Natural Product Discovery: Studies on the Phenolic Antioxidants from *Smilax Glyciphylla* and the Synthesis and Formation of Guaiane Sesquiterpenoids.

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by

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Abstract

The work within this thesis is positioned in the field of natural product (NP) chemistry and covers three main integrated studies along with some additional explorations. These studies not only included the isolation and characterisation of NP but also involved total syntheses of various NP and related derivatives and detailed mechanistic studies into potential routes of formation in nature. Given my naturally emerging zest for natural products, I have begun this thesis with a detailed discussion of the numerous syntheses of Taxol. This exemplar highlights not only why the field of natural products is so important, but also highlights the ever growing significance of total and semi-syntheses.

The first major study investigated the phenolic profile and antioxidant activity of the leaves of the Australian native plant *Smilax glyciphylla*. Along with the sweet principle glycyphyllin A, seven phenolic compounds including two new dihydrochalcone rhamnosides, glycyphyllin B and C, and five known flavonoids were isolated from the ethanolic extract of the leaves of *Smilax glyciphylla* for the first time. The structures of these compounds were characterised by spectroscopic methods including UV, HRMS, 1D and 2D NMR. *In vitro* antioxidant capacity tests employing the FRAP and DPPH assays indicated that three of the isolated compounds exhibited potent antioxidant activity and are the key phenolics responsible for the high antioxidant activity of the leaf extract of *S. glyciphylla*.

The second major study focused on the synthesis of guaiane type sesquiterpenoids via the diastereoselective epoxidation of guaiol and realized by manipulating the types of remote protecting groups on the isopropanoyl side chain, choice of solvent and epoxidising reagent. This stragety allowed for a concise stereoselective synthesis of a range of guaiane-type sesquiterpenoids including the natural products guaia-4(5)-en-11-ol, guaia-5(6)-en-11-ol, and aciphyllene and epimers of the recently isolated natural products, 1-*epi*-guaia-4(5)-en-11-ol, 1-*epi*-aciphyllene and 1-*epi*-melicodenone C and E in up to 31% yield within 11 steps.

The third study explored the autoxidation of α-guaiene and the mechanisms involved. Over a dozen sesquiterpenoids including natural rotundone, corymbolone and the C7 epimers of natural chabrolidione A and several unstable hydroperoxide intermediates were isolated from the autoxidation products of α-guaiene. Their structures were elucidated on the basis of spectroscopic data along with the synthesis of authentic compounds. Detailed mechanistic

studies have allowed many of the mechanisms involved in the formation of these downstream oxidation products to be elucidated.

Together with the above main studies, several deuterium labelled precursors including d_7-α-guaiene, d_5-(2*R*/2*S*)-rotundols, d_5-α-bulnesone, d_7-α-bulnesene and d_5-2*R*-bulnesol were synthesised and used as internal standards to develop a robust analytical method (SIDA) to monitor the transformation of certain precursors to the sesquiterpneoid fragrances rotundone and 2*R*-bulnesol.

A total of five publications support my research works herein and are included as the main research chapters of this thesis.

List of Publications

1. Huang, A.-C.; Wilde, A.; Ebmeyer, J.; Skouroumounis, G. K.; Taylor, D. K. Examination of the Phenolic Profile and Antioxidant Activity of the Leaves of the Australian Native Plant *Smilax glyciphylla*. *J. Nat. Prod*. 2013, *76* (10), 1930-1936.

2. Huang, A.-C.; Sumby, C. J.; Tiekink, E. R.T.; Taylor, D. K. Synthesis of Guaia-4(5)-en-11-ol, Guaia-5(6)-en-11-ol, Aciphyllene, 1-*ep*i-Melicodenone C and E and other Guaiane-type Sesquiterpenoids via the Diastereoselective Epoxidation of Guaiol. *J. Nat. Prod*. 2014, DOI: 10.1021/np500611z.

3. Huang, A.-C.; Burrett, S.; Sefton, M. A.; Taylor, D. K. Production of the Pepper Aroma Compound, (-)-Rotundone by Aerial Oxidation of α-Guaiene. *J. Agric. Food Chem*, 2014, *62* (44), 10809–10815.

4. Huang, A.-C.; Sefton, M. A.; Sumby, C. J.; Tiekink, E. R.T.; Taylor, D. K. Mechanistic Studies on the Autoxidation of α-Guaiene: The Structural Diversity of the Sesquiterpenoid Downstream Products. *J. Nat. Prod.* 2014, submitted.

5. Huang, A.-C.; Sefton, M. A.; Taylor, D. K. Rationalizing the Formation of Peppery and Woody Sesquiterpenes Derived from α-Guaiene and α-Bulnesene under Aerial Oxidative Conditions: Synthesis of Deuterium Analogues and SIDA/GC-MS Studies. *J. Agric. Food Chem*. 2014, submited.

Declaration

I certify that 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 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Ancheng Huang

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Abbreviations

Å	Angstroms
ABS	Absorbance
Ac	Acetyl
app. d	Apparent doublet
Ar	Aromatic
Bn	Benzyl
br	Broad
COSY	Correlation spectroscopy
cm	Centimetres
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DEAD	Diethyl azocaboxylate
DFT	Density function theory
DMSO	Dimethylsulfoxide
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
FRAP	Ferric cyanide reducing antioxidant power assay
g	Gram
GC	Gas chromatography
GCMS	Gas chromatography mass spectrometry
h	Hour
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
HPLC-DAD	High performance liquid chromatography- diode array detector
HRMS	High resolution mass spectrometry
Hz	Hertz
Hν	Light/irradiation
J	Coupling constant

L	Litre
LCMS	Liquid chromatography mass spectrometry
Lit.	Literature
LTMP	Lithium tetramethyl piperidide
m	Multiplet
M	Molar (moles/litre)
min.	Minute
m/z	Mass to charge ratio
MEOH	Methanol
Me	Methyl
mg	Milligram
$MgSO_4$	Magnesium sulphate
MHz	Megahertz
mL	Millilitre
mmol	Millimole
mol	Mole
mp	Melting point
MS	Mass spectrometry
nm	Nanometre
NMR	Nuclear magnetic resonance
Ph	Phenyl
ppm	Parts per million
PTSP	Pyridinium paratoluenesulfonate
psi	Pounds per square inch
q	Quartet
quint	Quintet
R_f	Retention factor
ROESY	Rotating frame overhauser effect spectroscopy
RT	Room temperature
s	Singlet
sext	Sextet
t	Triplet
TBS	*tert*-Butyldimethylsilyl
THF	Tetrahydrofuran

TLC	Thin layer chromatography
TPTZ	2,4,6-Tripyridyl-1,3,5-triazine
UV	Ultra-violet
μ	Micro