TETRAHYDROSWERTIANOLIN: A POTENT HEPATOPROTECTIVE AGENT FROM *SWERTIA JAPONICA* MAKINO

Koji HASE,[a] Shigetoshi KADOTA,*[,a] Purusotam BASNET,[a] Jianxin LI,[a] Shuichi TAKAMURA,[b] and Tsuneo NAMBA[a]

Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines),[a] Toyama Medical and Pharmaceutical University, 2630-Sugitani, Toyama 930-01, Japan and Health Science Laboratories,[b] Yamanouchi Pharmaceutical Co., Ltd., 2-3-11, Nihonbashi-Honcho, Chuo-ku, Tokyo 103, Japan

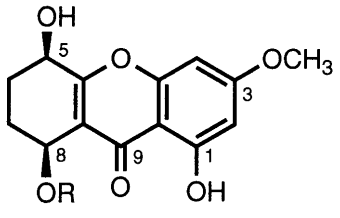
Tetrahydroswertianolin (**1**), a new tetrahydroxanthone glycoside, was isolated from the whole plant of *Swertia japonica* MAKINO (Gentianaceae). Its structure was determined by chemical and spectroscopic methods including 2D-NMR. This compound was found to be very effective in immunologically induced liver injury in mice.

KEY WORDS *Swertia japonica*; Gentianaceae; tetrahydroswertianolin; tetrahydroxanthone; D-galactosamine/lipopolysaccharide-induced liver injury

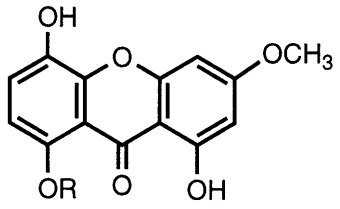
Swertiae Herba, the whole plant of *Swertia spp.*, has been widely used in Ayurvedic and Unani medicines as an anthelminthic, febrifuge, and liver tonic. In Japan, *Swertia japonica* MAKINO is a popular medicinal herb for stomach complaints. In serial research on hepatoprotective agents from natural sources,[1] *S. japonica* showed a significant hepatoprotective activity, and activity-guided fractionation yielded a new tetrahydroxanthone from this plant. This communication deals with the structure elucidation of tetrahydroswertianolin, a saturated analogue of swertianolin, and its hepatoprotective activity against immunological liver injury.

The shade-dried whole plant (5 kg) was extracted with 70% aqueous ethanol to obtain aqueous ethanolic extract (1330 g). This extract was partitioned into EtOAc (455.5 g)-, *n*-BuOH (438.7 g)-, and aqueous (495.0 g)-soluble fractions, respectively. Among the three fractions, the *n*-BuOH-soluble fraction showed strong protective activity against D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-induced liver injury. Therefore, a part (80 g) of this fraction was subjected to Sephadex LH-20 column chromatography and eluted with 30-80% MeOH in water to give eight fractions. Since the 30% MeOH eluate (5.5 g) exhibited the strongest activity of all fractions, it was further purified by silica gel column chromatography. Thus activity-guided fractionation finally gave tetrahydroswertianolin (**1**) (950 mg). The structure of **1** was determined by chemical and spectroscopic methods.

Tetrahydroswertianolin (**1**), a yellow amorphous solid, showed $[\alpha]_D^{20}$ +8.0° (MeOH, c = 0.2). The positive ion FAB-mass of **1** exhibited a quasi-molecular ion peak at *m/z* 441, and its molecular formula was determined to be $C_{20}H_{24}O_{11}$ {*m/z*: 441.1389 $[M+H]^+$, calcd 441.1397} by high-resolution FAB-MS. Its IR spectrum showed a sharp peak at λ_{max} 1660 cm^{-1} (CO). The ^{1}H-NMR data[2] coupled with detailed analysis of ^{1}H-^{1}H COSY indicated the presence of 19 proton signals: *meta*-coupled aromatic protons at δ_H 6.29 and 6.59 (each 1H, d, J = 2.0 Hz), methylene protons at δ_H 2.11 (2H, m) coupling with other methylene protons at δ_H 1.79 (1H, br tt, J = 13.5, 3.0 Hz) and 2.30



1 : R = β-D-glucopyranosyl
1a : R = H



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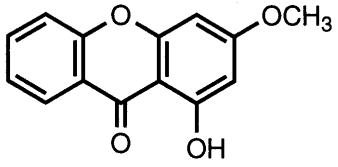
3 : R = H



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* To whom correspondence should be addressed.

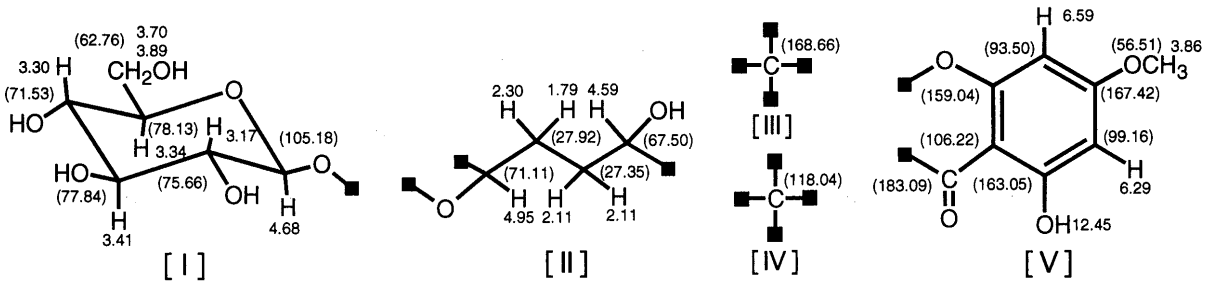

Fig. 1. Partial Structures Observed by ^{1}H and ^{13}C-NMR, ^{1}H-^{1}H COSY, and ^{1}H-^{13}C COSY Spectra of **1**

(1H, dq, J = 13.5, 3.0 Hz), two oxygen-bearing methine protons at δ_H 4.59 (1H, dd, J = 9.5, 7.0 Hz) and 4.95 (1H, t, J = 3.0 Hz), and seven protons due to the sugar part. In addition, methoxy protons at δ_H 3.86 (3H, s) and a hydroxy proton at δ_H 12.45 (1H, s) were observed. The ^{13}C-NMR and DEPT spectra[2] showed 20 carbon signals: eight carbon signals were similar to those of the partial structure of swertianolin (**2**) and its aglycone, bellidifolin (**3**),[3] except for two quaternary carbons (δ_C 118.04, 168.66), two methylene carbons (δ_C 27.35, 27.92), and two methine carbons bonded to oxygen (δ_C 67.50, 71.11).

Treatment of **1** with β-glucosidase gave **1a** and glucose. Acid hydrolysis of **1** with 5% HCl, 1-hydroxy-3-methoxyxanthone (**4**) was mainly obtained 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 with partial structures [I, II, III, IV, and V] as shown in Fig. 1.

The linkage of partial structures was confirmed by a ^{1}H-^{13}C long-range COSY experiment. The anomeric carbon at δ_C 105.18 in the sugar moiety showed a cross peak with the methine proton at δ_H 4.95 in structure [II]. Furthermore, this proton showed cross peaks with the carbonyl carbon at δ_C 183.09 in structure [V], and two quaternary carbons at δ_C 168.66 and 118.04 in structures [III] and [IV]. Also, some of the significant ^{1}H-^{13}C long-range correlations are indicated by arrows in the formula in Fig. 2-A. Thus the planar structure of this compound was shown to be **1**.

The relative configuration of **1** was determined by an NOE experiment. On irradiation of the 7-H_{ax} proton (δ_H 1.79), an NOE was observed at 5-H (δ_H 4.59) and 8-H (δ_H 4.95), while in contrast, on irradiation of the 7-H_{eq} (δ_H 2.30), no NOE was observed (Fig 2-B). These NOE data suggest that 5-H and 8-H were in *cis* configuration, which was further supported by the J-value of ^{1}H-NMR. 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, 7.3 Hz). 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 distorted half-chair conformation with equatorial 5-OH and axial 8-*O*-glucose.

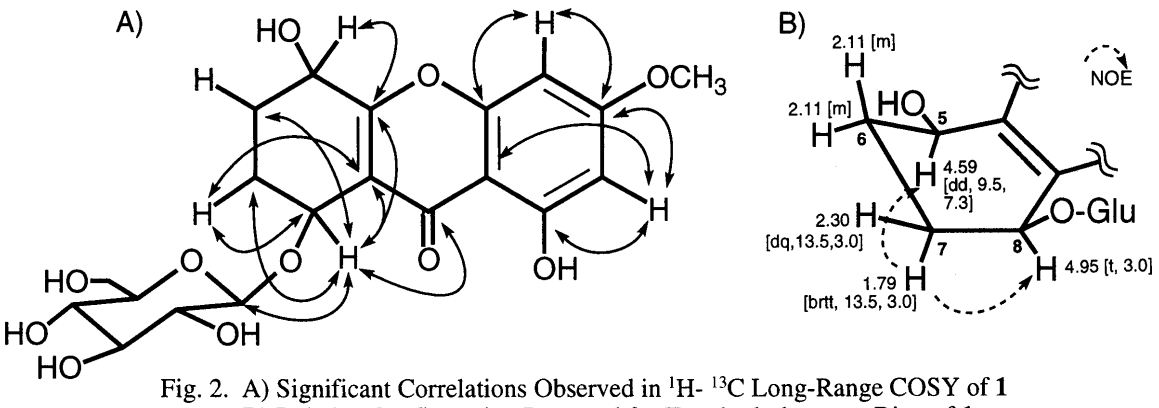

Fig. 2. A) Significant Correlations Observed in ^{1}H- ^{13}C Long-Range COSY of **1**
B) Relative Configuration Proposed for Tetrahydrobenzene Ring of **1**

Mosher's method[4] applied to α-methoxy-α-trifluoromethylphenylacetyl (MTPA) esters of **1** led to the confirmation of the (*R*)-configuration of the 5-position, since the chemical shift due to the 5-H signal of the (*S*)-MTPA ester was shifted upfield by 0.18 ppm compared with that of the (*R*)-MTPA ester, and that of the 6-Ha and 6-Hb signals of the (*S*)-MTPA ester was shifted downfield by 0.03 and 0.09 ppm compared with that of the (*R*)-MTPA ester. Based on these spectral data, the absolute configuration of tetrahydroswertianolin was determined as represented by formula **1**.

There are very few partially saturated xanthones as natural products, and this is the first report to find such a compound in *Swertia spp.* We investigated its hepatoprotective activity against immunologically induced liver injury. Co-administration of D-GalN (700 mg/kg) and LPS (10 μg/kg) is known to induce liver injury through the immune response in mice.[5] The extent of liver damage was expressed in terms of the alanine aminotransferase (ALT) level. In the normal group, the blood ALT level was 44.5 ± 2.3 (mean ± SE) U/l, whereas in the control group, it was elevated to 2843 ± 702 U/l 8 h after D-GalN/LPS challenge. When **1** (25 or 50 mg/kg, *s.c.*) was administered 18 and 2 h before D-GalN/LPS challenge, the blood ALT level was 942 ± 296 ($p<0.05$ vs. control) or 490 ± 121 U/l ($p<0.05$), respectively. On the other hand, in the glycyrrhizin (100 mg/kg, *s.c.*)-treated group, it was 1121 ± 453 U/l ($p<0.05$). These data clearly suggest that **1** showed a significant and dose-dependent hepatoprotective effect in this D-GalN/LPS-induced liver injury model. Its effect was stronger than that of glycyrrhizin as a positive control. Furthermore, the hepatoprotective activity of aglycone (**1a**) and its derivative (**4**) also investigated. In controls, the ALT level was 1989 ± 689 U/l, whereas in the **1a** (25 mg/kg, *s.c.*)-treated group, it was significantly suppressed to 477 ± 98 U/l ($p<0.05$). In contrast, **4** (25 mg/kg, *s.c.*) treatment did not show any activity (1865 ± 624 U/l). The results clearly suggest that the tetrahydrobenzene-ring structure is essential for the hepatoprotective activity of tetrahydroswertianolin (**1**). Further studies on the hepatoprotective mechanism are in progress in our laboratory.

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2) 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; ^{1}H-NMR (CD_3OD) δ: 1.79 (1H, br tt, J = 13.5, 3.0 Hz, 7-Hax), 2.11 (2H, m, 6-H), 2.30 (1H, dq, J = 13.5, 3.0 Hz, 7-Heq), 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-OC<u>H</u>$_3$), 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-O<u>H</u>); ^{13}C-NMR (CD_3OD) δ: 27.35 (t, 6-C), 27.92 (t, 7-C), 56.51 (q, 3-O<u>C</u>H$_3$), 62.76 (t, 6'-C), 67.50 (d, 5-C), 71.11 (d, 8-C), 71.53 (d, 4'-C), 75.66 (d, 2'-C), 77.84 (d, 3'-C), 78.13 (d, 5'-C), 93.50 (d, 4-C), 99.16 (d, 2-C), 105.18 (d, 1'-C), 106.22 (s, 9a-C), 118.04 (s, 8a-C), 159.04 (s, 4a-C), 163.05 (s, 1-C), 167.42 (s, 3-C), 168.66 (s, 4b-C), 183.09 (s, 9-C). 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.

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