A Cytotoxic Saponin from *Albizia julibrissin*

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A new triterpenoidal saponin (1: Julibroside J_{21}) with a xylopyranosyl moiety located at its C-21 side chain was isolated from *Albizia julibrissin* DURAZZ. (Leguminosae), and its structure was determined on the basis of comprehensive spectroscopic analyses. Compound 1 showed marked inhibitory action against Bel-7402 cancer cell line at 10 μg/ml.

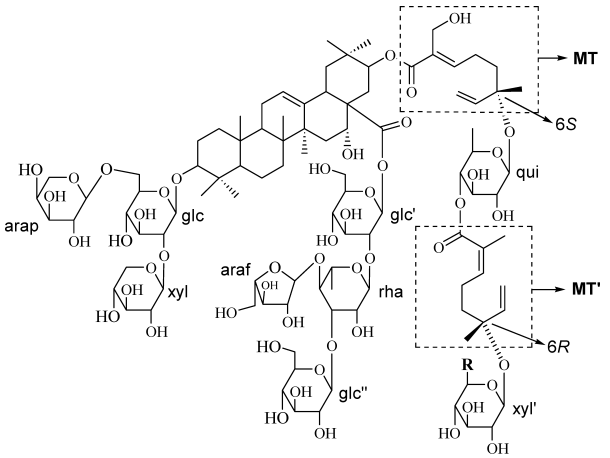
Key words *Albizia julibrissin*; Julibroside J_{21}; cytotoxicity; Leguminosae

The stem bark of *Albizia julibrissin* DURAZZ. (Leguminosae) is recorded in the Chinese Pharmacopoeia as a sedative and anti-inflammatory agent, and specified to treat injuries from falls and remove carbuncles.[1)] In the preceding studies,[2—4)] we reported the isolation and structure elucidation of some complicated and cytotoxic julibrosides from the stem bark of this plant. Recently, Haridas *et al.*,[5—7)] have reported the anti-cancer actions of avicins, a family of triterpenoid saponins obtained from the Australian desert tree *Acacia victoriae* (Leguminosae: Mimosoideae). Avicins showed strong inhibitory action towards human cancer cells and inducing action to cell apoptosis, partly *via* perturbing mitochondrial function. Their experiments also showed that avicins prevented chemical-induced carcinogenesis in mice, and strongly inhibited TNF-induced NF-kappa B. This paper reports the isolation and structure elucidation of compound **1**, an analogue of Julibroside J_1 (**2**). Compound **1** showed significant cytotoxic activity against the Bel-7402 cancer cell line by the SRB (Sulforhodamine B) method.[8)]

The 95% ethanol extract from stem barks of *A. julibrissin* was suspended over water and extracted successively with $CHCl_3$, EtOAc and *n*-BuOH, respectively. The *n*-BuOH soluble part was chromatographed on D_{101} macroporous resins and silica gels, giving rise to colorless powders (Frs. 41—43). A tridesmodic saponin (**1**) was obtained from Frs. 41—43 by Sephadex LH-20, C_{18} silica gel column chromatography and preparative HPLC.

Compound **1**, a white powder, gave a positive Liebermann–Burchard reaction. The FAB-MS mass spectrum ((Fast Atom Bombardment Mass Spectroscopy, positive ion) of **1** gave the quasi-molecular ion peak at *m/z* 2182 $[M+K+1]^+$ and 2167 $[M+Na+2]^+$, which was indicative of the formula $C_{100}H_{158}O_{49}$. The ^{1}H-NMR spectrum showed seven angular methyl signals at δ 1.28, 1.00, 0.96, 1.15, 1.86, 1.04, 1.06 (each 3H, s) and sugar proton signals at δ 3.4—6.3. The data suggested a triterpenoid saponin. On acidic hydrolysis, **1** furnished the aglycone which was identical with an authentic sample, acacic acid lactone on high-performance thin layer chromatography, and on PC the resulting sugars were identified as glucose, xylose, rhamnose, arabinose and quinovose. The ^{13}C-NMR spectrum gave nine anomeric carbon signals at δ 95.6, 99.2, 100.1, 101.7, 102.2, 105.7, 106.2, 106.7 and 111.1. The corresponding anomeric proton signals at δ 4.87 (1H, d, J=7.4 Hz, H-glc-1), 5.14 (1H, br s, H-arap-1), 4.98 (1H, d, J=6.3 Hz, H-xyl-1), 6.03 (1H, d, J=7.6 Hz, H-glc′-1), 5.88 (1H, s, H-rha-1), 6.24 (1H, s, H-araf-1), 5.31 (1H, d, J=7.9 Hz, H-glc″-1), 4.82 (1H, d, J=7.6 Hz, H-qui-1), 4.80 (1H, d, J=7.5 Hz, H-xyl′-1) were assigned according to HMQC (Heteronuclear Multiple Quantum Coherence) results. Based on the ^{1}H- and ^{13}C-NMR data of **1**, the anomeric configurations in the sugar moieties were determined as β-configuration for glucose, xylose, and quinovose moieties, and α-configuration for rhamnose and arabinose moieties.[9)] Except for the resonances of protons and carbons belonging to aglycone and sugar moieties, two groups of proton and carbon-13 signals due to monoterpenoids (MT, MT′) were observed in the ^{1}H- and ^{13}C-NMR spectra of **1** (see Experimental).

It was deduced by a careful comparison of the carbon-13 data of **1** with those of **2** that the signals of the aglycone, monoterpene and sugars moieties of **1** were almost superimposable on those of **2**, except that signals of **2** due to a quinovose moiety were different from those of **1** due to a sugar moiety. The correlation between an anomeric carbon-13 signal at δ 100.1 and an anomeric proton signal at δ 4.80 was observed in the HMQC spectrum of **1**. A marked spin coupling system was observed among proton signals at δ 4.80, 4.24, 4.19, 4.11, 3.95 and 3.66 in the TOCSY (Total Correlation Spectroscopy) spectrum of **1**. Corresponding to the above proton signals, five carbon-13 signals at δ 100.1, 75.4, 78.6, 70.8 and 66.9 were observed in the ^{13}C-NMR



1: Compound **1** R = H
2: Julibroside J_1 R = Me

Fig. 1. The Structures of Compound **1** and Julibroside J_1 (**2**)

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spectra of **1**, and their correlation peaks were observed in the HMQC spectrum of **1**, which suggested a xylopyranosyl moiety in **1**.[10] The correlation between the proton signal at δ 4.82 due to the anomeric proton of β-D-quinovopyranosyl moiety and the carbon-13 signal at δ 79.7 due to the C-6 of the inner MT moiety was observed in the HMBC (Heteronuclear Multiple-Bond Correlation) spectrum of **1**, which determined the linkage of β-D-quinovopyranosyl moiety to the inner MT moiety at C-21 side chain. Meanwhile, the correlation between the proton signal at δ 4.80 due to the anomeric proton of β-D-xylopyranosyl moiety and the carbon-13 signal at δ 79.4 due to the C-6 of the outer MT′ moiety was also observed in the HMBC spectrum of **1**, which suggested that the xylopyranosyl moiety be the terminal residue of C-21 side chain. This linkage mode was further conformed by its FAB-MS results (see Experimental).

Therefore, the structure of **1** was identified as 3-*O*-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]-21-*O*-{(6*S*)-2-*trans*-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(6*R*)-2-*trans*-2,6-dimethyl-6-*O*-(β-D-xylopyranosyl-2,7-octadienoyl)]-β-D-quinovopyranosyl-2,7-octadienoyl} acacic acid 28-*O*-β-D-glucopyranosyl-(1→3)-[α-L-arabinofuranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester, named as Julibroside J_{21} (see Fig. 1).

Compound **1** showed marked inhibitory action against Bel-7402 cells (human liver cancer cell line) (inhibition 80.8%) at 10 μg/ml in SRB method.

Experimental

Optical rotation was recorded with a Perkin-Elmer 241 spectropolarimeter. IR spectrum was measured on a Perkin-Elmer 983 FTIR instrument as samples in pressed KBr disks. 1D and 2D NMR spectra were recorded using Bruker AM 500 and Varian-300 instruments with Me_4Si as the intestinal standard. FAB mass spectra were recorded using a ZABspec mass spectrometer. HPLC was carried out using (1) a Gilson automatic system for preparative HPLC with an Alltima C_{18} column (5 μm, 60 Å, pore size, 22×250 mm i.d. and 10 μm, 60 Å, 22×250 mm i.d.), or (2) Waters 600 semipreparative HPLC with a μBondpak C_{18} column (6 μm, 60 Å, 7.8×300 mm i.d.). Macroporous resin D_{101} (Nankai), Silica Gel (10—40 μm, 200—300 mesh, Qingdao), Sephadex LH-20, RP C_{18} Silica Gel (100—200 mesh) (Ouya, Pharmacia) were used as packing materials for column chromatography.

Plant Material Dried stem bark of *A. julibrissin* was purchased from Mianyang Medicinal Company of Sichuan Province in October 1995. A sample has been deposited in the Division of Natural Medicinal Chemistry of Perking University.

Extraction and Isolation Air-dried powdered stem bark (13.5 kg) was extracted with 95% EtOH. The EtOH residues (1140 g) were suspended in water, and then extracted with $CHCl_3$, EtOAc and *n*-BuOH, respectively. The *n*-BuOH-soluble extract was dissolved in MeOH, and then poured dropwise into acetone. The resulting precipitate was chromatographed over a D_{101} resin column with gradient elution (100% water→100% MeOH). The fraction from the MeOH elution (248 g) was subjected to silica gel column chromatography using a gradient solvent system of $CHCl_3$–MeOH–water (100 : 0 : 0→6 : 4 : 1) to afford 68 fractions (500 ml/fraction). Frs. 41—43 was decolorized by activated charcoal in MeOH to give a white powder (22.5 g). The white powder (10.5 g) was subjected to repeated Sephadex LH-20, silica gel, RP C_{18} silica gel column chromatography, and preparative HPLC (43 : 57 MeCN–water, 8.0 ml/min, 216 nm detection) to afford **1** (64.8 mg).

Bioassay The cytotoxic activity of **1** was evaluated according to the same protocol as the literature.[8]

Acid Hydrolysis This experiment was carried out according to the procedure described in literature.[2]

Julibroside J_{21} (**1**): Amorphous white powder, $[\alpha]_D^{25}$ −28° (*c*=0.25, MeOH). FAB-MS (positive mode) *m/z*: 2182 $[M+K+1]^+$, 2167 $[M+Na+2]^+$, 2035 $[M+Na+2\text{-xyl}]^+$, 1845 $[M+Na+2-(xyl+MT')]^+$, 1564 $[M+Na+2-(2glc+rha+ara)]^+$, 1380 $[M+Na-(xy+MT'+qui+MT)-glc]^+$. IR (KBr) ν_{max} cm^{-1}: 3410, 2927, 1692, 1640, 1383, 1281, 1074 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.28, 1.00, 0.96, 1.15, 1.86, 1.04, 1.06 (3H×7, s, H-23, 24, 25, 26, 27, 29, 30), 5.61 (1H, br s, H-12), 4.87 (1H, d, *J*=7.4 Hz, H-glc-1), 5.14 (1H, br s, H-arap-1), 4.98 (1H, d, *J*=6.3 Hz, H-xyl-1), 6.03 (1H, d, *J*=7.6 Hz, H-glc′-1), 5.88 (1H, s, H-rha-1), 6.24 (1H, s, H-araf-1), 5.31 (1H, d, *J*=7.9 Hz, H-glc″-1), 4.82 (1H, d, *J*=7.6 Hz, H-qui-1), 4.80 (1H, d, *J*=7.5 Hz, H-xyl′-1), 1.75 (3H, d, *J*=5.5 Hz, H-rha-6), 1.33 (3H, d, *J*=6.1 Hz, H-qui-6), 7.02 (1H, t, *J*=7.0 Hz, H-MT-3), 2.64 (2H, m, H-MT-4), 1.71 (2H, m, H-MT-5), 6.17 (1H, dd, *J*=8.9, 18.3 Hz, H-MT-7), 5.19 (1H, d, *J*=8.9 Hz, H-MT-8a), 5.44 (1H, d, *J*=18.3 Hz, H-MT-8b), 4.71 (2H, s, H-MT-9), 1.49 (3H, s, H-MT-10), 7.05 (1H, t, *J*=7.2 Hz, H-MT′-3), 2.47 (2H, m, H-MT′-4), 1.81 (2H, m, H-MT′-5), 6.30 (1H, *J*=11.1, 17.5 Hz, H-MT′-7), 5.18 (1H, d, *J*=11.1 Hz, H-MT′-8a), 5.31 (1H, d, *J*=17.5 Hz, H-MT′-8b), 1.92 (3H, s, H-MT′-9), 1.46 (3H, s, H-MT′-10). ^{13}C-NMR (125 MHz, pyridine-d_5) δ: 39.6 (C-1), 26.9 (C-2) 88.7 (C-3), 40.1 (C-4), 56.0 (C-5), 18.4 (C-6), 33.6 (C-7), 40.4 (C-8), 47.1 (C-9), 37.1 (C-10), 23.7 (C-11), 123.1 (C-12), 143.3 (C-13), 42.0 (C-14), 35.9 (C-15), 73.8 (C-16), 51.6 (C-17), 40.9 (C-18), 47.9 (C-19), 35.4 (C-20), 77.0 (C-21), 36.4 (C-22), 28.2 (C-23), 17.1 (C-24), 15.8 (C-25), 17.3 (C-26), 27.2 (C-27), 174.5 (C-28), 29.2 (C-29), 19.1 (C-30), 106.7 (C-glc-1), 76.8 (C-glc-2), 78.4 (C-glc-3), 72.6 (C-glc-4), 77.2 (C-glc-5), 69.5 (C-glc-6), 102.2 (C-arap-1), 80.3 (C-arap-2), 72.5 (C-arap-3), 67.4 (C-arap-4), 64.2 (C-arap-5), 106.2 (C-xyl-1), 75.6 (C-xyl-2), 77.8 (C-xyl-3), 70.8 (C-xyl-4), 67.2 (C-xyl-5), 95.6 (C-glc′-1), 76.8 (C-glc′-2), 78.1 (C-glc′-3), 71.2 (C-glc′-4), 79.0 (C-glc′-5), 62.0 (C-glc′-6), 101.7 (C-rha-1), 70.5 (C-rha-2), 82.0 (C-rha-3), 78.9 (C-rha-4), 69.1 (C-rha-5), 18.8 (C-rha-6), 111.1 (C-araf-1), 84.4 (C-araf-2), 78.4 (C-araf-3), 85.4 (C-araf-4), 62.5 (C-araf-5), 105.7 (C-glc″-1), 75.3 (C-glc″-2), 78.4 (C-glc″-3), 71.8 (C-glc″-4), 78.4 (C-glc″-5), 62.8 (C-glc″-6), 99.2 (C-qui-1), 75.4 (C-qui-2), 78.4 (C-qui-3), 76.8 (C-qui-4), 73.0 (C-qui-5), 18.6 (C-qui-6), 100.1 (C-xyl′-1), 75.4 (C-xyl′-2), 78.6 (C-xyl′-3), 70.8 (C-xyl′-4), 66.9 (C-xyl′-5), 167.5 (C-MT-1), 133.8 (C-MT-2), 145.2 (C-MT-3), 23.5 (C-MT-4), 40.9 (C-MT-5), 79.7 (C-MT-6), 143.9 (C-MT-7), 115.0 (C-MT-8), 56.3 (C-MT-9), 23.9 (C-MT-10), 167.8 (C-MT′-1), 127.6 (C-MT′-2), 143.7 (C-MT′-3), 23.6 (C-MT′-4), 38.5 (C-MT′-5), 79.4 (C-MT′-6), 144.3 (C-MT′-7), 114.3 (C-MT′-8), 12.6 (C-MT′-9), 24.6 (C-MT′-10).

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