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Estrogenic activity of constituents from the rhizomes of *Rheum undulatum* Linné

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ABSTRACT

Stilbenes have been reported to be phytoestrogen compounds owing to its structural similarity to the estrogenic agent diethylstilbestrol. To find new stilbene-derivative phytoestrogens, isolation of stilbene-rich *R. undulatum* was performed and led to identify six new compounds (**1**–**5** and **28**), one newly determined absolute configurations compound (**27**) together with 21 previously reported compounds (**6**–**26**). The structures of compounds were determined on the basis of extensive spectroscopic methods including 1D and 2D NMR and CD spectra data. All the isolated compounds were tested for their estrogenic activities in HepG2 cells transiently transfected with ERα, ERβ and ERE-reporter plasmid. Among them, stilbene-derivatives, piceatannol 3′-*O*-β-D-xylopyranoside (**12**), *cis*-rhaponticin (**16**) and rhapontigenin 3′-*O*-β-D-glucopyranoside (**17**), showed the more potent binding affinity for estrogen receptors than 17β-estrodiol.

Estrogen plays an important role in the growth, differentiation and function of many bodily targets, including the female and male reproductive systems. Estrogens, including phytoestrogens, act via the estrogen receptor (ER), a member of the nuclear receptor superfamily. The biological responses triggered by estrogen are brought by binding of the hormone to a specific nuclear ER. These hormone-bound ER mediates biological response to initiate gene transcription.[1] Therefore, estrogen-like compounds can be potential candidates for drug development. Owing to its structural similarity to the estrogenic agent, diethylstilbestrol, several stilbenes such as resveratrol and *trans*-resveratrol have been reported to be phytoestrogen compounds.[2,3] In addition, an extract from the roots of stilbene-rich *Rheum rhaponticum* (Rhapontic Rhubarb) has been used in Germany as an herbal medicine for the treatment of menopausal symptoms since the 1950s.[4] It was developed as dietary supplement named Estrovera® for menopausal hot flash relief by providing stilbenes of rhaponticin and deoxyrhaponticin.

Stilbenes are considered to be important components in chemotaxonomy to discriminate rhubarbs based on the quantity of rhaponticin.[5] A number of varieties of rhubarb has been used both as medicinal plants and for human consumption. Among various rhubarbs, *R. undulatum* is especially known for having stilbene derivatives as the principal constituents.[6–9] *R. undulatum* Linné (Polygonaceae), a perennial herb commonly known as Korean rhubarb, is mainly distributed and cultivated in South Korea. It is a well-known medicinal plant that has been widely used as laxatives, anti-inflammatories and anti-blood stagnation agents in East Asia.[10,11] In Europe, the leaves of *R. undulatum* are widely used for alimentary purposes. Thus, as a part of an investigation to find new stilbene phytoestrogens, phytochemical investigation of *R. undulatum* was performed and led to the isolation of six new compounds (**1**–**5** and **28**), one newly determined absolute configurations compound (**27**) together with 21 previously reported compounds (**6**–**26**)[10] (Fig. 1). These isolated compounds were tested for their estrogenic activity in ERα, ERβ and the target ERE-dependent promoter-transfected HepG2 cells by measuring binding affinity for estrogen receptors.

The rhizomes of *R. undulatum* were purchased at Kyung-dong herbal market in Seoul, Korea in 2015 and authenticated by Dr. Rack-Seon Seong, a director of Center of Natural Resources Research, Jeonnam Bioindustry Foundation. A voucher specimen (RU201506) is deposited at the Herbarium of College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon, Korea.

The methanol extract of the rhizomes of *R. undulatum* was suspended in water and partitioned with $CHCl_3$ and EtOAc to obtain three layers. Using various chromatographic resin and isolation techniques, six new compounds along with 19 known compounds

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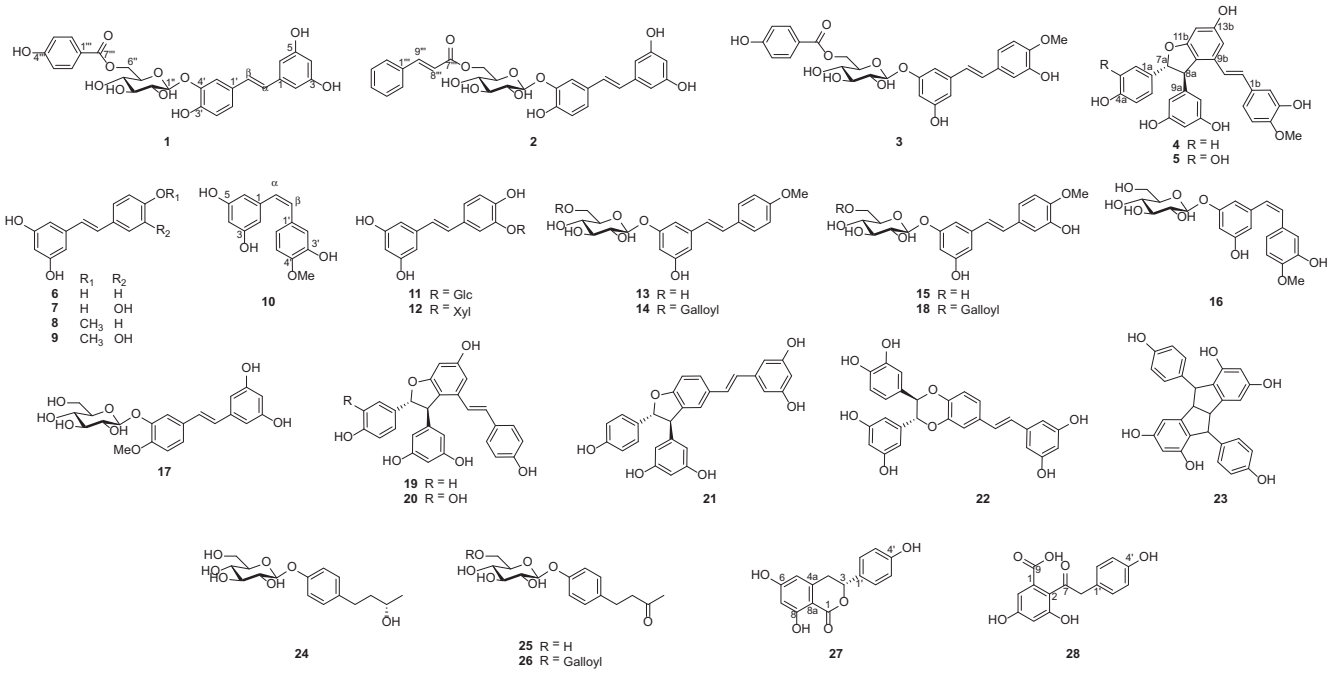

Fig. 1. Chemical structures of compounds **1**–**28**.

were isolated. The known compounds were compared with those reported ^{1}H, ^{13}C NMR and MS data in the literature and identified as resveratrol, piceatannol, deoxyrhapontigenin, rhapontigenin (**6**–**9**),[11] *cis*-rhapontigenin (**10**),[12] astringin, piceatannol 3′-*O*-β-D-xylopyranoside, deoxyrhaponticin, 6″-galloyl deoxyrhaponticin, rhaponticin, *cis*-rhaponticin, rhapontigenin 3′-*O*-β-D-glucopyranoside, 6″-galloyl rhaponticin (**11**–**18**),[5] (−)-ε-viniferin (**19**),[13] scirpusin A (**20**),[14] *trans*-δ-viniferin (**21**),[15] cassigarol E, pallidol (**22**–**23**),[16] (−)-rhododendrol 4′-*O*-β-D-glucopyranoside (**24**),[17] 4-(4′-hydroxyphenyl)-2-butanone-4′-*O*-β-D-glucopyranoside (**25**),[18] and lindleyin (**26**)[19] (Fig. 1). Among 19 known compounds, the absolute configuration of (3*R*)-thunberginol C (**27**) was determined for the first time in present study.

Compound **1**[20] was obtained as white amorphous powder and its molecular formula was determined as $C_{27}H_{26}O_{11}$ by HR-ESI-MS ion at *m*/*z* 525.1389 $[M-H]^-$ (calcd for $C_{27}H_{25}O_{11}$, 525.1397). The ^{1}H NMR spectrum of **1** displayed typical proton signals of piceatannol[11]: *trans*-olefinic protons at δ_H 6.77 (d, *J* = 15.9 Hz) and 6.87 (d, *J* = 15.9 Hz) and two aromatic rings with one ABX-type signals at δ_H 6.80 (d, *J* = 8.4 Hz), 7.07 (dd, *J* = 2.0, 8.4 Hz) and 7.29 (d, *J* = 2.0 Hz) and one AX_2-type signals at δ_H 6.14 (t, *J* = 2.2 Hz) and 6.39 (d, *J* = 2.2 Hz). The β-linkage of the glucosyl moiety was deduced from the coupling constant of 7.2 Hz of the anomeric proton signal at δ_H 4.90. In addition, another A_2B_2-type aromatic protons at δ_H 6.67 (d, *J* = 8.4 Hz) and 7.83 (d, *J* = 8.4 Hz) were exhibited (Table 1). The ^{13}C NMR and DEPT spectra of compound **1** revealed the signals 27 carbons including nine quaternary carbons, 17 methines and one methylene carbon (Table 1). The analysis NMR data of **1** indicated the structure of **1** was similar to those of piceatannol 3′-*O*-β-D-(6′′-*O*-galloyl)glucopyranoside[5] except for the replacement of galloyl group with a hydroxybenzoyl group. The HMBC correlations between H-1″ (δ_H 4.90) and C-3′ (δ_C 146.6) suggested the presence of β-glucopyranosyl sugar moiety at C-3′. In addition, the position of a hydroxybenzoyl group was verified by the HMBC correlation between H-6″ (δ_H 4.38 and 4.70)/H-2‴, 6‴ (δ_H 7.83) and C-7‴ (δ_C 168.1), concluding that the hydroxybenzoyl group is located at C-6″ (Fig. 2). The NMR data of hydroxybenzoyl sugar moiety was consistent with those of houttuynoside A.[21] Based on the above data, compound **1** was identified as piceatannol 3′-*O*-(6′′-4-hydroxybenzoyl)-β-D-glucopyranoside.

The molecular formula of **2**[22] was determined as $C_{29}H_{28}O_{10}$ by the HR-ESI-MS pseudoion at *m*/*z* 535.1651 $[M-H]^-$ (calcd for $C_{29}H_{27}O_{10}$, 535.1604). The ^{1}H NMR spectrum of **2** showed typical proton signals of piceatannol along with a *trans*-olefinic protons at δ_H 6.37 (d, *J* = 16.0 Hz) and 7.56 (d, *J* = 16.0 Hz) and a phenyl group at δ_H 7.17 (d, *J* = 8.8 Hz), 7.20 (d, *J* = 8.8 Hz) and 7.24 (m). The ^{13}C NMR and DEPT spectra of compound **2** showed the signals 29 carbons including eight quaternaries, 20 methines and one methylene carbon (Table 1). The analysis NMR data of **2** indicated the structure **2** was similar to those of **1** except for the replacement of the hydroxybenzoyl group with cinnamoyl group. The HMBC correlations between H-6″ (δ_H 4.36 and 4.59)/H-8‴ (δ_H 6.37) and C-7‴ (δ_C 168.6) suggested the cinnamoyl group located at C-6″ (Fig. 2). Consequently, the structure of **2** was determined to be piceatannol 3′-*O*-(6″-*E*-cinnamoyl)-β-D-glucopyranoside.

HR-ESI-MS of **3**[23] gave an ion peak at *m*/*z* 539.1561 $[M-H]^-$ (calcd for $C_{28}H_{27}O_{11}$, 539.1553), suggesting molecular formula of $C_{28}H_{28}O_{11}$. ^{1}H NMR spectrum of **3** presented the signals of rhapontigenin[5]: a methoxy signal at δ_H 3.84 (s), a *trans*-olefinic protons at δ_H 6.75 (d, *J* = 16.0 Hz) and 6.92 (d, *J* = 16.0 Hz) and two aromatic rings with one ABX-type signals at δ_H 6.85 (d, *J* = 8.4 Hz), 6.85 (dd, *J* = 2.2, 8.4 Hz) and 6.94 (d, *J* = 2.2 Hz) and one 1,3,5-substituted benzene ring with signals at δ_H 6.46 (t, *J* = 2.2 Hz), 6.61 (s) and 6.70 (s). In addition, the signals for hydroxybenzoyl group along with β-linkage of the glucosyl moiety were detected as those of compound **1**. The NMR data of **3** were similar to those of rhaponticin 6′′-*O*-gallate[5] except for the replacement of galloyl group with a hydroxybenzoyl group. The HMBC correlations between H-1″ (δ_H 4.99) and C-3 (δ_C 160.3) and between H-6″ (δ_H 4.31 and 4.74)/H-2‴, 6‴ (δ_H 7.86) and C-7‴ (δ_C 168.1) suggested the presence of β-glucopyranosyl sugar moiety and the hydroxybenzoyl group at C-3 and C-6″, respectively (Fig. 2). Thus, based on evidence above, compound **3** was elucidated as 6″-4-hydroxybenzoyl rhaponticin.

Compound **4**[24] was obtained as white amorphous powder and its molecular formula was confirmed as $C_{29}H_{24}O_7$ indicated by