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Note

High-performance liquid chromatographic analysis of Swertia herb

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Swertia herb is a gentianaceous plant, *Swertia japonica* Makino, which has been used in China and Korea since ancient times as a bitter digestive tonic. Several compounds of different types have been reported as the constituents of this herb: (i) bitter tasting, monoterpene glycosides (iridoid glycosides), sweroside, gentiopicroside, swertiamarin, amarogentin and amaroswerin[1–8]; (ii) flavonoids, such as isovitexin, swertiajaponin, homo-orientin and swertisin[9,10]; (iii) xanthones, swertianin, norswertianin, methylswertianin, swertianolin, bellidifolin, methylbellidifolin, desmethylbellidifolin[11–13].

Previously[14], we reported the high-performance liquid chromatographic (HPLC) separation of these bitter compounds using an Aquasil column. For the simultaneous analysis of the above types of constituents of Swertia herb[15–21], we have no attempted to use an ODS column and a gradient solvent system.

EXPERIMENTAL

Materials

The following Swertia herbs were employed for the present study: herbs commercially available and originally collected in the Yamanashi (at Lake Matsubara), Ibaragi (at Ooarai), Iwate and Fukushima districts; wild herbs growing and collected in the Aomori and Hiroshima districts; herbs cultivated in the Aomori and Kochi districts.

Instruments

An high-performance liquid chromatograph consisting of an SP-8700 solvent-delivery system (Spectra-Physics, U.S.A.), Rheodyne 7125 20-μl loop injector, SSC-Y-1000 UV detector (Senshu Scientific) and SIC-7000B integrator (System Instruments) coupled with an 8-in. floppy disk drive system was used. The column (Senshu Scientific) was a Senshu Pak SS-1251N (ODS, 25 cm × 4.6 mm I.D.). The gradient solvent system in Table I was employed as a mobile phase.

Extraction

Swertia herb (harvested at the flowering time) was powdered by a mixer. One gram of the powder was extracted twice with 10 ml of methanol for 30 min on an ultrasonic bath. The combined extracts were evaporated to dryness, and the residue

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TABLE I

GRADIENT SYSTEM

Time (min)	*3% Aqueous acetic acid(%)*	*Acetonitrile (%)*
0	98	2
8	85	15
20	85	15
55	0	100
60	0	100

was dissolved in 10 ml of methanol; 10 μl of the solution were injected for HPLC.

For extraction of individual parts of the herb, Swertia herb was divided into the flower, leaf, stem and root, which were powdered by a mixer. The procedure described above was applied to extract each plant portion for HPLC.

RESULTS AND DISCUSSION

All the papers[14–21] published so far on the chemical analysis of *Swertia japonica* mostly dealt with some special group of compounds, such as monoterpene glycosides (iridoid glycosides), flavonoids or xanthones. We have developed a general procedure to analyse the several types of components simultaneously by HPLC using an ODS column and a gradient solvent system.

For the most efficient separation of iridoid glycoside, swertiamarin (t_R 17.3 min) sweroside (t_R 19.4 min) and gentiopicroside (t_R 20.0 min), a linear increase in acetonitrile from 2 to 15% in 2% aqueous acetic acid solution should be performed within 8 min and then the final conditions held for another 12 min. One of the flavonoid components, isovitexin, had t_R 31.8 min at acetonitrile–2% acetic acid (44:56). In the final stage of gradient elution, where the amount of acetonitrile reached 100%, some iridoid components, amaroswerin (t_R 35.0 min) and amarogentin (t_R 36.3 min), and xanthones were separated (see Table II and Fig. 1), whereas gentisin and isogentisin were not clearly separated by this solvent system.

Using this HPLC system we tried to analyse Swertia herb samples collected in several different districts of Japan. As shown in Fig. 2, a well defined separation of the methanol extract was obtained within 60 min using authentic samples as the reference (Fig. 1). A quantitative analysis of the Swertia extracts is shown in Table II. As reported earlier, for *Swertia japonica* Makino, as well as other species of Swertia[12,19], the contents of iridoid glycosides, swertiamarin and gentiopicroside varied, largely depending on the different sources, such as commercial material[15–19], wild[17,19] or cultivated specimens collected in different places[15,16,18,20], or harvested in different seasons[20,21]. We obtained similar results, in the present study, finding various contents of the iridoid glycosides as well as of the flavonoids such as isovitexin, and the xanthone components.

Using HPLC, Takino[17] reported a quantitative analysis of the bitter principles of Swertia herb, amaroswerin, amarogentin and gentiopicroside. The highest content of amarogentin was observed in the flower, with smaller quantities in the leaf, root

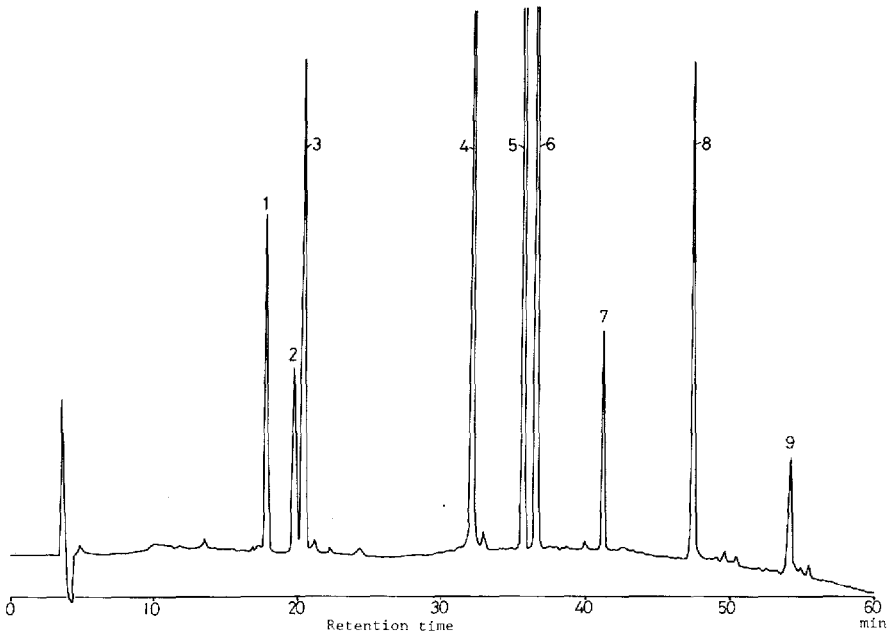

Fig. 1. HPLC profile of an authentic sample. Column: Senshu Pak SS-1251N (ODS, 250 mm × 4.6 mm I.D.). Flow-rate: 0.8 ml/min. Detector: UV, 254 nm. Peaks: 1 = swertiamarin; 2 = sweroside; 3 = gentipicroside; 4 = isovitexin; 5 = amaroswerin; 6 = amarogentin; 7 = gentisein; 8 = gentisin and isogentisin; 9 = 1-hydroxy-3,7-dimethoxyxanthone.

and stem. The highest content of amaroswerin was found in the flower, followed by the leaf and stem, while gentiopicroside was contained in larger quantity in the root than in the stem. By means of quantitative analysis using thin-layer chromatography

TABLE II

CONTENTS (μg/g HERB) OF THE PRINCIPLES OF *SWERTIA JAPONICA* (WHOLE PLANT) COLLECTED IN DIFFERENT LOCALITIES IN JAPAN

Compounds: A = swertiamarin; B = sweroside; C = gentiopicroside; D = isovitexin; E = amaroswerin; F = amarogentin; G = gentisin and isogentisin.

Locality	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Lake Matsusbara	1230	122	6.53	237	176	19.8	146
Ooarai	762	107	23.1	229	724	25.4	137
Ibaragi Pref.	36.7	82.7	20.3	429	352	30.4	14.7
Iwate Pref.	2030	117	37.4	547	612	22.1	149
Fukushima Pref.	1720	102	32.5	505	544	29.8	135
Aomori Pref. (wild)	749	139	—	121	268	28.2	149
Hiroshima Pref. (wild)	175	110	147	192	3.52	75.0	60.6
Aomori Pref. (cultivated)	525	144	4.01	240	122	32.1	68.6
Kochi Pref. (cultivated)	34.9	20.8	212	149	56.5	166	91.7

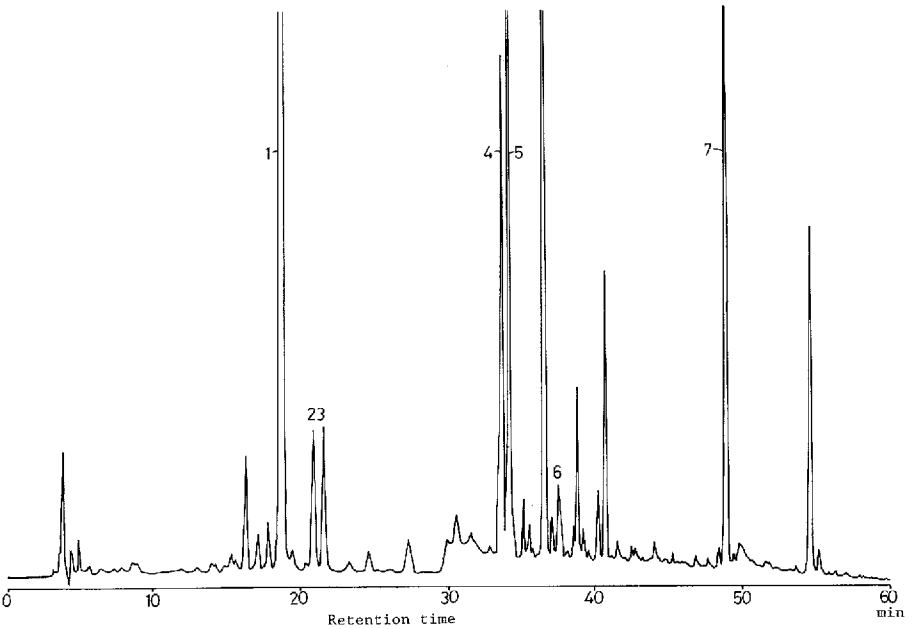

Fig. 2. HPLC profile of the methanol extract of *Swertia japonica* (whole plant, Fukushima district). Details as in Fig. 1. Peaks: 1–6 as in Fig. 1; 7 = gentisin and isogentisin.

(TLC), Hayashi[18] reported the content of swertiamarin as 1–2.5% in the dried herb from eleven species of Swertia which were commercially available, decreasing in quantity in the order: flower, leaf, stem and root.

Using the present HPLC procedure, we analysed the contents of iridoid glucoside, amarogentin, amaroswerin, gentiopicroside and swertiamarin in the various parts of Swertia herb, and obtained different results to those referred to above. Table III shows the contents of the above eight chemical constituents in the respective parts

TABLE III

CONTENTS OF THE PRINCIPLES IN THE DIFFERENT BOTANICAL PARTS OF *SWERTIA JAPONICA*

Details as in Table II.

Botanical part	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Iwate Pref. (wild)							
Flower	637	16.1	358	1150	1100	167	266
Leaf	208	1.22	2.95	–	202	18.9	39.2
Stem	194	112	35.5	126	161	26.7	39.2
Root	74.0	155	37.8	95.6	45.6	40.1	55.8
Fukushima pref.							
Flower	466	24.7	340	994	454	60.5	142
Leaf	352	17.5	31.2	306	383	17.1	53.9
Stem	251	114	18.7	162	174	–	16.3
Root	580	515	24.1	161	114	47.1	4.86

of the herb. Gentiopicroside was contained in largest amount in the flower. The amount of amarogentin decreased in the order flower, root, stem and leaf, while amaroswerin showed a higher content in the flower than the leaf and root. The content of swertiamarin showed no uniformity in trend from part to part. We also found flavonoids in the root, which were not reported earlier by Kubota *et al.*[20].

Accordingly, our HPLC procedure could usefully be applied to the evaluation of this herb with the reference of quantitative analytical data of the chemical constituents.

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