Triterpenoids and Flavonoids from the Leaves of *Astragalus membranaceus* and Their Inhibitory Effects on Nitric Oxide Production

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Four new cycloartane triterpenes, named huangqiyegenins V and VI and huangqiyenins K and L (**1**–**4**, resp.), together with nine known triterpenoids, **5**–**13**, and eight flavonoids, **14**–**21**, were isolated from a 70%-EtOH extract of *Astragalus membranaceus* leaves. The structures of the new compounds were elucidated by detailed spectroscopic analyses, and the compounds were identified as (9*β*,11*α*,16*β*,20*R*,24*S*)-11,16,25-trihydroxy-20,24-epoxy-9,19-cyclolanostane-3,6-dione (**1**), (9*β*,16*β*,24*S*)-16,24,25-trihydroxy-9,19-cyclolanostane-3,6-dione (**2**), (3*β*,6*α*,9*β*,16*β*,20*R*,24*R*)-16,25-dihydroxy-3-(*β*-D-xylopyranosyloxy)-20,24-epoxy-9,19-cyclolanostan-6-yl acetate (**3**), and (3*β*,6*α*,9*β*,16*β*,24*E*)-26-(*β*-D-glucopyranosyloxy)-16-hydroxy-3-(*β*-D-xylopyranosyloxy)-9,19-cyclolanost-24-en-6-yl acetate (**4**). All isolated compounds were evaluated for their inhibitory activities against LPS-induced NO production in RAW264.7 macrophage cells. Compounds **1**–**3**, **14**, **15**, and **18** exhibited strong inhibition on LPS-induced NO release by macrophages with IC_{50} values of 14.4–27.1 μM.

Introduction. – Dried roots of *Astragalus membranaceus* have been well used in traditional Chinese medicine as antiperspirants, diuretics, tonics, *etc.* [1]. A previous chemical investigation of this plant revealed that the major chemical constituents were saponins, flavonoids, and polysaccharides [2]. It also includes components such as alkaloids, amino acids, *β*-sitosterol, and metallic elements. In addition, the leaves of *A. membranaceus* have many biological functions including anticaducity, anti-inflammatory, and antioxidation activities [3], and protection against pancreatic injury [4]. Dried leaves of *A. membranaceus* (named beiqishencha) were authorized as functional food by the State Food and Drug Administration of P. R. China in 1998.

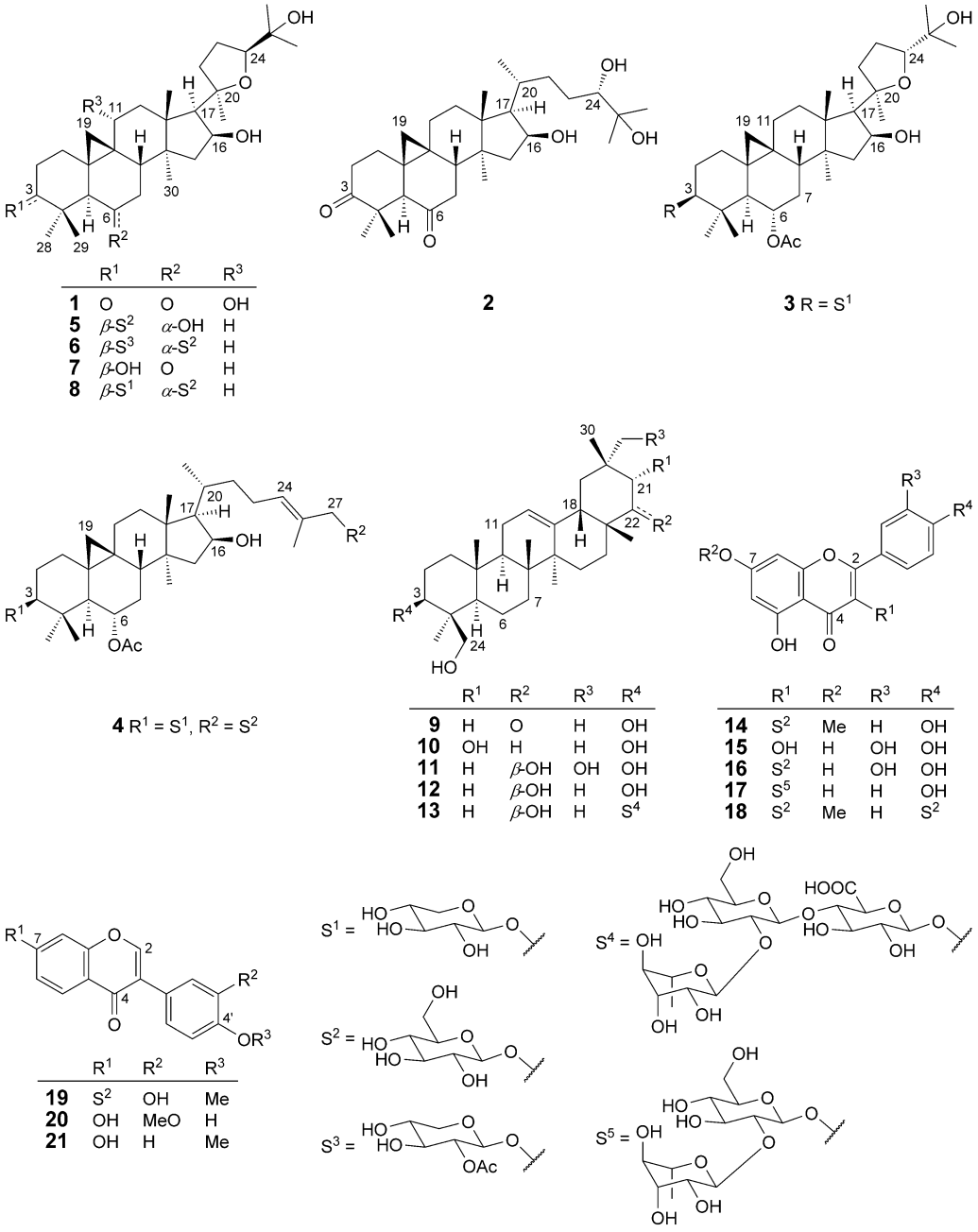
The structure of 9,19-cycloartane-type saponins has drawn more and more attention of chemists ever since cycloartane triterpenoids were first discovered in *Astragalus* plants [5]. Up to now, more than 150 uncommon cycloartane triterpenoids have been isolated from *Astragalus* plants [6]. The *Astragalus* saponins have been studied extensively during the past 30 years and have been reported to have a wide range of biological properties [7], such as hepatoprotection [7a], cardioprotection [7b], antidiabetic nephropathy [7c], anti-inflammatory [7d], gastroprotection [7e], and neuroprotection activities [7f]. In the process of our continuing efforts to study this

source, four new cycloartane-type triterpenoids, huangqiyegenin V (**1**), huangqiyegenin VI (**2**), huangqiyenin K (**3**), and huangqiyenin L (**4**), together with 17 known compounds, **5**–**21** (*Fig. 1*), were isolated from a 70%-EtOH extract of *A. membranaceus* leaves. These isolated compounds were evaluated for their inhibitory effects on lipopolysaccharide (LPS)-induced NO production in RAW264.7 cells. Here, we describe the structural elucidation of the four new cycloartane triterpenoids and NO inhibitory effects of all isolated compounds.

Results and Discussion. – *Structure Elucidation*. Huangqiyegenin V (**1**) was obtained as white amorphous powder. The molecular formula, $C_{30}H_{46}O_6$, was determined by positive-ion mode HR-ESI-MS (*m*/*z* 525.3194 ($[M+Na]^+$; calc. 525.3187)). The IR spectrum of **1** exhibited absorptions at 3393 (OH) and 1705 cm^{-1} (C=O). The ^{1}H-NMR spectrum (*Table 1*) of **1** revealed the presence of seven Me groups resonating at δ(H) 1.80, 1.59, 1.54, 1.34, 1.29, 1.26, and 0.94, and three CH–O groups at 5.08 (*ddd*, *J* = 14.0, 7.7, 2.8), 4.00 (br. *d*), and 3.85 (*dd*, *J* = 9.0, 5.0). The ^{13}C-NMR spectrum (*Table 1*) of **1** exhibited signals of two C=O groups at δ(C) 213.9 and 211.2. A comparative study of the NMR data (*Table 1*) of **1** with those of huangqiyegenin I indicated that the structures of these two compounds are very similar [8]. In the HMBC spectrum (*Fig. 2*), the correlations of CH_2(1) (δ(H) 1.78–1.86 and 1.98–2.07) with C(3) (δ(C) 213.9) and of H–C(8) (δ(H) 3.20) with C(6) (δ(C) 211.2) indicated that the two C=O groups are located at C(3) and C(6). Moreover, the correlation of CH_2(19) (δ(H) 1.22) with C(11) (δ(C) 68.2) in the HMBC spectrum (*Fig. 2*) showed that a OH group is attached to C(11). In the NOESY spectrum, the correlation between Me(21) (δ(H) 1.34 (*s*)) and H–C(17) (2.60 (*d*, *J* = 7.7)), and the correlation between H–C(17) and H–C(24) (3.85 (*dd*, *J* = 9.0, 5.0)) indicated that H–C(17) is α-oriented. Furthermore, the cross-peaks H–C(11) (δ(H) 4.00)/Me(18) (1.80) and H–C(16) (5.08)/Me(30) (0.94) indicated that H–C(11) is β- and H–C(16) is α-oriented. Therefore, **1** was established to be (9β,11α,16β,20*R*,24*S*)-11,16,25-trihydroxy-20,24-epoxy-9,19-cyclolanostane-3,6-dione.

Huangqiyegenin VI (**2**) was obtained as white amorphous powder with a molecular formula established as $C_{30}H_{48}O_5$ on the basis of HR-ESI-MS (*m*/*z* 511.3396 ($[M+Na]^+$; calc. 511.3394)). The IR spectrum revealed the presence of OH (3404 cm^{-1}) and C=O groups (1728 cm^{-1}). The ^{1}H-NMR spectrum (*Table 1*) of **2** revealed the presence of six quaternary Me groups resonating at δ(H) 1.60, 1.48, 1.45, 1.27, 1.06, and 0.86, and two CH–O groups at 4.69–4.70 and 3.93. The ^{13}C-NMR spectrum (*Table 1*) of **2** exhibited signals of two C=O groups at δ(C) 214.0 and 211.0. The NMR data were similar to those of huangqiyegenin II [8], except for the presence of an additional C=O group in **2**. In the HMBC spectrum (*Fig. 2*), the correlations of CH_2(1) (δ(H) 2.05–2.08 and 1.53–1.58) with C(3) (δ(C) 214.0) and of H–C(8) (δ(H) 2.68) with C(6) (δ(C) 211.0) indicated that the two C=O groups are located at C(3) and C(6). Thus, **2** was assigned as (9β,16β,24*S*)-16,24,25-trihydroxy-9,19-cyclolanostane-3,6-dione.

Huangqiyenin K (**3**), obtained as white amorphous powder, has the molecular formula of $C_{37}H_{60}O_{10}$, as established on the basis of HR-ESI-MS data (*m*/*z* 665.4261 ($[M+H]^+$; calc. 665.4259)). The IR spectrum indicated the presence of OH (3393 cm^{-1}) and C=O groups (1726 cm^{-1}). The NMR data of **3** (*Table 2*) were in good



	R^1	R^2	R^3
1	O	O	OH
5	β-S^2	α-OH	H
6	β-S^3	α-S^2	H
7	β-OH	O	H
8	β-S^1	α-S^2	H

2

3 R = S^1

4 R^1 = S^1, R^2 = S^2

	R^1	R^2	R^3	R^4
9	H	O	H	OH
10	OH	H	H	OH
11	H	β-OH	OH	OH
12	H	β-OH	H	OH
13	H	β-OH	H	S^4

	R^1	R^2	R^3	R^4
14	S^2	Me	H	OH
15	OH	H	OH	OH
16	S^2	H	OH	OH
17	S^5	H	H	OH
18	S^2	Me	H	S^2

	R^1	R^2	R^3
19	S^2	OH	Me
20	OH	MeO	H
21	OH	H	Me

Fig. 1. *Structures of* **1**–**21**

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; in C_5D_5N) *of* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.78–1.86 (*m*), 1.98–2.07 (*m*)	31.2	1.53–1.58 (*m*), 2.05–2.08 (*m*)	30.6
2	2.57–2.64 (*m*)	36.0	2.61–2.63 (*m*)	36.1
3		213.9		214.0
4		50.2		50.1
5	2.96 (*s*)	56.5	2.89 (*s*)	56.4
6		211.2		211.0
7	2.39 (*dd*, *J*=17.0, 7.8), 2.42 (*dd*, *J*=17.0, 3.4)	41.5	2.30–2.31 (*m*), 2.61–2.63 (*m*)	41.4
8	3.20 (*dd*, *J*=7.8, 3.4)	40.6	2.68 (*dd*, *J*=7.5, 3.4)	42.1
9		29.5		22.4
10		30.0		28.6
11	4.00 (br. *d*)	68.2	1.34–1.35 (*m*), 1.56–1.57 (*m*)	26.6
12	1.98–2.07 (*m*), 2.19–2.25 (*m*)	42.5	1.56–1.57 (*m*), 2.22–2.24 (*m*)	33.2
13		45.4		46.0
14		47.4		47.6
15	1.85 (*d*, *J*=6.4), 1.98–2.07 (*m*)	44.2	1.78–1.79 (*m*), 2.05–2.08 (*m*)	45.7
16	5.08 (*ddd*, *J*=14.0, 7.7, 2.8)	73.1	4.69–4.70 (*m*)	71.3
17	2.60 (*d*, *J*=7.7)	57.9	1.78–1.79 (*m*)	56.5
18	1.80 (*s*)	21.3	1.06 (*s*)	18.5
19	1.22 (*s*)	19.3	0.24 (*d*, *J*=4.8), 0.97 (*d*, *J*=4.8)	20.5
20		87.3	2.30–2.33 (*m*)	28.8
21	1.34 (*s*)	28.7	1.27 (*s*)	15.5
22	1.65–1.66 (*m*), 3.10–3.13 (*m*)	34.9	1.38–1.39 (*m*), 1.56–1.57 (*m*)	32.7
23	1.98–2.07 (*m*), 2.22–2.25 (*m*)	26.5	1.78–1.79 (*m*), 1.94–1.96 (*m*)	28.0
24	3.85 (*dd*, *J*=9.0, 5.0)	81.6	3.93 (*dd*, *J*=10.8, 2.4)	77.3
25		71.4		72.5
26	1.54 (*s*)	28.2	1.48 (*s*)	25.8
27	1.26 (*s*)	27.1	1.45 (*s*)	26.5
28	1.29 (*s*)	25.7	1.27 (*s*)	25.6
29	1.59 (*s*)	20.6	1.60 (*s*)	20.6
30	0.94 (*s*)	19.5	0.86 (*s*)	19.0

agreement with those of huangqiyenin D [9] except for the configurations at C(24) and the sugar moiety positioned at C(3). The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table 2*) indicated the presence of a pentose moiety with the anomeric H-atom resonating at δ(H) 4.85 (*d*, *J*=7.6). Acid hydrolysis of **3** afforded D-xylose detected by GC [10]. The coupling constant (*J*=7.6) of the anomeric H-atom suggested that the anomeric C-atom of the D-xylopyranose moiety is β-configured. In the HMBC spectrum (*Fig. 2*), the long-range correlations of H–C(6) (δ(H) 4.97–5.00) with the ester C=O C-atom (δ(C) 170.4) indicated that the AcO group is linked to C(6), and the significant HMB correlation of the anomeric H-atom resonating at δ(H) 4.85 (*d*, *J*=7.6) with C(3) (δ(C) 87.6) indicated that the sugar unit is located at C(3). In the NOESY spectrum, a cross-peak between Me(21) (δ(H) 1.42 (*s*)) and Me(26) (1.28 (*s*)) was observed. Moreover, the NOESY correlation between Me(21) and H–C(17) (2.28 (*d*, *J*=7.7)), as well as comparison with the NMR data of huangqiyenin D, suggested that the side chain at

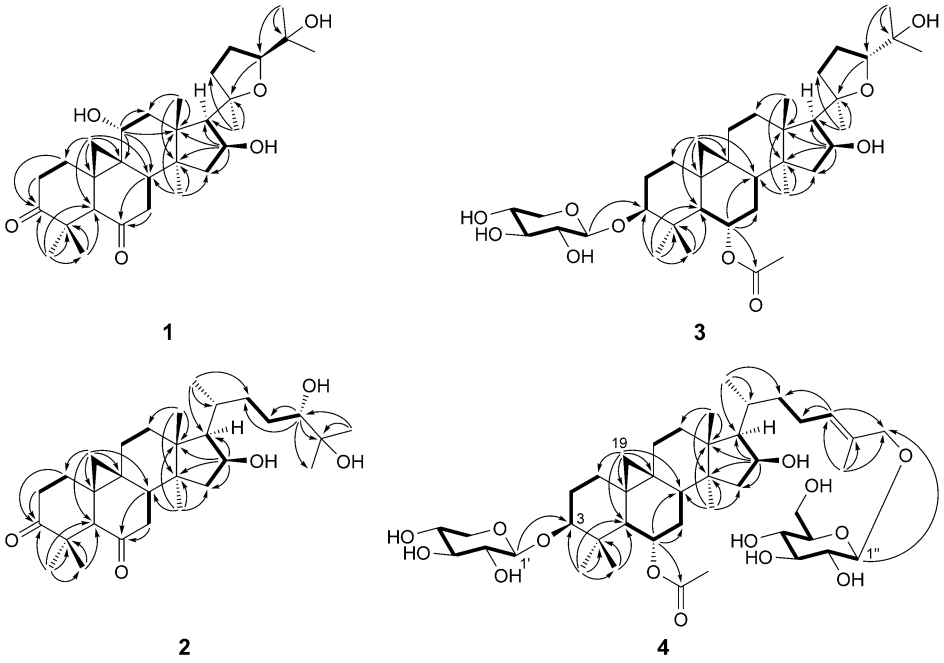


Fig. 2. *Key* $^{1}H,^{1}H$*-COSY* (—) *and selected HMB* (H → C) *correlations of* **1**–**4**

C(17) is β-oriented. Thus, the structure of **3** was elucidated as (3β,6α,9β,16β,20*R*,24*R*)-16,25-dihydroxy-3-(β-D-xylopyranosyloxy)-20,24-epoxy-9,19-cyclolanostan-6-yl acetate.

Huangqiyenin L (**4**) was obtained as white amorphous powder. Its molecular formula was determined as $C_{43}H_{70}O_{14}$ by positive-ion mode HR-ESI-MS (*m*/*z* 811.4847 ($[M+H]^+$; calc. 811.4838)). The IR spectrum indicated the presence of an ester C=O group (1728 cm^{-1}) and an olefinic moiety (1634 cm^{-1}). The ^{13}C-NMR spectrum (*Table 2*) of **4** revealed a total of 43 C-atom signals due to the aglycon moiety (32 C-atom signals) along with two sugar units (eleven C-atom signals). The NMR data (*Table 2*) of **4** were similar to those of kahiricoside V [11], except for the sugar moiety and the signals of one additional AcO group. Acid hydrolysis of **4** afforded D-xylose and D-glucose (GC evidence) [10]. The HMBCs (*Fig. 2*) of H–C(3) (δ(H) 3.53 (*dd*, *J* = 11.0, 4.0)) with C(1′) (δ(C) 107.8) and of CH_2(27) (δ(H) 4.25 (*d*, *J* = 11.6) and 4.46 (*d*, *J* = 11.6)) with C(1″) (δ(C) 103.5) suggested that D-xylopyranose and D-glucopyranose are located at C(3) and C(27), respectively. Moreover, it is obvious that the AcO group is located at C(6), based on comparison with the NMR data of **3**. Hence, **4** was elucidated as (3β,6α,9β,16β,24*E*)-26-(β-D-glucopyranosyloxy)-16-hydroxy-3-(β-D-xylopyranosyloxy)-9,19-cyclolanost-24-en-6-yl acetate.

The known triterpenoids were identified as huangqiyesaponin C (**5**) [12], astragaloside II (**6**) [13], huangqiyegenin I (**7**) [8], astragaloside IV (**8**) [14], soyasapogenol E (**9**) [15], (3β,21α)-olean-12-ene-3,21,24-triol (**10**) [16], (3β,22β)-olean-12-ene-3,22,24,29-tetrol (**11**) [17], soyasapogenol B (**12**) [18], and soyasapogenol B 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosi-

Table 2. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; in C_5D_5N) *of* **3** *and* **4**. δ in ppm, J in Hz.

Position	**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.20–1.22 (*m*), 1.64–1.66 (*m*)	32.1	1.23–1.26 (*m*), 1.62–1.64 (*m*)	32.1
2	1.96–1.97 (*m*), 2.35–2.41 (*m*)	30.1	1.94–1.97 (*m*), 2.37–2.42 (*m*)	30.1
3	3.53 (*dd*, *J*=11.3, 3.3)	87.6	3.53 (*dd*, *J*=11.0, 4.0)	87.7
4		42.3		42.3
5	1.80 (*d*, *J*=5.5)	50.2	1.85 (*d*, *J*=9.0)	50.1
6	4.97–5.00 (*m*)	70.8	4.99–5.01 (*m*)	70.8
7	1.44–1.45 (*m*), 1.74–1.76 (*m*)	33.3	1.48–1.53 (*m*), 1.71–1.73 (*m*)	33.4
8	1.99 (*dd*, *J*=10.4, 4.2)	45.4	1.94–1.97 (*m*)	45.5
9		20.9		21.1
10		28.6		28.3
11	1.85–1.87 (*m*), 2.09–2.15 (*m*)	25.6	1.23–1.26 (*m*), 1.85 (*d*, *J*=9.0)	26.0
12	1.44–1.45 (*m*), 1.73–1.74 (*m*)	33.4	1.62–1.64 (*m*)	33.1
13		46.5		45.8
14		46.8		46.7
15	1.77–1.79 (*m*), 2.07–2.09 (*m*)	48.8	1.69–1.70 (*m*), 2.08–2.11 (*m*)	48.9
16	4.69–4.73 (*m*)	73.0	4.57–4.63 (*m*)	71.1
17	2.28 (*d*, *J*=7.7)	56.6	1.80–1.82 (*m*)	56.8
18	1.49 (*s*)	20.9	1.32 (*s*)	18.7
19	0.23 (*d*, *J*=4.3), 0.50 (*d*, *J*=4.3)	29.0	0.22 (*d*, *J*=4.0), 0.50 (*d*, *J*=4.0)	28.6
20		87.7	2.22–2.27 (*m*)	30.7
21	1.42 (*s*)	29.3	1.03 (*d*, *J*=6.6)	18.2
22	1.83–1.85 (*m*), 2.57–2.62 (*m*)	38.4	1.24–1.25 (*m*), 2.08–2.11 (*m*)	36.6
23	1.90–1.93 (*m*), 2.12–2.15 (*m*)	26.1	2.13–2.15 (*m*), 2.22–2.27 (*m*)	25.6
24	4.10 (*dd*, *J*=10.4, 5.7)	87.7	5.72 (*t*, *J*=6.0)	129.5
25		70.3		131.9
26	1.28 (*s*)	26.4	1.80 (*s*)	14.3
27	1.41 (*s*)	27.2	4.25 (*d*, *J*=11.6), 4.46 (*d*, *J*=11.6)	75.2
28	1.16 (*s*)	27.0	1.17 (*s*)	27.0
29	1.39 (*s*)	16.6	1.42 (*s*)	16.6
30	0.96 (*s*)	20.2	0.99 (*s*)	20.0
1′	4.85 (*d*, *J*=7.6)	107.8	4.85 (*d*, *J*=7.5)	107.8
2′	4.03–4.05 (*m*)	75.6	4.02–4.08 (*m*)	75.6
3′	4.16–4.18 (*m*)	78.7	4.14–4.18 (*m*)	78.7
4′	4.23–4.28 (*m*)	71.3	4.22–4.28 (*m*)	71.2
5′	3.76 (*dd*, *J*=11.0, 10.1), 4.38 (*dd*, *J*=11.0, 5.0)	67.2	3.77 (*dd*, *J*=11.0, 10.1), 4.38 (*dd*, *J*=11.0, 5.0)	67.2
1″			4.89 (*d*, *J*=7.8)	103.5
2″			4.22–4.28 (*m*)	75.3
3″			4.22–4.28 (*m*)	78.7
4″			4.22–4.28 (*m*)	71.8
5″			3.93–3.98 (*m*)	78.5
6″			4.38 (*dd*, *J*=11.0, 5.0), 4.58 (*d*, *J*=11.0)	62.9
AcO	2.04 (s)	21.8, 170.4	2.03 (*s*)	21.8, 170.3

Table 3. *Inhibitory Effects of Compounds on NO Production in LPS-Stimulated RAW264.7 Cells*

Compound	$IC_{50} \pm$SD [μM]	Compound	$IC_{50} \pm$SD [μM]
1	21.5 ± 4.5	**14**	14.8 ± 3.8
2	14.7 ± 2.3	**15**	14.4 ± 2.5
3	27.1 ± 3.0	**17**	31.2 ± 3.6
4	64.9 ± 7.6	**18**	21.2 ± 4.8
5	73.6 ± 10.0	**20**	44.4 ± 11.0
9	63.0 ± 11.6	**21**	50.0 ± 12.6
10	98.5 ± 12.9	Hydrocortisone	23.1 ± 4.9
12	95.6 ± 11.6	Other compounds[a])	> 100

[a]) Specifically **6**–**8**, **11**, **13**, **16**, and **19**.

duronic acid (**13**) [19], and the eight known flavonoids were rhamnocitin 3-*O*-*β*-D-glucopyranoside (**14**) [9], quercetin (**15**) [20], quercetin 3-*O*-*β*-D-glucopyranoside (**16**) [21], rhamnocitin 3-*O*-*β*-neohesperidoside (**17**) [22], complanaruside (**18**) [23], calycosin 7-*O*-*β*-D-glucopyranoside (**19**) [24], 4′,7-dihydroxy-3′-methoxyisoflavone (**20**) [25], and formononetin (**21**) [26] (*Fig. 1*).

Biological Assays. All isolated compounds were examined for their inhibitory effects on the release of NO from macrophages using LPS-induced RAW264.7 cells as model system. Firstly, the noncytotoxic concentrations of the compounds toward macrophage RAW264.7 cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. Compounds **1**–**3**, **14**, **15**, and **18** exhibited strong inhibitory activities against NO production in noncytotoxic concentrations with IC_{50} values of 14.4–27.1 μM (*Table 3*), with hydrocortisone (IC_{50} 23.1 ± 4.9 μM) being used as positive control [27].

Conclusions. – Investigation of the EtOH extract of *A. membranaceus* leaves led to the isolation of 13 triterpenoids, **1**–**13**, and eight flavonoids, **14**–**21**, of which four, *i.e.*, huangqiyegenin V (**1**), huangqiyegenin VI (**2**), huangqiyenin K (**3**), and huangqiyenin L (**4**), are new cycloartane triterpenes. Compounds **1**–**3**, **14**, **15**, and **18** possess strong inhibitory effects on LPS-induced NO production in RAW264.7 cells. These results indicate that aglycons seem to show stronger activities than glycosides on the inhibition of NO production. Thus, this work provided valuable information on the structural features of cycloartane triterpenes and the potential anti-inflammatory activity of *A. membranaceus* leaves.

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Experimental Part

General. Thin layer chromatography (TLC): precoated silica gel F_{254} plates (SiO_2; *Merck*). Column chromatography (CC): SiO_2 (200–300 mesh; *Qingdao Marine Chemical Industry*), *ODS* (50 μm; *YMC*), and *Sephadex LH-20* (*Pharmacia*). Semi-prep. HPLC: *Sunfire C18* column (10 × 250 mm, 5 μm); *Waters Delata-600* pump; *Waters RID-2414* detector; flow rate, 3.0 ml min^{-1}. GC: *DM-5* column (30 m ×

0.25 mm, 0.25 μm; *Dikma*, P. R. China); *Fuli-9790* hydrogen flame detector. Optical rotations: *Jasco P-2000* polarimeter. IR Spectra: *Shimadzu FTIR-8400S* infrared spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker DPX-400* spectrometer (400 and 100 MHz, resp.); in C_5D_5N; at r.t.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS (pos.): *Waters Xero Q Tof* MS spectrometer; in *m*/*z*. Microplate reader: *PerkinElmer VICTOR® X3* multilabel plate reader.

Plant Material. Leaves of *A. membranaceus* were collected in July 2010 from Daxinganling, Heilongjiang Province, P. R. China. The botanical identification was made by Prof. *Zhen-Yue Wang*, School of Pharmacy, Heilongjiang University of Chinese Medicine. A voucher specimen (Herbarium No. 20100706) was deposited with the School of Pharmacy, Heilongjiang University of Chinese Medicine, P. R. China.

Extraction and Isolation. Dried leaves of *A. membranaceus* (10.0 kg), which were divided into four equal parts, were extracted three times under reflux conditions with 75% aq. EtOH (each 3.0 l, 2 h, 2.5 kg). The combined EtOH extracts were concentrated *in vacuo* to yield a syrup-like residue (1395.0 g), which was dissolved in H_2O (5.0 l) and then portioned between petroleum ether-soluble (50.0 g), AcOEt-soluble (190.0 g), BuOH-soluble (195.0 g), and H_2O-soluble (960.0 g) portions. The BuOH-soluble portion (120.0 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 100 : 1 → 0 : 1, gradient) to furnish ten fractions, *Frs. 1–10*. *Fr. 2* (5.8 g) was successively subjected to CC (ODS; MeOH/H_2O 3 : 7 → 7 : 3) to yield four fractions, *Frs. 2.1–2.4*. *Fr. 2.2* (1.6 g) was further separated by CC (*Sephadex LH-20*; MeOH) to afford **19** (12.5 mg) and **21** (23.2 mg). *Fr. 3* (7.5 g) was further subjected to CC (*ODS*; MeOH/H_2O 3 : 7 → 7 : 3) to afford six fractions, *Frs. 3.1–3.6*. Compound **14** (200.0 mg) was crystallized directly with MeOH from *Fr. 3.3* (1.8 g). The mother liquor of *Fr. 3.3* was purified by semi-prep. HPLC (MeOH/H_2O 68 : 32) to give **2** (12.5 mg). *Fr. 3.4* (680.0 mg) was further separated by semi-prep. HPLC (MeOH/H_2O 3 : 1) to afford **20** (15.8 mg). *Fr. 3.5* (527.0 mg) was further purified by semi-prep. HPLC (MeOH/H_2O 53 : 47) to give **3** (19.0 mg), **6** (12.5 mg), and **8** (7.1 mg). *Fr. 5* (3.6 g) was successively subjected to CC (*ODS*; MeOH/H_2O 3 : 7 → 7 : 3) to give five fractions, *Frs. 5.1–5.5*. *Fr. 5.2* (830.0 mg) was further separated by semi-prep. HPLC (MeOH/H_2O 3 : 1) to afford **9** (15.8 mg) and **12** (18.5 mg). Compounds **11** (7.1 mg) and **7** (18.0 mg) were obtained from *Fr. 5.3* (395.0 mg) after purification by semi-prep. HPLC (MeOH/H_2O 7 : 3). *Fr. 6* (1.2 g) was subjected to CC (*ODS*; MeOH/H_2O 3 : 7 → 7 : 3) to afford six fractions, *Frs. 6.1–6.6*. *Fr. 6.3* (450.0 mg) was further separated by CC (*Sephadex LH-20*; MeOH) to afford **15** (7.5 mg), **16** (8.9 mg), and a mixture, which was purified by semi-prep. HPLC (MeOH/H_2O 73 : 27) to give **5** (7.9 mg). *Fr. 6.5* (228.0 mg) was successively subjected to semi-prep. HPLC (MeOH/H_2O 55 : 45) to obtain **1** (15.6 mg) and **10** (8.5 mg). *Fr. 8* (1.0 g) was successively separated by CC (*ODS*; MeOH/H_2O 3 : 7 → 7 : 3) to give seven fractions, *Frs. 8.1–8.7*. *Fr. 8.3* (350.0 mg) was further separated by CC (*Sephadex LH-20*; MeOH) to afford **18** (39.7 mg) and a mixture. The mixture was subsequently purified by semi-prep. HPLC (MeOH/H_2O 73 : 27) to yield **13** (7.9 mg). *Fr. 8.5* (230.0 mg) was purified by semi-prep. HPLC (MeOH/H_2O 7 : 3) to afford **4** (8.4 mg) and **17** (17.7 mg).

Huangqiyegenin V (*=(9β,11α,16β,20*R,*24*S*)-11,16,25-Trihydroxy-20,24-epoxy-9,19-cyclolanostane-3,6-dione*; **1**). White amorphous powder. $[\alpha]_D^{25} = +46.7$ ($c = 0.39$, MeOH). IR: 3393, 2988, 1705, 1425, 1377, 1356, 1038. ^1H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 525.3194 ($[M+Na]^+$, $C_{30}H_{46}NaO_6^+$; calc. 525.3187).

Huangqiyegenin VI (*=(9β,16β,24*S*)-16,24,25-Trihydroxy-9,19-cyclolanostane-3,6-dione*; **2**). White amorphous powder. $[\alpha]_D^{25} = +41.5$ ($c = 0.40$, MeOH). IR: 3404, 2999, 1728, 1481, 1443, 1377, 1366, 1250, 1217, 1146. ^1H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 511.3396 ($[M+Na]^+$, $C_{30}H_{48}NaO_5^+$; calc. 511.3394).

Huangqiyenin K (*=(3β,6α,9β,16β,20*R,*24*R*)-16,25-Dihydroxy-3-(β-*D*-xylopyranosyloxy)-20,24-epoxy-9,19-cyclolanostan-6-yl Acetate*; **3**). White amorphous powder. $[\alpha]_D^{25} = +4.9$ ($c = 0.37$, MeOH). IR: 3393, 2968, 2939, 2872, 1726, 1439, 1379, 1362, 1032. ^1H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 665.4261 ($[M+H]^+$, $C_{37}H_{61}O_{10}^+$; calc. 665.4259).

Huangqiyenin L (*=(3β,6α,9β,16β,24*E*)-26-(β-*D*-Glucopyranosyloxy)-16-hydroxy-3-(β-*D*-xylopyranosyloxy)-9,19-cyclolanost-24-en-6-yl Acetate*; **4**). White amorphous powder. $[\alpha]_D^{25} = +38.18$ ($c = 0.33$, MeOH). IR: 3380, 3060, 3055, 3034, 1728, 1634, 1441, 1252, 1032. ^1H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 811.4847 ($[M+H]^+$, $C_{43}H_{71}O_{14}^+$; calc. 811.4838).

Acid Hydrolysis and Determination of Sugar Components. Compounds **3** and **4** (each 2.0 mg) were hydrolyzed in 1M aq. HCl soln. (1.0 ml) for 2 h at 85°. The mixture was cooled and partitioned between $CHCl_3$ (2.0 ml) and H_2O (2.0 ml). The aq. layer was washed with $CHCl_3$ (3 × 3.0 ml), neutralized with $Ba(OH)_2$, filtered, and evaporated under reduced pressure. The residue was dissolved in pyridine (1.0 ml) and 0.1M L-cysteine methyl ester hydrochloride in pyridine (2.0 ml) was added. The mixture was heated at 60° for 1 h. An equal volume of Ac_2O was added and heating was continued for 1 h. The acetylated thiazolidine derivatives were analyzed by GC using authentic samples as standards. Temperatures of injector and detector were both 280°. A temp. gradient system was used for the oven, starting at 160° and increasing up to 195° at a rate of 5°/min. Identification of D-xylose from **3** and D-xylose and D-glucose from **4** present in the aq. layer was carried out by comparison of t_R values with those of authentic samples (*NICPBP*, P. R.China; t_{R1} 10.2 min (D-xylose), t_{R2} 13.9 min (D-glucose)).

Determination of NO Production and Cell Viability Assay. Mouse monocyte-macrophage RAW264.7 cells (ATCC TIB-71) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, P. R. China). RPMI 1640 medium, penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from *HyClone* (N.Y., USA). LPS, DMSO, MTT, and hydrocortisone were purchased from *Sigma Co*. The cells were suspended in RPMI 1640 medium supplemented with penicillin (100 U ml^{-1}), streptomycin (100 U ml^{-1}), and 10% heat-inactivated FBS under a humidified atmosphere of 5% CO_2 at 37°. The cells were harvested with trypsin until they attained confluence and were used for assays during the exponential growth phase.

The cells were seeded in 96-well plates with 6×10^4 cells/well. After 1 h incubation, cells were treated with 1 μg ml^{-1} LPS and various concentrations of test compounds for 24 h [28]. Each compound was dissolved in DMSO, which was applied at a final concentration of 0.1% (*v*/*v*) in cell culture supernatants. Control groups received an equal amount of DMSO. NO Production was determined by adding 100 μl *Griess* reagent (1% sulfanilamide and 0.1% *N*-(naphthalen-1-yl)ethane-1,2-diamine in 5% H_3PO_4) to 100 μl supernatant from LPS or the compound-treated cells in triplicate. After 5 min of incubation, the absorbance was measured at 540 nm with a microplate reader. Cytotoxicity was determined by MTT colorimetric assay, after 24 h of incubation with test compounds. Concentrations of NO_2^- in the supernatant were calculated by a working line from 0, 1, 2, 5, 10, 20, 50, and 100 μM $NaNO_2$ solns. The inhibitory rate was calculated according to the formula:

$$\text{Inhibitory rate } [\%] = 100 \cdot \frac{[NO_2^-]_{LPS} - [NO_2^-]_{LPS+sample}}{[NO_2^-]_{LPS} - [NO_2^-]_{untreated}}$$

Every experiment was performed in triplicate; data are expressed as mean ± SD of three independent experiments.

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