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Triterpenoids from *Tripterygium wilfordii*

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Abstract

The extract (T_{II}) of *Tripterygium wilfordii* Hook f. afforded four triterpenoids: wilforic acid D (3β,24-epoxy-2α-hydroxy-24R*-ethoxy-29-friedelanoic acid); (E) 3β,24-epoxy-2-oxo-3α-hydroxy-29-friedelanoic acid; (F) 2β-hydroxy-3-oxo-friedelan-29-oic acid; 29-hydroxy-3-oxo-olean-12-en-28-oic acid and 17 known triterpenoids. Their structures were established on the basis of spectroscopic studies. In a bioactivity analysis, only the known dulcioic acid compound showed a significant inhibitory effect on cytokine production.

Keywords: *Tripterygium wilfordii*; Triterpenoids; Immunosuppressive activity

1. Introduction

The genus *Tripterygium* has been used as a source of traditional Chinese drugs for the treatment of cancer, and as an insecticide, for 100s of years. In the course of our studies on the bioactive metabolites of this genus, we have described several interleukin-1 inhibitors: the diterpene quinoids, tingenone and celastrol from *Tripterygium wilfordii* var. *regelii* (Shishido et al., 1994; Takaishi et al., 1997), and have reported on the isolation and structure determination of wilforic acids A, B and C from *T. wilfordii* Hook f. (Li, Duan, Kawazoe & Takaishi, 1997). Recently, the extract derived from a water/chloroform extract of the roots of *T. wilfordii* Hook f. (the so-called total multi-glycoside or T_{II} fraction) was used in the clinical treatment of rheumatoid arthritis, skin disorders, in male-fertility control and for other inflammatory and antoimmune diseases (Qian, 1987; Matlin et al., 1993; Qian, Xu & Zhang, 1995). The precise mechanism of the therapeutic effect of T_{II}, however, has not been completely delineated. In order to determine which of the components present in the extract T_{II} are responsible for such diverse activities, studies were initiated on the isolation of the active principles of the extract of *T. wilfordii* (T_{II} fraction). We report here the isolation and structure elucidation of four new triterpenoids, named wilforic acids D (**1**), E (**2**), and F (**3**), and 29-hydroxy-3-oxo-olean-12-en-28-oic acid (**4**), and 17 known triterpenoids (**5**–**21**) from the extract of *T. wilfordii* (T_{II} fraction), along with the immunosuppressive activity of these compounds.

2. Results and discussion

The powdered extract T_{II} of *T. wilfordii* Hook f. was subjected to repeated chromatography to afford wilforic acids D (**1**), E (**2**), and F (**3**), 29-hydroxy-3-oxo-olean-12-en-28-oic acid (**4**), and the known compounds **5**–**21**.

Compound **1** (wilforic acid D) revealed hydroxy and

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carbonyl bands in its IR spectrum (3423 and 1702 cm^{-1}). The ^1H NMR spectrum showed three methine proton signals [δ_H 5.02 (1H, *s*), 4.04 (1H, *br t*, J = 4.4 Hz) and 3.88 (1H, *d*, J = 4.9 Hz)], one ethoxy group [δ_H 3.79, 3.42 (each 1H, *dt*, J = 16.6, 7.3 Hz), 1.20 (3H, *t*, J = 7.3 Hz)], five methyl groups [δ_H 1.25, 1.08, 0.96, 0.92 and 0.86 (each 3H, *s*)], and one doublet methyl group [δ_H 1.05 (3H, *d*, J = 6.8 Hz)]. The ^{13}C NMR spectrum of **1** showed a carboxylic acid carbon at δ_C 184.6, three methine carbons at δ_C 103.4, 83.6 and 69.1, considered to be attached to oxygen functionalities, an oxygenated methylene carbon at δ_C 64.6, seven methyl groups, four methine carbon signals, nine methylene carbon signals, and six quaternary carbon signals. These observations agreed with a molecular formula $C_{32}H_{52}O_5$, which was supported by HR FABMS. Except for the ethoxy group, the C-skeleton containing 30 carbons, including five tertiary methyl and one secondary methyl groups, and was deduced to be a friedelane-type triterpene. Comparison of the ^{13}C NMR spectral data indicated that compound **1** was very similar to orthosphenic acid (**9**) (Gonzalez et al., 1983), except for ring A (Table 1). In the HMBC spectrum of **1**, the proton signal at δ_H 4.04 (H-2) was correlated with the carbon signals at δ_C 27.0 (C-1) and 83.6 (C-3), the proton signal at δ_H 3.88 (H-3) with the carbon signals at δ_C 49.6 (C-5), 14.2 (C-23) and 103.4 (C-24), and the proton signal at δ_H 5.02 (H-24) with the carbon signals at δ_C 52.8 (C-10), 83.6 (C-3), 44.1 (C-4) and 64.6 (ethoxy group). Therefore, compound **1** was considered to be 3-dehydroxy-24-ethoxy-orthosphenic acid. The coupling constant of H-2 (*br t*, J = 4.4 Hz) indicated that H-2 was coupled to H-3 (*d*, J = 4.9 Hz) and only one proton of H_a-1 (δ_H 1.73, *dt*, J = 4.1, 14.1 Hz). By studying the molecular model of **1**, H-2 was assigned as an equatorial proton with the hydroxy group having a 2α orientation. On the other hand, the proton signal at δ_H 5.02 (H-24) was correlated with the signal at δ_H 0.92 (H_3-25) in the NOESY spectrum, thus a 24R* configuration of the ethoxy group was proposed. Assignments of the ^1H and ^{13}C NMR spectral data were made on the basis of the 2D NMR spectra (see Section 3 and Table 1). Since ethanol solvent had not used in the extraction and isolation of T_{II}, we believe that compound **1** is a natural product.

Table 1
^{13}C NMR chemical shift for compounds **1**–**4**, **9** and **16**[a]

Carbon	1	2	3	4	9	16
1	27.0	34.2[c]	32.5	39.2	28.7	38.6
2	69.1	207.4	75.0	34.2	74.2	34.4
3	83.6	103.1	212.4	217.8	108.1	220.7
4	44.1	51.2	55.6	47.5	46.8	50.9
5	49.6	47.5	43.0	55.3	47.5	55.7
6	29.6	33.3	41.2	19.6	34.0	19.2
7	19.2	19.1	18.2	32.2	19.7	32.4
8	50.4	50.3	50.8	39.3	50.4	39.2
9	37.2	37.8	37.4	47.0	37.5	46.5
10	52.8	57.2	56.8	36.9	53.3	36.6
11	34.2	34.1[c]	35.3	23.0	34.7	22.9
12	29.1	29.0	29.5	122.8	29.5	122.2
13	39.1[b]	39.2[d]	39.3	143.4	39.6	143.7
14	39.0[b]	39.0[d]	39.3	41.8	39.3	41.7
15	29.6	29.5	29.4	27.7	29.7	27.7
16	36.1	36.0	36.1	23.6	36.7	23.7
17	30.1	30.1	30.1	46.9	30.5	46.5
18	44.2	44.0	44.2	40.3	44.8	41.2
19	30.3	30.3	30.3	40.1	30.9	45.9
20	40.4	40.4	40.4	35.8	40.7	30.7
21	29.5	29.4	29.7	28.3	30.5	33.8
22	36.6	36.7	36.7	31.6	37.4	32.3
23	14.2	7.2	6.6	21.5	8.4	22.1
24	103.4	72.7	14.7	26.5	72.1	65.7
25	16.9	16.8	18.5	15.1	16.9	16.0
26	16.6	16.7	16.4	17.0	16.8	16.8
27	18.1	17.9	18.1	26.0	18.1	25.8
28	31.9	31.8	31.9	183.1	32.1	182.8
29	184.6	184.4	184.4	74.3	181.3	33.1
30	31.5	31.4	31.4	19.0	32.3	23.6

[a] **1**: OEt, 64.6 (*t*), 15.4 (*q*); solvents **1**–**4** and **16**: $CDCl_3$; **9**: C_5D_5N, TMS as int. standard.
[b,c,d]Assignments may be interchangeable in each column.

Wilforic acid E (**2**) exhibited a molecular ion peak at *m/z* 486.3352 from HR EIMS. It showed IR absorption bands at 3436, 1736, and 1702 cm^{-1}, suggesting the presence of a hydroxy group, carboxylic acid, and carbonyl group. The ^{13}C NMR spectral data of **2** were very similar to those of orthosphenic acid (**9**), except for C-1, C-2, C-3 and C-4. It was concluded that compound **2** is 2-dehydroxy-2-oxo-orthosphenic acid. In the HMBC spectrum of **2**, the proton signal at δ_H 2.53 (H_α-1) was correlated with the carbon signals at δ_C 207.4 (C-2), 103.1 (C-3), 57.2 (C-10) and 47.5 (C-5), and the proton signal at δ_H 1.51 (H-4) was correlated with the ketone carbon signal at δ_C 207.4 (C-2). Moreover, the proton signal at δ_H 1.09 (H_3-23) was correlated with the signals at δ_C 103.1 (C-3), 51.2 (C-4) and 47.5 (C-5). Thus, the ketone group was located at position C-2.

Wilforic acid F (**3**), $C_{30}H_{48}O_4$, showed the presence of a hydroxy group, a carboxylic acid and a ketone group in the IR spectrum (3436, 1719 and 1703 cm^{-1}). The ^1H NMR spectrum of **3** revealed six tertiary methyl groups [δ_H 1.27, 1.10, 1.00, 0.70 (each 3H, *s*) and 0.88 (6H, *s*)], a secondary methyl group [δ_H 0.95 (3H, *d*, J = 6.7 Hz)], and a methine proton [δ_H 4.08 (1H, *br d*, J = 10.5 Hz)] attached to the oxygen function. Its ^{13}C NMR spectrum revealed a ketonic carbonyl carbon (δ_C 212.4), a carboxylic carbon (δ_C 184.4), a methine carbon (δ_C 75.0) attached to the oxygen function, and seven methyl carbons (δ_C 31.9, 31.4, 18.5, 18.1, 16.4, 14.7 and 6.6). In addition, it also showed ten methylenes, four methines, and six quaternary carbons. It is also a friedelane triterpene, pos-

sessing a hydroxy, a ketone and a carboxylic acid group (Sousa, Silva, Pedersoli & Alves, 1990), Moreover, the ^{13}C NMR spectral data were very similar to those of **2**, except for ring A (Table 1). In the HMBC spectrum, the proton signal at δ_H 2.44 (H_a-1) was correlated with the carbon signals at δ_C 75.0 (C-2), 212.4 (C-3) and 43.0 (C-5), and the methyl proton signal at δ_H 0.95 (H_3-23) was correlated with the signal at δ_C 212.4 (C-3), 55.6 (C-4) and 43.0 (C-5), while the proton signal at δ_H 2.25 (H-4) was correlated with the carbon signal at δ_C 75.0 (C-2). From these observations, it was concluded that the hydroxy and ketone groups were assigned at positions C-2 and C-3, respectively. Furthermore, the proton signal at δ_H 4.08 (H-2) was correlated with the proton signal at δ_H 2.25 (H-4) in the NOESY spectrum, indicating that the relative stereochemistry of the hydroxy group was 2β.

Compound **4**, $C_{30}H_{46}O_4$, possessed hydroxy, carboxylic and ketone groups from the IR spectrum (3443, 1720 and 1703 cm^{-1}). The ^1H NMR spectrum of **4** showed a methine proton [δ_H 5.28 (1H, *br s*)] attached to a double bond, methylene groups [δ_H 3.25 (2H, *s*)] attached to an oxygen functionality, and six methyl groups [δ_H 1.11, 1.04, 1.00, 0.99, 0.92 and 0.77 (each 3H, *s*)]. Its ^{13}C NMR spectrum was similar to that of compound **16** (Hart, Lamberton, Sioumis & Suares, 1976), with the assignments of the ^{13}C NMR spectral data being aided by examination of 2D NMR spectra. In this way, it was found that the difference between compounds **4** and **16** was the position of the hydroxy methylene group (Table 1). In the HMBC spectrum of **4**, the proton signal at δ_H 3.25 (H_2-29) was correlated with the carbon signals at δ_C 35.8 (C-20), 28.3 (C-21) and 19.0 (C-30). On the other hand, the proton signal at δ_H 2.84 (H-18β) showed NOESY correlations with the signals at δ_H 0.92 (H_3-30) and 5.28 (H-12). Based on the above observations and the conformation of the oleanene skeleton, a C-29 assignment of hydroxy methylene group is proposed. Thus, compound **4** is formulated as 29-hydroxy-3-oxo-olean-12-en-28-oic acid.

Known compounds **5**–**21** were identified from their spectral data upon comparison with values reported in the literature as 3-hydroxy-2-oxo-D:A-friedoolean-3-en-29-oic acid (**5**) (Morota et al., 1995a, 1995b), 3β-hydroxy-2-oxofriedelan-29α-carboxylic acid (**6**) (Sousa et al., 1990), polpunoic acid (**7**) and cangoronine (**8**) (Itokawa, Shirota, Ikuta, Morita, Takeya & Iitaka, 1991), orthosphenic acid (**9**) (Gonzalez et al., 1983), wilforic acid B (**10**) (Li et al., 1997), celastrol (**11**) (Morota et al., 1995a, 1995b), triptocallic acid D (**12**) (Nakano, Oose & Takaishi, 1997), 22α-hydroxy-3-oxo-olean-12-en-29-oic acid (**13**) and 22β-hydroxy-3-oxo-olean-12-en-29-oic acid (**14**) (Kutney, Hewitt, Lee, Piotrowska, Roberts & Rettig, 1992), 3-epikatonic acid (**15**) (Coxon & Wells, 1980), 24-hydroxy-3-oxo-olean-12-en-28-oic acid (**16**) (Hart et al., 1976), 23-hydroxy-3-oxo-olean-12-en-28-oic acid (**17**) (Verma, Singh & Nath, 1997), abruslactone A (**18**) (Chang, Chiang & Mak, 1982; Takaishi et al., 1997), dulcioic acid (**19**) (Takaishi et al., 1997), regelindiol A (**20**) (Pang, Zhao, Hori & Inayama, 1989), and hypodiol (**21**) (Duan, Kawazoe, Bando, Kido & Takaishi, 1997).

In a continuing study of immunosuppressive active principles, we have reported previously on the activity of celastrol (**11**) and 22β-hydroxy-tingenone (Takaishi et al., 1997). As a part of our work in search for immunosuppressive active principles, the inhibitory effect on cytokine (TNF-α, IL-1β, IL-2, 4, 8, and IFN-γ) production of compounds **1**, **5**, **7**–**10**, **12**–**15** and **18**–**20** were examined. The data for the isolated compounds which demonstrated an inhibitory effect are shown in Table 2. Compounds **7** and **13** showed an inhibitory effect on IL-2 release, compounds **10** and **15** inhibited IL-2, 4, 8 and THF-γ release, and dulcioic acid **19** showed a inhibitory effect on all of the cytokines produced by lipopolysaccharide-stimulated human peripheral mononuclear cells compared to the reference compound (prednisolone).

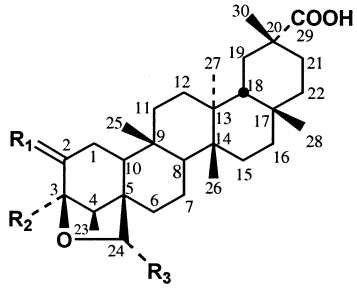
3. Experimental

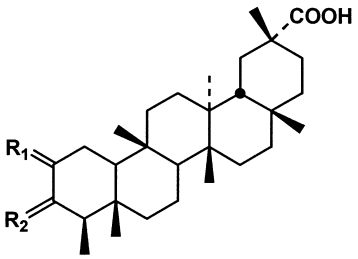
NMR spectra were acquired on a Bruker ARX-400 instrument. ^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz, using TMS as int. standard and MS were obtained on a JEOL JMSD-300 instrument. Chromatography column: silica gel 60 (Merck) and Sephadex LH-20 (Pharmacia); HPLC: GPC (Asahipak, GS-310 2G, MeOH), silica gel HPLC (YMC-Pack SIL-06 SH-043-5-06, 250 × 20 mm). IR spectra were recorded on a 1720 Infrared Fourier Transform Spectrometer (Perkin-Elmer), where UV spectra were obtained on a UV 2100 UV–Vis recording spectrometer (Shimadzu). Optical rotations were measured using a JASCO DIP-370 digital polarimeter.

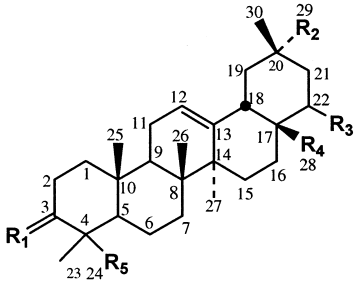
Table 2
The inhibition effect on cytokines of compounds **7**, **10**, **13**, **15** and **19**[a]

Compounds	Inhibition (%)					
	TNF-α	IL-8	IL-1β	IL-4	IL-2	IFN-γ
7	−67.1	−15.1	−27.5	40.6	77.1	−1.4
10	38.1	60.4	42.9	79.7	88.8	81.4
13	−9.4	−5.7	−15.3	27.0	66.7	11.2
15	19.1	66.2	18.8	72.9	85.3	65.9
19	77.5	97.9	82.3	98.5	100.0	99.3
Prednisolone	88.5	90.3	88.5	96.1	93.9	95.4

[a] Concentration: compounds **7**, **10**, **13**, **15** and **19**, 10 μg/ml; prednisolone, 0.3 μg/ml.



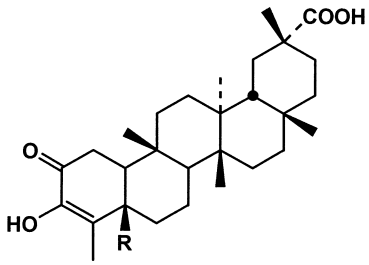


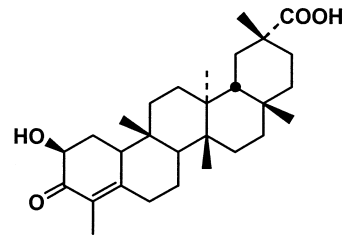


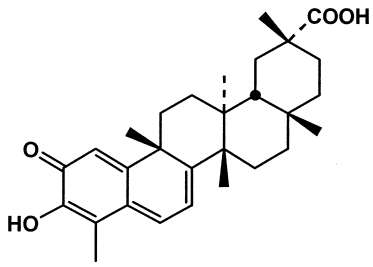
	R_1	R_2	R_3
1	α-OH, β-H	H	OEt
2	O	OH	H
9	α-OH, β-H	OH	H

	R_1	R_2
3	α-H, β-OH	O
6	O	α-H, β-OH
7	2H	O

	R_1	R_2	R_3	R_4	R_5
4	O	CH_2OH	H	COOH	Me
12	α-OH, β-H	COOH	α-OH	Me	Me
13	O	COOH	α-OH	Me	Me
14	O	COOH	β-OH	Me	Me
15	α-OH, β-H	COOH	H	Me	Me
16	O	Me	H	COOH	βCH_2OH
17	O	Me	H	COOH	αCH_2OH



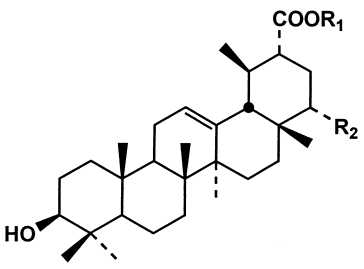


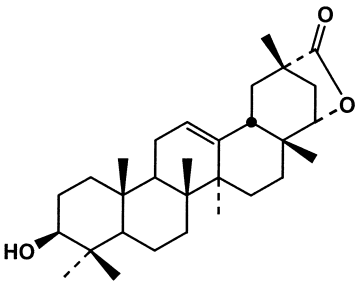


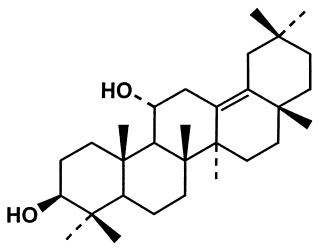
5 R=Me
8 R=CHO

10

11







	R_1	R_2
19	H	H
20	Me	OH

18

21

3.1. Isolation of compounds 1–21

The powdered extract T_{II} of *Tripterygium wilfordii* 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 water, then with chloroform and separated by column chromatography (silica gel, eluted with $CHCl_3$–MeOH, 95:5). Samples of T_{II} and the original plant (*T. wilfordii*) are deposited in the Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

The extract (T_{II}, 54 g) was subjected to chromatog-

raphy 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 frs (fr. 1–10). Fr. 2 was crystallized from MeOH to obtain **18** (52 mg). Fr. 5 (16.5 g) was chromatographed on a silica gel column (6 × 80 cm) and eluted with hexane–EtOAc (1:1, 1:2, 1:4) to give 12 frs (fr. 5.1–5.12). Combined fr. 5.2 + 5.3 (1.7 g) was chromatographed on Sephadex LH-20 to give three frs (fr. 5.2.1–5.2.3). Fr. 5.2.1 was separated by medium pressure liquid chromatography (MPLC, $CHCl_3$–MeOH, 99:1) and GPC (MeOH) to give **17** (14 mg) and **20** (37 mg). Fr. 5.2.2 was separated by MPLC ($CHCl_3$–MeOH, 99:1) and Si HPLC (hexane–EtOAc, 3:2) to give **2** (4.5 mg) and **5** (19 mg). Fr. 5.2.3 was separated by GPC (MeOH) and preparative TLC ($CHCl_3$–acetone) to give **8** (16 mg). Combined fr. 5.4 + 5.5 (1.7 g) was subjected to Sephadex LH-20 chromatography and medium-pressure liquid chromatography (MPLC, silica gel, $CHCl_3$–MeOH, 97:3) to give **15** (110 mg) and other six frs (fr. 5.4.1–5.4.6). Fr. 5.4.2 was separated by GPC (MeOH) and Si HPLC (hexane–EtOAc, 2:3) to give **6** (14 mg), **13** (11 mg) and **14** (4 mg). Fr. 5.4.5 was separated by GPC (MeOH) and Si HPLC (hexane–EtOAc–MeOH, 6.5:3:0.5) to give **4** (4.5 mg) and **10** (6 mg). Combined fr. 5.6 + 5.7 (2.7 g) was subjected to Sephadex LH-20 chromatography to obtain six frs (fr. 5.6.1–5.6.6). Fr. 5.6.2 was separated by GPC (MeOH) to give **1** (16 mg) and **9** (44 mg). Fr. 5.6.3 was separated by PTLC ($CHCl_3$–MeOH, 9:1) and Si HPLC (hexane–EtOAc, 1:3) to give **16** (4 mg), **19** (17 mg) and **21** (1.5 mg). Fr. 5.6.4 was separated by GPC (MeOH) and preparative TLC ($CHCl_3$–MeOH, 9:1) to give **3** (6 mg) and **7** (12 mg). Fr. 5.6.5 was separated by GPC (MeOH) to give **11** (40 mg) and **12** (31 mg).

3.2. Wilforic acid D (**1**)

Amorphous powder, $[\alpha]_D^{25}$ − 15.7° (MeOH, *c* 1.2). IR ν_{max}^{KBr} cm^{-1}: 3570, 3423, 2938, 2372, 1736, 1719, 1702, 1678, 1656, 1460, 1381, 1264, 1216, 1116, 1052, 1036, 963, 705. ^1H NMR ($CDCl_3$): δ 0.86 (3H, *s*, H_3-26), 0.92 (3H, *s*, H_3-25), 0.96 (3H, *s*, H_3-27), 1.05 (3H, *d*, *J* = 6.8 Hz, H_3-23), 1.08 (3H, *s*, H_3-28), 1.20 (3H, *t*, *J* = 7.3 Hz, OEt), 1.25 (3H, *s*, H_3-30), 1.73 (1H, *dt*, *J* = 4.1, 14.1 Hz, H_a-1), 1.97 (1H, *q*, *J* = 6.8 Hz, H-4), 2.15 (1H, *br d*, *J* = 14.2 Hz, H-6), 2.23 (1H, *br d*, *J* = 13.2 Hz, H-21), 2.32 (1H, *br d*, *J* = 14.2 Hz, H-19), 3.42 (1H, *dt*, *J* = 16.6, 7.3 Hz, OEt), 3.79 (1H, *dt*, *J* = 16.6, 7.3 Hz, OEt), 3.88 (1H, *d*, *J* = 4.9 Hz, H-3), 4.04 (1H, *br t*, *J* = 4.4 Hz, H-2), 5.02 (1H, *s*, H-24). ^{13}C NMR ($CDCl_3$), see Table 1. EI MS: *m*/*z* (rel. int.) 442 $[M - OEt]^+$ (100), 424 (50), 409 (13), 372 (14), 317 (24), 304 (20), 259 (14), 248 (25), 235 (35), 221 (20), 189 (30), 175 (32), 147 (41), 135 (25), 121 (42), 109 (56), 95 (54), 81 (38), 69 (29), 44 (50). FAB MS: *m*/*z* 515 $[M - H]^+$, HR FAB MS: *m*/*z* 515.3748 $[M - H]^+$, $C_{32}H_{51}O_5$ requires 515.3737.

3.3. Wilforic acid E (**2**)

Amorphous powder, $[\alpha]_D^{25}$ − 32.5° (MeOH, *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3469, 3436, 3369, 3307, 2930, 2873, 1736, 1702, 1678, 1656, 1630, 1459, 1451, 1389, 1191, 1121, 1005, 897, 700. ^1H NMR ($CDCl_3$): δ 0.88 (3H, *s*, H_3-26), 0.95 (3H, *s*, H_3-27), 1.04 (3H, *s*, H_3-25), 1.09 (3H, *d*, *J* = 7.5 Hz, H_3-23), 1.10 (3H, *s*, H_3-28), 1.26 (3H, *s*, H_3-30), 1.51 (1H, *q*, *J* = 7.5 Hz, H-4), 1.89 (1H, *br d*, *J* = 14.8 Hz, H-6), 1.98 (1H, *dt*, *J* = 14.0, 3.9 Hz, H-22), 2.15 (1H, *br d*, *J* = 14.6 Hz, H-21), 2.31 (1H, *br d*, *J* = 13.7 Hz, H-19), 2.53 (1H, *dd*, *J* = 16.4, 6.1 Hz, H_α-1), 2.74 (1H, *dd*, *J* = 16.4, 13.1 Hz, H_β-1), 3.87 (1H, *d*, *J* = 8.7 Hz, H′-24), 4.36 (1H, *d*, *J* = 8.7 Hz, H″-24). ^{13}C NMR ($CDCl_3$), see Table 1. EI MS: *m*/*z* (rel. int.) 486 $[M]^+$ (15), 458 (11), 332 (11), 304 (20), 289 (11), 235 (17), 221 (12), 189 (15), 175 (13), 163 (26), 155 (28), 147 (24), 135 (33), 125 (100), 121 (60), 109 (92), 95 (80), 81 (75), 67 (57), 55 (77), 41 (50). HR EIMS: *m*/*z* 486.3352 $[M]^+$, $C_{30}H_{46}O_5$ requires 486.3345.

3.4. Wilforic acid F (**3**)

Amorphous powder, $[\alpha]_D^{25}$ − 20.6° (MeOH, *c* 0.6). IR ν_{max}^{KBr} cm^{-1}: 3570, 3436, 2930, 1719, 1703, 1656, 1639, 1619, 1562, 1459, 1451, 1390, 1246, 1125, 1085, 981. ^1H NMR ($CDCl_3$): δ 0.70 (3H, *s*, H_3-24), 0.88 (6H, *s*, H_3-25, 26), 0.95 (3H, *d*, *J* = 6.7 Hz, H_3-23), 1.00 (3H, *s*, H_3-27), 1.10 (3H, *s*, H_3-28), 1.27 (3H, *s*, H_3-30), 1.99 (1H, *dt*, *J* = 14.1, 4.0 Hz, H-22), 2.16 (1H, *br d*, *J* = 14.4 Hz, H-21), 2.25 (1H, *q*, *J* = 6.7 Hz, H-4), 2.34 (1H, *br d*, *J* = 13.4 Hz, H-19), 2.44 (1H, *dd*, *J* = 10.6, 7.0 Hz, H-1), 4.08 (1H, *br d*, *J* = 10.5 Hz, H-2). ^{13}C NMR ($CDCl_3$), see Table 1. EI MS: *m*/*z* (rel. int.) 472 $[M]^+$ (43), 454 $[M - H_2O]^+$ (19), 318 (15), 289 (54), 264 (29), 250 (26), 235 (31), 209 (23), 189 (23), 163 (34), 155 (85), 147 (23), 135 (31), 121 (42), 109 (100), 95 (61), 81 (47), 69 (36), 55 (41), 43 (31). HR EIMS: *m*/*z* 472.3542 $[M]^+$, $C_{30}H_{48}O_4$ requires 472.3553.

3.5. 29-Hydroxy-3-oxo-olean-12-en-28-oic acid (**4**)

Amorphous powder, $[\alpha]_D^{25}$ + 66.0° (MeOH, *c* 0.4). IR ν_{max}^{KBr} cm^{-1}: 3652, 3570, 3530, 3443, 3327, 2944, 2376, 1720, 1703, 1667, 1664, 1656, 1639, 1619, 1562, 1389, 1032, 707, 605. ^1H NMR ($CDCl_3$): δ 0.77 (3H, *s*, H_3-26), 0.92 (3H, *s*, H_3-30), 0.99 (3H, *s*, H_3-23), 1.00 (3H, *s*, H_3-25), 1.04 (3H, *s*, H_3-24), 1.11 (3H, *s*, H_3-27), 2.33 (1H, *m*, H_α-2), 2.50 (1H, *m*, H_β-2), 2.84 (1H, *dd*, *J* = 13.8, 3.9 Hz, H-18), 3.25 (2H, *s*, H_2-29), 5.28 (1H, *br s*, H-12). ^{13}C NMR ($CDCl_3$), see Table 1. EI MS: *m*/*z* (rel. int.) 470 $[M]^+$ (14), 439 $[M - CH_2OH]^+$ (11),

424 (4), 251 (12), 233 (100), 219 (17), 201 (59), 187 (16), 159 (11), 145 (10), 119 (13), 105 (14), 95 (14), 81 (19), 69 (15), 55 (21), 41 (17). HR EIMS: m/z 470.3374 $[M]^+$, $C_{30}H_{46}O_4$ requires 470.3396.

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