

# The role of acetylenic and allenic precursors in the formation of $\beta$ -damascenone

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

By

**Carolyn Jane Puglisi**  
B. Sc (Hons.)

School of Chemistry, Physics and Earth Sciences,  
Flinders University, Adelaide, S.A.



*December 2007*



## **Dedication**

This Thesis is dedicated to my late husband and best friend Christopher 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

<b>Table of contents</b>	i
<b>Abstract</b>	iv
<b>Declaration</b>	vi
<b>Acknowledgements</b>	vii
<b>Publications</b>	ix
<b>Abbreviations</b>	x
<b>List of figures and tables</b>	xi
<b>CHAPTER 1: Introduction</b>	<b>1</b>
<b>1.1 A Short History of the Australian Wine Industry</b>	<b>2</b>
1.1.1 Australia and wine exports	4
1.1.2 Wine regions of Australia	5
1.1.3 Grape varieties	6
<b>1.2 Flavour and Aroma</b>	<b>8</b>
1.2.1 Different classes of aroma compounds	9
<b>1.3 The Rose Ketones</b>	<b>11</b>
<b>1.4 <math>\beta</math>-Damascenone</b>	<b>13</b>
1.4.1 Previous syntheses of $\beta$ -damascenone	14
1.4.1.1 Isoe et al. (1973)	14
1.4.1.2 Ohloff et al. (1973)	14
1.4.1.3 Kitahara et al. (1979)	15
1.4.1.4 Torii et al. (1979)	15
1.4.2 <i>In vivo</i> generation of $\beta$ -damascenone	17
1.4.2.1 Early work – Isoe and Ohloff	18
1.4.2.2 More recently – Skouroumounis et al.	21
<b>1.5 Glycosidic Precursors</b>	<b>24</b>
1.5.1 The role of the sugar in nature	24
1.5.2 The formation of flavour from glycoconjugated volatiles	25
1.5.3 The effect of the sugar on chemical reactivity	27
<b>1.6 Aims</b>	<b>29</b>

**RESULTS AND DISCUSSION**

<b>CHAPTER 2: Synthesis of 9-Hydroxymegastigma-3,5-dien-7-yne (36)</b>	<b>31</b>
2.1 Background on 9-Hydroxymegastigma-3,5-dien-7-yne (36)	32
2.2 Synthesis of 6,9-Dihydroxymegastigm-4-en-7-yne (8)	32
2.3 Attempted Dehydration of 8	34
2.3.1 ( <i>E</i> )-1-(2,3,6-Trimethylphenyl)buta-1,3-diene (TPB, 49)	34
2.4 Attempted Dehydration of 50	37
2.5 Successful Dehydration of 50	41
2.6 Authentication of 9-Hydroxymegastigma-3,5-dien-7-yne (36)	43
2.7 Products of Hydrolysis of 36	44
<b>CHAPTER 3: Synthesis and Hydrolysis of Glycoside 43</b>	<b>46</b>
3.1 Background on Glycosylation Reactions	47
3.2 Synthesis of Model Glycoside 65	48
3.3 Synthesis of Glycoside 43	50
3.4 Synthesis of Isomerically pure Glycosides 43	51
3.5 Hydrolytic Studies	53
3.5.1 Hydrolysis of aglycone 36	53
3.5.2 Hydrolysis of glycosides (9 <i>S</i> )-43 and (9 <i>R</i> )-43	56
<b>CHAPTER 4: Synthesis of 3,9-Dihydroxymegastigma-4,6,7-triene (35)</b>	<b>60</b>
4.1 General Strategy for the Synthesis of Allenic Diol 35	61
4.2 Synthesis of Optically Pure Diols 35	63
<b>CHAPTER 5: Hydrolysis of 3,9-Dihydroxymegastigma-4,6,7-triene (35)</b>	<b>69</b>
5.1 Hydrolysis of Diol 35	70
5.2 C <sub>3</sub> or C <sub>9</sub> Epimerisation?	74
5.3 Implications for the Mechanism of Formation of $\beta$ -Damascenone	79
5.4 Conclusions	83
5.4.1 Hydrolysis of the allene triol 31	83
5.4.2 The effect of glycosylation on the formation of damascenone	85

<b>CHAPTER 6: Experimental</b>	<b>89</b>
<b>6.1</b> General Experimental	90
<b>6.2</b> Procedures	93
6.2.1  Material relating to Chapter 2	93
6.2.2  Material relating to Chapter 3	108
6.2.3  Material relating to Chapter 4	123
6.2.4  Material relating to Chapter 5	128
<b>CHAPTER 7: References</b>	<b>131</b>

## ABSTRACT

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

**Chapter 3** outlines the synthesis and hydrolysis of the C<sub>9</sub> 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 °C) 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 (*9R*) 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 °C and pH 3.0. Careful analysis of the four product mixtures by chiral GC-MS revealed that this epimerisation was occurring exclusively at C<sub>3</sub>. 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

## ACKNOWLEDGEMENTS

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.

I thank all of the above for offering me such a fascinating and stimulating project.

I thank the staff and students at the Australian Wine Research Institute, especially Dimi Capone and Heather Smyth for their support and friendship.

I thank the AWRI and FUSA for hosting my project and the CRCV for funding my project. I thank Dr K Puntener of Hoffman-La Roche International for generously supplying me with a sample of *S*-Phorenol for use in my synthetic studies.

I thank my friends, Drs. Saba Jahangiri, Neil Trout, Kerry Wilkinson and Sean Graney, as well as my fellow PhD students in the organic corridor, for allowing me to grow not only as a scientist but as a person.

To my late husband Christopher Puglisi who, at times endured me and who encouraged and supported me during challenging times.

To my mother, Terry Muscat and mother in-law, Dorothy Puglisi for continued encouragement and support. To my father in-law, Joseph Puglisi for his encouragement, interest and curiosity in relation to my research.

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  $\beta$ -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  $\beta$ -Damascenone in Grapes and Wine:, *11th AWITC*, Adelaide, **2001**. 11<sup>th</sup> 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:, 19<sup>th</sup> 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	( <i>E</i> )-1-(2,3,6-trimethylphenyl)buta-1,3-diene

## LIST OF FIGURES AND TABLES

### Figures

<b>Figure 1.1</b>	Some common monoterpenes found in grapes and wine.	10
<b>Figure 1.2</b>	Some simple shikimate derived aroma volatiles found in wine.	10
<b>Figure 1.3</b>	Some common C <sub>13</sub> -norisoprenoids found in wine.	11
<b>Figure 1.4</b>	The Rose ketones.	12
<b>Figure 1.5</b>	Basic megastigmane skeleton, and numbering scheme.	12
<b>Figure 1.6</b>	Compounds utilised in the synthesis of <b>1</b> by Torii et al.	16
<b>Figure 1.7</b>	<i>Romalea Microptera</i> , from which was isolated grasshopper ketone.	19
<b>Figure 1.8</b>	Tentatively identified intermediates ( <b>35</b> and <b>36</b> ) in the hydrolysis of <b>31</b> .	22
<b>Figure 1.9</b>	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 <b>R</b> substituent.	26
<b>Figure 1.10</b>	Different modes of cleavage of glycosides by either enzyme or acid.	26
<b>Figure 1.11</b>	Glycosylated target compounds <b>43</b> - <b>45</b> .	29
<b>Figure 1.12</b>	Predicted conversions of allene diol <b>35</b> and its two glucosides. C <sub>3</sub> glycosylation would be expected to direct reaction towards <b>2</b> , whereas C <sub>9</sub> glycosylation would direct reaction towards <b>1</b> .	30
<b>Figure 2.1</b>	TPB, and its main precursor compounds in wine.	34
<b>Figure 2.2</b>	Mass spectra obtained for compound assigned as <b>36</b> from the original hydrolysate of <b>31</b> , and the sample synthesised in this study.	43
<b>Figure 3.1</b>	Neighbouring group assistance in the mechanism of glycosylation using <b>61</b> .	48
<b>Figure 3.2</b>	Oximate orthoester <b>62</b> , used as an alternative to <b>61</b> .	48
<b>Figure 3.3</b>	Model compounds chosen for synthesis.	49
<b>Figure 3.4</b>	Possible intermediates involved in the hydrolysis of <b>36</b> .	54
<b>Figure 3.5</b>	Formation of damascenone from aglycone <b>36</b> at pH 3.0, 25 °C.	55
<b>Figure 3.6</b>	Formation of damascenone from aglycone <b>36</b> at pH 3.2, 25 °C.	55
<b>Figure 3.7</b>	Formation of damascenone from (9 <i>S</i> )- <b>43</b> at pH 3.0, 25 °C.	57
<b>Figure 3.8</b>	Formation of damascenone from (9 <i>S</i> )- <b>43</b> at pH 3.2, 25 °C.	57
<b>Figure 3.9</b>	Formation of damascenone from (9 <i>R</i> )- <b>43</b> at pH 3.0, 25 °C.	58
<b>Figure 3.10</b>	Formation of damascenone from (9 <i>R</i> )- <b>43</b> at pH 3.2, 25 °C.	58

<b>Figure 4.1</b>	Compounds initially trialled in the synthesis of <b>35</b> .	61
<b>Figure 4.2</b>	Absolute stereochemistries of grasshopper ketone ( <b>30</b> ) and the corresponding allenic triol <b>31</b> .	63
<b>Figure 5.1</b>	Proposed divergence in the hydrolysis of <b>35</b> .	71
<b>Figure 5.2</b>	GC traces of the hydrolyses of each of the four isomers of <b>35</b> synthesised. From top to bottom they correspond to the <b>SR-1</b> , <b>SR-2</b> , <b>SS-1</b> , and <b>SS-2</b> isomers.	72
<b>Figure 5.3</b>	Products of interrupted hydrolysis of allene diol <b>35</b> at pH 3.0, 25 °C. The stereochemistry shown corresponds to the <b>SS-2</b> isomer.	73
<b>Figure 5.4</b>	Excerpt from the GC trace of the hydrolysis of the <b>SS-2</b> isomer after 24 hours. Peaks indicated are: 16.55 min. ( <b>SS-2</b> isomer); 16.61 min. (epimer of <b>SS-2</b> 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.	73
<b>Figure 5.5</b>	Stereochemical relationship between the <b>SS-2</b> and <b>SR-1</b> isomers, assuming that epimerisation takes place at C <sub>9</sub> .	75
<b>Figure 5.6</b>	Stereochemical relationship between the <b>SS-2</b> and <b>SR-1</b> isomers, assuming that epimerisation takes place at C <sub>3</sub> . The isomers in red are the synthesised starting materials, while those in black (ent.) are the enantiomers of the synthesised compounds.	76
<b>Figure 5.7</b>	Stereochemical relationship between the <b>SS-1</b> and <b>SR-2</b> isomers, assuming that epimerisation takes place at either A: C <sub>9</sub> , or B: C <sub>3</sub> .	76
<b>Figure 5.8</b>	Likely conjugation relationships between the two hydroxyl functions and the various olefins present in <b>35</b> .	79
<b>Figure 5.9</b>	Newly proposed route of <i>in vivo</i> generation of β-damascenone ( <b>1</b> ).	80
<b>Figure 5.10</b>	Proposed mode of formation of <b>2</b> directly from triol <b>31</b> .	81
<b>Figure 5.11</b>	Kinetics determined of the conversion of <b>35</b> and <b>36</b> into <b>1</b> . The value for <i>k</i> <sub>3</sub> is taken from the work of Daniel.	81
<b>Figure 5.12</b>	Proposed mode of formation of all the major hydrolysis products of allene triol <b>31</b> .	83
<b>Figure 5.13</b>	Model hydroxylated allenes <b>85</b> and <b>86</b> studied by Daniel.	84
<b>Figure 5.14</b>	Hydrolysis products of A: model allene <b>85</b> , and B: model allene <b>86</b> , as observed by Daniel.	84
<b>Figure 5.15</b>	Potential outcome of hydrolysis of C <sub>3</sub> glycoside <b>45</b> vs. aglycone <b>35</b> .	87

**Tables**

<b>Table 1.1</b>	Australian wine exports for the twelve months ending Oct 2007.	5
<b>Table 1.2</b>	Wine and grape production in Australia for 2005-2006 vintage.	6
<b>Table 1.3</b>	Common descriptors of wines produced from various grape varieties.	7
<b>Table 1.4</b>	Grape varieties used for production of various wine styles.	8
<b>Table 1.5</b>	Aroma descriptors and sensory thresholds, in air, of some Rose ketones.	13
<b>Table 2.1</b>	Attempted dehydration of <b>8</b> .	36
<b>Table 2.2</b>	Dehydration of <b>50</b> using <i>p</i> -TsOH.	38
<b>Table 2.3</b>	Attempted dehydration of <b>50</b> .	39
<b>Table 2.4</b>	Attempted elimination of HCl from <b>55</b> .	40
<b>Table 2.5</b>	Dehydration of <b>50</b> using P <sub>2</sub> O <sub>5</sub> .	42
<b>Table 3.1</b>	Methods for glycosylation of model compound <b>63</b> .	49
<b>Table 3.2</b>	Half-lives for the conversion of <b>36</b> , ( <i>9S</i> )- <b>43</b> and ( <i>9R</i> )- <b>43</b> into damascenone ( <b>1</b> ).	56
<b>Table 4.1</b>	Stereochemistries expected in allene diol <b>35</b> .	65
<b>Table 4.2</b>	<sup>1</sup> H NMR details, and optical rotations for the four isomers of <b>35</b> .	67
<b>Table 5.1</b>	Epimeric pairs obtained from hydrolyses of each isomer of <b>35</b> .	74
<b>Table 5.2</b>	Retention times of epimers produced during hydrolysis, and the newly assigned stereochemistries of the <b>SR-1</b> and <b>SR-2</b> isomers.	77
<b>Table 5.3</b>	Ratios of half-lives obtained for hydrolyses of <b>36</b> and <b>43</b> .	86
<b>Table 6.1</b>	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%.	119