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New $CuCl_2$-induced glucoside esters and other constituents from *Portucala oleracea*

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ABSTRACT

Two new glucoside esters **1** and **2** were produced as stress metabolites in the fresh leaves of *Portulaca oleracea*, in response to abiotic stress elicitation by $CuCl_2$. A new sugar ester (**3**) and two known compounds (**4** and **5**) were also isolated. Their structures were established by spectroscopic means. The antioxidative activities of stress metabolites and the related isolates were evaluated by DPPH assay. The results showed that new stress-driven adducts of monolignans and monoterpenes with a glucose bridge exhibited much stronger antioxidative activities than other compounds.

1. Introduction

Portulaca oleracea Linn. (machixian in Chinese and purslane in English), widely used as a potherb in the Mediterranean, Central European and Asian countries,[1] which is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea'.[2,3] Recent research has shown that the *P. oleracea* is a rich source of omega-3 fatty acids,[4] gallotannins, kaempferol, quercetin and apigenin,[5,6] carotenoids,[7] flavonoids,[8] monoterpenes,[9] and other phenolics.[10] The antioxidative assay showed that *P. oleracea* was capable of inhibiting lipid peroxidation.[11,12]

Copper is an essential trace element for all higher plants, and has several roles in metabolic processes in plants.[13,14] Copper concentrations in soil generally contain between 2 and 250 ppm and healthy plant tissues range from 20 to 30 $\mu g\ g^{-1}$ dry weight. However, excess concentrations are said to generate oxidative stress due to an increase in the levels of reactive oxygen species (ROS) within subcellular compartments.[15,16] Thus, for protection against oxidative damage, antioxidative strategies have been developed, and damage in the plant tissue is alleviated by the action of both enzymatic and non-enzymatic antioxidative metabolisms.[17] These antioxidative systems make the plant not only capable to deal with oxygen stress but also to use oxygen activation as a defense system.[18] In order to find stress metabolites from *P. oleracea* Linn., a stress application on the leaves of this plant was carried. In the present study, application of the abiotic stress agent $CuCl_2$ to the leaves and stems of *P. oleracea* resulted in the production of two additional spots in the extract of the treated plants in comparison with that of the corresponding control extract on the TLC plates. Two new compounds, 9-(6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-β-D-glucopyranosyloxy)-guaiacylglycerol (**1**) and 9-(6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-β-D-glucopyranosyloxy)-syringoylglycerol (**2**), produced in response to abiotic stress treatment, and one new compound 6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-(α-β)-D-glucopyranose (**3**), two known compounds, phlebotrichin (**4**) and guaiacylglycerol (**5**) were separated by preparative TLC, and purified by Sephadex LH-20 column chromatography. Their structures and configurations were established by spectroscopic means. The antioxidative activities of stress metabolites and the related isolates were evaluated by DPPH assay. The results showed that new stress-driven adducts of monolignans and monoterpenes with a glucose bridge exhibited much stronger antioxidative activities than other compounds.

2. Results and discussion

The ethyl acetate and *n*-butanol soluble fractions of the methanol extract of the leaves and roots of copper stressed *P. oleracea* Linn. were subject to repeated chromatography to afford new compounds **1**–**3** and two known compounds (Fig. 1). Two known compounds were identified as phlebotrichin (**4**)[19] and guaiacylglycerol (**5**),[20] by analysis of their NMR and MS data, and by comparison of their spectroscopic data with literature values.

Compound **1**, was obtained as a white powder with a positive optical rotation, and the HRFTICRMS exhibited a molecular ion

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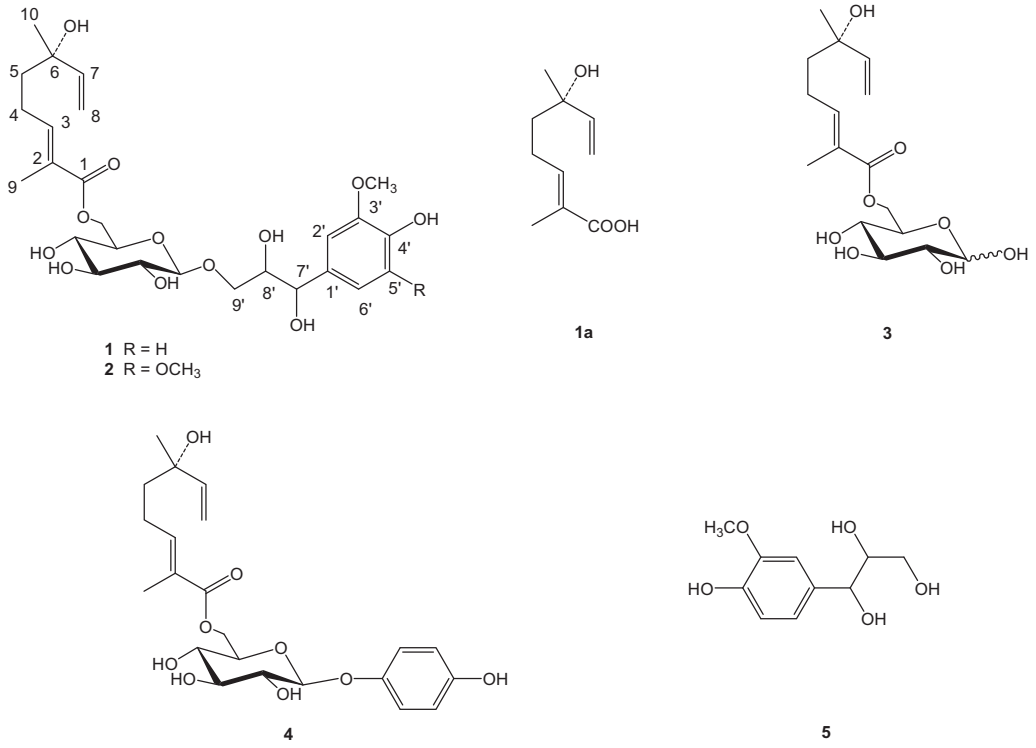


Figure 1. The structures of compounds **1**–**5** and **1a**.

peak at *m/z* 565.2245 $[M+Na]^+$ (calcd 565.2255), corresponding to the molecular formula, $C_{26}H_{38}O_{12}$. The IR spectrum of **1** showed the presence of carbonyl (1715 cm^{-1}), aromatic ring (1520 cm^{-1}) and hydroxyl group (3451 cm^{-1}). The ^1H NMR spectrum (Table 1) of **1** showed the presence of two methyl groups (δ_H 1.79 (s) and 1.27 (s)), one vinyl group (δ_H 5.90 (dd, *J* = 17.4, 10.8 Hz), 5.23 (dd, *J* = 17.4, 1.2 Hz) and 5.05 (dd, *J* = 10.8, 1.2 Hz)), ABX-type aromatic protons (δ_H 6.73 (d, *J* = 8.3 Hz), 6.82 (br d, *J* = 8.3 Hz) and 7.00 (d, *J* = 2.1 Hz)) and 1,2,3-propane-triol unit (δ_H 4.64 (d, *J* = 6.6 Hz), 3.35 (m), 3.67(m), and 3.56 (m)). The ^{13}C NMR spectrum showed the presence of six signals for a glucopyranose moiety, nine signals for a monolignan, with the remaining 10 resonances corresponding to a monoterpene skeleton. The gross structure of **1** was determined by detailed analysis of 1D (Table 1) and 2D NMR data (Fig. 2) and hydrolysis experiment. The 10 signals for the monoterpene unit comprised two methyls (δ_C 10.4 and 25.8), two methylenes (δ_C 22.5 and 39.6), a methine (δ_C 49.1), an oxygenated quaternary carbon (δ_C 72.9), a carbonyl carbon (δ_C 167.6), a tetrasubstituted double bond (δ_C 126.4 and 142.5) and terminal double bond (δ_C 144.0 and 110.4). In the COSY spectrum of **1** (Fig. 1), the methylene protons at δ_H 2.23 (m, H-4) were coupled with the methylene protons at δ_H 1.56 (t, *J* = 8.0 Hz, H-5) and olefin proton at δ_H 6.76 (t, *J* = 7.5 Hz, H-3). Another sequence of H_2-8/H-7 of the terminal double bond was also observed in the COSY spectrum. The terminal double bond moiety was assigned at C-6 from the observation of HMBC correlations from the olefin protons at δ_H 5.23 (dd, *J* = 17.4, 1.2 Hz, H-8a), 5.05 (dd, *J* = 10.8, 1.2 Hz, H-8b) to the oxygenated quaternary carbon (δ_C 72.9, C-6). The HMBC peaks from the methyl group at δ_H 1.79 (s, Me-9) to another olefin at δ_C 126.4 and 142.5 assigned this double bond to C-2 and C-3. The carbonyl group was assigned to C-1 from analysis of the HMBC cross peak of Me-9/C-1. After hydrolysis of **1** with 0.5 N sodium hydroxide, two main compounds **1a** and **5** were separated. Compound **5** was also isolated from the same plant, which was identified as guaiacylglycerol by analysis of its NMR and MS data, and by comparison of its spectroscopic data with the literature values.[20] The structure of **1a** was established as (*E*)-linalool-1-oic acid (2,6-dimethyl-6-hydroxyl-2-*trans*-2,7-octadienoic acid) by comparison of its spectroscopic data with natural and synthetic form of (*E*)-linalool-1-oic acid. The monoterpenes were proved to be attached at C-6 of the glucose unit with an ester linkage by analysis of the HMBC cross peak of H-6-Glu/C-1. The sugar obtained by the acid hydrolysis of **1** was identified by co-TLC and GC analysis, and were confirmed as D-glucose. The glucosylated position in the aglycone was deduced to be at C-9′ of guaiacylglycerol by analysis of the HMBC cross correlation between the anomeric proton and C-9′ (Fig. 2). The configuration of the glycosidic linkage of the glucopyranoside moiety in **1** was determined to be β based on the coupling constant of the anomeric proton at δ_H 4.23 (d, *J* = 7.5 Hz). When compared the molecular rotation (+25.8) of **1a** with the known *d*-linalool (+27.1), the C-6 position of **1** and **1a** had an *S*-configuration.[19] From the above information, **1** was elucidated as 9-(6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-β-D-glucopyranosyloxy)-guaiacylglycerol (Fig. 1).

Compound **2** was obtained as a white powder. The HRFTICRMS exhibited a molecular ion peak at *m/z* 595.2344 $[M+Na]^+$ (calcd 595.2361), corresponding to the molecular formula $C_{27}H_{40}O_{13}$. The UV and IR spectra of **2** exhibited the similar general patterns as those of **1**. The ^1H and ^{13}C NMR spectra in DMSO-d_6 of **2** (Table 1) showed similar chemical shifts and the same multiplicities to most carbons atoms as in **1**, except for an additional methoxyl for **2**, indicating that **2** was the methylated derivatives of **1**. After hydrolysis of **2**, two main compounds were separated. One compound was identified as syringoylglycerol by analysis of its NMR and MS data, and by comparison of its spectroscopic data with literature values.[21] The structure of the monoterpene unit was proved to be the same as in **1** and **1a**, (*E*)-linalool-1-oic acid. The HMBC correlation from the anomeric proton signal of a glucose moiety at δ_H 4.34 (d, *J* = 7.5 Hz) to the carbon resonance at δ_C 71.2 (C-9′) indicated that the glucose moiety was also attached at C-9′. Thus,

Table 1
NMR data (500 MHz) for compound **1–3** in DMSO-d_6

Position	1		2		3	
	δ_C[a,b]	δ_H[c] mult. (*J* in Hz)	δ_C[a,b]	δ_H[c] mult. (*J* in Hz)	δ_C[a,b]	δ_H[c] mult. (*J* in Hz)
1	167.6 (s)		168.2 (s)		168.3 (s)	
2	126.4 (s)		127.0 (s)		127.2 (s)	
3	142.5 (d)	6.76 (t, *J* = 7.5)	143.1 (d)	6.81 (t, *J* = 7.5)	143.0 (d)	6.76 (t, *J* = 7.5)
4	22.5 (t)	2.23 (m)	23.1 (t)	2.23 (m)	23.2 (t)	2.19 (m)
5	39.6 (t)	1.56 (t, *J* = 8.0)	40.3 (t)	1.61 (m)	40.3 (t)	1.58 (t, *J* = 8.0)
6	72.9 (s)		72.2 (s)		72.4 (s)	
7	144.0 (d)	5.90 (dd, *J* = 17.4, 10.8)	144.6 (d)	5.92 (dd, *J* = 17.4, 10.8)	144.6 (d)	5.88 (dd, *J* = 17.5, 10.5)
8	110.4 (t)	5.23 (dd, *J* = 17.4, 1.2), 5.05 (dd, *J* = 10.8, 1.2)	111.0 (t)	5.22 (dd, *J* = 17.4, 1.2), 5.06 (dd, *J* = 10.8, 1.2)	111.1 (t)	5.19 (dd, *J* = 17.5, 1.2), 5.03 (dd, *J* = 10.8, 1.2)
9	10.4 (q)	1.79 (s)	11.0 (q)	1.83 (s)	11.1 (q)	1.78 (s)
10	25.8 (q)	1.27 (s)	26.4 (q)	1.29 (s)	26.5 (q)	1.23 (s)
1′	132.4 (s)		132.4 (s)			
2′	109.6 (d)	7.00 (d, *J* = 2.1)	104.2 (d)	6.71 (s)		
3′	146.7 (s)		147.2 (s)			
4′	145.0 (s)		134.6 (s)			
5′	113.9 (d)	6.73 (d, *J* = 8.3)	147.2 (s)			
6′	118.7 (d)	6.82 (br d, *J* = 8.3)	104.2 (d)	6.71 (s)		
7′	73.1 (d)	4.64 (d, *J* = 6.6)	74.1 (d)	4.57 (d, *J* = 6.6)		
8′	69.8 (d)	3.35 (m)	70.3 (d)	3.32 (m)		
9′	69.7 (t)	3.67, 3.56 (2m)	71.2 (t)	4.01, 3.65 (2m)		
Glucose						
1	102.7 (d)	4.23 (d, *J* = 7.5)	103.7 (d)	4.34 (d, *J* = 7.5)	96.8 (d)/ 92.6 (d)	4.45 (d, *J* = 7.5)/ 5.05 (d, *J* = 3.6)
2	73.3 (d)	3.47 (m)	73.8 (d)	3.41 (m)	73.4 (d)	3.49 (m)
3	75.7 (d)	3.38 (m)	76.3 (d)	3.35 (m)	76.5 (d)	3.33 (m)
4	71.6 (d)	3.28 (m)	73.8 (d)	3.27 (m)	71.6 (d)	3.10 (m)
5	74.0 (d)	3.78 (m)	74.3 (d)	3.86 (m)	74.1 (d)	3.95 (m)
6	62.9 (t)	4.43 (d, *J* = 11.7), 4.19 (dd, *J* = 11.7, 6.2)	63.4 (t)	4.47 (dd, *J* = 11.7, 1.8), 4.24 (dd, *J* = 11.7, 6.2)	63.7/63.6 (t)	4.35 (dd, *J* = 11.9, 1.9)/4.40 (dd, *J* = 11.9, 1.9), 4.21(dd, *J* = 11.9, 5.2)/4.23(dd, *J* = 11.9, 5.2)
3-OCH_3	54.4 (q)	3.78 (s)	55.4 (q)	3.83 (s)		
3′-	OCH_3			55.4 (q)	3.83 (s)	

[a] Recorded on 125 MHz.
[b] Multiplicities inferred from DEPT and HMQC experiments.
[c] Recorded on 500 MHz.

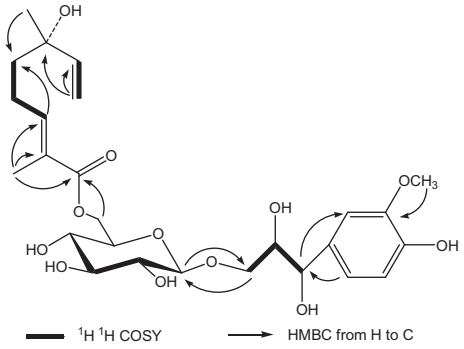
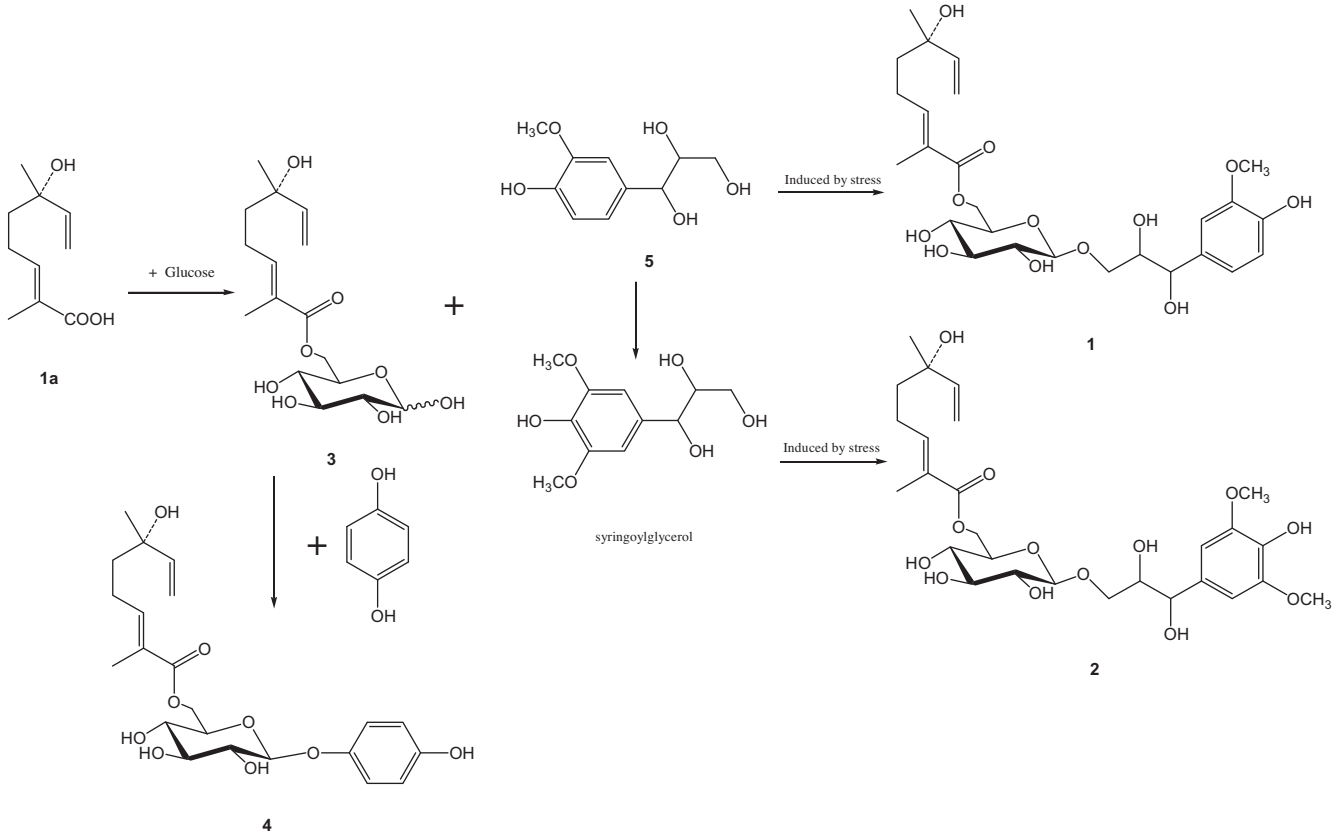


Figure 2. Key ^{1}H ^{1}H COSY and HMBC correlations of compound **1**.

2 was elucidated as 9-(6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-β-D-glucopyranosyloxy)-syringoylglycerol.

Compound **3** was obtained as yellow gum. The HRFTICRMS exhibited a molecular ion peak at *m/z* 369.1526 $[M+Na]^+$ (calcd 369.1520), corresponding to the molecular formula, $C_{16}H_{26}O_8$. After hydrolysis of **3** with 0.5 N sodium hydroxide, (*E*)-linalool-1-oic acid was obtained, the C-6 position of which was determined as *S*-configuration due to the similar molecular rotation (+29.3) with *d*-linalool, suggesting its structure to be a mono-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl glucose. The sugar obtained by the acid hydrolysis of **3** was identified by co-TLC and GC analysis, and was confirmed as D-glucose. The ^{1}H NMR spectrum (Table 1) of **3** revealed two different patterns of proton resonances belonging to an α/β anomeric mixture of monosubstituted glucose, whereby a pair of doublets, centered at δ_H 4.45 (d, *J* = 7.5 Hz) and 5.05 (d, *J* = 3.6 Hz) were recognized, indicating the presence of a free anomeric OH. The ^{1}H NMR spectrum also showed a pair of double doublets at δ_H 4.35 (dd, *J* = 11.9, 1.9 Hz, H-6a) and δ_H 4.40 (dd, *J* = 11.9, 1.9 Hz, H-6a) as well as a pair of double doublets at δ_H 4.21 (d, *J* = 11.9, 5.2 Hz, H-6b) and δ_H 4.23 (d, *J* = 11.9, 5.2 Hz, H-6b), attributable to the two H-6 methyleneic glucose protons in both anomers of **3**. Duplication of almost all of the ^{13}C NMR resonances reconfirmed that **3** formed an anomeric mixture due to the existence of a free anomeric hydroxyl group. To find out the esterified site of attachment of the (2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl moiety in the molecule of **3**, HMBC spectroscopic analysis was then performed. The observation of HMBC correlation from Glu-H-6 of the glucose moiety to the carbonyl C-1 indicated that Glu-H-6 of glucose moiety was esterified. Furthermore, the measured chemical shifts of the carbon resonances proved that sugar moiety existed in a pyranose form.[22] Thus, the structure of **3** was finally confirmed to be a new natural sugar ester, 6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-(α-β)-D-glucopyranose.

Since monoterpenes, monolignans, and sugar esters were found in the same plant, we could tentatively outline the plausible stress-driven biogenetic relationships of these isolates shown in Scheme 1. The monothepene, (*E*)-linalool-1-oic acid, which etherify with a



Scheme 1. Plausible biogenetic relationships of compounds **1**–**5** and **1a**.

glucose to form a new sugar ester 6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-(α-β)-D-glucopyranose (**3**), acts as starting compound. Methoxylation of guaiacylglycerol (**5**) gives syringoylglycerol (not isolated). Compound **3** reacts with two monolignans, guaiacylglycerol (**5**) and syringoylglycerol to form two new stress metabolites **1** and **2**, and reacts with hydroquinone to form phlebotrichin (**4**).

As important two classes of naturally occurring secondary metabolites, monoterpenes and lignans are well known for their antioxidative activities.[18] In order to determine the role of new adducts of monoterpene and lignan in the defense system in the plant, the antioxidative activities of compounds **1**–**5** and **1a** were evaluated by DPPH radical assay, which has been wildly used for evaluation of antioxidative activities of natural products.[23,24] The results showed that antioxidative activities of the new stress metabolites **1** and **2** with IC_{50} values of 11.6 ± 0.6 μM and 36.7 μM ± 5.7 were stronger than those of their precursive monoterpene **1a**, monolignan **5**, sugar ester **3** and phlebotrichin (**4**) with IC_{50} values of 122.8 μM ± 5.2, 75.4 μM ± 3.7, 57.2 μM ± 4.2 and 92.7 μM ± 7.8 (tocopherol as a control, IC_{50} = 14.8 μM ± 0.8).

In our previous studies on stress metabolites, an adduct of sesquiterpene and phenol was proved to be an active phytoalexin induced by copper stress.[25] We discovered that the plant could promote their anti-stress activity through the strategy of cyclizing the phenolic compounds,[23] hydroxylation of dibenz[*b*,*f*]oxepin[26] and glycosylation.[27] The result of this study showed that compounds **1** and **2** were the stress metabolites in response to copper toxicity. Antioxidative activities of secondary metabolites play a major role in their anti-stress abilities.[28] It has been reported that the antioxidative property of phenolics is due to their high tendency to chelate metals.[29] Many plants exposed to heavy metals exude high levels of phenolics.[30]

3. Experimental

3.1. General methods

The melting point (uncorrected) was obtained on a Reichert apparatus. Optical rotations were recorded on a Perkin–Elmer-341 polarimeter. UV spectra were measured with a JASCO UV-2200 UV-vs recording spectrophotometer. The IR spectra were run on a NicoletAvatar-360 FT-IR spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured on a Bruker AVANCE DMX 500 NMR spectrometer with TMS as internal standard (at 25 °C). HRFTICRMS were recorded on Bruker Apex III spectrometer. ESIMS were recorded on a Bruker Esquire-3000 ^{plus} spectrometer. TLC was performed using Merck precoated plates (silica gel 60 F254) of 0.25 mm thickness. A Waters 600 preparative HPLC, with a Shim-pack PREP-ODS (250 × 20 mm) column, was used for preparative HPLC. Sephadex LH-20 (Amersham) was used for column chromatography.

3.2. Plant material and stress applications

P. oleracea Linn. was collected in Linan County, Zhejiang Province, People's Republic of China, in August 2010 and identified by Professor Changxi Zhang (Jinhua Medical College, Jinhua, People's Republic of China). A voucher specimen (Vs17) is maintained at the Jinhua Medical College, Jinhua, People's Republic of China. The stress applications were carried on 100 plants according to our previous method.[26,27] All the plants were separated into

control (50 plants) and stressed groups (50 plants). To elicit the stress, plants were sprayed with 2% aq solution of $CuCl_2$. After 48 h, leaves of the control and sprayed plants were collected and dried at 60 °C, and finely powdered in an electronic blender and kept in separate containers for extraction.

3.3. Extraction and isolation

The dried, powdered $CuCl_2$ treated leaves and stems (708 g) of *P. oleracea* and untreated leaves (771 g) were extracted at room temperature with MeOH (3 × 5 L), respectively. The extracts were evaporated in vacuo to afford a gummy residue (68 g) for treated and a gummy residue (72 g) for the corresponding control. The residues were partitioned in H_2O (500 mL) and extracted with EtOAc (4 × 500 mL) and *n*-butanol (4 × 500 mL), successively. The EtOAc and *n*-butanol extracts of treated and the corresponding control were subjected to TLC examination on aluminium sheets precoated with Si Gel 60 F 254 (Merck). The spots were applied in as equal amounts as possible. The plates were developed in the following developing solvent systems: benzene–acetone (6:1), benzene–EtOAc (5:1) petroleum ether–EtOAc (5:1) for the EtOAc extract; $CHCl_3$–MeOH (3:1), CH_2Cl_2–MeOH (4:1) and benzene–$CHCl_3$–MeOH (1:3:1) for the *n*-butanol extract. After development, the plates were examined under UV light (254 nm) to locate any additional spots in the different extracts of the treatments in comparison with that of the corresponding control extracts. The spots on the plates were also visualized by spraying with an EtOH–H_2SO_4 solution. Several prep-TLC plates were prepared and the compounds were separated by preparative TLC in different solvent systems. The crude compounds were applied to a Sephadex LH-20 column (1 × 80 cm, 38 g, Amersham), and eluted with MeOH to yield pure compounds **1** (1.4 mg), **2** (1.2 mg), **3** (10.9 mg), **4** (8.1 mg) and **5** (10.3 mg). The extract of untreated leaves were separated by the same methods to afford **3** (12.0 mg), **4** (12.5 mg) and **5** (11.9 mg).

3.4. Identification

3.4.1. 9-(6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-β-D-glucopyranosyloxy)-guaiacylglycerol (1)White powder; $[\alpha]_D^{20}$ +46 (*c* 0.001, MeOH); mp 111–113 °C; UV (MeOH) λ_{max} (logε) 210 (4.56), 254 (3.88), 280 (3.91) nm; IR ν_{max}^{KBr} 3451, 1715, 1645, 1520, 787 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESI MS *m/z* 565 [M+Na]$^+$; HRFTICRMS *m/z* 565.2245 [M+Na]$^+$ (calcd 565.2255).

3.4.2. 9-(6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-β-D-glucopyranosyloxy)-syringoylglycerol (2)

White powder; $[\alpha]_D^{20}$ +32 (*c* 0.001, MeOH); mp 120–122 °C; UV (MeOH) λ_{max} (logε) 210 (4.09), 254 (3.32), 280 (3.89) nm; IR ν_{max}^{KBr} 3455, 1716, 1645, 1521, 756 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESI MS *m/z* 595 [M+Na]$^+$; HRFTICRMS *m/z* 595.2344 [M+Na]$^+$ (calcd 595.2361).

3.4.3. 6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl]-(α-β)-D-glucopyranose (3)

Yellowish gum: $[\alpha]_D^{20}$ +98 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (logε) 213 (4.33) nm; IR ν_{max}^{KBr} 3502, 1715, 1644, 987 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESI MS *m/z* 369 [M+Na]$^+$; HRFTICRMS *m/z* 369.1526 [M+Na]$^+$ (calcd 369.1520).

3.5. Acid hydrolysis of 1–3

Each compound (2.0 mg) in 10% HCl was stirred at 90 °C for 4 h. The reaction mixture was filtered, and examined by TLC together with authentic D-glucose. The dried filtrate was dissolved in dry pyridine,[31] to which was added L-cysteine methyl ester hydrochloride. The mixture was stirred at 60 °C for 1.5 h, then hexamethyldisilazane–trimethylchlorosilane (2:1) was added, and stirred for 0.5 h.[32] After centrifugation, the supernatant was directly subjected to GC analysis. The sugar derivatives obtained from **1**–**3** were detected in each case by co-injection of the D-glucose derivatives.

3.6. Determination of antioxidative activities

The antioxidative activities of all the compounds were screened by DPPH assay as previously described.[23] The purities of compounds used for the assay were above 95% checked by HPLC and ^{1}H NMR experiments. In brief, 100 μl test samples at different concentrations in MeOH and 8.0×10^{-5} M DPPH in MeOH (300 μl) were added to a 96-well microtiter plate. The plate was shaken for 1 min on a plate shaker, and incubated for 30 min at room temperature in the dark. After incubation, the absorbance was recorded at 517 nm. The tested samples at different concentrations without DPPH solution were used as a blank control to eliminate the influence of sample color. Vitamin E was used as a positive control. All tests were independently performed in triplicate and the definition of IC_{50} values in the tested compounds is the concentration required to scavenge 50% DPPH free radicals. The DPPH radical scavenging activity was calculated according to the following equation: DPPH radical scavenging activity (%) = [{$Abs_C - (Abs_S - Abs_B)/Abs_C$}] × 100.

Acknowledgments

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References

1. Chan, K.; Islam, M. W.; Kamil, M.; Radhakrishnan, R.; Zakaria, M. N. M.; Habibullah, M.; Attas, A. *J. Ethnopharmacol.* **2000**, *73*, 445–451.
2. Amin, E. S.; El-Deeb, S. M. *Carbohydr. Res.* **1977**, *56*, 123–128.
3. Samy, J.; Sugumaran, M.; Lee, K. L. W. *Herbs of Malaysia: An Introduction to the Medicinal, Culinary, Aromatic and Cosmetic Use of Herbs*; Times Edition: Kuala Lumpur, 2004.
4. Simopoulos, A. P. *Biol. Res.* **2004**, *37*, 263–277.
5. Cai, Y.; Luo, Q.; Sun, M.; Corke, H. *Life Sci.* **2004**, *74*, 2157–2184.
6. Radhakrishnan, R.; Zakaria, M. N. M.; Islam, M. W.; Chen, H. B.; Kamil, M.; Chan, K.; Al-Attas, A. *J. Ethnopharmacol.* **2001**, *76*, 171–176.
7. Graça Dias, M. G.; Filomena, M.; Camões, G. F. C.; Oliveira, L. *Food Chem.* **2009**, *113*, 808–815.
8. Andarwulan, N.; Batari, R.; Sandrasari, D. A.; Bolling, B.; Wijaya, H. *Food Chem.* **2010**, *121*, 1231–1235.
9. Sakai, N.; Inada, K.; Okamoto, M.; Shizuri, Y.; Fukuyam, Y. *Phytochemistry* **1996**, *42*, 1625–1628.
10. Huang, Z.; Wang, B.; Eaves, D. H.; Shikany, J. M.; Pace, R. D. *Food Chem.* **2007**, *103*, 1395–1402.
11. Lim, Y. Y.; Quah, E. P. L. *Food Chem.* **2007**, *103*, 734–740.
12. Chew, Y. L.; Goh, J. K.; Lim, Y. Y. *Food Chem.* **2009**, *116*, 13–18.
13. Maksymiec, W. *Photosynthetica* **1997**, *34*, 321–342.
14. McKenna, B. A.; Nicholson, T. M.; Wehr, J. B.; Menzies, N. W. *Carbohydr. Res.* **2010**, *345*, 1174–1179.
15. Mittler, R.; Vanderauwera, S.; Gollery, M.; Breusegem, F. V. *Trends Plant Sci.* **2004**, *9*, 490–498.
16. Ródio, R. T.; Pereira, E. M.; Tavares, M. F. M.; Ferreira, A. M. C. *Carbohydr. Res.* **1999**, *315*, 319–329.
17. Hasegawa, P. M.; Bressan, R. A.; Zhu, J. K.; Bohnert, H. J. *Annu. Rev. Plant Physiol Plant Mol. Biol.* **2000**, *51*, 463–499.
18. Grassmann, J.; Hippeli, S.; Elstner, E. F. *Plant Physiol. Biochem.* **2002**, *40*, 471–478.
19. Takido, M.; Fukuhara, K.; Yamanouchi, S.; Takahashi, S. *Phytochemistry* **1983**, *22*, 223–225.
20. Sakushima, A.; Ohno, K.; Maoka, T.; Coskun, M.; Guvenc, A.; Erdurak, C. S.; Ozkan, A. M.; Seki, K.; Ohkura, K. *Phytochem. Anal.* **2003**, *14*, 48–53.
21. Nakano, H.; Morita, S.; Shigemori, H.; Hasegawa, K. *Plant Growth Regul.* **2006**, *48*, 215–219.
22. Hanaya, T.; Yamamoto, H. *Carbohydr. Res.* **1998**, *306*, 157–162.
23. He, S.; Wu, B.; Pan, Y.; Jiang, L. *J. Org. Chem.* **2008**, *73*, 5233–5241.
24. Cerchiaro, G.; Sant'Ana, A. C.; Temperini, M. L. A.; Ferreira, A. M. C. *Carbohydr. Res.* **2005**, *340*, 2352–2359.
25. Wu, B.; Gan, L.; Qu, H. *J. Nat. Prod.* **2010**, *73*, 1069–1074.
26. Chen, J.; Zhang, H.; Chen, L.; Wu, B. *Nat. Prod. Commun.* **2011**, *6*, 53–54.

27. Wu, B.; Wang, K.; Wu, X. *Helv. Chim. Acta* **2011**, *94*, 1677–1684.
28. Lopes, G. K. B.; Schulman, H. M.; Hermes-Lima, M. *BBA Gen. Subj.* **1999**, *1472*, 142–152.
29. Marquez-Garcia, B.; Férnandez, M. A. *Bioresour. Technol.* **2009**, *100*, 446–451.
30. Winkel-Shirley, B. *Curr. Opin. Plant Biol.* **2002**, *5*, 218–223.
31. Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843–1845.
32. Fang, W.; Ruan, J.; Wang, Z.; Zhao, Z.; Zou, J.; Zhou, D.; Cai, Y. *J. Nat. Prod.* **2006**, *69*, 1641–1644.