SYNTHESIS OF ALLENIC KETONES AND THE ROLE OF YEAST IN THEIR CONVERSION TO DAMASCENONE

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A Thesis Submitted for the

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Dedication

This Thesis is dedicated to my dad, Robert Lloyd who passed away (03/10/1941 – 16/11/2009) and my wonderful mom, Maxine and brother, Ryan who are always there to support me. I am so grateful to have both of you in my life.

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Abstract

The thesis describes the formation of damascenone during fermentation conditions from potential ketone precursors. An introduction to the thesis and aims of the study are described in Chapter 1, the synthesis of the precursors is detailed in Chapter 2, identification of the precursors as natural products and hydrolysis studies are included in Chapter 3, fermentation studies are detailed in Chapters 4 and the damascenone stability studies are in Chapter 5.

Chapter 2 describes the synthesis of megastigma-4,6,7-triene-3,9-dione (**26**) and 3-hydroxymegastigma-4,6,7-trien-9-one (**27**) from the common starting material, 4-oxoisophorone as well as the synthesis of 9-hydroxymegastigma-4,6,7-trien-3-one (**28**) from diketone **26**. The allene 3-*tert*-butyldimethylsilyloxy-9-hydroxymegastigma-4,6,7-triene (**29**) was synthesised first and this was then used to produce ketones **26** and **27**. The synthesis of **26** occurred in two steps from the silylated allene diol **29** which involved deprotection, followed by a Dess-Martin oxidation. The synthesis of ketone **27** was achieved by a Dess-Martin oxidation of the silylated allene diol **29** followed by deprotection using TBAF. The ketone **28** was synthesised by a selective reduction of **26**.

Chapter 3 covers the identification of the ketones **26** and **28** as natural products. It describes the hydrolysis studies performed with ketone **27** in model wine and includes a discussion as to why **27** was not seen in grape juice or honey extracts, in contrast to the analogues **26** and **28**.

The fermentation study involving the synthesised ketones **26**, **27** and **28** is discussed in Chapter 4. The fermentation studies demonstrate that damascenone can be formed by the action of yeast during fermentation, from precursors **26**, **27** and **28**. The results emphasise that the presence of yeast is essential for the conversion. The concentration of damascenone at the end of fermentation was also shown to be dependent on the yeast strain. Yeast strain AWRI 796 showed to be more efficient in the formation of damascenone compared to yeast strain AWRI 1537.

The final section of the thesis (*Chapter 5*) details the stability of damascenone during fermentation. A substantial loss of damascenone was observed during fermentation and the factors involved were further explored. The possible contributing factors included the loss of damascenone through the action of yeast metabolism, loss from reaction with components in the model or real juice and loss via evaporation of damascenone via the ferment flask during fermentation.

Declaration

I, Natoiya Dee Rayette Lloyd certify that this work contains no material which as been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Natoiya Dee Rayette Lloyd

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Publications

Refereed Publications

1. Lloyd, N. D. R.; Capone, D. L.; Ugliano, M.; Taylor, D. K.; Skouroumounis, G. K.; Sefton, M. A.; Elsey, G. M., Formation of damascenone under both commercial and model fermentation conditions. *J. Agric. Food. Chem.* **2011**, 59, (4), 1338-1343.

2. Lloyd, N. D. R.; Capone, D. L.; Ugliano, M.; Taylor, D. K.; Skouroumounis, G. K.; Sefton, M. A.; Elsey, G. M. The role of yeast in the generation of the odorant damascenone in wine. *Proceedings of the 9th Wartburg Symposium on Flavor Chemistry and Biology*, Eisenach, Germany,13 – 16th April, **2010**, pp. 447 – 451.

Symposia

1. Lloyd, N. D. R.; Capone, D. L.; Ugliano, M.; Taylor, D. K.; Sefton, M. A.; Elsey, G. M. The role of yeast in the generation of damascenone in wine. Crush Conference, Adelaide, South Australia, **2011**.

2. Lloyd, N. D. R.; Capone, D. L.; Ugliano, M.; Taylor, D. K.; Sefton, M. A.; Elsey, G. M. The role of yeast in the generation of the odorant damascenone in wine. 14th Australian Wine Industry Technical Conference, Adelaide, South Australia, **2010**.

3. Lloyd, N. D. R.; Capone, D. L.; Ugliano, M.; Taylor, D. K.; Elsey, G. M. The formation of damascenone under fermentation conditions. The 23rd RACI Organic Division Conference, 'Organic 08', Hobart, Tasmania, **2008**.

Abbreviations

CDGJ medium	Chemically defined grape juice medium
CDMW	Chemically defined model wine (made from CDGJ medium)
COSY	Correlation spectroscopy
DCM	Methylene chloride
DMAP	4-Dimethylaminopyridine
DMSO	Dimethylsulfoxide
EIC	Extracted ion chromatogram
EtOAc	Ethyl acetate
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
hr	Hour
Hz	Hertz
KI	Kovats Index
LAH	Lithium Aluminium Hydride
MeOH	Methanol
mM	millimolar
NaOMe	Sodium methoxide
ppb	parts per billion, μg/L
ppm	parts per million, mg/L
ppt	parts per trillion, ng/L
RT	Room temperature
sat. aq.	Saturated aqueous
TBAF	Tetrabutylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TBDMSCl	*tert*-Butyldimethylsilyl chloride
THF	Tetrahydrofuran
TIC	Total ion chromatogram
TLC	Thin layer chromatography
μM	micromolar

List of Figures, Schemes and Tables