Immunosuppressive Diterpenoids from *Tripterygium wilfordii*

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A clinically used extract of *Tripterygium wilfordii* afforded three new diterpenoids—3β,19-dihydroxyabieta-8,11,13-triene (triptobenzene L) (**1**); 12,19-dihydroxy-3-oxoabieta-8,11,13-triene (triptobenzene M) (**2**); and 19-hydroxy-3,7-dioxo-abieta-8,11,13-triene (triptobenzene N) (**3**)—along with 14 known diterpenoids. The structures of **1**–**3** were established on the basis of spectroscopic studies. Of the known compounds, the stereochemistry at C-4 of triptonediol (**4**) was reassigned. Tripterifordin (**8**) and 13-*epi*-manoyl oxide-18-oic acid (**9**) showed significant inhibitory effects on cytokine production.

Tripterygium wilfordii Hook f. (Celastraceae) has been used as a Chinese traditional medicine for several hundred years. Recently, an extract, derived from a water–chloroform extract of the roots (the so-called "total multiglycoside" or "T_{II} extract") has been used in the clinical treatment of rheumatoid arthritis and other inflammatory and autoimmune diseases, for skin disorders, and in male-fertility control.[1–3] The precise mechanism of the therapeutic effect of T_{II}, however, has not been completely delineated. To determine which of the components are responsible for such diverse activities, we have started work on the isolation of the active principles of the T_{II} extract of *T. wilfordii*. In this paper, we report the isolation and structure elucidation of the three new diterpenoids, named triptobenzenes L (**1**), M (**2**), and N (**3**), and 14 known diterpenoids from the T_{II} extract of *T. wilfordii*. By studying the NOESY NMR spectrum, the stereochemistry at C-4 of the known compound triptonediol (**4**) was reassigned. In immunosuppressive bioassay performed on these diterpenoids, two compounds (**8** and **9**) exhibited significant inhibitory effects on cytokine production.

Results and Discussion

The powdered extract (T_{II}) of *T. wilfordii* was chromatographed on Si gel. The fractions were further chromatographed and afforded triptobenzenes L (**1**), M (**2**), and N (**3**) and 14 known compounds.

Compound **1** (triptobenzene L) had the molecular formula $C_{20}H_{30}O_2$ as found from its HREIMS (*m/z* 302.2251), and its IR spectrum showed hydroxy group (3401 cm^{-1}) absorption. The UV spectrum showed the presence of an aromatic moiety with maxima at 238 and 267 nm. The ^{1}H NMR spectrum of **1** revealed the presence of an isopropyl group [δ_H 1.22 (6H, d, $J = 6.9$ Hz); 2.81 (1H, sept, $J = 6.9$ Hz)], two methyl groups [δ_H 1.16, 1.32 (each 3H, s)], a pair of methylene protons [δ_H 3.43, 4.33 (each 1H, d, $J = 11.2$ Hz)] bearing an oxygen function, a methine proton [δ_H 3.53 (1H, dd, $J = 4.4, 11.7$ Hz)] attached to the oxygen function, and three aromatic protons [δ_H 6.99, 7.15 (each 1H, d, $J = 8.2$ Hz) and 6.89 (1H, s)]. The ^{13}C NMR spectrum of **1** confirmed the presence of a benzene ring, in addition to

four methyl, four methylene, two methine, and two quaternary carbon signals, as well as an additional methine carbon (δ_C 80.7) and a methylene carbon (δ_C 64.3) attached to an oxygen function, respectively. From this information, compound **1** was assumed to be an abietane-type diterpene, the same as triptobenzenes A–K isolated from *T. wilfordii* var. *regelii*[4] and *T. hypoglaucum*.[5]

In the HMBC spectrum of **1**, the methine proton signal (δ_H 2.81) of the isopropyl group correlated with the carbon signals at δ_C 146.0 (C-13) and 124.2 (C-12), and the proton signal at δ_H 6.99 (H-12) correlated with signals at δ_C 33.5 (C-15), 146.3 (C-9), and 126.9 (C-14). In turn, the proton signal at δ_H 6.89 (H-14) correlated with the signals at δ_C 33.5 (C-15), 146.3 (C-9), 124.2 (C-12), and 31.1 (C-7). From these observations, the location of the isopropyl group at C-13 was inferred. Further, the proton signal at δ_H 4.33 (H-19) correlated with the signals at δ_C 22.6 (C-18), 43.0 (C-4), and 80.7 (C-3), while the proton signal at δ_H 3.53 (H-3) correlated with the signals at δ_C 22.6 (C-18), 64.3 (C-19), and 43.0 (C-4). Therefore, these two hydroxyl groups in **1** could be assigned to positions C-3 and C-19. In the NOESY spectrum, the proton signal at δ_H 3.53 (H-3) showed correlations with the proton signals at δ_H 1.46 (H-5) and 1.32 (H$_3$-18), and the proton signal at δ_H 4.33 (H-19) showed a correlation with the methyl proton signals at δ_H 1.16 (H$_3$-20). Thus, the configurations of the hydroxyl group at C-3 and the hydroxy methylene at C-4 were confirmed to be β. Assignments of the ^{1}H and ^{13}C NMR spectral data were made on the basis of the 2D NMR spectra (see Experimental Section and Table 1).

Triptobenzene M (**2**), $C_{20}H_{28}O_3$ ([M]$^+$ at *m/z* 316.2028, HREIMS), showed hydroxyl and ketone IR bands (3406 and 1703 cm^{-1}) and the UV absorptions (220 and 283 nm) of an aromatic ring. The ^{1}H NMR spectrum of **2** showed a 1,3,4,6-tetrasubstituted benzene ring [δ_H 6.62, 6.85 (each 1H, s)], a methylene group attached to an oxygen function [δ_H 3.52, 4.07 (each 1H, d, $J = 11.3$ Hz)], and an isopropyl group [δ_H 3.13 (1H, sept, $J = 6.9$ Hz), 1.25 (6H, d, $J = 6.9$ Hz)], and two methyl groups [δ_H 1.24, 1.35 (each 3H, s)]. The ^{13}C NMR spectral data were similar to those of compound **1**, except for C-2, -3, -4, -8, -11, -12, and -13 (Table 1), so it was concluded that **2** showed the same skeleton as **1**. In the HMBC spectrum, the proton signal at δ_H 2.38 (H-1) correlated with the ^{13}C NMR signals at δ_C 25.7 (C-20), 34.8 (C-2), 220.7 (C-3), and 51.3 (C-5); the proton signal at δ_H 1.35 (H$_3$-18), with the signals at δ_C 220.7

Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**4**[a]

carbon	1	2	3	4
1	36.8 t	37.2 t	36.3 t	35.4 t
2	28.6 t	34.8 t	35.4 t	34.7 t
3	80.7 d	220.7 s	215.2 s	224.0 s
4	43.0 s	50.8 s	52.2 s	50.1 s
5	50.8 d	51.3 d	50.0 d	52.8 d
6	19.1 t	19.9 t	36.2 t	19.2 t
7	31.1 t	30.4 t	197.9 s	26.6 t
8	134.5 s	126.5 s	130.3 s	130.7 s
9	146.3 s	144.9 s	151.0 s	130.8 s
10	37.2 s	36.8 s	37.3 s	37.7 s
11	124.6 d	112.2 d	124.5 d	150.5 s
12	124.2 d	151.2 s	133.0 d	112.0 d
13	146.0 s	132.6 s	147.7 s	139.7 s
14	126.9 d	126.7 d	125.3 d	148.7 s
15	33.5 d	26.8 d	33.7 d	26.2 d
16	24.0 q	22.5 q	23.8 q	23.8 q
17	24.0 q	22.7 q	23.9 q	23.9 q
18	22.6 q	22.3 q	21.4 q	22.9 q
19	64.3 t	65.8 t	65.6 t	66.1 t
20	26.0 q	25.7 q	23.6 q	21.1 q
−OMe				60.8 q

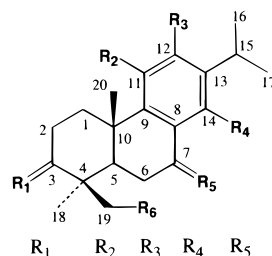[a] CDCl$_3$ was used as solvent, and TMS as internal standard.

(C-3), 51.3 (C-5), and 65.8 (C-19); and the proton signal at δ_H 4.07 (H-19), with the signals at δ_C 22.3 (C-18) and 220.7 (C-3). In addition, the proton signals at δ_H 6.85 (H-14) and 3.13 (H-15) correlated with the signal at δ_C 151.2 (C-12). Therefore, the ketone and hydroxyl groups were assigned to positions C-3 and C-19, respectively. In the NOESY spectrum, the methyl signal at δ_H 1.24 (H$_3$-20) correlated with the proton signals at δ_H 4.07 (H-19) and 6.62 (H-11); and the proton signal at δ_H 6.85 (H-14), with the signals at δ_H 2.87 (H-7) and 3.13 (H-15). It was confirmed that the configuration of the hydroxy methylene at C-4 was β, and the phenolic hydroxyl group was assigned to C-12. Thus, the structure of triptobenzene M was formulated as **2**.

Triptobenzene N (**3**), C$_{20}$H$_{26}$O$_3$, showed hydroxyl and two ketone groups in the IR spectrum (3498, 1693, and 1674 cm^{-1}) and an aromatic ring in the UV spectrum (253 and 301 nm). The ^{1}H NMR spectrum of **3** revealed three aromatic proton signals at δ_H 7.30 (1H, d, $J = 8.1$ Hz), 7.44 (1H, dd, $J = 1.7, 8.1$ Hz), and 7.90 (1H, d, $J = 1.7$ Hz); methylene proton signals at δ_H 3.69, 4.02 (each 1H, d, $J = 11.1$ Hz) attached to an oxygen function; an isopropyl group; and two methyl groups. The structural similarity between triptobenzenes N (**3**) and L (**1**) was indicated by the close resemblance of the ^{13}C NMR spectra of both compounds, except for C-2, -3, -4, -6, -7, and -8 (Table 1). It was concluded that compound **3** is 3,7-oxo-triptobenzene L due to the presence of two carbonyl carbon resonances in the ^{13}C NMR spectrum (δ_C 215.2 and 197.9), with the upfield resonance suggesting an aryl ketone. In the HMBC spectrum of **3**, the proton signal at δ_H 1.24 (H$_3$-18) correlated with the carbon signals at δ_C 215.2 (C-3), 52.2 (C-4), 65.6 (C-19), and 50.0 (C-5); the proton signal at δ_H 2.51 (H-5), with the signals at δ_C 52.2 (C-4), 23.6 (C-20), and 197.9 (C-7); and the proton signals at δ_H 7.90 (H-14), with the signals at δ_C 197.9 (C-7) and 33.7 (C-15). From the above facts, the two ketone groups were assigned to positions C-3 and C-7. The stereochemistry for C-4 of **3** was readily determined by NOE observations as described for **1** and **2**.
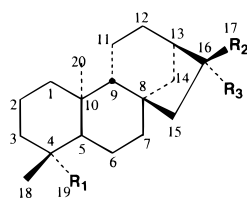
Compound **4**, C$_{21}$H$_{30}$O$_4$, contained an isopropyl group, one oxygenated methylene [δ_H 3.44, 4.18 (each 1H, d, $J = 11.5$ Hz)], four methyl groups, and one methoxy group, as indicated by the ^{1}H NMR spectrum. Its ^{13}C NMR spectral data were similar to those of triptobenzene M (**2**), except for the benzene ring (Table 1). Compound **4** showed the

same spectral data as triptonediol isolated earlier from *T. wilfordii*,[6] which was assigned with a 4α-hydroxy methyl-ene substituent. However, this assignment differs from the configuration at C-4 determined in the previous investigation for triptobenzenes L (**1**), M (**2**), and N (**3**). The original structure of triptonediol was determined only by using NMR chemical shift comparisons. In the NOESY spectrum of **4**, the proton signal at δ_H 4.18 (H-19) correlated with the signal at δ_H 1.30 (H$_3$-20), while the proton signal at δ_H 1.42 (H$_3$-18) correlated with the signal at δ_H 2.21 (H-5). Therefore, we propose the revised structure **4** for trip-tonediol, with the hydroxymethyl group having a β orienta-tion.

Known compounds were identified from their spectral data upon comparison with values reported in the litera-ture as triptobenzene A,[7] hinokiol,[8] triptinin B,[9] triptoben-zene H,[10] triptoquinone A,[4] triptoquinone C (**5**),[4] tripto-quinone B,[4] triptotin B,[11] 16α,19-dihydroxy-*ent*-kaurane,[12] (−)-16α-hydroxykauran-19-oic acid (**6**),[13] (−)-17-hydroxy-16α-kauran-19-oic acid (**7**),[14,15] tripterifordin (**8**),[16] and 13-*epi*-manoyl oxide-18-oic acid (**9**),[17] respectively. This is the first time these known diterpenoids have been isolated from the T$_{II}$ extract of *T. wilfordii*.

	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$	R$_6$
1	βOH, αH	H	H	H	H, H	OH
2	O	H	OH	H	H, H	OH
3	O	H	H	H	O	OH
4	O	OH	H	OMe	H, H	OH

	R$_1$	R$_2$	R$_3$
6	COOH	Me	OH
7	COOH	CH$_2$OH	H
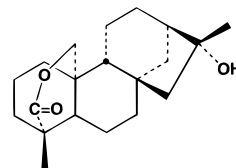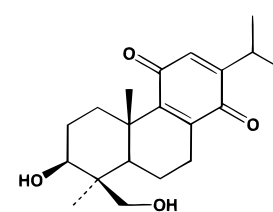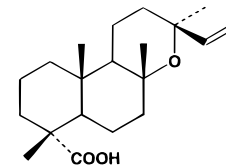
In a screen for immunosuppressive activity for these diterpene derivatives, we examined the inhibitory effect on cytokine production and show bioactivity data for com-pounds with an inhibitory effect in Table 2 (the other compounds were inactive in the test system). Compounds **8** and **9** showed a significant inhibitory effect on cytokine production from lipopolysaccharide-stimulated human pe-ripheral mononuclear cells when compared with the refer-

Table 2. Inhibitory Effects of Compounds **2**, **3**, **5**, **7**−**9** and Prednisolone on Cytokine Production[a]

compound	inhibition (%)					
	TNF-α	IL-8	IL-1β	IL-4	IL-2	IFN-γ
2	7.5	−29.1	13.5	40.1	55.6	54.1
3	13.3	15.4	37.9	−52.2	64.5	42.3
5	−29.5	−24.6	16.4	66.4	64.0	89.0
7	0.0	4.2	−6.1	56.2	35.0	34.0
8	76.8	96.6	75.7	95.7	97.9	98.6
9	49.9	65.2	36.1	95.4	100.0	94.0
prednisolone	88.5	90.3	88.5	96.1	93.9	95.4

[a] For protocols used, see Experimental Section. Concentration used: **2**, **3**, **5**, **7**−**9**, 10 μg/mL; prednisolone, 0.3 μg/mL.

ence compound (prednisolone),[18] and the immunosuppressive activity of these two 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 a 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. ^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz, both using TMS as internal standard. MS were obtained on a JEOL JMSD-300 instrument. Chromatographic columns: Si gel 60 (Merck) and Sephadex LH-20 (Pharmacia); HPLC; GPC (gel-permeation chromatography, Asahipak, GS-310 2G, MeOH), Si gel HPLC (YMC−pack SIL-06 SH-043−5−06, 250 × 20 mm).

Plant Material. A powdered extract of *Tripterygium wilfordii* (T$_{II}$) was purchased in 1997, from the School of Pharmacy, Shanghai Medical University, People's Republic of China. It was extracted from the root xylem with H$_2$O then with CHCl$_3$ and by column chromatographic separation (Si gel, CHCl$_3$−MeOH, 95:5). Samples of T$_{II}$ and the original plant (*T. wilfordii*) are deposited in Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

Extraction and Isolation. The extract (T$_{II}$, 54 g) was chromatographed on a Si gel column (1.0 kg, 11 × 90 cm) and eluted with solvent mixtures of increasing polarity [CHCl$_3$−MeOH (99:1, 95:5, 9:1, MeOH)] to give 10 fractions (fractions 1−10). Fraction 5 (16.5 g) was chromatographed over a Si gel column (6 × 80 cm) eluting with hexanes−EtOAc (1:1, 1:2, 1:4) to give 12 fractions (fractions 5.1−5.12). Combined fractions 5.4 and 5.5 (1.7 g) were chromatographed over Sephadex LH-20 to give four fractions (fractions 5.4.1−5.4.4), and fraction 5.4.3 (1.1 g) was then subjected to MPLC (Si gel, CHCl$_3$−MeOH, 97:3, 9:1) to give seven fractions (fractions 5.4.3.1−5.4.3.7). Fraction 5.4.3.3 (118 mg) was isolated by GPC (MeOH) to give three fractions, and the major fraction (85 mg) was purified by Si gel HPLC (hexane−EtOAc, 1:1) to give **3** (16 mg), triptobenzene H (21 mg), and triptoquinone A (15 mg). Fraction 5.4.3.4 (200 mg) was separated by GPC (MeOH) to give **5** (37 mg). Fraction 5.4.3.5 (507 mg) was chromatographed by GPC (MeOH) to give six fractions (fractions 5.4.3.5.1−5.4.3.5.6). Fraction 5.4.3.5.1 was separated by Si gel HPLC (hexanes−EtOAc−MeOH, 65:30:5) to yield 16α,19-dihydroxy-*ent*-kaurane (40 mg), **6** (8 mg), and **9** (3 mg). Fraction 5.4.3.5.3 was isolated by Si gel HPLC (hexanes−EtOAc−MeOH, 65:30:5) to give **2** (7 mg) and triptinin B (40 mg).

Fraction 2 (1.5 g) was chromatographed by Si gel MPLC eluting with hexanes−EtOAc (2:1), to obtain eight fractions (fractions 2.1−2.8). Fraction 2.5 was separated by GPC (MeOH) to give three fractions, and the major fraction was purified by Si gel HPLC (hexane−EtOAc, 1:1) to give triptoquinone B (33 mg) and triptotin B (3 mg). Combined fractions 5.2 and 5.3 (1.7 g) were chromatographed by Si gel MPLC eluting with CHCl$_3$−MeOH (99:1, 99:5), to afforded seven fractions (fractions 5.2.1−5.2.7). Fraction 5.2.4 (200 mg) was separated by GPC (MeOH) to give **4** (20 mg) and triptobenzene

A (92 mg). Fraction 5.2.5 was separated by Si gel HPLC (hexane−EtOAc, 3:2) to give **1** (4 mg) and hinokiol (5 mg). Fraction 5.2.6 was separated by GPC (MeOH) to give **7** (21 mg) and **8** (6 mg).

Triptobenzene L (3β,19-dihydroxyabieta-8,11,13-triene) (1): amorphous powder; [α] +30.4° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (3.79), 267 (2.87) nm; IR (KBr) ν_{max} 3570, 3401, 2954, 1708, 1656, 1639, 1499, 1459, 1381, 1261, 1090, 1039, 972, 823, 757, 626 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.15 (1H, d, J = 8.2 Hz, H-11), 6.99 (1H, d, J = 8.2 Hz, H-12), 6.89 (1H, s, H-14), 4.33, 3.43 (2H, d, J = 11.2 Hz, H$_2$-19), 3.53 (1H, dd, J = 4.4, 11.7 Hz, H-3), 2.83, 2.92 (2H, m, H$_2$-7), 2.81 (1H, sept, J = 6.9 Hz, H-15), 2.34 (1H, ddd, J = 3.4, 3.4, 13.3 Hz, H-1), 2.01 (1H, m, H-2), 1.97, 1.66 (2H, m, H$_2$-6), 1.91 (1H, m, H-2), 1.65 (1H, br s, −OH, D$_2$O exchange), 1.54 (1H, ddd, J = 3.5, 13.3, 13.3 Hz, H-1), 1.46 (1H, dd, J = 1.2, 12.3 Hz, H-5), 1.32 (3H, s, H$_3$-18), 1.22 (6H, d, J = 6.9 Hz, H$_3$-16, 17), 1.16 (3H, s, H$_3$-20); ^{13}C NMR (CDCl$_3$), see Table 1; EIMS m/z 302 [M]$^+$ (62), 287 [M − Me]$^+$ (34), 269 (100), 251 (57), 239 (44), 227 (32), 209 (49), 199 (23), 185 (41), 171 (29), 159 (36), 143 (41), 129 (47), 117 (31), 91 (30), 55 (19), 43 (48); HREIMS m/z 302.2251 (calcd for C$_{20}$H$_{30}$O$_2$, 302.2246).

Triptobenzene M (12,19-dihydroxy-3-oxoabieta-8,11,13-triene) (2): amorphous powder; [α] +42.4° (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (3.83), 283 (3.53) nm; IR (KBr) ν_{max} 3570, 3406, 2956, 2868, 2346, 1703, 1656, 1639, 1619, 1511, 1468, 1439, 1378, 1234, 1171, 1110, 1035, 911, 711 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 6.85 (1H, s, H-14), 6.62 (1H, s, H-11), 4.99 (1H, br s, −OH, D$_2$O exchange), 4.07, 3.52 (2H, d, J = 11.3 Hz, H$_2$-19), 3.13 (1H, sept, J = 6.9 Hz, H-15), 2.87 (1H, ddd, J = 1.3, 5.6, 12.2 Hz, H-7), 2.76 (1H, m, H-7), 2.70 (1H, m, H-2), 2.58 (1H, dt, J = 7.9, 16.0 Hz, H-2), 2.38 (1H, ddd, J = 4.6, 8.4, 13.2 Hz, H-1), 2.13 (1H, dd, J = 2.1, 12.9 Hz, H-5), 2.05 (1H, m, H-1), 1.86, 1.63 (2H, m, H$_2$-6), 1.35 (3H, s, H$_3$-18), 1.25 (6H, d, J = 6.9 Hz, H$_3$-16, 17), 1.24 (3H, s, H$_3$-20); ^{13}C NMR (CDCl$_3$), see Table 1; EIMS m/z 316 [M]$^+$ (100), 301 [M − Me]$^+$ (28), 286 (31), 271 (69), 255 (13), 241 (31), 213 (23), 199 (44), 187 (30), 173 (21), 159 (32), 147 (39), 141 (24), 115 (22), 91 (17), 43 (50); HREIMS m/z 316.2028 (calcd for C$_{20}$H$_{28}$O$_3$, 316.2038).

Triptobenzene N (19-hydroxy-3,7-dioxoabieta-8,11,13-triene) (3): amorphous powder; [α] −42.3° (*c* 1.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 253 (3.97), 301 (3.27) nm; IR (KBr) ν_{max} 3570, 3498, 2965, 1693, 1674, 1639, 1607, 1492, 1460, 1420, 1382, 1307, 1263, 1245, 1112, 1051, 842, 608 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.90 (1H, d, J = 1.7 Hz, H-14), 7.44 (1H, dd, J = 1.7, 8.1 Hz, H-12), 7.30 (1H, d, J = 8.1 Hz, H-11), 4.02, 3.69 (2H, d, J = 11.1 Hz, H$_2$-19), 2.94 (1H, sept, J = 6.9 Hz, H-15), 2.83, 2.76 (2H, m, H$_2$-6), 2.79, 2.71 (2H, m, H$_2$-2), 2.65, 2.09 (2H, m, H$_2$-1), 2.51 (1H, dd, J = 4.0, 14.0 Hz, H-5), 2.28 (1H, br s, −OH, D$_2$O exchange), 1.41 (3H, s, H$_3$-20), 1.25 (6H, d, J = 6.9 Hz, H$_3$-16, 17), 1.24 (3H, s, H$_3$-18); ^{13}C NMR (CDCl$_3$), see Table 1; EIMS m/z 314 [M]$^+$ (32), 296 [M − H$_2$O]$^+$ (56), 284 (60), 269 (66), 254 (26), 241 (73), 227 (59), 213 (66), 199 (100), 187 (52), 171 (36), 159 (37), 141 (66), 128 (57), 115 (49), 91 (31), 43 (62); HREIMS m/z 314.1900 (calcd for C$_{20}$H$_{26}$O$_3$, 314.1882).

Triptonediol (11,19-hydroxy-14-methoxy-3-oxoabieta-8,11,13-triene) (4): ^{1}H NMR (CDCl$_3$) δ 6.41 (1H, s, H-12), 5.35 (1H, br s, −OH, D$_2$O exchange), 4.18, 3.44 (2H, d, J = 11.5 Hz, H$_2$-19), 3.24 (1H, sept, J = 6.4 Hz, H-15), 3.10, 2.55 (2H, m, H$_2$-7), 3.08, 2.04 (2H, m, H$_2$-1), 2.76 (1H, ddd, J = 6.5, 11.0, 15.4 Hz, H-2), 2.40 (1H, m, H-2), 2.21 (1H, br d, J = 12.6 Hz, H-5), 1.84, 1.47 (2H, m, H$_2$-6), 1.42 (3H, s, H$_3$-18), 1.30 (3H, s, H$_3$-20), 1.18, 1.17 (6H, d, J = 6.4 Hz, H$_3$-16, 17); ^{13}C NMR (CDCl$_3$), see Table 1; EIMS m/z 346 [M]$^+$ (15), 285 (18), 272 (20), 257 (19), 243 (39), 232 (27), 229 (42), 217 (68), 203 (43), 189 (55), 175 (60), 165 (29), 153 (34), 141 (36), 129 (48), 117 (41), 91 (66), 67 (42), 43 (100), 31 (93); HREIMS m/z 346.2148 (calcd for C$_{21}$H$_{30}$O$_4$, 346.2144).

(−)-16α-Hydroxykauran-19-oic acid (6): ^{1}H NMR (C$_5$D$_5$N) δ 2.49 (1H, br d, J = 12.9 Hz, H-3), 2.28 (1H, m, H-2), 2.24 (1H, m, H-6), 2.13 (1H, br s, H-13), 1.97, 1.68 (2H, d, J = 13.7 Hz, H$_2$-15), 1.89 (1H, br d, J = 13.7 Hz, H-1), 1.78 (1H, br d, J = 12.8 Hz, H-7), 1.10 (1H, br d, J = 12.1 Hz, H-5), 0.88 (1H,

br t, $J = 13.2$ Hz, H-1), 1.58 (3H, s, H$_3$-17), 1.36 (3H, s, H$_3$-18), 1.22 (3H, s, H$_3$-20); ^{13}C NMR (C$_5$D$_5$N) δ 41.1 (t, C-1), 19.9 (t, C-2), 38.8 (t, C-3), 43.9 (s, C-4), 57.1 (d, C-5), 22.9 (t, C-6), 42.7 (t, C-7), 45.6 (s, C-8), 56.4 (d, C-9), 40.0 (s, C-10), 18.7 (t, C-11), 27.3 (t, C-12), 49.2 (d, C-13), 38.1 (t, C-14), 58.6 (t, C-15), 77.9 (s, C-16), 25.1 (q, C-17), 29.4 (q, C-18), 180.2 (s, C-19), 16.1 (q, C-20).

(−)-17-Hydroxy-16α-kauran-19-oic acid (7): ^{1}H NMR (CDCl$_3$) δ 3.40 (2H, d, $J = 8.3$ Hz, H$_2$-17), 2.14 (1H, br d, $J = 13.5$ Hz, H-3), 2.06 (1H, br s, H-13), 1.94 (1H, m, H-16), 1.22 (3H, s, H$_3$-18), 0.92 (3H, s, H$_3$-20); ^{13}C NMR (CDCl$_3$) δ 40.8 (t, C-1), 19.2 (t, C-2), 37.9 (t, C-3), 43.8 (s, C-4), 57.1 (d, C-5), 22.5 (t, C-6), 41.7 (t, C-7), 44.8 (s, C-8), 55.4 (d, C-9), 39.7 (s, C-10), 19.0 (t, C-11), 31.5 (t, C-12), 38.2 (d, C-13), 37.3 (t, C-14), 45.1 (t, C-15), 43.4 (d, C-16), 67.5 (t, C-17), 29.1 (q, C-18), 184.0 (s, C-19), 15.7 (q, C-20).

Bioassay Procedure. Whole blood from healthy volunteers (containing 20 U heparin/mL) was suspended in supplemented RPMI-1640 medium containing 100 U/mL penicillin and 100 μg/mL streptomycin to obtain a 30% solution. Lipopolysaccharide (LPS) was also dissolved in the supplemented RPMI-1640 media at a concentration of 3 μg/mL. The test sample was dissolved in DMSO at a concentration of 3 mg/mL and then diluted with the supplemented RPMI-1640 media (1:100). Only DMSO was contained in control suspension (1:100). Equal volumes from each of three solutions (whole blood, LPS, and test sample) were mixed, and the mixture was incubated at 37 °C in a humidified atmosphere of 5% CO$_2$−95% air for 18−24 h. The supernatant of culture prepared by centrifugation was stored at −20 °C until the assay of cytokine. The concentrations of six human cytokines (IL-1β, -2, -4, -8, TNF-α, and IFN-γ) were assayed using ELISA kits. The ratio (%) of inhibition of the cytokine release was calculated by the equation: Inhibition (%) = $100 \times (T/C)$, where T represents the concentration of the cytokine in the culture supernatant with the test compound, and C represents the concentration of the cytokine in the culture supernatant with the solvent.

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