Isolation, characterisation and properties of 8,8-methylmethine flavan-3-ol-malvidin-3-glucoside pigments found in red wines

by

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Abstract

This study concerns the isolation, characterisation and physio-chemical properties of 8,8-methylmethine-(epi)catechin-malvidin-3-glucoside compounds found in red wines. 8,8-Methylmethine-(epi)catechin-malvidin-3-glucoside compounds were isolated via chromatographic methods developed in this study. The compounds were characterised via nuclear magnetic resonance spectrometry which, with the aid of molecular modelling, afforded their possible 3-dimensional structures. Their physio-chemical properties including ionisation and hydration constants, colour parameters and chemical stabilities were determined. The formation of 8,8-methylmethine-flavan-3-ol-malvidin-3-glucoside compounds and other pigments in wines was also studied.

8,8-Methylmethine-(epi)catechin-malvidin-3-glucoside compounds were also synthesised by condensing malvidin-3-glucoside with (epi)catechin in the presence of acetaldehyde. Diastereomers of 8,8-methylmethine-(epi)catechin-malvidin-3-glucoside pigments were isolated from the reaction using size-exclusion liquid chromatography followed by cation-exchange liquid chromatography.

The structures of the four 8,8-methylmethine-catechin (and epicatechin)-malvidin-3-glucoside diastereomers were determined using mass spectrometry and one and two-dimensional nuclear magnetic resonance spectroscopy. It was found that for all four compounds, the methylmethine bridge occurs at the 8-positions of malvidin-3-glucoside and (epi)catechin and that the 3-dimensional structural differences between the diastereomers is the positioning of the (epi)catechin moiety with respect to the glucoside group. One diastereomer has the (epi)catechin on the same side, with respect to the malvidin entity whilst it is on the opposite side for the other diastereomer. The proposed structures also afforded the malvidin entity protection from nucleophilic attack via steric hindrance by the (epi)catechin moiety.

8,8-Methylmethine-(epi)catechin-malvidin-3-glucoside pigments have greater colour stability with regards to changes in pH and SO_2 bleaching compared to malvidin-3-glucoside providing evidence that little or no hydration in aqueous solutions is occurring for these compounds. Further evidence for little or no hydration occurring is the presence

of isosbestic points in the UV-vis spectra observed for the 8,8-methylmethine-(epi)catechin-malvidin-3-glucoside in the pH range 2 to 7. Although the 8,8-methylmethine-(epi)catechin-malvidin-3-glucoside pigments have greater colour stability to pH, SO_2 and oxidation, compared to malvidin-3-glucoside, they have lower temporal stabilities and under both aerobic and anaerobic conditions they have significantly higher degradation rate constants than malvidin-3-glucoside.

The ionisation constants of the 8,8-methylmethine-(epi)catechin-malvidin-3-glucoside compounds were determined using high voltage paper electrophoresis (HVPE) and UV-visible spectroscopy. The first ionisation constants (pKa_1) of the 8,8-methylmethine-(epi)catechin-malvidin-3-glucoside compounds were found to be higher than that of malvidin-3-glucoside whereas the second and third ionisation constants (pKa_2 and pKa_3) were found to be lower. The correlation of the ionisation constants between HVPE and UV-visible spectroscopy supports the proposal that there is little or no occurrence of hydration for the 8,8-methylmethine-(epi)catechin-malvidin-3-glucoside compounds in the pH range investigated.

8,8-Methylmethine-flavan-3-ol-malvidin-3-glucoside compounds were the major pigments formed during fermentations of chemically defined grape juice media containing malvidin-3-glucoside and various flavan-3-ols. The yeast strain used for fermentation had a major influence on the levels and rates of formation of these pigments during fermentation. The yeast strain used also has an important influence on wine pigment composition, concentration and evolution during maturation thereby affecting the colour density and hue of the resultant wines. The initial formation of 8,8-methylmethine-flavan-3-ol-malvidin-3-glucoside compounds and their subsequent gradual degradation during maturation, allowed a pool of malvidin-3-glucoside to be available for the formation of other colour stable and more temporally stable pigments.

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Declaration

This thesis describes my research carried out in the School of Agriculture, Food and Wine, the University of Adelaide and contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge this thesis contains no material previously published or written by any other person except where due reference is made in the text.

I give my consent for a copy of this thesis, when deposited in the Library of the University of Adelaide, to be available for the loan and photocopying.

David Lee

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List of Abbreviations

AM1	Austin Model 1
CDGJM	Chemically Defined Grape Juice Media
CY3G	Cyanidin-3-glucoside
DEPT	Distortionless Enhancement by Polarization Transfer
DP3G	Delphinidin-3-glucoside
DQCOSY	Double Quantum Correlated Spectroscopy
HCl	Hydrochloric Acid
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HSQC	Heteronuclear Single Quantum Coherence
HVPE	High Voltage Paper Electrophoresis
MC1	8,8-methylmethine-catechin-malvidin-3-glucoside diasteromer
MC2	8,8-methylmethine-catechin-malvidin-3-glucoside diasteromer
ME1	8,8-methylmethine-epicatechin-malvidin-3-glucoside diasteromer
ME2	8,8-methylmethine-epicatechin-malvidin-3-glucoside diasteromer
MOPAC	Molecular Orbital Package
MV3G	Malvidin-3-glucoside
MV3GACET	malvidin-3-(acetyl)glucoside
MV3GCOU	malvidin-3-(coumaryl)glucoside
NaCl	Sodium Chloride
NMR	Nuclear Magnetic Resonance
PE3G	Peonidin-3-glucoside
PT3G	Petunidin-3-glucoside
ROESY	Rotational Nuclear Overhauser Effect Spectroscopy
TFA	Trifluoroacetic Acid
TLC	Thin Layer Chromatography

List of publications arising from this thesis

Refereed journals

Lee, D. F.; Swinny, E. E.; Jones, G. P. NMR identification of ethyl-linked anthocyanin-flavanol pigments formed in model wine ferments. *Tetrahedron Lett.* **2004**, *45*, 1671-1674.

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