Immunosuppressive Sesquiterpene Alkaloids from *Tripterygium wilfordii*

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Nine new sesquiterpene pyridine alkaloids [wilfornines A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**8**), and G (**9**); wilfordinines I (**6**) and J (**7**)] and six known compounds (**10**−**15**) were isolated from a clinically used extract (T_{II}) of *Tripterygium wilfordii*. The structures of **1**−**9** were elucidated by spectroscopic and chemical methods. The inhibitory effects on cytokine production of **1**−**3** and several related compounds were evaluated. Compounds **10** and **14** showed significant inhibitory effects on cytokine production.

Tripterygium wilfordii Hook f. (Celastraceae) has been used in traditional Chinese medicine to treat cancer and also as an insecticide for hundreds of years. Recently, an extract (the so-called "total multi-glycoside" or "T_{II} extract") derived from a water and chloroform partitioning of the roots of *T. wilfordii* has been used in the clinical treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory and autoimmune diseases in China.[1−3] In previous papers, we have reported a number of immunosuppressive diterpenes and anti-HIV sesquiterpenes from *T. wilfordii*.[4,5] As a continuation of our studies on the bioactive principles from the genus *Tripterygium*, we have turned our attention to the immunosuppressive constituents and describe herein the isolation and structure determination of nine new (**1**−**9**) and six known sesquiterpene alkaloids (**10**−**15**) (Chart 1) from the clinically used extract of *T. wilfordii* (T_{II} extract), along with their inhibitory effects on cytokine production.

Results and Discussion

The powdered T_{II} extract of *T. wilfordii* was chromatographed repeatedly on Si gel to afford nine new sesquiterpene alkaloids, named wilfornines A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**8**), and G (**9**) and wilfordinines I (**6**) and J (**7**), as well as six known compounds (**10**−**15**).

Wilfornine A (**1**) was assigned the molecular formula $C_{45}H_{51}O_{20}N$ from its HRFABMS. Its IR spectrum showed hydroxyl and ester carbonyl bands (3483 and 1750 cm^{-1}), and the UV spectrum revealed the presence of an aromatic ring (228 and 267 nm). The ^1H NMR (Table 1) data revealed the presence of six acetyl, one benzoyl (Bz), and four sets of methylene groups. In addition, seven methine protons and a 2,3-substituted pyridine ring were detected. Due to the presence of seven ester groups and a pyridine ring, **1** was assumed to be a sesquiterpene alkaloid derivative, similar to the wilfordate-type sesquiterpenes.[6,12] The ^{13}C NMR (Table 2) spectral data of **1** were very similar to those of wilfordine (**16**),[7] except for the number of acetyl groups (**1**, six acetyls, one benzoyl; **16**, five acetyls, one benzoyl) and the downfield shift of the carbon signal attributed to C-9′ (δ_C 81.6). In the HMBC spectrum of **1**, the proton signals at δ_H 5.50 (H-1), 5.19 (H-2), 6.85 (H-5), 5.45 (H-7), 5.13 (H-8), and 4.43 (H-11b) correlated with the acetyl carbonyl carbon signals at δ_C 167.8, 168.1, 169.6, 169.8, 168.5, and 170.1, respectively. Thus, the six acetyl groups were assigned at positions C-1, C-2, C-5, C-7, C-8, and C-11, and the benzoyl group was located at position C-9′.

Benzoylation of alatusinine (**18**)[8] with benzoyl chloride and a catalytic amount of 4,4-(dimethylamino)pyridine afforded a product identical to **1**. In the NOESY spectrum of **1**, the proton signal at δ_H 5.15 (H-11a) correlated with the signals at δ_H 6.85 (H-5) and 1.54 (H_3-12), and the signal at δ_H 5.50 (H-1) with the signals at δ_H 5.19 (H-2) and 5.13 (H-8), while the signal at δ_H 5.13 (H-8) correlated with the signals at δ_H 5.45 (H-7) and 1.14 (H_3-14). Thus, the relative configurations of the ester groups were determined as 1β, 2β, 5α, 7β, and 8β. Therefore, the structure of **1** was assigned as 9′-benzoyloxyeuonine.

Wilfornine B (**2**), $C_{43}H_{49}O_{19}N$, had five acetyl groups and one benzoyl group from its ^1H NMR spectrum. On the basis of comparison of its ^{13}C NMR data to those of **1**, this compound could be assigned within the same structural type. A downfield carbon signal at position C-9′ was apparent in comparison with ^{13}C NMR data of **18**, and a proton signal at δ_H 5.25 (H-5) was apparent in a more upfield position than the same proton in **1** (δ_H 6.85, H-5). Compound **2** was therefore deduced as be 5-deacetylwilfornine A (**1**). Acetylation of **2** in the usual way afforded **1**. Thus, wilfornine B (**2**) was elucidated as 5-deacetyl-9′-benzoyloxyeuonine.

Wilfornines C (**3**) and D (**4**) were assigned with the same structural framework as **1** and **2** from their ^{13}C NMR spectral data. The difference between these compounds was the nature of the ester groups and their positions. The HMBC spectral data of **3** indicated that the five acetyl groups were located at C-1, C-2, C-7, C-8, and C-11, and one of the benzoyl groups was at C-5. The remaining benzoyl group was located at C-9′, as in the case of compounds **1** and **2**. For **4**, the acetyl groups were assigned at positions C-1, C-2, C-5, C-7, C-8, and C-11. The furanoyl group was deduced to be located at C-9′ from the downfield carbon signal at C-9′. Furanoylation of **18** afforded **4**. Thus, the structures of wilfornine C (**3**) and D (**4**) were determined as O^5-benzoyl-O^5-deacetyl-9′-benzoyloxyeuonine and 9′-furanoyloxyeuonine, respectively.

Wilfornine E (**5**) had a molecular formula $C_{36}H_{43}O_{18}N$. Its ^{13}C NMR spectral data revealed the presence of a carbonyl carbon at δ_C 196.7 and different chemical shift

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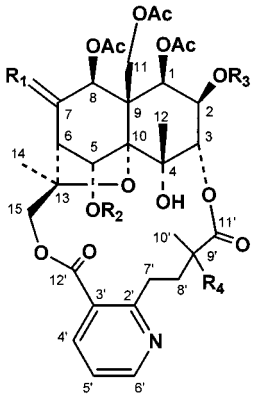
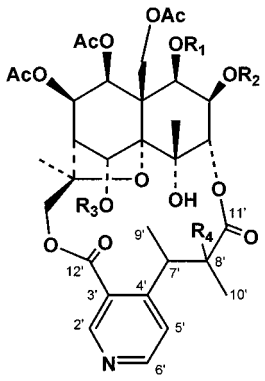
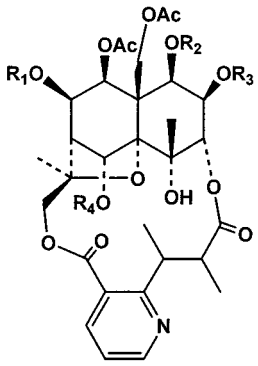
Chart 1



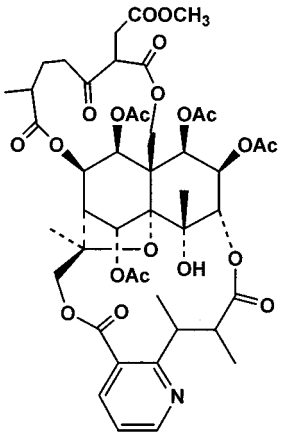
	R_1	R_2	R_3	R_4
1	β-OAc, α-H	Ac	Ac	OBz
2	β-OAc, α-H	H	Ac	OBz
3	β-OAc, α-H	Bz	Ac	OBz
4	β-OAc, α-H	Ac	Ac	OFu
5	O	Ac	Ac	OH
16	β-OAc, α-H	Ac	Bz	OH
17	β-OAc, α-H	Ac	Ac	H
18	β-OAc, α-H	Ac	Ac	OH
19	β-OAc, α-H	Ac	Bz	H
20	β-OAc, α-H	Ac	Fu	H



	R_1	R_2	R_3	R_4
6	Ac	Bz	Bz	OH
7	H	Ac	Ac	H
21	Ac	Bz	Ac	OH



	R_1	R_2	R_3	R_4
8	Ac	Ac	H	Bz
9	Nic	Ac	Ac	Ac
10	Ac	Bz	Ac	Bz
11	Ac	Ac	Nic	Bz
12	Ac	Bz	Ac	Ac
13	Ac	Ac	Ac	Ac
14	Ac	Ac	Ac	Bz



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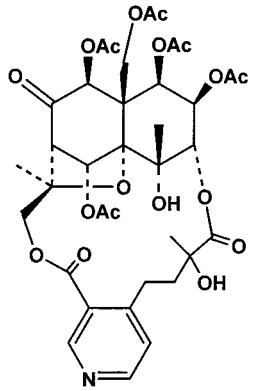

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values for C-6, C-7, and C-8 than those of compounds **1**−**4** and **16** (Table 2). In the HMBC spectrum of **5**, the proton signal at δ_H 6.78 (H-5) correlated with the carbon signals at δ_C 52.5 (C-9), 95.5 (C-10), 86.5 (C-13), and 196.0 (C-7), and the proton signals at δ_H 5.56 (H-8) and 3.05 (H-6) correlated with the carbonyl group (δ_C 196.0). Thus, compound **5** was characterized as 7-deacetoxy-7-oxoalatusinine.

Wilfordinine I (**6**) was assigned a molecular formula $C_{48}H_{51}O_{19}N$ from HRFABMS. Its ^{1}H NMR spectrum (Table 1) showed four acetyl groups, two benzoyl groups, two oxygenated methylene groups, and a secondary methyl group coupled with a methine. Comparison of the ^{13}C NMR spectral data of **6** with those of **1**−**4** indicated that **6** contained a dihydroagarofuran unit linked to a pyridyl moiety (Table 2). The coupling pattern suggested that the pyridine unit in **6** is 3,4-substituted [δ_H 8.99 (s), 8.71 and 7.80 (each 1H, d, J = 5.4 Hz)]. Thus, compound **6** was assumed to be an isomeric evoninate-type sesquiterpene similar to those found in *T. hypoglaucum*[6] and *Peritassa compta*.[17] Comparison of the ^{13}C NMR spectral data of **6** with those of hypoglaunine C (**21**)[6] indicated that the pyridyl moiety was a 2-hydroxy-2,3-dimethyl-3-(3′-carboxy-4′-pyridyl)propanoic acid unit. Furthermore, the proton signal at δ_H 4.84 (H-3) correlated with the signal at δ_C 175.2 (C-11′), and the proton signal at δ_H 5.42 (H-15a) with the signal at δ_C 167.9 (C-12′). These observations indicated that the pyridyl moiety was linked to the sesquiterpene unit at positions C-3 and C-15. In the same manner, two benzoyl groups were located at positions C-2 and C-5, and the four acetyl groups were assigned at C-1, C-7, C-8, and C-11, respectively. The stereochemistry of six ester groups was readily determined by the analysis of the NOESY spectrum as described for **1**. Thus, wilfordinine I (**6**) was formulated as O^2,O^5-dibenzoyl-O^2-deacetyl-O^5-deacetyl-8′-hydroxyperitassine A.

Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**9**[a]

proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	5.50 (d, 3.9)	5.57 (d, 3.6)	5.50 (d, 3.9)	5.59 (d, 3.8)	5.72 (d, 2.9)	5.76 (d, 3.8)	4.42 (d, 5.6)	5.49 (d, 3.7)	5.70 (d, 4.1)
2	5.19 (dd, 2.1, 3.9)	5.18 (dd, 3.6, 2.4)	5.12 (dd, 3.9, 2.2)	5.26 (dd, 3.8, 2.1)	5.17 (dd, 3.1, 2.9)	5.69 (dd, 2.4, 3.8)	5.10 (dd, 2.4, 5.6)	4.12 (br d, 3.2)	5.55 (dd, 2.2, 4.1)
3	4.93 (d, 2.1)	4.99 (d, 2.4)	4.87 (d, 2.2)	5.02 (d, 2.1)	5.01 (d, 3.1)	4.84 (d, 2.4)	4.77 (d, 2.4)	4.80 (d, 2.4)	4.85 (d, 2.2)
5	6.85 (s)	5.25 (d, 3.2)	6.84 (s)	7.00 (s)	6.78 (s)	7.23 (s)	6.76 (s)	7.19 (s)	7.05 (s)
6	2.24 (d, 4.1)	2.34 (d, 3.9)	2.27 (d, 3.7)	2.45 (d, 3.7)	3.05 (s)	2.60 (d, 3.8)	2.38 (d, 3.8)	2.52 (d, 4.0)	2.37 (d, 3.9)
7	5.45 (dd, 5.4, 4.1)	5.45 (dd, 4.6, 3.9)	5.48 (dd, 5.9, 3.7)	5.53 (dd, 5.2, 3.7)		5.59 (dd, 6.0, 3.8)	5.53 (dd, 3.8, 5.6)	5.58 (dd, 4.0, 6.1)	5.53 (dd, 3.9, 6.0)
8	5.13 (d, 5.4)	5.18 (d, 4.6)	5.16 (d, 5.9)	5.21 (d, 5.2)	5.56 (s)	5.45 (d, 6.0)	5.57 (d, 5.6)	5.41 (d, 6.1)	5.41 (d, 6.0)
11a	5.15 (d, 13.6)	5.19 (d, 13.0)	5.11 (d, 13.5)	5.19 (d, 13.5)	4.86 (d, 13.1)	5.53 (d, 13.4)	5.11 (d, 13.2)	4.73 (d, 13.6)	4.37 (d, 13.4)
11b	4.43 (d, 13.6)	4.47 (d, 13.0)	4.42 (d, 13.5)	4.55 (d, 13.5)	4.53 (d, 13.1)	4.48 (d, 13.4)	4.60 (d, 13.2)	5.34 (d, 13.6)	5.42 (d, 13.4)
12	1.54 (s)	1.89 (s)	1.52 (s)	1.60 (s)	1.65 (s)	1.87 (s)	1.53 (s)	1.60 (s)	1.64 (s)
14	1.14 (s)	1.27 (s)	1.34 (s)	1.16 (s)	1.54 (s)	1.68 (s)	1.71 (s)	1.69 (s)	1.71 (s)
15a	5.59 (d, 11.8)	5.87 (d, 12.5)	5.58 (d, 12.0)	5.71 (d, 11.9)	5.92 (d, 12.0)	5.42 (d, 11.4)	6.01 (d, 11.5)	3.61 (d, 11.5)	3.71 (d, 11.5)
15b	3.71 (d, 11.8)	3.62 (d, 12.5)	3.76 (d, 12.0)	3.60 (d, 11.9)	3.75 (d, 12.0)	4.19 (d, 11.4)	3.68 (d, 11.5)	6.04 (d, 11.5)	5.98 (d, 11.5)
2′						8.99 (s)	8.98 (s)		
4′	8.08 (dd, 7.9, 1.6)	8.15 (dd, 7.8, 1.6)	8.12 (dd, 7.8, 1.6)	8.06 (d, 7.8)	8.17 (br d, 7.8)			8.04 (dd, 1.6, 7.8)	8.08 (dd, 1.6, 7.9)
5′	7.29 (dd, 7.9, 4.6)	7.34 (dd, 7.8, 4.8)	7.31 (dd, 7.8, 4.8)	7.34 (dd, 7.8, 4.6)	7.24 (m)	7.80 (d, 5.2)	7.37 (d, 5.1)	7.26 (dd, 4.7, 7.8)	7.27 (dd, 4.7, 7.9)
6′	8.76 (dd, 4.6, 1.6)	8.77 (dd, 4.8, 1.6)	8.71 (dd, 4.8, 1.6)	8.81 (d, 4.6)	8.71 (br d, 3.2)	8.71 (d, 5.2)	8.71 (d, 5.1)	8.70 (dd, 1.6, 4.7)	8.70 (dd, 1.6, 4.7)
7a′	3.70 (m)	3.91 (m)	3.72 (m)	3.81 (m)	4.09 (m)	4.30 (q, 7.3)	4.67 (q, 7.1)	4.72 (q, 7.0)	4.67 (q, 7.0)
7b′	2.97 (m)	2.97 (m)	2.91 (m)	3.06 (m)	2.88 (m)				
8a′	2.90 (m)	2.60 (m)	2.68 (m)	2.95 (m)	2.47 (m)		2.41 (q, 7.2)	2.57 (q, 7.4)	2.60 (q, 7.1)
8b′	2.33 (m)	2.36 (m)	2.29 (m)	2.39 (m)	2.22 (m)				
9′						1.28 (d, 7.3)	1.37 (d, 7.1)	1.43 (d, 7.0)	1.40 (d, 7.0)
10′	1.79 (s)	1.79 (s)	1.75 (s)	1.84 (s)	1.49 (s)	1.39 (s)	1.07 (d, 7.2)	1.17 (d, 7.4)	1.22 (d, 7.1)
1-OAc	1.66 (s)	1.56 (s)	1.73 (s)	1.71 (s)	1.93 (s)	1.99 (s)		1.97 (s)	1.96 (s)
2-OAc	2.14 (s)	2.14 (s)	2.10 (s)	2.17 (s)	2.17 (s)		2.18 (s)		2.16 (s)
5-OAc	2.14 (s)		2.14 (s)		2.23 (s)		2.20 (s)		2.22 (s)
7-OAc	2.09 (s)	2.10 (s)	2.13 (s)	2.21 (s)		2.26 (s)	2.17 (s)	2.24 (s)	
8-OAc	1.89 (s)	1.85 (s)	1.90 (s)	1.94 (s)	2.13 (s)	1.99 (s)	1.98 (s)	2.04 (s)	1.82 (s)
11-OAc	2.23 (s)	2.11 (s)	2.22 (s)	2.27 (s)	2.05 (s)	2.30 (s)	2.26 (s)	2.34 (s)	2.32 (s)

[a] $CDCl_3$ was used as solvent, and TMS as internal standard.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**9** and **16**[a]

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**16**
1	72.1	72.1	72.3	72.1	71.9	72.9	72.7	75.5	73.1	73.2
2	69.3	69.9	69.5	69.5	68.7	68.4	73.3	69.8	69.8	69.3
3	78.4	78.5	78.7	78.6	76.2	77.9	75.9	78.4	75.6	76.9
4	69.6	71.9	69.9	69.8	70.2	70.7	70.7	70.7	70.4	69.8
5	73.7	74.2	74.9	73.9	73.5	74.9	73.2	75.1	73.7	73.6
6	50.7	52.3	50.7	50.9	62.4	50.7	50.5	50.7	50.6	51.1
7	68.4	68.8	68.7	68.8	196.0	69.1	69.7	69.4	68.9	69.0
8	71.7	72.0	72.2	71.8	78.3	70.7	71.8	71.3	70.5	70.8
9	52.0	50.7	52.3	52.2	52.5	52.3	51.9	51.9	52.1	51.9
10	93.3	92.8	93.2	93.4	95.5	93.5	94.0	94.3	94.0	94.0
11	60.1	61.1	60.4	60.2	60.3	60.5	61.4	60.5	60.3	60.6
12	23.3	24.3	23.5	23.2	23.4	22.7	23.4	23.3	23.3	23.0
13	84.2	85.4	84.5	84.4	86.5	83.8	84.5	84.2	84.3	85.0
14	17.2	17.7	17.3	17.3	18.8	18.7	18.5	18.6	18.6	17.9
15	69.7	71.0	69.8	70.1	70.0	69.8	70.3	70.2	69.9	69.8
2′	161.7	163.1	161.9	161.8	164.8	150.9	150.9	165.2	165.3	164.8
3′	125.6	124.8	125.9	125.5	125.3	127.7	125.4	126.1	125.0	125.5
4′	138.2	138.4	138.1	138.5	138.2	151.5	156.3	137.7	137.8	137.9
5′	120.9	121.4	121.0	121.4	120.9	123.4	121.6	121.3	121.2	120.6
6′	152.2	152.8	152.4	152.3	152.3	152.6	152.9	151.6	151.6	152.2
7′	30.6	31.1	30.8	30.6	31.4	41.8	33.4	36.5	36.4	31.4
8′	37.7	38.4	38.0	37.8	38.7	76.6	45.7	46.9	45.0	38.4
9′	81.6	82.1	81.9	81.4	77.7	16.9	11.5	12.0	11.9	77.7
10′	22.7	22.2	23.0	22.3	27.8	24.2	10.0	9.5	9.7	21.6
11′	171.7	172.0	172.0	171.6	172.3	175.2	173.6	174.6	174.0	172.5
12′	167.6	167.9	167.8	167.6	167.8	167.9	168.0	168.7	168.6	168.0

[a] $CDCl_3$ was used as solvent, and TMS as internal standard.

Wilfordinine J (**7**), $C_{36}H_{45}O_{17}N$, contained five acetyl groups, two secondary methyl groups, and two coupled methine protons. It also had a 3,4-disubstituted pyridine ring, as compound **6**. The ^{13}C NMR spectral data of **7** were similar to those of **6**, except for the number and nature of the ester groups and chemical shift values of C-7′, C-8′, C-9′, and C-10′. The pyridyl unit was assumed to be an isomeric form of evoninate acid, which already has been reported from *T. hypoglaucum*.[4] In the HMBC spectrum, the signal at δ_H 8.98 (H-2′) correlated with the carbon signals at δ_C 125.4 (C-3′), 156.3 (C-4′), 152.9 (C-6′), and 168.0 (C-12′), the signal at δ_H 1.07 (H-10′) with the signals at δ_C 33.4 (C-7′), 45.7 (C-8′), and 173.6 (C-11′), and the signal at δ_H 1.37 (H-9′) with the signals at δ_C 156.3 (C-4′), 33.4 (C-7′), and 45.7 (C-8′). From above observations, the presence of a 2,3-dimethyl-3(3′-carboxy-4′-pyridyl)propanoic acid unit was determined. Furthermore, the proton signals at δ_H 5.10 (H-2), 6.76 (H-5), 5.53 (H-7), 5.57 (H-8), and 5.11 (H-11a) correlated with the acetyl carbonyl carbon signals at δ_C 170.3, 169.9, 170.2, 169.9, and 169.8, respectively. Therefore, the five acetyl groups were located at C-2, C-5, C-7, C-8, and C-11. Thus, wilfordinine J (**7**) was determined as *O*[1]-deacetylperitassine A.

Wilfornine F (**8**) had a molecular formula of $C_{41}H_{47}O_{17}N$. The ^1H NMR spectral data revealed four acetyl methyl groups, two secondary methyls, two coupled methine protons, and one benzoyl group, as well as a 2,3-substituted pyridine moiety [δ_H 8.70 (1H, dd, 4.7, 1.6), 7.26 (1H, dd, 7.8, 4.7), and 8.04 (1H, dd, 7.8, 1.6)]. The ^{13}C NMR spectral data were similar to those of hyponine D[9] (**11**) except for the ester groups. In the HMBC spectrum, the proton signals at δ_H 5.49 (H-1), 5.58 (H-7), 5.41 (H-8), and 4.73 (H-11a) correlated with the acetyl carbonyl signals at δ_C 169.5, 170.4, 169.3, and 170.2, respectively. Thus, the positions of these four acetyl groups were assigned at C-1, C-7, C-8, and C-11. Further, the proton signal at δ_H 7.19 (H-5) correlated with the benzoyl carbonyl carbon at δ_C 166.0, so the benzoyl group was located at C-5. In the NOESY spectrum, the proton signal at δ_H 5.34 (H-11b) correlated with the signals at δ_H 7.19 (H-5) and 1.60 (H_3-12), the signal at δ_H 5.49 (H-1) with the signals at δ_H 4.12 (H-2) and 5.41 (H-8), and the signal at δ_H 5.41 (H-8) with the signals at δ_H 5.58 (H-7) and 1.69 (H_3-14). Thus, the relative configurations of the ester groups were determined as 1β, 2β, 5α, 7β, and 8β. Therefore, **8** was formulated as 7-(acetyloxy)-*O*[5]-benzoyl-*O*[2]-deacetyl-*O*[5]-deacetyl-7-deoxoevonine.

Table 3. Inhibitory Effects of Compounds **1**−**3**, **10**−**17**, **19**−**22**, and Prednisolone on Cytokine Production[a]

	inhibition (%)					
compound	TNF-α	IL-1β	IL-8	IL-2	IL-4	IFN-γ
1	0	−4	2	23	50	8
2	−6	−3	−23	22	100	7
3	−11	−6	−8	51	39	32
10	76	49	97	100	100	92
11	−9	7	−15	45	66	13
12	−9	2	2	55	14	36
13	55	47	31	53	11	28
14	37	43	84	100	100	99
15	62	55	43	33	−20	17
16	2	−6	8	35	50	4
17	20	14	60	50	77	71
19	79	64	1	67	16	41
20	64	53	7	22	−53	4
21	−28	2	−26	57	51	20
22	10	18	64	47	77	62
prednisolone	52	68	15	65	76	75

[a] For protocols used, see Experimental Section. Concentrations used: **1**−**3**, **10**−**17**, **19**−**22**, 10 μg/mL; prednisolone, 0.3 μg/mL.

Wilfornine G (**9**), $C_{42}H_{48}O_{18}N_2$, contained five acetyl groups and a nicotinoyl group [δ_H 9.32 (d, 1.5), 8.85 (dd, 4.8, 1.5), 8.38 (dt, 7.4, 1.5), 7.48 (dd, 7.4, 4.8)] from its ^1H NMR spectrum. It had the same skeleton as **8** by comparing the ^{13}C NMR spectral data of these two compounds (Table 2). The differences between **8** and **9** were the ester groups and their positions. By using the same strategy as above (the comparison of ^1H, ^{13}C NMR, and 2D NMR spectral data), the structure of wilfornine G (**9**) was determined as 7-(nicotinoyloxy)-7-deoxoevonine.

Several known compounds were identified from their spectral data upon comparison with values reported in the literature as ebenifoline E-11 (**10**),[10] hyponine D (**11**),[9]

mayteine (**12**),[11] euonymine (**13**),[12] congorinine E-1 (**14**),[13] and triptonine A (**15**).[14]

In a screen for immunosuppressive agents from the T_{II} extract of *T. wilfrodii*, we examined the inhibitory effect of isolated compounds and other reported compounds[6,15] (**16**, **17**, and **19**−**22**, from the same origin) on cytokine production. The activities of those compounds with an inhibitory effect are shown in Table 3 (the other tested compounds were inactive in the test system). Compounds **10** and **14** showed a significant inhibitory effect on cytokine production from lipopolysaccharide (or phytohemagglutinin)-stimulated human peripheral mononuclear cells compared with the reference compound (prednisolone).[16] Compounds **17** and **22** inhibited IL-4, IL-8, and IFN-γ production, and **19** (isolated from the T_{II} extract) inhibited TNF-α, IL-1β, and IL-2 production. The immunosuppressive activity of these known compounds has been determined for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. UV spectra were run on an UV 2100 UV−vis recording spectrometer (Shimadzu). IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin-Elmer). NMR experiments were run on a Bruker ARX-400 instrument using TMS as internal standard. NMR spectra were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C). Mass spectra were obtained on a JEOL JMSD-300 instrument, matrix: magic/thio12 [the mixture of magic (dithiothreitol−dithioerythritol, 3:1) and thio (thioglyserol), 1:2]. Column chromatography was performed using Si gel 60 (Merck). HPLC was carried out as follows: Si gel HPLC (Hibar RT 250-25, LiChrosorb Si 60), ODS (INERTSIL PREP ODS, 20.0 × 250 mm, GL Sciences Inc., Tokyo, Japan).

Plant Material. A powdered extract of *T. wilfordii* (T_{II}) was purchased in 1997 from the School of Pharmacy, Shanghai Medical University, Shanghai, People's Republic of China. This extract was prepared from the root xylem with water then with chloroform and by column chromatographic separation (Si gel, $CHCl_3$−MeOH, 95:5). Samples of T_{II} and the original plant (*T. wilfordii*, TW940930) are deposited in the Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

Extraction and Isolation. The extract (T_{II}, 54 g) prepared from *T. wilfordii* was chromatographed over a Si gel column (1.0 kg, 11 × 90 cm) and eluted with solvents of increasing polarity [$CHCl_3$−MeOH (99:2, 95:5, 9:1, MeOH)] to give 10 fractions (fractions 1−10). Fraction 3 (0.7 g) was chromatographed by medium-pressure liquid chromatography (MPLC) on a Si gel column (3 × 35 cm) with a hexane−EtOAc (hexane−EtOAc, 2:1, 1:2) system to give nine further fractions (fractions 3.1−3.9). Fraction 3.7 was separated by gel-permeation chromatography (eluting with MeOH) and then Si gel HPLC to give **10** (8 mg) and **14** (148 mg). Fraction 4 (7.5 g) was chromatographed by MPLC on a Si gel column (hexanes−EtOAc, 3:2, 1:2) to give 15 fractions (fractions 4.1−4.15). Fraction 4.12 was separated on ODS (MeOH−H_2O, 8:2) to give **12** (26 mg), **13** (133 mg), and another major fraction. This major fraction was further separated on HPLC (Si 60, $CHCl_3$−MeOH, 99:1) to give **1** (65 mg) and **15** (3 mg). Fraction 4.13 was separated on ODS (MeOH−H_2O, 8:2) to give **5** (5 mg), **11** (26 mg), and another five fractions (fractions 4.13.1−4.13.5). Fraction 4.13.3 was separated by HPLC (Si 60, $CHCl_3$−MeOH, 99:1) to give **4** (8 mg) and **7** (4.5 mg). Fraction 4.13.4 was separated by HPLC (Si 60, $CHCl_3$−MeOH, 99:1) to give **3** (14 mg) and **8** (4.5 mg). Fraction 5 (16.5 g) was chromatographed on a Si gel column (6 × 80 cm) by elution with hexanes−EtOAc (1:1, 1:2, 1:4) to give 12 further fractions (fractions 5.1−5.12). Fraction 5.9 was separated by ODS (MeOH−H_2O, 8:2, then 7:3) to give **2** (23 mg), **6** (5 mg), and **9** (28 mg).

Wilfornine A (**1**): amorphous powder; $[\alpha]_D$ −41.9° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 267 (3.68), 228 (4.27) nm; IR (KBr) ν_{max} 3483, 2361, 1750, 1577, 1442, 1375, 1232, 1135, 1052, 755, 716 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1 and OBz-9′, δ 7.69 (2H, d, J = 7.4 Hz, *ortho*), 7.46 (1H, br t, J = 7.3 Hz, *para*), 7.30 (2H, dd, J = 7.4, 7.3 Hz, *meta*); ^{13}C NMR ($CDCl_3$), see Table 2 and δ 20.1, 167.8 (OAc-1), 21.4, 168.1 (OAc-2), 20.8, 169.6 (OAc-5), 20.9, 169.8 (OAc-7), 20.3, 168.5 (OAc-8), 21.2, 170.1 (OAc-11), 165.6, 129.6, 130.2, 127.9, 132.9 (OBz-9′); FABMS *m/z* 926 $[M + H]^+$; HRFABMS *m/z* 926.3048 $[M + H]^+$ (calcd for $C_{45}H_{52}O_{20}N$, 926.3083).

Benzoylation of 18. Compound **18** (10 mg, isolated from *T. wilfordii*[15]) was dissolved in 1 mL of pyridine and a catalytic amount of 4,4-(dimethylamino)pyridine, and 1 mL of benzoyl chloride was added to the solution. The reaction mixture was stirred for 48 h at room temperature under N_2. Workup gave 25 mg of residue, which was purified by HPLC (GPC, $CHCl_3$) to give 7 mg of **1**.

Wilfornine B (**2**): amorphous powder; $[\alpha]_D$ −17.8° (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 265 (3.60), 228 (4.17) nm; IR (KBr) ν_{max} 3423, 2927, 2362, 1751, 1453, 1373, 1231, 1101, 1047, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1 and OBz-9′, δ 7.78 (2H, d, J = 7.3 Hz, *ortho*), 7.50 (1H, br t, J = 7.4 Hz, *para*), 7.35 (2H, br t, J = 7.5 Hz, *meta*); ^{13}C NMR ($CDCl_3$), see Table 2 and δ 20.1, 168.2 (OAc-1), 21.2, 168.3 (OAc-2), 21.4, 170.0 (OAc-7), 20.4, 168.7 (OAc-8), 21.0, 169.8 (OAc-11), 166.0, 129.6, 130.5, 128.1, 133.2 (OBz-9′); EIMS *m/z* 883 $[M]^+$ (31), 824 $[M - OAc]^+$ (16), 810 (39), 780 (12), 762 (66), 752 (22), 704 (22), 326 (16), 222 (13), 204 (53), 176 (53), 160 (31), 150 (15), 132 (25), 105 (100), 93 (24), 77 (13), 43 (23); HREIMS *m/z* 883.2927 $[M]^+$ (calcd for $C_{43}H_{49}O_{19}N$, 883.2899).

Acetylation of 2. Compound **2** (1.5 mg) was treated with Ac_2O (0.3 mL) and C_5H_5N (0.5 mL) at room temperature overnight. The reaction mixture on work up gave **1** (1 mg).

Wilfornine C (**3**): amorphous powder; $[\alpha]_D$ −50.0° (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 267 (3.74), 231 (4.51) nm; IR (KBr) ν_{max} 3446, 2923, 2853, 2362, 1752, 1722, 1636, 1453, 1373, 1254, 1233, 1133, 1097, 1052, 1004, 904, 715; cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1 and OBz-5, δ 8.27 (2H, d, J = 7.9 Hz, *ortho*), 7.48 (2H, dd, J = 7.9, 7.4 Hz, *meta*), 7.59 (1H, t, J = 7.4 Hz, *para*), OBz-9′: 7.71 (2H, d, J = 7.9 Hz, *ortho*), 7.33 (2H, br t, J = 7.8 Hz, *meta*), 7.49 (1H, br t, J = 7.5 Hz, *para*); ^{13}C NMR ($CDCl_3$), see Table 2 and δ 20.5, 168.0 (OAc-1), 21.1, 168.3 (OAc-2), 21.5, 170.1 (OAc-7), 20.3, 168.7 (OAc-8), 21.1, 170.1 (OAc-11), 165.7, 129.7, 130.4, 128.9, 133.7 (OBz-5), 165.7, 130.4, 129.4, 133.1, 128.1 (OBz-9′); EIMS *m/z* 987 $[M]^+$ (58), 866 (95), 822 (19), 754 (6), 675 (4), 616 (3), 326 (11), 253 (5), 176 (18), 105 (100), 28 (59); FABMS *m/z* 988 $[M + H]^+$; HRFABMS *m/z* 988.3203 $[M + H]^+$ (calcd for $C_{50}H_{54}O_{20}N$, 988.3239).

Wilfornine D (**4**): amorphous powder; $[\alpha]_D$ −21.1° (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (3.57), 216 (3.99) nm; IR (KBr) ν_{max} 3446, 2925, 2853, 2374, 1749, 1635, 1435, 1372, 1318, 1233, 1096, 1047, 1007, 875, 602 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1 and OFu-9′, δ 6.51 (1H, d, J = 1.1 Hz), 7.30 (1H, br s), 7.75 (1H, s); ^{13}C NMR ($CDCl_3$), see Table 2 and δ 20.2, 168.3 (OAc-1), 20.9, 170.2 (OAc-2), 21.5, 170.0 (OAc-5), 21.0, 168.5 (OAc-7), 20.3, 168.9 (OAc-8), 21.3, 170.4 (OAc-11), 162.4, 118.7, 110.2, 143.3, 149.1 (OFu-9′); FABMS *m/z* 916 $[M + H]^+$; HRFABMS *m/z* 916.2858 $[M + H]^+$ (calcd for $C_{43}H_{50}O_{21}N$, 916.2875).

Preparation of 3-Furoyl Chloride. A mixture of 3-furoic acid (3 g) and $SOCl_2$ (5 mL) in dry benzene (20 mL) was stirred for 2 h under reflux. Excess $SOCl_2$ was repeatedly removed by distillation with benzene at 100 °C. The reaction mixture was then concentrated to 5 mL under reduce pressure.

Esterification of 18 with 3-Furoyl Chloride. Compound **18** (10 mg) was dissolved in 5 mL of pyridine, and a catalytic amount of 4,4-(dimethylamino)pyridine and 1 mL of the reaction mixture prepared above were added to the solution. The reaction mixture was stirred for 18 h at room temperature under N_2. The usual workup gave 18 mg of residue, which was purified by HPLC (GPC, $CHCl_3$) to give 5 mg of **4**.

Wilfornine E (**5**): amorphous powder; $[\alpha]_D$ +1.1° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 267 (3.45), 222 (3.82) nm; IR (KBr) ν_{max} 3454, 2925, 2854, 1752, 1583, 1456, 1374, 1227, 1135, 1088, 1044 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR

($CDCl_3$), see Table 2 and δ 20.5, 169.6 (OAc-1), 21.1, 168.5 (OAc-2), 21.4, 169.3 (OAc-5), 20.2, 169.4 (OAc-8), 20.6, 169.7 (OAc-11); EIMS *m/z* 777 $[M]^+$ (25), 733 (31), 674 (29), 368 (11), 264 (16), 246 (32), 205 (13), 194 (27), 176 (29), 149 (27), 137 (21), 125 (31), 111 (50), 109 (42), 97 (80), 81 (50), 71 (87), 57 (96), 43 (100); HREIMS *m/z* 777.2495 $[M]^+$ (calcd for $C_{36}H_{43}O_{18}N$, 777.2480).

Wilfordinine I (**6**): amorphous powder; $[\alpha]_D$ +63.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 262 (3.77), 230 (4.53) nm; IR (KBr) ν_{max} 3464, 2927, 2855, 2362, 1746, 1599, 1453, 1372, 1313, 1264, 1156, 1097, 1051, 903, 713 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and OBz-2, 8.13 (2H, d, J = 7.5 Hz, *ortho*), 7.53 (2H, br t, J = 7.5 Hz, *meta*), 7.63 (1H, br t, J = 7.5 Hz, *para*), OBz-5: 8.22 (2H, d, J = 7.5 Hz, *ortho*), 7.52 (2H, br t, J = 7.5 Hz, *meta*), 7.63 (1H, br t, J = 7.5 Hz, *para*); ^{13}C NMR ($CDCl_3$), see Table 2 and δ 20.5, 169.4 (OAc-1), 21.2, 170.2 (OAc-7), 20.6, 169.1 (OAc-8), 21.4, 170.3 (OAc-11), 164.9, 129.2, 130.1, 128.9, 133.9 (OBz-2), 165.7, 129.2, 130.2, 129.0, 134.0 (OBz-5); EIMS *m/z* 945 $[M]^+$ (100), 886 (7), 872 (6), 824 (5), 763 (3), 720 (2), 571 (1), 469 (1), 352 (3), 222 (4), 150 (9), 105 (46), 44 (8); FABMS *m/z* 946 $[M + H]^+$; HRFABMS *m/z* 946.3148 $[M + H]^+$ (calcd for $C_{48}H_{52}O_{19}N$, 946.3134).

Wilfordinine J (**7**): amorphous powder; $[\alpha]_D$ −8.1° (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (3.46), 222 (3.85) nm; IR (KBr) ν_{max} 3482, 2936, 1747, 1650, 1591, 1456, 1372, 1233, 1187, 1158, 1120, 1048, 976, 603 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2 and δ 21.1, 170.3 (OAc-2), 21.7, 169.9 (OAc-5), 21.0, 170.2 (OAc-7), 20.8, 169.9 (OAc-8), 21.4, 169.8 (OAc-11); EIMS *m/z* 763 $[M]^+$ (100), 748 (31), 704 $[M - OAc]^+$ (50), 690 (24), 206 (44), 178 (52), 160 (32), 107 (98), 43 (26); HREIMS *m/z* 763.2682 $[M]^+$ (calcd for $C_{36}H_{45}O_{17}N$, 763.2687).

Wilfornine F (**8**): amorphous powder; $[\alpha]_D$ +18.2° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (3.54), 229 (4.16) nm; IR (KBr) ν_{max} 3480, 2939, 2361, 1721, 1650, 1541, 1456, 1374, 1255, 1116, 1057, 787, 715 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and OBz-5, 8.34 (2H, d, J = 7.4 Hz, *ortho*), 7.59 (1H, br t, J = 7.3 Hz, *para*), 7.50 (2H, br t, J = 7.5 Hz, *mata*); ^{13}C NMR ($CDCl_3$), see Table 2 and δ 21.0, 169.5 (OAc-1), 21.3, 170.4 (OAc-7), 20.7, 169.3 (OAc-8), 21.7, 170.2 (OAc-11), 166.0, 129.8, 130.5, 129.0, 133.7 (OBz-5); EIMS *m/z* 825 $[M]^+$ (100), 797 (6), 782 (9), 766 $[M - OAc]^+$ (14), 752 (5), 722 (6), 704 (11), 652 (29), 634 (15), 206 (57), 178 (22), 107 (8); HREIMS *m/z* 825.2834 $[M]^+$ (calcd for $C_{41}H_{47}O_{17}N$, 825.2844).

Wilfornine G (**9**): amorphous powder; $[\alpha]_D$ +11.0° (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 260 (3.80), 222 (4.26) nm; IR (KBr) ν_{max} 3482, 2988, 2360, 1745, 1647, 1590, 1454, 1432, 1371, 1314, 1275, 1232, 1171, 1114, 1059, 1023, 880, 740 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and ONic-7, 9.32 (1H, d, J = 1.5 Hz), 8.85 (1H, dd, J = 4.8, 1.5 Hz), 8.38 (1H, dt, J = 7.5, 1.5 Hz), 7.48 (1H, dd, J = 7.5, 4.8 Hz); ^{13}C NMR ($CDCl_3$), see Table 2 and δ 20.5, 169.0 (OAc-1), 21.0, 170.0 (OAc-2), 21.7, 169.9 (OAc-5), 20.5, 169.1 (OAc-8), 21.2, 170.5 (OAc-11), 163.7, 124.9, 137.4, 123.7, 154.6, 151.3 (ONic-7); EIMS *m/z* 868 $[M]^+$ (100), 825 (11), 809 $[M - OAc]^+$ (24), 718 (10), 686 (12), 572 (13), 206 (56), 178 (24), 161 (14), 124 (32), 107 (33), 43 (12); HREIMS *m/z* 868.2886 $[M]^+$ (calcd for $C_{42}H_{48}O_{18}N_2$, 868.2902).

Bioassay Procedure. The bioassay was performed as previously described in ref 4.

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