Structure elucidation of antiplasmodial sesquiterpene lactones from *Vernonia staehelinoides* and *Oncosiphon piluliferum*

by

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Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously, in its entirety or in part, submitted it at any university for a degree.

Signature: ……. **Date**: ……..

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SUMMARY

Malaria continues to be a major cause of mortality and morbidity especially in Sub-Saharan Africa. The emergence and spread of drug resistant parasites has highlighted the need for new chemically diverse, effective drugs. Historically, one of the major sources of antimalarial agents and novel template compounds has been higher order plants. The widespread use of medicinal plants for the treatment of malaria in South Africa represents a diverse resource of potential antimalarial drugs.

Two South African plants, *Vernonia staehelinoides* and *Oncosiphon piluliferum*, were identified as potential sources of new antimalarial drugs through a national multidisciplinary-consortium project aimed at scientifically validating South African medicinal plants for the treatment of malaria. The *in vitro* antiplasmodial activity of extracts of these plants warranted further investigation to identify the biologically active components. Bio-assay guided fractionation based on *in vitro* antiplasmodial activity against the D10 *P. falciparum* strain was used to identify the compounds responsible for the observed activity. Compounds were purified using silica gel column chromatography. The structures of the isolated compounds were elucidated using spectroscopic techniques.

Bioassay-guided fractionation of the organic extracts of *V. staehelinoides* leaves identified a pair of structurally-related hirsutinolides with significant *in vitro* antiplasmodial activity. The compounds were found to be cytotoxic at similar concentrations but proved to be interesting scaffolds for potential structure-activity relationship studies.

Three germacranolides and two eudesmanolides were identified through bioassay-guided fractionation of the organic *O. piluliferum* extract. Selected derivatizations were conducted in order to fully characterize the compounds. The absolute configuration of the major active germacranolide was determined using Mosher's method. The effect of the reduction of the α-methylene group of the major active

germacranolide on antiplasmodial activity and cytotoxicity was also investigated. The 5 compounds and the reduction product were found to possess varying degrees of *in vitro* antiplasmodial activity and cytotoxicity. None was sufficiently active or selective to be a viable drug candidate but the potential for further structure-activity relationship studies exists.

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Om Saraswati Namah

ABBREVIATIONS/FORMULAE COMMONLY USED

SANBI	South African National Biodiversity Institute
MRC	Medical Research Council
CSIR	Council for Scientific and Industrial Research
UCT	University of Cape Town
WHO	World Health Organisation
Ac	acetyl
Acetyl-CoA	acetyl coenzyme A
APAD	3-acetylpyridine adenine dinucleotide
$CDCl_3$	deuterated chloroform
C_6D_6	deuterated benzene
CHO	Chinese Hamster Ovarian
DMAP	4-(dimethylamino)pyridine
DMAPP	dimethylallyl pyrophosphate
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
FPP	farnesyl pyrophosphate
GGPP	geranylgeranyl pyrophosphate
GAP	glyceraldehyde 3-phoshate
GPP	geranyl pyrophosphate
H_2O	water
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HEPES	*N*-[2-hydroxyethyl]-piperazine-*N*'-[2-ethanesulphonic acid]
IPP	isopentenyl pyrophosphate
MVA	mevalonic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MeOH	methanol
MTPA	α-methoxy-α-trifluoromethylphenyl acetic acid

$NaBH_4$	sodium borohydride
$NaHCO_3$	sodium hydrogen carbonate
Na_2SO_4	sodium sulphate
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced form of nicotinamide adenine dinucleotide phosphate
NBT	nitroblue tetrazolium
Me	methyl
MeOH	methanol
pLDH	parasite lactate dehydrogenase
PES	phenazine ethosulphate
PBS	phosphate buffered saline
PRBC	packed red blood cells
RPMI	Roswell Park Memorial Institute medium
RBC	red blood cells
SH	thiol or sulfanil
TRIS	tris(hydroxymethyl)aminomethane

A	absorbance
br	broad resonance
COSY	correlated spectroscopy
c	concentration
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
dddd	doublet of doublets of doublets of doublets
ddq	doublet of doublets of quartets
ddt	doublet of doublets of triplets
DEPT	distortionless enhancement by polarisation transfer
dq	doublet of quartets
dt	doublet of triplets

EI- MS	electron ionization- mass spectrometry
^{1}H NMR	proton (^{1}H) nuclear magnetic resonance spectroscopy
^{13}C NMR	carbon-13 nuclear magnetic resonance spectroscopy
HR EI-MS	high resolution electron ionization- mass spectrometry
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum correlation
Hz	hertz
J	coupling constant
Lit.	Literature
IC_{50}	inhibitory concentration at which 50% inhibition is achieved
NOESY	nuclear Overhauser effect spectroscopy
m	multiplet
mp	melting point
m/z	mass-to-charge-ratio
ppm	parts per million
q	quartet
R_f	retention factor
s	singlet
t	triplet
RI	resistance index
SI	selectivity index
TLC	thin layer chromatography

cm	centimeters
h	hours
kV	kilovolts
mg	milligrams
mg/L	milligrams per liter
mm	millimeters
mmol	millimoles

ng/ml	nanograms per milliliter
nm	nanometers
μM	micromolar
μg/ml	micrograms per milliliter
g/L	grams per liter

spp.	species
subsp.	subspecies

et al.	and others
i.e.	that is
viz.	namely

CONTENTS

CHAPTER 2

Sesquiterpene lactones

CHAPTER 3

Antiplasmodial Activity of *Vernonia staehelinoides*

CHAPTER 4

Antiplasmodial Activity of *Oncosiphon piluliferum*

CHAPTER 5
Experimental