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TRITERPENOID INHIBITORS OF INTERLEUKIN-1 SECRETION AND TUMOUR-PROMOTION FROM *TRIPTERYGIUM WILFORDII* VAR. *REGELII*

YOSHIHISA TAKAISHI,* NORIKO WARIISHI, HIDEO TATEISHI, KAZUYOSHI KAWAZOE, KIMIKO NAKANO, YUKIHISA ONO,† HARUYUKI TOKUDA,‡ HOYOKU NISHINO‡ and AKIO IWASHIMA‡

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770, Japan; † Otsuka Pharmaceutical Co., Kagasuno, Tokushima 771-01, Japan; ‡ Department of Biochemistry, Kyoto Prefectural University of Medicine, Kyoto 602, Japan

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Key Word Index—*Tripterygium wilfordii*; Celastraceae; triterpene; regeol; interleukin-1; anti-tumour-promoting activity.

Abstract—Three new triterpenoids, 2,3,22β-trihydroxy-21-oxo-24, 29-nor-D:A-friedooleana-1,3,5(10)-triene, 2α, 6β-dihydroxy-3-oxo-24-nor-D:A-friedooleana-4-ene-29-oic acid and 2,3,7-trihydroxy-6-oxo-24-nor-D:A-friedooleana-1,3,5(10), 7-tetraene-29-oic acid, named regeol A, B and C, and nine known triterpenoides were isolated from *T. wilfordii* var. *regelii*. Their structures were established on the basis of the chemical reactions and spectroscopic evidence. Isolated compounds and derivatives were observed to inhibit Epstein–Barr virus early antigen activation and showed potent inhibitory activities against interleukin-1α and β release from human peripheral mononuclear cells.

INTRODUCTION

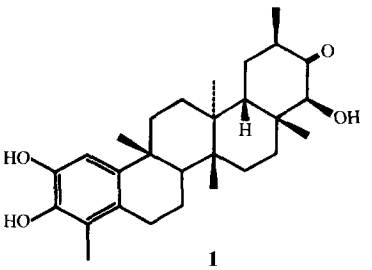
In the course of our search for bioactive metabolites from plants, we are investigating Celastraceae plants and have started a study of the active principles in *Tripterygium wilfordii* var. *regelii* Makino [1–3]. In a previous paper [4], we reported on the isolation of the diterpene quinoids, triptoquinone A–F which have interleukin-1 (IL-1) inhibitory activity. We have now isolated three novel triterpenes, regeol A (**1**), B (**2**) and C (**3**), and nine known triterpenes; 22β-hydroxy-tingenone (**4**) [5], wilforol A (**5**) [6], regelide (**6**) [7], salaspemic acid (**7**) [8], orthophenic acid (**8**) [9], celastrol (**9**) [10], dulcioic acid (**10**) [11], methyl-katonoate (**11**) [12], demethylregelin (**13**) [7] from this plant. This paper deals with the structural determination of the three novel triterpenes and an investigation of their bioactivity.

RESULTS AND DISCUSSION

Repeated column chromatography of the ethyl acetate-soluble fraction from the methanol extract of steam barks of *T. wilfordii* var. *regelii* yielded regerol A (**1**), B (**2**), C (**3**) and nine known triterpenoids **4**–**13**.

Regeol A (**1**) showed a carbonyl band at 1708 cm^{-1} and hydroxy band at 3436 cm^{-1} in its IR spectrum. Its UV spectrum contained absorption maxima at 240 and 286 nm due to an aromatic ring. The ^1H NMR spectrum revealed the presence of six methyls [δ 0.85, 0.98, 1.20, 1.20, 2.01 (each 3H, *s*), 1.05 (3H, *d*, $J = 5.9$ Hz)], and a methine [δ 4.55 (1H, *d*, $J = 3.4$ Hz)] attached to an oxygen function, and one methine proton [δ 6.65 (1H, *s*)] attached to the aromatic ring. The ^{13}C NMR spectrum of **1** showed one carbonyl signal at δ 214.2, benzene ring carbon signals at δ 108.3 (*d*), 122.5 (*s*), 126.3 (*s*), 139.8 (*s*), 141.4 (*s*) and 143.8 (*s*), six methyl carbon signals, one methine carbon signal (δ 77.6) attached to oxygen function, seven methylene carbon signals, three methine carbon signals, and four quaternary carbon signals. The mass spectrum of **1** showed the peak due to $[M]^+$ at *m/z* 440. These facts agreed with a molecular formula for **1** as $C_{28}H_{40}O_4$, which was supported by the high resolution mass spectral data. Many types of triterpene have been isolated from Celastraceae plants [13]; compound **1** was assumed to be of the tingenin type of triterpine from the carbon number (C_{28}) and the presence of six methyls. On comparison of the ^{13}C NMR spectrum of **1** with that of tingenin B (Table 1), the chemical shifts of C-14 to C-22 and C-26 to C-28 were found to be similar. This suggested that in the structure of regerol A, the C, D and E rings system were the same as in tingenin B (**4**). To confirm the structure of **1**, we

* Author to whom correspondence should be addressed.



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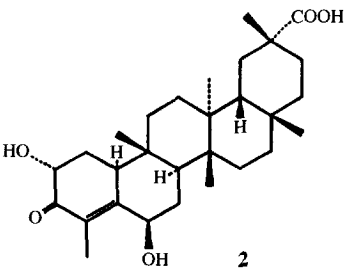

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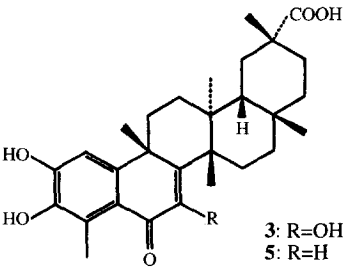

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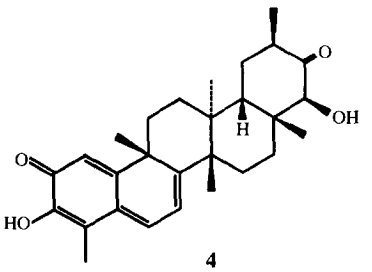

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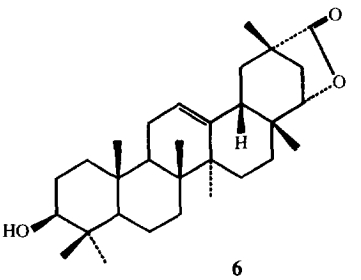

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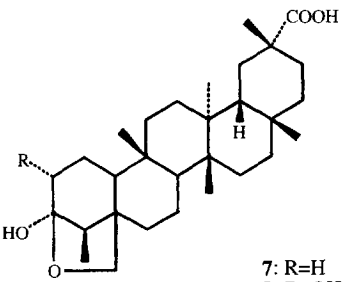

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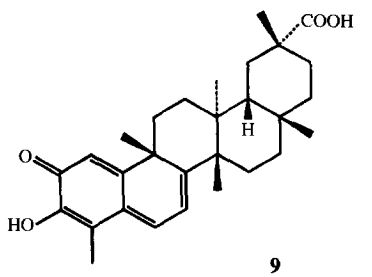
8: R=OH



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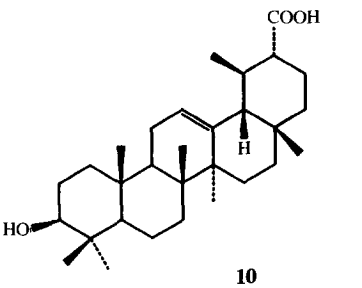

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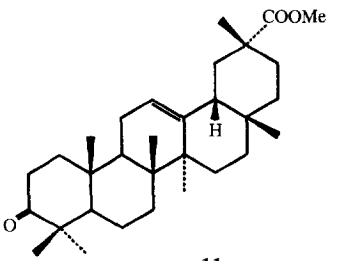

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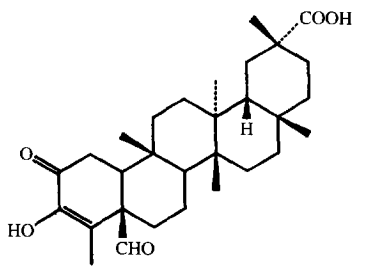

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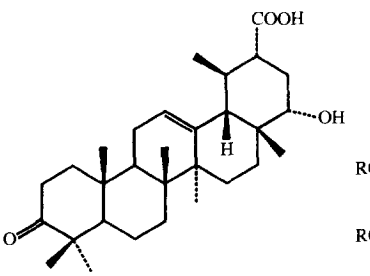

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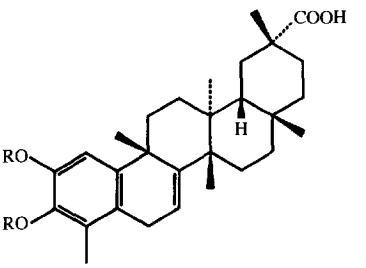

14: R=H
15: R=Ac

determined the 2D NMR spectra. From the ^{1}H-^{1}H COSY spectrum of **1**, the presence of partial structures, -CH_2-CH_2- × 3, -$CH_2CH(CH_3)$- were suggested. From the ^{1}H-^{13}C COSY and ^{1}H-^{13}C long range correlation spectra, the C, D and E rings system were confirmed to be the same as in tingenin B. In the ^{1}H-^{13}C long range correlation spectrum for the A and B rings of compound **1**, the proton signal at δ 6.65 (H-1) showed long range correlations with the carbon signals at δ 36.7 (C-9), 126.3 (C-5), 139.8 (C-3) and 141.4 (C-2), the proton signal at δ 2.75 (H-6) with the carbon signals at δ 43.3 (C-8), 126.3 (C-5) and 143.8 (C-10) and the proton signal at δ 2.01 (H-23) with the carbon signals at δ 139.8 (C-3), 122.5 (C-4) and 126.3 (C-5). These facts clearly indicated that the A ring is aromatic, as in the compound **1**. Acetylation of **1** gave a triacetate, whose ^{1}H NMR spectrum showed the presence of three acetyl methyl signals at δ 2.19, 2.28 and 2.31. These facts indicated that the structure of regerol A should be formulated as **1**.

Regeol B (**2**) showed carbonyl bands at 1704 and 1674 cm^{-1} and a hydroxy band at 3430 cm^{-1} in its IR spectrum. The ^{1}H NMR spectrum revealed the presence of six methyls [δ 0.87, 0.92, 1.11, 1.23, 1.44 and 2.76 (each 3H, *s*)] and two methines [δ 4.65 (1H, *dd*, J = 9.3, 4.9 Hz) and δ 4.73 (1H, *dd*, J = 10.3, 5.4 Hz]. The ^{13}C NMR spectrum of **2** showed an α,β-unsaturated carbonyl carbon signal at δ 201.0, one double bond carbon signals at δ 128.0 (*s*) and 158.7 (*s*), a signal for a methine carbon attached to an oxygen

Table 1. ^{13}C NMR spectral data for compounds **1**–**5** and **12**

	1	2	5	3	4	12
1	108.3	29.5	109.7	109.3	120.2	30.9
2	141.4	70.1	151.2	152.2	178.8	193.5
3	139.8	201.0	143.9	144.1	146.5	149.1
4	122.5	128.0	126.6	127.9	117.6	125.9
5	126.3	158.7	122.6	119.5	128.2	54.9
6	28.0	75.6	187.3	182.1	134.1	32.7
7	18.3	34.4	126.7	146.9	118.6	18.9
8	43.3	47.5	170.5	138.1	168.8	49.5
9	36.7	40.4	40.2	40.0	43.0	37.1
10	143.8	49.6	150.6	151.7	165.1	55.5
11	34.2	33.7	34.6	33.5	34.4	33.3
12	30.0	30.1	30.2	29.8	30.4	30.9
13	40.0	39.6	39.5	39.4	44.7	39.4
14	39.3	40.7	44.7	46.4	41.0	39.6
15	27.9	29.5	29.0	29.4	28.7	30.5
16	29.6	36.5	36.8	37.2	29.9	36.6
17	44.9	30.5	30.8	30.5	45.2	30.5
18	45.4	44.7	44.6	44.4	45.5	44.8
19	31.7	31.0	31.2	31.4	32.4	29.5
20	41.3	39.7	40.5	40.7	41.3	40.7
21	214.2	30.5	30.4	30.4	213.7	29.4
22	77.6	37.4	35.5	36.0	76.8	37.4
23	11.5	12.6	14.8	14.8	10.7	10.7
24	—	—	—	—	—	195.8
25	28.2	17.3	37.8	41.3	39.6	17.2
26	15.3	16.6	21.1	20.5	22.0	17.9
27	19.2	17.5	18.8	19.9	20.9	16.2
28	25.2	32.1	31.7	31.9	25.4	32.3
29	—	181.0	181.0	181.2	—	181.5
30	14.8	32.3	33.2	32.8	15.1	32.1

function (δ 70.1 and 75.6) and one carboxylic acid signal at δ 181.0 (Table 1). The ^{13}C NMR spectrum of **2** was similar to that of D:A-friedoolean-24-al-3-en-3-ol-2-one-29-oic acid (**12**) [15], except for the signals due to C-1 to C-10 and C-24. The FAB mass spectrum of **2** showed a peak due to $[M+H]^+$ at *m/z* 473. These facts agreed with a molecular formula for **2** as $C_{29}H_{44}O_5$. From the ^1H-^1H COSY and HOHAHA spectra of **2**, the partial structures $>CH(OH)-CH_2-CH<$ × 2 were suggested. From the ^1H-^{13}C COSY and ^1H-^{13}C long range correlation spectra, the C, D and E rings were confirmed to be the same as in compound **12** [15]. In the ^1H-^{13}C long range correlation spectrum, the proton signal at δ 2.76 (H-23) showed long range correlation with the carbon signals at δ 201.0, 128.0 and 158.7, the proton signal at δ 0.92 (H-25) with the carbon signals at δ 33.7, 40.4, 47.5 and 49.6, the proton signal at δ 0.87 (H-26) with the carbon signals at δ 29.5, 40.7 and 47.5. From these facts the carbon signals at δ 47.5 (*d*) and 49.6 (*d*) were assigned to C-8 and C-10, respectively, and the partial structure of $>CHCH_2CH(OH)-$ could be assigned to C-1, C-2, C-3 and $>CH(OH)-CH_2-CH<$ assigned to C-6, C-7 and C-8. Thus, the A ring contains an α,β-unsaturated ketone moiety. To confirm the relative stereochemistry of **2**, we measured the NOESY spectrum; the proton signal at δ 4.65 (H-2) was correlated with the methyl signal at δ 0.92 (H-25), and the proton signal at δ 4.75 (H-6) with the proton signals at δ 1.50 (H-8) and 2.12 (H-10). These facts clearly showed that the orientation of the hydroxy groups at C-2 and C-6 are α and β, respectively. The structure of regeol B (**2**) was thus determined as shown.

Compound **5**, $C_{29}H_{38}O_5$, showed a hydroxy band at 3420 cm^{-1} and carbonyl bands at 1700 and 1635 cm^{-1} in its IR spectrum. The UV spectrum contained bands (252 and 304 nm) due to an aromatic ring. The ^1H NMR spectrum showed six methyls [δ 1.05, 1.08, 1.21, 1.38, 1.54 and 3.25 (each 3H, *s*)]. From the 2D NMR spectra (^{13}C-^1H COSY, ^1H-^1H COSY, ^{13}C-^1H long range correlation and NOESY), compound **5** was identified as the known compound 23-nor-6-oxo-demethylpristimerol (= wilforol A) [5].

Regeol C (**3**) showed a hydroxy band at 3373 cm^{-1} and carbonyl bands at 1702 and 1602 cm^{-1} in its IR spectrum. Its UV spectrum contained bands at 283 and 322 nm due to an aromatic ring. The ^1H NMR spectrum of **3** revealed the presence of six methyls [δ 1.12, 1.26, 1.42, 1.49, 1.59 and 3.18 (each 3H, *s*)] and one methine proton [δ 7.25 (1H, *s*)] attached to an aromatic ring. The ^{13}C NMR spectrum of **4** showed six methyls, seven methylenes, four double bonds ($>C{=}C<$ × 3, $-CH{=}C<$ × 1), α, β, γ, δ unsaturated carbonyl carbon (δ 182.1) and a carboxylic acid (δ 181.2) and five quaternary carbons. The FAB mass spectrum of **3** showed the peak due to $[M+H]^+$ at *m/z* 483. From these facts, the molecular formula of **3** was deduced to be $C_{29}H_{38}O_6$. The ^1H NMR spectrum of **3** was very similar to that of compound **5** except for the methine signal at δ 7.25 (1H, *s*) and methylene signal at δ 3.22 (1H, *ddd*, J = 14.2, 3.9, 1.2 Hz) for compound **3** and methine signals at δ 7.21, 6.50 (each 1H, *s*) for compound **5**. The ^{13}C NMR spectral data (Table 1) of compound **3** were very similar to those of compound **5**, only different points are carbon signals assignable to C-6, C-7, C-8 and C-24. From the ^1H and ^{13}C NMR spectral data, in the structure of compound **3** it was concluded that the hydrogen on C-7 in compound **5** was replaced by hydroxy. In the ^1H NMR spectrum of **3**, the signal at δ 3.22 was assigned to H_β-15 which was shifted more down field than that of compound **5**. This fact is explained by the neighbouring effect of the hydroxy group on C-7. From these facts the structure of regeol C was formulated as **3**.

Seven other triterpenes were characterized as the known 22β-hydroxytingenine (**4**) [5], [6], regelide (**6**) [7], salaspemic acid (**7**) [8], orthophenic acid (**8**) [9], celastrol (**9**) [10], dulcioic acid (**10**) [11], methylkatonoate (**11**) [12], demethylregelin (**13**) [7] from comparisons of the spectra data.

T. wilfordii Hook has been used to treat rheumatoid arthritis and spondylitis in some Chinese clinics [15]. In rheumatoid arthritis, it is reported that there is a strong relationship between the production of interleukin-1 (IL-1) by the synovium and the degree of

Table 2. The activities of compounds **1–6**, **9**, **13** and predonisolone as inhibitors of IL-1α and IL-1β release

Compound	Inhibition (%) IL-1α	IL-1β
1	47	30
2	1	0
3	−10	2
4	100	100
5	−7	−2
6	5	1
9	98	100
13	2	0
14	98	99
15	95	99
Predonisolone	87	76

Concentration (**1–6**, **9**, **13**: 1×10^{-6} g m^{-1}, predonisolone: 3×10^{-7} g ml^{-1}).

Table 3. Inhibition of EBV-EA activation by constituents of *T. wilfordii* and their derivatives. Activity is presented relative to a positive control (100%).* Values in parentheses are % viability of Raji cells in the assay, >60% viability prevoting an inhibitory effect

Compound	Concentration† 1×10^3	5×10^2	1×10^2	1×10
2	0(70)	53.7(>80)	83.6	100
3	11.3(70)	65.4(>80)	84.5	100
4	13.6(70)	24.8(>80)	50.3	81.8
5	10.5(40)	34.7(>80)	75.4	92.6
6	26.4(70)	68.9(>80)	84.7	100
9	0(50)	49.4(>80)	78.6	95.7
13	26.2(70)	54.8(>80)	83.6	100
14	0(60)	0(>80)	42.4	75.2
15	0(60)	0(>80)	36.3	70.3

* Induction by TPA at 20 ng ml^{-1} = 32 pM.
† Mol ratio/TPA (20 ng ml^{-1} = 32 pM ml^{-1}).

inflammation of the arthricular synovial membrane [16–18]. Compounds **1–6**, **9** and **13–15** were tested for inhibitory activity for IL-1α and IL-1β release from lipopolysaccharide-stimulated human peripheral mononuclear cells compared to a reference compound (prednisolone) [2, 4] (Table 2). Tingenine B (**4**), celastrol (**9**) and the derivatives (**14** and **15**) from celastrol showed strong inhibitory activity.

Recently, the anti-tumour-promoting activities of several natural products [20] have been investigated for inhibition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced Epstein–Barr virus early antigen (EBV-EA) activation [21]. We have reported the inhibitory effects of dihydroagarofuran sesquiterpenes on EBV activation [3]. In continuation of our previous interest in this area, we examined the effect on EBV-EA activation of the isolated compounds **1–6**, **9** and **13–15**, using the methods described previously [22]. Their inhibitory effects on the activation of the early antigen and the viabilities of Raji cells are shown in Table 3. Compounds **2**, **9**, **14** and **15** showed stronger activities than the other compounds.

EXPERIMENTAL

^{1}H NMR; 270 and 400 MHz with TMS at int. stand; ^{13}C NMR: 100.2 MHz; CC: silica gel 60 (Merck), Sephadex LH-20 (Pharmacia) and TOYO pearl HW-40 (TOSHO); HPLC [GPC: H2002(Shodex), ODS: D-ODS-5 (YMC)].

Isolation of regerol A (**1**), *B* (**2**) *and C* (**4**) *and compounds* **3**, **5–11**

(i) The extraction and subsequent fr. (by silica gel CC) of the extract from dry stalks (108 kg) of *T. wilfordii* Hook fil. var. *regelii* is described in a previous paper [4]. Fr. 6 (168 g) was chromatographed on a silica gel column with solvents of increasing polarity to give 13 frs (6.1–6.13). An aliquot (11.5 g) of fr. 6.7 (25.0 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (19:1) to give 11 frs (6.7.1–6.7.11). Fr. 6.7.8 (2.16 g) was separated on silica gel with $CHCl_3$ (19:1), on Sephadex LH-20 with MeOH–$CHCl_3$ (9:1) and HPLC [ODS, MeOH–H_2O (9:1)] to give 6 mg of regeol B (**2**). Fr. 6.7.9 (3.25 g) was chromatographed on silica gel with $CHCl_3$–MeOH (19:1) to give 10 frs (6.7.9.1–6.7.9.10). Fr. 6.7.9.7 (0.48 g) and Fr. 6.7.9.8 (0.72 g) were sepd by HPLC [ODS, MeOH–0.1%H_3PO_4 (9:1)] to give 11 mg of regeol C (**3**) and 92 mg of **4**, respectively. Fr. 6.2 (23.0 g) was chromatographed on silica gel with $CHCl_3$–MeOH (19:1) (×2), and with hexane–EtOAc (1:1) to give 265 mg of **5**. Fr. 6.1 (44 g) was chromatographed on silica gel with $CHCl_3$–MeOH (19:1), then crystallized from MeOH to give 349 mg of **6**. Fr. 4.7 (19 g) was chromatographed on Sephadex LH-20 with MeOH to give 13.6 g of **9**.

(ii) The extraction and subsequent fractionation of the extract from the dry stalks (12.0 kg) of *T. wilfordii* Hook fil. var. *regelii* is described in a previous paper [4]. Fr. 10 (6.4 g) was sepd on Sephadex LH-20 with MeOH to give 6 frs (10.1–10.6). Fr. 10.5 (0.26 g) was crystallized from MeOH to give 43 mg of regeol A (**1**). Fr. 10.3 (2.6 g) was chromatographed on silica gel with $CHCl_3$–MeOH (19:1), silica gel with hexane–EtOAc (1:1), Sephadex-LH20 with MeOH, silica gel with $CHCl_3$–MeOH (49:1) and HPLC [ODS, MeOH–H_2O (9:1)] to give 30 mg of **11**. Fr. 13 (4.77 g) was chromatographed on silica gel with $CHCl_3$–MeOH (19:1, 9:1) to give 9 frs (13.1–13.9). Fr. 13.8 (1.53 g) was chromatographed on silica gel with $CHCl_3$–MeOH (19:1 and 9:1) and crystallized from MeOH to give 101 mg of **10**. Fr. 17 (5.83 g) was chromatographed on Sephadex LH-20 with MeOH and crystallized from MeOH to give 48 mg of **8**. Fr. 12 (11.66 g) was chromatographed on silica gel with

$CHCl_3$–MeOH (49:1, 19:1) to give 15 frs (12.1–12.5). Fr. 12.8 (1.03 g) was crystallized from MeOH to give 92 mg of **7**.

Regeol A (**1**). Amorphous powder, $[\alpha]_D^{25} -49.3°$ ($CHCl_3$ *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3436, 1708, 1616, 1455, 1382, 1290, 1220, 1117, 1016, 992, 857, 757; UV λ_{max}^{MeOH} nm (ε): 240 (200), 286 (2000), 313 (1000). ^1H NMR: δ ($CDCl_3$): 0.85 (3H, *s*, H-27), 0.98 (3H, *s*, H-25), 1.05 (3H, *d*, *J* = 5.9 Hz, H-28), 1.20 (3H × 2, *s*, H-24, 26), 2.01 (3H, *s*, H-23), 2.23 (1H, *dd*, *J* = 14.7, 7.1 Hz, H-19), 4.55 (1H, *d*, *J* = 3.4 Hz, H-22), 6.65 (1H, *s*, H-1); ^{13}C NMR ($CDCl_3$): Table 1; EI-MS *m/z* (rel. int.): 440 $[M]^+$ (73), 425 $[M-CH_3]^+$ (56), 409 (32), 205 (91), 203 (100), 189 (61), 177 (68). HR-MS *m/z* 440.2926 $[M]^+$ $C_{28}H_{40}C_4$ required 440.2927.

Regeol B (**2**). Amorphous powder, $[\alpha_D^{25} + 105.3°$ (MeOH *c* 0.23). IR ν_{max}^{KBr} cm^{-1}. 3430, 1704, 1674, 1459, 1384, 1275, 1106, 1058; ^1H NMR: δ (C_5D_5N): 0.87 (3H, *s*, H-25), 0.92 (3H, *s*, H-25), 1.11 (3H, *s*, H-27), 1.23 (3H, *s*, H-26), 1.44 (3H, *s*, H-29), 2.76 (3H, *s*, H-23), 1.72 (1H, *m*, H-8), 1.96, 2.26 (each 1H, *m*, H-7), 2.14, 2.29 (each 1H, *m*, H-2), 2.54 (1H, *br d*, *J* = 13.2 Hz, H-12), 2.68 (1H, *br d*, *J* = 15.1 Hz, H-19). 4.65 (1H, *dd*, *J* = 9.3, 4.9 Hz, H-2), 4.73 (1H, *dd*, *J* = 10.3, 5.4 Hz, H-6); ^{13}C NMR (C_5D_5N): Table 1; FAB-MS *m/z*: 473 $[M+H]^+$. FAB HR-MS *m/z* 473.2910 $[M+H]^+$ $C_{29}H_{45}O_5$ required 473.2927.

Regeol C (**3**). Amorphous powder, $[\alpha]_D^{25} -160.6°$ (MeOH, *c* 0.25). IR ν_{max}^{KBr} cm^{-1}: 3373, 1702, 1602, 1462, 1376, 1305, 1210, 1012; UV λ_{max}^{MeOH} nm (ε): 283 (5000), 320 (4000). ^1H NMR: δ (C_5D_5N): 1.12 (3H, *s*, H-26), 1.26 (3H, *s*, H-27), 1.42 (3H, *s*, H-25), 1.49 (3H, *s*, H-29), 1.59 (3H, *s*, H-24), 3.18 (3H, *s*, H-23), 3.22 (1H, *ddd*, *J* = 14.2, 3.9, 1.2 Hz, H-15), 7.25 (1H, *s*, H-1); FAB-MS *m/z*: 483 $[M+H]^+$; ^{13}C NMR (C_5D_5N): Table 1; FAB HR-MS *m/z* 483.2772 $[M+H]^+$ $C_{29}H_{39}O_6$ required 483.2736.

Compound **5**. Amorphous powder, $[\alpha]_D^{25} -65.1°$ (MeOH *c* 0.77). IR ν_{max}^{KBr} cm^{-1}: 3420, 1700, 1635, 1557, 1501, 1462, 1319, 1058, 1034, 877; UV λ_{max}^{MeOH} nm (ε): 252 (12 000), 304 (7000), 313 (1000); ^1H NMR: δ (C_5D_5N): 1.05 (3H, *s*, H-26), 1.08 (3H, *s*, H-27), 1.21 (3H, *s*, H-25), 1.38 (3H, *s*, H-29), 1.54 (3H, *s*, H-24), 3.25 (3H, *s*, H-23), 6.50 (1H, *s*, H-7), 7.21 (1H, *s*, H-1); ^{13}C NMR (C_5D_5N): Table 1; EI-MS *m/z* (rel. int.): 466 $[M]^+$ (84), 451 $[M-CH_3]^+$ (47), 218 (100); HR-MS *m/z* 466.2712 $[M]^+$ $C_{29}H_{38}O_5$ required 466.2719.

Compound **4**. Plates, mp 288–292°, $[\alpha]_D^{25} -167.8°$ ($CHCl_3$ *c* 1.2). IR ν_{max}^{KBr} cm^{-1}: 3402, 1709, 1594, 1553, 1440, 1378, 1284, 1215, 1189, 995, 869; UV λ_{max}^{MeOH} nm (ε): 250 (9000). ^1H NMR: δ ($CDCl_3$): 0.87 (3H, *s*, H-27), 0.99 (3H, *s*, H-26), 1.07 (3H, *d*, *J* = 5.9 Hz, H-28), 1.37 (3H, *s*, H-25), 1.52 (3H, *s*, H-24), 2.23 (3H, *s*, H-23), 4.55 (1H, *d*, *J* = 4.4 Hz, H-22), 6.40 (1H, *d*, *J* = 7.3 Hz, H-7), 6.54 (1H, *d*, *J* = 1.5 Hz, H-1), 7.05 (1H, *dd*, *J* = 7.3, 1.5 Hz, H-6).

Compound **6**. Needles, mp 256–612° $[\alpha]_D^{25} -116.5°$ (MeOH *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3490, 1750, 1456, 1364, 1173, 1099, 996; ^1H NMR: δ ($CDCl_3$): 0.79 (3H, *s*, H-24), 0.87 (3H, *s*, H-28), 0.93 (3H, *s*, H-26), 0.94 (3H, *s*, H-25), 0.99 (3H, *s* H-23), 1.08 (3H, *s*, H-27), 1.21 (3H, *s*, H-30), 3.22 (1H, *dd*, *J* = 11.7, 4.9 Hz, H-3), 4.15 (1H, *d*, *J* = 5.4, H-22), 5.30 (1H, *br t*, *J* = 3.4 Hz, H-12); HR-MS *m/z* 454.3463 $[M]^+$ $C_{30}H_{46}O_{53}$ required 453.3447.

Compound **7**. Amorphous powder, $[\alpha]_D^{25} +40.0°$ (MeOH *c* 0.25). IR ν_{max}^{KBr} cm^{-1}: 3310, 1692, 1456, 1384, 1262, 1225, 1192, 1146, 1085, 875; ^1H NMR: δ (C_5D_5N): 0.85 (3H, *s*, H-26), 0.92 (3H, *s*, H-25), 1.15 (3H, *s*, H-28), 1.21 (3H, *d*, *J* = 7.3, H-23), 1.529 (3H, *s*, H-27), 1.45 (3H, *s*, H-30), 3.72, 4.28 (each 1H, *d*, *J* = 8.3, H-24); ^{13}C NMR ($CDCl_3$): Table 1; EI-MS *m/z* (rel. int.): 472 $[M]^+$(12), 125 (100). HR-MS *m/z* 472.3550 $[M]^+$ $C_{30}H_{48}O_4$ required 472.3553.

Compound **8**. Amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3303, 1697, 1607, 1456, 1391, 1271, 1176, 1068, 989; ^1H NMR: δ (C_5D_5N): 0.85 (3H, *s*, H-26), 0.94 (3H, *s*, H-25), 1.14 (3H, *s*, H-28), 1.20 (3H, *s*, H-27), 1.24 (3H, *d*, *J* = 7.3 Hz, H-23), 1.43 (3H, *s*, H-30), 3.79 (1H, *d*, *J* = 7.8 Hz, H-24), 4.24 (1H, *d*, *J* = 7.8 Hz, H-24), 4.42 (1H, *d*, *J* = 3.9 Hz, H-2); EI-MS *m/z* (rel. int.): 488 $[M]^+$ (12), 412 (26), 155 (37), 125 (92), 109 (100), 95 (59); HR-MS *m/z* 488.3498 $[M]^+$ $C_{30}H_{48}O_5$ required 488.3502.

Compound **10**. Needles, mp 278–283°, $[\alpha]_D^{25} +92.0°$ (MeOH *c* 0.25). IR ν_{max}^{KBr} cm^{-1}. 3415, 1712, 1607, 1412, 1387, 1312, 1295, 1224, 1181, 1092, 1044, 998; ^1H NMR: δ (C_5D_5N): 0.92 (3H, *s*, H-28), 0.98 (3H, *s*, H-25), 1.05 (3H, *s*, H-26), 1.07 (3H, *s*, H-24), 1.19 (3H, *d*, *J* = 6.3 Hz, H-29), 1.23 (3H, *s*, H-27), 1.26 (3H, *s*, H-23), 3.46 (1H, *dd*, *J* = 10.7, 5.4 Hz, H-4), 5.27 (1H, *s*, H-12); EI-MS *m/z* (rel. int.): 456 $[M]^+$(4), 438 (10), 395 (10), 248 (100); HR-MS *m/z* 456.3605 $[M]^+$ $C_{30}H_{38}O_3$ required 456.3688.

Compound **13**. Amorphous powder, $[\alpha]_D^{25} +115.8°$ (MeOH *c* 1.01). IR ν_{max}^{KBr} cm^{-1}: 3542, 1718, 1681, 1461, 1381, 1250, 1172, 1030; ^1H NMR: δ (C_5D_5N): 1.00 (3H, *s*, H-25), 1.06 (3H, *s*, H-24), 1.08 (3H, *s*, H-26), 1.15 (3H, *s*, H-23), 1.17 (3H, *d*, *J* = 5.9 Hz, H-29), 1.25 (3H, *s*, H-27), 1.31 (3H, *s*, H-28), 3.74 (1H, *dd*, *J* = 9.8, 5.4 Hz, H-22), 5.30 (1H, *br t*, H-12). ^{13}C NMR δ (C_5D_5N): 39.4 (*t*, C-1), 34.8 (*t*, C-2), 216.2 (*s*, C-3), 47.4 (*s*, C-4), 55.2 (*d*, C-5), 19.8 (*t*, C-6), 32.5 (*t*, C-7), 40.3 (*s*, C-8), 47.1 (*d*, C-9), 36.7 (*s*, C-10), 23.8 (*t*, C-11), 125.2 (*d*, C-12), 139.3 (*s*, C-13), 43.1 (*s*, C-14), 26.5 (*t*, C-15), 21.4 (*t*, C-16), 39.7 (*s*, C-17), 58.3 (*d*, C-18), 34.8 (*d*, C-19), 51.2 (*d*, C-20), 34.4 (*t*, C-21), 77.6 (*d*, C-22), 26.7 (*q*, C-23), 21.6 (*q*, C-24), 15.4 (*q*, C-25), 16.9 (*q*, C-26), 23.8 (*q*, C-27), 25.2 (*q*, C-28), 19.0 (*q*, C-29), 178.2 (*s*, C-30); EI-MS *m/z* (rel. int.): 470 $[M]+$(14), 452 (20), 246 (100), 120 (58), 107 (33), 95 (34); HR-MS *m/z* 470.3406 $[M]^+$ $C_{30}H_{46}O_4$ required 470.3396.

Compounds **14** *and* **15**. Compounds **14** and **15** were derived from celastrol (**9**) using published methods [13].

IL-1α and β release inhibitory activity assay. Materials: Endotoxin (LPS) from *Escherichia coli* 055:B5 was obtained from Difco Laboratories, Detroit, MI, U.S.A. Heparin was purchased from

Takeda Chemical Industries, Ltd, Osaka, Japan. Method: the test compound dissolved (20 U heparine ml^{-1}) in whole blood from healthy volunteers, and LPS (1 $\mu g\ ml^{-1}$) were suspended in RPMI-1640 medium containing 100 U ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin, and incubated at 37° in a humidified atmosphere of 5% CO_2–95% air for 18–24 h. The final concn of DMSO was 0.3% (v/v). Only DMSO was contained in the control suspension. The supernatant of culture prepared by centrifugation was stored at −20° until the assay of cytokine. The concns of two human cytokines (IL-1α and β) were assayed using ELISA kits (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan). The ratio (%) of inhibition of cytokine release was calculated by the following equation: Cytokine release inhibitory ratio (%) = $100 \times (1 - T/C)$, where T is the concn of the cytokine in the culture supernatant with the test compound, and C is the concn of the cytokine in the culture supernatant with the solvent.

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