2008

Attempts to find the correct structure of uniflorine A

Andrew Stewart Davis
University of Wollongong

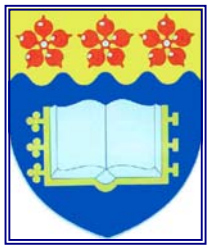
Attempts to find the correct structure of uniflorine A

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

Doctor of Philosophy

from

University of Wollongong



Andrew Stewart Davis

B. Sc (Hons)

School of Chemistry

May, 2008

Declaration

I, Andrew Stewart Davis, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless due reference is provided. This document has not been submitted for qualifications at any other academic institution.

Andrew Stewart Davis

May, 2008

TABLE OF CONTENTS

List of Figures

List of Schemes

List of Tables

List of Abbreviations

$[\alpha]_D$	specific rotation
Ac	acetyl
Ar	aromatic
ax	axial
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
br	broad
Bz	benzoyl
CI	chemical ionisation
Cy	cyclohexyl
d	doublet
δ	NMR chemical shift
DCM	dichloromethane
DEAD	diethylazadicarboxylate
DEPT	Distortionless Enhancement by Polarisation Transfer
DMAP	*N*,*N*-Dimethyl-4-aminopyridine
DMF	dimethylformamide
EI	Electron impact Ionisation
eq	equatorial
ESI+	electrospray ionisation (positive ion mode)
FCC	flash column chromatography
gCOSY	gradient Correlated Spectroscopy
gHSQC	gradient Heteronuclear Single Quantum Correlation
gHMBC	gradient Heteronuclear Multiple Bond Correlation
HR	high resolution
Hz	Hertz
LR	low resolution
MS	mass spectrometry
m	multiplet
m.p.	melting point
[M+]	molecular ion
m/z	mass/charge ratio
NMR	nuclear magnetic resonance
NMO	*N*-methylmorpholine-*N*-oxide
petrol	petroleum spirit bp 40-60 °C

ppm	parts per million
pyr	pyridine
q	quartet
R_f	relative mobility
rt	room temperature
s	singlet
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Tr	trityl, triphenylmethyl
Troc	(2,2,2-trichloroethoxy)carbonyl

ABSTRACT

The alkaloid uniflorine A was isolated in 2000 from the leaves of the tree *Eugenia uniflora* L, together with two other water soluble alkaloids, uniflorine B and the known alkaloid (+)-(3α,4α,5β)-1-methylpiperidine-3,4,5-triol piperidine. Uniflorine A was found to be an inhibitor of the α-glucosidases, rat intestinal maltase and sucrase, with IC_{50} values of 12 and 3.1 μM, respectively, and its structure was deduced from NMR analysis to be structure **1**. Uniflorine B was also found to be an inhibitor of the above α-glucosidases and its structure was determined from NMR analysis to be structure **2**.

The initial goal of this study was to complete the total synthesis of **1** and determine the validity of its proposed structure. In the event, an efficient 9-step diastereoselective synthesis of **1** was achieved by using the Petasis borono-Mannich reaction, ring-closing metathesis and stereoselective *cis*-dihydroxylation as key steps. The structure of our synthetic **1** was unequivocally established by a single-crystal X-ray crystallographic study of its pentaacetate derivative. However, the ^{1}H and ^{13}C NMR data for synthetic **1** did not match with those reported for uniflorine A; the latter showed many more downfield peaks in the ^{1}H NMR, perhaps consistent with the amine salt. The ^{1}H NMR of the hydrochloride salt of synthetic **1**, however, did not match the literature spectroscopic data either. We therefore concluded that the structure assigned to uniflorine A was not correct. We also found that the coupling constant $J_{1,8a}$ of 4.5 Hz for uniflorine A, was more consistent with the relative *syn*-H-8a, H-1 configuration, suggesting that uniflorine A, if it was an indolizidine alkaloid, had the same H-1 configuration as castanospermine. Our attempts to prepare 2-*epi*-**1** and 1,2-di-*epi*-**1** were unsuccessful due to unexpected competing side-reactions.

In addition, the diasteresoselective synthesis of the C-1, C-2 di-epimer of **1** was achieved. This synthesis employed a novel pyrrolo[1,2-*c*]oxazin-1-one precursor to allow for the reversal of π-facial diastereoselectivity in an osmium(VIII)-catalysed *syn*-dihydroxylation (DH) reaction. The NMR spectroscopic data of this epimeric compound and that of related isomers did not match that of the natural product. From a comparison of the NMR data of uniflorine A and uniflorine B with that of casuarine and the known synthetic 1,2,6,7-tetrahydroxy-3-hydroxymethylpyrrolizidine isomers we concluded unequivocally that uniflorine B is the known alkaloid casuarine. Although we cannot unequivocally prove the structure of uniflorine A, without access to the original material and data, the published data suggest that the natural product is also a 1,2,6,7-tetrahydroxy-3-hydroxymethylpyrrolizidine with the same relative C-7-C-7a-C-1-C-2-C-3 configuration as casuarine. We thus suggest that uniflorine A is 6-*epi*-casuarine.

Acknowledgments

First and foremost, I would like to extend a very sincere thankyou to Prof. Stephen Pyne for his expert supervision of this project. Steve has always been engaged with the project, giving direction and help with problem solving. His strong work ethic is always motivating and his response time for thesis editing cannot be surpassed.

Next I would like to acknowledge my family for their support. To my parents, Ken and Wendy, thankyou for giving me the financial support to undertake university studies, particularly at undergraduate level but also during the final stages of this PhD project. Your investment in my education has rewarded me in so many untold ways. Together with my sisters Katrina and Megan, you have taught me to appreciate the journey of life rather than be unnecessarily focussed on getting to some 'better' place. Such perspective has sustained me with the patience to endure 9 years of university study.

Throughout this project I have been richly blessed with some amazing friendships. For your support, encouragement and humour, thankyou Paul, Min and baby Ruby Sanders, Dan and Marg Nichols, De-Arne Brampton, Chris and Liza Hawley, Simon Barritt, Marty and Ali Barritt, Bill Hawkins, Dave Brennan, Steve Taylor, Tien Pham, Minyan Tang, Joseph Hartley, Jane Faragalla, Soli, David Ruffels, Bree and Michael, David and Pha and my ANSTO car-pool buddies Naomi, Guita and Honqin.

To the members of the Pyne Group that I have worked with, this project has been made easier by the diligence, sense of fun and camaraderie that you have brought to the laboratory. I would like to particularly acknowledge Thunwadee for her willingness to engage, learn and provide assistance in the latter stages of this project. I would also like to thank Ian Morgan for some helpful discussions on chemistry and for sparking my interest in computational chemistry, and Leena Burgess for graciously testing the biological activites of some of my compounds. Other School of Chemistry members I would like to thank are Wilford Lie for assistance with NMR spectroscopy, Karin Maxwell, Roger Kanitz and the late Larry Hick for the running of high-resolution mass spectra, and to John Korth, Peter Pavlik, Peter Sara and Steve Cooper for their own expert assistance.

Finally, I would like to state my gratitude to the sovereign Lord God for the majestic natural world He has created and the freedom He has given us to explore it with intellectual endeavour. The challenge of synthesizing a complex natural product in the laboratory, in constrast to the effortless way that nature does it, has certainly increased my appreciation of the greatness of God. To the members of St Marks Anglican Church, West Wollongong, thankyou for your reliable Christian fellowship and support during my PhD study.

Publications arising from this thesis

1. Davis, Andrew S.; Ritthiwigrom, Thunwadee; Pyne, Stephen G. Synthetic and spectroscopic studies on the structures of uniflorines A and B: structural revision to 1,2,6,7-tetrahydroxy-3-hydroxymethylpyrrolizidine alkaloids. *Tetrahedron*. **2008**, 64(21), 4868-4879.

2. Pyne, Stephen G.; Au, Christopher W. G.; Davis, Andrew S.; Morgan, Ian R.; Ritthiwigrom, Thunwadee; Yazici, Arife. Exploiting the borono-Mannich reaction in bioactive alkaloid synthesis. *Pure Appl. Chem*. **2008**, 80(4), 751-762.

3. Machan, Theeraphan; Davis, Andrew S.; Liawruangrath, Boonsom; Pyne, Stephen G. Synthesis of castanospermine. *Tetrahedron.* **2008**, 64(12), 2725-2732.

4. Pyne, Stephen G.; Davis, Andrew S.; Gates, Nicole J.; Hartley, Joseph P.; Lindsay, Karl B.; Machan, Theeraphan; Tang, Minyan. Asymmetric synthesis of polyfunctionalized pyrrolidines and related alkaloids. *Synlett*. **2004**, 15, 2670-2680.

5. Davis, Andrew S.; Pyne, Stephen G.; Skelton, Brian W.; White, Allan H. Synthesis of Putative Uniflorine A. *J. Org. Chem*. **2004**, 69(9), 3139-3143.

6. Davis, Andrew S.; Gates, Nicole J.; Lindsay, Karl B.; Tang, Minyan; Pyne, Stephen G. A new strategy for the diastereoselective synthesis of polyfunctionalized pyrrolidines. *Synlett*. **2004**, 1, 49-52.