Phytochemistry 71 (2010) 1925–1929

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Phenolic glycosides from *Agrimonia pilosa*

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ARTICLE INFO

Article history:
Received 15 March 2010
Received in revised form 30 July 2010

Keywords:
Agrimonia pilosa
Rosaceae
Flavonoids
3,4-Dihydroisocoumarins
Chromones

ABSTRACT

Phytochemical investigation of the methanolic extract from the aerial parts of *Agrimonia pilosa* led to the isolation of three compounds, (−)-aromadendrin 3-*O*-β-D-glucopyranoside (**1**), desmethylagrimonolide 6-*O*-β-D-glucopyranoside (**2**), and 5,7-dihydroxy-2-propylchromone 7-*O*-β-D-glucopyranoside (**3**), together with nine known compounds, agrimonolide 6-*O*-glucoside, takanechromone C, astragalin, afzelin, tiliroside, luteolin, quercetin, isoquercetrin, and quercitrin. Their structures were determined by various spectroscopic analysis and chemical transformations.

1. Introduction

The species of the genus *Agrimonia*, belonging to the Rosaceae, has about a dozen species, which are perennial herbaceous flowering plants, mainly distributed in the temperate regions of the Northern Hemisphere. Some species have been used in traditional medicine, e.g. *Agrimonia eupatoria* has been traditionally used in Europe as astringent, cholagogue, diuretic and antidiabetic agents (Shabana et al., 2003), *Agrimonia japonica* has also been used in Japan as an antidiarrheal and an hemostatic (Okuda et al., 1984), and *Agrimonia pilosa* has been used in Traditional Chinese Medicine (TCM). The roots and aerial parts of *A. pilosa* have also had different usage in TCM. Roots were used for treatment of cancer, and tannins were reported to be principal constituents for this usage (Miyanoto et al., 1988). The aerial parts of *A. pilosa* were also listed in the Chinese Pharmacopoeia as an astringent hemostatic for treating various kinds of bleeding, including bloody dysentery, and also to counteract toxins and reduce swelling for treating boils and sores (Xu et al., 2005). Pharmacological studies on the extracts prepared from the aerial parts of *A. pilosa* demonstrated broad biological properties, such as antihemorrhagic (Wang et al., 1984), antiplatelet (Wang et al., 1985), antioxidant (Zhu et al., 2009), nitric oxide scavenging (Taira et al., 2009), acetylcholinesterase inhibitory (Jung and Park, 2007), and α-glucosidase inhibitory (Asano et al., 2006) activities. Chemical studies on the aerial parts of *A. pilosa* showed the presences of flavonoids (Jung and Park, 2007; Pan et al., 2008; Su et al., 1984), 3,4-dihydroisocoumarins (Taira et al., 2009), and triterpenoids (An et al., 2005; Kouno et al., 1988). The potential medicinal importance and our ongoing interest in the chemistry of bioactive natural compounds from TCM thus prompted us to investigate the chemical constituents of this plant, resulting in the isolation of three new compounds, (−)-aromadendrin 3-*O*-β-D-glucopyranoside (**1**), desmethylagrimonolide 6-*O*-β-D-glucopyranoside (**2**), and 5,7-dihydroxy-2-propylchromone 7-*O*-β-D-glucopyranoside (**3**), together with nine known compounds (Fig. 1). In this paper, we report the isolation and structure elucidation of these compounds by various spectroscopic analysis and chemical transformations.

2. Results and discussion

The air-dried aerial parts of *A. pilosa* were extracted with MeOH. The extract was partitioned between EtOAc and H_2O. The EtOAc fraction was subjected to silica gel column chromatography, and further purification was carried out by reversed phase HPLC to afford three new compounds (**1**–**3**) and nine known compounds. The known compounds were identified as agrimonolide 6-*O*-glucoside (**4**) (Pei et al., 1989), takanechromone C (**5**) (Tanaka et al., 2009), astragalin (**6**) (Han et al., 2004), afzelin (**7**) (Chung et al., 2004), tiliroside (**8**) (Tsukamoto et al., 2004), luteolin (**9**) (Park et al., 2007), quercetin (**10**) (Awad et al., 2002), isoquercetrin (**11**) (Han et al., 2004), and quercitrin (**12**) (Pyo et al. 2002) by detailed NMR spectroscopic analysis and comparison with literature data.

Compound **1** was isolated as a pale yellow solid. Its molecular formula was determined to be $C_{21}H_{22}O_{11}$ by analysis of the HR–ESI–MS spectrum. The ^{1}H NMR spectrum of **1** (Table 1) showed

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doi:10.1016/j.phytochem.2010.08.007

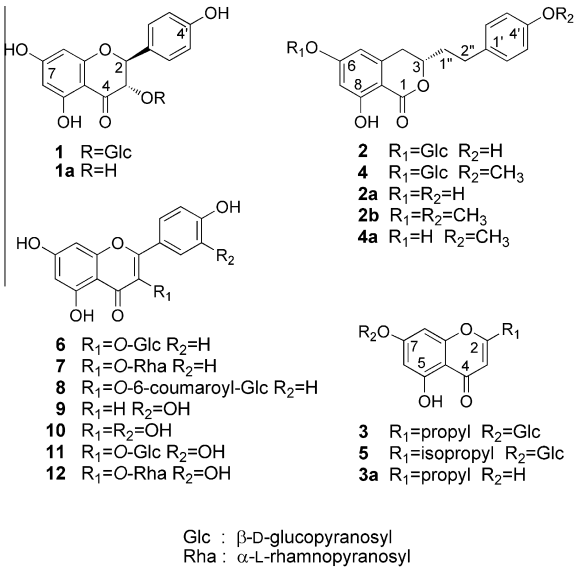

Fig. 1. Compounds isolated from *Agrimonia pilosa*.

Table 1
^{1}H and ^{13}C NMR spectroscopic data of **1**.

Position	δ_C	δ_H (J in Hz)
2	83.5	5.27 (d, 9.4)
3	77.7	4.95 (d, 9.4)
4	196.6	
5	165.6	
6	97.4	5.91 (d, 2.0)
7	169.1	
8	96.4	5.89 (d, 2.0)
9	164.2	
10	102.4	
1′	128.6	
2′ and 6′	130.6	7.31 (d, 8.6)
3′ and 5′	116.0	6.78 (d, 8.6)
4′	159.1	
Glc-1	104.6	4.68 (d, 7.8)
Glc-2	75.6	3.11 (dd, 9.4, 7.8)
Glc-3	77.9	3.33 (t, 9.4)
Glc-4	71.6	3.16[a]
Glc-5	78.0	3.16[a]
Glc-6	62.9	3.75 (dd, 11.7, 1.7) 3.53 (dd, 11.7, 5.5)

[a] Overlapping signals.

resonances assignable to two *meta*-coupled aromatic protons at δ_H 5.91 (1H, d, J = 2.0 Hz, H-6) and 5.89 (1H, d, J = 2.0 Hz, H-8), four A_2X_2-type aromatic ring protons at δ_H 7.31 (2H, d, J = 8.6 Hz, H-2′, 6′) and 6.78 (2H, d, J = 8.6 Hz, H-3′, 5′), two coupled oxymethine protons at δ_H 5.27 (1H, d, J = 9.4 Hz, H-2) and 4.95 (1H, d, J = 9.4 Hz, H-3), and a set of protons for a β-glucopyranosyl moiety, with its anomeric proton at δ_H 4.68 (1H, d, J = 7.8 Hz, Glc-H-1). The β-glucopyranosyl moiety was determined to be in the D-form by GLC analysis of its trimethylsilylthiazolidine derivative after acid hydrolysis of **1**. The ^{13}C NMR spectrum (Table 1) showed a typical downfield resonance at δ_C 196.6, characteristic of a C-4 carbonyl resonance of a flavanonol skeleton (Sakushima et al., 2002). All of the above data suggested **1** was a flavanonol β-D-glucopyranoside. The relationship of H-2 and H-3 was determined to be *trans* by their coupling constant values of 9.4 Hz. The β-D-glucopyranosyl moiety was determined at C-3 by the HMBC correlations between δ_H 4.68 (Glc-H-1) and δ_C 77.7 (C-3), and δ_H 4.95 (H-3) and δ_C 104.6 (Glc-C-1). Compound **1** showed superimpossible NMR spectroscopic data and the same relative configurations of C-2 and C-3 with the known compound, arthromerin B (2*R*,3*R*-5,7,4′-trihydroxyflavanonol 3-*O*-β-D-glucopyranoside), but differing in optical rotations, $[\alpha]_D^{25}$ − 84.1 (c 0.7, MeOH) for **1** and $[\alpha]_D$ + 35.7 (c 0.7, MeOH) for arthromerin B (Godecke et al., 2005). The CD spectrum of **1** exhibits a positive Cotton effect at 293 nm and a negative one at 256 nm, which is opposite to the Cotton effects for arthromerin B, suggesting the absolute configuration in **1** was 2*S*, 3*S*. Enzymatic hydrolysis of **1** with naringinase, afforded the aglycone **1a**, which gave identical spectroscopic data including NMR, $[\alpha]_D$ and CD data with the known compound (−)-aromadendrin (2*S*,3*S*-5,7,4′-trihydroxy flavanonol) (Takahashi et al., 1988; Prescott et al., 2002). Thus, structure **1** was unambiguously determined as (−)-aromadendrin 3-*O*-β-D-glucopyranoside (2*S*,3*S*-5,7,4′-trihydroxyflavanonol 3-*O*-β-D-glucopyranoside).

Compound **2** was isolated as a pale yellow solid. Its molecular formula was determined to be $C_{23}H_{26}O_{10}$ by analysis of the HR–ESI–MS spectrum. The IR spectrum showed absorption peaks at 1637 cm^{-1}, suggesting the presence of a lactone moiety in **2**, which was supported by the ^{13}C NMR resonance at δ_C 171.4 (C-1) (Table 2). The ^{1}H NMR spectrum of **2** had resonances assignable to two *meta*-coupled aromatic protons at δ_H 6.52 (1H, d, J = 2.3 Hz, H-7) and 6.50 (1H, d, J = 2.3 Hz, H-5), four A_2X_2-type aromatic ring protons at δ_H 7.04 (2H, d, J = 8.7 Hz, H-2′, 6′) and 6.71 (2H, d, J = 8.5 Hz, H-3′, 5′), and a set of protons of a β-glucopyranosyl moiety, with its anomeric proton at δ_H 4.98 (1H, d, J = 7.6 Hz, Glc-H-1). The β-glucopyranosyl moiety was determined to be in the D-form by GLC analysis of its trimethylsilylthiazolidine derivative after acid hydrolysis of **2**. In addition to the above proton resonances, it also had resonances for three methylenes at δ_H 2.97 (1H, dd, J = 16.7, 3.9 Hz, H-4a) and 2.92 (1H, dd, J = 16.7, 10.8 Hz, H-4b), 2.09 (1H, m, H-1″a) and 1.97 (1H, m, H-1″b), and 2.79 (1H, ddd, J = 14.3, 9.4, 5.3 Hz, H-2″a) and 2.70 (1H, ddd, J = 14.3, 8.9, 7.1 Hz, H-2″b), and one oxymethine at 4.51 (1H, m, H-3), suggesting the presence of a CH_2–CH–CH_2–CH_2 moiety by their correlations in the DQF–COSY spectrum. Connection of these structure units was determined by the HMBC data. Namely, the C-10 connecting to C-4 was determined by the correlations between H-4/C-3, H-4/C-10, H-4/C-9,

Table 2
^{1}H and ^{13}C NMR spectroscopic data of **2**.

Position	δ_C	δ_H (J in Hz)
1	171.4	
3	80.2	4.51 (m)
4	34.0	2.97 (dd, 16.7, 3.9) 2.92 (dd, 16.7, 10.8)
5	108.4	6.50 (d, 2.3)
6	165.2	
7	103.6	6.52 (d, 2.3)
8	165.2	
9	104.1	
10	143.4	
1'	133.2	
2′ and 6′	130.4	7.04 (d, 8.7)
3′ and 5′	116.4	6.71 (d, 8.5)
4′	156.8	
1″	37.9	2.09 (m) 1.97 (m)
2″	31.2	2.79 (ddd, 14.3, 9.4, 5.3) 2.70 (ddd, 14.0, 8.9, 7.1)
Glc-1	101.5	4.98 (d, 7.6)
Glc-2	74.8	3.47[a]
Glc-3	78.0	3.47[a]
Glc-4	71.3	3.38 (t, 9.1)
Glc-5	78.4	3.47[a]
Glc-6	62.5	3.89 (dd, 12.1, 2.0) 3.69 (dd, 12.1, 5.7)

[a] Overlapping signals.

the C-4 connecting to C-10 was determined by the correlations between H-4/C-5, H-4/C-9, H-3/C-10 and H-5/C-4, the C-2″ connecting to C-1′ was determined by the correlations between H-1″/C-1′, and H-2″/C-2′. The β-D-glucopyranosyl moiety at C-6 was determined by the HMBC correlation between δ_H 4.98 (Glc-H-1) and δ_C 165.2 (C-6). Although the HMBC correlation between H-3/C-1 could not be observed, taking account of the molecular formula, it was suggested the lactone moiety was connected to C-3. Thus, the structure of **2** was determined to be a 4′-demethylated compound of agrimonolide 6-*O*-glucoside (**4**), which was also isolated from this plant as a known compound. This was confirmed by permethylation of compounds **2** and **4**, affording the same product **2b**. Since **4** has not been reported its absolute configuration, enzymatic hydrolysis by naringinase of **2** and **4** was carried out to afford the aglycone **2a** (desmethylagrimonolide) (Yamato, 1958) and **4a** (agrimonolide) (Arakawa et al., 1968; Yamato and Hashigaki, 1976), respectively. Compounds **2**, **4** and their derivatives **2a**, **2b** and **4a** showed the same positive Cotton effects at 268 nm, which was identical with the CD data of **4a** in literature (Arakawa et al., 1968), suggesting they have the same absolute configuration 3*S*. On the basis of the above evidence, structure **2** was unambiguously determined as desmethylagrimonolide 6-*O*-β-D-glucopyranoside (3*S*-6,8-dihydroxy 3-(4-hydroxyphenylethyl)-3,4-dihydroisocoumarin 6-*O*-β-D-glucopyranoside).

Compound **3** was isolated as a colorless solid. Its molecular formula was determined to be $C_{18}H_{22}O_9$ by the HR–ESI–MS spectrum. The ^{1}H NMR spectrum of **3** (Table 3) showed resonances assignable to a pair of *meta*-coupled aromatic protons at δ_H 6.59 (1H, d, J = 2.2 Hz, H-8) and 6.39 (1H, d, J = 2.2 Hz, H-6), a separate olefinic proton at δ_H 6.03 (1H, s, H-3), a set of protons for vinyl propyl moiety at δ_H 2.55 (2H, t, J = 7.4 Hz, H_2-1′), 1.68 (2H, qt, J = 7.4, 7.4 Hz, H_2-2′), 0.94 (3H, t, J = 7.4 Hz, H_3-3′), and a set of protons for a β-glucopyranosyl moiety with its anomeric proton resonance at δ_H 4.94 (1H, d, J = 7.3 Hz, Glc-H-1). The ^{13}C NMR spectrum had typical downfield resonance at δ_C 184.2 (C-4) assignable to a conjugated carbonyl moiety. Comparison of the ^{1}H- and ^{13}C NMR spectroscopic data of **3** with those of the known compound takanechromone C (**5**), indicated similar resonances, except that the resonances for the isopropyl moiety at C-2 in **5** were replaced by the propyl moiety in **3**. The connection of the propyl moiety to C-2 was confirmed by the HMBC correlations between δ_H 6.03 (H-3) and δ_C 36.9 (C-1′), δ_H 2.55 (H_2-1′) and δ_C 108.9 (C-3), and δ_H 1.68 (H_2-2′) and δ_C 173.0 (C-2). Enzymatic hydrolysis of **3** with naringinase, afforded D-glucose and the aglycone **3a**, which was a known compound, 5,7-dihydroxy-2-propylchromone (**3a**) (Alves et al., 1999). The β-D-glucopyranosyl moiety was connected to C-7 by the HMBC correlation between δ_H 4.94 (Glc-H-1) and δ_C 164.8 (C-7), and NOESY correlations of Glc-H-1 with H-6 and H-8, respectively. Thus, the structure of **3** was unambiguously determined as 5,7-dihydroxy-2-propylchromone 7-*O*-β-D-glucopyranoside.

Table 3
^{1}H and ^{13}C NMR spectroscopic data of **3**.

Position	δ_C	δ_H (J in Hz)
2	173.0	
3	108.9	6.03 (s)
4	184.2	
5	163.0	
6	101.1	6.39 (d, 2.2)
7	164.8	
8	96.0	6.59 (d, 2.2)
9	159.5	
10	107.0	
1′	36.9	2.55 (t, 7.4)
2′	21.3	1.68 (qt, 7.4)
3′	13.7	0.94 (t, 7.4)
Glc-1	101.6	4.94 (d, 7.3)
Glc-2	74.7	3.39[a]
Glc-3	77.8	3.39[a]
Glc-4	71.2	3.31 (t, 9.1)
Glc-5	78.3	3.42 (ddd, 9.6, 5.8, 2.2)
Glc-6	62.4	3.62 (dd, 12.2, 5.8) 3.81 (dd, 12.2, 2.2)

[a] Overlapping signals.

Since the EtOAc fraction using in isolation showed inhibitory activity in α-glucosidase inhibition assay, compounds **1**–**12** were evaluated for their α-glucosidase inhibitory activity. Tiliroside (**8**) showed weak inhibitory activity against rat intestinal maltase with an IC_{50} value of 0.58 mM.

2.1. Concluding remarks

Compounds **1**–**12** can be classified into flavanonol glycosides (**1**), flavonols and their glycosides (**6**, **7**, **8**, **10**, **11**, and **12**), flavones (**9**), 3,4-dihydroisocoumarin glycosides (**2** and **4**) and 2-alkylated chromone glycosides (**3** and **5**), respectively. Agrimonolide 6-*O*-glucoside (**4**) is a characteristic constituent of *A. pilosa*. Further isolation of its demethylated derivate **2** suggests that **2** maybe a biosynthesis precursor of **4**. 2-Alkylated chromone glycosides are rare in nature, and have only been reported from the genus *Hypericum* (An et al., 2009; Tanaka et al., 2009), *Staphylea* (Sueyoshi et al., 2008) and *Aloe* (Lv et al., 2008). This is the first report of 2-alkylated chromone glycosides from the genus *Agrimonia*.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO P-2200 polarimeter in a 0.5-dm cell, whereas IR spectra were obtained on a JASCO FT/IR-4100 spectrometer by the KBr disk method. The ^{1}H- and ^{13}C NMR spectra were measured on a JEOL ECP-500 spectrometer with TMS as the internal reference, with chemical shifts expressed in δ (ppm). ESI–MS and HR–ESI–MS were conducted using a JEOL JMS-T100LP AccuTOF LC-plus mass spectrometer. For HPLC, a JASCO PU-2086 HPLC system, equipped with a JASCO RI-2301 Differential Refractometer detector, was used. Silica gel CC was carried out using Silica gel 60 N (Kanto Chemical Co. Inc., Tokyo, Japan), and TLC used Silica gel 60 F_{254} plates (E. Merck). GLC was carried out on a Perkin–Elmer Clarus 500 GC–MS instrument. Absorbance for bioactive assay was measured on microplate reader Immuno Mini NJ-2300 (Biotec Co. Ltd., Tokyo, Japan).

3.2. Plant material

Aerial parts of *A. pilosa* Ledeb. were collected in October, 2006, in Guyuan city, Ningxia Hui Autonomous Region, PR China, and identified by Professor Shirui Xing (Ningxia Institute for Drug control, PR China). A voucher specimen (TH206) has been deposited at Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Toho University.

3.3. Extraction and isolation

The air-dried aerial parts of *A. pilosa* (725 g) were extracted with MeOH (10 L × 3) at room temperature. Evaporation of the solvent under reduced pressure from the combined extract gave a MeOH extract (69.0 g), which was then partitioned between EtOAc and H_2O. The EtOAc fraction (29.6 g) was subjected to silica gel CC with a gradient of $CHCl_3$–MeOH–H_2O to give eight fractions (1–8). Fractions 4 (1.4 g), 5 (1.3 g) and 6 (4.5 g) were separated by preparative reversed phase HPLC to afford 12 compounds, **1** (7 mg), **2** (3 mg), **3**

(4 mg), **4** (30 mg), **5** (4 mg), **6** (9 mg), **7** (22 mg), **8** (64 mg), **9** (5 mg), **10** (8 mg), **11** (10 mg), and **12** (7 mg).

3.4. (−)-Aromadendrin 3-O-β-D-glucopyranoside (**1**)

Pale yellow solid. $[\alpha]_D^{25}$: −84.1 (*c* 0.7, MeOH). UV (MeOH): λ_{max} nm (logε) 213 (4.36), 225 (sh) (4.31), 291 (4.11), 330 (sh) (3.66). IR (KBr): ν_{max} 3423, 1638, 1543, 1522, 1459, 1384, 1260, 1170, 1082, 1026 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1. ESI–MS (positive) *m/z*: 473 $[M + Na]^+$. HR–ESI–MS (positive) *m/z*: Found: 473.1056; Calc. for $[C_{21}H_{22}O_{11}Na]^+$: 473.1060. CD (MeOH): $[\theta]^{25}$ (nm) −11759 (323), +13335 (293), +7574 (229), −18728 (216).

3.5. Desmethylagrimonolide 6-O-β-D-glucopyranoside (**2**)

Pale yellow solid. $[\alpha]_D^{20}$: −12.7 (*c* 0.3, MeOH). UV (MeOH): λ_{max} nm (logε) 216 (4.21), 263 (3.85), 303 (3.45). IR (KBr): ν_{max} 3445, 1637, 1518, 1459, 1384, 1255, 1205, 1175, 1078 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 2. ESI–MS (positive) *m/z*: 485 $[M + Na]^+$. HR–ESI–MS (positive) *m/z*: Found: 485.1427; Calc. for $[C_{23}H_{26}O_{10}Na]^+$: 485.1424. CD (MeOH): $[\theta]^{25}$ (nm) −3021 (302), +3849 (267), −4205 (227).

3.6. 5,7-Dihydroxy-2-propylchromone 7-O-β-D-glucopyranoside (3)

Colorless solid. $[\alpha]_D^{20}$: −63.9 (*c* 0.4, MeOH). UV (MeOH): λ_{max} nm (logε) 229 (4.18), 248 (4.19), 285 (3.75), 311 (sh) (3.57). IR (KBr): ν_{max} 3423, 1656, 1560, 1543, 1509, 1459, 1266, 1176, 1077 cm^{-1}. For ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 3. ESI–MS (positive) *m/z*: 405 $[M + Na]^+$. HR–ESI–MS (positive) *m/z*: Found: 405.1164; Calc. for $[C_{18}H_{22}O_9Na]^+$: 405.1161.

3.7. Enzymatic hydrolysis of **1–4**

Compound **1** (1.0 mg) was dissolved in 0.1 M NaoAc acetate buffer (pH 4.0, 1.0 ml), then naringinase (1.0 U, Sigma Chemical Co.) was added. After the reaction solution was stirred at 40 °C for 4 h, it was passed through a Sep-pak C_{18} cartridge, then eluted with H_2O (10 ml). Further elution by MeOH (10 ml) afforded the aglycone **1a** (0.6 mg), which was identified as (−)-aromadendrin by comparison with literature data (Takahashi et al., 1988; Prescott et al., 2002).

Enzymatic hydrolysis of **2** (1.0 mg), **3** (1.0 mg), and **4** (3.0 mg) were carried out by the same procedure as **1** to afford the aglycones **2a** (desmethylagrimonolide, 0.5 mg) (Yamato, 1958), **3a** (5,7-dihydroxy-2-propylchromone, 0.8 mg) (Alves et al., 1999) and **4a** (agrimonolide, 2.5 mg) (Arakawa et al., 1968; Yamato and Hashigaki, 1976).

Desmethylagrimonolide (**2a**): pale yellow solid. $[\alpha]_D^{23}$: −3.5 (*c* 0.1, MeOH). UV (MeOH): λ_{max} nm (logε) 215 (3.87), 269 (3.54), 302 (3.26). IR (KBr): ν_{max} 3445, 1637, 1518, 1459, 1384, 1255, 1205, 1175, 1078 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ 7.04 (2H, d, *J* = 8.5 Hz, H-2′ and 6′), 6.71 (2H, d, *J* = 8.7 Hz, H-3′ and 5′), 6.18 (1H, d, *J* = 2.3 Hz, H-7), 6.16 (1H, d, *J* = 2.3 Hz, H-5), 4.47 (1H, m, H-3), 2.87 (2H, m, H_2-4), 2.79 (1H, ddd, *J* = 14.7, 9.4, 5.3 Hz, H-2″a), 2.69 (1H, ddd, *J* = 14.0, 9.2, 7.1 Hz, H-2″b), 2.06 (1H, m, H-1″a), 1.96 (1H, m, H-1″b). ^{13}C NMR (CD_3OD, 125 MHz): δ 171.8 (qC, C-1), 166.8 (qC, C-8), 165.7 (qC, C-6), 156.7 (qC, C-4′), 143.4 (qC, C-10), 133.3 (qC, C-1′), 130.4 (CH, C-2′ and 6′), 116.3 (CH, C-3′ and 5′), 108.6 (CH, C-5), 102.5 (qC, C-9, CH, C-7), 79.9 (CH, C-3), 38.0 (CH_2, C-1″), 34.0 (CH_2, C-4), 31.2 (CH_2, C-2″). ESI–MS (negative) *m/z*: 299 $[M–H]^-$. CD (MeOH): $[\theta]^{25}$ (nm) −1133 (302), +2137 (269), −1500 (230).

5,7-Dihydroxy-2-propylchromone (**3a**): colorless solid. $[\alpha]_D^{23}$: +0.25 (*c* 0.1, MeOH). IR (KBr): ν_{max} 3447, 1647, 1560, 1508, 1499, 1474, 1459, 1420, 1357, 1167 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ 6.31 (1H, d, *J* = 2.3 Hz, H-8), 6.18 (1H, d, *J* = 2.3 Hz, H-6), 6.05 (1H, s, H-3), 2.61 (2H, t, *J* = 7.4 Hz, H-1′), 1.76 (2H, qt, *J* = 7.4 Hz, H-2′), 1.02 (3H, t, *J* = 7.4 Hz, H-3′). ^{13}C NMR (CD_3OD, 125 MHz): δ 184.1 (qC, C-4), 172.4 (qC, C-2), 166.2 (qC, C-7), 163.3 (qC, C-5), 160.0 (qC, C-9), 108.5 (CH, C-3), 105.4 (qC, C-10), 100.1 (CH, C-6), 95.0 (CH, C-8), 36.9 (CH_2, C-1′), 21.3 (CH_2, C-2′), 13.8 (CH_3, C-3′). ESI–MS (positive) *m/z*: 221 $[M + H]^+$.

Agrimonolide (**4a**): colorless solid. $[\alpha]_D^{24}$: −7.4 (*c* 0.3, MeOH). UV (MeOH): λ_{max} nm (logε) 217 (4.30), 268 (4.00), 300 (3.66). IR (KBr): ν_{max} 3425, 1656, 1631, 1510, 1383, 1245, 1168, 1117 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ 7.15 (2H, d, *J* = 8.8 Hz, H-2′ and 6′), 6.83 (2H, d, *J* = 8.7 Hz, H-3′ and 5′), 6.21 (1H, d, *J* = 2.3 Hz, H-7), 6.20 (1H, d, *J* = 2.0 Hz, H-5), 4.47 (1H, m, H-3), 3.75 (3H, s, OCH_3), 2.88 (2H, m, H_2-4), 2.82 (1H, ddd, *J* = 14.4, 9.6, 5.3 Hz, H-2″a), 2.73 (1H, ddd, *J* = 13.8, 9.2, 7.1 Hz, H-2″b), 2.07(1H, m, H-1″a), 1.97(1H, m, H-1″b). ^{13}C NMR (CD_3OD, 125 MHz): δ 171.7 (qC, C-1), 166.4 (qC, C-8), 165.7 (qC, C-6), 159.6 (qC, C-4′), 143.5 (qC, C-10), 134.5 (qC, C-1′), 130.4 (CH, C-2′ and 6′), 115.0 (CH, C-3′ and 5′), 108.0 (CH, C-5), 102.3 (qC, C-7), 101.6 (CH, C-9), 79.9 (CH, C-3), 55.7 (CH_3, OCH_3), 37.9 (CH_2, C-1″), 34.0 (CH_2, C-4), 31.2 (CH_2, C-2″). ESI–MS (positive) *m/z*: 337 $[M + Na]^+$. CD (MeOH): $[\theta]^{25}$ (nm) −3037 (301), +6789 (269), −4511 (231).

3.8. Methylation of **2** and **4**

Compounds **2** and **4** (each 0.5 mg) were individually dissolved in MeOH (0.1 ml), respectively. After $TMSCHN_2$ (0.6 M in ether, 20 μl) was added, the solutions stood for 6 h at room temperature. The residues were obtained by evaporation *in vacuo* and purified by preparative TLC with $CHCl_3$–MeOH–H_2O (60:15:1) to give a same compound **2b** (each 0.5 mg). The structure of **2b** was determined as methylagrimonolide 6-*O*-β-D-glucopyranoside.

Methylagrimonolide 6-*O*-β-D-glucopyranoside (**2b**): colorless solid. $[\alpha]_D^{22}$: −11.2 (*c* 0.1, MeOH). UV (MeOH): λ_{max} nm (logε) 216 (4.45), 259 (4.05), 298 (3.70). IR (KBr): ν_{max} 3397, 1708, 1610, 1514, 1252, 1102, 1082, 1057, 1038 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): δ 7.13 (2H, d, *J* = 8.8 Hz, H-2′ and 6′), 6.83 (2H, d, *J* = 8.7 Hz, H-3′ and 5′), 6.74 (1H, d, *J* = 2.0 Hz, H-7), 6.60 (1H, d, *J* = 2.0 Hz, H-5), 5.00 (1H, d, *J* = 7.5 Hz, Glc-H-1), 4.34 (1H, m, H-3), 3.92 (1H, dd, *J* = 12.1, 2.3 Hz, Glc-H-6a), 3.88 (3H, s, 8′-OCH_3), 3.75 (3H, s, 4′-OCH_3), 3.66 (1H, dd, *J* = 12.1, 6.4 Hz, Glc-H-6b), 3.48 (3H, m, Glc-H-2, 3 and 5), 3.34 (1H, m, Glc-H-4), 2.93 (1H, dd, *J* = 16.7, 4.3 Hz, H-4a), 2.88 (1H, dd, *J* = 16.1, 10.1 Hz, H-4b), 2.81 (1H, ddd, *J* = 14.5, 9.2, 5.3 Hz, H-2″a), 2.72 (1H, ddd, *J* = 13.9, 9.1, 7.1 Hz, H-2″b), 2.05 (1H, m, H-1″a), 1.95 (1H, m, H-1″b). ^{13}C NMR (CD_3OD, 500 MHz): δ 165.5 (qC, C-1), 164.5 (qC, C-8), 164.3 (qC, C-6), 159.6 (qC, C-4′), 145.7 (qC, C-10), 134.5 (qC, C-1′), 130.4 (CH, C-2′ and 6′), 115.0 (CH, C-3′ and 5′), 108.4 (qC, C-9), 108.3 (CH, C-5), 101.8 (CH, Glc-C-1), 101.0 (CH, C-7), 78.7 (CH, Glc-C-5), 78.5 (CH, C-3), 78.0 (CH, Glc-C-3), 74.8 (CH, Glc-C-2), 71.6 (CH, Glc-C-4), 62.7 (CH_2, Glc-C-6), 56.6 (CH_3, 8′-OCH_3), 55.7 (CH_3, 4′-OCH_3), 37.7 (CH_2, C-1″), 35.4 (CH_2, C-4), 31.2 (CH_2, C-2″). ESI–MS (positive) *m/z*: 513 $[M + Na]^+$.

3.9. Acid hydrolysis and determination of the absolute configuration of sugars in **1–4**

Acid hydrolysis of compound **1–4** (each 0.5 mg) was carried out by the same procedure as in our previous study (Li et al., 2009). Briefly, acid hydrolysis was carried out in 1 M HCl (dioxane-H_2O, 1:1, 200 μl) at 100 °C for 1 h under Ar. After the solution was extracted with EtOAc (1 ml × 3) to remove the aglycone, the aqueous layer was evaporated to give the sugar fraction. The solution of the

sugar fraction in pyridine (0.1 ml) was added to a solution of 0.08 M L-cysteine methyl ester hydrochloride in pyridine (1.5 ml), and kept at 60 °C for 1.5 h. The residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml) for 2 h, then partitioned between *n*-hexane and H_2O (0.3 ml each). GC–MS analyses of the *n*-hexane extracts and those derivatives from D-glucose and L-glucose standards, showed the presence of D-glucose in **1**–**4**.

3.10. α-Glucosidase inhibition assay

α-Glucosidase inhibitory activity of the isolated compounds was measured as follows. Rat intestine acetone powder (100 mg, Sigma–Aldrich Japan Co., Tokyo, Japan) in 56 μM maleate buffer (pH 6.0, 0.9 ml) was sonicated at 4 °C for 20 min, then centrifuged at 15,000*g* at 4 °C for 10 min. The supernatant was then diluted by adding a two fold volume of 56 μM maleate buffer which was used as sucrase solution, and by adding a forty fold volume of 56 μM maleate buffer which was used as maltase solution. Sucrose or maltose in 56 μM maleate buffer (20 mg/ml) was used as substrate solution. The reaction mixtures containing the above enzyme solution (0.1 ml), substrate solution (0.1 ml), samples of various concentrations in MeOH (0.01 ml) and 56 μM maleate buffer (0.04 ml) were incubated for 60 min at 37 °C, and heated at 102 °C for 10 min to stop the reaction. The glucose release was determined in a 96-well plate using a glucose assay kit (Glucose CII-test Wako, Wako Pure Chem. Co., Osaka, Japan) based on the glucose oxidase/peroxidase method. Acarbose was used as the positive control with the IC_{50} value of 7.5 μM to sucrose and 1.2 μM to maltase at this assay system.

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