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Triterpenoid saponins from *Albizia lebbeck* (L.) Benth and their inhibitory effect on the survival of high grade human brain tumor cells

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ABSTRACT

As part of our search of new bioactive triterpenoid saponins from Cameroonian Mimosaceae plants, phytochemical investigation of the roots of *Albizia lebbeck* led to the isolation of two new oleanane-type saponins, named lebbeckosides A–B (**1**–**2**). Their structures were established on the basis of extensive 1D and 2D NMR (^{1}H, ^{13}C NMR, DEPT, COSY, TOCSY, ROESY, HSQC, and HMBC) and HRESIMS studies, and by chemical evidence. Compounds **1**–**2** were evaluated for their inhibitory effect on the metabolism of high grade human brain tumor cells, the human glioblastoma U-87 MG cell lines and the glioblastoma stem-like TG1 cells isolated from a patient tumor, and known to be particularly resistant to standard therapies. The isolated saponins showed significant cytotoxic activity against U-87 MG and TG1 cancer cells with IC_{50} values of 3.46 μM and 1.36 μM for **1**, and 2.10 μM and 2.24 μM for **2**, respectively.

1. Introduction

The genus *Albizia* comprises about 150 species widely distributed in the tropics, with the greatest diversity in Africa and South America.[1] Triterpenoid saponins are commonly described in this genus. Adianthifoliosides, grandibracteosides, gummiferaosides, julibrosides, coriariosides, and albizosides are complex triterpenoid saponins isolated from the *Albizia* genus.[2] These glycosides, represent a class of very complex glycosides possessing a common aglycon unit, acacic acid, having various oligosaccharide moieties at C-3 and C-28 and an acyl group at C-21. They have been reported to inhibit the growth of tumor cells, and thus appear as a new potential class of anticancer natural triterpenoid saponins.[2] In order to discover new bioactive acacic acid glycosides, we screened the saponin content of Cameroonian Mimosaceae including *Albizia*, *Acacia*, and *Entada* genera. Three *Albizia* species, among which *Albizia lebbeck*, were selected on the basis of their HPLC-DAD, LC-NMR, and LC–MS profiles.

Albizia lebbeck (L.) Benth is a pantropical species distributed in Africa, Asia, America, and Australia. In West Africa, it is traditionally used against diarrhea, dysentery, hemorrhoids, bronchitis, asthma, eczema, and leprosy.[3] In South-East Asia and Australia, the stem bark is used as a folk remedy to treat abdominal tumors, boils, cough, eye disorders, and lung ailments. It is also reported to be astringent, pectoral, rejuvenating, and tonic.[4] Nootropic and anxiolytic activities of a saponin fraction isolated from *A. lebbeck* leaves have been reported.[5] Oral administration of the saponin fraction isolated from *A. lebbeck* bark to male rats has been reported to significantly reduce fertility through reduction of sperm mobility and density.[6] Previous phytochemical studies of *A. lebbeck* stem bark reported the presence of glycosides of acacic acid lactones.[7]

In the present investigation on *A. lebbeck* roots, we report the isolation and structural characterization of two new acacic acid glycosides, named lebbeckosides A–B (**1**–**2**). The isolated compounds were evaluated for their inhibitory effect on the

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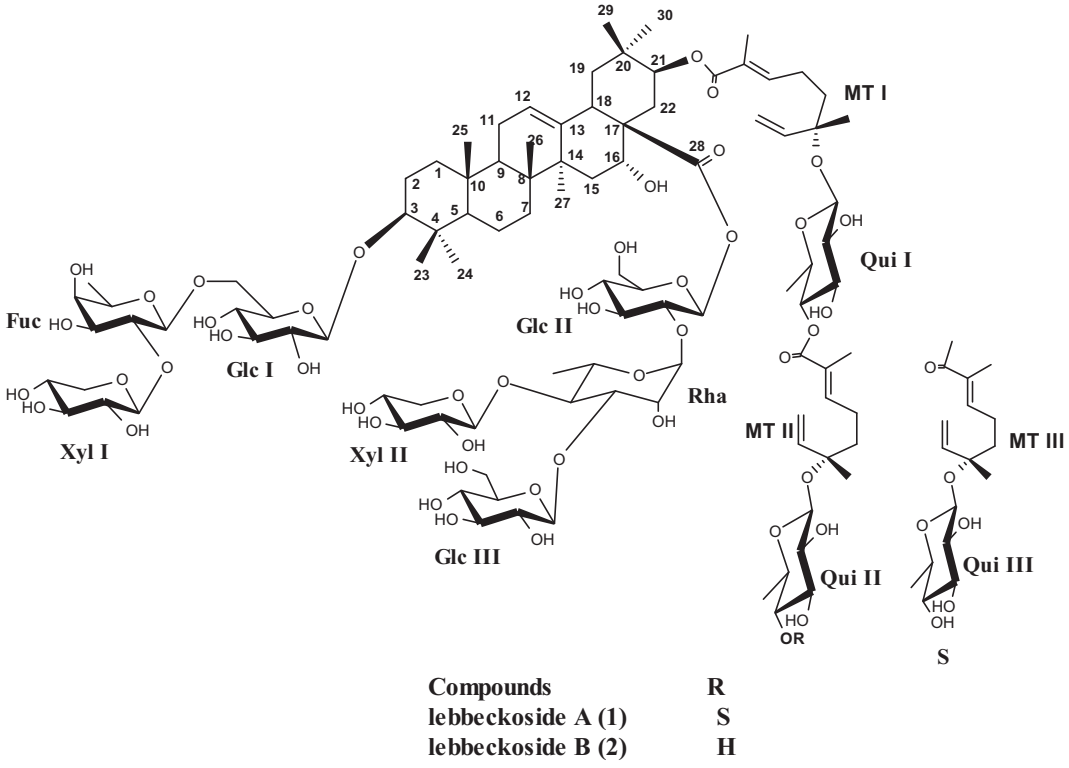
metabolism of high grade human brain tumor cells, namely the human glioblastoma U-87 MG cell lines and temozolomide-resistant glioblastoma stem-like cells isolated from a patient tumor, results are reported herein.

2. Results and discussion

The air-dried powdered roots of *A. lebbeck* (300 g) were extracted with aq-EtOH 70% using a soxhlet apparatus. After evaporation of the solvent, the resulting brown residue was partitioned between water and water-saturated *n*-BuOH. The *n*-BuOH fraction was then submitted to vacuum-liquid chromatography (VLC) on reversed-phase silica gel yielding three main fractions that were subjected to VLC on silica gel. Purification of the eluated subfractions by Semprep-HPLC afforded two new triterpenoid saponins (Chart 1).

Lebbeckoside A (**1**) was obtained as a white, amorphous powder. Its high-resolution electrospray ionization mass spectrometry (HRESIMS) (positive-ion mode) exhibited a pseudo-molecular ion peak at *m*/*z* 2486.2146 $[M+NH_4]^+$ (calcd 2485.2147), consistent with a molecular formula of $C_{118}H_{186}O_{54}$. Upon acid hydrolysis with 2.0 M HCl, **1** gave an acacic acid lactone unit, which was identified with an authentic sample, and compound **1** also gave glucose (Glc), xylose (Xyl), fucose (Fuc), rhamnose (Rha), arabinose (Ara), and quinovose (Qui), which were identified by co-TLC with authentic samples. The absolute configuration of these sugar residues was determined to be D for Glc, Xyl, and Fuc, and L for Ara and Rha based on GC analysis of their trimethylsilyl thiazolidine derivatives.[8] Its ^{1}H NMR spectrum showed seven angular methyl groups as singlets at δ_H 0.90, 1.01, 1.08, 1.10, 1.12, 1.31, and 1.84 (each 3H, s), one olefinic proton at δ_H 5.64 (1H, br s), and sugar proton signals at δ_H 4.89–6.42. ^{13}C NMR spectrum showed two olefinic carbon signals at δ_C 123.5 and 143.8, suggesting that **1** was an oleanane type triterpenoid saponin. 1D (^{1}H, ^{13}C NMR, DEPT) and 2D (COSY, HSQC and HMBC) NMR techniques permitted assignments of all ^{1}H and ^{13}C NMR signals of the aglycone of **1**. This aglycon was thus recognized to be acacic acid (3β,16α,21β-trihydroxyolean-12-ene-28-oic acid) by comparison of its ^{1}H and ^{13}C NMR signals with those reported in the literature.[9–21] The downfield position of the axial group at C-14 (Me-27, δ 1.84) in the ^{1}H NMR spectrum, implied an additional axial (α) hydroxyl group at C-16. The ROESY correlations observed between H-21 (δ 5.37) and H-29 (δ 1.01, s), suggested an α-axial orientation of H-21, as well as between H-3 (δ 3.49) and H-5 (δ 0.82) indicated the α-axial orientation of the two protons. The 3,21-hydroxy groups and 28-carbonyl group of the aglycon carried a sugar moiety, respectively, as evidenced by the glycosylation- and acylation-induced shifts observed at δ_C 89.0 (deshielded signal for C-3 of the aglycon), 174.9 (shielded signal for C-28 of the aglycon), and 77.6 (deshielded signal for C-21 of the aglycon), indicating that **1** was a 21-acyl 3,28-bidesmosidic acacic acid derivative with sugar chains linked to C-3 and C-28 of the aglycon through an ether and ester bond, respectively, and with an acyl group attached at C-21.

The ^{1}H NMR spectrum of **1** showed 10 anomeric protons at δ_H 4.91 [d, *J* = 8.0 Hz, glucose (Glc I)], 4.97 [d, *J* = 8.0 Hz, fucose (Fuc)], 5.09 [d, *J* = 7.1 Hz, xylose (Xyl I)], 6.16 [d, *J* = 8.0 Hz, glucose (Glc II)], 6.42 [br s, rhamnose (Rha)], 5.42 [d, *J* = 8.1 Hz, glucose (Glc III)], 5.30 [d, *J* = 7.1 Hz, xylose (Xyl II)], 4.90 [d, *J* = 8.0 Hz, quinovose (Qui I)], 4.89 [d, *J* = 8.0 Hz, quinovose (Qui II)], and 4.97 [d, *J* = 8.0 Hz, quinovose (Qui III)], which correlated with ten anomeric carbon atom resonances at δ_C 105.3, 103.7, 107.3, 95.6, 101.6, 106.4, 106.8, 99.7, 99.6, and 97.3, respectively, in the HSQC spectrum (Tables 1 and 2). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of the monosaccharide residues were assigned starting, either from the readily identifiable anomeric proton of each hexosyl or pentosyl unit, or from the CH_3-proton doublet of each 6-deoxyhexosyl unit, by means of TOCSY, HSQC, and HMBC spectra obtained for this compound. The anomeric centers of the D-glucose, D-fucose, D-quinovose, and D-xylose units were each determined to have a β-configuration based on large $^3J_{H\text{-}1,H\text{-}2}$ values. And the α-anomeric configuration of the L-rhamnose was judged by the broad singlet of the anomeric proton and the chemical shift value of C-5 (δ 68.8).[22] In



Compounds	R
lebbeckoside A (1)	S
lebbeckoside B (2)	H

Chart 1. Structures of triterpene saponins **1**–**2**.