Review:

Triterpenoid saponins from Cortex Albiziae

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ABSTRACT

Cortex Albiziae, the dried stem bark of a leguminous plant, *Albizia julibrissin* Durazz, was specified in Chinese Pharmacopoeia (1995 edit.) as a traditional Chinese medicine to be used to relieve melancholia and uneasiness of body and mind, to invigorate the circulation of blood and subside a swelling.[1] In a course of our quality assessment of traditional Chinese medicines, the *n*-BuOH soluble part of 95% EtOH extracts from the stem barks of *Albizia julibrissin* was subjected to a series of solvent treatment and chromatographic separations, including Rp-HPLC methods, giving rise to 28 pentacyclic triterpenoid saponins. Based on chemical methods and spectroscopic evidences, the structures of saponins were identified, which included six pairs of diastereoisomers, five pairs of position isomers, and 26 of them were new compounds. All the saponins are trisdesmosidic saponins, which are composed of an acacic acid moiety, 7-9 monosaccharide moieties, 1-2 monoterpenic acid moieties, and exhibited their molecular ion peaks around *m/z* 2000. Their carbon-13 signals and most of proton signals were assigned based on 1D and 2D NMR experiments. The proton and carbon signals of several known saponins were revised and reassigned. The spectroscopic properties related to these saponins were analyzed and summarized. The cytotoxic activity and other activities of these saponins and their analogues were discussed also.

INTRODUCTION

Saponins are widely distributed in plant species, being reported in nearly 100 families. They are naturally occurring glycosides which are found mainly, but not exclusively (lower marine animals) in the plant kingdom. They consist of aglycone coupled to sugar chain units. These sugars can be attached as one, two or three sugar chains and the terms monodesmoside, bidesmoside and tridesmoside have been given to these saponins, respectively. According to the nature of the aglycone, they can be classified into steroidal or triterpene groups. All classes of aglycones may have a number of functional groups (usually -OH, -COOH and $-CH_3$, some instances $-SO_3Na$ in marine creatures) causing big natural diversity only because of aglycone structures. Over 100 steroidal and probably even larger numbers of triterpene sapogenins have been identified.[2] This diversity can be further multiplied by the composition of sugar chains, sugar numbers, branching patterns and type of substitutions. It is well recognized that even one plant species may possess a number of individual saponins, e.g., alfalfa roots contain at least 25 medicagenic acid, hederagenin, zanhic acid, soyasapogenol and bayogenin glycosides, with the attached number of sugars ranging from one to seven.[3] These structures and their amounts may differ depending on the plant part studied. This structural diversity and resulting wide range of polarities makes fine purification and preparation of individual saponins very difficult, and structural elucidation much complicated.

Man has relied on plants as a source of medicinal agents for centuries. Today, with the specter of antibiotic resistance, emerging infectious diseases, and cancers, phytochemicals continue to provide new structural leads for the chemotherapeutic industry. A number of triterpenoids have shown promise as antineoplastic agents. Members of the cycloartane, lupane, ursane, oleanane, friedelane, dammarane, cucurbitacin, and limonoid triterpenoids, have demonstrated anti-proliferative activity on

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various cancer cell lines.[4] Saponins are common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and mollusks, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycemia, and to act as antifungal and antiviral agents. These compounds can thus affect animals in a host of different ways both positive and negative.[5]

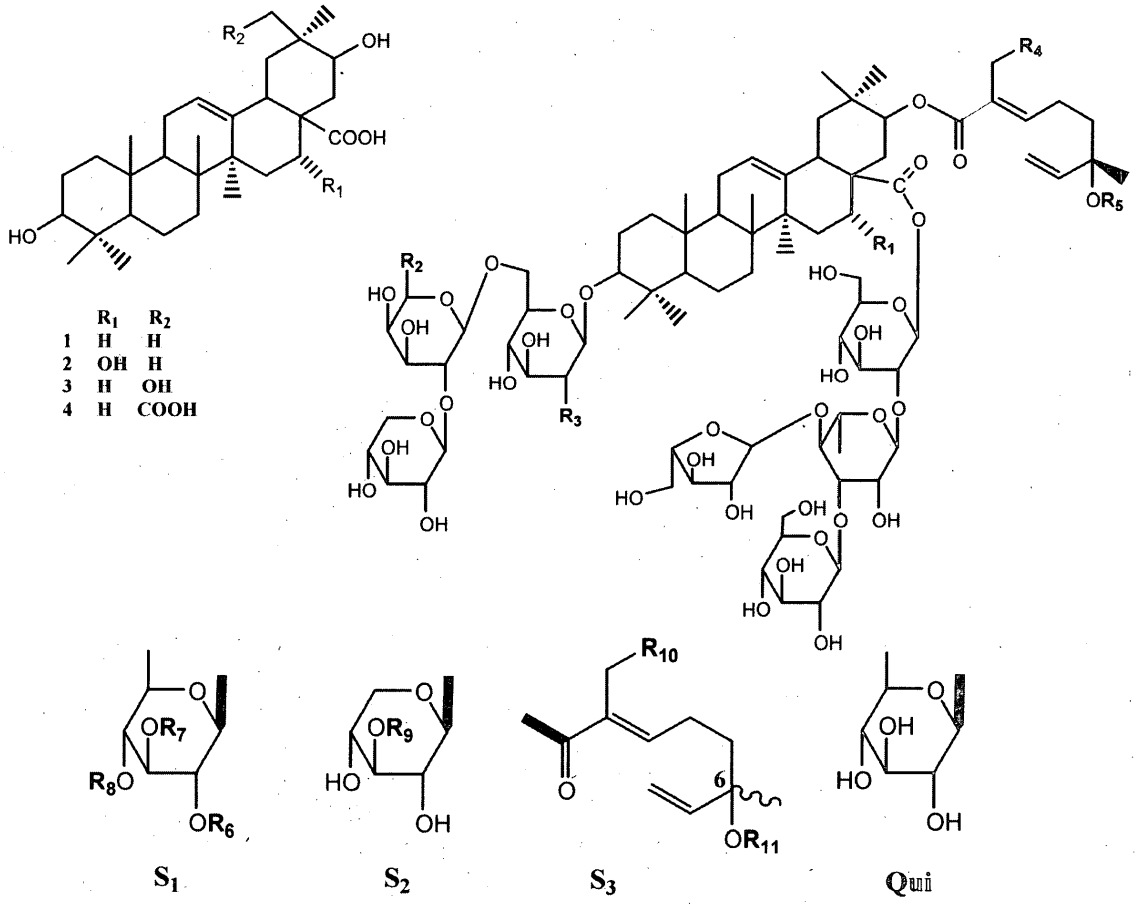
Over the centuries, traditional Chinese medicines (TCM) have served as a major source of medicines for the prevention and treatment of diseases. They uses a system of categorizing clusters of symptoms and signs to differentially assess the presence or absence of syndromes for which effective herbal formulas and methods are known. This system can be useful in detecting and counteracting some of the severe systemic and metabolic imbalances.[6] Interest in TCM is growing rapidly beyond China. This interest is driven by a combination of factors including recognition of potential benefits of TCM; dissatisfaction with the traditional Western medical model; an increasing commitment to holistic care and increasing evidence for the interaction of psychological factors and outcomes of disease and treatment and health consumer demand.[7] A brief survey on some reputed traditional Chinese medicines demonstrate that saponins are important principles responsible for their bioactivities, such as ginsenosides in Radix Ginseng,[8,9] glycyrrhizin in Radix Glycyrrhizae,[10] astragalosides in Radix Astragali,[11] onjisaponins in Radix Polygalae,[12] and saikosaponins in Radix Bupleuri,[13] etc.

Cortex Albiziae is the dried stem bark of *Albizia julibrissin* Durazz (Leguminosae), which is known as silk tree, mimosa, silky acacia, and Hehuan in Chinese, usually cultivated as an ornamental plant in China and Japan, also in the southeastern United States and Europe since the 18th century. *Albizia* species belongs to *Mimosa* subfamily of Leguminosae. Approximate 150 *Albizia* species are distributed all over the world and 16 of them grow in China,[14,15] The major species distributed in China include *A. julibrissin Durazz., A. chinensis* (O) Mert., and *A. lebbeck* (L) Benth. The dried stem barks of A. *julibrissin* Durazz were recorded in Chinese Pharmacopoeia as a sedative agent to dispel melancholy and soothe the nerves, and an agent to invigorate the circulation of blood and dissolve the stagnant. It was usually used to treat the uneasiness, melancholia, pulmonary abscess, sores, swelling and traumatic injuries from falls in traditional Chinese medicines. Recently, it was reported to show many pharmacological activities, such as antitumor, uterotonic, antagonic actions against PAF receptor, etc.[16] In the process of our study on the variety analysis and quality assessment of traditional herbal materials, we paid much attention to those with saponins as their principles, such as *Glyccyrhiza uralensis, G. inflata, G. glabra*,[10,17] *Bupleurum scorzonerifolium*,[18] *B. chinense* DC,[19] *B. smithii* var. *parvifolium*,[20] and *B. smithii* Wolff,[21] As a part of the ninth five-year research program for traditional Chinese medicines granted by China National Natural Science Foundation, the quality evaluation and the related of Cortex Albizae was carried out in our laboratory.[22-25]

Separation and isolation of triterpenoid saponins from Cortex Albiziae

95%, 90%, 85%, 75% and 60% ethanol solutions were preliminarily tested as solvents to extract crude total saponins from 50 grams of the crude drugs, respectively. The extracts were subjected to macroporous resin column chromatography to afford to 17.5 g, 14.3 g, 12.7 g, 10.8 g and 11.4 g of crude total saponins, respectively. The extracts were obtained for the largest amount by 95% ethanol, accordingly, 95% ethanol was chosen as the extraction solvent. Since scores of triterpenoid saponins with a relatively high content were found

to be present in this extract, and they share many common structure units, miscellaneous separation procedures were thus used for the isolation of saponins. Macroporous resins (D_{101}) were used to remove sugars, proteins, resins and some other highly-polar constituents. A white powder mainly composed of saponins was obtained by decolorizing crude saponins using activated charcoal. The combination of Sephadex LH-20 column chromatography, normal phase silica gel chromatography, reversed phase C_{18} silica gel chromatography and Rp-18 semi-preparative and preparative HPLC, especially the application of a quaternary solvent system composed of methanol, acetonitrile, tetrahydrofuran and water, allowed a successful isolation of 28 saponins, including 6 pairs of diasteroisomers and 5 pairs of position isomers, from the crude saponin fraction. Meanwhile, a weak ultra-violet absorption of Julibrosides at 216 nm derived from unsaturated monoterpenic acid moieties allowed a convenient detection for these saponins.[26, 27]



Scheme 1: Structures of Julibrosides from *Albizia julibrissin*

No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	C-6
5	OH	H	OH	OH	S_1	H	H	S_3		H	Qui	R
6	OH	H	OH	OH	S_1	H	H	S_3		H	Qui	S
7	OH	Me	OH	OH	S_1	H	H	S_3		H	Qui	R
8	OH	Me	OH	OH	S_1	H	H	S_3		H	Qui	S
9	OH	H	OH	OH	S_1	S_3	H	H		H	Qui	R
10	OH	H	OH	OH	S_1	S_3	H	H		H	Qui	S
11	OH	Me	NHAc	OH	S_1	H	H	S_3		H	Qui	R
12	OH	Me	NHAc	OH	S_1	H	H	S_3		H	Qui	S
13	OH	H	OH	H	S_1	H	H	S_3		H	Qui	R
14	OH	H	OH	H	S_1	H	H	S_3		H	Qui	S

15	OH	Me	OH	H	S_1	H	H	S_3		H	Qui	R
16	OH	Me	OH	H	S_1	H	H	S_3		H	Qui	S
17	OH	H	OH	OH	S_1	H	S_3	H		OH	H	
18	OH	H	OH	OH	S_1	H	H	S_3		OH	H	
19	OH	H	OH	OH	S_1	H	S_3	H		H	Qui	R
20	OH	Me	NHAc	OH	S_1	H	S_3	H		OH	H	
21	OH	Me	NHAc	OH	S_1	H	H	S_3		OH	H	
22	OH	H	OH	OH	S_2				H			
23	OH	H	OH	OH	S_2[1]	H	H	S_3	H	H	S_2	R
24	OH	H	OH	OH	S2				S_3	OH	H	
25	OH	Me	OH	OH	S_1	H	H	S_3	H	H	S_2	R
26	OH	H	NHAc	OH	S_1	H	H	H				
27	OH	H	NHAc	OH	S_1	H	H	S_3		OH	H	
28	OH	Me	NHAc	H	S_1	H	H	S_3		H	Qui	S
29	OH	H	NHAc	OH	S_1	H	H	S_3		H	Qui	R
30	H	H	OH	OH	S_1	H	H	H				
31	OH	H	OH	OH	S_1	H	H	H				
32	OH	Me	OH	OH	S_1	H	H	S_3		OH	H	
33	OH	Me	Glc	H	S_1	H	H	S_3		H	Qui	S

Structures of triterpenoid saponins from Cortex Albiziae

Triterpenoid saponins are composed of sugars and aglycones, i.e. triterpenes. In saponins from *A. julibrissin*, the aglycone is usually a triterpene with a skeleton of oleanolic acid: acacic acid (**1**), except for three other sapogenins: 16-deoxy acacic acid (**2**),[28] 30-hydroxyl acacic acid (**3**) and 30-carboxyl acacic acid (**4**).[29] To date, only three Julibrosides are revealed to be composed of aglycones **2**, **3** and **4**. The aglycones are linked to three sugar chains at their C-3, C-21 and C-28 positions in these saponins. Few saponin has been found to be attached to C-16 hydroxyl group of the aglycone.[29] The monosaccharide moieties in these saponins include β-D-glucopyranosyl, β-D-xylopyranosyl, β-D-quinovopyranosyl, β-D-fucopyranosyl, 2-deoxy-2-acetamido-β-D-glucopyranosyl, α-L-rhamnopyranosyl, α-L-arabinopyranosyl and α-L-arabinofuranosyl. Besides the above structure units, esterified monoterpenic acids were found to be popular in the saponins from *A. julibrissin*. The diversity of saponin structures in this plant seemed to be mainly derived from an alternative configuration at C-6 position of the outer monoterpenic acid moiety (S_3 in Scheme 1) in the substituted group at C-21 site of the aglycone,[26] different esterification positions of the inner β-D-quinovopyranosyl moiety (S_1 in Scheme 1) in the substituted group at C-21 site of the aglycone,[27] a substitution of acetamido group for 2-hydroxyl group of β-D-glucopyranosyl moiety (R_3 in Scheme 1) in C-3 sugar chain,[23, 30] etc. However, a sugar chain composed of four sugar moieties attached to C-28 position of the aglycone was little subjected to any modification when the carboxyl group in C-28 position of the aglycone was glycosylated. Although a variety of structural modifications were found all over the substituted group at the C-21 side chain of the aglycone, a monoterpenic acid moiety was showed to be linked directly to C-21 site of the aglycone for most of Julibrosides.[23-28, 30]

Structural elucidation of triterpenoid saponins from Cortex Albiziae

1. Molecular ions observed in their mass spectra

The mass spectra for most of Julibrosides were recorded on a FAB MS machine in a negative or positive mode. In such a case, an interesting phenomenon was observed in FAB mass spectra of

Julibrosides. The positive FAB mass spectra of Julibrosides usually showed quasi-molecular ion peaks at *m/z* $[M+Na+2]^+$, or $[M+Na+1]^+$, other than at *m/z* $[M+H]^+$, $[M+Na]^+$ or $[M+K]^+$. In order to obtain a normal molecular or quasi-molecular ion peak, ESI-MS and TOF MS were used to record their MS spectra. In case of Julibroside J_1, however, the quasi-molecular ion peak at *m/z* 1077.6 [M-2]- was observed in its ESI-MS, and the quasi-molecular ion peak at *m/z* 2195 $[M+K]^+$ was observed in its TOF-MS.[26] A possible explanation for this quasi-molecular ion peak at *m/z* $[M+Na+2]^+$, or $[M+Na+1]^+$ was derived from an introduction of carbon-13 isotope in these saponins, such as Julibroside II,[31] whose high resolution FAB mass spectrum gave a quasi-molecular ion peak at *m/z* 2179.0342 $[M+Na]^+$, corresponding to a molecular formula of $^{12}C_{101}{}^{13}CH_{162}O_{48}Na$.

2. Aglycones

Upon acidic hydrolysis with 2.0 M HCl, Julibrosides gave their sapogenins. In case of Julibroside J_1, the major saponin in this plant, its aglycone (acacic acid lactone) was identified by its ^{1}H NMR and EIMS data. The acacic acid moiety in Julibrosides was well represented by its ^{13}C-NMR data, which were subjected to little variation from one to another Julibroside. However, a complete elucidation of 1D and 2D NMR spectra for these saponins revealed a false assignment of carbon-13 signals for 24- and 25-methyl carbons given in many literatures,[23, 24, 31, 32] which was revised as δ 17.1 and 15.8 ppm, respectively.[28]

In case of 16-deoxy acacic acid moiety (**1**, **30**),[28] marked downfield shifts of 7.1 ppm for C-15, 49.1 ppm for C-16, and an upfield shift of 3.0 ppm for C-17 were observed in its ^{13}C-NMR spectrum. Furthermore, the signals of C-19, C-27, C-18 and C-28 undergo downfield shifts of 1.3 ppm, 1.4 ppm, and upfield shifts of 0.5 ppm, 0.5 ppm, respectively. It is worthwhile to be mentioned that the angular methyl proton signal at C-27 position displayed a distinct upfield shift of 0.52 ppm, from δ1.87 ppm to δ1.35 ppm, which afforded a characterization of 16-deoxy acacic acid moiety, since few other proton signals could be found nearby in the 1H NMR spectra of Julibrosides.

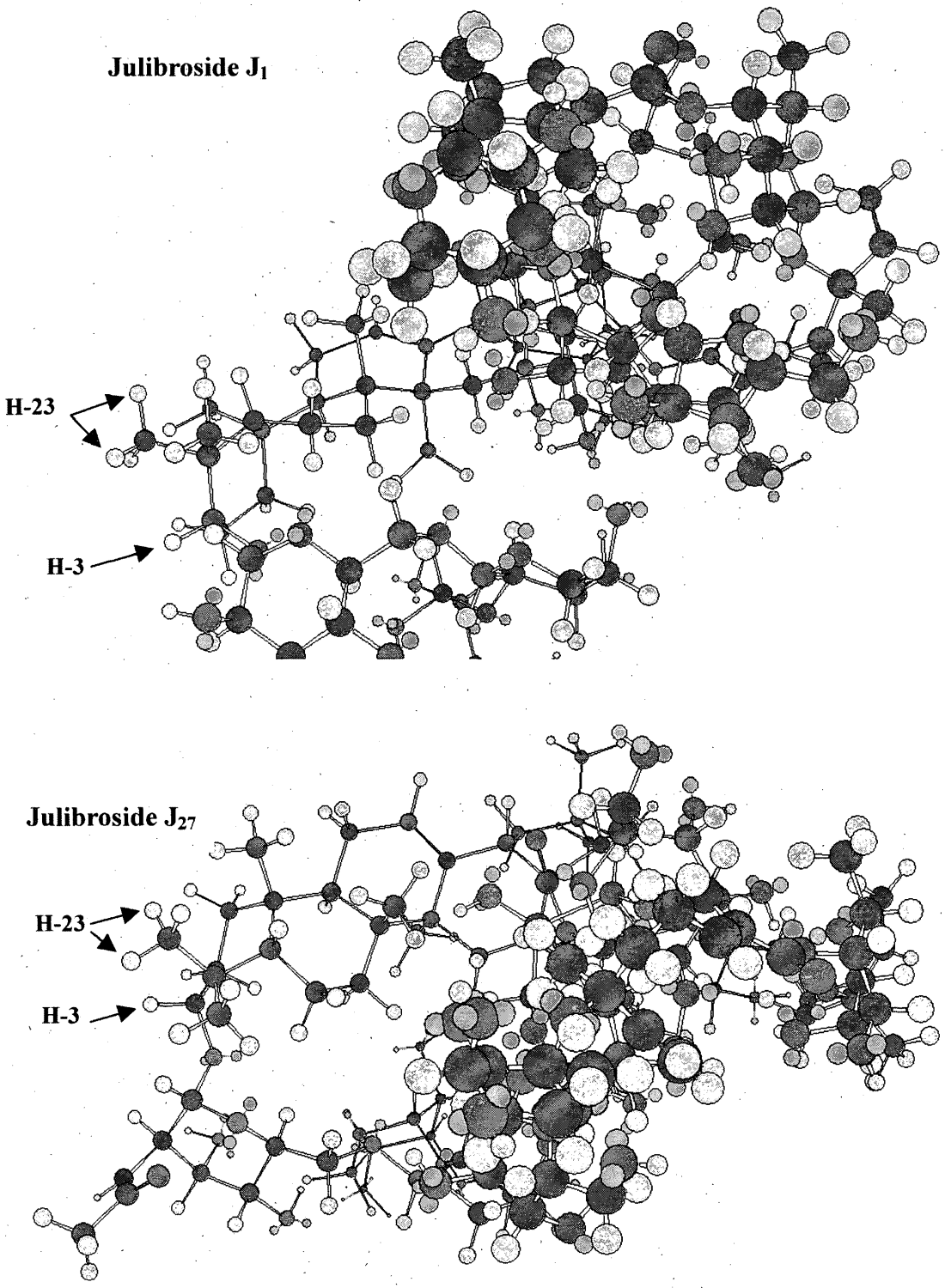
Generally, an introduction of 30-hydroxyl group (**3**) mainly influences the signals of carbons in the E-ring, i.e. C-19, 20, 21, 29 and 30, which showed -4.7 ppm, +4.8 ppm, -2.9 ppm, -1.5 ppm and +40.5 ppm of chemical shifts in comparison with those of acacic acid moiety.[29] Similarly, a substitution of a carbonyl group for a methyl group at C-30 of the aglycone gives rise to 5.0, 4.4 and 6.4 ppm of upfield shifts for C-19, -21 and -29, respectively, and 11.4 ppm of a downfield shift for C-20.[29]

3. Monosaccharides and sugar chains

Upon acidic hydrolysis of Julibrosides with 2.0 M HCl, monosaccharides were obtained from the hydrolyte, which were dried and subjected to derivation with hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine. The monosaccharides and their ratios were determined by the comparison of these derivatives with standards by GLC methods.[26]

2-Deoxy-2-acetamido-β-D-glucopyranosyl moiety was found to be present in many saponins isolated from *Albizia* and *Acacia* species, and showed good activities toward several cancer cell lines.[23, 31, 33, 34] This group is characteristic of its carbon-13 signals at δ 58.0 ppm for the amination carbon, δ 170.0 and 24.0 ppm for the acetyl carbons, also its proton signals at δ 2.10 ppm for the methyl group, δ 8.88 (1H, J = 7.8 Hz) for the amide proton. Sometimes, the doublet peak for the amide proton appeared as a broad single peak when the NMR condition was not functioned well.[35] Eight saponins with this moiety were isolated from *A. julibrissin* in our experiment (**11**, **12**, **20**, **21**, **26**-**29**). A comparison of ^{1}H-NMR spectrum of Julibroside J_1 (**5**) with that of Julibroside J_3 (**29**) revealed that the proton signal of H-23 in **29** undergoes an upfield shift of *ca.* 0.13 ppm, from δ 1.28 ppm to δ 1.15 ppm, where the 26-methyl protons is usually located, which make a distinguish between them from a view of methyl proton shapes in their proton NMR spectra.[30] A report on the

conformation of triperpene saponins in solutions pointed out that C-3 sugar chains had the most stable conformation among the sugar chains.[36] Therefore, the flexibility and distortion of C-3 sugar chains were greatly confined. When a huge group, such as an acetamido group, was introduced around the A-ring of the aglycone, the 23-methyl group was rejected by this space hindrance, resulting in an increase of the electron cloud density of 23-methyl protons, which was theoretically derived from a closer distance between H-23 and H-3 calculated by Chem3D software 5.0 for Julibroside J_3 (**29**) (2.21) than that for Julibroside J_1 (**5**) (2.45), although the distances between H-23 and H-24, H-23 and H-23 for these two compounds did not show marked differences (Scheme 2).[37]



Scheme 2: 3D structures of Julibrosides J_1 and J_{27}

The β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosyl-, β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-fucopyranosyl and β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1 → 6)-2-deoxy-2-acetamido-β-D-glucopyranosyl moieties were the main types of sugar chains glycosylated at 3-hydroxyl group of aglycones. A substitution of a β-D-fucopyranosyl moiety for a α-L-arabinopyranosyl moiety was easily recognized by a sharp methyl proton signal at δ 1.47 ppm and one more methyl carbon signal at δ 17.4 ppm, due to a methyl group of the β-D-fucopyranosyl moiety. Interestingly, this substitution was also represented by small, but distinct, downfield shifts of 0.80 ppm and 0.10 ppm, for the anomeric carbon and proton signals of β-D-xylopyranosyl moiety, respectively.[38]

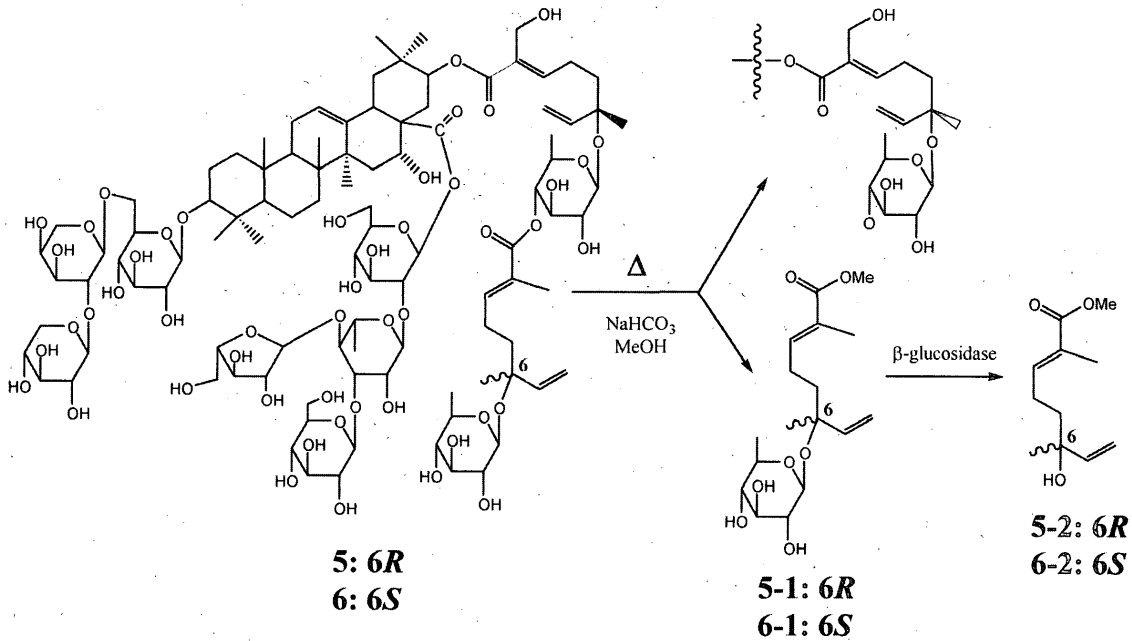
In several saponins (**22-25**), a β-D-fucopyranosyl-monoterpenic acid ester moiety was found to be linked to their C-21 side chains.[38,39] A similar side chain was found to be present in saponins isolated from the branches of *Calliandra anomala*.[40,41] It's typical of its anomeric carbon signal between δ 100.1 and 100.7, where no other peaks were located in the ^{13}C NMR spectra of Julibrosides.

4. Absolute configuration of C-6 in the outer monoterpenic acid moiety

Six pairs of diastereoisomers: **5** and **6**,[24,26] **7** and **8**,[37] **9** and **10**,[42] **11** and **12**,[35] **13** and **14**, **15** and **16**,[37] were obtained in our experiment, which appeared pairwise in their HPLC chromatograms. The common difference between a pair of diastereoisomer was derived from the absolute configuration of C-6 position in S_3 moiety (Scheme 1). In order to elucidate the absolute configuration of C-6 site in S_3 moiety, **5** and **6** were hydrolyzed with saturated $NaHCO_3$ in methanol, affording to **5-1** and **6-1** (Scheme 3), respectively. The products were further hydrolyzed with β-glucosidase at room temperature, giving rise to **5-2** and **6-2**, respectively, whose optical values were identical with those of (6*R*)-methiafolic acid and (6*S*)-methiafolic acid. Therefore, the absolute configurations of C-6 position in S_3 moieties for **5** and **6** were assigned as *R* and *S*, respectively.

Several plants were reported to contain constituents composed of S_3 moiety.[32-34,40,42-49] However, most of them only bear a 6*R* configuration, few plants were reported to contain compounds consisted of 6*R* and 6*S* configurations together.[42,44] In cases of **5**, **7**, **9**, **11**, **13** and **15**, (6*R*) and (6*S*)-methiafolic acid moieties are present in a compound together.

Saponins **5** and **6** possess markedly different ^{13}C NMR data due to differences in the S_3 moiety. A comparison of the ^{13}C-NMR data of **6** with those



5: 6*R*
6: 6*S*

5-1: 6*R*
6-1: 6*S*

5-2: 6*R*
6-2: 6*S*

Scheme 3: Degeneration of Julibrosides J_1 and J_9

of **5** showed that the signals of C-5 and C-10 sites in S_3 of **6** undergo a downfield shift of 1.8 ppm and an upfield shift of 1.3 ppm, respectively. The similar results were observed for all other five pairs of saponins, which were identical with the ^{13}C-NMR data of (6*R*)-methiafolic acid and (6*S*)-methiafolic acid, which were obtained from alkaline hydrolysis in H_2O, followed by β-glucosidase hydrolysis of **7** and **8**.[37]

5. Esterification positions of the inner ß-D-quinovopyranosyl moiety (S1)

There are three positions available for esterification in the S_1 moiety. Most of saponins bear S_1 moieties esterified at C-4 hydroxyl group, three of them (**17**, **19** and **20**) at C-3 hydroxyl group, and two of them (**9** and **10**) at C-2 hydroxyl group. Saponins with different esterification positions demonstrated markedly different chromatographic properties. When saponins share common structural units, they appear successively in Rp-18 HPLC in the order of C-3, C-4 and C-2 types, such as saponins **5**, **9** and **19**. The spectroscopic differences due to different esterification positions were well recognized from their proton and carbon data. Saponins **9** and **10** were characteristic of carbon-13 signals at δ 96.9 ppm, due to the anomeric carbon of the S_1 moiety.[50] ^{1}H-NMR spectra of saponins **17**, **19** and **20** showed a distinct triplet peak at δ 5.74 ppm due to H-3 proton of the S_1 moiety.[27, 51]

6. Monoterpenic acid moieties

When the R_4 group was substituted for a hydroxyl group in a saponin, its polarity increased greatly. Although a hydroxymethyl structure unit was found to be present in the inner monoterpenic acid moiety for most of saponins, seven of them (**17**, **18**, **20**, **21**, **24**, **27** and **32**) bear this unit in their outer monoterpenic acid moieties, i.e. S_3 group.[27, 30, 37, 52] This hydroxylation greatly shifted the signals of olefinic carbons neighboring to C-9, however, the signal due to the carbonyl carbon was little changed, despite of its locating nearby. In addition, the presence of a free hydroxyl group in C-6 of S_3 moiety was always accompanied with a hydroxylation of C-9 in the inner monoterpenic acid moiety. When it was subjected to glycosylation with a quinovose, besides some normal shifts of signals due to C-5, -6, -7, -8 and -10 carbons of S_3 moiety, an unordinary but marked shift of a proton signal due to H-3 proton of S_3 moiety was observed, from δ 6.32 to 6.04 ppm, which was probably attributed to a hindrance effect ascribed to an introduction of a huge size of substitution group, i.e. quinovose.[52]

Biological activities of triterpenoid saponins from Cortex Albiziae

1. Antitumor

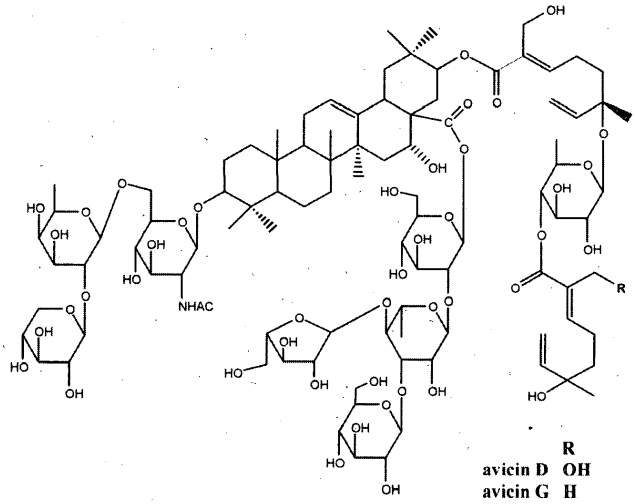
So far, most of activities reported for Julibrosides were related to the cytotoxicity against human cancer lines. The cytotoxicity of ten Julibrosides (**5**, **6**, **7**, **8**, **15**, **18**, **21**, **23**, **27** and **32**) against Bel-7402, KB, BGC and HL-60 human cancer lines were observed by SRB method. Although an α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1 → 3)]-a-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl ester unit and a monoterpene quinovopyranosyl moiety were thought to be the crucial groups for cytotoxicity among this class of compounds,[52] two of 10 saponins, which bear these crucial groups, did not showed any marked cytotoxicity towards the four human cancer cell lines mentioned above at 10.0 micromoles by the SRB method. All the Julibrosides tested in our experiment showed better activity against KB cell line than other three human cell lines, and showed no marked inhibitory action against HL-60 cell line even at 10.0 micromoles. The important role of 2-acetamide group in C-3-β-D-glucopyranosyl moiety was revealed by the fact that all the saponins with this group showed a good cytotoxicity, furthermore, the strongest activity was demonstrated by this kind of saponin.[26, 53]

The antitumour activity of a mixture of Julibrosides was observed *in vivo*. Several groups of Swiss rats were inoculated subcutaneously with S_{180} tumour, then fed orally with normal saline, cyclophosphamide and Julibroside mixture,

respectively. 0.7, 1.0 and 1.8 mg/kg of this mixture showed 25.4%, 35.1% and 40.5% inhibitory action against S_{180} growth, respectively, and the inhibitory action of 1.8 mg/kg of this mixture is similar to that of 10.0 mg/kg of cyclophosphamide.[54]

Recently, a detailed investigation on the anticancer action of two analogues to Julibrosides was carried out by Gutterman and his colleagues.[55] These analogues were named as Avicins D and G (Scheme **4**), and the structure of Avicin D was elucidated as the same as that of **21**, i.e. Julibroside J_{19}.[30, 56] The mixture of triterpenoid saponins and Avicins were demonstrated to induce apoptosis in the Jurkat human T cell line by affecting the mitochondrial function. Avicin G induced cytochrome c release within 30-120 min in whole cells and within a minute in the cell-free system. Caspase inhibitors DEVD or zVAD-fmk had no effect on cytochrome c release, suggesting the direct action of Avicin G on the mitochondria. Activation of caspase-3 and total cleavage of poly (ADP-ribose) polymerase (PARP) occurred between 2 and 6 h posttreatment with Avicins by zVAD-fmk. Interestingly, in the treated cells no significant changes in the membrane potential preceded or accompanied cytochrome c release. A small decrease in the generation of reactive oxygen species (ROS) was measured.

In order to evaluate the ability of Avicins to inhibit chemically induced mouse skin, carcinogenesis varying doses of Avicins were applied to shaved



Scheme 4: Structures of Avicins D and G

dorsal skin of SENCAR mice 15 min before application of 100 nmol of 7,12-dimethylbenz [α] anthracene (DMBA) twice a week for 4 weeks (complete carcinogenesis model). 57 The dorsal skin of a second group of mice was treated with one dose of 10 nmol of DMBA. Avicins were then applied 15 min before repetitive doses of 2 mg of phorbol 12-tetradecanoate 13-acetate (TPA) twice a week for 8 weeks (initiation/promotion model). At 12 weeks, Avicins produced a 70% decrease in the number of mice with papillomas and a greater than 90% reduction in the number of papillomas per mouse in both protocols. It was also observed: a 62% and 74% reduction by Avicins in H-*ras* mutations at codon 61 in the DMBA and DMBAy TPA models, respectively, as well as a significant inhibition of the modified DNA base formation (8-OH-dG) in both protocols. Marked suppression of aneuploidy occurred with treatment at 16 weeks in the initiation/promotion experiment. These findings, when combined with the proapoptotic property of these compounds and their ability to inhibit hydrogen peroxide (H_2O_2) generation, nuclear factor-κB (NF-κB) activation, and inducible nitric oxide synthase (iNOS) induction reported elsewhere, suggest that Avicins could prove exciting in reducing oxidative and nitrosative stress and thereby suppressing the development of human skin cancer and other epithelial malignancies.

The effect of F094 (an Avicin mixture) and Avicin G was examined on tumor necrosis factor (TNF)-induced activation of NF-κB in Jurkat cells (human T cell leukemia).[58] Both F094 and Avicin G were found to be potent inhibitors of TNF-induced NF-κB. Treatment of Jurkat cells with Avicin G resulted in a much slower accumulation of the p65 subunit of NF-κB into the nucleus whereas the degradation of IκBα was unaffected. Avicin G also impaired the binding of NF-κB to DNA in *in vitro* binding assays. Treatment of cells with DTT totally reversed the Avicin G-induced inhibition of NF-κB activity, suggesting that sulfhydryl groups critical for NF-κB activation were being affected. Avicin G treatment resulted in decreased expression of NF-κB-regulated proteins such as iNOS and

cyclooxygenase (COX-2). Thus, the Avicins may prove important for reducing both oxidative and nitrosative cellular stress and thereby suppressing the development of malignancies and related diseases. Another triterpene saponin mixture, named as F035,[47] and the Avicins markedly inhibited the growth of several tumor cell lines with minimum growth inhibition in human foreskin fibroblasts, mouse fibroblasts, and immortalized breast epithelial cells at similar concentrations. F035 and the Avicins induced cell cycle (G1) arrest of the human MDA-MB-453 breast cancer cell line and apoptosis of the Jurkat (T-cell leukemia) and the MDA-MB-435 breast cancer cell line. The triterpenoid saponins also partially inhibited phosphatidylinositol 3-kinase activity in Jurkat T cells in a time-dependent manner and phosphorylation in the downstream protein Akt, whereas no affect was seen on the Ras/mitogen-activated protein kinase cascade. These observations as well as other work from their previously demonstrating mitochondrial perturbation, chemoprevention, and inhibition of NF-kB suggest that triterpenoid saponins from *A. victoriae* have potential as novel anticancer agents. Recent work linking Akt signaling with glucose metabolism, stress resistance, and longevity suggests other potential applications of these compounds.

2. Antiinflammatory and antibacterial action

The antiinflamatory action of a mixture composed of Julibrosides was observed. The mouse ear edema induced by croton oil was markedly decreased by spreading this mixture on mouse ears at a concentration of 1.8 μg/ml, which is approximately equivalent to that of similar dosage of Aspirin. Meanwhile, inhibitory action of a mixture of Julibrosides against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* was examined. This saponin mixture showed a marked inhibitory action against *Pseudomonas aeruginosa,* a moderate strength of inhibition against *Staphylococcus aureus*, but little action against the other two bacteria.[59]

3. Others

The acute toxicity of total Julibrosides was examined according to the procedures described in Append V of "Assessment Procedures of New Drugs in China". The LD_{50} value of this mixture was measured as 1.132 g/kg by Bliss method. A bi-directional adjustment of a Julibroside mixture was observed in the splenocyte proliferation of mice, i.e. stimulation at low concentration (0.1 ng/ml), inhibition at high concentration (100.0 ng/ml).[59] Cortex Albizae is recorded as a sedation agent in Chinese Pharmacopoeia, and Julibrosides are its major constituents in composition, nevertheless, several research groups failed to prove a positive sedative activity of Julibroside mixtures, estimated from their prolonging sleep time of mice following sodium pentobarbital anesthesia, mixtures.[22, 60] Meanwhile, a sedative compound was isolated from this medicine, i.e. syringaresinol diglucoside, a lignan glucoside, other than a saponin.[61, 62]

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