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Phytochemistry 65 (2004) 2071–2076
PHYTOCHEMISTRY
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Kaurane and abietane diterpenoids from *Tripterygium doianum* (Celastraceae)

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Received 18 November 2003; received in revised form 1 April 2004
Available online 2 July 2004

Abstract

Extraction of *Tripterygium doianum* (Celastraceae) afforded five new diterpenoids and 11 known diterpenoids belonging to the *ent*-kaurane and abietane families. Their structures were established based on spectroscopic studies. The isolated compounds showed moderate cytotoxicity against human tumor cell assays.

Keywords: *Tripterygium doianum*; Celastraceae; Diterpenoid; *ent*-Kaurane; Abietane; Cytotoxicity

1. Introduction

The genus *Tripterygium* has been used in traditional Chinese medicine for treatment of cancer as well as an insecticide for hundreds of years. Recently, some Chinese clinics have used *Tripterygium wilfordii* Hook f. to treat rheumatoid arthritis and ankylosing spondylitis (Matlin et al., 1993; Qian, 1987; Qian et al., 1995). The chemical constituents of this genus have been investigated by many groups. In the course of our search for bioactive metabolites from plants, we became interested in the *Tripterygium* plants and began to study their chemical constituents. We previously examined constituents of *T. wilfordii* var. *regelii*, *T. wilfordii* and *Tripterygium hypoglaucum*, and reported their isolation and structure determination and biological activities (Shishido et al., 1994; Takaishi et al., 1997; Fujita et al., 2000; Duan et al., 1997a,b, 2000, 2001a,b). In a previous investigation, we also examined the constituents of *T. doianum* a scrubby deciduous vine found in southern Japan, and reported 12 triterpenoids and two sesquiterpenoids (Tanaka et al., 2002). We report herein the isolation and structure elucidation of 16 additional diterpenoids, including five new diterpenoids [doianoterpenes A–D (**1**–**4**), triptobenzene O (**5**)], and 11 known diterpenoids.

2. Results and discussion

Doianoterpene A (**1**) was assigned the molecular formula of $C_{20}H_{28}O_2$ based on its H REIMS, and analysis of its IR spectrum which showed a lactone carbonyl band (1727 cm^{-1}). The ^1H NMR spectrum of **1** indicated the presence of one methine attached to a double bond [δ_H 5.02 (1H, *brs*,)], one oxygenated methylene [δ_H 4.24 (1H, *dd*, $J = 11.5$, 2.3 Hz), 4.04 (1H, *dd*, $J = 11.5$, 0.9 Hz)], one doublet methyl [δ_H 1.71 (3H, *d*, $J = 1.3$ Hz)], and one singlet methyl [δ_H 0.91 (3H, *s*)]. The ^{13}C NMR spectrum of **1** (Table 1) showed the presence of one carbonyl carbon [δ_C 175.2], one double bond [δ_C 144.2, 132.7], one oxygenated methylene [δ_C 77.1] and three quaternary carbon, three methine, eight methylene, and two methyl functionalities. Based on these data, **1** was assumed to be an *ent*-kaurene diterpene. The ^{13}C NMR spectral data of **1** were similar to

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doi:10.1016/j.phytochem.2004.04.032

Table 1
^{13}C NMR spectral data for **1**–**5**

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[a]
1	39.6	39.4	40.6	40.1	37.3
2	20.9	20.9	18.3	21.3	35.0
3	41.1	40.9	35.7	42.9	220.7
4	33.2	33.2	38.7	34.2	51.0
5	50.1	50.2	56.5	57.1	51.9
6	21.6	22.7	19.1	21.2	19.1
7	37.4	31.1	43.0	42.5	24.8
8	49.1	44.1	51.0	46.0	123.0
9	45.8	53.2	47.1	55.5	147.8
10	48.4	48.1	39.8	48.8	37.0
11	19.9	19.3	18.6	18.9	116.3
12	22.5	20.9	25.3	25.9[c]	124.2
13	45.5	44.9	38.1	49.1	124.5
14	41.6	37.5	38.7	36.5[c]	152.9
15	132.7	48.4	162.0	59.0	80.1
16	144.2	156.3	148.6	77.9	26.3
17	15.3	102.6	189.6	25.0	26.6
18	23.9	23.9	27.1	33.5	22.2
19	77.1	77.1	65.6	23.0	65.8
20	175.2	174.8	18.2	179.1	25.6
OMe	–	–	–	–	50.9

[a] Measured in $CDCl_3$.
[b] Measured in pyridine-d_5.
[c] These values can be interchanged.

those of neotripterifordin (**6**) (Chen et al., 1995; Corey and Kun, 1997), except for the chemical shifts of C-8, C-9, C-13–C-17. In the HMBC spectrum of **1**, the following long-range correlations were observed: H-9 with C-15; H-15 with C-7, C-8, C-9, C-14 and C-17; H_2-14 with C-15 and C-16; H_3-17 with C-13, C-15 and C-16. These findings indicated that the double bond could be placed between C-15 and C-16. Based on these results, the structure of **1** was assigned to be *ent*-kaur-15-en-20,19-olide as shown in Fig. 1.

Doianoterpene B (**2**) was assigned the molecular formula of $C_{20}H_{28}O_2$ based on HREIMS. The ^1H NMR spectrum revealed the presence of an exocyclic methylene [δ_H 4.81, 4.71 (each 1H, *s*)], an oxygenated methylene [δ_H 4.24 (1H, *dd*, $J = 11.5$, 2.3 Hz), 4.05 (1H, *dd*, $J = 11.5$, 1.0 Hz)], and a methyl [δ_H 0.92 (3H, *s*)] groups. The ^{13}C NMR spectral data of **2** were very similar to those of **1**, except for the chemical shifts of C-14–C-17 (Table 1). From these data, **2** was considered to be an *ent*-kaurene diterpene having an exocyclic methylene and a six-membered lactone ring between C-20 and C-19. In the HMBC spectrum of **2**, the following long-range correlations were observed: H_2-17 with C-13 and C-15; H_2-15 with C-16 and C-17. This indicated that the double bond is located at C-16. Thus, the structure of **2** was determined as *ent*-kaur-16-en-20,19-olide as shown in Fig. 1.

Doianoterpene C (**3**) was assigned the molecular formula $C_{20}H_{30}O_2$ by HREIMS, and its ^1H NMR spectrum revealed one aldehyde proton [δ_H 9.73 (1H, *s*)], as well as one methine attached to a double bond [δ_H 6.57 (1H, *s*)], one oxygenated methylene [δ_H 3.73, 3.47 (each 1H, *d*, $J = 10.9$ Hz)], and two methyls [δ_H 1.07, 0.99 (each 3H, *s*)]. The ^{13}C NMR spectrum of **3** showed the presence of one carbonyl carbon (δ_C 189.6), an double bond (δ_C 162.0, 148.6), an oxygenated methylene (δ_C 65.6), three quaternary carbon, three methine, eight methylene, and two methyl groups. The ^{13}C NMR data spectra of **3** was similar to those of *ent*-kaur-15-en-17-al-19-oic acid (Harrigan et al., 1994) except for the chemical shifts of C-4, C-19 and C-20 [**3**: δ_C 38.7 (C-4), 65.6 (C-19), 18.2 (C-20); *ent*-kaur-15-en-17-al-19-oic acid: δ_C 43.9 (C-4), 182.6 (C-19), 15.5 (C-20)]. This difference suggested that **3** had a hydroxyl group on C-19. The analysis of the HMBC spectrum of **3** showed a long-range correlation between the proton signal of H_3-18 and the oxygenated methylene carbon. Furthermore, a NOE correlation between H_3-20 and the oxygenated methylene protons was observed in its NOESY spectrum. Based on these data, the oxygenated methylene can be placed on C-19 instead of carboxylic acid of *ent*-kaur-15-en-17-al-19-oic acid. Thus, **3** was elucidated as *ent*-19-hydroxykaur-15-en-17-al as shown in Fig. 1.

Doianoterpene D (**4**) has a molecular formula of $C_{20}H_{32}O_3$ based on HREIMS (*m*/*z* 320.2354, $[M]^+$). The IR spectrum of **4** showed hydroxyl and carbonyl bands at 3354 and 1677 cm^{-1}. Its ^1H and ^{13}C-NMR spectral data were similar to those of *ent*-16β-hydroxykaurane (**7**) (Fraga et al., 1987). However, **4** has three methyl signals in the NMR spectrum, and also showed the presence of a carboxylic acid in its IR and ^{13}C NMR spectrum (δ_C 179.1). In the HMBC spectrum, the proton

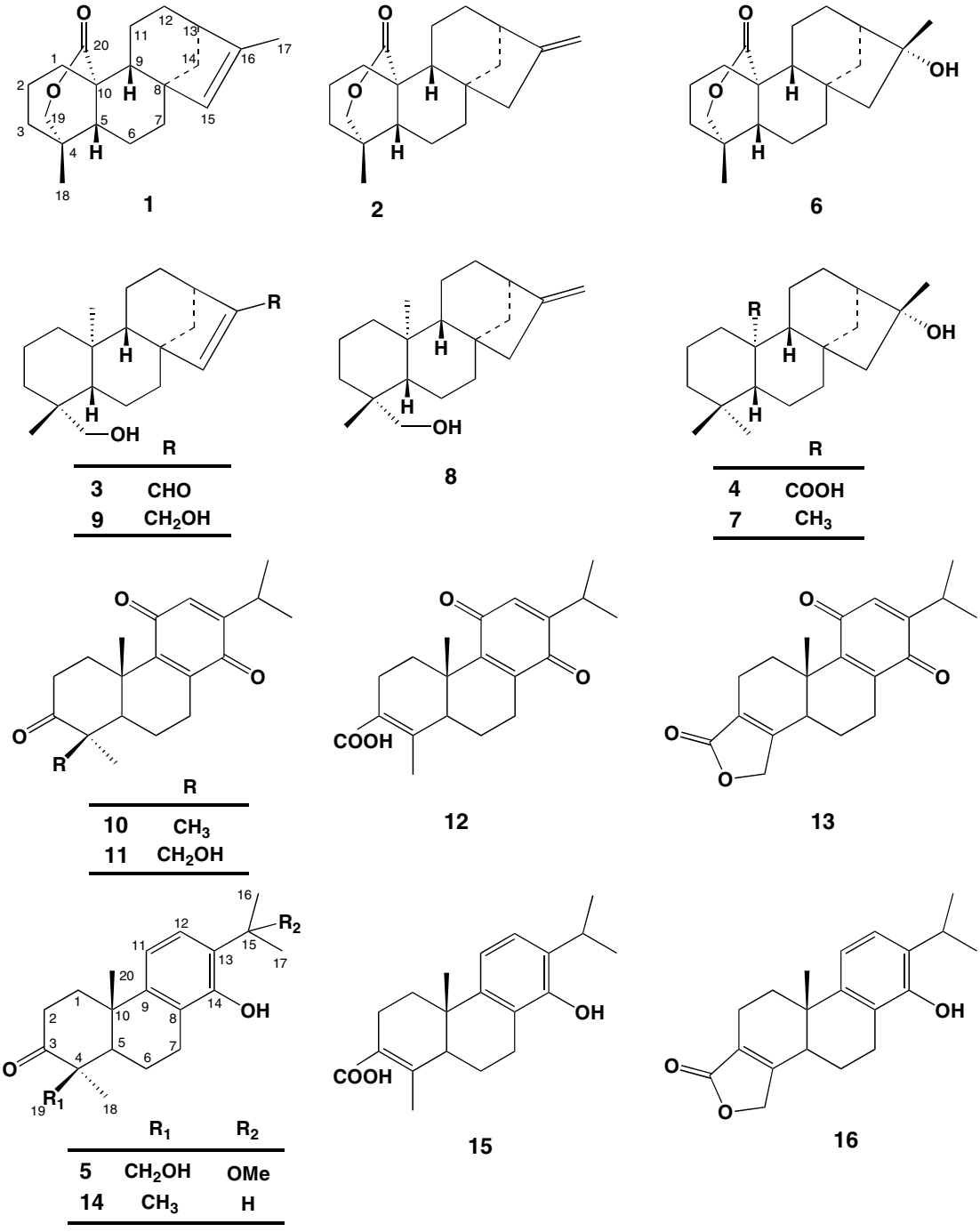

Fig. 1. The structures of compounds **1**–**16**.

signals of H-9 and H-5 were correlated with the carbon signal of the carboxylic acid. This fact indicated that the carboxylic acid was located at C-20. Thus, the structure of **4** was elucidated as *ent*-16α-hydroxykauran-20-oic acid as shown in Fig. 1.

Triptobenzene O (**5**) exhibits a molecular ion peak at *m/z* 346.2118 $[M]^+$ in HREIMS corresponding to a molecular formula of $C_{21}H_{30}O_4$. The ^1H NMR spectrum of **5** showed the presence of two aromatic protons [δ_H 6.89, 6.73 (each 1H, *d*, $J = 8.3$ Hz)], one oxygenated methylene [δ_H 4.09, 3.55 (each 1H, *d*, $J = 11.3$ Hz)], one methoxy group [δ_H 3.21 (3H, *s*)], and four methyls [δ_H 1.59, 1.58, 1.36, 1.28 (each 3H *s*)]. The ^{13}C NMR spectrum of **5** was similar to that of wilforol F (Morota et al., 1995) except for chemical shifts of C-11–C-17. In the HMBC spectrum of **5**, the following long-range correlations were observed: H-11 with C-8 and C-13; H-12 with C-9 and C-14; H_3-16 with C-13, C-15 and C-17;

H_3-17 with C-13, C-15 and C-16; H_3-OMe with C-15; H_3-20 with C-9. These facts showed that the hydroxymethyl group can be placed at C-18 or C-19. In the NOESY spectrum, the following NOE correlations were observed: H_3-20 with H_2-19 and H-11; H-12 with H_3-16 and H_3-17. These facts indicated the position of hydroxymethyl at C-19. Based on these data, the structure of **5** was assigned as 14,19-dihydroxy-15-methoxy-3-oxo-abieta-8,11,13-triene as shown in Fig. 1.

The following known compounds were identified by comparison with literature data: neotripterifordin (**6**) (*ent*-16α-hydroxykaurane-20,19-olide), *ent*-kauranol (**7**) (*ent*-17β-hydroxykaurane), *ent*-19-hydroxykaur-16-en (**8**) (Gonzalez et al., 1981), *ent*-17,19-dihydroxykaur-15-en (**9**) (Lloyd and Fales, 1967), triptoquinone H (**10**) (3,11,14-oxo-abieta-8,12-diene, Fujita et al., 2000), triptoquinone B (**11**) (19-hydroxy-3,11,14-oxo-abieta-8,12-diene, Shishido et al., 1994), triptoquinone A (**12**) [11,14-oxo-19-(4→3)-*abeo*-abieta-3,8,12-triene-19-oic acid, Shishido et al., 1994], quinone 21 (**13**) [11,14-oxo-19-(4→3)-*abeo* -abieta-3,8,12-triene-19,18-olide, Morota et al., 1995], triptonoterpene (**14**) (14-hydroxy-3-oxo-abieta-8,11,13-triene, Fu et al., 1994), triptinin B (**15**) [14-hydroxy-19(4→3)-*abeo*-abieta-3,8,11,13-tetraen-19-oic acid, Xu et al., 1997], triptophenolide (**16**) [14-hydroxy-19(4→3)-*abeo*-abieta-3,8,11,13-tetraen-19,18-olide, Zhou et al., 1982]. The immunosuppressive effects of triptoquinone A (**12**) and B (**11**) were reported (Shishido et al., 1994).

Compounds (**1**, **4**, **6**, **7**, **10**–**16**) were assayed for cytotoxicity using a reported procedure (Rubinstein et al., 1990). Compounds **1**, **10**, **11**, **13** and **16** were showed moderate activity as inhibitors of human tumor cell replication (Table 2). The isolated compounds (**1**, **4**, **6**, **7**, **10**–**16**) were also assayed for anti-HIV activity (Duan et al., 2000), but showed no effective results.

Table 2
Cytotoxicity data for compounds **1**, **4**, **6**, **7** and **10**–**16**

Compounds	Cell lines (IC_{50}, mcg/mL)[a]	
	A549[b]	MCF-7
1	13.1	7.8
4	>20 (48)[c]	>20 (21)
6	>20 (31)	>20 (20)
7	>20 (20)	>20 (25)
10	11.0	5.0
11	11.5	6.5
12	>20 (26)	>20 (27)
13	8.7	3.9
14	18.4	>20 (45)
15	>20 (12)	>20 (12)
16	9.7	9.6
VP-16 (etoposide)	1.9	>5(34)
Camptotherin	0.05	0.10
Tamoxijen citrate	10.0	5.0

[a] IC_{50} = Concentration that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using SRB assay.
[b] A549: lung, MCF-7: breast.
[c] If inhibition <50% at 20 mcg/mL, then percent inhibition observed is given as the bracketed value.

3. Experimental

3.1. General experimental procedures

NMR experiments were recorded on a Bruker ARX-400 instrument, ^{1}H NMR: 400 MHz, ^{13}C NMR 100 MHz, using TMS as int. stand. MS were obtained on a JEOL JMSD-300 instrument. Chromatography column used were: silica gel 60 (Merck, 63–210 μm), Sephadex LH-20 (pharmacia), and Toyopearl HW-40 (TOSOH); HPLC: GPC (Shodex H-2001, 2002, $CHCl_3$; Asahipak, GS-310 2G, MeOH), silica gel HPLC (YMC-Pack SIL-06 SH-043-5-06, 250 × 20 mm), detected with UV at 256 nm. 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 branches of *T. doianum* identified by Dr. Kotaro Murakami (University of Tokushima) were collected in October 1998 from Miyazaki Prefecture, the southern part of Kyushu Island, Japan, and specimen is deposited in the botanical garden of the University of Tokushima (Specimen No.: UTP98008).

3.3. Extraction and isolation of compounds

The branches of *T. doianum* (15.6 kg) were crushed and extracted (3 × 50 L) with MeOH at 60 °C for 6 h. The MeOH extracts were concentrated in vacuo to give a residue (1.08 kg), which was partitioned between EtOAc and H_2O (EtOAc: 10 L, H_2O: 5 L × 3). The EtOAc layer was concentrated to give a residue (238 g), which was subjected to silica gel CC eluted with solvents of increasing polarity *n*-hexane–EtOAc (3:1, 1:1, 1:2, 1:4 and MeOH) to give 14 fractions (fr. 1–14). Fr. 2 was subjected to a medium-pressure liquid column chromatography, eluting with $CHCl_3$, $CHCl_3$–MeOH (97:3, 94:6, 9:1) and MeOH to give four fractions (fr. 2.1–fr. 2.4). Fr. 2.1 was separated by Toyopearl CC with $CHCl_3$–MeOH (2:1) as eluent to give two fractions (fr. 2.1.1, fr. 2.1.2). Fr. 2.1.2 was subjected to a Si HPLC column with *n*-hexane–EtOAc (2:1) as eluent to give two fractions (fr. 2.1. 2.1, fr. 2.1. 2.2). Fr. 2.1. 2.1 on a Si HPLC column with *n*-hexane–EtOAc (3:1) yielded **1** (53 mg), **2** (5 mg) and **8** (2 mg). Fr. 2.1.2.2 was applied on a Si HPLC column with *n*-hexane–EtOAc (1:4) as eluent to give **10** (13 mg), **14** (59 mg). Fr. 2.2 was separated by a HPLC (GPC, $CHCl_3$), and isolated by a Si HPLC

column with *n*-hexane–EtOAc (2:1) to give **3** (3 mg) and **9** (3 mg). Fr. 2.3 was separated by a HPLC (GPC, $CHCl_3$) to give **7** (70 mg). Fr. 4 was subjected to a medium-pressure liquid chromatography, eluting with $CHCl_3$, $CHCl_3$–MeOH (99:1, 98:2, 96:4, 9:1) and MeOH to give five fractions (fr. 4.1–fr. 4.5) and **6** (489 mg). Fr. 4.2 was applied to a HPLC (GPC, $CHCl_3$) and recrystallized from MeOH to give **16** (47 mg). Fr. 4.3 was subjected to a HPLC (GPC, $CHCl_3$), further purified by a Si HPLC column with *n*-hexane–EtOAc (2:1) to give **5** (7 mg) and **11** (31 mg). Fr. 4.4 was subjected to a HPLC (GPC, $CHCl_3$) and isolated by a Si HPLC column with *n*-hexane–EtOAc (3:2) to give **12** (21 mg). Fr. 4.5 was separated by a HPLC (GPC, MeOH) to give **4** (161 mg). Fr. 5 was applied to a Sephadex LH-20 column chromatography eluted with MeOH to give three fractions (fr. 5.1–fr. 5.3). Fr. 5.1 was separated by a HPLC (GPC, MeOH) to give three fractions (fr. 5.1.1–fr. 5.1.3). Fr. 5.1.2 was isolated by a prep. TLC eluting with $CHCl_3$–MeOH (95: 5) to give **13** (16 mg). Fr. 5.3 was applied to a HPLC (GPC, $CHCl_3$), and isolated by a Si HPLC column with *n*-hexane–EtOAc (1:1) to give **15** (82 mg).

3.4. Doianoterpene A (*1, ent-kaur-15-en-20,19-olide*)

Amorphous powder. $[\alpha]_D$: −25.9° (*c* 0.9, MeOH); IR (KBr) ν_{MAX} cm^{-1}: 2924, 2859, 1727, 1442, 1141; HREIMS: *m/z* 300.2082, $[M]^+$ (calcd. for $C_{20}H_{28}O_2$, 300.2089); ^1H NMR ($CDCl_3$): 5.02 (1H, *brs*, H-15), 4.24 (1H, *dd*, $J = 11.5$, 2.3 Hz, H-19a), 4.04 (1H, *dd*, $J = 11.5$, 0.9 Hz, H-19b), 2.37 (1H, *m*, H-1), 2.34 (1H, *m*, H-13), 1.84 (2H, *m*, H_2-14), 1.71 (3H, *d*, $J = 1.3$ Hz, H_3-17), 1.21 (1H, *m*, H-9), 0.91 (3H, *s*, H_3-18); for ^{13}C NMR ($CDCl_3$), see Table 1.

3.5. Doianoterpene B (*2, ent-kaur-16-en-20,19-olide*)

Amorphous powder. $[\alpha]_D$: −41.9° (*c* 0.4, MeOH); IR (KBr) ν_{MAX} cm^{-1}: 2920, 2855, 1731, 1139; HREIMS: *m/z* 300.2070, $[M]^+$ (calcd. for $C_{20}H_{28}O_2$, 300.2089); ^1H NMR ($CDCl_3$): 4.81 (1H, *s*, H-17a), 4.71 (1H, *s*, H-17b), 4.24 (1H, *dd*, $J = 11.5$, 2.3 Hz, H-19a), 4.05 (1H, *dd*, $J = 11.5$, 1.0 Hz, H-19b), 2.16 (1H, *m*, H-15a), 2.11 (1H, *m*, H-15b), 0.92 (3H, *s*, H_3-18); for ^{13}C NMR ($CDCl_3$), see Table 1.

3.6. Doianoterpene C (*3, ent-19-hydroxykaur-15-en-17-al*)

Amorphous powder. $[\alpha]_D$: −23.8° (*c* 0.2, MeOH); IR (KBr) ν_{MAX} cm^{-1}: 3345, 2931, 2869, 1716, 1456; HREIMS: *m/z* 302.2229, $[M]^+$ (calcd. for $C_{20}H_{30}O_2$, 302.2246); ^1H NMR ($CDCl_3$): 9.73 (1H, *s*, H-17), 6.57 (1H, *s*, H-15), 3.73 (1H, *d*, $J = 10.9$ Hz, H-19a), 3.47 (1H, *d*, $J = 10.9$, H-19b), 1.07 (3H, *s*, H_3-20), 0.99 (3H, *s*, H_3-18); for ^{13}C-NMR ($CDCl_3$), see Table 1.

3.7. Doianoterpene D (*4, ent-16α-hydroxykauran-20-oic acid*)

White powder. $[\alpha]_D$: −34.3° (*c* 1.3, MeOH); IR (KBr) ν_{MAX} cm^{-1}: 3354, 2973, 2939, 1677, 1446, 1269; HREIMS: *m/z* 320.2354, $[M]^+$ (calcd. for $C_{20}H_{32}O_3$, 320.2351); ^1H NMR (pyridine-d_5): 2.82 (1H, H-14a), 2.30 (1H, *m*, H-11a), 2.10 (1H, *m*, H-13), 2.06 (1H, *m*, H-14b), 2.06 (1H, *m*, H-11b), 2.04 (1H, *d*, $J = 14.0$ Hz, H-15a), 1.74 (1H, *d*, $J = 14.0$ Hz, H-15b), 1.60 (3H, *s*, H_3-17), 1.10 (3H, *s*, H_3-18), 0.96 (H, *s*, H_3-19); for ^{13}C NMR (pyridine-d_5), see Table 1.

3.8. Triptobenzene O (*5, 14,19-dihydroxy-15-methoxy-3-oxo-abieta-8,11,13-triene*)

Yellow gum. $[\alpha]_D$: +13.3° (*c* 0.9, MeOH); IR (KBr) ν_{MAX} cm^{-1}: 3419, 2943, 1701, 1457, 1379, 1270, 1052; UV (MeOH) λ_{max} nm (log ε): 220 (0.9); HREIMS: *m/z* 346.2118, $[M]^+$ (calcd. for $C_{21}H_{30}O_4$, 346.2144); ^1H NMR ($CDCl_3$): 6.89 (1H, *d*, $J = 8.3$ Hz, H-12), 6.73 (1H, *d*, $J = 8.3$ Hz, H-11), 4.09 (1H, *d*, $J = 11.3$ Hz, H-19a), 3.55 (1H, *d*, $J = 11.3$ Hz, H-19b), 3.21 (3H, *s*, H_3-OMe), 3.04 (1H, *dd*, $J = 17.6$, 5.4 Hz, H-7a), 2.71 (1H, *m*, H-2a), 2.61 (1H, *m*, H-2b), 2.57 (1H, *m*, H-7b), 1.59 (3H, *s*, H_3-17), 1.58 (3H, *s*, H_3-16), 1.36 (3H, *s*, H_3-18), 1.28 (3H, *s*, H_3-20); for ^{13}C NMR ($CDCl_3$), see Table 1.

3.9. Cytotoxicity assay

All stock cultures were grown in T-25 flask (5 ml of RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin). Freshly trypsinized cell suspensions were seeded in 96-well microtitre plates at densities of 1500–7500 cells per well with test compounds from DMSO-diluted stock. After 3 days in culture, cells attached to the plastic substractum were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbancy at 562 nm was measured using a microplate reader after solubilizing the bound dye. The IC_{50} is the concentration of test compound that reduced cell growth by 50% over a 3-day assay period.

References

Chen, K., Shi, Q., Fujioka, T., Nakano, T., Hu, C.-Q., Jin, J.-Q., Kilkuskie, R.E., Lee, K.-H., 1995. Anti-AIDS agents—XIX. Neotripterifordin, a novel anti-HIV principle from *Tripterygium wilfordii*: isolation and structural elucidation. Bioorganic and Medicinal Chemistry 3, 1345–1348.

Duan, H., Kawazoe, K., Takaishi, Y., 1997a. Sesquiterpene alkaloids from *Tripterygium hypoglaucum*. Phytochemistry 45, 617–621.

Duan, H., Kawazoe, K., Bando, M., Kido, M., Takaishi, Y., 1997b. Di- and triterpenoids from *Tripterygium hypoglaucum*. Phytochemistry 46, 535–543.

Duan, H., Takaishi, Y., Imakura, Y., Jia, Y., Li, D., Cosentino, L.M., Lee, K-H., 2000. Sesquiterpene alkaloids from *Tripterygium hypoglaucum* and *Tripterygium wifordii*: a new class of potent anti-HIV agents. Journal of Natural Products 63, 357–361.

Duan, H., Takaishi, Y., Momota, H., Ohmoto, Y., Taki, T., Jia, Y., Li, D., 2001a. Immunosuppressive sesquiterpene alkaloids from *Tripterygium wilfordii*. Journal of Natural Products 64, 582–587.

Duan, H., Takaishi, Y., Momota, H., Ohmoto, Y., Taki, T., Tori, M., Takaoka, S., Jia, Y., Li, D., 2001b. Immunosuppressive terpenoids from extracts of *Tripterygium wilfordii*. Tetrahedron 57, 8413–8424.

Corey, E.J., Li, Kun., 1997. Enantioselective total synthesis of the potent anti-HIV agent neotripteryfordin. Reassignment of stereochemistry at C(16). Journal of the American Chemical Society 119, 9929–9930.

Fraga, B.M., Hernandez, M.G., Fernandez, C., Arteaga, J.M., 1987. Diterpenes from *Sideritis dendrochahorra* and *S. cystosiphon*. Phytochemistry 26, 775–777.

Fu, M., Zhou, X., Xie, D., Deng, F., Wang, H., 1994. Study of 2D NMR of two diterpenes from *Tripterygium wilfordii* Hook F. Chinese Journal of Magnetic Resonance 11, 165–172.

Fujita, R., Duan, H., Takaishi, Y., 2000. Terpenoids from *Tripterygium hypoglaucum*. Phytochemistry 53, 715–722.

Gonzalez, A.G., Fraga, B.M., Hernandez, M.G., Hanson, J.R., 1981. The ^{13}C NMR spectra of some *ent*-18-hydroxykaur-16-enes. Phytochemistry 20, 846–847.

Harrigan, G.G., Bolzani, V.S., Gunatilaka, A.A.L., Kingston, D.G.I., 1994. Kaurane and trachylobane diterpenes from *Xylopia aethiopica*. Phytochemistry 36, 109–113.

Lloyd, H.A., Fales, H.M., 1967. Terpene alcohols of *Helichrysum dendroideum*. Tetrahedron Letters 48, 4891–4895.

Morota, T., Qin, W.-Z., Takagi, K., Xu, L.-H., Maruno, M., Yang, B.-H., 1995. Diterpenoids from *Tripterygium wilfordii*. Phytochemistry 40, 865–870.

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., 1993. Male antifertility compounds from *Tripterygium wilfordii* Hook f. Contraception 47, 387–400.

Qian, S.Z., 1987. *Tripterygium wilfordii*, a Chinese herb effective in male fertility regulation. Contraception 36, 335–345.

Qian, S.Z., Xu, Y., Zhang, J.W., 1995. Recent progress in research on *Tripterygium*: a male antifertility plant. Contraception 51, 121–129.

Rubinstein, L.V., Shoemarker, R.H., Paull, K.D., Simon, R.M., Tosini, S., Skehan, P., Scudiero, D.A., Monks, A., Boyd, M.R., 1990. Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. Journal of National Cancer Institute 82, 1113–1118.

Shishido, K., Nakano, K., Wariishi, N., Tateishi, H., Omodani, T., Shibuya, M., Goto, K., Ono, Y., Takaishi, Y., 1994. Diterpene quinoides from *Tripterygium wilfordii* var. *regelii* which are Interleukin-1 inhibitors. Phytochemistry 35, 731–737.

Takaishi, Y., Wariishi, N., Tateishi, H., Kawazoe, K., Nakano, K., Ono, Y., Tokuda, H., Nishino, H., Iwashima, A., 1997. Triterpenoid inhibitors of Interleukin-1 secretion and tumour-promotion from *Tripterygium wilfordii* var. *regelii*. Phytochemistry 45, 969–974.

Tanaka, N., Duan, H., Takaishi, Y., Kawazoe, K., Goto, S., 2002. Terpenoids from *Tripterygium doianum* (Celastraceae). Phytochemistry 61, 93–98.

Xu, J., Ikekawa, T., Ohkawa, M., Yokota, I., Hara, N., Fujimoto, Y., 1997. Triptinins A and B, two leukotriene D_4 antagonistic 19(4→3)-*abeo*-abietanes from *Tripterygium wilfordii*. Phytochemistry 44, 1511–1514.

Zhou, B., Song, G., Hu, C., 1982. Studies on the chemical constituents of *Tripterygium wilfordii* Hook F. Acta Pharmaceutica Sinica 17, 146–150.