A Cytotoxic Saponin from *Albizia julibrissin*

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A new triterpenoidal saponin (1: Julibroside J₂₁) 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 μ g/ml.

Key words *Albizia julibrissin*; Julibroside J₂₁; 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- κ B. This paper reports the isolation and structure elucidation of compound **1**, an analogue of Julibroside J₁ (**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 CHCl₃, EtOAc and *n*-BuOH, respectively. The *n*-BuOH soluble part was chromatographed on D₁₀₁ 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, C₁₈ 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 C₁₀₀H₁₅₈O₄₉. The ¹H-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 ¹³C-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 ¹H- and ¹³C-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 ¹H- and ¹³C-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 ¹³C-NMR

Fig. 1. The Structures of Compound **1** and Julibroside J₁ (**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 $\rightarrow$ 2)- α -L-arabinopyranosyl-(1 $\rightarrow$ 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 $\rightarrow$ 3)-[α -L-arabinofuranosyl-(1 $\rightarrow$ 4)]- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl ester, named as Julibroside J₂₁ (see Fig. 1).

Compound **1** showed marked inhibitory action against Bel-7402 cells (human liver cancer cell line) (inhibition 80.8%) at 10 μ g/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 Me₄Si 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 C₁₈ column (5 μ m, 60 Å, pore size, 22 $\times$ 250 mm i.d. and 10 μ m, 60 Å, 22 $\times$ 250 mm i.d.), or (2) Waters 600 semipreparative HPLC with a μ Bondpak C₁₈ column (6 μ m, 60 Å, 7.8 $\times$ 300 mm i.d.). Macroporous resin D₁₀₁ (Nankai), Silica Gel (10–40 μ m, 200–300 mesh, Qingdao), Sephadex LH-20, RP C₁₈ 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 CHCl₃, 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 D₁₀₁ resin column with gradient elution (100% water $\rightarrow$ 100% MeOH). The fraction from the MeOH elution (248 g) was subjected to silica gel column chromatography using a gradient solvent system of CHCl₃–MeOH–water (100:0:0 $\rightarrow$ 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 C₁₈ 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 J₂₁ (**1**): Amorphous white powder, [α]_D²⁵ –28° (*c*=0.25, MeOH). FAB-MS (positive mode) *m/z*: 2182 [M+K+1]⁺, 2167 [M+Na+2]⁺, 2035 [M+Na+2-xy]⁺, 1845 [M+Na+2-(xy+MT')]⁺, 1564 [M+Na+2-(2glc+rha+ara)]⁺, 1380 [M+Na-(xy+MT'+qui+MT)-glc]⁺. IR (KBr) $\nu_{\max}$ cm^{–1}: 3410, 2927, 1692, 1640, 1383, 1281, 1074 cm^{–1}. ¹H-NMR (500 MHz, pyridine-*d*₅) δ : 1.28, 1.00, 0.96, 1.15, 1.86, 1.04, 1.06 (3H $\times$ 7, s, H-23, 24, 25, 26, 27, 29, 30), 5.61 (1H, brs, H-12), 4.87 (1H, d, *J*=7.4 Hz, H-gluc-1), 5.14 (1H, brs, H-arap-1), 4.98 (1H, d, *J*=6.3 Hz, H-xy-1), 6.03 (1H, d, *J*=7.6 Hz, H-gluc'-1), 5.88 (1H, s, H-rha-1), 6.24 (1H, s, H-araf-1), 5.31 (1H, d, *J*=7.9 Hz, H-gluc'-1), 4.82 (1H, d, *J*=7.6 Hz, H-qui-1), 4.80 (1H, d, *J*=7.5 Hz, H-xy-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). ¹³C-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-gluc-1), 76.8 (C-gluc-2), 78.4 (C-gluc-3), 72.6 (C-gluc-4), 77.2 (C-gluc-5), 69.5 (C-gluc-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-xy-1), 75.6 (C-xy-2), 77.8 (C-xy-3), 70.8 (C-xy-4), 67.2 (C-xy-5), 95.6 (C-gluc'-1), 76.8 (C-gluc'-2), 78.1 (C-gluc'-3), 71.2 (C-gluc'-4), 79.0 (C-gluc'-5), 62.0 (C-gluc'-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-gluc'-1), 75.3 (C-gluc'-2), 78.4 (C-gluc'-3), 71.8 (C-gluc'-4), 78.4 (C-gluc'-5), 62.8 (C-gluc'-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-xy-1), 75.4 (C-xy-2), 78.6 (C-xy-3), 70.8 (C-xy-4), 66.9 (C-xy-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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References

- 1) The Pharmacopoeia Committee of People's Republic of China (ed.), "Pharmacopoeia (I)," People Health Press, Beijing, 1995, pp. 119–120.
- 2) Zou K., Tong W. Y., Liang H., Cui J. R., Tu G. Z., Zhao Y. Y., Zhang R. Y., *Carbohydr. Res.*, **340**, 1329–1334 (2005).
- 3) Zou K., Zhao Y. Y., Tu G. Z., Zhang R. Y., Jia Z. H., *Carbohydr. Res.*, **324/3**, 182–188 (2000).
- 4) Zou K., Cui J. R., Wang B., Zhao Y. Y., Zhang R. Y., *J. Asian Nat. Prod. Res.*, **7**, 783–788 (2005).
- 5) Haridas V., Higuchi M., Jayatilake G. S., Bailey K. M., Blake M. E., Arntzen C. J., Gutterman J. U., *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 5821–5825 (2001).
- 6) Hanausek M., Ganesh P., Wasaszek Z., Arntzen C. J., Slaga T. J., *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 11551–11556 (2001).
- 7) Haridas V., Arntzen C. J., Gutterman J. U., *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 11557–11562 (2001).
- 8) Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R., *J. Natl. Cancer Institute*, **82**, 1107–1109 (1990).
- 9) Agrawal P. K., *Phytochemistry*, **31**, 3307–3330 (1992).
- 10) Kiuchi F., Gafur M. A., Obata T., Tachibana A., Tsuda Y., *Chem. Pharm. Bull.*, **45**, 807–812 (1997).