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A NOVEL EPOXY-TRITERPENE AND NORTRITERPENE FROM CALLUS CULTURES OF *TRIPTERYGIUM WILFORDII*

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Key Word Index—*Tripterygium wilfordii* var. *regelii*; Celastraceae; tissue culture; epoxy-triterpene; nortriterpene; triptocallic acid; CMR.

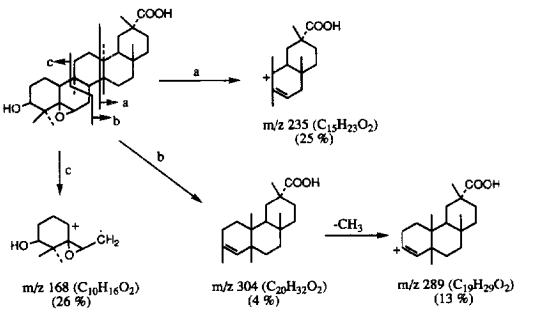
Abstract—The callus of *T. wilfordii* var. *regelii* afforded three new pentacyclic triterpenes D:B-friedoolean-3-ol-5,6-epoxy-29-oic acid, 3α, 22α-dihydroxy-olean-12-en-29-oic acid and 24, 29-dinor D:A-friedoolean 4-en-2β, 22β-dihydroxy-3, 21-dion, named triptocallic acid C, D and triptocalline A, respectively, in addition to five known triterpenes. These structures were determined by spectroscopic analyses. © 1997 Elsevier Science Ltd

INTRODUCTION

In the preceding paper [1], we reported on the isolation and structure elucidations of a new abietane diterpenoid (triptocallol) and two new pentacyclic triterpenoids (triptocallic acid A and B) from callus cultures of *Tripterygium wilfordii* var. *regelii* MAKINO. We now report on the isolation of novel epoxy-triterpene, D:B-friedoolean-3-ol-5,6-epoxy-29-oic acid (**1**), named triptocallic acid C, 3α, 22α-dihydroxy-olean-12-en-29-oic acid (**2**), named triptocallic acid D, and 24, 29-dinor D:A-friedoolean 4-en-2β,22β-dihydroxy-3, 21-dion, named triptocalline A (**3**), dulcioic acid (**4**), maytenoic acid (**5**), wilforlide A (**6**), wilfolor D (**7**) and salaspermic acid (**8**) from the same source, and the structural characterization of **1**, **2** and **3**.

RESULTS AND DISCUSSION

Triptocallic acid C (**1**) exhibited a molecular ion peak at *m/z* 472 in its mass spectrum and the molecular formula $C_{30}H_{48}O_4$ was determined by HR-MS. The IR spectrum of **1** showed hydroxyl (3380 cm^{-1}), carbonyl (1703 cm^{-1}) and epoxide (1260 cm^{-1}) adsorptions. These functional groups were further supported by the ^{13}C NMR data (δ 76.0, 182.7, 64.9 and 52.2). Seven methyls, 10 methylenes, five methines and eight quaternary carbons, were revealed by the DEPT spectra. The EI-mass spectrum of **1** (Scheme 1) showed major fragment ions at *m/z* 168 (26% of the base peak), 235 (25), 304 (8) and 289 (13). The peak at *m/z* 168 indicated that **1** had two oxygens in the A/B rings,



Scheme 1. Mass fragmentation pattern of compound **1**.

while the last three fragment ions indicated that a carboxylic acid was situated in the D/E rings.

The 2D NMR spectra suggested the partial structures A–F shown in Fig. 1. The HMBC and HOHAHA spectra were used to connect these partial structures to construct a pentacyclic triterpene skeleton (Table 1). The structure of **1** was established as the 5,6-epoxide based on the degree of unsaturation (seven) and the chemical shifts of C-5 (δ_C 64.9, C) and C-6 (δ_C 52.2, CH). In the NOESY spectrum the proton signals at δ 1.72 (H-10) were correlated with the proton signals at δ 1.04 (H_3-23), 1.40 (H-8) and 1.90 (H-2α), and the proton signal at δ 1.04 (H_3-23) were correlated with the proton signals at δ 1.72 (H-10), 1.90 (H-2α), 3.13 (H-6) and 3.68 (H-3). The coupling constants of H-3 showed the presence of an equatorial proton at C-3.

From the above evidence triptocallic acid C was shown to be 3β-hydroxy-D:B friedoolean-5β,6β-epoxy-29-oic acid (**1**).

Triptocallic acid D (**2**) showed $[M+Na]^+$ at *m/z* 495 (FAB-MS), suggesting the molecular formula of $C_{30}H_{48}O_4$. The ^1H NMR spectrum of **2** showed signals

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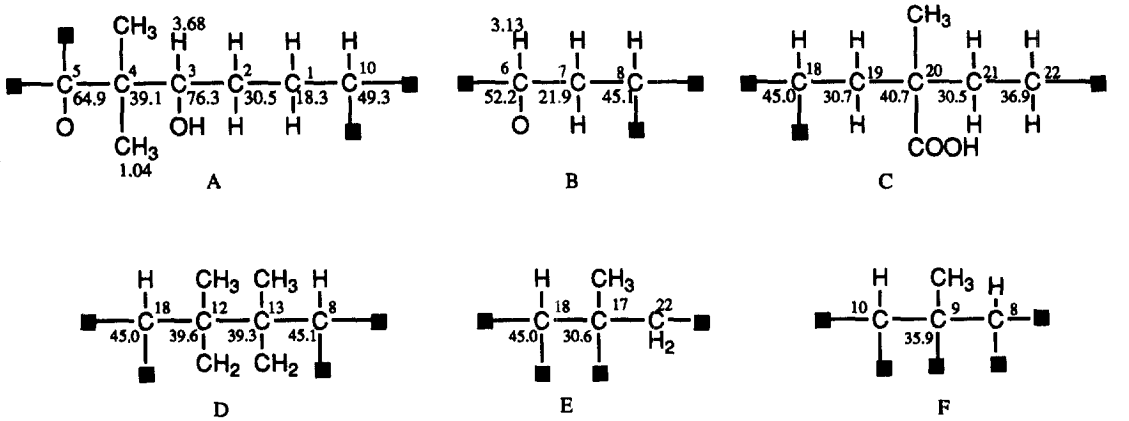
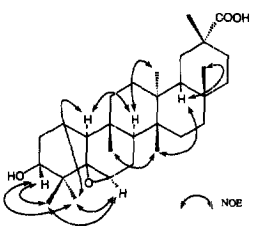


Fig. 1. Partial structures of triptocallic acid C (**1**)

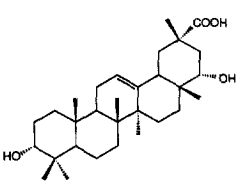
Table 1. One bond (H-C COSY) and multiple bond (HMBC) H-C correlation data of **1**

δ_H (ppm)	One bond correlation δ_C (ppm)	Multiple bond correlation δ_C (ppm)			
1.04 (23-Me)	25.4 (C-23)	20.9 (C-24)	39.1 (C-4)	64.9 (C-5)	76.0 (C-3)
1.07 (24-Me)	20.9 (C-24)	25.4 (C-23)	39.1 (C-4)	64.9 (C-5)	76.0 (C-3)
1.03 (25-Me)	18.3 (C-25)	35.1 (C-9)	45.1 (C-8)	49.3 (C-10)	35.3 (C-11)
0.88 (26-Me)	15.9 (C-26)	39.3 (C-14)	45.1 (C-8)	39.6 (C-13)	29.3 (C-15)
1.21 (27-Me)	17.8 (C-27)	39.6 (C-13)	29.4 (C-12)	39.3 (C-14)	45.0 (C-18)
1.11 (28-Me)	31.9 (C-28)	30.6 (C-17)	45.0 (C-18)	36.9 (C-22)	
1.44 (30-Me)	32.8 (C-30)	40.8 (C-20)	30.7 (C-19)	30.5 (C-21)	182.7 (C-29)
3.68 (3-αH)	76.0 (C-3)	64.9 (C-5)	18.3 (C-1)		
3.13 (6-αH)	52.2 (C-6)	21.9 (C-7)	45.1 (C-8)		
1.58 (18-H)	45.0 (C-18)	39.6 (C-13)	17.8 (C-27)	31.9 (C-28)	

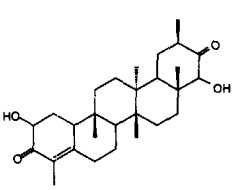
(in pyridine-d_5).



Triptocallic acid (1)



Triptocallic acid D (2)



Triptocalline A (3)

corresponding to one vinyl proton, seven tertiary methyl groups and two methine protons, indicating that **2** had a β-amyrin skeleton. The ^{13}C NMR spectrum of **2** (Table 2) showed one carboxylic acid (δ 186.3), two methines (δ 76.3, 76.5) attached to oxygen functions, one double bond (δ 123.2, 144.2), seven methyls, nine methylenes, three methines and six quaternary carbon signals. The ^1H and ^{13}C NMR data as well as the ^1H-^{13}C COSY, and ^1H-^{13}C long range COSY spectra were in full agreement with the structure **2**. The signal at δ 3.38 suggested an α configuration for the hydroxyl group at C-3, because its coupling constant was small and the C-24 carbon signal suggested a down-field shift (δ 22.8) relative to 3β-OH (δ 15.6) [2]. The second hydroxyl group of **2** was shown to be at C-22α from the NOESY spectrum. Cross-peaks were observed between H-22 and CH_3-28, and CH_3-30. Moreover, acetylation of **2** with acetic

Table 2. ^{13}C NMR spectral data of compounds **1–3**, wilforic acid B [3] and tingenine B

C	1	2	Wilforic acid B	3	Tingenine B
1	18.3	33.8	28.6	28.7	120.2
2	30.3	26.4	71.6	71.5	178.0
3	76.0	76.3	200.1	200.1	146.5
4	39.1	37.9	127.6	127.7	117.6
5	64.9	49.3	159.6	159.2	128.2
6	52.2	18.9	31.0	30.9	134.1
7	21.9	33.3	21.0	20.8	118.6
8	45.1	40.8	48.6	47.7	168.8
9	35.1	48.1	37.6	37.6	43.0
10	49.3	39.0	52.5	52.3	165.1
11	35.3	24.1	33.1	33.1	34.4
12	29.4	123.2	29.1	29.5	30.4
13	39.6	144.2	38.8	39.5	41.0
14	39.3	43.0	39.5	40.0	44.7
15	29.3	25.8	29.3	28.1	28.2
16	36.8	20.0	36.4	29.7	29.9
17	30.6	37.7	30.1	44.9	45.2
18	45.0	47.7	44.3	45.3	45.5
19	30.7	38.2	29.5	31.7	32.4
20	40.7	44.2	40.4	41.3	41.3
21	30.5	41.8	30.0	214.0	213.7
22	36.9	76.5	36.1	77.2	76.8
23	25.4	28.7	11.2	11.2	10.7
24	20.9	22.8			
25	18.3	15.7	16.7	15.6	39.6
26	15.9	17.3	16.2	19.1	22.6
27	17.8	26.3	17.7	17.5	20.9
28	31.9	25.1	30.2	25.2	25.4
29	32.8	186.3	184.7		
30	182.7	21.5	31.7	14.8	15.1

anhydride in pyridine at room temperature formed an acetyl γ-lactone (**2a**, $C_{32}H_{48}O_4$).

From the above evidence triptocallic acid D was shown to be 3α,22α-dihydroxy-olean-12-en-29-oic acid (**2**).

Triptocalline A (**3**) was found to contain six methyls [δ_H 0.82, 0.84, 0.98 (each 3H, *s*), 1.06 (3H, *d*, $J = 6.27$ Hz), 1.37, 1.85 (each 3H, *s*)] and two methines [δ_H 4.03 (1H, *dd*, $J = 5.73$, 13.9 Hz) and 4.59 (1H, *br s*)] attached to hydroxy groups. The ^{13}C NMR spectrum of **3** (Table 2) showed a α,β-unsaturated carbonyl carbon signals at δ 159.2, 127.6 and 200.1, a six membered ring carbonyl at δ 214.0, six methyls, eight methylenes, four methines and four quaternary carbon signals. The ^{13}C NMR spectral data of **3** were very similar to those of wilforic acid B [3] (Table 2) except for the signals due to the D, E rings, which were identical to those of 22β-hydroxytingenone (tingenin B) [4]. In the mass spectrum of **3**, the $[M]^+$ was at *m/z* 442.3068 which agreed with a molecular formula $C_{28}H_{42}O_4$.

From the above evidence **3** was shown to be 24, 29-dinor-D:A-friedoolean-4-en-2β,22β-dihydroxy-3,21-dion.

Compounds **4–8** were shown to be 3β-hydroxy-urs-12-en-oic acid (dulcioic acid) [5], maytenoic acid [6], wilforlide A [7], wilfolor D [8] and salaspermic acid [9] by comparing their physical and spectral data with those in the literature.

EXPERIMENTAL

The instrument used to obtain physical data and the experimental conditions for chromatography were the same as described in the preceding paper [1].

Isolation of compounds **1–8**. The remaining frs of cell extract which described in the preceeding paper [1] were repeatedly chromatographed on silica gel and Sephadex LH-20 columns to afford triptocallic acid C (**1**) (11 mg), D (**2**) (9 mg) and triptocalline A (**3**) (2 mg) along with known compounds **4** (10 mg), **5** (23 mg), **6** (13 mg), **7** (4 mg) and **8** (5 mg). **4–8** were identified by comparison with published data.

Triptocallic acid C (**1**). Needles, mp 259–260° (decomp.), $[\alpha]_D + 44.4°$ ($CHCl_3$–MeOH, *c* 0.27). EI-MS *m/z*: 472 $[M]^+$ (calcd for $C_{30}H_{48}O_4$: 472.3542, Found: 472.3505), 304, 289, 248, 235, 187, 168; IR ν_{max}^{KBr} cm^{-1}: 3380, 1703, 1455, 1385, 1260, 1213, 1170; ^1H NMR (d_5-Py) δ: 0.88, 1.03, 1.04, 1.07, 1.11, 1.21, 1.44 (each 3H, *s*), 1.58 (1H, *br d*, $J = 7.33$ Hz, 18-H), 1.72 (1H, *dd*, $J = 2.93$, 12.69 Hz, 10-H), 1.90 (1H, *br t d*, $J = 13.67$ Hz, 2-αH), 2.01 (1H, *br dd*, $J = 2.93$, 13.67 Hz, 2-βH), 2.19 (1H, *ddd*, $J = 3.42$, 12.7, 13.0

Hz, 1-βH), 2.41 (1H, *br dt*, $J = 3.42$, 13.67 Hz, 22-αH), 2.58 (1H, *br d*, $J = 14.16$ Hz, 21-βH), 2.70 (1H, *br d*, $J = 15.63$ Hz, 19-βH), 3.13 (1H, *d*, $J = 4.88$ Hz, 6-H), 3.68 (1H, *br s*, 3-H); ^{13}C NMR (Pyridine-d_5): Table 2.

Triptocallic acid D (**2**). Amorphous powder, $[\alpha]_D + 5.38°$ ($CHCl_3$–MeOH, *c* 0.52). FAB-MS *m/z*: 495 $[M+Na]^+$; ^{1}H NMR ($CHCl_3+CD_3OD$) δ: 0.85, 0.95, 0.97, 0.98, 0.99 (each 3H, *s*), 1.20 (6H, *s*), 3.38 (1H, *s*), 3.50 (1H, *dd*, $J = 4.40$, 12.21 Hz), 5.24 (1H, *br s*). ^{13}C NMR ($CDCl_3+CD_3OD$): Table 2. **2** (4 mg) was acetylated with Ac_2O–Pyridine at room temp. to give acetyl γ-lactone **2a** (2 mg); EI-MS *m/z*: 496 $[M]^+$ ($C_{32}H_{48}O_4$), 436, 246, 190; ^{1}H NMR ($CDCl_3$) δ: 0.86, 0.88, 0.90, 0.94, 0.97, 1.13, 1.21 (each 3H, *s*), 2.07 (3H, *s*), 4.16 (1H, *d*, $J = 5.4$ Hz), 4.46 (1H, *br s*), 5.30 (1H, *br s*).

Triptocalline A (**3**). Amorphous powder. EI-MS *m/z*: 442 $[M]^+$ (calcd for $C_{28}H_{42}O_4$: 442.3038, Found: 442.3063); ^{1}H NMR ($CDCl_3$) δ: 0.82, 0.84, 0.98, 1.37, 1.85 (each 3H, *s*), 1.06 (3H, *d*, $J = 6.27$ Hz), 2.74 (1H, *six*, $J = 6.35$ Hz), 2.95 (1H, *dd*, $J = 4.19$, 16.35 Hz), 4.03 (1H, *dd*, $J = 5.73$, 13.90 Hz), 4.59 (1H, *s*); ^{13}C NMR ($CDCl_3$): Table 2.

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