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-

130 glucopyranosyloxybenzyl-3-*trans*-cinnamoyl)-2-(*β*-D-glucopyranosyl-2-acetyl)-4-(*β*-D-
131 glucopyranosyloxybenzyl)-2R-benzylmalic acid.
132 The extracts of the plant have been shown to be composed of antibacterial compounds, an
133 activity mostly attributed to the phenolic and phenanthrenes (Yan et al., 2018, Hu et al., 2013). We
134 therefore wanted to affirm whether the antibacterial activity displayed by the extracts from *A.*
135 *graminifolia* can also attributed to other class of compounds present in the plant. Thus, the antibacterial
136 activity of all the isolated compounds were tested against *Bacillus cereus*, *Escherichia coli* strain O157:H7,
137 *Listeria monocytogenes* strain F2365 and *Staphylococcus aureus* strain ATCC 25923. However, all the
138 isolated compounds did not display any antibacterial effect.
139 In conclusion, we report the isolation and characterization of nine new glucosyloxybenzyl 2*R*-
140 benzylmalate derivatives from the underground part of *A. graminifolia*. It is noteworthy to indicate that
141 arundinoside R and S are the first monodesmosidic derivatives isolated in *A. graminifolia.* All the new
142 compounds were assayed for antibacterial activities, but unfortunately none of them showed any
143 activity.
144
145 **3. Materials and methods**
146 *3.1. General experimental procedures*
147 NMR spectra were acquired on a Bruker 500 MHz Avance III spectrometer fitted with a DCH
148 $^{13}C/^{1}H$ Cryoprobe (Bruker Biospin, Rheinstetten, Germany). Optical rotations were recorded on a
149 Jasco Perkin Elmer 341 polarimeter (Jasco, Lisses, France), while IR spectra were measured using a 380
150 FT-IR spectrophotometer (Thermo Electron Corporation, Saint Herblain, France). Centrifugal partition
151 chromatography (CPC) was performed using a FCPC200 instrument (Kromaton Technologies, Angers,
152 France) equipped with 20 circular partition disk rotors (1320 partition cells: 0.130 mL per cell; total
153 column capacity of 1000 mL).HR-ESI-MS analysis were performed using a 1200 Agilent Series fitted to
154 an Agilent 6520 Accurate Mass Q-TOF spectrometer (Agilent Technologies, Santa Clara, USA). Semi
155 preparative HPLC was performed on a Gilson LC system equipped with a Kinetex Axia C-18 column
156 (100 mm × 21.2 mm, 5μm).
157 *3.2. Plant material*
158 The whole of *A. graminifolia* was collected in September 2010 from an orchid farm in Chiang
159 Mai Province, Thailand. The plant was authenticated at the Faculty of Science, Chiang Mai University,
160 Thailand, where a voucher specimen was deposited (n° 05-563). The roots and rhizomes were dried and
161 imported to France in accordance to the specification of the Convention on International Trade in
162 Endangered Species (CITES).

163 *3.3. Extraction and isolation*

164 The dried roots and rhizomes of *A. graminifolia* were powdered and macerated with ethanol
165 thrice (1g powdered sample to 20 mL solvent). The ethanol extracts were pulled together and
166 concentrated under reduced pressure using a rotary evaporator. The crude EtOH extract was
167 reconstituted in water and furtherly partitioned with CH_2Cl_2 and EtOAc to afford the CH_2Cl_2 (10.40 g)
168 and EtOAc extracts (10.93 g) after evaporation. The EtOAc extract was purified by centrifugal partition
169 chromatography using *n*-heptane/EtOAc/MeOH/H_2O (0.25:5:1:5, v/v) as the solvent system. The
170 aqueous mobile phase was pumped into the column in the head to tail mode using a flow rate of 5
171 mL/min after the rotor has been filled with the organic stationary phase, and a gradual increase in
172 rotation speed from 0 to 1000 rpm until complete equilibrium between the organic and aqueous phases
173 was acheived. For each CPC run, the EtOAc extract (5 g) was dissolved in 40 mL of equal ratio of the
174 organic and aqueous phase mixture. The flow rate of the system was gradually increased to 11 mL/min
175 after sample injection and maintained for 140 mins. At the end of 200 min of head to tail mode, an
176 extrusion mode of the organic phase was performed at 70 mL/min and the rotation speed was reduced
177 to 500 rpm. Fourteen fractions (A-N) were collected and monitored at a wavelenght of 210 and 280
178 nm. Fractions E (617 mg), G (726 mg), H (301 mg) and I (540 mg) were further purified by Sephadex
179 LH-20 eluted with MeOH. Sub-fraction E-3 (290 mg) was purified on semi-preparative RP-HPLC (H_2O
180 (A)/CH_3CN (B) both containing 0.05% formic acid ; 40% B for 2 min, 40% to 50% B for 48 min, 50%
181 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)
182 and **10** (12.9 mg). Sub-fraction E-4 (120 mg) was purified using the same method as E-3 to yield
183 compounds **4** (2.1 mg) and **5** (19.1 mg).

184 Sub-fraction G-3 (224.9 mg) was purified by semi-preparative HPLC (H_2O (A)/CH_3CN (B)
185 both containing 0.05% formic acid ; 45% B for 5 min, 45% to 49% B for 25 min,49% to 100% B for 2
186 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-
187 fractions H-3 (101.1 mg), H-4 (107.2 mg) and I-4 (63.5 mg) were purified by semi preparative HPLC
188 to afford compounds **8** (18.8 mg), **1** (1.8 mg) and **2** (4.1 mg), respectively.

189 *3.3.1 Arundinoside R (**1**)*

190 White amorphous powder (4.1 mg);$[\alpha]_D^{25}$ -64 (*c* 0.4, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 218
191 (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.
192 $C_{23}H_{32}NO_{13}$ for 530.18682).

193 *3.3.2 Arundinoside S (2)*

194 White amorphous powder (1.8 mg);$[\alpha]_D^{25}$ -56 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 218
195 (3.05), 276 (1.82); ^{1}H NMR and ^{13}C NMR see Table 1; HR-ESI-MS: *m/z* 633.18336 [M-H]$^-$ (calcd.
196 $C_{30}H_{33}O_{15}$ for 633.18249).

197 *3.3.3 Arundinoside T* (**3**)

198 White amorphous powder (2.3 mg);$[\alpha]_D^{25}$ -99 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 217
199 (3.50), 268 (1.70); ^{1}H NMR and ^{13}C NMR see Table 2; HR-ESI-MS: *m/z* 779.24534 [M-H]$^-$ (calcd. for
200 $C_{36}H_{43}O_{19}$ for 779.24040).

201 *3.3.4. Arundinoside U* (**4**)

202 White amorphous powder (2.1 mg); $[\alpha]_D^{25}$ -58 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 216
203 (3.25), 271 (1.95); ^{1}H NMR and ^{13}C NMR see Table 2; HR-ESI-MS: *m/z* 779.24417 [M-H (calcd. for
204 $C_{36}H_{43}O_{19}$ for 779.24040).

205 *3.3.5 Arundinoside V* (**5**)

206 White amorphous powder (19.1 mg); $[\alpha]_D^{25}$ -62 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε):
207 210 (3.01), 267 (1.88); ^{1}H NMR and ^{13}C NMR see Table 2; HR-ESI-MS: *m/z* 779.24451 [M-H]$^-$ (calcd.
208 for $C_{36}H_{43}O_{19}$ for 779, 24040).

209 *3.3.6 Arundinoside W* (**6**):

210 White amorphous powder (2.7 mg); $[\alpha]_D^{25}$ -71 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 211
211 (3.04), 267 (1.82); ^{1}H NMR and ^{13}C NMR see Table 3; HR-ESI-MS: *m/z* 779.24433 [M-H]$^-$ (calcd. for
212 $C_{36}H_{43}O_{19}$ for 779.24040).

213 *3.3.7. Arundinoside X* (**7**)

214 White amorphous powder (1.2 mg); $[\alpha]_D^{25}$ -37 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 217
215 (3.10), 273 (1.98); ^{1}H NMR and ^{13}C NMR see Table 4; HR-ESI-MS: *m/z* 943.28596 [M-H]$^-$ (calcd. for
216 $C_{45}H_{51}O_{22}$ for 943.28775).

217 *3.3.8. Arundinoside Y* (**8**)

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

221 *3.3.9. Arundinoside Z* (**9**)

222 White amorphous powder (3.0 mg); $[\alpha]_D^{25}$ -69 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 217
223 (3.03), 269 (2.10), 277 (1.82); ^{1}H NMR and ^{13}C NMR see Table 5; HR-ESI-MS: *m/z* 1093.34878 [M-
224 H]$^-$ (calcd. for $C_{54}H_{61}O_{24}$ for 1093.35583).

225 *3.4. Determination of the sugar moiety*

226 The sugar moiety was determined according to the method of Simmler et al. (Simmler et al.,
227 2011). The compounds (1 mg) was dissolved in 2 M HCl (0.5 mL) and heated for 3 hours under reflux.
228 The solution was cooled and partitioned three times with *n*-butanol. After partitioning, the aqueous
229 phase was dried under reduced pressure and derivatization was performed with pyridine and 1-
230 (trimethylsilyl) imidazole (4:1 v/v) at 60°C for 1 hour. The identification of the derivatized sample was
231 conducted on a GC-MS Trace GC Ultra instrument equipped with a TR-5MS SQC column (0.25 µm,
232 15 m x 0.25 mm) and operated using the following set of conditions: 1 min at 40°C; a thermal ramp of
233 10°C until 250°C (helium flow rate 1 mL/min, injector temperature 250°C, transfer temperature
234 285°C). The detection was performed on a DSQII Thermo Scientific mass spectrometer, with a
235 detection mass range of *m/z* 0 to 500. The sugar (D-glucose) was identified based on the comparison of
236 the retention time of the derivative with that of the standard glucose treated in the same manner.

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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**)

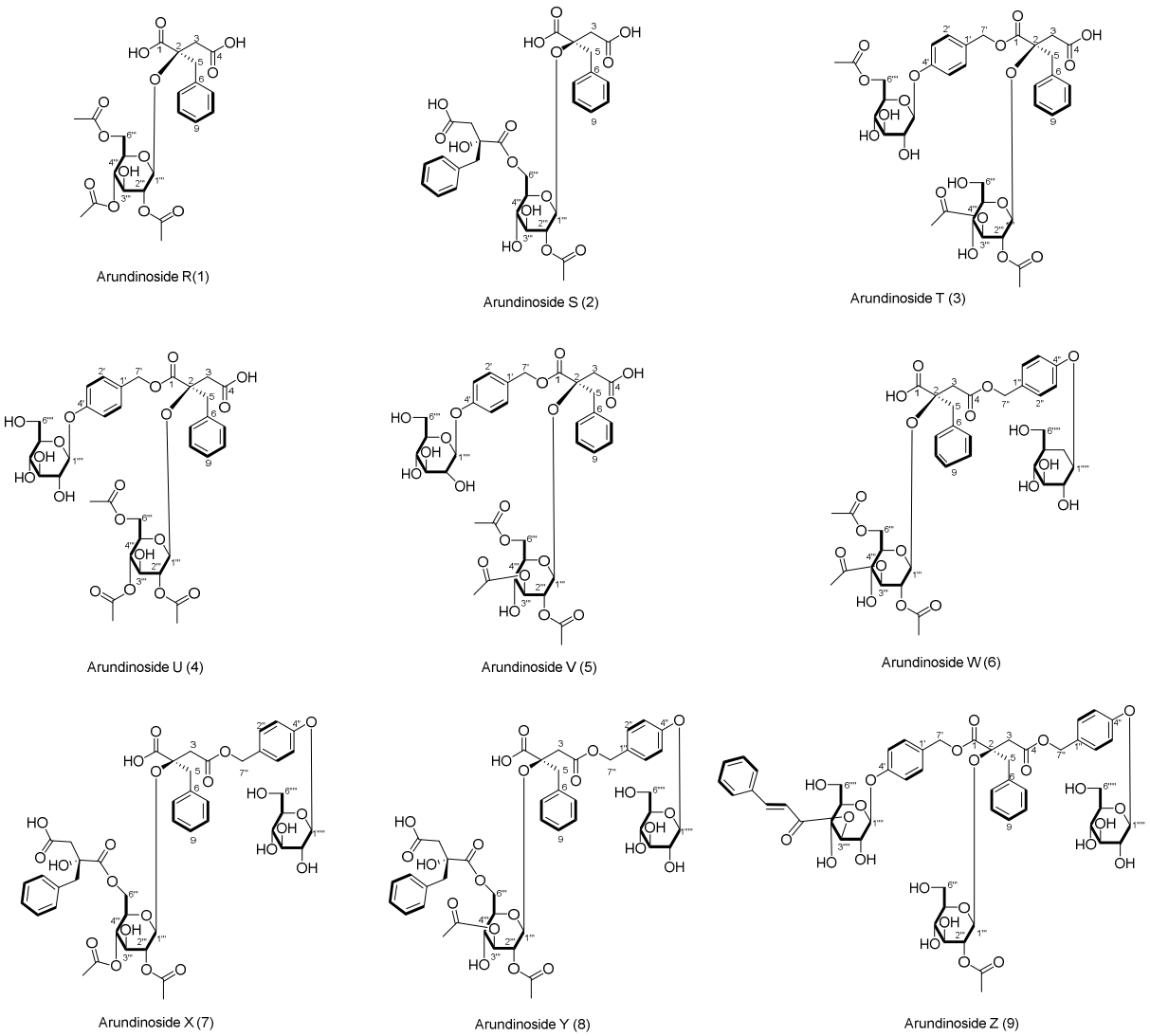


Figure 1. Chemical structures of the nine isolated compounds arundinosides R-Z

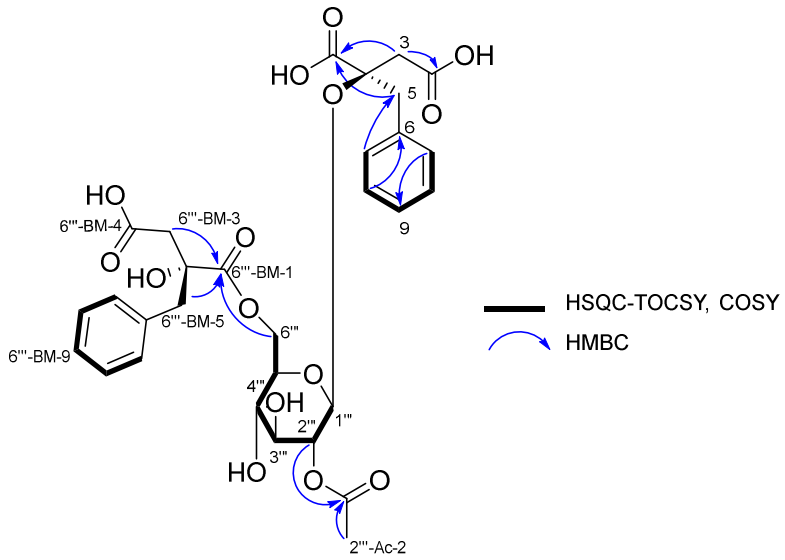


Figure 2. Key HSQC-TOCSY, COSY, HMBC correlations of arundinoside S (**2**)

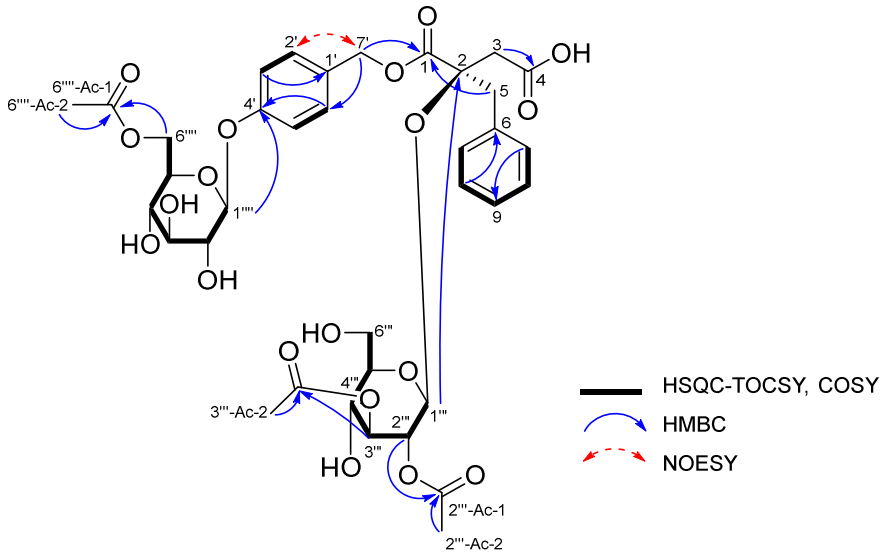


Figure 3. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside T (**3**)

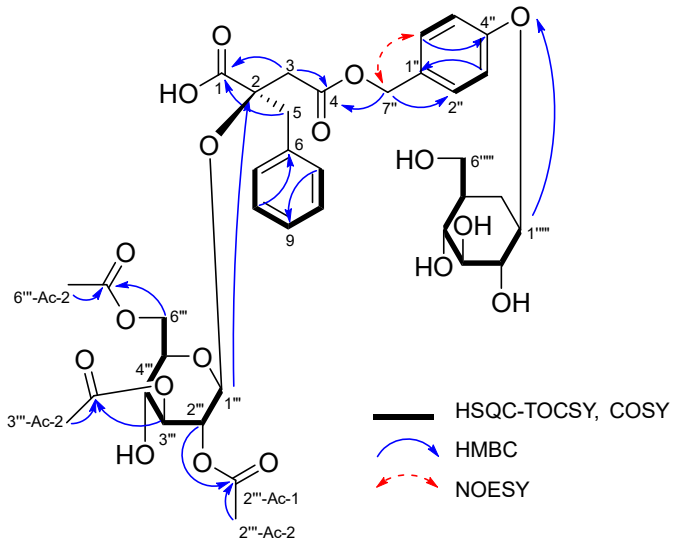


Figure 4. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside W (**6**)

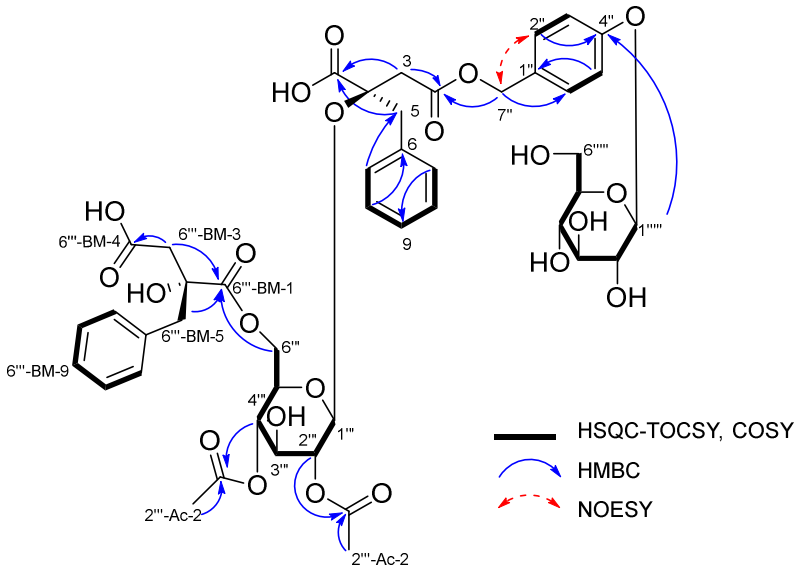


Figure 5. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside X (**7**)

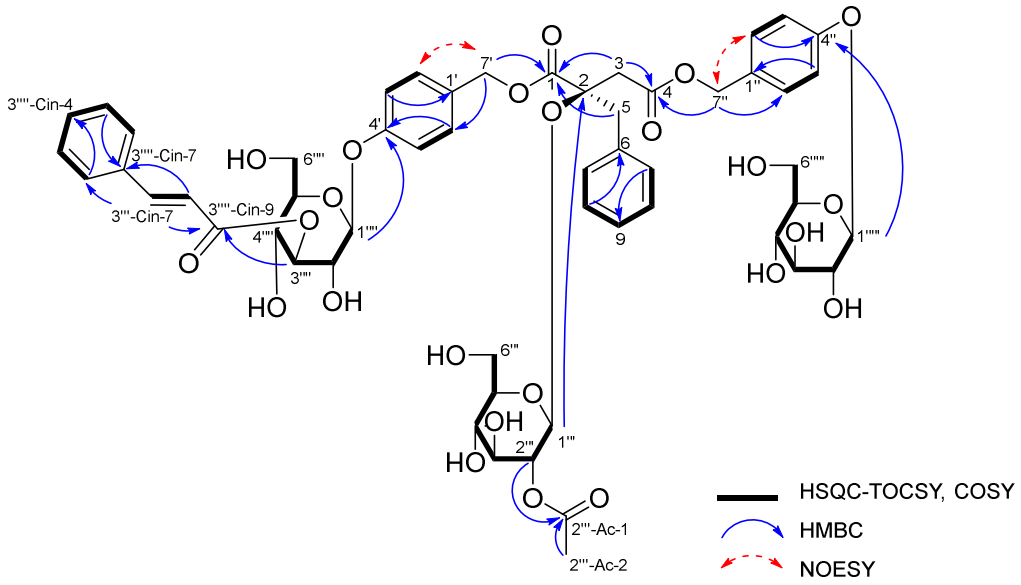


Figure 6. Key HSQC-TOCSY, COSY, HMBC and NOESY correlations of arundinoside Z (**9**)

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)

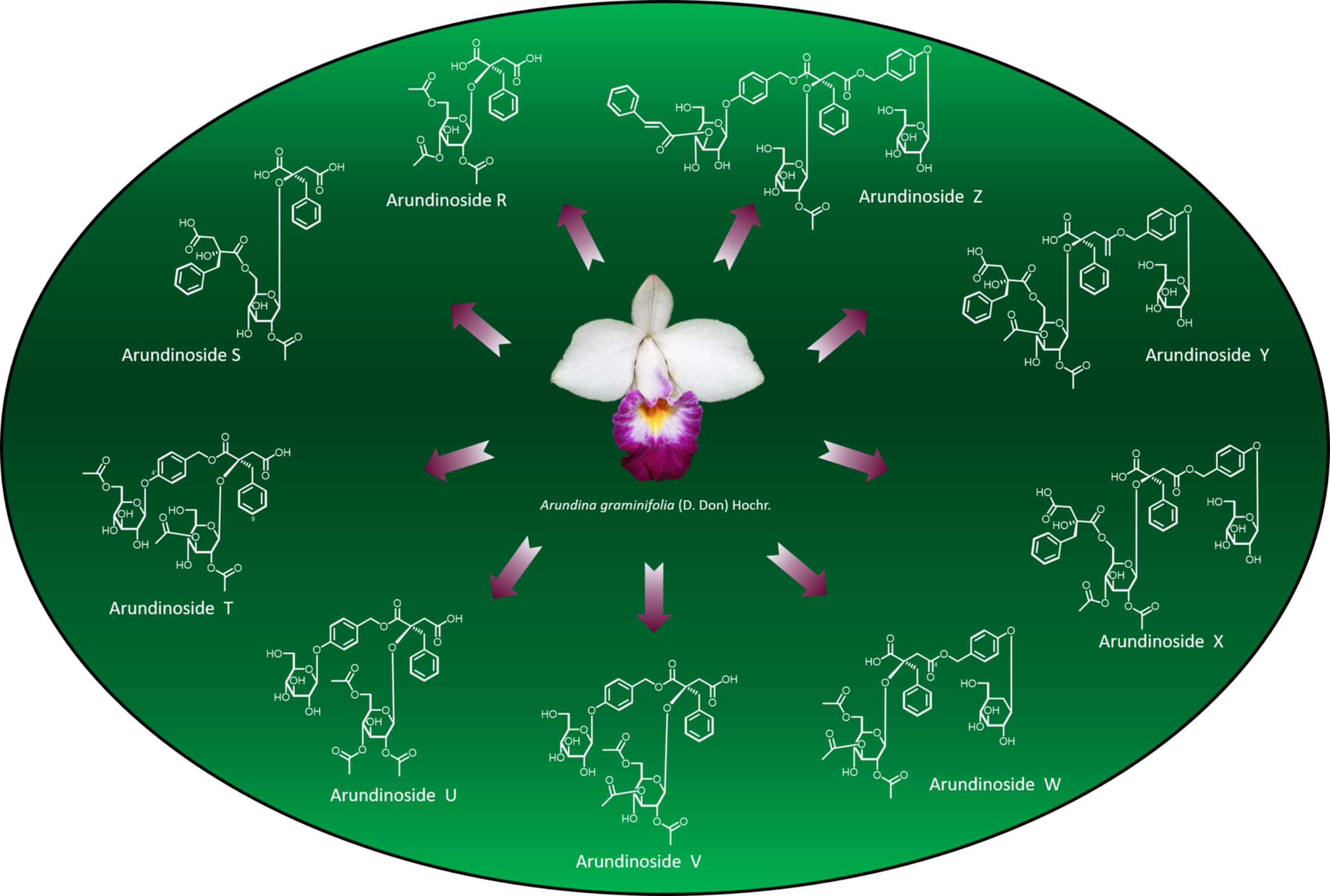
Table 4. ^{1}H (500 MHz, methanol-d_4) and ^{13}C (125 MHz, methanol-d_4) NMR data of arundinoside X-Y (**7**-**8**)

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
Arundinoside T
Arundinoside X
Arundinoside U
Arundinoside V
Arundinoside W
Arundina graminifolia (D. Don) Hochr.