Four New *ent*-Kaurane Diterpenoids from the Fruits of *Annona cherimola*

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Received February 16, 2010; accepted February 27, 2010; published online March 4, 2010

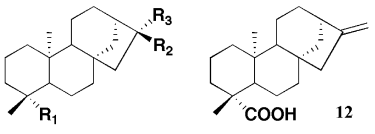
Four new *ent*-kaurane diterpenoids (16*R*)-*ent*-kauran-17,19-diol (1), (16*R*)-17-hydroxy-*ent*-kauran-19-oic acid (2), (16*S*)-17-hydroxy-*ent*-kauran-19-oic acid (3), and (16*R*)-17-dimethoxy-*ent*-kauran-19-oic acid (4) have been isolated from the fresh fruits of *Annona cherimola* together with eight known compounds. Their structures are determined on the basis of spectroscopic data and optical rotation.

Key words *Annona cherimola*; *ent*-kaurane; diterpenoid; Annonaceae

Annona cherimola Mill. (Annonaceae) is widely distributed in many tropical and subtropical regions of Africa, South America, and Asia. *A. cherimola* is also commercially cultivated in the United States, Chile, and Spain and is one of the popular edible fruits in the world. In the phytochemical studies on *A. cherimola*, the presence of various types of a cetogenin analogs in a seed has been shown[1] while that in a fruit has been scarcely reported. Therefore, we have investigated fresh fruits without seeds and identified four new *ent*-kaurane diterpenoids (16*R*)-*ent*-kauran-17,19-diol (**1**), (16*R*)-17-hydroxy-*ent*-kauran-19-oic acid (**2**), (16*S*)-17-hydroxy-*ent*-kauran-19-oic acid (**3**), and (16*R*)-17-dimethoxy-*ent*-kauran-19-oic acid (**4**) together with eight known compounds (**5**—**12**). The stereostructure of **2** and **3**, reported as a mixture,[2] has been elucidated for the first time. In this paper, we describe the isolation and structural elucidation on the basis of a two-dimensional (2D) NMR spectroscopic analysis.

Results and Discussion

The methanolic extract of the fresh fruits of *A. cherimola* was subjected to MCI gel CHP20P, octadecyl silica gel (ODS), and silica gel column chromatographies and finally to HPLC to obtain twelve compounds (**1**—**12**). Compounds



	R_1	R_2	R_3
1	CH_2OH	H	CH_2OH
2	COOH	H	CH_2OH
3	COOH	CH_2OH	H
4	COOH	H	$CH(OCH_3)_2$
5	CH_2OH	CH_2OH	H
6	COOH	OH	CH_2OH
7	COOH	CH_2OH	OH
8	$COOCH_3$	CH_2OH	OH
9	CH_3	OH	CH_2OH
10	CH_3	CH_2OH	OH
11	CH_3	CH_3	OH

5—**12** were identified as (16*S*)-*ent*-kauran-17,19-diol[3] (**5**), 16β,17-dihydroxy-*ent*-kauran-19-oic acid[4] (**6**), 16α,17-dihydroxy-*ent*-kauran-19-oic acid[4] (**7**), 16α,17-dihydroxy-*ent*-kauran-19-oic acid methyl ester[4] (**8**), *ent*-kauran-16β,17-diol[4] (**9**), *ent*-kauran-16α,17-diol[4] (**10**), *ent*-kauran-16α-ol[5] (**11**), and *ent*-kauran-16-en-19-oic acid[5] (**12**), respectively, on the basis of their optical rotation and spectral data. However, the NMR data for **5** were not reported in the literature, and therefore, we performed NMR data assignments.

Compound **1** was obtained as an amorphous powder, $[\alpha]_D$ −62.1°. The molecular formula, $C_{21}H_{34}O_2$, was established by the positive high-resolution (HR)-electron ionization-mass spectra (EI-MS) ($[M]^+$, *m*/*z* 306.2564). The analysis of the ^{1}H- and ^{13}C-NMR (Table 1) spectrum, and the heteronuclear multiple quantum coherence (HMQC) spectrum of **1** revealed four sp^3 methines, eleven sp^3 methylenes, three sp^3 quaternary carbons, and two methyl groups. Among the eleven methylene carbons, two methylene carbons (δ_C 63.9, 66.8) were ascribed to those bearing an oxygen atom. The molecular structure of **1** was deduced from extensive analyses of the 2D NMR data, including the ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) experiments. The ^{1}H–^{1}H COSY spectrum revealed connectivities of three partial structures, **a** (C-1—C-3), **b** (C-5—C-7), and **c** (C-9 and C-11—C-17) as shown in Fig. 1. The connectivities of C-3, C-5, C-18, and C-19 (δ_C 63.9) through a quaternary carbon C-4 (δ_C 39.4) were implied by the HMBC correlations for H_3-18 (δ_H 1.17) to C-3, C-4, C-5, and C-19, and H_2-19 (δ_H 3.60 and 3.98) to C-18. The HMBC crosspeaks for H_3-20 (δ_H 0.95) to C-1, C-5, C-9, and C-10 indicated that C-1, C-5, and C-9 were connected *via* the quaternary carbon at C-10 (δ_C 39.2). In addition, the HMBC correlations for H_2-15 (δ_H 1.07 and 1.61) to C-7, C-8, C-9, and C-14 suggested that C-7, C-9, C-14, and C-15 were connected through C-8 (δ_C 44.8) in an *ent*-kaurane-type skeleton. Therefore, the connectivities of the three partial structures **a**, **b**, and **c**, quaternary carbons (C-4, 8, and 10), and oxygen-bearing carbons (C-17 and C-19) of **1** were clarified, and the plane structure was concluded to be *ent*-kauran-17,19-diol. The stereostructure was characterized by a nuclear Overhauser and exchange spectroscopy (NOESY) experiment, which showed nuclear Overhauser effect (NOE) correlations between the following proton pairs [H-5 (δ_H

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Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of **1**—**5** (Pyridine-d_5)

Position	**1**		**2**		**3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	40.7	0.71 dt (4.0, 13.2) 1.72 br d (13.2)	41.0	0.80 dt (4.0, 13.2) 1.84 br d (12.6)	41.0	0.79 dt (4.0, 13.2) 1.80 br d (12.6)	41.0	0.79 dt (4.0, 13.8) 1.82[a)]	40.6	0.71 dt (3.5, 13.2) 1.70 br d (12.6)
2	18.7	1.36[a)] 1.65 br dd (3.4, 14.3)	19.7	1.49[a)] 2.22 tq (4.1, 13.7)	19.7	1.47[a)] 2.21 tq (4.0, 13.8)	19.7	1.47[a)] 2.20[a)]	18.6	1.36[a)] 1.66[a)]
3	36.1	0.94[a)] 2.16 br d (13.7)	38.5	1.07[a)] 2.43 br d (13.2)	38.5	1.04[a)] 2.41 br d (13.2)	38.5	1.03[a)] 2.42 br d (12.6)	36.0	0.95[a)] 2.15 br d (13.2)
4	39.4		43.7		43.7		43.7		39.1	
5	57.0	0.87 dd (1.7, 12.1)	56.9	1.03 dd (4.0, 13.2)	56.9	1.04[a)]	56.9	1.02[a)]	56.9	0.90 br d (12.1)
6	21.2	1.34 dq (3.5, 12.0) 1.65[a)]	23.0	2.01 br dd (2.3, 13.7) 2.14 dq (3.5, 13.8)	22.8	1.98 br dd (2.3, 13.8) 2.11 dq (2.9, 13.8)	23.0	2.01 br dd (1.8, 13.9) 2.12 dq (2.9, 13.9)	20.9	1.33 dq (3.5, 12.6) 1.64[a)]
7	42.3	1.40[a)] 1.40[a)]	41.9	1.46[a)] 1.46[a)]	42.4	1.42 dt (3.5, 13.2) 1.52[a)]	41.8	1.49[a)] 1.49[a)]	42.7	1.37 dt (3.4, 14.3) 1.44[a)]
8	44.8		44.8		44.3		45.1		44.2	
9	56.6	0.94[a)]	55.6	0.98 br d (7.5)	56.6	0.95 br d (8.0)	55.3	0.99 br d (8.1)	57.6	0.93[a)]
10	39.2		39.7		39.8		39.8		39.3	
11	18.9	1.54[a)] 1.54[a)]	19.0	1.58 m 1.58 m	19.3	1.51[a)] 1.57 m	18.8	1.53[a)] 1.53[a)]	19.1	1.45[a)] 1.53 m
12	31.9	1.41[a)] 1.53[a)]	31.7	1.45[a)] 1.45[a)]	26.2	1.38 m 1.74 m	31.6	1.43 m 1.43 m	26.1	1.47 m 1.75 m
13	38.8	2.34 br s	38.5	2.33 br d (2.9)	37.5	2.30 br t (4.0)	38.0	2.24 br d (2.9)	37.6	2.33 br s
14	37.3	1.03 dd (4.6, 11.5) 1.78 d (11.5)	37.2	1.09[a)] 1.85 br d (10.9)	40.7	0.99 dd (4.0, 13.2) 1.97 br d (11.5)	37.9	1.05 dd (4.0, 13.2) 1.82 br d (12.0)	40.5	0.96[a)] 1.92 br d (10.9)
15	45.8	1.07 dd (5.2, 13.2) 1.61 ddd (1.7, 8.6, 13.2)	45.6	1.12[a)] 1.63 ddd (1.8, 8.6, 13.2)	44.4	1.20 dd (6.9, 12.6) 1.65 t (12.6)	44.3	1.38 dd (5.7, 13.8) 1.59 ddd (1.7, 9.2, 13.8)	44.5	1.18 dd (6.9, 12.1) 1.63 t (12.6)
16	44.0	2.16[a)]	43.9	2.18[a)]	43.8	2.45 m	43.2	2.19[a)]	43.6	2.43 m
17	66.8	3.62 dd (10.3, 14.9) 3.64 dd (10.3, 13.2)	66.7	3.63 dd (10.3, 15.4) 3.64 dd (10.3, 13.8)	63.1	3.93 d (10.3) 3.97 d (10.3)	107.8	4.20 d (8.0)	63.1	3.92 d (10.3) 3.95 d (10.3)
18	28.0	1.17 s	29.2	1.32 s	29.2	1.30 s	29.2	1.30 s	27.9	1.16 s
19	63.9	3.60 d (10.9) 3.98 d (10.9)	179.9		180.0		179.4		63.8	3.60 d (10.9) 3.97 d (10.9)
20	18.2	0.95 s	15.8	1.12 s	15.9	1.10 s	15.8	1.09 s	18.3	0.95 s
OMe							52.6	3.31 d (1.2)		
OMe							52.8	3.32 d (1.2)		

a) Overlapped.

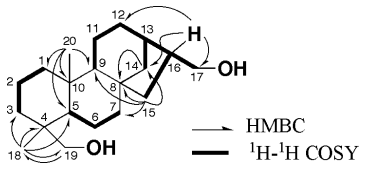

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of **1**

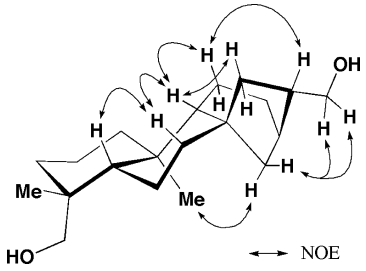

Fig. 2. NOE Correlations of **1**

0.87) and H-7 (δ_H 1.40); H-7 (δ_H 1.40) and H-9 (δ_H 0.94); H-9 (δ_H 0.94) and H-11 (δ_H 1.54); H-9 (δ_H 0.94) and H-15 (δ_H 1.61); H-11 (δ_H 1.54) and H-16 (δ_H 2.16); H-14 (δ_H 1.03) and H_2-17 (δ_H 3.62, 3.64); H-14 (δ_H 1.78) and H_3-20 (δ_H 0.95)] (Fig. 2). Accordingly, **1** was elucidated to be (16*R*)-*ent*-kauran-17,19-diol.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ −92.7°. The HR-electronspray ionization (ESI)-MS data ($[M-H]^-$, *m/z* 319.2286) indicated its molecular formula $C_{20}H_{31}O_3$ (Calcd for 319.2273). The ^{1}H-NMR spectrum of **2** was very similar to that of **1**, except for the disappearance of the oxygen-bearing methylene proton. In the ^{13}C-NMR spectrum, almost all signals were superimposable on those of **1**, although one carboxyl carbon appeared and one oxygenated methylene carbon disappeared. Furthermore, key HMBC correlations were observed between H_3-18 (δ_H 1.32) and the carboxyl carbon at C-19 (δ_C 179.9), resulting in the determination of the plane structure of **2**. In a NOESY experiment, NOE showed the same correlations as those of **1**. Therefore, **2** was identified as *ent*-kaurane diterpenoid possessing a hydroxyl group instead of the carboxyl group of **1** at the C-19 position.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ −36.1°, and its molecular formula was the same as that of **2**, $C_{20}H_{31}O_3$, based on the molecular ion peak $[M-H]^-$ at *m/z* 319.2278 (Calcd for 319.2273) in the HR-ESI-MS. The detailed analysis of its 1D and 2D NMR spectra elucidated that its plane structure was identical to that of **2**. However, slight differences in spectral data at C-12 (δ_C: 31.7, +5.5), C-14 (δ_C: 37.2, −3.6), and C-17 (δ_C: 66.7, +3.6) were observed, and **3** was predicted to be the stereoisomer of **2** at C-13 or C-16. From the results of the NOESY experiments, it was concluded that NOE correlations were observed between H-5 and H-9; H-9 and H-15; H-15 and H_2-17; H-14 and H_3-20, indicating that the stereochemistry of **3** was the same as that of **2**, except for H-16. Hence, **3** was determined to be a stereoisomer of **2** at the C-16 position.

Compound **4** was obtained as an amorphous powder, $[\alpha]_D$ −73.0°. The HR-ESI-MS showed an $[M-H]^-$ ion peak at *m/z* 363.2519, corresponding to the molecular formula $C_{22}H_{35}O_4$. The ^{1}H- and ^{13}C-NMR spectrum were analogous to those of **2** or **3**, except for the appearance of one acetal moiety and two methoxyl groups and the lack of one oxygenated methylene group. From the result of the detailed investigation of 1D and 2D NMR spectrum, it was found that **4** was very similar to **2**, except around the C-17 position. In the HMBC experiment, key correlations were observed between two methoxyl protons (δ_H 3.31, 3.32) and the C-17 acetal carbon (δ_C 107.8), resulting in the elucidation of the plane structure. The configuration was concluded to be the same as that of **2** on the basis of the NOESY experiment. Therefore, **4** was elucidated to be (16*R*)-17-dimethoxy-*ent*-kauran-19-oic acid. Previously, the *ent*-kaurane diterpenoid possessing an aldehyde group at C-17 was isolated as a natural product.[6] Therefore, the methoxyl groups at C-17 might be artificially formed *via* the reaction of the aldehyde group with MeOH during the extraction and/or isolation procedures.

Compound **5** was obtained as an amorphous powder, $[\alpha]_D$ −35.2°, with the molecular formula $C_{21}H_{34}O_2$ on the basis of the molecular ion peak $[M]^+$ at *m/z* 306.2562 (Calcd for $C_{20}H_{34}O_2$: 306.2559) by HR-EI-MS. Its ^{1}H- and ^{13}C-NMR spectral properties were closely related to those of **1**, except for some differences (C-12, 14, 17). In addition, 2D NMR experiments supported the conclusion that the plane structure of **5** was identical to that of **1**. From these observations, **5** was expected to be the stereoisomer of **1** at the C-16 position as well as a pair of **2** and **3**. In the NOESY spectrum, NOE correlations were observed between H-11 and H_2-17; H-12 and H_2-17; H-13 and H-14; H-13 and H-16; H-14 and H_3-20, indicating that the stereochemistry of **5** was the same as that of **3**. Consequently, **5** was identified as a stereoisomer of **1** at the C-16 position.

To the best of our knowledge, compounds **1**—**4** are new *ent*-kaurane diterpenoids, and the isolation of **5**—**12** from *A. cherimola* is described here for the first time.

Experimental

General Procedure Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The NMR spectra were measured with a JEOL ECA 500 NMR spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) and *J* values in Hz, using pyridine-d_5 for ^{1}H-NMR (7.20 ppm) and ^{13}C-NMR (123.5 ppm) as an internal standard. The MS were recorded with a JEOL JMS-T100LP spectrometer, and a JEOL JMS-DX-303HF instrument. HPLC was carried out using the Mightysil RP-18 (10.0 mm i.d.×250 mm, Kanto Chemical Co., Ltd., Tokyo, Japan); column with a Tosoh CCPM pump, and Tosoh RI-8010 detector. TLC was performed on pre-coated Kieselgel 60 F_{254} (Merck Ltd., Tokyo, Japan), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (230—400 mesh, Merck Ltd., Tokyo, Japan), MCI gel CHP20P (Mitsubishi Chemical Co., Tokyo, Japan), and ODS (PrePAK-500/C_{18}, Waters Co., Tokyo, Japan).

Plant Material The fruits of *Anona cherimola* were purchased from Nippi Trading Co., Ltd., Tokyo, Japan.

Extraction and Isolation The fresh fruits of *Annona cherimola* (1082 g) were extracted with MeOH at room temperature for one month. The MeOH extract (114 g) was subjected to MCI gel CHP20P column chromatography [MeOH–H_2O (10 : 90→30 : 70→40 : 60→90 : 10, v/v)→MeOH→aceton] to afford two fractions [Fractions 1 (597 mg), 2 (1.84 g)]. Fraction 1 (597 mg) was further separated by ODS column chromatography [MeOH–H_2O (60 : 40→70 : 30→80 : 20→90 : 10, v/v)] and silica gel column chromatography [$CHCl_3$–MeOH (20 : 1, v/v)], followed by HPLC [MeOH–H_2O (70 : 30, v/v)] to furnish compound **6** (5 mg), **7** (5 mg), and [MeOH–H_2O (80 : 20, v/v)] to furnish compound **1** (5 mg), **5** (7 mg). Fraction 2 (1.84 g) was further separated by ODS column chromatography [MeOH–H_2O (60 : 40→70 : 30→80 : 20→90 : 10, v/v)] and silica gel column chromatography [$CHCl_3$–MeOH (20 : 1, v/v)], followed by HPLC [MeOH–H_2O (70 : 30, v/v)] to furnish compound **8** (13 mg), [MeOH–H_2O (80 : 20, v/v)] to furnish compound **2** (10 mg), **3** (8 mg), [MeOH–H_2O (90 : 10, v/v)] to fur-

nish compound **4** (2 mg), **9** (9 mg), and [MeOH–H_2O (95 : 5, v/v)] to furnish compound **10** (19 mg), **11** (16 mg), **12** (22 mg).

Compound **1**: Amorphous powder; $[\alpha]_D$ −62.1° (c=0.39, pyridine); HR-EI-MS m/z: 306.2564 $[M]^+$ (Calcd for $C_{21}H_{34}O_2$, 306.2559); ^{1}H- and ^{13}C-NMR data (Table 1).

Compound **2**: Amorphous powder; $[\alpha]_D$ −92.7° (c=0.71, pyridine); HR-ESI-MS m/z: 319.2286 $[M-H]^-$ (Calcd for $C_{20}H_{31}O_3$, 319.2273); ^{1}H- and ^{13}C-NMR data (Table 1).

Compound **3**: Amorphous powder; $[\alpha]_D$ −36.1° (c=0.73, pyridine); HR-ESI-MS m/z: 319.2278 $[M-H]^-$ (Calcd for $C_{20}H_{31}O_3$, 319.2273); ^{1}H- and ^{13}C-NMR data (Table 1).

Compound **4**: Amorphous powder; $[\alpha]_D$ −73.0° (c=0.13, pyridine); HR-ESI-MS m/z: 363.2519 $[M-H]^-$ (Calcd for $C_{22}H_{35}O_4$, 363.2535); ^{1}H- and ^{13}C-NMR data (Table 1).

Compound **5**: Amorphous powder; $[\alpha]_D$ −35.2° (c=0.54, pyridine); HR-EI-MS m/z: 306.2562 $[M]^+$ (Calcd for $C_{21}H_{34}O_2$, 306.2559); ^{1}H- and ^{13}C-NMR data (Table 1).

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