Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Biochemical Systematics and Ecology 33 (2005) 201–205
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Flavonol 3,7-glycosides and dihydroxyphenethyl glycosides from *Aconitum napellus* subsp. *lusitanicum*

Jesús G. Díaz[a], Juan García Ruiz[a], Bianca Rachid Días[a], José A. Gavín Sazatornil[a], Werner Herz[b,*]

[a]*Instituto de Bio-Orgánica "A. González", Universidad de La Laguna, Ctra a la Esperanza 2, 38206 La Laguna, Tenerife, Spain*
[b]*Department of Chemistry and Biochemistry, The Florida State University, Tallahassee, FL 32306-4390, USA*

Received 14 April 2004; accepted 9 July 2004

Keywords: *Aconitum napellus* ssp. *lusitanicum*; *Ranunculaceae*; Flavonol glycosides

1. Subject and source

Aerial parts of *Aconitum napellus* L. ssp. *lusitanicum* Rory were collected on September 18, 1997 in San Emilio, Leon Province, Spain. The plant was identified by Professor Julián Molero, Department of Botany, Faculty of Pharmacy, Universidade de Barcelona. A voucher specimen (No. BCF 43708) is on deposit in the herbarium of that department.

* Corresponding author. Tel.: +1 850 644 2774; fax: +1 850 644 8281.
E-mail address: jdulin@chem.fsu.edu (W. Herz).

0305-1978/$ - see front matter
doi:10.1016/j.bse.2004.07.005

2. Previous work

Numerous studies have dealt with the diterpene alkaloids of *Aconitum* species (Ranunculaceae) but reports on other constituents are sparse. An early study dealt with the phenolic constituents of *A. variegatum* and *A. napellus* ssp. *skevisorae* (Sweykowski and Krzakowa, 1977a,b) and during the last decade articles have appeared on the flavonoids of several Korean *Aconitum* species (Whang et al., 1994; Kim et al., 1996; Dae et al., 1996; Jeong et al., 1997). Lim et al. (1999) studied flavonoid variations in members of the Korean *Aconitum jaluense* complex; subsequently Fico et al. (2001a,b) reported on flavone glycosides from the flowers of two other subspecies of *A. napellus* ssp. *tauricum* and ssp. *neomontanum*. The same group (Fico et al., 2003) very recently demonstrated that their flavonoid glycoside profiles characterize the two *A. napellus* subspecies studied by them earlier and that these differed in turn from the flavonoid profiles of *A. paniculatum* and *A. vulparia*. As a result they suggested that species recognition within this large genus might be tackled by using flavonoids as chemical molecular markers.

3. Present work

3.1. General procedures

IR spectra were determined using a Bruker IFS-55 spectrometer. ^{1}H and ^{13}C NMR spectra were measured using a Bruker AMX-400 or Bruker MAX-500 instruments. EIMS and exact mass measurements were determined using a Micromass Autospec instrument at 70 eV. Al_2O_3 Merck (neutral, 200–300 mesh) and Schleicher and Schuell 394 732 were used for column (CC) and thin layer (TLC) chromatography, respectively. Sephadex was LH-20, Pharmacia (ref. 17-0090-01). HPLC separations were performed on a JASCO Pu-980 series pumping system equipped with a JASCO UV-975 ultraviolet detector and with a Waters Kromasil® Si 5 μm (Wx 250 mm) column; flow rate of mobile phase 3 ml^{-1} with EtOAc–hexane.

3.2. Extraction and isolation of constituents

Air-dried powdered aerial parts (2.56 kg) of *A. napellus* ssp. *lusitanicus* were extracted with 90% EtOH at room temperature for 8 days. Filtration and removal of solvent at reduced pressure afforded 232 g of crude extract which was adsorbed on 550 g of Si gel and submitted to flash chromatography using hexane (10 l), hexane–EtOAc (1:1, 10 l), EtOAc (10 l), EtOAc–MeOH (3:1, 10 l), EtOAc–MeOH (1:1, 10 l) and MeOH (10 l) to furnish 30 g, 26 g, 141 g, 37.5 g and 92 g of residues in the respective eluates. A portion (15 g) of the EtOAc–MeOH (3:1) fraction were chromatographed on Sephadex LH-20 using MeOH–$CHCl_3$–hexane (1:1:9) as eluent to give 58 fractions of 100 ml each. These were combined into 12 fractions on the basis of TLC analyses (Si 60 F_{254} gel coated plastic sheets, eluent EtOAc–MeOH 1:2). Fractions 3–4 (75 mg) were combined and acylated with Ac_2O–pyridine in the