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Alkaloids of *Antizoma miersiana* (Menispermaceae)

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Abstract

The main alkaloids of *Antizoma miersiana* and one of two species of the southern African endemic genus *Antizoma* (Menispermaceae), have been studied for the first time. Eight isoquinoline alkaloids could be positively identified (structural type in brackets): crotsparine (proaporphine); bulbocapnine and dicentrine (aporphine); cissacapine, cycleaneonine, cycleanine, insulanoline and insularine (bisbenzyltetrahydroisoquinoline). Crotsparine and bulbocapnine were the main alkaloids in the leaves, while bulbocapnine, dicentrine, insulanoline and an unidentified alkaloid are the major compounds in stems. The rhizome contains small amounts of all except crotsparine. There are clear differences, not only between various plant parts, but also between the two species of *Antizoma*. Bulbocapnine, for example, was previously found to be absent from the leaves and stems of *Antizoma angustifolia*. Dicentrine is absent from the latter and therefore appears to be of further diagnostic value to

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distinguish between the two species. The rich variety and yield of alkaloids in *A. miersiana* provide a rationale for its value as a medicinal plant.

Keywords: Menispermaceae; *Antizoma miersiana*; *A. angustifolia*; Isoquinoline alkaloids; chemotaxonomy; Southern Africa

1. Introduction

Antizoma Miers is the only southern African endemic genus of the Menispermaceae. This large tropical and subtropical family comprises some 75 genera and 520 species (Watson and Dallwitz, 1992) and is of considerable medicinal interest. The biological activities are ascribed to a rich diversity of isoquinoline alkaloids. *Antizoma* comprises two species, *Antizoma angustifolia* (Burch.) Miers ex Harv. and *Antizoma miersiana* Harv. In a previous publication, the results of a study of the alkaloids of *A. angustifolia* (presence of seven identified and four unidentified alkaloids) were presented. In this paper, we report on the alkaloids of the second species, *A. miersiana*, as part of a chemotaxonomic and ethnopharmacological evaluation. The aim was to identify the main alkaloids in *A. miersiana* and to compare them with the alkaloids of *A. angustifolia* (De Wet et al., 2004a).

A. miersiana is a woody shrub of up to 1.5 m in height that is found in dry places in Namibia and the western parts of South Africa. In contrast to *A. angustifolia*, which has numerous medicinal uses in southern Africa (Watt and Breyer-Brandwijk, 1962; Von Koenen, 2001), there is only a single reference to a medicinal use for *A. miersiana* (Archer, 1994). In Namaqualand, the boiled root is drunk to treat stomach ulcers. No published information could be found on the alkaloids of *A. miersiana*.

2. Material and methods

2.1. General experimental procedures

NMR spectra (^{1}H and ^{13}C) were recorded on either a Varian Gemini 300 MHz or a Varian Inova 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard. Apart from ^{1}H and ^{13}C, COSY, NOESY, DEPT, HMQC and HMBC experiments were performed to elucidate the structures of compounds. EI-MS were recorded on a Shimadzu GCMS QP2010 apparatus. Optical rotations were measured on a JASCO DIP 370 digital polarimeter. Column chromatography was performed using silica gel 60 (230–400 mesh) using cyclohexane:chloroform:diethylamine (50:40:10) as the eluent. Analytical thin-layer chromatography of compounds or extracts was performed on Silica Gel 60 F_{245}, Merck plates, using the same eluent system as for column chromatography. HPLC analyses were performed on a Shimadzu 10A system with a binary gradient system and photodiode array detector.

Analytical HPLC analyses were performed on a Waters XTerra RP C18 column with the following linear gradient system: 0–90% acetonitrile in a 10-mM ammonium acetate solution (pH 9.5) (50 min).

2.2. Plant material

Bulk material and material for analytical studies were collected in Botterkloof Pass, between the towns of Clanwilliam and Calvinia in the western part of the Northern Cape Province of South Africa. In addition, small amounts of material from rich herbarium specimens were also analysed. The sources of plant material and voucher specimen details are given in Table 1.

2.3. Extraction, purification and identification of alkaloids

Air-dried plant material (maximum 40 °C) were separated into leaves (0.55 kg), stems (1.35 kg) and rhizomes (1.23 kg) and then finely ground. The material was suspended in a 0.05-M H_2SO_4 solution and left at room temperature for 1 h. The aqueous extracts were filtered under vacuum through a coarse grade celite-577 and the pH of the filtrates adjusted to 7 by adding a 25% ammonium hydroxide solution. The filtrates were extracted with CH_2Cl_2 in a separating funnel and the organic extracts filtered through a glass column, packed with a coarse celite-577. Removal of the solvent under reduced pressure yielded the alkaloidal extracts.

Table 1
Voucher specimens of the material of *Antizoma miersiana* used for alkaloid isolation and identification

Voucher specimens	Locality	Alkaloid yield (mg/g dry weight)
Van Wyk & De Wet 4067 (ZULU)	Botterkloof Pass [3119 CD (Calvinia)]	
Bulk leaf		12.2
Plant 2 leaves		19.6
Plant 3 leaves		12.9
Plant 4 leaves		21.6
Plant 2 stems		0.2
Plant 3 stems		0.1
Plant 4 stems		0.3
Bulk rhizomes		3.0
Pienaar 1167 (PRE)	Springbok [2917 DB]	
Leaves		9.5
Stems		1.7
Leistner 3396 (PRE)	Vioolsdrif [2817 CC]	
Leaves		14.9
Stems		1.7

The yields of crude alkaloidal extracts are also given.
ZULU = The Herbarium, University of Zululand, KwaZulu-Natal.
PRE = National Herbarium, Pretoria, (National Botanical Institute), Gauteng.

Alkaloids from the root and leaf bulk sample extracts were isolated by column chromatography. The leaf extract yielded pure samples of crotsparine (205 mg) and bulbocapnine (11 mg). Crotsparine was also isolated from *A. angustifolia* (De Wet et al., 2004a). Bulbocapnine had δ_H 7.00 (1H, s, OH), 6.81 (2H, s, H-8, 9), 6.62 (1H, s, H-3), 6.08 (1H, d, *J* 1.2 Hz, OCH_2O), 5.93 (1H, d, *J* 1.2 Hz, OCH_2O), 3.89 (3H, s, OCH_3), 3.16–2.90 (4H, m, H-4, 5, 6a, 7), 2.63 (1H, dd, *J* 16.2 and 3.6 Hz, H-4), 2.52 (3H, s, $N-CH_3$), 2.52–2.40 (2H, m, H-5, 7); δ_C 148.2 (C-10), 145.9 (C-2), 142.8 (C-11), 140.4 (C-1), 129.6 (C-7a), 128.8 (C-11a), 127.3 (C-3a), 119.2 (C-8), 118.4 (C-11a), 114.2 (C-11b), 110.8 (C-9), 107.6 (C-3), 100.2 (OCH_2O), 62.7 (C-6a), 56.1 (OMe), 53.0 (C-5), 44.0 ($N-CH_3$), 35.4 (C-7), 29.4 (C-4); EI-MS *m/z* 325 $[M]^+$ (100%), 310 (86), 282 (25), 280 (26), 224 (13), 152 (14).

The root sample gave cissacapine (222 mg), cycleaneonine (6 mg), cycleanine (3 mg), insulanoline (12 mg) and insularine (97 mg). The spectroscopic characteristics of cycleanine (NMR: Kashiwaba et al., 1998; MS: Baldas et al., 1972), cycleaneonine (Wang et al., 1993), insularine (NMR: Bick, et al., 1961; MS: Baldas et al., 1972) and insulanoline (Baldas et al., 1972) are in agreement with the published data. Although the structures of insularine and insulanoline have been known for many years, only limited NMR data are available in the readily-available literature. The spectral characteristics of these two compounds as well as the structure and spectral characteristics of the novel alkaloid cissacapine are described by Van Heerden et al. (submitted for publication).

Dicentrine, isolated from *Cissampelos capensis* leaves and *Cissampelos hirta* roots (De Wet et al., 2004b) and identified by MS and NMR, was detected by TLC and HPLC in *A. miersiana*. The spectropic data of dicentrine were in agreement with published values (Guinaudeau et al., 1979).

3. Results and discussion

The yield of alkaloids in *A. miersiana* varied from 0.1 to 21.6 mg/g dry weight (Table 1). Leaves yielded significantly more crude alkaloids (9.5–21.6 mg/g dry weight) than the stems and rhizomes (0.1–3.0 mg/g dry weight). As can be seen in Table 2, the leaves contain two major alkaloids (crotsparine and bulbocapnine) and one minor alkaloid (dicentrine). The stems gave five alkaloids, of which three could be positively identified as bulbocapnine, dicentrine and insulanoline (the unidentified alkaloids are listed as X_1 and X_2 in Table 2). The rhizome sample contained seven identified and two unidentified alkaloids (X_2 and X_3), including the same three alkaloids as in the stem, but in addition also cissacapine, cycleaneonine, cycleanine and insularine.

There is a clear pattern in the presence and absence of certain alkaloids in the three plant parts investigated (Table 3). Leaves, for example, contain crotsparine, while this alkaloid appears to be totally absent from stems and rhizomes. The presence of four bisbenzyltetrahydroisoquinoline alkaloids (cissacapine, cycleaneonine, cycleanine and insularine) in the rhizome and their total absence in the leaves and stems are noteworthy.

Table 2
Distribution of alkaloids (%) in *Antizoma miersiana* leaves (L), stems (S) and rhizomes (R)

Alkaloids	Bulk leaf	[a]2-L	3-L	4-L	SB-L	VD-L	2-S	3-S	4-S	SB-S	VD-S	Bulk R
Proaporphine												
Crotsparine	18	7	10	25	18	1	–	–	–	–	–	–
Aporphine												
Bulbocapnine	81	92	89	74	82	95	8	21	29	10	75	3
Dicentrine	1	1	1	1	–	4	32	18	13	tr	tr	8
Bisbenzyltetrahydroisoquinoline												
Cissacapine	–	–	–	–	–	–	–	–	–	–	–	5
Cycleaneonine	–	–	–	–	–	–	–	–	–	–	–	14
Cycleanine	–	–	–	–	–	–	–	–	–	–	–	25
Insulanoline	tr	–	–	–	–	tr	25	40	13	12	8	16
Insularine	–	–	–	–	–	–	–	–	–	–	–	12
Unidentified												
X_1 (15.513)	–	–	–	–	–	–	35	13	32	73	17	–
X_2 (20.192)	tr	–	tr	tr	–	–	tr	6	13	5	tr	8
X_3 (21.835)	–	–	–	–	–	–	–	–	–	–	–	9

For the unidentified alkaloids (X_1, X_2 and X_3), retention times (in min) are given in brackets.
[a] 2, 3 and 4 are different plants within the population from which the bulk sample was collected (SB = Springbok; VD = Vioolsdrif).

Table 4 shows a comparison of the alkaloids of *A. angustifolia* (De Wet et al., 2004a) and *A. miersiana*, the only two species in the genus *Antizoma*. Cissacapine, cycleaneonine, cycleanine and insularine are unique in their distribution within various plant parts of *A. angustifolia*, while dicentrine, cycleaneonine, cycleanine and insulanoline similarly show a unique pattern in various parts of *A. miersiana*. Although the two species are morphologically quite similar and both show xeromorphic adaptations, their alkaloid patterns are substantially different: crotsparine (exclusive to leaves of *A. miersiana*) is present in stems and roots of *A. angustifolia*. Glaziovine (sporadically present in *A. angustifolia*) was not detected in *A. miersiana*. Bulbocapnine, the main alkaloid of *A. miersiana*, is practically absent in *A. angustifolia*. A small amount (2% of total alkaloids) of bulbocapnine was detected in roots of *A. angustifolia* from Pretoria but in none of the 10 other samples studied. Dicentrine, a main stem alkaloid of *A. miersiana*, was not detected in *A. angustifolia*. The major stem alkaloid of *A. miersiana* (X_1 in Tables 2 and 4) could not be identified due to sample limitations. This alkaloid is present in *A. angustifolia*, but appears to be highly characteristic of *A. miersiana* stems. Other alkaloids such as cissacapine and insularine are sporadic in their distribution in both species and rarely occur in large amounts.

Bulbocapnine has been isolated from only one other Menispermaceae species, *Cissampelos parreira* (Barbosa-Filho et al., 2000). This compound is known to have antimicrobial, antihypertensive, cataleptic, sedative and adrenolytic properties. In low doses it depresses spinal motorneurone synaptic reflexes (Dictionary of Natural Products, 2004). The presence of this alkaloid may contribute to the biological activity of *A. miersiana*.

Table 3
Alkaloids isolated from different plant parts of *Antizoma miersiana*

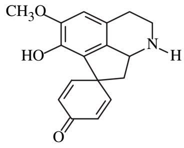
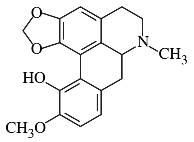
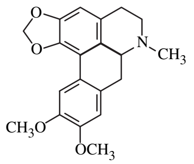
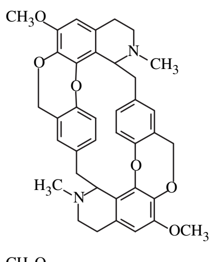
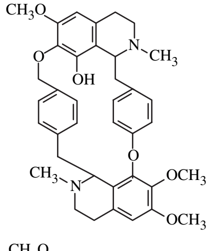
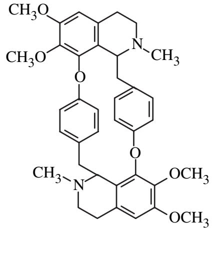
		Leaf (n = 6) % (range)	Stem (n = 5) % (range)	Rhizome (n = 1) %
Proaporphine Crotsparine		$\bar{X}$=13 (1–25)	–	–
Aporphine Bulbocapnine		$\bar{X}$=86 (74–95)	$\bar{X}$=29 (8–75)	3
Dicentrine		$\bar{X}$=1	$\bar{X}$=13	8
Bisbenzyltetrahydroisoquinoline Cissacapine		–	–	5
Cycleaneonine		–	–	14
Cycleanine		–	–	25

Table 3 (*continued*)

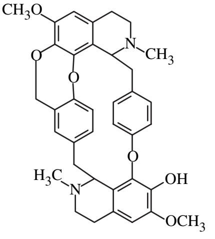
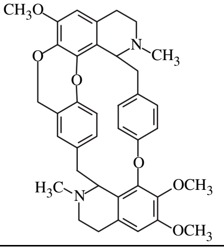
		Leaf (n = 6) % (range)	Stem (n = 5) % (range)	Rhizome (n = 1) %
Insulanoline		tr	$\bar{X}$=20 (17–73)	16
Insularine		–	–	12

Table 4
A comparison between the alkaloids identified in *A. angustifoialia* and *A. miersiana*, the only two species in the genus *Antizoma*

Alkaloids	*A. angustifolia*			*A. miersiana*		
	Leaves	Stem	Rhizome	Leaves	Stem	Rhizome
Proaporphine						
Crotsparine	++	++	++	++	–	–
Glaziovine	(+)	+	+	–	–	–
Pronuciferine	+	–	+	–	–	–
Aporphine						
Dicentrine	–	–	–	+	++	+
Bulbocapnine	–	–	+	++	++	+
Morpinane						
Salutaridine	–	–	+	–	–	–
Bisbenzyltetrahydroisoquinoline						
Cissacapine	+	+	–	–	–	+
Cycleaneonine	–	–	–	–	–	+
Cycleanine	–	–	–	–	–	+
Insulanoline	–	–	–	(+)	++	+
Insularine	+	+	–	–	–	+
X_1	–	+	–	–	++	–

++ = Main alkaloid; + = present; (+) = trace amounts; – = absent.

4. Conclusions

A. miersiana is similar to *A. angustifolia* in its rich diversity of different isoquinoline alkaloids. The presence of these compounds, and particularly the predominance of bisbenzyltetrahydroisoquinoline alkaloids in the rhizomes (i.e. the parts that are used in traditional medicine) suggests that *A. miersiana* may have some medicinal value in treating stomach ulcers. It may be interesting to test the main rhizome alkaloids for possible analgesic, antispasmodic, anti-inflammatory and antimicrobial activity. The main alkaloids in leaves were found to be crotsparine and bulbocapnine (a proaporphine and an aporphine alkaloid, respectively) while bisbenzyltetrahydroisoquinoline alkaloids were practically absent. Bulbocapnine predominates in the stems, together with dicentrine, insulanoline and an unidentified alkaloid. There are clear differences, not only between various plant parts, but also between the two species of *Antizoma*. Alkaloids such as dicentrine, bulbocapnine and insulanoline are potential diagnostic characters to distinguish between the two species.

Acknowledgments

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