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Pyrazole alkaloids from watermelon (*Citrullus lanatus*) seeds

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ABSTRACT

Three new compounds, *i.e.* 1-[2-(5-hydroxymethyl-1*H*-pyrrole-2-carbaldehyde-1-yl)ethyl]-1*H*-pyrazole (**1**), 1-({[5-(α-D-galactopyranosyloxy)methyl]-1*H*-pyrrole-2-carbaldehyde-1-yl}-ethyl)-1*H*-pyrazole (**2**), and (4-hydroxyphenyl)methanol 4-[β-D-apiofuranosyl(1→2)-*O*-β-D-glucopyranoside] (**3**), were isolated from an extract of watermelon seeds. Compounds **1** and **2** were pyrazole-alkaloids with a pyrrole ring. This is the first study to show compounds with pyrrole and pyrazole rings in a molecule isolated from natural products. In the evaluation for melanogenesis inhibitory, compound **1** exhibited modest inhibitory activity on melanogenesis without cytotoxicity. Meanwhile compound **2** showed some inhibitory activity accompanied by some cytotoxicity.

1. Introduction

Citrullus lanatus (Thunb.) Matsum. and Nakai (*Citrullus vulgaris* Schrad, Japanese name: suika) is cultivated throughout the world for use as a fruit as well as a medicine. Its fruits have been used as a diuretic, its seed coat has been used to treat hematemesis and melena, and its kernel has been used as an antihypertensive and in the treatment of acute cystisis (Shanghai Scientific and Technical Publishers, Shogakukan, Inc., 1985). Previous studies detected polyphenols (Lako et al., 2006), flavonols (Lako et al., 2006), carotenoids (Lako et al., 2006), amino acids (Shanghai Scientific and Technical Publishers, Shogakukan, Inc., 1985), including citrulline and arginine, and triterpenes (Ripperger and Seifert, 1975) in the fruits of *C. lanatus*. The apoptogenic effects of cucurbitacin L 2-*O*-glucoside on colon adenocarcinoma, and anti-inflammatory activities of cucurbitacin E, both isolated from *C. lanatus*, have been reported previously (Hassan et al., 2011). We recently isolated two new cucurbitane-type triterpenes, 24-hydroperoxycucurbita-5,25-dien-3β-ol and 25-hydroperoxycucurbita-5,23-dien-3β-ol, that had hydroperoxy groups on their side chains from the Et_2O fraction of a *C. lanatus* seed MeOH extract, and examined their cytotoxic activities against the HL-60, P388, and L1210 cell lines, and melanogenesis inhibitory activities in α-MSH-stimulated B16 4A5 cells (Kikuchi et al., 2013a). In the present study, we describe the isolation and structural elucidation of compounds **1**–**3** as well as the inhibitory effects of compounds from watermelon on melanogenesis in B16 4A5 cells.

2. Results and discussion

Three new compounds (**1**–**3**) were isolated from the seeds of *C. lanatus* (Fig. 1).

Compound **1** exhibited an $[M+H]^+$ ion in the HR-FAB-MS at *m*/*z* 220.1089, which was compatible with the molecular formula $C_{11}H_{14}N_3O_2$ (calcd 220.1086). Its IR spectrum showed absorption that indicated a hydroxy group (ν_{max} 3424 cm^{-1}) and carbonyl group (ν_{max} 1649 cm^{-1}). ^1H and ^{13}C NMR spectra indicated the presence of an oxymethylene [δ_H 4.02 (s); δ_C 55.9 (t)], pyrrole ring [δ_H 6.16 (d, J = 4.1 Hz), 7.03 (d, J = 4.1 Hz); δ_C 111.3 (d), 126.8 (d), 133.4 (s), 145.3 (s)], pyrazole ring [δ_H 6.19 (t, J = 2.0 Hz), 7.16 (dd, J = 0.6, 2.0 Hz), 7.49 (dd, J = 0.6, 2.0 Hz); δ_C 106.9 (d), 131.8 (d), 140.9 (d)], and formyl group [δ_H 9.45 (s); δ_C 181.1 (d)]. The following correlations were observed in the HMBC spectrum; H-1′ [δ_H 4.52 (t, J = 5.9 Hz)]/C-5 [δ_C 131.8 (d)]; H-2′ [δ_H 4.71 (t, J = 5.9 Hz)]/C-2″ [δ_C 133.4 (s)] and C-5″ [δ_C 145.3 (s)]; H-3″ [δ_H 7.03 (d, J = 4.1 Hz)]/C-7″ [δ_C 181.1 (d)]; H-6″ [δ_H 4.02 (s)]/C-4″ [δ_C 111.3 (d)] and 5″ [δ_C 145.3 (s)] (Fig. 2). In the ^1H–^1H COSY spectrum, H-3 [δ_H 7.49 (dd, J = 0.6, 2.0 Hz)]/H-4 [δ_H 6.19 (t, J = 2.0 Hz)], H-4/H-5 [δ_H 7.16 (dd, J = 0.6, 2.0 Hz)], and H-3″ [δ_H 7.03 (d, J = 4.1 Hz)]/H-4″ [δ_H 6.16 (d, J = 4.1 Hz)] were observed (Fig. 2). Therefore, **1** was established as 1-[2-(5-hydroxymethyl-1*H*-pyrrole-2-carbaldehyde-1-yl)ethyl]-1*H*-pyrazol (Table 1).

Compound **2** exhibited an $[M+H]^+$ ion in the HR-FAB-MS at *m*/*z* 382.1617, which was compatible with the molecular formula

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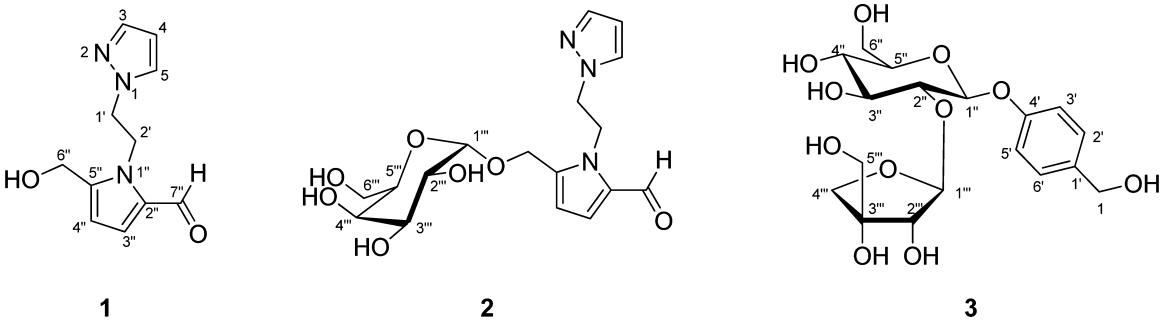

Fig. 1. Structures of compounds **1**–**3** from watermelon seeds.

$C_{17}H_{24}N_3O_7$ (calcd for 382.1609). Its IR spectrum showed absorption that indicated hydroxy groups (3408 cm^{-1}) and a carbonyl group (1656 cm^{-1}). ^1H NMR and ^{13}C NMR spectra suggested compound **2** was a glycoside of **1** since the signals of **2** were similar to those of **1**, except for the sugar moiety. This was confirmed by HMBC and ^1H–^1H COSY spectra (Fig. 2). The ^1H and ^{13}C NMR signals of the sugar moiety [δ_H 4.79 (d); δ_C 62.8 (t), 70.0 (d), 71.1 (d), 71.3 (d), 72.9 (d), 99.5 (d)] suggested the presence of a terminal D-galactopyranose, and its configuration at C-1‴ was established as the α-orientation due to the coupling constant of H-1‴ (J = 4.1 Hz). This was confirmed by acid hydrolysis, derivatization with L-cysteine methyl ester hydrochloride and *o*-tolylisothiocyanate, and HPLC analysis. Therefore, compound **2** was established as 1-({[5-(α-D-galactopyranosyloxy)methyl]-1*H*-pyrrole-2-carbaldehyde-1-yl}-ethyl)-1*H*-pyrazol (Table 1). Compounds **1** and **2** are rare pyrazole-alkaloids. Kumar et al. (2013) isolated L-α-amino-β-(pyrazolyl-*N*)-propanoic acid, a naturally occurring amino acid with a pyrazole ring, from the juice of watermelons. The pyrazole rings of **1** and **2** may be derived from this amino acid. This is the first study to show compounds with pyrrole and pyrazole rings in a molecule isolated from natural products; however, pyrrole–imidazole alkaloids have been isolated from the Mediterranean sponge (Fattorusso and Taglialatela-Scafati, 2000).

Compound **3** exhibited an $[M+Na]^+$ ion in the HR-FAB-MS at m/z 441.1375, which had the molecular formula of $C_{18}H_{26}O_{11}Na$ (calcd 441.1372). Its IR spectrum revealed absorption bands indicating hydroxy groups (ν_{max} 3392 cm^{-1}). The ^{13}C NMR spectrum of **3** displayed δ_C 62.5 (t), 71.4 (d), 78.0 (d), 78.6 (d), 78.7 (d), 101.0 (d) attributed to inner glucose, and δ_C 66.1 (t), 75.5 (t), 78.1 (d), 80.8 (s), 110.8 (d) attributed to terminal apiose. In the ^1H NMR spectrum, the A_2B_2-type aromatic proton signals, [δ_H 7.04 (d, 8.8) and 7.27 (d, 8.8)] and hydroxy methylene signal [δ_H 4.53 (s); δc 64.8 (t)] suggested the presence of a hydroxy methyl phenyl moiety. The following correlations were observed in the HMBC spectrum: H-1 [δ_H 4.53 (s)]/C-1′ [δ_c 136.5 (s)]; H-2′ [δ_H 7.27 (d)]/C-1′; H-3′ [δ_H 7.04 (d)]/C-4′ [δ_c 158.4 (s)]; H-1″ [δ_H 4.95 (d)]/C-4′; H-1‴ [δ_H 5.46 (d)]/C-2″ [δ_c 78.6 (d)], C-3‴; H-5″/C-1″, C-4″; H-6″/C-4″; H-2‴/C-3‴; H-4‴/C-3‴; H-5‴/C-3‴(Fig. 2). On acid hydrolysis, **3** afforded D-glucose and D-apiose, which were identified by derivatization with L-cysteine methyl ester hydrochloride and *o*-tolylisothiocyanate, and HPLC analysis. Thus, compound **3** was established as (4-hydroxyphenyl)methanol 4-[β-D-apiofuranosyl(1→2)-*O*-β-D-glucopyranoside] (Table 1).

Compounds **1**–**3** and the positive control, arbutin were evaluated for there melanogenesis inhibitory effects against α-MSH-induced melanogenesis in B16 4A5 melanomas (Table 2). To determine safe concentrations, the cytotoxicities of three compounds were examined using the MTT assay. Compound **1** did not exhibit cytotoxicity against B16 4A5 cells at 3–100 μM. It also modestly inhibited melanogenesis in B16 4A5 cells (melanin content: 80.3% at 30 μM, 64.1% at 100 μM). This compound may be valuable in the development of skin-whitening agents. Compound **2** exhibited some melanogenesis inhibitory activity (melanin content: 86.0% at 30 μM, 48.6% at 100 μM); however, this was attributed to its cytotoxic effects (cell viability: 92.7% at 30 μM, 70.4% at 100 μM).

3. Experimental

3.1. General

Chemicals and reagents were purchased as follows: fetal bovine serum (FBS) from Invitrogen Co. (Carlsbad, CA, USA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) from Sigma–Aldrich Japan Co. (Tokyo, Japan), Dulbecco's modified Eagle's medium (D-MEM), and antibiotics from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals and reagents were of analytical grade. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1720X FTIR spectrophotometer. The ^1H (600 MHz) and ^{13}C

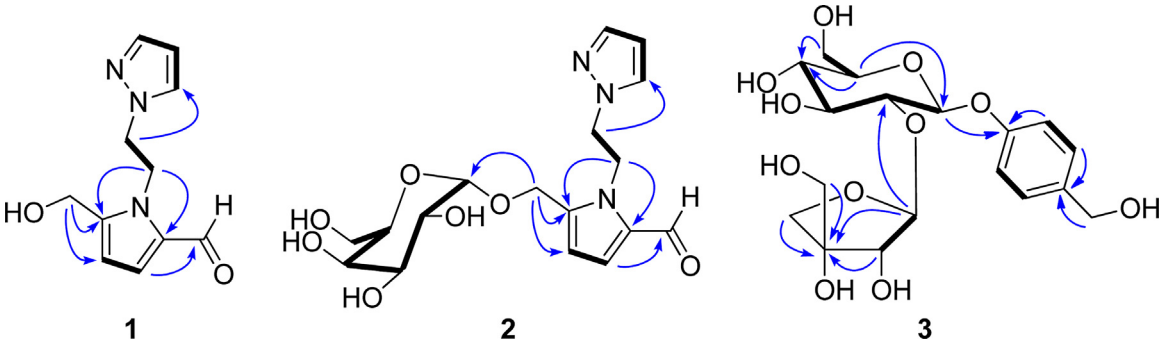

Fig. 2. Key HMBC (⟶) and ^1H–^1H COSY (▬) correlations of compounds **1**–**3**.