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NEW INDOLES FROM THE ROOTS OF *Brassica rapa* ssp. *campestris*

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*Two new indoles, 2-*C*-β-D-glucopyranosyl-1-methoxyindole-3-acetonitrile (**1**) and 6-hydroxy-1-methylindole-3-acetonitrile (**2**), along with two known indoles [caulilexin C (**3**) and arvelexin (**4**)], were isolated from turnip roots (*Brassica rapa *ssp.* campestris *L.). The structures of the compounds were established on the basis of NMR, FAB-MS, and IR spectroscopic data.*

Keywords: *Brassica rapa* ssp. *campestris* L., indole compounds, 2-*C*-*β*-D-glucopyranosyl-1-methoxyindole-3-acetonitrile, 6-hydroxy-1-methylindole-3-acetonitrile.

Brassica rapa ssp. *campestris* L. (Brassicaceae) is a conical, deep purple, edible root vegetable commonly called turnip. The consumption of *B. rapa* has been associated with reductions in the risks of certain chronic diseases, including cardiovascular problems and cancers [1, 2]. This association is often attributed to phytochemicals such as glucosinolates [3, 4], which induce a variety of physiological functions such as antioxidant activity, enzyme regulation, and control of apoptosis and the cell cycle. Indole glucosinolate metabolites are the major autolytic breakdown products of glucosinolates. High-performance liquid chromatography (HPLC) with fluorescence detection is an efficient method of identifying the indole compounds in plants [5]. This research was carried out by us to search for new glucosinolates of pharmaceutical value. In this paper, we describe the procedures for the isolation and structure determination of two new indole compounds, along with two known compounds.

Compound **1** (2-*C*-*β*-D-glucopyranosyl-1-methoxyindole-3-acetonitrile), brown powder (MeOH), negative FAB-MS *m/z* 347 $[M - H]^-$, HR-FAB-MS *m/z* 347.1243 $[M - H]^-$, indicating the molecular formula to be $C_{17}H_{19}N_2O_6$ (calcd 347.1244 for $C_{17}H_{19}N_2O_6$). IR spectra (KBr, ν, cm^{-1}) showed absorption bands from a nitrile group (2249 cm^{-1}) and an aromatic ring (1654 cm^{-1}). In the PMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz), four olefine methine proton signals at 7.68 (1H, d, J = 8.4, H-4), 7.47 (1H, d, J = 8.4, H-7), 7.32 (1H, dd, J = 8.4, 7.2, H-6), and 7.17 (1H, dd, J = 8.4, 7.2, H-5) due to a 1,2-disubstituted benzene ring, a *β*-hemiacetal proton signal at 4.47 (1H, d, J = 9.6, H-1′), and other proton signals at 3.81 (1H, dd, J = 12.4, 2.0, H-6′a), 3.75 (1H, dd, J = 12.4, 5.2, H-6′b), 3.70 (1H, dd, J = 9.2, 7.6, H-3′), 3.32 (1H, dd, J = 9.6, 7.6, H-4′), 3.21 (1H, m, H-5′), and 3.11 (1H, dd, J = 9.6, 9.2, H-2′) were observed. A methoxy proton at 4.17 (3H, s, OCH_3) and a methylene proton at 4.16 (2H, s, H-10) experienced a downfield signal shift due to the nitrogen and the cyano group. The ^{13}C NMR spectrum (100 MHz, CD_3OD, δ, ppm) identified four olefine quaternary carbon signals at δ 134.63 (C-8), 124.23 (C-2), 123.25 (C-9), and 111.31 (C-3); four olefine methine signals at δ 125.46 (C-6), 121.82 (C-5), 119.80 (C-4), and 109.76 (C-7); and one nitrile-carbon signal at δ 119.64 (C-11). An anomer carbon (C-1′) signal was observed at δ 90.12, indicating the presence of a *C*-glycosidic linkage. The sugar was identified as D-glucopyranose by four other oxygenated methine signals at δ_C 82.20 (C-5′), 79.31 (C-3′), 74.26 (C-2′), and 71.05 (C-4′) and an oxygenated methylene signal at δ_C 62.68 (C-6′) [6]. The configuration of the anomer carbon was determined to be *β* based on the coupling constant (J = 9.6 Hz) of the anomer proton signal at δ 4.47 in the PMR spectrum.

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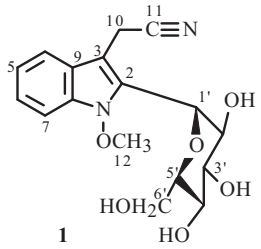

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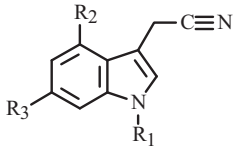

2 - 4

2: $R_1 = CH_3$, $R_2 = H$, $R_3 = OH$
3: $R_1 = OCH_3$, $R_2 = R_3 = H$
4: $R_1 = R_3 = H$, $R_2 = OCH_3$

One methoxy carbon signal at δ_C 66.99 (OCH_3, C-12) and one methylene carbon signal at 14.66 (C-10) were also observed. The chemical shifts due to the aglycon moieties were similar to those of 1-methoxyindole-3-acetonitrile, with the exception of an olefine quaternary carbon (C-2) and its neighboring carbons. In order to confirm the connections of the sugar molecule and the key functional group, a heteronuclear multiple bonding connectivity (gHMBC) experiment was performed. On the gHMBC spectrum, the methylene proton signal at 4.16 (H-10) was correlated with three olefine quaternary carbon signals at δ_C 124.23 (C-2), 111.31 (C-3), and 123.25 (C-9) and an acetylene quaternary carbon signal at 119.64 (C-11), indicating the connection position where an acetonitrile moiety was linked at C-3 of the indole structure. In addition, a cross-peak between the anomer proton signal at 4.47 (H-1′) and the olefine quaternary carbon signal at 124.23 (C-2) revealed the position where the glucopyranosyl unit is linked to C-2 of the aglycon. Therefore, compound **1** was established as 2-*C*-*β*-D-glucopyranosyl-1-methoxyindole-3-acetonitrile, which was named 2-*C*-*β*-D-glucopyranosylbrassicaindole.

Compound **2** (6-hydroxy-1-methylindole-3-acetonitrile), colorless powder (MeOH), negative FAB-MS *m/z* 185 $[M - H]^-$, HR-FAB-MS *m/z* 185.0794 $[M - H]^-$, indicating that the molecular formula was $C_{11}H_9N_2O$ (calcd 185.0795 for $C_{11}H_9N_2O$). IR spectra (KBr, ν, cm^{-1}) showed absorption bands due to hydroxyl (3340 cm^{-1}) and nitrile groups (2250 cm^{-1}), as well as an aromatic ring (1609 cm^{-1}). In the PMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz), three olefine methine proton signals of a 1,2,4-trisubstitution benzene ring at δ 7.15 (1H, d, J = 8.8, H-4), 6.91 (1H, d, J = 2.4, H-7), and 6.75 (1H, dd, J = 8.8, 2.4, H-5), along with one olefine methine signal at δ 7.02 (1H, s, H-2), were observed. In the high magnetic region, a methylene signal at δ 3.78 (2H, s, H-10) and a methyl signal at δ 3.66 (3H, s, H-12) were also identified; these were shifted downfield due to a cyano group and nitrogen, respectively. In the ^{13}C NMR spectrum (100 MHz, CD_3OD, δ, ppm), we observed four olefine methine carbon signals at δ 129.07 (C-2), 113.07 (C-5), 111.12 (C-4), and 103.20 (C-7); one oxygenated olefine quaternary carbon signal at δ 151.86 (C-6); three olefine quaternary carbon signals at δ 133.56 (C-8), 128.54 (C-9), and 103.12 (C-3); one nitrile-carbon signal at δ 119.82 (C-11); a *N*-methyl carbon signal at δ 32.91 (C-12); and a methylene carbon signal at δ 14.16 (C-10). gHMBC was carried out in order to determine the positions of key signals. In the gHMBC spectrum, an olefine proton signal at δ_H 6.91 (H-7) showed cross-peaks with an olefine methine carbon signal at δ_C 113.07 (C-5), an oxygenated olefine quaternary carbon signal at δ_C 151.86 (C-6), and a nitrogenated olefine carbon signal at δ_C 133.56 (C-8). No cross-peak was observed between the olefine proton signal at δ_H 6.75 (H-5) and the nitrogenated olefine quaternary carbon signal at δ_C 133.56 (C-8). Accordingly, the position of the hydroxyl group was revealed to be C-6. In addition, the methylene proton signal at δ_H 3.78 (H-10) was correlated with three olefine quaternary carbon signals at δ_C 129.07 (C-2), 103.12 (C-3), and 128.54 (C-9) and a quaternary carbon signal at δ_C 119.82 (C-11), indicating that the connection occurred between an acetonitrile moiety and C-3 of the indole structure. The structure of compound **2** was determined to be 6-hydroxy-1-methylindole-3-acetonitrile and the compound was named 6-hydroxybrassicaindole. Even though it has been previously reported as a chemical synthetic compound, there are no reported NMR data for 6-hydroxybrassicaindole [7]. This is the first report of the isolation of compound **2** from a natural source and identification with instrumental analysis.

Compounds **3** and **4** were identified as caulilexin C and arvelexin, respectively, by comparisons of the collected spectroscopic data with data for authentic samples and data reported in the literature [8].

EXPERIMENTAL

General Methods. Optical rotation was measured on a JASCO P-1010 digital polarimeter (Jasco, Tokyo, Japan). IR spectra were obtained using a Perkin–Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). FAB-MS was conducted on a JEOL JMSAX-700 (Tokyo, Japan). ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded

on a Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA, USA). SiO_2 (Kieselgel 60, Merck, Darmstadt, Germany) and ODS (LiChroprep RP-18, Merck) resins were used for column chromatography (c.c.), and TLC analysis was carried out using Kieselgel 60 F_{254} and RP-18 F_{254S} (Merck) plates. The compounds were detected using a Spectroline UV lamp model ENF-240 C/F (Spectronics Corporation, NY, USA) and a 10% H_2SO_4 solution.

Plant Material. *Brassica rapa* roots were provided by Ganghwa Agricultural R&D Center (Incheon, Korea) in May 2009 and were positively identified by Dr. Hae-Gon Chung of the Ganghwa Agricultural R&D Center. A voucher specimen (KHU050512) was deposited at the Laboratory of Natural Products Chemistry at Kyung Hee University (Yongin, Korea).

Extraction of *Brassica rapa* Roots and Isolation of Indole Compounds. Fresh *B. rapa* roots (77.1 kg) were chopped and extracted with 95% EtOH (770 L × 2) at room temperature for 24 h. The extracts were filtered through filter paper and concentrated in a rotary vacuum evaporator to yield a dark brown mass (295 g). The extract was suspended in 8 L water and successively partitioned with EtOAc (8 L × 2) and *n*-BuOH (6.4 L × 2), yielding concentrated extracts of EtOAc (BRE, 39 g), *n*-BuOH (BRB, 117 g), and H_2O (139 g) sequentially. The EtOAc fraction (BRE, 39 g) was fractionated into 16 fractions (BRE-1 to BRE-16) using silica gel (Kieselgel 60) c.c. (10 cm × 12 cm), followed by elution with *n*-hexane–EtOAc (15:1→12:1→9:1→7:1, each 15 L) and $CHCl_3$–MeOH (14:1→12:1→9:1→7:1→5:1→3:1→1:1, each 12 L). Each eluant was monitored by TLC. BRE-6 [13.5 g, v_e/v_t (elution volume/total volume) 0.31–0.44] was separated by chromatography on silica gel c.c. (8 cm × 18 cm) and was eluted with *n*-hexane–EtOAc (5:1, 15 L) to obtain 14 subfractions (BRE-6-1 to BRE-6-14). BRE-6-7 (3.2 g, v_e/v_t 0.32–0.38) was further separated on silica gel c.c. (6 cm × 15 cm) and eluted with *n*-hexane–$CHCl_3$–MeOH (45:23:1, 4 L) to obtain six subfractions (BRE-6-7-1 to BRE-6-7-6). BRE-6-7-3 (1.7 g, v_e/v_t 0.58–0.83) was separated by ODS c.c. (5 cm × 7 cm) and eluted with MeOH–H_2O (3:2→3:1, both 1 L) to yield purified compound **3** [BRE-6-7-3-2, 38 mg, v_e/v_t 0.04–0.09; TLC (RP-18 F_{254s}) R_f 0.6, MeOH–H_2O 6:1]. Subfraction BRE-6-9 (583 mg, v_e/v_t 0.43–0.68) was subjected to ODS c.c. (8 cm × 18 cm) and eluted with MeOH–H_2O (2:3→1:1→3:2→3:1, each 0.6 L) to yield purified compound **4** [BRE-6-9-5, 18 mg, v_e/v_t 0.20–0.29; TLC (RP-18 F_{254s}) R_f 0.5, MeOH–H_2O 4:1]. BRE-7 [268 mg, v_e/v_t 0.45–0.51] was separated by ODS c.c. (3 cm × 5 cm) and eluted with MeOH–H_2O (1:1, 0.5 L) to obtain compound **2** [BRE-7-3, 21 mg, v_e/v_t 0.24–0.41; TLC (RP-18 F_{254s}) R_f 0.5, MeOH–H_2O 2:1]. The *n*-BuOH fraction (BRB, 117 g) was fractionated into 14 subfractions (BRB-1 to BRB-14) using Diaion HP-20 c.c. (10 cm × 45 cm) and eluted with H_2O (4 L) → MeOH (2 L) → acetone (4 L) → H_2O (2 L). BRB-6 (6.1 g, v_e/v_t 0.39–0.46) was subjected to silica gel c.c. (6 cm × 15 cm) and eluted with $CHCl_3$–MeOH (6:1 → 2:1, each 8 L) and $CHCl_3$–MeOH–H_2O (6:4:1, 8 L) to yield 19 subfractions (BRB-6-1 to BRB-6-19). Subfraction BRB-6-2 (282 mg, v_e/v_t 0.06–0.08) was further purified by successive chromatography on a Sephadex LH-20 column (8 cm × 18 cm) and eluted with MeOH–H_2O (60% → 80%, each 0.5 L) to yield purified compound **1** [BRB-6-2-5, 12 mg, v_e/v_t 0.10–0.11; TLC (RP-18 F_{254s}) R_f 0.5, MeOH–H_2O 3:2].

2-*C*-β-D-Glucopyranosyl-1-methoxyindole-3-acetonitrile (**1**). Brown powder (MeOH); negative FAB-MS *m/z* 347 $[M - H]^-$; HR-FAB-MS *m/z* 347.1243 (calcd 347.1244 for $C_{17}H_{19}N_2O_6$); $[\alpha]_D^{18}$ –26° (*c* 0.2, MeOH). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 134.63 (C-8), 125.46 (C-6), 124.23 (C-2), 123.25 (C-9), 121.82 (C-5), 119.80 (C-4), 119.64 (C-11), 111.31 (C-3), 109.76 (C-7), 90.12 (C-1′), 82.20 (C-5′), 79.31 (C-3′), 74.26 (C-2′), 71.05 (C-4′), 66.99 (OCH_3, C-12), 62.68 (C-6′), 14.66 (C-10).

6-Hydroxy-1-methylindole-3-acetonitrile (**2**). Colorless powder (MeOH); negative FAB-MS *m/z* 185 $[M - H]^-$; HR-FAB-MS *m/z* 185.0794 (calcd 185.0795 for $C_{11}H_9N_2O$). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 151.86 (C-6), 133.56 (C-8), 129.07 (C-2), 128.54 (C-9), 119.82 (C-11), 113.07 (C-5), 111.12 (C-4), 103.20 (C-7), 103.12 (C-3), 32.91 (C-12), 14.16 (C-10).

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