Immunosuppressive Sesquiterpenes from *Tripterygium wilfordii*

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Five new sesquiterpenes, 1β-furanoyl-2β,3α,7α,8β,11-pentaacetoxy-4α,5α-dihydroxy-dihydroagarofuran (1), 1β,2β,3α,5α,7β,8β,11-heptaacetoxy-dihydroagarofuran (2), 1β-furanoyl-2β,3α,7α,8β,11-pentaacetoxy-5α-hydroxy-dihydroagarofuran (3), 1β,7β,8α-triacetoxy-2β-furanoyl-4α-hydroxy-11-isobutyryloxy-dihydroagarofuran (4), and 1β-nicotinoyl-2β,5α,7β-triacetoxy-4α-hydroxy-11-isobutyryloxy-8α-furanoyl-dihydroagarofuran (5), were isolated from the xylem of *Tripterygium wilfordii*, together with a known compound (6). Their structures were elucidated on the basis of spectroscopic studies. Compounds 2—5 showed significant immunosuppressive activities.

Key words *Tripterygium wilfordii*; xylem; sesquiterpene; immunosuppressive activity

Tripterygium wilfordii (Celastraceae) has been used in traditional Chinese medicine as a cancer treatment and also as an insecticide for hundreds of years. In recent years, its xylem extract has been used in the clinical treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory and autoimmune diseases.[1—3] In previous papers, we have reported the isolation of some anti-HIV agents, triptonines A and B, along with several related compounds from *Tripterygium wilfordii*.[4—6] This paper deals with the isolation and structure determination of five new (**1**—**5**) and one known (**6**) sesquiterpenes from the xylem of *T. wilfordii*. The *in vitro* immunosuppressive activity of isolated compounds was tested by lymphocyte transformation experiments. Stimulation of lymphocytes with concanavalin A will cause lymphocyte proliferation, which is the first step of the immune response. The inhibitory effect on lymphocyte transformation can express the immunosuppressive activity of the compounds tested. In this bioassay, we found that most of the sesquiterpenes showed a significant inhibitory effect on lymphocyte transformation.

The chloroform-soluble fraction of the xylem extracts was separated by repeated silica gel column chromatography, Sephadex LH-20 and preparative HPLC to give compounds **1**—**6**.

Compound (**1**) was obtained as a white powder, having the molecular formula $C_{30}H_{38}O_{16}$ from HR-FAB-MS. Its IR spectrum showed a hydroxyl and ester carbonyl carbon band (3459, 1753 cm^{-1}), and the UV spectrum revealed the presence of an aromatic moiety (235, 276 nm). The ^{1}H-NMR spectral data of **1** revealed the presence of five acetyl methyl groups [δ_H 2.24, 2.20, 2.13, 1.96, 1.60 (each 3H, s)], a furanoyl group [δ_H 7.93 (1H, s), 6.62 (1H, d, J=1.7 Hz), 7.40 (1H, d, J=1.7 Hz)], an oxygenated methylene [δ_H 5.12, 4.42 (each 1H, d, J=13.2 Hz)], and three tertiary methyl groups (δ_H 1.85, 1.66, 1.58), as well as six methine proton signals (δ_H 5.79, 5.75, 5.47, 5.36, 5.09, 4.87). The ^{13}C-NMR spectral data of **1** revealed the presence of eight methyls, one oxygenated methylene, six oxygenated methine carbons, and five ester carbonyl carbons (δ_C 170.2, 170.0, 169.5, 169.1, 168.1), in addition to four quaternary carbons, and one furanoyl group (δ_C 160.9, 147.7, 118.7, 109.4, 143.9). From the above information, compound **1** was deduced to be a sesquiterpene polyol ester having a dihydroagarofuran skeleton found in the same genus of *Tripterygium*.[7—9)]

The ^{1}H–^{1}H COSY spectrum of **1** revealed two separated spin-spin system (H-1/H-2/H-3, H-6/H-7/H-8) in the dihydroagarofuran skeleton. The remaining dihydroagarofuran proton signal at δ_H 5.09 (H-5) was correlated with the carbon signals at δ_C 52.9 (C-6), 73.8 (C-7), 51.0 (C-9), 91.6 (C-10) and 84.7 (C-13) in the HMBC spectrum.

From the HMBC spectrum, the signal at δ_H 5.80 (H-1) was correlated with the resonance at δ_C 160.9 (furanoyl), while the signals at δ_H 5.36 (H-2), 4.87 (H-3), 5.47 (H-7), 5.75 (H-8), and 5.12 (H-11a) were correlated with the acetyl carbonyl carbons at δ_C 168.1, 169.1, 170.0, 169.5 and 170.2, respectively. From the above observations, the furanoyl group was assigned at position C-1, and five acetyl groups were assigned at positions C-2, C-3, C-7, C-8 and C-11, respectively.

In the NOESY spectrum of **1**, the proton signal at δ_H 5.12 (H-11a) correlated with the signal at δ_H 1.85 (H_3-12) and δ_H 5.09 (H-5), the proton signal at δ_H 5.80 (H-1) with the signals at δ_H 5.75 (H-8) and 5.36 (H-2), while the proton at δ_H 5.75 (H-8) also correlated with the signal at δ_H 1.66 (H_3-14). Thus, the relative stereochemistries of the ester and hydroxyl groups were elucidated as having the 1β, 2β, 4α, 5α and 8β configurations (Fig. 1). On the other hand, the proton signal at δ_H 5.47 (H-7) correlated with the signal at δ_H 5.09 (H-5), and the proton signal at δ_H 4.87 (H-3) correlated with the signal at δ_H 1.85 (H_3-12) in the NOESY spectrum. Further-

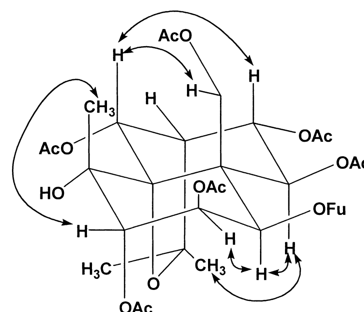

Fig. 1. The NOESY Data of Compound **1**

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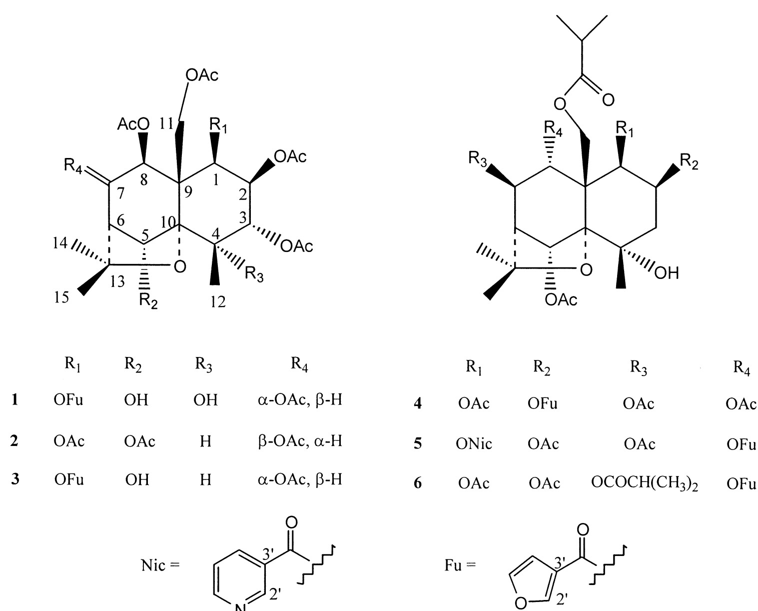

Fig. 2

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**5**

Position	**1**[a)]	**2**[a)]	**3**[a)]	**4**[b)]	**5**[a)]
1	5.80 (d, 3.6)	5.75 (d, 4.0)	5.98 (d, 3.7)	5.56 (d, 3.7)	5.84 (d, 3.8)
2	5.36 (m)	5.36 (m)	5.43 (m)	5.52 (m)	5.64 (m)
3	4.87 (m)	4.79 (m)	4.79 (m)	2.32, 1.93 (m)	2.32, 2.08 (m)
4	—	2.46 (q, 7.9)	2.62 (q, 7.9)	—	—
5	5.09 (br s)	6.54 (s)	5.04 (br s)	6.27 (s)	6.35 (s)
6	2.50 (d, 3.5)	2.34 (d, 3.8)	2.41 (d, 3.4)	2.40 (d, 2.9)	2.41 (d, 2.9)
7	5.47 (dd, 3.5, 9.8)	5.46 (dd, 3.8, 5.9)	5.38 (dd, 3.4, 9.7)	5.26 (d, 2.9)	5.25 (d, 2.9)
8	5.75 (d, 9.8)	5.38 (m)	5.76 (d, 9.7)	5.53 (s)	5.58 (s)
11	5.12, 4.42 (d, 13.2)	5.08, 4.45 (d, 13.1)	5.12, 4.50 (d, 13.0)	4.91, 4.78 (d, 12.9)	5.12, 4.77 (d, 13.1)
12	1.85 (s)	1.16 (d, 7.9)	1.42 (d, 7.9)	1.50 (s)	1.53 (s)
14	1.66 (s)	1.49 (s)	1.54 (s)	1.60 (s)	1.63 (s)
15	1.58 (s)	1.35 (s)	1.46 (s)	1.57 (s)	1.57 (s)

a) $CDCl_3$, *b*) CD_3OD.

more, the coupling constant of H-7 (1H, dd, $J=3.5$, 9.8 Hz) clearly indicated that the proton at position C-7 has an axial orientation. Thus, the remaining acetyl groups at position C-3 and C-7 were elucidated as having 3α and 7α configurations. The ^{1}H- and ^{13}C-NMR assignments were obtained by 2D NMR spectra including NOESY. Therefore, the structure of **1** was determined as shown (Fig. 2).

Compound (**2**), an amorphous powder, had the molecular formula of $C_{29}H_{40}O_{15}$ (HR-FAB-MS). Its ^{1}H-NMR spectral data revealed the presence of seven acetyl methyl groups (δ_H 2.26, 2.17, 2.12, 2.11, 2.09, 1.97, 1.91), three methyl groups [δ_H 1.49, 1.35 (each 3H, s), 1.16 (3H, d, $J=7.9$ Hz)], as well as eight methine proton signals (Table 1). The ^{13}C-NMR spectral data were similar to those of **1**, except for the C-4 and C-12 carbon signals and ester groups (Table 2). The carbon signal at δ_C 37.4 (d) suggested the absence of the hydroxyl group at C-4 by comparing with the signal at the same position in **1** [δ_C 70.5 (s)]. Compound **2** was also a dihydroagarofuran sesquiterpene poly ester, having seven acetyl groups. In the HMBC spectrum of **2**, the proton signals at δ_H 5.75 (H-1), 5.36 (H-2), 4.79 (H-3), 6.54 (H-5), 5.46 (H-7), 5.38 (H-8) and 5.08 (H-11a) correlated with the carbonyl carbon signals at δ_C 169.2, 169.0, 170.1, 169.6, 170.1, 168.9 and 170.1, respectively. Thus, seven acetyl groups were located at positions C-1, C-2, C-3, C-5, C-7, C-8 and C-11. In the NOESY spectrum, the proton signal at δ_H 5.08 (H-11a) correlated with the signals at δ_H 6.54 (H-5) and 1.16 (H_3-12), and the signal at δ_H 5.75 (H-1) correlated with the signals at δ_H 5.36 (H-2) and 5.38 (H-8), while the signal at δ_H 5.46 (H-7) correlated with the signals at δ_H 5.38 (H-8) and 1.49 (H_3-14). In turn, the signal at δ_H 4.79 (H-3) correlated with the signal at δ_H 1.16 (H_3-12). Therefore, the relative configurations of the ester groups of **2** were determined as 1β, 2β, 3α, 5α, 7β and 8β (Fig. 2).

Compound (**3**), $C_{30}H_{38}O_{15}$, showed five acetyl methyl groups (δ_H 2.21, 2.10, 2.10, 1.96, 1.68) and a furanoyl group [7.96 (1H, s), 6.66 (1H, d, $J=1.7$ Hz), 7.41 (1H, d, $J=1.7$ Hz)] in the ^{1}H-NMR spectrum. Its ^{13}C-NMR spectral data were similar to those of **2**, except for the ester groups (**2**: seven acetyls; **3**: five acetyls, one furanoyl). Compound **3** should be a dihydroagarofuran poly ester having five acetyl groups and one furanoyl group. In the HMBC spectrum of **3**, the proton at δ_H 5.43 (H-2), 4.79 (H-3), 5.38 (H-7), 5.76 (H-8) and 5.12 (H-11a) correlated with the carbonyl carbon sig-

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**6**

Position	**1**$^{a)}$	**2**$^{a)}$	**3**$^{a)}$	**4**$^{b)}$	**5**$^{a)}$	**6**$^{a)}$
1	71.7	74.2	73.1	72.4	71.9	70.7
2	68.5	69.8	70.0	69.5	68.1	68.0
3	74.6	74.6	73.9	41.9	42.0	42.1
4	70.5	37.4	37.0	71.3	69.9	69.9
5	77.0	75.2	75.7	77.2	75.8	75.5
6	52.9	52.4	53.4	54.5	53.0	53.0
7	73.8	69.7	74.3	77.9	76.6	76.1
8	74.8	71.2	75.1	73.3	72.2	71.5
9	51.0	50.6	50.4	55.9	54.5	54.0
10	91.6	89.3	90.9	92.5	91.5	91.4
11	60.0	60.2	60.0	66.7	65.7	65.5
12	23.5	14.4	16.0	25.4	24.6	24.5
13	84.7	81.4	82.9	84.7	83.3	83.5
14	26.3	24.6	26.3	25.8	25.5	25.4
15	30.1	30.2	30.9	29.7	29.5	29.6

a) $CDCl_3$, *b*) CD_3OD.

Table 3. Inhibitory Effects of Compounds **1**—**6**

Compounds	Inhibition (%)		
	80 μg/ml	20 μg/ml	5 μg/ml
1	17	15	10
2	34	11	10
3	44	30	14
4	28	12	10
5	28	19	12
6	4	3	1

Inhibition of dexamethasone=61% (50 μg/ml).

nals at δ_C 168.6, 170.0, 170.3, 169.4 and 170.1, respectively. The signals at δ_H 5.98 (H-1) and δ_H 6.66 (furanoyl) were correlated with the resonance at δ_C 160.9. Thus, the five acetyl groups were located at positions C-2, C-3, C-7, C-8 and C-11, and the furanoyl group was located at position C-1. In addition, the proton signal at δ_H 5.04 (H-5) was apparent in a more upfield position than the same proton in **2** (δ_H 6.54, H-5), and this observation indicated a hydroxyl group was located at C-5 instead of one acetyl group in **2**. The coupling constants of H-7 and H-8 were similar to those of **1** (Table 1), indicating that the proton at position C-7 has an axial orientation. Further analysis of the NOESY spectral data also supported the H-7β orientation. Thus, the structure of **3** was determined as shown (Fig. 2).

Compound (**4**), $C_{32}H_{42}O_{15}$, revealed the presence of four acetyl groups (δ_H 2.16, 2.11, 2.09, 1.62), a furanoyl group [8.08 (1H, s), 6.72 (1H, d, J=1.8 Hz), 7.60 (1H, d, J=1.8 Hz)] in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectral data of **4** were similar to those of **6**[10] (Table 2), except for the ester groups. From the ^{1}H–^{1}H, ^{13}C–^{1}H COSY and HMBC spectra, the presence of an isobutyryloxy group [2.80 (1H, sept., J=7.0 Hz), 1.26 and 1.27 (each 3H, d, J=7.0 Hz)] was deduced. In the HMBC spectrum of **4**, the proton signals at δ_H 5.56 (H-1), 6.27 (H-5), 5.26 (H-7), 5.53 (H-8) and 4.91 (H-11a) correlated with the carbonyl carbon signals at δ_C 171.4, 171.9, 171.2 and 171.5, respectively. Furthermore, the proton signals at δ_H 4.91 (H-11a), 1.27 (3H) and 2.80 (1H) (isobutyryloxy) were correlated with the signal at δ_C 178.4, and the proton signals at δ_H 5.52 (H-2) and 6.72 (furanoyl) were correlated with the signal at δ_C 162.5. From above observations, four acetyl groups were assigned at positions C-1, C-5, C-7 and C-8, respectively, the isobutyryloxy group was assigned at position C-11, and the furanoyl group was located at position C-2. The coupling pattern of H-7 (d, 2.9) and H-8 (s) showed a different configuration at position C-8 compared to that of **1**—**3** (Table 1). In the NOESY spectrum, the proton signals δ_H 4.78 (H-11b) correlated with the signals at δ_H 1.50 (H_3-12), 5.53 (H-8) and δ_H 6.27 (H-5). The signal at δ_H 5.52 (H-2) correlated with the signals at δ_H 5.56 (H-1) and 2.32 (H-3α), the proton signal at δ_H 1.93 (H-3β) correlated with the signal at δ_H 1.50 (H_3-12), and the signal at δ_H 5.26 (H-7) correlated with the signal at δ_H 1.60 (H_3-14). Thus, the ester groups of **4** were elucidated as having the 1β, 2β, 5α, 7β and 8α configurations (Fig. 2).

Compound (**5**), $C_{36}H_{43}O_{15}N$, revealed three acetyl groups, a furanoyl group, an isobutyryloxy group, and a nicotinoyl group [δ_H 8.78 (1H, d, J=1.5 Hz), 8.68 (1H, dd, J=1.7, 4.8 Hz), 7.85 (1H, br d, J=8.0 Hz), 7.25 (1H, m)] in the ^{1}H-NMR spectrum. Comparison of the ^{13}C-NMR spectral data of **5** with those of **4** and **6**, indicated **5** had the same structural framework, and the difference between these compounds was the nature of the ester groups and their positions. In the HMBC spectral data of **5**, the proton signals at δ_H 5.84 (H-1) and 7.85 (nicotinoyl) correlated with the signal at δ_C 164.1, and the signals at δ_H 5.53 and 6.36 (furanoyl) correlated with the signal at δ_C 160.7. Thus, the nicotinoyl group was at C-1, and the furanoyl group was at C-8. In the same manner, three acetyl groups were located at C-2, C-5, and C-7, and the isobutyryloxy group was assigned at position C-11. In the NOESY spectrum, the proton signal at δ_H 4.77 (H-11b) correlated with the signal at δ_H 1.53 (H_3-12), 5.58 (H-8) and δ_H 6.35 (H-5), and the signal at δ_H 5.64 (H-2) correlated with the signals at δ_H 5.84 (H-1) and 2.32 (H-3α), the signal at δ_H 2.08 (H-3β) correlated with the signal at δ_H 1.53 (H_3-12), while the proton at δ_H 5.25 (H-7) correlated with the signal at δ_H 1.63 (H_3-14). Thus, the stereochemistry of six ester groups was determined as having the 1β, 2β, 5α, 7β and 8α configurations (Fig. 2).

By comparing spectral data, the known compound **6** was identified as 4α-hydroxy-1β,2β,5α-triacetoxy-7β,11-diisobutyryloxy-8α-furanoyl-dihydroagarofuran (**6**).[10]

In a screen for immunosuppressive agents from the extract of *T. wilfordii*, we examined the inhibitory effect of the isolated compounds on lymphocyte transformation (Table 3). The values of inhibition percent of compounds **1**—**5** revealed a significant distinction compared to the concanavalin (Con A) control group ($p<0.01$, $n=6$), and showed an inhibitory effect on lymphocyte transformation by comparing with a reference compound (dexamethasone).

Experimental

General Experimental Procedures NMR experiments were run on a Bruker AVANCE 300 instrument. ^{1}H-NMR, 300 MHz; ^{13}C-NMR, 75 MHz, both with teramethylsilane as an internal standard. MS data were obtained on a JEOL JMS D-300 instrument. Chromatography column, Silica-gel (Qingdao Haiyang Chemical Co., Ltd.) and Sephadex LH-20 (Amersham Pharmacia Biotech); HPLC, JASCO Gulliver Series, PU-1580 (pump), RI-1530 and UV-1575 (detector). Column type, ODS (YMC-Pack ODS-A, SH-343-5), Si-HPLC (Hibar RT 250-25, Lichrosorb, Si60 7 μm); IR spectra were recorded on a 1710 Infrared Fourier Transform sepectrometer (PERKIN-ELMER), UV spectra were obtained on a UVIKON$_{XS}$ recording spectrometer (BIO-TEK). Optical rotation was measured with a MC 241 digital polarimeter (PERKIN-ELMER).

Plant Material The xylem rhizoma of *T. wilfordii* were purchased from Yueyang, Hunan province, and were identified by Prof. Wen-Yuan Gao. A voucher specimen (D20021018) was deposited at the School of Pharmacy, Tianjin Medical University, China.

Extraction and Isolation The xylem rhizome (10 kg) was refluxed three times with 95% EtOH (15 l each) for 2 h. The extract was concentrated under reduced pressure to give a residue (390 g) which was partitioned between chloroform and H_2O. The $CHCl_3$ layer was concentracted to give a residue (112 g). It was subjected to chromatographic separation on a silica gel column, and was eluted with solvents of increasing polarity [petroleum ether–EtOAc (8 : 1, 5 : 1, 3 : 1, 1 : 1, 1 : 2, 1 : 4), EtOAc, EtOAc–MeOH (19 : 1, 9 : 1, 4 : 1), MeOH] to give 16 frs. Fraction 9 (1.8 g) was chromatographed on Sephadex LH-20 (MeOH) to give three frs. (fr. 9.1—9.3). Fraction 9.2 (1.49 g) was chromatographed on a middle pressure silica gel column with $CHCl_3$–MeOH (97 : 3, 92 : 8) to give 4 frs. (fr. 9.2.1—9.2.4). Fraction 9.2.2 (890 mg) was separated by HPLC (ODS, MeOH–H_2O 8 : 2) to give 9 frs. (fr. 9.2.2.1—9.2.2.9). Fraction 9.2.2.1 (179 mg) was separated by HPLC (ODS, MeOH–H_2O 7 : 3), and then by Si-HPLC ($CHCl_3$–MeOH 98 : 2) to give **1** (6.5 mg) and **3** (8.7 mg), respectively. Fraction 9.2.2.2 (212 mg) was separated by HPLC (ODS, MeOH–H_2O 7 : 3) to give **2** (16.1 mg). Fraction 9.2.2.3 (76.0 mg) was separated by HPLC (ODS, MeOH–H_2O, 7 : 3) to give **4** (40.8 mg). Fraction 9.2.2.5 (79 mg) was separated by HPLC (ODS, MeOH–H_2O, 7 : 3) to give **6** (10.0 mg).

Fraction 10 (2 g) was chromatographed on Sephadex LH-20 (MeOH) to give three frs. (fr. 10.1—10.3). Fraction 10.1 (840 mg) was separated by HPLC (ODS, MeOH–H_2O 8 : 2) to give 12 frs. (fr. 10.1.1—10.1.12). Fraction 10.1.4 (67.0 mg) was separated by HPLC (ODS, MeOH–H_2O 7 : 3), and then by Si-HPLC ($CHCl_3$–MeOH 98 : 2) to give **5** (6.0 mg).

Compound (**1**): Amorphous powder, $[\alpha]_D^{25}$ $-12.2°$ (c=0.8, MeOH). UV λ_{max}^{MeOH} nm (log ε): 235 (3.39), 276 (2.32). IR ν_{max}^{KBr} cm^{-1}: 3459, 1753, 1579, 1507, 1371, 1303, 1229, 1140, 1078, 1043, 874, 760. ^1H-NMR ($CDCl_3$), see Table 1, δ 2.13 (2-OAc); 2.20 (3-OAc); 1.96 (7-OAc); 1.60 (8-OAc); 2.24 (11-OAc); 7.93 (1H, s), 6.62 (1H, d, J=1.7 Hz), 7.40 (1H, d, J=1.7 Hz), (1-OFu). ^{13}C-NMR ($CDCl_3$), see Table 2, δ: 20.7, 168.1 (2-OAc); 20.8, 169.1 (3-OAc); 20.8, 170.0 (7-OAc); 20.3, 169.5 (8-OAc); 21.3, 170.2 (11-OAc); 160.9, 147.7, 118.7, 109.4, 143.9 (1-OFu). FAB-MS: m/z 677 $[M+Na]^+$ (14), 637 (7), 230 (100), 214 (38), 115 (39), 58 (34), 43 (31). HR-FAB-MS m/z 677.2114 $[M+Na]^+$ Calcd for $C_{30}H_{38}O_{16}Na$, 677.2058.

Compound (**2**): Amorphous powder, $[\alpha]_D^{25}$ $-19.7°$ (c=2.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3477, 1749, 1435, 1372, 1234, 1147, 1093, 1043, 873, 757. ^1H-NMR ($CDCl_3$), see Table 1, δ: 1.99 (1-OAc); 2.11 (2-OAc); 2.09 (3-OAc); 1.91 (5-OAc); 2.26 (7-OAc); 2.12 (8-OAc); 2.17 (11-OAc). ^{13}C-NMR ($CDCl_3$), see Table 2, δ: 20.5, 169.2 (1-OAc); 21.2, 169.0 (2-OAc); 21.3, 170.1 (3-OAc); 20.5, 169.6 (5-OAc); 21.3, 170.1 (7-OAc); 21.0, 168.9 (8-OAc); 20.9, 170.1 (11-OAc). FAB-MS: m/z 629 $[M+H]^+$ (33), 569 (28), 509 (15), 467 (22), 307 (20), 245 (34), 227 (28), 137 (83), 43 (100). HR-FAB-MS m/z 629.2384 $[M+H]^+$ Calcd for $C_{29}H_{41}O_{15}$, 629.2445.

Compound (**3**): Amorphous powder, $[\alpha]_D^{25}$ $-32.3°$ (c=1.7, MeOH). UV λ_{max}^{MeOH} nm (log ε): 234 (3.49), 280 (2.42). IR ν_{max}^{KBr} cm^{-1}: 3491, 1751, 1579, 1510, 1371, 1304, 1224, 1137, 1076, 1032, 874, 760. ^1H-NMR ($CDCl_3$), see Table 1, δ 2.10 (2-OAc); 2.10 (3-OAc); 1.96 (7-OAc); 1.68 (8-OAc); 2.21 (11-OAc); 7.96 (1H, s), 6.66 (1H, d, J=1.7 Hz), 7.41 (1H, d, J=1.7 Hz) (1-OFu). ^{13}C-NMR ($CDCl_3$), see Table 2, δ: 21.2, 168.6 (2-OAc); 20.8, 170.0 (3-OAc); 20.9, 170.3 (7-OAc); 20.3, 169.4 (8-OAc); 21.4, 170.1 (11-OAc); 160.9, 147.6, 119.0, 109.6, 143.9 (1-OFu). FAB-MS: m/z 661 $[M+Na]^+$ (5), 525 (4), 481 (15), 307 (23), 245 (34), 289 (17), 154 (100), 136 (81), 91 (30), 71 (29). HR-FAB-MS m/z 661.2090 $[M+Na]^+$ Calcd for $C_{30}H_{38}O_{15}Na$, 661.2103.

Compound (**4**): Amorphous powder, $[\alpha]_D^{25}$ $-13.1°$ (c=1.7, MeOH). UV λ_{max}^{MeOH} nm (log ε): 233 (3.43), 280 (2.12). IR ν_{max}^{KBr} cm^{-1}: 3452, 1743, 1575, 1510, 1370, 1310, 1232, 1158, 1083, 1032, 875, 761. ^1H-NMR (CD_3OD), see Table 1, δ: 1.62 (1-OAc); 2.11 (5-OAc); 2.16 (7-OAc); 2.09 (8-OAc); 2.80 (1H, sept., J=7.0 Hz), 1.26 (3H, d, J=7.0 Hz), 1.27 (3H, d, J=7.0 Hz), [11-$OCOCH(CH_3)_2$]; 8.08 (1H, s), 6.72 (1H, d, J=1.8 Hz), 7.60 (1H, d, J=1.8 Hz), (2-OFu). ^{13}C-NMR (CD_3OD), see Table 2, δ: 20.9, 171.4 (1-OAc); 21.4, 171.9 (5-OAc); 21.3, 171.2 (7-OAc); 21.3, 171.5 (8-OAc); 35.4, 19.6, 19.5, 178.4 [11-$OCOCH(CH_3)_2$]; 162.5, 150.7, 119.4, 110.7, 145.8 (2-OFu). FAB-MS: m/z 689 $[M+Na]^+$ (32), 649 (19), 589 (8), 547 (7), 393 (9), 305 (18), 230 (79), 175 (36). 95 (99), 43 (100), 71 (88). HR-FAB-MS m/z 689.2406 $[M+Na]^+$ Calcd for $C_{32}H_{42}O_{15}Na$, 689.2416.

Compound (**5**): Amorphous powder, $[\alpha]_D^{25}$ $+9.2°$ (c=1.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 216 (3.97), 255 (3.56). IR ν_{max}^{KBr} cm^{-1}: 3437, 1373, 1592, 1510, 1370, 1310, 1232, 1137, 1083, 1030, 874, 741. ^1H-NMR ($CDCl_3$), see Table 1, δ: 2.05 (2-OAc); 2.12 (5-OAc); 2.18 (7-OAc); 2.85 (1H, sept., J=7.0 Hz), 1.32 (3H, d, J=7.0 Hz), 1.30 (3H, d, J=7.0 Hz), [11-$OCOCH(CH_3)_2$]; 7.76 (1H, s), 6.36 (1H, d, J=1.6 Hz), 7.29 (1H, d, J=1.6 Hz), (8-OFu), 8.78 (1H, d, J=1.5 Hz), 8.68 (1H, dd, J=1.7, 4.8 Hz), 7.85 (1H, br d, J=8.0 Hz), 7.25 (1H, m), (1-ONic). ^{13}C-NMR ($CDCl_3$), see Table 2, δ: 21.0, 169.5 (2-OAc); 21.4, 169.6 (5-OAc); 21.0, 169.5 (7-OAc); 34.0, 19.1, 19.0, 177.1 [11-$OCOCH(CH_3)_2$]; 160.7, 148.7, 117.6, 109.4, 143.7, (8-OFu); 164.1, 125.2, 136.4, 123.0, 153.4, 150.4, (1-ONic). FAB-MS: m/z 730 $[M+H]^+$ (11), 637 (8), 563 (12), 484 (16), 456 (24), 374 (23), 300 (54), 221 (35), 215 (81), 154 (100), 136 (96), 89 (90), 77 (87), 45 (81). HR-FAB-MS m/z 730.2758 $[M+H]^+$ Calcd for $C_{36}H_{44}O_{15}N$, 730.2711.

Bioassay Procedure The samples were prepared by dissolving the compounds from *T. wilfordii* with dimethyl sulfoxide, followed by dilution of solutions into different concentrations with Hank's solution. The solutions were then mixed with 0.2 ml of lymphocytes (5×10^6/ml) respectively and incubated (37 °C, 5% CO_2) for 72 h. Concanavalin A (Con A) was used as a control group. The OD values of the samples were measured at 490 nm.[11,12]

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References

1) Qian S.-Z., *Contraception*, **36**, 335—345 (1987).
2) Matlin S. A., Belenguer A., Stacey V. E., Qian S.-Z., Xu Y., Zhang J.-W., Sanders J. K. M., Amor S. R., Pearce C. M., *Contraception*, **47**, 387—400 (1993).
3) Qian S.-Z., Xu Y., Zhang J.-W., *Contraception*, **51**, 121—129 (1995).
4) Duan H.-Q., Takaishi Y., Momota H., Ohmoto Y., Taki T., Jia Y.-F., Li D., *J. Nat. Prod.*, **64**, 582—587 (2001).
5) Duan H.-Q., Takaishi Y., Imakura Y., Jia Y.-F., Li D., Lee K.-H., *J. Nat. Prod.*, **63**, 357—361 (2000).
6) Duan H.-Q., Takaishi Y., Jia Y.-F., Li D., *Chem. Pharm. Bull.*, **47**, 1664—1667 (1999).
7) Takaishi Y., Tokura K., Tamai S., Ujita K., Nakano K., Tomimatsu T., *Phytochemistry*, **30**, 1567—1572 (1991).
8) Takaishi Y., Tamai S., Nakano K., Tomimatsu T., *Phytochemistry*, **30**, 3027—3031 (1991).
9) Duan H.-Q., Takaishi Y., Momota H., Ohmoto Y., Taki T., Jia Y.-F., Li D., *Phytochemistry*, **53**, 805—810 (2000).
10) Liu J.-K., Becker H., Zapp J., Wu D.-G., *Phytochemistry*, **40**, 841—846 (1995).
11) Fletcher M. A., Klimas N., Morgan R., "Manual of Clinical Laboratory Immunology," 4th ed., American Society for Microbiology, Washington, 1992, pp. 213—219.
12) Zhang J.-T., "Modern Pharmacological Experimental Methods," Peking Union Medical College Press, Peking, 1998, pp. 701—722.