Further 2R-Benzylmalate derivatives from the undergrounds parts of Arundina graminifolia (Orchidaceae)

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Abstract

Nine new glucosyloxybenzyl 2*R*-benzylmalate derivatives, named arundinosides R-Z (**1**-**9**) were isolated from the underground parts of *Arundina graminifolia*. The structures of the new compounds were elucidated by in-depth spectroscopic data analysis including 1D and 2D NMR experiments, in combination with mass spectrometry data.

Keywords: *Arundina graminifolia*, Orchidaceae, Glucosyloxybenzyl 2*R*-benzylmalate derivatives, arundinosides R-Z

1. Introduction

Arundina graminifolia is a plant belonging to the Orchidaceae family and it is mainly distributed in tropical Asian countries (Hong et al., 1983). Popularly called the bamboo orchid, *A. graminifolia* has been used for treating arthritis, snake bite, jaundice, inflammation, lung infection and for detoxification (Hossain, 2009, 2011; Rajendran et al., 1997; Xiaohua et al., 2015; Zhang et al., 2012). Previous phytochemical studies on the plant have suggested the presence of phenanthrenes, bibenzyls and diphethylenes (Liu et al., 2005a; Liu et al., 2005b ; Du et al., 2014; Liu et al., 2004; Majumder and Ghosal, 1994 ; Gao et al., 2014; Li et al., 2013; Meng et al., 2014). The plant and the compounds isolated from it has been reported to displayed several pharmacological effects, including anti-liver fibrotic effect, antibacterial, anti-haemolytic, cytotoxic and antiviral properties (Yan et al., 2018; Liu et al., 2019; Hu et al., 2013). We reported the presence of a unique class of compound, the glucosyloxybenzyl 2*R*-benzylmalate derivatives in our previous studies on the chemistry of the ethyl acetate extracts obtained from the aerial and underground part of the plant (Auberon et al., 2018; Auberon et al., 2019). In continuing our efforts to identify additional structurally diverse constituents from the sub-fractions obtained from the crude ethyl acetate extract showing prolific chemical profile fingerprints from HPLC and ^{1}H NMR data were further investigated, leading to the isolation of nine additional new glucosyloxybenzyl 2*R*-benzylmalate derivatives, arundinosides R-Z (**1**-**9**), together with the known arundinoside C (**10**). We report herein details of the isolation, structure elucidation and antibacterial activity of the isolated compounds.

2. Results and discussion

Arundinoside R (**1**) had a molecular formula of $C_{25}H_{32}O_{13}$ as determined from HR-ESI-MS at *m/z* 530.18716 $[M+NH_4]^+$ (calcd. $C_{25}H_{36}NO_{13}$ for 558.21812). The ^{1}H and ^{13}C NMR spectra (Table 1) displayed signals of five aromatic protons and carbons, two methylene groups, one quaternary carbon and two carbonyl groups. HMBC correlations from H-3 to C-1 and C-4, H-5 to C-1, H-7 to C-9 and H-10 to C-6 as well as comparison of the spectrum data of **1** to those of arundinosides A-Q (Auberon et al., 2018; Auberon et al., 2019; Liu et al., 2019) indicated the existence of a 2-benzylmalic acid moiety. Additional signals belonging to an oxygenated methylene group, three acetyl groups and five methine groups indicated the presence of an acylated sugar moiety. The HMBC correlations of H-1''' to C-2, H-2''' to C-2'''-Ac-1, H-4''' to C-4'''-Ac-1 and H-6''' to C-6'''-Ac-1 established the connections between the 2-benzyl malic acid moiety and the acylated sugar moiety as well as the location of the carbonyl groups on the sugar moiety. Comparison of the optical rotation observed for arundinoside R $[\alpha]_D^{25}$ -64 (*c* 0.1, CH_3OH) with those of similar 2*R*-benzylmalate derivatives (Auberon et al., 2018; Auberon et al., 2019; Sahakitpichan et al., 2013; Tanaka et al., 1984) suggested a 2*R* absolute configuration. Therefore, arundinoside R was elucidated as 2-(*β*-D-glucopyranosyl-2,4,6-triacetyl)-2*R*-benzylmalic acid.

Arundinoside S (**2**) had a molecular formula of $C_{30}H_{34}O_{15}$ deduced from the HR-ESI-MS at *m/z* 633.18336 [M-H]$^-$ (calcd. $C_{30}H_{33}O_{15}$ for 633.18249). The similarity between the ^{1}H and ^{13}C NMR spectra data of **2** (Table 1) to arundinoside L (Liu et al., 2019) was obvious, suggesting that these two compounds share the same core structure of one benzylmalic acid, one *β*-D-glucose moiety connected to the C-2 of the benzylmalic acid, an acetyl-group at C-2''' and another benzymalic moiety at C-6'''. However, the two glucosyloxybenzyl moieties connected to C-1 and C-4 in arundinoside L were absent in arundinoside S. The 2*R* configuration was indicated by comparison of the optical rotation of arundinoside S $[\alpha]_D^{25}$ -56 (*c* 0.4, CH_3OH) to the ones reported for similar analogues with known absolute configuration (Auberon et al., 2018; Auberon et al., 2019). Thus, arundinoside S was described as 2-(*β*-D-glucopyranosyl-2-acetyl-6-›1–2*R*-benzylmalyl)-2*R*-benzylmalic acid.

The molecular formula of arundinoside T (**3**) was as deduced by HR-ESI-MS at *m/z* 779.24534 [M-H]$^-$ as $C_{36}H_{44}O_{19}$. The NMR spectroscopic data as well as optical rotation values were similar to those of arundinoside Q (Auberon et al., 2019). Detailed analysis of the 1 and 2D NMR spectroscopic data uncovered the similarities in the structural features of these two compounds, aside the existence of an additional acetyl moiety in arundinoside T. This acetyl moiety position was ascertained at C-6'''' according to correlations from H-6a/b'''' (δ_H 4.25, 4.41) and Ac-1 (δ_H 2.04) to C-6''''-Ac-1 (δc 172.8) in the HMBC spectrum. The 2*R* configuration was indicated a by comparison of the optical rotation values with previous reports (Auberon et al., 2019). Thus, compound **3** was elucidated as 1-(*β*-D-glucopyranosyloxybenzyl-6-acetyl)-2-(*β*-D-glucopyranosyl-2,4-diacetyl)-2*R*-benzylmalic acid.

Arundinosides U (**4**) and V (**5**) had the same molecular formula of $C_{36}H_{43}O_{19}$ according to the *m/z* peak at 779.24417 and 779.24451 [M-H]$^-$, respectively and they were also identified as 2*R*-benzyl malic acid derivatives. Their ^{1}H and ^{13}C NMR had a striking resemblance to those of arundinoside T (**3**), indicating that these compounds have the same basic structural features. HMBC, HSQC-TOCSY and COSY correlations were used for establishing the 2*R*-benzyl malic acid and the acylated sugar core structure of **4** and **5** as hitherto elucidated for arundinosides R and T. The main difference between these compounds were the attachment of the acetyl groups on the sugar moiety at C-4''' in arundinosides U and at C-3''' in arundinoside V. The position of these acetyl groups were established on the basis of their HMBC correlations from these protons to their corresponding carbons. Accordingly, arundinosides U (**4**) and V (**5**) were identified as 1-(*β*-D -glucopyranosyloxybenzyl)-2-(*β*-D-glucopyranosyl-2,4,6-triacetyl)-2*R*-benzylmalic acid and 1-(*β*-D -glucopyranosyloxybenzyl)-2-(*β*-D-glucopyranosyl-2,3,6-triacetyl)-2*R*-benzylmalic acid, respectively.

Based on the HR-ESI-MS and ^{13}C NMR data (Table 3), the molecular formula of arundinoside W (**6**) was determined as $C_{36}H_{44}O_{19}$ (*m/z* 779.24433 [M-H]$^-$), thus indicating that **6** is a regioisomer of

compound **5**. The ^{1}H and ^{13}C NMR spectroscopic data assigned to the 2-benzyl malic acid, glucosyloxybenzyl and β-D-glucopyranosyl and the three acetyl moieties of **6** were consistent with the ones reported for arundinoside V (**5**). The main difference between the two compounds is the position of the glucosyloxybenzyl moiety which was found esterified at C-1 in arundinoside V (**5**), whereas it was located at the carbonyl C-4 in arundinoside W (**6**). The position has been verified through HMBC correlations between H-7'' and H-3 to C-4. As deduced for the previous compounds the 2*R* configuration of **6** was based on comparison of the optical rotation value of $[\alpha]_D^{25}$ -71 (*c* 0.2, CH_3OH) with previous cited reports ((Auberon et al., 2018; Auberon et al., 2019)). Therefore, arundinoside W was identified as 4-(β-D-glucopyranosyloxybenzyl)-2-(β-D-glucopyranosyl-2,3,6-triacetyl)-2*R*-benzylmalic acid.

Arundinosides X (**7**) was assigned the molecular formula of $C_{45}H_{52}O_{22}$ and according to *m/z* peak at 943.28596 $[M-H]^-$ in the HR-ESI-MS. The 1D and 2D NMR spectral analyses of **7** indicated that the partial structure of **7** was the same as that of arundinoside M (Auberon et al., 2019). The presence of the two 2-benzyl malic acid, glucopyranosyloxybenzyl, β-D-glucopyranosyl and acetyl moieties was revealed when compared to arundinoside M. However, the absence of one glucosyloxybenzyl moiety attached to C-1 in arundinoside M was evident in compound **7**. The remaining glucosoyloxybenzyl moiety was positioned at C-4 with the help of HMBC correlations between H-3 and H-7'' to C-4. Therefore, arundinosides X was identified as 4-(β-D-glucopyranosyloxybenzyl)-2-(β-D-glucopyranosyl-2,4-diacetyl-6->1-2*R*-benzylmalyl)-2*R*-benzylmalic acid.

Arundinoside Y (**8**) displayed the same molecular formula ($C_{45}H_{52}O_{22}$) as arundinoside X based on the *m/z* peak at 943.28384 $[M-H]^-$, indicating that the two compounds are isomers. The main difference between arundinoside Y and arundinoside X is the position of the acetyl group attached to the glucopyranosyloxybenzy moiety. In arundinoside Y, the acetyl groups were attached to C-2''' and C-3''', while in arundinoside X, the acetyl groups were on positions C-2''' and C-4'''. Thus, the structure of arundinoside Y was established as 4-(β-D-glucopyranosyloxybenzyl)-2-(β-D-glucopyranosyl-2,3-diacetyl-6->1-2*R*-benzylmalyl)-2*R*-benzylmalic acid.

Arundinoside Z (**9**) was determined to have a molecular formula of $C_{54}H_{62}O_{24}$, based on its HR-ESI-MS $[M-H]^-$ ion peak at *m/z* 1093.34878 (calcd. for $C_{54}H_{61}O_{24}$ for 1093.35583). The ^{1}H and ^{13}C NMR spectral analyses of **9** indicated that its planar structure was closely related to arundinoside A (Auberon et al., 2018), except for the absence of resonances assigned to one *trans*-cinnamoyl ester moiety at C-4'''' and an acetyl group at C-6'''' in **9**. The absolute configuration of arundinoside Z was established based on comparing the observed optical rotation $[\alpha]_D^{25}$ -69 (*c* 0.2, CH_3OH) with previous reports (Auberon et al., 2019). Thus, arundinoside Z was elucidated as 1-(β-D-

glucopyranosyloxybenzyl-3-*trans*-cinnamoyl)-2-(β-D-glucopyranosyl-2-acetyl)-4-(β-D-glucopyranosyloxybenzyl)-2R-benzylmalic acid.

The extracts of the plant have been shown to be composed of antibacterial compounds, an activity mostly attributed to the phenolic and phenanthrenes (Yan et al., 2018, Hu et al., 2013). We therefore wanted to affirm whether the antibacterial activity displayed by the extracts from *A. graminifolia* can also attributed to other class of compounds present in the plant. Thus, the antibacterial activity of all the isolated compounds were tested against *Bacillus cereus*, *Escherichia coli* strain O157:H7, *Listeria monocytogenes* strain F2365 and *Staphylococcus aureus* strain ATCC 25923. However, all the isolated compounds did not display any antibacterial effect.

In conclusion, we report the isolation and characterization of nine new glucosyloxybenzyl 2*R*-benzylmalate derivatives from the underground part of *A. graminifolia*. It is noteworthy to indicate that arundinoside R and S are the first monodesmosidic derivatives isolated in *A. graminifolia*. All the new compounds were assayed for antibacterial activities, but unfortunately none of them showed any activity.

3. Materials and methods

3.1. General experimental procedures

NMR spectra were acquired on a Bruker 500 MHz Avance III spectrometer fitted with a DCH $^{13}C/^{1}H$ Cryoprobe (Bruker Biospin, Rheinstetten, Germany). Optical rotations were recorded on a Jasco Perkin Elmer 341 polarimeter (Jasco, Lisses, France), while IR spectra were measured using a 380 FT-IR spectrophotometer (Thermo Electron Corporation, Saint Herblain, France). Centrifugal partition chromatography (CPC) was performed using a FCPC200 instrument (Kromaton Technologies, Angers, France) equipped with 20 circular partition disk rotors (1320 partition cells: 0.130 mL per cell; total column capacity of 1000 mL).HR-ESI-MS analysis were performed using a 1200 Agilent Series fitted to an Agilent 6520 Accurate Mass Q-TOF spectrometer (Agilent Technologies, Santa Clara, USA). Semi preparative HPLC was performed on a Gilson LC system equipped with a Kinetex Axia C-18 column (100 mm × 21.2 mm, 5μm).

3.2. Plant material

The whole of *A. graminifolia* was collected in September 2010 from an orchid farm in Chiang Mai Province, Thailand. The plant was authenticated at the Faculty of Science, Chiang Mai University, Thailand, where a voucher specimen was deposited (n° 05-563). The roots and rhizomes were dried and imported to France in accordance to the specification of the Convention on International Trade in Endangered Species (CITES).

3.3. Extraction and isolation

The dried roots and rhizomes of *A. graminifolia* were powdered and macerated with ethanol thrice (1g powdered sample to 20 mL solvent). The ethanol extracts were pulled together and concentrated under reduced pressure using a rotary evaporator. The crude EtOH extract was reconstituted in water and furtherly partitioned with CH_2Cl_2 and EtOAc to afford the CH_2Cl_2 (10.40 g) and EtOAc extracts (10.93 g) after evaporation. The EtOAc extract was purified by centrifugal partition chromatography using *n*-heptane/EtOAc/MeOH/H_2O (0.25:5:1:5, v/v) as the solvent system. The aqueous mobile phase was pumped into the column in the head to tail mode using a flow rate of 5 mL/min after the rotor has been filled with the organic stationary phase, and a gradual increase in rotation speed from 0 to 1000 rpm until complete equilibrium between the organic and aqueous phases was acheived. For each CPC run, the EtOAc extract (5 g) was dissolved in 40 mL of equal ratio of the organic and aqueous phase mixture. The flow rate of the system was gradually increased to 11 mL/min after sample injection and maintained for 140 mins. At the end of 200 min of head to tail mode, an extrusion mode of the organic phase was performed at 70 mL/min and the rotation speed was reduced to 500 rpm. Fourteen fractions (A-N) were collected and monitored at a wavelenght of 210 and 280 nm. Fractions E (617 mg), G (726 mg), H (301 mg) and I (540 mg) were further purified by Sephadex LH-20 eluted with MeOH. Sub-fraction E-3 (290 mg) was purified on semi-preparative RP-HPLC (H_2O (A)/CH_3CN (B) both containing 0.05% formic acid ; 40% B for 2 min, 40% to 50% B for 48 min, 50% to 60% B for 2 min, 100% B for 5 min at a flow rate of 14 mL/min) to afford compounds **3** (2.3 mg) and **10** (12.9 mg). Sub-fraction E-4 (120 mg) was purified using the same method as E-3 to yield compounds **4** (2.1 mg) and **5** (19.1 mg).

Sub-fraction G-3 (224.9 mg) was purified by semi-preparative HPLC (H_2O (A)/CH_3CN (B) both containing 0.05% formic acid ; 45% B for 5 min, 45% to 49% B for 25 min,49% to 100% B for 2 min at a flow rate of 14 mL/min) to afford compounds **6** (2.7 mg), **7** (8.3 mg) and **9** (3.0 mg). Sub-fractions H-3 (101.1 mg), H-4 (107.2 mg) and I-4 (63.5 mg) were purified by semi preparative HPLC to afford compounds **8** (18.8 mg), **1** (1.8 mg) and **2** (4.1 mg), respectively.

*3.3.1 Arundinoside R (**1**)*

White amorphous powder (4.1 mg); $[\alpha]_D^{25}$ -64 (*c* 0.4, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 218 (3.16), 275 (1.80); ^{1}H NMR and ^{13}C NMR see Table 1; HR-ESI-MS: *m/z* 530.18716 $[M+NH_4]^+$ (calcd. $C_{23}H_{32}NO_{13}$ for 530.18682).

*3.3.2 Arundinoside S (**2**)*

White amorphous powder (1.8 mg);$[\alpha]_D^{25}$ -56 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 218 (3.05), 276 (1.82); ^1H NMR and ^{13}C NMR see Table 1; HR-ESI-MS: *m/z* 633.18336 $[M-H]^-$ (calcd. $C_{30}H_{33}O_{15}$ for 633.18249).

3.3.3 Arundinoside T (**3**)

White amorphous powder (2.3 mg);$[\alpha]_D^{25}$ -99 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 217 (3.50), 268 (1.70); ^1H NMR and ^{13}C NMR see Table 2; HR-ESI-MS: *m/z* 779.24534 $[M-H]^-$ (calcd. for $C_{36}H_{43}O_{19}$ for 779.24040).

3.3.4. Arundinoside U (**4**)

White amorphous powder (2.1 mg); $[\alpha]_D^{25}$ -58 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 216 (3.25), 271 (1.95); ^1H NMR and ^{13}C NMR see Table 2; HR-ESI-MS: *m/z* 779.24417 [M-H (calcd. for $C_{36}H_{43}O_{19}$ for 779.24040).

3.3.5 Arundinoside V (**5**)

White amorphous powder (19.1 mg); $[\alpha]_D^{25}$ -62 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 210 (3.01), 267 (1.88); ^1H NMR and ^{13}C NMR see Table 2; HR-ESI-MS: *m/z* 779.24451 $[M-H]^-$ (calcd. for $C_{36}H_{43}O_{19}$ for 779, 24040).

3.3.6 Arundinoside W (**6**):

White amorphous powder (2.7 mg); $[\alpha]_D^{25}$ -71 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 211 (3.04), 267 (1.82); ^1H NMR and ^{13}C NMR see Table 3; HR-ESI-MS: *m/z* 779.24433 $[M-H]^-$ (calcd. for $C_{36}H_{43}O_{19}$ for 779.24040).

3.3.7. Arundinoside X (**7**)

White amorphous powder (1.2 mg); $[\alpha]_D^{25}$ -37 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 217 (3.10), 273 (1.98); ^1H NMR and ^{13}C NMR see Table 4; HR-ESI-MS: *m/z* 943.28596 $[M-H]^-$ (calcd. for $C_{45}H_{51}O_{22}$ for 943.28775).

3.3.8. Arundinoside Y (**8**)

White amorphous powder (18.8 mg); $[\alpha]_D^{25}$ -45 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 219 (3.08), 266 (2.11); ^1H NMR and ^{13}C NMR see Table 4; HR-ESI-MS: *m/z* 943.28384 $[M-H]^-$ (calcd. for $C_{45}H_{51}O_{22}$ for 943.28775).

3.3.9. Arundinoside Z (**9**)

White amorphous powder (3.0 mg); $[\alpha]_D^{25}$ -69 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 217 (3.03), 269 (2.10), 277 (1.82); ^1H NMR and ^{13}C NMR see Table 5; HR-ESI-MS: *m/z* 1093.34878 $[M-H]^-$ (calcd. for $C_{54}H_{61}O_{24}$ for 1093.35583).

3.4. Determination of the sugar moiety

The sugar moiety was determined according to the method of Simmler et al. (Simmler et al., 2011). The compounds (1 mg) was dissolved in 2 M HCl (0.5 mL) and heated for 3 hours under reflux. The solution was cooled and partitioned three times with *n*-butanol. After partitioning, the aqueous phase was dried under reduced pressure and derivatization was performed with pyridine and 1-(trimethylsilyl) imidazole (4:1 v/v) at 60°C for 1 hour. The identification of the derivatized sample was conducted on a GC-MS Trace GC Ultra instrument equipped with a TR-5MS SQC column (0.25 µm, 15 m x 0.25 mm) and operated using the following set of conditions: 1 min at 40°C; a thermal ramp of 10°C until 250°C (helium flow rate 1 mL/min, injector temperature 250°C, transfer temperature 285°C). The detection was performed on a DSQII Thermo Scientific mass spectrometer, with a detection mass range of *m/z* 0 to 500. The sugar (D-glucose) was identified based on the comparison of the retention time of the derivative with that of the standard glucose treated in the same manner.

References

Auberon, F., Olatunji, O.J., Krisa, S., Herbette, G., Antheaume, C., Bonte, F., Merillon, J.-M., Lobstein, A., 2018. Arundinosides A-G, new glucosyloxybenzyl 2R-benzylmalate derivatives from the aerial parts of *Arundina graminifolia*. Fitoterapia 125, 199-207.

Auberon, F., Olatunji, O.J., Waffo-Teguo, P., Adekoya, A.E., Bonté, F., Mérillon, J.-M., Lobstein, A., 2019. New glucosyloxybenzyl 2R-benzylmalate derivatives from the undergrounds parts of *Arundina graminifolia* (Orchidaceae). Fitoterapia 135, 33-43.

Du, G., Shen, Y., Yang, L., Shu, L., Wen, M.-L., Hu, Q.-F., 2014. Bibenzyl derivatives of *Arundina graminifolia* and their cytotoxicity. Chem. Nat. Compd. 49, 1019-1022.

Gao, Y., Jin, Y., Yang, S., Wu, J., Gao, X., Hu, Q., Ma, Y., 2014. A new diphenylethylene from *Arundina graminifolia* and its cytotoxicity. Asian J. Chem. 26, 3903-3905.

Hong, D.Y., Lian, Y.S., Shen, L.D., 1983. Flora of China. Chinese Science Press, Beijing, China.

Hossain, M.M., 2009. Traditional therapeutic uses of some indigenous orchids of Bangladesh. Med Aromat Plant Sci Biotechnol 42, 101-106.

Hossain, M.M., 2011. Therapeutic orchids: traditional uses and recent advances--an overview. Fitoterapia 82, 102-140.

Hu, Q.F., Zhou, B., Huang, J.M., Gao, X.M., Shu, L.D., Yang, G.Y., Che, C.T., 2013. Antiviral phenolic compounds from *Arundina gramnifolia*. J Nat Prod. 76, 292-296.

Li, Y.-K., Zhou, B., Ye, Y.-Q., Du, G., Niu, D.-Y., Meng, C.-Y., Gao, X.-M., Hu, Q.-F., 2013. Two new diphenylethylenes from *Arundina graminifolia* and their cytotoxicity. Bull. Korean Chem. Soc. 34, 3257-3260.

Liu, M.F., Ding, Y., Zhang, D.M., 2005a. Phenanthrene constituents from rhizome of *Arundina graminifolia*. Zhongguo Zhong Yao Za Zhi 30, 353-356.

Liu, M.F., Han, Y., Xing, D.M., Shi, Y., Xu, L.Z., Du, L.J., Ding, Y., 2004. A new stilbenoid from *Arundina graminifolia*. J Asian Nat Prod Res 6, 229-232.

Liu, M.F., Han, Y., Xing, D.M., Wang, W., Xu, L.Z., Du, L.J., Ding, Y., 2005b. One new benzyldihydrophenanthrene from *Arundina graminifolia*. J Asian Nat Prod Res 7, 767-770.

Liu, Q., Sun, F., Deng, Y., Dai, R., Lv, F., 2019. HPLC-ESI-MSn identification and NMR characterization of glucosyloxybenzyl 2R-benzylmalate derivatives from *Arundina graminifolia* and their anti-liver fibrotic effects in vitro. Molecules 24, 525.

Majumder, P.L., Ghosal, S., 1994. Two stilbenoids from the orchid *Arundina bambusifolia*. Phytochemistry 35, 205-208.

Meng, C.-Y., Niu, D.-Y., Li, Y.-K., Zhou, B., Ye, Y.-Q., Du, G., Hu, Q.-F., Gao, X.-M., 2014. A new cytotoxic stilbenoid from *Arundina graminifolia*. Asian J. Chem. 26, 2411-2413.

Rajendran, A., Rao, N.R., Kumar, K.R., Henry, A.N., 1997. Some medicinal orchids of southern India. Anc Sci Life 17, 10-14.

Sahakitpichan, P., Mahidol, C., Disadee, W., Chimnoi, N., Ruchirawat, S., Kanchanapoom, T., 2013. Glucopyranosyloxybenzyl derivatives of (R)-2-benzylmalic acid and (R)-eucomic acid, and an aromatic glucoside from the pseudobulbs of *Grammatophyllum speciosum*. Tetrahedron 69, 1031-1037.

Simmler, C., Antheaume, C., Andre, P., Bonte, F., Lobstein, A., 2011. Glucosyloxybenzyl eucomate derivatives from *Vanda teres* stimulate HaCaT cytochrome c oxidase. J. Nat. Prod. 74, 949-955.

Tanaka, T., Suda, H., Naganawa, H., Hamada, M., Takeuchi, T., Aoyagi, T., Umezawa, H., 1984. Production of (*S*)-□-benzylmalic acid, inhibitor of carboxypeptidase A by actinomycetes. J. Antibiot. 37, 682-684.

Xiaohua, D., Jin, Z., Hui, W., Haifeng, C., Chao, Z., Zepu, Y., 2015. Effect of Yajieshaba, a preparation of Dai indigenous medicine, on enhanced liver detoxification. J Trad Chin Med. 35, 197-205.

Yan, X., Tang, B., Liu, M., 2018. Phenanthrenes from Arundina graminifolia and in vitro evaluation of their antibacterial and anti-haemolytic properties. Nat Prod Res. 32, 707-710.

286 Zhang, C., Chongsuvivatwong, V., Keawpradub, N., Lin, Y., 2012. Analysis of prescription database
287 extracted from standard textbooks of traditional Dai medicine. J Ethnobiol Ethnomed. 8, 34.

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292 **Legends to figures**

293 **Figure 1.** Chemical structures of the nine isolated compounds arundinosides R-Z

294 **Figure 2.** Key HSQC-TOCSY, COSY, HMBC correlations of arundinoside S (**2**)

295 **Figure 3.** Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside T (**3**)

296 **Figure 4**. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside W (**6**)

297 **Figure 5**. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside X (**7**)

298 **Figure 6**. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside Z (**9**)

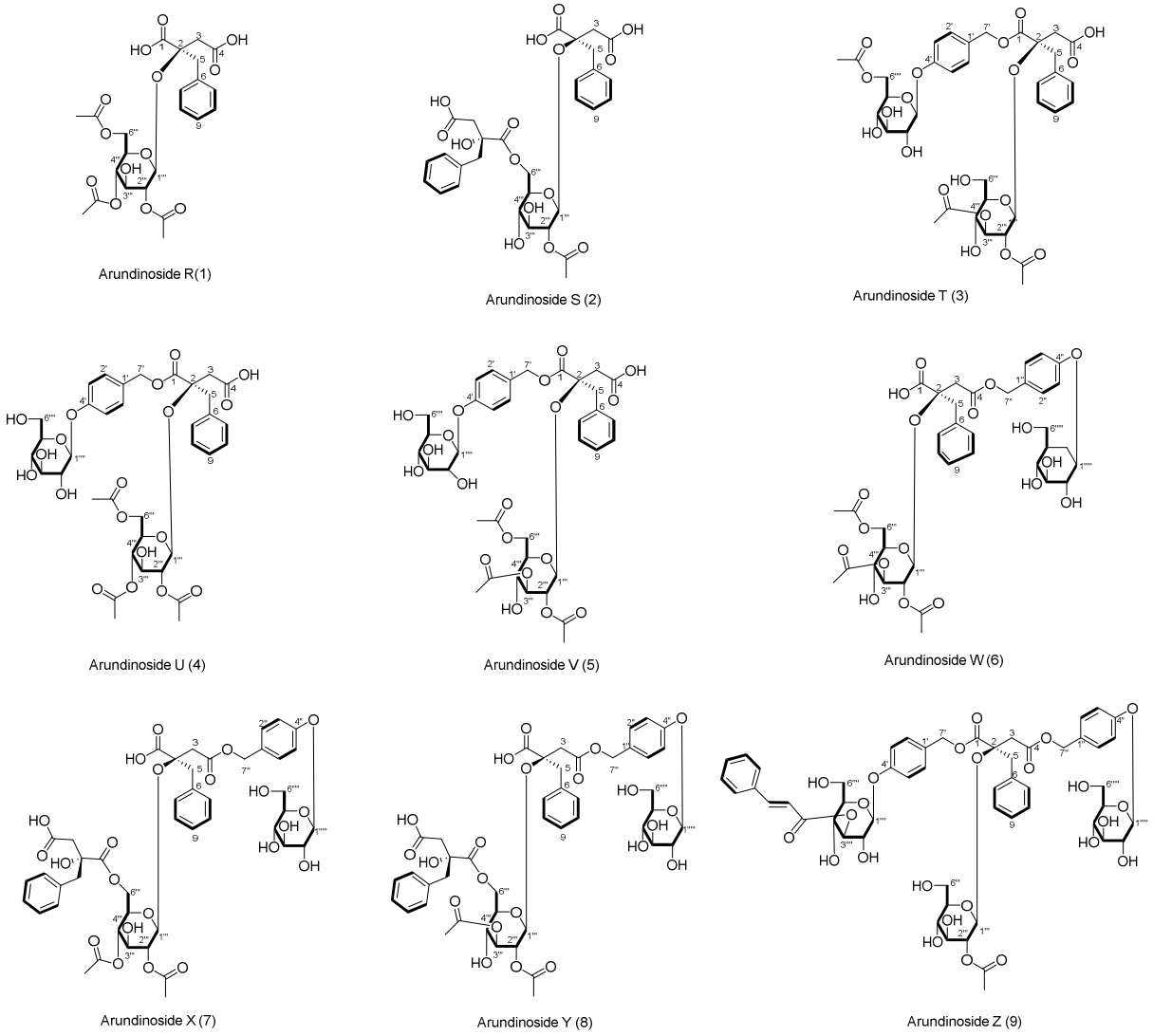


Figure 1. Chemical structures of the nine isolated compounds arundinosides R-Z

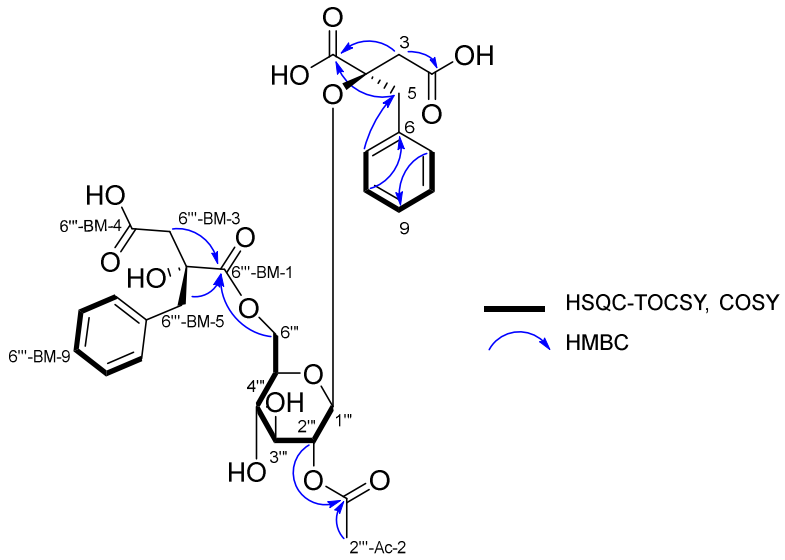


Figure 2. Key HSQC-TOCSY, COSY, HMBC correlations of arundinoside S (**2**)

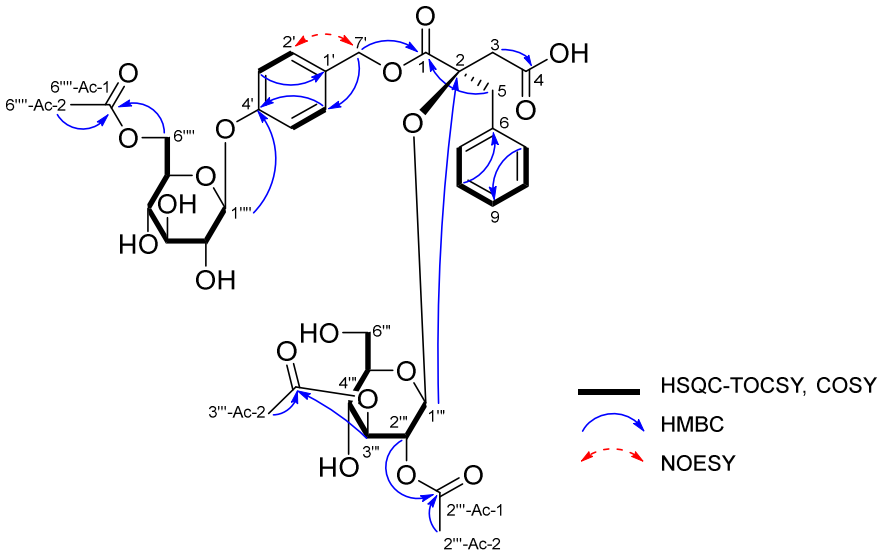


Figure 3. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside T (**3**)

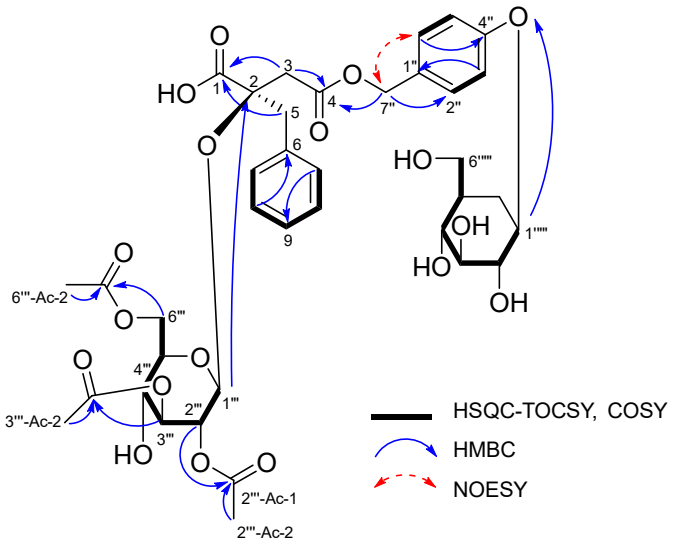


Figure 4. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside W (**6**)

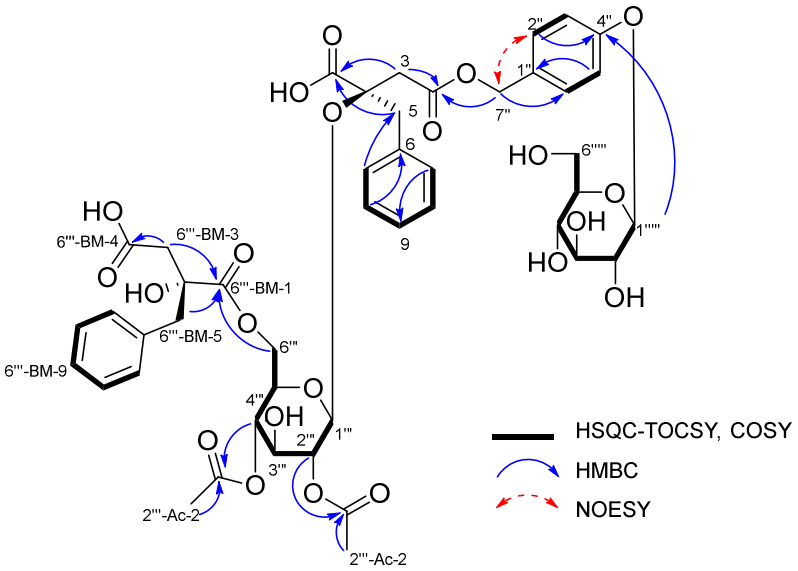
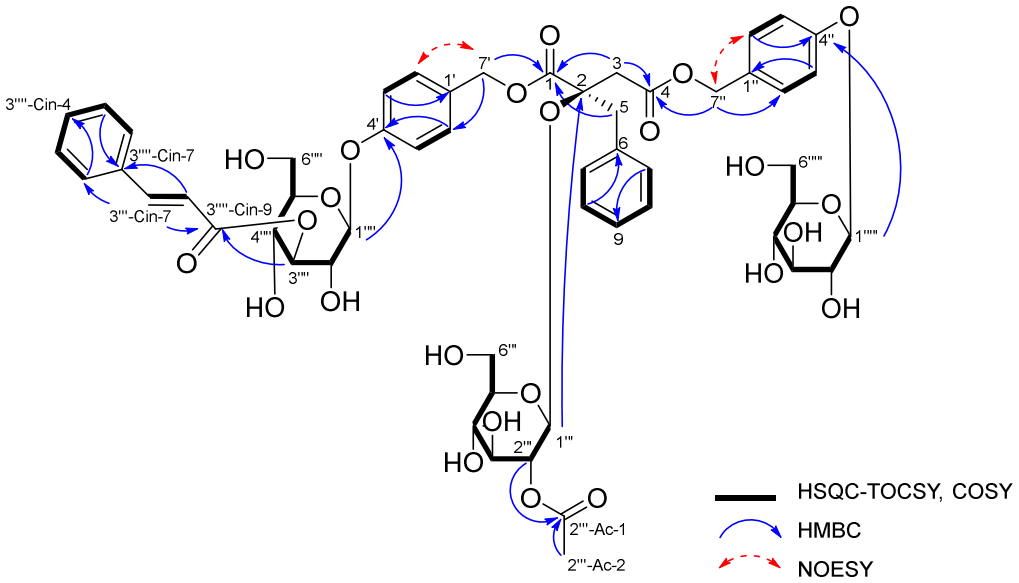


Figure 5. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside X (**7**)



335

336 **Figure 6**. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside Z (**9**)

Table 1. ^{1}H (500 MHz, methanol-d_4) and ^{13}C (125 MHz, methanol-d_4) NMR data of arundinosides R-S (**1-2**)

Position	Arundinoside R (**1**)		Arundinoside S (**2**)	
	δ_C (ppm)	δ_H (ppm; *J*, in Hz)	δ_C (ppm)	δ_H (ppm; *J*, in Hz)
1	174.0		174.5	
2	82.3		82.3	
3a	43.5	2.89 (d, 17.9)	42.9	2.94 (d, 17.9)
3b		3.07 (d, 17.9)		3.01 (d, 17.9)
4	174.4		174.3	
5a	47.1	3.00 (d, 13.3)	46.4	2.99 (d, 13.7)
5b		3.04 (d, 13.3)		3.08 (d, 13.7)
6	136.8		136.9	
7/11	132.1	7.21 (m)	132.1	7.16 (m)
8/10	129.0	7.21 (m)	129.0	7.19 (m)
9	127.8	7.20 (m)	127.8	7.19 (m)
2-*O*-glc-1'''	99.0	5.09 (d, 8.2)	98.8	5.01 (d, 8.2)
2'''	74.8	4.81 (dd, 8.2; 9.4)	75.0	4.79 (dd, 8.2; 9.3)
3'''	73.8	3.64 (dd, 9.6 ; 9.4)	76.0	3.45 (d, 9.3)
4'''	71.8	4.91 (dd, 9.6 ; 9.6)	71.0	3.45 (d, 9.3)
5'''	72.7	3.40 (ddd, 2.5 ;4.0 ; 9.6)	75.0	3.27 (m)
6'''a	63.3	3.99 (dd, 2.5; 12.1)	65.2	4.18 (dd, 1.8; 11.9)
6'''b		4.19 (dd, 4.0; 12.1)		4.37 (dd, 4.4; 11.9)
2'''-Ac-1	172.3		172.6	
2'''-Ac-2	21.2	1.80 (s)	21.4	1.85 (s)
4'''-Ac-1	171.8			
4'''-Ac-2	21.0	2.05 (s)		
6'''-Ac-1	172.7			
6'''-Ac-2	20.8	2.04 (s)		
6'''-BM-1			175.7	
6'''-BM-2			77.5	
6'''-BM-3a			44.1	2.56 (d, 16.0)
6'''-BM-3b				3.00 (d, 16.0)

6'''-BM-4			173.9	
6'''-BM-5a			46.4	2.94 (d, 18.1)
6'''-BM-5b				3.05 (d, 18.1)
6'''-BM-6			136.8	
6'''-BM-7/11			131.8	7.24 (m)
6'''-BM-8/10			129.1	7.25 (m)
6'''-BM-9			128.7	7.25 (m)

Table 2. ^{1}H (500 MHz, methanol-d_4) and ^{13}C (125 MHz, methanol-d_4) NMR data of arundinosides T-V (**3–5**)

Position	Arundinoside T (**3**)		Arundinoside U (**4**)		Arundinoside V (**5**)	
	δ_C (ppm)	δ_H (ppm; *J*, in Hz)	δ_C (ppm)	δ_H (ppm; *J*, in Hz)	δ_C (ppm)	δ_H (ppm; *J*, in Hz)
1	173.0		172.7		172.6	
2	82.6		82.4		82.4	
3a	44.9	3.00 (d, 17.8)	44.4	2.96 (d, 18.0)	44.1	2.97 (d, 18.1)
3b		3.12 (d, 17.8)		3.11 (d, 18.0)		3.10 (d, 18.1)
4	174.7		174.1		174.0	
5a	47.4	2.94 (d, 13.5)	47.4	2.95 (d, 13.5)	47.3	2.94 (d, 13.4)
5b		3.00 (d, 13.5)		3.01 (d, 13.5)		3.00 (d, 13.4)
6	136.6		136.5		136.4	
7/11	132.0	7.02 (m)	132.0	7.06 (m)	131.9	7.03 (m)
8/10	129.1	7.14 (m)	129.1	7.19 (m)	129.1	7.17 (m)
9	127.8	7.14 (m)	127.9	7.18 (m)	127.9	7.17 (m)
1′	131.1		130.9		130.7	
2′/6′	131.6	7.28 (d, 8.6)	131.6	7.27 (d, 8.7)	131.6	7.27 (d, 8.6)
3′/5′	117.9	7.09 (d, 8.6)	117.9	7.09 (d, 8.7)	117.8	7.09 (d, 8.6)
4′	159.2		159.4		159.3	
7a′	67.9	4.96 (d, 12.0)	67.9	4.95 (d, 12.1)	68.0	4.94 (m)

7b′		5.11 (d, 12.0)		5.09 (d, 12.1)		5.10 (d, 11.9)
2-*O*-glc-1‴	98.6	5.14 (d, 8.0)	98.9	5.09 (d, 8.1)	98.5	5.15 (d, 8.1)
2‴	73.1	4.79 (dd, 8.0; 9.9)	74.6	4.79 (dd, 8.1; 9.8)	72.7	4.80 (dd, 8.1; 9.8)
3‴	77.3	4.96 (m)	73.9	3.57 (dd, 9.8; 9.8)	76.7	4.96 (m)
4‴	68.9	3.67 (m)	71.7	4.92 (dd, 9.8; 9.8)	69.2	3.60 (dd, 9.6; 9.6)
5‴	77.2	3.11 (m)	72.8	3.39 (m)	74.6	3.31 (m)
6‴a	61.5	3.71 (dd, 3.5; 12.2)	63.2	4.00 (dd, 2.5; 12.3)	63.9	4.22 (dd, 4.1; 11.9)
6‴b		3.79 (dd, 2.1; 12.2)		4.20 (dd, 3.7; 12.3)		4.28 (dd, 2.2; 11.9)
2‴-Ac-1	172.0		172.2		171.9	
2‴-Ac-2	21.0	1.51 (s)	21.2	1.64 (s)	20.9	1.49 (s)
3‴-Ac-1	172.2				172.1	
3‴-Ac-2	21.0	2.02 (s)			21.0	2.02 (s)
4‴-Ac-1			172.6			
4‴-Ac-2			20.8	2.04 (s)		
6‴-Ac-1			171.8		172.9	
6‴-Ac-2			21.0	2.05 (s)	20.9	2.07 (s)
4′-*O*-glc-1⁗	102.3	4.92 (m)	102.4	4.92 (d, 7.7)	102.3	4.94 (m)
2⁗	75.0	3.47 (m)	75.0	3.47 (m)	74.9	3.48 (m)
3⁗	77.9	3.47 (m)	78.1	3.46 (m)	78.0	3.49 (m)
4⁗	71.6	3.39 (m)	71.5	3.40 (m)	71.3	3.42 (m)
5⁗	75.4	3.66 (m)	78.3	3.45 (m)	78.1	3.43 (m)

6a""	64.8	4.25 (dd, 6.1 ;11.9)	62.7	3.71 (dd, 5.4 ;12.1)	62.5	3.72 (dd, 5.0 ;11.8)
6b""		4.41 (dd, 2.2 ;11.9)		3.90 (dd, 2.3 ;12.1)		3.90 (dd, 1.8 ;11.8)
6""-Ac-1	172.8					
6""-Ac-2	20.9	2.04 (s)				

Table 3. ^{1}H (500 MHz, methanol-d_4) and ^{13}C (125 MHz, methanol-d_4) NMR data of arundinoside W (**6**)

Position	Arundinoside W (**6**)	
	δ_C (ppm)	δ_H (ppm; *J*, in Hz)
1	174.3	
2	82.9	
3a	42.5	2.97 (m)
3b		
4	172.1	
5a	46.1	3.06 (d, 13.3)
5b		3.12 (d, 13.3)
6	137.0	
7/11	132.0	7.18 (m)
8/10	129.0	7.18 (m)
9	127.8	7.16 (m)
1″	131.3	
2″/6″	131.5	7.34 (d, 8.7)
3″/5″	118.0	7.10 (d, 8.7)
4″	159.4	
7a″	71.5	5.04 (d, 11.8)
7b″		5.09 (d, 11.8)
2-*O*-glc-1‴	98.6	5.08 (d, 8.1)
2‴	73.1	4.80 (dd, 8.1; 9.8)
3‴	76.7	4.98 (dd, 9.8 ; 9.8)
4‴	69.5	3.53 (dd, 9.8; 9.8)
5‴	74.6	3.23 (m)
6‴a	63.9	4.04 (dd, 4.6; 12.0)
6‴b		4.08 (dd, 2.6; 12.0)
2‴-Ac-1	172.0	
2‴-Ac-2	21.0	1.79 (s)
3‴-Ac-1	172.1	
3‴-Ac-2	21.0	2.03 (s)
6‴-Ac-1	172.8	
6‴-Ac-2	20.9	1.97 (s)
4″-*O*-glc-1⁗	102.4	4.91 - 4.95 (m)
2⁗	75.0	3.47 (m)
3⁗	78.1	3.47 (m)
4⁗	71.5	3.40 (m)
5⁗	78.2	3.45 (m)
6a⁗	62.6	3.70 (dd, 5.4 ; 12.1)
6b⁗		3.90 (dd, 2.3 ; 12.1)

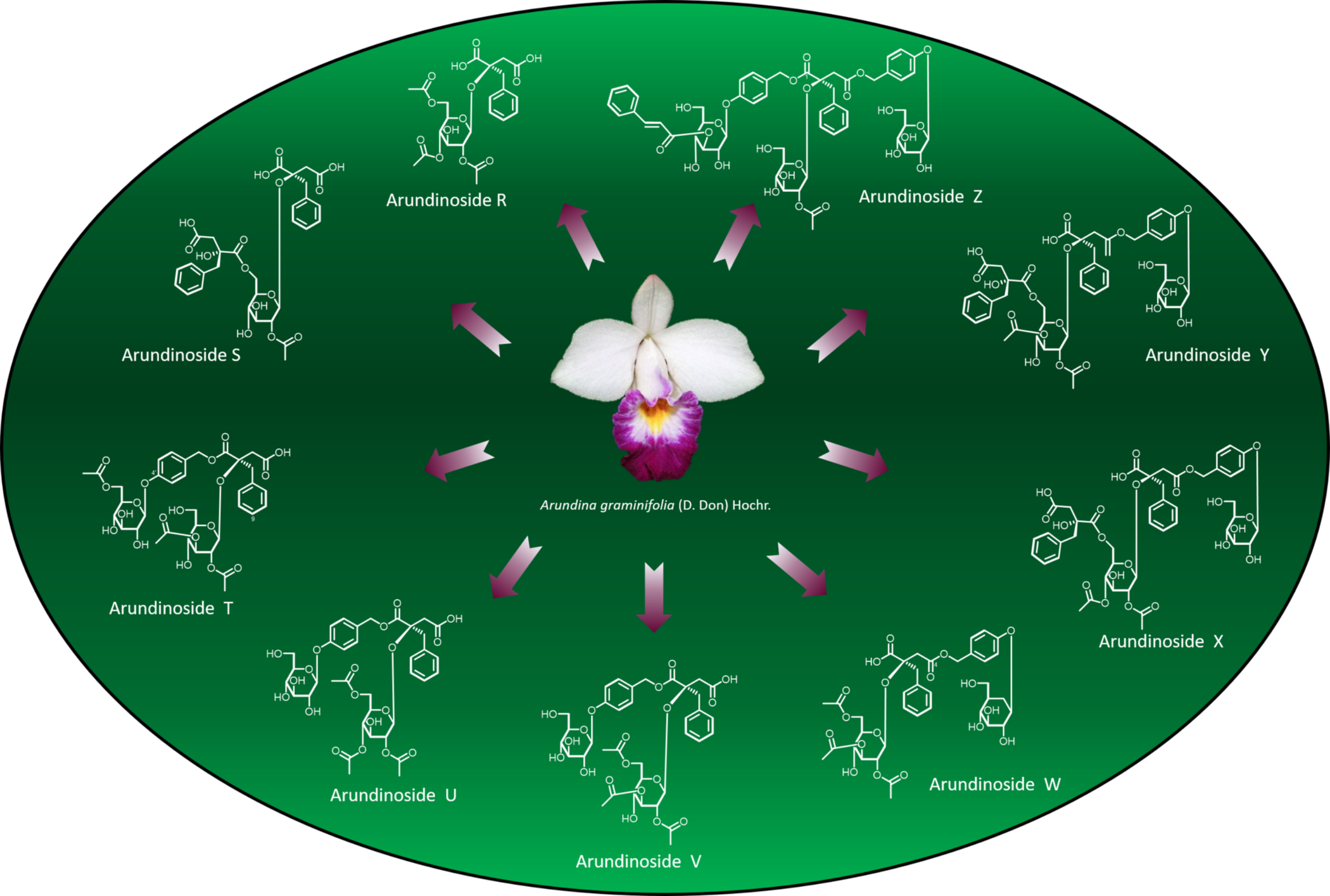
Table 4. ^{1}H (500 MHz, methanol-d_4) and ^{13}C (125 MHz, methanol-d_4) NMR data of arundinoside X-Y (**7**-**8**)

Position	Arundinoside X (**7**)		Arundinoside Y (**8**)	
	δ_C (ppm)	δ_H (ppm; *J*, in Hz)	δ_C (ppm)	δ_H (ppm; *J*, in Hz)
1	174.3		174.2	
2	82.9		82.8	
3a	41.9	2.89 (d, 17.2)	42.1	2.91 (d, 17.8)
3b		3.03 (d, 17.2)		3.01 (d, 17.8)
4	172.1		172.0	
5a	45.4	3.09 (d, 13.6)	45.5	3.07 (d, 13.7)
5b		3.17 (d, 13.6)		3.15 (d, 13.7)
6	137.0		136.9	
7/11	132.1	7.16 (m)	131.7	7.16 (m)
8/10	129.1	7.17 (m)	129.1	7.17 (m)
9	127.9	7.17 (m)	127.9	7.17 (m)
1′	131.3		131.2	
2′/6′	131.7	7.33 (d, 8.7)	132.0	7.32 (d, 8.7)
3′/5′	117.9	7.09 (d, 8.7)	118.1	7.09 (d, 8.7)
4′	159.4		159.5	
7a′	67.4	4.99 (d, 12.0)	67.4	5.01 (d, 11.8)
7b′		5.10 (d, 12.0)		5.07 (d, 11.8)
2-*O*-glc-1‴	98.6	4.93 (m)	98.4	5.01 (d, 8.3)
2‴	74.8	4.83 (dd, 8.1 ; 9.6)	73.1	4.84 (dd, 8.3 ; 9.5)
3‴	73.7	3.62 (dd, 9.6 ; 9.4)	76.6	4.97 (dd, 9.5 ; 9.5)
4‴	72.0	4.84 (dd, 9.5; 9.5)	69.3	3.50 (dd, 9.5; 9.5)
5‴	72.8	3.31 (m)	74.8	3.21 (ddd, 9.5 ; 5.2 ; 2.0)
6‴a	64.7	3.79 (dd, 5.3; 12.1)	65.0	3.96 (dd, 5.2; 11.9)
6‴b		4.04 (dd, 2.1; 12.1)		4.15 (dd, 2.0; 11.9)
2‴-Ac-1	172.2		172.0	
2‴-Ac-2	21.3	1.90 (s)	21.0	1.81 (s)
3‴-Ac-1			172.2	
3‴-Ac-2			21.0	2.03 (s)
4‴-Ac-1	172.1			
4‴-Ac-2	21.1	2.10 (s)		
6‴-BM-1	175.5		175.5	
6‴-BM-2	77.2		77.5	
6‴-BM-3a	44.2	2.48 (d, 16.2)	44.4	2.50 (d, 16.3)

6'''-BM-3b		2.96 (d, 16.2)		2.96 (d, 16.3)
6'''-BM-4	174.4		174.2	
6'''-BM-5a	46.2	2.83 (d, 13.6)	46.5	2.81 (d, 13.6)
6'''-BM-5b		2.94 (d, 13.6)		2.94 (d, 13.6)
6'''-BM-6	136.9		136.8	
6'''-BM-7/11	131.8	7.13 (m)	131.7	7.13 (m)
6'''-BM-8/10	129.3	7.21 (m)	129.3	7.21 (m)
6'''-BM-9	128.0	7.20 (m)	128.1	7.20 (m)
4'-*O*-glc-1''''	102.4	4.92 (m)	102.4	4.93 (m)
2''''	75.0	3.45 (m)	75.1	3.45 (m)
3''''	78.1	3.46 (m)	78.1	3.46 (m)
4''''	71.5	3.40 (m)	71.5	3.38 (m)
5''''	78.3	3.41 (m)	78.2	3.42 (m)
6a''''	62.6	3.72 (dd, 5.4 ; 12.1)	62.6	3.68 (dd, 5.6 ; 12.1)
6b''''		3.89 (dd, 2.3 ; 12.1)		3.87 (dd, 2.2 ; 12.1)

Table 5. ^{1}H (500 MHz, methanol-d_4) and ^{13}C (125 MHz, methanol-d_4) NMR data of arundinoside Z (**9**)

Position	Arundinoside Z (**9**)	
	δ_C (ppm)	δ_H (J, in Hz)
1	172.5	
2	82.2	
3a	43.9	3.09 (d, 18.0)
3b		3.16 (d, 18.0)
4	172.3	
5a	47.3	2.97 (d, 13.5)
5b		3.03 (d, 13.5)
6	136.5	
7/11	132.1	7.06 (m)
8/10	129.2	7.18 (m)
9	128.0	7.18 (m)
1′	130.2	
2′/6′	131.7	7.24 (d, 8.6)
3′/5′	118.0	7.10 (d, 8.6)
4′	159.3	
7a′	67.8	4.90 (m)
7b′		5.03 (d, 12.2)
1″	130.2	
2″/6″	131.5	7.30 (d, 8.6)
3″/5″	118.0	7.10 (d, 8.6)
4″	159.4	
7a″	67.5	4.92 (m)
7b″		5.08 (d, 12.2)
2-*O*-glc-1‴	98.8	4.99 (d, 8.1)
2‴	75.0	4.71 (dd, 8.1; 9.1)
3‴	76.2	3.38 (dd, 9.1; 9.1)
4‴	71.0	3.40 (dd, 9.1; 9.1)
5‴	77.5	2.93 (m)
6‴a	62.0	3.52 (dd, 11.9; 2.2)
6‴b		3.56 (dd, 11.9; 4.3)
2‴-Ac-1	172.6	
2‴-Ac-2	21.4	1.69 (s)
4′-*O*-glc-1⁗	102.3	5.06 (d, 8.0)
2⁗	69.6	3.68 (m)
3⁗	79.1	5.20 (dd, 9.4; 9.4)
4⁗	73.4	3.70 (m)
5⁗	78.1	3.59 (m)
6a⁗	62.3	3.75 (dd, 5.2; 12.0)
6b⁗		3.92 (dd, 2.1; 12.0)
3⁗-cin-1	136.0	
2/6	129.4	7.63 (m)
3/5	130.2	7.42 (m)
4	131.7	7.42 (m)
7	146.4	7.77 (d, 15.9)
8	119.3	6.64 (d, 15.9)
9	168.5	
6⁗-Ac-1	172.6	
6⁗-Ac-2	21.4	1.69 (s)
4″-*O*-glc-1⁗⁗	102.4	4.92 (m)
2⁗⁗	75.0	3.48 (m)
3⁗⁗	78.0	3.47 (m)
4⁗⁗	71.5	3.40 (dd, 9.1; 9.1)
5⁗⁗	78.2	3.45 (m)
6a⁗⁗	62.6	3.71 (dd, 5.5; 12.1)
6b⁗⁗		3.89 dd, 2.1; 12.1)


Arundinoside R
Arundinoside Z
Arundinoside S
Arundinoside Y
Arundina graminifolia (D. Don) Hochr.
Arundinoside T
Arundinoside X
Arundinoside U
Arundinoside V
Arundinoside W