A New Chromone, 11-Hydroxy-*sec-O*-glucosylhamaudol from *Ostericum koreanum*

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From the ethyl acetate fraction of the roots of *Ostericum koreanum*, a new chromone, 11-hydroxy-*sec-O*-glucosylhamaudol (1) along with the known compounds: four chromones, three coumarins, six phenolic compounds, and three quinic acids were isolated. These compounds were assessed for antioxidant activities in the DPPH radical and superoxide anion radical scavenging assay systems. Among isolates, 4-(2-hydroxy-vinyl)-benzene-1,2-diol (12) showed the most potent DPPH radical scavenging activity (IC_{50}=4.80±0.62 μg/ml) and superoxide anion radical scavenging activity (IC_{50}=11.05±0.83 μg/ml) in the xanthine/xanthine oxidase system. The antioxidant activities of 12 were comparable to those of quercetin and luteolin.

Key words *Ostericum koreanum* (Umbelliferae); 11-hydroxy-*sec-O*-glucosylhamaudol; chromone; coumarin; quinic acid; antioxidant activity

The roots of *Ostericum koreanum* MAX. (Umbelliferae) have long been used in the oriental medicine for the treatment of common cold, headache, neuralgia and arthralgia.[1)] Several coumarins, such as isoimperatorin, oxypeucedanin, bergapten, osthol, oxypeucedanin hydrate and marmesinin have been shown to be present in the extracts of this plant.[2)]

In this paper, we describe the isolation of a new chromone, 11-hydroxy-*sec-O*-glucosylhamaudol (**1**) along with the 15 known compounds: three chromones: *sec-O*-glucosylhamaudol (**2**) and *prim-O*-glucosylcimifugin (**3**),[3)] cimifugin (**4**),[4)] three coumarins: marmesinin (**5**),[5)] oxypeucedanin hydrate (**6**)[6)] and bergaptol-*O*-β-D-glucopyranoside (**7**),[7)] six phenolic compounds: ligustiphenol (**8**),[8)] 2-methoxy-2-(4′-hydroxyphenyl)-ethanol (**9**),[9)] 4-(2-hydroxy-vinyl)-2-methoxy-phenol (**10**),[10)] 3-methoxy-benzene-1,4-diol (**11**), 4-(2-hydroxy-vinyl)-benzene-1,2-diol (**12**) and protocatechuic acid (**13**) and three quinic acids: 5-caffeoylquinic acid methyl ester (**14**),[11)] 3,5-dicaffeoylquinic acid (**15**) and 4,5-dicaffeoylquinic acid (**16**).[12)] and their subsequent structural determination by spectroscopic analysis.

The EtOAc extract the roots of *O. koreanum* was purified by column chromatography using Sephadex LH-20, as well as a combination of chromatography over silica gel and RP-18, to yield a new chromone (**1**) along with the 15 known compounds (Fig. 1).

Compound **1** was obtained as a white powder. The ^{1}H- and ^{13}C-NMR spectra of **1** indicated a structure similar to those of *sec-O*-glucosylhamaduol from *Ledebouriella seseloides*[3)] except for the methylene peak at δ 4.47. The signals at δ 6.36 (s, 8) and 6.30 (s, 3) were characteristic for the chromone of **1**. The signals at δ 2.77 (dd, J=6.8, 17.2 Hz, H-4′a) and 3.01 (dd, J=5.2, 17.2 Hz, H-4′b), 4.13 (1H, dd, J=5.4, 6.4 Hz, H-3′) and 1.38, 1.42 (each 3H, s, gem-CH_3) can be assigned to disubstituted pyran ring. In addition, the signal of anomeric proton appeared at δ 4.48 (d, J=7.4 Hz) with coupling constants characteristic of a β-configuration. In the HMBC spectrum, the hydroxylated methylene signal at δ 4.47 (C-11) was correlated with the signal at δ 171.7 (C-2) and the signal of anomeric proton at δ 4.48 showed a ^{1}H–^{13}C long range correlation with a signal of the C-3′ at δ 74.6, respectively. The stereochemistry at C-3′ position was determined to be *S*-configuration because of very similar CD to those of *sec-O*-glucosylhamaudol and **1**, respectively.[3)] On the basis of the evidence obtained, compound **1** was assigned to 11-hydroxy-*sec-O*-glucosylhamaudol.

All of the isolates were assessed for antioxidant activities in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and superoxide anion radical scavenging assay systems (Table 1). For comparisons, quercetin, luteolin and resveratrol were in-

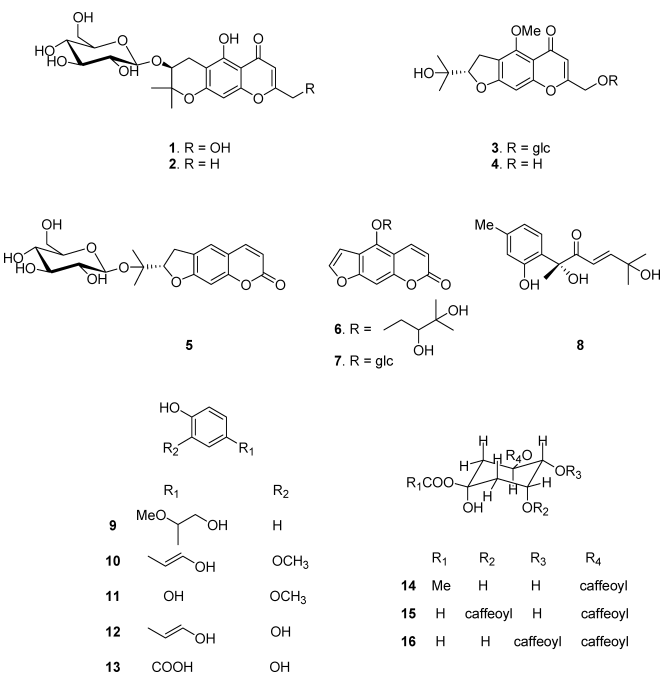

Fig. 1. Compounds Isolated from EtOAc Extract of the Roots of *O. koreanum*

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Table 1. DPPH and Superoxide Anion Radicals Scavenging Activities of Isolated Compounds from Roots of *O. pretearicum*[a)]

Compound	IC_{50} (μg/ml) on DPPH	IC_{50} (μg/ml) on superoxide anion
1—**9**	>50	>50
10	14.6±2.0	>50
11	49.6±0.1	>50
12	4.80±0.6	11.1±0.8
13	9.63±0.2	45.4±2.3
14	10.8±0.2	37.2±2.4
15	10.3±0.1	19.9±1.0
16	12.9±0.5	23.2±1.1
Quercetin	4.10±0.1	18.0±1.8
Luteolin	5.77±0.1	9.46±0.3
Resveratrol	21.8±1.3	>50

a) All values are averages of at least three runs.

cluded as positive controls. Among tested, chromones including 11-hydroxy-*sec*-*O*-glucosylhamaudol (**1**) and coumarins did not exhibit noticeable antioxidant activities, while phenolics and caffeoylquinic acids showed potent antioxidant activities on two assay systems. 4-(2-Hydroxy-vinyl)-benzene-1,2-diol (**12**) showed the most potent DPPH radical scavenging activity (IC_{50}=4.80±0.62 μg/ml) and superoxide anion radical scavenging activity (IC_{50}=11.05±0.83 μg/ml) in the xanthine/xanthine oxidase system among isolates. The antioxidant activities of **12** were comparable to those of quercetin and luteolin, and more potent than those of caffeoylquinic acid derivatives (**14**—**16**).

Experimental

Optical rotations were determined on an Autopol III Automatic polarimeter (Rudolph Research Flanders, NJ, U.S.A.). IR spectra were recorded on a Midac High Resolution FT-IR spectrometer in KBr disks. NMR spectra were recorded on a Bruker 300 and 400 spectrometer. ^{1}H–^{1}H COSY, DEPT, HMQC and HMBC NMR spectra were obtained with the usual pulse sequences.

Plant Material The roots of *O. koreanum* were purchased from a local Korean herbal drug market in June 2004 and identified by Emeritus Professor Chang Soo Yook at Kyung Hee University in Korea. Voucher specimens (952-31) have been deposited in the laboratory of Korea Institute of Science and Technology (KIST).

Extraction and Isolation The dried roots of *O. koreanum* (6 kg) were cut into small pieces and extracted three times with MeOH at room temperature. The methanol extract (935 g) was suspended in water and then partitioned in turns with CH_2Cl_2, EtOAc, and *n*-butanol. The EtOAc extract (4.9 g) was first subjected to column chromatography using Sephadex LH-20 eluting with MeOH to yield fourteen fractions (Fr. 1—Fr. 14). Fraction 4 (2.0 g) was divided by column chromatography using Sephadex LH-20 and EtOH as an eluent. Fraction 4a (1.5 g) was divided by column chromatography over silica gel using CH_2Cl_2 : MeOH (20 : 1→10 : 1) and CH_2Cl_2 : MeOH : H_2O (5 : 1 : 0.1→5 : 1.3 : 0.2) system to give fourteen fractions (Fr. 4a1—4a14). Fraction 4a6 (366.2 mg) was purified by column chromatography on silica gel eluting with EtOAc : CH_2Cl_2 (5 : 1→7 : 1) and CH_2Cl_2 : MeOH (15 : 1) system to yield compounds **4** (189.5 mg), **6** (4.2 mg), **8** (4.4 mg) and **9** (28.9 mg). Fraction 4a10 (389.5 mg) was purified by column chromatography on LiChroprep RP-18 eluting with MeOH : H_2O (3 : 7→7 : 3) gradient system to yield compounds **1** (4.4 mg), **2** (41.9 mg), **3** (5.7 mg) and **5** (9.6 mg), respectively. Fraction 5 (1.1 g) was divided by column chromatography over silica gel using CH_2Cl_2 : MeOH (20 : 1→10 : 1) and CH_2Cl_2 : MeOH : H_2O (5 : 1 : 0.1→2 : 1.5 : 0.2) gradient system to give ten fractions (Fr. 5a—5j). Fraction 5b (148.9 mg), 5g (184.4 mg) was purified by column chromatography on LiChroprep RP-18 column eluting with MeOH : H_2O (4 : 6→7 : 3) gradient system to give compounds **10** (59 mg), **11** (8.2 mg) and **13** (6.6 mg), respectively. Fraction 5d was purified by column chromatography on LiChroprep RP-18 column eluting with MeOH : H_2O (3 : 7→6 : 4) gradient system to afford compounds **7** (5.1 mg), **12** (27.3 mg) and **14** (34.7 mg). Fraction 7 (572.6 mg) was purified by column chromatography on LiChroprep RP-18 column eluting with MeOH : H_2O (3 : 7→7 : 3) gradient system to yield compounds **15** (5.3 mg) and **16** (4.1 mg), respectively.

11-Hydroxy-*sec*-*O*-glucosylhamaudol (**1**): White powder; mp 139.5—145 °C; $[\alpha]_D^{24}$ −70° (*c*=0.22, MeOH); CD [MeOH, nm ($\Delta(\varepsilon)$)]; 225 (−5.96), 255 (+9.10); UV (MeOH) λ_{max} (log ε): 255 (1.79), 215 (3.06) nm; IR bands (KBr): 3430, 2924, 2861, 1632, 1313, 1158, 1077, 809, 621 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD): δ 1.38, 1.42 (each 3H, s, gem-CH_3), 2.77 (1H, dd, *J*=6.8, 17.2 Hz, 4′a), 3.01 (1H, dd, *J*=5.2, 17.2 Hz, 4′b), 3.18 (1H, dd, *J*=7.8, 9.1 Hz, 2″), 3.32—3.42 (3H, m, 2″,3″,4″, overlapped with solvent), 3.67 (1H, dd, *J*=5.5, 11.8 Hz, 6″a) 3.91 (1H, dd, *J*=1.8, 11.6 Hz, 6″b), 4.13 (1H, dd, *J*=5.4, 6.4 Hz, 3″), 4.47 (2H, s, 11), 4.48 (1H, d, *J*=7.6 Hz, 1″), 6.30 (1H, s, 3), 6.36 (1H, s, 8); ^{13}C-NMR (100 MHz, CD_3OD): δ 22.2 (gem-CH_3), 22.4 (C-4′), 25.9 (gem-CH_3), 61.4 (C-11), 62.9 (C-6″), 71.8 (C-4″), 74.6 (C-3′), 74.9 (C-2″), 78.1 (C-3″ and 5″), 79.3 (C-2′), 95.8 (C-8), 101.7 (C-1″), 105.1 (C-6), 105.4 (C-10), 106.4 (C-3), 157.4 (C-9), 160.5 (C-5), 161.0 (C-7), 171.8 (C-2), 184.2 (C-4); HR-FAB-MS *m*/*z*: 455.1546 ($[M+H]^+$, Calcd for $C_{21}H_{27}O_{11}$: 455.1553).

Scavenging of DPPH Radicals The potential antioxidant activity of pure compounds was assessed on the basis of scavenging activity of the DPPH free radical. Reactions mixtures containing test samples (dissolved in EtOH) and 100 μM DPPH ethanolic solution in 96-well microtiter plates were incubated at 37 °C for 30 min. Absorbances were measured at 515 nm. Percent inhibition was determined by comparison with an ethanol-treated control group. IC_{50} values denote the concentration of samples required to scavenge 50% DPPH free radicals.[13)]

Assay for Inhibitory Activity of Superoxide Anion Radical by Xanthine Oxidase The reaction mixture consisted of 40 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.1 mM EDTA, 50 μg protein/ml of bovine serum albumin, 25 mM NBT, and 1.4×10^{-8} unit xanthine oxidase (EC 1.2.3.2) in final volume of 200 μl. After incubation at 25 °C for 20 min, the reaction was terminated by the addition of 6.6 μl of 6 mM $CuCl_2$. The absorbance of formazan produced was determined at 560 nm, and IC_{50} values donate the concentration of samples required to scavenge 50% superoxide anion radicals.[14)]

Acknowledgments The authors are grateful to Emeritus Professor Chang Soo Yook at Kyung Hee University in Korea for identification of the specimen. This research was supported by a grant (PF0320202-01) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government.

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