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Norcucurbitane Triterpenoids from the Fruits of *Momordica charantia* var. *abbreviata*

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Two new 27-norcucurbitane triterpenoids, 27-nor-3 β -hydroxy-7 β -methoxycucurbita-5,23(*E*)-dien-25-one (**1**) and 27-nor-3 β -hydroxy-5 β ,19-epoxycucurbita-6,23(*E*)-dien-25-one (**2**), together with two known cucurbitane triterpenes, 23(*E*)-7 β -methoxycucurbita-5,23,25-trien-3 β -ol (**3**) and 5 β ,19-epoxy-25-methoxycucurbita-6,23(*E*)-dien-3 β -ol (**4**), were isolated from the fruits of *Momordica charantia* var. *abbreviata*. Their structures were determined by analysis of spectroscopic data and comparison with the data of known analogues.

Keywords: *Momordica charantia* var. *abbreviata*, Cucurbitaceae, Fruit, Triterpenoid, Norcucurbitane.

Momordica charantia Linn. var. *abbreviata* Ser. (Cucurbitaceae), a wild variety of bitter melon, is native to tropical areas of Asia and is commonly found growing in the wild in Taiwan. The size of its fruit is only about one-fifth that of the cultivated bitter melon and usually consumed not only as a vegetable but also as a popular folk medicine for the treatment of liver diseases and diabetes [1]. Previous investigations have showed that the extracts of fruits of *M. charantia* var. *abbreviata* possess antioxidant [2,3], anti-inflammatory [4,5], and anti-bacterial activities [6], and activate the peroxisomal proliferator-activated receptor α [7]. Although *M. charantia* var. *abbreviata* has been widely used as a health food and in folk medicine, little information is available on the phytochemical components of this plant. In this study, we examined the secondary metabolites of the fruits of *M. charantia* var. *abbreviata* that led to the purification and identification of two new 27-norcucurbitane triterpenoids, 27-nor-3 β -hydroxy-7 β -methoxycucurbita-5,23(*E*)-dien-25-one (**1**) and 27-nor-3 β -hydroxy-5 β ,19-epoxycucurbita-6,23(*E*)-dien-25-one (**2**), together with two known cucurbitane triterpenes, 23(*E*)-7 β -methoxycucurbita-5,23,25-trien-3 β -ol (**3**) [8] and 5 β ,19-epoxy-25-methoxycucurbita-6,23(*E*)-dien-3 β -ol (**4**) [9] (Figure 1). Herein, we report the isolation, and structure elucidation of compounds **1** and **2**.

The molecular formula of compound **1**, C₃₀H₄₈O₃, was determined by HR-EI-MS, which showed a molecular ion at *m/z* 456.3604. In the IR spectrum absorption bands typical for hydroxyl (3510 cm⁻¹), olefinic double bond (3027, 1650 cm⁻¹), and α,β -unsaturated ketone (1671 cm⁻¹) were observed. The ¹H and ¹³C NMR spectra of **1** (Table 1) showed similar resonances to the known compound, 23(*E*)-7 β -methoxycucurbita-5,23,25-trien-3 β -ol (**3**) [8], including a methoxy [δ_{H} 3.33 (3H, s)], two oxymethines [δ_{H} 3.39 (1H, brd, *J*=5.6 Hz), 3.49 (1H, brs)], and a trisubstituted double bond

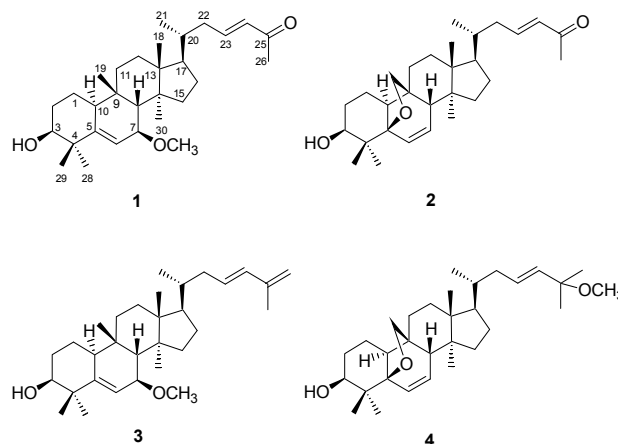


Figure 1: Structures of compounds 1-4 from *M. charantia* var. *abbreviata*.

[δ_{H} 5.80 (1H, d, *J* = 5.6 Hz); δ_{C} 120.8 (d), 146.8 (s)]. Thirty carbon signals were observed in the ¹³C NMR spectrum of **1**, and were resolved by a DEPT experiment into seven aliphatic methyls, seven aliphatic methylenes, four aliphatic methines, four aliphatic quaternary, three olefinic methines, one quaternary olefinic, two tertiary oxygenated, one conjugated ketone carbonyl, and one methoxy carbons. Detailed comparison of spectral data of **1** with those of 23(*E*)-7 β -methoxycucurbita-5,23,25-trien-3 β -ol (**3**) showed that both compounds exhibited the same 7 β -methoxycucurbita-5-en-3 β -ol skeleton but were different from each other in the side chain part.

The HMBC correlations between H-3 (δ_{H} 3.49) and C-1 (δ_{C} 21.1) and C-5 (δ_{C} 146.8), and between H-7 (δ_{H} 3.39) and C-5, C-6

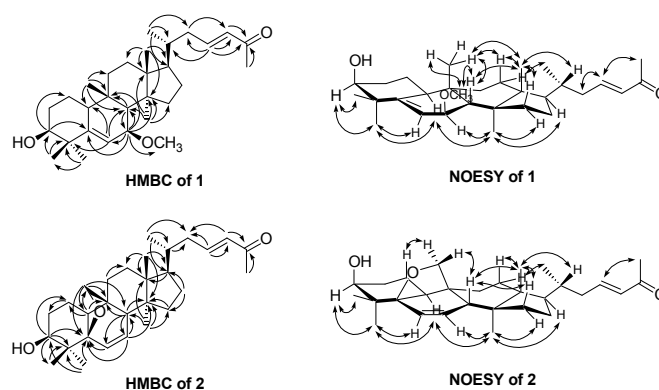
Table 1: ^1H and ^{13}C NMR data for **1** and **2** (400 MHz and 100 MHz in CDCl_3).

| No. | 1 | | 2 | |
|---------------------|---------------------|----------------------------|---------------------|------------------------------|
| | δ_{C} | δ_{H} | δ_{C} | δ_{H} |
| 1 | 21.1 | 1.48 m, 1.56 m | 17.6 | 1.44 m |
| 2 | 28.6 | 1.72 m, 1.86 m | 27.3 | 1.78 m |
| 3 | 76.7 | 3.49 brs | 76.1 | 3.37 brd (9.6) |
| 4 | 41.7 | | 37.2 | |
| 5 | 146.8 | | 87.5 | |
| 6 | 120.8 | 5.80 d (5.6) ^{a)} | 131.9 | 6.01 dd (2.0, 9.6) |
| 7 | 77.3 | 3.39 brd (5.6) | 131.3 | 5.60 dd (3.2, 9.6) |
| 8 | 47.9 | 2.02 brs | 51.9 | 2.30 brs |
| 9 | 34.0 | | 45.3 | |
| 10 | 38.7 | 2.28 brd (12.0) | 38.8 | 2.25 m |
| 11 | 32.6 | 1.42 m, 1.58 m | 23.5 | 1.44 m, 1.75 m |
| 12 | 30.0 | 1.46 m, 1.62 m | 30.7 | 1.62 m |
| 13 | 46.2 | | 45.4 | |
| 14 | 47.9 | | 48.6 | |
| 15 | 34.6 | 1.32 m | 33.1 | 1.33 m |
| 16 | 27.8 | 1.33 m, 1.89 m | 28.1 | 1.40 m, 2.00 m |
| 17 | 50.1 | 1.48 m | 50.3 | 1.48 m |
| 18 | 15.4 | 0.91 s | 14.9 | 0.86 s |
| 19 | 28.8 | 0.97 s | 79.8 | 3.49 d (8.4) 3.65 d (8.4) |
| 20 | 36.0 | 1.64 m | 35.9 | 1.64 m |
| 21 | 19.0 | 0.90 d (6.0) | 18.9 | 0.90 d (6.4) |
| 22 | 39.6 | 1.92 m, 2.34 m | 39.6 | 1.94 m, 2.34 m |
| 23 | 147.4 | 6.75 ddd (6.0, 8.8, 16.0) | 147.3 | 6.75 ddd (6.0, 8.4, 15.2) |
| 24 | 132.6 | 6.04 d (16.0) | 132.6 | 6.05 d (15.2) |
| 25 | 198.6 | | 198.5 | |
| 26 | 26.9 | 2.23 s | 26.9 | 2.23 s |
| 28 | 27.8 | 1.02 s | 24.5 | 0.87 s |
| 29 | 25.4 | 1.19 s | 20.5 | 1.18 s |
| 30 | 18.0 | 0.68 s | 20.0 | 0.85 s |
| 3-OH | | | | 3.97 d (9.6) |
| 23-OCH ₃ | 56.3 | 3.33 s | | |

^{a)} Coupling constants are presented in Hz.

(δ_{C} 120.8), C-8 (δ_{C} 47.9) and 7-OCH₃ (δ_{C} 56.3) confirmed that the hydroxyl and methoxyl groups are attached at C-3 and C-7, respectively (Figure 2). Except for the ^{13}C NMR signal of the methoxyl group, the skeleton of compound **1** contained 29 carbons and was thus presumed to be a norcucurbitane triterpene with a C₇ side chain. The ^1H NMR signals of the side chain included a secondary methyl [δ_{H} 0.90 (3H, d, $J = 6.0$ Hz)], an aliphatic methine [δ_{H} 1.64 (1H, m)], one methylene [δ_{H} 1.92 m, 2.34 m], an acetyl methyl [δ_{H} 2.23 (3H, s)], and a *trans*-oriented disubstituted double bond [δ_{H} 6.04 (1H, d, $J = 16.0$ Hz), 6.75 (1H, ddd, $J = 6.0, 8.8, 16.0$ Hz)]. Comparing the ^1H and ^{13}C chemical shifts of the side chain of **1** with those of 27-*nor*-3 β -hydroxycycloart-23-en-25-one [10] hinted that both compounds exhibited the same C₇ side chain structure, 23(*E*)-en-25-one. The UV absorption of 233 nm and the HMBC correlations between Me-21 (δ_{H} 0.90) and C-17 (δ_{C} 50.1), C-20 (δ_{C} 36.0) and C-22 (δ_{C} 39.6); between H-23 (δ_{H} 6.75) and C-20, C-22, C-24 (δ_{C} 132.6) and C-25 (δ_{C} 198.6); and between Me-26 (δ_{H} 2.23) and C-24 and C-25 confirmed this proposed side chain structure. Thus, compound **1** was formulated as 27-*nor*-3 β -hydroxy-5 β ,19-epoxycucurbita-6,23(*E*)-dien-25-one. Complete ^1H and ^{13}C NMR chemical shifts were established by ^1H - ^1H COSY, HMQC, HMBC, and NOESY spectra (Figure 2).

The HR-FAB-MS spectrum of compound **2** showed an $[\text{M}+\text{H}]^+$ ion at m/z 441.3359, which was consistent with the molecular formula C₂₉H₄₄O₃. The IR spectrum indicated the presence of hydroxyl (3481 cm⁻¹), α,β -unsaturated ketone (1670 cm⁻¹) and *cis* and *trans*-disubstituted double bond (3032, 1652, 977, 734 cm⁻¹) functionalities. The ^1H and ^{13}C NMR data of **2** (Table 1) showed close resemblance with that of the known compound, 5 β ,19-epoxycucurbita-6,23(*E*)-diene-3 β ,25-diol [11], except for that of the side chain part from C-20 to C-26. Thus, compound **2** was tentatively proposed to exhibit a basic skeleton of 5 β ,19-epoxycucurbit-6-en-3 β -ol. The HMBC spectrum of **2** (Figure 2) showed long-range correlations between H-3 (δ_{H} 3.37) and C-1 (δ_{C} 17.6), C-4 (δ_{C} 37.2), and C-5 (δ_{C} 87.5), and this suggested that one

**Figure 2:** Selected HMBC and NOESY correlations of compounds **1** and **2**.

hydroxyl group was located at C-3. The HMBC correlations between H-6 (δ_{H} 6.01) and C-5 (δ_{C} 87.5); between H-7 (δ_{H} 5.60) and C-5, C-8 (δ_{C} 51.9), and C-14 (δ_{C} 48.6); and between H-19 (δ_{H} 3.49, 3.65) and C-5, C-8, C-9 (δ_{C} 45.3), C-10 (δ_{C} 38.8), and C-11 (δ_{C} 23.5) confirmed an ether linkage between C-19 and C-5. The ^{13}C NMR spectrum of **2** revealed 29 carbon signals. The ^{13}C chemical shifts from C-20 to C-26 in the side chain portion of **2** were identical to those of **1**, which permitted the assignment of the C₇ side chain structure of **2** as 23(*E*)-en-25-one. Accordingly, compound **2** was determined to be 27-*nor*-3 β -hydroxy-5 β ,19-epoxycucurbita-6,23(*E*)-dien-25-one. Complete ^1H and ^{13}C NMR chemical shifts were established by ^1H - ^1H COSY, HMQC, HMBC, and NOESY spectra (Figure 2).

Experimental

General experimental procedures: Optical rotations were measured using a JASCO DIP-180 digital spectropolarimeter. The IR spectra were obtained on a Nicolet 510P FT-IR spectrometer. UV spectra were measured in MeOH on a Shimadzu UV-1601PC spectrophotometer. The NMR spectra were recorded in CDCl_3 at room temperature on a Varian Mercury plus 400 NMR spectrometer, and the solvent resonance was used as internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EI-MS and HR-EI-MS were recorded on Finnigan TSQ-700 and JEOL SX-102A spectrometers, respectively. FAB-MS and HR-FAB-MS were recorded on a Finnigan/Thermo Quest MAT 95XL spectrometer. TLC was performed using silica gel 60 F₂₅₄ plates (Merck). Diaion HP-20 (Mitsubishi) and silica gel (230–400 mesh ASTM, Merck) were used for column chromatography. HPLC was performed on a Hitachi L-7000 chromatograph with a LiChrosorb Si 60 column (7 μm , 250 \times 10 mm, Merck).

Plant material: The fruits of *Momordica charantia* var. *abbreviata* were collected in Pingtung County, Taiwan, in July 2008. The plant material was identified by Prof. Sheng-Zehn Yang, Curator of Herbarium, National Pingtung University of Science and Technology. A voucher specimen (no. BT205) was deposited in the Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan.

Extraction and isolation: The fresh fruits of *Momordica charantia* var. *abbreviata* (3.5 kg) were dried in a hot air circulating oven at 50°C and then were extracted 5 times with methanol (10 L) at room temperature (7 days each). The MeOH extract was evaporated *in vacuo* to afford a black residue, which was suspended in H₂O (1 L), and then partitioned sequentially using EtOAc and *n*-BuOH (1L \times 5). The EtOAc fraction (181 g) was chromatographed on silica gel (7 \times 45 cm) using *n*-hexane and EtOAc of increasing polarity as

eluent to obtain 12 fractions. Fr. 3 (7.6 g) from *n*-hexane/EtOAc (30/1) elution was further chromatographed on a silica gel column (3×45 cm) eluted with CH₂Cl₂/EtOAc (400/1) to obtain 7 frs (each 400 mL), fr. 3A–3G. HPLC of fr. 3B (0.8 g) on a Merck LiChrosorb Si 60 column with *n*-hexane/acetone (10/1) as eluent, 2 mL/min, yielded **4** (3.1 mg, *t_R* = 25.1 min). HPLC of fr. 3C (0.5 g) on a Merck LiChrosorb Si 60 column with *n*-hexane/acetone (9/1) as eluent, 2 mL/min, yielded **3** (5.2 mg, *t_R* = 27.2 min). Fr. 4 (12.3 g) from *n*-hexane/EtOAc (19/1) elution was further chromatographed on a silica gel column (3×45 cm) eluted with CH₂Cl₂/EtOAc (200/1) to obtain 8 frs (each 300 mL), fr. 4A–4H. HPLC of fr. 4E (0.6 g) on a Merck LiChrosorb Si 60 column with *n*-hexane/acetone (15/1) as eluent, 2 mL/min, yielded **2** (6.0 mg, *t_R* = 28.1 min). Fr. 9 (10.5 g) from *n*-hexane/EtOAc (1/1) elution was further chromatographed on a silica gel column (3×45 cm) eluted with CH₂Cl₂/MeOH (100/1) to obtain 7 frs (each 400 mL), fr. 9A–9G. HPLC of fr. 9C (1.1 g) on a Merck LiChrosorb Si 60 column with *n*-hexane/acetone (10/1) as eluent, 2 mL/min, yielded **1** (7.6 mg, *t_R* = 21.4 min).

27-nor-3β-Hydroxy-7β-methoxycucurbita-5,23(E)-dien-25-one (1)

Amorphous white powder.

$[\alpha]_{\text{D}}^{25}$: +80.1 (*c* 0.30, CHCl₃).

IR (KBr) ν_{max} : 3510, 3027, 2960, 2926, 2872, 2814, 1737, 1671, 1650, 1460, 1411, 1372, 1153, 1114, 1080, 1046, 973, 944, 925 cm⁻¹.

UV λ_{max} (MeOH) nm (log ϵ): 233 (3.9).

¹H and ¹³C NMR: Table 1.

EI-MS *m/z* 456 [M]⁺ (56), 438 (83), 423 (80), 391 (40), 223 (78), 203 (45), 173 (39), 164 (50), 149 (100), 133 (98), 121 (52), 105 (41), 95 (40), 81 (17), 55 (37), 43 (100).

HR-EI-MS: *m/z* 456.3604 (calcd for C₃₀H₄₈O₃ 456.3605, [M]⁺).

27-nor-3β-Hydroxy-5β,19-epoxycucurbita-6,23(E)-dien-25-one (2)

Amorphous white powder.

$[\alpha]_{\text{D}}^{25}$: -54.4 (*c* 0.24, CHCl₃).

IR (KBr) ν_{max} : 3481, 3032, 2940, 2876, 1727, 1698, 1670, 1652, 1626, 1465, 1436, 1416, 1377, 1250, 1080, 977, 944, 910, 734 cm⁻¹.

UV λ_{max} (MeOH) nm (log ϵ): 231 (3.7).

¹H and ¹³C NMR: Table 1.

FAB-MS *m/z* (rel. int.): 441 [M+H]⁺ (10), 393 (8), 323 (3), 307 (19), 289 (15), 235 (9), 219 (10), 171 (18), 153 (100), 137 (95), 106 (68), 89 (73), 77 (72), 63 (36), 51 (40).

HR-FAB-MS: *m/z* 441.3359 (calcd for C₂₉H₄₅O₃ 441.3371, [M+H]⁺).

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