Pergamon

PII: S0031–9422(96)00860–6

Phytochemistry, Vol. 45, No. 5, pp. 975–978, 1997

0031–9422/97 $17.00+0.00

TERPENOIDS FROM *TRIPTERYGIUM WILFORDII* VAR. *REGELII*

YOSHIHISA TAKAISHI,* KANAME MIYAGI, KAZUYOSHI KAWAZOE, KIMIKO NAKANO, KUNHUA LI and HONGQUAN DUAN

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770, Japan

(*Received in revised form* 6 *November* 1996)

Key Word Index—*Tripterygium wilfordii*; Celastraceae; triterpene; diterpene; regelone.

Abstract—The methanol extract of dried stem barks of *Tripterygium wilfordii* Hook fil. var *regelii* afforded two new triterpenes: 22-hydroxy-3, 19, 24-trinor-D:A-friedoolenane-1(10), 5, 7-triene-2-oic acid methyl ester, named regelone, and 3-methyl-23-ol-oxotingenol; a new diterpene—8β, 19-dihydroxy-3-oxopimar-15-ene; and three known triterpenes—3-methyl-22-β, 23-diol-6-oxotingenol, tingenin B and cangoronin. Their structures were established on the basis of chemical and spectroscopic studies. ©1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of our search for bioactive metabolites from plants, we are investigating members of the Celastraceae and have isolated active principles from *Tripterygium wilfordii* var. *regelii* Makino [1–4]. In our previous paper, we reported the isolation of three new triterpenes regeol A, B and C, which have biological activities [5]. We now report on the isolation of two new triterpenes, regelone (**1**) and 3-methyl-23-ol-6-oxotingenol (**3**), one new diterpene, 8β, 19-dihydroxy-3-oxopimar-15-ene (**4**) and three known triterpenes, 3-methyl-22β, 23-diol-6-oxotingenol (**2**), tingenin B (**5**), cangoronine (**6**) from the bark of this plant. This paper deals with the structural investigations of these compounds.

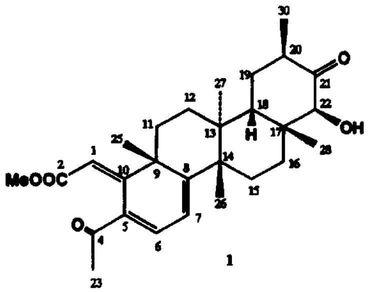
RESULTS AND DISCUSSION

Repeated column chromatography of the ethyl-acetate-soluble fraction from the methanol extract of stem barks of *T. wilfordii* var. *regelii* yielded regelone (**1**), 3-methyl-23-ol-6-oxotingenol (**3**), 8β, 19-dihydroxy-3-oxopimar-15-ene (**4**) and the known triterpenes **2**, **5** and **6**.

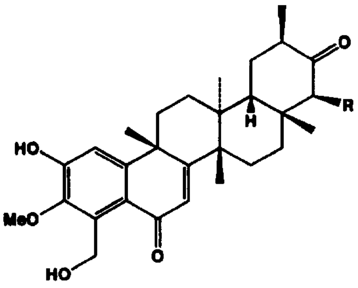
Regelone (**1**), obtained as a powder, showed a molecular ion peak at m/z 454 $[M]^+$ in the EI-mass spectrum. Its IR spectrum contained absorption bands due to hydroxy (3425 cm^{-1}) and carbonyl (1708 and 1640 cm^{-1}) groups. Its UV spectrum showed absorption bands at 230 and 388 nm. The ^{1}H NMR spectrum revealed the presence of five tertiary methyls [δ 0.85, 1.10, 1.23, 1.37, 2.38 (each 3H, *s*)] one secondary methyl [δ 1.06 (3H, *d*, $J = 6.3$ Hz)], one methoxy [δ 3.65 (3H, *s*)], three olefinic protons [δ 5.95 (1H, *s*), 6.03, 6.74 (each 1H, *d*, $J = 6.0$ Hz)] and one methine [δ 4.55 (1H, *br s*)] attached to an oxygen function. The ^{13}C NMR spectrum of **1** showed three carbonyl carbon signals at δ 167.6, 197.8 and 213.7, three double bond carbon signals at δ 115.4 (*d*), 116.4 (*d*), 132.1 (*d*), 133.7 (*s*), 157.9 (*s*), 162.4 (*s*), seven methyl carbon signals, five methylene carbon signals, three methine carbon signals and four quaternary carbon signals. The high resolution mass spectrum gave a molecular formula of $C_{28}H_{38}O_5$. Thus, compound **1** had one methoxy or carbomethoxy group, and its C-skeleton contained 27 carbons. Many triterpenes have been isolated from Celastraceae plants, i.e. C$_{30}$-type (oleanane, ulsane, fridelin), C$_{29}$-type (pristimerin, celastrol), C$_{28}$-type (tingenone, iguesterin), but C$_{27}$-type triterpenes from Celastraceae plants have never been described [6]. The ^{13}C NMR spectral data for the D- and E-rings of **1** were similar to those of tingenin B (**5**), indicating a ketone group at C-20 and hydroxy group at C-21. To confirm the position of the carbomethoxy group, unsaturated ketone and other functionalities, the 2D NMR spectra of **1** were measured. From the ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectra, the partial structures, I: >CH–CH$_2$–CH(CH$_3$)–, II, III: –CH$_2$–CH$_2$– and IV: >CH=CH< were suggested and the partial structures of I, II and III could be placed on the C, D and E rings. In the ^{13}C-^{1}H long range correlation spectrum, the proton signal at δ 6.03 (1H, *d*, $J = 6.0$ Hz) showed a long range correlation with the carbon signals at δ 40.6 (C-9), 44.7 (C-14) and 133.7; the proton signal at δ 6.74 (1H, *d*, $J = 6.0$ Hz) with the carbon signals at δ 157.9, 162.4 and

---

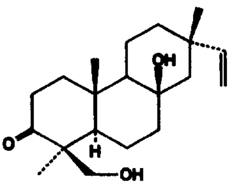
* Author to whom correspondence should be addressed.



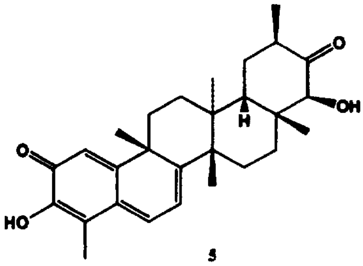
1



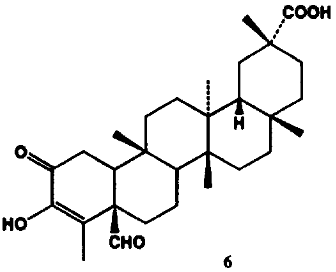
2: R=OH
3: R=H



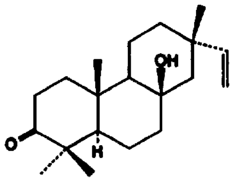
4



5



6



7

197.8; the proton signals δ at 5.95 (1H, *s*) with the carbon signals at δ 40.6 (C-9), 133.7 and 157.9; the proton signal of the methylene at δ 2.38 with the carbon signal at δ 197.8; the proton signal of the methoxy at δ 3.65 with the carbon signals at δ 167.6; the proton signal of the methyl at δ 1.37 with the carbon signals at δ 32.3 (C-11), 40.6 (C-9), 157.9 and 162.4. From these correlations, the carbon signals at δ 115.4, 197.8, 133.7, 132.1, 116.4, 162.4, 40.9 and 157.9 could be assigned to C-1, C-4, C-5, C-6, C-7, C-8, C-9 and C-10, respectively (Table 1). The remaining carbonyl carbon signal at δ 167.6 could be assigned to C-2. Thus, the structure of regelone was formulated as **1**, which is a unique structure in which the pentacyclic triterpene skeleton lacks C-3. Regelone (**1**) lacks the typical quinonemethide chromophore. It is probably formed biogenetically from a quinone methide precursor as a result of ring A oxidation. More than 100 celastrolides have been isolated from Celastraseae plants or derived from the congeners [6], but the only example of a compound related to regelone is celastrahydride, which was isolated from *Kokoona zeylanica* [7].

Compound **2** $C_{29}H_{38}O_6$, showed a hydroxy band at 3470 cm^{-1} and ketone bands at 1713, 1679 and 1609 cm^{-1} in its IR spectrum. Its UV spectrum showed absorption at 250 and 312 nm. The ^{1}H NMR spectrum showed the presence of four tertiary methyls, one secondary methyl, one methoxy methyl, one methylene [δ 4.76, 4.92 (each 1H, *d*, J = 12.1 Hz)] and one methine [δ 4.54 (1H, *s*)] attached to an oxygen function, and two olefinic protons [δ 6.38, 7.08 (each 1H, *s*)]. A total of 29 carbon signals observed in the ^{13}C NMR spectrum of **2** were identified as Me $\times$ 6, CH$_2$ $\times$ 5, –CH$_2$O–, >CH– $\times$ 2, >CHO–, –CH = $\times$ 2, C $\times$ 4, >C = $\times$ 6, and >CO $\times$ 2 with the help of the DEPT spectrum. The ^{13}C NMR spectrum of **2** was similar to that of **1** and tingenin B (**5**) for the signals due to C-11 to C-23 (Table 1). It was concluded that compound **2** was based on the same skeleton as **1** and **5** in the C, D and E rings. From the 2D NMR spectrum, the structure of compound **2** was identified as 3-methyl-

Table 1. ^{13}C NMR spectral data for compounds **1**–**5** and **7**

	1	**2**	**3**	**5**	**4**	**7**
1	115.4	111.4	117.2	120.2	37.8	37.9
2	167.6	153.3	153.1	178.8	34.3	34.1
3	—	144.7	144.6	146.5	220.8	217.2
4	197.8	134.8	134.7	117.6	50.9	47.4
5	133.7	123.5	123.4	128.2	55.5	55.2
6	132.1	187.7	187.6	134.1	18.3	18.8
7	116.4	125.5	125.3	118.6	42.9	42.9
8	162.4	172.9	173.1	168.6	71.9	72.0
9	40.6	40.9	40.5	43.0	55.5	55.8
10	157.9	155.8	155.8	165.1	36.4	36.7
11	32.3	34.4	35.5	34.4	17.8	17.5
12	29.6	30.2	30.1	30.4	38.0	38.3
13	39.0	40.5	40.4	44.7	36.6	36.5
14	44.7	45.2	44.7	41.0	51.5	51.5
15	28.1	28.3	28.5	28.7	151.1	151.0
16	29.6	29.6	32.0	29.9	108.9	108.6
17	45.0	44.9	38.2	45.2	24.3	24.3
18	44.9	45.2	43.5	45.5	22.2	26.6
19	32.3	32.0	34.1	32.4	65.8	21.3
20	40.8	41.0	41.9	41.3	16.4	15.4
21	213.7	213.5	213.6	213.7		
22	76.5	76.7	52.6	76.8		
23	26.6	57.1	57.0	10.7		
24	—	—	—	—		
25	30.0	38.6	38.4	39.6		
26	21.3	21.0	20.7	22.0		
27	22.6	20.7	19.7	20.9		
28	25.0	25.2	32.6	25.4		
29	—	—	—	—		
30	14.8	14.9	15.1	15.1		
OMe	51.5	63.6	63.6	—		

22β,23-diol-6-oxotingenol, which had already been isolated from the South American medicinal plants *Maytenus ilicifolia* and *M. chuchuasca* (Celastraseae) [8].

Compound **3**, $C_{29}H_{38}O_5$, showed the presence of hydroxy and ketone absorptions in its IR spectrum. Its UV spectrum indicated the same absorptions as compound **2** at 250 and 315 nm. The ^{1}H NMR spectrum of **3** showed four tertiary methyl signals at δ 1.01, 1.02, 1.40 and 1.62 (each 3H, *s*), one secondary methyl signal at δ 1.00 (3H, *d*, $J = 6.4$ Hz), one methoxy signal at δ 3.95 (3H, *s*), one methylene signal attached to oxygen function at δ 4.75, 4.99 (each 1H, *Abq*, $J = 11.7$ Hz) and two olefinic proton signals at δ 6.41, 7.10 (each 1H, *s*). The ^{1}H NMR spectrum of **3** was very similar to that of compound **2**, except for the methine proton attached to an oxygen function at δ 4.54 (1H, *s*) in compound **2**. The ^{13}C NMR spectrum was also similar to that of compound **2**, especially the chemical shifts of C-1 to C-15 and C-23 to C-26, which were almost the same, differing only in the number of methylene and methine signals (Table 1). On the basis of the ^{13}C NMR spectra and molecular formula, the structure of compound **3** was deduced to be 3-methyl-23-ol-6-oxotingenol.

Compound **4**, $C_{20}H_{32}O_3$, showed a hydroxy band at 3415 and a carbonyl band at 1694 cm^{-1}. The ^{1}H NMR spectrum showed three tertiary methyls at δ 1.04, 1.24 and 1.28 (each 3H, *s*), one methylene attached to an oxygen function at δ 3.47, 4.02 (each 1H, *d*, $J = 11.3$ Hz), and one vinyl group at δ 4.83 (1H, *d*, $J = 10.7$ Hz), 4.87 (1H, *d*, $J = 17.4$ Hz) and 5.74 (1H, *dd*, $J = 17.4$, 10.7 Hz). The ^{13}C NMR spectrum of **4** indicated the presence of three methyls, eight methylenes, one vinyl, two methines and five quaternary carbons including one carbonyl. These functional groups accounted for two of the five sites of saturation deduced from the molecular formula. This suggested that compound **4** had three rings. From the ^{1}H-^{1}H-COSY, ^{13}C-^{1}H COSY and ^{13}C-^{1}H long range correlation spectra, the presence of partial structures $-CH_2CH_2C(CH_3)CH<$, $-COC(CH_3)-CH<$, $-CH_2C(CH_3)CH{=}CH_2$ were suggested. The above data were consistent with a primarane or isoprimarane skeleton. The ^{13}C NMR spectrum data of **4** was very similar to that of 8β-hydroxy-3-oxopimar-15-ene (**7**) [9], except for the carbon signals at C-4, 18 and 19 (Table 1). In the NOESY spectrum of **4**, the proton signal at δ 1.04 (H-20) was correlated with the hydroxy methylene signals at δ 3.47 and 4.02 (H-19). Thus, the structure of this new diterpenoide was finally established as 8β, 19-dihydroxy-3-oxopimar-15-ene (**4**).

The known triterpenoids tingenin B (**5**) [10] and cangoronin (**6**) [11] were identified by comparisons of their physicochemical and spectral data with those in the literature.

EXPERIMENTAL

^{1}H NMR; 270 and 400 MHz with TMS as int. stand; ^{13}C NMR: 100.2 MHz; CC: silica gel 60 (Merck), Sephadex LH-20 (Pharmacia) and TOYO pearl HW-40 (TOSO); HPLC GPC (H-2002, SHODEX).

Isolation of regelone (**1**) *and compounds* **2–6**

The dry bark (3.8 kg) of *T. wilfordii* Hook fil. var. *regelii* were collected in August 1991 on Mt Turugi (Tokushima Prefecture, Japan) and extracted with MeOH (20 l) at 60°. The MeOH extracts were concd *in vacuo* to give a residue, which was partitioned between EtOAc and H_2O. The EtOAc layer was concd to give a residue (409 g), to which CH_2Cl_2 was added and sepd into CH_2Cl_2-soluble and -insoluble frs. The CH_2Cl_2-soluble fraction was concd *in vacuo* to give a residue (255 g), which was chromatographed on a silica gel column. The column was eluted with solvents of increasing porarity (CH_2Cl_2–MeOH) to give 17 frs (frs 1–7). Fr. 14 (167 g) was chromatographed on a silica gel column with CH_2Cl_2–MeOH to give 12 frs (14.1–14.12). Fr. 14.6 (11.6 g) was chromatographed on silica gel with hexane–Et_2O (1:1 to 1:2) to give 12 frs (14.6.1–14.6.12). Fr. 14.6.4 (0.23 g) was chromatographed on Sephadex LH-20 with MeOH, silica gel with $CHCl_3$–MeOH (19:1) and HPLC (GPC, $CHCl_3$) to give 14 mg of **1**. Fr. 14.7 (22.9 g) was chromatographed on silica gel with $CHCl_3$–MeOH (97:3) to give six frs (14.7.1–14.7.6). Fr. 14.7.2 (2.91 g) was chromatographed on Sephadex LH-20 with MeOH to give five frs (14.7.2.1–14.7.2.5). Fr. 14.7.2.3 (0.49 g) was sepd on silica gel with $CHCl_3$–MeOH (19:1) and HPLC (GPC, $CHCl_3$) to give 14 mg of **2**. Fr. 14.7.2.2 was sepd on silica gel with $CHCl_3$–MeOH (19:1) and repetitive HPLC (GPC, $CHCl_3$) to give 4 mg of **3**. Fr. 14.6.7 (0.98 g) was crystallized from MeOH to give 40 mg of **6**, the mother liquid was concd *in vacuo* and chromatographed on Sephadex LH-20 with MeOH to give 9 frs (14.6.7.1–14.6.7.9). Fr. 14.6.7.6 (174 mg) was chromatographed on silica gel with hexane–EtOAc (1:1) and HPLC (GPC, $CHCl_3$) to give 10 mg of **4**. Fr. 14.6.3 (1.06 g) was chromatographed on Sephadex LH-20 with MeOH to give 404 mg of **5**.

Regelone A (**1**). Amorphous powder, $[\alpha]_D^{25}$ +208.5° ($CHCl_3$ *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3425, 1708, 1640, 1579, 1454, 1381, 1308, 1193, 1121, 1063, 1030; UV λ_{max}^{MeOH} nm (ε): 263 (4200), 338 (6800); ^{1}H NMR: δ ($CDCl_3$): 0.85 (3H, *s*, H-27), 1.06 (3H, *d*, $J = 6.3$ Hz, H-28), 1.10 (3H, *s*, H-25), 1.23 (3H, *s*, H-26), 1.37 (3H, *s*, H-24), 2.38 (3H, *s*, H-23), 3.65 (3H, *s*), 4.55 (1H, *s*, H-22), 5.95 (1H, *s*, H-1), 6.03 (1H, *d*, $J = 6.0$ Hz, H-7), 6.74 (1H, *d*, $J = 6.0$ Hz, H-6); ^{13}C NMR ($CDCl_3$): Table 1; EI-MS *m/z* (rel. int.): 454 $[M]^+$ (7), 439 $[M-Me]^+$ (100), 201 (5); HR-MS *m/z* 454.2705 $[M]^+$ $C_{28}H_{38}O_5$ required 454.2719.

Compound **2**. Amorphous powder, $[\alpha]_D^{25}$ −55.9° ($CHCl_3$ *c* 1.8). IR ν_{max}^{KBr} cm^{-1}: 3470, 1713, 1679, 1609, 1561, 1456, 1378, 1252, 1196, 1119; UV λ_{max}^{MeOH} nm (ε): 259 (12 600), 315 (7800); ^{1}H NMR: δ ($CDCl_3$): 0.88 (3H, *s*, H-27), 1.02 (3H, *s*, H-25), 1.06 (3H, *d*, $J = 6.3$ Hz, H-28), 1.42 (3H, *s*, H-26), 1.63 (3H, *s*, H-24), 3.94

(3H, *s*), 4.54 (1H, *s*, H-22), 4.76, 4.92 (each 1H, *d*, $J = 12.1$ Hz, H-23), 6.38 (1H, *s*, H-7), 7.08 (1H, *s*, H-1); ^{13}C NMR ($CDCl_3$): Table 1; EI-MS *m/z* (rel. int.): 482 $[M]^+$ (43), 467 $[M-Me]^+$ (100), 245 (10), 231 (58); HR-MS *m/z* 482.2677 $[M]^+$ $C_{29}H_{38}O_6$ required 482.2668.

Compound **3**. Amorphous powder, $[\alpha]_D^{25}$ −33.8° ($CHCl_3$ *c* 0.77). IR ν_{max}^{KBr} cm^{-1}: 3417, 1708, 1640, 1579, 1456, 1381, 1311, 1031; UV λ_{max}^{MeOH} nm (ε): 250 (9900), 315 (5600). ^{1}H NMR: δ ($CDCl_3$): 1.00 (3H, *d*, $J = 6.5$ Hz, H-28), 1.01 (3H, *s*, H-26), 1.02 (3H, *s*, H-27), 1.40 (3H, *s*, H-25), 1.62 (3H, *s*, H-24), 3.95 (3H, *s*), 3.95 (3H, *s*), 4.75, 4.99 (each 1H, *d*, $J = 11.7$ Hz, H-23), 6.41 (1H, *s*, H-7), 7.10 (1H, *s*, H-1); ^{13}C NMR ($CDCl_3$): Table 1; EI-MS *m/z* (rel. int.): 466 $[M]^+$ (48), 451 $[M-Me]^+$ (100), 248 (18), 231 (45), 95 (11); HR-MS *m/z* 466.2692 $[M]^+$ $C_{29}H_{38}O_6$ required 466.2719.

Compound **4**. Amorphous powder, $[\alpha]_D^{25}$ +19.5° ($CHCl_3$ *c* 0.51). IR ν_{max}^{KBr} cm^{-1}: 3415, 1694, 1650, 1579, 1035; UV λ_{max}^{MeOH} nm (ε): 250 (9900), 315 (5600); ^{1}H NMR: δ ($CDCl_3$): 1.04 (3H, *s*, H-20), 1.24 (3H, *s*, H-17), 1.28 (3H, *s*, H-18), 1.98 (1H, *ddd*, $J = 15.2$, 8.3, 4.7 Hz, H-1), 2.45 (1H, *ddd*, $J = 16.1$, 9.1, 4.7 Hz, H-2), 2.57 (1H, *ddd*, $J = 16.1$, 8.2, 8.1 Hz, H-2), 3.47, 4.02 (each 1H, *ABq*, $J = 11.3$ Hz, H-19), 4.83 (1H, *d*, $J = 10.7$ Hz, H-16), 4.87 (1H, *d*, $J = 17.4$ Hz, H-16), 5.72 (1H, *dd*, $J = 17.4$, 10.7 Hz, H-15); ^{13}C NMR ($CDCl_3$): Table 1; EI-MS *m/z* (rel. int.): 320 $[M]^+$ (30), 305 (72), 290 (71), 272 (58), 203 (33), 189 (55), 137 (56), 109 (79), 81 (100), 55 (90), 41 (72); HR-MS *m/z* 320.2358 $[M]^+$ $C_{20}H_{32}O_3$ required 320.2351.

REFERENCES

1. Takaishi, Y., Tamai, S., Nakano, K., Murakami, K. and Tomimatsu, T., *Phytochemistry*, 1991, **30**, 3027.
2. Takaishi, Y., Shishido, K., Wariishi, N., Shibuya, M., Goto, K., Kido, M., Takai, M. and Ono, Y., *Tetrahedron Letters*, 1992, **33**, 7177.
3. Ujita, K., Takaishi, Y., Tokuda, H., Nishino, H., Iwashima, A. and Fujita, T., *Cancer Letters*, 1993, **68**, 129.
4. Shishido, K., Nakano, K., Wariishi, N., Tateishi, H., Omodani, T., Shibuya, M., Goto, K., Ono, Y. and Takaishi, Y. *Phytochemistry*, 1994, **35**, 731.
5. Takaishi, Y., Wariishi, N., Tateishi, H., Kawazoe, K., Nakano, K., Ono, Y., Tokuda, H., Nishino, H. and Iwashima, A., *Phytochemistry*, 1997, **45**, 969.
6. Gunatilaka, A. A. L., Tamm, Ch. and Walser-Volken, P., *Fortschritte der Chemie Organischer Naturstoffe*, Vol. 67. Springer, Wien, 1996, p. 1.
7. Gamlath, C. B., Gunatilaka, A. A. L., Tezuka, Y., Kikuchi, T. and Balasubramaniam, *Phytochemistry*, 1990, **29**, 3189.
8. Shirota, O., Morita, H., Takeya, K., Itokawa, H. and Iitaka, Y., *Journal of Natural Products*, 1994, **57**, 1675.
9. Li, Y. and Chen, Y., *Phytochemistry*, 1990, **29**, 3033.
10. Bavovada, R., Blasko, G., Shieh, H. L., Pezzuto, J. M. and Cordell, G. A., *Planta Medica*, 1990, **56**, 380.
11. Itokawa, H., Shiroto, O., Ikuta, H., Morita, H., Takeya, K. and Iitaka, Y., *Phytochemistry*, 1991, **30**, 3713.