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Alkaloids of *Antizoma angustifolia* (Menispermaceae)

Helene De Wet [a,1], Fanie R. van Heerden [b], Ben-Erik van Wyk [a,*]

[a] *Department of Botany, Rand Afrikaans University, PO Box 524, Auckland Park, Johannesburg 2006, South Africa*
[b] *Department of Chemistry and Biochemistry, Rand Afrikaans University, PO Box 524, Auckland Park, Johannesburg 2006, South Africa*

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Abstract

The main alkaloids in leaves, stems and rhizomes of *Antizoma angustifolia*, a traditional medicinal plant of the family Menispermaceae, were isolated and identified. The main compound in leaves is almost invariably crotsparine, while stems and rhizomes have several other alkaloids (glaziovine, pronuciferine, bulbocapnine, salutaridine, cissacapine, insularine) in lower yields. These results do not agree with a previous study, where salutaridine was identified as the main alkaloid. Salutaridine was detected as a minor constituent of the rhizomes of only one of the samples. Alkaloids appear to be quite variable within different plant parts and different provenances of *A. angustifolia*, a fact that may explain the apparent absence of salutaridine in our samples.

Keywords: Menispermaceae; *Antizoma angustifolia*; Major alkaloids; Crotsparine; Glaziovine; Pronuciferine; Bulbocapnine; Salutaridine; Cissacapine; Insularine

1. Introduction

The Menispermaceae Juss. comprises some 75 genera and 520 species (Watson and Dallwitz, 1992) and is widespread in tropical and subtropical countries, with

* Corresponding author. Tel.: +27-11-489-2412; fax: +27-11-489-2411.
E-mail address: bevw@na.rau.ac.za (B.-E. van Wyk).
[1] Present address: Department of Botany, University of Zululand, PO Box X1001, Kwa-Dlangezwa 3880, South Africa.

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relatively few species in temperate regions. According to Troupin (1962), approximately 25 genera with 101 species are found in Africa, of which seven genera with 13 species and two varieties are found in southern Africa. The genus *Antizoma* with its two species, *A. angustifolia* (Burch.) Miers ex Harv. and *A. miersiana* Harv., is the only endemic genus in southern Africa. *A. angustifolia* is an evergreen shrubby climber that grows in dry places in Namibia, Botswana and the northern parts of South Africa. This species has medicinal value and is used mainly to treat stomach ailments and diarrhoea (Watt and Breyer-Brandwijk, 1962; Von Koenen, 2001). Numerous other uses have been recorded in Namibia (Von Koenen, 2001): infusions and decoctions of the roots are used against pain, stomachache and cough; leaf and root infusions are said to be useful as antenatal medicine to ensure an easy delivery and have also been used as bitter tonics and digestive medicines. Despite all these traditional uses, very little is known about the alkaloids of *A. angustifolia*. Dekker et al. (1988) isolated a morphinandienone-type isoquinoline alkaloid, sinoacutine [(−)salutaridine] from a whole plant extract in high yield as the only alkaloid present. Isoquinoline alkaloids are known for their medicinal properties and a more thorough investigation of *A. angustifolia* was undertaken as a first step towards an improved understanding of the value of the plant in traditional medicine. The study was also undertaken to reconfirm the identity of the main alkaloid in *A. angustifolia*. A further aim was to show possible geographical variation in alkaloids by comparing different populations (provenances) and different plant parts to determine if alkaloidal pathways are different in leaves, stems and rhizomes. In view of the report by Dekker et al. (1988) an unexpected diversity of main alkaloids was found.

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. EIMS 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 with a Waters XTerra RP C18 column and the following linear gradient system: 0–90% acetonitrile in a 10 mM ammonium acetates solution (pH 9.5) (50 min).

2.2. Collecting of plant material

Bulk material and material for analytical studies were collected near Pretoria, Gauteng Province, South Africa. Herbarium samples were also used in the analytical studies. The sources of plant material and voucher specimen details are listed in Table 1. The bulk sample used for the isolation of main alkaloids was collected near Pretoria, close to the locality where the material used by Dekker et al. (1988) was obtained.

2.3. Extraction, purification and identification of alkaloids

Plant material was air-dried at maximum 40 °C, separated into leaves (0.857 kg) and rhizomes (1.237 kg) and then finely ground. The material was then 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.

The proaporphines crotsparine and glaziovine were isolated from the bulk sample of *A. angustifolia* alkaloids by column chromatography. Glaziovine had δ_H 6.96 (1H, dd, *J* 9.9 and 3.0 Hz, H-8), 6.81 (1H, *J* 9.9 and 2.7 Hz, H-12), 6.54

Table 1
Voucher specimens of the material of *A. angustifolia* used for alkaloid isolation and identification. The yields of crude alkaloidal extracts are also given

Voucher specimens	Locality	Alkaloid yield mg/g dry weight
Van Wyk and De Wet 4059 (UZ)	16 km south from Pienaarsriver [2528 AD (Pretoria)]	
Bulk leaf		4.8
Plant 1 leaves		6.0
Plant 2 leaves		8.3
Plant 3 leaves		10.5
Plant 1 stems		0.6
Plant 2 stems		0.8
Plant 3 stems		0.6
Bulk rhizomes		1.4
Kreulen 497 (PRE)	Northern Botswana [2321 BA]	
Leaves		4.7
Stems		1.8
Watt and Breyer-Brandwijk 1251 (PRE)	Brits [2527 DB (Rustenburg)]	
Stems		0.8

UZ, The Herbarium, University of Zululand, KwaZulu-Natal.
PRE, National Herbarium, Pretoria, (National Botanical Institute), Gauteng.

(1H, s, H-3), 6.34 (1H, dd, *J* 9.9 and 1.8 Hz, H-9), 6.26 (1H, dd, *J* 9.9 and 1.8 Hz, H-11), 3.78 (3H, s, OCH_3), 3.42 (1H, dd, *J* 10.5 and 6 Hz, H-6a), 3.09 (1H, dd, *J* 12.0 and 6.0 Hz, H-5_{eq}), 2.91 (1H, ddd, *J* 16.8, 11.4 and 6.6 Hz, H-4_{ax}), 2.73 (1H, dd, *J* 16.8 and 4.5 Hz, H-4_{eq}), 2.46 (1H, td, *J* 11.7 and 5.4, H-5_{ax}), 2.34 (3H, s, *N*–CH_3), 2.42 (1H, dd, *J* 12.0 and 6.3 Hz, H-7), 2.19 (1H, dd, *J* 11.7 and 10.8 Hz, H-7); δ_C 186.4 (C-10), 153.4 (C-8), 149.7 (C-12), 147.4 (C-2), 140.9 (C-1), 134.5 (C-3a), 128.7 (C-9), 127.5 (C-11), 124.0 (C-7b or 7c), 122.9 (C-7b or 7c), 109.8 (C-3), 65.7 (C-6a), 56.5 (OCH_3), 55.0 (C-5), 50.7 (C-7a), 47.1 (C-7), 43.5 (N–Me), 27.1 (C-4); EIMS *m*/*z* 297 (100%, M^+), 268 (53), 252 (13), 83 (28). Crotsparine had δ_H 6.96 (1H, dd, *J* 9.9 and 2.7 Hz, H-8), 6.84 (1H, dd, *J* 9.9 and 3.0 Hz, H-12), 6.56 (1H, s, H-3), 6.36 (1H, dd, *J* 9.9 and 1.8 Hz, H-9), 6.26 (1H, dd, *J* 9.9 and 1.8 Hz, H-11), 4.25 (1H, dd, *J* 10.2 and 6.0 Hz, H-6a), 3.80 (3H, s OCH_3), 3.42 (1H, ddd, *J* 12.9, 6.3 and 1.8 Hz, H-5_{eq}), 3.12 (1H, ddd, *J* 12.9, 10.8 and 6.3 Hz, H-5_{ax}), 2.69 (2H, m, H-4), 2.36 (1H, dd, *J* 12.0 and 6.6 Hz, H-7), 2.16 (1H, dd, *J* 12.0 and 10.8 Hz, H-7); δ_C 186.1 (C-12), 153.2 (C-8), 149.4 (C-12), 147.2 (C-2), 140.7 (C-1), 135.7 (C-3a), 128.7 (C-9), 127.5 (C-11), 123.7 (C-7b or 7c), 123.4 (C-7b or 7c), 110.2 (C-3), 57.9 (C-6a), 56.6 (OMe), 50.8 (C-7a), 48.2 (C-7), 45.2 (C-5), 26.1 (C-4); EIMS *m*/*z* 283 (53%, M^+), 154 (100), 136 (85), 107 (41). The structures of these compounds were confirmed by comparison with published spectroscopic data (MS: Baldwin et al., 1967; NMR glaziovine: Dasgupta et al., 1979; crotsparine: Stuart and Cava, 1968).

In the analytical samples, the identity of the compounds was confirmed by comparison of R_t and UV spectra (HPLC-DAD) with reference compounds. Bulbocapnine, insularine and cissacapine were isolated from *A. miersiana* (De Wet et al., 2004a) and pronuciferine and salutaridine from *Cissampelos capensis* (De Wet et al., 2004b). The structures of pronuciferine (MS: Baldwin et al., 1967; NMR: Stuart and Cava, 1968) and salutaridine (MS: Wheeler et al., 1967; NMR: Szántay et al., 1982) were confirmed by comparison with literature data.

3. Results and discussion

A. angustifolia contains high yields of alkaloids, ranging from 0.6 to 10.5 mg/g dry weight (Table 1). These relatively high values compare favourably with the yield of 2.8% for unspecified plant parts reported by Dekker et al. (1988). The yields of the crude alkaloid extracts were significantly higher in the leaves (4.7–10.5 mg/g dry weight) than in stems and rhizomes (0.6–1.8 mg/g dry weight).

The diversity of alkaloids present in *A. angustifolia* was surprising, considering that only one main alkaloid was reported previously, namely salutaridine (Dekker et al., 1988). As can be seen in Table 2, the leaves, stems and rhizomes yielded one major alkaloid (crotsparine) and six minor alkaloids (glaziovine, pronuciferine, bulbocapnine, salutaridine, cissacapine and insularine). The pattern in leaves is relatively uniform, with crotsparine as the only major alkaloid in most samples. In contrast, the pattern in stems appears to be more erratic, with one of the three Pretoria samples containing cissacapine and insularine as major alkaloids rather

Table 2
Alkaloids isolated from different plant parts of *A. angustifolia*, given as a percentage of total alkaloids

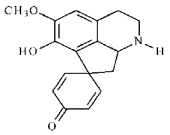
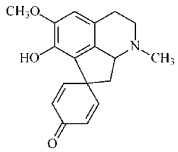
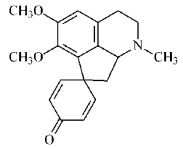
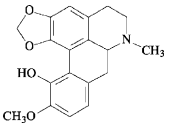
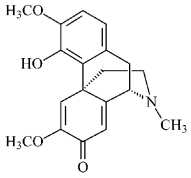
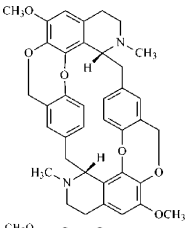
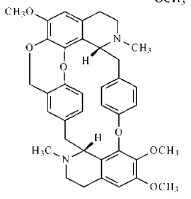
		Leaf ($n = 5$) mean and range (%)	Stem ($n = 5$) mean and range (%)	Rhizome ($n = 1$) (%)
Proaporphine				
Crotsparine		$\bar{X} = 72,\ 0–93$	$\bar{X} = 9,\ 0–31$	71
Glaziovine		Tr	$\bar{X} = 4,\ 0–18$	8
Pronuciferine		$\bar{X} = 2,\ 0–6$	–	12
Aporphine				
Bulbocapnine		–	–	2
Morphinane				
Salutaridine		–	–	6
Bisbenzyltetrahydro-isoquinoline				
Cissacapine		$\bar{X} = 6,\ 0–13$	$\bar{X} = 10,\ 0–48$	–
Insularine		$\bar{X} = 3,\ 0–7$	$\bar{X} = 6,\ 0–29$	–

Table 3
Distribution of alkaloids (%) in *A. angustifolia* leaves (L), stems (S) and rhizomes (R)

Alkaloids	Bulk L	[a]1-L	2-L	3-L	Botswana L	1-S	2-S	3-S	Brits S	Botswana S	Bulk R
Proaporphine											
Crotsparine	93	89	91	85	–	5	31	10	–	–	71
Glaziovine	Tr	Tr	Tr	Tr	–	1	1	Tr	–	18	8
Pronuciferine	6	1	1	2	1	–	–	–	–	–	12
Aporphine											
Bulbocapnine	–	–	–	–	–	–	–	–	–	–	2
Morphinane											
Salutaridine	–	–	–	–	–	–	–	–	–	–	6
Bisbenzyltetrahydroisoquinoline											
Cissacapine	–	5	4	8	13	–	–	48	–	–	–
Insularine	–	3	3	4	7	–	–	29	–	–	–
Unidentified compounds											
X_1	–	–	–	–	–	7	4	2	41	25	–
X_2	–	–	–	–	–	19	55	7	7	–	–
X_3	–	2	1	2	4	12	9	3	52	52	Tr
X_4	–	–	–	–	75	–	–	–	–	4	–

[a] 1, 2 and 3 are different plants within the population from which the bulk sample was collected.

than crotsparine. A few other, as yet unidentified alkaloids were detected in the analytical samples but quantities were insufficient to allow for their isolation and identification.

Salutaridine was detected in only one of the 11 samples investigated, namely the bulk rhizome sample from Pretoria. This is unexpected, as the material came from the same vicinity as the sample used by Dekker et al. (1988). It is possible that this is another example of the rather sporadic patterns observed in the stems. A possible explanation for the variation can be seasonal differences, especially in xerophytic plants growing in an area of strongly seasonal rainfall. Moisture stress may possibly play a role, as our Pretoria collection was made in a particularly dry period (February, 2001). At least four main types of isoquinoline alkaloids are present but no obvious differences or discontinuities in biosynthetic pathways could be detected between plant parts.

4. Conclusions

In conclusion, it seems that crotsparine should be considered the main alkaloid of *A. angustifolia* (at least in leaves) and that other plant parts appears to be highly variable with regards to their main alkaloids. The diversity of known traditional uses of the leaves and roots may therefore be a result of regional differences in the main alkaloids. Isolated alkaloids should be tested for possible analgesic, antispasmodic, oxytocic and antimicrobial activities, in order to validate some of the recorded medicinal uses listed in the Introduction. It may be interesting to compare *A. angustifolia* with *A. miersiana*, the only other species of *Antizoma*. It also seems important to account for possible geographical and genetic variation in the sampling, as the few populations of *A. angustifolia* represented in our study differed quite substantially in their main alkaloids (Table 3)

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