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Iridoids in Hydrangeaceae

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Abstract

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The content of glycosides in *Kirengeshoma palmata* and *Jamesia americana* (Hydrangeaceae) have been investigated. The former contains loganin and secoiridoids, including the alkaloid demethylalangiside. The latter contains no iridoids, but the known glucosides arbutin, picein and prunasin. In order to further investigate the chemotaxonomy of the family Hydrangeaceae, the distribution of the iridoid and secoiridoid glucosides as well as the known biosynthetic pathways to these compounds have been reviewed. However, only a few genera of the family has been investigated. Loganin, secologanin, and derivatives of these are common. The genus *Deutzia* is characteristic in containing more structurally simple iridoids in which C-10 has been lost during biosynthesis. Such compounds have so far only been reported from the genus *Mentzelia* (Loasaceae). The taxonomic relationships between Hydrangeaceae and the closely related Cornaceae and Loasaceae is discussed and found to agree well with recent DNA sequence results.

**Keywords:**

*Kirengeshoma*

*Jamesia*

Hydrangeaceae

Iridoid glycosides

Chemotaxonomy
1. Introduction

de Jussieu (1789) originally placed Hydrangeaceae in Saxifragaceae and this association was followed by most taxonomists (Bentham and Hooker, 1862-83; Engler, 1928; Takhtajian, 1969; 1980; Cronquist, 1988). Dahlgren (1975; 1980; 1989) put a great weight on embryology was impressed by the correlation with the occurrence of iridoid compounds, and he was one of the first to place the family in his Cornales. Thorne (1992) and Hufford (1992) followed this concept, and with the advent of DNA sequencing techniques (Downie and Palmer, 1992; Hufford et al., 2001; Albach et al., 2001) Loasaceae was also placed in Cornales. The relationships between the genera of Hydrangeaceae has primarily been analyzed by Hufford (1997; Hufford et al., 2001). The DNA sequencing phylogenetic relationships are depicted in Fig. 1.

Only the genera Hydrangea and Deutzia appears to have been much investigated chemically, probably due the common use of these two genera as garden ornaments. The family consists of 17 genera in the two subfamilies (Hufford et al., 2001): Hydrangeoideae, include tribe Hydrangeeae (Hydrangea, Platycrater, Decumaria, Pileostegia, Schizophragma, Dichroa, Broussaisia, Deinanthe, Cardiandra) and tribes Philadelphae (Philadelphus, Carpenteria, Deutzia, Kirengeshoma, Whipplea, Fendlerella), and Jamesioideae (Fendlera, Jamesia). In order to extend these studies we have investigated a member of each of the last two tribes, namely Kirengeshoma palmata Yatabe and Jamesia americana Torr. & A. Gray.

2. Materials and Methods

2.1 Plant material

Fresh material of Kirengeshoma palmata from two different plants (IOK 8-1999 and IOK 1-2010) and Jamesia americana (IOK 13-2003) was provided by the staff of the Botanical Garden of The University of Copenhagen. The vouchers were authenticated by one of us (HQL) and deposited in the herbarium of East China Normal University (HSNU).

2.2. General

$^{1}H$, $^{13}C$ spectra were recorded Varian Mercury-300 MHz or a Varian Unity plus 500 MHz instrument in D$_2$O or CD$_3$OD using the solvent peak as the internal standard. The isolated compounds were identified by their $^{1}H$ and $^{13}C$ NMR spectra by comparison with spectra of known standards. Preparative HPLC was performed on a Merck Lobar RP-18 column size B or C.

2.3. Extraction and isolation

2.3.1 Kirengeshoma palmata

a) Fresh, aerial parts collected in June (65 g) were blended with EtOH (300 ml), filtered and taken to dryness. Partitioning in H$_2$O-Et$_2$O gave from the aq. phase after drying 2.5 g which was chromatographed on a C column to give in order of elution: chlorogenic acid (5, 250mg); salidroside (80 mg); 6-$\beta$-hydroxysweroside (3, 22 mg); sweroside (2, 740 mg); loganin (1, 80 mg).

b) Fresh, frostbitten aerial parts collected in October (58 g) were cut into small pieces, blended with EtOH (250 ml), boiled for 10 min and left to extract overnight. The extract was filtered, dried (3.05 g) and partitioned in H$_2$O-Et$_2$O (2:5). The aq. phase
was collected, dried (1.70g) and subsequently subjected to preparative chromatography. The initial solvent used was H₂O, then mixtures of H₂O-MeOH of decreasing polarity, and finally MeOH. The following compounds were obtained in order of elution: chlorogenic acid (5, 120mg); 6-β-hydroxysweroside (3, 20 mg); sweroside (2, 70 mg); loganin (1, 80 mg) and fraction A (50 mg) was obtained.

Fraction A was further purified using the solvent mixture (2:1) to yield demethylalangiside (4, 10 mg).

2.3.2 Jamesia americana

Frozen aerial parts (23 g) were blended with EtOH (150 ml). After filtering, the extract was taken to dryness and partitioned in H₂O-Et₂O. The concentrated aq. phase (1.50 g) was chromatographed as above to give: sugars (mainly glucose; 310 mg); arbutin (6; 30 mg); picein (7; 35 mg) and prunasin (8; 150 mg).

2.3.3 Perspective and outline

In order to extend the results obtained from the two plants we have undertaken to make a review of the iridoids reported from the Hydrangeaeceae including the reported data on the biosynthesis of the compounds found in the family. Furthermore, we have discussed the distribution of relevant compounds in relation to the phylogenetic results shown in the cladogram (Fig. 1).

3. Results and Discussion

The aqueous extract of the aerial parts of K. palmata after chromatography on preparative reverse phase gave loganin (1) and three secoiridoids, namely sweroside (2), 6-β-hydroxysweroside (3) (Rodríguez et al., 2002) and demethylalangiside (4) (Itoh et al., 2001) as well as chlorogenic acid (5).

J. americana has been investigated by Plouvier (1959) who discovered the presence of a cyanogenic glycoside. In the present work, we have isolated the compounds Arbutin (6), picein (7) (Johansen et al., 2007) and prunasin (8) (Hübel et al., 1981), the latter is most likely the cyanogenic compound detected by Plouvier. No iridoids were found.

The compounds isolated were identified by comparison with spectra of authentic compounds or published data.
3.1 Biosynthesis

3.1.1 Secoiridoids

The iridoids are of terpenoid origin and their biosynthesis (Fig. 2) has been well investigated (Inouye et al., 1977a, Inouye and Uesato, 1986; Jensen, 1991, 1992), although new information about the early steps have recently been discovered (Salim et al., 2014; Miettinen et al., 2014).

Fig. 2. Biosynthesis of secologanin.

Thus, it has been shown that these compounds are not of mevalonoid origin as first believed, but that they arise by the recently discovered triose phosphate/pyruvate pathway via 1-deoxy-D-xylulose in Mentha (Eisenreich et al., 1997) and in Swertia (Wang et al., 2001) in the case of geraniol. Using cell-free extracts from Lonicera japonica Thunb. and Hydrangea macrophylla (Thunb.) Ser., it has furthermore been shown that the initial glucosylation in the pathway take place in the formation of deoxyloganic acid (11) (Yamamoto et al., 2002). Secologanin (14) has also been shown to derive from the triose phosphate/pyruvate pathway in Catharanthus roseus (L.) G. Don cell-cultures (Contin et al., 1998; Miettinen et al., 2014).

3.1.2 Ipecac alkaloids

A group of tetrahydroisoquinoline alkaloids of limited distribution are derived from secologanin (14). This has been shown in Cephaelis ipecacuanha (Brot.) A. Rich.
(Rubiaceae) by Battersby and Gregory (1968) where labelled loganin (1) was incorporated in ipecoside (15) which is likely to be the precursor of the ipecac alkaloids.

![Biosynthesis of ipecac alkaloids](image1)

3.1.3 *Deutzia* iridoids

The iridoid glucosides found in *Deutzia* species are peculiar since they lack the C-10 carbon atom, a feature rarely seen in the compounds from other plants. The biosynthesis (Inouye et al., 1977b, Uesato et al, 1986) is different from that above (Fig. 4) since glucosylation apparently takes place earlier, namely at the iridodial stage. Thus, it has been shown by feeding deuterium labelled precursors (Frederiksen et al., 1999) to proceed from iridodial glucoside (9a) via decapetaloside (16) to the compounds present in the plants, deutzioside (18) and scabroside (19). The compound loasaside (17) has not been tested as an intermediate, but seems the logical step prior to 18 and 19.

![Biosynthesis of Deutzia compounds](image2)

3.2 Iridoids and some other glycosides in Hydrangeaceae

3.2.1 *Hydrangeae*; Hydrangea

Loganin (1) was first reported (Plouvier, 1964) from three species of *Hydrangea*, namely *H. aspera* D. Don, *H. bretschneideri* Dippel and *H. xanthoneura* Diels. This compound has later been reported from *H. hortensis* Sm. (Khalifa et al., 2001) and *H. macrophylla* (Yamamoto et al, 2002). Recently, the β-glucopyranosyl ester of deoxyloganic acid (11), namely 11a, was isolated from *H. macrophylla* subsp. *serrata* (Thunb.) Makino (Kikuchi et al., 2008a).
Also, a number of aromatic esters of loganic acid (12) as well as loganin (1) and three diglucosides of this has been reported from *H. paniculata* Sieb. (Shi et al., 2012). Examples are (12a) and (1a).

Loganin (1), secologanic acid (13), secologanin (14) and sweroside (2) as well as four derivatives of (14) named hydrangeosides A-D (20-23) were found in *H. macrophylla* var. *macrophylla* (Inouye et al., 1980; Uesato et al., 1981).

A year later, three similar compounds, namely hydrangeosides E-F (24-26) were reported from *H. scandens* (L. f.) Ser. (Uesato et al., 1982). In addition, in a final paper (Uesato et al., 1984) the absolute structures of the seven compounds (20-26) were elucidated. Hydrangeoside A (20) has also been reported from *H. chinensis* Maxim. (Patnam et al., 2001), *H. hortensis* (Khalifa et al., 2001) together with the dimethyl acetal of secologanin (14), a typical artifact formed from 14 when extracting the plant material with methanol.

More recently, another variety of the plant, namely *H. macrophylla* var. *thunbergii* Makino was investigated (Yoshikawa et al., 1994; Matsuda et al., 1999) to give the somewhat different lactones hydramacroside A (27) and B (28). This study was later
extended by an investigation of the flowers of the same variety and gave hydrangeoside A (20) as well as 27 and 28, and in addition the two alkaloids derived from 28, namely the two epimers hydrangeamine A (29) and B (30) (Liu et al., 2013).

From a third taxon, *H. macrophylla* subsp. *serrata* was isolated hydrangeoside A (20) and C (22) as well as the dimethyl acetal of 20 and a number of methyl and/or *n*-butyl acetal derived from secologanin (14), secologanic acid (13) or morroniside (31) (Sakai et al., 2007; Kanno et al., 2007). Such acetals are usually artifacts formed with the solvents used during work-up. Further work with this plant provided the four macrophyllanosides A-D (32-35), cyclic acetals of 14 with glycerol D-galactopyranoside, myo-inositol and shikimic acid (Kikuchi et al., 2008b).

An additional acetal of secologanin, this time with the cyanogenic glucoside taxiphyllin, namely hydracyanoside D (36), was later reported from the parent *H. macrophylla* (Wang et al., 2010) together with hydracyanoside F (37), secoxyloganin (38) and 8-epiloganin (39).
Finally, two esters of demethylsecologanol were isolated from *H. paniculata* (Shi et al., 2014), namely hydrangeside B (40a) and methylgrandifloroside (40b).

3.2.2 Hydrangeae; Others

*Platycrater, Decumaria, Pileostegia, Broussaisia, Deinanthe, Cardiandra* appears not to have been properly investigated; of these, *Decumaria* and *Broussaisia* have only been examined for flavonoids (Bohm et al., 1985). *Dichroa febrifuga* Lour., on the other hand, is much used in Chinese medicine and has been well investigated. However, no iridoids have been reported.

3.2.3 Philadelphæae; *Deutzia*

The compound scabroside (19) was the first iridoid glucoside isolated from *D. scabra* Thunb. (Esposito and Guiso, 1973), quickly followed by deutzioside (18) (Bonadies et al., 1974) and deutziol (41) (Esposito et al., 1976). Later, work was continued on this plant and gave rise to scabrosidol (42) (Esposito et al., 1983) and the two aglucones deutziogenin (18a) and scabrogenin (19a) (Esposito et al., 1984). The compounds 18 and 19 have also been isolated from *D. crenata* Sieb. et Succ. (Inouye et al., 1977) and *D. schneideriana* Rehder (Frederiksen et al., 1999).

3.2.4 Philadelphæae; *Kirengeshoma*

In the present work we found loganin (1), sweroside (2), 6-β-hydroxysweroside (3) and demethylalangiside (4).
3.2.5 Philadelphaceae; Others

The genera *Philadelphus, Carpenteria, Whipplea, Fendlerella* have all been investigated for their flavonoid content (Bohm et al, 1985), but apparently not for iridoids.

3.2.6 Jamesioideae; Jamesia

In the present work, we have isolated the compounds Arbutin (6), picein (7) and prunasin (8), and no iridoids were present.

3.2.7 Jamesioideae; Fendlera

Apparently, no chemical work has been done on this genus.

3.3 Chemosystematics

The phylogenetic relationships based on the published DNA sequencing results are shown in Fig. 1. From the above information, it is difficult to deduce much about the chemical relationships within the Hydrangeaceae, since only six of the seventeen genera appears to have been investigated properly for their content of iridoid glycosides. However, some useful taxonomic information about the interfamilial relationships can be drawn from the published chemical data. Thus, the iridoids in *Deutzia* are closely related to those found in *Mentzelia* (Loasaceae) (Jensen et al., 1981; El-Naggar et al., 1982) and they share a peculiar biosynthetic route (Frederiksen et al., 1999) in formation of the 10-decarboxylated compounds (Fig. 3) so far solely found in these two genera. In addition, the common occurrence of the iridoid glucoside 6-β-hydroxywseroside (3) in *Kirengeshoma* and in *Gronovia scandens* L. (Loasaceae) (Rodríguez et al., 2002) may be indicative, since 3 is a compound that has never been reported from other taxa, and furthermore, hydroxylation of the 6-position of secoiridoids appears to be almost unknown otherwise. Taken together, this indicate a common ancestry of the two families Hydrangeaceae and Loasaceae, as it is also found in the DNA sequence investigations (Fig. 1). On the other hand, many of the simple iridoid glucosides shown in Fig. 2, namely compounds 1, 11-14 and also 31 and 38 are shared between *Hydrangea* and Loasaceae, but these compounds are common in most other families in Dipsacales and Gentianales.

Hydrangeaceae also share many of the simple compounds above with Cornaceae, however, the occurrence of the rare esters of loganin (1a and 12a) in *Hydrangea paniculata* are particularly interesting since these same compounds are also reported from Cornaceae, namely 1a from *Alangium planatifolium* (Sieb. & Zucc.) Harms (Otsuka et al., 1996) and 5a from *A. lamarckii* Thwaites (Itoh et al., 2001). Similarly, the finding of demethylalangiside (4) in *Kirengeshoma* is paralleled by the occurrence of this and many similar ipecac alkaloids in *Alangium* (Itoh et al., 2001). Otherwise, compounds from this biosynthetic pathway (Fig. 3) is solely found in a few genera of Rubiaceae.
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Fig. 1. Cladogram of Hydrangeaceae based on maximum parsimony analyses of the combined matK and rbcL data set. Sequences are downloaded from GenBank. Numbers above branches are bootstrap values.