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A DITERPENOID AND TRITERPENES FROM TISSUE CULTURES OF *TRIPTERYGIUM WILFORDII*

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Key Word Index—*Tripterygium wilfordii* var. *regelii*; Celastraceae; tissue culture; abietane diterpenoid; ursane-triterpenoid; multiflorenol 29-oic acid; nortriterpenoids.

Abstract—Explants of *Tripterygium wilfordii* var. *regelii* were induced to form a callus on a Gamborg B5 medium. Three new compounds were isolated from the callus extracts and their structures were established on the basis of spectroscopic and chemical evidence. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Tripterygium wilfordii Hook f. has been used as an anticancer drug and an insecticide by the Chinese for hundreds of years. Two antileukaemic principles, triptolide and tripdiolide, have been isolated from this plant [1] and have been shown to be produced by plant tissue cultures [2–4].

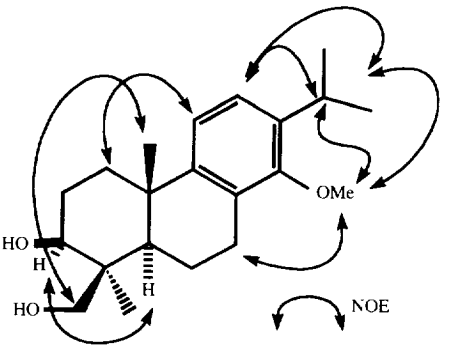
In a previous paper, we reported on the isolation of triptoquinones A–F, which are interleukin-1 inhibitors, from the stems of *T. wilfordii* var. *regelii* Makino [5]. In a continuation of our studies on this plant, we induced callus formation in order to produce these diterpenes. However, culture extracts did not appear to contain triptoquinones when examined by TLC. Investigation of the products present led to the isolation of a new abietane diterpenoid, named triptocallol (**1**), and two new pentacyclic triterpenoids, named triptocallic acid A (**2**) and B (**3**), together with five known compounds **4**–**8**. This paper deals with the isolation and structure elucidation of the new compounds.

RESULTS AND DISCUSSION

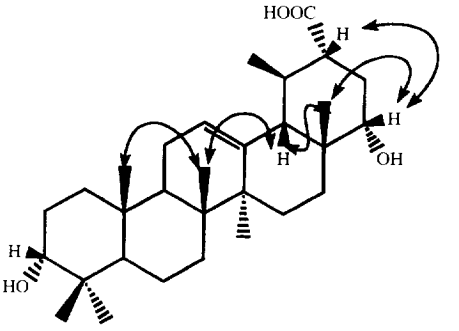
Leaf explants from *T. wilfordii* var. *regelii* plants maintained under sterile conditions were used to establish callus cultures. The callus material was subcultured monthly and after 8 months appeared green, black and white. The crude methanol extract of the callus was partitioned between ethyl acetate and water. Repeated column chromatography of the ethyl acetate fraction yielded triptocallol (**1**), triptocallic acid A and B (**2** and **3**), ifflaionic acid (**4**) [6], katononic acid (**5**) [7], epikatonic acid (**6**) [7] and aromatic nortriterpenes (**7** and **8**) [8, 9], along with quinoid triterpenes (celastol, tingenone and 22β-hydroxytingenone).

The molecular formula, $C_{21}H_{32}O_3$ of **1** was indicated by EI-mass spectrometry ($[M]^+$ *m/z* 332). The ^1H NMR spectrum showed the presence of one isopropyl group [δ 1.19, 1.20 (each 3H, *d*, J = 6.84 Hz), 3.27 (1H, *sep*, J = 6.84 Hz)], two methyl groups [δ 1.15, 1.32 (each 3H, *s*)], one methoxyl group [δ 3.71 (3H, *s*)], one hydroxy methylene [δ 3.43, 4.31 (each 1H, ABq, J = 11.23 Hz)], a hydroxy methine [δ 3.52 (1H, *dd*, J = 4.39, 11.72)] and two aromatic protons [δ 6.99, 7.04 (each 1H, *d*, J = 8.79 Hz)]. The ^{13}C NMR spectrum showed 21 carbon signals including those assignable to a tetra-substituted aromatic ring [δ 120.6 (*d*), 123.9 (*d*), 128.3 (*s*), 138.3 (*s*), 147.9 (*s*), 154.8 (*s*)]. Comparison of the ^1H and ^{13}C NMR spectral data for **1** with those of triptoquinone C [5] indicated the same abietane skeleton the difference between the two compounds being in ring C. It was assumed that the methoxyl group was at C-14, and the isopropyl group at C-13. The 4β axial native of the hydroxyl methyl group was based on cross peaks between Me-20 and the hydroxy methyl group in a 2D NOESY experiment. Thus, the structure of triptocallol was established as 14-methoxyabieta-8,11,13-trien-3β,19-diol (**1**).

Triptocallic acid A (**2**) showed $[M+Na]^+$ at *m/z* 495 (FAB-MS) suggesting the molecular formula of $C_{30}H_{48}O_4$. The IR data showed the presence of a hydroxyl group (3442 cm^{-1}) and a carboxylic group (1692 cm^{-1}). The ^1H NMR spectrum of **2** indicated the presence of six tertiary and one secondary methyl group and two secondary hydroxyl methines. Compound **2** gave the diacetate **2a** on acetylation with acetic anhydride–pyridine, thus confirming the presence of two hydroxyl groups in the molecule. The HR-mass spectrometry of **2a** showed $[M]^+$ at *m/z* 556.3749



Triptocallol (**1**)



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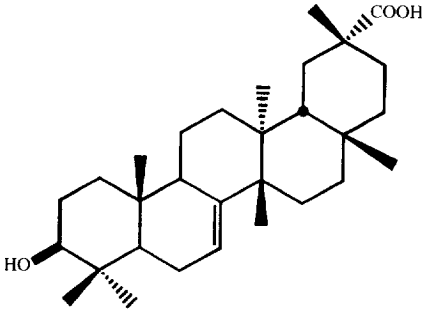
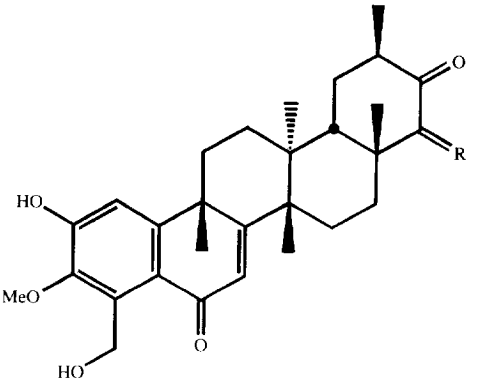


Triptocallic acid B (**3**)



7 R=H

8 R= 

(calculated for $C_{34}H_{52}O_6$, 556.3764). Other important fragments at *m/z* 306 and 250 could be explained by the typical fragmentation of a 12-en-triterpenoid [10] with a carboxyl group and an acetate group located on the D/E ring and another acetate group on the A/B ring.

The ^1H and ^{13}C NMR data as well as the ^1H-^{13}C COSY and ^1H-^{13}C Long Range COSY spectra were fully in agreement with the structure **2**. The ^1H NMR signal of H-3 at δ 3.63 (*br s*) and the small coupling constant between H-3 and H-2 indicated the α-configuration of 3-OH in **2**. Furthermore, the C-24 methyl group showed a down-field shift (δ 22.5) relative to α-amyrin (δ 15.6) [11]. 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, H-22 and H-20, CH_3-28 and H-18. Thus, the structure of triptocallic acid A was assigned as 3α,22α-dihydroxy-urs-12-en-30-oic acid (**2**).

Triptocallic acid (**3**) showed $[M]^+$ at *m/z* 456.3600 (HR-MS) suggesting the molecular formula $C_{30}H_{48}O_3$ (calculated 456.3603). The ^1H NMR spectrum exhibited, in addition to signals for seven tertiary methyl groups (δ 0.75, 0.85, 0.95, 0.97, 1.00, 1.02, 1.21), a one-proton doublet-like signal (δ 5.46) for a vinylic proton of a trisubstituted double bond and a carbinyl proton signal of an equatorial hydroxyl group at δ 3.19 (*dd*, J = 6.83, 8.79 Hz). The ^{13}C NMR spectrum of **3** showed 30 carbon atoms; the multiplicities of which were determined by using DEPT experiments. This revealed the presence of 7 methyl, 10 methylene, 4 methine, 1 olefine (δ 117.6, 145.7), 1 carbonyl (δ 182.8) and 6 quaternary carbons. The EI-mass spectrum of **3** showed intense peaks at *m/z* 456 $[M]^+$, 441, 423, 259, 247, 235, 220, 189 and 135, which were consistent with the fragmentation pattern characteristic for D:C-friedoolean-7-en pentacyclic triterpenes [10, 12]. Moreover, the mass spectral fragmentations suggested that the carboxyl group was located in ring D/E, and that the hydroxyl group was

in ring A/B. The ^{13}C NMR data of **3** were similar to those of wilforic acid C [13] for C-15 to C-22 and C-28 to C-30, and swertenol [14] for C-1 to C-14 and C-23 to C-25. These data suggested that the A/B ring systems were the same as in swertenol and the D/E ring system as in wilfolic acid C. Thus, triptocallic acid B was shown to be 3β-hydroxymultiflor-7-en 29-oic acid (**3**). This is the first example of a migrated multifloren type triterpenoid acid representing a D:C-friedooleane skeleton.

Compounds **4**–**8**, were identified as ifflaionic acid, katononic acid, 3-epikatonic acid and aromatic nor-triterpenes by comparing their physical and spectral data with those in the literature.

EXPERIMENTAL

^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) with TMS as int. standard; CC: silica gel 60 (Merck), silanized silica gel 60 (Merck) and Sephdex LH-20 (Pharmacia).

Plant and cell culture. Tripterygium wilfoldii var. *regelii* Makino plants and seeds were collected in July 1993 on Mt Turugi (Tokushima Prefecture, Japan). Cultures were started from leaf explants which were derived from the aseptically germinated seedling. The medium used was Gamborg B5 medium containing 2 mg l^{-1} NAA, 0.02 mg l^{-1} BAP and 0.8% Agar (Kanto Kagaku). The cultures were grown at 23±2°, 2000 lux, 16 hr light day^{-1}. Calluses were routinely transferred at 4 week intervals.

Isolation of products. Fresh cells (750 g) were extracted with MeOH at room temp overnight. After filtration, the residue was further extracted with hot MeOH for 2 hr. The combined filtrates were evapd and the residue (13.5 g) partitioned between EtOAc and H_2O. The EtOAc layer (4.3 g) was repeatedly chromatographed on a silica gel column (hexane–Me_2CO hexane–EtOAc and $CHCl_3$–MeOH), a silanized silica gel column (60% MeOH) and a Sephadex LH-20 column (MeOH, 80% MeOH and $CHCl_3$–MeOH) to afford **1** (3 mg), **2** (25 mg), **3** (5.2 mg), **4** (2.2 mg), **5** (3.6 mg), **6** (29.5 mg), **7** (11 mg), **8** (6 mg), tingenone and 22β-hydroxy tingenone (143 mg) and celastrol (331 mg). The known compounds **4-8** were identified by comparison with published spectral data. Since no ^{13}C NMR data for **4** was included in the literature, the data which was rigorously coincident with the structure is described below.

Triptocallol (14-*methoxyabieta*-8,11,13-*trien*-3β,19-*diol* **1**). Amorphous powder, $[\alpha]_D$ +42.5° ($CHCl_3$; *c* 0.4). EI-MS *m/z* (rel. int.): 332 $[M]^+$ (100), 299 (46), 239 (37); ^1H NMR ($CDCl_3$): δ 1.15 (3H, *s*, Me-20), 1.19, 1.20 (each 3H, *d*, *J* = 6.84 Hz, Me-16, 17), 1.32 (3H, *s*, Me-18), 3.27 (1H, *sep*, *J* = 6.84 Hz, H-15), 3.43 (1H, *d*, *J* = 11.2 Hz, Ha-19), 3.52 (1H, *dd*, *J* = 4.39, 11.72 Hz, H-3), 3.78 (3H, *s*, OMe), 4.31 (1H, *d*, *J* = 11.2 Hz, Hb-19), 6.99, 7.05 (each 1H, *d*, *J* = 8.79 Hz, H-11, 12); ^{13}C NMR ($CDCl_3$): δ 36.9, 28.5, 80.6, 42.9, 50.4, 18.5, 25.6, 128.3, 147.9, 37.3, 120.6, 123.9, 138.3, 154.8, 25.9, 23.9, 24.0, 22.5, 64.2, 26.1 (C_1-C_{20}), 60.5 (OMe).

Triptocallic acid A (3α,22α-*dihydroxy-urs*-12-*en*-30-*oic acid* **2**). Needles, mp 230–235°, $[\alpha]_D$ +63.5° ($CHCl_3$; *c* 1.09). FAB-MS *m/z*: 495 $[M+Na]^+$, $C_{30}H_{48}O_4$; IR ν_{max}^{KBr} cm^{-1}: 3440, 1692, 1461, 1389, 1255, 1213, 1068, 1025, 994, 671; ^1H NMR (C_5D_5N): δ 0.93, 1.02, 1.11, 1.18, 1.24, 1.33 (each 3H, *s*), 1.17 (3H, *d*, *J* = 5.37 Hz), 3.63 (1H, *br s*, H-3), 3.74 (1H, *dd*, *J* = 4.88, 10.74 Hz, H-22), 5.31 (1H, *br d*, H-12); ^{13}C NMR (C_5D_5N): δ 33.9, 26.4, 75.2, 37.9, 18.4, 33.1, 40.6, 47.8, 37.3, 23.4, 125.6, 139.2, 43.0, 26.5, 21.4, 39.7, 58.2, 34.9, 51.2, 34.9, 77.2, 29.3, 22.8, 15.8, 17.1, 23.6, 25.3, 19.0, 178.3 (C_1-C_{30}). **2** (8 mg) was acetylated with Ac_2O–Py to give acetate **2a** (3 mg), EI-MS *m/z* (rel. int.): 556 $[M]^+$ ($C_{34}H_{52}O_6$) (14.5), 306 $[C_{18}H_{26}O_4]^+$ (47.8), 246 $[C_{16}H_{22}O_2]^+$ (100), 190 $[C_{14}H_{22}]^+$ (96), 120 (74); ^1H NMR ($CDCl_3$): δ 0.85 (3H, *d*, *J* = 7.81 Hz), 0.87, 0.88, 0.90, 0.97, 1.01, 1.26 (each 3H, *s*), 2.05, 2.08 (each 3H, *s*, OAc), 4.59 (1H, *dd*, *J* = 4.88, 11.7 Hz, H-22), 4.65 (1H, *br s*, H-3), 5.21 (1H, *br t*, H-12).

Triptocallic acid B (3β-*hydroxymultiflor*-7-*en*-29-*oic acid*, **3**). Amorphous powder, $[\alpha]_D$ −15.9° ($CHCl_3$–MeOH; *c* 0.7). EI–MS *m/z* (rel. int.): 456 $[M]^+$ ($C_{30}H_{48}O_3$) (88), 423 (42), 259 $[C_{18}H_{27}O]^+$ (65.8), 235 $[C_{15}H_{23}O_2]^+$ (97), 220 $[C_{15}H_{24}O]^+$ (76), 189 $[C_{14}H_{21}]^+$ (71), 95 (100); ^1H NMR ($CDCl_3$): δ 0.75, 0.85, 0.95, 0.97, 0.99, 1.02, 1.21 (each 3H, *s*), 3.19 (1H, *dd*, *J* = 8.79, 6.83 Hz, H-3), 5.46 (1H, *br d*, H-7); ^{13}C NMR ($CDCl_3$): δ 37.6, 27.6, 79.3, 39.2, 50.7, 24.5, 117.6, 145.7, 48.3, 35.5, 17.5, 33.3, 37.3, 42.5, 30.9, 37.3, 31.6, 45.8, 30.9, 40.6, 29.5, 36.0, 27.8, 15.0, 13.4, 25.2, 27.8, 31.5, 182.8, 33.5 (C_1-C_{30}).

Compound **4**. Amorphous powder, $[\alpha]_D$ +17.5° ($CHCl_3$; *c* 0.2), EI-MS *m/z* (rel. int.): 454 $[M]^+$ ($C_{30}H_{46}O_3$) (11), 248 $[C_{16}H_{24}O_2]^+$ (100), 207 (3), 187 (13); ^1H NMR ($CDCl_3$): δ 0.80 (3H, *d*, *J* = 5.86 Hz), 0.83 (3H, *s*), 1.06 (6H, *s*), 1.08, 1.10, 1.12 (each 3H, *s*), 5.17 (1H, *br s*, H-12); ^{13}C NMR ($CDCl_3$): δ 39.5, 34.2, 217.7, 47.7, 55.3, 19.6, 32.4, 40.0, 46.9, 36.6, 23.5, 124.7, 138.7, 42.1, 25.6, 27.9, 33.6, 58.0, 34.4, 53.1, 26.4, 40.6, 26.5, 21.5, 15.4, 16.7, 23.4, 28.4, 182.0, 18.7 (C_1-C_{30}). Identified with ifflaionic acid [6].

Compound **5**. Amorphous powder, $[\alpha]_D$ +28.0° ($CHCl_3$; *c* 0.11). EI-MS *m/z* (rel. int.): 454 $[M]^+$ ($C_{30}H_{46}O_3$) (7.6), 410 (8.7), 248 $[C_{16}H_{24}O_2]^+$ (100), 187 (42), 173 (30); ^1H NMR ($CDCl_3$): δ 0.87 (6H, *s*), 1.02, 1.06, 1.07, 1.10, 1.15 (each 3H, *s*), 5.25 (1H, *br s*, H-12); ^{13}C NMR ($CDCl_3$): δ 39.3, 34.2, 217.9, 47.5, 55.2, 19.6, 32.4, 39.8, 46.9, 23.5, 122.6, 144.0, 41.6, 25.9, 26.9, 32.4, 46.0, 40.2, 42.5, 28.9, 35.7, 26.5, 21.5, 15.2, 16.7, 25.8, 28.2, 182.1, 19.1 (C_1-C_{30}). Identified with katononic acid [7].

Compound **6**. Colourless needles, $[\alpha]_D$ +66.7° ($CHCl_3$–MeOH; *c* 0.3). IR ν_{max}^{KBr} cm^{-1}: 3380, 2935, 1708, 1380; EI-MS *m/z* (rel. int.): 456 $[M]^+$ ($C_{30}H_{48}O_3$) (7.8), 248 $[C_{16}H_{24}O_2]^+$ (100), 207 $[C_{14}H_{23}O]^+$ (9), 187 $[C_{14}H_{19}]^+$ (26), 173 (18). Identified with epikatonic acid [7].

Compound **7**. Amorphous powder, $[\alpha]_D$ −33.7°

($CHCl_3$; *c* 0.77). IR ν_{max}^{KBr} cm^{-1}: 3417, 1708, 1640, 1579, 1456, 1381, 1311, 1031, 875, 757, 566; UV λ_{max}^{MeOH} nm (ε): 250 (9900), 314 (5600); EI-MS *m/z* (rel. int.): 466 $[M]^+$ ($C_{29}H_{38}O_5$) (49), 451 $[C_{28}H_{35}O_5]^+$ (100), 248 (7.6), 231 (45). Identified with 3-methyl-23-hydroxy-6-oxo-tingenol [8].

Compound **8**. Amorphous powder, $[\alpha]_D$ −55.5 ($CHCl_3$; *c* 1.8). IR ν_{max}^{KBr} cm^{-1}: 3470, 1713, 1679, 1609, 1561, 1456, 1378, 1252, 1196, 1119, 994, 841, 758, 669; UV λ_{max}^{MeOH} nm (ε): 250 (12 600), 315 (7800); EI-MS *m/z* (rel. int.): 482 $[M]^+$ ($C_{29}H_{38}O_6$) (15), 467 $[C_{28}H_{35}O_6]^+$ (100), 231 (68). Identified with 3-methyl-22β,23-dihydroxy-6-oxo-tingenol [8, 9].

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