SHORT REPORT

A C G publications

Rec. Nat. Prod. 10:4 (2016) 513-518

records of natural products

Chemical Constituents and Acetylcholinesterase Inhibition of *Senecio ventanensis* Cabrera (Asteraceae)

Natalia Paola Alza and Ana Paula Murray*

INQUISUR-CONICET, Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, B8000CPB Bahía Blanca, Argentina

(Received September 27, 2013; Revised November 04, 2014; Accepted November 10, 2014)

Abstract: Chemical constituents and acetylcholinesterase inhibition was investigated in both essential oil and dichloromethane subextract obtained from the aerial parts of *Senecio ventanensis*, an endemic Asteraceae. The chemical composition of the oil was determined by GC and GC-MS. The major constituents were α-terpinene (12.2%), limonene (11.9%), □-humulene (10.5%), sabinene (9.1%), terpinolene (8.8%), *p*-cymene (8.1%) and α-ocimene (7.3%). The bioassay guided isolation of the constituents of the active dichloromethane subextract (IC_{50} = 361.6 μg/mL) led us to the isolation of two diastereoisomers of a sesquiterpene endoperoxide 3,6-epidioxy-1,10-bisaboladiene (1 and 2), reported here for the first time in *S. ventanensis.* The identification was achieved by comprehensive analyses of its ^{1}H and ^{13}C NMR spectroscopic data (including 2D experiments) and mass spectrometric data. Their absolute configuration is proposed on the basis of AM1 calculations and NOESY experiments. This is the first time that compounds 1 and 2 have been separated as well as the first phytochemical investigation of *S. ventanensis.*

Keywords: *Senecio ventanensis*; essential oil; sesquiterpene endoperoxide; acetylcholinesterase inhibition.

1. Plant Source

Senecio ventanensis Cabrera (Asteraceae) (Sin.: *Senecio pinnatus* Poir. var. *glandulosus* Ball) is an endemic species that grows wild in the Southwest of Buenos Aires province, Argentina. The genus *Senecio* is known to be a source of pyrrolizidine alkaloids (PAs), eremophilanolides, and furanoeremophilanes [1]. *Senecio* species were used in the folk medicine for the treatment of wounds and as antiemetic, anti-inflammatory, and vasodilatory preparations [1].

Flowering plants of *S. ventanensis* were collected at Cerro Calvario, Tornquist, Buenos Aires province in October 2009. Voucher specimen was identified by Dr. Maria Gabriela Murray and deposited in the Herbarium of Universidad Nacional del Sur (BBB) in Bahía Blanca, Argentina, under the number *Murray M.G. 486*.

2. Previous Studies

To the best of our knowledge, there are no phytochemical and biological reports on *S. ventanensis*. The sesquiterpene endoperoxide 3,6-epidioxy-1,10-bisaboladiene has been obtained previously, always as a mixture of two stereoisomers, from several species, mainly from the

* Corresponding author: E- Mail:apmurray@uns.edu.ar (A.P. Murray), Phone +54-291-4595101 ext 3538.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 01/01/2016 EISSN: 1307-6167

Asteraceae family [2-5]. Antimicrobial, antifungal, antitumor and antimalarial activities have been reported for this mixture in the past [2-4, 6, 7].

3. Present Study

As part of our ongoing investigations of acetylcholinesterase (AChE) inhibitors from endemic and native plants [8, 9], we have studied the essential oil and the dichloromethane (DCM) subextract of *S. ventanensis*, their ability to inhibit AChE and chemical composition.

The fresh plant material was grounded into small pieces and the essential oil from the aerial parts of *S. ventanensis* (88.1 g) was isolated by hydrodistillation in a Clevenger-type apparatus (3-4 h) (yield: 0.015 %). The chemical composition of the oil was determined by GC-MS (See Supplementary material). The compounds were identified by comparison of their retention indices (Kovats Indices) with those of known compounds [10-15] and also by comparison of their MS with those stored in the MS database (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas. Results are summarized in Table 1.

Table 1. Chemical composition of the essential oil from *Senecio ventanensis*.

Constituents[a]	RI [b]	Percentage [c]	Identification [d]
1-nonene	892	0.9	Ri, MS, S
α-thujene	938	0.4	Ri, MS
α-pinene	939	3.6	Ri, MS, S
sabinene	972	9.1	Ri, MS
β-pinene	981	6.0	Ri, MS
β-myrcene	992	2.3	Ri, MS, S
α-phellandrene	1006	1.3	Ri, MS
α-terpinene	1012	12.2	Ri, MS
p-cymene	1027	8.1	Ri, MS
limonene	1030	11.9	Ri, MS, S
trans β-ocimene	1038	0.7	Ri, MS
α-ocimene	1056	7.3	Ri, MS
γ-terpinene	1074	2.6	Ri, MS
terpinolene	1088	8.8	Ri, MS
terpinen-4-ol	1179	3.4	Ri, MS
α-cubebene	1349	0.2	Ri, MS
α-copaene	1377	0.4	Ri, MS
β-cubebene	1391	0.3	Ri, MS
caryophyllene	1421	2.2	Ri, MS
aromadendrene	1440	0.8	Ri, MS
α-humulene	1454	10.5	Ri, MS
δ-cadinene	1518	2.0	Ri, MS
spathulenol	1578	5.1	Ri, MS

[a] Components are listed in order of elution on HP-5 column. [b]RI retention index determined on HP-5column. [c] Determined by GC-FID integration. [d] Ri = retention index identical to bibliography, MS = identification based comparison of mass spectra , S = retention time identical to authentic compound

Twenty three components representing the 98.5% of the total oil were identified. The major constituents were α-terpinene (12.2%), limonene (11.9%) and α-humulene (10.5%). Monoterpene hydrocarbons were the main constituents of the essential oil of *S. ventanensis* (66.2%), followed by sesquiterpene hydrocarbons (16.3%), phenylpropanoids (8.1%), oxygenated sesquiterpenes (5.1%) and oxygenated monoterpenes (3.4%).

It was reported that α-terpinene was the predominant compound in other two Argentinian *Senecio* species oils, *S. filaginoides* var. *lobulatus* (4.4%) from the province of Chubut [16] and *S. graveolens* (60%) from the province of Jujuy [17]. This compound was also one the main components of the essential oils of *S. atacamensis* (30.0 and 20.6% for leaves and stems, respectively) from Chile [18] and *S. nutants* aerial parts oils (15.1-12.2%) from Peru [19]. Limonene was one of the

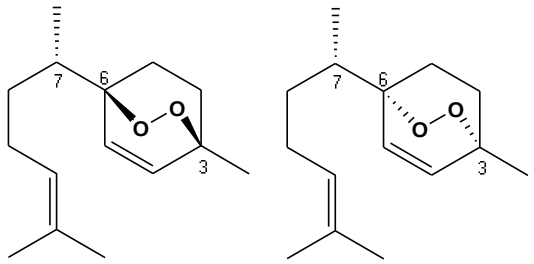
major constituents of the essential oils of *S. vernalis* (6.2%) from Turkey [20] and of the South African species *S. polyanthemoides* (3.1-43%) [21] and *S. pterophorus* (10.3-30.2%) [22]. The essential oils of *S. bombayensis* [23] and *S. longipenicillatus* [24] were reported to contain the sesquiterpene α-humulene (5.2 and 15.8%, respectively) as one the main constituents of the oils.

These components of *S. ventanensis* essential oil, α-terpinene, limonene and α-humulene, have not been detected as main compounds of the oils of other five *Senecio* species from Argentina (*S. mustersii, S.subpanduratus, S. subulatus, S. viridis, S. argophylloides*) although limonene was detected as a minor constituent of all the studied oils (0.2-5.3%) [25, 26].

Dichloromethane (DCM) subextract (14 g) was obtained from fresh material (1.3 kg) by maceration in ethanol, evaporation to dryness and further partition with H_2O:MeOH (9:1) and DCM.

We found that the DCM sub-extract from aerial parts of *S. ventanensis* inhibited AChE, with IC_{50} = 361.6 μg/mL in a spectrophotometric assay using Ellman´s colorimetric method [27]. On the other hand, the essential oil resulted to be inactive against AChE (8.9% of AChE inhibition at 166 μg/mL).

In order to identify the AChE inhibitors present in the DCM subextract a bioassay guided fractionation was carried out. A portion of this subextract (5 g) was subjected to column chromatography over Silicagel, eluting with mixtures of hexane:AcOEt of increasing polarity. Active fractions, eluted with hexane:AcOEt 98:2, were combined and submitted to flash chromatographic separation with hexane:Et_2O 98:2. Compound 1 (2.5 mg, $[\alpha]_D^{25}$ = -180 (*c* 0.1, $CHCl_3$)) and a mixture of 1 and 2 (4.2 mg, 54:46 ratio determined by GC) were obtained. Repeated and careful chromatographic separation of 1 and 2 afforded a mixture enriched in compound 2 (75%, 1.9 mg). From de NMR spectra of these compounds, provided in the supplementary material, the presence of a bisabolane sesquiterpenoid endoperoxide was established. Comparison of these data with the literature revealed that 1 and 2 are two diastereomers of 3,6-epidioxy-1,10-bisaboladiene that have been obtained previously, always as a mixture of two diastereoisomers (Figure 1). This is the first time that one of these diastereomers has been isolated.



1 **2**

Figure 1. Structure of compounds **1** and **2** isolated from *S. ventanensis.*

The stereochemistry of C-7 in 1 and 2 has been already established as *S* by a biosynthetic approach [2]. The stereochemistry of C-3 and C-6 is either 3*R*,6*R* or 3*S*,6*S*. In the NOESY spectrum of 1 we observed cross peaks between CH_3-14 (0.99 ppm) and H-5a (2.02 ppm) and H-5b (1.50 ppm), while in the NOESY spectrum of the mixture of 1 and 2 we also observed that for compound 2 the signal of CH_3-14 (1.00 ppm) correlates only with H-5a (2.02 ppm). AM1 calculations optimizing the dihedral angle between C-14 and the peroxy bridge showed that the stereoisomer 3*S*,6*S* has one conformation of minimal energy that could explain the NOE observed between CH_3-14 and one of the H-5 protons, while the stereoisomer 3*R*,6*R* has two conformations of minimal energy that show how CH_3-14 could give a cross peak with H-5a and H-5b in the NOESY experiment (Figure 2). These observations lead us to propose that the endoperoxide **1** has the 3*R*,6*R*,7*S* stereochemistry while **2** is the 3*S*,6*S*,7*S* isomer.

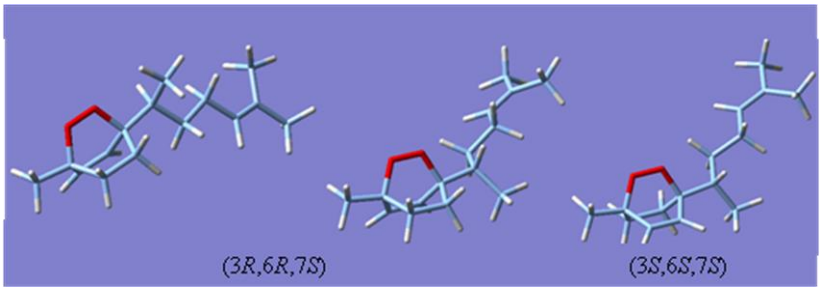


Figure 2. Minimal energy conformation of (3*R*,6*R*,7*S*)- and (3*S*,6*S*,7*S*)-3,6-epidioxy-1,10-bisaboladiene.

Table 2. Summary of acetylcholinesterase inhibition results

Inhibitor [a]	**Inhibition (%)**[b] **/ IC_{50}**
Essential oil	8.9 ± 0.7 (166 μg/mL)
DCM subextract	361.6 ± 1.1 μg/mL
1	10.6 ± 1.4 (200 μM)
1:2 (54:46)	36.4 ± 0.1 (200 μM)
1:2(25:75)	49.4 ± 0.2 (200 μM)
eserine [c]	99.0 ± 0.2 (200 μM)

[a] Samples were dissolved in buffer with MeOH as cosolvent (final conc. 2.5 %).
[b] Values represent the mean of three replicates ± standard deviation, inhibitor concentration between brackets. [c] Positive control.

Results suggest that endoperoxide 2 is a better inhibitor than 1 since the highest inhibition was observed for the mixture enriched in 2. The IC_{50} determined for the 54:46 mixture was 432.3 ± 0.6 μM (IC_{50} = 102.1 ± 0.1 μg/mL). Unfortunately, further attempts to obtain pure 2 have been unsuccessful until now. These results confirm the importance of knowing the stereochemistry of the inhibitor.

This study represents the first analysis of the secondary metabolites present in *S. ventanensis* with AChE inhibition and the first phytochemical record for this species. Even though the DCM subextract showed a moderated enzymatic inhibition (IC_{50} = 361.6 μg/mL), compounds 1 and 2 proved to be weak inhibitors, which suggest that others AChE inhibitors, that couldn`t be isolated, should be responsible for the observed activity of the DCM subextract. Finally, this is the first time that compounds 1 and 2 have been separated, allowing to propose an absolute configuration for each one.

Acknowledgements

This work was supported by CONICET, ANPCYT and Universidad Nacional del Sur. The authors wish to thank UMYMFOR (CONICET-FCEN) for some of the NMR spectra, Dra. María G. Murray for plant identification and Dra. Viviana Dorn for AM1 calculations. N.P.A thanks CONICET for a Doctoral fellowship. A.P.M. is a Research Member of CONICET.

References

[1] Y. Yang, L. Zhao, Y.F. Wang, M.L. Chang, C.H. Huo, Y.C. Gu, Q.W. Shi and H. Kiyota (2011). Chemical and pharmacological research on plants from the genus *Senecio*. *Chem. Biodiv.* **8**, 13-72.

[2] H.T. Simonsen, A. Adsersen, P. Bremner, M. Heinrich, U.W. Smitt and J.W. Jaroszewski (2004). Antifungal constituents of *Melicope borbonica*, *Phytother. Res.* **18**, 542-545.

[3] K. Nishikawa, N. Aburai, K. Yamada, H. Koshino, E. Tsuchiya and K. Kimura (2008). The bisabolane sesquiterpenoid endoperoxide, 3,6-epidioxy-1,10-bisaboladiene, isolated from *Cacalia delphiniifolia*

inhibits the growth of human cancer cells and induces apoptosis, *Biosci. Biotechnol. Biochem.* **72**, 2463-2466.

[4] A.F. Barrero, M.M. Herrador, J.F. Quilez, R. Alvarez-Manzaneda, D. Portal, J.A. Gavin, D.G. Gravalos, M.S.J. Simmonds and W.M. Blaney (1999). Bioactive sesquiterpenes from *Santolina rosmarinifolia* subsp. *canescens*. A conformational analyses of the germacrene ring, *Phytochemistry* **51**, 529-541.

[5] E.P. Schenkel, G. Rücker, D. Manns, M.B Falkenberg, N.I. Matzenbacher, M. Sobral, L.A. Mentz, S.A.L. Bordignon and B.M. Heinzmann (2002). Screening of Brazilian plants for the presence of peroxides, *Braz. J. Pharm. Sci.* **38**, 191-196.

[6] G. Rücker, R.D. Walter, D. Manns and R. Maier (1991). Antimalarial activity of some natural peroxides, *Planta Med.* **57**, 295-296.

[7] G. Rücker, E. Breitmaier, D. Manns, W. Maier, A. Marek, B. Heinzmann, K. Heiden and S. Seggewies (1997). Antimalarial activity of 1,4-epidioxy-bisabola-2,12-diene derivatives, *Arch. Pharm. Pharm. Med. Chem.* **330**, 12-16.

[8] A.P. Murray, M.S. Vela Gurovic, S.A. Rodriguez, M.G. Murray and A.A. Ferrero (2009). Acetylcholinesterase inhibition and antioxidant activity in essential oils from *Schinus areira* L. and *Schinus longifolia* (Lindl.) Speg., *Nat. Prod. Comm.* **4**, 873-876.

[9] M.S. Vela Gurovic, M.J. Castro, V. Richmond, M.B. Faraoni, M.S. Maier and A.P. Murray (2010). Triterpenoids with acetylcholinesterase inhibition from *Chuquiraga erinacea* D. Don. subsp. *erinacea* (Asteraceae), *Planta Med.* **76**, 607-610.

[10] R.P. Adams (2001). Identification of essential oils components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, IL, USA. 1-455.

[11] D. Machiels, S.M. Van Ruth, M.A. Posthumus and L. Istasse (2003). Gas chromatography-olfactometry analysis of the volatile compounds of two commercial Irish beef meats, *Talanta* **60**, 755-764.

[12] L. Jirovetz, G. Buchbauer, M.B. Ngassoum and M. Geissler (2002). Aroma compounds analysis of *Piper nigrum* and *Piper guineense* essential oils from Cameroon using solid-phase microextraction-gas chromatography, solid-phase microextraction-gas chromatography-mass spectrometry and olfactometry, *J. Chromatogr. A* **976**, 265-275.

[13] M.J Jordan, C.A. Margaria, P.E. Shaw and K.L. Goodner (2003). Volatile components and aroma active compounds in aqueous essence and fresh pink guava fruit puree (*Psidium guajava* L.) by GC-MS and multidimensional GC/GC-O, *J. Agric. Food Chem.* **51**, 1421-1426.

[14] J.A. Sotomayor, R.M. Martinez, A.J. García and M.J. Jordan (2004). Thymus zygis subsp. Gracilis: watering level effect on phytomass production and essential oil quality, *J. Agric. Food Chem.* **52**, 5418-5424.

[15] E. Mancini, N.A. Arnold, L. De Martino, V. De Feo, C. Formisano, D. Rigano and F. Senatore (2009). Chemical composition and phytotoxic effects of essential oils of *Salvia hierosolymitana* Boiss. and *Salvia multicaulis* Vahl. var. *simplicifolia* Boiss. growing wild in Lebanon, *Molecules* **14**, 4725-4736.

[16] A. Arancibia, A. Marchiaro, M. Arce, and V. Balzaretti (1999) *Senecio filaginoides* var. *lobulatus* essential oil, *Acta Hortic.* **500**, 127-128.

[17] C. Pérez, A.M. Agnese and J.L. Cabrera (1999). The essential oil of *Senecio graveolens* (Compositae): chemical composition and antimicrobial activity tests, *J. Ethnopharmacol.* **66**, 91-96.

[18] J. Benites, F. Bravo, M. Rojas, R. Fuentes, C. Moiteiro and F. Venâncio (2011). Composition and antimicrobial screening of the essential oil from the leaves and stems of *Senecio atacamensis* Phil. from Chile, *J. Chil. Chem. Soc.* ***56***, 712-714.

[19] V. De Feo, E. Urrunaga Soria, R. Urrunaga Soria and F. Senatore (2003). Chemical composition of essential oils of *Senecio nutans* Sch.-Bip. (Asteraceae), *Flavour Fragr. J.* **18**, 234-236.

[20] E. Bagci and O. Kilic (2012). Chemical composition of essential oil of *Senecio vernalis* Waldst. Et Kit. (Asteraceae) from Turkey, *J. Essent. Oil Bear. Pl.* **15**, 399-404.

[21] L.A. Oladipupo and O.O. Adebola (2009). Chemical composition of the essential oils of the flowers, leaves and stems of two *Senecio polyanthemoides* Sch. Bip. samples from South Africa, *Molecules* **14**, 2077-2086.

[22] O.A. Lawal and A.O. Oyedeji (2010). Volatile constituents of *Senecio pterophorus* (African daisy) DC. from South Africa, *Nat. Prod. Commun.* **5**, 1811-1814.

[23] R.K. Joshi (2014). Essential Oil of *Senecio bombayensis* from Western Ghats Region of India, *Chem. Nat. Compd.* **50**, 382-383.

[24] M. Rondón, M. Araque, A. Morales, M. Gualtieri, J. Rojas, K. Veres and I. Máthé (2006). Chemical composition and antibacterial activity of the essential oil of *Lasiocephalus longipenicillatus* (*Senecio longipenicillatus*), *Nat. Prod. Commun.* **1**, 113-115.

[25] J.S. Dambolena, M.P. Zunino, E. Banchio, E.I. Lucini, F.N. Biurrun, A. Rotman, O. Ahumada and J.A Zygadlo (2008). Essential oil composition of three species of *Senecio* from Argentina, *J. Essent. Oil Bear. Pl.* **11**, 623-627.
[26] L. Arancibia, C. Naspi, G. Pucci and M. Arce (2010). Aromatic plants from Patagonia: Chemical composition and antimicrobial activity of the essential oil of *Senecio mustersii* and *S. subpanduratus*, *Bol. Latinoam. Caribe* **9**, 123-126.
[27] G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherstone (1961) A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* **7**, 88-95.