Hepatoprotective Principles of *Swertia japonica* MAKINO on D-Galactosamine/Lipopolysaccharide-Induced Liver Injury in Mice

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The *n*-BuOH extract of *Swertia japonica* showed a significant hepatoprotective effect on D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-induced liver injury in mice. The activity-guided fractionation led to the isolation of a new tetrahydroxanthone derivative, tetrahydroswertianolin (1), as well as two known iridoids, gentiopicroside (2) and sweroside (3). Their structures were elucidated by spectroscopic methods and chemical reactions. Of the three compounds, 2 and 3 possessed mild hepatoprotective activity at a dose range of 25—50 mg/kg, whereas, 1 exhibited potent activity in a dose-dependent manner. The hepatoprotective effect of tetrahydroswertianolin (1) was stronger than that of glycyrrhizin which was used as a positive control.

Key words *Swertia japonica*; Gentianaceae; tetrahydroswertianolin; gentiopicroside; sweroside; hepatoprotective effect

Various factors have been reported to induce hepatic injury. Since endotoxemia is commonly seen in patients with some liver diseases and animals with experimental liver injuries,[1] endotoxin is thought to play an etiologic role in several liver diseases. An active component of endotoxin is lipopolysaccharide (LPS) which is derived from the cell wall of gram-negative bacteria. Administration of LPS to animals is known to induce lethal shock and multiorgan failure including liver injury.[2] In the study of LPS-induced liver injury, D-galactosamine (D-GalN) sensitization is frequently employed. D-GalN highly sensitizes the host response of mice to LPS by inhibiting transcription and protein synthesis selectively in the liver. Therefore, co-administration of D-GalN and LPS to mice produces liver failure and consequently causes fulminant hepatitis within 8 h.[3] This immunological liver injury model has been used to evaluate the efficacy of hepatoprotective agents.[4]

Swertia is a popular medicinal herb in Japan as well as in South-Asia. It has been widely used in Ayurvedic and Unani medicine as an anthelminthic, febrifuge, and liver tonic. In Japan, *Swertia japonica* MAKINO is used for stomach complaints[5] because of its characteristic bitter taste. There are a number of reports on the bitter principles of this plant.[6] In addition, the whole plant is reported to show hair tonic, antimicrobial and antitumor activity.[7] We previously reported that the aqueous ethanolic extract of *S. japonica* exhibited a potent hypoglycemic effect in streptozotocin (STZ)-induced diabetic rats,[8] and the active principle is a xanthone, bellidifoline.[9] As to its antihepatotoxic effect, only one report describes the *in vitro* antihepatotoxic activity.[10] In our serial research on hepatoprotective agents from natural sources,[11] we found that the *n*-BuOH extract of *S. japonica* showed a significant hepatoprotective activity in an *in vivo* immunological liver injury model. In this paper, we wish to report in detail the hepatoprotective effect of *S. japonica* as well as the isolation and structural elucidation of its active principle.[12]

Experimental

IR spectra were taken in KBr discs on a Hitachi 260-10 IR spectrophotometer and UV spectra in MeOH on a Shimadzu UV 2200 UV-visible spectrophotometer. Optical rotation was measured at 20 °C on a JASCO DIP-4 automatic polarimeter. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL GX-400 and Fourier-transform NMR spectrometer with tetramethylsilane (TMS) as an internal standard and chemical shifts are expressed in δ-values. ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C COSY, ^{1}H–^{13}C long-range COSY and nuclear Overhauser effect (NOE) spectra were obtained using the usual pulse sequences, and data processing was performed with standard JEOL software. Mass spectra

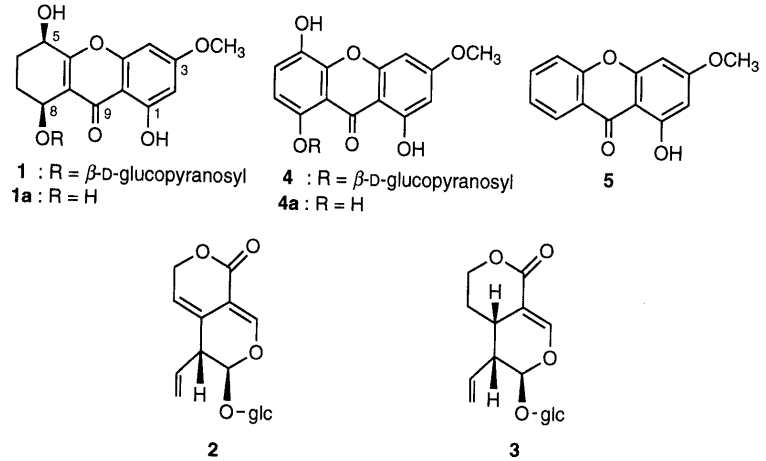

Chart 1

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and high-resolution mass spectra were taken on a JEOL JMS-SX 102A (ionization voltage, 70 eV; accelerating voltage, 5.0 kV). Column chromatography was performed using Sephadex LH-20 (Pharmacia, Uppsala, Sweden), Wako gel C-200 (Wako Pure Chemical Industries, Co., Ltd., Japan) and Iatrobeads (Iatron Laboratories, Inc., Tokyo, Japan). D-GalN was obtained from Wako Pure Chemical Industry, Osaka, Japan. LPS (*Escherichia coli* serotype 055: B5) was purchased from Difco Laboratories, U.S.A. Serum alanine aminotransferase (ALT) levels in mice were measured on a Reflotron S system (Boeringer Mannheim Co., Ltd., Osaka, Japan). The plant material was collected from the Tohoku district of Japan, supplied by Uchida Pharmaceutical Co. Ltd., Japan, and identified as *Swertia japonica* MAKINO (Gentianaceae). The voucher sample (TMPW No. 13769) was preserved in the Museum for Materia and Medica, Analytical Research Center for Ethnomedicines of Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation The shade-dried whole plant (5 kg) was chopped into small pieces and refluxed with 70% aqueous ethanol for 3 h for the first extraction and 2 h each subsequent extraction. The total filtrate (120 l) was evaporated under reduced pressure to obtain the dark green viscous mass of the aqueous ethanolic extract (1330 g). This extract was suspended in distilled water (5 l) and stirred with a magnetic stirrer overnight with ethyl acetate (EtOAc; 6 l × 4). Concentration of the EtOAc layer (24 l) under reduced pressure yielded the EtOAc-soluble fraction (455.5 g). The aqueous layer was successively extracted with *n*-butanol (*n*-BuOH; 6 l × 4) by a similar method to that above to give the *n*-BuOH-soluble fraction (438.7 g). The aqueous layer was then lyophilized to obtain the aqueous-soluble fraction (495.0 g). Of the three fractions, the *n*-BuOH-soluble fraction showed strong protective activity against D-GalN/LPS-induced liver injury. Part of this fraction (80 g) was subjected to Sephadex LH-20 column chromatography (7.4 × 55 cm) and gradient elution with 30% (2 l), 30% (0.5 l), 30% (0.5 l), 30% (0.5 l), 30% (2.5 l), 60% (2 l), 80% (2 l), and 100% (4 l) MeOH in water to give fr. 1 (2 g), fr. 2 (15 g), fr. 3 (16.5 g), fr. 4 (15.2 g) fr. 5 (5.5 g), fr. 6 (2.6 g) fr. 7 (9.6 g) and fr. 8 (4 g). Fr. 5 exhibited the strongest activity of all fractions and was further purified by silica-gel column chromatography (4 × 30 cm) with 5—15% MeOH in $CHCl_3$ as eluant to give tetrahydroswertianolin (**1**) (950 mg). The structure of **1** was determined by chemical and spectroscopic methods. A portion (2 g) of fr. 4, which showed mild activity, was also subjected to rechromatography on an Iatrobead column (2.5 × 30 cm) and eluted with 10—20% MeOH in $CHCl_3$ to obtain **2** (230 mg) and **3** (714 mg). These were identified as gentiopicroside (**2**) and sweroside (**3**) by comparison of their ^{1}H- and ^{13}C-NMR spectroscopic data, and other physiochemical data with the literature.[13]

Tetrahydroswertianolin (1) A yellow amorphous solid, $[\alpha]_D^{20}$ +8.0° (MeOH, c=0.2), UV λ_{max} nm (log ε): 210 (3.93), 233 (4.03), 252 (4.19), 258 (4.18), 293 (3.77), 325 (3.51), IR ν_{max} (KBr) cm^{-1}: 3380, 2890, 1660, 1450, 1070, Positive ion FAB-MS m/z: 441 $[M+H]^+$, Molecular formula $C_{20}H_{24}O_{11}$ from High-resolution FAB-MS (Found 441.1389, Calcd 441.1397). ^{1}H-NMR (CD_3OD) δ: 1.79 (1H, br tt, J=13.5, 3.0 Hz, 7-H$_{ax}$), 2.11 (2H, m, 6-H), 2.30 (1H, dq, J=13.5, 3.0 Hz, 7-H$_{eq}$), 3.17 (1H, dd, J=9.0, 8.0 Hz, 2′-H), 3.30 (1H, m, 4′-H), 3.34 (1H, m, 5′-H), 3.41 (1H, t, J=9.0 Hz, 3′-H), 3.70 (1H, dd, J=12.0, 5.0 Hz, 6′-Ha), 3.86 (3H, s, 3-OCH$_3$), 3.89 (1H, dd, J=12.0, 2.0 Hz, 6′-Hb), 4.59 (1H, dd, J=9.5, 7.0 Hz, 5-H), 4.68 (1H, d, J=8.0 Hz, 1′-H), 4.95 (1H, t, J=3.0 Hz, 8-H), 6.29 (1H, d, J=2.0 Hz, 2-H), 6.59 (1H, d, J=2.0 Hz, 4-H), 12.45 (1H, s, 1-OH), ^{13}C-NMR (CD_3OD) in Table 1. Assignment of ^{1}H- and ^{13}C-NMR signals were confirmed by ^{1}H–^{1}H, ^{1}H–^{13}C, and ^{1}H–^{13}C long-range COSY.

Enzymatic Hydrolysis of 1 A mixture containing **1** (20 mg), ethanol (5 ml), 0.2 M K_2HPO_4–0.1 M citric acid buffer (pH 4.0) (10 ml) and 0.5% nariginase (10 ml) was incubated for 17 h with gentle stirring at 37 °C. The reaction mixture was partitioned with $CHCl_3$ (10 ml × 3). The $CHCl_3$ layer was evaporated and purified by preparative TLC with 80% EtOAc/benzene as developing solvent to give compound **1a** (7.2 mg).

Tetrahydrobellidifolin (1a) A pale yellow amorphous powder, $[\alpha]_D^{20}$ +13.5° (MeOH, c=0.2), EI-MS m/z: 278 $[M]^+$. ^{1}H-NMR (CD_3OD) δ: 1.84 (1H, br tt, J=13.5, 3.5 Hz, 7-H$_{ax}$), 1.98 (1H, br dd, J=13.5, 3.5 Hz, 7-H$_{eq}$), 2.06 (1H, m, 6-H$_{eq}$), 2.13 (1H, m, 6-H$_{ax}$), 3.87 (3H, s, 3-OCH$_3$), 4.59 (1H, dd, J=6.0, 9.0 Hz, 5-H), 4.93 (1H, t, J=3.5 Hz, 8-H), 6.32 (1H, d, J=2.2 Hz, 2-H), 6.52 (1H, d, J=2.2 Hz, 4-H), ^{13}C-NMR (CD_3OD) in Table 1. Assignment of ^{1}H- and ^{13}C-NMR signals was confirmed by ^{1}H–^{1}H, ^{1}H–^{13}C, and ^{1}H–^{13}C long-range COSY.

Table 1. ^{13}C-NMR Data of Tetrahydroswertianolin (**1**), Tetrahydrobellidifolin (**1a**), Bellidifolin (**4a**), and 1-Hydroxy-3-methoxyxanthone (**5**)

Carbon	**1**[a]	**1a**[a]	**4a**[a]	**5**[b]
1	163.05 (s)	163.13 (s)	164.15 (s)	157.50 (s)
2	99.16 (d)	99.06 (d)	98.47 (d)	97.03 (d)
3	167.42 (s)	167.39 (s)	169.07 (s)	163.58 (s)
4	93.50 (d)	93.40 (d)	93.98 (d)	92.83 (d)
4a	159.04 (s)	159.04 (s)	159.42 (s)	156.03 (s)
4b	168.66 (s)	167.52 (s)	145.15 (s)	166.75 (s)
5	67.50 (d)	62.10 (d)	138.42 (s)	117.56 (d)
6	27.35 (t)	27.39 (t)	124.98 (d)	134.98 (d)
7	27.92 (t)	28.78 (t)	110.65 (d)	123.99 (d)
8	71.11 (d)	67.34 (d)	154.40 (s)	125.87 (d)
8a	118.04 (s)	119.62 (s)	108.83 (s)	120.64 (s)
9	183.09 (s)	183.18 (s)	186.05 (d)	180.81 (s)
9a	106.22 (s)	106.37 (s)	103.55 (s)	103.60 (s)
1′	105.18 (d)	—	—	—
2′	75.66 (d)	—	—	—
3′	77.84 (d)	—	—	—
4′	71.53 (d)	—	—	—
5′	78.13 (d)	—	—	—
6′	62.76 (t)	—	—	—
3-OCH$_3$	56.51 (q)	56.45 (q)	56.63 (q)	55.81 (q)

Chemical shifts in δ ppm, measured in *a*) CD_3OD or *b*) $CDCl_3$. The multiplicities of carbon signals were determined by means of the DEPT method, and indicated as s, d, t, q for singlet, doublet, triplet and quartet, respectively.

Acid Hydrolysis of 1 Compound **1** (100 mg) was hydrolyzed in 5% HCl (3 ml) for 2 h at 100 °C. The reaction mixture was partitioned with EtOAc (10 ml × 3). Using preparative TLC as above, compound **5** (7.7 mg) was obtained from the EtOAc layer. The aqueous layer was lyophilized *in vacuo* to give a residue. The residue was developed with EtOAc–MeOH–H_2O–AcOH (13 : 3 : 3 : 6) on TLC and identified as glucose by comparing its *Rf* value with that of an authentic sample.

Preparation of α-Methoxy-α-trifluoromethylphenylacetyl (MTPA) Ester of 1 *S*-(−)- or *R*-(+)-MTPA-Cl (91 mg) was added to a solution containing **1** (20 mg) and pyridine (300 μl). After stirring for 12 h at room temperature, the reaction mixture was purified by preparative TLC with 5% EtOAc/Benzene to give an *R*-MTPA ester (**1b**; 4.4 mg) or *S*-MTPA ester (**1c**; 13 mg) of **1**.

R-MTPA Ester (**1b**): ^{1}H-NMR (CD_3OD): 2.05 (1H, dq, J=13.0, 2.0 Hz, 6-H$_\alpha$), 2.13 (1H, m, 6-H$_\beta$), 6.76 (1H, d, J=2.5 Hz, 4-H).

S-MTPA Ester (**1c**): ^{1}H-NMR (CD_3OD): 2.08 (1H, dq, J=13.0, 2.0 Hz, 6-H$_\alpha$), 2.22 (1H, m, 6-H$_\beta$), 6.58 (1H, d, J=2.5 Hz, 4-H).

Animals and Treatments Male ddY mice, 6 weeks old, weighing 30—32 g were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan, and maintained under a 12 h light/dark cycle in a temperature- and humidity-controlled room. The animals were allowed free access to laboratory pellet chow (CE2; CLEA Japan Inc., Tokyo, Japan) and water *ad libitum* before the experiment. Liver injury was produced in the 12 h fasted mice by intraperitoneal injection of 700 mg/kg D-GalN and 10 μg/kg LPS as previously described.[11] The samples derived from *S. japonica* were administered orally or subcutaneously before D-GalN/LPS challenge. Physiological saline and glycyrrhizin were used as a negative and positive control, respectively. The ALT was measured at 8 h after D-GalN/LPS challenge.

Statistical Analysis All values were expressed as means ± S.E. for n experiments. Student's t-test for unpaired observations between control and tested samples was carried out to identify statistically significant differences; a p value less than 0.05 was considered statistically significant.

Results and Discussion

The hepatoprotective activity of the EtOAc-, *n*-BuOH- and aqueous-soluble fractions from the 70% ethanol extract of *S. japonica* were examined in the D-GalN/LPS-induced liver injury model (Fig. 1). These samples were administered s.c. at a dose of 200 mg/kg, 18 and 2 h, before D-GalN/LPS challenge. Of the three fractions, the *n*-

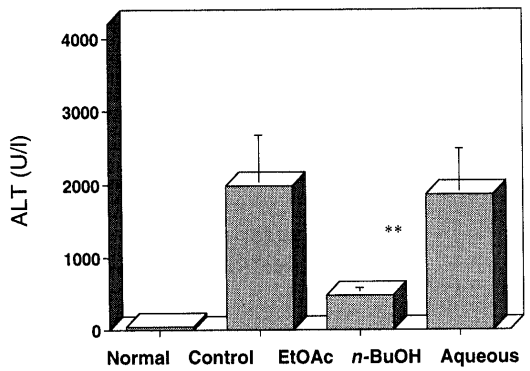

Fig. 1. Effect of EtOAc, *n*-BuOH and Aqueous Soluble Fraction from *S. japonica* on D-GalN/LPS-Induced Liver Injury in Mice

The results are expressed as means ± S.E., $n=3$ (normal) or 8. Significantly different from control, ** $p<0.01$. Each sample was administered s.c., at a dose of 200 mg/kg, 18 and 2 h, before D-GalN/LPS challenge.

Table 2. Effect of the Fr. 1 to Fr. 8 on D-GalN/LPS-Induced Liver Injury in Mice

Group	*n*	ALT level (U/l)	ALT decrease (%)
Normal	3	66 ± 17	—
Control 1	8	6423 ± 1247	—
Fr. 1	8	6201 ± 1301	3.5
Fr. 2	8	6063 ± 658	5.7
Fr. 3	8	3987 ± 1190	38.3
Fr. 4	8	3388 ± 1094*	47.7
Fr. 5	8	1627 ± 532**	75.4
Control 2	10	3401 ± 865	—
Fr. 6	9	3172 ± 865	6.9
Fr. 7	9	4763 ± 1543	<0.0
Fr. 8	9	2741 ± 916	19.8

Significantly different from control, * $p<0.05$, ** $p<0.01$. Each fraction of *S. japonica* was administered s.c. at a dose of 100 mg/kg, 18 and 2 h, before D-GalN/LPS challenge.

BuOH-soluble fraction significantly inhibited ALT elevation, an indicator of liver injury, by 72.7%. Therefore, chemical analysis of this fraction was carried out. Part of the *n*-BuOH-soluble fraction was separated into eight fractions (fr. 1 to fr. 8) by Sephadex LH-20 column chromatography. As shown in Table 2, fr. 4 and fr. 5 (each 100 mg/kg, s.c. × 2) exhibited significant activity. Fr. 4 and fr. 5 were further purified as described in Materials and Methods and, thus, hepatoprotective activity-guided fractionation finally gave us three compounds, **1**, **2** and **3**, as active constituents. The structure of these compounds was determined by chemical and spectroscopic methods. Two of them, **2** and **3**, were identified as gentiopicroside and sweroside by comparing their ^{1}H- and ^{13}C-NMR spectra and other physiochemical data with the literature.[13] Compound **1** was found to be a new tetrahydroxanthone derivative.

Tetrahydroswertianolin (1) Compound **1** had a quasi-molecular ion peak at *m/z* 441 in positive ion FAB-MS. The molecular formula was determined to be $C_{20}H_{24}O_{11}$ by high-resolution FAB-MS. There was IR absorption at 1660 cm^{-1} due to the presence of a carbonyl group. The ^{1}H-NMR data coupled with detailed analysis of ^{1}H–^{1}H COSY indicated the presence of 19 proton signals: a set of *meta*-coupled aromatic protons at δ 6.29 and 6.59 (each 1H, d), methylene protons at δ 2.11 (2H, m) coupling with other methylene protons at δ 1.79 (1H, br tt) and 2.30 (1H, dq), two oxygen-bearing methine protons at δ 4.59 (1H, dd) and 4.95 (1H, t), and seven protons due to the sugar moiety. In addition, methoxy protons at δ 3.86 (3H, s) and a hydroxy proton at δ 12.45 (1H, s) were observed. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed 20 carbon signals: eight carbon signals were similar to those of the partial structure of swertianolin (**4**)[14] and its aglycone, bellidifolin (**4a**),[9] except for two quaternary carbons (δ 118.04, 168.66), two methylene carbons (δ 27.35, 27.92), and two methine carbons bonded to oxygen (δ 67.50, 71.11) (Table 1).

Treatment of **1** with β-glucosidase gave an aglycone (**1a**) and glucose. Acid hydrolysis of **1** with 5% HCl mainly gave 1-hydroxy-3-methoxyxanthone (**5**) instead of **1a**, together with glucose.

These data and detailed ^{1}H- and ^{13}C-NMR studies of **1** with the aid of ^{1}H–^{1}H and ^{1}H–^{13}C COSY led us to conclude that **1** may be a tetrahydroxanthone glucoside. However, the assignment of the tetrahydrobenzene ring and the connecting position of the glucose were still unclear. Therefore, a ^{1}H–^{13}C long-range COSY experiment was performed. The anomeric carbon at δ 105.18 in the sugar moiety showed a cross peak with the methine proton at δ 4.95, which was assigned to be 8-H due to the cross peak with the carbonyl carbon at δ 183.09; another methine proton at δ 4.59 was 5-H. Furthermore, the quaternary carbon at δ 168.66 was assigned as C4b due to the cross peak with 5-H and the quaternary carbon at δ 118.04 was C8a due to the cross peak with 7-H and 8-H. Thus, the planar structure of this compound was indicated to be **1**.

The relative configuration of **1** was determined by analysis of the *J*-value from ^{1}H-NMR and an NOE experiment. The coupling constant between 7-H and 8-H was small ($J=3.0$ Hz) and, thus, 8-H is quasi-equatorial and the 8-*O*-glucosyl moiety is quasi-axial. On the other hand, 5-H is quasi-axial because it has a large coupling constant (dd, $J=9.5$, 7.0 Hz). Irradiation of the 7-H_{ax} proton (δ 1.79) enhanced the signal intensity of 5-H (δ 4.59) and 8-H (δ 4.95) while, on irradiation of the 7-H_{eq} (δ 2.30), no NOE effect was observed. These NOE data suggest that 5-H and 8-H were in the *cis* configuration. Thus, Dreiding model analysis together with the analysis of the *J*-value from ^{1}H-NMR and NOE experiments suggest that the tetrahydrobenzene ring in **1** might be in a distorted half-chair conformation with an equatorial 5-OH and axial 8-*O*-glucose.

To determine the absolute configuration of **1**, Mosher's method[15] was applied. Esterification of **1** with *R*-(+)-MTPA or *S*-(−)-MTPA chloride afforded an MTPA ester (**1b** or **1c**) (Chart 2). The chemical shift due to the 6-H_α and 6-H_β signals of the S-MTPA ester was shifted downfield by 0.03 and 0.09 ppm compared with that of the *R*-MTPA ester, otherwise, the 4-H signal of the *S*-MTPA ester was shifted upfield by 0.18 ppm compared with that of the *R*-MTPA ester. Based on these spectral data, the absolute configuration of C-5 was concluded to be (*R*).

From the above spectral data, the structure was determined to be as represented by the formula **1**.

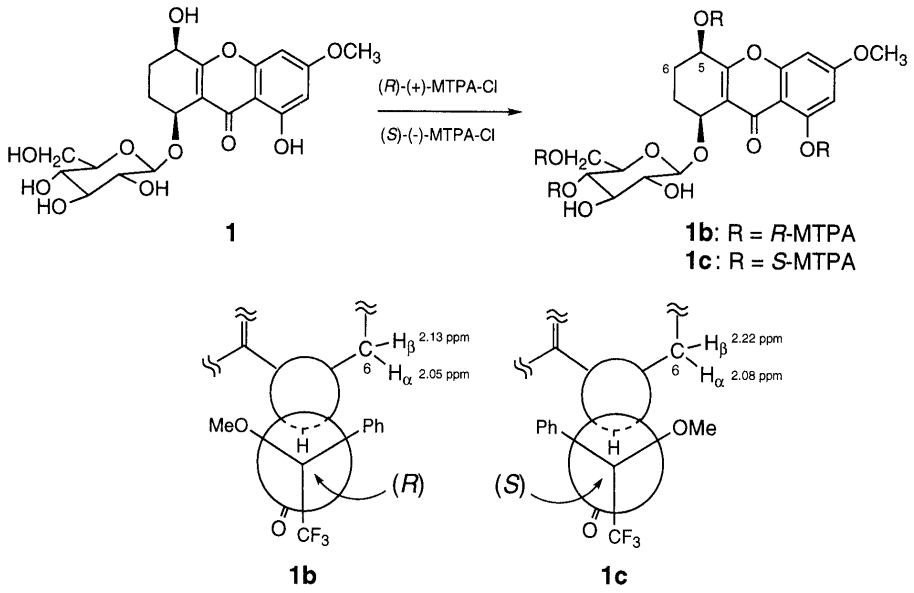

Chart 2

Table 3. Effect of Compounds **1**, **2** and **3** on D-GalN/LPS-Induced Liver Injury in Mice

Group	Dose (mg/kg)	ALT level (U/l)	ALT decrease (%)
Normal	—	45± 21	—
Control 1	—	2843±702	—
Glycyrrhizin	100	1122±453*	61.5
Tetrahydroswertianolin (**1**)	25	942±296*	67.9
	50	490±121**	84.1
Control 2	—	2347±554	—
Gentiopicroside (**2**)	25	1285±318	46.1
	50	1965±522	16.6
Sweroside (**3**)	25	855±194*	64.8
	50	1425±637	40.0

Significantly different from control, $*p<0.05$, $**p<0.01$. Each sample was administered s.c., 18 and 2 h, before D-GalN/LPS challenge.

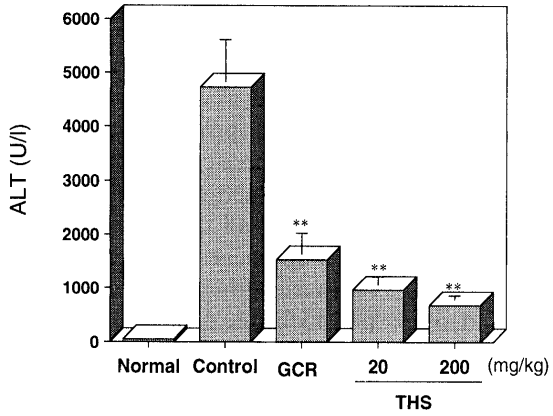

Fig. 2. Effect of Tetrahydroswertianolin (THS; **1**) and Glycyrrhizin (GCR) on D-GalN/LPS-Induced Liver Injury in Mice

The results are expressed as means±S.E., $n=3$ (normal), 9 or 10 (control). Significantly different from control, $**p<0.01$. Each sample was administered *p.o.*, once a day for one week before D-GalN/LPS challenge. Glycyrrhizin was administered at a dose of 200 mg/kg as a positive control.

Hepatoprotective Activity of 1, 2 and 3 The hepatoprotective activity of **1**, **2** and **3** was examined using host response-dependent liver injury induced by D-GalN/LPS in mice. Each sample was administered subcutaneously, 18 and 2 h, before D-GalN/LPS challenge. The ALT level was measured at 8 h after intoxication. Compounds **2** and **3**, two representative iridoids in *Swertia* spp.[6,16] have been reported to exhibit a mild hepatoprotective effect against some experimental liver injury models induced by LPS/bacillus Calmette-Guerin, carbon tetrachloride or Cd.[17] As shown in Table 3, they also exerted a moderate hepatoprotective activity in the present D-GalN/LPS-liver injury model. In contrast, compound **1**, which has a very rare, partially saturated, xanthone structure exhibited very strong activity. Subcutaneous administration of **1** at doses of 25 and 50 mg/kg suppressed ALT elevation by 67.9 and 84.0%. This effect was significant and dose-dependent (Table 3). Moreover, oral administration of **1** at doses of 20 and 200 mg/kg once a day for one week also proved to be very effective (Fig. 2) inhibiting the ALT elevation by 80.3 and 90.7%. The hepatoprotective effect of **1** was stronger than that of 100 (s.c.) or 200 (*p.o.*) mg/kg glycyrrhizin, a drug used clinically to treat liver diseases in Japan (Table 3, Fig. 2).[18]

S. japonica is well known to be rich in xanthones such as swertianolin and bellidifolin.[9,14] However, the EtOAc soluble fractions, fr. 6, fr. 7 and fr. 8 which contain a high proportion of xanthones showed only weak activity. Thus, a further study was designed to find the active center of tetrahydroswertianolin (**1**). The hepatoprotective activity of the aglycone (tetrahydrobellidifolin; **1a**) and its derivative (1-hydroxy-3-methoxyxanthone; **5**) was investigated. In the control group, the ALT level was 1989±689 U/l, whereas in **1a** (25 mg/kg, s.c., ×2)-treated group, it was significantly reduced to 477±98 U/l (Fig. 3). In contrast, the same dosage of **5** showed no activity (ALT=1865± 624 U/l). These results suggest that the tetrahydrobenzene-ring moiety is important for the hepatoprotective activity of tetrahydroswertianolin (**1**).

The present study provides the first report of the *in vivo*

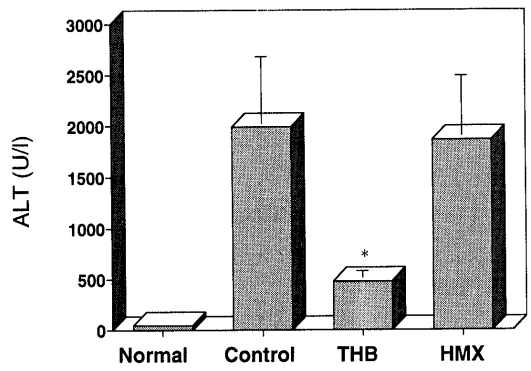

Fig. 3. Effect of Tetrahydrobellidifolin (THB; **1a**) and 1-Hydroxy-3-methoxyxanthone (HMX; **5**) on D-GalN/LPS-Induced Liver Injury in Mice

The results are expressed as means ± S.E., $n=3$ (normal) or 8. Significantly different from control, $* p<0.01$. Each sample was administered s.c., at a dose of 25 mg/kg, 18 and 2 h, before D-GalN/LPS challenge.

hepatoprotective activity of *S. japonica* in an immunological liver injury model. A new tetrahydroxanthone, tetrahydroswertianolin (**1**), which has a rare, partially saturated, xanthone frame, was isolated and proved to be effective in this model. The whole plants of *Swertia japonica* and its active constituent, tetrahydroswertianolin (**1**) may be useful for the clinical treatment of liver diseases. Further pharmacological studies on the hepatoprotective mechanism are in progress in our laboratory.

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