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Profiles of alkylresorcinols in *Iris* plants

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1. Subject and source

The Iridaceae family is comprised of a large number of flowering plants. Several species of this family have been used as folk medicines. Major phytochemical studies of *Iris* genus were focused on flavonoids (Wang et al., 2010; Baser et al., 2011), but only a limited number of studies have been performed targeting other metabolites. The present study aims to compare the resorcinolic lipid profiles in the *Iris* genus. Eight species of *Iris* were cultivated in a greenhouse at Konkuk University, Seoul Korea. Voucher specimens (Table 1) have been deposited at the Department of Bioresources and Food Technology, College of Life and Environmental Sciences, Konkuk University, Seoul, Korea.

2. Previous work

Numerous secondary metabolites were discovered in previous chemical investigations from *Iris* plants, including terpenes and organic acids (Baser et al., 2011), flavonoids (Wang et al., 2010; Kukula-Koch et al., 2014), anthocyanins (Kitahara et al., 2014), and xanthones (Wei et al., 2012). The most comprehensive studies were performed with polymethoxyflavones and isoflavones in *Iris domestica* (syn. *Belamcanda chinensis*), a well-known Chinese medicine (Woo and Woo, 1993; Zhang et al., 2011). Profiling of resorcinolic lipids have been performed mainly with *Iris* seeds (Kato et al., 1990; Fukuyama et al., 1991; Seki et al., 1995; Kukula-Koch et al., 2014). Similar studies with leaves or roots have never been reported. Moreover, only a limited number of reports are available regarding the chemo-taxonomy of the species (Marner and Horper, 1992; Wang et al., 2010), while molecular phylogenetic analyses of *Iris* have already been provided by many research groups (Wilson, 2004; Lee and Park, 2013).

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Table 1
Iris plants and voucher specimen numbers in this study.

Scientific name	Voucher specimen#	Tissues	Amount[b]
Iris domestica (*Belamcanda chinensis*)[a]	KUBF-NP-IR-0001	Leaf	17.2 ± 5.3
Iris ensata	KUBF-NP-IR-0002	Leaf, root	30.7 ± 10.4/8.2 ± 4.7
Iris germanica	KUBF-NP-IR-0003	Leaf	25.3 ± 7.1
Iris japonica	KUBF-NP-IR-0004	Leaf	34.3 ± 13.6
Iris odaesanensis	KUBF-NP-IR-0005	Leaf, root	5.1 ± 2.0/3.1 ± 1.4
Iris pseudoacorus	KUBF-NP-IR-0006	Leaf	5.0 ± 3.7
Iris sanguinea	KUBF-NP-IR-0007	Leaf	4.2 ± 1.8
Iris setosa	KUBF-NP-IR-0008	Leaf, root	8.1 ± 3.3/3.2 ± 2.3

[a] Synonym for *I. domestica*.
[b] Amount of fresh leaf and root (g). In general, 3–4 plants were used.

3. Present study

In this study, 5-alkylresorcinol (AR) profiles from eight species of *Iris* were determined from leaf and root tissues. The samples were cut into small pieces, mixed with 50 volume of methanol (150–1500 mL), and finely macerated with a Waring blendor. After filtration, the solvent was removed under reduced pressure, leaving an oily residue (1.3–28.7 g, depending on species and tissues). Aliquots of the residues (1–5 g) were suspended in water (300 mL) and sequentially extracted with *n*-hexane (300 mL*1), ethyl acetate (300 mL*3), and *n*-butanol (300 mL). The residues of the ethyl acetate extracts were further purified by silica gel column chromatography with a mixture of dichloromethane and methanol (Fraction 1–6). Based on the results of a preliminary study with ARs from rye seeds (*Secale cereale*), resorcinolic lipids were eluted from fraction 5 (Fr5, 5% MeOH/dichloromethane). The residues in Fr5 were converted to trimethylsilyl (TMS) derivatives and analyzed with gas chromatography-mass spectrometry (GC–MS). GC–MS conditions are described in Appendix S2.

For quantitative analysis, three synthetic ARs with 5-pentadecyl-, 5-heptadecyl-, or 5-nonadecyl groups were prepared (Appendix S1, Fig. S1). Concentrations of ARs were determined from the standard curves of these-ARs. Briefly, standard solutions of synthetic-AR were prepared (1, 5, 10, 50, 100 mg/L, ethyl acetate). Aliquot amount of the solution (100 μL) was derivatized with N,N-bistrimethylsilyl trifluoroacetamide–trimethyl chlorosilane (BSTFA–TMCS, 100 μL) in pyridine (800 μL). The reaction mixture (2 μL) was injected to GC–MS. The standard curves were calculated from the peak area of the AR TMS derivatives. The concentrations of the above ARs in plant samples were determined from the standard curves, while the amounts of other ARs were estimated from the standard curve of 5-nonadecyl-AR derivative.

From MS analysis of the synthetic ARs, several characteristic ions were identified (Fig. S2). For example, 3,5-bis-trimethylsilyloxybenzyl ion (*m*/*z* 268) appeared as the base peak in all ARs, while the consecutive removal of methyl ($M^+ - 15$), followed by a methylene group (−14 for each unit) indicated that the substituent at the 5th ring-carbon is a straight-chained alkyl group.

Approximately seventeen 5-alkylresorcinols (ARs) were identified from the leaves and roots of the *Iris* plants studied (Figs. S3–S5 and Table 2).

In general, alkyl chain-lengths of ARs were between 15 and 31. ARs with even-numbered alkyl groups were found in trace quantities, while those with odd-numbered alkyl analogues were observed in higher quantities. Overall concentrations of ARs in leaves varied depending on the species. For example, *I. domestica* and *I. germanica* contained trace amounts of ARs, while large amounts were observed in *I. odaesanensis* and *I. setosa*. One of the most notable differences between *I. odaesanensis* and other species was the presence of **15**, **16** and **17** (Table 1). In *I. odaesanensis*, **15** was one of the most abundant metabolites but it was either absent (*I. domestica*, *I. ensata*, *I. germanica*, *I. japonica*, and *I. sanguinea*) or found in trace quantities (*I. pseudoacorus* and *I. setosa*) in the other species. The mass spectra indicated that these three metabolites (**15**–**17**) were closely related to synthetic ARs, though their molecular weights were different. They were identified as nonacosyl- (**15**), triacontyl-(**16**), and hentriacontyl-ARs (**17**) (Figs. S3 and S5). The leaf AR profiles of *I. setosa* were also largely different from other species. For example, heptadecyl analogue was the major AR in this species, while heneicoyl-AR was the dominant metabolite in *I. domestica*, *I. ensata*, *I. germanica*, and *I. sanguinea*. The AR concentrations in the roots were determined in three species (*I. ensata*, *I. odaesanensis*, and *I. setosa*). The amounts of ARs in the roots were far less than in the leaves (Fig. S4).

4. Chemotaxonomic significance

The present study reports for the first time a comparative analysis of leaf ARs in eight *Iris*. The taxanomic significance of these metabolites is discussed. In addition, three ARs with long chain substituents were isolated.

ARs are widely distributed in plants and microorganisms (Kozubek and Tyman, 1999). Simple 5-alkylresorcinols without additional substituents (simple ARs) are the most common resorcinolic lipids in most plants. In general, simple ARs with saturated alkyl groups are more abundant than unsaturated analogues (Kozubek and Tyman, 1999). *Iris* leaves contain only ARs with saturated alkyl chains. The alkyl chain length of ARs is in the range of 15–27 in Gramineae and other plant taxa (Kozubek and Tyman, 1999), while some *Iris* species contain ARs with higher molecular weights. Metabolites **15**–**17** in *Iris*

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