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# Five D:C-Friedo-Oleanane Triterpenes from the Seeds of *Trichosanthes kirilowii* MAXIM. and Their Anti-inflammatory Effects

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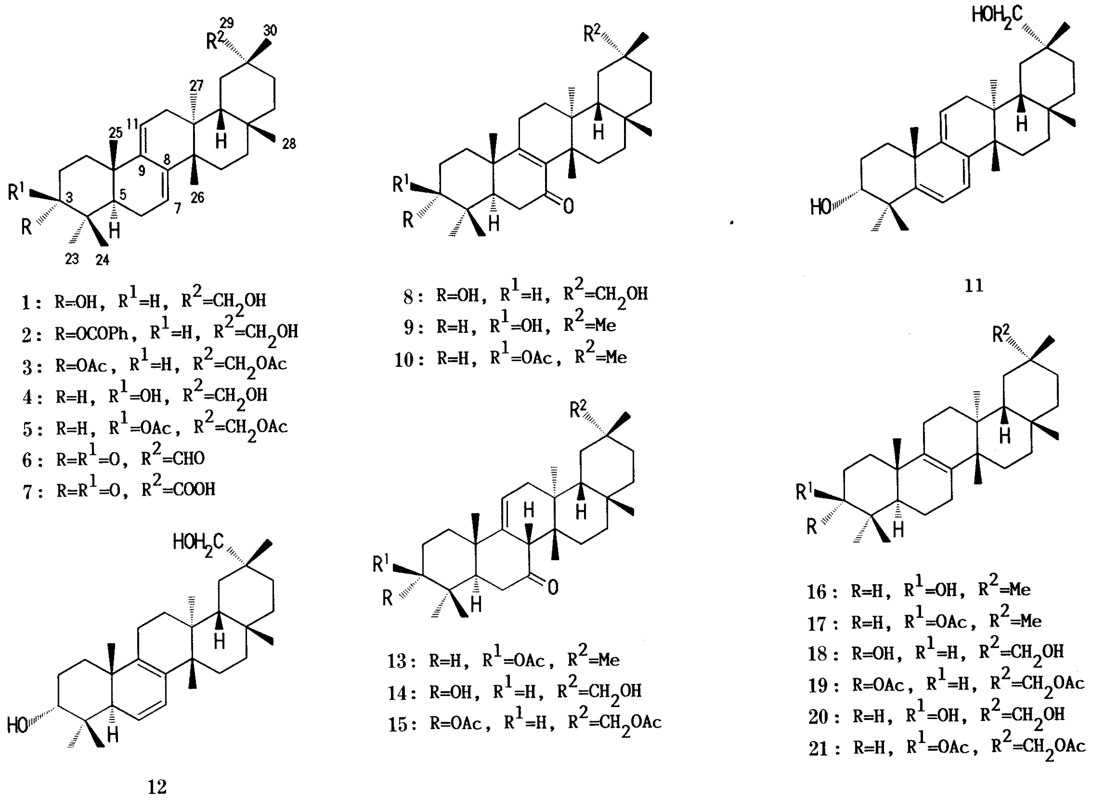
**Five triterpenes with a D:C-friedo-oleanane skeleton, D:C-friedo-oleana-7,9(11)-diene-3β,29-diol (3-epikarounidiol), 7-oxo-D:C-friedo-olean-8-en-3β-ol (7-oxoisomultiflorenol), 7-oxo-8β-D:C-friedo-olean-9(11)-ene-3α,29-diol, D:C-friedo-olean-8-ene-3α,29-diol (3-epibryonolol), and D:C-friedo-olean-8-ene-3β,29-diol (bryonolol), the first four of which are new naturally occurring compounds, were isolated from the seeds of *Trichosanthes kirilowii* MAXIM. The structures were determined by spectral and chemical methods. 3-Epikarounidiol, 7-oxoisomultiflorenol, and 3-epibryonolol showed marked inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in mice. The 50% inhibitory dose of these triterpenes for TPA-induced inflammation (1 μg) was 0.2—0.6 mg/ear.**

**Keywords** *Trichosanthes kirilowii*; seed; D:C-friedo-oleanane triterpenes; antioedema; TPA-induced ear oedema

The seeds of *Trichosanthes kirilowii* MAXIM. (Cucurbitaceae) have been used in Chinese medicine as an anti-inflammatory agent, a cough medicine, and an expectorant.[1)] In previous papers[2—5)] we reported the isolation and structure elucidation of five D:C-friedo-oleanane triterpenes: karounidiol (**1**),[2)] its 3-*O*-benzoate (**2**),[2)] 7-oxodihydrokarounidiol (**8**),[3)] 5-dehydrokarounidiol (**11**),[4)] and isokarounidiol (**12**).[5)] This paper describes our continued study on the *T. kirilowii* seed extract which led to the isolation and characterization of five other D:C-friedo-oleanane triterpenes: D:C-friedo-oleana-7,9(11)-diene-3β,29-diol (3-epikarounidiol; **4**), 7-oxo-D:C-friedo-olean-8-en-3β-ol (7-oxoisomultiflorenol; **9**), 7-oxo-8β-D:C-friedo-olean-9(11)-ene-3α,29-diol (**14**; this was isolated and identified as its acetyl derivative, **15**), D:C-friedo-olean-8-ene-3α,29-diol (3-epibryonolol; **18**), and D:C-friedo-olean-8-ene-3β,29-diol (bryonolol; **20**), the former four being novel naturally occurring compounds.



1: R=OH, R$^1$=H, R$^2$=$CH_2OH$
2: R=OCOPh, R$^1$=H, R$^2$=$CH_2OH$
3: R=OAc, R$^1$=H, R$^2$=$CH_2OAc$
4: R=H, R$^1$=OH, R$^2$=$CH_2OH$
5: R=H, R$^1$=OAc, R$^2$=$CH_2OAc$
6: R=R$^1$=O, R$^2$=CHO
7: R=R$^1$=O, R$^2$=COOH

8: R=OH, R$^1$=H, R$^2$=$CH_2OH$
9: R=H, R$^1$=OH, R$^2$=Me
10: R=H, R$^1$=OAc, R$^2$=Me

13: R=H, R$^1$=OAc, R$^2$=Me
14: R=OH, R$^1$=H, R$^2$=$CH_2OH$
15: R=OAc, R$^1$=H, R$^2$=$CH_2OAc$

16: R=H, R$^1$=OH, R$^2$=Me
17: R=H, R$^1$=OAc, R$^2$=Me
18: R=OH, R$^1$=H, R$^2$=$CH_2OH$
19: R=OAc, R$^1$=H, R$^2$=$CH_2OAc$
20: R=H, R$^1$=OH, R$^2$=$CH_2OH$
21: R=H, R$^1$=OAc, R$^2$=$CH_2OAc$

Chart 1

In the course of our study on the anti-inflammatory effect of plant extracts,[6-9] we have demonstrated that some sterols and triterpenes,[7] including **1**[9] and **2**,[7] possess marked inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in mice. The anti-inflammatory effect of the five D:C-friedo-oleananes was examined in this study: **4**, **9**, **15** (acetyl derivative), **18**, and **20** were isolated from *T. kirilowii* seeds, and **4**, **9**, and **18** were found to have a marked inhibitory effect.

Acetyl derivatives of five triterpenes: **5**, **10**, **15**, **19**, and **21**, were isolated from the saponified extract of the seeds of *T. kirilowii* by silica gel column chromatography followed by acetylation of the separated triterpene fraction, and subsequent reversed phase HPLC of the acetate fraction. Triterpene **15** was identified as the diacetate of 7-oxo-8$\beta$-D:C-friedo-olean-9(11)-ene-3$\alpha$,29-diol (**14**) by chromatographic and spectral comparison with synthetic **15**.[3] Alkaline hydrolysis of **5**, **10**, **19**, and **21** yielded the corresponding free alcohols, **4**, **9**, **18**, and **20**, respectively. Triterpenes **18** and **20** were identified as 3-epibryonolol and bryonolol, respectively, by chromatographic and spectral comparison with the corresponding synthetic compounds.[3] The structures of **4** and **9** were established as described below.

The molecular formula of **4** was determined as $C_{30}H_{48}O_2$ on the basis of the high-resolution mass spectrum (HR-MS) [*m/z* 440.3676 ($M^+$)]. The compound had two hydroxyl groups [the acetyl derivative **5** was a diacetate; *m/z* 524.3842 ($M^+$), $C_{34}H_{52}O_4$], and two double bonds as revealed by $^{13}$C-NMR spectroscopy (Table I). Thus, in combination with the molecular formula, it was indicated that **4** was pentacyclic. The UV spectrum ($\lambda_{max}$ 232, 237, 247 nm) and the shifts of the olefinic proton signals in the $^1$H-NMR spectrum (Table II) are consistent with $\Delta^{7,9(11)}$-conjugated diene system.[2] Triterpene **4** was confirmed to have the basic skeleton, double bonds, and hydroxyl groups in the same position as karounidiol (**1**), with the exception of the orientation of the hydroxyl group at C-3, by spectral comparison and detailed analysis of the $^{13}$C- and $^1$H-NMR and mass spectral data (see Tables I and II, and Experimental section) in the manner described previously.[2-5] The $^1$H-NMR of **4** showed the H-3 proton signal at $\delta$ 3.23 as a double doublet ($J=4.4$, 11.0 Hz), which suggested that H-3 oriented axially with the equatorial hydroxyl group pointing toward $\beta$-face[3]; we propose the structure D:C-friedo-oleana-7,9(11)-diene-3$\beta$,29-diol (3-epikarounidiol) for **4** which was confirmed by chemical correlation with karounidiol (1) isolated from the same source. Thus, Sarrett oxidation of **1** afforded a keto-aldehyde (**6**) and a keto-carboxylic acid (**7**). $NaBH_4$ reduction of **6** yielded **4**, in addition to **1**, which was identical by chromatographic and spectral comparison with natural **4**.

Table I. $^{13}$C-NMR Spectral Data ($\delta$/ppm) for the Triterpenes from *Trichosanthes kirilowii* Seeds ($CDCl_3$, 100.62 MHz)[a]

| $^{13}$C | **4** | **9** | **15**[b] | **18** |
|---|---|---|---|---|
| 1 | 35.6 (t) | 34.4 (t) | 30.5 (t) | 29.5 (t) |
| 2 | 27.7 (t) | 27.4 (t) | 23.4 (t) | 25.8 (t) |
| 3 | 79.1 (d) | 78.2 (d) | 77.5 (d) | 75.9 (d) |
| 4 | 39.0 (s) | 38.7 (s) | 37.3 (s) | 37.5 (s) |
| 5 | 47.8 (d) | 47.5 (d) | 47.4 (d) | 44.6 (d) |
| 6 | 23.9 (t) | 36.5 (t) | 40.5 (t) | 19.1 (t) |
| 7 | 118.3 (d) | 198.3 (s) | 208.0 (s) | 27.0 (t) |
| 8 | 144.5 (s) | 142.4 (s) | 59.8 (d) | 135.1 (s) |
| 9 | 142.1 (s) | 163.0 (s) | 144.0 (s) | 133.6 (s) |
| 10 | 36.4 (s) | 39.3 (s) | 39.1 (s) | 37.5 (s) |
| 11 | 114.4 (d) | 22.5 (t) | 116.3 (d) | 20.7 (t) |
| 12 | 39.3 (t) | 29.7 (t) | 38.6 (t) | 30.8 (t) |
| 13 | 37.4 (s) | 38.0 (s) | 36.9 (s) | 37.5 (s) |
| 14 | 40.0 (s) | 39.3 (s) | 39.2 (s) | 40.7 (s) |
| 15 | 27.5 (t) | 29.2 (t) | 28.3 (t) | 26.7 (t) |
| 16 | 36.7 (t) | 36.1 (t) | 36.5 (t) | 36.3 (t) |
| 17 | 31.7 (s) | 30.8 (s) | 32.3 (s) | 31.1 (s) |
| 18 | 44.5 (d) | 42.2 (d) | 45.0 (d) | 42.9 (d) |
| 19 | 27.1 (t) | 35.4 (t) | 29.7 (t) | 28.7 (t) |
| 20 | 32.7 (s) | 28.5 (s) | 31.4 (s) | 33.1 (s) |
| 21 | 29.9 (t) | 33.0 (t) | 28.8 (t) | 28.8 (t) |
| 22 | 34.3 (t) | 38.4 (t) | 33.9 (t) | 37.5 (t) |
| 23 | 27.6 (q) | 27.3 (q) | 27.2 (q) | 28.0 (q) |
| 24 | 15.3 (q) | 15.0 (q) | 21.0 (q) | 22.3 (q) |
| 25 | 20.7 (q) | 18.2 (q) | 19.9 (q) | 19.6 (q) |
| 26 | 22.0 (q) | 26.5 (q) | 24.3 (q) | 25.9 (q) |
| 27 | 19.2 (q) | 18.5 (q) | 21.2 (q) | 18.0 (q) |
| 28 | 31.0 (q) | 30.7 (q) | 30.3 (q) | 31.3 (q) |
| 29 | 71.1 (q) | 34.2 (q) | 72.8 (q) | 72.8 (q) |
| 30 | 30.0 (q) | 31.8 (q) | 29.7 (q) | 27.6 (q) |
| 3-OCO$\underline{C}H_3$ | — | — | 21.3 (q) | — |
| 29-OCO$\underline{C}H_3$ | — | — | 21.1 (q) | — |
| 3-O$\underline{C}OCH_3$ | — | — | 170.7 (s) | — |
| 29-O$\underline{C}OCH_3$ | — | — | 171.4 (s) | — |

*a*) Multiplicity in parentheses determined by DEPT experiment. *b*) Acetyl derivative.

The molecular formula of triterpene **9** was determined as $C_{30}H_{48}O_2$ based on the HR-MS [*m/z* 440.3631 ($M^+$)]. $^{13}$C-NMR spectroscopy revealed that **9** has a hydroxyl group [the acetyl derivative **10** was a monoacetate; *m/z* 482.3738 ($M^+$), $C_{32}H_{50}O_3$], a carbonyl group and a double bond (Table I). The hydroxyl group was suggested to be located at C-3 with equatorial orientation pointing toward $\beta$-face, by $^1$H-NMR spectroscopy which exhibited a double doublet at $\delta$ 3.30 ($J=4.8$, 11.7 Hz) due to H-3$\alpha$ being axially oriented.[3] Triterpene **9** exhibited absorptions at 254 nm in the UV spectrum and 1654 and 1585 cm$^{-1}$ in the IR spectrum, suggesting the presence of a conjugated enone. The conjugated enone has to be a 7-oxo-8-ene because of the presence of diagnostic fragments $C_{19}H_{27}O_2^+$ (*m/z* 287, ABC ring + C-26, C-27) and $C_{15}H_{24}O_2^+$ (*m/z* 236, AB ring + C-10–C-12; this is characteristic of 7-oxo triterpenes with a $\Delta^8$ or $\Delta^{9(11)}$ double bond) in the mass spectrum.[10] The ion $C_{19}H_{27}O_2^+$ further suggested that **9** possesses methyl groups at C-13 and C-14, but no methyl groups at C-8 and C-9.[2,10] The $^{13}$C-NMR spectrum confirmed the presence of a tetrasubstituted double bond (Table I). Taking these and the fact that the $^1$H-NMR spectrum exhibited only the methyl singlets into consideration, triterpene **9** was proposed to have a D:C-friedo-oleanane skeleton and, thus, to possess the structure 7-oxo-D:C-friedo-olean-8-en-3$\beta$-ol (7-oxoisomultiflorenol). The proposed structure was confirmed by chemical correlation with known D:C-friedo-olean-8-en-3$\beta$-ol (isomultiflorenol, **16**).[11] Oxidation of isomultiflorenol acetate (**17**) with chromyl chloride yielded 7-oxo-8$\beta$-D:C-friedo-olean-9(11)-en-3$\beta$-ol acetate (**13**), which was isomerized by treatment with $BF_3$

Table II. $^1$H-NMR Spectral Data ($\delta$/ppm) for the Triterpenes from *Trichosanthes kirilowii* Seeds ($CDCl_3$, 400 MHz)[a]

| Proton | **4** | **9** | **15**[b] | **18** |
|---|---|---|---|---|
| 1 | 1.51, 1.80 (dt; 12.6, 3.3) | 1.40, 1.86 (dt; 13.2, 3.3) | 1.50, 1.84 | 1.48, 1.53 |
| 2 | 1.63, 1.76 | 1.68, 1.75 (ddd; 3.7, 7.7, 15.8) | 1.83, 2.04 | 1.60, 1.94 |
| 3 | 3.23 (dd; 4.4, 11.0) | 3.30 (dd; 4.8, 11.7) | 4.73 (dd; 2.7, 2.9) | 3.43 (br s) |
| 5 | 1.24 | 1.65 | 1.76 | 1.55 |
| 6 | 2.10 (br dd; 5.5, 17.9)<br>2.24 (dt; 18.1, 4.9) | 2.41, 2.41 | 2.22 (dd; 3.7, 13.9)<br>2.41 (dd; 13.9, 13.9) | 1.41, 1.61 |
| 7 | 5.50 | — | — | 2.05, 2.05 |
| 8 | — | — | 2.82 (br s) | — |
| 11 | 5.22 (br d; 5.2) | 2.12, 2.27 (br dd; 8.4, 11.0) | 5.35 (br d; 5.4) | 1.87, 2.03 |
| 12 | 1.72, 2.09 | 1.34, 1.56 | 1.58, 1.92 (dd; 5.9, 17.6) | 1.32, 1.53 |
| 15 | 1.37, 1.71 | 1.52, 2.40 | 1.07, 2.87 (dt; 13.9, 7.3) | 1.46, 1.72 |
| 16 | 1.46, 1.64 | 1.39, 1.58 | 1.05, 1.55 | 1.44, 1.57 |
| 18 | 1.59 | 1.55 | 1.53 | 1.56 |
| 19 | 1.35, 1.68 | 1.19 (dd; 5.5, 13.9), 1.38 | 1.40, 1.51 | 1.24, 1.56 |
| 21 | 1.26, 1.48 | 1.27 (dt; 12.5, 2.9), 1.46 | 1.41, 1.60 | 1.36, 1.36 |
| 22 | 0.87, 1.67 | 0.93, 1.44 | 0.92, 1.78 | 0.94, 1.55 |
| 23 | 0.98 (3H, s) | 0.99 (3H, s) | 0.83 (3H, s) | 0.97 (3H, s) |
| 24 | 0.88 (3H, s) | 0.89 (3H, s) | 0.95 (3H, s) | 0.86 (3H, s) |
| 25 | 0.91 (3H, s) | 1.01 (3H, s) | 1.24 (3H, s) | 0.97 (3H, s) |
| 26 | 0.91 (3H, s) | 1.34 (3H, s) | 0.94 (3H, s) | 1.11 (3H, s) |
| 27 | 0.85 (3H, s) | 0.95 (3H, s) | 0.90 (3H, s) | 0.98 (3H, s) |
| 28 | 1.08 (3H, s) | 1.15 (3H, s) | 1.07 (3H, s) | 1.13 (3H, s) |
| 29 | 3.21 (d; 10.4), 3.53 (d; 10.7) | 0.96 (3H, s), 0.96 (3H, s) | 3.82 (d; 11.0), 3.90 (d; 10.3) | 3.25 (d; 10.4), 3.40 (d; 10.4) |
| 30 | 0.98 (3H, s) | 0.98 (3H, s) | 1.00 (3H, s) | 0.90 (3H, s) |
| 3-OCOMe | — | — | 2.08 (3H, s) | — |
| 29-OCOMe | — | — | 2.07 (3H, s) | — |

*a*) Figures in parentheses denote *J* values (Hz). If not otherwise specified in parentheses, the multiplicity of $^1$H signals was not determined. *b*) Acetyl derivative.

etherate[3,12] into a conjugated ketone (**10**) which, upon alkaline hydrolysis, yielded **9**. The semi-synthetic **9** was identical by chromatographic and spectral comparison with natural **9**.

The proton at C-8 of two $\Delta^{9(11)}$-unsaturated D:C-friedo-oleananes, **13** and **15**, was demonstrated to be $\beta$-oriented from examination by two-dimensional (2D) nuclear Overhauser effect (NOE) correlation spectroscopy (2D NOESY). Thus, the 2D NOESY spectrum of **13** showed the clear NOE correlation of H-8 broad singlet ( $\delta$ 2.76, 1H) with the methyl singlets of both $H_3$-25 ($\delta$ 1.24; 10$\beta$-Me) and $H_3$-26 ($\delta$ 0.91; 14 $\beta$-Me) which unambiguously suggested the $\beta$-orientation of H-8. The H-8 broad singlet ($\delta$ 2.82, 1H) (see Table II) of **15** also exhibited the definite NOE correlated peaks coupled with $H_3$-25 ($\delta$ 1.24) and $H_3$-26 ($\delta$ 0.94) singlets in the 2D NOESY spectrum.[13]

Among the five triterpenes, **4**, **9**, **15** (acetyl derivative), **18**, and **20**, isolated from the *T. kirilowii* seed extract, the first four are new naturally occurring compounds, although **15** and **18** were synthesized recently by us.[3] Triterpene **20**, in addition to **16** and several other D:C-friedo-oleanane triterpenes, was recently shown to be biosynthesized in cell suspension cultures of *Citrullus lanatus* and *Luffa cyclindrica* (Cucurbitaceae).[14] Triterpene acetate **15** possesses a $\beta,\gamma$-enone system, and was anticipated to be labile under acidic and basic conditions.[15] We were unable to get **14** from **15** by alkaline hydrolysis but obtained its $\Delta^8$-isomer, **8**, a conjugated enone, as a sole product. Whether triterpene **14** occurs as the seed constituent of *T. kirilowii* or whether it is an artefact formed from **18** by autoxidation during the isolation procedure was not clear.

Tables I and II show the $^{13}$C- and $^1$H-NMR spectra, respectively, of triterpenes **4**, **9**, **15** (acetyl derivative), and **18**, which were newly characterized in the *T. kirilowii* seed extract. The signal assignments were performed by comparison with the literature data for the relevant D:C-friedo-oleanane triterpenes[4,5,16] and 3$\alpha$-hydroxy triterpenes,[17] and further with the aid of several NMR experiments described in the Experimental section.

The five D:C-friedo-oleanane triterpenes isolated in this study were examined for their inhibitory effects on TPA-induced inflammation in mice. The inhibitory effects were compared with those of **2**,[7] and glycyrrhetinic acid (18$\beta$-olean-12-ene-3$\beta$,29-diol)[18] and quercetin (3,3′,4′,5,7-pentahydroxyflavone),[19] known inhibitors of TPA-induced inflammation in mice, and the commercially available anti-inflammatory drugs, indomethacin and hydrocortisone (Table III). Three (**4**, **9**, **18**) of the triterpenes markedly inhibited the TPA-induced inflammation with 0.2—0.6 mg/ear of the 50% inhibitory dose whose inhibitory effect was nearly equal to that of **2**.[7] Whereas the inhibitory effects of the three D:C-friedo-oleananes were weaker than that of hydrocortisone, the inhibition was at a level comparable to that of indomethacin, and was more inhibitive than quercetin. The lower inhibitory effect of **20** was considered to be partly due to its being less solubile in the solvent system ($MeOH-CHCl_3$, 1:1) used for the assay in this study. The inhibitory activities against TPA-induced inflammation have been demonstrated to be almost in parallel with those against tumor promotion,[6,7,20] and further study is necessary on the correlation between the structural features of triterpenes and their anti-inflammatory and anti-tumor-promoting activities.

TABLE III. Inhibitory Effect of Some D:C-Friedo-Oleanane Triterpenes and Reference Compounds on TPA-Induced Inflammation in Mice a)

| Compound | $ID_{50}$ (mg/ear) | I.R. c) (%) |
|---|---|---|
| 3-Epikarounidiol (**4**) | 0.6 | 48 d) |
| 7-Oxoisomultiflorenol (**9**) | 0.2 | 96 d) |
| 7-Oxo-8$\beta$-D:C-friedo-olean-9(11)-ene-3$\alpha$,29-diol diacetate (**15**) | 0.8 | 36 d) |
| 3-Epibryonolol (**18**) | 0.2 | 94 d) |
| Bryonolol (**20**) | — | 25 d) |
| Karounidol 3-*O*-benzoate (**2**) b) | 0.2 | 95 e) |
| Glycyrrhetinic acid | 0.1 | 97 e) |
| Quercetin | 1.6 | 40 f) |
| Indomethacin | 0.3 | 96 f) |
| Hydrocortisone | 0.03 | 99 f) |

*a*) Compounds were applied 30 min before TPA (1 $\mu$g). Ear thickness was determined at 8 h after TPA treatment. *b*) Taken from ref. 7. *c*) $p<0.01$ by Student's *t*-test as compared to the control group. *d*) I.R.: Inhibition ratio at 0.5 mg/ear. *e*) I.R. at 2.0 mg/ear. *f*) I.R. at 1.0 mg/ear.

## Experimental

Melting points were measured with a Yanagimoto melting point apparatus, and are uncorrected. All triterpenes were crystallized as fine needles from acetone–MeOH. Preparative HPLC was carried out on an octadecyl silica column (Altex Ultrasphere ODS 5$\mu$ column, 25 cm × 10 mm i.d.; Beckman Instruments, Inc., San Ramon, California) with MeOH (4 ml/min) using an SSC Flow System 3100K (Sehshu Scientific Co., Tokyo) and an ERC-7520 refractive index detector (Erma Optical Works, Ltd., Tokyo). Gas-liquid chromatography (GLC) was run on a Shimadzu GC-14A apparatus using a DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temp. 275 °C). Karounidiol diacetate (**3**) was the standard for the determination of $Rt_R$ in HPLC [$Rt_R$: 1.00; cholesterol (cholest-5-en-3$\beta$-ol) acetate has $Rt_R$: 3.26 under the above HPLC conditions] whereas cholesterol acetate was the standard ($Rt_R$: 1.00) in GLC. UV spectra were recorded on a Shimadzu UV-300 spectrometer in EtOH, and IR spectra were recorded in KBr with a JASCO IR-810 IR spectrometer. Electron-impact (EI) MS and HR-MS were taken on a Hitachi M-80B double focusing gas chromatograph-mass spectrometer (70 eV) using a direct inlet system. NMR spectra were recorded with JEOL GX-400 and GSX-400 spectrometers at 400 MHz ($^1$H-NMR) and 100.62 MHz ($^{13}$C-NMR) in $CDCl_3$ with tetramethylsilane ($^1$H-NMR) and $CDCl_3$ at $\delta$ 77.0 ($^{13}$C-NMR) as internal standards, and chemical shifts were recorded in $\delta$ values. Acetylation was performed in $Ac_2O$–pyridine at room temperature overnight. Hydrolysis of triterpene acetates was done in 5% KOH in MeOH at room temperature overnight. The seeds of *T. kirilowii* were purchased from Kinokuniya Kan-Yaku Kyoku Co. (Tokyo). Triterpenes **1** and **3**,2) **15** and **18**—**21**,3) and **16** and **17**11) were used as authentic triterpenes in this study. Glycyrrhetinic acid was isolated from licorice root,8) and quercetin from Tokyo Kasei Kogyo Co. (Tokyo), indomethacin and hydrocortisone from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.), and TPA from Chemicals for Cancer Research Inc. (Minnesota, U.S.A.) were purchased. Female ICR mice were obtained from Japan SLC Inc. (Shizuoka, Japan). The NMR experiments used for signal assignments were $^{13}$C distortionless enhancement by polarization transfer (DEPT), $^1$H–$^1$H correlated spectroscopy (COSY), $^1$H–$^1$H NOESY, $^1$H–$^{13}$C COSY, $^1$H–$^{13}$C correlation spectroscopy for long-range couplings (COLOC), and heteronuclear multiple-bond correlation (HMBC) experiments.

**Isolation Procedure** Air-dried and ground seeds of *T. kirilowii* (4 kg) were extracted with $CH_2Cl_2$ in a Soxhlet extractor. Unsaponifiable lipids (17 g) were obtained from the extract (1.2 kg) by alkaline hydrolysis (5% KOH in MeOH, reflux, 3 h). The unsaponifiables were chromatographed over silica gel (700 g) as described previously.2) The residue (2.13 g) of the most polar of the 9 fractions2) was acetylated, and the resultant acetate fraction (1.75 g) was subjected to repeated preparative HPLC which eventually yielded **3** (296 mg),2) **5** (15 mg), **8**-diacetate (179 mg),3) **10** (2 mg), **11**-diacetate (13 mg),4) **12**-diacetate (50 mg),5) **15** (12 mg), **19** (2 mg), and **21** (2 mg). Hydrolysis of **5**, **10**, **15**, **19**, and **21** yielded **4**, **9**, **8**, **18**, and **20**, respectively.

**3-Epikarounidiol Diacetate (5)** mp 189—191 °C. $Rt_R$: 1.24 (HPLC), 4.93 (GLC). UV $\lambda_{max}$ nm (log $\varepsilon$): 232 (4.13), 238 (4.17), 245 (3.97). MS *m/z* (%): 524 ($M^+$, 2), 509 (1), 464 (4), 449 (5), 389 (3), 313 (5), 287 (1), 253 (13), 229 (4), 227 (6), 213 (5), 199 (5), 185 (6), 43 (100). HR-MS *m/z*: 524.3842 [Calcd for $C_{34}H_{52}O_4$ ($M^+$): 524.3862]. $^1$H-NMR $\delta$: 0.77 (3H, s, H-27), 0.86 (3H, s, H-23), 0.90 (3H, s, H-26), 0.93 (3H, s, H-25), 0.96 (3H, s, H-24), 0.99 (2H, s, H-30), 1.07 (3H, s, H-28), 2.06 (3H, s, 3$\beta$-OCO$\underline{\text{Me}}$), 2.09 (3H, s, 29-OCO$\underline{\text{Me}}$), 3.79, 3.90 (each 1H, d, $J=11.0$ Hz, H-29), 4.50 (1H, dd, $J=4.4$, 11.0 Hz, H-3$\alpha$), 5.23 (1H, br d, $J=5.5$ Hz, H-11), 5.48 (1H, m, H-7).

**3-Epikarounidiol (4)** mp 225—226 °C. $Rt_R$: 0.41 (HPLC). UV $\lambda_{max}$ nm (log $\varepsilon$): 232 (4.05), 236 (4.10), 247 (3.91). IR $\nu_{max}$ cm$^{-1}$: 3414, 3340 (OH), 1619 (conjugated C=C), 813 (C=CH). MS *m/z* (%): 440 ($M^+$, 100), 425 (9), 409 (7), 407 (7), 353 (2), 311 (3), 300 (5), 285 (3), 271 (27), 259 (5), 257 (4), 253 (11), 246 (7), 245 (7), 239 (5), 231 (7), 227 (11), 213 (8). HR-MS *m/z*: 440.3676 [Calcd for $C_{30}H_{48}O_2$ ($M^+$): 440.3652]; 271.2007 [Calcd for $C_{19}H_{27}O$: 271.2060]; 257.1947 [Calcd for $C_{18}H_{25}O$: 257.1904]; 245.1932 [Calcd for $C_{17}H_{25}O$: 245.1905]. The prominent fragmentations $C_{19}H_{27}O^+$ (formed by cleavage at C-13–C-18 and C-14–C-15 with 1H loss), $C_{18}H_{25}O^+$ (formed by cleavage at C-12–C-13 and C-15–C-16 with 2H loss), and $C_{17}H_{25}O^+$ (formed by cleavage at C-11–C-12 and C-15–C-16), supported the presence of a $\Delta^{7,9(11)}$-diene system in the D:C-friedo-oleanane skeleton.10)

**7-Oxoisomultiflorenol Acetate (10)** mp 268—272 °C. $Rt_R$: 0.57 (HPLC), 3.51 (GLC). UV $\lambda_{max}$ nm: 245. MS *m/z* (%): 482 ($M^+$, 46), 467 (18), 422 (5), 316 (6), 303 (17), 278 (46), 271 (4), 265 (3), 243 (14), 218 (6), 205 (64), 191 (12), 43 (100). HR-MS *m/z*: 482.3738 [Calcd for $C_{32}H_{50}O_3$ ($M^+$): 482.3756]. $^1$H-NMR $\delta$: 0.88 (3H, s, H-23), 0.94 (3H, s, H-27), 0.96 (6H, s, H-24, H-29), 0.98 (3H, s, H-30), 1.03 (3H, s, H-25), 1.15 (3H, s, H-28), 2.07 (3H, s, 3$\beta$-OCO$\underline{\text{Me}}$), 4.53 (1H, dd, $J=4.4$, 11.7 Hz, H-3$\alpha$).

**7-Oxoisomultiflorenol (9)** mp 214—216 °C. $Rt_R$: 0.38 (HPLC). UV $\lambda_{max}$ nm: 245 (4.03). IR $\nu_{max}$ cm$^{-1}$ : 3436 (OH), 1654, 1585 (conjugated enone). MS *m/z* (%): 440 ($M^+$, 98), 425 (33), 411 (7), 300 (15), 287 (12), 273 (20), 261 (41), 243 (18), 236 (86), 205 (100), 121 (69), 69 (90). HR-MS *m/z*: 440.3631 [Calcd for $C_{30}H_{48}O_2$ ($M^+$): 440.3651]; 287.2065 [Calcd for $C_{19}H_{27}O_2$: 287.2010]; 261.1843 [Calcd for $C_{17}H_{25}O_2$: 261.1852]; 236.1786 [Calcd for $C_{15}H_{24}O_2$: 236.1775]; 205.1924 [Calcd for $C_{15}H_{25}$: 205.1954]. The prominent fragmentations $C_{19}H_{27}O_2^+$ (formed by cleavage at C-13–C-18 and C-14–C-15 with 1H loss), $C_{17}H_{25}O_2^+$ (formed by cleavage at C-11–C-12, C-13–C-14, and C-15–C-16), $C_{15}H_{24}O_2^+$ (formed by cleavage at C-12–C-13 and C-8–C-14 with 1H transfer from the residue), and $C_{15}H_{25}^+$ ($M^+ - C_{15}H_{24}O_2$) supported a 7-oxo-$\Delta^8$-ene structure in the D:C-friedo-oleanane skeleton.10)

**7-Oxo-8$\beta$-D:C-friedo-olean-9(11)-ene-3$\alpha$,29-diol Diacetate (15) and Its Hydrolysis Product (8)** **15**: mp 198—202 °C (lit.3) 198—200 °C). $Rt_R$: 0.33 (HPLC), 7.26 (GLC). UV $\nu_{max}$ nm: 295 (weak). HR-MS *m/z*: 540.2787 [Calcd for $C_{34}H_{52}O_5$ ($M^+$): 540.3811]. Hydrolysis of **15** afforded 7-oxodihydrokarounidiol (**8**) instead of the expected 7-oxo-8$\beta$-D:C-friedo-olean-9(11)-ene-3$\alpha$,29-diol (**14**). **8**: mp 282—285 °C (lit.3) 287—289 °C). $Rt_R$: 0.08 (HPLC). MS *m/z*: 456 ($M^+$). UV $\lambda_{max}$ nm: 253. IR $\nu_{max}$ cm$^{-1}$: 1727, 1709 (C=O), 1247 (C-O), 800 (C=O).

**3-Epibryonolol Diacetate (19) and Its Hydrolysis Product (18)** **19**: mp 205—207 °C (lit.3) 206—208 °C). $Rt_R$: 1.08 (HPLC), 4.25 (GLC). HR-MS *m/z*: 526.4020 [Calcd for $C_{34}H_{54}O_4$ ($M^+$): 526.4019]. 3-Epibryonolol (**18**): mp 271—275 °C (**18** prepared from semi-synthetic **19**3) by hydrolysis showed mp of 270—274 °C). $Rt_R$: 0.51 (HPLC). IR $\nu_{max}$ cm$^{-1}$: 3348 (OH). HR-MS *m/z*: 442.3795 [Calcd for $C_{30}H_{50}O_2$ ($M^+$): 442.3807].

**Bryonolol Diacetate (21) and Its Hydrolysis Product (20)** **21**: 214—215 °C (lit.3) 215—217 °C, lit.21) 206—209 °C). $Rt_R$: 1.32 (HPLC),22) 4.94 (GLC). MS *m/z*: 526 ($M^+$). Bryonolol (**20**): mp 271—281 °C (lit.3) 284—287 °C, lit.21) 285—288 °C). $Rt_R$: 0.39 (HPLC). MS *m/z*: 442 ($M^+$). The $^1$H- and $^{13}$C-NMR data for **21** were consistent with the literature data.16)

**Partial Synthesis of 3-Epikarounidiol (4) from Karounidiol (1)** A solution of **1** (200 mg) in pyridine (3 ml) was added to a well-stirred ice-cold suspension of $CrO_3$ (0.9 g) and pyridine (10 ml). After being stirred overnight the mixture was worked up and the product (192 mg) was subjected to HPLC which yielded 3-oxo-D:C-friedo-oleana-7,9(11)-dien-29-al (**6**) (10 mg) and 3-oxo-D:C-friedo-oleana-7,9(11)-dien-29-oic acid (**2**) (30 mg). **6**: mp 160—162 °C. $Rt_R$: 0.54 (HPLC). MS *m/z* (%): 436 ($M^+$, 100), 421 (13), 407 (4), 403 (3), 393 (4), 314 (4), 298 (4), 297 (4), 269 (49), 259 (13), 243 (16), 218 (8), 205 (19). HR-MS *m/z*: 436.3371 [Calcd for $C_{30}H_{44}O_2$ ($M^+$): 436.3339]. $^1$H-NMR $\delta$: 0.59, 0.89, 0.96, 1.04, 1.05, 1.12, 1.15 (each 3H, s), 5.24 (1H, br d, $J=4.9$ Hz, H-11), 5.47 (1H, br s, H-7), 9.34 (1H, d, $J=1.7$ Hz, H-29). **7**: mp 241—245 °C. R$t_R$: 0.43 (HPLC). MS *m/z* (%): 452 ($M^+$, 100), 437 (16), 422 (2), 391 (8), 367 (3), 366 (3), 314 (3), 297 (3), 283 (3), 271 (17), 269 (28), 258 (10), 257 (11), 243 (17), 229 (9), 221 (4), 209 (7). HR-MS *m/z*: 452.3261 [Calcd for $C_{30}H_{44}O_3$ ($M^+$): 452.3288]. $^1$H-NMR $\delta$: 0.71, 0.88, 1.02, 1.03, 1.12, 1.14, and 1.23 (each 3H, s), 5.25 (1H, br s, $J=5.0$ Hz, H-11), 5.48 (1H, br s, H-7). Reduction of **6** (10 mg) with $NaBH_4$ (20 mg) in MeOH (10 ml) at room temperature overnight followed by the usual work-up and HPLC yielded 3-epikarounidiol (**4**) (8.8 mg) and karounidiol (**1**) (1.6 mg). Semi-synthetic **4** (mp 226—228 °C) was identical by chromatographic and spectral comparison with the natural product (**4**).

**Partial Synthesis of 7-Oxoisomultiflorenol (9) from Isomultiflorenol Acetate (17)** Chromyl chloride (15 $\mu$l) in $CH_2Cl_2$ (0.8 ml) was added to a stirred solution of **17** (50 mg) in $CH_2Cl_2$ (2.5 ml) at $-60$ °C. Aqueous $NaHSO_3$ was added after stirring for 2 h at $-30$ °C. The crude product was isolated by ether extraction. The residue (51 mg) of the extract was subjected to HPLC which yielded 7-oxo-8$\beta$-D : C-friedo-olean-9(11)-en-3$\beta$-ol acetate (**13**) (13 mg). **13**: mp 195—197 °C. R$t_R$: 0.74 (HPLC), 2.98 (GLC). UV $\lambda_{max}$ nm: 293 (weak). MS *m/z* (%): 482 ($M^+$, 35), 467 (8), 329 (9), 316 (4), 303 (11), 278 (67), 271 (9), 243 (4), 206 (32), 205 (24), 191 (41), 43 (100). $^1$H-NMR $\delta$: 0.83 (3H, s, H-23), 0.91 (6H, s, H-24, H-26), 0.93 (3H, s, H-27), 0.96 (3H, s, H-29), 1.00 (3H, s, H-30), 1.02 (3H, s, H-28), 1.24 (3H, s, H-25), 2.06 (3H, s, 3$\beta$-OCO$\underline{Me}$), 2.76 (1H, br s, H-8$\beta$), 4.49 (1H, dd, $J=5.1$, 10.6 Hz, H-3$\alpha$), 5.34 (1H, m, H-11). $BF_3$ etherate (0.13 ml) was added dropwise to a solution of **13** (10 mg) in $C_6H_6$ (6 ml). The mixture was kept at room temperature for 2 d. Aqueous work-up and ether extraction afforded 7-oxoisomultiflorenol acetate (**10**) which was purified by HPLC. Purified **10** (6 mg, mp 276—278 °C) and its hydrolysis product **9** (mp 215—218 °C) were identical by chromatographic and spectral comparison with the acetyl derivative (**10**) and free natural triterpene (**9**), respectively.

**Assay of TPA-Induced Inflammation in Mice** Female ICR mice were housed in an air-condition roomed (22—23 °C) lit from 08:00 to 20:00. Food and water were available *ad libitum*. 1 nM TPA dissolved in acetone (20 $\mu$l) was applied to the right ear only of ICR mice by a micropipette. A volume of 10 $\mu$l was delivered to both the inner and outer surface of the ear. The sample or its vehicle, MeOH–$CHCl_3$ (1 : 1, 20 $\mu$l), as a control, was applied topically about 30 min before each TPA treatment. Application of the sample completely inhibited TPA-induced inflammation and this inhibitory activity was reduced in a dose-dependent manner. For ear thickness determinations, a pocket thickness gauge (Mitsutoyo Co., Ltd., Tokyo, Japan) with a range of 0—9 mm, graduated at 0.01 mm intervals and modified so that the contact surface area was increased, thus reducing the tension, was applied to the tip of the ear.

The ear thickness was measured before treatment (*a*). The oedema was measured 6 h after TPA-treatment (*b*: TPA alone; *b*': TPA plus sample). The inhibition ratio (I.R.) was calculated as follows, where oedema *A*: oedema was induced by TPA alone ($b-a$), oedema *B*: oedema was induced by TPA plus sample ($b'-a$).

$$\text{I.R.} = \frac{A-B}{A} \times 100$$

Each value was the mean of individual determinations for 5 mice, and 50% inhibitory dose ($ID_{50}$) values were determined by the method of probit-graphic interpolation for at least four dose levels.

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