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Structure elucidation of secondary metabolites isolated from the leaves of *Ixora undulate* and their inhibitory activity toward advanced glycation end-products formation

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ABSTRACT

Three aromatic glycosides (**1**–**3**), two sulfur and nitrogen-containing compound glucosides (**4**, **5**), and one flavonoid glycoside (**6**) were isolated from the leaves of *Ixora undulata*. Their structures were established by extensive 1D, 2D NMR, and HRESIMS experiments, and structure **4** was further confirmed by single crystal X-ray diffraction analysis. Of the assayed compounds, **7**, **11** and **12** showed strong inhibitory activity toward advanced glycation end-products formation with IC_{50} values of 86.0 μM, 76.6 μM and 98.6 μM, respectively.

1. Introduction

Ixora is a genus in the family Rubiaceae, consisting of tropical evergreens and shrubs. *Ixora* species are native to tropical Asia, where over 400 species exist. People of the region have been using *Ixora* species for generations, not only for ornamental purposes, but more importantly because of their medicinal values. In southern China, one of the most common native species is *Ixora chinensis*, and it has already been reported that its leaves contain iridoid glucosides (Takeda et al., 1975). It is widespread in southeast Asian flower gardens, and is used to treat various ailments like rheumatism and wounds. *Ixora coccinea*, a dense shrub with scarlet flowers, is native to India, where it is widely used in traditional medicine as well. The leaves possess an antiseptic property, and the roots can be used to treat diarrhea and fever (Yasmeen et al., 2010). The present study on the constituents of *Ixora undulata*, collected in Egypt, afforded six new compounds, **1**–**6**, along with seven known compounds, and the structure of **4** including its absolute configuration was also determined by X-ray crystallographic analysis. This paper deals with structural elucidation and evaluation of the inhibitory activity toward advanced glycation end-products (AGEs) formation *in vitro* of these compounds.

2. Results and discussion

The leaves of *I. undulata* were extracted three times with EtOH at room temperature for three days. The EtOH extract (112 g) was subjected to Diaion HP-20 column chromatography to give H_2O- (95 g), MeOH- (13 g), and acetone-eluted fractions (4 g). The MeOH-eluted fraction was subjected to normal- and reversed-phase silica gel column chromatographies, and repetitive HPLC separations to give six new compounds, 1-(*R*)-phenyl ethanol *β*-gentiobioside (**1**), 2-methylphenylmethanol *β*-gentiobioside (**2**), 3,4-dimethylphenol *β*-gentiobioside (**3**), (5*R*,6*R*,*Z*)-5,6-dihydroxy-5,6-dihydro-2*H*-thiopyran-2-one *O*-methyl oxime *β*-D-glucopyranoside (**4**), (5*R*,6*R*,*Z*)-5,6-dihydroxy-5,6-dihydro-2*H*-thiopyran-2-one *O*-methyl oxime *β*-gentiobioside (**5**), and kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1 → 6)-(4″-*trans*-*p*-coumaroyl) *β*-D-galactopyranoside 7-*O*-*α*-L-rhamnopyranoside (**6**), respectively (Chart 1). Seven known compounds were also isolated, corchoionoside C (**7**) (Yoshikawa et al., 1997), icariside B_1 (**8**) (Miyase et al., 1987), 3-methoxy-4-hydroxyphenol 1-*O*-*β*-D-glucopyranoside (**9**) (Ishimura et al.,

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1987), kaempferol 3-*O*-robinobioside (**10**) (Brasseur and Angenot, 1986), quercetin 3-*O*-robinobioside (**11**) (Brasseur and Angenot, 1986), variabiloside E (**12**) (Brasseur and Angenot, 1988), and acteoside (**13**) (Jia et al., 1991) (Chart 2). Their structures were elucidated by extensive inspection of spectroscopic data, including those obtained with ESI-MS, and 1D and 2D NMR spectroscopies, as well as chemical and biochemical methods.

Compound **1** was isolated as an amorphous powder exhibiting negative optical rotation ($[\alpha]_D^{27}$ −58.7 (*c* = 0.54, MeOH)). Its IR spectrum showed absorption bands at 3394, 1605 and 1074 cm^{-1} ascribable to hydroxy, aromatic ring and ether functional groups, respectively. In its UV spectrum, absorption maxima were observed at 257 (log ε 3.87) and 213 (log ε 4.11) nm. The molecular formula, $C_{20}H_{30}O_{11}$, of **1** was determined by high-resolution (HR)-electrospray ionization (ESI)-MS analysis (*m/z* 469.1677 $[M+Na]^+$, calcd 469.1680). Its ^1H NMR (methanol-d_4) spectrum displayed five aromatic proton signals corresponding to a mono-substituted benzene ring [δ 7.22 (1H, m), 7.28 (2H, t, *J* = 7.4 Hz), 7.45 (2H, dd, *J* = 1.4, 7.4 Hz)], methyl protons [δ 1.47 (3H, d, *J* = 6.6 Hz)], two anomeric protons of β-glucopyranosyl moieties [δ 4.10 (1H, d, *J* = 7.4 Hz), 4.46 (1H, d, *J* = 7.8 Hz)], and a methine proton [δ 5.06 (1H, q, *J* = 6.6 Hz)]. Of the 18 ^{13}C NMR resonances, six signals corresponded to aromatic carbons [δc 127.9 (2CH), 128.6 (CH), 129.4 (2CH), 144.1 (C)], a methine carbon bearing an oxygen atom at δc 76.3, and a methyl carbon at δc 24.7 (CH_3). The remaining twelve resonances were attributable to two β-glucopyranosyl moieties (Table 1). Acid hydrolysis of **1** in 1 M HCl liberated D-glucose, which was identified by HPLC using an optical rotation detector together with an authentic sample. Furthermore, enzymatic hydrolysis of **1** with β-glucosidase furnished 1-(*R*)-phenylethanol, which was identified with an authentic sample. The linkages of the two glucosyl moieties and the methyl group were determined by 2D NMR experiments. The HMBC experiment on **1** showed long-range correlations between the methyl protons and C-1 and C-7, H-2 and C-7, H-3 and C-1, H-4 and C-2, H-1′ and C-7, and H-1″ and C-6′. The structure of compound **1** was therefore determined to be 1-(*R*)-phenylethanol β-gentiobioside.

Compound **2** was also obtained as an amorphous powder exhibiting negative optical rotation ($[\alpha]_D^{28}$ −34.7, MeOH). Its molecular formula, $C_{20}H_{30}O_{11}$, was determined by positive-ion HR-ESI-MS measurement (*m/z* 469.1680 $[M+Na]^+$, calcd 469.1680). The ^1H and ^{13}C NMR spectra of **2** were essentially the same as those of **1**, except for the presence of methyl protons, which appeared as a singlet at δ_H 2.73, oxygenated methylene signals at [δ_H 4.64 (1H, d, *J* = 11.6 Hz) and δ_H 4.64 (1H, d, *J* = 11.6 Hz)], and four aromatic protons. These findings indicated that the methyl group of **1** at C-7 was shifted to C-2 in **2**, and this was confirmed by the HMBC correlations of the methyl protons with C-1, 2 and 3, as well as between H_2-7 and C-1, 2 and 6. On the basis of the above mentioned evidence, the structure of **2** was elucidated to be 2-methylphenylmethanol β-gentiobioside.

Compound **3** was also obtained as an amorphous powder exhibiting negative optical rotation ($[\alpha]_D^{28}$ −42.5 in MeOH) with the same molecular formula as that of compounds **1** and **2**. The ^1H NMR spectrum showed three aromatic protons coupled in an ABX system [δ_H 6.95 (1H, dd, *J* = 2.0, 8.0 Hz), δ 6.97 (1H, d, *J* = 2.0 Hz), and δ_H 7.05 (1H, d, *J* = 8.0 Hz)], and two methyl singlets at δ_H 2.24 and δ_H 2.25, together with two anomeric protons at δ_H 4.86 (d, *J* = 7.6 Hz) and δ_H 4.39 (d, *J* = 7.7 Hz) for a β-gentiobiosyl moiety (Table 1). The connectivity of the two methyl groups in **3** was

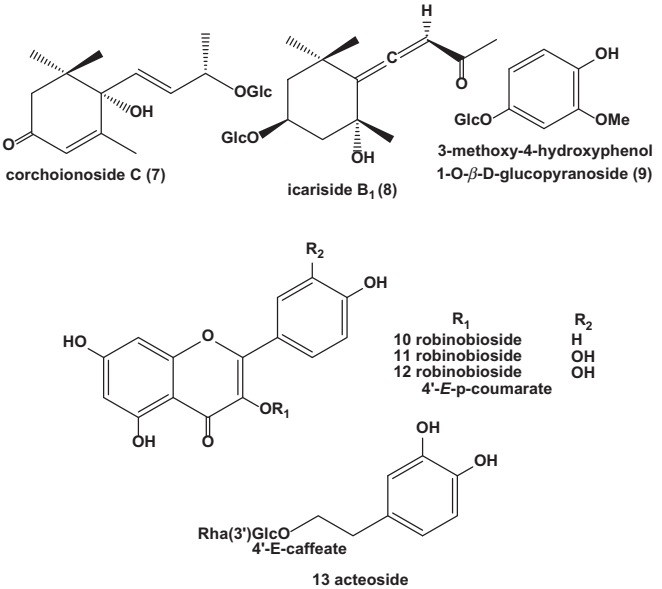

Chart 2. Structures of known compound.

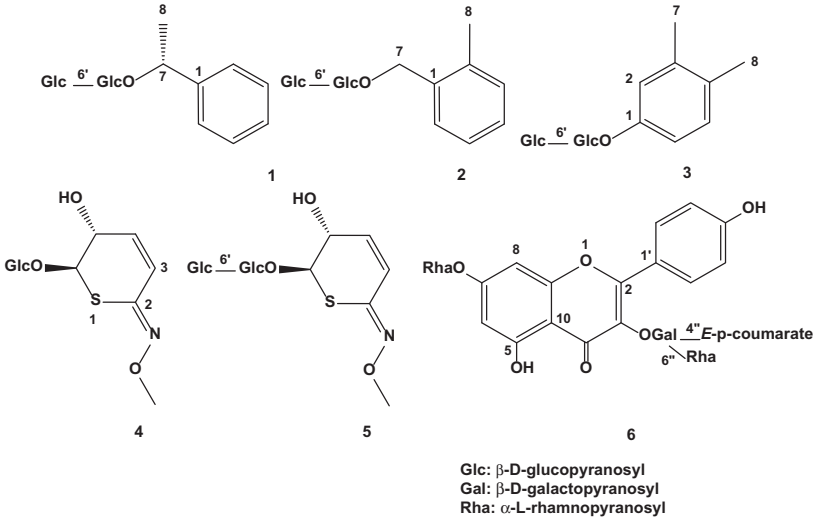

Chart 1. Structures of new compounds.

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