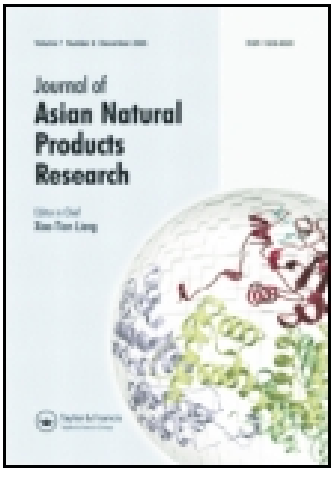


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Zhi Yu[a], Xie-Shu Wang[a] & Ze-Hua Hu[b]

[a] College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan, 430070, China

[b] School of Medicine, Hubei University for Nationalities, Enshi, 445000, China

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# Dammarane-type saponins from *Ziziphus jujube*

Zhi Yu[a]*, Xie-Shu Wang[a] and Ze-Hua Hu[b]

[a]*College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan 430070, China;* [b]*School of Medicine, Hubei University for Nationalities, Enshi 445000, China*

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Two new dammarane-type saponins jujubosides D and E, together with three known compounds, were isolated from the seeds of *Ziziphus jujube*. Their structures were elucidated on the basis of chemical and spectroscopic evidence. Compounds **1**–**5** showed lipoxygenase-inhibiting activity.

**Keywords:** *Ziziphus jujube*; dammarane-type saponins; jujuboside D; jujuboside E; lipoxygenase-inhibiting activity



## 1. Introduction

Plants of the *Ziziphus* genus (Rhamnaceae) are commonly used as edible or medicinal plants in warm and tropical regions [1]. They are well known for its content of triterpenes, saponins, and cyclopeptide alkaloids [2]. *Ziziphus jujube* Mill. is a thorny rhamnaceous plant which is widely distributed in northern China. Its seeds have been used as a traditional Chinese medicine for treatment of insomnia. In previous phytochemical investigation, a series of dammarane saponins have been isolated [3,4]. Some of them showed inhibitory activity against histamine release and anti-sweet activity [4,5]. In the course of our studies on the bioactive saponins of natural medicines, we investigated the chemical constituents and biological activities of this plant. In this article, we report the isolation and structure elucidation of two new saponins jujubosides D and E (Figure 1), as well as their lipoxygenase-inhibiting activity.

## 2. Results and discussion

The EtOH extract of dried seeds of *Z. jujube* was partitioned between $H_2O$ and *n*-BuOH. The *n*-BuOH extract was separated by repeated column chromatography using silica gel, $C_{18}$ silica gel, and HPLC to give two new compounds jujuboside D (**1**) and jujuboside E (**2**), together with three known compounds, jujuboside A (**3**) [6], jujuboside C (**4**) [6], and jujuboside $A_1$ (**5**) [5]. Their structures were elucidated on the basis of chemical and spectroscopic evidence.

Compound **1** was isolated as a white, amorphous powder. The HR-ESI-MS (positive-ion mode) experiment revealed a pseudo-molecular ion peak $[M + Na]^+$ at *m/z* 1391.6466, in agreement with the molecular formula $C_{64}H_{104}O_{31}$. The aglycone was assigned as jujubogenin on the basis of the $^1H$ and $^{13}C$ NMR spectra and homo- and heteronuclear correlations observed in $^1H$–$^1H$ COSY, HSQC, and HMBC experiments. Most of the $^{13}C$ NMR signals were assigned through $^2J_{H-C}$ and $^3J_{H-C}$ couplings of the seven methyls and are in agreement with literature data [4–6] (Table 1). The downfield shifts of C-3 at $\delta_C$ 89.8 suggested that **1** was a monodesmosidic saponin. The ROESY correlations between H-17, Me-21, and H-22b and between H-22a and H-23 and

*Corresponding author. Email: yuzhi198011@126.com

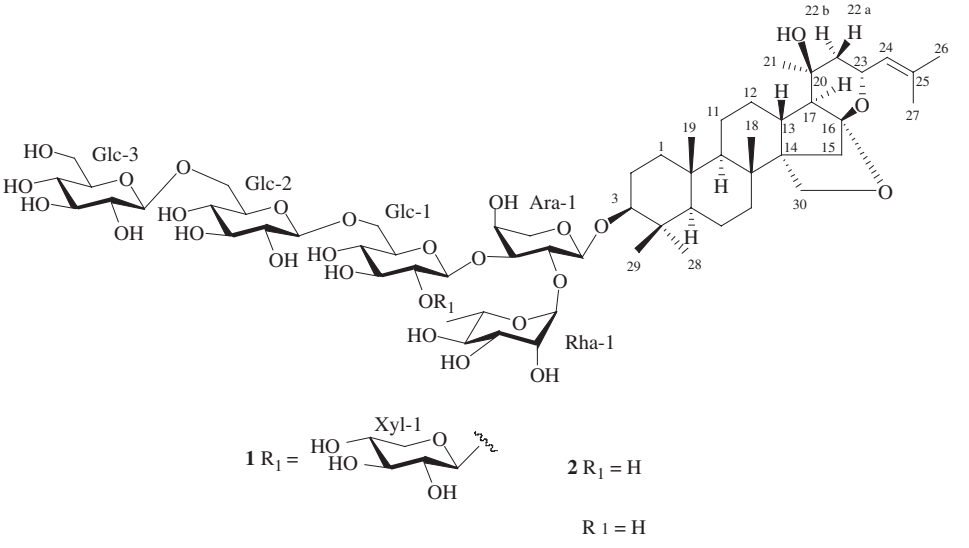


Figure 1. Structures of compounds **1** and **2**.

the magnitude of the coupling constant between H-13 and H-17 ($J = 7.0$ Hz) are in agreement with the configuration of rings D–F of jujubogenin [6,7]. The monosaccharides obtained after aqueous acid hydrolysis of **1** were identified as glucose, rhamnose, arabinose, and xylose by TLC comparison with authentic samples. The absolute configuration of the monosaccharides was determined to be D for glucose and xylose and L for rhamnose and arabinose by GC analysis of chiral derivatives of the monosaccharides in the hydrolysate of each compound (see Section 3). Complete assignment of each sugar proton system was achieved by considering $^{1}$H–$^{1}$H COSY and TOCSY spectra, while glycosidic carbons were assigned from the HSQC spectrum (Tables 1 and 2). Evaluation of spin-spin couplings and chemical shifts allowed the identification of three β-glucopyranosyl (Glc), one α-rhamnopyranosyl (Rha), one β-xylopyranosyl (Xyl), and one α-arabinopyranosyl moieties (Ara). In the HMBC spectrum, the anomeric proton signals at $\delta_H$ 4.42 (Ara-1), 5.12 (Rha-1), 4.50 (Glc-1), 4.62 (Xyl-2), 4.31 (Glc-2), and 4.36 (Glc-3) showed cross-peaks with the carbon signals at $\delta_C$ 89.8 (Aglycone-C-3), 75.8 (Ara-1-C-2), 68.6 (Ara-1-C-4), 82.6 (Glc-1-C-2), 69.8 (Glc-1-C-6), and 68.2 (Glc-2-C-6), respectively. These signals provide evidence to determine the linkages between the sugars, and between the sugar and the aglycone. From the above evidence, the structure of **1** was established as 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-[(1 → 2)-β-D-xylopyranosyl)]-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl jujubogenin, which has been named jujuboside D.

Compound **2** was isolated as a white, amorphous powder. The HR-ESI-MS (positive-ion mode) experiment revealed a pseudo-molecular ion peak $[M + Na]^+$ at *m/z* 1259.6041, in agreement with the molecular formula $C_{59}H_{96}O_{27}$. The spectroscopic properties of **2** were closely related to those of **1**; however, signals due to the xylopyranosyl moiety linked to Glc-1-C-2 in **1** were absent for **2**. This observation was supported by a relative upfield shift of Glc-1-C-2 of **2** at $\delta_C$ 75.1 (Glc-1-C-2 of **1** at $\delta_C$ 82.6). Thus, the structure of **2** was determined as 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-gluco-

Table 1. $^{13}C$ NMR spectral data for the aglycone and sugar moieties of compounds **1** and **2** (125 MHz, in $CD_3OD$).

| No. | **1** | **2** | No. | **1** | **2** |
|---|---|---|---|---|---|
| Aglycone | | | Ara-1 | | |
| 1 | 39.8 | 39.8 | 1 | 104.6 | 104.6 |
| 2 | 27.2 | 27.2 | 2 | 75.8 | 75.7 |
| 3 | 89.8 | 89.8 | 3 | 82.2 | 82.1 |
| 4 | 40.2 | 40.1 | 4 | 68.6 | 68.7 |
| 5 | 57.4 | 57.4 | 5 | 63.8 | 63.7 |
| 6 | 19.0 | 19.0 | | | |
| 7 | 36.7 | 36.7 | Rha-1 | | |
| 8 | 38.2 | 38.2 | 1 | 101.7 | 101.5 |
| 9 | 54.0 | 54.0 | 2 | 72.1 | 71.9 |
| 10 | 37.9 | 37.8 | 3 | 72.0 | 72.1 |
| 11 | 22.3 | 22.3 | 4 | 73.8 | 73.4 |
| 12 | 30.3 | 30.3 | 5 | 70.4 | 70.5 |
| 13 | 37.9 | 37.9 | 6 | 18.1 | 18.2 |
| 14 | 54.2 | 54.2 | Glc-1 | | |
| 15 | 37.0 | 37.0 | 1 | 103.4 | 104.2 |
| 16 | 111.2 | 111.2 | 2 | 82.6 | 75.1 |
| 17 | 54.2 | 54.2 | 3 | 77.6 | 78.2 |
| 18 | 19.2 | 19.2 | 4 | 71.4 | 71.6 |
| 19 | 16.8 | 16.8 | 5 | 76.5 | 77.1 |
| 20 | 69.3 | 69.3 | 6 | 69.8 | 69.4 |
| 21 | 29.5 | 29.5 | Xyl-1 | | |
| 22 | 45.2 | 45.2 | 1 | 105.6 | |
| 23 | 70.1 | 70.1 | 2 | 75.3 | |
| 24 | 126.2 | 126.2 | 3 | 78.0 | |
| 25 | 136.5 | 136.5 | 4 | 70.8 | |
| 26 | 25.6 | 25.6 | 5 | 67.9 | |
| 27 | 18.2 | 18.2 | Glc-2 | | |
| 28 | 28.3 | 28.3 | 1 | 104.6 | 104.5 |
| 29 | 17.0 | 17.0 | 2 | 75.2 | 75.2 |
| 30 | 66.7 | 66.6 | 3 | 77.9 | 77.9 |
| | | | 4 | 71.2 | 71.1 |
| | | | 5 | 76.5 | 76.4 |
| | | | 6 | 68.2 | 68.1 |
| | | | Glc-3 | | |
| | | | 1 | 104.8 | 104.8 |
| | | | 2 | 74.8 | 74.9 |
| | | | 3 | 78.0 | 78.1 |
| | | | 4 | 71.2 | 71.1 |
| | | | 5 | 78.0 | 78.0 |
| | | | 6 | 62.7 | 62.6 |

pyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl jujubogenin, which has been named jujuboside E.

The inhibitory activity of **1**–**5** against lipoxygenase was determined using the method described in Section 3 (Table 3). These compounds are potent inhibitors of lipoxygenase.

## 3. Experimental

### *3.1 General experimental procedures*

Optical rotations were determined on a JASCO P-1020 digital polarimeter (JASCO

Table 2. $^1$H NMR spectral data for the aglycone and sugar moieties of compounds **1** and **2** (500 MHz, in $CD_3OD$).

| No. | | No. | **1** | **2** | No. | **1** | **2** |
|---|---|---|---|---|---|---|---|
| Aglycone | | Ara-1 | | | Xyl-1 | | |
| 1 $CH_2$ | 1.65, 0.94 2 m | 1 | 4.42 d 5.0 | 4.41 d 4.5 | 1 | 4.62 d 8.0 | |
| 2 $CH_2$ | 1.87, 1.74 2 m | 2 | 3.95 dd 7.0, 5.0 | 3.94 dd 7.0, 4.5 | 2 | 3.42 dd 9.0, 8.0 | |
| 3 CH | 3.07 dd 12.5, 4.0 | 3 | 3.97 dd 7.0, 4.5 | 3.98 dd 7.0, 4.5 | 3 | 3.61 m | |
| 5 CH | 0.78 d 8.5 | 4 | 4.01 m | 4.03 m | 4 | 3.51 m | |
| 6 $CH_2$ | 1.58, 1.50 2 m | 5a | 3.50 d 10.0 | 3.51 d 10.0 | 5a | 3.90 dd 10.5, 4.5 | |
| 7 $CH_2$ | 1.57, 1.51 2 m | 5b | 3.84 d 10.0, 5.0 | 3.83 d 10.0, 5.0 | 5b | 3.20 d 10.5 | |
| 9 CH | 0.91 m | Rha-1 | | | | | |
| 11 $CH_2$ | 1.67, 1.52 2 m | 1 | 5.12 br s | 5.11 br s | Glc-2 | | |
| 12 $CH_2$ | 1.84, 1.69 2 m | 2 | 4.10 d 3.0 | 4.12 m | 1 | 4.31 d 8.0 | 4.34 d 8.0 |
| 13 CH | 2.50 m | 3 | 3.71 dd 8.5, 3.0 | 3.70 dd 8.0, 4.0 | 2 | 3.41 m | 3.28 m |
| 15 $CH_2$ | 2.10, 1.21 d 8.5 | 4 | 3.50 dd 8.5, 9.0 | 3.42 dd 8.0, 9.0 | 3 | 3.35 m | 3.20 m |
| 17 CH | 0.98 d 7.0 | 5 | 3.81 m | 3.77 m | 4 | 3.29 m | 3.32 m |
| 18 Me | 1.12 s | 6 | 1.20 d 6.0 | 1.21 d 6.0 | 5 | 3. 51 m | 3. 42 m |
| 19 Me | 0.89 s | Glc-1 | | | 6a | 4.10 dd 11.0,5.0 | 4.07 dd 10.0,4.5 |
| 21 Me | 1.13 s | 1 | 4.50 d 7.0 | 4.42 d 7.5 | 6b | 4.51 d 11.0 | 4.48 d 10.0 |
| 22 $CH_2$ | 1.45, 1.39 2 m | 2 | 3.51 m | 3.27 m | Glc-3 | | |
| 23 CH | 4.71 m | 3 | 3.41 m | 3.37 m | 1 | 4.36 d 8.0 | 4.37 d 7.5 |
| 24 CH | 5.12 m | 4 | 3.34 m | 3.30 m | 2 | 3.30 m | 3.32 m |
| 26 Me | 1.71 s | 5 | 3.62 m | 3.52 m | 3 | 3.36 m | 3.34 m |
| 27 Me | 1.70 s | 6a | 3.69 dd 10.0, 6.0 | 3.71 dd 10.0, 6.0 | 4 | 3.31 m | 3.33 m |
| 28 Me | 1.08 s | 6b | 4.20 d 10.0 | 4.13 d 10.0 | 5 | 3.29 m | 3.28 m |
| 29 Me | 0.83 s | | | | 6a | 3.53 br d 12.0 | 3.51 br d 12.0 |
| 30 $CH_2$ | 4.01, 3.95 d 12.0 | | | | 6b | 3.81 br d 12.0 | 3.82 br d 12.0 |

Table 3. Inhibitory effects of compounds **1**–**5** against lipoxygenase.

| Compound | $IC_{50}$ (μM) |
|---|---|
| **1** | 56.7 |
| **2** | 53.9 |
| **3** | 61.2 |
| **4** | 48.9 |
| **5** | 71.3 |
| Baicalein | 21.4 |



Corporation, Tokyo, Japan). IR spectra were recorded on an Avater-360 spectrometer (Thermo Scientific, Waltham, MA, USA). NMR spectra were recorded on a Bruker INOVA 500 spectrometer (Bruker, Waltham, MA, USA) with tetramethylsilane as internal standard. The HR-TOF-MS were acquired using an Agilent 6210 mass spectrometer (Agilent, Santa Clara, CA, USA). GC was employed on an Agilent 6890N gas chromatograph (Agilent). Chromatography was carried out on silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan), and C18 silica gel (150–200 mesh, Merck, Darmstadt, Germany), respectively. HPLC separation was carried out on an ODS column (250 × 10 mm, i.d. 5 μm, YMC, Kyoto, Japan) with an Alltech ES 2000 evaporative light scattering detector (Grace, Crutis Bay, MD, USA).

### 3.2 Plant material

The roots and rhizomes of *Z. jujube* were collected in August 2010 in Chengdu city, Sichuan Province of China. Identification of the plant was done by one of the authors (Z. Yu). A voucher specimen (ZJ 4403) is maintained in our laboratory.

### 3.3 Extraction and isolation

The dried seeds (4 kg) of *Z. jujube* were extracted with EtOH. The EtOH extract was concentrated under reduced pressure, and the viscous concentrate (491 g) was passed through a Diaion HP-20 column, successively eluting with 20% EtOH, 70% EtOH, and 90% EtOH. The 70% EtOH eluate fraction (224 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH–$H_2O$ (10:1:0, 5:1:0.1, 1:1:0.2) to afford fractions 1–4 (41, 37, 66, and 55 g, respectively). Fraction 2 was subjected to $C_{18}$ silica gel column chromatography and eluted with MeOH–$H_2O$ in a gradient of MeOH (MeOH–$H_2O$, 10:90 → 100:0%), to give subfractions 2a–2d. Subfraction 2c (1.6 g) was isolated by HPLC (MeOH–$H_2O$, 32:68, 2.0 ml/min; tube temperature 120°C, gas flow 2.5 l/min) to yield compounds **1** (r.t. 16.3 min, 51 mg) and **4** (r.t. 20.6 min, 27 mg). Subfraction 2d (2.5 g) was isolated by HPLC (MeOH–$H_2O$, 34:66, 2.0 ml/min; tube temperature 120°C, gas flow 2.5 l/min) to yield compounds **2** (r.t. 17.9 min, 61 mg), **3** (r.t. 19.5 min, 31 mg), and **5** (r.t. 21.7 min, 24 mg).

#### 3.3.1 *Jujuboside D (**1**)*

White amorphous power, $[\alpha]_D^{20}$ −46, ($c = 0.5$, MeOH); IR(KBr) $\nu_{max}$ ($cm^{-1}$): 3421, 1724, 1072; for $^1H$ and $^{13}C$ NMR spectral data, see Tables 1 and 2. HR-ESI-MS: *m/z* 1391.6466 $[M + Na]^+$ (calcd for $C_{64}H_{104}O_{31}Na$, 1391.6459).

#### 3.3.2 *Jujuboside E (**2**)*

White amorphous power, $[\alpha]_D^{20}$ −52 ($c = 0.5$, MeOH); IR(KBr) $\nu_{max}$ ($cm^{-1}$): 3421, 1724, 1072; for $^1H$ and $^{13}C$ NMR spectral data, see Tables 1 and 2. HR-ESI-MS: *m/z* 1259.6041 $[M + Na]^+$ (calcd for $C_{59}H_{96}O_{27}Na$, 1259.6037).

### 3.4 Acid hydrolysis

Compound **1** or Compound **2** (5.0 mg each) was heated in 5 ml of 10% HCl–dioxane (1:1) at 80°C for 4 h. After the dioxane was removed, the solution was extracted with

EtOAc (3 ml × 3) to yield the aglycone and the sugar, respectively. The aqueous layer was evaporated and dissolved in anhydrous pyridine (100 ml), 0.1 M L-cysteinemethyl ester hydrochloride (200 ml) was added, and the resultant mixture was warmed at 60°C for 1 h. The trimethylsilylation reagent (hexamethyldisilazane–trimethylchlorosilane–pyridine, 2:1:10; Acros Organics, Geel, Belgium) was added and warmed at 60°C for 30 min. To the mixture, the thiazolidine derivatives were analyzed by GC for sugar identification [8]. The retention times of L-arabinose (6.42 min), L-rhamnose (7.83 min), D-xylose (8.68 min), and D-glucose (11.36 min) were confirmed by comparison with those of authentic standards.

### 3.5 *Assay of lipoxygenase inhibition*

Lipoxygenase-inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed by Tappel [9]. Lipoxygenase type I-B and linoleic acid were purchased from Sigma Chemicals (Saint Louis, MO, USA). A mixture of 160 μl of 0.1 mM sodium phosphate buffer (pH 7.0), 10 μl of test compound solution, and 20 μl of lipoxygenase solution was incubated for 5 min at 25°C. The reaction was then initiated by the addition of linoleic acid 10 μl (substrate) solution, with the formation of (9Z, 11E, 13S)-13-hydroperoxyoctadeca-9,11-dienoate. The change in absorbance was followed for 10 min. Test compounds and the control were dissolved in 50% EtOH. All the reactions were carried out in triplicate. The $IC_{50}$ values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific, Inc., Amherst, MA, USA). Baicalein was used as a positive control.

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