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ORIGINAL PAPER

Novel phenolic glycosides, adenophorasides A–E, from Adenophora roots

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Abstract Five novel phenolic glycosides, adenophorasides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), were isolated from commercial Adenophora roots, together with vanilloloside (**6**), 3,4-dimethoxybenzyl alcohol 7-*O*-*β*-D-glucopyranoside (**7**), and lobetyolin (**8**). The structures of the new compounds (**1**–**5**) were characterized as 4-hydroxy-3-methoxyphenylacetonitrile 4-*O*-*β*-D-glucopyranoside (**1**), 4-hydroxy-3-methoxyphenylacetonitrile 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**2**), 4-hydroxy-3-methoxyphenylacetonitrile 4-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**3**), 4-hydroxyphenylacetonitrile 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**4**), and 4-hydroxy-3-methoxybenzyl alcohol 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**5**), respectively, by means of spectroscopic and chemical analyses.

Keywords Adenophora roots · Adenophorae Radix · Campanulaceae · Phenolic glycoside · Phenylacetonitrile · Adenophoraside

Introduction

The family of Campanulaceae comprises about 80 genera such as Platycodon, Codonopsis, and Adenophora. Phytochemical investigations of Platycodon roots (the dried roots of *Platycodon grandiflorum*) have revealed many saponin constituents [1–5]. It is widely thought that these saponins play an important role in the antitussive and expectorant activities of Platycodon roots in empirical medicine and Kampo medicine [6]. Adenophora roots (the dried roots of Adenophora species) have been used as antitussives and expectorants in the same manner as Platycodon roots [7, 8]. However, in contrast to Platycodon roots, the chemical constituents and bioactive components of Adenophora roots have not yet been revealed. Therefore, we initiated this study to elucidate the constituents of Adenophora roots, specifically focusing on the fraction that included the glycosides, in order to identify the bioactive components.

The origin of Adenophora roots is the dried roots of *Adenophora tetraphylla* and the allied plants such as *A. triphylla* and *A. stricta* [7–9]. As for the constituents of *A. tetraphylla*, α-spinasterol [10], nonacosan-10-ol [10], 24-methylene cycloartenol [10], lupenone [10], *β*-sitosterol [10], 3-*O*-palmitoyl-*β*-sitosterol [10], *β*-sitosterol 3-*O*-*β*-D-glucopyranoside [10, 11], eicosanoic acid [10], linoleic acid [11], methyl stearate [11], syringinoside [11], and shashenosides I–III [11] have been reported. And, essential oils [12, 13], pyrrolidine and piperidine derivatives [14], and triterpenoids [15] have been isolated from *A. triphylla* and *A. stricta*. In this paper, we describe the isolation and characterization of five novel compounds including phenylacetonitriles, regarded as the precursors of cyanogenic glycosides, from Adenophora roots.

Results and discussion

Adenophora roots (6.9 kg) were extracted with 70% aqueous methanol at room temperature. After concentration, the extract was partitioned between water and diethyl ether. The aqueous portion, after concentration, was subjected to column chromatography over silica gel and octadecylsilanized silica gel (ODS) repeatedly to give eight

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compounds, as described in the "Experimental". Three of these compounds were identified as vanilloloside (**6**), 3,4-dimethoxybenzyl alcohol 7-*O*-*β*-D-glucopyranoside (**7**), and lobetyolin (**8**) by comparison of their physical and spectral data with those reported [16, 17]. The remaining five compounds were found to be novel phenylacetonitrile glycosides and a benzyl alcohol glycoside, as discussed below (Fig. 1).

Adenophoraside A (**1**), a white amorphous powder, showed a $[M+H]^+$ peak at *m/z* 326.1245 in HR FAB-MS, suggesting its molecular formula to be $C_{15}H_{19}NO_7$. The IR spectrum of **1** exhibited characteristic absorption bands due to hydroxyl groups (3408 cm^{-1}), a nitrile group (2249 cm^{-1}), and an aromatic ring (1597 and 1518 cm^{-1}). The ^{13}C-NMR spectroscopic features of **1** were close to those of **6**, except for the presence of the sp quaternary carbon signal (δ 119.5) and the methylene group signal (δ 21.9) instead of the hydroxymethyl carbon signal in **6** and some differences in the chemical shifts of the C-1, C-2, and C-6 signals (−11.9, +1.4, +1.6 ppm, respectively) from that in **6** (Table 1).

On enzymatic hydrolysis, **1** afforded a sugar portion and an aglycone (**1a**), the latter as an orange solid. The sugar portion was identified as D-glucose by HPLC. The aglycone **1a** showed a $[M]^+$ peak at *m/z* 163.0631 in HR FAB-MS corresponding to the molecular formula of $C_9H_9NO_2$. The IR spectrum of **1a** exhibited absorption bands due to similar functional groups as in **1**. The NMR spectrum of **1a** indicated the presence of a methoxyl group (δ_H 3.75; δ_C 55.6), a methylene group (δ_H 3.85; δ_C 21.9), a 1,2,4-trisubstituted benzene (δ_H 6.72, 6.75, 6.87; δ_C 112.3, 115.7, 120.5, 121.6, 146.0, 147.8), a nitrile group (δ_C 119.7), and a phenolic hydroxyl group (δ_H 9.07). In the HMBC spectrum of **1a**, correlations were observed between a phenolic hydroxyl proton (δ 9.07) and C-3 (δ 147.8), C-4 (δ 146.0), and C-5 (δ 115.7); and H-7 (δ 3.85) and C-1 (δ 121.6), C-2 (δ 112.3), C-6 (δ 120.5), and C-8 (δ 119.7); and a methoxyl proton (δ 3.75) and C-3 (δ 147.8). These data suggested **1a** to be 4-hydroxy-3-methoxyphenylacetonitrile, and this was proved by direct comparison (TLC, NMR, and MS) with an authentic sample synthesized by Schwartz's method [18]. On the other hand, the anomeric proton signal of **1** appeared as a doublet (J = 7.5 Hz) at δ 4.89, and was shown to correlate with the C-4 carbon signal (δ 145.9) in the HMBC spectrum. These results demonstrated that adenophoraside A (**1**) was 4-hydroxy-3-methoxyphenylacetonitrile 4-*O*-*β*-D-glucopyranoside.

Adenophoraside B (**2**), a white amorphous powder, showed a $[M+H]^+$ peak at *m/z* 488.1755 in HR FAB-MS, suggesting its molecular formula to be $C_{21}H_{29}NO_{12}$, 162 mass units ($C_6H_{10}O_5$) larger than that of **1**. The ^{13}C-NMR spectroscopic features of **2** were similar to those of **1**, except for the appearance of an additional six signals (δ 61.1, 70.1, 73.6, 76.6, 76.9, 103.3) and the observation of the lower field shift (+7.8 ppm) of the C-6′ carbon signal from that in **1** because of the glycosylation shift (Table 1). On enzymatic hydrolysis, **2** afforded a sugar portion and an aglycone, which were identified as D-glucose and **1a**, respectively, by a similar method to that used for **1**. A correlation was observed between H-1″ (δ 4.16, d, J = 8.1 Hz) and C-6′ (δ 68.4) in the HMBC spectrum of **2** which indicated that the additional glucopyranosyl moiety was attached to C-6′ of **2**. These facts indicated that adenophoraside B (**2**) was 4-hydroxy-3-

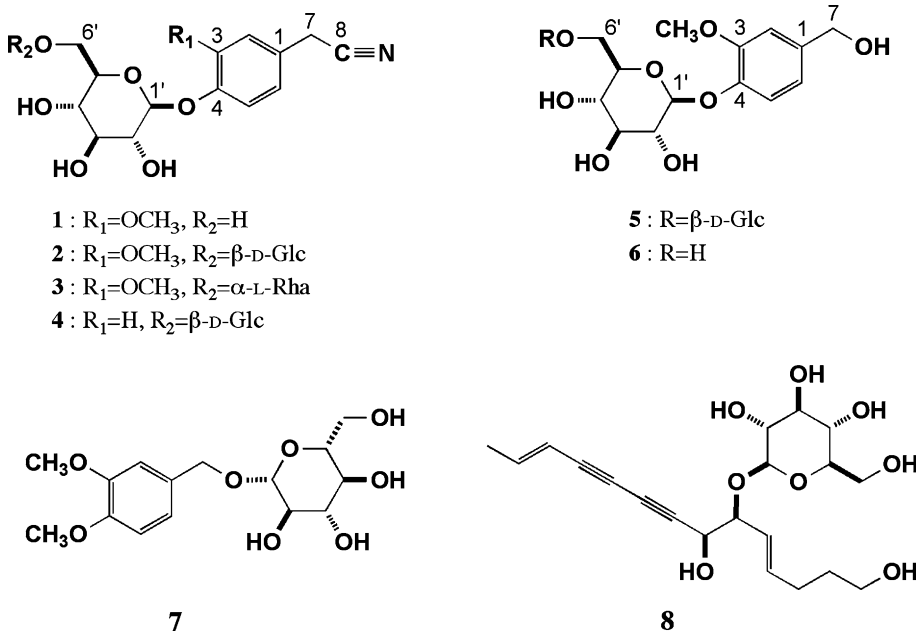

Fig. 1 Structures of **1**–**8** isolated from Adenophora roots

Table 1 ^{13}C- and ^{1}H-NMR spectral data of **1–5** (125 MHz for ^{13}C and 500 MHz for ^{1}H)

	1			**2**			**3**			**4**			**5**		
	δ_C	δ_H		δ_C	δ_H		δ_C	δ_H		δ_C	δ_H		δ_C	δ_H	
1	124.5	–		124.6	–		124.8	–		124.3	–		136.3	–	
2	112.5	6.95	(1H, d, 1.7)	112.5	6.94	(1H, d, 1.7)	112.6	6.96	(1H, d, 1.7)	129.3	7.25	(1H, d, 8.6)	111.0	6.91	(1H, d, 1.7)
3	149.1	–		149.0	–		149.2	–		116.9	7.08	(1H, d, 8.6)	148.7	–	
4	145.9	–		145.9	–		145.9	–		156.9	–		145.2	–	
5	115.6	7.07	(1H, d, 8.0)	116.1	7.16	(1H, d, 8.0)	116.0	7.04	(1H, d, 8.6)	116.9	7.08	(1H, d, 8.6)	115.5	7.09	(1H, d, 8.6)
6	120.2	6.83	(1H, dd, 1.7, 8.0)	120.5	6.85	(1H, dd, 1.7, 8.0)	120.3	6.84	(1H, dd, 1.7, 8.6)	129.3	7.25	(1H, d, 8.6)	118.8	6.80	(1H, dd, 1.7, 8.6)
7	21.9	3.92	(2H, s)	22.0	3.91	(2H, s)	22.0	3.92	(2H, s)	21.7	3.92	(2H, s)	62.8	4.40	(2H, d, 5.7)
8	119.5	–		119.5	–		119.5	–		119.6	–		–		
OCH_3	55.7	3.75	(3H, s)	55.7	3.75	(3H, s)	55.7	3.76	(3H, s)	–	–		55.6	3.73	(3H, s)
7-OH	–	–		–	–		–	–		–	–		–	5.09	(1H, t, 5.7)
Inner glucose															
1′	99.9	4.89	(1H, d, 7.5)	100.0	4.87	(1H, d, 7.5)	100.3	4.83	(1H, d, 7.5)	100.4	4.82	(1H, d, 6.9)	100.2	4.83	(1H, d, 7.5)
2′	73.2	3.23[a]		73.2	3.24[a]		73.2	3.24[a]		73.2	3.22[a]		73.2	3.23[a]	
3′	76.9	3.23[a]		76.8	3.24[a]		76.8	3.25[a]		76.5	3.25[a]		76.8	3.23[a]	
4′	69.6	3.14	(1H, m)	69.6	3.15	(1H, m)	69.9	3.09	(1H, m)	69.8	3.14	(1H, m)	69.6	3.15	(1H, m)
5′	77.0	3.28[a]		75.9	3.55[a]		75.6	3.42[a]		75.9	3.57[a]		75.9	3.51	(1H, m)
6′	60.6	3.43[a]		68.4	3.55[a]		66.5	3.38[a]		68.6	3.57[a]		68.2	3.57	(1H, dd, 6.3, 10.9)
		3.64	(1H, m)		3.93	(1H, br d, 9.8)		3.81	(1H, br d, 10.3)		3.98	(1H, m)		3.93	(1H, br d, 10.9)
Terminal glucose															
1″	–	–		103.3	4.16	(1H, d, 8.1)	–	–		103.4	4.18	(1H, d, 8.1)	103.2	4.16	(1H, d, 8.1)
2″	–	–		73.6	2.93[a]		–	–		73.6	2.95[a]		73.6	2.93[a]	
3″	–	–		76.6	3.05	(1H, m)	–	–		76.7	3.09	(1H, dd, 8.6, 8.6)	76.6	3.06[a]	
4″	–	–		70.1	3.01	(1H, m)	–	–		70.2	3.02[a]		70.1	3.01[a]	
5″	–	–		76.9	2.96[a]		–	–		76.9	2.99[a]		76.8	2.96[a]	
6″	–	–		61.1	3.39[a]		–	–		61.1	3.40[a]		61.1	3.38[a]	
					3.63	(1H, m)					3.64	(1H, br d, 10.9)		3.62	(1H, dd, 5.7, 10.3)
Rhamnose															
1″	–	–		–	–		100.6	4.51	(1H, d, 1.2)	–	–		–	–	
2″	–	–		–	–		70.4	3.57	(1H, br s)	–	–		–	–	
3″	–	–		–	–		70.7	3.42[a]		–	–		–	–	
4″	–	–		–	–		72.0	3.16	(1H, m)	–	–		–	–	
5″	–	–		–	–		68.4	3.40[a]		–	–		–	–	
6″	–	–		–	–		17.9	1.09	(3H, d, 6.3)	–	–		–	–	

Measured in DMSO-d_6

δ in ppm from TMS (coupling constants (J) in Hz)

[a] Overlapped signal

methoxyphenylacetonitrile 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

Adenophoraside C (**3**), a white amorphous powder, showed a $[M+H]^+$ peak at *m/z* 472.1868 in HR FAB-MS, suggesting its molecular formula to be $C_{21}H_{29}NO_{11}$, 16 mass units smaller than that of **2**. The ^{13}C-NMR spectrum of **3** exhibited similar signals to that of **2**, except for the presence of six signals (δ 17.9, 68.4, 70.4, 70.7, 72.0, 100.6) instead of the terminal glucosyl moiety in **2** (Table 1). On enzymatic hydrolysis, **3** afforded a sugar portion and an aglycone. The former was identified as D-glucose and L-rhamnose, the latter was identified as **1a** by a similar method to that used for **1**. The configuration of C-1″ was determined to be α from the chemical shifts of C-5″ (**3**: δ 68.4; shatavaroside A [19]: δ 68.7) in the ^{13}C-NMR spectrum [19, 20]. The observation of the cross peak between H-1″ (δ 4.51) and C-6′ (δ 66.5) in the HMBC spectrum of **3** indicated that an α-L-rhamnopyranosyl moiety was located at C-6′ of **3**. Thus, the structure of adenophoraside C (**3**) was determined to be 4-hydroxy-3-methoxyphenylacetonitrile 4-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside.

Adenophoraside D (**4**), a white amorphous powder, showed a $[M+H]^+$ peak at *m/z* 458.1611 in HR FAB-MS, suggesting its molecular formula to be $C_{20}H_{27}NO_{11}$, 30 mass units (CH_2O) smaller than that of **2**. The ^{13}C-NMR spectrum of **4** showed 20 signals, twelve of which were similar to those due to the disaccharide moiety of **2** (Table 1). On enzymatic hydrolysis, **4** afforded a sugar portion and an aglycone (**4a**), the latter as an orange solid. The sugar portion was identified as D-glucose by HPLC. The aglycone **4a** showed a $[M]^+$ peak at *m/z* 133.0534 (C_8H_7NO) in HR FAB-MS. The NMR spectrum of **4a** indicated the presence of a methylene group (δ_H 3.85; δ_C 21.5), a 1,4-disubstituted benzene (δ_H 6.75, 6.75, 7.12, 7.12; δ_C 115.6, 115.6, 121.1, 129.2, 129.2, 156.8), a nitrile group (δ_C 119.7), and a phenolic hydroxyl group (δ_H 9.49). The HMBC spectrum of **4a** revealed correlations between H-7 (δ 3.85) and C-1 (δ 121.1), C-2 (δ 129.2), C-6 (δ 129.2), and C-8 (δ 119.7); and a phenolic hydroxyl proton (δ 9.49) and C-3 (δ 115.6), C-4 (δ 156.8), and C-5 (δ 115.6). These results indicated that **4a** was 4-hydroxyphenylacetonitrile, i.e., corresponding to the demethoxyl derivative of **1a**, and this was proved by direct comparison (TLC, NMR, and MS) with an authentic sample synthesized by Schwartz's method [18]. On the other hand, the observation of the cross peak between H-1′ (δ 4.82) and C-4 (δ 156.9) in the HMBC spectrum of **4** indicated that the sugar moiety was located at C-4 of **4**. Consequently, adenophoraside D (**4**) was characterized as 4-hydroxyphenylacetonitrile 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

Adenophoraside E (**5**), a white amorphous powder, showed a $[M+H]^+$ peak at *m/z* 479.1750 in HR FAB-MS, suggesting its molecular formula to be $C_{20}H_{30}O_{13}$. This result indicated that the molecular weight of **5** was 162 ($C_6H_{10}O_5$) larger than that of vanilloloside (**6**). The IR spectrum of **5** exhibited characteristic absorption bands due to hydroxyl groups (3408 cm^{-1}) and an aromatic ring (1597 and 1516 cm^{-1}). The ^{13}C-NMR spectroscopic features of **5** were similar to those of **6**, except for the appearance of additional six signals (δ 61.1, 70.1, 73.6, 76.6, 76.8, 103.2) and the observation of the lower field shift (+7.5 ppm) of the C-6′ carbon signal from that in **6** because of the glycosylation shift (Table 1). On enzymatic hydrolysis, **5** afforded an aglycone, which was identified as vanillyl alcohol by comparison with commercial authentic samples, and D-glucose identified by HPLC. On the other hand, the observation of the cross peak between H-1″ (δ 4.16, d, $J = 8.1$ Hz) and C-6′ (δ 68.2) in the HMBC spectrum of **5** indicated that the additional glucopyranosyl moiety was located to the C-6′. Thus, the structure of adenophoraside E (**5**) was characterized as 4-hydroxy-3-methoxybenzyl alcohol 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

As mentioned above, we isolated eight compounds from Adenophora roots, including five novel compounds (**1**–**5**). Among them, **1**–**4** are phenylacetonitrile glycosides. It is well known that cyanogenic glycosides are found in many natural sources such as plants of the family Rosaceae and Gramineae and are biosynthesized from their corresponding amino acids via phenylacetonitrile. To our knowledge, only a few phenylacetonitriles have been isolated from natural sources: phenylacetonitrile [21], 4-[*β*-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]-phenylacetonitrile [22], niazirin [23], niazirinin [23], and niaziridin [24]. This is the first report of phenylacetonitrile isolation from plants of the family Campanulaceae. In this study, saponin was not isolated from Adenophora roots. This is mere conjecture, but the antitussive and expectorant effects of Adenophora roots might therefore not be due to saponins.

Additionally, we evaluated the cytotoxic activity of novel phenolic glycosides (**1**–**5**) against human breast cancer (MCF-7) and leukemia (U937) cell lines by using the MTT assay. The results demonstrated that these compounds had little inhibitory effect on cell growth even at 100 μM (data not shown).

Experimental

General

Optical rotations were obtained on a P-1020 polarimeter (JASCO, Tokyo, Japan). IR spectra were measured on an FT/IR-410 spectrometer (JASCO). NMR spectra were recorded on a JNM ECA-500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C; JEOL, Tokyo, Japan), and the

chemical shifts were given in ppm scale from TMS used as an internal standard. The signals were assigned by DEPT and 2D NMR techniques (^{1}H–^{1}H COSY, HMQC, HMBC). MS spectra were obtained on a JMS-700 spectrometer (JEOL), with the matrix used for FAB-MS shown in parentheses. HPLC was carried out on a Mightysil RP-18GP (20 mm i.d. × 250 mm, 5 μm; Kanto Chemical, Tokyo, Japan) with a UV-8011 (Tosoh, Tokyo, Japan) for preparative HPLC or a COSMOSIL Sugar-D (4.6 mm i.d. × 250 mm; Nacalai Tesque, Kyoto, Japan) with an RI-2031 Plus and an OR-2090 Plus detector (JASCO) for the D/L determination of monosaccharides. TLC was performed on pre-coated silica gel 60 F_{254} or RP-18 WF_{254} plates (Merck Ltd., Darmstadt, Germany) with detection achieved by spraying with 10% H_2SO_4 followed by heating. Column chromatography (CC) was performed on silica gel 60 (63–200 μm; Merck Ltd.) or ODS (chromatorex DM-1020T; Fuji-Silysia Chemical Ltd., Aichi, Japan). Other reagents, unless otherwise stated, were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Extraction and isolation

Commercial Adenophora roots (6.90 kg, Lot. No. 028107003: imported from Hubei Province in China; Tochimoto-Tenkaido Co., Osaka, Japan; voucher specimens are deposited at the Herbarium of Showa University) were powdered and percolated with 70% MeOH (67 L) at room temperature. After being concentrated in vacuo at 40°C, the 70% MeOH extract (1.75 kg) was suspended in H_2O (4.8 L) and successfully extracted with diethyl ether (3 × 1.10 L) to yield a diethyl ether extract (20.9 g). The residual H_2O layer (1.73 kg) was purified by ODS CC, eluting with water containing increasing amounts of methanol, to give seven fractions (Fr): Frs. A (H_2O, 1.68 kg), B (H_2O, 10.5 g), C (30% MeOH, 17.2 g), D (30% MeOH, 2.5 g), E (70% MeOH, 8.2 g), F (70% MeOH, 1.4 g), and G (MeOH, 0.7 g).

Fr. B was purified by ODS CC, eluting with water containing increasing amounts of methanol, to give five fractions: Frs. B-1 (H_2O, 3.8 g), B-2 (30% MeOH, 3.1 g), B-3 (30% MeOH, 248 mg), B-4 (70% MeOH, 91 mg), and B-5 (MeOH, 131 mg). Fr. B-2 was purified by ODS CC (25% MeOH) to give six fractions: Frs. B-2-1 (377 mg), B-2-2 (150 mg), B-2-3 (59 mg), B-2-4 (557 mg), B-2-5 (299 mg), and B-2-6 (1.0 g). Fr. B-2-1 was purified by silica gel CC [$CHCl_3$–MeOH (4:1)] to give **1** (67 mg). B-2-2 was purified by silica gel CC [$CHCl_3$–MeOH–H_2O (38:12:1)] to give **6** (56 mg). Fr. B-2-4 was purified by silica gel CC [$CHCl_3$–MeOH–H_2O (34:16:3)] and then ODS CC (10% MeOH) to give **2** (307 mg). Frs. B-2-5 and B-2-6 were purified by silica gel CC [$CHCl_3$–MeOH–H_2O (14:6:1 and 16:9:2, respectively)] to give **4** (79 mg) and **5** (109 mg), respectively. Fr. C was purified by ODS CC, eluting with water containing increasing amounts of methanol, to give five fractions: Frs. C-1 (H_2O, 2.1 g), C-2 (30% MeOH, 6.6 g), C-3 (30% MeOH, 5.8 g), C-4 (70% MeOH, 630 mg), and C-5 (MeOH, 86 mg). Fr. C-2 was purified by silica gel CC [$CHCl_3$–MeOH–H_2O (14:6:1)] and then ODS CC (15% CH_3CN) to give **3** (26 mg) and **7** (6 mg). Fr. D was purified by silica gel CC [$CHCl_3$–MeOH–H_2O (38:12:1)] to give four fractions: Frs. D-1 (183 mg), D-2 (241 mg), D-3 (291 mg), and D-4 (1.34 g). Fr. D-2 was purified by ODS CC (40% MeOH) to give **8** (56 mg).

Adenophoraside A (**1**)

A white amorphous powder. $[\alpha]_D^{25}$ −55.9° (*c* 1.00, MeOH). IR (KBr) cm^{-1}: 3408, 2916, 2249, 1597, 1518. Positive HR FAB-MS (NBA) *m/z*: 326.1245 ($[M+H]^+$, $C_{15}H_{20}NO_7$: 326.1240). ^{13}C- and ^{1}H-NMR: Table 1. Selected HMBC correlations: H-7/C-1, C-2, C-6, C-8; O$\underline{CH_3}$/C-3; H-6/C-4; H-1′/C-4.

Adenophoraside B (**2**)

A white amorphous powder. $[\alpha]_D^{25}$ −57.6° (*c* 1.00, H_2O). Positive HR FAB-MS (NBA) *m/z*: 488.1755 ($[M+H]^+$, $C_{21}H_{30}NO_{12}$: 488.1768). IR (KBr) cm^{-1}: 3396, 2941, 2251, 1599, 1520. ^{13}C- and ^{1}H-NMR: Table 1. Selected HMBC correlations: H-7/C-1, C-2, C-6, C-8; O$\underline{CH_3}$/C-3; H-6/C-4; H-1′/C-4; H-1″/C-6′.

Adenophoraside C (**3**)

A white amorphous powder. $[\alpha]_D^{25}$ −35.4° (*c* 0.13, H_2O). Positive HR FAB-MS (NBA) *m/z*: 472.1868 ($[M+H]^+$, $C_{21}H_{30}NO_{11}$: 472.1819). IR (KBr) cm^{-1}: 3433, 2924, 2252, 1595, 1514. ^{13}C- and ^{1}H-NMR: Table 1. Selected HMBC correlations: H-7/C-1, C-2, C-6, C-8; O$\underline{CH_3}$/C-3; H-6/C-4; H-1′/C-4; H-1″/C-6′.

Adenophoraside D (**4**)

A white amorphous powder. $[\alpha]_D^{25}$ −66.1° (*c* 1.00, H_2O). Positive HR FAB-MS (NBA) *m/z*: 458.1611 ($[M+H]^+$, $C_{20}H_{28}NO_{11}$: 458.1662). IR (KBr) cm^{-1}: 3435, 2920, 2251, 1591, 1514. ^{13}C- and ^{1}H-NMR: Table 1. Selected HMBC correlations: H-7/C-1, C-2, C-6, C-8; H-1′/C-4; H-1″/C-6′.

Adenophoraside E (**5**)

A white amorphous powder. $[\alpha]_D^{25}$ −60.5° (*c* 1.00, H_2O). Positive HR FAB-MS (NBA) *m/z*: 479.1750 ($[M+H]^+$,

$C_{20}H_{31}O_{13}$: 479.1765). IR (KBr) cm^{-1}: 3408, 2925, 1597, 1516. ^{13}C- and ^{1}H-NMR: Table 1. Selected HMBC correlations: H-7/C-1, C-2, C-6; OC$\underline{H}_3$/C-3; H-6/C-4; H-1′/C-4; H-1″/C-6′.

Known compounds isolated

Vanilloloside (**6**) [16]: A white amorphous powder. $[\alpha]_D^{25}$ −47.7° (*c* 0.50, MeOH). Positive HR FAB-MS (NBA) *m/z*: 316.1148 ($[M]^+$, $C_{14}H_{20}O_8$: 316.1158). IR (KBr) cm^{-1} : 3433, 2922, 1597, 1514. ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 55.6 (O$\underline{C}H_3$), 60.7 (C-6′), 62.7 (C-7), 69.7 (C-4′), 73.3 (C-2′), 76.9 (C-5′), 77.0 (C-3′), 100.2 (C-1′), 111.1 (C-2), 115.2 (C-5), 118.6 (C-6), 136.4 (C-1), 145.3 (C-4), 148.8 (C-3). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.15 (1H, m, H-4′), 3.23 (1H, overlapped, H-2′), 3.23 (1H, overlapped, H-3′), 3.26 (1H, overlapped, H-5′), 3.43 (1H, ddd, J = 5.8, 5.8, 12.1 Hz, H-6′a), 3.64 (1H, br dd, J = 4.6, 12.1 Hz, H-6′b), 3.73 (3H, s, OC$\underline{H}_3$), 4.40 (2H, d, J = 5.2 Hz, H-7), 4.85 (1H, d, J = 7.5 Hz, H-1′), 5.09 (1H, t, J = 5.2 Hz, 7-O$\underline{H}$), 6.78 (1H, dd, J = 1.8, 8.0 Hz, H-6), 6.92 (1H, d, J = 8.0 Hz, H-5), 7.01 (1H, d, J = 1.8 Hz, H-2). Selected HMBC correlations: H-7/C-1, C-2, C-6; OC$\underline{H}_3$/C-3; H-6/C-4; H-2/C-4; 7-O$\underline{H}$/C-1, C-7; H-1′/C-4.

3,4-Dimethoxybenzyl alcohol 7-*O*-β-D-glucopyranoside (**7**) [16]: A white amorphous powder. $[\alpha]_D^{25}$ −39.9° (*c* 0.33, MeOH).

Lobetyolin (**8**) [17]: A brown syrup. $[\alpha]_D^{25}$ −26.6° (*c* 1.89, MeOH).

Enzymatic hydrolysis of **1**–**5**

Each glycoside (10.0 mg) was incubated with emulsin (β-glucosidase from almonds: G-0395: Lot No. 119H4029; 6.8 units; Sigma, MO, USA) in McIlvaine buffer (pH 5.0) at 37°C for 4 h. The reaction mixture was diluted with H_2O and passed through a Sep-pak ODS cartridge (Waters, MA, USA), eluting with H_2O and MeOH, to afford an H_2O eluate and a MeOH eluate, respectively. In the case of **1**–**4**, the MeOH eluate was purified by reverse-phase preparative HPLC (40% MeOH). Finally, **1a** was obtained as aglycone in the case of **1**–**3**, and **4a** and **5a** were obtained in the case of **4** and **5**, respectively. The H_2O eluate was analyzed by HPLC under the following conditions: column, COSMOSIL Sugar-D; solvent, 70% acetonitrile; flow rate, 1.0 ml/min; detectors, RI-2031 and OR-2090 plus. Consequently, the constituent of the H_2O eluate was detected to be D-glucose (t_R 5.9 min, positive) in the case of **1**, **2**, **4**, and **5**, and D-glucose and L-rhamnose (t_R 4.2 min, negative) in the case of **3** by comparison with an authentic sample.

4-Hydroxy-3-methoxyphenylacetonitrile (**1a**)

An orange solid. Positive HR FAB-MS (NBA) *m/z*: 163.0631 ($[M]^+$, $C_9H_9NO_2$:163.0633). IR (Nujor) cm^{-1} : 3390, 2925, 2252, 1603, 1518. ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 21.9 (C-7), 55.6 (O$\underline{C}H_3$), 112.3 (C-2), 115.7 (C-5), 119.7 (C-8), 120.5 (C-6), 121.6 (C-1), 146.0 (C-4), 147.8 (C-3). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.75 (3H, s, OC$\underline{H}_3$), 3.85 (2H, s, H_2-7), 6.72 (1H, dd, J = 1.7, 8.0 Hz, H-6), 6.75 (1H, d, J = 8.0 Hz, H-5), 6.87 (1H, d, J = 1.7 Hz, H-2), 9.07 (1H, s, 4-OH). Selected HMBC correlations: H-7/C-1, C-2, C-6, C-8; OC$\underline{H}_3$/C-3; H-6/C-4; O$\underline{H}$/C-3, C-4, C-5.

4-Hydroxyphenylacetonitrile **(4a)**

An orange solid. Positive HR FAB-MS (NBA) *m/z*: 133.0534 ($[M]^+$, C_8H_7NO:133.0528). IR (Nujor) cm^{-1}: 3326, 2925, 2252, 1610, 1516. ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 21.5 (C-7), 115.6 (C-3), 115.6 (C-5), 119.7 (C-8), 121.1 (C-1), 129.2 (C-2), 129.2 (C-6), 156.8 (C-4). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.85 (2H, s, H_2-7), 6.75 (2H, d, J = 8.6 Hz, H-3 and H-5), 7.12 (2H, d, J = 8.6 Hz, H-2 and H-6), 9.49 (1H, s, 4-OH). Selected HMBC correlations: H-7/C-1, C-2, C-6, C-8; O$\underline{H}$/C-3, C-4, C-5.

Synthesis of 4-hydroxy-3-methoxyphenylacetonitrile **(1a)**

4-Hydroxy-3-methoxyphenylacetonitrile (**1a**) was synthesized as described elsewhere but with slight modifications [18]. Briefly, a mixture of 4-hydroxy-3-methoxybenzyl alcohol (1.23 g, 8.0 mmol) and NaCN (472 mg, 9.6 mmol) in DMF (20 ml) was stirred under nitrogen at 120°C for 24 h. The reaction mixture was cooled and 0.4 ml of water was added, the mixture was then alkalized with solid NaOH to more than pH 10, followed by evaporation under vacuum. Water (1 ml) was added and neutralized with acetic acid. After the mixture was extracted with $CHCl_3$, the extract was washed with H_2O three times and dried over anhydrous Na_2SO_4, followed by evaporation under vacuum. The extract (1.26 g) was purified by silica gel CC [hexane–acetone (2:1)] and then ODS CC (30% MeOH) to give **1a** (315 mg, 1.9 mmol) as an orange solid.

Synthesis of 4-hydroxyphenylacetonitrile **(4a)**

4-Hydroxyphenylacetonitrile (**4a**) was synthesized similarly to **1a** but with slight modifications. Briefly, a mixture of 4-hydroxybenzyl alcohol (1.01 g, 8.15 mmol) and NaCN (483 mg, 9.9 mmol) in DMF (25 ml) was stirred under nitrogen at 120°C for 26 h. The reaction mixture was cooled and 0.5 ml of water was added, then the mixture

was alkalized with solid NaOH to more than pH 10, followed by evaporation under vacuum. After the addition of water (17.5 ml) and neutralization with acetic acid, the mixture was extracted with $CHCl_3$. The extract was washed with H_2O three times and dried over anhydrous Na_2SO_4, followed by evaporation under vacuum. The extract (964 mg) was purified by silica gel CC [hexane–acetone (2:1)] to give **4a** (546 mg, 4.1 mmol) as an orange solid.

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