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Chromones from the tubers of *Eranthis cilicica* and their antioxidant activity

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ABSTRACT

Chemical studies on the constituents of *Eranthis cilicica* led to isolation of ten chromone derivatives, two of which were previously known. Comprehensive spectroscopic analysis, including extensive 1D and 2D NMR data, and the results of enzymatic hydrolysis allowed the chemical structures of the compounds to be assigned as 8,11-dihydro-5-hydroxy-2,9-dihydroxymethyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one, 5,7-dihydroxy-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-2-methyl-4*H*-1-benzopyran-4-one, 5,7-dihydroxy-2-hydroxymethyl-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-4*H*-1-benzopyran-4-one, 7-[(β-D-glucopyranosyl)oxy]-5-hydroxy-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-2-methyl-4*H*-1-benzopyran-4-one, 7-[(β-D-glucopyranosyl)oxy]-5-hydroxy-2-hydroxymethyl-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-4*H*-1-benzopyran-4-one, 9-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]methyl-8,11-dihydro-5,9-dihydroxy-2-methyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one, 8,11-dihydro-5,9-dihydroxy-9-hydroxymethyl-2-methyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one, and 7-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]methyl-4-hydroxy-5*H*-furo[3,2-*g*][1]benzopyran-5-one, respectively. The isolated compounds were evaluated for their antioxidant activity.

1. Introduction

The genus *Eranthis* belonging to the family Ranunculaceae is composed of seven species, including *Eranthis cilicica, E. hyemalis* and *E. pinnatifida* (Tsukamoto, 1989). *Eranthis cilicica* Schott et Kotschy is indigenous to Turkey and Afghanistan. Previously, we have reported the structural determination of two new bisdesmosidic triterpene glycosides, namely eranthisaponins A and B, along with four known triterpene glycosides from *E. cilicica* tubers (Watanabe et al., 2003). A literature survey showed that *E. hyemalis* (Kopp et al., 1991; Junior, 1979) and *E. pinnatifida* (Wada et al., 1974) contained a variety of chromone derivatives, which prompted us to make a phytochemical examination of *E. cilicica* tubers with particular attention to chromones. As a result, eight new chromones (**3**–**10**), along with two known chromones (**1** and **2**), were isolated. This paper deals with their structural determination on the basis of spectroscopic analysis, including extensive 1D and 2D NMR data, and the results of enzymatic hydrolysis. The antioxidant activity of the isolated compounds is also described.

2. Results and discussion

The dried tubers of *E. cilicica* (1.3 kg) were extracted with hot MeOH, and the MeOH extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column. The MeOH–H_2O (1:1, v/v) eluate fraction was subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, giving compounds **1** (127 mg), **2** (172 mg), **3** (127 mg), **4** (42.4 mg), **5** (78.1 mg), **6** (25.3 mg), **7** (16.3 mg), **8** (297 mg), **9** (42.5 mg), and **10** (13.0 mg).

Compounds **1** and **2** were identified as 2,3-dihydro-7-hydroxymethyl-2-(1-hydroxy-1-methylethyl)-4-methoxy-5*H*-furo-[3,2-*g*]-[1]-benzopyran-5-one (Sasaki et al., 1982) and 9-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]methyl-8,11-dihydro-5-hydroxy-2-methyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one [eranthin 9-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] (Kopp et al., 1991), respectively.

Compound **3** was obtained as a yellow amorphous solid. Its molecular formula was determined to be $C_{15}H_{14}O_6$ on the basis of the HRESI-TOFMS data, showing an accurate $[M+H]^+$ ion at *m/z* 291.0877. The UV spectrum of **3** had absorption maxima at 324 and 258 nm. The IR spectrum was consistent with the presence of hydroxy groups (3272 cm^{-1}) and a conjugated carbonyl group (1652 cm^{-1}). The ^1H NMR spectrum of **3** (DMSO–d_6) displayed signals for a chelated proton of a hydroxy group at δ 12.76 (*s*), an aromatic proton at δ 6.40 (*s*), two olefinic protons at δ 6.32 (*s*) and 5.92 (*t*-like, *J* = 5.7 Hz), three oxymethylene groups at δ 4.69 (2H, *s*), 4.47

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(2H, *s*), and 3.85 (2H, *s*), and a deshielded methylene group at δ 3.58 (2H, *d*, *J* = 5.7 Hz). These NMR spectroscopic data were closely related to those of the aglycone moiety of **2**. However, the methyl singlet signal at δ 2.44 ($C_{(2)}$–Me) observed in the ^{1}H NMR spectrum of **2** was absent from that of **3** and was replaced by the oxymethylene resonace at δ 4.47. In the HMQC spectrum of **3**, the oxymethylene proton signal was associated with the one-bond coupled carbon resonance at δ 60.6. These data indicated that the methyl group attached to C-2 of the chromone skeleton in **2** is modified to a hydroxymethyl group in **3**. This was supported by long-range correlations between the oxymethylene signal at δ 4.47 and the C-2 (δ 172.0) and C-3 (δ 106.5) carbon resonances. Thus, structure **3** was identified as 8,11-dihydro-5-hydroxy-2,9-dihydroxymethyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one.

Compound **4** was shown to have the molecular formula $C_{15}H_{16}O_5$ on the basis of the HRESI-TOFMS (*m/z* 277.1064 $[M+H]^+$) data. The UV spectrum of **4** had absorption maxima at 299 and 258 nm. The IR spectrum showed absorption bands of hydroxy groups at 3228 cm^{-1} and a conjugated carbonyl group at 1658 cm^{-1}. The ^{1}H NMR spectrum exhibited signals for a chelated proton of a hydroxy group at δ 12.79 (*s*), an aromatic proton at δ 6.27 (*s*), two olefinic protons at δ 6.15 (*s*) and 5.37 (*t*-like, *J* = 7.4 Hz), two methyl groups attached to double bonds at δ 2.36 and 1.73 (each 3H, *s*), an oxymethylene group at δ 3.76 (2H, *s*), and a deshielded methylene group at δ 3.33 (2H, *d*, *J* = 7.4 Hz). The above spectral features were essential analogous to those of the aglycone moiety of **2**. However, the signal due to the H_2-8 oxymethylene group in the oxepin moiety, which was observed at δ 4.74 (2H, *s*) in the ^{1}H NMR spectrum of **2**, could not be detected in **4**. In the HMBC spectrum of **4**, the olefinic proton at δ 5.37 (H-2') showed long-range correlations with the hydroxymethyl carbon at δ 67.1 (C-4') and the methyl carbon at δ 14.4 (C-5'), whereas the deshielded methylene protons at δ 3.33 (H_2-1') were correlated with the aromatic carbons of the chromone nucleus at δ 162.4 (C-7), 106.5 (C-8), and 155.9 (C-8a), together with the quaternary olefinic carbon at δ 136.2 (C-3') (Fig. 1). The H_2-1' methylene protons showed proton spin-coupling correlations with the H-2' olefinic proton in the ^{1}H–^{1}H COSY spectrum. Furthermore, the aromatic proton at δ 6.29 (H-6) and a chelated proton of the C-5 hydroxy group at δ 12.70 showed long-range correlations with the aromatic carbon at δ 159.5 (C-5). These data implied that the oxepin ring is opened and that a 4'-hydroxy-3'-methylbut-2'-enyl group is located at the C-8 position in **4**. In the PHNOESY spectrum of **4**, an NOE correlation between the protons of H-2' and H_2-4' provided evidence for the (2'*E*)-geometry. Thus, structure **4** was established as 5,7-dihydroxy-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-2-methyl-4*H*-1-benzopyran-4-one.

Compound **5**, obtained as an amorphous yellow solid, was analyzed for $C_{15}H_{16}O_6$ on the basis of HRESI-TOFMS (*m/z* 293.1037 $[M+H]^+$), which was higher than that of **4** by one oxygen atom. Comparison of the ^{1}H and ^{13}C NMR spectra of **5** with those of **4** completed the following information: The methyl group located at C-2 position of **4**, of which the ^{1}H and ^{13}C NMR signals were observed at δ_H 2.36 and δ_C 20.8, was missing in **5**. Instead, resonances for an oxymethylene group showed up at δ_H 4.42 (*s*) and δ_C 60.6. Moreover, the HMBC spectrum of **5** featured cross-peaks from the oxymethylene protons to the C-2 (δ 171.4) and C-3 (δ 105.9) carbons, which implied the locus of a hydroxymethyl group at C-2. Therefore, **5** was established as 5,7-dihydroxy-2-hydroxymethyl-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-4*H*-1-benzopyran-4-one.

Compound **6** was obtained as an amorphous yellow solid with a molecular formula $C_{21}H_{26}O_{10}$, as determined by HRESI-TOFMS (*m/z* 439.1637 $[M+H]^+$). As in the case of **4**, the ^{1}H NMR spectrum exhibited signals for a chelated proton of a hydroxy group at δ 12.79 (*s*), an aromatic proton at δ 6.58 (*s*), two olefinic protons at δ 6.24 (*s*) and 5.40 (*t*-like, *J* = 7.3 Hz), two methyl groups attached to double bonds at δ 2.39 and 1.75 (each 3H, *s*), an oxymethylene group at δ 3.76 (2H, *s*), and a deshielded methylene group at δ 3.55 (*dd*, *J* = 14.1, 7.3 Hz) and 3.36 (*dd*, *J* = 14.1, 7.3 Hz). In addition, a resonance due to an anomeric proton of a hexopyranosyl group could be observed at δ 4.97 (*d*, *J* = 7.3 Hz). Enzymatic hydrolysis of **6** with naringinase gave **4** and D-glucose. Identification of D-glucose, including its absolute configuration, was carried out by direct HPLC analysis of the hydrolysate. In the HMBC spectrum of **6**, the anomeric proton showed a long-range correlation with the oxygenated aromatic carbon at δ 160.6, which exhibited long-range correlations with the H-6 (δ 6.58) and H_2-1' (δ 3.55 and 3.36) protons and was assigned to C-7 of the aglycone (Fig. 2). Thus, structure **6** was defined as 7-[(β-D-glucopyranosyl)oxy]-5-hydroxy-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-2-methyl-4*H*-1-benzopyran-4-one.

Compound **7** had the molecular formula $C_{21}H_{26}O_{11}$ on the basis of HRESI-TOFMS (*m/z* 455.1572 $[M+H]^+$), which differed from **6** by containing one more oxygen atom. When the ^{1}H NMR spectrum of **7** was compared with that of **6**, the signal for the $C_{(2)}$–methyl group, which was observed at δ 2.39 in **6**, disappeared in **7**, and a resonance for a hydroxymethyl group was detected at δ 4.44 (2H, *s*). These ^{1}H NMR spectroscopic data, along with the hydroxymethyl carbon signal at δ 59.5, allowed the structure of **7** to be assigned as 7-[(β-D-glucopyranosyl)oxy]-5-hydroxy-2-hydroxymethyl-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-4*H*-1-benzopyran-4-one.

Compound **8** was obtained as an amorphous yellow solid. Its molecular formula was determined as $C_{27}H_{34}O_{16}$ by HRESI-TOFMS, showing an $[M+H]^+$ peak at *m/z* 615.1920. The spectroscopic properties of **8** were similar to those of **2** and were

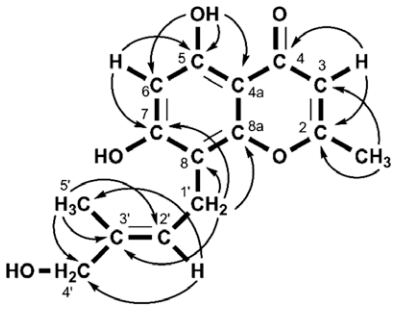


Fig. 1. HMBC correlations of **4**.

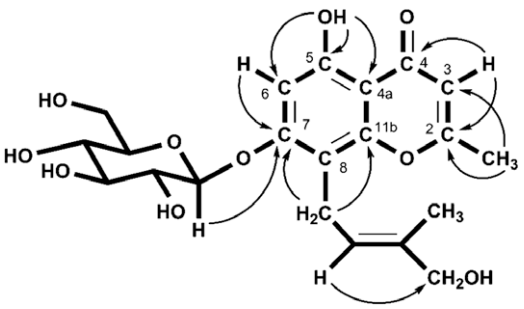


Fig. 2. HMBC correlations of **6**.

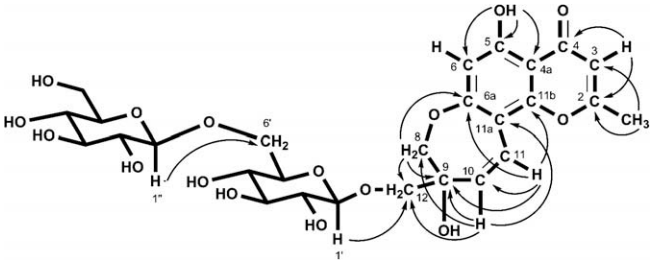
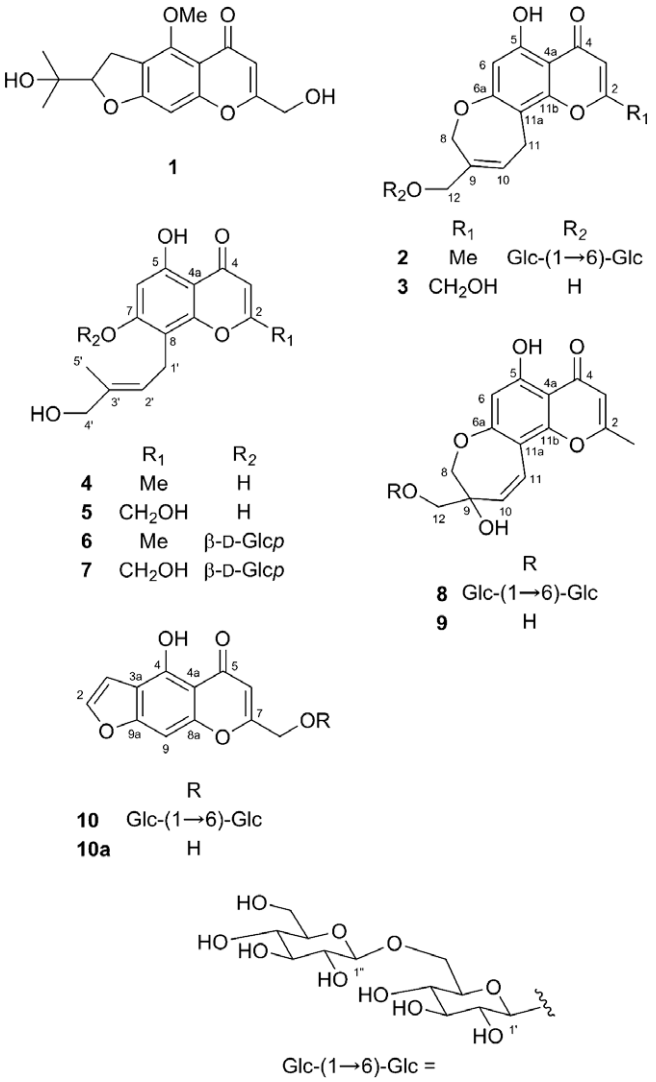


Fig. 3. HMBC correlations of **8**.

suggestive of a chromone diglucoside related to **2**. However, differences between **8** and **2** were observed in the NMR signals arising from the oxepin moiety, which is connected through C-6a and C-11a. On the basis of the ^{1}H NMR spectroscopic data, the oxepin moiety of **8** was composed of two oxymethylene groups [δ 4.34 (*d*, *J* = 11.6 Hz, H-8a) and 3.93 (*d*, *J* = 11.6 Hz, H-8b); δ 3.82 (*d*, *J* = 10.6 Hz, H-12a) and 3.46 (*d*, *J* = 10.6 Hz, H-12b)] and a pair of olefinic protons [δ 6.80 (*d*, *J* = 12.4 Hz, H-11); δ 5.87 (*d*, *J* = 12.4 Hz, H-10)]. Furthermore, the ^{13}C NMR spectrum showed signal due to a quaternary carbon bearing a hydroxy group at δ 73.5 (C-9). In the HMBC spectrum, long-range correlations were observed between H-11 and C-6a (δ 164.3)/C-11b (δ 155.0)/C-9 (δ 73.5)/C-10 (δ 132.7), and between H-10 and C-8 (δ 74.2)/C-9 (δ 73.5)/C-11a (δ 105.8)/C-12 (δ 73.0) (Fig. 3). Thus, the oxepin moiety with a double bond at C-10(11), and a hydroxy and an oxymethylene group at C-9 was shown to be attached to the chromone ring through C-6a and C-11a. A linkage of a β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl group to C-12 as in **2** was ascertained by HMBC correlations between the H-1 proton of the terminal glucosyl moiety at δ 4.22 and C-6 of the inner glucosyl moiety at δ 68.5, and between the H-1 proton of the inner glucosyl at δ 4.24 and C-12 at δ 73.0. On the basis of the above evidence, structure **8** was determined as 9-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]methyl-8,11-dihydro-5,9-dihydroxy-2-methyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one.

Compound **9** had the molecular formula $C_{15}H_{14}O_6$ on the basis of HRESI-TOFMS (*m/z* 291.0877 $[M+H]^+$). The ^{1}H and ^{13}C NMR spectra of **9** strongly suggested that it is identical to the aglycone of **8**. Enzymatic hydrolysis of **8** with naringinase resulted in the production of **9** and D-glucose. The structure **9** was elucidated as 8,11-dihydro-5,9-dihydroxy-9-hydroxymethyl-2-methyl-4*H*-pyrano-[2,3-*g*][1]benzoxepin-4-one.

Compound **10**, isolated as an amorphous yellow solid, showed an $[M+H]^+$ ion at *m/z* 577.1525 in HRESI-TOFMS, corresponding to the empirical molecular formula $C_{24}H_{28}O_{15}$. Analysis of the ^{1}H and ^{13}C NMR spectra of **10** implied that it was a chromone diglycoside whose sugar sequence is the same as that of **2** and **8**. On comparison of the ^{1}H NMR spectrum of the aglycone moiety of **10** with that of **5**, the AX pattern signals at δ 8.05 and 7.12 (each *d*, *J* = 2.2 Hz), assignable to H-2 and H-3 of a furan ring, appeared in **10** instead of the signals for the (2*E*)-4-hydroxy-3-methylbut-2-enyl unit in **5**. Enzymatic hydrolysis of **10** with naringinase liberated 4-hydroxy-7-hydroxylmethyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (**10a**, norkhellol) (Cao et al., 2005) and D-glucose. The diglycoside *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl group was ascertained to be linked to C-7 of the aglycone by analysis of the HMBC spectrum of **10**. Thus, the structure of **10** was characterized as 7-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]methyl-4-hydroxy-5*H*-furo[3,2-*g*][1]benzopyran-5-one.



The antioxidative property of the isolated compounds was examined against superoxide anion using a chemiluminescence assay. Although the chromone glycosides **2**, **6**, **7**, **9**, and **10** did not show any apparent superoxide anion scavenging activity even at a sample concentration of 1000 μg/ml, the 2-hydroxymethylchromone derivatives **1**, **3**, and **5** were slightly active with IC_{50} values of 179, 198, and 274 μg/ml, respectively. In **1**, **3**, and **5**, the 2-hydroxymethyl group may contribute to the appearance of their antioxidant activity. Epigallocatechin gallate used as a positive control had an IC_{50} value of 3.0 μg/ml.

3. Concluding remarks

Eight new chromone derivatives (**3**–**10**), along with two known ones, were isolated from the tubers of *E. cilicica*, and the structures of the new chromones were determined by spectroscopic analysis, including extensive 1D and 2D NMR data, and the results of enzymatic hydrolysis. Chromones are a group of natural products with the 4*H*-1-benzopyran-4-one skeleton and are considered to be distributed in the relatively limited species of higher plants belonging to the family Ranunculaceae, Umbelliferae, Leguminosae, and Cneoraceae on the basis of the facts reported up to the present (Harborne et al., 1999). In plants of the family Ranunculaceae, chromones have been identified only in genus *Eranthis* (Kopp

et al., 1991; Junior, 1979; Wada et al., 1974) and *Cimicifuga* (Cao et al., 2005; Kondo and Takemoto, 1972). This is the first comprehensive report on chromone constitusion of *E. cilicica*.

4. Experimental

4.1. General

Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimater. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ-value with reference to tetramethylsilane (TMS) as internal standard. HRESI-TOFMS data were obtained on a Waters-Micromass LCT mass spectrometer (Manchester, UK). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography (CC). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh) or a Shodex OR-2 detector (Showa Denko, Tokyo, Japan), and a Rheodyne injection port. A Capcell Pak C_{18} UG120 column (10 mm i.d. $\times$ 250 mm, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC. The following reagents were obtained from the indicated companies: xanthine oxidase (Sigma, St. Luis, MO, USA); hypoxantnine (Wako, Osaka, Japan); MPEC (2-methyl-6-*p*-methoxyphenylethynylimidazopyrazinone) (ATTO, Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

4.2. Plant material

Eranthis cilicica was purchased from a nursery in Heiwaen, Japan, in October 2000 and was identified by Dr. Yutaka Sashida, emeritus professor of the Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (voucher No. 00-7-EC, Laboratory of Medicinal Pharmacognosy).

4.3. Extraction and isolation

The dry tubers of *E. cilicica* (1.3 kg) were extracted with MeOH (10 l $\times$ 2) under reflux for 2 h. Following removal of MeOH, the residue (135 g) suspended in MeOH–H_2O (3:7, v/v) was applied to a Diaion HP-20 column [MeOH–H_2O (3:7, v/v), MeOH–H_2O (1:1, v/v), MeOH–H_2O (4:1, v/v), MeOH, EtOH, and EtOAc (each 3 l)]. The MeOH–H_2O (1:1, v/v) eluate portion (35 g) was subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (20:10:1) and MeOH alone, to give five fractions (I–V). Fraction II was purified by ODS silica gel CC eluted with MeOH–H_2O (11:9) into three subfractions (II-1–II-3). Fraction II-1 was applied to a silica gel column, this being eluted with $CHCl_3$–MeOH (49:1; 19:1) to yield **4** (42.4 mg). Fraction II-2 was subjected to silica gel CC eluted with $CHCl_3$–MeOH (49:1) to give **3** (127 mg) and **8** (297 mg). Fraction II-3 was applied to a silica gel column eluted with $CHCl_3$–MeOH (14:1; 9:1) to yield **1** (127 mg) and **5** (78.1 mg), and **6** with a few impurities, which was further purified by preparative HPLC using MeOH–H_2O (1:1) to furnish **6** (25.3 mg). Fraction III was subjected to ODS silica gel CC eluted with MeOH–H_2O (11:9; 1:1) and silica gel CC with $CHCl_3$–MeOH–H_2O (20:10:1) to yield **7** (16.3 mg). Compounds **2** (172 mg), **9** (42.5 mg), and **10** (13.0 mg) were isolated from fraction IV via preparative HPLC using MeCN–H_2O (1:4).

4.4. Compound **3**

8,11-Dihydro-5-hydroxy-2,9-dihydroxymethyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one (**3**); amorphous yellow solid; HRESI-TOFMS (positive-mode) *m/z*: 291.0877 $[M+H]^+$ (calculated for $C_{15}H_{15}O_6$, 291.0869); UV λ_{max} (log ε) nm: 324 (3.68), 258 (4.33); IR ν_{max} (film) cm^{-1}: 3272 (OH), 1652 (C=O); ^{1}H NMR (DMSO–d_6): δ 12.76 (1H, *s*, $C_{(5)}$–O*H*), 6.40 (1H, *s*, H-6), 6.32 (1H, *s*, H-3), 5.92 (1H, *t*-like, *J* = 5.7 Hz, H-10), 4.69 (2H, *s*, H-8), 4.47 (2H, *s*, $C_{(2)}$–CH_2OH), 3.85 (2H, *s*, H-12), 3.58 (2H, *d*, *J* = 5.7 Hz, H-11); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.5. Compound **4**

5,7-Dihydroxy-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-2-methyl-4*H*-1-benzopyran-4-one (**4**); amorphous yellow solid; HRESI-TOFMS (positive-mode) *m/z*: 277.1064 $[M+H]^+$ (calculated for $C_{15}H_{17}O_5$, 277.1076); UV λ_{max} (log ε) nm: 299 (3.80), 258 (4.39); IR ν_{max} (film) cm^{-1}: 3228 (OH), 1658 (C=O); ^{1}H NMR (DMSO–d_6): δ 12.79 (1H, *s*, $C_{(5)}$–O*H*), 6.27 (1H, *s*, H-6), 6.15 (1H, *s*, H-3), 5.37 (1H, *t*-like, *J* = 7.4 Hz, H-2'), 3.76 (2H, *s*, H-4'), 3.33 (2H, *d*, *J* = 7.4 Hz, H-1'), 2.36 (3H, *s*, $C_{(2)}$–*Me*), 1.73 (3H, *s*, Me-5'); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.6. Compound **5**

5,7-Dihydroxy-2-hydroxy-methyl-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-4*H*-1-benzopyran-4-one (**5**); amorphous yellow solid; HRESI-TOFMS (positive-mode) *m/z*: 293.1037 $[M+H]^+$ (calculated for $C_{15}H_{17}O_6$, 293.1025); UV λ_{max} (log ε) nm: 301 (3.72), 258 (4.30); IR ν_{max} (film) cm^{-1}: 3276 (OH), 1669 (C=O); ^{1}H NMR (DMSO–d_6): δ 12.78 (1H, *s*, $C_{(5)}$–O*H*), 6.29 (1H, *s*, H-6), 6.22 (1H, *s*, H-3), 5.37 (1H, *t*-like, *J* = 7.2 Hz, H-2'), 4.42 (2H, *s*, $C_{(2)}$–*Me*), 3.75 (2H, *s*, H-4'), 3.34 (2H, *m*, H-1'), 1.72 (3H, *s*, Me-5'); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.7. Compound **6**

7-[(β-D-Glucopyranosyl)oxy]-5-hydroxy-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-2-methyl-4*H*-1-benzopyran-4-one (**6**); amorphous yellow solid; $[\alpha]_D^{25}$ −38.3° (*c* 0.05; MeOH); HRESI-TOFMS (positive-mode) *m/z*: 439.1637 $[M+H]^+$ (calculated for $C_{21}H_{27}O_{10}$, 439.1640); UV λ_{max} (log ε) nm: 325 (3.66), 254 (4.36); IR ν_{max} (film) cm^{-1}: 3375 (OH), 1657 (C=O); ^{1}H NMR (DMSO–d_6): δ 12.79 (1H, *s*, $C_{(5)}$–O*H*), 6.58 (1H, *s*, H-6), 6.24 (1H, *s*, H-3), 5.40 (1H, *t*-like, *J* = 7.3 Hz, H-2'), 4.97 (1H, *d*, *J* = 7.3 Hz, H-1''), 3.76 (2H, *s*, H-4'), 3.71 (1H, *br d*, *J* = 11.9 Hz, H-6"a), 3.55 (1H, *dd*, *J* = 14.1, 7.3 Hz, H-1'a), 3.46 (1H, *dd*, *J* = 11.9, 5.7 Hz, H-6"b), 3.36 (1H, *dd*, *J* = 14.1, 7.3 Hz, H-1'b), 2.39 (3H, *s*, $C_{(2)}$–*Me*), 1.75 (3H, *s*, H-5'); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.8. Enzymatic hydrolysis of **6**

Compound **6** (9.4 mg) was dissolved in AcOH–NaOAc buffer (pH 5.0, 5 ml) with naringinase (Sigma, EC 232-962-4, β-glucosidase activity: 69 units/g) (15.0 mg) and incubated at room temperature for 14 h. The crude reaction mixture was applied to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (7:4:1) to yield **4** (3.9 mg) and a sugar fraction (1.5 mg). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex Sugar SC1011 (8.0 mm i.d. $\times$ 300 mm, 5 μm, Showa Denko); solvent, H_2O; flow rate, 1.0 ml/min; column temperature, 80 °C; detection, RI and OR. Identification of D-glucose was carried out by comparison of its retention time and optical rotation with those of authentic samples. R_t (min): 7.61 (D-glucose, positive polarity).

Table 1
^{13}C NMR spectroscopic data for **3**–**10** in DMSO–d_6.

Position	3	8	9	Position	4	5	6	7	Position	10
2	172.0	168.7	171.4	2	168.3	171.4	168.3	171.4	2	147.3
3	106.5	108.6	105.5	3	108.5	105.9	108.1	105.5	3	104.9
4	183.5	182.6	182.5	4	182.9	183.0	182.5	182.5	3a	113.4
4a	107.3	106.1	105.4	4a	104.2	104.7	105.0	105.4	4	159.2
5	160.0	160.3	159.5	5	159.9	160.0	159.5	159.5	4a	106.3
6	104.6	102.5	98.4	6	99.1	99.2	98.3	98.4	5	184.7
6a	165.1	164.3	165.0	7	162.4	162.6	160.6	160.8	6	107.2
8	70.8	74.2	75.1	8	106.5	106.6	108.1	108.2	7	168.7
9	140.4	73.5	66.2	8a	155.9	155.6	154.5	154.1	8a	155.2
10	123.1	132.7	134.7	1'	21.5	21.4	21.0	20.9	9	92.0
11	21.6	117.3	117.5	2'	121.7	121.7	121.1	121.0	9a	154.5
11a	111.4	105.8	106.7	3'	136.2	136.2	135.6	135.7	$C_{(2)}$–CH_2O–	66.3
11b	154.1	155.0	155.6	4'	67.1	67.1	66.5	66.5		
12	63.8	73.0	66.2	5'	14.4	14.4	13.8	13.7		
$C_{(2)}$–CH_2–	60.6	20.1	20.8	$C_{(2)}$–CH_2–	20.8	60.6	20.2	59.5		
1'		103.9		1''			100.7	100.7	1'	104.4
2'		73.7		2''			73.5	73.5	2'	74.2
3'		76.5		3''			76.7	76.7	3'	77.6
4'		70.9		4''			69.8	69.8	4'	70.9
5'		76.0		5''			77.3	77.3	5'	76.7
6'		68.5		6''			60.8	60.8	6'	69.5
1''		103.4							1''	103.3
2''		73.7							2''	74.4
3''		76.9							3''	77.4
4''		70.0							4''	70.9
5''		77.0							5''	77.7
6''		61.1							6''	61.9

4.9. Compound **7**

7-[(β-D-Glucopyranosyl)oxy]-5-hydroxy-2-hydroxymethyl-8-[(2*E*)-4-hydroxy-3-methylbut-2-enyl]-4*H*-1-benzopyran-4-one (**7**), amorphous yellow solid; $[\alpha]_D^{25}$ −38.3° (*c* 0.05; MeOH); HRESI-TOFMS (positive-mode) *m/z*: 455.1572 $[M+H]^+$ (calculated for $C_{21}H_{27}O_{11}$, 455.1553); UV λ_{max} (log ε) nm: 327 (3.59), 255 (4.30); IR ν_{max} (film) cm^{-1}: 3375 (OH), 1659 (C=O); ^{1}H NMR (DMSO–d_6): δ 12.74 (1H, *s*, $C_{(5)}$–OH), 6.60 (1H, *s*, H-6), 6.29 (1H, *s*, H-3), 5.39 (1H, *t*-like, *J* = 7.4 Hz, H-2'), 4.98 (1H, *d*, *J* = 7.5 Hz, H-1''), 4.44 (2H, *s*, $C_{(2)}$–CH_2OH), 3.75 (2H, *s*, H-4'), 3.70 (1H, *br d*, *J* = 11.5 Hz, H-6''a), 3.54 (1H, *dd*, *J* = 14.2, 7.4 Hz, H-1'a), 3.47 (1H, *dd*, *J* = 11.5, 5.0 Hz, H-6''b), 3.35 (1H, *dd*, *J* = 14.2, 7.4 Hz, H-1'b), 1.74 (3H, *s*, H-5'); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.10. Compound **8**

9-[(*O*-β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]methyl-8,11-dihydro-5,9-dihydroxy-2-methyl-4*H*-pyrano[2,3-*g*][1]benzoxepin-4-one (**8**); amorphous solid; $[\alpha]_D^{25}$ −5.57° (*c* 0.10; MeOH); HRESI-TOFMS (positive-mode) *m/z*: 615.1920 $[M+H]^+$ (calculated for $C_{27}H_{35}O_{16}$, 615.1925); UV λ_{max} (log ε) nm: 338 (3.57), 258 (4.54); IR ν_{max} (film) 3376 (OH), 1660 (C=O); ^{1}H NMR (DMSO–d_6): δ 13.01 (1H, *s*, $C_{(5)}$–O*H*), 6.80 (1H, *d*, *J* = 12.4 Hz, H-11), 6.43 (1H, *s*, H-6), 6.27 (1H, *s*, H-3), 5.87 (1H, *d*, *J* = 12.4 Hz, H-10), 4.34 (1H, *d*, *J* = 11.6 Hz, H-8a), 4.24 (1H, *d*, *J* = 7.4 Hz, H-1'), 4.22 (1H, *d*, *J* = 7.5 Hz, H-1''), 3.97 (1H, *br d*, *J* = 10.3 Hz, H-6'a), 3.93 (1H, *d*, *J* = 11.6 Hz, H-8b), 3.82 (1H, *d*, *J* = 10.6 Hz, H-12a), 3.63 (1H, *dd*, *J* = 11.4, 5.2 Hz, H-6''a), 3.57 (1H, *dd*, *J* = 11.5, 6.3 Hz, H-6'b), 3.46 (1H, *d*, *J* = 10.6 Hz, H-12b), 3.40 (1H, *dd*, *J* = 11.4, 5.8 Hz, H-6''b), 2.44 (3H, *s*, $C_{(2)}$–*Me*); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.11. Compound **9**

8,11-Dihydro-5,9-dihydroxy-9-hydroxymethyl-2-methyl-4*H*-pyrano-[2,3-*g*][1]benzoxepin-4-one (**9**); amorphous yellow solid; $[\alpha]_D^{25}$ −2.6° (*c* 0.10; MeOH); HRESI-TOFMS (positive-mode) *m/z*: 291.0877 $[M+H]^+$ (calculated for $C_{15}H_{14}O_6$, 291.0869); UV λ_{max} (log ε) nm: 334 (3.51), 257 (4.68); IR ν_{max} (film) cm^{-1}: 3387 (OH), 1660 (C=O); ^{1}H NMR (DMSO–d_6): δ 13.00 (1H, *s*, $C_{(5)}$–O*H*), 6.74 (1H, *d*, *J* = 12.4 Hz, H-11), 6.39 (1H, *s*, H-6), 6.31 (1H, *s*, H-3), 5.85 (1H, *d*, *J* = 12.4 Hz, H-10), 4.29 (1H, *d*, *J* = 11.5 Hz, H-8a), 3.89 (1H, *d*, *J* = 11.5 Hz, H-8b), 3.41 (2H, *s*, H-12), 2.44 (3H, *s*, $C_{(2)}$–*Me*); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.12. Enzymatic hydrolysis of **8**

Compound **8** (20.0 mg) was subjected to enzymatic hydrolysis with naringinase as described for **6** to give an aglycone **9** (6.4 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of that of **6** showed the presence of D-glucose. R_t (min): 7.73 (D-glucose, positive polarity).

4.13. Compound **10**

7-[(*O*-β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]methyl-4-hydroxy-5*H*-furo[3,2-*g*][1]benzopyran-5-one (**10**); amorphous solid; $[\alpha]_D^{25}$ −41.8° (*c* 0.10; MeOH); HRESI-TOFMS (positive-mode) *m/z*: 557.1525 $[M+H]^+$ (calculated for $C_{24}H_{29}O_{15}$, 557.1506); UV λ_{max} (log ε) nm: 340 (3.45), 252 (4.49); IR ν_{max} (film) cm^{-1}: 3376 (OH), 1659 (C=O); ^{1}H NMR (DMSO–d_6): δ 13.65 (1H, *s*, $C_{(5)}$–O*H*), 8.05 (1H, *d*, *J* = 2.2 Hz, H-2), 7.39 (1H, *s*, H-8), 7.12 (1H, *d*, *J* = 2.2 Hz, H-3), 6.65 (1H, *s*, H-6), 4.77 and 4.68 (each 1H, *d*, *J* = 15.7 Hz, $C_{(2)}$–CH_2O–), 4.34 (1H, *d*, *J* = 7.8 Hz, H-1''), 4.25 (1H, *d*, *J* = 7.8 Hz, H-1'), 4.02 (1H, *br d*, *J* = 11.5 Hz, H-6'a), 3.66 (1H, *dd*, *J* = 11.6, 4.9 Hz, H-6''a), 3.57 (1H, *dd*, *J* = 11.5, 7.1 Hz, H-6'b), 3.42 (1H, *d*, *J* = 11.6, 5.8 Hz, H-6''b); for ^{13}C NMR (DMSO–d_6) spectroscopic data, see Table 1.

4.14. Enzymatic hydrolysis of **10**

Compound **10** (6.5 mg) was subjected to enzymatic hydrolysis with naringinase as described for **6** to give norkhellol (**10a**,

1.8 mg) and sugar fractions (3.0 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **6** showed the presence of D-glucose. R_t (min): 7.73 (D-glucose, positive polarity).

4.15. Assay for antioxidant activity

Superoxide anion production was measured using the chemiluminescent probe, 2-methyl-6-*p*-methoxyphenylethynylimidazopyrazinone (MPEC) (Shimomura et al., 1998; Ishii et al., 2005). Each test sample (10 ml) dissolved in DMSO was added to a solution containing 10 μl of MPEC (300 μM), 170 μl of buffer (100 μM KH_2PO_4–NaOH, pH 7.5, 50 μM EDTA), 60 μl of xanthine oxidase (0.1 unit/ml), and 50 μl of hypoxanthine (3.6 mM) to give the final concentrations of 1–1000 μg/ml, which was then stirred at 30 °C for 20 s. The intensity of chemiluminescence of the mixture was automatically determined at 30 °C for 30 s using an AB-2200-R Luminescencer-PSN (ATTO). The superoxide anion radical scavenging activity was expressed as the sample concentration (μg/ml) necessary to give a 50% reduction in the sample intensity of luminescence (IC_{50}).

References

Cao, P., Pu, X.F., Peng, S.L., Zhang, X.R., Ding, L.S., 2005. Chemical constituents from *Cimicifuga foetida*. J. Asian Nat. Prod. Res. 7, 145–149.

Tsukamoto, Y. (Ed.), 1989. In: The Grand Dictionary of Horticulture, vol. 3. Shogakukan, Tokyo, pp. 63–64 (in Japanese).

Harborne, J.B., Baxter, H., Moss, G.P., (Eds.), 1999. In: Phytochemical Dictionary. Taylor and Francis, London, pp. 381-389.

Ishii, T., Yasuda, K., Akatsuka, A., Hino, O., Hartman, P.S., Ishii, N., 2005. A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis. Cancer Res. 65, 203–209.

Junior, P., 1979. Eranthin and eranthin-β-D-glucoside: two new chromones from *Eranthis hiemalis*. Phytochemistry 18, 2053–2054.

Kondo, Y., Takemoto, T., 1972. The structure of Cimifugin, a new bitter principle from *Cimicifuga simplex* WORMSK. Chem. Pharm. Bull. 20, 1940–1944.

Kopp, B., Kubelka, E., Reich, C., Robien, W., Kubelka, W., 1991. 4*H*-Chromenone glycosides from *Eranthis hyemalis* (L.) Salisbury. Helv. Chim. Acta 74, 611–616.

Sasaki, H., Taguchi, H., Endo, T., Yosioka, I., 1982. The constituents of *Ledebouriella seseloides* Wolff I. Structures of three new chromones. Chem. Pharm. Bull. 30, 3555–3562.

Shimomura, O., Wu, C., Murai, A., Nakamura, H., 1998. Evaluation of five imidazopyrazinone-type chemiluminescent superoxide probe and their application to the measurement of superoxide anion generated by *Listeria monocytogenes*. Anal. Biochem. 258, 230–235.

Wada, H., Gaino, M., Saito, S., 1974. Furochromones of *Eranthis pinnatifida*. Phytochemistry 13, 297–299.

Watanabe, K., Mimaki, Y., Sakuma, C., Sashida, Y., 2003. Eranthisaponins A and B, two new bisdesmosidic triterpene saponins from the tubers of *Eranthis cilicica*. J. Nat. Prod. 66, 879–882.