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Phytochemical Constituents of Bitter Melon (*Momordica charantia*)

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Abstract – Phytochemical constituents were isolated from bitter melon (the fruits of *Momordica charantia*) through open column chromatography. Their structures were identified as β-sitosterol (**1**), (23*E*)-5β,19-epoxycucurbita-6,23-diene-3β,25-diol (**2**), daucosterol (**3**), uracil (**4**), and allantoin (**5**) by interpretation of spectroscopic analysis including MS and ^{1}H- & ^{13}C-NMR. Among them, allantoin (**5**) was isolated from this plant for the first time.

Keywords – *Momordica charantia*, Bitter melon, Column chromatography, Allantoin

Introduction

Momordica charantia, belonging to the family of Cucurbitaceae, is an indigenous medicinal and vegetable plant found in the tropical and subtropical regions of the world and is commonly known as bitter gourd or bitter melon (Lee *et al*., 2009). It is distributed in Asian countries and widely cultivated as a vegetable crop. The fruits, vines, leaves, and roots of *M. charantia* have been used to treat toothache, diarrhea, furuncle, and diabetes in China. All parts of the plant including the fruit taste bitter. The fruit looks usually rectangle and resembles a cucumber (Basch *et al*., 2003; Krawinkel and Keding, 2006). Bitter melon contains biologically active chemicals such as essential oil, flavonoids, phenolic acids, glycosides, triterpenes, and alkaloids. The immature fruits are a good source of vitamin C and also provide vitamin A (Xie *et al*., 1998; Braca *et al*., 2008; Chen *et al*., 2008; Parichat and Artiwan, 2009; Zhang *et al*., 2009; Choi *et al*., 2012).

A number of studies have reported the effects of bitter melon unrelated to diabetes. Bitter melon has some interesting biological and pharmacological activities, e.g. anti-cancer, anti-viral, anti-bacterial, analgesic, anti-inflammatory, hypotensive, anti-fertility, and anti-oxidant (Zafar and Neerja, 1991; Ng *et al*., 1992; Scartezzini and Speroni, 2000; Grover and Yadav, 2004; Beloin *et al*., 2005; Sin *et al*., 2011; Cho *et al*., 2012a; Choi *et al*., 2012; Mahmood *et al*., 2012; Sin *et al*., 2012). Bitter melon has been used for the treatment of diabetes mellitus due to their effective constituents such as charantin and peptides which are similar to insulin and several alkaloids (Lee *et al*., 2009). This paper describes the procedure for the isolation of phytochemical constituents from bitter melon by repeated column chromatography, and structure determination by spectral analyses such as NMR and MS.

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Experimental

Plant materials – Bitter melon (*Momodica charantia* fruits) was grown and collected at the Experimental Field of Farming Cooperation Hamyang (Hamyang, Korea) in October, 2009.

General experimental procedures – Mass spectrometry (MS) was measured using a Jeol JMS-600W (Tokyo, Japan) mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 300, 400, or 500 NMR (Germany) spectrometer in $CDCl_3$, pyridine, or DMSO using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in Hertz. TLC analysis was conducted with Kiesel gel 60 F254 (Art. 5715, Merck Co., Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 10% H_2SO_4 followed by charring at 60°C. Open column chromatography was conducted with a silica gel (200 - 400 mesh ASTM; Merck Co., Germany). All other chemicals and reagents were analytical grade.

Extraction and isolation – The dried and finely powdered bitter melon (1,150.1 g) was extracted with methanol (MeOH) for 3 hr (6 L × 5) under reflux at 65 - 75°C and solvent was evaporated *in vacuo* to give brown residue (351.4 g). The residue was suspended in H_2O and partitioned with *n*-hexane (12.4 g), CH_2Cl_2 (MC) (11.2 g), ethyl acetate (EtOAc) (5.6 g), and *n*-butanol (*n*-BuOH) (10.3 g), successively. A portion of the *n*-hexane fraction (10.1 g) was chromatographed on a Si gel (6 × 80 cm, No. 7734) column, packed in *n*-hexane, eluting with a step gradient of *n*-hexane/EtOAc followed by EtOAc, all fractions being monitored by TLC. Elution of the Si gel column with *n*-hexane/EtOAc (9 : 1) and *n*-hexane/EtOAc (8 : 2) afforded compounds **1** and **2**, respectively. A portion of the MC fraction (9.5 g) was chromatographed on a Si gel (6 × 80 cm, No. 7734) column, packed in *n*-hexane, eluting with a step gradient of *n*-hexane/EtOAc followed by EtOAc, all fractions being monitored by TLC. Elution of the Si gel column with EtOAc afforded compound **3**. A portion of the *n*-BuOH fraction (9.5 g) was chromatographed on a Si gel (6 × 80 cm, No. 7734) column, packed in CH_2Cl_2, eluting with a step gradient of MC/MeOH followed by MeOH, all fractions being monitored by TLC. Elution of the Si gel column with MC/MeOH (1 : 9) and MC/MeOH (2 : 8) afforded compounds **4** and **5**, respectively.

Compound 1 – White powder; EI-MS *m/z*: 414 $[M]^+$ (100.0), 396 (53.1), 381 (25.8), 329 (28.3), 303 (31.0), 289 (11.8), 273 (20.8), 255 (26.3), 231 (15.8), 213 (23.2), 159 (17.0), 145 (21.4); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.52 (1H, m, H-3), 2.27 (2H, m, H-4), 5.35 (1H, m, H-6), 1.99 (2H, m, H-11), 0.68 (3H, s, H-18), 1.01 (3H, s, H-19), 0.96 (3H, d, *J* = 6.3 Hz, H-21), 0.83 (3H, d, *J* = 6.3 Hz, H-26), 0.80 (3H, d, *J* = 3.3 Hz, H-27), 0.91(3H, t, *J* = 6.3 Hz, H-29); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 37.4 (C-1), 29.8 (C-2), 72.0 (C-3), 39.9 (C-4), 141.1 (C-5), 122.2 (C-6), 32.0 (C-7), 31.8 (C-8), 50.3 (C-9), 36.6 (C-10), 21.2 (C-11), 40.7 (C-12), 42.4 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.2 (C-17), 11.9 (C-18), 19.1 (C-19), 36.3 (C-20), 18,9 (C-21), 34.1 (C-22), 26.2 (C-23), 46.0 (C-24), 29.3 (C-25), 19.9 (C-26), 19.5 (C-27), 23.2 (C-28), 12.1 (C-29).

Compound 2 – Amorphous white powder; EI-MS *m/z*: 456 $[M]^+$ (8), 438 (45), 390 (80), 309 (100), 281 (65); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.45 (1H, m, H-1), 1.83 m (1H, m, H-2), 3.40 (1H, m, H-3), 6.04 (1H, dd, *J* = 2.0, 10.0 Hz, H-6), 5.63 (1H, dd, *J* = 10.0, 3.5 Hz, H-7), 2.34 (1H, br s, H-8), 2.27 (1H, dd, *J* = 2.8 Hz, H-10), 1.46, 1.80 (1H, m, H-11), 1.64 (1H, m, H-12), 1.35 (1H, m, H-15), 1.42, 2.00 (1H, m, H-16), 1.48 (1H, m, H-17), 0.86 (3H, s, H-18), 3.67 (1H, d, *J* = 8.5 Hz, H-19), 3.52 (1H, d, *J* = 8.5 Hz, H-19), 1.45 (1H, m, H-20), 0.89 (3H, s, *J* = 6.5 Hz, H-21), 1.80, 2.14 (1H, m, H-22), 5.58 (1H, m, H-23) 5.58 (1H, m, H-24), 1.31 (3H, s, H-26), 1.31 (3H, s, H-27), 1.20 (3H, s, H-28), 0.89 (3H, s, H-29), 0.86 (3H, s, H-30), 4.01 (1H, d, *J* = 10.0 Hz, OH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 17.8 (C-1), 27.6 (C-2), 76.4 (C-3), 37.4 (C-4), 87.8 (C-5), 132.0 (C-6), 131.7 (C-7), 52.3 (C-8), 45.7 (C-9), 39.1 (C-10), 23.8 (C-11), 31.0 (C-12), 45.5 (C-13), 48.8 (C-14), 33.4 (C-15), 28.2 (C-16), 50.3 (C-17), 15.1 (C-18), 80.1 (C-19), 36.4 (C-20), 18.8 (C-21), 39.3 (C-22), 125.4 (C-23), 139.9 (C-24), 70.9 (C-25), 30.1 (C-26), 30.2 (C-27), 20.7 (C-28), 24.8 (C-29), 20.3 (C-30).

Compound 3 – Amorphous white powder; FAB-MS *m/z*: 599 $[M+Na]^+$; ^{1}H-NMR (400 MHz, pyridine): δ 4.03 (1H, m, H-3), 5.38 (1H, br d, *J* = 4.8 Hz, H-6), 0.69 (3H, s, H-18), 1.03 (3H, s, H-19), 0.92 (3H, d, *J* = 0.68 Hz, H-21), 0.86 (3H, d, *J* = 6.3 Hz, H-26), 0.89 (3H, d, *J* = 6.6 Hz, H-27), 0.88 (3H, t, *J* = 7.8Hz, H-29), 4.91 (1H, d, *J*=7.2 Hz, H-1'), 4.12 (1H, t, *J*=8.7 Hz, H-2'), 4.33 (1H, *J*=8.7 Hz, H-3'), 4.36 (1H, t, *J*=8.4 Hz, H-4'), 4.48 (1H, dd, *J* = 5.7, 11.7 Hz, H-6'a), 4.84 (1H, dd, *J*=2.4, 11.7 Hz, H-6'b); ^{13}C-NMR (100 MHz, pyridine): δ 37.4 (C-1), 30.8 (C-2), 72.2 (C-3), 40.5 (C-4), 141.4 (C-5), 123.5 (C-6), 32.6 (C-7), 32.7 (C-8), 50.8 (C-9), 37.98 (C-10), 21.8 (C-11), 39.8 (C-12), 43.0 (C-13), 25.0 (C-15), 57.3 (C-14), 29.0 (C-16), 56.7 (C-17), 12.5 (C-18), 19.9 (C-19), 36.3 (C-20), 19.4 (C-21), 34.5 (C-22), 27.3 (C-23), 50.3 (C-24), 30.5 (C-25), 18.3 (C-26), 20.5 (C-27), 23.4 (C-28), 12.8 (C-29), 103.1 (C-1'), 78.6 (C-2'), 79.1 (C-3'), 75.9 (C-4'), 79.0 (C-5'), 63.3 (C-6').

Compound 4 – Amorphous white powder; EI-MS *m/z*: 112 $[M]^+$ (100.0), 69 (47); ^{1}H-NMR (500 MHz, pyridine): δ 5.80 (1H, d, *J* = 7.5 Hz, H-5), 7.51 (1H, d, *J* = 7.5 Hz, H-6).

Compound 5 – White crystal; EI-MS *m/z*: 158 $[M]^+$ (9.8), 141 (10.9), 130 (100.0), 115 (31.4), 87 (80.8), 70 (10.8) 60 (22.4); ^{1}H-NMR (500 MHz, DMSO): δ 10.53 (1H, s, H-1), 8.04 (1H, s, H-3), 6.89 (1H, d, *J* = 8.2 Hz, N<u>H</u>CONH$_2$), 5.77 (2H, s, NHCON<u>H</u>$_2$), 5.24 (1H, d, *J* = 8.2 Hz, H-4).

Results and Discussion

An open column chromatography of the MeOH extract of bitter melon led to the isolation of compounds **1** - **5**.

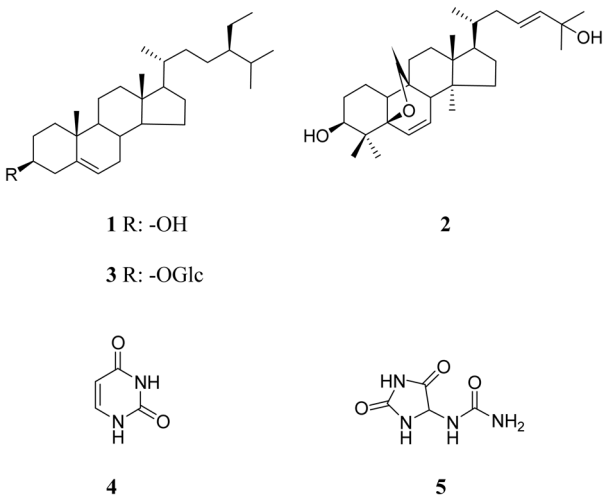
Compound **1** was obtained as white powders from the *n*-hexane fraction and it showed a molecular ion peak at *m/z* 414 $[M]^+$ in the EI-MS. The ^{1}H-NMR spectrum of **1** showed existence of sterol skeleton. The two angular methyl singlets of 18- and 19-Me at δ 0.68 and 1.01, and

the three doublets of 21-, 26-, and 27-Me at δ 0.96, 0.83, and 0.80, and the one triplet of 29-Me at δ 0.91 were observed. The olefinic proton broad doublet one signal at δ 5.35 was showed H-6. The ^{13}C-NMR spectrum of **1** showed 27 resonances, and C-5 and -6 signals were noticed at δ 141.1 and 122.2, respectively. Accordingly, the structure of **1** was elucidated as β-sitosterol (stigmast-5-en-3-ol) by comparison of the spectral data in the literature (Rubinstein *et al*., 1976; Lee *et al*., 2013).

Compound **2** was obtained as amorphous white powder from the *n*-hexane fraction and it showed a molecular ion peak at *m/z* 456 $[M]^+$ in the EI-MS. The ^{1}H- and ^{13}C-NMR data indicated the presence of six tertiary methyl signals at δ 0.89, 1.20 (3H each, s), 0.86 (3H × 2), and 1.31 (3H × 2), a secondary methyl signal at δ 0.89 (3H, d, J = 6.5 Hz), an oxomethylene signal at δ 3.53 (1H, d, J = 8.5 Hz), 3.67 (1H, d, J = 8.5 Hz), and δ 80.1, and a mutiplet oxymethine (δ 3.40) coupling to hydroxyl group (δ 4.01). In addition, the NMR signals for an allylic ABX system of *cis*-oriented cyclohexene signal at δ 6.04 (1H, dd, J = 2.0, 10.0 Hz, H-6), 5.63 (1H, dd, J = 10.0, 3.5 Hz, H-7), 2.34 (1H, br s, H-8); δ 132.0, 131.7, 52.3 were also found. Accordingly, the structure of **2** was elucidated as (23*E*)-5β,19-epoxycucurbita-6,23-diene-3β,25-diol by comparison of the spectral data in the literature (Chang *et al*., 2006).

Compound **3** was obtained as white powders from the MC fraction and it showed a molecular ion peak at *m/z* 599 $[M + Na]^+$ in the FAB-MS. The ^{1}H-NMR spectrum showed the two angular methyl singlets of 18- and 19-Me at δ 0.69 and 1.03, and the three doublets of 21-, 26-, and 27-Me at δ 0.92, 0.86, and 0.89, respectively. The olefinic proton of a broad doublet signal at δ 5.38 showed H-6. The anomeric proton of **3** produced a peak at δ 5.07 (d, J = 7.2 Hz), and the glucose position was at C-3 (β-linkage) of the aglycon. The ^{13}C-NMR spectrum of **3** showed C-5 and -6 signals noticed at δ 141.1 and 123.5, respectively. Accordingly, the structure of **3** was daucosterol (β-sitosterol 3-*O*-glucoside) by comparison of the spectral data in the literature (Huh *et al*., 2011; Cho *et al*., 2012b, Lee *et al*., 2013).

Compound **4** was obtained as white crystals from the *n*-BuOH fraction and showed a molecular ion peak at *m/z* 112 $[M]^+$ in the EI-MS, which corresponds to a molecular formula of $C_4H_4N_2O_2$. In the ^{1}H-NMR spectrum of **4**, doublets at δ 7.51 (J = 7.5 Hz) and 5.80 (J = 7.5 Hz) assigned H-6 and -5 of pyrimidine, respectively. Accordingly, the structure of **4** was elucidated as uracil by comparison of the spectral data in the literature (Ko *et al*., 1992; Lee *et al*., 2002).



1 R: -OH **2**

3 R: -OGlc

4 **5**

Fig. 1. Structures of compounds **1** - **5**.

Compound **5** was obtained as white crystals from the *n*-BuOH fraction. In the EI-MS, molecular peak showed at *m/z* 158 $[M]^+$ corresponding to the molecular formula $C_4H_6N_4O_3$. In the ^{1}H-NMR spectrum, it was obtained relating the peak height of the down-field proton at ä 10.53. The broad amino signal at δ 5.77 was detected and a sharp singlet at δ 5.23 was presumably -NHCHNH-. Accordingly, the structure of **5** was elucidated as allantoin (2,5-dioxo-4-imdazolidinylurea) by comparison of the spectral data in the literature (Siegfried and Johannes, 1975; Kim *et al*., 2009).

β-Sitosterol (**1**) and daucosterol (**3**) showed anti-inflammatory, anti-neoplastic, and immune modulating activities (Patrick and Lamprecht, 1999). Allantoin (**5**) has the effect of urease inhibitory activity and prevents inflammation and ulcers in human (Weber *et al*., 1981; Leem *et al*., 2005; Fu *et al*., 2006).

In conclusion, β-sitosterol (**1**), (23*E*)-5β,19-epoxy-cucurbita-6,23-diene-3β,25-diol (**2**), daucosterol (**3**), uracil (**4**), and allantoin (**5**) were isolated from bitter melon (Fig. 1). Among them, allantoin (**5**) was isolated from this plant for the first time.

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