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Cytotoxic Triterpenoid Saponins of *Albizia gummifera* from the Madagascar Rain Forest[‖]

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Abstract

Bioassay-guided fractionation of an EtOH extract obtained from the roots of the Madagascan plant *Albizia gummifera* led to the isolation of three new cytotoxic oleanane-type triterpenoid saponins, gummiferaosides A-C (**1**-**3**). The structures of these new compounds were elucidated using 1D and 2D NMR experiments and mass spectrometry. Compounds **1**-**3** showed cytotoxicity against the A2780 human ovarian cancer cell line with IC_{50} values of 0.8, 1.5, and 0.6 μg/mL, respectively.

In our continuing search for bioactive molecules from the Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[1] we obtained an extract of the roots of *Albizia gummifera* (J. F. Gmel.) C. A. Sm. var. *gummifera* (Fabaceae). This extract, designated MG 1012, showed reproducible cytotoxicity to the A2780 ovarian cancer cell line, with an IC_{50} value of 7.2 μg/mL. The extract was selected for bioassay-guided fractionation based on this activity.

The genus *Albizia* comprises about 150 species widely distributed in the tropics, with the greatest diversity in Africa and South America.[2] Alkaloids,[3] flavonoids,[4] sterols,[5] and triterpenoid saponins[6-11] have been isolated from *Albizia* species, and *Albizia gummifera* in particular has been studied for its alkaloids[3a,3c] and triterpenoids.[5,9] It has been reported that alkaloids from *Albizia adinocephala* inhibit plasmepsin II, an aspartyl proteinase crucial to the survival of the malaria parasite.[3c] Albiziasaponin B from *Albizia myriophylla* was found to show a potent sweetness intensity relative to sucrose.[6b] Some triterponoid saponins from *Albizia* species exhibited in vitro cytotoxicity against various cancer cell lines.[2,6a,7,8]

In this paper, we report the isolation, structure elucidation, and cytotoxicity of three new bioactive triterpenoid saponins (**1**-**3**) obtained from the roots of *Albizia gummifera*.

Results and Discussion

Liquid-liquid partitioning of a portion of an EtOH extract of the roots of *Albizia gummifera* into hexane, CH_2Cl_2 and aqueous MeOH fractions indicated that the aqueous MeOH fraction

[‖]Dedicated to the late Dr. Kenneth Rinehart of the University of Illinois at Urbana-Champaign for his pioneering work on bioactive natural products.

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(1 g) was the most active fraction, with an IC_{50} value of < 6.25 μg/mL (the lowest concentration tested). Purification of the aqueous MeOH fraction using a C_{18} open column, followed by preparative HPLC on a phenyl bonded column, and final purification by HPLC on an analytical size C_8 bonded column, led to the isolation of compounds **1**-**3**.

Compound **1** was obtained as a white solid. Its HRFABMS (positive-ion mode) exhibited a quasimolecular ion peak at *m/z* 2177.9998, consistent with a molecular composition of $C_{102}H_{162}O_{48}Na$ (calcd for $C_{102}H_{162}O_{48}Na^+$, 2178.0128).[12] The aglycon of **1** (fragment I) was identified as acacic acid by analysis of ^1H and ^{13}C NMR spectra (Tables 1 and 2) and from observation of connectivities in the COSY, TOCSY, ROESY, HSQC, and HMBC NMR spectra. The NMR spectra indicated the presence of one trisubstituted double bond (12-position) and three oxygenated methines (3-, 16-, and 21-positions) in the oleanane-type aglycon of **1**. Out of the seven methyl groups in the aglycon, only H_3-27 (δ_H 1.42, s) exhibited a ^3J HMBC correlation to C-13 (δ_C 143.7), which confirmed the location of the double bond. In the HMBC spectrum of **1**, H_3-23 (δ_H 1.09, s) and H_3-24 (δ_H 0.86, s) showed correlations to C-3 (δ_C 90.3). The H_2-22 (δ_H 1.67 and δ_H 2.07) signals correlated to both C-16 (δ_C 74.1) and C-21 (δ_C 78.6), while H_2-22, H_3-29 (δ_H 0.85, s), and H_3-30 (δ_H 1.03, s) exhibited ^2J, ^3J, and ^3J HMBC correlations to C-21, respectively. ROESY correlations between H_3-23 and H-3 (δ_H 3.33), and H_3-25 (δ_H 0.96, s) and H_3-24 revealed that H-3 has an α-axial orientation. In turn, H-16 (δ_H 4.45, dd, J = 5.0 and 5.0 Hz) of **1** was assigned as a β-equatorial proton from its coupling constants. In corroboration of this assignment, H-16α of the 16β-oxygenated triterpenoid gymnemagenin is a doublet of doublets with coupling constants of 11.5 and 5.0 Hz, respectively. [13] The orientation of H-21 (δ_H 5.43, dd, J = 10.8 and 5.5 Hz) was determined as α-axial, which was confirmed by a ROESY correlation between H_3-29 and H-21. Further, the NMR data of fragment I of **1** were in full agreement with those reported in the literature for acacic acid, supporting an acacic acid aglycon.[10,14]

Analysis of the ^1H, ^{13}C NMR, and HSQC spectra of **1** indicated the presence of nine sugar units and additional unsaturated ester units in three fragments designated II, III, and IV. 1D TOCSY spectra were initially obtained in CD_3OD to elucidate the structures of these fragments, but overlapping signals in the ^1H NMR spectrum prevented complete spectroscopic interpretation. The use of C_5D_5N containing three drops of CD_3OD as the NMR solvent was found to reduce the overlap problem, and the following discussion is based on the NMR data collected in this mixed solvent system (see Experimental Section for ^1H NMR data, and Table 2 for ^{13}C NMR data).

Protons H-21, H-MT-3 (δ_H 6.87, t, J = 7.3 Hz) and H-MT-9 (δ_H 1.88, s) of **1** all showed HMBC correlations to C-MT-1 (δ_C 168.1) (Figure 1), indicating that C-21 is esterified. The HMBC correlations between H-MT-10 (δ_H 1.54, s) and C-MT-5/C-MT-6/C-MT-7 (δ_C 40.7/80.1/144.4), and the COSY correlations between H_2-MT-4 (δ_H 2.40, m) and H-MT-3/ H_2-MT-5 (δ_H 6.87, t, J = 7.3 Hz/1.74, m), and between H-MT-7 (δ_H 6.21, m) and H_2-MT-8 (δ_H 5.44, br d, J = 15.8 Hz; δ_H 5.27, br d, J = 11.0 Hz) indicated that the ester unit is a 6-*O*-2,6-dimethylocta-2,7-dienoyl monoterpenoid moiety.[10] The trisubstituted double bond in the inner monoterpenyl unit was assigned the *E* configuration, as evidenced by a ROESY correlation between H_3-MT-9 and H_2-MT-4.

The H-Qui-1 proton (δ_H 4.84, d, J = 8 Hz) showed a ^3J HMBC correlation to C-MT-6 (δ_C 80.1), establishing the connectivity from the 6-position of the inner monoterpenyl moiety to the anomeric position of the quinovopyranose unit. The spin system from the anomeric proton to the other protons of the inner quinovopyranose was clearly exhibited in a 1D TOCSY spectrum [H-Qui-1 (selected): δ_H 4.84, d, J = 8.0 Hz; H-Qui-2: δ_H 3.99, d, J = 8.0, 8.8 Hz; H-Qui-3: δ_H 4.17, dd, J = 8.8, 8.8 Hz; H-Qui-4: δ_H 5.32, dd, J = 8.8, 9.2 Hz; H-Qui-5: δ_H 3.66, m; H-Qui-6: δ_H 1.34, d, J = 6.0 Hz] and the HSQC-TOCSY spectrum (correlations from H-

Qui-1 to C-Qui-1–6: C-Qui-1, δ_C 99.6; C-Qui-2, δ_C 75.8; C-Qui-3, δ_C 75.8; C-Qui-4, δ_C 77.5; C-Qui-5, δ_C 70.5; C-Qui-6, δ_C 19.1) of **1**.

The outer monoterpenoid moiety (MT′) and outer quinovopyranosyl unit (Q′) of **1** were determined of being identical to the corresponding inner ones by the same methods. The HMBC correlations from H-Qui-4 to C-MT′-1, and H-Qui′-1 to C-MT′-6 established the connectivities from the 4-position of the inner quinovopyranose to the 1-position of the outer monoterpenyl moiety, and from the 6-position of the outer monoterpenyl moiety to the anomeric position of the outer quinovopyranosyl unit. H-Qui-1 and H-Qui′-1 showed ROESY correlations to H-MT-10 and H-MT′-10, respectively. The ^{13}C NMR chemical shifts of C-MT-5/C-MT′-5 and C-MT-10/C-MT′-10 of compound **1** were nearly identical to those of C-MT-5 (δ_C 41.3) and C-MT-10 (δ_C 23.8) of the related compound kinmoonoside B, which has the *S* configuration at the C-MT-6 and C-MT′-6 position.[14a] In contrast, these chemical shifts were different from those of C-MT-5 (δ_C 39.5) and C-MT-10 (δ_C 24.5) of kinmoonoside A, which has the *R* configuration at the C-MT-6 and C-MT′-6 position.[14a] These facts indicated that compound **1** has the *S* stereochemistry at the 6-positions of the monoterpenoid moieties.[14a] These observations were used to establish the structure of fragment III.[10]

Starting from the anomeric and/or the sixth protons of each of the other seven sugar units, all the protons within each spin system of **1** were assigned using COSY NMR spectra with the aid of ROESY, 1D and 2D TOCSY spectra. The ^{13}C NMR resonances of each of these seven sugar units were assigned by HSQC-TOCSY, HSQC, and HMBC spectra. One of the seven sugars was found to be a β-fucopyranosyl unit, as indicated by the presence of a methyl group at δ_H 1.48 (H_3-F-6, d, *J* = 6.0 Hz). The coupling constants of H-Fuc-1 (δ_H 4.94, d, *J* = 7.8 Hz), H-Fuc-2 (δ_H 4.40, dd, *J* = 7.8, 9.6 Hz) and H-Fuc-3 (δ_H 4.11, dd, *J* = 4.0, 9.6 Hz) indicated axial positions for these three protons. The proton signal of H-Fuc-4 (δ_H 4.00) was broad, indicating an α-equatorial orientation. An α-axial position for H-Fuc-5 (δ_H 3.75, m) was required by the ROESY correlations between H-Fuc-1 and H-Fuc-3/H-Fuc-5. The carbon signal at δ_C 82.6 assigned to C-Fuc-2 suggested that it was a glycosidic linkage site for another sugar.[15] The second of the seven sugars was identified as an α-rhamnopyranosyl unit. The proton signals of both H-Rha-1 (δ_H 6.36) and H-Rha-2 (δ_H 4.78) had small coupling constants, suggesting that both were equatorial. The coupling patterns of H-Rha-3 (δ_H 4.72, dd, *J* = 3.6, 8.8 Hz), H-Rha-4 (δ_H 4.43, dd, *J* = 8.8, 9.2 Hz), H-Rha-5 (δ_H 4.54, m), and H-Rha-6 (δ_H 1.74, d, *J* = 6.5 Hz) indicated a rhamnopyranosyl unit. The downfield chemical shift of C-Rha-4 (δ_C 83.4) indicated its connectivity to another anomeric position.[16] Two of the seven sugars were identified as β-xylopyranosyl units, as evidenced by their proton and carbon chemical shifts [Xyl-1–5 (δ_H/δ_C): 5.03/107.3, 4.05/76.3, 4.05/78.1, 4.05/71.0, 3.58 and 4.45/67.6; Xyl ′-1–5 (δ_H/δ_C): 5.25/106.7, 3.86-4.20/76.5, 3.86-4.20/78.7, 3.86-4.20/71.2, 3.47 and 4.23/67.5 (Experimental Section and Table 2)]. The ^{13}C NMR chemical shifts of these two xylopyranosyl units were in good agreement with literature data.[17] The final three sugars were found to be β–glucopyranosyl units. The unit at the 3-position of the aglycon was determined to be a β–glucopyranosyl moiety, the chemical shifts [C-Glc-1–6 (δ_H/δ_C): 4.87, d, *J* = 7.6 Hz/105.3; 4.00, dd, *J* = 7.6, 9.0 Hz/83.2; 4.12, dd, *J* = 9.0, 9.0 Hz/78.5; 4.50/71.8; 4.25/77.0; 4.40 and 4.60/70.2 of which were assigned by 1D and 2D NMR spectra (Experimental Section and Table 2)]. The axial orientations of H-Glc-3 and H-Glc-5 were determined by the observation of ROESY correlations between H-Glc-1 and H-Glc-3/H-Glc-5. The significant downfield signals for C-Glc-2 and C-Glc-6 indicated that they attached to two other sugars.[18] The coupling patterns of H-Glc′-1 (δ_H 5.35, d, *J* = 7.6 Hz) and H-Glc′-2 (δ_H 4.08, dd, *J* = 7.6, 8.4 Hz), and the ROESY correlations between H-Glc′-1 and H-Glc′-3/H-Glc′-5 (δ_H 4.19/3.90, br d, *J* = 8.4 Hz) indicated a β–glucopyranosyl unit, which was supported by a set of typical carbon chemical shifts for this unit [C-Glc′-1–6 (δ_C): 106.3, 76.1, 77.5, 72.3, 78.5, 62.8 (Table 2)].[17] Similar to the previously described β–glucopyranosyl units, the chemical shifts of a third β–glucopyranosyl unit [Glc″-1–6 (δ_H/δ_C): 6.11/95.5, 4.23/76.7, 4.23 or 3.96/79.4, 4.23 or 3.96/71.4, 4.23 or

3.96/79.4, 4.23/62.1 (Experimental Section and Table 2)] matched literature values, especially the ^{13}C NMR chemical shifts.[19]

The connectivities of these seven glycosidic units in fragments II and IV were determined by analysis of HMBC and ROESY experiments (Figure 1). The anomeric proton of Glc-1 showed ROESY and HMBC correlations to H-3 and C-3, indicating a linkage of this β–glucopyranosyl unit to the 3-position of the aglycon. H-Glc′-1 correlated to C-Glc-2 in the HMBC spectrum, and H-Xyl-1 and H-Fuc-1 showed an HMBC and a ROESY correlation to C-Fuc-2 and H-Glc-6, respectively. The structure of fragment II was thus assigned as shown.[10]

Both H-18 and H-Glc″-1 exhibited ^{3}J HMBC correlations to C-28 (δ_C 175.0), suggesting that position-28 is esterified. The interglycosidic correlations of Glc″, Xyl′, and Rha were evident from the ROESY [H-Rha-1 to H-Glc″-2] and HMBC [H-Xyl′-1 to C-Rha-4] cross-peaks. Hence, fragment IV[20] and thus the final structure of **1** were determined as shown.

Compound **1** is structurally related to similar complex saponins with monoterpenoid esters at C-21 such as the julibrosides from *Albizia julibrissin*. Three close analogs are julibrosides I, II and J_{14},[7,10] which differ from **1** in the nature and position of some of the sugar units. Similar compounds were also isolated from other genera of the legume family, for example the avicins from *Acacia victoriae* Benth.[13,14] and the elliptosides from *Archidendron ellipticum* (Blume) I. C. Nielsen,[21] all of which share the same acacic acid aglycon with monoterpenoid glycosides at the 21-position and oligosaccharides at the 3- and 28-positions.

Compound **2** was obtained as a white solid. Comparison of the NMR data (Tables 1 and 2) of **1** and **2** in CD_3OD indicated that the xylopyranosyl unit at the 2-position of the fucopyranosyl unit in fragment II of **1** was replaced by an arabinopyranosyl residue in **2**, while fragments I, III, and IV of **2** were the same as those of **1**. The arabinopyranosyl residue was attached to the 2-position of the fucopyranosyl unit, as deduced from an HMBC correlation between H-Ara-1 (δ_H 4.55) and C-Fuc-2 (δ_C 81.2), and the ^{13}C NMR chemical shifts of the arabinopyranosyl residue [C-Ara-1–6 (δ_C): 106.1, 71.6, 73.1, 70.0, 66.3 (Tables 1 and 2)] were in good agreement with the literature data,[11] suggesting that fragment II of **2** is A^{1}-$^{2}F^{1}$-$^{6}G(^{1}$-^{3}aglycon$)^{2}$-$^{1}G'$. Therefore, the structure of **2** was determined as shown.

Compound **3** was also isolated as a white solid, and its aglycon was shown to be the same as that of **1** by comparison of NMR spectra. The major difference between compounds **1** and **3** was the presence of an extra monoterpenoid moiety in **3**, as was evidenced by its mass spectrum and the HSQC spectrum. There were nine sugar units assigned in **3**, including three quinovopyranoses, three glucopyranoses, one rhamnopyranose, one fucopyranose, and one xylopyranose. Fragments I and IV of **3** were the same as those of **1**, as indicated by a comparison of their NMR spectra. There were only three sugars in fragment II [F^{1}-$^{6}G(^{1}$-^{3}aglycon$)^{2}$-$^{1}G'$][17] of **3** (Tables 1 and 2), as opposed to four in **1**, but these three were the same as three of the four sugars in **1**, and so this unit was identified by a comparison of NMR spectra. Protons H-Q′-4 and H-Q″-1 showed HMBC correlations to the carbons C-MT″-1 and C-MT″-6, respectively, of the additional monoterpenoid unit, indicating that fragment III is (aglycon)– $^{1}MT^{6}$-$^{1}Q^{4}$-$^{1}MT'^{6}$-$^{1}Q'^{4}$-$^{1}MT''^{6}$-$^{1}Q''$. The connectivities of these units were determined by the same methodology used in the structure elucidation of **1**. The structure of **3** was thus determined as shown.

Some acacic acid-type saponins have been evaluated for their cytotoxicity. It was reported that the monoterpene-quinovopyranosyl moiety at C-21 and the oligosaccharide ester at C-28 of the acacic acid-type aglycon are crucial substituents required for the cytotoxicity of julibroside III and prosapogenins 8-10 against KB cells, and their hydroxyl group at C-16 may also be important for the cytotoxicity.[6a] Neither monodesmonoterpenyl elliptoside A nor any of the anatoliosides A-E (monoterpene glycosides) produced distinctive cytotoxicity in the NCI 60-

cell line screen, which supported the apparent requirement for both the terminal monoterpenoid unit and the acacic acid portion of such active molecules.[14, 21] It was reported that the trisaccharide unit at C-3 of kinmoonosides A-C were not crucial for their cytotoxicity,[14a] but oligosaccharides at 3-positions may intensify the cytotoxicity of acacic acid derivatives. The apoptotic properties of avicins from *Acacia victoriae* have been studied by Gutterman et al.[22] The same group also reported the thioesterification of avicins by a thioester linkage between Cys-199 of OxyR and the outer monoterpene side chain; such derivatization can induce an adaptive response that protects cells against oxidative or nitrosative stress.[23] In a cytotoxicity assay using the A2780 human ovarian cancer cell line, compounds **1**-**3** showed cytotoxicity with IC_{50} values of 0.8, 1.5, and 0.6 μg/mL, respectively. Compounds **1**-**3** possess structural features essential for cytotoxicity, similar to the cytotoxic julibrosides, prosapogenins, elliptoside, and avicins, mentioned above.

Experimental Section

General Experimental Procedures

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 for ^{1}H, ^{13}C, HMQC, and HMBC and an INOVA 400 spectrometer for TOCSY, COSY, ROESY, and HSQC-TOCSY. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on a JEOL JMS-HX-110 instrument, in the positive-ion mode. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative C_8 Varian Dynamax column (5 μm, 250 × 10 mm) and a preparative phenyl Varian Dynamax column (8 μm, 250 × 21.4 mm).

Cytotoxicity Bioassays

Cytotoxicity measurements were performed at Virginia Polytechnic Institute and State University against the A2780 ovarian cancer cell line, as described previously. The A2780 cell line is a drug-sensitive human ovarian cancer cell line.[24]

Plant Material

Roots of *Albizia gummifera* (J.F. Gmel.) C.A. Sm. var. *gummifera* (Fabaceae) were collected in November 2001 as collection RFA 579. The collection was made by Fidy Ratovoson et al., 3 km northwest of the village of Nosivola. The plant was growing in a dense humid forest adjacent to Zahamena National Park, in Toamasina Province, Madagascar (17. 41 01S; 48. 38. 28E, elevation 900 m). The specimen accessed was a small tree 9 m in height and trunk diameter 14 cm, with pale green sepals, white petals, and 10 dark red stamens. The vernacular name of this species in this area is "*volomborona*". Duplicate voucher specimens were deposited at Centre National d'Application des Recherches Pharmaceutiques (CNARP) and the Departement des Recherches Forestieres et Piscicoles Herbarium in Antananarivo, Madagascar (TEF), at Missouri Botanical Garden, St. Louis, Missouri (MO), and the Museum National d'Histoire Naturelle in Paris, France (P).

Extraction and Isolation

Dried roots of *A. gummifera* (430.9 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 1012 (10.14 g), of which 7.44 g was shipped to Virginia Polytechnic Institute and State University (VPISU) in triplicate vials for distribution to Eisai Research Institute (2.79 g), Dow AgroSciences (2.19 g) and VPISU (2.46 g). Extract MG 1012 (1.49 g, IC_{50} 7.2 μg/mL) was suspended in aqueous MeOH (MeOH-H_2O, 9:1, 100 mL) and extracted with hexanes (3 × 100 mL portions). The aqueous layer was

then diluted to 70% MeOH with H_2O and extracted with CH_2Cl_2 (3 × 100 mL portions). The aqueous MeOH extract (1 g) was active with an IC_{50} less than 6.25 μg/mL, while both the hexane and CH_2Cl_2 extracts were inactive. The aqueous MeOH fraction was chromatographed on an open C_{18} column (130 × 22 mm) using H_2O-MeCN (80:20 to 40:60, then 0:100) to yield the three fractions A [296 mg (polar, inactive)], B [516 mg, IC_{50} less than 6.25 μg/mL], and C [73 mg, nonpolar, inactive]. Fraction B furnished 15 subfractions after HPLC separation on a phenyl-bonded column (35% MeOH/H_2O, 10 mL/min). HPLC of subfraction 4 (28 mg) on a C_8 bonded phase column eluted with 70% MeOH/H_2O (2 mL/min) yielded compounds **1** (t_R 30 min, 6 mg) and **2** (t_R 34 min, 3 mg). Compound **3** (t_R 37 min, 3 mg) was obtained by HPLC of subfraction 14 (18 mg) also using C_8 HPLC (72% MeOH/H_2O, 2 mL/min).

Gummiferaoside A (1): white solid; $[\alpha]^{26}_D$ -15 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.6) nm; IR (film) ν_{max} 3339, 2945, 2833, 1744, 1730, 1432, 1364, 1343, 1304, 1253, 1200, 1153, 1076, 1009, 1025 cm^{-1}; ^1H NMR (500 MHz, CD_3OD), see Table 1; ^{13}C NMR (125 MHz, CD_3OD and C_5D_5N with three drops of CD_3OD), see Table 2; ^1H NMR (500 MHz, C_5D_5N with three drops of CD_3OD): 7.02 (1H, t, *J* = 7.5 Hz, H-MT′-3), 6.87 (1H, t, *J* = 7.3 Hz, H-MT-3), 6.36 (1H, br s, H-R-1), 6.21 (1H, m, H-MT-7), 6.21 (1H, m, H-MT′-7), 6.18 (1H, m, H-21), 6.11 (1H, d, *J* = 7.6 Hz, H-G″-1), 5.70 (1H, br s, H-12), 5.44 (1H, br d, *J* = 15.8 Hz, H-MT-8a), 5.44 (1H, br d, *J* = 15.8 Hz, H-MT′-8a), 5.35 (1H, d, *J* = 7.6 Hz, H-G′-1), 5.32 (1H, dd, *J* = 8.8, 9.2 Hz, H-Q-4), 5.30 (1H, m, H-16), 5.27 (1H, br d, *J* = 11.0 Hz, H-MT-8b), 5.27 (1H, br d, *J* = 11.0 Hz, H-MT′-8b), 5.25 (1H, d, *J* = 7.0 Hz, H-X′-1), 5.03 (1H, d, *J* = 6.4 Hz, H-X-1), 4.94 (1H, d, *J* = 7.8 Hz, H-F-1), 4.87 (1H, d, *J* = 7.6 Hz, H-G-1), 4.84 (1H, d, *J* = 8.0 Hz, H-Q-1), 4.84 (1H, d, *J* = 8 Hz, H-Q′-1), 4.78 (1H, br s, H-R-2), 4.72 (1H, dd, *J* = 3.6, 8.8 Hz, H-R-3), 4.60 (1H, m, H-G-6b), 4.54 (1H, m, H-R-5), 4.50 (1H, m, H-G-4), 4.45 (1H, dd, *J* = 4.0, 11.4 Hz, H-X-5b), 4.43 (1H, dd, *J* = 8.8, 9.2 Hz, H-R-4), 4.40 (1H, dd, *J* = 7.8, 9.6 Hz, H-F-2), 4.40 (1H, m, H-G-6a), 4.29 (1H, m, H-G′-6b), 4.25 (1H, m, H-G-5), 4.23 (1H, m, H-X′-5b), 4.23 (1H, m, H-G″-2), 4.23 or 3.96 (2H, m, H_2-G″-6), 4.23 or 3.96 (1H, m, H-G″-3), 4.23 or 3.96 (1H, m, H-G″-4) 4.23 or 3.96 (1H, m, H-G″-5), 4.22 (1H, m, H-G′-6a), 4.20-3.86 (1H, m, H-X′-2), 4.20-3.86 (1H, m, H-X′-3), 4.20-3.86 (1H, m, H-X′-4), 4.19 (1H, m, H-G′-3), 4.19 (1H, m, H-G′-4), 4.17 (1H, dd, *J* = 8.8, 8.8 Hz, H-Q-3), 4.12 (1H, dd, *J* = 9.0, 9.0 Hz, H-G-3), 4.11 (1H, dd, *J* = 4.0, 9.6 Hz, H-F-3), 4.08 (1H, dd, *J* = 7.6, 8.4 Hz, H-G′-2), 4.07 (1H, m, H-Q′-3), 4.07 (1H, m, H-Q′-4), 4.05 (1H, m, H-X-2), 4.05 (1H, m, H-X-3), 4.05 (1H, m, H-X-4), 4.00 (1H, dd, *J* = 7.6, 9.0 Hz, H-G-2), 4.00 (1H, br s, H-F-4), 3.99 (1H, dd, *J* = 8.0, 8.8 Hz, H-Q-2), 3.95 (1H, dd, *J* = 8.0, 8.8 Hz, H-Q′-2), 3.90 (1H, br d, *J* = 8.4 Hz, H-G′-5), 3.75 (1H, m, H-F-5), 3.66 (1H, m, H-Q-5), 3.66 (1H, m, H-Q′-5), 3.58 (1H, dd, *J* = 10.0, 11.4 Hz, H-X-5a), 3.47 (1H, dd, *J* = 9.6, 11.2 Hz, H-X′-5a), 3.45 (1H, m, H-3), 3.45 (1H, m, H-18), 2.91 (1H, dd, *J* = 14.0, 13.1 Hz, H-19a), 2.82 (1H, dd, *J* = 14.2, 3.9 Hz, H-22a), 2.40 (2H, m, H_2-MT-4), 2.40 (2H, m, H_2-MT′-4), 2.26 (1H, m, H-2a), 2.20 (1H, m, H-22b), 2.07 (1H, m, H-11a), 2.04 (2H, m, H_2-15), 1.91 (1H, m, H-2b), 1.88 (3H, s, H_3-MT-9), 1.86 (1H, m, H-9), 1.82 (3H, s, H_3-MT′-9), 1.80 (3H, s, H_3-27), 1.74 (2H, m, H_2-MT-5), 1.74 (2H, m, H_2-MT′-5), 1.74 (3H, d, *J* = 6.5 Hz, H_3-R-6), 1.63 (1H, m, H-1), 1.61 (1H, m, H-11b), 1.60 (1H, m, H-6a), 1.58 (2H, m, H_2-7), 1.57 (1H, d, *J* = 6.0 Hz, H_3-Q′-6), 1.54 (3H, s, H_3-MT-10), 1.54 (3H, s, H_3-MT′-10), 1.48 (3H, d *J* = 6.0 Hz, H-F-6), 1.41 (1H, m, H-19b), 1.34 (1H, d, *J* = 6 Hz, H_3-Q-6), 1.30 (1H, m, H-6b), 1.28 (3H, s, H_3-23), 1.09 (3H, s, H_3-24), 1.07 (3H, s, H_3-30), 1.05 (3H, s, H_3-25), 0.98 (3H, s, H_3-29), 0.88 (3H, s, H_3-26), 0.85 (1H, br d, *J* = 10.0 Hz, H-5); HRFABMS *m/z* 2177.9998 (calcd for $C_{102}H_{162}O_{48}Na$, 2178.0128).

Gummiferaoside B (2): white solid; $[\alpha]^{26}_D$ -11 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.6) nm; IR (film) ν_{max} 3344, 2931, 1678, 1439, 1360, 1309, 1280, 1246, 1201, 1181, 1134, 1063, 998 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; HRFABMS *m/z* 2178.0071 (calcd for $C_{102}H_{162}O_{48}Na$, 2178.0128).

Gummiferaoside C (3): white solid; $[\alpha]^{26}_{D}$ -24 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.7) nm; IR (film) ν_{max} 3328, 2943, 2833, 1745, 1732, 1598, 1431, 1364, 1342, 1304, 1252, 1195, 1152, 1067, 1022, 1007 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; HRFABMS *m/z* 2358.1262 (calcd for $C_{113}H_{178}O_{50}Na$, 2358.1278).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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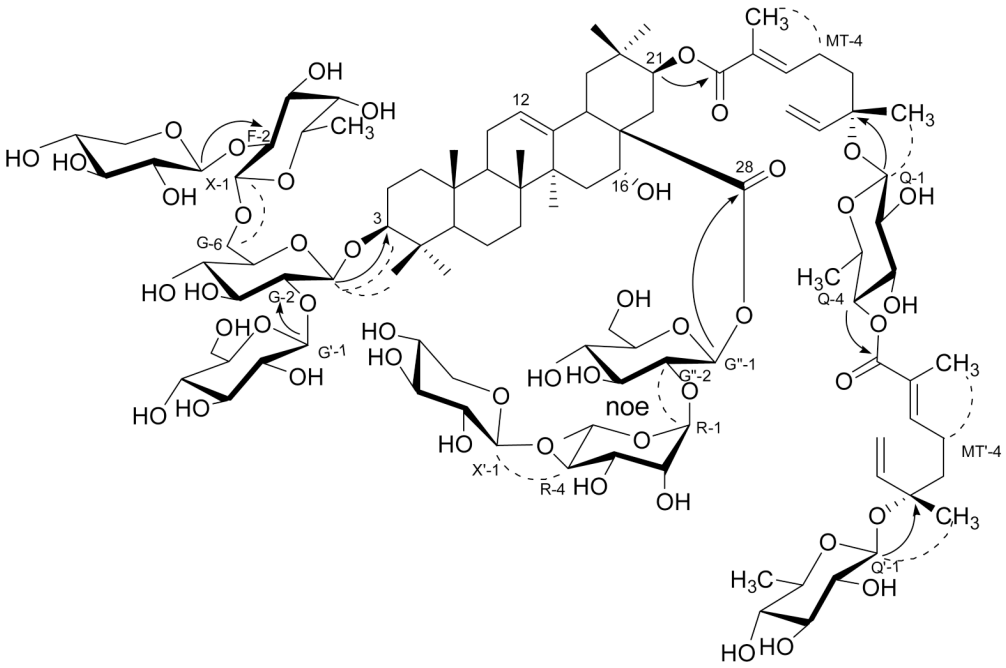

Figure 1.
Key HMBC (arrows) and ROESY (dashed) correlations for compound **1**

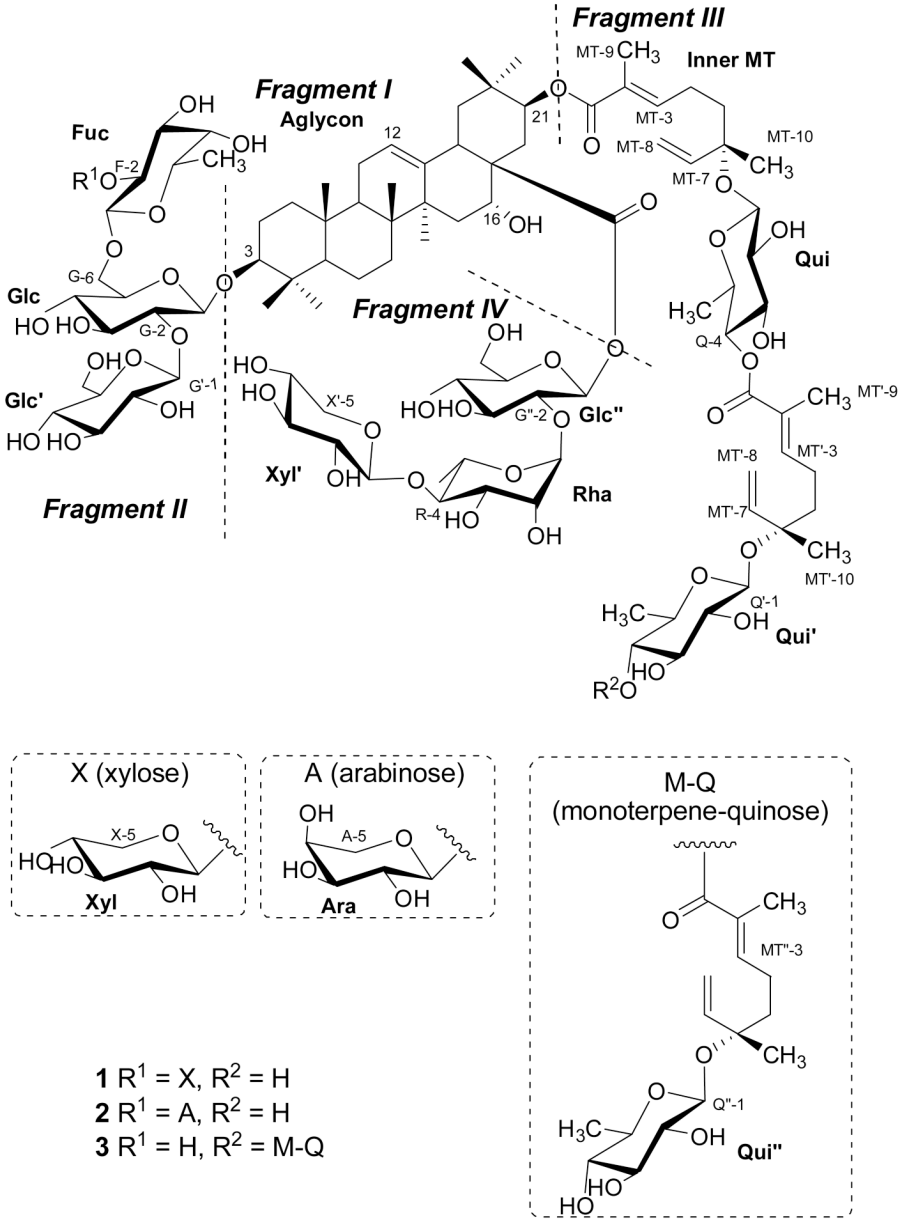

Table 1

^{1}H NMR Data of Compounds **1**-**3**[a,b]

position	1	2	3	position	1	2	3
1	1.62 m	1.62 m	1.62 m	MT′-4	2.30 m	2.30 m	2.30 m
2	1.85 m	1.85 m	1.85 m	-5	1.70 m	1.70 m	1.70 m
	1.70 m	1.70 m	1.70 m				
3	3.33 m	3.33 m	3.33 m	-7	5.93 dd (17.7, 11.0)	5.92 dd (17.7, 11.0)	5.92 dd (17.7, 11.0)
5	0.78 br d (10.0)	0.78 m	0.78 m	-8	5.27 dd (17.7, 1.2)	5.27 dd (17.7, 1.2)	5.27 dd (17.7, 1.2)
					5.20 dd (11.0, 1.2)	5.20 dd (11.0, 1.2)	5.21 m
6	1.50 m	1.50 m	1.52 m	-9	1.89 s	1.82 s	1.82 s
	1.28 m	1.28 m	1.38 m				
7	1.36 m	1.38 m	1.38 m	-10	1.38 s	1.38 s	1.38 s
9	1.68 m	1.66 m	1.66 m	MT″-3			6.73 t (7.3)
11	1.92 m	1.90 m	1.90 m	-4			2.30 m
12	5.34 br s	5.34 br s	5.35 br s	-5			1.70 m
15	1.50 m	1.45; 1.55	1.50 m	-7			5.73 dd (17.7, 11.0)
16	4.45 dd (5.0, 5.0)	4.48 br s	4.48 br s	-8			5.21 m
18	2.96 dd (10.5, 5.5)	2.97 m	2.97 m	-9			1.79 s
19	2.50 dd (12.0, 10.5)	2.50 dd (14.0, 14.0)	2.50 dd (14.0, 14.0)	-10			1.33 s
	1.18 dd (12, 5.5)	1.17 dd (13.1, .5.0)	1.17 dd (13.1, 5.0)				
21	5.43 dd (10.8, 5.5)	5.44 dd (11, 5.5)	5.43 dd (11, 5.5)	Q-1	4.42 d (7.6)	4.41 d (7.8)	4.42 d (8.0)
22	2.07 dd (12.0, 5.5)	2.10 dd (13.8, 5.5)	2.10 dd (13.8, 5.5)	-6	1.10 d (6.1)	1.09 d (6.0)	1.09 d (6.2)
	1.67 m	1.67 m	1.67 m				
23	1.09 s	1.09 s	1.09 s	Q′-1	4.35 d (7.6)	4.34 d (7.8)	4.54 d (8.0)
24	0.86 s	0.85 s	0.85 s	-6	1.23 d (6.1)	1.22 d (6.2)	1.23 d (6.2)
25	0.96 s	0.96 s	0.96 s	Q″-1			4.35 d (7.8)
26	0.75s	0.76s	0.76s	-6			1.26 d (6.2)
27	1.42 s	1.41 s	1.40 s	G-1	4.47 m	4.43 d (7.6)	4.43 d (7.6)
29	0.85 s	0.85 s	0.85 s	G′-1	4.66 d (7.8)	4.66 d (7.7)	4.66 d (7.8)
30	1.03 s	1.03 s	1.03 s	F-1	4.47 m	4.46 d (7.1)	4.32 d (7.1)
MT-3	6.75 t (7.3)	6.75 t (7.3)	6.75 t (7.3)	-6	1.26 d (6.4)	1.26 d (6.2)	1.26 d (6.2)
-4	2.30 m	2.30 m	2.30 m	X-1	4.47 m		
-5	1.70 m	1.70 m	1.70 m	A-1		4.55 d (6.4)	
-7	5.95 dd (17.7, 11.0)	5.93 dd (17.7, 11.0)	5.93 dd (17.7, 11.0)	G″-1	5.32 d (7.8)	5.32 d (7.8)	5.32 d (7.8)
-8	5.29 dd (17.7, 0.8)	5.29 dd (17.7, 1.2)	5.29 dd (17.7, 1.2)	R-1	5.41 d (1.4)	5.40 d (1.5)	5.40 d (1.5)
	5.21 dd (11.0, 0.8)	5.21 dd (11.0, 1.2)	5.21 m				
-9	1.83 s	1.82 s	1.84 s	-6	1.31 d (6.4)	1.30 d (6.2)	1.30 d (6.2)
-10	1.36 s	1.36 s	1.37 s	X′-1	4.47 m	4.50 d (7.7)	4.50 d (7.6)
MT′-3	6.80 t (7.3)	6.80 t (7.3)	6.83 t (7.3)				

[a] δ (ppm) 500 MHz; multiplicities; *J* values (Hz) in parentheses.

[b] In CD_3OD.

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Table 2

^{13}C NMR Data of Compounds **1-3**[a,b]

carbon	1[c]	1[d]	2[c]	3[c]	carbon	1[c]	1[d]	2[c]	3[c]
1	39.9	39.9	40.2	40.1	Q-1	99.3	99.6	99.5	99.4
2	27.4	27.1	27.6	27.4	-2	76.4	75.8	76.6	76.5
3	90.3	89.0	91.2	91.6	-3	76.4	75.8	76.4	76.7
4	40.6	40.3	40.7	40.6	-4	77.9	77.5	78.1	78.0
5	57.1	56.4	57.2	57.3	-5	70.9	70.5	71.1	71.3
6	18.3	18.7	18.5	18.5	-6	18.3	19.1	18.4	18.4
7	34.3	33.7	34.5	34.5	Q′-1	99.3	99.6	99.5	97.6
8	40.8	40.8	41.0	41.0	-2	75.5	75.7	75.8	76.1
9	48.0	47.4	48.3	48.0	-3	78.3	78.5	78.5	76.3
10	37.9	37.3	38.1	38.1	-4	77.3	77.0	77	78.1
11	24.6	24.0	24.8	24.7	-5	72.6	73.3	73.3	73.3
12	124.0	124.0	124.3	124.3	-6	18.2	19.1	18.4	18.4
13	143.7	144.3	143.9	143.3	Q″-1				99.4
14	42.6	42.3	42.8	42.8	-2				75.8
15	35.9	36.2	36.1	36.1	-3				78.5
16	74.1	73.8	74.3	74.3	-4				77.5
17	52.3	52.0	52.5	52.5	-5				73.0
18	41.5	41.3	41.8	41.9	-6				18.4
19	48.0	48.0	48.3	48.0	G-1	104.6	105.3	105.5	105.5
20	36.3	35.6	36.6	36.5	-2	81.2	83.2	81.2	81.3
21	78.6	77.4	78.8	78.7	-3	78.2	78.5	78.7	78.6
22	36.1	36.7	36.4	36.3	-4	71.8	71.8	72.0	72.0
23	28.4	28.5	28.6	28.7	-5	77.5	77.0	78.1	78.1
24	16.9	16.1	17.2	17.1	-6	69.8	70.2	69.4	69.0
25	16.2	17.2	16.4	16.3	G′-1	105.2	106.3	104.7	105.3
26	17.6	17.5	17.9	17.8	-2	76.2	76.1	75.9	75.8
27	27.4	27.5	27.5	27.4	-3	77.9	77.5	77.5	77.3
28	175.4	175.0	175.6	175.6	-4	71.9	72.3	72.1	72.1
29	29.4	29.5	29.6	29.5	-5	78.3	78.5	78.4	78.3
30	19.4	19.5	19.6	19.5	-6	63.1	62.8	63.4	63.3
MT-1	169.1	168.1	169.3	169.2	F-1	104.0	103.7	104.1	104.7
-2	129.1	128.9	129.3	129.2	-2	82.4	82.6	81.2	72.4
-3	144.0	142.5	144.2	144.1	-3	75.4	75.4	75.7	75.6
-4	24.4	23.9	24.7	24.5	-4	72.9	72.9	72.5	72.5
-5	41.0	40.7	41.2	41.3	-5	71.7	71.6	71.4	72.1
-6	81.0	80.1	81.3	81.2	-6	16.7	17.7	16.9	16.9
-7	144.1	144.4	144.3	144.2	X-1	107.1	107.3		
-8	115.9	115.4	116.2	116.1	-2	75.6	76.3		
-9	12.6	13.0	12.8	12.8	-3	77.8	78.1		
-10	23.6	23.9	23.8	23.8	-4	71.1	71.0		
MT′-1	169.6	168.0	169.8	169.8	-5	67.3	67.6		
-2	128.4	128.3	128.6	128.8	A-1			106.1	
-3	144.1	143.7	144.2	144.2	-2			71.6	
-4	24.5	23.9	24.6	24.6	-3			73.1	
-5	41.0	40.7	41.2	41.3	-4			70.0	
-6	80.9	79.8	81.1	81.1	-5			66.3	
-7	144.7	144.4	144.9	144.8	G″-1	95.2	95.5	95.4	95.4
-8	115.9	115.2	116.1	116.1	-2	77.1	76.7	77.3	77.2
-9	12.6	13.0	12.8	12.8	-3	79.5	79.4	79.6	79.6
-10	23.6	24.0	23.8	23.8	-4	71.4	71.4	71.3	71.3
MT″-1				168.8	-5	79.5	79.4	79.8	79.6
-2				128.8	-6	62.2	62.1	62.4	62.3
-3				143.8	R-1	101.1	101.6	101.3	101.3

carbon	1[c]	1[d]	2[c]	3[c]	carbon	1[c]	1[d]	2[c]	3[c]
-4				24.3	-2	72.2	72.7	72.0	72.1
-5				41.2	-3	72.9	72.9	73.0	73.2
-6				81.0	-4	84.0	83.4	84.2	84.0
-7				144.5	-5	68.8	68.8	69.0	69.0
-8				116.5	-6	18.3	19.0	18.5	18.3
-9				12.8	X′-1	106.9	106.7	107.1	107.0
-10				24.2	-2	77.1	76.5	77	77.0
					-3	78.4	78.7	78.6	78.5
					-4	71.1	71.2	71.1	71.0
					-5	67.3	67.5	67.5	67.5

[a] δ (ppm) 125 MHz.

[b] The signals of the sugar carbons were assigned by HSQC-TOCSY and ^{13}C NMR.

[c] In CD_3OD.

[d] In C_5D_5N with three drops of CD_3OD.