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TETRAHEDRON

# Immunosuppressive terpenoids from extracts of *Tripterygium wilfordii*

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**Abstract**—The clinically used extract ($T_{\Pi}$) of *Tripterygium wilfordii* Hook f. give 19 new compounds, including five kaurane diterpenes (**1**–**5**), one manoyl oxide diterpene (**6**), and one abietane diterpene (**7**), three ursene triterpenes (**8**, **9** and **15**), six oleanane triterpenes (**10**–**13**, **16** and **19**), and three friedelane triterpenes (**14**, **17** and **18**), as well as 15 known compounds (**20**–**34**). Their structures were elucidated by spectroscopy and X-ray analysis. Based on the screening of isolated compounds and other compounds reported in previous papers [J. Nat. Prod. 62 (1999) 1522; J. Nat. Prod. (2001) in press; Phytochemistry 53 (2000) 805], we identified the main components that are responsible for the therapeutic effect of $T_{\Pi}$. 

## 1. Introduction

*Tripterygium wilfordii* Hook f. (Celastraceae) has been used as a traditional medicine in China for several hundred years. Recently, an extract derived from a water/chloroform extract of the roots (the so-called 'total multi-glycoside' or '$T_{\Pi}$ extract') has been used for the clinical treatment of rheumatoid arthritis, other inflammatory and autoimmune diseases, and skin disorders, as well as in male-fertility control.[4–6] However, the precise mechanism of the therapeutic effect of $T_{\Pi}$ has not been completely delineated. To determine which of its components are responsible for such diverse activities, we began to isolate the active principles of the $T_{\Pi}$ extract of *T. wilfordii*.

For the in vitro assay system, we used macrophage-derived mediators, interleukins-1, 2, 4 and 8, tumor necrosis factor (TNF-α), and interferon gamma (IFN-γ), which are considered to play a key role in inflammatory and immune responses based on their presence at inflammatory sites and their ability to induce many of the hallmarks of inflammatory response. Inhibition of these cytokines could be used to evaluate anti-inflammatory effect.[7] In rheumatoid arthritis, it has been reported that there is a strong relationship between the production of IL-1 by the synovium and the degree of inflammation of the arthritic synovial membrane.[8]

In previous papers, we have reported some immunosuppressive diterpenes,[1] sesquiterpenes,[2] and triterpenes[3] from the extract ($T_{\Pi}$) of *Tripterygium wilfordii*. As a continuation of our previous studies in this area, we further examined the components of $T_{\Pi}$ extract and report here the isolation and structure elucidation of 19 new terpenoids (**1**–**19**), as well as the results of a bioassay for all of the components from $T_{\Pi}$ in the screening experiment.

## 2. Result and discussion

Compound **1** had a molecular formula of $C_{22}H_{36}O_3$ based on HREIMS. The IR spectrum revealed a hydroxyl group (3473 $cm^{-1}$). Its $^1H$ NMR spectrum showed one oxygenated methylene [$\delta_H$ 4.44 and 3.33 (each 1H, d, $J$=10.9 Hz)], one acetal methine [$\delta_H$ 4.25 (1H, s)], two tertiary methyls [$\delta_H$ 0.86 and 1.38 (each 3H, s)], and one ethyl group [$\delta_H$ 3.71, 3.37 (each 1H, dt, $J$=14.2, 7.1 Hz), 1.20 (3H, t, $J$=7.1 Hz)]. The $^{13}C$ NMR spectrum of **1** showed three methyls, two oxygenated methylenes, one acetal methine ($\delta_C$ 104.4), and nine methylenes. In addition, three quaternary carbons, one oxygenated quaternary carbon, and three methines were also observed. Compound **1** was assumed to be a kaurane-type diterpene from the same origin,[1] and its $^{13}C$ NMR spectrum was similar to that of tripterifordin[9] except for one methine carbon ($\delta_C$ 104.4) and one ethyl group. In the HMBC spectrum, the proton signal at $\delta_H$ 0.86 ($H_3$-18) correlated with the carbon signals at $\delta_C$ 49.5 (C-5) and 104.4 (C-19), the signal at $\delta_H$ 3.33 (H-20a) correlated with the signals at $\delta_C$ 104.4 (C-19) and 49.5 (C-5), and the signal

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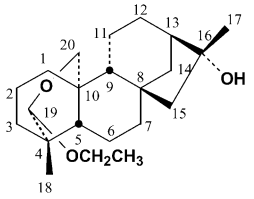
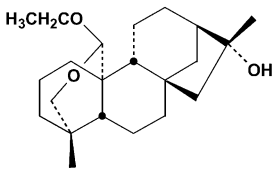


**1** 19-R*

**2** 19-S*



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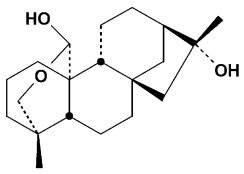

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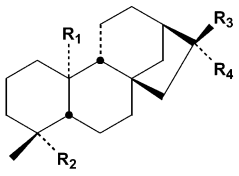
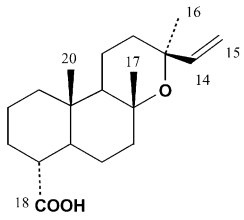


| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **5** | COOH | COOH | Me | OH |
| **20** | Me | COOH | H | COOH |
| **21** | $CH_2OH$ | $CH_2OH$ | Me | OH |



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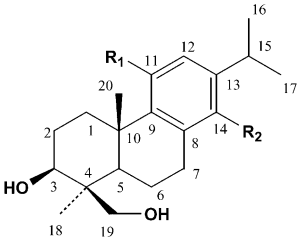
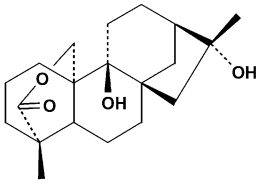


| | $R_1$ | $R_2$ |
|---|---|---|
| **7** | OH | OMe |
| **22** | H | OH |



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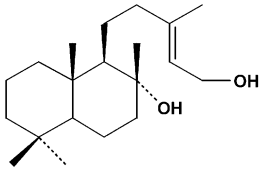

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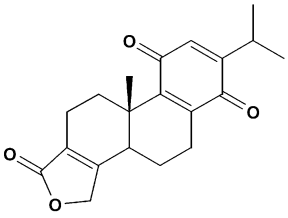

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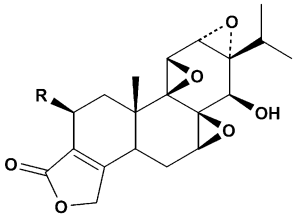
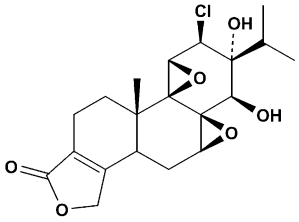
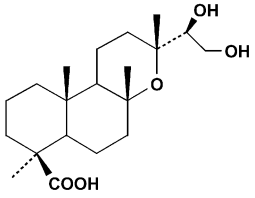


**26** R = H

**27** R = OH



**28**



**29**

**Figure 1.** The structure of compounds **1**–**7** and **20**–**29**.

at $\delta_H$ 4.25 (H-19) correlated with the carbon signal at $\delta_C$ 62.8 (ethyl group). Thus, C-19 was assigned to be an acetal carbon, bonded to an ethoxy group and linked to C-20 by an ether bond (Fig. 1). A colorless crystal was obtained for X-ray crystallographic analysis, which confirmed the determined structure. The ORTEP drawing suggested that the configuration of C-19 was $R^*$ (Fig. 2). Compound **2**, which had a molecular ion peak at *m/z* 348, had the same molecular formula $C_{22}H_{36}O_3$ as **1** based on HREIMS. The $^1H$ and $^{13}C$ NMR spectra were very similar to those of **1**, except for H-19 ($\delta_H$ 4.42, in **2**; 4.25, in **1**). Therefore, compound **2** was thought to have the same framework and an acetal group as **1**. Analysis of the HMBC spectrum of **2** suggested that **2** had the same structure as **1** except for the configuration of C-19. Furthermore, the proton signal at $\delta_H$ 3.87 (–OEt) showed NOESY correlation with the signal at $\delta_H$ 2.05 (H-3a). Therefore, **2** was a diastereomer of **1**, and C-19 was proposed to have an $S^*$ configuration.

Compound **3**, $C_{22}H_{36}O_3$, had one ethoxy group, one oxygenated methylene, one acetal methine, and two tertiary methyl groups based on its $^1H$ NMR spectrum. Its $^{13}C$ NMR spectrum was similar to that of **1**, except for C-1, 19 and 20 (Table 1). In its HMBC spectrum, the proton signal at $\delta_H$

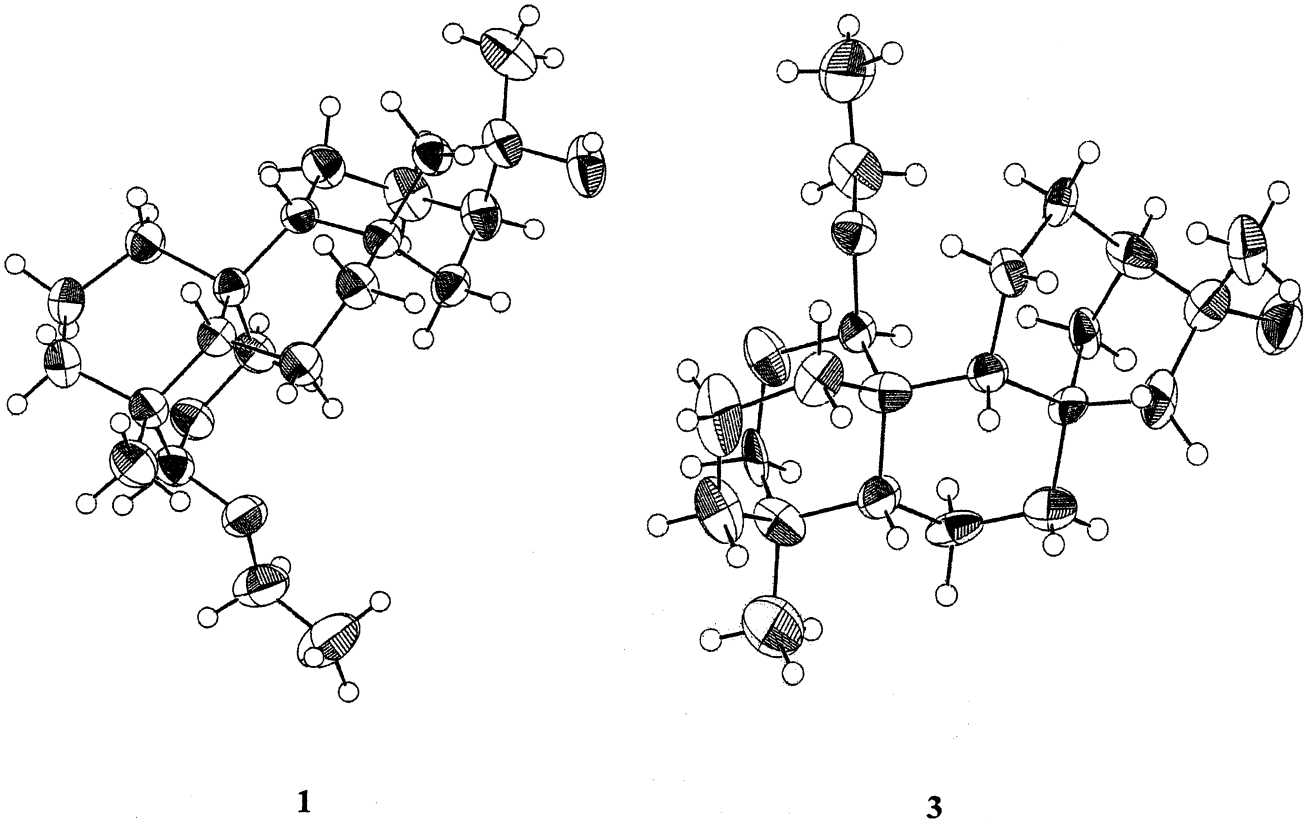



**Figure 2.** ORTEP drawings of compounds **1** and **3**.

0.69 ($H_3$-18) correlated with the carbon signals at $\delta_C$ 71.8 (C-19) and 52.3 (C-5), the signal at $\delta_H$ 3.48 (H-19) correlated with the signals at $\delta_C$ 52.3 (C-5) and 101.8 (C-20), and the proton signal at $\delta_H$ 5.22 (H-20) correlated with the signal at $\delta_C$ 64.1 (ethoxy group). Thus, **3** was assigned to be 16α-hydroxy-20-ethoxy-19,20-epoxykauran. X-Ray analysis confirmed the structure of **3**, and its ORTEP drawing suggested that C-20 had an $R^*$ configuration (Fig. 2). The $^{13}C$ NMR spectrum of **4** closely matched that of **3**, except for the chemical shift value of C-20 and the ethoxy group in **3**. Compound **4** was assumed to be a 20-deethoxy compound of **3**. Moreover, the proton signal of H-20 ($\delta_H$ 5.98) showed a NOESY correlation with that of H-14b ($\delta_H$ 1.93). Based on the skeleton of this kaurane-type compound (obtained

**Table 1.** $^{13}C$ NMR spectral data of compounds **1**–**7** (400 MHz, δ, ppm, $CDCl_3$ as solvents)

| Carbon | 1 | 2 | 3 | 4[a] | 5 | 6[a] | 7 |
|---|---|---|---|---|---|---|---|
| 1 | 40.9 | 40.8 | 35.9 | 35.5 | 40.5 | 37.8 | 35.0 |
| 2 | 22.6 | 22.3 | 22.5 | 23.0 | 22.4 | 20.4 | 28.4 |
| 3 | 40.6 | 35.2 | 41.4 | 41.7 | 39.4 | 30.7 | 80.3 |
| 4 | 36.8 | 36.7 | 33.4 | 33.4 | 44.8 | 44.7 | 43.2 |
| 5 | 49.5 | 52.1 | 52.3 | 52.4 | 57.0 | 49.3 | 53.1 |
| 6 | 22.2 | 19.9 | 20.0 | 20.2 | 23.6 | 23.9 | 18.5 |
| 7 | 40.6 | 40.2 | 40.8 | 41.2 | 37.1 | 42.2 | 27.7 |
| 8 | 45.1 | 44.9 | 45.0 | 45.3 | 46.2 | 75.5 | 131.0 |
| 9 | 52.7 | 52.0 | 54.5 | 54.9 | 55.6 | 56.5 | 132.8 |
| 10 | 39.2 | 39.5 | 42.0 | 42.5 | 49.7 | 35.9 | 38.7 |
| 11 | 18.2 | 18.0 | 18.7 | 19.3 | 19.4 | 16.0 | 151.2 |
| 12 | 26.9 | 26.7 | 25.7 | 26.3 | 26.2 | 34.7 | 111.5 |
| 13 | 48.5 | 48.4 | 49.2 | 49.5 | 49.4 | 73.1 | 138.9 |
| 14 | 38.5 | 38.6 | 38.8 | 38.9 | 42.8 | 148.0 | 148.3 |
| 15 | 58.0 | 57.7 | 58.2 | 59.0 | 58.7 | 109.5 | 26.0 |
| 16 | 79.5 | 79.6 | 79.5 | 78.2 | 78.2 | 32.6 | 23.8 |
| 17 | 24.5 | 24.6 | 24.4 | 24.9 | 25.3 | 24.0 | 23.8 |
| 18 | 23.4 | 23.3 | 23.3 | 23.4 | 30.1 | 178.3 | 22.8 |
| 19 | 104.4 | 104.9 | 71.8 | 71.9 | 179.3 | – | 64.3 |
| 20 | 62.8 | 68.6 | 101.8 | 96.2 | 178.6 | 12.9 | 20.3 |
| OEt | 62.8, 15.4 | 65.1, 15.4 | 64.1, 15.2 | – | – | – | 60.7 (OMe) |

[a] **4** and **6**: $C_5D_5N$.

**Table 2.** $^{13}C$ NMR spectral data of compounds **8**–**19** ($CDCl_3$)

| C | 8 | 9 | 10 | 11 | 12 | 13[a] | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 39.5 | 39.5 | 46.6 | 39.3 | 38.5 | 33.6 | 31.7 | 39.4 | 128.4 | 109.7 | 109.6 | 41.5 |
| 2 | 34.2 | 34.2 | 69.0 | 34.2 | 26.7 | 26.2 | 74.4 | 34.2 | 143.9 | 142.7 | 142.7 | 175.2 |
| 3 | 217.9 | 217.8 | 84.0 | 217.9 | 78.7 | 75.2 | 209.7 | 217.7 | 201.2 | 140.5 | 141.0 | 180.3 |
| 4 | 47.5 | 47.5 | 39.3 | 47.5 | 38.6 | 37.8 | 52.4 | 47.5 | 44.0 | 121.6 | 120.1 | 46.0 |
| 5 | 55.3 | 55.3 | 55.4 | 55.3 | 55.1 | 49.1 | 54.2 | 55.4 | 53.9 | 125.3 | 125.2 | 48.9 |
| 6 | 19.7 | 19.7 | 18.4 | 19.7 | 18.2 | 18.6 | 36.7 | 19.7 | 18.8 | 44.7 | 43.9 | 20.7 |
| 7 | 32.3 | 32.4 | 33.1 | 32.1 | 32.5 | 32.9 | 19.4 | 32.2 | 32.7 | 119.8 | 116.6 | 32.1 |
| 8 | 40.2 | 40.1 | 39.4 | 39.8 | 39.7 | 40.3 | 49.6 | 39.9 | 40.5 | 150.3 | 152.6 | 39.3 |
| 9 | 46.9 | 46.9 | 47.6 | 46.8 | 47.5 | 47.7 | 37.5 | 46.9 | 43.1 | 37.8 | 37.7 | 39.4 |
| 10 | 36.7 | 36.7 | 38.3 | 36.7 | 36.8 | 37.3 | 55.7 | 36.7 | 38.4 | 142.2 | 142.4 | 41.6 |
| 11 | 23.7 | 23.7 | 23.6 | 23.7 | 23.4 | 23.8 | 34.5 | 23.8 | 23.6 | 36.8 | 36.9 | 23.9 |
| 12 | 125.5 | 125.8 | 124.5 | 123.3 | 123.1 | 123.2 | 29.4 | 123.6 | 123.0 | 30.5 | 30.6 | 124.6 |
| 13 | 138.0 | 137.9 | 140.5 | 143.2 | 143.2 | 144.4 | 40.2 | 142.5 | 143.4 | 38.0 | 38.2 | 140.1 |
| 14 | 42.6 | 42.2 | 42.6 | 41.9 | 41.5 | 42.0 | 39.4 | 41.9 | 41.9 | 44.1 | 44.3 | 43.3 |
| 15 | 26.3 | 25.9 | 24.4 | 25.4 | 25.2 | 25.9 | 28.3 | 25.6 | 25.7 | 29.1 | 29.3 | 24.4 |
| 16 | 27.5 | 23.3 | 25.3 | 22.2 | 21.8 | 23.0 | 29.6 | 23.6 | 28.1 | 36.8 | 36.8 | 25.3 |
| 17 | 38.0 | 37.9 | 35.3 | 36.9 | 36.7 | 37.6 | 44.8 | 39.6 | 37.4 | 30.5 | 30.7 | 35.4 |
| 18 | 52.8 | 53.0 | 43.5 | 41.1 | 41.1 | 41.7 | 45.5 | 48.0 | 43.9 | 44.4 | 44.5 | 43.6 |
| 19 | 34.0 | 34.1 | 33.9 | 39.9 | 40.1 | 41.4 | 31.6 | 41.4 | 39.9 | 30.5 | 30.5 | 39.8 |
| 20 | 45.1 | 51.8 | 39.6 | 42.4 | 42.1 | 42.8 | 41.4 | 151.6 | 42.5 | 40.1 | 40.2 | 39.6 |
| 21 | 32.4 | 25.1 | 39.9 | 28.5 | 28.4 | 29.5 | 214.2 | 69.1 | 36.2 | 29.8 | 29.8 | 33.9 |
| 22 | 74.8 | 34.3 | 83.2 | 29.8 | 29.7 | 30.8 | 77.5 | 45.9 | 75.8 | 34.8 | 34.9 | 83.2 |
| 23 | 26.7 | 26.7 | 28.7 | 21.6 | 27.9 | 29.2 | 7.9 | 26.6 | 27.3 | 12.7 | 12.0 | 28.5 |
| 24 | 21.6 | 21.6 | 16.9 | 26.6 | 15.4 | 22.7 | 174.2 | 21.6 | 21.9 | – | – | 23.4 |
| 25 | 15.5 | 15.5 | 17.0 | 15.3 | 15.3 | 15.7 | 16.8 | 15.3 | 20.0 | 35.5 | 35.8 | 19.2 |
| 26 | 16.8 | 16.8 | 17.1 | 16.7 | 16.5 | 17.0 | 15.5 | 16.8 | 17.5 | 22.1 | 22.2 | 17.2 |
| 27 | 23.8 | 23.5 | 24.1 | 25.4 | 25.7 | 26.0 | 19.4 | 25.8 | 25.4 | 18.3 | 18.3 | 23.7 |
| 28 | 21.7 | 69.3 | 25.0 | 69.0 | 68.6 | 68.3 | 25.4 | 68.8 | 19.9 | 31.5 | 31.5 | 25.2 |
| 29 | 18.5 | 18.0 | 182.5 | 184.5 | 181.7 | 181.5 | – | – | 184.0 | 181.6 | 181.6 | 182.4 |
| 30 | 182.1 | 181.6 | 21.1 | 19.2 | 19.1 | 20.1 | 15.0 | 103.6 | 23.8 | 32.8 | 32.9 | 20.7 |
| OMe | – | – | – | – | – | – | 51.7 | – | – | – | – | 52.2 |

[a] **13**: $C_5D_5N$.

from X-ray analysis of **3**), C-20 was proposed to have an $R^*$ configuration. Thus, **4** was determined to be 16α-hydroxy-19,20-epoxy-20$R^*$-hydroxy-kaurane (Fig. 1).

The positive HRFABMS of compound **5** gave a quasi-molecular ion peak at *m/z* 373.1934, which suggested the molecular formula $C_{20}H_{30}O_5Na$. Its $^{13}C$ NMR spectrum was similar to those of **1** and **2**, except for C-18–C-20 (Table 1). It was also thought to be a kaurane-type diterpene, with two carboxylic groups and one hydroxyl group. In the HMBC spectrum, the proton signals at $\delta_H$ 1.51 ($H_3$-18) and 1.33 (H-5) correlated with the carboxylic carbon signal at $\delta_C$ 179.3 (C-19), and the signal at $\delta_H$ 1.45 (H-9) correlated with the signal at $\delta_C$ 178.6 (C-20). Furthermore, the proton signal at $\delta_H$ 1.51 ($H_3$-18) showed a NOESY correlation with that at $\delta_H$ 1.33 (H-5). Thus, two carboxylic groups were located at C-19 and C-20. Methylation of **5** [$(CH_3)_3SiCHN_2$] afforded dimethylate **5a**. Therefore, compound **5** was elucidated to be 16α-hydroxykauran-19,20-dioic acid.

Compound **6** had a molecular formula of $C_{19}H_{30}O_3$ based on HREIMS. Its $^1H$ NMR spectrum showed one vinyl group [$\delta_H$ 6.10 (1H, dd, $J$=17.9, 11.0 Hz), 5.03 (1H, d, $J$=17.9 Hz), and 4.95 (1H, d, $J$=11.0 Hz)], and three tertiary methyl groups [$\delta_H$ 1.27, 1.21, and 0.65 (each 3H, s)]. The $^{13}C$ NMR spectrum of **6** showed one carboxylic group, one vinyl group, two oxygenated quaternary carbons, three methyls, and seven methylene groups. These observations suggested a manoyl oxide acid-related structure.[10] In the HMBC spectrum, the methyl signal at $\delta_H$ 1.21 ($H_3$-16) correlated with the carbon signals at $\delta_C$ 73.1 (C-13) and 148.0 (C-14), and the methine proton signal at $\delta_H$ 2.47 (H-4) correlated with the carboxylic carbon signal at $\delta_C$ 178.3. Moreover, in the NOESY spectrum, the proton signal at $\delta_H$ 0.65 ($H_3$-20) correlated with that at $\delta_H$ 2.47 (H-4), and the signal at $\delta_H$ 6.10 (H-14) correlated with that at $\delta_H$ 1.27 ($H_3$-17). Thus, **6** was determined to be 13-*epi*-19-normanoyloxide-18-oic acid.

Compound **7**, $C_{21}H_{32}O_4$, showed aromatic ring absorptions at 225 and 276 nm in its UV spectrum. The $^1H$ NMR spectrum of **7** revealed an isopropyl group [$\delta_H$ 3.18 (1H, sept., $J$=6.9 Hz), 1.14 (6H, d, $J$=6.9 Hz)], one oxygenated methylene [$\delta_H$ 4.28, 3.34 (each 1H, d, $J$=11.1 Hz)], and one aromatic methine [$\delta_H$ 6.37 (1H, s)]. In addition, two methyl groups [1.27, 123 (each 3H, s)] and one methoxy group ($\delta_H$ 3.62) were also observed. In addition to one methoxy group, its $^{13}C$ NMR spectrum showed 20 carbon signals, including a benzene ring, four methyls, an oxygenated methine ($\delta_C$ 80.3), and an oxygenated methylene ($\delta_C$ 64.3). Based on this information, **7** was assumed to be an abietane-type diterpene, the same as triptobenzenes A–K isolated from *T. wilfordii* var. *regelii*[11] and *T. hypoglaucum*.[12] In the HMBC spectrum of **7**, the proton signal at $\delta_H$ 3.34 (H-19a) correlated with the carbon signals at $\delta_C$ 80.3 (C-3) and 53.1 (C-5), and the proton signal at $\delta_H$ 1.27 ($H_3$-18) correlated with the signal at $\delta_C$ 80.3 (C-3) and with the hydroxyl methylene carbon signal (64.3). Thus, the hydroxyl and hydroxyl methylene groups were assigned to be located at C-3 and C-4, respectively. In the NOESY spectrum, the methoxy group at $\delta_H$ 3.62 correlated with the

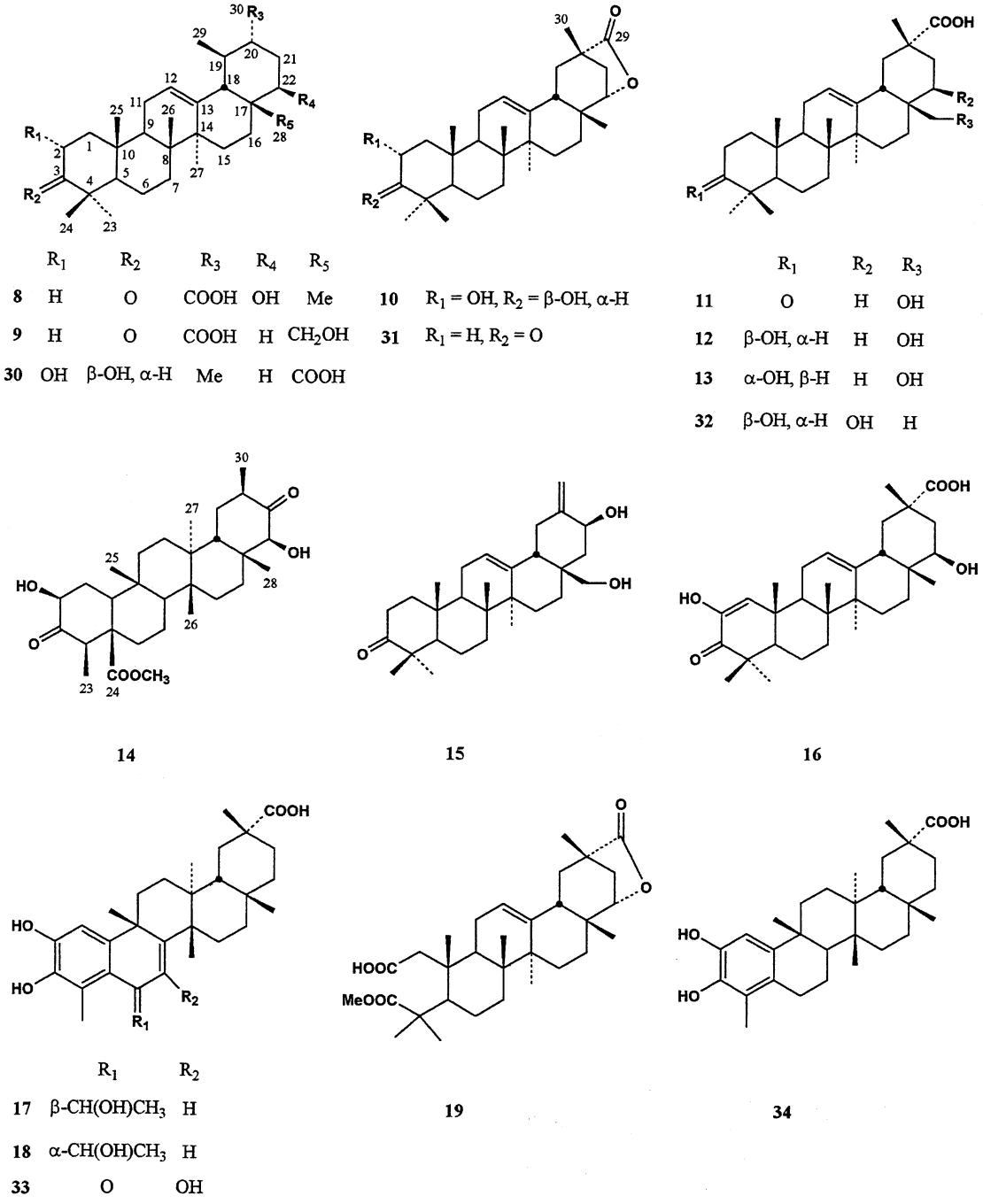



**Figure 3.** The structure of compounds **8**–**19** and **30**–**34**.

signals at $\delta_H$ 2.97 (H-7a), 2.57 (H-7b) and 3.18 (H-15), the signal at $\delta_H$ 6.37 (H-12) correlated with the signal at $\delta_H$ 1.14 ($H_3$-16 or 17), and the hydroxyl methylene proton signal at 4.28 (H-19) correlated with the signal at 1.23 ($H_3$-20). These observations clearly indicated that the hydroxyl and methoxy groups could be assigned to C-11 and C-14, respectively, and the configuration of the hydroxyl methylene was assigned to be 4β (Fig. 1).

Compound **8** was assigned the molecular formula $C_{30}H_{46}O_4$ based on HREIMS. The IR spectrum revealed hydroxyl and carbonyl absorption bands (3427 and 1702 $cm^{-1}$). Its $^1H$ NMR spectrum showed an olefinic proton [$\delta_H$ 5.22 (1H, br s)], an oxygenated methine [3.51 (1H, br s)], six tertiary methyls [$\delta_H$ 1.13, 1.10, 1.08, 1.07, 1.06, and 0.93 (each 3H, s)], and one secondary methyl group [$\delta_H$ 0.86 (3H, d, $J$=6.2 Hz)]. The $^{13}C$ NMR spectrum revealed 30 carbons, including a ketone ($\delta_C$ 217.9), a carboxylic carbon ($\delta_C$ 182.1), one double bond, one oxygenated methine, and seven methyl groups (Table 2). Compound **8** was an ursene-type triterpene, and showed $^{13}C$ NMR data similar to those of 2α-hydroxy-ursolic acid (**30**).[13] In the HMBC spectrum, the methyl proton signals at $\delta_H$ 1.10 ($H_3$-23) and 1.06 ($H_3$-24) correlated with the ketone carbon signal at $\delta_C$ 217.9 (C-3), the signal at $\delta_H$ 0.93 ($H_3$-28) correlated with the signals at $\delta_C$ 74.8 (C-22) and 52.8 (C-18), the signal at $\delta_H$

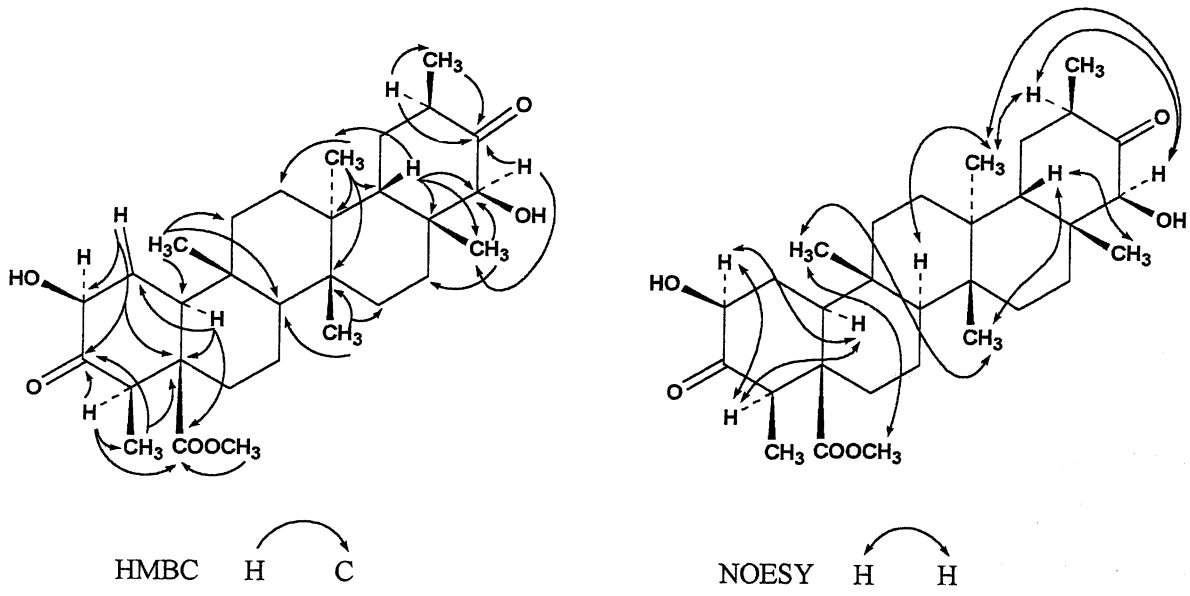



**Figure 4.** The HMBC and NOESY spectral data of **14**.

0.86 ($H_3$-29) correlated with the signals at $\delta_C$ 52.8 (C-18) and 45.1 (C-20), and the signal at $\delta_H$ 2.47 (H-20) correlated with the signal at $\delta_C$ 182.1 (C-30). Furthermore, the coupling constant of H-22 (1H, br s) indicated that H-22 had an equatorial configuration. Therefore, **8** was elucidated to be 22β-hydroxy-3-oxo-12-ursen-30-oic acid. The $^{13}C$ NMR spectrum of **9** was very similar to that of **8**, except for one oxygenated methylene in **9** and one oxygenated methine in **8** (Table 2). Compound **9** was proposed to be a 22-dehydroxy-28-hydroxy of **8**. Further 2D NMR spectral analysis confirmed that **9** was 28-hydroxy-3-oxo-12-ursen-30-oic acid (Fig. 3).

Compound **10**, $C_{30}H_{46}O_4$, had an olefinic proton signal [$\delta_H$ 5.31 (br s)], three hydroxyl methine proton signals at $\delta_H$ 4.15 (1H, d, $J$=5.4 Hz), 3.70 (1H, m) and 3.01 (1H, d, $J$=9.5 Hz), and seven tertiary methyl signals at $\delta_H$ 1.21, 1.08, 1.04, 1.02, 0.93, 0.87 and 0.83, based on its $^1H$ NMR spectrum. Its $^{13}C$ NMR spectrum showed 30 carbons, including seven tertiary methyls, one double bond and one carbonyl carbon. Compound **10** was assumed to be a 12-ene oleanane-type triterpene. In its HMBC spectrum, the proton signals at $\delta_H$ 1.04 ($H_3$-23) and 0.83 ($H_3$-24) correlated with the signals at $\delta_C$ 84.0 (C-3) and 55.4 (C-5), the proton signal at $\delta_H$ 3.01 (H-3) correlated with the signals at $\delta_C$ 16.9 (C-24), 39.3 (C-4) and 69.0 (C-2), and the proton signal at $\delta_H$ 2.00 (H-1a) correlated with the signals at $\delta_C$ 69.0 (C-2), 84.0 (C-3) and 55.4 (C-5). Thus, the two hydroxyl groups were assigned to C-2 and C-3. Furthermore, the proton signal at $\delta_H$ 4.15 (H-22) correlated with the signals at $\delta_C$ 43.5 (C-18), 39.6 (C-20) and 182.5 (C-29), the methyl proton signal at $\delta_H$ 1.21 ($H_3$-30) correlated with the signal at $\delta_C$ 182.5 (C-29), and the signal at $\delta_H$ 0.87 ($H_3$-28) correlated with the signals at $\delta_C$ 43.5 (C-18) and 83.2 (C-22). Based on these findings, a C-22, 29 or 30-lactone ring was deduced, and this was further supported by accounting for the degrees of unsaturation suggested by its molecular formula. Moreover, in the NOESY spectrum, the proton signal at $\delta_H$ 0.87 (H-28) correlated with those at $\delta_H$ 4.15 (H-22) and 2.14 (H-18), the signal at $\delta_H$ 3.01 (H-3) correlated with those at $\delta_H$ 1.04 ($H_3$-23) and 0.83 (H-5), and the proton signal at $\delta_H$ 3.70 (H-2) correlated with that at $\delta_H$ 1.02 ($H_3$-25). Therefore, **10** was determined to be 2α,3β-dihydroxy-olean-12-ene-22,29-lactone (Fig. 3).

Compounds **11**, **12** and **13** were also 12-ene oleane-type triterpenes based on comparison of their $^{13}C$ NMR spectra with that of **10** (Table 2). They differed with regard to the substituted functions at C-3 (Fig. 2). The HMBC correlation between C-3 ($\delta_C$ 217.9) and $H_3$-23 indicated that **11** was 28-hydroxy-3-oxo-olean-12-en-29-oic acid. In the same way, the coupling constant of H-3 [3.14 (1H, br d, $J$=10.5 Hz, in **12**); 3.58 (1H, m, in **13**)] and analysis of the NOESY spectrum suggested that **12** and **13** were 3β,28-dihydroxy-olean-12-en-29-oic acid and 3α,28-dihydroxy-olean-12-en-29-oic acid, respectively (Fig. 2).

Compound **14** had a molecular formula of $C_{30}H_{46}O_6$, and had hydroxyl, carboxylic and ketone groups based on its IR spectrum (3446, 1719 and 1640 $cm^{-1}$). Its $^1H$ NMR spectrum showed two oxygenated methine signals [$\delta_H$ 4.58 (1H, d, $J$=3.9 Hz), 4.14 (1H, br t, $J$=9.0 Hz)], two secondary methyl groups [$\delta_H$ 1.07 (3H, d, $J$=6.3 Hz), 0.97 (3H, d, $J$=6.7 Hz)], four methyl groups [$\delta_H$ 1.36, 0.93, 0.84, and 0.79 (3H, s)], and one methoxy group [$\delta_H$ 3.62 (3H, s)]. The $^{13}C$ NMR spectrum showed two ketone carbons, one carboxylic carbon, two oxygenated methines, one methoxy, and six methyl carbon signals (Table 2). It was deduced to be a friedelane-type triterpene, like polpunoic acid, which was also isolated from the same plant.[14] Further HMBC and NOESY spectral analysis (Fig. 4) confirmed that compound **14** was 2β,22β-dihydroxy-3,21-dioxo-24-carboxyl-29-norfriedelan methyl ester (Fig. 3).

The positive HRFABMS of compound **15** gave a molecular ion peak at *m/z* 463.3143, which suggested a molecular formula of $C_{29}H_{44}O_3Na$. Its $^{13}C$ NMR spectrum was similar to that of **11**, except for the E-ring (Table 2). In the HMBC spectrum, the proton signal at $\delta_H$ 3.53 (H-28a) correlated with the signals at $\delta_C$ 48.0 (C-18) and 45.9 (C-22), the signal

**Table 3.** Inhibitiory effects of the isolated compounds on cytokine production

| Compound | Inhibition (%) | | | | | |
|---|---|---|---|---|---|---|
| | IL-1β | IL-8 | TNF-α | IL-2 | IL-4 | IFN-γ |
| **1** | 2 | 54 | −7 | 76 | 25 | 26 |
| **3** | 16 | −337 | −6 | 85 | 51 | 39 |
| **8** | −69 | −84 | −7 | 70 | 36 | 20 |
| **10** | −10 | 40 | −1 | 100 | 56 | 68 |
| **11** | −4 | 46 | 9 | 53 | 34 | 1 |
| **13** | −25 | −28 | 24 | 63 | 38 | 10 |
| **18** | 92 | 83 | 100 | 100 | 100 | 100 |
| **20** | 9 | −76 | −15 | 79 | 32 | 16 |
| **22** | −8 | 71 | −2 | 100 | 47 | 66 |
| **26** | 100 | 100 | 100 | 100 | 100 | 100 |
| **27** | 100 | 100 | 100 | 100 | 100 | 100 |
| **28** | 100 | 100 | 100 | 100 | 100 | 100 |
| **34** | 90 | −204 | 45 | 82 | 29 | 71 |
| Prednisolone | 68 | 15 | 52 | 65 | 76 | 75 |

Concentration: isolated compounds, 10 μg/ml; prednisolone, 0.3 μg/ml.

at $\delta_H$ 2.04 (H-22) correlated with the signals at $\delta_C$ 48.0 (C-18) and 151.6 (C-20), the signal at $\delta_H$ 2.21 (H-19a) correlated with the signals at $\delta_C$ 48.0 (C-18), 151.6 (C-20) and 69.1 (C-21), and the signal at $\delta_H$ 4.77 (H-30a) correlated with the signal at $\delta_C$ 69.1 (C-21). Moreover, the coupling constant of H-21 (dd, $J$=11.7, 4.9 Hz) indicated that it had an axial configuration. Thus, **15** was determined to be 21β,28-dihydroxy-3-oxo-olean-12,20(30)-diene.

Compound **16** had a molecular formula of $C_{30}H_{44}O_5$ based on HREIMS. The $^1$H NMR spectrum showed two olefenic protons [$\delta_H$ 6.36 (1H, s) and 5.37 (1H, br s)], an oxygenated methine [$\delta_H$ 3.58 (1H, dd, $J$=7.0, 3.1 Hz)], and seven tertiary methyl groups. Its $^{13}$C NMR spectrum was similar to that of triptotriterpenic acid B (**32**),[15] except for the A-ring. In the HMBC spectrum, the proton signal at $\delta_H$ 6.36 (H-1) correlated with the carbon signals at $\delta_C$ 143.9 (C-2), 201.2 (C-3) and 53.9 (C-5), the signals at $\delta_H$ 1.42 ($H_3$-24) and 1.24 ($H_3$-23) correlated with the signal at $\delta_C$ 201.2 (C-3), and the methyl proton signal at $\delta_H$ 1.27 ($H_3$-25) correlated with the signal at $\delta_C$ 128.4 (C-1). Thus, **16** was determined to be 2,22β-dihydroxy-3-oxo-olean-1,12-dien-29-oic acid (Fig. 3).

Compound **17** showed $[M+Na]^+$ (HRFABMS) at $m/z$ 519.3062, which was consistent with a molecular formula of $C_{31}H_{44}O_5Na$. Its UV spectrum revealed the presence of an aromatic ring (280 and 223 nm). The $^1$H NMR spectrum of **17** revealed two methines [$\delta_H$ 6.70 (1H, s) and 5.72 (1H, d, $J$=6.3 Hz)] attached to the double bond, two methine protons [$\delta_H$ 3.58 (1H, q, $J$=6.4 Hz), 3.47 (1H, t, $J$=6.8 Hz)], one secondary methyl [$\delta_H$ 1.11 (3H, d, $J$=6.8 Hz)], and six tertiary methyls ($\delta_H$ 2.19, 1.46, 1.16, 1.13, 1.02, and 0.62). The $^{13}$C NMR spectrum was similar to that of wilforic acid (**34**),[14] except for the B-ring and the $CH_3CH(OH)$– unit. In the HMBC spectrum, the proton signal at $\delta_H$ 6.70 (H-1) correlated with the signals at $\delta_C$ 140.5 (C-3), 125.3 (C-5) and 37.8 (C-9), the signal at $\delta_H$ 5.72 (H-7) correlated with the signals at $\delta_H$ 125.3 (C-5), 37.8 (C-9) and 150.3 (C-8), the signal at $\delta_H$ 3.47 (H-6) correlated with the signals at $\delta_C$ 150.3 (C-8), 121.6 (C-4), 142.2 (C-10), and 73.3 (C-31), and the signal at $\delta_H$ 1.11 ($H_3$-32) correlated with the signals at 44.7 (C-6) and 73.3 (C-31). Furthermore, the proton signal at $\delta_H$ 3.58 (H-31) showed NOESY correlation with $H_3$-25 ($\delta_H$ 1.46). Therefore, **17** was determined to be 2,3-dihydroxy-1,3,5(10),7-tetraene-6β(1′-hydroxyethyl)-24-nor-D:A-friedooleane-29-oic acid (Fig. 2). The $^{13}$C NMR spectrum of **18** closely matched that of **17** (Table 2); the only difference was in the chemical shift values at H-6, H-31 and $H_3$-32 in the $^1$H NMR spectrum [**18**: $\delta_H$ 3.55 (dd, $J$=5.6, 2.6 Hz, H-6), 4.00 (m, H-31), 1.30 (d, $J$=6.4 Hz, $H_3$-32); **17**: $\delta_H$ 3.47 (t, $J$=6.8 Hz, H-6), 3.58 (q, $J$=6.4 Hz, H-31), 1.11 (d, $J$=6.4 Hz, $H_3$-32)]. Thus, **18** was proposed to be a 6-epimer of **17** (Fig. 3).

Compound **19** was assigned the molecular formula $C_{31}H_{46}O_6Na$ based on HRFABMS. Its $^{13}$C NMR spectrum was similar to that of **10**, except for the A-ring. Thus, it was also a 22,29-lactone-oleane-12-ene-type triterpene. In the HMBC spectrum, the methyl proton signal at $\delta_H$ 1.04 ($H_3$-25) correlated with the signals at $\delta_C$ 41.5 (C-1) and 48.9 (C-5), the signals at $\delta_H$ 1.28 ($H_3$-23) and 1.24 ($H_3$-24) correlated with the signals at $\delta_C$ 180.3 (C-3) and 48.9 (C-5), the methoxy proton signal at $\delta_H$ 3.66 (–OMe) correlated with the carboxyl carbon signal at $\delta_C$ 180.3 (C-3), and the signals at $\delta_H$ 2.44 and 2.28 ($H_2$-1) correlated with

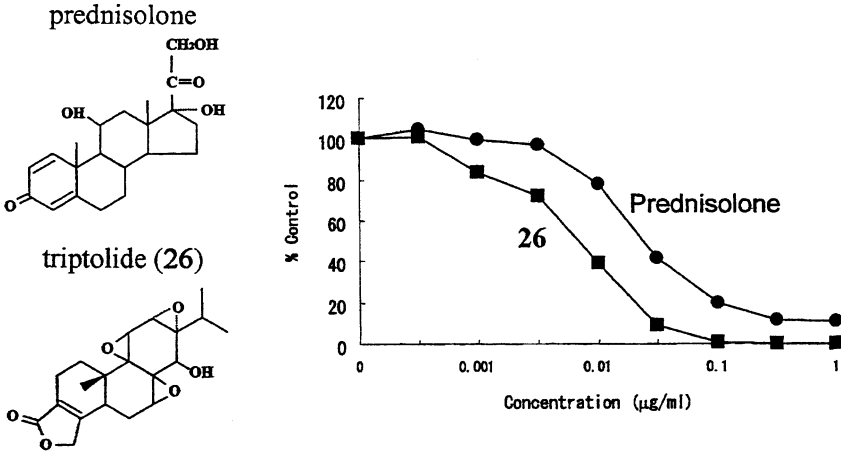



**Figure 5.** The inhibitory effects of comparison on IL-1 release.

the signal at $\delta_C$ 175.2 (C-2). The degrees of unsaturation of **19** indicated that the A-ring was disunited between C-2 and C-3. Thus, **19** was elucidated to be 2,3-*seco*-22,29-lactone-oleane-12-ene-2,3-dioic acid 3-methyl ester (Fig. 3).

Known compounds were identified based on comparison of their spectral data with values reported in the literature: 16a-(−)-kauran-17,19-dioic acid (**20**),[16] kaurane-16,19,20-triol (**21**),[17] triptobenzene J (**22**),[12] tripterinin (**23**),[18] labd-13(*E*)-ene-8α,15-diol (**24**),[19] quinone 21 (**25**),[20] triptolide (**26**),[21] triptodiolide (**27**),[21] triptochlorolide (**28**),[22] 14,15-dihydroxy-8,13-epoxy-labd-14-en-19-oate (**29**),[23] 2α-hydroxyursolic acid (**30**),[13] wilforlide A (**31**),[24] triptotriterpenic acid B (**32**),[15] regeol C (**33**),[25] and wilforic acid A (**34**).[14]

In screening the isolated compounds (**1**–**34**) from the $T_\Pi$ extract of *T. wilfordii* for immunosuppressive activity, we examined their inhibitory effects on cytokine production: the bioactivity data for compounds with an inhibitory effect are shown in Table 3 (other compounds were inactive in this test system). Compounds **18** and **26**–**28** had a significant inhibitory effect on cytokine production from lipopolysaccharide-stimulated human peripheral mononuclear cells compared with the reference compound (prednisolone).[26]

The chemical constituents of $T_\Pi$ extract of *T. wilfordii* have been studied and reported by other groups.[15,27–30] However, no previous detailed reports have associated the chemical constituents and immunosuppressive activity. In our previous papers, we have reported 36 sesquiterpenes,[3,31–33] 17 diterpenes,[1] and 21 triterpenes[2] from $T_\Pi$, along with their immunosuppressive activities. In this paper, we report the further chemical study of $T_\Pi$, and an analysis of isolated immunosuppressive active components. In a secondary screening experiment, we discovered a potent immunosuppressive diterpene (triptolide, **26**), its inhibitory effects on IL-1 production in comparison with those of prednisolone are shown in Fig. 5. In summary, we believe that we have identified the main active principle of $T_\Pi$: compound **26** completely inhibited IL-1 release at 0.1 μg/ml, and showed more potent activity than prednisolone (compound **26** comprised 0.65 μg/mg in $T_\Pi$).

## 3. Experimental

### 3.1. General experimental procedures

NMR spectra (400 MHz for $^1$H NMR, 100 MHz for $^{13}$C NMR, both with TMS as internal standard) were measured on a Bruker ARX-400 instrument, and MS spectra were measured on a JEOL JMSD-300 instrument. CC: Silica gel 60 (Merck) and Sephadex LH-20 (Pharmacia); HPLC: GPC (Gel-Permeation Chromatography: Shodex H-2001, 2002, $CHCl_3$; Asahipak, GS-310 2G, MeOH), silica gel HPLC ($Si_1$: YMC-Park SIL-06 SH-043-5-06, 250×20 mm, hexane–EtOAc system; $Si_2$: Hibar RT 250-25, LiChrosorb Si 60, $CHCl_3$–MeOH system). ODS (Hibar RT 250-25, LiChrosorb RP 18). IR spectra were recorded on a 1720 Infrared Fourier Transform spectrometer (Perkin–Elmer), and UV spectra were measured on a UV 2100 UV–VIS recording spectrometer (Shimadzu). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

### 3.2. Plant material

The powdered extract of *Tripterygium wilfordii* ($T_\Pi$) 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 and then chloroform, and by column chromatographic separation. Samples of $T_\Pi$ and the original plant (*T. wilfordii*, TW940930) were deposited at the Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

### 3.3. Extraction and isolation of compounds

The extracts ($T_\Pi$, 54 g) of *T. wilfordii* were chromatographed on a silica gel column (1.0 kg, 11×90 cm) and eluted with solvents of increasing polarity [$CHCl_3$–MeOH (99:1, 95:5, 9:1, MeOH)] to give 10 fractions (fr. 1–10). Fraction 2 (1.5 g) was chromatographed over a medium-pressure liquid chromatography (MPLC) column and eluted with hexane–EtOAc (3:1, 1:1) to give eight fractions (fr. 2.1–2.8). Fraction 2.5 was separated by GPC ($CHCl_3$) and HPLC ($Si_1$, hexane–EtOAc, 2:1) to give **1** (15 mg), **2** (11 mg), **3** (3.5 mg) and 25 (3 mg). Fraction 2.6 was separated by GPC ($CHCl_3$) and HPLC ($Si_1$) to give **4** (24 mg) and **21** (9 mg). Fraction 5 (16 g) was chromatographed on MPLC to give 12 fractions (fr. 5.1–5.12). Combined fractions 5.4+5.5 were chromatographed over LH-20 (MeOH) to give four fractions (fr. 5.4.1–5.4.4). Fraction 5.4.3 was separated by GPC (MeOH) and HPLC ($Si_1$) to give **19** (5 mg), **20** (8 mg), **22** (25 mg) and **23** (4 mg). Fraction 6 (11 g) was chromatographed over MPLC and eluted with hexane–EtOAc (1:1, 1:2, 1:4) to give 11 fractions (fr. 6.1–6.11). Fraction 6.5 (2.5 g) was chromatographed on LH-20 (MeOH) to afford **34** (570 mg) and four other fractions (fr. 6.5.1–6.5.4). Fraction 6.5.1 was separated by GPC ($CHCl_3$) and HPLC ($Si_1$) to give **24** (9 mg), **26** (29 mg) and another major fraction, which was further separated by preparative TLC (PTLC) to give **6** (5 mg). Fraction 6.7 was chromatographed over LH-20 (MeOH) to give four fractions (fr. 6.7.1–6.7.4). Fraction 6.7.2 was separated by GPC (MeOH) and HPLC ($Si_2$) to give **10** (11 mg), **11** (57 mg) and **14** (3 mg). Combined fractions 6.7.3 and 6.7.4 were separated by GPC (MeOH) and HPLC ($Si_2$) to give **29** (6 mg), **31** (9 mg) and **32** (49 mg). Fraction 6.9 was separated by LH-20 (MeOH) and HPLC ($Si_2$) to give **7** (2 mg), **27** (3 mg) and **28** (5 mg).

Combined fractions 7+8 (16.5 g) were chromatographed over MPLC (hexane–EtOAc system) to give 13 fractions (fr. 7.1–7.13). Combined fractions 7.3+7.4 were chromatographed over LH-20 to give four fractions (fr. 7.3.1–7.3.4). Fraction 7.3.3 was separated by GPC (MeOH) and HPLC ($Si_1$) to give **17** (6 mg), **18** (15 mg) and **33** (3 mg). Fraction 7.7 was chromatographed on GPC (MeOH) to give six fractions (fr. 7.7.1–7.7.6). Fraction 7.7.1 was separated by HPLC ($Si_2$, $CHCl_3$–MeOH, 94:6) to give **5** (7 mg) and **12** (8 mg). Fraction 7.7.2 was separated by ODS (MeOH–$H_2O$, 85:15) to give **9** (17 mg), **13** (17 mg) and **15** (4 mg). Fraction 7.11 was separated by GPC (MeOH) and ODS (MeOH–$H_2O$, 85:15) to give **8** (20 mg) and **30** (9 mg).

**3.3.1. 16α-Hydroxy-19,20-epoxy-19*R**-ethoxy-kaurane (1).** Obtained as colorless needles, mp 163.0–163.5°C,

$[\alpha]_D^{25}=-158°$ (*c* 0.8; $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3473, 2927, 2362, 1633, 1460, 1371, 1155, 1117, 1047, 1030, 970, 904; HREIMS: *m/z* 348.2620 (calcd for $C_{22}H_{36}O_3$, 348.2664); EIMS: *m/z* 348 $[M]^+$ (14), 303 $[M-OEt]^+$ (57), 291 (65), 274 (94), 256 (92), 241 (97), 216 (97), 200 (51), 187 (95), 159 (96), 147 (82), 131 (78), 122 (95), 108 (97), 91 (94), 79 (95), 55 (88), 47 (100); $^1H$ NMR spectral data of **1** (400 MHz, $\delta$, ppm, $CDCl_3$): 4.44 (1H, d, *J*=10.9 Hz, H-20a), 4.25 (1H, s, H-19), 3.71 (1H, dq, *J*=14.2, 7.1 Hz, –OEt), 3.37 (1H, dq, *J*=14.2, 7.1 Hz, –OEt), 3.33 (1H, d, *J*=10.9 Hz, H-20b), 2.25 (1H, m, H-2a), 2.17 (1H, m, H-1a), 1.96 (1H, m, H-6a), 1.81 (1H, m, H-13), 1.38 (3H, s, $H_3$-17), 1.20 (3H, t, *J*=7.1 Hz, –OEt), 0.86 (3H, s, $H_3$-18); $^{13}C$ NMR data see Table 1.

**3.3.2. 16α-Hydroxy-19,20-epoxy-19*S**-ethoxy-kaurane (2).** Obtained as an amorphous powder. $[\alpha]_D^{25}=-36°$ (*c* 0.5; $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3450, 2933, 2360, 1649, 1458, 1375, 1157, 1120, 1070; HREIMS: *m/z* 348.2656 (calcd for $C_{22}H_{36}O_3$, 348.2664); EIMS: *m/z* 348 $[M]^+$ (47), 303 $[M-OEt]^+$ (23), 275 (89), 259 (91), 242 (85), 228 (91), 214 (94), 205 (79), 199 (89), 185 (96), 174 (100), 157 (95), 149 (91), 135 (82), 118 (99), 106 (84), 94 (75), 85 (96), 59 (62), 45 (97); $^1H$ NMR spectral data of **1** (400 MHz, $\delta$, ppm, $CDCl_3$): 4.44 (1H, d, *J*=11.5 Hz, H-20a), 3.87 (1H, dq, *J*=16.6, 7.1 Hz, –OEt), 3.68 (1H, d, *J*=11.5 Hz, H-20b), 3.48 (1H, dq, *J*=16.6, 7.1 Hz, –OEt), 2.36 (1H, m, H-2a), 2.17 (1H, m, H-1a), 2.05 (1H, m, H-3a), 1.83 (1H, m, H-13), 1.38 (3H, s, $H_3$-17), 1.19 (3H, t, *J*=7.1 Hz, –OEt), 1.12 (1H, m, H-5), 0.80 (3H, s, $H_3$-18); $^{13}C$ NMR data see Table 1.

**3.3.3. 16α-Hydroxy-19,20-epoxy-20*R**-ethoxy-kaurane (3).** Obtained as colorless needles, mp 132.0–132.5°C. $[\alpha]_D^{25}=-61.7°$ (*c* 1.1; $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3464, 2972, 2924, 2866, 2360, 1631, 1458, 1375, 1147, 1119, 1063, 991, 928; HREIMS: *m/z* 348.2653 (calcd for $C_{22}H_{36}O_3$, 348.2664); EIMS: *m/z* 348 $[M]^+$ (4), 303 $[M-OEt]^+$ (4), 274 (99), 256 (97), 241 (49), 227 (46), 213 (38), 200 (37), 187 (99), 173 (32), 159 (77), 147 (33), 122 (98), 107 (41), 95 (38), 85 (100), 67 (40), 55 (45), 43 (84); $^1H$ NMR spectral data of **1** (400 MHz, $\delta$, ppm, $CDCl_3$): 5.22 (1H, s, H-20), 3.97 (1H, dq, *J*=16.2, 7.1 Hz, –OEt), 3.63 (1H, d, *J*=11.3 Hz, H-19a), 3.48 (1H, d, *J*=11.3 Hz, H-19b), 3.42 (1H, dq, *J*=16.2, 7.1 Hz, –OEt), 2.53 (1H, m, H-1a), 2.38 (1H, m, H-2a), 2.01 (1H, H-11a), 1.82 (1H, m, H-13), 1.38 (3H, s, $H_3$-17), 1.22 (3H, t, *J*=7.1 Hz, –OEt), 1.08 (1H, m, H-5), 0.69 (3H, s, $H_3$-18); $^{13}C$ NMR data see Table 1.

**3.3.4. 16α-Hydroxy-19,20-epoxy-20*R**-hydroxy-kaurane (4).** Obtained as an amorphous powder. $[\alpha]_D^{25}=-62.4°$ (*c* 1.0; $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3408, 3275, 2910, 2862, 2362, 1651, 1454, 1381, 1157, 1126, 1057, 974, 906; HREIMS: *m/z* 320.2351 (calcd for $C_{20}H_{32}O_3$, 320.2351); EIMS: *m/z* 320 $[M]^+$ (5), 274 (62), 256 (90), 227 (36), 216 (41), 187 (48), 173 (17), 159 (33), 145 (28), 131 (32), 122 (90), 105 (43), 95 (28), 85 (100), 67 (27), 47 (73), 41 (38); $^1H$ NMR spectral data of **1** (400 MHz, $\delta$, ppm, $C_5D_5N$): 5.98 (1H, s, H-20), 3.75 (1H, d, *J*=10.9 Hz, H-19a), 3.59 (1H, d, *J*=10.9 Hz, H-19b), 2.95 (1H, m, H-1a), 2.87 (1H, m, H-2a), 2.39 (1H, m, H-11a), 2.36 (1H, m, H-12a), 2.23 (1H, m, H-13), 2.19 (1H, d, *J*=10.9 Hz, H-14a), 1.98 (1H, d, *J*=13,9 Hz, H-15a), 1.93 (1H, br d, *J*=10.7 Hz, H-14b), 1.73 (1H, d, *J*=13.9 Hz, H-15b), 1.62 (3H, s, $H_3$-17), 1.12 (1H, br d, *J*=9.6 Hz, H-5), 0.63 (1H, s, $H_3$-18); $^{13}C$ NMR data see Table 1.

**3.3.5. 16α-Hydroxykauran-19,20-dioic acid (5).** Obtained as an amorphous powder. $[\alpha]_D^{25}=-9°$ (*c* 0.3; $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3423, 2939, 1757, 1699, 1452, 1381, 1254, 1122, 1003, 955; HRFABMS: *m/z* 373.1934 (calcd for $C_{20}H_{30}O_5Na$, 373.1991); $^1H$ NMR spectral data of **1** (400 MHz, $\delta$, ppm, $C_5D_5N$): 3.20 (1H, m, H-6a), 3.03 (1H, m, H-1a), 2.03 (1H, m, H-2a), 2.56 (1H, m, H-7a), 2.48 (1H, m, H-3a), 2.44 (1H, m, H-11a), 2.23 (1H, m, H-6b), 1.59 (3H, s, $H_3$-17), 1.51 (3H, s, $H_3$-18), 1.45 (1H, m, H-9), 1.33 (1H, m, H-5); $^{13}C$ NMR data see Table 1.

**3.3.6. Methylation of 5.** One mg of **5** was dissolved in MeOH (1 ml) and treated with $(CH_3)_3SiCHN_2$ (0.3 ml) for 1 h at room temperature. The reaction mixture was worked up in the usual way to give 0.5 mg of dimethylate **5a**. $^1H$ NMR data of **5a** (400 MHz, $\delta$, ppm, $CDCl_3$): 3.67 (3H, s, –COOMe), 3.63 (3H, s, –COOMe), 2.60 (1H, m), 2.31 (1H, m), 2.12 (1H, m), 1.36 (3H, s), 1.23 (3H, s).

**3.3.7. 13-*epi*-19-Nor-Manoyloxide-18-oic acid (6).** Obtained as an amorphous powder. $[\alpha]_D^{25}=-8.4°$ (*c* 0.8; $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3427, 2935, 2868, 2362, 1703, 1454, 1286, 1221, 1163, 1076, 962, 912; HREIMS: *m/z* 306.2176 (calcd for $C_{19}H_{30}O_3$, 306.2195); EIMS: *m/z* 306 $[M]^+$ (9), 291 (90), 273 (98), 245 (60), 227 (34), 208 (100), 193 (85), 175 (30), 147 (22), 121 (31), 107 (57), 95 (49), 81 (99), 67 (54), 55 (76), 43 (98); $^1H$ NMR (400 MHz, $\delta$, ppm, $C_5D_5N$): 6.10 (1H, dd, *J*=17.9, 11.0 Hz, H-14), 5.03 (1H, d, *J*=17.9 Hz, H-15a), 4.95 (1H, d, *J*=11.0 Hz, H-15b), 2.47 (1H, m, H-4), 2.21 (1H, m, H-12a), 2.03 (1H, m, H-3a), 1.83 (1H, m, 6a), 1.79 (1H, m, H-3b), 1.27 (3H, s, $H_3$-17), 1.21 (3H, s, $H_3$-16), 0.65 (3H, s, $H_3$-20); $^{13}C$ NMR data see Table 1.

**3.3.8. 3β,11,19-Trihydroxy-14-methoxy-abieta-8,11,13-triene (7).** Obtained as an amorphous powder. $[\alpha]_D^{25}=+71°$ (*c* 0.1; MeOH); UV $\lambda_{max}^{MeOH}$ nm (log $\epsilon$)225 (4.05), 276 (3.50); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3648, 3614, 3566, 2930, 2361, 1742, 1699, 1650, 1541, 1516, 1458, 1035; HREIMS *m/z*: 348.2306 (calcd for $C_{21}H_{32}O_4$ 348.2301); EIMS *m/z*: 348 $[M]^+$ (100), 315 (14), 297 (12), 285 (8), 257 (11), 243 (6), 229 (10), 205 (19), 189 (10), 163 (7), 133 (5), 101 (6), 91 (6), 69 (8), 57 (10), 43 (12); $^1H$ NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 6.37 (1H, s, H-12), 4.45 (1H, dd, *J*=11.6, 4.7 Hz, H-3), 3.62 (3H, s, –OMe), 3.34, 4.28 (each 1H, d, *J*=11.1 Hz, $H_2$-19), 3.18 (1H, sept., *J*=6.9 Hz, H-15), 2.57, 2.97 (each 1H, m, $H_2$-7), 1.26 (1H, br d, *J*=12.5 Hz, H-5), 1.27 (3H, s, $H_3$-18), 1.24 (6H, d, *J*=6.9 Hz, $H_3$-16, 17), 1.23 (3H, s, $H_3$-20); $^{13}C$ NMR data see Table 1.

**3.3.9. 22β-Hydroxy-3-oxo-12-ursen-30-oic acid (8).** Obtained as an amorphous powder. $[\alpha]_D^{25}=+71.1°$ (*c* 1.0; $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3427, 2954, 2871, 2362, 1703, 1458, 1383, 1255, 1207, 1115, 1034, 756; HREIMS *m/z*: 470.3385 (calcd for $C_{30}H_{46}O_4$, 470.3396); EIMS *m/z*: 470 $[M]^+$ (19), 455 (5), 340 (5), 264 (100), 246 (48), 221 (10), 205 (19), 187 (10), 163 (10), 148 (16), 135 (22), 121 (18),

107 (19), 95 (17), 81 (17), 69 (14), 55 (20); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 5.22 (1H, br s, H-12), 3.51 (1H, br s, H-22), 2.54 (1H, m, H-2a), 2.47 (1H, m, H-20), 2.40 (1H, m, H-2b), 2.09 (1H, m, H-21a), 2.07 (1H, m, H-19), 1.72 (1H, d, $J$=11.2 Hz, H-18), 1.62 (1H, m, H-9), 1.13 (3H, s, $H_3$-27), 1.10 (3H, s, $H_3$-23), 1.08 (3H, s, $H_3$-25), 1.07 (3H, s, $H_3$-26), 1.06 (3H, s, $H_3$-24), 0.93 (3H, s, $H_3$-28), 0.86 (3H, d, $J$=6.2 Hz, $H_3$-29); $^{13}$C NMR data see Table 2.

**3.3.10. 28-Hydroxy-3-oxo-12-ursen-30-oic acid (9).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=+66.9° (*c* 1.0; $CHCl_3$); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3444, 2945, 1701, 1460, 1385, 1180, 1038, 756; HREIMS *m/z*: 470.3350 (calcd for $C_{30}H_{46}O_4$, 470.3396); EIMS *m/z*: 470 $[M]^+$ (12), 439 (26), 264 (72), 246 (20), 233 (80), 221 (19), 215 (83), 205 (30), 187 (100), 173 (20), 159 (20), 145 (23), 131 (27), 119 (27), 105 (29), 81 (35), 69 (32), 55 (41); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 5.21 (1H, br s, H-12), 3.55 (1H, d, $J$=10.9 Hz, H-28a), 3.24 (1H, d, $J$=10.9 Hz, H-28b), 2.53 (1H, m, H-2a), 2.38 (1H, m, H-2b), 2.06 (1H, m, H-19), 1.46 (1H, d, $J$=12.1 Hz, H-18), 1.33 (1H, m, H-5), 1.15 (3H, s, $H_3$-27), 1.10 (3H, s, $H_3$-23), 1.07 (3H, s, $H_3$-25), 1.06 (3H, s, $H_3$-24), 1.04 (3H, s, $H_3$-26), 0.86 (3H, d, $J$=6.3 Hz, $H_3$-29); $^{13}$C NMR data see Table 2.

**3.3.11. 2α,3β-Dihydroxy-olean-12-ene-22,29-lactone (10).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=+35.7° (*c* 1.0; MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3648, 3566, 2931, 2361, 1771, 1650, 1541, 1510, 1456, 1391, 1172, 958; HREIMS *m/z*: 470.3383 (calcd for $C_{30}H_{46}O_4$, 470.3396); EIMS *m/z*: 470 $[M]^+$ (17), 452 $[M-H_2O]^+$ (21), 246 (100), 233 (45), 218 (34), 205 (41), 185 (38), 159 (33), 145 (40), 131 (53), 119 (56), 107 (46), 95 (46), 81 (37), 69 (36), 55 (40), 43 (33); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 5.31 (1H, br s), 4.15 (1H, d, $J$=5.4 Hz, H-22), 3.70 (1H, m, H-2), 3.01 (1H, d, $J$=9.5 Hz, H-3), 2.27 (1H, d, $J$=11.8 Hz, H-19), 2.14 (1H, dd, $J$=12.5, 8.6 Hz, H-18), 2.00 (1H, dd, $J$=12.3, 4.4 Hz, H-1a), 1.21 (3H, s, $H_3$-30), 1.08 (3H, s, $H_3$-27), 1.04 (3H, s, $H_3$-23), 1.02 (3H, s, $H_3$-25), 0.93 (3H, s, $H_3$-26), 0.87 (3H, s, $H_3$-28), 0.83 (3H, s, $H_3$-25), 0.83 (1H, m, H-5); $^{13}$C NMR data see Table 2.

**3.3.12. 28-Hydroxy-3-oxo-olean-12-en-29-oic acid (11).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=+48.7° (*c* 1.1; MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3449, 2942, 2870, 2362, 1698, 1462, 1384, 1234, 1116, 1002, 753, 666; HREIMS *m/z*: 470.3379 (calcd for $C_{30}H_{46}O_4$, 470.3396); EIMS *m/z*: 470 $[M]^+$ (8), 225 (6), 191 (10), 179 (7), 165 (10), 149 (10), 136 (15), 119 (18), 111 (30), 97 (47), 83 (52), 71 (63), 57 (100), 43 (79); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 5.28 (1H, br t, $J$=3.3 Hz); 3.28, 3.55 (each 1H, d, $J$=11.0 Hz, $H_2$-28), 2.38, 2.54 (each 1H, m, $H_2$-2), 2.25 (1H, br t, $J$=13.6 Hz, H-19a), 1.25 (3H, s, $H_3$-30), 1.19 (3H, s, $H_3$-27), 1.10 (3H, s, $H_3$-24), 1.07 (3H, s, $H_3$-25), 1.06 (3H, s, $H_3$-23), 1.00 (3H, s, $H_3$-26); $^{13}$C NMR data see Table 2.

**3.3.13. 3β,28-Dihydroxy-olean-12-en-29-oic acid (12).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=+43.1° (*c* 1.0; MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3427, 2931, 2871, 2362, 1701, 1466, 1383, 1259, 1043, 997, 756; HREIMS *m/z*: 472.3532 (calcd for $C_{30}H_{48}O_4$, 472.3553); EIMS *m/z*: 472 $[M]^+$ (13), 441 (9), 264 (46), 233 (85), 221 (13), 207 (28), 187 (100), 173 (13), 159 (14), 145 (13), 135 (19), 119 (20), 105 (19), 95 (20), 81 (24), 69 (20), 43 (20); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 5.16 (1H, t, $J$=2.9 Hz, H-12), 3.45 (1H, d, $J$=11.0 Hz, H-28a), 3.14 (1H, br d, $J$=10.5 Hz, H-3), 3.12 (1H, d, $J$ =11.0 Hz, H-28b), 2.14 (1H, t, $J$=13.6 Hz, H-19a), 1.93 (1H, dd, $J$=13.6, 3.3 Hz, H-18), 1.63 (1H, m, H-15a), 1.14 (3H, s, $H_3$-29), 1.10 (3H, s, $H_3$-27), 0.91 (3H, s, $H_3$-23), 0.87 (3H, s, $H_3$-26), 0.86 (3H, s, $H_3$-25), 0.71 (3H, s, $H_3$-24), 0.66 (1H, br d, $J$=10.8 Hz, H-5); $^{13}$C NMR data see Table 2.

**3.3.14. 3α,28-Dihydroxy-olean-12-en-29-oic acid (13).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=−1.3° (*c* 1.1; $C_5H_5N$); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3427, 2941, 2868, 2362, 1691, 1460, 1387, 1248, 1068, 1026, 997; HREIMS *m/z*: 472.3493 (calcd for $C_{30}H_{48}O_4$, 472.3553); EIMS *m/z*: 472 $[M]^+$ (7), 424 (9), 354 (9), 264 (47), 246 (16), 233 (76), 221 (22), 207 (41), 187 (100), 175 (31), 161 (23), 148 (15), 133 (23), 122 (17), 105 (23), 93 (23), 81 (29), 69 (27), 55 (30), 43 (24); $^1$H NMR (400 MHz, $\delta$, ppm, $C_5D_5N$): 5.30 (1H, br s, H-12), 3.85 (1H, d, $J$=10.7 Hz, H-28a), 3.59 (1H, d, $J$=10.7 Hz, H-28b), 3.58 (1H, m, H-3), 2.60 (1H, t, $J$=13.5 Hz, H-19a), 2.40 (1H, br d, $J$=10.8 Hz, H-18), 2.23 (1H, m, H-21a), 1.63 (1H, br d, $J$=12.1 Hz, H-5), 1.50 (3H, s, $H_3$-29), 1.18 (3H, s, $H_3$-23), 1.17 (3H, s, $H_3$-27), 0.97 (3H, s, $H_3$-26), 0.93 (3H, s, $H_3$-25), 0.87 (3H, s, $H_3$-24); $^{13}$C NMR data see Table 2.

**3.3.15. 2β,22β-Dihydroxy-3,21-dioxo-24-carboxyl-29-nor-friedelan methyl ester (14).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=+5° (*c* 0.4; MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3649, 3446, 2924, 2854, 2362, 1719, 1650, 1456, 1382, 1208, 1117, 991; HREIMS *m/z*: 502.3262 (calcd for $C_{30}H_{46}O_6$, 502.3294); EIMS: *m/z* 502 $[M]^+$ (100), 484 $[M-H_2O]^+$ (8), 415 (17), 401 (16), 333 (17), 273 (8), 215 (6), 175 (9), 159 (10), 147 (12), 135 (16), 121 (35), 109 (34), 95 (23), 81 (19), 55 (21), 43 (17); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 4.58 (1H, d, $J$=3.9 Hz, H-22), 4.14 (1H, 1H, br t, $J$=9.0 Hz, H-2), 3.62 (3H, s, –OMe), 2.74 (1H, m, H-20), 2.59 (1H, dt, $J$=13.2, 3.1 Hz, H-6), 2.47 (1H, m, H-1a), 2.26 (1H, q, $J$=6.7 Hz, H-4), 2.20 (1H, m, H-19a), 1.89 (1H, dd, $J$=13.1, 3.1 Hz, H-10), 1.77 (1H, br d, $J$=7.5 Hz, H-18), 1.36 (3H, s, $H_3$-26), 1.07 (3H, d, $J$=6.3 Hz, $H_3$-30), 0.97 (3H, d, $J$=6.7 Hz, $H_3$-23), 0.93 (3H, s, $H_3$-27), 0.84 (3H, s, $H_3$-28), 0.79 (3H, s, $H_3$-25); $^{13}$C NMR data see Table 2.

**3.3.16. 21β,28-Dihydroxy-3-oxo-olean-12,20(30)-diene (15).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=+101° (*c* 0.2; $CHCl_3$); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3427, 2933, 2362, 1701, 1458, 1387, 1257, 1043, 808; HRFABMS: *m/z* 463.3143 (calcd for $C_{29}H_{44}O_3Na$, 463.3188); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 5.34 (1H, br s, H-12), 4.89 (1H, s, H-30a), 4.77 (1H, s, H-30b), 4.26 (1H, dd, $J$=11.7, 4.9 Hz, H-21), 3.53 (1H, d, $J$=11.0 Hz, H-28a), 3.23 (1H, d, $J$=11.0 Hz, H-28b), 2.66 (1H, t, $J$=13.5 Hz, H-19a), 2.54 (1H, m, H-2a), 2.39 (1H, m, H-2b), 2.21 (1H, dd, $J$=13.5, 5.0 Hz, H-19b), 1.66 (1H, m, H-9), 1.20 (3H, s, $H_3$-27), 1.10 (3H, s, $H_3$-23), 1.08 (3H, s, $H_3$-25), 1.06 (3H, s, $H_3$-24), 1.00 (3H, s, $H_3$-26); $^{13}$C NMR data see Table 2.

**3.3.17. 2,22β-Dihydroxy-3-oxo-olean-1,12-dien-29-oic acid (16).** Obtained as an amorphous powder. $[\alpha]_D^{25}$=+64.0° (*c* 1.1; MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$:

3437, 2947, 2360, 1701, 1668, 1460, 1385, 1238, 1055, 756; HREIMS *m/z*: 484.3178 (calcd for $C_{30}H_{44}O_5$, 484.3189); EIMS: *m/z* 484 $[M]^+$ (43), 314 (16), 274 (24), 264 (100), 246 (43), 233 (25), 217 (58), 205 (34), 187 (46), 173 (33), 159 (38), 147 (46), 135 (74), 122 (64), 107 (65), 95 (63), 81 (67), 69 (43), 55 (66), 43 (72); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 6.36 (1H, s, H-1), 5.37 (1H, br s, H-12), 3.58 (1H, dd, $J$=7.0, 3.1 Hz, H-22), 2.28 (1H, t, $J$=13.5 Hz, H-19a), 2.17 (1H, m, H-18), 1.89 (1H, m, H-9), 1.41 (3H, s, $H_3$-29), 1.27 (3H, s, $H_3$-25), 1.24 (3H, s, $H_3$-23), 1.42 (3H, s, $H_3$-24), 1.38 (3H, s, $H_3$-27), 1.04 (3H, s, $H_3$-26), 0.91 (3H, s, $H_3$-28); $^{13}$C NMR data see Table 2.

**3.3.18. 2,3-Dihydroxy-1,3,5(10),7-tetraene-6β(1′-hydroxyethyl)-24-nor-D:A-friedooleane-29-oic acid (17).** Obtained as an amorphous powder. $[\alpha]_D^{25}=-40°$ (*c* 0.2; MeOH); UV $\lambda_{max}^{MeOH}$ nm (log $\epsilon$) 280 (3.46), 223 (3.91); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3427, 2927, 2362, 1703, 1624, 1460, 1377, 1273, 1207, 1074, 877; HRFABMS: *m/z* 519.3062 (calcd for $C_{31}H_{44}O_5Na$, 519.3086); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$–$CD_3OD$, 3:1): 6.70 (1H, s, H-1), 5.72 (1H, d, $J$=6.3 Hz, H-7), 3.58 (1H, q, $J$=6.4 Hz, H-31), 3.47 (1H, t, $J$=6.8 Hz, H-6), 2.35 (1H, br d, $J$=15.6 Hz, H-19a), 2.19 (3H, s, $H_3$-23), 1.46 (3H, s, $H_3$-25), 1.16 (3H, s, $H_3$-26), 1.13 (3H, s, $H_3$-29), 1.11 (3H, d, $J$=6.4 Hz, $H_3$-32), 1.02 (3H, s, $H_3$-28), 0.62 (3H, s, $H_3$-27); $^{13}$C NMR data see Table 2, $\delta$ 73.3 (C-31), 20.4 (C-32).

**3.3.19. 2,3-Dihydroxy-1,3,5(10),7-tetraene-6α(1′-hydroxyethyl)-24-nor-D:A-friedooleane-29-oic acid (18).** Obtained as an amorphous powder. $[\alpha]_D^{25}=-63°$ (*c* 0.7; MeOH); UV $\lambda_{max}^{MeOH}$ nm (log $\epsilon$) 282 (3.40), 224 (3.83); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3365, 2931, 2362, 1699, 1460, 1377, 1286, 1209, 1101, 1022, 876; HRFABMS: *m/z* 519.3062 (calcd for $C_{31}H_{44}O_5Na$, 519.3086); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$–$CD_3OD$, 3:1): 6.67 (1H, s, H-1), 5.73 (1H, d, $J$=6.1 Hz, H-7), 4.00 (1H, m, H-31), 3.55 (1H, dd, $J$=5.6, 2.6 Hz, H-6), 2.43 (1H, br d, $J$=15.5 Hz, H-19a), 2.16 (3H, s, $H_3$-23), 1.55 (3H, s, $H_3$-25), 1.30 (3H, d, $J$=6.4 Hz, $H_3$-32), 1.25 (3H, s, $H_3$-26), 1.16 (3H, s, $H_3$-29), 1.08 (3H, s, $H_3$-28), 0.65 (3H, s, $H_3$-27); $^{13}$C NMR data see Table 2, $\delta$ 701. (C-31), 21.1 (C-32).

**3.3.20. 2,3-*seco*-22,29-Lactone-oleane-12-ene-2,3-dioic acid 3-methyl ester (19).** Obtained as an amorphous powder. $[\alpha]_D^{25}=+31°$ (*c* 0.3; $CHCl_3$); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3435, 2927, 1770, 1724, 1639, 1458, 1389, 1236, 1142, 1099, 957, 804; HRFABMS: *m/z* 537.3229 (calcd for $C_{31}H_{46}O_6Na$, 537.3192); $^1$H NMR (400 MHz, $\delta$, ppm, $CDCl_3$): 5.31 (1H, br s, H-12), 4.15 (1H, d, $J$=5.4 Hz, H-22), 3.66 (3H, s, –OMe), 2.59 (1H, m, H-9), 2.44 (1H, d, $J$=18.4 Hz, H-1a), 2.42 (1H, m, H-5), 2.26 (1H, m, H-21a), 2.28 (1H, d, $J$=18.4 Hz, H-1b), 1.28 (3H, s, $H_3$-23 or 24), 1.24 (3H, s, $H_3$-23 or 24), 1.21 (3H, s, $H_3$-29), 1.09 (3H, s, $H_3$-27), 1.04 (3H, s, $H_3$-25), 0.95 (3H, s, $H_3$-26), 0.87 (3H, s, $H_3$-28); $^{13}$C NMR data see Table 2.

**3.3.21. X-Ray crystallographic analysis data of 1.** An orthorhombic crystal was obtained from a solvent system of $CHCl_3$–MeOH (2:1). Crystal data: $C_{22}H_{36}O_3$, Mr=348.00, orthorhombic. Crystal size=0.45×0.4×0.25 mm. Cell parameters: $a$=8.367000 (0), $b$=9.42900 (0), $c$=25.591999 (0) Å, $V$=2007.099976 (0) Å$^3$, space group $P2_12_12_1$ ($Z$=4). Data collection was performed on a DIP Image plate, the structure was resolved by direct methods (maXus SIR92), and the final $R$ and $Rw$ values were 0.037 and 0.093 for 1754 observed reflections.

**3.3.22. X-Ray crystallographic analysis data of 3.** A monoclinic crystal was obtained from a solvent system of $CHCl_3$–MeOH (2:1). Crystal data: $C_{22}H_{36}O_3$, Mr=348.00, monoclinic. Crystal size=0.5×0.35×0.1 mm. Cell parameters: $a$=18.676 (8), $b$=9.819 (5), $c$=11.481 (5) Å, $V$=2027.670044 (2) Å$^3$, space group $P2_1$ ($Z$=4). Data collection was performed on a MXC (MAC Science), the structure was resolved by direct methods (CRYSTAN SIR92), and the final $R$ and $Rw$ values were 0.074 and 0.107 for 1325 observed reflections.

## References


1. Duan, H. Q.; Takaishi, Y.; Momota, H.; Ohmoto, Y.; Taki, T.; Jia, Y. F.; Li, D. *J. Nat. Prod.* **1999**, *62*, 1522–1525.
2. Duan, H. Q.; Takaishi, Y.; Momota, H.; Ohmoto, Y.; Taki, T.; Jia, Y. F.; Li, D. *J. Nat. Prod.* **2001**, *64*, 582–587.
3. Duan, H. Q.; Takaishi, Y.; Momota, H.; Ohmoto, Y.; Taki, T.; Jia, Y. F.; Li, D. *Phytochemistry* **2000**, *53*, 805–810.
4. Qian, S. Z. *Contraception* **1987**, *36*, 335–345.
5. 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* **1993**, *47*, 387–400.
6. Qian, S. Z.; Xu, Y.; Zhang, J. W. *Contraception* **1995**, *51*, 121–129.
7. Durum, S. K.; Opprenheim, J. J. In *Fundamental Immunology*; Paul, W. E., Ed.; 2nd ed, Raven: New York, 1989.
8. Miyasaka, N.; Sato, K.; Goto, M.; Sasano, M.; Natsuyama, M.; Inoue, K.; Nishioka, K. *Arthritis Rheum.* **1988**, *31*, 480–486.
9. Chen, K.; Shi, Q.; Fujioka, T.; Nakano, T.; Hu, C. Q.; Jin, J. Q.; Kilkuskie, R. E.; Lee, K. H. *Bioorg. Med. Chem.* **1995**, *3*, 1345–1348.
10. Tsichritzis, F.; Jakupovic, J. *Phytochemistry* **1991**, *30*, 211–213.
11. Takaishi, Y.; Wariishi, N.; Tateishi, H.; Kawazoe, K.; Miyagi, K.; Li, K. H.; Duan, H. Q. *Phytochemistry* **1997**, *45*, 979–984.
12. Duan, H. Q.; Kawazoe, K.; Bando, M.; Kido, M.; Takaishi, Y. *Phytochemistry* **1997**, *46*, 535–543.
13. Seo, S.; Tomita, Y.; Tori, K. *J. Chem. Soc., Chem. Commun.* **1975**, 954–955.
14. Li, K.; Duan, H. Q.; Kawazoe, K.; Takaishi, Y. *Phytochemistry* **1997**, *45*, 791–796.
15. Zhang, C.-P.; Zhang, Y.-G.; Lu, X.-Y.; Chen, Y.; Ma, P.-C.; Yu, D.-Q.; He, C.-H.; Shen, F.-L.; Yang, J.-J. *Acta Acad. Med. Sin.* **1989**, *11*, 322–325.
16. Gao, F.; Miski, M.; Gage, D. A.; Norris, J. A.; Mabry, T. J. *J. Nat. Prod.* **1985**, *48*, 489–490.
17. Pradhan, B. P.; Chakraborty, S.; Ghosh, R. K.; Roy, A. *Phytochemistry* **1995**, *39*, 1399–1402.
18. Xu, R.-S.; Wiedmann, T. W. PCT Int. Appl. WO 94 20488, 15 Sep 1994, US Appl. 31288, 10 Mar 1993, 46pp.
19. Calabuig, M. T.; Cortes, M.; Francisco, C. G.; Hernandez, R.; Suarez, E. *Phytochemistry* **1981**, *20*, 2255–2258.
20. Morota, T.; Qin, W.-Z.; Takagi, K.; Xu, L.-H.; Maruno, M.; Yang, B.-H. *Phytochemistry* **1995**, *40*, 865–870.
21. Kutney, J. P.; Han, K. *Recl. Trav. Chim. Pay B* **1996**, *115*, 77–93.

22. Ma, P.-C.; Liu, X.-Y.; Yang, J.-J.; Zheng, Q.-T. *Acta Pharm. Sin.* **1991**, *26*, 759–763.
23. Feliciano, A. S.; Corral, J. M. D.; Lopea, J. L.; Pascual-Teresa, B. D. *Phytochemistry* **1992**, *31*, 1719–1722.
24. Qin, G.-W.; Yang, X.-M.; Gu, W.-H.; Wang, B.-D.; Chen, Z.-X.; Guo, R.-X.; Shao, K.-W. *Huaxue Xuebao* **1982**, *40*, 637–647.
25. Takaishi, Y.; Wariishi, N.; Tateishi, H.; Kawazoe, K.; Nakano, K.; Ono, Y.; Tokuda, H.; Nishino, H.; Iwashima, A. *Phytochemistry* **1997**, *45*, 969–974.
26. Kita, M.; Omoto, Y.; Hirai, Y.; Yamaguchi, N.; Imanishi *J. Microbiol. Immunol.* **1992**, *36*, 507–516.
27. Zheng, J.-R.; Fang, J. L.; Gu, K.-X.; Yi, Y.-P.; Xu, L.-F.; Gao, J.-W.; Guo, H.-Z.; Yu, Y.-H.; Sun, H.-Z. *Acta Acad. Med. Sin.* **1987**, *9*, 323–328.
28. Lu, X.-Y.; Ma, P.-C.; Chen, Y.; Zhang, C.-P.; Zhang, Y.-G.; Zhang, Z.-X.; Sheng, L.-S.; Li, S.-Z.; An, D.-K.; He, C.-H.; Zheng, Q.-T. *Acta Acad. Med. Sin.* **1990**, *12*, 157–161.
29. Ma, P.-C.; Lu, X.-Y.; He, C.-H.; Zheng, Q.-T. *Acta Bot. Sin.* **1991**, *33*, 370–377.
30. Zhang, C.-P.; Yan, Z.; Chen, Y.; Zhang, Y.-G.; Lu, X.-Y. *Acta Acad. Med. Sin.* **1994**, *16*, 466–468.
31. Duan, H. Q.; Takaishi, Y.; Jia, Y. F.; Li, D. *Chem. Pharm. Bull.* **1999**, *47*, 1664–1667.
32. Duan, H. Q.; Takaishi, Y.; Imakura, Y.; Jia, Y. F.; Li, D.; Cosentino, L. M.; Lee, K. H. *J. Nat. Prod.* **2000**, *63*, 357–361.
33. Duan, H. Q.; Takaishi, Y.; Jia, Y. F.; Li, D. *Phytochemistry* **2001**, *56*, 341–346.