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Homoisoflavonoid derivatives from the roots of *Ophiopogon japonicus* and their in vitro anti-inflammation activity

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ABSTRACT

Three new homoisoflavonoids (**1**–**3**) were isolated from the roots of *Ophiopogon japonicus* (Liliaceae). The structures of new metabolites were determined on the basis of spectroscopic analyses including 2D NMR. The anti-inflammatory activities of new compounds (**1**–**3**) were investigated by their effects on the release of the inflammatory chemokine eotaxin, stimulated by IL-4 and the combination of IL-4 and TNF-α in BEAS-2B cells, which mimics the in vivo conditions in bronchial allergic asthma.

Asthma is an allergic inflammatory disease of the airways. The interaction between bronchial epithelial cells and eosinophils is an important feature of an asthma attack. Most evidence suggests that eosinophilic infiltration and activation may account for the unique, spasmodic, and cyclic nature of hyperreactive airways.[1] The mechanisms underlying the selective recruitment of eosinophils are complex, and include multistep processes, probably mediated by the cooperative action between cytokines that cause eosinophil priming and increased survival interleukin [IL]-3, IL-5, granulocytemacrophage colony stimulating factor, and those that activate the endothelium IL-1, tumor necrosis factor (TNF)-α, IL-4, IL-13, and eosinophilselective chemoattractant molecules, especially C-C chemokines.[2] Eotaxin is a C–C chemokine implicated in the recruitment of eosinophils in a variety of inflammatory disorders and, unlike all other eosinophil chemoattractants, is eosinophil-specific.[3] This characterizes eotaxin as a key mediator in allergic diseases of which eosinophilic infiltration is characteristic.[3,4] Cultured bronchial epithelial cells, including normal bronchial epithelium, have been observed to produce eotaxin after stimulation with TNF-α and T helper (Th) type 2 cytokines.[5]

During a screening procedure on higher plants to find novel candidates as anti-inflammatory agents, the 70% EtOH extract of the roots of *Ophiopogon japonicus* Ker-Gawler (Liliaceae) was shown to exhibit considerable inhibitory activity. *O. japonicus* is an evergreen perennial. Its tuber is sweet with a slightly bitter aftertaste and have been employed in traditional Chinese medicine as an expectorant, antitussive, and tonic agent as well as showing pharmacological effects on the cardiovascular system.[6] It is recommended for latent heat in the lungs due to 'yin'-asthenia, fever in consumptive disease or general debility, dehydration of febrile disease, and dry mouth.[7] In the folk medicine of Vietnam it serves as expectorant, anti-cough and tonic agent.[8] Previous phytochemical studies of the tuber derived from *O. japonicus* resulted in the isolation of homoisoflavonoids,[9,10] saponins,[11] and amides,[12] as well as monoterpene glycosides.[10b,13] Homoisoflavonoids have been shown to potential anti-oxidation activities in vitro.[14] Recently, the anti-inflammatory effect of the aqueous extract from radix *O. japonicus* was examined in mouse and rat models, and results demonstrate that the aqueous extract presents remarkable anti-inflammatory activity, ruscogenin and ophiopogonin D are two of its active components, which supported its traditional use in the

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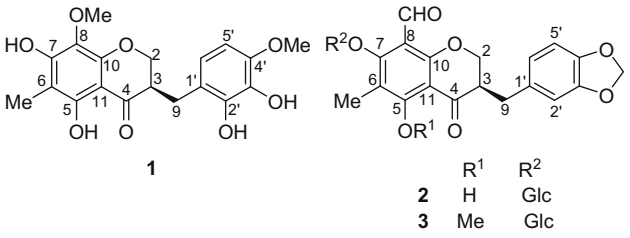

Figure 1. Chemical structures of isolated compounds **1–3**.

treatment of various diseases associated with inflammation.[15] However, no data is available on the influence of the components on eotaxin expression in bronchial epithelial cells, BEAS-2B, in vitro. In our present study, three compounds including a new homoisoflavonoid (**1**), and two new homoisoflavonoid glycosides (**2** and **3**) were isolated from the ethyl acetate fraction of the roots of *O. japonicus*. This paper reports the isolation, structure elucidation of the new isolated metabolites. In addition, the ability to suppress eotaxin expression stimulated by the Th2 cytokine IL-4 alone, and/or in combination with TNF-α in BEAS-2B of these homoisoflavonoids were determined.

Repeated chromatography of the EtOAc-soluble fraction of the 70% EtOH extract of *O. japonicus* on silica gel, YMC gel, Sephadex LH-20, and C_{18} columns led to the isolation of three new compounds (**1**–**3**).[16]

Compound **1** was obtained as colorless needles with the molecular formula $C_{19}H_{20}O_8$, as established by positive HR-FAB-MS with a $[M+Na]^+$ ion at *m*/*z* 399.1058. The IR spectrum indicated the presence of a phenolic hydroxy at 3395 and carbonyl group at 1640 cm^{-1}. In the UV spectrum, λ_{max} value at 280 nm was observed.[17a] The ^1H NMR signals at δ 4.25 (1H, dd, *J* = 7.8, 11.5 Hz, H-2ax), 4.43 (1H, dd, *J* = 5.0, 11.5 Hz, H-2 eq), and 3.14 (1H, m, H-3ax) showed the protons of the γ-dihydropyrone moiety of a homoisoflavanone.[9,10] The ^1H NMR spectrum of **1** also indicated the presence of a methyl group at δ 2.05 (3H, s), and two methoxy groups attached to an aromatic nucleus at δ 3.85 (3H, s) and 3.76 (3H, s). In addition, two benzylmethylene protons appeared at δ 2.84 (1H, dd, *J* = 10.5, 14.5 Hz, H-9a) and 3.89 (1H, dd, *J* = 4.5, 14.5 Hz, H-9b), and a pair of proton signals at δ 6.54 (1H, d, *J* = 8.2 Hz, H-5′) and 6.81 (1H, d, *J* = 8.2 Hz, H-6′) was evidence for two aromatic protons. The ^{13}C NMR and DEPT spectrum of **1** showed 19 carbon signals in the molecule, which supported for the homoisoflavanone structure. The full NMR assignments and connectivities of **1** were determined by the HMQC and HMBC spectroscopic data analysis. The HMBC spectrum confirmed the correlations between methyl protons (δ_H 2.05, s) and carbon signals at δ_C 103.5 and 158.2 indicated for the location of this methyl group at position C-6. The diagnostic long-range correlations were observed for two methoxy protons (δ_H 3.85, s) and (δ_H 3.76, s) to δ_C 128.1, and 159.8, which confirmed the location of these methoxy at C-8, and C-4′, respectively. In addition, the diagnostic long-range correlations were observed for the protons of the γ-dihydropyrone moiety and C-4 (δ 195.8), the benzylmethylene protons and C-3 (δ 46.7), C-4 (δ 195.8), and C-1′ (δ 117.5) (Fig 2). Based on the above analysis, the structure of compound **1** was elucidated as 5,7,2′,3′-tetrahydroxy-6-methyl-8-methoxy-3-(4′-methoxybenzyl)chroman-4-one, a new compound named ophiopogonanone G (Table 1).

Compound **2** was obtained as colorless needles. Its positive HR-FAB-MS showed a $[M+Na]^+$ ion at *m*/*z* 541.1320, which established the molecular formula $C_{25}H_{26}O_{12}$ of **2**. The IR spectrum showed absorption bands at 3440 (OH) and 1618 (C=O) cm^{-1}.[17b] The ^1H NMR spectrum of **2** indicated the presence of a methylenedioxy group at δ 5.95 (2H, s), an aldehyde group at δ 10.05 (1H, s), and a methyl group attached to an aromatic nucleus at δ 2.10. Also in the ^1H NMR spectrum, the proton signals at δ 4.28 (1H, dd, *J* = 8.0, 11.0 Hz, H-2ax), δ 4.45 (1H, dd, *J* = 4.8, 11.0 Hz, H-2 eq), and δ 3.20 (1H, m, H-3ax) were assigned for the γ-dihydropyrone moiety of a homoisoflavanone.[9,10] In addition, two benzylmethylene protons appeared at δ 2.67 (1H, dd, *J* = 10.5, 14.0 Hz, H-9a), and δ 3.82 (1H, dd, *J* = 4.5, 14.0 Hz, H-9b), and three ABX aromatic protons appeared at δ 6.75 (1H, d, *J* = 1.4 Hz, H-2′), 6.72 (1H, d, *J* = 8.0 Hz, H-5′), and 6.82 (1H, dd, *J* = 1.4, 8.0 Hz, H-6′). Furthermore, the ^1H NMR spectrum of **2** showed the presence of signals corresponding to an anomeric proton of a sugar moiety appeared at δ 4.95 (1H, d, *J* = 7.8 Hz, H-1″). The ^{13}C NMR and DEPT spectrum of **2** showed 25 carbon signals in the molecule. Among them, six signals at δ 105.4, 75.5, 77.4, 71.8, 78.3 and 62.9 belonged to a glucose unit, and the other 19 signals appearing belonged to a chroman-4-one skeleton. These data suggested that compound **2** was a monoglucoside of ophiopogonanone C.[9,10] All ^1H and ^{13}C NMR signal assignments of **2** were confirmed by the present study from the HMQC and HMBC spectra (Fig. 2). The sugar was assigned as glucopyranose on the basis of NMR data and the R_f value compared with authentic glucose after enzymatic (naringinase) hydrolysis of **2**.[18] The $J_{H,H}$ value (7.8 Hz) of the anomeric proton (H-1″) indicated that glucose was linked via a β-linkage. In addition, the position of the glucose linkage in **2** was established at the C-6 of the homoisoflavonoid moiety by the HMBC technique (Fig. 2). Thus, the structure of the **2** was established as 5-hydroxy-6-methyl-7-[*O*-β-D-glucopyranoside]- 8-aldehydo-3-(3′,4′-methylenedioxybenzyl)chroman-4-one, a new homoisoflavonoid glycoside named as ophiopogoside A.

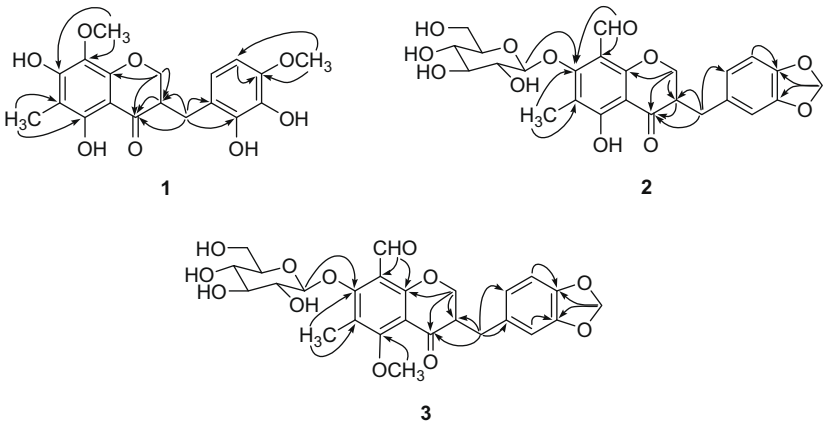

Figure 2. Selected HMBC correlations of **1–3**.