

A study of Mesembryanthemaceae alkaloids

by

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Appendix 1:

Plant Material Tables.

Table A.1: Genus and species, voucher numbers, and plant parts studied of all species investigated.

Genus and species (Author)	Voucher specimen number (JRAU)	Collectors	Plant parts studied
APTENIA			
<i>Aptenia cordifolia</i> (L.f.) Schwantes	FR van Heerden A (JRAU)	BEVW	Fruit and flowers, Leaves, Roots, Stems
<i>Aptenia lancifolia</i> L.Bolus	FR van Heerden B (JRAU)	CDG	Fruit and flowers, Leaves, Roots, Stems
<i>Aptenia cordifolia</i> (L.f.) Schwantes	FR van Heerden 2 (JRAU)	FRVH and CDG	Fruit and flowers, Leaves, Roots, Stems
ARIDARIA			
<i>Aridaria serotina</i> L.Bolus	FR van Heerden 55 (JRAU)	FRVH and CDG	Leaves, Roots, Stems
BROWNANTHUS			
<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	FR van Heerden 16 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	FR van Heerden 5 (JRAU)	FRVH and CDG	Mixed plant parts
MESEMBRYANTHEMUM			
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 7 (JRAU)	FRVH and CDG	Leaves, Roots, Stems
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 8 (JRAU)	FRVH and CDG	Leaves, Roots, Stems
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 9A (JRAU)	FRVH and CDG	Mixed plant parts
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 1 (JRAU)	FRVH and CDG	Fruit and flowers, Leaves, Stems
<i>Mesembryanthemum</i> cf. <i>guerichianum</i> Pax	FR van Heerden 67 (JRAU)	Ian Oliver, FRVH and CDG	Mixed plant parts
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 24 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 54 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 61 (JRAU)	FRVH and CDG	Leaves, Roots, Stems
<i>Mesembryanthemum excavatum</i> L.Bolus	FR van Heerden 84 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Mesembryanthemum guerichianum</i> Pax	FR van Heerden 44B (JRAU)	FRVH and CDG	Leaves, Roots
<i>Mesembryanthemum nodiflorum</i> L.	FR van Heerden 78 (JRAU)	FRVH and CDG	Roots, Stems

<i>Mesembryanthemum nodiflorum</i> L.	FR van Heerden 9B (JRAU)	FRVH and CDG	Mixed plant parts
<i>Mesembryanthemum</i> sp.	FR van Heerden 60 (JRAU)	FRVH and CDG	Leaves, Roots

PHYLLOBOLUS

<i>Phyllobolus</i> cf. <i>anrathus</i> N.E.Br.	FR van Heerden 73 (JRAU)	Ian Oliver, FRVH and CDG	Mixed plant parts
<i>Phyllobolus congestus</i> (L.Bolus) Gerbaulet	FR van Heerden 74 (JRAU)	Ian Oliver, FRVH and CDG	Mixed plant parts
<i>Phyllobolus digitatus</i> (Aiton) Gerbaulet subsp. <i>digitatus</i>	FR van Heerden 41 (JRAU)	FRVH and CDG	Green stems, Papery stems, Roots
<i>Phyllobolus</i> c.f. <i>longispinulus</i> N.E.Br.	FR van Heerden 71 (JRAU)	Ian Oliver, FRVH and CDG	Mixed plant parts
<i>Phyllobolus nitidus</i> (Haw.) Gerbaulet	FR van Heerden 49 (JRAU)	FRVH and CDG	Fruit and flowers, Leaves, Roots, Stems
<i>Phyllobolus rabiei</i> (L.Bolus) Gerbaulet	FR van Heerden 72 (JRAU)	Ian Oliver, FRVH and CDG	Mixed plant parts
<i>Phyllobolus spinuliferus</i> (Haw.) Gerbaulet	FR van Heerden 52B (JRAU)	FRVH and CDG	Mixed plant parts

PRENIA

<i>Prenia</i> cf. <i>pallens</i> (Aiton)	FR van Heerden 66 (JRAU)	Ian Oliver, FRVH and CDG	Mixed plant parts
<i>Prenia englishiae</i> (L.Bolus) Gerbaulet	FR van Heerden 29 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	FR van Heerden 37 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	FR van Heerden 39 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Prenia</i> sp.	FR van Heerden 40 (JRAU)	FRVH and CDG	Mixed plant parts

PSILOCAULON

<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 11 (JRAU)	FRVH and CDG	Leaves, Stems
<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 12 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 28 (JRAU)	FRVH and CDG	Fruit and flowers, Leaves, Stems
<i>Psilocaulon bicornis</i> (Sond.) Schwantes	FR van Heerden 21 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon</i> cf. <i>junceum</i> (Haw.) Schwantes	FR van Heerden 22 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon coriarium</i> (Burch. ex N.E.Br.) N.E.Br.	FR van Heerden 62 (JRAU)	FRVH and CDG	Stems
<i>Psilocaulon coriarium</i> (Burch. ex N.E.Br.) N.E.Br.	FR van Heerden 65 (JRAU)	FRVH and CDG	Fruit and flowers, Leaves, Stems
<i>Psilocaulon dinteri</i> (Engl.) Schwantes	FR van Heerden 44A (JRAU)	FRVH and CDG	Mixed plant parts

<i>Psilocaulon granulicaule</i> (Haw.) Schwantes	FR van Heerden 46 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon granulicaule</i> (Haw.) Schwantes	FR van Heerden 53 (JRAU)	FRVH and CDG	Leaves, Stems
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 14 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 20 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 26 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 27 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 50 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 77 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 81 (JRAU)	FRVH and CDG	Mixed plant parts

SCELETIUM

<i>Sceletium crassicaule</i> (Haw.) L.Bolus	FR van Heerden 23 (JRAU)	FRVH and CDG	Leaves, Roots, Stems
<i>Sceletium rigidum</i> L.Bolus	FR van Heerden 69 (JRAU)	Ian Oliver, FRVH and CDG	Mixed plant parts
<i>Sceletium tortuosum</i> (L.) N.E.Br.	FR van Heerden 68 (JRAU)	Ian Oliver, FRVH and CDG	Leaves, Stems
<i>Sceletium tortuosum</i> (L.) N.E.Br.	FR van Heerden 70 (JRAU)	Ian Oliver, FRVH and CDG	Leaves, Stems

Subfamily: Ruschoideae.

<i>Drosanthemum</i> sp.	FR van Heerden 10 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Drosanthemum</i> sp.	FR van Heerden 42 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Drosanthemum</i> sp.	FR van Heerden 82 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Malephora</i> sp.	FR van Heerden 83 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Monilaria moniliformis</i> (Thunb.) Ihlenf. & Jörg.	FR van Heerden 59 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Ruschia</i> sp.	FR van Heerden 33 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Ruschia</i> sp.	FR van Heerden 58 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Ruschia</i> sp. (<i>Uncinata</i> group)	FR van Heerden 18 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Ruschia</i> sp. (<i>Uncinata</i> group)	FR van Heerden 4 (JRAU)	FRVH and CDG	Mixed plant parts
<i>Pleiospilos compactus</i> (Aiton) Schwantes	M2	FRVH	Mixed plant parts
<i>Pleiospilos compactus</i> (Aiton) Schwantes	M3	FRVH	Mixed plant parts
<i>Eberlanzia ferox</i> Schwantes	M4	FRVH	Mixed plant parts
<i>Trichodiadema</i> sp.	M5	FRVH	Mixed plant parts

<i>Malephora</i> sp.	M6	FRVH	Mixed plant parts
<i>Ruschia</i> sp.	M7	FRVH	Mixed plant parts

Table A.2: All species screened, according to subfamily - plant parts studied and alkaloid status.

Subfamily: Mesembryanthemoideae.

Genus and species (Author)	Voucher specimen number (JRAU)	Plant parts studied	Alkaloid positive or negative, screened using TLC	Alkaloid positive or negative, screened using GC-MS
<i>APTENIA</i>				
<i>Aptenia cordifolia</i> (L.f.) Schwantes	FR van Heerden A (JRAU)	Fruit and flowers, Leaves, Roots, Stems	+	+
<i>Aptenia lancifolia</i> L.Bolus	FR van Heerden B (JRAU)	Fruit and flowers, Leaves, Roots, Stems	+	+
<i>Aptenia cordifolia</i> (L.f.) Schwantes	FR van Heerden 2 (JRAU)	Fruit and flowers, Leaves, Roots, Stems	+	+
<i>ARIDARIA</i>				
<i>Aridaria serotina</i> L.Bolus	FR van Heerden 55 (JRAU)	Leaves, Roots, Stems	+	+
<i>BROWNANTHUS</i>				
<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	FR van Heerden 16 (JRAU)	Mixed plant parts	-	NAD
<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	FR van Heerden 5 (JRAU)	Mixed plant parts	-	NAD
<i>MESEMBRYANTHEMUM</i>				
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 7 (JRAU)	Leaves, Roots, Stems	+	+
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 8 (JRAU)	Leaves, Roots, Stems	+	+

<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 9A (JRAU)	Mixed plant parts	+	NAD
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 1 (JRAU)	Fruit and flowers, Leaves, Stems	+	NAD
<i>Mesembryanthemum</i> cf. <i>guerichianum</i> Pax	FR van Heerden 67 (JRAU)	Mixed plant parts	-	NAD
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 24 (JRAU)	Mixed plant parts	-	NAD
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 54 (JRAU)	Mixed plant parts	-	NAD
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 61 (JRAU)	Leaves, Roots, Stems	+	NAD
<i>Mesembryanthemum excavatum</i> L.Bolus	FR van Heerden 84 (JRAU)	Mixed plant parts	-	NAD
<i>Mesembryanthemum guerichianum</i> Pax	FR van Heerden 44B (JRAU)	Leaves, Roots	+	+
<i>Mesembryanthemum nodiflorum</i> L.	FR van Heerden 78 (JRAU)	Roots, Stems	+	NAD
<i>Mesembryanthemum nodiflorum</i> L.	FR van Heerden 9B (JRAU)	Mixed plant parts	-	NAD
<i>Mesembryanthemum</i> sp.	FR van Heerden 60 (JRAU)	Leaves, Roots	+	NAD
PHYLLOBOLUS				
<i>Phyllobolus</i> cf. <i>anrathus</i>	FR van Heerden 73 (JRAU)	Mixed plant parts	+	NAD
<i>Phyllobolus congestus</i> (L.Bolus) Gerbaulet	FR van Heerden 74 (JRAU)	Mixed plant parts	-	NAD
<i>Phyllobolus digitatus</i> (Aiton) Gerbaulet subsp. <i>digitatus</i>	FR van Heerden 41 (JRAU)	Green stems, Papery stems, Roots	+	+
<i>Phyllobolus</i> c.f. <i>longispinulus</i>	FR van Heerden 71 (JRAU)	Mixed plant parts	-	NAD
<i>Phyllobolus nitidus</i> (Haw.) Gerbaulet	FR van Heerden 49 (JRAU)	Fruit and flowers, Leaves, Roots, Stems	+	+
<i>Phyllobolus rabiei</i> (L.Bolus) Gerbaulet	FR van Heerden 72 (JRAU)	Mixed plant parts	-	NAD
<i>Phyllobolus spinuliferus</i> (Haw.) Gerbaulet	FR van Heerden 52B (JRAU)	Mixed plant parts	+	NAD
PRENIA				
<i>Prenia</i> cf. <i>pallens</i> (Aiton)	FR van Heerden 66 (JRAU)	Mixed plant parts	-	NAD
<i>Prenia englishiae</i> (L.Bolus) Gerbaulet	FR van Heerden 29 (JRAU)	Mixed plant parts	-	NAD
<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	FR van Heerden 37 (JRAU)	Mixed plant parts	-	NAD
<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	FR van Heerden 39 (JRAU)	Mixed plant parts	-	NAD

<i>Prenia</i> sp.	FR van Heerden 40 (JRAU)	Mixed plant parts	-	NAD
PSILOCAULON				
<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 11 (JRAU)	Leaves, Stems	+	NAD
<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 12 (JRAU)	Mixed plant parts	+	NAD
<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 28 (JRAU)	Fruit and flowers, Leaves, Stems	+	NAD
<i>Psilocaulon bicornis</i> (Sond.) Schwantes	FR van Heerden 21 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon</i> cf. <i>junceum</i> (Haw.) Schwantes	FR van Heerden 22 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon coriarium</i> (Burch. ex N.E.Br.) N.E.Br.	FR van Heerden 62 (JRAU)	Stems	+	+
<i>Psilocaulon coriarium</i> (Burch. ex N.E.Br.) N.E.Br.	FR van Heerden 65 (JRAU)	Fruit and flowers, Leaves, Stems	+	+
<i>Psilocaulon dinteri</i> (Engl.) Schwantes	FR van Heerden 44A (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon granulicaule</i> (Haw.) Schwantes	FR van Heerden 46 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon granulicaule</i> (Haw.) Schwantes	FR van Heerden 53 (JRAU)	Leaves, Stems	+	NAD
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 14 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 20 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 26 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 27 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 50 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 77 (JRAU)	Mixed plant parts	-	NAD
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 81 (JRAU)	Mixed plant parts	-	NAD
SCELETIUM				
<i>Sceletium crassicaule</i> (Haw.) L.Bolus	FR van Heerden 23 (JRAU)	Leaves, Roots, Stems	+	+
<i>Sceletium rigidum</i> L.Bolus	FR van Heerden 69 (JRAU)	Mixed plant parts	-	NAD
<i>Sceletium tortuosum</i> (L.) N.E.Br.	FR van Heerden 68 (JRAU)	Leaves, Stems	+	+

<i>Sceletium tortuosum</i> (L.) N.E.Br.	FR van Heerden 70 (JRAU)	Leaves, Stems	+	+
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NAD = No alkaloids detected.

Genus and species (Author)	Voucher specimen number (JRAU)	Plant parts studied	Alkaloid positive or negative, screened using TLC
<i>Drosanthemum</i> sp.	FR van Heerden 10 (JRAU)	Mixed plant parts	-
<i>Drosanthemum</i> sp.	FR van Heerden 42 (JRAU)	Mixed plant parts	-
<i>Drosanthemum</i> sp.	FR van Heerden 82 (JRAU)	Mixed plant parts	-
<i>Malephora</i> sp.	FR van Heerden 83 (JRAU)	Mixed plant parts	-
<i>Monilaria moniliformis</i> (Thunb.) Ihlenf. & Jörg.	FR van Heerden 59 (JRAU)	Mixed plant parts	-
<i>Ruschia</i> sp.	FR van Heerden 33 (JRAU)	Mixed plant parts	-
<i>Ruschia</i> sp.	FR van Heerden 58 (JRAU)	Mixed plant parts	-
<i>Ruschia</i> sp. (<i>Uncinata</i> group)	FR van Heerden 18 (JRAU)	Mixed plant parts	-
<i>Ruschia</i> sp. (<i>Uncinata</i> group)	FR van Heerden 4 (JRAU)	Mixed plant parts	-
<i>Pleiospilos compactus</i> (Aiton) Schwantes	M2 (JRAU)	Mixed plant parts	-
<i>Pleiospilos compactus</i> (Aiton) Schwantes	M3 (JRAU)	Mixed plant parts	-
<i>Eberlanzia ferox</i> Schwantes	M4 (JRAU)	Mixed plant parts	-
<i>Trichodiadema</i> sp.	M5 (JRAU)	Mixed plant parts	-
<i>Malephora</i> sp.	M6 (JRAU)	Mixed plant parts	-
<i>Ruschia</i> sp.	M7 (JRAU)	Mixed plant parts	-

Appendix 2:

GC spectra of selected samples.

Chapter 1

Introduction

Since the dawn of time plants have provided for all of Man's needs varying from shelter to clothing, food, dyes, fragrances, flavours, and medicines. There is ample archaeological evidence indicating that medicinal plants were regularly employed by people in prehistoric times. Through extensive experimentation and research, the biodynamic activities of the phytochemical plant constituents were gradually discovered and exploited for specific medical and psychiatric applications. Several diverse lines indicate that medicinal plants and their respective chemical constituents represent the oldest and most widespread form of medication.

Until the last century most medicines were derived directly from plant or animal sources. In spite of the increasing use of factory-made synthetic drugs, natural products have persisted as the 'treatment of choice' for a multitude of health problems in populations throughout the world. The use of plants as medicine was probably brought about as a result of their availability, and the need for healing the ill and the injured.

The art of medicine is thought to have been cultivated in Egypt where the offices of priest and physician were combined in the same person. The priests were the physicians and their treatment was mainly aimed at promoting cleanliness and preventing contagion.¹ Ancient medicinal practices are known to have their roots in all parts of the world extending from Europe to the Middle East to Asia to Africa.^{1,2}

Ayurveda originated in India and it is one of the most ancient of all medicinal traditions, possibly even older than traditional Chinese medicine. It is considered to be the origin of systemized medicine.² The term 'Ayurveda' means science of

life in Sanskrit, and it is derived from the words '*ayur*' (meaning life) and '*veda*' (meaning knowledge or science).^{2,3,4}

In India, medicinal knowledge and wisdom were handed down from one generation to the next by way of songs and poems and are also contained in a four-part series of ancient texts known as *The Veda* which date back to as early as 2000 years BC.^{2,5} The first school known to teach Ayurvedic medicine was at the University of Banaras in 500 BC and the great Samhita (or encyclopaedia of medicine) was written around this time. Approximately 700 years later a second great encyclopaedia of medicine was written and together these two form the basis of Ayurveda.² Famous Ayurvedic medicinal plants include *Centella asiatica* (L.) Urban (Gotu Kola or Indian Pennywort), *Cinnamomum camphora* (L.) J. Presl. (Camphor), *Elettaria cardamomum* (L.) Maton (*ela* or cardamon), *Cinnamomum verum* J. Presl. (cinnamon) and *Santalum album* L. (Sandalwood).² *Centella asiatica* (Fig. 1.1 and 1.2) has been used as a traditional medicine for thousands of years and is said to have a positive effect on skin conditions such as psoriasis, leprosy, sores, ulcers, infections and inflammations. Included as well are upper respiratory tract infections, diarrhoea, fevers and diseases of the female reproductive system.⁶ Many of the Ayurvedic herbs are also used for culinary purposes.⁷ Cardamon was used for the treatment of fever, digestive upsets, urinary complaints, and pulmonary disease. It is currently listed in the British Herbal Pharmacopoeia as a specific for flatulent dyspepsia.⁸ Cinnamon (Fig. 1.1 and 1.2) is used as a stimulant, carminative, astringent, anti-infective, antifungal and digestive aid. It is also used to mask the unpleasant tastes of other medicinal plants. Recent research has shown that cinnamon is a powerful anti-diabetic that enhances the activity of insulin.⁷

The ancient system of Chinese traditional medicine is believed to be more than 5000 years old and is based on two different theories about the natural laws that govern good health and longevity: namely *yin* and *yang*, together with the five elements (*wu xing*).



<http://www.umm.edu/altmed/ConsHerbs/GotuKolach.html>



http://www.uni-graz.at/~katzner/engl/generic_frame.html?Cinn_zey.html

Figure 1.1: *Centella asiatica* and *Cinnamomum verum*.

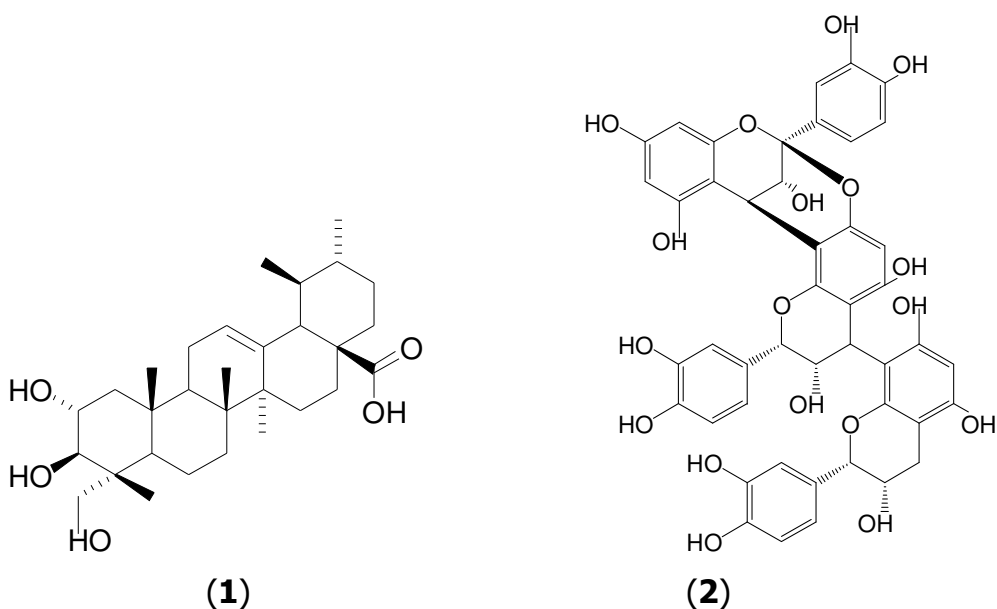
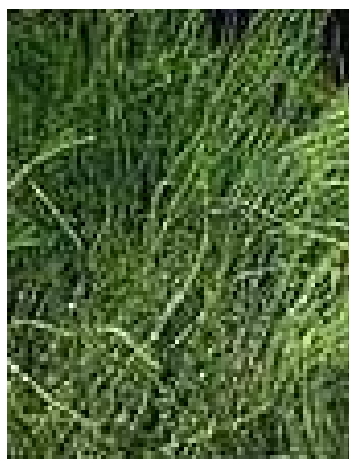


Figure 1.2: Structures of asiatic acid (1) from *Centella asiatica*,⁶ and a doubly linked procyanidin type A polymer (2) that enhances insulin activity found in *Cinnamomum verum*.⁷

The legendary Chinese emperor Shen Nung discussed herbs with medicinal uses in his works which were probably written approximately 2500 years BC.^{2,5} Between 100 and 200 BC traditional Chinese medicine was systematised and documented. The Modern Day Encyclopaedia of Chinese *materia medica* was published in 1977. It lists nearly 6000 drugs, of which 4800 are of plant origin, and is the most complete reference to Chinese herbal medicines.² Examples of

famous Chinese medicinal herbs include *Ephedra sinica* Stapf (ma huang) and *Panax ginseng* C.A. Mey. (ren shen) (Fig. 1.3 and 1.4).^{2,9} A vegetable alkaloid extracted from *Ephedra* (ephedrine) is still used to treat asthma.² *Panax ginseng* has been commercialised and is a widely used supplement and tonic for the treatment of diabetes as well as being an immune booster.⁹



<http://www.ibiblio.org/herbmed/faqs/medi-2-19-ma-huang.html>



http://alternativehealing.org/ren_shen.htm



Figure 1.3: *Ephedra sinica* and *Panax ginseng*.

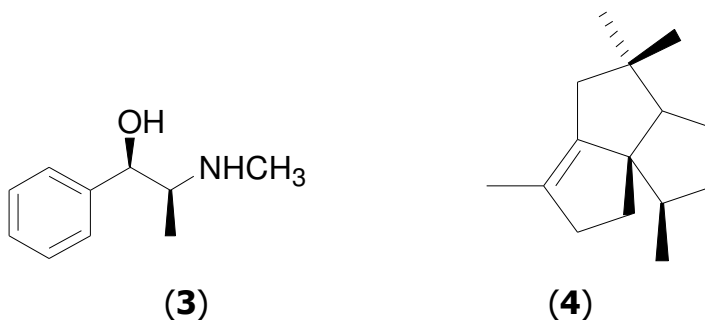


Figure 1.4: The structures of ephedrine **(3)** from *Ephedra sinica*¹⁰ and panaginsene **(4)** from *Panax ginseng*.¹¹

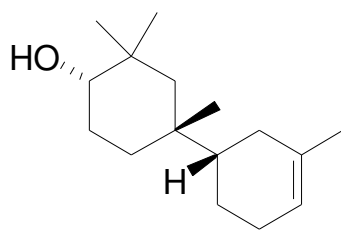
The Middle East is known as the cradle of civilization. The Assyrians, Babylonians and Sumerians recorded herbal treatments and remedies in cuneiform writing on numerous clay tablets. The Code of Hammurabi (ca. 1700 BC) is of special interest. The King of Babylon commissioned it, and it contains a comprehensive set of civil laws carved in stone, along with a list of several medicinal herbs.²

The Egyptians documented their knowledge (including medicinal and pharmaceutical) in wall paintings of tombs dating from the Old Kingdom as well as on papyrus. Some of this documentation has survived several millennia.² The most important of the papyrus writings is the Ebers Papyrus which is a medical handbook covering all sorts of illnesses and which originates from around 1500 BC. It is reported to contain ancient medicinal knowledge from before 3000 BC, and includes empirical as well as symbolic forms of treatment and a compilation of around 700 drugs (mostly of plant and animal origin).^{2,12} The diagnostic precision documented in this text is reported to be impressive.² These 'medicines' were administered in a variety of forms such as snuffs, poultices, gargles and infusions with wine, milk, beer or honey.¹²

In the ancient Western world the Greeks contributed significantly to the development of the use of herbal drugs.^{1,2} The European healing system is known as "Galenic medicine" as the principles were originally thought to have been defined by Claudius Galenus, commonly known as Galen of Pergamus, in AD 131 - 199.^{1,5} It most likely originated, however, from Hippocrates (460–377 BC) and Aristotle (384-322 BC) whose own ideas were rooted in ancient beliefs from Egypt and India.^{2,5} The natural scientist and philosopher, Theophrastus (±300 BC), dealt with the medicinal qualities of herbs in his *History of Plants*.^{1,2} Dioscorides, the famous Greek physician (100 AD), wrote one of the first books on medicinal herbs and titled it *De Materia Medica*.² Galen, who practiced and taught pharmacy and medicine in Rome, published no less than 30 books on these subjects.^{1,2} Herbal medicine, taken mainly in the form of teas, is still very popular in Europe today.^{2,13} Chamomile (*Matricaria recutita* L.) is known for its pleasant rich apple cinnamon aroma and is used as a general tonic, an antispasmodic and a calmative (Fig. 1.5).¹³



<http://altnature.com/gallery/chamomile.htm>



(5)



Figure 1.5: Chamomile (*Matricaria recutita*) and the structure of bisabololoxide A (5) from *Matricaria recutita*.¹⁴

The Arabs were the first to establish privately-owned drug stores in the 8th century AD.^{2,12} The famous Persian pharmacist, physician, philosopher and poet, Avicenna, contributed much to the sciences and medicine through his compilation *Canon medicinae*.^{1,2} This compilation is regarded as the “final codification of all Greco-Roman medicine” and includes elements of other healing cultures, forming the basis for a distinct Islamic healing system that is known today as *Unani Tibb*. Amongst the famous medicinal plants of the Middle East and Egypt are: *Allium cepa* L. (onion), *Carum carvi* L. (caraway), *Papaver somniferum* L. (opium poppy) (Fig. 1.6), and *Trigonella foenum-graecum* L. (fenugreek).²



http://www.uni-graz.at/~katzner/engl/generic_frame.html?Papa_som.html

Figure 1.6: *Papaver somniferum* (opium poppy).

African traditional medicine is perhaps the most diverse of all the medicine systems.^{2,15,16,17} Africa is considered to be the cradle of Mankind and is blessed

with a rich biological and cultural diversity marked by regional differences in healing practices.² Unfortunately, the various systems are poorly recorded and remain so to date, making it virtually impossible to trace the way in which the knowledge was gathered.^{2,15,16} Famous African medicinal plants include *Agathosma betulina* (Berg.) Pillans (buchu)¹⁷, *Aloe ferox* Mill. (Cape Aloe)¹⁷ and its products such as 'lewensessens' (Fig. 1.7).² *Aspalathus linearis* (Burm.f.) Dahlg. (rooibos tea), *Boswellia sacra* Flueckiger (frankincense) and *Commiphora myrrha* (Nees) Engl. (myrrh) are also well-known medicinal plants in the African continent (Fig. 1.8).²

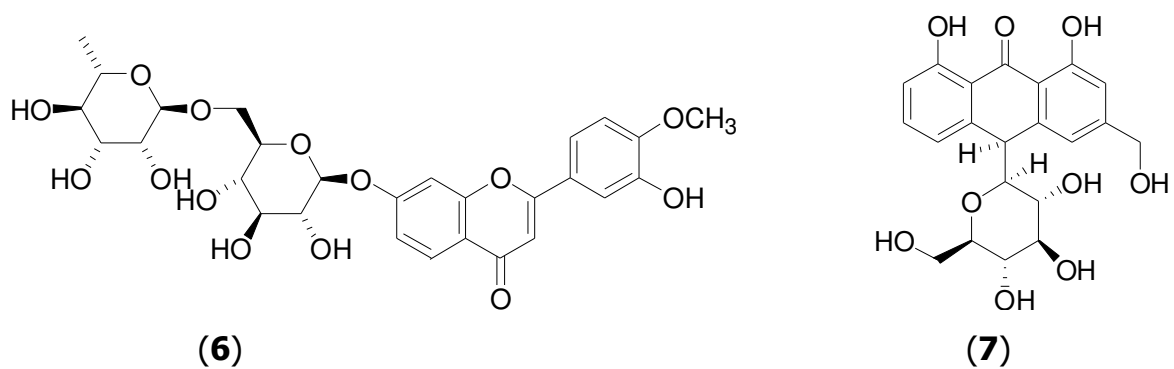


Figure 1.7: The structures of diosmin **(6)**¹⁸ from *Agathosma betulina* and aloin **(7)** from *Aloe ferox*.¹⁹



Figure 1.8: *Boswellia sacra*, Frankincense and Myrrh, and *Commiphora myrrha*.

The term "natural product" refers to compounds isolated from plants, animals, marine organisms, microbes or fungi.²⁰ The active ingredients in medicinal plants

are the chemical compounds that act either directly or indirectly to prevent disease or heal injury and maintain health.^{2,20} The great structural diversity of these compounds provides evidence of nature's enormous combinatorial capabilities.²⁰ Ethnopharmacology and drug discovery using natural products remain important issues in the current target-rich, lead-poor scenario.⁴ Natural products have played a vital role in the pharmaceutical industry for decades as a source of drugs and drug leads.²¹ More than 50% of all drugs in clinical use in the world are represented by natural products and their derivatives, with up to 74% of the anticancer drugs and 78% of antibacterials on the market being either natural products or of a natural origin.^{2,21} Of the 877 small-molecule drugs introduced between 1981-2002, 61% can be traced to natural products.²²

The Khoi-San people of southern Africa are considered to be one of the most ancient cultures. Over the many years of their existence, they have developed a remarkably broad range of remedies that are used to treat a great variety of complaints and diseases.^{5,21} Many of the current inhabitants of the Karoo region of South Africa are direct descendents of the Khoi-San lineage. This region is renowned for its large variety of medicinal plants, which include: *Aptosimum procumbens* (Lehm.) Steud. (Scrophulariaceae) (Fig. 1.9), also known as the Karoo violets (Karooviooltjies); *Garuleum bipinnatum* (Thunb.) Less. (Asteraceae), also known as 'slanghoutjie' (Fig. 1.9); *Hoodia gordonii* (Masson) Sweet ex Decne, also known as ghaap (Fig. 1.10); *Sutherlandia frutescens* (L.) R.Br. (Fabaceae), widely known as 'cancer bush' (Fig. 1.10); and a preparation derived from *Sceletium tortuosum* (L.) N.E. Br. and other species (Mesembryanthemaceae) known as 'kougoed'.¹⁷



<http://www.plantzafrica.com/plantab/aptosimprocum.htm>



<http://www.elsenburg.com/info/els/090/090e.html>

Figure 1.9: *Aptosimum procumbens* and *Garuleum bipinnatum*.

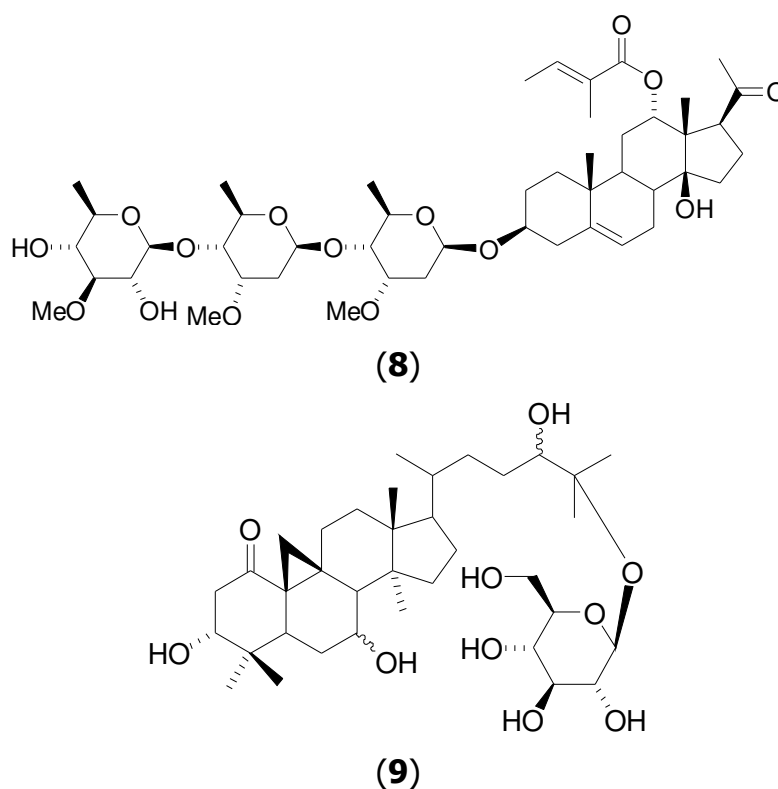


Figure 1.10: The structures of P57, the active compound of *Hoodia gordonii* (**8**)²³, and SU-1 (**9**), main triterpenoid of *Sutherlandia microphylla* Burch. ex DC.²⁴

This study focuses on the family Mesembryanthemaceae (sometimes included in the Aizoaceae). The Mesembryanthemaceae is a large family of approximately 123 genera and ca. 1680 species almost endemic to southern Africa.^{25,26} Members of the family have various traditional uses: *Sceletium* species are used to make “kougoed” which is a traditional hypnotic and which has interesting commercial potential as tranquillisers and anti-depressants; *Khadia* and *Trichodiadema* species are used in rural areas as ferment in beer and are said to improve the potency of the beer; *Pleiospilos* species are used as snuff.^{27,28} The alkaloids of *Sceletium* are well studied, but very little research is known to have been performed on the other genera within the Mesembryanthemaceae. In 1998, Smith et. al.²⁹ investigated selected taxa of the Mesembryanthemaceae for the distribution of mesembrane alkaloids. They investigated twenty species from nine genera of the Mesembryanthemaceae using gas chromatography (GC) with a nitrogen-

phosphorous detector (NPD), and reported the presence of alkaloids in 12 of the species and six of the genera they studied.

In the past, a simple screening has revealed the possible presence of alkaloids in several genera. Although Smith et. al did investigate selected taxa of the Mesembryanthemaceae for the distribution of mesembrane alkaloids in 1998, there are a few matters of concern regarding the results obtained by them. During the course of their investigations, Smith and colleagues analysed extracts of *Sceletium tortuosum* on a GC/MS, and recorded the retention times as well as the spectra for three of the mesembrane alkaloids present in the extracts. By comparison with mass spectral data published by Martin et al.³⁰, they assigned the structures of the compounds as 4'-*O*-demethylmesembrenol (RT 11.5 min), mesembrine (12 min) and mesembrenone (RT 12.5 min). Relying on the retention times and MS spectra obtained for these mesembrane alkaloids, their presence in other species was confirmed by comparing the retention times of compounds. It is risky to identify mesembrane alkaloids in complex mixtures solely on the basis of GC retention times and mass spectral data as many of these alkaloids have very similar molecular masses, GC/MS fragmentation patterns, and retention times on GC. It is also a worry for researchers that in the past, false positive results for the presence of mesembrane alkaloids have been known to occur due to contaminated GC inlets.

The aims of this study were:

- To screen a representative sample of the Mesembryanthemaceae genera and species for the possible presence of alkaloids, with emphasis on the subfamily Mesembryanthemoideae (*Sceletium*, *Aptenia*, and related genera);
- To record all ethnobotanical information on the medicinal and culinary uses of the Mesembryanthemaceae;
- To isolate and identify alkaloids from selected taxa.

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Chapter 2

The Mesembryanthemaceae and their alkaloids

2.1 Introduction

“Mesembs” is a popular and well-known term for succulent members of the family Aizoaceae. These succulent members are sometimes placed in a separate family of their own, the Mesembryanthemaceae.^{1,2,3} All the genera with axile placentation are members of the subfamily Mesembryanthemoideae, and are also known as “Weedy Mesembs”.^{1,3} On the basis of several morphological characters this subfamily has been considered to form a monophyletic group, which is supported by the molecular data shown by molecular systematics.⁴ Historically, the subfamily Mesembryanthemoideae are the main source of previous reports of alkaloids.

The Mesembryanthemaceae is South Africa’s largest succulent plant family, and it constitutes a major part (ca. 63%) of the southern African succulent flora.^{2,3,5,6} With approximately 123 genera and ca. 1680 species, it is also South Africa’s second largest plant family, accounting for approximately 10% of South Africa’s flora.^{2,3,5} The family is almost entirely endemic to southern Africa, although a few species are known to occur naturally outside this region.^{2,3,5,6}

The original name *Mesembrianthemum* was proposed by Breyne in 1689 and originated from the name ‘midday flower’, which refers to the opening of the flowers around noon.^{3,5} Other common names include ice plant, fig-marigold, iqina (Xhosa) and mesemb.^{3,5,7} Most South Africans know plants which belong to the Mesembryanthemaceae as *vygies*. Often this Afrikaans vernacular name is qualified by a descriptive prefix, as in *brakvygie*, *skerpioenvygie* or *soutvygie*.⁵ For convenience and to avoid confusion, the term *mesemb* is used to refer to any member of the Mesembryanthemaceae throughout this thesis.

Members of the subfamily Mesembryanthemoideae are usually found as weeds in disturbed places such as ploughed lands or roadsides; the leaves and stems are usually soft in texture and glistening or greyish-green in appearance. The glistening effect is due to the presence of prominent, water-containing bladder cells. The fruit capsules of the weedy mesembs have a corky rather than a woody texture and are pale in colour. The seeds are attached to the central axis of the fruit, i.e. they have an axile placenta. The large seeds are clearly observable when the capsule opens and may become wedged beneath the broad wings that are fused to the valves. Fruit of this type are typically known to have four, sometimes five locules (rarely six, as in the case of some *Sceletium* species).¹

A variety of growth forms can be observed in the Mesembryanthemoideae.^{1,2} Most species have flat or subcylindrical leaves and are rather woody. Those species that have large flat leaves are herbaceous and are covered with gleaming water cells. The group also incorporates stem succulents with articulated, green stems and short-lived leaves. As well as the above, there are some species with fleshy, thickened underground parts (geophytes). The weedy mesembs comprise 11 genera and 95 species.¹

2.2 Literature Review

2.2.1 Introduction to alkaloids.

An alkaloid is a nitrogenous organic molecule that has a pharmacological effect on humans and other animals.⁸ The term 'alkaloid' is made up out of the words 'alkali' and 'εἶδος' (Greek 'type' or 'similarity') or 'εἶδω' ('to see', 'to appear'). In essence, it means a substance with an alkali-like character.⁹ Originally the term was used to describe any nitrogen-containing base (an amine in modern terms). Alkaloids are found in plants, animals and fungi, and can be extracted from their sources by treatment with acids (usually hydrochloric acid or sulphuric acid). When administered to animals most alkaloids produce striking physiological effects, and the effects vary greatly from alkaloid to alkaloid. Even though many

alkaloids are poisonous, some are used in medicine as analgesics (pain relievers) or anaesthetics. Most alkaloids have a very bitter taste.⁸

Alkaloids are secondary metabolites. Such compounds are only found in specific organisms, or groups of organisms, and are recognised as an expression of the individuality of species. Secondary metabolites are not necessarily produced under all conditions, and in the vast majority of cases the function of these compounds as well as their benefit to the organism is yet to be determined.¹⁰ Levels of secondary plant products, including alkaloids, are known to be strongly influenced by factors such as plant age, season and growing conditions and genetically different chemotypes also occur.^{11,12} Some secondary metabolites are produced for easily appreciated reasons, for example as toxic materials providing defence against predators, or as volatile attractants to attract pollinators, and it is logical to assume that all do play some vital role for the well-being of the producer.^{10,11} It is secondary metabolism that provides most of the pharmacologically active natural products.¹⁰

2.2.2 Structural classification of mesembrane alkaloids.

Although reference has been made to the alkaloids in *Sceletium* being either cocaine-like or hyoscyamine-like, more correctly they show structural similarity to the Amaryllidaceae alkaloids of the crinane class.^{12,13}

There are three basic structural types of mesembrane alkaloids:

- 1) The mesembrine-type alkaloids are based on the 3a-aryl-*cis*-octahydroindole ring system, and are the most common type of mesembrine alkaloids (Fig. 2.1).^{12,13,14,15} This group is exemplified by the structure of mesembrine. The mesembrane alkaloids that belong in this group are mesembrine (**10**), mesembrenone (**11**), mesembrenol (**12**), mesembranol (**13**), sceletenone (**14**), 4'-*O*-demethylmesembrenol (**15**), *O*-acetylmesebrenol (**16**), Δ^7 -mesembrenone (**17**), 4'-*O*-demethylmesembrenone (**18**), 4'-*O*-demethylmesembranol (**19**), 4'-*O*-

methylsceletenone (**20**), 4,5-dihydro-4'-*O*-methylsceletenone (**21**), *N*-demethylmesembrenol (**22**) and *N*-demethylmesembranol (**23**).

- 2) The second type is known as the seco-mesembranes, and this group is exemplified by the structure of joubertiamine (**24**) (Fig. 2.2).^{13,14,16} The mesembrane alkaloids that belong in this group are joubertiamine (**24**), dehydrojoubertiamine (**25**), dihydrojoubertiamine (**26**), joubertinamine (**27**), 3'-methoxy-4'-*O*-methyljoubertiaminol (**28**), 4-(3,4-dimethoxyphenyl)-4-[2-(acetylmethylamino)ethyl]cyclohexanone (**29**), and 4-(3-methoxy-4-hydroxyphenyl)-4-[2-(acetylmethylamino)ethyl]cyclohexadienone (**30**) .
- 3) The third type of mesembrane alkaloids contains a 2,3-disubstituted pyridine moiety, and contains two nitrogen atoms in the molecular formula (Fig. 2.3).^{13,14,17} This group is exemplified by the structure of the alkaloid sceletium A₄ (**31**). The mesembrane alkaloids that belong in this group are sceletium A₄ (**31**), tortuosamine (**32**), *N*-formyltortuosamine (**33**), an unnamed alkaloid (**34**) isolated from *S. namaquense* in 1979 by Jeffs and Capps, and *N*-acetyltortuosamine (**35**).^{18,19}

Hordenine (**36**) (Fig. 2.4) is a non-mesembrane alkaloid present in *Sceletium* species.^{16,19}

2.2.3 Biosynthesis of mesembrane alkaloids.

Most of the mesembrane alkaloids belong to the mesembrine-type group that are based on an octahydroindole ring system.^{12,13,14} The general structure of this group contains a ring system which in principle may be constructed from an aromatic C₆ unit and a familiar C₆C₂N unit. The close structural similarity of mesembrine and related alkaloids to the Amaryllidaceae alkaloids of the crinane class prompted Jeffs et al. (1971)¹³ to consider that biosynthesis of the two groups might follow a parallel course. The results obtained led to the conclusion that the biosynthesis of mesembrine and related octahydroindole alkaloids involves the utilization of the two aromatic amino acids, phenylalanine and tyrosine, via separate pathways for the construction of the basic ring system in

this family. It was established by them that the aromatic ring of phenylalanine provides the aromatic C₆ unit and that tyrosine is utilized for the elaboration of the C₆C₂N unit (Scheme 2.1).¹³

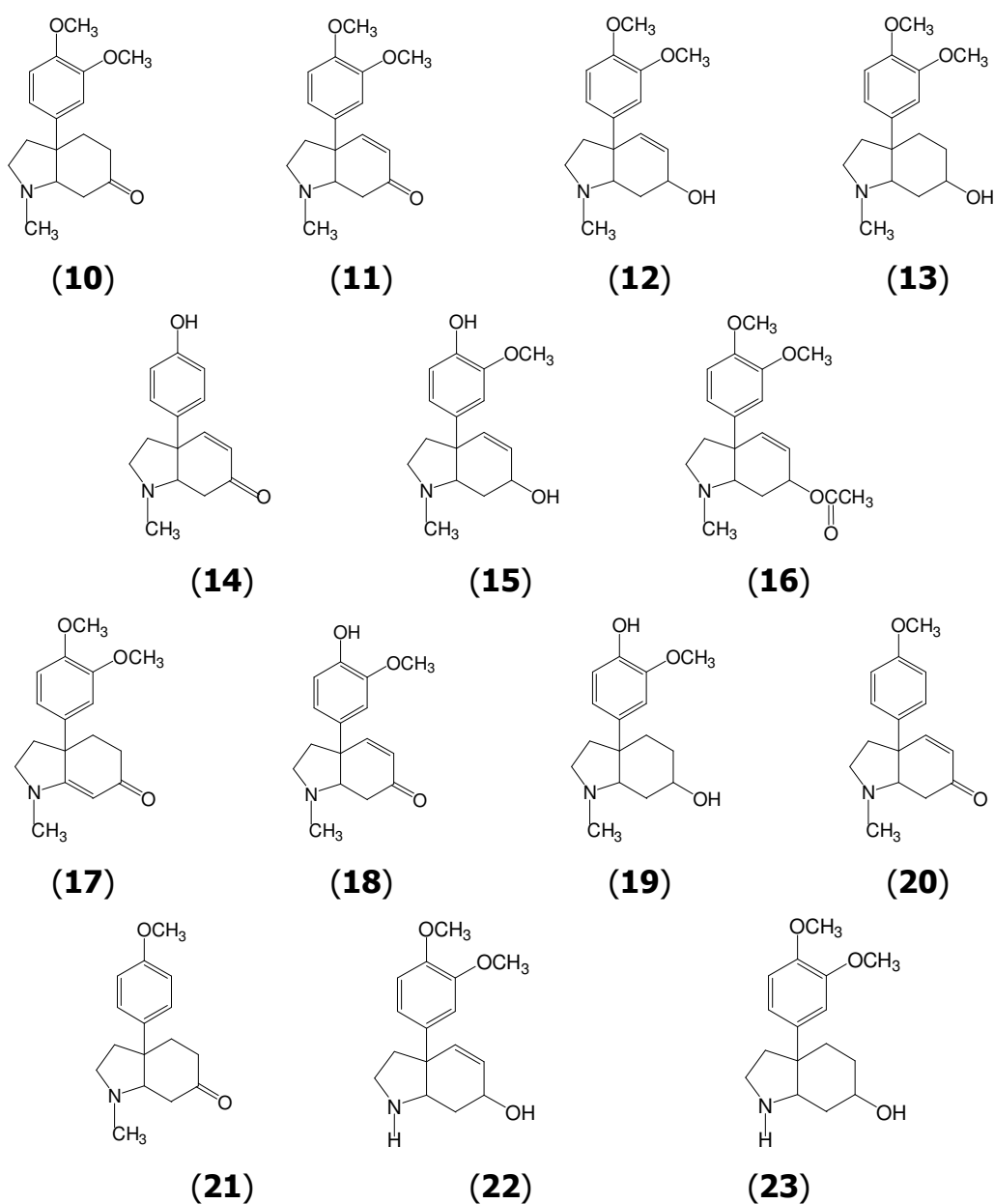


Figure 2.1: The structures of the mesembrine-type mesembrane alkaloids: mesembrine (10), mesembrenone (11), mesembrenol (12), mesembranol (13), sceletenone (14), 4'-*O*-demethylmesembrenol (15), *O*-acetylmesebrenol (16), Δ⁷-mesembrenone (17), 4'-*O*-demethylmesembrenone (18), 4'-*O*-demethylmesembranol (19), 4'-*O*-methylsceletenone (20), 4,5-dihydro-4'-*O*-methylsceletenone (21), *N*-demethylmesembrenol (22), *N*-demethylmesembranol (23).^{13,14,15,16,20}

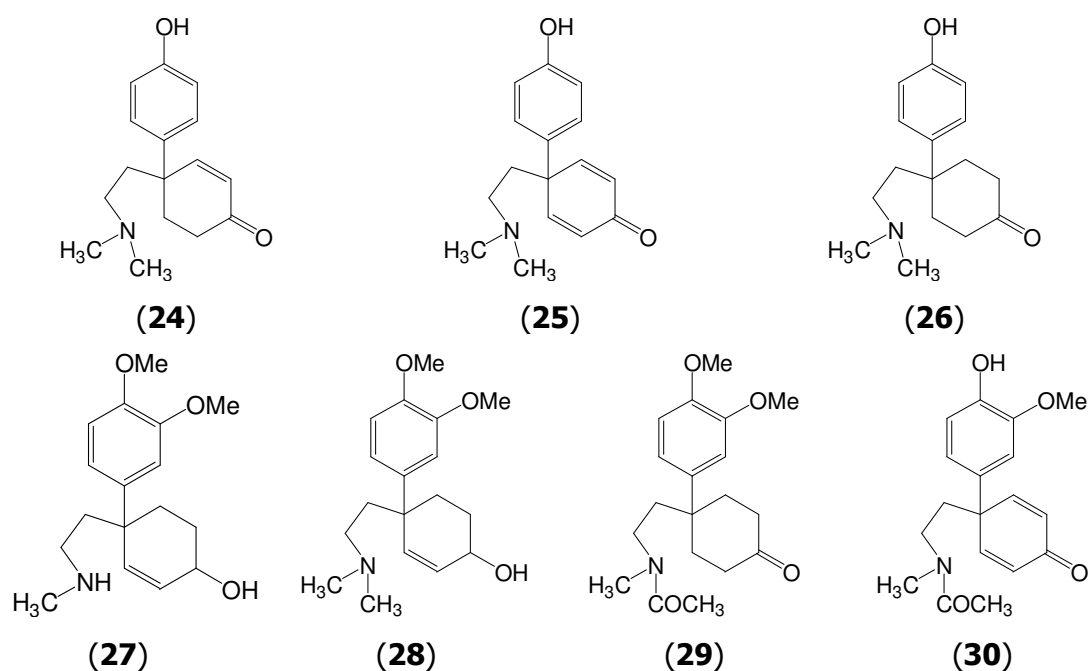


Figure 2.2: The structures of the seco-mesembrane alkaloids: joubertiamine (**24**), dehydrojoubertiamine (**25**), dihydrojoubertiamine (**26**), joubertinamine (**27**), 3'-methoxy-4'-O-methyljoubertiaminol (**28**), 4-(3,4-dimethoxyphenyl)-4-[2-(acetylmethylamino)ethyl]cyclohexanone (**29**), 4-(3-methoxy-4-hydroxyphenyl)-4-[2-(acetylmethylamino)ethyl]cyclohexadienone (**30**).^{16,21,22}

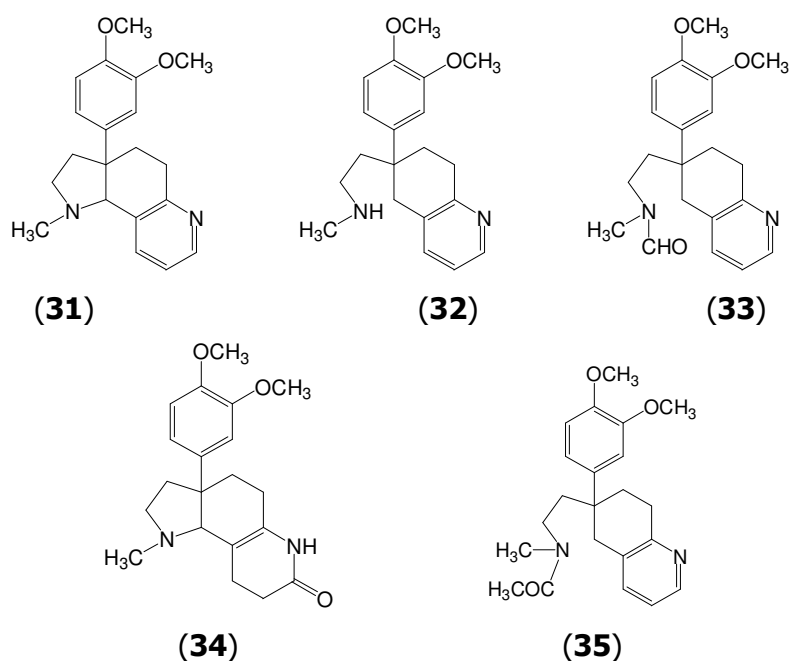


Figure 2.3: The structures of the third type of mesembrane alkaloids: scelletium A₄ (**31**), tortuosamine (**32**), *N*-formyltortuosamine (**33**), Un-named alkaloid (**34**) isolated in 1979, *N*-acetyltortuosamine (**35**).^{13,14,17,18,22}

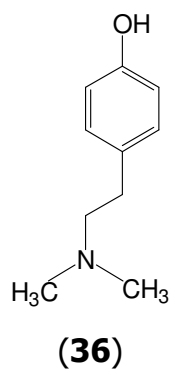
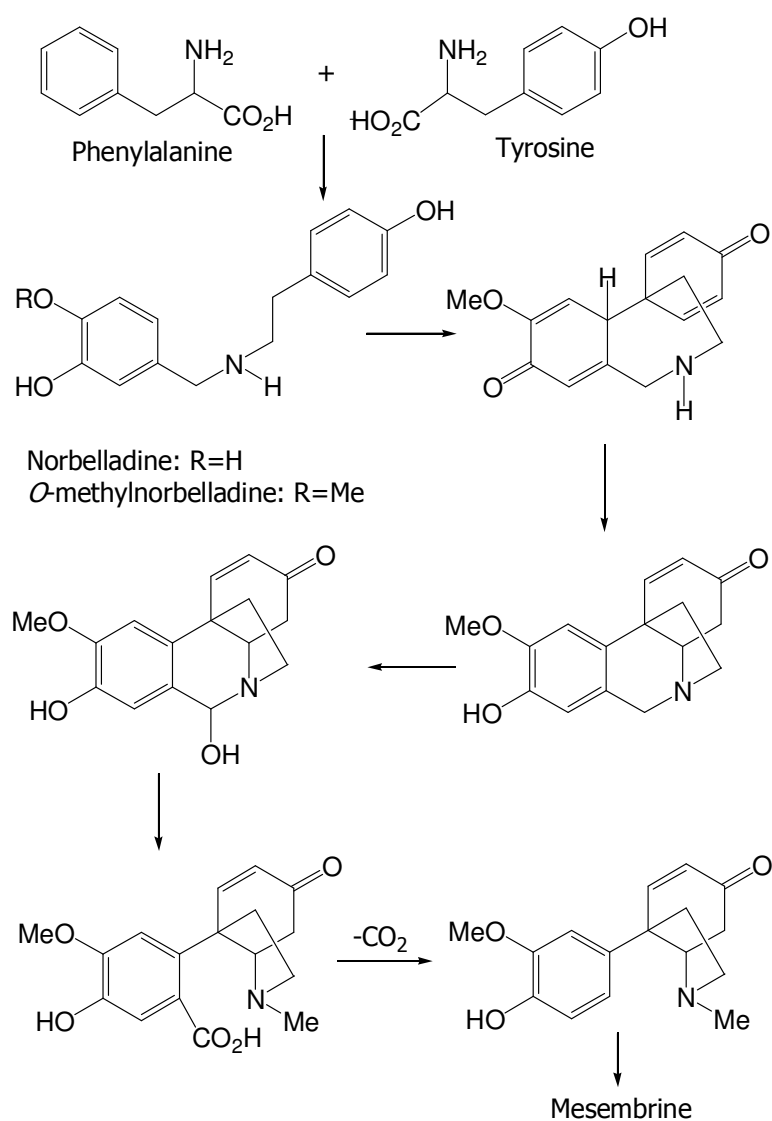
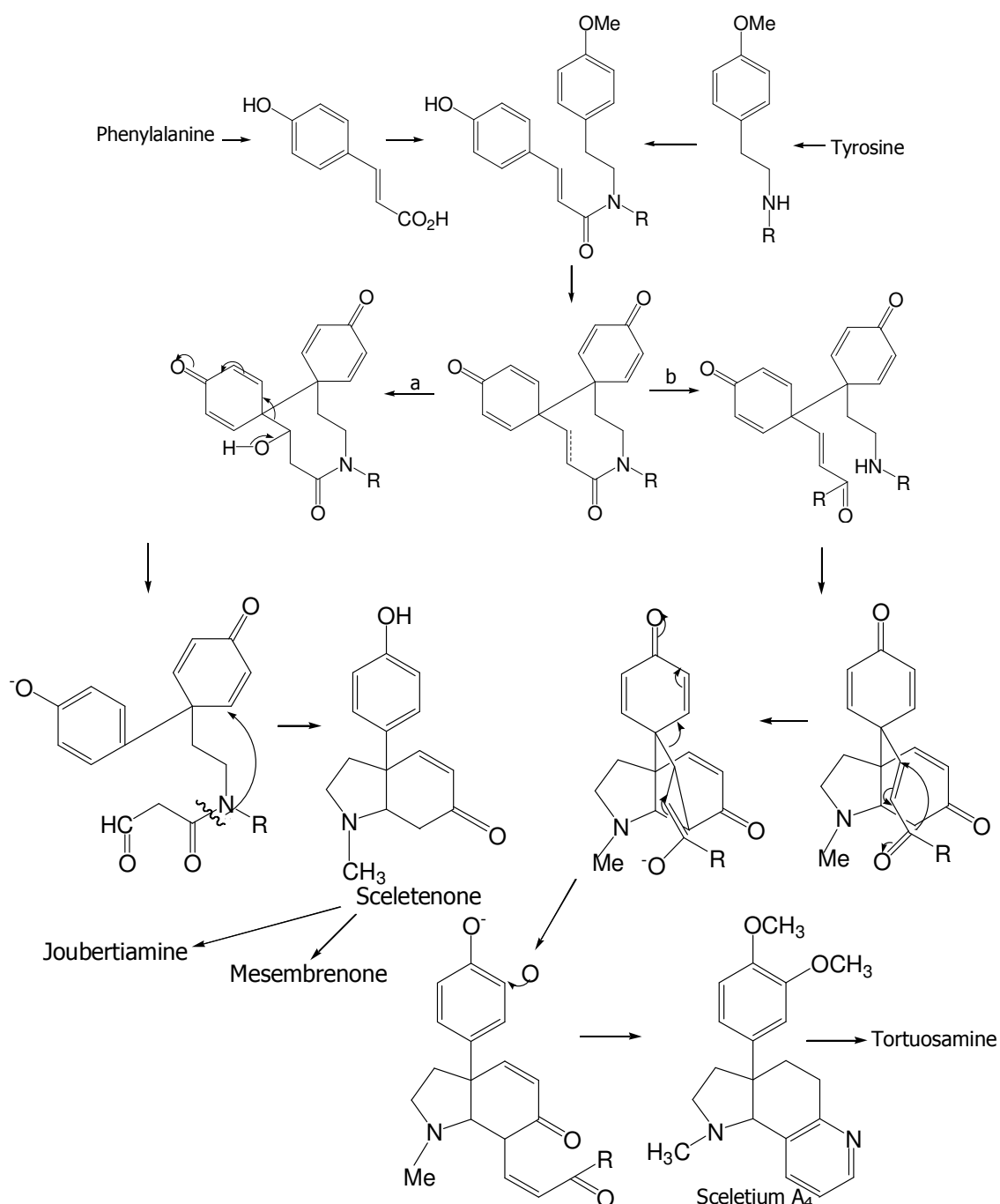


Figure 2.4: The structure of hordenine **(36)**.^{16,19}



Scheme 2.1: The proposed biogenetic pathway for mesembrine as suggested by Jeffs et al. (1971).¹³

In 1974, Jeffs et al.¹⁴ proposed a revised unified biogenetic scheme for the biosynthesis of the mesembrane alkaloids which accounts for the origins of the various ring systems of the different classes of mesembrane alkaloids, including the mesembrine-type, seco-mesembranes, and the third type of mesembrane alkaloids.¹⁴



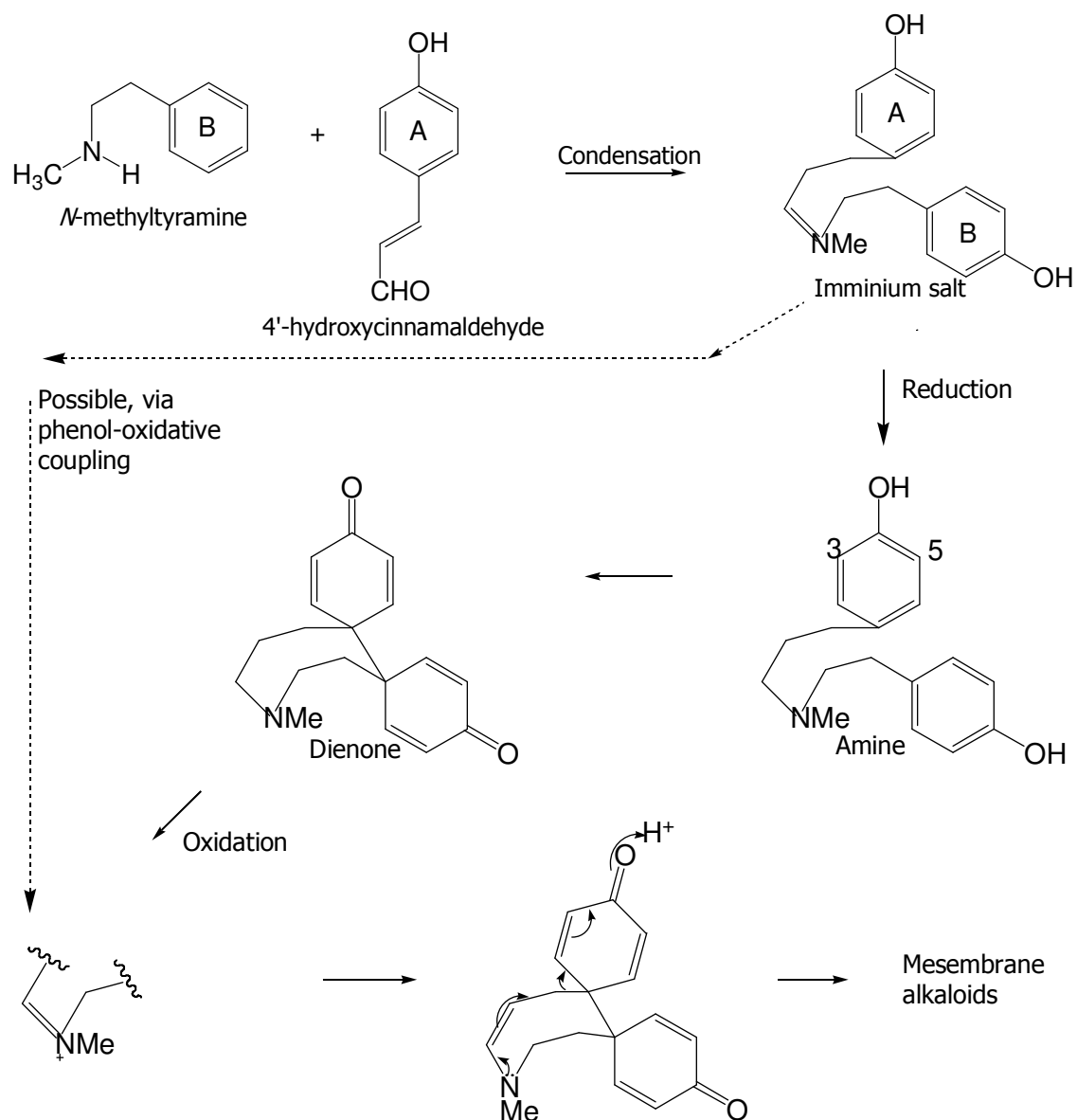
Scheme 2.2: The biogenetic pathway for the mesembrane alkaloids as proposed by Jeffs et al. (1974).¹⁴

By 1978, Jeffs et al.²⁴ had concluded that although the aromatic amino acid precursors of the Amaryllidaceae alkaloids and the mesembrane alkaloids are the same, the biosynthesis of the two classes of alkaloid are fundamentally different. During the course of their studies, they ruled out the possibility that the biosynthesis of the mesembrane alkaloids proceeds via an extension of the crinine pathway.²³

In 1989 Herbert and Kattah¹⁹ reported that the biosynthetic origins of the mesembrane alkaloids lie in the amino acids tyrosine via tyramine and *N*-methyltyramine, and phenylalanine by way of 4-hydroxyphenylpropionic acid and closely related compounds. In 1990, Herbert and Kattah²⁴ followed up on their proposed biosynthetic scheme by providing more information on the intermediates involved. The proposed biosynthesis are summarised in Scheme 2.3.

2.2.4 Biological properties of mesembrane alkaloids.

Mesembrine and related compounds are recognised as serotonin-uptake inhibitors. Pharmaceutical compositions containing such compounds are recommended for use in the treatment of psychological or psychiatric disorders with an anxiety component, depressive states, alcohol and drug dependence, bulimia nervosa, and obsessive-compulsive disorders.²⁶



Scheme 2.3: The biogenetic pathway for the mesembrane alkaloids as proposed by Herbert and Kattah (1989 and 1990).^{19,24}

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Chapter 3

Investigation into the genus *Sceletium*

3.1 Introduction

The genus *Sceletium* N.E.Br. is classified as part of the family Mesembryanthemaceae and belongs to the sub-family Mesembryanthemoideae.¹ The genus name *Sceletium* is derived from the Latin word *sceletus*, meaning skeleton. Two common names that are sometimes used when referring to plants of this genus are living skeletons or skeleton plants. The name *Sceletium* refers to the prominent leaf veins that persist as a skeleton-like structure in the dry leaves of the plants, making them easily recognisable. During dry periods, these skeletonised leaves enclose the young leaves, thus protecting them from adverse environmental conditions.^{1,2} Eight species of *Sceletium* are currently recognised, *i.e.* *S. crassicaule* (Haw.) L.Bolus, *S. emarcidum* (Thunb.) L.Bolus ex H.Jacobsen, *S. exalatum* Gerbaulet, *S. expansum* (L.) L.Bolus, *S. rigidum* L.Bolus, *S. strictum* L.Bolus, *S. tortuosum* (L.) N.E.Br., and *S. varians* (Haw.) Gerbaulet.^{1,3}

Kanna and *kougoed* (meaning something to chew) are two well-known vernacular names for *Sceletium* species (Fig. 3.1).^{1,2,4} *Kougoed* refers to a traditional preparation made from *S. emarcidum* or *S. tortuosum*, which is used as a stimulant with an effect not unlike that of tobacco.^{1,5} *S. varians* is known as *soutvygie* (salt mesemb).¹

The traditional method for the preparation of *Sceletium*-derived *kougoed* is as follows:

The plant material (usually a whole plant, including the roots) is crushed between stones following harvest, following which it is then placed into closed containers for several days to 'ferment'. Historically, a skin or canvas bag was used as a fermentation vessel, but plastic bags have replaced these in recent times. The bag

of crushed '*kougoed*' is then left in the sun to get warm and sweat. It is reportedly not necessary to put the bag in the shade, as 'it gets shade at night, and the sun doesn't harm it'. After 2-3 days the bag is opened, the '*kougoed*' is mixed around, and then the bag is tightly sealed again. On the eighth day after crushing, the bag is opened and the *kougoed* is spread out to dry in the sun. It is left out until it is completely dry. The finished product is light brown and stringy, and reportedly 'unattractive in appearance'.^{2,4,6}

An alternative *kougoed* preparation technique is reported, which is employed when the user seeks to prepare *kougoed* rapidly:

A small fire is made over sand, and when it dies down, the ashes are scraped aside and a hollow is made in the sand. A freshly picked whole *Sceletium* plant is placed in this excavation, and is covered with hot sand. An hour later the baked plant product is recovered from the sand, reputedly with acquired properties similar to the conventionally prepared material.⁴



Figure 3.1: *Kougoed* (left), prepared from *Sceletium tortuosum* (right).

Photograph from Smith et al 1998.¹

The Khoisan of South Africa first devised the preparation of the *Sceletium*-derived product *kougoed*, which is reported in the literature to have psychoactive properties.^{4,6} Plants of the genus *Sceletium* have been shown to contain alkaloids, which are suspected to be responsible for the medicinal attributes of plants of this

genus.^{4,5,6,7} The impression is given in the literature that *Sceletium* is a richer source of alkaloids than any other Mesembryanthemaceae.^{4,6}

The flowering time for *Sceletium* is from autumn to early summer (May to November in South Africa). Plants of the genus occur in karroid areas of the Eastern, Western, and Northern Cape Provinces of South Africa. Plants are frequently found beneath other shrubs into which they may sometimes scramble. *Sceletium* occurs in both summer and winter rainfall areas. They generally prefer rocky inland sites to coastal habitats.¹

3.2 Literature Review

3.2.1 Historical record of the genus *Sceletium*.

Plants of the genus *Sceletium* were first observed to contain an alkaloid by Meiring in 1898, who isolated the then unknown mesembrine from *Sceletium tortuosum*.^{4,6} He demonstrated a quick physiological response in frogs to subcutaneous injection of a 'few drops' of the alkaloid.⁴ Some sixteen years later, in 1914, E. Zwicky (a student of Professor C. Hartwick in Zurich) produced a dissertation titled 'Über channa' in which *S. expansum* and *S. tortuosum* were reported to contain alkaloids, to which the name mesembrin (now mesembrine) was given.^{4,6}

The use by the Khoisan of South Africa of *Sceletium* plants in psychoactive preparations has often been mentioned in the literature.^{2,4} Plants of the genus *Sceletium* were reportedly highly valued and sought after by both Khoi pastoralists and San (Bushmen) hunter-gatherers.^{4,8} Apart from its psychoactive properties, kougoed is reported to ward off thirst.⁶

In 1662 van Riebeeck bartered with the local inhabitants, in turn receiving sheep and 'kanna'.^{4,9} The Europeans prized this plant as a ginseng-like herb.⁴ It is recorded in 1685 by van der Stel, the second colonial governor of the Dutch Cape colony, in his journal:

'They chew mostly a certain plant which they call *Canna* and which they bruise, roots as well as the stem, between the stones and store and preserve in sewn-up sheepskins. When we came to the Coperbergh in October, it was being gathered from the surrounding hills by everybody (to serve as a supply for the whole year). They use it as the Indians use betel or areca, and are of a very cheerful nature.⁵ Accompanying the illustration in van der Stel's journal, which leaves little doubt that the plant was a species of *Sceletium*, was the caption:

'This plant is found with the Namaquaas and then only on some of their mountains... It is held by them and surrounding tribes in as great esteem as the betel or areca with the Indians. They chew its stem as well as the roots, mostly all day, and become intoxicated by it, so that on account of this effect and its fragrance and hearty taste one can expect some profit from its cultivation.⁴

Van der Stel's observation is probably the first to attribute intoxicating psychoactive properties to *Sceletium*.⁴ In 1773, Thunberg also noted the value of *Sceletium* as a trade item and its value in suppressing hunger and thirst:

'The Hottentots (Khoi People) come far and near to fetch this shrub with the root, leaves and all, which they beat together, and afterwards twist them up like pig-tail tobacco; after which they let the mass ferment and keep it by them for chewing, especially when they are thirsty. If chewed immediately after fermentation, it intoxicates. The Hottentots, who live near this spot hawk it about, frequently to a great distance, and exchange it for cattle and other commodities.⁴ From these accounts, it is evident that there was an annual almost ritualistic gathering of *Sceletium* at a specific locality, and that the value of the material warranted such collection, as well as storage and barter.^{4,6}

3.2.2 Chemistry of *Sceletium* alkaloids.

Meiring first isolated the alkaloid mesembrine from *S. tortuosum* in 1898.⁶ Chemists in the USA initially, followed later by chemists in South Africa, conducted research on the mesembrane alkaloids isolated by Zwicky (1914) and revealed a complex array of such compounds in a number of *Sceletium* species.¹⁰ *S.*

tortuosum was reported by Watt and Breyer-Brandwijk in 1932 to contain 0.3% mesembrine in the leaves and 0.86% mesembrine in the stems.⁸ Popelak and Lettenbauer (1967)¹¹ reported that levels of alkaloids in '*kougoed*' ranged from 1-1.5%, while mesembrine and mesembrenone levels were 0.7% and 0.2% respectively. While these results suggest that mesembrine may often be the most abundant alkaloid, it is important to note that the phenolic constituents of the plant represent a highly complex, multi-component mixture.⁴

Prior to 1970, studies on *S. namaquense* (now classified as part of *S. tortuosum*), *S. tortuosum*, and *S. anatomicum* (now classified as part of *S. emarcidum*) had led to the isolation and characterization of the alkaloids mesembrine (**10**), mesembrenone (**11**), and mesembranol (**13**). In 1970, Jeffs et al.¹² investigated *S. strictum* and isolated four new alkaloids, one major and three minor constituents. These new alkaloids were given the names mesembrenol (**12**) (the major component isolated), 4'-*O*-demethylmesembrenol (**15**), *O*-acetylmesebrenol (**16**) and 4'-*O*-demethylmesembranol (**19**). The presence of the three previously recognised alkaloids, mesembrine (**10**), mesembrenone (**11**), and mesembranol (**13**), was also observed.⁴ All the mesembrane alkaloids isolated and identified up until 1970 time were based upon the 3-aryl-*cis*-octahydroindole skeleton, and belong to the mesembrine-type group of mesembrane alkaloids.¹³

In 1970, Arndt and Kruger investigated *S. joubertii* (now classified as part of *S. tortuosum*) and isolated a further three new mesembrane alkaloids which are based upon a different skeleton to the known mesembrane alkaloids, and belonging to a group identified as the seco-mesembranes.^{5,13} They named these alkaloids joubertiamine (**24**), dehydrojoubertiamine (**25**) and dihydrojoubertiamine (**26**). They also isolated a known non-mesembrane alkaloid, hordenine (**36**), from this species.⁵

Jeffs et al.¹⁴ reported on the structure of the alkaloid scelletium A₄ (**31**), isolated from *S. namaquense* (now classified as *S. tortuosum*), in 1971. It was reported

that sceletium A₄ was of neither the mesembrine-type of mesembrane alkaloids nor the seco-mesembranes, but represented a new structural type of *Sceletium* alkaloids that contains a 2,3-disubstituted pyridine moiety.¹⁴ Snyckers and colleagues isolated sceletium A₄ (**31**) and tortuosamine (**32**) from *S. tortuosum* in 1971.¹⁵

In 1974, Jeffs et al. continued their investigations into the *Sceletium* alkaloids and expanded their studies to include attempts to elucidate the biosynthetic route to the octahydroindole alkaloids of the mesembrine series. They undertook a study of the minor alkaloids of *S. strictum* and *S. tortuosum* (as *S. namaquense*) with the view that characterization of new structural types may prove useful in the elucidation of previously unsuspected biosynthetic relationships in the mesembrine series. They isolated mesembrine (**10**), mesembrenone (**11**), sceletium A₄ (**31**), as well as two new alkaloids, Δ^7 -mesembrenone (**17**) and *N*-formyltortuosamine (**33**), from *S. tortuosum* (= *S. namaquense*). Their studies also resulted in the isolation and characterization of the alkaloids sceletenone (**14**), 4'-*O*-demethylmesembrenol (**15**), 4'-*O*-demethylmesembrenone (**18**) and 4'-*O*-demethylmesembranol (**19**) from *S. strictum*.¹³ In 1979, a new alkaloid base, which constitutes the second member of the same structural class as sceletium A₄, was isolated from *S. tortuosum* (as *S. namaquense*). This alkaloid (**34**), which was shown to have the structural formula C₂₀H₂₆N₂O₃, was not given a name in the article.¹⁶ The alkaloids isolated from *Sceletium* species are summarised in Table 3.1.

In 1998, Smith et al. investigated *S. tortuosum* and the *Sceletium*-derived kougoed along with several other mesembs for the distribution of mesembrane alkaloids within selected taxa of the Mesembryanthemaceae. *S. tortuosum* was found to contain the mesembrane alkaloids mesembrine (**10**), mesembrenone (**11**) and 4'-*O*-demethylmesembrenol (**15**), all in the highest levels of all the taxa studied. Following the fermentation process, kougoed was studied and a definite change in alkaloid levels was observed. The levels of mesembrine (**10**) and 4'-*O*-demethylmesembrenol (**15**) showed a marked decline; in fact the levels of

mesembrine (**10**) were observed to be half those in untreated *S. tortuosum* and the levels of 4'-*O*-demethylmesembrenol (**15**) were almost completely diminished. The levels of mesembrenone (**11**) showed a significant increase in *kougoed*, where they were double those in untreated *S. tortuosum*.⁶

Table 3.1: Alkaloids isolated from *Sceletium* species

Mesembrine-type	Seco-mesembranes	Third type of mesembranes
Mesembrine (10)	Joubertiamine (22)	Sceletium A4 (25)
Mesembrenone (11)	Dehydrojoubertiamine (23)	Tortuosamine (26)
Mesembrenol (12)	Dihydrojoubertiamine (24)	<i>N</i> -Formyltortuosamine (27)
Mesembranol (13)	Joubertinamine (27) ¹⁶	Un-named alkaloid (28)
Sceletenone (14)	3'-Methoxy-4'- <i>O</i> '-methyl joubertiaminol (28) ¹⁷	<i>N</i> -Acetyltortuosamine (35) ¹⁷
4'- <i>O</i> -Demethylmesembrenol (15)	4-(3,4-Dimethoxyphenyl)-4-[2-(acetylmethylamino)ethyl]cyclohexanone (29) ¹⁷	Other
<i>O</i> -Acetylmesebrenol (16)	4-(3-Methoxy-4-hydroxyphenyl)-4-[2-(acetylmethylamino)ethyl]cyclohexadienone (30) ¹⁷	Hordenine (36)
Δ^7 -Mesembrenone (17)		
4'- <i>O</i> -Demethylmesembrenone (18)		
4'- <i>O</i> -Demethylmesembranol (19)		
<i>N</i> -Demethylmesembrenol (22) ¹⁵		
<i>N</i> -Demethylmesembranol (23) ¹⁵		

Mesembrine is known today as a naturally occurring serotonin uptake inhibitor and has become an interesting lead compound for the preparation of antidepressants.^{10,20,21} The *Sceletium* alkaloids in general have continued to interest synthetic organic chemists as attractive molecular targets.¹⁰

3.2.3 Ethnopharmacology of *Sceletium*

During his studies, Zwicky (1914) studied the effects of *kougoed* and reported feelings of discomfort, analgesia and a slight headache following ingestion of *kougoed*, swallowing a decoction, or taking the hydrochloride salt of the alkaloid. No stimulatory action was observed.⁴ In 1928, Laidler⁹ noted that *kougoed* was 'chewed (by the indigenous peoples) and retained in the mouth for a while, when their spirits would rise, eyes brighten and faces take on a jovial air, and they

would commence to dance. But if indulged in to excess it robbed them of their senses and they became intoxicated'.^{4,9}

It has been reported that *S. tortuosum* was used as a narcotic by the African people in the Queenstown district, although it is said to be narcotic only after fermentation. In earlier times farmers in the Cape used a decoction or tincture of *S. tortuosum* as a sedative. The plant has also been reported to be used as an intoxicant and the Khoi people are said to have prized it for its effect in increasing strength. Watt and Breyer-Brandwijk reported that the Khoi people chew the leaf in order to provide relief for toothache and pains in the abdomen.^{8,9} The Nama use kougoed for the relief of hunger as well as pain of any kind.⁸ A Nama mother reportedly chews the root of *Sceletium* and ejects her saliva into the mouth of her child from an early age.^{6,8,9} In the literature there is also mention of the juice of the plant being used, on account of its alkalinity, for washing clothes during shortage of soap.⁸

Several individuals have self-experimented with *kougoed*, and have described the effects thereof. Most users found that *kougoed* caused a marked anxiolytic effect. One user has reported that the use of the product prior to giving a lecture induced a relaxed feeling with no cognitive impairment. Many users felt that the use of *kougoed*, on its own or in addition to alcohol, enhanced social interaction at functions and said they felt considerably less inhibited and self-conscious, making them more open than usual in conversation with strangers.⁴

Although the primary form of ingestion of *kougoed* is reported to be by chewing the product, there are also reports of it being taken as a tea and also as a snuff.^{4,6,22} Reputed negative side effects of *kougoed* include headaches, depression, loss of appetite and listlessness.⁴

3.3 Results and Discussion

In this study, three different species of *Sceletium*, *S. crassicaule*, *S. rigidum* and *S. tortuosum* (two different plants), were investigated for the presence of mesembrane alkaloids. Limited amounts of material of the two *S. tortuosum* plants as well as *S. rigidum* were available. Where sufficient material was available to be separated into plant parts, extracts of the various parts of the plants were prepared, i.e. extracts of fruit and flowers, leaves, stems, and roots. Where insufficient material was available, the material that could be obtained was extracted as a single extract and named 'mixed plant parts'. Comprehensive information on all plants screened in this study can be found in Tables 7.1 and 7.2 of Chapter 7, as well as Tables A.1 and A.2 in Appendix 1. Although the alkaloid chemistry of *S. tortuosum* has been investigated extensively,^{4-6,8,10,13-20} this is the first report on the alkaloids of *S. crassicaule* and *S. rigidum*.

It must be emphasised that the limited amount of material available throughout this study has placed certain constraints on the project. Earlier workers had large amounts of plant material available [Nieuwenhuis et al.²³ (5.7 kg), Arndt and Kruger⁶ (3.3 kg), Jeffs et al.¹³ (3.5 kg)] and to obtain this much material for extraction they would have had to obtain a fair-size population of *Sceletium*. Almost all of the plants used in this investigation are not cultivated and to obtain these amounts of material from the wild was not considered feasible or advisable. Therefore, we have started with much smaller amounts of plant material and the result of that was that we were forced to rely on analytical methods (thin-layer chromatography and GC/MS) for the identification of the compounds. Only in a few instances were we able to isolate the compounds by chromatography and subject it to rigorous identification by NMR spectroscopy.

Alkaloid extracts of the four *Sceletium* plants were prepared as described in Chapter 7. The extracts were then studied on thin-layer chromatography (TLC) using a solvent system of cyclohexane:chloroform:diethylamine in the ratio 10:6:1. Following a drying period of an hour, the TLC plates were visualised with

the aid of UV radiation (UV_{254}) followed by spraying with an iodoplatinate reagent that reacts with and allows the identification of nitrogen-containing compounds. Gas chromatography/mass spectrometry (electron impact ionisation) was then carried out on the extracts. A mesembrine (**10**) standard was obtained and used to obtain the retention time of mesembrine on the GC. The identification of the alkaloid mesembrine in the standard was confirmed by NMR spectroscopy (Fig 3.2, 3.3 and 3.4).

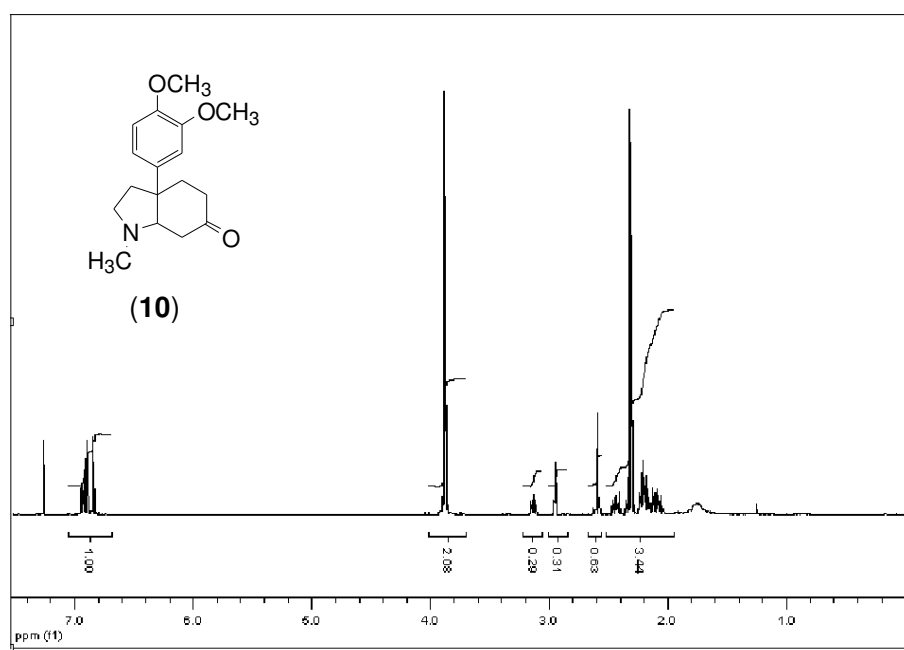


Fig 3.2. 300 MHz ^1H NMR spectrum of mesembrine (**10**).

The most prominent feature of the ^1H NMR spectrum of mesembrine (**10**) is the presence of three sharp three-proton signals at δ_{H} 3.89, 3.87 and 2.31 corresponding to the two methoxy groups and one N-methyl group, respectively (Fig. 3.2). Furthermore, there is also evidence for a trisubstituted aromatic ring with a typical AMX spin system (Fig. 3.3). The remainder of the protons resonate between δ 3.2 and 2.0, and cannot be analysed in detail due to the substantial overlap of the signals.

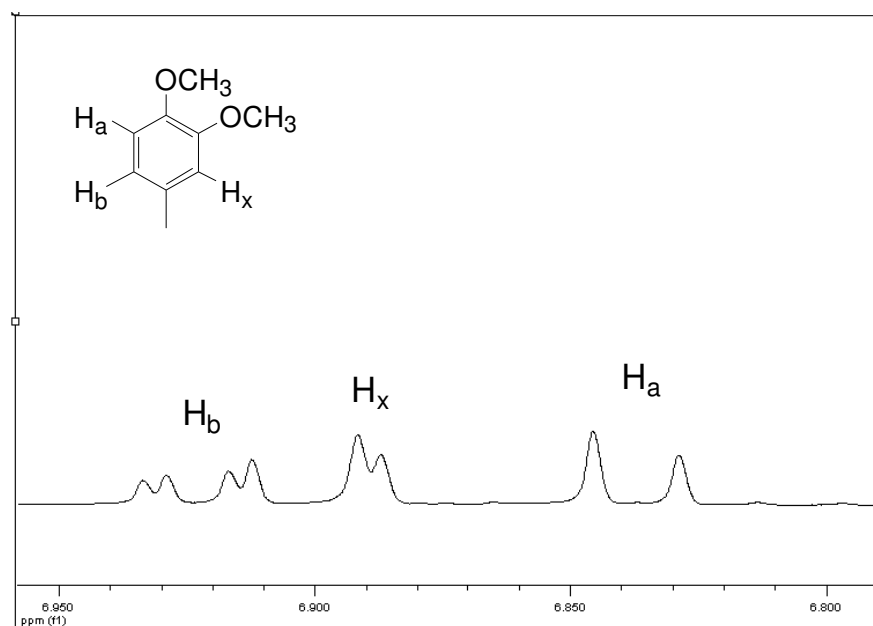


Fig 3.3. Expansion of the aromatic region of the ^1H NMR spectrum of mesembrine **(10)**

The ^{13}C NMR spectrum (Fig. 3.4) of mesembrine **(10)** shows the presence of a carbonyl carbon (δ_{C} 211.5) and six carbons in the aromatic region corresponding to the benzene ring. The NMR data of mesembrine **(10)** are in close agreement with the published values.²⁴

Mesembrine was run prior to the other samples on the GC/MS, and the retention time of mesembrine was identified as between 24.0 and 24.2 minutes. Structures of the other compounds were assigned based on their respective mass spectra.

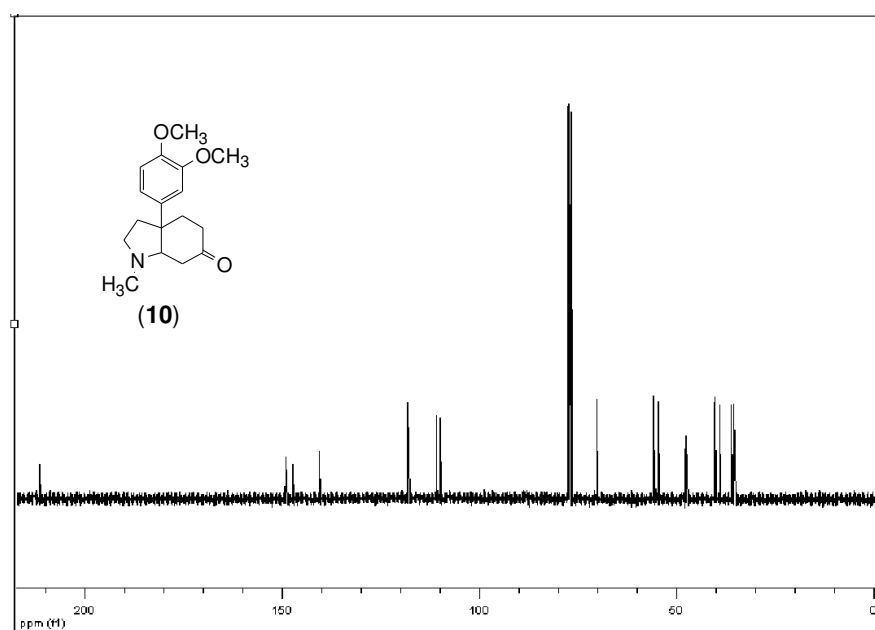


Fig 3.4. ^{13}C NMR spectrum of mesembrine (**10**).

From the literature it is evident that four fragments are often observed to be present in the MS spectra of mesembrane alkaloids. Peaks are observed at m/z 219, m/z 72, m/z 58 and m/z 44 and the corresponding structures of the four fragments are given in Figure 3.5. The ion that results in a peak at m/z 219 is considered to be characteristic of the mesembrine-type of mesembrane alkaloids.^{4,13} The ions at m/z 58 and m/z 72 resulted from fragmentation of *N,N*-dimethyl seco-mesembrane alkaloids such as joubertiamine (**24**), whereas the fragment with m/z 44 is derived from a *N*-methyl seco-mesembrane alkaloid such as joubertinamine (**27**). To a lesser extent, library searches from the commercial library of the mass spectrometer were also used.

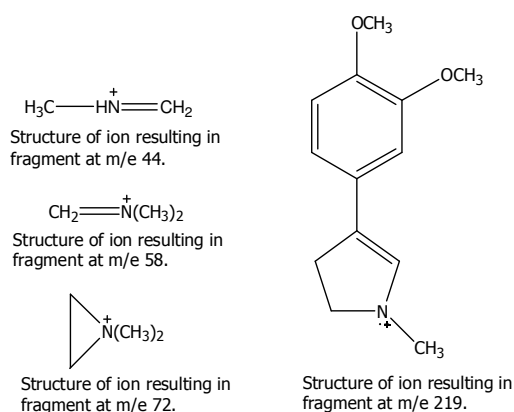


Figure 3.5: The structures of the ions that result in peaks at m/z 44, m/z 58, m/z 72 and m/z 219 on GC/MS spectra.^{5,14}

Three of the four *Sceletium* plants investigated, *S. crassicaule* and the two *S. tortuosum* plants, were observed to contain nitrogen-containing compounds when screened using TLC. No nitrogen-containing compounds could be detected by TLC in the *S. rigidum* extract. GC/MS of the four *Sceletium* extracts were performed to confirm the presence or absence of any mesembrane alkaloids, and to investigate the identities of any mesembrane alkaloids present. It confirmed the presence of mesembrane alkaloids in the extracts of *S. crassicaule* and the two *S. tortuosum* and that the *S. rigidum* extract did not contain any mesembrane alkaloids, nor did it contain hordenine (**36**). This is not surprising, as it must be emphasised that very little material of *S. rigidum* was available for extraction (~200mg), and it is also a recognised fact that within the genera of the Mesembryanthemaceae different species of the same genus have variable levels of alkaloids.

S. crassicaule was observed to contain up to four of the mesembrine-type of mesembrane alkaloids, as well as hordenine in its various parts. One of the two *S. tortuosum* plants investigated in this study was identified as being the Namaqualand variety of *S. tortuosum* (previously known as *S. namaquense*). Both of the *S. tortuosum* extracts contained mesembrane alkaloids of the mesembrine-type, but not the alkaloid hordenine. The distribution of the mesembrane alkaloids between the various species the genus *Sceletium* can be seen in Table 3.2. The

retention times of hordenine and the mesembrane alkaloids can be found in Table 3.3.

It is widely recognised that the alkaloid constituent of plants of the genus *Sceletium* and of the other genera of the Mesembryanthemoideae represent a highly complex, multi-component mixture.¹³ Hordenine is present in the leaves, stems and roots of *S. crassicaule*, but it is not present in either of the other two species investigated. Mesembrenone is the major alkaloid present in all three *S. crassicaule* extracts, followed by mesembrenol in the leaves and stems (mesembrenol is absent in the roots), and mesembrine in the roots. The third most prevalent alkaloid in the leaves of *S. crassicaule* is mesembrine, followed by hordenine. In *S. crassicaule* stems, the third most prevalent alkaloid is hordenine, followed by mesembranol and then mesembrine. In the roots of *S. crassicaule*, hordenine is only present in trace amounts.

It was observed that while the leaves of *S. tortuosum* contain several mesembrane alkaloids, the stems of the same plant contain no mesembrane alkaloids, and both the leaves and the stems of *S. tortuosum* ("*S. namaquense*" sample) contain only mesembrine. This is not unexpected as alkaloids are secondary metabolites in plants, and the levels of secondary plant products are known to be strongly influenced by several factors including geographical race. The alkaloids present in the leaves of *S. tortuosum*, ranging from most prevalent to least prevalent, are mesembrine, mesembranol, mesembrenone and mesembrenol. In 1998, Smith et al. investigated the distribution of the mesembrine alkaloids in selected taxa of the Mesembryanthemaceae.⁶ Among several genera, they investigated *S. tortuosum* using GC/MS and found that it contained the alkaloids mesembrine (highest levels), mesembrenone (second highest levels) and 4'-*O*-demethylmesembrenol (low levels). This is in keeping with the results obtained in this study.

Table 3.2 :Alkaloids present and prevalence observed in the genus *Sceletium*. (For voucher specimens, see chapter 8)

Genus and species	Alkaloids and retention times on GC/MS.						
	Hordenine 14.8 - 15.5 min	4,5-dihydro-4'- <i>O</i> -methyl sceletenone 22.6 - 22.8 min	4'- <i>O</i> -methyl sceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>Sceletium</i>							
<i>S. crassicaule</i> leaves	4	-	-	2	-	3	1
<i>S. crassicaule</i> roots	Trace	-	-	-	-	2	1
<i>S. crassicaule</i> stems	3	-	-	2	4	5	1
<i>S. rigidum</i> leaves	-	-	-	-	-	-	-
<i>S. tortuosum</i> (" <i>S. namaquense</i> ") leaves	-	-	-	-	-	1	-
<i>S. tortuosum</i> (" <i>S. namaquense</i> ") stems	-	-	-	-	-	Trace	-
<i>S. tortuosum</i> leaves	-	-	-	4	2	1	3
<i>S. tortuosum</i> stems	-	-	-	-	-	-	-

The numbers given under each alkaloid indicates how dominant the alkaloid is in the sample, with 1 indicating most dominant alkaloid.

"Trace" indicates that only trace levels of the alkaloid(s) were detected.

"-" indicates non-detectable levels of alkaloids.

Table 3.3: Hordenine and Mesembrane Alkaloids Retention Times on GC/MS.

Retention time (min)	Alkaloid identified
14.8 - 15.5	Hordenine (36)
22.6 - 22.8	4,5-dihydro-4'- <i>O</i> -methylsceletenone (21)
22.7 - 23.0	4'- <i>O</i> -methylsceletenone (20)
23.8 - 23.9	Mesembrenol (12)
23.9 - 24.0	Mesembranol (13)
24.0 - 24.2	Mesembrine (10)
24.2 - 24.4	Mesembrenone (11)

It is interesting to observe that only mesembrane alkaloids of the mesembrine-type were observed in all the *Sceletium* plants investigated in this study. No seco-mesembranes or alkaloids of the third type of mesembrane alkaloids were detected in any of the *Sceletium* plants studied. This may be due to the seco-mesembranes being present in plants of the genus *Sceletium* in trace levels.

3.4 Conclusion

Investigations into three different species of *Sceletium*, including *S. crassicaule*, *S. rigidum* and two different *S. tortuosum* plants, revealed that *S. rigidum* was the only species found to be negative for alkaloids. *S. tortuosum* ("*S. namaquense*") was observed to contain mesembrine only. *S. crassicaule* and *S. tortuosum* were found to contain a number of mesembrane alkaloids distributed within the various parts of the plants.

While the mesembrane alkaloids mesembrenone, mesembrenol, mesembranol, and mesembrine were all found to be present in various amounts in the leaf and stem extracts of *S. crassicaule* and the leaf extracts of *S. tortuosum*, the retention times of these alkaloids on GC/MS are so close that it was not possible to obtain any of the compounds in pure form. The plants of the genus *Sceletium* investigated in this study were found to contain mesembrane alkaloids of the mesembrine-type only.

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Chapter 4

Investigation into the genus *Aptenia*

4.1 Introduction

The genus name *Aptenia* is derived from the Greek word *apten*, meaning wingless. The name *Aptenia* refers to the wingless fruit capsules of plants of this genus. *Aptenia* is one of the 11 genera of “weedy mesembs”, i.e. it is a member of the family Mesembryanthemaceae and belongs to the subfamily Mesembryanthemoideae.^{1,2} Four species of *Aptenia* are currently recognised:³

Aptenia cordifolia (L.f.) Schwantes, *A. geniculiflora* (L.) Bittrich ex Gerbaulet, *A. haeckeliana* (A.Berger) Bittrich ex Gerbaulet, and *A. lancifolia* L.Bolus.^{1,3}

The species *A. cordifolia* is commonly known as brakvygie (brack mesemb).¹

Plants of the genus *Aptenia* are small climbers, shrubs, or grow flat on the ground. They are evergreen and rarely deciduous.^{1,2} Sometimes the roots become thick and fleshy. *Aptenia* is characterized by green succulent stems with closely arranged water cells, as well as its seeds which are always blackish brown and rather large.¹ Plants of this genus typically bloom from spring through to autumn, and their flowers, which range in colour from magenta to rosy pink to sulphur, are self-fertile.^{1,2}

Aptenia naturally occur in the summer rainfall areas of Southern Africa. *A. cordifolia* and *A. lancifolia* are often found in shady places on rocky outcrops in regions of higher rainfall. In South Africa, the natural distribution of *A. cordifolia* is unclear as, due to its cultivation both by traditional healers and more generally in rockeries and domestic gardens, it often becomes easily established in areas beyond its natural distribution.^{1,2}

Aptenia cordifolia and *A. lancifolia* (Fig. 4.1) are very popular garden plants around the world, and are particularly useful as attractive ground covers on dry slopes as they rapidly form lush green carpets with decorative reddish purple flowers. *A. cordifolia* has cordate or heart-shaped leaves, while the leaves of *A. lancifolia* are lance-shaped, tapering gradually towards their bases.^{1,2} Leaf shape is used as a rather tenuous character in distinguishing *A. cordifolia* from its nearest relative, *A. lancifolia*.²



Figure 4.1: *A. lancifolia* (left) and *A. cordifolia* (right).

Photographs from Smith et al 1998.¹

Note: These two photographs are labelled incorrectly in reference 1. The correct identities were confirmed by Priscilla Burgoyne at the National Herbarium of the National Botanical Institute in Pretoria.

4.2 Literature Review

Despite the fact that the Mesembryanthemaceae are the best-represented succulent family in southern Africa's flora, members of this family are seldom encountered in ethnomedicine. Relatively few documented accounts of the growth and use of mesemb taxa by traditional medicinal practitioners, or as trade items in marketplaces, exist.² The genus *Sceletium* was discussed in Chapter 3. In this chapter, *Sceletium's* sister genus⁴ *Aptenia* is discussed.

Of the ca. 1680 species of mesembs in southern Africa, only a small proportion has come into everyday medicinal use, which may perhaps reflect the limited presence of efficacious compounds within this family. It is, however, more likely that the limited ethnobotanical reports mirror the restricted distribution and thus the inaccessibility of most taxa in this family. Crouch et al. (2000) proposed that the reason why useful mesembs were not cultivated across the country lies in the fact that many winter-rainfall species of mesembs are not easy to cultivate, and that some require very specialised growing conditions. They suggest that if such species were grown successfully, they may show depauperate levels of their potentially useful secondary metabolites.²

Aptenia cordifolia is recognised as the most medicinally useful species of *Aptenia* in the literature.^{2,5} Many people have had access to this plant through centralized muthi (medico-magical herb) markets in South Africa. In the muthi markets *A. cordifolia* is known as 'ibohlolob' (Zulu), a name that is reputedly derived from 'bohla', meaning to subside or deflate, a likely allusion to its anti-inflammatory properties. In Zulu the word 'ibohlolob' is also a reference to the 'calm after the storm', reported as conceivably being a reference to the efficacy of treatments.²

In 1939, Gerstner was likely the first to document the use of *Aptenia* in the treatment of swellings, applied as a poultice. He also reported the use of *Aptenia* as a mild enema to infants. Traditional healers have described the use of *A. cordifolia* for many medical treatments. One healer described how leaves and stems of *Aptenia* plants are burned before being applied to aching joints to promote the anti-inflammatory effects. Another related how he places ±50 g of fresh plant material in a litre of boiling water, and encourages patients to keep their swollen feet in this infusion for as long as they can handle the heat. 'Uncolosi omncane' or 'ungcolosi', a black powder that is considered to possess great magical powers against sorcery ('umsizi'), is also prepared from the plant. Apart from its anti-inflammatory uses, *Aptenia* is also reportedly used as an anti-perspirant, for which property the plant is known as 'umjuluka'. This name may be derived from the Zulu verb 'juluka', meaning to perspire, sweat, or exude

moisture. A market trader recommended to Crouch et al. (2000) the drinking of a lukewarm infusion of the bruised leaves of *A. cordifolia* for the relief of sore throats and tonsillitis. Other reported uses of this versatile taxon include the treatment of nervous and sexual complaints, as well as pleurisy and dropsy. In the treatment of nervous complaints, leaf and stem preparations are reputedly rubbed into scarifications.² Given the broad array of prospective uses, it is somewhat surprising that only limited chemical analysis has been performed on this plant.

Oxalic acid (oxalate), a simple organic acid that can render plants unpalatable or potentially fatal to livestock, is known to be present in mesembs (including *Aptenia*) in variable amounts.^{2,6} The reported anti-perspirant nature of *Aptenia* is more likely to be deodorant in effect as oxalic acid may be bacteriostatic, limiting the metabolism of sweat components, thus reducing body odour production. The presence of antimicrobial agents has also been indicated in *Aptenia*, likely providing additional deodorant action.² The anti-inflammatory components as well as the constituents responsible for counteracting tonsillitis and throat infections await determination.

Interest in the potential value of the mesembrane alkaloids has risen in recent years, and the race appears to be on for finding new pharmacological functions as well as new plant sources for these "old" alkaloids.² Zwicky (1914) reported that *A. cordifolia* tested positive for alkaloid in general alkaloidal tests.⁷ In 1998, Smith et al. investigated *Aptenia cordifolia* along with several other mesembs for the distribution of mesembrane alkaloids within selected taxa of the Mesembryanthemaceae. *A. cordifolia* was found to have the second highest levels of 4'-*O*-demethylmesembrenol (**15**) (next to *Sceletium tortuosum*), and significant concentrations of mesembrine (**10**) - in fact, the second highest levels of mesembrine were detected in *A. cordifolia* (although they were only 13,6% those in *Sceletium tortuosum*). No mesembrenone (**11**), however, could be detected.^{2,8} Given the fact that to date very little is known of the pharmacological action of the mesembrane alkaloids, the presence of mesembrine and 4-*O*-demethylmesembrenol in *Aptenia* may yet account for the observed medicinal

efficacy of preparations of this plant.^{2,6} The activity may also be partly due to the recently reported cinnamic acid amides, lignanamides and oxyneolignans.^{9,10}

Recently, the isolation of six oxyneolignans with phytotoxic activity (apteniols A-F)⁹ and six amides¹⁰ were reported from *A. cordifolia* cultivated in Italy. The structure of two of these compounds, apteniol A (**37**) and a cinnamic acid amide (**38**), are given in Fig 4.2. From the structures it is clear that the apteniols and the amides are biogenetically derived from tyrosine, which is also the biogenetic precursor of the mesembrine alkaloids. Neither of these two classes of compounds will be extracted under the conditions that we have used for the extraction of the mesembrine alkaloids.

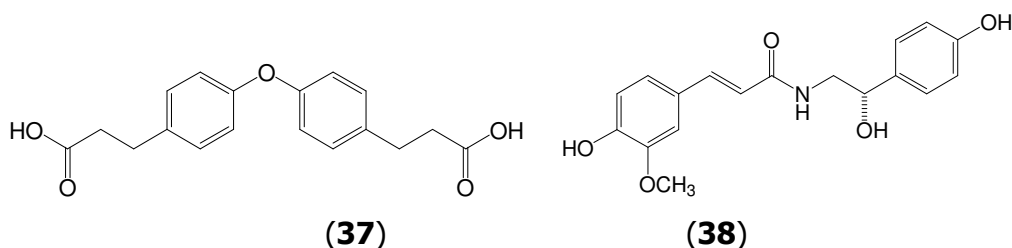


Fig 4.2: Structure of apteniol A (**37**) and cinnamic acid derivative **38**.

4.3 Results and Discussion

In this study, two species of *Aptenia*, *A. cordifolia* and *A. lancifolia*, were investigated for the presence of mesembrine alkaloids, as well as two *Aptenia cordifolia* plants of different origin. Comprehensive information on all plants screened in this study can be found in Tables 7.1 and 7.2 Chapter 7, as well as Tables A.1 and A.2 in Appendix 1.

Alkaloid extracts of the various parts of the three *Aptenia* plants were prepared and analysed by TLC and GC-MS as described in Chapter 7. As bulk extractions of the *A. lancifolia* and one of the *A. cordifolia* being studied had yielded larger amounts of extracts, the crude alkaloid extracts of these samples were separated into various fractions using a chromatotron. A major compound (5 mg) was isolated from *A. cordifolia* root extract and in smaller amounts from *A. lancifolia*

leaves, stems and roots. This compound was identified as 4'-*O*-methylsceletenone (**20**), the 3'-demethoxy analogue of mesembrenone (**11**) (Fig. 4.3). Sceletenone (**14**) has been isolated from *S. strictum* by Jeffs et al.¹¹ Although, 4'-*O*-methylsceletenone (**20**) was obtained by these authors by methylation of sceletenone (**14**) and was also subsequently prepared by total synthesis¹², our work is the first report of the isolation of 4'-*O*-methylsceletenone (**20**) from a natural source.

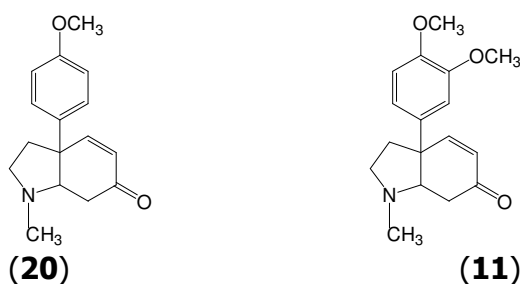


Fig 4.3: Structures of 4'-*O*-methylsceletenone (**20**) and mesembrenone (**11**).

The ¹H NMR spectrum of 4'-*O*-methylsceletenone (**20**) is given in Fig. 4.4. Structural features evident from this spectrum are:

- a methoxy group (three-proton singlet at δ_{H} 3.7)
- a *N*-methyl (three-proton broadened singlet at 2.33)
- a *para*-disubstituted benzene ring (two two-proton doublets at δ_{H} 7.26 and 6.88 with a mutual coupling constant of 7.3 Hz)
- an α,β -unsaturated carbonyl (two one-proton doublets at δ_{H} 6.72 and 6.10 with a mutual coupling of 10.0 Hz)

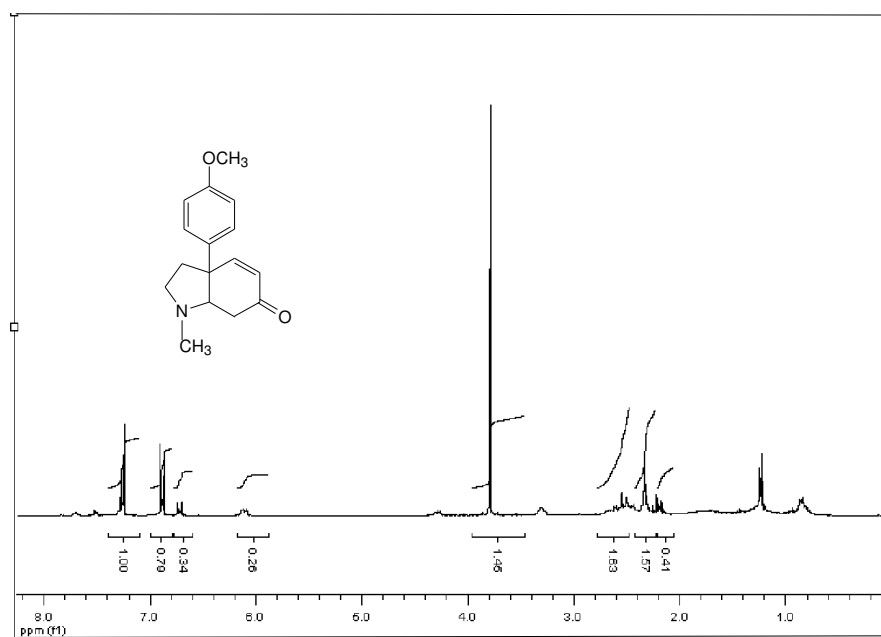


Fig. 4.4: ^1H NMR spectrum of 4'-*O*-methylsceletenone (**20**).

The structure of 4'-*O*-methylsceletenone (**20**) was confirmed by comparing the MS spectrum of this compound with that of the closely related mesembrenone (**11**) (Fig 4.5). 4'-*O*-Methylsceletenone has a M^+ at m/z 257 compared to that of mesembrenone (**11**) at m/z 287 (30 mass units lower). Mesembrenone has the characteristic peak at m/z 219 associated with fragment **39**, whereas 4'-*O*-methylsceletenone displayed once again a peak at 30 mass units lower at m/z 189 obtained from fragment **40**.

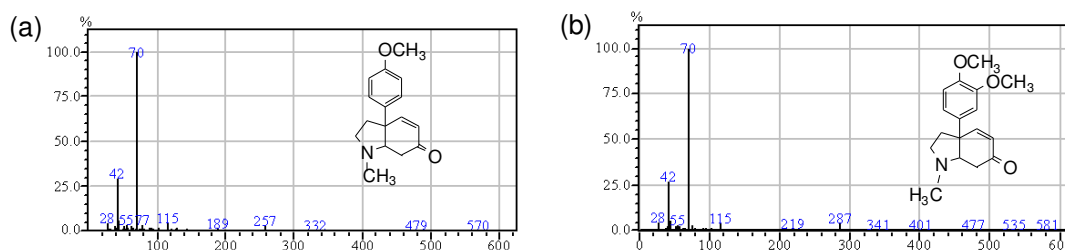


Fig. 4.5: Mass spectra of (a) 4'-*O*-methylsceletenone (**20**) isolated from *A. cordifolia* and (b) mesembrenone (**11**) present in *S. tortuosum* extract

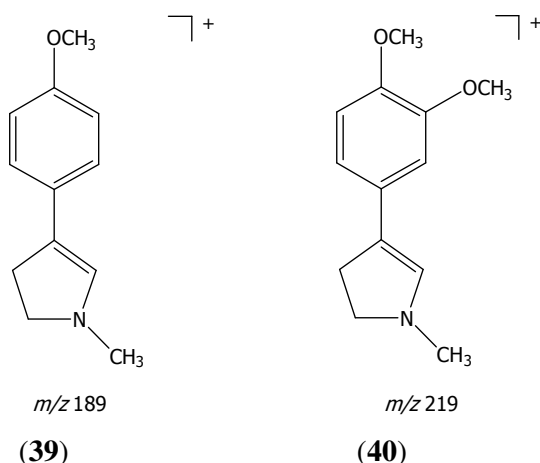


Fig. 4.6: Fragments observed in the mass spectra of 4'-*O*-methylsceletenone (**20**) and mesembrone (**11**).

A second minor compound in the extract of *A. lancifolia* leaves was identified as 4,5-dihydro-4'-*O*-methylsceletenone (**21**), the 3-demethoxy analogue of mesembrine (**10**). This compound has never been isolated from a natural source and has only once been described in a synthetic paper.¹² The NMR data of 4,5-dihydro-4'-*O*-methylsceletenone (**21**) was similar to those of 4'-*O*-methylsceletenone (**20**), with the major difference being the absence of the two olefinic protons adjacent to the 3-ketone. Once again, mass spectrometry provided proof for the structure of 4,5-dihydro-4'-*O*-methylsceletenone (**21**). The MS spectra of 4,5-dihydro-4'-*O*-methylsceletenone (**21**) and mesembrine (**10**) was virtually identical with the exception of the peaks at higher m/z where the peaks resulted from 4,5-dihydro-4'-*O*-methylsceletenone (**21**) are 30 mass units lower than those of mesembrine (**10**) (Fig. 4.7).

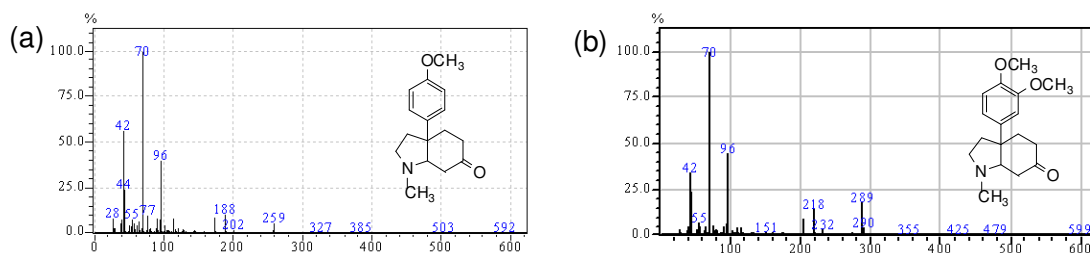


Fig. 4.7: Mass spectra of (a) 4,5-dihydro-4'-*O*-methylsceletenone (**21**) (isolated from *A. cordifolia*) (b) mesembrine (**10**) present in *S. tortuosum* extract.

Gas chromatography/mass spectrometry (electron impact ionisation) was carried out on all the *Aptenia* extracts. Once again, the mesembrine (**10**) standard was run prior to the other samples on the GC/MS, and the retention time of mesembrine was identified as between 24.0 and 24.2 minutes. Structures of the compounds were elucidated based on their respective mass spectra.

All three of the *Aptenia* plants investigated during the course of this study were found to test positive for nitrogen-containing compounds when screened using TLC. The three extracts were run on GC/MS to confirm the presence of any mesembrane alkaloids, and to investigate the identities of any mesembrane alkaloids present. The GC/MS spectra confirmed that the *A. lancifolia* extract and both of the *A. cordifolia* extracts did contain mesembrane alkaloids, as well as the alkaloid hordenine (**36**) in certain samples. The distribution of the mesembrane alkaloids between the various species the genus *Aptenia* can be seen in Table 4.1.

The mesembrine-type mesembranes 4,5-dihydro-4'-*O*-methylsceletenone (**21**) and 4'-*O*-methylsceletenone (**20**) were observed to be the mesembrane alkaloids present in various parts of all three of the *Aptenia* studied. In *A. cordifolia* sample 1, which was collected near Nature's Valley, no mesembrane alkaloids were observed to be present in any of the plant parts except for the stems. In the stems of *A. cordifolia* sample 1, the predominant alkaloid found was 4'-*O*-methylsceletenone (**20**), followed by hordenine (**36**) and then 4,5-dihydro-4'-*O*-methylsceletenone (**21**). In *A. cordifolia* sample 2, which was collected from the garden of Professor Ben-Erik van Wyk in Johannesburg, no alkaloids were found to be present in the fruit and flowers of the plant. Only the non-mesembrane alkaloid hordenine (**36**) was observed to be present only in the leaves of the plant in trace amounts, while the predominant alkaloid in both the stems and the roots of the plant was 4'-*O*-methylsceletenone (**20**), followed by 4,5-dihydro-4'-*O*-methylsceletenone (**21**).

In the *A. lancifolia* samples investigated, all of the plant parts were observed to contain mesembrane alkaloids. In the stems of *A. lancifolia*, 4'-*O*-methylsceletenone (**20**), 4,5-dihydro-4'-*O*-methylsceletenone (**21**) and hordenine (**36**) were all found to be present in trace amounts. 4,5-dihydro-4'-*O*-methylsceletenone (**21**) was the main alkaloid observed to be present and hordenine (**36**) was present in trace amounts in *A. lancifolia* leaves. In *A. lancifolia* roots, the predominant alkaloid present was 4'-*O*-methylsceletenone (**20**), followed by hordenine (**36**), and trace amounts of 4,5-dihydro-4'-*O*-methylsceletenone (**21**). The fruit and flowers of *A. lancifolia* yielded mainly 4,5-dihydro-4'-*O*-methylsceletenone (**21**) followed by 4'-*O*-methylsceletenone (**20**), and the stems of *A. lancifolia* yielded predominantly 4'-*O*-methylsceletenone (**20**) followed by 4,5-dihydro-4'-*O*-methylsceletenone (**21**).

Table 4.1 :Alkaloids present and prevalence observed in the genus *Aptenia*.

(For voucher specimens, see chapter 8)

Genus and species	Alkaloids and retention times on GC/MS.						
	Hordenine 14.8 - 15.5 min	4,5-Dihydro-4'- O-methyl sceletenone 22.6 - 22.8 min	4'-O-Methylsceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>Aptenia</i>							
<i>A. cordifolia</i> 1 leaves	-	-	-	-	-	-	-
<i>A. cordifolia</i> 1 roots	-	-	-	-	-	-	-
<i>A. cordifolia</i> 1 fruit and flowers	-	-	-	-	-	-	-
<i>A. cordifolia</i> 1 stems	2	3	1	-	-	-	-
<i>A. cordifolia</i> 2 leaves	Trace	-	-	-	-	-	-
<i>A. cordifolia</i> 2 roots	-	2	1	-	-	-	-
<i>A. cordifolia</i> 2 fruit and flowers	-	-	-	-	-	-	-
<i>A. cordifolia</i> 2 stems	-	2	1	-	-	-	-
<i>A. lancifolia</i> leaves	Trace	1	-	-	-	-	-
<i>A. lancifolia</i> roots	2	Trace	1	-	-	-	-
<i>A. lancifolia</i> fruit and flowers	-	1	2	-	-	-	-
<i>A. lancifolia</i> stems	Trace	Trace	Trace	-	-	-	-
<i>A. lancifolia</i> woody stems	-	2	1	-	-	-	-

The numbers given under each alkaloid indicates how dominant the alkaloid is in the sample, with 1 indicating most dominant alkaloid.

"Trace" indicates that only trace levels of the alkaloid(s) were detected.

"-" indicates non-detectable levels of alkaloids.

"NR" indicates that the extract tested alkaloid negative on TLC and was thus not run on GC/MS

The alkaloid constituents of the genera of the Mesembryanthemoideae represent a highly complex, multi-component mixture.⁶ It is interesting that the mesembrane alkaloids observed to be present in the *Aptenia* samples investigated are 4,5-dihydro-4'-*O*-methylsceletenone (**21**) and 4'-*O*-methylsceletenone (**20**), which are both-mesembrine type mesembrane alkaloids. These are slightly different alkaloids to those observed in the GC/MS spectra of the *Sceletium* extracts, but they are of the same type of mesembrane alkaloids as those found in *Sceletium*.

In 1998, Smith et al. investigated selected taxa of the Mesembryanthemaceae for the distribution of mesembrane alkaloids.⁸ In their studies they investigated *Sceletium tortuosum*, *Aptenia cordifolia* and *kougoed*, as well as several species of the subfamily Ruschiodeae. They analysed extracts of *Sceletium tortuosum* on a GC/MS, and recorded the retention times as well as the spectra for three of the mesembrane alkaloids present in the extracts. By comparison with mass spectral data published by Martin et al.¹³, the structures of the compounds were assigned as 4'-*O*-demethylmesembrenol (**15**) (RT 11.5 min), mesembrine (**10**) (12 min) and mesembrenone (**11**) (RT 12.5 min). Once the retention times and MS spectra for these mesembrane alkaloids had been determined, their presence in other species was confirmed by comparing the retention times of compounds using a GC with a NPD (nitrogen-phosphorous specific detector). Where appropriate, peak identity was confirmed using GC/MS.⁸

Using the program developed for this study, six of the mesembrane alkaloids and the alkaloid hordenine (**36**) were found among the extracts investigated, with retention times between 22,6 and 24,4 minutes for the mesembrane alkaloids, and 15 minutes for hordenine. Table 4.2 shows how close the retention times of some of the mesembrane alkaloids are.

Table 4.2: Hordenine and Mesembrane Alkaloids Retention Times on GC/MS.

Retention time (min)	Alkaloid identified
14.8 - 15.5	Hordenine (36)
22.6 - 22.8	4,5-dihydro-4'- <i>O</i> -methylsceletenone (21)
22.7 - 23.0	4'- <i>O</i> -methylsceletenone (20)
23.8 - 23.9	Mesembrenol (12)
23.9 - 24.0	Mesembranol (13)
24.0 - 24.2	Mesembrine (10)
24.2 - 24.4	Mesembrenone (11)

It is of concern that Smith et al. ran a short GC/MS separation program when they investigated selected taxa of the Mesembryanthemaceae for the distribution of mesembrine alkaloids. The retention times they obtained for 4'-*O*-demethylmesembrenol (**15**), mesembrine (**10**) and mesembrenone (**11**) are given as 11.5 min, 12 min and 12.5 min respectively. These values are very close together. In their article, Smith et al. reported the presence of mesembrine (**10**) and 4'-*O*-demethylmesembrenol (**15**) in *Aptenia cordifolia*. It is worth considering that perhaps some of the alkaloids identified by Smith et al. as being present in certain samples may have been mis-identified due to the compounds not separating fully as a result of the short GC/MS program run.

4.4 Conclusion

Investigations into *A. cordifolia* and *A. lancifolia* revealed the presence of the mesembrine-type of mesembrane alkaloids 4,5-dihydro-4'-*O*-methylsceletenone (**21**) and 4'-*O*-methylsceletenone (**20**), as well as the non-mesembrane alkaloid hordenine (**36**), in both species. This is the first report of these two mesembrane alkaloids from a natural source. The presence of only these two compounds (and none of the other alkaloids commonly found in *Sceletium*) suggest that (1) the two genera are different in their alkaloid profiles (and not similar, as existing literature suggests) and (2) that *Aptenia* species be reinvestigated for possible false positive alkaloid records and wrongly identified alkaloids. As a result of only mesembrane alkaloids of the mesembrine-type being detected in *Sceletium*, and

also in *Aptenia*, it is proposed that within the Mesembryanthemoideae the secomesembranes may be present in plants, but at very low levels.

4.5 References

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Chapter 5

Investigation into the genera within the subfamily Mesembryanthemoideae, excluding *Sceletium* and *Aptenia*

5.1 Introduction

The sub-family Mesembryanthemoideae comprise 11 genera and 95 species.^{1,2} The genus *Sceletium* was discussed in Chapter 3 and the genus *Aptenia* in Chapter 4. According to Klak et al.³ (2003) these two genera are closely related. In this chapter, the remaining nine genera of “weedy mesembs” are discussed. It is mentioned in Klak et al. (2003) that the relationships and generic circumscriptions of these genera are likely to change. The genus *Mesembryanthemum* L., for example, comprises at least three unrelated lineages. These remaining nine genera are:

Aridaria N.E.Br., *Aspazoma* N.E.Br., *Brownanthus* Schwantes, *Caulipsolon* Klak, *Mesembryanthemum*, *Phyllobolus* N.E.Br., *Prenia* N.E.Br., *Psilocaulon* N.E.Br., and *Synaptophyllum* N.E.Br.^{1,4}

The genus name *Aridaria* is derived from the Latin word *aridus*, meaning dry, in allusion to the arid natural habitat of plants of this genus.¹ Four species of *Aridaria* are currently recognised. These include *A. brevicarpa* L. Bolus (Fig. 5.1), *A. noctiflora* (L.) Schwantes (Fig. 5.1), *A. serotina* L. Bolus (Fig. 5.1), and *A. vespertina* L. Bolus.^{1,4} The species *A. noctiflora* is commonly known as *brakveldwitvygie* (brack field white mesemb).¹ The flowering season for plants of this genus is mainly between spring and early summer and depending on the species, the flowers open during the day, evening or night.¹ One of the main characters used to distinguish between species within the genus is the time at

which the flowers open, and this may vary from during the day only (*A. brevicarpa*) to only at night (*A. noctiflora*).¹



Figure 5.1: *A. noctiflora*, *A. brevicarpa* and *A. serotina*.

Photographs from Smith et. al. 1998.¹

The Greek word *aspazomai* (to clasp) is the origin of the genus name *Aspazoma*, and refers to the way in which the leaf sheath clasps the stem in plants of this genus.¹ Only one species of *Aspazoma* is currently recognised, *A. amplexans* (L. Bolus) N.E.Br., and no vernacular names appear to have been recorded.^{1,4} Flowering time for *Aspazoma* is during spring. It is to be noted that *Aspazoma* is closely related to *Brownanthus*, the main distinguishing characters being the arrangement of the leaves and the very large seeds in *Aspazoma*. It is considered that these two genera may eventually be combined.¹



Figure 5.2: Growth form, flowers and leaves of *Aspazoma amplexans*.

Photographs from Smith et. al. 1998.¹

The genus *Brownanthus* was named after the mesemb expert Dr. N.E. Brown (1849-1934) who worked at Kew.^{1,5} Dr. Brown's name is combined with the Latinised form of the Greek word *anthos*, meaning flower.¹ Ten species of *Brownanthus* are currently recognised, and no vernacular names are recorded for any of these species.^{1,4} The flowers of plants of this genus appear from spring to early summer, and the flowers open during daylight hours. Originally *Brownanthus* was included in *Psilocaulon*, but was later shown to be different in respect of fruit, flowers and leaf surfaces.¹ Figure 5.3 shows *Brownanthus pubescens* (N.E.Br. ex Maas) Bullock, *B. marlothii* (Pax) Schwantes, and the flowers of *B. ciliatus* (Aiton) Schwantes.



Figure 5.3: *Brownanthus pubescens*, *B. marlothii*, and flowers of *B. ciliatus*. Photographs from Smith et. al. 1998.¹

The genus name *Caulipsolon* is an anagram of *Psilocaulon*, the genus in which it was previously placed.¹ One species is currently recognised, *C. rapaceum* (Jacq.) Klak (Fig. 5.4), and no vernacular names seem to have been recorded.^{1,4} The genus *Caulipsolon* is characterized by creeping annual stems and a tuberous root stock. The stems and leaves have smooth surfaces, and the fruit have a distinctively funnel-shaped lower part.¹ The plant flowers between late winter and early spring.¹



Figure 5.4: *Caulipsolon rapaceum* and its tuberous rootstock.

Photographs from Smith et. al. 1998.¹

The genus name *Mesembryanthemum* has a complicated derivation. The name *Mesembryanthemum* is derived from the Greek words *mesos* (in the centre), *embryon* (pistil or embryo), and *anthemon* (flower), meaning “a flower with a pistil or embryo in the centre”.¹ However, the name *Mesembrianthemum*, from which the genus name originated, referred to the opening of the flowers of many mesemb species during midday.^{1,5} Fifteen species of *Mesembryanthemum* are currently recognised.^{1,4} Recorded common names for plants of this genus include ice plant, *brakslai* (brack salad), *brakvy* (brack mesemb), *olifantslai* (elephant salad), *volstruisslai* (ostrich salad), *slaaibos* (salad bush), *soutslai* (salt salad), *kama*, *nuta*, and *rosa-de-jericho*. *Mesembryanthemums* are often large-leaved fleshy herbs, and most species are covered with prominent water cells. The lettuce-like appearances of some species, as well as their abundance at some localities, make them easy to identify. The flowering season is mainly in the spring and summer months, with the flowers opening in the morning and closing at night.¹ Figure 5.5 shows *Mesembryanthemum guerichianum* Pax and a *Mesembryanthemum sp.* with the water cells clearly visible.



Figure 5.5: *Mesembryanthemum guerichianum* and *Mesembryanthemum sp.* clearly showing water cells.

Photographs from Smith et. al. 1998.¹

The genus name *Phyllobolus* is derived from the Greek word *phyllon* (meaning leaf) and from the Latin word *bolus* (meaning to throw or cast) in reference to the deciduous leaves of some species. Thirty-two species of *Phyllobolus* are currently recognised. *Phyllobolus digitatus* (Aiton) Gerbaulet (Fig. 5.6) is commonly known as *vingerkanna* (finger kanna) and *vingertjie-en-duimpie* (finger and thumb). In the USA, this species is also known as the hitchhiker plant due to its distinct growth form. The common name *brakveldvygie* is used to refer to *P. splendens* (L.) Gerbaulet, and *P. rabiei* (L. Bolus) Gerbaulet is known as *oumasepram* (*vygie*). Plants of the genus *Phyllobolus* flower mainly from mid-winter to mid-summer. The flowers close at night in most species, although some are known to bear night-opening flowers and others never close.¹



Figure 5.6: *Phyllobolus digitatus* subsp. *digitatus*, also known as vingertjie-en-duimpie or the hitchhiker plant.

Photograph: Candice. D. Gaffney.

The Greek word *prenes*, meaning prostrate, is the origin of the genus name *Prenia* in reference to the creeping habit of these plants.¹ Six species of *Prenia* are currently recognized.^{1,4} These include *P. englishiae* (L.Bolus) Gerbaulet, *P. pallens* Aiton) N.E.Br. (Fig. 5.7), *P. radicans* (L. Bolus) Gerbaulet, *P. sladeniana* L. Bolus (Fig. 5.7), *P. tetragonal* (Thunb.) Gerbaulet, and *P. vanrensburgii* L. Bolus.^{1,4} Only two of these species have known common names. *P. vanrensburgii* is known as *seepampoen* (sea pumpkin), and *P. sladeniana* is known as *skotteloor* (disc ear). The flowers of plants of the genus *Prenia* open by day, and they flower from spring through summer to autumn.¹



Figure 5.7: *Prenia sladeniana* and *P. pallens* subsp. *lutea*.

Photographs from Smith et. al 1998.¹

The leafless stems of plants of the genus *Psilocaulon* led to the genus name, which is derived from the Greek words *psilos* (meaning bare, bald, or smooth) and *kaulos* (meaning stalk).¹ Within the genus, 13 species are currently recognized.^{1,4} Commonly used vernacular names for plants of this species include *asbos* (ash bush), *loogasbossie* (lye ash bush), *seepbossie* (little soap bush), or *grootlidjies* (large jointlets). *P. dinteri* (Engler) Schwantes is commonly known as *skerpioenwygie* (scorpion mesemb), due to its resemblance to a scorpion. Young stems of *Psilocaulon* plants are jointed, succulent and green, while old stems become woody. Flowering time for plants of this genus is from early to mid-summer.¹ *Psilocaulon junceum* (Haw.) Schwantes and the flowering stems of *P. articulatum* (Thunb.) N.E.Br. can be seen in Figure 5.8.



Figure 5.8: *Psilocaulon junceum* and flowering stems of *P. articulatum*.
Photographs from Smith et. al. 1998.¹

The genus name *Synaptophyllum* is derived from the Greek words *synaptos* (meaning gnarled) and *phyllos* (meaning leaf).¹ Only one species of *Synaptophyllum*, *S. juttae* (Dinter & A.Berger) N.E.Br., is currently recognized and no vernacular names appear to have been recorded.^{1,4} *Synaptophyllum* is a particularly interesting mesemb in that it has a very restricted distribution. It is only known to grow in the fog zone of the southern Namib desert, near Lüderitz in south-western Namibia. The single species of *Synaptophyllum* is a desert specialist, which flowers in midsummer.¹



Figure 5.9: Flowering form of *Synaptophyllum juttae*, and young plant of *S. juttae* showing the large basal leaves.

Photographs from Smith et. al. 1998.¹

5.2 Literature Review

Very little information is available in the literature on the chemistry of the genera of the Mesembryanthemoideae, with the exception of *Aptenia* and of *Sceletium*.⁶ In 1914, Zwicky tested several Mesembryanthemoideae for the presence of alkaloids using general alkaloidal tests.⁷ Only five of six genera of Mesembryanthemoideae tested thus far have tested positive for alkaloids.⁸ These are *Aptenia*, *Sceletium*, *Aridaria*, *Prenia* and *Psilocaulon*.^{7,8} It should be stressed that the tests performed, however, were non-quantitative general alkaloid tests and do not necessarily indicate only mesembrine-like alkaloids.⁸ Hordenine (**36**) is a non-mesembrane alkaloid whose presence in plants of the subfamily Mesembryanthemoideae has previously been reported, as is the case with piperidine.^{7,8,9,10} The presence of oxalic acid and oxalates have also previously been reported in the Mesembryanthemoideae.^{6,7,8,11,12} It is also significant to note that within the genera of the Mesembryanthemaceae, different species have tested both alkaloid positive and negative.⁸

In 1928, Laidler reported that the plant *Mesembryanthemum crystallinum*, also known as slaai, was used for removing hair off animal skins and was still in use among farmers at the time.^{8,11} In the same article, Laidler reported that a plant named *Mesembryanthemum stellatum*, also known as karremoer, was one of the beer-making roots, a deliriant and intoxicant with stimulant action.¹¹ Watt and Breyer-Brandwijk reported in 1962 that *Mesembryanthemum aitonis* has been suspected of producing field poisoning of cattle in the Grahamstown district.⁷ They wrote:

'Experimentally the administration of 4 kg. to a sheep has resulted in listlessness, anorexy, tympanites, dyspnoea, quickening and weakening of the pulse, slight diarrhoea and arching of the back, with recovery.'⁷

It is reported in the literature that several species of *Mesembryanthemum* (which have not been fully determined) are traditionally used in a variety of ways.⁷

Thunberg reported that the early colonists used to administer a concoction of the fruit of *Mesembryanthemum sp.* to expectant women near term to ease delivery. A *Mesembryanthemum sp.* was used by the Swati in the treatment of cystitis. An enema of a *Mesembryanthemum sp.* is used by the Zulu people to treat stomach upsets. A similar preparation is sometimes drunk to quell nausea, and the Zulus rub the ash of this species into lacerations made over various parts of the body that are sore. The Europeans used to drink an infusion of the leaf of *Mesembryanthemum sp.* and they applied the leaf locally for the treatment of skin sores. The Zulu use an infusion of the leaf of *Mesembryanthemum sp.* to alleviate the nightmares that apparently accompany heart weakness. In the old Transvaal, a mixture of the juice of the leaf of a *Mesembryanthemum sp.* and the juice of a *Tragia sp.* were taken to treat syphilis.⁷

Watt and Breyer-Brandwijk reported in 1962 that *Psilocaulon absimile* N.E.Br. had caused stock poisoning in the Willowmore district, and that sun drying did not diminish the toxicity of the plant.⁷ They reported that the plant contains two lethal compounds, piperidine and oxalic acid.^{7,8} The ash of *Psilocaulon parviflorum* Schwantes is reported to have been employed by the African people and by the Khoi people in soap-making.⁷ The ash has also reportedly been used in the Worcester district as a lye in which grapes are steeped when making raisins.⁷ The ash of another two species, *P. junceum* and *P. coriarium*, has been used as lye in soap-making.¹ In the north-western Cape, *Psilocaulon* species are used to erect a *asbosskerm* or *kookskerm* (a traditional shelter around the fireplace) by stacking the plants up to a height of around 2,5 m.¹

5.3 Results and Discussion

In this chapter, six of the nine remaining genera of the Mesembryanthemoideae are investigated for the presence of mesembrane alkaloids. The genera investigated are *Aridaria* (1 species, 1 plant), *Brownanthus* (1 species, 2 plants), *Mesembryanthemum* (5 or 6 species, 13 plants), *Phyllobolus* (7 species, 7 plants), *Prenia* (between 2 and 4 species, 5 plants) and *Psilocaulon* (6 species, 17 plants).

Where sufficient material was available, extracts of the various parts of the plants were prepared, *i.e.* extracts of fruit and flowers, leaves, stems, and roots. Where insufficient material was available to be separated into plant parts, the material that could be obtained was extracted as a single extract and named 'mixed plant parts'. Comprehensive information on all plants screened in this study can be found in Tables 7.1 and 7.2 of Chapter 7, as well as Tables A.1 and A.2 in Appendix 1.

Alkaloid extracts of all of the Mesembryanthemoideae plants investigated in this study were prepared, and analysed by TLC and GC-MS as described in Chapter 7. As the bulk extraction of the roots of *Phyllobolus digitatus* subsp. *digitatus* roots yielded a fair amount of extract, the crude alkaloid extract of this sample was separated into various fractions using a chromatotron. All the Mesembryanthemoideae extracts that had screened positive for nitrogen-containing compounds on TLC were then analysed using Gas Chromatography /Mass Spectrometry (electron impact ionisation). Once again, a mesembrine (**10**) standard was used to ascertain the retention time of mesembrine on the GC. by NMR spectroscopy (Fig 3.2) allowed the identification of the alkaloid mesembrine in the standard. Structures of the other compounds were elucidated based on their respective mass spectra.

As mentioned in Chapters 3 and 4, it is evident from the literature that four fragments are often observed to be present on the GC/MS spectra of the mesembrane alkaloids. The peaks of these four fragments are at m/z 44, m/z 58, m/z 72 and m/z 219. The ion that results in a peak at m/z 219 is considered to be characteristic of the mesembrine-type of mesembrane alkaloids. The structures of the four ions are given in Figure 3.5.

Only one species of *Aridaria* could be obtained to investigate the presence of mesembrane alkaloids. The *Aridaria serotina* plant was separated into leaves, roots and stems. The extracts of all three plant parts screened positive for nitrogen-containing compounds on TLC and were subsequently analysed using

GC/MS, where they were found to contain mesembrane alkaloids. The distribution of the mesembrane alkaloids between the various parts of *Aridaria serotina* can be seen in Table 5.1.

The four alkaloids observed to be present in the various plant parts of *Aridaria serotina* are mesembrine (**10**), mesembrenone (**11**), mesembranol (**13**) and hordenine (**36**). The main alkaloid present in the leaves of *A. serotina* was mesembrine (**10**), followed by mesembranol (**13**) and then mesembrenone (**11**). No hordenine (**36**) was found in the leaves. In the stems of *A. serotina*, mesembrine (**10**) was once again the most predominant alkaloid, followed by mesembrenone (**11**), hordenine (**36**) and then mesembranol (**13**). Mesembrenone (**11**) was the main alkaloid found in the roots of *A. serotina*, followed by mesembrine (**10**), and then mesembranol (**13**) and hordenine (**36**) both in trace amounts.

Brownanthus ciliatus subsp. *ciliatus* was the only species of *Brownanthus* that could be obtained to investigate for the presence of mesembrane alkaloids, however, two different plants were obtained. Both plants were extracted as mixed plant parts, and both extracts were screened negative for nitrogen-containing compounds on TLC. The extracts were thus not run on GC/MS.

Five or six species and thirteen plants of *Mesembryanthemum* were investigated for the presence of mesembrane alkaloids. Four plants of *M. aitonis*, three of *M. crystallinum*, one *M. excavatum*, one confirmed *M. guerichianum*, one *M. cf. guerichianum*, two *M. nodiflorum* and one *M. species* were all screened for the presence of nitrogen-containing compounds using TLC. However, all the extracts except for the *M. excavatum* mixed plant parts and the *M. nodiflorum* 2 mixed plant parts (which both screened negative) were analysed by GC/MS to investigate the presence or absence of mesembrane alkaloids in the genus as well as the various species.

No mesembrane alkaloids were detected in any of the *Mesembryanthemum* samples. The only alkaloid detected on any of the *Mesembryanthemum* extracts' spectra following GC/MS analysis was hordenine (**36**). Hordenine (**36**) was observed to be present in the leaves of *M. aitonis* 2, the roots and stems of *M. aitonis* 3, and the leaves and roots of the confirmed *M. guerichianum*. All the other *Mesembryanthemum* extracts showed non-detectable levels of hordenine and of the mesembrane alkaloids.

Seven species of *Phyllobolus* were investigated for the presence of mesembrane alkaloids. As four of the species screened tested positive for the presence of nitrogen-containing compounds on TLC, all the *Phyllobolus* species were analysed by GC/MS. Of all the species investigated in this study, only *P. digitatus* subsp. *digitatus* showed the presence of any alkaloids on its GC/MS spectra. Extraction of a bulk sample of *P. digitatus* and subsequent fractionation of the extract by chromatotron resulted in the isolation of hordenine (**36**). The structure of hordenine was confirmed by NMR spectroscopy. In the ^1H NMR spectrum the typical AA'BB' [δ_{H} 6.97 (d, $J=8.4\text{Hz}$, 2H), 6.63 (d, $J=8.5\text{Hz}$, 2H)] pattern of a *p*-substituted phenol could be observed (Fig. 10 and Fig 11). The other elements of this rather simple spectrum were two pairs of multiplets characteristic of two adjacent methylene groups [δ_{H} 2.70 (m, 2H), 2.60 (m, 2H)] and a six-proton singlet [δ_{H} 2.33 (s, 6H)] indicating the presence of a dimethylamino [$\text{N}(\text{CH}_3)_2$] functionality. The ^{13}C NMR spectrum was in agreement with this assignment, with the evidence of a phenolic carbon (δ_{C} 155.2), a non-prototnated aromatic carbon (δ_{C} 130.3), two methylene carbons (δ_{C} 61.4, 32.6) and two N-methyl carbons (δ_{C} 44.9). In the MS spectrum, hordenine is characterised by a base peak at m/z 58, corresponding to a $\text{CH}_2=\text{N}(\text{CH}_3)_2^+$ fragment.

Hordenine (**36**) was found to be present in the green stems, the papery stems and the roots of *P. digitatus* subsp. *digitatus*. Hordenine (**36**) was not observed to be present in any of the other *Phyllobolus* species investigated, and no mesembrane alkaloids were detected in any of the *Phyllobolus* species

investigated. The alkaloid status of the genus *Phyllobolus* can be seen in Table 5.3.

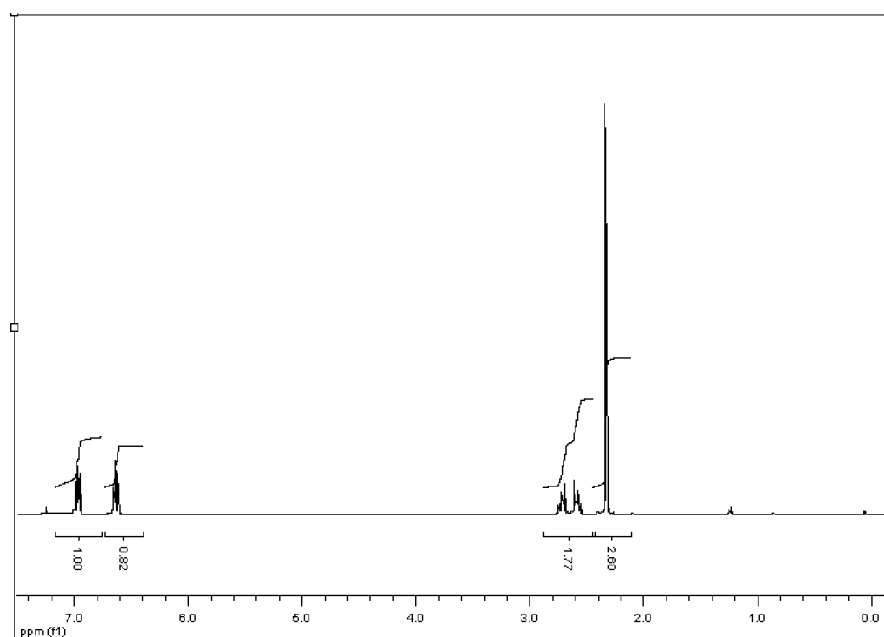


Figure 10: 300MHz ¹H NMR spectrum of hordenine (**36**).

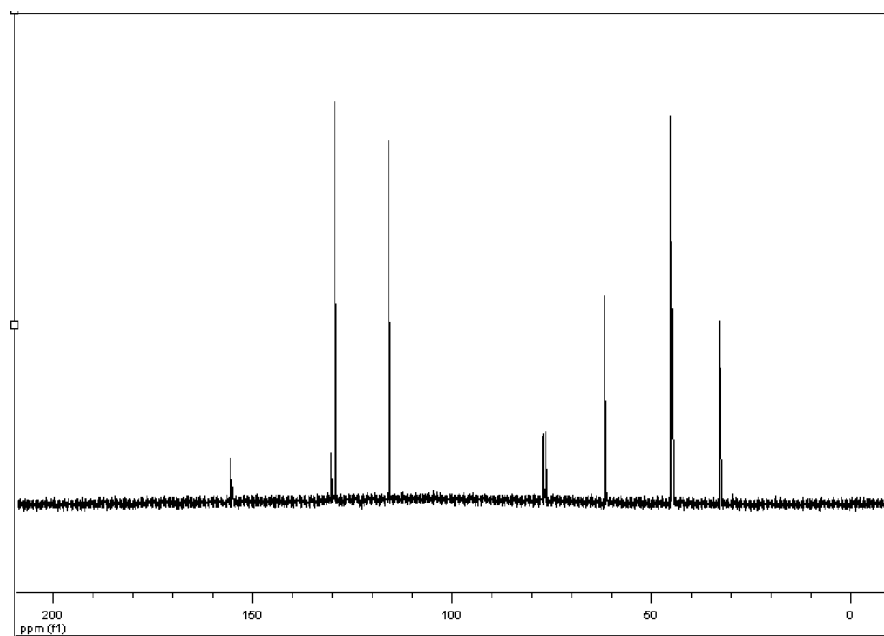


Figure 11: ¹³C NMR spectrum of hordenine (**36**).

Between two and four species of *Prenia* were investigated for the presence of mesembrane alkaloids. All five of the plants were extracted as mixed plant parts,

and all the extracts screened negative for nitrogen-containing compounds on TLC. The extracts were thus not studied by GC/MS.

Six species and seventeen plants of the genus *Psilocaulon* were investigated for the presence of mesembrane alkaloids. The species studied include *P. articulatum*, *P. bicornae*, *P. coriarium*, *P. dinteri*, *P. granulicaule*, and *P. junceum*. Some of the species investigated tested negative when screened for nitrogen-containing compounds using TLC. These are *P. bicornae*, *P. dinteri* and *P. junceum*. These species were not analysed by GC/MS. Other species of *Psilocaulon* screened positive for nitrogen-containing compounds. These species are *P. articulatum*, *P. coriarium* and *P. granulicaule*. The extracts of these three species were examined by GC/MS. Only *Psilocaulon coriarium* showed the presence of an alkaloid on its GC/MS spectra, and the alkaloid detected was hordenine (**36**). *P. coriarium* 1 stems, *P. coriarium* 2 stems and *P. coriarium* 2 leaves indicated detectable levels of the alkaloid hordenine (**36**). No mesembrane alkaloids were detected in the *P. coriarium* extracts. Neither hordenine nor any of the mesembrane alkaloids were detected in any of the other species of *Psilocaulon* investigated. The alkaloid status of the genus *Psilocaulon* can be seen in Table 5.4.

A bulk extract of *P. coriarium* was made, and the major alkaloid was isolated by chromatotron. By comparison of the ^1H and ^{13}C NMR spectra and the MS data, it was concluded that this compound was identical to the hordenine (**36**) isolated from *Phyllobolus digitatus*.

Within the genera of the Mesembryanthemoideae, only one of the genera investigated (other than *Aptenia* and *Sceletium*) displayed detectable levels of mesembrane alkaloids. This genus is *Aridaria*, which was observed to contain the mesembrine-type alkaloids mesembrine (**10**), mesembrenone (**11**) and mesembranol (**13**), as well as the tyrosine-derived non-mesembrane alkaloid hordenine (**36**). The *Brownanthus* investigated in this study screened negative for nitrogen-containing compounds, as did the *Prenia*. Within the genus *Mesembryanthemum*, hordenine (**36**) was the only alkaloid detected and it was

only found to be present in *M. aitonis* and *M. guerichianum*. In all of the other species of *Mesembryanthemum* investigated, neither hordenine (**36**) nor any mesembrane alkaloids were detected. Of the seven species of *Phyllobolus* investigated for the presence of mesembrane alkaloids, only the species *Phyllobolus digitatus* subsp. *digitatus* was found to contain any alkaloids, with the alkaloid detected being hordenine (**36**). No mesembrane alkaloids were detected in any of the other six species of *Phyllobolus* studied. *Psilocaulon bicornis*, *Psilocaulon dinteri* and *Psilocaulon junceum* screened negative for nitrogen-containing compounds. Of the remaining three species of *Psilocaulon* investigated in this study, *P. articulatum* showed non-detectable levels of mesembrane alkaloids and hordenine (**36**), as did *P. granulicaule*. *P. coriarium* was the only species to show detectable levels of alkaloid when run on GC/MS, with the alkaloid detected being hordenine (**36**).

5.4 Conclusion

The genera of the Mesembryanthemoideae are diverse in their growth forms and their habitats. They are also very interesting to botanists and chemists due to their complex phytochemical make-up. However, the extremely low concentration of alkaloids present in the majority of these species makes the identification of these alkaloids a formidable task. In this study, it was found that the genus *Aridaria* is the most interesting genus of the Mesembryanthemoideae from a phytochemical viewpoint (other than *Aptenia* and *Sceletium*) due to the array of mesembrane alkaloids found to be present within its species. *Aridaria*, like *Sceletium*, was found to contain mesembrane alkaloids of the mesembrine-type only, not sceletenone derivatives like *Aptenia*.

None of the other genera of the Mesembryanthemoideae investigated were found to contain any mesembrane alkaloids. The tyrosine-derived non-mesembrane alkaloid hordenine appears to be widespread in the Mesembryanthemoideae and was found to be present in some species of *Mesembryanthemum*, one species of *Phyllobolus* and one species of *Psilocaulon*. Both the *Brownanthus* and the *Prenia*

investigated were found to contain no detectable alkaloids. The results presented here indicate that there may be numerous false positive reports of alkaloids in the Mesembryanthemaceae (as reported in Watt & Breyer-Brandwijk⁶ 1962 and Smith et al.⁵ 1998) and that many of the so-called mesembrine-containing genera may in fact produce only hordenine and nothing more. In an unsophisticated TLC screening hordenine may easily be mistaken for mesembrine especially if one does not run a known sample of mesembrine at the same time, and this is due to the similarity of colour that develops for both compounds when the plate is sprayed with a dragendorff reagent. Hordenine has an R_f approximately half that of mesembrine.

Table 5.1 :Alkaloids present and prevalence observed in *Aridaria serotina*.

(For voucher specimens, see Chapter 8)

Genus and species	Alkaloids and retention times on GC/MS.						
	Hordenine 14.8 - 15.5 min	4,5-dihydro-4'-O-methylsceletenone 22.6 - 22.8 min	4'-O-methylsceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>Aridaria</i>							
<i>A. serotina</i> leaves	-	-	-	-	2	1	3
<i>A. serotina</i> roots	Trace	-	-	-	Trace	2	1
<i>A. serotina</i> stems	3	-	-	-	4	1	2

The numbers given under each alkaloid indicates how dominant the alkaloid is in the sample, with 1 indicating most dominant alkaloid.

"Trace" indicates that only trace levels of the alkaloid(s) were detected.

"-" indicates non-detectable levels of alkaloids.

Table 5.2 :Alkaloids present and prevalence observed in the genus *Mesembryanthemum*.

(For voucher specimens, see Chapter 8)

Genus and species	Alkaloids and retention times on GC/MS						
	Hordenine 14.8 - 15.5 min	4,5-dihydro-4'-O-methylsceletenone 22.6 - 22.8 min	4'-O-methylsceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>Mesembryanthemum</i>							
<i>M. aitonis</i> 1 leaves	-	-	-	-	-	-	-
<i>M. aitonis</i> 1 fruit and flowers	-	-	-	-	-	-	-
<i>M. aitonis</i> 1 stems	-	-	-	-	-	-	-

Genus and species	Alkaloids and retention times on GC/MS.						
	Hordenine 14.8 - 15.5 min	4,5-dihydro-4'-O-methylsceletenone 22.6 - 22.8 min	4'-O-methylsceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>M. aitonis</i> 2 leaves	1	-	-	-	-	-	-
<i>M. aitonis</i> 2 roots	-	-	-	-	-	-	-
<i>M. aitonis</i> 2 stems	-	-	-	-	-	-	-
<i>M. aitonis</i> 3 leaves	-	-	-	-	-	-	-
<i>M. aitonis</i> 3 roots	1	-	-	-	-	-	-
<i>M. aitonis</i> 3 stems	1	-	-	-	-	-	-
<i>M. aitonis</i> 4 mixed plant parts	-	-	-	-	-	-	-
<i>M. crystallinum</i> 1 mixed plant parts	-	-	-	-	-	-	-
<i>M. crystallinum</i> 2 mixed plant parts	-	-	-	-	-	-	-
<i>M. crystallinum</i> 3 leaves	-	-	-	-	-	-	-
<i>M. crystallinum</i> 3 roots	-	-	-	-	-	-	-
<i>M. crystallinum</i> 3 stems	-	-	-	-	-	-	-
<i>M. excavatum</i> mixed plant parts	NR						
<i>M. guerichianum</i> leaves	1	-	-	-	-	-	-
<i>M. guerichianum</i> roots	1	-	-	-	-	-	-
<i>M. cf. guerichianum</i> mixed plant parts	-	-	-	-	-	-	-

Genus and species	Alkaloids and retention times on GC/MS.						
	Hordenine 14.8 - 15.5 min	4,5-dihydro-4'-O-methylsceletenone 22.6 - 22.8 min	4'-O-methylsceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>M. nodiflorum</i> 1 leaves	-	-	-	-	-	-	-
<i>M. nodiflorum</i> 1 roots	-	-	-	-	-	-	-
<i>M. nodiflorum</i> 1 stems	-	-	-	-	-	-	-
<i>M. nodiflorum</i> 2 mixed plant parts	NR						
<i>M. sp</i> leaves	-	-	-	-	-	-	-
<i>M. sp</i> roots	-	-	-	-	-	-	-

The numbers given under each alkaloid indicates how dominant the alkaloid is in the sample, with 1 indicating most dominant alkaloid.

"-" indicates non-detectable levels of alkaloids.

"NR" indicates that the extract tested alkaloid negative on TLC and was thus not run on GC/MS

Table 5.3 :Alkaloids present and prevalence observed in the genus *Phyllobolus*.

(For voucher specimens, see Chapter 8)

Genus and species	Alkaloids and retent ion times on GC/MS.						
	Hordenine	4,5-dihydro-4'- <i>O</i> -methylsceletenone	4'- <i>O</i> -methylsceletenone	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
	14.8 - 15.5 min	22.6 - 22.8 min	22.7 - 23.0 min				
<i>Phyllobolus</i>							
<i>P. digitatus</i> subsp. <i>digitatus</i> stems	1	-	-	-	-	-	-
<i>P. digitatus</i> subsp. <i>digitatus</i> roots	1	-	-	-	-	-	-
<i>P. nitidus</i> fruit and flowers	-	-	-	-	-	-	-
<i>P. nitidus</i> leaves	-	-	-	-	-	-	-
<i>P. nitidus</i> roots	-	-	-	-	-	-	-
<i>P. nitidus</i> stems	-	-	-	-	-	-	-
<i>P. spinuliferus</i> mixed plant parts	-	-	-	-	-	-	-
<i>P. longispinulus</i> mixed plant parts	-	-	-	-	-	-	-
<i>P. rabiei</i> mixed plant parts	-	-	-	-	-	-	-
<i>P. cf. anrathus</i> mixed plant parts	-	-	-	-	-	-	-
<i>P. congestus</i> mixed plant parts	-	-	-	-	-	-	-

The numbers given under each alkaloid indicates how dominant the alkaloid is in the sample, with 1 indicating most dominant alkaloid.

"-" indicates non-detectable levels of alkaloids.

Table 5.4 :Alkaloids present and prevalence observed in the genus *Psilocaulon*.

(For voucher specimens, see Chapter 8)

Genus and species	Alkaloids and retent ion times on GC/MS.						
	Hordenine 14.8 - 15.5 min	4,5-dihydro-4'-O- methylsceletenone 22.6 - 22.8 min	4'-O- methylsceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>Psilocaulon</i>							
<i>P. articulatum</i> 1 leaves	-	-	-	-	-	-	-
<i>P. articulatum</i> 1 stems	-	-	-	-	-	-	-
<i>P. articulatum</i> 2 mixed plant parts	-	-	-	-	-	-	-
<i>P. articulatum</i> 3 fruit and flowers	-	-	-	-	-	-	-
<i>P. articulatum</i> 3 leaves	-	-	-	-	-	-	-
<i>P. articulatum</i> 3 stems	-	-	-	-	-	-	-
<i>P. bicornis</i> mixed plant parts	NR						
<i>P. granulicaule</i> 1 mixed plant parts	-	-	-	-	-	-	-
<i>P. granulicaule</i> 2 leaves	-	-	-	-	-	-	-
<i>P. granulicaule</i> 2 stems	-	-	-	-	-	-	-
<i>P. coriarium</i> 1 stems	1	-	-	-	-	-	-
<i>P. coriarium</i> 2 fruit and flowers	-	-	-	-	-	-	-
<i>P. coriarium</i> 2 leaves	1	-	-	-	-	-	-
<i>P. coriarium</i> 2 stems	1	-	-	-	-	-	-
<i>P. dinteri</i> mixed plant parts	NR						

Genus and species	Alkaloids and retention times on GC/MS.						
	Hordenine 14.8 - 15.5 min	4,5-dihydro-4'-O-methylsceletenone 22.6 - 22.8 min	4'-O-methylsceletenone 22.7 - 23.0 min	Mesembrenol 23.8 - 23.9 min	Mesembranol 23.9 - 24.0 min	Mesembrine 24.0 - 24.2 min	Mesembrenone 24.2 - 24.4 min
<i>P. junceum</i> 1 mixed plant parts	NR						
<i>P. junceum</i> 2 mixed plant parts	NR						
<i>P. junceum</i> 3 mixed plant parts	NR						
<i>P. junceum</i> 4 mixed plant parts	NR						
<i>P. junceum</i> 5 mixed plant parts	NR						
<i>P. junceum</i> 6 mixed plant parts	NR						
<i>P. junceum</i> 7 mixed plant parts	NR						
<i>P. cf. junceum</i> mixed plant parts	NR						

The numbers given under each alkaloid indicates how dominant the alkaloid is in the sample, with 1 indicating most dominant alkaloid.

"-" indicates non-detectable levels of alkaloids.

"NR" indicates that the extract tested alkaloid negative on TLC and was thus not run on GC/MS

5.5 References

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Chapter 6

Investigation into the subfamily Ruschioideae

6.1 Introduction

Within South Africa's largest succulent plant family, the Mesembryanthemaceae, two subfamilies are currently recognised: the Mesembryanthemoideae and the Ruschioideae.^{1,2,3} The subfamily of the Ruschioideae is the larger of the two subfamilies, comprising approximately 101 genera and 1563 species, and thus constituting around 85% of the Aizoaceae.⁴ On the basis of several morphological characters this subfamily has been considered to form a monophyletic group, which is supported by the molecular data shown by molecular systematics.⁵ The subfamilies Mesembryanthemoideae and Ruschioideae are both almost endemic to southern Africa, members of the two subfamilies often share similar habitats, and the distribution area of the two subfamilies coincides largely.⁵ As a result of these facts and literature reports of members of the Ruschioideae being utilised for ethnomedicinal purposes, a brief investigation into the subfamily of the Ruschioideae was included in this study.

6.2 Literature Review

The Mesembryanthemaceae is highly under-investigated with respect to the alkaloids present within the family as well as the distribution of the alkaloids within the genera and subfamilies.² In 1998, Smith et al. investigated selected taxa of the Mesembryanthemaceae for the distribution of mesembrane alkaloids.⁶ In their studies, Smith and colleagues investigated *Sceletium tortuosum*, *Aptenia cordifolia* and kougloed, as well as several species of Ruschioideae, namely *Bergeranthus scapiger*, *Delosperma minimum* and seven other species of *Delosperma*, *Drosanthemum hispidum*, *D. bicolour*, *Glottiphyllum longum*,

Lampranthus aureus, *L. spectabilis*, *L. roseus*, *L. blandus*, *L. coccineus*, *L. deltoides*, and *Ruschia lineolata*. They analysed extracts of *Sceletium tortuosum* on a GC/MS, and recorded the retention times as well as the spectra for three of the mesembrane alkaloids present in the extracts. By comparison with mass spectral data published by Martin et al.⁷, the structures of the compounds were assigned as 4'-*O*-demethylmesembrenol (**15**) (RT 11.5 min), mesembrine (**10**) (12 min) and mesembrenone (**11**) (RT 12.5 min). Once the retention times and MS spectra for these mesembrane alkaloids had been determined, their presence in other species was confirmed by comparing the retention times of compounds using a GC with a NPD (nitrogen-phosphorous specific detector). Where appropriate, peak identity was confirmed using GC/MS.⁶

Smith et al. found that seven of the species of *Delosperma* they investigated showed negligible or non-detectable levels of mesembrane alkaloids, the exception being *D. minimum* which was found to contain 4'-*O*-demethylmesembrenol (**15**) at a level of 15% of that observed in *Sceletium tortuosum*. *Glottiphyllum longum* and *Ruschia lineolata* reportedly showed a complete absence of the mesembrane alkaloids. *Lampranthus roseus*, *L. blandus*, *L. coccineus* and *L. deltoides* were observed to contain very low levels of mesembrenone, while *L. aureus* and *L. spectabilis* were both reported to contain mesembrenol. *Bergeranthus scapiger* was reported to contain low levels of both mesembrenone (**11**) and 4'-*O*-demethylmesembrenol (**15**). *Drosanthemum bicolor* and *D. hispidum* were both observed to contain 4'-*O*-demethylmesembrenol (**15**) (*D. hispidum* contained the fourth highest levels of this alkaloid), and both species were reported to contain mesembrenone (**11**) at levels equivalent to half their respective levels of 4'-*O*-demethylmesembrenol (**15**).⁶

6.3 Results and Discussion

In this study, a few members of the subfamily Ruschioideae were extracted on analytical scale and the extracts were screened for the presence of alkaloids by thin-layer chromatography (TLC), using an alkaloid-specific spray reagent. Comprehensive information on all plants screened in this study can be found in Tables 7.1 and 7.2 Chapter 7, as well as Tables A.1 and A.2 in Appendix 1.

The Ruschioideae screened included:

- Three species of *Drosanthemum* Schwantes,
- Two species of *Malephora* N.E.Br.,
- Five species of *Ruschia* Schwantes (two of which belong to the Uncinata group),
- *Pleiospilos compactus* (Aiton) Schwantes (2 different plants),
- *Eberlanzia ferox* Schwantes,
- *Trichodiadema* sp. Schwantes and
- *Monilaria moniliformis* (Thunb.) Ihlenf. & Jörg.

All of the plants of the subfamily Ruschioideae that were screened in this study tested negative for alkaloids on TLC. This was an interesting result, as Smith et al. (1998) had also investigated two species of *Drosanthemum* during the course of their research and had found the two species to be alkaloid positive. They too, however, could not detect alkaloids in the species of *Ruschia* they investigated. It must be mentioned that there are a large number of species in this subfamily, and although we have not detected any alkaloids in the plants we investigated, there are a large number of species that we did not investigate.

It is known that within genera of the Mesembryanthemaceae, different species have in the past tested both alkaloid positive and negative. It is also known that the levels of secondary plant products, including alkaloids, are strongly influenced by factors such as plant age, season, growing conditions and genetic chemotype.²

One should be very careful when reporting the presence of alkaloids in a species as it has been observed that a contaminated inlet on the GC/MS can result in false positive alkaloid detection in extracts which do not actually contain alkaloids. It is also important to optimise the GC/MS method to be used as the mesembrane alkaloids do not separate well under all conditions, and often compounds from this family have very close retention times. As a result, some mesembrane alkaloids may be mis-identified due to the compounds not being fully separated on the GC/MS.

Using the program developed for this study, six of the mesembrane alkaloids and the alkaloid hordenine were found among the extracts separated, with retention times between 22,6 and 24,4 minutes for the mesembrane alkaloids, and 15 minutes for hordenine. Table 6.1 shows how close the retention times of some of the mesembrane alkaloids are.

Table 6.1: The Retention Times of Hordenine and the Mesembrane Alkaloids on GC/MS.

Retention time (min)	Alkaloid identified
14.8 - 15.5	Hordenine (28)
22.6 - 22.8	Joubertiamine (21)
22.7 - 23.0	Dehydrojoubertiamine (22)
23.8 - 23.9	Mesembrenol (12)
23.9 - 24.0	Mesembranol (13)
24.0 - 24.2	Mesembrine (10)
24.2 - 24.4	Mesembrenone (11)

It is of concern that Smith et al.⁶ ran a short GC program when they investigated selected taxa of the Mesembryanthemaceae for the distribution of mesembrane alkaloids. The retention times they obtained for 4'-O-demethylmesembrenol (**15**), mesembrine (**10**) and mesembrenone (**11**) are given as 11.5, 12 and 12.5 min, respectively. These values are very close together. It is worth considering that perhaps some of the alkaloids identified by Smith et al. as being present in certain samples may have been mis-identified due to the compounds not separating fully as a result of the short GC run.

Conclusion

Determining the presence or absence of alkaloids in Mesembryanthemum is complicated, as different species have in the past tested both alkaloid positive and negative. It is also difficult as alkaloids are secondary metabolites and their levels are strongly influenced by many factors. The fifteen Ruschioideae screened for the presence of alkaloids in this study all tested negative for alkaloids on TLC. This could be due to the specific plants extracted not containing alkaloids, or possibly the specific species extracted and screened in this study are alkaloid negative. It may be worthwhile to carefully re-examine all species previously reported to contain alkaloids with the aim of determining the frequency of false positive reports.

6.4 References

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Chapter 7

Experimental Procedures

All glassware was washed prior to use and then rinsed with acetone, following which it was dried in an oven at ~100 °C. All organic solvents were distilled prior to use.

7.1 Alkaloid Extraction Procedures

Depending on the amount of plant material to be extracted, one of two alkaloid extraction methods was followed. The initial alkaloid extracts for the alkaloid screening process were analytical extracts. The bulk material of plants which tested alkaloid positive in the screening process was then extracted using the bulk extraction method.

7.1.1 Analytical Alkaloid Extraction Procedure

Plant material (1 g, oven-dried at maximum 40 °C) was finely ground using a pestle and mortar. It was then mixed with 15 ml 0.05 M H₂SO₄ and left standing at room temperature for between 20 and 30 minutes.

A cylindrical glass column (27 cm X 2.5 cm) was plugged with cotton wool, then 10 g anhydrous sodium sulphate and 24 g of coarse grade celite-577 were placed consecutively in the column. A small amount of acid washed sand was placed on top of the celite to prevent the celite from floating. A 250 ml flask was placed under the column to collect the extract. Filter paper (Whatman No. 4) was placed in a funnel above the column. The acidic plant extract was then filtered onto the column. In order to speed up the process and minimize loss of extract, the filter paper may be squeezed gently to force out the partly filtered solution. After the

acidic filtrate had begun to filter through the column, approximately 4 ml of ammonia was added to the column. The column was then washed with 100 ml of dichloromethane (DCM, CH_2Cl_2) and the extract was collected in the flat-bottomed flask. Evaporation under vacuum at 60 °C yielded the alkaloid extract. The crude alkaloid extract was transferred to a pre-weighed screw-top sample bottle, and its yield was determined.

7.1.2 Bulk Alkaloid Extraction Procedure

Plant material (oven-dried at maximum 40 °C) was finely ground using a rotary beater mill. Approximately 0.5 – 1.5 kg of the ground plant material was weighed and mixed with enough 0.05 M H_2SO_4 to cover the dry plant material. After being mixed thoroughly, it was left standing at room temperature for 1 hour (during which it was stirred a further two times).

A Büchner funnel was prepared by placing a piece of filter paper (Whatman No. 4) in the funnel followed by coarse grade celite-577 (enough to create a layer approximately 2 cm thick). A small amount of acid washed sand was placed on top of the celite to prevent the celite from floating. The funnel was placed on top of a 5 L Büchner flask and the aqueous plant material was filtered under vacuum. The pH of the filtrate was adjusted to 7.00 by adding 25% ammonia solution. The filtrate was poured into a separating funnel until it was three-quarters full, and 100 ml DCM was added to it. It was shaken well and left to separate into two layers. The aqueous layer was rinsed three times with DCM, and the organic layers were collected and pooled.

A cylindrical glass column (60 cm X 5 cm) was plugged with cotton wool, which was then covered with an approximately 1 cm thick layer of anhydrous sodium sulphate and packed with coarse grade celite-577 until three-quarters full. A small amount of acid washed sand was placed on top of the celite to prevent the celite from floating. The organic extract was then filtered onto the column and allowed to filter through. The column was rinsed with two to three column volumes of

dichloromethane (DCM, CH_2Cl_2) and the extract was collected in a flask. Evaporation under vacuum at 60 °C yielded the alkaloid extract. The crude alkaloid extract was transferred to a pre-weighed screw-top sample bottle, and its yield was determined.

7.2 Thin-Layer Chromatography

The alkaloid screening process as well as the subsequent phytochemical investigation of each plant was performed using thin-layer chromatography (TLC). Aluminium backed 0.2 mm silica gel plates were used (Merck 0.20 mm 60 F₂₅₄). The optimal solvent system for the extracts was found to be cyclohexane:chloroform:diethylamine in the ratio 10:6:1. The TLC plates were dried using a hairdryer for an hour prior to visualisation; otherwise the diethylamine in the developing system would react with the iodoplatinate detection reagent, resulting in a high background reading. The TLC plates were visualised with the aid of UV radiation (UV₂₅₄) followed by spraying with an iodoplatinate detection reagent.

Iodoplatinate detection reagent for alkaloids

A: Dissolve 0.15 g potassium chloroplatinate (K_2PtCl_6 , FW 486.0) in 5 ml concentrated hydrochloric acid and add to 45 ml water.

B: Dissolve 3 g of potassium iodide in 5 ml concentrated hydrochloric acid and add to 45 ml water.

Mix A and B in a dark bottle and keep in a fridge. Add a few drops of concentrated hydrochloric acid to the atomizer just before spraying the plates. Use reagent sparingly as it is very expensive.

7.3 Isolation of Compounds on a Chromatotron

Selected crude alkaloid extracts of bulk samples of *Aptenia cordifolia* as well as *A. lancifolia* were separated into individual compounds using a Chromatotron Model 7924T (year 1987), purchased from Harrison Research, 840 Moana Court, Palo Alto, California, USA.

The bulk alkaloid extract was dissolved in a minimum volume of DCM. Approximately 200 ml of the developing system cyclohexane: chloroform: diethylamine in the ratio 10:6:1 was prepared prior to the sample being loaded onto the chromatotron plate.

It was ensured that the grooves and the back of the chromatotron instrument were clean prior to the use of the instrument. The plate size was selected according to the amount of crude alkaloid extract to be separated as well as the number of compounds present in the sample. The appropriate size plate was lowered into the instrument and locked firmly but gently into place, being careful not to disturb the silica gel surface. The rotation was switched on and the sample was carefully applied in a drop-wise manner to the innermost base line of the plate. Once the base line was dry, the plate lid was shut gently and clamped into place. The appropriate size flow rate adaptor was selected according to the plate size in use, and was attached to the plate lid. The solvent pipe was attached to the flow rate adaptor, and the door of the instrument was closed. The UV lamp was then switched on to aid in the identification and separation of the individual compounds. With the tap of the separating funnel firmly closed, the developing solution was poured into the separating funnel. The tap of the separating funnel was then opened to allow the solvent to flow to the plate. Fractions of eluent were collected. Once all the desired fractions had been collected, the plate was rinsed using 100 ml methanol. The rinse fractions were also collected. Following use, the plate was removed and placed in an oven to dry for 1 hour before its next use.

TLC was performed on all the fractions collected in order to determine which ones to pool. The pooled fractions were then evaporated to dryness *in vacuo* at 60 °C.

7.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy of the isolated compounds was performed on either the Varian Gemini 300 MHz or the Varian Inova 300 MHz spectrophotometer. All spectra were recorded at 25 °C and the chemical shifts were recorded in parts per million (ppm) referenced to tetramethylsilane (TMS) or the solvent shift. Deuterated chloroform (CDCl₃) was used as the solvent.

7.5 Gas Chromatography/Mass Spectrometry

GC/MS (electron impact ionisation) was carried out using a GCMS-QP2010 gas chromatograph/mass spectrometer fitted with a 5% phenyl – 95% dimethylpolysiloxane ZB-5 column (dimensions 30m x0.25mm x25µm, Phenomenex). The column program was from 50 °C to 300 °C at 10 °C per minute. Structures of the compounds were elucidated based on their respective mass spectra and comparison of retention times with standards.

7.6 Plant Material

Authorities for the names of all taxa as well as the collection locality of all species investigated are given in Table 7.1. Table 7.2 indicates the percentage yields of alkaloids from all plants investigated in this study. Voucher specimens of all the plants screened in this thesis have been deposited in the University of Johannesburg Herbarium (JRAU), with the exceptions of specimens M1 to M7 of which only small quantities of material were collected. All plants screened in this thesis, with the exceptions FR van Heerden 66 to FR van Heerden 74 and M1 to M7, were identified by Priscilla Burgoyne at the National Herbarium (PRE) of the South African National Biodiversity Institute (SANBI), formerly the National

Botanical Institute (NBI) in Pretoria. Specimens FR van Heerden 66 to FR van Heerden 74 (inclusive) were obtained from and identified by Ian Oliver at the Karoo Desert National Botanical Garden in Worcester.

7.7 GC/MS analysis of selected plant extracts

GC/MS data for extracts where alkaloids were detected by TLC, was obtained.

The GC/MS data for the alkaloids present in *S. crassicaule* is given below. RT indicates the retention time of the compound.

S. crassicaule leaves:

(1) Hordenine: RT 14.858 min; m/z 165 (10%, M^+), 69 (27%), 57 (100%), 29 (47%); (2) Mesembrenol: RT 23.908 min; m/z 289 (1%, M^+), 219 (6%), 115 (3%), 70 (100%), 58 (7%), 42 (21%) 28 (5%); (3) Mesembranol: RT 24.010 min; m/z 290 [5%, $(M-1)^+$], 219 (10%), 70 (100%), 57 (42%), 28 (29%); (4) Mesembrine: RT 24.167 min; m/z 289 (8%, M^+), 218 (8%), 96 (29%), 70 (100%), 55 (11%), 42 (25%), 28 (14%); (5) Mesembrenone: RT 24.367 min; m/z 287 (2%, M^+), 219 (1%), 115 (2%), 70 (100%), 42 (23%).

S. crassicaule roots:

(1) Hordenine: RT 14.942 min; m/z 165 (8%, M^+), 69 (20%), 43 (78%), 28 (100%); (2) Mesembrine: RT 24.200 min; m/z 289 (10%, M^+), 218 (6%), 96 (33%), 70 (100%), 44 (28%), 28 (45%); (3) Mesembrenone: RT 24.375 min; m/z 287 (4%, M^+), 219 (1%), 115 (2%), 70 (100%), 42 (23%).

S. crassicaule stems:

(1) Hordenine: RT 14.850 min; m/z 165 (10%, M^+), 69 (17%), 57 (100%), 43 (68%), 29 (33%); (2) Mesembrenol: RT 23.883 min; m/z 289 (1%, M^+), 219 (5%), 115 (2%), 70 (100%), 42 (19%); (3) Mesembranol: RT 24.010 min; m/z 290 [8%, $(M-H)^+$], 219 (10%), 115 (5%), 70 (100%), 57 (18%), 44 (38%), 28 (15%); (4) Mesembrine: RT 24.150 min; m/z [289 (5%, M^+), 219 (10%), 129 (8%), 96 (21%), 70 (100%), 42 (37%); (5) Mesembrenone: RT 24.358 min; m/z 287 (3%, M^+), 219 (1%), 115 (3%), 70 (100%), 42 (22%).

The GC/MS data for the alkaloids present in *S. tortuosum* is given below. RT indicates the retention time of the compound.

S. tortuosum (ex *S. namaquense*) leaves:

(1) Mesembrine: RT 24.250 min; m/z 289 (3%, M^+), 218 (3%), 160 (2%), 96 (14%), 70 (35%), 43 (26%), 28 (100%).

S. tortuosum leaves:

(1) Mesembrenol: RT 23.900 min; m/z 289 (1%, M^+), 219 (11%), 204 (3%), 70 (100%), 58 (10%), 42 (27%), 29 (6%); (2) Mesembranol: RT 24.033 min; m/z 290 [6%, $(M-H)^+$], 204 (3%), 70 (20%), 58 (100%), 44 (23%), 29 (7%); (3) Mesembrine: RT 24.142 min; m/z 289 (11%, M^+), 274 (1%), 218 (11%), 96 (56%), 70 (100%), 58 (25%), 42 (56%), 28 (12%); (4) Mesembrenone: RT 24.375 min; m/z 287 (4%, M^+), 219 (1%), 115 (4%), 70 (100%), 58 (51%), 42 (41%), 28 (20%).

The GC/MS data for the alkaloids present in both *A. cordifolia* is given below. RT indicates the retention time of the compound.

Aptenia cordifolia 1 stems:

(1) 4'-*O*-methylsceletenone: RT 23.042 min; m/z 257 (4%, M^+), 189 (2%), 115 (5%), 70 (100%), 42 (27%); (2) Hordenine: RT 14.842 min; m/z 165 (11%, M^+), 57 (100%), 43 (66%), 29 (37%); (3) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.850 min; m/z 259 (8%, M^+), 202 (5%), 189 (19%), 115 (10%), 96 (32%), 70 (100%), 58 (67%), 42 (55%), 27 (8%).

Aptenia cordifolia 2 leaves:

(1) Hordenine: RT 14.875 min; m/z 165 (8%, M^+), 67 (21%), 57 (100%), 29 (54%).

Aptenia cordifolia 2 roots:

(1) 4'-*O*-methylsceletenone: RT 23.050 min; m/z 257 (3%, M^+), 189 (1%), 115 (3%), 70 (100%), 42 (28%), 28 (3%); (2) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.858 min; m/z 259 (10%, M^+), 202 (3%), 174 (16%), 96 (40%), 70 (100%), 28 (72%).

Aptenia cordifolia 2 stems:

(1) 4'-*O*-methylsceletenone: RT 23.075 min; m/z 257 (2%, M^+), 189 (1%), 115 (3%), 70 (100%), 42 (24%), 28 (2%); (2) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.842 min; m/z 259 (6%, M^+), 174 (12%), 115 (10%), 96 (42%), 70 (100%), 42 (65%), 28 (24%).

The GC/MS data for the alkaloids present in *A. lancifolia* is given below. RT indicates the retention time of the compound.

Aptenia lancifolia stems:

(1) 4'-*O*-methylsceletenone: RT 23.050 min; m/z 257 (2%, M^+), 189 (1%), 115 (3%), 70 (100%), 58 (6%), 42 (18%); (2) Hordenine: RT 14.858 min; m/z 165 (6%, M^+), 67 (15%), 57 (100%), 43 (52%), 29 (37%); (3) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.850 min; m/z 259 (6%, M^+), 202 (2%), 188 (10%), 96 (37%), 70 (100%), 42 (58%), 27 (18%).

Aptenia lancifolia leaves:

(1) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.833; m/z 259 (4%, M^+), 188 (10%), 96 (40%), 70 (100%), 42 (56%), 28 (8%); (2) Hordenine: RT 14.867 min; m/z 165 (5%, M^+), 67 (17%), 57 (100%), 43 (53%), 29 (39%).

Aptenia lancifolia roots:

(1) 4'-*O*-methylsceletenone: RT 23.100 min; m/z 257 (2%, M^+), 189 (1%), 115 (2%), 70 (100%), 42 (20%), 28 (1%); (2) Hordenine: RT 14.850 min; m/z 165 (7%, M^+), 57 (100%), 43 (56%), 29 (31%); (3) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.850 min; m/z 259 (2%, M^+), 188 (5%), 96 (33%), 70 (100%), 42 (59%), 28 (13%).

Aptenia lancifolia woody stems:

(1) 4'-*O*-methylsceletenone: RT 23.058 min; m/z 257 (2%, M^+), 189 (1%), 115 (3%), 70 (100%), 42 (25%), 28 (2%); (2) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.867 min; m/z 259 (2%, M^+), 188 (5%), 96 (28%), 70 (100%), 42 (52%), 28 (26%).

Aptenia lancifolia fruit and flowers:

(1) 4,5-dihydro-4'-*O*-methylsceletenone: RT 22.833 min; m/z 259 (5%, M^+), 202 (2%), 188 (10%), 115 (7%), 96 (41%), 70 (100%), 42 (52%), 28 (9%);

(2) 4'-*O*-methylsceletenone: RT 23.100 min; m/z 257 (2%, M^+), 189 (1%), 115 (4%), 70 (100%), 58 (7%), 42 (27%), 28 (17%).

The GC/MS data for the alkaloids present in *A. serotina* is given below. RT indicates the retention time of the compound.

Aridaria serotina leaves:

(1) Mesembrine: RT 24.250 min; m/z 289 (17%, M^+), 218 (14%), 96 (43%), 70 (100%), 55 (6%), 42 (32%), 28 (14%); (2) Mesembranol: RT 24.042 min; m/z 290 [25%, $(M-1)^+$], 274 (5%), 204 (10%), 96 (14%), 70 (63%), 57 (38%), 44 (100%); (3) Mesembrenone: RT 24.367 min; m/z 287 (4%, M^+), 219 (1%), 115 (2%), 70 (100%), 55 (3%), 42 (26%), 28 (4%).

Aridaria serotina roots:

(1) Mesembrenone: RT 24.475 min; m/z 287 (4%, M^+), 219 (1%), 115 (1%), 70 (100%), 55 (1%), 42 (17%); (2) Mesembrine: RT 24.175 min; m/z 289 (12%, M^+), 218 (11%), 96 (50%), 70 (100%), 55 (8%), 42 (44%), 28 (6%); (3) Mesembranol: RT 24.025 min; m/z 290 [25%, $(M-1)^+$], 276 (6%), 219 (10%), 91 (9%), 70 (62%), 57 (42%), 44 (100%), 28 (69%); (4) Hordenine: RT 14.893 min; m/z 165 (5%, M^+), 57 (100%), 43 (46%), 29 (36%).

Aridaria serotina stems:

(1) Mesembrine: RT 24.183 min; m/z 289 (12%, M^+), 218 (11%), 96 (48%), 70 (100%), 55 (7%), 42 (40%); (2) Mesembrenone: RT 24.367 min; m/z 287 (3%, M^+), 219 (1%), 115 (3%), 70 (100%), 55 (3%), 42 (26%), 28 (3%); (3) Hordenine: RT 14.833 min; m/z 165 (7%, M^+), 67 (12%), 57 (100%), 43 (49%), 29 (37%); (4) Mesembranol: RT 23.983 min; m/z 290 [26%, $(M-1)^+$], 274 (6%), 204 (12%), 96 (13%), 70 (56%), 57 (43%), 44 (100%), 28 (18%).

The GC/MS data for the alkaloids present in *Mesembryanthemum* is given below. RT indicates the retention time of the compound.

M. aitonis 2 leaves:

(1) Hordenine: RT 16.833 min; m/z 165 (2%, M^+), 71 (100%), 59 (43%), 43 (98%), 28 (20%).

M. aitonis 3 roots:

(1) Hordenine: RT 15.100 min; m/z 165 (1%, M^+), 77 (2%), 58 (100%), 42 (4%).

M. aitonis 3 stems:

(1) Hordenine: RT 15.100 min; m/z 165 (1%, M^+), 77 (2%), 58 (100%), 42 (3%).

M. guerichianum leaves:

(1) Hordenine: RT 15.258 min; m/z 165 (1%, M^+), 77 (3%), 58 (100%), 42 (7%), 28 (19%).

M. guerichianum roots:

(1) Hordenine: RT 15.467 min; m/z 77 (1%), 58 (100%), 42 (2%), 28 (33%).

The GC/MS data for the alkaloids present in *Phyllobolus* is given below. RT indicates the retention time of the compound.

P. digitatus subsp. *digitatus* green stems:

(1) Hordenine: RT 15.033 min; m/z 165 (1%, M^+), 77 (3%), 58 (100%), 42 (5%).

P. digitatus subsp. *digitatus* papery stems:

(1) Hordenine: RT 15.533 min; m/z 165 (1%, M^+), 77 (5%), 58 (100%), 42 (10%).

P. digitatus subsp. *digitatus* roots:

(1) Hordenine: RT 15.533 min; m/z 165 (5%, M^+), 107 (25%), 77 (55%), 58 (100%), 42 (62%), 30 (44%).

P. digitatus subsp. *digitatus* roots: chromatotron fractions 9-15:

(1) Hordenine: RT 15.442 min; m/z 165 (5%, M^+), 107 (25%), 77 (55%), 58 (100%), 42 (64%), 30 (47%).

The GC/MS data for the alkaloids present in *Psilocaulon* is given below. RT indicates the retention time of the compound.

P. coriarium 1 stems:

(1) Hordenine: RT 15.325 min; m/z 165 (5%, M^+), 107 (24%), 77 (58%), 58 (100%), 42 (66%), 30 (45%).

P. coriarium 2 leaves:

(1) Hordenine: RT 15.500 min; m/z 165 (10%, M^+), 121 (18%), 107 (28%), 77 (67%), 58 (100%), 42 (75%), 30 (57%).

P. coriarium 2 stems:

(1) Hordenine: RT 14.242 min; m/z 78 (1%), 58 (100%), 30 (4%).

7.8 Isolation of alkaloids from *Aptenia cordifolia* and *A. lancifolia*

Bulk plant material of *Aptenia cordifolia* was collected in the garden of Professor Ben-Erik van Wyk. Bulk plant material of *A. lancifolia* was collected on the University of Johannesburg Kingsway Campus. The plant material was separated into the various plant parts and dried in an oven at 37 °C, following which the dry weight of the material was determined. The bulk alkaloid extraction procedure described in section 7.1.2 was then followed, and the weight as well as the percentage yield of crude alkaloid extract was determined.

Aptenia cordifolia:

45.040 g of fruit and flowers yielded 13 mg of crude alkaloid extract, with a percentage yield of 0.029%.

11.083 g of roots yielded 26 mg of crude alkaloid extract, with a percentage yield of 0.235%.

45.281 g of leaves yielded 59 mg of crude alkaloid extract, with a percentage yield of 0.066%.

220.869 g of stems yielded 197 mg of crude alkaloid extract, with a percentage yield of 0.089%.

Aptenia lancifolia:

19.430 g of fruit and flowers yielded 3 mg of crude alkaloid extract, with a percentage yield of 0.015%.

83.154 g of roots yielded 158 mg of crude alkaloid extract, with a percentage yield of 0.191%.

110.868 g of leaves yielded 63 mg of crude alkaloid extract, with a percentage yield of 0.057%.

224.198 g of green stems yielded 91 mg of crude alkaloid extract, with a percentage yield of 0.041%.

117.259 g of woody stems yielded 242 mg of crude alkaloid extract, with a percentage yield of 0.206%.

The crude alkaloid extracts were screened for the presence of alkaloids using the thin-layer chromatography procedure described in section 7.2. Isolation of individual compounds where possible was performed using a Chromatotron as described in section 7.3. Nuclear Magnetic Resonance Spectroscopy (NMR) was then performed on the pure compounds, as described in section 7.4.

Chromatotron separation of extracts led to the isolation of:

2 mg of 4'-*O*-methylsceletenone from *A. lancifolia* roots,

4 mg of 4,5-dihydro-4'-*O*-methylsceletenone from *A. lancifolia* leaves,

2 mg of 4'-*O*-methylsceletenone from *A. lancifolia* stems,

5 mg of 4'-*O*-methylsceletenone from *A. cordifolia* roots.

4'-*O*-methylsceletenone (**20**) has:

^1H NMR: δ_{H} 7.26 (2H, d, J 7.3 Hz, H-2', 6'), 6.88 (2H, d, J 7.3 Hz, H-3', 5'), 6.72 (1H, br. d, J 10.1 Hz, H-4), 6.10 (1H, d, J 10.0 Hz, H-5), 3.79 (3H, s, OMe), 2.33 (3H, s, NCH₃).

MS: m/z 257 (2%, M⁺), 189 (1%), 115 (4%), 70 (100%), 58 (7%), 42 (27%), 28 (17%).

4,5-dihydro-4'-*O*-methylsceletenone (**21**) has:

^1H NMR: δ_{H} 7.09 (2H, br. d, J 8.5 Hz), 6.88 (2H, J 8.8 Hz, H-3',5'), 3.79 (3H, s, OCH₃).

MS: m/z 259 (5%, M^+), 202 (2%), 188 (10%), 115 (7%), 96 (41%), 70 (100%), 42 (52%), 28 (9%)

Mesembrine (**10**) has:

^1H NMR: δ_{H} 6.92 (1H, dd, J 8.3 and 2.3 Hz, H-6'), 6.40 (1H, d, J 2.3 Hz, H-6'), 6.84 (1H, d, J 8.4 Hz, H-5'), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 2.31 (3H, s, NMe).

^{13}C NMR: δ_{C} 211.5 (C-6), 149.0, 147.4 (C-3',4'), 140.2 (C-1'), 117.9, 111.0, 110.0 (C-2',5',6'), 70.4 (C-7a), 56.0, 55.9 (2 x OCH_3), 54.8, 47.5, 40.5, 40.1, 38.8, 36.2, 35.3.

MS: m/z 289 (12%, M^+), 218 (11%), 96 (50%), 70 (100%), 55 (8%), 42 (44%), 28 (6%).

7.9 Isolation of hordenine from *Psilocaulon coriarium* 2 (FVH 65) leaves and from *Phyllobolus digitatus* subsp. *digitatus* (FVH 41) roots.

Bulk plant material of *Psilocaulon coriarium* (FVH 65) leaves and of *Phyllobolus digitatus* subsp. *digitatus* (FVH 41) roots was collected. The plant material was dried in an oven at 37 °C, following which the dry weight of the material was determined. The bulk alkaloid extraction procedure described in section 7.1.2 was performed on the dry plant material, and the weight as well as the percentage yield of crude alkaloid extracts was determined. 6.243 g of *Psilocaulon coriarium* (FVH 65) leaves yielded 24 mg of crude alkaloid extract, with a percentage yield of 0.384%. 95.517 g of *Phyllobolus digitatus* subsp. *digitatus* (FVH 41) roots leaves yielded 62 mg of crude alkaloid extract, with a percentage yield of 0.065%.

The crude alkaloid extracts were screened for the presence of alkaloids using the thin-layer chromatography procedure described in section 7.2. In the crude alkaloid extract of the leaves of *Psilocaulon coriarium* (FVH 65), only one alkaloid

was detected. The alkaloid present in the extract was identified from the NMR spectra as hordenine (**36**).

In the crude alkaloid extract of the roots of *Phyllobolus digitatus* subsp. *digitatus* (FVH 41), only one alkaloid was detected. 29 mg of the crude extract (i.e. approximately half of the 62 mg of original crude extract) was then separated on a Chromatotron as described in section 7.3 to isolate the pure compound. 23 mg of pure compound was isolated. The alkaloid present in the extract was identified from the NMR spectrum as hordenine (**36**).

Hordenine (**36**) has:

^1H NMR: δ_{H} 6.97 (2H, d, $J=8.4$ Hz, H-2',6'), 6.63 (2H, d, $J=8.4$ Hz, H-3',5'), 2.74-2.66 (2H, m, H-2), 2.63-2.55 (2H m, H-1), 2.33 (6H, s, 2 x NCH_3).

^{13}C NMR: δ_{C} 155.3 (C-4'), 130.3 (C-1'), 129.5 (C-2',6'), 115.7 (C-3',5'), 61.44 (C-1), 44.9 (NMe), 32.6 (C-2).

MS: m/z 165 (1%, M^+), 77 (3%), 58 (100%), 42 (7%), 28 (19%).

Table 7.1: Genus and species, voucher number and collection locality of all species investigated.

Genus and species (Author)	Voucher specimen number (JRAU)	Locality
<i>Aptenia cordifolia</i> (L.f.) Schwantes	FR van Heerden A (JRAU)	Prof Ben-Erik van Wyk's home garden, JHB
<i>Aptenia lancifolia</i> L.Bolus	FR van Heerden B (JRAU)	UJ Kingsway Campus, in front of the postal division.
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 1 (JRAU)	Keurboomstrand rd approx. 200m from the turn-off from the N2 (first rd after bridge from Knysna); GPS: S 34.00.504 E 023.24.726 elevation 5.6m
<i>Aptenia cordifolia</i> (L.f.) Schwantes	FR van Heerden 2 (JRAU)	Grootriver Pass on R102 heading towards Nature's Valley from Plettenberg Bay; GPS: S 33.58.554 E 023.33.490
<i>Ruschia</i> sp. (<i>Uncinata</i> group)	FR van Heerden 4 (JRAU)	On the road from Uniondale to Willowmore; GPS: S 33.20.862 E 023.26.251 elevation 967m
<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	FR van Heerden 5 (JRAU)	On the road from Uniondale to Willowmore, approx 5km from Willowmore; GPS: S 33.20.331 E 023.26.638 elevation 939.2m
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 7 (JRAU)	On the road from Uniondale to Willowmore, approx 5km from Willowmore; GPS: S 33.20.331 E 023.26.638 elevation 939.2m
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 8 (JRAU)	On the road from Uniondale to Willowmore, approx 5km from Willowmore; GPS: S 33.20.331 E 023.26.638 elevation 939.2m
<i>Mesembryanthemum aitonis</i> Jacq.	FR van Heerden 9A (JRAU)	On the road from Uniondale to Willowmore, approx 5km from Willowmore; GPS: S 33.20.331 E 023.26.638 elevation 939.2m
<i>Mesembryanthemum nodiflorum</i> L.	FR van Heerden 9B (JRAU)	On the road from Uniondale to Willowmore, approx 5km from Willowmore; GPS: S 33.20.331 E 023.26.638 elevation 939.2m
<i>Drosanthemum</i> sp.	FR van Heerden 10 (JRAU)	In Willowmore, near the train station; GPS: S 33.18.070 E 023.29.047 elevation 842.9m
<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 11 (JRAU)	In Willowmore, near the train station; GPS: S 33.18.070 E 023.29.047 elevation 842.9m

<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 12 (JRAU)	In Willowmore, near the train station; GPS: S 33.18.070 E 023.29.047 elevation 842.9m
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 14 (JRAU)	On the rd from Willowmore to Klaarstroom; GPS: S 33.17.559 E 023.28.501
<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	FR van Heerden 16 (JRAU)	Approx 4km from Willowmore on rd to Klaarstroom; GPS: S 33.16.966 E 023.26.108
<i>Ruschia</i> sp. (<i>Uncinata</i> group)	FR van Heerden 18 (JRAU)	On the rd btw Willowmore and de Rust, 29km from the turn-off; GPS: S 33.31.951 E 023.57.049
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 20 (JRAU)	On rd from Willowmore to de Rust; approx 15km from turn-off; GPS: S 33.28.500 E 023.05.263
<i>Psilocaulon bicornis</i> (Sond.) Schwantes	FR van Heerden 21 (JRAU)	On rd from Willowmore to de Rust; approx 15km from turn-off; GPS: S 33.28.500 E 023.05.264
<i>Psilocaulon</i> cf. <i>junceum</i> (Haw.) Schwantes	FR van Heerden 22 (JRAU)	On rd from George to Oudtshoorn (N12 or R062); 15km from Oudtshoorn; GPS: S 33.42.223 E 022.17.307 elevation 523.4m
<i>Sceletium crassicaule</i> (Haw.) L.Bolus	FR van Heerden 23 (JRAU)	On rd from George to Oudtshoorn (N12 or R062); 14km from Oudtshoorn; GPS: S 33.42.223 E 022.17.307 elevation 523.4m
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 24 (JRAU)	On rd from George to Oudtshoorn (N12 or R062); 14km from Oudtshoorn; GPS: S 33.42.223 E 022.17.307 elevation 523.4m
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 26 (JRAU)	Approx 2km outside of Ladismith on the R062; GPS: S 33.30.872 E 021.15.366 elevation 510.9m
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 27 (JRAU)	Approx 11km outside of Ladismith on the R062; GPS: S 33.34.228 E 021.13.142 elevation 395.4m
<i>Psilocaulon articulatum</i> (Thunb.) N.E.Br.	FR van Heerden 28 (JRAU)	Approx 11km outside of Ladismith on the R062; GPS: S 33.34.228 E 021.13.142 elevation 395.4m
<i>Prenia englishiae</i> (L.Bolus) Gerbaulet	FR van Heerden 29 (JRAU)	19km from Ladismith on the R62; just before the Barrydale turn-off; GPS: S 33.35.862 E 021.08.314 elevation 325.4m
<i>Ruschia</i> sp.	FR van Heerden 33 (JRAU)	Between Klawer and Clanwilliam on the N7; GPS: S 32.05.229 E 018.49.398 elevation 94.5m
<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	FR van Heerden 37 (JRAU)	15km S of Klawer on the N7; GPS: S 31.54.994 E 018.39.798
<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	FR van Heerden 39 (JRAU)	15km S of Klawer on the N7; GPS: S 31.54.994 E 018.39.800

<i>Prenia</i> sp.	FR van Heerden 40 (JRAU)	7km S of Klawer on the N7
<i>Phyllobolus digitatus</i> (Aiton) Gerbaulet subsp. <i>digitatus</i>	FR van Heerden 41 (JRAU)	Knersvlakte*
<i>Drosanthemum</i> sp.	FR van Heerden 42 (JRAU)	Knersvlakte*
<i>Psilocaulon dinteri</i> (Engl.) Schwantes	FR van Heerden 44A (JRAU)	Knersvlakte*
<i>Mesembryanthemum guerichianum</i> Pax	FR van Heerden 44B (JRAU)	Knersvlakte*
<i>Psilocaulon granulicaule</i> (Haw.) Schwantes	FR van Heerden 46 (JRAU)	Knersvlakte*
<i>Phyllobolus nitidus</i> (Haw.) Gerbaulet	FR van Heerden 49 (JRAU)	Knersvlakte*
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 50 (JRAU)	Knersvlakte*
<i>Phyllobolus spinuliferus</i> (Haw.) Gerbaulet	FR van Heerden 52B (JRAU)	Knersvlakte*
<i>Psilocaulon granulicaule</i> (Haw.) Schwantes	FR van Heerden 53 (JRAU)	Knersvlakte*
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 54 (JRAU)	Knersvlakte*
<i>Aridaria serotina</i> L.Bolus	FR van Heerden 55 (JRAU)	Knersvlakte*
<i>Ruschia</i> sp.	FR van Heerden 58 (JRAU)	Knersvlakte*
<i>Monilaria moniliformis</i> (Thunb.) Ihlenf. & Jörg.	FR van Heerden 59 (JRAU)	Knersvlakte*
<i>Mesembryanthemum</i> sp.	FR van Heerden 60 (JRAU)	Knersvlakte*
<i>Mesembryanthemum crystallinum</i> L.	FR van Heerden 61 (JRAU)	Knersvlakte*

<i>Psilocaulon coriarium</i> (Burch. ex N.E.Br.) N.E.Br.	FR van Heerden 62 (JRAU)	Knersvlakte*
<i>Psilocaulon coriarium</i> (Burch. ex N.E.Br.) N.E.Br.	FR van Heerden 65 (JRAU)	On the road from Wellington to Worcester
<i>Prenia</i> cf. <i>pallens</i> (Aiton)	FR van Heerden 66 (JRAU)	Karoo Botanical Gardens, Worcester, on the hillside
<i>Mesembryanthemum</i> cf. <i>guerichianum</i> Pax	FR van Heerden 67 (JRAU)	Karoo Botanical Gardens, Worcester, on the pathway
<i>Sceletium tortuosum</i> (L.) N.E.Br.	FR van Heerden 68 (JRAU)	Karoo Botanical Gardens, Worcester, in the cultivated gardens
<i>Sceletium rigidum</i> L.Bolus	FR van Heerden 69 (JRAU)	Karoo Botanical Gardens, Worcester, in the greenhouse
<i>Sceletium tortuosum</i> (L.) N.E.Br.	FR van Heerden 70 (JRAU)	Karoo Botanical Gardens, Worcester, in the greenhouse
<i>Phyllobolus</i> cf. <i>longispinulus</i>	FR van Heerden 71 (JRAU)	Karoo Botanical Gardens, Worcester, in the greenhouse
<i>Phyllobolus rabiei</i> (L.Bolus) Gerbaulet	FR van Heerden 72 (JRAU)	Karoo Botanical Gardens, Worcester, in the greenhouse
<i>Phyllobolus</i> cf. <i>anrathus</i>	FR van Heerden 73 (JRAU)	Karoo Botanical Gardens, Worcester, in the greenhouse
<i>Phyllobolus congestus</i> (L.Bolus) Gerbaulet	FR van Heerden 74 (JRAU)	Karoo Botanical Gardens, Worcester, in the greenhouse
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 77 (JRAU)	4.5km on rd to Sutherland from Karooport; GPS: S 33.12.738 E 019.43.419 elevation 758.6m
<i>Mesembryanthemum nodiflorum</i> L.	FR van Heerden 78 (JRAU)	At the fork in the road on the Sutherland turn-off from Karooport
<i>Psilocaulon junceum</i> (Haw.) Schwantes	FR van Heerden 81 (JRAU)	3km from the fork in the rd to Sutherland from Karooport; GPS: S 33.11.478 E 019.45.435 elevation 706.0m
<i>Drosanthemum</i> sp.	FR van Heerden 82 (JRAU)	3km from the fork in the rd to Sutherland from Karooport; GPS: S 33.11.478 E 019.45.435 elevation 706.0m

<i>Malephora</i> sp.	FR van Heerden 83 (JRAU)	3km from the fork in the rd to Sutherland from Karooport; GPS: S 33.11.478 E 019.45.435 elevation 706.0m
<i>Mesembryanthemum excavatum</i> L.Bolus	FR van Heerden 84 (JRAU)	3km from the fork in the rd to Sutherland from Karooport; GPS: S 33.11.478 E 019.45.435 elevation 706.0m
<i>Pleiospilos compactus</i> (Aiton) Schwantes	M2	Bakensklip (kruitpomp), Murraysburg, Orange Free State
<i>Pleiospilos compactus</i> (Aiton) Schwantes	M3	Garden of R van der Walt (Bloemfontein). Original from Bakensklip, Murraysburg, Orange Free State
<i>Eberlanzia ferox</i> (Aiton)	M4	Bakensklip (kruitpomp), Murraysburg, Orange Free State
<i>Trichodiadema</i> sp.	M5	Bakensklip (kruitpomp), Murraysburg, Orange Free State
<i>Malephora</i> sp.	M6	Kareebosch, Murraysburg, Orange Free State; next to road near oil drilling hole.
<i>Ruschia</i> sp.	M7	Garden of R van der Walt (Bloemfontein). Original from Murraysburg, Orange Free State

*All the plants whose locality is identified as "Knersvlakte" were collected around the location Knersvlakte, turn-off "Douse-the-Glim", where the left farm road forks for the first time approx 1 km from the main gate on the road from Van Rhynsdorp to Nuwerus (+/- 22 km N of Van Rhynsdorp, on the farm of Mr. Buys Wiese.)

Table 7.2: Percentage yields of final extracts of alkaloid positive Cape Mesembs.

Sample identity	Genus	Percentage yield of alkaloids (%)
FVH A	<i>A. cordifolia</i> fruit and flowers	0.029
	<i>A. cordifolia</i> roots	0.235
	<i>A. cordifolia</i> leaves	0.660
	<i>A. cordifolia</i> stems	0.089
FVH B	<i>A. lancifolia</i> green stems	0.041
	<i>A. lancifolia</i> woody stems	0.206
	<i>A. lancifolia</i> roots	0.191
	<i>A. lancifolia</i> fruit+flowers	0.015
	<i>A. lancifolia</i> leaves	0.057

FVH 1 Leaves	<i>Mesembryanthemum aitonis</i>	0.014
FVH 1 Seeds	<i>Mesembryanthemum aitonis</i>	0.013
FVH 1 Stems	<i>Mesembryanthemum aitonis</i>	0.007
FVH 2 Leaves	<i>Aptenia cordifolia</i>	0.294
FVH 2 Roots	<i>Aptenia cordifolia</i>	0.244
FVH 2 Seeds	<i>Aptenia cordifolia</i>	1.740
FVH 2 Stems	<i>Aptenia cordifolia</i>	0.125
FVH 5	<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	0.007
FVH 7 Leaves	<i>Mesembryanthemum aitonis</i>	0.148
FVH 7 Roots	<i>Mesembryanthemum aitonis</i>	0.117
FVH 7 Stems	<i>Mesembryanthemum aitonis</i>	0.119
FVH 8 Leaves	<i>Mesembryanthemum aitonis</i>	0.285
FVH 8 Roots	<i>Mesembryanthemum aitonis</i>	0.184
FVH 8 Stems	<i>Mesembryanthemum aitonis</i>	0.279
FVH 9A	<i>Mesembryanthemum aitonis</i> Crude	0.218
FVH 9B	<i>Mesembryanthemum nodiflorum</i> L.	0.143
FVH 11 Leaves	<i>Psilocaulon articulatum</i>	0.257
FVH 11 Stems	<i>Psilocaulon articulatum</i>	0.293
FVH 12	<i>Psilocaulon articulatum</i>	0.215
FVH 14	<i>Psilocaulon junceum</i> (Haw.) Schwantes	0.116
FVH 16	<i>Brownanthus ciliatus</i> (Aiton) Schwantes subsp. <i>ciliatus</i>	0.004

FVH 20	<i>Psilocaulon junceum</i> (Haw.) Schwantes	0.034
FVH 21	<i>Psilocaulon bicornis</i> (Sond.) Schwantes	0.030
FVH 22	<i>Psilocaulon</i> cf. <i>junceum</i> (Haw.) Schwantes	0.019
FVH 23 Leaves	<i>Sceletium crassicaule</i>	0.660
FVH 23 Roots	<i>Sceletium crassicaule</i>	0.127
FVH 23 Stems	<i>Sceletium crassicaule</i>	0.177
FVH 24	<i>Mesembryanthemum crystallinum</i>	0.114
FVH 26 Leaves	<i>Psilocaulon junceum</i>	0.010
FVH 26 Roots	<i>Psilocaulon junceum</i>	0.011
FVH 26 Stems	<i>Psilocaulon junceum</i>	0.006
FVH 27	<i>Psilocaulon junceum</i> (Haw.) Schwantes	0.175
FVH 28 Fruit + Flowers	<i>Psilocaulon articulatum</i>	0.111
FVH 28 Leaves	<i>Psilocaulon articulatum</i>	0.031
FVH 28 Stems	<i>Psilocaulon articulatum</i>	0.018
FVH 29	<i>Prenia englishiae</i> (L.Bolus) Gerbaulet	0.189
FVH 37	<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	0.299
FVH 39	<i>Prenia pallens</i> (Aiton) N.E.Br. subsp. <i>pallens</i>	0.196
FVH 40	<i>Prenia</i> sp.	0.239
FVH 41 Green stems	<i>Phyllobolus digitatus</i> subsp. <i>Digitatus</i>	0.050
FVH 41 Papery stems	<i>Phyllobolus digitatus</i> subsp. <i>Digitatus</i>	0.012
FVH 41 Roots	<i>Phyllobolus digitatus</i> subsp. <i>Digitatus</i>	0.065
FVH 44A	<i>Psilocaulon dinteri</i> (Engl.) Schwantes	0.194
FVH 44B Leaves	<i>Mesembryanthemum guerichianum</i>	0.485
FVH 44B Roots	<i>Mesembryanthemum guerichianum</i>	0.893
FVH 46	<i>Psilocaulon granulicaule</i>	0.049
FVH 49 F + F	<i>Phyllobolus nitidus</i>	0.117
FVH 49 Leaves	<i>Phyllobolus nitidus</i>	0.120
FVH 49 Roots	<i>Phyllobolus nitidus</i>	0.329
FVH 49 Stems	<i>Phyllobolus nitidus</i>	0.029
FVH 50	<i>Psilocaulon junceum</i> (Haw.) Schwantes	0.083
FVH 52B	<i>Phyllobolus spinuliferus</i>	0.152
FVH 53 Leaves	<i>Psilocaulon granulicaule</i>	0.167
FVH 53 Stems	<i>Psilocaulon granulicaule</i>	0.118
FVH 54	<i>Mesembryanthemum crystallinum</i>	0.125
FVH 55 Leaves	<i>Aridaria serotina</i> Leaves	0.142
FVH 55 Roots	<i>Aridaria serotina</i> Roots	0.131
FVH 55 Stems	<i>Aridaria serotina</i> Stems	0.153

FVH 60 Leaves	<i>Mesembryanthemum</i> sp. Leaves	0.519
FVH 60 Roots	<i>Mesembryanthemum</i> sp. Roots	1.695
FVH 61 Leaves	<i>Mesembryanthemum crystallinum</i>	0.394
FVH 61 Roots	<i>Mesembryanthemum crystallinum</i> Roots	0.503
FVH 61 Stems	<i>Mesembryanthemum crystallinum</i> Stems	0.263
FVH 62 Stems	<i>Psilocaulon coriarium</i>	0.181
FVH 65 Fruit + Flowers	<i>Psilocaulon coriarium</i>	0.408
FVH 65 Leaves	<i>Psilocaulon coriarium</i>	0.384
FVH 65 Stems	<i>Psilocaulon coriarium</i>	0.023
FVH 66	<i>Prenia</i> cf. <i>pallens</i> (Aiton)	0.217
FVH 67	<i>Mesembryanthemum</i> cf. <i>guerichianum</i>	0.199
FVH 68 Leaves	<i>Sceletium namaquense</i>	1.874
FVH 68 Stems	<i>Sceletium namaquense</i>	0.435
FVH 69 Leaves	<i>Sceletium rigidum</i>	0.308
FVH 70 Leaves	<i>Sceletium tortuosum</i>	1.097
FVH 70 Stems	<i>Sceletium tortuosum</i>	0.478
FVH 71	<i>Phyllobolus longispinulus</i>	0.787
FVH 72	<i>Phyllobolus rabiei</i>	2.941
FVH 73	<i>Phyllobolus</i> cf. <i>anrathus</i>	0.244
FVH 74	<i>Phyllobolus congestus</i>	1.190
FVH 77	<i>Psilocaulon junceum</i> (Haw.) Schwantes	0.154
FVH 78 Roots	<i>Mesembryanthemum nodiflorum</i>	0.276
FVH 78 Stems	<i>Mesembryanthemum nodiflorum</i>	0.053
FVH 81	<i>Psilocaulon junceum</i> (Haw.) Schwantes	0.168
FVH 84	<i>Mesembryanthemum excavatum</i> L.Bolus	0.164

7.10 References

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4. The use of PRECIS (PREtoria Computerized Information System) data is gratefully acknowledged.

Chapter 8

Conclusions

During the course of this study, one of the aims was to screen a representative sample of the Mesembryanthemaceae genera and species for the possible presence of alkaloids, with emphasis on the subfamily Mesembryanthemoideae (*Sceletium*, *Aptenia*, and related genera). This was achieved by investigating eight of the eleven genera as well as twenty-seven of the ninety-five species recognised to comprise the Mesembryanthemoideae. The genera investigated include *Aptenia*, *Aridaria*, *Brownanthus*, *Mesembryanthemum*, *Phyllobolus*, *Prenia*, *Psilocaulon* and *Sceletium*. Sixteen plants belonging to the subfamily Ruschioideae were also screened in this study. These include the genera *Drosanthemum*, *Malephora*, *Ruschia* (two from the *Uncinata* group), *Pleiospilos*, *Eberlanzia*, *Trichodiadema* and *Monilaria*.

A second aim of this study was to record all ethnobotanical information on the medicinal and culinary uses of the Mesembryanthemaceae. This has been accomplished by sorting the ethnobotanical data available concerning the various genera according to genus, and including it in the chapter in which the genus concerned is discussed.

The third aim of this study was to isolate and identify alkaloids from selected taxa. The genera of the Mesembryanthemoideae are very interesting to botanists and chemists due to their complex phytochemical make-up. However, the extremely low concentration of alkaloids present in the majority of these species makes the identification of these alkaloids a formidable task. During the course of this study, the mesembrane alkaloids mesembrine, 4'-*O*-methylsceletenone and 4,5-dihydro-4'-*O*-methylsceletenone have been successfully isolated and characterised, as has the non-mesembrane tyrosine-derived alkaloid hordenine.

The mesembrane alkaloids have proven to be incredibly challenging to isolate due to their low concentrations, the similarity of their structures, the complexity of the alkaloid mixtures present in the extracts, and the very close retention times of these compounds on GC/MS. Mesembrane alkaloids of the mesembrine-type were only detected in three of the genera investigated, namely *Aptenia*, *Aridaria* and *Sceletium*.

In the genus *Aptenia*, the sceletenone derivatives 4'-*O*-methylsceletenone and 4,5-dihydro-4'-*O*-methylsceletenone were detected. Sceletenone has previously been isolated from plants within the subfamily Mesembryanthemoideae, but this appears to be the first time that these sceletenone derivatives have been isolated from a natural source. The mesembrine-type alkaloids mesembrine, mesembranol and mesembrenone, as well as hordenine, were detected in *Aridaria*. In *Sceletium*, the mesembrine-type alkaloids mesembrine, mesembrenone, mesembrenol and mesembranol, as well as hordenine, were detected. Amongst the genera *Mesembryanthemum*, *Phyllobolus* and *Psilocaulon*, there is a random distribution of the non-mesembrane alkaloid hordenine within the species. The genera *Brownanthus* and *Prenia* investigated in this study both screened alkaloid negative, as did all the Ruschioideae investigated.

The very low percentage of genera and species that proved in this study to have detectable quantities of alkaloids seems to indicate that the literature record of alkaloids in the family should be re-examined for the possible presence of false positive results. The erratic presence or absence of hordenine may account for at least some of the false positive (and false negative) results. This study (and especially the results for *Aptenia* species) has also highlighted the dangers of identifying mesembrane alkaloids in complex mixtures solely on the basis of GC retention times and mass spectral data.

Following the results obtained during the course of this study, the following conclusions were reached:

- The mesembrane alkaloids occur in Mesembryanthemaceae species other than *Sceletium*.
- In our investigation mesembrine alkaloids were detected only in the subfamily Mesembryanthemoideae and not in the subfamily Ruschioideae.
- In all cases extremely low levels of alkaloids were observed.
- Hordenine is present in several of the genera of the Mesembryanthemoideae.
- The mesembrine alkaloids, 4'-O-methylsceletenone and 4,5-dihydro-4'-O-methylsceletenone were isolated for the first time from a natural source.

Appendix 1: Plant Material Tables.

Authorities for the names of all taxa are given in Table A.1. Table A.2 contains all the species investigated as well as their alkaloid status. Voucher specimens of all the plants screened in this thesis have been deposited in the University of Johannesburg Herbarium (JRAU), with the exceptions of specimens M1 to M7 of which only small quantities of material were collected. All plants screened in this thesis, with the exceptions FR van Heerden 66 to FR van Heerden 74 and M1 to M7, were identified by Priscilla Burgoyne at the National Herbarium (PRE) of the South African National Biodiversity Institute (SANBI), formerly the National Botanical Institute (NBI) in Pretoria. Specimens FR van Heerden 66 to FR van Heerden 74 (inclusive) were obtained from and identified by Ian Oliver at the Karoo Desert National Botanical Garden in Worcester.

Opsomming

Sedert prehistoriese tye is plante 'n waardevolle bron van medisinale preparate vir mense as gevolg van die chemiese verbindings teenwoordig in die plante. Die familie Mesembryanthemaceae is feitlik endemies tot suider Afrika en die subfamilie Mesembryanthemoideae is wel bekend vir sy gebruike as medisine en voedsel. In hierdie verhandeling is 'n verteenwoordigende monster van die genera van die Mesembryanthemaceae, met 'n klem op die subfamilie Mesembryanthemoideae, fitochemies ondersoek. Die doelwitte van hierdie ondersoek was om al die etnobotaniese inligting ten opsigte van medisinale en voedsel-gebruike op te som en om alkaloiëde van geselekteerde taksa (veral dié van medisinale waarde) te identifiseer en te isoleer, met 'n spesifieke klem op die mesembraanalkaloïde.

'n Oorsig van die strukturele klassifikasie, biosintese en biologiese eienskappe van die mesembraanalkaloïede word in die verhandeling aangebied. Eksperimenteel is die ekstrakte van die 67 plante, wat 15 genera en omtrent 26 spesies insluit, op een van twee alkaloiëde-spesifieke ekstraksie procedures berei en vervolgens is die ekstrakte dan met behulp van dunlaagchromatografie vir die teenwoordigheid van alkaloiëde geanaliseer. Ekstrakte wat positief getoets het vir die teenwoordigheid van alkaloiëde is verder m.b.v. gaschromatografie -massaspektrometrie geanaliseer. Waar moontlik, is die suiwer alkaloiëde uit die ru-ekstrak geïsoleer en gekarakteriseer deur van ^1H en ^{13}C kernmagnetiese resonansspektroskopie gebruik te maak.

Dit is gevind dat ses mesembraanalkaloïede sowel as die tirosienafgeleide nie-mesembraanalkaloïed hordenien in verskeie genera en spesies van die Mesembryanthemoideae voorkom. Die mesembraanalkaloïede wat waargeneem is sluit in mesembrien, mesembrenoon, mesembrenol, mesembranol, 4'-*O*-metielskeletenoon en 4,5-dihidro-4'-*O*-metielskeletenoon. In die genus *Sceletium* is die alkaloiëde mesembrien, mesembrenoon, mesembrenol, mesembranol en hordenien geïdentifiseer. Mesembrien, mesembrenoon, mesembranol en hordenien is ook in *Aridaria* waargeneem. Die skeletenoonderivate 4'-*O*-metielskeletenoon, 4,5-

dihidro-4'-*O*-metielskeletenoon en hordenien is geïdentifiseer as die hoof alkaloiëde in die genus *Aptenia*. Geen alkaloiëde is waargeneem in die spesies van *Brownanthus* en *Prenia* wat in hierdie ondersoek bestudeer is nie. Gedurende hierdie ondersoek is 4'-*O*-metielskeletenoon en 4,5-dihidro-4'-*O*-metielskeletenoon van *Aptenia cordifolia* en *Aptenia lancifolia*, sowel as die nie-mesembraanalkaloïed hordenien van 'n aantal spesies geïsoleer. Eersgenoemde twee mesembraanalkaloïede is reeds vantevore sinteties berei, maar hierdie is die eerste maal dat die isolasie van die verbindings uit 'n natuurlike bron gerapporteer word.

Summary

Since prehistoric times medicinal plants have been an invaluable source of medicinal preparations to mankind due to their respective chemical constituents. The family of the Mesembryanthemaceae is almost entirely endemic to southern Africa, and the subfamily Mesembryanthemoideae is a key source of previous reports of plants with traditional uses, both medicinal and culinary. In this thesis, phytochemical investigations were performed on a representative sample of the Mesembryanthemaceae genera and species, with emphasis on the subfamily Mesembryanthemoideae. The aims of this study were to record all ethnobotanical information on the medicinal and culinary uses of the Mesembryanthemaceae, and to isolate and identify alkaloids from selected taxa (especially those of medicinal interest) with specific emphasis on mesembrane alkaloids

A review on the structural classification, biosynthesis and biological properties of the mesembrane alkaloids is presented in this dissertation. Experimentally, extracts of the 67 plants encompassing 15 genera and approximately 26 species which were investigated were prepared using one of two alkaloid extraction procedures, following which the extracts were screened for the presence of alkaloids by thin-layer chromatography. The extracts in which alkaloids were detected were analysed further using gas chromatography/mass spectrometry. Where possible, pure alkaloids were isolated from the crude alkaloid extracts and the pure compounds characterised using ^1H and ^{13}C nuclear magnetic resonance spectroscopy.

Six of the mesembrane alkaloids as well as the tyrosine-derived non-mesembrane alkaloid hordenine were found to be distributed amongst the various genera and species of the Mesembryanthemoideae. The mesembrane alkaloids detected include mesembrine, mesembrenone, mesembrenol, mesembranol, 4'-*O*-methylsceletenone and 4,5-dihydro-4'-*O*-methylsceletenone. Within the genus *Sceletium* mesembrine, mesembrenone, mesembrenol, mesembranol and hordenine were identified. Mesembrine, mesembrenone, mesembranol and

hordenine were also detected in *Aridaria*. The sceletenone derivatives 4'-*O*-methylsceletenone and 4,5-dihydro-4'-*O*-methylsceletenone as well as hordenine were identified as the major alkaloid constituents of the genus *Aptenia*. No alkaloids were detected in the species of *Brownanthus* and *Prenia* investigated in this study. Amongst the genera *Mesembryanthemum*, *Phyllobolus* and *Psilocaulon*, a random distribution of the alkaloid hordenine was observed. During the course of this study, the isolation and characterisation of 4'-*O*-methylsceletenone and 4,5-dihydro-4'-*O*-methylsceletenone from both *Aptenia cordifolia* and *Aptenia lancifolia*, as well as the non-mesembrane alkaloid hordenine from a number of species, was achieved. The two mesembrane alkaloids have been prepared synthetically, but this is the first report of the presence of the compounds in a natural source.