Cucurbitane-Type Triterpenoids from the Fruit of *Momordica charantia*

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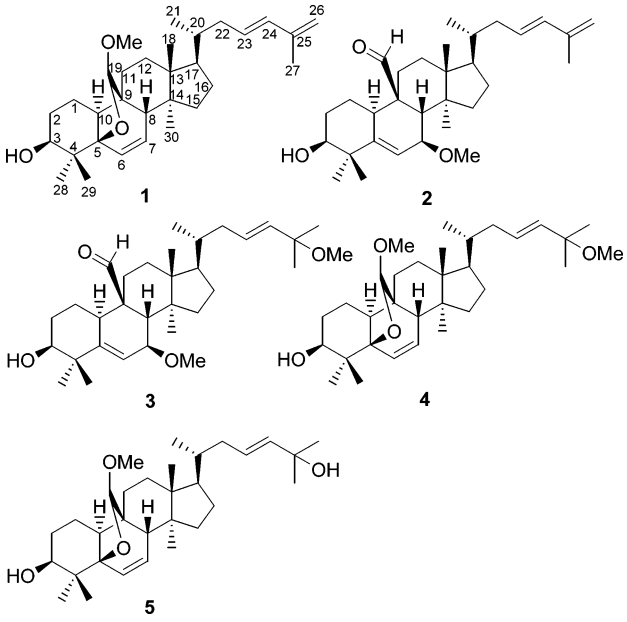
The structures of three new cucurbitane-type triterpenoids isolated from the methanol extract of the fruit of Japanese *Momordica charantia* were established as (19*R*,23*E*)-5*β*,19-epoxy-19-methoxycucurbita-6,23,25-trien-3*β*-ol (**1**), (23*E*)-3*β*-hydroxy-7*β*-methoxycucurbita-5,23,25-trien-19-al (**2**), and (23*E*)-3*β*-hydroxy-7*β*,25-dimethoxycucurbita-5,23-dien-19-al (**3**) on the basis of spectroscopic methods. These compounds were accompanied by the known (19*R*,23*E*)-5*β*,19-epoxy-19,25-dimethoxycucurbita-6,23-dien-3*β*-ol (**4**) and (19*R*,23*E*)-5*β*,19-epoxy-19-methoxycucurbita-6,23-diene-3*β*,25-diol (**5**). This is the first report of the isolation of tetracyclic triterpenoids possessing a $\Delta^{23,25}$-conjugated diene system, viz., **1** and **2**, from a natural source.

The plant *Momordica charantia* L. (Cucurbitaceae) is cultivated as a vegetable in Asian countries. In Chinese, Indian Ayurvedic, and Indonesian Jamu traditional medicines, the fruit of this plant has been used as a bitter stomachic, a laxative, an antidiabetic, and an anthelmintic for children. Antidiabetic properties of *M. charantia* have been reviewed,[1] and the favorable effects on the concentration of serum and hepatic lipids in rats fed with the fruit powder have been demonstrated.[2] Various cucurbitane-type triterpenoid glycosides have been reported as chemical constituents of the fruit.[1,3−6] In the course of our search for potential antitumor promoters from natural sources,[7,8] we were especially interested in *M. charantia*. In this paper, we present the isolation and structure elucidation of three new cucurbitane-type triterpenoids, **1**−**3**, along with the isolation and identification of two known cucurbitane-type triterpenoids, **4** and **5**, from the MeOH extract of the dried fruit of Japanese *M. charantia*, which is commonly called "nigauri" or "goya".

Compound **1** possesses the molecular formula $C_{31}H_{48}O_3$ as determined from the HRFABMS ($[M + K]^+$ *m/z* 507.3244) as well as from its ^{13}C NMR DEPT, which suggested that it was a triterpenoid with eight degrees of unsaturation. The ^{13}C and ^1H NMR spectra of **1** showed the presence of four tertiary methyls, a secondary methyl, a vinylic methyl, an *O*-methyl, a secondary hydroxyl, two disubstituted double bonds, a terminal methylene, and an acetal methine group (Table 1). The NMR data are in good agreement with those of (19*R*,23*E*)-5*β*,19-epoxy-19-methoxycucurbita-6,23-diene-3*β*,25-diol (**5**),[9] except for the signals arising from the side-chain moiety, which suggested that **1** possesses a 19-methoxy-5*β*,19-epoxy-3*β*-hydroxy-10α-cucurbitan-6-ene ring-system.[4,9,10] The UV absorptions at λ_{max} 240 (ϵ 4.08), 231 (ϵ 4.33), and 225 (ϵ 4.30) nm, along with the MS fragmentation at *m/z* 299 ([M − HCOOMe − side-chain (C_8H_{13})]$^+$) and the NMR signals from the side-chain moiety (Table 1), suggested the presence of a (23*E*)-$\Delta^{23,25}$-conjugated diene system in the side-chain of **1**.[11] The above evidence coupled with the analysis of $^1H-^1H$ COSY, HMQC, and HMBC data (Supporting Information) indicated that **1** possesses a (19*R*,23*E*)-5*β*,19-epoxy-19-methoxycucurbita-6,23,25-trien-3*β*-ol structure. The relative configuration was deduced from NOESY data (Figure 1) and molecular modeling.[12]



Compound **2** showed $[M]^+$ at *m/z* 468.3605 ($C_{31}H_{48}O_3$) in the HREIMS. The ^{13}C and ^1H NMR spectra of **2** showed the presence of four tertiary methyl, a secondary *O*-methyl, a secondary hydroxyl, a trisubstituted double bond, and an aldehyde group in the ring system of the molecule (Table 1). Compound **2** possesses the same side-chain as that of **1**, viz., a $\Delta^{23,25}$-di-unsaturated C_8 moiety, by the UV absorptions [λ_{max} 239 (ϵ 4.13), 231 (ϵ 4.29), 224 (ϵ 4.28)][11] and the diagnostic MS fragmentation at *m/z* 299 ([M − OMe − CHO − side-chain (C_8H_{13})]$^+$) as well as by the NMR data (Table 1). Spectroscopic comparison of **2** with (23*E*)-3*β*,7*β*,25-trihydroxycucurbita-5,23-dien-19-al and (23*E*)-3*β*,7*β*-dihydroxy-25-methoxycucurbita-5,23-dien-19-al[5,13] suggested that **2** possesses a 3*β*-hydroxy-7*β*-methoxycucurbit-5-en-19-al tetracyclic ring system. The above evidence and the analysis of $^1H-^1H$ COSY, HMQC, and HMBC data (Supporting Information) hence confirmed **2** as (23*E*)-3*β*-hydroxy-7*β*-methoxycucurbita-5,23,25-trien-19-al. The rela-

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Table 1. ^{13}C and ^{1}H NMR Data (δ values; C_5D_5N) for Triterpenoids **1**−**3**

	1[a]			**2**			**3**		
C no.	δ_C		δ_H[b]	δ_C		δ_H[b]	δ_C		δ_H[b]
1	17.4	t	1.49 (α), 1.53 (*β*)	21.6	t	1.69 (α), 2.01 (*β*)	21.6	t	1.69 (α), 2.01 (*β*)
2	27.2	t	1.78 (2H)	29.8	t	1.91 (*β*), 2.06 (α)	29.8	t	1.90 (*β*), 2.05 (α)
3	76.2	d	3.41 (br d, 6.9)	75.6	d	3.81 (br s)	75.6	d	3.81 (br s)
4	37.3	s		42.0	s		42.0	s	
5	86.8	s		147.7	s		147.7	s	
6	131.0	d	5.99 (dd, 2.0, 9.8)	121.1	d	6.14 (d 5.4)	121.1	d	6.15 (d, 5.4)
7	132.8	d	5.65 (dd, 3.2, 9.8)	75.7	d	3.54 (dd 5.4, 9.7)	75.7	d	3.55 (br d, 5.4)
8	41.7	d	2.89 (dd, 3.2, 3.2)	45.8	d	2.22 (s)	45.8	d	2.23 (s)
9	48.0	s		50.3	s		50.3	s	
10	40.5	d	2.41 (dd, 7.6, 10.7)	36.7	d	2.63	36.8	d	2.65
11	23.2	t	1.60, 1.76	22.6	t	1.54 (α), 2.65 (*β*)	22.6	t	1.54 (α), 2.64 (*β*)
12	30.6	t	1.62 (2H)	29.3	t	1.61 (2H)	29.4	t	1.60 (2H)
13	45.1	s		45.9	s		45.9	s	
14	48.3	s		48.0	s		47.9	s	
15	33.5	t	1.34, 1.40	35.1	t	1.35 (2H)	35.1	t	1.35 (2H)
16	28.1	t	1.39 (α), 1.97 (*β*)	27.8	t	1.36 (α), 1.95 (*β*)	27.7	t	1.36 (α), 1.95 (*β*)
17	50.3	d	1.46	50.5	d	1.57	50.3	d	1.56
18	14.7	q	0.88 (s)	15.0	q	0.94 (s)	15.0	q	0.95 (s)
19	112.1	d	4.65 (s)	207.2	d	10.30 (s)	207.2	d	10.31 (s)
20	36.6	d	1.55	36.8	d	1.55	36.4	d	1.55
21	18.8	q	0.91 (d, 6.3)	18.9	q	0.98 (d, 5.9)	19.0	q	1.00 (d, 5.9)
22	39.8	t	1.81, 2.26	40.1	t	1.88, 2.32	39.7	t	1.85, 2.24
23	129.3	d	5.61 (br d, 15.6)	129.7	d	5.78 (ddd, 6.1, 7.7, 15.6)	128.4	d	5.66 (ddd, 5.4, 8.3, 15.8)
24	134.2	d	6.12 (d, 15.6)	134.8	d	6.32 (d, 15.6)	137.8	d	5.57 (d, 15.8)
25	142.2	s		142.5	s		74.9	s	
26	114.1	t	4.86 (2H, s)	114.7	t	4.98 (s), 5.03 (s)	26.1	q	1.34 (s)
27	18.7	q	1.84 (s)	19	q	1.92 (s)	26.5	q	1.34 (s)
28	24.1	q	0.85 (s)	27.3	q	1.17 (s)	27.3	q	1.17 (s)
29	20.5	q	1.22 (s)	26.2	q	1.50 (s)	26.2	q	1.50 (s)
30	19.8	q	0.86 (s)	18.3	q	0.81 (s)	18.2	q	0.81 (s)
3-O*H*			3.95 (br d, 9.5)						
7-OMe				55.9	q	3.28 (s)	55.9	q	3.29 (s)
19-OMe	58.2	q	3.44 (s)						
25-OMe							50.2	q	3.23 (s)

[a] Determined in $CDCl_3$. [b] *J* values (Hz) determined are shown in parentheses.

tive configuration of **2** was deduced from NOESY data (Figure 1) and molecular modeling.[12]

The molecular formula of compound **3** was determined as $C_{32}H_{52}O_4$ from its HREIMS ($[M - H_2O]^+$, *m/z* 482.3759) and FABMS ($[M + K]^+$, *m/z* 539). The ^{13}C and ^{1}H NMR signals for the ring system of **3** were very similar to those of **2** (Table 1), suggesting that it also possesses a 3*β*-hydroxy-7*β*-methoxycucurbit-5-en-19-al tetracyclic ring system. The NMR data for the side-chain moiety (Table 1) of compound **3** showed the presence of two tertiary methyls, a secondary methyl, an *O*-methyl, and a *trans*-oriented disubstituted double bond, which are consistent with a (23*E*)-25-methoxy-Δ^{23}-unsaturated C_8 moiety.[9] Thus, the structure of **3** is (23*E*)-3*β*-hydroxy-7*β*,25-dimethoxycucurbita-5,23-dien-19-al, which was supported from the EIMS fragmentation and the analysis of $^{1}H-^{1}H$ COSY, HMQC, and HMBC data (Supporting Information) as well as from the phase-sensitive NOESY experiment (Figure 1).

Two other triterpenoids, **4** and **5**, isolated in this study were identified as (19*R*,23*E*)-5*β*,19-epoxy-19,25-dimethoxycucurbita-6,23-dien-3*β*-ol and (19*R*,23*E*)-5*β*,19-epoxy-19-methoxycucurbita-6,23-diene-3*β*,25-diol, respectively, on the basis of comparison with the literature data.[9]

Although a sterol possessing a $\Delta^{23,25}$-conjugated diene system is known as a synthetic (23*E*)-cholesta-5,23,25-trien-3*β*-ol,[11] this is the first report of the isolation of triterpenoids possessing a $\Delta^{23,25}$-conjugated diene system, viz., **1** and **2**, from a natural source.

Experimental Section

General Experimental Procedures. Crystallizations were performed in MeOH, and melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1030 polarimeter in acetone or in $CHCl_3$ at 25 °C. UV spectra on a Shimadzu UV-2200 spectrometer and IR spectra on a JASCO FTIR-300E spectrometer were recorded in EtOH and KBr disks, respectively. NMR spectra were recorded with a JEOL ECA-600 spectrometer at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR) in C_5D_5N or in $CDCl_3$ with tetramethylsilane as internal standard. EIMS (70 eV) and HREIMS were recorded on a JEOL JMS-BU20 spectrometer using a direct inlet system. FABMS and HRFABMS were obtained with a JEOL JMS-BU20 spectrometer using glycerol as a matrix. Silica gel (Kieselgel 60, 230−400 mesh, Merck) was used for open column chromatography. Reversed-phase preparative HPLC was carried out on an octadecyl silica column (Pegasil ODS II column, 25 cm × 10 mm i.d.; Senshu Scientific Co., Ltd., Tokyo, Japan) at 25 °C with MeOH−H_2O−acetic acid (99:1:1, v/v/v) as mobile phase at 2 mL/min.

Materials. Sliced and dried fresh whole fruit of "nigauri" (*M. charantia*), cultivated in Okinawa prefecture, Japan, in the summer of 2002, used in this study was purchased from Taiyo Co., Ltd. (Osaka, Japan).

Isolation. Sliced and dried fruit material of *M. charantia* (1.5 kg) was extracted with MeOH, which yielded the extract (108 g) after evaporation of the solvent in vacuo. The extract was partitioned between H_2O and EtOAc, giving the EtOAc-soluble fraction (15 g). The EtOAc fraction was further partitioned between *n*-hexane−MeOH−H_2O (19:19:1), which yielded *n*-hexane (4.2 g) and MeOH−H_2O (9.8 g) soluble fractions. Column chromatography on silica gel (400 g) of the *n*-hexane fraction, eluted with *n*-hexane−EtOAc [1:0 (0.5 L), 19:1 (2.4 L), 9:2 (7.2 L), 4:1 (2.3 L), 7:3 (0.2 L), 1:1 (0.4 L), 1:4 (0.9 L), 0:1 (0.5 L)], afforded seven fractions (A−G): A (21 mg), B (380 mg), C (267 mg), D (1100 mg), E (517 mg), F (113 mg), and G (1930 mg). Fraction D, on further chromatography on silica gel (100 g), yielded six fractions (Da−Df). The second

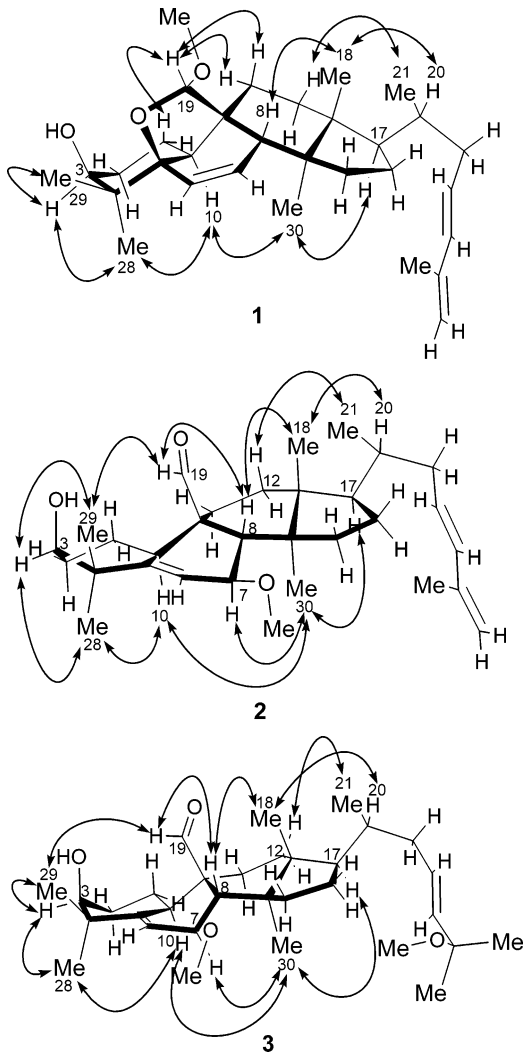

Figure 1. Major NOE correlations (↔) for triterpenoids **1**−**3**.

fraction Db (73 mg) was subjected to preparative HPLC, which yielded compound **1** (8.5 mg; retention time (t_R) 14.2 min). Fraction E was further chromatographed on silica gel (50 g) to yield nine fractions (Ea−Ei). Preparative HPLC of the fourth eluted fraction Ed (69 mg), the fifth eluted fraction Ee (21 mg), and the seventh eluted fraction Eg (102 mg) eventually afforded compounds **4** (4.5 mg; t_R 13.3 min), **5** (4.6 mg; t_R 14.8 min), and **2** (2.9 mg; t_R 24.8 min) and **3** (2.8 mg; t_R 14.4 min), respectively.

(23*E*)-5*β*,19-Epoxy-19-methoxycucurbita-6,23,25-trien-3*β*-ol (1): fine needles, mp 166−169 °C; $[\alpha]^{25}_D$ −74.0° (acetone; *c* 0.10); UV (EtOH) λ_{max} 240 (ϵ 4.08), 231 (ϵ 4.33), 225 (ϵ 4.30); IR (KBr) ν_{max} 3479 (OH), 2941, 1650, 1605 (conjugated diene), 879 (>C=CH_2) cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; EIMS *m/z* 408 [M − HCOOMe]$^+$ (100), 393 (*m/z* 408 − Me) (14), 390 (*m/z* 408 − H_2O) (20), 389 (22), 375 (*m/z* 408 − Me − H_2O) (17), 327 (*m/z* 408 − (C-22-C-27)) (10), 309 (*m/z* 327 − H_2O) (43), 299 (*m/z* 408 − side-chain (C_8H_{13})) (32), 281 (*m/z* 299 − H_2O) (38), 172 (59), 109 [C_8H_{13}]$^+$ (57); FABMS *m/z* 469 [M + H]$^+$, 507 [M + K]$^+$; HRFABMS *m/z* 507.3244 [M + K]$^+$ (calcd for $C_{31}H_{48}O_3$·K, 507.3240).

(23*E*)-3*β*-Hydroxy-7*β*-methoxycucurbita-5,23,25-trien-19-al (2): fine needles, mp 127−130 °C; $[\alpha]^{25}_D$ +19.1° ($CHCl_3$; *c* 0.21); UV (EtOH) λ_{max} 239 (ϵ 4.13), 231 (ϵ 4.29), 224 (ϵ 4.28); IR (KBr) ν_{max} 3445 (OH), 2928, 1713 (−CHO), 1670, 1610 (conjugated diene), 880 (>C=CH_2), 820 (>C=CH−) cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; EIMS *m/z* 468 [M]$^+$ (12), 450 [M − H_2O]$^+$ (7), 436 [M − MeOH]$^+$ (4), 408 [M − OMe − CHO]$^+$ (100), 393 (*m/z* 408 − Me) (10), 390 (10), 375 (*m/z* 408 − Me − H_2O) (12), 327 (*m/z* 408 − (C-22−C-27)) (9), 309 (*m/z* 327 − H_2O) (35), 299 (*m/z* 408 − side-chain (C_8H_{13})) (17), 281 (*m/z* 299 − H_2O) (23), 172 (61), 109 [C_8H_{13}]$^+$ (75); HREIMS *m/z* 468.3605 [M]$^+$ (calcd for $C_{31}H_{48}O_3$, 468.3603).

(23*E*)-3*β*-Hydroxy-7*β*,25-dimethoxycucurbita-5,23-dien-19-al (3): fine needles, mp 104−107 °C; $[\alpha]^{25}_D$ +25.9° ($CHCl_3$; *c* 0.26); IR (KBr) ν_{max} 3444 (OH), 2929, 1712 (−CHO), 845, 820 (>C=CH−) cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; EIMS *m/z* 482 [M − H_2O]$^+$ (6), 440 [M − H_2O − MeOH]$^+$ (74), 421 (22), 408 [M − H_2O − 2MeOH]$^+$ (100), 393 (*m/z* 408 − Me) (11), 389 (15), 375 (*m/z* 408 − Me − H_2O) (16), 309 (25), 299 (*m/z* 408 − side-chain (C_8H_{13})) (17), 293 (13), 281 (*m/z* 299 − H_2O) (19), 172 (94), 109 (86), 99 (100); HREIMS *m/z* 482.3759 [M − H_2O]$^+$ (calcd for $C_{32}H_{50}O_3$, 482.3760); FABMS *m/z* 539 [M + K]$^+$, corresponding to the formula $C_{32}H_{52}O_4$·K.

Supporting Information Available: ^{13}C and ^1H NMR and HMBC NMR data for **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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