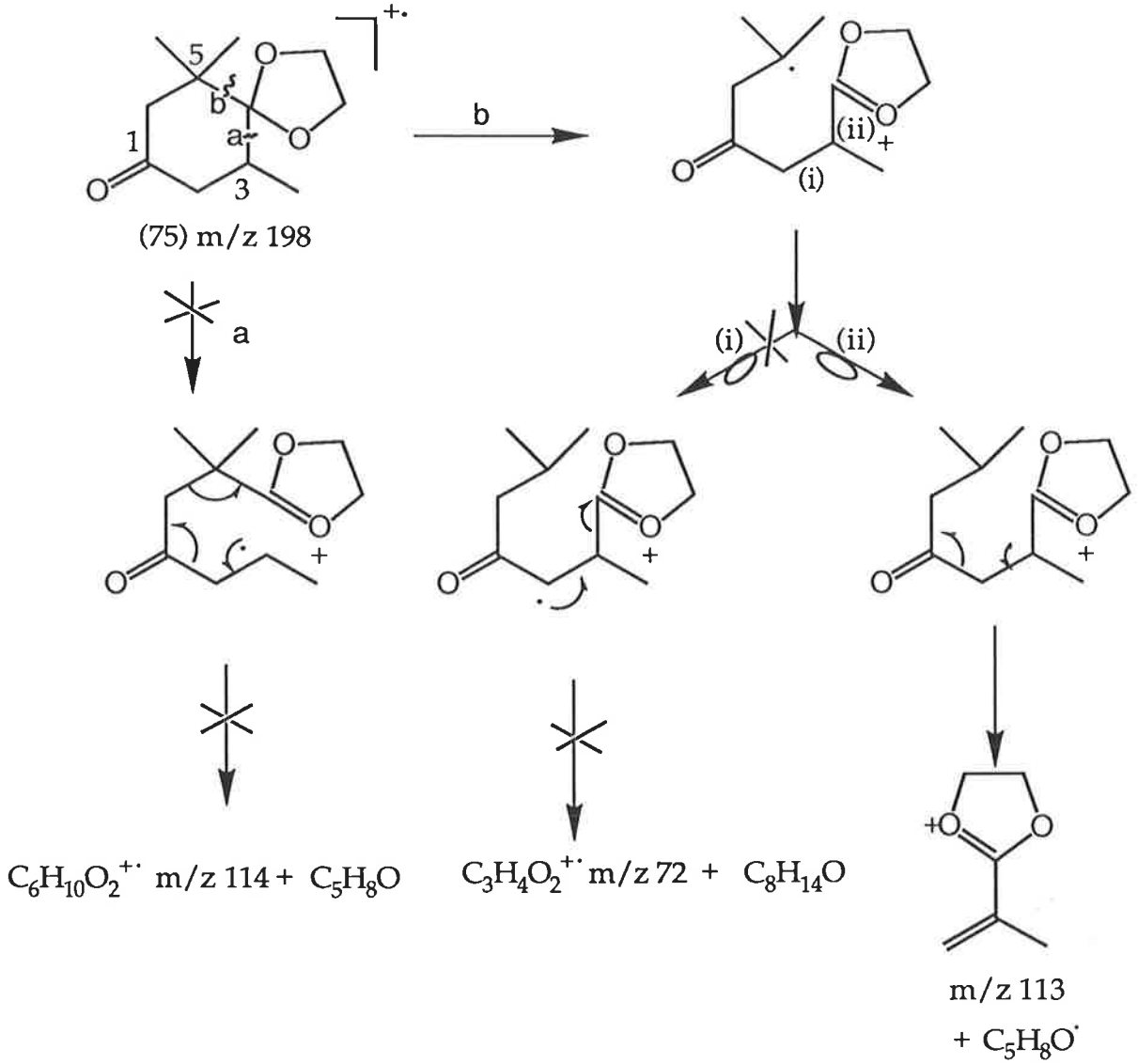
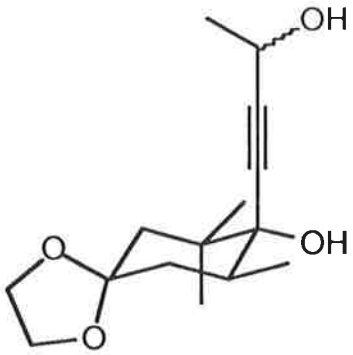


Figure 2.

Addition of the acetal mixture, composed largely of ketone (73), to the Grignard reagent of but-3-yn-2-ol (prepared by the treatment of two equivalents of ethylmagnesium bromide with but-3-yn-2-ol) gave the diol (76)[52] (Scheme 6.). The crude product (76), obtained as a yellow oil, showed two peaks in a ratio of 9:1 by GC / MS. Later transformations and a crystal structure determination of the derived triol (55)[66] showed that the main product (76a) was formed from axial addition of the Grignard reagent, from the same side as the secondary methyl group.

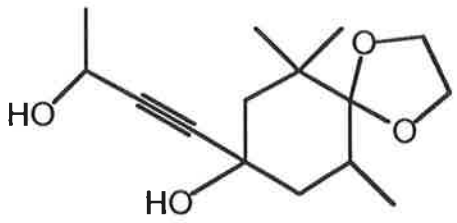


+ (76b)

(76a)

The mass spectrum of the minor component (76b) was identical with that of the major product and was therefore presumably a diastereoisomer.

When starting material containing the ketone (75) was utilized, two additional adducts were seen in a ratio of 1:9. These were shown to be isomers of (77) by an independent synthesis from the pure ketone (75) which was prepared from the bis acetal (74) as described above.



(77) + (77a) diastereoisomer

The two isomers, (77) and (77a), had identical mass spectra and they differed from the mass spectra of the isomers (76a) and (76b). In particular, the former pair gave spectra which included a base peak at m/z 100, not seen in the spectra of the latter, while ions at m/z 86 and 127 seen in (76a) and (76b) were not evident in the isomers of (77). See Figure 3a and 3b.

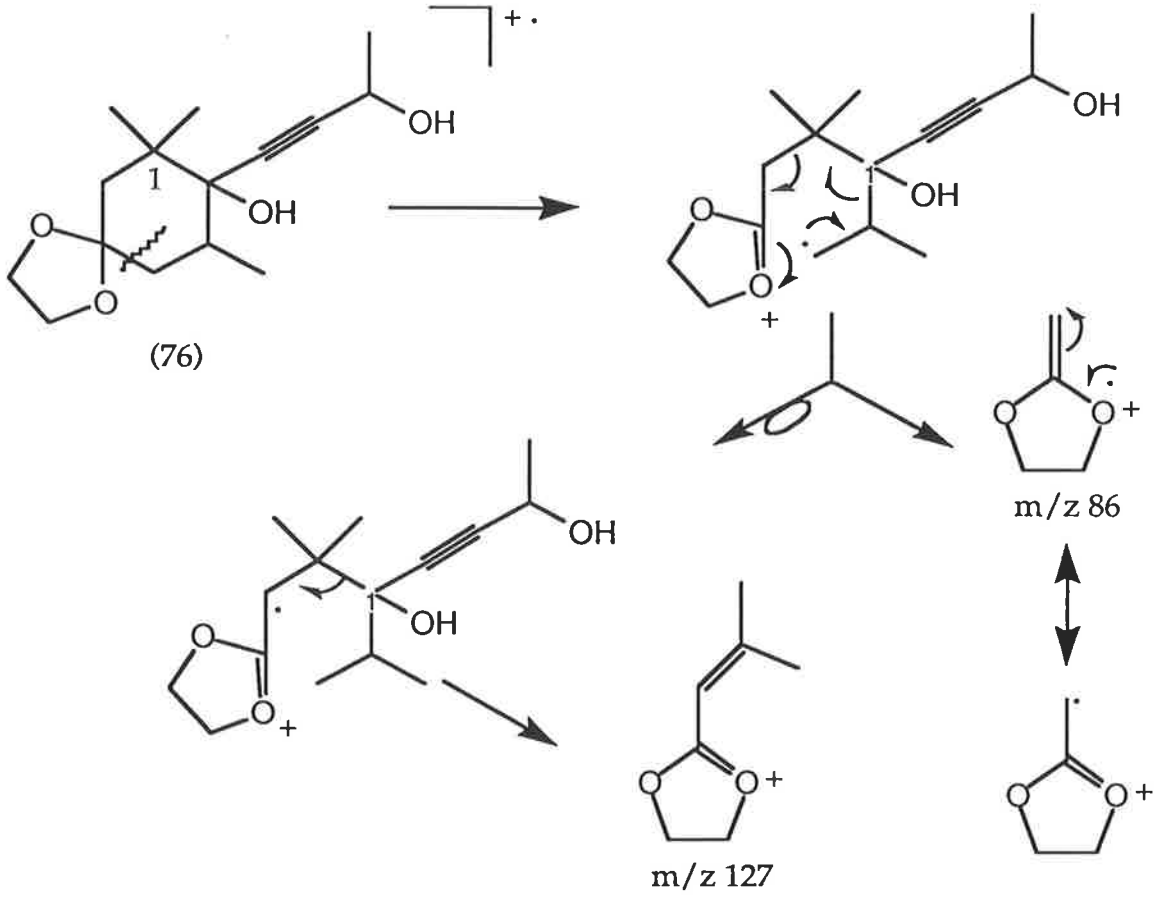


Figure 3a

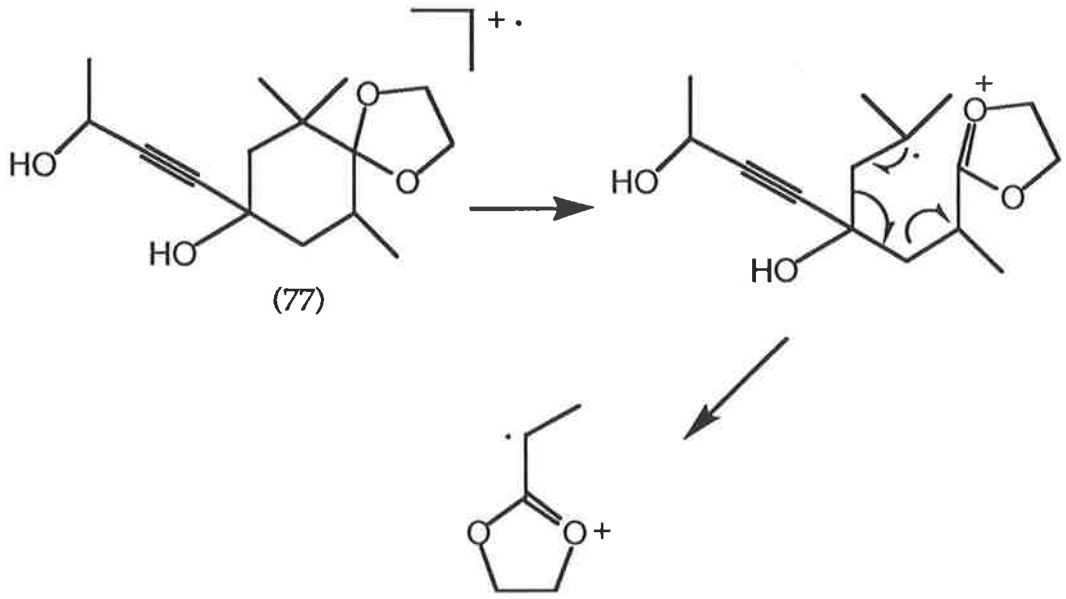
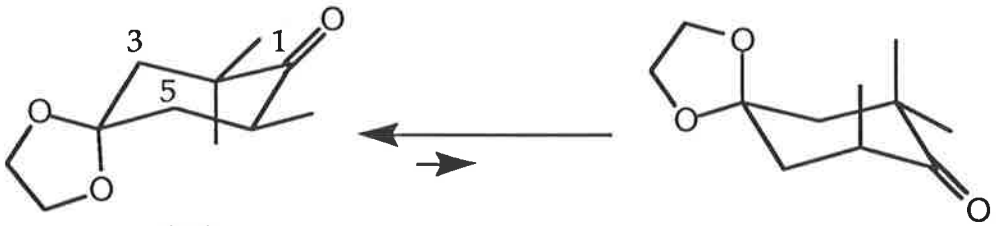


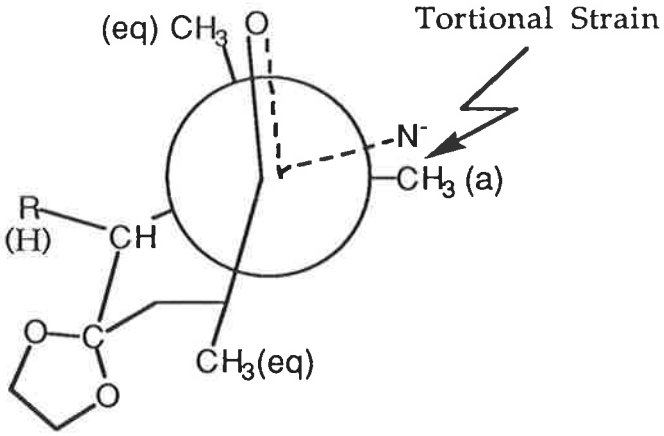
Figure 3b.

The addition of Grignard reagents to ketones is considered to be irreversible and kinetically controlled and thus occurs from the less hindered direction. Models suggest that the addition of the Grignard reagent of but-3-yn-2-ol to the ketone (73) should also be selective for one face of the carbonyl group, that is, via axial attack on the ketone, which is assumed to be in its favoured conformation, as shown below. In practice, this result was observed.

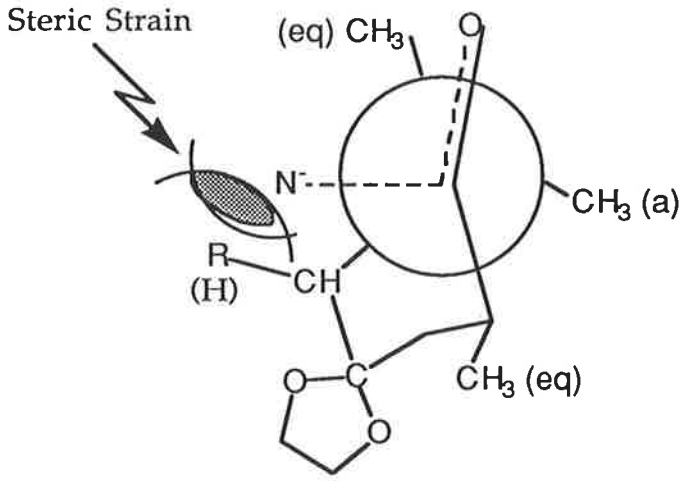


(73)

This observed mode of addition to form the major isomer(76a) is consistent with the hypothesis of Cherest,[67] according to which, the stereochemical outcome of attack of the Grignard reagent is determined by consideration of the transition state. According to these authors, the stereoselectivity of the addition of Grignard reagent to cyclohexanones is influenced by two factors. Axial attack is hindered by 1,3-diaxial steric interactions between the incoming nucleophile and the C3 and C5 axial substituents, whereas equatorial attack is hindered by the torsional interaction between the nucleophile-C1 bond and the C2 and C6 axial bonds. In the absence of bulky axial substituents at C3 and C5, as in the case of ketone (73), axial attack is favoured. (R = H, Figure 4.)



$A^{\#}$ -Equatorial attack t.s.



$E^{\#}$- Axial attack t.s.

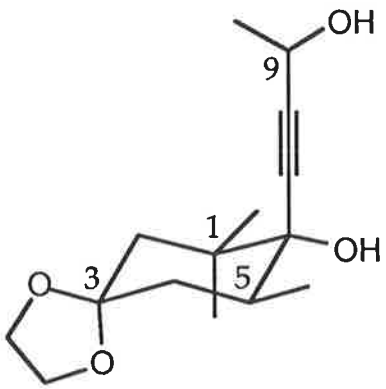
Figure 4.

In addition, the Grignard reagent in this case might be expected to have a low steric bulk, as alkyne anions are known to be small and linearly elongated groups and to show a predominance of axial attack to cyclohexanone[68]. However, because of the O-MgBr in the Grignard reagent, prediction about the size is more unreliable.

Cieplak[69] has suggested that the stereochemistry of nucleophilic addition to

cyclohexanones is determined by a combination of steric and stereoelectronic effects. He reports that electronic factors resulting from both axial and equatorial substituents favourably influence the axial mode of approach of nucleophiles on cyclohexanones.

The axial addition product (76a), arising from the reaction of the Grignard reagent with the ketone (73), would be expected to be a mixture of two diastereoisomers (epimeric at C9) in approximately equal proportions. However, only one substantial peak was observed during GC analysis of the products, which suggested that predominantly one diastereoisomer may have been formed. The 300 MHz ^{1}H n.m.r. spectrum of the crude product (76) supported this interpretation. Only one major signal attributable to the C10 methyl protons was seen, as was the case for the proton at H9, C1 axial methyl, C1 equatorial methyl, H2 axial proton, H2 equatorial proton and the C5 methyl. Additional small signals were attributed to a minor epimer. Treatment of the crude diastereoisomeric mixture (76a) with increasing concentrations of the reagent, known as Eu(fod)$_3$[70], only gave broadening of the methyl side chain signal in the ^{1}H n m r spectrum and was not diagnostic.

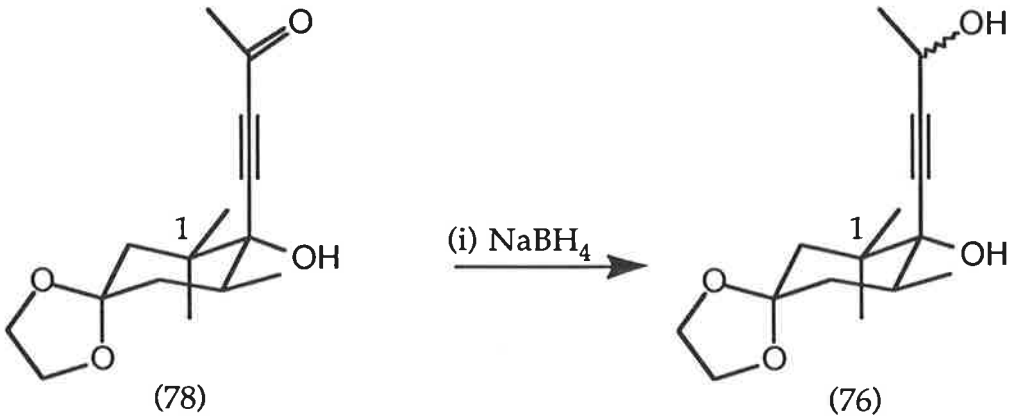


(76a)

To confirm whether compound (76a) was in fact a single diastereoisomer oxidation of the side chain hydroxyl with manganese dioxide[71] followed by reduction with a non stereoselective reducing agent (sodium borohydride) was investigated.

Oxidation of the purified major Grignard adduct (76a) gave one product (78a), which showed one peak by G.C., whereas oxidation of the 9:1 mixture of adducts (76a) and (76b) gave two products (78) (9:1), [epimeric at C6], as determined by G.C. These two products had identical mass spectra and were therefore diastereoisomers, differing only in the relative configuration at C5 and C6. Therefore the secondary methyl and acetylenic side chain are trans, and presumably diequatorial in the minor adduct.

Reduction of the pure ketone (78a) with sodium borohydride gave a product which was apparently homogeneous by GC and 300MHz ^{1}H n m r spectroscopy. Therefore the C9 epimers are not distinguishable by these techniques and the main product (76a) of the Grignard addition was therefore presumably also a diastereoisomeric mixture of these isomers.



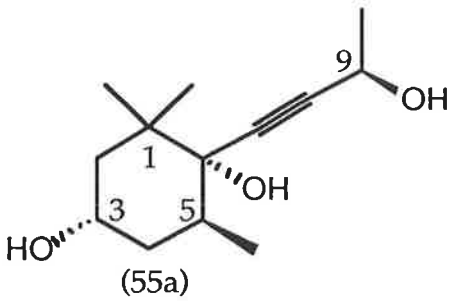
(78) (76)

2.1.3.1 Alternative Approach to the Synthesis of 3,3-Ethylenedioxy-7-megastigmyne-6,9-diol (76)

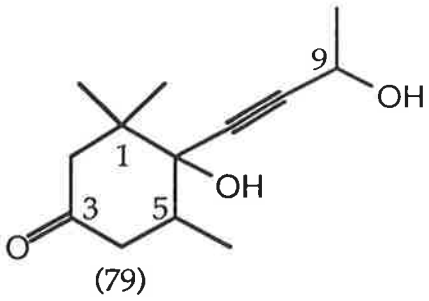
An attemped synthesis of diol (76) using the method of Saimoto[72] which involved treatment of the acetal (73) with a mixture of potassium hydroxide in 55% aqueous 3-butyn-2-ol at 40°C for thirty minutes, resulted in only starting material being recovered. This procedure was repeated at higher temperatures, however no formation of the diol (76), (diastereoisomers), was evident.

2.1.4 Synthesis of *(3S',5S',6R',9R')*-7-megastigmyne-3,6,9-triol (55a)

Mild hydrolysis of the crude acetal (76) in acidic methanol gave the crude dihydroxyketone (79), Scheme 6. Recrystallization of this product from dichloromethane / petroleum ether gave a 29% yield of colourless cubic crystals. GC analysis of the crude ketone before crystallization gave only one peak; however, comparison of the ^{1}H n.m.r. spectrum (300 MHz) of the crystalline ketone (79) with that of the mother liquors revealed minor signals in the latter, presumably attributable to the other diastereoisomer. The major signals seen in the ^{1}H n m r spectrum of (79) were a quartet at δ 4.66, J = 6.8 Hz (H9); an AB quartet for the methylene protons at C2, the axial hydrogen at δ 2.67, and the equatorial hydrogen, δ 2.09, with a further small coupling, presumably the 4 sigma-bond W long-range coupling with equatorial hydrogen at C4. A three proton doublet at δ 1.5, J = 6.8 Hz was assigned to the methyl group (H10) in the side chain, and another doublet at δ 1.14, J = 6.0 Hz to the methyl at C5.



(55a)

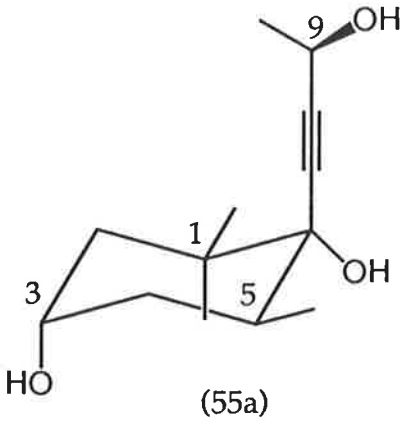


(79)

Reduction of the recrystallized ketone (79) with sodium borohydride was highly stereoselective, giving two triols in an approximate ratio of 95:5 as determined by GC / MS.

The stereochemistry of the major recrystallized triol (55a) (m p 145-147°C / $CHCl_3$ or EtOH) was partly determined from its 300 MHz ^1H n.m.r. spectrum, assigned with the aid of decoupling experiments. The spectrum comprised a quartet at δ 4.47, J = 6.7 Hz assigned to H9 ; one proton multiplets at δ 3.9 and δ 2.22 for (H3 and H5); a four proton multiplet at δ 1.58 for (H2 and H4); a three proton doublet at δ 1.34, J = 6.7 (C9 methyl); two three proton singlets at δ 1.17 and 1.01 for geminal methyls, and a three proton doublet at δ 0.98 (C5 methyl). Proton decoupling experiments verified these assignments. The one proton multiplet at δ 2.22 collapsed to a doublet of doublets when the methyl doublet at δ 0.98 was irradiated. The width of this doublet of doublets (15.5 Hz) suggests that the proton at H5 is axial. The signal at δ 3.9, coupled to the four-proton multiplet centred at 1.58, had a total width of less than 23Hz, comprising four vicinal coupling constants plus an additional H3-OH coupling (measured as 3.0 Hz). This suggests that H3 is equatorial.

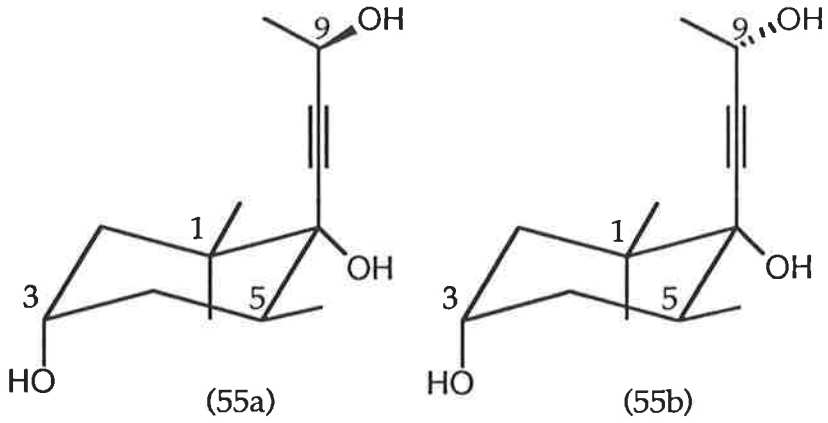
The complete stereochemistry of (55a) and therefore the relative configuration of C5 and C6 and C9 of the acetal (76a) was established by X-ray crystallography[66] and is shown below.



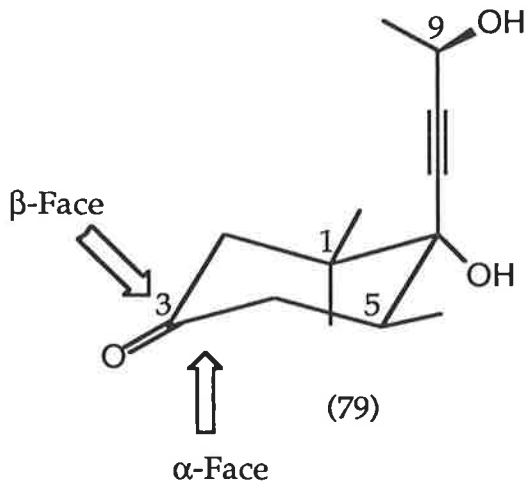
(55a)

The unit cell comprised two conformers of (55a) stabilized by H bonding at the oxygen at C9. The conformers differed by rotation about the C8 - C9 bond.

The product from the mother liquors from crystallization of the crude ketone (79) was also reduced with sodium borohydride to give a clear oil, which upon fractional crystallization from dichloromethane gave several crops. The melting points of these fractions differed from that of the pure triol (55a). The variation in the melting points is attributed to the presence of diastereoisomers, which are in fact detected by GC / MS. Reduction of the crude ketone (79) and subsequent GC / MS analysis revealed four diastereoisomers of triol (55); however, one diastereoisomer, present as greater than 90% of the mixture had the same retention time as the crystalline triol (55a) but a different melting point. Recrystallization of the crude triols (55) from ethyl acetate gave a crystalline triol (55b) with a sharp melting point (118°C), identical to that of a compound described by Loeber[52]. This has a different melting point from the crystalline triol (55a) (m p 144-147°C) which crystallized from the crude mixture (55) ($CHCl_3$). Both (55a) and (55b) had identical 300MHz ^1H n m r spectra and retention times by GC / MS.



The stereochemistry of this solid triol (55b) is considered to differ from (55a) only at C9. The observed stereochemistry of reduction, with hydride attack occurring from the β- face, is expected because the axial methyl at C1 hinders axial α- attack.



α-Face Shielded by C1 Axial Methyl Group

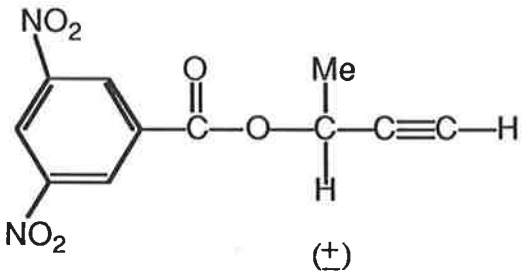
2.1.5 Attempted Synthesis of Optically Pure *(5R, 6S, 9S)*-6,9-Dihydroxy-megastigman-3-one (79 a)

Preliminary studies were done towards the synthesis of the optically active diol (79a) in order to obtain one enantiomer. This would enable further transformations to other norisoprenoids in the optically active series and, in particular, enable single diastereoisomers to be produced on glycosylation.

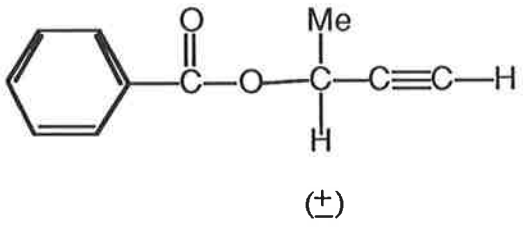
2.1.5.1 Resolution of 3-Butyn-2-ol (80)

Before carrying out ~~a resolution~~ the preparation of optically pure 3-butyn-2-ol (80) methods for determining the optical purity of the component were examined.

High pressure liquid chromatography (h.p.l.c.) is a useful tool used for the separation of diastereoisomers and for the determination of compound purity. Another important application of h.p.l.c.is the use of chiral columns for the analysis of enantiomers and diastereoisomers. The 3,5-dinitrobenzoyl and benzoyl esters of 3-butyn-2-ol (81) and (82) were synthesized for h.p.l.c. analysis on a chiral column (using a UV detector) to determine whether the enantiomers were resolvable. However, resolution by h.p.l.c. of the racemic esters (81) and (82) was unsuccessful.

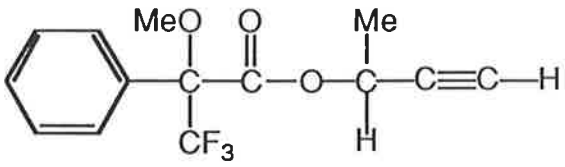


(81)



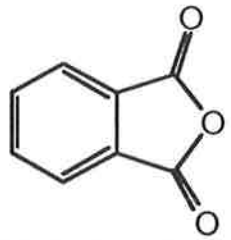
(82)

^{1}H and ^{19}F n m r spectroscopy is another means used to determine diastereoisomer ratios. The Mosher ester[73] of 3-butyn-2-ol (83) was synthesized and the methyl doublets of the two diastereoisomers were easily resolved by ^{1}H n m r spectroscopy at 60 MHz. The methyl groups were centred at δ 1.52 and δ1.4 ppm. ^{19}F n m r spectroscopy was also used and the fluorine resonances of the two diastereoisomers were found to be separated by 0.26 ppm.



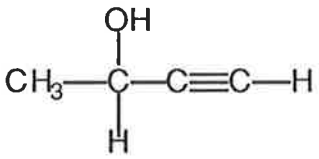
(83)

3-Butyn-2-ol (80) was resolved via the (S)-(-)-α-methylbenzylammonium salt of phthalic acid half ester[74,75]. The salt was recrystallized from acetone until a constant melting point solid 135-138°C was obtained. Saponification followed by distillation afforded the (S)-(–)-3-butyn-2-ol (80a) (70-73°C / 18·5 mm) in low overall yield. Scheme 7.



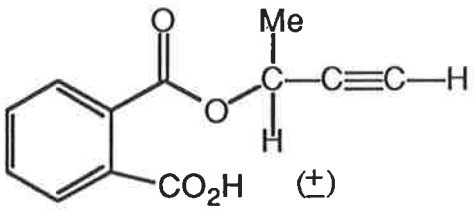
Phthalic anhydride

+

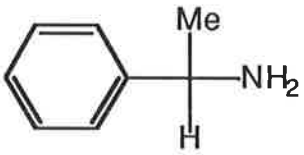


(±)- 3-Butyn-2-ol (80)

DMAP, NEt_3, CH_2Cl_2



Recrystallized from Chloroform / Petroleum Ether

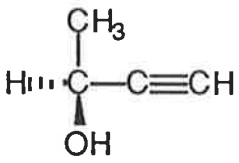


(S)-(–)-α-Methylbenzylamine

Salt, Recrystallized from Acetone.

Acidification

Saponification



(S)-(–)-3-Butyn-2-ol (80a)

Scheme 7.

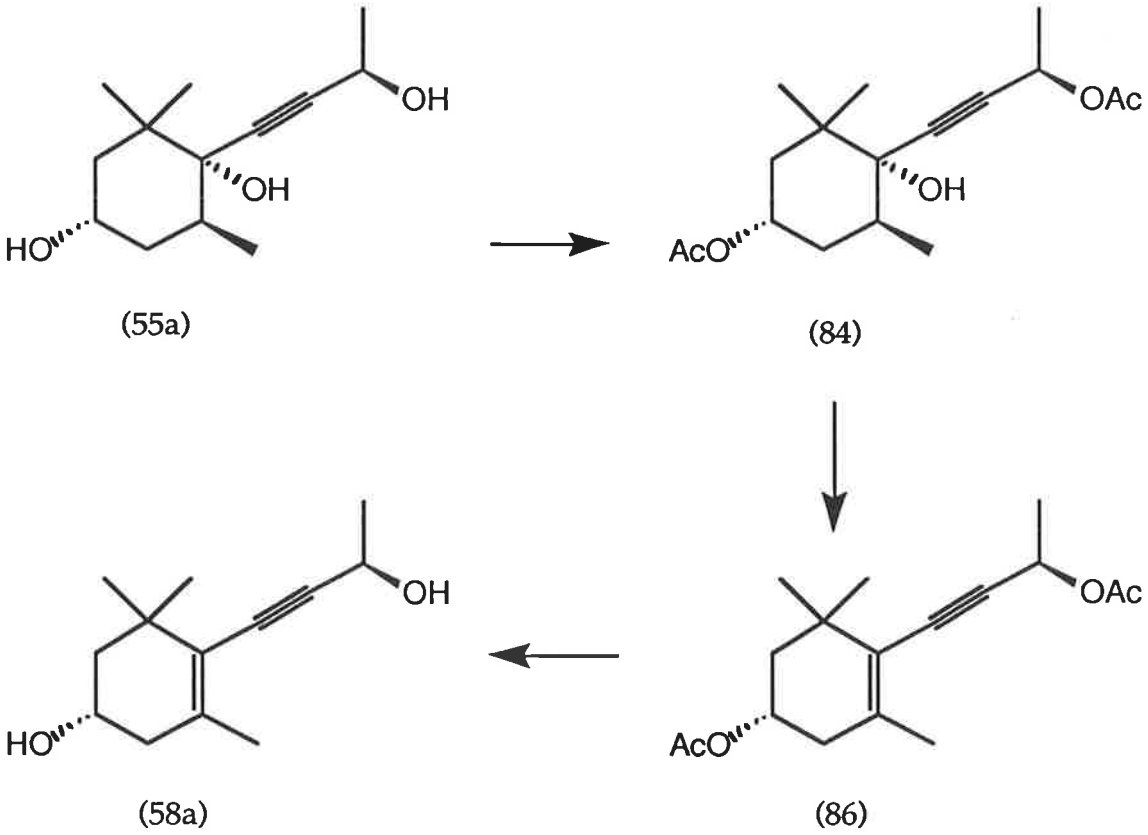
300MHz ^1H n m r spectra were recorded for both the racemate and resolved salt of the phthalic acid half ester (Scheme 7.). Both spectra clearly exhibited the signals resulting from the methyl resonances as well as that due to the alkyne proton. Therefore, ^1H n m r of the phthalic acid half ester was used as a means of determining diastereoisomeric purity(99%d.e). Examination of the Mosher ester was not necessary. Finally, the optical rotation of the resolved alcohol was measured in dioxane and found to be $[\alpha]^{22}_D$ = -52 4°. A wide range of values have been reported[75], however Olsson[75] quotes -51.8° in dioxane at the same concentration (c 3.8).

2.1.5.2 Attempted Synthesis of Optically Pure *(5R, 6S, 9S)*-6,9-Dihydroxy-megastigman-3-one (79 a).

The synthesis of the above compound (79a) using optically pure 3-butyn-2-ol was performed as shown in scheme 6. Addition of the acetal (73) to the Grignard reagent of but-3-yn-2-ol (prepared by treatment of ethylmagnesium bromide with 3-butyn-2-ol) gave the diol (76a). Purification of the adduct by chromatography afforded pure diol (76a). Mild hydrolysis gave a clear oil (79a) which could not be crystallized from dichoromethane / petroleum ether. Further purification by chromatography and seeding the oil with solid ketone (79) proved fruitless. Because of the time constraints this approach was not pursued any further.

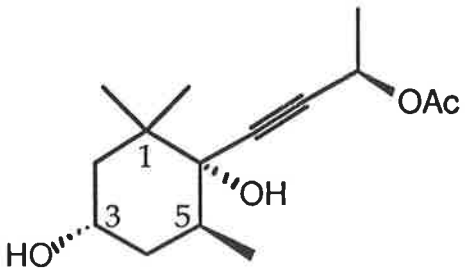
2.2 Synthesis of *(3S', 9R')*-5-Megastigmen-7-yne-3,9-diol (58a)

The synthesis of the enyne diol (58a) from the triol (55a) was carried out in three steps as described by Loeber.[52] Scheme 8. However an alternative and improved method for the second step was utilized.



Scheme 8.

Treatment of the triol (55a) with acetic anhydride in pyridine at room temperature gave diacetate (84). Also isolated from the reaction mixture was some minor amount of the monoacetylated product (85).



(85)

2.2.1 Synthesis of *(3S',9R')*-5-Megastigmen-7-yne-3,9-diyl diacetate (86).

A number of standard methods were attempted for the synthesis of compound (58a). The overall process requires the syn elimination of water. The approach outlined by Loeber[52] used phosphorus oxychloride in which they obtained compound (86) in 56% yield. Attempts to improve the yield of this key intermediate were undertaken by investigating by the following approaches:

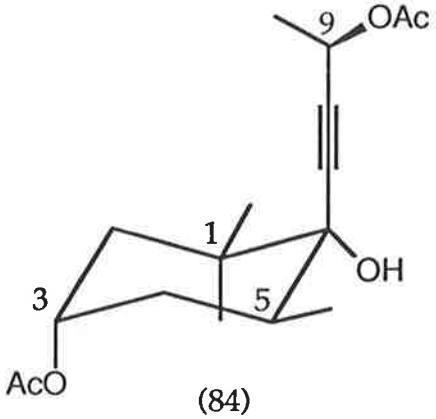
1 Formation of a leaving group commonly used for effecting ionization, i.e. through treatment with phosphorus oxychloride, thionyl chloride and mesyl chloride,

2 The attempted formation of leaving groups suitable for a thermal elimination, favourable for compounds with syn stereochemistry, e.g. acetate, carbonate and sulphamate ester.

2.2.1.1 Reaction with Phosphorus Oxychloride[52].

When the diacetate (84) was treated with phosphorus oxychloride in pyridine and heated at 90°C for twenty four hours, a relatively low yield (30-38%) of the enyne diacetate (86) was obtained[52]. The conditions were then changed by heating at 40°C and monitoring the elimination reaction by GC / MS. Elimination was seen to proceed smoothly and was apparently complete after 5 days as implied by the disappearence of diacetate (84), however, the yield of the enyne diacetate (86) after work up was only slightly higher. The low yield is presumably due to the unfavourable syn stereochemistry of the hydrogen at C5 and the leaving group at C6. The dehydration of diacetate (84) is assumed to proceed via a carbocation

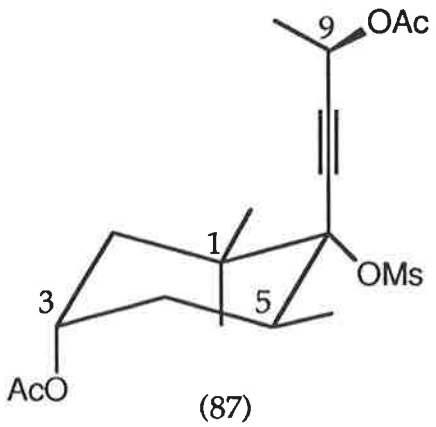
intermediate which can rearrange to form a number of undesirable products in addition to (86).



(84)

2.2.1.2 Attempted Dehydration Using the Mesylate (87).

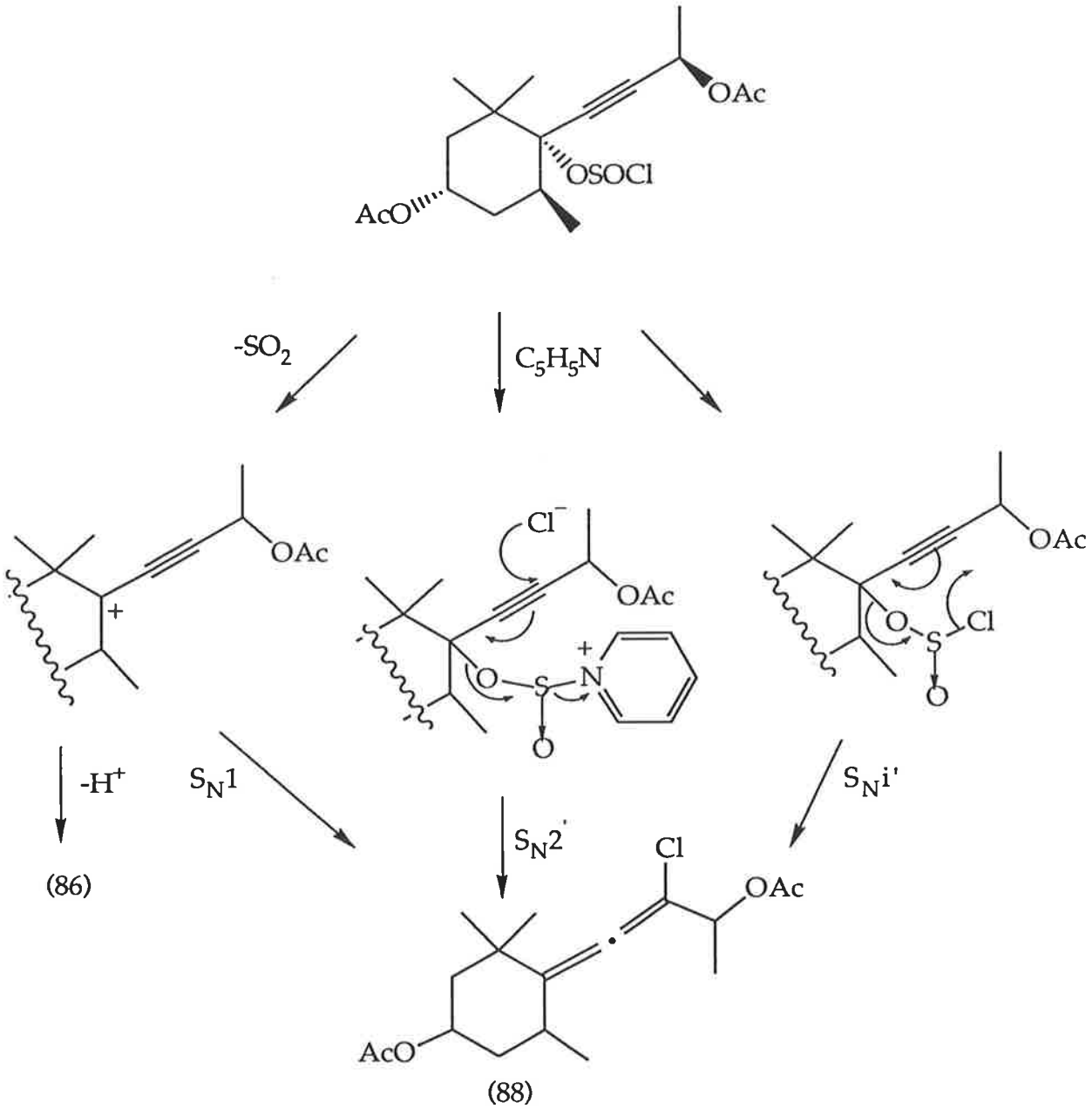
Treatment of the diacetate (84) at low temperature (-78°C) with methanesulphonyl chloride in the presence of triethylamine[76] yielded only starting material. The reaction was repeated at room temperature with only trace amounts of the tertiary mesylate being formed. When the reaction temperature was increased to 70°C for twenty four hours a mixture of compound (84), tertiary mesylate (87) and enyne diacetate (86) was obtained. This method was abandoned due to the poor recovery of products.



(87)

2.2.1.3 Treatment of Diacetate with Thionyl Chloride.[77]

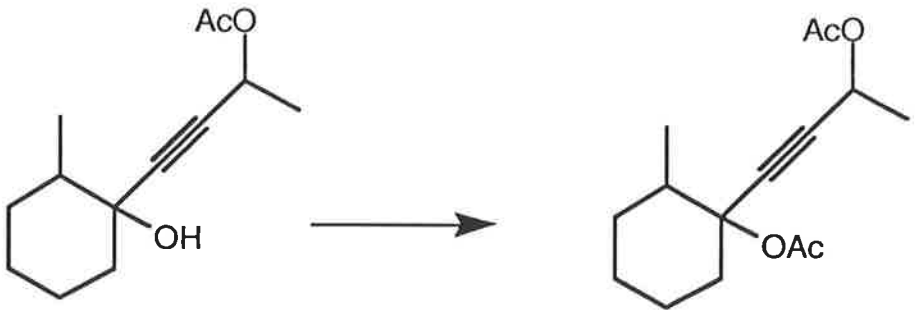
Attempted dehydration of compound (84) with thionyl chloride at low temperature (-10°C) resulted in the formation of approximately 17% enyne diacetate (86). However, the major products were the diastereoisomeric chloroallenes (88) formed in 82% yield. The chloroallenes were identified from their infrared spectrum in which the allenic carbon stretch diagnostic for allenes was seen at 1960 cm^{-1} and a C–Cl stretch at 735 cm^{-1}. Also, in the mass spectrum, masses of 226 and 228 (M^+-60-42) were seen in the ratio of 3:1 characteristic of chlorine incorporation. Their formation can be rationalised as shown below[78]. Because of the preferred formation of the chloroallenes (88) is approach for the preparations of the enyne diacetate was discontinued.



2.2.1.4 Formation of the Triacetate (89) and Its Elimination[79]

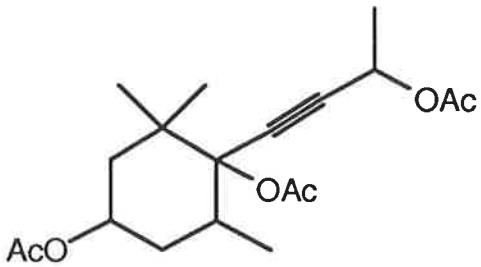
Formation of the tertiary acetate, with the required syn stereochemistry for easy thermal elimination to the enyne diacetate (86) was undertaken. In a preliminary study, the model alcohol (90) was successfully converted to the tertiary acetate (91) by treatment of (90) with acetic anhydride, pyridine and

a catalytic amount of 4-dimethylaminopyridine at room temperature for 63 hours.



(90) (91)

However, under the same conditions, the more hindered diacetate (84) did not react. to give the tertiary acetate (89). Even at 100°C in a sealed tube only trace amounts of tertiary acetate could be detected, with mainly starting material being recovered.

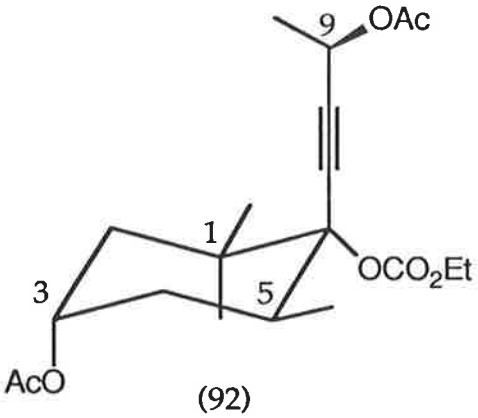


(89)

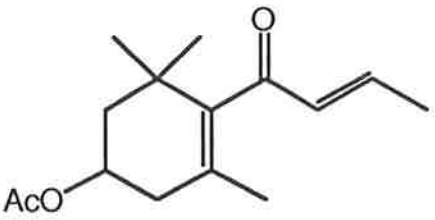
When acetylenic triol (55a) was heated in refluxing acetic anhydride by the method of Davies[80], however, even after twenty four hours, only diacetate (84) was obtained.

2.2.1.5 Formation of Tertiary Carbonate (92)

Formation of the tertiary ethyl carbonate by the patented method of Hoffmann[81], involved treatment of the diacetate (84) with n-butyllithium at -40°C followed by addition of chloroethyl formate. The resultant carbonate (92) was obtained in good yield.



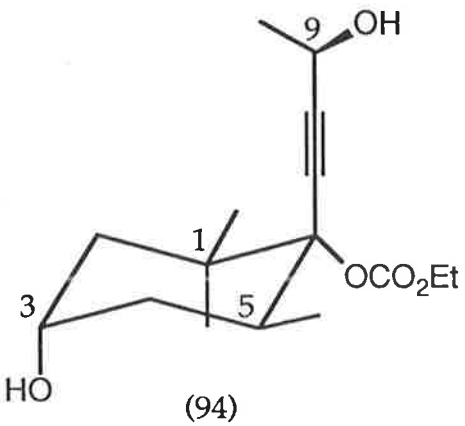
(92)



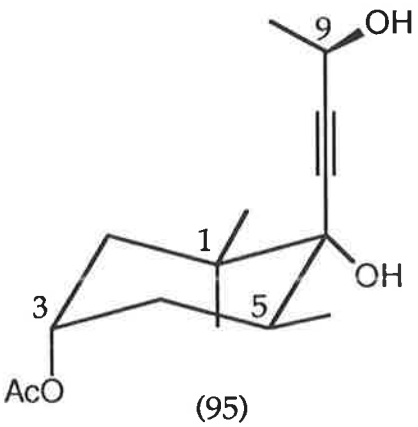
(93)

Treatment of the carbonate (92) at 60°C in the presence of a catalytic amount of pyridinium p-toluenesulphonate in dimethylformamide resulted in no elimination and starting material was recovered. At 80°C, again, no alkene formation occurred but at 100°C a higher Rf UV active component was seen. After refluxing the carbonate (92) in acetic acid for three days GC / MS revealed that the major components were the alkene (86) together with rearranged material (93) and starting material (92).

An attempt to convert the carbonate diacetate (92) to the carbonate diol (94) with 2 equivalents of sodium hydroxide in tetrahydrofuran failed, with compound (95) being obtained. This was confirmed by ^{1}H n m r where the quartet of the ethyl group had completely disappeared but the multiplet due to the hydrogen in the carbon bearing the ring acetate was still evident at δ 4.9 ppm as well as an acetate methyl group at δ 1.98 ppm. The tertiary carbonate method was abandoned for a different procedure.



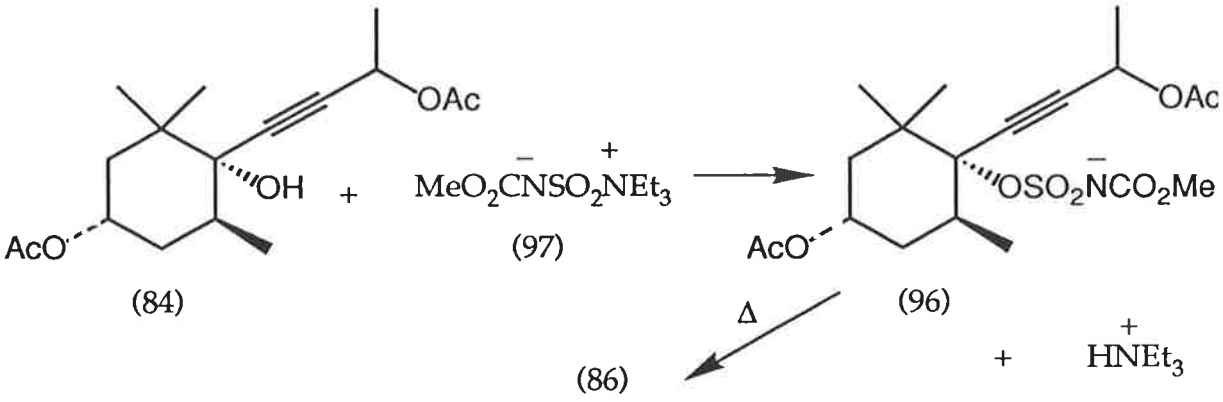
(94)



(95)

2.2.1.6 Use of N,N,N-Triethylammonio-N'-methoxycarbonylsulphamidate (97)

The most successful method of elimination involved the use of N,N,N-triethylammonio-N'-methoxycarbonylsulphamidate (97)



The reaction conditions described by Burgess[82,83] involved treatment of the alcohol with 2.5 equivalents of reagent (97) at 0°C in benzene followed by an increase in temperature to 60°C for one hour. Also, the use of potassium or sodium hydride to prevent the anion (96) being protonated was also investigated.

Treatment of this inner salt (97) with the acetylenic diacetate (84) in benzene gave the N-carbomethoxysulfamate ester salt (96) which upon reflux yielded the enyne diacetate (86) in good yield via thermal syn elimination. The optimum amount of reagent (97) was 2.2 mole equivalents. Thermal elimination at low temperature (60°C) was unsuccessful for the intermediate (96). Modifications to the counter ion were also examined as it was considered that the initially formed anion (96) might be protonated by its triethylammonium counter cation thereby favouring cleavage to give a

carbonium ion[84]. Use of sodium hydride[85] for preformation of the alkoxide from the alcohol (84) should circumvent this problem, however, dehydration of alcohol (84) still required refluxing conditions and no improvement in yields was observed with this modification

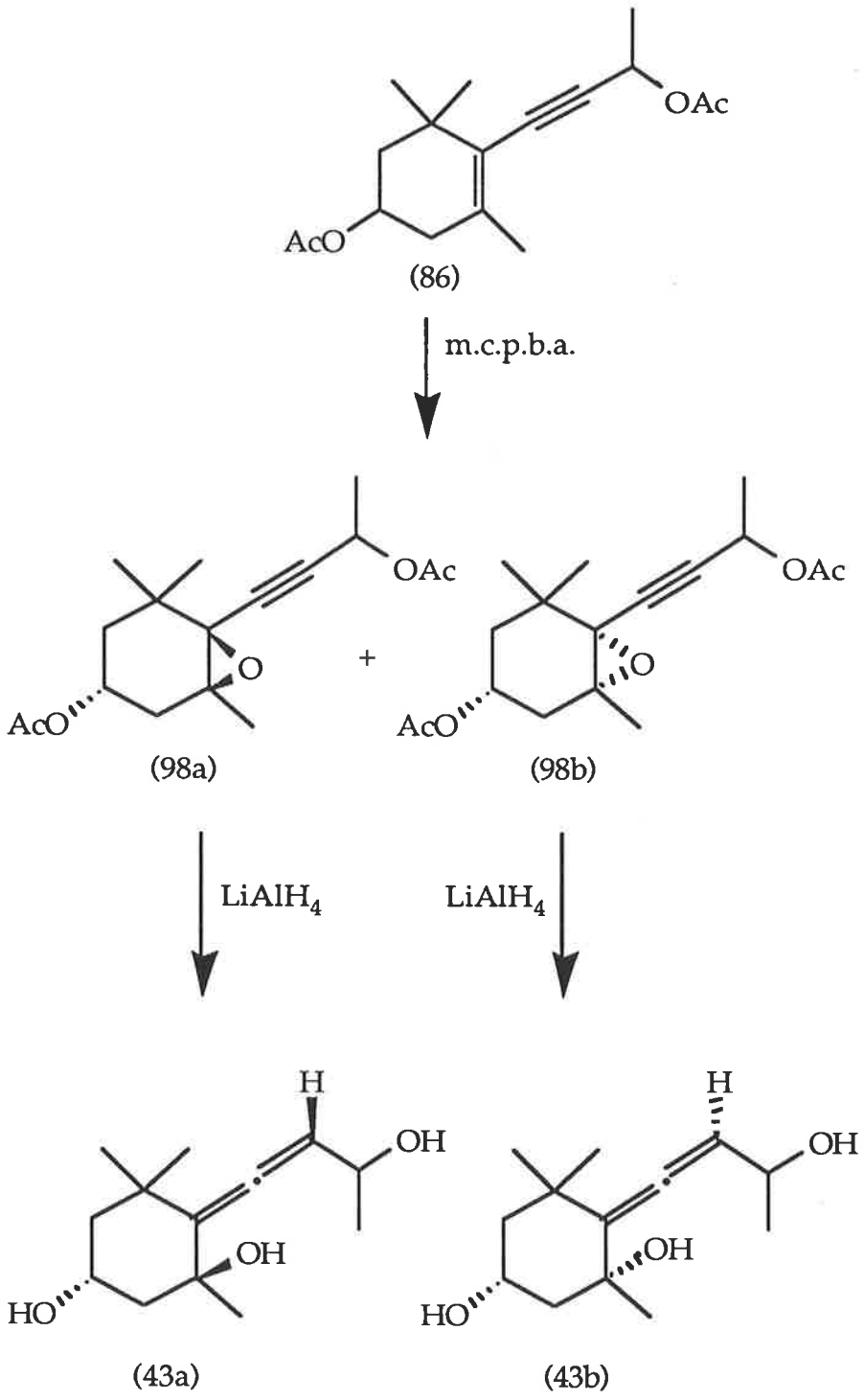
2.2.2 Synthesis of *(3S', 9R')*-5-Megastigmen-7-yne-3,9-diol (58a)

Conversion of the enyne diacetate (86) to the enyne diol (58a) was achieved by two different methods. Reduction of the enyne diacetate (86) with lithium aluminium hydride in ether[52], and hydrolysis of enyne diacetate (86) with potassium hydroxide in methanol[77], both gave the enyne diol (58a), with known relative configuration, in good yields. Enyne diol (58a) was obtained as an opaque oil with a sweet scent. It was found to be unstable to flash chromatography[86] and to readily undergo autoxidation.

After this reference material was available, the enyne diol (58a) was identified as both an aglycone and as glycoconjugate in grape juice samples.[18]

2.3 Synthesis of Allenes, 6,7-Megastigmadiene-3,5,9-triol (43)

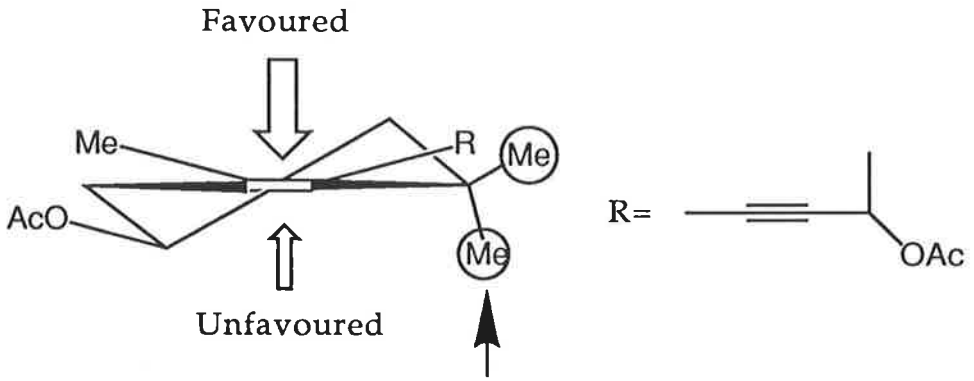
The route of Russell [87] to the allenic triols is shown in Scheme 9



Scheme 9.

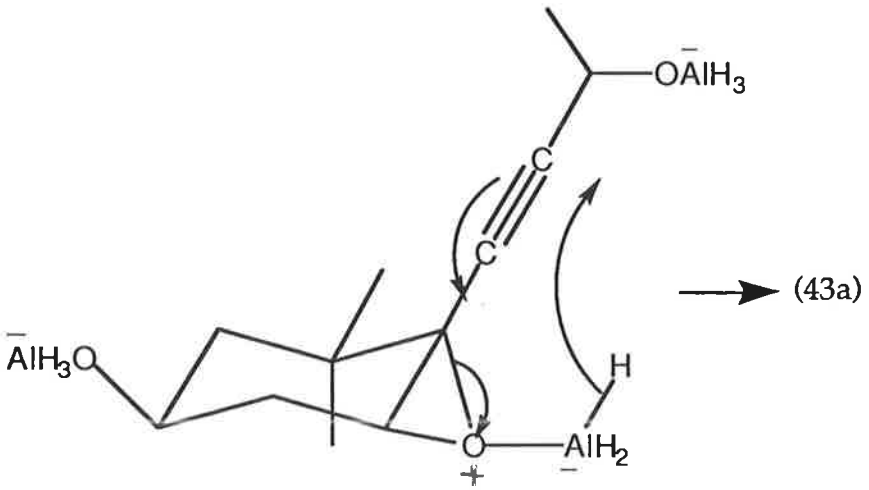
Treatment of enyne diacetate (86) with m-chloroperoxybenzoic acid (m.c.p.b.a.) in chloroform at 5°C yielded two epoxides (98a) and (98b) with the cis (acetate and epoxide) epoxide (98b) as the major isomer.[88] The observed stereoselectivity of epoxidation was accounted for by considering

the mode of addition of the peroxyacid to the enyne diacetate (86).[88] The favoured approach of the peroxyacid is cis to the acetate group as shown below. Approach from this direction avoids steric interaction with the quasi axial methyl group. DeVille [89] obtained a crystal structure for an allenic ketone which was synthesized from the trans epoxy acetate (98a), thereby proving the stereochemistry of the latter. It is known that other functional groups, such as the hydroxy group, in close proximity to the double bond exert a directing effect upon epoxidation[90]; however the acetoxy group has been reported to exhibit little influence.[91]



Quasi-Axial Methyl Group

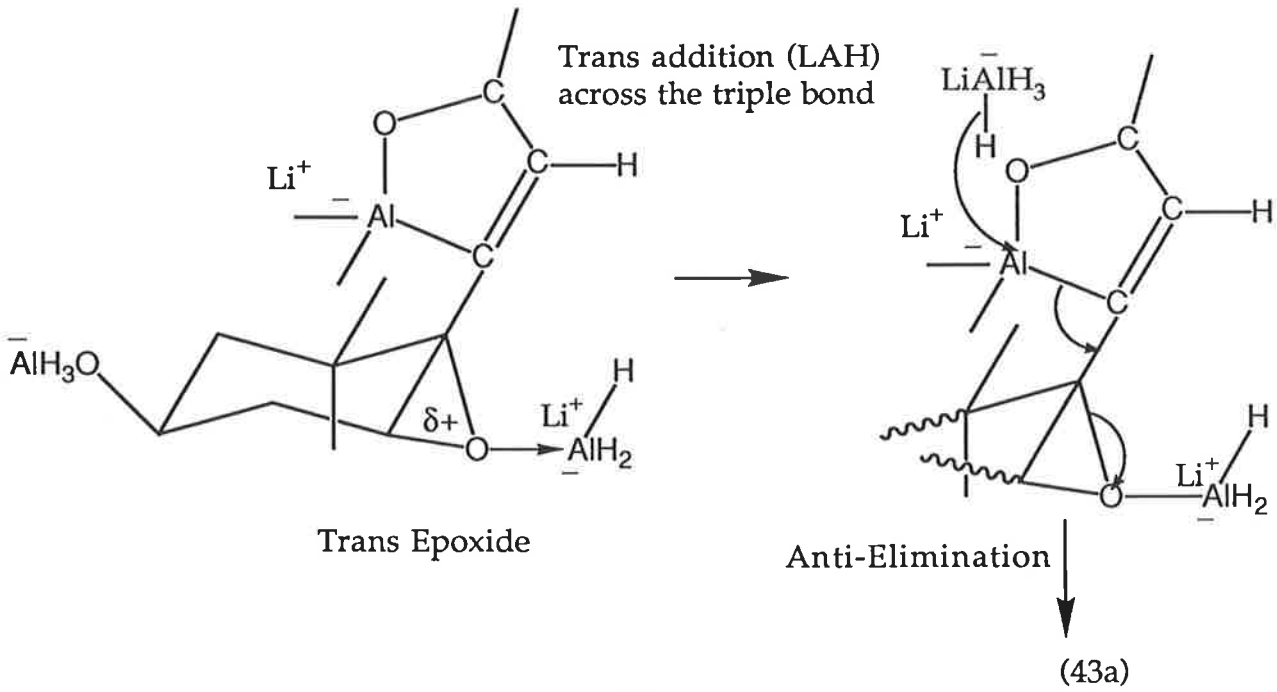
Synthesis of the allenic triols was then achieved by treatment of each of the epoxides, (98a) and (98b), with lithium aluminium hydride in tetrahydrofuran. The proposed mechanism for the allene formation is shown below[92].



Trans Epoxide

Hydride addition and epoxide bond breaking is believed to occur in a stereospecific fashion, with hydride delivery occurring as indicated to give the allene (43a) from epoxide (98a). The X-ray crystal structure[89] showed the cis orientation of the allenic hydrogen and tertiary hydroxyl group.

Olsson[73] has suggested that lithium aluminium hydride adds across the acetylenic triple bond in a trans fashion to give an organometallic intermediate having the indicated stereochemistry (Scheme 10). The addition of lithium aluminium hydride to the triple bond is followed by a 1,2-elimination with the overall stereochemical course of the reaction being a predominant attack of hydride on the triple bond from the direction in which the leaving group departs.

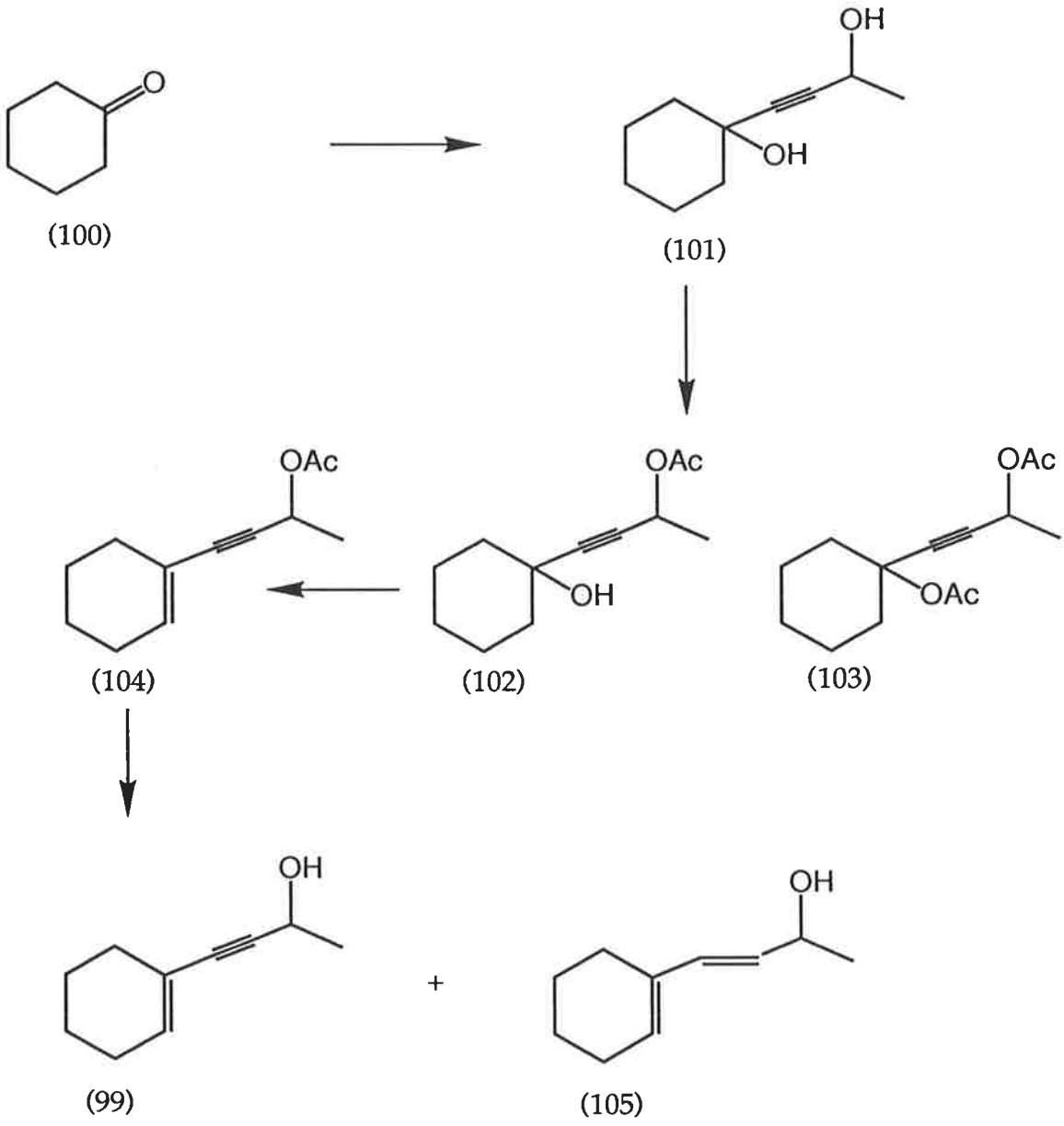


Scheme 10.

The stereochemistry of the trans allenic triol (43a) follows from an X-ray crystal structure of an identical allene triol isolated from the froth produced as a defensive mechanism by the flightless grasshopper *Romalea microptera* [93].

2.4 Synthesis of Model Enyne Alcohol (99)[94]

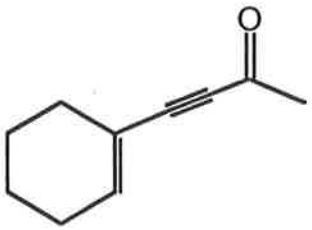
Alcohol (99) was synthesized by treatment of cyclohexanone (100) with the Grignard reagent prepared from 3-butyn-2-ol to give the diol (101)[71]. Scheme 11.



Scheme 11.

The diol (101) was then acetylated in pyridine / acetic anhydride at room temperature overnight to give the mono acetate (102) accompanied by a small amount of diacetate (103). Dehydration of the acetate (102) with phosphorus oxychloride in triethylamine proceeded in quantitative yield. Initial attempts to dehydrate compound (102) in acetic acid / acetic anhydride at reflux for two hours failed. The mixture appeared to polymerise.

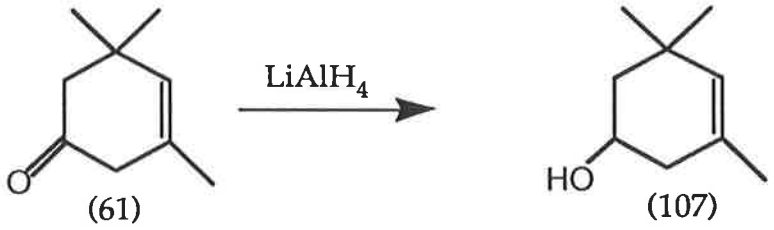
The enyne acetate (104) was then reduced with lithium aluminium hydride under nitrogen to give the model compound (99),accompanied by a substantial amount of the over-reduced product (105). The diene (105) was separated from (99) by chromatography and identified by ^{1}H n m r spectroscopy. The desired compound (99) was also found to autoxidise, presumably to the ketone (106). The structure of the ketone (106) was tentatively assigned from its mass spectrum which was similar to that of the alcohol (99).



(106)

2.5 Synthesis of Model Alcohol 3,5,5-Trimethyl-3-cyclohexen-1-ol (107).

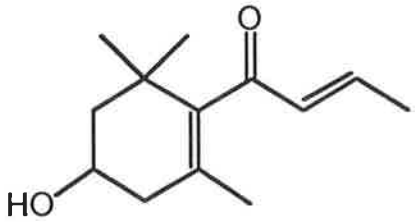
The alcohol (107)[59] was prepared by reduction of β-phorone (61) with lithium aluminium hydride.



(61) (107)

2.6 Synthesis of 3-Hydroxy-β-damascone (39).

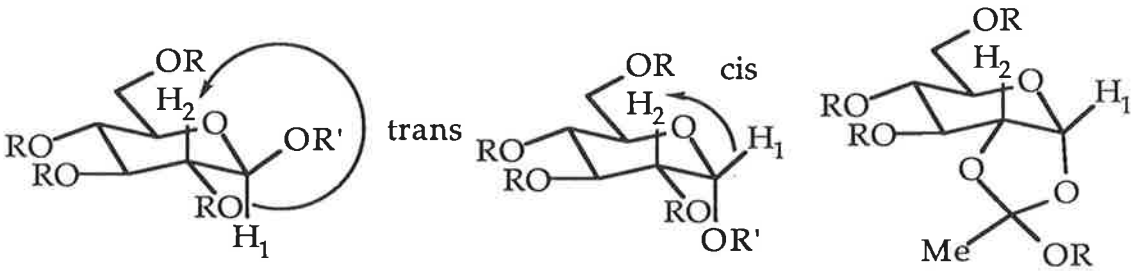
3-Hydroxy-β-damascone (39) was prepared by the acid catalyzed rearrangement of acetylenic triol (55a) in aqueous ethanol and was accompanied by β-damascenone (38) as a minor product. 3-Hydroxy-β-damascone (39) was obtained pure after chromatography in good yield. 3-Hydroxy-β-damascone (39) was found to have a rose floral and sweet aroma, as described by Ohloff.[25]



(39)

3.1 Introduction to β-Glycosidation Methods.

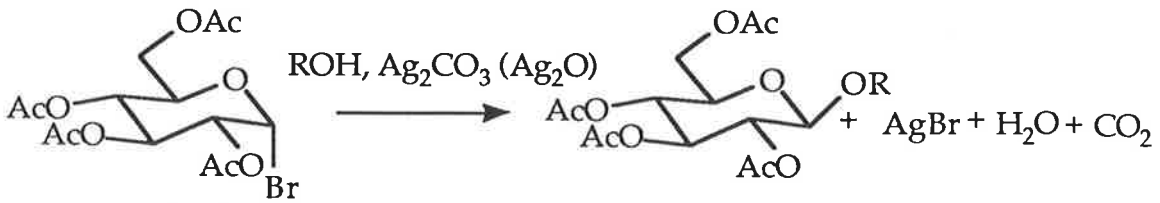
This section reviews the development of methods for the formation of β-glycosides during the last twenty years. The methods which appeared to be most suitable for glycosylating the aglycones in chapter 2 are emphasized. The large number of methods developed for β-glycoside synthesis reflects problems of yield and by-product formation, particularly α-glycosides and ortho esters. The α and β-glycosides can be distinguished by ^1H n.m.r. spectroscopy where the anomeric proton has a coupling constant of 2-5Hz for alpha (α) and 6-8Hz for beta (β) glycosides. In the β-glycoside H1 and H2 have a trans diaxial arrangement whereas in the α-glycoside H1 and H2 are in a cis arrangement.



β-glycoside α-glycoside Ortho ester

3.1.1 Heterogeneous Systems.

In 1901 Koenigs and Knorr[95] prepared β-glycosides (109) from the corresponding sugar bromide (108) with silver carbonate, silver nitrate, or pyridine as the acid acceptor.



(108) (109)

The reaction proceeded with inversion of configuration at the anomeric carbon atom. In general, this procedure, which is still widely used, involves treatment of a per-O-acetylated glycosyl halide with an alcohol in the presence of a heavy metal salt or an organic base as the acid acceptor. This method is referred to as the Koenigs-Knorr synthesis.

Since 1901, the synthesis of β-glycosides has undergone several variations; this section reviews some of the major changes from 1975.

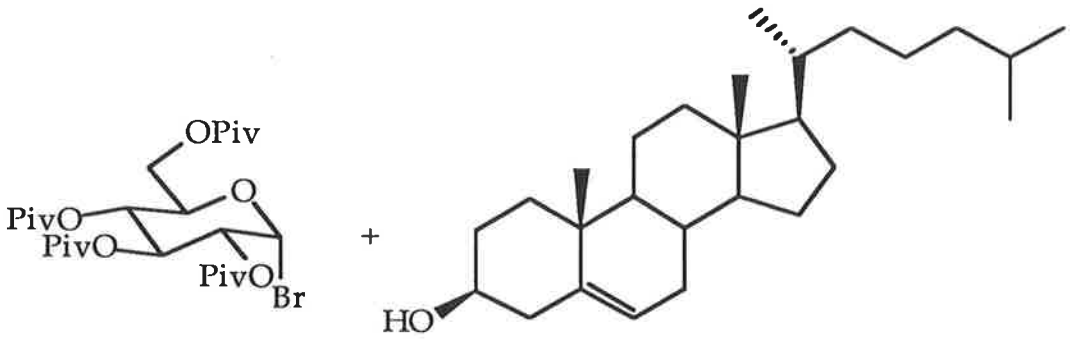
In 1977 Kikuo-Igarashi[96] reviewed the synthesis of 1,2-trans-glycopyranosides and highlighted some of the variations of the Koenigs-Knorr reaction. In the classical Koenigs-Knorr reaction, as shown above, water is formed during the reaction, and this can react with the halide to give a by-product. Kreider[97] introduced Drierite (anhydrous calcium sulphate) which is inert and acts as a good dehydrating agent. It was found that addition of iodine[96] may improve the yields.

Other workers[98] have used a variety of silver salts for glycosidation. However, a number of mercury (11) salts[99] such as mercury (11) oxide, mercury (11) acetate , mercury (11) cyanide and mercury (11) bromide can be used satisfactorily in place of the silver salts in the Koenigs-Knorr reactions.

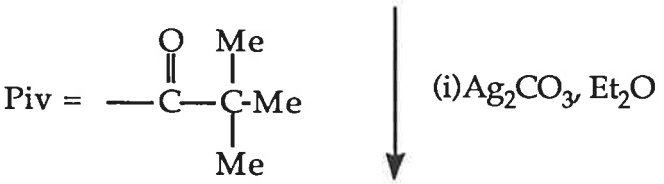
In 1977 Hanessian[100] produced a β-glycoside using tetraacetyl-α-D-bromopyranose in the presence of silver triflate as the Lewis acid and an acid acceptor 1,1,3,3 tetramethylurea in dichloromethane.

In 1979 Bochkov[101] reviewed the chemistry in the formation of the O-glycosidic bond. The review introduces the synthesis of oligosaccharides, polysaccharides, the use of ortho esters as intermediates for β-glycoside formation and conditions where cleavage of O-glycosidic bond occurs.

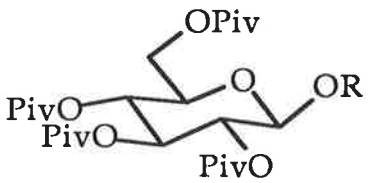
In 1982, Kunz's group[102] introduced the use of a different halopyranose. They used the tetrapivaloyl-α-D-bromopyranoside (110) which has the added effect of directing glycosidation via neighbouring group participation as well as steric hindrance to give exclusively the β-glycoside. The bulkiness of the pivaloyl group restricts ortho ester formation. In addition, they used various solvents and silver salts and found they achieved the best result using silver carbonate in diethyl ether. An example was the synthesis of cholesterol β-glycoside (112) in 78% yield.



(110) (111)



(i)Ag_2CO_3, Et_2O



(112) R = Cholesteryl

A modification of Kunz's 1982 procedure was the use of molecular sieves 3Å, silver carbonate and the tetrapivaloyl-α-D-bromopyranose by Vlakov in 1983[103]. The idea was to obtain stereoselectivity due to pivaloyl groups, anhydrous conditions using molecular sieves and a moderate Lewis acid in silver carbonate.

In 1983, an interesting paper by Magnus[104] reported solvent effects in the Koenigs-Knorr reaction with silver oxide as the heavy metal salt, and Drierite to keep the mixture anhydrous.These workers studied a two-solvent system, dichloromethane / diethyl ether and looked at the ratio of ortho ester to β-glycoside formation. The results they deduced were (i) in diethyl ether or dichloromethane less ortho ester formation was apparent, (ii) a mixture of the two solvents increased the dielectric constant thus making the medium more polar. This led to a substantial increase in ortho ester formation, most evident at the ratio of 25 : 75 diethyl ether / dichloromethane, (iii) no ortho esters were detected in chloroform / diethyl ether or carbon tetrachloride / diethyl ether mixtures, or when bulky dibutyl or diisopropyl ethers were used.

The introduction of Zeolites[105] became popular in the early 1980's. Molecular sieves (4Å, 3Å or 4Åsieve powder) were used instead of Drierite. Other insoluble promoters of the O-glycosylation reaction involved thallium, cobalt and cadmium zeolites (4Å and 13X). The best results observed were with the use of thallium zeolite which also replaced the use of silver carbonate as it acts as both a Lewis acid and a drying agent.

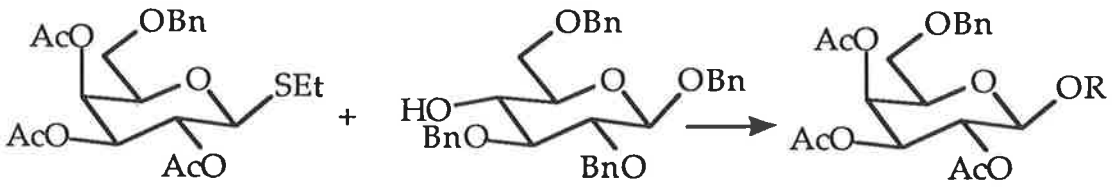
In 1985 Paulsen[106] used the more reactive catalyst silver silicate as well as pivaloyl pyranose halide which enabled the synthesis of primary, secondary and tertiary glycosides. 4Å sieves were employed as the drying agent in the reaction.

At the same time Kleine[107] reported the use of phase transfer catalysts for glycosylating phenols. These reactions used α-D-glycopyranylbromide, benzyltriethylammonium bromide (phase transfer catalyst) in chloroform / water in the presence of a base (sodium hydroxide). The base was used to generate the aryloxy anion. Note that this procedure is selective for alcohols

with acidic protons only. However, it provides an important method for the synthesis of aromatic glycosides.

In 1986, Kimura[108] noted that glycoside formation using trimethylsilyl triflate as a catalyst was inhibited during their attempts to glycosylate 4-demethoxy anthracyclinones using 4Å molecular sieve powder. However, a substantial increase in yield was seen when molecular sieves (4Å) were dried under a free flame in a stream of argon. They also noted that the use of 1,1,3,3 tetramethylurea and various other bases, which were used to remove trifluoromethanesulphonic acid, produced no glycosylation. Glycosylation occurred in the presence of 4Å sieves only.

In the same year Sato[109] reported the use of 1-β-thioglycosides which were activated with the aid of cupric bromide in the presence of a catalytic amount of tetrabutylammonium bromide. This constituted an efficient approach to the synthesis of glycosides, provided that silver triflate, together with powdered 4Å sieves, was used. An example was the synthesis of the secondary β-glycoside (115), from 1-β-thioglycoside (113) and alcohol (114), in 90% overall yield. Although this glycoside is a galactopyranoside the methodology may well be applicable to glucopyranosides.

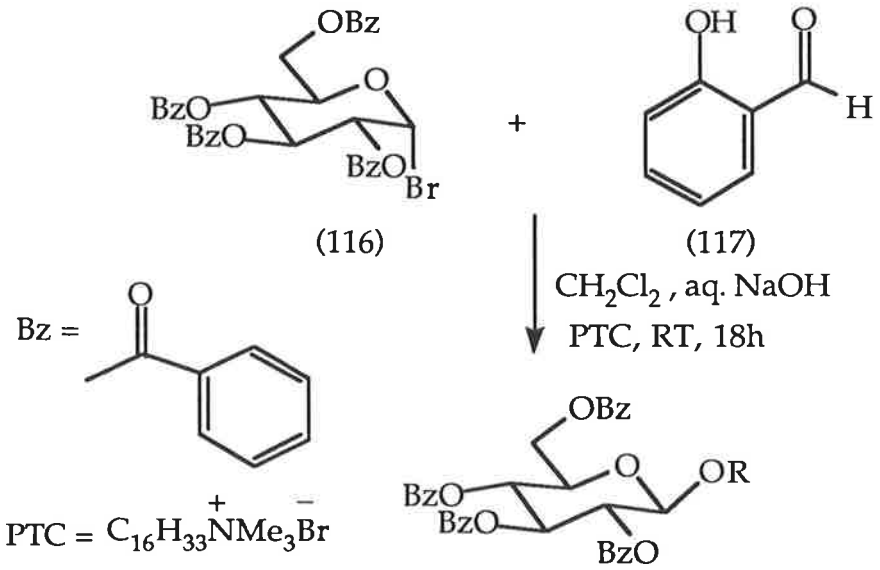


(113) (114) (115) R = (114)

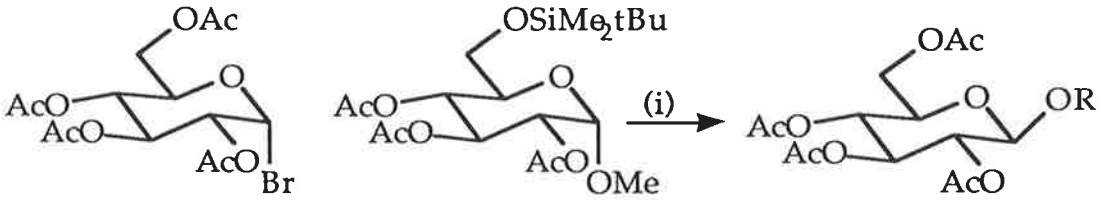
Note how this is contradictory to the work of Kimura[108] regarding the use of powdered molecular sieves. An explanation for the difference may lie in the binder used in molecular sieves / powder[110]. Yields were generally found to be greater when silver triflate was used in nitromethane as a solvent. It is interesting to note that the use of silver zeolite in nitromethane gave a

substantial yield of the corresponding glycoside. However, this method was found to yield less than the silver triflate / molecular sieve powder reaction. Also evident was the fact that silver triflate is a better Lewis acid than mercury (11) bromide, which was discussed earlier.

Subsequent glycosylations using phase transfer synthesis were published by Kleine[107] and Loganathan[111] in 1987 but using slightly different reagents. These were 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranylbromide (116) and aqueous sodium hydroxide with a phase transfer catalyst in dichloromethane, which were added to phenolic and cinnamate aglycones. The moderate yields indicated that an alternative mechanism was operating, in addition to the expected β-glycosylation pathway, and gave several minor by-products, one of which could not be identified. One minor by-product arose from elimination. An example of successful glycosylation by this method is the biphasic system employing cetyltrimethylammonium bromide as the phase transfer catalyst which afforded the β-glycoside (118) of o-hydroxy benzaldehyde (117) in 46% yield as shown below.



In 1987, Vijay Nair[112] described a novel procedure for the synthesis of oligosaccharides and aryl glycosides involving the glycosylation of silyl ethers. The use of silver triflate and Drierite in nitromethane as solvent followed by treatment of the silyl ether (119) with the sugar bromide (108) gave the β-glycoside. This reaction gave very high yields of the glycoside and the method was also applicable to phenols. An example was the formation of β-glycoside (120) in 89% yield.



(108) (119) (120) R = (119)

(i) Silver triflate, Drierite, Nitromethane. 0°C

Later, in 1989, Ackermann[113] modified the traditional Koenigs-Knorr method with the introduction of a soluble silver catalyst, silver trifluoroacetate, in the presence of a mild base, sodium hydrogen carbonate and a drying agent, calcium sulphate. The resultant yields for selected monoterpene glycosides made by this modified method, compared to the traditional Koenigs-Knorr method, showed i) a 1.2-2.0 fold increase for primary acyclic alcohols and ii) a 10-15 fold increase for tertiary and cyclic monoterpenols.

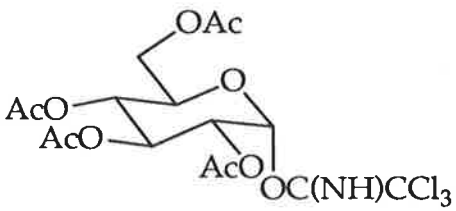
From the above review of papers on glycosylation in heterogeneous systems, it becomes obvious that there are a number of parameters which require consideration. Some require Drierite, molecular sieves, sieve powder, different solvent conditions , use of different Lewis acids and so on. In the earlier experiments, glycosylation occurred but yields were small. It was found that the primary alcohols glycosylated more readily than the secondary and tertiary alcohols which gave very low yields. This has

remained a problem until the present. However , since 1987 ,very high yields of secondary glycosides have been obtained through the use of a dry medium, soluble silver catalysts and a stable coupling sugar under milder conditions than those which prevailed in the earlier experiments. With respect to tertiary alcohols , glycosylation still remains a problem. However, the most recent paper by Ackermann[113] has shed some further light for its development.

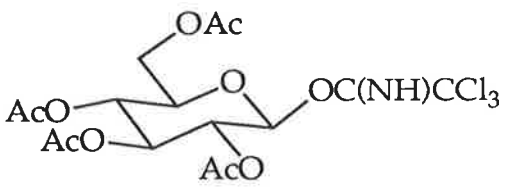
The following section reviews developments in homogeneous glycosylation which was first reported in 1980.

3.1.2 Homogeneous Systems

Homogeneous β-glycosidation has been developed in the last ten years and is becoming increasingly popular. The first report of homogeneous β-glycosidation was by Schmidt[114] in 1980 which described the synthesis of imidates as a new class of glycosylating reagents. The reagents (121) and (122) undergo nucleophilic substitution with inversion of configuration to give the corresponding glycoside with excellent control of stereochemistry at the anomeric carbon.



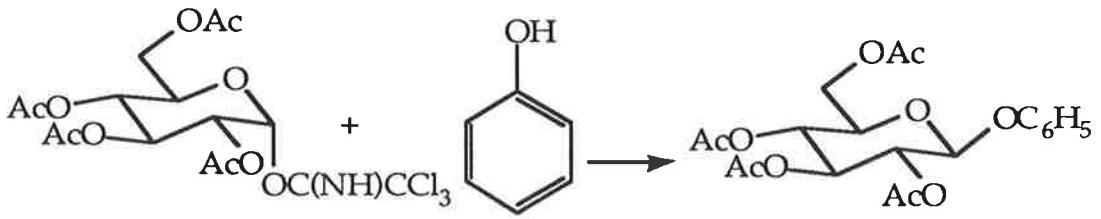
(121)



(122)

No α-glycosides can be formed from reagent (121) as the reaction proceeds irreversibly. Boron trifluoride etherate is used to activate the anomeric center and reactions are conducted in dichloromethane and at low temperatures

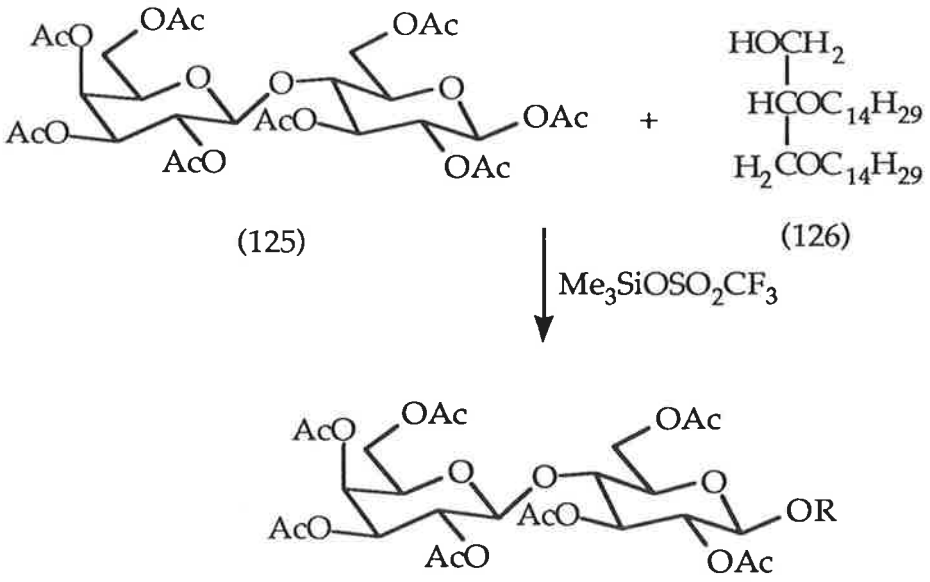
(-40 to 10 °C). The driving force for the reaction is the formation of trichloroacetamide as a stable leaving group and is assisted by neighboring group participation. However, the reaction is thought to proceed via an SN2 mechanism. An example is the synthesis of the phenolic β-glycoside (124) from phenol (123) in 44% yield.



(121) (123) (124)

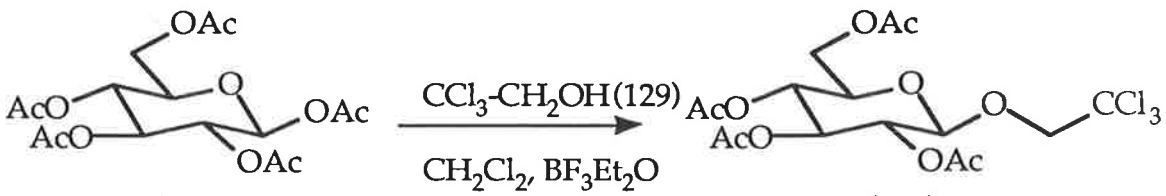
Schmidt's work[114] was a development towards the synthesis of β-glycosides from imidates. Sinay[115] had earlier developed the use of imidates, especially the N-methylacetamide, but the work was directed at the synthesis of α-glycosides and was not applicable to β-glycosidation.

In 1981 Ogawa[116] employed Lewis acids as efficient catalysts for the activation of the C-O bond at the anomeric carbon in the synthesis of trans-1, 2-glycosides. The Lewis acid used by Ogawa[116] was trimethysilyl trifluoromethanesulphonate. Treatment of a trans-1,2-diacetate with the Lewis acid in 1,2-dichloroethane lead to the trans-1,2-glycoside, (i.e.the β-glycoside). The disaccharide (125) when treated with the primary alcohol (126) in the presence of the above mentioned Lewis acid, gave exclusively the β-disaccharide glycoside (127) in 70% yield. The paper of Ogawa[116] presented many different examples of glycoside formation.



(127) R = (126)

In 1981 Magnusson[117] showed that the activation of the anomeric carbon (masked as an acetate) with equal amounts of boron trifluoride etherate was just as effective. Reactions were carried out in dichloromethane, whereas Ogawa[116] used 1,2 dichloroethane, and the temperature of the reaction was kept between -25 and 25 °C depending on the type of alcohol used. In general, primary alcohols were glycosylated at lower temperatures than secondary alcohols. Yields also ranged dramatically with higher yields being obtained for the less sterically hindered alcohols. An example was the synthesis of β-glycoside (130) in 89% yield from trichloroethanol (129).



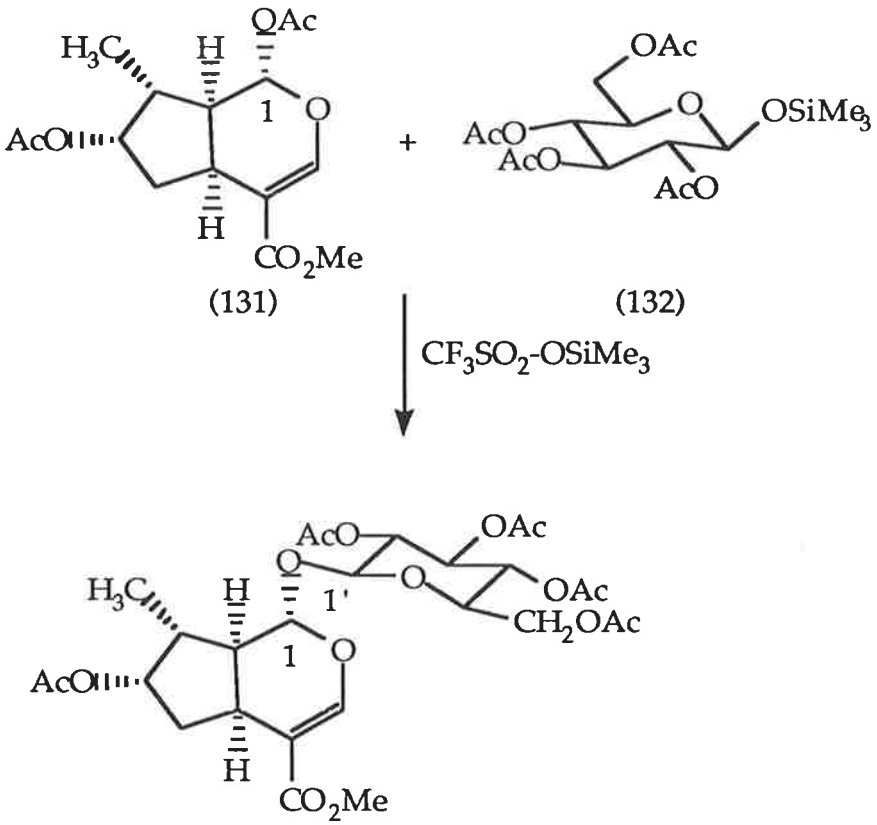
(128) (130)

In 1983 Dahmen[118] used the method of Magnusson in preparing 2-bromoethyl glycosides. They found that prolonged reaction time usually gave small proportions of deacetylated compounds. They also reported an

alternative method using heterogeneous glycosylation with silver triflate as a Lewis acid but no reason was given for the adoption of this procedure. This method has already been outlined in the discussion of heterogeneous β-glycosylation.

Paulsen[119], in 1983 treated a pentaacetylpyranose with trimethylsilyl triflate as outlined by Ogawa[116] and found the synthesis of O-glycopeptides / oligosaccharides to occur readily with primary and secondary alcohols of varying complexity. In 1984 Paulsen[119] discussed the reactivity of the pyranosyl halide and noted that ether-substituted compounds are always more reactive than acyl-substituted compounds. Introduction of a 1-O-acetate group instead of a halide in the glucopyranose requires activation by use of equimolar amounts of a Lewis acid. Paulsen also noted that the use of Lewis acids can cause side reactions to take place with reactive pyranosyl halides and should only be used for acyl type pyranoside derivatives.

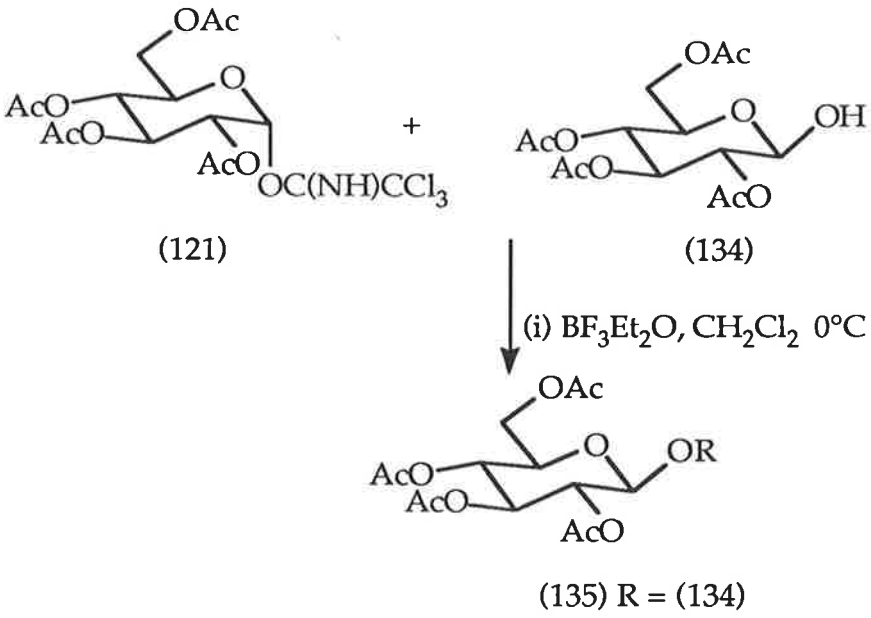
In 1983 Tietze[120] synthesized the iridoid glycoside (133), which contains the unusual diacetal moiety, with the trimethylsilyl-β-glucoside (132) and a catalytic amount of trimethylsilyl triflate. The yield of the β-glycoside (133) was 91%.



(133)

The results indicate that these acetals are stable to Lewis acids and therefore less reactive acetates should be even more stable.

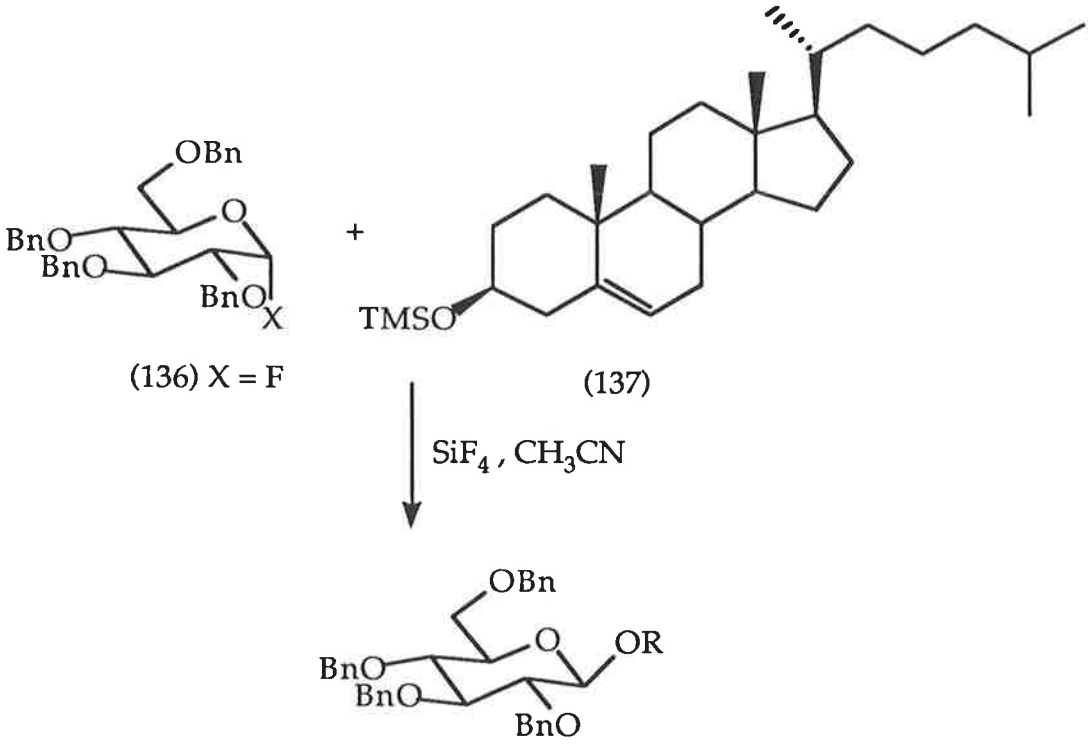
In 1984 Cook[121] used the method of Schmidt for the formation of the secondary glycoside, β,β-octaacetyltrehalose (135), in 58% isolated yield.



(121) (134)

(135) R = (134)

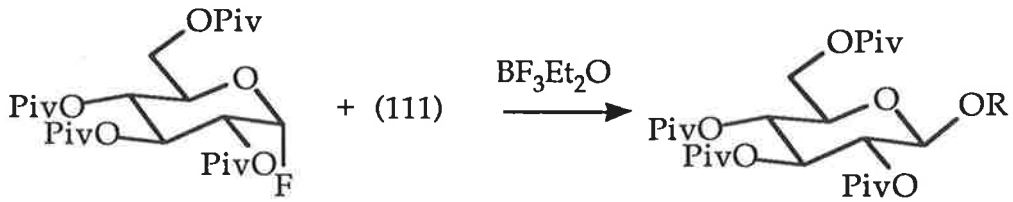
At the same time Hashimoto[122] reported a new class of glycosylating agents. They used glucopyranosyl fluorides activated with tetrafluorosilane or trimethylsilyl triflate (less than one molar equivalent) which catalyzed effectively the condensation of the appropriately protected tetra-O-benzylglucopyranosyl fluoride (136) and the trimethylsilyl ethers of alcohols such as cholesterol (137) to the β-glycoside (138). They obtained the β-glycoside (138) in 69%, however, α-glycoside was also detected in 16% yield.



(136) X = F (137)

(138) R = Cholesteryl

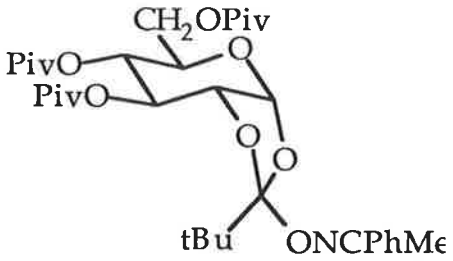
Kunz[123], in 1985, used the same approach as Hashimoto[122], by introducing more sterically hindered substituents in the 2,3,4,5 positions of the pyranose ring, using the pivaloate derivative (139). The Lewis acids used were tetrafluorosilane or trimethylsilyl triflate with the alcohol directly protected as a trimethylsilyl ether as in Hashimoto 's procedure. Kunz also used boron trifluoride etherate with successful results. Reaction of cholesterol (111) and fluoro pivaloate (139) in dichloromethane and boron trifluoride etherate yielded the β-glycoside (112) in 84% yield.



(139) (112) R = Cholesteryl

Kunz[124] later published an alternative method in 1986 dealing with ortho ester formation. Ortho ester formation is widely known to give unwanted side

products[125]. In this paper the α- bromopyranose (tetrapivaloate) was treated with acetophenone oxime in the presence of silver triflate[126] at low temperatures, in dichloromethane, to give the corresponding ortho ester (140). This ortho ester was activated with four equivalents of boron trifluoride etherate in the presence of the alcohol in dichloromethane to yield the β-glycosides. Reactions were carried out at room temperature and reaction times varied between five to thirty minutes, depending on the alcohol. The paper reports yields from secondary alcohols between 60-78%.



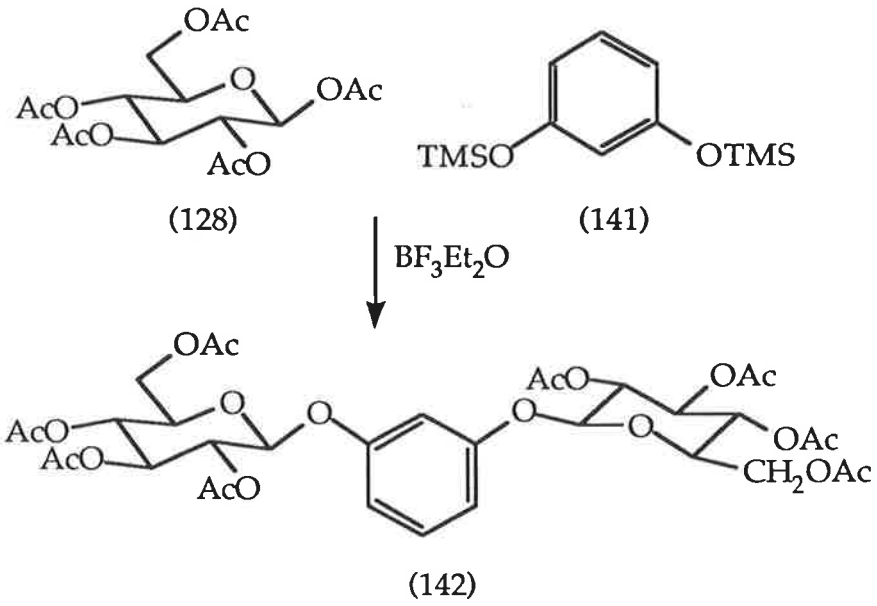
(140)

At the same time Schmidt[127] wrote a review on synthesis of glycosides and oligosaccharides detailing some of the aspects already mentioned above, especially the trichloroacetimidate method, and on activation through the formation of glycosylsulfonium salts and glycosyl fluorides.

Nakagawa,[128] in 1987, synthesized (+)-cerebroside B by the method of Schmidt[114], however he introduced 4Å molecular sieves into the reaction mixture.

In 1987 Nair[129] also modified a procedure for glycosylating alcohols from a heterogeneous to a homogeneous system. The modification used the β-glycosyl acetates instead of the glycosyl bromides. Boron trifluoride etherate was used to generate the glycosyl cation which then reacts with the silyl ether of the phenolic compound to give the required β-glycoside. The

synthesis of resorcinol diglucoside octaacetate (142) provides an illustration of the procedure.

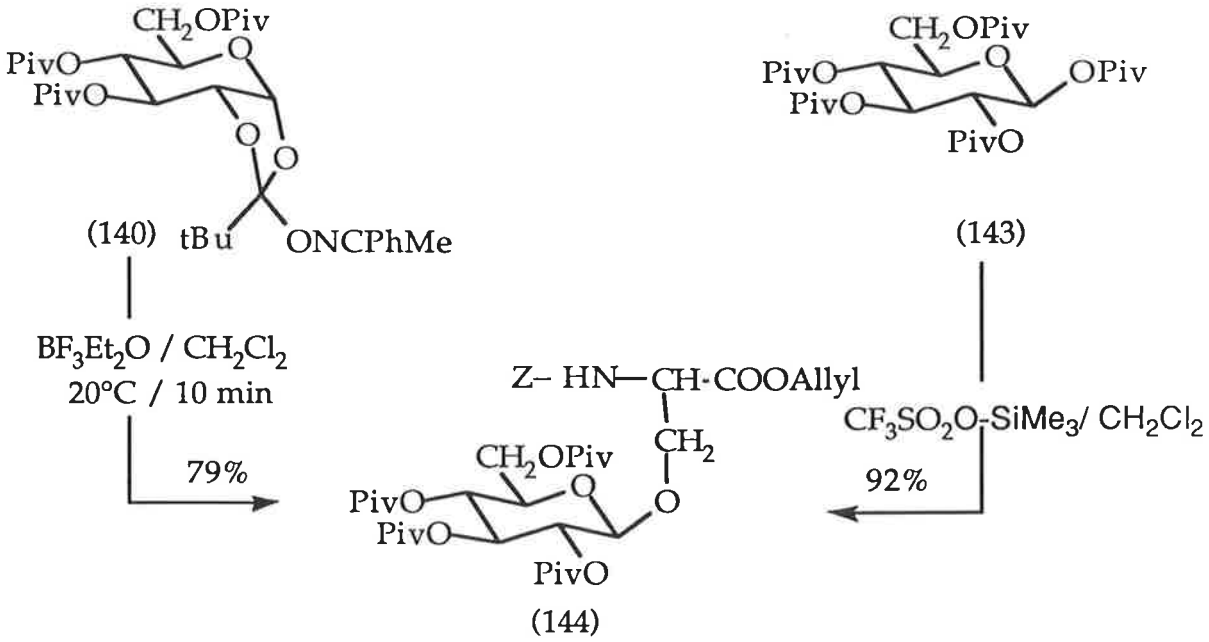


(128) (141)

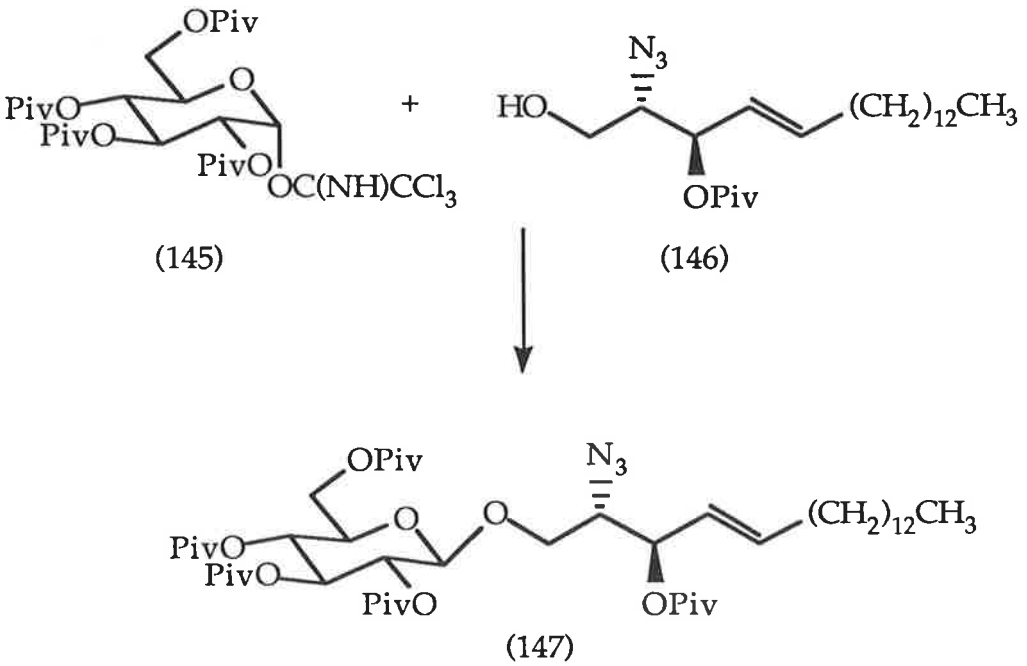
(142)

At the same time Teitze[130] produced two papers detailing the formation of acetal β-glycosides from cytotoxic aldehydes and an extention to iridoid glycoside formation using the methodology of his previous paper. It also was an extension to hemiacetals not previously encountered.

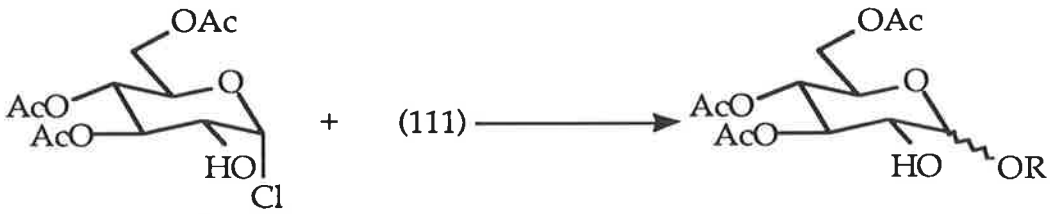
Kunz[131], in 1987, published a review on the synthesis of glycopeptides highlighting the ortho ester work as well as a modified extension with the use of the pentapivaloate compound (143) in which he compared the results of the two methods in the synthesis of serine ester β-glycoside (144). The glycoconjugate (144) formed stereoselectively and in almost quantitative yield. Experimental details for the formation of (144) from (143) were not reported when our syntheses were done.



Zimmermann[132], in 1988, used pivaloate instead of acetate protecting groups because of their effectiveness in reducing ortho ester formation when attempting to prepare the β-glycoside (147) by glycosylation of (146) with the imidate (145). They found the reaction proceeded smoothly to give compound (147) in 94% yield.



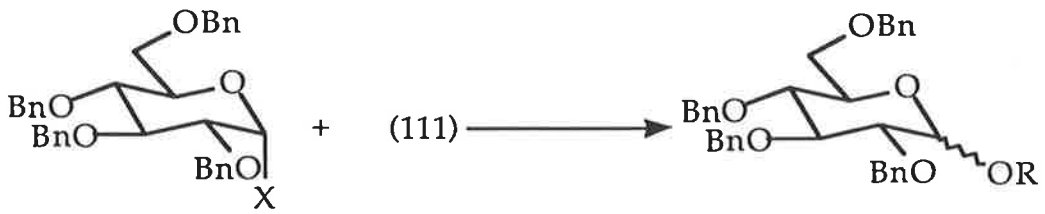
In the same year Nishizawa[133] synthesized cholesterol glycoside in 73% yield with an (α,β) ratio of 52:48. This approach involved using thermal conditions in the absence of solvents. The chloropyranoside (148) was heated at 110°C in the presence of cholesterol (111) and tetramethylurea to yield the glycosides (149).



(148) (149) R = Cholesteryl

Nishizawa[134] later (1989) reported the general applicability of thermal glycosidation using 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl chloride and obtained a 92% yield of α and β glycosides of cholesterol (111).

Finally, Sasaki[135], in 1990, described the development of another glycosidation technique involving high pressure. Reaction of the bromide (116) with cholesterol (111) in dichloromethane, in the presence of a sterically hindered base 2,6-lutidine, under high pressure yielded the α and β-glycosides in 88% yield.



(136a) X = Br (138) R =Cholesteryl

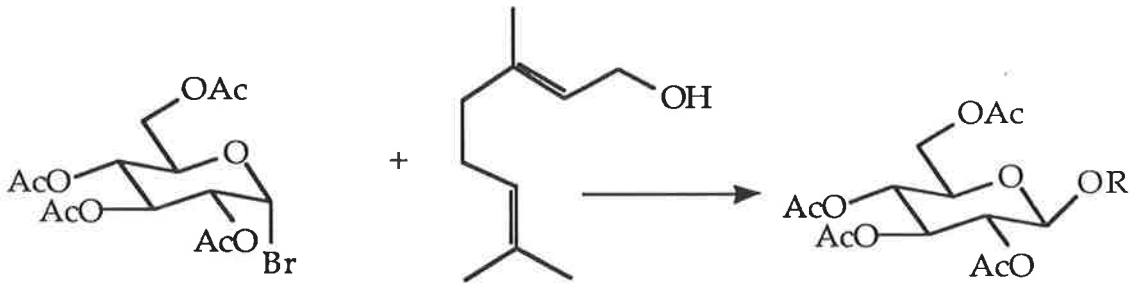
The brief survey above outlined the glycosidation techniques available. Methods involving the use of both heterogeneous and homogeneous conditions were chosen for the synthesis of glycosides of the aglycones described in chapter 2. Five glycosidation methods were studied: the

traditional Koenigs-Knorr with slight modification and the precedures described by Ogawa, Kunz, Schmidt, and Ackermann.

3.2 Synthesis of Geranyl-β-D-glucopyranoside (1a)

3.2 1 Synthesis of Geranyltetra-O-acetyl-β-D-glucopyranoside (1b)

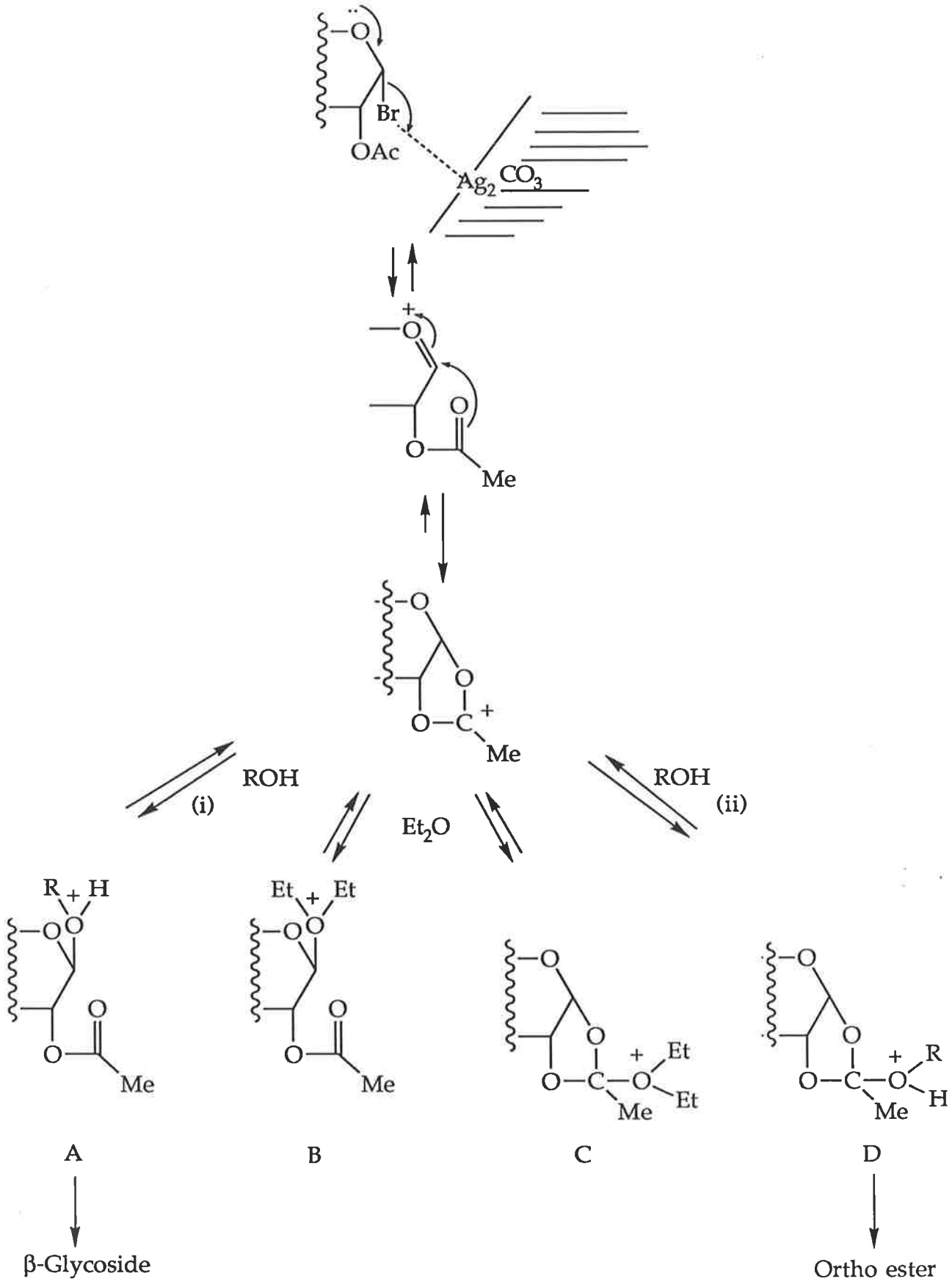
Synthesis of geranyl-β-D-glucopyranoside (1b)was achieved by the traditional Koenigs-Knorr method[95] with some slight modifications. Treatment of geraniol (1) with acetobromoglucose tetraacetate (108), silver carbonate and a drying agent (Drierite calcium sulphate) in dry diethyl ether under nitrogen at 20°C yielded the glucopyranoside (1b). The ^{1}H n m r spectrum of (1b) showed the anomeric proton as a doublet at δ 4.5. The 7.9Hz coupling is typical of the β-configuration.



(108) (1) (1b) R = (1)

The mechanism for the glycoside formation is a complicated series of equilibria and is shown schematically in scheme 12. Initial loss of bromide, initiated by the presence of silver carbonate, leads to the formation of a resonance stabilized secondary carbocation. This exists in equilibrium with the acetoxonium ion via neighbouring group participation. The carbocation can be trapped either by solvent, in this case diethyl ether, or the alcohol. Shown in scheme (12) are four product intermediates[104] which ultimately give rise to two products, the β-glycoside from intermediate A [route (i)] or the ortho ester from intermediate B [route (ii)]. The free carbocations are assumed to be in solvent-dependent equilibria with the corresponding intimate ion pairs involving the bromide ion[104]. For the case of geranyl

glucopyranoside (1b) pathway (i) eventuates as the dominant route. Ortho ester suppression is affected by an excess of aglycone alcohol, and this is commonly carried out for Koenigs-Knorr reactions.


Br
OAc
Ag_2CO_3
Me
ROH
(i)
ROH
(ii)
Et_2O
R
H
Et
Et
Me
Et
Et
Me
R
H
Me
A
B
C
D
β-Glycoside
Ortho ester

Scheme 12.

3.2.2. Synthesis of Geranyl-β-D-glucopyranoside (1a)

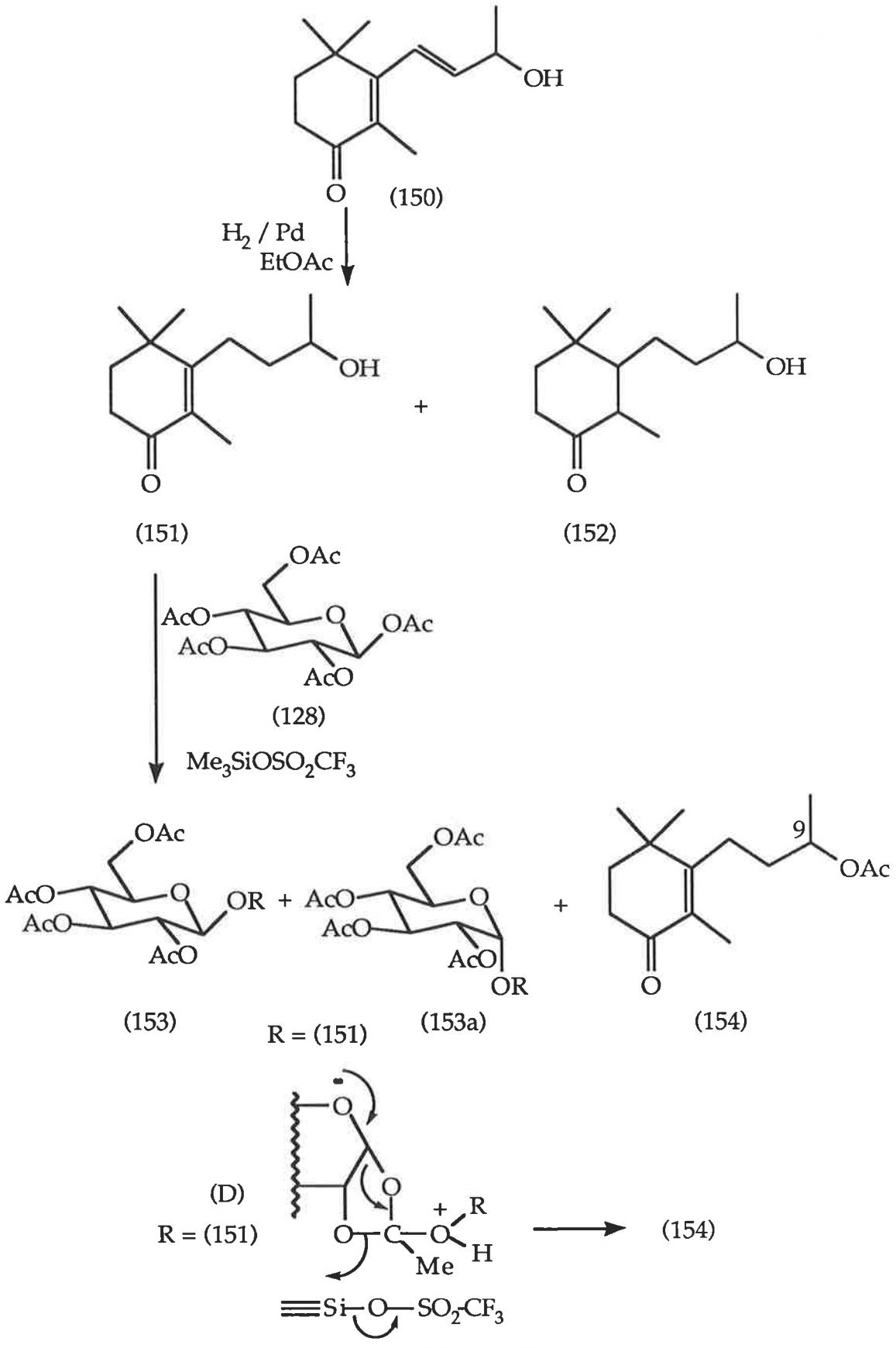
Treatment of the β-glycoside tetraacetate (1b) with sodium methoxide in methanol (Zemplen method)[136] overnight yielded the corresponding geranyl-β-D-glucopyranoside (1a) in 80% yield.

3.3 Synthesis of 4-Oxo-5-megastigmen-9-yl-tetra-O-acetyl-β-D-glucopyranoside (153).

The synthesis of glycoside (153) was attempted as this was a readily available secondary alcohol which could be used as a model for glycosylation by the method of Ogawa[116]. The aglycone (151) was prepared from the (E)-9-hydroxy-5,7-megastigmadien-4-one (150), which was available in our laboratory, by hydrogenation with palladium black in ethyl acetate. A minor amount of the over-reduced compound (152) was also isolated and obtained as a white solid. The glycosidation of the racemic 9-hydroxy-5-megastigmen-4-one (151) involved the treatment of this alcohol in 1,2-dichloroethane with penta-O-acetyl-β-D-glucopyranoside (128), molecular 4Å sieves and trimethylsilyl triflate at 20°C for three hours. Two major components were isolated and identified as the acetylated compound (154) (50% yield) and 4-oxo-5-megastigmen-9-yl-tetra-O-acetyl-β-D-glucopyranoside (153) (21% yield). A third and minor product was identified as the α-glycoside (153a) (9% yield). Electron impact MS gave the molecular ions for compounds (151,152,153 and 154). The β-glycoside (153) was obtained as a mixture of two diastereoisomers as evidenced by the chemical shift of the anomeric protons for the two diastereisomers. Each was observed as a doublet of 8.0Hz, at δ 4.60 and 4.57 and appeared as an apparent triplet. The anomeric proton of the minor α-glycoside (153a), also a mixture of two diastereoisomers, also occurred as two doublets centred at δ 5.48 with couplings of approximately 3.5Hz. The other major

compound (154) showed a broad multiplet centreed at δ 5.0, baseline width of 32Hz. The signal is due to the C9 hydrogen deshielded by the acetoxy group. This has a chemical shift similar to that of the C9 proton observed for the diacetate of the acetylenic triol (84). Its formation probably involved breakdown of an ortho ester type intermediate D [route (ii)], initiated by the Lewis acid, Scheme 13. Suppression of ortho ester type intermediate D can occur when an excess of alcohol is used, however in our case equimolar amounts were used.

Because many of the aglycones synthesized in chapter two involved multistep pathways and were not available in large quantities, an excess of the alcohol was undesirable, other methods were used in subsequent glycosylations.


OH
O
(150)
H_2 / Pd
EtOAc
OH
O
OH
O
(151)
(152)
OAc
AcO
O
OAc
AcO
AcO
(128)
$Me_3SiOSO_2CF_3$
OAc
AcO
O
OR
AcO
AcO
(153)
R = (151)
OAc
AcO
O
AcO
AcO
OR
(153a)
9
OAc
O
(154)
(D)
R = (151)
O
O
+
R
O
C
O
H
Me
(154)
Si
O
SO_2CF_3

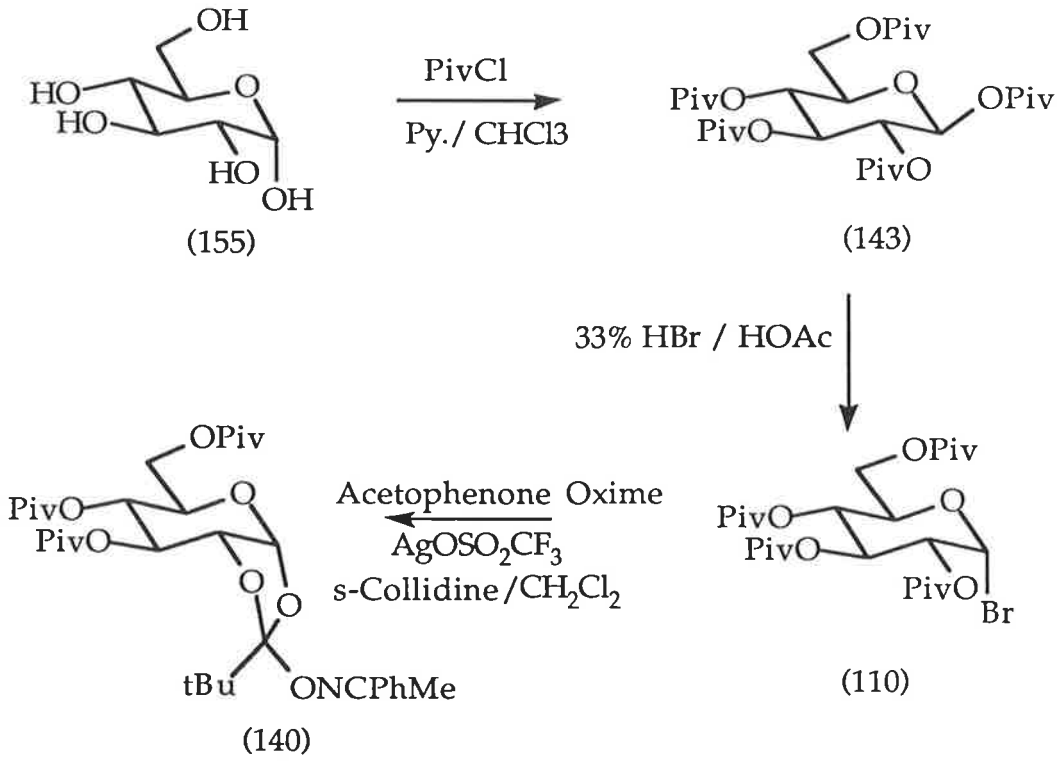
Scheme 13.

3.4 Synthesis of 3,5,5-Trimethyl-3-cyclohexenyl-β-D-glucopyranoside (107a).

The synthesis of the glucoside (107a) was carried out using the method of Kunz using ortho ester (140). It was envisaged that the bulky pivaloyl protecting groups would reduce the problems of both transesterification and α-glycosidation encountered in section 3.3.

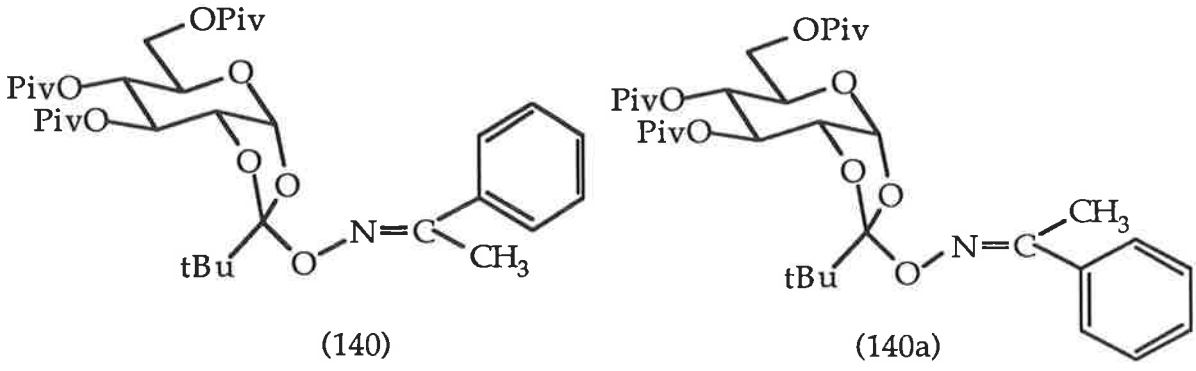
3.4.1 Synthesis of 1,2-O-(1-N-1-phenylethylideneaminooxy)-2,2-dimethylpropylidene-3,4,6-tri-O-pivaloyl-α-D-glucopyranose (140).

Treatment of α-D-glucose (155) with 6.6 equivalents of pivaloyl chloride in pyridine and chloroform for five days yielded the penta-O-pivaloyl-β-D-glucopyranoside (143)[102] in 69% yield (recrystallized). Compound (143), in dichloromethane, when treated with a 33% hydrogen bromide in anhydrous acetic acid solution at low temperature (-10 to 0°C) overnight gave the α-D-bromopyranoside (110). The ortho ester (140) was prepared by treatment of the α-bromide (110) at low temperature (-40°C) with silver trifluoromethanesulphonate[137] (silver triflate), acetophenone oxime and s-collidine.[126] Scheme 14.



Scheme 14.

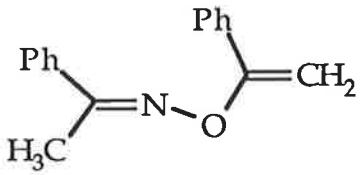
Chromatography and ^{1}H n m r spectroscopy showed two isomers (a major and a minor). Only the major isomer (140) was reported by Kunz. These were presumed to be the (E) and (Z) isomers (140) and (140a).



The ^{1}H n m r spectrum indicated that the minor isomer comprised 8% of the mixture and this was isolated by column chromatography. The anomeric proton in (140) (the major isomer (E)) occurs downfield at δ 6.2 and has a

large H1-H2 cis coupling of 6.0Hz in the glucone ring. It has been suggested by Kunz[124] that a twisted conformation exists for the crowded oximate ortho ester and consequently a small H2-H3 trans coupling of 3.0Hz was seen for the H2 proton at δ 4.67. The anomeric proton is deshielded by the aromatic ring. The minor isomer isolated from ortho ester formation had the anomeric proton slightly upfield at δ 5.9 and also a large H1-H2 cis coupling of 6.0Hz. The methyl group on the double bond was slightly downfield in (140a) compared to (140) by approximately 0.017ppm. Each isomer gave a molecular ion (m/z 633) on electron impact.

Kunz stated that formation of the ortho ester was also accomplished by use of silver carbonate. In the present study use of silver carbonate failed to give ortho ester formation. Instead many other products were obtained and identification of one of these products revealed a condensation reaction had occurred between acetophenone oxime and acetophenone, to give compound (156).

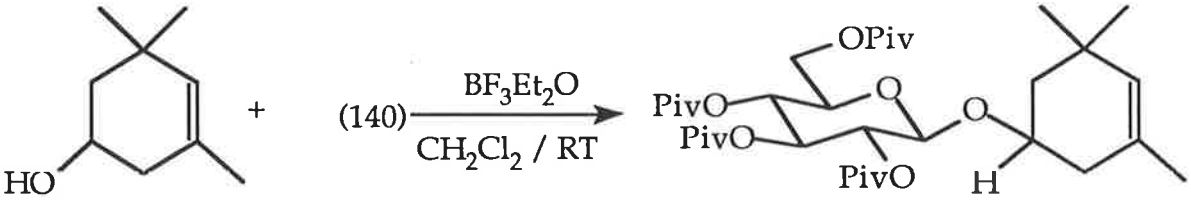


(156)

The infrared spectrum of (156) showed no hydroxyl or carbonyl stretches but a strong alkene stretch at 1625, a medium stretch at 1670 characteristic of a C=N and a C-O stretch at 1100 cm^{-1}. Mass spectroscopy gave a molecular ion of m/z 237. The ^{1}H n m r spectrum had a singlet for the vinyl methyl at δ 2.5 suggesting it is deshielded by the aromatic ring. The vinyl protons appeared as an AB system at δ 5.10 and 4.84 with a 2Hz coupling. The downfield vinyl proton at δ 5.15 is probably cis to the aromatic ring. The data support the structure (156). It was isolated as a white solid and recrystallized from ethanol. However it decomposed on standing.

3.4.2 Synthesis of 3,5,5-Trimethyl-3-cyclohexenyl-tetra-O-pivaloyl-β-D-glucopyranoside(107b)

Treatment of one equivalent of 3,5,5-trimethyl-3-cyclohexen-1-ol (107) with one equivalent of ortho ester (140) and four equivalents of boron trifluoride etherate in dichloromethane at room temperature for ten minutes yielded the β-glycoside (107b) in 90% yield. Recrystallization from methanol yielded one diastereoisomer whose molecular ion (638) was observed by mass spectroscopy.



(107) (107b)

The anomeric proton for the β-glycoside (107b), recrystallized from methanol, resonated as a doublet of 8.1Hz at δ 4.66. The corresponding proton for the other diastereoisomer, an 8.0Hz doublet, was further downfield at δ 4.69. The ^1H n m r spectrum of (107b) was fully assigned with the aid of a COSY spectrum.

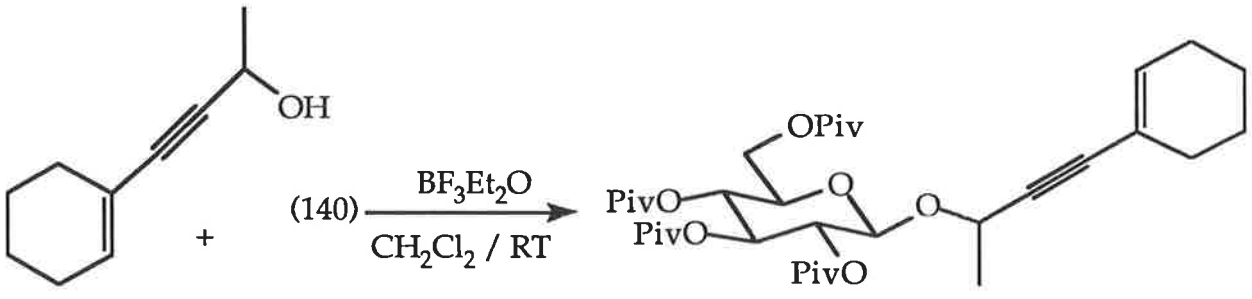
3.4.3 Synthesis of 3,5,5-Trimethyl-3-cyclohexenyl-β-D-glucopyranoside (107a)

Reaction of compound (107b) by the Zemplen method[136], which involves treatment with sodium methoxide in methanol, yielded the compound (107a) in 90% yield. The product (107a) was isolated by using reverse phase chromatography(C18 column). The ^{13}C n m r spectrum had no carbonyl resonances downfield, indicating that depivaloylation was complete.

3.5 Synthesis of 3-(1'-cyclohexenyl)-1-methyl-2-propynyl-β-D-glucopyranoside (99a).

3.5.1 Synthesis of 3-(1'-cyclohexenyl)-1-methyl-2-propynyl-tetra-O-pivaloyl-β-D-glucopyranoside (99b)

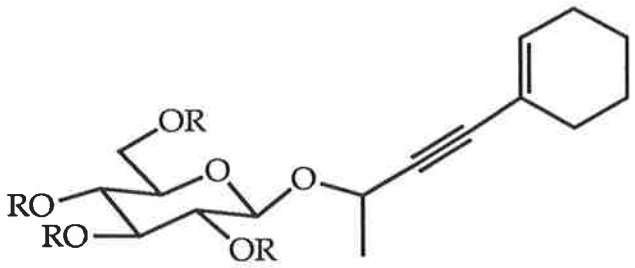
Treatment of 4-(1'-cyclohexenyl)-3-butyn-2-ol (99), using the method of Kunz[124] yielded the glucopyranoside tetrapivaloate (99b). The reaction was performed using oxygen free nitrogen, at low temperature (-20°C) , using 1.5 equivalents of ortho ester (140) and under strictly anhydrous conditions. After ten minutes no starting material was evident and the reaction mixture was worked up by the addition of a saturated sodium hydrogen carbonate solution. The β-glycoside tetrapivaloate (99b) was isolated by chromatography in 60% yield and an antioxidant, 2,6-ditertiary butyl-p-cresol, was added to avoid any autoxidation. The two diastereoisomers were partially separated by high pressure liquid chromatography. The ^{1}H n m r spectrum of the higher Rf material (t.l.c.) revealed that the anomeric proton was deshielded by the triple bond and resonated at δ 4.80 with a coupling of 8.3Hz. For the lower Rf diastereoisomer the anomeric proton, an 8.1Hz doublet at δ 4.76, was also deshielded. The molecular ion (m/z 648) was obtained with FAB mass spectroscopy.



(99) (99b)

3.5.2 Synthesis of 3-(1'-cyclohexenyl)-1-methyl-2-propynyl-β-D-glucopyranoside (99a).

Treatment of the β-glycoside tetrapivaloate (99b) (both diastereoisomers) with sodium methoxide in methanol followed by a standard work-up yielded the 3-(1'-cyclohexenyl)-1-methyl-2-propynyl-β-D-glucopyranoside (99a).in 41% yield. The molecular ion was not observed with electron impact and chemical ionization also proved unsuccessful. The ^{13}C n m r spectrum had no carbonyl resonances downfield. The glycoside (99a) was also acetylated and characterized as its tetra acetate (99c).



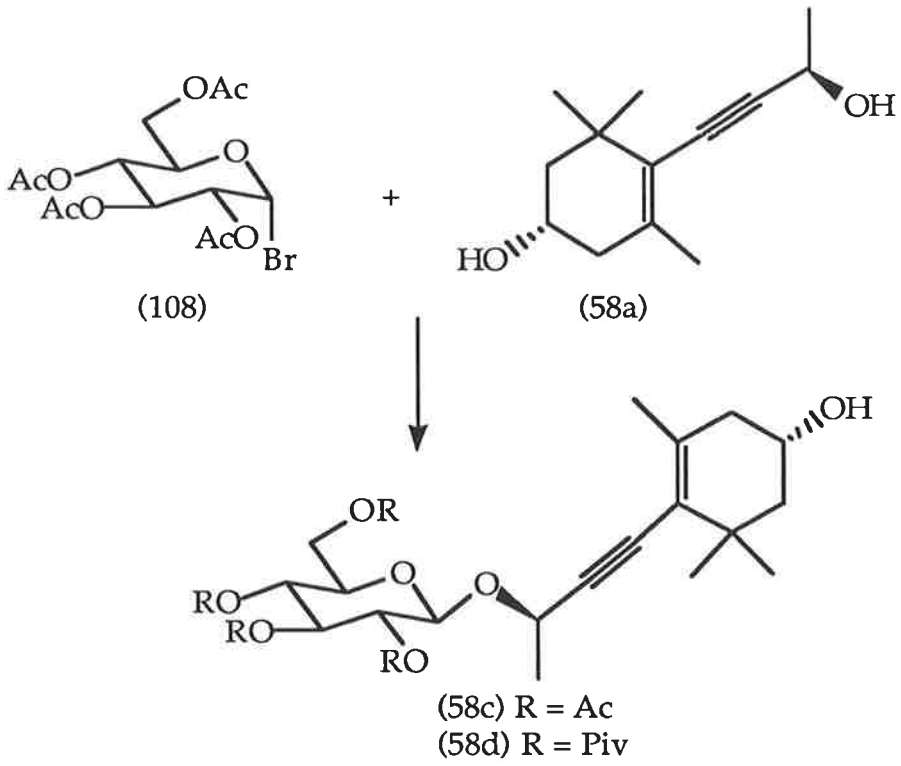
(99a) R = H
(99c) R = Ac

3.6 Synthesis of *(3SR,9RS)*-3-hydroxy-5-Megastigmen-7-yn-9-yl-β-D-glucopyranoside (58b)

3.6.1 Attempted Synthesis of *(3SR,9RS)*-3-hydroxy-5-Megastigmen-7-yn-9-yl-tetra-O-acetyl-β-D-glucopyranoside (58c)

The Koenigs-Knorr glycosylation method was first attempted because the product β-glycoside (58c) could then be analyzed by gas chromatography (tetra-O-acetyl-β-D-glucopyranosides can be detected by GC). This material could then have been compared to the glycosidic mixture obtained from the grape juice via C18 RP chromatography to see if it existed as a natural product. In the pivaloate series the glycosides could not be analyzed by

GC.as they are involatile. However, attempted syntheses of the β-glycoside (58c), the bis β-glycoside and the other monoglycoside by using the traditional Koenigs-Knorr method was unsuccessful. The enynediol (58a) was treated with the glucopyranosyl bromide (108) (5 equivalents), calcium sulphate and silver carbonate in anhydrous diethyl ether. The reaction was performed under three separate conditions. 1) at low temperature (5°C) for 24hours, 2) at room temperature for one week, and 3) at reflux for two days. T.l.c. indicated a mixture of products in addition to starting material, however, no trace of β-glycoside (58c) was evident.



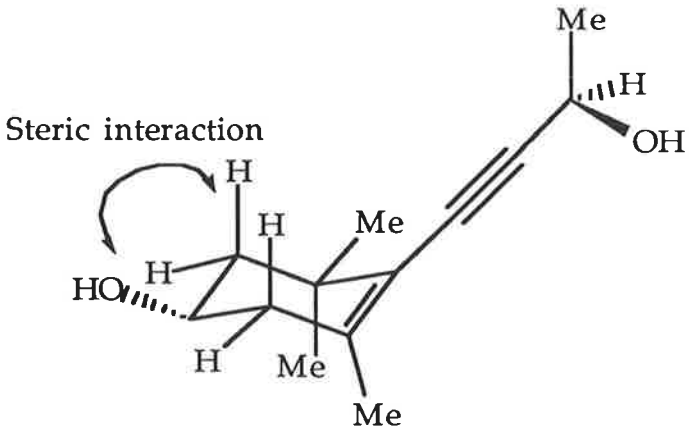
(108) (58a)

(58c) R = Ac
(58d) R = Piv

3.6.2 Synthesis of (3SR, 9RS)-3-hydroxy-5-Megastigmen-7-yn-9-yl-tetra-O-pivaloyl-β-D-glucopyranoside (58d)

The Koenigs-Knorr method was abandonded for the more reliable method of Kunz[124]. Treatment of enynediol (58a) with ortho ester (140) for four hours at low temperature (-10°C) using only 1.2 equivalents of ortho ester (140) yielded the β-glycoside (58d) in 54% yield . Inspection of a model of the

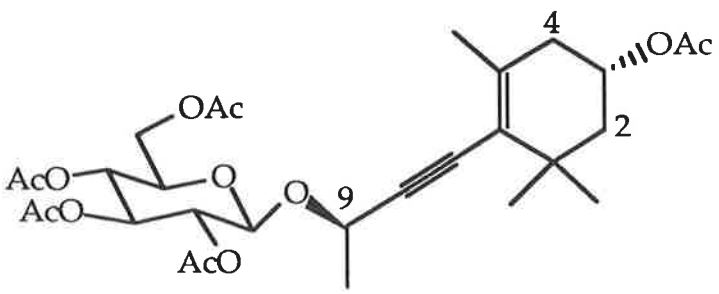
enynediol (58a) suggests that glycosidation would occur at the C9-hydroxyl as it appears there are less steric interactions involved.



(58a)

^{1}H n m r spectroscopy of the product revealed that the anomeric proton was deshielded, presumably by the triple bond which would tend to suggest that glycosidation had occurred at the C9-hydroxyl group. The anomeric proton in the β-glycoside (58d) had a similar chemical shift with that encountered of the model enyne system (99b) and was found as a three line (1:2:1) signal centred at δ 4.91, i.e. two doublets with an average coupling of 8.2Hz for each of the two diastereoisomers.

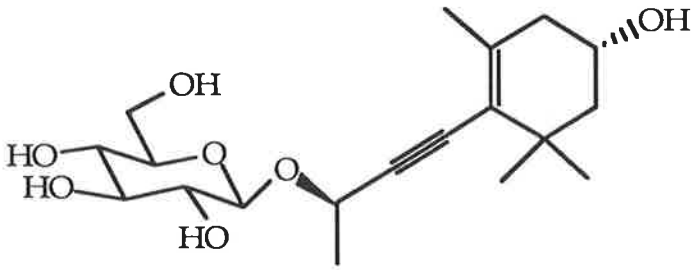
In order to establish which of the two hydroxyls of compound (58a) had been glycosylated it was decided to depivaloate the β-glycoside (58d) and acetylate the product (58b) to give (58e) in order to determine the structure by ^{1}H n m r spectroscopy.



(58e)

3.6.3 Synthesis of *(3SR,9RS)*-3-hydroxy-5-Megastigmen-7-yn-9-yl-β-D-glucopyranoside (58b)

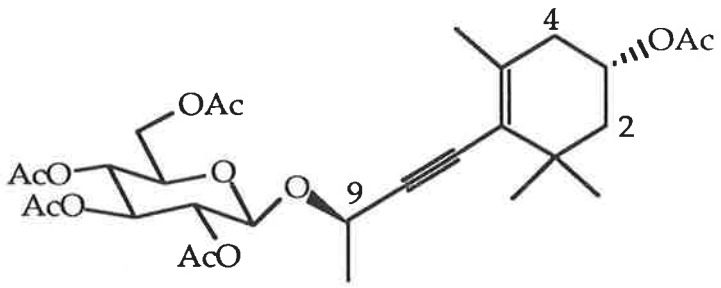
Depivaloylation was performed by the method described before using sodium methoxide in methanol. This was done over seven days at room temperature to yield the β-glycoside (58b) in 88% yield.



(58b)

3.6.4 Confirmation of Structure (58b).

Treatment of the β-glycoside (58b) with an excess of acetic anhydride in pyridine at room temperature overnight yielded the acetylated β-glycoside (58e). The 300 MHZ ^{1}H n m r spectrum showed ten acetate groups (as eight singlets, at approximately 2 ppm, of which two were superimposed). This was consistent with the product comprising two diastereoisomers. The anomeric proton in the acetylated β-glycoside (58e) was centred at δ 4.82 and appeared as a three line signal (1:2:1) for the expected two doublets of each diastereoisomer. An obscured multiplet for the aglycone moeity was observed at δ 5.0 for C3.



(58e)

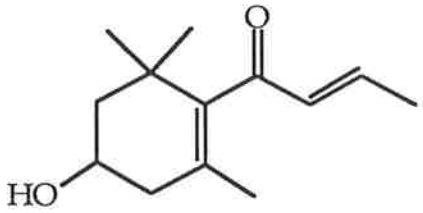
The C4 and C2 hydrogens resonate at δ 2.5-2.4 as a pair of complex multiplets. Decoupling at δ 5.0 resulted in collapse of both signals. This shows that the downfield signal at δ 5.0 was an acetoxy methine proton at C3 thus confirming the position of glycosylation at C9.

The glucoside pentaacetate (58e) chromatographed on GC / MS capillary column, but has not so far been observed as a juice constituent in our laboratory.

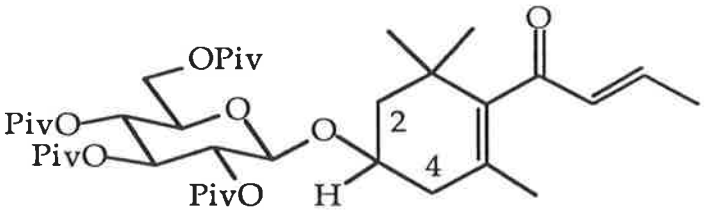
3.7 Synthesis of (E)-7-Oxo-5,8-megastigmadien-3-yl-β-D-glucopyranoside (39a)

3.7.1 Synthesis of (E)-7-Oxo-5,8-megastigmadien-3-yl-tetra-O-pivaloyl-β-D-glucopyranoside (39b)

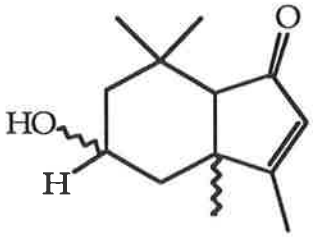
Treatment of 3-hydroxy-β-damascone (39) in dichloromethane with 1.2 equivalents of ortho ester (140) and 4 equivalents of boron trifluoride etherate for 15 minutes yielded two β-glycosides (39b) and (157b), the cyclized product (157) and starting material. The β-glycoside (39b) and the cyclized glycoside (157b) were each a mixture of diastereoisomers which were obtained as white solids after chromatography.



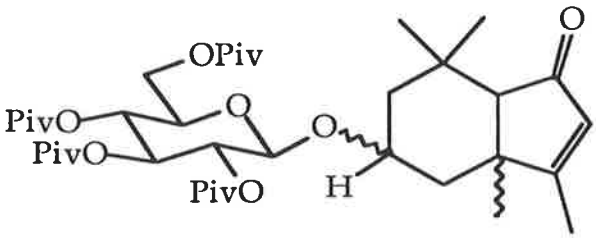
(39)



(39b)



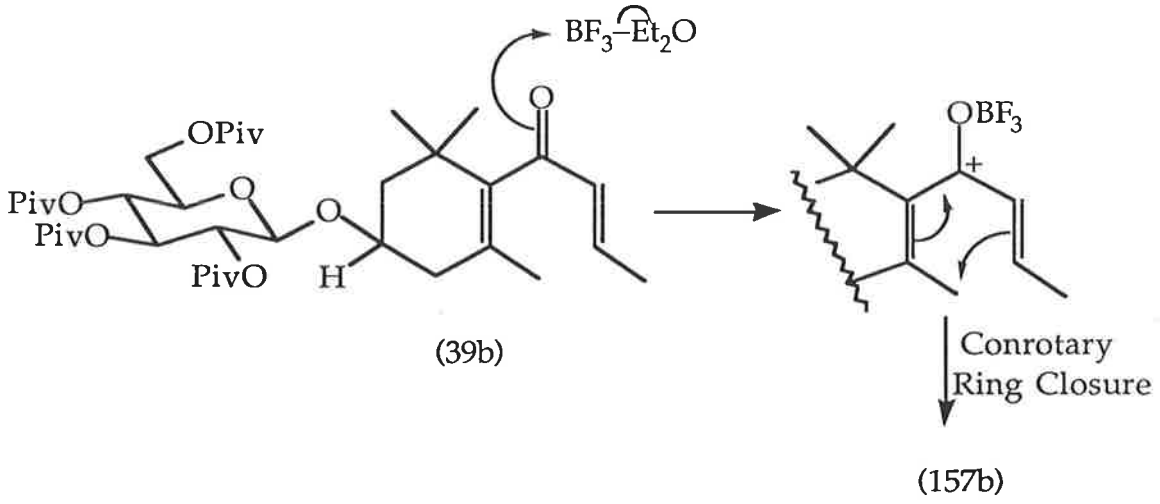
(157)



(157b)

^{1}H n m r spectroscopy of compound (39b) showed the anomeric proton as a three line signal centred at 4.68Hz (1:2:1) for the two diastereoisomers [(similar to that of the model compound (107b)] with an average coupling of 7.5Hz. The ^{1}H n m r spectrum of (39b) was complex, especially in the upfield region due to the coupling of the C2 and C4 hydrogens of the aglycone in the two diastereoisomers. However, with the aid of a COSY spectrum the axial and equatorial hydrogens at C2 and C4 were assigned and differentiated by the long range W coupling between the equatorial hydrogens evident in the COSY spectrum.

^{1}H n m r spectroscopy of the bicyclic compound (157b) revealed the anomeric proton for each diastereoisomer as a doublet almost superimposed at δ 4.62 and δ 4.61 and with couplings of 8.0Hz. The overall yield of the β-glycoside (39b) was low (10% yield). Formation of the bicyclic compound (157) can be explained as a Nazarov cyclization product[50,39]. When the reaction was repeated for a longer period more of the β-glycoside (157b) formed and less of (39b). The proposed mechanism for its formation involves a conrotary ring closure (phase dislocation).

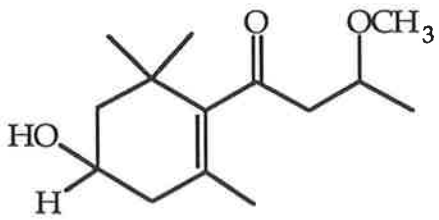


(39b)

(157b)

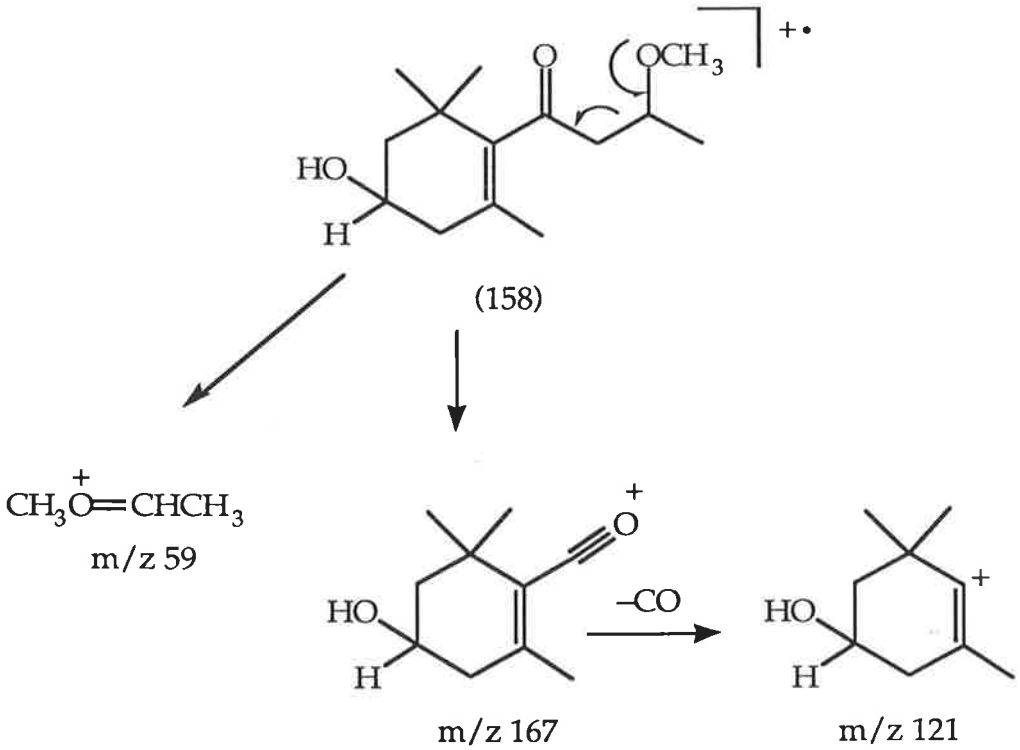
3.7.2 Attempted Synthesis of (E)-7-Oxo-5,8-megastigmadien-3-yl-β-D-glucopyranoside (39a)

Compound (39b) was treated under the conditions described for the depivaloylation of compounds [(58d), (99b), (107b)]. Treatment with sodium methoxide in methanol yielded a product that was UV active but ^{13}C n m r spectroscopy showed the absence of two of the four vinyl carbons. It was decided to try the same reaction on 3-hydroxy-β-damascone (39) and the results revealed that conjugate addition was occurring to give compound (158). This was confirmed by ^{1}H n m r spectroscopy with the appearance of the methoxy group at δ 3.33 as well as the disappearance of the ABX system of the vinyl protons.



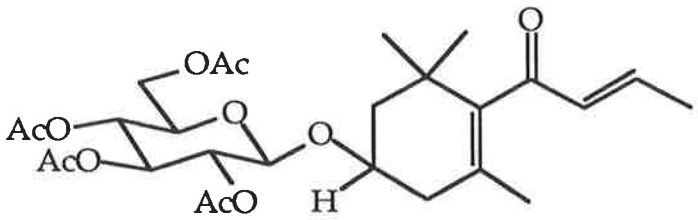
(158)

Mass spectral evidence was also obtained with the molecular ion (240) being observed, in addition to three major fragment ions at 59 , 167 and 121.



3.7.3 Synthesis of (E)-7-Oxo-5,8-megastigmadien-3-yl-tetra-O-acetyl-β-D-glucopyranoside (39c)

Because conjugate addition posed a major concern in synthesizing compound (39a) it was decided to use the acetate group as the protecting group in the glycoside synthesis in preference to the sterically more crowded pivaloate group. It is known that acetyl groups are easily removed in the presence of base, and that conjugate addition could be circumvented.



(39c)

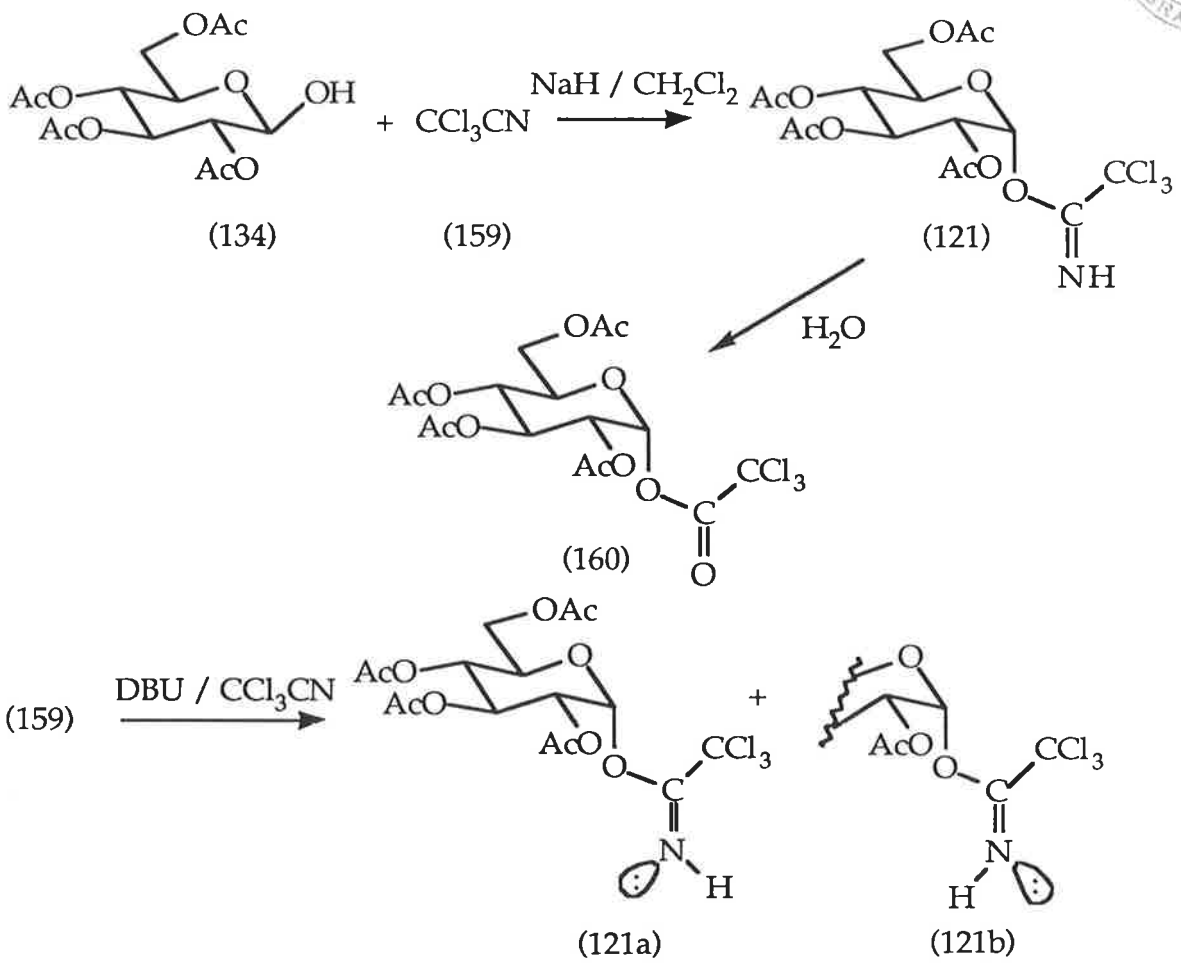
For this purpose the recently reported method of Schmidt[114] was employed, in preference to the method of Ogawa[116] due to problems of transesterification and α-glycosidation already encountered in the latter. In

order to minimize the undesirable acid catalyzed Nazarov cyclization, a lesser amount of boron trifluoride etherate was employed for the Schmidt reaction.

3.7.3.1 Synthesis of 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (121).

Synthesis of compound (121)[138] (Scheme 15.) involved the treatment of 2,3,4,6-tetra-O-acetyl-β-D-glucose (134)[139] and trichloroacetonitrile (159) in dichloromethane with sodium hydride for twenty minutes. Chromatography yielded the imidate (121). It is important to note that the imidate (121) is hygroscopic and for chromatography the solvents and the silica must be dry otherwise the by-product compound (160) was obtained as a white crystalline solid. The absence of the imine hydrogen (by ^{1}H n m r spectroscopy) and a positive test for chlorine with copper wire led to compound (160) being postulated.

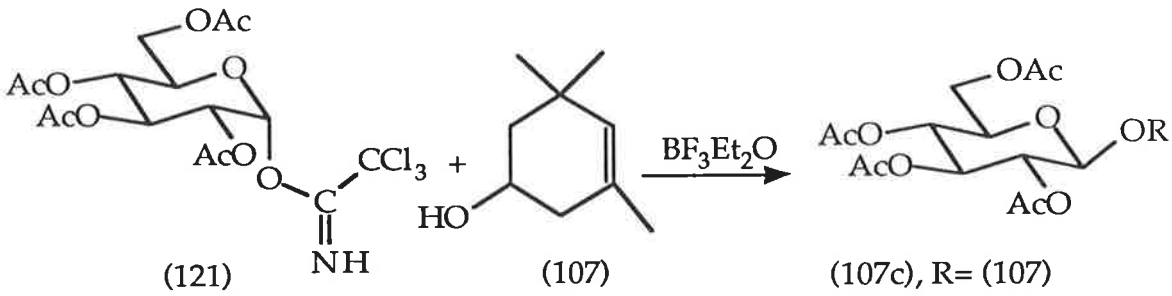
An alternative method for the synthesis of imidate (121) involving treatment of compound (134) with a sterically hindered base D.B.U. (1,8-diazabicyclo[5.4.0]-7-undecene[140]) in trichloroacetonitrile (159) instead of sodium hydride was preferred. .This was because when trace amounts of sodium hydroxide were present in the sodium hydride an explosive reaction occurred with trichloroacetonitrile. The reaction with D.B.U (3 equivalents) and trichloroacetonitrile (159) (6 equivalents) proceeded efficiently (-20 to 0°C) over one hour to give the imidate (121) as a 4:1 mixture of (E) and (Z)isomers (121a : 121b). ^{1}H n m r spectroscopy showed the NH protons at δ 8.70 and δ 8.58 and the anomeric protons at δ 6.64 and δ 6.57 both as 3.7Hz doublet.



Scheme 15.

3.7.3.2 Synthesis of 3,5,5-Trimethyl-3-cyclohexenyl-β-D- tetra-O-acetyl-glucopyranoside(107c)

Before carrying out glycosylation of 3-hydroxy-β-damascone (39) with imidate (121) the suitability of that reagent for glycosylating homoallylic cyclohexanols was tested with the model aglycone (107). Treatment of 1.4 equivalents of compound (121) and one equivalent of alcohol (107) in dichloromethane with one equivalent of boron trifluoride etherate at room temperature for thirty minutes yielded the β-glycoside (107c) plus starting material.

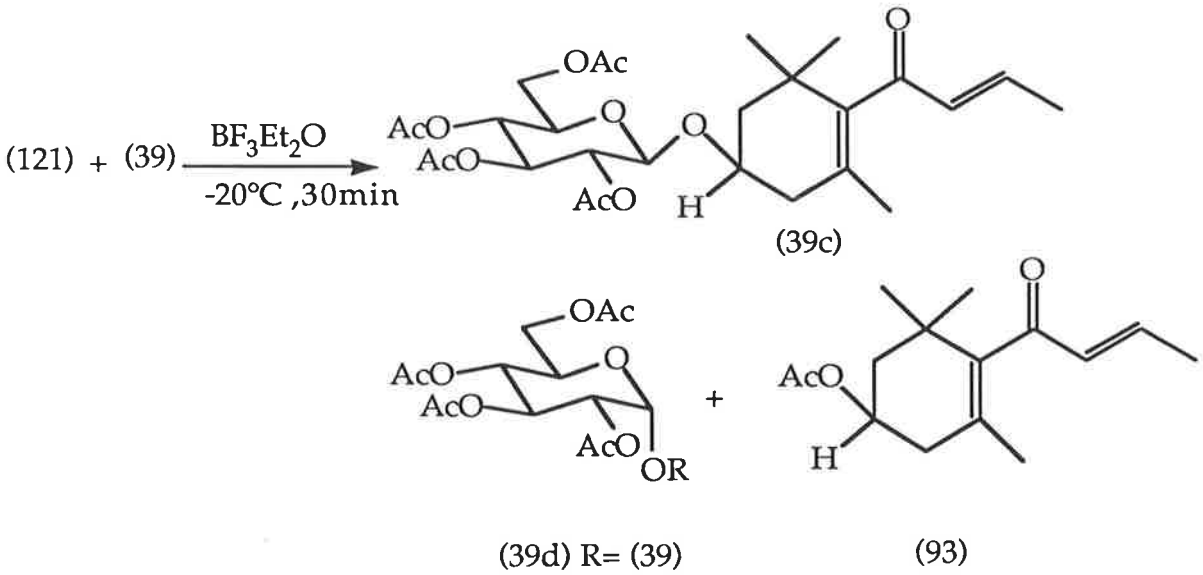


(121) (107) (107c), R= (107)

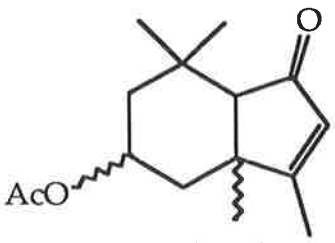
The overall yield of the β-glycoside (107c) was 21%. ^{1}H n m r spectrum showed the signal for the anomeric protons of the two diastereoisomers at δ 4.63 (7.9Hz) and δ 4.61 (8.1 Hz) respectively.

3.7.3.3 Synthesis of (E)-7-Oxo-5,8-megastigmadien-3-yl-tetra-O-acetyl-β-D-glucopyranoside (39c)

Treatment of 3-hydroxy-β-damascone (39) and imidate (121) in dichloromethane at room temperature for one hour with one equivalent of boron trifluoride yielded a number of products. The three products identified were β-glycoside (39c) (6%), α-glycoside (39d) and transesterification product (93). Starting material was recovered but no Nazarov cyclization products were detected under these conditions. Fridfrich-Bochnitschek[141] also reported α-glycoside formation in his use of the imidate (121) on a different alcohol. The anomeric protons of the β-glycoside (39c), a mixture of two diastereoisomers (each a doublet), were at δ 4.62 and δ 4.60 both with couplings of 7.9Hz. The anomeric proton of the α-glycoside (39d), also a mixture of two diastereoisomers (observed as a 1:2:1 three line pattern), was centred at δ 5.39 with an average coupling of 3.5Hz. Milder conditions (0.2 equivalents of boron trifluoride etherate) gave only trace amounts of the desired glycoside (39c).



Because the yield of the β-glycoside was quite low it was decided to repeat the reaction for a longer period (six hours) at -10°C with an excess of imidate (121) (1.5 equivalents) and an equimolar amount of boron trifluoride etherate. The order of addition was reversed in a parallel experiment where 3-hydroxy-β-damascone (39) was added to imidate (121) containing the Lewis acid at low temperature. The t.l.c. for both experiments revealed that no β-glycoside (39c) formation had occurred, however, starting material had disappeared. Two new U.V. active spots were present.and after chromatography the higher Rf material was found to be the bicyclic acetate (157c), produced via a Nazarov cyclization as described earlier.

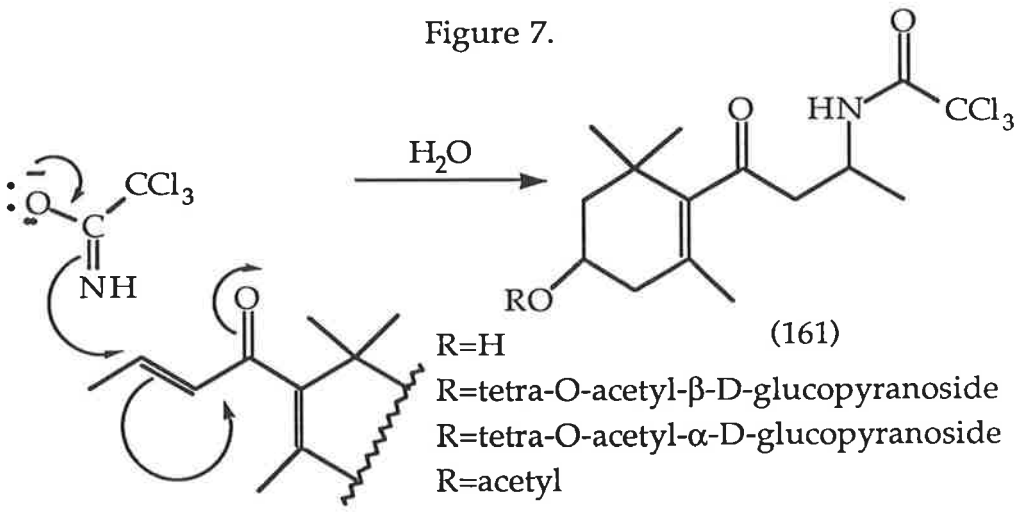


(157c)

The other product revealed an absence of vinylic protons, and it is believed that it arose from conjugate addition of the trichloroacetamide anion to the

side chain of either 3-hydroxy-β-damascone (39) or its acetate (93), or of any of the two possible glycosides (α and β). See Figure 7. Because of the complexity of the reaction and diversity of possible products the method was abandoned.

Figure 7.



(161)

3.7.3.4 Alternative Synthesis of (E)-7-Oxo-5,8-megastigmadien-3-yl-tetra-O-acetyl-β-D-glucopyranoside (39c)

An alternative method, employed by Ackermann[113], that does not require the use of Lewis acids, was considered. The Ackermann[113] procedure, a modification of the traditional Koenigs-Knorr but with the use of a soluble silver salt, silver trifluoroacetate[142],was next investigated. Treatment of 2.6 equivalents of 3-hydroxy-β-damascone (39) with one equivalent of glucopyranosyl bromide (108) in the presence of silver trifluoroacetate, calcium sulphate and sodium hydrogen carbonate in anhydrous ether at 0°C yielded the β-glycoside (39c) in 15.2% yield. ^{1}H n m r revealed trace amounts (less than 5% yield) of α-glycoside (39d) also. This method involved the use of an excess of alcohol to prevent any ortho ester formation and the presence of base to reduce the acidity of the reaction medium.

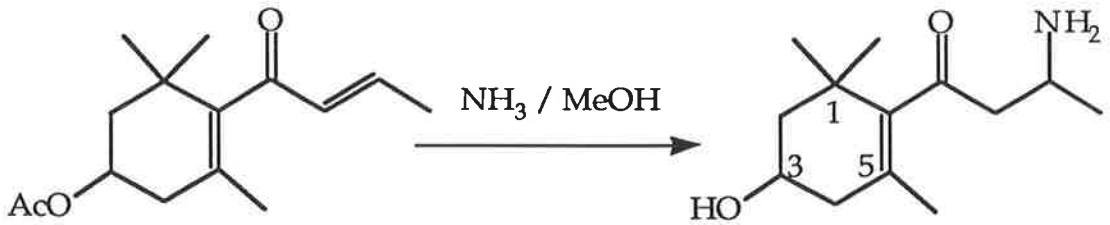
Using this product as a reference, GC / MS analysis confirmed the presence of the glucoside tetraacetate (39c) in acetylated glycosidic fractions isolated from a Riesling wine.

3.7.4 Deacetylation Conditions.

Due to the reactivity of the enone side chain in 3-hydroxy-β-damascone (39) and its derivatives it was important that mild conditions for deacetylation were developed such that the β-glycoside (39c) could be successfully converted to the β-glycoside (39a).

3.7.4.1 Attempted Deacetylation using Ammonia.

Deacetylation with ammonia[143] was investigated with the acetate (93) as a model substrate but gave the deacetylated adduct (162) as the only product.



(93) (162)

^{1}H n m r spectroscopy showed the absence of the ABX system for the vinyl protons and the multiplet for H3 was at δ 3.9, characteristic of a hydroxy methine hydrogen. No acetyl methyl group was present by ^{1}H n m r spectroscopy. Compound (162) was readily converted to 3-hydroxy-β-damascone (39) in refluxing 0.1M hydrochloric acid solution.

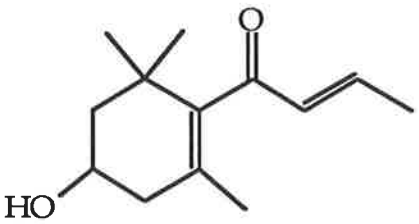
3.7.4.2 Attempted Deacetylation using Methoxide.

An attempt at deacetylating (93) with potassium carbonate / aqueous methanol failed also as conjugate addition of methanol occurred to give

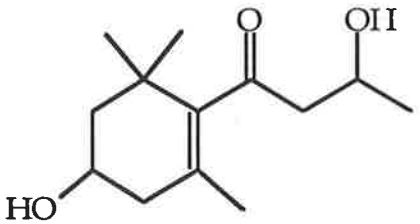
compound (158). The same result was seen when sodium methoxide in methanol was used as described earlier.

3.7.4.3 Use of Hydroxide.

In chapter four hydrolytic studies of glycosides and aglycones are discussed. An interesting observation was that 3-hydroxy-β-damascone (39) was in equilibrium with the hydroxy adduct (163) but its formation never exceeded 15% yield at low pH. It was decided to use four equivalents of sodium hydroxide in aqueous tetrahydrofuran on 3-hydroxy-β-damascone (39). Analysis at time intervals by GC / MS revealed that 3-hydroxy-β-damascone (39) was the predominant product with the adduct less than 20%.



(39)

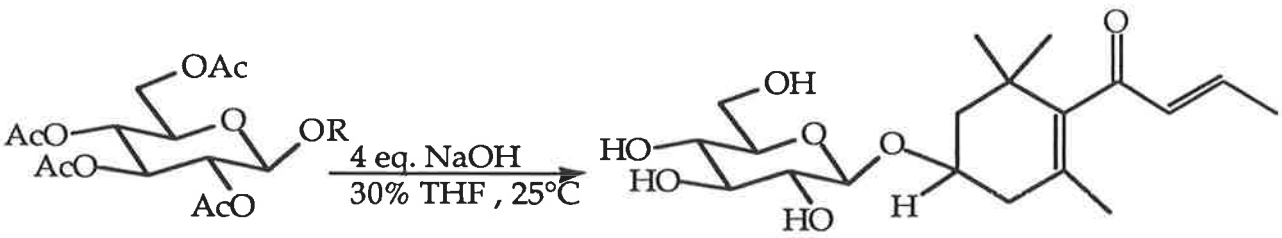


(163)

A competition experiment between hydrolysis of geranyl-β-D-tetra-O-aceyl-glucopyranoside (1b) and conjugate addition to 3-hydroxy-β-damascone (39) using slightly greater than four equivalents of base was performed. Geranyl-β-D-tetra-O-aceyl-glucopyranoside (1b) was insoluble in 10% aqueous tetrahydrofuran but was soluble in 30% aqueous tetrahydrofuran. The reaction was performed in a constant temperature water bath at 24-26°C. Aliquots were taken after 0.5, 1, 1.5, 2, 2.5 and 4hours. ^{1}H n m r spectroscopy showed that deacetylation occurred in preference to conjugate addition and was complete after 1hour. Even after 4hours less than 5% yield of conjugate addition could be seen. Therefore, hydroxide appeared to be suitable for the required ester hydrolysis.

3.7.4.4 Synthesis of (E)-7-Oxo-5,8-megastigmadien-3-yl-β-D-glucopyranoside (39a)

Compound (39c), dissolved in 30% aqueous tetrahydrofuran, was treated with four equivalents of sodium hydroxide at 25°C for 2hours, the reaction being monitored by t.l.c. Isolation of 3-β-D-glucopyranosyl-β–damascone (39a) was achieved by reverse phase chromatography in 40% yield. The mass and ^{13}C n m r spectra confirmed the structure of (39a).

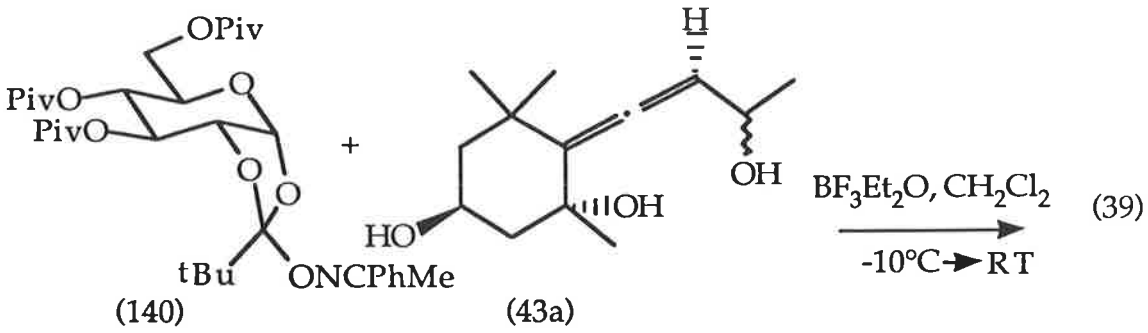


(39c) , R= (39) (39a)

3.8 Synthesis of 3,5-dihydroxy-6,7-Megastigmadien-9-yl-β-D-glucopyranoside (43c)

3.8.1 Attempted Synthesis of Compound (43a) Using the Kunz Method.

Glycosylation of the allenic triol (43a) using the method of Kunz[124] [one equivalent of ortho ester (140) and four equivalents of boron trifluoride etherate in dichloromethane at low temperature (-10°C) for ten minutes, followed by room temperature for ten minutes] yielded 3-hydroxy-β-damascone (39) as the sole product.

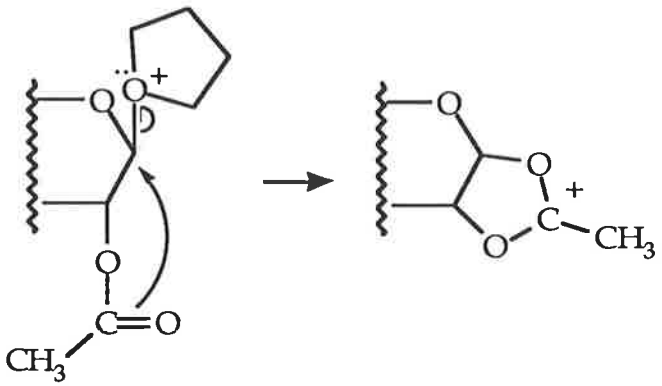


Formation of 3-hydroxy-β-damascone (39) is puzzling because anhydrous conditions were used in the reaction medium. Due to the high reactivity of the allenic triol (43a) with acid it was decided to glycosylate using the method of Ackermann[113].

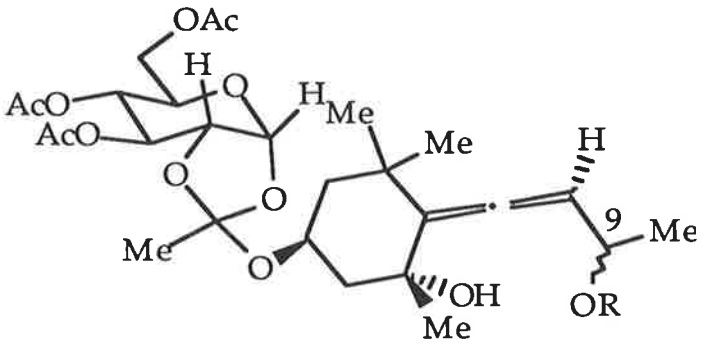
3.8.2 Attempted Synthesis of Compound (43c) Using the Ackermann Method.

Treatment of a suspension of the allenic triol (43a) in ether with tetra-O-acetyl-α-D-bromo-glucopyranoside (108), calcium sulphate, sodium hydrogen carbonate and silver trifluoroacetate at low temperature (0°C) for one hour yielded mainly starting material due presumably to the insolubility of the allenic triol (43a). Changing the solvent to tetrahydrofuran and conducting the experiment initially at 0°C for thirty minutes followed by room temperature for four hours resulted in the formation of four products. Flash chromatography followed by high pressure liquid chromatography gave two of the products [a major (164) and a minor (165)] at Rf 0.5 and 0.4, respectively. Of the other two products at a higher Rf (0.6 and 0.7) only the compound at Rf 0.7 was identified as an allenic substrate but the structure could not be determined. From a comparison of the ^{1}H n m r spectrum of (164) with its corresponding polyacetate (164a), combined with decoupling experiments, the anomeric proton was identified at δ 5.69 in the acetate

[essentially unchanged form the alcohol (164)] and this occurred as a 5.4Hz doublet. This coupling and chemical shift was typical of ortho esters.[126,144] The methyl group attached to the ortho ester carbon was observed at δ 1.75. Ortho esters are often obtained in the presence of a strongly nucleophilic solvent such as tetrahydrofuran which cannot form a covalent bond but merely an oxonium ion[144] thereby promoting ortho ester formation.



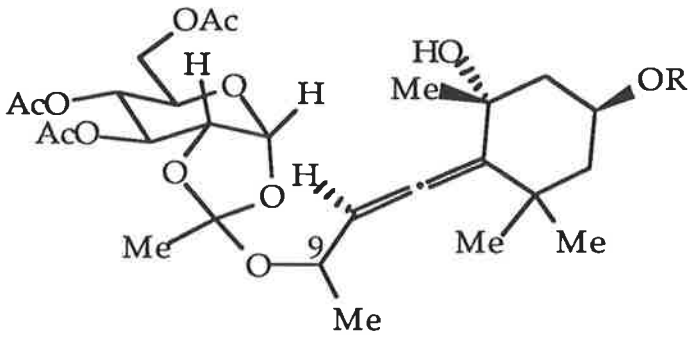
Decoupling of the multiplet at δ 5.35 of the polyacetate (164a) collapsed the three proton doublet (methyl group split by H9) to a singlet which suggests that the C9 hydroxyl was acetylated and therefore the ortho ester involves the ring hydroxyl group. The ortho ester compound (164) is a mixture of two diastereoisomers.



(164) R=H
(164a) R= AcO

The minor component (165) which has a slightly lower Rf than the major component (Rf 0.4),was also an ortho ester (two diastereoisomers) with a 1:2:1 three line pattern for the anomeric proton centred at δ 5.67 with an

average coupling of 4.9Hz. The ortho ester methyl group was also found at 1.73Hz. The ^{1}H n m r spectrum was very similar to that of the major component, however, acetylation of the minor component yielded many products. From the data available it is believed that the minor component is probably the ortho ester of the C9 hydroxyl. Both ortho esters (164) and (165) gave the expected molecular ions by fast atom bombardment.



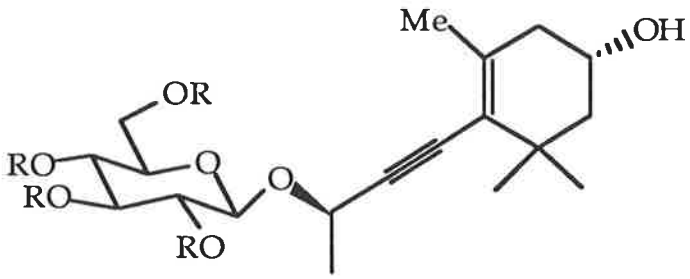
(165) R=H
(165a) R= AcO

The ^{1}H n m r spectrum of the trace component (Rf 0.7) suggested that it was an α -glycoside with the anomeric proton deshielded and downfield at δ 6.22 ppm with a coupling of 3.4 Hz. This apparent deshielding (from the normal position around δ 5.4 ppm) suggested that the glycoside was linked to the C9 with deshielding of the anomeric proton by the allenic group. However this assignment is only tentative.

3.8.3 Synthesis of 3,5-dihydroxy-6,7-Megastigmadien-9-yl-β-D-glucopyranoside (43c) Via an Indirect Route.

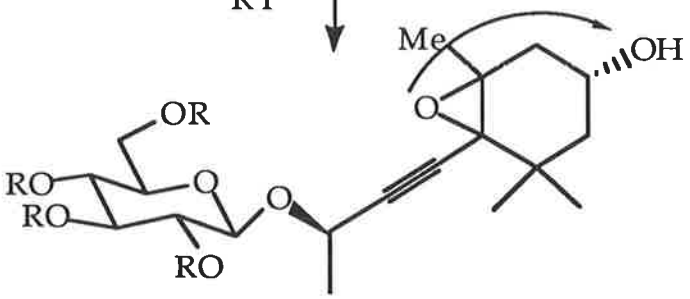
Treatment of the β-glycoside (58d) with m-chloroperoxybenzoic acid in dichloromethane for twelve hours gave a quantitive yield of the trans and cis epoxides (166) and (167), respectively. The inseparable mixture of epoxides (one spot by t.l.c.) was reduced with lithium aluminium hydride (ten equivalents) in tetrahydrofuran for six hours. Two allenic glycosides, (43c)

and isomer (43d) (derived from the two isomeric epoxides), were isolated in low yield but could not be separated.



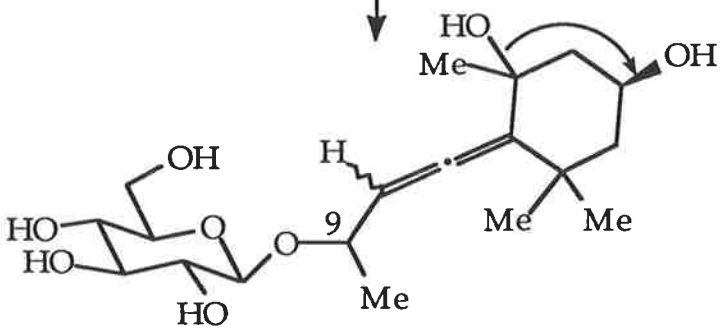
(58d) R = Piv
(58b) R = H

m.c.p.b.a
RT



(166) R = Piv, (168) R = H : epoxide oxygen and hydroxyl trans
(167) R = Piv, (169) R = H : epoxide oxygen and hydroxyl cis

LiAlH4 / THF
Reflux 6Hrs



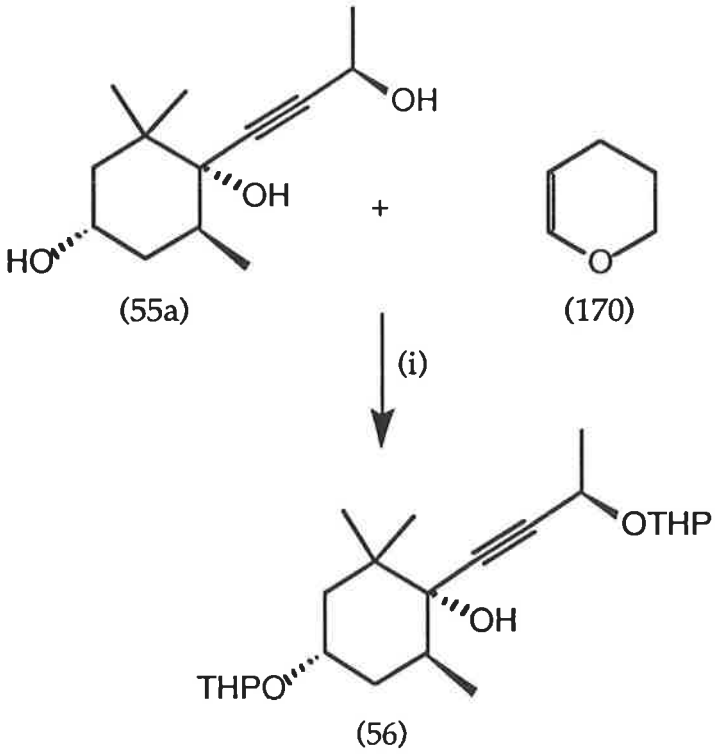
(43c) Trans
(43d) Cis } with respect to the hydroxyl groups

The same series of reactions on the β-glycoside (58b) were also performed. The β-glycoside (58b), however, was insoluble in dichloromethane and

tetrahydrofuran was used instead. Epoxidation yielded the trans and cis epoxides (168) and (169), respectively. Isolation was accomplished with the use of a reverse phase column. Reduction with lithium aluminium hydride in refluxing THF for six hours resulted in the formation of the allenic β-glycosides (43c) and (43d). A molecular ion was observed by chemical ionization giving the M + NH_4^+ ion. Isolation of the allenic β-glycosides (43c) and (43d) from the aluminium salts was difficult and the resulting yield was too low to allow proper characterization.

3.9 Synthesis of Bis Tetrahydropyranyl ether (56) of Acetylenic Triol (55a).

At an early stage of this work the bis-tetrahydropyranyl ether (56) of triol (55a) was synthesized as it is known that acetals are useful model compounds in mimicing glycosides[145]. The synthesis of (56) was achieved by treatment of triol (55a) in dichloromethane with dihydropyran (170) and a catalytic amount of pyridinium p-toluenesulphonate (PPTS) at room temperature. The formation of 4 diastereoisomers is possible as dihydropyran (170) is prochiral. After purification, we obtained a mixture of two of these isomers, which was used in the hydrolytic studies. Scheme 16.



(i) PPTS , CH_2Cl_2 , RT.

Scheme 16.

This concludes the chapter on β-glycoside and acetal formation and the next chapter describes the results of their hydrolytic behaviour.

4.1 Hydrolytic Studies of Geraniol (1) and its β-D-Glucopyranoside (1a)

4.1.1 Introduction .

Before undertaking synthesis and hydrolytic studies on likely precursors to β-damascenone (39), we decided to investigate the hydrolytic behaviour of a simple allylic alcohol, i.e. geraniol (1), to compare its hydrolytic rate with respect to its β-D-glucopyranoside (1a) to establish differences in reaction rates. Hydrolyses were carried out at a typical wine pH (pH 3) and at temperatures of 50°C and 80°C. Results are shown in Tables [(4.1),(4.2), (4.3), (4.4)].

4.1.2. Geraniol (1) at pH 3 and 50°C.

After one hour the major components of the reaction mixture were geraniol (1), linalool (3), α-terpineol (5) and trace amounts of (Z)-3,7-dimethyl-2-octene-1,7-diol (179), 2,6-dimethyl-7-octene-2,6-diol (11), nerol (2) and myrcene (172). When the reaction time was increased to twenty-four hours a substantial increase in linalool (3), α-terpineol (5), nerol (2), (Z)-3,7-dimethyl-2-octene-1,7-diol (179), 2,6-dimethyl-7-octene-2,6-diol (11) as well as trace amounts of another seven compounds were observed. These included rearranged, dehydrated and hydrated compounds (171), (172-176) and (179-180) respectively. (Structures shown in Figure 8 are presented in order of elution and appear on the page after the Table).

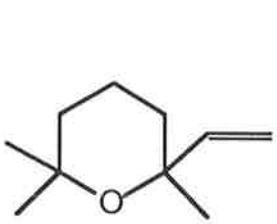
The hydrolyses of many monoterpenes under a variety of acid conditions have been studied; they have been reviewed by Coates.[146] The products shown in Table 4.1 are consistent with the earlier results. Over 40% of geraniol (1) was converted to linalool (3) via allylic rearrangement. The

other major product, α-terpineol (5), has been postulated to form either directly from geraniol (1) or indirectly via linalool (3)[5]. Figure 9

Table 4.1 : Hydrolysis of Geraniol (1) at 50°C, pH 3 in 10% Aqueous Ethanol

Entry	Products[a]	Yields (%)			
		1h	4h	8h	24h
1	2,2,6-Trimethyl-2-vinyl-tetrahydropyran (171)[b]	n.d.	n.d.	n.d.	+
2	Myrcene (172)[b]	+	0.1	0.1	0.2
3	Limonene (173)[b]	n.d.	n.d.	n.d.	+
4	Z-Ocimene (174)[b]	n.d.	+	0.1	0.2
5	E-Ocimene (175)[b]	n.d.	0.1	0.1	0.2
6	Terpinolene (176)[b]	n.d.	n.d.	n.d.	+
7	Linalool (3)	2.5	6.2	12.5	27.5
8	α-Terpineol (5)	0.25	0.4	0.5	1.4
9	Nerol (2)[b]	+	0.1	0.4	0.7
10	Geraniol (1)	97	97.2	85	67
11	2,6-dimethyl-7-octene-2,6-diol (11)[b]	+	0.1	0.2	0.6
12	Trans-1,8-Terpin (177)[b]	n.d.	n.d.	n.d.	n.d.
13	Cis-1,8-Terpin (178)[b]	n.d.	n.d.	n.d.	n.d.
14	(Z)-3,7-dimethyl-2-octene-1,7-diol (179)[b]	+	+	0.1	0.1
15	(E)-3,7-dimethyl-2-octene-1,7-diol (180)[b]	n.d.	n.d.	0.7	1.6

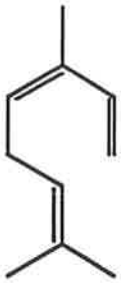
a Assignments of all compounds have been established previously in this laboratory by Strauss[147]. b concentration based on the assumption that components give the same GC / FID peak area / mg as the internal standard.
n.d., not detected + less than 0.1%



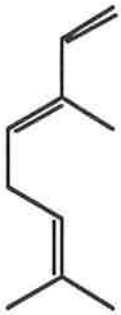
1. (171)

2. (172)

3. (173)

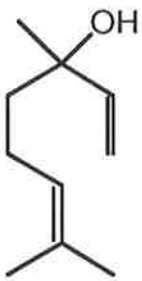


4. (174)

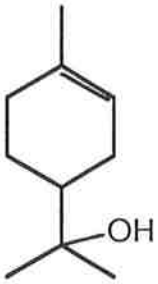


5. (175)

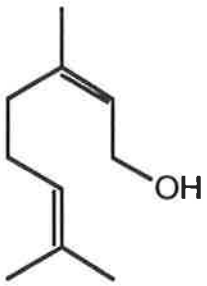
6. (176)



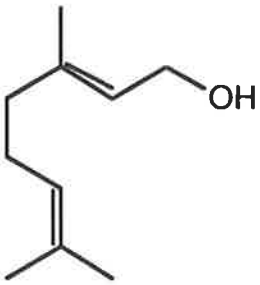
7. (3)



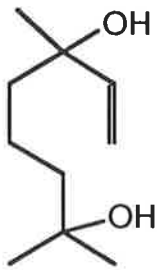
8. (5)



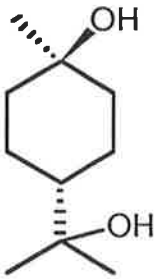
9. (2)



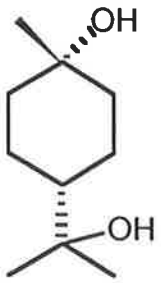
10. (1)



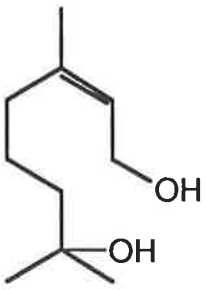
11. (11)



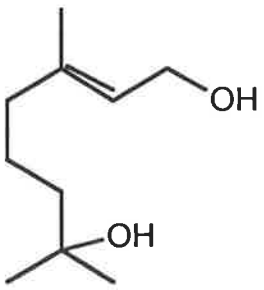
12. (177)



13. (178)



14. (179)



15. (180)

Figure 8. Compound number is preceded by entry number.

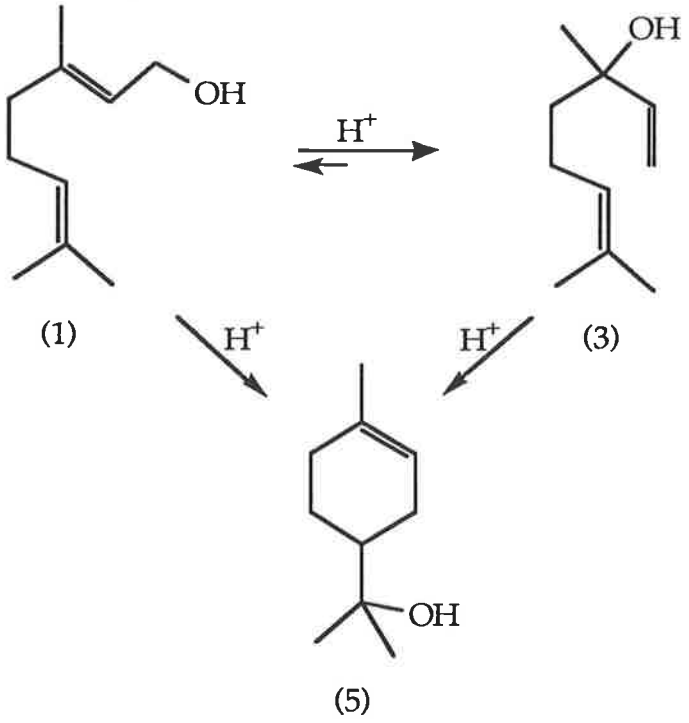


Figure 9

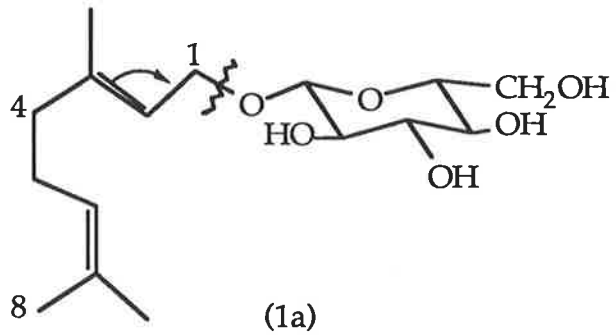
4.1.3. Geranyl-β-D-glucopyranoside (1a) at pH3 and 50°C.

As a control geranyl-β-D-glucopyranoside (1a) was dissolved in 10% ethanol and the solution extracted with dichloromethane (2ml). Analyses by GC / MS revealed that 0.16% free geraniol (1) was present. The results from Table (4.2) show that only three products were present compared to the thirteen compounds identified in the aglycone hydrolysis. These were linalool (3), a larger amount of α- terpineol (5) and 2,6-dimethyl-7-octene-2,6-diol (11). No additional geraniol (1) was observed other than that present in the control run. This suggests that the hydrolysis of the glucoside (1a) occurs with initial bond cleavage at the C1-O bond rather than the glycosidic bond. Figure 10.

Table 4.2. Geranyl-β-D-glucopyranoside (1a) at pH 3 and 50°C.

Entry	Products[a]	Yields (%) 1h	4h	8h	24h
7	Linalool (3)	0.1	0.2	0.5	1.4
8	α-Terpineol (5)	0.4	0.4	0.5	0.5
10	Geraniol (1)	0.15	0.15	0.1	n.d.
11	2,6-dimethyl-7-octene-2,6-diol (11)[b]	n.d.	0.4	0.3	0.2

a Assignments of all compounds have been established previously in this laboratory by Strauss[147]. b concentration based on the assumption that components give the same GC / FID peak area / mg as the internal standard.
n.d., not detected



(1a)

Figure 10

4.1.4 Geraniol (1) at pH 3 and 80°C.

At eighty degrees a substantial amount of geraniol (1) was converted to fifteen products (Figure 8.). After twenty-four hours only 3.2% of geraniol (1) remained. No ethyl ethers were detected, in particular geranyl ethyl ether, although 10% ethanol was used as the hydrolytic medium. Results are shown in Table 4.3. below. It is obvious from the data that the early high

concentrations of linalool (3) eventually decreases as the quantity of α-terpineol (5) increases (See Figure 9.).

Table 4.3 Geraniol, 80°C, pH 3.

Entry	Products[a]	Yields (%) 1h	4h	8h	24h
1	2,2,6-Trimethyl-2-vinyltetrahydropyran (171)[b]	0.1	0.3	0.9	3.7
2	Myrcene (172)[b]	0.2	0.5	0.8	1.7
3	Limonene (173)[b]	0.1	0.3	0.9	2.2
4	Z-Ocimene (174)[b]	0.3	0.6	1.1	1.7
5	E-Ocimene (175)[b]	0.3	1.0	1.8	2.7
6	Terpinolene (176)	0.1	0.3	1.1	1.8
7	Linalool (3)	25.0	45.5	43.3	13.3
8	α-Terpineol (5)	1.1	7.5	19.7	44.0
9	Nerol (2)[b]	0.8	2.0	3.3	4.2
10	Geraniol (1)	71.0	36.7	16.9	2.7
11	2,6-dimethyl-7-octene-2,6-diol (11)[b]	0.3	2.5	6.2	11.3
12	Trans-1,8-Terpin (177)[b]	n.d.	n.d.	0.5	4.3
13	Cis-1,8-Terpin (178)[b]	n.d.	n.d.	n.d.	0.5
14	(Z)-3,7-dimethyl-2-octene-1,7-diol (179)[b]	+	0.2	0.7	2.9
15	(E)-3,7-dimethyl-2-octene-1,7-diol (180)[b]	0.6	2.3	2.5	3.0

a Assignments of all compounds have been established previously in this laboratory by Strauss[147]. b concentration based on the assumption that components give the same GC / FID peak area / mg as the internal standard.
n.d., not detected + less than 0.1%

4.1.5 Geranyl-β-D-glucopyranoside (1a) at pH3 and 80°C

Again the same pattern of products is seen with significant amounts of linalool (3) and α-terpineol (5) as the major products. Table 4.4.

Table 4.4 Geranyl-β-D-glucopyranoside (1a) at pH3 and 80°C

Entry	Products[a]	Yields (%) 1h	4h	8h	24h
1	2,2,6-Trimethyl-2-vinyltetrahydropyran (171)[b]	n.d.	n.d.	0.1	0.6
2	Myrcene (172)[b]	n.d.	n.d.	0.1	n.d.
3	Limonene (173)	n.d.	n.d.	0.1	0.3
4	Z-Ocimene (174)[b]	n.d.	n.d.	0.1	0.2
5	E-Ocimene (175)[b]	n.d.	0.1	0.1	0.3
6	Terpinolene (176)	n.d.	n.d.	n.d.	0.3
7	Linalool (3)	1.5	2.9	4.6	3.8
8	α-Terpineol (5)	1.0	0.8	1.8	5.3
9	Nerol (2)[b]	n.d.	0.1	0.3	0.4
10	Geraniol (1)	0.2	n.d.	n.d.	n.d.
11	2,6-dimethyl-7-octene-2,6-diol (11)[b]	n.d.	n.d.	0.6	2.2
12	Trans-1,8-Terpin (177)[b]	n.d.	n.d.	n.d.	0.3
13	Cis-1,8-Terpin (178)[b]	n.d.	n.d.	n.d.	n.d.
14	(Z)-3,7-dimethyl-2-octene-1,7-diol (179)[b]	n.d.	n.d.	n.d.	0.3
15	(E)-3,7-dimethyl-2-octene-1,7-diol (180)[b]	n.d.	n.d.	n.d.	n.d.

a Assignments of all compounds have been established previously in this laboratory by Strauss[147]. b concentration based on the assumption that components give the same GC / FID peak area / mg as the internal standard.
n.d., not detected + less than 0.1%

Again no additional geraniol (1) other than that observed in the control is seen, confirming the results from the 50°C study.

4.1.6 Conclusion

Earlier studies reported by Strauss[148] on the hydrolysis of geranyl-β-D-glucopyranoside (1a), under different conditions, showed that a substantial amount of geraniol (1) was formed. However, no controls or full experimental details were reported in that study. Their results suggest that breakage of both the C1-O and the glycosidic bond occurred a higher temperature. It was also noted by the authors that the proportion of total monoterpene ethers to alcohols was approximately equal to the molar ratio of ethanol to water present in the reaction mixture. No ethyl ethers, in particular geranyl ethyl ether, were detected in our study when the hydrolyses were conducted at lower temperatures. This tends to suggest that regioselective hydrolysis of geranyl-β-D-glucopyranoside (1a) occurs at lower temperatures whereby loss of the glucopyranoside group is accompanied by direct double bond migration to form linalool (3) as shown in Figure 10.

When one considers the amounts of linalool (3) plus α-terpineol formed at 80°C after one hour from geraniol (1) and its glucoside(1a), the aglycone geraniol (1) reacts at a rate approximately ten times faster than its glucoside (1a). Data from Table (4.1) and Table (4.2) at 50°C also clearly indicate that hydrolysis of geraniol (1) is far more rapid than its glucoside (1a). This is contrary to the belief of Williams[7,12,16] that glycosylation of allylic alcohols should facilitate this ionization. Thus glycosylation of flavour compounds in grapes reduces the flavour and inhibits development of acid generated flavourants during bottle ageing.

4.2 Hydrolysis of 3,5,5-Trimethylcyclo-3-hexen-1-ol (107), 3-Hydroxy-β-damascone (39) and their β-D-glucopyranosides [(107a) and (39a) Respectively].

It has been postulated that 3-hydroxy-β-damascone (39) is an immediate precursor of β-damascenone (38) in nature[26]. However, Ohloff[39] has demonstrated that 3-hydroxy-β-damascone (39) does not give β-damascenone (38) under strongly acidic conditions but rather products derived by Nazarov type cyclization[50]. Nevertheless, nothing is known about the behaviour of the corresponding glucoside (39a) under mild acid conditions. In addition to studying this hydrolysis here we have also studied the hydrolysis of 3,5,5-trimethylcyclo-3-hexen-1ol (107) and its glucoside (107a), as a model for unactivated homoallylic cyclohexenols at wine pH, as these functional groups are common to many C13 norisoprenoids.

4.2.1 Hydrolytic Studies of 3,5,5-Trimethylcyclo-3-hexen-1-ol (107) and its β-D-glucopyranoside (107a).

Hydrolytic studies were performed on compounds (107) and (107a) over a range of temperatures and pH values.

4.2.1.1 Aglycone (107) and β-glucoside (107a) at pH 3 and 100°C.

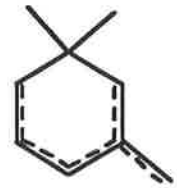
Results are shown in Table 4.5. At pH 3 the aglycone (107) was stable and even after 24hours at 100°C it was found that only 13% had reacted to give trace amounts of isomeric dienes of undetermined structure (181a-d), some isomeric alcohols (182a,b) and trace amounts of oxidation products (60), and (183). The mass spectra and retention times of these two ketones (60), (183) were identical to those of authentic materials.[18] The β-glucoside (107a) was almost inert under these conditions, however, some of the

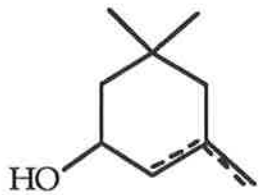
aglycone (107) was seen and only one diene (181a) was detected in trace quantities.

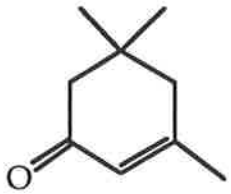
Table 4.5. Aglycone (107) and Glucoside (107a) at pH 3 and 100°C.

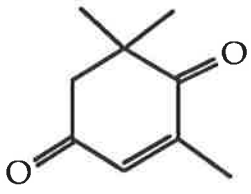
Conditions[a] Aglycone (107)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
100°C,4h	+ : + : + : +	96	+: 1.0	+	+
24h	+ : + : + : +	87	0.4 : 1.4	1	+
Glucoside (107a)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
100°C, 4h	n.d	+	n.d	n.d	n.d
24h	+ : n.d. : n.d. : n.d	0.8	n.d	n.d	n.d

[a] pH=3, n.d. not detected +. trace amount









Diene ratio of 3:1:1.5

(181a,b,c,d) (182a,b) (60) (183)

These results indicate that simple homoallylic alcohols and their glucosides are stable at wine pH. Therefore the reaction of these compounds under more forcing conditions, i.e. pH 1, was also investigated

4.2.1.2 Aglycone (107) and β-Glucoside (107a) at pH 1 and 50°C

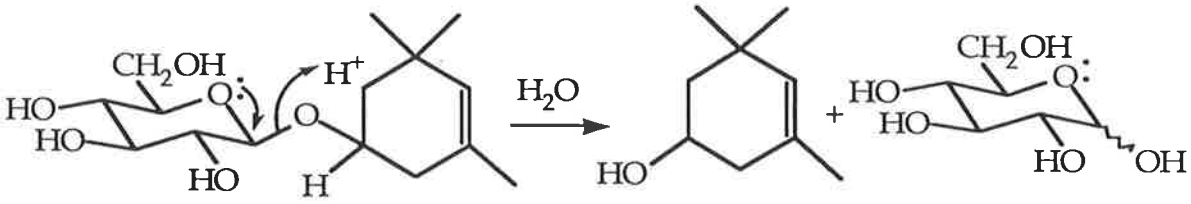
Compound (107) was found to be relatively stable at 50°C at pH 1. The results are shown in Table 4.6

Table 4.6. Aglycone (107) and glucoside (107a) at pH 1 and 50°C.

Conditions[a] Aglycone(107)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
1h	+: n.d : + : n.d	99	+: +	+	+
4h	0.4 : n.d : 0.2 : n.d	95	+: +	5.5	+
24.5h	1.4: 0.4: 0.7 : n.d	82	0.8: 2.6	7.9	0.3
Glucoside (107a)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
24.5h	n.d	+	n.d	n.d	n.d
48h	n.d	1.8	n.d	n.d	n.d

[a] 50°C, pH=1, n.d. not detected +. trace amount

The β-glucoside was found to be relatively stable under the same conditions and only trace amounts of the alcohol (107) were seen after 24hours. No dienes (181a,b,c.d) nor any oxidation products (60), (183) were detected. This tends to suggest that hydrolysis of the glycosidic bond occurs in preference to the homoallylic C-O bond, as shown below. Even, after 48hours only 1.8% of aglycone (107) was detected with the β-glucoside again being more stable than the alcohol (107).



(107a) (107)

4.2.1.3 Aglycone (107) and β-Glucoside (107a) at pH 1 and 80°C.

Results of the hydrolyses are shown in Table 4.7.

Table 4.7. Aglycone (107) and β-Glucoside (107a) at pH 1 and 80°C

Conditions[a] Aglycone (107)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
1h	1.4: 0.4 : 0.7 : +	64	0.5 : 1.7	4.3	0.5
4h	3.8 : 1.0 : 1.8 :0.4	39.6	1.2 : 1.3	4.2	0.5
24h	19.5 : 6 : 8.8: 3.9	11.6	1.9 : 1.9	4.6	0.8
Glucoside (107a)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
1h	1 : n.d.: 0.4 : n.d	1.3	0.3 : n.d	n.d	n.d
4h	5 : 1.3 : 2.3 : +	5.8	0.9 : n.d	n.d	n.d
24h	19.1 : 5.8 : 8.8 : 3.5	11.4	3.2 : 1	n.d.	n.d.

[a] pH 1, 80°C ; n.d. not detected ; + trace amount

At 80°C we find an increase in the rate of hydrolysis of the aglycone (107) and formation of an additional isomeric diene (181d). Approximately 36% of aglycone (107) had reacted to give the products shown in Table 4.7 after one hour. Those listed in the above Table are the main products identified

from their mass spectra. It is interesting to note that the ratio of the isomeric dienes remains constant for the first four hours at approximately 3.5 : 1 : 1.8 for the dienes (181a,b,c) although this ratio changes to approximately 3.2 : 1 : 1.4 as the diene (181d) appears. Again oxidation products (60), (183) are seen although they never exceed a total of 6%. At 80°C, the β-glucoside (107a) hydrolyzes at a rate similar to that of the aglycone (107). The diene formation almost parallels that of the aglycone hydrolysis. After 4hours a ratio of 3.8 : 1 : 1.8 is found but drops to 3.3 : 1 : 1.5 after 24hours. There is also an increase of aglycone (107) formation from the β-glucoside (107a) over 24hours.

4.2.1.4 Aglycone (107) and β-Glucoside (107a) at pH 1 and 100°C.

At 100°C there is no real difference between aglycone (107) and β-glucoside (107a) in that they are both very reactive and almost all of the aglycone (107) has reacted to give products similar to those already discussed. Again, Table 4.8 is a representation only of the main components from the hydrolyses.

Table 4.8 Aglycone (107) and Glucoside (107a) at pH 1 and 100°C.

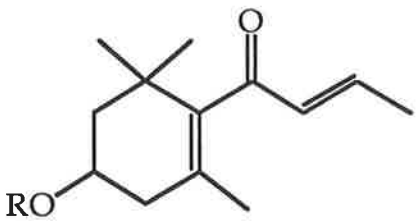
Conditions[a]					
Aglycone (107)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
4h	34.6 : 13 : 17.3 : 8.7	8.4	3.1: 3.7	7.7	1.1
Glucoside (107a)	(181a,b,c,d)	(107)	(182a,b)	(60)	(183)
4h	39 : 13 : 19.3 : 10.8	9.7	3.5 : 4.3	n.d	n.d

[a] pH 1, 100°C n.d. not detected

This concludes the discussion of the hydrolysis of the model alcohol (107) and its β-glucoside (107a). What is interesting is that at the lower temperature (50°C) the alcohol is slightly more reactive. At higher temperatures the difference is less evident. The homoallylic alcohol (107) and its β-glucoside (107a) are both relatively stable at pH 3.

4.2.2 Hydrolysis of 3-Hydroxy-β-Damascone (39) and its β-D-Glucopyranoside-(39a).

Although the results given in the preceding section showed that simple homoallylic cyclohexenols were inert at wine pH it was conceivable that the additional conjugation with the enone side chain could enhance the reactivity of that functional group. Dehydration of the vinylogous hydroxy enone could occur under acid conditions.



(39) R = H
(39a) R = β-D-glucopyranoside

4.2.2.1 Hydrolysis of β-D-glucopyranoside (39a)

Results are shown in Table 4.9 for two different pH values.

Table 4.9 Hydrolysis of β-D-glucopyranoside (39a) at varying pH

Conditions[a]	% (39)
pH=3, 24h	16
pH=1.1, 8h	35

[a] 100°C

4.2.2.2 pH 3, 100°C

When the hydrolysis of β-D-glucopyranoside (39a).was performed at pH 3 and 100°C for twenty-four hours only 16% of aglycone, 3-hydroxy-β-damascone (39) was obtained but no trace of β-damascenone (38) could be detected.

4.2.2.3 pH 1.1, 100°C

At pH 1.1 and 100°C for eight hours only 3-hydroxy-β-damascone (39) was obtained in 35% yield. Thus, although the hydrolysis of the glycosidic linkage in (39a) is faster than the model compound (107a), no elimination products were seen with the former. The reason for these differences is unknown. It is possible that an increase in steric interaction between the side chain and the methyl groups of (39a) is associated with diene formation and that this is responsible for the lack of β-damascenone (38) formation, even under forcing conditions.

The observed faster rate of hydrolysis of the glycoside of 3-hydroxy-β-damascone (39) compared to the model glucoside (107a) might be attributed to the formation of enolic intermediates in the former. To check this hypothesis, hydrolysis of 3-hydroxy-β-damascone (39) was carried out in deuterated solvents.

4.2.2.4 3-Hydroxy-β-damascone (39) at pH 3 and 100°C

Hydrolysis of 3-hydroxy-β-damascone (39) was performed at pH 3 in D_2O. After four hours and 100°C 3-hydroxy-β-damascone (39) was found to be completely stable and there was no deuterium incorporation.

4.2.2 5 pH 1 100°C

At pH 1 and 100°C for 80 minutes the major product obtained was 3-hydroxy-β-damascone (39) with incorporation of deuterium only in the side chain methyl and not in the ring. This was demonstrated by high field ^1H n m r spectroscopy where the vinyl methyl doublet completely disappeared and the vinyl signals collapsed to just two broad singlets , each integrating for one proton. This suggests that the enone side chain is rotated out of conjugation with the ring double bond, presumably to relieve steric interaction with the methyl groups. Thus the hydrolytic behaviour of 3-hydroxy-β-damascone glucoside (39a) was not influenced by enolization in the cyclohexenol ring. Thus, in agreement with Ohloff's[39] result, no β-damascenone (38) was observed. It is worth noting that exchange of the vinyl hydrogen did not occur, indicating selective protonation of the expected side chain dienol intermediate.

4.3 Hydrolysis of Acetylenic Alcohols and their β-D-Glucopyranosides.

Ohloff[39] has shown that the acetylenic triol (55) gave β-damascenone (38) and 3-hydroxy-β-damascone (39) upon strong acid hydrolysis and suggested that the enyne diol (58) was an intermediate. As a result of our work, the enyne diol (58) has been confirmed as a grape constituent both in free and glycoconjugated forms[18]. We have therefore investigated the hydrolytic behaviour of enyne diol (58a) and its glucoside (58b) as well as the related model compound (99) and its glucoside(99a). Although the acetylenic triol (55) has not yet been identified as a natural product we have also investigated its hydrolysis under milder conditions, i.e. at wine pH.

4.3.1 Hydrolytic studies of Model Enyne (99) and its β-D-Glucoside (99a)

Compounds (99) and (99a) were subjected to identical hydrolytic conditions to monitor the difference in reactivities of the aglycone and the β-D-glucoside at pH 3. A summary of the results is shown in Table 4.10.

4.3.2. Aglycone (99) at pH 3 and 100°C

At pH 3 and 100°C the reactivity of compound (99) was low. After forty-eight hours, approximately 26% of compound (99) had reacted to give two major products. One is the ketone (184), formally derived via a Meyer Schuster rearrangement, while the other product (185) is tentatively identified as a hydrate of ketone (184). The ketone (184) was confirmed by an independent synthesis described below.

Table 4.10 Aglycone (99) and Glucoside (99a) at pH 3 and 100°C

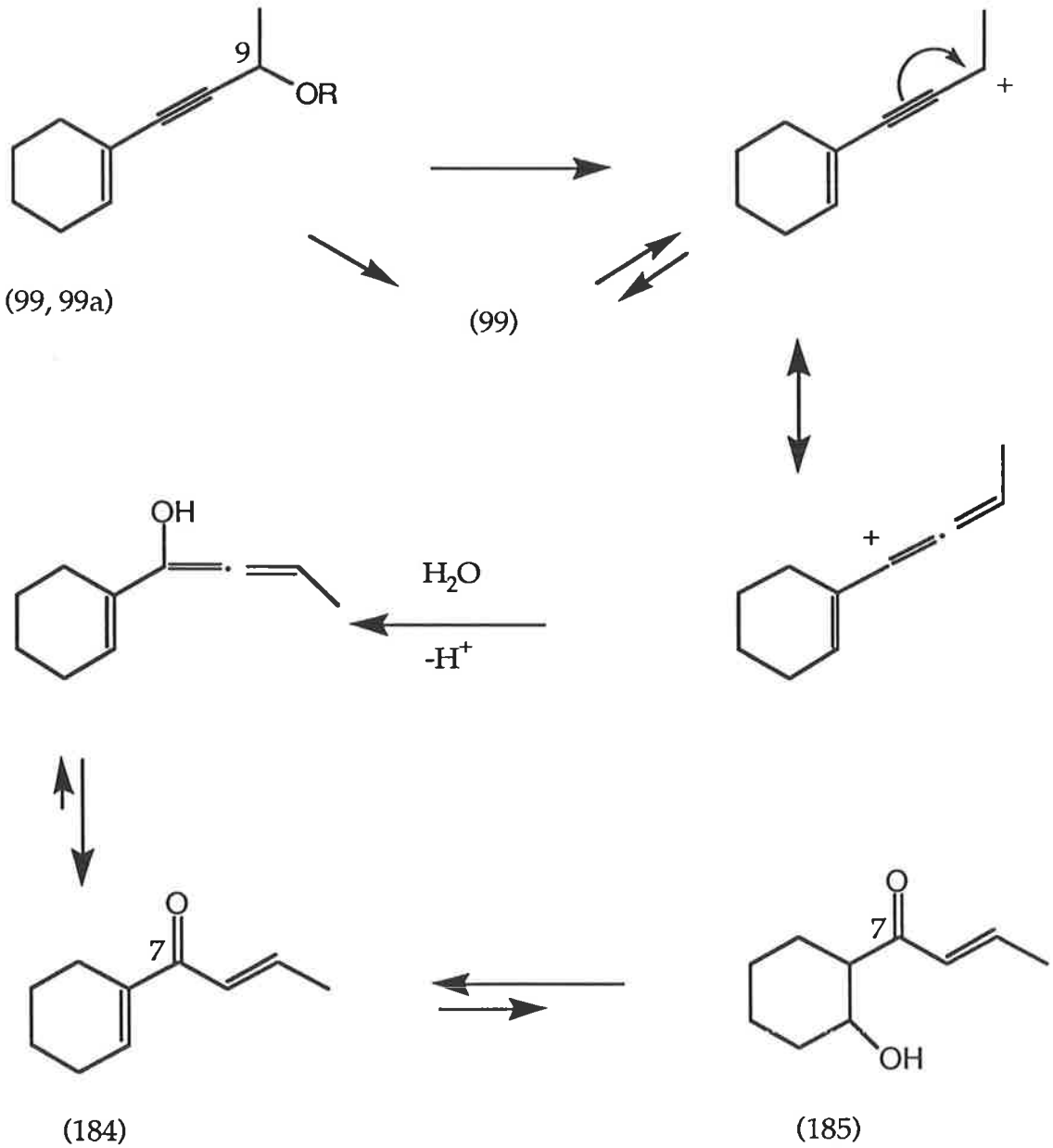
Conditions[a] Aglycone	(99)	(184)	(185)
4h	98.5	1.0	0.5
8h	94.8	3.9	1.3
24h	83.5	13.6	2.9
46h	74.0	21.5	4.8
Glucoside[a] (99a)	(99)	(184)	(185)
4h	0.9	0.5	trace
8h	1.9	1.4	0.2
24h	5.3	5.6	0.7
46h	5.9	7.4	0.9

[a] 100°C, pH 3

4.3.3 Glucoside (99a) at pH 3 and 100°C.

The hydrolysis of the C9 glucoside (99a) was found to be slower than that of the aglycone (99). After forty-six hours only 14% of products were detected compared to 26% for the aglycone. The major products were the aglycone (99), ketone (184) and β-hydroxy ketone (185). It is uncertain whether the aglycone formation comes about via hydrolysis of the glycosidic linkage to give the alcohol (99) directly or via breakage of the C9-O bond followed by hydration of the cation (megastigmane numbering). Thus the magnitude of the reduction in rate of C9-O bond breakage associated with glycosylation of aglycone (99) cannot be determined. However the formation of

rearrangement products (184) and (185) was approximately three times faster with the aglycone (99) than with the glucoside (99a). The formation of ketone (184) which involves a Meyer Schuster rearrangement,[49] occurs through initial ionization at C9. The β-hydroxy enone (185) would then arise from acid catalysed conjugate addition of water to the ketone (184). See scheme 17.

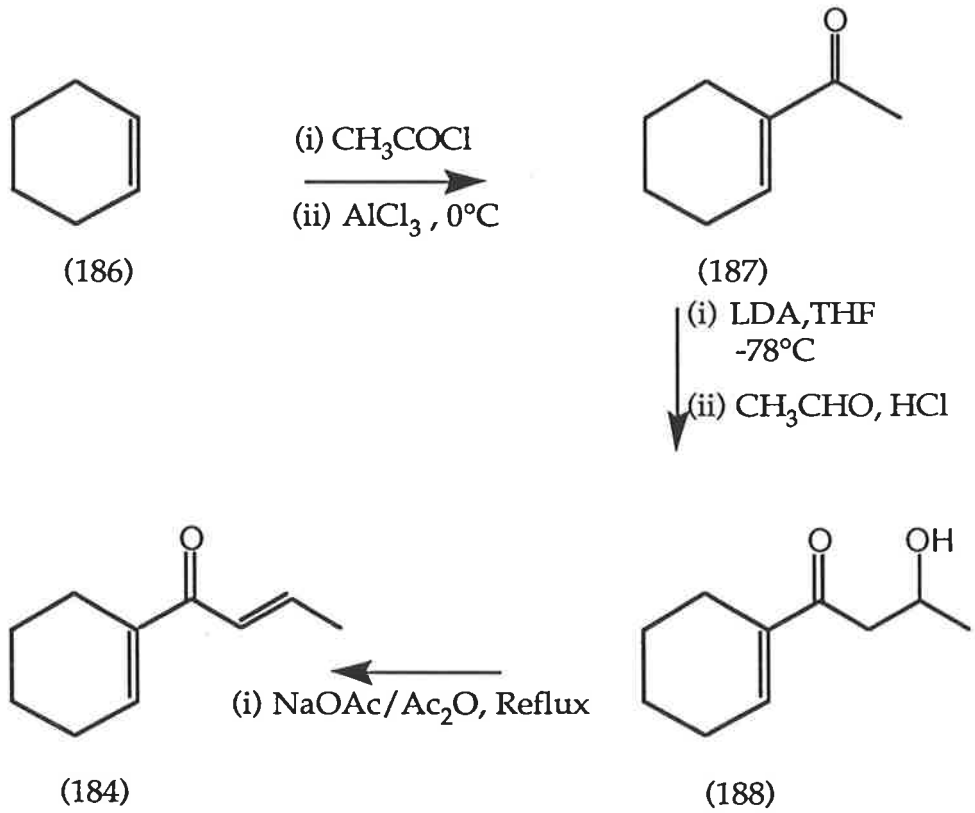


R = β-D-glucopyranosyl, H

Scheme 17.

4.3.4 Synthesis of Ketone (184)

The method used for the synthesis of ketone (184) in three steps was that described by Richter[149] as shown in scheme 18.



Scheme 18.

The first step involves a Lewis acid catalysed acylation of cyclohexene (186) with acetyl chloride and aluminium trichloride to give compound (187)[150]. Generation of the kinetic enolate ion at low temperature (-78°C) with lithium diisopropylamide followed by addition of acetaldehyde leads to the aldol product (188). This was efficiently dehydrated with sodium acetate in refluxing acetic anhydride to yield the ketone (184).

4.3.4.1 Interpretation of Mass Spectra (Electron Impact).

The mass spectrum and retention time of the synthetic ketone (184) were identical with those of the compound obtained from the hydrolytic

experiments. Compound (185) gave a molecular ion which was tentatively assigned from its mass spectrum. It gave no characteristic m/z 109 ion peak via loss of 59 ($C_3H_7O\cdot$) expected for the C9 hydroxy ketone (188), as was seen for compound (163) and (189) in section 4.3.5, but gave instead a base peak of 98, a strong m/z 70 (37%) and a significant 69 (9%) ion. See Figure 11.

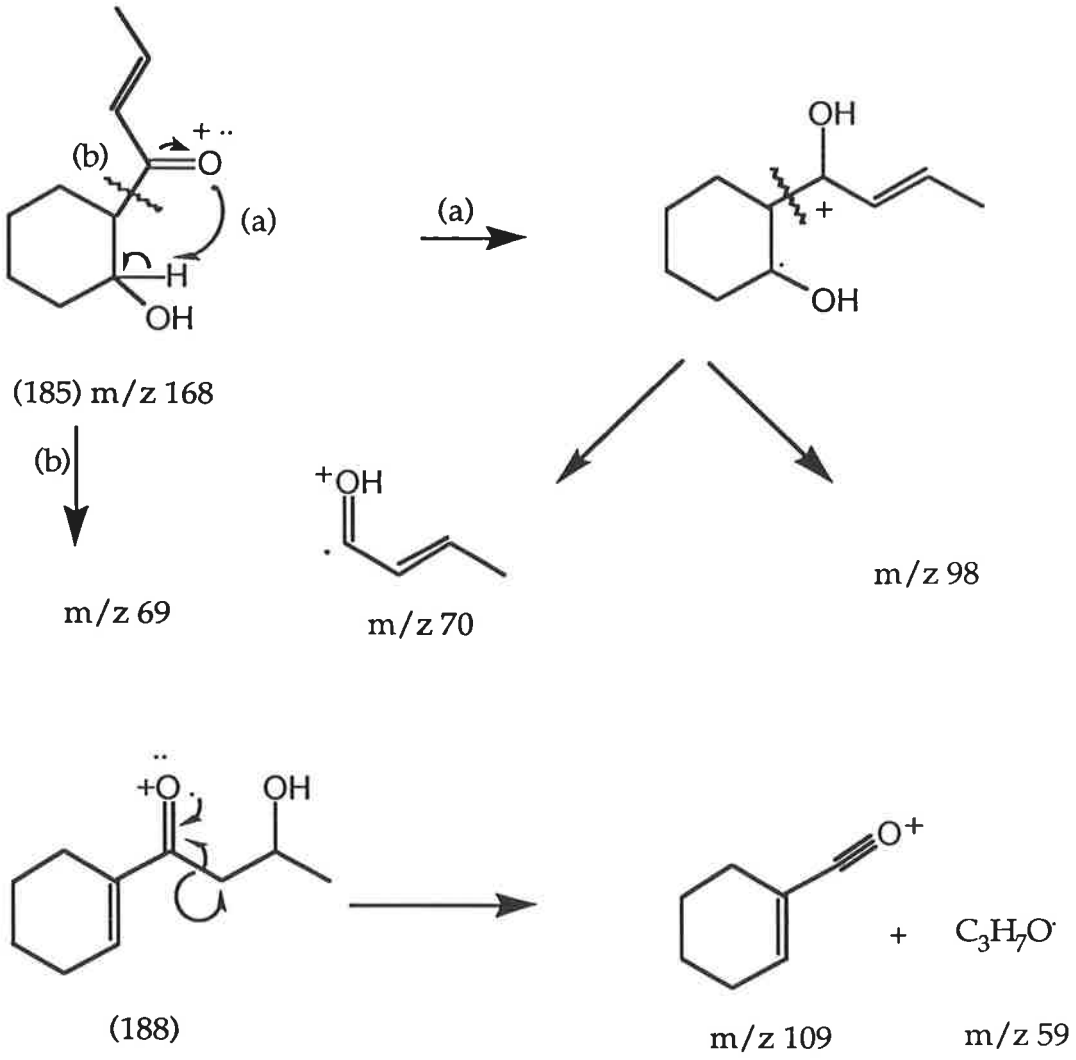


Figure 11

4.3.5 Conclusion

The hydrolytic studies clearly indicate that the model acetylenic alcohol (99) and its β-glucoside (99a) undergo relatively slow hydrolysis. Again the results indicate that an allylic glucoside reacts slower than its corresponding aglycone. This is in direct agreement with the results obtained in the hydrolysis of geraniol (1), model cyclohexenol (107) and their glucosides. Although the model enyne C9 glucoside (99a) is not a true allylic glucoside compared with geraniol glucoside (1a) the results show similarity in that they are only slowly hydrolysed at pH 3.

4.3.6 Hydrolysis of Enyne diol (58a) and Enyne diol C9 Glucoside (58b)

Hydrolytic studies were conducted on the enyne diol (58a) and its glucoside (58b) under various conditions. The relative configuration of enyne diol (58a) and its C9 glucoside (58b) were known because they were synthesized from the triol (55a) of known configuration (Section 2 2). Tables 4.11 and 4.12 show the various conditions employed in the hydrolyses of the two compounds.

4.3.6.1 Hydrolysis of Enyne Diol (58a) at pH 1 and 80°C.

At pH 1 and 80°C the enyne diol (58a) was found to be reactive and after twenty minutes only approximately 3% remained. The predominant products formed were 3-hydroxy-β-damascone (39), β-damascenone (38) and conjugate addition products of (39) and (38) labelled (163) and (189). The compounds (163) and (189) were tentatively assigned on the basis of their mass spectra. See Figure 12.

Table 11 Hydrolysis of Enyne diol (58a).

Conditions Aglycone (58a)	% Conversion	(38)	(39)	(58a)	(189)	(163)
pH=1, 80°C, 0.33h	97	5.1	84	2.8	2.2	5.7
1h	100	5.2	82	n.d.[a]	2.9	10
4h	100	4.2	82	n.d.	2.4	12
pH=3, 100°C, 4h	23	2.8	20	77	n.d.	n.d.
1d[b]	81	10	71	19	trace	trace
2d	97.5	14	83	2.5	trace	trace
pH=3, 50°C 1d	2	0.1	1.8	98	n.d.	n.d.
7d	13	0.8	12.3	87	n.d.	n.d.
28d	44	2.3	41	56	n.d.	n.d.
pH=3, 20°C, 28d	2.6	trace	2.6	97	n.d.	n.d.
90d	6.4	0.18	6.2	93.6	n.d.	n.d.
356d	22	0.9	21	78	n.d.	n.d.

a n.d., not detected b d, day

Table 4.12 Hydrolysis of C9 Glucopyranoside (58b) at pH 3.

Glucoside(58b)[a]	(38)	(39)	(58a)
100°C, 4h	0.6	2.1	1.9
2d	5.0	33.4	1.8
50°C,7d	0.14	1.6	1.1
28d	1.04	11.0	3.8

a pH 3 d, day

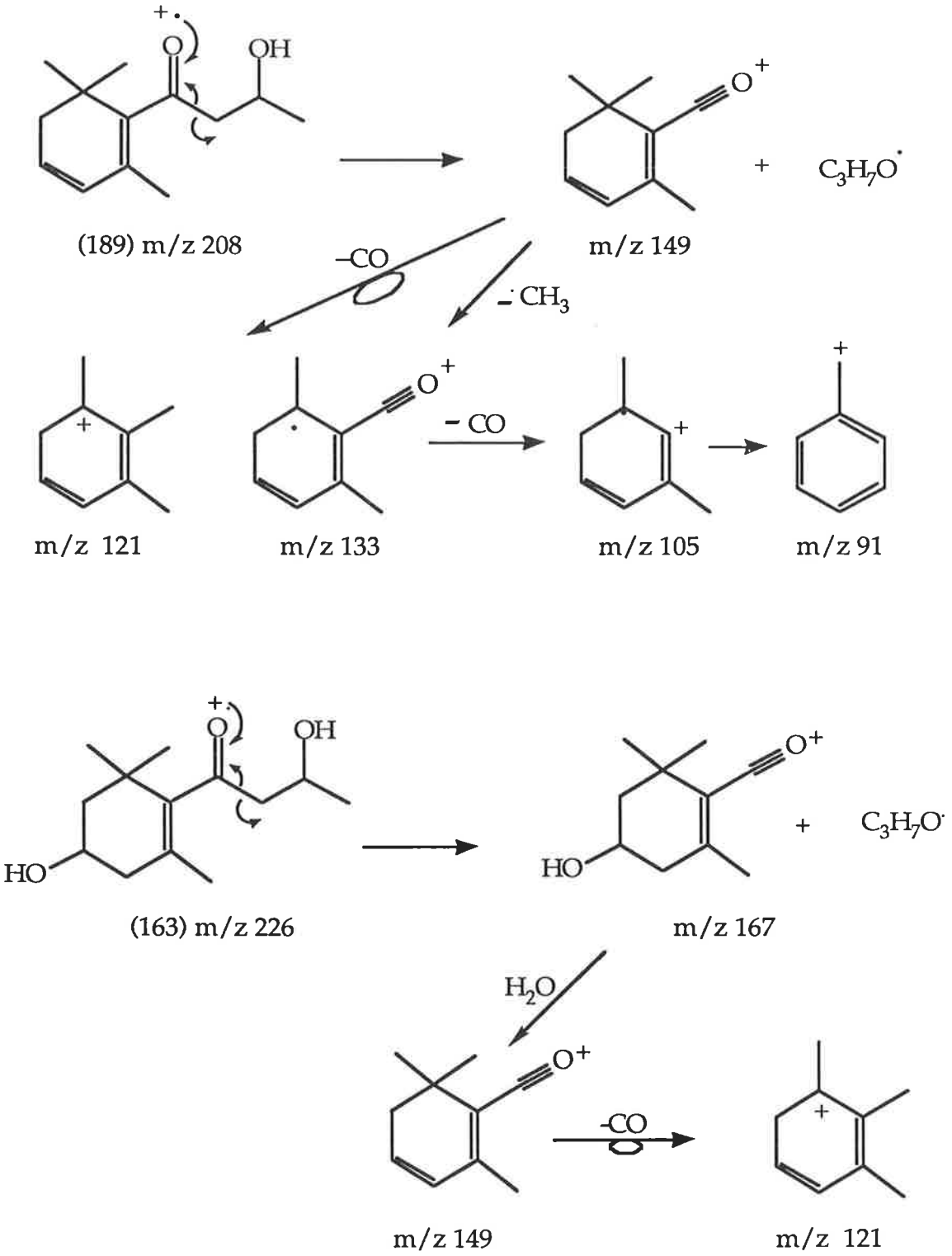


Figure 12

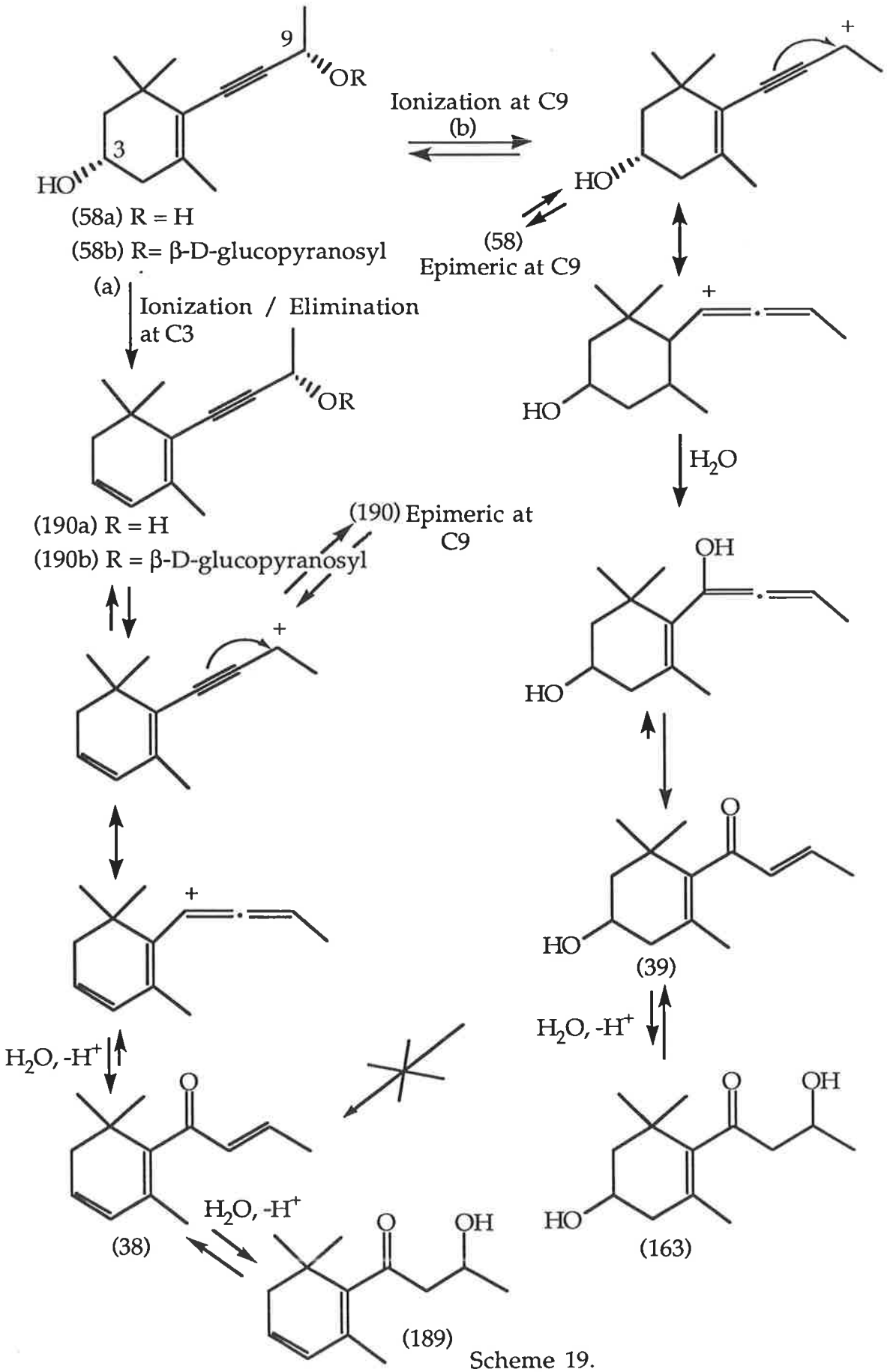
The major fragmentation ions of (189) are the 121, 149, 105 and 91 ions shown in Figure 12. Similarly, the major fragmentation ions of hydrate (163) are 167 and 121 ions. The ions 149 and 167 can be attributed to the

loss of a C_3H_7O radical. Scheme 19 is an outline of how the formation of the products may have occurred. Route (a) outlines the possible pathway in the formation of β–damascenone (38) and its hydrate (189). Route (b) outlines the possible pathway in the formation of 3-hydroxy-β–damascone (39) and its hydrate (163). In both instances a Meyer Schuster rearrangement is involved in the formation of an α,β-unsaturated ketone. Scheme 19 suggests that compound (190) and (190a) would be possible precursors to β-damascenone (38), however, these compounds were not detected by GC / MS analysis.

It is evident that 3-hydroxy-β–damascone (39) is in equilibrium with its conjugate adduct (163) and the relative amount of adduct never exceeds 12%. After one hour only 10% of adduct (163) was observed which remains relatively unchanged after four hours. A similar position occurs for β-damascenone (38) where its conjugate adduct (189) never exceeds 5%, see Table 4.5. The approximate ratio of 3-hydroxy-β-damascone (39) to β-damascenone (38) was found to be 20:1 at pH 1 at 80°C.

4.3.6.2 Enyne diol (58a) at pH 3 and 100°C.

At 100°C only two significant products other than enyne diol (58a) were seen. Again, the major products are compounds (39) and (38) and found to be in the approximate ratio of 7:1. After twenty-four hours 81% of (58a) had reacted to give the compounds (39) and (38) in the same ratio with trace amounts of hydrates (163) and (189) being detected. After forty-eight hours only 2.5% of enyne diol (58a) remained and the ratio of the four products discussed above remain unchanged, Table 4.11.
In going from pH 3 to 1 at 100°C the ratio of (39) to (38) changed significantly from 7:1 to 20:1, respectively. Scheme 19.


9
OR
3
HO
Ionization at C9
(b)
+
HO
(58a) R = H
(58b) R= β-D-glucopyranosyl
(58)
Epimeric at C9
(a)
Ionization / Elimination
at C3
OR
+
HO
H2O
(190a) R = H
(190b) R = β-D-glucopyranosyl
(190) Epimeric at
C9
OH
HO
+
+
O
HO
(39)
H2O, -H+
H2O, -H+
O
O
OH
HO
(38)
H2O, -H+
O
OH
HO
(163)
(189)

Scheme 19.

4.3.6.3. Enyne diol (58a) at pH 3 and 50°C.

At pH 3 and 50°C the enyne diol (58a) was less reactive and only two products were seen, (39) and (38). These were found to be in an approximate ratio of 17:1. Only 44% of enyne diol (58a) had reacted to give 3-hydroxy-β-damascone (39) as the major product and 2.3% of β-damascenone (38). No hydrates of compounds (38) and (39) were seen.

4.3.6.4..Enyne diol (58a) at pH 3 and 20°C.

After one year at 20°C the ratio of (39) to (38) increased to 20:1. The enyne diol (58a) was quite stable under these conditions with only 22% being converted into products. This result, however, is important as the hydrolysis of enyne diol (58a) can account in part for β-damascenone (38) formation in wines, albeit slowly.

4.3.6.5..C9 Glucopyranoside (58b) at pH 3 and 100°C.

At 100°C for 2 days the C9 glucoside (58b) gave 40% of product formation consisting of compounds (58a), (39) and (38). The C9 glucoside (58b) can react either directly to give compound (38) and compound (39) or via the aglycone (58) (Epimer at C9). The inference from the hydrolytic data is that both processes are occurring. Also, it should be noted that compound (39) does not give compound (38) under these conditions. The relative ratio of (39) to (38) did not alter during the course of the reaction at 100°C.

4.3.6.6 C9 Glucopyranoside (58b) at pH 3 and 50°C.

At 50°C the C9 glucopyranoside (58b) gave (39) and (38) in an approximate ratio of 11:1 and noticeably different from the ratio of 18:1 for the enyne diol (58a) hydrolysis under identical conditions. This result indicates that although β-damascenone (38) is formed more slowly, it comprises a larger

proportion of the total products. It appears that the allylic-type glucopyranosyl group, although ionizing slowly, is also acting as a stabilizing group, directing other chemical transformations to occur within the molecule. This is contrary to the suggestion of Williams[7,12,16] that an allylic glucoside would facilitate ionization of the glycoconjugate.

4.3.7 Conclusions on Hydrolytic Studies.

In conclusion, the results show that enyne diol (58a), identified as a natural product in grape juice / wine[18], is one of many precursors of β–damascenone (38). Similarly, the glycoconjugate (58b) can also act as a precursor although it reacts more slowly. In the long term however, the glycoconjugate (58b) would be expected to yield a greater amount of β-damascenone (38). The enyne diol (58a) was found to be approximately eight times more reactive than its corresponding C9 glucoside (58b) at pH 3. The routes and mechanisms for β-damascenone (38) formation are not clear at this stage but a suggestion for its formation has been shown in scheme 19. Results from Tables 4.11 and 4.12 show that the reactivity of compounds (58a) and (58b) are significantly greater than those noted for the model enyne compounds (99) and (99a) and this is attributed to the extra methyl substituents which can stabilize incipient carbocations via the inductive effect.

4.3 8 Acid Hydrolysis of Acetylenic Triol (55a)

Acid hydrolyses were carried out on acetylenic triol (55a) of known relative configuration, and also on its bis-tetrahydropyranyl ethers (56), which were used to mimic glucosides. Two different temperatures and pH values were used for the acetylenic triol (55a). The bis tetrahydropyranyl ethers (56) were dissolved in aqueous tetrahydrofuran (25%) because of solubility problems associated with the aqueous systems. Similarly, aqueous

tetrahydrofuran (25%) was used as well as aqueous ethanol for the acetylenic triol (55a) hydrolysis for comparison with the THP ethers. The conditions used by Ohloff[39] on the acetylenic triol (55) were also re-examined.

Table 4.13 shows the results of the hydrolyses of acetylenic triol (55a) in aqueous ethanol only. At pH 3 and 100°C for four hours the acetylenic triol (55a) was found to be stable and no products were detected.

Table 4.13 Hydrolysis of Triol under varying conditions

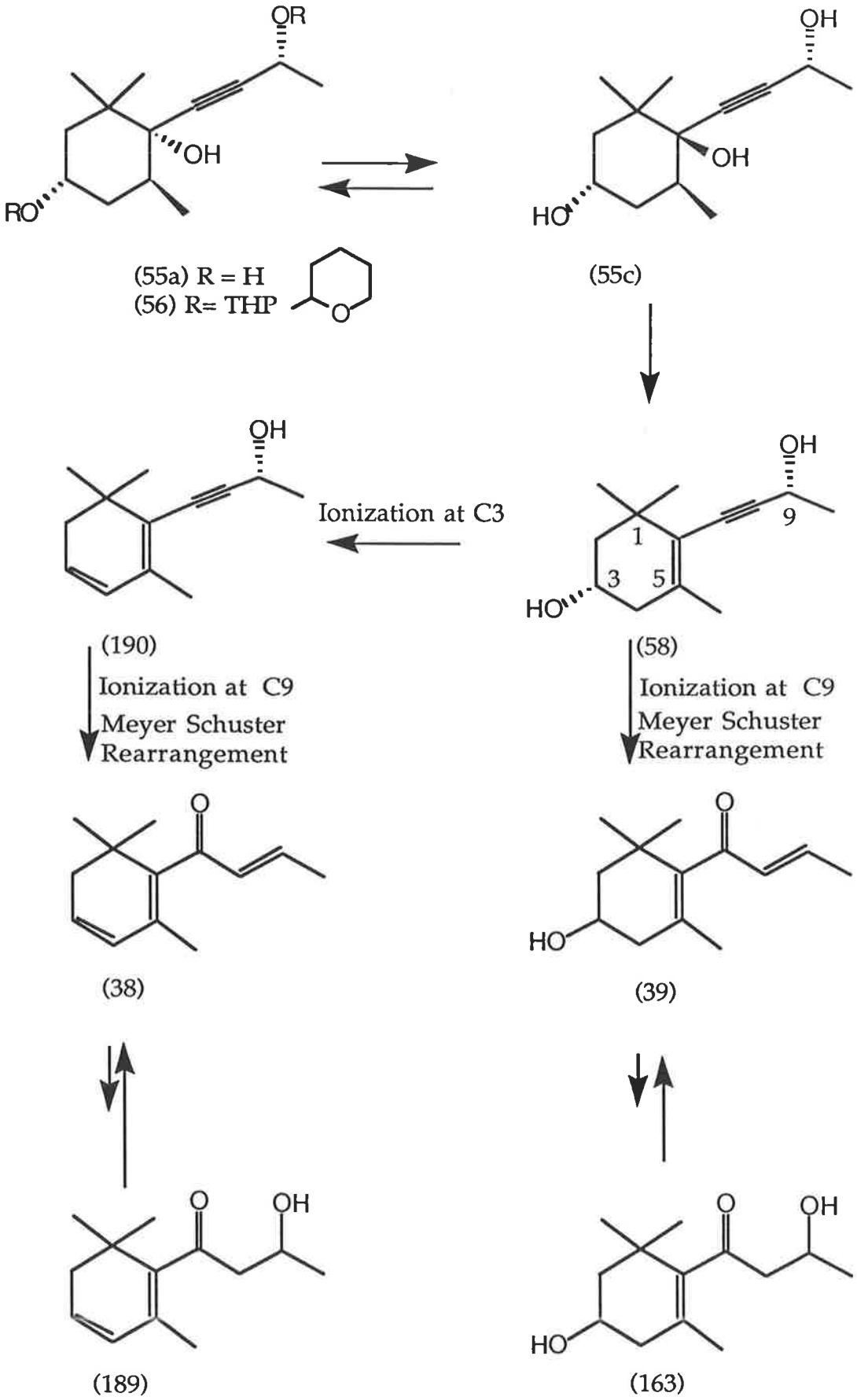
Conditions Triol (55a)	Conversion %	(38)	(39)	(58)	(55c)[a]	(189)	(163)
pH 3, 100°C, 4h	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
pH 1, 100°C 0.33h	68	4	52	1.4	3	2	5
1.33h	97	5	78	n.d.	n.d.	2.5	11
pH 1, 80°C, 1h	40	2	28	2	5.5	0.8	1.6
4h	73	3.7	58	n.d	n.d.	2	9

[a]The recovery of the triol (55) from the hydrolytic medium with dichloromethane is inefficient. Yields of the corresponding epimer (55c) may therefore be higher than recorded here. n.d., not detected.

4.3.8.1 Acetylenic Triol (55a) at pH 1 and 100°C

The reaction of the acetylenic triol (55a) at pH 1 and 100°C was quenched after twenty and eighty minutes. It was found that after twenty minutes six products were detected. They were 3-hydroxy-β-damascone (39), β-damascenone (38), conjugate addition products (163) and (189), enyne diol (58), and a further product which had an identical mass spectrum but shorter retention time than the triol (55a) and was presumably

the C6 epimer (55c). When the hydrolysis of the acetylenic triol (55a) was carried out at 100°C in 25% tetrahydrofuran the reaction was found to be significantly slower than in 10% ethanol. However, the first observable products were the epimer (55c) and enyne diol (58). Scheme 20 outlines the probable routes for the formation of the products.


OR
OH
RO
(55a) R = H
(56) R= THP
O
OH
OH
HO
(55c)
OH
Ionization at C3
OH
1
9
3
5
HO
(190)
Ionization at C9
Meyer Schuster
Rearrangement
(58)
Ionization at C9
Meyer Schuster
Rearrangement
O
HO
(38)
(39)
O
OH
O
OH
HO
(189)
(163)

Scheme 20.

Epimerization of the C6 hydroxyl group to give the acetylenic triol (55c) could dehydrate more readily to the enyne diol (58) because the elements of water at C5 and C6 are now trans-disposed. The Meyer Schuster[49] type rearrangements occur to give the products shown. Compounds (189) and (163) appear to reach equilibrium with β-damascenone (38) and 3-hydroxy-β-damascone (39) after prolonged reaction times. This indicates that (189) and (163) are not obligatory intermediates in the formation of β-damascenone (38) and 3-hydroxy-β-damascone (39) but may arise as end products from these two compounds. Tentative assignments of (189) and (163) were based on their mass spectral data. See section 4.3.5.

4.3.8.2 Acetylenic Triol (55a) at pH 1 and 80C.

At lower temperatures (80°C) similar products were obtained. It is interesting to note that data from the Table 4.13 suggest that the extent of the reaction and the proportion of β-damascenone(38) decrease at lower temperatures.

When Olhoff[39] conditions were employed, (stirring the acetylenic triol (55a) in 30% sulphuric acid at room temperature for ten hours), we observed by GC / MS the formation of 3-hydroxy-β-damascone (39), β-damascenone (38), and an additional twenty-seven products in minor amounts.

4.3.8.3..Bis-tetrahydropyranyl Ether (56) at pH 3 and 100°C.

The bis tetrahydropyranyl ethers (56) (a diastereoisomeric mixture) were also subjected to hydrolysis. At pH 3 and 100°C hydrolysis occurred within five minutes to give solely the acetylenic triol (55), with no products which could have arisen form C9-O bond fission. Work on the tetrahydropyranyl ethers (56) was abandoned due to their lability in dilute acid. These are clearly poor models for glucoside study.

4.3.8.4 .Conclusion.

The acetylenic triol (55a) is unlikely to be a natural constituent in grape juice and wine because it has not been detected even though it is stable and chromatographs by GC / MS readily. However, its transformation to enyne diol (58) has been observed. The reactivity of the acetylenic triol (55a) is low, particularly at grape juice / wine pH, and rearranged products are seen only at low pH and high temperature. The observation of the enyne diol (58) formation during these hydrolyses and the fact that the ratios of β-damascenone (38) to 3-hydroxy-β-damascone (39) under the same conditions are approximately the same for both enyne diol (58) and acetylenic triol (55a) suggests that the former is an intermediate in the hydrolysis of the latter.

4.4 Hydrolysis of Allene.(43a)

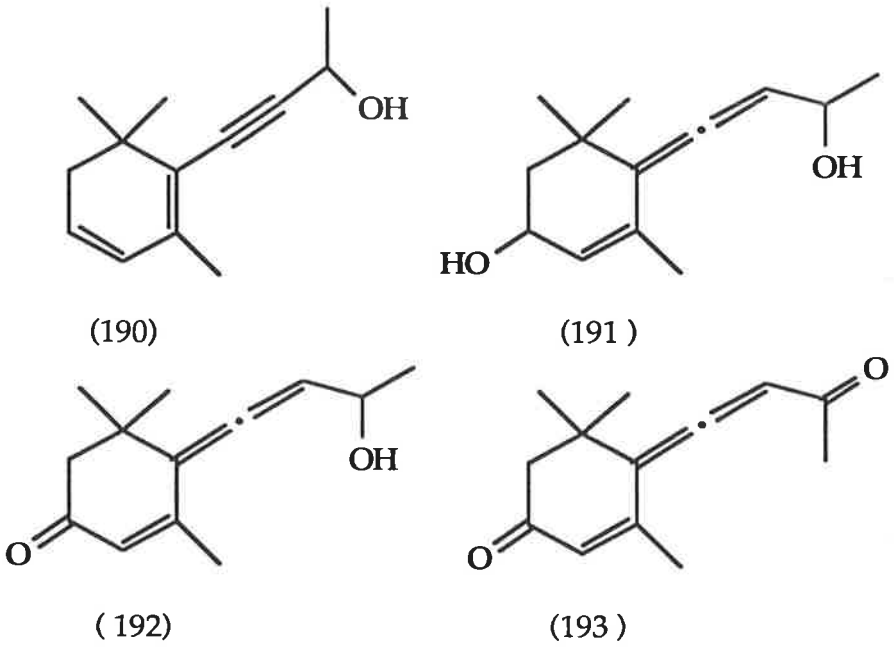
4.4.1 Hydrolysis Products (Introduction)

Olsson[152] has studied the acid catalysed rearrangements of some isomeric α–allenic and α-acetylenic tertiary alcohols to α,β-unsaturated ketones and found that allenic alcohols were far more reactive than the acetylenic alcohols. As a consequence of their results we investigated the hydrolysis of the allene (43a) under milder conditions than those used for the corresponding acetylenic triol (55) (each of the same oxidation state.) The results are shown in Table 4.14.

4.4.2 Reaction Conditions.

4.4.2.1 pH 3, 24°C

After one hour at 24°C only one product was detected which was 3-hydroxy-β-damascone (39). After four hours the allene (43a) had yielded an additional four products, β-damascenone (38), enyne diol (58), 3,5-megastigmadien-7-yn-9-ol (190) and 4,6,7-megastimatriene-3,9-diol (191). Product (190) had a mass spectrum which was the same as that reported[152] and is therefore tentatively identified as such. The final product (191) did not chromatograph readily and was only seen during injections of more concentrated samples. It was tentatively identified on the bases of its retention time, molecular weight and major mass spectral fragmentation. Significant ions at m/z 146 and 131 (100%) were attributed to dehydration of the molecular ion, followed by loss of 44 (the C9 and C10 carbons) and subsequent loss of a methyl group. The ion m/z 149 was attributed to M - 44 - 15, without loss of water. Similar fragmentations have been observed for the ketone (192)[18] and diketone (193)[153]. Both of these compounds gave ions at m/z 162 and 147 as the two major fragments.



(190) (191)

(192) (193)

Figure 13.

β-Damascenone (38) and compound (190) were identified in trace amounts in a ratio of 1:2 with the major product being 3-hydroxy-β–damascone (39). After a further twenty four hours over 70% of allene (43a) had reacted to give the same products in proportions different from those observed after four hours. β–Damascenone (38) and compound (190) were seen in trace amounts in a ratio of 2.8:1. Under these reaction conditions it appears that compound (190) reacted to give β–damascenone (38). Some possible modes of formation of the products are depicted in scheme 21.

Table 4.14 Hydrolysis of Allene (43a) at pH 3.

Conditions[a]	(38)	(190)	(39)	(191)	(58)
24°C, 1 h	none	none	2.0	none	none
4 h[b]	trace	trace	12.5	1.0	1.7
24 h[c]	trace	trace	55.7	5.0	10
50°C, 0.33 h[d]	trace	trace	16.8	trace	5.3
1 h[e]	trace	trace	48.8	trace	10.2
4 h	4.0	none	76.3	trace	19.7
80°C, 0.33 h	4.5	2.1[f]	69.8	none	23.5
1 h	7.6	trace	71.5	none	20.8
4 h	7.5	none	71.1	none	21.3

a pH = 3.0 in 10% aqueous ethanol

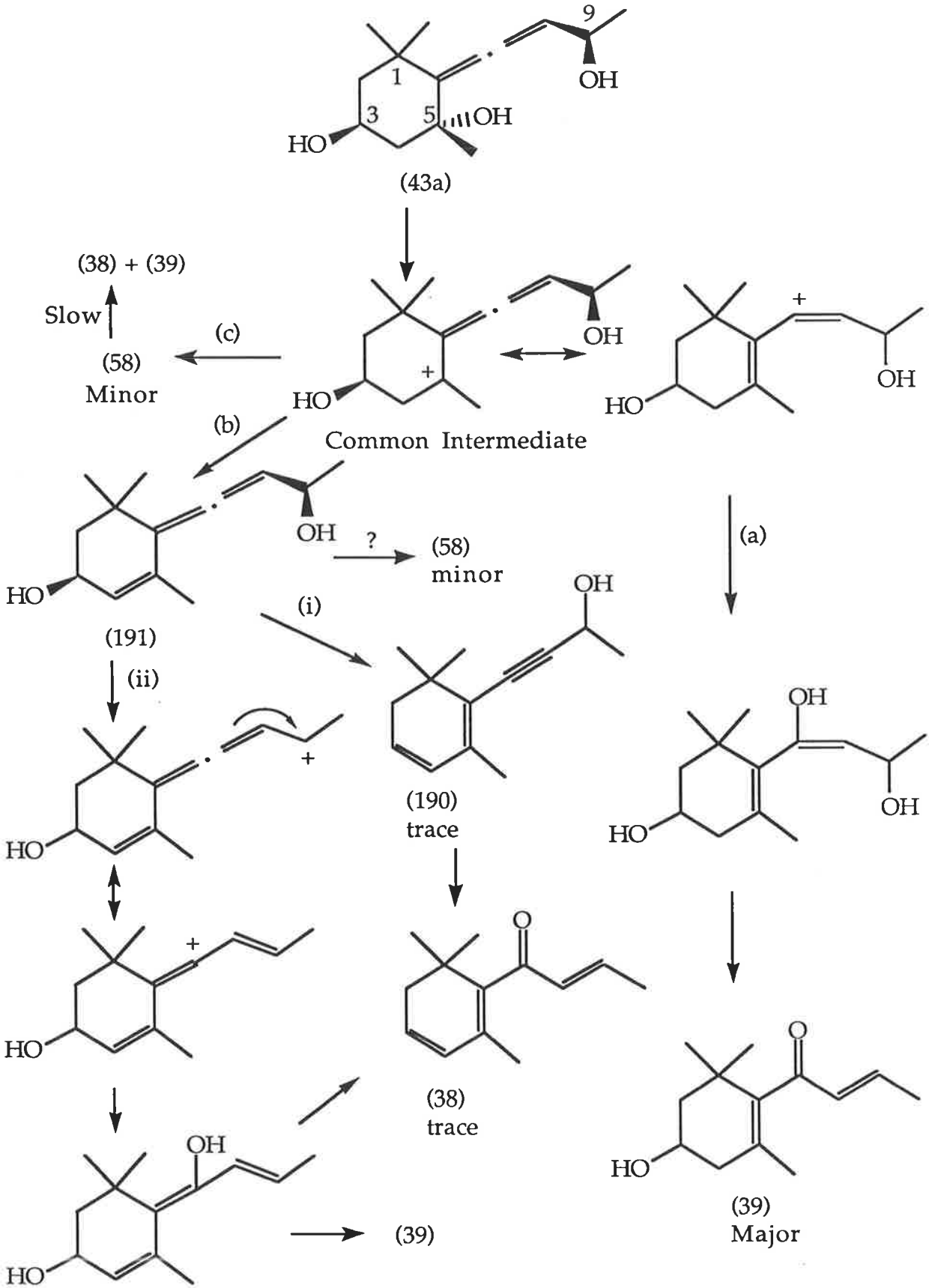
b (38) and (190) occur in the ratio 1:2

c (38) and (190) occur in the ratio 2.8:1

d (38) and (190) occur in the ratio 3:1

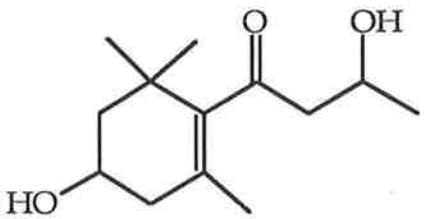
e (38) and (190) occur in the ratio 7:1

f Response ratio of 1 / mg internal standard was used


9
OH
1
3
5
OH
HO
(43a)
(38) + (39)
Slow
(c)
(58)
Minor
OH
+
HO
Common Intermediate
+
OH
HO
(b)
OH
?
(58)
minor
HO
(191)
(a)
(i)
OH
(ii)
+
HO
(190)
trace
OH
OH
HO
+
O
HO
(38)
trace
O
OH
HO
(39)
(39)
Major

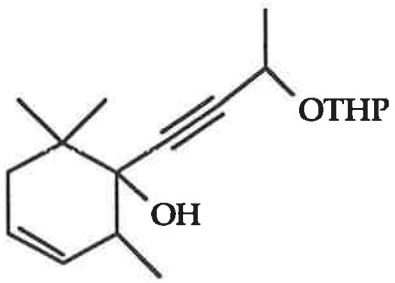
Scheme 21.

This scheme depicts three different pathways (a, b and c) involving a common intermediate ion. Pathway (a) seems to be the favoured route which involves a resonance stabilized carbocation which upon hydration and deprotonation generates the enol form of a β–hydroxy ketone. Elimination of water would then yield the α,β–unsaturated ketone (39). The β-hydroxy ketone (163) was not detected. This indicates that under the conditions used, the equilibrium lies in favour of the formation of α,β–unsaturated ketone (39).



(163)

Pathway (b) can explain the formation of compounds (190) and (191). Loss of an H4 hydrogen gives the allenic diol (191), which can then follow either of the two pathways outlined in scheme 21. Path (i) involves dehydration by the loss of the H8 hydrogen and the allylic hydroxyl group at C3 to give compound (190) which can undergo a Meyer Schuster rearrangement [49] to give β-damascenone (38). In support of this last step Fankauser[154] has shown that compound (194) gives damascenone (38) in 93% yield under acidic conditions.



(194)

The alternative pathway (ii) involves loss of the allylic hydroxyl group at C9, followed by hydration of the resonance stabilized carbocation and deprotonation to generate the enol form of an α,β-unsaturated ketone. This can either lose the allylic hydroxyl group at C3 to give β-damascenone (38) or tautomerize at C4 to give 3-hydroxy-β-damascone (39).
Finally, route (c) involves loss of hydrogen at H8 to give directly compound (58). Compound (58) is known to give β-damascenone (38) and 3-hydroxy-β-damascone (39) but under these reaction conditions this can only occur very slowly (see section 4.3).

4.4.2.2 pH 3, 50°C

When the reaction was carried out at higher temperature (50°C), after a reaction time of one hour only a trace amount of allenic diol (191) was detected. The ratio of β-damascenone (38) to compound (190), after twenty minutes, was 3:1, increasing to 7:1 after one hour. Compound (190) is a conjugated acetylenic alcohol and should be less reactive than the allenic diol (191).
Finally, 4% of β-damascenone (38) was seen after four hours with approximately 20% of compound (58) and approximately 76%of 3-hydroxy-β-damascone (39). The formation of β-damascenone (38) would favour route (b) as it is known that 3-hydroxy-β-damascone (39) and β-damascenone (38) are produced only slowly from compound (58)[18], thereby excluding route (c).

4.4.2.3 pH 3, 80°C

At 80°C more β-damascenone (38) was formed and it appeared that the reaction was complete after one hour. Compound (190) (2.1%) was detected after twenty minutes but this amount decreases substantially as

more β-damascenone (38) was formed. The allenic diol (191) was absent demonstrating that the allenic diol (191) is sensitive to higher temperatures and low pH.

4.4.3 Conclusions and predictions.

From Table 4.13 certain trends are apparent. At low temperature (24°C) the ratio of 3-hydroxy-β-damascone (39) to β-damascenone (38) is large. Also, the ratio of 3-hydroxy-β-damascone (39) to compound (58) is approximately five suggesting pathways (a) and (b) are favoured. At 50°C the ratio of 3-hydroxy-β-damascone (39) to β-damascenone (38) is 19:1 and the ratio (approximately 4:1) of 3-hydroxy-β-damascone (39) to compound (58) shows that more of compound (58) was being formed. At 80°C the ratio of 3-hydroxy-β-damascone (39) to β-damascenone (38) is further reduced to 9:1,.also indicating greater β-damascenone (38) formation. Similarly, the ratio of 3-hydroxy-β-damascone (39) to compound (58) is reduced to 3.2:1. It is known that 3-hydroxy-β-damascone (39) does not give β-damascenone (38)[39] and compound (58) does not give much 3-hydroxy-β-damascone (39) and β-damascenone (38) under the conditions[18] used. Therefore, under these conditions, pathway (c) cannot account for the amounts of 3-hydroxy-β-damascone (39) being formed in the reaction. Little is known about the reactivity of allenic diol (191) with respect to enynediol (58) formation . It appears from these results that there are many pathways leading to the formation of β-damascenone (38).

The β-glucosides of the allenic triol (43a) would be expected to react more slowly than (43a), however the ratio of products can also be affected depending on the pathway the hydrolysis follows. The allenic glucoside at C9 (side chain) would be interesting as it would assist in slowing the ionization reaction at C9, thereby favouring the C9 glucoside of

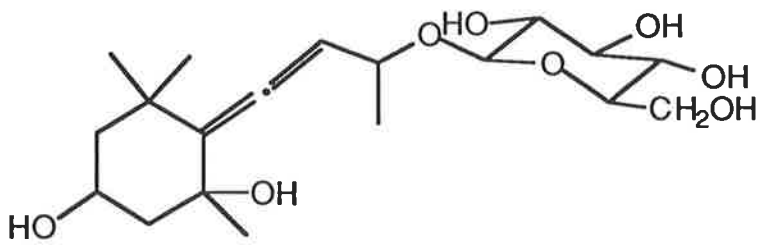
compound (191). Other chemical transformations could then occur which would affect the ratio of products already seen for the allene (43a).

Although the allenic glucoside (43c) was synthesized in this work the low yields and mixture of diastereoisomers prevented its hydrolysis being studied.

From the hydrolytic studies of the glucosides and their alcohols one can predict likely precursors to β-damascenone (38). Work in this thesis demonstrates that glucosides are slower reacting than the corresponding alcohols. The C9 (side chain) glucoside of enynediol (58) was shown to give a slight increase of β-damascenone (38) at 50° after one month compared to its aglycone . If one had an appropriate allylic glucoside such as allenic diol (191) glycosylated at C9 (side chain) then one would expect a higher relative reactivity of the C3 allylic hydroxyl group compared to the glucopyranosyl bond at C9. This would lead to an increase in either β-damascenone (38) or the glucoside of enynediol (58), via route (b) path (i), because, path (ii) would be reduced considerably. Therefore, one can ultimately predict that β-damascenone (38) probably arises from a glycoconjugate of compound (190), with damascenone (38) arising via route (b) path (i).

In addition to our work, it has been shown and postulated that there are many precursors to β-damascenone (38)[155] which contain glycones more complicated than simple glucosides, e.g. disaccharides[155]. Naf[156] has recently reported the isolation of a β-damascenone (38) precursor which was the allenic triol (43e) glycosylated at C9 (side chain). It was reported that the allenic glucoside polyacetate, under acidic conditions, gave both 3-hydroxy-β-damascone (39) and β-damascenone (38). However, no report of the ratios nor of any other hydrolysis products were documented. Finally,

during the conclusion of this work, the allene triol (43a) has been identified in our laboratories as an unknown glycoconjugate in Riesling wine by Marinos.[157]



(43e)

Conclusion

The synthesis of several compounds in this work has led to the identification of the postulated β-damascenone (38) precursors, enyne diol (58a), the allenic triol (43a) (as a glycoconjugate) and the 3-β-D-glucopyranoside of 3-hydroxy-β-damascone (39a) as natural products in grape juice / wine for the first time.

The study has shown that enyne diol (58a), its glucoconjugate (58b) and the allenic triol (43a), indeed give β-damascenone (38) via mild acid hydrolysis but neither 3-hydroxy-β-damascone (39) nor its 3-β-D-glucopyranoside (39a) give this product. The original hypothesis of Ohloff and Isoe receives strong support from this work, i.e. β-damascenone (38) ultimately arises from the degradation of the carotenoid neoxanthin (24) to grasshopper-ketone (31) which is reduced and probably glycosylated to an allenic glucoside. This glucoside can then undergo several chemical transformations which generate β-damascenone (38) by different pathways.

β-Glycosylation under homogeneous conditions using Lewis acids has proved to be an efficient technique for the synthesis of β-glucosides. In particular, the method of Kunz, which involves the use of the ortho ester (140) for achieving stereospecific glycosylation in high yield, was the most effective method, especially for the less reactive secondary alcohols. However this method was only applicable to alcohols stable to boron trifluoride etherate. For acid sensitive alcohols the method of Ackermann was a useful alternative.

This is the first study in which hydrolyses of reactive β-glucosides have been compared to the corresponding aglycones. In each case the β-glucoside hydrolyzed at a slower rate than the aglycone at wine pH. This is contrary to previously held notions that glycosylation of allylic alcohol flavour precursors in wines should facilitate their acid catalysed reactions.

At higher temperatures or for relatively unreactive aglycones little or no differences in rates of hydrolyses were observed. However, reactive alcohols such as geraniol (1) and enyne diol (58a) hydrolyzed approximately ten times faster than their corresponding β-glucosides at lower temperatures. Thus for the multifunctional and highly reactive β-damascenone (38) precursor, allenic triol (43a), which hydrolyzes to give several products via different pathways, glycosylation may greatly reduce the important pathways which give products other than β-damascenone (38). Although the rates of hydrolysis of such glucosides are significantly lower than for the aglycones (possibly orders of magnitudes lower), they may nevertheless yield much greater quantities of β-damascenone (38). Evidence for the comparative stability of glucosides of allenic triol (43a) is the observation of such β-glucosides in a one year old Riesling wine, even though the corresponding aglycone (43a) has a half life of less than 24hours at wine pH. Similarly, increases in the yield of β-damascenone (38) from the enyne diol (58a) hydrolysis resulting from glycosylation at C9 have been demonstrated here. Thus glycosylation of these compounds in the fruit potentially enhances wine flavour. Additionally, because the proportion of β-damascenone (38) is greater at higher temperatures, one could increase the amount of β-damascenone (38) by gentle heating of the juice to speed its formation and increase overall yield.

In the case of geraniol (1), glycosylation is a process that lowers the flavour of the grape juice / wine, not only because geraniol (1) is itself an important flavourant in some wines but because the rate of formation of other flavour compounds from geraniol (1) is also reduced.

5.1 EXPERIMENTAL.Chapter 2.

General

All melting and boiling points are uncorrected. Melting points were determined using Kofler hot-stage apparatus under a Reichert microscope.

Elemental analyses were carried out by the Canadian Microanalytical Service Ltd., New Westminster, Canada.

Infrared Spectra were recorded on a Jasco A-102 spectrophotometer using the 1603 cm^{-1} band of polystyrene as internal reference and as Nujol mulls unless otherwise stated.

NMR Spectroscopy

^{1}H n m r were recorded on either a Jeol JNM-PMX 60 MHz spectrometer, Bruker WP80DS Fourier Transform spectrometer operating at 80 MHz or CXP300 spectrometer operating at 300 MHz. ^{13}C n m r spectra were recorded on the Bruker WP80 DS Fourier Transform operating at 20.1 MHz or CXP300 instrument operating at 75.47 MHz. Chemical shifts have been quoted in parts per million (ppm) downfield from tetramethylsilane.

Multiplicities have been abbreviated to s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad, obs, obscure.

All thin layer chromatography was performed on Merck DC-Alufolien Kieselgel 60 F_{254} Art 5554 or DC Alufolien, Aluminium oxid 150 F_{254} neutral (Type T) Art 5551. T.l.c. plates were developed using either of the following systems only if UV inactive: a) 5% phosphomolybdic acid in ethanol; b) 10% w / v ammonium molybdate in water containing 6% v /v concentrated sulphuric acid in water; c) 0.5% w / v vanillin in ethanol containing 0.5% v /v concentrated sulphuric acid in ethanol and a few drops of glacial acetic acid; d) use of iodine vapour; followed by heating, or detected using an Oliphant UV lamp at 2537A. Column

chromatography was performed on Sorbsil or Merck silica gel 60, (70-230 mesh) Art 7734. Flash chromatography was performed on Amicon Matrex Silica Si medium Pore Dia. 60Å, particle size 50 m). Dry column chromatography was performed on Merck Kieselgel 60 PF_{254} gipshaltig, Art 7749, unless stated otherwise.

CHAPTER 2

GC / MS was performed on a Finnigan 4000 gas chromatograph / mass spectrometer using the following column QSBP10, I.D. 0.33mm, O.D. 0.45mm, length 25m, film thickness 0.5microns operating at 70eV. Standard program run was 100° (1min)–250° @ 4° / min, split injection, unless otherwise stated.

4-Bromo-3,5,5-trimethyl-2-cyclohexen-1-one [4-Bromoisophorone (69)].

This compound was prepared in 50% yield by the method of Marx.[61] ^1H n m r (60MHz / $CDCl_3$); δ: 5.87, s, 1H, H2; 4.33, s, 1H, H4; ABq, (2.63, 2.12), J = 16Hz, 2H, H6; 2.13, s, 3H ; 1.27, s, 3H; 1.16, s, 3H. m p 55-57°C.

3,5,5-Trimethyl-3-cyclohexen-1-one (61).

This compound was prepared in 65% yield according to the known method of Meinwald[55], however reproducibility was poor. Compound (61) was synthesized in consistent yield by the method of Marx.[61,62] Reductive elimination of 4-bromoisophorone yielded β-phorone (61) after fractional distillation in 45%yield. b p : 52-54° / 4.5 mm. Rf=0.34 50% ether / petroleum ether. ^1H n m r (60MHz / $CDCl_3$); δ: 5.35, m, 1H; 2.67, m, 2H; 2.27, S, 2H; 1.67, s, 3H; 1.02, s, 6H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 209.31, C1; 132.1 C4; 128.8, C3; 52.8, C2; 43.2, C6; 35.9, C5; 29.1, C5-Me; 22.0, C3-Me. m/z 138 (42), 123 (50), 96 (88), 95 (73), 82 (24), 81 (100), 79 (21), 77 (14), 67 (36), 55 (29), 53 (25), 41 (67). ν_{max}; 3500, 2950-2850, 1720, 1670, cm^{-1}.

For the synthesis of β-phorone (61), several procedures were investigated. The compounds characterized included (62) and (63), identified by Babler.[53] The structure of compound (64) was confirmed by
^{1}H n m r. (60MHz / $CDCl_3$); δ: 5.26, br m, W1/2 5Hz, 1H; 1.67, br s, 5H; 1.55, brs, 2H; 1.23, s, 3H; 1.03, s, 3H; 0.95, s, 3H. ν_{max}; 3350, 1670, 1370, 1185 cm $^{-1}$.
Compounds (65) and (66) had been characterized by Meinwald[55] and compound (67) was obtained as the major product from the method of Krafft.[56] Confirmation of structure was obtained from the ^{1}H n m r spectrum. (60MHz / $CDCl_3$); δ: 5.6, br,1H; 4.6, br, 2H; 2.15-1.88, m, 4H; 0.98, s, 3H; 0.97,s,3H; 0.95, s, 3H.
Compound (68) was also isolated; it had been characterized by Shiloff[59] in earlier studies.

3,4-Epoxy-3,5,5-trimethylcyclohexan-1-one (70)[63].

m-Chloroperoxybenzoic acid (200 mg; 1.15 mmol) in ether (10ml) was added to β-phorone (61) (76 mg; 0.55mmol) at 5° and the mixture allowed to stir overnight at 5°. After 19h the solution was washed with saturated sodium metabisulphite solution (2 x 20 ml); saturated sodium hydrogen carbonate solution (2 x 20 ml), water (1 x 50 ml) and brine (1 x 20ml). After the ethereal layer was dried (MgSO4) evaporation gave (70) as a white solid (70mg; 83%). Rf = 0.35, 50% ether / petroleum ether. ^{1}H n m r (300 MHz / $CDCl_3$); δ: 2.9, s, H; ABq, 2H, (2.77, 2.51); ABq, (2.36, 1.96), 2H; 1.4, s, 3H; 1.2, s, 3H; 1.05, s, 3H. ^{13}C n.m.r (75.47 MHz / $CDCl_3$); δ: 207.6, s; 67.1, d; 57.1, s; 48.3, t; 42.4, t; 33.2, s; 27.4, q; 24.6, q; 22.7, q. m/z; 154 (<1), 139 (1), 112 (10), 97 (8), 85 (5), 72 (11), 69 (31), 56 (19), 43 (100), 41 (56), 39 (39).

4-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (71)

The following modification of the published procedure[63] was used. m-Chloroperoxybenzoic acid (18.6g, 1.07 mol, 85%) was added to a cooled solution of β-phorone (61) (10g, 64.9 mmol) in chloroform (240 ml) and the temperature maintained at 5° overnight. The resultant solution from which m-chlorobenzoic acid precipitated, was filtered and the filtrate was evaporated under reduced pressure. Petroleum ether was added to the residue, another crop of crystals was removed by filtration, and the filtrate was evaporated. The product was dissolved in ether (40ml) and water (40ml) and the pH adjusted to 11-12 by the addition of sodium hydroxide. The mixture was allowed to stir for 1h at room temperature and then worked up in the usual manner. However, it was found that compound (71) was reasonably water soluble and it was necessary to saturate the aqueous phase with sodium chloride. Rf=0.06, 50% ether / petroleum ether.

^{1}H n m r (60 MHz / $CDCl_3$); δ: 5.9, br, 1H; 4.08, br, 1H; 3.8, br, 1H; OH; ABq, (2.43, 2.23), *J* =16Hz, 2H; 2.15, S 3H; 1.2, S, 3H; 1.13, S, 3H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 200.9; 163.7; 126.5; 77.0; 49.6; 39.1; 27.5; 22.2; 22.0. m/z; 154 (1),112 (33), 98 (99), 70 (72), 69 (57), 55 (18), 53 (10), 43 (38), 42 (93), 41 (100), 40 (19), 39 (79). ν_{max}; 3400, 1660, 1640 cm^{-1}.

2,2,6-Trimethylcyclohexane-1,4-dione (72).

This compound was prepared using a known method[63], except that the reaction time was shortened to 2h. Progress was monitored by t.l.c. Yield (83%). Rf=0.22, 50% ether / petroleum ether.

^{1}H n m r (80 MHz / $CDCl_3$); δ: 3.3-2.3, m, 5H; 1.15, d, *J* = 6Hz, 3H; 1.22, S, 3H; 1.12, s, 3H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 214, s, ; 207.7, s; 52.3, d; 44.6, s; 43.8, t; 39.5, t; 26.1, q; 25.1, q; 14.2, q. ν_{max}; 3400, 1725, 1705 cm^{-1}. m/z; 154

(4), 139 (10), 70 (16), 69 (17), 56 (75), 55 (14), 43 (8), 42 (100), 41 (78), 40 (10), 39 (44).

4,4-Ethylenedioxy-2,2,6-trimethylcyclohexan-1-one (73)[64].

A mixture of compound (72) (13.5, 87.7 mmol), ethylene glycol (7.14g, 115.2 mmol) and p-toluenesulphonic acid (75mg) in benzene (40ml) was refluxed using a Dean and Stark apparatus for the removal of water. After 2.5h, ether (100ml) was added to the benzene solution and the mixture was washed with sodium hydrogen carbonate solution (5%, 20ml) and then with water (10ml). After drying ($MgSO_4$), evaporation yielded a yellow oil (16.8g). Distillation at 50-60° / 0.05mm afforded compound (73) (11.4g; 66%) and a second fraction (4.8g) which was a mixture of acetals with starting material. Separation of acetals from starting material was achieved via dry column chromatography[158] using a solvent gradient of ethyl acetate / petroleum ether. This gave an additional 2g of (73). (Total yield, 13.4g; 77%). However, GC / MS revealed a minor amount of 4,4-ethylenedioxy-3,3,5- trimethylcyclohexan-1-one (75) and the bis acetal, 2,2,5,5- bis-ethylenedioxy-1,1,3-trimethylcyclohexane (74) which could not be separated by distillation. Separation was achieved by adoption of the bisulphite addition method.[65] A ten fold excess of sodium meta bisulphite in 30% aqueous methylated spirits was added to the mixture [(73-75)] and the mixture was stirred for 2h. The dichloromethane extract (2x20ml) was dried ($MgSO_4$) and evaporated to yield a mixture of the two acetals (73) and (74). Separation of compound (73) (b p 50° / 0.05mm) from (74) was achieved by fractional distillation. The aqueous layer was treated with sodium hydroxide and adjusted to pH 11-12. Extraction with dichloromethane, dried and evaporated yielded compound (75).

Compound (73) Rf=0.41, 50% ether / petroleum ether.

^1H n m r (60 MHz / $CDCl_3$); δ: 3.95, m, 4H; 3.35-2.12, m, 1H; 1.88, br, 4H; 1.27, s, 3H; 1.05, s, 3H; 0.97, d, 3H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 215.5, s; 106.9,

s; 64.2, t; 63.5, t; 47.4, t, 43.7, t; 43.0, s; 37.0, d; 26.6, q; 25.7, q; 14.1, q. ν_{max}; 1715, 1100 cm^{-1}. m/z; 198 (<1), 127 (46), 114 (23), 113 (72), 99 (66), 69 (27), 56 (28), 55 (53), 41 (100).

2,2,5,5-Bis-ethylenedioxy-1,1,3-trimethylcyclohexane (74).

Rf=0.52, 50% ether / petroleum ether.

^1H n m r (60MHz / $CDCl_3$); δ: 4.17-3.67,m, 8H; 2.03-1.43, m, 4H; 1.3-1.0, m, 4H; 1.0-0.83, m, 6H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 114.7, C2; 108.5, C5; 67.1; 64.4, 63.5; 45.4, C3; 41.2, C4; 40.8, C1; 34.3, C6; 25.2; 24.1; 14.7. ν_{max}; 2950, 2860, 1710, 1085 cm^{-1}. m/z; 242 (<1); 129 (100), 114 (15), 113 (10), 100 (33), 99 (25), 69 (13), 55 (27), 41 (65).

Note: Compound (74) was also synthesized by treatment of compound (72) with an excess of ethylene glycol under reflux for 40 h using a Dean and Stark apparatus for removal of water, followed by a standard work up.

4,4-Ethylenedioxy-3,3,5-trimethylcyclohexan-1-one (75).

A solution of bis acetal (74) (3.5g, 14.5 mmol) in methanol (25ml) was stirred with sulphuric acid (10ml, 0.25 M) for 22h at room temperature. The solution was extracted with dichloromethane (2 x 20 ml) and the dichloromethane was then washed with saturated sodium hydrogen carbonate solution (2 x 20ml) and brine (1 x 20 ml). The solution was dried ($MgSO_4$) and evaporated to yield oil (75) (2.64g). Distillation afforded a colourless liquid, b.p. 50° / 0.04mm. (2.45g, 86%). (Found : C, 66.3; H, 8.8. $C_{11}H_{18}O_3$ requires C, 66.6, H, 9.2%). Rf=0.38, 50% ether / petroleum ether.

^1H n m r (300 MHz / $CDCl_3$); δ: 4.17-4.10, m, 4H; ABq (2.71 H axial, 2.02 H equatorial), J = 13.8, 2.8Hz, 2H; 2.4-2.2, m, 3H; 1.04, s, 3H; 0.96, s, 3H; 0.95, d, J =7.5Hz, 3H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 209.4, s; 113.0, s; 67.0, t; 52.0, t; 46.3, t; 42.7, s; 36.2, d; 23.6, q; 14.4, q. ν_{max}; 1718, 1095 cm^{-1}. m/z; 198

(<1), 183 (1), 114 (19), 113 (65), 99 (59), 69 (17), 56 (18), 55 (34), 43 (20), 42 (34), 41 (100).

(*5S',6R',9R'*)-3,3-Ethylenedioxy-7-megastigmyne-6,9-diol (76).

This compound was prepared according to a known method.[52] Separation of the bis acetal (74) from the crude mixture (18.2g) was achieved via column chromatography (Sorbsil), using ether / dichloromethane gradient. Isolation gave (76) (17.0g, 80%) and also the bis acetal (74) (1.0g). GC / MS of (76) revealed a pair of diastereoisomers in a ratio of approximately 9:1. Rf 0.24, 50% ethylacetate / petroleum ether.0.47, 80%
Major diastereoisomer (76a), ^{1}H n.m.r (300 MHz / $CDCl_3$); δ: 4.51, q, *J* =6.7Hz; 3.92-3.77, m, 4H; 2.5-2.0, br, 2H; 1.8-1.5, m, 5H; 1.4, d, *J* =7Hz, 3H; 1.27-1.0, br, 6H; 1.03, d, *J* =5Hz, 3H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 108.2, s; 89.6, s; 83.0, s; 77.7, s; 64.4, t; 63.3, t; 57.8, q; 45.3, t; 41.4, t; 39.3, s; 34.4, d; 27.1, q; 24.4, q; 20.9, q; 15.9, q. ν_{max}; 3400, 1090 cm^{-1}. m/z; 129 (14), 127 (22), 113 (74), 99 (8), 87 (35), 86 (42), 69 (19), 55 (25), 53 (16), 45 (20), 43 (100).
Minor diastereoisomer (76b), m/z; 29 (10), 127 (23), 113 (85), 99 (5), 87 (40), 86 (49), 69 (17), 55 (25), 53 (15), 45 (20), 43 (100).

4,4-Ethylenedioxy-1-[3'-hydroxybut-1'-ynyl]-3,3,5-trimethylcyclohexan-1-ol (77).

This compound was prepared in 90% yield by the method described by Loeber[52] from ethylmagnesium bromide and 4,4-ethylenedioxy-3,3,5-trimethylcyclohexanone (75). Rf = 0.19, 70% ether / petroleum ether GC / MS analysis revealed two diastereoisomers in an approximate ratio of 1:9.
^{1}H n m r (60 MHz / $CDCl_3$); δ: 4.45,q, *J* =7Hz, 1H; 4.0, s,4H; 1.4, d, *J* =7Hz,3H; 1.22,s,3H; 107-0.6,m,6H. Major diastereoisomer (77), m/z; 207 (<1), 115 (2), 114 (25), 113 (58), 101 (4), 100 (100), 99 (30), 69 (12), 56 (12), 55 (20), 53 (13), 45 (16), 43 (57), 41 (53), 39 (16). Minor diastereoisomer (77a), m/z; 115 (5),

114 (26), 113 (46), 112 (6), 101 (9) 100 (100), 99 (32), 91 (9), 79 (8), 77 (9), 69 (15), 67 (6), 57 (7), 56 (16), 55 (23), 53 (16), 51 (11), 45 (19), 43 (68), 41 (53), 39 (23).

3,3-Ethylenedioxy-6-hydroxy-7-megastigmyn-9-one (78).

A mixture of compound (23a) (230 mg, 0.75 mmol), manganese dioxide[71] (600 mg, Activity A) in benzene (10 ml) was refluxed and reaction progress was monitored by t.l.c. The reaction was found to be complete after 50 min. The reaction mixture was cooled and filtered through a sintered glass funnel partially filled with $MgSO_4$ (anhydrous). The magnesium sulphate was then washed with ethyl acetate (2 x 50 ml) and the organic layers were combined and evaporated to yield a yellow oil (25) (180 mg, 79%). The yellow oil was further purified by dry column chromatography[158] using ethyl acetate / petroleum ether gradient to remove any trace amounts of starting material. The product (78) (120 mg, 53%) was obtained. (Found : C, 67.6; H, 8.5. $C_{15}H_{22}O_3$ requires C, 67.7; H, 8.3%). Rf 0.58, 70% ethyl acetate / petroleum ether. ^{1}H n m r (60 MHz / CDCl3); δ: 3.95, m, 4H; 2.4, S, 3H; 2.2-1.4, m, 5H; 1.2, S, 6H; 1.05, d, 3H. ^{13}C n m r (75.47MHz / $CDCl_3$); δ: 184.0; 107.6; 91.5; 86.8; 78.2; 64.5; 63.5; 45.3; 41.3; 39.6; 34.9; 32.8; 27.1; 21.0; 15.8. ν_{max}; 3450, 1660, 1210 cm^{-1}. m/z; 251 (<1), 221 (<1), 210 (<1), 195 (<1), 184 (<1), 165 (1), 127 (22), 113 (86), 87 (32), 86 (100), 69 (15), 55 (16), 53 (18), 43 (81).
The above synthesis was also performed on the diastereoisomeric mixture (76a,b). Two isomeric products were observed by GC / MS in a ratio of 9:1 confirming an axial preference for the Grignard addition. Minor diastereoisomer (78); m/z; 127 (27), 113 (100), 87 (10), 86 (100), 43 (72), 42 (20), 41(37).

(*5S'6R'9R'*)-6,9-Dihydroxy-7-megastigmyn-3-one (79).

A solution of (76) (9.4g, 35.1 mmol) in methanol (80ml) was stirred at room temperature with sulphuric acid (8ml, 0.25M). The reaction was monitored by

gas chromatography (5% 0V-17 column, 200°, Isothermal). After 6h 83% conversion was observed and complete conversion after 22h. GC/MS revealed only one isomer. Dichloromethane (100ml) was added and the solution was washed with saturated sodium hydrogen carbonate solution (2 x 30ml). The organic layer was separated, dried ($MgSO_4$) and evaporated to give a liquid (7.8g, 99%). On treatment with hot dichloromethane / petroleum ether (bp 65-69°), some of the dihydroxyketone (79) crystallized (2.28g, 29%) as colourless cubes. The mother liquors were evaporated to give a liquid fraction of the dihydroxyketone (76) (5.52g, 71%). This oil is composed primarily of one product with the same ^1H n m r spectrum as that of the solid fraction (76). Rf=0.47, 80% ethyl acetate / petroleum ether. m p. 132-134°. ^1H n m r (300 MHz / $CDCl_3$); δ: 4.66, q, J = 6.8 Hz, 1H; ABq (2.67, H2 axial, 2.09, H2 equatorial) 2H; 2.4-2.2, m, 3H; 1.50, d, 3H, J =6.8Hz, 1.20, s, 3H; 1.14,d, J = 6Hz, 3H; 0.97, s, 3H. ^{13}C n m r .(20.1 MHz / $CDCl_3$); δ: 209.5, s; 90.4, s; 82.4, s; 76.8, s; 58.3, d; 52.9, t; 47.0, t; 42.0, s; 37.3, d; 25.8, q; 24.6, q; 20.7, q; 16.5, q. ν_{max}; 3300, 1718, 1075 cm^{-1}. m/z; 150 (8), 126 (17), 111 (16), 83 (12), 80 (27),79 (13), 69 (12), 56 (10), 55 (26), 53 (21), 43 (100).

(*3S'5S'6R'9R'*)-1-[3'-hydroxybut-1'ynyl]-2,2,6-trimethylcyclohexane-1,4-diol (55a).

This compound was prepared according to the known method.[52] The reduction was performed on the recrystallized ketone (79) and the crude ketone (79), before any recrystallization (i.e. the mixture of diastereoisomers).
a) Reduction of the recrystallized ketone (79), m.p. 132-134°. This yielded a white solid in 98% yield, (2.28g (79) gave 2.3g (55a)). GC / MS analysis revealed two diastereoisomers, in a ratio of 95:5. Crystallization from $CHCl_3$ gave a sample of (55a) with m p 145-147°. X-ray studies showed the stereochemistry of the solid triol to have the relative configuration shown in (55a). (Found : C, 69.0; H, 9.7. $C_{13}H_{22}O_3$ requires C, 69.0; H, 9.8%). Major

component Rf=0.22 70% ethylacetate / petroleum ether. Minor component Rf=0.18.

b) Reduction of crude dihydroxyketone (79). The dihydroxyketone (79) (2.0g, 8.9 mmol) gave triols (55) 2.0g, 99%). GC / MS revealed 4 diastereoisomers, with one major isomer present (>90%).The major peak has the same retention time as that obtained in (a). Recrystallization of the crude triols from ethyl acetate yielded a solid triol (55b) of m p 118-120°. Solid triol (55b) has the same retention time and ^{1}H n m r spectrum as that of the X-ray triol (55a) and is presumably epimeric at C9 with the relative configuration of (*3S',5S',6R',9S''*). Further recrystallization ($CHCl_3$) yielded a higher m p triol analogous to the X-ray triol.

Triol (55a) and (55b) ^{1}H n m r (300 MHz / d_6 Acetone / D_2O); δ: 4.47, q, 1H; 3.91-3.88, m, 1H; 2.26-2.17, m, 1H; 1.68-1.49, m, 4H; 1.34, d, *J* =6.7Hz, 3H; 1.17, s, 3H; 1.01, s, 3H; 0.98, d, *J* =6.7Hz, 3H. ^{13}C n m r (20.1 MHz / D_2O); δ: 91.7, s; 85.6, s; 81.7, s; 69.2, d; 60.5, d; 46.0, t; 41.6, t; 41.0, s; 34.3, d; 29.5, q,; 26.1, q; 25.1, q; 16.3, q. Tertiary butanol was used as an external reference.

m/z; In order of elution by GC / MS (a): 208 (2), 152 (6), 126 (14), 122 (22), 111 (11), 95 (5), 82 (12), 80 (13), 69 (12), 55 (23), 43 (100).

(b): X-ray solid triol (55a) and epimer at C9 (55b): 226 (<1) 208 (6), 152 (14), 126 (22), 122 (28), 111 (17), 95 (8), 83 (11), 82 (20), 80 (20), 79 (16), 71 (11), 69 (9), 57 (9), 55 (24), 53 (16), 45 (16), 43 (100).

(c): 208 (<1), 152 (8), 140 (9), 126 (20), 122 (47), 111 (17), 95 (7),82 (14), 80 (20), 69 (19), 55 (24), 43 (100).

(d): 208 (1), 150 (13), 135 (6), 126 (48), 122 (25), 111 (25), 82 (19), 80 (34), 69 (15), 55 (12), 43 (100).

(*S*)-(-)-3-Butyn-2-ol (80a)

This compound was synthesized by the method of Weidmann.[74]

$[\alpha]^{22}_D$ =-52.4°.(c 3.8, Dioxane). Lit.[75] $[\alpha]^{22}_D$ = -51.8° (c 3.8, Dioxane).

(*5R,6S,9S*)-6,9-Dihydroxy-7-megastigmyn-3-one (79a)

Compound (79a) was synthesized according to the procedure described above for ketone (79). However, no crystallization could be induced using hot dichloromethane / petroleum ether. Because further purification by chromatography[86] and attempted crystallization failed, this approach to the preparation of an optically pure analogous to the X-ray triol, was discontinued.

1-Methyl-2-propynyl 3,5-Dinitrobenzoate (81)

Under anhydrous conditions and a nitrogen atmosphere, a solution of 3,5-dinitrobenzoyl chloride (1.65g, 7.1mmol) in chloroform (20ml), pyridine (0.58ml) was added to a solution of 3-butyn-2-ol (0.5g, 7.1mmol) in chloroform (70ml) at 20°C. The mixture was allowed to stand for 2days. The organic layer was washed with hydrochloric acid (2.5M, 10ml), sodium hydrogen carbonate solution (2 x 10ml), water (10ml), brine (10ml), then dried ($MgSO_4$) and evaporated to yield a yellow oil 1.4g (74%). On standing the oil crystallized. m p 98-102° ^{1}H n m r (60MHz / $CDCl_3$); δ: 9.4, s, 3H; 5.9, dq, *J* =2Hz, 6.5Hz, 1H; 2.75, d, *J* = 2Hz, 1H; 1.85, d, *J* = 6.5Hz, 3H. m/z; 264 (26), 249 (5), 219 (8), 196 (19), 195 (100), 189 (4), 179 (3), 166 (9), 149 (34), 145 (2), 119 (3), 103 (9), 91 (2), 76 (3), 75 (44), 74 (20), 73 (4), 69 (21), 63 (4), 53 (63), 52 (23), 51 (8). ν_{max}; 3200, 3000, 1710, 1250 cm^{-1}.

1-Methyl-2-propynyl Benzoate (82)

This was made according to the method used for the preparation of compound (81). Yield 98%, as a clear oil which crystallized on standing.
^{1}H n m r (60MHz / $CDCl_3$); δ: 8.2, m, 2H; 7.6, m, 3H; 5.9, dq, *J* =2Hz, 6.5Hz, 1H; 2.5, d, *J* = 2Hz, 1H; 1.61, d, *J* = 6.5Hz, 3H. m p 122-123°. m/z; 174 (15), 129 (5), 123 (5), 122 (5), 106 (10), 105 (100), 78 (4), 77 (24), 53 (12), 51 (9). ν_{max}; 3250, 1715, 1660, 1360, 1245 cm^{-1}.

Synthesis of compounds (81) and (82) for h.p.l.c. analysis on a chiral column proved unsuccessful in separation of enantiomers.

1'-Methyl-2'-propynyl 2-Methoxy-2-phenyl-3,3,3-trifluoropropionate (83)

Under anhydrous conditions and a nitrogen atmosphere, a solution of α-methoxy-α-trifluoromethylphenylacetyl chloride (88.4mg, 0.35mmol), dry pyridine (1ml) and carbon tetrachloride (1ml) was added to 3-butyn-2-ol (20mg, 0.29mmol). The mixture was stirred at room temperature overnight. (Crystalline pyridinium hydrochloride separated). The reaction mixture was diluted with carbon tetrachloride (10ml) which was washed with cold water (1ml), saturated copper sulphate (1ml), water (2ml), dried ($MgSO_4$) and evaporated to yield a clear oil. (99%). ^1H n m r (300MHz / $CDCl_3$); δ:7.54, bs, 2H; 7.41, bs, 3H; 5.6, m, 1H; 3.58, s, 3H; 2.54, d, J = 2Hz, 1H; 2.49, d, J = 2Hz, 1H, 1.60, d, J = 6.8Hz, 3H; 1.52, d, J = 6.7Hz, 3H. ^{19}F (282.4MHz / $CDCl_3$); δ; 4.91,s,3F; 4.65, s, 3F. m/z; Diastereoisomer 1: 286 (<1), 219 (<1), 190 (9), 189 (100), 141 (4), 139 (7), 127 (6), 119 (12), 105 (22), 91 (6), 77 (12), 69 (7), 53 (16), 51 (4). Diastereoisomer 2: 286 (<1), 219 (<1), 190 (9), 189 (100), 141 (4), 139 (7), 127 (6), 119 (13), 105 (22), 91 (7), 77 (12), 69 (7), 53 (16), 51 (4). ν_{max}; 3230, 1735, 1240, 1100, 1000 cm^{-1}.

(*3S',5S',6R',9R'*)-6-hydroxy-7-Megastigyne-3,9-diyl diacetate (84)[52]

This compound was prepared according to the known method[52], using triol (55a), but a slight modification in the work-up was made. The product was poured onto water and extracted with ether. The ethereal layer was successively washed with saturated copper sulphate solution (blue colour turning violet as it complexes with pyridine) until the blue colour persisted. The ethereal layer was washed with water (2 x 20ml), dried ($MgSO_4$) and

evaporated to yield a yellow oil. This oil was chromotagraphed by dry column chromatography[158] using a solvent gradient (ethyl acetate / petroleum ether). Two fractions of different Rf values were obtained. The major fraction [higher Rf, 0.36, 40% ether / petroleum ether] was the diacetate (84). The lower Rf product [0.13, same solvent system] was the monoacetate, (*3S',5S',6R',9R'*)-3,6-dihydroxy-7-Megastigyn-9-yl acetate (85). The triol (55a) (4.8g, 21.2 mmol) yielded diacetate (86) (5.1g, 78%) and monoacetate (85) as a solid (100mg). When triol (55b) was utilized, a solid diacetate was obtained.
^{1}H n m r (300MHz / $CDCl_3$); δ: 5.5-5.4, m, 1H; 5.0-4.9, m, 1H; 2.07, s, 3H; 2.04, s, 3H; 1.81-1.65, m, 4H; 1.5; d, *J* =6.8 Hz, 3H; 1.12, s, 3H; 1.07, s, 3H; 1.03, d, *J* =6.1Hz, 3H. ^{13}C n m r (20.1 MHz / $CDCl_3$); δ: 170.2; 169.6; 85.4; 84.2; 77.4; 69.3; 60.0; 40.6; 38.3; 36.4; 32.1; 26.6; 21.9; 20.9; 20.4; 15.4; 13.7. m/z; 310 (<1); 250 (2), 208 (4), 190 (4), 175 (5), 168 (7), 152 (16), 134 (8), 126 (9), 108 (14), 91 (7), 82 (23), 69 (11), 55 (18), 43 (100).
^{1}H n m r monoacetate (300 MHz / $CDCl_3$); δ: 5.44, q, *J* =6.5 Hz, 1H; 4.04-4.0, br, 1H; 2.4-2.25, m, 1H; 2.17, s, 1H; 2.06, S, 3H; 1.8-1.52, m, 4H; 1.49, d, *J* =6.5Hz, 3H; 1.21, s, 3H; 1.07, s, 3H; 1.04, d, *J* =6.8Hz, 3H. m p 88-94°; ν_{max} 3500, 1740 cm^{-1}.

(1S',3'R')-4-[3'-acetoxybut-1'-ynyl]-3,5,5-trimethyl-3-cyclohexenyl Acetate (86)[52].

Diacetate (84) (610mg, 1.97mmol) in benzene (40ml) was treated with N,N,N-triethylammonio-N'-methoxycarbonylsulphamidate (97)[83] (1.05g, 4.4mmol) in benzene (40ml) at reflux for 30 minutes. The solvent was evaporated and the resultant yellow oil was chromatographed directly to yield a clear oil (311mg) 54%, bp. 105-110° / 0.03 mm (block). Rf = 0.47, 50% ether / petroleum ether. ^{1}H n m r (60MHz / $CDCl_3$); δ: 5.4, q, *J* =7Hz, 1H; 5.0, br, 1H; 2.07, S, 3H; 2.03, S, 3H; 1.9, S, 3H;1.53, d, *J* =7Hz, 3H; 1.17, S, 6H; 2.5-1.0, m, approximately 22H.

^{13}C n m r (20.1 MHz/$CDCl_3$); δ: 170.3, 169.6, 137.7, 122.9, 91.8, 82.1, 77.0, 75.4, 67.6, 41.9, 36.9, 35.5, 29.5, 28.1, 21.7, 21.2, 20.8. m/z; 232 (6), 189 (6), 175 (18), 157 (26), 142 (8), 131 (5), 115 (3), 105 (5), 91 (7), 77 (7), 69 (5), 55 (6), 43 (100). ν_{max}; 1741, 1631 cm^{-1}.

Other methods which were used to dehydrate the tertiary alcohol were not as successful. Compound (87) was seen as part of a mixture when attempting mesylation by ^1H n m r. Compound (88) was obtained as a diastereoisomeric pair with compound (86). [(86) 14%, (89) 86%]. Compound (88) ^1H n m r (60MHz / $CDCl_3$); δ: 5.5, m, 1H; 5.0, 1H; 2.0, s, 3H; 0.93, s, 3H; 1.3, d, *J* =6Hz, 3H; 1.2-0.9, m, 14H. m/z; Diastereoisomer 1: 228 (0.8), 226 (2.9), 211 (0.7), 193 (0.6), 191 (1.0), 187 (0.5), 185 (2.1), 145 (0.8), 105 (2.2), 91 (3.9), 82 (4.7), 81 (2.0), 56 (1.0), 50 (10), 44 (2.0), 43 (100). Diastereoisomer 2: 228 (1.2), 226 (5.0), 211 (2.0), 193 (0.7), 185 (1.0), 145 (0.9), 105 (2.4), 91 (3.8), 82 (3.2), 81 (2.1), 56 (1.0), 50 (6.1), 44 (2.4), 43 (100). ν_{max}; 1960, 1740, 1240, 735 cm^{-1}. Compound (89) was obtained in trace amounts as a mixture by ^1H n m r.

1-[3'-Acetoxybut-1'-ynyl]-2-methylcyclohexyl Acetate (91).

Under a nitrogen atmosphere 1-[3'-acetoxy-but-1'-ynyl]-2-methylcyclohexan-1-ol (90) (200 mg, 0.9 mm) was stirred in acetic anhydride (0.4 ml), triethylamine (0.19 ml) and 4-dimethylaminopyridine (11 mg) at room temperature. Progress of the reaction was monitored by t.l.c. and found to be complete after 63 h. The solution was taken up in ether (10 ml), washed with brine (50%, 1 x 5 ml) then water (1 x 5 ml) and dried ($MgSO_4$). Evaporation of the solvent yielded a crude oil (210 mg, 81%) of compound (91). ^1H n m r (60 MHz / $CDCl_3$); δ: 5.4, q, *J* =8Hz, H; 2.0, s, 3H; 1.97, s, 3H; 1.5, d, *J* =6Hz, 3H; 1.0, d, *J* =8Hz, 3H. m/z; 207 (15), 191 (5), 182 (73), 167 (16), 164 (78), 149 (100), 147 (18), 135 (10), 135 (17), 123 (7), 122 (8), 121 (14), 107 (14), 97 (12), 79 (14), 69 (23), 55 (10). ν_{max}; 1740, 1220 cm^{-1}.

3,9-Diacetoxy-7-megastigmyn-6-yl Ethyl Carbonate (92)[81].

Under anhydrous conditions and a nitrogen atmosphere n-butyllithium (1.6M, 528µl) was added dropwise at -10° to a solution of diacetate (84) (200mg, 0.65mmol) in tetrahydrofuran (10ml). Ethyl chloroformate (94µl) was then added and the reaction mixture allowed to attain room temperature slowly. The reaction mixture was then treated with saturated ammonium chloride solution (3ml), and the organic layer was then washed with water (10ml), brine (10ml), dried ($MgSO_4$) and evaporated to yield a clear oil (91%). Rf 0.7, 70% Ethyl acetate / Petroleum ether.

^{1}H n m r (60MHz / $CDCl_3$); δ: 5.48, q, *J* =6Hz, 1H; 5.0, m, 1H; 4.17, q, *J* = 6Hz, 2H; 2.1, s, 3H; 2.07, s, 3H; 1.77, b, 2H; 1.52, d, *J* =6Hz, 3H; 1.3, s, 3H; 1.17, s, 3H; 1.1, t, *J* =6Hz, 3H; 1.05, d, 3H. ν_{max}; 2240, 1730, 1440, 1240 cm^{-1}.

(E)-7-Oxo-5-8-megastigmadien-3yl Acetate (93)[156]

This compound was obtained from acid hydrolysis of carbonate (92) in acetic acid.

^{1}H n m r (60MHz / $CDCl_3$); δ: 6.72, dd, *J* =15Hz, 7Hz, 1H; 6.16, d, *J* =15Hz, 1H; 5.2, m, ; 2.1, s, 3H; 1.92, dd, *J* =7Hz, 2Hz, 3H; 1.54, s, 3H; 1.18, s, 3H; 1.01, s, 3H. m/z; 250 (<1), 208 (<1), 190 (28), 175 (8), 149 (5), 133 (5), 122 (10), 121 (100), 120 (18), 118 (7), 105 (12), 91 (9), 78 (5), 69 (100), 55 (5), 44 (5). ν_{max}; 1750, 1740, 1640, 1230 cm^{-1}.

6,9-Dihydroxy-7-megastigmyn-3-yl Acetate (95)

This was obtained as a by product from deacetylation of carbonate (92).

^{1}H n m r (60MHz / $CDCl_3$); δ: 4.9, m, 1H; 4.5, q, *J* =7Hz, 1H; 2.0, s, 3H; 2.2, m, 1H; 1.69, m, 4H, 1.45, d, *J* =7Hz, 3H; 1.05, brs, 9H.

(*3S',9R'*)-5-Megastigmen-7-yne-3,9-diol (58a)[52]

Method 1

Lithium aluminium hydride (5 mg, 0.13 m mol) was added to enyne diacetate (86) (110 mg, 0.38 m mol) in ether (7 ml) and the mixture was refluxed for 20 h. The mixture was cooled and dropwise addition of potassium sodium tartrate (20%) was used to quench the reaction. The aqueous layer was saturated with solid sodium chloride and then extracted with ether (4 x 30 ml). The ethereal layer was dried ($MgSO_4$) and evaporated to yield a clear oil (74 mg). Flash chromatography[86] using 20% ether / dichloromethane gave pure enynediol (58a), (36 mg, 46%). Rf=0.36, 100% ether.

Method 2

Enyne diacetate (86) (70 mg, 0.24 m mol) in methanol (0.3 ml) was added to potassium hydroxide (40 mg, 0.71 m mol) in methanol (0.3 ml) at room temperature. Progress of the reaction was monitored by t.l.c. (silica) and found to be complete after 10 min. The reaction was poured into water, the solution saturated with solid sodium chloride and the product was extracted with Freon 11 (2 x 20 ml). Evaporation yielded crude enynediol (58a) (50 mg). Dry column chromatography (Matrex silica) using ethyl acetate / dichloromethane yielded [(58a), 26 mg, 33%] as a sweet scented, opaque oil. It was found that the enynediol (58a) was unstable to silica (flash chromatography on matrex silica) and readily underwent autoxidation.

^1H n m r. (60 MHz / $CDCl_3$); δ: 4.65, q, *J* =7Hz, 1H; 4.07, m, 1H; 2.2-2.0, m, 2H; 1.8,s, 3H; 1.50,d, *J* =7Hz, 3H; 1.13, s, 3H; 1.1, s, 3H. ^{13}C n m r. (20.1 MHz / $CDCl_3$); δ: 137.9, s; 123.3, s; 95.6, s; 81.7, s; 64.4, d; 58.5, d; 46.4, t; 41.0, t; 36.2, s; 30.1, q; 28.3, q; 24.6, q; 22.1, q. m/z; 208 (7), 193 (12), 190 (5), 175 (8), 172 (3), 157 (12), 149 (7), 142 (10), 133 (7), 131 (15), 121 (5), 119 (7), 115 (8), 105

(15), 91 (25), 79 (15), 77 (18), 43 (100), 41 (45), 39 (30). The ions at m/z 190, 172, 157, 142 and 115 were diminished in intensity in some spectra. ν_{max} 3600, 3300, 2200, 1625 cm^{-1}.

(*3S',5R',6R',9R'*)-5,6-Epoxy-7-megastigmyne-3,9-diyl Diacetate (98a), (*3S',5S',6S',9R'*)-5,6-epoxy-7-megastigmyne-3,9-diyl Diacetate (98b) [(98a) is referred to as <u>trans</u> (3-OH and epoxide) and (98b) as <u>cis</u>][87]

A solution of <u>m</u>-chloroperoxybenzoic acid (150 mg, 0.74 mmol, 85%) in chloroform (2-5 ml) was added to a solution enyne diacetate (86) (170 mg, 0.58 m mol) in chloroform (2 ml) at 5°. The mixture was allowed to stand overnight at 5°. Saturated sodium metabisulphite solution (5 ml) was added and the mixture stirred for 30 min. Ether (1 x 20 ml) was added and the organic layer was washed with sodium hydrogen carbonate solution (2 x 10 ml), water (1 x 10 ml), brine (1 x 10 ml), dried ($MgSO_4$) and evaporated to yield a yellow oil (75 mg, 46%). Rf cis (98b) 0.24, trans (98a) 0.16. 0.5% ethyl acetate / dichloromethane. Separation was achieved by flash chromatography[86] or with the chromatotron. The cis isomer (98b) was obtained pure, but the trans compound (98a) was contaminated with minor amounts of cis isomer. Cis epoxide ^{1}H n m r (300 MHz / $CDCl_3$); δ: 5.4, q, *J* =6.0Hz, 1H; 4.9-4.74,m, 1H; 2.25, H equatorial, ddd, *J* =14.9, 7.6, 1.8Hz, 1H; 2.0, s, 3H; 1.94, s, 3H; 1.97, s, 3H; 1.75, H axial, dd, *J* =14.9, 9.6Hz, 1H; 1.5-1.3, m, 2H; 1.43, d, *J*=7.0Hz; 1.39, s, 3H; 1.16, s, 3H; 1.13, s, 3H. ^{13}C n m r (75.47 MHz / $CDCl_3$); δ: 170.5, 169.6, 84.6, 81.0, 66.1, 65.8, 63.9, 63.6, 60.2, 37.5, 34.8, 34.5, 26.7, 24.3, 22.6, 21.2 20.9. m/z; 266 (<1), 248 (<1), 224 (<1), 206 (<1), 192 (1), 173 (1), 149 (4), 123 (8), 105 (5), 91 (4), 77 (3), 65 (2), 55 (4), 43 (100), 41 (9). Trans epoxide ^{1}H n m r (300 MHz / $CDCl_3$); δ: 5.40, q, *J* =6.2Hz, 1H; 4.85-4.7, m, 1H; 2.29, H equatorial, ddd, *J* =15.0, 5.9, 1.3Hz, 1H; 1.99, s, 3H; 1.92, s, 3H; 1.71, H axial, dd, *J* =15.0, 6.5, 1H; 1.57-1.46, m, 1H; 1.42, d, *J* =6.2Hz, 3H; 1.41, s, 3H; 1.38-1.31, m, 1H; 1.19, s, 3H;

1.06, s, 3H.

^{13}C n m r (75.47 MHz / $CDCl_3$); δ: 170.0, s; 169.7, s; 84.6, s; 81.3, s; 67.9, d; 67.0, s; 65.2, s; 60.2, d; 39.8, t; 35.7, t; 33.9, s; 28.5, q; 26.0, q; 21.8, q; 21.2, q (2xCH_3, C10); 20.9, q. m/z; 265 (<1), 248 (<1), 224 (<1), 192 (1), 149 (4), 123 (7), 105 (4), 91 (4), 77 (4), 65 (2), 55 (4), 43 (100), 41 (9).

(3S',5R',6R',9R')-6,7-Megastigmadiene-3,5,9-triol (43b)[87]

Lithium aluminium hydride (25 mg, 0.66 m mol) was added to the cis epoxydiacetate (98b) (22 mg, 0.07 m mol) in tetrahydrofuran (10 ml) and the mixture refluxed for 6 h. The mixture was cooled and dropwise addition of potassium tartrate solution (20%) was used to quench the reaction. The aqueous layer was saturated with solid sodium chloride and then extracted with ether (2 x 20 ml). The ethereal layer was dried ($MgSO_4$) and evaporated to yield 16 mg crude oil. Flash chromatography using ethyl acetate yielded the allenic triol (43b) (12 mg, 66%). Rf 0.17, 100% Ethyl acetate

^1H n m r (300 MHz / d_6 Acetone); δ: 5.2, d, *J* =5.8 Hz, 1H; 1.27, d, *J* =7 Hz, 3H; 1.18, d, *J* =7Hz, 3H; 1.01, s, 3H. MS solid probe m/z; 208 (4), 193 (1), 175 (2), 166 (2), 151 (3), 135 (10), 125 (21), 109 (16), 107 (22), 93 (13), 91 (15), 79 (17), 45 (40), 43 (100).

(3S',5S',6S',9R')-6,7-Megastigmadiene-3,5,9-triol (43a) [This is referred to as trans (3OH and 5OH)]

This allenic triol was synthesized according to the method described above. M p 166° (Lit[87] 166) ^1H n m r (300MHz / d_6 Acetone); δ: 5.25, d, *J* =5.8Hz, 1H; 4.27-4.11, m, 2H; 3.74-3.71, br, OH; 3.48-3.46, br, OH; 2.88, s, OH; 2.14-2.0, m, 1H; 1.85-1.79, m, 1H; 1.30,s,3H; 1.3-1.17, m, 2H; 1.28, s, 3H; 1.20, d, *J* =6.3Hz, 1.03, s, 3H.

^{13}C n m r (75.47Mz / D_2O); δ: 199.2; 121.5; 102.4; 68.1; 67.7; 66.7; 63.4; 50.0; 49.8; 48.9; 33.5; 31.6; 30.9; 29.7; 23.5. Tertiary butanol was used as an

external reference. m/z; 208 (37), 166 (19), 151 (16), 135 (48), 133 (16), 125 (100), 124 (18), 123 (15), 109 (59), 107 (77), 105 (22), 93 (28), 91 (31), 82 (21), 81 (23), 79 (31), 77 (22), 69 (25), 67 (16), 55 (16).

1-[3-Hydroxybutynyl]-cyclohexan-1-ol (101)[72]

The synthesis of the diol (101) was performed under conditions identical to those described for the compound (76). Cyclohexanone (100) (27.2g, 0.28m) yielded 45g of diol (101). (96%) B p 104° / 0.07mm.
^{1}H n m r (60MHz / $CDCl_3$); δ: 4.55, q, *J* =7Hz, 1H; 3.3, br, 2H, OH; 2.1-1.0, m, 10H; 1.45, d, *J* =7Hz, 3H. ν_{max} 3300, 1072 cm^{-1}.

3-(1'-Hydroxylcyclohexyl)1-methyl-2-propynyl Acetate (102)

The diol (101) (20g, 0.12m), in pyridine (40ml) and acetic anhydride (40ml, 0.2m), was stirred at room temperature overnight. Ether (100ml) was then added and the organic layer was washed with water (2x50ml), copper sulphate (2x50ml), water (2x50ml), brine (20ml), dried ($MgSO_4$) and evaporated to yield a yellow oil. (21g, 78%). ^{1}H n m r revealed trace amounts of diacetylated product (103) which was separated by chromatography.
Compound (102) ^{1}H n m r (60MHz / $CDCl_3$); δ: 5.58, q, *J* =7Hz, 1H; 3.0, br, 1H, OH; 2.1, s, 3H; 1.8-1.2, m, 10H; 1.55, d, *J* =7Hz, 3H. Compound (103) ^{1}H n m r (60MHz / $CDCl_3$); δ: 5.57, q, *J* =7Hz, 1H; 2.08, s, 3H; 2.05, s, 3H; 1.8-1.2, m, 10H; 1.5, d, *J* =7Hz, 3H.

3-(1'-Cyclohexenyl)-1-methyl-2-propynyl Acetate (104)

Dropwise addition of phosphorus oxychloride (2.8ml, 0.03m) to compound (102) (5g, 0.02mmol) in triethylamine (10ml) was performed at room temperature. After 12h a solid had precipitated and the reaction mixture was stirred overnight. Ether (100ml) was added and the ether extract was washed with ice cold water (10ml), saturated sodium hydrogen carbonate (30ml), dried

($MgSO_4$) and evaporated to yield a brown oil. Distillation at 85° / 0.5mm yielded (104) as a yellow oil (4g, 88%).

^1H n m r (60MHz / $CDCl_3$); δ: 6.2, br, 1H; 5.6, q, *J* =6Hz, 1H; 2.03, s, 3H; 2.4-2.0, m, 4H; 1.8-1.4, m, 4H; 1.5, d, *J* =6Hz, 3H. m/z; 192 (8), 135 (25), 133 (5), 132 (5), 131 (6), 121 (15), 117 (25), 115 (8), 107 (11), 105 (9), 93 (7), 91 (22), 79 (17), 78 (8), 77 (15), 67 (12), 66 (5), 65 (9), 55 (13), 53 (7), 52 (5), 43 (100), 41 (19), 39 (21). ν_{max}; 2240, 1720, 1600, 1240 cm^{-1}.

4-(1'-Cyclohexenyl)-3-butyn-2-ol (99)[94]

The synthesis of enyne alcohol (99) was achieved under conditions identical with those described for the compound (58a). Enyne acetate (104) (1.2g, 5.8mmol) was reduced with lithium aluminium hydride to yield enyne alcohol (99) (0.8g,84%), (Found : C, 79.6; H, 9.4. $C_{10}H_{14}O$ requires C, 80.0, H, 9.4%). A minor amount of over reduced product (105) was also obtained. The alcohol was found to undergo autoxidation readily to yield the ketone (106), identified tentatively by MS.

The alcohol (99) ^1H n m r (60MHz / $CDCl_3$); δ: 6.05, m, 1H; 4.6, m, 1H; 2.6, br, 1H, OH; 2.2, m, 4H; 1.8-1.5, m, 4H; 1.5, d, *J* =6.5Hz, 3H. ^{13}C n m r (20.1MHz / $CDCl_3$); δ: 134.7; 120.1; 88.4; 85.4; 58.3; 28.9; 25.4; 24.3; 22.0; 21.2. m/z; 150 (11), 135 (11), 121 (29), 107 (16), 105 (6), 95 (9), 92 (6), 91 (19), 81 (6), 80 (6), 79 (37), 78 (8), 77 (20), 73 (6), 67 (13), 65 (11), 63 (6), 55 (22), 53 (12), 51 (18), 50 (8), 45 (13), 43 (100), 41 (35), 39 (40). ν_{max}; 3400, 2250, 1660, 1080 cm^{-1}.

Compound (105) ^1H n m r (60MHz / $CDCl_3$); δ: 6.19, d, *J* =15Hz, 1H; 5.63, dd, *J* =15, 6 Hz, 1H; 4.4, dq, *J* =6, 3 Hz, 1H; 2.3-1.9, m, 4H; 1.8-1.4, m, 4H;, 1.25, d, *J* =6Hz, 3H. ν_{max}; 3300, 1600, 1260 cm^{-1}.

Ketone (106) m/z; 148 (45), 134 (8), 133 (100), 105 (40), 103 (13), 91 (10), 79 (29), 78 (8), 77 (30), 65 (6), 63 (7), 53 (6), 51 (17), 50 (6), 43 (40), 41 (9), 39 (19).

3,5,5-Trimethyl-3-cyclohexenol (107)

This was prepared by the method of Haubenstock.[60] Compound (61) (1g, 7.2mmol) yielded 1.0g, 99% of compound (107).
^{1}H n m r (300MHz / $CDCl_3$); δ: 5.09, br, 1H; 4.0, dddd, *J* = 3.8, 5.5, 9.4, 11.6 Hz, 1H; 2.22, dd, *J* =5.5, 16.2 Hz, 1H; 1.91-1.61, m, 2H; 1.65, s, 3H; 1.32, t, *J* =11.8Hz, 1H, 1.0, s, 3H; 0.97, s, 3H. ^{13}C n m r (20.1MHz / CDCl3); δ : 131.7; 128.6; 65.8; 45.9; 39.6; 33.9; 31.2; 29.4; 23.1. m/z; 140 (16), 125 (28), 122(18), 108 (10), 107 (100), 105 (6), 97 (6), 96 (19), 91 (19), 81 (30), 79 (22), 77 (8), 69 (9), 67 (7), 65 (5), 55 (34), 53 (11), 43 (26), 41 (39), 39 (28). ν_{max}; 3350, 1660, 1355 cm^{-1}.

3-Hydroxy-β-damascone (39)[39]

Acetylenic triol (55) (700mg, 0.3mmol) in 1M HCl (30ml) was refluxed for one hour. Solution was cooled and aqueous layer was extracted with dichloromethane (3x30ml), the dichloromethane was then washed with saturated sodium hydrogen carbonate (3x20ml), water (10ml), brine (10ml), dried ($MgSO_4$) and evaporated to yield a yellow oil. Chromatography[86] using 100% diethyl ether yielded 3-hydroxy-β-damascone (39) (490mg, 70%) and β-damascenone (38) (100mg, 16%).
3-Hydroxy-β-damascone (39): ^{1}H n m r (300MHz / $CDCl_3$); δ: 6.73, dq, *J* =15.7, 6.9Hz, 1H; 6.15, dd, *J* =15.7, 1.5Hz, 1H; 4.08, dddd, *J* =11.8, 9.5, 5.9, 3.7Hz, 1H; 2.36, dd, *J* =16.8, 5.9, 1H; 2.05, dd, *J* =16.8, 9.5Hz, 1H; 1.93, dd, *J* =6.9, 1.5Hz, 3H; 1.78-1.72, m, 2H; 1.55, s, 3H; 1.19, s, 3H, 0.99, s, 3H. ^{13}C n m r (20.1MHz / $CDCl_3$); δ: 201.7, s; 146.2, d; 140.0, s; 134.5, d; 128.1, s; 64.6, d; 47.7, t; 40.8, t; 36.2, s; 29.5, q; 28.9, q; 20.9, q; 18.2, q. m/z; 209 (4), 208 (34), 193 (35), 175 (41), 176 (5), 149 (20), 147 (16), 139 (11), 137 (8), 134 (10), 133 (11), 123 (10), 122 (12), 121 (62), 119 (13), 169 (11), 107 (12), 105 (23), 95 (10), 93 (18), 91 (20), 81 (10), 79 (20), 77 (6), 69 (100), 67 (7), 57 (7),

55 (26), 53 (12), 50 (5), 43 (24), 41 (84), 39 (29).

β-Damascenone (38): m.z; 190 (8), 122 (4), 121 (33), 120 (9), 105 (12), 91 (12), 79 (9), 77 (9), 70 (4), 69 (100), 65 (4), 53 (5), 41 (44), 40 (3), 39 (20).

5.2 Experimental Chapter 3.

General

Glycosides were analyzed by GC / MS on a Finnigan 4021 TSQ mass spectrometer using either a J & W DB1701, 15metre column (column A) or a J & W DB5, 30metre column (column B), both of which are fused silica columns (0.25 mm i.d. and 0.25 μm film thickness) with helium carrier gas at linear velocity of 40 cm / sec. Injections were made with a split injector at 200°C and a split ratio of 1:10. Both columns were held at 100°C for five minutes, then programmed at 5°C / min to 320°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV. They were also analyzed using FAB (Fast Atom Bombardment) and CI (Chemical Ionization, using ammonia as ionizing gas) on the Finnigan 4021 TSQ mass spectrometer. Accurate mass measurements were performed on DS90 mass spectrometer.

Many of the spectra for the synthetic β-D-glucopyranosides, which were all diastereoisomeric mixtures, exhibited complex ^{1}H n m r spectra in the up field region. However, in the downfield region where the glucose ring hydrogens (G1-G6) resonate, many of the resonances for each of the diastereoisomers were found to be superimposed and interpretation was simplified.

The following general procedure for glycosylation using the ortho ester (140) was used. Under strictly anhydrous conditions and under a nitrogen atmosphere, boron trifluoride etherate (4.8 equivalents) was added to a solution of alcohol (freshly distilled or pumped dry under high vacuum at 0.01mm at room temperature for 8 hours, one equivalent) and ortho ester (140) (pumped dry under high vacuum at 0.01mm at room temperature for

8hours, 1.2 equivalents) in dichloromethane (freshly distilled from calcium hydride)[161] at room temperature. Progress of the reaction was monitored by t.l.c. Upon completion of reaction the solution was then treated with a saturated solution of sodium hydrogen carbonate ensuring the aqueous layer was basic after stirring. The organic layer was then separated and washed with water (10ml), brine (10ml), dried ($MgSO_4$) and evaporated under reduced pressure to give generally clear oils which were chromatographed[86, 158] to isolate the pure tetra-O-pivaloyl-β-D-glucopyranosides.

The general procedure used for depivaloylation involved use of the Zemplén[136] method. Under a nitrogen atmosphere, the tetra-O-pivaloyl-β-D-glucopyranosides were treated with a 0.01M sodium methoxide / anhydrous methanol[161] for several days (7days generally).
Isolation of the β-D-glucopyranosides involved the following procedure. The solution from the hydrolysis was evaporated to dryness under reduced pressure. The salts were then dissolved in water and the aqueous layer was extracted with diethyl ether (2x10ml). The aqueous layer was retained and then evaporated to dryness and the weight of the salts was measured. [This is important as a SEP-PAK C18 R.P cartridge (Waters Associates, Patent no. 509,338, PART NO. 51910) was used to isolate the β-D-glucopyranoside]. The salts were then redissolved in water (water added such that 10mg of salts is dissolved in 1ml of water) and the pH adjusted by addition of ammonium chloride to neutral pH. The SEP-PAK cartridge was then used to isolate the β-D-glucopyranoside by the following method. The aqueous layer (1ml, containing approximately 10mg of salts) was loaded onto the SEP-PAK column and the column was eluted with water (20ml) to remove inorganic salts. The SEP-PAK cartridge was then eluted with 2ml of methanol which was kept separately. This procedure was then repeated till

all the aqueous layer containing the salts was exhausted. The methanol extract was then evaporated under reduced pressure to yield the β-D-glucopyranoside. The purity of the β-D-glucopyranoside was also monitored by t.l.c. and further purification was performed by chromatography if required. For larger quantities of salts the Bond Elut LRC [C18 Octadecyl (Catalog no. 607101) from Analytichem International, a division of Varian] was used where up to 100mg of salts can be loaded and the use of an aspirator ensured rapid separation.

Geranyl-tetra-O-acetyl-β-D-glucopyranoside (1b).

Compound (1b) was synthesized by the method of Strauss.[147]
^{1}H n m r (300MHz / CDCl3); δ; 5.2, brt, *J* =6.5Hz, vinyl H, 1H; 5.17, dd, G3, *J* =9.5, 9.3Hz, 1H; 5.0, dd, *J* =9.8, 9.3Hz, G4, 1H; 5.0, obs, vinyl H, 1H; 4.96, dd, *J* =9.8, 8.0, G2, 1H; 4.5, d, *J* =7.9, G1, 1H; 4.2, m, (G6a, + 2H on Geranyl C1), 3H; 4.1, dd, *J* =12.2, 2.5Hz, G6b, 1H; 3.6, ddd, *J* =12.5, 9.8, 4.9, G5, 1H; 2.05, s, 3H; 2.00, s, 3H; 1.99, s, 3H; 1.66, s, 3H; 1.62, s, 3H; 1.57, s, 3H. (Column A) m/z; 331 (<1), 139 (5), 136 (5), 121 (4), 115 (4), 110 (4), 109 (10), 98 (4), 93 (13), 81 (15), 80 (5), 69 (32), 68 (15), 67 (6), 55 (4), 43 (100).

9-Hydroxy-5-Megastigmen-4-one (151)

7(E)-9-Hydroxy-5-Megatigmadien-4-one (150)[159] (100mg, 0.48mmol) in ethyl acetate (20ml) was degassed four times with the introduction of hydrogen each time. Hydrogenation was achieved using palladium black (catalytic) at room temperature for 90 minutes. The mixture was filtered and the solvent removed under reduced pressure to yield a clear oil (100mg). GC / MS revealed two peaks in a ratio of 9:1. [10% over reduced product (152)]. Chromatographic separation using 25% ethyl acetate / petroleum ether yielded compound (151) (90mg, 89%) as a white solid, m p 55-57°C (Lit.[159] 55-57°C).

^{1}H n m r (60MHz / $CDCl_3$); δ: 3.9, m, H9, 1H; 2.6-2.1, m, methylene H, 4H; 2.0-1.5, m, methylene H, 4H; 1.8, s, 3H; 1.26, d, *J* =6Hz, H10, 3H; 1.2, s, H11+H12, 6H. m/z; 210 (8), 185 (20), 165 (27), 152 (48), 137 (64), 109 (75), 93 (38), 79 (37), 67 (45), 55 (65), 43 (100). ν_{max}; 3600, 3500, 1700, 1650, 1600 cm^{-1}.

Compound (152): ^{1}H n m r (300MHz / $CDCl_3$); δ: 3.79-3.69, m, H9, 1H; 2.53-2.42, m, 2H; 2.29-2.16, m, 2H; 1.6-1.24, m, 6H; 1.18, d, *J* =6.1Hz, 3H; 1.03, s, 3H; 0.97, s, 3H. ν_{max}; 3600, 1695 cm^{-1}.

4-Oxo-5-megastigmen-9-yl-tetra-O-acetyl-β-D-glucopyranoside (153)

Under strictly anhydrous conditions and a nitrogen atmosphere, 9-hydroxy-5-megastigmen-4-one (151) (90mg, 0.43mmol), penta-O-acetyl-β-D-glucopyranoside (128) (170mg, 0.44mmol) in 1,2-dichloroethane (10ml) was refluxed over 4Å molecular sieves for 1hour[133]. The solution was then cooled to room temperature (20°C) and trimethysilyl triflate (99mg, 86µl, 0.44mmol) was added to the reaction. After stirring for 3hours the reaction mixture was diluted with dichloromethane (20ml), washed with saturated sodium hydrogen carbonate (2x20ml), water (10ml), brine (10ml), dried ($MgSO_4$) and solvent evaporated to yield a clear oil. Chromatography[158], ethyl acetate / petroleum ether, yielded three products, 4-oxo-5-megastigmen-9-yl acetate (154) (50%), 4-oxo-5-megatigmen-9-yl-tetra-O-acetyl-β-D-glucopyranoside (153) (21%) (Found 540.2567. $C_{27}H_{40}O_{11}$ requires 540.2570) and its α-D-glucopyranoside (153a) (9%). Rf (154) 0.63, (153) 0.46, (153a) 0.25, 50% ethyl acetate / petroleum ether.

Compound (154): ^{1}H n m r (60MHz / $CDCl_3$); δ: 5.0, m, H9, 1H; 2.7-2.1, m, methylene H, 4H; 2.1, s, acetoxy methyl, 3H; 1.8, s, H13, 3H; 1.32, d, *J* =7Hz, H10, 3H; 1.2, s, H11+H12, 6H. ν_{max}; 1720, 1650, 1600, 1250 cm^{-1}.

Compound (153): ^{1}H n m r (300MHz / $CDCl_3$); δ: 5.22, dd, *J* =9.5, 9.4Hz,

G3, 1H; 5.10, dd, *J* =9.7, 9.4Hz, G4, 1H; 4.98, dd, *J* =9.5, 8.0Hz, G2, 1H; 4.58, d, *J* =8.0Hz, G1, 1H; 4.27-4.11, m, G6a+b, 2H; 3.9-3.6, m, G5+H9, 2H; 2.48-2.42, m, 2H; 2.06, 2.04, 2.03, 2.02, each, s, acetoxy methyl, 3H; 2.01, s, 6H; 2.00, s, 6H; 1.82-1.79, m, 2H; 1.78, s, 3H; 1.69-1.63, m, 2H; 1.30, d, *J* =6.1Hz, 3H; 1.14, s, 6H, 2.6-1.3, methylene hydrogens were obscure. (Column A) Diastereoisomer 1: m/z; 540 (<1), 420 (<1), 360 (3), 331 (8), 271 (4), 193 (21), 169 (38), 152 (16), 109 (100), 81 (23), 55 (20). (Column A) Diastereoisomer 2: m/z; 540 (<1), 420 (<1), 360 (3), 331 (7), 271 (4), 193 (21), 169 (38), 152 (23), 109 (100), 81 (23), 55 (18). ν_{max}; 1750, 1220, 1650, 1038 cm^{-1}. Compound (153a) ^{1}Hn m r (300MHz / $CDCl_3$); δ: 5.54, dd, *J* =10.1, 9.8Hz, G3, 1H; 5.48, dd, *J* =3.5Hz, G1, 1H; 5.09, dd, *J* =9.8, 9.7Hz, G4, 1H; 4.92, dd, *J* =10.1, 3.5 Hz, G2, 1H; 4.3-4.0, m, G5+G6a,b, 3H; 2.46, appeared as a triplet, *J* =6.9Hz, 2H; 3.91-3.80, m, 1H; 2.23-2.16, m, 2H; 2.10, 2.09, 2.04, 2.02, each, s, acetoxy methyl, 3H; 1.82, appeared as a triplet, *J* =6.9Hz, 2H; 1.78, s, 3H; 1.26, d, *J* =6.2Hz, 3H; 1.74-1.54, m, 2H; 1.17, s, 6H. (Column A) Diastereoisomer 1: m/z; 540 (<1), 485 (<1), 429 (<1), 355 (<1), 331 (10), 271 (5), 207 (10), 193 (18), 169 (33), 152 (18), 109 (100), 81 (30), 55 (33). (Column A) Diastereoisomer 2: m/z; 540 (<1), 484 (<1), 429 (<1), 355 (<1), 331 (10), 207 (6), 193 (22), 169 (28), 152 (20), 109 (100), 81 (30), 55 (28). ν_{max}; 1750, 1220, 1650, 1038 cm^{-1}.

1,2-O-(1-N-1-Phenylethylideneaminooxy)-2,2-dimethylpropylidene-3,4,5-tri-O-pivaloyl-α-D-glucopyranoside (140)[124].

The ortho ester (140) was synthesized by the method of Kunz.[124] When silver carbonate was used as the halophile no ortho ester formation occurred. A minor by-product (156)[160] was obtained by chromatography,[158] ethyl acetate / petroleum ether, as a white solid (recrystallized from ethanol, m p 118-119°C), however, on standing readily

decomposed.

Compound (156): ^{1}H n m r (300MHz / $CDCl_3$); δ: 7.80-7.66, m, aromatic H, 4H; 7.43-7.34, m, aromatic H, 6H; 5.10, d, *J* =1.5Hz, vinyl H, 1H; 4.84, d, *J* =1.5Hz, vinyl H, 1H; 2.46, s, vinyl methyl, 3H. ^{13}C n m r (75.47MHz / $CDCl_3$); δ: 159.4, s; 129.6, d; 128.6, d; 128.3, d; 126.6, d; 125.5, s; 96.2, s; 86.8, t; 13.6, q. m/z; 237 (100), 220 (25), 219 (22), 165 (14). ν_{max}; 3100, 3030, 2900, 1670, 1620, 1600, 1480, 1310, 1270, 1100 cm^{-1}.

The ortho ester (140) was successfully synthesized when silver trifluoromethanesulphonate was used as the halophile in 80% yield. m p 112°C (Lit.[124] 112-113°C). A small amount of cis ortho ester (140a) (m p 105-108°C) was also isolated.

Ortho ester (140): ^{1}H n m r (300MHz / $CDCl_3$); δ: 7.58-7.54, m, aromatic H, 2H; 7.36-7.34, m, aromatic H, 3H; 6.21, d, *J* =6.2Hz, G1, 1H; 5.25, dd, *J* =6.1, 3.1Hz, G3, 1H; 5.03, dd, *J* =9.6, 6.1Hz, G4, 1H; 4.71, dd, *J* =6.2, 3.1Hz, G2, 1H; 4.27-4.16, m, G5+G6a+G6b, 3H; 2.24, s, vinyl methyl, 3H; 1.25, 1.23, each, s, tert -butyl methyl, 9H; 1.19, s, tert-butyl methyl, 18H.
^{13}C n m r (20.1MHz / $CDCl_3$); δ: 177.8; 176.6; 159.4; 156.6; 136.2; 129.2; 128.3; 126.7; 125.6; 99.4; 99.0; 77.7; 77.4; 73.0; 67.8; 67.2; 62.2; 38.3; 26.7; 25.6; 24.7; 12.5. 633 (<1), 499 (7), 314 (4), 296 (6), 226 (56), 118 (30). ν_{max}; 1730, 1480, 1385, 1360, 1280, 1120 cm^{-1}.

Ortho ester (140a): ^{1}H n m r (300MHz / $CDCl_3$); δ: 7.70-7.67, m, aromatic H, 2H; 7.40-7.34, m, aromatic H, 3H; 5.97, dd, *J* =9.8, 4.8Hz, G3, 1H; 5.78, d, *J* =6.6Hz, G1, 1H; 5.11, dd, *J* =10.0, 9.8Hz, G4, 1H; 4.67, ddd, *J* =10.0, 2.9, 1.7Hz, G5, 1H; 4.07, dd, *J* =12.5, 1.7Hz, G6a, 1H; 3.93, dd, *J* =12.5, 2.9Hz, G6b, 1H; 2.30, s, vinyl methyl, 3H; 1.16, 1.14, 1.08, 1.05, each, s, tert-butyl methyl, 9H. ν_{max}; 1730, 1480, 1385, 1360, 1280, 1120 cm^{-1}.

3,5,5-Trimethyl-3-cyclohexenyl-tetra-O-pivaloyl-β-D-glucopyranoside (107b).

This was synthesized by the method of Kunz.[124] The reaction mixture was stirred for 10minutes before standard work-up. Compound (107) (200mg) yielded the β-glycoside (107b) (820mg, 90%). Rf 0.64, 50% diethyl ether / petroleum ether. Recrystallization from methanol yielded one diastereoisomer, m p 137-139°C. (Found C, 66.0; H 9.0. $C_{35}H_{58}O_{10}$ requires C, 65.8; H 9.1%)

Recrystallized diastereoisomer: ^{1}H n m r (300MHz / $CDCl_3$); δ: 5.30, dd, *J* =9.7, 9.5Hz, G3, 1H; 5.07, dd, *J* =9.7, 9.5Hz, G4, 1H; 5.06, obs, vinyl H, 1H; 5.01, dd, *J* =9.7, 8.1Hz, G2, 1H; 4.66, d, *J* =8.1Hz, G1, 1H; 4.25, dd, *J* =12.1, 1.8Hz, G6a, 1H; 4.01, dd, *J* =12.1, 6.7Hz, G6b, 1H; 3.90, m, H1, 1H; 3.75, ddd, *J* =9.7, 6.7, 1.8Hz, G5, 1H; 2.24, dd, *J* =16.8, 5.8Hz, H2 equatorial, 1H; 2.00, dd, *J* =16.8, 9.7Hz, H2 axial, 1H; 1.70-1.64, obs, 1H; 1.61, s, vinyl methyl, 3H; 1.43-1.24, obs, 1H; 1.22, s, tert-butyl methyl, 9H; 1.17, s, tert-butyl methyl, 18H;,1.11, s, tert-butyl methyl, 9H; 0.97, 0.93, each, s, C5+C5 methyl, 3H. ^{13}C n m r (20.1MHz / $CDCl_3$); δ: 178.0; 177.2; 176.6; 176.4; 131.4; 128.7; 99.9; 74.8; 72.3; 71.4; 68.4; 62.4; 42.7; 38.7; 37.8; 33.7; 31.3; 29.4; 27.1; 23.2. ν_{max}; 1735, 1390, 1280, 1140 cm^{-1}. The ^{1}H n m r of the diastereoisomer 2 was assigned from the mixture of diastereoisomers in the mother liquors. Diastereoisomer 2: ^{1}H n m r (300MHz / $CDCl_3$); δ: 5.33, dd, *J* =9.7, 9.5Hz, G3, 1H; 5.07, dd, *J* =9.7, 9.5Hz, G4, 1H; 5.06, obs, vinyl H, 1H; 5.01, dd, *J* =9.7, 8.0Hz, G2, 1H; 4.69, d, *J* =8.0Hz, G1, 1H; 4.25, dd, *J* =12.1, 1.8Hz, G6a, 1H; 4.01, dd, *J* =12.1, 6.7Hz, G6b, 1H; 3.90, m, H1, 1H; 3.75, ddd, *J* =9.7, 6.7, 1.8Hz, G5, 1H; 2.15, dd, *J* =16.5, 5.6Hz, 1H; 1.84, obs, 1H; 1.70-1.64, obs, 1H; 1.62, s, vinyl methyl, 3H; 1.43-1.24, obs, , 1H; 1.22, s, tert-butyl methyl, 9H; 1.16, s, tert-butyl methyl, 18H;,1.11, s, tert-butyl

methyl, 9H; 0.98, 0.95, each, s, C5+C5 methyl, 3H. ν_{max} (mixture of diastereoisomers); 1735, 1390, 1280, 1140 cm^{-1}.

3,5,5-Trimethyl-3-cyclohexenyl-β-D-glucopyranoside (107a).

Depivaloylation using the Zemplén method[136] yielded the β-D-glucopyranoside (107a) in 90% as a white solid, m p 118-124°C. Diastereoisomer 1: ^{13}C n m r (20.1MHz / D_2O); δ: 133.4; 131.3; 102.2; 77.3; 76.4; 71.1; 62.2; 43.1; 38.4; 34.6; 31.8; 29.9; 23.8. Dioxane was used as an external standard. Diastereoisomer 2: ^{13}C n m r (20.1MHz / D_2O / Dioxane); 133.4; 130.7; 102.1; 77.3; 76.3; 75.8; 71.1; 62.2; 44.5; 37.1; 34.8; 31.8; 29.9; 23.8. FAB m/z; 303 (35), 163 (40), 145 (42), 124 (66), 107 (100).

3-(1'-Cyclohexenyl)-1-methyl-2-propynyl-tetra-O-pivaloyl-β-D-glucopyranoside (99b).

This was synthesized by the method of Kunz. An oxygen trap[162] was used as a precaution because the enyne alcohol (99) was found to readily undergo autoxidation. The reaction mixture was stirred for 10minutes at -20°C and then worked-up as described previously. Enyne alcohol (99) (200mg, 1.3mmol) yielded β-D-glucopyranoside (99b) (514mg, 60%). Rf 0.35, 0.28, 20% diethyl ether / petroleum ether. UV_{max} 243.5nm ($CDCl_3$). Diastereoisomer 1, Rf 0.35: ^1H n m r (300MHz / $CDCl_3$); δ: 6.01,brs, vinyl H, 1H; 5.28, dd, *J* =9.8, 9.3Hz, G3; 1H; 5.03, dd, *J* =9.8, 9.6Hz, G4, 1H; 4.94, dd, *J* =9.3, 8.3Hz, G2, 1H; 4.80, d, *J* =8.3Hz, G1, 1H; 4.62, q, *J* =6.6Hz, H1, 1H; 4.16, dd, *J* =11.8, 1.0Hz, G6a, 1H; 3.98, dd, *J* =11.8, 5.6Hz, G6b, 1H; 3.67, ddd, *J* =9.8, 5.6, 1.0Hz, G5, 1H; 2.1-2.0, m, methylene H, 4H; 1.6-1.5, m, methylene H, 4H; 1.33, d, *J* =6.8Hz, C1 methyl, 3H; 1.15, 1.11, 1.08, 1.04, each, s, tert-butyl methyl, 9H. Diastereoisomer 2 was contaminated with

higher Rf material , however , the vinyl hydrogen at δ 5.95 and the anomeric proton at δ 4.76 were distinguishable. ^{13}C n m r (20.1MHz / $CDCl_3$); δ: 178.3; 178.1; 177.3; 176.6; 135.7; 120.0; 98.6; 87.8; 84.8; 72.3, 2C; 71.0; 68.0; 66.8; 62.1; 38.6; 28.9; 27.0; 25.4; 22.3; 22.1; 21.2. CI (NH_4^+): m/z; 666 (9), 634 (4), 535 (4), 534 (13), 533 (11), 532 (30), 500 (24), 499 (56), 265 (24), 211 (39), 195 (10), 194 (21), 181 (22), 160 (7), 149 (8), 146 (7), 136 (14), 134 (36), 133 (92), 132 (78), 131 (13), 121 (7), 120 (29), 119 (19), 118 (26), 117 (15), 110 (7), 109 (9), 106 (12), 105 (35), 104 (9), 103 (16), 102 (13), 97 (13), 92 (7), 91 (30), 86 (9), 85 (85), 81 (12), 79 (9), 78 (9), 77 (18), 69 (13), 65 (7), 58 (19), 57 (100), 56 (15), 55 (20), 53 (9), 51 (8). ν_{max}; 2240, 1740, 1390, 1365, 1280, 1040 cm^{-1}.

3-(1'-Cyclohexenyl)-1-methyl-2-propynyl-β-D-glucopyranoside (99a)

Depivaloylation by the Zemplén method yielded the β-D-glucopyranoside (99a) in 41%.

^{13}C n m r (75.47MHz / D_2O); δ: 138.7, d; 121.5, s; 101.4, d; 90.1, s; 87.3, s; 78.0, d; 77.9, d; 74.9, d; 71.5, d; 66.6, d; 62.7, t; 30.6, t; 27.3, t; 23.8, q; 23.5; 23.0. Tertiary butyl alcohol was used as an external standard.

3-(1'-Cyclohexenyl)-1-methyl-2-propynyl-tetra-O-acetyl-β-D-glucopyranoside (99c)

Acetylation of the β-D-glucopyranoside (99a) (5mg, 0.016mmol) in acetic anhydride (10μl), and pyridine (1ml) yielded the tetra-O-acetyl-β-D-glucopyranoside (99c) (5mg, 64%).

^1H n m r (300MHz / $CDCl_3$); δ: 6.09, brs, vinyl H, 1H; 5.23, dd, *J* =10.0, 9.5Hz, G3, 1H; 5.07, dd, *J* =9.8, 9.5Hz, G4, 1H; 4.98, dd, *J* =10.0, 7.8Hz, G2, 1H; 4.83, d, *J* =7.8, G1, 1H; 4.68, q, *J* =6.6Hz, H1, 1H; 4.25, dd, *J* =12.3, 4.7Hz, G6a, 1H; 4.10, dd, *J* =12.3, 2.1, G6b, 1H; 3.69, ddd, *J* =9.8, 4.7,

2.1Hz, G5, 1H; 2.09-2.05, m, methylene H, 4H; 2.06, 2.02, 2.01, 2.00, each, s, acetoxy methyl, 3H; 1.65-1.59, m, methylene H, 4H; 1.4, d, *J* =6.6Hz, 3H. ^{13}C n m r (75.47MHz / $CDCl_3$); δ: 170.6, s; 170.3, s; 169.4, s; 135.6, d; 120.0, s; 97.8, d; 87.8, s; 84.9, s; 73.0, d; 71.9, d; 68.7, d; 64.4, d; 62.0, t; 29.2, t; 25.6, t; 22,2, t; 21.4, q; 20.6,q. (Column B) m/z; 480 (3), 439 (5), 420 (6), 331 (3), 245 (4), 176 (5), 169 (28), 139 (23), 132 (100), 105 (30), 91 (28). ν_{max}; 2920, 2250, 1750, 1700, 1600, 1360, 1230, 1220, 1040 cm^{-1}.

***(3SR, 9RS)* -3-Hydroxy-5-megastigmen-7-yn-9-yl-tetra-O-pivaloyl-β-D-glucopyranoside (58d).**

This compound was also synthesized by the method of Kunz. The enyne diol (58a) (90mg, 0.43mmol) was stirred for 4hours (-10° to 25°C) and yielded the β-D-glucopyranoside (58d) (168mg, 54%) as a mixture of two diastereoisomers. Rf 0.69, 100% diethyl ether. Note oxygen free [162] nitrogen was used.

^1H n m r (300MHz / $CDCl_3$); δ: 5.31, dd, *J* =9.4, 9.4Hz, G3, 1H; 5.16-5.01, m, G4+G2, 2H; 4.91, t, *J* =8.2Hz, G1, (two doublets appearing as a triplet) 1H; 4.83, q, *J* =6.6Hz, H9 of diastereoisomer A, 0.5H; 4.72, q, *J* =6.7Hz, H9 of diastereoisomer B, 0.5H; 4.2-3.6, m, G5+G6a+G6b+H3, 4H; 2.4-2.3, and, 2.1-2.0, each, m, methylene H, 1H; 1.88, s, H13, 3H; 1.37, d, *J* =6.6Hz, H10, 3H; 1.6-1.0, m, tert-butyl methyls , H11, H12 and two methylene H, 44H. The β-D-glucopyranoside (58d) was found to readily undergo autoxidation to give a peroxide. FAB m/z; 739 (10) [$M+H^+ + O_2$], 707 (<1), 539 (2), 499 (26), 415 (3), 345 (46), 313 (3), 223 (43), 211 (64), 191 (100), 185 (85), 150 (100), 133 (60), 115 (20). ν_{max}; 3400, 1730, 1390, 1275, 1140 cm^{-1}.

***(3SR, 9RS)* -3-Hydroxy-5-megastigmen-7-yn-9-yl-β-D-glucopyranoside (58b).**

Depivaloylation at room temperature by the Zemplén method yielded after 7days the β-D-glucopyranoside (58b) in 88% as a mixture of two diastereoisomers.

^{13}C n m r (75.47MHz / D_2O); δ: 142.4; 124.2; 103.2; 101.5; 94.5; 86.5; 78.1; 77.9; 77.7; 75.3; 74.9; 71.7; 71.3; 69.3; 66.7; 66.2; 62.7; 62.6; 46.9; 41.8; 37.8; 31.6; 29.7; 23.8; 23.6; 23.5. Tertiary butyl alcohol was used as an external standard. FAB: m/z; 371 (10), 209 (42), 191 (100), 173 (25), 149 (20), 135 (35), 125 (30), 109 (18), 85 (10), 81 (7).

***(3SR, 9RS)* -3-Acetoxy-5-megastigmen-7-yn-9-yl-tetra-O-acetyl-β-D-glucopyranoside (58e).**

Treatment of the β-D-glucopyranoside (58b) with an excess of acetic anhydride in pyridine yielded the poly acetate-β-D-glucopyranoside (58e). (Found 598.2851. $C_{29}H_{40}O_{12}NH_4^+$ requires 598.2863). ^1H n m r (300MHz / $CDCl_3$); δ: 5.23-5.14, m, H3, 1H; 5.19, dd, *J* =9.5, 9.2Hz, G3, 1H; 5.07, dd, *J* =9.9, 9.5Hz, G4, 1H; 5.01, dd, *J* =9.2, 7.7Hz, G2, 1H; 4.77, q, *J* =6.5Hz, 4,3-4.0, m, G6a+G6b, 2H; 3.7, m, G5, 1H; 2.5-2.4, m, methylene H, 2H; 2.07, s, 1.5H; 2.04, s, 1.5H; 2.03, s, 1.5H; 2.02, s, 1.5H; 2.01, s, 1.5H; 2.00, s, 3H; 1.99, s, 1.5H; 1.95, s, 1.5H, all acetoxy methyls of two diastereoisomers; 1.86, s, H13, 3H; 1.58-1.52, m, methylene H, 2H; 1.47, d, *J* =6.5Hz, H10, 3H; 1.16, 1.15, 1.14, 1.12, each,s, H11+H12 of each diastereoisomer, 1.5H. (Column b) Diastereoisomer 1: m/z; 520 (<1), 460 (<1), 358 (<1), 347 (<1), 331 (<1), 188 (17), 175 (30), 173 (36), 172 (20), 159 (100), 157 (42), 131 (18), 109 (17), 91 (8). (Column b) Diastereoisomer 2: m/z; 520 (<1), 460 (<1), 358 (<1), 347 (<1), 331 (<1), 188 (8), 175 (25), 173 (42), 172 (38),

159 (100), 157 (50), 131 (20), 109 (17), 91 (10). ν_{max}; 2900, 2840, 2250, 1750, 1600, 1360, 1250, 1230 cm^{-1}.

(E)-7-Oxo-5,8-megastigmadien-3-yl-tetra-O-pivaloyl-β-D-glucopyranoside (39b).

Attempted synthesis of the β-D-glucopyranoside (39b).by the method of Kunz yielded many products depending on the reaction time. After 15 minutes at room temperature two β-D-glucopyranosides were obtained, (39b) and (157b), together with Nazarov cyclization product (157) and starting material. After 50 minutes, a trace of starting material was obtained, however, the β-D-glucopyranoside (157b) and its aglycone (157), were both obtained as the major products and as white solids. Compounds were isolated using the chromatotron and a solvent gradient of diethyl ether / petroleum ether. Compound (157) has been fully characterized and identified by Ohloff[39]. ^1H n m r (60MHz / $CDCl_3$); δ: 5.8, b, 1H; 4.4-3.8, m, 1H; 2.8, b, 1H; 2.1, s, 3H; 1.9-1.4, m, 4H; 1.3, s, 3H; 1.2, s, 3H; 1.01, s, 3H; 0.8, s, 3H. m p 85-90°C, (Lit[39] 109-110°C for cis and 95-96°C for trans isomer). (Column a) compound (157), diastereoisomer 1: m/z; 210 (<1), 209 (4), 208 (21), 193 (14), 176 (7), 175 67), 147 (7), 123 (41), 111 (31), 110 (100), 109 (55), 108 (17), 105 (12), 95 (34), 91 (18), 81 (24), 79 (24), 77 (13), 67 (9). (Column a) compound (157) diastereoisomer 2: m/z; 210 (<1), 209 (3), 208 (16), 193 (10), 175 (42), 147 (8), 123 (33), 111 (24), 110 (100), 109 (50), 108 (18), 105 (12), 95 (26), 91 (19), 82 (18), 81 (39), 79 (22), 77 (13), 67 (10). ν_{max}; 3350, 1675, 1620 cm^{-1}.

β-D-Glucopyranoside (39b) as a mixture of two diastereoisomers: ^1H n m r (300MHz / $CDCl_3$); δ: 6.70, dq, J =16.2, 6.7Hz, H9, 1H; 6.14, d, J =16.2Hz, H8, 1H; 5.32, dd, J =9.5, 9.5Hz, G3, 1H; 5.08, dd, J =9.8, 9.5Hz, G4, 1H; 4.98, dd, J =9.5, 7.5Hz, G2, 1H; 4.68, appears as a triplet,, J =7.5Hz, G1, 1H; 4.27, d, J =11.9Hz, G6a, 1H; 4.04-3.96, m, G6b+H3, 2H; 3.78-3.72, m,

G5, 1H; 2.35-1.85, m, methylene H, 2H; 1.91, d, *J* =6.7Hz, H10, 3H; 1.85-1.54, m, methylene H, 2H; 1.22, 1.17, 1.16, 1.13, 1.12, each, s, tert-butyl methyl, 9H; 1.13, 0.97, 0.95, each, s, H11+H12, 1.5H. The fourth 1.5H signal was obscured. Solid probe m/z; 706 (<1), 550 (1), 500 (6), 499 (20), 210 (33), 192 (11), 191 (53), 190 (39), 149 (13), 137 (6), 134 (7), 133 (8), 126 (8), 122 (7), 121 (40), 120 (13), 119 (9), 118 (22), 108 (13), 107 (9), 105 (10), 104 (7), 103 (11), 98 (7), 97 (11), 95 (6), 91 (11), 86 (10), 85 (100), 83 (10), 82 (6), 81 (16), 79 (6), 77 (15), 71 (7), 70 (12), 69 (75), 59 (6), 58 (22). β-D-Glucopyranoside (157b): (Found 706.4286. $C_{39}H_{62}O_{11}$ requires 706.4292), m p 135-138°C. ^{1}H n m r (300MHz / $CDCl_3$); δ: 5.76, s, H8, 1H; 5.31, dd, *J* =9.6, 9.3Hz, G3, 1H; 5.04, dd, *J* =9.8, 9.3Hz, G4, 1H; 4.99, dd, *J* =9.6, 8.0Hz, G2, 1H; 4.62, and 4.61, each, d, *J* =8.0Hz, G1, 0.5H; 4.30, dd, 12.2, 1.7Hz, G6a, 1H; 4.2-4.1, m, H3, 1H; 3.99, dd, *J* =12.2, 6.3Hz, G6b, 1H; 3.71, ddd, *J* =9.8, 6.3, 1.7Hz, G5, 1H; (the spectrum above δ 2.1 was too complicated to be assigned as it consists of a mixture of four diastereoisomers). ν_{max}; 2960, 1740, 1680, 1620, 1480, 1395, 1365, 1280, 1140 cm^{-1}.

3-Hydroxy-9-methoxy-5-megastigmen-7-one (158).

This compound was obtained as the major product from the reaction of 3-hydroxy-β-damascone (39) with sodium methoxide in methanol (0.1M) at room temperature for 42hours. The proton spectrum was complex and only the major peaks are recorded.

^{1}H n m r (60MHz / $CDCl_3$); δ: 3.3, s,methoxy H, 3H; 1.9, s, H13, 3H; 1.4, d, *J* =6Hz, H10, 3H; 1.2, s, 6H. m/z; 240 (5), 225 (8), 208 (8), 193 (5), 175 (4), 168 (13), 167 (75), 151 (18), 121 (47), 107 (8), 105 (10), 99 (8), 95 (22), 93 (24), 79 (16), 69 (25), 59 (100), 55 (28), 43 (40). ν_{max}; 3600, 2840, 2820, 1720, 1680,1360 cm^{-1}.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (121)[138]

The imidate (121) was prepared by both the method of Schmidt[138] and also by that of Tavecchia.[140] Two isomers were evident by high field ^{1}H n m r with the (Z) isomer existing as the minor isomer.
^{1}H n m r (300MHz / $CDCl_3$); δ: 8.7, s, NH, (E), 0.6H; 8.58, s, NH, (Z), 0.4H; 6.64, d, *J* =3.7Hz, G1, (Z), 0.4H; 6.57, d, *J* =3.7Hz, G1, (E), 0.6H; 5.57, dd, *J* =10.1, 9.7Hz, G3, 1H; 5.19, dd, *J* =9.9, 9.7Hz, G4, 1H; 5.14, dd, *J* =10.1, 3.7Hz, G2, 1H; 4.34-4.19, m, G6a+G5, 2H; 4.16-4.11, m, G6b, 1H; 2.2-2.0, seven singlets representing eight acetate groups. FAB: m/z; 498(<1), 496 (<1), 494 (14), 492 (14), 438 (18), 436 (18), 434 (50), 432 (50), 331 (100). ν_{max}; 3500, 3475, 3400, 3350, 1740, 1670, 1580, 1360, 1220, 1140, 1040, 820, 790 cm^{-1}.

2,3,4,6-Tetra-O-acetyl-O-trichloroacetyl-α-D-glucopyranose (160)[163]

This product was obtained as a solid by-product because imidate (121) was found to be very hygroscopic. m p 131°C (Lit. [163] 131°C). ^{1}H n m r (60MHz / $CDCl_3$); δ: 6.4, d, J =4Hz, 1H; 5.9-5.0, m, G3+G4+G2, 3H; 4.6-4.0, m, G5+G6a+G6b,3H; 2.17, s, 6H; 2.12, s, 6H. It tested positive for halogen with copper wire heated under a naked flame.

3,5,5-Trimethyl-3-cyclohexenyl-tetra-O-β-D-acetyl-glucopyranoside (107c).

The alcohol (107) (14mg, 0.1mmol) and imidate (121) (70mg, 0.14mmol) in dichloromethane (3ml) were heated under reflux over 4Å sieves for one hour.[133] The reaction mixture was cooled to room temperature and boron trifluoride etherate (17μl, 0.1mmol) was added and the mixture was stirred

for a further 30 minutes. GC was used to monitor the reaction and no change was seen after 2hours. The reaction mixture was diluted with dichloromethane (10ml) which was washed with a saturated solution of sodium hydrogen carbonate (2x20ml), water (10ml), brine (10ml), dried ($MgSO_4$) and evaporated to yield a clear oil. Chromatography[158], ethyl acetate / petroleum ether, yielded the solid tetra-O-acetyl-β-D-glucopyranoside (107c).(10mg, 21%), m p 95-97°C. ^1H n m r (300MHz / $CDCl_3$); δ: 5.19, dd, *J* =9.8, 9.3Hz, G3, 1H; 5.05, obs, H4, 1H; 5.04, dd, *J* =9.8, 9.8Hz, G4, 1H; 4.94, dd, *J* =9.8, 8.3Hz, G2, 1H; 4.59, d, *J* =8.3Hz, G1, 1H; 4.22, dd, *J* =12.2, 2.7Hz, G6a, 1H; 4.11, dd, *J* =12.2, 5.4Hz, G6b, 1H; 3.93-3.83, m, H1, 1H; 3.68, ddd, *J* =9.8, 5.4, 2.7Hz, G5, 1H; 2.19, dd, *J* =16.6, 5.4Hz, H2, 1H; 2.05-2.00, m, methylene H, 1H; 2.05, 2.01, 1.99, 1.97, s, acetoxy methyl, 3H; 1.59, s, vinyl methyl, 3H;,1.29-1.08, m, methylene H, 2H; 0.96, 0.93, each, s, C5+C5 methyl, 3H. ^{13}C n m r (75.47MHz / $CDCl_3$); δ: 170.6; 170.3; 169.4; 169.2; 131.3; 128.8; 99.8; 75.1; 72.9; 71.8; 71.6; 68.7; 62.2; 42.5; 37.7; 33.7; 31.3; 29.5; 23.2; 20.7; 20.6. ν_{max}; 1740, 1430, 1360, 1240, 1160, 1040 cm^{-1}.

(E)-7-Oxo-5,8-megastigmadien-3-yl-tetra-O-acetyl-β-D-glucopyranoside (39c).

Under strictly anhydrous conditions and a nitrogen atmosphere, 3-hydroxy-β-damascone (39) (130mg, 0.63mmol) in dichloromethane (1ml) was added slowly over 1hour to a solution of imidate (121) (370mg, 0.75mmol) and boron trifluoride etherate (79μl) in dichloromethane (2ml) at (-10-0°C). The reaction mixture changed from a colourless to red during addition. After addition, the reaction mixture was washed with saturated solution of sodium hydrogen carbonate (2x20ml), water (20ml), brine (10ml), dried ($MgSO_4$) and evaporation under reduced pressure yielded a clear oil. Chromatographic[86] separation, 100% diethyl ether, yielded transesterified

product (93), transesterified cyclized product (157c) and β-glycoside (39c) (20mg, 6%) (Found 538.2403. $C_{27}H_{38}O_{11}$ requires 538.2414). The order of addition was also reversed, however, the result was identical. An alternative and more consistent method involved the Ackermann procedure[113] where no Lewis acid is used.

3-Hydroxy-β-damascone (39) (600mg, 2.9mmol) yielded the β-glycoside (39c) (73.0mg, 15%). Work-up involved filtering the mixture through a pad of Kenite, then, washing the organic ethereal layer with a saturated solution of sodium hydrogen carbonate (2x20ml), water (20ml), brine (10ml), dried ($MgSO_4$) and evaporation under reduced pressure to yield a clear oil which was chromatographed directly. GC / MS revealed that in both methods α-glycoside (39d) was also formed, fortunately, in low yield.

β-glycoside (39c): ^1H n m r (300MHz / $CDCl_3$); δ: 6.69, dq, *J* =15.7, 6.9Hz, H9, 1H; 6.12, dd, *J* =15.7, 1.5Hz, H8, 1H; 5.20, dd, *J* =9.7, 9.6Hz, G3, 1H; 5.06, dd, *J* =10.1, 9.6Hz, G4, 1H; 4.96, dd, *J* =9.7, 7.9Hz, G2, 1H; 4.62, and 4.60, each, d, *J* =7.9Hz, G1, 0.5H; 4.23, dd, *J* =12.3, 5.3Hz, G6a, 1H; 4.13, dd, *J* =12.3, 2.5Hz, G6b, 1H; 4.05-3.9, m, H3, 1H; 3.67, ddd, *J* =10.1, 5.3, 2.5Hz, G5, 1H; 2.4-2.1, m, methylene H, 2H; 2.05, 2.04, 2.02, 2.01, each, s, acetoxy methyl, 1.5H; 1.99, 1.97, each, s, acetoxy methyl, 3H; 1.9, dd, *J* =6.9, 1.5Hz, H10, 3H; 1.51, s, H13, 3H; 1.48-1.35, m, methylene H, 2H; 1.13, 0.95, each s, H11+H12, 3H. ^{13}C n m r (75.47MHz / $CDCl_3$); δ: 201.1; 170.5; 170.2; 169.3; 169.1; 146.1; 140.5; 139.6; 134.3; 128.1; 127.1; 99.7; 73.4; 73.3; 72.8; 71.8; 71.5; 68.6; 62.3; 62.1; 61.4; 45.2; 44.2; 38.8; 37.5; 36.0; 35.8; 29.5; 28.9; 20.9; 20.5; 18.3. (Column a) Diastereoisomer 1: m/z; 538 (<1), 331 (1), 190 (42), 175 (15), 121 (98), 107 (39), 69 (100). (Column a)Diastereoisomer 2: m/z; 538 (<1), 331 (1), 190 (38), 175 (13), 169 (18), 121 (82), 107 (50), 69 (100). ν_{max}; 2950, 2930, 2850, 1750, 1640, 1430, 1360, 1220, 1040 cm^{-1}

α-glycoside (39d) (obtained as a mixture with the glucose tetra acetate):

^{1}H n m r (300MHz / $CDCl_3$); δ: 6.69, dq, *J* =15.7, 6.9Hz, H9, 1H; 6.10, dd, *J* =15.7, 1.6Hz, H8, 1H; 5.31, apparent triplet, *J* =3.5Hz, G1, 1H; 4.05-3.9, m, H3, 1H; 1.89, dd, *J* =6.9, 1.6, H13, 3H; 1.10, 0.93, each, s, H11+H12, 3H. were the major signals observed. (Column a) Diastereoisomer 1: m/z; 538 (<1), 497 (<1), 331 (1), 190 (36), 149 (18), 121 (100), 110 (38), 69 (78). (Column a) Diastereoisomer 2: m/z; 538 (<1), 497 (<1), 331 (2), 190 (38), 149 (18), 121 (100), 110 (50), 69 (100).

Compound (157c) was obtained as a by-product, tentatively assigned from its retention time and interpretation of its mass spectral data. It exits as a diastereoisomeric mixture. (Column a) Diastereoisomer 1: m/z; 235 (1), 190 (12), 175 (62), 161 (10), 149 (10), 147 (18), 135 (8), 123 (12), 110 (100), 109 (33), 81 (75), 55 (8). (Column a) Diastereoisomer 2: m/z; 235 (1), 190 (12), 175 (48), 161 (8), 147 (19), 133 (5), 122 (16), 110 (100), 109 (32), 82 (15), 81 (78), 55 (5).

(E)-7-Oxo-5,8-megastigmadien-3-yl-β-D-glucopyranoside (39a).

The β-glycoside (39c) (37.8mg, 0.07mmol) in tetrahydrofuran (6ml) was kept at 25°C. Sodium hydroxide (0.1M, 2.6ml, 0.26mmol) was added and the mixture was allowed to stand for 2hours. The solvent was evaporated off under reduced vacuum and the standard work-up, described for isolation of β-glycosides, yielded the β-glycoside (37a) (11.9mg, 40%).

^{13}C n m r (75.47MHz / $CDCl_3$); δ: 201.3; 146.4; 142.8; 140.4; 134.3; 130.5; 127.4; 101.6; 101.2; 76.3; 75.6; 73.5; 73.3; 72.5; 69.8; 61.7; 45.5; 44.3; 39.2; 37.6; 36.0; 29.7; 29.2; 21.0; 18.4. FAB: m/z; 371 (4), 222 (4), 209 (38), 209 (100), 191 (57), 173 (16), 149 (7), 135 (10), 125 (7), 107 (5). Negative ion: m/z; 369 (100), 324 (6), 221 (10), 179 (25), 161 (15), 99 (9), 89 (10), 33 (5). Attempted deacetylation using the method of Fiandor[143], which involves ammonia, was attempted on (E)-7-oxo-5,8-megastigmadien-3yl acetate (93). However, this failed as conjugate addition occurred to and

deacetylation. The product was tentatively identified as the conjugate adduct, because the retention time on t.l.c. (Rf) was different from that of 3-hydroxy-β-damascone (39). ^{1}H n m r revealed total upfield shift of H3 due to deacetylation. Major peaks only were identified.

^{1}H n m r (60MHz / $CDCl_3$); δ: 4.0, m, 1H; 3.4, m, 1H; 2.4-1.8, m, 2H; 1.6, s, 3H; 1.2, s, 3H; 1.07, s, 3H.

Synthesis of 3,5-dihydroxy-6,7-megastigadien-9-yl-β-D-glucopyranoside (43c)

Attempted glycosylation of allenic triol (43a) using the Kunz method failed. The predominant product obtained was 3-hydroxy-β-damascone (39) in 78% yield.

Use of the Ackermann method was also unsuccessful due to solubility problems in diethyl ether. A change of solvent to tetrahydrofuran overcame the solubility problems, however, the major products obtained were ortho esters.

The allene (43a) (100mg, 0.44mmol) in tetrahydrofuran (3.0ml) was added to anhydrous calcium sulphate (20.1mg, 0.15mmol), α-acetobromoglucose (108) (60.6mg, 0.15mmol), silver triflate (31,7mg, 0.14mmol) and sodium hydrogen carbonate (12.4mg, 0.15mmol) and the mixture was stirred at room temperature for 30 minutes. Additional tetrahydrofuran (5ml) was added and stirring was continued at room temperature for 30 minutes. The reaction mixture was filtered through a pad of Kenite and diethyl ether (20ml) was added to the solution. This solution was washed with a saturated solution of sodium hydrogen carbonate (2x10ml), water (10ml), the ethereal layer was dried ($MgSO_4$) and evaporated to yield an oil. Four products were observed by t.l.c. using the vanillin spray reagent described in the general section of the experimental. Rf 0.7, 0.6, 0.5, 0.4, 100% ethyl acetate. Further purification by h.p.l.c. and high field ^{1}H n m r studies revealed that two of the

four components were ortho esters.Rf 0.5 ortho ester (164) (12mg, 5%)and Rf 0.4 ortho ester (165) (7.4mg, 3%). The product at Rf 0.7 was tentatively been characterized as an unknown α-glycoside.

Ortho ester (164): ^{1}H n m r (300MHz / $CDCl_3$); δ: 5.69, d, *J* = 5.4Hz, G1, 1H; 5.35, d, *J* =5.9Hz, H8, 1H; 5.16, m, G3, 1H; 4.87, dd, *J* =9.8, 2.5Hz, G4, 1H; 4.35-4.25, m, G2+H9, 2H; 4.17-4.16, m, G6a+G6b, 2H; 3.91, ddd, *J* =9.8, 3.9, 3.9, G5, 1H; 2.09, 2.075, 2.071, each, s, acetoxy methyl, 3H; 1.75, s, ortho ester methyl, 3H; 1.32, 1.30, 1.04, each, s, tertiary methyl, 3H; 1.25, d, *J* =6.4Hz, H10, 3H. ^{13}C n m r (75.47MHz / $CDCl_3$); δ: 197.5; 170.7; 169.6; 169.2; 121.7; 117.5; 100.0; 97.0; 73.2; 70.3; 68.2; 67.1; 66.6; 66.3; 63.2; 47.6; 47.1; 35.1; 32.2; 31.2; 29.3; 23.3; 22.0; 21.8; 19.8. FAB m/z; 579 (<1), [M+Na], 539 (<1), 479 (<1), 331 (10), 271 (8), 242 (5), 229 (8), 211 (5), 191 (11), 169 (75), 150 (100), 127 (23), 109 (47). ν_{max}; 3600, 3450, 2950, 2850, 1950, 1740, 1440, 1360, 1220, 1040 cm^{-1}

Ortho ester (165): ^{1}H n m r (300MHz / $CDCl_3$) (This spectrum was complex and could not be fully assigned but included) ; δ: 5.67, d, *J* =4.4Hz, G1, 1H; 5.29, d, *J* =5.8Hz, H8, 1H; 5.1, m, G3, 1H; 4.87,brd, *J* =9.8Hz, G4, 1H; 4.3-4.1, m, 4H; 3.9-3.8, m, G5, 1H; 2.22, m, 1H; 2.18, m, 1H; 2.10, 2.08, 2.07, each, s, acetooxy methyl, 3H; 1.73, s, ortho ester methyl, 3H; 1.35, 1.33, 1.27, each s, 3H; 1.24, m; 1.06, s; 1.05, s; 1.04, s; ^{13}C n m r (75.47MHz / $CDCl_3$); δ: 198.1; 170.6; 169.6; 169.2; 121.4; 117.1; 98.2; 98.1; 96.9; 73.1; 73.0; 72.6; 70.2; 68.3; 67.1; 64.2; 63.1; 49.4; 48.8; 35.5; 35.3; 32.4; 32.1; 31.4; 31.3; 29.7; 29.3; 22.2; 22.1; 21.9; 21.7; 21.5; 20.7. FAB m/z; 579 (<1), [M+Na], 539 (<1), 515 (<1), 493 (<1), 459 (<1), 421 (<1), 331 (43), 289 (5), 271 (15), 229 (10), 191 (13), 169 (100), 127 (25), 109 (53). ν_{max}; 3600, 3450, 2950, 2900, 2850, 1950, 1740, 1440, 1360, 1220, 1040 cm^{-1}.

α-allenic glycoside (43f): ^{1}H n m r (300MHz / $CDCl_3$) This spectrum was complex and was not totally assigned. Signals included; δ:6.22, d, *J* =3.4Hz, G1, 1H; 5.25, dd, *J* =9.8, 9.8Hz, G4, 1H; 5.09, dd, *J* =10.3, 9.8Hz,

G3, 1H; 4.85, dd, J =10.3, 3.4Hz, G2, 1H; 4.26, dd, J =12.2, 4.4, G6, 1H; 2.18, 2.08, 2.06, 2.03, each, s, acetoxy methyl, 3H; 1.35 s; 1.34,s; 1.27, d, J =6.4Hz, H10, 3H; 1.26, s; 1.23,s; 1.07,s. ν_{max}; 3600, 3450, 1950, 1240, 1360, 1250, 1220, 1040 cm^{-1}.

Acetylation of Ortho Esters (164) and (165)

Ortho ester (164) (4.9 mg, 8.8 µmol) was acetylated using 1.5 equivalents of acetic anhydride (1.25 µl, 1.32 µmol) in pyridine (1.42 µl, 17.6 µmol) overnight . Chromatographic separation yielded the product (164a). Rf 0.54, 70% ethyl acetate / petroleum ether.

Ortho ester (164a): ^{1}H n m r (300MHz / $CDCl_3$); δ: 5.70, d, J =5.4Hz, G1, 1H; 5.37-5.33, m, H8+H9, 2H; 5.16, 5.17, each, dd, J =2.6, 2.7, G3, 0.5H; 4.88, dd, J =9.5, 2.7, G4, 1H; 4.38, 4.37, each, dd, J =5.4, 2.6, G2, 0.5H; 4.3-4.19, m, H3, 1H; 4.18-4.17, m, G6a+G6b, 2H; 3.93, ddd, J =4.2, 4.2, 9.5, G5, 1H; 2.15, 2.10, 2.08, 2.00, each, s, acetoxy methyl 3H; 1.75, s, ortho ester methyl, 3H; 1.32, s, 3H; 1.30, s, 3H; 1.27, d, J =6.4, 3H; 1.23, s, 3H; 1.03, s, 3H. FAB m/z; 599 (17), 539 (5), 461 (5), 390 (5), 331 (17), 289 (5), 251 (63) 232 (7), 209 (100), 191 (30), 169 (10), 133 (12), 117 (5).

Acetylation of ortho ester (165) under identical conditions gave many products and was discontinued.

3-Hydroxy-5,6-epoxy-7-megastigmyn-9yl-tetra-O-pivaloyl-β-D-glucopyranoside [(166) and (167)]

The β-D-Glycoside (58d) (20mg, 39.5 µmol)in dichloromethane (2ml) was treated with m-chloroperoxybenzoic acid (6.8mg) in dichloromethane (2ml) at room temperature and the reaction mixture was stirred overnight. Sodium hydrogen carbonate (0.5g) was added to the mixture followed by addition of sodium thiosulphate (20mg) and the mixture was stirred for 25 minutes ensuring the pH remained basic. The solid residues were filtered off and the

dichloromethane layer was separated, dried ($MgSO_4$) and evaporated to yield of an oil (20mg, quantitative). Rf 0.3, 40% ethyl acetate / petroleum ether, developing a chocolate brown spot with vanillin spray. This material was used directly in the next step as only one spot was evident by t.l.c. ^1H n m r (60MHz / $CDCl_3$); δ: 4.2, m; 2.0-1.8, m; 1.5,s, 3H; 1.27, s, 9H; 1.23, s, 9H; 1.17, s, 9H; 1.13, s, 9H. ν_{max}; 3400, 1730, 1600, 1390, 1360, 1260, 1140, 850, 800 cm^{-1}.

3-Hydroxy-5,6-epoxy-7-megastigmyn-9yl-β-D-glucopyranoside [(168) and (169)]

β-D-Glycoside (58b) (14.3mg, 38.6 μmol) in tetrahydrofuran (2ml) was treated with m-chloroperoxybenzoic acid (7.0mg) in tetrahydrofuran (2ml) at room temperature and the mixture was allowed to stir overnight. Sodium hydrogen carbonate (0.5g) was added followed by a saturated solution of sodium thiosulphate (20mg) and the mixture was stirred for 25 minutes ensuring the aqueous layer remained basic. The solution was filtered and the aqueous tetrahydrofuran was evaporated to dryness. Isolation of product by using the SEP-PAK yielded 12.5mg, 84% of cis and trans epoxides [(168) and (169)]. This derivative also gave a chocolate brown colour with vanillin spray.

3,5-Dihydroxy-6,7-megastigmadien-9-yl-β-D-glucopyranoside (43c).

Method 1.

The epoxide mixture (168) and (169) (12.5mg, 32.4μmol) was refluxed in tetrahydrofuran (2ml) containing 5 equivalents of lithium aluminium hydride for 6 hours. The mixture was then cooled and the excess of lithium aluminium hydride was quench by the addition of wet sodium sulphate. The

pH was adjusted to 7 and isolation of the glycoside (43c) in low yield (3mg, 24%) was achieved by use of SEP-PAK. The material gave a characteristic pink colour (characteristic of allenes) on t.l.c. with vanillin spray. Rf 0.26, 0.21, using the lower phase of the following mixture: chloroform / methanol / water ratio of 7 : 13 : 8.

Method 2

The epoxide mixture (166) and (167) (20.5mg, 40.5µmol) was refluxed in tetrahydrofuran (2ml) containing 10 equivalents of lithium aluminium hydride for 6 hours. The mixture was then cooled and the excess of lithium aluminium hydride was quench by the addition of wet solid sodium sulphate. The pH was adjusted to 7 and isolation of the glycoside (43c) was achieved by use of the SEP-PAK. However the yield was low, (4mg, 25%). The material gave the characteristic pink colour on t.l.c. with the use of vanillin spray. Rf 0.26, 0.21, Using the lower phase of the following mixture: chloroform / methanol / water ratio of 7 : 13 : 8. Both methods gave identical products by t.l.c.

CI (NH_4^+): m/z; 388 (1), 246 (10), 244 (9), 210 (14), 193 (38), 175 (23), 160 (15), 156 (4), 110 (9), 52 (100). Daughter ion 407, [$M+H_2O$]: m/z; 406 (10), 371 (5), 353 (7), 227 (26), 209 (48), 192 (26), 191 (50), 175 (100), 173 (20), 149 (5), 133 (5), 119 (5). Daughter ion 389: m/z; 209 (5), 191 (100), 175 (20), 173 (10), 149 (5), 133 (5).

A small amount of the diastereoisomeric mixture was acetylated by the standard method. Although the ^1H n m r spectrum of the product was very complex the anomeric protons which appeared as a 1:2:1 signal centred at δ 4.70 with an average coupling of 8.1Hz. ν_{max}; 1750, 1360, 1250, 1220, 1030 cm^{-1}. Naf[156] reported the acetylated allenic glucopyranoside (43e) at 4.60 with a coupling of 8.0Hz.

3,5-Bis-tetrahydropyranyloxy-7-megastimyn-6-ol (56)

The acetylenic triol (55a) (50mg, 0.22mmol) in dichloromethane (4ml) was stirred with dihydropyran (56mg, 0.67mmol) and pyridinium p-toluenesulphonate[164] (12mg, 0.044mmol) at reflux for 3hours. The reaction mixture was cooled and diethyl ether (10ml) was then added and the organic layer was washed with brine (50%, 10ml) and then water (5ml). The organic layer was dried ($MgSO_4$) and evaporation yielded a colourless oil (70mg, 80%). Two spots were present by t.l.c. Rf 0.35, 0.43, 50% diethyl ether / petroleum ether. Separation was achieved by column chromatography using an ethyl acetate / petroleum ether solvent gradient. Isolation of the two diastereoisomers was achieved.

^{1}H n m r (60MHz / $CDCl_3$); δ: 5.0-4.3, m, 3H; 4.25-3.0, m, 6H; 2.5-0.7,m,29H.

Diastereoisomer 1: m/z; 292 (<1), 209 (1), 135 (2), 122 (5), 121 (2), 109 (2), 107 (2), 95 (2), 91 (2), 86 (3), 85 (100), 83 (2), 81 (2), 79 (2), 69 (3), 67 (7), 57 (10), 56 (3), 55 (10), 53 (2), 43 (25), 41 (22), 40 (9), 39 (2).

Diastereoisomer 2: m/z; 292 (<1), 152 (2), 135 (2), 123 (2), 122 (5), 121 (2), 109 (2), 108 (2), 107 (2), 95 (2), 93 (2), 91 (2), 86 (3), 85 (100), 83 (2), 81 (2), 79 (2), 69 (3), 67 (8), 57 (10), 56 (3), 55 (10), 53 (2), 43 (25), 41 (24), 39 (2).

ν_{max}; 3450, 1020 cm^{-1}.

5.3 Experimental. Chapter 4.

General

Hydrolytic studies involved the reaction of the alcohol or glycoside in a 10% aqueous ethanolic solution. The hydrolyses were conducted at pH 1 or 3, in sealed ampoules. Before sealing the ampoules, the solutions were deoxygenated by a stream of oxygen-free[162] dry nitrogen for 15 seconds. All hydrolyses were done in duplicates. A constant temperature oven was used for experiments conducted at 50° over a long period of time. Constant temperature oil baths were used at 50, 80 and 100°C for hydrolyses carried out over shorter periods of time.

The pH solutions were made up as follows:

pH=1 (+)- Tartaric acid was dissolved in water and the pH adjusted with sulphuric acid (1M).*

pH=3 Potassium hydrogen tartrate was dissolved in water and the pH adjusted with sulphuric acid (1M).

* Measurement was obtained with a pH meter, standardized with a reference solution[165].

The following amounts of alcohol, glycoside and internal standard were used:

Geraniol (1) and its β-D-glucopyranoside (1a) (1mg / ml) with addition of 2-phenyl ethanol (1mg / ml) after hydrolysis, but before any work-up.

Model enyne alcohol (99) and its β-D-glucopyranoside (99a) (1mg / ml) with addition of butyl benzene (1mg / ml) after hydrolysis, but before any work-up.

3,5,5-Trimethyl-3-cyclohexen-ol (107) and its β-D-glucopyranoside (107a) (1mg / ml) with addition of butyl benzene (1mg / ml) after hydrolysis.

Acetylenic triol (55a) (0.5mg / ml) with addition of octadecanol (0.5mg / ml) after hydrolysis. This reaction was also done in 25% aqueous

tetrahydrofuran solution.

Enynediol (58a) (0.5mg / ml) with addition of octadecanol (0.5mg / ml) and its β-D-glucopyranoside (58b) (2mg / ml) with addition of octadecanol (0.5mg / ml) after hydrolysis.

Allenic triol (43a) (0.5mg / ml) with addition of octadecanol (0.5mg / ml) after hydrolysis.

3-Hydroxy-β-damascone (39) (5mg / ml) was treated at pH 1 and 3 in deuterium oxide (D_2O). Stock solutions of D_2O at pH 1 and 3 were made up as follows: Tartaric acid was dissolved in D_2O and the water was evaporated under reduced pressure. This was repeated twice. Finally, tartaric acid (d_4) was dissolved in D_2O and the pH adjusted with D_2SO_4 / potassium carbonate to pH 3. The pH 1 solution was prepared similarly. The alcohol (39) was dissolved in deuterated ethanol (EtOD) and the solvent evaporated. This was repeated three times before a 10% D_2O / EtOD solution at the adjusted pH was prepared for hydrolysis.

The β-D-glucopyranoside (39a) (1mg / ml) was treated at pH 1.1 and 3 in 10% aqueous ethanolic solution followed by addition of octadecanol (1mg / ml) after hydrolysis.

Bis tetrahydropyranyl ethers (56) (1mg / ml) followed by addition of octadecanol (1mg / ml) after hydrolysis.

Standard work-up of hydrolyses involved cooling the ampoules followed by addition of internal standard. The solution was then extracted with dichloromethane (2x1ml) and the dichloromethane layer was washed with saturated sodium hydrogen carbonate (1ml), dried ($MgSO_4$), filtered and injected directly into the GC / MS for analysis. Only for the hydrolyses of acetylenic triol (55a) and enynediol (58a) was the dichloromethane extract concentrated through a column of Fenske's helices prior to analysis by GC / MS.

Analyses were performed on a Varian 3300 gas chromatogram / Finnigan

4021 TSQ mass spectrometer.

The columns used were either: a) J & W DB 1701 or b) J & W DB5 each of which is a 30metre fused silica column (0.25 mm i.d. and 0.25 μm film thickness) with helium carrier gas at linear velocity of 40 cm / sec. Injections were made with a split injector at 200°C and a split ratio of 1:10. Flame ionization detection was used for compound analysis with a Hewlett Packard auto integration system. In addition, GC / MS ion volume peak areas were used in most cases.

Hydrolysis of Geraniol (1) and its β-D-Glucopyranoside (1a)

Geraniol (1) and its β-D-glucopyranoside (1a) were analyzed using column a). The column was held at 50°C for one minute, then programmed at 4°C / min to 250°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV.

All compounds in Table 4.1 have been identified by Strauss[147] and their mass spectra are shown below.

Entry

1. (171): m/z; 139 (80),121 (36), 109 (30), 93 (20), 81 (50), 71 (100), 69 (45), 68 (90), 67 (30), 56 (38), 55 (20).

2. (172): m/z; 136 (12), 93 (100), 91 (25), 84 (19), 80 (15), 69 (98), 67 (19), 49 (14).

3. (173): m/z; 136 (35), 121 (30), 107 (28), 94 (30), 93 (61), 92 (25), 91 (22) 81 (22)79 (27), 68 (100), 67 (75), 53 (18)

4. (174): m/z; 136 (11), 121 (13), 105 (15), 93 (100), 92 (38), 91 (45), 80 (10),79 (30), 77 (33), 53 (10).

5. (175): m/z; 136 (10), 121 (16), 105 (10), 94 (15), 93 (100), 92 (26), 91 (61), 81 (10), 80 (38), 79 (40), 77 (37), 67 (10), 53 (10).

6. (176): m/z; 136 (88), 121 (100), 105 (18), 93 (100), 91 (47), 79 (50), 77 (30), 49 (25).
7. (3): m/z; 154 (<1), 136 (10), 121 (18), 93 (60), 92 (10), 91 (9), 83 (15), 80 (27), 71 (100), 69 (40), 68 (10), 67 (12), 55 (40).
8. (5): m/z; 139 (14), 136 (68), 121 (65), 95 (18), 93 (76), 92 (50), 91 (16), 81 (50), 79 (16), 68 (16), 67 (22), 59 (100), 55 (16).
9. (2): m/z; 136 (18), 123 (20), 121 (26), 99 (18), 93 (20), 81 (18), 71 (19), 69 (100), 68 (30), 67 (21), 57 (19).
10. (1): m/z; 154 (<1), 136 (5), 123 (11), 111 (8), 93 (10), 80 (5), 70 (6), 69 (100), 68 (18), 55 (5).
11. (11): m/z; 139 (10), 121 (18), 84 (16), 83 (10), 81 (29), 71 (100), 69 (26), 68 (50), 67 (12), 56 (29), 55 (10).
12. Trans (178): m/z; 139 (20), 96 (50), 81 (100), 71 (20), 69 (17), 59 (38), 55 (12).
13. Cis (177): m/z; 157 (8), 139 (35), 125 (18), 121 (22), 97 (24), 96 (75), 82 (22), 81 (100), 71 (40), 69 (22), 67 (22), 59 (85), 55 (23).
14. (179): m/z; 136 (40), 121 (37), 93 (50), 84 (35), 83 (68), 81 (62), 71 (48), 70 (37), 69 (100), 68 (70), 59 (68), 55 (28).
15. (180): m/z; 136 (30), 121 (35), 93 (30), 83 (50), 81 (40), 71 (35), 70 (35), 69 (100), 67 (52), 59 (60).

Hydrolysis of 3,5,5-Trimethyl-3-cyclohexen-ol (107) and its β-D-Glucopyranoside (107a)

3,5,5-Trimethyl-3-cyclohexen-ol (107) and its β-D-glucopyranoside (107a) were analyzed using column a). The column was held at 50°C for one minute, then programmed at 4°C / min to 250°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV.

The compounds (181a,b,c,d) and (182a,b) were tentatively assigned based on interpretation of their mass spectral data.

Compound

(181a): m/z; 122 (37), 107 (100), 91 (50), 79 (14), 49 (17).

(181b): m/z; 122 (38), 107 (100), 105 (18), 91 (52), 84 (25), 79 (17), 77 (12), 51 (12).

(181c): m/z; 122 (40), 107 (100), 93 (47), 91 (58), 86 (45), 79 (85), 77 (35), 66 (30), 49 (58).

(181d): m/z; 122 (15), 107 (100), 105 (33), 92 (16), 91 (65), 86 (18), 84 (33), 79 (16),77 (15), 68 (17), 49 (25).

(182a): m/z; 140 (8), 125 (32), 109 (8), 107 (18), 97 (25), 82 (100), 69 (22), 55 (25).

(182b): m/z; 140 (10), 125 (20), 109 (100), 107 (17), 83 (64), 81 (33), 55 (38).

Compounds (60) and (183) were identified by comparison with authentic materials.

Compound (60): m/z; 138 (23), 83 (8), 82 (100), 54 (12).

Compound(183): m/z; 152 (55), 137 (10), 109 (13), 96 (87), 69 (10), 68 (100).

Hydrolysis of 3-Hydroxy-β-damascone (39) and its β-D-Glucopyranoside (39a)

3-Hydroxy-β-damascone (39) and its β-D-glucopyranoside (39a) were analyzed using column a). The column was held at 100°C for one minute, then programmed at 4°C / min to 250°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV.

3-Hydroxy-β-damascone (39) at pH 1.1 for 8hours at 100°C in D_2O / 10% EtOD, ^1H n m r (300MHz / $CDCl_3$); δ: 6.75-6.65, b, 1H; 6.14, d, J =15.8Hz, 1H; 4.1, m, 1H; 2.34, ddd, J =16.6, 5.9, 1Hz, 1H; 2.0, dd, J =16.6, 9.9Hz, 1H;

1.72, m, 1H; 1.55, s, 3H; 1.48-1.44, m, 1H; 1.14, s, 3H; 0.97, s, 3H. The C10 methyl doublet was absent and underwent total exchange with deuterium.
m/z; 210 (27), 209 (38), 194 (32), 193 (42), 177 (29), 176 (54), 175 (39), 150 (25), 149 (27), 122 (29), 121 (100), 107 (19), 105 (30), 93 (27), 91 (20), 79 (19), 71 (62), 70 (95), 69 (54), 55 (31).
3-Hydroxy-β-damascone (39) after reaction at pH 3.0 at 100°C for 4hours in D_2O / 10% EtOD
m/z; 208 (7), 194 (7), 193 (10), 175 (22), 149 (9), 147 (7), 133 (7), 121 (31), 119 (9), 105 (15), 93 (11), 91 (9), 79 (11), 77 (8), 70 (7), 69 (100), 67 (8), 57 (8), 55 (20), 53 (8).

Hydrolysis of Model Enyne Alcohol (99) and its β-D-Glucopyranoside (99a)

Model enyne alcohol (99) and its β-D-glucopyranoside (99a) were analyzed using column a). The column was held at 50°C for one minute, then programmed at 4°C / min to 250°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV.

1-(1'-Cyclohexenyl)-2-buten-1-one (184)[149]

The ketone (184) was synthesized by the method of Richter.[149]
^{1}H n m r (60MHz / $CDCl_3$); δ: 7.1-6.6, m, 3H; 2.4-2.2, m, 4H; 1.9, d, *J* =5Hz, 3H; 1.8-1.6, m, 4H. m/z; 151 (8), 150 (46), 149 (8), 136 (9), 135 (100), 122 (6), 121 (11), 117 (11), 109 (9), 108 (6), 107 (17), 93 (6), 91 (7), 81 (19), 79 (24), 77 (6), 69 (45), 53 (5), 41 (38), 39 (14). Compound (185) was tentatively assigned based on interpretation of its mass spectral data.
Compound (185): m/z; 168 (23), 153 (5), 150 (8), 126 (4), 125 (36), 124 (7), 99 (7), 98 (100), 83 (25), 81 (3), 79 (5), 77 (3), 71 (3), 70 (37), 69 (9), 67 (4), 56 (3), 55 (15), 53 (3).

Hydrolysis of Enynediol (58a) and its β-D-Glucopyranoside (58b)

Enynediol (58a) and its β-D-glucopyranoside (58b) were analyzed using column a). The column was held at 100°C for one minute, then programmed at 4°C / min to 250°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV.
β-Damascenone (38): m.z; 190 (8), 122 (4), 121 (33), 120 (9), 105 (12), 91 (12), 79 (9), 77 (9), 70 (4), 69 (100), 65 (4), 53 (5), 41 (44), 40 (3), 39 (20).
Compounds (163) and (189) were also tentatively assigned based on interpretation of their mass spectral data.
Compound (163): m/z; 208 (5), 175 (2), 149 (35), 133 (30), 122 (30), 121 (100), 105 (40), 91 (30), 87 (18), 79 (20), 77 (20), 45 (55), 43 (80).
Compound (189): m/z; 226 (4), 211 (4), 190 (1), 175 (3), 167 (100), 151 (18), 149 (10), 121 (55), 107 (10), 105 (12), 95 (23), 93 (25), 79 (22), 69 (30), 55 (35), 45 (40), 43 (70), 41 (40).

Hydrolysis of Acetylenic triol (55a) and its Bis tetrahydropyranyl ether (56)

Acetylenic triol (55a) and its bis tetrahydropyranyl ether (56) were analyzed using column a). The column was held at 100°C for five minutes, then programmed at 5°C / min to 320°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV.
Triol (55c) had the same retention time and identical mass spectrum as the first eluted triol. (See experimental chapter 2 in the synthesis of triol 55a)
Epimer (55c): m/z; 208 (2), 152 (6), 126 (14), 122 (22), 111 (11), 95 (5) 82 (12), 80 (13), 69 (12), 55 (23), 43 (100).

Hydrolysis of Allenic triol (43a)

Allenic triol (43a) was analyzed using column b). The column was held at 100°C for five minutes, then programmed at 5°C / min to 320°C and held at that temperature for 20 minutes. Electron impact spectra were taken at 70 eV. Compound (190) was found to exhibit a spectrum identical with that received through a personal communication[152]

Compound (190): m/z; 190 (63), 175 (100), 157 (26), 142 (13), 131 (20), 129 (20), 119 (20), 115 (18), 91 (30), 79 (21), 69 (15), 47 (10).

The compound (191) was also tentatively assigned based on interpretation of its mass spectral data and comparison with spectral data published for compounds (192)[18] and (193).[153]

Compound (191): m/z; 208 (13), 190 (18), 175 (45), 149 (40), 147 (32), 146 (50), 133 (30), 131 (100), 122 (27), 109 (35), 105 (32), 91 (38), 77 (18), 69 (38), 55 (8).

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Appendix

Bond Distances (Å) for Acetylenic Triol (55a)

C(2A)	---	C(1A)	1.553(3)	C(6A)	---	C(1A)	1.533(4)
C(7A)	---	C(1A)	1.476(4)	O(1A)	---	C(1A)	1.441(3)
C(3A)	---	C(2A)	1.532(4)	C(11A)	---	C(2A)	1.530(4)
C(12A)	---	C(2A)	1.538(4)	C(4A)	---	C(3A)	1.510(4)
H(5A)	---	C(3A)	1.035(32)	H(6A)	---	C(3A)	0.959(40)
C(5A)	---	C(4A)	1.502(5)	O(3A)	---	C(4A)	1.437(4)
H(7A)	---	C(4A)	1.013(36)	C(6A)	---	C(5A)	1.523(4)
H(8A)	---	C(5A)	0.924(40)	H(9A)	---	C(5A)	1.071(33)
C(13A)	---	C(6A)	1.524(4)	H(10A)	---	C(6A)	0.911(30)
C(8A)	---	C(7A)	1.195(3)	C(9A)	---	C(8A)	1.466(4)
C(10A)	---	C(9A)	1.498(4)	O(2A)	---	C(9A)	1.407(4)
H(4A)	---	C(9A)	0.992(45)	H(1A)	---	O(1A)	1.023(61)
H(2A)	---	O(2A)	1.129(59)	H(3A)	---	O(3A)	0.942(46)
C(2B)	---	C(1B)	1.560(4)	C(6B)	---	C(1B)	1.541(3)
C(7B)	---	C(1B)	1.479(3)	O(1B)	---	C(1B)	1.437(3)
C(3B)	---	C(2B)	1.538(3)	C(11B)	---	C(2B)	1.543(4)
C(12B)	---	C(2B)	1.540(4)	C(4B)	---	C(3B)	1.515(4)
H(5B)	---	C(3B)	1.059(31)	H(6B)	---	C(3B)	1.012(29)
C(5B)	---	C(4B)	1.511(4)	O(3B)	---	C(4B)	1.432(4)
H(7B)	---	C(4B)	1.032(28)	C(6B)	---	C(5B)	1.519(4)
H(8B)	---	C(5B)	1.025(32)	H(9B)	---	C(5B)	1.032(31)
C(13B)	---	C(6B)	1.530(4)	H(10B)	---	C(6B)	0.985(25)
C(8B)	---	C(7B)	1.201(3)	C(9B)	---	C(8B)	1.473(4)
C(10B)	---	C(9B)	1.509(4)	O(2B)	---	C(9B)	1.414(3)
H(4B)	---	C(9B)	1.043(28)	H(1B)	---	O(1B)	0.800(42)
H(2B)	---	O(2B)	0.773(42)	H(3B)	---	O(3B)	0.852(39)

Bond Angles (deg.) for Acetylenic Triol (55a)

C(6A)	C(1A)	C(2A)	111.7(2)
C(7A)	C(1A)	C(6A)	108.6(2)
O(1A)	C(1A)	C(6A)	109.6(2)
C(3A)	C(2A)	C(1A)	109.3(2)
C(11A)	C(2A)	C(3A)	108.9(2)
C(12A)	C(2A)	C(3A)	111.1(2)
C(4A)	C(3A)	C(2A)	116.8(2)
H(5A)	C(3A)	C(4A)	108.7(16)
H(6A)	C(3A)	C(4A)	104.2(21)
C(5A)	C(4A)	C(3A)	111.0(2)
O(3A)	C(4A)	C(5A)	107.2(2)
H(7A)	C(4A)	C(5A)	113.7(21)
C(6A)	C(5A)	C(4A)	113.5(3)
H(8A)	C(5A)	C(6A)	111.8(23)
H(9A)	C(5A)	C(6A)	108.4(17)
C(5A)	C(6A)	C(1A)	110.8(2)
C(13A)	C(6A)	C(5A)	111.9(3)
H(10A)	C(6A)	C(5A)	109.6(17)
C(8A)	C(7A)	C(1A)	174.4(3)
C(10A)	C(9A)	C(8A)	110.5(3)
O(2A)	C(9A)	C(10A)	108.3(3)
H(4A)	C(9A)	C(10A)	117.0(23)
H(11A)	C(10A)	C(9A)	109.7(2)
H(14A)	C(11A)	C(2A)	109.5(1)
H(13A)	C(10A)	H(9A)	109.6(2)
H(17A)	C(12A)	C(2A)	109.5(2)
H(22A)	C(13A)	C(6A)	109.4(2)
H(20A)	C(13A)	C(6A)	109.5(2)
H(1A)	O(1A)	C(1A)	114.3(31)
H(3A)	O(3A)	C(4A)	118.5(26)
C(7B)	C(1B)	C(2B)	110.1(2)
O(1B)	C(1B)	C(2B)	110.8(2)
O(1B)	C(1B)	C(7B)	108.8(2)
C(11B)	C(2B)	C(1B)	111.0(2)
C(12B)	C(2B)	C(1B)	110.1(2)
C(12B)	C(2B)	C(11B)	107.5(2)
H(5B)	C(3B)	C(2B)	107.7(15)
H(6B)	C(3B)	C(2B)	110.6(16)
H(6B)	C(3B)	H(5B)	105.0(21)
O(3B)	C(4B)	C(3B)	112.2(2)
H(7B)	C(4B)	C(3B)	104.4(15)
H(7B)	C(4B)	O(3B)	108.4(15)
H(8B)	C(5B)	C(4B)	108.6(17)
H(9B)	C(5B)	C(4B)	104.7(16)
H(9B)	C(5B)	H(8B)	107.6(22)
C(13B)	C(6B)	C(1B)	112.6(2)
H(10B)	C(6B)	C(1B)	102.4(14)
H(10B)	C(6B)	C(13B)	106.6(14)
C(9B)	C(8B)	C(7B)	179.3(3)
O(2B)	C(9B)	C(8B)	111.6(2)
H(4B)	C(9B)	C(8B)	106.0(15)
H(4B)	C(9B)	O(2B)	109.2(14)
H(12B)	C(10B)	C(9B)	109.6(2)
H(13B)	C(10B)	C(9B)	109.5(1)
H(15B)	C(11B)	C(2B)	109.5(1)
H(16B)	C(11B)	C(2B)	109.5(1)
H(18B)	C(12B)	C(2B)	109.4(1)
H(19B)	C(12B)	C(2B)	109.5(1)
H(2B)	O(2B)	C(9B)	111.3(29)

C(7A)	C(1A)	C(2A)	110.7(2)
O(1A)	C(1A)	C(2A)	106.0(2)
O(1A)	C(1A)	C(7A)	110.1(2)
C(11A)	C(2A)	C(1A)	110.3(2)
C(12A)	C(2A)	C(1A)	109.6(2)
C(12A)	C(2A)	C(11A)	107.7(2)
H(5A)	C(3A)	C(2A)	107.6(16)
H(6A)	C(3A)	C(2A)	110.3(21)
H(6A)	C(3A)	H(5A)	109.1(26)
O(3A)	C(4A)	C(3A)	113.3(3)
H(7A)	C(4A)	C(3A)	104.7(20)
H(7A)	C(4A)	O(3A)	106.9(20)
H(8A)	C(5A)	C(4A)	105.8(24)
H(9A)	C(5A)	C(4A)	108.6(17)
H(9A)	C(5A)	H(8A)	108.5(28)
C(13A)	C(6A)	C(1A)	112.4(2)
H(10A)	C(6A)	C(1A)	102.9(18)
H(10A)	C(6A)	C(13A)	108.9(18)
C(9A)	C(8A)	C(7A)	173.7(3)
O(2A)	C(9A)	C(8A)	112.1(2)
H(4A)	C(9A)	C(8A)	125.1(24)
H(4A)	C(9A)	O(2A)	77.8(24)
H(12A)	C(10A)	C(9A)	109.1(2)
H(15A)	C(11A)	C(2A)	109.5(2)
H(16A)	C(11A)	C(2A)	109.4(2)
H(18A)	C(12A)	C(2A)	109.4(2)
H(19A)	C(12A)	C(2A)	109.5(1)
H(21A)	C(13A)	C(6A)	109.6(2)
H(2A)	O(2A)	C(9A)	108.5(32)
C(6B)	C(1B)	C(2B)	111.6(2)
C(7B)	C(1B)	C(6B)	109.6(2)
O(1B)	C(1B)	C(6B)	105.7(2)
C(3B)	C(2B)	C(1B)	109.2(2)
C(11B)	C(2B)	C(3B)	107.7(2)
C(12B)	C(2B)	C(3B)	111.4(2)
C(4B)	C(3B)	C(2B)	115.5(2)
H(5B)	C(3B)	C(4B)	110.9(15)
H(6B)	C(3B)	C(4B)	106.6(15)
C(5B)	C(4B)	C(3B)	111.1(2)
O(3B)	C(4B)	C(5B)	107.9(2)
H(7B)	C(4B)	C(5B)	112.9(15)
C(6B)	C(5B)	C(4B)	113.3(2)
H(8B)	C(5B)	C(6B)	110.5(16)
H(9B)	C(5B)	C(6B)	112.0(16)
C(5B)	C(6B)	C(1B)	110.9(2)
C(13B)	C(6B)	C(5B)	111.4(2)
H(10B)	C(6B)	C(5B)	112.6(14)
C(8B)	C(7B)	C(1B)	179.5(2)
C(10B)	C(9B)	C(8B)	111.8(2)
O(2B)	C(9B)	C(10B)	107.0(2)
H(4B)	C(9B)	C(10B)	111.4(15)
H(11B)	C(10B)	C(9B)	109.4(2)
H(14B)	C(11B)	C(2B)	109.5(1)
H(17B)	C(12B)	C(2B)	109.5(1)
H(20B)	C(13B)	C(6B)	109.4(2)
H(1B)	O(1B)	C(1B)	107.0(27)
H(22B)	C(13B)	C(6B)	109.5(1)
H(21B)	C(13B)	C(6B)	109.5(1)
H(3B)	O(3B)	C(4B)	112.4(25)

Two Conformers of Acetylenic Triol (55a)