PERGAMON

Phytochemistry 51 (1999) 1087–1093

PHYTOCHEMISTRY

Phenolic glycosides from *Eriosema tuberosum*

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Received 11 September 1998; received in revised form 31 December 1998; accepted 31 December 1998

Abstract

Three new phenolic glycosides named eriosemasides A–C, one novel (named eriosematin F) and four known phenolic constituents were isolated from the *n*-BuOH-soluble fraction of the roots of *Eriosema tuberosum*. Their structures were established by spectroscopic analyses and chemical methods. All the phenolic compounds were fungitoxic except the phenolic glycosides.

Keywords: Eriosema tuberosum; Leguminosae; Chromone glycoside; Phenolics; Fungitoxic activity

1. Introduction

The roots of *Eriosema tuberosum* (Leguminosae) have been used as an ethnic medicinal agent in Yunnan province (P.R. China) since ancient times. Their medical uses and the isolation of less polar antifungal phenolic compounds were reported in our previous papers (Ma et al., 1995; Ma, Fuzzati, Xue, Yang, & Hostettmann, 1996; Ma, Fuzzati, Lu, Gu, & Hostettmann, 1996). Recently, the polar isoflavonoid constituents, have been reported in the literature (Ma, Fukushi, Hostettman, & Tahara, 1998). However, antifungal compounds in the methanolic extracts were not identified. This paper deals with the isolation and structure elucidation of four phenolic glycosides and four fungitoxic phenolic compounds from the methanolic extract.

2. Results and discussion

Repeated chromatographic purification of the *n*-BuOH-soluble fraction of the methanolic extract of *E. tuberosum* afforded three new phenolic glycosides (**1**–**3**) and, one novel (**4**) and four known non-glycosidic phenolic constituents (**5**–**8**). The structures of new compounds were established by spectroscopic analyses and chemical methods as 5,7-dihydroxy-8-γ, γ-dimethylallylchromone 7-*O*-rutinoside (**1**), 4-hydroxyphenyl β-D-apiofuranosyl-(1 → 2)-*O*-β-D-glucopyranoside (**2**), 5-*O*-methylgenistein 7-*O*-β-D-apiofuranosyl-(1 → 2)-*O*-β-D-glucopyranoside (**3**) and 3,4 dihydro-4-methoxynaphthalene-2-carboxylic acid (**4**). The known compounds were found to be arbutin (**5**), hydroquinone (**6**), 4-hydroxybenzoic acid (**7**) and vanillic acid (**8**). Antifungal activity of all the isolates was tested on silica gel TLC plates against *Cladosporium herbarum*. The results revealed that only phenolic constituents (**6**–**8**) were fungitoxic.

Compound **1** was obtained as a colourless amorphous powder after freeze drying. Its molecular formula was deduced as $C_{26}H_{34}O_{13}$ from the FD mass

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0031-9422/99/$ - see front matter
PII: S0031-9422(99)00180-6

Table 1
Proton NMR spectral data for compounds **1**, **1a**, **2**–**4** (500 MHz in CD_3OD)[a]

	1	**1a**	**2**	**3**	**4**
Aglycone					
1					7.18 *s*
2	8.09 *d* (6.4)	7.75 *d* (6.4)	6.93 *d* (8.4)	8.06 *s*	
3	6.22 *d* (6.4)	6.18 *d* (6.4)	6.85 *d* (8.4)		3.31–3.44 *m*
4					3.88 *dd* (7.09; 6.3)
5			6.85 *d* (8.4)		7.33 *d* (7.9)
6	6.62 *s*	6.23 *s*	6.93 *d* (8.4)	6.66 *d* (1.5)	7.08 *t* (7.8)
7					7.04 *t* (7.8)
8				6.71 *d* (1.5)	7.62 *d* (7.9)
9					
10					
1′	3.32 *d* (6.4)				
2′	5.21 *t* (6.4)			7.35 *d* (8.8)	
3′				6.82 *d* (8.8)	
4′	1.81 *s*	1.84 *t* (6.2)			
5′	1.65 *s*	2.72 *t* (6.2)		6.82 *d* (8.8)	
6′		1.36 *s* ×2		7.35 *d* (8.8)	
OMe				3.91 *s*	3.25 *s*
Sugar moieties					
Glucose					
1	4.95 *d* (8.1)		4.78 *d* (8.2)	4.79 *d* (8.4)	
2	3.54[b]		3.75[b]	3.72[b]	
3	3.63[b]		3.37[b]	3.29[b]	
4	3.35[b]		3.35[b]	3.34[b]	
5	3.51[b]		3.56[b]	3.64[b]	
6a	3.59 *dd* (12.4; 1.1)		3.65 *dd* (12.2; 6.1)	3.61 *dd* (12.2; 5.4)	
6b	4.03 *dd* (12.4; 0.8)		3.86 *dd* (12.2; 1.4)	3.85 *dd* (12.2; 1.2)	
Apiose					
1			5.45 *d* (1.2)	5.44 *d* (1.2)	
2			3.92 *d* (1.2)	3.92 *d* (2.1)	
3					
4a			3.77 *d* (12.2)	3.75 *d* (12.6)	
4b			4.07 *d* (12.2)	4.04 *d* (12.6)	
5			3.54 *s*	3.54 *s*	
Rhamnose					
1	4.68 *d* (0.8)				
2	3.92 *dd* (9.4; 1.0)				
3	3.75 *dd* (9.4; 2.5)				
4	3.34[b]				
5	3.62[b]				
6	1.17 *d* (7.4)				

[a] Chemical shifts δ in ppm relative to TMS; J values (in Hz) in parenthesis.
[b] Signal pattern unclear due to overlapping.

($[M]^+$ m/z 554), negative FAB mass ($[M-H]^-$ m/z 553), and ^1H and ^{13}C NMR spectral data (Tables 1 and 2). The UV spectrum of this compound in MeOH was characteristic of chromone type compounds previously isolated from this plant (Ma et al., 1996a). The ^1H NMR spectrum of **1** revealed clearly the presence of a preny group [δ 1.65, 1.81 ($2 \times$ Me); 3.32 ($1 \times CH_2$) and 5.21 ($1 \times$ CH)], a trisubstituted chromone moiety [two doublets at δ 8.09 and 6.22, $J = 6.4$ Hz for two protons, a single at 6.62 for one proton] and two hexoses [anomeric protons appeared at δ 4.95 ($J = 8.1$ Hz) and 4.86 ($J = 0.8$ Hz)]. Comparing its ^{13}C NMR spectral data with those of the known eriosematin A (Ma et al., 1996a) suggested compound **1** to be a disaccharide glycoside of eriosematin A. Acid hydrolysis of **1** with 1 N HCl afforded glucose and rhamnose as sugar moieties. Further cleavage of the molecule deserved from mass spectral (m/z 408 $[M\text{-rha}]^+$ and m/z 246 $[M\text{-rha-glc}]^+$ from FD mass spectrum; m/z 407 $[M-H\text{-rha}]^-$ and m/z 245 $[M-H\text{-rha-glc}]^-$ from FAB mass spectrum) suggested that the disaccharide unit may be rutinose. Enzymatic hydrolysis of **1** with β-glucosidase provided the aglycone which was identified as eriosematin A by comparing its NMR data with those reported (Ma et al., 1996b). A pseudo aglycone (**1a**) was obtained from the lipophilic residue after strong acid hydrolysis with HCl, and identified through the analysis of its FD mass ($[M]^+$ m/z 246), ^1H and ^{13}C

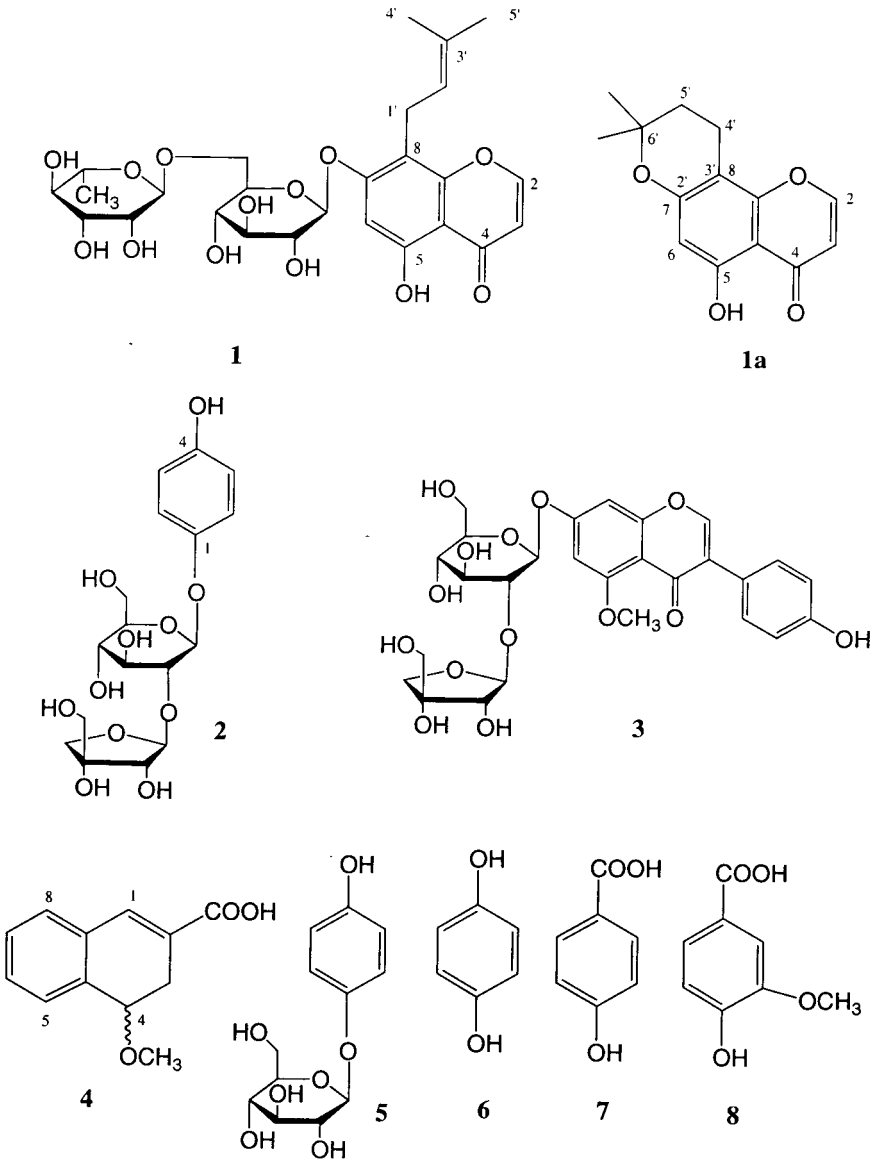
Table 2
^{13}C NMR spectral data for compounds **1**, **1a**, **2**–**4** (125 MHz; in CD_3OD)

	1	1a	2	3	4
Aglycone					
1			152.4		125.1
2	158.6	155.1	119.2	153.0	109.0
3	111.6	111.5	116.7	127.3	24.7
4	184.1	181.3	153.7	178.1	80.6
5	161.2	159.8	116.7	163.6	112.5
6	100.0	101.2	119.2	98.6	122.5
7	162.2	160.4		162.6	120.1
8	111.0	108.5		97.5	119.1
9	156.2	155.6		161.1	128.3
10	108.2	107.5		111.5	138.1
1′	22.7			124.4	
2′	123.2	16.1		131.6	
3′	132.7	31.6		116.1	
4′	18.1	76.1		158.6	
5′	25.9	26.6		116.1	
6′		26.6		131.6	
OMe				56.8	52.8
COOH					171.2
Sugar moieties					
Glucose					
1	102.0		102.3	102.3	
2	74.9		75.5	75.5	
3	77.3		78.1	78.1	
4	71.5		71.5	71.5	
5	78.4		78.7	78.7	
6	67.8		62.6	62.6	
Apiose					
1			110.8	110.8	
2			78.1	78.2	
3			80.8	80.8	
4			75.5	75.3	
5			66.1	66.1	
Rhamnose					
1	102.3				
2	72.1				
3	72.4				
4	74.1				
5	69.9				
6	18.9				

NMR spectral data (Tables 1 and 2). The carbon data of C-7 of **1**, compared with eriosematin A, showed a large down-field shift (+10 ppm) which indicated that the sugar chain was located at C-7 of the aglycone. The results of the HMBC experiment of **1** (Fig. 1) confirmed that the glycosylation position was at C-7, and also that the disaccharide unit was rutinose (terminal rhamnose linked to the C-6 of glucose). Therefore, compound **1** was established as 5,7-dihydroxy-8-γ-γ-dimethylallychromone-7-*O*-rutinoside (eriosemaside A).

Compound **2** was obtained as a colourless amorphous powder after freeze drying. The UV spectrum in MeOH was similar to compound **5** (arbutin), and suggested compound **2** may be a derivative of arbutin. This hypothesis was supported firstly by its FD mass spectrum, which readily demonstrated that it was a simple phenolic disaccharide glycoside from the molecular ion at m/z 404 $[M]^+$, and, successive loss of sugar moiety fragments at m/z 295 $[M+Na\text{-pentose}]^+$, 272 $[M-\text{pentose}]^+$, 133 $[M+Na\text{-pentose-hexose}]^+$ and 110 $[M-\text{pentose-hexose}]^+$. It was clear that the pentose was the terminal sugar moiety of the disaccharide unit. The molecular formula of $C_{17}H_{25}O_{11}$ was deduced from its FD mass and from the ^1H and ^{13}C NMR spectral data (Tables 1 and 2). The ^1H NMR spectrum of **2** demonstrated a hydroquinone as its aglycone through the A_2B_2 coupling system of the benzene ring (δ 6.93, $J=8.4$ for H-2, 4; 6.85, $J=8.4$ for H-3, 5). The other proton signals belonged to the sugar moieties were similar to the known compound (5-*O*-methylgenistein 7-*O*-β-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside) previously isolated from the same source, named here as eriosemaside (Ma et al., 1998). This might suggest that apiose and glucose were the components of sugar units of **2**. Indeed acid hydrolysis of **2** with 0.1 N H_2SO_4 and 1 N HCl (same treatment as eriosemaside) proved the sugar components were apiose and glucose. Careful comparison of its ^1H and ^{13}C NMR spectral data with those of eriosemaside revealed that the configurations of apiose and glucose of compound **2** were the same as eriosemaside. The glycosylation position of the aglycone and the location of the terminal apiose unit to the inner glucose were clearly determined by the relevant correlation between the protons and the carbons observed from its HMBC experiment (Fig. 1). Thus, based on the above analysis, compound **2** was established as 4-hydroxyphenyl β-D-apiofuranosyl-(1 → 2)-*O*-β-D-glucopyranoside, named eriosemaside B.

Compound **3** was obtained from the same fraction from which eriosemaside had been isolated and determined (Ma et al., 1998). Its molecular formula as $C_{27}H_{30}O_4$ was determined from the combination analysis of its FD mass, and ^1H and ^{13}C NMR spectral data (Tables 1 and 2). The UV spectrum and the characteristics of its FD mass, ^1H and ^{13}C NMR spectra were similar to eriosemaside. Careful comparison of its ^{13}C NMR spectral data with those of eriosemaside revealed that the terminal apiose unit of **3** was not connected to C-6 of the inner glucose moiety. The terminal apiose located at the C-2 of the inner glucose in compound **3** was suggested by an obvious glycosylation shift (δ ab. 2 ppm) in its ^{13}C NMR spectrum. In order to confirm this suspicion, an HMBC experiment of **3** was next carried out. The HMBC correlation between anomeric proton of apiose and C-2 of glucose, as well as H-2 of glucose to anomeric carbon of apiose confirmed that the apiose was located at the C-2 position of the glucose (Fig. 1). Therefore, compound **3** was established as 5-*O*-methylgenistein 7-*O*-β-D-apiofuranosyl-(1 → 2)-*O*-β-D-glucopyranoside, named eriosemaside C.



Compound **4** was isolated from the less polar subfraction of the *n*-BuOH soluble fraction. After freezing dry it presented as a pale yellow powder. From its FD mass (quasi-molecular ion at *m/z* 205 $[M+H]^+$), ^1H and ^{13}C NMR spectral data (Tables 1 and 2), a molecular formula as $C_{12}H_{12}O_3$ was given to the compound **4**. In the ^1H NMR spectrum, a set of signals coming from a 1,2-disubstituted aromatic ring were observed. A singlet signal resonating at δ 7.18 was apparently coming from the conjugated system. A methylene proton signal (δ 3.31, *m* for H-3), a methine proton signal (δ 3.88, *dd, J*=7.1, 6.3 for H-4), as well as a methoxyl group signal (δ 3.25, *s*) were also observed from its ^1H NMR spectrum. The COSY experiment of **4** confirmed the *ortho* substitution pattern of the benzene ring and the adjacent relationship between C-3 and C-4. HMBC experiment of **4** revealed that the methoxyl group was located at C-4 through the methine proton (H-4) directly correlated with the oxygenated carbon (C-4). The signal resonated at δ 171.2 ppm in ^{13}C NMR spectrum, together with the absorptions observed from its IR spectrum (3387–2750 cm^{-1}, O-H, stretching absorption; 1710 and 1680 cm^{-1}, carbonyl stretching absorption) indicated that the presence of a carboxyl group in the molecule of **4**. The key correlations between the relevant protons and the carbons observed from its HMBC experiment (Fig. 1) finally established the structure of compound **4** as 3,4 dihydro-4-methoxylnaphthalene-2-carboxylic acid, named eriosematin F.

All the isolates (**1**, **1a**, **2**–**8**) were tested for their antifungal activity against the growth of *Cladosporium herbarum* using silica gel TLC plate bioassay technique (Homans & Fuchs, 1970). Compounds **1a**, **4** and **6**–**8** were found to be active compounds. The minimum inhibitory dose needed to inhibit fungal growth on TLC

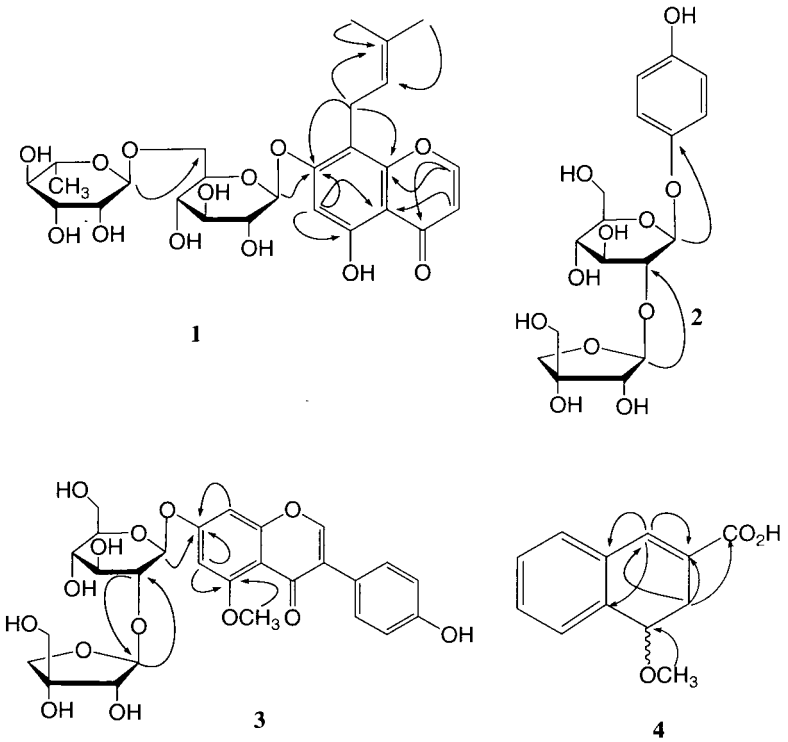


Fig. 1. Key HMBC correlations for establishing sugar sequence and positions of attachments on the aglycone of compounds **1**–**3**. Locations of carboxyl and methoxyl groups of compound **4** were determined through application of the HMBC technique. Each arrow indicates a correlation from the relevant proton to carbon.

was 1 μg for **1a**, 2 μg for **4** and 3 μg in each for compounds **6**–**8**, respectively. As reported previously, the aglycone of compound **1** (eriosematin A) was also an antifungal constituent (Ma et al., 1996a). Its minimum inhibitory dose was 3 μg. Simple phenolic constituents were found to be responsible for the fungitoxic activity of the MeOH extract. However, their corresponding glycosides i.e. compounds **1**, **2** and **5**, were not active against the fungus used in our experiment. It is reasonable to propose that simple fungitoxic phenolic compounds are released from the corresponding glycosides through the enzymatic hydrolysis caused by microbial invasion or herbivore attack on foliage (Harborne, 1988), or due to the isolation procedures. The glycoside form of these components are present in the plant as precursors of 'post-inhibitin'. Although there were some cases that demonstrated that, if the sugar moiety of arbutin were esterified by butyric acid, it will become an antimicrobial and cytotoxic compound. However, the active toxins are also released from such esterified simple phenolic glycoside by the glycoside hydrolysis (Perry & Brennan, 1997). From the root of *E. tuberosum* flavonoids, isoflavonoids and chromones, as well as simple phenolics, together with their corresponding glycosides were isolated and determined. It is clear that simple phenolic compounds are major fungitoxic components. The other types of constituents should be responsible for its medicinal uses as many cases of leguminous medicinal plants (Southon et al., 1994).

3. Experimental

3.1. General

Mps: uncorr. NMR: Bruker-AM 500 spectrometer; Mass spectra: JEOL JMS-SX 102 A instrument; UV: Hitachi U-3210 instrument; Optical rotation: JASCO DIP-370 polarimeter (in MeOH); IR: KBr; TLC: Merck HPTLC RP-18 WF254 plates; Detection: UV 254 and 366 nm and by spraying with Godin reagent followed 5% H_2SO_4 in MeOH; Saccharide identification was carried out on Merck silica gel TLC plates. The information on the plant material studied has been reported previously (Ma et al., 1995).

3.2. Extraction and isolation

Subsequent to the procedure previously described in Ma et al. (1998), sub-fr. 5 was passed through Lobar Diol CC with $CHCl_3$–MeOH (9:1), followed by separ-

ation on Lobar RP-18 CC MeOH–H_2O (8:2) and then repeated gel filtration on Sephadex LH-20 with MeOH to give compound **1** (10 mg). Sub fr. 1 was separated on Lobar Diol CC with $CHCl_3$–MeOH (9:1–7:3) affording pure compound **2** (15 mg) and sub fr. 1a–e. Sub fr. 1a was purified on silica gel CC with $CHCl_3$–MeOH (8:2) followed by gel filtration on Sephadex LH-20 with MeOH to provide compound **5** (20 mg). Sub fr. 1b was chromatographed on Lobar RP-18 CC MeOH–H_2O (1:1) and then subjected to repeated gel filtration on Sephadex LH-20 with MeOH to give compound **4** (3 mg). Sub fr. 1c was repurified by gel filtration on Sephadex LH-20 with MeOH to give compound **8** (5 mg). Sub fr. 1 d was repurified by gel filtration on Sephadex LH-20 with MeOH to give compound **6** (8 mg). Sub fr 1e was repurified by silica gel CC with $CHCl_3$–MeOH (9:1) to afford compound **7** (7 mg). Sub fr. 2a was passed through a Lobar RP-18 CC with MeOH–H_2O (3:7), followed by separation on Lobar Diol CC ($CHCl_3$–MeOH, 8:2) and repeated gel filtration on Sephadex LH-20 with MeOH to give compound **3** (3 mg).

3.3. Bioassay

Bioautography with *C. herbarum* for evaluation of antifungal activity of the samples was performed by TLC bioautography (Homans & Fuchs, 1970).

3.4. Acid hydrolysis of compound *1*

1 (3 mg) was refluxed in 1 N HCl (10 ml) for 2 h. The mixture was cooled and then extracted with EtOAc. The organic layer was evaporated to dryness in vacuo. The residue was dissolved in $CHCl_3$ and repurified on a preparative Si-gel TLC plate developing with $CHCl_3$–MeOH (95:5) to afford an aglycone derivative (**1a**, 1 mg). The aqueous layer was neutralized with $NaHCO_3$ followed by freeze drying. The residue was dissolved in 1 ml MeOH, filtered and concentrated to 0.5 ml. Comparison on TLC with standard sugars indicated those to be glucose and rhamnose (two times development with $CHCl_3$–MeOH–H_2O, 70:30:5, R_fs. 0.18 for glucose and 0.33 for rhamnose).

3.5. Enzymatic hydrolysis of compound *1*

1 (4 mg) was dissolved in 5 ml acetate buffer (pH 5.0) to which 5 mg β-glucosidase (Toyobo Co., Ltd) was added. The soln was then incubated at 32°C for 20 h. Extraction with EtOAc provided the aglycone which was found to be the known eriosematin A (Ma et al., 1996a).

3.6. Acid hydrolysis of compound *2*

2 (5 mg) was dissolved in 10 ml 0.1 N H_2SO_4, with the solution heated until reflux began, this being maintained for 20 min. The mixture was then cooled and H_2O (10 ml) was added. The aqueous layer was extracted with EtOAc, then neutralized with $NaHCO_3$ followed by freeze drying. The water soluble residue was dissolved in 1 ml MeOH, filtered and concentrated to 0.5 ml. Comparison on TLC with an authentic sample gave apiose ($CHCl_3$–MeOH–H_2O, 70:30:5; R_f: 0.38). From the EtOAc extract, arbutin (**5**) was compared with an authentic sample, as determined by TLC plate chromatography ($CHCl_3$–MeOH–H_2O, 70:30:5; R_f: 0.25).

3.7. Hydrolysis of *2* with 1 N HCl

Compound **2** (2 mg) was hydrolyzed as described in Ma et al. (1998).

3.8. Acid hydrolysis of compound *3*

Compound **3** (2 mg) was hydrolyzed as described in Ma et al. (1998).

3.9. Compound *1* (5,7-dihydroxy-8-γ,γ-dimethylallylchromone 7-O-rutinoside, eriosemaside A)

Colourless amorphous powder, mp 119–122°. HPTLC RP-18 (MeOH–H_2O, 7:3) R_f 0.65; $[\alpha]_D^{21}$ −65.0° (*c* 0.20, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 261 (4.25), 314 (3.47), 326 (3.50); IR (KBr) ν_{max} cm^{-1} 3510, 2954, 1670, 1410, 1280, 1108, 830; FD-MS *m/z*: 554 $[M]^+$, 408 $[M\text{-rha}]^+$ 246 $[M\text{-rha-glc}]^+$; negative FAB-MS *m/z*: 553 $[M-H]^-$, 407 $[M-H\text{-rha}]^-$, 245 $[M-H\text{-rha-glc}]^-$; ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

3.10. Compound *1a* (5-hydroxy-6′-dimethyl-6′-dihydropyrano (2,3:7,8) chromone)

Pale yellowish needles from MeOH, mp 193–195°. Silica gel TLC (Hexane–EtOAc, 6:4) R_f 0.55; FD-MS *m/z*: 246 $[M]^+$; ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

3.11. Compound *2* (4-hydroxyphenyl β-D-apiofuranosyl-(1→2)-O-β-D-glucopyranoside, eriosemaside B)

Yellowish amorphous powder, mp 152–156°. Silica gel HPTLC ($CHCl_3$–MeOH–H_2O, 7:3:0.5) R_f 0.25; $[\alpha]_D^{21}$ −76.8° (*c* 0.40, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 223 (4.54), 287 (3.15); IR (KBr) ν_{max} cm^{-1} 3334, 1628, 1517, 1455, 1252, 1050, 972; FD-MS *m/z*: 427 $[M+Na]^+$, 404 $[M]^+$, 295 $[M+Na\text{-apiose}]^+$, 272

[M-apiose]$^+$, 133 [M + Na-apiose-glucose]$^+$, 110 [M-apiose-glucose]$^+$; ^{1}H and ^{13}C NMR spectra: see Tables 1 and 2.

3.12. Compound **3** (5-O-methylgenistein 7-O-β-D-apiofuranosyl-(1→2)-O-β-D-glucopyranoside, eriosemaside C)

Yellowish amorphous powder, mp 159–161°. HPTLC RP-18 (MeOH–H_2O, 1:1) R_f 0.56; $[\alpha]_D^{21}$ −72.5° (*c* 0.22, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 258 (4.34), 282 sh (3.52), 310 sh (3.33); IR (KBr) ν_{max} cm^{-1} 3335, 1640, 1520, 1465, 1275, 1112, 945; FD-MS *m/z*: 579 [M + H]$^+$, 446 [M-apiose]$^+$, 284 [M-apiose-glucose]$^+$; ^{1}H and ^{13}C NMR spectra: see Tables 1 and 2.

3.13. Compound **4** (3,4-dihydro-4-methoxylnaphthalene-2-carboxylic acid, eriosematin F)

Yellowish amorphous powder, mp 169–173°. HPTLC RP-18 (MeOH–H_2O, 1:1) R_f 0.44; $[\alpha]_D^{21}$ −34.2° (*c* 0.038, MeOH); IR (KBr) ν_{max} cm^{-1} 3387, 1710, 1680, 1625, 1459, 748; FD-MS *m/z*: 205 [M + H]$^+$; ^{1}H and ^{13}C NMR spectra: see Tables 1 and 2.

Acknowledgements

The authors are grateful to Mr Kenji Watanabe and Dr Eri Fukushi (GC-MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University) for measuring 2-D NMR and mass spectra.

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