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# Bioactive constituents from the rhizomes of *Dioscorea septemloba* Thunb

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ABSTRACT

Eight new compounds, dioscorosides G (**1**), $H_1$ (**2**), $H_2$ (**3**), dioscorol B (**4**), dioscorosides I (**5**), J (**6**), $K_1$ (**7**), and $K_2$ (**8**), together with twelve known ones (**9**–**20**) were obtained from the rhizomes of *Dioscorea septemloba*. Their structures were elucidated by chemical and spectroscopic methods. Among the known isolates, **12**–**14**, **18**, and **20** were isolated from the Dioscoreae genus for the first time. While, **9**–**11**, **15**, and **16** were firstly obtained from the plant. Moreover, all the isolates were evaluated for in vitro anti-inflammatory potential using LPS-stimulated RAW 264.7 murine macrophages, and compounds **7**, **11**, **15**, and **16** were found to display significant inhibition of nitrite production.



## 1. Introduction

In the process of continuing to study anti-inflammatory constituents from the 70% EtOH extract of *Dioscorea spongiosa* rhizomes [1], eight new compounds, named as dioscorosides G (**1**), $H_1$ (**2**), $H_2$ (**3**), dioscorol B (**4**), dioscorosides I (**5**), J (**6**), $K_1$ (**7**), and $K_2$ (**8**), together with twelve known ones, albiflorin (**9**) [2], benzoyl paeoniflorin (**10**) [3], isopsoralen (**11**) [4], isodemethylfuropinarine (**12**) [5], annulatomarin (**13**) [6,7], icariside $E_5$ (**14**) [8], (+)-syringaresinol 4-*O*-β-D-glucopyranoside (**15**) [9,10], dioscorealide B (**16**) [11], zizyvoside I (**17**) [12], gusanlungionoside D (**18**) [13], 2,5,6-trihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (**19**) [14], 5,7-dihydroxy-2-heneicosyl chromone (**20**) [15] were obtained. Here, the isolation and identification of these compounds, along with their inhibitory effect evaluations on nitrite production are reported.

## 2. Results and discussion

The 70% EtOH extract from the rhizomes of *D. spongiosa* was subjected to D101 macroporous resin column chromatography (CC) and eluted with $H_2O$, 95% EtOH, and acetone, successively. Then 95% EtOH eluted fraction was isolated by Silica gel, Sephadex LH-20 and preparative HPLC to obtain compounds **1**–**20** (Figs. 1 and 2).

Dioscoroside G (**1**) was obtained as a white powder with positive optical rotation ($[\alpha]_D^{25}$ + 52.5°, in MeOH). The molecular formula, $C_{22}H_{22}O_{12}$, of **1** was established by negative-ion HRESI-TOF-MS (*m/z* 477.1053 $[M-H]^-$, calcd for $C_{22}H_{21}O_{12}$, 477.1038). Its IR spectrum suggested the presences of hydroxyl (3356 $cm^{-1}$), α,β-unsaturated carboxyl group (1673 $cm^{-1}$), aromatic ring (1634, 1504, 1458 $cm^{-1}$), *O*-glycoside linkage (1072 $cm^{-1}$), and methylenedioxyl (927 $cm^{-1}$). Acid hydrolysis of **1** with 1 M HCl afforded D-glucose, whose absolute configuration was determined by HPLC analysis [1]. The analysis of the $^1H$, $^{13}C$ (Table 1) and 2D ($^1H$ $^1H$ COSY, HSQC, HMBC) spectra revealed the presences of one ABX-type aromatic ring [δ 6.74 (1H, dd, *J* = 3.0, 8.5 Hz, H-4′), 6.94 (1H, d, *J* = 3.0 Hz, H-6′), 7.18 (1H, d, *J* = 8.5 Hz, H-3′)], one pentasubstituted aromatic ring [δ 6.42 (1H, s, H-5)], one oxygenated methine [δ 6.03 (1H, dd, *J* = 3.0, 12.0 Hz, H-3)], one methylenedioxyl [δ 6.04 (2H, s, $H_2$-9)], one methylene group [δ 3.05 (1H, dd, *J* = 12.0, 16.0 Hz, H-4ax), 3.36 (1H, dd, *J* = 3.0, 16.0 Hz, H-4eq)], together with one β-D-glucopyranosyl [δ 4.75 (1H, d, *J* = 7.0 Hz, H-1″)]. The $^1H$ $^1H$ COSY experiment on **1** indicated the presences of three partial structure written in bold bonds (Fig. 3). The planar structure of its aglycone was determined based on the key HMBC correlations from H-3 to C-1, 4, 4a, 1′, 2′, 6′; $H_2$-4 to C-4a, 5, 8a, C-1′; H-5 to C-1, 4a, 6, 7, 8a; $H_2$-9 to C-6, C-7; H-3′ to C-1′, 5′; H-4′ to C-2′, C-6′; H-6′ to C-2′, 4′ (Fig. 3), which was a dihydroisocoumarin. Furthermore, the absolute configuration at C-3 of **1** was elucidated to be *R* by comparing its Cotton effect with those of (+)-phyllodulcin [7]. Finally, according to the long-range correlation from H-1″ to C-2′ observed in the HMBC spectrum, the linkage position of β-D-glucopyranosyl was elucidated to be C-2′.

Dioscoroside $H_1$ (**2**), white powder, its molecular formula was $C_{26}H_{32}O_{11}$ [*m/z* 519.1874 $[M-H]^-$ (calcd for $C_{26}H_{31}O_{11}$, 519.1872)]. D-glucose was yielded after treated **2** with 1 M HCl [1]. The $^{13}C$ NMR spectrum (Table 2) displayed twenty-six carbon signals. In addition to the carbon signals represented by the above mentioned β-D-glucopyranosyl and two methoxy groups, the other eighteen ones

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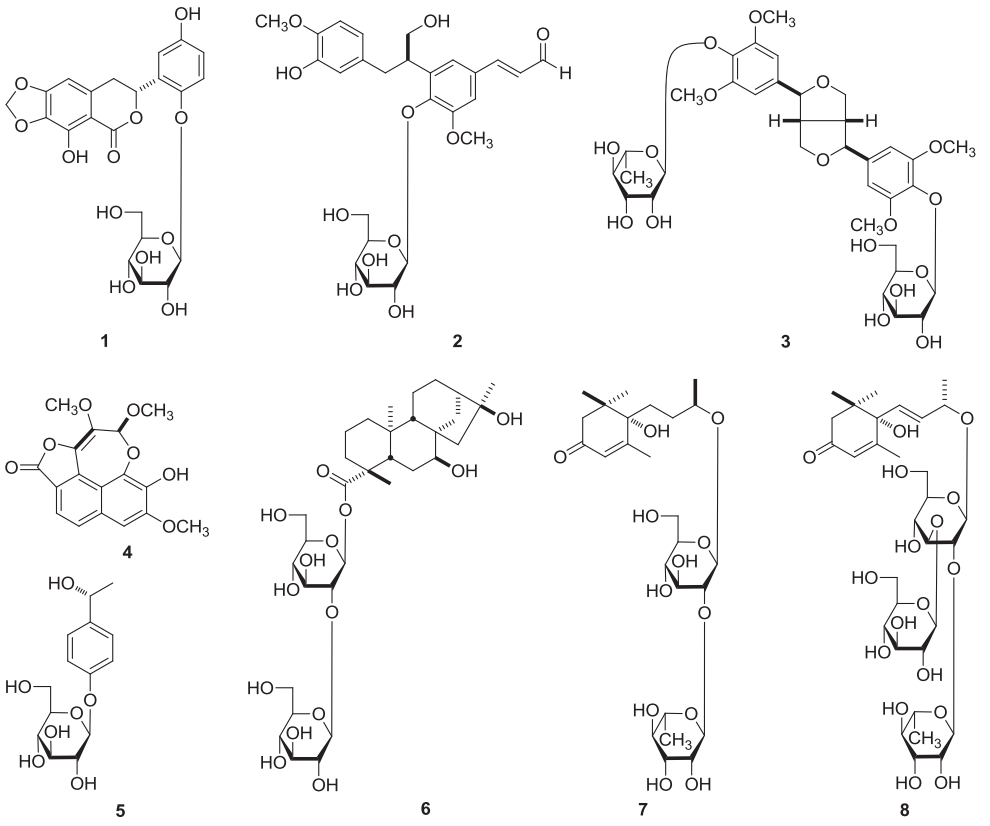


**Fig. 1.** Structures of new compounds **1**–**8**.

indicated **2** was a lignan. The $^{1}H$, $^{13}C$, $^{1}H$ $^{1}H$ COSY, HSQC, and HMBC NMR spectra suggested the presences of one ABX-type aromatic ring [$\delta$ 6.57 (1H, d, $J$ = 8.0 Hz, H-5), 6.61 (1H, d, $J$ = 2.0 Hz, H-2), 6.49 (1H, dd, $J$ = 2.0, 8.0 Hz, H-6)]; one 1,3,4,5-tetrasubstituted aromatic ring [$\delta$ 7.19 (1H, d, $J$ = 1.5 Hz, H-6′), 7.21 (1H, d, $J$ = 1.5 Hz, H-2′)]; one 1-hydroxypropyl {$\delta$ [2.76 (1H, dd, $J$ = 9.0, 14.0 Hz), 2.97 (1H, dd, $J$ = 6.0, 14.0 Hz), $H_2$-7], [3.73 (1H, dd, $J$ = 9.0, 10.5 Hz), 3.80 (1H, dd, $J$ = 6.0, 10.5 Hz), $H_2$-9], 3.99 (1H, m, H-8)}, along with one acryl [6.74 (1H, dd,

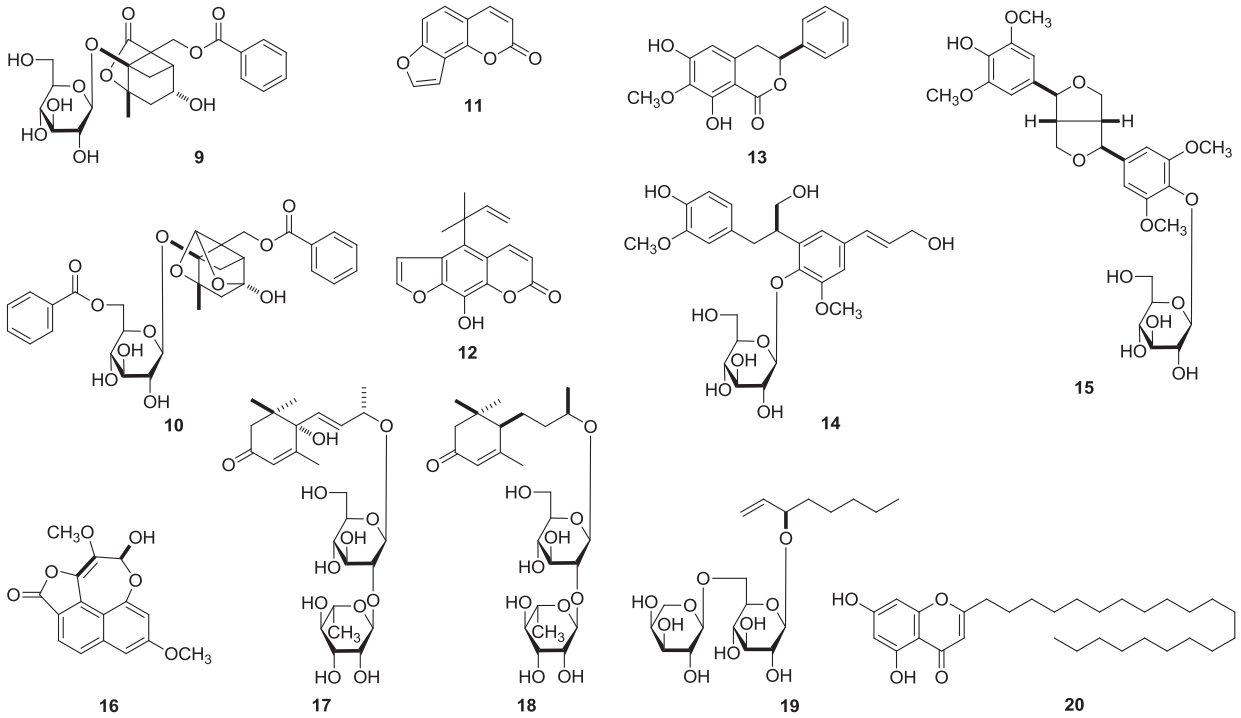


**Fig. 2.** Structures of known compounds **9**–**20**.

**Table 1**
$^1$H and $^{13}$C NMR data for **1** in $CD_3OD$.

| No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) | No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
|---|---|---|---|---|---|
| 1 | 171.5 | – | 2′ | 148.9 | – |
| 3 | 77.3 | 6.03 (dd, 3.0, 12.0) | 3′ | 119.6 | 7.18 (d, 8.5) |
| 4ax | 35.2 | 3.05 (dd, 12.0, 16.0) | 4′ | 117.0 | 6.74 (dd, 3.0, 8.5) |
| 4eq | | 3.36 (dd, 3.0, 16.0) | 5′ | 154.3 | – |
| 4a | 138.0 | – | 6′ | 113.8 | 6.94 (d, 3.0) |
| 5 | 101.5 | 6.42 (s) | 1″ | 104.5 | 4.75 (d, 7.0) |
| 6 | 155.6 | – | 2″ | 75.0 | 3.40 (dd, 7.0, 8.0) |
| 7 | 134.4 | – | 3″ | 78.2 | 3.42 (dd, 8.0, 8.0) |
| 8 | 147.0 | – | 4″ | 71.4 | 3.36 (m, overlapped) |
| 8a | 105.6 | – | 5″ | 78.3 | 3.36 (m, overlapped) |
| 9 | 103.9 | 6.04 (s) | 6″ | 62.6 | 3.68 (dd, 4.5, 11.5) |
| 1′ | 131.1 | – | | | 3.87 (br. d, ca. 12) |

**Table 2**
$^1$H and $^{13}$C NMR data for **2** in $CD_3OD$.

| No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) | No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
|---|---|---|---|---|---|
| 1 | 132.9 | – | 4′ | 148.0 | – |
| 2 | 113.7 | 6.61 (d, 2.0) | 5′ | 139.9 | – |
| 3 | 148.5 | – | 6′ | 110.7 | 7.19 (d, 1.5) |
| 4 | 145.5 | – | 7′ | 155.3 | 7.63 (d, 16.0) |
| 5 | 115.7 | 6.57 (d, 8.0) | 8′ | 129.0 | 6.74 (dd, 8.0, 16.0) |
| 6 | 122.6 | 6.49 (dd, 2.0, 8.0) | 9′ | 196.0 | 9.64 (d, 8.0) |
| 7 | 39.1 | 2.76 (dd, 9.0, 14.0) | 3′-$OCH_3$ | 56.5 | 3.87 (s) |
| | | 2.97 (dd, 6.0, 14.0) | 1″ | 104.9 | 4.82 (d, 8.0) |
| 8 | 42.9 | 3.99 (m) | 2″ | 75.9 | 3.48 (dd, 8.0, 9.0) |
| 9 | 66.4 | 3.73 (dd, 9.0, 10.5) | 3″ | 77.8 | 3.41 (dd, 9.0, 9.0) |
| | | 3.80 (dd, 6.0, 10.5) | 4″ | 71.2 | 3.39 (dd, 8.5, 9.0) |
| 4-$OCH_3$ | 56.3 | 3.73 (s) | 5″ | 78.1 | 3.13 (m) |
| 1′ | 132.4 | – | 6″ | 62.4 | 3.67 (dd, 6.0, 12.0) |
| 2′ | 122.6 | 7.21 (d, 1.5) | | | 3.74 (dd, 2.5, 12.0) |
| 3′ | 154.0 | – | | | |

$J$ = 8.0, 16.0 Hz, H-8′), 7.63 (1H, d, $J$ = 16.0 Hz, H-7′), 9.64 (1H, d, $J$ = 8.0 Hz, H-9′)]. According to the long-range correlations from H-2 to C-4, 6; H-5 to C-1, 3; H-6 to C-2, 4; 4-$OCH_3$ to C-4 observed in the HMBC spectrum, the ABX-type aromatic ring was elucidated, which was substituted with hydroxyl and methoxyl at C-3 and C-4, respectively. Using the same method, 1,3,4,5-tetrasubstituted aromatic ring and its substituted groups were clarified, too. On the other hand, the linkage positions of above mentioned moieties were determined by the correlations observed from H-7 to C-1, 2, 6, 5′; H-8 to C-1, 4′, 6′; H-9 to C-5′; H-7′ to C-2′, 6′; H-8′ to C-1′; H-1″ to C-4′. The structure of **2** was similar to that of icariside $E_5$, except for the substitution position of hydroxyl and methoxyl at ABX-type aromatic ring. Meanwhile, the optical rotation value ($[\alpha]_D^{25}$–105.5° for **2**; $[\alpha]_D^{22}$–109.0° for icariside $E_5$ [8], both in MeOH) and $^{13}$C NMR data at C-7 and 8 [$\delta_C$ 39.1 (C-7), 42.9 (C-8) for **2**; $\delta_C$ 39.1 (C-7), 42.7 (C-8) for icariside $E_5$ [8], both in $CD_3OD$] were very similar to each other. Then, the absolute configuration at C-8 was elucidated to be *R*.

Dioscoroside $H_2$ (**3**), $[\alpha]_D^{25}$–54.9° (in MeOH). Its molecular formula was revealed to be $C_{34}H_{46}O_{17}$ by HRESI-TOF-MS [*m/z* 725.2668 $[M-H]^-$ (calcd for $C_{34}H_{45}O_{17}$, 725.2662)]. The $^1$H, $^{13}$C (Table 3) and 2D ($^1$H $^1$H COSY, HSQC, HMBC) NMR spectra suggested the following moieties presented in **3**: two methoxyl signals [$\delta$ 3.83 (6H, s, 3,5-$OCH_3$), 3.85 (6H, s, 3′,5′-$OCH_3$)]; one $\beta$-D-glucopyranosyl [$\delta$ 4.85 (1H, d, $J$ = 7.5 Hz, H-1″)], one $\alpha$-L-rhamnopyranosyl [$\delta$ 5.21 (1H, br. s, H-1‴)], together with one 7,9′:7′,9-diepoxylignane aglycone [$\delta$ 3.12 (2H, m, H-8 and 8′), 3.92, 4.29 (2H each, both m, $H_2$-9 and 9′), 4.76 (2H, d, $J$ = 3.0 Hz, H-7 and 7′), 6.68 (2H, s, H-2′,6′), 6.71 (2H, s, H-2,6)]. Furthermore, the planar structure of **3** was elucidated by the long-range correlations from H-2,6 to C-1, 3,5, 4, 7; H-2′,6′ to C-1′, 3′,5′, 4′, 7′; H-1″ to C-4, H-1‴ to C-4′; 3,5-$OCH_3$ to C-3,5; 3′,5′-$OCH_3$ to C-3′,5′ found in its HMBC spectrum. Its NMR data of C-7–9, 7′–9′ were almost the same as those

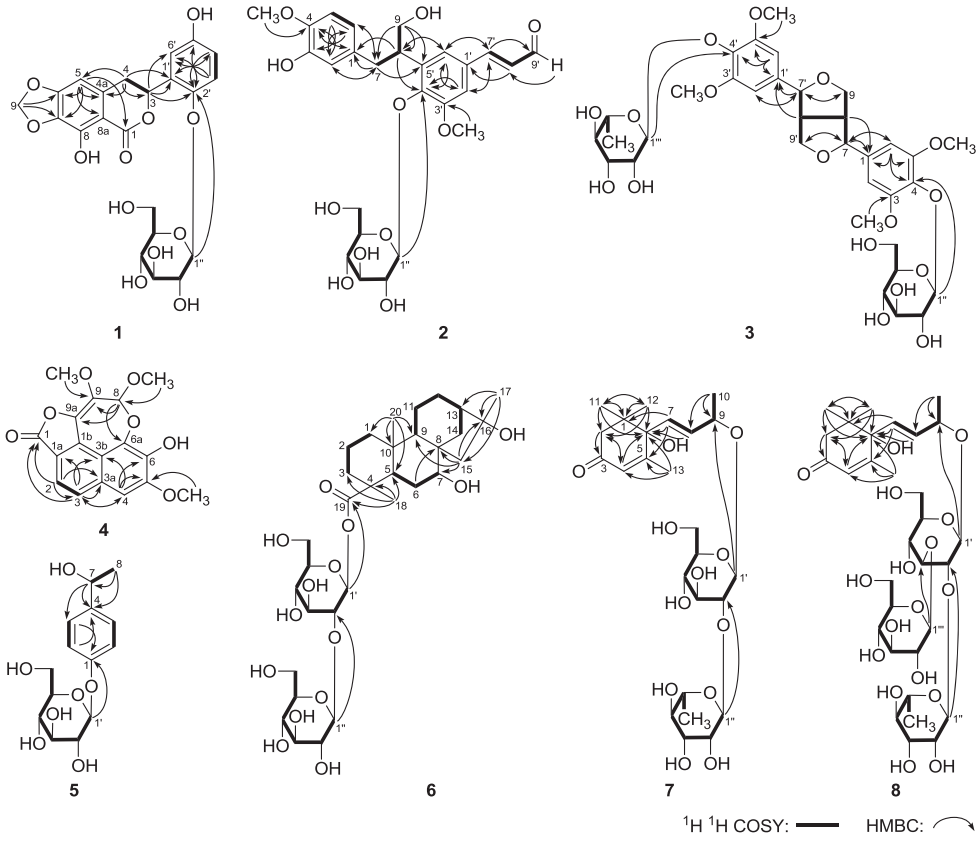


**Fig. 3.** The main $^1$H $^1$H COSY and HMBC correlations of **1**–**8**.

**Table 3**
$^1$H and $^{13}$C NMR data for **3** in $CD_3OD$.

| No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) | No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
|---|---|---|---|---|---|
| 1 | 139.5 | – | 3′,5′-$OCH_3$ | 57.1 | 3.85 (s) |
| 2,6 | 104.9 | 6.71 (s) | 1″ | 105.4 | 4.85 (d, 7.5) |
| 3,5 | 154.4 | – | 2″ | 75.7 | 3.48 (dd, 7.5, 9.0) |
| 4 | 135.6 | – | 3″ | 77.8 | 3.41 (dd, 9.0, 9.0) |
| 7 | 87.3 | 4.76 (d, 3.0) 2.35 (m) | 4″ | 71.3 | 3.41 (dd, 9.0, 9.0) |
| 8 | 55.7 | 3.12 (m) | 5″ | 78.3 | 3.20 (m) |
| 9 | 73.0 | 3.92 (m), 4.29 (m) | 6″ | 62.6 | 3.66 (dd, 5.0, 12.0) |
| 1′ | 139.2 | – | | | 3.77 (dd, 2.0, 12.0) |
| 2′,6′ | 104.0 | 6.68 (s) | 1‴ | 103.6 | 5.21 (br. s) |
| 3′,5′ | 154.8 | – | 2‴ | 72.1 | 4.10 (br. d, ca. 4) |
| 4′ | 135.2 | – | 3‴ | 72.3 | 3.89 (dd, 3.5, 9.0) |
| 7′ | 87.2 | 4.76 (d, 3.0) | 4‴ | 73.8 | 3.42 (dd, 9.0, 9.0) |
| 8′ | 55.7 | 3.12 (m) | 5‴ | 71.1 | 4.29 (m) |
| 9′ | 73.0 | 3.92 (m), 4.29 (m) | 6‴ | 17.9 | 1.21 (d, 6.0) |
| 3,5-$OCH_3$ | 56.6 | 3.83 (s) | | | |

of (−)-(7*R*,7′*R*,8*S*,8′*S*)-4′-hydroxy-3,3′,4,5,5′-pentamethoxy-7,9′:7′,9-diepoxylignane (a) [10]. But the Cotton effects of them [Δε (nm): +1.7 (277), +14.5 (240), +95.7 (209) for **3**; −0.72 (274), −0.86 (239), −3.79 (214) for a, both in MeOH] were just opposite. On the basis of above mentioned evidence, the absolute configuration dioscoroside $H_2$ (**3**) was determined as 7*S*,7′*S*,8*R*,8′*R*.

Dioscorol B (**4**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{25}$ + 97.7°, in $CHCl_3$). The molecular formula of it, as suggested by its HRESI-TOF-MS spectra, was deduced to be $C_{17}H_{14}O_7$ [*m*/*z* 329.0667 $[M - H]^-$ (calcd for $C_{17}H_{13}O_7$, 329.0667)]. The $^1$H, $^{13}$C NMR (Table 4) spectra indicated the presences of two aromatic spin systems {One was two ortho protons of a tetrasubstituted benzene ring [δ 7.58 (1H, d, $J$ = 8.0 Hz, H-2), 7.82 (1H, d, $J$ = 8.0 Hz, H-3)], and the other was one proton of another pentasubstituted benzene [δ 7.39 (1H, br. s, H-4)]}, together with one hemiacetal group [δ 5.80 (1H, s, H-8)]. The above mentioned moieties were connected with each other by the long-range correlations observed in the HMBC spectrum (Fig. 3). Which indicated it was a naphthofuranoxepin. On the other hand, there were three methoxy groups [δ 3.51, 3.99, 4.11 (3H each, all s, 8, 5, 9-$OCH_3$)] in **4**, whose linkage positions with aglycone were elucidated by the long-range correlations from 5-$OCH_3$ to C-5; 8-$OCH_3$ to C-8; 9-$OCH_3$ to C-9 and NOE correlations (Fig. 4) between H-4 and 5-$OCH_3$; H-8 and 8-$OCH_3$, 9-$OCH_3$ observed in the HMBC and NOESY spectra, respectively. The structure of **4** was similar to that of dioscorealide A [11]. The main difference lay in C-6 was substituted by hydroxyl in compound **4**. On the other hand, the Cotton effects of them were similar to each other, which suggested the oxepin ring actually adopted the *M* conformation [11]. Furthermore, **4** displayed positive optical rotation in $CHCl_3$, which indicated it possessed 8*S* configuration [11].

Dioscoroside I (**5**) was obtained as a white powder. Its molecular formula was proposed to be $C_{14}H_{20}O_7$ as rationalized from the negative HRESI-TOF-MS determination [*m*/*z* 301.1043 $[M - H]^-$ (calcd for $C_{14}H_{19}O_7$, 301.1035)] of it. After treating it with 1 M HCl, D-glucose was yielded [1]. In its $^1$H NMR spectrum (Table 5), one AA′BB′ aromatic spin coupling system [δ 7.07 (2H, d, $J$ = 8.0 Hz, H-2,6), 7.29 (2H, d, $J$ = 8.0 Hz, H-3,5)]; one (O)CH-$CH_3$ moiety [δ 1.41 (3H, d, $J$ = 6.5 Hz, $H_3$-8), 4.78 (1H, q, $J$ = 6.5 Hz, H-7)], along with one β-D-glucopyranosyl [δ 4.88 (1H, d, $J$ = 7.0 Hz, H-1′)] were observed. Furthermore, its planar structure was elucidated by the long-range correlations from $H_3$-8 to C-4; H-1′ to C-1 observed in the HMBC spectrum. Finally, the aglycone (**5a**) of **5** was yielded by enzymatic hydrolysis with β-glucosidase, whose absolute configuration was clarified as 7*R* by comparison its optical rotation ($[\alpha]_D^{25}$–20.6°, in MeOH) with those of diethyl 2-hydroxypropylphosphonate (*R* configuration: $[\alpha]_D^0$–6.0°; *S* configuration: $[\alpha]_D^0$ + 7.5°, in MeOH) [16].

**Table 4**
$^1$H and $^{13}$C NMR data for **4** in DMSO-$d_6$.

| No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) | No. | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
|---|---|---|---|---|---|
| 1 | 167.0 | – | 6 | 139.8 | – |
| 1a | 115.0 | – | 6a | 133.4 | – |
| 1b | 134.1 | – | 8 | 100.9 | 5.80 (s) |
| 2 | 117.4 | 7.58 (d, 8.0) | 9 | 139.8 | – |
| 3 | 127.9 | 7.82 (d, 8.0) | 9a | 130.9 | – |
| 3a | 129.6 | – | 5-$OCH_3$ | 56.0 | 3.99 (s) |
| 3b | 116.8 | – | 8-$OCH_3$ | 57.0 | 3.51 (s) |
| 4 | 103.6 | 7.39 (br. s) | 9-$OCH_3$ | 60.0 | 4.11 (s) |
| 5 | 152.3 | – | | | |

The molecular formula, $C_{32}H_{52}O_{14}$, of dioscoroside *J* (**6**) was established by negative-ion HRESI-TOF-MS [*m*/*z* 705.3348 $[M + COOH]^-$ (calcd for $C_{33}H_{53}O_{16}$, 705.3339)]. The $^{13}$C NMR (Table 6) spectrum displayed thirty-two carbon signals, consisting of five quaternary carbons, fourteen methines, ten methylenes, together with three methyls. Combining with its $^1$H NMR spectrum, the presences of two β-D-glucopyranosyl groups [δ 4.84 (1H, d, $J$ = 8.0 Hz, H-1″), 5.55 (1H, d, $J$ = 8.0 Hz, H-1′)] were deduced. In addition to the carbon signals represented by the above mentioned sugars, the other twenty ones indicated **6** was a diterpenoid glycoside. The $^1$H $^1$H COSY spectrum of **6** suggested the presence of five partial structures written in bold lines (Fig. 3). The planar structure of the aglycone was determined based on the key HMBC correlations from $H_2$-15 to C-7–9, 16; $H_3$-17 to C-13, 15, 16; $H_3$-18 to C-3–5, 19; $H_3$-20 to C-1, 5, 9, 10. On the other hand, the $^1$H and $^{13}$C NMR data on A, B rings in **6** were superimposable on those of 7β,17-dihydroxy-16α-*ent*-kauran-19-oic acid 19-*O*-β-D-glucopyranoside ester [17], which indicated 7-OH of compound **6** was in β configuration, which was further clarified by the NOE correlations observed from H-5 to H-9, $H_3$-18; H-6α to H-7, $H_3$-20 in the NOESY spectrum. Furthermore, the NOE correlations observed between H-14α to H-7, H-13; H-13 to $H_3$-17 suggested 16-OH was in β configuration, too. Furthermore, the connectivity of oligoglycoside moiety to the aglycone part was characterized by HMBC experiment, in which long-range correlations were found from H-1′ to C-19; H-1″ to C-2′. On the basis of above mentioned evidences, the structure of **6** was elucidated to be 7β,16β-dihydroxy-*ent*-kauran-19-oic acid 19-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranoside ester.

Dioscoroside $K_1$ (**7**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{25}$–42.9°, in MeOH). The IR spectrum of **7** showed absorption bands at 3364, 1647, 1069 $cm^{-1}$ ascribable to hydroxyl, α,β-unsaturated carbonyl, and ether functions. Its molecular formula, $C_{25}H_{42}O_{12}$, was determined from negative-ion HRESI-TOF-MS [*m*/*z* 579.2664 $[M + COOH]^-$, calcd for $C_{26}H_{43}O_{14}$, 579.2658)]. Acid hydroxysis of **7** with 1 M HCl liberated D-glucose and L-rhamnose [1]. The $^1$H, $^{13}$C NMR (Table 7) spectra indicated the presences of one β-D-glucopyranosyl [δ 4.40 (1H, d, $J$ = 8.0 Hz, H-1′)] and one α-L-rhamnopyranosyl [δ 5.23 (1H, d, $J$ = 1.5 Hz, H-1″)], together with the characteristic signals for megastigmane aglycone [δ 1.02, 1.11 (3H each, both s, $H_3$-12 and 11), 1.22 (3H, d, $J$ = 6.0 Hz, $H_3$-10), 2.06 (3H, d, $J$ = 1.5 Hz, $H_3$-13), 2.16, 2.62 (1H each, both d, $J$ = 18.0 Hz, $H_2$-2), 5.87 (1H, br. s, H-4)]. The side chain structure was determined by the correlations between $H_2$-8 and $H_2$-7, H-9; H-9 and $H_3$-10 observed its $^1$H $^1$H COSY spectrum. Furthermore, the long-range correlations from the proton to carbon pairs were observed in the HMBC spectrum: $H_2$-2 to C-3, 4, 11, 12; $H_2$-7 to C-1, 5, 6; $H_2$-8 to C-6; $H_3$-11 to C-1, 2, 6, 12; $H_3$-12 to C-1, 2, 6, 11; $H_3$-13 to C-4-6; H-1′ to C-9; H-1″ to C-2′. Then, the planar structure of **7** was elucidated, which was similar to that of dihydrovomifoliol-*O*-β-D-glucopyranoside [18], the difference was that C-2′ was substituted by α-L-rhamnopyranosyl in **7**. Meanwhile, the CD spectral data for **7** was essentially identical to that of dihydrovomifoliol-*O*-β-D-glucopyranoside [18], which suggested the absolute configuration at C-6 was *S*. On the other hand, the $^1$H NMR data for 11- and 12-position were deduced from NOE correlation between H-7 and H-11 observed in the NOESY spectrum. Finally, the absolute configuration at C-9 was elucidated to be *R* by the comparison of its $^{13}$C NMR data ($\delta_C$ 75.9) with that of dihydrovomifoliol-*O*-β-D-

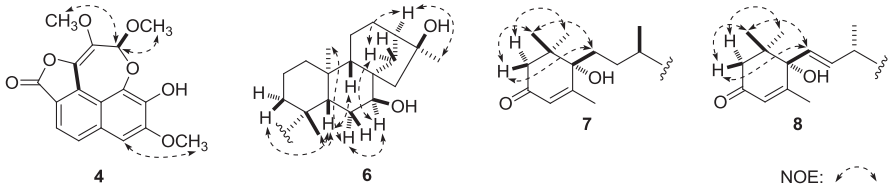


**Fig. 4.** The main NOE correlations of **1**, **6**–**8**.

glucopyranoside ($\delta_C$ 76.3, 9*R*) and icariside $B_5$ ($\delta_C$ 77.9, 9*S*) [18] determined in $CD_3OD$. Then, the structure of **7** was clarified as (6*S*,9*R*)-6,9-dihydroxymegastigman-4-en-3-one 9-*O*-*α*-L-rhamnopyranosyl(1 → 2)-*β*-D-glucopyranoside.

The molecular formula of dioscoroside $K_2$ (**8**) was deduced as $C_{31}H_{50}O_{17}$ from the negative-ion HRESI-TOF-MS [*m*/*z* 739.3033 $[M + COOH]^-$ (calcd for $C_{32}H_{51}O_{19}$, 739.3030)]. The $^1H$, $^{13}C$, $^1H$ $^1H$ COSY, HSQC, and HMBC NMR spectra analysis revealed the planar structure of its aglycone was the same as that of (6*S*,9*S*)-vomifoliol [12]. On the other hand, there were three sugar moieties, consisting of two *β*-D-glucopyranosyl [*δ* 4.33 (1H, d, *J* = 7.5 Hz, H-1′), 4.45 (1H, d, *J* = 7.5 Hz, H-1‴)] and one *α*-L-rhamnopyranosyl [*δ* 5.28 (1H, br. s, H-1″)]. Furthermore, the linkage positions between aglycone and sugar, and between sugar moiety each other were elucidated by the long-range correlations observed from H-1′ to C-9; H-1″ to C-2′; H-1‴ to C-3′. On the other hand, the absolute configuration at C-6 was deduced to be *S* by the CD determination and the comparison with that of (6*S*,9*S*)-vomifoliol [12]. Moreover, by comparing its NMR data (Table 7) with those of (6*S*,9*R*)-, (6*R*,9*R*)-, (6*S*,9*S*)-, and (6*R*,9*S*)-roseoside [(6*S*,9*R*)-, and (6*R*,9*R*)-roseoside: $\delta_H$ ~ 5.85 (2H, m, H-7 and H-8); $\delta_C$ ~ 77 (C-9), ~21 (C-10); (6*S*,9*S*)-, and (6*R*,9*S*)-roseoside: $\delta_H$ ~ 5.70 (1H, dd, *J* = 8, 16 Hz, H-8), ~5.97 (d, *J* = 16 Hz, H-7); $\delta_C$ ~ 75 (C-9), ~22 (C-10), all determined in $CD_3OD$] [12], the absolute configuration at C-9 was revealed as *S*.

The potential anti-inflammatory effects of all obtained compounds (**1**—**21**) on LPS-stimulated NO production were examined in RAW 264.7 macrophages by using the same method as that reported before [1]. As shown in Table 8, NO production was markedly induced in LPS-stimulated RAW 264.7 macrophages when compared to unstimulated normal (negative control). As the results, compounds **7**, **11**, **15**, and **16** displayed significant inhibitory activities on LPS-induced NO production at 30 μM, but **4** showed slight activities on it at same concentration, which suggested that compounds **7**, **11**, **15**, and **16** may have potent anti-inflammatory activity.

## 3. Experimental

### 3.1. General

The following instruments were used to obtain physical data: UV and IR spectra were determined on a Varian Cary 50 UV–Vis and Varian 640-IR FT-IR spectrophotometer, respectively. Optical rotations were determined on a Rudolph Autopol® IV automatic polarimeter. NMR spectra were recorded on a Bruker 500 MHz NMR spectrometer at 500 MHz for $^1H$ and 125 MHz for $^{13}C$ NMR (internal standard: TMS). Negative-ion HRESI-TOF-MS were made on an Agilent Technologies 6520 Accurate-Mass Q-Tof LC/MS spectrometer.

And the following experimental conditions were used for chromatography: Column chromatographies (CC) were performed on macroporous resin D101 (Haiguang Chemical Co., Ltd., Tianjin, China), Silica gel (74–149 μm, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), and Sephadex LH-20 (Ge Healthcare Bio-Sciences, Uppsala, Sweden). Preparative high performance liquid chromatography (PHPLC) {pump: LC-8A (Shimadzu) Preparative liquid chromatograph; detector: RIA-10A (Shimadzu) Refractive index detector; column: Cosmosil $5C_{18}$-MS-II (20 mm i.d. × 250 mm, Nakalai Tesque, Inc., Tokyo, Japan) was used to isolate the compounds.

**Table 5**
$^1H$ and $^{13}C$ NMR data for **5** in $CD_3OD$.

| No. | $\delta_C$ | $\delta_H$ (*J* in Hz) | No. | $\delta_C$ | $\delta_H$ (*J* in Hz) |
|---|---|---|---|---|---|
| 1 | 158.2 | – | 2′ | 75.0 | 3.46 (m, overlapped) |
| 2,6 | 117.6 | 7.07 (d, 8.0) | 3′ | 78.0 | 3.47 (m, overlapped) |
| 3,5 | 127.6 | 7.29 (d, 8.0) | 4′ | 71.4 | 3.41 (m, overlapped) |
| 4 | 141.5 | – | 5′ | 78.0 | 3.42 (m, overlapped) |
| 7 | 70.4 | 4.78 (q, 6.5) 2.35 (m) | 6′ | 62.5 | 3.70 (dd, 5.0, 12.5) |
| 8 | 25.5 | 1.41 (d, 6.5) | | | 3.89 (br. d, ca. 13) |
| 1′ | 102.4 | 4.88 (d, 7.0) | | | |

**Table 6**
$^1H$ and $^{13}C$ NMR data for **6**.

| No. | In $CD_3OD$ | | In $C_5D_5N$ | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| 1*α* | 41.6 | 1.83 (m) | 40.8 | 1.80 (m, overlapped) |
| 1*β* | | 0.89 (ddd, 3.5, 13.5, 13.5) | | 0.98 (ddd, 4.0, 13.5, 13.5) |
| 2*α* | 20.4 | 1.42 (m) | 20.1 | 1.47 (m) |
| 2*β* | | 1.94 (m) | | 2.19 (m, overlapped) |
| 3*α* | 38.9 | 1.07 (ddd, 3.5, 13.5, 13.5) | 38.1 | 1.19 (ddd, 4.5, 14.0, 14.0) |
| 3*β* | | 2.32 (m) | | 2.77 (m) |
| 4 | 44.8 | – | 44.1 | – |
| 5 | 49.0 | 1.74 (br. d, ca. 13) | 48.0 | 2.27 (br. d, ca. 14) |
| 6*α* | 30.2 | 2.13 (dd, 13.0, 13.0) | 30.2 | 2.33 (br. d, ca. 14) |
| 6*β* | | 1.96 (m) | | 2.56 (dd, 14.0, 14.0) |
| 7 | 78.1 | 3.62 (br. s) | 76.8 | 4.07 (br. s) |
| 8 | 50.2 | – | 50.0 | – |
| 9 | 51.2 | 1.39 (d, 7.5) | 50.4 | 1.79 (m, overlapped) |
| 10 | 40.5 | – | 39.8 | – |
| 11 | 18.8 | 1.57 (m, overlapped) | 18.4 | 1.51 (m, overlapped) |
| 12 | 28.2 | 1.57 (m, overlapped) | 27.8 | 1.52 (m, overlapped) |
| 13 | 49.5 | 1.85 (m) | 49.1 | 2.16 (m) |
| 14*α* | 38.1 | 1.66 (dd, 4.0, 11.5) | 37.4 | 1.96 (m, overlapped) |
| 14*β* | | 1.79 (br. d, ca. 12) | | 2.19 (m, overlapped) |
| 15*α* | 54.8 | 1.54 (d, 14.5) | 55.5 | 1.96 (m, overlapped) |
| 15*β* | | 1.86 (d, 14.5) | | 2.57 (d, 13.5) |
| 16 | 79.9 | – | 78.0 | – |
| 17 | 24.4 | 1.34 (s) | 25.0 | 1.57 (s) |
| 18 | 29.3 | 1.22 (s) | 29.2 | 1.46 (s) |
| 19 | 178.1 | – | 176.4 | – |
| 20 | 16.9 | 0.95 (s) | 16.8 | 1.15 (s) |
| 1′ | 94.1 | 5.55 (d, 8.0) | 93.5 | 6.28 (d, 8.0) |
| 2′ | 79.1 | 3.85 (dd, 8.0, 8.5) | 80.8 | 4.42 (dd, 8.0, 9.0) |
| 3′ | 78.7 | 3.68 (dd, 8.5, 9.0) | 78.5 | 4.32 (dd, 9.0, 9.0) |
| 4′ | 71.1 | 3.44 (dd, 9.0, 9.0) | 70.9 | 4.27 (dd, 9.0, 9.0) |
| 5′ | 78.6 | 3.39 (m) | 79.1 | 3.94 (m) |
| 6′ | 62.4 | 3.70 (m, overlapped) | 62.2 | 4.35 (dd, 5.0, 12.0) |
| | | 3.83 (dd, 2.0, 12.0) | | 4.43 (br. d, ca. 12) |
| 1″ | 104.0 | 4.84 (d, 8.0) | 105.5 | 5.51 (d, 7.5) |
| 2″ | 75.9 | 3.20 (dd, 8.0, 8.5) | 76.3 | 4.03 (dd,7.5, 9.0) |
| 3″ | 77.9 | 3.37 (dd, 8.5, 9.0) | 78.2 | 4.21 (dd, 9.0, 9.0) |
| 4″ | 72.0 | 3.26 (dd, 9.0, 9.0) | 72.0 | 4.23 (dd, 9.0, 9.0) |
| 5″ | 78.3 | 3.28 (m) | 78.5 | 3.96 (m) |
| 6″ | 63.2 | 3.71 (dd, 5.0, 11.5) | 63.0 | 4.41 (dd, 5.0, 12.0) |
| | | 3.89 (dd, 2.0, 11.5) | | 4.50 (br. d, ca. 12) |

**Table 7**
$^{1}H$ and $^{13}C$ NMR data for **7** and **8** in $CD_3OD$.

| No. | 7 | | 8 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| 1 | 42.9 | – | 42.4 | – |
| 2 | 51.1 | 2.16 (d, 18.0)<br>2.62 (d, 18.0) | 50.8 | 2.22 (d, 17.0)<br>2.52 (d, 17.0) |
| 3 | 201.1 | – | 201.1 | – |
| 4 | 126.9 | 5.87 (br. s) | 127.4 | 5.93 (s) |
| 5 | 171.5 | – | 167.0 | – |
| 6 | 79.3 | – | 80.0 | – |
| 7 | 34.7 | 1.83 (m) 2.35 (m) | 135.3 | 5.90 (d, 15.5) 2.35 (m) |
| 8 | 32.2 | 1.63 (m), 1.72 (m) | 133.5 | 5.71 (dd, 9.0, 15.5) |
| 9 | 75.9 | 3.89 (m) | 74.7 | 4.54 (m) |
| 10 | 21.2 | 1.22 (d, 6.0) | 22.1 | 1.31 (d, 6.0) |
| 11 | 24.2 | 1.11 (s) | 24.7 | 1.02 (s) |
| 12 | 24.7 | 1.02 (s) | 23.5 | 1.05 (s) |
| 13 | 22.0 | 2.06 (d, 1.5) | 19.7 | 1.96 (s) |
| 1′ | 101.0 | 4.40 (d, 8.0) | 99.1 | 4.33 (d, 7.5) |
| 2′ | 78.4 | 3.35 (dd, 8.0, 8.0) | 77.9 | 3.54 (dd, 7.5, 9.0) |
| 3′ | 79.4 | 3.46 (dd, 8.0, 8.5) | 88.8 | 3.56 (dd, 9.0, 9.0) |
| 4′ | 71.9 | 3.26 (dd, 8.5, 8.5) | 70.2 | 3.38 (dd, 9.0, 9.0) |
| 5′ | 77.8 | 3.23 (m) | 77.8 | 3.15 (m) |
| 6′ | 62.8 | 3.65 (dd, 5.5, 12.0)<br>3.84 (dd, 2.0, 12.0) | 62.7 | 3.65 (dd, 5.0, 11.5)<br>3.86 (br. d, ca. 12) |
| 1″ | 101.9 | 5.23 (d, 1.5) | 102.4 | 5.28 (br. s) |
| 2″ | 72.1 | 3.89 (dd, 1.5, 3.0) | 72.0 | 3.91 (br. d, 3.0) |
| 3″ | 72.3 | 3.63 (dd, 3.0, 9.5) | 72.2 | 3.58 (dd, 3.0, 9.0) |
| 4″ | 73.9 | 3.38 (dd, 9.5, 9.5) | 73.9 | 3.40 (dd, 9.0, 9.0) |
| 5″ | 69.8 | 4.04 (m) | 69.9 | 4.09 (m) |
| 6″ | 18.0 | 1.22 (d, 6.0) | 18.0 | 1.23 (d, 6.0) |
| 1‴ | | | 104.5 | 4.45 (d, 7.5) |
| 2‴ | | | 75.1 | 3.25 (dd, 7.5, 8.0) |
| 3‴ | | | 78.1 | 3.38 (dd, 8.0, 9.0) |
| 4‴ | | | 71.5 | 3.27 (dd, 9.0, 9.0) |
| 5‴ | | | 78.3 | 3.36 (m) |
| 6‴ | | | 62.6 | 3.63 (dd, 4.5, 11.5)<br>3.90 (br. d, ca. 12) |

### 3.2. Plant material

The rhizomes of *D. spongiosae* were purchased at a market in Anguo country, Hebei province, China, and identified by Dr. Li Tianxiang. The voucher specimen was deposited at the Academy of Traditional Chinese Medicine of Tianjin University of TCM.

### 3.3. Extraction and isolation

The dried rhizomes of *D. spongiosae* (7.8 kg) were dealt with the same method as that reported in the reference [1] to furnish $H_2O$ (725.2 g), 95% EtOH (245.5 g), and acetone (1.9 g) eluent for D101 CC, respectively.

The 95% EtOH eluent (150.0 g) was subjected to $SiO_2$ gel CC [$CHCl_3$–MeOH (100:1 → 100:3 → 100:7, *v*/*v*) → $CHCl_3$–MeOH–$H_2O$ (10:3:1 → 7:3:1 → 6:4:1, *v*/*v*/*v*, lower layer)] to yield twelve fractions (Fr. 1–12). Fraction 2 (11.0 g) was isolated by $SiO_2$ gel CC [petroleum ether–EtOAc (50:1 → 30:1 → 20:1 → 10:1 → 5:1, *v*/*v*) → $CHCl_3$–MeOH (100:1 → 100:5, *v*/*v*)] to afford sixteen fractions (Fr. 2-1–2-16). Fraction 2–7 (299.4 mg) was further separated by $SiO_2$ gel CC [$CHCl_3$–MeOH (100:0 → 100:1, *v*/*v*)], and five fractions (Fr. 2-7-1–2-7-5) were given. Fraction 2-7-1 (81.3 mg) was purified by PHPLC [$CH_3CN$–$H_2O$ (40:60, *v*/*v*)] to yield isopsoralen (**11**, 5.7 mg, Rt 26.3 min). Fraction 2–9 (540.8 mg) was recrystallized with MeOH, and 5,7-dihydroxy-2-heneicosyl chromone (**20**, 24.3 mg) was produced. Fraction 2–11 (1210.1 mg) was separated by PHPLC [$CH_3CN$–$H_2O$ (40:60, *v*/*v*)] to afford eighteen fractions (Fr. 2-11-1–2-11-18). Fraction 2-11-7 (16.0 mg) was purified by PHPLC [$CH_3CN$–$H_2O$ (40:60, *v*/*v*)] to give isodemethylfuropinarine (**12**, 6.9 mg, Rt 24.5 min). Fraction 2-11-12 (118.0 mg) was isolated by PHPLC [$CH_3CN$–$H_2O$ (42:58, *v*/*v*)], and annulatomarin (**13**, 48.5 mg, Rt 37.4 min) was furnished. Fraction 2–14 (241.1 mg) was subjected to Sephadex LH-20 CC [$CHCl_3$–MeOH (1:1, *v*/*v*)] to afford four fractions (Fr. 2-14-1–2-14-4). Fraction 2-14-2 (81.5 mg) was separated by PHPLC [$CH_3CN$–$H_2O$ (38:62, *v*/*v*) + 1% HAc], and dioscorol B (**4**, 17.0 mg, Rt 31.4 min) was obtained. Fraction 2–15 (480.9 mg) was precipitated in MeOH and centrifugated at 4000 rpm to yield dioscorealide B (**16**, 34.7 mg). Fraction 5 (5.5 g) was subjected to ODS CC [MeOH–$H_2O$ (40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, *v*/*v*)], and eleven fractions (Fr. 5-1–5-11) were given. Fractions 5–3 (237.5 mg) and 5–4 (382.8 mg) were purified by PHPLC [$CH_3CN$–$H_2O$ (21:79, *v*/*v*)] to produce (+)-syringaresinol 4-*O*-*β*-D-glucopyranoside (**15**, 11.8 mg, Rt 12.9 min) and benzoyl paeoniflorin (**10**, 19.8 mg, Rt 16.0 min), respectively. Fraction 6 (14.0 g) was separated by ODS CC [MeOH–$H_2O$ (20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, *v*/*v*)], and eighteen fractions (Fr. 6-1–6-18) were yielded. Fraction 6–1 (1170.0 mg) was isolated by PHPLC [$CH_3CN$–$H_2O$ (3:97, *v*/*v*)] to furnish seventeen fractions (Fr.6-1-1–6-1-17). Fraction 6-1-14 (90.9 mg) was purified by Sephadex LH-20 CC (MeOH), and dioscoroside I (**5**, 29.4 mg) was produced. Fraction 6–4 (200.0 mg) was prepared by PHPLC [$CH_3CN$–$H_2O$ (10:90, *v*/*v*) + 1% HAc] to obtain albiflorin (**9**, 43.5 mg, Rt 48.2 min). Fraction 6–7 (585.8 mg) was subjected to Sephadex LH-20 CC (MeOH) to furnish four fractions (Fr. 6-7-1–6-7-4). Fraction 6-7-1 (375.2 mg) was further purified by PHPLC [$CH_3CN$–$H_2O$ (15:85, *v*/*v*)], and dioscorosides $H_1$ (**2**, 2.2 mg, Rt 34.5 min), $H_2$ (**3**, 20.4 mg, Rt 57.5 min), together with icariside $E_5$ (**14**, 3.0 mg, Rt 36.8 min) were yielded. Fraction 6–8 (666.5 mg) was separated by PHPLC [$CH_3CN$–$H_2O$ (18:82, *v*/*v*)] to give dioscoroside G (**1**, 8.0 mg, Rt 50.1 min) and gusanlungionoside D (**18**, 11.6 mg, Rt 36.6 min). Fraction 6–10 (1379.6 mg) was isolated by Sephadex LH-20 CC (MeOH) and four fractions (Fr. 6-10-1–6-10-4) were furnished. Fraction 6-10-2 (729.4 mg) was further purified by PHPLC [MeOH–$H_2O$ (50:50, *v*/*v*) + 1% HAc] to obtain 3-*O*-[*α*-L-arabinopyranosyl(1 → 6)-*β*-D-glucopyranosyl]oct-1-ene-3-ol (**19**, 21.6 mg, Rt 35.4 min). Fraction 7 (8.0 g) was isolated by ODS CC [MeOH–$H_2O$ (20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, *v*/*v*)], and fourteen fractions (Fr. 7-1–7-14) were obtained. Fraction 7–4 (190.9 mg) was prepared by Sephadex LH-20 CC (MeOH) to give three fractions (Fr. 7-4-1–7-4-3). Fraction 7-4-1 (49.6 mg) was purified by PHPLC [$CH_3CN$–$H_2O$ (10:90, *v*/*v*) + 1% HAc] to furnish dioscoroside $K_1$ (**7**, 4.2 mg, Rt 46.9 min) and zizyvoside I (**17**, 15.4 mg, Rt 42.5 min). Fraction 9 (8.3 g) was subjected to ODS CC [MeOH–$H_2O$ (30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, *v*/*v*)] to yield fifteen fractions (Fr. 9-1–9-15). Fraction 9–4 (222.0 mg) was isolated by PHPLC [$CH_3CN$–$H_2O$ (12:88, *v*/*v*) + 1% HAc] to obtain dioscoroside $K_2$ (**8**, 14.0 mg, Rt 28.1 min). Fraction 9–9 (427.9 mg) was purified by PHPLC [MeOH–$H_2O$ (50:50, *v*/*v*)] to furnish dioscoroside J (**6**, 12.1 mg, Rt 26.4 min).

**Table 8**
Inhibitory effects of positive control and compounds obtained from *D. spongiosa* on NO production in RAW 264.7 macrophages.

| | NRC (%) | | NRC (%) | | NRC (%) | | NRC (%) |
|---|---|---|---|---|---|---|---|
| Normal | 11.0 ± 0.7** | 4 | 43.0 ± 0.2* | 10 | 97.7 ± 1.5 | 16 | 29.0 ± 1.6** |
| Control | 100.0 ± 1.2 | 5 | 96.1 ± 1.8 | 11 | 29.2 ± 0.8** | 17 | 100.6 ± 1.6 |
| L-NIL | 12.6 ± 0.8** | 6 | 98.4 ± 0.2 | 12 | 87.9 ± 1.1 | 18 | 91.0 ± 0.6 |
| 1 | 98.1 ± 1.0 | 7 | 27.8 ± 1.0** | 13 | 100.2 ± 1.1 | 19 | 88.0 ± 0.7 |
| 2 | 97.5 ± 1.1 | 8 | 95.7 ± 1.8 | 14 | 97.5 ± 1.1 | 20 | 95.2 ± 1.1 |
| 3 | 94.0 ± 1.6 | 9 | 96.5 ± 1.6 | 15 | 9.5 ± 0.3** | | |

Positive control: L-NIL (L-$N^6$-(1-iminoethyl)lysine). Nitrite relative concentration (NRC): percentage of control group, which set as 100%. Values represent the mean ± SD of three determinations. *P < 0.05; **P < 0.01 (differences between compound-treated group and control group). N = 6. L-NIL and **1**–**20** was 30 μM.

#### 3.3.1. Dioscoroside G (*1*)

White powder; $[\alpha]_D^{25}$ + 52.5° (*conc.* 0.4, MeOH); CD (MeOH, *c.* 0.0017 M) $\Delta\varepsilon$ ($\lambda_{nm}$): +0.04 (258), −24.5 (238); UV $\lambda_{max}$ (MeOH) nm

(log ε): 284 (3.93), 235 (4.26); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3356, 2918, 1673, 1634, 1504, 1458, 1344, 1304, 1259, 1209, 1143, 1072, 1030; $^1H$ and $^{13}C$ NMR data, see Table 1; HRESI-TOF-MS Negative-ion mode *m/z* 477.1053 $[M–H]^-$ (calcd for $C_{22}H_{21}O_{12}$, 477.1038).

*3.3.2. Dioscoroside $H_1$ (**2**)*

White powder; $[\alpha]_D^{25}$–105.5° (*conc.* 0.11, MeOH); UV $\lambda_{max}$ (MeOH) nm (log ε): 304 (3.30); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3354, 2918, 2840, 1665, 1621, 1581, 1516, 1426, 1392, 1307, 1273, 1205, 1155, 1128, 1067, 820; $^1H$ and $^{13}C$ NMR data, see Table 2; HRESI-TOF-MS Negative-ion mode *m/z* 519.1874 $[M–H]^-$ (calcd for $C_{26}H_{31}O_{11}$, 519.1872).

*3.3.3. Dioscoroside $H_2$ (**3**)*

White powder; $[\alpha]_D^{25}$–54.9° (*conc.* 0.85, MeOH); CD (MeOH, *c.* 0.0017 M) Δε ($\lambda_{nm}$): +1.7 (277), +14.5 (240), +95.7 (209); UV $\lambda_{max}$ (MeOH) nm (log ε): 317 (3.29, sh), 280 (3.60, sh), 255 (3.94), 235 (4.17); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3390, 2937, 1596, 1507, 1463, 1421, 1374, 1330, 1229, 1126, 1067, 915, 833; $^1H$ and $^{13}C$ NMR data, see Table 3; HRESI-TOF-MS Negative-ion mode *m/z* 725.2668 $[M - H]^-$ (calcd for $C_{34}H_{45}O_{17}$, 725.2662).

*3.3.4. Dioscorol B (**4**)*

White powder; $[\alpha]_D^{25}$ + 97.7° (*conc.* 0.77, $CHCl_3$); CD (MeOH, *c.* 0.0019 M) Δε ($\lambda_{nm}$): +14.4 (311), +5.7 (293), +26.2 (241); CD ($CH_3CN$, *c.* 0.0019 M) Δε ($\lambda_{nm}$): +16.9 (305), +4.4 (286), +25.5 (242); UV $\lambda_{max}$ (MeOH) nm (log ε): 381 (3.57), 286 (4.31), 223 (4.08); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3408, 3358, 2942, 2842, 1755, 1679, 1625, 1598, 1529, 1486, 1432, 1391, 1285, 1259, 1200, 1117, 1052, 978, 897, 834; $^1H$ and $^{13}C$ NMR data, see Table 4. HRESI-TOF-MS Negative-ion mode *m/z* 329.0667 $[M - H]^-$ (calcd for $C_{17}H_{13}O_7$, 329.0667).

*3.3.5. Dioscoroside I (**5**)*

White powder; $[\alpha]_D^{25}$–75.4° (*conc.* 0.89, MeOH); UV $\lambda_{max}$ (MeOH) nm (log ε): 265 (3.21), 220 (3.90); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3367, 2970, 2924, 1611, 1511, 1398, 1232, 1181, 1074, 837; $^1H$ and $^{13}C$ NMR data, see Table 5. HRESI-TOF-MS Negative-ion mode *m/z* 301.1043 $[M - H]^-$ (calcd for $C_{14}H_{19}O_7$, 301.1035).

*3.3.6. Dioscoroside J (**6**)*

White powder; $[\alpha]_D^{25}$–39.3° (*conc.* 0.30, MeOH); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3370, 2928, 2841, 1727, 1446, 1372, 1224, 1073; $^1H$ and $^{13}C$ NMR data, see Table 6. HRESI-TOF-MS Negative-ion mode *m/z* 705.3348 $[M + COOH]^-$ (calcd for $C_{33}H_{53}O_{16}$, 705.3339).

*3.3.7. Dioscoroside $K_1$ (**7**)*

White powder; $[\alpha]_D^{25}$–42.9° (*conc.* 0.21, MeOH); CD (MeOH, *c.* 0.0015 M) Δε ($\lambda_{nm}$): +4.6 (310), −24.7 (255), +37.7 (221); UV $\lambda_{max}$ (MeOH) nm (log ε): 243 (2.76); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3364, 2966, 2922, 1647, 1069, 1043; $^1H$ and $^{13}C$ NMR data, see Table 7. HRESI-TOF-MS Negative-ion mode *m/z* 579.2664 $[M + COOH]^-$ (calcd for $C_{26}H_{43}O_{14}$, 579.2658).

*3.3.8. Dioscoroside $K_2$ (**8**)*

White powder; $[\alpha]_D^{25}$ + 3.7° (*conc.* 0.53, MeOH); CD (MeOH, *c.* 0.0014 M) Δε ($\lambda_{nm}$): −1.52 (312), +32.8 (246); UV $\lambda_{max}$ (MeOH) nm (log ε): 235 (3.00); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3365, 2965, 2928, 1653, 1069, 1044; $^1H$ and $^{13}C$ NMR data, see Table 7. HRESI-TOF-MS Negative-ion mode *m/z* 739.3033 $[M + COOH]^-$ (calcd for $C_{32}H_{51}O_{19}$, 739.3030).

*3.4. Acid hydrolysis for **1**–**3** and **5**–**8***

Compounds **1**–**3** and **5**–**8** (each 1.5 mg) were refluxed with 1 M HCl (1.0 mL) on a water bath for 3 h, respectively. The reaction mixture was neutralized with Amberlite IRA-400 ($OH^-$ form) and removed by filtration. The aqueous layer was subjected to HPLC analysis using the similar method as that reported before [1], D-glucose was identified from **1**–**3** and **5**–**8**, and L-rhamnose was found from **3**, **7**, and **8**. All of them were identified by comparison of their retention times and optical rotations with those of authentic samples [L-rhamnose (8.8 min, negative optical rotation) and D-glucose (17.0 min, positive optical rotation)].

*3.5. Enzymatic hydrolysis of **5***

A solution of **5** (5.0 mg) in $H_2O$ (1.0 mL) was treated with β-glucosidase (5.0 mg, Almond, Sigma-Aldrich, Co. 3050 Spruce Street, St. Louis, MO, 63103 USA), which was stirred at 37 °C for 12 h. The reaction mixture was extracted with EtOAC. Evaporation the solvent under reduce pressure to furnish **5a**.

$^1H$ NMR ($CD_3OD$, 500 MHz) of **5a**: δ 6.73 (2H, d, $J$ = 8.5 Hz, H-2,6), 7.17 (2H, d, $J$ = 8.5 Hz, H-3,5), 4.73 (1H, q, $J$ = 6.5 Hz, H-7), 1.39 (3H, d, $J$ = 6.5 Hz, $H_3$-8); $^{13}C$ NMR ($CD_3OD$, 125 MHz) δ: 157.6 (C-1), 116.0 (C-2,6), 127.8 (C-3,5), 138.4 (C-4), 70.4 (C-7), 25.5 (C-8).

*3.6. In vitro anti-inflammatory assay*

The in vitro anti-inflammatory assay was performed by using the same method as described in the reference [1]. And RAW 264.7 macrophages (IBMS, CAMS/PUMC, Beijing, China) were maintained in DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere containing 5% $CO_2$ at 37 °C. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide [MTT, Sigma Chemical (St. Louise, MO)]) assay was used to determine cell viability.

As nitrite is a major stable product of NO, the concentration of NO in culture supernatants was determined by measuring the nitrite levels by using Griess reagent (St. Louise, MO). Cells were pretreated with different compounds from St. Louise, for 1 h, and then stimulated with LPS [500 ng/mL, Sigma Chemical (St. Louise, MO)] for 24 h. After incubation, aliquots of 100 μL of each culture medium was mixed with an equal volume of Griess reagent. Nitrite levels were determined using an ELISA plate reader at 540 nm, and concentrations were calculated by reference to a $NaNO_2$ standard calibration curve.

## Conflict of interest

We declare that we have no conflict of interest.

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