Flavonoid Pattern in Pteridaceae, I Flavonoid Glycosides Obtained from the Fronds of *Adiantum aethiopicum* and *A. monochlamys**

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Abstract

Flavonoid glycosides contained in *Adiantum aethiopicum* and *A. monochlamys* which resemble morphologically each other are compared. In *A. aethiopicum*, flavonoids detected are prunin (naringenin 7-glucoside) and naringin (naringenin 7-rhamnoglucoside) as flavanone glycosides, and astragalin (kaempferol 3-glucoside), isoquercitrin (quercetin 3-glucoside) and an unidentified kaempferol glycoside as flavonol glycosides. *A. monochlamys* contains prunin, astragalin, trifolin (kaempferol 3-galactoside), isoquercitrin, hyperin (quercetin 3-galactoside) and unidentified kaempferol and quercetin glycosides. As regards flavonol glycosides, the components of *A. monochlamys* are additive to those of *A. aethiopicum*, and the sugars attached to the 3-position of the main flavonol glycosides contained in both species are different from each other.

Introduction

One of the present authors¹⁾ reported previously that each of 6 subgenera belonging to *Prunus* had a specific flavonoid pattern in its wood. Flavonoid compounds in *Pinus* wood were investigated in detail by Erdtman *et al.*²⁾, who found that flavonoid pattern in subgenera Haploxylon and Diploxylon differed from each other. According to McClure and Alston³⁾, the flavonoid association of each species in Lemnaceae was found to be unique in the family with the exception of two species. Also, some species of *Baptisia* have been analysed, and they are divided into three types according to their flavonoid patterns⁴⁾. Concerning flavonoid glycosides in Ranunculaceae, Egger and Keil⁵⁾ reported that the glycoside types in corresponding organs have some bearing on the taxonomical groups. Therefore, it is quite likely that flavonoids play some significant role in chemical taxonomy of plants.

The surveys on flavonoid components in ferns were carried out by Harada and Saiki⁶⁾ and also by Harborne⁷⁾. Harada and Saiki described a wider distribution of kaempferol, quercetin, apigenin and luteolin in ferns. Harborne studied especially on the distribution of anthocyanidin derivatives in 6 genera. In spite of the efforts of many investigators we have only a short knowledge on the distribution of flavonoid glycosides in ferns at present.

Considering taxonomic situation of both species, flavonoid glycosides in the fern species, *A. aethiopicum* and *A. monochlamys* were comparatively studied in this work.

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Experiments and Results

Adiantum monochlamys was collected at Mt. Ōyama, Kanagawa Prefecture in August, and 80 g fresh fronds were used. *A. aethiopicum* was collected in November in the suburb of Melbourne, Australia, and 320 g dried fronds were treated. Fronds were extracted with boiling 80% ethanol (ca. 6 times the weight of fronds) until green color was thoroughly removed. The combined ethanolic extract was evaporated under reduced pressure and the residue was extracted with hot water, whereby an insoluble matter was removed by filtration. The filtrate was shaken with ether and then thoroughly with ethyl acetate in a separatory funnel. The ethyl acetate fraction was concentrated in a rotary evaporator and the residue was dissolved in a small amount of ethanol. The ethanolic solution was applied to silica gel chromatography. In the case of *A. monochlamys*, hyperin was precipitated from this ethanolic solution on standing for a few days. After removal of hyperin by filtration, the mother liquor was subjected to silica gel column chromatography. The elution of the column was effected by a mixture of benzene and ethyl acetate with increasing ethyl acetate ratio.

The eluate was fractionated into 14 to 16 fractions by checking the fluorescence under UV light of long wave-length. Each fraction was evaporated to dryness. During the course of evaporation, the fractions, No. 7, No. 10 and No. 11, which were obtained from *A. aethiopicum*, gave rise to the crystallization of astragalin (238 mg), isoquercitrin (16 mg) and naringin (276 mg), respectively. All the flavonoids in each fraction were detected by paper chromatography using *n*-butanol/acetic acid/water (6:1:2, v/v) (BAW) and 6% acetic acid (6% AA) as developing solvents. Each flavonoid detected was then separated by large scale paper chromatography with BAW and then with 6% AA. The purity of individual flavonoids was tested again with the solvent systems, BAW, 6% AA and 70% phenol. Thin layer chromatography (TLC) using silica gel G (Merck) was applied, if necessary, using the solvent system of ethyl acetate/chloroform/formic acid/water (19:1:1:1, v/v). The glycosides isolated by paper chromatography were hydrolyzed with 5% hydrochloric acid, and the resulted aglycones and sugars were determined by comparison with the authentic specimens on the chromatograms, using BAW, 70% phenol, 6% AA and also the solvent systems, *m*-cresol/acetic acid/water (25:1:24, v/v) (*m*-cresol) and *n*-butanol/pyridine/water (6:4:3, v/v) (pyridine). The R_f values of the glycosides isolated and the aglycones and sugars obtained therefrom were shown in Table 1 and 2.

Astragalin was recrystallized from dilute ethanol into yellow crystals of m. p. 208–210°. Upon hydrolysis with 5% hydrochloric acid, kaempferol and glucose were formed. The UV spectrum of the glycoside showed the absorption maxima at 268 m μ and 354 m μ (lit. 265 m μ and 354 m μ)⁸⁾. On admixture with the authentic astragalin, no depression of melting point was observed. The IR spectrum was identical with that of the authentic specimen.

Naringin was recrystallized from dilute ethanol giving colorless needles of m. p. 169–173°. After hydrolysis with 5% hydrochloric acid, it gave naringenin, glucose and rhamnose, which were clearly detected on the paper chromatograms. The UV and IR spectra and the mixed melting point test proved the identity with the authentic sample.

Hyperin was recrystallized from dilute ethanol into yellow crystals of m. p. 225–227°. This was identified as authentic hyperin by the mixed melting point test, UV

Table 1. R_f values of flavonoid glycosides.

Plant materials	Flavonoids	R_f values $\times 100$ in			TLC
		BAW	6% AA	70% Phenol	
<i>Adiantum aethiopicum</i>	Prunin	69(68)*	54(53)	81(81)	—
	Astragalin	66(66)	25(25)	81(81)	—
	Isoquercitrin	56(56)	20(21)	53(53)	—
	Naringin	52(52)	20(21)	76(76)	—
	Kaempferol glycoside	61	48	67	—
<i>Adiantum monochlamys</i>	Prunin	63(63)	52(52)	79(79)	—
	Astragalin	64(64)	28(29)	73(73)	32(32)
	Trifolin	67(67)	22(21)	71(70)	26(26)
	Isoquercitrin	53(52)	18(19)	59(57)	—
	Hyperin	50(50)	19(18)	63(62)	18(18)
	Kaempferol glycoside	69	55	67	—
	Quercetin glycoside	50	42	63	—

* Figures in parentheses indicate the R_f values of authentic samples.

Table 2. R_f values of aglycones and sugars.

Aglycones	BAW	R_f values $\times 100$ in		
		70% Phenol	6% AA	m-Cresol
Naringenin	92(92)*	95(95)	26(26)	—
Kaempferol	84(84)	59(59)	—	46(44)
Quercetin	60(60)	37(37)	—	—
Sugars	BAW	70% Phenol	Pyridine	
Glucose	11(11)	36(36)	33(33)	
Galactose	—	40(40)	29(29)	
Rhamnose	29(29)	49(49)	—	

* Figures in parentheses indicate the R_f values of authentic samples.

and IR spectra. The aglycone and sugar obtained by hydrolysis with 5% hydrochloric acid were determined paperchromatographically as quercetin and galactose, respectively.

Discussion

A. monochlamys and *A. aethiopicum* resemble closely in most of their morphological characters, therefore Christ⁹⁾ classified them into the same group by the characters of pinnate compound leaves and small and wedge-shaped leaflets. Here, we have made a comparative study on flavonoid glycosides contained in both species.

The substances in etherial fraction were not examined in this study, since no indication of specific chemotaxonomical significance was obtained with this fraction (unpublished data). The glycoside patterns in both species are different, especially in the patterns of flavonol glycosides. Astragalin (kaempferol 3-glucoside) is dominant in *A. aethiopicum*. Trifolin (kaempferol 3-galactoside) and hyperin (quercetin 3-gala-

ctoside) are characteristic in *A. monochlamys*. Prunin and isoquercitrin are common to both species. Besides, a trace of naringin was found in *A. monochlamys* in another experiment, so that it seems that the glycoside constitution of *A. monochlamys* might be additive to that of *A. aethiopicum*. This fact reminds us of the previous findings that a hybrid between pear and apple¹⁰⁾ and also a hybrid of *Baptisia*⁴⁾ had a chemical pattern which was additive for the two parents.

The sugar residue attached to the 3-position of astragalin is different from that of trifolin, in which galactose is attached to the 3-position of kaempferol. It seems to be a dominant character in *A. monochlamys* that galactose is linked to the 3-position of kaempferol and quercetin. The isolation of prunin, naringin, trifolin and hyperin from ferns has not been reported in the previous literatures.

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摘 要

長谷川正男*・赤堀洋子*：イノモトソウ科におけるフラボノイドのパターン I.

Adiantum aethiopicum およびハコネシダの葉のフラボノイド配糖体

形態の似ている *Adiantum* 属 2 種, *A. aethiopicum* と *A. monochlamys* (ハコネシダ) のフラボノイド配糖体を同定し, その比較を行った. *A. aethiopicum* からはプルニン, ナリンギン, アストラガリン, イソクエルチトリンおよび未同定のケンペロール配糖体, ハコネシダからはプルニン, アストラガリン, トリホリン, イソクエルチトリン, ヒペリンと未同定のケンペロールおよびクエルナチンの配糖体を検出した. 2 種類の植物のちがいは特に結合糖に現われ, *A. aethiopicum* ではブドウ糖, ハコネシダでは主にガラクトースであり, ブドウ糖もみられた. ハコネシダのフラボノイドは *A. aethiopicum* のフラボノイドにさらにガラクトースの配糖体加わった型のものであった. (*東京都立大学理学部生物学教室)