Isolation of amylase regulators from the leaves of *Ixeridium dentatum*

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ABSTRACT

Two new compounds, one sesquiterpene lactone (1) and one phenylethanoid tautomer (2), together with eleven known compounds (3–13) were isolated from the leaves of *Ixeridium dentatum*. Their structures were determined by extensive spectroscopic methods, including 1D-, 2D-NMR, and mass spectrometry. All compounds were evaluated for their amylase secretion activity in human salivary gland cells after treatment in 40 mM of high glucose. All compounds showed increased amylase secretion activity. Moreover, previously undescribed compounds (1–2), luteolin 7-O-β-D-glucopyranoside (10), quercimeritrin (11), and quercetin 3-O-β-D-xylopyranoside (13) exhibited significant amylase activity, which is comparable to the positive control.

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ARTICLE HISTORY

Received 29 November 2018
Accepted 20 March 2019

KEYWORDS

Ixeridium dentatum; sesquiterpene lactone; phenylethanoid; amylase secretion activity

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1599885.

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1. Introduction

Saliva plays an important role in maintaining oral health. Inadequate volumes of saliva cause dry mouth (xerostomia), difficulty swallowing food, and increased susceptibility to opportunistic infections (Amerongen and Veerman 2002). A number of physiological circumstances, including age, reduce salivary secretion. Dry mouth is the most common complaint among the elderly population (Petersen and Yamamoto 2005; Turner and Ship 2007). In our previous studies, we tried to investigate the molecular mechanism as a regulator of amylase secretion in salivary glands using the roots of *Ixeridium dentatum* extract. In addition, we isolated two new compounds together with twelve known sesquiterpene lactone glycosides from the roots of *I. dentatum* (Park et al. 2015). From the isolated sesquiterpene, 8-epiisolipidiol-3-β-D-glucopyranoside and ixerin F were proven to be useful to prevent and treat xerostomia (Lee et al. 2013; Bhattarai et al. 2018).

In our continuing project to develop *I. dentatum* as a supplements for dry mouth, a phytochemical investigation from the leaves of *I. dentatum* was performed and obtained one new sesquiterpene lactone (1) and one new phenylethanoid tautomer (2), along with eleven known compounds (3–13), 11β,13-dihydrolactucin (3) (Kim et al. 2008), cichorioside B (4) (Seto et al. 1988), crepidiaside B (5) (Adegawa et al. 1985), lactuside A (6) (Nishimura et al. 1986), dihydrosyringin and 3-(4-hydroxy-3,5-dimethoxyphenyl)propyl β-D-glucopyranoside (7–8) (Dou et al. 2003), astragalin (9) (Kim et al. 1994), luteolin 7-O-β-D-glucopyranoside (10) (Chiruvella et al. 2007), quercimeritrin (11) (Zheng et al. 2006), isoquercetin (12) (Vvedenskaya et al. 2004), and quercetin 3-O-β-D-xylopyranoside (13) (Kadota et al. 1990) (Figure 1). All the isolated compounds were evaluated for their amylase secretion activity in human salivary gland (HSG) cells after treatment with high glucose to make an in vitro model of xerostomia. This paper mainly deals with the isolation, structural characterisation and evaluation of the isolated compounds as amylase regulators.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder and its molecular formula was determined as C_{21}H_{29}NO_{7}, by the HR-ESI-MS [M–H_{2}O]^{+} ion at m/z 389.1825 (calcd for C_{21}H_{27}NO_{6}, 389.1838). The 1H-NMR spectrum showed three methyl resonances at δ_{H} 0.96, 1.03 and 2.41 (each 3H, s). The 13C-NMR and HSQC spectra of 1 revealed the signals of 22 carbons, including six quaternary, eight methines, four methylenes, and three methyl carbons. The analysis of 1H- and 13C-NMR suggested the structure of 1 was a lactucin derivative with an amino acid moiety. The NMR data of 1 suggested the presence of 11β,13-dihydrolactucin (Kim et al. 2008) as the backbone. The HMBC correlations between H-14 (δ_{H} 2.41) and C-1 (δ_{C} 133.7)/C-9 (δ_{C} 48.2)/C-10 (δ_{C} 149.0) suggested the position of a methyl group at C-10. The presence of hydroxymethylene at C-4 was confirmed by HMBC correlations between H-15 (δ_{H} 4.37 and 4.81) and C-3 (δ_{C} 133.4)/C-4 (δ_{C} 176.1)/C-5 (δ_{C} 49.5). In addition, 13C-NMR chemical shifts of C-1' (δ_{C} 172.3), C-2' (δ_{C} 69.4), C-3' (δ_{C} 37.5), C-4' (δ_{C} 26.5), C-5' (δ_{C} 15.8), and C-6' (δ_{C} 12.2) implied the presence of isoleucine (Röper et al. 1983). The position of isoleucine was verified by the HMBC correlation from H-2' (δ_{H} 3.41) to C-13 (δ_{C} 48.8), indicating that
the isoleucine was located at C-13. The COSY experiment established the spin systems –CH–CH(CH₃)–CH–CH₂–CH₃ of isoleucine in 1 (Figure S1). Many guaianolides have been reported from *I. dentatum*. As a biogenetic derivative, compound 1 is supposed to have an *α*-configuration at H-5. The large coupling constants of H-5/H-6 (*J* = 10.5 Hz) and H-6/H-7 (*J* = 11.0 Hz), suggested *trans*-configurations of H-5 (**α**), H-6 (**β**) and H-7 (**α**). These were further confirmed by CD spectrum with a negative Cotton effect at 264 nm (Figure S2) (Miyase et al. 1985). The configuration of the methylene group at C-11 was determined to be **α**, which was confirmed by the large coupling constant of H-7/H-11 (*J* = 11.1 Hz) (Park et al. 2015). Finally, **α**-configuration of the hydroxyl group at C-8 was defined by ROESY correlations between H-8 (δH 3.75) and H-11 (δH 3.16) (Figure S3). Based on the evidence above, compound 1 was established as isoleucilactucin.

Compound 2, a white amorphous powder, was obtained as a mixture of sugar epimers, which showed two separate peaks in HPLC, but soon after isomerised into a mixture. The structures of the two inseparable compounds were thus elucidated in the form of a mixture. The negative HR-ESI-MS exhibited [M–H]⁻ signal at 447.1317 (calcd for C₂₂H₂₃O₁₀, 447.1297), suggesting a molecular formula of C₂₂H₂₄O₁₀. The **α**- and **β**-anomers of the glucosyl moiety was deduced from the coupling constant (*J* = 3.6 and 7.2 Hz) of the anomeric proton signal at δH 5.22 and 4.67, respectively. In addition, ¹H-NMR spectrum exhibited the signals of two *p*-hydroxyphenylacetyl

Figure 1. Chemical structures of compounds 1–13.
group: $A_2B_2$-type signals at $\delta_H 6.72$ (d, $J = 8.4$ Hz) and $7.00$ (d, $J = 8.4$ Hz) and at $\delta_H 3.19$ and $3.30$ (2H each). The $^{13}$C-NMR and HSQC spectra of 2 showed 22 carbons signals, including six quaternary carbons, thirteen methines, and three methylene carbons. These carbons of the symmetrical benzene ring once again confirmed the presence of two $p$-hydroxyphenylacetyl groups. $^{1}$H- and $^{13}$C-NMR data of 2 were very similar to those of 2,3,4-tri-$($4-hydroxyphenylacetyl$)$glucopyranose except for the disappearance of one $p$-hydroxyphenylacetyl group (Sessa et al. 2000). The positions of $p$-hydroxyphenylacetyl groups at C-2 and C-3 were confirmed by HMBC correlation between H-2 ($\delta_H 4.64/4.72$) and carbonyl carbon ($\delta_C 173.4$); between H-3 ($\delta_H 5.39/5.05$) and carbonyl carbon ($\delta_C 173.2$), respectively (Figure S1). Thus, the structure of 2 was elucidated as 2,3-di-$O$-$($4-hydroxyphenylacetyl$)$glucopyranoside.

The amylase secretion activity was measured after treating the isolated compounds in high glucose-treated HSG cells (Lee et al. 2013; Bhattarai et al. 2018). Our previous studies suggested that high glucose treatment in HSG cells reduce amylase secretion activity, which depends on salivary secretion. Amylase secretion activity was determined by measuring levels of nitrophenol, a chromogenic product hydrolysed by $\alpha$-amylase. It is considered to be a simple, direct kinetic method for determining salivary $\alpha$-amylase. All compounds increased amylase secretion activity and reverted back to low glucose treated cells except compound 3. Moreover, compounds 1, 2, 10, 11, and 13 exhibited the most potent amylase secretion activity that is comparable to the positive control, 8-episoliolipidiol-3-$O$-$\beta$-$D$-glucopyranoside (Lee et al. 2013) (Table S1). These results suggest that those active compounds could play potential roles in the secretion of saliva and can ameliorate oral dryness.

3. Plant material and compounds data

3.1. Plant material

The leaves of I. dentatum were collected in Jeonju-si, Jeonbuk, Korea in May 2017 and authenticated by Dr. Sang-Won Lee, National Institute of Horticultural and Herbal Science, Rural Development Administration, Korea (RDA). A voucher specimen (ID201705) is deposited at the Herbarium of College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon, Korea.

3.2. Compounds data

3.2.1. Isoleucilactucin (1)

White amorphous powder; $\left[\alpha\right]_{D}^{20} = -11.4$ (c 0.02, MeOH); CD (c $= 1 \times 10^{-3}$, MeOH) $\Delta \varepsilon$ (nm) $-3.86$ (262); C$_{21}$H$_{29}$NO$_{7}$, HR-ESI-MS m/z: 389.1825 [M–H$_2$O]$^+$ (calcd for C$_{21}$H$_{27}$NO$_{6}$, 389.1838); $^1$H (methanol-$d_4$, 400 MHz) $\delta$: 6.40 (1H, s, H-3), 4.81 (1H, d, $J = 18.6$ Hz, H-15), 4.37 (1H, d, $J = 18.6$ Hz, H-15), 3.82 (1H, d, $J = 10.5$ Hz, H-6), 3.75 (1H, m, H-8), 3.71 (1H, d, $J = 10.5$ Hz, H-5), 3.66 (1H, overlapped, H-13), 3.41 (1H, d, $J = 3.7$ Hz, H-2’), 3.16 (1H, m, H-11), 3.07 (1H, m, H-13), 2.82 (1H, dd, $J = 11.1$, 13.5 Hz, H-9), 2.51 (1H, d, $J = 11.0$ Hz, H-7), 2.44 (1H, overlapped, H-9), 2.41 (3H, s, H-14), 1.91 (1H, m, H-3’), 1.61 (1H, dq, $J = 7.2$, 12.4 Hz, H-4’), 1.28 (1H, tt, $J = 7.2$, 16.5 Hz, H-4’), 15.8 (3H, d, $J = 6.9$ Hz, H-5’), 0.96 (3H, t, $J = 7.3$ Hz, H-6’) and $^{13}$C NMR (methanol-$d_4$, 100 MHz)
δ: 197.0 (C-2), 176.1 (C-4), 174.5 (C-12), 172.3 (C-1'), 149.0 (C-10), 133.7 (C-1), 133.4 (C-3), 82.2 (C-6), 69.4 (C-2'), 68.8 (C-8), 63.1 (C-15), 60.9 (C-7), 49.5 (C-5), 48.8 (C-13), 48.2 (C-9), 45.2 (C-11), 37.5 (C-3'), 26.5 (C-4'), 21.8 (C-14), 15.8 (C-5'), 12.2 (C-6').

3.2.2. 2,3-Di-O-(4-hydroxyphenylacetyl)glucopyranoside (2)
White amorphous powder; C_{22}H_{24}O_{10}, HR-ESI-MS m/z: 447.1317 [M−H]− (calcd for C_{22}H_{23}O_{10}, 447.1297); 1H (methanol-d_4, 400 MHz) δ: 7.00 (8H, d, J = 8.4 Hz, H_α-3', 5', 3'', 5''/H_β-3', 5', 3', 5''), 6.72 (4H, d, J = 8.4 Hz, H_α-2', 6', 2'', 6''/H_β-2', 6', 2'', 6''), 5.22 (1H, d, J = 3.6 Hz, H_α-1), 5.39 (1H, t, J = 9.8 Hz, H_α-3), 5.05 (1H, t, J = 9.3 Hz, H_β-3), 4.72 (1H, overlapped, H_β-2), 4.68 (1H, d, J = 7.8 Hz, H_β-1), 4.64 (1H, overlapped, H_α-2), 3.84 (1H, overlapped, H_α-5), 3.82 (1H, overlapped, H_β-6), 3.70 (1H, overlapped, H_α-6), 3.65 (1H, overlapped, H_β-6), 3.56 (2H, overlapped, H_α-4/H_β-4), 3.47 (1H, overlapped, H_α-6), 3.37 (1H, overlapped, H_α-5), 3.30 (2H, overlapped, H_α-7''/H_β-7''), 3.19 (2H, d, overlapped, H_α-7'/H_β-7') and 13C NMR (CD_3OD, 100 MHz) δ: 173.4 (C_β-8/C_β-8'), 173.2 (C_α-8/C_β-8'), 157.5 (C_α-4/C_β-4''), 157.4 (C_α-4'/C_β-4'), 131.5 (C_α-3', 5', 3'', 5''/C_β-3', 5', 3', 5''), 126.1 (C_α-1'/C_β-1'), 125.9 (C_α-1'/C_β-1'), 116.2 (C_β-2', 6', 2'', 6''/C_β-2', 6', 2'', 6''), 96.0 (C_β-1), 92.0 (C_α-1), 77.9 (C_β-5), 76.9 (C_β-3), 74.6 (C_β-2), 73.9 (C_α-3), 73.5 (C_α-2), 72.8 (C_α-5), 69.7 (C_α-4/C_β-4), 62.3 (C_β-6), 62.1 (C_α-6), 40.9 (C_α-7''/C_β-7''), 40.6 (C_α-7'/C_β-7').

Acknowledgments
This work was supported by the “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01158104)” Rural Development Administration, Republic of Korea and Basic Research Infrastructure Support Program (University-Centered Labs-2018R1A6A1A03023718) through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning, Republic of Korea.

Disclosure statement
No potential conflict of interest was reported by the authors.

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