Cyclokirilodiol and Isocyclokirilodiol: Two Novel Cycloartanes from the Seeds of *Trichosanthes kirilowii* MAXIM.

Yumiko KIMURA,*[,a] Toshihiro AKIHISA,[b] Ken YASUKAWA,[a] Sei-ichi TAKASE,[c] Toshitake TAMURA,[b] and Yoshiteru IDA[d]

College of Pharmacy, Nihon University,[a] 7–7–1, Narashinodai, Funabashi-shi, Chiba 274, Japan, College of Science and Technology, Nihon University,[b] 1–8, Kanda Surugadai, Chiyoda-ku, Tokyo 101, Japan, College of Science and Technology (Funabashi Campus), Nihon University,[c] 7–24–1, Narashinodai, Funabashi-shi, Chiba 274, Japan, and School of Pharmaceutical Sciences, Showa University,[d] 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142, Japan.
Received August 22, 1996; accepted October 18, 1996

Two novel cycloartane-type triterpene alcohols possessing a monohydroxy-tetrahydrofuran ring in the side chain, cyclokirilodiol and isocyclokirilodiol, were isolated from the nonsaponifiable lipid fraction obtained from the methanolic extract of *Trichosanthes kirilowii* seeds. Their structures were determined as (22*S*,24*S*)-22,25-epoxy-24-hydroxy-5α-cycloartan-3β-ol and (22*R*,24*S*)-22,25-epoxy-24-hydroxy-5α-cycloartan-3β-ol, respectively, by detailed spectroscopic analyses.

Key words *Trichosanthes kirilowii*; seed; cycloartane triterpene; cyclokirilodiol; isocyclokirilodiol; Cucurbitaceae

The seeds of *Trichosanthes kirilowii* MAXIM. (Cucurbitaceae) are a Chinese medicines used as an anti-inflammatory agent, a cough medicine and an expectorant.[1] We reported previously the isolation and structural elucidation of ten triterpenes[2–5] and four sterols[6] from the nonsaponifiable lipid fraction obtained from the methanolic extract of *T. kirilowii* seeds. These compounds showed marked inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in mice.[4,5,7,8] Further phytochemical investigation on the lipid fraction led us to isolate two novel dihydroxy cycloartane-type triterpenes, cyclokirilodiol (**1**) and isocyclokirilodiol (**2**). This paper describes their characterization.

The two triterpenes, **1** and **2**, were isolated as diacetates (**1a** and **2a**, respectively) from the most polar part of the nonsaponifiable lipid fraction[5] by acetylation followed by reverse phase HPLC.

Cyclokirilodiol diacetate (**1a**) was found to have a molecular formula of $C_{34}H_{54}O_5$ by high-resolution electron ionization mass spectrometry (HR-EI-MS) with a molecular ion (M^+) at *m/z* 542.3987. Its IR spectrum indicated the presence of an ester group (1735 cm^{-1}), a cyclopropyl group (3040 cm^{-1}) and no hydroxyl group. The ^{1}H-NMR spectrum showed two acetoxy methyls (3H s at δ 2.05 and 2.06) and two methine proton signals (dd at δ 4.57 and 4.95), suggesting the presence of two secondary acetoxyl groups, in addition to the signals due to an oxygenated methine (δ 4.04), a secondary methyl (δ 0.89), six tertiary methyls and a cyclopropyl methylene group (ABq at δ 0.34 and 0.57, J=4 Hz) (Table 1) . On alkaline hydrolysis, **1a** yielded an alcohol, cyclokirilodiol (**1**), with M^+ at *m/z* 458.3775 ($C_{30}H_{50}O_3$) in the HR-EI-MS. Its IR spectrum indicated the presence of hydroxyl (3419 cm^{-1}) and cyclopropyl groups (3040 cm^{-1}). The AB quartet appeared at δ 0.34 and 0.55 (J=4 Hz), a typical feature of cycloartenol, and the ^{13}C-NMR spectrum showed signals assignable to the carbon atoms on its A, B and C rings (Table 2), so that **1** was suggested to be a triterpene with a cycloartane skeleton.[9–12] This was supported by the presence of the diagnostic fragment peak at *m/z* 315 ($C_{22}H_{35}O^+$) formed by loss of the side chain moiety from 3β-hydroxy-cycloartanes. The structure of the side chain moiety, formulted as $C_8H_{15}O_2$, was confirmed as follows. ^{1}H–^{1}H correlations between H_3-21/H-20, H-20/H-22, H-20/H-17, H-22/H_2-23, H_2-23/H-24 were observed in the ^{1}H-NMR spectra of **1** and **1a**, and their heteronuclear multiple-bond connectivity (HMBC) spectra revealed correlations between H_3-21/C-17, H_3-21/C-20, H_3-21/C-22, H_2-23/C-20, H_2-23/C-22, H_2-23/C-24, H_2-23/C-25, H_3-26/C-24, H_3-26/C-25, H_3-27/C-24 and H_3-27/C-25. The chemical shifts of C-22, C-24 and C-25 of **1** at δ 76.7, 78.2 and 81.7, respectively, indicated that they are linked to an oxygen atom, and comparison of the proton signals at C-22 and C-24 between **1** and **1a** led us to conclude that the third oxygen atom forms an ether linkage between C-22 and C-25 and that the C-24 has a hydroxyl group in **1**, as shown in the figure. Thus, **1** and **1a** were established as 22,25-epoxy-24-hydroxy-5α-cycloartan-3β-ol and its 3,24-diacetate.

Isocyclokirilodiol diacetate (**2a**) was found to have a molecular formula of $C_{34}H_{54}O_5$ by HR-EI-MS. On alkaline hydrolysis, **2a** afforded isocyclokirilodiol (**2**), which was formulated as $C_{30}H_{50}O_3$. Spectral data of **2** and **2a** were quite similar to those of **1** and **1a**, respectively, suggesting that their structures resemble each other. Furthermore, the ^{1}H–^{1}H correlation spectroscopy (COSY) and HMBC spectra of **2** and **2a** showed their planar structure to be the same as **1** and **1a**, respectively.

In order to establish the stereostructures of **1** and **2**, difference nuclear Overhauser effect (NOE) experiments were carried out. For **1**, an NOE was observed between H-20/H_3-18, H-20/H-22, H-22/H_2-16, H-22/H-23b, H-22/H-24 and H-22/H_3-26. This indicates that H-20, H-23b, H-24 and H_3-26 are located on the same face with H-22 while this proton is located near the C-16 methylene group. Further NOE correlations were also observed between H_3-21/H-17, H_3-21/H-23b as shown in the figure. Therefore, the absolute stereostructure of cyclokirilodiol (**1**) was characterized as (22*S*,24*S*)-22,25-epoxy-24-hydroxy-

* To whom correspondence should be addressed.

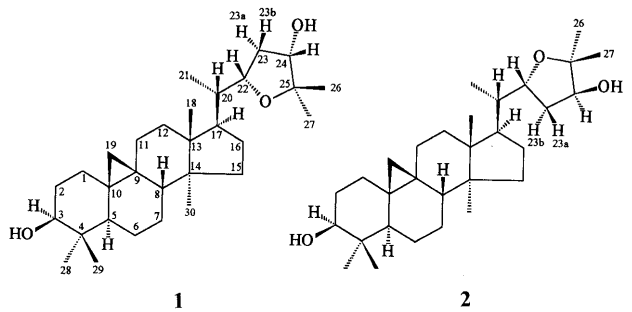
Table 1. ^{1}H-NMR Data (δ/ppm, 400 MHz, $CDCl_3$) of Cyclokirilodiol (**1**), Isocyclokirilodiol (**2**) and Their Diacetyl Derivatives, **1a** and **2a**[a)]

Proton	1	1a	2	2a
1	1.58 (α), 1.25 (β)	1.61 (α), 1.27 (β)	1.58 (α), 1.25 (β)	1.61 (α), 1.26 (β)
2	1.76 (α), 1.57 (β)	1.78 (α), 1.62 (β)	1.76 (α), 1.57 (β)	1.78 (α), 1.62 (β)
3	3.29 (dd, 4.4, 11.0)	4.57 (dd, 5.1, 10.6)	3.29 (dd, 4.4, 11.0)	4.57 (dd, 5.1, 10.6)
5	1.30 (dd, 3.7, 12.6)	1.39 (dd, 4.0, 12.0)	1.30 (dd, 4.0, 12.6)	1.39 (dd, 4.4, 12.5)
6 (α)	1.60	1.59	1.60	1.58
6 (β)	0.79 (dq, 2.6, 12.5)	0.80 (dq, 2.6, 12.5)	0.79 (dq, 2.2, 12.1)	0.79 (dq, 2.2, 12.8)
7	1.08 (α), 1.33 (β)	1.09 (α), 1.34 (β)	1.09 (α), 1.33 (β)	1.09 (α), 1.34 (β)
8	1.50 (dd, 4.5, 12.6)	1.51 (dd, 4.8, 11.2)	1.51 (dd, 4.5, 12.5)	1.51 (dd, 4.8, 12.5)
11	2.02 (α), 1.13 (β)	2.00 (α), 1.15 (β)	2.01 (α), 1.11 (β)	2.00 (α), 1.15 (β)
12	1.70 (α), 1.61 (β)	1.70 (α), 1.59 (β)	1.70 (α), 1.62 (β)	1.70 (α), 1.61 (β)
15	1.31 (2H)	1.32 (2H)	1.32 (2H)	1.31 (2H)
16	1.98 (α), 1.34 (β)	1.98 (α), 1.35 (β)	1.98 (α), 1.35 (β)	1.97 (α), 1.35 (β)
17	1.95	1.99	1.94	1.93
18	0.96 (s)	0.95 (s)	0.95 (s)	0.95 (s)
19 (*exo*)	0.34 (d, 4.0)	0.34 (d, 4.0)	0.33 (d, 4.0)	0.34 (d, 4.0)
19 (*endo*)	0.55 (d, 4.0)	0.57 (d, 4.0)	0.55 (d, 4.0)	0.57 (d, 4.0)
20	1.45	1.47	1.41	1.44
21	0.92 (d, 7.3)	0.89 (d, 7.2)	0.85 (d, 7.0)	0.87 (d, 7.2)
22	4.01 (ddd, 1.8, 8.1, 8.1)	4.04 (ddd, 1.0, 7.3, 7.3)	4.22 (ddd, 1.8, 7.0, 7.0)	4.19 (ddd, 1.0, 7.0, 7.0)
23a	1.67 (ddd, 4.8, 8.1, 13.6)	1.72 (ddd, 4.0, 7.3, 13.6)	1.77 (ddd, 4.8, 7.0, 12.8)	1.74 (ddd, 3.7, 7.0, 13.4)
23b	2.25 (ddd, 7.0, 8.1, 13.6)	2.33 (ddd, 6.6, 7.3, 13.6)	2.04 (ddd, 7.0, 7.0, 12.8)	2.13 (ddd, 7.0, 7.0, 13.4)
24	3.91 (dd, 4.8, 7.0)	4.95 (dd, 4.0, 7.3)	3.89 (dd, 4.8, 7.0)	4.90 (dd, 3.7, 7.0)
26	1.15 (s)	1.18 (s)	1.19 (s)	1.22 (s)
27	1.21 (s)	1.18 (s)	1.18 (s)	1.16 (s)
28	0.97 (s)	0.85 (s)	0.97 (s)	0.85 (s)
29	0.81 (s)	0.89 (s)	0.81 (s)	0.89 (s)
30	0.93 (s)	0.93 (s)	0.93 (s)	0.93 (s)
3-OCOMe	—	2.05 (s)	—	2.05 (s)
24-OCOMe	—	2.06 (s)	—	2.08 (s)

a) Figures in parentheses denote *J* values (Hz). If not otherwise specified in parentheses, multiplicity of ^{1}H signals was not determined.

Table 2. ^{13}C-NMR Data (δ/ppm, 100.6 MHz, $CDCl_3$) of Cyclokirilodiol (**1**), Isocyclokirilodiol (**2**) and Their Diacetyl Derivatives, **1a** and **2a**

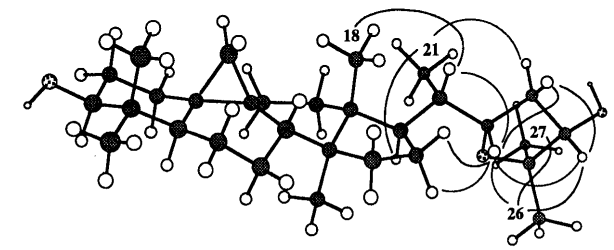
Carbon	1	1a	2	2a	Carbon	1	1a	2	2a
1	32.0	31.6	32.0	31.6	18	17.9	17.8	17.9	17.8
2	30.4	26.8	30.4	26.8	19	29.9	29.8	29.9	29.7
3	78.8	80.7	78.8	80.7	20	39.3	39.3	39.8	39.3
4	40.5	39.5	40.5	39.5	21	12.2	12.2	12.2	12.3
5	47.1	47.2	47.1	47.2	22	76.7	77.2	77.2	78.4
6	21.1	20.9	21.2	20.9	23	38.7	36.1	37.9	35.5
7	26.1	25.9	26.1	25.9	24	78.2	79.1	78.4	79.7
8	48.1	48.0	48.2	48.0	25	81.7	81.2	81.6	81.2
9	20.0	20.2	20.0	20.2	26	25.0	25.0	27.3	27.7
10	26.1	26.0	26.1	26.0	27	22.4	22.6	20.9	21.8
11	26.5	26.5	26.5	26.5	28	25.4	25.4	25.4	25.4
12	32.8	32.7	32.8	32.8	29	14.0	15.1	14.0	15.1
13	45.4	45.5	45.4	45.5	30	19.6	19.6	19.6	19.6
14	48.7	48.7	48.7	48.7	3-COMe	—	170.7	—	171.0
15	35.7	35.7	35.7	35.6	3-COMe	—	21.3	—	21.3
16	27.8	27.8	27.8	27.7	24-COMe	—	171.0	—	171.1
17	49.1	49.1	48.9	49.0	24-COMe	—	21.1	—	21.1



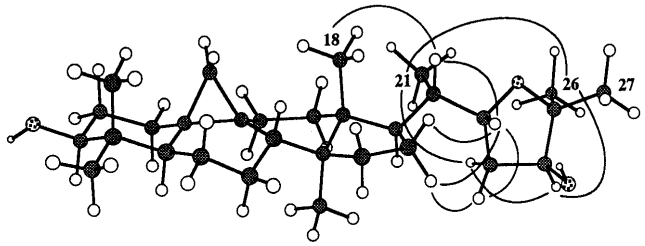
1 **2**

5α-cycloartan-3β-ol.

For **2**, the NOE correlations observed were H_3-21/H_3-26, H-22/H_2-16, H-22/H-20, H-22/H-23a, H-23a/H-16α, H-23b/H_3-21, H-23b/H-24, H-24/H_3-26 and those shown in the figure. Thus, the stereostructure of isocyclokirilodiol (**2**) was determined as (22*R*,24*S*)-22,25-epoxy-24-hydroxy-5α-cycloartan-3β-ol. The most stable conformations of **1** and **2** with minimum steric energy were simulated by CAChe[13)] as shown in the figure. The simulated conformers were mostly consistent with the NOE correlation mentioned above.



1



2

Fig. 1. CAChe Drawings and Some Representative NOE Correlations (—) for Cyclokirilodiol (**1**) and Isocyclokirilodiol (**2**)

This study is the first instance of the isolation of triterpenes from natural products possessing a monohydroxy-tetrahydrofuran ring in their side chain.

Experimental

General Procedure General procedures were the same as described in previous papers.[5,6]

Isolation Procedure The acetylated product (1.75 g) of the most polar of the fractions[6] was repeatedly chromatographed on preparative HPLC to give new compounds, **1a** (8 mg) and **2a** (3 mg), together with the acetyl derivatives of four hydroxylated sterols as reported previously.[6] On alkaline hydrolysis with 5% KOH in MeOH, **1a** and **2a** afforded free alcohols, **1** (96% yield) and **2** (98% yield), respectively.

Cyclokirilodiyl Diacetate (1a) Fine needles (acetone–MeOH), mp 171—175 °C. Rt_R(II): 0.11 (HPLC), 5.06 (GLC). IR (KBr) cm^{-1}: 3040, 1735. MS *m/z* (%): 542 (M^+, 2), 482 (16), 467 (5), 439 (3), 422 (2), 407 (2), 360 (5), 325 (2), 297 (4), 255 (2), 229 (2), 215 (1), 203 (5), 185 (3), 175 (7), 157 (17), 124 (15), 97 (100). HR-EI-MS *m/z*: 542.3987 (M^+, Calcd for $C_{34}H_{54}O_5$: 542.3968), 482.3730 ($C_{32}H_{50}O_3$: 482.3757), 467.3505 ($C_{31}H_{47}O_3$: 467.3522), 422.3532 ($C_{30}H_{46}O$: 422.3535). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

Cyclokirilodiol (1) Fine plates (acetone–MeOH), mp 220—224 °C. Rt_R(I): 0.11 (HPLC), 5.13 (GLC). IR (KBr) cm^{-1}: 3419, 3040, 2931, 1451, 1379, 1100, 1049. MS *m/z* (%): 458 (M^+, 3), 443 (2), 440 (7), 425 (3), 398 (1), 325 (1), 318 (7), 315 (2), 297 (1), 260 (1), 255 (1), 229 (2), 203 (2), 175 (4), 142 (17), 115 (100), 97 (8), 71 (95). HR-EI-MS *m/z*: 458.3775 (M^+, Calcd for $C_{30}H_{50}O_3$: 458.3757), 443.3556 ($C_{29}H_{47}O_3$: 443.3523), 440.3643 ($C_{30}H_{48}O_2$: 440.3651), 425.3426 ($C_{29}H_{45}O_2$: 425.3417), 115.0759 ($C_6H_{11}O_2$: 115.0758), 71.0497 (C_4H_7O: 71.0496). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

Isocyclokirilodiyl Diacetate (2a) Amorphous powder. Rt_R(II): 0.10 (HPLC), 4.88 (GLC). IR (KBr) cm^{-1}: 3040, 1738. MS *m/z* (%): 542 (M^+, 1), 482 (7), 467 (2), 439 (1), 407 (1), 360 (3), 297 (1), 229 (1), 215 (1), 203 (2), 187 (2), 185 (1), 184 (2), 175 (3), 157 (10), 124 (10), 97 (100). HR-EI-MS *m/z*: 542.4001 (M^+, Calcd for $C_{34}H_{54}O_5$: 542.3968), 482.3737 ($C_{32}H_{50}O_3$: 482.3757). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

Isocyclokirilodiol (2) Fine needles (acetone–MeOH), mp 210—213 °C. Rt_R(II): 0.11 (HPLC), 5.05 (GLC). IR (KBr) cm^{-1}: 3422, 3040, 2925, 1457, 1379, 1099. MS *m/z* (%): 458 (M^+, 4), 443 (4), 440 (4), 425 (5), 398 (1), 318 (5), 315 (1), 297 (1), 260 (1), 255 (1), 229 (2), 203 (3), 175 (4), 142 (16), 115 (100), 97 (13), 71 (90). HR-EI-MS *m/z*: 458.3749 (M^+, Calcd for $C_{30}H_{50}O_3$: 458.3757), 443.3570 ($C_{29}H_{47}O_3$: 443.3523), 440.3687 ($C_{30}H_{48}O_2$: 440.3651), 425.3395 ($C_{29}H_{45}O_3$: 443.3523), 115.0751 ($C_6H_{11}O_2$: 115.0758), 71.0490 (C_4H_7O: 71.0496). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

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13) CAChe with extended MM2 parameters (CAChe Scientific Inc., Beaverton, Oregon, U.S.A.). Conformation with minimum steric energy was obtained by potential energy map using the "Sequential Search" option. The minimum steric energy calculated was: **1** (191.90 kcal/mol) and **2** (190.79 kcal/mol). Drawings were performed by Chem3D program (Cambridge Scientific Computing Inc., Cambridge, Massachusetts, U.S.A.).