5-Dehydrokarounidiol [D:C-Friedo-oleana-5,7,9(11)-triene-3α,29-diol],[1] a Novel Triterpene from *Trichosanthes kirilowii* MAXIM.

Toshihiro AKIHISA,*,[a] Wilhelmus C. M. C. KOKKE,[b] Jeanette A. KRAUSE,[c] Drake S. EGGLESTON,[c] Shin-ichi KATAYAMA,[a] Yumiko KIMURA,[d] and Toshitake TAMURA[a]

College of Science and Technology, Nihon University,[a] 1–8, Kanda Surugadai, Chiyoda-ku, Tokyo 101, Japan, College of Business and Administration, Graduate MBA, St. Joseph's University,[b] Philadelphia, PA 19131, U.S.A., Department of Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals,[c] King of Prussia, PA 19406, U.S.A., College of Pharmacy, Nihon University,[d] 7–7–1, Narashinodai, Funabashi, Chiba 274, Japan.
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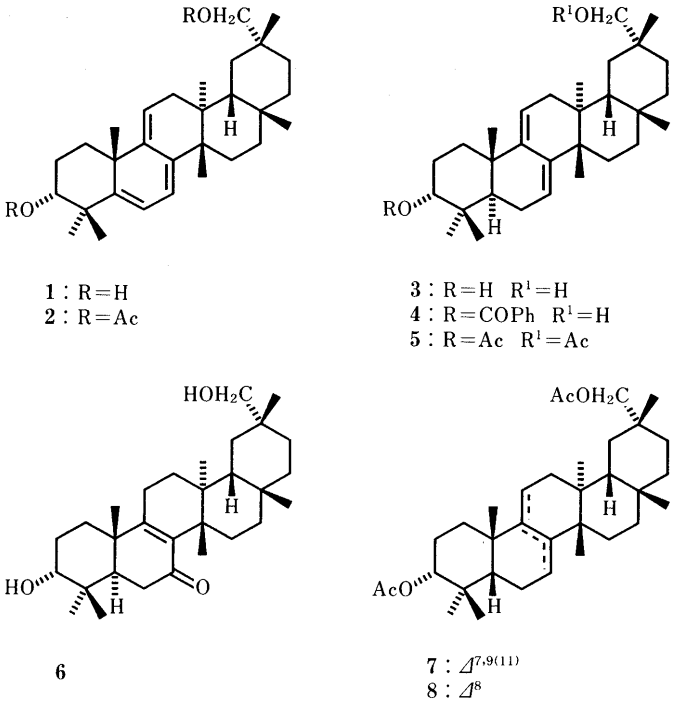
A new triterpene isolated from the seeds of *Trichosanthes kirilowii* (Cucurbitaceae) was proposed to be 5-dehydrokarounidiol [D:C-friedo-oleana-5,7,9(11)-triene-3α,29-diol] on the basis of spectroscopic evidence and because three closely related compounds, D:C-friedo-oleana-7,9(11)-diene-3α,29-diol, its 3-*O*-benzoate, and 7-oxo-D:C-friedo-olean-8-ene-3α,29-diol, had previously been isolated from the same source. The structure was confirmed by X-ray analysis of the corresponding diacetate. This is the first report of a naturally occurring triterpene possessing a $\Delta^{5,7,9(11)}$-conjugated triene system.

Keywords *Trichosanthes kirilowii*; Cucurbitaceae; seed; triterpene; 5-dehydrokarounidiol; D:C-friedo-oleana-5,7,9(11)-triene-3α,29-diol; X-ray analysis; ^{1}H-NMR; ^{13}C-NMR

The seeds of *Trichosanthes kirilowii* MAXIM. (Cucurbitaceae) have been used in Chinese medicine as an anti-inflammatory agent, a cough medicine, and expectorant.[2] In previous papers[3,4] we reported the isolation and structure elucidation of D:C-friedo-oleana-7,9(11)-diene-3α,29-diol (**3**), its 3-*O*-benzoate (**4**), and 7-oxo-D:C-friedo-olean-8-ene-3α,29-diol (**6**) from the seeds of *T. kirilowii*. Triterpenes **3** and **6** were given the trivial names karounidiol and 7-oxodihydrokarounidiol, respectively, after karounin, the Japanese word for the seeds of *T. kirilowii*. In this paper we report the results of further work on the *T. kirilowii* seed extract which resulted in the isolation and structure elucidation of another novel pentacyclic triterpene, *viz.* D:C-friedo-oleana-5,7,9(11)-triene-3α,29-diol (5-dehydrokarounidiol) (**1**).

A dioxygenated triterpene **1** was isolated by silica gel column chromatography followed by reversed phase HPLC from the saponified extract of the seeds of *T. kirilowii*. The molecular formula of **1** was determined as $C_{30}H_{46}O_2$ on the basis of the high-resolution mass spectrum (HR-MS) [*m/z*: 438.3514 (M^+)]. The compound had two hydroxyl groups [it gave a diacetate (**2**); *m/z*: 522.3695 (M^+), $C_{34}H_{50}O_4$], and three double bonds as has been revealed by carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy. This, in combination with the molecular formula, indicated that **1** was pentacyclic. The ultraviolet (UV) spectrum (λ_{max} 303, 314, 330 nm) and the shifts of the olefinic proton signals in the proton (^{1}H)-NMR spectrum (Table I) were consistent with a $\Delta^{5,7,9(11)}$-conjugated triene system.[5] Triterpene **1** was confirmed to have the basic skeleton and hydroxyl groups in the same position as karounidiol (**3**)[3] and 7-oxodihydrokarounidiol (**6**)[4] by spectral comparison and detailed analysis of the ^{1}H- and ^{13}C-NMR and mass spectral (MS) data (see Table I and Experimental section) in the manner described previously,[3,4] and we proposed the structure D:C-friedo-oleana-5,7,9(11)-triene-3α,29-diol (5-dehydrokarounidiol) for **1**.



1 : R=H
2 : R=Ac

3 : R=H R^1=H
4 : R=COPh R^1=H
5 : R=Ac R^1=Ac

6

7 : $\Delta^{7,9(11)}$
8 : Δ^8

Chart 1

Attempted chemical correlation of **1** with the known triterpenes such as **3** had failed,[6,8] and, thus, we confirmed the structure of **1** by X-ray crystallography. Single crystals of the quality required for X-ray analysis were prepared using 5-dehydrokarounidiol diacetate (**2**). The C-5–C-6, C-7–C-8, and C-9–C-11 distances of 1.324 (5), 1.343 (5), and 1.354 (5) Å, respectively, are consistent with double bonds at these positions. A perspective view of the molecular structure of **2** is shown in Fig. 1.

Using the results of ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H correlated spectroscopy (COSY), ^{1}H–^{1}H nuclear Overhauser and exchange spectroscopy (NOESY), ^{1}H–^{13}C COSY, and ^{1}H–^{13}C correlation spectroscopy for long-range couplings (COLOC) experiments the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** were completely assigned (see Table I).

Triterpene **1** is the first example of a naturally occurring triterpene with a $\Delta^{5,7,9(11)}$-conjugated triene system. There are several known $\Delta^{5,7,9(11)}$-unsaturated sterols. They have been isolated from diverse sources[11] such as fungi (including yeasts),[12] vegetable oils,[13] a nematode,[14] a

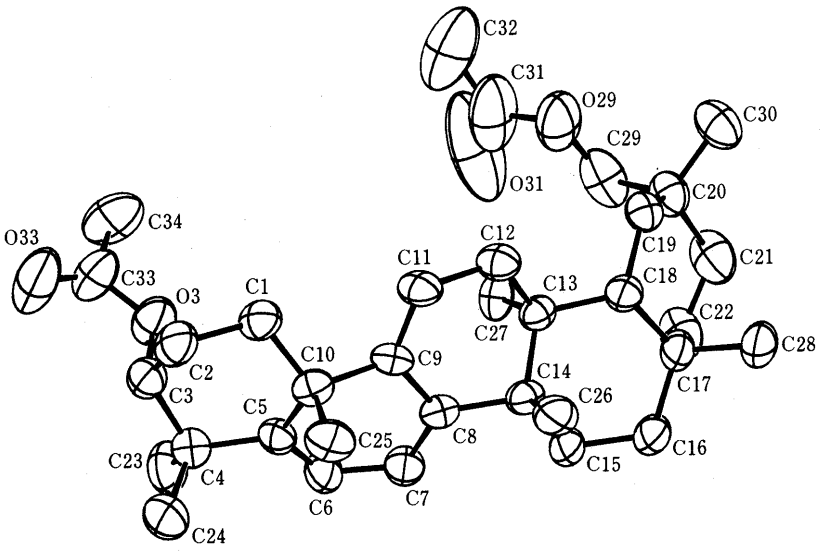


Fig. 1. Overall Conformation of 5-Dehydrokarounidiol Diacetate (**2**) as Determined by X-Ray Analysis Showing Crystallographic Numbering Scheme

TABLE I. ^{1}H- and ^{13}C-NMR Spectral Signal Assignments for 5-Dehydrokarounidiol (**1**) and Its Diacetate (**2**)[a]

C No.	Diol (**1**)		Diacetate (**2**)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	30.2	1.78 (m), 1.88 (m)	30.4	1.74 (2H, m)
2	25.2	1.78 (m), 2.13 (m)	22.8	1.84 (m), 2.08 (m)
3	75.6	3.54 (dd, 3, 4)	77.4	4.78 (dd, 3, 4)
4	40.5	—	38.9	—
5	148.6	—	148.9	—
6	119.6	5.92 (d, 6)	118.0	5.86 (d, 6)
7	114.2	5.61 (br d, 6)	114.4	5.61 (d, 6)
8	144.8	—	144.7	—
9	141.7	—	141.1	—
10	39.1	—	39.2	—
11	119.0	5.43 (m)	118.4	5.38 (m)
12	40.0	1.79 (m), 2.21 (m)	40.0	1.81 (m), 2.15 (m)
13	38.2	—	38.1	—
14	40.0	—	40.0	—
15	26.8	1.35 (m), 1.75 (m)	26.7	1.34 (m), 1.76 (m)
16	36.6	1.49 (m), 1.67 (m)	36.6	1.50 (m), 1.67 (m)
17	31.7	—	31.5	—
18	44.4	1.63 (dd, 4, 9)	44.5	1.66 (m)
19	26.9	1.49 (m), 1.66 (m)	27.7	1.46 (dd, 7, 7), 1.65 (dd, 3, 10)
20	32.7	—	31.1	—
21	29.9	1.25 (m), 1.49 (m)	30.0	1.35 (m), 1.52 (m)
22	34.2	0.88 (m), 1.70 (m)	33.9	0.90 (m), 1.72 (m)
23	27.3[b]	1.20 (s)	26.8[b]	1.10 (s)[b]
24	31.5[b]	1.20 (s)	31.6[b]	1.23 (s)[b]
25	31.0	1.19 (s)	30.6	1.18 (s)
26	21.2	1.04 (s)	21.0	1.03 (s)
27	19.4	0.82 (s)	19.6	0.79 (s)
28	31.0	1.09 (s)	31.1	1.10 (s)
29	70.9	3.22 (d, 11), 3.52 (d, 11)	72.1	3.82 (d, 11), 3.89 (d, 11)
30	30.1	0.99 (s)	30.5	1.00 (s)
31	—	—	171.4	—
32	—	—	21.0	2.08 (s)
33	—	—	171.0	—
34	—	—	21.3	2.05 (s)

a) Determined at 400 MHz (^{1}H-NMR) and 100.62 MHz (^{13}C-NMR) in $CDCl_3$ with tetramethylsilane (^{1}H-NMR) or $CDCl_3$ at δ 77.0 (^{13}C-NMR) as internal standard. Figures in parentheses on ^{1}H-NMR denote *J* values (Hz). *b*) Assignment in each column may be interchanged.

green alga,[15] and marine sponges.[5,16]

Experimental

X-Ray Analysis[17] Crystals were obtained by vapour diffusion of a MeOH–benzene (10 : 1) solution of the diacetate (**2**) (*ca.* 30 mg). Crystal data: $C_{34}H_{50}O_4$, M_r 522.78, monoclinic, space group $P2_1$, $a=14.589$ (6), $b=7.409$ (4), $c=15.336$ (7) Å, $\beta=111.07°$ (3), $V=1546.9$ (11) Å^3 (by least squares refinement of the angular settings from 25 reflections lying in a 2θ range of 30—34°), $\lambda(MoK_\alpha)=0.71073$ Å, $Z=2$, $D_c=1.122$ gcm^{-3}, clear, colorless rods, approximate dimentions $0.40\times0.20\times0.20$ mm, $\mu=0.667$ cm^{-1}, $F(000)=572$.

Data Collection and Processing Enraf-Nonius CAD-4 diffractometer, $T=296$ K, ω–2θ mode, graphite-monochromated MoK_α radiation, 4027 unique reflections measured (merging $R=0.024$), giving 2238 reflections with $I\geq3\sigma(I)$, no evidence of decay. Data were corrected for Lorentz and polarization effects as well as absorption effects (correction: min. 0.565, max. 1.105) based on DIFABS algorithm of Walker and Stuart.[18] The structure was solved with MULTAN[19] and refined by full-matrix least squares on F minimizing the function $\Sigma w(|F_o|-|F_c|)^2$. Non-hydrogen atoms were refined with anisotropic displacement parameters; hydrogen atom positions were calculated based on geometrical criteria at a distance of 1.0 Å from the adjacent atom and assigned isotropic temperature factors. Large thermal factors for the acetoxy group at C-29 suggested a possible disorder, however no suitable model could be refined. An extinction coefficient of the form proposed by Zachariasen[20] was applied and refined, $g=3.50\times10^{-7}$. Weights were assigned as $w=4F_o^2/\sigma^2(I)$ with $\sigma^2(I)$ defined as $[\sigma^2(I_c)+(0.07F_o)^2]$. The final refinement converged with $R=0.057$, $R_w=0.077$, $(\Delta/\sigma)_{max}=0.01$. A final Fourier map showed maximum positive residual electron density of 0.205 eÅ^{-3}. All programs were from the locally modified SDP package.[21] Neutral atom scattering factors were taken from the International Tables for X-Ray Crystallography[22]; hydrogen scattering factors were those of Stewart *et al.*[23] Fractional atomic coordinates and equivalent isotropic temperature factors for non-hydrogen atoms are given in Table II.

General Methods Melting points were measured with a Yanagimoto melting point apparatus, and are uncorrected. Thin-layer chromatography (TLC) on silica gel (Kieselgel 60G, Merck, 0.5 mm thick) was developed using hexane–EtOAc (6 : 1, v/v). Preparative high-performance liquid chromatography (HPLC) was carried out on an octadecyl silica column (Altex Ultrasphere ODS 5 μ column, 25 cm × 10 mm i.d., Beckman Instruments, Inc., San Ramon, California) with MeOH–water (98 : 2, v/v) (4 ml/min) using a SSC Flow System 3100K (Senshu Scientific Co.) and an ERC-7520 refractive index detector (Erma Optical Works, Ltd.). Karounidiol diacetate (**5**) was the standard for the determination of relative retention times (Rt_R) in HPLC [Rt_R: 1.00; cholesterol (cholest-5-en-3β-ol) acetate has Rt_R: 3.26 under the above HPLC conditions]. UV spectra were recorded on a Shimadzu UV-300 spectrometer in EtOH. Electron-impact (EI) MS and HR-MS were taken on a Hitachi M-80B double focusing

TABLE II. Atomic Positional Parameters and Equivalent Isotropic Thermal Parameters of 5-Dehydrokarounidiol Diacetate (**2**)

Atom	x	y	z	B_{eq} (Å^2)
O3	−0.3191 (2)	0.9383 (6)	0.1166 (2)	5.43 (8)
O29	0.2331 (3)	1.2538 (8)	0.1127 (3)	8.0 (1)
O31	0.1319 (4)	1.117 (2)	−0.0160 (3)	18.3 (3)
O33	−0.4692 (3)	1.0574 (8)	0.0671 (3)	8.9 (1)
C1	−0.1851 (3)	1.0502 (7)	0.2949 (3)	4.12 (9)
C2	−0.2932 (3)	1.0145 (8)	0.2751 (3)	5.0 (1)
C3	−0.3314 (3)	0.8727 (7)	0.2014 (3)	4.5 (1)
C4	−0.2778 (3)	0.6901 (7)	0.2266 (3)	4.3 (1)
C5	−0.1660 (3)	0.7153 (6)	0.2680 (3)	3.51 (9)
C6	−0.1058 (3)	0.5871 (7)	0.2607 (3)	4.2 (1)
C7	−0.0004 (3)	0.5998 (6)	0.3016 (3)	4.1 (1)
C8	0.0449 (3)	0.7569 (6)	0.3343 (3)	3.21 (8)
C9	−0.0164 (3)	0.9139 (6)	0.3286 (2)	3.30 (8)
C10	−0.1206 (3)	0.8816 (6)	0.3283 (3)	3.44 (9)
C11	0.0215 (3)	1.0808 (6)	0.3291 (3)	3.89 (9)
C12	0.1248 (3)	1.1138 (6)	0.3360 (3)	3.82 (9)
C13	0.1787 (3)	0.9476 (6)	0.3197 (3)	3.13 (8)
C14	0.1555 (3)	0.7866 (6)	0.3735 (3)	3.35 (9)
C15	0.2136 (3)	0.6213 (7)	0.3633 (4)	4.7 (1)
C16	0.3237 (3)	0.6510 (7)	0.4085 (4)	5.6 (1)
C17	0.3628 (3)	0.8240 (7)	0.3726 (4)	5.0 (1)
C18	0.2912 (3)	0.9878 (6)	0.3568 (3)	3.48 (9)
C19	0.3248 (3)	1.1438 (6)	0.3079 (3)	3.61 (9)
C20	0.3715 (3)	1.1093 (8)	0.2325 (3)	4.5 (1)
C21	0.4312 (3)	0.9352 (8)	0.2520 (4)	6.3 (1)
C22	0.3824 (3)	0.7787 (7)	0.2833 (4)	6.0 (1)
C23	−0.3147 (3)	0.5985 (9)	0.2992 (3)	5.7 (1)
C24	−0.3087 (3)	0.5662 (9)	0.1408 (4)	5.8 (1)
C25	−0.1063 (3)	0.8402 (8)	0.4321 (3)	4.6 (1)
C26	0.1829 (3)	0.8297 (8)	0.4791 (3)	5.0 (1)
C27	0.1351 (3)	0.9109 (7)	0.2137 (3)	4.1 (1)
C28	0.4614 (4)	0.8727 (8)	0.4512 (5)	6.7 (2)
C29	0.2948 (3)	1.097 (1)	0.1333 (3)	6.5 (1)
C30	0.4385 (3)	1.2688 (9)	0.2338 (4)	5.8 (1)
C31	0.1514 (5)	1.240 (2)	0.0324 (4)	11.7 (3)
C32	0.0928 (7)	1.423 (2)	0.0227 (8)	16.4 (4)
C33	−0.3928 (4)	1.0339 (9)	0.0575 (3)	5.8 (1)
C34	−0.3668 (5)	1.105 (1)	−0.0206 (4)	7.8 (2)

gas chromatograph-mass spectrometer (70 eV) using a direct inlet system. The MS data do not include peaks having m/z <200. The NMR spectrometers (400 MHz for ^{1}H; 100.62 MHz for ^{13}C) used were a JEOL GSX-400 and Bruker AM400 (Karlsruhe, Germany). NMR spectra were recorded in $CDCl_3$ with tetramethylsilane (for ^{1}H-NMR) or $CDCl_3$ (δ 77.0; for ^{13}C-NMR) as internal standard. Chemical shifts are recorded in δ values. Acetylation was performed in Ac_2O–pyridine at room temperature overnight. Unless stated otherwise, all other methods used in this study have been described previously.[3] The seeds of *T. kirilowii* were purchased from Kinokuniya Kan-Yaku Kyoku Co. (Tokyo).

Isolation Procedure Air-dried and ground seeds of *T. kirilowii* (10 kg) were extracted with CH_2Cl_2 in a Soxhlet extractor. Unsaponifiable lipids (25 g) were obtained from the extract (2120 g) by saponification (1 N KOH in MeOH, reflux, 3 h). The unsaponifiables were chromatographed over silica gel (1 kg) as described previously.[4] The residue (5 g) of the most polar of the 9 fractions[3] (*Rf* 0.07 on TLC, 2 developments) was rechromatographed over silica gel (300 g, eluent hexane–EtOAc 1 : 1, v/v) and further purified by reversed phase HPLC to yield 5-dehydrokarounidiol (**1**) (49 mg), karounidiol (**3**)[3] (700 mg), 7-oxodihydrokarounidiol (**6**)[4] (440 mg), and an unidentified triterpene (100 mg).

5-Dehydrokarounidiol (1) Colorless needles (from MeOH–water), mp 201—203 °C. Rt_R: 0.49 (HPLC). UV λ_{max} nm (log ε): 303 (4.05), 314 (4.07), 330 (3.86). MS m/z (%): 438 (M$^+$, 20), 423 (4), 420 (7), 405 (22), 269 (11), 255 (37), 251 (17), 243 (100), 237 (11), 229 (17), 225 (23). HR-MS m/z: 438.3514 [Calcd for $C_{30}H_{46}O_2$ (M$^+$): 438.3496]; 269.1890 (Calcd for $C_{19}H_{25}O$: 269.1904); 255.1715 (Calcd for $C_{18}H_{23}O$: 255.1747); 243.1764 (Calcd for $C_{17}H_{23}O$: 243.1748). The prominent fragmentations $C_{19}H_{25}O^+$ (formed by cleavage at C-13–C-18 and C-14–C-15 with 1H loss), $C_{18}H_{23}O^+$ (formed by cleavage at C-12–C-13 and C-15–C-16 with 2H transfer), and $C_{17}H_{23}O^+$ (formed by cleavage at C-11–C-12 and C-15–C-16) supported the presence of a $\Delta^{5,7,9(11)}$-triene system in the D:C-friedo-oleanane skeleton.[24]

5-Dehydrokarounidiol Diacetate (2) This was prepared from **1** by acetylation. Colorless needles (from MeOH–acetone), mp 164—166 °C. Rt_R: 0.76 (HPLC). MS m/z (%): 522 (M$^+$, 26), 507 (4), 462 (80), 449 (31), 447 (26), 395 (11), 387 (19), 311 (4), 297 (22), 295 (15), 285 (80), 271 (19), 251 (48), 237 (37), 225 (100), 211 (26). HR-MS m/z: 522.3695 [Calcd for $C_{34}H_{50}O_4$ (M$^+$): 522.3706].

Partial Hydrogenation of 5-Dehydrokarounidiol Diacetate (2) Hydrogenation of **2** (9.0 mg) in AcOH (12 ml) using pre-reduced PtO_2 (15 mg) as catalyst (atm. press., room temperature, overnight) followed by HPLC fractionation yielded two major products: 5β-D:C-friedo-oleana-7,9(11)-diene-3α,29-diol diacetate (5-epikarounidiol diacetate) (**7**) (3.6 mg) and 5β-D:C-friedo-olean-8-ene-3α,29-diol diacetate (5-epidihydrokarounidiol diacetate) (**8**) (3.0 mg).

5-Epikarounidiol Diacetate (7) Fine needles (from MeOH–water), mp 105—108 °C. Rt_R: 0.92 (HPLC). UV λ_{max} nm (log ε): 233 (4.03), 240 (4.07), 248 (3.89) [characteristic for $\Delta^{7,9(11)}$-diene system].[3,5] MS m/z (%): 524 (M$^+$, 69), 464 (60), 449 (29), 435 (10), 389 (17), 382 (10), 380 (10), 313 (4), 307 (11), 253 (51), 239 (40), 227 (100), 213 (29), 211 (29). HR-MS m/z: 524.3860 [Calcd for $C_{34}H_{52}O_4$ (M$^+$): 524.3862]; 253.1975 (Calcd for $C_{19}H_{25}$: 253.1955); 239.1814 (Calcd for $C_{18}H_{23}$: 239.1799); 227.1812 (Calcd for $C_{17}H_{23}$: 227.1799). The prominent fragmentations $C_{19}H_{25}^+$, $C_{18}H_{23}^+$, and $C_{17}H_{23}^+$ supported the presence of a $\Delta^{7,9(11)}$-diene system in the D:C-friedo-oleanane skeleton.[3,24] ^{1}H-NMR δ: 0.80, 0.83, 0.85, 0.91, 0.96, 1.00, 1.09, 2.04, and 2.09 (each 3H and s), 3.82 (1H, d, J=11 Hz), 3.90 (1H, d, J=11 Hz), 4.56 (1H, dd, J=4, 12 Hz), 5.38 (1H, m), 5.41 (1H, m).

5-Epidihydrokarounidiol Diacetate (8) Fine needles (from MeOH), mp 176—178 °C. Rt_R: 1.04 (HPLC). MS m/z (%): 526 (M$^+$, 10), 511 (29), 466 (44), 451 (75), 391 (11), 329 (7), 301 (71), 289 (29), 255 (20), 241 (93), 229 (92), 203 (100). HR-MS m/z: 526.4040 [Calcd for $C_{34}H_{54}O_4$ (M$^+$): 526.4020]; 255.2135 (Calcd for $C_{19}H_{27}$: 255.2111); 241.1930 (Calcd for $C_{18}H_{25}$: 241.1954); 229.1974 (Calcd for $C_{17}H_{25}$: 229.1955). The presence of a Δ^8-double bond was supported by the prominent fragmentations[3,24] $C_{19}H_{27}^+$, $C_{18}H_{25}^+$, and $C_{17}H_{25}^+$. ^{1}H-NMR δ: 0.93 (3H), 0.98 (6H), 1.01 (3H), 1.02 (3H), 1.10 (3H), 1.12 (3H), 2.05 (3H), and 2.09 (3H) (each s), 3.80 (1H, d, J=10 Hz), 3.86 (1H, d, J=11 Hz), 4.49 (1H, dd, J=4, 12 Hz).

Acknowledgements We are grateful to Professor Emeritus Toshio Nambara, Tohoku University, for his generous encouragement.

References and Notes

1) The C-20α substituent in these compounds has been assigned the locant 29 in accordance with the more common convention.
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6) ^{1}H-NMR spectra showed that partial catalytic hydrogenation of 5-dehydrokarounidiol diacetate (**2**) did not yield the expected compounds but, apparently, their 5β-diastereoisomers, *viz.*, 5-epikarounidiol diacetate [5β-D:C-friedo-oleana-7,9(11)-diene-3α,29-diol diacetate] (**7**) and 5-epidihydrokarounidiol diacetate (5β-D:C-friedo-olean-8-ene-3α,29-diol diacetate) (**8**) (thier 5α-epimers are known).[3] The stereochemical assignment as 5β for **7** and **8** was based on the examination of stereomodels. These 5β diastereoisomers (**7**, **8**) have 3β-H oriented axially [with the equatorial hydroxy (or acetoxy) group pointing toward the α-face] which broadens the shape of the 3-H signal[7] as was observed for **7** (dd, J=4, 12 Hz) and **8** (dd, J=4, 12 Hz).
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8) Catalytic reduction of a Δ^5 double bond in a sterol is expected to give a product with a 5α-configuration.[9] Thus, the products of Raney Ni reduction of 4,4-dimethylcholesta-5,7,9(11)-trien-3β-ol are 4,4-dimethyl-5α-cholesta-7,9(11)-dien-3β-ol and 4,4-dimethylcholesta-5,7-dien-3β-ol.[10]
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