Localization of Prenylated Flavonoids in *Sophora flavescens* var. *angustifolia* Plants

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A histochemical analysis was carried out on the distribution of flavonoid derivatives in *Sophora flavescens* var. *angustifolia* plant. Prenylated flavonoids such as kushenol I, kurarinone, sophoraflavanone G and des-*O*-methylanhydroicaritin were mainly localized in the periderm (cork layers, and cork layer-like tissues scattered in parenchymatous tissues of the root system). Pterocarpan derivatives, maackiain, maackiain-3-*O*-glucoside (trifolirhizin) and its 6′-*O*-malonyl ester, were distributed in the cortex, cambium and pith as an ester form. In the aerial parts of the plant, neither pterocarpan nor prenylated flavonoid derivatives, but only flavone monoglucosides such as luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside were detected.

Introduction

We have previously reported that *Sophora flavescens* var. *angustifolia* callus cultures established on MS [1] gellan gum medium produced both prenylated flavonoids (sophoraflavanone G and lehmannin) and pterocarpans (maackiain derivatives) [2]. In that paper, we also reported that the production of these flavonoid derivatives was changed by transfer to different media; prenylated flavonoids were increased on White's [3] or M-9 [4] medium, whereas pterocarpans were produced predominantly on Nitsch & Nitsch medium [5]. Moreover, time-course experiments suggested that the production of pterocarpans was closely related with cell growth whereas there existed an inverse relationship between the production of prenylated flavonoids and cell growth. These results indicated that in *S. flavescens* var. *angustifolia* cells, the production of prenylated flavonoids and pterocarpans was differentially regulated, and that similar control mechanisms may also exist in intact plants. In the present study, to elucidate the control mechanism of flavonoid production in *S. flavescens* var. *angustifolia,* we investigated the localization of prenylated flavonoids and pterocarpans in the root system of intact plants. We also examined the differences in the pattern of flavonoid constituents in the aerial parts of this plant by use of HPLC and photodiode array.

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Materials and Methods

Plant materials

Plants of *Sophora flavescens* var. *angustifolia* were collected in Kawashima-cho, Gifu, Japan in May 1991. Surface sterilized seeds collected in September 1990 were germinated aseptically on MS [1] agar medium without phytohormones and grown for one month under illumination at 25 °C. The origin and the subculture of callus cultures were described previously [2].

Histochemical analysis

To analyze the distribution of flavonoids in root tissues, a transverse section of fresh roots (*ca.* 5 cm diameter and *ca.* 1 cm thickness) was divided concentrically into several fractions as shown in Table II. Transverse sections of each sample prepared with a freezing microtome were examined under a microscope to determine their histological characteristics. Lignification was detected by a solution of 5% phloroglucinol and suberization by a solution of 0.5% sudan III.

Quantitative analysis

Lyophylized samples (100 mg) were Soxhlet-extracted with MeOH for 6 h and assayed by reverse phase HPLC to measure the quantities of flavonoids on the basis of an absorption peak area at 294 and 350 nm by Chromatopak C-R6A (Shimadzu, Japan), respectively. The conditions of HPLC were as follows; column: Capcellpak C_{18} AG-120A (5 μm, 4.6 mm I.D. × 250 mm, Shiseido, Japan), oven temp. 40 °C, solvent: MeCN/H_2O gradient of 20% MeCN to 70% in 50 min, flow rate: 1 ml/min. Compounds in each sample were identified by use of Shimadzu photodiode array SPDM-6A system (Shimadzu, Japan).

Results

Fresh *Sophora flavescens* var. *angustifolia* plants were separated into leaves, stems and underground parts, lyophylized, extracted with MeOH and analyzed by HPLC to determine the content of flavonoid derivatives. As shown in Table I, the aerial parts characteristically contained flavone monoglucosides such as luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside, but neither prenylated flavonoids nor pterocarpans. On the other hand, in the underground parts, both prenylated flavonoids such as kushenol I, kurarinone, sophoraflavanone G and des-*O*-methylanhydroicaritin, and pterocarpans maackiain-3-*O*-glucoside (trifolirhizin) were accumulated, while flavone monoglucosides were hardly detected. As reported previously [6], maackiain was not found in the fresh roots, and was accumulated as the 6′-malonyl ester of its 3-*O*-glucoside. In the present study, however, to simplify the analysis, the malonate ester was hydrolyzed to trifolirhizin by Soxhlet-extraction and the content of the latter was determined. Recently, Weidermann *et al.* [7] isolated malonyl esters of pterocarpan glucosides from cell suspension cultures of *Cicer arientinum*. They also reported that the pterocarpan glucoside malonyl conjugates were not found in the healthy *C. arientinum* plant, and were accumulated only after infection by pathogenic fungi [7]. Different from their results, *S. flavescens* var. *angustifolia* accumulated the pterocarpan glucoside malonate conjugate in roots constitutively without infection by fungi (Table III).

In order to clarify the distribution of prenylated flavonoids and pterocarpans in different tissues of the main root, fresh samples were divided into 6 parts as shown in Table II. Three replicates were analyzed and typical data are shown in Table II. The prenylated flavonoids were mainly localized in periderm such as cork layer (**1**) and fiber-like yellowish tissues (**6**) scattered in parenchymatous tissues. By microscopic observation, this tissue was morphologically similar to the cork layer tissues, and was positive to both sudan III (positive to lipophilic compounds) and phloroglucin-HCl reaction (positive to lignification), which suggested that this tissue was physiologically the same as cork layers. Pterocarpans were not detected in these tissues, but were accumulated in cortex, cambium and pith (**2**–**5**) as glucoside malonyl esters.

We also examined the distribution of flavonoids in roots with different diameters which represent the root age. Main roots (15 mm in diameter), lateral roots (1–10 mm in diameter) and rootlets (lower than 1 mm in diameter) obtained from one mature plant root system were assayed and results

Table I. Contents of flavonoid derivatives in *Sophora flavescens* var. *angustifolia* plant.

Compound [mg/g dry wt]	Leaves	Stems	Underground parts
luteolin-7-*O*-glucoside	23.33	3.58	–
apigenin-7-*O*-glucoside	5.39	0.38	–
kushenol I	–	–	16.83
kurarinone	–	–	23.32
sophoraflavanone G	–	–	1.86
des-*O*-methylanhydroicaritin	–	–	2.86
maackiain	–	–	–
trifolirhizin	–	–	4.33

–: not detected.

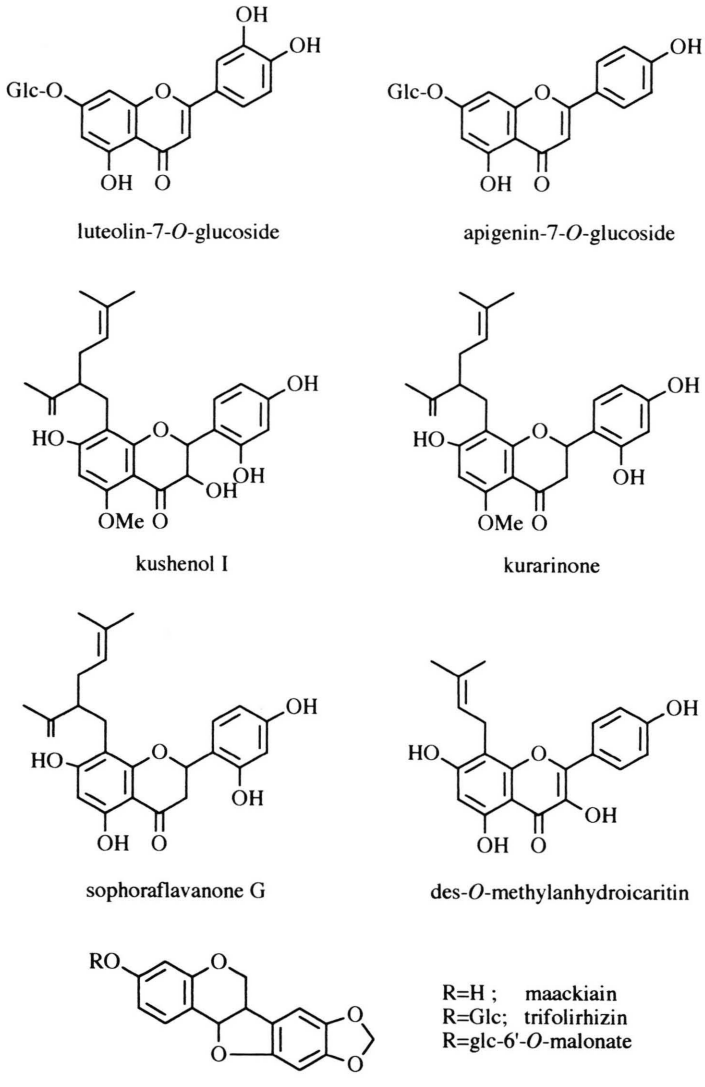


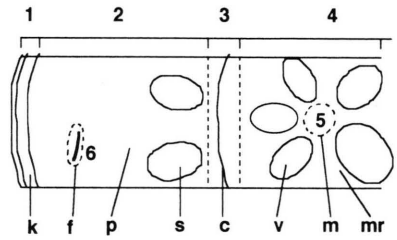
Fig. 1. Flavonoids isolated from *Sophora flavescens* var. *angustifolia* plants.

are shown in Table III. The contents of prenylated flavonoids were almost the same in lateral roots and rootlets, but those in main roots were lower than in the other roots, because of the low ratio of cork layer against other root constituents. Interestingly, radicles of aseptically germinated plantlet (5 cm long) contained higher amounts of trifolirhizin malonate and lower amounts of lavandulyl flavonoids such as kushenol I and kurarinone compared with the mature roots. In radicles, cork layers are underdeveloped whereas the growth of parenchymatous cells is predominant. These results therefore also suggested that the production of lavandulyl flavonoids was closely correlated with the development of cork layers whereas that of trifolirhizin malonate was correlated with parenchymatous cells as we had suggested previously [2]. Moreover, the ratio of the content of sophoraflavanone G against the total contents of 8-lavandulyl flavonoid derivatives in radicles was larger than that in mature roots. Methylation of a 5-OH group may also be closely correlated with the development of cork layers. In radicles, the content of des-*O*-methylanhydroicaritin, which

Table II. Contents of flavonoid derivatives in different tissues of *Sophora flavescens* var. *angustifolia* roots.

	Trifolirhizin [mg/g]	Maackiain [mg/g]	Kushenol I [mg/g]	Kurarinone [mg/g]	Des-*O*-methyl-anhydroicaritin [mg/g]	Sophora-flavanone G [mg/g]
1	–	–	89.81	77.31	15.22	10.22
2	1.61	–	1.87	1.81	0.33	0.20
3	1.69	–	0.23	0.22	0.07	0.01
4	1.01	–	0.05	0.08	0.28	0.10
5	1.03	–	0.12	0.27	–	–
6	–	–	27.29	33.90	4.06	3.08

–: not detected



Abbreviations: c, cambium; f, fiber-like yellowish tissue; k, cork layer; m, pith; mr, medullary ray; p, cortex parenchymatous cells; s, phloem; v, xylem.

Table III. Contents of flavonoid derivatives in roots of different sizes and in callus* of *Sophora flavescens* var. *angustifolia*.

	Trifolirhizin [mg/g]	Maackiain [mg/g]	Kushenol I [mg/g]	Kurarinone [mg/g]	Des-*O*-methyl-anhydroicaritin [mg/g]	Sophora-flavanone G [mg/g]
main roots (15 mm)**	3.21	–***	10.89	15.91	1.66	1.24
lateral roots						
first (10 mm)	6.60	–	17.36	26.37	2.91	1.98
second (3–5 mm)	4.51	–	18.12	23.29	2.61	2.11
third (1 mm)	3.74	–	19.48	24.44	3.76	1.83
rootlets	3.60	–	18.31	26.58	3.37	2.16
radicles	26.03	–	3.24	1.10	31.35	5.81
callus	6.80	1.34	–	–	–	0.87

* Callus tissue was derived from hypocotyls on MS medium solidified with 0.3% gellan gum containing 1 μM 2,4-D and 1 μM kinetin in the dark, and subcultured over 3 years [2].
** Root diameter.
*** Not detected.

has a γ,γ-dimethylallyl group at the C-8 position, was higher than that in other parts. Des-*O*-methylanhydroicaritin has not been isolated from callus cultures [2], so some unknown control mechanisms should exist in the production of des-*O*-methylanhydroicaritin in *S. flavescens* var. *angustifolia* cells.

Discussion

Several workers have reported on the localization of secondary metabolites in plant tissues. In the case of flavonoids, Stafford reviewed the compartmentation of flavonoids in plant tissues [8], but there are few papers discussing the localization

of flavonoids in root systems. Tani *et al.* investigated the localization of flavones in *Scutellaria baicalensis* roots [9] and of isoflavones in *Pueraria lobata* roots [10], and reported that the flavonoid glycosides were distributed in inner tissues such as cortex, phloem, xylem and pith, whereas the aglycones were localized in the outer periderm. In the present study, we also demonstrated that the flavonoid aglycones were localized in the outer cork layer, whereas the flavonoid glucosides were distributed in inner tissues in *S. flavescens* var. *angustifolia* roots. It is noteworthy that the prenylated flavonoids in *S. flavescens* var. *angustifolia* roots belong to the 5-hydroxyflavonoids whereas the pterocarpan glucosides were derived from 5-deoxyflavonoids. In the cases of *Scutellaria* and *Pueraria* roots, flavonoid derivatives contained in outer and inner tissues have the same aglycones. The variety of flavonoid distribution patterns would be caused by the differences in the activities of the final biosynthetic enzymes such as glycosyltransferases or glycosidases. Whereas 5-hydroxy- and 5-deoxyflavonoids biosyntheses branch off at the early stage of chalcone synthesis [11, 12, 13], in *S. flavescens* var. *angustifolia* roots, these two branches of flavonoid metabolism may be strictly compartmentalized at early biosynthetic steps. Not only sites of accumulation, but also sites of synthesis of these two flavonoid groups may be different in *S. flavescens* var. *angustifolia* roots. It is necessary to investigate the localization of key enzymes of prenylated flavonoids and pterocarpans production in *S. flavescens* var. *angustifolia* roots.

Both prenylated flavonoids and pterocarpans have antimicrobial [14] and antifungal [15] activities. Moreover, *S. flavescens* var. *angustifolia* contains toxic quinolizidine alkaloids in all tissues, especially in aerial parts [16]. *S. flavescens* var. *angustifolia* plants may use these compounds as chemical defence systems against various invaders in different manners.

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