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XANTHONES FROM *SWERTIA PUNICEA*

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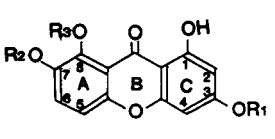
ABSTRACT.—Two known xanthones, decussatin [**1**] and gentiacaulein [**2**], were isolated from the aerial parts of *Swertia punicea* by means of cc and preparative tlc. Three known compounds, decussatin [**1**], methyl swertianin [**3**], and 1-hydroxy-3,5,7,8-tetramethylxanthone [**4**], and a new compound, 1,8-dihydroxy-2,4,6-trimethoxyxanthone [**5**], were also isolated from the cultured tissue of the same plant. Their structure elucidations were carried out by spectral data and chemical transformation to known compounds. Compound **5** was converted into two new xanthones, 1-hydroxy-2,4,6,8-tetramethoxyxanthone [**6**] and 1,2,4,6,8-pentamethoxyxanthone [**7**].

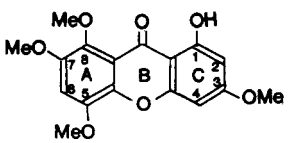
Swertia punicea Hemsl. (Gentianaceae) grows in moist, mountainous areas in the central district of mainland China. Although it has been used in folk medicine as a bitter stomachic, the constituents of this plant have not been isolated. We were interested in the chemical constituents of the plant, especially xanthones, but a major difficulty is to collect sufficient material for study because the plant cultivation is not easy. To circumvent this problem, tissue culture investigation and its component analysis were carried out. In this report we describe the isolation and the structure elucidation of two known compounds, decussatin [**1**] and gentiacaulein [**2**], from the plant and those of three known compounds, decussatin [**1**], methyl swertianin [**3**], and 1-hydroxy-3,5,7,8-tetramethoxyxanthone [**4**], and the new compound 1,8-dihydroxy-2,4,6-trimethoxyxanthone [**5**] from the cultured tissue. The new compound **5** was methylated with CH_2N_2 to give a known compound **4** and two new methylated derivatives **6** and **7**.

RESULTS AND DISCUSSION

The MeOH extract of the whole dried plant of *S. punicea* was subjected to Si gel cc



1 $R_1=R_2=R_3=Me$
2 $R_1=R_3=Me$, $R_2=H$
3 $R_1=R_2=Me$, $R_3=H$



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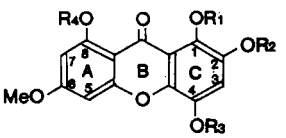

5 $R_1=R_4=H$, $R_2=R_3=Me$
6 $R_1=H$, $R_2=R_3=R_4=Me$
7 $R_1=R_2=R_3=R_4=Me$

and preparative tlc to afford decussatin [**1**] (1) and gentiacaulein [**2**] (2), both as yellow needles, in yields of 0.56% and 0.77%, respectively. They were identified by comparison of their uv, ir, ^{1}H-nmr (Table 1), and eims spectral data with those of the authentic compounds.

TABLE 1. ^{1}H Chemical Shifts of Compounds **1–7**.

Proton	Compound						
	1	**2**	**3**	**4**	**5**	**6**	**7**
H-2, -4	6.26 d (2) 6.32 d (2)	6.34 d (2) 6.38 d (2)	6.34 d (2) 6.42 d (2)	6.30 d (2) 6.44 d (2)			
H-3					7.02 s	6.99 s	6.89 s
H-5, -6	7.17 d (9) 7.38 d (9)	7.17 d (9) 7.40 d (9)	6.68 d (9) 7.19 d (9)	6.95 s			6.31 d (2) 6.52 d (2)
H-5, -7					6.32 d (2) 6.49 d (2)	6.33 d (2) 6.57 d (2)	
OH	13.30 s	13.10 s	11.98 s 12.11 s	13.18 s	11.56 s 11.95 s	12.78 s	
OMe	3.89 s 3.91 s 4.01 s	3.90 s 4.05 s	3.91 s 3.96 s	3.86 s 3.93 s 3.94 s 3.99 s	3.88 s 3.95 s 3.97 s	3.91 s 3.94 s 3.97 s 3.99 s	3.88 s 3.92 s 3.95 s 3.96 s 4.00 s

[a]Coupling constants in Hz are given in parentheses. All compounds were measured at 200 MHz in $CDCl_3$.

The MeOH extract of the whole cultured tissue of *S. punicea* was subjected to Si gel cc and preparative tlc to afford decussatin [**1**] (1), methyl swertianin [**3**] (2), 1-hydroxy-3,5,7,8-tetramethoxyxanthone [**4**] (3), and a new compound, 1,8-dihydroxy-2,4,6-trimethoxyxanthone [**5**], all as yellow needles, in yields of 0.038%, 0.0071%, 0.0064%, and 0.017%, respectively. The compounds **3** and **4** were identified by comparing their uv, ir, ^{1}H-nmr (Table 1), and eims spectral data with those of the authentic compounds.

Compound **5** was analyzed for $C_{16}H_{14}O_7$ by hrms. Its uv spectrum (237, 258, and 332 nm) was closely similar to that of 1,3,5,8-tetraoxygenated xanthone (4) and different from that of 1,3,5,6-tetraoxygenated xanthone (5). Its ir spectrum indicated the presence of hydroxyl (3400 cm^{-1}) and α,β-unsaturated ketone (1660 cm^{-1}). The eims of **5** showed an $[M]^+$ peak at *m/z* 318 ($C_{16}H_{14}O_7$) and fragment ion peaks at *m/z* 303 $[M-Me]^+$ and 275 $[M-Me-2CH_2]^+$.

The ^{1}H-nmr analysis of **5** (Table 1) suggested the presence of two chelated hydroxyl protons (δ 11.56 and 11.95), three methoxyl groups (δ 3.88, 3.95, and 3.97), and three aromatic protons (δ 6.32, 6.49, and 7.02, 1H each). Therefore, **5** is concluded to be a penta-substituted xanthone consisting of three methoxyl groups and two hydroxyl groups. The chelated hydroxyl proton signals, the foregoing two doublet meta coupling signals (δ 6.32 and 6.49, 1H each, $J = 2$ Hz), and the similarity of the meta coupling signals in **1–4** suggested that the A-ring structure of **5** is the same as the C-ring structure of **1–4**.

The NOESY spectrum of **5** (Figure 1) indicated that the methoxyl group (δ 3.88) enhanced the two aromatic proton signals (δ 6.32 and 6.49), and the aromatic proton (δ 7.02) enhanced the two methoxyl signals (δ 3.95 and 3.97).

Methylation of **5** with CH_2N_2 afforded three methylated derivatives, which were isolated by preparative tlc and one of which was identified to be 1-hydroxy-3,5,7,8-tetramethoxyxanthone [**4**] by comparison of spectral data, Rt, and R_f values of the cultured tissue product **4**. Other methylated derivatives were determined to be 1-hydroxy-

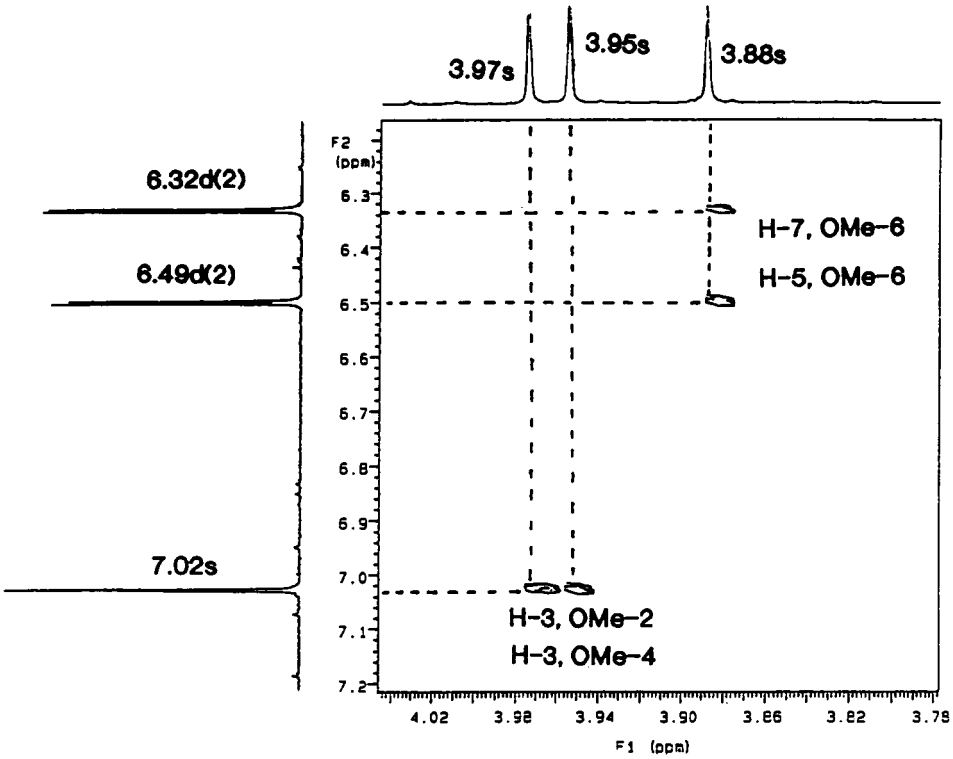

FIGURE 1. NOESY spectrum of compound **5**.

2,4,6,8-tetramethoxyxanthone [**6**] and 1,2,4,6,8-pentamethoxyxanthine [**7**] by ^{1}H-nmr (Table 1) and ir spectral data.

It is worthy of note that (a) the cultured tissue has produced pentaoxygenated xanthones, **4** and **5**, in addition to tetraoxygenated ones, **1** and **3**, and (b) the plant has produced only tetraoxygenated xanthones, **1** and **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Mp's were determined on an MRK air-bath type melting point apparatus and are uncorrected. Ir and uv spectra were recorded on a Jasco IR-810 spectrometer and a Hitachi 320S spectrometer, respectively. ^{1}H-nmr spectra were determined on a Varian XL-200 (200.06 MHz for ^{1}H nmr) using TMS as an internal standard, and the results are shown in Table 1. The NOESY spectrum of **5** (Figure 1) was obtained by a Varian VXR-500 spectrometer (499.84 MHz for ^{1}H nmr). All the samples for nmr analyses were dissolved in $CDCl_3$. Ei and ci mass spectra were recorded on a Hitachi M80 instrument. Analytical hplc was performed on a Waters Associates Model ALC/GPC 244 liquid chromatograph equipped with a Tosoh ODS-80TM column (4.6 mm × 15 cm), eluting with MeOH-H_2O (7:3), and a Waters Model 440 uv monitor (used at 254 nm). Si gel (Merck, type 60, 70–230 mesh) was used for cc, and precoated Si gel plates (Merck, 60F-254, 0.25 mm) were used for analytical tlc. Detection of components was made by use of a uv lamp and heating after spraying with 10% H_2SO_4. Precoated Si gel plates (Merck, 60F-254, 2 mm) were used for preparative tlc. Solvent A [C_6H_6-EtOAc-hexane (14:5:6)] was used for cc, analytical tlc, and preparative tlc.

PLANT MATERIAL.—Second-year plants of the biennial *S. punicea* were cultivated in the experimental garden of Hiroshima University and utilized in this study. The plant was identified by Professor K. Kondo, Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University. A voucher specimen was deposited in the above department.

CULTURED TISSUE.—A cultured tissue was also deposited in the above department. Their apical meristems (0.2 mm long), leaf pieces (9 mm area), internodes of stems (5 mm long), and roots (5 mm long) were aseptically excised for primary culture. The Linsmaier and Skoog (SL) medium (6) was supplemented

with indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and N6-benzyladenine (BA) at various concentrations and in various combinations. The pH of the media was adjusted to 5.7, and 2 g/liter gelrite was added to each medium. The cultures were maintained under a condition of 2000 lx (luminous intensity) illumination with the daylight of 12 h at a relative humidity of 65% and at a temperature of 25°. The calli induced under culture conditions were cut into 2 × 2-cm pieces and subcultured in the above media at intervals of 20 days.

Tissue segments from stalked second-year plants of the biennial *S. punicea* induced calli when they were cultured on the LS gelrite media containing 1 μM NAA and 1 μM BA, or 1 μM NAA and 10 μM BA, or 1 μM 2,4-D and 1 μM BA. Those cells were rapidly propagated on the LS gelrite medium containing 0.5 μM NAA or 2,4-D at 25°, at a relative humidity of 65%, and under an illumination of 2000 lx for 12 h. After approximately 60 days of the callus culture in the LS gelrite medium containing 0.5 μM IAA and 0.5 μM BA, the calli began to produce numerous adventitious buds which later regenerated plantlets (7).

EXTRACTION AND ISOLATION OF DECUSSATIN [**1**] AND GENTIACAULEIN [**2**] FROM THE PLANT.—Dried aerial parts of *S. punicea* (2.96 g) were cut into small pieces and extracted with MeOH (450 ml) by standing for 60 days at room temperature (18–23°). Evaporation of the solvent gave a dark brown residue (1.274 g) that was subjected to cc (Si gel 100 g, 4 cm × 30 cm), using solvent A (1400 ml). Eluate was collected with monitoring tlc to afford 7 fractions. Fractions 2 and 3 both showed two major spots in tlc. They were evaporated and subjected to preparative tlc to afford compounds **1** (19 mg) and **2** (29 mg). They were recrystallized by using Me_2CO/hexane to afford pure compounds **1** (16.5 mg) and **2** (22.7 mg) both as yellow needles.

EXTRACTION AND ISOLATION OF DECUSSATIN [**1**], METHYL SWERTIANIN [**3**], 1-HYDROXY-3,5,7,8-TETRAMETHOXYXANTHONE [**4**], AND 1,8-DIHYDROXY-2,4,6-TRIMETHOXYXANTHONE [**5**] FROM THE CULTURED TISSUE.—Cultured tissues (342 g) of the plant were cut into small pieces and extracted with MeOH (5200 ml) by standing for 60 days at room temperature. The MeOH solution was evaporated to afford a dark brown residue (16.2 g). The residue was subjected to cc (Si gel 600 g, 6 cm × 100 cm) with solvent A (4700 ml). The eluate was collected with monitoring tlc to give 12 fractions.

Fraction 5 showed a major spot in tlc, and it was subjected to preparative tlc to give pure compound **1** (129 mg) as yellow needles.

Fractions 6 and 7 showed two major spots in tlc, and they were subjected to preparative tlc to give two crude compounds, which were purified by recrystallization using EtOH to afford two pure compounds, **3** (24.3 mg) and **5** (53.9 mg), both as yellow needles.

Fraction 9 showed a major spot in tlc and evaporated to give a yellow residue, which was subjected to preparative tlc followed by recrystallization using EtOH to give pure compound **4** (22 mg) as yellow needles.

1,8-DIHYDROXY-2,4,6-TRIMETHOXYXANTHONE [**5**].—Mp 179–181°; uv λ max (EtOH) 237 (ε 17,400), 258 (ε 17,700), 278 (ε 22,200), 332 (ε 10,200); ir (KBr) 3400 (OH), 1660 (C=O), 1635, 1585, 1490, 1160, 1102, 1050, 822, 810 cm^{-1}; ^1H nmr see Table 1; eims *m/z* $[M]^+$ 318 (100%), 303 (89%), 275 (18%); hreims *m/z* 318.0782 (calcd for $C_{16}H_{14}O_7$, *m/z* 318.0797).

METHYLATION OF **5** INTO **4**, **6**, AND **7**.—An MeOH solution of **5** (8 mg) was added to an Et_2O solution of CH_2N_2 prepared from nitrosomethylurea and KOH. The mixed solution was stirred at 0° for 12 h and evaporated to give a yellow residue that showed three spots in tlc. The residue was subjected to preparative tlc to afford three pure compounds, **4** (1.4 mg), **6** (4.4 mg), and **7** (2.0 mg). ^1H-nmr and ir spectra, mp (196–197°), R_f (0.38, solvent A), and Rt (14.4 min) values of **4** coincided with those of the natural compound **4**.

1-HYDROXY-2,4,6,8-TETRAMETHOXYXANTHONE [**6**].—Compound **6** was obtained as yellow needles: mp 178–180°; ir (KBr) 3400 (OH), 1618, 1600, 1318, 1220, 1115, 985, 818 cm^{-1}; ^1H nmr see Table 1.

1,2,4,6,8-PENTAMETHOXYXANTHONE [**7**].—Compound **7** was obtained as pale yellow needles: mp 179–181°; ir (KBr) 1662 (C=O), 1600, 1292, 1110, 1060, 970, 815 cm^{-1}; ^1H nmr see Table 1.

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