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Estrogenic and anti-estrogenic compounds from the Thai medicinal plant, *Smilax corbularia* (Smilacaceae)

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ABSTRACT

From the rhizomes of *Smilax corbularia* Kunth. (Smilacaceae), 11 compounds, (2*R*,3*R*)-2″-acetyl astilbin, (2*R*,3*R*)-3″-acetyl astilbin, (2*R*,3*R*)-4″-acetyl astilbin, (2*R*,3*R*)-3″-acetyl engeletin, (2*R*,3*S*)-4″-acetyl isoastilbin, 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*R*)-2H,8H-benzo [1,2-b:3,4-b′] dipyran-8-one, 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*S*)-2H, 8H-benzo [1,2-b:3,4-b′] dipyran-8-one, 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxyphenyl)-5-[(1*E*)-2-(4-hydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one, 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxy-phenyl)-5-[(1*E*)-2-(3,4-dihydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one, 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-5-[(1*E*)-2-(4-hydroxyphenyl) ethenyl]-2H-1-benzopyran-2-one, and 5,7,3′,4′-tetrahydroxy-3-phenylcoumarin along with 34 known compounds were isolated and characterized as 19 flavonoids, 14 catechin derivatives, 6 stilbene derivatives, and 6 miscellaneous substances. All isolates had their estrogenic and anti-estrogenic activities determined using the estrogen-responsive human breast cancer cell lines MCF-7 and T47D. The major constituents were recognized as flavanonol rhamnosides by the suppressive effect on estradiol induced cell proliferation at a concentration of 1 μM. Meanwhile, flavanonol rhamnoside acetates demonstrated estrogenic activity in both MCF-7 and T47D cells at a concentration of 100 μM, and they enhanced the effects of co-treated E2 on T47D cell proliferation at concentrations of more than 0.1 μM.

1. Introduction

As a part of our continued research on estrogenic compounds from Thai medicinal plants, a methanol extract of *Smilax corbularia* Kunth. (Smilacaceae) was investigated. *Smilax corbularia* is a climbing vine distributed in South-East Asia, and its rhizomes have mostly been used as a Thai folk medicine not only for the treatment of female diseases, such as breast and ovary cancers but for AIDS treatment (Tewtrakul et al., 2006). However, no scientific reports about the constituents of *S. corbularia* have been published.

This paper deals with the isolation of constituents from these plants, their structural characterization by spectroscopic methods, and the assessment of the estrogenic and anti-estrogenic activity of the isolates using the estrogen responsive MCF-7 and T47D cell lines.

2. Results and discussion

The methanol extract of rhizomes of *S. corbularia* (70 g) was partitioned with ethyl acetate and water. The ethyl acetate-soluble and water-soluble fractions had their estrogenic and anti-estrogenic activities tested in MCF-7 and T47D cells. As a result, it was found that the ethyl acetate-soluble fraction enhanced proliferation of T47D at 10 μg/mL and suppressed activity of co-treated estradiol in both cell lines, especially in MCF-7 cells, at 1 μg/mL. Due to these results, the ethyl acetate-soluble fraction was subjected to silica gel column chromatography and eluted with chloroform–methanol–water as a gradient solvent system to afford 15 combined fractions. Further purification of the active fractions was carried out using preparative HPLC to give 45 pure compounds including 11 new compounds (**1**–**11**). The following 34 known compounds were identified by comparisons of their spectroscopic data and optical rotations with the values reported in the literature: astilbin (**12**) (De Britto et al., 1995), neoastilbin (**13**) (De Britto et al., 1995), isoastilbin (**14**) (Gaffield et al., 1975), neoisoastilbin (**15**) (De Britto et al., 1995), engeletin (**16**) (Gaffield et al., 1975), isoengeletin (**17**) (Gaffield et al., 1975), (+) taxifolin (**18**) (Nonaka et al., 1987), (+) dihydrokaempferol (**19**) (Xu et al., 2005), naringenin (**20**) (Perry et al., 1999), eriodictyol (**21**) (Gaffield et al., 1975), homoeriodictyol (**22**) (Ibrahim et al., 2003), quercetin (**23**) (Lee et al., 2004), quercitrin (**24**) (Fukunaga et al., 1988), luteolin (**25**) (Sugamoto et al., 2008), (−) catechin (**26**) (Kumar and Rajapaksha, 2005), (−) epicatechin (**27**) (Ban et al., 2006), cinchonain Ia (**28**) (Chen et al., 1993), catechin-(7,8-b,c)-4b-(3,4-dihydroxyphenyl)-2(3H)-pyranone (**29**) (Chen et al., 1993), cinchonain Ib (**30**) (Chen

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et al., 1993), rhinchoin Ia (**31**) (Foo, 1987), cinchonain Id (**32**) (Chen et al., 1993), (4*S*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2H,8H-benzo[1,2-b:3,4-b′]dipyran-2-one (**33**) (Chen et al., 1993), cinchonain Ic (**34**) (Chen et al., 1993), (4*R*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2H,8H-benzo[1,2-b:3,4-b′]dipyran-2-one (**35**) (Chen et al., 1993), phyllocoumarin (**36**) (Foo, 1989), epiphyllocoumarin (**37**) (Foo, 1989), *trans*-resveratrol (**38**) (Nakajima et al., 1978), piceatannol (**39**) (Yao et al., 2005), isorhapontigenin (**40**) (Silayo et al., 1999), eucryphin (**41**) (Tschesche et al., 1979), (−) syringaresinol (**42**) (Deyama, 1983), 5-*O*-caffeoylshikimic acid (**43**) (Silayo et al., 1999), caffeic acid (**44**) (Flamini et al., 2001), and protocatechuic acid (**45**) (Wu et al., 2007) (Fig. 1).

The spectroscopic features of the new compounds (**1**–**5**) were very similar to one another, suggesting they were flavonoid glycosides and 2″-acetyl astilbin (**1**) was assigned the molecular formula, $C_{23}H_{24}O_{12}$, as determined from its molecular ion $[M+H]^+$ peak at *m*/*z* 493.1355 in HRFABMS. The ^{1}H NMR spectrum of **1** showed a pair of doublet aromatic proton signals [δ 5.90 (d, J = 2 Hz) and 5.92 (d, J = 2 Hz)] and ABX-type aromatic proton signals [δ 6.76 (d, J = 8 Hz), 6.79 (dd, J = 8, 2 Hz) and 6.93 (d, J = 2 Hz)], and it was suggested that the aglycone moiety was a 2,3-*trans* flavanonol from the characteristic signals for H-2 and 3 observed at δ 5.11 (d, J = 10.5 Hz) and 4.53 (d, J = 10.5 Hz), respectively. The ^{1}H NMR spectrum also indicated the presence of a rhamnose moiety from the following signals: [δ 3.97 (d, J = 2 Hz), 1.18 (d, J = 6 Hz)], which are unique for the H-1″ and Me-6″ of the sugar, respectively. Acid hydrolysis of **1** gave (+)-taxifolin (**18**) as an aglycone and a sugar moiety, which was identified by GC analysis as L-rhamnose. The HMBC spectrum of **1** indicated the bonding position of L-rhamnose to be C-3 according to the H–C long-range connectivity of H-1″ [δ 3.97 (d, J = 2 Hz)] to C-3 (δ 78.3). The spectrum supported the NMR assignments since the aromatic proton signals at δ 6.79 (dd, J = 8, 2 Hz) and 6.93 (d, J = 2 Hz) showed correlations with C-2 (δ 83.7). Furthermore, a correlation of the proton resonance of rhamnose at δ 4.87 (H-2″) with the carbonyl carbon signal at δ 171.5 (Me<u>C</u>O-2″) suggested that the bonding position of the acetyl moiety was C-2″. The absolute configuration of the C-2 of **1** was determined as 2*R* due to the detection of a positive Cotton effect ($[\theta]_{328}$ + 6900, $[\theta]_{295}$ − 39,400) in the CD spectrum (Gaffield et al., 1975). On the basis of the above spectroscopic evidence, **1** was found to be a new compound with the structure (2*R*,3*R*)-5,7,3′,4′-tetrahydroxyflavanonol 2″-acetyl rhamnoside or (2*R*,3*R*)-2″-acetyl astilbin.

3″-Acetyl astilbin (**2**), 4″-acetyl astilbin (**3**), and 4″-acetyl isoastilbin (**5**) were assigned the molecular formula $C_{23}H_{24}O_{12}$, which is same as that of **1**, as determined from their molecular ion $[M+Na]^+$ peaks at *m*/*z* 515.1174, 515.1146, and 515.1188 in HRFABMS, respectively. Their ^{1}H NMR spectra indicated that they shared a pair of doublet aromatic proton signals around the δ 5.9, ABX-type aromatic proton signal at δ 6.8–7.0 and rhamnose sugar moieties. From their ^{1}H NMR spectra, **2** and **3** were deduced to be as 2,3-*trans* flavanonol rhamnosides from their H-2 coupling constants [**2**: δ 5.09 (d, J = 11 Hz), **3**: 5.06 (d, J = 11 Hz)], whereas **5** was found to be a 2,3-*cis* flavanonol rhamnoside [**5**: δ 5.42 (d, J = 2 Hz)]. Acid hydrolysis of these compounds (**2**,**3**, and **5**) gave (+)-taxifolin (**18**) as an aglycone with a common sugar moiety, which was identified by GC analysis as L-rhamnose. The HMBC spectra indicated that these compounds commonly bore an L-rhamnose moiety at their C-3 position, as determined by the H–C long range correlations of H-1″ [**2**: δ 4.01 (d, J = 1.5 Hz), **3**: 4.05 (d, J = 1.5 Hz), **5**: 4.96 (d, J = 1.5 Hz)] to C-3 (**2**: δ 75.7, **3**: 78.7, **5**: 74.7). Additionally, the bonding positions of their acetyl moieties were confirmed from the correlations between their rhamnose proton signals [**2**: δ 4.90 (dd, J = 10, 3, H-3″), **3**: 4.85 (t, J = 10, H-4″), **5**: 4.65 (t, J = 10, H-4″)] and carbonyl carbon signals [**2**: δ 172.7 (Me<u>C</u>O-3″), **3**: 172.6 (Me<u>C</u>O-4″), **5**: 172.6 (Me<u>C</u>O-4″)]. The absolute configurations of the C-2 of **2**, **3**, and **5** were determined to be 2*R* due to the detection of a positive Cotton effect at 330 nm and a negative Cotton effect at 295 nm in their CD spectra. From these results, the structures of 3″-acetyl astilbin (**2**), 4″-acetyl astilbin (**3**), and 4″-acetyl isoastilbin (**5**) were determined to be (2*R*,3*R*)-5,7,3′,4′-tetrahydroxyflavanonol 3″-acetyl rhamnoside or (2*R*,3*R*)-3″-acetyl astilbin (**2**), (2*R*,3*R*)-5,7,3′,4′-tetrahydroxyflavanonol 4″-acetyl rhamnoside or (2*R*,3*R*)-4″-acetyl astilbin (**3**), and (2*R*,3*S*)-5,7,3′,4′-tetrahydroxyflavanonol 4″-acetyl rhamnoside or (2*R*,3*S*)-4″-acetyl isoastilbin (**5**), respectively.

3″-Acetyl engeletin (**4**) was assigned the molecular formula $C_{23}H_{24}O_{11}$, as determined from its molecular ion $[M+Na]^+$ peak at 499.1237 in HRFABMS. The ^{1}H NMR spectrum of **4** indicated the structure to be a flavanonol rhamnoside like **2** from the similarity between the two spectra, and **4** was recognized to have a 1,4-disubstituted B-ring from its A_2B_2-type aromatic proton signals [δ 6.82 (d, J = 8 Hz), 7.36 (d, J = 8 Hz)] while ABX-type aromatic proton signals were observed in **2**. That is the only difference between these two compounds. Acid hydrolysis of **4** gave (+)-dihydrokaempferol (**19**) as an aglycone and a sugar moiety, which was identified as L-rhamnose by GC analysis. In the HMBC spectrum of **4**, correlations were observed (i) from the anomeric proton of rhamnose [δ 4.08 (d, J = 1.5 Hz) to C-3 (δ 79.2) and (ii) from H-3″ [δ 4.91 (dd, J = 10, 3 Hz)] to the carbonyl carbon (δ 172.6), which suggested its structure to be (2*R*,3*R*)-5,7,4′-trihydroxyflavanonol 3″-acetyl rhamnoside or (2*R*,3*R*)-3″-acetyl engeletin (**4**).

Corbulain Ia (**6**) and Ib (**7**) were assigned the molecular formula $C_{24}H_{20}O_8$, as determined from their molecular ion $[M+H]^+$ peak at *m*/*z* 437.1242 and the $[M]^+$ peak at *m*/*z* 436.1187 in HRFABMS, respectively. The ^{1}H and ^{13}C NMR spectroscopic data of **6** and **7** were similar to those of cinchonain Ia (**28**) and Ib (**30**), respectively. However, the ^{1}H NMR spectra showed the presence of a 1,4-disubstituted phenyl group [**6**: δ 7.09 (2H, d, J = 8.5 Hz), 6.68 (2H, d, J = 8.5 Hz); **7**: δ 7.28 (2H, d, J = 8.5 Hz), 6.77 (2H, d, J = 8.5 Hz)] and a 1,3,4-trisubstituted phenyl group [**6**: δ 6.60 (1H, d, J = 2 Hz), 6.69 (1H, d, J = 8 Hz), 6.51 (1H, dd, J = 8, 2 Hz); **7**: δ 6.52 (1H, d, J = 2 Hz), 6.60 (1H, d, J = 8 Hz), 6.42 (1H, dd, J = 8, 2 Hz)], instead of the two 1,3,4-trisubstituted phenyl groups found in the cinchonain I series. In the HMBC spectra of **6** and **7**, correlations were observed from the H-7″ [**6**: δ 4.44 (dd, J = 7,1.5); **7**: δ4.52 (dd, J = 7.5,1.5)] to C-2″ and 6″ [**6**: δ115.3 (C-2″), 119.4 (C-6″); **7**: δ115.0 (C-2″), 119.2 (C-6″)], which suggested the bonding position of the catechol group to be C-7″. The NOE spectrum of **7** indicated the configuration of C-7″ from enhancement of the H-2′ and H-6′ proton signals of its B-ring (δ 7.28) when H-7″ (δ 4.52) was irradiated, and no NOE enhancement was observed in the case of **6**. The CD spectra of **6** and **7** also showed good accordance to the corresponding compounds (**28** and **30**) (Chen et al., 1993). From these results, corbulain Ia (**6**) and Ib (**7**) were defined as 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*R*)-2H,8H-benzo [1,2-b:3,4-b′] dipyran-8-one (**6**) and 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxy phenyl)-(2*R*,3*R*,10*S*)-2H,8H-benzo [1,2-b:3,4-b′] dipyran-8-one (**7**).

Gnetumontanin E (**8**), F (**9**), and G (**10**) were assigned the molecular formula $C_{23}H_{18}O_6$, $C_{23}H_{18}O_7$, and $C_{24}H_{20}O_6$, respectively, as determined from their molecular ion peaks at *m*/*z* 390.1111 $[M]^+$, 406.1071 $[M]^+$, and 404.1288 $[M]^+$ in HRFABMS, respectively. The spectroscopic features of these compounds were very similar to one another and shared many features with those of gnetumontanin C, a stilbene analogue (Li et al., 2004). The ^{1}H NMR spectrum of **8** showed a pair of *meta*-coupled aromatic proton signals [δ 6.48 (d, J = 2.5 Hz) and 6.90 (d, J = 2.5 Hz)], aromatic proton resonances from a 1,3,4-trisubstituted phenyl group [δ 6.47 (dd, J = 8, 2 Hz), 6.53 (d, J = 2 Hz), and 6.70 (d, J = 8 Hz)], aromatic proton