South African Journal of Botany 2005, 71(2): 228–230
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SOUTH AFRICAN JOURNAL
OF BOTANY
ISSN 1727–9321

A novel alkylamide from the leaves of *Acmella caulirhiza* (Asteraceae), a traditional surface analgesic

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Received 17 June 2004, accepted in revised form 30 September 2004

Dried leaves of the widespread African ethnomedicinal herb *Acmella caulirhiza* (Asteraceae) have been phytochemically characterised. The hexane extract yielded two unsaturated alkylamides, spilanthol and a novel compound tentatively identified as *N*-isobutylnona-2*E*, 4*E*-dien-8-ynamide based on ^{1}H, ^{13}C, COSY and HETCOR spectra. The occurrence of these amides supports the validation of documented traditional usage patterns.

Introduction

Published accounts of the widespread African annual *Acmella caulirhiza* Delile in Caillaud (Asteraceae: Heliantheae) have almost invariably appeared under the misapplied and invalid name *Spilanthes mauritiana* (Pers.) DC. (Jansen 1985). In South Africa the Zulu use *A. caulirhiza* (syn. *Spilanthes africana* DC.) as an oral local analgesic for the relief of toothache, applying the moistened and powdered leaf to dental caries (Watt and Breyer-Brandwijk 1962) and to reduce the sensitivity to pain of gums during dental extractions. The Zulu name *isisinini* applied to plants of this species derives from the noun for gums (*izinsini*) (Doke *et al*. 1958). The Xhosa chew the flowers in treating pyorrhea (pus discharges) (Watt and Breyer-Brandwijk 1962) and the Tsonga, who refer to the plant by the vernacular *xixwene-lamhofu*, rub leaves on mouth ulcers to ease pain (Liengme 1981). In South Africa, its use as an analgesic has also been recorded for the Vhavenda (*P. Watson s.n*., PRE), who know the plant as *tshishengelaphofu* (*Netshiungani 886*, PRE). Its use in southern Africa was first recorded by Wood (1897), who noted its name as the 'electric' plant on account of its peculiar pungent taste. Bundles of whole flowering plants are purveyed in the medicinal plant markets of Durban, traders recommending a cold water infusion of the crushed plant as a remedy for severe chest coughs. Elsewhere in Africa *A. caulirhiza* is similarly employed in the relief of painful sores of the mouth, gums and throat, as well as stomach ache (Watt and Breyer-Brandwijk 1962, Kokwaro 1976). In eastern Tanzania and the Great Lakes region, pulped or ground leaves and twigs are applied as dressings for dislocations, sprains and broken limbs (Chhabra *et al*. 1989, Neuwinger 2000). Decoctions of leafy twigs are further used as compresses for rheumatism (Neuwinger 2000).

Chhabra *et al*. (1984) qualitatively screened whole plants of *A. caulirhiza* and reported the presence only of anthocyanins and coumarins. The first detailed phytochemical investigation (Jondiko 1986) of fresh aerial parts sourced in Kenya, isolated *N*-isobutyl-2*E*,4*E*,8*E*,10*Z*-dodecatetra-2,4,8,10-amide, a potent mosquito larvicide. This alkylamide induced 100% mortality in the third instar larvae of *Aedes aegypti* at concentrations of 10^{-2}µgml^{-1}. An unsaturated amide spilanthol (affinin; *N*-isobutyldecatriene-2,6,8-amide), previously reported from the related *Acmella uliginosa* (Sw.) Cassini (syn. *Spilanthes uliginosa* Sw.), has also been attributed with mosquito larvicidal, as well as local anaesthetic, properties. Broader activities for whole plant extracts of West African *A. uliginosa* have been reported and include antibacterial, antifungal, antiviral, antiprotozoal and anthelmintic effects (Oliver-Bever 1986). Extracts of *Acmella oleracea* (L.) R.K. Jansen have shown both *in vitro* and *in vivo* (mice) activity against malarial parasites (Gasquet *et al*. 1993). The *in vitro* antiplasmodial activity (IC_{50} = 5.3µgml^{-1}) of dichloromethane/methanol extracts of the stems of *A. caulirhiza* has recently been demonstrated (Clarkson *et al*. 2004). Spilanthol has been found to possess strong molluscicidal activity against the freshwater snail *Physa occidentalis* (LD_{50} = 100µM) and the cercariae of schistosome flukes (Johns *et al*. 1982).

In view of the historical confusion surrounding the naming and identity of *A. caulirhiza*, the current investigation aimed to provide a clear phytochemical profile of South African material of this widespread medicinal species.

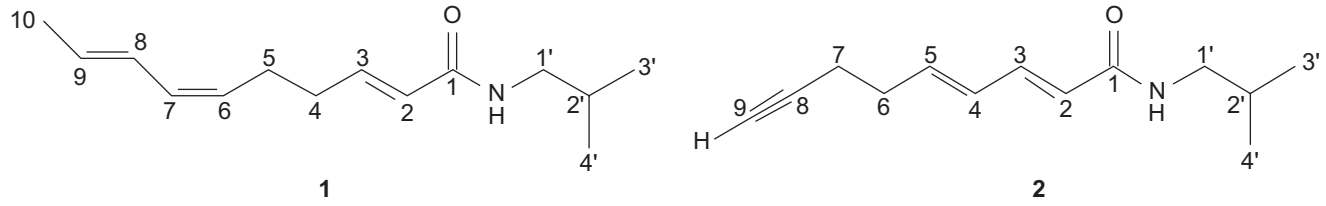

Figure 1: Alkylamides isolated from the leaves of *Acmella caulirhiza*

Materials and Methods

Plant material

Cultivated material of *Acmella caulirhiza* Delile in Caillaud was obtained from Silverglen Medicinal Plant Nursery, Durban, South Africa in March 1999, and a voucher lodged for verification purposes (*Crouch 788*, NH).

Extraction and fractionation

The dried leaves (611g) were extracted with 1l hexane at room temperature for 48h on a shaker. Gravity column chromatography over silica gel (Merck 9385) of the hexane extract (5.1g) using a 20% ethyl acetate in hexane solvent system yielded compound 1 (5mg) and using a 30% ethyl acetate in hexane system, compound 2 (4.0mg).

Structure elucidation

Both compounds 1 and 2 were found to be extremely unstable, even when stored in a freezer. Thus no IR or mass spectra could be obtained, for the compounds decomposed during NMR analysis. However, ^{1}H, ^{13}C, COSY and HETCOR spectra were available to elucidate these structures and were recorded on a Varian Gemini 300 MHz NMR spectrometer.

Results

Following comparison with literature NMR data (Yasuda *et al*. 1980, Martin and Becker 1984) the identity of compound 1 was determined as spilanthol (Figure 1). The presence of a *N*-isobutylamide group in compound 2 as in spilanthol (compound 1), was indicated by an amide carbonyl carbon resonance at δ169.4 in the ^{13}C NMR spectrum (Table 1) and coupled resonances in the COSY spectrum assignable to 2H-1'(2H, δ3.10, d, *J* = 6.8Hz), H-2' (δ1.87, m) and the equivalent 3H-3' and 3H-4' proton resonances (6H, δ0.96, d, *J* = 6.8Hz).

The H-2 alkene proton resonance occurred as a doublet at δ6.02 (*J* = 15.1Hz) and this was seen to be coupled to a double doublet at δ7.15 (1H, *J* = 15.1, 10.7Hz), which was assigned to H-3. The $J_{2,3}$ coupling constant of 15.1 Hz indicated that H-1 and H-2 were *trans* to each other. The COSY spectrum showed that H-3 was also coupled to a double-doublet resonance at 6.30, ascribed to H-4, (1H, *J* = 15.3, 10.7Hz), which was, in turn, seen to be coupled to a multiplet at δ6.16, which was assigned to H-5. Again, the $J_{3,4}$-coupling constant of 15.3 Hz indicated a *trans*-substituted double bond.

The H-5 alkene proton was seen to be coupled to protons of a methylene group at δ2.43 (2H-6) whose corresponding ^{13}C NMR carbon resonance occurred at δ19.3 (C-6). This methylene group proton resonance was superimposed on a second methylene group (2H-7) whose corresponding ^{13}C NMR carbon resonance occurred at δ32.4 (C-7). The 2H-7 resonance was seen to be coupled, in the COSY spectrum, with a one proton singlet at δ2.51, which was assigned to the terminal alkyne proton, H-9. The HETCOR spectrum showed that the corresponding C-9 resonance occurred at δ66.8, and the remaining non-protonated C-8 signal was seen to occur at δ77.5 in the ^{13}C NMR spectrum. Thus compound 2 was tentatively assigned as the novel structure *N*-isobutylnona-2*E*,4*E*-dien-8-ynamide (Figure 1).

Discussion

The known spilanthol (*N*-isobutyldecatriene-2,6,8-amide), compound 1, and the novel *N*-isobutylnona-2*E*,4*E*-dien-8-ynamide (compound 2), were isolated from the hexane extract of the dried leaves of *A. caulirhiza*. These alkylamide findings are consistent with earlier reports for the genus (Martine and Becker 1984, Oliver-Bever 1986). Spilanthol (compound 1), has previously been identified from several asteraceous taxa: *Heliopsis longipes* (A. Gray) S.F. Blake (roots) (Johns *et al*. 1982), *Acmella paniculata* (Wallich ex DC.) R.K. Jansen (as *Spilanthes acmella* (L.) Murr.) (Martin and Becker 1984), *A. oleracea* (leaves) (Yasuda *et al*. 1980) and *Wedelia parviceps* Blake (flowers) (Johns *et al*. 1982). Although spilanthol was reported by Watt and Breyer-Brandwijk (1962) to have been earlier isolated from the flowers of *A. caulirhiza* (*as S. mauritiana*), the reference cited (Asano and Kanematsu 1927) refers rather to its isolation from the Asian *A. paniculata* (as *S. acmella*) (Jansen 1985). Accordingly, the current report represents the first authentic isolation of spilanthol from *Acmella caulirhiza*.

Based on the traditional usage profile of *A. caulirhiza* in East Africa, Fabry *et al*. (1998) investigated the bacteristatic and bactericidal properties of a methanolic extract of the roots and flowers against 105 strains of bacteria, including *Staphylococcus aureus*. The resultant MICs and MBCs (≥ 8mgml^{-1}) indicated very low antibacterial activity. However, when the less polar hexane extract of *A. caulirhiza* was later investigated by Matu and Van Staden (2003) it was found to be highly active against *S. aureus* (1.03 ± 0.03; ratio of inhibition zone of extract (100mgml^{-1}) to inhibition

Table 1: NMR data for *N*-isobutylnona-2*E*,4*E*-dien-8-ynamide (compound 2) (CD_3OD, 300 MHz)

Carbon number	^{1}H NMR data (300 MHz)	^{13}C NMR data (75 MHz)	COSY correlations
1	–	169.4 (C)	–
2	6.02 (1H, d, *J* = 15.1 Hz)	124.2 (CH)	H-3
3	7.15 (1H, dd, *J* = 10.7, 15.1 Hz)	141.5 (CH)	H-2, H-4
4	6.30 (1H, dd, *J* = 10.7, 15.3 Hz)	131.2 (CH)	H-3, H-5
5	6.16 (1H, m)	140.6 (CH)	H-4, H-6
6	2.43 (2H, m)	32.4 (CH_2)	H-5, H-7
7	2.43 (2H, m)	19.3 (CH_2)	H-6
8	–	77.5 (C)	–
9	2.51 (1H, s)	66.8 (CH)	–
1’	3.10 (2H, d, *J* = 6.8 Hz)	48.1 (CH_2)	H-2’
2’	1.87 (1H, m)	29.8 (CH)	H-1’, H-3’, H-4’
3’	0.96 (3H, d, *J* = 6.8 Hz)	20.5 (CH_3)	H-2’
4’	0.96 (3H, d, *J* = 6.8 Hz)	20.5 (CH_3)	H-2’

zone of neomycin (500µgml^{-1}) in agar diffusion test). From a similar hexane extract we report the isolation of two alkylamides. Accordingly, these amides may directly inhibit bacterial pathogens, besides providing localised pain relief to patients. The observed antibacterial activity validates the traditional use of this plant species for chest complaints amongst the Zulu, and for treating gum, mouth and throat sores in East and southern Africa. Hexane extracts of *A. caulirhiza* have also been shown to reduce cyclooxygenase activity by 76% *in vitro* (COX-1 assay) (Matu and Van Staden 2003). This anti-inflammatory effect at least partly accounts for the documented analgesic effect of the amides and their use in the traditional treatment of rheumatism. The value of alkylamides as anti-inflammatory agents warrants further investigation, although their remarkably broad-based activities may preclude pharmaceutical development.

Acknowledgements — Silverglen Medicinal Plant Nursery (Durban) is thanked for providing plant material for analysis. The Department of Environmental Affairs and Tourism (DEAT) has funded the development of MEDBASE, the National Medicinal Plants Database for South Africa. We thank D Jagjivan for running NMR spectra. JJ Nair gratefully acknowledges a post-doctoral grant from the University of Natal and A Langlois a postgraduate bursary from the National Research Foundation (NRF), who also funded this research. Mrs E Retief of the National Herbarium (PRE) and Dr H Glen of the KZN Herbarium (NH) are thanked for helpful taxonomic discussions. The staff of the Mary Gunn Library (National Herbarium) kindly facilitated access to literature.

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Edited by PJ Houghton