The role of acetylenic and allenic precursors in the formation of β-damascenone

A thesis submitted in fulfillment of the requirements of the degree of Doctor of Philosophy

By

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Dedication

This Thesis is dedicated to my late husband and best friend Christoper Stephen Puglisi (16/12/1971-14/3/2006) and my two beautiful daughters Amelie Mae and Jada Lilly Puglisi. Thank you for giving me the strength to continue.

TABLE OF CONTENTS

RESULTS AND DISCUSSION

ABSTRACT

This thesis describes an investigation into the role of acetylenic and allenic precursors in the formation of the important aroma compound β-damascenone (**1**).

Chapter 1 provides an introduction to the subject, beginning with a brief history of the Australian wine industry which began with the first fleet's arrival in 1788. Many of the various volatile compounds found in wine are then discussed, with particular emphasis on β-damascenone (**1**). Some previous syntheses of **1** are summarised, as well as the *in vivo* generation of this compound, and also the role of glycoconjugation in nature. The chapter concludes with the aims of the present work.

Chapter 2 covers the synthesis of the suspected acetylenic precursor 9-hydroxymegastigma-3,5-dien-7-yne (**36**), which was prepared by the addition of 3-butyn-2-ol to 2,6,6-trimethylcyclohex-2-en-1-one, followed by a conjugate dehydration reaction. The synthetic sample of **36** was shown to be identical to a compound previously observed in the hydrolysate of 3,5,9-trihydroxymegastigma-6,7-diene (**31**). Upon acid hydrolysis, **36** produced >90% β-damascenone (**1**).

Chapter 3 outlines the synthesis and hydrolysis of the C_9 glycoside **43**, which was prepared by a modified Koenigs-Knorr procedure on aglycone **36**. Diastereomerically pure samples of each of the two possible glycosides were synthesised from corresponding enantiomerically pure samples of **36**, which in turn were prepared by the use of either (*R*) or (*S*) 3-butyn-2-ol. Detailed hydrolytic studies (at 25 ºC) were conducted on both the aglycone and the two glycosides: the half lives of conversion of **36** into **1** were 40 hours and 65 hours at pH 3.0 and pH 3.2 respectively; the (9*R*) diastereomer of **43** had half-lives of 3 days and 6 days,

respectively at the same pH values, whereas the (9*S*) diastereomer had half lives of 3.5 days and 6.5 days, respectively at the same pH values.

The synthesis of the other suspected precursor, megastigma-4,6,7-triene-3,9-diol (**35**) is covered in **Chapter 4**. This allene was prepared by addition of 3-butyn-2-ol to phorenol, with the allene function generated by reaction with lithium aluminium hydride. By using (3*S*)-phorenol and both (*R*) and (*S*) 3-butyn-2-ol, four different diastereomers of **35** were prepared and characterised. The (3*S*, 6*R*, 9*S*)-isomer of **35** was also found to be identical to a compound previously observed in the hydrolysate of (**31**).

A detailed hydrolytic study of the four synthetic isomers of **35** is contained within **Chapter 5**. This study revealed that each of the four isomers underwent rapid epimerisation at 25 ºC and pH 3.0. Careful analysis of the four product mixtures by chiral GC-MS revealed that this epimerisation was occurring exclusively at C_3. The complete absence of 3-hydroxydamascone (**2**) from any of the hydrolysates required a re-appraisal of the mechanism of *in vivo* formation of β-damascenone (**1**), which forms the focus of the second half of this chapter.

The experimental procedures (materials and methods) for all work covered in chapters 2-5 are located in **Chapter 6.**

DECLARATION

"I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any University; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text."

Carolyn J. Puglisi

"I believe that this thesis is properly presented, conforms to the necessary specifications, and is of sufficient standard to be, *prima facie*, worthy of examination."

Gordon M. Elsey

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I thank my principal supervisor Dr. Gordon Elsey, who has given me an enormous amount of support and encouragement throughout my PhD, particularly in the final years. Thank you for teaching me, by example, the attributes of a good and honourable scientist. I thank my co-supervisors Dr. Mark Sefton and Professor Rolf Prager, and Institute colleagues Dr. George Skouroumounis and Prof.. Peter Høj for many discussions and advice regarding my project.

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To all of my friends, some of whom have passed out of my life, and some of whom have remained constant, for all having played a special role in my life.

PUBLICATIONS

Refereed Publications

1. **C.J. Puglisi**, G.M. Elsey, R.H. Prager, G.K. Skouroumounis, and M.A. Sefton. Identification of a precursor to naturally occurring β-damascenone. *Tetrahedron Lett.,* **2001**, *42*, 6937-6939.

2. A. Janusz, D.L. Capone, **C.J. Puglisi**, M.V. Perkins, G.M. Elsey, and M.A. Sefton. (*E*)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene – a potent grape-derived odorant in wine. *J. Agric. Food Chem.,* **2003**, *51*, 7759-7763.

3. **C.J. Puglisi**, M.A. Daniel, D.L. Capone, G.M. Elsey, R.H. Prager and M.A. Sefton. Precursors to damascenone: synthesis and hydrolysis of four isomeric 9-dihydroxymegastigma-4,6,7-trienes. *J. Agric. Food Chem.,* **2005**, *53***,** 4895-4900.

4. M.A. Daniel, **C.J. Puglisi**, G.M. Elsey, M.V. Perkins and M.A. Sefton. Rationalising the formation of damascenone: Synthesis and hydrolysis of damascenone models and precursors and their analogues, in both aglycone and glycoconjugate form. *J. Agric. Food Chem., in preparation.*

Symposia

1. **C.J. Puglisi**, G.M. Elsey, G.K. Skouroumounis, M.A. Sefton and R.H. Prager. On The Formation of Naturally Occurring *β*-Damascenone in Grapes and Wine:, *11th AWITC*, Adelaide, **2001**. 11th Australian Wine Industry Technical Conference, Adelaide, **2001**.

2. **C.J. Puglisi**, G.M. Elsey, R.H. Prager and M.A. Sefton. On the Formation of Naturally Occurring Damascenone:, 19th Royal Australian Chemical Institute Organic Chemistry Symposium, Lorne, **2003**.

ABBREVIATIONS

DMAP	4-dimethylaminopyridine
ee	enantiomeric excess
FVP	flash vacuum pyrolysis
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
HMBC	heteronuclear multiple bond correlation
NMR	nuclear magnetic resonance spectroscopy
PTFE	polytetrafluoroethylene, ie. ‘teflon’
RT	room temperature
SCC	short column chromatography
SIDA	stable isotope dilution assay
TBAF	tetrabutylammonium fluoride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMOF	trimethyl orthoformate
TMS	trimethylsilyl
TPB	(*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene

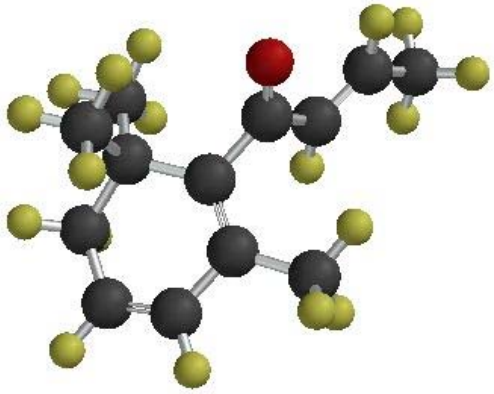
LIST OF FIGURES AND TABLES

Figures

Tables

CHAPTER ONE

Introduction



1.1 A Short History of the Australian Wine Industry

The first grape vine cuttings were brought to Australia with the first fleet on January 26, 1788; these vines were planted by Captain Arthur Phillip just days after the arrival of the fleet and although attempts at wine making from these first vines failed, the vines were replanted and successfully cultivated for winemaking.[1] Very soon there were many successful vineyards established in what are now suburban areas of Sydney[2] and by the 1820's Australian wine was available for domestic sale.[3]

In 1833 James Bushby returned from France and Spain with a very good selection of grape varieties including most classic French varieties and a good selection of varieties for fortified wine production. The production and quality of wine was greatly improved with the arrival of the free settlers, their skills and knowledge of wine making resulting in some of Australia's premier wine regions.[4]

Some of the earliest vine plantings occurred in the 1820's in Tasmania and in the Hunter Valley in 1830. Plantings in South Australia occurred from the 1830's to the 1860's with German settlers in the mid 1850's establishing South Australia's Barossa Valley as a premium wine making region. Western Australian commenced vine plantings in the 1840's and Queensland followed in the 1860's.[4,5]

There were many great predictions about wine from Australia very early on, with Australia being referred to as a virtual paradise for viticulture.[6] Gregory Blaxland was the first to export Australian wine in 1822 and was the first to win an international award.[2]

Considerable success was achieved very early on with many awards being awarded for Australian wines, particularly in France. In 1882 an Australian wine won "first class" at the Bordeaux International Exhibition and another won a gold medal at the

1889 Paris International Exhibition.[7] However there were difficulties, particularly due to the unfamiliar Australian climate; many vineyards would soon disappear, wiped out by Phylloxera, overtaken by dairy farming or suffering as the consumer developed a taste for beer and fortified wines.[5,6,8]

Prior to the 1950's fortified wines were the main focus of production in Australia. Additional levels of alcohol provided wine with protection against microbial attack and made it more suitable for storage and transport. Fortified wine production for the 1950 vintage comprised no less than 86% of total production with the centre of production being in South Australia. Fortified wine amounted to 76% of the total wine exported with the majority being shipped to the United Kingdom. The wine industry of that time bore very little resemblance to the current Australian wine industry.[5,6,8]

The 1960's and 70's saw a shift in the focus of wine production from fortified wines to table wines.[9] In the late 1960's, sparked by a strong economy and a boom in immigration, production of red wines became popular.[10] The 1970's saw a trend toward white table wines[11] and the invention of the wine 'cask' ('bag-in-box') by a group of Australian companies. The increased consumption of white wine was driven by sales of 'bag-in-box' wine rather than bottled wine during this period.[11]

As the next decade began the industry found itself suffering considerably; imports had exceeded exports each year from 1975 to 1980. Fortunately, the per capita consumption of wine had more than doubled during the 10 years from 1970 to 1980 and strong domestic growth was maintained through to 1984/1985. The export market however, remained one of continuing stagnation until 1986/1987 when exports increased by 95.5% and for the first time exceeded the 1937 record of 18 million litres.[12] During the period from 1988 to 1991 domestic sales declined but

exports remained static as the country abruptly left the boom of the 1980's and entered a severe economic recession. 1990 saw the export industry recommence its climb and by 1994 the market had matured to the point where it was unlikely to see a decline.[12]

1.1.1 Australia and Wine Exports

Australia is now the fourth largest exporter of wine worldwide after France, Italy and Spain. The biggest markets for Australian wine are the UK and the USA followed by Canada, whose market share is expanding rapidly (Table 1.1).[13]

The Australian wine industry's growth has been driven by Australia's strong export performance. The volume of wine exports for the 2004-2005 period grew at an average annual rate of 18.2 %. Australia now exports to more than 100 countries, some of which are listed in Table 1.1. Wine exports to the USA rose from 578,000 cases in 1990 to 20,000,000 cases in 2004 and in the year 2000 Australia exported more wine to the UK than did France for the first time in history, a situation which continues to the present day.[14]

Table 1.1: Australian wine exports for the twelve months ending Oct 2007.[13]

Country	Litres	$A	$A/L
United Kingdom	290 026 590	981 993 666	$3.38
United States	216 447 152	952 787 988	$4.40
Canada	50 580 160	286 886 632	$5.67
Germany	38 540 713	62 370 268	$1.62
New Zealand	33 723 949	102 690 357	$3.05
Netherlands	25 972 921	75 256 296	$2.90
China	21 302 729	54 459 797	$2.56
Ireland	16 619 843	49 419 999	$3.33
Sweeden	9 669 508	14 415 271	$1.49
France	9 256 446	25 762 420	$2.78
Belgium	9 256 446	25 762 420	$2.78
Japan	9 061 850	47 603 109	$5.25
Singapore	5 535 089	45 977 011	$8.31
Finland	4 751 456	16 432 538	$3.46
Rest of world	39 512 266	198 297 116	$5.02
World	805 920 343	3 038 317 205	$3.77

Today the export boom continues and the industry continues to thrive. The industry aims to become the world's most profitable wine exporter by increasing its wine production 3-fold by 2025.

1.1.2 Wine Regions of Australia

There are almost 2000 wineries spread across Australia with vineyards in every state and territory. The top three producers account for 78.5% of the total market revenue, with the next largest four accounting for 12% combined. The 40 largest operators each process more than 10,000 tonnes annually, with many small, family owned wineries crushing less than 100 tonnes per year.[14]

There are 60 designated wine regions across the country, which produce over 100 different varieties of wine. The warm inland grape growing regions include the

Riverina and Murray-Darling areas of NSW and Victoria and the Riverland region of South Australia. These regions are characterized by stable warm conditions and access to good irrigation, and account for approximately 60% of national production. These regions service the basic wine and lower price brands of the premium wine market. The cooler climate wine regions produce a variety of wine types but generally service the higher end quality product in the premium and super premium wine market.

South Australia is the centre of grape and wine production in Australia with approximately 50% of the national wine production during the 2005-2006 vintage. NSW and ACT combined produced some 33%, with Victoria following third at around 12% (Table 1.2).[15]

Table 1.2: Wine and grape production in Australia for 2005-2006 vintage.

State	Grapes Crushed (%)	Wine Production (%)	Total Wineries (%)
SA	48.0	50.6	29.9
NSW/ACT	34.6	33.7	21.1
Vic	13.6	12.4	24.8
WA	3.5	3.0	18.9
Qld	0.2	0.1	3.0
TAS	0.2	0.1	2.3

1.1.3 Grape Varieties

Australia is renowned for the variety and quality of wines it produces. There are about 130 different grape varieties used by commercial winemakers in Australia. The major grape varieties grown in Australia include Shiraz, Cabernet Sauvignon, Chardonnay, Sauvignon Blanc, Semillon and Riesling.[4] Table 1.3 below includes some common descriptors for the wines produced from these varieties.

Table 1.3: Common descriptors of wines produced from various grape varieties.[4]

Grape Variety	Common Descriptors
Chardonnay	Cucumber, grapefruit, apple, lime, rockmelon, fruit salad, tropical fruit, tobacco, gooseberry, melon, peach, fig.
Riesling	Floral, apple, pear, citrus, lime, passionfruit, tropical fruit.
Sauvignon Blanc	Asparagus, capsicum, herbaceous, grassy, gooseberry, tropical fruit,
Semillon	Herbaceous, straw, gooseberry, apple, lemon, lime, passionfruit, grassy, quince, citrus.
Cabernet Saunignon	Herbaceous, capsicum, tomato bush, leafy, minty, dusty, black olive, blackcurrant.
Merlot	Herbaceous, leafy, cherry, violets, plum, blackcurrant, raspberry, beetroot.
Pinot Noir	Cherry, strawberry, violets, raspberry, plum, stewed plum
Shiraz	Herbs, spice, raspberry, plum, pepper, blackberry, mulberry, licorice, black olive, jammy.

In recent years winemakers have begun exploring the use of some alternative varieties for use in wine making, many of these have come from France, Italy and Spain and include Petit Verdot, Pinot Grigio, Sangiovese, Tempranillo and Viognier.

The styles of wines produced in Australia may be loosely placed into 4 categories; they are sparkling wines, dry white table wines, semi-sweet and sweet white wines and dry red table wines. Table 1.4 below lists some of the grape varieties used for each wine style.[5]

Table 1.4: Grape varieties used for production of various wine styles.

Wine Style	Grape Variety
Sparkling wines	Chardonnay, Muscat Gordo Blanco, Riesling, Semillon, Pinot Noir, Pinot Meunier, Muscadelle, Colombard, Chenin Blanc, Ondenc, Trebbiano
Dry white table wines	Chardonnay, Chenin Blanc, Colombard, Frontignac, Gewürztraminer, Marsanne, Riesling, Sauvignon Blanc, Semillon, Trebbiano, Verdelho, Viognier
Semi-sweet and sweet white wines	Muscat de Frontignan, Gewurztraminer, Muscat Gordo Blanco, Rielsing, Semillon
Dry red table wines	Barbera, Cabernet Franc, Cabernet Sauvignon, Chambourcin, Durif, Grenache, Malbec, Merlot, Mourvedre, Nebbiolo, Petit Verdot, Pinot Noir, Ruby Cabernet, Sangiovese, Shiraz, Tarrango, Zinfandel.

1.2 Flavour and Aroma

It has been suggested that flavour is the most important factor contributing to the enjoyment of wine. Flavour results from the interaction of chemical constituents with the sense of taste and smell and is a combination of the effect of both volatile and non-volatile compounds.[16,17] The non-volatile compounds are responsible for taste sensations, which impart bitterness, sweetness, sourness and saltiness. The compounds in wine that actually cause taste sensations are sugars, organic acids, polymeric phenols and mineral substances.[16]

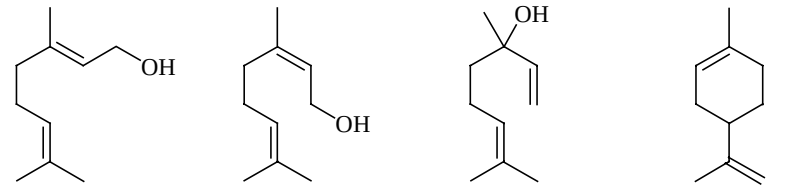
Volatile compounds are responsible for the aroma of wine and are inhaled by air into the olfactory epithelium.[18] Because the sense of smell is extremely sensitive, volatile components can sometimes be perceived in very low concentrations. The concentration of such compounds range from 10^{-1} to 10^{-10} g/kg. To date there have

been more than 800 volatile compounds identified in grapes and wine. Wine aroma results from the balance of many of these components.[19-21]

The aroma of wine is a product of both biochemical and technological processes. It may originate from the grape, grape processing (juice production, ie. crushing), fermentation or from transformations that occur during wine maturation.[17,21] The quantities of the various individual aroma components are influenced by a variety of factors including climate, soil, cultivar, fruit condition, conditions during fermentation such as pH, temperature, micro-flora and any post-fermentation treatments such as clarification and blending.[16]

1.2.1 Different classes of aroma compounds

Previous authors have divided wine and flavour compounds derived from grape secondary metabolites into three major classes: the monoterpenes, the shikimates and the norisoprenoids.[22,23] The monoterpenes are found predominantly in Muscat varieties and are responsible for their characteristic aroma.[17] Monoterpenes (C_{10}) consist of two isoprene units. Some of the more prominent monoterpene compounds which occur in high concentration in Muscat and aroma related grapes and wines are linalool, geraniol, nerol, α-terpineol and hotrienol. There are also some terpene compounds, which occur in lower concentrations that can nevertheless have a significant effect on aroma. Some typical aroma descriptions of some important monoterpenes include floral, rose like (geraniol, nerol),[24] coriander/cilantro (linalool),[25,26] and citrus (limonene)[27,28] (Figure 1.1). Monoterpenes are also characteristic components of the essential oils produced by many plants.[29]

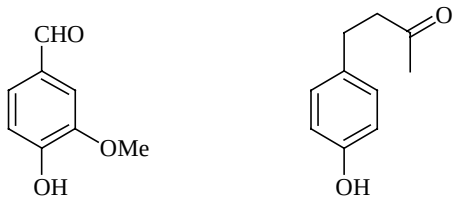


Geraniol Nerol Linalool Limonene

Figure 1.1: Some common monoterpenes found in grapes and wine.

In addition to the volatile monoterpenes of floral grape varieties mentioned above, there exists a large pool of other, less volatile terpene derivatives, comprising polyols and monoterpene glycosides.[29] Although these polyols and glycosides are flavourless they can sometimes be an important source of aroma compounds, formed under mildly acidic conditions.[30,31] Alternatively, enzymatic hydrolysis of glycosides is an attractive way of increasing the pool of volatile monoterpenes in wine in significant quantities.[17]

Shikimates have been observed in Chardonnay and Semillon varieties and are derived *via* the shikimic acid biochemical pathway.[32] These are predominantly benzenoid compounds. Examples include vanillin and raspberry ketone (Figure 1.2).

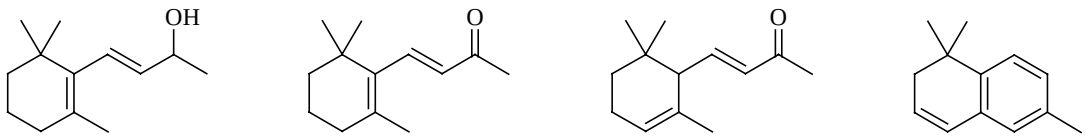


Vanillin Raspberry ketone

Figure 1.2: Some simple shikimate derived aroma volatiles found in wine.

C_{13}-Norisoprenoids are 13-carbon compounds that accumulate in grapes in the form of glycoconjugates.[31,33] C_{13}-norisoprenoids are thought to be derived from carotenoid degradation and are common components of many plants. They are believed to result from oxidative cleavage of the polyene chain followed by chemical

transformations mediated by enzyme or acid.[34] The total level of carotenoids in grapes during maturation has been shown to decrease steadily. Levels at maturity compared with initial levels have shown a reduction by a factor of 6 in Sirah (Shiraz) and 8 in Carignan and Grenache varieties.[35] Some common examples of C_{13}-norisoprenoids include β-ionol, β-ionone, α-ionone, 1,1,6-trimethyl-1,2-dihydronapthalene (TDN) (Figure 1.3) and the Rose Ketones, which are discussed below. TDN exhibits a distinctive, kerosene-like odour, which develops in many aged Riesling wines.[36,37]



Ionol β-Ionone α-Ionone TDN

Figure 1.3: Some common C_{13}-norisoprenoids found in wine.

1.3 The Rose Ketones

The Rose ketones are an important group of flavour compounds, which have routinely been used in the perfume industry since they were discovered more than 30 years ago. Their importance is substantiated by the existence of over 350 scientific and patent publications and an annual production measuring in the tonnes.[38-40]

The rose ketone family consists of β-damascenone (**1**), 3-hydroxy-β-damascone (**2**), β-damascone (**3**), α-damascenone (**4**), α-damascone (**5**) and γ-damascone (**6**) (Figure 1.4).

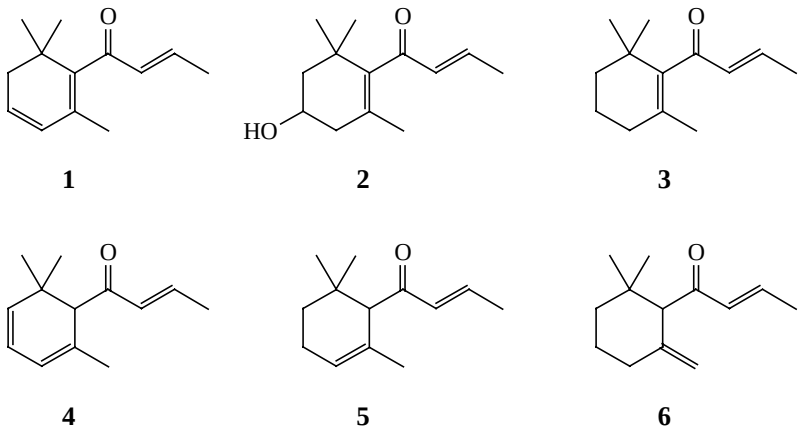


Figure 1.4: The Rose ketones

Their structures and nomenclature are based on the megastigmane carbon skeleton, which contains 13 carbon atoms, and is numbered as shown in Figure 1.5. β-damascenone (**1**) is also known as megastigma-3,5,8-trien-7-one. In recent times, β-damascenone (**1**) has also more commonly been referred to within the chemical literature as simply 'damascenone'.

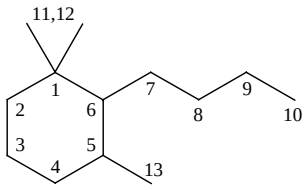


Figure 1.5: Basic megastigmane skeleton, and numbering scheme. This numbering scheme is used throughout the remainder of this thesis.

Some common descriptors associated with their aroma, and their detection thresholds (in air) are collected in Table 1.5.

Table 1.5: Aroma descriptors and sensory thresholds, in air, of some Rose ketones.[39]

Rose Ketone	Aroma Descriptors	Sensory Threshold in air (μg/L at 25ºC)
β-damascenone (**1**)	Floral, fruity, rose, honey-like, ionone-like, stewed apple	1.3×10^{-5}
α-damascone (**5**)	Rose-apple	1.4×10^{-4}
β-damascone (**3**)	Blackcurrnat, plum, rose	1.9×10^{-4}
γ-damascone (**6**)	Fruity, floral, rosy, green	2.1×10^{-5}

1.4 β-Damascenone

β-Damascenone (**1**) is one of the most frequently occurring C_{13}-norisoprenoids found in nature and since its discovery has greatly influenced flavour and fragrance chemistry.[41] β-Damascenone was first isolated from Bulgarian rose oil (*Rosa damascena*) in 1970 by Demole and co-workers[42] and has since been identified in fruit,[43,44] various beverages such as tea,[45] coffee[46] and both red[47] and white wine.[48,49]

β-Damascenone was first identified in grapes and wine in 1974 by Schreier and Drawert.[50] It has a very low odour threshold, having been reported as 2 ng/L in water[44] and 50 ng/L in 10% aqueous ethanol.[49] Its aroma, which has been variously described as flowery,[51] sweet and honey-like,[52] is complex and varies depending on concentration. The concentration of β-damascenone in wine appears to vary considerably. Values as high as 980 ng/L and 840 ng/L have been found in Scheurebe and Gewurztraminer wines, respectively,[49] and approximately 0.2 to 1.3 μg/L have been reported in Merlot wines.[53]

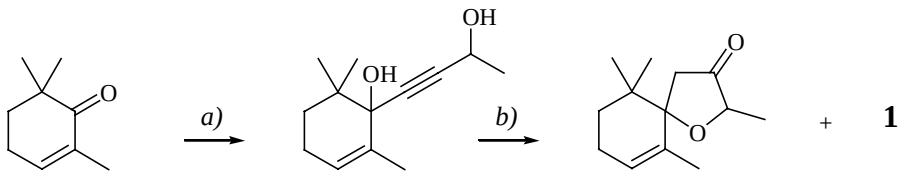
β-Damascenone has also been used extensively in the perfume industry, having been used in perfume for the first time in 1979 in Guerlain's Nahema® and since then, featuring in fragrances such as Poison® (1986) and Drakkar Noir® (1982).[54]

1.4.1 Previous syntheses of β-damascenone (1)

As the quantities of β-damascenone (**1**) available from natural sources are extremely limited, much effort has been devoted to the synthesis of this very important natural product. Detailed below is a selection of published syntheses of **1**.

1.4.1.1 Isoe et al. (1973)

The Isoe group in 1973 conducted a synthesis of **1** (although yields were not stated) from 2,6,6-trimethylcyclohex-2-enone (**7**)[55] (Scheme 1.1). The synthesis involved reaction of the enone **7** with the dilithio derivative of but-3-yn-2-ol in liquid ammonia to produce **8**, which was subsequently treated with 80% formic acid under reflux to produce β-damascenone (**1**) and the spiroketoether **9**.



7 **8** **9**

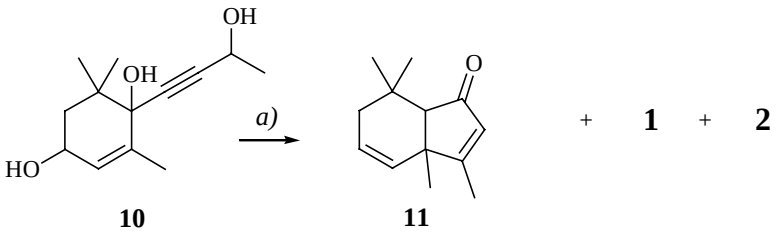
a) NH_3, $LiC{\equiv}CCH(OLi)CH_3$ **b)** 80% HCOOH, Δ

Scheme 1.1

1.4.1.2 Ohloff et al. (1973)

In an almost identical manner to that described above, Ohloff et al. treated **8** with 66% formic acid at 45ºC over 48 hours to produce both **1** and **9** in a ratio of 1:9 in a 60% combined yield (scheme 1.1 above).[56]

Ohloff et al. also synthesized β-damascenone using the acetylenic triol **10** (Scheme 1.2). The triol was stirred for 10h in 30% aqueous sulphuric acid, giving a 20% yield of β-damascenone. Also obtained were 70% 3-hydroxy-β-damascone (**2**) and 5% of the bicyclic compound **11**.



10 **11**

a) 30 % H_2SO_4

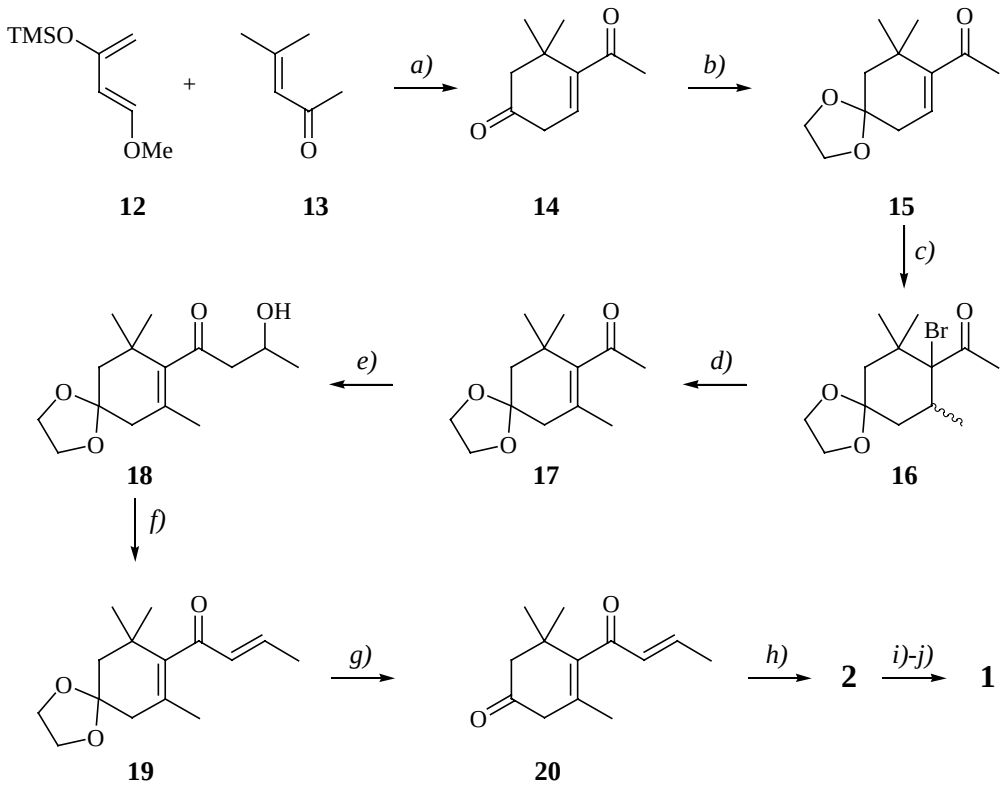
Scheme 1.2

1.4.1.3 Kitahara et al. (1979)

In 1979 Kitahara et al. published a rather long and convoluted synthesis of β-damascenone (**1**) (Scheme 1.3).[57] The synthesis employed a Diels-Alder reaction between the very reactive 1-methoxy-3-trimethylsilyloxy-butadiene (**12**) and the enone **13** in order to produce the key intermediate **14**, which was then ultimately converted into **1**. This synthesis produced β-damascenone in a 7.2% yield from mesityl oxide (**13**).

1.4.1.4 Torii et al. (1979)

In the same year Torii et al. published a synthesis of β-damascenone from dimedone (**21**).[58] (Scheme 1.4). This synthesis made use of 3-ethoxy-6-[(*E*)-1-hydroxy-2-butenyl]-5,5-dimethyl-2-cyclohexen-1-one (**22**), which was prepared from condensation of the kinetic enolate anion of 3-ethoxy-5,5-dimethyl-2-cyclohexen-1-one (**23**), with (*E*)-2-butenal (**24**) (Figure 1.6).[59]



a) i) 180 °C, 24 h; ii) 10% HCl, THF
b) $(CH_2OH)_2$, p-TsOH
c) i) Me_2CuLi, Et_2O, 0 °C; ii) –40 °C, Br_2, C_6H_6
d) LiCl-Li_2CO_3, DMF, Δ
e) LDA, CH_3CHO, -78 °C
f) Ac_2O-AcONa, 120 °C
g) 40% $HClO_4$, THF
h) L-Selectride, THF, -78 °C
i) MsCl, Pyridine, 0 °C
j) DBU, NaI, toluene, Δ

Scheme 1.3

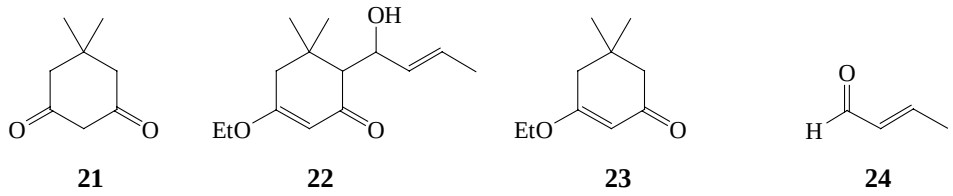
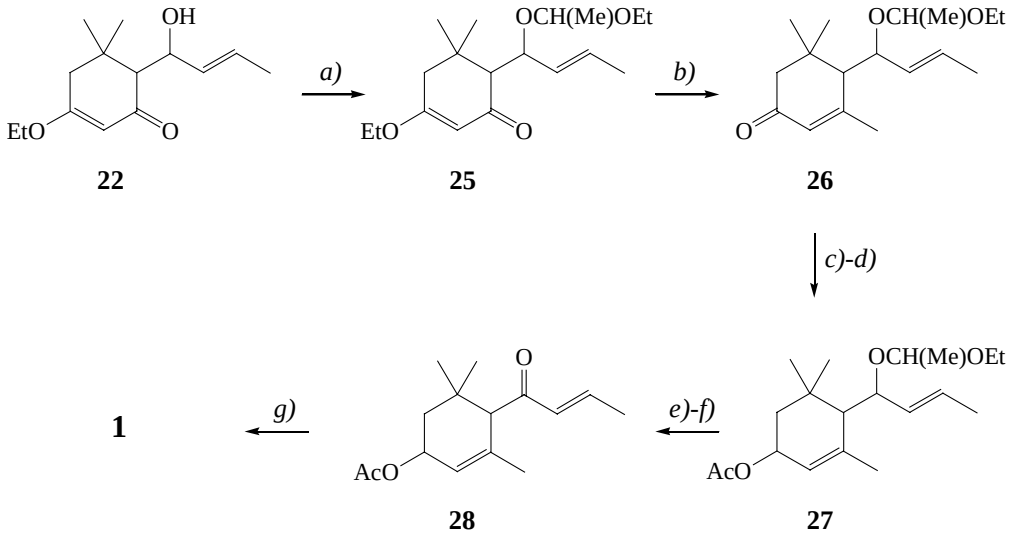


Figure 1.6: Compounds utilised in the synthesis of **1** by Torii et al.

Torii et al. (Scheme 1.4) thus utilised a C_4 plus C_9 union to prepare β-damascenone. Their synthesis used enol ether **22** and produced damascenone (**1**) in seven steps from **22**, with an overall yield of 14%.



a)  , PPTS, CH_2Cl_2, 7h **b)** CH_3Li, 3-4 °C, 40 min. **c)** $LiAlH_4$, 0-5 °C, 1h

d) Ac_2O, pyr, 0-5 °C, 30 min. **e)** PPTS, EtOH, 40 °C, 3h **f)** MnO_2, CH_2Cl_2, 5h

g) DBU, Δ, 20 s

Scheme 1.4

1.4.2 *In vivo* generation of β-damascenone

The *in vivo* generation of β-damascenone has been investigated for some 30 years, yet the complete pathway has yet to be fully elucidated. Our interest in this field is concerned with β-damascenone precursors and the formation of naturally occurring β-damascenone in wine. There exist very strict guidelines regarding the use of chemical additives during wine production in Australia.[4] Winemakers are not permitted to modify the aroma or flavour of wines by the use of additives, hence our interest in the natural formation of β-damascenone. If β-damascenone precursors can be identified and quantified in the grape, its juice or in a young wine, the potential for β-damascenone formation in a particular wine may be predicted.

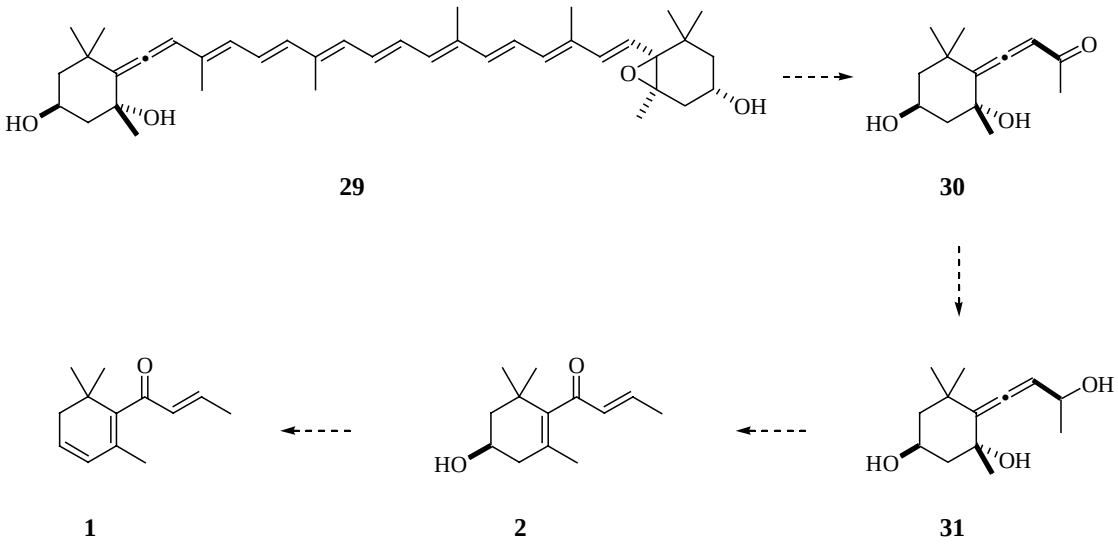
Eventually manipulation of the growing conditions or indeed the winemaking process might allow for selective formation of β-damascenone in wines.

The development of flavour in wine during maturation is an important aspect of wine flavour; under acidic conditions certain non-volatile compounds (including sugar derivatives) undergo chemical change to release volatile components, which contribute to the overall flavour and complexity of the wine. For this reason, the work described in this thesis includes the investigation of glycosylation and its effect on the formation of β-damascenone in wine.

1.4.2.1 Early work- Isoe and Ohloff

Early work on the *in vivo* generation of β-damascenone came independently in 1973 from two groups. Isoe et al. hypothesised that the damascenones were formed from carotenoids or their metabolites.[55] It was suggested that the generation of β-damascenone (**1**) involved oxidative cleavage of neoxanthin (**29**), a plant carotenoid, to form 'grasshopper ketone' (**30**). It was proposed that reduction of **30** followed by rearrangement of 3,5,9-trihydroxymegastigma-6,7-diene (**31**) and dehydration of 3-hydroxy-β-damascone (**2**) (which is odourless) would give β-damascenone (**1**) (Scheme 1.5).

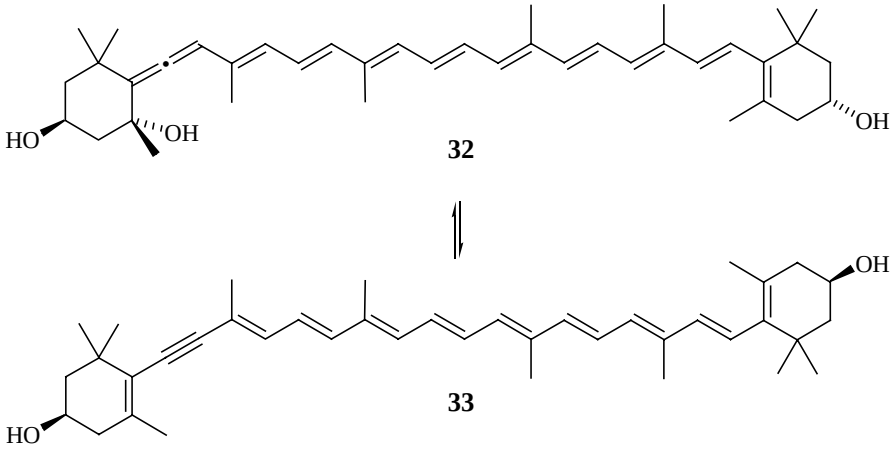
Grasshopper ketone (**30**) was first isolated from salivary secretions of the large flightless grasshopper, *Romalea microptera* (Figure 1.7).[60-64] Its structure and stereochemistry were confirmed by Russell and Weedon.[65] **30** has also been identified in grape and wine grape extracts,[66-69] as well as in such extracts treated with a glycosidase enzyme preparation.[70] All the compounds displayed in scheme 1.5 have been identified as grape products.



Scheme 1.5

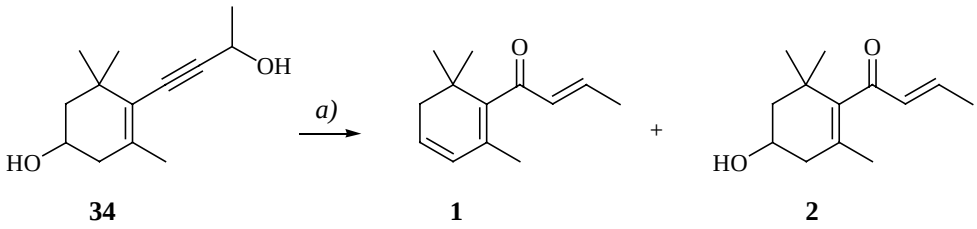
Figure 1.7: *Romalea Microptera*, from which was isolated grasshopper ketone.

Work carried out by Egger et al. had shown that the allenic carotenoid deepoxineoxanthin (**32**) could be isomerised under acidic conditions to give the acetylenic carotenoid diatoxanthin[71] (**33**) (Scheme 1.6). Based on this work, Ohloff et al. suggested a similar pathway, concentrating instead on the acetylenic diol **34**, as this could be envisaged as being produced *via* oxidative cleavage of **33**, followed by reduction of the C_9 carbonyl.[56]



Scheme 1.6

Under acidic conditions (30% sulfuric acid solution), Ohloff et al. showed that 3,9-dihydroxymegastigm-5-en-7-yne (**34**) produced a 1:4 ratio of **1** and **2** (Scheme 1.7).[56] They also demonstrated that transformation of 3-hydroxy-β-damascone (**2**) to β-damascenone (**1**) as previously proposed by Isoe et al.[55] does not occur, even under such strongly acidic conditions as 30% H_2SO_4.



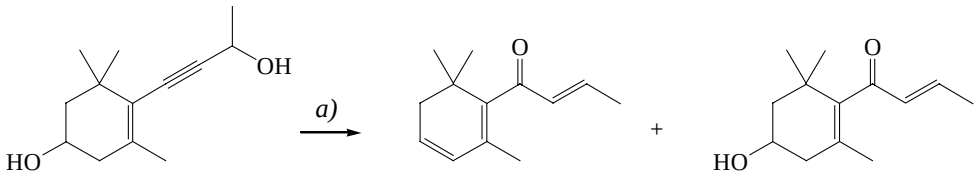
34 **1** **2**

a) 30% H_2SO_4

Scheme 1.7

1.4.2.2 More recently- Skouroumounis et al.

More recently, Skouroumounis and Sefton conducted a thorough study into the hydrolytic behaviour of suspected β-damascenone precursors.[72] They showed that after one year at room temperature and wine pH, **34** produced only 1% of β-damascenone (Scheme 1.8). Although this chemical transformation may contribute a small amount of **1** to aged bottled wines, it was thought unlikely to account for the total amount of **1** seen in young wines.

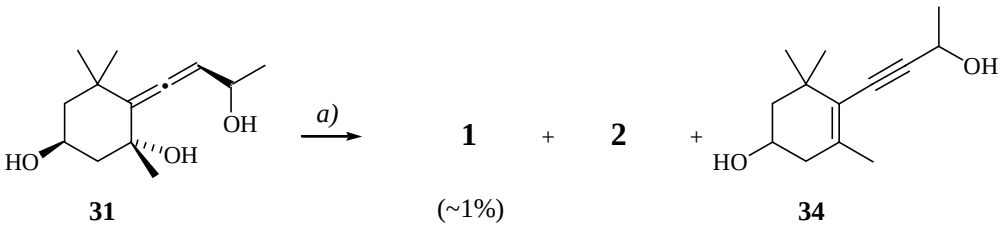


34 **1** (~1%) **2**

a) RT, pH 3.0, 1 yr.

Scheme 1.8

Skouroumounis et al. had previously shown that the allenic triol **31** can give **1** rapidly at wine pH and room temperature, albeit in low yield (1% at RT, pH 3, 24 hrs).[73] The two major products of the reaction were **2** and **34** in a ratio of 5.5:1. Thus, whilst the acetylene **34** produced 1% of β-damascenone over the period of one year, the allene **31** produced the same amount of β-damascenone, under the same conditions, in 24 hours. They concluded that **34** could not have been an intermediate in the conversion of the allenic triol **31** into **1** and **2**, because of this low reactivity at room temperature (Scheme 1.9).

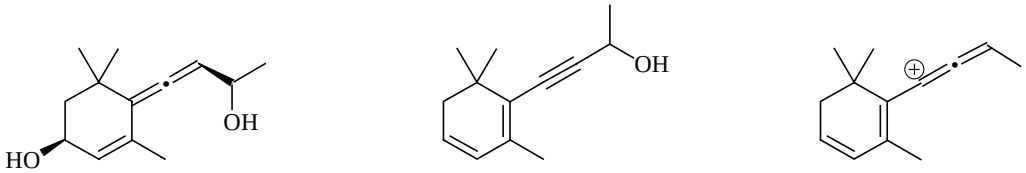


31 (~1%) **34**

a) RT, pH 3.0, 24 hr.

Scheme 1.9

Close scrutiny of the hydrolysate of **31** revealed the presence of two minor, unknown compounds, which were tentatively identified as being megastigma-4,6,7-triene-3,9-diol (**35**) and megastigma-3,5-dien-7-yn-9-ol (**36**) (Figure 1.8). **35** can be envisaged as being produced from allene triol **31** by simple dehydration. Further dehydration accompanied by rearrangement would produce **36**, which could then undergo a Meyer-Schuster rearrangement to give β-damascenone (**1**).



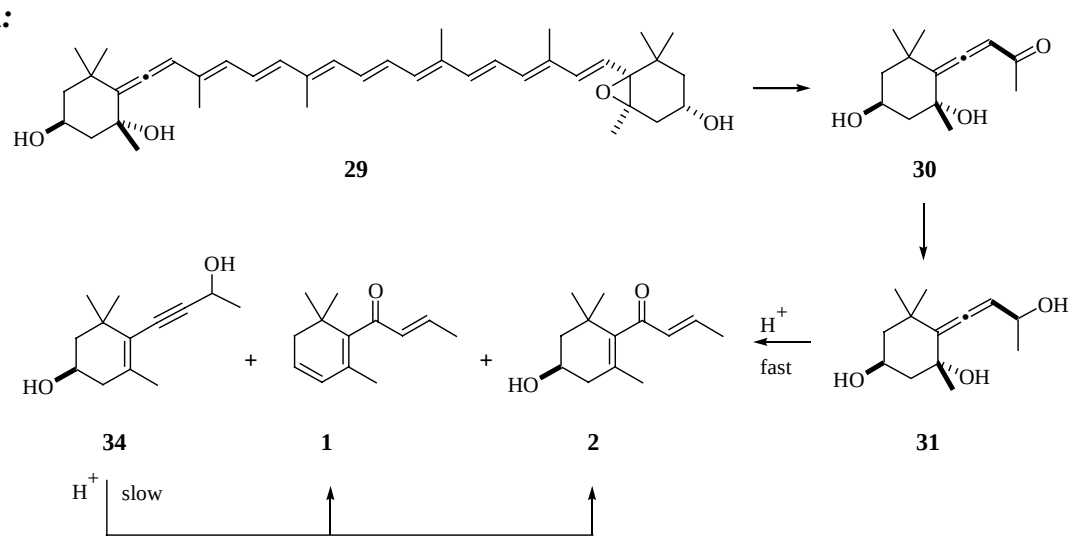
35 **36** **37**

Figure 1.8: Tentatively identified intermediates(**35** and **36**) in the hydrolysis of **31**.

The Meyer-Schuster rearrangement is the isomerisation, by 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 **37** (Figure 1.8).[74]

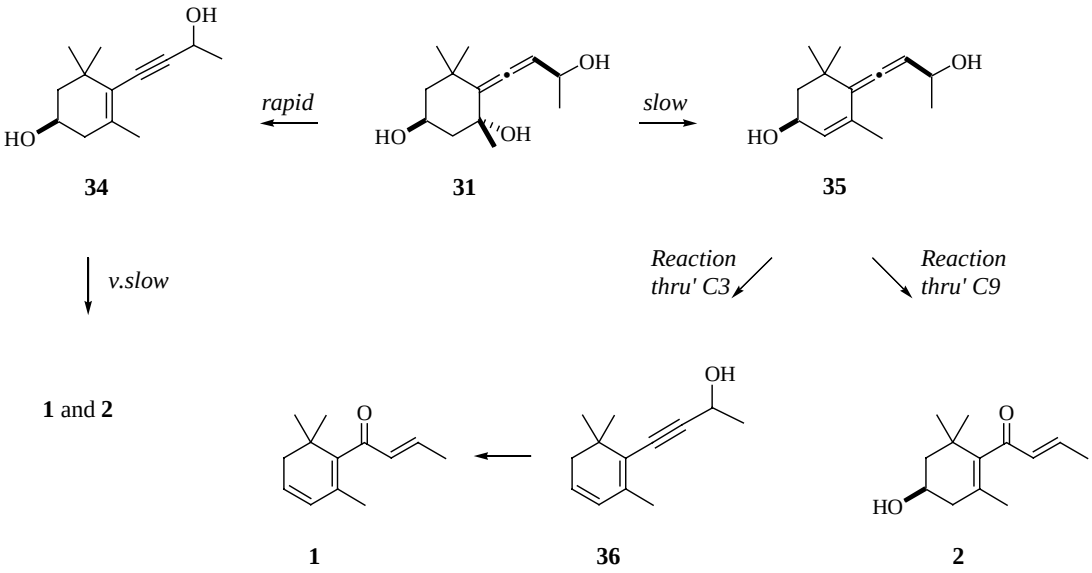
Skouroumounis and Sefton[72] proposed a refined scheme for the formation of β-damascenone, beginning with neoxanthin (**29**). Oxidative cleavage of **29** produces grasshopper ketone (**30**); this undergoes subsequent reduction to produce **31**, which as shown previously under acidic hydrolytic conditions, produces **1**, **2** and **34**. (Scheme 1.10A). They suggested that dehydration of **31** first produces **35**, which can then behave in one of two ways: it can (i) undergo rearrangement to form **2** *via* reaction through the C_9 hydroxyl, or (ii) undergo dehydration accompanied by rearrangement to give **36**, *via* reaction through the C_3 hydroxyl. Under acidic conditions **36** would be expected to produce β-damascenone exclusively (Scheme 1.10B).

A:



Scheme 1.10A

B:



Scheme 1.10B

1.5 Glycosydic Precursors

1.5.1 The Role of Glycoconjugation in Nature

Glycosidically bound volatiles have been found in a wide variety of fruits and vegetables including grapes,[75] raspberry,[76] pineapple,[77] celery[78] and tomato.[79] They occur in all parts of the plant and there are several hypotheses as to the role of glycosylation in nature; glycoconjugates are considered to play an important role in the accumulation and storage of volatile compounds within the plant. They are also thought to play an important role in transport and more generally as intermediates in the formation of secondary metabolites.[75,80]

In fruit and vegetables the level of bound volatiles has been found to be considerably higher than the corresponding unbound volatile fractions. Chemically, glycoconjugated aroma compounds show enhanced water solubility and reduced reactivity, which may explain the high levels of glycoconjugates in fruits and vegetables. Storage of low molecular weight metabolites in the form of

glycoconjugates protects the plant from any toxicity exhibited by the free aglycones.[81]

Transport functions of glycoconjugates have been suggested in certain fruits. Experiments in which various substrates were added exogenously, were later found in glycosylated form in a different part of the plant. One such experiment focused on geraniol metabolism in apples. After injection of geraniol into the core of the fruit, it was rapidly glycosylated before being transported to the flesh of the fruit, where it was metabolized further.[82]

1.5.2 The Formation of Flavour from Glycoconjugated Volatiles

The aglycones can sometimes be liberated from their glycosides by slow chemical hydrolysis, catalysed, for example, by the acidic conditions of wine, or by the action of enzymes (from grapes, yeast, bacteria or commercial glycosidase preparations). Watanabe et al.[83,84] have proposed that aroma volatiles are biosynthesised in the leaves, glycosylated and transported (*via* phloem) to the flower buds, where they accumulated as odourless precursors. The aroma volatiles were then subsequently released upon opening of the flower when sugars were cleaved by enzymes. It has been shown, in support of this hypothesis, that the enzymatic activities of the glycosidase enzymes in *Gardenia jasminoides* reached their maximum levels during the opening of the flowers.[83]

β-Damascenone (**1**) is formed in wine by hydrolysis of multiple precursors including both glycoconjugates and non-glycosidic (aglycone) compounds.[85] The generic structure of a β-D-glycoside is shown below (Figure 1.9); a general feature of glycosidically bound aroma compounds is that the directly bound sugar is β-D-glucose. The sugar may or may not be further substituted with one or more

additional sugar units. If further substitution is a feature, substitution occurs at the $C_{6'}$ position as shown below.[30]

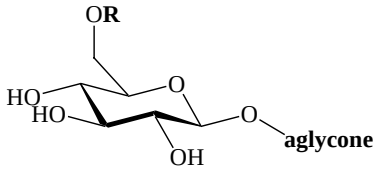


Figure 1.9: Generic structure of β-D-glycosides. The first attached sugar is invariably β-D-glucose; further carbohydrate substitution is possible at the 6'-position, indicated by the **R** substituent.

As indicated above, the release, in wine, of volatile compounds from the glucose moiety can occur by acid hydrolysis as a result of acids present in the wine, or by glycosidase activity through the use of enzyme preparations. The composition and sensory characteristics of the resultant pool of aroma compounds released by the two processes differ considerably.[67] In the presence of enzymes the release of volatile compounds (mostly from glycosides of alcohols) result from the breakage of the glycosidic linkage, often without further chemical transformation (Figure 1.10, left).

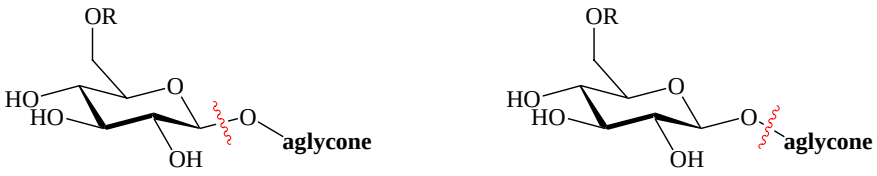


Figure 1.10: Different modes of cleavage of glycosides by either enzyme (left) or acid (right).

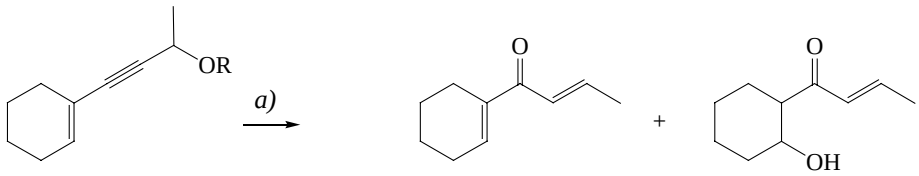
The release of volatile compounds from glycoconjugated alcohols by mild acid hydrolysis apparently only takes place with glycosides of activated alcohols (eg. allylic alcohols).[72] Acidic cleavage is believed to occur at the ether linkage rather

than at the glycosidic linkage (Figure 1.10, right). This results in the formation of a carbocation, which often reacts in a number of ways to give a range of products.[67]

It has been shown that the pool of volatile compounds resulting from mild acid hydrolysis of glycosidic extracts is generally much smaller than that generated by the enzymatic process.[68] The reduced pool of volatile compounds is a result of a smaller number of glycoconjugates that can be hydrolysed under mild acid conditions.

1.5.3: The Effect of Glycoconjugation on Chemical Reactivity

A reduction in chemical reactivity of aglycones towards acid, as a result of glycoconjugation, has been demonstrated by Skouroumounis et al.[86] An acetylenic, model compound **38** and its β-D-glucoside **39** (scheme 1.11) demonstrated a lower rate of reactivity on the part of the glycoconjugated species. The hydrolysis of aglycone **38** (pH 3.0, 100°C, 46 hours) produced **40** and **41** as the two major products, with approximately 26% consumption of the aglycone. Hydrolysis of the glycoside **39** under the same conditions produced the same two products; however the glycoside was shown to react at approximately one-third the rate of the aglycone.



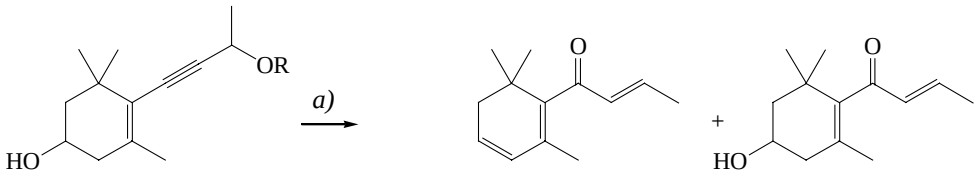
38, R = H
39, R = Glucose

40 **41**

a) 100 °C, pH 3.0, 46 hr.

Scheme 1.11

Later work by Skouroumounis and Sefton (Scheme 1.12) showed that, at 50 °C and pH 3.0, hydrolysis of the acetylenic diol **34** was eight times more rapid than its corresponding C_9 glycoside **42**.[72] In addition, the study showed that the proportion of β-damascenone formed from the glycoside **42** was higher than for the aglycone **34**. This suggests that the relative reactivities of the two positions (C_3 and C_9) had been altered, in favour of C_3. It was proposed that the presence of the C_9 glycoside acted to inhibit acid catalysed ionization of the propargyl alcohol function, thus allowing the alternative loss of the C_3 hydroxyl to become more competitive.



34, R = H
42, R = Glucose

1 **2**

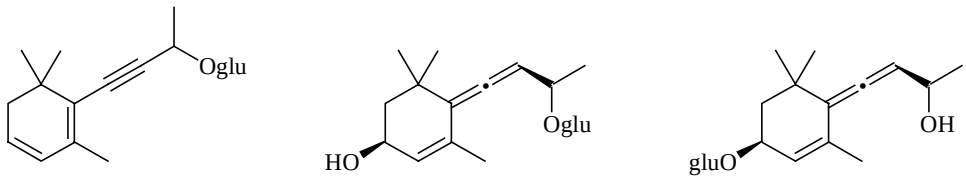
S.M.	Product ratio (**1**):(**2**)
34, R = H	**1 : 18**
42, R = Glucose	**1 : 11**

a) 50 °C, pH 3.0, 28 d.

Scheme 1.12

1.6 Aims

The aims of the work described in this thesis were to synthesise the suspected damascenone precursors megastigma-4,6,7-triene-3,9-diol (**35**) and megastigman-3,5-dien-7-yn-9-ol (**36**) and confirm their identities as the tentatively identified compounds from the previously reported allene triol hydrolysate. We then wished to conduct hydrolytic studies to investigate the potential intermediacy of **35** and **36** (and where possible their glycoconjugates **43**-**45**) in the formation of **1**. Thorough analysis of the products of hydrolysis of these compounds, especially **35**, was considered crucial as this information should furnish valuable information as to the precise mechanism of formation of **1**.



43 **44** **45**

Figure 1.11: Glycosylated target compounds **43** - **45**.

It had been predicted[72] that C_9 glycosylation of **35** (as in **44**) would result in a proportionally higher production of β-damascenone (**1**) relative to 3-hydroxy-β-damascone (**2**). It was thought that under these circumstances, ionisation at C_9 would be retarded and would allow dehydration on the ring to occur before rearrangement of the side chain. Alternatively, the C_3 glycoside **45** was expected to produce a higher proportion of **2** for similar reasons; reaction at the C_3 position being retarded, allowing reactions on the side chain to occur (Figure 1.12).

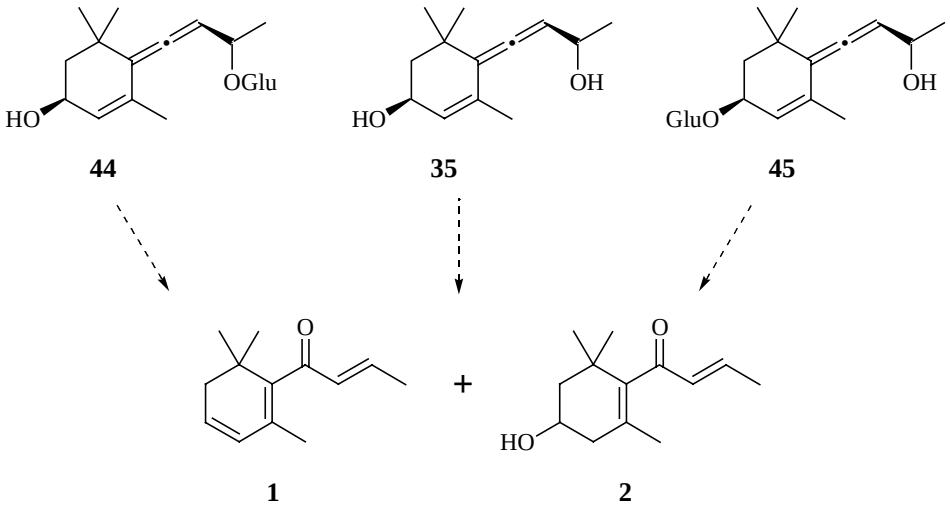
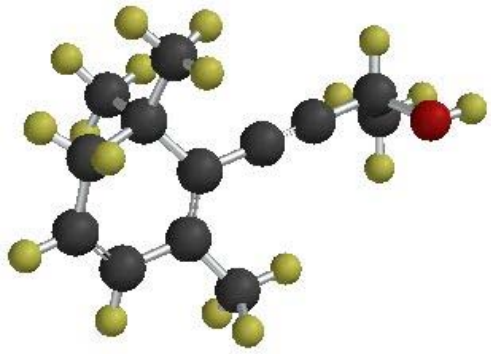


Figure 1.12: Predicted conversions of allene diol **35** and its two glucosides: C_3 glycosylation would be expected to direct reaction towards **2**, whereas C_9 glycosylation would direct reaction towards **1**.

CHAPTER TWO

Synthesis of 9-Hydroxymegastigma-3,5-dien-7-yne (36)



2.1: Background on 9-Hydroxymegastigma-3,5-dien-7-yne (36)

An inspection of the literature has revealed that 9-hydroxymegastigma-3,5-dien-7-yne (**36**) has been reported as a flavour compound in rum extracts.[87] However, no chemical evidence was presented to support the finding. To date **36** has not been reported in wine or grapes.

Skouroumounis et al.[72,73] had previously tentatively identified compound **36** from an acid hydrolysate of 3,5,9-trihydroxymegastigma-6,7-diene (**31**). One of the major aims of the work described in this thesis was to synthesise and characterise **36** and confirm its identity as that intermediate in the hydrolysis of **31**. Also, its proposed role as precursor in the generation of β-damascenone was to be investigated, as well as that of its corresponding β-D-glucopyranoside. This chapter concerns the synthesis and characterisation of **36**, while the glycoside **43** forms the focus of Chapter 3.

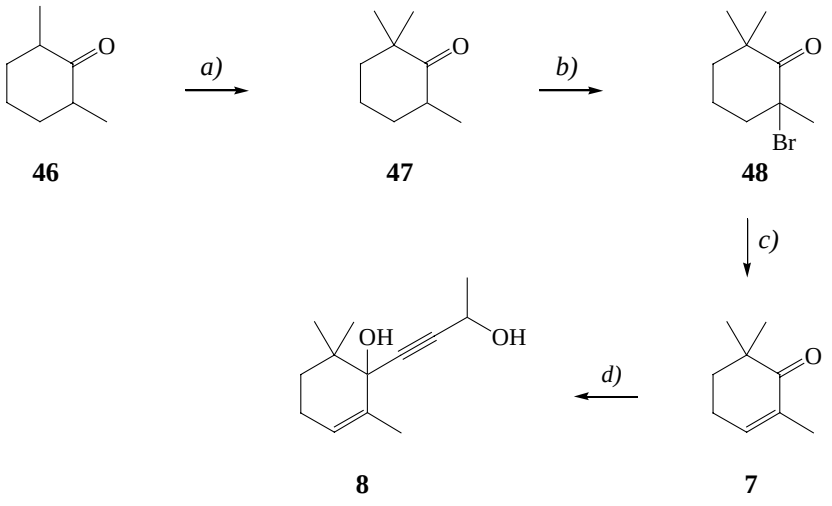
2.2: Synthesis of 6,9-Dihydroxymegastigm-4-en-7-yne (8)

Our first goal in the synthesis of **36** was the development of a reliable, high yielding synthesis of the enone **7**. This compound would have multiple uses as starting material for a range of reactions in a range of studies.

Synthesis of **7** (scheme 2.1) began with methylation of 2,6-dimethylcyclohexanone (**46**) in the presence of LDA and MeI to produce 2,2,6-trimethylcyclohexanone (**47**). The resulting crude reaction mixture was used in the next step without further purification. Although a small amount of **46** was still present in the product mixture of **47**, it was converted into 2,6-dimethylphenol following the bromination and dehydrobromination steps, and was therefore easily removed by washing with NaOH solution.

Bromination of **47** produced **48** and subsequent dehydrobromination resulted in the production of **7**. Alkylation of **7** was achieved with the use of the dilithio derivative of but-3-yn-2-ol[88] to produce **8** (scheme 2.1). Due to the partial insolubility of the dilithio species of butynol, excess solvent was required to allow efficient stirring. This reaction also required several days to reach completion. The product obtained was a mixture of diastereomers; however, one of the diastereomeric products was crystallised, with the remaining liquors comprising a mixture. Given that the final product **36** had only one stereocentre, it was decided not to attempt separation of the individual isomers of the bulk of the product.

The ^{13}C NMR spectrum of the single diastereomer obtained of **8** showed 2 alkene signals at 134.4 and 124.4 ppm as well as two alkyne signals at 87.5 and 85.3 ppm. The ^{1}H NMR spectrum included a signal at 4.58 ppm arising from the C_9 proton, which was split by both the C_{10} methyl and the C_9 hydroxyl. Also evident was the C_4 alkene signal at 5.48 ppm.



a) LDA, CH_3I (99%) **b)** Br_2, HOAc (100%) **c)** Pyridine, Δ (79%)

d) $LiC{\equiv}CCH(OLi)CH_3$, H_3O^+ (100%)

Scheme 2.1

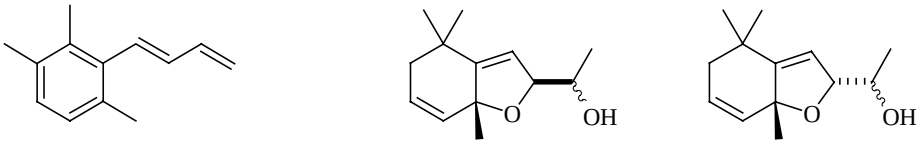
2.3: Attempted Dehydration of 8

A number of methods for the dehydration of **8** were investigated and are detailed in Table 2.1 (methods A-D).

METHOD A

Initial attempts to dehydrate **8** involved the use of *p*-TsOH. These reactions were carried out in NMR tubes for easy monitoring of reaction progress. The first reaction was heated at 50ºC for 21 hours; monitoring after both 1.5 and 4 hours revealed very complicated NMR spectra, but showed complete consumption of the starting material. However, after standing overnight under the same conditions, the NMR was very much simplified and showed the presence of only one product, in a high state of purity.

2.3.1 (*E*)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene (TPB, 49)



TPB, 49 **The Actinidols**

Figure 2.1: TPB, and its main precursor compounds in wine.

The product was identified as 1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB, **49**).[89] This compound had previously been suggested as a flavour compound in rum and cognac (incidentally without presentation of *any* supporting evidence,[90]) and had also been implicated in the staling of beer.[91] As a result of this synthesis, TPB was identified as an aroma compound in wine for the first time.[92] Its aroma detection threshold was measured as 40 ng/L in white wine, placing it on a par with the most potent odorants. It was found by Cox et al. to be present in a wide variety of white

wines, at concentrations ranging from barely detectable (<10 ng/L) to well in excess of the measured threshold.[93] A curious observation was made in that TPB was totally absent in all red wines studied but was observed (in substantial quantities) in the hydrolysates of glycoside samples sourced from red varieties. Detailed studies established that certain wine components (in particular the polyphenolic compounds, ie. tannins commonly associated with red wines) were responsible for its absence in the final wines.[93]

TPB belongs, as does damascenone (**1**), to the class of compounds known as the C_{13}-norisoprenoids.[94] Its origin within grapes was examined and found to begin with common grape components such as the actinidols[95-97] (Figure 2.1), which under the acidic conditions of wine, undergo various hydrolytic and rearrangement processes to produce, *inter alia*, TPB.

METHOD B

The use of acetic anhydride and pyridine resulted in the formation of **50** from **8,** rather than the desired acetate of **36**. Important changes in the ^{1}H NMR spectrum in the transformation of **8** to **50** included a downfield shift of the H_9 signal, and the appearance of the acetyl methyl at 2.04 ppm. Two signals, one at 169.8 ppm and another at 20.9 ppm confirmed the presence of the acetyl group in the ^{13}C NMR.

METHOD C

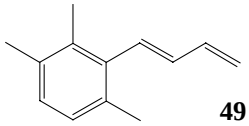
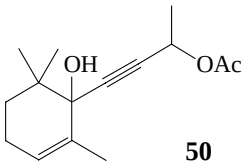
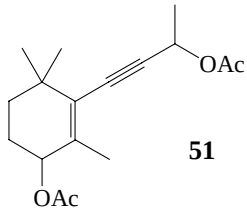
The use of sodium acetate, DMAP and acetic anhydride resulted in production of the allylically rearranged species **51**. Formation of compound **51** was confirmed by analysis of the NMR spectra. In the proton spectrum, the peak corresponding to the alkene proton at C_4 was now absent but inspection of the carbon spectrum revealed that two alkene signals (at 137.8 and 129.1 ppm) were still present, consistent with the allylically rearranged structure. In addition to the hydrogen at C_9 (5.60 ppm, J =

6.6 Hz), a second signal adjacent to oxygen (5.22 ppm, J = 4.4 Hz) was assigned to the lone H_4 proton. These general spectral features were observed in all of the allylically rearranged products obtained during the attempted dehydrations of **8** and **50**, and proved to be most useful in assigning the structures of the products obtained.

METHOD D

Flash vacuum pyrolysis was also investigated for the dehydration of **8**, however this method proved to be unsuccessful in our hands, giving only very messy mixtures of products, with no evidence of successful formation of **36**. FVP was not investigated further.

Table 2.1: Attempted dehydration of **8**.

Conditions	**T** (°C)	**Time**	**Product**
p-TsOH (xs), $CDCl_3$ (method **A**)	50	21 hr.	 **49**
Pyridine (3 eq.), Ac_2O (2 eq.) (method **B**)	110	O/N	 **50**
NaOAc (2 eq.) DMAP (cat.), Ac_2O (method **C**)	110	45 min.	 **51**
F.V.P. (method **D**)	various	completion	decomp.

Due to the apparent instability of **8**, all further attempts to prepare **36** utilised **50** as the starting material.

2.4: Attempted dehydration of 50

The next series of reactions involved the use of **50** as starting material in an attempt to reduce rearranged products as a result of having a free propargylic alcohol at the C_9 position.[98] The use of *p*-TsOH was again investigated under a variety of conditions as shown in Table 2.2.

METHOD E1

The use of 5% *p*-TsOH at 50ºC for one hour in $CDCl_3$ produced a number of products, including the allylically rearranged alcohol **52** (~56%), desired compound **53** (~44%) and β-damascenone itself. However, alternative procedures were sought in an attempt to improve the yield of **53** and reduce the amount of allylically rearranged product. ^{1}H NMR spectral data for compound **52** included a quartet at 5.60 ppm (H_9) and a broad triplet at 4.01 ppm (H_4) indicating the loss of the H_4 alkene. The acetate group appeared to remain with signals present in both the ^{1}H and ^{13}C NMR spectra. Two alkene and two alkyne resonances also appeared in the ^{13}C NMR spectrum.

METHOD E2

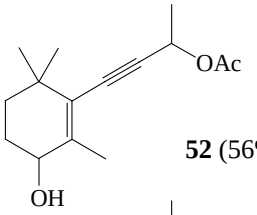
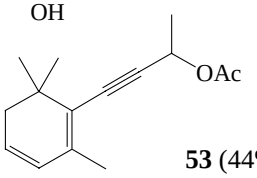
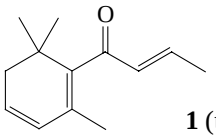
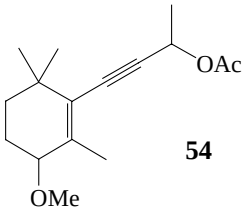
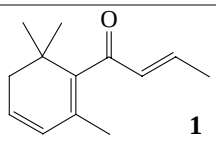
Trimethylorthoformate (TMOF) was employed to remove water produced during the reaction, however, due to the concomitant formation of methanol, formation of the allylically rearranged methoxy adduct **54** became a problem. ^{1}H NMR spectral data for **54** included a quartet at 5.60 ppm arising from H_9, a broad triplet at 3.53 ppm resulting from H_4, now residing adjacent to a methoxy functionality and a singlet at 3.35 ppm as a result of the methoxy group itself. The C_9 acetate remained in the

spectrum of **54** and again, two alkene and two alkyne resonances were present in the ^{13}C spectrum of **54**.

METHOD E3

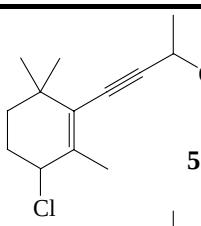
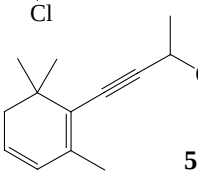
The use of 5% p-TsOH at 50ºC for one hour in CH_2Cl_2 produced (**1**) as the major product. The quantities quoted in the following tables do not refer to isolated yields of compounds but rather to the relative proportions of products, as determined by NMR spectroscopy.

Table 2.2: Dehydration of **50** using *p*-TsOH.

Conditions	**T (°C)**	**time**	**Product**
p-TsOH (cat.), $CDCl_3$ (method **E1**)	50	1 hr.	 **52** (56%)  **53** (44%)  **1** (trace)
p-TsOH (cat.), CH_2Cl_2, TMOF (method **E2**)	50	1 hr.	 **54**
p-TsOH (cat.), CH_2Cl_2, (method **E3**)	50	1 hr.	 **1**

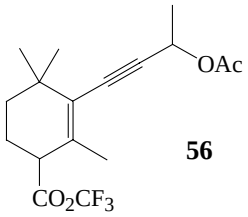
The next set of reactions also made use of **50** as starting material and involved the use of a variety of different dehydrating agents (Table 2.3, Methods F-H). Reagents investigated were MsCl, $SOCl_2$ and $POCl_3$; the major product formed in each of these reactions was the allylically rearranged chloride **55**. The use of MsCl in pyridine also produced **53**; however, again the yield was small and so alternative procedures were investigated. ^{1}H NMR spectral data for the chloride **55** included a quartet at 5.59 ppm, resulting from H_9, and a broad triplet at 4.44 ppm, resulting from H_4, now residing adjacent to the chlorine functionality. The acetate resonances remained in both the ^{1}H and ^{13}C spectra and again, there were two alkene and two alkyne resonances in the ^{13}C spectrum.

Table 2.3: Attempted dehydration of **50**.

Conditions	**T (°C)**	**time**	**Product**
MsCl, pyridine (method **F**)	50	1 hr.	 **55** (63%)  **53** (37%)
$SOCl_2$, pyridine (method **G**)	50	1 hr.	**55**
$POCl_3$, pyridine (method **H**)	50	2 hr.	**55**

Under the above conditions **55** was produced with relative ease; this presented a possible alternative pathway to the target, **36**, by elimination of HCl under basic conditions. These reactions were unsuccessful (Table 2.4).

Table 2.4: Attempted elimination of HCl from **55**.

Conditions	**T (°C)**	**Time**	**Product**
Pyridine, $CHCl_3$ (method **I1**)	Δ	2 hr.	**55**
Alumina, THF (method **I2**)	RT	1 d.	**55** (50%), **53** (50%)
KO^tBu, ^tBuOH (method **I3**)	RT	2 hr.	decomp.
NEt_3, CF_3CO_2Ag, THF (method **I4**)	RT	45 min.	 **56**

The use of triethylamine and silver trifluoroacetate resulted in the formation of the allylically rearranged trifluoroacetate adduct **56**. ^1H NMR spectral data for compound **56** included a quartet at 5.58 ppm (H_9) and a broad triplet at 5.39 ppm (H_4). The acetate group remained in both ^1H and ^{13}C spectra and, as with all of the allylically rearranged series, two alkene and two alkyne resonances were observed in the ^{13}C spectra.

2.5: Successful Dehydration of 50

The use of P_2O_5 produced some interesting results with a variety of conditions investigated (Table 2.5). The use of P_2O_5 eliminated the formation of all side products, including allylically rearranged adducts; when product was not formed, starting material was invariably isolated. The physical nature of P_2O_5 under these conditions was to form a solid mass on the bottom of the flask, thus producing a two-phase system making stirring and extraction very difficult. The use of a commercial product Sicapent® [102] (phosphorus pentoxide on an inert mineral support) reduced the formation of the solid mass. However, Sicapent® resulted in only a very poor yield (6%) of desired acetate **53**, therefore so its use was subsequently abandoned.

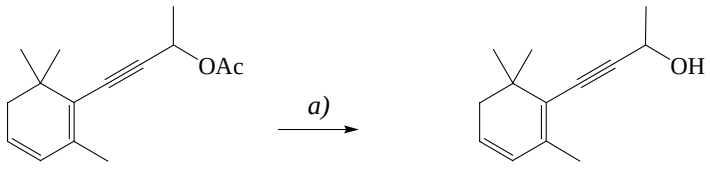
The use of ground pumice stone was investigated in an attempt to keep the P_2O_5 finely dispersed in solution. This method did not reduce formation of solid mass significantly and made extraction of the product very difficult.

The most successful reaction (54%) involved the adsorption of P_2O_5 onto dried celite[103] in pyridine and toluene. Celite allowed the P_2O_5 to be more finely dispersed in the reaction medium and resulted in improved extraction and isolation of the product.

Table 2.5: Dehydration of **50** using P_2O_5.

Conditions	**T (°C)**	**Time**	**Product**
P_2O_5 (3 eq.), pyr, $CHCl_3$ (method **J1**)	Δ	2 d.	**53** (15%)
Sicapent® (12 eq.), pyr (method **J2**)	Δ	2 d.	**53** (6%)
P_2O_5 (10 eq.), pumice, pyr, toluene (method **J3**)	Δ	3 d.	**53** (16%)
P_2O_5 (10 eq.), celite, pyr, toluene (method **J4**)	Δ	3d	**53** (54%)

With a method in place for producing **53** in acceptable yield, deprotection of **53** was investigated and achieved in aqueous ethanol solution (50%) with the use of KOH (Scheme 2.2). **36** was produced in excellent yield (95%) and fully characterised. The NMR data for **36** showed a 6H singlet at 1.06 ppm assigned to H_{11} and H_{12}, a 3H doublet at 1.52 ppm (J=6.6 Hz) resulting from H_{10}, a 3H broad, singlet at 1.93 ppm resulting from H_{13}, a 2H dd at 2.10 ppm (J=1.4, 3.9 Hz) resulting from H_2, a 1H quartet at 4.75 ppm (J=6.6 Hz) resulting from H_9 and a complex 2H multiplet from 5.86-5.76 ppm resulting from H_3 and H_4.



53 **36**

a) KOH (10 eq.), 50% aq. EtOH (95%)

Scheme 2.2

2.6: Authentication of 9-Hydroxymegastigma-3,5-dien-7-yne (36)

The first issue to be addressed was the identity of the compound previously tentatively identified in the hydrolysate of the allenic triol **31**. A sample of this hydrolysate was analysed by GC-MS for compound **36**.

The peak assigned as **36** in the chromatogram of this hydrolysate (Figure 2.2) had an identical GC retention time and mass spectrum to that of the authentic synthetic sample, and was symmetrically enhanced when the two samples were co-injected.

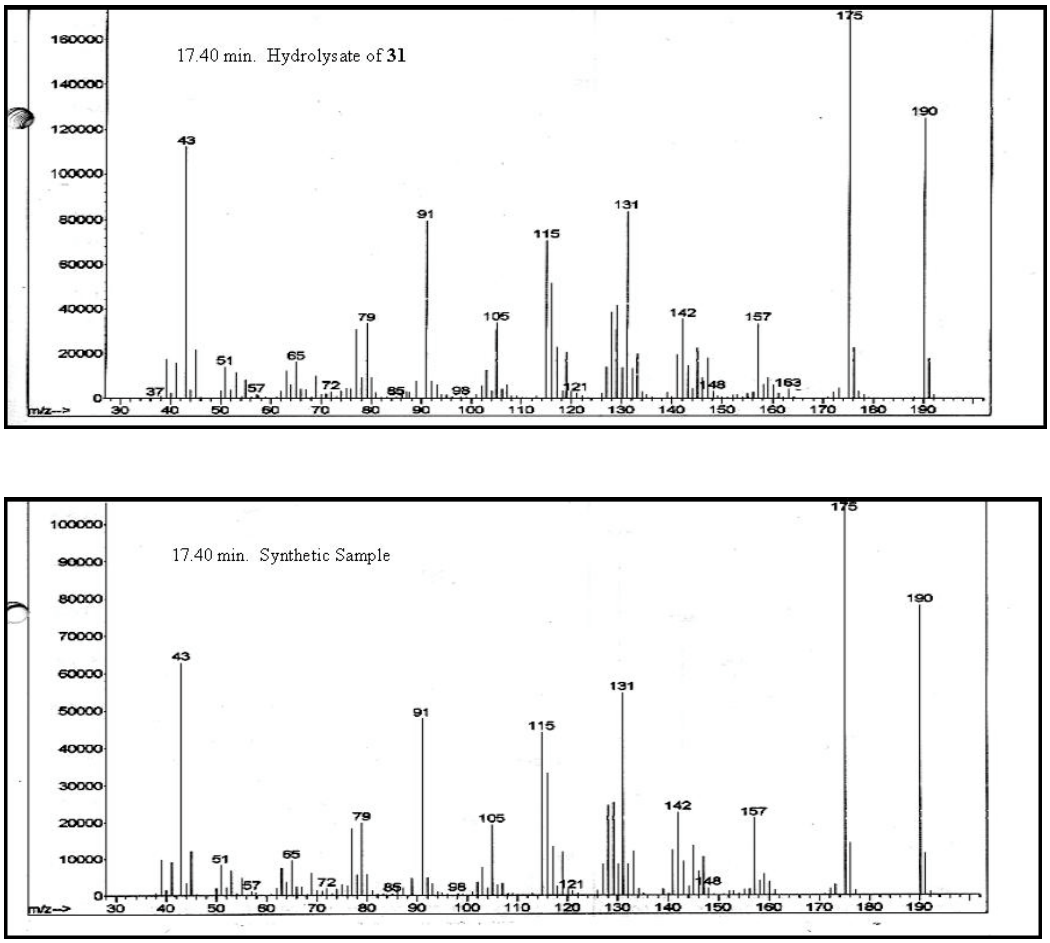
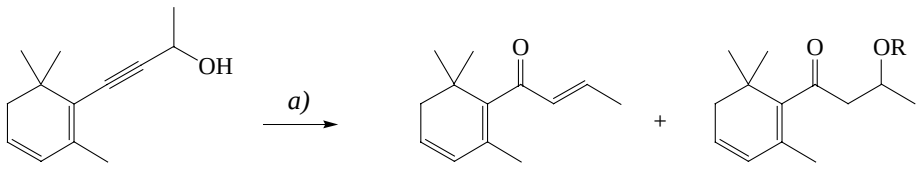


Figure 2.2: Mass spectra obtained for compound assigned as **36** from original hydrolysate of **31**[73] (upper); and the sample synthesised in this study (lower).

2.7: Products of Hydrolysis of (36)

The next issue to be resolved was whether the target **36** was actually a precursor in the formation of β-damascenone (**1**) under acidic conditions. **36** underwent hydrolysis in model wine (pH 3.0) at 45 ºC, in sealed ampoules, under an inert atmosphere for 18 hours. The temperature was elevated so as to mimic long-term studies of this nature. The products of hydrolysis, after this time, were β-damascenone (**1**) (84.3%), starting material **36** (9.3%), a compound tentatively identified as the hydrate of β-damascenone **57** (3.3%) and another compound tentatively identified as the ether adduct of β-damascenone **58** (3.1%) (Scheme 2.3). Thus, β-damascenone comprised >90% of the product mixture. That β-damascenone was the major product in the hydrolysis of **36** confirms its role as immediate precursor in the production of **1**. Hydrolytic studies conducted at 25 ºC are described in the following chapter.



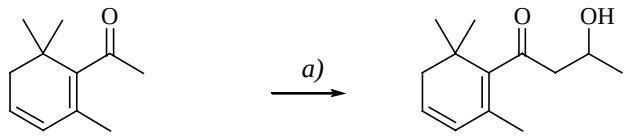
36 **1** **57**, R = H
58, R = Et

a) pH 3.0, 45 °C, 2 d.

Scheme 2.3

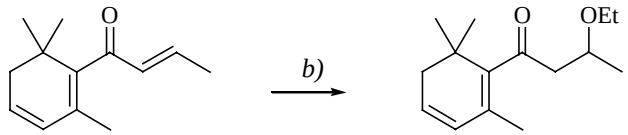
The identities of the two latter compounds have since been confirmed through synthetic means.[101] Compound **57** was synthesized by the addition of LiHMDS to a solution of 2,5,5-trimethyl-1-acetylcyclohexa-1,3-diene (**59**) at -78ºC, followed by reaction with acetaldehyde (scheme 2.4A). Compound **58** was synthesized more directly by the addition of sodium ethoxide to β-damascenone in solution (scheme 2.4B).

A:



59 **57**

B:



1 **58**

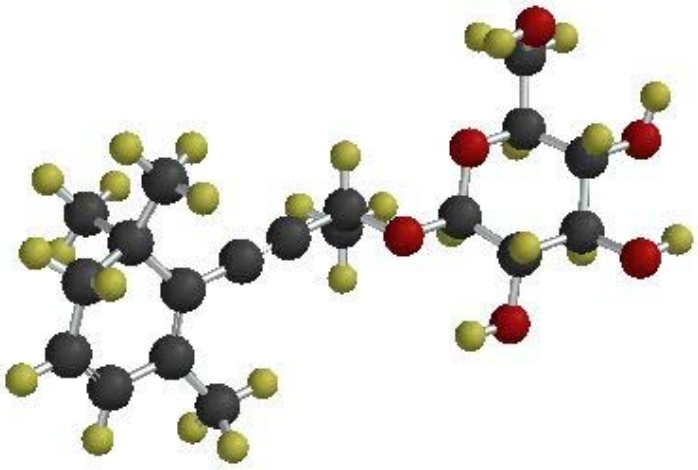
a) i) LiHMDS, -78 °C
ii) CH_3CHO, H_3O^+

b) NaOEt, EtOH

Scheme 2.4

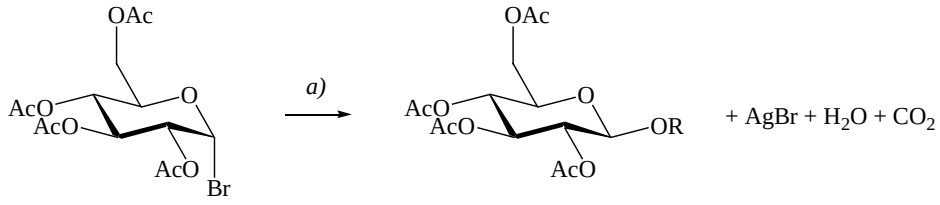
CHAPTER THREE

Synthesis and Hydrolysis of Glycoside (43)



3.1: Background on Glycosylation Reactions

The most well known early preparation for β-D-glycosides is that of Koenigs and Knorr[102] in 1901 using a tetraacetatylated bromoglucose (**60**) with silver carbonate or silver nitrate, and pyridine as the acid acceptor (Scheme 3.1).



60

a) ROH, Ag_2CO_3

Scheme 3.1

This reaction has been modified over the years to maximise yield and minimise side products, most often the formation of the α-glucoside and orthoester by-products. These modifications are briefly discussed here.

A variety of silver salts, including silver oxide, silver carbonate and silver nitrate, have been used in the Koenigs-Knorr glycosylation with tetraacetyl-α-D-bromopyranose. In 1979 Banoub and Bundle[103] used silver triflate as the Lewis acid, with collidine or N,N-tetramethylurea in dichloromethane to synthesise either orthoesters or glycosides.

Early glycosylation reactions were seen to give a mixture of the α-glucoside, β-glucoside and orthoester by-product formation. In 1982 Kunz and co-workers introduced the use of tetrapivaloyl-α-D-bromopyranoside (**61**),[104] which directs glycosylation through neighbouring group participation (Figure 3.1), and with added steric hindrance gave exclusively the β-glucoside. It also reduced orthoester formation, further increasing the yield.

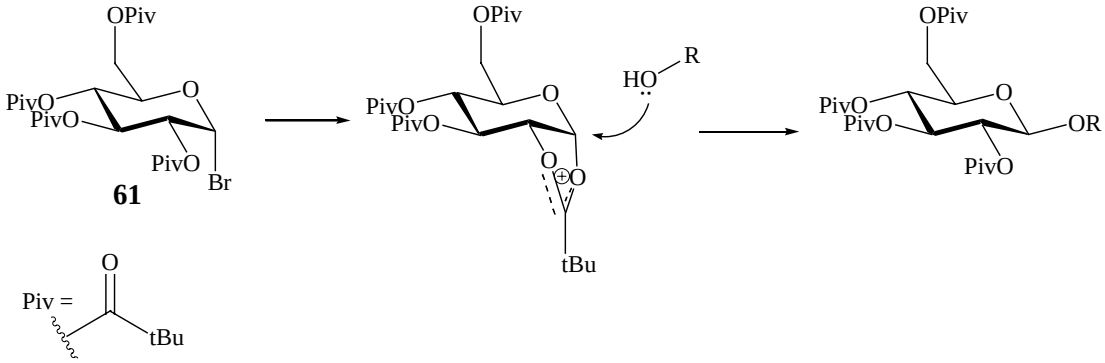
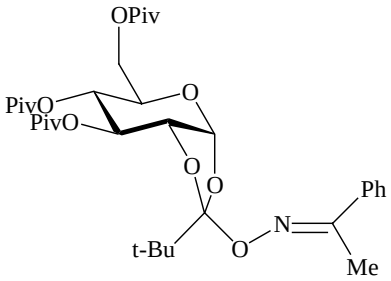


Figure 3.1: Neighbouring group assistance in the mechanism of glycosylation using **61**.

Kunz and Pfrengle[105] also published an alternate method to further reduce orthoester by-product formation. It involved synthesis of orthoester **62** (Figure 3.2) from tetrapivaloyl α-bromoglucopyranose treated with acetophenone oxime in the presence of silver triflate. This orthoester, in the presence of boron trifluoride etherate gave β-glycosides of secondary alcohols in excellent yields.



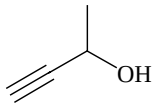
62

Figure 3.2: Oximate orthoester **62**, used as an alternative to **61**.

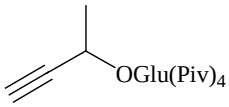
3.2: Synthesis of Model Glycoside 65

Due to the difficulties traditionally associated with carbohydrate chemistry (mixed stereoselectivity, low yielding reactions and competing orthoester formation) and the difficulty involved in long-term storage and production of **36**, a model system (**63**) (Figure 3.3) was investigated before glycosylation of **36** was attempted. **63** was felt

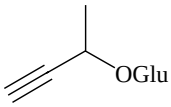
to be a good model as it also contains a propargyllic alcohol function, exactly as found in **36**. In fact, the side chain in **36** was introduced by addition of **63** as its lithium acetylide.



63



64



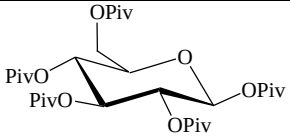
65

Figure 3.3: Model compounds chosen for synthesis.

In 1987 Kunz published a review comparing the use of pentapivaloate **66** against the orthoester **62** in the glycosylation of the primary hydroxyl in serine.[106] The glyconconjugate was formed steroeselectively in almost quantitative yield. Both methods were investigated as detailed in Table 3.1. In our hands, the use of **66** was not as successful as other methods with minor amounts of orthoester and α-glycoside formed.

A modified Koenigs-Knorr[107] synthesis, using tetrapivaloyl-α-D-bromopyranose (**61**), silver triflate and s-collidine as the base was found to be the most practical and reliable method for introducing glucose, and was employed for the synthesis of the target glycoconjugates.

Table 3.1: Methods for glycosylation of model compound **63**.

Method	**Conditions**	**Time**	**Product/ Yield**
 **66**	TMS-Triflate, Acetonitrile	15 min.	orthoester (minor) + **64** (67%) + α-glyc (minor)
Kunz Orthoester (**62**)	$BF_3.Et_2O$, DCM	2.5 hr.	**64** (61% isol)
Modified Koenigs-Knorr (**61**)	Silver triflate, S-collidine, DCM, RT	O/N	**64** (92% crude)

Glycosylation of the model compound **63** using the pentapivaloate **66** produced the desired product, however it also produced minor amounts of orthoester and the unwanted α-glycoside of **63**.

The use of orthoester **62** required 4 equivalents of $BF_3.Et_2O$ to activate the orthoester in the presence of the alcohol in dichloromethane with good yields and selectivity being reported. This method was successful in our hands and produced the desired adduct **64** in an isolated yield of 61%.

The use of tetra-*O*-pivaloyl-α-D-bromopyranoside (**61**) in conjunction with silver triflate and s-collidine was also successful in our hands. The reaction, conducted in chloroform overnight, gave **64** in a very clean state with yields as high as 92%. The use of racemic **63** resulted in the production of two diastereomeric products, which were separable but whose absolute stereochemistry could not be assigned directly. A pure sample of (*S*)-**63** was glycosylated and by comparison with this pure glycoside, the stereochemistries of the earlier prepared compounds were able to be assigned.

Deprotection of the pivaloylated form of **64** was successfully achieved using sodium methoxide in methanol in high yields.

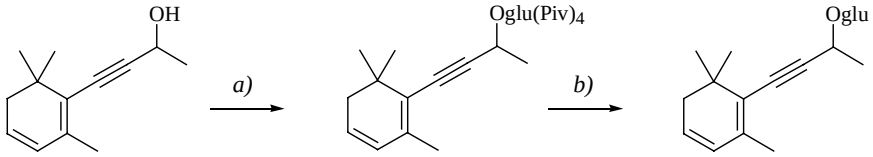
Due to the success of the above reactions, the glycosylation of **36** was attempted without further investigation of model reactions.

3.3: Synthesis of Glycoside 43

The first method investigated was the highest yielding for the model system, the modified Koenigs Knorr[107] (Scheme 3.2). The reaction made use of **36** in the presence of **61**, s-collidine and silver triflate in dichloromethane at room temperature. Reaction with **36** produced **67** in 72% yield, a good yield for glycosylation of a secondary alcohol. Purification by column chromatography produced the two

expected diastereomers of the pivaloylated glycoside, with the exact stereochemistries unknown at the actual time of their synthesis. This issue was resolved by using optically active starting reagents, as discussed in section 3.4.

The two remaining methods of introducing glucose were also attempted; however the addition of $BF_3.Et_2O$ or TMS-triflate resulted in decomposition of starting material as evidenced by an immediate blackening of the solution, and were consequently abandoned.



36 **67** **43**

a) **61**, s-collidine, AgOTf, CH_2Cl_2, (72%) **b)** NaOMe (10eq), MeOH, RT, 1hr (99%)

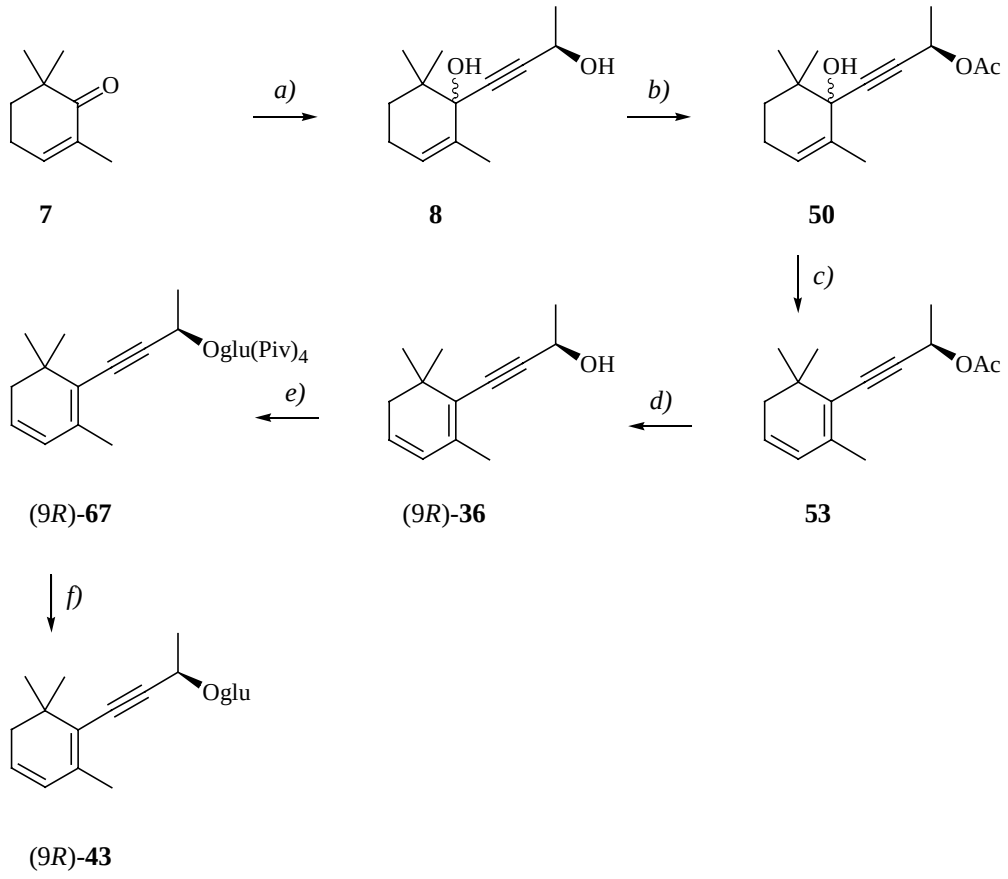
Scheme 3.2

A variety of methods were investigated for the deprotection of **67**. The best results were obtained using excess NaOMe in methanol over one hour, with short column chromatography (SCC) being an ideal method for purification.

3.4: Synthesis of Isomerically Pure Glycosides 43

The above describes the synthesis of the glycoside beginning with racemic dienyne alcohol. While this works well, and gives both of the expected diastereomers, which were separable by column chromatography, the question of assigning stereochemistry remained. It was considered that synthesis of enantiomerically pure dienyne alcohol by the use of enantiomerically pure alcohol **63** was necessary in order to determine the stereochemistries of the two glycosides. The synthesis was

carried out using the synthetic methods previously described but, as mentioned, involved alkylation of the ketone **7** using both enantiomerically pure (*R*) and (*S*) but-3-yn-2-ol (**63**). As dehydration of the tertiary alcohol (ie. in intermediate **8**) was a part of the synthesis, it was not necessary to consider the stereochemistry at this carbon atom as the hydroxyl was eliminated during the dehydration step. Glycosylation of **36** produced the diastereomerically pure protected glycosides and comparison with the racemic work showed that the first eluting diastereomer of the protected glycoside **67** corresponded to the (9*S*) diastereomer, with the second eluting isomer corresponding to the (9*R*). Scheme 3.3 depicts the synthesis of the (9*R*) glycoside; the (9*S*) glycoside was prepared in an identical manner, but using (*S*) but-3-yn-2-ol.



a) (*R*) LiC≡CCH(OLi)CH_3 (or (*S*) LiC≡CCH(OLi)CH_3) **b)** Ac_2O, pyr, Δ **c)** P_2O_5, pyr, tol., Δ

d) KOH, aq. EtOH **e)** **61**, s-collidine, AgOTf, CH_2Cl_2 **f)** NaOMe, MeOH

Scheme 3.3

3.5: Hydrolytic Studies

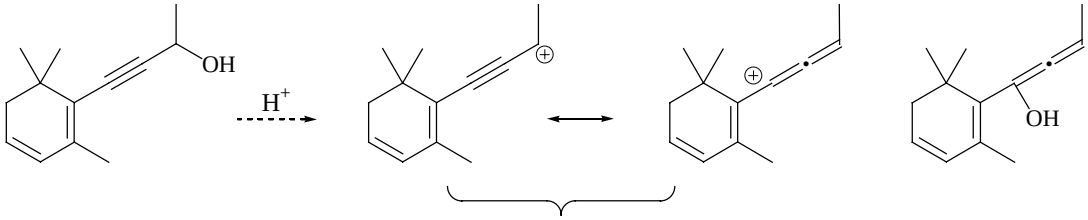
3.5.1: Hydrolysis of 9-hydroxymegastigm-3,5-dien-7-yne (36)

Preliminary hydrolytic studies were carried out in order to obtain an approximate half-life for the conversion of **36** into damascenone (**1**); this was done to enable appropriate sampling times to be chosen for the accurate study. A half-life of the order of one day for the alcohol **36** at room temperature was indicated.

The accurate hydrolytic work utilised the racemate of the aglycone. The hydrolytic study was carried out at 25 ºC, at both pH 3.0 and pH 3.2 with each experiment performed in duplicate to ensure reproducibility of results. In order to avoid sample to sample variation caused by small differences in the solvents, a large batch of model wine solution was prepared and used for all experiments. The starting material **36** was synthesized and purified using short column chromatography and used immediately to eliminate degradation of the starting materials as a factor that might influence the results. The concentration of damascenone in the hydrolysates was determined by GC-MS by a method closely based on that of Kotseridis et al.,[47] using d_4-damascenone as internal standard.

Figures 3.5 and 3.6 show the formation of damascenone (from the racemic aglycone) over time at pH 3.0 and pH 3.2, respectively. The half-life of the aglycone, defined as the time required to reach half the final observed concentration, was measured at approximately 40 hours for the pH 3.0 study, and approximately 65 hours for the pH 3.2 study. Importantly, the sigmoidal shape of the curve indicates the presence of a chemical intermediate. This could be either a cationic species, (**68**, figure 3.4) which likely results from protonation and loss of the hydroxyl function, or the C_7-hydrated (enol) form **69**. This is known as the Meyer-Schuster rearrangement;[74] a 1,3-

hydroxyl shift of, in this case, a secondary α-acetylenic alcohol to give the α-β-unsaturated carbonyl compound, in this case, β-damascenone.



36 **68** **69**

Figure 3.4: Possible intermediates involved in the hydrolysis of **36**.

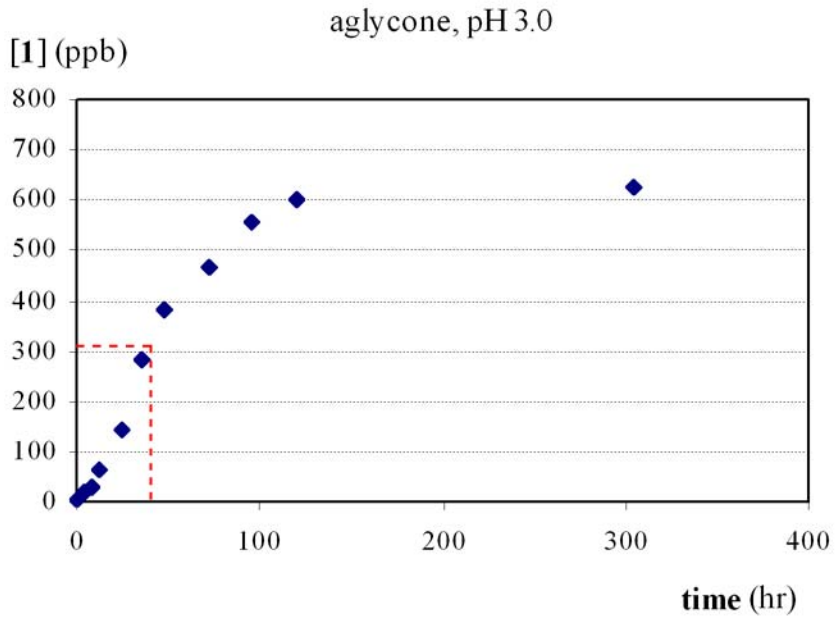


Figure 3.5: Formation of damascenone from aglycone **36** at pH 3.0, 25 ºC.

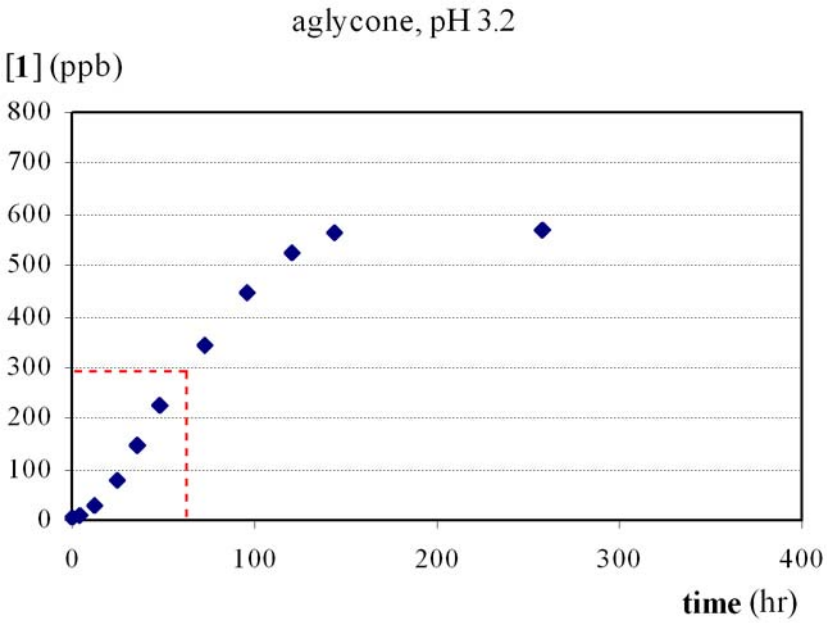


Figure 3.6: Formation of damascenone from aglycone **36** at pH 3.2, 25 ºC.

3.5.2: Hydrolysis of glycosides (9*S*)-43 and (9*R*)-43

Again, preliminary hydrolytic studies were carried out for the glycoside, these indicated a half-life of approximately 5 days for the glycoside **43**. As before, these studies gave an indication of appropriate sampling times for the more detailed study.

The detailed hydrolytic work was conducted with both (9*S*)-**43** and (9*R*)-**43** (Figures 3.7/3.8 and 3.9/3.10, respectively). It was our expectation that the rate of formation of **1** from the two glycosides would not vary significantly from each other. However, it was expected that β-damascenone would be formed at a higher rate from the aglycone than from the glycosides, and this was found to be the case. As with the aglycone hydrolyses, the experiments were carried out at 25 ºC, at pH 3.0 and 3.2 and each experiment was again done in duplicate. The model wine solution was made fresh and the same batch used for each experiment. Both (9*S*)-**43** and (9*R*)-**43** were synthesised, purified using short column chromatography and used immediately to eliminate degradation of the starting materials. The half-lives of conversion of **36**, (9*S*)-**43** and (9*R*)-**43** are collected in Table 3.2.

Table 3.2: Half-lives for the conversion of **36**, (9*S*)-**43** and (9*R*)-**43** into damascenone (**1**).

Compound	$t_{½}$ (pH 3.0)	$t_{½}$ (pH 3.2)
aglycone (**36**)	40 hr	65 hr
(9*R*)-(**43**)	3.0 d	6 d
(9*S*)-(**43**)	3.5 d	6.5 d

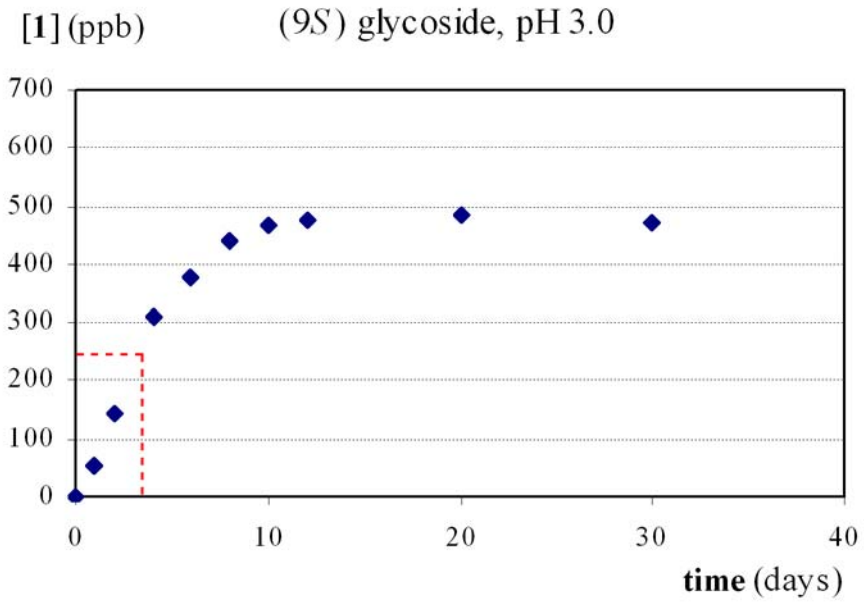


Figure 3.7: Formation of damascenone from (9*S*)-**43** at pH 3.0, 25 ºC.

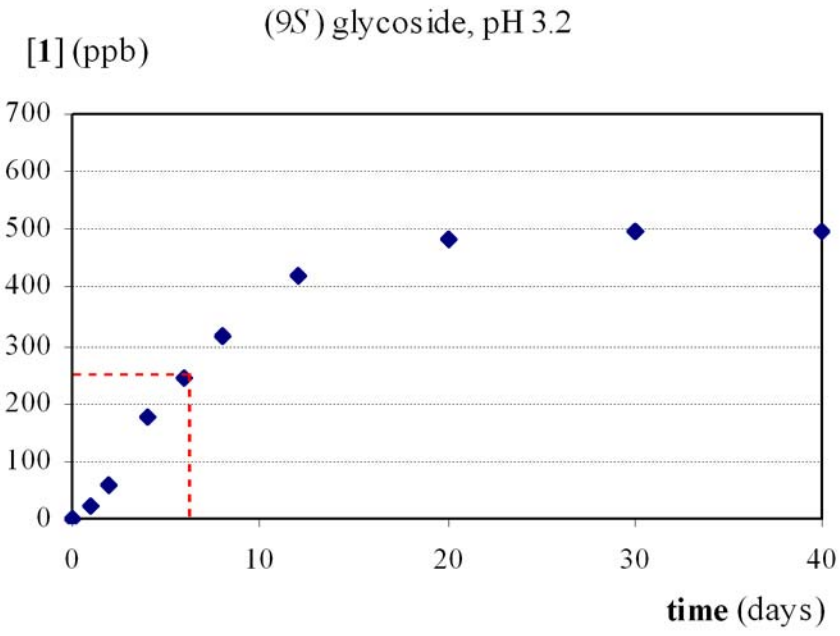


Figure 3.8: Formation of damascenone from (9*S*)-**43** at pH 3.2, 25 ºC.

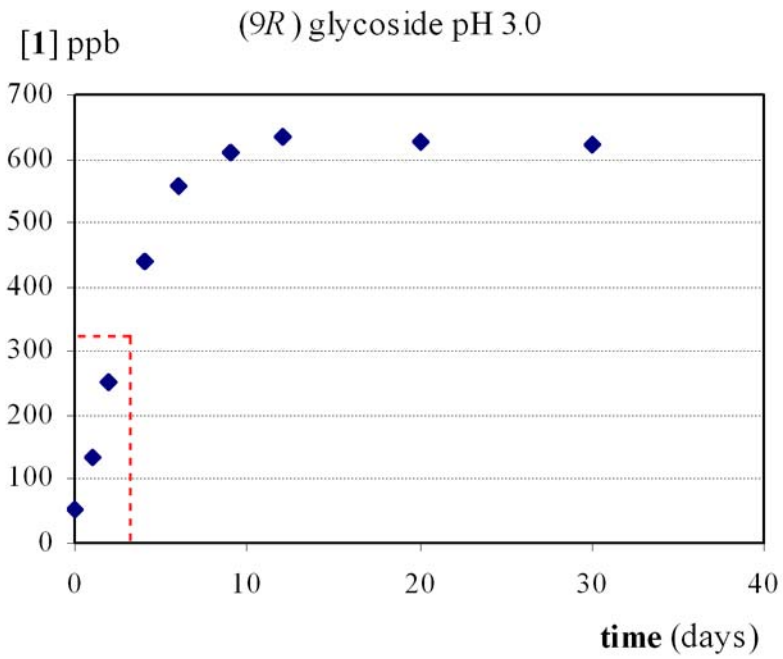


Figure 3.9: Formation of damascenone from (9*R*)-**43** at pH 3.0, 25 ºC.

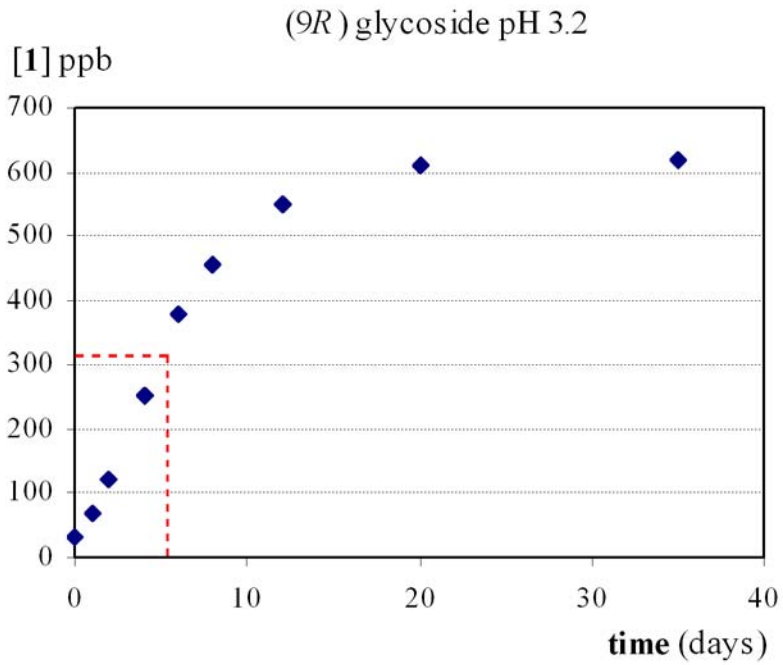


Figure 3.10: Formation of damascenone from (9*R*)-**43** at pH 3.2, 25 ºC.

The concentrations of compounds in the hydrolytic study were: **36** (1 mg/L) and **43** (1.7 mg/L for each diastereomeric glycoside.) The latter concentrations were chosen so that the final concentration of damascenone produced would be the same as that produced from the aglycone, in each case a theoretical maximum of approximately 900-1000 ppb. As can be seen by inspection of Figures 3.5-3.10, the final concentration of damascenone produced in each case is 500-600 ppb, equating to a yield in each case of approximately 60%.

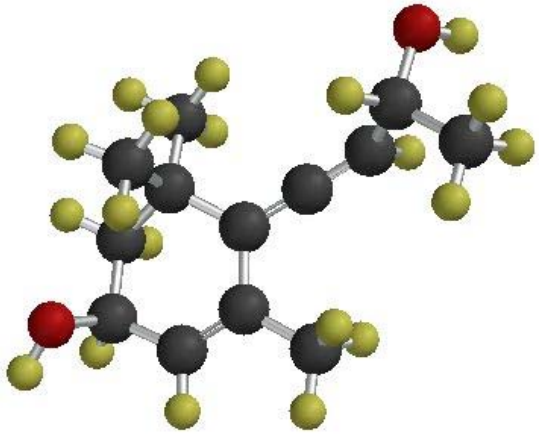
It should be pointed out that the method employed for the quantification of damascenone did that and that alone; the complete composition of the product mixtures was not investigated in detail. However, from earlier experiments (chapter 2) it was observed that C_9 adducts of damascenone form with reasonable facility. Also, the hydrolytic work conducted on the allenic diol (Chapter 5) showed that small (but not negligible) amounts of other extraneous compounds are quite easily formed. Together, these factors can account for the less than quantitative conversion into damascenone.

As expected, the half-life of the aglycone is shorter than that of either of the glycosides. This occurs as a result of glycosylation and is consistent with previous observations.[72] The formation of β-damascenone from the aglycone at pH 3.0 was 2.1 fold faster than from the (*S*)-glycoside and 2.4 fold faster than from the (*S*)-glycoside at pH 3.2. Formation of β-damascenone from the aglycone was 1.8 fold faster than from the (*R*)-Glycoside at pH 3.0 and 2.2 fold faster than from the (*R*)-Glycoside at pH 3.2.

A fuller discussion of the implications of these results towards the mechanism of formation of damascenone can be found in Chapter 5.

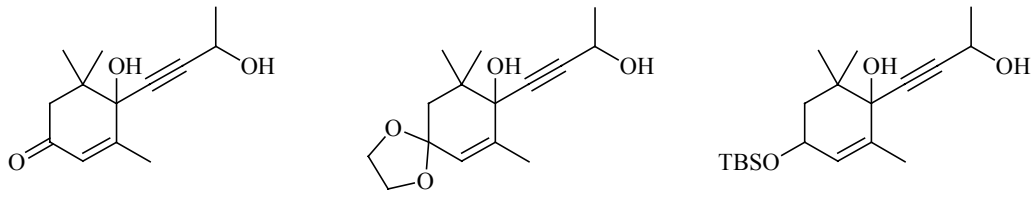
CHAPTER FOUR

Synthesis of 3,9-Dihydroxymegastigma-4,6,7-triene (35)



4.1 General Strategy for the Synthesis of Allenic Diol 35

Two compounds **70** and **71** (Figure 4.1), which had previously been synthesised by others within the group were utilized in initial attempts to produce **35**.

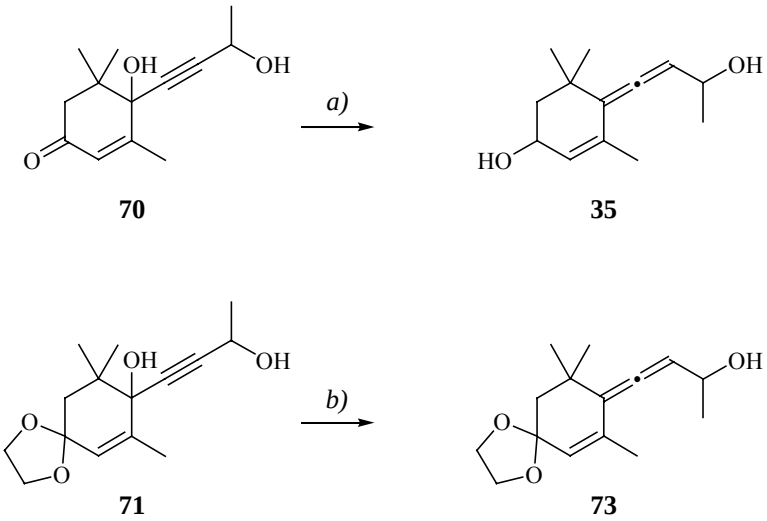


70 **71** **72**

Figure 4.1: Compounds initially trialled in the synthesis of **35**.

These initial reactions (scheme 4.1) were preliminary reactions chosen to provide an indication of the success (or otherwise) of various reducing conditions; the products of these reactions were not purified and characterised but simply used as a guide for future reactions with more appropriate starting materials. The reactions included simple reduction (with the use of $LiAlH_4$) of **70** and **71**. Both reactions produced promising results, with apparent formation of the allene moiety indicated in the ^{1}H NMR spectra by signals in the region between 5.5 and 5.7 ppm, and in the ^{13}C NMR spectrum with peaks present at around 200 ppm.

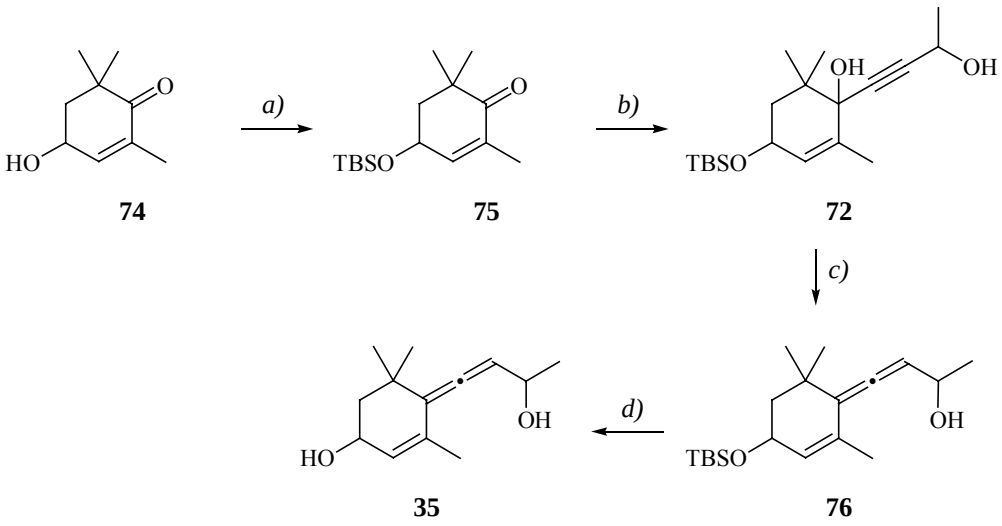
Although the first of these trial reactions appeared to produce the desired compound **35**, the stereochemistry at the relevant positions (in particular C_3) was not addressed. The second trial reaction appeared to produce the acetal **73**, which might have proved problematic when it came to deprotection. It was considered unlikely that the acetal protecting group could be removed, even under extremely mild acidic conditions, without causing substantial rearrangement of the allene function.



a) $LiAlH_4$ (10 eq.), THF, Δ, 5 hr. ***b)*** $LiAlH_4$ (10 eq.), THF, Δ, 4.5 hr.

Scheme 4.1

Consequently, the *t*-butyl dimethylsilyl ether **72** was synthesised (figure 4.1) and employed as a starting reagent to avoid any such potential problems in the final stage of synthesis.



a) TBSCl, pyr., (92%) ***b)*** $LiC{\equiv}CCH(OLi)CH_3$ (80%)

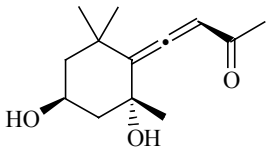
c) $LiAlH_4$, THF, Δ, 6 hr. (55%) ***d)*** TBAF (65%)

Scheme 4.2

The general synthetic strategy devised for the synthesis of allene diol **35** is shown in scheme 4.2. In order to minimise wastage of stereochemically pure reagents, all the reactions were first optimised using both racemic phorenol **74** and racemic but-3-yn-2-ol **63**. Protection of **74** was achieved with the use of *t*-BDMSCl in pyridine to give **75** in good yield.[108] Alkylation of the protected alcohol **75** with **63** produced **72**, this was then reduced with $LiAlH_4$ to produce the protected allene **76**. Deprotection of **76** produced a mixture of allenes **35**, which upon purification by column chromatography separated into two distinct allene fractions. Both fractions produced ^1H NMR signals consistent with the structure of **35**, these included signals between 5.5 ppm and 5.7 ppm resulting from H_4 and H_8 and signals between 4.2 ppm and 4.4 ppm resulting from H_3 and H_9.

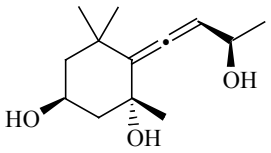
4.2 Synthesis of Optically Pure Diols 35

Once a synthetic pathway had been established, it was necessary to consider the stereochemistry of both the allene triol **31** and grasshopper ketone **30** (Figure 4.2).



30

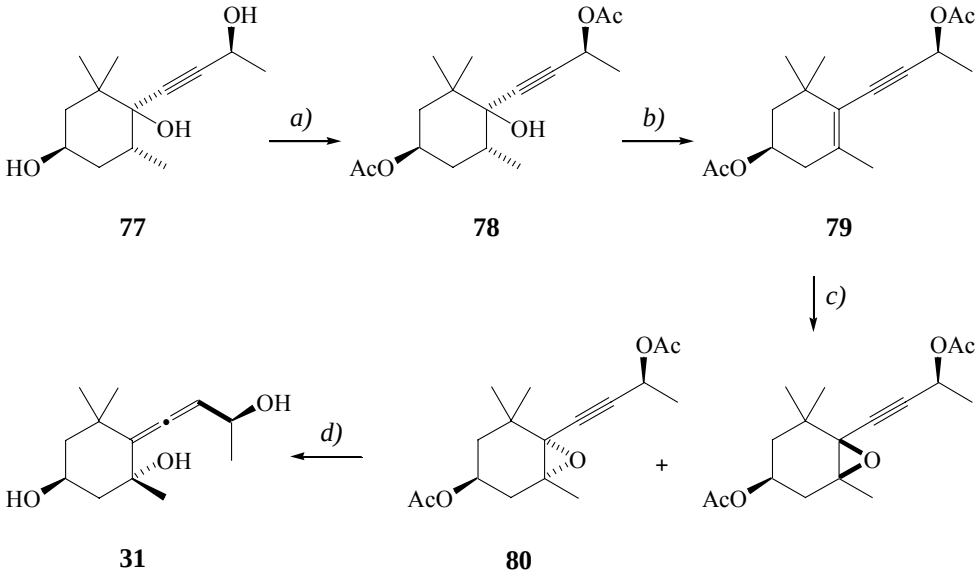
(3*S*, 5*R*, 6*R*)



31

(3*S*, 5*R*, 6*R*, 9*S*)

Figure 4.2: Absolute stereochemistries of grasshopper ketone (**30**) and the corresponding allenic triol **31**.



a) Ac_2O, 24h, Δ ***b)*** $MeO_2CNSO_2NEt_3$, Benzene Δ, 30 min.

c) MCPBA, $CHCl_3$, 5°C ***d)*** $LiAlH_4$, THF, Δ, 6h.

Scheme 4.3

The original allene triol utilised by Skouroumounis et al. was synthesised from the diastereomerically pure acetylenic triol **77**, which was fractionally crystallised from a mixture, and whose relative stereochemistry was established by X-ray crystallography.[109,110] This diastereomer was used for the conversion to allene triol (scheme 4.3), with the relative stereochemistry at C_3 and C_9 remaining untouched. The relative stereochemistry at C_3, C_5 and C_6 was established by oxidation of the C_9 hydroxyl and comparison with authentic grasshopper ketone (**30**). Given that natural **30** has only (3*S*, 5*R*, 6*R*) stereochemistry, our synthetic targets for the full study were thus defined as those stereoisomers of **35** possessing (3*S*) stereochemistry.

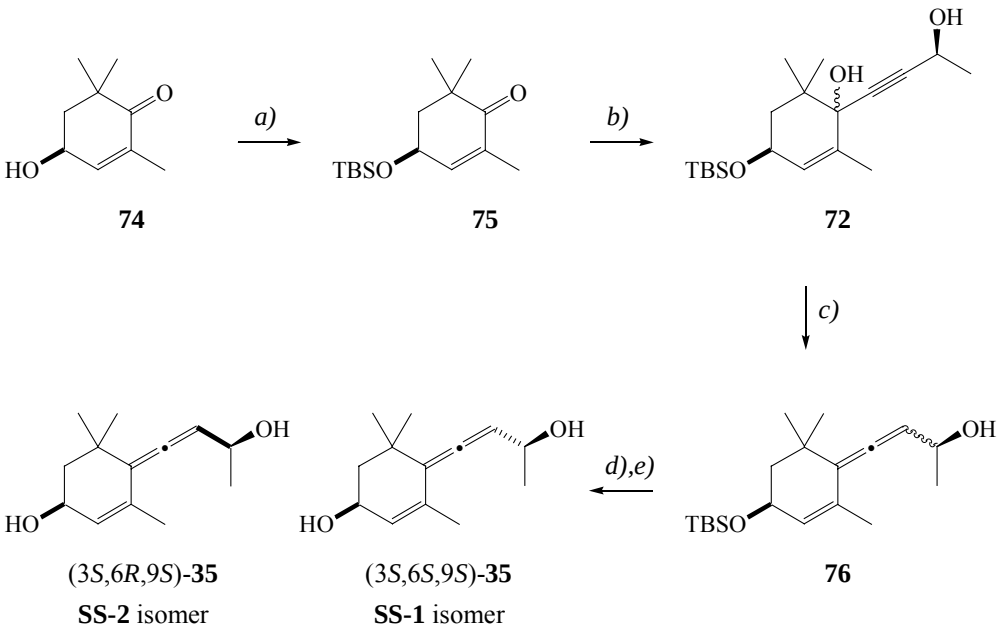
The synthetic strategy devised employs an alkylation reaction (**75** → **72**, scheme 4.2), which gives a pair of diastereomers as products. The reaction of this mixture with $LiAlH_4$ will therefore lead to the production of two isomeric allenes.[111] Thus, use of (*S*) phorenol (**74**), in conjunction with either (*S*) or (*R*) but-3-yn-2-ol (**63**) should provide access to four of the possible eight stereoisomers of **35**. The

stereochemical relationships between the starting reagents and the expected products are shown in Table 4.1. It was not deemed necessary to prepare any of the isomers possessing (3*R*) stereochemistry, as it was thought that having possession of the four stereoisomers listed in Table 4.1 would allow for a full evaluation of the hydrolysis. Ultimately, this proved to be the case.

Table 4.1: Stereochemistries expected in allene diol **35**.

74 (config.)		**63** (config.)		Expected config. of **35**
S	+	*S*	→	(3*S*, 6*S*, 9*S*) + (3*S*, 6*R*, 9*S*)
S	+	*R*	→	(3*S*, 6*S*, 9*R*) + (3*S*, 6*R*, 9*R*)

Schemes 4.4 and 4.5 outline the synthesis of **35** using (*R*)-but-3-yn-2-ol and (*S*)-but-3-yn-2-ol, respectively. Attempts to separate the individual allenes as their TBS ethers (as in **76**) were unsuccessful. However, after deprotection with TBAF, separation became possible and was duly achieved. As the stereochemistries at the allene positions was at the time unknown, the two diastereomers resulting from addition of (*S*)-butynol were labelled as the **SS-1** and **SS-2** isomers, with the numeral simply referring to the order in which they eluted off a silica chromatography column. In an identical manner, the two diastereomers resulting from addition of (*R)*-butynol were labeled as the **SR-1** and **SR-2** isomers. The ^{1}H NMR details, as well as optical rotations for the four isomers of **35** are collected in Table 4.2. Inspection of this table reveals that the isomers are all *extremely* similar spectrally. However, the optical rotations revealed that the four isomers were indeed separate and distinct. Also, all four isomers were distinguishable from one another by GC-MS, with the order of elution on a DB-1701 column being **SR-2**, **SS-2**, **SS-1** and **SR-1**.



74 **75** **72** **76**

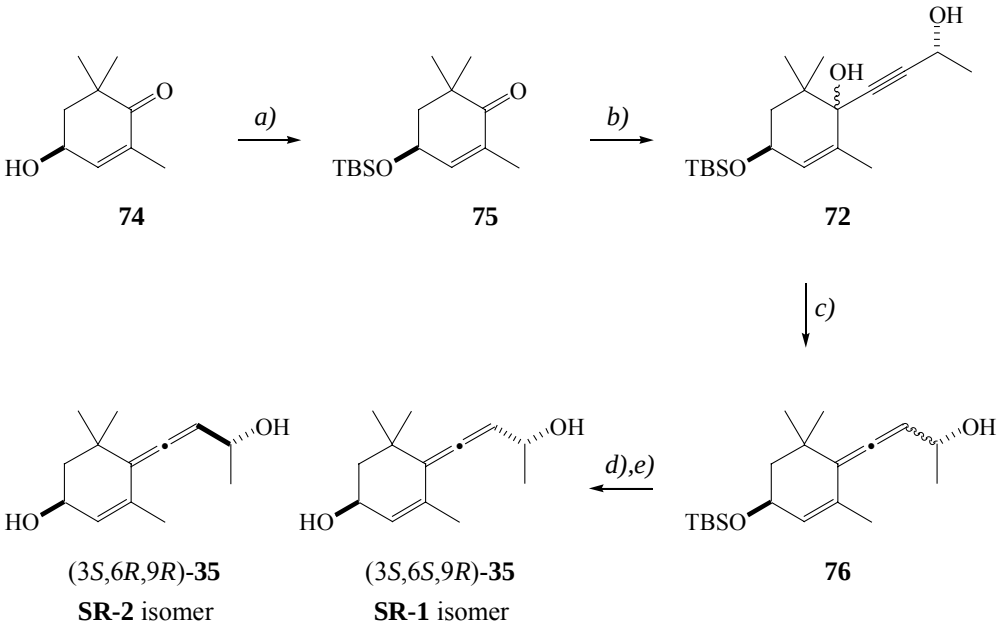
(3*S*,6*R*,9*S*)-**35** **SS-2** isomer

(3*S*,6*S*,9*S*)-**35** **SS-1** isomer

a) TBDMSCl, pyr (92%) ***b)*** (*S*) LiC≡CCH(CH_3)OLi (69%) ***c)*** $LiAlH_4$, 4h (36%)

d) TBAF (87%) ***e)*** SiO_2

Scheme 4.4



74 **75** **72** **76**

(3*S*,6*R*,9*R*)-**35** **SR-2** isomer

(3*S*,6*S*,9*R*)-**35** **SR-1** isomer

a) TBDMSCl, pyr (92%) ***b)*** (*R*) LiC≡CCH(CH_3)OLi (69%) ***c)*** $LiAlH_4$, 4h (23%)

d) TBAF (97%) ***e)*** SiO_2

Scheme 4.5

Table 4.2: ^{1}H NMR details, and optical rotations for the four isomers of **35**.

Isomer	**SS-1**	**SS-2**	**SR-1**	**SR-2**
$[\alpha]_D^a$	-22.6	+45.0	+12.8	+35.2
H_{2a}	1.91, ddd (J = 12.5, 5.8, 1.1)	1.92, ddd (J = 12.3, 5.8, 1.1)	1.91, ddd (J = 12.5, 5.8, 1.1)	1.92, ddd (J = 12.3, 5.7, 1.1)
H_{2b}	1.43, dd (J = 12.5, 9.8)	1.45 (J = 12.3, 9.6)	1.42, dd (J = 12.5, 9.6)	1.45, dd (J = 12.3, 9.6)
H_3	4.42-4.28, m	4.42-4.26, m	4.41-4.29, m	4.41-4.30, m
H_4	5.60, m	5.61, m	5.60, m	5.62-5.55, m
H_8	5.66, br d (J = 5.3)	5.58, br d (J = 6.0)	5.66, br d (J = 5.4)	5.62-5.55, m
H_9	4.42-4.26, m	4.42-4.26, m	4.41-4.29, m	4.41-4.30, m
H_{10}	1.31, d (J = 6.3)	1.31, d (J = 6.3)	1.31, d (J = 6.3)	1.31, d (J = 6.4)
$H_{11,12}$	1.12, 1.07, 2s	1,12, 1.05, 2s	1.11, 1.07, 2s	1.13, 1.05, 2s
H_{13}	1.74, app. t (J ≈ 1.6)	1.75, app. t (J ≈ 1.6)	1.75, app. t (J ≈ 1.6)	1.74, app. t (J ≈ 1.6)

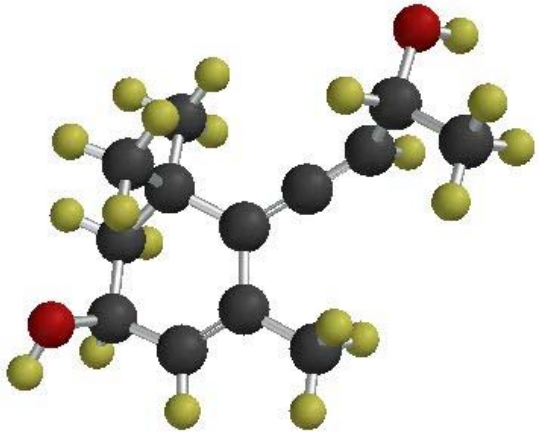
a) all optical rotations were conducted as solutions in chloroform.

The first issue to be resolved was the identity of the compound tentatively identified as **35** from the original hydrolysate of **31**. Given that the original allene triol hydrolysis experiments were conducted on material that was diastereomerically homogeneous, and that the stereochemistry at all positions was known then a match with one of the authentic (3*S*,9*S*) isomers (ie. **SS-1** or **SS-2**) was expected. GC-MS analysis of both the authentic and synthetic samples revealed an exact match (retention time, mass spectrum and symmetrical enhancement upon co-injection) with the **SS-2** isomer. The stereochemistry of this isomer was thus able to be assigned as (3*S*, 6*R*, 9*S*)-**35**. As its partner from the chromatography, namely the **SS-**

1 isomer differs only in the configuration at the allene position, then it too can be assigned, as being (3*S*, 6*S*, 9*S*)-**35**. At this point in proceedings, the absolute stereochemistries of the two **SR** isomers were still unassigned. That will form a large part of the focus of the next chapter.

CHAPTER FIVE

Hydrolysis of 3,9-Dihydroxymegastigma-4,6,7-triene (35)



5.1 Hydrolysis of Megastigma-4,6,7-triene-3,9-diol (35)

As described earlier, previous hydrolytic studies involving triol **31** showed that **1** was produced only as a minor product, with 3-hydroxydamascone (**2**) and the acetylenic diol **34** being the major products.[73] Also, previous studies had shown that whilst the hydrolysis of **34** at room temperature did produce a small amount of β-damascenone over a period of 1 year, the allene triol produced approximately the same amount of β-damascenone over a 24 hour period. It was therefore concluded that **34** could not be an intermediate in the conversion of **31** into **1** and **2** because of its low reactivity at room temperature.

Further scrutiny of the hydrolysate indicated the presence of two intermediates tentatively assigned as the dienyne alcohol **36** and allene diol **35**. Based on these studies, Skouroumounis and Sefton proposed that dehydration of **31** would produce **35**, which can behave in one of two ways; it can undergo rearrangement to form **2**, or it can dehydrate and undergo rearrangement to form **36**, and eventually **1** (Figure 5.1).[72]

Based on the previous hydrolytic studies which showed that **2** was the major product produced from **31**, the former pathway was expected to be the dominant one. In order to investigate this proposal, it was necessary to perform hydrolytic studies on each of the newly synthesised and identified compounds **35** and **36**. Chapter 3 covered the hydrolysis of **36**; the remainder of this chapter concerns the hydrolysis of **35**.

Preliminary hydrolytic studies indicated a half-life of approximately 1-2 days for the allene diol **35** at room temperature and pH 3. The hydrolytic study was qualitative,

focussing on product distribution. The studies were carried out at a concentration of 1 mg /L **35** at 25 ºC in model wine at pH 3.0.

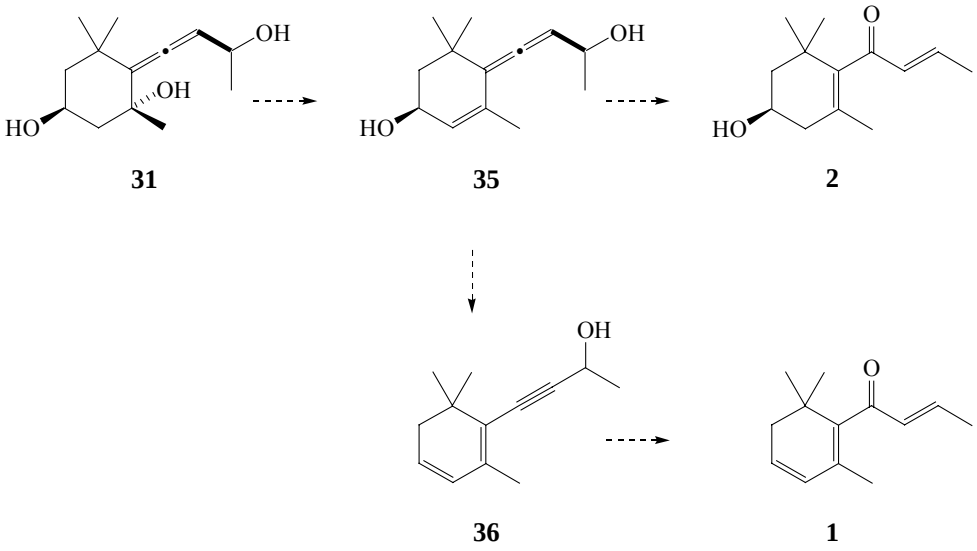


Figure 5.1: Proposed divergence in the hydrolysis of **35**.

Samples were taken at 24 and 48 hours and analysed by GC-MS. The GC-MS traces for each of the four hydrolysates at 48 hours are shown in Figure 5.2.

When each of the four allene diols **35** was hydrolysed (pH 3.0, 24 hr) separately and the hydrolysates analysed by GC-MS, some general features were observed in the products. The major products at the same oxidation level as β-damascenone were examined in detail, and in the specific case of the **SS-2** isomer, were: β-damascenone **1** (12.65 min.), dienyne alcohol **36** (13.11 min.), unreacted allene diol (plus a newly formed epimer) (16.55/16.61 min.), two compounds tentatively identified as epimers of C_3 ethoxy allene diol **81** (15.89/15.97 min.), and minor amounts of β-damascenone addition products **57** and **58** (14.33 and 14.37 min, respectively). Minor amounts of oxidised products were also observed. Noteworthy was the complete absence of 3-hydroxydamascone (**2**), even in trace amounts. The products obtained from the hydrolysis of the other three isomers of **35** were identical (albeit

with different stereochemistries). The individual **SS-2** case is illustrated graphically in Figure 5.3.

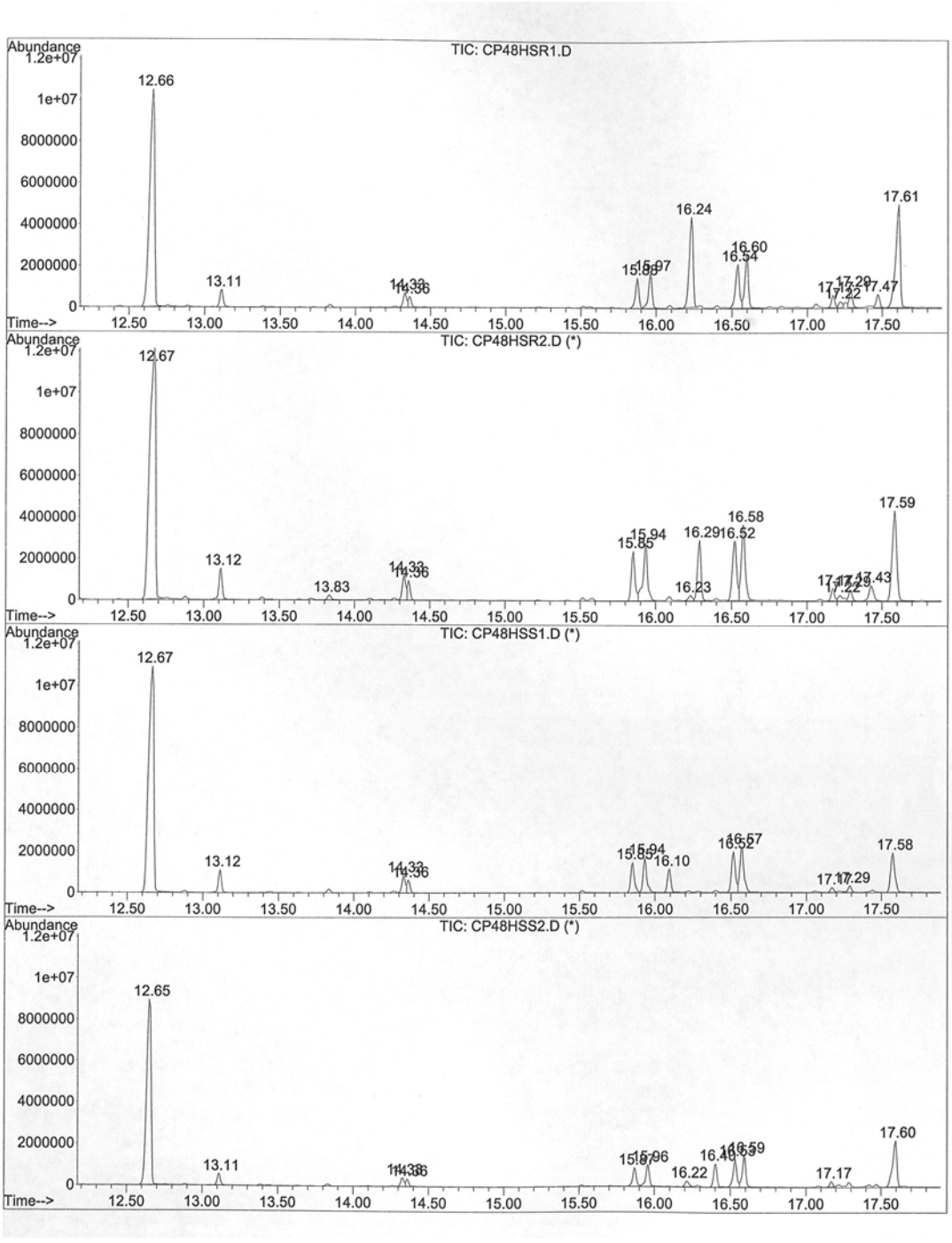


Figure 5.2: GC traces of the hydrolyses of each of the four isomers of **35** synthesised. From top to bottom they correspond to the **SR-1**, **SR-2**, **SS-1**, and **SS-2** isomers.

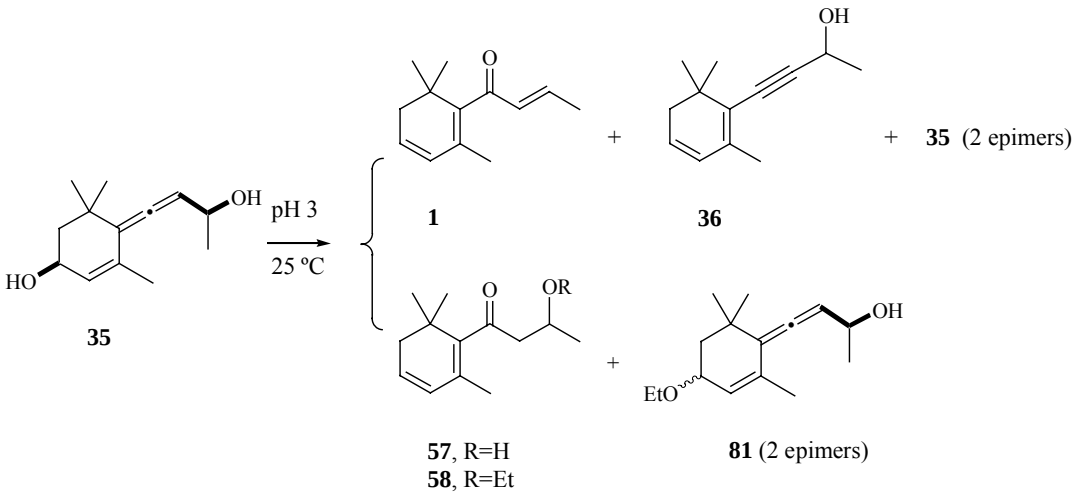


Figure 5.3: Products of interrupted hydrolysis of allene diol **35** at pH 3.0, 25 ºC. The stereochemistry shown corresponds to the **SS-2** isomer.

Each allene diol showed considerable reaction progress after 24 hours with unconsumed allene diol now accompanied by an essentially equivalent amount of compound, which was clearly epimeric based on mass spectral analysis. Figure 5.4 shows an expanded excerpt from the **SS-2** isomer, but it was the case that each isomer of **35** gave only a single new epimer of **35**. In the case of the **SS-2** isomer, this new epimer proved to be indistinguishable from the **SR-1** isomer by GC-MS.

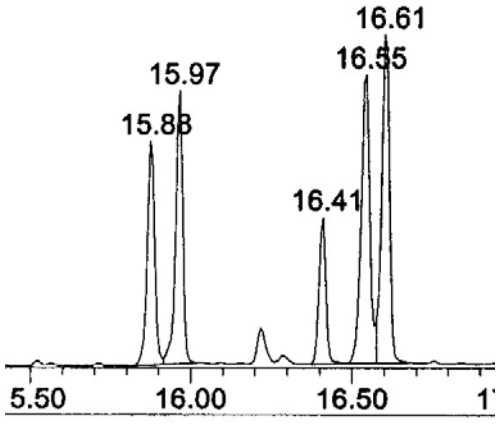


Figure 5.4: Excerpt from the GC trace of the hydrolysis of the **SS-2** isomer after 24 hours. Peaks indicated are: 16.55 min. (**SS-2** isomer); 16.61 min. (epimer of **SS-2** isomer); 15.88 and 15.97 min. (epimeric ethyl ethers of the two epimeric diols); the peaks observed between the two pairs of epimers are due to small amounts of oxidised material.

This pairing of the **SS-2** with the **SR-1** isomer was observed in the converse reaction also. Similarly, the **SS-1** isomer gave an epimer identical to the **SR-2** isomer and *vice versa*. Table 5.1 lists the epimeric pairs obtained after hydrolysis of each of the four allene diastereomers.

Table 5.1: Epimeric pairs obtained from hydrolyses of each isomer of **35**.

Starting Isomer	After Epimerisation
SS-1 (3*S*,6*S*,9*S*)	**SS-1 + SR-2**[a]
SS-2 (3*S*,6*R*,9*S*)	**SS-2 + SR-1**[a]
SR-1	**SR-1 + SS-2**[a]
SR-2	**SR-2 + SS-1**[a]

a) or its enantiomer

5.2 C_3 or C_9 Epimerisation?

It was clear from the hydrolyses that whichever epimer of **35** was used, it was undergoing epimerisation at only one of the hydroxyl functions. Had epimerisation taken place at both centres, then four isomers would have been produced from each epimer of the diol **35**. Whether this epimerisation was occurring at C_3 or C_9 was impossible to determine on these data alone. For instance, if epimerisation were to occur at C_9 of the **SS-2** isomer, which has (3*S*,6*R*,9*S*) stereochemistry, then the new epimer (*identical* to **SR-1**) must have (3*S*,6*R*,9*R*) stereochemistry. Similarly for the **SR-1** isomer (with (3*S*,6*R*,9*R*) stereochemistry), C_9 epimerisation must produce the (3*S*,6*R*,9*S*) epimer. Thus, the **SS-2** and **SR-1** isomers would produce the same pair of isomers (equation 1). This is shown graphically in Figure 5.5.

SS-2 $\Rightarrow$ **SRS** and **SRR** $\Leftarrow$ **SR1** (equation 1)

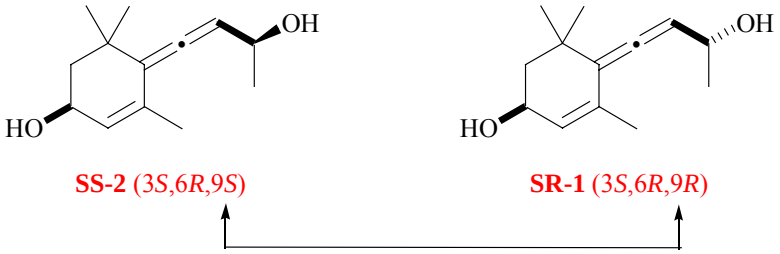


Figure 5.5: Stereochemical relationship between the **SS-2** and **SR-1** isomers, assuming that epimerisation takes place at C_9.

If the two product mixtures were analysed by chiral-phase GC, then if C_9 epimerisation was occurring, the **SS2**/**SR1** pair would be expected to give the same two isomers. That is the two different product mixtures combined must contain the same two enantiomers of **35**.

However, if epimerisation were occurring at C_3, then, again considering the specific instance of the **SS-2** isomer with (3*S*,6*R*,9*S*) stereochemistry, the new epimer (*equivalent* to **SR-1** on achiral GC) must be the (3*R*,6*R*,9*S*) isomer, ie. the enantiomer of **SR-1**, which was not actually synthesised as part of this study. Therefore, under these circumstances (C_3 epimerisation), the **SR-1** isomer must be the enantiomer of that having (3*R*,6*R*,9*S*) stereochemistry; in other words the **SR-1** isomer would actually have (3*S*,6*S*,9*R*) stereochemistry. By extension, the **SR-1** isomer (with (3*S*,6*S*,9*R*) stereochemistry), would be transformed by C_3 epimerisation into the (3*R*,6*S*,9*R*) isomer, which would be the enantiomer of the isomer having (3*S*,6*R*,9*S*) stereochemistry. Again, by achiral GC the two are indistinguishable. In summary, by achiral GC the **SS-2** isomer would be seen as producing the **SR-1** isomer when in fact it produces the enantiomer of the **SR-1** isomer. Similarly, the **SR-1** isomer would be seen as giving the **SS-2** isomer, when in fact it too gives the enantiomer (equation 2). This is shown graphically in Figure 5.6.

SS-2 ⇒**SRS** and **RRS**; **SR-1** ⇒ **SSR** and **RSR** (equation 2)

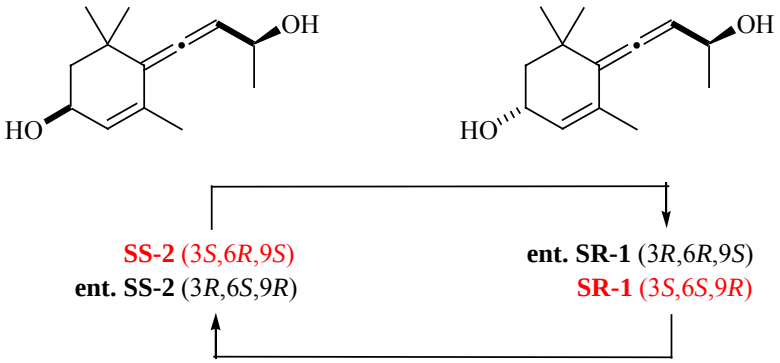


Figure 5.6: Stereochemical relationship between the **SS-2** and **SR-1** isomers, assuming that epimerisation takes place at C_3. The isomers in red are the synthesised starting materials, while those in black (ent.) are the enantiomers of the synthesised compounds.

The above arguments are equally valid for the **SS-1**/**SR-2** pair of isomers, and the various stereochemical relationships are shown graphically in Figure 5.7.

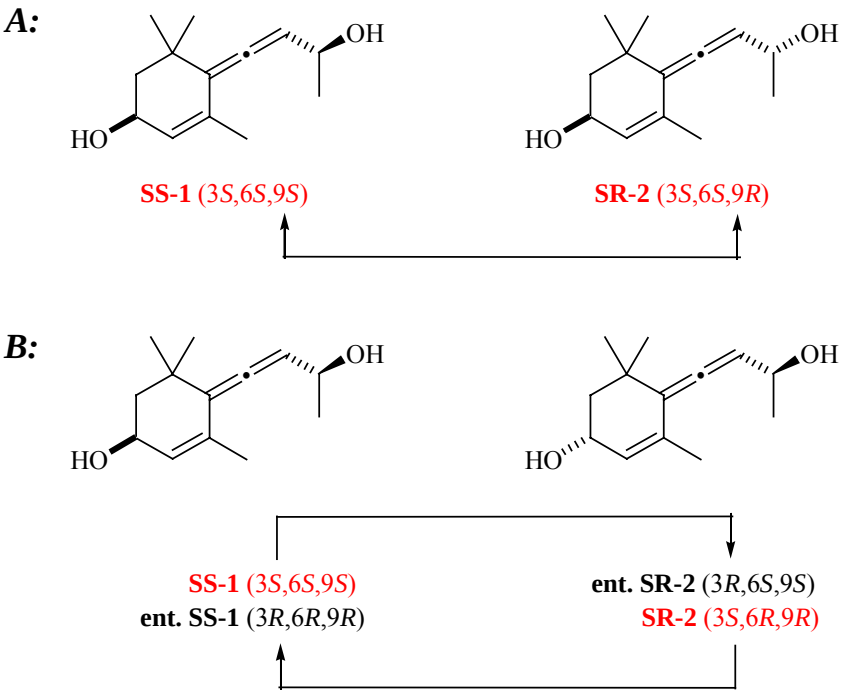


Figure 5.7: Stereochemical relationship between the **SS-1** and **SR-2** isomers, assuming that epimerisation takes place at either A: C_9, or B: C_3.

In summary, chiral-phase GC would provide the answer to this question of C_3 vs. C_9 epimerisation: the **SS-2** and **SR-1** isomers must give a combined total of two epimers (equation 1) if epimerisation is occurring at C_9, or a combined total of four epimers (equation 2) if C_3 is the site of epimerisation. In a similar vein, the **SS-1** and **SR-2** isomers must give either a combined total of two epimers (epimerisation at C_9) or a combined total of four epimers (epimerisation at C_3). Therefore, in order to deduce what was actually happening, and to be able to unambiguously assign the stereochemistry of the **SR-1** and **SR-2** isomers, chiral phase GC was employed. The GC retention times of the isomers of **35**, formed during hydrolysis of the various isomers of **35,** on a chiral Cyclosil B column are shown in Table 5.2. The combined total of epimers of **35** from the **SS-2** and **SR-1** hydrolysates is four (three clear peaks, including one coincident pair). This result is only possible from C_3 epimerisation. Also, the same result was obtained from the combined **SS-1** and **SR-2** hydrolysates. To confirm that these results were not due to minor variation in chromatographic behaviour, all samples were re-run in a different order on a separate occasion, with identical outcomes. The absolute stereochemistries of the remaining isomers (**SR-1** and **SR-2**) could now be assigned and are as shown in Table 5.2.

Table 5.2: Retention times of epimers produced during hydrolysis, and the newly assigned stereochemistries of the **SR-1** and **SR-2** isomers.

Starting Isomer	**GC Retention Time on Chiral Column** (Cyclosil B)
SS1 (3*S*, 6*S*, 9*S*)	2 peaks 18.09
SS2 (3*S*, 6*R*, 9*S*)	2 peaks 18.10, 18.17
SR1 (3*S*, 6*S*, 9*R*)	2 peaks 18.13
SR2 (3*S*, 6*R*, 9*R*)	2 peaks 18.07, 18.15

The identity of the epimeric ethyl ethers observed in the hydrolyses can now be confirmed as those shown in Figure 5.3. As epimerisation is occurring only at C_3 it is inconceivable that the ether could form at any other position within the molecule.

5.3: Implications for the Mechanism of Formation of Damascenone (1)

Examination of the two hydroxy functionalities of the allene diol **35** shows them to be chemically different. The cation formed by loss of the C_3 hydroxyl is conjugated to the 4,5 carbon-carbon double bond as well as the left hand side of the allene (Figure 5.8A), whilst that formed by loss of the C_9 hydroxyl is conjugated only to the right hand side of the allene (Figure 5.8B).

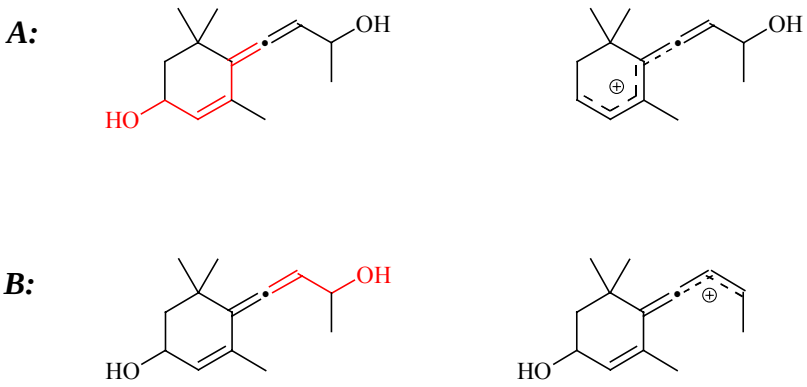


Figure 5.8: Likely conjugation relationships between the two hydroxyl functions and the various olefins present in **35**.

The preference for reactivity at C_3 over C_9 is therefore not surprising. Although protonation and loss of either hydroxyl would produce a stabilized cationic intermediate, that produced from loss of the C_3 hydroxyl (Figure 5.A) would be expected to be more highly delocalised and therefore more stable than the alternative (Figure 5.8B).

The fact that epimerization is taking place only at C_3 under the conditions used in this study (conditions chosen for their similarity to normal wine maturation) has implications for the mechanism of formation of β-damascenone. The proposal of Skouroumounis and Sefton[72] was consistent with the available data at the time, but now needs to be revised in light of these new findings. The suggestion that both

dienyne alcohol **36** and 3-hydroxydamascone (**2**) are formed from a common intermediate, allene diol **35** is contradicted by the finding that the hydrolysates produced from each of the authentic diols **35** are completely devoid of **2**. This last compound, the major product in the hydrolysis of allenic triol **31** appears to be formed directly from the triol as indicated below (Figure 5.9). It appears from the new data that the presence of the $C_{4,5}$ olefin (as in **35**) is necessary for the facile loss of the C_3 hydroxyl.[72]

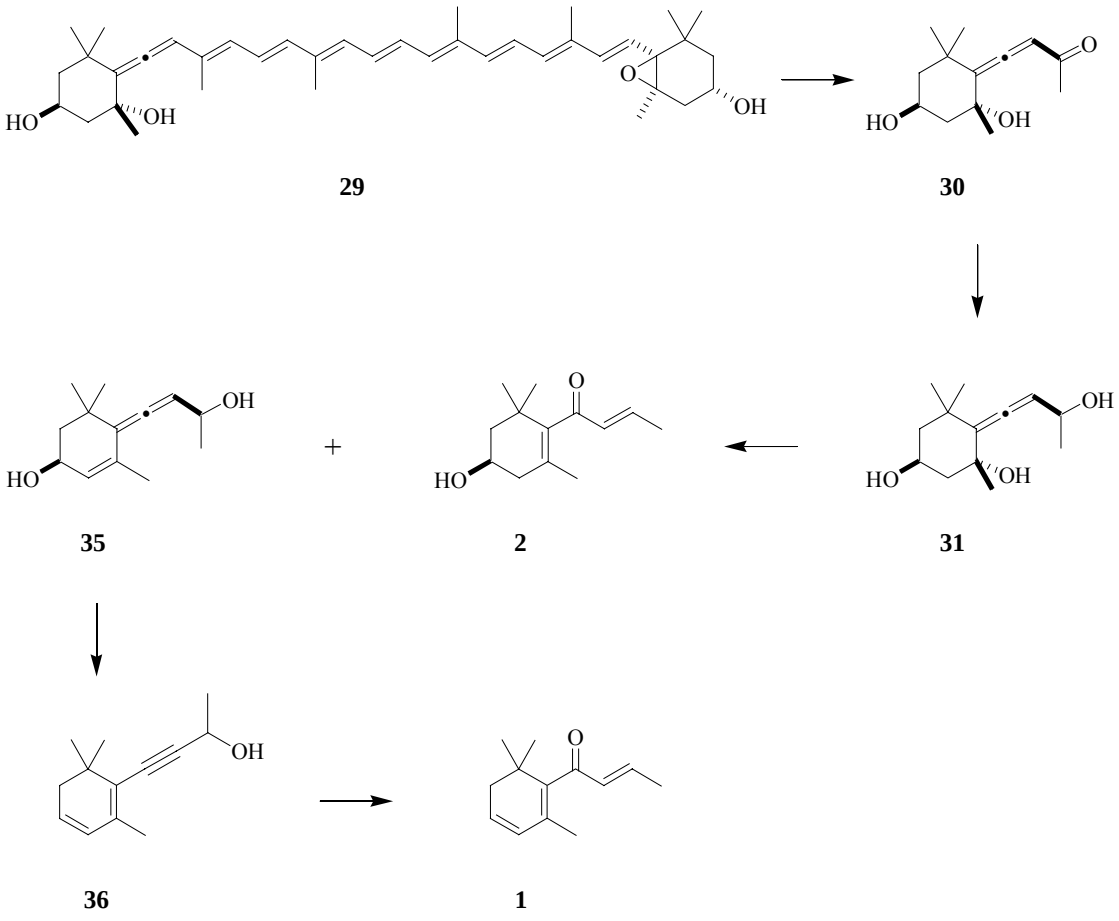


Figure 5.9: Newly proposed route of *in vivo* generation of β-damascenone (**1**).

Although the process by which 3-hydroxydamascone (**2**) is formed is as yet undetermined, it is possible that it is formed *via* the pathway shown in Figure 5.10. Loss of the tertiary (and allylic) hydroxyl at C_5 is likely, and would produce the

cationic intermediate **82**. Trapping of this cation by water would give **83,** which could then produce **2** either directly, or *via* the intermediacy of the hydrated form **84**.

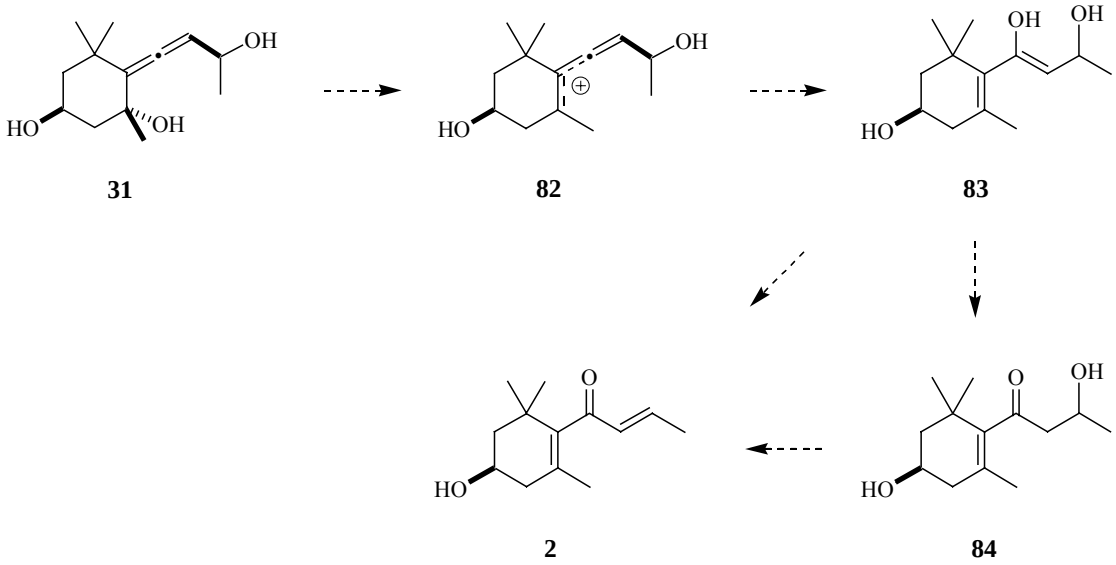


Figure 5.10: Proposed mode of formation of **2** directly from triol **31**.

Comparison of the two hydrolytic studies of **35** and **36** also reveal some interesting results, these are detailed below and include some mechanistic considerations. As detailed in Chapter 4, the interrupted hydrolyses of **35** revealed the presence of a small amount of acetylenic alcohol **36**. Figure 5.11 shows the half-lives of formation of damascenone directly from both **35** and **36** at 25 ºC and pH 3.0.

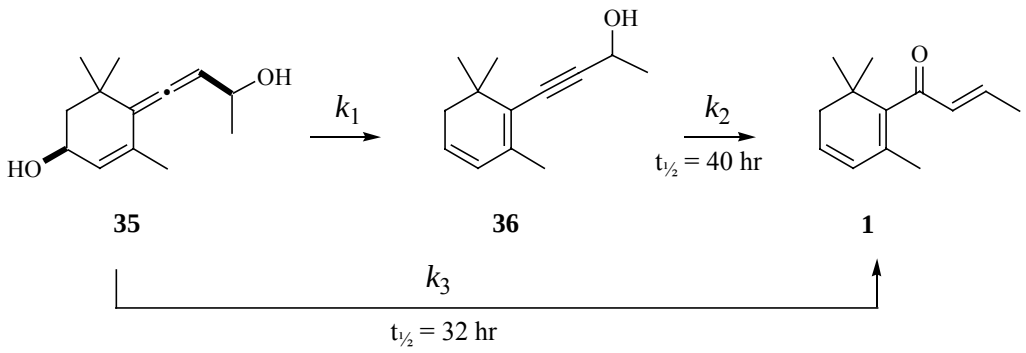


Figure 5.11: Kinetics determined of the conversion of **35** and **36** into **1**. The value for k_3 is taken from the work of Daniel.[101]

Interestingly, the rate of formation of damascenone from the acetylenic alcohol is slightly lower than the formation of damascenone from the allene ($k_2 < k_3$). The presence of a small amount only of the dienyne alcohol **36**, compared to the starting material **35** and β-damascenone (**1**), in the interrupted hydrolysis of the allene suggests that k_1 is slightly lower than k_2. If the opposite were true we would expect to see large amounts of **36** accumulate during the reaction. If the acetylenic alcohol **36** is an obligatory intermediate in the sequence, we would expect $k_3 \leq k_1 < k_2$.

Our results show the opposite to be the case, with measured half-lives of 32 hours and 40 hours for k_3 and k_2, respectively (Figure 5.11). Given the inverse relationship between k and $t_{½}$, this means that k_3 is actually slightly higher than k_2. This result would indicate that there are at least two separate pathways for the formation of damascenone from the allenic diol, only one of which involves the intermediacy of the acetylenic alcohol **36**.

5.4: Conclusions

5.4.1: Hydrolysis of the allene triol 31

The combined hydrolytic work on the allene and acetylene and previous work carried out by others provides a more complete picture of the formation of the various end products from the allene triol **31**. As illustrated in Figure 5.12, the formation of all such products appears to be initiated exclusively by acid catalysed breakage of the tertiary C_5 C-O bond to form the delocalised cation **82**.

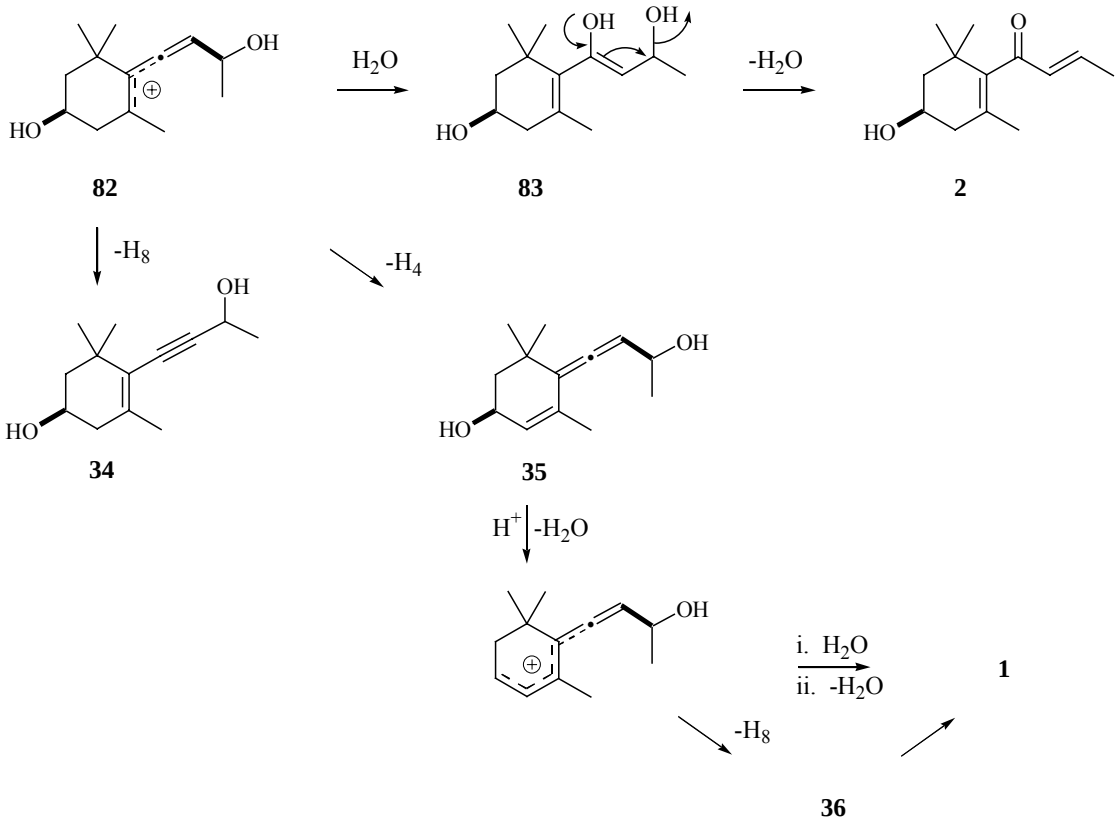


Figure 5.12: Proposed mode of formation of all the major hydrolysis products of allene triol **31**.

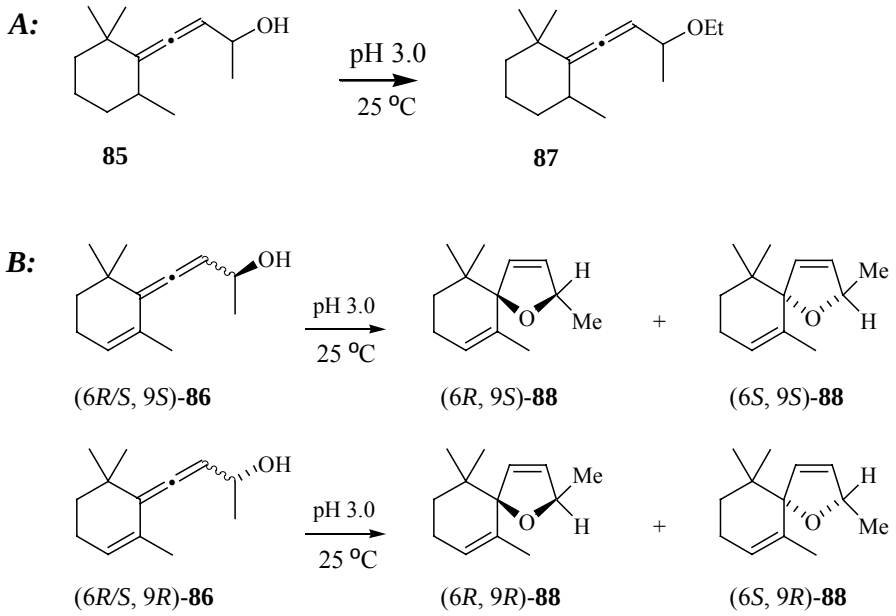
Loss of the H_8 proton leads to formation of the enyne **34**; hydration at C_7 followed by dehydration at C_9 gives **2**; loss of the H_4 proton produces **35** whose C_3 hydroxyl is only now activated towards ionisation and loss. The resultant cation can then either

produce β-damascenone (**1**) directly, in a process comparable to that which produces **2**, or it can lose the H_8 proton to produce dienyne alcohol **36** which has been shown (chapter 2) to produce **1** almost exclusively.

85 **86**

Figure 5.13: Model hydroxylated allenes **85** and **86** studied by Daniel.

Daniel[101] conducted studies into the hydrolysis of the model allenes **85** and **86** (Figure 5.13), as well as the glycoside **45**. Hydrolytic studies with **85** and **86** indicated that conversion of simple secondary α-hydroxy allenes to α,β-unsaturated ketones under the same acidic conditions employed in this study is considerably slower than the reaction of the damascenone precursors **31**, **35** and **36**.



85 **87**

(6*R/S*, 9*S*)-**86** (6*R*, 9*S*)-**88** (6*S*, 9*S*)-**88**

(6*R/S*, 9*R*)-**86** (6*R*, 9*R*)-**88** (6*S*, 9*R*)-**88**

Figure 5.14: Hydrolysis products of A: model allene **85**, and B: model allene **86**, as observed by Daniel.

When reaction at C_9 does occur, as in the case of **85**, no transposition of oxygen from C_9 to C_7 was seen (Figure 5.14A); the exclusive product obtained was the ethyl ether **87**. The other model compound, **86**, produced the isomeric theaspiranes (**88**)[112,113] as almost exclusive (>90%) products. Chiral GC-MS analysis (Figure 5.14B) revealed the reaction to have been highly stereospecific, with no indication of any reaction at C_9 having taken place, as indicated by the complete retention of stereochemistry at that position.

The 1,3-transposition of oxygen seems only to occur with highly conjugated acetylenic precursors such as **36**, or with *tertiary* allenic alcohols such as **31**, in keeping with earlier work on α-hydroxy allenes described by Olsson et al.[114]

5.4.2 The effect of glycosylation on the formation of damascenone

In attempting to determine the effect of glycosylation on the formation of damascenone, it was our aim to investigate (i) if C_9 glycosylation would result in a reduced rate of damascenone formation and, (ii) time permitting, investigate whether C_9 vs C_3 glycosylation of **35** would result in a shift in product distribution between damascenone and 3-hydroxydamascone.

As already discussed, and in keeping with previous studies the effect of glycosylation on **36** produced an approximate 2-fold reduction in the final rate of damascenone formation (Table 5.3)

Table 5.3: Ratio of half-lives obtained for the hydrolyses of **36** and **43**.

Compound	$t_{½}$ (pH 3.0)[a]	Ratio[b]	$t_{½}$ (pH 3.2)	Ratio
aglycone (**36**)	40		65	
(9*R*)-(**43**)	72	1.8	144	2.2
(9*S*)-(**43**)	84	2.1	156	2.4

a) all half-lives in hours; b) $t_{½\,(\mathbf{43})} / t_{½\,(\mathbf{36})}$

It is not known whether cleavage of the C_9 oxygen is the rate-determining step in damascenone formation from **36** and **43**, and it is therefore not possible to estimate the effect that glycosylation has on the formation of the C_9 cation. Given that both the aglycone **36** and the corresponding glucosides **43** produced **1** in the same yield (~60%), then the rate difference is of little practical significance.

In parallel to the work described in this thesis, hydrolytic studies conducted by Daniel on the allenic diol **35** and its C_3 glycoside **45** showed a rate reduction for the glycoside of approximately 1.6 compared to the aglycone **35**.[101] But, as was the case with **36**/**43**, the yields of damascenone formed from **35**/**45** were the same. Again, this rate difference is of little practical significance in the context of wine-making.

Lastly, no trace of 3-hydroxydamascone (**2**) was observed in the hydrolysis of glucoside **45**. This was in contrast to what was expected (Figure 5.15); it was thought that glycosylation at C_3 might inhibit reaction at that site to the point where reaction at C_9 would become competitive, and thus produce 3-hydroxydamascone (**2**).

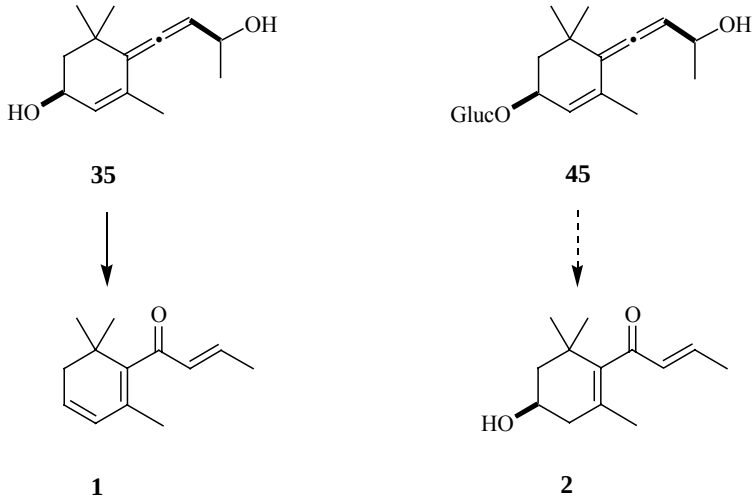


Figure 5.15: Potential outcome of hydrolysis of C_3 glycoside **45** vs. aglycone **35**.

The effect of glycosylation on cation formation from **35** is hard to assess, as this cation rehydrates rapidly, as indicated by the rapid epimerisation at C_3 observed in the hydrolysis of all four stereoisomers of **35** investigated. In the case of **35**, the steps subsequent to ionisation are rate determining, ie. $k_{\text{epimerisation}} >> k_{\text{damascenone formation}}$.

However, in the case of **45**, where damascenone formation is measurably slower, cation formation is at least partially rate-determining. Therefore, the effect on the actual rate of cation formation could be very large.

Previous work had shown that the effect of glycosylation on allylic alcohols can be quite significant. In the case of geraniol and its *O*-glycoside, the rate of formation of linalool observed upon introduction of the sugar is nearly ten-fold lower.[72] In the present study, results suggest that the effect of glycosylation (at either C_3 or C_9) on the formation of damascenone from allenic triol **31**, is only likely to be limited to modifying the proportions of product formed from the C_5 cation (Figure 5.12), perhaps by no more than steric effects.

However, in other fruits, or flowers, with higher pH levels, and therefore lower reactivity, differential glycosylation may yet play a significant role on the rate of formation of damascenone (**1**). But, given the larger time frames involved in wine-making and especially wine maturation, any effects are likely to only be marginal.

CHAPTER SIX

Experimental

6.1 General Experimental

Solvents:

Solvents for synthesis were BDH AnalaR or distilled. X4 solvent is a fraction obtained from distillation of petroleum, containing *n*-hexane as the major component. Diethyl ether and THF were distilled from sodium/benzophenone immediately prior to use; dichloromethane was distilled from CaH_2 immediately prior to use.

Reagents:

Reagents of the highest available purity were purchased from Sigma-Aldrich-Fluka. Labelled (d_4) damascenone was prepared as described in Kotseridis et al.[47] (*S*)-phorenol (ee > 96%) was generously provided by Dr. K. Puntener, Hoffmann-La Roche International.

Model wines:

10% redistilled ethanol (*v/v*) in MilliQ water was buffered to the required pH with potassium hydrogen tartrate and L (+)-tartaric acid (10%). Model wine was prepared freshly. pH measurements were made with an EcoScan pH 5/6 meter (Eutech Instruments, Singapore) which was calibrated before use.

Glassware:

Volumetric glassware and pipettes were used for all quantitative work. All volumetric glassware was solvent rinsed (two organic solvents), washed with a detergent (pyroneg) and rinsed with MilliQ water. Syringes and volumetric pipettes were cleaned with three different organic solvents. All acid hydrolysis experiments were conducted in glass ampoules which were sealed under nitrogen.

Vials and ampoules:

Vials with aluminium lined caps (various volumes ranging from 2 mL to 15 mL) were purchased from Supelco, USA. GC-MS vials with PTFE-lined crimp caps (2 mL) were purchased from Agilent. Pre-scored ampoules ranging in volumes from 1-50 mL were purchased from Sigma-Aldrich. New vials and ampoules were used for all analyses.

NMR spectroscopy:

Routine NMR spectra were run on a Varian Gemini spectrometer operating at either 300 MHz (^{1}H) or 75 MHz (^{13}C). HMBC experiments were performed at 600 MHz on a Varian Unity Inova spectrometer using Varian vnmr6.1c software. Chemical shifts (δ) are reported in parts per million (ppm) downfield. The following abbreviations are used in the assignment of ^{1}H spectra: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; bs = broad singlet; dd = doublet of doublets; Ar = aromatic; J = coupling constant (Hz).

Polarimetry:

Optical rotations were recorded on a PolAAr 21 polarimeter.

Chromatography:

Column chromatography was performed with silica gel 60 F_{254} (230-400 mesh) obtained from Merck. Analytical thin layer chromatography was performed with aluminium backed silica gel 60 F_{254} sheets from Merck.

Melting Points:

Melting points were measured with a Reichert hot-stage apparatus and are uncorrected.

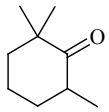
Elemental Analysis:

Microanalyses were performed by Microanalytical Services, University of Otago, New Zealand.

6.2 Procedures

6.2.1 Material relating to Chapter 2.

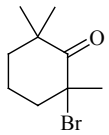
2,2,6-Trimethylcyclohexanone (47)



To a cooled (-78 ºC), stirred solution of di-isopropylamine (10.42 g, 0.10 mol) in THF (100 mL), was added *n*-butyllithium (2.5 M in hexanes, 40 mL, 0.10 mol). After stirring for 5 minutes, 2,6-dimethylcyclohexanone (**46**) (10 g, 0.08 mol) was added and the solution was stirred at -40 ºC for 1hour. The reaction was re-cooled to -78 ºC and methyl iodide (17.94 g, 0.13 mol, 1.6 eq) was added slowly. The solution was allowed to warm to room temperature over 45 min and quenched with water. The THF was removed *in vacuo* and the residue was extracted with hexane. The organic layer was washed with water and brine before being dried (Na_2SO_4) and concentrated *in vacuo* to yield a clear liquid (**47**) (11.3 g, 99%), whose purity by NMR was sufficient for further use.

^{1}H NMR: (δ ppm, $CDCl_3$): 2.64 (m, 1H, H_6); 2.06-1.45 (m, 5H, $H_{3,4,5a}$); 1.30 (m, 1H, H_{5b}); 1.16 (d, 3H, J=6.5 Hz, H_9); 1.02, 0.98 (2 x s, 6H, $H_{7,8}$).

2,6,6-Trimethyl-2-bromocyclohexanone (48)

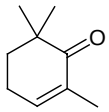


To a solution of 2,2,6-trimethylcyclohexanone (**47**) (11.33 g, 0.08 mol) and acetic acid (0.5 mL) in $CHCl_3$ (100 mL), at 0 ºC was added approximately 1 mL of bromine solution (18.61 g, 0.12 mol bromine in 20mL $CHCl_3$). After the initial red colour had dissipated, the bromine solution was added dropwise until the red colour

persisted. The mixture was then stirred at room temperature for 30 minutes before being quenched with water. The organic layer was extracted with ethyl acetate, washed with sodium thiosulphate solution (10%), sodium bicarbonate solution and brine. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to yield an orange liquid (**48**) (17.67 g, 100 %), which was used without further purification.

^{1}H NMR: (δ ppm, $CDCl_3$): 2.48-2.39 (m, 1H, H_{3a}); 2.24-2.08 (m, 1H, H_{3b}); 1.97-1.80 (m, 2H, H_4); 1.82 (s, 3H, H_9); 1.72-1.50 (m, 2H, H_5); 1.48, 1.09 (2 x s, 6H, $H_{7,8}$).

2,6,6-Trimethylcyclohex-2-en-1-one (7)

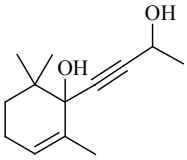


A solution of 2,6,6-trimethyl-2-bromocyclohexanone (**48**) (14.25 g, 0.07 mol) and pyridine (39.20 g, 0.5 mol) in $CHCl_3$ (200 mL) was heated at reflux overnight. The reaction was quenched with water, extracted with dichloromethane then washed with 3M HCl solution, NaOH solution (10%) and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to yield an orange liquid. The crude product was distilled (125 mmHg @130-135 ºC) to yield (**7**) as a colourless liquid (12.01 g, 79%).

^{1}H NMR: (δ ppm, $CDCl_3$): 6.58 (m, 1H, H_3); 2.28 (ddt, 2H, J=6.0, 12.3, 8.1 Hz, H_4); 1.76 (t, 2H, J=6.0 Hz, H_5); 1.70 (dt, 3H, J=1.2, 2.1 Hz, H_9); 1.05, (s, 6H, $H_{7,8}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 204.75 (C_1); 143.48, 133.75 ($C_{2,3}$); 41.21 (C_6); 36.64 (C_5); 24.29, 24.29 ($C_{7,8}$); 23.04 (C_4); 16.46 (C_9).

6,9-Dihydroxymegastigm-4-en-7-yne (8)



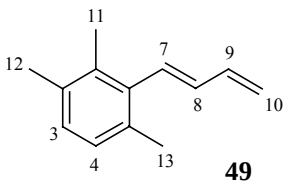
To a solution of but-3-yn-2-ol (**63**) (7.6 g, 0.11 mol) in ether (200mL) at -78 ºC was added *n*-butyllithium (1.6 M in hexanes, 130 mL, 0.21 mol). The thick, viscous solution was stirred at room temperature for 1 hour then 2,6,6-trimethylcyclohex-2-enone (**7**) (5 g, 0.04 mol) was added. The mixture was stirred at room temperature for 2 days and quenched with saturated ammonium chloride solution (100 mL). The organic layer was washed with brine solution (100 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield 8.78 g of crude material. Recrystallisation (ethyl acetate/ hexane) produced 1.80 g (24%) of one diastereomer (white powder) and 5.69 g (76%) recovered mother liquor, which was composed of a mixture of diastereoisomers. m.p (single diastereomer): 122-124°C (lit.[88] 123-124.5°C).

^{1}H NMR: (δ ppm, $CDCl_3$): (single diastereomer) 5.49 (m, 1H, H_4); 4.58 (dq, 1H, H_9, J=6.5, 6.5 Hz); 2.01 (m, 2H, H_3); 1.87 (m, 5H, 2 x OH**,** H_{13}); 1.62-1.49 (m, 2H, H_2); 1.46 (d, 3H, J=6.5 Hz, H_{10}); 1.06, 1.04 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): (single diastereomer) 134.38, 124.36 ($C_{4,5}$); 87.45, 85.30 ($C_{7,8}$); 74.35 (C_6); 58.45 (C_9); 37.30 (C_1); 31.66 (C_2); 24.41 (C_{10}); 24.24, 22.77 ($C_{11,12}$); 22.60 (C_3); 19.37 (C_{13}).

Attempted dehydration of 6,9-dihydroxymegastigm-4-en-7-yne (8)

Method A (*p*-TsOH)



49

To a solution of 6,9-dihydroxymegastigm-4-en-7-yne (**8**) (11.8 mg, 0.06 mmol) in $CDCl_3$ (0.7 mL) was added *p*-toluene sulphonic acid (10 mg). The reaction was heated to 50 ºC for 21h, at which time analysis by NMR revealed the product to be (*E*)-1-(2,3,6-trimethylphenyl)-1,3-butadiene (**49**). The reaction was quenched with $NaHCO_3$ solution, washed with water and brine before being dried and concentrated *in vacuo*. The crude material was purified by column chromatography (eluant: hexane) to yield (*E*)-1-(2,3,6-trimethylphenyl)-1,3-butadiene (**49**) as a colourless liquid (~10 mg, ~100%), which was found to be 94% pure by GC-MS.

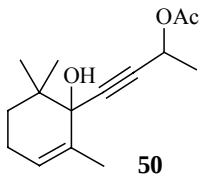
(*E*)-1-(2,3,6-Trimethylphenyl)-1,3-butadiene (**49**)

^{1}H NMR: (δ ppm, $CDCl_3$): 6.98, 6.96 (AB quartet) (d, 1H, J=7.8 Hz; d, 1H, J=7.8 Hz, H_4, H_5); 6.71 (br d, 1H, J=16.1, 0.8 Hz, H_7); 6.28 (dddd, 1H, J=16.1, 10.4, 0.8, 0.8 Hz, H_8); 6.64 (dddd, 1H, J=10.4, 10.4, 16.9, 0.8 Hz, H_9); 5.31 (dddd, 1H, J=16.9, 1.9, 0.8, 0.8 Hz, H_{10a}); 5.18 (dddd 1H, J=10.4, 1.9, 0.8, 0.8 Hz, H_{10b}); 2.19 (s, 1H, H_{11}); 2.22 (s, 1H, H_{12}); 2.22 (s, 1H, H_{13}).

^{13}C NMR: (δ ppm, $CDCl_3$): 137.4, 136.9, 135.2, 134.4, 134.2, 133.5, 131.8, 128.3, 127.1, 116.7, 20.9, 20.4, 16.9.

MS m/z (%): 172 (M^+,35), 158 (13), 157 (100), 156 (10), 143 (15), 142 (85), 141 (30), 129 (13), 128 (15), 127 (8), 115 (20), 91 (5), 77 (6).

Method B (Ac_2O)



50

To a solution of 6,9-dihydroxymegastigm-4-en-7-yne (**8**) (1.8 g, 8.68 mmol) in THF (25 mL) was added pyridine (2.06 g, 26.0 mmol) and acetic anhydride (1.77 g, 17.4 mmol). The mixture was heated at reflux overnight, diluted with ethyl acetate and quenched with 3M HCl solution. The organic layer was washed with $NaHCO_3$ and

brine, dried (Na_2CO_3) and concentrated *in vacuo* to yield an orange liquid (2.1 g), which was distilled (~0.3 mmHg @ 150º-175 ºC) to yield a yellow oil (1.91 g, 88%) identified as 6-hydroxy-9-acetoxymegastigma-4-en-7-yne (**50**).

6-Hydroxy-9-acetoxymegastigm-4-en-7-yne (**50**)

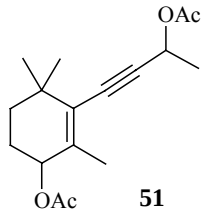
^{1}H NMR: (δ ppm, $CDCl_3$): 5.45 (m, 2H, H_4, H_9); 2.04 (s, 3H, OAc); 2.02-1.94 (m, 2H, H_3); 1.83 (dd, 3H, J=3.6, 2.1 Hz, H_{13}); 1.62-1.4 (m, 2H, H_2); 1.46 (d, 3H, J=6.9 Hz, H_{10}); 1.01 (s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 169.82 (**C**=O); 134.16 (C_5); 124.32 (C_4); 86.04, 83.86 ($C_{7,8}$); 74.18 (C_6); 60.38 (C_9); 37.26 (C_1); 31.47 (C_2); 24.04, 22.79 ($C_{11,12}$); 22.54 (C_3); 21.29 (C_{10}); 20.95 (Ac); 19.30 (C_{13}).

MS m/z (%): 232 (M^+-18) (1), 207 (1), 194 (6), 175 (5), 152 (100), 142 (3)134 (24), 123 (58), 119 (30), 115 (8), 105 (23), 91 (80), 82 (38), 57 (24), 43 (50).

Anal: Calcd for $C_{15}H_{22}O_2$: C 71.97, H 8.86; Found: C: 71.89, H 9.09.

Method C (Ac_2O, DMAP)



51

Diol **8** (0.12 g, 0.6 mmol), 4-DMAP (6 mg, 5%) and sodium acetate (0.17 g, 1.19 mmol) were added to acetic anhydride (10 mL). The mixture was heated at reflux for 45 min, poured over ice and stirred for 2 hr. The residue was extracted with ethyl acetate, washed with 3M HCl, $NaHCO_3$ solution, brine and concentrated *in vacuo* to yield 0.15 g (86%) crude material, which was identified as 4,9-diacetoxymegastigm-5-en-7-yne (**51**). The crude product was purified by short column chromatography (ethyl acetate/ hexane).

4,9-Diacetoxymegastigm-5-en-7-yne (**51**)

^{1}H NMR: (δ ppm, $CDCl_3$): 5.60 (q, 1H, J=6.6 Hz, H_9); 5.22 (br t, 1H, J=4.8 Hz, H_4); 2.06, 2.05 (2 x s, 6H, 2 x OAc); 1.82 (d, 3H, J=0.7 Hz, H_{13}); 1.74-1.39 (m, 4H, $H_{2,3}$); 1.52 (d, 3H, J=6.6 Hz, H_{10}); 1.12, 1.06 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 170.75, 169.89 (2 x C=O); 137.79, 129.11 ($C_{5,6}$); 93.39, 82.24 ($C_{7,8}$); 70.79 (C_4); 60.92 (C_9); 34.23 (C_1); 33.15 (C_3 or C_2); 28.80, 27.45 ($C_{11,12}$); 24.98 (C_2 or C_3); 21.54 (C_{10}); 21.19, 21.04 (2 x Ac); 19.10 (C_{13}).

MS m/z (%): 292 (M^+) (1), 232 (11), 217 (2), 190 (100), 175 (27), 157 (16), 147 (10), 134 (12), 119 (7), 105 (9), 91 (12), 77 (6), 43 (25).

HRMS: $[M+H]^+$ Calcd for $C_{17}H_{24}O_4$: 293.1713; Found: 293.1612.

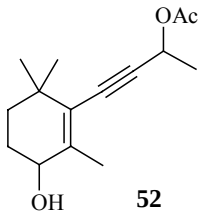
Method D (Flash Vacuum Pyrolysis)

i) Flash vacuum pyrolysis was carried out on diol **8** (50 mg, 0.24 mmol) at 0.4 mmHg, with a furnace temperature of 400°C, and with the sublimation temperature ramped from 100°C-175°C. A yellow oil containing some white solid was collected, analysis of which indicated the presence of a substantial quantity of starting material (white solid), and some unidentified material.

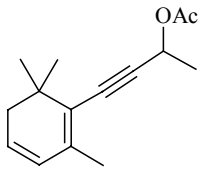
ii) Flash vacuum pyrolysis was carried out on **8** (1 g, 4.8 mmol) at 0.4 mmHg, with a furnace temperature of 540°C, and a sublimation temperature of 100°C. A yellow oil was collected from the cold trap, analysis of which indicated complete degradation of the organic material.

Attempted dehydration of (50) with *p*-TsOH

Method E1



52



53

To a solution of 9-acetoxy-6-hydroxymegastigm-4-en-7-yne (**50**) (25 mg, 0.10 mmol) in $CDCl_3$ (0.7 mL), was added *p*-TsOH (1.3 mg, 5%). The solution was heated at 50 ºC for 1hr, at which time analysis by NMR revealed the product to be a mixture of 9-acetoxy-4-hydroxymegastigm-5-en-7-yne (**52**) (56%) along with the desired 9-acetoxymegastigma-3,5-diene-7-yne (**53**) (44%), as well as a trace (<1%) of β-damascenone (**1**). Pure samples of **52** and **53** were obtained by silica chromatography (ethyl acetate/hexane).

4-Hydroxy-9-acetoxymegastigm-5-en-7-yne (**52**)

^{1}H NMR: (δ ppm, $CDCl_3$): 5.60 (q, 1H, J=6.7 Hz, H_9); 4.01 (br t, 1H, J=5.1 Hz, H_4); 2.07 (s, 3H, OAc); 1.96 (d, 3H, J=0.6 Hz, H_{13}); 1.74-1.38 (m, 4H, $H_{2,3}$); 1.53 (d, 3H, J=6.7 Hz, H_{10}); 1.12, 1.05 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 169.98 (**C**=O); 141.61, 126.93 ($C_{5,6}$); 92.73, 82.65 ($C_{7,8}$); 68.80 (C_4); 61.02 (C_9); 34.36 (C_1); 33.12 (C_2 or C_3); 28.85, 28.20 ($C_{11,12}$); 27.71 (C_2 or C_3); 21.61 (C_{10}); 21.10 (OAc); 19.21 (C_{13}).

HRMS: $[M-H_2]^+$ Calcd for $C_{15}H_{20}O_3$: 248.1412; Found 248.1408.

9-Acetoxymegastigma-3,5-dien-7-yne (**53**)

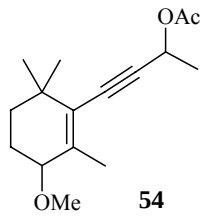
^{1}H NMR: (δ ppm, $CDCl_3$): 5.88-5.76 (m, 2H, H_3, H_4); 5.66 (q, 1H, J=6.7 Hz, H_9); 2.10-2.07 (m, 2H, H_2); 2.08 (s, 3H, OAc); 1.92 (s, 3H, H_{13}); 1.54 (d, 3H, J=6.7 Hz, H_{10}); 1.06, 1.05 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 169.9 (**C**=O); 137.4, 127.6, 126.8, 122.6 ($C_{3,4,5,6}$); 94.7, 83.2 ($C_{7,8}$); 61.3 (C_9); 38.2 (C_2); 32.7 (C_1); 26.9, 26.9 ($C_{11,12}$); 21.8 (C_{10}); 21.2 (OAc); 20.5 (C_{13}).

MS m/z (%): 232 (M^+) (39), 217 (2), 189(16), 175 (60), 157 (100), 142 (79), 128 (31), 115 (29), 105 (12), 91 (9), 77 (13), 43 (28).

HRMS (ESI): $[M+Na]^+$ Calcd for $C_{15}H_{20}O_2Na$: 255.1361; Found: 255.1357.

Method E2



54

To a solution of acetate **50** (25 mg, 0.10 mmol) in $CDCl_3$ (0.7 mL) was added *p*-TsOH (1.3 mg, 5%) and trimethylorthoformate (10 mg, 0.10 mmol). The reaction mixture was heated at 50 ºC for 1hr and quenched with $NaHCO_3$ solution (5 mL). The organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo.* The crude material was purified by column chromatography to yield 25mg (100%) of 4-methoxy-9-acetoxymegastigm-5-en-7-yne (**54**).

4-Methoxy-9-acetoxymegastigm-5-en-7-yne (**54**)

^{1}H NMR: (δ ppm, $CDCl_3$): 5.60 (q, 1H, J=6.7 Hz, H_9); 3.53 (br t, 1H, J=4.7 Hz, H_4); 3.35 (s, 3H, OMe); 2.06 (s, 3H, OAc); 1.91 (s, 3H, H_{13}); 1.84-1.32 (m, 4H, H_2, H_3); 1.52 (d, 3H, J=6.7 Hz, H_{10}); 1.10, 1.04 (2 x s, 6H, $H_{11,12}$).

MS m/z (%): 264 (M^+) (29), 249 (23), 233 (1), 221 (2), 207 (11), 189 (25), 175 (12), 166 (25), 157 (9), 148 (100), 133 (35), 119 (15), 110 (13), 91 (26), 77 (16), 43 (33).

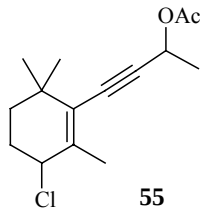
HRMS: $[M+H]^+$ Calcd for $C_{16}H_{24}O_3$: 264.1725; Found 264.1714.

Method E3

To a solution of acetate **50** (0.1g, 0.4mmol) in CH_2Cl_2 (5mL) was added *p*-TsOH (5 mg). The mixture was heated at 50°C for 1hr, cooled and concentrated *in vacuo* to yield 0.12g of crude material. The residue, which was analysed immediately by NMR, was identified as β-damascenone (**1**) (~80%), plus a small amount of unknown material, which was neither isolated nor identified.

Other attempted dehydrations of (50)

Method F (MsCl)



55

To a solution of acetate **50** (30 mg, 0.119 mmol) in $CDCl_3$ (0.7 mL), was added pyridine (19 mg, 0.24 mmol) and mesyl chloride (27 mg, 0.24 mmol). The solution was stirred at 50 ºC for 1hr, after which time NMR revealed the presence of 4-chloro-9-acetoxymegastigm-5-en-7-yne (**55**) (~63%) and the desired compound (**53**) (~37%). The reaction was quenched with 3M HCl solution with the organic layer washed with $NaHCO_3$ solution and brine before being dried (Na_2CO_3) and concentrated *in vacuo* to yield crude material (30 mg). Purification was achieved by short column chromatography (5% EtOAc/Hexane).

4-Chloro-9-acetoxymegastigm-5-en-7-yne (**55**)

^{1}H NMR: (δ ppm, $CDCl_3$): 5.59 (q, 1H, J=6.7 Hz, H_9); 4.44 (br t, 1H, J=3.5 Hz, H_4); 2.06 (s, 3H, O**Ac**); 2.23-1.42 (m, 4H, $H_{2,3}$); 1.96 (br s, 3H, C_{13}); 1.52 (d, 3H, J=6.7 Hz, H_{10}); 1.14, 1.04 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 169.87 (**C**=O); 138.96, 128.48 ($C_{5,6}$); 93.86, 82.30 ($C_{7,8}$); 60.88 (C_9); 60.14 (C_4); 34.31 (C_1); 31.88, 29.55, 28.49, 26.91 ($C_{2,3,11,12}$); 21.47 (C_{10}); 21.02 (OAc); 20.50 (C_{13}).

HRMS: $[M+H]^+$ Calcd for $C_{15}H_{23}O_2{}^{35}Cl$: 269.1308; Found 269.1306.

Anal: Calcd for $C_{15}H_{22}O_2Cl$: C 67.03, H 7.88; Found: C: 67.05, H 8.19.

Method G ($SOCl_2$)

To a solution of acetate **50** (30 mg, 0.12 mmol) in $CDCl_3$ (0.7 mL), was added pyridine (19 mg, 0.24 mmol) and thionyl chloride (37 mg, 0.24 mmol). The solution was stirred at 50 ºC for 1hr and quenched with aqueous 3M HCl solution. The organic layer was washed with $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated *in vacuo* to again yield the chloride **55** (30 mg, 93% crude).

Method H ($POCl_3$)

To a solution of acetate **50** (50 mg, 0.2 mmol) in $CDCl_3$ (0.6 mL) was added $POCl_3$ (61 mg, 0.4 mmol) and pyridine (32 mg, 0.4 mmol). The mixture was heated for 2 hours at 50°C, cooled (water bath) and analysed immediately by NMR to again reveal almost exclusively chloride **55**.

Attempted Elimination of HCl From (55)

Method I1

A solution of chloride **55** (32 mg, 0.12 mmol) and pyridine (11 mg, 0.15 mmol, 1.2 eq) in $CHCl_3$ (3 mL) was heated at reflux for 2 hours. The mixture was cooled and diluted with diethyl ether before being washed with 3M HCl solution, water and brine. The organic material was dried (Na_2CO_3) and concentrated *in vacuo* to yield 30 mg of starting material.

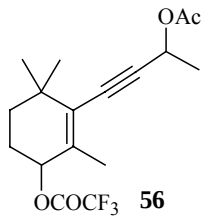
Method I2

To dried alumina (1 g) was added a solution of chloride **55** (100 mg, 0.37 mmol) in enough THF to cover the alumina. The mixture was stirred for 1 day at RT, the alumina filtered off and rinsed with ethyl acetate. The organic material was concentrated *in vacuo* to yield 0.11 g crude material which, by NMR was found to consist of approximately equal proportions of unreacted **55** and 4-hydroxy-9-acetoxymegastigm-5-en-7-yne (**52**).

Method I3

In a solution of *t*-butanol (ca. 5mL) was dissolved a small piece of metallic sodium (c. 10 mg). 4-Chloro-9-acetoxymegastigm-5-en-7-yne (**55**) (50 mg, 0.19mmol) was added and the mixture stirred at room temperature for 2 hours. The reaction was quenched with saturated ammonium chloride solution, diluted with diethyl ether and the organic layer washed with water. The organic material was dried (Na_2SO_4) and concentrated *in vacuo* to yield 60mg of crude material, which by NMR showed multiple unidentified products.

Method I4



56

To a solution of chloride **55** (100 mg, 0.37 mmol) in THF (2 mL) was added triethylamine (51 mg, 0.5 mmol, 1.35 eq). The solution was stirred at room temperature for 30 min. In the absence of light, silver trifluoroacetate (91 mg, 0.44 mmol, 1.2 eq) in THF was added dropwise. The mixture was stirred for 45 min., during which time an off-white precipitate formed. The mixture was filtered through celite and washed with diethyl ether and then ethyl acetate. The organic material was

concentrated *in vacuo* to yield 0.14 g of brown liquid. Analysis of the crude material by NMR showed the presence of 4-trifluoroacetoxy-9-acetoxymegastigma-5-en-7-yne (**56**).

4-Trifluoroacetoxy-9-acetoxymegastigma-5-en-7-yne (**56**)

^{1}H NMR: (δ ppm, $CDCl_3$): 5.58 (q, 1H, J=6.7 Hz, H_9); 5.39 (br t, 1H, J=4.3 Hz, C_4); 2.06 (s, 3H, O**Ac**); 1.84 (s, 3H, H_{13}); 1.67-1.36 (m, 4H, $H_{2,3}$); 1.52 (d, 3H, J=6.7 Hz, C_{10}); 1.14, 1.07 (2 x s, 6H, $H_{11,12}$).

Dehydration of (50) with P_2O_5

Method J1

To a pre-dried flask containing P_2O_5 (0.34 g, 2.4 mmol, 3 eq.) under an inert atmosphere was added a solution of 9-acetoxy-6-hydroxymegastigm-4-en-7-yne (**50**) (0.2 g, 0.8 mmol) and pyridine (190 mg, 2.4 mmol, 3 eq) in chloroform (2 mL). The mixture was heated at reflux for 2 days, cooled and quenched with saturated Na_2CO_3 solution. The residue was extracted with chloroform, washed with brine, dried (Na_2SO_4) and the organic layer concentrated *in vacuo* to yield 0.19 g of crude material. Analysis by NMR revealed a mixture of (**53**) (~15%), some unidentified material (~15%) and a substantial amount of starting material (~70%).

Method J2

To a solution of acetate **50** (0.71 g, 2.8 mmol) in pyridine (13 mL) was added Sicapent® (4.8 g, 34 mmol, 12 eq). The mixture was heated at reflux for 2 days. The mixture was then cooled, the residue diluted (CH_2Cl_2) and the solid material rinsed (CH_2Cl_2). The two organic fractions were combined and filtered, before being concentrated *in vacuo* to yield 0.21 g of crude material. The crude material was purified by column chromatography (eluant: 5% ethyl acetate in hexane) to yield a pure fraction of compound (**53**) (40 mg, 6%) and a mixed fraction (30 mg), which by

NMR indicated the presence of approximately equal proportions of starting material (**50**) and the desired acetate (**53**).

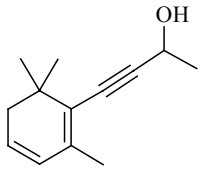
Method J3

To a pre-dried flask containing P_2O_5 (2.5 g, mmol, eq.) under an inert atmosphere was added dried, ground pumice stone (1.5 g). The two solids were stirred vigorously and to the mixture was added a solution of acetate **50** (0.5 g, 2 mmol) and pyridine (5 mL) in toluene (20 mL). The mixture was heated at reflux for 3 days, cooled, filtered and the solids washed with diethyl ether and ethyl acetate. The combined organic fractions were concentrated *in vacuo* to yield 1.3 g of crude material, which was purified by column chromatography (5% ethyl acetate/ hexane) to yield a pure fraction of (**53**) (0.07 g, 16%).

Method J4

To a pre-dried flask containing P_2O_5 (570 mg, 4 mmol, 10 eq.) under an inert atmosphere was added celite (1.19 g). The two solids were stirred vigorously and to the mixture was added a solution of acetate **50** (100 mg, 0.4 mmol) and pyridine (0.32 g, 4 mmol, 10 eq.) in toluene (20 mL). The mixture was heated at reflux for 3 days, cooled, filtered and the solids washed with diethyl ether and ethyl acetate. The combined organic fractions were concentrated *in vacuo* to yield 0.15 g of crude material, which was purified by column chromatography (5% ethyl acetate/ hexane) to yield a pure fraction of (**53**) (50 mg, 54%).

9-Hydroxymegastigma-3,5-dien-7-yne (36)



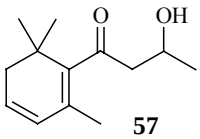
To a 50% aqueous ethanol solution (3 mL) was added KOH (0.075 g, 1.35 mmol, 2 eq) and acetate **53** (0.16 g, 0.67 mmol,). The mixture was stirred for 45 minutes at room temperature, after which time the organic residue was extracted with dichloromethane. The organic layer was washed with water and brine, dried (Na_2SO_4) and concentrated *in vacuo* to yield 0.12 g (95%) of 9-hydroxymegastigma-3,5-diene-7-yne (**36**).

^{1}H NMR: (δ ppm, $CDCl_3$): 5.86-5.76 (m, 2H, $H_{3,4}$); 4.75 (q, 1H, J=6.6 Hz, H_9); 2.11-2.07 (m, 2H, H_2); 1.93 (br s, 3H, H_{13}); 1.52 (d, 3H, J=6.6 Hz, H_{10}); 1.06 (s, 6H, $H_{11,12}$).

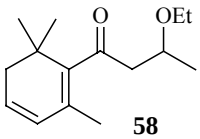
^{13}C NMR: (δ ppm, $CDCl_3$): 136.5, 127.6, 126.4, 122.9 ($C_{3,4,5,6}$); 98.5, 82.1 ($C_{7,8}$); 58.8 (C_9); 38.2 (C_2); 32.6 (C_1); 26.8, 26.8 ($C_{11,12}$); 24.7 (C_{10}); 20.3 (C_{13}).

MS m/z (%): 190 (M^+) (70), 175 (100), 157 (20), 147 (8), 142 (19), 131 (47), 115 (42), 105 (17), 91 (41), 77 (17), 65 (8), 51 (7), 43 (53).

Hydrolysis of (36) in buffered model wine solution



57



58

9-Hydroxymegastigma-3,5-diene-7-yne (**36**) (24 mg, 0.12 mmol) was added to a solution of model wine (pH 3.0, 25 mL) and heated at 45 ºC for 18 hours before being analysed by GC-MS to reveal 84.3% conversion to β-damascenone (**1**). Other products, which were identified by GC-MS, included starting material (~9.3%), the

C_9 hydrate of β-damascenone (**57**) (3.3%) and the C_9 ethyl ether of β-damascenone (**58**) (3.1%). The latter two compounds were identified by comparison with authentic spectra, as described in Daniel et al.[115]

9-Hydroxymegastigma-3,5-dien-7-one (**57**)

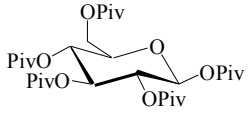
MS m/z (%): 208(4), 149(39), 133(31), 122(31), 121(100), 107(20), 105(42), 91(25), 77(15), 43(38).

9-Ethoxymegastigma-3,5-dien-7-one (**58**)

MS m/z (%): 236(5), 221(7), 192(3), 175 (6), 149(73), 133(49), 122(100), 121(85), 107(60), 105(54), 91(40), 73(76), 45(72).

6.2.2 Material relating to Chapter 3.

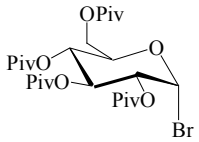
1,2,3,4,6-Pentapivaloyl-β-D-glucopyranose (66)



D-Glucose (4.0 g, 22.2 mmol) was added in several portions to a solution of pivaloyl chloride (27.35 mL, 222.0 mmol), pyridine (18.0 mL, 222.0 mmol) in chloroform (100 mL). The solution was then heated at reflux for 72 hours, after which the solvent was evaporated, the residue dissolved in water (100 mL) and extracted with ethyl acetate (5 x 60 mL). The combined organic extracts were washed with water (100 mL), hydrochloric acid (1M, 100 mL), saturated sodium bicarbonate (100 mL) and brine (100 mL). The solution was dried, concentrated and the crude product recrystallised from ethanol to give **66** as a white crystalline material (11.2 g, 85%); m.p. 155-158 ºC (lit.[104] m.p. 156-158 ºC).

^{1}H NMR: (δ ppm, $CDCl_3$): 5.70 (d, 1H, J=8.4 Hz, H_1); 5.37 (dd, 1H, J=9.3, 9.3 Hz, H_3); 5.21 (dd, 1H, J=9.3, 8.4 Hz, H_2); 5.16 (dd, 1H, J=9.3, 9.3 Hz, H_4); 4.18-4.07 (m, 2H, H_6); 3.89-3.83 (m, 1H, H_5); 1.20, 1.17, 1.15, 1.11, 1.11 (5 x s, 45H, CMe_3).

2,3,4,6-Tetrapivaloyl-α-D-bromoglucopyranose (61)

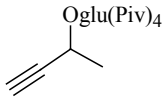


48% Hydrobromic acid solution in acetic acid (2.5 mL) was added dropwise to a solution of **66** (4.40 g, 0.73 mmol) in dichloromethane at 0 ºC. The mixture was then stirred at room temperature for 16 hours, before being concentrated, with co-evaporation with toluene (2 x 30 mL) and ether (2 x 30 mL). The residue was dissolved in diethyl ether (40 mL) and washed with a saturated solution of sodium

bicarbonate (3 x 30 mL) and water (40 mL), before being dried and concentrated. The crude product was recrystallised from ethanol to give **61** as a white crystalline solid (4.04 g, 95%); m.p. 143-145 ºC (lit.[104] m.p. 142-143 ºC).

^{1}H NMR: (δ ppm, $CDCl_3$): 6.56 (d, 1H, J=4.1 Hz, H_1); 5.56 (dd, 1H, J=9.3, 9.3 Hz, H_3); 5.20 (dd, 1H, J=9.3, 9.3 Hz, H_4); 4.76 (dd, 1H, J=9.3, 4.1 Hz, H_2); 4.35-4.25 (m, 1H, H_5); 4.20-4.15 (m, 2H, H_6); 1.21, 1.19, 1.17, 1.13 (4 x s, 36H, CMe_3).

2-O-(2',3',4',6'-tetrapivaloyl-β-D-glucopyranosyl)-but-3-yne (64)



i) To a mixture containing but-3-yn-2-ol (**63**) (10 g, 0.14 mol) and 1,2,3,4,6-pentapivaloyl-β-D-glucopyranose (**66**) (8.57 g, 0.13 mol) in acetonitrile (150 mL) was added TMS triflate (2.58 mL, 0.014 mol). The reaction was monitored by TLC and after stirring at room temperature for 15 minutes was quenched with saturated $NaHCO_3$ solution. The organic layer was diluted with ethyl acetate, washed with brine and dried (Na_2SO_4) before being concentrated *in vacuo* to yield 5.31 g of crude material which, after recrystallisation from ethanol produced 5.36 g (67%) of pure crystalline material. The crystals were composed of two epimers with differing physical properties. These were separated manually to produce pure samples of each. Assignment of absolute stereochemistry to each of the individual diasteromers was based on the success of the corresponding reaction undertaken with pure (*S*)-**63**.

(*R*)-2-O-(2',3',4',6'-tetrapivaloyl-β-D-glucopyranosyl)-but-3-yne (*R*)-**64**

^{1}H NMR: (δ ppm, $CDCl_3$): 5.33 (app. t, 1H, J=9.5 Hz, $H_{3'}$); 5.06 (m, 2H, $H_{2',4'}$); 4.83 (d, 1H, J=8.0 Hz, $H_{1'}$); 4.53 (dq, 1H, J=2.2, 6.5 Hz, H_2); 4.23 (dd, 1H, J=1.9, 12.3 Hz, $H_{6'}$); 4.02 (dd, 1H, J=6.2, 12.3 Hz, $H_{6'}$); 3.8-3.73 (m, 1H, $H_{5'}$); 2.47 (d, 1H, J=2.2 Hz, H_4); 1.46 (d, 1H, J=6.5 Hz, H_1); 1.22, 1.16, 1.15, 1.11 (4 x s, 36H, OPiv).

^{13}C NMR: (δ ppm, $CDCl_3$): 178.1, 177.2, 176.5, 176.4 (C=O); 98.9 ($C_{1'}$); 82.4, 74.2 ($C_{3,4}$); 72.5, 72.3, 71.1, 68.1, 66.0, 62.2 ($C_{2',3',4',5',6',2}$); 38.8, 38.8, 38.7, 38.7 (quat. *t*-Bu); 27.1, 27.1, 27.1, 27.0 (Pivs); 22.5 (C_1).

(*S*)-2-O-(2',3',4',6'-tetrapivaloyl-β-D-glucopyranosyl)-but-3-yne, (*S*)-**64**

^{1}H NMR: (δ ppm, $CDCl_3$): 5.35 (app. t, 1H, J=9.5 Hz, $H_{3'}$); 5.13-4.99 (m, 2H, $H_{2'}H_{4'}$); 4.88 (d, 1H, J=8.10 Hz, $H_{1'}$); 4.58 (dq, 1H, J=2.1, 6.6 Hz, H_2); 4.23 (dd, 1H, J=1.8, 12.2 Hz, $H_{6'}$); 4.04 (dd, 1H, J=5.7, 12.2 Hz, $H_{6'}$); 3.79-3.73 (m, 1H, $H_{5'}$); 2.43 (d, 1H, J=2.1 Hz, H_4); 1.42 (d, 1H, J=6.6 Hz, H_1); 1.22, 1.16, 1.15, 1.11, (4 x s, 36H, OPiv).

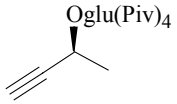
^{13}C NMR: (δ ppm, $CDCl_3$): 178.1, 177.2, 176.6, 176.5 (C=O); 97.7 ($C_{1'}$); 82.1, 74.1 ($C_{3,4}$); 72.3, 72.2, 70.8, 68.1, 62.9, 61.9 ($C_{2',3',4',5',6',2}$); 38.9, 38.8, 38.8, 38.8 (quat. *t*-Bu); 27.1, 27.1, 27.1, 27.0 (Pivs); 21.8 (C_1).

ii) To a solution containing but-3-yn-2-ol (**63**) (25 mg, 0.36 mmol) and orthoester (**62**)[105,109] (0.23 g, 0.36 mmol) in dichloromethane (2 mL) was added $BF_3.Et_2O$ (180 μL, 1.4 mmol). The mixture was stirred at room temperature for 35 min. before being quenched with $NaHCO_3$ solution. The mixture was diluted with dichloromethane, washed with water and brine and concentrated *in vacuo* to yield 157 mg of crude material. The crude material was purified by column chromatography to yield 134 mg (61%) of **64**.

iii) To a mixture containing butynol (**63**) (0.025 g 0.36 mmol), silver triflate (0.061 g, 0.24 mmol) and S-collidine (0.03 mL, 0.24 mmol) in dry dichloromethane was added tetra-O-pivaloyl-α-D-bromopyranoside (**61**) (0.14 g, 0.24 mmol). The reaction was stirred overnight at room temperature, quenched with saturated

$NaHCO_3$ solution, washed with brine, dried (Na_2SO_3) and concentrated *in vacuo* to yield 0.12 g (92%) of **64**.

(*S*)-2-O-(2',3',4',6'-tetrapivaloyl-β-D-glucopyranosyl)-but-3-yne (64)

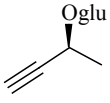


To a mixture containing (*S*)-but-3-yn-2-ol (**63**) (0.5 g, 7.13 mmol) and 1,2,3,4,6-pentapivaloyl-α-D-glucopyranose (**66**) (4.29 g, 7.13 mmol) in acetonitrile (75 mL) was added TMS triflate (1.29 mL, 7.13 mmol). The reaction was monitored by TLC and after stirring at room temperature for 3 hours was quenched with saturated $NaHCO_3$ solution. The organic layer was diluted with ethyl acetate, washed with brine and dried (Na_2SO_4), before being concentrated *in vacuo* to yield 1.89 g of crude material which, after recrystallisation from ethanol produced 1.07 g (27%) of pure crystalline material, plus a further 0.82 g recovered from the mother liquor.

^{1}H NMR: (δ ppm, $CDCl_3$): 5.35 (app. t, 1H, J=9.5 Hz, $H_{3'}$); 5.13-4.99 (m, 2H, $H_{2'}H_{4'}$); 4.88 (d, 1H, J=8.10 Hz, $H_{1'}$); 4.58 (dq, 1H, J=2.1, 6.6 Hz, H_2); 4.23 (dd, 1H, J=1.8, 12.2 Hz, $H_{6'}$); 4.04 (dd, 1H, J=5.7, 12.2 Hz, $H_{6'}$); 3.79-3.73 (m, 1H, $H_{5'}$); 2.43 (d, 1H, J=2.1 Hz, H_4); 1.42 (d, 1H, J=6.6 Hz, H_1); 1.22, 1.16, 1.15, 1.11, (4 x s, 36H, OPiv).

^{13}C NMR: (δ ppm, $CDCl_3$): 178.1, 177.2, 176.6, 176.5 (C=O); 97.7 ($C_{1'}$); 82.1, 74.1 ($C_{3,4}$); 72.3, 72.2, 70.8, 68.1, 62.9, 61.9 ($C_{2',3',4',5',6',2}$); 38.9, 38.8, 38.8, 38.8 (quat. *t*-Bu); 27.1, 27.1, 27.1, 27.0 (Pivs); 21.8 (C_1).

(*S*)-(But-3-yn-2-ol)-O-β-D-glucopyranoside (65)

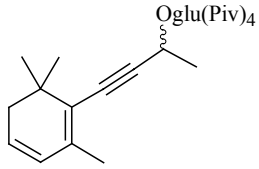


To a solution of (*S*)-**64** (0.1 g, 0.18 mmol) in dry methanol (1 mL) was added a solution of sodium methoxide (0.24 g Na dissolved in 4 mL MeOH). The reaction was stirred at room temperature with the reaction progress monitored by TLC. Upon completion (1 hour), the reaction was quenched with solid NH_4Cl. The supernatant was removed from the solid material and concentrated *in vacuo*. Short column chromatography (SCC) was conducted on silica gel (ethyl acetate/hexane) to yield 0.07 g (70%) of the desired glycoside.

^{1}H NMR: (δ ppm, acetone-d_6): 4.71 (dq, 1H, J= 6.6, 2.1 Hz, H_2); 4.60 (d, 1H, J=7.8 Hz, $H_{1'}$); 3.83 (dd, 1H, J=11.8, 2.1 Hz, $H_{6a'}$); 3.65 (dd, 1H, J=11.8, 5.4 Hz, $H_{6b'}$); 3.50-3.18 (m, 4H, $H_{2',3',4',5'}$); 2.99 (d, 1H, J=2.2 Hz, H_4); 1.39 (d, 1H, J=6.6 Hz, H_2).

^{13}C NMR: (δ ppm, acetone-d_6): 100.1 ($C_{1'}$); 83.4 (C_3); 77.0 (C_4); 76.9, 74.6, 73.7, 70.6 ($C_{2',3',4',5'}$); 62.4 (C_2); 61.9 ($C_{6'}$); 21.9 (C_1).

(9*R* and 9*S*) 9-O-(2',3',4',6'-tetrapivaloyl-β-D-glucopyranosyl)-megastigma-3,5-dien-7-yne (67)

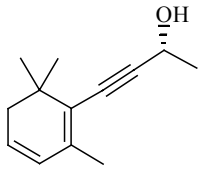


To a solution of 9-hydroxymegastigma-3,5-diene-7-yne (**36**) (0.12 g, 0.65 mmol) in dry dichloromethane (3 mL) was added silver triflate (0.03 g, 1.1 mmol, 1.6 eq), S-collidine (0.14 mL, 1.1 mmol, 1.6 eq) and glucosyl bromide (**61**) (0.57 g, 0.98 mmol, 1.5 eq). The reaction was stirred overnight at room temperature with the exclusion of light. The reaction was quenched with $NaHCO_3$ solution and the organic layer diluted with dichloromethane. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to yield 0.66 g of a brown toffee-like substance. The crude material was purified by column chromatography (5% ethyl acetate in X4) to yield a fraction (0.04 g) which contained mainly one stereoisomer and another

fraction (0.28 g) which contained a mixture of the two stereoisomers. The combined yield was 72%. Assignment of stereochemistry to the two diastereomers was made after each was synthesised individually using optically pure starting materials.

Anal: Calcd for $C_{39}H_{60}O_{10}$: C 68.00, H 8.78; Found: C: 67.60, H 8.71.

(*9R*) 9-Hydroxymegastigma-3,5-dien-7-yne (36)



a) *addition of side chain:* To a solution of (*R*)-but-3-yn-2-ol (2.1 g, 0.03 mol) in cold (-78°C) ether (40 mL) was added *n*-BuLi (2.5 M in hexanes, 23 mL, 0.058 mol) dropwise. The reaction mixture was allowed to warm to room temperature before **7** (2.78 g, 0.02 mol) was added dropwise. The reaction was stirred at room temperature for 2 days before being quenched with saturated NH_4Cl. The organic layer was diluted with ethyl acetate and washed with brine, before being dried (Na_2SO_4) and purified by silica column chromatography to produce 3.63 g of (9*R*)-**8** (87%).

^{1}H NMR: (δ ppm, acetone-d_6): 5.54-5.43 (m, 2H, $H_{4,9}$); 4.58 (q, 1H, J=6.6 Hz, H_9); 2.01 (m, 2H, H_3); 1.86 (m, 5H, 2 x OH, H_{13}); 1.62-1.49 (m, 2H, H_2); 1.46 (d, 3H, J=6.6 Hz, H_{10}); 1.05, 1.04 (2 x s, 6H, $H_{11,12}$)

b) *acetylation at C_9:* To a solution of (9*R*)-**8** (3.63 g, 17.5 mmol) in THF (20 mL) was added pyridine (4.15 g, 52.5 mmol, 4.24 mL) and acetic anhydride (3.57 g, 35 mmol, 3.3 mL). The mixture was heated at reflux overnight, cooled and diluted with ethyl actate. The organic layer was washed with 10% HCl solution, aqueous Na_2SO_4 and brine before being dried and concentrated *in vacuo*. The crude material

was purified by column chromatography (15% ethyl acetate in hexane) to produce 3.2 g (73%) (9*R*)-**50**.

^{1}H NMR: (δ ppm, acetone-d_6): 5.54-5.43 (m, 2H, $H_{4,9}$); 2.10-1.96 (m, 2H, H_3) 2.05 (s, 3H, OAc); 1.85 (m, 3H, H_{13}); 1.64-1.42 (m, 2H, H_2); 1.48 (d, 3H, J=6.7 Hz, H_{10}); 1.04 (s, 6H, $H_{11,12}$).

c) *dehydration:* To a pre-dried flask containing P_2O_5, (16 g) and celite (8 g), under an inert atmosphere was added toluene (50 mL) and pyridine (10 mL). The mixture was stirred to form a slurry and a solution of (9*R*)-**50** (1.0 g, 4.8 mmol) in toluene (5 mL) was then added. The mixture was heated at reflux for 2 days, cooled, filtered and washed with diethyl ether followed by ethyl acetate. The residue was concentrated *in vacuo* and purified by column chromatography to yield (9*R*)-**53** (0.14g, 22%)

^{1}H NMR: (δ ppm, $CDCl_3$): 5.88-5.76 (m, 2H, H_3, H_4); 5.66 (q, 1H, J=6.7 Hz, H_9); 2.10-2.07 (m, 2H, H_2); 2.08 (s, 3H, OAc); 1.92 (s, 3H, H_{13}); 1.54 (d, 3H, J=6.7 Hz, H_{10}); 1.06, 1.05 (2 x s, 6H, $H_{11,12}$).

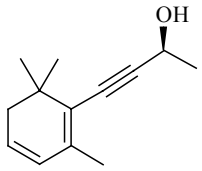
HRMS (ESI): $[M+Na]^+$ Calcd for $C_{15}H_{20}O_2Na$: 255.1361; Found: 255.1357.

d) *deprotection:* To a 50% aqueous ethanol solution (2 mL) was added KOH (0.015 g, 0.14 mmol) and (9*R*)-**53** (0.03 g, 0.13 mmol,). The mixture was stirred for 2 hours at room temperature and the residue was then extracted with dichloromethane. The organic layer was washed with water and brine, dried (Na_2SO_4) and concentrated *in vacuo* to yield (*9R*)-**36** (0.0247 g, 99%). As expected, the spectral properties of the pure (*9R*)-enantiomer were indistinguishable from the racemate prepared earlier.

^{1}H NMR: (δ ppm, $CDCl_3$): 5.86-5.76 (m, 2H, $H_{3,4}$); 4.75 (q, 1H, J=6.6 Hz, H_9); 2.11-2.07 (m, 2H, H_2); 1.93 (br s, 3H, H_{13}); 1.52 (d, 3H, J=6.6 Hz, H_{10}); 1.06 (s, 6H, $H_{11,12}$).

$[\alpha]_D$ +24.7 (c. 0.46, $CHCl_3$).

(*9S*) 9-Hydroxymegastigma-3,5-dien-7-yne (36)



The (9*S*) enantiomer of **36** was prepared in an identical manner to that described above for the (9*R*) enantiomer, with the only difference being the use of (*S*)-but-3-yn-2-ol to introduce the stereochemistry on the side-chain. As described above, the pure (9*S*)-enantiomer was indistinguishable from the racemate prepared earlier.

a) (9*S*)-**8:** (yield 71%)

^{1}H NMR: (δ ppm, acetone-d_6): 5.48 (m, 1H, H_4); 4.56 (m, 1H, J=6.6 Hz, H_9); 2.00 (m, 2H, H_3); 1.86 (m, 5H, 2 x OH, H_{13}); 1.61-1.47 (m, 2H, H_2); 1.46 (dd, 3H, J=0.3, 6.6 Hz, H_{10}); 1.05, 1.04 (2 x s, 6H, $H_{11,12}$).

b) (9*S*)-**50:** (yield 88%)

^{1}H NMR: (δ ppm, acetone-d_6): 5.51-5.44 (m, 2H, $H_{4,9}$); 2.07-1.96 (m, 2H, H_3) 2.06 (s, 3H, OAc); 1.85 (m, 3H, H_{13}); 1.63-1.42 (m, 2H, H_2); 1.49 (dd, 3H, J=0.6, 6.6 Hz, H_{10}); 1.04 (s, 6H, $H_{11,12}$).

c) (9*S*)-**53:** (yield 12%)

^{1}H NMR: (δ ppm, $CDCl_3$): 5.88-5.76 (m, 2H, $H_{3,4}$); 5.66 (q, 1H, J=6.7 Hz, H_9); 2.10-2.07 (m, 2H, H_2); 2.08 (s, 3H, OAc); 1.92 (s, 3H, H_{13}); 1.54 (d, 3H, J=6.7 Hz, H_{10}); 1.06, 1.05 (s, 3H, $H_{11,12}$).

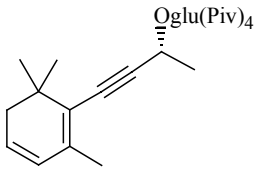
HRMS (ESI): $[M+Na]^+$ Calcd for $C_{15}H_{20}O_2Na$: 255.1361; Found: 255.1359.

d) (9*S*)-**36:** (yield 96%)

^{1}H NMR: (δ ppm, $CDCl_3$): (*R*)-epimer: 5.86-5.76 (m, 2H, H_3, H_4); 4.75 (q, 1H, J=6.6 Hz, H_9); 2.11-2.07 (m, 2H, H_2); 1.93 (br s, 3H, H_{13}); 1.52 (d, 3H, J=6.6 Hz, H_{10}); 1.06 (s, 6H, $H_{11,12}$).

$[\alpha]_D$ -25.5 (c. 0.19, $CHCl_3$).

(9*R*) 9-O-(2',3',4',6'-tetrapivaloyl-β-D-glucopyranosyl)-megastigma-3,5-dien-7-yne (67)



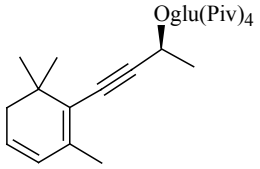
(*9R*)-**36** was converted into the corresponding protected glycoside in a manner identical to that described above for racemic **36**.

^{1}H NMR: (δ ppm, $CDCl_3$): 5.88-5.78 (m, 2H, $H_{3,4}$); 5.30 (app. t, J=9.5 Hz, $H_{3'}$); 5.16-5.03 (m, 2H, $H_{2',4'}$); 4.92 (app d, 1H, J=7.9 Hz, $H_{1'}$); 4.77 (q, 1H, J=6.7 Hz, H_9); 4.19 (dd, 1H, J=12.2, 1.8 Hz, $H_{6a'}$); 4.04 (dd, 1H, J=12.2, 5.5 Hz, $H_{6b'}$); 3.73 (m, 1H, $H_{5'}$); 2.10 (br d, 2H, J=2.9 Hz, H_2); 1.92 (br s, 3H, H_{13}); 1.51 (d, 3H, J=6.7 Hz, H_{10}); 1.21, 1.16, 1.15, 1.11, (4 x s, 36H, OPiv's); 1.06, 1.06 (s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 178.1, 177.3, 176.5, 176.4 (C=O); 137.1, 127.6, 126.8, 122.8 ($C_{3,4,5,6}$); 99.0 ($C_{1'}$); 94.7, 85.1 ($C_{7,8}$); 72.4, 72.4, 71.2, 67.9, 67.4, 62.1

($C_{2',3',4',5',6',9}$); 38.8, 38.7, 38.7, 38.1 (quat. tBu); 38.1 (C_2); 32.7 (C_1); 27.1, 27.1, 27.1, 27.1, 27.0, 27.0 (Pivs, $C_{11,12}$); 23.4 (C_{13}); 20.6 (C_{10}).

(9*S*) 9-O-(2',3',4',6'-tetrapivaloyl-β-D-glucopyranosyl)-megastigma-3,5-dien-7-yne (67)

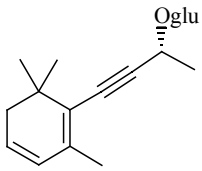


(*9S*)-**36** was also converted into the corresponding protected glycoside in a manner identical to that described above for racemic **36**.

^{1}H NMR: (δ ppm, $CDCl_3$): 5.90-5.79 (m, 2H, $H_{3,4}$); 5.30 (app. t, J=9.4 Hz, $H_{3'}$); 5.13-5.02 (m, 2H, $H_{2'}$, $H_{4'}$); 4.94 (app d, 1H, J=8.1 Hz, $H_{1'}$); 4.83 (q, 1H, J=6.6 Hz, H_9); 4.21 (dd, 1H, J=12.3, 2.0 Hz, $H_{6a'}$); 4.07 (dd, 1H, J=12.3, 7.7 Hz, $H_{6b'}$); 3.71 (m, 1H, $H_{5'}$); 2.12 (br d, 2H, J=2.9 Hz, H_2); 1.93 (br s, 3H, H_{13}); 1.45 (d, 3H, J=6.6 Hz, H_{10}); 1.21, 1.15, 1.14, 1.10, (4 x s, 36H, OPiv's); 1.06, 1.05 (s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 178.1, 177.2, 176.6, 176.5 (C=O); 137.2, 127.6, 126.9, 122.7 ($C_{3,4,5,6}$); 97.6($C_{1'}$); 94.4, 84.8 ($C_{7,8}$); 72.3, 72.3, 70.9, 68.2, 63.8, 62.1 ($C_{2',3',4',5',6',9}$); 38.8, 38.7, 38.7, 38.7 (quat. tBu); 38.1 (C_2); 32.7 (C_1); 27.1, 27.1, 27.1, 27.0, 27.0, 27.0 (Pivs, $C_{11,12}$); 22.3 (C_{13}); 20.6 (C_{10}).

(9*R*) 9-O-(β-D-glucopyranosyl)-megastigma-3,5-dien-7-yne (43)



To a solution of (9*R*)-**67** (0.02 g, 0.36 mmol) in methanol (2 mL) was added sodium methoxide (4 eq.) in methanol (2 mL). The mixture was stirred at room temperature overnight (monitored by TLC) and purified using column chromatography (Merck

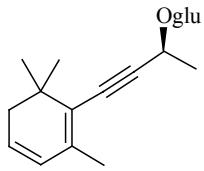
silica 60 HF_{254}) (10% MeOH in dichloromethane.) to yield pure material (9*R*)-**43** (9.7 mg, 75%)

^{1}H NMR: (δ ppm, acetone-d_6): 5.93-5.82 (m, 2H, $H_{3,4}$); 4.88 (q, 1H, J=6.6 Hz, H_9); 4.54 (d, 1H, J=7.8, $H_{1'}$); 3.87-3.78 (m, 1H, $H_{6'}$); 3.72-3.62 (m, 1H, $H_{6'}$); 3.47-3.18 (multiple overlapping signals, 4H, $H_{2',3',4',5'}$); 2.12 (dd, 2H, H_2); 1.93 (s, 3H, H_{13}); 1.50 (d, 3H, J=6.6 Hz, H_{10}); 1.07 (s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, acetone-d_6): 137.72, 129.16, 127.97, 124.95 ($C_{3,4,5,6}$); 103.06 ($C_{1'}$); 98.96, 84.35 ($C_{7,8}$); 78.85, 78.29, 75.80, 72.46, 67.03, 63.79 ($C_{2',3',4',5',6',9}$); 39.55 (C_2); 34.20 (C_1); 28.16, 28.10 ($C_{11,12}$); 23.69 (C_{13}); 21.44 (C_{10}).

$[\alpha]_D$ +41.1 (c. 0.16, MeOH).

(9*S*) 9-O-(β-D-glucopyranosyl)-megastigma-3,5-dien-7-yne (43)



To a solution of (9*S*)-**67** (0.04 g, 0.59 mmol) in methanol (2 mL) was added sodium methoxide (4 eq.) in methanol (2 mL). The mixture was stirred at room temperature over night before being concentrated *in vacuo*. Column chromatography as described for the (9*R*) compound gave pure compound (9*S*)-**43** (0.15 g, 67%).

^{1}H NMR: (δ ppm, acetone-d_6): 5.96-5.85 (m, 2H, $H_{3,4}$); 4.99 (q, 1H, J=6.6 Hz, H_9); 4.74 (d, 1H, J=7.8 Hz, $H_{1'}$); 3.96-3.84 (m, 1H, $H_{6'}$); 3.74-3.64 (m, 1H, $H_{6'}$); 3.48-3.20 (multiple overlapping signals, 4H, $H_{2',3',4',5'}$); 2.14 (m, 2H, H_2); 1.95 (s, 3H, H_{13}); 1.48 (d, 3H, J=6.6 Hz, H_{10}); 1.10 (s, 6H, $H_{12,13}$).

^{13}C NMR: (δ ppm, acetone-d_6): 137.83, 129.15, 128.06, 124.85 ($C_{3,4,5,6}$); 101.63 ($C_{1'}$); 97.81, 85.14 ($C_{7,8}$); 78.98, 78.57, 75.51, 72.53, 64.55, 63.80 ($C_{2',3',4',5',6',9}$); 39.52 (C_2); 34.16 (C_1); 28.16, 28.11 ($C_{11,12}$); 23.49 (C_{13}); 21.48 (C_{10}).

$[\alpha]_D$ –103.9 (c. 0.14, MeOH).

General hydrolysis procedure for 9-Hydroxymegastigma-3,5-dien-7-yne (36) and (Megastigma-3,5-diene-7-yn-9-yl)-O-β-D-Glucopyranose (43)

Solutions containing one of **36**, (9*R*)-**43** or (9*S*)-**43** (1 mg/L and 1.7 mg/L respectively) in model wine (buffered 10% aqueous ethanol) at pH 3.0 or 3.2 were sealed in ampoules in triplicate and heated at 25 °C in a water bath. Ampoules were removed periodically, opened and to 1mL was added internal standard (d_4-damascenone[47]), the solution was extracted with a 2:1 mix pentane/ ethyl acetate and subjected to GC-MS analysis (ZB-Wax, 30 m column) to determine the formation rates of β-damascenone (see below).

The quantified amounts of damascenone are collected in Table 6.1, on the following page.

Table 6.1: Levels of damascenone measured during hydrolytic study. These data correspond to the graphs found in Chapter 3. All values are averages of two measurements, with the variation between individual replicates <5%.

Aglycone (**36**)			
pH 3.0, 25 ºC		**pH 3.2, 25 ºC**	
Time (hrs)	[**1**] (ppb)	Time (hrs)	[**1**] (ppb)
0	5.8	0	5.8
4	17.9	4	10.4
8	31.7	12	31.7
12	64.3	24	78.7
24	143	36	145.3
36	281.7	48	226.7
48	380.4	72	344.7
72	468.2	96	445.0
96	557.2	120	525.7
120	603.5	144	563.0
305	628.3	257	571.1

(9*S*) Glycoside (**43**)			
pH 3.0, 25 ºC		**pH 3.2, 25 ºC**	
Time (days)	[**1**] (ppb)	Time (days)	[**1**] (ppb)
0	0.8	0	0
1	53.1	1	23.2
2	144.0	2	57.6
4	309.9	4	175.8
6	375.5	6	244.9
8	439.2	8	314.0
10	466.4	12	421.9
12	473.7	20	481.9
20	484.3	30	497.9
30	471.9	40	499.0

(9*R*) Glycoside (**43**)			
pH 3.0, 25 ºC		**pH 3.2, 25 ºC**	
Time (days)	[**1**] (ppb)	Time (days)	[**1**] (ppb)
0	54.0	0	31.0
1	135.5	1	68.5
2	250.5	2	121.0
4	441.5	4	252.5
6	557.5	6	377.0
9	611.5	8	433.5
12	633.0	12	548.0
20	628.5	20	610.5
30	622.0	35	617.5

Preparation of samples for analysis of damascenone:

An aliquot (100 μL) of a solution of $^{2}H_4$-damascenone in ethanol (5 μg/mL) was added to the hydrolysate samples (5 mL or 10 mL) in a 15 mL glass screw cap vial with an aluminium-lined cap (Supelco) using a glass syringe (100 μL, SGE). Pentane:ethyl acetate (2:1, 3 mL) was added and the mixture was shaken briefly. A portion of the organic layer was then transferred to a vial for GC-MS analysis. For calculating the concentration of the analytes in the wine samples, replicate standards were prepared at the same time as the wine samples, by adding the internal standard solution (100 μL, 5 μg/mL) and a solution of damascenone in ethanol (100 μL, 5 μg/mL) to dichloromethane (1.8 mL), and analysing this mixture by the GC-MS method (see below) to calculate the relative response factors.

GC-MS analysis:

Samples were analysed with a Hewlett-Packard (HP) 6890N gas chromatograph fitted with a Gerstel MPS2 autosampler and coupled to an HP 5973N mass spectrometer. The liquid injector was operated in fast liquid injection mode with a 10 μL syringe (SGE, Australia) fitted. The gas chromatograph was fitted with an approx. 30 m x 0.25 mm I.D. J &W fused silica capillary column DB-WAX, 0.25 μm film thickness. The carrier gas was helium (BOC gases, Ultra High Purity), and the flow rate was 1.2 mL/min. The oven temperature, started at 50°C, was held at this temperature for 1 minute, then increased to 220°C at 10°C/min, and held at this temperature for 10 minutes. The injector was held at 200°C and the transfer line at 250°C. The sample volume injected was 2 μL and the splitter, at 42:1, was opened after 36 sec. Fast injection was done in pulsed splitless mode with an inlet pressure of 25.0 psi maintained until splitting. The glass liner (Agilent Technologies) was

borosilicate glass with a plug of resilanised glass wool (2-4 mm) at the tapered end to the column. Positive ion electron impact spectra at 70 eV were recorded in the range *m/z* 35-350 for scan runs. For quantification of damascenone, mass spectra were recorded in the Selective Ion Monitoring (SIM) mode. The ions monitored in SIM runs were: *m/z 69, 175 and* <u>*190*</u> for damascenone, and *73,193 and* <u>*194*</u> for [$^{2}H_{4}$]-damascenone. Selected fragment ions were monitored for 20 ms each. The underlined ion for each compound was the ion typically used for quantitation, having the best signal to noise and the least interference from other components. The other ions were used as qualifiers.

Validation:

The method was validated by a series of duplicate standard additions of unlabelled damascenone (1.0 to 200 μg/L, n = 9 x 2 for compound) to a dry white wine (Australian Chenin Blanc, 11.5 % alc/vol, pH =3.40). The standard addition curve obtained was linear throughout the concentration range, with excellent coefficients of determination (r^2) > 0.996. To ensure that the accuracy of the analysis was maintained, duplicate control model wines, each with and without spiked standard addition of 100 μg of damascenone per litre of wine, were analysed with every set of samples.

6.2.3 Material relating to Chapter 4

All reactions conducted using optically pure reagents were first optimized using both racemic phorenol (**74**) and racemic but-3-yn-2-ol (**63**). The products of these reactions were necessarily mixtures of diastereomers, but gave entirely satisfactory spectroscopic and microanalytical data:

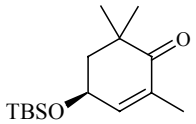
Diastereomeric mixture of silyl protected acetylene **72:**

Anal: Calcd. for $C_{19}H_{34}O_3Si$: C, 67.4; H 10.1. Found :C, 67.2; H 10.0.

Diastereomeric mixture of silyl protected allene **76:**

Anal: Calcd. for $C_{19}H_{34}O_2Si$: C, 70.7; H 10.6. Found : C, 70.7; H 10.7.

(4*S*) 4-*t*-Butyldimethylsilyloxy-2,6,6-trimethylcyclohex-2-enone (*S*)-75

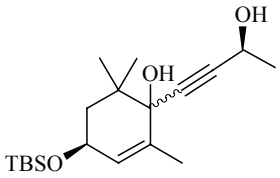


To a solution of (*S*)-phorenol (**74**) (1.0 g, 6.40 mmol) in pyridine (10 mL) was added *t*-butyldimethylsilyl chloride (1.47 g, 9.60 mmol). The mixture was stirred at room temperature overnight, quenched with water and extracted with ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4) before being concentrated *in vacuo* to yield (*S*)-**75** (1.60 g, 92%), which was used without further purification.

^{1}H NMR: (δ ppm, $CDCl_3$): 6.49 (m, 1H, H_3); 4.56 (m, 1H, H_4); 1.99 (ddd, 1H, J=13.0, 5.5, 1.9 Hz, H_{5a}); 1.87 (dd, 1H, J =13.0, 9.6 Hz, H_{5b}); 1.78-1.76 (m, 3H, H_9); 1.14, 1.11 (2 x s, 6H, $H_{7,8}$); 0.92 (s, 9H, t-Bu); 0.13, 0.11 (2 x s, 6H, SiMe).

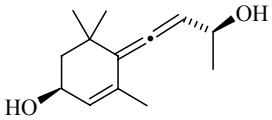
$[\alpha]_D$ –49.0 (c. 0.40, $CHCl_3$); lit.[108] -57 (c 0.44, $CHCl_3$).

(3S,9S) 3-*t*-Butyldimethylsilyloxy-6,9-dihydroxymegastigma-4,6,7,-triene (3S,9S)-72

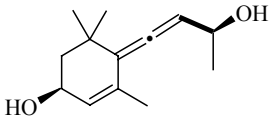


(*S*)-But-3-yn-2-ol (**63**) (0.31 g, 4.50 mmol) in ether (200 mL) was treated with n-BuLi (2.5M, 3.5 mL, 8.6 mmol) at 0 ºC and stirred at room temperature for 2 h. Protected (*S*)-phorenol (**75**) (0.40 g, 1.6 mmol) was then added and the mixture stirred at room temperature for 48 h before being quenched with saturated NH_4Cl solution. The residue was extracted with diethyl ether, washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude material was purified by column chromatography (30% ethyl acetate/hexanes) to yield (3*S*,9*S*)-**72** as a colourless oil (0.35 g, 69%). NMR analysis revealed the product to be a mixture of two diastereomers.

(3*S*,9*S*) 3,9-Dihydroxymegastigma-4,6,7-triene (3*S*,9*S*)-35



(3*S*,6*S*,9*S*)-**35**
SS-1 isomer



(3*S*,6*R*,9*S*)-**35**
SS-2 isomer

(3*S*,9*S*)-**72** (0.25 g, 0.73 mmol) in ether (10 mL) was treated with $LiAlH_4$ (0.14 g, 3.7 mmol) and the mixture stirred at reflux for 4 h. The reaction was quenched by addition of a solution of saturated sodium sulfate, and the product extracted with ethyl acetate. The organic extracts were then washed with 10% NaOH solution and brine, before being dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (10% ethyl acetate/hexane) to yield (3*S*,9*S*)-**76** as a pair of diastereomers (85 mg, 36%). Attempted chromatography on the mixture of **76** was unsuccessful, and the product was subsequently deprotected as the

mixture. The product was treated with t-butyl ammonium fluoride (t-BAF) (0.53 mL of 1.0M solution in THF, 0.53 mmol) in dichloromethane (10 mL) overnight at room temperature, before being quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The extract was washed with brine, dried and concentrated *in vacuo* to yield 80 mg of crude (3*S*,9*S*)-**35**. Purification by chromatography (40% ethyl acetate, hexane) yielded: pure **SS-1** isomer (12 mg, 23%), followed by a mixed fraction (27 mg, 50%), followed by a fraction containing pure **SS-2** isomer (8 mg, 14%).

(**SS-1** isomer) (3*S*,6*S*,9*S*)-**35**

^{1}H NMR: (δ ppm, $CDCl_3$): 5.66 (br d, 1H, J =5.3 Hz, H_8); 5.60 (m, 1H, H_4); 4.42-4.28 (m, 2H, $H_{3,9}$); 1.91 (ddd, 1H, J =12.5, 5.8, 1.1 Hz, H_{2a}); 1.74 (app. t, 3H, J ~1.6 Hz, H_{13}); 1.60 (br s, 2H, OH); 1.43 (dd, 1H, J =12.5, 9.8 Hz, H_{2b}); 1.31 (d, 3H, J =6.3 Hz, H_{10}); 1.12, 1.07 (2 x s, 6H, $H_{11,12}$)

^{13}C NMR: (δ ppm, $CDCl_3$): 199.9, 130.3, 128.0, 116.3, 101.6, 66.2, 65.9, 45.9, 33.8, 29.7, 28.3, 23.5, 20.9

MS *m/z*(%): 208(4), 193(5), 190(22), 175(43), 157(16), 149(35), 146(24), 131(100), 121(26), 115(35), 109(32), 105(30), 91(53), 77(26), 69(24).

$[\alpha]_D$ –22.6 (c. 0.02, $CHCl_3$).

(**SS-2** isomer) (3*S*,6*R*,9*S*)-**35**

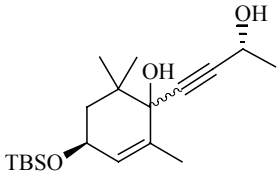
^{1}H NMR: (δ ppm, $CDCl_3$): 5.61 (m, 1H, H_4); 5.58 (br d, 1H, J =6.0 Hz, H_8); 4.42-4.28 (m, 2H, $H_{3,9}$); 1.92 (ddd, 1H, J =12.3, 5.8, 1.1 Hz, H_{2a}); 1.75 (app. t, 3H, J ~1.6 Hz, H_{13}); 1.60 (br s, 2H, OH); 1.45 (dd, 1H, J =12.3, 9.6 Hz, H_{2b}); 1.31 (d, 3H, J =6.3 Hz, H_{10}); 1.12, 1.05 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 200.1, 130.1, 127.9, 116.1, 101.4, 66.4, 65.9, 46.1, 33.9, 30.1, 27.7, 23.5, 20.8.

MS *m/z*(%): 208(3), 193(4), 190(11), 175(20), 157(12), 149(20), 146(28), 131(100), 121(16), 115(29), 109(28), 105(19), 91(44), 77(21), 69(16).

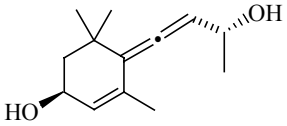
$[\alpha]_D$ +45.0 (c. 0.02, $CHCl_3$).

(3S,9R) 3-*t*-Butyldimethylsiloxy-9-hydroxymegastigma-4,6,7,-triene (3S,9R)-72

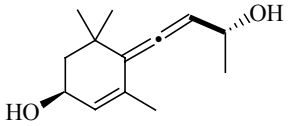


The above reaction was repeated using (*R*)-but-3-yn-2-ol (0.31 g, 4.5 mmol) and (*S*)-Phorenol (**74**) (0.40 g, 1.6 mmol). Identical work-up gave (3*S*,9*R*)-**72** as a colourless oil (0.35 g, 69%), again as a pair of diastereomers.

(3*S*,9*R*) 3,9-Dihydroxymegastigma-4,6,7-triene (3*S*,9*R*)-35



(3*S*,6*S*,9*R*)-**35**
SR-1 isomer



(3*S*,6*R*,9*R*)-**35**
SR-2 isomer

(3*S*,9*R*)-**72** (0.25 g, 0.73 mmol) was treated with $LiAlH_4$ (0.14 g, 3.7 mmol) in ether (10 mL) as described above. Work-up gave, after chromatography (3*S*,9*R*)-**76** (64 mg, 23%) as a colourless oil. Deprotection with *t*-BAF (0.40 mL of 1.0M solution in THF, 0.40 mmol) provided 74 mg of crude (3*S*,9*R*)-**35**, which was chromatographed as before to give, in order of elution, pure **SR-1** isomer (16 mg, 40%), a mixed fraction (19 mg, 47%), and pure **SR-2** isomer (4 mg, 10%).

(**SR-1** isomer) (3*S*,6*S*,9*R*)-**35**

^{1}H NMR: (δ ppm, $CDCl_3$): 5.66 (br d, 1H, J =5.4 Hz, H_8); 5.60 (m, 1H, H_4); 4.41-4.29 (m, 2H, $H_{3,9}$); 1.91 (ddd, 1H, J =12.5, 5.8, 1.1 Hz, H_{2a}); 1.75 (app. t, 3H, J ~1.6

Hz, H_{13}); 1.62 (br s, 2H, OH); 1.42 (dd, 1H, J =12.5, 9.6 Hz, H_{2b}); 1.31 (d, 3H, J =6.3 Hz, H_{10}); 1.11, 1.07 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 199.7, 130.1, 128.0, 116.0, 101.5, 66.2, 65.8, 45.8, 33.7, 29.6, 28.2, 23.5, 20.9

MS *m/z*(%): 208(5), 193(10), 190(23), 175(45), 157(15), 149(29), 146(30), 131(100), 121(30), 115(39), 109(26), 105(27), 91(45), 77(28), 69(22).

$[\alpha]_D$ +12.8 (c. 0.25, $CHCl_3$).

(**SR-2** isomer) (3*S*,6*R*,9*R*)-**35**

^{1}H NMR: (δ ppm, $CDCl_3$): 5.62-5.55 (2H, m, $H_{4,8}$); 4.41-4.30 (m, 2H, $H_{3,9}$); 1.92 (ddd, 1H, J =12.3, 5.7, 1.1 Hz, H_{2a}); 1.74 (dd, 3H, J =1.6, 1.3 Hz, H_{13}); 1.61 (br s, 2H, OH); 1.45 (dd, 1H, J =12.3, 9.6 Hz, H_{2b}); 1.31 (d, 3H, J =6.4 Hz, H_{10}); 1.13, 1.05 (2 x s, 6H, $H_{11,12}$).

^{13}C NMR: (δ ppm, $CDCl_3$): 199.9, 130.2, 128.0, 116.2, 101.5, 66.4, 65.9, 46.1, 33.9, 30.0, 27.6, 23.5, 20.8.

MS *m/z*(%): 208(4), 193(3), 190(15), 175(30), 157(9), 149(25), 146(34), 131(100), 121(20), 115(37), 109(22), 105(23), 91(48), 77(27), 69(20).

$[\alpha]_D$ +35.2 (c. 0.06, $CHCl_3$).

6.2.4 Material relating to Chapter 5

General Hydrolysis Procedure

Solutions of **35** (1 mg/L) in buffered 10% aqueous ethanol at pH 3.0 were sealed in ampoules and heated at 25 ºC in a water bath for 24 h. After this time, ampoules were removed, opened and the contents extracted with ether and subjected to GC-MS analysis as described in section 6.2.2, on either an achiral DB-1701 column, or a chiral Cyclosil B column, using the conditions reported by Wilkinson et al.[107]

Hydrolysis of 35 (SS-2 isomer)

A solution of (3*S*,6*R*,9*S*)-**35** (1 mg/L, 10 mL aliquots) was prepared, heated and extracted as described above. The product mixture was shown to contain **1**, **36**, hydrate **57**, ethanol adduct **58**, unreacted **SS-2** isomer, a second isomer of **35** which proved indistinguishable from the synthetic **SR-1** isomer on achiral GC, and two isomers of a compound whose mass spectra were in accord with the assigned structure **81**.

(3*S/R*,6*R*,9*S*) 3-Ethoxy-9-hydroxymegastigma-4,6,7-triene (**81**)

isomer 1 (RT 15.88 min.) ***m/z*(%):** 236(24), 221(100), 203(10), 191(21), 177(50), 149(26), 147(31), 146(30), 137(94), 131(77), 121(35), 109(63), 105(41), 91(45), 77(28), 69(21).

isomer 2 (RT 15.97 min.) ***m/z*(%):** 236(23), 221(100), 203(10), 191(19), 177(49), 149(26), 147(28), 146(23), 137(91), 131(57), 121(34), 109(60), 105(38), 91(41), 77(25), 69(21).

Compounds **1**,[73] **36**, **57**[101,115] and **58**[101,115] were identified by comparison of retention times and mass spectra with authentic samples.

Hydrolysis of 35 (SS-1 isomer)

A solution of (3*S*,6*S*,9*S*)-**35** (1 mg/L, 10 mL aliquots) was prepared, heated and extracted as described above. The product mixture was shown to contain **1**, **36**, hydrate **57**, ethanol adduct **58**, unreacted **SS-1** isomer, a second isomer of **35** which proved indistinguishable from the synthetic **SR-2** isomer on achiral GC, and two isomers of a compound whose mass spectra were in accord with the assigned structure **81**.

(3*S/R*,6*S*,9*S*) 3-Ethoxy-9-hydroxymegastigma-4,6,7-triene (**81**)

isomer 1 (RT 15.86 min.) ***m/z*(%):** 236(24), 221(100), 203(10), 191(20), 177(51), 163(25), 149(27), 147(30), 146(30), 137(95), 131(77), 121(36), 109(63), 105(41), 91(46), 77(27), 69(22).

isomer 2 (RT 15.95 min.) ***m/z*(%):** 236(19), 221(100), 203(10), 191(20), 177(49), 163(25), 149(26), 147(29), 146(23), 137(90), 131(58), 121(34), 109(61), 105(38), 91(41), 77(25), 69(20).

Hydrolysis of 35 (SR-2 isomer)

A solution of (3*S*,6*R*,9*R*)-**35** (1 mg/L, 10 mL aliquots) was prepared, heated and extracted as described above. The product mixture was shown to contain **1**, **36**, hydrate **57**, ethanol adduct **58**, unreacted **SR-2** isomer, a second isomer of **35** which proved indistinguishable from the synthetic **SS-1** isomer on achiral GC, and two isomers of a compound whose mass spectra were in accord with the assigned structure **81**.

(3*S/R*,6*R*,9*R*) 3-Ethoxy-9-hydroxymegastigma-4,6,7-triene (**81**)

isomer 1 (RT 15.85 min.) ***m/z*(%):** 236(24), 221(100), 203(10), 191(21), 177(50), 149(26), 147(31), 146(30), 137(94), 131(77), 121(35), 109(63), 105(41), 91(45), 77(28), 69(21).

isomer 2 (RT 15.94 min.) ***m/z*(%):** 236(23), 221(100), 203(10), 191(19), 177(49), 149(26), 147(28), 146(23), 137(91), 131(57), 121(34), 109(60), 105(38), 91(41), 77(25), 69(21).

Hydrolysis of 35 (SR-1 isomer)

A solution of (3*S*,6*S*,9*R*)-**35** (1 mg/L, 10 mL aliquots) was prepared, heated and extracted as described above. The product mixture was shown to contain **1**, **36**, hydrate **57**, ethanol adduct **58**, unreacted **SR-1** isomer, a second isomer of **35** which proved indistinguishable from the synthetic **SS-2** isomer on achiral GC, and two isomers of a compound whose mass spectra were in accord with the assigned structure **81**.

(3*S/R*,6*S*,9*R*) 3-Ethoxy-9-hydroxymegastigma-4,6,7-triene (**81**)

isomer 1 (RT 15.89 min.) ***m/z*(%):** 236(22), 221(95), 203(10), 191(20), 177(50), 163(19), 149(28), 147(32), 146(36), 137(100), 131(88), 121(39), 109(75), 105(46), 91(50), 77(32), 69(25);

isomer 2 (RT 15.97 min.) ***m/z*(%):** 236(22), 221(96), 203(10), 191(19), 177(49), 163(24), 149(27), 147(30), 146(25), 137(100), 131(64), 121(39), 109(69), 105(45), 91(46), 77(27), 69(24).

CHAPTER SEVEN

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