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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

Sachiko Sugimoto [a], Amira Samir Wanas [b,e], Tsutomu Mizuta [c], Katsuyoshi Matsunami [a], Mohamed Salah Kamel [b], Hideaki Otsuka [a,d,*]

[a] Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan
[b] Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia 61519, Egypt
[c] Department of Chemistry, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan
[d] Department of Natural Products Chemistry, Faculty of Pharmacy, Yasuda Women's University, 6-13-1 Yasuhigashi, Asaminami-ku, Hiroshima 731-0153, Japan
[e] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

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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.,

* Corresponding author at: Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan. Tel.: +81 0 8043004387; fax: +81 0 828789540.
E-mail addresses: hotsuka@hirsohima-u.ac.jp, otsuka-h@yasuda-u.ac.jp (H. Otsuka).

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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

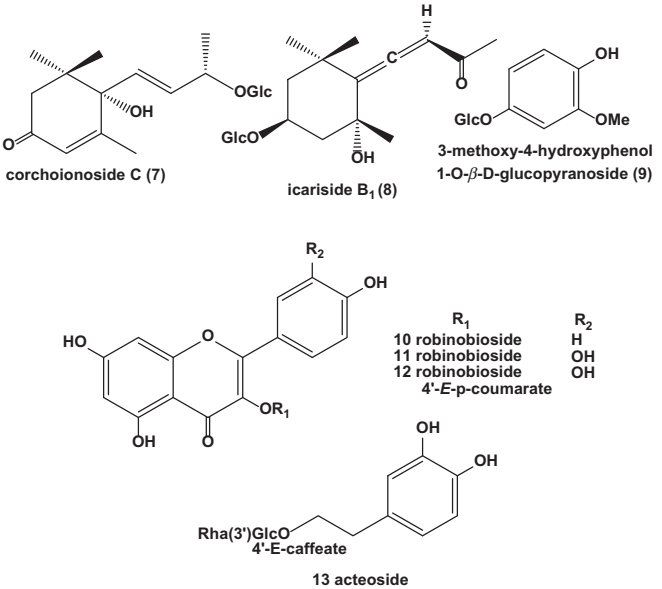

Chart 2. Structures of known compound.

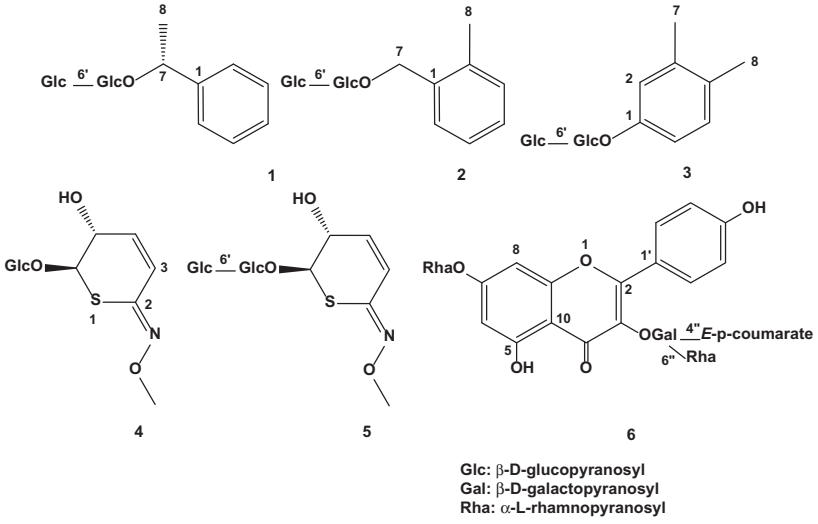

Chart 1. Structures of new compounds.

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Table 1
NMR spectroscopic data for compounds **1**–**3** (150 MHz for ^{13}C, 600 MHz for ^{1}H, methanol-d_4), m: multiplet.

Position	Compound **1**		Compound **2**		Compound **3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	144.1	–	137.1	–	155.3	–
2	127.9	7.45 (1H, dd, *J* = 1.4, 7.4 Hz)	138.1	–	128.6	6.97 (1H, d, *J* = 2.0 Hz)
3	129.4	7.28 (2H, t, *J* = 7.4 Hz)	131.3	7.16 (1H, dd, *J* = 1.6, 7.3 Hz)	128.9	–
4	128.6	7.22 (1H, m)	129.3	7.18 (1H, dd, *J* = 1.6, 7.3 Hz)	132.9	–
5	129.4	7.28 (2H, d, *J* = 7.4 Hz)	127.0	7.13 (1H, dd, *J* = 1.6, 7.3 Hz)	116.6	7.05 (1H, d, *J* = 8.0 Hz)
6	127.9	7.45 (1H, dd, *J* = 1.4, 7.4 Hz)	130.6	7.40 (1H, d, *J* = 7.3 Hz)	132.5	6.95 (1H, d, *J* = 2.0, 8.0 Hz)
7	76.3	5.06 (1H, q, *J* = 6.6 Hz)	70.7	4.64 (1H, d, *J* = 11.6 Hz) 4.95 (1H, d, *J* = 11.6 Hz)	16.8	2.24 (3H, s)
8	24.7	1.47 (3H, d, *J* = 6.6 Hz)	19.4	2.37 (3H, s)	20.9	2.25 (3H, s)
Glc 1′	101.3	4.10 (1H, d, *J* = 7.4 Hz)	103.7	4.35 (1H, d, *J* = 7.9 Hz)	103.1	4.86 (1H, d, *J* = 7.6 Hz)
2′	75.15	3.28 (1H, dd, *J* = 7.4, 8.5 Hz)	75.4	3.24 (1H, m)	75.5	3.23 (1H, dd, *J* = 7.6, 8.0 Hz)
3′	78.09	3.40 (1H, dd, *J* = 8.5, 8.9 Hz)	78.31	3.36 (1H, m)	78.2	3.35 (1H, dd, *J* = 8.0, 8.9 Hz)
4′	71.57	3.37 (1H, dd, *J* = 8.9, 9.0 Hz)	71.9	3.27 (1H, m)	71.7	3.44 (1H, dd, *J* = 8.9, 9.2 Hz)
5′	77.1	3.31 (1H, ddd, *J* = 2.0, 5.5, 9.0 Hz)	77.5	3.46 (1H, ddd, *J* = 2.0, 5.9, 9.0 Hz)	77.6	3.64 (1H, ddd, *J* = 1.9, 6.0, 9.2 Hz)
6′	69.6	3.82 (1H, dd, *J* = 5.5, 11.7 Hz)	70.2	3.81 (1H, dd, *J* = 5.9, 11.6 Hz)	70.0	3.83 (1H, dd, *J* = 5.9, 11.6 Hz)
	–	4.17 (1H, dd, *J* = 2.0, 11.7 Hz)	–	4.18 (1H, dd, *J* = 2.0, 11.6 Hz)	–	4.17 (1H, dd, *J* = 1.9, 11.6 Hz)
Glc 1″	104.9	4.46 (1H, d, *J* = 7.8 Hz)	105.2	4.41 (1H, d, *J* = 7.9 Hz)	104.9	4.39 (1H, d, *J* = 7.7 Hz)
2″	75.21	3.29 (1H, dd, *J* = 7.8, 9.2 Hz)	75.4	3.24 (1H, m)	75.3	3.49 (1H, dd, *J* = 7.7, 8.3 Hz)
3″	78.13	3.32 (1H, m)	78.33	3.34 (1H, m)	78.4	3.48 (1H, dd, *J* = 8.3, 8.6 Hz)
4″	71.64	3.33 (1H, dd, *J* = 8.8, 9.8 Hz)	71.9	3.33 (1H, m)	71.9	3.30 (1H, dd, *J* = 8.6, 9.0 Hz)
5″	77.8	3.25 (1H, ddd, *J* = 1.5, 5.5, 9.8 Hz)	78.4	3.26 (1H, m)	78.2	3.21 (1H, ddd, *J* = 2.3, 5.8, 9.0 Hz)
6″	62.8	3.70 (1H, dd, *J* = 5.5, 11.8 Hz) 3.90 (1H, dd, *J* = 1.5, 11.8 Hz)	63.1	3.67 (1H, dd, *J* = 5.6, 11.9 Hz) 3.88 (1H, dd, *J* = 2.0, 11.9 Hz)	63.0	3.67 (1H, dd, *J* = 5.8, 12.0 Hz) 3.87 (1H, dd, *J* = 2.3, 12.0 Hz)

Table 2
NMR spectroscopicdata for compounds **4** and **5** (150 MHz for ^{13}C, 600 MHz for ^{1}H, a: pyridine-d_5, b: methanol-d_4, c: DMSO-d_6), m: multiplet.

Position	Compound **4**[a]		Compound **5**[b]		Position	Compound **4**[c]	
	^{13}C	^{1}H	^{13}C	^{1}H		^{13}C	^{1}H
1	–	–	–	–	1	–	–
2	148.7	–	149.9	–	2	147.7	–
3	122.9	6.47 (1H, dd, *J* = 1.4, 10.8 Hz)	123.7	6.24 (1H, dd, *J* = 1.8, 10.8 Hz)	3	121.9	6.26 (1H, dd, *J* = 1.5, 10.7 Hz)
4	135.8	6.41 (1H, dd, *J* = 3.3, 10.8 Hz)	135.8	6.13 (1H, dd, *J* = 3.1, 10.8 Hz)	4	135.0	6.18 (1H, dd, *J* = 3.7, 10.7 Hz)
5	68.9	5.05 (1H, ddd, *J* = 1.4, 3.3, 6.8 Hz)	69.6	4.53 (1H, ddd, *J* = 1.8, 3.1, 7.4 Hz)	5	66.5	4.44 (1H, ddd, *J* = 1.5, 3.7, 5.0, 6.5 Hz)
6	84.1	5.74 (1H, d, *J* = 6.8 Hz)	84.1	5.12 (1H, d, *J* = 7.4 Hz)	6	82.5	5.10 (1H, d, *J* = 6.5 Hz)
2-OMe	62.5	3.91 (3H, s)	63.0	3.94 (3H, s)	2-OMe	62.1	3.91 (3H, s)
					5-OH		5.68 (1H, d, *J* = 5.0 Hz)
Glc 1′	105.6	5.32 (1H, d, *J* = 7.9 Hz)	105.1	4.58 (1H, d, *J* = 7.8 Hz)	Glc 1′	103.7	4.49 (1H, d, *J* = 7.8 Hz)
2′	75.4	4.05 (1H, dd, *J* = 8.2, 8.6 Hz)	75.1	3.27 (1H, dd, *J* = 7.8, 8.9 Hz)	2′	73.6	3.04 (1H, ddd, *J* = 4.4, 7.8, 8.6 Hz)
3′	78.4	4.22 (1H, dd, *J* = 8.9, 9.1 Hz)	77.7	3.34–3.42 (1H, m)	3′	76.3	3.21 (1H, ddd, *J* = 4.8, 8.6, 8.9 Hz)
4′	71.2	4.27 (1H, dd, *J* = 9.1, 9.2 Hz)	71.4	3.33 (1H, m)	4′	69.6	3.13 (1H, ddd, *J* = 5.2, 8.9, 9.2 Hz)
5′	79.0	3.95 (1H, ddd, *J* = 2.3, 5.0, 9.2 Hz)	77.8	3.54 (1H, ddd, *J* = 1.9, 6.2, 9.7 Hz)	5′	77.1	3.18 (1H, ddd, *J* = 1.7, 5.5, 9.2 Hz)
6′	62.5	4.38 (1H, dd, *J* = 5.0, 11.9 Hz) 4.51 (1H, dd, *J* = 2.3, 11.9 Hz)	69.7	3.87 (1H, dd, *J* = 6.2, 12.1 Hz) 4.14 (1H, dd, *J* = 1.9, 12.1 Hz)	6′	60.8	3.52 (1H, br dd, *J* = 5.5, 11.5 Hz) 3.71 (1H, ddd, *J* = 1.7, 5.3, 11.5 Hz)
Glc 1″			104.8	4.63 (1H, d, *J* = 7.7 Hz)	2′-OH		5.22 (1H, d, *J* = 4.4 Hz)
2″			75.4	3.22 (1H, dd, *J* = 7.7, 9.2 Hz)	3′-OH		5.06 (1H, d, *J* = 4.8 Hz)
3″			77.8	3.34–3.42 (1H, m)	4′-OH		5.00 (1H, d, *J* = 5.2 Hz)
4″			71.7	3.33 (1H, m)	6′-OH		4.47 (1H, d, *J* = 5.3 Hz)
5″			78.0	3.34–3.42 (1H, m)			
6″			62.8	3.70 (1H, dd, *J* = 5.7, 11.9 Hz) 3.90 (1H, dd, *J* = 2.2, 11.9 Hz)			

clarified by the HMBC experiment, which showed long-range correlations between H_3-7 and C-2, 3, and 4, H_3-8 and C-3, 4 and 5, and H-5 and C-1, 3. Consequently, the structure of **3** was determined to be 3,4-dimethylphenol β-gentiobioside.

Compound **4** was isolated as a major compound with colorless crystals. The IR spectrum of **4** showed absorption bands at 3308, 1648, and 1028 cm^{-1} ascribable to hydroxy, double bond, and ether functional groups, respectively. The molecular formula, $C_{12}H_{19}O_8NS$, of **4** was determined by positive-ion HR-ESI-MS measurement (*m/z* 360.0726 $[M+Na]^+$, calcd 360.0724). In the UV spectrum of **4**, absorption maxima were observed at 308 ($\log\varepsilon$ 1.51) and 249 ($\log\varepsilon$ 1.42) nm. Compound **4** was hydrolyzed with 1 M HCl to afford a sugar moiety, which was identified as D-glucose on HPLC with an optical rotation detector. The ^{13}C NMR (DMSO-d_6) spectrum displayed six signals attributable to one β-glucopyranosyl moiety, and the remaining six signals consisted of those of one methoxy group (δ_C 62.5), two oxygenated methines (δ_C 68.9 and 84.1), one double bond (δ_C 122.9 and 135.8), and one fairly deshielded carbon (δ_C 148.7) (Table 2). Structural elucidation of the partial structural units was performed based on the ^{1}H–^{1}H COSY spectrum. The positions of the substitutions were determined mainly based on HMBC correlations between the methoxy protons and C-2, H-4 and C-2, H-5 and C-3, H-6 and C-2 and C-1′, and OH-5 and C-4, 5 and 6. The lonely and fairly deshielded sp^2 carbon may be connected with a nitrogen atom to form an imine functional group and thus a sulfur atom must be involved in the ring

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system to form a thiopyran ring. To confirm this assumption, crystalline **4** was subjected to X-ray analysis for unambiguous corroboration of the stereochemistry using glucose as a chiral probe. An ORTEP drawing of **4** is shown in Fig. 1, and the absolute stereo chemistries of the two chiral centers were determined to be 5*R* and 6*R*. Finally, the structure of **4** was elucidated to be (5*R*,6*R*,*Z*)-5,6-dihydroxy-5,6-dihydro-2*H*-thiopyran-2-one *O*-methyl oxime 6-*O*-*β*-D-glucopyranoside, as shown in Chart 1.

Compound **5** was obtained as an amorphous powder and its molecular formula was determined to be $C_{18}H_{29}NO_{13}S$ (*m/z* 522.1252 $[M+Na]^+$, calcd 522.1252). The ^1H and ^{13}C NMR spectra (methanol-d_4) of **5** were superimposable on those of compound **4**, except for the sugar moieties. The ^{13}C NMR spectrum indicated the presence of two glucopyranosyl units (δc 105.1, 75.1, 77.7, 71.4, 77.8, 69.7, 104.8, 75.4, 77.8, 71.7, 78.0, 62.8 ppm) (Table 2). The attachment of the terminal glucosyl unit to C-6 of the internal unit was deduced in the HMBC experiment, which showed long-range correlations between the H-1′ and C-6, and H-1″ and C-6′. Consequently, the structure of **5** was determined to be (5*R*,6*R*,*Z*)-5,6-dihydroxy-5,6–2H-thiopyran-2-one *O*-methyl oxime *β*-gentiobioside (Chart 1).

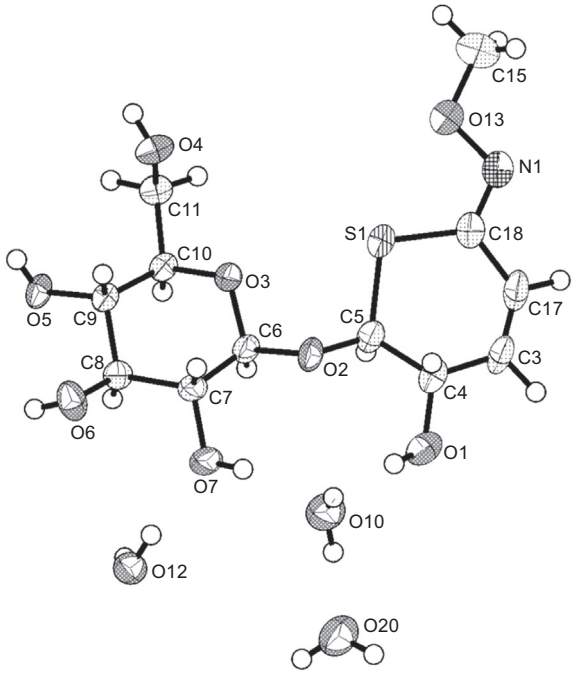

Fig. 1. ORTEP drawing of compound **4**. The crystal structure has crystallographic numbering.

Compound **6** was isolated as a pale yellow powder with negative optical rotation ($[\alpha]_D^{29}$ −125, MeOH). Its elemental composition was determined to be $C_{42}H_{46}O_{21}$. In the UV spectrum of **6**, absorption maxima were observed at 335 (log ε 4.35), 291 (4.44), and 240 (4.47) nm, suggestive of a flavonol structure (Yoshikawa et al., 2008). Acid hydrolysis of **6** with 1 M HCl afforded D-galactose and L-rhamnose. The ^1H NMR and ^{13}C NMR spectra of **6** were similar to those of quercetin 3-*O*-rhamnosyl(1 → 6)(4″-*trans*-*p*-coumaroyl) galactoside 7-*O*-rhamnoside (Schaufelberger et al., 1987). The ^1H NMR spectra of **6** showed two downfield doublets at δ 6.51 (1H, *J* = 2.0 Hz) and 6.78 (1H, *J* = 2.0 Hz) corresponding to H-6 and H-8 of kaempferol, respectively. Moreover, they contained deshielded signals at δ_H 7.51 (2H, d, *J* = 8.6 Hz) and 6.86 (2H, d, *J* = 8.6 Hz) corresponding to H-2‴″ and 6‴″, and H-3‴″ and 5‴″, respectively, of a *p*-substituted benzene ring, and δ_H 6.41 and 7.68 of a *trans* double bond together with δ_C 168.7, indicating the presence of a coumaroyl group. Three anomeric proton signals at δ_H 5.33 (1H, d, *J* = 7.1 Hz, H-1″), 4.48 (1H, d, *J* = 1.2 Hz, H-1‴), and 5.59 (1H, brs, H-1⁗), together with three corresponding anomeric carbon resonances at δ_C 104.5, 102.2 and 99.9, indicated that **6** is a flavonol triglycoside, and acid hydrolysis of **6** indicated that the sugar units comprised one *β*-D-galactopyranosyl and two *α*-L-rhamnopyranosyl moieties (Table 3). One of the sugar linkages was substantiated by the HMBC experiment, which that showed a correlation between H-1⁗ (δ_H 5.59) and C-7 (δ_C 163.7). Furthermore, the HMBC experiment showed long-range correlations between the following protons and carbons: H-1″ (δ_H 5.33) and C-3 (δ_C 135.6), H-1‴ (δ_H 4.48) and C-6″ (δ_C 67.1). The position of the *E*-*p*-coumaroyl group was also determined in the HMBC experiment, which showed a long-range correlation between H-4″ and the

Table 3
NMR spectroscopicdata for compound **6** (150 MHz for ^{13}C, 600 MHz for ^1H, methanol-d_4).

Position	^{13}C	^1H	Position	^{13}C	^1H
1	–	–	3-Gal 1″	104.5	5.33 (1H, d, *J* = 7.1 Hz)
2	160.1	–	2″	73.36	3.82 (1H, m)[a]
3	135.6	–	3″	73.41	3.82 (1H, m)[a]
4	179.6	–	4″	71.4	5.38 (1H, dd, *J* = 0.8, 3.0 Hz)
5	162.9	–	5″	74.0	3.88 (1H, m)
6	100.7	6.51 (1H, d, *J* = 2.0 Hz)	6″	67.1	3.24 (1H, dd, *J* = 6.0, 12.0 Hz)
7	163.7	–			3.54 (1H, dd, *J* = 2.9, 12.0 Hz)
8	95.8	6.78 (1H, d, *J* = 2.0 Hz)			
9	158.1	–	Rha 1‴	102.2	4.48 (1H, d, *J* = 1.2 Hz)
10	107.4	–	2‴	72.07	3.60 (1H, m)
1′	122.7	–	3‴	72.6	3.52 (1H, m)
2′, 6′	132.5	8.16 (2H, d, *J* = 8.8 Hz)	4‴	73.8	3.25 (1H, t-like, *J* = 9.5 Hz)
3′, 5′	116.2	6.95 (2H, d, *J* = 8.8 Hz)	5‴	69.9	3.40 (1H, dd, *J* = 6.3, 9.5 Hz)
4′	161.4	–	6‴	17.9	1.08 (3H, d, *J* = 6.3 Hz)
Coumaroyl group			7-Rha 1⁗	99.9	5.59 (1H, brs)
1⁗	127.2	–	2⁗	71.7	4.05 (1H, brs)
2⁗, 6⁗	131.4	7.51 (2H, d, *J* = 8.6 Hz)	3⁗	72.09	3.87 (1H, m)
3⁗, 5⁗	116.9	6.86 (2H, d, *J* = 8.6 Hz)	4⁗	73.6	3.51 (1H, dd, *J* = 9.5, 9.7 Hz)
4⁗	161.9	–	5⁗	71.3	3.63 (1H, dd, *J* = 6.2, 9.5 Hz)
7⁗	147.3	7.68 (1H, d, *J* = 15.8 Hz)	6⁗	18.1	1.28 (3H, d, *J* = 6.2 Hz)
8⁗	115.0	6.41 (1H, d, *J* = 15.8 Hz)			
9⁗	168.7	–			

m: multiplet.
[a] overlapped.

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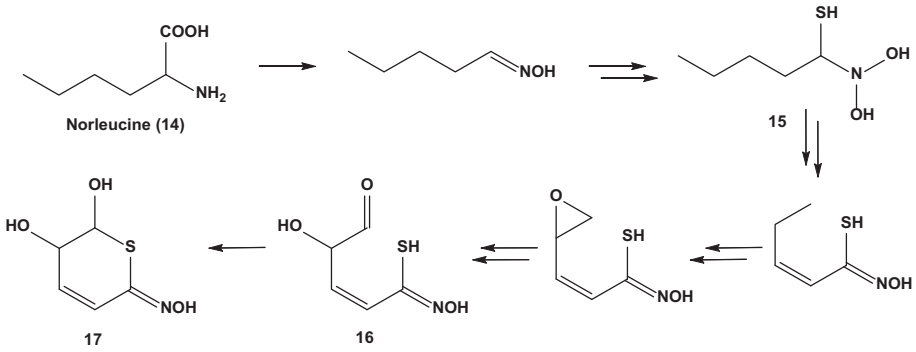

Fig. 2. Plausible biosynthetic pathway of the aglycone of compounds **4** and **5**.

p-coumaroyl carbonyl carbon (δ_C 168.7). On the basis of these findings, compound **6** was determined to be kaempherol 3-*O*-α-L-rhamnopyranosyl(1 → 6)(4″-*p*-coumaroyl) β-D-galactopyranoside 7-*O*-α-L-rhamnopyranoside.

Advanced glycation end products (AGEs), which readily form and accumulate with sustained hyperglycemia, contribute to the development of diabetic complications and, as such, are considered a potential therapeutic target. All the isolated compounds (**1**–**13**) were subjected to *in vitro* bioassaying of AGEs inhibitory activity. The new compounds did not show significant activity, but compounds **7**, **11** and **12** showed strong inhibitory activity toward AGEs formation with IC_{50} values of 86.0 μM, 76.6 μM and 98.6 μM, respectively, with the inhibitory activity of a positive control, aminoguanidine, being 2.48 mM. The current results indicate that, in comparison with the reference compound, aminoguanidine, which once entered phase II clinical trials, but was withdrawn because of its side-effects (Reddy and Beyaz, 2006), one megastigmane glucoside (**7**) and two flavonoid glycosides (**11**, **12**) were clearly more efficient in inhibiting the formation of AGEs. Thus, these compounds seem to be worthy of consideration as potential therapeutic agents for diabetic complications and related diseases though additional biological evaluation.

3. Concluding remarks

In conclusion, three new aromatic glycosides (**1**–**3**), two new sulfur and nitrogen-containing compound glucosides (**4**, **5**), and one new flavonoid glycoside (**6**) were isolated from the leaves of *I. undulata*, and their structures were determined on the basis of chemical and physicochemical evidence, and X-ray crystallography. Compounds **4** and **5** have a relatively rare aglycone, which contains three hetero atoms, such as oxygen, nitrogen and sulfur. A plausible biosynthetic pathway is shown in Fig. 2. Norleucine (**14**) is converted to an oxime derivative, which is conjugated with a sulfur donor (presumably cysteine) and then cysteine derivative is converted to compound **15** catalyzed by a C-S lyase (Redovnikovic et al., 2008). Finally, ring-closing of intermediate **16** would produce an intermediate **17**, which then undergoes glucosylation and methylation to form compounds **4** and **5**.

4. Experimental

4.1. General methods

The following instruments were used to obtain physical and spectroscopic data: specific rotations, JASCO P-1030 polarimeter; IR spectra, Shimadzu FT-710 spectrometer; HR-ESI mass spectra, LTQ Orbitrap XL; and ^1H and ^{13}C NMR spectra, Bruker Avance III spectrometer at 600 MHz and 150 MHz, respectively, with tetramethylsilane as an internal standard. A highly-porous synthetic resin, Diaion HP-20, was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Column chromatography(CC) was performed on silica gel 60 [(E. Merck, Darmstadt, Germany), 70–230 mesh]. Reversed-phase ODS open CC (RPCC) used Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [ϕ = 50 mm, L = 25 cm, linear gradient: MeOH–H_2O]. HPLC was performed on an ODS-3 column (Inertsil; GL Science, Tokyo, Japan; ϕ = 10 mm, L = 25 cm, flow rate: 2.00 mL/min), and the eluate was monitored with a refractive index monitor, RID-6A (Shimadzu, Kyoto, Japan). Precoated silica gel 60 F_{254} plates (E. Merck; 0.25 mm in thickness) were used for TLC monitoring with visualization by spraying with a 10% solution of H_2SO_4 in EtOH and heating to around 150 °C on a hotplate. (*R*)-(+)-1-Phenylethanol and β-glucosidase from almond were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

4.2. Plant material

The leaves of *I. undulata* were collected in the Al Zohreya Botanical Garden, Giza, Egypt in 2013. This plant was identified by the botanists of that garden and it is mentioned in the book containing all this gardens plants. A voucher specimen of the plant was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Egypt (Mn-Ph-Cog-Iu).

4.3. Extraction and isolation

Air-dried leaves of *I. undulata* (1.0 kg) were extracted three times with EtOH (5 L × 3) at room temperature for one week and then concentrated. The ethanol extract (112 g) was subjected to a Diaion HP-20 column [2.0 kg, H_2O (20 L) → MeOH (16 L) → acetone (6 L)] to give H_2O- (95 g), MeOH- (13 g), and acetone-soluble fractions (4 g). The methanol-soluble fraction (13 g), was subjected to silica gel CC [400 g, $CHCl_3$ (4 L) → $CHCl_3$-MeOH [19:1 (4 L) → 9:1 (4 L) → 7:1 (4 L) → 5:1 (4 L) → 3:1 (4 L)] → $CHCl_3$-MeOH–H_2O (15:6:1) (4 L) → (6:4:1) (4 L) → MeOH (6 L)] to give ten fractions. Fraction 5 (1.38 g) was separated by reversed-phase silica gel CC [120 g, MeOH–H_2O (1:9 → 1:4 → 3:7 → 2:3 → 1:1) → MeOH] into eight fractions. Fr. 5–1 (522 mg) was crystallized from H_2O/MeOH to give **4** (200 mg) as colorless needles. Fr. 5–2 (200 mg) was purified by HILIC–HPLC [MeCN–H_2O (19:1, v/v)] to give **7** (6.7 mg) from the peak at 26 min. Fr. 6 (520 mg) was separated by reversed-phase silica gel CC [120 g, MeOH–H_2O (1:9 → 1:4 → 3:7 → 2:3 → 1:1 → 3:2 → 7:3 → 4:1) → MeOH] to yield ten fractions. Fr. 6–1 (106 mg) was purified by HPLC [MeOH–H_2O (3:17, v/v)] to give **9** (5.4 mg). Fr. 6–2 (115 mg) was purified by HILIC–HPLC [MeCN–H_2O (19:1, v/v)] to give (6.4 mg) from the peak at 23 min. Fr. 7 (1.3 g) was separated by reversed-phase silica gel CC [120 g, MeOH–H_2O

(1:9 → 1:4 → 3:7 → 2:3 → 1:1 → 3:2 → 7:3 → 4:1) → MeOH] to yield 11 fractions. Fr. 7–4 (200 mg) was purified by HPLC [MeOH–H_2O (9:11, v/v)] to give **13** (30.6 mg), **11** (44.6 mg), and **10** (10.4 mg) from the peaks at 12 min, 26 min, and 67 min, respectively. Fr. 7–7 (28.3 mg) was purified by HPLC [MeOH–H_2O (11:9, v/v)] to give **6** (6.0 mg) from the peak at 18 min. Fr. 8 (1.0 g) was separated by reversed-phase silica gel CC [120 g, MeOH–H_2O (1:9 → 1:4 → 3:7 → 2:3 → 1:1 → 3:2 → 7:3) → MeOH] into eight fractions. Fr. 8–1 (300 mg) was purified by HPLC [MeOH–H_2O (1:9, v/v)] to give compound **5** (6.0 mg) from the peak at 80 min. Fr. 8–2 (178 mg) was purified by HPLC [MeOH–H_2O (35:65, v/v)] to give **1** (6.4 mg), **2** (4.3 mg), and **3** (11.3 mg) from the peaks at 30 min, 32 min and 46 min, respectively.

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, MS, ^{1}H NMR, and ^{13}C NMR) with the values reported in the literature.

4.4. Compound **1**

Amorphous powder, $[\alpha]_D^{27}$ −58.7 (c = 0.54, MeOH); IR (film) ν_{max} 3394, 2928, 1634, 1605, 1447, 1373, 1074, 1036 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 257 (3.87), 213 (4.11); For ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_4) spectroscopic data, see Table 1; positive-ion HR-ESI-MS m/z 469.1677 [M+Na]$^+$ (Calcd for $C_{20}H_{30}O_{11}Na$: 469.1680).

4.5. Compound **2**

Amorphous powder, $[\alpha]_D^{28}$ −34.7 (c = 0.38, MeOH); IR (film) ν_{max} 3360, 2928, 1605, 1547, 1466, 1073, 1039 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 260 (3.56), 217 (3.80); For ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_4) spectroscopic data, see Table 1; positive-ion HR-ESI-MS m/z 469.1680 [M+Na]$^+$ (Calcd for $C_{20}H_{30}O_{11}Na$: 469.1680).

4.6. Compound **3**

Amorphous powder, $[\alpha]_D^{28}$ −42.5 (c = 1.13, MeOH); IR (film) ν_{max} 3372, 2924, 1635, 1501, 1377, 1218, 1070 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 258 (2.83), 221 (4.03); For ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_4) spectroscopic data, see Table 1; positive-ion HR-ESI-MS m/z 469.1678 [M+Na]$^+$ (Calcd for $C_{20}H_{30}O_{11}Na$: 469.1680).

4.7. Compound **4**

Colorless crystals, m.p. 207–215 °C; $[\alpha]_D^{28}$ +107 (c = 1.16, pyridine); IR (film) ν_{max} 3308, 2934, 1648, 1537, 1418, 1261, 1075, 1028 cm^{-1}; UV λ_{max} (THF) nm (log ε): 308 (1.51), 249 (1.42); For ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) spectroscopic data, see Table 2; positive-ion HR-ESI-MS m/z 360.0726 [M+Na]$^+$ (Calcd for $C_{12}H_{19}O_8NSNa$: 360.0724).

4.8. Compound **5**

Amorphous powder, $[\alpha]_D^{19}$ +43.3 (c = 1.03, MeOH); IR (film) ν_{max} 3368, 2937, 1636, 1537, 1420, 1372, 1258, 1158, 1075, 1031 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 293 (1.79), 248 (1.76); For ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_4) spectroscopic data, see Table 2; positive-ion HR-ESI-MS m/z 522.1252 [M+Na]$^+$ (Calcd for $C_{18}H_{29}O_{13}NSNa$: 522.1252);

4.9. Compound **6**

Pale yellow amorphous powder, $[\alpha]_D^{29}$ −125 (c = 0.60, MeOH); IR (film) ν_{max} 3360, 2935, 1715, 1698, 1653, 1601, 1513, 1490, 1172, 1069, 1019 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 335 (4.35), 291 (4.44), 240 (4.47); For ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_4) spectroscopic data, see Table 3; positive-ion HR-ESI-MS m/z 909.2412 [M+Na]$^+$ (Calcd for $C_{42}H_{46}O_{21}Na$: 909.2429).

4.10. Acid hydrolysis of compounds **1**–**6**

Solutions of **1**–**6** (1 mg) in 1 M HCl (1.0 mL) were heated under conditions of reflux for 3 h. After cooling, the reaction mixtures were neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrates were extracted with EtOAc. The aqueous layers were subjected to HPLC analysis [column: Shodex Asahipak NH 2P-50 4E, 250 × 4.6 mm i.d.; mobile phase: MeCN–H_2O (3:1, v/v); detection: optical rotation (JASCO 2090*Plus* Chiral); flow rate: 1.0 mL/min] to identify D-galactose from **6**, D-glucose from **1** to **5**, and L-rhamnose from **6**, which were identified by comparison of their retention times with those of authentic samples; t_R: 8.2 min (D-glucose, positive optical rotation); t_R: 7.9 min (D-galactose, positive optical rotation); and t_R: 6.5 min (L-rhamnose, negative optical rotation).

4.11. Enzymatic hydrolysis of compound **1**

A solution of **1** (3.5 mg) in 0.2 M acetate buffer (1.0 mL, pH 3.8) was treated with β-glucosidase (7.8 mg), and the solution was stirred at 37 °C for 4 h. After the addition of 1.0 mL of EtOH, the mixture was centrifuged at 1400 g for 10 min and the supernatant solution was concentrated under reduced pressure to give a residue. The residue was purified by HPLC [column: Inertsil; GL Science, Tokyo, Japan, 250 × 4.6 mm i.d.; mobile phase: MeOH–H_2O (2:3, v/v); detection: optical rotation (JASCO 2090*Plus* Chiral); flow rate: 1.0 mL/min] to furnish 1-(*R*)-phenylethanol (**1a**) (1.0 mg). The obtained compound, 1-(*R*)-phenylethanol (**1a**), was identified by comparison with an authentic sample.

4.12. X-ray crystallographic analysis of **4**

A crystal of $C_{12}H_{19}NO_8S{\cdot}3H_2O$, with approximate dimensions of 0.20 mm × 0.20 mm × 0.30 mm, was used for X-ray crystallographic analysis. The X-ray intensity data were measured at −100 °C using a Bruker APEX-II Ultra CCD-based diffractometer. The integration of the data using a monoclinic unit cell yielded a total of 5448 reflections to a maximum θ angle of 27.96° (0.76 Å resolution), of which 3603 were independent (average redundancy 1.512, completeness = 97.8%, R_{int} = 4.68%, R_{sig} = 8.31%), and 3163 (87.79%) were greater than $2\sigma(F^2)$. The final cell constants of a = 9.262(3) Å, b = 4.6152(17) Å, c = 21.773(8) Å, β = 96.477(4)°, V = 924.8(6) Å^3 were based upon refinement of the XYZ-centroids of reflections above 20 $\sigma(I)$. The calculated minimum and maximum transmission coefficients (based on crystal size) were 0.9383 and 0.9583. The structure was solved and refined using the Bruker SHELXTL Software Package, using space group $P2_1$ with Z = 2 for the formula unit, $C_{12}H_{25}NO_{11}S$. The final anisotropic full-matrix least-squares refinement on F^2 with 312 variables converged at R_1 = 5.14% for the observed data, and wR_2 = 13.35% for all data. The goodness-of-fit was 1.044. The largest peak in the final difference electron density synthesis was 0.317 e$^-$/Å^3 and the largest hole was 0.320 e$^-$/Å^3 with an RMS deviation of 0.053 e$^-$/Å^3. On the basis of the final model, the calculated density was 1.269 g/cm^3 and F(000) 372 e$^-$. The absolute configuration of the molecule was determined on the basis of the stereochemistry of the glucose moiety. The correct configuration was also proved by the Flack parameter x = −0.02(12) being close to zero (Parsons and Flack, 2004).

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4.13. Determination of AGEs formation in vitro

According to the method of Séro et al. (2013)), the reaction mixture, 10 mg/mL of bovine serum albumin (SIGMA) in 50 mM phosphate buffer (pH 7.4) containing 0.02% sodium azide, was added to a 0.5 M ribose solution. The reaction mixture was then mixed with test compounds. After incubation at 37 °C for 24 h, the fluorescent reaction products were assayed with a spectrofluorometric detector (EnSpire, PerkinElmer Japan; Ex: 370 nm, Em: 440 nm). Measurements were performed in triplicate, and the concentrations required for 50% inhibition (IC_{50}) of the intensity of fluorescence were determined graphically. Aminoguanidine hydrochloride (TCI, Japan) was used as a reference compound.

Supplementary material

CCDC 992109 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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