Flavonoid Pattern in the Pteridaceae II.

Flavonoid Constituents in the Fronds of *Adiantum capillus-veneris* and *A. cuneatum*

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Abstract

Flavonoid glycosides in *Adiantum capillus-veneris* and *A. cuneatum* were investigated to see if any relation exists among the flavonoid patterns in the genus *Adiantum*. Identified flavonoids in *A. capillus-veneris* fronds were astragalin (kaempferol 3-glucoside), isoquercitrin (quercetin 3-glucoside), kaempferol 3-glucuronide, querciturone (quercetin 3-glucuronide), nicotiflorin (kaempferol 3-rhamnoglucoside) and rutin (quercetin 3-rhamno glucoside), but a flavanone and two flavonols were not identified. Astragalin, kaempferol 3-glucuronide and querciturone were found in *A. cuneatum*. Therefore these two species have three common flavonoid glycosides.

Introduction

In the previous paper¹⁾ we reported on the flavonoid constituents of two species of *Adiantum*, *A. aethiopicum* and *A. monochlamys*. It was found that their flavonoid constituents differ, especially in their main flavonoid glycosides. *A. aethiopicum* has kaempferol and quercetin 3-glucoside as main glycosides, while *A. monochlamys* has kaempferol and quercetin 3-galactosides. Although these two species resemble each other morphologically, their flavonoid pattern is different in glycosidic sugar.

In this paper, we will report on the flavonoid patterns of *A. capillus-veneris* and *A. cuneatum* which resemble each other morphologically and are classified into the same group "cuneata" by Christ²⁾.

Experiments and Results

Fronds of *A. capillus-veneris* were collected at Kamakura, Kanagawa Prefecture in November, 1967 and 235 g (fresh weight) were used. Fresh fronds of *A. cuneatum* were generously supplied by National Park Shinjukugyoen and 118 g of them grown in green-house were subjected to extraction.

The method of extraction was almost the same as described in the previous paper¹⁾. The ethyl acetate soluble portion was chromatographed using silica gel (Merck) column. And as the eluting solvents, the following systems were employed successively, benzene: ethyl acetate=2:1, 1:1, 1:2, ethyl acetate alone and ethyl

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acetate: ethanol=4:1, 2:1. Fractionation was made by the aid of fluorescence under the ultra-violet light (3650 Å). Furthermore, all eluates were checked by paper chromatography with solvent systems of 6% acetic acid (AA) and *n*-butanol: acetic acid: water=6:1:2 (BAW), and the eluates which showed the same chromatographic pattern were combined. Each fraction was developed with the solvent of AA or BAW by one dimensional large-scale paper chromatography and brown bands under UV light were eluted with 50% ethanol in the eluting cabinet.

In the case of *A. capillus-veneris*, eleven fractions were obtained and the fraction 1 (solvent system, benzene: ethyl acetate=2:1) contained no flavonoid. Astragalin and isoquercitrin were found in the fraction 4 (solvent system, benzene: ethyl acetate=1:2), nicotiflorin and kaempferol 3-glucuronide were in the fractions 6 and 7, rutin was in the fraction 9 (solvent, ethyl acetate alone) and quercitrone was in the fractions 10 and 11 (solvent systems, ethyl acetate: ethanol=4:1, 2:1).

The eluates obtained from *A. cuneatum* were treated in the same manner. Astragalin was detected in the fraction 4, kaempferol 3-glucuronide and quercitrone were in the fraction 7 and 8, respectively.

Table 1. Flavonoid glycosides isolated from *A. capillus-veneris* and *A. cuneatum* and their Rf values and absorption maxima in ethanol.

Plant material	Flavonoid glycoside	Absorption maxima in EtOH		Rf value×100 in			
				BAW*	Phe*	AA*	ECFW*
<i>Adiantum capillus-veneris</i>	Astragalin	268,	355	72(72)**	75(75)	26(26)	—
	Isoquercitrin	260,	364	64(64)	57(57)	23(24)	—
	Kaempferol 3-glucuronide	267.5,	353	72	39	29	47
	Quercitrone	259,	364	52(52)	31(31)	19(19)	33(33)
	Nicotiflorin	268,	355	58(58)	65(65)	38(38)	11(11)
	Rutin	260,	365	45(45)	50(50)	37(37)	10(11)
<i>A. cuneatum</i>	Astragalin	267.5,	355	75(75)	79(80)	32(32)	—
	Kaempferol 3-glucuronide	267.5,	354	70(70)***	45(44)	29(29)	—
	Quercitrone	260,	364	51(51)	26(26)	25(26)	—

* BAW; *n*-butanol: acetic acid: water=6:1:2 (v/v), Phe; phenol saturated with water, AA; 6% acetic acid, (paper chromatography), ECFW; ethyl acetate: chloroform: formic acid: water=19:1:1:1, (thin layer chromatography). ** Figures in parentheses indicate the Rf values of authentic samples. *** Figures in parentheses indicate the Rf values of kaempferol 3-glucuronide from *A. capillus-veneris*.

Identification of glycosides was made by hydrolysis, ultra-violet absorption in ethanol, and cochromatography with authentic specimens using paper or thin layer chromatography (Table 1). After concentration of the eluates from paper, each glycoside was hydrolyzed with 5% hydrochloric acid for one hour on a water bath and aglycones and sugars were identified on paper chromatograms by comparing with authentic samples which were developed at the same time in several solvent systems (Table 2). Since the authentic sample of kaempferol 3-glucuronide was not available, the structure was assumed by the behaviors on chromatograms of the hydrolysis products, absorption maximum in ethanol, and zirconium oxychloride test³⁾.

Authentic quercitrone⁴⁾ was kindly given by Professor T. Sasaki and nicotiflorin⁵⁾ was isolated from the leaves of *Ginkgo biloba*.

Table 2. Rf values of aglycones and sugars obtained from glycosides.

Glycoside	Aglycone	Rf value $\times 100$ in		Sugar	Rf value $\times 100$ in		
		BAW	Phe		Pyr	Phe	BAW
Astragalin	K	81(80)*	64(64)	Gl	33(32)	35 (35)	—
Isoquercitrin	Q	65(65)	36(37)	Gl	32(32)	37 (36)	—
Kaempferol 3-glucuronide	K	82(83)	67(67)	GA	—	55**(55)	31**(31)
					—	10 (11)	11 (11)
Querciturone	Q	73(72)	29(29)	GA	—	62**(63)	31**(31)
Nicotiflorin	K	82(80)	65(65)	Gl	32(32)	35 (35)	—
				Rham	52(53)	56 (56)	—
Rutin	Q	67(67)	38(37)	Gl	—	38 (39)	16 (16)
				Rham	—	60 (60)	34 (34)

Abbreviations ; K=kaempferol, Q=quercetin, Gl=glucose, GA=glucuronic acid, Rham =rhamnose, Pyr=(*n*-buthanol: pyridine: water=6:4:3 (v/v)).

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

** Rf values of lactonized form.

Discussion

From the results it will be thought that kaempferol and quercetin 3-glucuronide which are main components of the fronds are common to these species. Due to small amounts of material of *A. cuneatum*, we think we could not find minor flavonoid components. We could, however, say that the flavonoid patterns of these two species resemble each other closely.

In some species of genus *Baptisia*, Alston⁶⁾ reported that there were three types of flavonoid patterns and each species could be immediately roughly classified to have one of these three patterns. Egger and Keil⁷⁾ pointed out that much variation occurred in sugar components and their position in glycosides contained in plants belonging to Ranunculaceae. Harborne⁸⁾ also indicated that some species of *Pisum* could be divided into three groups from their glycosidic types.

Although few species in *Adiantum* were investigated, it might be possible that the genus is divided into some groups from the viewpoint of flavonoid glycosidic pattern. The four species treated so far are divided into three groups by the flavonol 3-monoglycosides which exist as main flavonoids in the fronds. Namely, the first is *A. aethiopicum* which contains mainly 3-glucosides, the second is *A. monochlamys* which contains 3-glucosides and 3-galactosides and it is characterized by 3-galactosides. The last one is the group of *A. capillus-veneris* and *A. cuneatum* which are represented by 3-glucuronides. Now we of course are ignorant of the meaning on differences of glycosidic patterns among the groups, but these results would give some suggestions in taxonomy.

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