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Histochemistry. VII.[1] Flavones in Scutellariae Radix

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Histochemical studies were performed on the distribution of bioactive flavones, baicalein, baicalin, wogonin and wogonin 7-*O*-glucuronide, in various parts and tissues of Scutellariae Radix and cultivated *Scutellaria baicalensis* (Labiatae) root.

By means of high-performance liquid chromatography, it has been clarified that the flavone glucuronides are mainly distributed in the cortex, phloem and xylem of the root, and the aglycones are present in the outer periderm at high concentrations.

It has been found that Scutellariae Radix from China is morphologically different from the drugs from Japan, Korea and North Korea.

Keywords——histochemistry; Scutellariae Radix; *Scutellaria baicalensis*; Labiatae; crude drug; flavone; baicalein; baicalin; wogonin; HPLC

In order to evaluate various kinds of herbal drugs the authors have been histochemically investigating the locations of bioactive components in crude drugs from morphological and chemical viewpoints.[1a] In the present paper the results of a histochemical investigation of the distribution of some bioactive flavones in Scutellariae Radix by means of high-performance liquid chromatography (HPLC) are described.

Scutellariae Radix, which is called "Huang-gin" in China and "Ogon" in Japan, is a crude drug prepared from the dry and peeled root of *Scutellaria baicalensis* GEORGI (Labiatae). It has been said traditionally that a brilliant yellow, substantial and bitter Scutellariae Radix is of good quality, and the drug having decayed parts (decayed xylem and pith: Anko or Anke in Japanese) is inferior.

Results

HPLC Analysis of Flavones in Scutellariae Radix

Four flavones, baicalein, wogonin and their glucuronides, baicalin and wogonin 7-*O*-glucuronide, in Scutellariae Radix can be well separated by the reversed-phase system on a ZORBAX C-8 column. Calibration curves for the four flavones can be established from the peak areas. The equations of the least-squares regression are given below:

y signifies the quantity in μg and x the peak area.

$y(\text{baicalin}) = 3.23 \times 10^{-5}x - 0.385$ (correlation coefficient $r = 0.999$)

$y(\text{wogonin glucuronide}) = 3.47 \times 10^{-5}x - 0.254$ ($r = 0.999$)

$y(\text{baicalenin}) = 1.76 \times 10^{-5}x + 0.034$ ($r = 0.999$)

$y\ (\text{wogonin}) = 8.86 \times 10^{-6}x - 0.012$ ($r = 0.999$)

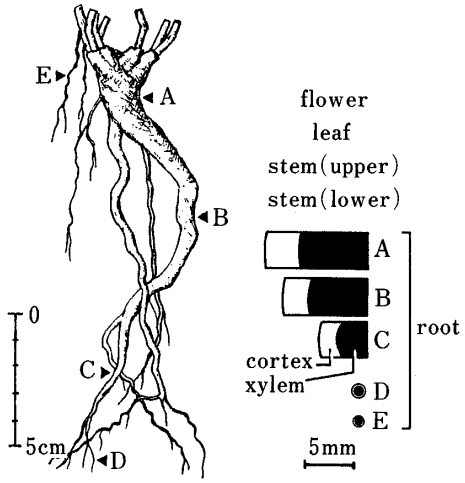
Flavones in Different Parts of Fresh *Scutellaria baicalensis* (Fig. 1)

Fresh *S. baicalensis* cultivated for four years was divided into 9 parts, namely flower, leaf, stem (upper and base), root (A—D) and adventitious root (E) (Fig. 1). The four flavones were detected in the stem and root, but were hardly detected in the flower or the leaf.

There were larger amounts of baicalein and wogonin in the lower stem with a yellowish-colored surface than in the upper stem and green stem.

Regarding flavones contents in the individual parts of the root, baicalin was distributed almost uniformly in each part of the root: ($10.25 \pm 0.62\%$, average $\pm$ standard deviation, coefficient variation (C.V.) $= 0.06$). The variation of wogonin 7-*O*-glucuronide content in roots ($3.46 \pm 0.86\%$, C.V. $= 0.25$) was about 4 times greater than that of baicalin.

In contrast the aglycone contents were lower than those of glucuronides and varied among the parts of the root: baicalein, $0.32 \pm 0.48\%$, C.V. $= 1.50$; wogonin, $0.22 \pm 0.31\%$, C.V. $= 1.41$. It is apparent that thinner roots (D and E in Fig. 1) contain larger amount of aglycones than thicker roots (A, B and C).



	baicalin (%)	wogonin 7-*O*-glucuronide (%)	baicalein (%)	wogonin (%)
flower	—	—	—	—
leaf	—	—	—	—
stem(upper)	0.58	0.07	0.01	0.01
stem(lower)	4.27	3.32	0.24	0.21
A	10.36	4.43	0.04	0.06
B	10.54	3.70	0.01	0.01
C	10.50	3.87	0.01	0.01
D	10.69	3.17	0.40	0.31
E	9.16	2.15	1.12	0.73
		A, B, C	D, E	
baicalein		0.020 ± 0.017	0.76 ± 0.51	
		$p < 0.025$		
wogonin		0.027 ± 0.029	0.52 ± 0.30	
		$p < 0.025$		

Fig. 1. Flavones in Different Parts of Fresh *Scutellaria baicalensis* (Sample [A])

Flavones in Different Tissues of Fresh *S. baicalensis* Main Root (Fig. 2) and Chinese Scutellariae Radix (Fig. 3)

In order to clarify the distribution of flavones in different tissues of the main root, a fresh sample [B] was divided into 12 tissues as shown in Fig. 2.

The contents of glucuronide flavones varied remarkably in different tissues. Thus, the cortex and phloem (cx (2), (3)) and xylem (xy (2), (3), (4)) contained the two glucuronides in 3—6 times greater amount than the outermost layer (pm) and innermost tissues (m: completely decayed xylem and pith (Anko)). Higher concentrations of aglycones were found in the outermost layer (pm) (baicalein: 0.41%, wogonin: 0.43%) than in other tissues (trace $-$ 0.12%).

Scutellariae Radix from China (sample [I]) was also divided into 8 concentric tissues. The distribution pattern and content of the flavones in the Chinese drugs were in accord with those of the Japanese cultivated plant, but the content of aglycones was remarkably higher than in the Japanese species.

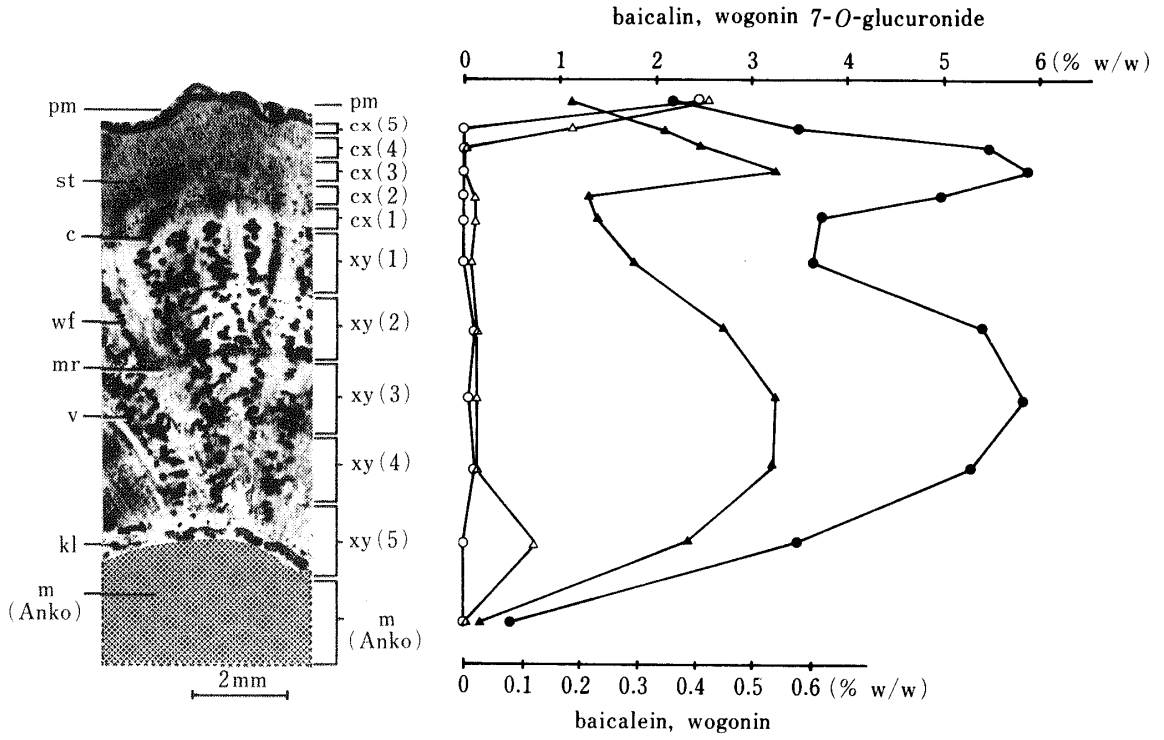


Fig. 2. Flavones in Different Tissues of Fresh *Scutellaria baicalensis* (Sample [B])

pm, periderm; st, stone cell; c, cambium; wf, wood fiber; mr, medullary ray; v, vessel; kl, cork layer; m, pith; cx, cortex and phloem; xy, xylem and xylem ray.
—●—, baicalin; —▲—, wogorin 7-*O*-glucuronide; —○—, baicalein; —△—, wagonin.

Anatomically, the Chinese Scutellariae Radix differed in the arrangement of wood fibers (wf) from that of *S. baicalensis* cultivated in Japan. The Chinese crude drug possesses concentrically arranged wood fibers, and the Japanese species has radially arranged ones.

The morphological characteristics and distribution of the flavones in Scutellariae Radix from Korea (sample [C]) and from North Korea (sample [D]) were the same as those of the Japanese drug (sample [B]).

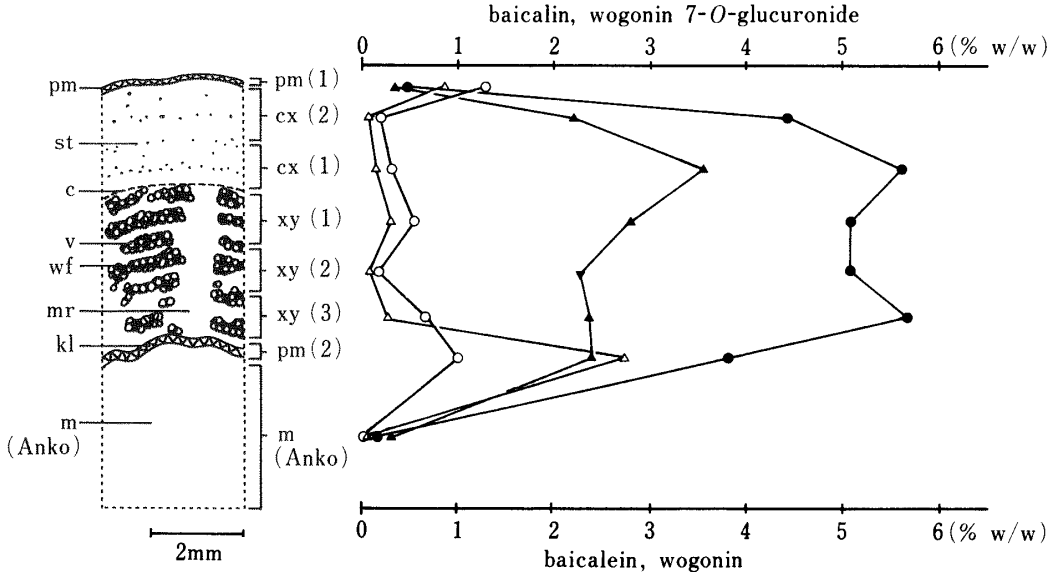


Fig. 3. Flavones in Different Tissues of Scutellariae Radix from China (Sample [I])

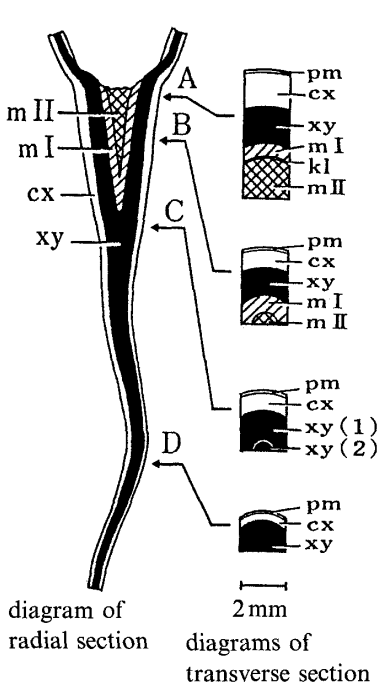
Symbols are the same as in Fig. 2.

Flavones in Different Parts of Scutellariae Radix (Figs. 4 and 5)

Four parts of cultivated *S. baicalensis* root (sample [A]) were histochemically analyzed:

diagrams of the radial and transverse sections are shown in Fig. 4.

It was found that the concentrations of baicalin and wogonin 7-*O*-glucuronide in the cortical layer (cx) and xylem (xy) were higher than in the other tissues, periderm (pm) and decayed xylem and pith (Anko). However, the aglycones were hardly detected in cortex, phloem and xylem. They were present in high content in the outermost periderm.



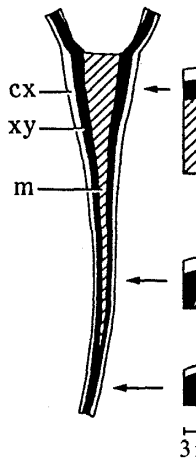
	baicalin (%)	wogonin 7-*O*-glucuronide (%)	baicalein (%)	wogonin (%)
A pm	0.25	0.15	0.02	1.28
A cx	5.09	1.81	—	—
B xy	4.18	1.81	—	—
B mI	0.65	0.24	4.69	1.64
B kl	0.59	0.39	3.98	1.17
B mII	0.19	0.05	0.08	0.13
C pm	3.19	1.79	0.75	0.26
C cx	4.38	1.87	—	—
C xy	3.60	2.07	—	—
C mI	1.30	0.38	3.07	1.40
C mII	1.60	0.71	0.08	0.42
D pm	0.44	0.31	0.24	0.87
D cx	2.24	1.19	—	—
D xy(1)	4.42	2.93	—	—
D xy(2)	3.70	1.65	—	—
pm	0.56	0.06	0.16	1.31
cx	6.18	2.80	—	—
xy	4.96	3.04	—	—

Fig. 4. Flavones in Different Parts of Fresh *Scutellaria baicalensis* Root (Sample [A])

mI, slightly decayed xylem and pith; mII, completely decayed xylem and pith.

As noted in Fig. 5, decayed xylem and pith (Anko) was distributed from thick to thin roots of Scutellariae Radix from China, whereas Anko was found only in the root-head part in the drug from Japan, Korea and North Korea.

In the Chinese drug, glucuronide flavones were also present in cortex, phloem and xylem at high concentrations. However, aglycone components of the Chinese drug were found not only in the periderm and Anko but also in the cortex, phloem and xylem, in contrast to the Japanese species.



	baicalin (%)	wogonin 7-*O*-glucuronide (%)	baicalein (%)	wogonin (%)
cx	7.10	3.03	0.12	0.13
xy	7.78	3.11	0.66	1.09
m	0.72	0.82	0.01	0.13
cx	5.81	2.61	0.21	0.21
xy	4.73	1.76	0.48	0.21
m	0.32	0.50	1.94	1.22
cx	5.87	2.70	0.13	0.05
xy	6.19	2.27	0.46	0.18

Fig. 5. Flavones in Different Parts of Scutellariae Radix from China (Sample [I])

TABLE I. Flavones in Several Kinds of Commercial Scutellariae Radix

	Baicalin (%)		Wogonin 7-*O*-glucuronide (%)		Baicalein (%)		Wogonin (%)	
[A] *Scutellaria baicalensis* ($n=4$) (cultivated at Osaka)	8.62±1.26		4.58±0.36		0.04±0.02		0.08±0.04	
[C] Scutellariae Radix ($n=5$) (Korea)	8.56±0.57		4.48±0.12		0.22±0.04		0.11±0.02	
[D] Scutellariae Radix ($n=4$) (North Korea, with peel)	8.51±0.60		4.14±0.41		0.37±0.03		0.18±0.01	
[E] Scutellariae Radix ($n=5$) (China, Hen-ogon)	6.97±0.75		2.34±0.31		0.52±0.05		0.14±0.03	
[F] Scutellariae Radix ($n=5$) (China, Hen-ogon)	6.83±1.07		2.66±0.36		0.33±0.11		0.11±0.05	
[G] *Scutellariae Radix* ($n=5$) (China, Sen-ogon)	7.29±0.16		2.76±0.34		0.29±0.03		0.10±0.02	
[H] Scutellariae Radix ($n=5$) (China, Sen-ogon)	8.56±0.89		2.72±0.41		0.62±0.07		0.16±0.02	
[I] Scutellariae Radix ($n=6$) (China, Sen-ogon)	7.01±1.69		2.45±0.76		0.58±0.02		0.31±0.17	
[A]—[D]	8.67±0.79	$t=3.27$ $p<0.05$ (df=37)	4.40±0.35	$t=11.27$ $p<0.05$ (df=37)	0.20±0.14	$t=4.56$ $p<0.05$ (df=37)	0.12±0.05	$t=1.52$ $p<0.20$ (df=37)
[E]—[I]	7.31±1.20		2.71±0.42		0.48±0.18		0.18±0.12	

Values are expressed as the mean±standard deviation. df: degree of freedom.

Flavones in Commercial Scutellariae Radix (Fig. 6)

Flavone contents of 7 commercial samples of Scutellariae Radix were quantitatively analyzed. The experimental results (shown in Table I) are the average of four or six measurements.

Two- or 3-fold variations in the contents of flavones were noted between individual samples: baicalin (4.25—10.45%, 7.75±1.22%, C.V.=0.16), wogonin 7-*O*-glucuronide (1.96—6.35%, 3.31±0.91%, C.V.=0.27), baicalein (0.02—0.71%, 0.38±0.21%, C.V.=0.55) and wogonin (0.03—0.54%, 0.15±0.11%, C.V.=0.66).

Chinese products ([E]—[I]), which are mainly used in Japan, have lower total flavones contents (7.68—13.31%, 10.71±1.48%) than Japanese cultivated product [A] (12.5—14.6%, 13.39±0.91%), Korean products [C] (12.7—15.1%, 13.39±0.62%) or North Korean product [D] (12.4—14.4%, 13.30±0.93%).

Discussion

In order to clarify the contents and compositions of bioactive flavones in various kinds and parts of Scutellariae Radix, an HPLC determination method with gradient elution for the four flavones has been developed. The results show that the method can be used satisfactorily as a simple means for the determination of the four flavones and can trace exactly where the flavones are located in Scutellariae Radix.

It has been confirmed that glucuronides and aglycones of flavones are distributed in different parts of Scutellariae Radix. Thus, baicalin and wogonin 7-*O*-glucuronide are distributed in the cortex, phloem and xylem at high concentrations, whereas the aglycones, baicalein and wogonin, are localized in the outermost periderm and inner slightly decayed xylem and pith. Further work is in progress to investigate the part-specific accumulation of flavones during the development of the plant.

Scutellariae Radix is usually prepared by removing the outer peel from fresh roots. Removal of the peel, which includes the periderm and outer cortex containing the flavones, may result in an appreciable loss of bioactive constituents from the original plant material. This finding is in good accord with our previous histochemical result on ginseng.[2)]

The contents and compositions of flavones and the anatomical characteristics varied depending on the kinds of Scutellariae Radix. Efforts are being made to characterize how the decayed xylem and pith (Anko) develop in the cultivated plant.

As regards anatomical characteristics, Scutellariae Radix from China has concentrically arranged wood fibers, and extensively decayed xylem and pith (Anko), in which there is only a small amount of flavones. This finding gives support to the traditional evaluation of Scutellariae Radix, *i.e.*, that the drug having a decayed cavity is inferior.

Experimental

Materials——*Scutellaria baicalensis* root [A], cultivated for 4 years and [B] for 2 years in the herbarium of the Faculty of Pharmaceutical Sciences of Kinki University.

Scutellariae Radix (Ogon, commercially available in Osaka) [C], from Korea (collected in 1977); [D], from North Korea (in 1975); [E], [F], from China (in 1974 and 1983, respectively: Hen-ogon in Japanese, with extensively decayed parts (Anko)); [G], [H] and [I], from China (collected in 1976, 1977 and 1983: Sen-ogon in Japanese).

Morphological Analysis——Cross-sectioned slices of the fresh root, [A], [B], and the crude drugs, [C]—[I] were prepared by means of a freezing microtome (model MA-101, Komatsu Electronics Inc.). Diagrams of the radial and cross sections were drawn in the usual manner.

The cross-sectioned slices obtained from each sample were divided concentrically into several parts: the outermost cork layer (kl), periderm (pm), cortex and phloem (cx), xylem (xy), and decayed xylem or pith (Anko) (Fig. 2).

Quantitative Analysis of Flavones——Baicalein, wogonin and their glucuronides, baicalin and wogonin 7-*O*-

glucuronide, were isolated from Scutellariae Radix by the reported method.[3] Finely pulverized individual whole root (1 g) or divided parts of fresh root and commercial drugs were extracted with refluxing MeOH (100 ml × 4) to afford the extractive, which was redissolved in *N*,*N*-dimethylformamide (DMF) and MeOH mixture (1 : 1) containing naphthalene as an internal standard.

An aliquot was subjected to HPLC (Shimadzu LC-2 with an ultraviolet detector, Shimadzu SPD-1). A stainless-steel column (25 cm × 4 mm) packed with ZORBAX C-8 (Shimadzu) was used. The mobile phase was a mixture of solution A (0.5% H_3PO_4: $(CH_3)_2NH$ = 1000 : 7) and solution B (aq. CH_3CN: 30%—(2%/min)—50%—(10%/min)—100%). The flow rate was 1 ml/min, and the detection wavelength was 254 nm. The peak area was measured using a computing integrator, Shimadzu C-R1A.

The suitability of the conditions was tested by injection of a standard solution containing known amounts of the four flavones. The possible presence of the flavones in each sample was checked by co-chromatography with the standard solution. Four or six samples of the same batch were measured to calculate the average values and standard deviation in the cases of the commercial drugs.

References and Notes

1) *a*) Part VI: T. Tani, T. Katsuki, M. Kubo, S. Arichi and I. Kitagawa, *Chem. Pharm. Bull.*, **33**, 3834 (1985); *b*) This paper also forms Part XI in the series of Studies on Scutellariae Radix. Part X: M. Kubo, H. Matsuda, Y. Kimura, H. Okuda and S. Arichi, *ibid.*, **32**, 5051 (1984).
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