Precursors to the Potent Odorant Wine Lactone

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Doctor of Philosophy

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Abstract

Summary

This thesis describes the synthesis, hydrolysis and analysis of menthiafolic acid, a precursor to wine lactone in wine. (*R*)-Menthiafolic acid was synthesised and then taken through acid hydrolyses to confirm its conversion to wine lactone under wine-like conditions and to determine the chirality of the resultant product. A Gas Chromatography/ Mass Spectrometry (GC/MS) Stable Isotope Dilution Assay (SIDA) method was developed to analyse for this compound in grapes and wine. Chiral analysis was also carried out on wine extracts to confirm which enantiomers of menthiafolic acid and wine lactone are present in real wine samples. Bioconversion of the glucose ester of menthiafolic acid utilising three different microorganisms was evaluated in order to determine if menthiafolic acid is produced and hence if this compound is an indirect precursor to wine lactone through initial degradation to menthiafolic acid.

Chapter 1 comprises an introduction and literature review.

Chapter 2 concerns the synthesis and acid hydrolysis of (*R*)-menthiafolic acid. The synthesis gave a mixture of 95% (*R*)-enantiomer and 5% (*S*)-enantiomer menthiafolic acid. Hydrolysis was carried out under mild, wine-like conditions and under harsh Simultaneous Distillation Extraction (SDE) conditions. These hydrolyses showed that this compound is, in fact, converted to wine lactone under wine-like conditions but both the ‘natural’ (-)-isomer of wine lactone and its enantiomer are produced in varying proportions depending on the hydrolytic conditions. This work has been published; Giaccio *et al*. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 660.

Chapter 3 describes the development of a SIDA method for the analysis of menthiafolic acid in grapes and wine. Extraction methods were investigated for model wine solutions and then transferred to white wine. A d_5-analogue of menthiafolic acid was prepared for use in later quantifications. Grapes and wines were analysed and menthiafolic acid was found in the wines in varying concentrations ranging from < 10 μg/L to 342 μg/L with the highest concentration found in a Lexia wine. Wines analysed showed menthiafolic acid in significant concentrations which could potentially produce wine lactone in concentrations

above its aroma threshold. Grape analyses were also carried out and menthiafolic acid was observed in concentrations ranging from 16 μg/L to 235 μg/L. Gerwütztraminer grapes contained the greatest concentration of this precursor. Chiral analysis of menthiafolic acid present was also carried out on grape and wine samples. The analyses showed that the (*S*)-enantiomer of menthiafolic acid is the more prevalent enantiomer in these particular grape and wine samples.

Chapter 4 concerns fermentation studies of the glucose ester of menthiafolic acid. The SIDA method discussed in Chapter 3 was used to analyse for menthiafolic acid in these samples in order to determine if menthiafolic acid is released from the glucose ester *via* fermentation with various yeast and bacteria. Approximately 15% bioconversion of the glucose ester to menthiafolic acid was observed when fermenting with *Saccahormyces cerevisiae* (strain AWRI 838). Bioconversion occurred to a lesser extent (approx. 5%) when fermenting with the lactic acid bacteria *Oenococcus oeni* (strain VP-41) and even less of the glucose ester was converted to menthiafolic acid when fermenting with spoilage yeast *Dekkera bruxellensis* (strain AWRI 1499). Menthiafolic acid was not observed in a concentration above the limit of quantification in *D.bruxellensis* fermentations.

Chapter 5 details an attempt to develop a quantification method for wine lactone in model wine. Extraction of wine lactone from a white wine was also attempted. Chiral analysis of wine lactone extracted from wine by continuous liquid extraction was also conducted showing that the predominant enantiomer of wine lactone present in the wine analysed was, in fact, the (+)-enantiomer which has not previously been reported in wine. The (-)-enantiomer of wine lactone was also observed and the ratios of the two wine lactone enantiomers correlated with what was expected when taking into account the ratios of menthiafolic acid also present in the wine.

Chapter 6 comprises the experimental methods, materials and instrumentation utilised in these studies.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Joanne Giaccio 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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Publications and Symposia

Publications:

Giaccio, J.; Capone, D. L.; Håkansson, A. E.; Smyth, H. E.; Elsey, G. M.; Sefton, M. A.; Taylor, D. K., The Formation of Wine Lactone from Grape-Derived Secondary Metabolites. *Journal of Agricultural and Food Chemistry* **2011**, 59, (2), 660-664.

Giaccio, J.; Sefton, M. A.; Taylor, D. K., Quantitative Analysis of a Wine Lactone Precursor in Wine. *Proceedings of the 13th Weurman Flavour Research Symposium*. *In Press.*

Giaccio, J.; Håkansson, A. E.; Curtin, C.; Sefton, M. A.; Taylor, D. K., The Relationship Between Menthiafolic Acid and Wine Lactone in Wine. *Journal of Agricultural and Food Chemistry*. *In draft.*

Symposia:

Giaccio, J.; Sefton, M. A.; Taylor, D. K., Quantitative analysis of a wine lactone precursor in wine. **Poster and flash oral presentation** presented at the *13th Weurman Flavour Research Symposium*, Zaragosa, Spain, September 2011.

Giaccio, J.; Elsey, G. M.; Sefton, M. A.; Taylor, D. K., Precursors to the potent odorant wine lactone. **Poster** presented at the *14th Wine Industry Technical Conference*, Adelaide, Australia, July 2010.

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Abbreviations

^{1}H NMR	proton nuclear magnetic resonance
μ	micro
Ac	acetyl
aq.	aqueous
AWRI	Australian Wine Research Institute
br.s	broad singlet
ca.	*circa*
CDGJM	chemically defined grape juice medium
cm	centimetres
d	doublet
d. bruxellensis	*Dekkera bruxellensis*
dd	doublet of doublets
DMAP	dimethylaminopyridine
dt	doublet of triplets
ee	enantiomeric excess
EtOAc	ethyl acetate
g	grams
GC/MS	gas chromatography / mass spectrometry
Glu	glucose
HPLC	high pressure liquid chromatography
Hz	hertz
ID	internal diameter
IR	infra red
J	coupling constant
L	litre
LC	liquid chromatography
LC-FD	liquid chromatography-fluorescence detector
Lit.	literature
LOD	limit of detection
LOQ	limit of quantitation
M	moles per litre
m	multiplet
m/z	mass to charge ratio

MDGC	multi dimensional gas chromatography
mg	milligram
MHz	mega hertz
min.	minutes
mL	millilitre
MLCCC	multi layer countercurrent chromatography
μm	micrometers
mm	millimetre
mmol	millimole(s)
MS	mass spectrometer
ng/L	nanograms per litre
NIST	national institute of standards and technology
nm	nanometre
NMR	nuclear magnetic resonance
ppm	parts per million
PVPP	polyvinyl (poly) pyrollidone
q	quartet
QC	quality control
r^2	correllation coefficient
R_f	retention factor
rpm	revolutions per minute
rt	room temperature
s	singlet
sat.	saturated
SBSE	stir bar sorptive extraction
sec	seconds
SDE	simultaneous distillation extraction
SIDA	stable isotope dilution assay
SIDA-GC/MS	stable isotope dilution assay-gas chromatography/mass spectrometry
SIDA-LC/MS/MS	stable isotope dilution assay- liquid chromatography tandem mass spectrometry
SIM	selected ion monitoring
SPE	solid phase extraction
SPME	solid phase microextraction
t	triplet
THF	tetrahydrofuran

TLC	thin layer chromatography
tq	triplet of quartets
UV	ultra violet
v.vinifera	*vitis vinifera*
v/v	volume to volume
W	watts
w/v	weight to volume
X4	hexane fraction
YM	yeast malt

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