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# Triterpenoidal glycosides from *Justicia betonica*

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**Abstract**

From the aerial portion of *Justicia betonica* L., four triterpenoidal glycosides (justiciosides A–D) were isolated. Their structures were established through chemical and NMR spectroscopic analyses as olean-12-ene-1β,3β,11α,28-tetraol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, olean-12-ene-1β,3β,11α,28-tetraol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, respectively.
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*Keywords:* *Justicia betonica*; Acanthaceae; Justiciosides A–D; Triterpenoidal glycoside; Olean-12-ene-1β; 3β; 11α; 28-tetraol; 11α-Methoxy-olean-12-ene-1β, 3β; 28-triol

## 1. Introduction

*Justicia betonica* L. (Acanthaceae; Thai name: Tri-Cha-Va, Hang-Kra-Rok) is an ornamental plant, commonly grown in Northeast of Thailand, but for which there is no ethnopharmacological use in Thai traditional medicine. However, its aerial parts are used in Indian traditional medicine as an anti-diarrhea medicine as well as an anti-inflammatory agent. Preliminary studies on plants in the genus *Justicia* have led to the isolation of several compounds such as lignans (Okigawa et al., 1970; Ghosal et al., 1979, 1980; Trujillo et al., 1990; Asano et al., 1996; Rajasekhar et al., 1998; Rajasekhar and Subbaraju, 2000) and an amide (Lorenz et al., 1999). However, there have been no reports on triterpenoidal glycosides of *Justicia* species, although a number of triterpenoidal glycosides were isolated from other genera in the family Acanthaceae. In our continuing studies on the chemical constituents of Acanthaceous plants (Kanchanapoom et al., 2001, 2002), we isolated four new triterpenoidal glycosides, justiciosides A–D (**1**–**4**, Scheme 1), from the aerial portion of this plant. The present paper deals with the isolation and structural elucidation of these compounds.

## 2. Results and discussion

Justicioside A (**1**) was obtained as an amorphous powder and determined as $C_{42}H_{70}O_{14}$ by HR-FAB mass spectrometry. Inspection of the $^{13}C$ NMR spectral data revealed the presence of two sugar units (anomeric carbons at $\delta$ 103.5 and 105.9) in addition to 30 carbon signals for the aglycone moiety. The appearance of seven tertiary methyl groups ($\delta$ 0.79, 0.80, 1.01, 1.05, 1.21,

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1.26 and 1.33) and one trisubstituted olefinic proton (δ 5.51) of the aglycone moiety in the $^1$H NMR spectrum, together with information from the $^{13}$C NMR spectrum (seven sp$^3$ carbon at δ 13.6, 16.0, 18.2, 23.7, 25.7, 28.6 and 33.2, and two sp$^2$ olefinic carbons at δ 126.1 and 147.0) indicated that the aglycone has an olean-12-ene skeleton (Doddrell et al., 1974). Enzymatic hydrolysis of **1** with crude hesperidinase afforded a new aglycone (**1a**) with a molecular formula $C_{30}H_{50}O_4$, and D-glucose which was identified by TLC and comparison of its optical rotation with an authentic sample. The structure of **1a** was established by analyzing the 1D- and 2D-NMR spectra (including HSQC and HMBC) in addition to the coupling constants in the $^1$H NMR spectrum. In the $^1$H NMR spectrum of **1a**, the signals due to seven methyls, three oxymethine protons, two oxymethylene protons, and one olefinic proton were clearly observed. The $^{13}$C NMR spectrum coupled with the DEPT experiments indicated the presence of seven methyls, nine methylenes, seven methines and seven quaternary carbons. All protonated carbons were determined by HSQC spectral analyses (Table 1). The oxymethylene protons at δ 3.56 and 3.81 (each *d*, *J* = 10.7 Hz) were assigned to H-28 on the basis of correlations with C-16 (δ 22.7), C-17 (δ 37.3) and C-18 (δ 41.8) in the HMBC spectrum (Fig. 1). The oxymethine proton at δ 3.94, which showed long-range correlations to C-25 (δ 13.6) and C-9 (δ 57.3) could be assigned to H-1. The proton signal at δ 3.58 was diagnostic for H-3, deducing from an HMBC correlation between H-23 (δ 1.24), H-24 (δ 1.08) and C-3 (δ 75.4). The methine proton signal at δ 4.56, which had the significant correlations to C-9 (δ 57.3), C-12 (δ 126.1) and C-13 (δ 147.4), was assigned to H-11. The occurrence of H-1 as a doublet of doublets having coupling constants with H-2$_{ax}$ (*J* = 11.5 Hz) and H-2$_{eq}$ (*J* = 4.4 Hz), indicated that H-1 was in the axial position; this in turn suggested a β-configuration of the hydroxyl group at C-1. For the same reason, the hydroxyl group at C-3 was also determined to have a β-configuration, this being supported by the splitting pattern of H-3 (*dd*, *J* = 12.9, 4.4 Hz). The hydroxyl group at C-11 was concluded to be in the α-configuration from the coupling constant between H-11 (δ 4.56) and H-9 (δ 2.05) with *J* = 8.1 Hz, and also from H-11 (δ 4.56) and H-12 (δ 5.58) with *J* = 3.7 Hz (Calis et al., 1993). Therefore, structure **1a** was assigned as olean-12-ene-1β,3β,11α,28-tetraol. Comparison of the $^{13}$C NMR spectral data of **1** with those of **1a** revealed glycosylation shifts for C-28 (Δδ + 7.8) and C-17 (Δδ − 0.6) on going from **1a** to **1**, demonstrating that the sugar moiety was linked to C-28. The sugar sequence was identified to be a β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl unit by comparison of the chemical shifts with that of reported data (Kasai et al., 1988). Moreover, negative FAB-MS of **1** exhibited significant fragment ions at *m*/*z* 635 [M–162]$^-$ and 473 [M–162–162]$^-$. Consequently, structure **1** was olean-12-ene-1β,3β,11α,28-tetraol28-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside.

Table 1
$^1$H and $^{13}$C NMR spectral data for aglycone (**1a**, in $C_5D_5N$)

| Position | DEPT | $\delta_C$ | $\delta_H$ |
|---|---|---|---|
| 1 | CH | 77.7 | 3.94 (1H, *dd*, *J* = 11.5, 4.4 Hz) |
| 2 | $CH_2$ | 37.3 | 2.40 (1H, *ddd*, *J* = 12.9, 4.4, 4.4 Hz) |
| | | | 2.34 (1H, *m*) |
| 3 | CH | 75.4 | 3.58 (1H, *dd*, *J* = 12.9, 4.4 Hz) |
| 4 | C | 39.6 | |
| 5 | CH | 52.9 | 0.86 (1H, *m*) |
| 6 | $CH_2$ | 18.3 | 1.65 (1H, *m*) |
| | | | 1.55 (1H, *m*) |
| 7 | $CH_2$ | 31.7 | 1.94 (1H, *m*) |
| | | | 1.65 (1H, *m*) |
| 8 | C | 41.9 | |
| 9 | CH | 57.3 | 2.05 (1H, *d*, *J* = 8.1 Hz) |
| 10 | C | 44.8 | |
| 11 | CH | 66.3 | 4.56 (1H, *dd*, *J* = 8.1, 3.7 Hz) |
| 12 | CH | 126.1 | 5.58 (1H, *d*, *J* = 3.7 Hz) |
| 13 | C | 147.4 | |
| 14 | C | 44.1 | |
| 15 | $CH_2$ | 26.3 | 1.88 (1H, *m*) |
| | | | 1.01 (1H, *m*) |
| 16 | $CH_2$ | 22.7 | 1.99 (1H, *m*) |
| | | | 1.51 (1H, *m*) |
| 17 | C | 37.3 | |
| 18 | CH | 41.8 | 2.31 (1H, *br d*, *J* = 12.4 Hz) |
| 19 | $CH_2$ | 46.4 | 1.75 (1H, *dd*, *J* = 13.6, 12.4 Hz) |
| | | | 1.14 (1H, *dd*, *J* = 13.6, 4.2 Hz) |
| 20 | C | 31.1 | |
| 21 | $CH_2$ | 34.5 | 1.44 (1H, *m*) |
| | | | 1.22 (1H, *m*) |
| 22 | $CH_2$ | 33.4 | 1.53 (1H, *m*) |
| | | | 1.22 (1H, *m*) |
| 23 | $CH_3$ | 28.8 | 1.24 (3H, *s*) |
| 24 | $CH_3$ | 16.0 | 1.08 (3H, *s*) |
| 25 | $CH_3$ | 13.6 | 1.29 (3H, *s*) |
| 26 | $CH_3$ | 18.2 | 1.04 (3H, *s*) |
| 27 | $CH_3$ | 25.8 | 1.39 (3H, *s*) |
| 28 | $CH_2$ | 68.7 | 3.81 (1H, *d*, *J* = 10.7 Hz) |
| | | | 3.56 (1H, *d*, *J* = 10.7 Hz) |
| 29 | $CH_3$ | 33.2 | 0.83 (3H, *s*) |
| 30 | $CH_3$ | 23.6 | 0.88 (3H, *s*) |

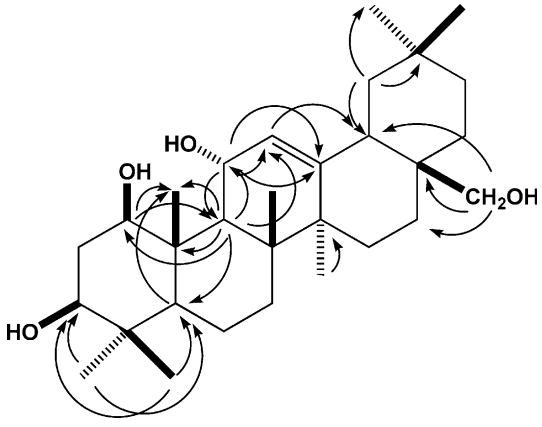
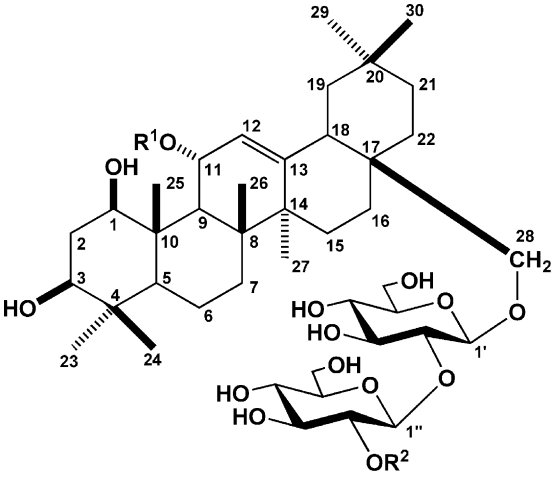



Fig. 1. Significant HMBC correlations for aglycone (**1a**).

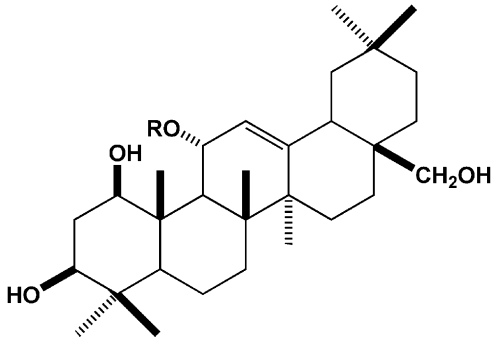
Justicioside B (**2**) was isolated as an amorphous powder. Its molecular formula was determined as $C_{48}H_{80}O_{19}$ by HR-FAB mass spectrometric analyses. The $^{1}H$ and $^{13}C$ NMR spectra showed the presence of three sugar units from the anomeric proton signals at $\delta$ 4.48 (*d*, $J=7.6$ Hz), 5.27 (*d*, $J=7.6$ Hz) and 5.43 (*d*, $J=7.6$ Hz), and from carbon signals at $\delta$ 103.0, 103.4 and 106.1. Enzymatic hydrolysis of **2** with crude hesperidinase gave **1a** and D-glucose. The negative FAB-MS displayed characteristic fragment ions of a linear sugar unit at *m/z* 797 $[M–162]^-$, 635 $[M–162–162]^-$, 473 $[M–162–162–162]^-$. The chemical shifts of **2** were closely related to those of justicioside A (**1**), except for a set of additional signals arising from a β-D-glucopyranosyl unit. This additional unit was assigned to be attached to C-2″ of the inner sugar because the chemical shifts of C-2″ ($\delta$ 85.3), C-1″ ($\delta$ 103.0) and C-3″ ($\delta$ 77.6) changed by +8.4, −2.9 and −0.6, respectively. Thus, **2** was elucidated as olean-12-ene-1β,3β,11α,28-tetraol 28-*O*-β-D-glucopyran osyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Justicioside C (**3**) was obtained as an amorphous powder. Its molecular formula, $C_{43}H_{72}O_{14}$, was determined by HR-FAB mass spectrometric analyses. Negative FAB-MS exhibited fragment ions at *m/z* 811 $[M–H]^-$, 649 $[M–162]^-$, 487 $[M–162–162]^-$. The $^{1}H$ and $^{13}C$ NMR spectral data revealed that **3** contains the same sugar moiety as justicioside A (**1**) with a different aglycone. Enzymatic hydrolysis of **3** provided D-glucose and an aglycone (**3a**) with a molecular formula $C_{31}H_{52}O_4$. The structure of **3a** was established by comparison of its chemical shifts to those of **1a**, in which the additional signal due to a methoxyl group was observed in the spectra ($\delta$ 3.27 in the $^{1}H$ NMR spectrum and $\delta$ 51.3 in the $^{13}C$ NMR spectrum). This additional group was located at C-11 of the aglycone since the chemical shifts of C-9, C-11, C-12 and C-13 were significantly changed by −7.1, +8.0, −4.2 and +4.6, respectively, when compared to **1a** (Calis et al., 1993). Thus, **3a** was concluded to be an 11α-methoxy derivative of **1a**. Accordingly, **3** was elucidated as 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Justicioside D (**4**) was obtained as an amorphous powder and determined as $C_{49}H_{82}O_{19}$ by HR-FAB mass spectrometric analyses. The $^{1}H$ and $^{13}C$ NMR spectra indicated the presence of three sugar units, one methoxyl group and signals for the aglycone moiety. The chemical shifts of the aglycone moiety were superimposable with those of **3**, as well as the chemical shifts of three sugar units were the same as those of **2**. Enzymatic hydrolysis of **4** afforded **3a** and D-glucose. Besides, negative




| | $R^1$ | $R^2$ |
|---|---|---|
| 1 | H | H |
| 2 | H | β-D-Glc |
| 3 | Me | H |
| 4 | Me | β-D-Glc |




| | R |
|---|---|
| 1a | H |
| 3a | Me |

Scheme 1.

FAB-MS showed the significant fragment ions at *m/z* 973 $[M–H]^-$, 811 $[M–162]^-$, 649 $[M–162–162]^-$, 487 $[M–162–162–162]^-$. Consequently, **4** was identified to 11α-methoxy-olean-12-ene-1β, 3β, 28-triol 28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (see Scheme 1).

## 3. Experimental

### 3.1. General

NMR spectra were recorded in $C_5D_5N$ using a JEOL JNM α-400 spectrometer (400 MHz for $^1H$ NMR and 100 MHz for $^{13}C$ NMR) with tetramethylsilane (TMS) as internal standard, whereas MS obtained using a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. For CC, silica gel G (Scharlau GE0049, 70-230 mesh ASTM), YMC-gel ODS (50 μm, YMC) and highly porous copolymer resin of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd.) were used. HPLC (Waters 515 HPLC pump) was carried on a column of ODS (150×20 mm i.d., YMC) with a Shimadzu refractive index (RID-6A) detector.

### 3.2. Plant material

The aerial portion of *J. betonica* L. was collected from Kalasin Province, Thailand, in November, 2002, and identified by Mr. Bamrung Tavinchiua of the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. A voucher sample (KKU-0045) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

### 3.3. Extraction and isolation

The dried aerial portion (1.8 kg) of *J. betonica* was extracted with hot EtOH-$H_2O$ (95:5,v/v) under reflux 4 times (each 8 l, 3 h, 70 °C). The EtOH extract was concentrated to dryness (256.6 g) and partitioned between $Et_2O$ and $H_2O$, with the aqueous soluble applied to a column of highly porous copolymer resin of styrene and divinylbenzene, and eluted with $H_2O$, MeOH–$H_2O$ (3:2), MeOH and $Me_2CO$, successively. The fraction eluted with MeOH (17.5 g) was applied to a silica gel column using solvent systems EtOAc–MeOH (9:1, 5 l), EtOAc–MeOH–$H_2O$ (40:10:1, 5 l) and EtOAc–MeOH–$H_2O$ (70:30:3, 3.5 l) to give ten fractions. Fraction 6 (2.9 g) was applied to a column of ODS using a gradient system [MeOH–$H_2O$ (2:3, 1 l) to MeOH (1 l)] to afford six fractions. Fraction 6-3 was purified by prep. HPLC-ODS (37% MeCN in $H_2O$) to provide compounds **1** (113 mg) and **3** (42 mg). Fraction 7 (3.8 g) was similarly applied to a column of ODS using a gradient system [MeOH–$H_2O$ (2:3, 1 l) to MeOH (1 l)] to give eight fractions. Fraction 7-2 was purified by prep. HPLC-ODS (33% MeCN in $H_2O$) to afford compound **2** (589 mg) and **4** (330 mg).

### 3.4. Justicioside A (*1*)

Amorphous powder, $[\alpha]_D^{27}$ − 1.9° (MeOH, *c* 2.65); $^1H$ NMR ($C_5D_5N$): aglycone moiety δ 5.51 (1H, *d*, *J* = 3.0 Hz, H-12), 4.53 (1H, *dd*, *J* = 8.1, 3.0 Hz, H-11), 3.80 (1H, *d*, *J* = 9.3 Hz, H-28a), 3.71 (1H, *d*, *J* = 9.3 Hz, H-28b), 3.55 (1H, *dd*, *J* = 11.7, 3.9 Hz, H-3), 1.33 (3H, *s*, H-27), 1.26 (3H, *s*, H-25), 1.21 (3H, *s*, H-23), 1.05 (3H, *s*, H-26), 1.01 (3H, *s*, H-24), 0.80 (3H, *s*, H-30), 0.79 (3H, *s*, H-29), sugar moiety δ 5.37 (1H, *d*, *J* = 7.6 Hz, H-1″), 4.89 (1H, *d*, *J* = 7.3 Hz, H-1′); $^{13}C$ NMR ($C_5D_5N$): Table 3; Negative FAB-MS *m/z* 797 $[M–H]^-$, 635 $[M–Glc]^-$, 473 $[M–Glc–Glc]^-$; Negative HR-FAB-MS, *m/z*: 797.4681 $[M–H]^-$ (calcd for $C_{42}H_{69}O_{14}$, 797.4687).

### 3.5. Olean-12-ene-1β,3β,11α,28-tetraol (*1a*)

Amorphous powder, $[\alpha]_D^{27}$ + 37.5° (MeOH, *c* 0.13); $^1H$ and $^{13}C$ NMR ($CD_3OD$): Table 1; Negative HR-FAB-MS, *m/z* : 476.3639 $[M–H]^-$ (calcd for $C_{30}H_{49}O_4$, 473.3630).

### 3.6. Justicioside B (*2*)

Amorphous powder, $[\alpha]_D^{27}$ − 4.2° (MeOH, *c* 2.61); $^1H$ NMR ($C_5D_5N$): aglycone moiety δ 5.54 (1H, *d*, *J* = 3.2 Hz, H-12), 4.51 (1H, *dd*, *J* = 8.1, 3.2 Hz, H-11), 3.76 (1H, *d*, *J* = 9.5 Hz, H-28a), 3.71 (1H, *d*, *J* = 9.5 Hz, H-28b), 3.51 (1H, *dd*, *J* = 11.7, 3.9 Hz, H-3), 2.15 (1H, *dd*, *J* = 12.9, 2.9 Hz, H-18), 1.99 (1H, *d*, *J* = 8.1 Hz, H-9), 1.33 (3H, *s*, H-27), 1.29 (3H, *s*, H-25), 1.15 (3H, *s*, H-23), 1.08 (3H, *s*, H-26), 1.02 (3H, *s*, H-24), 0.78 (3H, *s*, H-30), 0.75 (3H, *s*, H-29), sugar moiety δ 5.43 (1H, *d*, *J* = 7.6 Hz, H-1″), 5.27 (1H, *d*, *J* = 7.6 Hz, H-1″), 4.48 (1H, *d*, *J* = 7.6 Hz, H-1′); $^{13}C$ NMR ($C_5D_5N$): Table 3; Negative FAB-MS *m/z* 959 $[M–H]^-$, 797 $[M–Glc]^-$, 635 $[M–Glc–Glc]^-$, 473 $[M–Glc–Glc–Glc]^-$ ; Negative HR-FAB-MS, *m/z*: 959.5224 $[M–H]^-$ (calcd for $C_{48}H_{79}O_{19}$, 959.5215).

### 3.7. Justicioside C (*3*)

Amorphous powder, $[\alpha]_D^{27}$ + 10.1° (MeOH, *c* 3.57); $^1H$ NMR ($C_5D_5N$): aglycone moiety δ 5.27 (1H, *d*, *J* = 3.2 Hz, H-12), 3.82 (1H, *d*, *J* = 10.0 Hz, H-28a), 3.63 (1H, *d*, *J* = 10.0 Hz, H-28b), 3.52 (1H, *dd*, *J* = 11.7, 3.4 Hz, H-3), 1.95 (1H, *d*, *J* = 9.0 Hz, H-9), 1.26 (3H, *s*, H-27), 1.20 (3H, *s*, H-23), 1.16 (3H, *s*, H-25), 1.02 (3H, *s*, H-24), 0.94 (3H, *s*, H-26), 0.89 (6H, *s*, H-29, 30), sugar

moiety $\delta$ 5.38 (1H, *d*, $J$=7.6 Hz, H-1″), 4.88 (1H, *d*, $J$=7.6 Hz, H-1′); $^{13}$C NMR ($C_5D_5N$): Table 3; Negative FAB-MS *m/z* 811 $[M–H]^-$, 649 $[M–Glc]^-$, 487 [M–Glc–Glc]$^-$; Negative HR-FAB-MS, *m/z*: 811.4851 $[M–H]^-$ (calcd for $C_{43}H_{71}O_{14}$, 811.4843).

### 3.8. 11α-Methoxy-olean-12-ene-1β,3β,28-triol (*3a*)

Amorphous powder, $[\alpha]_D^{27}$ + 46.7° (MeOH, *c* 0.71); $^1$H and $^{13}$C NMR ($CD_3OD$): Table 2; Negative HR-FAB-MS, *m/z*: 487.3796 $[M–H]^-$ (calcd for $C_{31}H_{51}O_4$, 487.3787).

### 3.9. Justicioside D (*4*)

Amorphous powder, $[\alpha]_D^{27}$ + 13.6° (MeOH, *c* 7.79); $^1$H NMR ($C_5D_5N$): aglycone moiety $\delta$ 5.27 (1H, *d*, $J$=3.2 Hz, H-12), 3.77 (1H, *d*, $J$=9.5 Hz, H-28a), 3.65 (1H, *d*, $J$=9.5 Hz, H-28b), 3.49 (1H, *dd*, $J$=12.0, 3.8 Hz, H-3), 1.94 (1H, *d*, $J$=803 Hz, H-9), 1.26 (3H, *s*, H-27), 1.16 (3H, *s*, H-23), 1.15 (3H, *s*, H-25), 1.00 (3H, *s*, H-24), 0.99 (3H, *s*, H-26), 0.86 (6H, *s*, H-30), 0.82 (3H, *s*, H-29), sugar moiety $\delta$ 5.42 (1H, *d*, $J$=7.6 Hz, H-1″), 5.32 (1H, *d*, $J$=7.6 Hz, H-1‴), 4.88 (1H, *d*, $J$=7.6 Hz, H-1′); $^{13}$C NMR ($CD_3OD$) spectra: Table 1; Negative FAB-MS *m/z* 973 $[M–H]^-$, 811 $[M–Glc]^-$, 649 [M–Glc–Glc]$^-$, 487[M–Glc–Glc–Glc]$^-$; Negative HR-FAB-MS, *m/z*: 973.5267 $[M–H]^-$ (calcd for $C_{49}H_{81}O_{19}$, 973.5371).

Table 2
$^1$H and $^{13}$C NMR spectral data for aglycone (**3a**, in $C_5D_5N$)

| Position | DEPT | $\delta_C$ | $\delta_H$ |
|---|---|---|---|
| 1 | CH | 77.2 | 3.63 (1H, *dd*, $J$=11.6, 4.4 Hz) |
| 2 | $CH_2$ | 37.2 | 2.34 (1H, *ddd*, $J$=14.2, 4.4, 4.4 Hz) |
| | | | 2.23 (1H, *ddd*, $J$=14.2, 12.2, 11.6 Hz) |
| 3 | CH | 75.2 | 3.56 (1H, *dd*, $J$=12.2, 4.4 Hz) |
| 4 | C | 39.6 | |
| 5 | CH | 52.8 | 0.78 (1H, *br d*, $J$=9.7 Hz) |
| 6 | $CH_2$ | 18.2 | 1.67 (1H, *m*) |
| | | | 1.50 (1H, *m*) |
| 7 | $CH_2$ | 31.6 | 1.99 (1H, *m*) |
| | | | 1.67 (1H, *br d*, $J$=15.4 Hz) |
| 8 | C | 41.7 | |
| 9 | CH | 50.2 | 2.00 (1H, *d*, $J$=8.5 Hz) |
| 10 | C | 45.1 | |
| 11 | CH | 74.3 | 4.41 (1H, *dd*, $J$=8.5, 3.7 Hz) |
| 12 | CH | 121.9 | 5.31 (1H, *d*, $J$=3.7 Hz) |
| 13 | C | 152.0 | |
| 14 | C | 44.1 | |
| 15 | $CH_2$ | 26.5 | 1.86 (1H, *br d*, $J$=13.2 Hz) |
| | | | 1.01 (1H, *m*) |
| 16 | $CH_2$ | 22.7 | 2.01 (1H, *m*) |
| | | | 1.50 (1H, *m*) |
| 17 | C | 37.3 | |
| 18 | CH | 42.4 | 2.40 (1H, *dd*, $J$=14.1, 4.4 Hz) |
| 19 | $CH_2$ | 47.0 | 1.88 (1H, *dd*, $J$=13.7, 13.2 Hz) |
| | | | 1.14 (1H, *dd*, $J$=13.7, 4.2 Hz) |
| 20 | C | 31.3 | |
| 21 | $CH_2$ | 34.5 | 1.41 (1H, *br dd*, $J$=13.7, 3.7 Hz) |
| | | | 1.26 (1H, *m*) |
| 22 | $CH_2$ | 33.3 | 1.48 (1H, *br dd*, $J$=12.7, 3.2 Hz) |
| | | | 1.25 (1H, *m*) |
| 23 | $CH_3$ | 28.6 | 1.23 (3H, *s*) |
| 24 | $CH_3$ | 15.9 | 1.05 (3H, *s*) |
| 25 | $CH_3$ | 13.5 | 1.18 (3H, *s*) |
| 26 | $CH_3$ | 18.2 | 0.96 (3H, *s*) |
| 27 | $CH_3$ | 24.5 | 1.33 (3H, *s*) |
| 28 | $CH_2$ | 68.6 | 3.76 (1H, *d*, $J$=10.5 Hz) |
| | | | 3.55 (1H, *d*, $J$=10.5 Hz) |
| 29 | $CH_3$ | 33.3 | 0.94 (3H, *s*) |
| 30 | $CH_3$ | 23.7 | 0.97 (3H, *s*) |
| MeO-11 | $CH_3$ | 51.3 | 3.27 (3H, *s*) |

Table 3
$^{13}$C NMR spectral data for justiciosides A–D (**1**–**4**, in $C_5D_5N$)

| Position | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| 1 | 77.8 | 77.8 | 77.2 | 77.1 |
| 2 | 37.3 | 37.0 | 37.1 | 36.8 |
| 3 | 75.4 | 75.3 | 75.2 | 75.1 |
| 4 | 39.6 | 39.4 | 39.5 | 39.4 |
| 5 | 52.9 | 52.9 | 52.8 | 52.7 |
| 6 | 18.2 | 18.2 | 18.1 | 18.1 |
| 7 | 32.3 | 32.0 | 32.2 | 31.8 |
| 8 | 41.8 | 41.7 | 41.5 | 41.2 |
| 9 | 57.2 | 57.1 | 50.2 | 50.1 |
| 10 | 44.8 | 44.6 | 45.0 | 44.9 |
| 11 | 66.2 | 66.2 | 74.3 | 74.2 |
| 12 | 126.1 | 126.1 | 122.0 | 121.9 |
| 13 | 147.0 | 147.0 | 151.6 | 151.5 |
| 14 | 44.1 | 44.1 | 44.2 | 44.1 |
| 15 | 26.6 | 26.5 | 26.9 | 26.7 |
| 16 | 21.8 | 22.0 | 21.9 | 22.0 |
| 17 | 36.7 | 36.6 | 36.7 | 36.6 |
| 18 | 42.2 | 41.9 | 42.7 | 42.3 |
| 19 | 46.0 | 45.9 | 46.6 | 46.5 |
| 20 | 30.9 | 30.8 | 31.1 | 31.0 |
| 21 | 34.3 | 34.2 | 34.3 | 34.2 |
| 22 | 33.2 | 33.2 | 33.1 | 33.2 |
| 23 | 28.6 | 28.5 | 28.6 | 28.5 |
| 24 | 16.0 | 15.9 | 15.9 | 15.8 |
| 25 | 13.6 | 13.5 | 13.5 | 13.4 |
| 26 | 18.2 | 18.2 | 18.2 | 18.2 |
| 27 | 25.7 | 25.8 | 24.6 | 24.5 |
| 28 | 76.5 | 76.8 | 76.4 | 76.6 |
| 29 | 33.2 | 33.1 | 33.2 | 33.2 |
| 30 | 23.7 | 23.7 | 23.8 | 23.8 |
| MeO-11 | | | 51.2 | 51.2 |
| Glc-1′ | 103.5 | 103.4 | 103.5 | 103.3 |
| 2′ | 82.7 | 82.6 | 82.7 | 82.6 |
| 3′ | 77.6 | 77.5 | 77.9 | 77.5 |
| 4′ | 71.6 | 71.0 | 71.6 | 71.0 |
| 5′ | 78.1 | 77.8 | 78.1 | 77.8 |
| 6′ | 62.6 | 62.4 | 62.6 | 62.4 |
| Glc-1″ | 105.9 | 103.0 | 105.9 | 103.0 |
| 2″ | 76.9 | 85.3 | 76.9 | 85.2 |
| 3″ | 78.2 | 77.6 | 78.2 | 77.6 |
| 4″ | 71.5 | 70.6 | 71.5 | 70.6 |
| 5″ | 78.4 | 77.6 | 78.4 | 77.8 |
| 6″ | 62.6 | 62.3 | 62.7 | 62.3 |
| Glc-1‴ | | 106.1 | | 106.1 |
| 2‴ | | 76.2 | | 76.2 |
| 3‴ | | 78.7 | | 78.2 |
| 4‴ | | 71.3 | | 71.3 |
| 5‴ | | 78.9 | | 78.9 |
| 6‴ | | 62.7 | | 62.7 |

### 3.10. Enzymatic hydrolysis of justiciosides A–D

Each sample of justicioside A (**1**, 25 mg) and B (**2**, 65 mg) was dissolved in 0.5 ml of MeOH. A solution of crude hesperidinase (Kohda and Tanaka, 1975) (100 mg in 20 ml of $H_2O$) was added in each experiment. After stirring at 37 °C for 1 week, the mixtures were extracted with EtOAc, concentrated to dryness, and then purified by prep. HPLC-ODS using MeOH–$H_2O$ (9:1) as solvent system to give **1a** (8 and 22 mg, respectively). The aqueous layer of each sample was concentrated to dryness and applied to a silica gel column [EtOAc–MeOH–$H_2O$ (40:10:1)], affording D-glucose (7 and 18 mg, respectively) in comparison of its optical rotation with an authentic sample. By the same method, justicioside C (**3**, 20 mg) and D (**4**, 50 mg) provided **3a** (4 and 17 mg, respectively) and D-glucose (5 mg and 9 mg, respectively).

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