FULL PAPER

Megastigmane Glycosides from *Docynia indica* and Their Anti-inflammatory Activities

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Using various chromatographic methods, three new megastigmane glycosides, docynicasides A – C (**1** – **3**) and ten known, (6*S*,9*R*)-vomifoliol 9-*O*-*β*-D-xylopyranosyl-(1″→6′)-*O*-*β*-D-glucopyranoside (**4**), hyperin (**5**), quercitrin (**6**), quercetin 3-α-L-arabinofuranoside (**7**), naringenin 7-*O*-*β*-D-glucopyranoside (**8**), phloridzin (**9**), phloretin 2′-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**10**), pinosylvin 3-*O*-*β*-D-glucopyranoside (**11**), tormentic acid (**12**), and chlorogenic acid methyl ester (**13**) were isolated from the fruits of *Docynia indica*. Their chemical structures were elucidated by physical and chemical methods. All the isolated compounds were evaluated for the inhibitory activity on NO production in LPS-stimulated BV2 cells. As the results, compounds **3** – **5** showed significant inhibitory activity on LPS-stimulated NO production in BV2 cells with the IC_{50} values ranging from 21.0 to 29.3 μM.

Keywords: *Docynia indica*, Rosaceae, Docynicasides A – C, Anti-inflammatory activities.

Introduction

Inflammation is a part of the complex response of body tissues to injury or infection with foreign organisms such as bacteria and viruses. NO mediates a variety of biological actions from vasodilatation, neurotransmission, inhibition of platelet adherence and aggregation, as well as the macrophage and neutrophil-mediated killing of pathogens [1]. However, excessive chronic inflammation causes the basis of inflammatory diseases including rheumatoid arthritis [2], diabetes [3], and chronic hepatitis [4]. Nitric oxides synthases (NOSs) are the family of enzymes catalyzing the production of NO from L-arginine. NO produced in large amounts by inducible nitric oxide synthase (iNOS) has been identified as important biological molecules involving the immune responses and inflammation.

Docynia indica (W.) DECNE (Rosaceae) were distributed throughout India, Myanmar, China, Thailand, and Vietnam. Its fruits have been used in traditional remedies for the treatment of infectious diseases, digestive disorders, dyhypeslipidemia, and hypertension [5]. In addition, the chemical investigation confirmed the presence of flavonoids in this plant [6]. We report, herein, the isolation, structural elucidation, and evaluation of inhibitory activity on NO production of three new megast igmane glycosides (**1** – **3**) and ten known compounds (**4** – **13**) from the fruits of *D. indica* (*Fig. 1*).

Results and Discussion

Compound **1** was obtained as an amorphous powder and its molecular formula was determined to be $C_{24}H_{40}O_{11}$ on the basic of HR-ESI-MS at *m*/*z* 527.2482 $[M + Na]^+$ (calc. for $C_{24}H_{40}NaO_{11}^+$, 527.2463). The ^{1}H-NMR spectrum of **1** showed signals of four Me groups at *δ*(H) 1.02 (*s*), 1.10 (*s*), 1.19 (*d*, *J* = 6.4), and 2.06 (*s*), indicating the presence of megastigmane aglycone; two anomeric H-atoms at *δ* (H) 4.29 (*d*, *J* = 8.0) and 4.32 (*d*, *J* = 8.0) suggesting the presence of two sugar units, as listed in *Table 1*. The ^{13}C-NMR and DEPT spectra of **1** revealed the signals of 24 C-atoms, including one CO group, two nonprotonated C-atoms, twelve CH groups, five CH_2 groups, and four Me C-atoms. The ^{1}H- and ^{13}C-NMR spectral data of **1** indicated that the structure of **1** was similar to those of gusanlungionoside D [7], except for the sugar moieties at C(9). The HMBCs between H–C(11) (*δ*(H) 1.02)/H–C(12) (*δ*(H) 1.12) and C(1) (*δ*(C) 37.4)/C(2) (*δ*(C) 48.1)/C(6) (*δ*(C) 52.3) indicated the positions of two Me groups at C(1). Moreover, the HMBCs from H–C(4) (*δ*(H) 5.81) to C(2) (*δ*(C) 48.1)/C(3) (*δ*(C) 202.5) and from H–C(13) (*δ* (H) 2.06) to C(4) (*δ*(C) 125.3)/C(5) (*δ*(C) 170.3)/C(6) (*δ* (C) 52.3) confirmed the presence of the C=C bond at C(4)/C(5) and CO group at C(3). The absolute configuration of C(6) was confirmed by CD data. The CD spectrum of **1** showed a positive *Cotton* effect at 214 nm

DOI: 10.1002/hlca.201600125

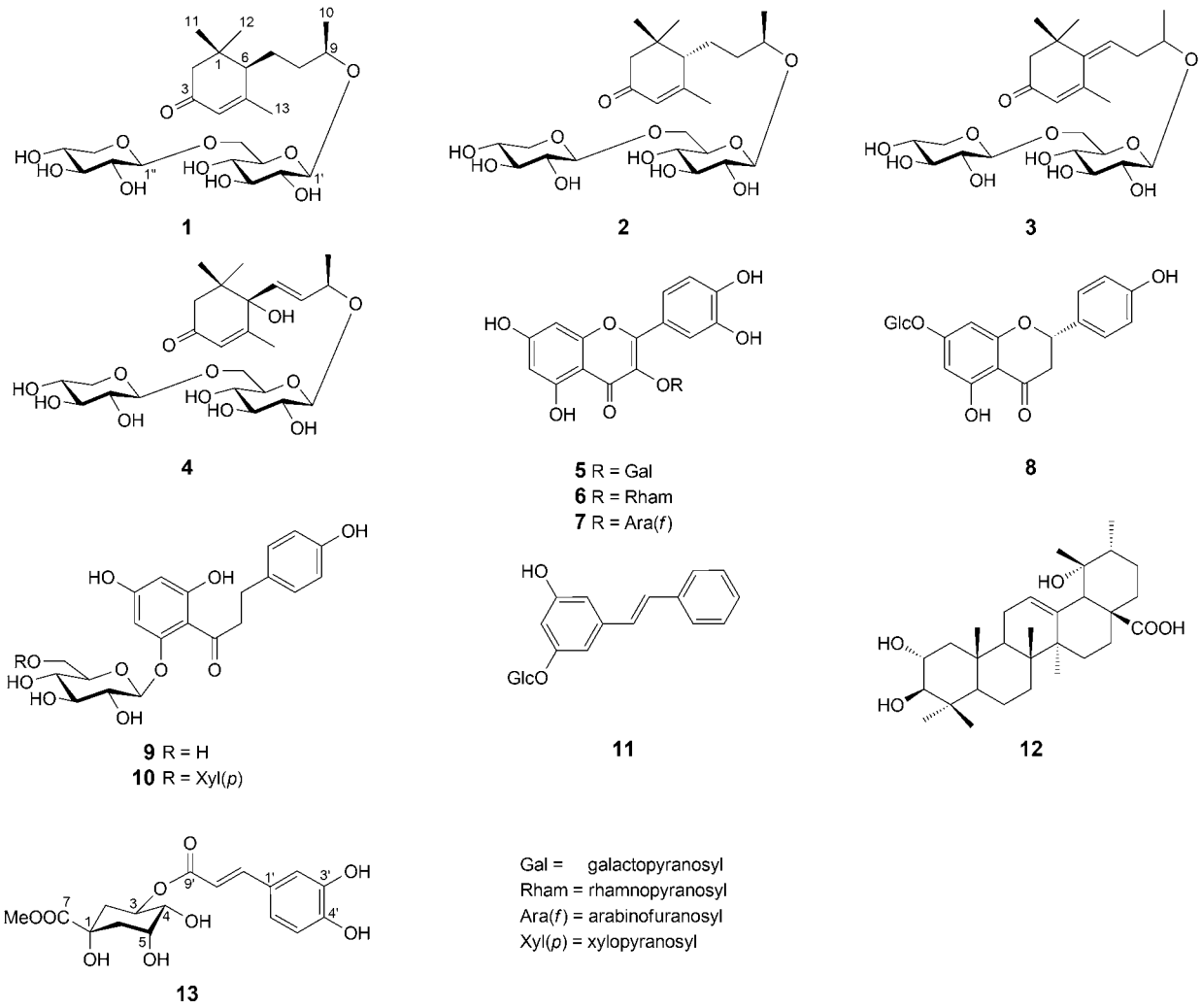

Fig. 1. Chemical structures of **1** – **13** from the fruits of *Docynia indica.*

($\Delta\varepsilon$ + 20.8 mdeg). Comparison of the CD spectra of (6*S*) compound (gusanlungionoside C: $\Delta\varepsilon$ (nm) −15.6 (212)) and (6*R*) compound (gusanlungionoside D: (nm) +25.9 (210)) [7] proved the C(6) configuration of **1** to be (*R*). Furthermore, acid hydrolysis of **1**, which were further identified as TMS derivatives by a gas chromatography method, revealed D-xylose and D-glucose as sugar moieties [8]. In addition, the ^{1}H- and ^{13}C-NMR spectral data of **1** showed the presence of one *β*-D-xylopyranose and one *β*-D-glucopyranose moieties [9]. The HMBCs between glc H–C(1′) (δ(H) 4.32) and C(9) (δ(C) 75.7); xyl H–C(1″) (δ(H) 4.29) and glc C(6′) (δ(C) 69.8); and between glc H–C(6′) (δ(H) 3.70 and 4.08) and xyl C(1″) (δ(C) 105.3) were observed (*Fig. 2*). These observations indicated the sequence of sugar linkages of **1** as *O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside and its position at C(9) of the aglycone. The ^{13}C-NMR chemical shifts of C (9) (δ(C) 75.7) and C(10) (δ(C) 20.11) confirmed the configuration at C(9) to be (*R*) by comparing them with those of (6*R*,9*S*)-9-hydroxymegastigman-4-en-3-one 9-*O*-*β*-D-glucopyranoside (C(9) (δ(C) 77.7) and C(10) (δ(C) 22.0)) and (6*R*,9*R*)-9-hydroxymegastigman-4-en-3-one 9-*O*-*β*-D-glucopyranoside (C(9) (δ(C) 75.7) and C(10) (δ(C) 19.9)) (in the same CD_3OD solvent) [10]. Consequently, the structure of **1** was determined to be (6*R*,9*R*)-9-hydroxymegastigman-4-en-3-one 9-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside, named as docynicaside A.

The molecular formula of **2** was determined as $C_{24}H_{40}O_{11}$ by the HR-ESI-MS *pseudo*-molecular ion at *m*/*z* 527.2486 $[M + Na]^+$ (calc. for $C_{24}H_{40}NaO_{11}^+$, 527.2463) resulted in the same molecular formula as that of **1**. The ^{1}H- and ^{13}C-NMR data of **2** showed almost the same as those of docynicaside A (**1**), suggesting the possibilities of different configurations at the chiral C-atoms of the aglycone. In line with this, ^{1}H- and ^{13}C-NMR, HSQC, HMBC, and COSY spectral analyses showed that compound **2** had the same constitution as **1**. The CD spectra of **1** and **2** were opposite from 200 to 260 nm, suggesting that the absolute configuration of C(6) of **2** is (6*S*). The configuration at C(9) of **2** was determined as the same

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data for Compounds **1** – **3**[a]) in CD_3OD

C	**1**		**2**		**3**	
	δ(H) (*J*, mult., Hz)	δ(C)	δ(H) (*J*, mult., Hz)	δ(C)	δ(H) (*J*, mult., Hz)	δ(C)
Aglycone						
1	–	37.4	–	37.4	–	41.9
2	2.48 (*d*, *J* = 17.6), 1.96 (*d*, *J* = 17.6)	48.1	2.49 (*d*, *J* = 17.6), 1.98 (*d*, *J* = 17.6)	48.2	2.27 (*s*)	53.7
3	–	202.5	–	202.5	–	202.0
4	5.81 (*s*)	125.3	5.81 (*s*)	125.4	5.86 (*s*)	128.9
5	–	170.3	–	170.3	–	159.9
6	1.96 – 2.00 (*m*)	52.3	1.97 – 2.01 (*m*)	52.5	–	144.4
7	1.97 – 1.88 (*m*), 1.48 – 1.50 (*m*)	26.9	1.80 – 1.84 (*m*), 1.65 – 1.69 (*m*)	27.1	5.94 (*t*, *J* = 6.4)	130.4
8	1.61 – 1.66 (*m*)	37.9	1.62 – 1.66 (*m*)	37.9	2.58 – 2.62 (*m*), 2.49 – 2.51 (*m*)	38.7
9	3.84 – 3.88 (*m*)	75.7	3.85 – 3.89 (*m*)	75.8	3.96 – 4.00 (*m*)	75.8
10	1.19 (*d*, *J* = 6.4)	20.1	1.19 (*d*, *J* = 6.4)	20.0	1.21 (*d*, *J* = 6.0)	20.1
11	1.02 (*s*)	29.1	1.02 (*s*)	29.1	1.16 (*s*)	28.5
12	1.10 (*s*)	27.7	1.10 (*s*)	27.6	1.16 (*s*)	28.4
13	2.06 (*s*)	25.1	2.05 (*s*)	25.2	2.24 (*s*)	25.2
9-*O*-Glc						
1′	4.32 (*d*, *J* = 8.0)	102.3	4.32 (*d*, *J* = 8.0)	102.2	4.33 (*d*, *J* = 7.6)	102.5
2′	3.18 – 3.21 (*m*)	75.1	3.15 – 3.19 (*m*)	75.1	3.13 – 3.17 (*m*)	75.0
3′	3.30 – 3.34 (*m*)	78.0	3.29 – 3.33 (*m*)	78.0	3.36 – 4.00 (*m*)	78.0
4′	3.35 – 3.39 (*m*)	71.4	3.36 – 3.40 (*m*)	71.4	3.25 – 3.29 (*m*)	71.3
5′	3.30 – 3.32 (*m*)	76.8	3.30 – 3.32 (*m*)	76.8	3.36 – 4.00 (*m*)	76.8
6′	4.08 (br. *d*, *J* = 11.2), 3.70 (*dd*, *J* = 5.2, 11.2)	69.8	4.07 (br. *d*, *J* = 11.2), 3.71 (*dd*, *J* = 5.2, 11.2)	69.9	4.05 (br. *d*, *J* = 11.2), 3.69 (*dd*, *J* = 4.8, 11.2)	69.8
6′-*O*-Xyl						
1″	4.29 (*d*, *J* = 8.0)	105.3	4.29 (*d*, *J* = 8.0)	105.6	4.27 (*d*, *J* = 7.6)	105.5
2″	3.18 – 3.21 (*m*)	74.8	3.16 – 3.20 (*m*)	74.8	3.13 – 3.17 (*m*)	74.8
3″	3.30 – 3.34 (*m*)	77.7	3.29 – 3.33 (*m*)	77.7	3.36 – 3.40 (*m*)	77.7
4″	3.35 – 3.37 (*m*)	71.1	3.35 – 3.39 (*m*)	71.1	3.25 – 3.29 (*m*)	71.1
5″	3.84 (*dd*, *J* = 5.6, 11.6), 3.17 (*dd*, *J* = 6.4, 11.6)	66.9	3.84 (*dd*, *J* = 5.6, 11.6), 3.17 (*dd*, *J* = 6.4, 11.6)	66.9	3.82 (*dd*, *J* = 5.2, 11.6), 3.15 (*dd*, *J* = 6.8, 11.6)	66.9

[a]) Assignments were done by HSQC, HMBC, and COSY experiments.

with those of **1** due to the similar chemical shifts of C(9) (δ(C) 75.7) and C(10) (δ(C) 20.1) for **1** and C(9) (δ(C) 75.7) and C(10) (δ(C) 20.0) for **2**. Consequently, compound **2** was determined to be (6*S*,9*R*)-9-hydroxymegastigman-4-en-3-one 9-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside and named docynicaside B.

The molecular formula of **3** was determined to be $C_{24}H_{38}O_{11}$ by HR-ESI-MS at *m*/*z* 525.2305 $[M + Na]^+$ (calc. for $C_{24}H_{38}NaO_{11}^+$, 525.2306). The ^{1}H-NMR spectrum of **3** (in CD_3OD) showed the signals for two olefinic H-atoms at δ(H) 5.86 (br. *s*) and 5.94 (*t*, *J* = 6.4), four Me groups at δ(H) 1.16 (*s*, 6 H), 1.21 (*d*, *J* = 6.0), and 2.24 (*s*), and two anomeric H-atoms at δ(H) 4.27 (*d*, *J* = 7.6) and 4.33 (*d*, *J* = 7.6). The analytical NMR data of **3** indicated that it was very similar to those of **1** and **2**, except for the additional C=C bond at C(6)/C(7). The position of this C=C bond was confirmed by the HMBCs between H–C(11)/H–C(12) (δ(H) 1.16)/H–C(13) (δ(H) 2.24) and C(6) (δ(C) 144.4). The (*Z*) configuration at C(6)/C(7) was confirmed by NOE observation between H–C(7) (δ(H) 5.94) and H–C(11)/H–C(12) (δ(H) 1.16). Acid hydrolysis of **3** yielded D-xylose and D-glucose (same components as of **1** and **2**). The sugar linkages of *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside and the position of these sugars at C(9) were confirmed by the HMBCs from xyl H–C(1″) (δ(H) 4.27) to glc C(6′) (δ(C) 69.8) and glc H–C(1′) (δ(H) 4.33) to C(9) (δ(C) 75.8). Based on the above evidence, compound **3** was defined as 9-hydroxymegastigma-4,6*Z*-dien-3-one 9-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside and named docynicaside C.

The known compounds were identified as (6*S*,9*R*)-vomifoliol 9-*O*-*β*-D-xylopyranosyl-(1″→6′)-*O*-*β*-D-glucopyranoside (**4**) [11], hyperin (**5**) [12], quercitrin (**6**) [13], quercetin-3-*α*-L-arabinofuranoside (**7**) [14], naringenin 7-*O*-*β*-D-glucopyranoside (**8**) [15], phloridzin (**9**) [16], phloretin 2′-*O*-*β*-D-xylopyranoside (1→6)-*β*-D-glucopyranoside (**10**) [16], pinosylvin 3-*O*-*β*-D-glucopyranoside (**11**) [17], tormentic acid (**12**) [18], and chlorogenic acid methyl ester (**13**) [19] (*Fig. 1*) by the comparison of their NMR and MS data with the reported values in the literature. All these compounds were reported from *D. indica* for the first time.

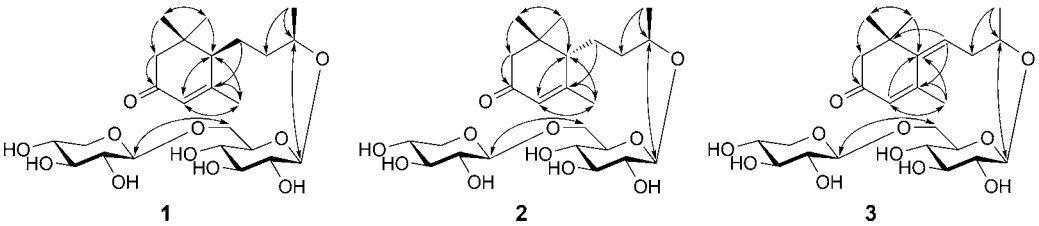

Fig. 2. The key HMBCs of compounds **1** – **3**.

Nitrite concentrations were measured in the supernate of BV2 cells by the *Griess* reaction [20]. All the isolated compounds were evaluated for the inhibitory activity on NO production in LPS-stimulated BV2 cells. First, the compounds were examined at the concentration of 50 μM to screen their cytotoxicity and inhibitory activity on NO production. None of them showed cytotoxic activities (cell viability > 95%). All the tested compounds showed inhibitory NO production in BV2 cells with the inhibitory percentages > 50%. Thus, compounds were chosen to evaluate inhibitory NO production at the concentrations of 10, 20, and 40 μM to get IC_{50} values. Based on the results, compounds **1**, **3**, **4**, and **7** – **10** showed significant inhibitory activity on LPS-stimulated NO production in BV2 cells with the IC_{50} values ranging from 21.0 to 29.3 μM (*Table 2*), which were similar to L-NMMA, the positive control, with IC_{50} value of 22.1 μM. The remaining compounds showed moderate inhibitory activities on NO production. Thus, this study proved these compounds could be important anti-inflammatory constituent of the fruits of *D. indica.*

Table 2. Inhibitory NO effect of compounds in LPS-stimulated BV2 cells

Compound	IC_{50}[a]) [μM]	Cell viability[a]) [%]
1	29.3 ± 3.1	107.6 ± 2.4
2	47.2 ± 3.2	116.8 ± 6.6
3	22.8 ± 1.5	114.0 ± 4.4
4	29.5 ± 2.0	109.4 ± 3.0
5	51.5 ± 4.4	111.1 ± 4.2
6	46.6 ± 3.2	127.5 ± 6.1
7	24.7 ± 1.9	112.5 ± 3.1
8	29.3 ± 1.7	111.1 ± 4.2
9	21.0 ± 2.3	114.0 ± 4.4
10	26.2 ± 1.8	102.5 ± 2.6
11	46.8 ± 3.6	126.3 ± 8.8
12	49.7 ± 3.3	124.3 ± 7.5
13	37.4 ± 1.4	111.8 ± 0.9
L-NMMA[b])	22.1 ± 1.2	95.5 ± 4.1

[a]) Cell viability after treatment with 50 μM of each compounds.
[b]) L-NMMA as a positive control.

This research was supported by '*National Program for Tay Bac, VNU Hanoi*' under grant number KHCN-TB.04C/13-18.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/hlca.201600125.

Experimental Part

General

For thin layer chromatography (TLC), a precoated SiO_2 *60 F254* (0.25 mm, *Merck*) and *RP-18 F254S* plates (0.25 mm, *Merck*) were used. Column chromatography (CC) was performed over SiO_2 (*Kieselgel 60*, 70 – 230 mesh and 230 – 400 mesh, *Merck*) or *YMC RP-18* resins (30 – 50 μm, *Fujisilisa Chemical Ltd.*). Prep. HPLC was carried out using an *AGILENT 1200* HPLC system. Optical rotations were determined on a *Jasco DIP-370* automatic polarimeter. All NMR spectra were recorded on an *Agilent 400-MR*-NMR spectrometer operated at 400 and 100 MHz for ^1H and ^{13}C, resp. Data processing was carried out with the MestReNova ver.6.0.2 program. Chemical shifts are reported in parts per million from TMS. HR-ESI-MS spectra were obtained using an *AGILENT 6550 iFunnel Q-TOF* LC/MS system.

Plant Material

The branches with leaves and fruits of *D. indica* were collected in Lao Cai province, Vietnam in September 2014 and identified by Prof. *Vu Xuan Phuong*, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (HVD-006-15) was deposited with the Department of Herbal specimen, Institute of Ecology and Biological Resources.

Extraction and Isolation

The dried fruits of *D. indica* (2.5 kg) were extracted with hot MeOH using a sonicator three times (3 × 8 l, each 2 h) to yield 300 g extract after evaporation of the solvent. This extract was suspended in H_2O and successively partitioned with hexane and AcOEt to obtain the hexane (*DI1*, 10.0 g), AcOEt (*DI2*, 40.0 g), and H_2O (*DI3*,

250.0 g) extracts after removal of the solvents *in vacuo*. The *DI2* fraction was chromatographed over a SiO_2 column and eluting with a gradient of hexane/acetone (40:1 → 0:1, *v*/*v*) to obtain six subfractions, *DI2A* (7.0 g), *DI2B* (4.0 g), *DI2C* (2.0 g), *DI2D* (5.0 g), *DI2E* (4.0 g), and *DI2F* (10.0 g). The *DI2D* fraction was chromatographed over a SiO_2 column eluting with $CHCl_3$/acetone (5:1, *v*/*v*) to give four smaller fractions, *DI2D1* – *DI2D4*. The *DI2D2* fraction was chromatographed over a *RP-18* column eluting with MeOH/H_2O (6:1, *v*/*v*) to yield compound **12** (200.0 mg). The *DI2E* fraction was chromatographed over a SiO_2 column eluting with $CHCl_3$/MeOH (8:1, *v*/*v*) to give four smaller fractions, *DI2E1* – *DI2E4*. The *DI2E1* fraction was chromatographed over a SiO_2 column eluting with hexane/AcOEt (5:1, *v*/*v*) to give three fractions, *DI2E1A* – *DI2E1C*. The *DI2E1A* fraction was chromatographed over a *RP-18* column eluting with MeOH/H_2O (3.5:1, *v*/*v*) to yield **13** (26.0 mg) and **7** (13.0 mg). The *DI2E1B* fraction was chromatographed over a HPLC column (*J'sphere*, *ODS H-80*, 250 × 20 mm) with flow rate of 4 ml/min eluting 30% MeCN to yield **8** (40.0 mg) and **9** (12.0 mg). The *DI2E1C* fraction was chromatographed over a *RP-18* column eluting with MeOH/H_2O (1:1, *v*/*v*) to yield compounds **6** (7.5 mg) and **11** (7.5 mg). The *DI2E4* fraction was chromatographed over a *RP-18* column eluting with MeOH/H_2O (1:1, *v*/*v*) to obtain two fractions, *DI2E4A* and *DI2E4B*. Compound **10** (20.0 mg) was obtained from *DI2E4A* fraction, using *J'sphere ODS H-80* column (250 × 20 mm), 25% MeCN with flow rate of 4 ml/min.

The H_2O-soluble fraction (*DI3*, 250 g) was chromatographed over a *Diaion HP-20* column eluting with H_2O to remove sugar components then increasing concentrations of MeOH (25, 50, 75, and 100%) to obtain four subfractions, *DI3A* (190.0 g), *DI3B* (15.0 g), *DI3C* (25.0 g), and *DI3D* (20.0 g). The *DI3C* fraction was chromatographed over a SiO_2 column eluting with gradient solvents of $CHCl_3$/MeOH (20:1 → 2:1, *v*/*v*) to give four smaller fractions, *DI3C1* – *DI3C4*. *DI3C2* was chromatographed over a *RP-18* column eluting with MeOH/H_2O (1:1, *v*/*v*) then HPLC using *J'sphere ODS H-80* 250 × 20 mm, 20% MeCN, and flow rate of 4 ml/min to yield compounds **1** (24.0 mg), **2** (19.0 mg), **3** (18.0 mg), **4** (25.0 mg), and **5** (16.0 mg).

Docynicaside A (= **(2*R*)-4-[(1*R*)-2,6,6-Trimethyl-4-oxocyclohex-2-en-1-yl]butan-2-yl 6-*O*-*β*-D-Xylopyranosyl-*β*-D-glucopyranoside**; **1**). White amorphous powder. $[\alpha]_D^{20}$ = +82.1 (*c* = 0.1, MeOH). CD (MeOH): +20.8 (214). ^{1}H- and ^{13}C-NMR (CD_3OD): see *Table 1*. HR-ESI-MS: 527.2482 ($[M + Na]^+$, $C_{24}H_{40}NaO_{11}^+$; calc. 527.2463).

Docynicaside B (= **(2*R*)-4-[(1*S*)-2,6,6-Trimethyl-4-oxocyclohex-2-en-1-yl]butan-2-yl 6-*O*-*β*-D-Xylopyranosyl-*β*-D-glucopyranoside**; **2**). White amorphous powder. $[\alpha]_D^{20}$ = +63.0 (*c* = 0.1, MeOH). CD (MeOH): −22.7 (215). ^{1}H- and ^{13}C-NMR (CD_3OD): see *Table 1*. HR-ESI-MS: 527.2486 ($[M + Na]^+$, $C_{24}H_{40}NaO_{11}^+$; calc. 527.2463).

Docynicaside C (= **(4*Z*)-4-(2,6,6-Trimethyl-4-oxocyclohex-2-en-1-ylidene)butan-2-yl 6-*O*-*β*-D-Xylopyranosyl-*β*-D-glucopyranoside**; **3**). White amorphous powder. $[\alpha]_D^{20}$ = +50.7 (*c* = 0.1, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD): see *Table 1*. HR-ESI-MS: 525.2305 ($[M + Na]^+$, $C_{24}H_{38}NaO_{11}]^+$; calc. 525.2306).

Acid Hydrolysis

Each compound (**1** – **3**, 2.0 mg) was separately dissolved in 1.0N HCl (dioxane/H_2O, 1:1, *v*/*v*, 1.0 ml) and heated to 80 °C in a H_2O bath for 3 h. The acidic soln. was neutralized with Ag_2CO_3 and the solvent thoroughly driven out under N_2 overnight. After extraction with $CHCl_3$, the aq. layer was concentrated to dryness using N_2. The residue was dissolved in 0.1 ml of dry pyridine, followed by addition of L-cysteine methyl ester hydrochloride in pyridine (0.06M, 0.1 ml). The mixture was heated at 60 °C for 2 h. Trimethylsilylimidazole soln. (0.1 ml) was then added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with hexane and H_2O (0.1 ml each), and the org. layer was analyzed by gas chromatography (GC): column *SPB-1* (0.25 mm × 30 m), detector FID, column temp. 210 °C, injector temp. 270 °C, detector temp. 300 °C, carrier gas He (2 ml/min). Under these conditions, the standard sugars gave peaks at t_R (min) 8.55 and 9.25 for D- and L-glucose; 4.02 and 9.17 for D- and L-xylose, resp. Peaks at t_R (min) 8.55 and 4.02 for compounds **1** – **3** were observed, confirming the presence of D-glucose and D-xylose in compounds **1** – **3**.

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Received May 3, 2016
Accepted June 13, 2016

