

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

*A thesis submitted in fulfilment of the
requirements for the degree of*

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 $\mu\text{g/L}$, 172 $\mu\text{g/L}$, 132 $\mu\text{g/L}$ and 305 $\mu\text{g/L}$, while in the latter medium the thresholds were calculated to be 57 $\mu\text{g/L}$, 380 $\mu\text{g/L}$, 175 $\mu\text{g/L}$ and 285 $\mu\text{g/L}$, 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 $\mu\text{g/L}$, 285 $\mu\text{g/L}$, 34 $\mu\text{g/L}$ and 8 $\mu\text{g/L}$ for the (*R*)-enantiomers, while the thresholds were calculated to be 135 $\mu\text{g/L}$, 91 $\mu\text{g/L}$, 47 $\mu\text{g/L}$ and 39 $\mu\text{g/L}$ 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:

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Abbreviations and glossary

| | |
|--------------------------------------|---|
| °C | degrees Celsius |
| Δ | heat |
| Å | angstroms |
| ACCN | 1,1'-azo- <i>bis</i> -(cyclohexanecarbonitrile) |
| AcOH | acetic acid (glacial) |
| Ac ₂ O | acetic anhydride |
| AIBN | 2,2'-azo- <i>bis</i> -(2-methylpropionitrile) |
| a.k.a | also known as |
| Al ₂ O ₃ | aluminium oxide |
| app. | apparent |
| BET | best estimate threshold |
| BH ₃ .Me ₂ S | borane-dimethyl sulfide |
| bpt | boiling point |
| brine | saturated aqueous sodium chloride solution |
| <i>c</i> | concentration |
| cat. | catalytic |
| CCl ₄ | carbon tetrachloride |
| C ₆ H ₆ | benzene |
| (CH ₃) ₂ CHBr | <i>iso</i> -propyl bromide |
| CH ₂ Cl ₂ | dichloromethane |
| cm | centimetres |
| COSY | correlation spectroscopy |
| CuSO ₄ | copper sulfate |
| δ | chemical shift (parts per million) |
| DCC | <i>N,N'</i> -dicyclohexylcarbodiimide |
| DCl | deuterium chloride |
| DIBAL | di- <i>iso</i> -butylaluminium hydride |
| DMAP | 4-(<i>N,N</i> -dimethylamino)pyridine |
| DME | 1,2-dimethoxyethane |
| DMF | <i>N,N</i> -dimethylformamide |
| DMSO | dimethyl sulfoxide |
| D ₂ O | deuterium oxide |

| | |
|-------------------|--|
| ee | enantiomeric excess |
| EI | electron impact |
| Et ₃ N | triethyl amine |
| EtOAc | ethyl acetate |
| Et ₂ O | 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 |
| <i>J</i> | coupling constant (Hz) |
| KOH | potassium hydroxide |
| L | litre |
| LDA | lithium di- <i>iso</i> -propylamide |
| lit. | literature |
| LOD | limit of detection |
| <i>m</i> -CPBA | <i>m</i> -chloroperbenzoic 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 |
| <i>m/z</i> | 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 ₂ SO ₄ | sodium sulfate |
| NH ₄ Cl | 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 | <i>tert</i> -butyldimethylsilyl |
| TFA | trifluoroacetic acid |
| THF | tetrahydrofuran |
| TLC | thin layer chromatography |
| TMEDA | <i>N,N,N',N'</i> -tetra-methylethylenediamine |
| TsCl | <i>p</i> -toluenesulfonyl chloride |
| W | watts |