7-Oxodihydrokarounidiol [7-Oxo-D:C-friedo-olean-8-ene-3α,29-diol],[1] a Novel Triterpene from *Trichosanthes kirilowii*

Toshihiro AKIHISA,*,[a] Wilhelmus C. M. C. KOKKE,[b] Toshitake TAMURA,[a] and Toshio NAMBARA[c]

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, Pennsylvania 19131, U.S.A., and Pharmaceutical Institute, Tohoku University,[c] Aobayama, Sendai, Miyagi 980, Japan. *Received September 17, 1991*

The structure of 7-oxodihydrokarounidiol [7-oxo-D:C-friedo-olean-8-ene-3α,29-diol], isolated from the seeds of *Trichosanthes kirilowii* (Cucurbitaceae), was determined by chemical correlation with karounidiol [D:C-friedo-oleana-7,9(11)-diene-3α,29-diol] from the same source. Two other natural products, *viz.* bryonolic acid (3β-hydroxy-D:C-friedo-olean-8-en-29-oic acid) and bryononic acid (3-oxo-D:C-friedo-olean-8-en-29-oic acid), were also correlated with karounidiol.

Keywords *Trichosanthes kirilowii*; Cucurbitaceae; seed; triterpene; 7-oxodihydrokarounidiol; 7-oxo-D:C-friedo-olean-8-ene-3α,29-diol; ^{1}H-NMR spectrum; ^{13}C-NMR spectrum

We have recently reported the isolation and structure determination of D:C-friedo-oleana-7,9(11)-diene-3α,29-diol (**1**) and its 3-*O*-benzoate from the seeds of *Trichosanthes kirilowii* MAXIM. (Cucurbitaceae).[2] Triterpene **1** was given the trivial name, karounidiol, after karounin, the Japanese word for the seeds of *T. kirilowii*. Our continuing study on the seed extract of the same plant has resulted in the isolation of a structurally related compound to which we will refer as 7-oxodihydrokarounidiol (**3**). In this paper we report the structure elucidation of **3** by chemical correlation with **1** and with two other structurally related natural products, bryonolic acid (3β-hydroxy-D:C-friedo-olean-8-en-29-oic acid) (**14**)[3] and bryononic acid (3-oxo-D:C-friedo-olean-8-en-29-oic acid) (**12**).[4]

7-Oxodihydrokarounidiol (**3**) was isolated by silica gel column chromatography from the saponified extract of the seeds of *T. kirilowii*. The molecular formula of **3** was determined as $C_{30}H_{48}O_3$ on the basis of the high-resolution mass spectrum (HR-MS) [*m/z*: 456.3601 (M^+)]. Similarities in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum and MS indicated that karounidiol (**1**)[2] and **3** were structurally related. Triterpene **3** has two hydroxyl groups [it gives a diacetate (**5**), $C_{34}H_{52}O_5$], a double bond and a carbonyl group as has been revealed by ^{13}C-NMR spectroscopy. Both **1** and **3** have a 3α-OH, a hydroxymethylene and a 4,4-geminal dimethyl group [*cf.* ^{1}H-NMR data of the diacetates, karounidiol diacetate (**2**) and **4**, Table I]. Triterpene **3** exhibited absorptions at 252 nm in the ultraviolet (UV) spectrum and 1643 cm^{-1} in the infrared (IR) spectrum which suggested the presence of a conjugated enone. The compound had to be a 7-oxo-8-ene because of the presence of diagnostic fragments $C_{19}H_{27}O_2^+$

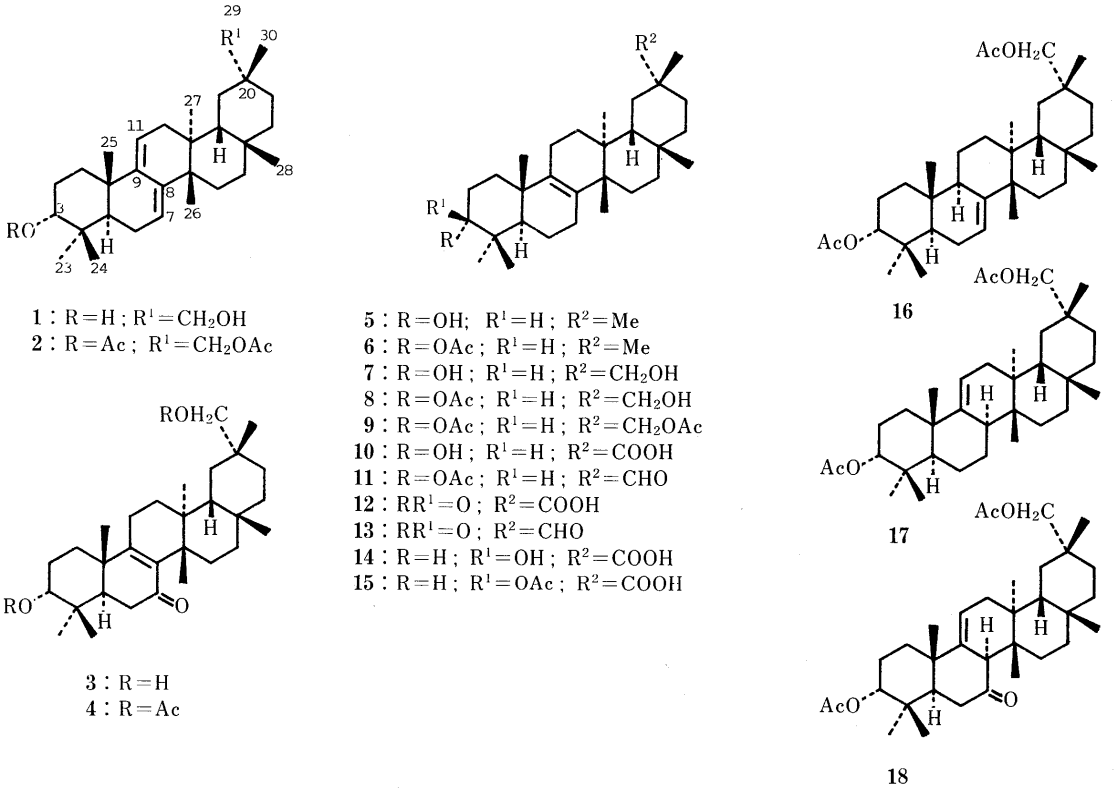

Chart 1

TABLE I. ^{1}H-NMR Data of Selected Triterpenes Described in This Paper (400 MHz, $CDCl_3$, Shifts are δ Values)

Compound	3-H[a]	29-H or -H_2[b]		7-H[c]	11-H[d]	3-OAc/29-OAc[e]		Methyl singlets (unassigned)
2	4.693	3.832	3.900	5.482	5.229	2.081	2.083	0.829 0.855 0.911 0.933 0.992 0.999 1.080
3	3.491	3.268	3.322	—	—	—	—	0.941 0.959 0.964 1.015 1.015 1.187 1.377
4	4.714	3.763	3.797	—	—	2.084	2.087	0.872 0.974 0.998 1.034 1.040 1.185 1.372
5	3.429	—	—	—	—	—	—	0.863 0.955 0.963 0.969 0.969 0.997 1.060 1.072
6	4.667	—	—	—	—	2.065	—	0.867 0.907 0.962 0.970 0.978 1.035 1.064 1.097
7	3.426	3.249	3.398	—	—	—	—	0.863 0.965 0.970 0.978 0.989 1.112 1.127
8	3.667	3.248	3.431	—	—	2.065	—	0.867 0.908 0.972 0.992 1.017 1.112 1.130
9	4.668	3.831 (s)		—	—	2.081	2.085	0.867 0.907 0.970 0.970 1.003 1.091 1.119
10	3.416	—	—	—	—	—	—	0.852 0.904 0.950 0.956 0.961 1.027 1.241
11	4.653	9.378	—	—	—	2.056	—	0.797 0.855 0.896 0.955 0.959 0.970 1.046
12	—	—	—	—	—	—	—	0.880 0.977 1.040 1.040 1.051 1.087 1.241
13	—	9.370[f]	—	—	—	—	—	0.750 0.954 0.984 1.039 1.046 1.049 1.087
14	3.224	—	—	—	—	—	—	0.797 0.879 0.947 0.957 0.988 1.031 1.237
15	4.481	—	—	—	—	2.048	—	0.864 0.870 0.870 0.959 0.977 1.038 1.244
16	4.689	3.780	3.847	5.482	—	2.081	2.092	0.775 0.849 0.973 1.016 1.071 1.101 1.112
17	4.669	3.862 (2H, s)		—	5.271	2.069	2.078	0.875 0.891 0.913 0.966 0.980 1.087 1.107
18	4.724	3.838	3.896	—	5.344	2.070	2.079	0.826 0.898 0.937 0.943 1.002 1.065 1.239

a) dd, *J* typically 2.7, 2.7 Hz and 4.4, 11.5 Hz for 3α and 3β compounds, respectively. *b*) In most cases the C-29 protons appear as an AB system with 10—11 Hz between the lines of each of the pairs. In some cases the quartet is collapsed to a singlet. *c*) m, half width 10 Hz. *d*) d, $J=5$ Hz. *e*) Signals unassigned in the case of diacetates. *f*) d, $J=1.7$ Hz.

(*m/z* 287, ABC ring+C-26, C-27) and $C_{15}H_{24}O_2^+$ (*m/z* 236, AB ring+C-10, C-11, characteristic of 7-oxo triterpenes with a Δ^8 or $\Delta^{9(11)}$ double bond) in the mass spectrum.[5] The ^{13}C-NMR spectrum confirmed the presence of a tetrasubstituted double bond. The formation of the fragments $C_{19}H_{27}O_2^+$ and $C_{15}H_{25}O_1^+$ (*m/z* 221, DE ring+C-26, C-27) also implied that the primary alcohol group was located either at C-28, C-29, or C-30.[5] Chemical correlation (two methods: A and B) with karounidiol (**1**), isolated from the same source, confirmed the structure of the skeleton and showed that the primary hydroxyl group is located at C-20α (C-29). Thus, the novel triterpene ketodiol (**3**) possesses the structure 7-oxo-D:C-friedo-olean-8-ene-3α,29-diol.

Method A Lithium and ethylenediamine reduction[6] of karounidiol diacetate (**2**) followed by acetylation afforded a diacetoxy-8-ene (**9**) [D:C-friedo-olean-8-ene-3α,29-diol (3-epibryonolol) diacetate] in addition to three other reduction products, *viz.* D:C-friedo-olean-8-ene ((isomultiflorene), D:C-friedo-olean-8-en-3α-ol acetate (3-epiisomultiflorenol acetate) (**6**), and D:C-friedo-olean-8-en-29-ol acetate (3-deoxybryonolol acetate). Oxidation of **9** with chromyl chloride yielded 7-oxo-D:C-friedo-olean-9(11)-ene-3α,29-diol diacetate (**18**), which was isomerized by treatment with BF_3 etherate[7-9] into a conjugated ketone (**4**) which was identical by spectral comparison with the diacetate.

Method B Reduction of 7-oxodihydrokarounidiol diacetate (**4**) under Huang–Minlon conditions[9,10] followed by acetylation afforded D:C-friedo-olean-7-ene-3α,29-diol diacetate (**16**) together with two double bond isomers, *viz.* **9** and D:C-friedo-olean-9(11)-ene-3α,29-diol diacetate (**17**). Selenium dioxide dehydrogenation of the individual monoenes (**9**, **16**, **17**) yielded a diacetoxy-7,9(11)-diene which was identical with karounidiol diacetate (**2**).

We decided to also synthesize bryonolic acid (**14**) and bryononic acid (**12**) because these natural products are structurally related to karounidiol (**1**) and 7-oxodihydrokarounidiol (**3**). Thus Sarrett oxidation of 3-epibryonolol (**7**) [prepared from **2** by lithium and ethylenediamine reduction (*vide supra*)] afforded **12** and a keto aldehyde, 3-oxo-D:C-friedo-olean-8-en-29-al (**13**). $NaBH_4$ reduction of **12** yielded **14** which was identical by NMR comparison with **14** isolated from the roots of *Bryonia dioica*.[3]

Kamisako *et al.*, who published the X-ray structure of bryonolol diacetate,[11,12] prepared from bryonolic acid (**14**), and also of bryonolic acid methyl ester[13,14] did not properly identity their "bryonolic acid" with authentic bryonolic acid obtained from *Bryonia dioica*.[3] They were the first to report the triterpenes from *Luffa cylindrica* (Cucurbitaceae), but, in principle, the possibility existed that their *L. cylindrica* compound was a C-20 epimer of bryonolic acid. Comparison of the ^{1}H-NMR data of two compounds (**14**, **15**) (Table I) with data reported by Kamisako *et al.*[12,16] confirmed correctly that these compounds both have a (20*R*/α)-configuration.[17]

Whereas we previously concluded[2] that 3α-hydroxy-D:C-friedo-oleana-7,9(11)-dien-29-oic acid and bryocoumaric acid [3α-(*p*-hydroxycinnamoyloxy)-D:C-friedo-oleana-7,9(11)-dien-29-oic acid][21,22] isolated from *B. dioica* possessed a (20*S*/β)-configuration, the present study has proven our previous conclusion to be erroneous based on the ^{1}H-NMR evidence which supported the (20*R*)-configuration of **14** from *B. dioica*. The two triterpenes from *B. dioica* mentioned above were chemically correlated with **14**.[21,22]

Experimental

Melting points were measured with a Yanagimoto melting point apparatus, and are uncorrected. Preparative thin-layer chromatography (TLC) on silica gel (Kieselgel 60G, Merck; 0.5 mm thick) was developed using hexane–EtOAc (6:1, v/v). Argentation preparative TLC plates (silica gel–$AgNO_3$, 4:1, w/w) were developed with CCl_4–CH_2Cl_2 (5:1, v/v). Preparative high-performance liquid chromatography (HPLC) was carried out on an Altex Ultrasphere ODS 5μ 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.). Gas-liquid chromatography (GLC) was run on a Shimadzu GC-4CM apparatus using an OV-17 glass capillary column (30 m × 0.3 mm i.d., column temperature 255 °C). Karounidiol diacetate (**2**) was the standard for the determination of relative retention times (Rt_R) in HPLC

[R_tR: 1.00; cholesterol (cholest-5-en-3β-ol) acetate has R_tR: 3.26 under the above HPLC conditions), whereas cholesterol acetate was the standard in GLC (R_tR: 1.00). UV spectra were determined on a Shimadzu UV-300 spectrometer in EtOH, and IR spectra in KBr on a Type IRA-2 IR spectrophotometer (Japan Spectroscopic Co.). Electron-impact mass spectrum (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. The MS data do not include peaks having $m/z<180$. NMR spectra were recorded with a JEOL GX-400 for ^{1}H-NMR (400 MHz) and a Bruker AM400 (Karlsruhe, Germany) for ^{13}C-NMR (100.62 MHz) in $CDCl_3$ with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. The ^{1}H-NMR data of 13 new and 4 known (**2**, **12**, **14**, **15**) compounds are listed in Table I. Acetylation was performed in Ac_2O–pyridine at room temperature overnight. The standard procedure for saponification of acetates was reflux with 1 N KOH in MeOH for 3 h. All other methods used in this study have been described previously.[2] The seeds of *T. kirilowii* were purchased from Kinokuniya Kan-Yaku Kyoku Co. (Tokyo). Karounidiol (**1**) was isolated from these seeds[2] and bryonolic acid (**14**), isolated from the roots of *B. dioica*,[3] was generously donated by Professor L. Cattel, University of Turin, Italy.

Isolation of 7-Oxodihydrokarounidiol (3) Air-dried and ground seeds of *T. kirilowii* (5 kg) were extracted with CH_2Cl_2 in a Soxhlet extractor. Unsaponifiable lipids (12 g) were obtained from the extract (1600 g) by saponification (1 N KOH in MeOH). The unsaponifiables were chromatographed over silica gel (500 g) as described in our previous paper.[2] The residue (2.745 g) of the most polar of the 9 fractions[2] (*Rf* 0.07, TLC, 2 developments) was rechromatographed over silica gel [175 g, eluent hexane–EtOAc (1 : 1, v/v)] and karounidiol (**1**) (357 mg) and 7-oxodihydrokarounidiol (**3**) (212 mg) were obtained. Acetylation of **3** yielded the diacetate **4**.

7-Oxodihydrokarounidiol (3) mp 287—289 °C (MeOH–acetone). R_tR: 0.08 (HPLC). UV λ_{max} nm: 252. IR ν_{max} cm^{-1}: 3350 (OH), 1643 and 1580 (conjugated enone). MS *m/z* (%): 456 (M^+, 57), 441 (34), 426 (18), 425 (22), 316 (8), 315 (6), 302 (6), 301 (6), 287 (13), 273 (11), 261 (21), 244 (44), 236 (100), 221 (64). HR-MS *m/z*: 456.3601 [Calcd for $C_{30}H_{48}O_3$: (M^+): 456.3600]; 287.2024 (Calcd for $C_{19}H_{27}O_2$: 287.2009); 236.1811 (Calcd for $C_{15}H_{24}O_2$: 236.1775); 221.1923 (Calcd for $C_{15}H_{25}O$: 221.1904). ^{13}C-NMR δ (multiplicity): 198.27 (s, C-7), 163.68 (s, C-9), 142.64 (s, C-8), 74.97 (d, C-3), 74.23 (t, C-29), 41.39 (d), 41.24 (d), 39.21 (s), 39.00 (s), 38.77 (t), 38.08 (s), 37.40 (s), 36.40 (t), 35.83 (t), 33.43 (s), 31.25 (s), 30.50 (q), 29.83 (t), 29.77 (2 × t), 28.56 (t), 27.95 (t), 27.03 (q), 26.97 (q), 25.70 (q), 25.31 (t), 22.21 (t), 21.72 (q), 18.27 (q), 17.99 (q).

7-Oxodihydrokarounidiol Diacetate (4) mp 190—192 °C (MeOH–water). R_tR: 0.18 (HPLC). UV λ_{max} nm: 251. IR ν_{max} cm^{-1}: 1740 and 1240 (acetate C=O), 1660 and 1600 (conjugated enone). MS *m/z* (%): 540 (M^+, 77), 525 (18), 480 (24), 465 (21), 451 (6), 358 (15), 343 (6), 329 (15), 316 (9), 303 (18), 278 (100), 263 (59), 243 (79), 218 (15), 203 (56). HR-MS *m/z*: 540.3803 [Calcd for $C_{34}H_{52}O_5$ (M^+): 540.3811]. ^{1}H-NMR δ: 0.872 (3H, s, H-24), 0.974 (3H, s, H-27), 0.998 (3H, s, H-23), 1.034 (3H, s, H-25), 1.040 (3H, s, H-30), 1.185 (3H, s, H-28), 1.372 (3H, s, H-26), 2.084 (3H, s, 3α-OAc), 2.087 (3H, s, 29-CH_2OAc), 3.763 (1H, d, J=10.9 Hz) and 3.797 (1H, d, J=10.5 Hz) (H-29), 4.714 (1H, dd, J=2.8, 2.8 Hz, H-3β). ^{13}C-NMR δ (assignment): 198.28 (C-7), 171.58 (acetate C=O at C-29) and 170.58 (acetate C=O at C-3), 163.31 (C-9), 142.60 (C-8), 76.79 (C-3), 74.66 (C-29), 42.65 (C-5), 41.47 (C-18), 39.19 (2 × C; C-10, C-14), 38.44 (C-22), 38.03 (C-13), 36.59 (C-4), 36.25 (C-6), 35.87 (C-16), 32.01 (C-20), 31.16 (C-17), 30.61 (C-28), 30.17 (C-19), 29.81 (C-12), 29.53 (C-15), 29.40 (C-1), 28.22 (C-21), 26.84 (C-26), 26.75 (C-24), 26.27 (C-30), 22.93 (C-2), 22.24 (C-11), 21.49 (C-23), 21.31 (acetate methyl at C-3), 21.09 (acetate methyl at C-29), 18.35 (C-27), 18.03 (C-25). To make the above NMR assignments we used 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.

Partial Synthesis of 7-Oxodihydrokarounidiol Diacetate (4) from Karounidiol Diacetate (2) Step 1: To a solution of **2** (900 mg) in ethylenediamine (135 ml) under N_2 was added with stirring lithium wire (200 mg) cut in small pieces. The mixture was refluxed for 2 h. The crude product (700 mg), isolated by ether extraction, was acetylated. Yield, 770 mg. Fractionation of the mixture of acetylation products by TLC (1 development) afforded three fractions: A (69 mg, *Rf* 0.79, isomultiflorene), B [270 mg, *Rf* 0.54, mixture of two components (*vide infra*)], and C [297 mg, *Rf* 0.21, 3-epibryonolol diacetate (**9**)]. Work-up of fraction B by argentation TLC (3 developments) afforded two subfractions: B1 (163 mg, *Rf* 0.69, 3-deoxybryonolol acetate) and B2 (16 mg, *Rf* 0.55, 3-epiisomultiflorenol acetate) (**6**). The free alcohols **5**, **7** and 3-deoxybryonolol, were obtained by saponification of **6**, **9** and 3-deoxybryonolol acetate, respectively. Saponification of **9** yielded, in addition, its 3-monoacetate **8**.

Isomultiflorene[23] mp 184—186 °C (MeOH–acetone) (lit.[6] 178—182 °C; lit.[24,25] 188—189 °C). R_tR: 0.58 (GLC). HR-MS *m/z*: 410.3927 [Calcd for $C_{30}H_{50}$ (M^+): 410.3910].

3-Epiisomultiflorenol (5) and Its Acetate (6) **5**: mp 236—238 °C (MeOH–acetone). R_tR: 1.53 (HPLC). MS *m/z* (%): 426 (M^+, 22), 411 (17), 393 (6), 309 (2), 273 (6), 272 (6), 259 (100), 247 (20), 241 (32), 229 (29), 218 (19), 206 (26), 205 (23), 191 (17), 189 (17). HR-MS *m/z*: 426.3887 [Calcd for $C_{30}H_{50}O$ (M^+): 426.3859]. **6**: mp 192—194 °C (MeOH–acetone). R_tR: 2.75 (HPLC); 1.48 (GC). MS *m/z* (%): 468 (M^+, 44), 453 (29), 408 (2), 393 (19), 301 (39), 289 (19), 272 (10), 257 (13), 255 (13), 241 (84), 229 (76), 218 (26), 205 (100), 191 (39), 189 (45). HR-MS *m/z*: 468.3953 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3964].

3-Deoxybryonolol mp 209—211 °C (MeOH–acetone) (lit.[4] 207—210 °C). R_tR: 1.61 (HPLC). HR-MS *m/z*: 426.3885 [Calcd for $C_{30}H_{50}O$: 426.3860]. See[16] for assigned ^{13}C-NMR data.

3-Deoxybryonolol Acetate mp 142—144 °C (MeOH–acetone) (lit.[4] 137—138 °C). R_tR: 3.27 (HPLC); 1.85 (GLC). HR-MS *m/z*: 468.3949 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3964]. See[16] for assigned ^{13}C-NMR data.

3-Epibryonolol (7) and Its 3-Acetate (8) A solution of **9** (50 mg) and KOH (2 g) in MeOH (80 ml) was stirred at room temperature for 4 h. Standard work-up followed by HPLC fractionation yielded **7** (6 mg) and **8** (34 mg). **7**: mp 270—274 °C (MeOH–acetone). R_tR: 0.49 (HPLC). MS *m/z* (%): 442 (M^+, 30), 427 (32), 411 (59), 409 (17), 394 (4), 393 (4), 309 (6), 288 (15), 273 (10), 259 (100), 247 (65), 241 (87), 229 (70), 222 (68), 221 (42), 207 (49), 203 (56), 189 (51). HR-MS *m/z*: 442.3795 [Calcd for $C_{30}H_{50}O_2$ (M^+): 442.3807] **8**: mp 190—193 °C (MeOH). R_tR: 0.61 (HPLC). MS *m/z* (%): 484 (M^+, 19), 469 (19), 453 (22), 424 (3), 409 (25), 394 (13), 301 (50), 288 (19), 264 (6), 255 (9), 241 (100), 229 (47), 222 (47), 221 (47), 203 (59), 189 (56). HR-MS *m/z*: 484.3910 [Calcd for $C_{32}H_{52}O_3$ (M^+): 484.3913].

3-Epibryonolol Diacetate (9) mp 206—208 °C (MeOH). R_tR: 1.08 (HPLC). MS *m/z* (%): 526 (M^+, 21), 511 (18), 466 (7), 453 (18), 451 (25), 393 (4), 391 (4), 330 (7), 315 (4), 301 (32), 289 (14), 264 (21), 263 (18), 241 (78), 229 (65), 203 (100), 189 (39). HR-MS *m/z*: 526.4022 [Calcd for $C_{34}H_{54}O_4$ (M^+): 526.4019].

Step 2: Chromyl chloride (15 μl) in CH_2Cl_2 (0.8 ml) was added to a stirred solution of **9** (50 mg) in CH_2Cl_2 (1 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. Yield 48 mg. TLC (2 developments) afforded a fraction (11 mg, *Rf* 0.17) which contained 7-oxo-D:C-friedo-olean-9(11)-ene-3α,29-diol diacetate (**18**) as the major component. HPLC purification afforded pure **18** (6 mg).

7-Oxo-D:C-friedo-olean-9(11)-ene-3α,29-diol Diacetate (18) mp 198—200 °C (MeOH). R_tR: 0.28 (HPLC). UV λ_{max} nm: 295. MS *m/z* (%): 540 (M^+, 41), 525 (3), 480 (8), 465 (5), 329 (10), 304 (10), 278 (100), 269 (8), 264 (15), 249 (13), 243 (13), 204 (23), 203 (21). HR-MS *m/z*: 540.3848 [Calcd for $C_{34}H_{52}O_5$ (M^+): 540.3812].

Step 3: BF_3 etherate (0.11 ml) was added dropwise to a solution of **18** (6 mg) in C_6H_6 (3 ml). The mixture was kept at room temperature for 2 d. Aqueous work-up and ether extraction afforded crude 7-oxodihydrokarounidiol diacetate (**4**) which was purified by HPLC. Purified **4** [3 mg, mp 189—190 °C (MeOH–water)] was identical by spectral comparison with the diacetate (**4**) of the natural product (**3**).

Partial Synthesis of Karounidiol Diacetate (2) from 7-Oxodihydrokarounidiol Diacetate (4) Step 1: A mixture of **4** (110 mg), hydrazine hydrate (80%, 1.0 ml), and diethylene glycol (7 ml) was refluxed (oil bath, 130 °C) for 1.5 h. Unreacted hydrazine hydrate was removed by distillation. KOH (110 mg) was added. Standard work-up after refluxing under N_2 for 3 h gave a residue (65 mg) which was acetylated. TLC of the acetates afforded a mixture of three components (29 mg). D:C-Friedo-olean-7-ene-3α,29-diol diacetate (**16**) (2 mg), 3-epibryonolol diacetate (**9**) (4 mg), and D:C-friedo-olean-9(11)-ene-3α,29-diol diacetate (**17**) (9 mg) were obtained by HPLC separation of this mixture.

D:C-Friedo-olean-7-ene-3α,29-diol Diacetate (16) mp 166—168 °C (MeOH). R_tR: 1.28 (HPLC). MS *m/z* (%): 526 (M^+, 11), 511 (11), 466 (7), 453 (11), 451 (22), 315 (4), 301 (22), 289 (15), 271 (22), 262 (85), 241 (48), 229 (59), 203 (100), 202 (59). HR-MS *m/z*: 526.3996 [Calcd for $C_{34}H_{54}O_4$ (M^+): 526.4018].

D:C-Friedo-olean-9(11)-ene-3α,29-diol Diacetate (17) mp 227—229 °C (MeOH). Rt_R: 1.16 (HPLC). MS *m/z* (%): 526 (M^+, 38), 511 (15), 466 (23), 451 (23), 315 (8), 301 (15), 289 (31), 276 (31), 271 (31), 215 (31), 229 (85), 203 (100). HR-MS *m/z*: 526.4035 [Calcd for $C_{34}H_{54}O_4$ (M^+): 526.4018].

Step 2: A solution of SeO_2 (30 mg) in 96% AcOH (1 ml) was added to a solution of the diacetate (**16**) (8 mg) in glacial AcOH (5 ml). Standard work-up after refluxing for 1 d and HPLC purification gave karounidiol diacetate (**2**) [3.7 mg, mp 222—224 °C (MeOH–acetone)].[26] This procedure was also used to convert the other two mono-enes (**9** and **17**) obtained in step 1 into **2**.

Bryononic Acid (12) A solution of 3-epibryonolol (**7**) (200 mg, see Step 1, synthesis of **2**, above) in pyridine (3 ml) was added to a well-stirred, ice cold suspension of CrO_3–pyridine complex prepared from CrO_3 (1.2 g) and pyridine (12 ml). After being left overnight at room temperature the mixture was worked-up. HPLC of the crude mixture (133 mg) gave bryononic acid (**12**) (14 mg) and 3-oxo-D:C-friedo-olean-8-en-29-al (**13**) (17 mg). **12**: mp 241—242 °C (MeOH) (lit.[4] 244—245 °C; lit.[21,22] 222—225 °C). Rt_R: 0.44 (HPLC). HR-MS *m/z*: 454.3450 [Calcd for $C_{30}H_{46}O_3$ (M^+): 454.3445].

3-Oxo-D:C-friedo-olean-8-en-29-al (13) mp 191—193 °C (MeOH–acetone). Rt_R: 0.57 (HPLC). IR ν_{max} cm^{-1}: 1730, 2686 and 2788 (aldehyde), 1700 (ketone). MS *m/z* (%): 438 (M^+, 18), 423 (18), 409 (24), 395 (6), 271 (13), 257 (100), 245 (96), 232 (10), 220 (34), 219 (44), 205 (21), 201 (25), 191 (82). HR-MS *m/z*: 438.3500 [Calcd for $C_{30}H_{46}O_2$ (M^+): 438.3495].

Bryonolic Acid (14) and Its Acetate (15) A mixture of bryononic acid (**12**) (10 mg) and $NaBH_4$ (10 mg) in MeOH (5 ml) was left at room temperature overnight. The excess reagent was decomposed with dilute HCl. HPLC purification of the residue (8 mg) of the ether extract gave bryonolic acid (**14**) (4 mg). **14**: mp 302—306 °C (MeOH) (lit.[3] 303—305 °C) Rt_R: 0.43 (HPLC). HR-MS *m/z*: 456.3571 [Calcd for $C_{30}H_{48}O_3$ (M^+): 456.3600]. See[12] for assigned ^{13}C-NMR data. **15**: This was obtained from **14** by acetylation. mp 261—264 °C (MeOH–water). Rt_R: 0.78 (HPLC). HR-MS *m/z*: 498.3676 [Calcd for $C_{32}H_{50}O_4$ (M^+): 498.3705]. See[12] for assigned ^{13}C-NMR data.

3-Epibryonolic Acid (10), Its 3-Acetate and 3α-Hydroxy-D:C-friedo-olean-8-en-29-al Acetate (11) A solution of **8** (30 mg) in pyridine (1 ml) was added to a well-stirred ice-cold suspension of CrO_3–pyridine complex prepared from CrO_3 (200 mg) and pyridine (2 ml). The mixture was left overnight at room temperature. Standard work-up and HPLC separation yielded 3-epibryonolic acid acetate (6 mg) and **11** (4 mg). **10** was obtained from its corresponding 3-acetate by hydrolysis. **10**: mp 262—264 °C (MeOH–water). Rt_R: 0.59 (HPLC). MS *m/z* (%): 456 (M^+, 54), 441 (49), 438 (4), 423 (56), 395 (4), 377 (3), 302 (19), 287 (12), 259 (63), 247 (41), 241 (100), 235 (41), 229 (92), 221 (41), 215 (18), 203 (45), 189 (79). HR-MS *m/z*: 456.3604 [Calcd for $C_{30}H_{48}O_3$ (M^+): 456.3601]. 3-Epibryonolic acid acetate: mp 230—232 °C (MeOH–water) (lit.[21] 220—222 °C). Rt_R: 0.65 (HPLC). HR-MS *m/z*: 498.3734 [Calcd for $C_{32}H_{50}O_4$ (M^+): 498.3706]. **11**: mp 178—180 °C (MeOH). Rt_R: 0.87 (HPLC). MS *m/z* (%): 482 (M^+, 58), 467 (28), 453 (17), 422 (6), 407 (58), 379 (6), 301 (33), 289 (19), 271 (8), 263 (8), 255 (4), 241 (100), 229 (89), 220 (28), 203 (58), 191 (42), 189 (50). HR-MS *m/z*: 482.3793 [Calcd for $C_{32}H_{50}O_3$ (M^+): 482.3757].

Bryonolol This was prepared by reduction of **13** (6 mg) with $NaBH_4$ (10 mg) in MeOH (5 ml) and purified by HPLC. Yield 4 mg. mp 284—287 °C (MeOH) (lit.[4] 285—288 °C). Rt_R: 0.39 (HPLC). HR-MS *m/z*: 442.3842 [Calcd for $C_{30}H_{50}O_2$ (M^+): 442.3809].

Bryonolol Diacetate This was prepared by acetylation of bryonolol. mp 216—217 °C (MeOH–acetone) (lit.[4] 206—209 °C). Rt_R: 0.76 (HPLC). HR-MS *m/z*: 526.4010 [Calcd for $C_{34}H_{54}O_4$ (M^+): 526.4018].

References and Notes

1) The C-20α substituent in these compounds has been assigned the locant 29 in accordance with the more common convention.
2) T. Akihisa, T. Tamura, T. Matsumoto, D. S. Eggleston, W. C. M. C. Kokke, and N. Shimizu, *J. Chem. Soc., Perkin Trans. 1*, **1988**, 439.
3) G. Biglino, L. Cattel, O. Caputo, and G. Nobili, *Gaz. Clim. Ital.*, **99**, 830 (1969).
4) K. Y. Sim and H. T. Lee, *Phytochemistry*, **11**, 3341 (1972).
5) H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
6) P. Sengupta, M. Sen, and S. N. Rao, *Indian J. Chem.*, **16B**, 738 (1978).
7) E. V. Lassak, J. Y. Pinhey, and J. J. H. Simes, *Aust. J. Chem.*, **26**, 1051 (1973).
8) R. B. Boar, J. F. McGhie, and D. A. Lewis, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 2590.
9) M. Naora, T. Murae, T. Tsuyuki, and T. Takahashi, *Bull. Chem. Soc. Jpn.*, **59**, 1767 (1986).
10) P. Sengupta and H. N. Khastgir, *Tetrahedron*, **19**, 123 (1963).
11) W. Kamisako, K. Isoi, H. Nakai, and M. Shiro, *Acta Cryst., Sect. C*, **40**, 1013 (1984).
12) W. Kamisako, K. Suwa, K. Morimoto, and K. Isoi, *Org. Magn. Reson.*, **22**, 93 (1984).
13) H. Nakai, M. Shiro, W. Kamisako, C. Honda, and K. Isoi, *Acta Cryst., Sect. C*, **43**, 1779 (1987).
14) Crystal structures of a bryonolic acid derivative [bis(3β-acetoxy-D:C-friedo-olean-8-en-29-oic)anhydride] have been reported by Nakai.[15]
15) H. Nakai, *Acta Cryst., Sect. C*, **45**, 1465, 1479 (1989).
16) W. Kamisako, K. Suwa, C. Honda, K. Isoi, H. Nakai, M. Siro, and K. Machida, *Magn. Reson. Chem.*, **25**, 838 (1987).
17) This possibility is not far-fetched because there are several examples in the literature of friedo-oleanane type triterpenoids with a 20β-configuration. In *Catha cassinoides*,[18] *Maytenus diversifolia*,[19] and *M. nemerosa*[20] the 20α, β isomers of 29- and 30-hydroxy-friedelan-3-one even co-occur in the same plant.
18) C. Betancor, R. Freire, A. G. Gonzalez, J. A. Salazar, C. Pascard, and T. Prange, *Phytochemistry*, **19**, 1989 (1980).
19) H. Nakai, H. Suzuki, T. Hirayama, R. Kasai, R.-Y. Wu, and K.-H. Lee, *Phytochemistry*, **25**, 479 (1986).
20) S. D. Fang, D. E. Berry, D. G. Lynn, S. M. Hecht, J. Campbell, and W. S. Lynn, *Phytochemistry*, **23**, 631 (1984).
21) P. J. Hylands, E.-S. S. Mansour, and M. T. Oskoui, *J. Chem. Soc., Perkin Trans. 1*, **1980**, 2933.
22) P. J. Hylands and M. T. Oskoui, *Phytochemistry*, **18**, 1843 (1979).
23) Isomultiflorene has been isolated from ferns.[24]
24) H. Ageta and Y. Arai, *Phytochemistry*, **22**, 1801 (1983).
25) H. Ageta, K. Shiojima, and Y. Arai, *Chem. Pharm. Bull.*, **35**, 2705 (1987).
26) The previously reported mp of **2** (197—200 °C)[2] is incorrect.