Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1839–1842

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Phytoestrogens from the Roots of *Polygonum cuspidatum* (Polygonaceae): Structure-Requirement of Hydroxyanthraquinones for Estrogenic Activity

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Received 8 March 2001; accepted 28 April 2001

Abstract—The methanolic extract from the roots of *Polygonum* (*P.*) *cuspidatum* was found to enhance cell proliferation at 30 or 100 μg/mL in MCF-7, an estrogen-sensitive cell line. By bioassay-guided separation from *P. cuspidatum* with the most potent activity, emodin and emodin 8-*O*-β-D-glucopyranoside were isolated as active principles. The methanolic extracts from *Polygonum*, *Cassia*, *Aloe*, and *Rheum* species, which were known to contain anthraquinones, also showed the MCF-7 proliferation. As a result of the evaluation of various anthraquinones from plant sources and synthetic anthraquinones, aloe-emodin, chrysophanol, chrysophanol 8-*O*-β-D-glucopyranoside, and 1,8-dihydroxyanthraquinone showed weak activity. On the other hand, alizalin and 2,6-dihydroxyanthraquinone as well as emodin having the 2- and/or 6-hydoxyl groups showed potent activity. These results show that the unchelated hydroxyl group is essential for strong activity. Emodin and 2,6-dihydroxyanthraquinone also inhibited 17β-estradiol binding to human estrogen receptors (ERs) with K_i values of 0.77 and 0.31 μM for ERα and 1.5 and 0.69 μM for ERβ. These findings indicate that hydroxyanthraquinones such as emodin are phytoestrogens with an affinity to human estrogen receptors.

Insufficiency of endogenous estrogen secretion is known to cause several physical disorders in postmenopausal women, such as osteoporosis, hypercholesteremia, and symptoms of menopause (hot flush and depression). Synthetic estrogen-replacement therapy has been reported to be effective for these diseases. Recently, the estrogenic activity of isoflavones, lignans, and coumarins, which are widely distributed in vegetables, fruits, and medicinal plants, have been well investigated and these compounds are generally called phytoestrogens. In our study of new phytoestrogens guided by a proliferative activity of MCF-7,[1] an estrogen-sensitive cell line, several flavone glycosides (6″-acetylapiin, apigetrin, and apiin) isolated from *Petroselinum crispum* (parsley) were found to show estrogenic activity.[2]

In our continuous study of new types of phytoestrogen, we evaluated the estrogenic activity of the methanolic extracts from several medicinal herbs. MCF-7 suspended (2000 cells/100 μL) in phenol red-free Dulbecco's modified Eagle medium (D-MEM) containing 5% estrogen-free fetal calf serum (charcoal and dextran-treated FCS)[3] were seeded in a 96-well culture plate. After 24-h culture, whole medium was changed for fresh medium containing a test sample, and the cells were continuously cultured for 4 days. Proliferation of the cells was assessed by MTT assay. As shown in Table 1, *Cassia obtusifolia*, *Aloe* (*A.*) *arborescens*, *A. ferox*, *Polygonum* (*P.*) *multiflorum* and *Rheum* (*R.*) *palmatum* enhanced proliferation of MCF-7 at 100 μg/mL. On the other hand, *P. cuspidatum*, *R. tanguticum*, *R. officinale*, *R. coreanum*, *R. undulatum*, and *R. rhabarbarum* showed the activity at 30 μg/mL. These herbs with estrogenic activity are known to contain anthraquinones, and also many natural medicines containing anthraquinones have been traditionally used for prevention and palliation of menoxenia and postmenopausal disease.

Among these herbs, *P. cuspidatum* (Polygonaceae), which has been traditionally used for menoxenia, skin burn, gallstone, hepatitis, inflammation, and osteomyelitis in China, was found to show potent estrogenic activity. By bioassay-guided separation, emodin (**1**) and emodin 8-*O*-β-D-glucopyranoside (**2**) were isolated as active principles. Briefly, the methanolic extract of

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0960-894X/01/$ - see front matter
PII: S0960-894X(01)00318-3

P. cuspidatum was subjected to LH-20 column, which was successively eluted with water, methanol, and acetone. On evaluation of the estrogenic activities of these three eluates, the methanol and acetone eluates enhanced the MCF-7 proliferation. Continuously, the methanolic eluate was subjected to silica gel (SiO_2) column chromatography [$CHCl_3$–MeOH (10:1)→$CHCl_3$–MeOH–H_2O (10:3:1, lower layer)→MeOH] to give seven fractions. From active fractions 1–4, emodin (**1**, 0.086% from natural medicine) and emodin 8-*O*-β-D-glucopyranoside (**2**, 0.11%) were isolated by $AgNO_3$–SiO_2 column chromatography [*n*-hexane–AcOEt (8:1→4:1→1:1)→AcOEt] or by crystallization.

Emodin (**1**) and emodin 8-*O*-β-D-glucopyranoside (**2**) enhanced proliferation of MCF-7 from 1 to 10 μM in a concentration-dependent manner (Table 2). Next, we obtained several anthraquinones from the rhizome of *Rheum* species[4] and evaluated their activity (Chart 1). Physcion (**5**), rhein (**6**), and sennosides A (**7**) and B (**8**) did not show any activity, while aloe-emodin (**3**) and chrysophanol (**4**) showed weak activity. Among anthraquinone glycosides, aloe-emodin 1-*O*-β-D-glucopyranoside (**3a**) and chrysophanol 1-*O*-β-D-glucopyranoside (**4a**) did not show any activity, while emodin 8-*O*-β-D-glucopyranoside (**2**) and chrysophanol 8-*O*-β-D-glucopyranoside (**4b**) showed weak activity. By comparison of the activities of **1**–**8**, the 6-hydroxyl group seemed to be essential for enhancement of the estrogenic activity. On the other hand, the glucopyranosyl moiety at the 1- or 8-position seemed to reduce the activity.

To evaluate the structure–activity relationships of hydroxyanthraquinones for the estrogenic activity, we examined the activity of several synthetic anthraquinones. Alizarin (**10**) and 2,6-dihydroxyanthraquinone

Table 1. Estrogenic activities of methanolic extracts from various herbs

	Part	MCF-7 proliferation (% of control)	
		30 μg/mL	100 μg/mL
Cassia obtusifolia	Seed	98.2±3.3	133.6±4.9*
Aloe arborescens	Leaf	98.2±3.3	125.7±4.2**
Aloe ferox	Leaf	96.5±3.7	161.5±4.7**
Polygonum cuspidatum	Root	170.7±4.0**	276.7±4.0**
Polygonum multiflorum	Root	102.4±3.2	149.3±1.8**
Rheum palmatum	Rhizome	101.4±4.7	125.3±7.0**
Rheum tanguticum	Rhizome	113.5±5.3**	145.8±7.8**
Rheum officinale	Rhizome	150.9±6.8**	—
Rheum coreanum	Rhizome	121.3±3.4**	147.2±4.2**
Rheum undulatum	Rhizome	133.9±2.4**	—
Rheum franzenbachii	Rhizome	102.8±1.5	115.7±1.2
Rheum rhabarbarum	Stem	126.8±5.6**	152.6±7.3**

Each value represents the mean±SEM of six experiments. Asterisks denote significant differences from the control at * $p<0.05$, ** $p<0.01$.

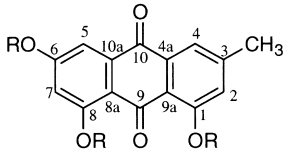
Table 2. Estrogenic activities of anthraquinones

	MCF-7 proliferation (% of control)		
	1 μM	3 μM	10 μM
Emodin (**1**)	126.6±1.2**	165.0±5.7**	196.6±4.3**
Emodin trimethyl ether (**1a**)	99.1±3.0	109.3±3.5	127.2±5.1**
Emodin 8-*O*-β-D-glucopyranoside (**2**)	113.1±3.5*	118.5±3.2**	159.8±3.0**
Emodin 8-*O*-β-D-glucopyranoside hexamethyl ether (**2a**)	88.0±3.2	91.0±3.5	98.3±4.0
Aloe-emodin (**3**)	99.2±3.7	102.9±4.7	126.9±4.7**
Aloe-emodin 1-*O*-β-D-glucopyranoside (**3a**)	95.0±2.4	102.3±2.3	107.0±1.7
Chrysophanol (**4**)	118.5±4.8**	111.0±2.7	124.1±4.3**
Chrysophanol 1-*O*-β-D-glucopyranoside (**4a**)	105.8±3.4	104.9±4.1	114.4±4.3
Chrysophanol 8-*O*-β-D-glucopyranoside (**4b**)	123.7±2.8**	117.8±4.9*	111.1±4.4
Physcion (**5**)	104.2±1.4	94.0±2.1	99.2±4.2
Rhein (**6**)	96.0±1.9	97.3±4.6	99.5±3.2
Sennoside A (**7**)	90.6±5.7	108.1±5.8	104.6±4.9
Sennoside B (**8**)	83.5±4.5	92.3±3.1	97.1±2.0
Anthraquinone (**9**)	112.7±5.4	109.8±4.3	114.0±5.1
Alizarin (**10**)	130.1±4.8**	157.5±3.9**	143.0±2.8**
Alizarin dimethyl ether (**10a**)	100.0±4.7	113.0±4.9	108.4±2.3
1,4-Dihydroxyanthraquinone (**11**)	109.0±6.5	106.6±6.0	104.1±5.9
1,5-Dihydroxyanthraquinone (**12**)	105.7±2.6	107.0±4.2	113.9±5.8*
1,8-Dihydroxyanthraquinone (**13**)	121.0±3.9**	114.3±1.4*	122.4±2.1**
2,6-Dihydroxyanthraquinone (**14**)	170.0±4.2**	164.0±1.5**	167.4±6.7**
2,6-Dimethoxyanthraquinone (**14a**)	85.0±4.0	97.0±2.2	108.3±2.0
Daidzein	170.9±3.7**	—	170.1±7.4**
Genistein	171.5±5.8**	—	197.2±12.4**

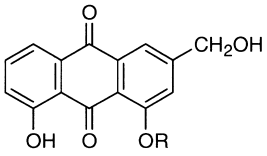
Each value represents the mean±SEM of six experiments. Asterisks denote significant differences from the control at * $p<0.05$, ** $p<0.01$.

(**14**) having the 2- and/or 6-hydroxyl groups showed potent estrogenic activity at 1–10 μM. The activities of methylated derivatives (**1a**, **2a**, **10a**, and **14a**) were weaker than those of **1**, **2**, **10**, and **14**. These results suggested that the unchelated hydroxyl group was essential for the strong estrogenic activity.

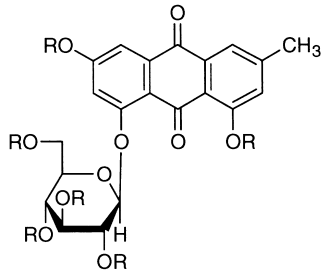
From the evaluation of estrogen receptors (ERα and ERβ) knockout mice, ERα, but not ERβ, is recognized to mediate skeleton growth.[5] To clarify the affinity of the active anthraquinones [emodin (**1**), emodin 8-*O*-β-D-glucopyranoside (**2**), and 2,6-dihydroxyanthraquinone (**14**)] to ERα and ERβ, a competitive binding assay was performed using 17β-estradiol and human estrogen receptors (Ligand Screening System: Estrogen, Toyobo Co., Ltd., Osaka, Japan). Aloe-emodin (**3**) and chrysophanol (**4**) did not compete with ERα and ERβ for 17β-estradiol binding at a high concentration (40 μM). Emodin (**1**) and 2,6-dihydroxyanthraquinone (**14**) competed for 17β-estradiol binding with both ERα and ERβ. Their IC_{50} (K_i) values were 2.7 and 1.1 μM (0.77 and 0.31 μM) for ERα and 5.2 and 2.4 μM (1.5 and 0.69 μM) for ERβ, respectively (Table 3). The ranking of the estrogenic potency of the compounds for both human



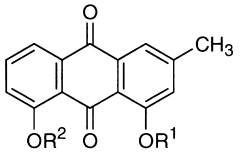
emodin (**1**) : R=H
emodin trimethyl ether (**1a**) : R=CH_3



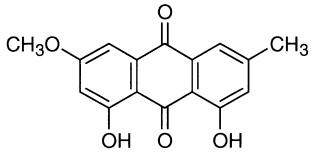
aloe-emodin (**3**) : R=H
aloe-emodin 1-*O*-β-D-glucopyranoside (**3a**) : R=Glc



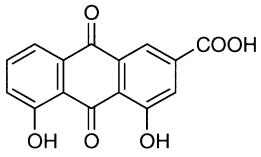
emodin 8-*O*-β-D-glucopyranoside (**2**) : R=H
emodin 8-*O*-β-D-glucopyranoside hexamethyl ether (**2a**) : R=CH_3



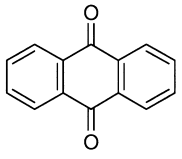
chrysophanol (**4**) : R^1=R^2=H
chrysophanol 1-*O*-β-D-glucopyranoside (**4a**) : R^1=Glc, R^2=H
chrysophanol 8-*O*-β-D-glucopyranoside (**4b**) : R^1=H, R^2=Glc



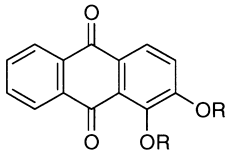
physcion (**5**)



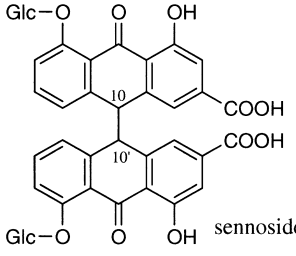
rhein (**6**)



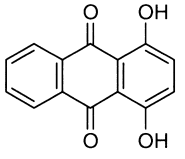
anthraquinone (**9**)



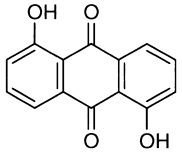
alizarin (**10**) : R=H
alizarin dimethyl ether (**10a**) : R=CH_3



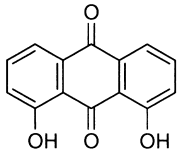
sennoside A (**7**) : *threo*
sennoside B (**8**) : *erythro*
10—10'



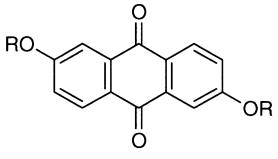
1,4-dihydroxyanthraquinone (**11**)



1,5-dihydroxyanthraquinone (**12**)



1,8-dihydroxyanthraquinone (**13**)



2,6-dihyrdoxyanthraquinone (**14**) : R=H
2,6-dimethoxyanthraquinone (**14a**) : R=CH_3

Glc : β-D-glucopyranosyl

Chart 1.

Table 3. Inhibitory activities of phytoestrogens on 17β-estradiol binding to human estradiol receptors (ERα and ERβ)

	IC_{50} (K_i) (μM)	
	ERα	ERβ
Emodin (**1**)	2.7 (0.77)	5.2 (1.5)
Emodin 8-*O*-β-D-glucopyranoside (**2**)	20 (5.7)	62 (18)
2,6-Dihydroxyanthraquinone (**14**)	1.1 (0.31)	2.4 (0.69)
Daidzein	6.3 (1.8)	1.1 (0.30)
Genistein	1.3 (0.37)	0.083 (0.024)

K_i values were calculated by the following formula: $K_i = \frac{IC_{50}}{1 + [L]/K_d}$ $K_d = 4 \times 10^{-9}$ M, $[L] = 1 \times 10^{-8}$ M (L = 17β-estradiol).

ER subtypes is different; that is, 2,6-dihydroxyanthraquinone (**14**), genistein > emodin (**1**) > daidzein≫emodin 8-*O*-β-D-glucopyranoside (**2**) for ERα and genistein≫daidzein > 2,6-dihydroxyanthraquinone (**14**) > emodin (**1**) > emodin 8-*O*-β-D-glucopyranoside (**2**) for ERβ.

In conclusion, the methanolic extracts from *Polygonum*, *Cassia*, *Aloe*, and *Rheum* species were found to enhance the proliferation of MCF-7, an estrogen-sensitive cell line. Emodin (**1**, 1–10 μM) and emodine 8-*O*-β-D-glucopyranoside (**2**, 1–10 μM) from *P. cuspidatum* enhanced MCF-7 proliferation, and this is the first report for estrogenic activity of anthraquinones. Concerning structural requirements of anthraquinones for the activity, the unchelated hydroxyl group is essential for the strong estrogenic activity. The findings that emodin (**1**) bound to human ERα and ERβ may be useful for replacement therapy for human menoxenia and postmenopausal diseases.

References and Notes

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