Iridoid glucosides of *Paederota bonarota* and the relationships between *Paederota* and *Veronica*.

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Abstract – In a chemical investigation of the water soluble compounds in *Paederota bonarota* five known iridoid glucosides were isolated together with a compound with an 8,9-double bond, namely bonarotoside (10-*O*-benzoyl arborescosidic acid). The known iridoid glucosides were aucubin, catalpol and the 6-*O*-esters of catalpol: verproside, catalposide, amphicoside and veronicoside, compounds that are characteristic for many species of *Veronica*. The only other species in the genus *Paederota*, namely *P. lutea* contains an iridoid glucoside analogous to bonarotoside but with an additional arabinosyl moiety present. This common chemical attribute of *Paederota* distinguishes the genus from *Veronica*; it is in good agreement with the results from recent investigations of the phylogeny based on plastid DNA sequences of *Veronica* and its closest relatives, where *Paederota* is placed as a sister-group next to *Veronica* and not included in *Veronica* as previously suggested.

Keywords: Chemotaxonomy; *Paederota bonarota*; *Veronica*; Plantaginaceae; Scrophulariaceae; Iridoid glucosides; Bonarotoside

1. Introduction

Paederota is a genus of two species from the southeastern European Alps; *P. lutea* L. f. with yellow flowers and oval-lanceolate leaves with highly serrated margins, and *P. bonarota* (L.) L. with blue-violet flowers and orbicular leaves with laxly serrated margins (Pignatti 1982). It is included in the part of the former Scrophulariaceae that has recently been moved to Plantaginaceae (APG 2003). The genus is closely related to *Veronica*, in which it at times has been included (von Wettstein, 1895). Analyses of DNA sequence data have recently helped to elucidate the relationship between *Paederota* and *Veronica*. Thus, nuclear ribosomal DNA sequences showed *Paederota* to be derived from within *Veronica* (Albach and Chase, 2001), whereas analyses of plastid DNA sequences revealed a sister-group relationship between the two genera which, based on analytical grounds, was considered as more reliable (Albach and Chase, 2004).

Chemical investigations of the genus are scanty. The first were those of Grayer-Barkmeijer (1973, 1979) who in her comprehensive paper chromatographic study of *Veronica* and its relatives also examined the two species of *Paederota*. Both species were reported to contain aucubin (**1**), catalpol (**2**) and the 6-O-catalpol esters veronicoside (**3**), catalposide (**4**) and amphicoside (**5**); in addition to these, *P. lutea* also contained verproside (**6**) as well as a so-called unidentified '*Veronica spicata* ester' (Grayer-Barkmeijer, 1973). Recently, we have reported on the isolation of the iridoid glucosides of *P. lutea* (Albach et al., 2004) and we confirmed the presence of the 6-*O*-catalpol esters typical for *Veronica*, which were already detected by Grayer-Barkmeijer. However, in addition we found the iridoid glucoside paederotoside (**8**), a derivative of arborescosidic acid. We have now investigated *P. bonarota* and report the results.

2. Materials and methods

2.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova-500 MHz or Mercury-300 MHz instruments in MeOH-d_4 using the solvent peak (δ 3.31 and 49.0, respectively) as the internal standard. LC-HR ESIMS was performed on an Agilent HP 1100 Liquid Chromatograph equipped with a BDS-C18 reversed phase column running a water-acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the positive electrospray ion mode using 5-leucineenkephalin as lock mass. The compounds isolated were identified by their NMR data by comparison with standards **(**Albach et al., 2004).

2. 2 Plant material

Material of *P. bonarota* was collected in August 2004 at 1645 m a.s.l. at Dosso Alto, Brescia (N 45°48.946' E 010°24.753') in Northern Italy and the determination confirmed using the identification key of Pignatti (1982); a voucher (IOK-3/2005) has been deposited at the herbarium of The Botanical Museum of Copenhagen (C).

2.3 Work-up

Dry flowering stems (7.5 g) was brought to boiling with EtOH (50 ml), cooled and blended, and left to stand for 7 days. After filtering, the extract was taken to dryness and partitioned in H_2O–Et_2O. Finally, the aqueous layer was concentrated to give the crude extract (480 mg). This was chromatographed on a Merck Lobar (RP-18) reverse-phase column (size B) eluting with H_2O-MeOH (25:1 to 1:1). This gave a sugar fraction (ca. 210 mg; with mainly α- and β-glucose, as seen by ^{13}C NMR in D_2O), a fraction with catalpol (**8**; 30 mg), aucubin (**1**; 30 mg), verproside (**6**; 30 mg), catalposide (**4**; 30 mg), amphicoside (**5**; 20 mg), veronicoside (**3**; 10 mg) and bonarotoside (**9**; 5 mg).

2.5 Bonarotoside (7)

$[\alpha]_D^{20}$ = - 3 ° (*c* 0.2, MeOH); LC-HR ESIMS *m/z*: 501.1383 $[M+Na]^+$ ($C_{23}H_{26}O_{11}Na$ requires 501.1373); NMR data in Table 1.

3. Results and discussion.

The water-soluble part of an EtOH extract of *P. bonarota* collected in the Italian Alps in 2004, was subjected to reversed phase column chromatography and gave glucose and a number of known iridoid glucosides, namely aucubin (**1**) and catalpol (**2**) as well as the catalpol esters **3-6** previously detected in the plant. In addition was isolated a new iridoid glucoside which we have named bonarotoside (**7**).

Compound (**7**) was isolated as a glass $[\alpha]_D^{20}$ - 3 ° with the molecular formula $C_{23}H_{26}O_{11}$, as established by HR-ESIMS. In agreement with this, the ^{13}C NMR spectrum (Table 1) showed 23 signals; of these, ten could be assigned to an iridoid aglucone very similar to that of paederotoside (**8**), and seven signals could likewise be allocated to a benzoyl group as in **8** (Albach et al., 2004). The remaining 6 signals could be assigned to a β-glucopyranosyl moiety. In fact, when compared to the reported data for **8** a convincing correspondence was seen (Table 1) when allowing for the low field shift of C-6' caused by the α-arabinopyranosyl moiety in the latter. The assignment of the ^1H NMR chemical shifts (Table 1) was partly based on DQF-COSY and HSQC spectra and was in full agreement with this analysis. Thus, the low field position of the singlet at δ 6.31 assigned to C-1 of the iridoid aglucone proved the presence of an 8,9-double bond (Jensen et al., 1996). The C-3 peak, however, was found at an unusually high field (δ 7.24) when compared to the usual value for an iridoid (i.e. δ 7.5-7.6), but this is a known effect for iridoid acids, which are partly ionised (von Poser et al., 1998; Iavarone et al., 1983). The low field position of the signals assigned to the C-10

methylene group, an AB system centred at δ 5.01, indicated that this was the position of the benzoyloxy group, and a correlation between CO (δ 167.5) of the benzoyl group and the H-10 signals confirmed this.

Aucubin (**1**) and catalpol (**2**) together with the 6-*O*-catalpol esters **3**-**6** have been found to be present in most species of *Veronica* investigated and is a chemical marker for the genus (Grayer-Barkmeijer, 1973; 1979; Taskova et al., 2002; Jensen et al., 2005). The finding of the carbocyclic iridoid glucoside with an 8,9-double bond in both species of *Paederota* is also taxonomically interesting. Compounds with this structural feature have a very limited distribution: they have been reported previously from *Veronica*, namely *V. anagallis-aquatica* L. (Lahloub, 1992), *V. cymbalaria* Bod. (Taskova et al., 1999), and *V. polita* Fries (Afiffi et al., 2001) as well as from *Veronica* section *Hebe*, from *Wulfenia, Erinus* and *Globularia* (Taskova et al., 2005; 2006, and references cited). Finally, compounds of this type appear to be present in about half of the fifty *Plantago* species investigated for iridoid glucosides (Rønsted et al., 2003). All these taxa are members of the new Plantaginaceae (Veronicaceae *sensu* Olmstead et al. 2001), which has recently been separated from Scrophulariaceae s. str., based on DNA gene sequencing results (Olmstead et al. 2001; Albach et al., 2005; Oxelman et al. 2005). With this distribution pattern, iridoid glucosides with an 8,9-double bond are therefore potential taxonomic markers although they apparently only occur sporadically within each genus.

The present finding in *P. bonarota* of bonarotoside (**7**), a benzoyl ester of arborescosidic acid similar to paederotoside (**8**) previously known from *P. lutea* provides a good chemical character to distinguish the genus *Paederota* from *Veronica*. As noted in the introduction the two genera are closely related and this is also seen for the chemical markers where the characteristic 6-*O*-catalpol

esters are present in most species of *Veronica*. The finding of glucose as the main free carbohydrate in *P. bonarota* may also be significant. *P. lutea* contained approximately 25 % mannitol (Albach et al., 2004) as opposed to most species of *Veronica* which have mannitol as the main carbohydrate present (Jensen et al., 2005; Taskova et al., 2006).

Acknowledgements

We thank Kristian Fog Nielsen, BioCentrum, The Technical University of Denmark, for recording the mass spectrum, and Roberta M. Ceriani, The Native Flora Centre (Galbiate, Lecco, Italy), for confirming our species identification.

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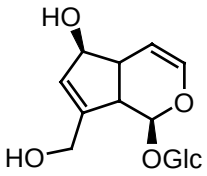
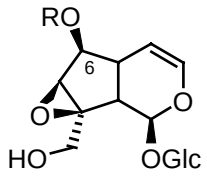
Table 1. NMR data (CD_3OD; 500 MHz) for bonarotoside (**7**) and model compound **8**.

Atom	Bonarotoside (**7**) [a]			Paederotoside [b] (**8**)
	^1H	^{13}C	HMBC	^{13}C
Aglucon				
1	6.31 (*s*)	91.5	3, 5, 8, (9), 1'	91.6
3	7.24 (*br. s*)	149.2	1, 4, 5, 11	149.4
4		117.2		115.6
5	3.64 (*m*)	39.8		39.8
6	2.63 (*m*), 1.50 (*m*)	32.1	(8)	32.2
7	2.59 (2H, *m*)	35.2	9	35.2
8		136.6		136.8
9		136.2		135.8
10	4.95 (*d d*, 13.2, 2.0)	61.4	8, (CO)	61.6
	5.08 (*d*, 13.2)		7, (8), 9, CO	
11		173.1		167.9
Glc				
1'	4.70 (*d*, 7.9)	99.8	1	100.1
2'	3.17 (*dd*, 8.1, 8.9)	74.7	1', 3'	74.7
3'	3.37 (*t*, 8.9)	78.0	2', 4'	77.9
4'	3.3 obsc.	71.5	3'	71.7
5'	3.3 obsc.	78.3		77.2
6'	3.66 (*dd*, 11.9, 5.4)	62.7		69.5
	3.86 (*dd*, 11.9, 1.9)			
Ara				
1"				105.5
2"				72.5
3"				74.1
4"				69.9
5"				66.7
Benzoyl				
1"'		131.4		131.3
2"'/6"'	8.01 (2H, *d*, 7,1)	130.6	4"', CO	130.7
3"'/5"'	7.48 (2H, *t*, 7.8)	129.7	1"'	129.7
4"'	7.60 (*t*, 7.5)	134.4	2"', 6"'	134.4
CO		168.0		165.6

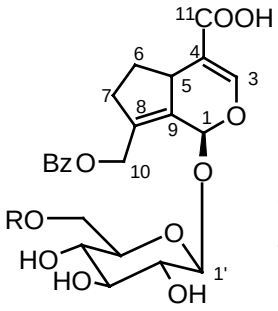
[a]) Signals were assigned using DQF-COSY and HSQC spectra. [b]) Data from Albach et al. (2004).



1



2 R = H
3 R = Benzoyl
4 R = 4-OH-Benzoyl
5 R = Vanilloyl
6 R = 3,4-di-OH-Benzoyl



7 R = H
8 R = α-Ara-p