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Investigation on the flavonoid composition of *Aconitum angustifolium* Bernh. flowers and leaves

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ABSTRACT

A new flavonol glycoside, kaempferol 7-*O*-(6-*E*-*p*-coumaroyl)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside 3-*O*-β-D-galactopyranoside (**1**), together with other five known compounds (**2**–**6**), were identified from the flowers and leaves of *Aconitum angustifolium* Bernh. Their chemical structures were elucidated by extensive NMR spectral studies, as well as by ESI-MS analysis.

1. Introduction

The *Aconitum* genus (Ranunculaceae), distributed from Eurasia to North America, grows on the Italian Alps and Appennines presenting 12 taxa. A great deal of variations, perhaps as a result of hybridization, occurs in many species and subspecies, especially in those belonging to the *A. napellus* group such as *Aconitum angustifolium* Bernh. It is an allopolyploid (6*n*) species endemic in North-Eastern Italy, Croatia and Slovenia, probably derived from *A. variegatum*/*A. paniculatum* × *A. napellus* ssp. *tauricum* (Pignatti, 1982). *A. angustifolium* is characterized by stems up to 130 cm, glabrous inflorescence and seeds with transverse lamellae on each side (Pignatti, 1982; Akeroyd and Charter, 1993).

Aconitum species have been used in China as an essential drug in traditional medicine for 2000 years. Their tubers and roots are commonly applied for various diseases, such as collapse, syncope, rheumatic fever, painful joints, gastroenteritis, diarrhea, oedema, bronchial asthma, various tumors, and some endocrinal disorders like irregular menstruation (Singhuber et al., 2009). In the West, *Aconitum* has been employed, particularly in the past, as antineuralgic for the trigeminal nerve and sciatica treatment, soothing cough, and cardioregulator. Now, this genus, because of its high toxicity, is used only in homeopathy (Heinrich et al., 2012). A literature survey revealed that about 50 species of *Aconitum* have been chemically investigated and many alkaloids in addition to β-sitosterol and some flavonoid glycosides have been isolated from various plant parts (Singhuber et al., 2009). These secondary metabolites, especially nor- and diterpene alkaloids, are responsible for the biological activities of this genus (Srivastava et al., 2010). Our previous studies reported the antioxidant activity of some flavonol glycosides from Italian *Aconitum* species (Braca et al., 2003; Mariani et al., 2008; Vitalini et al., 2010). These were also used to provide chemotaxonomic information on the critical species (Fico et al., 2003).

In order to complete the investigation on flavonoid content of all Italian *Aconitum* species, we carried out the phytochemical study of *A. angustifolium*. In this paper we describe the isolation and structural identification of one new compound, kaempferol 7-*O*-(6-*E*-*p*-coumaroyl)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside 3-*O*-β-D-galactopyranoside (**1**), together with other five known flavonol glycosides (**2**–**6**) (Fig. 1) and their antioxidant activity.

Our further interest was to investigate whether the isolated compounds could clarify the taxonomical position of the species under study into the complex *Aconitum* genus.

2. Results and discussion

2.1. Morphological analysis

The morphological characters used for the identification of *A. angustifolium* are the following: tuberous rootstock, leaf with fine lacinia, violet flower, forward curved nectary, glabrous helmet, glabrous follicles, seed with membranous side lamellae and one

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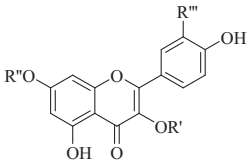

Compound	R'	R''	R'''	*Species
1	Gal	(6-*E*-*p*-coumaroyl)Glc(1→3)Rha	H	-
2	Glc	(6-*E*-caffeoyl)Glc(1→3)Rha	H	*A. n. neomontanum*
3	Glc	(6-*E*-*p*-coumaroyl)Glc(1→3)Rha	H	*A. n. neomontanum*
4	(2"-acetyl)Gal	Ara	OH	*A. paniculatum*
5	Glc	Glc(1→3)Rha	OH	*A. burnatii*
6	Glc	(6-*E*-caffeoyl)Glc(1→3)Rha	OH	*A. n. neomontanum*

*Species in which the compound was already isolated
Glc = β-D-glucopyranose, gal = β-D-galactopyranose, ara = α-L-arabinopyranose, rha = α-L-rhamnopyranose

Fig. 1. Flavonols identified in *A. angustifolium.*

winged margin; straight plant stalk with simple to much-branched inflorescence (Pignatti, 1982; Akeroyd and Charter, 1993). The check of these markers on the population from Monte Matajur (Udine, Italy) has confirmed it as *A. angustifolium* species.

2.2. Phytochemical study

The methanol crude extracts of *A. angustifolium* flowers and leaves were chromatographed on Sephadex LH-20 and RP-HPLC (C-18) to yield pure compounds **1–6** (Fig. 1).

Compound **1** was isolated as a yellow amorphous powder. Its molecular formula was established as $C_{42}H_{46}O_{22}$ by means of HRESIMS ($[M-H]^-$ peak at *m/z* 901.2458). Analysis of ^{13}C NMR spectra (600 MHz) suggested a flavonoid skeleton for **1** (Table 1). The ^1H NMR spectrum (Table 1) indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled doublets at δ 6.29 and 6.34, J = 2.0 Hz) and a 4′-hydroxylation pattern for ring B (AA'XX' system signals at δ 6.89, *d*, J = 8.0 Hz; 8.08, *d*, J = 8.0 Hz), allowing the aglycon to be recognized as kaempferol (Fico et al., 2001a). The ^1H NMR spectrum of **1** also showed signals ascribable to sugar moieties and a *p*-coumaroyl residue (Table 1). Three anomeric protons arising from the sugar moieties appeared at δ 4.53 (*d*, J = 7.5 Hz), 5.15 (*d*, J = 7.0 Hz), and 5.38 (*d*, J = 1.8 Hz) which correlated respectively with signals at δ 98.8, 105.0, and 98.8 ppm in the HSQC spectrum. All the ^1H and ^{13}C NMR signals of **1** were assigned using 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments. Complete assignments of proton and carbon chemical shifts of the sugar portion were accomplished by DQF-COSY and 1D-TOCSY experiments and allowed the identification of the sugars as one terminal β-galactopyranosyl, one α-rhamnopyranosyl, and one β-glucopyranosyl unit. The configurations of the sugar units were assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilylated, and GC retention times compared with those of authentic sugar samples prepared in the same manner. The lower field shifts of H_2-6_{glc} (δ 4.52, 4.54) of the glucosyl unit suggested the substitution site of the acyl moiety. Unequivocal information could be obtained by 2D-NMR spectra; the HMBC experiment indicated correlations between δ 5.15 (H-1_{gal}) and 135.5 (C-3), δ 5.38 (H-1_{rha}) and 164.0 (C-7), δ 4.53 (H-1_{glc}) and 83.2 (C-3_{rha}), δ 4.52, 4.54 (H_2-6_{glc}) and 168.9 (COO). Thus, the structure of **1** was determined as kaempferol 7-*O*-(6-*E*-*p*-coumaroyl)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside 3-*O*-β-D-galactopyranoside.

Four known flavonols were also isolated and identified by spectral analysis and comparison with published spectroscopic data: kaempferol 7-*O*-(6-*E*-caffeoyl)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside-3-*O*-β-D-glucopyranoside (**2**) (Fico et al., 2001b), kaempferol 7-*O*-(6-*E*-*p*-coumaroyl)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside-3-*O*-β-D-glucopyranoside (**3**) (Fico et al., 2001b), quercetin 7-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside-3-*O*-β-D-glucopyranoside (**5**) (Vitalini et al., 2010), and quercetin 7-*O*-(6-*E*-caffeoyl)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside-3-*O*-β-D-glucopyranoside (**6**) (Fico et al., 2001b). Quercetin 7-*O*-α-L-arabinopyranoside-3-*O*-(2″-acetyl)-β-D-galactopyranoside (**4**) was identified by spectral library comparison (see Section 3). Compounds **2** and **3** were isolated both from flowers and leaves, **5** and **6** from leaves only. Compound **4** was identified in the extract from flowers of *A. angustifolium*. Flavonols **2**, **3**, and **6** were identified before in *A. napellus* ssp. *neomontanum* (Wulfer) Gáyer (Fico et al., 2001b), **4** in *A. paniculatum* Lam. (Fico et al., 2000), and **5** in *A. burnatii* (Vitalini et al., 2010). Thus, the flavonoidic profile of *A. angustifolium* is similar to that of *A. napellus* ssp. *neomontanum* (Fig. 1). Moreover, compound **5**, in common with *A. burnatii*, differs in the glycosidic moiety, from **2**, **3**, and **6** only in the absence of caffeoyl and *p*-coumaroyl group.

As reported in literature, hybrids tend to produce the characteristic compounds of the parental taxa (Harborne and Turner, 1984), so the presence of compound **4** could support *A. angustifolium* as a cross between *A. paniculatum* and *A. napellus.* The discussion on the presence of *A. napellus* ssp. *neomontanum* instead of *A. napellus* ssp. *tauricum* in the parental taxa is more critical. Our