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ORIGINAL PAPER

# Tetrahydroxanthones from Mongolian medicinal plant *Gentianella amarella* ssp. *acuta*

**Shuangxin Lu[1] · Naonobu Tanaka[1,2] · Kazuyoshi Kawazoe[1] · Kotaro Murakami[3] · Davaadagva Damdinjav[4] · Enkhjargal Dorjbal[4] · Yoshiki Kashiwada[1]**

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**Abstract** Two tetrahydroxanthones, 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and six new tetrahydroxanthone glycosides, amarellins A–F (**3**–**8**), were isolated from the aerial parts of a Mongolian medicinal plant *Gentianella amarella* ssp. *acuta* (Gentianaceae). The structures of **1**–**8** were elucidated on the basis of spectroscopic analysis, chemical conversion, and ECD calculation. Amarellins A–C (**3**–**5**) were assigned as 8-*O*-β-D-glucoside, 8-*O*-β-D-xyloside, and 1-*O*-β-D-glucoside of **1**, respectively, while amarellins D–F (**6**–**8**) were elucidated to be 8-*O*-β-D-xyloside, 1-*O*-β-D-glucoside, and 3-*O*-β-D-glucoside of **2**, respectively.

**Keywords** Tetrahydroxanthone · *Gentianella amarella* ssp. *acuta* · Gentianaceae · Amarellins A–F

## Introduction

The plants of the genus *Gentianella* (Gentianaceae), comprising about 250 species, of which many have a bitter taste, are distributed in temperate regions throughout the world [1]. *Gentianella amarella* ssp. *acuta* (synonym *Gentiana acuta*) is an annual herb distributed in East Asia, Siberia, and North America, and has been used as a traditional remedy for the treatment of headache, fever, hepatitis, and gallbladder disorders in Mongolia [2]. Xanthones, flavonoids, iridoids, triterpenoids, and steroids have been isolated from this species [2, 3]. As part of our research on Mongolian traditional medicines [4], aiming at searching natural product-based lead compounds for therapeutic agents, the constituents of the aerial parts of *G. amarella* ssp. *acuta* were investigated, resulting in the isolation of two tetrahydroxanthones, 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and six new tetrahydroxanthone glycosides, amarellins A–F (**3**–**8**). Herein, we describe the isolation and structure elucidation of **1**–**8**.

## Results and discussion

The MeOH extract of the aerial parts of *G. amarella* ssp. *acuta* was partitioned with *n*-BuOH and water. Chromatographic separations of the *n*-BuOH-soluble material gave two tetrahydroxanthones, 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and six new tetrahydroxanthone glycosides, amarellins A–F (**3**–**8**) (Fig. 1), as well as ten known compounds, campestroside [5], bellidin [6], corymbiferin 3-*O*-glucopyranoside [7], swertianolin [8], norswertianolin [9], isomangiferin [10], sweroside [11], swertiamarin [12], swertiaside A [13], and lariciresinol 4′-*O*-β-D-glucoside [14]. The structures of known compounds were identified by comparison of their spectroscopic data with the literature data.

✉ Yoshiki Kashiwada
kasiwada@tokushima-u.ac.jp

1 Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan

2 Graduate School of Bioscience and Bioindustry, Tokushima University, Tokushima 770-8513, Japan

3 Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto 862-0082, Japan

4 School of Pharmaceutical Biomedical Sciences, Mongolian National University of Medical Sciences, Ulaanbaatar 14210, Mongolia

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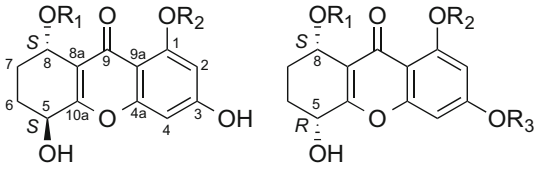

| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | H | H |
| **3** | Glc | H |
| **4** | Xyl | H |
| **5** | H | Glc |



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **2** | H | H | H |
| **6** | Xyl | H | H |
| **7** | H | Glc | H |
| **8** | H | H | Glc |

**Fig. 1** Structures of 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**), 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and amarellins A–F (**3**–**8**)

Compounds **1** and **2** were obtained individually as optically active pale yellow amorphous solids {$[\alpha]_D^{20}$ +78.1 (*c* 0.34, MeOH) for **1**; $[\alpha]_D^{20}$ +9.1 (*c* 0.15, MeOH) for **2**}. The HRESIMS revealed that **1** and **2** had the same molecular formula of $C_{13}H_{12}O_6$ (*m/z* 287.0526 $[M+Na]^+$, Δ −0.6 mmu for **1**; *m/z* 287.0528 $[M+Na]^+$ Δ −0.4 mmu for **2**). The $^1$H NMR spectrum of **1** measured in $CD_3OD$ showed the signals of two *meta*-coupled aromatic protons, two oxygenated $sp^3$ methines, and two $sp^3$ methylenes (Table 1), while the resonance due to a hydrogen-bonded hydroxy proton ($\delta_H$ 12.83, s) was observed in the $^1$H NMR spectrum in DMSO-$d_6$. The $^{13}$C NMR spectrum of **1** revealed the presence of 13 carbons, including one carbonyl carbon, five aromatic carbons, and two olefinic carbons. Though the feature of the 1D NMR spectra of **2** was similar to that of **1**, subtle differences were found for the chemical shifts of H-5, $H_2$-6, and $H_2$-7. The 1D NMR spectra of **2** also resembled those of tetrahydrobellidifolin (1,5*R*,8*S*-tetrahydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone) [15], except for the absence of the methoxy signal. These observations implied **1** and **2** to be demethyl derivatives of tetrahydrobellidifolin, with a diastereomeric relationship between **1** and **2**. The planar structures of **1** and **2** were confirmed to be 1,3,5,8-tetrahydroxy-5,6,7,8-tetrahydroxanthone by analysis of the $^1$H–$^1$H COSY and HMBC spectra (Fig. 2). Though 1,3,5,8-tetrahydroxy-5,6,7,8-tetrahydroxanthone was reported as a constituent of Gentianaceous plants *Swertia punicea* [16] and *Lomatogonium carinthiacum* [17], the reported 1D NMR data [17] resembled neither those of **1** nor **2**.

The relative and absolute configurations of **1** and **2** were assigned as follows. The pseudochair conformation of the cyclohexene ring (C-8a, C-5–C-8, and C-10a) and the H-5/H-8-*anti* relationship for **1** was suggested by NOESY correlations for H-8/$H_2$-7 and H-5/$H_2$-6 (Fig. 3A) and small $^3J$ values of the vicinal couplings for H-8/$H_2$-7 and H-5/$H_2$-6. NOESY correlations of H-8/$H_2$-7 and H-5/H-7β for **2** indicated the orientations of H-8 and H-5 to be pseudoequatorial and pseudoaxial, respectively (Fig. 3B). Thus, the H-5/H-8-*syn* relationship for **2** was assigned. This assignment was supported by resemblance of the chemical shifts of H-5, $H_2$-6, $H_2$-7, and H-8 for **2** with those for tetrahydrobellidifolin [15].

Since **1** displayed a quite weak cotton effect in the ECD spectrum, **1** was converted into the 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**1b**). The ECD spectrum of **1b** was similar to the calculated spectrum of (5*S*,8*S*)-**1b**, rather than that of (5*R*,8*R*)-**1b** (Fig. 4), indicating the 5*S* and 8*S* configurations of **1**. Confirmation of the 5*R* and

**Table 1** $^1$H and $^{13}$C NMR data for 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**) in $CD_3OD$

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| 1 | 163.5 | – | 163.4 | – |
| 2 | 100.0 | 6.17 (1H, brs) | 100.1 | 6.16 (1H, brs) |
| 3 | 166.3 | – | 166.6 | – |
| 4 | 95.0 | 6.32 (1H, brs) | 95.1 | 6.33 (1H, brs) |
| 4a | 159.4 | – | 159.3 | – |
| 10a | 165.9 | – | 167.1 | – |
| 5 | 65.5 | 4.49 (1H, brs) | 67.3 | 4.56 (1H, brt, 7.4) |
| 6 | 27.0 | 2.27 (1H, brt, 13.1) | 27.4 | 2.09, 2.03 (each 1H, m) |
| | | 1.85 (1H, brd, 13.1) | | |
| 7 | 26.5 | 2.04 (1H, brt, 13.4) | 28.8 | 1.95 (1H, m) |
| | | 1.80 (1H, brd, 13.4) | | 1.83 (1H, brt, 13.3) |
| 8 | 61.4 | 4.92 (1H, brs) | 62.2 | 4.91 (1H, t, 3.9) |
| 8a | 119.1 | – | 119.3 | – |
| 9 | 183.0 | – | 183.0 | – |
| 9a | 105.6 | – | 105.3 | – |

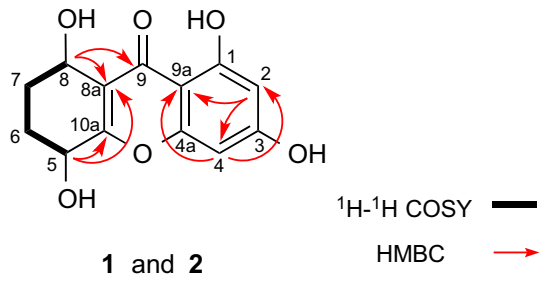


**Fig. 2** Selected $^1$H–$^1$H COSY and HMBC correlations for **1** and **2**

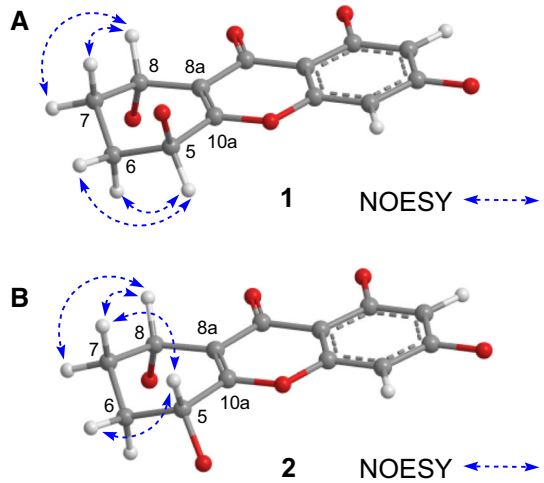


**Fig. 3** Selected NOESY correlations and relative configuration for **A** 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and for **B** 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**) (the protons of hydroxy groups are omitted)

8*S* configurations of **2** was also obtained by comparison of the ECD spectrum of 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**2b**) of **2** with the calculated spectra for a pair of enantiomers in the same manner (Fig. 5).

Amarellin A (**3**) was isolated as an optically active pale yellow amorphous solid {$[\alpha]_D^{20}$ +34.4 (*c* 0.14, MeOH)}. The molecular formula of **3** was assigned as $C_{19}H_{22}O_{11}$ by the HRESIMS (*m/z* 449.1076 $[M+Na]^+$, Δ +1.6 mmu). The $^1$H NMR spectrum displayed the resonances due to a tetrahydroxanthone moiety similar to those of **1**, along with the signals arising from a sugar moiety (Table 2). The sugar moiety was deduced to be glucose based on the $^1$H NMR coupling patterns and the $^{13}$C chemical shifts. The glycosidic linkage at C-8 was revealed by HMBC analysis (Fig. 6). The HRESIMS suggested that amarellins B (**4**) and C (**5**) had the molecular formulae $C_{18}H_{20}O_{10}$ and $C_{19}H_{22}O_{11}$, respectively (*m/z* 419.0946 $[M+Na]^+$, Δ −0.8 mmu for **4**; *m/z* 449.1061 $[M+Na]^+$, Δ +0.1 mmu for **5**). Interpretations of the 2D NMR spectra indicated that **4** and **5** were 8-*O*-xyloside and 1-*O*-glucoside of **1**, respectively. In the $^1$H NMR spectrum of **5** measured in $C_5D_5N$, the characteristic signal due to a hydrogen-bonded hydroxy proton was absent, which supported the connectivity of C-1 to the glucose for **5**. Analysis of the HRESIMS and 1D NMR spectra (Table 3) showed that amarellins D–F (**6**–**8**) were also glycosides of tetrahydroxanthone. The 1D NMR data suggested that the sugar moiety of **6** was xylose, while those of **7** and **8** were glucose. In contrast to **3**–**5**, the $^1$H signals of the aglycone moieties for **6**–**8** resembled that of **2**. The locations of the sugar moieties in **6**–**8** were assigned by HMBC analysis.

Identifications of the sugar moieties in amarellins A–F (**3**–**8**) were elucidated as follows. Acid hydrolysis of amarellin A (**3**) gave the sugar moiety, which was treated with L-cysteine methyl ester and *o*-tolylisothiocyanate [18]. HPLC analysis of the reaction mixture showed a peak, whose retention time was identical to that of the derivative of authentic D-glucose prepared in the same procedure, suggesting the sugar moiety of **3** to be D-glucose. In the same way, the sugar moieties of amarellins C (**5**), E (**7**), and F (**8**) were also assigned as D-glucose, whereas those of

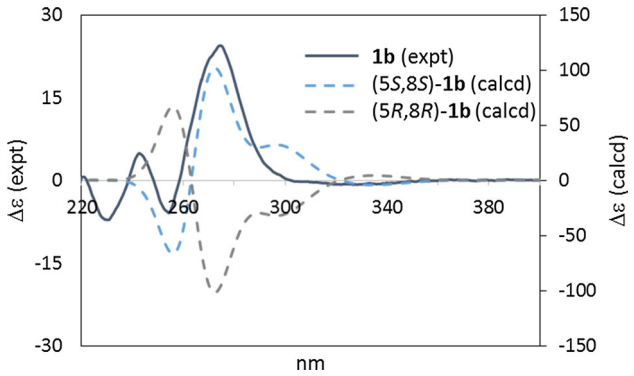


**Fig. 4** Experimental and calculated ECD spectra of 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**1b**) of 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**)

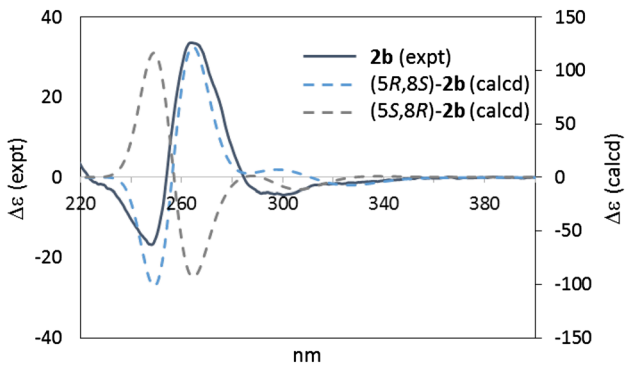


**Fig. 5** Experimental and calculated ECD spectra of 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**2b**) of 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**)

**Table 2** $^{1}H$ and $^{13}C$ NMR data for amarellins A–C (**3**–**5**) in $CD_3OD$

| Position | **3** | | **4** | | **5** | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
| 1 | 163.3 | – | 162.2[a] | – | 160.2 | – |
| 2 | 100.1 | 6.16 (1H, brs) | 100.3[a] | 6.18 (1H, brs) | 106.0 | 6.69 (1H, d, 2.2) |
| 3 | 166.3 | – | 165.6[a] | – | 168.2[a] | – |
| 4 | 95.1 | 6.32 (1H, brs) | 95.1[a] | 6.33 (1H, brs) | 99.8 | 6.47 (1H, d, 2.2) |
| 4a | 159.3 | – | 159.6[a] | – | 160.9 | – |
| 10a | 166.8 | – | 167.1[a] | – | 163.9 | – |
| 5 | 65.3 | 4.50 (1H, brs) | 65.3 | 4.49 (1H, dd, 3.5, 1.8) | 65.5 | 4.50 (1H, t, 4.1) |
| 6 | 26.9 | 2.28 (1H, brt, 14.2) | 26.8 | 2.27 (1H, tt, 14.1, 3.5) | 27.2 | 2.26 (1H, tt, 14.0, 4.1) |
| | | 1.87 (1H, brd, 14.2) | | 1.86 (1H, dd, 14.1, 1.8) | | 1.83 (1H, m) |
| 7 | 24.7 | 2.17 (1H, brd, 14.2) | 24.7 | 2.08 (1H, dd, 14.2, 3.1) | 26.6 | 2.04 (1H, dd, 14.0, 3.6) |
| | | 2.00 (1H, brt, 14.2) | | 1.99 (1H, tt, 14.2, 2.3) | | 1.78 (1H, m) |
| 8 | 71.1 | 4.93 (1H, brs) | 71.2 | 4.91 (1H, brs) | 62.5 | 4.91 (1H, t, 3.6) |
| 8a | 117.4 | – | 117.4 | – | 120.5 | – |
| 9 | 183.1 | – | 183.1[a] | – | 179.3 | – |
| 9a | 105.7 | – | 105.9[a] | – | 108.4[a] | – |
| 1′ | 105.2 | 4.69 (1H, d, 7.8) | 105.9 | 4.63 (1H, d, 7.9) | 104.8 | 4.82 (1H, d, 7.8) |
| 2′ | 75.7 | 3.16 (1H, dd, 9.0, 7.8) | 75.6 | 3.11 (1H, dd, 9.6, 7.9) | 74.8 | 3.59 (1H, dd, 9.0, 7.8) |
| 3′ | 77.8 | 3.42 (1H, t, 9.0) | 77.7 | 3.36 (1H, t, 9.6) | 77.4 | 3.48 (1H, m) |
| 4′ | 71.5 | 3.30 (1H, m) | 71.2 | 3.46 (1H, m) | 71.2 | 3.44 (1H, m) |
| 5′ | 78.1 | 3.35 (1H, m) | 67.2 | 3.87 (1H, dd, 11.1, 4.7) | 78.5 | 3.44 (1H, m) |
| | | | | 3.28 (1H, m) | | |
| 6′ | 62.7 | 3.70 (1H, dd, 12.0, 5.5) | | | 62.5 | 3.75 (1H, dd, 12.0, 3.5) |
| | | 3.89 (1H, dd, 12.0, 2.1) | | | | 3.92 (1H, brd, 12.0) |

[a] Signal was detected by the HMBC spectrum

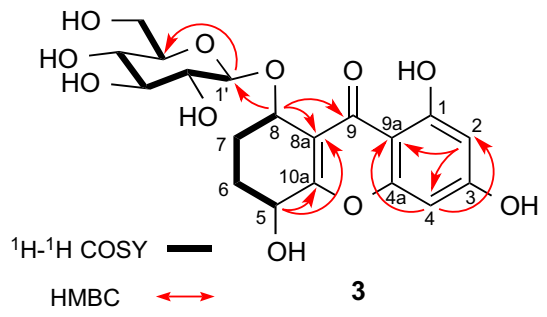


**Fig. 6** Selected $^{1}H$–$^{1}H$ COSY and HMBC correlations for amarellin A (**3**)

amarellins B (**4**) and D (**6**) were elucidated to be D-xylose. The β-glycosidic linkages between the sugar moieties of **3**–**8** and respective aglycones were concluded based on the coupling constants of their anomeric protons (Tables 2 and 3). Enzymatic hydrolyses of amarellins A (**3**), C (**5**), E (**7**), and F (**8**) with β-glucosidase were carried out to give their aglycones (**3a**, **5a**, **7a**, and **8a**, respectively), while amarellins B (**4**) and D (**6**) were hydrolyzed with cellulase to afford the aglycones **4a** and **6a**, respectively. The spectroscopic data of **3a**, **4a**, and **5a** including the HRESIMS data, $^{1}H$ NMR spectra, and optical rotations were identical to those of 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**), whereas **6a**, **7a**, and **8a** were assigned as 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**). Consequently, the structures of amarellins A–F (**3**–**8**) were assigned as shown in Fig. 1.

Though a variety of plant-derived xanthones have been reported so far, 5,6,7,8-tetrahydroxanthones have been isolated limited from Gentianaceous plants [5, 15–17, 19, 20]. In this research, the aerial parts of a Mongolian medicinal plant *Gentianella amarella* ssp. *acuta* were investigated to give two tetrahydroxanthones, 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and six new 5,6,7,8-tetrahydroxanthone glycosides, amarellins A–F (**3**–**8**). On the basis of spectroscopic analysis, chemical conversion, and ECD calculation, the structures of amarellins A–C (**3**–**5**) were assigned as 8-*O*-β-D-glucoside, 8-*O*-β-D-xyloside, and 1-*O*-β-D-glucoside of **1**, respectively, while amarellins D–F (**6**–**8**) were elucidated to be 8-*O*-β-D-

**Table 3** $^{1}H$ and $^{13}C$ NMR data for amarellins D–F (**6**–**8**) in $CD_3OD$

| Position | **6** | | **7** | | **8** | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
| 1 | 163.7[a] | – | 160.2 | – | 163.1 | – |
| 2 | 100.3 | 6.16 (1H, brs) | 105.3 | 6.73 (1H, brs) | 101.0 | 6.47 (1H, d, 2.1) |
| 3 | 166.8 | – | 166.9[a] | – | 164.9 | – |
| 4 | 95.1 | 6.33 (1H, brs) | 99.4 | 6.54 (1H, brs) | 95.9 | 6.68 (1H, d, 2.1) |
| 4a | 159.3 | – | 160.6 | – | 158.8 | – |
| 10a | 168.2 | – | 165.2 | – | 167.9 | – |
| 5 | 67.5 | 4.56 (1H, dd, 9.3, 7.3) | 67.2 | 4.57 (1H, dd, 8.7, 6.0) | 67.4 | 4.59 (1H, dd, 8.9, 6.2) |
| 6 | 27.3 | 2.08, 2.04 (each 1H, m) | 27.5 | 2.08, 2.03 (each 1H, m) | 27.3 | 2.11, 2.05 (each 1H, m) |
| 7 | 27.8 | 2.19 (1H, brd, 15.0) | 28.7 | 1.95 (1H, brd, 14.1) | 28.8 | 1.97, 1.84 (each 1H, m) |
| | | 1.77 (1H, brt, 15.0) | | 1.83 (1H, brt, 14.1) | | |
| 8 | 71.3 | 4.88 (1H, brs) | 63.1 | 4.90 (1H, m)[b] | 61.9 | 4.93 (1H, t, 3.5) |
| 8a | 117.6 | – | 120.9 | – | 119.7 | – |
| 9 | 182.8 | – | 179.3 | – | 183.2 | – |
| 9a | 105.3[a] | – | 108.7[a] | – | 107.1 | – |
| 1′ | 105.9 | 4.61 (1H, d, 7.6) | 104.8 | 4.82 (1H, d, 7.7) | 101.6 | 5.03 (1H, d, 7.3) |
| 2′ | 75.6 | 3.14 (1H, dd, 9.0, 7.6) | 74.7 | 3.59 (1H, dd, 8.9, 7.7) | 74.7 | 3.47 (1H, m) |
| 3′ | 77.7 | 3.36 (1H, t, 9.0) | 77.3 | 3.47 (1H, m) | 77.8 | 3.47 (1H, m) |
| 4′ | 71.2 | 3.48 (1H, m) | 71.2 | 3.45 (1H, m) | 71.1 | 3.41 (1H, m) |
| 5′ | 67.2 | 3.87 (1H, dd, 11.4, 5.4) | 78.5 | 3.45 (1H, m) | 78.3 | 3.50 (1H, m) |
| | | 3.26 (1H, m) | | | | |
| 6′ | | | 62.5 | 3.75 (1H, dd, 11.5, 3.8) | 62.4 | 3.71 (1H, dd, 12.2, 5.5) |
| | | | | 3.93 (1H, brd, 11.5) | | 3.90 (1H, dd, 12.2, 2.2) |

[a] Signal was detected by the HMBC spectrum

[b] Signal was overlapped with that of HOD

xyloside, 1-*O*-β-D-glucoside, and 3-*O*-β-D-glucoside of 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone, respectively (**2**).

## Experimental

### General

Optical rotations were measured by a JASCO P-2200 digital polarimeter. UV, ECD, and IR spectra were recorded on a Hitachi U-3900H, a JASCO J-1500, and a JASCO FT/IR-6200 spectrophotometers, respectively. MS were obtained on a Waters LCT Premier 2695. NMR spectra were measured by a Bruker AVANCE 500 instrument using tetramethylsilane as an internal standard. Column chromatography was performed with silica gel 60N (63–210 μm, Kanto Chemical), MCI gel CHP-20P (75–150 μm, Mitsubishi Chemical), YMC gel ODS-A (S-50 μm, YMC Co., Ltd.), and Sephadex LH-20 (25–100 μm, GE Health Care). HPLC was performed on Mightysil RP-18 GP (5 μm, $\phi$20 × 250 mm; Kanto Chemical), Mightysil RP-18 GP-II (5 μm, $\phi$20 × 250 mm), COSMOSIL πNAP (5 μm, $\phi$20 × 250 mm, Nacalai Tesque), and COSMOSIL $5C_{18}$-AR-II (5 μm, $\phi$20 × 250 mm).

### Plant material

*Gentianella amarella* ssp. *acuta* was collected at Khövsgöl province, Mongolia in August 2011. A voucher specimen (11JM0077) was deposited at the herbarium of Tokushima University.

### Extraction and isolation

The dried aerial parts of *Gentianella amarella* ssp. *acuta* (213 g) were extracted with MeOH at room temperature to give the extract (68.4 g), which was partitioned between *n*-BuOH and $H_2O$. The *n*-BuOH-soluble material (36.9 g) was subjected to silica gel column chromatography (*n*-hexane/EtOAc, 40:1–0:1 and then EtOAc/MeOH/$H_2O$, 40:3:2–5:3:2) to give 22 fractions (frs. 1–22). Fr. 16 was fractionated by an MCI gel CHP-20P column (MeOH/$H_2O$, 0:1–1:0) to afford 15 fractions (frs. 16.1–15). Fr. 16.2 was

applied to a silica gel column ($CHCl_3$/MeOH, 50:1–10:1) to give eight fractions (frs. 16.2.1–8). 1,3,5*S*,8*S*-Tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**, 6.0 mg) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**, 5.0 mg) were isolated from fr. 16.2.4 by HPLC on COSMOSIL πNAP (MeOH/$H_2O$, 38:62). Fr. 18 was separated by an MCI gel CHP-20P column (MeOH/$H_2O$, 0:1–1:0), a silica gel column ($CHCl_3$/MeOH/$H_2O$, 10:1:0.1– 6:4:1), and an ODS column (MeOH/$H_2O$, 0:1–1:0) to give amarellin A (**3**, 13.3 mg). Fr. 18.5 was chromatographed over a silica gel column ($CHCl_3$/MeOH/$H_2O$, 10:1:0.1–6:4:1) and a Sephadex LH-20 column (MeOH/$H_2O$, 0:1–1:0), and purified by HPLC on COSMOSIL πNAP (MeOH/$H_2O$, 40:60) and Mightysil RP-18 GP (MeOH/$H_2O$, 25:75) to give amarellins B (**4**, 2.0 mg) and D (**6**, 6.8 mg). Fr. 18.3 was applied to a silica gel column ($CHCl_3$/MeOH/$H_2O$, 10:1:0.1–6:4:1) to yield amarellin F (**8**, 3.3 mg). Fr. 18.2 was separated by an ODS column (MeOH/$H_2O$, 0:1–1:0) and HPLC on COSMOSIL πNAP (MeOH/$H_2O$, 40:60) to give amarellin C (**5**, 3.6 mg). Fr. 21 was loaded on a sequence of column chromatographies, including an MCI gel CHP-20P column (MeOH/$H_2O$, 0:1–1:0), a Sephadex LH-20 column (MeOH/$H_2O$, 0:1–1:0), a silica gel column ($CHCl_3$/MeOH/$H_2O$, 10:1:0.1–6:4:1), and HPLC on COSMOSIL πNAP (MeOH/$H_2O$, 25:75) to afford amarellin E (**7**, 2.0 mg).

### 1,3,5*S*,8*S*-Tetrahydroxy-5,6,7,8-tetrahydroxanthone (1)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ +78.1 (*c* 0.34, MeOH); HRESIMS *m*/*z* 287.0526$[M+Na]^+$ (calcd for $C_{13}H_{12}O_6Na$, 287.0532); UV (MeOH) $\lambda_{max}$ 260 (ε 13000) and 317 (ε 5400) nm; IR (KBr) $\nu_{max}$ 3392, 2360, 2335, and 1653 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 1); $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 12.83 (1H, s, 1-OH), 6.29 (1H, brs, H-4), 6.13 (1H, brs, H-2), 4.73 (1H, brs, H-8), 4.37 (1H, brs, H-5), 2.16 (1H, brt, $J$ = 13.9 Hz, H-6a), 1.87 (1H, brt, $J$ = 13.9 Hz, H-7a), 1.70 (1H, brd, $J$ = 13.9 Hz, H-6b), and 1.63 (1H, brd, $J$ = 13.9 Hz, H-7b); $^1H$ NMR ($C_5D_5N$) $\delta_H$ 13.46 (1H, s, 1-OH), 6.69 (1H, brs, H-4), 6.54 (1H, brs, H-2), 5.40 (1H, brs, H-8), 4.80 (1H, brs, H-5), 2.68 (1H, brt, $J$ = 13.4 Hz, H-6a), 2.42 (1H, brt, $J$ = 13.4 Hz, H-7a), 2.16 (1H, m, H-6b), and 2.11 (1H, m, H-7b).

### 1,3,5*R*,8*S*-Tetrahydroxy-5,6,7,8-tetrahydroxanthone (2)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ +9.1 (*c* 0.15, MeOH); HRESIMS *m*/*z* 287.0528 $[M+Na]^+$ (calcd for $C_{13}H_{12}O_6Na$, 287.0532); UV (MeOH) $\lambda_{max}$ 259 (ε 12000) and 318 (ε 4700) nm; IR (KBr) $\nu_{max}$ 3414, 2357, and 1659 $cm^{-1}$;$^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 1); $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 12.84 (1H, s, 1-OH), 6.29 (1H, brs, H-4), 6.13 (1H, brs, H-2), 4.73 (1H, brs, H-8), 4.47 (1H, dd, $J$ = 8.5, 7.4 Hz, H-5), 2.01 (1H, m, H-6a), 1.90 (1H, m, H-6b), 1.75 (1H, m, H-7a), and 1.65 (1H, m, H-7b); $^1H$ NMR ($C_5D_5N$) $\delta_H$ 13.44 (1H, s, 1-OH), 6.67 (1H, d, $J$ = 2.7 Hz, H-4), 6.54 (1H, d, $J$ = 2.7 Hz, H-2), 5.35 (1H, t, $J$ = 3.3 Hz, H-8), 4.89 (1H, dd, $J$ = 9.1, 5.8 Hz, H-5), 2.71 (1H, m, H-6a), 2.27 (1H, m, H-6b), 2.25 (1H, m, H-7a), and 1.93 (1H, tt, $J$ = 13.3, 1.9 Hz, H-7b).

### Preparation of 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (1b and 2b) of 1 and 2

A mixture of 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**, 2.0 mg), $CH_3I$ (100 μL), and $K_2CO_3$ (65 mg) in dry acetone (2.0 mL) was stirred at room temperature for 2 h. After removal of inorganic salts by filtration, the filtrate was concentrated under reduced pressure to give 1,5*S*,8*S*-trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (**1a**). To a solution of **1a**, 4-dimethylaminopyridine (23.8 mg), and triethylamine (70 μL) in $CH_2Cl_2$ (5 mL) was added 4-methoxybenzoyl chloride (19.6 mg), and the mixture was stirred at room temperature for 4 h. After addition of water (10 mL), the mixture was extracted with EtOAc (10 mL × 3). The EtOAc-soluble material was purified by a silica gel column (*n*-hexane/EtOAc, 8:2–7:3) to afford 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**1b**, 2.3 mg) of **1**. Methylation of 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**, 1.5 mg) as for **1** gave 1,5*R*,8*S*-trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (**2a**), whose 1,5,8-tris-4-methoxybenzoate (**2b**, 2.1 mg) was obtained by the same procedure as described above.

### 1,5*S*,8*S*-Trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (1a)

White amorphous solid; HRESIMS *m*/*z* 301.0679 $[M+Na]^+$ (calcd for $C_{14}H_{14}O_6Na$, 301.0688); $^1H$ NMR ($CD_3OD$) $\delta_H$ 6.53 (1H, d, $J$ = 2.0 Hz, H-4), 6.33 (1H, d, $J$ = 2.0 Hz, H-2), 4.94 (1H, t, $J$ = 3.1 Hz, H-8), 4.52 (1H, dd, $J$ = 4.4, 3.3 Hz, H-5), 3.86 (3H, s, 3-OMe), 2.28 (1H, tt, $J$ = 13.6, 3.1 Hz, H-6a), 2.05 (1H, tt, $J$ = 13.6, 3.3 Hz, H-7a), 1.87 (1H, m, H-6b), and 1.81 (1H, m, H-7b).

### 3-*O*-Methyl-1,5,8-tris-4-methoxybenzoate (1b) of 1

White amorphous solid; HRESIMS *m*/*z* 703.1799 $[M+Na]^+$ (calcd for $C_{38}H_{32}O_{12}Na$, 703.1791); $^1H$ NMR ($CDCl_3$) $\delta_H$ 8.11 (2H, d, $J$ = 8.7 Hz, Bz), 8.01 (2H, d, $J$ = 8.7 Hz, Bz), 7.90 (2H, d, $J$ = 8.7 Hz, Bz), 6.94 (2H, d, $J$ = 8.7 Hz, Bz), 6.93 (2H, d, $J$ = 8.7 Hz, Bz), 6.86 (2H, d, $J$ = 8.7 Hz, Bz), 6.77 (1H, d, $J$ = 2.5 Hz, H-4),

6.70 (1H, d, $J = 2.5$ Hz, H-2), 6.31 (1H, t, $J = 2.8$ Hz, H-8), 6.12 (1H, dd, $J = 3.8$, 1.6 Hz, H-5), 3.87 × 3 (each 3H, s, OMe ×3), 3.84 (3H, s, 3-OMe), 2.36 (1H, tt, $J = 14.4$, 3.8 Hz, H-6a), 2.22 (1H, dd, $J = 14.4$, 2.8 Hz, H-7a), 2.11 (1H, m, H-6b), and 2.10 (1H, m, H-7b); ECD (MeOH) $\Delta\varepsilon$ (nm) −7.2 (230), +4.9 (243), −5.9 (254), and +24.5 (275).

### 1,5*R*,8*S*-Trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (2a)

White amorphous solid; HRESIMS *m/z* 361.0688 $[M+Na]^+$ (calcd for $C_{14}H_{14}O_6Na$, 361.0688); $^1H$ NMR ($CD_3OD$) $\delta_H$ 6.54 (1H, d, $J = 2.3$ Hz, H-4), 6.33 (1H, d, $J = 2.3$ Hz, H-2), 4.92 (1H, t, $J = 4.6$ Hz, H-8), 4.60 (1H, dd, $J = 9.0$, 6.4 Hz, H-5), 3.86 (3H, s, 3-OMe), 2.12 (1H, m, H-6a), 2.05 (1H, m, H-6b), 1.96 (1H, m, H-7a), and 1.87 (1H, m, H-7b).

### 3-*O*-Methyl-1,5,8-tris-4-methoxybenzoate (2b) of 2

White amorphous solid; HRESIMS *m/z* 703.1790 $[M+Na]^+$ (calcd for $C_{38}H_{32}O_{12}Na$, 703.1791); $^1H$ NMR ($CDCl_3$) $\delta_H$ 8.12 (2H, d, $J = 9.1$ Hz, Bz), 8.08 (2H, d, $J = 9.1$ Hz, Bz), 7.95 (2H, d, $J = 9.1$ Hz, Bz), 6.99 (2H, d, $J = 9.1$ Hz, Bz), 6.90 (2H, d, $J = 9.1$ Hz, Bz), 6.88 (2H, d, $J = 9.1$ Hz, Bz), 6.70 (1H, d, $J = 2.4$ Hz, H-2), 6.69 (1H, d, $J = 2.4$ Hz, H-4), 6.22 (1H, t, $J = 3.6$ Hz, H-8), 6.14 (1H, dd, $J = 9.1$, 6.7 Hz, H-5), 3.90 (3H, s, 3-OMe), 3.85 × 3 (each 3H, s, OMe ×3), 2.32 (1H, m, H-7a), 2.27 (1H, m, H-6a), 2.25 (1H, m, H-6b), and 2.00 (1H, m, H-7b); ECD (MeOH) $\Delta\varepsilon$ (nm) −16.7 (248), +33.6 (264), and −4.3 (301).

### Calculation of ECD spectra of 1b and 2b

Conformational searches and DFT calculations were carried out on Spartan'10 [21] and Gaussian 09 [22], respectively. The enantiomers (5*S*,8*S*-**1b**, 5*R*,8*R*-**1b**, 5*R*,8*S*-**2b**, and 5*S*,8*R*-**2b**) were separately subjected to conformational searches using MMFFaq as the force field. The initial low-energy conformers with Boltzmann distributions over 1 % were further optimized by DFT calculations at the B3LYP/6-31G(d) level in the presence of MeOH with a polarizable continuum model (PCM). The low-energy conformers for 5*S*,8*S*-**1b**, 5*R*,8*R*-**1b**, 5*R*,8*S*-**2b**, and 5*S*,8*R*-**2b** with Boltzmann distributions over 1 % (16, 16, 13, and 15 conformers, respectively) were subjected to TDDFT calculations at the B3LYP/6-31G(d) level in the presence of MeOH with a PCM. The resultant rotatory strengths of the lowest 30 excited states for each conformer were converted into Gaussian-type curves with half-bands (0.2 eV) using SpecDis v1.61 [23]. The calculated ECD spectra were composed after correction based on the Boltzmann distribution of the stable conformers. The calculated ECD spectra of 5*S*,8*S*-**1b** and 5*R*,8*R*-**1b** (Fig. 4) were red-shifted by 15 nm, respectively.

### Amarellin A (3)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ +34.4 (*c* 0.14, MeOH); HRESIMS *m/z* 449.1076 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) $\lambda_{max}$ 220 ($\varepsilon$ 15700), 258 ($\varepsilon$ 13000), and 300 ($\varepsilon$ 5200) nm; IR (KBr) $\nu_{max}$ 3393, 2360, 2342, and 1659 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 2).

### Amarellin B (4)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ +25.7 (*c* 0.09, MeOH); HRESIMS *m/z* 419.0946 $[M+Na]^+$ (calcd for $C_{18}H_{20}O_{10}Na$, 419.0954); UV (MeOH) $\lambda_{max}$ 260 ($\varepsilon$ 10300) and 299 ($\varepsilon$ 4400) nm; IR (KBr) $\nu_{max}$ 3407, 2361, 2337, and 1638 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 2).

### Amarellin C (5)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ −14.4 (*c* 0.15, MeOH); HRESIMS *m/z* 449.1061 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) $\lambda_{max}$ 226 ($\varepsilon$ 12800), 254 ($\varepsilon$ 11300), and 298 ($\varepsilon$ 5500) nm; IR (KBr) $\nu_{max}$ 3384, 2361, 2337, and 1651 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 2); $^1H$ NMR ($C_5D_5N$) $\delta_H$ 7.43 (1H, d, $J = 2.0$ Hz, H-2), 6.71 (1H, d, $J = 2.0$ Hz, H-4), 5.40 (1H, d, $J = 7.5$ Hz, H-1′), 5.35 (1H, brs, H-8), 4.81 (1H, t, $J = 3.8$ Hz, H-5), 4.41 (1H, m, H-2′), 4.37 (1H, m, H-6′a), 4.34 (1H, m, H-6′b), 4.35 (1H, m, H-4′), 4.34 (1H, m, H-3′), 4.01 (1H, m, H-5′), 2.64 (1H, tt, $J = 13.7$, 3.8 Hz, H-6a), 2.36 (1H, tt, $J = 13.7$, 3.3 Hz, H-7a), 2.10 (1H, m, H-6b), and 2.07 (1H, m, H-7b).

### Amarellin D (6)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ +7.7 (*c* 0.15, MeOH); HRESIMS *m/z* 419.0963 $[M+Na]^+$ (calcd for $C_{18}H_{20}O_{10}Na$, 419.0954); UV (MeOH) $\lambda_{max}$ 252 ($\varepsilon$ 16800), 260 ($\varepsilon$ 17800), and 298 ($\varepsilon$ 6300) nm; IR (KBr) $\nu_{max}$ 3408, 2361, 2336, and 1654 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 3).

### Amarellin E (7)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ −51.8 (*c* 0.22, MeOH); HRESIMS *m/z* 449.1066 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) $\lambda_{max}$ 227 ($\varepsilon$ 13700), 254 ($\varepsilon$ 11000), and 297 ($\varepsilon$ 5600) nm; IR (KBr) $\nu_{max}$ 3410, 2361,

2336, and 1638 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 3).

### Amarellin F (8)

Pale yellow amorphous solid; $[\alpha]_D^{20}$ −30.5 (*c* 0.23, MeOH); HRESIMS *m/z* 449.1069 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) $\lambda_{max}$ 231 ($\varepsilon$ 11500), 245 ($\varepsilon$ 12100), 250 ($\varepsilon$ 12300), and 289 ($\varepsilon$ 4500) nm; IR (KBr) $\nu_{max}$ 3400, 2360, 2336, and 1646 $cm^{-1}$; $^1H$ and $^{13}C$ NMR ($CD_3OD$) (Table 3).

### Acid hydrolysis of amarellins A–F (3–8)

Amarellin A (**3**, 0.5 mg) was treated with 1 M HCl (3.0 mL) at 80 °C for 2 h. The reaction mixture was neutralized with an anion-exchange resin (IRA-400, Organo Co.) and evaporated to give a residue. The residue and L-cysteine methyl ester hydrochloride (0.5 mg) was dissolved in pyridine (0.1 mL) and heated at 60 °C for 1 h, and then *o*-tolylisothiocyanate (10 μL) was added to the mixture and heated at 60 °C for 1 h. The reaction mixture was analyzed by ODS HPLC [COSMOSIL $5C_{18}$-AR-II ($\phi$4.6 × 250 mm); solvent, $CH_3CN$/50 mM $H_3PO_4$ aq. (25:75); flow rate, 0.8 mL/min; UV detection, 250 nm; column temperature, 35 °C] to give a peak at 17.5 min. The retention time was identical to that of the derivative of authentic D-glucose prepared by the same procedure as described above. Acid hydrolysis followed by HPLC analyses for amarellins C (**5**), E (**7**), and F (**8**) were carried out in the same manner as for **3**, suggesting the sugar moieties of **5**, **7**, and **8** to be D-glucose. Similarly, the sugar moieties of amarellins B (**4**) and D (**6**) were assigned as D-xylose by comparison of the retention time for the derivatives of **4** and **6** with that for the derivative of authentic D-xylose ($t_R$ 20.4 min).

### Enzymatic hydrolysis of amarellins A–F (3–8)

Amarellin A (**3**, 1.5 mg) was treated with β-glucosidase (from almonds, 1.5 mg, Sigma Chemical Co.) in water (2 mL) at 38 °C for 2 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL × 3). The EtOAc-soluble material was purified by HPLC on COSMOSIL πNAP (5 μm, $\phi$10 × 250 mm; MeOH/$H_2O$, 38:62) to furnish an aglycone (**3a**, 0.4 mg) of **3**. Similarly, amarellins C (**5**), E (**7**), and F (**8**) were separately hydrolyzed by β-glucosidase to give their aglycones (**5a**, **7a**, and **8a**, respectively). A mixture of amarellin B (**4**, 1.5 mg) and cellulase (from *Trichoderma longibrachiatum*, 9.0 mg, Sigma Chemical Co.) in water (2 mL) was kept standing at 38 °C for 24 h. After dilution with water (10 mL), the reaction mixture was extracted with EtOAc (10 mL × 3), and the EtOAc-soluble material was purified by HPLC on COSMOSIL πNAP ($\phi$10 × 250 mm; MeOH/$H_2O$, 45:55) to afford an aglycone (**4a**) of **4**. Enzymatic hydrolysis for amarellin D (**6**) was carried out in the same manner as for **4** to give an aglycone (**6a**) of **6**. Spectroscopic data, including the HRESIMS data, $^1H$ NMR spectra, and optical rotations for the aglycones **3a**, **4a**, and **5a**, were identical to those for 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**), while spectroscopic data for the aglycones **6a**, **7a**, and **8a** were coincident with those for 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**).

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