

## Chemical Constituents of *Senecio procumbens*

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**Abstract.** A chemical study of *Senecio procumbens* afforded the 12-membered macrocyclic diester pyrrolizidine alkaloids senecionine (**1**), senecivernine (**2**), retrorsine (**3**) and their *N*-oxides, together with 13<sup>2</sup>-hydroxyphaeophytin a (**4**), caffeic acid methyl ester (**5**), and cacticin (**6**). The identification of these compounds was accomplished by comparison of their spectral features with those reported in literature. The structures of compounds **1**, and those of the *N*-oxides of **1** and **3** were confirmed by comparison with authentic samples. In addition, the <sup>1</sup>H and <sup>13</sup>C NMR spectroscopies of **2** *N*-oxide are reported since they were not available in the literature.

**Keywords:** *Senecio procumbens*, Asteraceae, Senecioneae, Pyrrolizidine alkaloids.

**Resumen.** De un estudio químico de *Senecio procumbens* se aislaron los alcaloides pirrolizidínicos diésteres macrocíclicos de 12 miembros: senecionina (**1**), senecivernina (**2**), retrorsina (**3**) y sus *N*-óxidos, junto con 13<sup>2</sup>-hidroxifeofitina a (**4**), cafeato de metilo (**5**) y cacticina (**6**). Estos metabolitos fueron identificados por comparación de sus datos espectroscópicos con los descritos. La estructura de **1**, y de los *N*-óxidos de **1** y **3** fue confirmada por comparación con muestras auténticas. Adicionalmente se incluyen los datos de RMN de <sup>1</sup>H y <sup>13</sup>C del *N*-óxido de **2**, los cuales no han sido descritos.

**Palabras clave:** *Senecio procumbens*, Asteraceae, Senecioneae, alcaloides pirrolizidínicos.

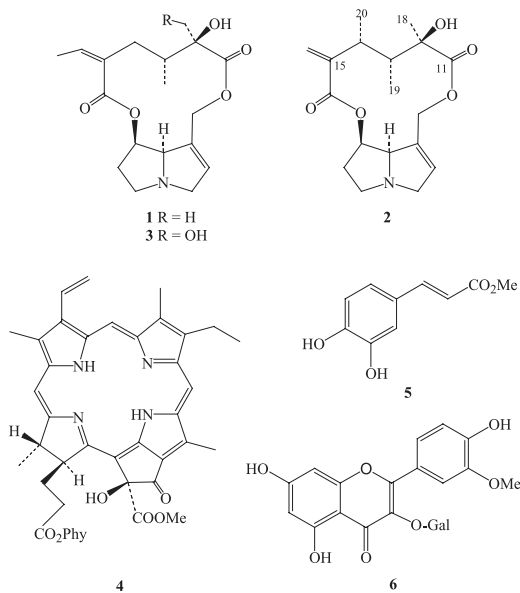
### Introduction

The genus *Senecio* is known as an important source of pyrrolizidine alkaloids (PAs) [1, 2], many of them being toxic to livestock and humans [3]. Sesquiterpenes, mainly those with an eremophilane skeleton, and flavonoids are also among their characteristic metabolites [4]. As a continuation of our research on this genus [5], we studied the chemical composition of *Senecio procumbens* Kunth which, to our knowledge, has no previous chemical studies. In this paper we report the isolation of three 12-membered macrocyclic diester (PAs) (**1-3**), one phaeophytin (**4**), one phenylpropanoid (**5**) and one flavonoid glycoside (**6**).

### Results and Discussion

The methanolic extract of roots of *S. procumbens*, which gave positive Dragendorff test, was submitted to a reductive process (Zn/aq. H<sub>2</sub>SO<sub>4</sub>) [6]. The reduced extract produced senecionine (**1**) [7a, 8], a (4:3) mixture of senecionine (**1**) and senecivernine (**2**) [7, 8], retrorsine (**3**) *N*-oxide [7a, 8], and a (4:3) mixture of the *N*-oxides of **1** and **2**. The reduction of the mixture of *N*-oxides of **1** and **2** by Zn/aq. H<sub>2</sub>SO<sub>4</sub> produced a mixture of free bases. The presence of *N*-oxides, in spite of the reductive treatment, could be due to a partial reduction, to a transformation of the free alkaloids into their *N*-oxides on standing with CHCl<sub>3</sub> at room conditions [9], or both. The methanolic extract of the aerial parts of *S. procumbens* gave positive Dragendorff test and produced senecionine (**1**) and its *N*-oxide. The acetone-

ic extract afforded 13<sup>2</sup>-hydroxyphaeophytin a (**4**) [10], caffeic acid methyl ester (**5**) [11], cacticin (**6**) [12, 13], and β-sitosterol glucopyranoside. From the hexanic extract only waxes and a mixture of β-sitosterol-stigmasterol were isolated. Compounds **1-6** were identified by comparison of their spectral features with those reported in literature. The structures of senecionine (**1**), its *N*-oxide, retrorsine (**3**) *N*-oxide, β-sitosterol, stigmasterol, and β-sitosterol β-D-glucopyranoside were confirmed by comparison with authentic samples. We report the <sup>1</sup>H and <sup>13</sup>C NMR data of the *N*-oxide of senecivernine (**2**), since they were not available in literature. Assignments were achieved by means of 1D and 2D NMR experiments, in which the characteristic deshielding effect of



*N*-oxides on the 3, 5 and 8 positions, was evident [14]. Assignment of the Me-18, Me-19 and Me-20 signals were confirmed by an HMBC experiment. This showed the interaction of H-18 with C-11; the three bond correlation of H-19 with C-12 and C-14 and that of H-20 with the vinylic singlet of C-15.

The 1-dehydro twelve membered macrocyclic pyrrolizidine alkaloids found in *S. procumbens* are commonly isolated from species of this genus and are known to be potentially toxic [3]. Flavonoids and phenylpropanoids are also frequent in *Senecio* species; nevertheless, phaeophytins have not been previously reported for this genus.

## Experimental

### General Experimental Procedures

Melting points were determined on a Fisher Jones melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-360 digital polarimeter. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. EIMS data were determined on a JEOL JMS-AX505HA mass spectrometer at 70 eV.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data were obtained on a Varian Unity 300 instrument. Chemical shifts were referred to TMS ( $\delta$  0). Standard Varian programs were used for COSY spectra at 300 MHz. HETCOR experiments were obtained for  $^1J_{\text{CH}} = 140$  Hz at 75 MHz. COLOC experiments were obtained for  $^nJ_{\text{CH}} = 9$  Hz at 75 MHz. Vacuum column chromatographies (VCCs) were performed using Silica gel 60 G Merck.

### Plant Material

*Senecio procumbens* Kunth (Asteraceae, Senecioneae) is an herbaceous, perennial, alpine species, 5-18 cm tall [15]. It was collected in November 2002 near to the first lake of the crater of the Nevado de Toluca, State of Mexico, Mexico, at an altitude of ca. 4000 m. A voucher specimen was deposited at the Herbarium of the Instituto de Biología, UNAM, Mexico (MEXU 1045507).

### Extraction and Isolation

Dried and ground roots (130 g) of *S. procumbens* were extracted with MeOH until the extract gave a negative test with Dragendorff reagent. The extract was concentrated and treated with Zn/aq.  $\text{H}_2\text{SO}_4$  [6] to give 2.2 g of alkaloidal residue. This was purified by vacuum column chromatography (VCC) and eluted with hexane- $\text{CHCl}_3$ -MeOH mixtures of increasing polarity. From fractions obtained with  $\text{CHCl}_3$ -MeOH 19:1, senecionine (**1**, 58 mg), mp 235-7°C and a (4:3) mixture of senecionine and senecivernine (**2**, 120 mg) [7, 8], were isolated. Fractions eluted with  $\text{CHCl}_3$ -MeOH 9:1 afforded senecionine and senecivernine *N*-oxides as a (4:3) mixture (245 mg). Reduction of this mixture by Zn/aq.  $\text{H}_2\text{SO}_4$  produced

the free bases. Fractions collected with  $\text{CHCl}_3$ -MeOH 7:3 were further purified by VCC, eluted with  $\text{CHCl}_3$ -MeOH 4:1, to afford retrorsine *N*-oxide (**3-N**-oxide), mp 145-7°C [7, 8].

Dried and ground aerial parts (700 g) of *S. procumbens* were extracted successively with hexane, acetone and MeOH. Only the methanolic extract gave positive test with Dragendorff reagent. After purification by VCC the hexane extract (12 g) produced waxes and a  $\beta$ -sitosterol-stigmasterol mixture (150 mg). The acetonic extract (13 g) was worked up by VCC eluted with hexane-EtOAc mixtures of increasing polarity. Fractions collected with hexane-EtOAc 4:1 (2.5 g), after two successive VCCs eluted with hexane-EtOAc 17:3 and hexane- $\text{Me}_2\text{CO}$  9:1, afforded 125 mg of 13<sup>2</sup>-hydroxyphaeophytin a (**4**), mp 110-112°C,  $[\alpha]_{\text{D}}^{20} -52.9$  (c 0.01,  $\text{CHCl}_3$ ) [10]. Fractions obtained with EtOAc were further fractionated by VCC eluted with  $\text{CH}_2\text{Cl}_2$ -MeOH 4:1 to afford caffeic acid methyl ester (**5**, 104 mg), mp 155-6°C [11], cacticin (**6**, 80 mg), mp 212-4°C,  $[\alpha]_{\text{D}}^{20} -51.6$  (c 0.2, pyridin) [12, 13], and  $\beta$ -sitosteryl  $\beta$ -D-glucopyranoside (450 mg). The methanolic extract was submitted to a reductive process to obtain the alkaloidal portion (1.9 g). Purification of this portion by VCC eluted with  $\text{CHCl}_3$ -MeOH mixtures of increasing polarity produced senecionine (**1**, 13 mg) and senecionine *N*-oxide (40 mg) from fractions collected with  $\text{CHCl}_3$ -MeOH 19:1 and 9:1, respectively.

**Senecivernine N-oxide (2 N-oxide):**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.26 (1H, br d,  $J = 2.0$  Hz, H-2), 4.70 (1H, br d,  $J = 12.5$  Hz, H-3a), 4.29 (1H, dd,  $J = 12.5, 1.0$  Hz, H-3b), 3.76 (1H, m, H-5a), 3.76 (1H, m, H-5b), 2.86 (1H, ddd,  $J = 12.0, 7.0, 4.5$  Hz, H-6a), 2.39 (1H, dddd,  $J = 12.0, 6.0, 2.5, 2.5$  Hz, H-6b), 5.53 (1H, ddd,  $J = 6.0, 4.5, 2.5$  Hz, H-7), 4.84 (1H, br s, H-8), 5.36 (1H, dd,  $J = 12.0, 1.5$  Hz, H-9a), 4.31 (1H, br d,  $J = 12.0$  Hz, H-9b), 1.71 (3H, qd,  $J = 7.0, 1.5$  Hz, H-13), 2.62 (1H, q.d,  $J = 7.5, 1.5$  Hz, H-14), 1.34 (3H, s, H-18), 0.82 (3H, d,  $J = 7.0$  Hz, H-19), 1.09 (3H, d,  $J = 7.5$  Hz, H-20), 5.91 (1H, br s, H-21a), 5.36 (1H, br s, H-21b);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  131.1 (C-1), 131.5 (C-2), 78.7 (C-3), 69.4 (C-5), 33.3 (C-6), 75.1 (C-7), 96.6 (C-8), 60.5 (C-9), 178.3 (C-11), 78.7 (C-12), 42.3 (C-13), 37.4 (C-14), 148.1 (C-15), 169.3 (C-16), 26.6 (C-18), 5.9 (C-19), 12.5 (C-20), 123.1 (C-21).

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