

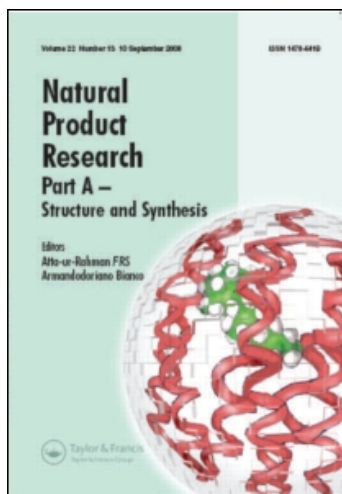
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Two new alkamides from roots of *Acmella decumbens*

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From the roots of *Acmella decumbens* (Sm) R.K. Jansen three compounds belonging to the alkamide family were isolated: (2*E*, 4*E*)-*N*-hydroxyphenylethyl-2,4-decadien-9-inamide (**1**); (4*E*, 6*E*)-*N*-isobutyl-4,6-undecen-10-inamide; (**2**) and (2*Z*)-*N*-phenylethyl-2-nonane-6,8-diinamide (**3**). The structures were determined by means of IR, MS, ¹H NMR, ¹³C NMR, DEPT 135 and COSY. Compounds **1** and **2** are reported for the first time, while **3** was previously isolated from *Spilanthes acmella* L.

Keywords: *Acmella decumbens*; alkamides; acmelline; decumbine

1. Introduction

Acmella decumbens (Sm) R.K. Jansen (= *Spilanthes decumbens* (Smith) A.H. Moore) (Asteraceae) is a species whose habitat extends from southeastern Uruguay to Rio Grande do Sul in Brazil. In Argentina, its distribution corresponds to the northeastern and central regions of the country. This plant grows in very different soils, varying in density and dimensions of foliage (Cabrera, 1971, 1974, 1978; Jansen, 1985; Zuloaga & Morrone, 1999). Its roots produce a pungent effect accompanied by a sleepy sensation of the tongue when they are bitten. For this reason, in folk medicine analgaesic properties are attributed to this plant, and it is used as an odontalgic agent (Cabrera, 1978). The species belongs to a genera that is characterised by the presence of alkamides (Greger, 1984). These compounds have been biologically studied, showing that several of them have insecticidal properties; others are immunostimulatory or antiviral, develop inhibitory activity of the synthesis of RNA, or cause inhibitory activity of the arachidonic acid metabolism. Alkamides in general are characterised by a pungent effect that they produce when they are bitten, as well as a sialagogue effect (Bauer & Reminger, 1989; Greger, 1984, 1988; Nagashima & Nakatani, 1992; Ramsewak, Erickson, & Nair, 1999).

In the present work, we report the isolation and identification of two new alkamides from the hexane root extract of *A. decumbens*, (2*E*, 4*E*)-*N*-hydroxyphenylethyl-2,4-decadien-9-inamide (acmelline, **1**), (2*E*, 4*E*)-*N*-isobutyl-4,6-undecen-10-inamide (decumbine, **2**), as well as a third already known compound (2*Z*)-*N*-phenylethyl-2-nonane-6,

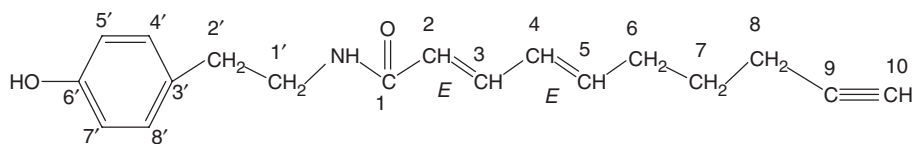
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8-diinamide (**3**). The methodology used for isolation and purification is described, together with the spectroscopic data obtained and a discussion on the structure elucidation.

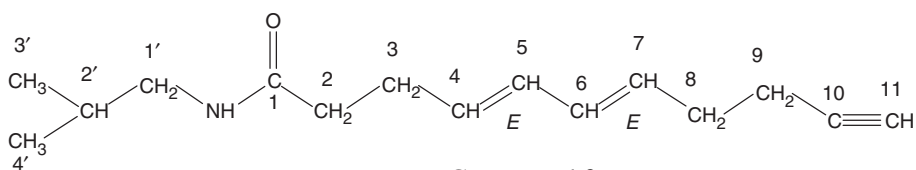
2. Results and discussion

Three compounds (**1–3**) (Figure 1) belonging to the alkamide family were obtained by purification of the hexane root extract of *A. decumbens*. Compounds **1** and **2** are described for the first time in the present work and their structures were determined by IR, MS, ^1H RMN, COSY H–H, ^{13}C NMR, DEPT and elemental analysis, while **3** has been isolated previously from *Spilanthes acmella* L. (Nagashima & Nakatani, 1992).

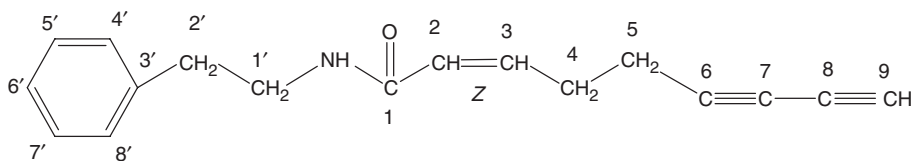
Compound **1** was isolated as yellow oil. The IR spectra showed absorption bands at 3304, 1632 and 1532 cm^{-1} , the characteristics of a secondary amide, at 2221 cm^{-1} an absorption band due to a triple bond presence in the structure and at 2982 cm^{-1} a band that corresponds to aromatic hydrogens (Nagashima & Nakatani, 1992; Martin & Becker, 1984; Saadali, Boriky, Blaghen, Vanhaelen, & Talbi, 2001). The MS presents an ion at m/z 284 that corresponds to $[\text{M} + 1]^+$, as well as the following ions that correspond to other breakdown fragments of the compound at m/z 255 ($\text{C}_{16}\text{H}_{17}\text{NO}_2$), 164 ($\text{C}_9\text{H}_{10}\text{NO}_2$), 93 ($\text{C}_6\text{H}_5\text{O}$) and 58 (C_2HNO) (Saadali et al., 2001). The ^1H NMR spectrum presented a wide singlet at δ_{H} 5.49 (1H), which corresponded to the H of an amide group, and a multiplet at δ_{H} 7.21–7.35 (4H, H 4', 5', 7', 8'), attributable to four aromatic protons. The ^{13}C NMR spectrum showed the signals of aromatic ring carbons at δ_{C} 138.42, 126.56, 128.75, 146.83, 128.75 and 126.56. The triple bond of the structure was confirmed by the



Compound **1**



Compound **2**



Compound **3**

Figure 1. Alkamides from *A. decumbens*. Compound **1**: (2*E*,4*E*)-*N*-hydroxyphenylethyl-2,4-decadien-9-inamide. Compound **2**: (4*E*,6*E*)-*N*-isobutyl-4,6-undecen-10-inamide. Compound **3**: (2*Z*)-*N*-phenylethyl-2-nonane-6,8-diinamide.

presence of a wide singlet at δ_{H} 1.95 (1H, H10) in the ^1H NMR spectrum, and by two signals in the ^{13}C NMR spectrum assignable to the sp carbons at δ_{C} 77.8 and 64.8. Four signals were observed in the ^1H NMR spectrum at 5.79 (1H; H4; J :15), 5.82 (1H; H2; J :15,7), 6.23 (1H; H5; J :15,7) and 7.24 (1H; H3; J :15,7) that were assigned to four olefinic protons (Nagashima & Nakatani, 1992; Martin & Becker, 1984). In the ^{13}C NMR spectrum, four signals that correspond to the carbons involved in the double bonds at δ_{C} 123.83, 142.14, 124.85 and 143.92 were observed. Double bond *trans*-configuration (*E*) of the molecule was determined by the coupling constant (J) value of 15 Hz (Greger & Hofer, 1989). By means of the homonuclear correlation experiment COSY, it was possible to establish all the proton–proton correlations of the molecule. Methylene and methine groups present in the structure were identified by means of the DEPT experiment. From all of the above data, compound **1** was identified as (2*E*, 4*E*)-*N*-hydroxyphenylethyl-2,4-decadien-9-inamide (acmelline) (Table 1).

Compound **2** was isolated as yellow oil. The MS presented a molecular ion $[\text{M}]^+$ at m/z 233, consistent with the molecular formula ($\text{C}_{15}\text{H}_{23}\text{NO}$), and another ion at m/z 57 that corresponded to a terminal isobutyl fragment (Greger & Hofer, 1989; Nagashima & Nakatani, 1992; Martin & Becker, 1984). The IR spectrum revealed a secondary amide group presence (3310, 1633 and 1531 cm^{-1}) and a triple bond in the structure at 2221 cm^{-1} . The ^1H NMR spectrum presented a wide singlet at δ_{H} 1.96 (1H; H11) that was attributable to the triple bond, confirmed by two signals in the ^{13}C NMR spectrum corresponding to the sp carbons at δ_{C} 77.7 and 64.8, respectively. The ^1H NMR spectrum presented a wide singlet at δ_{H} 5.42 (1H; NH) that corresponded to the H of the amide group. The presence of a terminal isobutyl group was attributed to a quadruplet at δ_{H} 3.14 (2H; H1'; J :7), a multiplet at δ_{H} 1.77 (1H; H2') and a doublet at δ_{H} 0.93 (6H; H3', 4'; J :7),

Table 1. Compound **1**: ^1H NRM, COSY (200.13 MHz, CDCl_3), ^{13}C NRM, DEPT (50.03 MHz, CDCl_3).

H no.	δ_{H} ppm (J Hz)	COSY	δ_{C} ppm	DEPT
1			165.59	
2	5.82 dt (15,7)	H ₃	123.83	CH
3	7.24 d (15)	H ₂ H ₄	142.14	CH
4	5.79 d (15)	H ₃ , H ₅	124.85	CH
5	6.23 dd (15,7)	H ₆ , H ₄	143.92	CH
6	2.85 dd (7,7)	H ₅ , H ₇	30.39	CH ₂
7	2.41 d (7)	H ₆ , H ₈	20.08	CH ₂
8	1.49 t	H ₇	26.94	CH ₂
9	–	–	77.83	–
10	1.95 s	–	64.89	CH
NH	5.49 s	–	–	–
1'	3.58 m	H ₂ '	40.38	CH ₂
2'	2.85 dd (7,7)	H ₁ '	35.63	CH ₂
3'	–	–	138.42	–
4'	7.21–7.35 m	H ₅ ', 7', 8'	126.56	CH
5'	7.21–7.35 m	H ₄ ', 7', 8	128.75	CH
6'	–	–	146.83	–
7'	7.21–7.35 m	H ₄ ', 5', 8	128.75	CH
8'	7.21–7.35 m	H ₄ ', 5', 7'	126.56	CH
OH	9.27 s, b	–	–	–

and three signals in the ^{13}C NMR spectrum at 46.8, 28.5 and 18.9, respectively (Nagashima & Nakatani, 1992). In the ^1H NMR spectrum, three double triplets at δ_{H} 5.81 (2H; H_{5,7}; *J*:15,7), 6.08 (1H; H₄; *J*:15,7) and 6.83 (1H; H₆; *J*:15,7) were assigned to the olefinic protons confirmed by the ^{13}C NMR spectrum, which were four signals corresponding to the carbons involved in the double bonds at δ_{C} 124.3, 143.5, 124.0 and 137.1 (Greger & Hofer, 1989; Nagashima & Nakatani, 1992; Martin & Becker, 1984; Saadali et al., 2001). The *trans*-configuration (*E*) of the double bonds was determined by the coupling constant (*J*) value of 15 Hz (Greger & Hofer, 1989). By means of the homonuclear correlation experiment COSY, all the proton–proton correlations of the molecule were established. Primary, secondary and tertiary carbon assignation (groups CH₃, CH₂ and CH) was corroborated by means of DEPT. According to all these antecedents, compound **2** was identified as (*2E, 4E*)-*N*-isobutyl-4,6-undecen-10-inamide (decumbine) (Table 2).

Compound **3** was obtained as pale yellow oil. It was previously isolated from *S. acmella* L.; its structure was assigned by comparison to the spectroscopic data of IR, MS and ^1H NMR cited in the literature (Nagashima & Nakatani, 1992).

3. Experimental

3.1. General

^1H NMR and COSY spectra were measured on a Bruker AC spectrometer at 200.13 MHz. ^{13}C NMR and DEPT were measured on the same equipment at 50.03 MHz, using CDCl_3 as solvent. IR spectra were obtained with a Nicolet 5 SXC FTIR infrared spectrophotometer. EIMS were obtained on a Variant Mat CH 7-A at 70 eV. Microanalysis of CHNS was recorded on a CARLO ERBA EA 1108. Column chromatography (CC) was performed on silica gel (Merck 70–230 mesh). Thin layer chromatography (TLC) was performed on precoated silica gel plates (Merck Kiessigel 60 F254). Spots were revealed

Table 2. Compound **2**: ^1H NMR, COSY (200.13 MHz, CDCl_3), ^{13}C NMR, DEPT (50.03 MHz, CDCl_3).

H no.	δ_{H} (ppm)	COSY	δ_{C} ppm	DEPT
1	–	–	165.0	–
2	2.34 dt (7.7)	H ₃	26.9	CH ₂
3	2.89 dd (15.7)	H ₂ , H ₄	27.4	CH ₂
4	6.08 dt (15.7)	H ₃ , H ₅	124.3	CH
5	5.81 dt (15.7)	H ₆ , H ₄	143.5	CH
6	6.86 dt (15.7)	H ₅ , H ₇	124.0	CH
7	5.81 dt (15.7)	H ₆ , H ₈	137.1	CH
8	1.81 dd (15.7)	H ₇ , H ₉	30.9	CH ₂
9	2.34 dt (7.7)	H ₈	46.6	CH ₂
10	–	–	77.7	–
11	1.96 s	–	64.8	CH
NH	5.42 s	–	–	–
1'	3.14 c (7)	H _{2'}	46.8	CH ₂
2'	1.77 m	H _{1',3',4'}	28.5	CH ₂
3'4'	0.93 d (7)	H ₂	18.9	CH ₃

under UV light by spraying the plates with Dragendorff reactive. Preparative TLC was performed on plates prepared with silica gel 60 GF254.

3.2. Plant material

Roots of *A. decumbens* were collected in Embalse de Río Tercero, Los Cocos, Athos Pampa (Córdoba Province, Argentina) between the end of December 2002 and the beginning of January 2003. Plant material was identified by Prof. Dr Luis Ariza Espinar (Instituto Multidisciplinario de Biología Vegetal, CONICET-Universidad Nacional de Córdoba). A voucher specimen has been deposited at the Museo Botánico de Córdoba (UNC) as CORD 775.

3.3. Extraction and isolation

Plant material was dried at room temperature. Fragmented *A. decumbens* roots (1180 g) were extracted for 72 h using *n*-hexane in a Soxhlet apparatus. The hexane extract (12 g) was submitted to CC eluted with trichloroethylene : EtOAc (9 : 1) in increasing proportions of EtOAc, up to 100%. All the fractions were monitored by TLC, using toluene : EtOAc (7 : 3). Two main fractions were obtained and both were posteriorly purified by CC with petrol : EtOAc (8 : 2) as mobile phase. The eluents were monitored by TLC with *n*-hexane : acetone (3 : 1) as mobile phase. Final separation and purification of **1** (7.4 mg), **2** (9.3 mg) and **3** (2.5 mg) were achieved by several successive preparative TLC with the same last mobile phase.

Acmelline (*2E, 5E*)-*N*-hydroxyphenylethyl-2,5-decadien-9-inamide (**1**): Yellow oil. IR (KBr) ν_{\max} cm⁻¹: 3304, 2982, 2952, 2221, 1668, 1632, 1532, 1300. MS: 284 [M + 1]⁺ (C₁₈H₂₂NO₂), 255 (C₁₆H₁₇NO₂), 164 (C₉H₁₀NO₂), 107 (C₇H₇O), 93 (C₆H₅O), 58 (C₂H₄NO). Elemental analysis Calcd for C₁₈H₂₁NO₂: C 76.33%; H 7.42%, N 4.94%, found C 75.09%, H 7.01%, N 4.20%. ¹H NMR, COSY, DEPT and ¹³C NMR (Table 1).

Decumbine (*4E, 6E*)-*N*-isobutyl-4,6-undecen-10-inamide (**2**): Yellow oil. IR ν_{\max} cm⁻¹: 3310, 2966, 2926, 2850, 2221, 1682, 1633, 1531, 1373. MS: 233 (C₁₅H₂₃NO), 219 (C₁₄H₂₁NO), 131 (C₁₀H₁₁), 77 (C₆H₅), 57 (C₄H₉). Elemental analysis Calcd for C₁₅H₂₃NO: C 77.25%, H 9.87%, N 6.0%, found C 75.92%, H 10.07%, N 6.71%. ¹H NMR, COSY, DEPT and ¹³C NMR (Table 2).

(*2Z*)-*N*-phenylethyl-2-nonane-6,8-diinamide (**3**): IR ν_{\max} cm⁻¹: 3320, 3021, 2930, 2904, 2250, 1665, 1634, 1550, 1330. MS: 251 (C₁₇H₁₇NO), 131 (C₉H₇O), 105 (C₈H₉), 91 (C₇H₇), 77 (C₆H₅). All the NMR spectroscopic data are coincident with those reported in the literature (Nagashima & Nakatani, 1992).

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