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Xanthone Glucosides of *Swertia japonica* MAKINO and a Related Plant: Structure of a New Glucoside, Isoswertianolin and Structure Revision of Swertianolin and Norswertianolin

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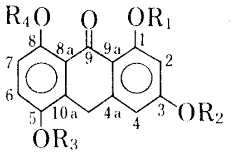
A new xanthone glucoside named isoswertianolin (**2**) was isolated from *Swertia japonica* and its structure was elucidated to be 5-*O*-β-glucopyranoside of bellidifolin by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. In connection with this study, the structure of swertianolin (**1**), a constituent of this plant, was reinvestigated by NMR spectroscopy, leading to the revision of its structure (the location of its glucosyl linkage) from 1-*O*-β-glucopyranoside of bellidifolin (**3**) to 8-*O*-β-glucopyranoside of **3**. It follows that **1** is identical with the glucoside isolated from *Gentiana campestris* by Kaldas *et al.* in 1974. Analogously, the structure of norswertianolin (**6**), previously isolated from *Swertia randaiensis* HAYATA, was also revised, being formulated as 8-*O*-β-glucopyranoside of desmethylbellidifolin (**7**).

Keywords——*Swertia japonica*; *Swertia randaiensis*; Gentianaceae; xanthone glucoside; isoswertianolin; swertianolin; structure revision; norswertianolin; ^{1}H NMR; ^{13}C NMR

Swertiae Herba (*Swertia japonica* MAKINO, Gentianaceae) is a well-known Japanese folk medicine named "Senburi or Toh-yaku." From a glycoside fraction of this plant, several bitter secoiridoid glucosides,[1] flavone-*C*-glucosides[2] and a xanthone glucoside, swertianolin (**1**),[3] have been isolated. During our study on the separative analysis of these glucosides by high performance liquid chromatography,[4] a new xanthone glucoside (**2**) was isolated from this plant. Further, in connection with the structure determination of **2**, the structure of **1** was reinvestigated.

An aqueous suspension of the methanolic extract of Swertiae Herba was washed with ether and then extracted with ethyl acetate. The ethyl acetate layer was concentrated to dryness and the resulting residue was chromatographed repeatedly to give **2** in a yield of 0.015%, pale yellow needles, mp 259°C, $C_{20}H_{20}O_{11}\cdot 2H_2O$ (by elemental analysis and FD mass spectroscopy) along with **1**. On acid hydrolysis, **2** afforded glucose and a yellow pigment which was identified as bellidifolin (**3**), the aglycone of **1**.[3] In the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of **2** (Tables I and II), an anomeric proton signal at δ 4.90 (1H d, $J=6.0$ Hz) and a set of signals between δ 60.8 and 102.3 indicated the presence of a β-glucopyranosyl unit. By reference to Markham's study on the ^{1}H NMR of hydroxy xanthone derivatives,[5] the proton signals of **3** were unambiguously assigned as shown in Table I. On going from **3** to **2**, the proton signal due to H-6 was displaced downfield by 0.39 ppm and the signals due to H-4 and -7 were slightly deshielded, while the signals attributable to H-2 and the 3-methoxyl group remained almost unshifted. This indicated that the β-glucopyranosyl group of **2** must be located at its 5-hydroxyl group. The formulation of **2** as 5-*O*-β-glucopyranoside of **3** was further substantiated by its ^{13}C NMR spectrum (Table II). Carbon signal assignments of a number of hydroxyxanthone derivatives have been investigated by Westerman *et al.*[6] and Chaudhuri *et al.*[7] By reference to these papers, carbon signals of **3** were assigned as shown in Table II and compared with those of **2**. It is known that glycosylation of a phenolic hydroxyl group results in the downfield shift of the carbon resonances due to the *ortho-* and *para-*carbons.[8] On going from **3** to **2**, signals due to C-6, -8 and -10a were deshielded by 2.1—2.6 ppm, while other signals were almost unaffected. The name "isoswertianolin" is now proposed for **2**.

Previously, the glucosyl group of **1** was reported to be located at its 1-hydroxyl group (**1′**) by the classical procedure, *i.e.*, on the basis of methylation of **3** and demethylation of trimethylbellidifolin (**4**).[9] The structure of **1** was reinvestigated by means of ^1H and ^{13}C NMR spectroscopy. A comparison of the ^1H NMR spectrum of **1** with that of **3** (Table I) showed that the signal due to H-7 was significantly deshielded, while all of the other signals remained almost unaffected. In the ^{13}C NMR spectrum, on going from **3** to **1**, signals due to C-5, -7, -8a, -9a and -10a were deshielded and those due to C-6, -8 and -9 were somewhat shielded, while no significant displacements were observed for signals assignable to C-1—C-4 and -4a. These findings are inconsistent with the former proposal for the structure (**1′**) of **1**, leading to a revision of the location of the glucosyl group to the 8-hydroxyl group. In 1974, Kaldas *et al.* isolated a xanthone glucoside from *Gentiana campestris* L. (Gentianaceae) and assigned the structure as 8-*O*-β-glucopyranosyl bellidifolin,[10] the same structure as that proposed for **1** in



	R_1	R_2	R_3	R_4
1	H	Me	H	glc
1′	glc	Me	H	H
2	H	Me	glc	H
3	H	Me	H	H
4	Me	Me	Me	Me
5	Ac	Me	Ac	$glc(Ac)_4$
6	H	H	H	glc
7	H	H	H	H

glc: β-glucopyranosyl.
Ac: acetyl.

Chart 1

TABLE I. ^1H NMR Chemical Shifts in DMSO-d_6

	H-2 H-4	H-6 H-7	C-3-OMe	Anomeric H	Phenolic OAc
1	6.36, 6.57 (d, $J=2.4$ Hz)	7.30, 7.13 (d, $J=9.0$ Hz)	3.89	4.83 (d, $J=6.0$ Hz)	
2	6.39, 6.69 (d, $J=2.4$ Hz)	7.65, 6.72 (d, $J=9.0$ Hz)	3.89	4.90 (d, $J=6.0$ Hz)	
3	6.34, 6.56 (d, $J=2.4$ Hz)	7.26, 6.62 (d, $J=9.0$ Hz)	3.89		
5	6.55, 6.68 (d, $J=2.4$ Hz)	7.31, 6.96 (d, $J=9.0$ Hz)	3.89		2.43, 2.50[a] (3H each)
	6.53, 6.66[b] (d, $J=2.5$ Hz)	7.30, 6.95 (d, $J=9.5$ Hz)	3.87		2.40, 2.49[a] (3H each)
6	6.19, 6.39 (d, $J=2.2$ Hz)	7.25, 7.12 (d, $J=9.0$ Hz)		4.78 (d, $J=6.4$ Hz)	
7	6.22, 6.41 (d, $J=2.2$Hz)	7.24, 6.62 (d, $J=8.8$ Hz)			

[a] In $CDCl_3$.
[b] Reported by M. Kaldas, K. Hostettmann and A. J-Guillarmod.[10]

the present paper. The identification of **1** with Kaldas's glucoside was confirmed by comparison of the physical constants including the ^1H NMR signals of the peracetate (**5**) of **1** with those reported by Kaldas *et al.*[10] (Table I).

From *Swertia randaiensis* HAYATA (Japanese name: Niitakasenburi), Tomimori and Komatsu isolated a xanthone glucoside (**6**) named norswertianolin, for which the structure 1-*O*-β-glucopyranosyl-desmethylbellidifolin has been proposed.[11] A reinvestigation of the location of the glucosyl linkage of **6** was conducted by comparison of its ^1H and ^{13}C NMR spectra with those of its aglycone (desmethylbellidifolin (**7**)) in the same way as for **1** (see Tables I and II), leading to a revision of the structure of **6** to 8-*O*-β-glucopyranoside of **7**.

TABLE II. ^{13}C NMR Chemical Shifts in DMSO-d_6

Carbon No.	1	2	3	6	7
1	162.6	161.7	161.8	162.9	162.1
2	97.1	97.7	97.3	98.2	98.4
3	166.2	167.1	166.9	165.4	166.3
4	92.1	93.1	92.8	93.5	94.3
4a	156.3	157.3	157.2	156.4	157.3
10a	144.9	145.3	143.2	144.8	143.2
5	141.0	137.2	137.3	140.9	137.1
6	121.0	125.9	123.7	120.9	123.6
7	112.4	109.3	109.3	112.6	109.3
8	149.3	154.3	151.7	149.4	151.7
8a	111.8	107.3	107.4	111.9	107.3
9	180.9	183.7	183.9	180.7	183.5
9a	103.2	102.1	101.9	102.6	101.2
OMe	56.0	56.2	56.1		
G-1	103.4	102.3		103.4	
2	73.5	73.3		73.6	
3	76.0[a]	76.5[a]		76.1[a]	
4	69.7	69.7		69.8	
5	77.4[a]	77.2[a]		77.5[a]	
6	60.8	60.8		60.9	

[a] These signals may be reversed in each column.

Experimental

General Procedures——NMR spectra were taken in DMSO-d_6 on JEOL PFT-100 and Varian XL-100A spectrometers (internal standard: tetramethylsilane (TMS)), ^1H NMR at 100 MHz and ^{13}C NMR at 25.15 MHz. Melting points were taken on a micro hot stage and are uncorrected. EI and FD mass spectra (MS) were taken on a JEOL D-300 equipped with a combined electron impact/field ionization/FD ion source and a JEOL JMA-2000 data system. For FD mass spectrometry, commercially available silicone emitters were used at an ion source potential of +3 kV for the field anode and −6 kV for the slotted cathode plate. The ion source pressure was 3×10^{-7} Torr at room temperature; the sample was desorped by direct heating by using the supplied emitter heating current (0—27 mA) and controlling the emission of FD ions roughly by means of the ion monitor.

Extraction and Separation——Commercial Swertiae Herba (205 g) was extracted repeatedly with hot MeOH and the MeOH solution was concentrated to dryness. A suspension of the resulting MeOH extract (48.6 g) in H_2O was washed with Et_2O and then extracted with EtOAc. The EtOAc layer was concentrated to dryness and the residue (1.1 g) was subjected to column chromatography on polyamide by elution with H_2O and then with MeOH. The MeOH eluate was further chromatographed on silica gel (elution with $CHCl_3$–MeOH (95: 5) to give secoiridoid glucosides (amarogentin and amaroswerin), **2** (yield: 0.015%), **1** (yield: 0.12%) and finally the flavone-*C*-glucoside (swertisin).

The New Xanthone Glucoside (**2**): Pale yellow needles (from MeOH), mp 259°C, $[\alpha]_D^{10}$: −33.2° (c=0.16, C_5H_5N). *Anal.* Calcd for $C_{20}H_{20}O_{11}\cdot 2H_2O$: C, 50.84; H, 5.08. Found: C, 50.55; H, 4.86. FD MS *m/z*: 475 $[M+K]^+$, 459 ($[M+Na]^+$, base peak) and 437 $[M+H]^+$. UV λ_{max}^{EtOH} nm (ε): 333 (4600), 308 (sh, 5300), 273

(5500), 254 (9800), 228 (7100) and 208 (6800). A solution of **2** (22 mg) in 10% H_2SO_4/H_2O–EtOH (3: 1) (10 ml) was refluxed for 3 h and the reaction mixture was extracted with Et_2O. The Et_2O layer was washed with H_2O and then concentrated to dryness and the residue was recrystallized from EtOH to give **3** (5 mg), mp 274—277°C, which was identified by mixed mp determination and by comparison of the EI-mass spectrum (M^+ m/z: 274) and IR (KBr tablet) with those of an authentic sample. The aqueous layer was neutralized with Amberlite MB-3 and concentrated to dryness. In the residue, glucose was identified by GLC (YANACO-2800 gas chromatograph; column, 3% SE-30 on Chromosorb W AW DMCS 80—100 mesh 1 m × 3 mm, column temperature, 180°C, injection temperature, 210°C, N_2 gas at 25 ml/min).

Extraction and Purification of 6——See previous paper.[11]

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