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TRITERPENES FROM *TRIPTERIGIUM WILFORDII**

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Key Word Index—*Tripterigium wilfordii*; Celastraceae; wilforols; oleanane; D:B-friedooleanane; triterpenes.

Abstract—The acetone extract of dried root bark of *Tripterigium wilfordii* afforded two novel oleanane and D:B-friedooleanane triterpenes: wilforol C and D. Their structures were established on the basis of chemical and spectroscopic studies.

INTRODUCTION

In our previous paper, we reported the isolation of D:A-friedo-24-noroleanane triterpenoids from root bark of the *Tripterigium wilfordii* Hook f. [1]. In continuing studies on the chemical components of root bark of this plant, we have isolated two novel triterpenes, 3α,23-dihydroxy-olean-12-en-28-oic acid (**1**) and 3β-hydroxy-D:B-friedoolean-5-en-29-oic acid (**2**), which we have named wilforols D and E, along with four known triterpenes [3α, 24-dihydroxy-olean-12-en-28-oic acid (**3**); 3β, 29-dihydroxy-D:B-friedoolean-5-en (**4**); 3-hydroxy-D:A-friedoolean-24-al-3-en-2-on-29-oic acid (cangoronine, **5**) and 3-hydroxy-D:A-friedoolean-3-en-2-on-29-oic acid (**6**)]. Compounds **3**–**6** were identified by comparing their physicochemical and spectral data with those in the literature [2–5]. This paper deals with the structural investigations of the two novel triterpenes.

RESULTS AND DISCUSSION

An acetone extract of root bark of *T. wilfordii* afforded two novel triterpenes: an oleanane triterpene (wilforol C, **1**) and a D:B-friedooleanane triterpene (wilforol D, **2**).

Compound **1** was obtained as needles and gave a positive response to the Liebermann–Burchard test for triterpenes. Its molecular formula, $C_{30}H_{48}O_4$, was determined by high-resolution (HR) mass spectrometry and was the same as that of **3**.

The IR spectrum of **1** was very similar to that of **3**, including the adsorption bands due to the hydroxyl (3428 cm^{-1}) and carboxyl (1696 cm^{-1}) groups. The EI-mass spectrum of **1** showed the same fragmentation pattern as **3**, with important fragments at m/z 472 [M^+], 248, 203 (248 − COOH), which arose from retro Diels–Alder cleavage around ring C. It was a characteristic fragmentation pattern for an olean-12-ene triterpene [6, 7]. The ^1H and ^{13}C NMR data of **1** were also very similar to those of **3**. Thus, **1** was presumed to be a structural isomer of **3**.

The location of the carboxyl group was decided as follows. Among the possible positions (C-28, C-29, C-30) of the carboxyl group suggested by the mass spectral data of **1** described above, C-28 was thought to be the most reasonable because of the good agreement of the carbon signals ascribable to the ring B, C, D and E portions in the ^{13}C NMR spectra of **1** and **3** (Table 1). Furthermore, the presence of the *gem*-dimethyl group (C-29, C-30) at C-20 was confirmed by the correlation cross-peaks in the HMBC data of **1** (Fig. 1). Thus, the carboxyl group was determined to be at the C-28 position.

The locations of the two hydroxyl groups in **1** were decided as follows. One hydroxyl group was thought to be primary and the other to be secondary because of the signals in the ^1H NMR spectrum (δ3.94 (1H, *t*-like, J = 2.6 Hz), δ3.68 and 3 .88 (each 1H, *d*, J = 10.9 Hz)). The location of the secondary hydroxyl group at the C-3 position was highly probable on a biogenetic basis and its α-orientation was confirmed by the J-value of the H-3 methine signal (*t*, J = 2.6 Hz) in the ^1H NMR spectrum of **1**. The position of the primary hydroxyl group was established to be at C-23. The signals ascribable to C-3, C-4, C-5, C-6, C-23 and C-24 in the ^{13}C NMR spectrum of **1** were different from those of **3** and in particular the hydroxymethyl signal (δ71.3) showed a lower shift (5.5 ppm, axial → equatorial) and the methyl signal (δ18.2) attached to the C-4 position showed a higher shift (5.4 ppm, equatorial → axial, Table 1). The locations of the C-23 hydroxymethyl and the C-3α-hydroxyl groups

*Part 2 in the series 'Chemical Studies on the Root Bark of *T. Wilfordii*'. For Part 1 see [1].

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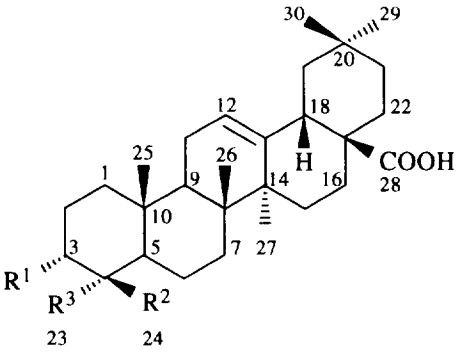
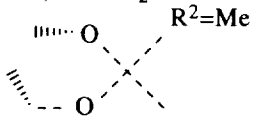
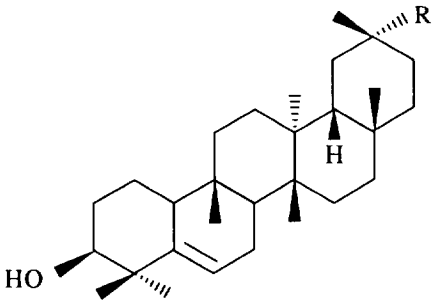


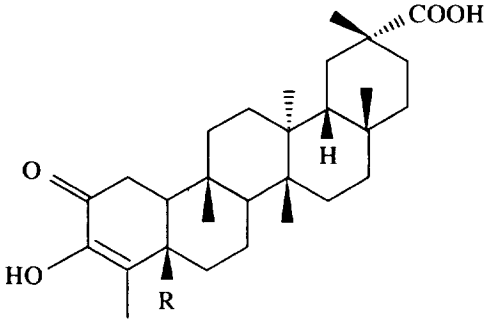
1 R^1= OH, R^2=Me, R^3=CH_2OH

1a R^1, R^3: 

3 R^1=OH, R^2=CH_2OH, R^3=Me



R
2 COOH
4 CH_2OH



R
5 CHO
6 Me

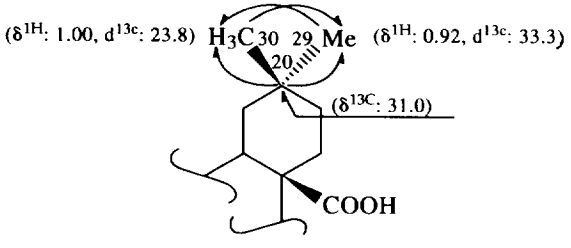


Fig. 1. HMBC data of compound **1**.

were confirmed by the physicochemical properties of the monoacetonide (**1a**) prepared from **1**. Thus, the structure of wilforol C was established as 3α-23-dihydroxy-olean-12-en-28-oic acid (**1**).

Wilforol D (**2**) was obtained as needles whose molecular formula was determined as $C_{30}H_{48}O_3$ by HR-mass spectrometry. Its IR spectrum showed hydroxyl (3400 cm^{-1}) and carboxyl (1700 cm^{-1}) adsorption. The ^1H NMR spectrum of **2** showed seven tertiary methyl, one oxygen-bearing methine (δ3.69, *t*, J = 2.8 Hz) and one vinyl proton (δ5.75, *d*, J = 5.8 Hz) signals. The EI-mass spectrum of **2** showed intense peaks at m/z 456 (M^+), 304, 259, 235, 152, 134, which were consistent with the fragmentation pattern characteristic for D:B-friedoolean-5-ene pentacyclic triterpenes (Fig. 2) [6]. The mass spectral fragmentations also suggested that the hydroxyl group was located in ring A or B and that the carboxyl group was on ring D or E. In the ^1H NMR spectrum of **2**, a methine signal at δ3.69 (1H, *t*, J = 2.8 Hz) showed the presence of the same 3β-substituted secondary hydroxyl group in **2** as in **4**. It was also supported by the good agreement of the signals due to the ring A portion of **2** and **4** in their ^{13}C NMR spectra (Table 1). The location of the carboxyl group was pre-

Table 1. ^{13}C NMR spectral data of triterpenoids from *T. wilfordii* (125 MHz, in pyridine-d_5 TMS as int. standard)

C	1	1a*	3	2	4	5†	6
1	33.4	32.5	33.9	18.9	19.0	32.7	34.5
2	26.5	23.0[a]	26.5	29.1	28.8	193.8	194.7
3	75.7	73.0	70.0	75.5	75.5	149.0	144.4
4	40.7	34.9	43.9	41.0	41.1	125.9	139.9
5	43.6	42.9	50.2	144.0	144.0	54.9	39.5[a]
6	18.4	17.8	19.1	119.9	119.9	33.3	38.6
7	33.0	32.5	33.8	24.1	23.9	18.9	18.2
8	39.9	39.6	40.0	45.5	48.3	49.5	50.2
9	48.1	47.5	48.2	35.0	35.2	37.1	36.9
10	37.3	36.8	37.5	50.6	50.7	55.5	55.7
11	23.7	23.3[a]	24.0	34.7	35.0	36.6	33.9
12	122.6	122.7	122.6	36.8	36.3	30.9	36.6
13	144.9	143.6	144.8	39.4	38.0[a]	39.6	39.7[a]
14	42.3	41.7	42.2	39.0	39.9[a]	39.4	39.4[a]
15	28.3	27.7	28.3	29.9	32.7	29.5	29.6
16	23.9	23.5[a]	23.7	30.5	30.8	30.5	30.5
17	46.7	46.6	46.7	30.6	30.8	30.5	30.5
18	42.0	41.0	42.0	45.0	42.6	44.8	44.8
19	46.5	45.9	46.5	31.0	30.2	30.9	30.9
20	31.0	30.7	31.0	40.7	33.7	40.7	40.7
21	34.2	33.9	34.3	29.4	29.3	29.4	29.4
22	33.3	32.9	33.2	37.0	39.9	37.4	37.4
23	71.3	68.4	23.6	26.2	26.3	10.7	10.6
24	18.2	17.3[b]	65.8	29.5	29.6	195.8	18.9
25	15.8	15.8	15.9	17.0	16.4	17.2	18.0[b]
26	17.5	17.4[b]	17.4	16.2	20.4	17.9	18.1[b]
27	26.2	26.0	26.1	17.6	18.2	16.2	16.4
28	180.2	184.1	180.2	31.9	32.2	32.1	32.1[c]
29	33.3	33.1	33.2	181.3	73.8	181.4	181.3
30	23.8	23.6	23.8	32.7	27.1	32.3	32.3[c]
		O | O – C $(CH_3)_2$ 19.3 29.3 98.1					

Assignments of **1**–**4** were based on the 2D NMR data (H–H, C–H, HMBC).
*Recorded at 100 MHz.
†Assignments were different from those in the literature [4].
[a-c]Assignments may be interchangeable in each column.

sumed to be at C-28, C-29 or C-30 by the EI-mass spectral data of **2**. In the HMBC data of **2**, the signal of the methyl at δ1.42 showed two significant correlation peaks between that of the carboxyl at δ181.3 and that of the quarternary carbon atom at δ40.7 (C-20), indicating that the carboxyl group was attached to C-20. Furthermore, in the comparisons of the ^{13}C NMR spectrum of **2** with those of **5** and **6**, which had the same substitution groups at the C-20 and the D/E ring structure, the signals assignable to ring D/E and their substitution groups agreed very closely with those of **5** and **6**. Thus, the carboxyl group was determined to be located at C-29. From the results mentioned above, wilforol D was formulated as 3β-hydroxy-D:B-friedoolean-5-en-29-oic acid (**2**). The structure was further confirmed by the chemical conversion of **4** into **2** as shown in Scheme 1.

Wilforol D (**2**) has the same skeleton as **4**. However, the ^1H and ^{13}C NMR data of **2** showed some disagreement with those of **4**. This was caused by the difference of the C/E ring conformation which resulted from the substitution of a group at C-20 [4, 8].

Compound **3** has been isolated previously from *Salvia nicolsoniana* (Labiatae) [2], and **4**, **5** and **6** have been isolated from various plants belonging to the Celastraceae [3–5]. This is the first report of isolation of these compounds from *Tripterigium wilfordii*.

EXPERIMENTAL

General. Mps: uncorr. ^1H NMR: 500 MHz; ^{13}C NMR: 125 MHz with TMS as int. standard; 2D NMR: 500 MHz in common conditions; EIMS: 70 eV. Silica gel

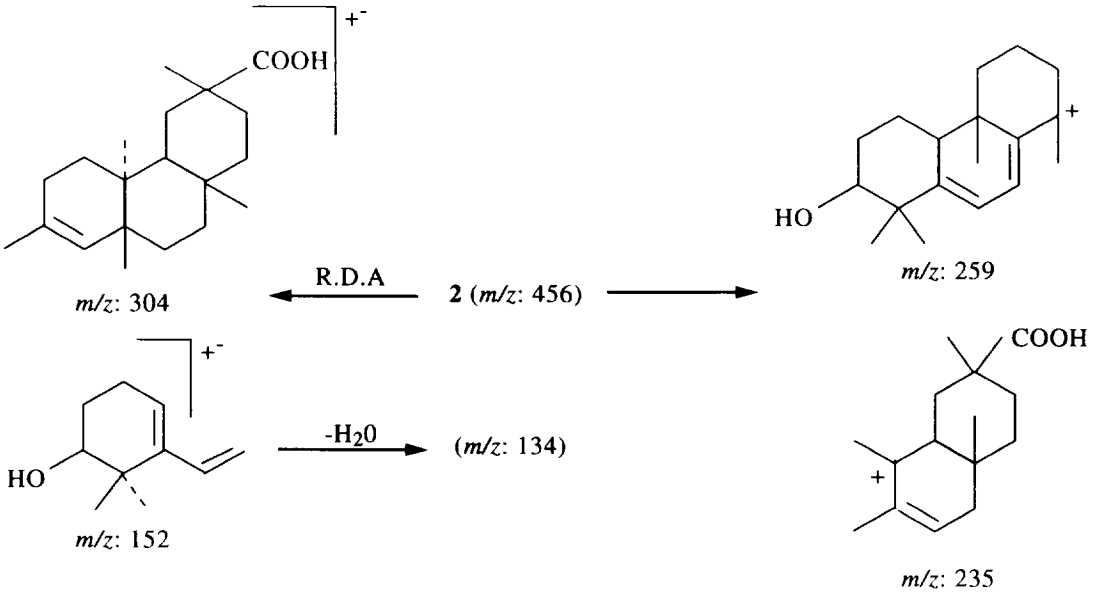
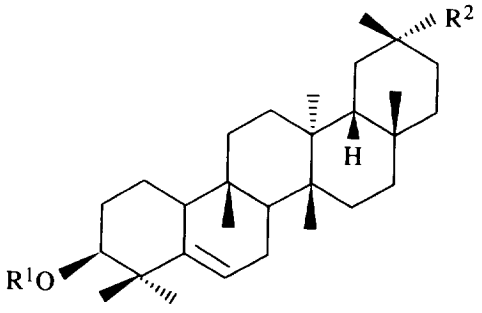


Fig. 2. Mass spectral fragments of compound **2**.



4 R^1=H, R^2=CH_2OH

↓ Py, Ac_2O

4a R^1 = Ac, R^2 = CH_2OAc

↓ 0.5N KOH, MeOH

4b R^1 = Ac, R^2 = CH_2OH

↓ CrO_3/20% H_2SO_4

4c R^1 = Ac, R^2 = COOH

↓ 4% KOH, 95% EtOH, Δ

2: R^1 = H, R^2 = COOH

Scheme 1. Chemical conversion of **4** into **2**.

column chromatography (CC) was carried out on Kieselgel 60 (70–230 mesh); prep. HPLC: column, CIG Si-10 (silica gel, 1.5 i.d. × 30 cm).

Plant material. See [1].

Isolation procedure. Among the frs afforded by the Sephadex LH-20 CC of fr. L3 [1], those which did not show a positive response to $FeCl_3$ were combined (1.2 g). This material was chromatographed on silica gel CC and eluted with 20% Me_2CO in benzene to afford four frs (1–4). Fr. 2 was crystallized from EtOH to give **4** (210 mg). Fr. 3 was crystallized from MeOH to give wilforol C (**1**, 56 mg). The Me_2CO in *n*-hexane (2:23) eluate (18.5 g) of silica gel CC of M3 [1] was further chromatographed over silica gel with an increasing amount of EtOAc in *n*-hexane to give 8 frs. Fr. 5 (2.1 g) was crystallized from MeOH to afford **3** (460 mg). Fr. 6 (5.3 g) was further purified with prep. HPLC (EtOAc–*n*-hexane, 1:4) and the rich fraction was crystallized from MeOH to give wilforol D (**2**, 35 mg). The mother liquor of celastrol [1] gave mixed crystals of two compounds (860 mg) with MeOH. They were separated by silica gel CC and each of the compounds obtained was crystallized from MeOH to give **5** (170 mg) and **6** (520 mg).

Wilforol C (3α, 23-*dihydroxy-olean*-12-*en*-28-*oic acid*, **1**). Needles (EtOH), mp > 300°. $[\alpha]_D^{26}$ + 84° (pyridine, *c* 0.18). IR ν_{max}^{KBr} cm^{-1}: 3428, 2940, 1696. EIMS *m/z* (rel. int.): 472 $[M]^+$ (0.4, calc. for $C_{30}H_{48}O_4$; 472.35526, found: 472.35687), 454 (0.5), 248 (100), 207 (5), 203 (77), 189 (13). ^1H NMR (pyridine-d_5): δ 0.79 (3H, *s*, H-23), 0.92 (3H, *s*, H-29), 0.96 (3H, *s*, H-25), 1.00 (3H, *s*, H-30), 1.07 (3H, *s*, H-26), 1.23 (3H, *s*, H-27), 3.30 (1H, *dd*, *J* = 13.7 and 4.6 Hz, H-18), 3.68 (1H, *d*, *J* = 10.9 Hz, H-23), 3.88 (1H, *d*, *J* = 10.9 Hz, H-23), 3.94 (1H, *t*, *J* = 2.6 Hz, H-3), 5.60 (1H, *t*, *J* = 3.7 Hz, H-12). ^{13}C NMR: see Table 1.

Acetonidation of **1**. A soln of **1** (25 mg) in dry Me_2CO (3 ml) was treated with 2,2-dimethoxypropane (50 μl) and a catalytic amount of *p*-toluene sulphonic acid (*p*-TsOH), and the mixture was stirred at 20° for 2 hr. After adding H_2O to the soln, it was neutralized with Na_2CO_3.

The product was then dissolved in H_2O and extracted with CH_2Cl_2. Work-up of the CH_2Cl_2 extract in the usual manner yielded the product, which was purified by silica gel CC with Me_2CO in *n*-hexane (2:23) to furnish **1a** (13.4 mg):amorphous powder. EIMS *m/z* (rel. int.): 512 $[M]^+$ (1), 454 (2), 248 (100), 207 (11), 203 (73), 189 (16). ^1H NMR (pyridine-d_5): δ0.69 (3H, *s*), 0.76 (3H, *s*), 0.90 (3H, *s*), 0.93 (3H, *s*), 0.94 (3H, *s*), 1.17 (3H, *s*), 1.39 (3H, *s*), 1.40 (3H, *s*), 2.82 (1H, *dd*, *J* = 13.6 and 3.9 Hz), 3.25 (1H, *d*, *J* = 12.4 Hz), 3.63 (1H, *t*, *J* = 2.6 Hz), 3.65 (1H, *d*, *J* = 12.4 Hz), 5.28 (1H, *t*, *J* = 3.4 Hz). ^{13}C NMR: see Table 1.

Wilforol D (3β-*hydroxy-D:B-friedoolean-5-en-29-oic acid*, **2**). Needles (EtOH), mp 280–282°. $[\alpha]_D^{26}$ + 47° (pyridine; *c* 0.53). IR ν_{max}^{KBr} cm^{-1}: 3400, 3036, 2952, 1700. EIMS *m/z* (rel. int.): 456 $[M]^+$ (10, calc. for $C_{30}H_{48}O_3$: 456.36035; found: 456.36152), 438 (5), 304 (100), 289 (94), 275 (15), 259 (39), 235 (44), 152 (45), 134 (96). ^1H NMR (pyridine-d_5): δ0.98 (3H, *s*, H-26), 1.00 (3H, *s*, H-25), 1.07 (3H, *s*, H-24), 1.11 (3H, *s*, H-28), 1.25 (3H, *s*, H-27), 1.39 (3H, *s*, H-23), 1.42 (3H, *s*, H-30), 2.37 (1H, *dt*, *J* = 14.0 and 3.9 Hz, H-22), 2.54 (1H, *d*, *J* = 14.0 Hz, H-21), 2.70 (1H, *d*, *J* = 15.2 Hz, H-19), 3.69 (1H, *t*, *J* = 2.8 Hz, H-3), 5.75 (1H, *d*, *J* = 5.8 Hz, H-6). ^{13}C NMR: see Table 1.

3β, 29-*Dihydroxy-D:B-friedoolean-5-en diacetate* (**4a**). An amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 2932, 1736. EIMS *m/z* (rel. int.): 526 $[M]^+$ (3), 466 (21), 332 (82), 317 (35), 259 (70). 134 (78). ^1H NMR ($CDCl_3$): δ0.85 (3H, *s*), 0.98 (3H, *s*), 1.04 (3H, *s*), 1.05 (3H, *s*), 1.07 (3H, *s*), 1.13 (3H, *s*), 1.20 (3H, *s*), 2.01 (3H, *s*, Ac), 2.07 (3H, *s*, Ac), 3.76 (2H, *s*), 4.69 (1H, *dd*, *J* = 3.5 and 2.3 Hz), 5.89 (1H, *d*, *J* = 5.9 Hz).

Selective deacetylation of **4a**. Compound **4a** (174 mg) was dissolved in 0.5 M KOH in MeOH and left for 1.5 hr at 22°. Usual work-up gave a residue (172 mg) which was purified with prep. HPLC (Me_2CO–*n*-hexane, 1:9) to give 120 mg 29-hydroxy-D:B-friedoolean-5-en-3β-yl (**4b**), needles (MeOH), mp 221–222°. IR ν_{max}^{KBr} cm^{-1}: 3500, 2932, 1712. EIMS *m/z* (rel. int.): 484 $[M]^+$ (3), 424 (11), 290 (82), 275 (43), 259 (100), 134 (56). ^1H NMR ($CDCl_3$):δ0.85 (3H, *s*), 1.00 (3H, *s*), 1.02 (3H, *s*), 1.04 (3H, *s*), 1.07 (3H, *s*), 1.09 (3H, *s*), 1.21 (3H, *s*), 2.01 (3H, *s*, Ac), 3.23 (1H, *d*, *J* = 10.3 Hz), 3.29 (1H, *d*, *J* = 10.3 Hz), 4.69 (1H, *dd*, *J* = 3.6 and 2.4 Hz), 5.56 (1H, *d*, *J* = 6.0 Hz).

Oxidation of **4b**. Several drops of 25% CrO_3 (in H_2SO_4–H_2O, 1:9) was added to the Me_2CO soln of **4b** (50 mg) and the mixture stirred for 3 hr at room temp., then *iso*-PrOH was added. The reaction mixture was partitioned between $CHCl_3$ and 1% HCl. Usual post-treatment of the $CHCl_3$ layer gave a residue (65 mg) which was purified with prep. HPLC (Me_2CO–*n*-hexane, 1:9) to give 55 mg of D:B-friedoolean-5-en-3-β-yl-29-oic acid (**4c**), needles (MeOH), mp 241–243°. IR ν_{max}^{KBr} cm^{-1}: 3256, 2932, 1726. EIMS *m/z* (rel. int.): 498 $[M]^+$ (2), 438 (20), 304 (78), 289 (73), 259 (30), 235 (38), 134 (90). ^1H NMR ($CDCl_3$): δ0.86 (3H, *s*), 0.93 (3H, *s*), 0.97 (3H, *s*), 1.036 (3H, *s*), 1.042 (3H, *s*), 1.08 (3H, *s*), 1.25 (3H, *s*), 2.01 (3H, *s*, Ac), 4.68 (1H, *t*, *J* = 3.1 Hz), 5.53 (1H, *d*, *J* = 6.0 Hz).

Hydrolysis of **4c**. Compound **4c** (55 mg) was hydrolysed with 4% KOH (3 ml, in 95% EtOH) under reflux for 1 hr. Usual post-treatment gave a residue (49 mg), which was purified with prep. HPLC (Me_2CO–*n*-hexane: 3:17) to give 39 mg 3-β-hydroxy-D:B-friedoolean-5-en-29-oic acid (**4d**). Its physicochemical and spectral data (mp, mmp, TLC, IR, MS, ^1H and ^{13}C NMR) were identical to those of **2**.

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