Two New Triterpenoid Glycosides Isolated from Aesculus assamica Griff

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Phytochemical study of the ethanol extract of the seeds of Aesculus assamica led to the isolation of two new triterpenoid saponins. The structure of the new compounds were elucidated on the basis of spectral data to be 28-O-acetyl-21-O-(4-O-angeloyl)-6-deoxy-β-glucopyranosyl-3-O-[β-glucopyranosyl(1—2)-O-[β-glucopyranosyl(1—4)]-β-glucuronopyranosyl]protoaesigenin (1), and 21-O-(4-O-angeloyl)-6-deoxy-β-glucopyranosyl-3-O-[β-glucopyranosyl(1—2)-O-[β-glucopyranosyl(1—4)]-β-glucuronopyranosyl]protoaesigenin (2). Their in vitro bioactivity against plant pathogenic fungus Pyricularia oryzae and cytotoxicity against K562 and HCT-15 cell lines were evaluated.

Key words triterpenoid glycoside; Aesculus assamica; anti-fungal activity; cytotoxicity

The plants of Aesculus genus (Family Hippocastanaceae) have attracted much interests of phytochemists for many years due to the discovery of acylated triterpenoid saponins—escins with extraordinary anti-inflammatory activity from horse chestnut (Aesculus hippocastanum L.) seeds and the escins-like compounds used as herbal medicines for the treatment of distention and pain in the chest and abdomen. A number of escins-like saponins have been isolated from plants of this genus,1—11) and of which various biological activities have been studied and reported, such as anti-inflammatory, hypoglycemic activities, inhibitory effects on ethanol absorption, as well as anti-HIV-1 protease activities. During our search for a substitute in China, we did physicochemical investigation on the seeds of A. assamica Griff, a plant distributed in Yunnan Province of China. Two novel triterpenoid saponins with insulin-like activity, termed assamicins I and II, have been isolated from A. assamica.12) Herein, we describe the isolation, structure determination, and bioactivities of two new triterpenoid saponins, designated as assaminic III and IV.

Results and Discussion

The 95% EtOH extracts of A. assamica were separated as described in Experimental to yield compounds 1 and 2, 28-O-acetyl-21-O-(4-O-angeloyl)-6-deoxy-β-glucopyranosyl-3-O-[β-glucopyranosyl(1—2)-O-[β-glucopyranosyl(1—4)]-β-glucuronopyranosyl]protoaesigenin (1) and 21-O-(4-O-angeloyl)-6-deoxy-β-glucopyranosyl-3-O-[β-glucopyranosyl(1—2)-O-[β-glucopyranosyl(1—4)]-β-glucuronopyranosyl]protoaesigenin (2) (Fig. 1).

Compound 1 was isolated as a white amorphous powder. Its IR spectrum showed absorption bands due to hydroxyl, carbonyl, ester, and olefin functions at 3409, 1724, 1720, 1678, 1654 and 1648 cm−1, and two broad absorption bands at 3409 and 1049 cm−1 suggested the possible existence of a glycosidic structure. High resolution FAB-MS revealed the molecular formula of 1 to be C61H90O26. In the positive ESI-MS of 1, quasimolecular ion peak was observed at m/z 1299 [M+Na]⁺. The positive-ion ESI-MS spectrum of m/z 1299 gave ions at m/z 1137 [M+Na−60−162]⁺, 1077 [M+Na−60−162−162]⁺, 739 [M+Na−60−162−162−176]⁺, 511 [M+Na−60−162−162−176−228]⁺, indicating the sequential losses of hexose units. The 1H- and 13C-NMR signals of compound 1 were assigned by 2D-NMR methods. The further acid hydrolysis of the deacylated saponin obtained was too little to get the good NMR data, it was only analyzed by ESI-MS/MS spectrum (1157 [M+Na]⁺, 995 [M+Na−162]⁺, 833 [M+Na−60−162−162−176]⁺, 511 [M+Na−60−162−162−162−176−146]⁺). The further acid hydrolysis of the deacylated saponin liberated aglycone and sugars. 6-Deoxy-glucose, glucose and glucuronic acid were identified by paper chromatography and GC in comparison with authentic samples. The mixture of aglycones including protoaesigenin were detected by TLC with different solvents.

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together with HMBC correlation observed between CH$_3$-6 and C-4 indicated the presence of 6-deoxy-β-glucose, which was also confirmed by comparison with NMR data of assamicin II. The position of sugar moiety at C-3 and C-21 and interlinking among sugar moiety were deduced from HMBC experiments, in which H-1, H-1", H-1", and H-1" were correlated with C-3 (90.8), C-2' (78.4), C-4' (82.0), and C-21 (90.8). The position of acetyl and angeloyl groups at C-28 and C-4 were also deduced from HMBC spectrum as indicated in Fig. 2. The relative stereochemistry of 1 was confirmed to be the same as that of assamicin I and II by NOESY experiment. In its NOESY spectrum, Me-29 showed a strong correlation with H-21, while Me-30 correlated strongly with H-22 at 4.60 (d, J = 9.6 Hz). These indicated the α configuration of H-21 and β configuration of H-22. The coupling constant of H-21 and H-22 (J = 10.0 Hz) also supported the proposed structure. On the basis of the above evidence, the structure of compound 1 was determined to be 28-O-acetyl-21-O-(4-O-angeloyl)-6-deoxy-β-glucopyranosyl-3-O-[β-glucopyranosyl(1→2)-O-[β-glucopyranosyl(1→4)]-β-glucuronopyranosyl]protoaescigenin, and designated as assamicin III.

Compound 2 was isolated as amorphous powder. The molecular formula, C$_{59}$H$_{94}$O$_{27}$, was determined on the basis of the HR-FAB-MS spectrum. In the positive ESI-MS of 1, quasi-molecular ion peak was observed at m/z 1257 [M+Na]$^+$. The positive-ion ESI-MS$^2$ spectrum of m/z 1257 gave ions at m/z 1095 [M+Na−162]$^+$. 793 [M+Na−162−162]$^+$. 757 [M+Na−162−162−176]$^+$. 529 [M+Na−162−162−176−228]$^+$. indicating the sequential losses of hexose units. The IR, $^1$H- and $^13$C-NMR spectrum of 2 showed very similarity to that of 1, except for the loss of acetyl group at C-28. In summary, the characteristic signals of protoaescigenin skeleton including five methyl groups at δ [0.66 (Me-25), 0.82 (Me-26), 1.24 (Me-23), 1.51 (Me-29), 1.35 (Me-30) and a broad signal at δ 5.37 (H-12)], four anomeric protons of sugar moieties at δ [4.88 (d, J = 8.0 Hz, H-1'), 5.51 (d, J = 7.6 Hz, H-1''), 5.18 (d, J = 7.6 Hz, H-1'''), and 4.86 (d, J = 8.0 Hz, H-1''''), and a angeloyl signals at δ [1.89 (d, 3H, J = 1.6 Hz, Me-3'''), 1.99 (dd, 3H, J = 1.6, 7.2 Hz, Me-5'''), and 5.93 (dq, J = 1.6, 7.2 Hz, H-4''')] were observed in its $^1$H-NMR spectrum, and so was their corresponding carbon signals in the $^13$C-NMR spectrum of 2. The whole structure of 2 was established from HMBC analysis, and signal assignments were accomplished in combination of COSY, TOCSY, HMBC, and NOESY spectral analysis. Compound 2 possess the same stereochemistry as that of 1, which can be deduced from NOESY experiments. Based on the above analysis and comparison with the NMR data of 1, compound 2 was identified as 21-O-(4-O-angeloyl)-6-deoxy-β-glucopy-
1H-NMR, along with 2D NMR spectra were obtained on a spectrometer. ESI-MS was conducted using Bruker esquire 2000 mass spectrometer. Negative-ESI-MS (m/z): 1275 [M–H]−. 1R (KBr) cm−1: 3409, 2947, 1724, 1720, 1678, 1654, 1648, 1380, 1238, 1049. 1H-NMR (pyridine-d5): δ: 3.29 (1H, m, H-3), 5.38 (1H, br s, H-6), 9.31 (1H, m, H-16), 2.96 (2H, d, J=10.8 Hz, H-18), 4.17 (1H, m, H-23), 4.6 (1H, d, J=9.6 Hz, H-22), 1.24 (3H, s, H-29), 3.29 (1H, m, H-24), 4.22 (1H, m, H-24), 0.63 (3H, s, H-25), 0.82 (3H, s, H-25), 1.48 (2H, d, J=4.0, 11.6 Hz, H-28), 3.79 (1H, d, J=9.6 Hz, H-27), 3.90 (dq, J=6.9, 6.9 Hz, H-5), 3.22 (1H, m, H-24), 1.92 (3H, s, H-29), 0.20 (3H, s, H-25), 0.94 (3H, m, H-24). The product was redissolved in H2O (1 ml) and treated with 20% aqueous H2SO4 (1 ml). The mixture was heated under reflux for 4 h, then neutralized with saturated NaHCO3 and extracted three times with EtOAc. TLC (CHCl3: MeOH, 95:5; Rf=0.35) analysis showed the presence of protoaescigenin in both 1 and 2 by comparison with authentic protoaescigenin.

The water layer was then condensed and divided into two parts. One part was subjected to paper chromatography and GC analysis. Glucuronic acid (Rf=0.66), glucose (Rf=0.19), and deoxy-glucose (Rf=0.40) were detected by paper chromatography [n-BuOH:EtOH:H2O:conc. NH4OH, 45:5:49:1]. For GC analysis, the residue was trimethylsilylated with hexamethyldisilazane and trimethylchlorosilane in a 2:1 ratio at room temperature. Alkaline and Acid Hydrolysis of Saponins A solution of saponin (1 and 2, 4 mg each) in 10% aqueous KOH−50% aqueous dioxane (1:1, v/v, 2 ml) was stirred at 37°C for 1 h. The reaction mixture was neutralized with 10% acetic acid. After removal of the solvent from the filtrate under reduced pressure, the residue was dissolved in H2O (5 ml) and extracted with EtOAc: BuOH (1:1). The organic solvents were evaporated and purified by preparative TLC on silica gel (CHCl3: MeOH: H2O, 70:30:5) to give the deoxy saponin (about 1 mg for 1 and 2). The product was redissolved in H2O (1 ml) and treated with 20% aqueous H2SO4 (1 ml). The mixture was heated under reflux for 4 h, then neutralized with saturated NaHCO3 and extracted three times with EtOAc. TLC (CHCl3: MeOH, 95:5; Rf=0.35) analysis showed the presence of protoaescigenin in both 1 and 2 by comparison with authentic protoaescigenin.

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