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Verbesinosides A–F, 15,27-Cyclooleanane Saponins from the American Native Plant *Verbesina virginica*

Wen-Hui Xu[†,‡], **Melissa R. Jacob**[†], **Ameeta K. Agarwal**[†], **Alice M. Clark**[†,§], **Zong-Suo Liang**[‡], and **Xing-Cong Li**[*,†]

National Center for Natural Products Research and Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677

College of Life Sciences, Northwest A & F University, Yangling, Shaanxi 712100, People's Republic of China

Abstract

Verbesinosides A–F (**1**–**6**), six new 15,27-cyclooleanane-type triterpenoid saponins carrying different aromatic acyl moieties on the aglycon, were isolated from the leaves and flowers of *Verbesina virginica*. Their structures were established by interpretation of spectroscopic data and chemical methods. The representative major saponin, verbesinoside A, (**1**) has the structure, 21-trimethoxybenzoyl 15α,27-cyclooleanan-12-en-3β,21β-diol-28-oic acid 3-*O*-β-D-xylopyranosyl (1→4)-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside. This is the first report of triterpenoid saponins possessing the unique 15,27-cyclooleanane skeleton. The anisotropic effects of the aromatic acyl moieties to the triterpenoid skeleton are discussed.

Several species of the plant genus *Verbesina* (Asteraceae) have been studied chemically. Characteristic compounds include sesquiterpenoids,[1–4] diterpenoids,[5] and triterpenoids[6] derivatives. *Verbesina virginica* L. (white crownbeard) is one of 18 species of *Verbesina* distributed in the North American continent that has been used ethnobotanically as a gastrointestinal and urinary aid, as well as for antirheumatic, emetic, and laxative, and ceremonial purposes.[7] Fractionation of the organic extract of the combined leaves and flowers of *V. virginica* yielded column fractions containing saponins, as evident by their frothing property in aqueous solution. This prompted us to investigate the saponin chemistry in this species since there is only one previous report of a triterpenoid saponin, copteroside E, from the genus *Verbesina*.[8] Our studies have led to the identification of six new triterpenoid saponins (**1**–**6**) possessing a unique cyclopropane ring between C-14 and C-15 on the oleanane skeleton. This is the first report of the 15,27-cyclooleanane skeleton.

Results and Discussion

The 95% EtOH extract of the leaf and flower of *V. virginica* was partitioned between hexanes and MeOH–H_2O (9:1). The MeOH/H_2O layer was further partitioned with $CHCl_3$. The organic layer was evaporated and the resultant residue was chromatographed on silica gel. The saponin-

*To whom correspondence should be addressed. Tel.: 662-915-6742. Fax: 662-915-7989. E-mail: xcli7@olemiss.edu.
†National Center for Natural Products Research, The University of Mississippi.
‡College of Life Sciences, Northwest A & F University.
§Department of Pharmacognosy, The University of Mississippi.

Supporting Information Available: NMR spectra of compounds **1**–**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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containing chromatographic fractions were subjected to reversed-phase silica gel column chromatography and HPLC to yield compounds **1**–**6**, which were designated as verbesinosides A–F.

Verbesinoside A (**1**) was obtained as an amorphous white powder and is the major saponin in this plant. The negative-ion high-resolution ESIMS of **1** showed a pseudomolecular peak at *m*/*z* 1089.5245 $[M-H]^-$. In conjunction with the ^{13}C NMR spectrum displaying 56 resonances, its molecular formula was thus determined as $C_{56}H_{82}O_{21}$. A DEPT NMR experiment permitted differentiation of the 56 resonances into six methyls, 12 methylenes, 23 methines, 12 quaternary carbons, and three methoxy carbons. The ^1H NMR spectrum of **1** in pyridine-d_5 indicated that the six methyl groups resonating at δ_H 0.80, 1.02, 1.10, 1.12, 1.25, and 1.36 (3H each, s), which correlated with the carbons at δ_C 15.1, 16.3, 28.8, 19.5, 27.6, and 18.9, respectively, in the HMQC spectrum, were tertiary methyl groups. Three anomeric protons of monosaccharide units at δ_H 5.13 (d, *J* = 7.0 Hz), 4.87 (d, *J* = 7.0 Hz), 4.84 (d, *J* = 7.0 Hz), which were better resolved into δ_H 4.57 (d, *J* = 7.2 Hz), 4.44 (d, *J* = 7.2 Hz), and 4.34 (d, *J* = 7.2 Hz) in the ^1H NMR spectrum measured in MeOH-d_4, correlated with three anomeric carbons at δ_C 106.3, 103.6, and 104.5, respectively, in the HMQC spectrum. The presence of a trimethoxybenzoyl moiety in **1** was evident from the ten resonances at δ 165.7 (qC), 153.5 (qC, 2C), 142.8 (CH), 126.0 (qC), 107.3 (CH, 2C), 60.5 (CH_3, OMe), and 56.0 (CH_3, OMe × 2),[9] which was further supported by the long-range H–C correlations between the aromatic protons at *δ* 7.52 (2H, brs) and the carbons at δ 165.7, 153.5, 142.8, and 126.0 in the HMBC spectrum. The IR spectrum of **1** also showed a strong aromatic ester absorption at 1699 cm^{-1}, in accordance with the UV maxima at 214 and 261 nm.[10] The free trimethoxybenzoic acid, namely, eudesmic acid, was isolated previously from *Verbesina* species.[4] Based on the presence of the carboxylic carbon (δ 178.1), two olefinic carbons (δ 137.8, qC and 121.1, CH), and one oxygen-bearing methine carbon (δ 88.9), resonating in the downfield region that are typical of oleanolic acid derivatives,[11] we believed initially compound **1** to be an oleanane-like triterpenoid skeleton attached to a sugar moiety of three monosaccharides and a trimethoxybenzoyl moiety.

Upon acid hydrolysis with 2 N HCl in 50% aqueous dioxane, **1** afforded D-glucose and D-xylose in a ratio of 1:2 and a complex mixture of aglycons. Given that the sugar moiety was composed of one β-D-glucopyranosyl moiety and two β-D-xylopyranosyl units identified from the 2D NMR of **1**, the aglycon should thus have a molecular composition of $C_{30}H_{46}O_4$. A distinguishing feature for the aglycon moiety was that the ^1H NMR spectrum of **1** showed a significantly upfield proton at δ 0.34 (brs), which correlated with a methylene carbon at δ 20.6 in the HMQC spectrum, indicating the presence of a cyclopropane ring system in the skeleton.[12–14] Thus, considering that there are only six tertiary methyl groups in the molecule, the aglycon was proposed as being an oleanane skeleton with one cyclopropane ring system, one carboxylic group, one double bond, and two hydroxy groups.

The 2D NMR spectra were in turn used to determine the structure of the aglycon and the linkage of the trimethoxybenzoyl moiety to the aglycon. In the HMBC spectrum, the two methyl protons at δ 1.02 (Me-24) and 1.25 (Me-23) showed correlations with signals at *δ* 88.9 (CH, C-3), 39.3 (qC, C-4), and 55.7 (CH, C-5), while another two methyl protons at *δ* 1.10 (Me-29) and 1.36 (Me-30) exhibited cross peaks with *δ* 35.7 (qC, C-20), 45.4 (CH_2, C-19), and 76.6 (CH, C-21). These chemical shift values and HMBC correlation patterns represent a typical oleanane skeleton with 3β,21β-dihydroxy substitutions.[15] The cyclopropane ring appeared to be located between C-14 and C-15, since a clear spin-spin coupling network was observed from the most upfield proton at δ 0.34 (brs, H-27α) to H-15 at δ 1.47 and then to H-16β at δ 2.85 in the DQF-COSY spectrum. The assignments of these two protons were facilitated with the HMQC spectrum. The very upfield methine C-15 at δ 15.2 was due to strong shielding effects of the cyclopropane ring. In addition, H-27α showed critical long-range correlations with C-13 and C-16 in the HMBC spectrum, confirming the double bond between C-12 and

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C-13. Thus, the structure of the aglycon was determined as 15α,27-cycloolean-12-en-3β,21β-diol-28-oic acid. The key HMBC correlation between the significantly downfield shifted H-21 at δ 5.32 (brd, J = 7.0 Hz) and the ester carbon at δ 165.7 indicated that the trimethoxybenzoyl moiety was attached to the hydroxy group of C-21 of the aglycon.

The sequence and interglycosidic linkages of the sugar moiety of **1** were established as follows. The positive-ion ESIMS exhibited a pseudomolecular ion peak at *m/z* 1091.5 $[M + H]^+$ and fragmentation peaks at *m/z* 959.5 $[M - 132 + H]^+$, 827.3 $[M - 132 \times 2 + H]^+$, and 665.4 $[M - 132 \times 2 - 162 + H]^+$, indicating the glucosyl unit was directly attached to the hydroxy group of C-3 of the aglycon. This was supported by the HMBC correlations between the anomeric proton at δ 4.84 and C-3 of the aglycon at δ 88.9 and between H-3 of the aglycon at δ 3.30 (dd, J = 12.0, 4.0 Hz) and the anomeric carbon at δ 104.5. One xylosyl unit was proven to be attached to the hydroxy group of C-2 of the glucosyl unit, as its anomeric proton at δ 5.13 showed an HMBC correlation with a carbon at δ 83.9 (CH), which was assigned as C-2 of the glucosyl unit by DQF-COSY and HMQC correlations starting from the anomeric proton of the glucosyl unit at δ 4.84. In addition, the anomeric proton at δ 5.13 produced a clear three-bond HMBC correlation with the upfield shifted C-5 (CH_2) signal at δ 64.7 within the xylosyl unit, indicating that the hydroxy group of C-4 of this xylosyl unit is glycosylated. Confirmation was made by the HMBC correlation between the anomeric proton at δ 4.87 of the remaining xylosyl unit and a carbon at δ 77.6 (CH), which undoubtedly was C-4 of the inner xylosyl unit. Thus, the sugar sequence was established as xylopyranosyl(1→4)-xylopyranosyl(1→2)-glucopyranoside. The upfield shift of the anomeric carbon of the terminal β-D-xylopyranosyl unit in such a 4-*O*-glycosylation of a β-D-xylopyranosyl derivative is consistent with those reported in the literature.[16,17]

Analysis of the ROESY data of compound **1** provided important information on the relative configuration. The NOE correlations from H-3 to H-5 confirmed the β-orientation of the hydroxy group at C-3 of the aglycon. Other key NOE correlations were observed from H-18 to Me-30 and H-12 and from H-27α to H-19α, indicating that the cyclopropane ring must be located below the plane of the pentacyclic ring system. Based on this information, a possible conformation of the aglycon of **1** was generated by Chem3D Pro 11.0, in which the A, B, and E rings adopt chair conformations, while the C and D rings take chair-like and boat conformations, respectively (Figure 1). The calculated distances between H-12 and H-18, between H-18 and Me-30, and between H-27α and H-19α are 2.452, 2.267, and 2.257 Å, respectively, which supports the aforementioned NOE correlations. Interestingly, a strong NOE correlation between Me-30 and the two aromatic protons of the trimethoxybenzoyl moiety was also observed. This not only confirmed the β-orientation of the 21-hydroxy group but also indicated that this acyl moiety is very close spatially to the triterpenoid skeleton. It should be pointed out that this conformation has not been energy-optimized by advanced molecular modeling methods and does not represent the lowest-energy conformer. However, it appears that the Chem3D Pro 11.0 program can generate helpful 3D structural information that is consistent with the experimental NMR data of compound **1**. We have also noted that some of the NMR signals, e.g., C-18, C-19, C-21, C-22, C-28, H-21, Me-26, and H-27, were broad and weak in the 1D NMR spectrum of **1** acquired at room temperature, which is probably due to minor rotational restriction of the bulky aromatic acyl moiety. A significantly improved HMBC spectrum was obtained at 40 ºC. The detailed HMBC and NOE correlations shown in Table 1 fully support the structure shown.

Based on the above evidence, the structure of **1** was elucidated as 21-trimethoxybenzoyl 15α, 27-cycloolean-12-en-3β,21β-diol-28-oic acid 3-*O*-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl(1→2)-β-D-glucopyranoside. This is the first report of a triterpenoid saponin possessing a unique 15,27-cyclooleanane skeleton for which the structure has been confirmed by 2D NMR spectroscopic data. Although the well-established cycloartane class of triterpenes

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possesses a cyclopropane ring system between the two quaternary C-9 and C-10 carbons on the tetracyclic skeleton,[18–21] it is rare that such a system is present in pentacyclic triterpenoid skeletons. Phyllanthol is the first pentacyclic ursane triterpenoid with a cyclopropane ring between C-13 and C-14.[22] Monacanthic acid was reported to possess a possible structure of (18ξ)-15α,27-cycloolean-12-en-3β-ol-28-oic acid based on limited spectroscopic data.[23] Another analogue is 3-hydroxy-12,13-cyclotaraxerene-14-en-28-oic acid that was isolated from *Paeonia suffruticosa*.[24] It should be mentioned that the strained cyclopropane ring system on triterpenoid skeletons are labile to acid treatment, in particular for those with a double bond[25] or a ketone functionality[26] located nearby, resulting in cyclopropane ring opening and dehydrogenation. In the case of compound **1**, the double bond between C-12 and C-13 may have facilitated this process during the acid hydrolysis to produce a complex mixture of unidentified aglycons, explaining our failure to obtain a single sapogenin in pure form.

Verbesinoside B (**2**) was obtained from a less polar column fraction. Its ^{13}C NMR spectrum resembled that of **1**, with identical chemical shifts due to the aglycon and acyl moieties. The primary difference was the lack of a set of resonance signals corresponding to a β-D-xylopyranosyl unit in **2**. This was supported by the positive-ion high-resolution ESIMS, which showed a pseudomolecular ion peak at *m*/*z* 981.4839 $[M+ Na]^+$, in accordance with an empirical molecular formula of $C_{51}H_{74}O_{17}$. The ^{1}H NMR spectrum of **2** showed two anomeric protons at δ_H 4.92 (d, J = 7.0 Hz) and 4.57 (d, J = 8.5 Hz). The Me-24 resonance was slightly shifted upfield to δ_H 1.10, as opposed to δ_H 1.02 in **1**. This is likely due to the steric effect of a disaccharide moiety in **2** instead of a trisaccharide moiety in **1**. The ^{13}C NMR chemical shifts due to the disaccharide moiety are consistent with those of glycosides possessing a β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl sequence.[27,28] Thus, the structure of **2** was determined as 21-trimethoxybenzoyl 15α,27-cycloolean-12-en-3β,21β-diol-28-oic acid 3-*O*-β-D-xylopyranosyl(1→4)-β-D-glucopyranoside.

Verbesinoside C (**3**) has a molecular formula of $C_{54}H_{78}O_{19}$ on the basis of its negative-ion high-resolution ESIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **3** were very similar to those of **1** except for the resonances corresponding to the acyl moiety. Careful inspection of the NMR data indicated that a *p*-methoxybenzoyl structural moiety is present in **3**, as evident by the resonances at δ_H 8.26 and 7.11 (2H each, d, J = 8.9 Hz), 3.73 (3H, s) and δ_C 165.8 (qC), 163.7 (qC), 131.8 (CH, 2C), 123.3 (qC), 114.1(CH, 2C), 55.6 (qC).[30] Thus, the structure of **3** was established as 21-*p*-methoxybenzoyl 15α,27-cycloolean-12-en-3β,21β-diol-28-oic acid 3-*O*-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl(1→2)-β-D-glucopyranoside.

Verbesinoside D (**4**) has a molecular formula of $C_{58}H_{84}O_{21}$, deduced from its negative-ion high-resolution ESIMS and ^{13}C NMR data. The NMR spectra of **4** were also very similar to those of **1** except for the presence of additional resonances in the ^{1}H NMR spectrum at δ_H 8.05 and 6.92 (1H each, d, J = 16.0 Hz) and corresponding ^{13}C NMR spectroscopic data at δ_C 145.0 (CH) and 118.2 (CH). In conjunction with other resonances at δ_H 7.15 (s, 2H), 3.92 (s, 3H), and 3.83 (s, 6H), and δ_C 166.7 (qC), 153.9 (qC, 2C), 140.7 (CH), 130.5 (qC), 106.1(CH, 2C), 60.5 (CH_3, OMe), and 56.0 (CH_3, OMe × 2), the presence of a *trans*-trimethoxycinnamoyl moiety[30] in **4** was confirmed. Thus, the structure of **4** is 21-*E*-trimethoxycinnamoyl 15α,27-cycloolean-12-en-3β,21β-diol-28-oic acid 3-*O*-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl (1→2) -β-D-glucopyranoside.

Verbesinoside E (**5**) produced ^{1}H and ^{13}C NMR spectra similar to those of **4**, with differences being the aromatic acyl moieties at the hydroxy group of C-21. The presence of a *E*-*p*-methoxycinnamoyl moiety was evident from the resonances at δ_H 8.03 (1H, d, J = 16.0 Hz), 7.69 (2H, d, J =8.0 Hz), 7.03 (2H, d, J =8.0 Hz), 6.75 (1H, d, J = 16.0 Hz), and 3.68 (s, 3H), and δ_C 166.6 (qC), 162.0 (qC), 144.9 (CH), 130.2 (CH, 2C), 127.3 (qC), 116.6 (CH, 2C,), 114.7 (CH), and 55.2 (CH_3).[31] The high-resolution ESIMS data also supported the molecular formula

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of **5** as $C_{56}H_{80}O_{19}$. Thus, compound **5** is 21-*E*-*p*-methoxycinnamoyl 15α,27-cycloolean-12-en-3β,21β-diol-28-oic acid 3-*O*-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl(1→2) -β-D-glucopyranoside.

Verbesinoside F (**6**) exhibited ^{1}H and ^{13}C NMR spectra similar to those of compound **5**, except for the substitution of different acyl moieties at the hydroxy group of C-21. The presence of an *E*-cinnamoyl moiety derived from the resonances at δ_H 8.03 (1H, d, *J* = 16.5 Hz), 7.70 (2H, d, *J* = 7.0 Hz), 7.40 (3H, m), and 6.82 (1H, d, *J* = 16.5 Hz), and δ_C 166.1 (qC), 144.9 (CH), 130.4 (qC), 129.5 (CH, 2C), 128.6 (CH, 2C), 123.3 (qC), 118.8 (CH), was consistent with those reported for the cinnamoyl eudesmane derivatives isolated from *Verbesina* species.[5] The high-resolution ESIMS data of **6** were in accordance with an empirical molecular formula of $C_{55}H_{78}O_{18}$. Thus, the structure of **6** was established as 21-*E*-cinnamoyl 15α,27-cycloolean-12-en-3β,21β-diol-28-oic acid 3-*O*-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl(1→2) -β-D-glucopyranoside.

It has been noted that anisotropic effects of different aromatic acyl moieties in compounds **1**–**6** have a significant effect on the ^{1}H NMR chemical shifts of Me-29 and Me-30. As mentioned above, the trimethoxylbenzoyl moiety in **1** is close to the triterpenoid skeleton and thus would have a strong deshielding effect on the protons nearby. For compounds **4**–**6**, the α,β-unsaturated ester system increases the length between the phenyl ring and the triterpenoid skeleton, and would be expected to have less such effects. This is evident by the difference of the chemical shifts of Me-29 and Me-30 in these compounds. For example, the relatively deshielding Me-29 and Me-30 resonances were observed at δ_H 1.10 and 1.36, respectively, in compound **1**, as opposed to δ_H 1.04 and 1.22, respectively, in compound **4**. It is important to note, however, that the anisotropic effects of these aromatic acyl moieties on the ^{13}C NMR chemical shifts of the triterpenoid skeleton are minimal. The ^{13}C NMR chemical shifts due to the aglycon and sugar moieties of compounds **1**–**6** remained consistently close, which formed the comparative basis for the structure elucidation of these compounds.

The purified major saponin, verbesinoside A (**1**), was found to be inactive at the highest test concentration of 20 μg/mL used against the pathogenic fungi *Candida albicans*, *Cryptococcus neoformans*, *and Aspergillus fumigatus*, and the bacteria *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa*, and *Mycobacterium intracellulare*, using methods described in previous publications.[32,33]

Experimental Section

General Experimental Procedures

Optical rotations were measured on an Autopol IV polarimeter. UV was obtained from an HP 8453 diode array spectrophotometer. IR spectra were recorded using a Thermo Nicolet IR 300 FT/IR spectrometer. The 1D and 2D NMR (DQF-COSY, HMQC, HMBC, ROESY) spectra using standard pulse programs were recorded at room temperature on a Varian Oxford AS400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz, or on a Bruker Avance DRX 500 FT spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz. An elevated temperature of 40 ºC was utilized for compound **1** in the HMBC experiment optimized for a $J_{H\text{-}C}$ value of 8 Hz. The chemical shift values are expressed relative to the internal standard, TMS. HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was performed using normal-phase silica gel (J. T. Baker, 40 μm) and reversed-phase silica gel (RP-18, J. T. Baker, 40 μm). Semi-preparative HPLC was conducted on a C_{18} column (Gemini, 250 × 10 mm, 5 μm) with UV detection at 254 nm. Analytical HPLC was performed on a C_{18} column (Gemini, 4.6 × 150 mm, 5 μm) with a diode array detector at 250 nm. TLC was carried out on silica gel sheets (Alugram® Sil G/UV$_{254}$, Macherey-Nagel, Germany) and

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reversed-phase plates (RP-18 F_{254S}, Merck, Germany), with visualization by10% H_2SO_4, followed by heating.

Plant Material

The leaves and flowers of *Verbesina virginica* were collected by Sharon Bodine & Gretchen Walters in Ha Ha Tonka State Park, on Hwy D-144, Camden County, Missouri (coordinates: 37°57′15″N 092°46′03″W) in August, 2001, and identified by Gretchen Walters. A voucher specimen (no. 5318394) is deposited in the Herbarium of the Missouri Botanical Garden, St Louis, MO.

Extraction and Isolation

Powdered, air-dried leaves and flowers of *V. virginica* (117 g) were extracted exhaustively with 95% EtOH (1 L × 3) at room temperature for 24 h. Removal of the solvent by evaporation in vacuo afforded a residue (12 g), which was dissolved in MeOH–H_2O (9:1, 200 mL) and defatted with hexanes (5 × 100 mL). The combined hexanes layers were evaporated to dryness to afford a dark residue (3.3 g). To the MeOH/H_2O layer was added 40 mL of H_2O, and the solution was partitioned with $CHCl_3$ (200 mL). The organic layer was evaporated to dryness and the resultant residue (6.0 g) was subjected to silica gel chromatography using a stepwise gradient elution of $CHCI_3$–MeOH (50:1 to 3:1) and finally with MeOH to afford 14 pooled fractions (Fr. A-Q) according to TLC. Fr. L (130 mg) was chromatographed on a C_{18} reversed-phase column, using 70% CH_3CN/H_2O, and then further purified on a semi-preparative C_{18} reversed-phase HPLC column, using 47% CH_3CN/H_2O, at a flow rate of 4 mL/min, to afford compound **2** (4.6 mg, t_R 17.4 min). Fr. O (317 mg) was chromatographed on a semi-preparative C_{18} reversed-phase HPLC column, using 50% CH_3CN/H_2O, at a flow rate of 4 mL/min, to give compounds **1** (70.0 mg, t_R 9.6 min), **3** (4.4 mg, t_R 11.4 min), **4** (11.1 mg, t_R 12.7 min), **5** (3.0 mg, t_R 17.1 min), and **6** (2.2 mg, t_R 19.7 min).

Verbesinoside A (1): amorphous white powder; $[\alpha]^{25}_D$ +36.2 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log e) 214 (4.62), 261 (4.23) nm; IR (NaCl) ν_{max} 3351, 2839, 1699, 1560, 1457, 1330, 1221, 1037, 752 cm^{-1}; NMR data (pyridine-d_5), see Table 1; ^1H NMR (MeOH-d_4, 400 MHz) δ 7.29 (2H, brs, H-2′,6′), 5.51 (1H, brs, H-12), 4.90 (H-21, overlapped by H_2O residue in the NMR solvent), 4.57 (1H, d, *J* = 7.2 Hz, H-1‴), 4.44 (1H, d, *J* = 7.2 Hz, H-1⁗), 4.34 (1H, d, *J* = 7.2 Hz, H-1″), 3.87 (3H, s, H-9′), 3.85 (6H, s, H-8′,10′), 1.21 (3H, s, Me-30), 1.04 (3H, s, Me-23), 0.96 (3H, s, Me-29), 0.89 (6H, s, Me-25,26), 0.84 (3H, s, Me-24), 0.73 (1H, br d, *J* = 10.8 Hz, H-5), 0.18 (1H, brs, H-27α); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 166.8 (qC, C-7′), 154.0 (qC, C-3′,5′), 143.2 (qC, C-4′), 137.9 (qC, C-13), 126.5 (qC, C-1′), 124.7 (CH, C-12), 107.6 (CH, C-2′,6′), 105.8 (CH, C-1‴), 105.0 (CH, C-1″), 103.7 (CH, C-1⁗), 90.7 (CH, C-3), 83.2 (CH, C-2″), 78.0 (CH, C-3″), 77.8 (CH, C-5″), 77.5 (CH, C-21′), 77.2 (CH, C-4‴), 77.0 (CH, C-3‴), 75.6 (CH, C-3‴), 75.4 (CH, C-2‴), 73.9 (CH, C-2⁗), 70.9 (CH, C-4″), 70.6 (CH, C-4⁗), 66.7 (CH_2, C-5⁗), 64.5 (CH_2, C-5‴), 62.3 (CH_2, C-6″), 61.1 (CH_3, C-9′), 56.8 (CH, C-5), 56.5 (CH_3, C-8′,10′), 53.7 (CH, C-9), 48.2 (qC, C-17), 46.0 (CH_2, C-19), 40.5 (CH, C-18), 40.0 (qC, C-4), 39.3 (CH_2, C-1), 37.6 (qC, C-10), 36.4 (CH_2, C-24), 36.3 (qC, C-20), 35.8 (qC, C-8), 33.6 (CH_2, C-7), 33.5 (qC, C-14), 29.4 (CH_3, C-29), 28.1 (CH_3, C-23), 26.9 (CH_2, C-2), 24.7 (CH_2, C-11), 21.1 (CH_2, C-27), 19.9 (CH_3, C-26), 19.4 (CH_3, C-30), 18.8 (CH_2, C-6), 16.6 (CH_3, C-24), 15.8 (CH, C-15), 16.7 (CH_3, C-25) (the carboxylic C-28 resonance did not appear, and the assignments of the ^1H and ^{13}C NMR chemical shifts were facilitated with DEPT, HMQC, and HMBC spectra and comparison with the data assigned in pyridine-d_5); HRESIMS *m*/*z* 1089.5245 (calcd for $[C_{56}H_{82}O_{21} - H]^-$, 1089.5276); ESIMS *m*/*z* 1091.5 $[M + H]^+$, 959.5 $[M - (xylose) + H]^+$, 827.3 $[M - (xylose) \times 2 + H]^+$, 665.4 $[M - (xylose) \times 2 - glucose + H]^+$.

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Verbesinoside B (2): amorphous white powder; $[\alpha]^{25}_{D}$ +43.3 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log e) 213 (4.54), 262 (4.05) nm; IR (NaCl) ν_{max} 3323, 2941, 1699, 1590, 1504, 1415, 1336, 1228, 1129, 757 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 7.59 (2H, brs, H-2′,6′), 5.71 (1H, brs, H-12), 5.36 (1H, m, H-21), 4.92 (1H, d, *J* = 7.0 Hz, H-1‴), 4.57 (1H, d, *J* = 8.4 Hz, H-1″), 3.94 (3H, s, H-9′), 3.81 (6H, s, H-8′,10′), 3.44 (1H, brd, H-18), 3.33 (1H, brd, H-3), 1.34 (3H, s, Me-30), 1.29 (3H, s, Me-23), 1.10 (9H, s, Me-24,26,29), 0.80 (3H, s, M-25), 0.71 (1H, brd, *J* = 11.0 Hz, H-5), 0.34 (1H, s, H-27α); ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 981.4839 (calcd for $[C_{51}H_{74}O_{17} + Na]^+$, 981.4818), 850.2549 $[M + Na - xylose + H]^+$, 993.4520 (calcd for $[C_{51}H_{74}O_{17} + Cl]^-$, 993.4620).

Verbesinoside C (3): amorphous white powder; $[\alpha]^{25}_{D}$ +22.5 (*c* 0.08, MeOH); IR (NaCl) ν_{max} ca. 3300 (weak), 2872, 1698, 1558, 1457, 1337, 1240, 1039, 749 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 8.26 (2H, d, *J* = 8.0 Hz, H-2′, 6′), 7.11 (2H, d, *J* = 8.0 Hz, H-3′,5′), 5.71 (1H, brs, H-12), 5.21 (1H, brd, *J* = 7 Hz, H-21), 5.19 (1H, d, *J* = 7.0 Hz, H-1‴), 4.94 (1H, d, *J* = 7.0 Hz, H-1⁗), 4.90 (1H, d, *J* = 8.0 Hz, H-1′), 3.73 (3H, s, H-9′), 3.48 (1H, brd, H-18), 3.32 (1H, brd, H-3), 1.31 (3H, s, Me-30), 1.27 (3H, s, Me-23), 1.15 (3H, s, Me-26), 1.05 (6H, s, Me-24,29), 0.80 (3H, s, Me-25), 0.71 (1H, brd, *J* = 11.0 Hz, H-5), 0.34 (1H, s, H-27α); ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 1029.5004 (calcd for $[C_{54}H_{78}O_{19} - H]^-$, 1029.5059).

Verbesinoside D (4): amorphous white powder; $[\alpha]^{25}_{D}$ +27.7 (*c* 0.07, MeOH); IR (KBr) ν_{max} ca. 3300 (weak), 2921, 1693, 1580, 1461, 1382, 1242, 1165, 1047, 751 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) δ 8.05 (1H, d, *J* = 16.0 Hz, H-7′), 7.15 (2H, brs, H-2′,6′), 6.92 (1H, d, *J* = 16.0 Hz, H- 8′), 5.70 (1H, brs, H-12), 5.26 (1H, d, *J* = 7.0 Hz, H-21), 5.19 (1H, d, *J* = 7.0 Hz, H-1‴), 4.92 (1H, d, *J* = 8.4 Hz, H-1⁗), 4.90 (1H, d, *J* = 8.4 Hz, H-1″), 3.92 (3H, s, H-11′), 3.83 (s, 6H, H-10′,12′), 3.43 (1H, br d, H-18), 3.31 (1H, brd, H-3), 1.26 (3H, s, Me-23), 1.22 (3H, s, Me-30), 1.14 (3H, s, Me-26), 1.04 (6H, s, Me-24,29), 0.80 (3H, s, Me-25), 0.71 (1H, brd, *J* = 12.0 Hz, H-5), 0.34 (1H, brs, H-27α); ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 1115.5402 (calcd for $[C_{58}H_{84}O_{21} - H]^-$, 1115.5432).

Verbesinoside E (5): amorphous white powder; $[\alpha]^{25}_{D}$ +31.9 (*c* 0.06, MeOH); IR (KBr) ν_{max} ca. 3300 (weak), 2917, 1692, 1581, 1461, 1244, 1157, 1040, 824, 752 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 8.03 (1H, d, *J* = 16.0 Hz, H-7′), 7.69 (2H, d, *J* = 8.5 Hz, H-2′,6′), 7.03 (2H, d, *J* = 8.0 Hz, H-3′,5′), 6.75 (1H, d, *J* = 16.0 Hz, H-8′), 5.70 (1H, brs, H-12), 5.26 (1H, brd, *J* = 7.0 Hz, H-21), 5.20 (1H, d, *J* = 7.0 Hz, H-1″), 4.94 (1H, d, *J* = 8.0 Hz, H-1⁗), 4.90 (1H, d, *J* = 8.0 Hz, H-1″), 3.68 (3H, s, H-10′), 3.44 (1H, brd, H-18), 3.32 (1H, brd, H-3), 1.27 (6H, s, Me-23,30), 1.14 (3H, s, Me-26), 1.05 (6H, s, Me-24,29), 0.80 (3H, s, Me-25), 0.70 (1H, brd, *J* = 12.0 Hz, H-5), 0.34 (1H, brs, H-27α); ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 1055.5147 (calcd for $[C_{56}H_{80}O_{19} - H]^-$, 1055.5221).

Verbesinoside F (6): amorphous white powder; $[\alpha]^{25}_{D}$ +24.2 (*c* 0.05, MeOH); IR (KBr) ν_{max} ca. 3300 (weak), 2850, 1691, 1581, 1479, 1250, 1164, 1041, 754 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 8.03 (1H, d, *J* = 16.5 Hz, H-7′), 7.70 (2H, d, *J* = 7.0 Hz, H-3′,5′), 7.39 (3H, m, H-2′,4′,6′), 6.82 (1H, d, *J* = 16.5 Hz, H- 8′), 5.71 (1H, brs, H-12), 5.32 (1H, H-21, overlapped), 5.20 (1H, d, *J* = 7.5 Hz, H-1‴), 4.95 (1H, d, *J* = 8.0 Hz, H-1⁗), 4.91 (1H, d, *J* = 8.5 Hz, H-1″), 3.44 (1H, brd, H-18), 3.32 (1H, brd, *J* = 12 Hz, H-3), 1.47 (1H, m, H-9), 1.27 (6H, s, Me-23,30), 1.14 (3H, s, Me-26), 1.05 (6H, s, Me-24,29), 0.80 (3H, s, Me-25), 0.71 (1H, brd, *J* = 12.0 Hz, H-5), 0.34 (1H, brs, H-27α); ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 1049.5150 (calcd for $[C_{55}H_{78}O_{18} + Na]^+$, 1049.5080).

Acid Hydrolysis of 1

A solution of compound **1** (30 mg) in 2 N HCl/dioxane (1:1, 10 mL) was heated at 90 °C for 5 h. After cooling, the reaction mixture was diluted with H_2O (5 mL) and extracted with

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$CHCl_3$ (6 mL × 3). The $CHCl_3$ extract turned out to contain an inseparable complex mixture. The aqueous layer was neutralized by passing through an Amberlite MB-150 column eluting with H_2O. The eluent was concentrated to dryness to yield a sugar residue (5 mg). The sugar residue was analyzed by normal-phase silica gel TLC with the solvent system $CHCl_3$–MeOH–AcOH–H_2O (70:30:10:5) in comparison with standard samples. Glucose (R_f = 0.19) and xylose (R_f = 0.28) were detected in a ratio of 1:2. Determination of the absolute configuration of the sugars followed a recently reported procedure.[34] Briefly, the sugar residue (1 mg) and L-cysteine methyl ester (1 mg) was dissolved in pyridine (0.2 mL) and heated at 60 °C for 1 h, and then *o*-tolyl isothiocyanate (1 mg) was added to the mixture and heated at 60 °C for another 1 h. The reaction mixture (10 μL) was analyzed by analytical HPLC using gradient elution of 15 to 85% aqueous CH_3CN containing 0.1% acetic acid, at a flow rate of 1 mL/min, over a 35 min-run. D-Glucose (t_R 17.15 min) and D-xylose (t_R 17.90 min) were identified by comparing their retention times with those of authentic samples, while L-glucose and L-xylose showed different retention times at 16.90 and 17.62 min, respectively.

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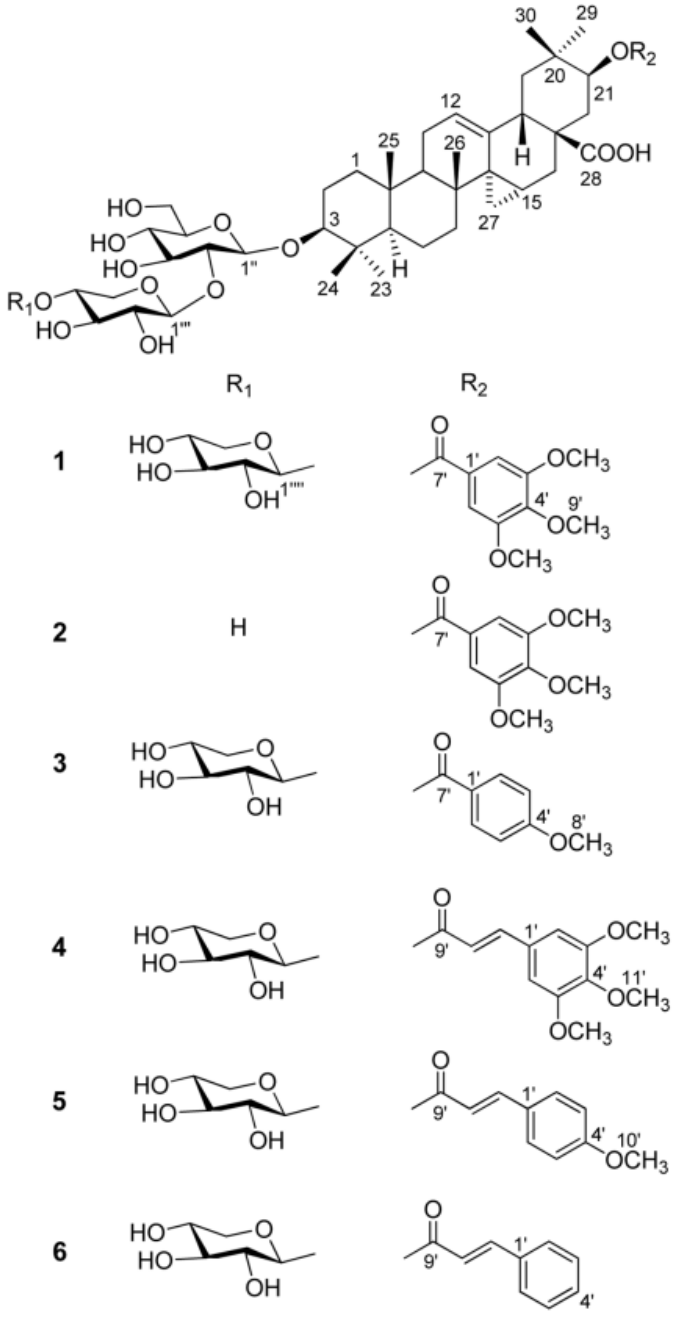

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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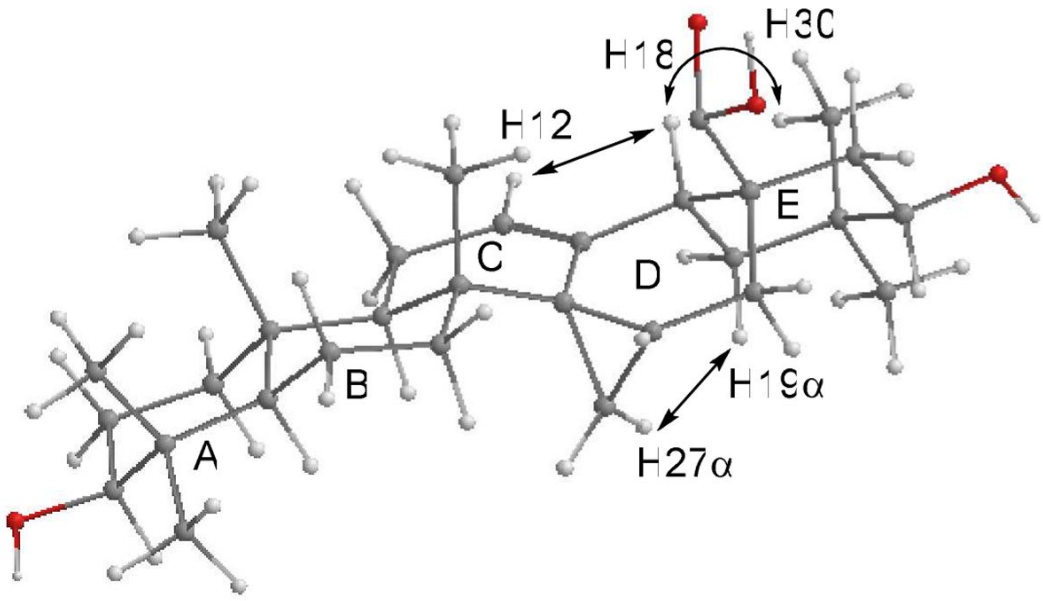

Figure 1.
Possible conformation of the aglycon of compound **1** generated by Chem3D Pro 11.0, showing key NOE correlations.

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Table 1

NMR Spectroscopic Data of **1** in pyridine-d_5 (δ, ppm)[a]

position	δ_C, mult.	δ_H (*J* in Hz)	HMBC	ROESY
aglycon-1	38.3, CH_2	0.9 β		
		1.4 α	C: 3, 25	
2	26.4, CH_2	1.76 β	C: 4	
		2.23 α		H: 3
3	88.9, CH	3.30, dd (12, 4.0)	C: 4, 23, 24	H: 2α, 5, 1″
4	39.3, qC			
5	55.7, CH	0.71, brd (11)	C: 4, 10, 24	H: 1α, 3, 9
6	18.0, CH_2	1.12		
		1.36		
7	32.7, CH_2	0.88 β	C: 26	
		1.02 α		H: 9
8	35.2, qC			
9	52.9, CH	1.47	C: 11, 14, 25, 26	H: 5, 7α, 11α
10	36.8, qC			
11	24.0, CH_2	2.11 β	C: 12	H: 25
		2.20 α	C: 13	H: 9
12	123.7, CH	5.71, s	C: 9, 14, 18	H: 11β, 18
13	137.8, qC			
14	32.8, qC			
15	15.2, CH	1.47	C: 14	
16	26.7, CH_2	2.03 α		
		2.85 β		
17	48.0, qC			
18	39.8, CH	3.44, brs		H: 12, 30
19	45.4, CH_2	1.47 β		H: 19α
		1.74 α	C: 29	H: 19β, 27α
20	35.7, qC			
21	76.6, CH	5.32, brd (7.0)	C: 30, 7′	
22	36.3, CH_2	2.11		
		2.25		
23	27.6, CH_3	1.25, s	C: 3, 4, 5, 24	
24	16.3, CH_3	1.02, s	C: 3, 4, 5, 23	
25	15.1, CH_3	0.80, s	C: 1, 5, 9, 10	
26	19.5, CH_3	1.12, s	C: 7, 8. 9, 14	
27	20.6, CH_2	0.34 α brs	C: 13, 15, 16	H: 19α, 27β
		1.36 β		H: 27α
28	178.7, qC			
29	28.8, CH_3	1.10, s	C: 19, 20, 21, 30	
30	18.9, CH_3	1.36, s	C: 19, 20, 21, 29	H: 18, 2′, 6′

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position	δ_C, mult.	δ_H (*J* in Hz)	HMBC	ROESY
Ar-1′	126.0, qC			
2′, 6′	107.3, CH	7.52, brs	C: 1′, 3′, 4′, 7′	H: 30, 8′, 10′
3′, 5′	153.4, qC			
4′	142.8, qC			
7′	165.7, qC			
8′, 10′	56.0, CH_3	3.85, s	C: 3′	
9′	60.5, CH_3	3.95, s	C: 4′	
Glc-1″	104.5, CH	4.84, d (7.0)	C: 3	H: 3, 2″, 5″
2″	83.9, CH	4.05	C: 4″, 1‴	H: 1‴
3″	78.1, CH	4.28	C: 2″, 4″	H: 1″
4″	71.1, CH	4.13	C: 6″	
5″	77.7, CH	3.85		H:1″
6″	62.4, CH_2	4.31	C: 4″	
		4.50	C: 5″	
Xyl-1‴	106.3, CH	5.13, d (7.0)	C: 2″, 5‴	H: 2″, 5‴
2‴	75.5, CH	4.13	C: 4‴	
3‴	76.0, CH	4.04	C: 1‴, 4‴	
4‴	77.6, CH	4.11	C: 2‴	
5‴	64.7, CH_2	3.58 α		H: 1‴
		4.45 β	C: 1‴, 3‴	
Xyl-1⁗	103.6, CH	4.87, d (7.0)	C: 4‴, 3⁗, 5⁗	H: 4‴, 3⁗, 5⁗
2⁗	73.6, CH	3.96	C: 1⁗	
3⁗	76.6, CH	4.06		H: 1⁗
4⁗	70.6, CH	4.11	C: 5⁗	
5⁗	67.1, CH_2	3.64 α	C: 1⁗	H: 1⁗
		4.26 β	C: 3⁗	

[a] Data recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. Assignments were based on DEPT and 2D NMR methods including DQF-COSY, HMQC, HMBC, and ROESY. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses. For overlapped signals, only chemical shift values are given. Some geminal protons were denoted with α or β orientation based on NOE evidence.

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Table 2

^{13}C NMR Spectroscopic Data of Compounds **2**–**6** in pyridine-d_5 (δ, ppm)[a]

position	2	3	4	5	6
aglycon					
1	38.3	38.3	38.3	38.3	38.3
2	26.4	26.4	26.4	26.4	26.4
3	88.8	88.8	88.8	88.8	88.9
4	39.4	39.3	39.3	39.4	39.4
5	55.6	55.3	55.6	55.6	55.5
6	18.0	18.0	18.0	18.0	18.1
7	32.7	32.7	32.7	32.7	32.7
8	35.2	35.2	35.2	35.2	35.2
9	52.9	52.9	52.9	52.9	52.8
10	36.8	36.8	36.8	36.8	36.8
11	24.1	24.1	24.1	24.1	24.0
12	123.5[b]	123.5[b]	123.5[b]	123.5[b]	123.5[b]
13	137.7	137.8	137.9	137.7	137.6
14	32.8	32.8	32.8	32.8	32.9
15	15.2	15.3	15.3	15.2	15.3
16	26.7	26.7	26.7	26.6	26.6
17	48.0	48.0	48.0	48.0	48.3
18	39.9	39.8	39.8	39.8	39.8
19	45.4	45.4	45.5	45.4	45.4
20	35.7	35.6	35.5	35.5	35.5
21	76.4	76.7	76.2	76.2	76.3
22	36.3	36.4	36.4	36.3	36.3
23	27.7	27.6	27.7	27.7	27.7
24	16.3	16.3	16.3	16.3	16.4
25	15.1	15.1	15.1	15.1	15.1
26	19.5	19.5	19.5	19.5	19.5
27	20.7	20.6	20.6	20.6	20.6
28	_[c]	178.8	_[c]	178.3	_[c]

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position	2	3	4	5	6
29	28.8	28.8	28.8	28.7	28.7
30	19.0	19.0	18.9	18.9	18.8
Ar					
1′	126.0	123.5[b]	130.5	127.3	135.8[d]
2′, 6′	107.3	131.8	106.1	130.2	128.6
3′, 5′	153.5	114.1	153.9	114.7	129.5
4′	142.9	163.7	140.7	162.0	130.4
7′	165.7	165.8	145.0	144.9	144.9
8′	55.9	55.6	118.2	116.6	118.8
9′	60.5		166.7	166.6	166.1
10′	55.9		56.0	55.2	
11′			60.5		
12′			56.0		
Glc					
1″	104.8	104.5	104.6	104.6	104.6
2″	83.7	83.9	84.2	84.2	84.2
3″	78.7	78.1	78.2	78.3	78.3
4″	71.3	71.1	71.2	71.2	71.2
5″	77.9	77.7	77.9	77.9	78.0
6″	62.5	62.4	62.5	62.5	62.5
Xyl					
1‴	106.7	106.3	106.6	106.6	106.6
2‴	76.6	75.5	75.6	75.4	75.6
3‴	78.0	76.0	75.9	75.7	76.2
4‴	70.9	77.6	77.8	77.9	77.9
5‴	67.3	64.7	64.8	64.8	64.9
Xyl					
1‴′		103.6	103.7	103.8	103.8
2‴′		73.6	73.6	73.7	73.7
3‴′		76.6	76.7	76.7	76.7
4‴′		70.6	70.7	70.8	70.8

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position	2	3	4	5	6
5‴′		67.1	67.2	67.3	67.2

[a] Data recorded at 100 MHz for **2** and **4** and 125 MHz for **3**, **5**, and **6**. Assignments were based on comparison with compound **1**.

[b] Overlapped with the pyridine-d_5 signals at δ 123.5.

[c] Low intensities or not evident.

[d] Overlapped with the pyridine-d_5 signals at δ 138.8.