γ-Lactones in wine:
Synthesis, quantification and sensory studies

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Doctor of Philosophy

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Abstract

γ-Lactones are found in a wide variety of food and beverage products, in particular grapes and wine. This thesis details the work completed on some γ-lactones in wine: their synthetic preparation, development of quantification methodologies and sensory studies.

Chapter 1 outlines the history of the Australian wine industry from the arrival of the first vines on the First Fleet in 1788 with Captain Arthur Philip. This chapter provides: an overview of Australia's position in the world of grape and wine production; an analysis of the export arm of the industry; and a look at the different wine producing regions around the country. The latter part of the chapter focuses on the different volatile compounds found in wine.

Part A:

Chapter 2 provides an overview on the history of barrel manufacture and the use of oak wood in cooperage, with an emphasis on oak's well known ability to impart desirable characteristics to wine through the extraction of volatile aroma compounds. This chapter provides a summary of these odorants with a particular emphasis on the oak lactones. Previous sensory studies and synthetic work are discussed. Of great importance to this work are the recent advancements in 1,2-dioxine chemistry, highlighted in this chapter.

Chapter 3 details the synthetic work completed for the preparation of all four possible oak lactone stereoisomers. A suitably substituted racemic 1,2-dioxine featured as the common intermediate and enabled preparation of the γ-lactone moiety upon reaction with a chiral malonate diester and separation of the diastereomers by column chromatography. A key step involved the decarboxylation of the ester cleaved γ-lactone diastereomers, which could be directed to give either the *cis*- or *trans*-products. Standard chemical transformations were then utilised to produce the desired stereoisomers of oak lactone.

Chapter 4 describes the results from the sensory studies that were completed on the synthetic oak lactone samples. Odour detection thresholds were measured in both a white and a red wine. The thresholds in the former medium were calculated to be 24 μg/L, 172 μg/L, 132 μg/L and 305 μg/L, while in the latter medium the thresholds were calculated to be 57 μg/L, 380 μg/L, 175 μg/L and 285 μg/L, for (4*S*,5*S*)-*cis*-, (4*S*,5*R*)-*trans*-, (4*R*,5*R*)-*cis*- and (4*R*,5*S*)-*trans*-oak lactone, respectively. Difference testings were completed on the pairs of enantiomers and also on mixtures of the nature-identical isomers: between the *cis*-enantiomers a significant difference was found at the 99% confidence level, while between the *trans*-enantiomers and also the mixtures of *cis*- and *trans*-isomers little difference was observed.

Chapter 5 contains the experimental procedures for ***Part A***.

Part B:

Chapter 6 discusses the sensory properties of some γ- and δ-lactones, with the focus on a series of five-alkyl substituted γ-lactones: γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone. Topics covered in this chapter include chirality, biosynthetic pathways and quantification results in wine from previous studies for these γ-lactones.

Chapter 7 concerns the method development for the quantification of γ-lactones in wine using a stable isotope dilution assay (SIDA). Deuterated analogues were prepared from commercially available racemic γ-lactones for use as internal standards. Initially a head space solid-phase microextraction (HS SPME) method was developed using d_5-standards; however, analysis of bottled wine samples revealed the presence of co-eluting compounds that contained several of the selected ions. Thus an alternative method was developed using d_7-standards, with a specific focus on sample clean-up, *via* solid-phase extraction (SPE). Using this procedure, 44 white and 120 red wines were analysed for their γ-lactone content. The lactones were found to be significantly more common in the red wines, with γ-nonalactone the most abundant lactone in this series.

Chapter 8 deals with the extension of the SIDA method, as developed in Chapter 7, for use with a chiral gas chromatography column. Optically pure standards were prepared, from either L- or D-glutamic acid, and used to determine the order of elution of the enantiomers. A method was developed for the quantification of the individual enantiomers of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone. The enantiomeric distribution of γ-nonalactone was investigated in 34 red wines; the (*R*)-stereoisomer was found to be dominant with an average of 59%, although there were wines analysed that did contain the (*S*)-stereoisomer in greater amounts.

Chapter 9 describes the results from the sensory studies that were completed on the individual enantiomers of the γ-lactones. Odour detection thresholds were measured in a red wine. The thresholds were calculated to be 238 μg/L, 285 μg/L, 34 μg/L and 8 μg/L for the (*R*)-enantiomers, while the thresholds were calculated to be 135 μg/L, 91 μg/L, 47 μg/L and 39 μg/L for the (*S*)-enantiomers, of γ-octalactone, γ-nonalactone, γ-decalactone and γ-dodecalactone, respectively.

Chapter 10 contains the experimental procedures for ***Part B***.

Chapter 11 contains the appendices, followed by the references in **Chapter 12**.

Declaration

'I certify that this thesis does not incorporate without acknowledgment 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.'

Rachel C. Brown
16 November 2007

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

Gordon M. Elsey
16 November 2007

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'*The important thing is not to stop questioning. Curiosity has its own reason for existing*.' Albert Einstein (1879-1955)

Publications

Refereed journal articles:

Brown, R.C.; Capone, D.L.; Sefton, M.A.; Elsey, G.M. Quantification of γ-lactones in grapes and wine: Method development and application, *Analytica Chimica Acta*, *in preparation.*

Brown, R.C.; Sefton, M.A.; Taylor, D.K.; Elsey, G.M. An odour detection threshold determination of all four possible stereoisomers of oak lactone in a white and a red wine, *Australian Journal of Grape and Wine Research*, **2006**, *12*(2), 115-118.

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Elsey, G.M.; **Brown, R.C.**; Pardon, K.H.; Capone, D.L.; Sefton, M.A.; Herderich, M.J.; Pretorius, I.S. Chirality and its effect on wine aroma: Recent experiences in aroma research at the AWRI, *Australia and New Zealand Wine Industry Journal*, *in press*.

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Abbreviations and glossary

°C	degrees Celsius
Δ	heat
Å	angstroms
ACCN	1,1'-azo-*bis*-(cyclohexanecarbonitrile)
AcOH	acetic acid (glacial)
Ac_2O	acetic anhydride
AIBN	2,2'-azo-*bis*-(2-methylpropionitrile)
a.k.a	also known as
Al_2O_3	aluminium oxide
app.	apparent
BET	best estimate threshold
$BH_3.Me_2S$	borane-dimethyl sulfide
bpt	boiling point
brine	saturated aqueous sodium chloride solution
c	concentration
cat.	catalytic
CCl_4	carbon tetrachloride
C_6H_6	benzene
$(CH_3)_2CHBr$	*iso*-propyl bromide
CH_2Cl_2	dichloromethane
cm	centimetres
COSY	correlation spectroscopy
$CuSO_4$	copper sulfate
δ	chemical shift (parts per million)
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCl	deuterium chloride
DIBAL	di-*iso*-butylaluminium hydride
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
D_2O	deuterium oxide

ee	enantiomeric excess
EI	electron impact
Et_3N	triethyl amine
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
g	grams
GC-MS	gas chromatography-mass spectrometry
HCl	hydrochloric acid
HMBC	heteronuclear multiple bond connectivity
HMQC	heteronuclear multiple quantum coherence
HRMS	high resolution mass spectrometry
hrs	hours
HS SPME	head space solid-phase microextraction
Hz	hertz
J	coupling constant (Hz)
KOH	potassium hydroxide
L	litre
LDA	lithium di-*iso*-propylamide
lit.	literature
LOD	limit of detection
m-CPBA	*m*-chloroperbenozic acid
Me	methyl
MeCN	acetonitrile
MeOH	methanol
mg	milligrams
μg	microgram
$MgSO_4$	magnesium sulfate
MHz	megahertz
mins	minutes
mL	millilitre
mmol	millimole
μm	micrometer
mol	mole

mpt	melting point
m/z	mass to charge ratio
$NaBD_4$	sodium borodeuteride
$NaBH_4$	sodium borohydride
NaH	sodium hydride
$NaHCO_3$	sodium hydrogen carbonate
$NaHSO_4$	sodium hydrogen sulfate
$NaIO_4$	sodium periodate
$NaNO_2$	sodium nitrite
Na_2SO_4	sodium sulfate
NH_4Cl	ammonium chloride
nm	nanometre
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
o/n	overnight
Pd-$BaSO_4$	palladium on barium sulfate
Ph	phenyl
ppm	parts per million
pyr	pyridine
R_f	retention factor
rt	room temperature
$RuCl_3$	ruthenium trichloride
sat.	saturated
SIDA	stable isotope dilution assay/analysis
SIM	selected ion monitoring
SPE	solid-phase extraction
SO_2	sulfur dioxide
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	*N*,*N*,*N*',*N*'-*tetra*-methylethylenediamine
TsCl	*p*-toluenesulfonyl chloride
W	watts