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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December 2007

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.

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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

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 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

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