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Studies on the Mutagenicity of Swertiae Herba. II.[1] Quantitative Analysis of Mutagenic Components by Thin Layer Chromatograph Densitometry

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Good separation of seven mutagenic xanthones present in Swertiae Herba was accomplished by thin-layer chromatography (TLC) on silica gel plates. The conditions for quantitative analysis of these xanthones by TLC-densitometry were investigated, and this TLC-densitometry was applied to the analysis of the constituents of the commercial Swertiae Herba and to the determination of the time courses of their extraction with hot water. The relationship between xanthone content and mutagenicity is discussed.

Keywords——Swertiae Herba; *Swertia japonica*; xanthone derivative; mutagenicity; thin-layer chromatograph densitometry; quantitative analysis

Swertiae Herba (*Swertia japonica* MAKINO, Gentianaceae; Japanese name: Senburi or Toh-yaku) is a well-known Japanese folk medicine and has been used as a bitter stomachic. Previously, we reported a structure study of its xanthone glucosides[2] and a quantitative analysis of the bitter seco-iridoid glucosides in this plant.[3] It was also shown that the mutagenic principles of this plant are represented by seven tetraoxygenated xanthones, methylbellidifolin (**1**), methylswertianin (**2**), swertianin (**3**), bellidifolin (**4**), norswertianin (**5**), desmethylbellidifolin (**6**) and a new compound, 5,8-dimethylbellidifolin (**7**). As a continuation of this series of studies, the present paper reports a quantitative analysis of the relationship between the xanthone content and the mutagenicity of this plant.

	R1	R2	R3	R4		R1	R2	R3	R4
6	H	H	H	H	**5**	H	H	H	H
4	H	Me	H	H	**3**	H	Me	H	H
1	H	Me	Me	H	**2**	H	Me	Me	H
7	H	Me	Me	Me					

Chart 1

Experimental

Plant Materials——Five specimens of commercial Swertiae Herba (sample Nos. 1—5) were purchased in

Hiroshima market in 1982—1983.

Authentic Samples——Authentic samples of xanthone derivatives, **1**—**7**, were extracted and purified according to the previous paper.$^{1)}$

Extraction and Separation for Analysis——Swertiae Herba (2 g) was extracted with MeOH (100 ml) for 8 h three times to ensure complete extraction of the xanthones. The combined MeOH-extracts were concentrated to dryness *in vacuo*. A suspension of the residue in H_2O (20 ml) was extracted with Et_2O (15 ml) three times and the combined Et_2O layers were evaporated to dryness. The residue was tentatively named "the xanthone fraction." For the analysis of **1**, **2**, **3** and **4**, a solution of the xanthone fraction in MeOH–EtOAc (1 : 1, 10 ml) was subjected to thin-layer chromatography (TLC)-densitometry without further separation. For the analysis of **5**, **6** and **7**, 5 ml of the xanthone fraction was chromatographed on a column of Sephadex LH-20 (1.2 i.d. × 22 cm) using EtOAc as an eluting solvent. The first 10 ml of eluent was rejected, then the next 10 ml was collected, concentrated to 2 ml, and used for TLC-densitometric analysis of **7**. The next 30 ml of the subsequent eluent was rejected, and the final eluent (150 ml) was concentrated to 2 ml and analyzed for **5** and **6** by TLC-densitometry.

H_2O Extract——The material (1.5 g) (sample No. 1) was cut into 2—5 cm pieces, and added to boiling H_2O (400 ml). The mixture was kept at 90 °C for 5—120 min on a hot plate and filtered to give the H_2O extract. After addition of NaCl (5 g), this H_2O extract was extracted with Et_2O (100 ml) three times. The combined Et_2O layers were evaporated to dryness and a solution of the residue in MeOH–EtOAc (1 : 1, 3 ml) was subjected to analysis for **1**, **2**, **3** and **4** by TLC-densitometry. All values were the averages of three determinations.

TLC-Densitometry——TLC plate: Kieselgel 60 F-254 (20 × 20 cm; 0.25 mm Merck). Solvent: toluene–HCOOEt–HCOOH (12 : 4 : 1). Densitometer: dual-wavelength TLC scanner (CS-910, Shimadzu). Detection: ultraviolet (UV) at 254 nm (reference 600 nm). Mode: zig-zag scanning. Scanning speed: 20 mm/min.

Mutagenicity Test——See the previous paper.$^{1)}$

Results and Discussion

TLC on a silica gel using toluene–HCOOEt–HCOOH (12 : 4 : 1) as the solvent gave excellent separation of all of the xanthones, **1**—**7**, as shown in Fig. 1. As illustrated in Fig. 2, calibration plots for integrated value *vs.* concentration were found to be linear up to a concentration of 1.0 μg/spot. Calibration curves could be extrapolated through the origin. Quantitative analysis of **1**—**7** in commercial plant materials was conducted by means of the present procedure, and the results are summarized in Table I.

The mutagenicity of each xanthone in 1 mg of the methanolic extract was obtained as a function of the number of His^+ revertant colonies and calculated as mutagenicity/$\mu g^{1)}$ × its

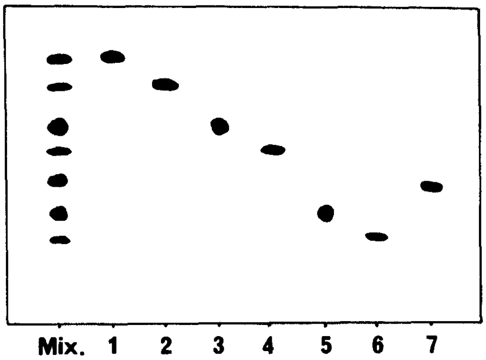

Fig. 1. Thin Layer Chromatograms of Xanthone Derivatives

Plate: Kieselgel 60 F-254. Solvent: toluene–ethyl formate–formic acid (12 : 4 : 1). Detection: UV at 254 nm.

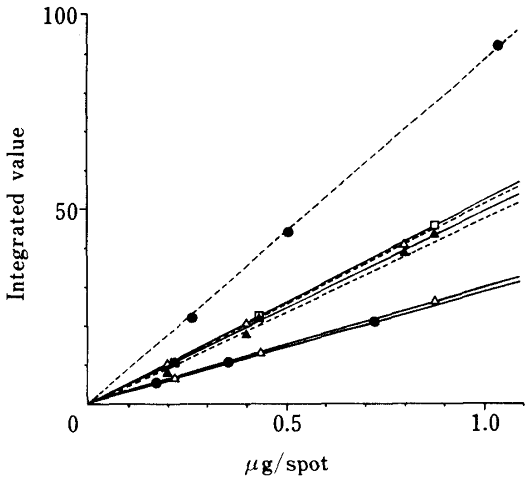

Fig. 2. Calibration Curves

●—●, methylbellidifolin (**1**); ●---●, methylswertianin (**2**); ▲---▲, swertianin (**3**); ▲—▲, bellidifolin (**4**); △---△, norswertianin (**5**); △—△, desmethylbellidifolin (**6**); □—□, 5,8-dimethylbellidifolin (**7**).

Plate: Kieselgel 60 F-254. Solvent: toluene–ethyl formate–formic acid (12 : 4 : 1). Detection: UV, λ_S = 254 nm, λ_R = 600 nm. Mode: zig zag scanning.

Table I. Contents of Xanthone Derivatives (%)

Compound	Samples				
	1	2	3	4	5
1	0.042	0.043	0.033	0.042	0.045
2	0.095	0.066	0.033	0.107	0.087
3	0.145	0.125	0.108	0.100	0.092
4	0.548	0.544	0.470	0.320	0.422
5	0.007	0.006	0.004	0.007	0.003
6	0.023	0.031	0.022	0.034	0.031
7	0.012	0.013	0.012	0.011	0.013

Table II. The Mutagenicities of Methanolic Extract and Xanthone Derivatives Present in the Extract (1 mg in Each Case)

		Mutagenicity (His^+ revertant colonies)				
		Samples				
		1	2	3	4	5
MeOH ext.		1290	1185	1140	1065	1250
1	(7.9)[a]	9	10	11	12	12
2	(3.4)	9	7	4	16	12
3	(16.5)	78	70	65	70	61
4	(53.3)	951	960	900	697	915
5	(16.5)	3	3	4	4	4
6	(47.5)	36	50	36	66	61
7	(8.2)	3	3	4	4	4
Sum		1089	1103	1024	851	1069

a) His^+ revertant colonies per μg.

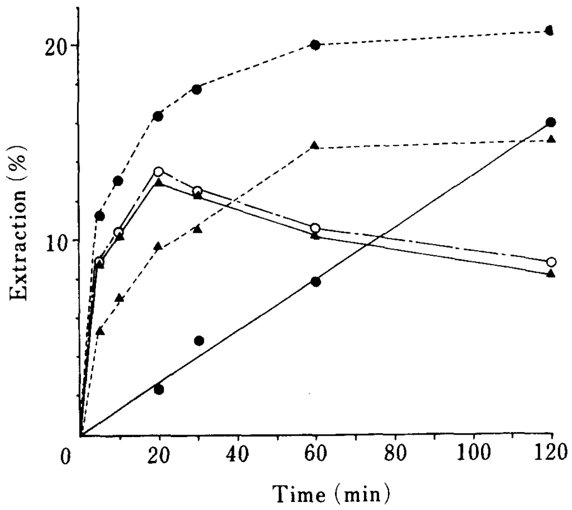

Fig. 3. Time Courses of Extraction of Xanthone Derivatives with Hot Water

●—●, methylbellidifolin (**1**); ●---●, methylswertianin (**2**); ▲---▲, swertianin (**3**); ▲—▲, bellidifolin (**4**); ○—-—○, sum of **1**—**4**.

content in the methanolic extract, as summarized in Table II. It can be concluded that about 80—90% of the mutagenicity of the methanolic extract of Swertiae Herba is accounted for by the sum of the activities of the seven xanthones in the extract. In particular, the bulk of the mutagenicity of the methanolic extract may be due to **4**.

When Swertiae Herba is used as a medicine, the percolate extracted from 0.3—1.5 g with hot water for 30 min is drunk. The time course of xanthones extraction with hot water is illustrated in Fig. 3. After 30 min, about 11% of the xanthones was extracted. The extraction of **1**—**3** increased till 120 min, but that of **4** reached a maximum at 20 min and then decreased slowly. It appears that **4** is more unstable to heating than **1**—**3**.

Recently, Morimoto *et al.* reported the mutagenic activity of gentisin and isogentisin from Gentiana Radix (Gentianaceae).[4)] Many xanthone derivatives other than glucosides (swertianolin, isoswertianolin[1)] and mangiferin[5)]) show mutagenic activity. Further, like flavonol glucosides,[6)] xanthone glucosides would be hydrolyzed by glucosidase in the animal body to form mutagenic xanthones.

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